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COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

STATUS AND TRENDS OF THE CONSERVATION AND SUSTAINABLE USE OF MICROORGANISMS IN AGROINDUSTRIAL PROCESSES

By

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This document has been prepared at the request of the Secretariat of the FAO Commission on Genetic Resources for Food and Agriculture, and in close collaboration with the FAO Rural Infrastructure and Agro-Industries Division, to facilitate the Commission's discussions when it will review key issues in micro-organisms and invertebrates at its Fourteenth Regular Session.

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Abbreviations and Acronyms

AMF:	Arbuscular Mycorrhizal Fungi
ASPAC:	Asian-Pacific
ATMA:	Agricultural Technology Management Agency
B.:	*Bacillus*
BDAs:	Biodiversity Development Agreements
BIS:	Bureau of India Standards
BRCs:	Biological Resource Centers
CAGR:	Compound Annual Growth Rate
CAP:	Common Agricultural Policy
DNA:	Deoxyribonucleic Acid
E. coli:	*Escherichia coli*
EM:	Effective Microorganisms
EPA:	Environmental Protection Agency
EPS:	extracellular polymeric substances
FAO:	Food and Agriculture Organization of the United Nations
FOS:	Fructooligosaccharides
GHG:	Greenhouse Gas
GIA:	Global Industry Analysis
GMOs:	Genetically Modified Rumen Microorganisms
ICAR:	Indian Council of Agricultural Research
ICARDA:	International Centre for Agricultural Research in Dry Areas
INM:	Integrated Nutrient Management
IOC:	International Oil Council
IPM:	Integrated Pest Management
ISOPOM:	Integrated Scheme of Oilseeds, Pulses, Maize and Oil palm
LAB:	Lactic Acid Bacteria
LGT:	Lateral Gene Transfer
MDA:	Multiple Displacement Amplification
mRNA:	messenger Ribonucleic Acid
NABARD:	National Bank for Agricultural and Rural Development
NBDC:	National Biofertilizer Development Centre
NFM:	Nitrogen Fixing Microbes
NFSM:	National food Security Mission
NGO:	Non-Governmental Organization
NGS:	Next-Generation Sequencing
NPOF:	National Project on Organic Farming
OECD:	Organization for Economic Cooperation and Development
P.:	*Pseudomonas*
PCR:	polymerase chain reaction
PGPMs:	plant growth-promoting microorganisms
PGPRs:	plant growth-promoting regulators
PSBs:	Phosphate Solubilizing Bacteria
PSM:	Phosphorus Solubilizing Microbes
qPCR:	quantitative real-time Polymerase Chain Reaction
RCOF:	Regional Climate Outlook Forums
RGPR:	plant growth promoting Rhizobacteria

RNA:	Ribonucleic Acid
rRNA:	ribosomal Ribonucleic Acid
RT-PCR:	Reverse Transcription Polymerase Chain Reaction
SAGs:	Single Amplified Genomes
SIP:	Stable-Isotope Probing
SmF:	Submerged Fermentation
SOM:	Soil Organic Matter
SSF	Solid State Fermentation
TFAC:	Tokachi Federation of Agricultural Cooperatives
TMOP:	Technology Mission on Oilseeds
WDCM:	World Data Center for Microorganisms
WFCC:	World Federation of Culture Collections

EXECUTIVE SUMMARY

Microorganisms play an important role in plant health, soil fertility and agricultural sustainability. The use of chemicals (fertilizers, pesticides) in agriculture cause critical problems such as soil-water pollution, marine eutrophication, and killing of beneficial insects (as natural enemies of harmful insects) including pollinators. Sustainable agriculture promotes the activity of beneficial soil microorganisms engaged in the nutrient cycles, supports biodiversity by reducing nitrogen and other forms of chemical nutrient pollution, and provides other benefits via rotation practices and planting of leguminous crops to supply nitrogen. It also includes the use of composting methods and of biological control agents.

Microorganisms are used in agro-industry as bio-fertilizers and/or bio-inoculants, Effective Microorganisms (EM), bio-pesticides and bio-remediating indicators.

Biofertilizers/bioinoculants are preparations containing living cells of efficient microbial strains, artificially multiplied, and able to colonise the rhizosphere or the interior of the plant. When applied on seed, soil or plant surfaces, they promote growth by increasing the supply or availability of nutrients in a form easily assimilated by plants. Several microorganisms exploited in the production of biofertilizers are mentioned in this study. Research should focus on improving the efficacy of biofertilizers/biopesticides by manipulating/modulating the biological agents, by adapting formulations, and by applying advanced technologies for developing new strains. This should enable farmers to provide economically feasible new products beneficial to public health. Effective Microorganisms comprise a mixture of beneficial, naturally occurring microorganisms, used as inoculants to the soil/plant ecosystem for increasing the microbial diversity of soils, for improving soil quality, soil health, and for enhancing the growth, yield and quality of crops.

Biopesticides are pest management agents based on living microorganisms or natural products, with the aim to protect agricultural crops against insects, fungal, bacterial and viral diseases, weeds and nematodes. The current commercial status of biopesticides has been reviewed in this study. In addition, important technical barriers towards improving the effectiveness of biopesticides, and contemporary opportunities promoting the development of biopesticides for Integrated Pest Management (by combining ecology with post-genomic technologies), are discussed.

Organic wastes from agro-industry (crop residues and animal manure) may cause serious environmental and health problems. Agro-industrial residues are abundant sources of sugar rich lignocellulose that can be converted into fermentable sugars as potential energy sources for microbial fermentation. By this transformation, agro-industrial residues are converted into value added products such as biofertilizers (compost), functional metabolites (enzymes, food additives, organic acids, pigments) and bio-fuels. Members of different genera of microorganisms are involved in these processes.

Bio-remediation is the degradation of environmental contaminants (xenobiotics) into less toxic forms, using naturally occurring microorganisms (bacteria, fungi, algae) to degrade or detoxify substances hazardous to human health and the environment.

This report provides an assessment of the current status and trends on the use and conservation of microorganisms in agro-industrial processes. Furthermore, the relevance of protecting microbial biodiversity, and the impact of climate change on the diversity of microorganisms and their use in agro-industrial processes, were explored.

Moreover, the implemented policy in a number of developing and developed countries has been recorded. Resulting from e-mailed questionnaires, information was obtained on market trends,

aspects and views of companies' executives and scientists, and on potential future prospects for the use of microorganisms and microbial products in agro-industrial processes.

I. INTRODUCTION

I.1. Background and rationale

This study has been prepared by Handong Global University at request of the Secretariat of the FAO Commission on Genetic Resources for Food and Agriculture (the Commission) and in close collaboration with the FAO Rural Infrastructure and Agro-Industries Division. The Commission, at its Twelfth Regular Session in July 2011, emphasized the need for assessing the status and trends of microorganisms relevant to food and agriculture. In this context, it requested, *inter alia*, the preparation of an analytical study on the status and trends of microorganisms in agro-industrial processes.

I.2. The demand for an environmental friendly agriculture

Agro-industry is an industry dealing with the supply, processing and distribution of farm products. It is related to the large-scale production, processing and packaging of food using modern equipment and methods. The agro-industrial sector is a component of the manufacturing sector, where value is added to agricultural raw materials, derived from agriculture, fisheries, forestry, and intermediate products through processing and handling operations. Most agricultural, fisheries and forestry production is subject to some form of transformation prior to eventual end use.

While global demand for food increases, pressure is increasing on natural resources either already under stress or scarce, or on crops that may also be used as sources of bio-energy and for other industrial purposes. Climate change drives more nations in converting crops into bio-fuels as an alternative to fossil fuels. However, in the future, bio-fuel refineries should depend less on food crops and more on organic wastes and residues, like corn stalks, rice hulls, sawdust, or waste paper. Other sources of renewable biomass include drought-resistant grasses, fast-growing trees, and several other energy crops that will grow on marginal lands unsuitable for raising food plants.

Agriculture may adapt and contribute to the mitigation of climate change (global warming and increased climate variability) thereby raising new parameters (e.g., solar radiation, temperature, precipitation and the consequences of increasing drought or flooding). Climate changes cause a shift in agricultural zones towards the poles, lead to changes in production and precipitation patterns, and may cause extreme weather events and higher vulnerability of crops to infection, pests and weeds. Climate change is a stress factor for ecosystems, putting their structure and functioning at risk, but is also likely to have a negative effect on agricultural productivity, particularly in the tropical regions, and could directly affect poor people's assets, including access to water and natural resources, homes and infrastructure. Most developing countries are highly dependent on subsistent agriculture and other climate sensitive natural resources for income and well-being, while coincidently lacking sufficient financial and technical capacities to manage climate risk (Skoufias *et al.*, 2011) (http://ww.wds.worldbank.org/servlet/WDSContentServer/WDSP/IB/2011/04/04/000158349_20110404100435/Rendered/PDF/WPS5622.pdf).

Agriculture remains the most important economic activity in the world, employing 45% of the working population whereas in some parts of Asia and Africa over 80% of the labour force is dedicated to agriculture.

The major types of agriculture are:

- **Industrialised agriculture**, which demands mechanisation and consequently non renewable fossil fuel energy (mostly oil and natural gas), heavy water use for irrigation (leaving behind salts in topsoil), single crops/monoculture of selected cultivars (causing loss of diversity),

conventional and genetically modified seeds, and commercial agro-chemicals (inorganic fertilizers and sewage sludge to supply plant nutrients and synthetic pesticides). As industrialised agriculture is globally export-oriented, the tendency is toward intensive production, thereby driving out small producers (Horrigan *et. al.,* 2002).

- **Plantation agriculture**, a form of industrial agriculture in tropical developing countries, dealing with cash crops.
- **Traditional subsistence agriculture.**
- **Traditional intensive agriculture** which refers to the production of enough food for a farm family's survival and a surplus that can be sold. It uses higher inputs of labor, fertilizer, and water than traditional subsistence agriculture but a much lower scale than industrialized agriculture.
- **Urban agriculture**, implemented by migrants to cities in developing countries (FAO, 2007, ftp://ftp.fao.org/docrep/fao/010/a1471e/a1471e00.pdf).

It has been estimated that net investments of 83 billion US$ per year must be made in agriculture in developing countries, in order to retain enough food for all people by 2050. To obtain increased yields, intensive application of inorganic nitrogen and other chemical fertilizers is needed, in addition to increased irrigation, greater application of pesticides and herbicides, and the use of hybrid seeds or genetically modified crops. As a result, the farmers are drawn into the control of international corporations that own the patents of those crops. In fact, the trend away from smaller family farms to larger farms has resulted in economic immobility of many rural areas. Generally, the use of better seeds, fertilizer, farming methods and equipment, contributes to soil degradation and loss of plant diversity. The use of heavy machinery in soil preparation has led to compaction and other detrimental changes in soil structure. Desertification is attributed to erosion, soil compaction, forest removal, overgrazing, salinization, climate change and depletion of water sources. Many arid and semiarid lands suffer and this induces a productivity loss of more than 10%. Desertification affects 1/3 of the planet's land area in over 100 countries and induces costs of 10 billion US$/year. China loses over 6.5 billion US$/year (from goat overgrazing) and in Kenya 80% of the land is vulnerable to desertification from overgrazing and deforestation. At present, agriculture is forced to compete for land and water with urban sprawl and is facing scarcity of land and water. Moreover, intensive agriculture is leading to a depletion of water resources. Industrialised farming agribusiness means increasing the number of giant multinational corporations, subsidized through taxes, also resulting in prices being kept artificially low, in the diversion of food crops to bio-fuels production, and in the commercial behaviour in commodity markets responsible for almost half of the increase in the prices of the major food crops.

I.2.1 The use of microorganisms in agro-industrial processes

Types of microorganisms and their application in agro-industrial processes are depicted in Table 1, and may be summarised as follows:

- **Bio-fertilizers**, usually defined as preparations containing living cells of efficient strains of microorganisms artificially multiplied, which colonise the rhizosphere or the interior of the plant, when applied through seed, soil or plant surfaces and they promote growth.
- **Effective Microorganisms (EM) Technology**, based on mixed cultures of beneficial and naturally occurring microorganisms that are used in many systems pertaining to sustainable practices in agriculture and environmental management.

- **Composting as a process of agro-industrial wastes.** Organic residues**,** wastes from human, animal, agricultural and industrial establishments, can be bio-converted into value added products as biofertilizers and biofuels. Additional composting processes are: (i) Vermicomposting, (ii) Production of compost using mushroom-bed, (iii) Regeneration of energy (methane, heat, etc), (iv) Using organic and biodegradable substrates (wastes) for generating gaseous mixtures (mainly methane, but also carbon dioxide, nitrogen, hydrogen, hydrogen sulfide), referred as "Biogas", and (v) Production of bio-fuels, food additives, organic acids, enzymes, and pigments.
- **Lignocellulose conversion into valuable products** using microorganisms for Solid State Fermentation.
- **Bioremediation** by degradation of environmental contaminants (xenobiotics) into less toxic forms, using living organisms, primarily microorganisms.

Table 1. Microorganisms and their specific functions in agro-industrial processes

Agro-industrial processes	Microorganisms	Specific function
	Azotobacter, Beijerinckia, Clostridium, Klebsiella, Anabaena, Nostoc	**Free living N_2 fixers, beneficial to a wide array of crops**
	Rhizobium, Frankia, Anabaena azollae	**Symbiotic N_2 fixers, fix nitrogen in symbiotic association with certain legumes**
	Azospirillum	**Associative N_2 fixers**
	Bacteria: *Bacillus megaterium* var. *phosphaticum, Bacillus subtilis, Bacillus circulans, Pseudomonas striata* Fungi: *Penicillium* sp., *Aspergillus awamori*	**Phosphate solubilizers**
	Arbuscular mycorrhizae: *Glomus* sp., *Gigaspora* sp., *Acaulospora* sp., *Scutellospora* sp., *Sclerocystis* sp., Ectomycorrhizae: *Laccaria* sp., *Pisolithus* sp., *Boletus* sp., *Amanita* sp. Ericoid mycorrhizae: *Pezizella ericae* Orchid mycorrhizae: *Rhizoctonia solani*	**Phosphate mobilizers or absorbers**
Biofertilizers/ Bioinoculants	*Pseudomona, Bacillus, Rhizobium, Serratia, Azotobacter, Paenibacillus, Azospirillum, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Agrobacterium radiobacter, Mycobacterium, Streptomyces griseoviridis* Biocontrol agents: *Pseudomonas fluorescens, Bacillus pumilus, Bacillus subtilis, Bacillus cereus, Azospirillum* Mycofungicides: *Ampelomyces quisqualis, Trichoderma harzianum, trichoderma polysporum, Trichoderma viride, Fusarium oxysporum, trichoderma* spp., *Coniothyrium minitans, Choetomium cupreum, Chaetomium globosum, Pythium oligandrum, gliocladium catenulatum, Gliocladium virens, Candida oleophila* PGPRs protect from stress: *Agrobacterium genomovars, Azospirillum lipoferum, Alcaligenes, Bacillus, Burkholderia, Enterobacter, Methylobacterium fujisowaense, Pseudomonas, Ralstonia solanacearum, Rhizobium, Rhodococcus, Sinorhizobium meliloti, Variovorax paradoxus*	**Plant growth promoting Rhizobacteria affecting plant growth and development, directly or indirectly**
	Ectomycorrhizae: *Pisolithus tinctorus*	**Plant growth promoting fungi able to promote plant growth**

	Arbuscular mycorrhizae: *Glomus intraradices* *Trichoderma harzianum, Trichoderma hamatum, Trichoderma* spp. *Phoma* sp., *Penicillium simplicissimum, Alternaria, Cladosporium, Dematium, Gliocladium, Helminthosporium, Humicola, Metarhizium*	
Biopesticides	Bacteria: *Bacillus thuringiensis, Pseudomonas fluorescens, Bacillus popilliae, Serratia entomophila* Fungi: *Verticillium lecanii, Chondrosterum purpureum, Metarhizium anisopilae, Beauveria bassiana, Paecilomyces fumosoroseus* Viruses: *Cydia pomonella, Lymantria dispar, Neodiprion sertifer*	**Ability to protect crops against insect pests, fungal and bacterial diseases and weeds**

I.3. Scope of the study

The main aim of the study was to assess the current status and trends of the use and conservation of microorganisms in agro-industrial processes. The study explored technologies associated with the use of microorganisms, and investigates the technological needs required for enhancing the utilisation of microorganisms in agro-industrial processes.

Beneficial microorganisms are of extreme importance in agro-industrial processes, but current status and trends in their uses and conservation are not well documented, especially for the countries with developing economies. This study aims to gather existing information that is expected to contribute by enhancing the understanding of the use of microorganisms in agro-industrial processes such as crop fertilization, the improvement of soil fertility dealing with pests, the treatment of agro-industrial wastes, bioremediation, and the production of bio-energy, and also their use in biotechnology processes (e.g., the production of enzymes, fragrances and additives). There is also a need for collecting information on the diversity of microorganisms in order to develop strategies for expanding and enhancing their utilisation in agro-industrial processes, where appropriate. While the use of microorganisms in agro-industrial processes such as in fermentation is well established, their potential uses and the challenges for enhancing other agro-industrial processes, require better understanding and documentation. In addition, there is a need for exploring the possible effects of climate change on the diversity of microorganisms used in agro-industrial processes, as a basis for developing appropriate mitigation strategies.

I.3.1. Objectives

The specific objectives of the present study are to:

- Identify and document the **current status and trends** in the use of microorganisms in agroindustrial processes.
- Explore the relevancy of the **need for diversity** of microorganisms.
- **Identify the main microorganisms** that are used in agro-industrial processes and identify their **specific uses.**
- Explore and identify any **conservation practices**, including traditional management practices that are used to **safeguard this diversity.**
- Explore any **gaps** in terms of **knowledge, technologies and policies,** which could contribute to the improved use of microorganisms in agro-industrial processes.
- Identify possible **threats or opportunities** to the use of microorganisms in agro-industrial processes.
- Explore the past, current and potential future **impact of climate change** on the use of microorganisms in agro-industrial processes.

I.3.2 Methodology

This study is based on the collection of existing information. Initially, an extensive and in-depth review of the available information was conducted. The review included available reports, project documents and other literature and data relevant to the study. Information was gathered from both private and public sectors. Additional information and data from previous work experience in this domain derived from the Agricultural University of Athens, and, furthermore, information and data originating from other institutions or stakeholders were used. A few relevant examples of the on-going or recently completed activities towards the enhancement of the use of microorganisms in agro-industrial processes are reported. Field and agro-industry visits were made in order to gather important information. Questionnaires were prepared and distributed to agro-industrial companies, in order to obtain a complete view on uses of microorganisms in agro-industrial processes.

II. THE CURRENT STATUS AND TRENDS IN THE USE OF MICROORGANISMS IN AGRO-INDUSTRIAL PROCESSES

II.1. Microbes and microbial products

According to BCC (Business Communication Co.) Research Report (2011) the total global market for microbes and microbial products was worth more than 144 billion US$ in 2010, 156 billion US$ in 2011, and was projected to amount to 259 billion US$ in 2016 (Fig. 1), with a Compound Annual Growth Rate (CAGR) of 10.7%. Microbial products comprise the largest segment of the market, with a value of 151 billion US$ in 2011 and an expected 252 billion US$ in 2016, with an increase at a CAGR of 10.8%. BCC estimated markets for microbes (e.g. bio-fertilizers, bio-pesticides and probiotics) amounted to nearly 4.5 billion US$ in 2010, 4.9 billion US$ in 2011 and were projected to approach 6.8 billion US$ in 2016, representing a CAGR of 6.9% over the forecast period. The healthcare sector accounts for more than 60% of the total market for microbes and microbial products. The market for microbes and microbial products appears to have a significant and bright commercial potential in the future.

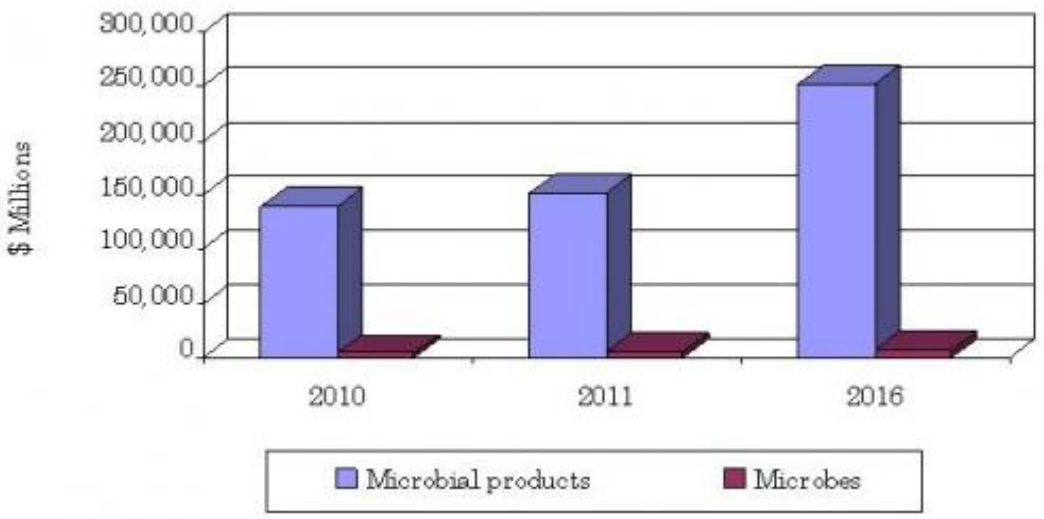

Figure 1. Trends in the global market for microbes and microbial products, 2010-2016 (in US$ Millions) (BCC Research, April 2011, *http://www.bccresearch.com/report/microbial-products-markets-bio086a.html*)

II.2. Bio-fertilizers

A bio-fertilizer or microbial inoculant is a substance that contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonise the rhizosphere or the interior of the plant, and promote growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Bio-fertilizers are essential components of organic agriculture and play a vital role in maintaining long-term soil fertility and sustainability, but also ensure the production of safe and healthy food. Bio-fertilizers are not fertilizers that give nutrition directly to crop plants. They are cultures of microorganisms like bacteria, fungi, algae alone or in combination, packed in a carrier material. Therefore, the critical component in bio-fertilizers is the microorganism(s) (Mahdi *et al*., 2010).

Bio-fertilizers are low cost, effective and renewable sources of plant nutrients that potentially supplement chemical fertilizers, and, in this way, provide an economically viable support to small and marginal farmers for realising the ultimate goal of increasing productivity (Boraste *et al.*, 2009). Some inoculants can improve plant uptake of nutrients and thereby increase the use efficiency of applied chemical fertilizers and manures. As a natural method it does not cause any problems like salinity, alkalinity and soil erosion.

The contribution of bio-fertilizers depends upon the efficacy of the microbial strains used. Efficient strains, suitable for a given soil and climatic conditions, have to be isolated and identified, multiplied (grown/cultivated) in the laboratory, and packed in carrier materials (peat, lignite powder) in such a way to guarantee sufficient shelf life and distribution.

Important parameters in bio-fertilizer technology are:

- the use of a proper formulation of inocula preparations,
- the selection of an adequate carrier,
- the design of correct delivery methods.

Mass production techniques and formulation development protocols have to be standardised to increase the shelf life of the formulation.

II.2.1. Microorganisms

A successful microbial strain should possess features such as high rhizosphere competence, high competitive saprophytic ability, the potential for enhancing plant growth, ease for mass multiplication, a broad spectrum of action, enabling excellent and reliable control, safety to the environment, compatibility with other rhizobacteria, and tolerance to desiccation, heat, oxidizing agents and UV radiation (Nakkeeran *et al.*, 2005).

Beneficial microorganisms belong to a wide array of genera, classes and phyla, ranging from bacteria to yeasts and fungi, with the ability to support plant nutrition by different mechanisms (Fuentes-Ramirez and Caballero-Mellado, 2005; Mahdi *et al.*, 2010; Ahemad and Khan, 2011).

The main genera of beneficial microorganisms and their impact on plant growth are summarised in Table I of the Annex.

Nitrogen fixers

Biological nitrogen fixation occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. The process is coupled with the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H_2. In free-living diazotrophs, the nitrogenase-generated ammonium is assimilated into glutamate through the glutamine/glutamate synthase pathway.

Nitrogen fixers can be divided into the following groups:

a) Symbiotic nitrogen fixing bacteria, which include members of family *Rhizobiaceae* (*Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium* and *Allorhizobium*), able to enter a symbiotic relationship with leguminous plants. *Frankia* is a nitrogen fixing Actinomycete infecting and nodulating a group of eight families of mainly woody plants. (Gentili and Jumpponen, 2006).

b) Non-symbiotic (free-living, associative and endophytic) nitrogen fixing bacteria (e.g., *Acetobacter diazotrophicus, Herbaspirillum* sp., *Azoarcus* spp., *Alcaligenes, Azospirillum, Bacillus, Enterobacter, Klebsiella, Pseudomonas, Rhizobium, Azotobacter, Burkholderia, Beiferinckia* and *Clostridium*), and

c) Cyanobacteria (formerly called "blue-green algae") including *Aulosira, Trichodesmium, Anabaena, Cylindrospermum, Nostoc plectonema* and *Tolypothrix.* Cyanobacterial nitrogen fixation has been essential in the cultivation of rice and is most important for rice-field fertility (Anand and Pereira, 2011).

Phytohormones and plant growth-regulator producers

The majority of soil microorganisms can produce plant growth promoting regulators (PGPR), such as auxins (IAA), cytokinins, gibberellins, ethylene, absisic acid and enzymes. The auxins are synthesized from microorganisms and improve plant growth and development. Various PGPR species belonging to the genera *Azospirillum, Pseudomonas, Xanthomonas, Rhizobium, Bradyrhizobium, Alcaligenes, Enterobacter, Acetobacter* and *Klebsiella*, and also the species *Bacillus pumilus, B. licheniformis* and *Paenibacillus polymyxa*, possess the ability to excrete phytohormones. Similarly, strains of plant-associated phototrophic purple bacteria, *Methylobacterium* sp. and different other bacterial species such as *Proteus mirabilis, P. vulgaris, Klebsiella pneumoniae, Bacillus megaterium, B. cereus* and *Escherichia coli*, have been reported to synthesize plant growth regulators.

Microorganisms able to produce indole-3-acetic acid (IAA) in the presence of the precursor tryptophan or peptone are: *Erwinia herbicola, Bradyrhizobium, Klebsiella* and *Enterobacter, Agrobacterium tumefaciens, Pseudomonas syringae, Pseudomonas putida, P. fluorescens, Rhizobium* spp., *Bradyrhizobium* spp. and *Azospirillum* (Ahemad and Khan, 2011). IAA is produced during all stages of culture growth but maximum amounts are formed after the stationary phase, or during steady-state growth (Khalid *et al.*, 2009; Bashan and de-Bashan, 2010; Ahemad and Khan, 2011).

Phosphate solubilizers and absorbers

Solubilization of insoluble inorganic phosphorus is an environment-friendly and economically feasible strategy for improving crop production in low P soils. Organic forms of P are found in humus or other organic materials, including decayed plant, animal and microbial tissues. These constitute an important reservoir of immobilised P, accounting for about 20-80% of total soil phosphorus. A large portion of soluble inorganic P is applied to the soil as fertilizer. Due to its rapid rate of fixation and

complex formation with other elements of soils it is speedily immobilised and becomes unavailable to plants.

Several reports refer to different bacterial species endowed with phosphate solubilizing activity, often termed Phosphate Solubilizing Bacteria (PSB) that may provide the available forms of phosphorus to plants and consequently a viable substitute to chemical phosphatic fertilizers. Among the bacterial genera with this activity are: *Pseudomonas, Bacillus, Rhizobium, Azospirillum halopraeferens* and strains of *A. brasilense* and *A. lipoferum, Burkhodleria, Achromobacter, Agrobacterium, Micrococcus, Aerobacter, Flavobacterium* and *Erwinia*. Arbuscular Mycorrhizal Fungi (AMF) are able to solubilize soil phosphorus.

A substantial improvement in crop productivity has been noticed when PSB is applied singly or in combination with other rhizosphere microbes (Adesemoye and Kloepper, 2009; Mahdi *et al.*, 2010; Bashan and de-Bashan 2010; Ahemad and Khan, 2011).

Other element solubilizers

Many PGPR such as *Pseudomonas* and *Acinetobacter* strains enhance uptake of Fe, Zn, Mg, Ca, and K by crop plants. Chickpea and barley inoculated with *Mesorhizobium mediterraneum* had increased contents of K, Ca, Mg, and N. Beneficial effects of PGPR (*Pseudomonas mendocina*) and AMF (*Glomus inrtaradices* and *G. mosseae*) on uptake of N, Fe, Ca, and Mn in lettuce were mentioned. Furthermore, a significant increase in Mg concentrations has been noticed in seedlings of *Sesbania aegypriaca* and *S. grandiflora* after inoculation of AMF *Glomus macrocarpum.* Mycorrhizal maize has displayed an increase in acquisition of Fe, Zn, Cu and Mn. Sulfur (S) and Fe uptake have been achieved from sulfur oxidizing bacteria and siderophore-producing bacteria respectively. Lowland rice inoculated with *Rhizobium leguminosarum* var. *trifolii* indicated a significant increase in Fe uptake. Zinc can be solubilised by microorganisms like *Bacillus subtilis, Thiobacillus thioxidans* and *Saccharomyces* sp., which can be used as bio-fertilizers. Inoculation with *Azospirillum brasilense* increased the number and length of roots of *Sorghum bicolor* by 33-40%. These changes were directly attributed to positive effects on mineral (NO_3^-, NH_4^+, PO_4^{2-}, K^+, Rb^+, and Fe^{2+}) and several micronutrients uptake by the plant (Adesemoye and Kloepper, 2009; Mahdi *et al.*, 2010; Bashan and de-Bashan, 2010).

Siderophore producers

Iron is an essential micronutrient of plants as it serves as a co-factor of many enzymes with redox activity. It is also important for rhizobacteria as it has a dominant role in the nitrogen fixation and assimilation. A large portion of iron in soils is insoluble, thus iron acts as a limiting factor for plant growth even in iron rich soils. Several soil microorganisms (bacteria and fungi) produce siderophores. They are low molecular weight iron chelating compounds that bind Fe^{3+} that can be taken up by active transport mechanisms, and aids in iron uptake. Most isolates able to produce siderophores belong to Gram-negative bacteria such as *Pseudomonas* and *Enterobacter*, and Gram-positive bacteria (of the genera *Bacillus* and *Rhodococcus*.

Numerous microorganisms show ability to produce siderophores, and include *Pseudomonas* spp., *Rhizobium* strains, *Mesorhizobium* sp., *Ustilago sphaerogena, Streptomyces pilosus, Streptomyces coelicolor, Streptomyces coelicolor, Fusarium roseum Burkholderia cepacia, Acinetobacter calcoaceticus, Escherichia coli, Bacillus subtilis, Bacillus anthracis, Bacillus cereus, Bacillus megaterium, Vibrio cholerae, Azotobacter vinelandii, Pseudomonas aeruginosa,*

Pseudomonas fluorescens, P. putida and *Yersinia pestis.* The majority of endophytic Actinomycetes strains produce antibiotic siderophores.

Moreover, siderophore mediated growth promoting activity of PGPR is associated with the suppression of root pathogens by competitive exclusion. PGPR increase plant growth by antagonisms to potentially deleterious microorganisms, because they produce extracellular siderophores that complex environmental iron, making it less available to certain native microbiota (Podile and Kishore, 2006; Ahemad and Khan, 2011; Saharan and Nehra, 2011).

Mycorrhizae

There are two major types of mycorrhizae: Ectomycorrhizal fungi, and Endomycorrhizal fungi or Arbuscular mycorrhizae fungi (AMF). They are found among gymnosperms and angiosperms, including members of Pine, Oak and Beech families. Ectomycorrhizal fungi are usually specific to a certain host species. The AM fungi are a group of endophytes that constitute the phylum Glomeromycota. AMF associate in a symbiotic relationship with the roots of approximately 80% of all vascular plant species, including many important crop species such as maize, wheat, rice and potato.

Mycorrhizae contribute significantly to plant nutrition, particularly to phosphorus uptake. They contribute to the selective absorption of immobile (P, Zn and Cu) and mobile (S, Ca, K, Fe, Mn, Cl, Br and N) elements from plants, and to water uptake. Mycorrhizae improve soil structure, leading to increased soil stability and quality as well as decreased erosion. AMF help plants to resist and overcome pathogen infections, and, in addition, induce disease resistance in plants. For example, AMF *Glomus irregulare* significantly inhibits *Fusarium sambucinum* growth.

The use of AMF has not been widely integrated in the intensive agriculture of Europe and North America yet. A Canadian company produces and sells AMF inoculants for horticulture and agriculture in North America (Canada, USA and Mexico) and in Europe (Spain and France). Advances have been made by developing countries such as Cuba, India and Mexico, where chemical fertilizers are prohibitively expensive. In India, commercial inoculants are used on a large-scale rice production and have resulted in yield increases of around 10% with a 25-50% reduction of fertilizer, considering India's low phosphorous soils.

Synergistic effects between AMF (*Acaulospora spinosa, Entrophospora infrequens, Scutellospora fulgida, Glomus claroideum, G. lamellosum* and *G. mosseae*) and nitrogen fixers (*Azospirillum brasilense*) on plant communities haved been reported (Bauer *et al.,* 2012; Roy-Bolduc and Hijri, 2010; Antoun and Prevost, 2005).

Other fungi

The benefits are similar to those reported for mycorrhizal symbiosis. The root-associated fungi benefit plants by promoting plant growth and crop yield and by reduction of root pathogen infection. These fungi may also improve plant nutrient uptake or allow plant access to otherwise unavailable nutrient sources. Very little effort has been made thus far for the development of inoculum production of these fungi (Gentili and Jumpponen, 2006).

II.3. Bio-pesticides

As a result of monocropping and irrigation practices, creating ideal conditions for pests, modern agriculture is relying on chemical pesticides to control these pests. Pesticides create a number of

problems such as: 1) killing beneficial insects and plants, 2) causing pollution and runoff into irrigation water and then into rivers, damaging wildlife habitats, and killing fish, 3) impacting human health, causing (e.g.) cancer (organophosphates), 4) disrupting the natural ecosystem and natural biodiversity, and killing both target and non-target species, 5) inducing chemical resistance of pests, and 6) accumulation of pesticide residues in food.

Bio-pesticides are used to control harmful organisms, insects, nematodes, bacteria and fungi. Major types of bio-pesticides include bio-insecticides, bio-fungicides, bio-herbicides and bio-nematicides. They are used for various applications such as seed treatment, on farm application and post harvested application. Bio-pesticides are used in food crops, turf and ornamentals, forage and pastureland, public health and forestry as shown in Table II of the Annex.

II.3.1. Global bio-pesticides market

Bio-pesticide is a rapidly growing market and is expected to increase with the demand for residue free crop products, which have less or no negative impact on environmental safety.

The size of the global pesticide market was approximately 40 billion US$ in 2008. It increased to nearly 43 billion US$ in 2009 and is expected to grow at a CAGR of 3.6% to reach 51 billion in 2014 (Fig. 2). Global demand for pesticides is estimated to approach 57 billion US$ in 2016. The global market for Bio-pesticides is strong and developing global at a CAGR of 15.6% from 1.6 billion US$ in 2009 to 3.3 billion US$ to 2014. The report "Global Bio-pesticides Market-Trends & Forecasts (2012-2017)" published by Markets and Markets (http://www.marketsandmarkets.com) estimated the global bio-pesticides market at 1.3 billion US$ in 2011 and expects it to reach 3.2 billion US$ by 2017 at a CAGR of 15.8% from 2012 to 2017. The global report on Bio-pesticides market released by Global Industry Analysts, Inc. (GIA) forecast the market to reach 3.4 billion US$ by the year 2017. It is noticed that widely divergent statistics have been broadcasted for the size of the bio-pesticides market. The reasons are first the diversity of bio-pesticide products (microbes, bio-chemicals, plant growth regulators, insect growth regulators, beneficials, essential oils, pheromones, minerals, etc) and second the criteria used to define the market.

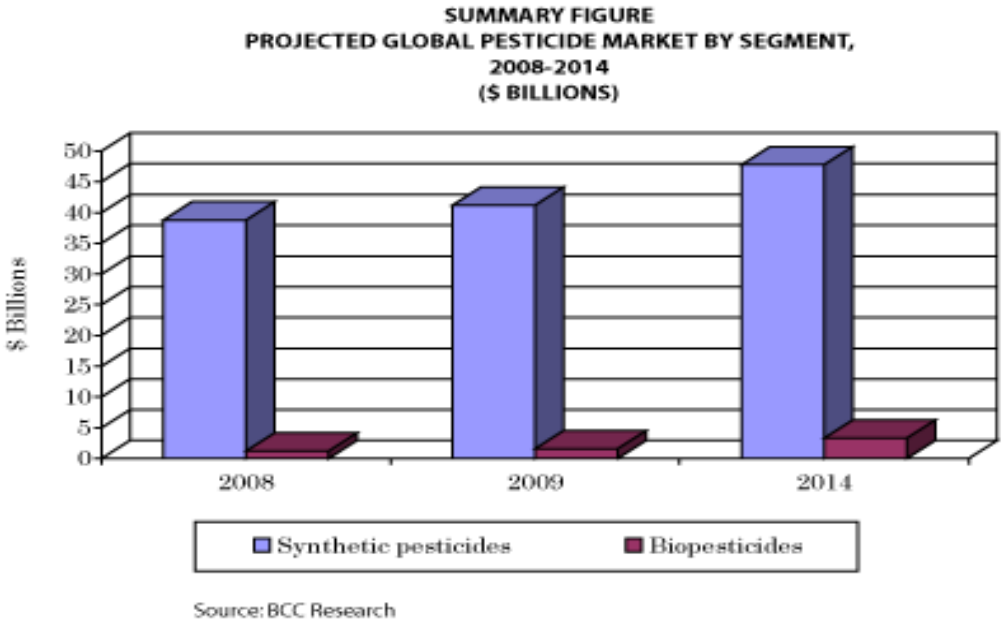

Figure 2. Bio-pesticides: The Global Market (February 2010) (BCC Research Corporation

http://www.bccresearch.com/report/biopesticides-market-chm029c.html)

In 2011, North America dominated the global bio-pesticides market, accounting for around 40% of the global bio-pesticides demand. Europe is expected to be the fastest growing market in the near future owing to the stringent regulation for chemical pesticides and an increasing demand for organic products.

Asia-Pacific and Europe remain two of the fastest growing markets, each projected to grow at a CAGR of 14.2% and 16% respectively. Both regions offer great opportunities for the use and development of microbial bio-pesticides. Products based on *Bacillus thurigiensis* dominate the market. The market for nematode and microbial based pesticides increased noticeably in the Asia-Pacific region. Other major products include fungal based products such as *Metarhizium, Bauveria* and *Trichoderma*, endomopathogenic viruses, mainly *Spodoptera litura* NPV and *Helicoverpa armigera* NPV, bacteria based products such as *Pseudomonas fluorescens, Bacillus subtilis* and other *Bacillus* derived products for protection from plant diseases and *Salmonella*-based rat poison. China is the largest market for bio-pesticides in the region followed by India and Japan. Market participants in Europe and the Asia-Pacific region are determined to gain market share. To this end, , they started focusing on new product registrations and innovations.

A great number of patents are getting registered in Europe and North America (USA). Regional companies, instead of a few large global players, cover the global biopesticides market. The majority of these companies are establishing licensing agreements with start-up companies or acquiring products rather than investing in R & D projects. To capitalize on the growth trend in the global bio-pesticides market, several leading crop protection chemical companies try to develop and manufacture bio-pesticides offering them as crop protection products. Growth in the organic food market and the easy registration of bio-pesticides compared to chemical pesticides are also positively influencing the growth the bio-pesticides market.Other factors for an increasing bio-pesticides industry globally are: the toxicity of crop protection products, environmentally friendly products, innovative production practices, new product offerings, increased availability and advent of new pests. An important factor in the growth of the bio-pesticides market is the advancements in bio-pesticides technology, including improvements in formulation techniques, mass production of bio-pesticides, increased storage and shelf life and improved application methods. Finally, increased knowledge among end users has also contributed to the increased use of biopesticides.

II.3.2 Biological control agents

PGPRs

The PGPRs play a major role in the biological control of plant pathogens and they can suppress a broad spectrum of bacterial, fungal, nematode and viral diseases. Some microbes produce secondary metabolites locally, or near the plant surface, where they act. Moreover, the molecules of biological origin are biodegradable as compared with many agrochemicals. Biological control is also used to control diseases occurring during the storage of fruits (postharvest control). The main mechanisms of biological control are the following (Podile and Kishore, 2006; Lugtenberg and Kamilova, 2009):

- Antagonism,
- Competition for ferric iron ions,
- Signal interference,
- Predation and parasitism (lysis),
- Competition for nutrients and niches,

- Inhibition of pathogen produced enzymes or toxins,
- Interference with activity, survival, germination and sporulation of the pathogen, and
- Induced systemic resistance.

Chandler *et al.* (2011) informed us about the existence of around 67,000 different crop pest species that cause an estimated 40% reduction in the world's crop yield. Those crop losses undermine food security in combination with inclement weather, poor soils and farmers' limited access to technical knowledge. While the application of synthetic pesticides have a number of disadvantages, bio-pesticides, in comtrast, are less harmful and more environmentally friendly. Biopesticides are designed to affect only one specific pest or a few target organisms; they are effective in very small quantities and often decompose quickly. While they are more expensive than synthetic pesticides, they need to be applied less (Gupta and Dikshit, 2010; Chandler *et al.*, 2011).

Bio-pesticides

- **Bio-pesticides based on bacteria** have been used to control plant diseases, nematodes, insects and weeds. The most widely used bacteria is the insect pathogenic bacterium *Bacillus thurigiensis* (Bt). During spore formation Bt produces insecticidal proteins (the Bt δ-endotoxin), a highly specific endotoxin, that binds to and destroys the cellular lining of the insect digestive tract, causing the insect to stop feeding and die. The δ-endotoxin crystals are mass produced in fermentation tanks and formulated as a sprayable product. The protein kills caterpillar pests, fly and mosquito larvae, and beetles. Bt sprays are used on fruit and vegetable crops, on broad-acre crops such as maize, soya bean and cotton. *Bacillus sphaericus* is another insecticidal bacterium that has been used to control mosquito species. Certain strains of *Bacillus subtilis, Bacillus pumilus, Pseudomonas fluorescens, Pseudomonas aureofaciens* and *Streptomyces* spp. prevent plant diseases by outcompeting plant pathogens in the rhizosphere, producing anti-fungal compounds and promoting plant and root growth. They are used against a range of plant pathogens including damping-off and soft rots. The K84 strain of *Agrobacterium radiobacter* is used to control crown gall caused by *Agrobacterium tumefaciens*.
- **Fungal bio-pesticides** can be used to control plant diseases caused by fungi, bacteria or nematodes, as well as some insect pests and weeds. The modes of action are through competitive exclusion, mycoparasitism and production of metabolites. The most common commercial fungal bio-pesticides used in the nursery, ornamental, vegetable, field crop and forestry industry, are *Trichoderma* spp. and *Beauveria bassiana*. *Trichoderma* is able to colonise plant roots and out-compete pathogenic fungi for food and space, or attack and parasitize plant pathogens under certain environmental conditions. In the process it can stimulate plant host defense and affect root growth. *Trichoderma harzianum* is an antagonist of *Rhizoctionia*, *Pythium*, *Fusarium* and other soil-borne pathogens. *Beauveria bassiana* and *Metarhizium anisopliae* are parasitic fungi found on many insect species. *Beauveria bassiana* has proved effective in controlling crop pests such aphids, thrips and whitefly pesticide resistant strains. *Metarhizium anisopliae* is used against spittlebugs on sugarcane and grassland and furthermore for the control of locust and grasshopper pests in Africa

and Australia. *Coniothyrium minitans* is a mycoparasite applied against *Sclerotinia sclerotiorum* (Chandler *et al.,* 2011).

- **Baculoviruses** are a family of naturally occurring viruses that infect only insects and some related arthropods. They infect and kill *Lepidoptera* larvae (caterpillars). The granulovirus of the coding moth *Cydia pomonella* (CpGV) is a commercially successful viral insecticide used in the USA and Europe. Most CpGV applications occur in conventional orchards where its mode of action can minimise the risk of resistance to chemical insecticides. In Brazil the nucleopolyhedrovirus is used to control the soya bean caterpillar *Anticarsia gemmatalis*.
- **Non-pathogenic yeasts** like *Cryptococcus* and *Candida* species naturally occur on plant tissues and in water. *Candida oleophila* strain O has been developed into a pesticide for the control of post harvest fruit rots. It is applied to apples and pears after harvest, but before storage, to control particular fungal pathogens. The yeast acts as an antagonist to fungal pathogens such as gray mould (*Botrytis cinerea*) and blue mould (*Penicillium expansum*), which cause post harvest decay. *Candida oleophila* strain O works through competition for nutrients and pre-colonisation of plant wound sites. It produces enzymes to degrade fungal cell walls and stimulates plant host defense pathways.
- **Plant pathogens** have been used as bio-herbicides. There are no products currently available in Europe; however, "Collego" (*Colletotrichum gloesporioides*) and "DeVine" (*Phytophthora palmivora*) have been applied in the USA (Chandler *et al.,* 2011).
- **Biochemical bio-pesticides** are divided into plant growth regulators, insect growth regulators, organic acids, plant extracts, pheromones and minerals.

II.4. Effective Microorganisms (EM)

The concept of effective microorganisms (EM) was developed by Professor Teruo Higa, a microbiologist from the University of the Ryukyus, Okinawa, Japan, who made an accidental discovery while investigating the beneficial aspects of isolated strains of microorganisms on soil composition and plant growth (Higa and Parr, 1994).

II.4.1. Applications of EM

Effective microorganisms (EM) are a mixed culture of beneficial microorganisms, primarily photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes and fermenting fungi, that can be applied as an inoculant to increase the microbial diversity of soil, which in turn can improve soil quality and health, and enhances the growth, yield and quality of crops. The increase of photosynthetic bacteria (phototrophic bacteria) in the soil enhances other effective microorganisms. They are the pivot of EM activity. Lactic acid bacteria (LAB) have the ability to suppress *Fusarium* propagation, which is a harmful microorganism causing diseases during continuous cropping. Yeasts produce bioactive substances such as hormones and enzymes to promote active cell and root division. Actinomycetes produce antimicrobial substances and suppress harmful fungi and bacteria. Fungi such as *Aspergillus* and *Penicillium* decompose organic matter rapidly to produce alcohol, esters and antimicrobial substances, suppressing odors and preventing infestation of harmful insects and maggots. Some of the EMs include members of the genera *Rhodopseudomonas, Rhodospirillum,*

Rhodobacter, *Lactobacillus*, *Candida*, *Saccharomyces*, *Streptomyces*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Xanthomonas*, *Aspergillus*, *Penicillium*, *Trichoderma* and *Phanerochaete*. When EMs increase as a community in soils, populations of native effective microorganisms are also enhanced, the microbiota is enriched, and microbial ecosystems in the soil become well balanced. Thus, soil-borne diseases are suppressed. Beneficial influences of EMs include the promotion of germination, flowering, fruiting and ripening in plants, the improvement of physical, chemical and biological environments of the soil and suppression of soil borne pathogens and pests, the enhancement of the photosynthetic capacity of crops, the insurance of better germination and plant establishment, and increasing the efficacy of organic matter as fertilizers.

EM is not a substitute for other management practices, but it optimizes the soil and crop management practices (crop rotations, use of organic amendments, conservation tillage, crop residue recycling, biological control of pests), and enhances the beneficial effects of these practices.

The main product, EM-1, is a liquid bacterial product. All products using EM Technology contain EM-1 in some part of their manufacturing process. The EM Technology is marketed and applied around the world by a number of producers and vendors. Worldwide, the two largest license holders for EM technology operate under the trademark names EMRO and EMCO, both headquartered in Japan. Other licenseholders are the Tropical Plant Research Institute (aka, TPRI, or TPRI, or TPRR) in Japan, which produces EM-X liquid nutritional supplement, and Asia Pacific Natural Agriculture Network (APNAN), an organisation of scientists representing 15 countries of the Asia-Pacific Region conducting research on nature farming practices and technologies, including EM. APNAN produces and distributes EM in many parts of Asia. A number of smaller regional organisations and companies are also kown to operate in Asia, some of which are particularly active in the region's leastdeveloped countries, like for example the Community Welfare and Development Society (CWDS) in Nepal.

EM can be applied as:

(1) EM1 stock solution, a yellow-brown liquid with a pleasant odor and sweet- sour taste which is used

for watering into the soil (by watering cans, sprinklers or irrigation systems) or spraying onto plants (foliar spray) by sprayer or watering can,

(2) EM Bokashi (EM fermented organic matters) prepared by fermenting organic matter such as rice bran, corn bran, wheat bran, maize flour, rice husk, bean husk, rice straw, cotton deed cake, oil cake, press mud, bagasse, chopped weeds, sawdust, coconut fiber and husks, and crop residues (e.g., empty fruit bunches in oil palm, molasses, fish meal, bone meal, dung of any animal species, kitchen garbage, seaweed, crab shells). The best of all is rice bran with EM Bokashi. Bokashi is normally found as a powder or as granules. When mixed with organic wastes it is activated and proliferates to produce rich compost filled with nutrients and antioxidants. Bokashi works very well when added to the finishing phase of aerobic compost piles. It can be used 3-14 days after treatment (fermentation) and can be applied in crop production, even when the organic matter has not decomposed. It has been used by Japanese farmers as traditional soil amendment to increase the microbial diversity of soils and supply nutrients to crops,

(3) EM5 (EM fermented solutions) also known in Japan as Sutochu, is a fermented mixture of vinegar, spirits (alcohol), molasses and EM-1. It is used to spray the plant to suppress pathogens and keep away insect pests,

(4) EM Fermented Plant Extract (EM-F.P.E.) is a mixture of fresh weeds fermented with molasses and EM-1, which supplies nutrients to crops, suppresses pathogens and keeps away insects, and

II.5 Composting agro-industrial wastes

In 2008, agriculture and forestry accounted for 1.7% of the total waste generation in the EU-27 by economic activity (NACE Rev. 2)% (Fig. 3).

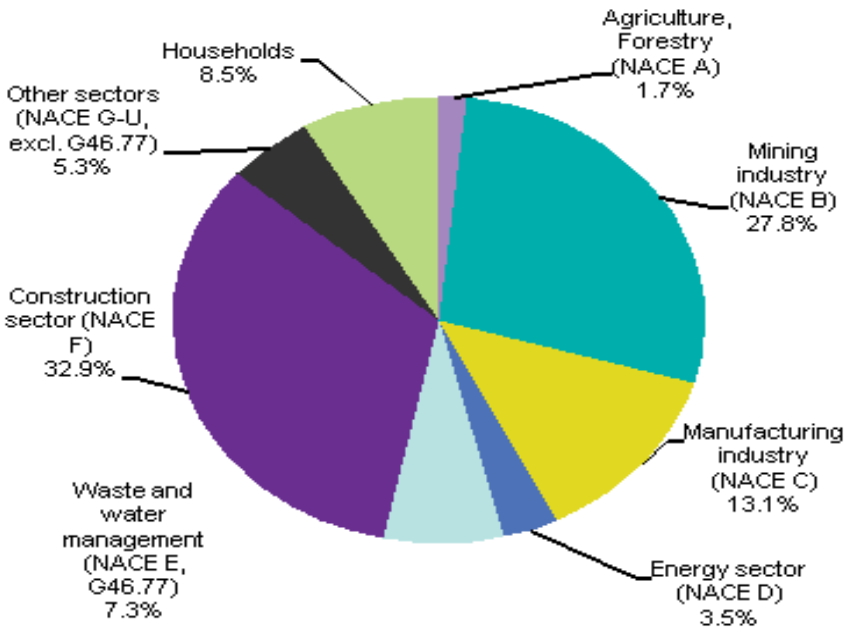

Figure 3. Total waste generation in the EU-27 by economic activity. **Source:** Eurostat (NACE: Nomenclature Generale des Activites economiques dans l' Union Europeennes

http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Waste_statistics)

In both rich and poor countries, the vast majority of waste goes into landfills, where it is often covered up. A very small share of waste is recycled or composted.

II.5.1. Composition of agro-industrial wastes

Large amounts of wastes are generated worldwide every year from the industrial processing of agricultural raw materials and animal products. Most waste materialis used either as animal feed or burned. Wastes derived from agricultural activities include straw, stem, stalk, leaves, husk, shell, peel, lint, seed/stones, pulp or stubble from fruits, legumes and cereals (e.g. rice, wheat, corn, sorghum, barley, etc), bagasses from sugarcane, sweet sorghum milling, spent coffee grounds, brewer's spent grains, and others. Sugars, fibres, proteins, and minerals represent important components of waste. Their large availability and rich composition render them attractive substrates for re-use in other processes. Such wastes may be used as low-cost raw materials for the production of other value-added compounds, with the purpose of reducing the production costs. Large amounts of the agro-industrial wastes of plant origin are mainly composed of cellulose, hemicellulose and lignin, in varying amounts

according to waste, plant species, and the process to which the agricultural material is subjected. The ratios in a single plant may also vary, e.g., with age and stage of growth. The presence of sugars, proteins, minerals and water make the agro-industrial wastes suitable for the growth of microorganisms, mainly fungal strains, adapted to these wastes. The major groups of fungi with the ability to decompose cellulose include *Fusarium oxysporum, Trichoderma reesei, Trichoderma viride* and members of genus *Penicillium, Aspergillus, Alternaria, Hormodendrum, Phanerochaete, Chaetomium, Pythium, Mortierella, Agaricul,* and brown rot fungus *Oligoporus placenta. Phanerochaete chrysosporium, Phlebia radiata, Trametes versicolor, Polyphorus varsicolor* and *Pleurotus sajor caju* are some of the fungi that can degrade lignin. The agro-industrial wastes may also contain phenolic compounds or other potentially toxic compounds, which may cause deterioration of the environment when the waste is discharged.

II.5.2 Uses of agro-industrial wastes

Compost

Compost is a soil amendment produced by a controlled decomposition process in which aerobic microorganisms degrade and transform organic material into a range of increasingly complex organic substances, some of which are referred to as humus. Compost is a stable, biologically active material of organic origin that can vary in texture, has a typical dark brown colour, and an earthy appearance and smell. Compost is made from a mixture of organic materials that are blended to achieve an appropriate carbon to nitrogen ratio, and the composting process is managed to maintain temperature, oxygen and moisture within acceptable levels. In the composting process, organic substrates are utilized, with heat and CO_2 emission, while the remaining carbon skeletons are recalcitrant humic substances that are responsible for the soil amending ability of compost.

In biologically active soils any available nutrients will stimulate additional microbial growth that further aids nutrient retention. Thus, the crops require less fertilizer while fewer nutrients are leached into the groundwater. Compost utilisation improves the security of soil and water resources. Also, the use of compost leads to improved crop performance (better yields, product quality and storage life, more efficient and reduced use of fertilizers and pesticides, better utilisation of irrigation, increased crop resistance to pests and diseases) and reduction in the production costs. Even the soil quality is improved due to higher levels of organic matter, increased availability of water, increased nutrient availability and nutrient-holding capacity of the soil, improved structure, and reduced soil-borne plant pathogens and pests. Members of the genera *Bacillus, Enterobacter, Pseudomonas* and *Flavobacterium* (*F. balustinum*), *Streptomyces, Penicillium, Trichoderma* and *Gliocladium* (*G. virens*) have been identified as biocontrol agents in composts. Pathogens sensitive to compost inhibitory capacity include *Pythium ultimum, Rhizoctonia solani, Venturia inaequalis, Fusarium oxysporum, Verticillium dahliae, Phytophthora nicotianae, Phytophthora cinnamomi* and *Cylindrocladium spathiphyll.* Beneficial bacterial genera such as *Pseudomonas, Bacillus* and *Pantoea* recolonise compost rapidly after the thermophilic phase of composting, and have shown general disease suppression. Some beneficial fungal genera like *Trichoderma* and *Penicillium* show suppression of *Phytophthora, Rhizoctonia,* and *Fusarium.* Beneficial fungal strains recolonise compost during maturation or the curing phase of composting. *Gliocladium,* a beneficial saprophytic fungus, produces a broad spectrum antibiotic called gliotoxin. This antibiotic effectively suppresses a series of soil pathogens and of damping off, caused by *Pythium ultimum.*

In the final report of the European Compost Network on “Compost production and use in the EU” (2008), the authors concluded that more mature markets are expected, which will lead to higher

compost qualities and more compost mix products for special application. The resulting higher prices will allow longer transport distances and thus more cross border business (Barth *et al.*, 2008).

In conclusion, the success of composting is determined by the balance between production costs and the returns from the benefits. Costs include raw material assembly, processing, distribution and spreading. Around the world, agricultural use of compost varies enormously and success usually reflects the market development approaches adopted. Compost production has grown significantly in recent years and is likely to accelerate in the coming years.

Production of mushrooms using agro-industrial residues

Mushroom cultivation is a prominent biotechnological process for the exploitation of agro-industrial residues. A huge amount of lignocellulosic agricultural crop residues and agro-industrial by-products are annually generated. These products are rich in organic compounds that can be recovered and upgraded to higher value and useful products. A number of these residues can be converted by solid-state fermentation (SSF) into various different value-added products, including mushrooms, using basidiomycetes fungi. Mushroom cultivation has proved its economic strength and ecological importance for efficient utilisation, value-addition and biotransformation of agro-industrial residues.

Among mushroom fungi, *Agaricus bisporus, Lentinula edodes* and *Pleurotus* species (*P. ostreatus, P. sajor-caju, P. pulmonarius, P. eryngii, P. cornucopiae, P. tuer-regium, P. citrinopileatus* and *P. flabellatus*) are highly efficient in the degradation of a wide range of lignocellulosic residues such as wheat straw, cotton wastes, coffee pulp, corn cobs, sunflower seed hulls, wood chips and sawdust, peanut shells, and vine pruning into mushroom protein (Philippoussis, 2009).

Vermicomposting of agro-industrial waste

Vermicomposting is generally defined as the solid phase decomposition of organic residues in the aerobic environment by exploiting the optimum biological activity of earthworm and microorganisms. The process depends upon the earthworms to fragment, mix and promote microbial activity in the organic waste material. The earthworms ingest organic solids and convert a portion of it into earthworm biomass, respiration products and a peat like material termed as vermicompost. As compared to the thermal composting, vermicomposting generates a product with lower mass, high humus content, lower processing time, less likely phytotoxicity, usually greater fertilizer value, and an additional product (earthworms) with additional potential uses. The vermicomposting process takes place within the mesophilic temperature range (35-40 ^{o}C). The passage of material through the earthworms' intestine rapidly converts the locked up minerals (e.g., nitrogen, potassium, phosphorus and calcium) into more accessible forms to plants, with the help of various enzymes that are present in the gut and of some ingested microorganisms,. The earthworms seem to have developed a mutualistic relationship with microorganisms ingested for decomposition of organic matter present in their food. Also, earthworms release coelomic fluids, which contain mucocytes, vacuolocytes, granulocytes and lymphocytes that kill the bacteria and parasites that are present in waste material, resulting into odour and pathogen free vermicompost.

Eventually, the vermicompost is an excellent product of homogeneous and odourless nature, with reduced levels of contaminants, rich in microbial population and containing more nutrients (such as nitrates, phosphates, calcium, potassium and magnesium) over a longer period, which does not adversely impact the environment. Vermicompost has high porosity, water holding capacity and a large surface area that provides abundant sites for microbial activity and the retention of nutrients.

Plant growth regulators and other compounds (auxins, cytokinins and humic substances), may be produced by the microbes in vermicompost (Garg and Gupta, 2009).

Earthworms constitute more than 80% of soil invertebrate biomass. The earthworm species most often used are red wigglers *Eisenia fetida* or *E. andrei*, *Lunbricus rubellus* (red earthworm of dilong in China), *Eisenia hortensis* (European nightcrawlers, dendrobaenas, dendras, Belgian nightcrawlers). *Perionyx excavatus* (blueworms) and also *P. sansibaricus* and *Eudrilus eugeniae* may be used in the tropics, (Gajalakshmi *et al.*, 2001; Suthar and Singh, 2008; Padmavathiamma *et al.*, 2008; Nithya and Lekeshmanaswamy, 2010).

Various agroindustrial wastes have been tested as feedstock for vermicomposting, e.g., sugar refinery industrial waste, winery waste, crop residues, textile industry sludge, coir pith, cassava roots, pulp and paper mill sludge, coffee pulp, woodchips, oil industry waste and food industry waste.

Textile mill waste was vermicomposted by mixing it in the range of 20-30% with cow dung and inoculating with nitrogen fixing strains of *Azotobacter chroococcum* and *Azospirillum brasilense* and, moreover, with phosphate solubilising *Pseudomonas maltrophila*. Nitrogen fixing bacteria helped to increase the nitrogen content of vermicompost by activating the nitrification bacteria and reducing denitrification of the substrate. In addition, *Pseudomonas maltrophila* increases the phosphorus content by solubilising phosphate during the inoculation period (Kaushik *et al.*, 2008).

Vermicomposting produces a leachate as a result of the addition of moisture contents through the column of worm action. Draining of this water or leachate is important to prevent saturation of the vermicomposting unit and attraction of pests. The leachate is termed as vermiwash and, when collected, it can be used as a liquid fertilizer containing large amounts of plant nutrients. If used as fertilizer, it is better diluted to avoid plant damage, but this decreases its nutrient content, and thus has to be combined with other mineral fertilizers. Commercial formulations of liquid fertilizers are sometimes complemented with certain chemical compounds in order to increase nutrient availability for plants.

II.6. Production of microbial metabolites

Numerous organic materials and products are derived from microbes, often when cultured on agro-industrial residues with great commercial significance. Products of microbial origin include organic acids, chemical additives, pigments, enzymes, food additives, antibiotics, biofuels, solvents, bioplastics, mushrooms, compost and vermicompost. The development of these sectors has different social impacts related to job creation and rural development. The global market for agricultural biotechnology wasUS$13.7 billion in 2011 and is estimated to grow toUS$14.4 billion in 2012, growing at a CAGR of 11.4% to reach a forecast value of nearlyUS$24.8 billion in 2017, the bulk of which is made up by transgenic seeds (Fig. 4).

Products of environmental biotechnology have enormous potential in overseas markets. For example, the USA market for environmental biotechnology products for waste treatment increased fromUS$166.8 million in 2007, toUS$180.3 million by the end of 2008 and it should reachUS$261.3 million by 2013 with a CAGR of 7.7% (Fig. 4).

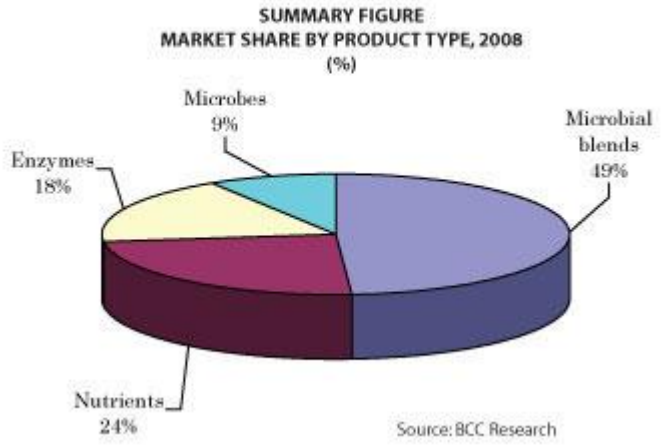

Figure 4. Market share of products derived from microbes. **(Source:** BCC Research *http://bccresearch.blogspot.gr/2012_09_01_archive.html*)

II.6.1. Substrate and methods

Agro-industrial residues are the most abundant renewable resources on earth, as they constitute a significant proportion (amounting to over 30%) of the worldwide agricultural productivity. Accumulation of this biomass in large quantities every year results not only in the deterioration of the environment, but also in the loss of potentially valuable material which can be processed to yield a number of valuable added products such as food, fuel, feed and a variety of chemicals.

Pre-treatment is an important tool for breakdown of the structure of these residues mainly composed of cellulose, hemicellulose and lignin, with over 300 million tons of lignocellulose produced worldwide annually. Various physical, chemical and biological pre-treatment methods and their combinations are available. Major biological methods exploit the enzymatic potential of microbial strains of, e.g., *Aspergillus niger, A. awamori, Phanerohaete chrysosporium, P. sajor-caju, Bjerkendra adusta, Cyathus stercoreus, Pleurotus ostreatus, Trametes reesei* and *T. versicolor*.

Using biotechnological innovations, sugar-rich lignocellulosic residues (cellulose, hemicellulose and lignin) are being converted by fermentation processes into valuable products. Microorganisms utilise the sugars for growth and production of value added compounds such as ethanol, food additives, organic acids and enzymes (Alonso Bocchini Martins *et al.*, 2011; Joyce and Stewart Jr., 2012) (Table 2), (Barclay *et al.*, 1994; Soomro *et al.*, 2002; Vandamme, 2003; Liang *et al.*, 2004; Nitschke *et al.*, 2004; Simon, 2005; Brandelli, 2008; Mussatto and Teixeira, 2010; Elshahed, 2010; Sidkey *et al.*, 2010; Bacha *et al.*, 2011; Jang *et al.*, 2012; Sarma, 2012).

Agro-industrial residues (crop residues, forest litter, grass and animal garbage) are directly burnt as fuel in developing countries.

Metabolites are being produced using Solid State Fermentation (SSF) with two potential areas of application:

(1) for environmental control such as for the production of compost, ensiling and animal feed conversion from solid wastes, bioremediation and biodegradation of hazardous compounds, and biological detoxification of agro-industrial wastes, and

(2) to obtain value added compounds such as enzymes, mushrooms, amino acids, bio-pesticides, bio-fuels, bio-surfactants, organic acids, flavours, colourants, aromatic compounds, biologically active secondary metabolites, and other substances of interest to the food industry.

Table 2. Conversion of lignocellulose into valuable products using Solid State Fermentation (SSF).

Agro-industrial processes	Microorganisms	Specific function
	Saccharomyces cerevisiae, Zymomonas mobilis, Pichia stipitis	**Bio-ethanol production from cellulose and fermentation of hemicellulose hydrolysates**
	Clostridium acetobutylicum, Clostridium beijerinckii	**Butanol production from cellulose and fermentation of hemicellulose hydrolysates**
Biofuels	*Bacillus polymyxa, Klebsiella pneumoniae, Bacillus subtilis, Serratia marcescens, Aerobacter hydrophila*	**Liquid fuel production from cellulose and fermentation of hemicellulose hydrolysates**
	Photosynthetic cyanobacteria: *Synechococcus elongates, Botryococcus braunii, Nannochloropsis* sp., *Schizochytrium* sp.	**Biodiesel, non-petroleum fuel consisting of alkanes**
	Algae and cyanobacteria: *Rhodopseudomonas palustris, Chlamydomonas reinhardtii* *Halanaerobium saccharolyticum, Klebsiella* sp. HE1, Enterobacter aerogenes HU-101, NBRC 12010, *Thermotoga neapolitana* DSM 4359, *Rhodopseudomonas palustris*	**Hydrogen. Crude glycerol generated during biodiesel manufacturing processes can be used as a feedstock for hydrogen production**
Lactic acid	*Lactobacillus pentosus, Lactobacillus delbrueckii, Lactobacillus brevis* Fungi: *Rhizopus* sp.,	**Fermentation of lignocellulose hydrolysates**
Acetic acid	*Acetobacter, Aspergillus wentii, Aspergillus clavatus, Mucor piriformis, Citromyces*	**Fermentation of lignocellulose hydrolysates**
Citric acid	*Penicillium luteum, Penicillium citrinum, Aspergillus niger, Aspergillus wentii, Aspergillus clavatus, Mucor piriformis, Citromyces pfefferianus, Paecilomyces divaricatum, Trichoderma viride, Yarrowia lipolytica, candida guilliermondii*	**Fermentation of lignocellulose hydrolysates**
Succinic acid	*Mannheimia succiniciproducens, Actinobacillus succinogenes, Anaerobiospirillum succiniciproducens*	**Fermentation of lignocellulose hydrolysates**
Butyric acid	*Clostridial* species (*Clostridium tyrobutyricum*)	**Fermentation of lignocellulose hydrolysates**
Xylitol	*Candida guilliermondii, Candida entomaee, Pichia guilliermondii*	**Fermentation of xylose present in hemicellulosic hygrolysates**
	Bacillus subtilis, Bacillus polymyxa, Bacillus mesentericus, Bacillus vulgaris, Bacillus megaterium, Bacillus licheniformis Fungi: *Gibberella fujikuroi, Aspergillus oryzae, Aspergillus flavus*	**α-amylase from lignocellulosic materials by SSF**

	Streptomyces sp.	**Cellulases from lignocellulosic materials by SSF**
	Aspergillus terreus, thermoascus aurantiacus, Bacillus sp.	**Xylanases from lignocellulosic materials by SSF**
Enzymes	*Aspergillus oryzae,*	**Proteases from lignocellulosic materials by SSF**
	Aspergillus oryzae	**Fructosyl transferase from lignocellulosic materials by SSF**
	Penicillium aculeatum	**Chitinase from lignocellulosic materials by SSF**
	Aspergillus sp.	**Tannase from lignocellulosic materials by SSF**
	Aspergillus niger	**Pectinase from lignocellulosic materials by SSF**
	Streptomyces, Bacillus, Arthrobacter sp., *Microbacterium* sp., *Kocuria rosea*	**Keratinases from lignocellulosic materials by SSF**
Pigments	*Xanthophyllomyces dendrorhous*	**Carotenoids (astaxanthin) production by fermentation of wood hydrolysate**
Flavours	*Ceratocystis fimbriata, Kluyveromyces marxianus, Kluyveromyces lactis, Sporidiobolus salmonicolor, geotrichum klebahnii, Penicillium* sp., *Botryodiplodia* sp., *Aspergillus niger, Pycnoporus cinnabarinus, Phanerochaete chrysosporium, Zygosaccharomyces rouxii, Torulopsis bombicola, Candida tropicalis*	**Feedstock of plant or animal origin using SSF**
Single cell protein	*Chaetomium cellulolyticum, Pleurotus sajor-caju, Aspergillus, Penicillium* spp.	**Lignocellulosic wastes (potato, orange, carrot, apple peels) convert**
Biosurfactants	*Bacillus* sp.	**Substrates: molasses, milk whey, cassava flour wastewater (manipueira)**
Bioactive compounds (gebberellic acid, oxytetracyclin, destruxins A, B, or cycclodepsipeptides, ellagic acid)	*Giberella fujikuroi, Fusarium moniliforme, Streptomyces rimosus, Metarhizium anisopliae, Aspergillus niger*	**SSF of lignocellulose wastes (corn cobs, rice husk, pomegranate peel, creosote bush leaves)**
Polysaccharides (xanthan, dextran)	*Xanthomonas campestris, Leuconostoc mesenteroides*	
Nutrient (lysine, vitamin B_{12}	*Corynebacterium glutamicum, Brevibacterium flavum, Pseudomonas denitrificans, Propionibacterium*	

II.6.2. Organic acids

Organic acids are widely used in food and beverage industries because they prevent deterioration and extend the shelf life of food. SSF has been successfully employed for many years to produce citric and lactic acid under large-scale production processes. The production of oxalic acid, gluconic acid and gallic acid by SSF has also been reported. Strain selection is very important,

because the microorganism must have relatively stable characteristics and the ability to grow rapidly and vigorously. The selected microorganism should also be non-pathogenic and suitable for the studies related to the optimisation of parameters. The most important economic characteristic in the selection of a microbe is its ability to produce high yields of the desired product (Singh nee' Nigam, 2009)

- **Citric acid** is the most important organic acid produced at bio-industrial level and is extensively used in food, beverages, cosmetics, pharmaceutical and chemical products. In the food industry, citric acid has been applied as an additive, and is used as preservative, flavour enhancer, antifoam agent, or antioxidant. In the chemical industry, it is exploited as plasticizer, softener, and for the treatment of textiles. This acid has also widely been used in the detergent industry for replacement of polyphosphates, thereby decreasing the production costs. *Aspergillus niger* is one of the microbial species commercially used for production of citric acid. Under SSF conditions, being cultivated on agro-industrial wastes such as corncob, sugarcane bagasse, coffee husts, kiwi fruit peels, wheat bran, rice bran, pineapple waste, mixed fruit waste, maosmi waste sugar beet molasses, sawdust with rice hulls, cassava, fibrous residue, apple pomace, and potato starch residue.
- **Lactic acid** plays an important role in various biochemical processes. It is used as acidulant and preservative of many food products such as cheese, meat, beer and jellies. Lactic acid has also wide uses in pharmaceutical, leather and textile industries, in the synthesis of biodegradable plastics and coating, but it is also used in the manufacture of cellophane, resins and some herbicides and pesticides. Lactic acid production by SSF has been carried out using fungal and bacterial strains. Strains of *Rhizopus* spp. and *Lactobacillus* spp. are the most commonly utilised microorganisms, and substrates used in these processes are sugarcane bagasse, sugarcane press mud and carrot processing wastes.
- **Other acids** include oxalic acid, which is produced by *Aspergillus oryzae* using wheat kernels as support. The main application of oxalic acid is for cleaning or bleaching, especially for the removal of rust. It is also used in the restoration of old wood and is an important reagent in the lanthanide chemistry. Gluconic acid is produced by *Aspergillus niger* using tea waste as support and sugarcane molasses as carbon source. Gluconic acid is utilized as a food additive, acting as acidity regulator. It is also used in cleaning products where it dissolves mineral deposits especially in alkaline solution.

II.6.3. Flavour and aroma compounds

Aroma compounds can be found in food, wine, spices, perfumes and essential oils**,** but over a quarter of these are used in the food industry. These compounds play an important role in the production of flavours, which are used to improve food quality and add value. Most of the flavouring compounds are presently produced via chemical synthesis or extraction from natural materials, but since consumers prefer food free from chemical additives, microbial biosynthesis or bioconversion systems avail themselves as promising substitutes for producing aroma compounds. Both fungi and bacteria have the ability to synthesize aroma compounds by SSF (Table III, ANNEX) (Dastager, 2009).

The worldwide flavour and fragrance market wasUS$21.8 billion in 2011 and is projected to exceedUS$23 billion in 2012 andUS$30 billion in 2017 with a GAGR of 5.6% between 2012 and 2017. The flavour and fragrance industry's total demand for ingredients was an estimatedUS$7.6 billion in 2011 and is growing at a CAGR of 5.75 to reachUS$8.1 billion in 2012 and an

expectedUS$10.7 billion by 2017 **(**BCC Research http://bccresearch.blogspot.gr/2012/06/global-markets-for-flavors-and.html).

On overall, the demand for ingredients used in fragrances will continue to be driven by ongoing consumer preferences for natural ingredients and rising consumer interests in more complex and authentic fragrances. Asia-Pacific, Eastern Europe and Latin America are demonstrating a steady growth.

II.6.4. Enzymes

Enzymes are the most important products obtained from microbial sources. They have application in a variety of areas including food biotechnology, environment, animal feed, pharmaceutical, textile, paper and other technical and chemical industries. Due to the large application and significant cost, there is a necessity to develop processes able to minimize the production costs. The production of enzymes by SSF has the potential to promote higher yields than the production by Submerged Fermentation (SmF), for the same microbial strain. Additionally, a variety of agro-industrial wastes may be used as support material for the production of different enzymes by SSF (Table III, ANNEX). In this way, a variety of low cost wastes are reused for the production of a value added product, with decreased production costs (Mussatto *et al.*, 2012).

The market demand for industrial enzymes is growing steadily as most enzymes are produced by biotechnology using microorganisms in submerged cultures.

With an increasing demand for economical production, new product functionalities, improved safety and an increasing will to reduce the environmental pollution, the use of enzymes tends to replace traditional chemical transformation processes.. To meet the rising demand for enzymes, most new enzymes are produced from fungi or bacteria grown in large-scale fermenters using agro-industrial waste products. There are different strategies that can be employed to obtain efficient enzymes with the desired properties for industrial applications. A valuable option is the exploitation of microbial diversity to provide microorganisms that produce enzymes well-suited for various applications. Strain improvement by either conventional mutagenesis or through recombinant DNA technology is another option. Protein engineering can be employed to improve the yield, stability and the catalytic properties of an enzyme.

II.6.5. Fructooligosaccharides (FOS)

FOS can be used as artificial or alternative sweeteners and are considered as a small dietary fibre, with low caloric value. Besides, FOS serves as a "prebiotic" substrate for beneficial microbiota (e.g., *Bifidobacterium* spp.) in the large intestine, improving, at the same time, the overall health of the gastrointestinal tract. FOS promotes also the calcium and magnesium absorption in the animal and human gut, and reduces the levels of phospholipids, triglycerides and cholesterol. Different strains of the fungal genera *Aspergillus*, *Aureobasidium* and *Penicillium* produce FOS. In the past years, *Aspergillus japonicus* has been considered a potential strain for industrial production of FOS by SSF. Agroindustrial wastes like corncobs, coffee silverskin, and cork oak have been used as support and nutrient source. Further research should be conducted to develop a viable and economic process for obtaining high productivity.

II.6.6. Bioactive compounds

Bioactive compounds are extra nutritional constituents used as ingredients in food and cosmetic industries. Most common bioactive compounds include secondary metabolites such as

mycotoxins, bacterial endotoxins, alkaloids, steroids, plant growth factors, antibiotics, immuno-suppressive drugs, food grade pigments, and phenolic compounds. Secondary metabolites are excreted by microbial cultures at the end of primary growth and during the stationary phase of growth and represent some of the most economically important industrial products. In the last decades, there has been an increasing trend towards the utilisation of the SSF to produce bioactive compounds, since this process has been shown to be more efficient than SmF. Besides the higher yields by SSF compared to SmF, it has been reported that SSF produce secondary metabolites in shorter times than SmF, with significantly lower capital costs.

A variety of agricultural residues such as wheat straw, rice hulls, spent cereal grains, various brans such as wheat and rice bran and corncobs are available globally which can be considered as the cheaper and often cost free substrates for the commercial production of secondary metabolites. Some examples of SSF production of bioactive compounds include alkaloids, synthesized usually from amino acids. Total ergot alkaloids can be produced by *Claviceps fusiformis*. Antibiotics produced by SSF are penicillin, actinorhodin, methylenomycin and monorden. Important factors in antibiotic production by SSF include the type of strain used, the fermenter design, the general methodology, and control of parameters. Microbes used for antibiotics production are *Streptomyces rimossus, Penicillium chrysogenum, Amycolatopsis mediterranei, Streptomyces viridifaciens* and *Bacillus subtilis.* Phenolic compounds with anti-inflammatory, antimicrobial and antioxidant activities have also been efficiently produced by SSF.

A number of microbes produce antibiotics and other bioactive metabolites. These include bacteria such as *Bacillus* spp., *Pseudomonas* spp., *Mycobacter* and *Cyanobacter,* Actinomycetes like *Streptomyces* spp., and fungi of the genera *Penicillium* and *Aspergillus,* and also Basidiomycetes, yeasts and slime moulds (Berdy, 2005).

Surfactants

Surfactants are molecules that concentrate at interfaces and decrease surface and interfacial tension. These compounds find application in a wide variety of industrial processes involving emulsification, foaming, detergency, wetting, dispersing or solubilization. Currently, almost all the surfactants are chemically derived from petroleum. The naturally occurring surface-active compounds derived from microorganisms are called bio-surfactants and they hold several advantages over chemical surfactants, such as low toxicity, inherent good biodegradability and ecological acceptability. Most bio-surfactants are complex molecules, comprising different structures that include peptides, glycolipids, glycopeptides, fatty acids and phospholipids. Even though interest in bio-surfactants is increasing, they do not compete economically with synthetic surfactants. The choice of inexpensive raw materials is important for an economic process, because they account for 50% of the final product cost and also reduce the expenses for waste treatment. Agro-industrial wastes with a high content of carbohydrates or lipids meet the requirements for use as substrates for bio-surfactant production. Peat hydrolysate, olive oil mill effluent, lactic whey, soybean curd residue, potato process effluent and molasses are possible substrates for bio-surfactant production. In Brazil, the readily available agro-industrial wastes or by-products that have a high content of carbohydrates are cassava flour wastewater (manipueira), cheese whey and molasses. These agro-industrial wastes may be used for production of bio-surfactants using bacterial isolates identified as *Bacillus* sp. (Nitschke *et al.*, 2004).

II.6.7. Microbial pigments

The recent increasing interest in the use of edible colouring agents is a response to the potential carcinogenicity and teratogenicity of various synthetic colouring agents that have meanwhile been banned. Thus, the demand for safe and naturally occurring (edible) colouring agents has inevitably increased. There are no reliable published statistics on the size of the colour market, however, on a global scale a reasonable estimate would be US$940 million. Currently, the cost of the natural colours is higher than that of synthetic colours, but this hurdle can be overcome by the mass biotechnical production of the natural colours, thereby bringing down the cost.

Several non-carotenoid pigments (quinones) are produced by filamentous fungi. Anthraquinone pigments are produced by *Eurotium* spp., *Fusarium* spp., *Curvularia lunata* and *Drechslera* spp. The yellow pigments epurpurins A to C were isolated from *Emericella falconensis* and *Emericella fructiculosa.* Moreover, *Monascus* spp. produce azophilone pigments. A red colourant of the anthraquinone class, it may be produced by a variety of *Penicillium oxalicum.* Currently, the pigments produced by microorganisms and commercially used, are riboflavin (vitamin B2), a yellow pigment permitted in most countries and produced by *Eremothecium ashbyii* and *Ashbya gossypi*, and the pigments from *Monascus purpureus* and *M. ruber.* Carotenoids (yellow pigments) are being produced by several microorganisms, but to this moment commercial production is only from microalgae, such as β-carotene using *Dunaliella salina* and *D. bardawil*, and astaxanthin by *Haematococcus pluvialis.* Ficobiliproteins such as phycocianin (blue pigment), used in food and cosmetics, are produced by *Spirulina* sp. The pigments with potential use in the future could be indigoids, anthraquinones and naphtoquinones.

Microbial pigments are advantageous in terms of production, when compared to similar pigments extracted from vegetables or animals. The development of superior plant or animal organisms is slower than that of microorganisms and micro-algae. Therefore, the production of the pigments by bioprocesses involving microorganisms, with high growth rate, is expected to be more competitive in industrial productivity. Furthermore, the isolation and development of new strains may provide new, different pigments (Babitha, 2009).

II.6.8. Production of protein enriched feed

Technologies available for protein enrichment of agro-industrial wastes include SSF, ensiling and high solid or slurry processes. The utilised agro-industrial wastes could be lignocellulosic wastes, slaughter house wastes and manure, fish and fishery industrial wastes, animal wastes, cassava and other roots and tuber crops wastes like cocoyam, potato and sweet potato, fruit industry and vegetable wastes, olive mill and other lipid wastes. Instances of residues used as principal substrates for enrichment are: cassava waste, coffee pulp, wheat bran and straw, corn stover, millet, sugar beet pulp, citrus waste, mustard straw, agave bagasse, perennial grass, apple pomace and apple pulp. Others are grape waste, pineapple waste, cactus pear and waste fibre, rice polishing, rice bran and straw, viticulture waste, corn straw, cane bagasse, sawdust, mango waste, palm kernel cake, cabbage and Chinese cabbage wastes. Microorganisms used for protein enrichment are *Saccharomyces cerevisiae, Lactobacillus* spp., *Rhizopus oryzae, Aspergillus niger, Cephalosporium eichhorniae, Pleurotus* spp., *Lentinus* spp., *Brevibacterium divaricatum, Geotrichum fragrance, Streptomyces, Microsphaeropsis,* various Bacidiomycetes, *Coprinus fimetarius, Micromycetes, Phanerochaete chrysosporium, Pleurotus ostreatus, Thamnidium elegans,* cellulolytic bacteria, *Neurospora sitophila, Rhodotorula gracilis, Trametes* spp., *Ganoderma* spp., *Coriolus versicolor, Trichoderma* spp., *Lentinus edode, Cellulomonas biazoteain, Candida utilis, Saccharomyces cerevisiae, Pleurotus sajor-caju,* silage population, *Trichoderma reesei, T. aureoviride,* various yeasts, *Sclerotium rolfsii, Trichoderma*

harzianum, Pichia stipitis, Kluyveromyces marxianus, indigenous microbes, and many others (Ugwuanyi *et al.,* 2009).

Single cell protein extracted from cultivated microbial biomass.

Bioconversion of agricultural and industrial wastes to protein-rich food and fodder stocks has an additional benefit of making the final product cheaper. Single cell protein is produced on various agro-industrial residues by fungi *Aspergillus niger* AS 101, *Sporotrichum pulverulentum, Candida krusei* SO1, *Saccharomyces* spp. KL3G, *Candida tropicalis ceppo* 571, *Chaetomium cellulolyticum, Chrysonilia sitophilia, Fusarium graminearum, paecilomces variolii, Penicullium cyclopium,* marine yeast, mixed cultures of yeasts, *Penicillium roqueforti, Penicillium camemberti, Pichia pastoris, Schwanniomyces occidentalis, Scytalidium acidophilum, Trichoderma album, Trichoderma reesei, Kluyveromyces marxianus,* white rot fungi, *Saccharomyces cerevisiae,* or from bacteria *Methylococcaceae, Brevibacterium* spp., *Cellulomonas* spp., *Methanomonas methanica, Methylophilus methanotrophus, Pseudomonas fluorescens, Rhodopseudomonas gelatinosus* and *Streptomyces* spp. Since ancient times, people in Africa and in Mexico have been harvesting *Spirulina* from the waters and using it as food after drying. Single cell protein can be produced from the micro-algae and cyaobacteria *Caulerpa rocemosa, Chlorella salina* CU-1(28), *Chlorella* spp., *Chlorella* spp. (M109, M121, M122, M138, M150), *Dunaaliella, Chlorella* and diatoms, *Laminaria, Porphyra, Sargassum, Spirulina maxima, Spirulina* spp. (Ravindra, 2000; Bacha *et al.,* 2011).

The edible mushroom, *Pleurotus* spp., is able to bio-convert lignocellulosic agro-industrial residues to increase nutritional values and digestibility for use as animal feed (Albores *et al.,* 2006).

II.6.9. Polysaccharides

Biologically active polysaccharides have been extensively studied for their applications in the health, food and medicine sectors. Many strains of bacteria, yeasts and fungi have been selected and are used commercially because they have been found to produce enough extracellular polysaccharides in broth culture to be of economic interest. Pullulan is a homopolysaccharide of industrial interest and economic importance; it is produced from agro-industrial waste by the yeast-like fungus *Aureobasidium pullulans* (Israilides *et al.,* 1999). Among microbial polysaccharides, exopolysaccharides (EPSs) from *Aureobasidium pullulans* have attracted particular attention because they can be used as food additives, in pharmaceutical formulations and as cosmetic ingredients. However, in spite of the benefits of *A. pullulans* EPS, industrial production has been limited due to the production cost of medium constituents, which has been optimized, meanwhile, by Yoon et al. (2012).

II.6.10. Bio-plastics

Three major degradable polymer groups may be found in the market, polyhydroxyalkanoate or PHA or poly 3-hydroxy butyric acid (PHB), polylactides (PLA) and starch based polymers. Other materials used commercially for degradable plastics are lignin, cellulose, polyvinyl alcohol and poly-e-caprolactone. Molecules from renewable natural resources can be polymerised for use in the manufacture of biodegradable plastics. Lactic acid is produced through fermentation of sugar feedstocks such as beets and by converting starch in corn, potatoes or other sources. It can be polymerized to produce polylactic acid, a polymer that is used to produce plastic.

Bio-plastics are made from a compound called polyhydroxyalkanoate or PHA. Bacteria accumulate PHA in the presence of excess carbon source. Poly 3-hydroxy butyric acid (PHB) is the

most common microbial PHA. There are two ways of fermentation for creating biopolymers and bioplastics.

1. Lactic acid fermentation: lactic acid is the by-product of sugar fermentation, which is further treated using traditional polymerization processess to convert it to polylactic acid.
2. Bacterial polyester fermentation: bacteria (e.g. *Ralstonia eutropha, Bacillus megaterium, Pseudomonas putida, Pseudomonas* spp., *Bacillus mycoides, Alcanivorax borkumensi, Rhodococcus ruber* etc.) are used in a fermentation process in which they make use of the carbohydrates of harvested plants, such as corn, to fuel their cellular process. The by-product of these cellular processes is a polyester biopolymer, which is then separated from the bacterial cells.

In the manufacturing process, corn is first milled to extract starch, which is then processed to produce unrefined dextrose. Fermentation is then used to convert dextrose to lactic acid. The lactic acid is condensed to produce lactide that is used as the monomer for the biopolymer. Lactide is purified and then polymerisation occurs to produce polylactic acid.

Production of Bio-plastics

The two largest agricultural crops supplying biomass for bio-plastics are soybean and corn. They are globally available and are being grown on every continent. China is globally number two in corn production and fourth in soybean production. The use of biomass in plastics production supports the economic health of rural communities. Soybean and corn are the most important crops grown in the USA and are a major source of income to agricultural communities.

Although *Pseudomonas putida* S12 has been successfully applied for breaking down sugars obtained by the hydrolysis of lignocellulose, they have not been able to process xylose and arabinose. The result was that 20% of the material was left unused. Genetic modification of *Pseudomonas putida* S12 by inserting two genes from *E. coli* produced enzymes that transform xylose into a molecule that could be digested. Even this method proved inefficient and only 20% of the xylose was utilised; it was used as an "evolutionary" process to "train" the bacteria, by selecting only the most efficient individuals for further tests.

In 2009 world bio-plastics production was heavily concentrated in the industralised countries of North America, Western Europe and Japan. A dramatic change is expected by 2013 when Brazil will become the world's leading producer of bio-plastics. Bio-plastics demand in Japan will advance nearly six fold to 178,000 metric tons in 2013. Furthermore, China plans to open over 100,000 metric tons of new bio-plastics capacity by 2013, and thus become a major player in the global industry. The bio-plastics market in Southern Asia is in an emerging stage and in the preliminary development phase. The market is expected to grow at a compounded annual growth rate of 129.8% in the next years until 2015. According to BCC Research Report the usage of bio-plastics is expected to grow at an annual rate of 41% through 2015 (Fig. 5). This means that the global market for bio-plastics that was 640,000 metric tons in 2010, will reach 3,230,660 metric tons in 2015 and 3,700,000 metric tons in 2016. The European market already uses a higher percentage of bio-plastics but they still expect to see an almost 34% growth in bio-plastic use. Europe and North America remain interesting as locations for research and development and also important as sales market, however, establishment of new production capacities is favoured in South America and Asia.

A large number of companies, particularly in Western Europe, USA, Japan and China, are either engaged in active manufacture or self-positioning towards manufacturing of degradable plastics. Bio-based and biodegradable plastics are highly promising innovation for both industry and economy.

In the entire value added chain, agriculture is involved by cultivation of renewable raw materials and use of products.

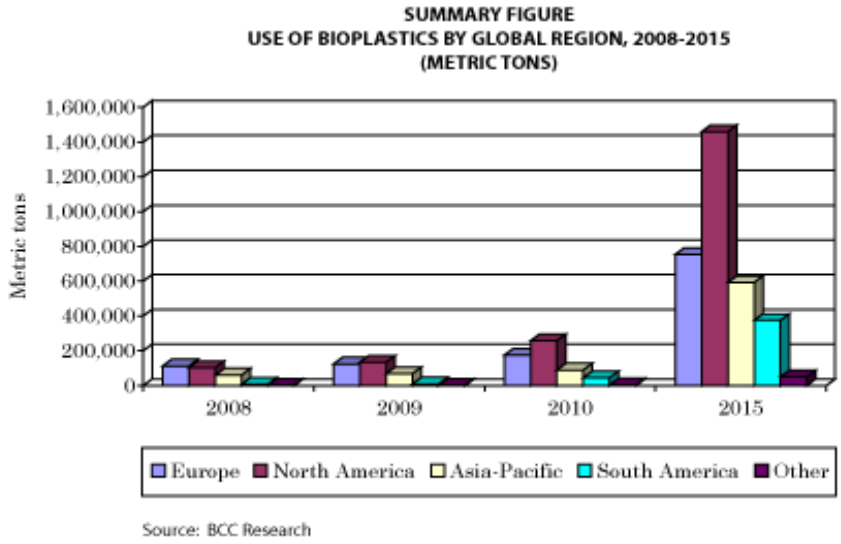

Figure 5. Use of bio-plastics by region, 2008-2015 (**Source:** BCC Research Report "Bioplastics: Technologies and Global Markets" 2010. *http://plasticsandtheplanet.com/archives/309*).

II.6.11. Bio-fuels

Bio-energy and bio-fuels are of growing public and private interest at a time of rapidly rising world energy demand and high oil prices. Increased concerns raised by global environmental challenges, including climate change are calling for "cleaner" alternatives to fossil fuels. As a result, the world biofuel production (ethanol and biodiesel) has significantly increased during the last decade, particularly in South, Central and North America (Fig. 6).

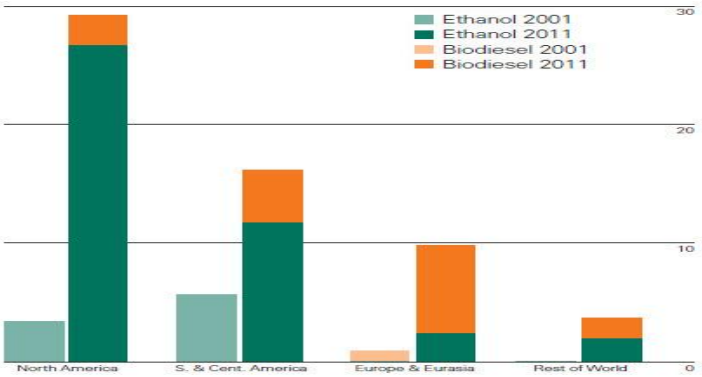

Figure 6. World bio-fuel production **(**Million tons oil equivalent) (*http://www.bp.com/sectiongenericarticle800.do?categoryId=9037217&contentId=7068633*).

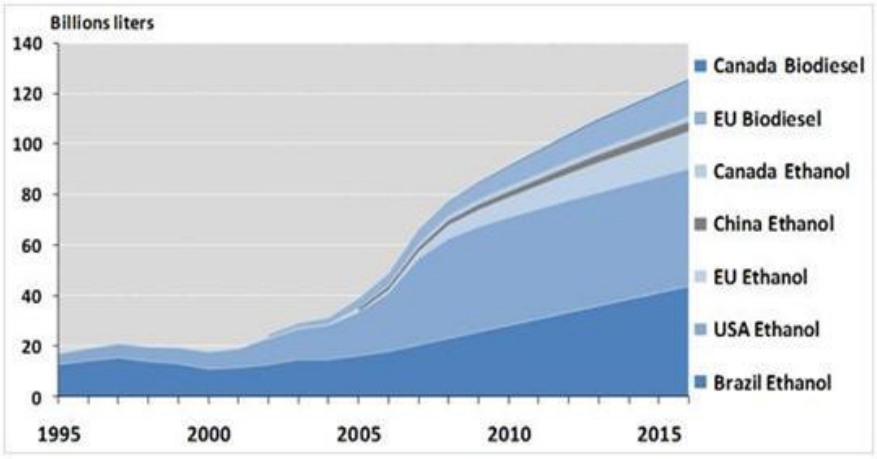

Figure 7. Bio-fuel production in selected countries - Projection up to 2016 ***(OECD-FAO Agricultural Outlook*** *site: www.agri-outlook.org*).

, The USA and Brazil, are the two largest bio-fuel producing countries, with a share of 43 and 27%, respectively, of the world's total bio-fuel production. The USA produces 54% and Brazil 34% of the world's total bio-ethanol production (Fig. 7). Regarding biodiesel, Germany contributes 16%, France 12%, the USA 11%, Brazil 9.5% and Argentina 7% of the total world production.

Feedstocks used to produce bio-fuels

Agro-industrial residues offer a cheap and abundant availability of options as raw materials for bio-fuel production. Bio-fuel use will continue to represent an important share of global cereal, sugar and vegetable oil production by 2020. Bio-fuels can be classified into two major types, *i.e.,* gaseous and liquid.

- **Liquid Bio-fuels**

Bio-ethanol is a bio-fuel used as a petroleum substitute. It is produced by simple fermentation processes involving cheaper and renewable agricultural carbohydrate feedstock (e.g. sugar-cane stalks, sugar beet tubers and sweet sorghum) and yeasts as biocatalysts. Agricultural residues and wastes have several advantages, as they do not require any additional lands because they are collected into piles at large agricultural and forestry facilities. Major raw materials used in bioethanol production are lignocellulosic materials and non-lignocellulosic materials like thippi (composed of starch, pectin, fiber and protein) and switchgrass (*Panicum virgatum*).

In Brazil, first generation ethanol is produced from sugarcane broth, a readily fermentable material where the substrate (sucrose) is directly used by the fermentative agent *Saccharomyces cerevisiae*. In the USA alcohol fuel is produced from corn starch, whereas in Europe (except for France, which uses sugar beet) wheat and barley starch are mainly used.

The second-generation ethanol is produced by saccharification of lignocellulosic material and the conversion of sugars into ethanol. The pre-treatment stage is of crucial importance to increase enzymatic conversion efficiency. Ethanol production by SSF using grape, apple and sugar beet pomaces as solid substrates, has recently been evaluated. When grape pomace and sugar beet pomace were used for cultivation of yeast *Saccharomyces cerevisiae*, the ethanol production yields were greater than those obtained by SmF. Therefore, and considering the importance of the ethanol production in the actual world economy, it is expected to observe an increase in the development of a

suitable process for ethanol production by SSF. Other fermentative agents for ethanol production are bacterial strains of *Zymomonas mobilis* and *Clostridium thermocellum* and fungal strains of *Saccharomyces cerevisiae* and *Candida utilis.*

It is estimated that 12% of the global production of coarse grains will be used to produce ethanol compared to 11% on average over the 2008-10 period. Also, 16% of the global production of vegetable oil will be used to produce biodiesel compared to 11% on average over the 2008-10 period, and 33% of the global production of sugar compared to 21% on average over the 2008-10 period. Over the projection period, 21% of the global coarse grains production increase, 29% of the global vegetable oil production increase and 68% of the global sugar cane production increase are expected to be used for the production of bio-fuels (Fig. 8).

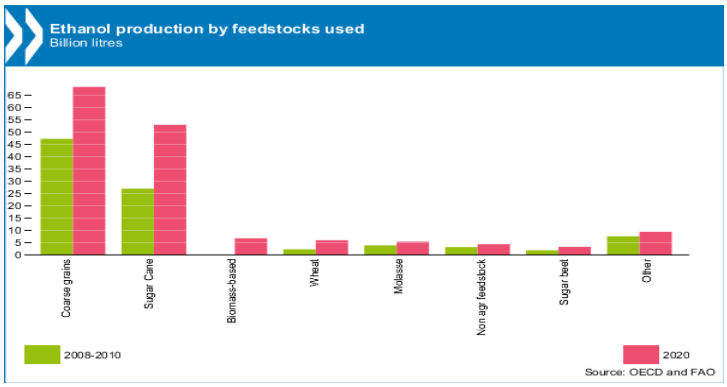

Figure 8. Ethanol production by feedstocks used. Periods 2008-2010 and 2020 (OECD-FAO Agricultural Outlook 2012-2021**,** *http://www.oecd.org/site/oecd-faoagriculturaloutlook/biofuels-oecd-faoagriculturaloutlook2011-2020.htm#analysis*).

In developed countries, the share of corn-based ethanol of the total volume of ethanol produced should decrease from 89% on average during the 2008-10 period to 78% in 2020. Wheat based ethanol should account for 6% of ethanol production in developed countries compared to 3% during the base period, most of this increase will take place in the EU. Sugar beet based ethanol should account for about 4% of ethanol production throughout the projection period. Cellulosic ethanol production is expected to become increasingly important in developed countries from 2017 onwards, to represent about 8% of total ethanol production by 2020.

Bio-diesel is generally produced from vegetable oils or animal fats. Various oils like palm oil, soybean oil, sunflower oil, rice bran oil and rapeseed oil, are used. The choice of the type of vegetable oil depends on its availability and relative abundance in the country where the biodiesel is produced. There are no reports available on the use of agro-industrial residues for biodiesel production (Chattopadhyay *et al.*, 2009; Alonso Bocchini Martins *et al*., 2011; Mussatto *et al.*, 2012).

In developed countries, the share of vegetable oil based biodiesel over total biodiesel produced should decrease from 85% on average over the 2008-10 period to 75% in 2020. Biodiesel produced from non-agricultural sources such as fat and tallow, as well as from waste oils and by-products of ethanol production should represent about 15% of the total biodiesel produced in the developed world over the projection period. Second generation biodiesel production is expected to grow in developed countries from 2018 and to represent about 10% of total biodiesel in 2020.

The most important biodiesel feedstock in the developing world should remain vegetable oils based on palm or soybean oil. This will be a result of the strong production increase in Argentina and Brazil, where biodiesel is produced predominately from soybean oil. The share of jatropha is expected to only account for 10% (19% when excluding Brazil and Argentina) of biodiesel produced in 2020 in the developing world due to the slow growth of cultivation capacities. Rapeseed oil is of minor importance for biodiesel production in developing countries, with the exception of Chile where the climatic conditions allow for rapeseed cultivation. Biodiesel production from rapeseed oil is also expected to develop in transition countries like Ukraine and Kazakhstan. Less important from a global perspective but notable from a national perspective is the production of biodiesel based on tallow in Paraguay and Uruguay, as a result of the large livestock sector in these countries.

Butanol is a very competitive renewable bio-fuel for use in internal combustion engines. Butanol (acetone, ethanol and isopropanol) is naturally formed by a number of clostridia. The fermentation substrate is one of the most important factors that influence the price of butanol. Some renewable and economically feasible substrates like starch based packaging materials, corn fiber hydrolysate, soy molasses, fruit processing industry waste and whey permeate, were investigated. Metabolic engineering of *Clostridium acetobutylicum* will improve the fermentation process and butanol recovery (Jin *et al.*, 2011).

- **Gaseous Bio-fuels**

Biogas production technology has been used for decades in developing countries specifically using animal manure for cheap production of fuel for heating and cooking from agro-residues. More developed countries have followed suit with production from a wide range of agro-wastes. The conventional biogas, which is produced in biogas plants employing anaerobic digestion of organic wastes including manures by mixed microbial cultures, is composed primarily of methane and carbon dioxide approximately 90% and may also include smaller amounts of hydrogen sulfide, water vapor, oxygen and various trace hydrocarbons. Due to its lower methane content and therefore lower heating value, compared to natural gas, biogas use is generally limited to engine-generator sets and boilers (Chattopadhyay *et al.*, 2009).

Bio-methane is upgraded or sweetened biogas after the removal of the bulk of the carbon dioxide, water, hydrogen sulfide and other impurities from raw biogas. Anaerobic digestion has proved to be the most feasible strategy for biogas production from agro-industrial wastes (Chattopadhyay *et. al.*, 2009). Methanogenesis comprises methane production by methanogens via microbial decomposition of organic matter in anaerobic environments. The process is not carried out solely by a single microorganism but by syntrophic associations. There are a number of stages in the production of methane from agricultural residues. The initial stage of depolymerization of the residues is carried out by a large number of bacteria including obligate anaerobes such as clostridia and facultative anaerobes such as streptococci and enteric bacteria. The less complex end products of hydrolysis are used as substrates by fermentive microorganisms in this stage and organic acids (e.g., acetic, propionic, butyric and other short chain fatty acids, alcohols, hydrogen and carbon dioxide) are produced. Due to the large numbers of species of bacteria involved in both stages, several organic acids and alcohols are produced. Obligately hydrogen producing bacteria further degrade propionic and butyric acid to acetate, formate, CO_2 and H_2. Various types of digesters are used, such as two-phase, plug flow, packed bed and fluidized bed digesters.

In developing countries, the technology has been used for decades only at small scale. In rural parts of India, anaerobic digestion of manure in small digestion facilities produce what is known as “Gobar” or “Gober Gas”. There have been approximations of over 2 million of these home facilities

that provide energy for cooking or possible on-site electric generation. The design of the facility is that of an airtight circular pit made of concrete with a pipe connection. The manure is usually directed to the digester directly from the cattle shed.With vast numbers of grass species, crops, vegetable wastes and also livestock manure, there are numerous feedstocks for production of Biogas. Primarily, manure has been the mainstay of these digesters in the developing world. Research is also being directed toward the production of the "Energy Crops" which are not grown for food consumption, but rather specifically for use in digesters.

Gross energy output of the bio-methane system was 17% higher than that of the bioethanol. Maybe of even greater significance was that of the cost of biomethane production dipping to 76% of its counterpart. This demonstrates the production of biomethane from such energy crops is superior to that of bioethanol.

Biogas has its uses in developing countries as a cheap energy sources especially on a small scale. The larger scale production of bio-methane has the attention of organisations such as the EU (CROPGEN 2007). From such directives as the 6th framework the production of biomethane could possible lead to the powering of cars, buses, etc. (Ward and Singh nee' Nigam, 2009).

Bio-hydrogen is a high energy yielding fuel in comparison to methane or ethanol, and produces water instead of greenhouse gases, when combusted. Autotrophically growing bacteria and micro-algae utilise light as primary energy source to split water into hydrogen and oxygen by the enzyme hydrogenase. Several forms of organic waste streams, ranging from solid wastes like rice straw and black strap molasses to waste water from sugar factories and rice wineries, have been successfully used for hydrogen production (Chattopadhyay *et al.,* 2009).

Hydrogen is a clean source of energy with no harmful by-products produced during its combustion. Crude glycerol generated during the biodiesel manufacturing process can also be used as a feedstock for hydrogen production using microbial processes. Microorganisms used for glycerol bioconversion are *Thermotoga neapolitana, Halanaerobium saccharolyticum* subsp. *saccharolyticum, Halanaerobium saccharolyticum* subsp. *senegalensis, Enterobacter aerogenes* HU-101, *Rhodopseudomonas palustris*, and *Klebsiella* sp. HE1. Genetic and metabolic engineering may be seen as potential tools for improvement of hydrogen production by bioconversion of crude glycerol (Sarma *et al.,* 2012).

Photosynthetic microorganisms such as cyanobacteria and green micro-algae may be used for biohydrogen production. Also, several groups of microorganisms are known to produce hydrogen as an end product of fermentation, e.g. *E. coli*, *Enterobacter aerogenes* and *Clostridium butyricum* (Elshahed, 2010).

- **Market current status and trends**

World bio-ethanol prices increased by more than 30% in 2010. In that year, the USA became a net exporter of ethanol for the first time, while exports from Brazil were significantly reduced due to sky-high raw sugar prices and the relatively more competitive corn-based ethanol.

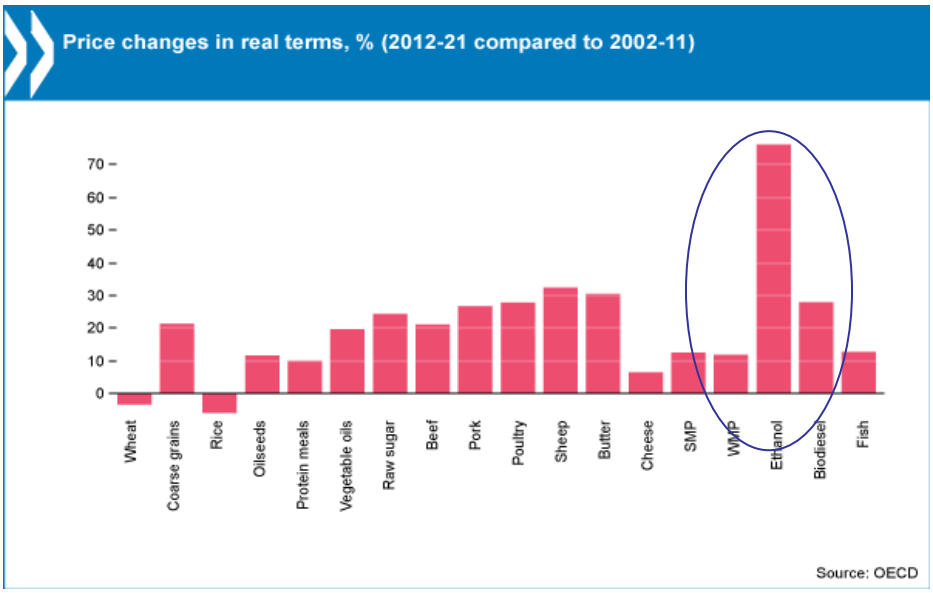

Figure 9. Estimated change in prices of bio-ethanol and bio-diesel between the forthcoming and the previous decade (**Source:** OECD).

World ethanol and biodiesel prices are expected to continue to increase (Fig. 9). Compared to the previous decade, the prices for ethanol and biodiesel are projected to rise by approximately 80 and 45%, respectively, in the 2011-2020 period (OECD-FAO Agricultural Outlook 2011-2020). The ethanol and biodiesel prices are expected to remain firm as policies promoting bio-fuel use are being implemented and crude oil prices are expected to remain strong.

Global ethanol and biodiesel production are projected to continue to expand rapidly over the next ten years. The expansion of bio-fuel production and use should be mainly driven by policy support in the forms of use mandates or other targets that impact use, tax relief for producers and consumers of bio-fuels, broader protection measures and fuel quality specifications as well as by investment capacities in leading producing countries (Fig. 10). Driven by policy mandates and renewable energy goals around the world, global ethanol and biodiesel production are projected to continue their rapid increases and reach respectively some 155 billion litres and 42 billion litres by 2020. First generation biofuels are sugarcane ethanol, starch-based or corn ethanol and biodiesel. The feedstock for producing these biofuels either consists of sugar, starch and oil crops or animal fats, which in most cases can also be used as food and feed or consists of food residues. Second generation biofuels are produced from cellulose, hemicellulose or lignin. Examples of second-generation biofuels are cellulosic ethanol and Fischer-Tropsch fuels.

The US is expected to remain the largest ethanol producer and consumer. As raw sugar prices are projected to fall, sugarcane based ethanol should become more competitive than in 2010 and exports from Brazil should recover. Ethanol use for fuel is expected to reach almost 71 billion litres by 2020. Research and development on cellulosic ethanol does not yet allow for large-scale production. Second generation ethanol production is thus only projected to expand to reach 4.3 billion litres in 2020. The USA biodiesel use continues to increase to reach 4.8 billion litres by 2020. Biodiesel production from tallow or other animal fat, waste oils as well as from corn oil by-products of ethanol plants is expected to represent more than 60% of USA biodiesel production (OECD-FAO Agricultural Outlook, 2011-2020).

Total biodiesel use in the EU is projected to increase by almost 85% and reach around 20 billion litres by 2020. The European Union is expected to be by far the major producer and user of biodiesel. European ethanol production mainly wheat, coarse grains and sugar beet based is projected to increase to almost 16.5 billion litres in 2020. The production of second generation ethanol is assumed to increase and reach 1.6 billion litres by 2020 (OECD-FAO Agricultural Outlook, 2011-2020).

In Canada, the mandate calls for an ethanol share of 5% in volume, in gasoline type fuel. Canadian ethanol consumption is projected to grow in line with fuel consumption. Domestic production is expected to rise to reach almost 2.4 billion litres in 2020.

In Australia, the ethanol share in gasoline type fuel use is expected to remain almost unchanged at about 1.6%. The biodiesel share in diesel type fuel use should remain at around 2.7%. Most biodiesel production should be based on animal tallow.

According to the analysis from Bloomberg New Energy Finance (2012), if 17.5% of the agricultural waste available in eight regions is converted into next-generation ethanol, the amount of fuel generated (115 billion liters/year) could replace about half of the global gasoline demand by 2030. The waste could be harvested without affecting the food supply or current land-use patterns.

By 2020, Brazil, India and China should account for 85% of the 71 billion litres of ethanol production in the developing world. Asian and South-American regions should also become notable ethanol producers. Brazil is projected to become the second largest ethanol producer, with a projected 33% share of the global production, in 2020. In Brazil biodiesel production based on soybean oil or possibly palmoil is also expected to increase beyond 3 billion litres by 2020 as a result of an increasing domestic demand.

Some developing countries (Argentina, Malaysia and Thailand) could play a significant role in biodiesel exports. In Thailand, production is expected to grow by 1.5 billion litres to reach about 2.2 billion litres by 2020. Investments in ethanol producing capacities are expected to continue to occur and ethanol production derived from sugar cane is expected to rapidly expand, growing by almost 6% per year. The largest biodiesel producer in the developing world will still be Argentina. By 2020, the country is expected to account for about 25% (3.2 billion litres) and 8% of the total volume of biodiesel produced bydeveloping countries and at the global level, respectively. In Malaysia biodiesel production should further increase to about 1.3 billion litres in 2020. Other East Asian countries like Indonesia and India are also expected to significantly increase their domestic biodiesel production,by about 1-1.5 billion litres each.

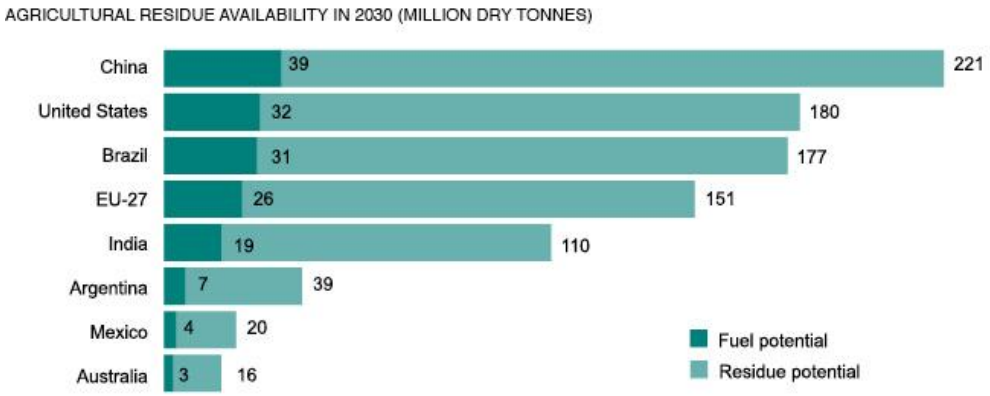

Figure 10. Trends in ethanol production from conversion of agricultural waste **(Source:** Bloomberg New Energy Finance (January 2012): "Moving Towards a Next generation ethanol Economy",

http://thecleanrevolution.org/quick-facts/analysis-second-generation-bio-fuel-from-agricultural-waste-could-replace-half-of-the-worlds-transport-fuels/).

Bio-fuel production projections in many developing countries are quite uncertain following little or no production increases in recent years. The cultivation of new feedstocks, like jatropha or cassava, does not yet allow for large-scale bio-fuel production.

There are many uncertainties concerning the future of bio-fuel policies. An important issue concerns the policy options faced by the US Environmental Protection Agency (EPA) in the implementation of the US bio-fuel policy.

II.7. Bioremediation

Bioremediation is the use of microorganism metabolism to remove pollutants. The indigenous microorganisms normally carry out bioremediation, and their activity can be enhanced by a more suitable supply of nutrients, or by enhancing their population. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. The main advantage of bioremediation is its reduced cost compared to thermal and physico-chemical remediation such as incineration. In addition, bioremediation is often a permanent solution (providing complete transformation of the pollutant), rather than a remediation method that transfer wastes from one phase to another.

The methods of microbial bioremediation are:

1. *in situ:* include bio-sparging, bio-venting, bio-augmentation and biodegradation,
2. *ex situ:* include landfarming, composting and bio-piles
3. bioreactors: include slurry reactors and aqueous reactors.

Another *in situ* treatment method is phyto-remediation using living green plants for the removal of contaminants and metals from soil. Terrestrial, aquatic and wetland plants and algae can be used for the phyto-remediation process under specific cases and conditions.

Microorganisms and processes used for bioremediation are:

- aerobic: requiring sufficient oxygen (e.g. *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus, Mycobacterium*), resulting in degradation of pesticides and hydrocarbon, both alkanes and polyaromatic compounds, with bacteria using the contaminant as the sole source of carbon and energy; with no methane generation it is a faster process;
- anaerobic: conducted in the absence of oxygen and with a concomitant slow energy input, the anaerobic bacteria are not as frequently used as aerobic bacteria; the anaerobic bacteria are used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), and chloroform and may generate methane;
- lignolytic fungi: with the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants (e.g.: white rot fungi *Phanerochaete chrysosporium*), the most common substrates include straw, saw dust, and corn cobs; and
- methylotrophs: utilising methane for carbon and energy, they are active against a wide range of compounds, including the chlorinated, aliphatics trichloroethylene and 1,2-dichloroethane (Robles-Gonzalez, *et. al.*, 2008; Wood, 2008; Gavrilescu, 2010).

II.7.1. Bioremediation of olive mill wastewater

Mediterranean countries produce more than 98% of the world's olive oil, which is estimated at over 2.5 million metric tons per year. According to the International Olive Oil Council, the European Union (EU) is the major producer of olive oil (74.3%), with the EU/27 accounting for 60% of the World total (IOC, 2012). Within the EU, the main production of olive oil comes from Spain (46.2%), Italy (15.2%), Greece (10.6%) and Portugal (1.9%). In the rest of the world, olive oil production comes from Morocco (5.9%), Syria (5.0%), Tunisia (5.0%) and Turkey (4.9%). The EU/27, together with Egypt, Turkey, Argentina, Algeria, Syria and Morocco were the top producers in 2009/2010, comprising 87% of the world production.

During the seasonal olive oil production that occurs in a relatively short period of the year, a large amount of liquid waste is generated (e.g., from $7x10^6$ to $3x10^7$ m^3 per year in the Mediterranean area). This liquid waste, called olive mill wastewater (OMW), constitutes a major environmental problem. Another environmental impact of olive oil production is the utilisation of large amounts of water and the production of large amounts not only of wastewater but sludge also. OMW has a very high organic load (COD: chemical oxygen demand and BOD: biological oxygen demand) and also contains high levels of phytotoxic and microbial inhibitory compounds, such as phenolics and long-chain fatty acids. The main organic constituents of OMW are sugars (e.g., fructose, mannose, glucose, sucrose and pentose) and polyphenols. Phenolic compounds that are present in olive stones and pulp tend to be more soluble in the aqueous phase than oily phase, resulting in high concentrations of OMW. More than 30 different phenolic compounds have been identified in OMW and their types and concentration vary tremendously. The variety of components of OMW (carbohydrates, polysaccharides, sugars, lipids, and phenolic compounds) is extremely recalcitrant, making its treatment a difficult process. The presence of phytotoxic phenolics generally prohibits the use of untreated OMW for irrigation purposes in agricultural production.

At present, OMW is treated by storage in evaporation ponds that quickly become anaerobic resulting in a dark, toxic wastewater that develops odours. The toxicity of OMW to plants and soil microorganisms, due to phenolics and lipids, prevents its reuse without treatment and it is not readily amenable to degradation by most environmental microorganisms. Often, the wastewater is poured into fresh and coastal waters or directly onto soil or in sewage, thus becaming a source of pollution . Discharge of OMW directly onto soil affects soil physical and chemical properties such as porosity and pH. The high concentration of phenolics, which are phytotoxic, can inhibit plant seed germination, when OMW is used for direct irrigation. Even, the untreated OMW may change the microbial composition of the soil through its antibacterial activity. The OMW has a high concentration of darkly coloured polyphenols that can discolour streams and rivers, when discharged directly into surface water. Furthermore, the high content of reduced sugars is able to stimulate microbial respiration, lowering dissolved oxygen concentrations, while the high phosphorus matter can lead to eutrophication (Laconi, *et. al.*, 2007; McNamara, *et. al.*, 2008; Mann, *et. al.*, 2010).

OMW treatment processes employ physical, chemical, biological and combined technologies. Untreated OMW and other agricultural wastes have been proposed for the production of animal feed, although digestability and nutritional values were found to be unsatisfactory. A number of different microorganism (Archaea, Bacteria and Fungi) and processes (aerobic or anaerobic bioreactors, composting) have been tested to treat OMW. The OMW can be used as a culture medium for growing a cocktail of microbial strains, with the purpose to degrade the polyphenolics of this material and, at the same time, produce microbial biomass potentially useful as animal feed integrators, or to bioremediate of minimally supplemented OMW at near-ambient temperatures in the presence of

autochthonous microorganisms. Bioremediation of OMW may produce valuable products such as an excellent fertilizer. OMW can also serve as a substrate for nitrogen-fixing bacteria or polymer production (Balis *et. al.,* 1996).

- **Microbial treatment of OMW**
  - **Treatment by aerobic microorganisms:**

    Bacteria. A number of different bacteria have been tested for aerobic treatment of OMW, including *Bacillus pumilus, Arthrobacter* sp., *Azotobacter vinelandii, Pseudomonas putida* and *Ralstonia* sp., as well as various bacterial consortia. Aerobic bacteria have been tested for removal of phytotoxic compounds (i.e. monoaromatic or simple phenolics). A 50% reduction in the phenolic content of OMW by *B. pumilis* and a complete transformation of tyrosol to 4-hydroxyphenylacetic acid by *Arthrobacter* sp., have been reported. A strain of *Azotobacter vinelandii* removed more than 90% of phytotoxic compounds from OMW. OMW as well as industrial wastewater (textile, pharmaceutical) require treatment before delivery to municipal treatment plants or direct discharge into surface water. These effluents were previously treated by *Pseudomonas putida* mt-2. Toxicity was totally removed when mice were treated by the bioremediated effluent. This indicates that *Pseudomonas putida* was able to completely detoxify the toxic industrial effluent (Ben Mansour, *et. al.,* 2012).

    Fungi. Fungal bioremediation of OMW have been studied using white rot fungi (including the edible mushrooms *Lentinula* and *Pleurotus*), *Aspergillus* sp., and several different yeasts. In general, a variety of white rot fungi have been used for OMW remediation, including *Coriolus versicolor, Funalia trogii, Goetrichum candidum, Lentinula edodes* and *Phanerochaete* sp. Moreover, strains of the ligninolytic basidiomycetes *Pleurotus floridae, P. eryngii, P. ostreatus* and *P. safor-caju* have been used together with the yeast strains *Saccharomyces cerevisiae* and *Kluyveromyces lactis.* They were also used species of filamentous fungi *Oidodendron* spp. and *Penicillium* spp. as inocula in treated OMW (Laconi *et. al.,* 2007). In Australia 220 fungi were screened for their ability to produce detoxifying enzymes and grow in OMW. Four isolates from the species of *Cerrena, Byssochlamys, Lasiodiplodia* and *Bionectria* were selected and compared against *Phanerochaete chrysosporium* for their ability to bioremediate OMW in the presence of a competing indigenous microflora. The bioremediation capacity of all the fungal isolates reported in the initial screen improved markedly after acclimation to OMW an increase in the age of the inoculum (Mann, *et. al.,* 2010). Other fungi used for bioremediation of OMW are *Aspergillus niger, Aspergillus terreus* and the yeasts *Trichosporon cutaneum, Candida tropicalis* and *Saccharomyces* sp. (McNamara, *et. al.,* 2008). Another treatment strategy is based on fungi (*Trametes versicolor* or *Pleurotus sajor caju*) encapsulation, on silica-alginate for removal of organic compounds, COD, colour, and toxicity in Portuguese and Moroccan OMW, thus decreasing its potential impact in the environment (Duarte, *et. al.,* 2012). The co-composting of spent coffee grounds, olive mill wastewater sludge and poultry manure, which are some of the many agro-industrial by-products generated in Tunisia and other Mediterranean countries, was investigated on a semi-industrial scale. In order to reduce the toxicity of the phenolic fraction and to improve the degree of composting humification, composts were inoculated with the white-rot fungus *Trametes versicolor* in the early stages of the maturation phase (Hachicha, *et. al.,* 2012).

    Combined bacterial-fungal strains such as the yeast *Yarrowia lipolytica* and *Pseudomonas putida,* are used for aerobic treatment.

  - **Treatment by anaerobic microorganisms:**

Anaerobic bioremediation of OMW has employed uncharacterised microbial consortia derived from municipal and other waste facilities. A significant advantage of anaerobic processes over aerobic may be the generation of methane, which could be used in remediation or as an energy source for other processes.

- **Treatment by combined aerobic-anaerobic systems:**
  Fungi like *Aspergillus niger* have been used effectively in the pre-treatment of OMW resulting in more than double methane production in subsequent anaerobic digestion. Aerobic pre-treatment with *Aspergillus terreus* reduced the concentration of phenolics and increased methane production. The yeast *Candida tropicalis* has been used for aerobic pre-treatment of OMW prior to anaerobic digestion. Two different white rot fungi (*Phanerochaete chrysosporium* and *Geotrichum candidum*) have been used in the pre-treatment of OWM prior to anaerobic digestion, with quite different result (McNamara, *et. al.,* 2008). In addition to the use of bioreactors, composting has been used to treat OMW. Cotton waste or maize straw, have been used as bulking agents for treating OMW with poultry manure, municipal waste or industrial waste from orange juice extraction as nutrient sources.

II.8. Ensiling

The application of lactic acid bacteria (LAB) to crops for ensiling to improve silage quality is a common practice in the USA and Europe. Homofermentative LAB such as *Lactobacillus plantarum, Enterococcus faecium,* and *Pediococcus* spp., are used, with the goal of providing a faster fermentation, lower final pH, raised lactate/acetate ratios, lower ethanol and ammonia, nitrogen concentrations, and improved dry matter recovery. A heterofermentative LAB inoculant species, *Lactobacillus bucheri,* has become available commercially and produces high concentrations of acetic acid in silage, which inhibit fungi and thus preserve silages susceptible to spoilage upon exposure to air. It has been tested alone or in combination with homofermentative lactic acid bacteria in alfalfa and corm silage. These inoculants help direct silage fermentation toward a more heterolactic type of fermentation, reduce the number of yeasts and thus increase the time that the silage remains stable upon exposure to air (Muck, *et. al.,* 2007; Tabacco, *et. al.,* 2011).

II.9. Impact of climate change on the sustainable use and conservation of microorganisms in agro-industrial processes

In 2020, temperature rise will probably no longer be an issue for debate. Climate change is considered a fact, and has affected global agriculture. Although a higher temperature, in combination with an increased CO_2 concentration, may even have increased the yield of vegetable production on a global scale, the regional variations are large. The effect of climate change on microbes will be complex and highly variable and will be dependent on interactions with other organisms for each ecosystem.

Global warming mostly causes climate change that affects agriculture by increasing the temperature, and modifying the rate of rainfall, water-preservation and soil fertility. Climate change impact on agriculture is different depending on the agro-ecosystem condition, but based on a number of studies, the most affected part of the world would seem to be the tropical region. Today, global warming is a major and controversial issue all over the world. It affects many aspects of life, agriculture, plant and animal biodiversity, environment and socio-economic wellbeing.

Reviews about terrestrial respiration in broad geographical regions (Raich and Schlesinger, 1992; Schlesinger, 1997; Schimel, 1995; Peng and Apps, 2000; Luo and Zhou, 2006); in particular geographic regimes and biomes (Townsend *et al*., 1992; Bekku *et al*., 2003; Bond-Lamberty and

Thomson, 2010; Anderson, 2010a) and in relation to soil decomposition processes (Tate *et al*., 1993; Adl, 2003) provide evidence of global climate change, (global temperature and patterns of precipitation) with significant effects on the dynamics of microbial communities and respiratory CO_2 emissions, especially at higher latitudes where thawing of the permafrost may release substantial stored-up carbon compounds, thus increasing microbial respiration and efflux of CO_2 into the atmosphere.

Microorganisms have survived major climate changes, such that the current projected scenarios of climate change are not likely to precipitate novel irreversible configurations of the microbial world. This hypothesis is based not only on the natural historical record of microorganisms but also on their prolific and extremely effective adaptive strategies. For example, the elevated atmospheric concentration of carbon dioxide is likely to result in changes in plant growth characteristics, affecting root systems, exudates, and litter production. Changes in vegetation cover will in turn affect the growth and distribution of free-living fungi, mychorrhizal relationships, soil bacterial diversity, and the occurrence of plant diseases. These simple interactions may then cascade to modify the activities of fungivores, bacteriovores, and omnivores. All of these changes are likely to be accompanied by dramatic fluctuations in local nitrogen cycling and in the efficiency of other biogeochemical cycles (Wall *et al*., 2001).

The uncertainty involved in making predictions about the climate is one source of uncertainty in predicting the role of microorganisms in future cropping systems. Important aspects of these changes, as they influence the contributions of microorganisms to system resilience, include the following (Beed *et al*., 2011; FAO, BSP 57):

- Changes in **average** conditions will influence cropping systems, for example allowing some systems to move into regions that were previously too cold, and restricting others through drought. Microorganisms may need to be introduced with cropping systems as they move, and may be used to support drought tolerance.

- Changes in the **variability** of conditions may have important impacts on microorganism communities. Their short generation times allow microorganisms to respond more quickly than most plants or animals to new environmental conditions. Thus, when extreme conditions occur, beneficial or detrimental microorganisms may respond rapidly.

II.9.1. Role of microorganisms in buffering of climate change

Ecologists studying plants and animals have long recognised that genetic diversity across a landscape is central to understanding the impact of environmental factors. Microorganisms offer valuable insights into relative influence of dispersal limitations and environmental heterogeneity, as well as environmental and evolutionary changes, in shaping the structure of ecological communities. In some cases, the functional adaptability of microorganisms to environmental changes hides changes in the community structure. It is clear that microorganisms are highly adaptable to different ecosystems. This can be partly explained by their short reproduction (generation) time (from hours to days), which allows them to respond to introduced selection pressures very quickly. Microorganisms live in all environments ranging from thermophilic to halophilic conditions, illustrating the highly dynamic response of microorganism genetic resources to different human or climatic impacts (Averhoff and Muller, 2010).

Microorganisms play a very important role in buffering climate change in the soil, contributing to the rate of production and consumption of CO_2, CH_4 and nitrogen. Increased biodiversity confers increased ecosystem resilience, which can buffer and stabilize climate change impacts.

Microorganisms other than those fulfilling soil functions can also play a key role in climate change buffering. For example, biological control microorganisms can stabilize population levels of plants and herbivores, making them more resilient to climate change (Hoover and Newman, 2004).

II.9.2. Microbial biodiversity leads to ecosystem resilience and sustainability

Resilience is the tendency of a system to return to its original state after a perturbation. Sustainability is the tendency of a system not to degrade from an original state. Natural resilience and sustainability are inherent in every ecosystem. Microbial biodiversity contributes to ecosystem resilience and sustainability, although understanding of this relationship is still developing.

The concept of "complex adaptive systems" can be applied to microorganism communities (Levin, 2005) based on the following characteristics:

1. "Sustained diversity and individuality of components" – microorganism communities meet this criterion by exhibiting high diversity, as well as individuality in the sense of more-or-less distinct species;
2. "Localised interactions among those components" - a large number of microorganisms generally interact only with other microorganisms that are close enough in space to compete for resources and experience the same chemical environment;
3. "An autonomous system that selects from among those components, based on the results of local interactions, a subset for replication or enhancement" - those microorganisms that can reproduce most successfully in a small local environment (such as a leaf or root) will become more abundant there and may then successfully disperse to other environments (FAO, BSP 57).

II.9.3. Impact of climate change on microbes

Bacteria are more able to respond to moisture pulses regardless of temperature, while fungi only respond to moisture pulses during cooler periods. These organisms are involved in carbon and nitrogen cycling, and changes in their activity thus ultimately disrupt aboveground processes (Bell *et al*., 2008). Changes in the timing and magnitude of precipitation will be a key-limiting factor regulating primary productivity, soil microbial activity, and ecosystem dynamics in arid and semi-arid regions. Water availability is most closely associated with structural and functional changes in the microbial community, which has implications for seasonal effects of rainfall (Williams, 2007). The composition and function of soil microbial communities is directly affected by water-related stress conditions (Schimel *et al*., 2007). Droughts may have significant implications for fungal community diversity and, therefore, the potential to interfere with ecosystem processes such as organic matter decomposition (Toberman *et al*., 2008). Extended dry periods (and heat stress) can be associated with increased nematode damage in rain-fed and upland rice areas as well as in dryland cereal systems. Although aflatoxin-producing fungi are common throughout the soils, air, and on crop surfaces, grain colonization is significantly increased when host crops are drought stressed (Cotty and Jaime-Garcia, 2007). As climate warms and weather patterns become more erratic and drought events more intense, aflatoxin contamination could further restrict the area over which crops may be economically grown. Areas in Australia with both dry and hot climates have suffered an increased probability of higher aflatoxin risk compared with locations having either dry or hot conditions alone (Chauhan *et al*., 2008). Soil biota thus plays a role in climate stabilisation and regulation. An estimated 70-140 million tons of nitrogen are fixed by microbes worldwide annually (worth an equivalent of US $90 billion compared to nitrogen fertilizer use). Microbes provide the potential to rely on biological processes

rather than on external inputs and climate change inducing inputs such as synthetic fertilizer for sustainable farming systems.

For altered moisture regimes due to climate change, as with temperature, the geographic shifts of pests, pathogens, vectors, as well as predators, parasites, and insect pathogens and interactions among them and with crops – will increase the need for knowledge of microbe function and access to living specimens to facilitate the adaptation of farming systems.

II.9.4. Adaptability of microorganisms to a changing ecosystem – Impact of climate changes

The precise impact of climate change on microorganism genetic resources for agriculture is uncertain because some changes will favour certain organisms and inhibit others. Caution is required against a simple approach of predicting the effects of individual environmental factors on single microorganisms without adopting an ecological approach. It is the combined interactions among microorganisms and their relationship with crops in an ecosystem that will dictate the impact of changes in climate. Climate change must be considered as a selection pressure that is additional to existing selection pressures, such as changes in agricultural practices and land use, and adds further complexity and uncertainty to the sustainability of farming systems. As such, climate change should not be studied in isolation but rather as an intrinsic part within a holistic ecosystem.

The adaptability of microorganisms to different pressures is clear in the behaviour of extremophilic prokaryotes. They are able to live in environments characterised by extremely high or low pH, temperature, salinity, pressure and various combinations thereof. Ever since extremophiles were discovered, their physiology and their adaptation to the hostile environment have attracted wide interest, and led to their exploitation in novel biotechnological tools (e.g., thermo-active enzymes) (Moreno et al., 2005).

II.9.5. Microbial resistance to climate change

The influence of climate changes on natural or managed ecosystem soil processes, including disease pressure, is likely to be inversely related to species diversity present within successive trophic levels. Effects of environmental changes will also vary depending upon whether a given soil food web is regulated mainly from the top down or from the bottom up (Tylianakis *et al*., 2008). Applying emerging genetic techniques holds great promise for understanding how soil organisms will respond to global changes (Roesch *et al*., 2007; Chakraborty *et al*., 2008). Sequencing entire rhizosphere communities in conjunction with the transcriptome of the associated root holds great promise for linking plant physiological status to rhizosphere activities and community dynamics. This will be a complicated and expensive undertaking because the physiology of roots varies substantially by order (Guo *et al*., 2008a, b; Pritchard and Strand, 2008) and because quantity and quality of rhizodeposits, and resulting effects on other soil organisms, may vary significantly during the lifespan of a root (Pritchard and Rogers, 2000). One might imagine that the rhizosphere community may undergo succession on a microscopic scale as a root differentially alters the chemistry of the rhizosphere during its ontogeny. Effects of the environment on such processes are therefore a moving target. Understanding these processes will require collaboration among plant physiologists, soil ecologists, geneticists and bioinformaticists. It is unclear on what time-scales changes in soil organism functioning must be studied to get a satisfactory view of what future environmental changes portend for interrelated soil and plant processes. Variable effects of environmental changes on different members of soil food webs may be manifested over different time-scales and so short-term effects of environmental changes (as are observed in most simulation experiments) on a given trophic level may

change in direction and magnitude over longer time periods. Obviously, experimental approaches to understanding the effects of warming and the associated environmental changes on soil organisms must become more realistic in terms of complexity and duration (Klironomos *et al*., 2005; Tylianakis *et al*., 2008; Kimball and Conley, 2009).

Although attempts to heat vegetation or soil without heating the air have been made in field settings (Ineson *et al*., 1998; Shen and Harte, 2000), these approaches effectively uncouple atmospheric and soil conditions (Kimball *et al*., 2008). These sorts of manipulative experiments raise questions about the validity of extrapolating to field conditions. Pritchard, (2011), advocates the initiation of a new generation of climate-change experiments designed to study interactive effects of multiple climate-change factors over timescales of decades or longer.

Some plausible direct effects are that, due to warmer temperatures, some microbes will exploit geographic locations to the North and South and in the tropics to higher altitudes. Unexpected impacts could be that, as a consequence of the increased diversity of microbes, adaptation or natural selection based on mutations may be more rapid than for other taxa. Several factors associated with climate change will affect microbes (nematodes and insects), including elevated temperature, extreme rain events, elevated carbon dioxide, and wind.

II.9.6. Elevated temperatures - global warming and its effect on severity of microbial pathogens

The existing link between temperature and microorganisms is obvious. Under experimental conditions, when wheat is grown in sterilised soil and inoculated with the take-all fungal pathogen (*Gaeumannomyces graminis*), the severity of the disease increases when the temperature is raised from 13 to 27 °C. In natural, unsterile soil, however, the disease declines when the temperature exceeds 18 °C because higher temperatures promote other microorganisms such as *Pseudomonas* spp., which antagonize the take-all fungus (Henry, 1932). Although microorganisms tend to adapt to specific environmental conditions, many can tolerate one sub-optimal factor, if all other factors are near optimal, while a combination of sub-optimal factors can prevent growth. *Diel* temperature oscillations (various time scales, including regular day and night fluctuations) affect organisms that respond quickly to temperature changes (Dang et al., 2009). These organisms include bacteria, fungi, and micro algae, which together drive a large portion of global biogeochemical cycles. The specific temperature-growth-response patterns of dominant species result in responses of ecosystem processes to temperature changes that are more complex than is generally acknowledged in large-scale models. While microbial communities would by and large be expected to favour warmer conditions (if other growth parameters are non-limiting), the composition, biomass, respiration, and function as measured by enzyme activities, decrease when exposed to increased soil temperatures (Waldrop and Firestone, 2006). Furthermore, while warm temperatures speed up biochemical reactions (catabolism and anabolism) that can result in increased activity, growth, development, and reproduction, this acceleration comes at a cost. Higher food consumption rates are required to maintain a positive energy balance (Mtui, 2011). Losses to plant diseases caused by microbes are most severe in the subtropics and tropics because of warmer temperatures, longer growing seasons, and, in some regions, year-round production that creates favorable conditions for pathogen survival. It is possible that risk due to disease will increase with increased temperatures because there will be a reduction in the number of frost days that normally reduce over wintering survival of pathogens. In contrast, lignifications of cell walls increase in forages at higher temperatures to confer increased resistance (Dahal *et al*., 1998).

II.9.7. Effect of climate and environmental conditions on liquid inocula

Previous studies have noted that liquid efficiency of Biofertilizers is almost the same in all environments, but efficiency may be reduced by 20-25% in different climatic conditions in case of

solid base. Normally the RCOF liquid formulation of an organism remains active for a time after application, ideally throughout the period of the crop, or in soil throughout the crop cycle. Microbes are inactivated by several environmental factors, like high temperature, humidity, leaf surface exudates and competitors. For example, inactivation by sunlight is the most important factor reducing persistence of microbes applied to foliage, whereas field temperatures and humidity have relatively little effect except on fungi. Liquid formulations contain organic additives to protect them against adverse effect on the environmental conditions (Pindi, 2012).

- **Effect of temperature on liquid inocula.** Temperature is important for the shelf life of microbial products and it can affect their activity before or after application. However, the optimal temperature varies with the microorganism. The microbial colonization proceeds well at field temperatures during the cropping season, but slow at lower temperature. Strains used in liquid formulation normally grow at 37 ^{o}C, (Chandra *et al*., 1999) and are able to tolerate temperature up to 45 ^{o}C for two year or more. On the other hand, solid base shelf life is hardly up to 3 months since temperatures beyond 35 ^{o}C, lead to rapid decline of organisms.
- **Effect of sunlight on liquid inocula.** Microbial inoculum must be kept away from direct sunlight. The most harmful wavelengths reaching the Earth's surface are between 280 and 320 nm (UVB). However, there may be sensitivities of some organism to wavelengths outside this range. To counter harmful effects, sunscreens are added to the formulations. Sunscreens act by physically reflecting and scattering, or by selectively absorbing radiation, converting short wavelengths to harmless longer ones. However, no such type of material is present in the solid base to avoid the effect of sunlight.
- **Effect of humidity-water availability on liquid inocula.** The moisture content also tends to affect the storage stability of the innocula. Some organisms may need moisture for its activity and thus need is fulfilled by liquid inoculum but in case of carrier base inoculum bacteria tend to get stressed, when carrier become dry during transport and storage. Bacteria used for plant growth need the plant surface to be wet in order to establish them. These needs can be overcome by only liquid formulation as product contains humectant. In general, there is little direct effect of relative humidity on the spore forming bacteria in liquid form. This implies that climate change is likely to have an effect on the storageability of some of the innocula.

II.10. Can the use/application of microorganisms in agro-industrial processes mitigate the effects of climate change in any way?

II.10.1. Involvement of biotechnology in climate change adaptation and mitigation: Improving agricultural yield

Conventional agricultural biotechnology methods such as energy-efficient farming, use of biofertilizers, tissue culture and breeding for adaptive varieties are among feasible options that could positively address the potential negative effects of climate change and thereby contributing to carbon sequestration initiatives. On the other hand, the adoption of modern biotechnology through the use of genetically modified stress-tolerant, energy-efficient and high-yielding transgenic crops also stand to substantially counter the negative effects of climate change. Safe application of biotechnology will greatly complement other on-going measures being taken to improve agricultural productivity and food security. Both conventional and modern agricultural biotechnologies will significantly contribute to the current and future worldwide climate change adaptation and mitigation efforts.

In general the following practices will help to reduce the effect of climate change:

- Greenhouse gas reduction,
- Use of environmentally friendly fuels,
- Less fuel consumptions, Carbon sequestration,
- Reduced artificial fertilizer use,
- Use of biofertilizers composting and
- Use of animal manure (Treasury, 2009; Powlson *et al*., 2011).

II.10.2 Strategies for using microorganisms for mitigating climate change

Strategies that could be used to manage microbial communities in the soil so that they contribute towards mitigating the effects of climate change are:

- **Managing microbial communities to improve carbon budget:** The carbon sink capacity of the soil, which is mediated by microorganisms, has important implications for climatic change. As soil is such a large store of global carbon, one of the major roles of microorganisms in climate change lies in their sequestration of carbon in soil organic matter (SOM) and their role in releasing carbon as CO_2 from the decomposition of SOM. Climate change alters soil carbon storage through increasing mineralization of SOM, altering the deposition of SOM, and influencing soil erosion and respiration. The quantity of CO_2 released from the respiration of soil organisms is dependent on how efficient their respiration processes are, which depends on local environmental conditions and the profile of microorganisms present. Hence, global optimal conditions for soil respiration by microorganisms cannot be defined.

  Agronomic management practices that improve the soil carbon sink will mitigate global climatic changes. Such practices include amendment of soil with organic fertilizers (manure, compost, slurries, etc.), proper crop-residue management (type and mix, C/N ratio, lignin content, etc.), no-tillage, maintenance of cover crops on the soil surface, avoidance of flood irrigation, and use of mineral fertilizers according to absolute uptake by crops and of types that are environmentally friendly (slow-release, coated, precision agriculture, etc.). Some inorganic nitrogenous fertilizers provide microorganisms with easy-to-use nitrogen, thereby boosting the activity of these chemical engineers. This increases the rate of decomposition of low-quality organic inputs and SOM, resulting in continuing decline of SOM content. This in turn causes a loss in the structure of soil, and with it the ability of soil to retain water, air and nutrients. Conversely, high levels of SOM amendments stimulate the immobilization of carbon and nitrogen in microorganism biomass, leading to high turnover of SOM into humic substances (FAO, BSP 57).
- **Managing microbial communities to reduce carbon dioxide emissions:** Currently, soils contain about 2,000 Pg of organic carbon, which is twice the amount of carbon in the atmosphere and three times the quantity found in vegetation (Smith, 2004; Solomon, 2007). The capacity of different land types (for example, woodland, pasture and arable land), to store carbon differs, and it has been suggested that land use can be managed to sequester a further 1 Pg of carbon per year in soils. This potential has received considerable scientific attention (Lal, 2008; De Deyn *et al.*, 2009; Smith, 2008a*;* Busse, 2009).
- **Prediction of geographic shifts of pests:** Overall, the (not easily predicted) geographic shifts of pests, pathogens, vectors, as well as predators, parasites, and disease-causing organisms of insects due to elevated temperatures will determine the extent to which countries are increasingly reliant on microbial genetic resources from sources beyond their own borders, as part of their responses to these threats.

II.11. Future research on climate change and responses of microbial communities

The manipulation of terrestrial ecosystems offers a potentially powerful means by which the effects of anthropogenic climate change could be mitigated.

II.11.1. Managing microbial communities to reduce carbon dioxide emissions.

Complex biological mechanisms control the incorporation of organic carbon into soil, as well as the influence of changing abiotic factors, such as moisture, temperature, land use and nitrogen enrichment, which also affect soil carbon pools (Smith, 2008a*;* Busse, 2009, Reay *et al.*, 2008). It can be argued that manipulating land use (for example, changing from arable land to forestry) and land management practices (for example, using low-nitrogen-input agriculture) may promote the growth of oligotrophic communities. However, the ecological strategies of other dominant microbial taxa need to be understood. It is true that not all taxa in a phylum will be either copiotrophic or oligotrophic (Monson, 2006), and thus phyla alone may not be a predictor of carbon loss from the soil (Fierer *et al.*, 2007). It is therefore essential that we use rapidly developing technologies such as high-throughput sequencing to better understand soil microbial diversity. Moreover, emerging technologies such as metagenomics, metatranscriptomics, metaproteomics and stable-isotope probing (SIP) must be used to examine the physiological abilities and roles of individual taxa in a given ecosystem. There is contradictory evidence about the effects of nitrogen enrichment on soil carbon stocks, and therefore it is not possible to make sweeping statements about how soil carbon sinks will respond to nitrogen enrichment. Moreover, to realize the real potential of soils to sequester carbon in the long term, we need to further expand our understanding of the interactions between different climatic conditions (temperature, moisture level and water table level), soil (pH, moisture content and structure) and biotic (bacterial, fungal and archaeal soil fauna, and plants and their consumers) properties that influence soil carbon cycling, which is currently limited.

II.11.2 Managing microbial communities generation of bio-fuels

Perhaps the most enticing and controversial area of microbial climate engineering is the substitution of fossil fuel energy sources with biofuels. As strong sources of methane (CH_4) production, landfill sites are increasingly being used for heat and electricity generation. Numerous large-scale sites across the developed world now routinely collect the CH_4 produced and either pipe it directly into the gas supply network or use it on-site for electricity generation and space heating. Such use of landfill CH_4 provides the double climate benefit of avoided CH_4 emissions and substitution of fossil fuels (Themelis and Villoa, 2007). An extension of this technology is the use of anaerobic digestion of manure, sewage and other organic wastes to maximize methanogenesis for methane collection and use. Such optimized systems also help to avoid the more diffuse emissions of nitrous oxide (N_2O) and CH_4 to the atmosphere that occur when such wastes are applied directly to soils (Tafdrup, 1995). For liquid biofuels generated from agricultural crop and residue feedstocks, microorganisms are again at the heart of current efforts to increase production and reduce fossil fuel use (Searchinger, 2008). Some of the suggested solutions to some of these problems include the use of cellulosic crop and forest residues as the feedstock for biofuel production: recent advances include the discovery of a fungus that can convert woody material into biodiesel (Strobel, 2008) and the production of ethanol by a modified *Escherichia coli* (Keasling and Chou, 2008). Such discoveries have prompted further optimism that extant or engineered microorganisms can be used to improve the net climate benefits of biofuels (Stephanopoulos, 2007). Similarly, the production of algal biomass under controlled conditions and its subsequent conversion to biodiesel or ethanol also helps to avoid

the land-use changes, food price increases and N_2O penalties that are associated with many first-generation biofuels such as corn ethanol (Chisti, 2008).

II.11.3. Managing microbial communities to reduce methane emissions.

Our understanding of the microbiology of greenhouse gas cycling is more complete for CH_4 than for CO_2 or N_2O, as the pathway is simple and specialized microorganisms are involved. However, many of the above uncertainties also apply to the management of terrestrial CH_4 fluxes. This is because most atmospheric CH_4 is produced by microorganisms, it is theoretically feasible to control a substantial proportion of CH_4 emissions from terrestrial ecosystems by managing microbial community structure and processes. This knowledge can also be applied to the reduction of CH_4 emissions by changing land use and management. In rice cultivation, for example, methanotrophs have long played a crucial part in absorbing a proportion of the CH_4 produced and, as a result, improved management of flooding frequency and duration could reduce net emissions by increasing oxygen availability in soils (Yagi, 1996). There is also great potential to make effective use of inhibitors of methanogenesis, such as ammonium sulphate fertilizers, in managed systems to promote the growth of sulphate reducers at the expense of methanogens (Neue, 2007). To reduce methane emissions from ruminant livestock, strategies include improving feed quality and directly inhibiting methanogen communities in the rumen using antibiotics, vaccines and alternative electron acceptors (Smith, 2008a).

It is likely that more than one strategy will be required to enable ruminant production systems to lower methane emissions significantly, and different mitigation strategies may be suitable for different farming practices and systems. It should also be noted that any strategy aiming at improving the animal productivity will lead to a decrease in methane production per kg of animal product (McSweeney and Mackie, 2012, FAO, BSP61).

Recent studies using denaturing gradient gel electrophoresis and qRT-PCR analysis indicate that supplementation of diets with dry corn distillers grain with solubles, condensed tannin, extruded linseed alters the diversity of rumen methanogens without affecting total methanogen numbers (Mohammed et al., 2011; Popova et al., 2011). By contrast supplementation of cattle with soya oil resulted in decrease in abundance of methanogens but diversity did not change (Lillis *et al.*, 2011).

II.11.4. Managing microbial communities to reduce N_2O emissions.

A major source of anthropogenic N_2O emission is the use of nitrogen fertilizers in agriculture. As a substantial proportion of applied fertilizers is emitted in the form of N_2O, better targeted fertilizer applications, which reduce the availability of nitrogen to microorganisms, can substantially decrease N_2O emissions. Potential strategies include reducing the amount of fertilizer and applying it at an appropriate time (when crop demand for nitrogen is high and leaching-loss rates are low), using slow-release fertilizers, and avoiding nitrogen forms that are likely to produce large emissions or leaching losses (such as nitrate in wet soil). Similarly, improved land drainage and better management practices to limit anaerobic conditions in soils (for example, land compaction and excessive wetness) could reduce denitrification rates and, thus, N_2O emissions. Finally, for the mitigation of N_2O fluxes from agriculture, the use of nitrification inhibitors in fertilizers to limit nitrate production and subsequent leaching or denitrification losses is now a well-established strategy (Smith, 2008b). These and similar microorganism-mediated strategies have great potential to reduce greenhouse gas emissions from the land use and agricultural sectors.

There is consensus among scientists about a continuous global climate change that increases in global average temperatures since 1900, are largely attributed to human activities. However, there

remains much uncertainty about predictions of future greenhouse gas emissions and the response of these emissions to further changes in the global climate and atmospheric composition. To help tackle this uncertainty, there is a need to better understand terrestrial microbial feedback responses and the potential to manage microbial systems for the mitigation of climate change. There is an urgent need to improve the mechanistic understanding of microbial control of greenhouse gas emissions and the interactions between the different abiotic and biotic components that regulate them. This understanding will help to remove large uncertainties about the prediction of feedback responses of microorganisms to climate change and will enable the knowledge to be incorporated into future models of climate change and terrestrial feedbacks. Singh *et al.* (2010) proposed several research topics that need to be prioritised for developing microorganism-mediated approaches to mitigate climate change:

- Better understanding and quantification of microbial responses to climate change and to future ecosystem functioning.
- Classification of microbial taxa in terms of their functional and physiological capabilities and to link this information to the level of ecosystem function.
- Improving our mechanistic understanding of microbial control of greenhouse gas emissions and microbial responses to simultaneous climatic factors, such as warming, altered precipitation and increased CO_2 levels, across different ecosystems.
- Developing a framework to incorporate microbial data (biomass, community, diversity and activity) into climate models to reduce uncertainty and to improve estimation and prediction.
- Better understanding of the effect of climate change on above-ground and below-ground interactions and nutrient cycling, as well as the role of these interactions in modulating the response of ecosystems to global change.
- Developing a framework based on the above five points to potentially manage natural microbial systems to enhance carbon sequestration and/or reduce net greenhouse gas emissions.
- Using an interdisciplinary approach that includes microbial ecology, environmental genomics, soil and plant science, and ecosystem modelling. There have been substantial advancements in the technologies that can be used to examine microbial communities and to relate them to ecosystem functions. These technologies should be applied to study how particular taxa respond to individual and multiple climate variables and how such responses influence ecosystem functions.

However, to further improve predictions, we need to incorporate data on microbial diversity, community structure and physiological capabilities of various taxa. Only after we have such an improved understanding of microbial responses can a framework on management of microbial systems for reduced greenhouse gas emission be developed. Microorganisms could either greatly help in climate change mitigation, or prove disastrous by exacerbating anthropogenic climate change through positive-feedback mechanisms. No academic article is complete without a call for 'more research', but seldom is there an area such as microbiology and climate change that urgently requires so much more research effort and that has so much at stake (Fig. 11). Microorganisms may be out of sight, but we cannot afford them to be out of our mind (Singh *et al*., 2010).

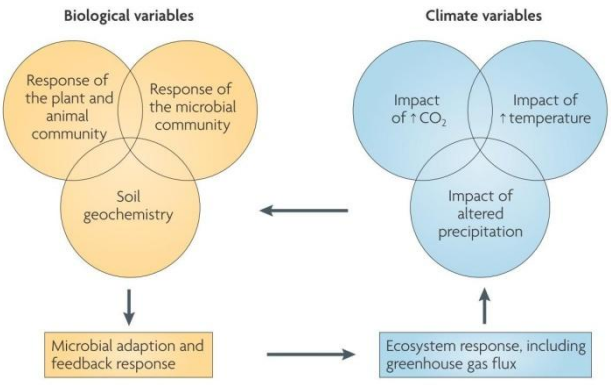

Figure 11. A proposed framework for future research on climate change and ecological responses. It is important to understand the responses of individual microbial species and whole microbial communities, as well as their interactions with other soil biota and plants, to single climatic variables (such as increased levels of carbon dioxide (CO_2) and changes in temperature and precipitation) and in multifactorial experimental conditions. This approach should then be tested in contrasting ecosystems differing in climatic, nutritional, chemical and physical properties. Such an integrated approach is essential for gaining a mechanistic understanding of microbial adaptation and feedback responses to individual and interacting global changes. This understanding then can be exploited to predict the feedback response at the ecosystem level using various climate models (Sing *et al.*, 2011).

II.11.5 Microorganisms, process rates and climate models

The relationship between global changes (altered temperature, CO_2 levels and precipitation) and the rate of processes such as denitrification and respiration can change according to the response of microbial communities. For example, a soil process (such as the decomposition of organic carbon) converts a component from state 1 to state 2 at a rate *k*, and it is assumed that the process is mediated by the soil biota present. In the first scenario global change directly influences the functioning of existing microbial communities without altering the community structure. This may cause a shift in the process rate, but its behavior and controls remain unchanged. However, as in the second scenario, a shift in microbial community structure caused by global change could also alter the fundamental control mechanism of the process. Most ecosystem models and all climate models that include a description of microbial processes use first-order rate kinetics, which assume that the microbial population is sufficient to carry out the function (for example, decomposition) and that the rate of the process is modified by environmental factors such as temperature and moisture.

This approach works well within the parameterized limits of the model, and process rates largely follow trajectories that are mimicked well by such formulations. What is not known, however, is what happens if the climate changes beyond the parameterized limits. For example, if the structure of the microbial community changes in such a way that the function also changes, a discontinuity in the response may occur and the response could move to a different trajectory. Such threshold effects cannot be represented in the current structure of ecosystem and coupled-climate models. Understanding these potential threshold effects and identifying the systems and processes for which they are likely to be of greatest importance remain key challenges for microbiology (Singh *et al*., 2010).

II.12. Impacts of Climate Change - Gaps in knowledge - Recommendations

There is abundant evidence and agreement that the degree to which organisms will tolerate new conditions imposed by climate change will vary across species and populations, but we cannot yet predict the extent to which phenotypic plasticity, evolutionary adaptation, and non-genetic parental effects will allow species to adjust. Basic information on species and population traits ranging from physiology to behavior, life history characteristics, current distributions, dispersal abilities, and ecological relationships is needed to understand why some species and populations are able to adjust to the impacts of climate change (while others decline), and will be critical for building better models to forecast future biological responses and vulnerabilities (McMahon *et al.*, 2011).

Projecting climate change impacts on biodiversity involves many uncertainties (Pereira *et al.*, 2010; Bellard *et al.*, 2012) stemming from variability in climate projections (particularly precipitation patterns), uncertainties in future emissions, and assumptions and uncertainties in the models used to project species responses and extinctions (He and Hubbell, 2011). Some of these uncertainties are

inevitable given that we are trying to predict the future; nonetheless, techniques and modeling approaches are becoming more sophisticated and able to evaluate myriad influences such as biotic interactions and dispersal abilities that were previously deficient. Projections are also complicated by uncertainty about where and how human responses to climate change are likely to impact biodiversity. Sustainable energy development and infrastructure, changes in agricultural practices, human migrations, and changes in water extraction and storage practices in response to climate change are all very likely to have impacts on biodiversity. Predicting where these mitigation and adaptation responses will occur, and how they will impact biodiversity will be a critical step in developing credible future climate change impact scenarios. Although many tools for forecasting climate change impacts on ecosystem services exist (Kareiva *et al.*, 2011), fewer methods for anticipating how people will respond to those impacts have been developed or incorporated into projected impacts on biodiversity (Staudinger *et al.*, 2012).

The specific impacts of climate change on biodiversity will largely depend on the ability of species to migrate and cope with more extreme climatic conditions. Ecosystems have adjusted to relatively stable climate conditions, and when those conditions are disrupted, the only options for species are to adapt, move or die.

It is expected that many species will be unable to keep up with the pace and scale of projected climate change, and as a result will be at an increased risk of extinction, both locally and globally. In general climate change will test the resilience of ecosystems, and their capacity for adaptation will be greatly affected by the intensity of other pressures that continue to be imposed. Those ecosystems that are already at, or close to, the extremes of temperature and precipitation tolerances are at particularly high risk.

Over the past 200 years, the oceans have absorbed approximately a quarter of the carbon dioxide produced from human activities, which would otherwise have accumulated in the atmosphere. This has caused the oceans (which on average are slightly alkaline) to become more acidic, lowering the average pH value of surface seawater by 0.1 units. Because pH values are on a logarithmic scale, this means that water is 30 per cent more acidic.

The impact on biodiversity is that the greater acidity depletes the carbonate ions, positively charged molecules in seawater, which are the building blocks needed by many marine organisms, such as corals, shellfish and many planktonic organisms, to build their outer skeletons. Concentrations of carbonate ions are now lower than at any time during the last 800,000 years. The impacts on ocean biological diversity and ecosystem functioning will likely be severe, though the precise timing and distribution of these impacts are uncertain.

II.12.1. Gaps in key knowledge

- Debate in science, policy and public domains about suitable objectives for conservation in the face of climate change, informed by an understanding of social values associated with biodiversity.
- Regionally specific information about impacts and their implications, combining local ecological expertise with modelling and published information.
- A richer body of science-policy knowledge to enable managers to determine and seek the information that will be useful to them, and to help researchers develop analysis tools and monitoring.
- Knowledge and tools to help managers balancing worthy but competing demands, such as the protection of habitat and management of threats.
- More understanding and better use of tools to deal with uncertainty. Establishing new alliances between science and conservation agencies would ensure research was focused on

priority policy and management knowledge gaps, and help facilitate rapid flow of information into conservation agencies' decision making.

- More work is required in the emerging discipline of climate change biogeography to understand how high levels of environmental change, as predicted for many continents, will translate into ecological change, vulnerability and the likelihood of loss in values.
- Helping policymakers and managers respond to climate change, debate is needed in science, policy and public domains about suitable objectives for conservation in the face of climate change how to effectively minimise loss while accommodating substantial ecological change. This needs to be informed by an understanding of the many different attributes of species, ecosystems, landscapes and patterns in diversity, how they may change, and the social values associated with them.
- Understanding the implications of climate change at regional scales and reassessing objectives will require good information about future changes at appropriately fine scales. This can come from a combination of collating existing information (e.g. the continental analyses of this project, and many reviews of ecological impacts), existing regionally specific ecological knowledge and the results of monitoring and new research. Site-specific collaboration between researchers and managers may help address this.
- More information is required about landscape processes and features that might give rise to persistence and adaptability of biodiversity at various scales. Relevant ecological factors may include interactions between species, climatic variability, extreme events, disturbances, connectivity, environmental buffering, refuges, access to variable ecological resources and the value of restored habitat.
- A richer body of science-policy knowledge is required to enable managers to determine and seek the information that will be useful to them, and to help researchers develop more useful analysis tools and monitoring. This needs to incorporate social and institutional factors such as biodiversity values, information availability and resources (Dunlop *et al.,* 2012).

II.12.2. Proposed key actions

- Identify a core set of widely recognized, policy-relevant questions about impacts on biodiversity and ecosystem services.
- Establish a broader ecosystem assessment process and framework.
- Align monitoring, modeling, and assessment activities for climate with those for biodiversity and ecosystem services.
- Identify and convey clear connections between biodiversity loss, reduced ecosystem services, and societal benefits (Staudinger *et al.,* 2012).

II.12.3. Conclusions and implications

- **Climate change is likely to lead to very significant and widespread ecological impacts.** Over most of the continent climate change will lead to a significant mismatch between local biodiversity, as it is distributed today, and future environments.
- **Spatial environmental heterogeneity may help buffer the impact for some species.** Ecological analysis and modelling indicate that the ecological impacts of climate change at any given location may potentially be reduced by local and regional scale environmental heterogeneity, including the presence of refuges, and that this buffering is likely to be widespread. However, the level of buffering afforded by such heterogeneity

will vary substantially between regions, depending especially on topographic relief and elevation gradients. The modelling suggests that, in many areas, it is possible that the capacity for local buffering may be swamped by the overall magnitude of environmental change. However, analysis with finer-scaled biotic and environmental information is required before conclusions can confidently be drawn about future, local and regional environmental buffering and refuges. Even where the magnitude of change does exceed buffering for current species, environmental variability will still provide critical habitat heterogeneity and buffering (from temporal variability) for new species, thereby contributing to future landscape-level species richness.

- **Many threats to biodiversity will increase as a result of climate change.** Without careful planning, adaptation to climate change in other sectors — including grazing, cropping, forestry, water supply and settlements — is potentially a significant threat to biodiversity, and could readily affect protected areas by changing the landscape context, including broad ecological processes.
- **Climate change will affect how we conserve biodiversity.** The magnitude and pervasive extent of future climate change means that conservation programs are facing much greater levels of ecological change and losses in species and other biodiversity values than previously anticipated. This suggests significant changes to current conservation strategies may be required. These should be underpinned by a good appreciation of the ecological impacts of climate change, including the multiple types of change. Climate change increases the risk of reductions in crop and livestock yields. Within a given region, different crops and livestock are subject to different degrees of impacts from current and projected climate change Lobell *et al.* (2008). In light of this, the adoption of specific crops, livestock or varieties in areas and farms where they were not previously grown are among the adaptation options available to farmers (Bryan *et al.* 2009; Chigwada, 2005; Chatterjee and Das 2005). Further, the use of currently under-utilized crops and livestock can help to maintain diverse and more stable agroecosystems (Bowe, 2007). Conserving crop and livestock diversity often helps maintain microbial communities.
- **Changes in agricultural practice.** Given the above-mentioned impacts of climate change on agricultural systems, practices that enhance soil conservation and sustainable use and maintainance of favorable microclimates are important for adaptation in agriculture. These practices can include methods such as: terracing and stone bunding, (Shiferaw *et al.*, 2007) the use of organic fertilizers, and changes to tillage practices, (WRI, World Resources 2008); crop rotation and the use of vegetation buffer strips; (Lal *et al.*, 2007) and maintaining cover through plantings or mulches (Miller *et al*., 2009). In drylands, agricultural practices such as the use of shadow crops can enhance resilience by providing protection against extreme rainfall, and increasing infiltration into the soil. (Blanco, 2004) Many of these measures reduce the need for nutrient inputs and use of heavy machinery. They also decrease vulnerability to extreme weather events. For example, in Thailand, the sustainable economy project is encouraging diversification within previous mono-cropping practices (largely rice paddies) with positive impacts on poverty alleviation, carbon sequestration and agricultural biodiversity.
- **Agroforestry** is a promising option for increasing the resilience of rural communities in the face of climate change. Agroforestry involves the integration of trees into crop and animal production areas and includes a diverse range of systems, such as silvopastoral systems, shade-grown perennial crops (e.g., coffee, cocoa, and rubber), windbreaks, alley cropping, and improved fallows. Inclusion trees within agricultural systems leads to increased soil conservation, microclimatic buffering and more efficient water use, (Rao *et*

al., 2007) and thereby helps buffer the impacts of climate change. Agroforestry systems provide important microbial biodiversity benefits and also serve an important role in climate change mitigation by enhancing carbon stocks within the agricultural landscape and, in some cases, reducing pressure on nearby forests, thereby reducing emissions from deforestation.

II.12.4. Monitoring and surveillance

Our current lack of knowledge makes it is impossible to predict precisely how climate change will affect the outcomes of interactions between microorganisms, crops and other components of the ecosystem. Therefore, integrated systems for routinely monitoring the influence of climate change, alongside other drivers such as land-use management practices, are required. Based on the knowledge accrued from practical scenarios, better-informed decisions can be made on how to adapt to climate change and ensure that crop-based sustainable agriculture thrives. A few soil-monitoring initiatives have been established, and include France's Soil Quality Measurement Network, Germany's network of 800 soil-monitoring sites, the Netherlands' Soil Quality Network, and the soil section of the European Commission's Joint Research Centre. While these discrete activities recognise the importance of soil, there is no global system to evaluate and share genetic and functional information on soil biodiversity. An optimal system would be to select key sites based on climate (continental, Mediterranean, arid, temperate; etc.), soil type, podogenetics and cropping system. The sites would be selected strategically so that they represent larger areas, allowing interpolation of the results to scale (Beed *et al.*, 2011). Any monitoring programme will be based on indicators selected for specific purposes.

For the use of microbial indicators in a terrestrial monitoring programme the following is recommended:

- **Identification of specific minimum data sets for specific end points.** A minimum data set (MDS), that is a limited number of indicators, will be required in the development of a monitoring programme due to costs and labour.
- **Establishment of baseline values.** Baseline values on the selected microbial indicators, including information on both spatial and temporal variations, have to be known or developed within the first year of monitoring to define reference and threshold values for repeated monitoring activities.
- **Improvement of the scientific basis.** It is recommended that further scientific knowledge should be developed through research activities included in the monitoring programme to provide part of the scientific base for new management policy at the national and international level. Specifically, research on microbial biodiversity should be in focus.
- **Implementation of new indicators.** Implementation of new indicators is recommended as soon as these are applicable for soil monitoring purposes. These new indicators should be based on continuous development of microbial methods within the scientific community and will provide more precise, detailed and integrated results, and give a dynamic up-to-date monitoring programme. Implementation is recommended in parallel with existing measurements to assure the quality and comparability of the new indicators as the old indicators are phased out. The data sets of the new indicator can be used as the baseline for future monitoring activities (Nielsen and Winding, 2002). For plant pathogens, monitoring programmes have been established as a means to ensure that genetic resistance of crop plants or control through pesticides are not overcome. When crop resistance is overcome or pesticide resistance occurs, efforts are made to screen for new forms of resistance or develop new pesticides with different modes of action. However, plant-pathogen monitoring programmes, such as the National Plant Diagnostic Network in the

United States of America (Miller *et al.*, 2009), are limited to large-scale agricultural production in technologically advanced countries. As crop deployment responds to climate change, it is likely that many crops, often with a narrow genetic base, will be grown far from their centres of origin and apart from the pathogens that have co-evolved with them. In such circumstances, it will be even more critical to have monitoring and surveillance systems that share global knowledge on how to diagnose causal agents of disease and to monitor their global distribution, spread and hence the risk that they will become established in surrounding areas. Despite all the tiers of likely interactions at any one site, the aim of monitoring and surveillance is to help clarify which microorganism species could be used as indicators for key ecosystem roles (nutrient acquisition, biological control, disease, food spoilage, etc.) for a given crop system, ultimately leading to ecosystem resilience and therefore sustainability of the agricultural system.

II.12.5. Methods for characterising microbial species, communities and their functions

Genomic and other "omics" techniques provide new ways for microbial diagnostics and studying functions.

II.12.6. Standardised methods and metadata

The standardization of methods used to characterize designated parameters is critical if data from different locations, collected by different individuals, are to be compared. The need to compare data is especially important as the climate changes and lessons learned in one location need to be applied elsewhere. Because assumptions and recommendations will be made based on analysis of the data collected, it is imperative that the data are of the utmost quality. Data about data are called metadata and are used to describe data sets in order to support their synthesis. For example, publication of microarray data needs to follow MIAME (Minimum Information About a Microarray Experiment) standards, to enable the unambiguous interpretation of the results of the experiment and to create a good potential to reproduce the experiment (Brazma *et al.*, 2001). There are particularly stringent requirements for metadata that define microorganism within ecosystems. Microorganism data will include observations that may not be of immediate interest to experimenters collecting the data, but are important for synthetic analyses across data sets (Nielsen and Winding, 2002).

II.12.7. Models for microbial function

Models allow examination of complex interactions from different perspectives by using combinations of factors based on available data and considered assumptions. Microorganism community function is so varied that models can be used to simplify a range of interactions and to focus only on those that are critical to crop-based agriculture. Modelling offers the opportunity to test hypotheses developed based on controlled environment studies or field-based observations, and to determine whether specific relationships are robust when ecosystem complexity is increased. Modelling also offers the opportunity to test whether inferences made from field studies are correct when other factors are varied.

II.13. Conclusions (climate)

The complexity of microbial communities living belowground and the various ways they associate with their surroundings make it difficult to pinpoint the various feedback responses that soil microbes may have to global warming. Whether a positive feedback response results, in which microbial processes further contribute to climate change, or whether a negative feedback response

slows its effects, it is clear that microbes can have a huge impact on future climate scenarios and ecosystem-level responses to climate change. Soil respiration plays a pivotal role in these effects due to the large amount of CO_2 and CH_4 emissions produced during respiration, the reliance of carbon stocks in soils on rates of respiration, and the initial sensitivity of soil respiration to increased atmospheric temperatures. Further studies in long-term feedback effects of soil respiration on climate change can contribute to our understanding of the overall impacts of climate change; including the ability of terrestrial forests to uptake excess CO_2 from the atmosphere. As we attempt to mitigate greenhouse gas emissions and adapt to predicted climate change effects, turning towards microscopic life that lies below the surface can perhaps help us to become better equipped for future changes at the macroscopic and even global scale.

Microbes can play a role in climate change mitigation. Soil organic matter is the major global storage reservoir for carbon (and not forests as is commonly thought). Microbe (and invertebrate) diversity is responsible for breaking this material down and making it available to plants while, at the same time, contributing to the rate of production and consumption of carbon dioxide, methane, and nitrogen. Genetic resources held by discrete projects and microbe collections across the world need to be understood in terms of functionality. For agriculture, such capacities will help screening programs to select appropriate crop germplasm for different agro-ecological zones or farming systems.

In support of all of these efforts, it is already important, and it will be increasingly so with the impact of climate change, to be able to access, characterize, and pool, representative samples of the genetic diversity of microbial species and strains.

III. THE CONSERVATION OF MICROORGANISMS USED IN AGRO-INDUSTRIAL PROCESSES

III.1. Importance of Biodiversity

Microorganisms are important sources of knowledge about the strategies and limits of life and therefore they should be preserved. They are of critical importance to the sustainability of life on our planet. The untapped diversity of microorganisms is a resource for new genes and organisms of value to biotechnology. Diversity patterns of microorganism can be used for monitoring and predicting environmental change. Furthermore, microorganisms play a role in conservation and restoration biology of higher organisms. Microbial communities are excellent models for understanding biological interactions and evolutionary history. Genetic diversity enables researchers to develop improved strains for human needs. The conservation of biodiversity is fundamental to achieving sustainable development.

Figure 12, below, shows the number of species of all kinds of living organisms currently known.

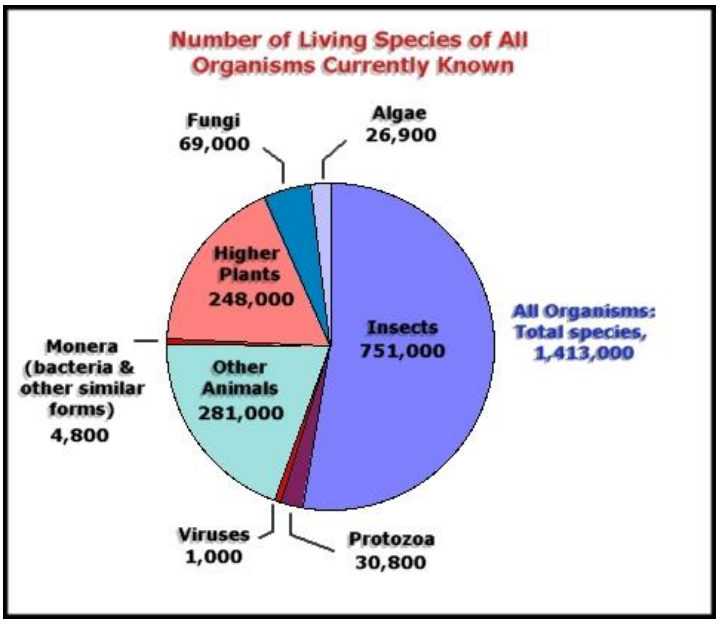

Figure 12. Number of currently known living species of all organisms (*http://www.globalchange.umich.edu/globalchange2/current/lectures/biodiversity/biodiversity.html).*

Although extensive information has been generated on plant and animal biodiversity, little is known about microbial diversity, comprising an estimated 50% of all living populations on earth. There may be 1.5 million species of fungi, but only 5% are described and as many as 1-2 million species of bacteria, but only about 5,000 have been described.

Microbial diversity has driven and impacted life on Earth as well as the nutrient cycle, which are keys to the operation of biosphere. Microbes evolve more quickly than we can study them, providing an ever-increasing diversity of function for industrial application. In the soil profile, the microbial population mostly occurs within 40 cm of topsoil. Bacteria are predominant followed by the actinomycetes (belonging to the Gram-positive bacterial Phylum *Actinobacteria*) and fungi. High variation can be found in abundance of microbes between different soil types, seasons and land uses.

The greatest uncertainty in population counts is our inability to recover all the organisms in a culture or sample, as only about 5-10% of the soil organisms can be recovered. Large fractions of the microbial assemblage of any ecosystem are unculturable, and thus remain unchartered. The ability to obtain genomic libraries of uncultured microorganisms will further increase our knowledge and access to nature's diversity, and supply future industry with new raw materials. Microbial diversity is a great source for biotechnological exploration of novel organisms, products and processes. Developed countries having the technology and resources to patent and develop commercial biological products, will have the benefits of biodiversity through the collected and conserved biological material (Tripathi *et. al.,* 2007; van der Heijden *et. al.,* 2008).

III.1.1. Role of diversity in the use and conservation of microbes

Biodiversity loss continues, in part, because local benefits from wildland preservation are limited. Biodiversity development agreements (BDAs) intend, through bioprospecting efforts, to distribute benefits of biodiversity to those who bear preservation costs. Monetary returns from bioprospecting could be substantial, though realization of returns is uncertain and likely to take time. Considerable non-monetary benefits from BDAs have included training and increased infrastructure

and institutional capacity. BDAs probably will not finance desired land preservation, nor is it certain they can influence land use. Nonetheless, carefully structured BDAs can be useful components of biodiversity conservation programs (Day-Rubenstein and Frisvold, 2001).

III.1.2. Biodiversity: conservation and collections of microorganisms

Conservation of microorganisms is an open scientific field. It is not yet known what processes lead to the extinction of microorganism taxa or significant loss of genetic variability.

Plant pathogens are so difficult to diagnose in the first place, so a systematic approach to the characterization of biodiversity is required. However, the ever-shrinking human and technical capacity in this area means that work needs to be concentrated at key reference collection sites for the benefit of the global community. Microorganism collections preserve type of strains that represent key genetic entities by serving as living references for each functional group. Live reference collections at centralised and open-access facilities are needed in order to characterize the taxonomy and function of microorganisms as a prerequisite to the development of tools for diagnosis and detection (Barba *et al.*, 2010). Such collections can also be used to increase awareness of the available living material through databases and web-based portals, and to provide access for research and capacity building. If submission of strains becomes a condition of acceptance for scientific publications, a system similar to the GenBank (www.ncbi.nlm.nih.gov/genbank/) could be created to ensure the completeness of collections. Also, the conservation of all variants of any particular microorganism is necessary to permit the development of a diagnostic tool that encompasses all the known variants. This is critical as microorganism pathogens adapt rapidly in the face of selection pressure such as the mass deployment of resistant crop germplasm, and this evolution must be captured in tools used to monitor the presence or spread of pathogen species.

All data accrued on the variance of microorganism pathogens could also be sourced via centralised collections under the auspices of the World Federation for Culture Collections (www.wfcc.info/).

In situ conservation also has an important role to play. For example, *ex situ* conservation of wild crop relatives depends on maintenance of appropriate microorganism communities, so that co-evolution among plants and microorganisms can continue. Determining what microorganism communities are most appropriate is challenging because of our limited knowledge of these interactions. Furthermore, because microorganisms are highly adaptive to new scenarios, such as those likely to be induced by climate change, *ex situ* collections of microorganisms may become outdated. Efforts are therefore required to advance *in situ* conservation methods for microorganisms.

Keeping the appropriate conservation of microorganisms is necessary in order to:

- Characterise the biodiversity at the ecosystem level using biochemical and molecular techniques,
- Characterise the resilience and sustainable ecosystems for use in research,
- Prioritise key species for enhanced use based on socio-economic assessment and evaluation against major biotic and abiotic stresses,
- Consolidate biobanks for microbial genetic resources for agriculture, thereby providing more comprehensive insight on biodiversity, including DNA, BAC libraries and genetic stocks,
- Develop and implement methods and strategies for conserving microorganism genetic resources for agriculture in natural ecosystems and protected areas, and

- Promote and encourage participatory research for sustaining production in the face of climate change and other threats.

III.2. Conservation practices

Organic fertilizers have long been used to address declining fertility while pests and disease were initially controlled through crop rotation. After modern agriculture has come to tropical countries, local biodiversity has been degraded and pesticides have accumulated. The compacted soil by modern agriculture machinery restricts water penetration and increases the amount of surface water, causing runoff to carry away greater volumes of soil. The opening up of semi-arid land for grazing and cropping has resulted in subsurface salt rising to the surface. Very large areas of Australian soils have been severely degraded by salinisation and similar problems trouble the agricultural landscapes of India and Pakistan.

Biodiversity for agriculture has two elements:

- Biodiversity of crops both planted and wild and livestock,
- Soil microorganisms and pest predators.

Microorganisms are a major part of ecological processes that sustain the functioning ecosystems, including a range of associations between plants and microorganisms that fix atmospheric nitrogen, and extract phosphorus, various micronutrients and water under very low moisture conditions, and through mycorrhizal associations.

In recent decades scientists have responded to this threat by developing a worldwide network of gene banks for conserving genetic resources. Protected areas for biodiversity throughout the agricultural landscape are needed to support the agriculture sector and contribute to the biodiversity by maintaining wild relatives, traditional cultivars and farming systems. Traditional knowledge has been practised and passed on from one generation to another and is intertwined with cultural and spiritual values. For example, traditional agriculture in Sri Lanka is an integrated system based on ecological principles that include trees, crops, livestock and fish. Beliefs about supernatural beings play an important role. In Africa and elsewhere, traditional knowledge and people participation are necessary. Moreover, the traditions of indigenous people and farmers in the management of biodiversity need to be strengthened to continue and develop. To fulfil this, national governments and farmers should collaborate and the subject of biodiversity and traditional knowledge needs to be introduced in schools to educate the younger generation (Natarajan, 2002).

Agroecosystems currently occupy 30% of the earth's surface and include the earth's most productive soils. Effective management of agroecosystems is critical to improving the conservation and viability of biodiversity. Sustainable agriculture must work toward identification and implementation of practices that minimize energy loss throughout the system, while maintaining productivity. Diversity is a key issue in energy flux. Diversity in crops, cropping systems and management practices will enhance the stability of agriculture and affect the microbial portion of the agroecosystem, as it is essential to sustainable agriculture and the maintenance of viable, diverse populations and functioning microbial communities in the soil (Kennedy and Smith, 1995).

Sustainable agriculture can raise poor farmers' incomes and increase yields by conserving resources, maintaining healthy soils, reducing water pollution, increasing farm biodiversity and by energy efficiency. Sustainable agriculture includes the following practices: rotating crops and using grass in rotations, growing "cover" crops, application of integrated pest management and natural pest regulation, implementation of integrated soil fertility management, using of livestock manure, planting legumes, promoting a diversity farm landscape, using minimum tillage farming, use of conservation

tillage, choice of crops with high efficiency in nutrient use, returns of farmyard manures and household wastes.

In arid and semi-arid environments the key problem of agricultural production is the steady decline in water availability and soil fertility, which is closely correlated to duration of soil use. Implementing agricultural practices that reduce soil degradation has the potential to increase agricultural sustainability and soil conservation. Managing the frequency and type of tillage can stop soil degradation maintaining soil organic matter, as tillage disrupts soil aggregates exposing organic matter to microbial degradation. Changes in soil structure can affect soil water, temperature, aeration, or increase soil erosion. Soil disturbance can cause significant modifications of soil habitat, which affect the microbial diversity (Al-Ouda, 2010).

III.2.1. Organic agriculture

Lower-input farming practices of organic agriculture typically require more information, trained labour, time and management skills per unit of production than conventional farming. Thus, extension services and farmer-to-farmer sharing of information are of major importance. Organic agriculture is perhaps one of the oldest farming systems in the world and has been in practice for millennia in Asia. Significant portions of Chinese agriculture still use organic farming system. The prevention of the use of chemicals has a positive effect on microorganisms responsible for nourishment of soil, which are killed from the application of pesticides and chemical fertilizers. Soil fauna and flora is encouraged, improving soil formation and structure and creating more stable systems. The management techniques play an important role in soil erosion control and promote biodiversity on which organic agriculture is based (Letourneau and Bothwell, 2008; Perffecto and Badgley, 2007; http://www.infonet-biovision.org/res/res/files/488.OrgFarm.pdf).

III.2.2. Integrated pest management

Alongside the demands on agricultural increased production and crop protection, there are needs for sustainable cultivation practices and considering the impacts of climate variability, the conservation of biodiversity for a sustainable use is necessary to provide food, improving people's economic, social and environmental conditions and meeting the needs of future generations, in particular the rural poor. The conservation and enhancement of biodiversity in cropping system both above and belowground are part of the foundation of sustainable farming practices.

Nowadays, there is a demand for alternative systems to make crop protection more sustainable. Integrated pest management (IPM) is an approach that combines different crop protection practices with careful monitoring of pests and their natural enemies. The aim is to manage pest populations below levels that cause economic damage. The main IPM components are 1) synthetic pesticides with high levels of selectivity and low-risk compounds, 2) breed of crop cultivars with pest resistance, 3) cultivation practices (crop rotation, intercropping, undersowing), 4) physical methods (mechanical weeders), 5) natural products (semiochemical, biocidal plant extracts), 6) biological control with natural enemies and microbial pathogens, 7) inform farmers when it is economically beneficial to apply pesticides (timing of pest activity and scouting) and other controls. An analysis of 62 IPM research and development projects in 26 countries, covering over 5 million farm households, showed that IPM leads to substantial reductions in pesticide applications (Chandler *et. al.*, 2011). Biopesticides are useful tools used in IPM for crop protection.

Soil management practices and those that influence the fertility have an immediate impact on microbial population. Tillage, crop rotations, manuring, burning and pesticide application are the major most studied agricultural practices. The impact of land use is highly variable.

- Each kind of vegetation (natural or crops) provides a particular substrate that encourages some microbial species over others in the rhizosphere. Cultivation increases the population and diversity in soils, but there are some reports of increased population under minimum tillage with residue incorporation as compared to conventional tillage. However, this superiority is normally restricted to surface soil (0-75 mm).
- Accelerated erosion and loss of clay and organic carbon can cause significant decline in microbial diversity, especially in Alfisols and Vertisols. Manuring and fertilizer application have a significant impact on the microbial species diversity.
- Fertilization cause significant changes in microbial populations through changes in soil pH. Application of nitrogen fertilizers, like ammonium sulphate increase the fungal population whereas manure and NPK application increase the population of fungi, bacteria and actinomycetes. Certain species of microorganisms like *Azotobacter* are sensitive to soil acidity while *Nitrosomonas* and *Nitrobacter* are more sensitive to erosion of topsoil.
- Soil microbes are also affected by a wide range of other soil biota, especially by their consumers such as protozoa *Collembola* and nematodes (Venkateswarlu and Srinvasarao, 2005).
- The application of organic fertilizers increases the organic carbon content of the soil and thereby increased the microbial diversity. On the other hand, the use of inorganic fertilizers (urea, phosphate and potash) may result in low organic carbon content and negative impact on soil microbial diversity (Nakhro and Dkhar, 2010; Das and Dkhar, 2011). The organic amendments on soil shift the microbial diversity, depending on the amended soil (Perez Piqueres *et al.*, 2006).

Practices for microbial biodiversity conservation are:

Crop rotation

- Crop rotation avoids a decrease in soil fertility, as growing the same crop repeatedly in the same place eventually depletes the soil of various nutrients
- Crop that leaches the soil of one kind of nutrients is followed during the next growing reason by a dissimilar crop that returns that nutrients in the soil or draws a different ratio of nutrients for example paddy followed by cotton.
- Crop rotation farmers can keep their fields under continuous production without the need to let them lie fallow and thus reducing the need for artificial fertilizers, both of which can be expensive.

Green manure

- Green manures usually perform functions that include soil improvement and soil protection.
- Leguminous green manures contain nitrogen-fixing symbiotic bacteria in root nodule that fix atmospheric nitrogen in a form that plants can use.
- Green manures increase the percentage of organic matter (biomass) in the soil, thereby improving water retention, aeration and other soil characteristics.
- Green manure crops are: leguminous crops, soybean**,** winter cover crops such as oats and rye**,** mustard, clover, fenugreed (Telugh-*Menthulu*)**,** lupin, Sunn hemp (a tropical legume)**,** vetch or Winter tares**,** winter field beans**,** alfalfa (which sends roots deep to bring nutrients to the surface)**,** buckwheat (a rapidly growing green manure in temperate regions)**,** ferns of the genus *Azolla* (used as a green manure in southern Asia)**,** and velvet bean (*Mucuna pruriens*, common in the southern US during the early part of the 20th century, before being replaced by soybeans).

Types of traditional practices for composting

- Biodung compost: Green Biomass (Monsoon weed hedge plants and leaves of fast growing trees) is soaked with cattle dung slurry and polythene coverage over biomass provides optimum conditions for temperature, moisture and aeration for microbial activity;
- Vermicompost: use of earthworms for composting organic residues; and
- Phospho-compost: crop residues, cattle dung, urine are mixed with rock phosphate or pyrite and enriched with phosphorus solubilizing microbes.

III.2.3. Biological control

Biological control is a method of controlling pests (including insects, mites, weeds and plant diseases) that relies on predation, parasitism, herbivory, or other natural mechanisms. It can be an important component of integrated pest management programs (IPM). Biological control agents include predators, parasitoids, pathogens, herbivores and plant pathogens.

Some practices of traditional farmers for disease management are: altering of plant and crop architecture, biological control, burning, adjusting crop density or depth or time of planting, planting diverse crops, fallowing, flooding, mulching, multiple cropping, planting without tillage, using organic amendments, planting in raised beds, rotation, sanitation, manipulating shade, and tillage. Most, but not all, of these practices are sustainable in the long term.

III.2.4. Compost and soil organic matter

Degraded soils are often characterised by low organic matter status, poor microbial diversity and activity of microbial population, low water retention capacity, and low nutrient content and declined soil fertility. Soil with low organic matter has a poor microbial diversity that leads to poor plant productivity. Healthy soil is inhabited by microscopic and macroscopic organisms that convert dead and decaying matter as well as minerals to plant nutrients. A degraded soil can be restored within 4-5 years of its production by following improved management/farming practices. A combination of soil fertility restoration technologies and conservation tillage practices can offer opportunities for sustainable land use. Amendment of soil with additives such as compost, animal manure, biosolids, fly ash, green manure, organic wastes and sewage and paper sludge are some of the appropriate strategies to improve the organic matter status of soil that supports the biological activity of different organisms. Application of compost reduces soil bulk density; increases soil aggregation, water retention capacity and soil macro porosity. Organic matter added through compost has been found to be more effective in controlling the soil temperature by reducing the thermal conductivity and heat capacity of soil. Increase in organic matter content results in improved soil microbial biomass, increased biodiversity and biological activity of soil organisms and plant nutrient availability. The peak in microbial activity may be attributed to preferential stimulation of microbes by different constituents of organic residues in the soil. Beneficial microorganisms compete with pathogens for space and nutrients. They may also produce lytic enzymes, antibiotics as well as siderophores that can be implicated in the biological control of plant diseases. Conservation agriculture practices through combining no tillage or minimum tillage with a protective crop cover and crop rotations maintains surface residues, roots and soil organic matter, helps control weeds, and enhances soil aggregation and intact large pores. Therefore, by practicing the proper soil management technology along with organic amendments as compost, productivity of soil can be improved.

III.2.5. Culture Collections

Culture Collections are fundamental to the harnessing and preservation of the world's biodiversity and genetic resources, as well as being part of the key infrastructure supporting biotechnology, bioprocessing and the development of new approaches in the prevention, diagnosis and treatment of disease. They also have a vital role in ensuring the safe and regulated use of organisms that are known pathogens to humans, plants or animals. Culture collections are key repositories of biodiversity.

As microbiology evolves, there is a great demand to integrate genome science with ecology, systematics, molecular evolution and microbial chemistry. There is also a need for innovative research in information management (e.g., databases, information processing technology, information networks, etc.), rapid identification techniques, and large volume handling capacity (strain vectors, genetic material, etc.).

Agriculture and biodiversity are closely linked to biotechnology. In traditional agricultural areas, modern biotechnology offers an opportunity not only to increase productivity and significantly reduce the use of off-farm chemical inputs, but also to enhance natural agro-biological systems. Recent advances in genomic, proteomic and matabolomic research offer unprecedented opportunities for the search, identification, and commercial utilisation of biological products and molecules in the pharmaceutical, nutraceutical, agricultural and environmental sectors. Genetic diversity analysis and, using molecular markers, has improved the characterization, conservation and utilisation of important microorganisms. Integral to studies in microbial genome research is the accumulation, analysis and communication of information about genomes, and the collection, definition and preservation of genetic stocks allowing comparison of genetic diversity to be made and subsequently utilized in applied research.

Culture collections, herbaria, museums and libraries are all critical components of the scientific infrastructure. Culture collections have been developed to:

1. Establish repositories of interesting, rare or useful organisms,
2. Provide the research community with "taxonomic type strains", 'control/reference strains' for experimental use and standardised testing, or "specialised genetic or clinical strains" and
3. Establish a repository of organisms necessary to permit enablement of patented inventions. Selection criteria are novelty, taxonomic and physiological significance (type strains) and anticipated use. Uniqueness is very important. Some collections have wild-type organisms that are unique or are from special, extreme or endangered environments, which are valuable. Other collections have genetic stock strains, which are well characterized, and represent the end points of research.

Federal funding support for culture collections is justified because:

- They serve important national interests (technological and commercial competitiveness),
- The necessity for advancing the missions of many Federal agencies in health, education, agriculture, commerce, etc.,
- They are considered as an important national infrastructure for the biological sciences,
- They are advancing the protection of intellectual property rights and hence commercialization of new technologies,
- Adequate revenues cannot be obtained from user fees or service charges,
- No other source of independent public funds are available, and

- The research community, both academic and industrial, is dependent upon open and equitable access to cultures and their selection for archival.

These collections have been of tremendous benefit to science and society in the past and will continue to be so in the future. They have countless contributions to basic and applied research in agriculture, biotechnology, ecology, and medicine. In addition to maintaining and disseminating genetic material harvested from decades of research in microbiology, they preserve the knowledge gained and promote its advancement. Importance of collections to commercial use concerns a source of strains for screening, especially a source of carefully selected material that might not otherwise be available. Without culture collection we will never be able to exploit biodiversity. Culture collections have an important role in the development of the bioeconomy needed to alleviate poverty and improve human welfare because they conserve living organisms and cells, supply material and related information for teaching, research and industry, offer services related to their activities, apply quality management and insecurity control and perform innovative research.

The diversity of bacteria, protozoa, fungi, unicellular algae, constitutes the most extraordinary reservoir of life in the biosphere, and one that we have only begun to explore. If we want to compare a 'pyramid of known species' with a 'pyramid of existing species' we observe that in both pyramids, animals were placed at the bottom, plants above them, and microorganisms at the top. The striking fact is that one pyramid is pointed upward, the other downward. Known animal species formed the base of the upward-pointing pyramid, being more numerous than known plant species and much more numerous than known bacterial species (only about 5,000). In the pyramid of existing species, microorganisms constitute the base of the pyramid. Microorganisms remaining to be discovered outnumber by far the existing plant and animal species. They are adapted to extremely diverse environments, and examples of practically all known metabolic pathways are represented.

The molecular genetics revolution has renewed the systematic investigation of the world's microbial potential. Molecular ecology enhances the bacterial classification and helps to define conditions for culturing unknown organisms *in vitro* which is key to exploring the microbial world. Molecular genetics is also providing tools for studying microbial communities, additionaly with classical microorganism isolations. New approaches are based on the analysis of 'meta-genomes' comprising all the genomes present in a microbial ecosystem. Interactions in such communities are complex, and very little is known about them.

Current erosion of microbial biodiversity is a disturbing fact; its seriousness is illustrated by reported effects on wild strains, which is mainly blamed on genetically modified organisms (GMOs). Under an optimistic scenario valuing biodiversity is expected to become a driving force in our society, and our approach to molecular genetics would become balanced.

We have to assess the risks associated with applications of molecular genetics and recombinant DNA technology. The current state of knowledge worldwide allows a purely scientific assessment. There are some conditions that scientists must have in mind. We should not overemphasize the role of genetics, which is only a part of scientific knowledge; we should leave all options open and weigh their respective merits despite the fact that the process will be long and difficult.

There is no doubt that the study of the microbial proteome according to changes in environmental conditions will improve the industrial use of microorganisms. The selection of new industrial microorganisms will almost certainly require multiple approaches depending on the state of progress of genome and proteome characterisation, while their use will require efficient production methods. The membrane technology should contribute to the improvement of bioreactor performance by increasing the cell density and their specific activities.

IV. LOOKING FORWARD: PREPARING FOR THE FUTURE

Effective strategies for initial selection and screening of microbial isolates to use in the agriculture, food or pharmaceutical industry, are required. It is important to consider host plant specificity or adaptation to a particular soil, climatic conditions, and pathogen interactions. The introduced microorganism should have compatibility with local indigenous microbial isolates for prompt and effective plant growth as well stability and the ability to survive in the carrier system. Additional features include survival on coated seeds and even under adverse climatic conditions, a wide range of host applications, ability to maintain genetic stability, absence of harmful contaminants, and prolonged shelf life.

IV.1. Organic agriculture

Organic agriculture is the fastest growing agricultural based industry in the world. Practicing organic farming requires radical changes that may be costly in terms of both time spent learning and initial crop response. Adjustments will be necessary in cultivation methods, the production and use of organic inputs such as fertilizers and pesticides, and the use of labour.

In the decade 2001 to 2011, the total worldwide organic agricultural hectares have grown by 135%, which equates to an 8.9% per annum compound growth over the decade. The organic hectares for 2011in 160 countries accounted for a total of 37.2 million. Also, 71 countries out of these had an increase from 15.8 million to 35.3 million that is 94.8% of the total global organic agriculture area and 58.2% of the total global agriculture area. For the remaining 89 countries, the data showed that their organic agriculture accounted for only 1.9 million hectares representing 5.2% of the global organic agriculture surface. At global scale the growth appears to have had a steady incremental growth over the decade, but it is highly uneven when disaggregated by country. The decadal increase in organic hectares ranges from Australia's gain of 4.3 million organic hectares and China's gain of 1.9 million organic hectares through to Costa Rica's decrease of 1549 organic hectares. Globally, the organic hectares total has multiplied by 2.3 in the decade from 2001 to 2011, but this has varied greatly by country. Uruguay increased its organic hectares dramatically with a hectares-multiplier of 716.1, India with a multiplier of 689.7, China with a multiplier of 214.99, Philippines with a multiplier of 553.12, Columbia with a multiplier of 209.08, Romania with a multiplier of 168.29 and Croatia with a multiplier of 118.28. In contrast, Denmark had barely increased with a hectares-multiplier of 1.07, while Suriname and Mauritius exhibited the greatest shrinkage with a multiplier of 0.03. Cameroon had a shrink of 0.41, Zimbabwe of 0.42, Papua New Guinea of 0.78 and Costa Rica of 0.84. China and India are the only countries that rank among the top ten for both of the indices of organic growth, namely, the decadal organic hectares increase and the decadal organic hectares-multiplier. Both India and China have the capacity for substantial and rapid increase with currently just 0.66% of India's and 0.35% of China's agricultural land managed organically (Fig. 13). The great diversity within the 71 countries demonstrates that the organic projects are able of breaching barriers of politics, geography, language and culture (Paull, 2011).

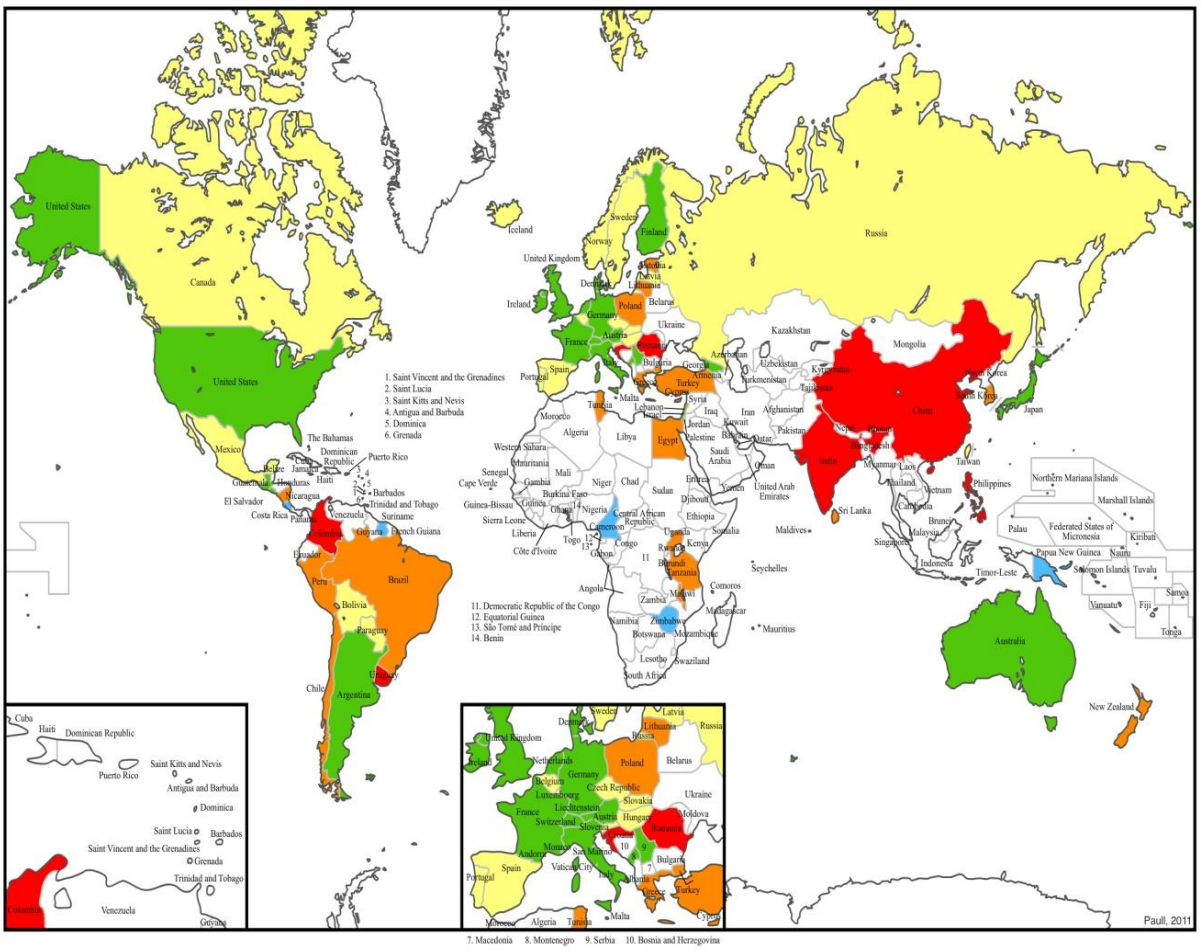

Figure 13. World map of organic agriculture growth rates for 71 countries over a decade (2001-2011). Triple digit growth=red, Double digit growth=orange, Single digit growth (above average)=yellow, Single digit growth (below average)=green, Negative growth=blue (Paull , 2011).

The three main crop types grown organically are arable land crops (mainly cereals, fresh vegetables, green fodder and industrial crops), permanent crops (mainly fruit trees and berries, olive groves and vineyards) and pastures and meadowland. Arable land constitutes 17% of the organic agricultural land. Most of the organic arable land is located in Europe followed by North America and Latin America. Most of this land is used for cereals including rice, followed by green fodder from arable land and vegetables. Africa has a large proportion of permanent crops, which are mainly cash crops (coffee, tropical fruit and olives). Europe and North America use about half of their organic agricultural land as grassland and the other half as arable land. In Europe the share of permanent crops is higher than in North America, mainly due to olives and grapes grown in the Mediterranean countries. Latin America owns little arable land compared to the large grazing areas (Uruguay and Argentina), but it has a comparatively high portion of permanent crops (mainly coffee). Oceania is characterised by the large grazing areas of Australia. The Pacific Islands produce a large range of tropical crops and New Zealand produces a considerable amount of decidious fruit (Letourneau and Bothwell, 2008; Perffecto and Badgley, 2007; http://www.infonet-biovision.org/res/res/files/488.OrgFarm.pdf).

The promise of higher prices is often the primary driver used to induce adoption of organic methods. The more successful farmers appear to apply organic agriculture for several reasons that go beyond earning a higher price for their crops. There are at least five major reasons for this, being to:

1. Earn more for their production,
2. Reduce or eliminate the need for purchased inputs,
3. Avoid potentially harmful agrochemicals,
4. Reduce their risks through crop deversification and improved soil quality/stability,
5. Maintain or improve valuable local natural resources and biodiversity.

IV.1.1. Knowledge, skills and support

A few cases demonstrated that farm groups or communities converted their entire production to organic (Jianxi, China and Maharashtra, India). The early adopters tend to be the more resourceful, better skilled, and typically better educated farmers (Madhya Pradesh cotton, Uttaranchal). They also tend to have a higher tolerance for risk and may be leaders in the community. The strong institutional support for some projects clearly facilitated the adoption. The complete converters shared having both the firm leadership of a strong organization and its full financial support and guidance. Organisational forms and institutional arrangements are helping farmers to reach organic markets.

The forms of organisational structures supporting smallholders under organic agriculture are:

1. Farmers organised by a company.
2. Farmers organised through a non-governmental organization (NGO).
3. Farmers organised by government.
4. Farmers forming their own organisation. Farmers' organisations have required considerable support on a number of levels. The farmers' association creates a platform for farmers to exchange experiences and ideas, improves quality control, serves as an information and technology centre for local organic production, illiterate or poorly educated farmers can receive technical support from the association, introduces useful techniques and varieties, plays an important role in organic products marketing, ensures that farmers own the benefits of their labour (the association has registered a brand for its organic products) (http://www.ifad.org/evaluation/public_html/eksyst/doc/thematic/organic/toc.htm)

Reasons for conversion to organic systems include farmer and community health, environmental benefits and community solidarity. The conversion to organic also requires a fundamental shift, which includes systematic training in the use of a new technology and investment in certification and secure (non-contaminated) storage, processing and transport. When switching from intensive forms of agriculture to organics, negative consequences occur in terms of yields or output. Little direct financial help is necessary to bridge conversion.

The recycling of farm nutrients is a primary feature of organic agriculture and significantly reduces input costs. This cost reduction is partly covered by an increase in labour to produce the inputs needed. In most of the monitored cases, organic farminghas led to significant improvements in soil conditions and fertility were noted.. Organic practices also positively contribute to reducing waterlogging in the fields during heavy rains. Besides, in many cases, the soil structure and organic content improved, the soil water holding capacity increased, and as a result, the irrigation requirements dropped. In China (Yunnan) the introduction of methods such as interplanting with different varieties rather than monocropping have helped farmers to reduce the spread of disease and nearly double their yields. By interplanting different varieties, farmers were able to overcome serious problems with rice blast that did not respond to conventional agro-chemical methods.

Organic agriculture can increase productivity through a number of mechanisms. Developing the biodiversity in the farm through crop rotation, intercropping and polyculture tends to lower the risk of heavy pest and disease related losses, while improving fertility. Moreover, intercropping and appropriate cover crops can reduce erosion, improve soil moisture, reduce the need for weeding and provide fodder and additional sources of income. Optimizing resources such as forest area, livestock and water and recycling of farm nutrients by composting improves soil fertility and consequently reduce both costs and the farmer's vulnerability.

IV.1.2. Biofertilizers

An increasing number of farmers are choosing biofertilizers as they are found to be gentler on the soil. The value of biofertilizers has further increased in an increasingly eco-conscious world. Soil quality is also improved through the uptake of environmental friendly fertilizers. Biofertilizers also contributes in reducing the negative impact of global warming. It could be useful to hold various seminars and workshops on the application of biofertilizer, so that farmers would have the opportunity to understand the effects of biofertilizers and finally use them. In order to improve and maintain the productivity of agricultural lands, an integrated approach to screen out more favourable biofertilizers is urgently needed. Commercialization of this technology still demands extensive optimizations. The prospects of biofertilizers technology are promising if we take into consideration the rising cost and declining reserves of fossil fuels in the world, as well as pollution problems.

Advantages of Biofertilizers

- A wider range of nutrients, particularly micronutrients,
- Helping to increase soil organic matter content,
- Relatively inexpensive alternatives,
- Absence (or negligible amounts) of harmful materials such as heavy metals.

Disadvantages of Biofertilizers

- Biofertilizers complement other fertilizers, but they cannot totally replace them.
- Much lower nutrient density -- requires large amounts to get enough for most crops
- Requires a different type of machine to apply than chemical fertilizers
- Sometimes they are hard to locate in certain areas
- Biofertilizers require special care for long-term storage because they are alive. They must be used before their expiry date.
- Biofertilizers lose their effectiveness if the soil is too hot or dry.
- If other microorganisms contaminate the carrier medium or if growers use the wrong strain, they are not as effective.
- The soil must contain adequate nutrients for biofertilizer organisms to thrive and work.
- Excessively acidic or alkaline soils also hamper successful growth of the beneficial microorganisms; moreover, they are less effective if the soil contains an excess of their natural microbiological enemies.
- Shortages of particular strains of microorganisms or of the best growing medium reduce the availability of some biofertilizers.

IV.1.3. Use of biofertilizers - recommendations

More information and research is needed on the interactions among plants and rhizophere's microorganisms. The rhizosphere is a highly dynamic system with a vast number of microorganisms interacting simultaneously. Concerning nitrogen fixation understanding of the ecological factors that

control the fate and performance of nitrogen fixation systems in crop fields is essential for promotion and successful adoption of these technologies.

- **Competitive ability over other strains.** Local or regional strains should preferably be selected and used for the target crops. The microorganism should be antagonistic to indigenous soil microbiota, to have an effective establishment. In the case of mycofungicides the needed biological properties of the isolates to be considered when selecting strains for potential biological control agents are: laboratory virulence, field performance, genetic stability, productivity, stability of conidia in storage, stability in formulation, field persistence and tolerance to environmental factors (e.g., UV, temperature, desiccation), mammalian safety, low environmental impact and capacity to persist in the environment.

- **The persistence of the biofertilizer after inoculation.** If inoculum potential can be built into agricultural soil, the interval between biofertilizer applications could be increased and costs lowered. Multiple inoculations can aim to stimulate nitrogen fixation, phosphorus uptake and mineral nutrition in general. The positive effects must be repeatably shown in practical application, unless commercial viability of inoculation programs will be uncertain.

- **The need for field trials with multiple strain inoculations.** Inoculum combinations may be of greatest value for securing the effectivity and impact of an application.

- **Quality control.** Quality control is necessary because it must be ensured that the product is of standard quality. For mass production of biofertilizers, critical benchmarks at all stages of inoculum development covering all possible parameters desirable for ensured production, are required to be identified. These include viability checks from the processing stage till the formulation stage, ranging from the colonization of host roots, weight of dried inoculum at harvest, propagule estimations, infectively potential of crude and formulated diluted inoculum, formulation conditions like temperature and suitable storage conditions. The production should also be monitored by microbiologists (Gentili and Jumpponen, 2006; FNCA, 2006; http://www.ipni.net/ipniweb/portal.nsf/0/94cfd5a0ed0843028525781c0065437e/$FILE/16%20China.Cheng.Issues%20related%20to%20development%20of%20biofertilizers%20in%20China.pdf).

- **Collaboration between research facilities and biotechnology industry.** This issue comprises of the connection between the research sector and industry to produce inocula for field trials, but also for industrial scale testing of the inoculum production for direct marketing.
- **Establishing federal and international guidelines for inoculum production and trade.** To protect the end user of inoculum and to promote safe choice of commercial inocula collaboration among research facilities, federal agencies, farmers and the inoculum producers are of pivotal importance. Such collaborative approaches will allow a heal start for commercial and economically viable production of biofertilizer inocula from marketing directly to primary target consumer. The **economics** of labour cost, agricultural inputs and gross returns of the farmer under organic farming, will lead to a profitable combination for specified areas. Maximum benefits could be achieved from the careful combination of host-microbe-organic amendment.

- **Strain improvement.** For commercial bioprocesses it is recommended using wild strains isolated directly from nature/"natural" habitats, with the exception of Food Biotechnology.

- What to improve: increased yield, faster growth, improved fermentation properties, better tolerance of process conditions, decreased formation of by-products, better bacteriophage resistance, new or modified activity, regulation of enzyme synthesis,
- Genetic improvement: The biotechnological methods for enhancing colonization and effectiveness may involve addition of one or more beneficial traits. Monitoring should consider the survival of the transgenic microbial inoculant population, and also positive and negative interactions with indigenous microbial populations. This process should also include the exchange of cultures between countries of similar climatic conditions and evaluating their performance for selecting better strains for a particular crop, as well as checking the activity of cultures during storage to avoid natural mutants.

  Mutagenesis: This involves genome mutations, chromosome mutations, gene or point mutations, spontaneous mutations (rate 10^{-7} to 10^{-6}), mutagenic agents in molecular technology.

  Directed mutagenesis and protein engineering: specific changes in amino acids encoded by cloned genes, oligonucleotide-directed mutagenesis, knowledge of 3D structure.

- **Developing suitable alternative formulations.** Developing suitable alternative formulations (liquid inoculants, granular formulations) for all bioinoculants to carrier based inoculants. Standardizing the media, method of inoculation, etc for the new formulations. Identifying two or three common carrier materials in different countries based on availability and recommending them to the producers is also important. Unavailability of suitable carrier due to which shelf life of biofertilizers is short is a major constraint. Good quality carrier must have good moisture holding capacity, free from toxic substances, sterilizable and readily adjustable. Under extreme soil and weather conditions there must be suitable carrier material. Technologies used for the production of living hybrids materials could be a new frontier in the development of **carriers for PGPMs**. Silica has appeared as a promising host for microorganisms' encapsulation: immobilization pathways are based on immobilization of population bacteria dispersed into a silica gel. Bacteria can be either entrapped into alginate microbeads coated with silica membranes or intomacrocavities created inside the silica matrix. Such material improves the mechanical properties of the alginate bead, reduces cell leakage, and enhances cell viability. The application of bionanotechnologies could also provide new avenues for the development of carrier-based microbial inocula.

- **Possible knowledge gap of farmers using biofertilizers.** As demonstrated above, the use of biofertilizers as a potential alternative fertilizing method has many advantages but also some disadvantages. For example, the use of biofertilizers requires good farmers' education level and needs flexibility in updating and advising which may render sustainable agriculture complicated. Suggestions, either for improvement or as areas for special focusing, are summarised below.

  1. Better links between science and practice including local and traditional knowledge.
  2. Adopting indigenous territorial management: an ecosystem approach for climate change resilience.
  3. Documentation and testing of nutritional and therapeutic properties of indigenous varieties of seeds.
  4. Document and organize indigenous knowledge and wisdom on weather forecast for better adaptation to climate change.

5. Priority to defend knowledge systems of small-scale food providers (farmers, livestock keepers, pastoralists, fisher folk).
6. Facilitate development of knowledge and skills of small scale food providers: seeds, livestock breeds, aquatic organisms, soils, waters, landscapes, coastal waters, commons.
7. Increase recognition of knowledge of small-scale food producers by others, e.g., consumers, NGO's, and policy makers.
8. Help organisations and social movements of small scale food providers to defend and develop their knowledge and technologies in the framework of food sovereignty.
9. Develop strategies to mitigate market impacts on local knowledge and agricultural biodiversity and ecological food provision.

- **Farming techniques**

1. Improve on production through green houses
2. Mechanisms for technology assessments at local, national and regional levels
3. Technology assessments/observation platforms at local, national and regional level
4. Document link between researchers, extension staff and farmers

- **Ecosystems:** Ecosystems functions – information sharing and exchange
- **Climate change adaptation**

1. Think tank on how we can equip farmers to adapt to continuous changes in climate
2. How to transition to resilient agriculture (learn, develop, discuss)

- **Farmers' Services**

- Radio talks and TV programs
- Demonstration of latest technology on farmer's field
- Arranging short-term training programs on use and importance of Biofertilizers / Microbial Pesticides for extension workers, scientists, farmers etc.
- Publication of press notes, popular articles, news items, folders etc.
- Tapping people based knowledge. Processing the knowledge through co-creation
- Sharing of knowledge (formal and informal)
- Codification of knowledge
- Provide for passing on of indigenous knowledge
- Interrogations of indigenous knowledge in the context of technology advancement (internet, media, facebook, twitter)
- Dialogue as a basis for passing on knowledge (concept traditional courts)
- Create an info bank accessible to various organisations for comparison and expansion
- Language used in development of knowledge programme should be as simple as possible for all users to participate and use it
- How do we translate the knowledge to be useful to farmers
- Platform for sharing of community technology innovations
- Dissemination through network organisations
- Documenting and sharing knowledge
- Compile available indigenous knowledge and fill in gaps
- Publish in journal and information bulletins

- Global knowledge networks: agric-cultures, APC, GRP
- Training workshops, dialogues, meetings with elders, youth, women how they use information
- Intergenerational knowledge transfer
- Enhanced collaboration and networking
- Networking and policy action
- Information technology: community radio for agricultural extensions
- Development education
- Adapting practical knowledge to influence policy
- Social media: blogs, video clips, twitter (Report of the Thika Meeting, 10 – 12 October 2011).

- **Farmers' acceptance and utilisation of biofertilizers.** To convince farmers to utilise biofertilizers three major programs should be fulfilled. Inoculants production programs, extension programs so that farmers can apply inoculants on to their farms and demonstration and awareness programs to show farmers the benefits of inoculated plots.

- **Research on biofertilizers.** Excess nutrients are accumulated in soils, particularly phosphorus as a result of over application of chemical fertilizers by farmers during intensive agricultural practices. Hence, major research focus should be on the production of efficient and sustainable biofertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems. In view of overcoming this bottleneck, it will be necessary to undertake short-term, medium, and long-term research, in which soil microbiologists, agronomists, plant breeders, plant pathologists, and even nutritionists and economists must work together.

  The most important and specific research needs should highlight on following points:
  1. Selection of effective and competitive multi-functional biofertilizers for a variety of crops
  2. Quality control system for the production of inoculants and their application in the field, to ensure and explore the benefits of plant-microorganism symbiosis
  3. Study of microbial persistence of biofertilizers in soil environments under stressful conditions
  4. Agronomic, soil, and economic evaluation of biofertilizers for diverse agricultural production systems
  5. Transferring technological know-how on biofertilizer production to the industrial level and for optimum formulation
  6. Strict regulation for quality control in markets and application (Chien *et al*., 2007).
- **Natural production of bacterial biofilms.** Another interesting new technology is proposing the **exploitation of the natural production of bacterial biofilms** as a possible carrier, and not only for the production of the inoculum, of defined bacterial or fungi-bacteria consortia. Biofilm production is already used for different industrial applications (e.g., wastewater treatment, production of chemical compounds). Two types of biofilms are employed in that case: biofilms growing onto inert supports (charcoal, resin, concrete, clay brick, sand particles) and biofilms that are formed as a result of aggregate formation. In the first case, biofilms grow all around the particles, and the size of the biofilm particles grows with time

usually to several mm in diameter. Biofilm formed by aggregation is called granular biofilm; granule formation may take several weeks to several months.

- **Beneficial biofilms** developed in *in vitro* cultures containing both fungal and bacterial strains have been used as biofertilizers for nonlegume species with good efficacy results. Application of a biofilmed inoculant containing a fungal-rhizobia consortium significantly increased N_2-fixation in soybean compared to a traditional rhizobium inoculant. Wheat seedlings inoculated with biofilm-producing bacteria exhibited an increased yield in moderate saline soils. Biofilms seem also to help the microorganisms to survive after inoculation even under stress conditions: this is a key aspect for the effectiveness of PGPM inoculation under agricultural conditions. Inocula made with biofilms were shown to allow their rhizobia survive at high salinity (400 mM NaCl) by 105-fold compared to rhizobial monocultures. Interestingly, beneficial endophytes were observed to produce higher acidity and plant growth-promoting hormones than their mono- or mixed cultures with no biofilm formation (Malus*a et al*., 2012).

IV.2. Bio-pesticides - recommendations

The global biopesticides market accounts of $3.3 billion and is expected to continue to grow rapidly and a huge biodiversity of sources remains unexploited.

Changes in political and social attitudes towards safer and more environmental friendly pest control alternatives have increased opportunities for biopesticides, which have to be more effective, more diverse and more economical. To enhance delivery and persistence of biopesticides, bioactive molecules and endophytic microorganisms that have systemic activity in plants should be used. Increased persistence of activity after application, will also drive biopesticide uptake in the market. To achieve the desired level of persistence, a combination of microorganisms with a high rhizosphere or phyllosphere competence, novel formulations or other methods could be applied.

More research is needed for the improvement in biopesticide formulations and better understanding of modes of action. Whole or partial genome sequencing will be useful tools for selection of superior isolates with a known mode of action, such as antibiotics' production or novel variations of toxins. Strategic selection of target pests and markets would provide biopesticides with the public profile for their widespread acceptance as safe, cheap and sustainable by consumers and retailers (Nakkeeran *et al.,* 2005; Kaewchai *et al.,* 2009; Malusa *et al*., 2012).

- **Continued investment in expertise for the discovery, development and implementation of biopesticides.** Support in industry research and development (R & D) is necessary to support the development and registration of more biopesticides. The early development research is often conducted in universities and government research institutes. Collaboration of public and private sector is needed to educate farmers, retailers and public on the use and the merits of biopesticides. Registration and legislative changes will be crucial to boost development of new biopesticides. Biopesticides have not yet reached their potential and they have a future. The economics of mass production, formulation and application have, in the past, greatly limited biopesticides reaching the market place because the sale price needed to recover costs was prohibitive. Often, the cost of fermentation of microbes is higher than the cost of making a synthetic chemical. Microbial isolates must have high potency against the pest, or high yielding capacity during production, so as to be competitive in the market. Biopesticides are currently developed and provided by a range of organisations, from public institutions to small or medium-sized companies. Until biopesticide sales are able to support

large companies, it is difficult to develop the needed level of R & D to produce new, or improve existing products. The market for biopesticides remains relatively small, while the cost of R & D is high. More targeted public or private partnerships are needed. Increasing interest in biopesticides has been prevented by larger companies, which are involved in agreements that allow them to distribute and sell biopesticides produced by smaller companies. These large companies bring new marketing channels to biopesticides and larger R & D budgets. A threat to increasing market of biopesticides is the potential development of competing technologies. Many of the microbial isolates used in biopesticide products are the result of public sector research, and are not patented. Protection of International Patent during the development of a biopesticide is often necessary to attract investors, but overemphasis can restrict knowledge sharing and result in high legal costs. There is a mistaken belief that biopesticides are less active than chemical pesticides. Genetic manipulation can offer a powerful technique for enhancing the intrinsic virulence of a biopesticide agent in those cases where the mechanism of action is well understood. However, even if intrinsic virulence can be increased, it still remains to achieve this virulence in the field. Environmental parameters, which can influence both activity and persistence of biopesticides probably, have the greatest impact on the performance of biopesticides in the field. Selection of novel strains that are less sensitive to extremes of temperature can be successful. Another major factor is the rapid decline in the bacterial population on the leaf surface after application. Formulates can delay this decline, but further formulation development is required in order to maintain the leaf surface population at effective levels, particularly after rainfall. Inactivation by ultraviolet light is one of the major factors responsible for the rapid loss in activity of biopesticides after application to leaf surfaces. Rainfastness is another problem for leaf surface applications of biopesticides, but promising progress is being made using starch encapsulation technology. Some innovative approaches to protection of biopesticides from ultraviolet light inactivation have been made using genetic manipulation. Humidity is another important environmental influence on the efficacy of foliar applied biopesticides, particularly fungi, which can require high relative humidity for spore germination and subsequent infection of weeds or insect pests. The use of formulations such as invert emulsions, which retain water that is then available to fungal spores for germination, can offer a solution to this problem. An understanding of the biology of fungi can be combined with formulation developments to reduce environmental limitations on the use of fungal biological control agents. The activity spectra of biopesticides tend to be very selective in comparison to those of agrochemicals. However, strain isolation and selection has proved to be a very powerful technique for finding new strains of organisms with novel activities. The host range of biological control agents can be broadened by using conjugal mating for transferring large plasmids containing the endotoxin genes, to other strains. Thereby, a conjugate strain can be produced that has the host range of the two parent strains. An alternative approach to the issue of narrow specificity, which is under investigation for mycoherbicides, is to select a virulent pathogen with a broad host range and then to restrict the spectrum of activity. Attempts have been made to improve the relatively slow speed of action of some biopesticides (Nakkeeran *et al.,* 2005; Kaewchai *et al.*, 2009; Malusa *et al*., 2012).

- **Improving the efficacy of biopesticides by manipulating the biological control agent itself or by adapting formulation and application technology**. **Registration regime**. It is important to realize that in many cases biopesticides are not used on their own, but in combination with conventional pesticides. Even if the field efficacy of a biopesticide is satisfactory the production process must be cost effective if use of the product is to be

economically attractive. For biopesticides such as bacteria that can be produced on a large scale by fermentation, production costs are not a barrier to commercialization. Conventional distribution chains for agrochemicals accept that shelf lives of one to two years are required for products stored under ambient conditions. The available technology allows storage of vegetative bacterial cells (in the presence of protective polymers) and fungal conidia (in the form of dry granules) for periods of at least 12 and 6 months respectively at temperatures up to 25^{o}C. Such shelf life does not allow full use of a conventional distribution chain, but it does allow an adequate degree of flexibility in product storage and dispatch. Biopesticides have to compete with highly effective and often relatively cheap chemical pesticides. An alternative strategy is to target at markets where the available chemical pesticides have relatively poor efficacy, as for example the development of biopesticides for control of slug pests. In horticulture there is an existing demand for new biopesticides. The environmental challenges are less in horticulture than in arable crops, and therefore the likelihood of success is greater. The use of biological control products could be wider in horticultural Integrated Pest Mnagement (IPM) programmes and then spread to field crop IPM programmes.

Another major constraint on the development of biopesticides could be the regulations governing the registration and release. Microbial pesticides (bacteria, fungi and viruses) are subject to registration requirements in almost all countries, whereas biopesticides based on higher organisms (e.g. nematodes and beneficial insects) are excluded from these requirements in most countries. However, there are significant differences between the USA, where a tier approach to biopesticide registration has been adopted and the European Community. In the USA the first tier of toxicology and ecological/environmental data requirements is relatively simple, compared to the requirements for an agrochemical, and if the data for a new biopesticide are satisfactory the registration is granted. In contrast, the data required for a new biopesticide in EU are much closer to those for a new agrochemical. The final result is that registration of a new biopesticide is quicker and less expensive in the USA than in EU. Considering the genetically modified organisms, the differences become even greater. In the USA the product is regulated, not the method by which it was generated, therefore, a biopesticide containing a genetically modified organism does not need greater data requirements than one containing a naturally occurring isolate. In contrast, the EU regulatory framework for genetically modified organisms is still under discussion but probably there will be extra data requirements over those for biopesticides containing naturally occurring isolates. The impact of these differences will be that significant developments in biopesticides will occur more rapidly in the USA. Within the EU biopesticides that do not require registration will be favoured particularly for small markets. Small biotechnology companies have been the source of innovation for biopesticides, because those companies have the flexibility to respond quickly to advances in science and technology. Worldwide there are about 1400 biopesticides products being sold. Companies would develop biopesticides products if they have a profit. A farmer would adopt a novel technology thinking of the cost benefit to be made from using it. The guidance of the OECD is that biopesticides should only be authorized if they pose minimal or zero risk. The biopesticide registration data portfolio includes information about mode of action, toxicological and eco-toxicological evaluation, host range testing, etc. This information is expensive for companies to produce and it can avert them from commercialising biopesticides that are usually niche market products.

The EU passed a package of legislative measures in 2009 based around IPM, including the Framework Directive on the Sustainable Use of Pesticides (EU DG Environment). IPM principles do not become mandatory until 2014, but member states have been encouraged to use rural development programmes, funded under the Common Agriculttural Policy, to provide financial incentives to farmers to start implementing IPM before this date. Biopesticides should qualify as low-risk active substances under the legislation. Low-risk substances are granted initial approval for 15 years rather than the standard 10, and a reduced dossier can be submitted, but this has to include a demonstration of sufficient efficacy. One

more requirement is that their half-life in the soil should be less than 60 days and this may cause problems for some microbial biopesticides, such as rhizosphere competent antagonists of soil borne plant pathogens. In the EU having a system of mutual recognition of plant protection products, it is possible for one member state to engage in regulatory innovation and gain a first mover advantage over other member states (Nakkeeran *et al.,* 2005; Kaewchai *et al.,* 2009; Malusa *et al*., 2012).

- **Research and knowledge**. Exploiting knowledge of the genomes of pests and their natural enemies is going to prove the biggest advance in biopesticides' development. This information will give new insights into the ecological interactions of pests and biopesticides and lead to new possibilities for improving biopesticide efficacy, through strain improvement of microbial natural enemies. Some other opportunities are, plant inoculation with endophytic strains of entomopathogenic fungi to prevent infestation by insect herbivores, or exploit the volatile alarm signals emitted by crop plants so that they recruit microbial natural enemies as bodyguards against pest attack and use novel chemicals to impair the immune system of crop pests to make them more susceptible to microbial biopesticides. The biopesticide products that will result from new scientific advances may stimulate the adoption of different policies in different countries. In the USA, Canada, China, India and Brazil, farmers have been quickly adopted transgenic broad acre crops expressing Bt δ-endotoxin genes. The US Environmental Protection Agency includes transgenes in the category of biopesticides In EU there is widespread resistance among consumers to GM crops and the EU excludes them from the biopesticide regulatory process. IPM is adopted more on horticultural crops and is correlated with farmer education and experience. The biocontrol based IPM has been adopted widely by the labour intensive and technically complex greenhouse crops industry, and by growers with already high level of knowledge and used to technological innovation. The IPM based biological control is not used much by growers of broad acre crops. Another proposal is to develop a "total system" approach to pest management in which the farm environment become resistant to the build up of crop pests, and therapeutic treatments are used as a second line of defense (Nakkeeran *et al.,* 2005; Kaewchai *et al.,* 2009; Malusa *et al*., 2012).

- **Use of fungi**. The use of fungi as fungicides and biofertilizers is not new, although most have been developed in the last two decades. Although, there have been little investment in the research and development of fungal products, because they may have poor effect in the field. There is still a wide gap between the research carried out in laboratories and the use in the field, therefore the future research should develop fungal products with significant effects in field applications and storage stability. Aspects to be considered include: the selection of fungal strains that should be used, the possibility to be reliable and cheap to produce on a large scale, the existence of detrimental impact to the environment, their safety to humans and to the environment and possibility to patent the formulation. Integration of combination of inocula or combination with other beneficial fungi should be considered since combinations may be more effective. Greater communication is needed between researchers and industry in the early stages of development (Nakkeeran *et al.,* 2005; Kaewchai *et al.,* 2009; Malusa *et al.*, 2012).

- **Restriction of limiting factors**. Factors that limit the development and utilization of biopesticides worldwide are, the lack of suitable screening protocol for the selection of promising microorganisms, the lack of sufficient knowledge on the microbial ecology of biocontrol agents and plant pathogens, the optimization of fermentation technology and mass production of biopesticides, the inconsistent performance and poor shelf life, the lack of patent protection, the prohibitive registration cost, the deficit of awareness, training and

education, the lack of multi disciplinary approach, the technology constraints, the understanding and use of delivery system, the product quality and stability, since contamination of inoculum is a common problem in small scale production and the long term shelf life of the product is essential to attract multinational companies to invest on a large scale (Nakkeeran *et al.*, 2005; Kaewchai *et al.*, 2009; Malusa *et al.*, 2012).

- **Commercialisation**. To promote the commercialization of biopesticides it is necessary to motivate farmers through publicity, field demonstrations, farmers days, conducting periodical trainings for commercial producers and farmers to increase and improve the supply. Also, technical support is needed by the entrepreneurs on quality control and registration, regular monitoring to maintain the quality, constant research support to standardize the dosage, storage, and delivery systems and naturally support policy from government to use more biopesticides in crop protection (Nakkeeran *et al.*, 2005; Kaewchai *et al.*, 2009). Although there are many biological control products, there are still many problems to overcome to achieve successful commercialization of other potential biological control products. There are biological control agents working well in the laboratory, but not in the field. Biological control of plant diseases by fungal antagonists remains a challenge for future research and development. There are many species of fungi that have been formulated and registered as commercial products, but their products have been used mainly at small scale due to their ineffectiveness in controling plant diseases in the field, as compared to synthetic fungicides.

- **Microbial metabolites for pest control.** Many biological control agents produce secondary metabolites that have properties to control plant diseases. Those metabolites should be tested and studied and must be harmless to humans and the environment. Advances in the study of molecular genetics of biological control agent have provided a powerful tool that will help to improve the effectiveness of biological contol activity.

- **Market trend**. The demand for biopesticides and biofertilizers has been rising steadily, due to their advantages. While some Asian countries have made significant advances in the development and use of biopesticides and biofertilizers, their potential remains largely underutilized, with diverse efforts and experiences in different countries. There are a lot of technological and policy gaps. The technological gaps are: inconsistency in efficacy of the product, toxicology and general safety including allergenic risks in inhaling proteinaceous materials, the degree of stringency of regulation, location, characterization and indexing of agents and creation of repositories, characterisation of agro-ecological conditions and regions for key traits and raising the threshold of desired trains, standard and stable products, quality control, matching performance with synthetics, bioprospecting and allied chemical profiling, scientifically sound use package; well defined role in integrated pest and nutrient management systems and joint use with the synthetics.

  Considering policy aspects, there must be extensive experts' consultation about:

  1. Review the current status of research, development and use of biopesticides and biofertilizers in agriculture at the global, regional and national level,
  2. Develop consensus on place of biopesticides and biofertilizers in the conventional agriculture and issues of quality requirements, quality control, regulatory management, commercialization and marketing,
  3. Identify the role of public and private sector organisations and public-private participation in promoting the use of bioagents in agriculture,
  4. Promote stewardship, regional cooperation, public awareness and stakeholders' participation,

5. Policy framework and advocacy for promotion of their use in greater proportion in future.

The development of biopesticides and biofertilizers market worldwide is limited by:

- A lack of suitable screening protocols for the selection of promising strains,
- Lack of sufficient knowledge on the microbial ecology of PGPR strains and plant pathogens, optimization of fermentation technology and mass production of efficient strains,
- Inconsistent performance and poor shelf life,
- Lack of patent protection,
- Prohibitive registration cost,
- Awareness, training and education shortfalls,
- Lack of multi-disciplinary approach,
- Technology constraints.

The lack of awareness among stakeholders about the potential value of biopesticides and biofertilizers is a major setback. There is a need for:

- Raising the awareness among the policy makers on the potential for biopesticides/ biofertilizers their efficacy and their effect in reducing the health and environmental problems,
- Promotion of the opportunities offered by the commercialization in terms of generation of wealth and employment,
- Providing information to investors about the opportunities that exist for establishing commercial companies to manufacture market and sell biopesticides/biofertilizers,
- Training of government extension workers in biopesticides/biofertilizers and intensification of the communication between research and extension sectors,
- Explanation to the farmers, who are used to chemicals, of the nature and mode of action of biopesticides/biofertilizers.

IV.3. Bio-fuels - recommendations

Rapid advances in biofuel technology will create a dynamic market. Projected further increases in biofuel production over the coming decades involve the greater use of potentially lower cost biomass (lignocellulosics) as feedstock. Achieving this will require significant technological breakthroughs (pre-treatment, enzymes, recombinant microorganisms), while opening up the opportunity for the use of agricultural and forestry residues, new high biomass yielding energy crops and associated higher value fermentation products.

- **Global bioethanol** production is increasing rapidly. Bioethanol, conventionally produced from cane molasses by yeast fermentation, can also be produced from various agroindustial residues and plant wastes. Efficient process optimization and integration by combining production and recovery processes may lead to economic production of bioethanol. Projections by the US Department of Energy are that by 2020, the volume of ethanol produced from the conversion of lignocellulosic materials (biomass) will be twice that produced from corn. To achieve this goal, a number of technological obstacles should be overcome:
    1. Development of cost effective pre-treatment strategies for the various lignocellulosic materials,
    2. Reduction in the costs of producing cellulase enzymes,

3. Availability of robust recombinant microbes (yeasts, bacteria) for high ethanol yields from the C5 (xylose, arabinose) and C6 (glucose) sugars from lignocellulosic hydrolysates,
4. Product and market development for the non-reactive lignin by-product (approximately 15%) with potential for its use in paints and adhesives.

Brazil has played a leading role and it continues to be a global leader-although ethanol production from corn in the USA is now approaching Brazilian levels. A major difference between Brazil and the USA is that the industry cost structure and the use of sugarcane in Brazil as raw material (instead of corn) result in most advantageous cost. In Brazil, half of the current sugar crop is converted to fuel ethanol. The USA Department of Energy has announced major financial support for producing ethanol from lignocellulosics. Much more R&D input needs to be supported in Australia for second-generation processes for biomass to ethanol conversion, including the use of flexible pre-treatment plant design to facilitate use of a range of lignocullulosic raw material from both energy crops and agricultural forestry residues.

- **Biodiesel,** on the other hand, is generally produced from vegetable oils. Agroindustiral residues are still not used as a substrate for biodiesel, though the residual oil present in oil cake, a waste product of oil extraction units, holds great potential. In future, suitable residues with high lipid content may be used as potential raw materials for biodiesel production. Biotechnological procedures to produce biofuels from agroindustrial wastes and residues may be effective in reducing the emission of toxic pollutants and greenhouse gases, and in partly solving the worldwide fuel crisis.
- **Biogas** originates from bacteria during the process of biodegradation of organic materials under anaerobic conditions. All homogenous and liquid organic materials can be fermented or digested (e.g. faeces and urine from cattle, pigs and possibly poultry and wastewater from toilets). Waste and wastewater from food processing industries are suitable if they are homogenous and in liquid forms. Biogas is mainly composed of: 40-70% methane, 30-60% carbon dioxide, 1-5% other gases (0-1% hydrogen, 0-3% hydrogen sulfide). The biogas produced by a digester can be used in the same way as any other combustible gas and it yields a range of benefits for users, which are in general, the production of energy (heat, light, electricity), the transformation of organic wastes into high-quality fertilizer, the improvement of hygienic conditions through reduction of pathogens, etc., the reduction of workload, mainly for women, the positive environmental impact through protection of soil, water, air and woody vegetation and the economic benefits through energy and fertilizer substitution.

A barrier to the large-scale introduction of biogas technology is the fact that the poorer strata of rural populations often cannot afford the initial investment cost for a biogas plant. Financial support from the government can be seen as an investment to hold future costs due to the importation of petrol products and inorganic fertilizers, increasing costs for health and hygiene, as well as natural resource degradation. China is one of the leading countries in biogas construction in rural areas.

The development of biogas technology depends on the political will. It is the task of the government and administrative authorities to provide access to the technology and to secure and organize the requisite materials, financial resources and legal basis. Governments can play a supportive role in biogas research, information dissemination and the regulations for funding, subsidies or tax waiving.

In more general terms, it is essential to develop high performance microbes that are capable of producing biofuels with very high efficiency in order to compete with the fossil fuel. The strategies for developing microbial strains by systems metabolic engineering, which can be considered as metabolic engineering integrated with systems biology and synthetic biology, have been developed. Systems metabolic engineering allows successful development of microbes that are capable of producing several different biofuels including bioethanol, biobutanol, alkane, biodiesel an even hydrogen. Some examples of systems metabolic engineering approaches are strain development of *Saccharomyces cerevisiae, Escherichia coli, Zymomonas mobilis* for ethanol production, *Clostridium acetobutylicum, C. beijerinckii* and *E. coli* for butanol and isobutanol production.

In order to develop economical and sustainable processes for biofuel production, the metabolic pathways of biofuel producers need to be optimally redesigned to achieve improved product yields, higher product concentration and productivity and product tolerance. Also, the whole process should be operationally inexpensive. It is expected that this combined strategy will result in the development of microorganisms capable of producing various biofuels cost effectively on industrial scale (Jang *et al.,* 2012; Liu *et al.* 2012).

IV.4. Compost - recommendations.

- There is a need for promotion and improved disseminaton of information to farmers on the benefits of vermicompost. The demand will double or triple in the near future.
- There is good scope for new entrepreneurs.

IV.5. Microbial Metabolites - General Recommendations

The knowledge on new microbes and any information on their genomics, or about their communities, will pose an enormous potential to provide industry with novel products and processes based on the use of microbial resources. Because of their biotech impact, numerous efforts are being undertaken worldwide, with an ultimate goal to deliver new usable substances of microbial origin to the markets. The direct isolation of microbes always reveals that the majority cannot be cultured. There are four strategies to increase microbial performance, according to the primary biotechnology goal:

- Use of wild-type microbes that can be further improved by systems biology approaches,
- Rational design or protein engineering of the enzymes of interest, which can be further hyper-produced in a well-characterized microbial host working properly under physical chemical conditions relevant to the desired industrial process,
- Combination of different genetic pathways into a single cell factory to establish an organism capable of producing quickly and cost-efficiently the products of interest,
- Utilisation of a systems biotechnology approach that considers cells as a whole.

Examples of recent developments in the systems biology approaches have been achieved for a number of microorganisms of biotechnological relevance, such as *Corynebacterium glutamicum, Clostridium acetobutylicum, E. coli, Saccharomyces cerevisiae, Aspergillus terreus* and *Aspergillus niger* for the production of high-value substances such as methinonine, L-lysine, IGF-1, fussion protein, poly-hydroxyalkanoates, human leptin, riboflavin, L-threonine and lovastatin. A broad range of different *E. coli* strains have been constructed, to express industrially important enzymes, such as benzaldehyde lyases, benzolformate, decarboxylases, hydroxynitrile lyases and alcohol

dehydrogenases. An esterase from uncultured microorganisms is able to degrade terephtalate esters, important component of bio-plastics (Beloqui *et. al.,* 2008).

Genetically modified microorganisms (GMO) are now used to produce pharmaceuticals, vaccines, special chemicals, feed additives and processing agents for the food industry. All systems that use GMOs must be reported to governmental agencies. There are EU laws governing the facilities and microorganisms used for genetic engineering as well as the safety and quality of the resulting products. Additives that are produced with the help of GMOs do not require labeling or special regulations, because the microorganisms are not directly associated with the final product, which is carefully purified. In some cases, amino acids and enzymes are not legally considered foods, but they are known as processing aids and there is no legal requirement to declare them on the list of ingredients.

Modern biotechnology in the form of genetic engineering has the potential to provide important benefits if used carefully and ethically. Scientists, farmers, food manufacturers and policy makers recognize that the use of transgenic organisms should be considered very carefully to ensure that they pose no environmental and health risks, or at least no more than the use of current crops and practices (Beyer, *et. al.,* 2002; Wieczorek, 2003).

IV.5.1. Agricultural biotechnology

Agricultural biotechnology is widely viewed as one of the key technological advancements that would enable agricultural innovation systems to meet more efficiently the needs of farmers. A question to many developing country governments, including the donor community, is to what extent agricultural biotechnology can contribute to poverty reduction and sustainable development. Biotechnology definition refers to any technique that uses living organisms or substances from these organisms to make or modify a product improve plants or animals or develop microorganisms for specific uses. Biotechnology is not a separate science, but rather a mixture of disciplines, such as biochemistry, genetics, molecular biology, and cell biology. Agricultural biotechnology is becoming a progressively more important factor in shaping agricultural production systems worldwide, including in the developing countries. Using advanced biotechnology tools, genetic resources can be more precisely characterized, efficiently improved and tailored to specific needs. The technologies can be used to support the development of sustainable production systems for food, feed and crops for industrial purposes, such as biofuel. Novel agro-processing techniques using biotechnology can add downstream value to crops and their by-products. The diversity of techniques that constitute modern biotechnology offers much promise to serve the pressing needs of sustainable development in the agriculture, industrial and health sector. For developing countries the challenge will be to develop biotechnology based innovation systems that are able to adapt relevant knowledge and technologies that can contribute to economic growth and also improve environment, health and livelihoods. Modern agricultural biotechnology, which includes disciplines such as genetic engineering, bioinformatics, structural and functional genomics, and synthetic genomics, is a comparatively young field of science. thus we have so far only seen the beginning of what promises to be a very exciting and possibly also revolutionary technology. It is important for developing countries to be part of this bioscience revolution, with its spectrum of techniques and opportunities. Countries without basic know-how will continue to be dependent on global actors and would miss the opportunity to steer technological development and to adapt and develop technologies to their own needs. Biotechnology opportunities in the development of agriculture represent a complex and also vast topic with many uncertainties and there is rapid generation of new information. The possibilities that biotechnology offers may not yet be fully apparent, and it is likely that progress will take place far more quickly than current popular belief (Ivar *et al*., 2007).

IV.5.2. New sequencing technologies in characterisation of microbial diversity

Microbial diversity has been studied by applying phylogenetic markers such as the rRNA genes. Such work revealed that the vast majority of microbial diversity had been missed by cultivation-based methods and that natural diversity was far more complex than formerly expected. It is estimated that about 95-99% of the microorganisms in nature are typically not culturable by standard techniques (Hugenholtz and Tyson, 2008). A bar-coded pyrosequencing approach targeting some hypervariable region of the bacterial 16S rRNA gene has allowed studies of the genetic diversity at significantly higher resolution compared to traditional fingerprinting methods (Miller *et al*., 2009). However, a single phylogenetic marker does not allow studies of whole genetic diversity, as phylogeny based on a single gene is not directly associated with the metabolism. Today, the aim to characterise complete microbial ecosystems by combining metagenomics, meta-transcriptomics and meta-metabolics to study microbial systems at the ecosystem level (eco-systems biology) may be achieved soon (Raes and Bork, 2008). Providing a view not only of the community structure (species phylogeny, richness, and distribution) but also of the functional (metabolic) potential of a community, would require virtually all genes to be captured and sequenced. Above mentioned approaches are largely facilitated by the ongoing revolution in sequencing technologies allowing a massive sequencing producing millions of bases in a single day (Metzker, 2010). The increased throughput enables increased sampling frequency for metagenomics, and even rapid sequencing of several environmental microbial genomes. Moreover, it is expected that in the near future sequencing techniques on the individual organism level will be available.

IV.5.3. Nanotechnology

Nanotechnology employs nanoparticles that are made of inorganic or organic materials that are defined by having one or more dimensions in the order of 100 nm or less. The integration of whole cells with nanostructures leads to hybrid systems that have numerous applications in many fields including agriculture. Indeed, even though nanoscale constructs are smaller than cells, macroscopic filters, made of radically aligned carbon nanotube walls, able to absorb *Escherichia coli*, were fabricated. The same technology could therefore be applied to collect bacterial cells from fermentation processes and deliver them to the plant. The physical stability and the high surface area of nanotubes, together with the ease and cost-effective fabrication of nanotube membranes, may thus expand their use in the production of biofertilizers. The use of nanoformulations may enhance the stability of biofertilizers and biostimulators with respect to dessication, heat, and UV inactivation. For example, the addition of hydrophobic silica nanoparticles of 7–14 nm to the water-in-oil emulsion formulation of the biopesticide fungus *Lagenidium giganteum* reduced the desiccation of the mycelium. The physical features of the formulation were improved and the microorganism was still effective after 12 weeks of storage at room temperature (Malusa *et al*., 2012).

IV.5.4. Applications for new sequencing technologies

Approaches, which are driven by whole genome sequencing and high-throughput functional genomics data, are revolutionising studies on microbial functionality and diversity. High throughput sequencing technologies are the base for many applications, e.g., metagenomics, metatranscriptomics, metaproteomics, and single amplified genomes.

IV.5.5. Meta-approaches: metagenomics, metatranscriptomics, metaproteomics

Metagenomics is the analysis of genomic DNA obtained directly from whole community of organisms inhabiting environment (Handelsman *et al.*, 1998). To date, the approach has been applied mostly to microbial communities (Hugenholtz and Tyson, 2008; Wang *et al*., 2011). Metagenomics provides a view not only of the community structure (species phylogeny, richness, and distribution) but also of the functional (metabolic) potential of a community because virtually about all genes are captured and sequenced. Metagenomic protocols begin with the extraction of genomic DNA from cellular organisms and/or viruses in an environmental sample; the DNA is then randomly sheared,

these many short fragments are cloned, sequenced in either a random or targeted fashion and reconstructed into a consensus sequence. Many genes may go unnoticed due to their "unclonability" in a heterologous or non-native host like *Escherichia coli* (most commonly used host for cloning libraries). Failure to produce clones representing these novel genes is primarily due to their toxicity on *E. coli.* Basically, these genes may be too "foreign," and their expressed protein may cause failures in the operation of their host cell. New sequencing technologies like 454 pyrosequencing can address this problem because they eliminate the cloning step by direct sequencing of extracted DNA. In principle, any environment is amenable to metagenomic analysis provided that nucleic acids can be extracted from sample material and that they are of good quality.

Meta-transcriptomics refers to the analysis of the collective transcriptomes of a given habitat. Poretsky *et al*. (2005) developed an environmental transcriptomic approach based on the direct retrieval and analysis of microbial transcripts from marine and freshwater bacterioplankton communities. They suggested that their environmental transcriptomic procedure might be a promising tool for exploring functional gene expression within natural microbial communities without bias towards known sequences. This approach has not been tested yet for the analysis of microbial communities in contaminated sites. Another approach for environmental metatranscriptome analysis is by using DNA microarrays (Gao *et al*., 2007). While microarray-based metatranscriptome analysis undoubtedly provides valuable information about the response of microorganisms to environmental parameters, the information remains restricted to the number and nature of the probes spotted on the array. Frias-Lopez *et al.* (2008) report on a global analysis of expressed genes in a naturally occurring microbial community. Although many transcripts detected were highly similar to genes previously detected in ocean metagenomic surveys, a significant fraction (approximately 50%) was unique. Microbial community transcriptomic analyses revealed not only indigenous gene and taxon-specific expression patterns but also gene categories undetected previously.

Environmental ***metaproteomics,*** i.e. the study of the entire protein content of a given habitat is still in its infancy and faces great challenges in terms of protein extraction procedures (Maron *et al*., 2007), protein separation and identification, and bioinformatic tools to archive and analyse the huge amount of data generated by this approach (Wilke *et al*., 2003). Moreover the interpretation of protein expression levels in environmental organisms is a challenge due to the high genetic variability, the dependence on the nutritional and reproductive state of the organisms, and climatic and seasonal variations in the environment itself (Nesatyy and Suter, 2007). In metaproteomics, complex mixtures of proteins from an environmental sample are typically separated with two-dimensional (2D) gel electrophoresis or high performance liquid chromatography. Following protein separation, fractions of interest (e.g., protein spots on a 2D gel) are analyzed by high-throughput mass spectrometry based analytical platforms (Domon and Aebersold, 2006). Protein prediction and subsequent identification are greatly facilitated by available relevant metagenomic sequence data. So far, only a few environmental metaproteomic studies have been conducted (Wilmes and Bond, 2004, 2006; Ram *et al*., 2005; Bastida *et al*., 2009; Wu *et al*., 2011; Wang *et al*., 2011; Benndorf *et al*., 2007).

Single amplified genomes. Direct sequencing from community DNA (i.e. metagenomics) is unsuitable for genome assemblies and metabolic reconstruction of the members of complex (i.e., most natural communities) even with very large sequencing efforts. Fortunately, DNA from individual cells can be amplified and analysed by various means. Such new emerging strategy is called the ‘single amplified genomes’ (SAGs) approach (Ishoey *et al*., 2008). The multiple displacement amplification (MDA) method generates micrograms of DNA from the several femtograms present in a typical bacterial cell. MDA is based on isothermal (at 30 °C) strand displacement synthesis in which the highly productive phi29 DNA polymerase repeatedly extends random primers on the template as it concurrently displaces previously synthesized copies (Dean *et al*., 2001). Depending on the desired throughput, the environment and organisms targeted, single cells have been isolated for use in MDA

reactions by dilution, fluorescence activated cell sorting (FACS), micromanipulation, and microfluidics). Sorting by FACS has the best potential for high throughput technologies by which thousands of cells can be isolated in minutes. Potentially, single-cell sorting can be combined with fluorescent *in situ* hybridization (FISH) to enrich for specific taxa (Ishoey *et al*., 2008). Cells can be sorted into micro-plates, thereby facilitating automation.

Massive parallel sequencing encompasses several high-throughput approaches to DNA sequencing; it is also called 'next-generation sequencing' (NGS) or **second-generation sequencing**. This is an inexpensive production of large volumes of sequence data and has primary advantages over conventional methods. Some of these technologies emerged in late 1996 and became commercially available since 2005. These technologies use miniaturised and parallelised platforms for sequencing of 1–100 million of short reads (50-400 bases) (Anderson and Schrijver, 2010). Next-generation sequencing (NGS) technologies aim to sequence genomes in a shorter time and at a lower cost than traditional Sanger sequencing. These methods have different underlying biochemical basics. They bypass the cloning of DNA fragments before sequencing, a necessary step for most Sanger sequencing, and this has resulted in the discovery of new microorganisms that previously had been missed because of cloning difficulties and biases (Blow, 2008).

Pyrosequencing was among the first of the so-called "next-generation" sequencing methods developed by Life Sciences (Ronaghi, 2001). Pyrosequencing gives us a unique opportunity to determine any bacterial species. It generates 1 million fragments (reads) that are shorter than the conventional Sanger technique but, compared to most of other technologies, produces the longest read length (presently up to 400 bp). This method provides the energy for the generation of light. The light emitted is recorded as an image for analysis. ***Illumina****/Solexa GA* developed by Illumina was released in 2007. Solexa GA technology produces more nucleotides per run (1 Gbp data) with better accuracy (more than 99%) compared to pyrosequencing but with a read length of 30-35 bp.

Solid sample preparation. After amplification, the beads are immobilised onto a custom substrate. A primer that is complementary to the adaptor sequence (green), random oligonucleotides with known 3' dinucleotides (blue) and a corresponding fluorophore (colored circles), are hybridised sequentially along the sequence and image data collected. After five repeats, the complementary strand is melted away and a new primer is added to the adaptor sequence, ending at a position one nucleotide upstream of the previous primer. Second-strand synthesis is repeated, allowing two-color encoding and double reading of each of the target nucleotides. Repeats of these cycles ensure that nucleotides in the gap between known dinucleotides are read. Knowledge of the first base in the adaptor reveals the dinucleotide using the color-space scheme.

Helioscope from Applied Biosystems released its own technology Heliscope that sequences single molecules. The output consists of 50 nucleotides, 30 - 90 million reads and 500Mb with high accuracy (99.4%).

HeliScope sequencing. Unamplified DNA is immobilised with ligated adaptors to a substrate. Each species of dNTP with a bright fluorophore attached is used sequentially to create second-strand DNA; a 'virtual terminator' prevents the inclusion of more than one nucleotide per strand and cycle, and background signal is reduced by removal of 'used' fluorophore at the start of each cycle.

Pacific Biosciences is developing not yet commercially available sequencing method. The method is expected to be commercially released in 2010. Output (read length is expected to be several thousand bps) and data quality are not known yet. They had used szero-mode waveguides to perform real-time observation of ribosomal translation (Uemura *et al*., 2010).

Pacific Biosciences sequencing occurs in zeptolitre wells that contain an immobilised DNA polymerase. DNA and dNTPs are added for synthesis. Fluorophores are cleaved from the complementary strand as it grows and diffuse away, allowing single nucleotides to be read. Continuous detection of fluorescence in the detection volume and high dNTP concentration allow extremely fast and long reading.

***Data analysis*.** "Next-generation" sequencing technologies bring up a huge amount of sequenced information. Until recently such genome or metagenome sequencing was almost entirely restricted to large genome centers, now it is feasible for individual laboratories. Next to computational resources, uncharacterized gene products with unknown function are likely to be the biggest bottleneck for the foreseeable future. The major public database of genome nucleotide sequences is maintained by NCBI Entrez. Sequence data are stored in Entrez Genome (as complete chromosomes, plasmids, organelles, and viruses) and Entrez Nucleotide (as chromosome or genomic fragments such as contigs). The Genome Project database provides an umbrella view of the status of each genome project, links to project data in the other Entrez databases, and links to a variety of other NCBI and external resources associated with a defined genome project. Sequences associated with a defined organism can also be retrieved in the taxonomy browser. Due to massive release of NGS data (sort read sequences, SRSs) the major databases needed to be restructured, and new databases appeared. The main goal of resequencing projects is generally to identify SNPs and other types of polymorphism, such as short insertions and deletions, collectively called indels. SNP discovery is essential for genetic mapping in eukaryotic organisms, as they possess large genomes. However, SNP approach might be useful in ecological studies of microbes which otherwise need vast sequencing due to the high number of individual organisms. Comparisons of microbial genomes widen possibilities to identify chromosomal rearrangement events such as gene acquisition, duplications, and deletions. On the other hand, using the complete genomes in phylogenetic analysis, might lead to loss of phylogenetic signal – mainly due to lateral gene transfer (LGT). LGT results in variable phylogenetic histories across genes and is suggested to lead to complicated or even completely defeating attempts to reconstruct bacterial evolution. High level of LTG may cause elusive phylogeny at organism level because we do not know which genes represent the true history of the cell lineages. However, the existence of core genes resistant to LGT has been proposed and is supported by some studies. Using complete genomes for phylogeny needs sufficient taxon sampling within a clade – yet, the rapidly increasing number of fully sequenced microbial genomes enables such taxon sampling (Lerat *et al.*, 2003).

IV.6. Policies, Main Gaps

The policies supporting sustainable agricultural production and extensive research to improve the effectiveness and consistency of microbial inocula have resulted in the registration of several strains for both biological control and biofertilization. Yet, a wider use of microbial inoculants, especially those acting as phytostimulators and biofertilizes, has been frequently hampered due to the variability and inconsistency of results between laboratory, greenhouse, and field studies. The reason is the incomplete understanding of the complex relationships established between the plant, the microorganism and the environmental conditions, particularly those of soil. Furthermore, the lack of correct formulations and the expensive and time consuming registration procedures are among the factors holding back the use of biofertilizers on a wider scale.

Small farmers are crucial for the agricultural sector of developing countries. Around 70% of the world's poor live in rural areas and engage primarily in subsistence agriculture. It is important to note that many of the small scale farmers in developing countries are women. Traditional farming systems,

especially in the tropics, can be characterized by their high level of diversity, giving them a high degree of stability, resilience and efficiency, ensuring, above all, their sustainability. The disease resistance of traditional cultivars or landraces selected over millennia also needs to be mentioned. Landraces are usually genetically diverse and in balance with their environment and endemic pathogens; and although not necessarily high yielding, growing landraces always ensure some harvest under all but the worse conditions. Pesticides are generally being used in small amounts by traditional farmers, primarily because of their cost.

Organic agriculture is perhaps one of the oldest farming systems in the world. In this context, questions that are frequently being asked, include:

- Are traditional agricultural practices sustainable?
- Can a traditional practice be continued for a long period of time without environmental degradation, serious reduction of crop productivity, and the addition of heavy fossil fuel inputs?

Most traditional practices are sustainable, although some of them require high external inputs, and many have high labour requirements, which are not necessarily undesirable in settings where land, energy and capital are more limiting than labour. Traditional farmers' knowledge of many aspects of agriculture is often broad, detailed, and comprehensive, although this is not fully accepted.

For example, traditional farmers have been practicing integrated pest management for centuries, using various cultural controls, resistant varieties and biological control. Therefore, strengthening research on methods of pest management in traditional agriculture, with the aim of improving their use, may provide a sound basis on which to initiate realistic improvements in traditional agriculture systems. Furthermore, such studies will provide lessons and information of value to modern agriculture. Traditional agricultural practices must be understood and conserved before they are lost with the rapid advance of modern agriculture in developing countries.

IV.6.1. Relative instances

It would be interesting to look at cases mainly in developing countries. Increasing cost of chemical fertilizers, declining yield in response to fertilizer application and degradation of soil, and limit the soil fertility. This model of agriculture has led to resource degradation, depletion of natural resources, increased erosion and loss of natural fertility of soils, decrease in soil organic matter pool, increased incidence of new pests and diseases, reduction of biomass production and biodiversity, modification of soil microbial composition and balance, increase in soil compaction and soil quality deterioration, with an overall impact over the sustainability of various production systems.

There is an urgent need to break the vicious spiral between environmental degradation and poverty in developing countries. When only the young men migrate to the urban areas to eke out a living, the young women are compelled to take over the responsibility of management of the subsistence farming and seasonal labour. The mass exodus of farming families to the urban areas leads to fast development of urban slums and civic problems. Even with food availability, millions of marginal farming, fishing and landless rural families have either very low or no access to food due to lack of income generating livelihoods. Approximately 200 million rural women, children and men in India alone fall in this category.

The deleterious consequences of the human induced changes in climate, like global warming, which is due to the increase in the concentrations of greenhouse gases (GHG), has begun to cause sea level rise and an increase in hydro-meteorological natural disasters, and could aggravate the poverty and miseries of these farmers. In many developing countries, agriculture has always been a "gamble with monsoon", and the present climate change makes it even more so.

In this regard, intensification of agriculture to meet the future demands for commodities would be necessary in order to avoid further expansion onto marginal lands, forest areas and fragile ecosystems. Also, the increased use of external inputs and development of specialised production and farming systems tend to increase vulnerability to environmental stresses and market fluctuations. There is a need to intensify agriculture by diversifying the production systems for maximum efficiency in the use of local resources, while minimising environmental and economic risks.

For these reasons, the evergreen revolution (pro-nature, pro-poor, pro-women and pro-employment/livelihood oriented eco-agriculture) which is founded on the principles of environmental and social sustainability and economic viability needs to be promoted further. It is a system of agriculture that involves sustainable management of natural resources and progressive enhancement of soil quality, biodiversity and productivity.

Annex II provides additional detailed information on cases from different countries and regions regarding the current status of the agro industrial uses of microorganisms with emphasis on the aspects of policies including the main gaps.

IV.7. Questionnaires and analysis of interviews

Questionnaire were sent to 211 companies, distributors, scientists and Ministries of Agriculture. The synthesized results based on the responses received from company experts, are listed in Table 3.

Table 3. Questionnaire analysis (company experts)

Does your company produce or import bio-inoculants/biofertilizers or any other product of microorganisms in different form (cultures, enzymes, flavours, fragrances and additives)?	**Yes**	**No**			
	9	0			
Do you export bio-inoculants/biofertilizers or any other products of microorganisms in different forms (cultures, enzymes, flavours, fragrances and additives) concerning agro-industry processes?	**Yes**	**No**			
	7	2			
What kind of bioinoculants-biofertilizers/or related products are most popular (PGPRs, algae, fungi etc?)	**Fungi**	**PGPR**	**Bacteria**	**Algae**	**Effective Microorganisms**
	6	4	3	1	2
Does your company use genetically engineered microorganism (GEM) applied as bioinoculants-biofertilizers /or related products?	**Yes**	**No**			
	0	9			
Which sector is consulting the farmers on bioinoculants-biofertilizers/or related products?	**Public**	**Private**	**Both**		
	1	3	5		
Who is the supervisor of the farmers during bioinoculants-biofertilizers/or related products application?	**Farmers**	**Scientists**	**Public Agencies**	**None**	
	3	2	3	1	
Is the education level and the technical training of	**Yes**	**No**			

the farmers crucial for the proper application of bioinoculants-biofertilizers/or related products?	4	5			
Are bioinoculants and biofertilizers used simultaneously with fertilizers or pesticides?	**Yes**	**No**			
	8	1			
Is microbial diversity influenced by bioinoculants application?	**Yes**	**No**			
	5	4			
Are there any field trials of new products in progress?	**Yes**	**No**			
	9	0			

As shown in the first question, all the answers were positive. This was to be expected as each of these companies sells bio-inoculants/biofertilizers.

77.8% of the respondents replied positively to the second question. The results of the third question are shown in Fig. 14.

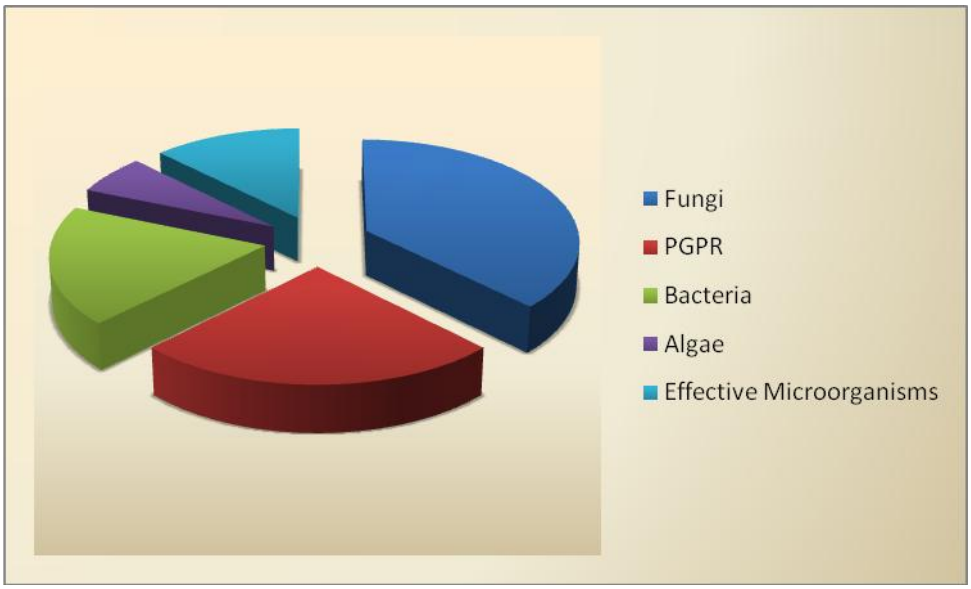

Figure 14. Which kind of bio-inoculants/biofertilizers or related products is most popular?

None of the companies responding to the questionnaire used genetically engineered microorganisms in their formulations.

The answers to the fifth question are shown in the following diagram (Fig. 15). We observed that in most cases farmers sought consultation on bio-inoculants/biofertilizers or related products, in both the public and the private sector.

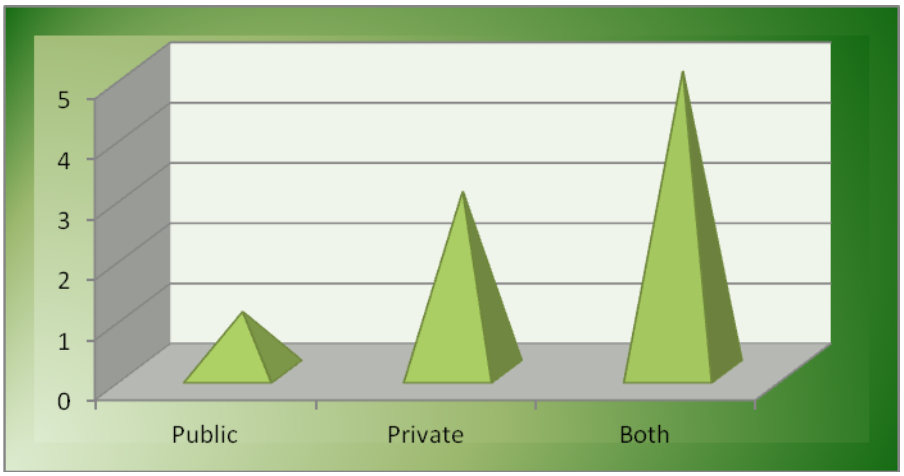

Figure 15. Which sectors are advising farmers on the application of bio-inoculants/biofertilizers or related products?

The sixth question tried to identify who provides advice to the farmers when they apply bio-inoculants/biofertilizers or related products. The responses to this question are reflected in the diagram below (Fig. 16).

In most cases, either the farmer or the public sector is responsible for the application.

This can be explained by the fact that the application rates of bio-inoculants/biofertilizers and related products are not as critical as those of chemical fertilizers/pesticides. Moreover, the public sector, like for example the Ministry of Agriculture and Food Safety, also trains farmers in the application of such formulations.

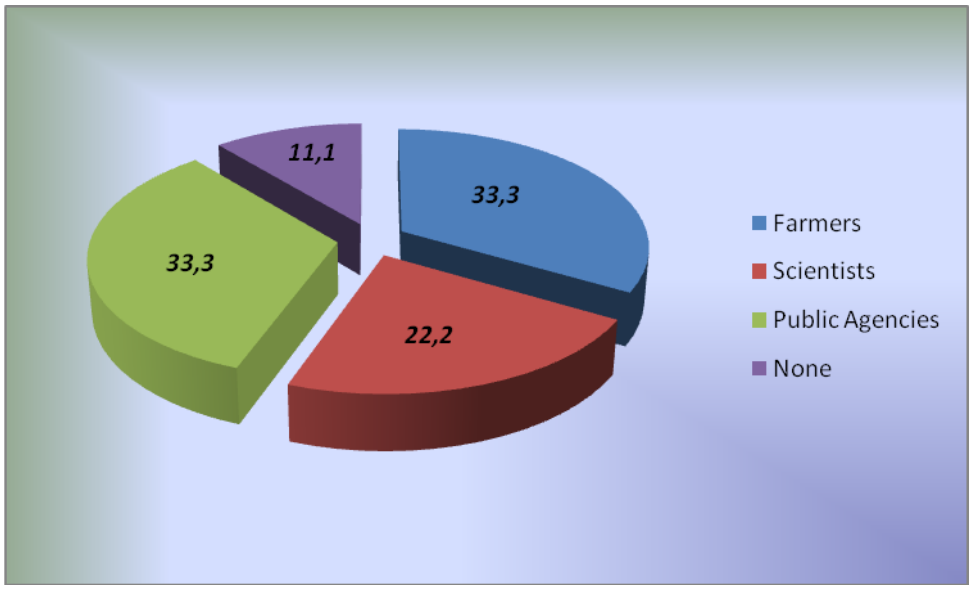

Figure 16. Who advices farmers when they apply bioinoculants/biofertilizers and/or related products?

When the experts were asked whether farmers' education level and technical training was crucial for the proper application of bio-inoculants/biofertilizers/or related products, the opinions varied. In most cases, experts claim that there is no necessity for farmers' training or education, as the products are easy to apply and can be implemented in a wide variety of crop production systems. The minority view argues that farmers' training and education is desirable, since they are savvier to the benefits of using such products.

The overwhelming majority of respondents indicated that bio-inoculants and biofertilizers are being applied simultaneously with chemical fertilizers and pesticides.

The prevailing view of experts is that bio-diversity is affected by the application of bio-inoculants.

Finally, in all questionnaires submitted by companies, experts admitted that there are ongoing field trials for new products.

The questionnaires that were addressed to other receivers contained quite similar aspects.

V. CONCLUSIONS AND FUTURE PROSPECTS

V.1. Conclusions

The extensive study of published reports and references, as well as the analysis of the questionnaires, has demonstrated that there is a broad use of microorganisms in agroindustrial processes.

Agricultural production needs to increase by 60% over the next 40 years in order to meet the rising demand for food. Additional increase in production will also be necessary to provide feedstock for expanding biofuel production. Increasing agricultural productivity will be central to containing food prices in a context of rising resource constraints. At the same time, there is a growing need to improve the sustainable use of available land, water, marine ecosystems, fish stocks, forests, and biodiversity. Around 25% of all agricultural land is highly degraded, while critical water scarcity in agriculture is a fact for many countries. Moreover, several fish stocks are over-exploited or at risk.

There is a growing consensus that extreme weather events are becoming more frequent and climatic patterns are changing in many parts of the world. Global agricultural output grew by 2.6% p.a. over the last decade, led by growth in Brazil, China, India and the Russian Federation. In the late 1960s an agricultural explosion took place, due to the widespread use of inexpensive chemical fertilizer and market reforms. Especially the yields of rice and corn increased rapidly. At present, production of these grains faces troubles, because of land shortages and soaring prices for fertilizer. The second issue constitutes a major factor contributing to a rise in food prices and thereby threatening to push millions of poor people into malnutrition. For that reason some farmers tried to replenish nutrients in the soil and revert to older methods of fertilization by spreading manure on fields. According to Heffer and Prud'homme (2012) agricultural production will grow steadily in order to supply the food, feed, fibre and bioenergy markets. The cropped area would continue to expand in Latin America, Sub-Saharan Africa and South-east Asia. Developing countries are projected to increase their share of global crop and livestock production.

Many studies in greenhouses and fields have assessed the effect of rhizobacteria and endophytic species on plant growth, grain yield of annual crops, and the cultivars of different crops to save fertilizers, or to diminish pollution caused by agrochemicals, or, both.

Microbial inoculants have long been incorporated into field practices worldwide, with satisfactory results, especially for rhizobia. Compared with chemical applications in agriculture, their present impact on the agromarket is smaller than expected. However, the agrochemical industry is more sympathetic now to the concept of bacterial inoculants than it has been previously. There is a genuine interest in developing bacterial products that are reliable and that can act as complements to

chemicals already on the market. Research and limited field trials of PGPB over the last two decades have opened up new horizons for the inoculation industry.

PGPR have gained worldwide importance and acceptance for agricultural benefits. These microorganisms are the potential tools for sustainable agriculture and the trend for the future. Scientific researches involve multidisciplinary approaches to understand adaptation of PGPR to the rhizosphere, mechanisms of root colonization, effects on plant physiology and growth, biofertilization, induced systemic resistance, biological control of plant pathogens, production of determinants etc. Biodiversity of PGPR and mechanisms of action for the different groups: diazotrophs, bacilli, pseudomonads, and rhizobia, are shown. Effects of physical, chemical and biological factors on root colonization and the proteomics perspective on biocontrol and plant defence mechanism is discussed. Visualization of interactions of pathogens and biocontrol agents on plant roots using autofluorescent protein markers has provided more understanding of biocontrol process. Commercial formulations and field applications of PGPR are detailed in this report.

Most inoculants today are used for legumes and to a lesser extent for cereals. The market dictates that the inoculants must be as cheap as possible. The cost of developing new inoculant materials quickly moves the price out of a practical range for agriculture, especially in developing countries. However, there are several high-value specialty markets such as flowers, fresh organic fruits and vegetables, where chemicals are undesirable or become difficult to use because of restrictions. Greenhouse crops are also primary targets for commercial inoculants. Since they are often grown in disinfected soils or even without soil but with high input costs, the additional inoculation costs will not cause an unacceptable economic burden to the grower. At the same time, this type of cultivation avoids all the difficulties originating from the interaction of the inoculants with the soil.

V.2. Future prospects

One concern still remains, even with the latest approaches mentioned before. PGPB may function through multiple mechanisms, but the transfer of a single mechanism may not provide significant benefits. With engineered crops, most of the technical difficulties inherent to bacterial inoculants are removed because the grower simply purchases the "modified" seeds, which certainly will be more expensive. During the last century, peat formulations have been developed into effective and accepted carriers, but their development has almost reached its limits. Synthetic carriers, which have yet to be transferred from experimental concepts into commercial inoculants, offer greater potential and flexibility for the inoculant industry. Due to the shortage of information about new developments from inoculant companies, it is premature to view these carriers as potentially universal, even though they may overcome many of the deficiencies of peat-based inoculants. While it is true that in contemporary agricultural practices synthetic inoculants are frequently too expensive for the target crop, and therefore companies are reluctant to develop them, the bioremedation industry might support development of such advanced inoculants. Many types of encapsulated forms of microorganisms have been developed for use in bioremedation. Moreover, numerous bioremedation projects are supported by governments in developing countries or by large contaminating industries in developed countries which are forced to "clean up", both of which are more resourceful than an individual farmer. More efficient inoculants will undoubtedly be used for bioremedation processes, especially in emergencies, regardless of their higher costs. This use may provide agriculture with an opportunity for the development of novel inoculant materials and formulations. A wider use in non-agricultural applications may help these materials become cost competitive for agriculture.

When developing PGPR biofertilizers, the strain(s), the inoculum production and, in general, the development of appropriate formulations as well as strategies of field experimentations are

fundamental conditions for a successful application of PGPR species. However, the extensive commercialisation of PGPR biofertilizers has been limited worldwide.

In most countries there are ongoing projects for the conservation of microbial diversity. Such projects usually relate to the establishment of culture collections of agricultural and industrial microorganisms, and to the evaluation and mass multiplication of potential biological control agents. Most of the scientists consider the maintenance of microbial culture collections as crucial to the safeguarding of microbial diversity. Traditional management practices for safeguarding microbial diversity include:

1. Organic farming;
2. Integrated Pest Management practices;
3. Rice intensification systems, which is a method of rice cultivation using less water and other inputs;
4. Use of organic materials; and
5. Composting.

The following knowledge gaps regarding technologies and policies which are needed to improve the use of microorganisms have been identified:

- Quality control of microbial products;
- Proper regulatory mechanisms;
- Separate registration policies for microorganisms based products;
- Appropriate extension and demonstration in the farmer's fields;
- Public-private partnerships;
- Gap between the availability of beneficial strains and their use, due to insufficient technology for their distribution to field crops;
- Technologies to select and preserve best microorganisms in culture collection facilities centers for later industrial use .

Some scientists seem to be doubtful about the possible threats of imported microbial products (biopesticides) to human and or animal health. Thus any release of such products should be thoroughly evaluated and monitored. It should also be pointed out that several bioproducts, especially in Southeast Asia, are fake, causing pathogenic contamination of plants, animals and/or humans. Moreover, many microbial spores could be potential allergens. Other threats from the use of microorganisms in agroindustrial processes, could be the lack of availability of quality products, accidental contaminants during mass multiplication of microbes, the lack of large-scale production technologies to meet the demand and the timely supply of quality products to users. Many bacteria, which are potentially pathogenic to humans, are not recognised by registration offices as threats (risks). Such bacteria may be accepted as biofertilizers, while being harmful both to humans who work in the field and to the consumers of the produces such as fruit and vegetables.

Interesting aspects have been outlined on the potential impacts of climate change on microbial diversity. It is believed that climate changes will influence microbial diversity and modulate microbial community composition. Also, certain sub-sets of microbes could develop larger diversity as noticed in *Trichoderma* and *Beauveria bassiana* that are used as biocontrol against coffee root diseases and coffee berry borer pest. Microorganisms are expected to acquire various different physiological properties because of climate change.

Composting from all kinds of farm wastes (plant or animal organic material) seems to be widespread. Oyster mushroom cultures are used to hasten the composting. Leaf litter in plantations is used extensively in vermicomposting. Farmyard manure production is a regular practice especially in India. Composts often are applied in soil to improve soil fertility or are used for the preparation of substrates. The prices of compost products is not encouraging and governments have to often intervene through subsidies.

The biofuels market is a rising and dynamic market that is expected to further increase over the coming decades. Biofuels involves the greater use of potentially lower cost biomass as feedstock. For producing biofuels with high efficiency, it is essential to develop high performing microbes. The adoption of new technologies has to be enhanced in order to improve the quality and image of these products.

Biofertilizer manufacturers need to address segments where adoption can be hastened, such as cash crops, fruits and vegetables and export oriented crops. The communication should focus on commercial advantages of adopting this technology (such as improvement in quality of produce leading to better prices, lesser residues leading to greater acceptance in export markets), rather than an environmental one (soil fertility and preserving the biosphere). Biofertilizers, as a product category, should create an identity that is distinct from organic fertilizers. Biofertilizer manufacturers need to make the product simpler to use so as to increase its application. Currently, small and medium enterprises are producing biofertilizers but , they do not have adequate resources for extension activities. Therefore, there is a case for large-scale enterprises to enter into the manufacturing of biofertilizers, which would lead to economies of scale and make resources available for extension activities. This could solve the problem of availability, awareness and quality. From a realistic perspective, one has to accept that, in the foreseeable future, chemicals will continue to dominate the market. Only a gradual and modest increase in the use of bacterial inoculants is to be expected. Agriculture in developed countries is definitely the major promoter of microbial inoculants that are "environmentally friendly". Nevertheless, special attention should be paid to the needs and constraints of developing countries that need easy-to-use and inexpensive formulations. For the short- and medium-term future, more research should focus on the development of better and more economical feasible, synthetic inoculant carriers, while sustaining peat-based inoculant production for agriculture. The other options should be considered as long-term goals.

Organic agriculture is a fast growing sector of agriculture within the scope of sustainable practices. Therefore, the demand forinputs for organic agriculture (biofertilizers and bioinoculants) is expected to rise. The implementation of sustainable practices in agriculture requires more labour, time, knowledge, and the encouragement from the governments through appropriate policies and advice from experts. Initially, the farmers could be doubtful, because of the possibly reduced yields and profits.

Companies, the competent Ministries, Universities and Institutes should continue to invest in research for the innovation, development and application of new biofertilizers/biopesticides. The farmers need to be educated and trained for the application of microbial products, such as biofertilizers and biopesticides, and should also be informed on their possible limiting factors. While commercialisation is important, traditional practices are key to sustainable agriculture and their role in safeguarding soil health, soil microbial diversity, and effective pests control needs to be acknowledged.

The study also found that farmers seek consultation on biofertilizers/biopesticides in both the public and the private sector. Equally, farmers and public agencies are responsible for the use of biofertilizers/biopesticides. 55.5% of the experts claim that there is no need for farmers' training or

education, as the products are easy to apply and can be implemented by a wide variety of equipment, while 44.5% believe farmers' training and education to be desirable. They claim that farmers have to be educated for using biofertilizers to convince them about the results and cost effectiveness when compared to the use of chemical fertilizers.

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REFERENCES

Adesemoye, A.O. and Kloepper, J.W., 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. *Appl Microbiol Biot* **85:** 1-12.

Adl, S.M., 2003. The Ecology of Soil Decomposition. CAB International, Wallingford, ISBN 0-85199-661-2, pp. 270-294.

Ahemad, M. and Khan, M.S., 2011. Functional Aspects of Plant Growth Promoting Rhizobacteria: Recent Advancements. *Insight Microbiology* **1:** 39-54.

Albores, S., Pianzzola, M.J., Soubes, M. and Cerdeiras, M.P., 2006. Biodegradation of agroindustial wastes by *Pleurotus* spp. For its use as ruminant feed. *Electron J Biotechn* **9:** 215-220.

Alonso Bocchini Martins, D., Ferreira Alves do Prado, H., Simoes Ribeiro Leite, R., Ferreira, H. and de Souza, M.M., 2011. Agroindustrial wastes as substrates for microbial enzymes production and source of sugar for bioethanol production. In: Integrated Waste Management, vol. 2, Chapter 17, (Ed.) Sunil Kumar, InTech, Croatia, ISBN 978-953-307-447-4, pp 319-360.

Al-Ouda A., 2010. The role of improved regional cultural practices in the implementaiton of conservation agriculture in Arab countries. *Options Mediterraneennes A* **96:** 107-116.

Anand, T. and Pereira, G.N., 2011. *Azolla* as a biofertilizer in coffee plantations http://www.ineedcoffee.com/06/azolla/.

Anderson, M.W. and Schrijver, I., 2010. Next Generation DNA Sequencing and the Future of Genomic Medicine. *Genes* **1**: 38-69.

Anderson, O.R., 2010a. The Reciprocal Relationships between High Latitude Climate Changes and the Ecology of Terrestrial Microbiota: Emerging Theories, Models, and Empirical Evidence, Especially Related to Global Warming. In: Gutierrez, B. and Pena, C. (Eds.), Tundras: Vegetation, Wildlife and Climate Trends. Nova Science Publ, New York, ISBN 978-160-876-588-1, pp. 47-79.

Antoun, H. and Prevost, D., 2005. Ecology of plant growth promoting rhizobacteria. In: Siddiqui, Z.A. (Ed.), PGPR: Biocontrol and Biofertilization**.** Springer, The Netherlands, ISBN 978-1-4020-4152-5, pp. 1-38.

Averhoff, B., Müller, V., 2010. Exploring research frontiers in microbiology: recent advances in halophilic and thermophilic extremophiles. *Res Microbiol* **161:** 506-514.

Babitha, S., 2009. Microbial pigments. In: Singh nee' Nigam, P. and Pandey, A. (Eds.), Biotechnology for Agroindustrial Residues Utilisation. Springer & Business Media B.V., ISBN 978-1-4020-9942-7, pp. 147-162.

Bacha, U., Nasir, M., Khaliqu,e A., Anjum, A.A. and Jabbar, M.A., 2011. Comprative assessment of various agroindustrial wastes for *Saccharomyces cerevisiae* biomass production and its quality evaluation as single cell protein. *JAPS* **21:** 844-849.

Balis, C., Chatzipavlidis, J., Flouri, F. 1996. Olive mill waste as a substrate for nitrogen fixation. *Int. Biodeter. Biodegr.* **38:** 169-178.

Barba, M., Van Den Bergh, I., Belisario, A. and Beed, F. 2010. The need for culture collections to support plant pathogen diagnostic networks. *Research in Microbiology* **161**:472-479.

Barclay, W.R., Meager, K.M. and Abril, J.R., 1994. Heterotrophic production of long chain omega-3-fatty acids utilizing algae and algae-like microorganisms. *J. Appl. Phycol.* **6:** 123-129.

Barth, J., Amlinger, F., Favoino, E., Siebert, S., Kehres, B., Gottschall, R., Bieker, M., Lobig, A. and Bidlingmaier, W., 2008. Compost production and use in the EU, Final Report, Organic

Recovery & Biological Treatment (ORBIT)/ European Compost Network (ECN), European Commission.

Bashan, Y., and de-Bashan, L.E., 2010. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth-a critical assessment. *Adv Agron* **108:** 77-136.

Bastida, F., Moreno, J.L., Nicolás, C., Hernández, T. and García, C., 2009. Soil metaproteomics: a review of an emerging environmental science. Significance, methodology and perspectives. *Eur J of Soil Sci* **60:** 845–859.

Bauer, J.T., Kleczewski, N.M., Bever, J.D., Clay, K. and Reynolds, H.L., 2012. Nitrogen-fixing bacteria, arbuscular mycorrhizal fungi, and the productivity and structure of prairie grassland communities *Oecologia* http://www.springerlink.com/content/x7tj4x271837t70w/fulltext.pdf.

Beed, F., Benedetti, A., Cardinali, G., Chakraborty, S., Dubois, T., Garrett K. and Halewood M., 2011. Climate change and microorganism genetic resources for food and agriculture: state of knowledge, risks and opportunities. *Background study paper* **no. 57**, FAO.

Bekku, Y.S., Nakatsubo, T., Kume, A., Adachi, M. and Koizumi, H., 2003. Effects of warming on the temperature dependence of soil respiration rate in Arctic, temperate and tropical soils. *Appl Soil Ecol* **22**: 205-210.

Bell, T. L., Rosenfeld, D., Kim, K.M., Yoo, J.M., Lee, M.I. and Hahnenberger, M., 2008. Midweek increase in U.S. summer rain and storm heights suggests air pollution invigorates rainstorms. *J. Geophys. Res.* **113**, D02209, doi:10.1029/2007JD008623.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. and Courchamp F. 2012. Impacts of climate change on the future of biodiversity. *Ecol. Lett.* **15**: 365–377.

Beloqui, A., Dominguez de Maria, P., Golyshin, P.N. & Ferrer, M. (2008). Recent trends in industrial microbiology. *Curr. Opin. Microbiol.* **11:** 240-248.

Ben Mansour, H., Dellai, A. & Ayed, Y. (2012). Toxicities effects of pharmaceutical, olive mill and textile wastewaters before and after degradation by *Pseudomonas putida* mt-2. *Cancer Cell Int.* **12:** 1-4.

Benndorf, D., Balcke, G.U., Harms, H. and von Bergen, M., 2007. Functional metaproteome analysis of protein extracts from contaminated soil and groundwater. *Isme J* **1**: 224-234.

Berdy, J., 2005. Bioactive microbial metabolites. *J Antibiot* **58:** 1-26.

Beyer, P., Al-Babili, S., Ye, X., Lucca, P., Schaub, P., Welsch, R., & Potrykus, I. (2002). Golden Rice: Introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *J. Nutr.* **132(3):** 506S-510S.

Blanco, A. V. R. 2004. Comprehensive environmental projects: linking adaptation to climate change, sustainable land use, biodiversity conservation and water management. ENDS

Blow, N., 2008. Metagenomics: exploring unseen communities. *Nature* **453:** 687-90.

Bond-Lamberty, B. and Thomson, A., 2010. Temperature- associated increases in the global soil respiration record. *Nature* **464:** 579–582.

Boraste, A., Vamsi, K.K., Jhadav, A., Khairnar, Y., Gupta, N., Trivedi, S., Patil, P., Gupta, G., Gupta, M., Mujapara, A.K. and Joshi, B., 2009. Biofertilizers: A novel tool for agriculture. *Int J Microbiol Res* **1:** 23-31.

Bowe, C., 2007. Potential answers to the adaptation to and mitigation of climate change through the adoption of underutilised crops. *Tropical Agriculture Association Newsletter* **27**: 9-13

Brandelli, A., 2008. Bacterial keratinases: useful enzymes for bioprocessing agroindustrial wastes and beyond. *Food Bioprocess Tech* **1:** 105-116.

Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W., Ball, C.A., Causton, H.C., Gaasterland, T., Glenisson P., Holstege, F.C., Kim, I.F., Markowitz, V., Matese, J.C., Parkinson, H., Robinson, A., Sarkans, U., Schulze-Kremer, S., Stewart, J., Taylor, R., Vilo, J. and Vingron, M. *2001*. Minimum information about a microarray experiment (MIAME)—toward standards for microarray data. *Nature Genetics* **29**: 365 - 371.

Bryan, E., Deressa, T. T., Gbetibouo, G. A. and Ringler,C. 2009. Adaptation to climate change in Ethiopia and South Africa: options and constraints. *Environmental Science and Policy*

Busse, M.D., 2009. Soil carbon sequestration and changes in fungal and bacterial biomass following incorporation of forest residues. *Soil Biol Biochem.* **41**: 220–227.

Chakraborty, S., Luck, J. and Hollaway, G., 2008. Impacts of globalchange on diseases of agricultural crops and forest trees. *Nutr Natl Resour* **3**: 1–15.

Chandler, S., Bailey, A.S., Tatchell, G.M., Davidson, G., Greaves, J., and Grant W.P., 2011. The development, regulation and use of biopesticides for integrated pest management. *Philos T Roy Soc B* **366:** 1987-1998.

Chandra, S., Ziemke, J. R. and Stewart R. W. 1999. An 11-year solar cycle in tropospheric ozone from TOMS measurements. *Geophys. Res. Lett*. **26**:185–188.

Chatterjee, K., Chatterjee, A. and Das, S. 2005. Case study 2: India community adaptation to drought in Rajasthan. *Ids Bulletin-Institute of Development Studies* **36**: 33-52

Chattopadhyay, S., Mukerji, A. and Sen, R., 2009. Biofuels. In: Singh nee' Nigam, P. and Pandey, A. (Eds.), Biotechnology for Agroindustrial Residues Utilisation, Springer & Business Media B.V., ISBN 978-1-4020-9942-7, pp. 62-76.

Chauhan Y.S., Wright G.C. and Rachaputi N.C. 2008. Modelling climatic risks of aflatoxin contamination in maize. *Australian Journal of Experimental Agriculture* **48**(3): 358–366

Chien, S-Y., Young, C-C., & Wang, C-L., 2007. Current status of biofertilizers development, farmers' acceptance and utilization, and future perspective in Taiwan. Food and Fertilizer Tecnology Center, http://www.agnet.org/htmlarea_file/library/20110804152212/eb603.pdf.

Chigwada, J. 2005. Case study 6: Zimbabwe -Climate proofing infrastructure and diversifying livelihoods in Zimbabwe. *Ids Bulletin-Institute of Development Studies* **36**:103-116.

Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol.* **26**: 126–131.

Cotty, P.J. and Jaime-Garcia R., 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination International. *J. Food Microbiol.* **119:**109-15.

Dahal, G., Hughes, J.d'A., Thottapilly, G. and Lockheart, B.L., 1998. Effect of temperature on symptom expression and reliability of banana streak badnavirus detection in naturally infected plantain and banana (*Musa* spp.). *Plant Dis* **82**: 16–21.

Dang, C. K., S Harrison, M. M., Sturt, P. S., Giller, M. A. and Jansen, K., 2009. Is the elemental composition of stream invertebrates a determinant of tolerance to organic pollution? *J N A Benthol Soc* **28(4):** 778–784.

Das, B.B. and Dkhar, M.S., 2011. Rhizosphere microbial populations and physico chemical properties as affected by organic and inorganic farming practices. *AEJAES* **10:** 140-150.

Dastager, S.G., 2009. Aroma compounds. In: Singh nee' Nigam, P. and Pandey, A. (Eds.), Biotechnology for Agroindustrial Residues Utilisation, Springer & Business Media B.V., ISBN 978-1-4020-9942-7, pp. 105-127.

Day-Rubenstein, K. and Frisvold, G., 2001. Genetic prospecting and biodiversity development

agreements. *Land Use Policy* **18:**205–219.

De Deyn, G.B., Biere, A., van der Putten, W.H., Wagenaar, R., Klironomos, J.N., 2009. Chemical defense, mycorrhizal colonization and growth responses in *Plantago lanceolata L*. *Oecologia* **160(3):**433-442.

Dean, F.B., Nelson, J.R., Giesler, T.L. and Lasken, R.S., 2001. Rapid amplification of plasmid and phage DNA using Phi29 DNA polymerase and multiply-primed rolling circle amplification. *Genome Res* **11**: 1095-1099.

Domon, B. and Aebersold, R., 2006. Mass spectrometry and protein analysis. *Science* **312:** 212-217.

Duarte, K.R., Freitas, A.C., Pereira, R., Pinheiro, J.C., Concalves, F., Azaari, H., El Azzouzi, M., Zrineh, A., Zaydoun, S., Duarte, A.C. and Rocha-Santos, T.A.P., 2012. Treatment of olive oil mill wastewater by silica-alginate-fungi biocomposites. *Water Air Soil Poll* **223:** 4307-4318.

Dunlop, M., Hilbert, D.W., Ferrier, S., House, A., Liedlof,f A., Prober, S.M., Smyth, A., Martin, T.G., Harwood, T., Williams, K.J., Fletcher, C., and Murphy H., 2012 The Implications of Climate Change for Biodiversity Conservation and the National Reserve System: Final Synthesis. A report prepared for the Department of Sustainability, Environment, Water, Population and Communities, and the Department of Climate Change and Energy Efficiency. CSIRO Climate Adaptation Flagship, ISBN 978-0-643-10954-4, Canberra.

Elshahed, M.S., 2010. Microbiological aspects of biofuel production: Current status and future directions. *J Adv Res* **1:** 103-111.

Environmental Indicator Report 2012, European Environment Agency, http://www.eea.europa.eu/publications/environmental-indicator-report-2012.

FAO, 2007. Adaptation to climate change in agriculture, forestry and fisheries: perspective, framework and priorities.

FAO, 2007. Profitability and Sustainability of Urban and Peri-urban Agriculture ftp://ftp.fao.org/docrep/fao/010/a1471e/a1471e00.pdf.

Fatah, L., 2007. The Potentials of Agro-Industry for Growth Promotion and Equality Improvement in Indonesia. *AJAD* **4:** 57-73.

Fierer, N., Bradford, M.A. and Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. *Ecology* **88**: 1354–1364.

FNCA (Forum for Nuclear Cooperation in Asia), 2006. Biofertilizer Manual, FNCA Biofertilizer Project Group, Coordinator Dr. Sueo Machi, Japan Atomic Industial Forum (JAIF), Japan. http://www.fnca.mext.go.jp/english/bf/bfm/e_bfm.html.

Frias-Lopez, J., Shi, Y., Tyson, G.W., Coleman, M.L., Schuster, S.C., Chisholm, S.W. and DeLong, E.F., 2008. Microbial community gene expression in ocean surface waters. *PNAS* **105**: 3805-3810.

Fuentes-Ramirez, L.E. and Caballero-Mellado, J., 2005. Bacterial Biofertilizers In: Siddiqui, Z.A. (Ed.), PGPR: Biocontrol and Biofertilization. Springer, Dordrecht, The Netherlands, ISBN 978-1-4020-4152-5, pp. 143-172.

Gajalakshmi, S., Ramasamy, E.V. and Abbasi, S.A., 2001. Potential of two epigeic and two anecic earthworm species in vermicomposting of water hyacinth. *Bioresource Technol* **76:** 177-181.

Gao, H., Yang, Z.K., Gentry, T.J., Wu, L., Schadt, C.W. and Zhou, J., 2007. Microarray-based analysis of microbial community RNAs by whole-community RNA amplification. *Appl Environ Microb* **73:** 563-71.

Garg, V.K. and Gupta, R., 2009. Vermicomposting of agroindustrial processing waste. In: Singh

nee' Nigam, P. and Pandey, A. (Eds.), Biotechnology for Agroindustrial Residues Utilisation. Springer & Business Media B.V., ISBN 978-1-4020-9942-7, pp. 431-456.

Gavrilescu, M., 2010. Environmental Biotechnology: Achievements, opportunities and challenges. *In: Dyn. Biochem. Process Botech. Mol. Biol.*, (Ed)Texeira da Silva, J.A., pp. 1-36, Global Science Books, Ltd.

Gentili, F. and Jumpponen, A., 2006. Potential and possible uses of bacterial and fungal biofertilizers. In: Rai, M.K. (Ed.), Handbook of Microbial Biofertilizers. The Haworth Press, Inc., New York, ISBN 978-1-56022-269-9, pp. 1-28.

Guo, D.L., Mitchell, R.J., Withington, J.M., Fan, P.P. and Hendricks, J.J., 2008a. Endogenous and exogenous controls of root lifespan, mortality and nitrogen flux in a longleaf pine forest: root branch order predominates. *J Ecol* **96**: 737–745.

Gupta, S. and Dikshit, A.K., 2010. Biopesticides: an ecofriendly approach for pest control. *J Biopesticides* **3 (1 special issue):** 186-188.

Hachicha, R., Rekik, O., Hachicha, S., Ferchichi, M., Woodward, S., Moncef, N., Gegarra, J. and Mechichi, T., 2012. Co-composting of spent coffee ground with olive mill wastewater sludge and poultry manure and effect of *Trametes versicolor* inoculation on the compost maturity. *Chemosphere* **88:** 677-682.

Handelsman, J., Rondon, M.R., Brady, S.F., Clardy, J. and Goodman, R.M., 1998. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chem Biol* **5**: R245-249.

He, F. and Hubbell, S.P., 2011. Species-area relationships always overestimate extinction rates from habitat loss. *Nature* **473**: 368-371.

Henry, A.W., 1932. Influence of soil temperature and soil sterilization on the reaction of wheat seedlings to *Ophiobolus graminis* Sacc. *Can J Res* **7**: 198–203.

Heffer, P. and Prud'homme, M. 2012. Fertilizer Outlook 2012-2016. International Fertilizer Industry Association (IFA) Paris – France (http://www.fertilizer.org/ifacontent/download/81563/1196065/version/2/file/2012_doha_ifa_summary.pdf).

Higa, T. and Parr, J.F., 1994. Beneficial and effective microorganism for a sustainable agriculture and environment. http://www.agriton.nl/higa.html.

Hoover, J.K. and Newman, J.A., 2004. Tritrophic interactions in the context of climate change: a model of grasses, cereal aphids and their parasitoids. *Glob. Change Biol.* 10:1197-1208.

Horrigan, L., Lawrence, R.S. and Walker, P., 2002. How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environ Health Persp* **110:** 445-456.

Hugenholtz, P. and Tyson, G.W., 2008. Microbiology: metagenomics. *Nature* **455**: 481-483.

Ineson, P., Benham, D.G., Poskitt, J., Harrison, A.F., Taylor, K. and Woods, C., 1998. Effects of climate change on nitrogen dynamics in upland soils. 2. A warming study. *Glob. Change Biol.***4**: 153–161.

IOC (International Olive Council), 2012. Olivae (English Edition), No. 112. Príncipe de Vergara, 154, E- 28002 Madrid, Spain, ISSN: 0255-996X.

IPNI, 2011. A summary of the status of biofertilizers, International Plant Nutrition Institute http://www.ipni.net/ipniweb/portal.nsf/0/94cfd5a0ed0843028525781c0065437e/$FILE/00%20IPNI%20Biofertilizers%20Report%20110125.pdf

Ishoey, T., Woyke, T., Stepanauskas, R., Novotny, M. and Lasken, R.S., 2008. Genomic sequencing of single microbial cells from environmental samples. *Curr Opin Microbiol* **11**:

198-204.

Israilides, C., Smith, A., Scanlon, B. and Barnett, C., 1999. Pullulan from agroindustrial wastes. *Biotechnol Genet Eng* **16:** 309-324.

Ivar, V., Bhagaran, M., Komen, J. Kullaya, A., Louwaars, N., Monis, E.J., Okori, P. and Persley, G. 2007. Agricultural Biotechnology and Small-Scale Farmers in Eastern and Southern Africa. Stockholm Environment Institute, Sweden.

Janssens, L., De Pooter, H. L., Schamp, N. M., and Vandamme, E. J. 1992. Production of flavours by microorganisms. *Process Biochem.* **27:** 195-215

Jang, Y.S., Park, J.M., Choi, S., Choi, Y.J., Seung, D.Y., Cho, J.H. and Lee, S.Y., 2012. Engineering of microorganisms for the production of biofuels and perspectives based on systems metabolic engineering approaches. *Biotechnol Adv* **30:** 989-1000.

Jee, H-J., 2009. Current status of biofertilizers and bio-pesticides development, farmer's acceptance and their utilization in Korea. http://www.agnet.org/library.php?func=view&id=20110712072318&type_id=4

Jin, C., Yao, M., Liu, H., Lee, C.F. and Ji, J., 2011. Progress in the production and application of n-butanol as a biofuel. *Renew Sust Energ Rev* **15:** 4080-4106.

Joyce, B.L. and Stewart Jr., C.N., 2012. Designing the perfect plant feedstock for biofuel production: Using the whole buffalo to diversify fuels and products. *Biotechnol Adv* **30:** 1011-1022.

Kaewchai, S., Soytong, K. and Hyde, K.D., 2009. Mycofungicides and fungal biofertilizers. *Fungal Divers* **38:** 25-50.

Kareiva, P., Daily G., Ricketts T., Tallis H. and Polasky S., 2011. Natural Capital, Theory and Practice of Mapping Ecosystem Services: Oxford University Press. ISBN 9780199589005, 432 pp.

Kaushik, P., Yadav, Y.K., Dilbaghi, N. and Garg, V.K., 2008. Enrichment of vermicompost prepared from cow dung spiked solid textile mill sludge using nitrogen fixing and phosphate solubilizing bacteria. *Environmentalist* **28:** 283-287.

Keasling, J.D. and Chou, H., 2008. Metabolic engineering delivers next-generation biofuels. *Nat Biotechnol* **26**: 298–299.

Kennedy, A.C. and Smith, K.L., 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil* **170:** 75-86.

Khalid, A., Srshad, M., Shaharoona, B. and Mahmood, T., 2009. Plant growth promoting rhizobacteria and sustainable agriculture. In: Khan, M.S., Zaidi, A. and Musarrat, J. (Eds.), Microbial Strategies for Crop Improvement. Springer-Verlag, Berlin Heidelberg, ISBN 978-3-642-01979-1, pp. 133-160.

Kimball, B.A. and Conley M.M., 2009. Infrared heater arrays for warming field plots scaled up to 5-m diameter. *Agr. Forest Meteorol.* **149:** 721–4.

Kimball, B.A., Conley, M.M. and Wang, S., 2008. Infrared heater arrays for warming ecosystem field plots. *Glob Change Biol* **14**: 309–320.

Klironomos, J.N., Allen, M. and Rillig, M.C., 2005. Abrupt rise in atmospheric CO_2 overestimates community response in a model plant–soil system. *Nature* **433**: 621–624.

Laconi, S., Molle, G., Cabiddu, A. & Pompei, R. (2007). Bioremediation of olive oil mill wastewater and production of microbial biomass. *Biodegradation* **18:** 559-566.

Lal, R., 2008. Carbon sequestration. *Phil Trans R Soc B Biol Sci* **363**: 815–830.

Lal, R., Follett, R.F., Stewart, B.A. and Kimble, J.M., 2007. Soil carbon sequestration to mitigate

climate change and advance food security. *Soil Sci.* **172:** 943-956.

Lerat, E., Daubin, V. and Moran, N.A., 2003. From gene trees to organismal phylogeny in prokaryotes: the case of the gamma-Proteobacteria. *PLoS Biol* **1**: E19.

Letourneau, D.K,, & S. G. Bothwell, 2008. Comparison of organic and conventional farms: challenging ecologists to make biodiversity functional. *Front. Ecol. Environ.* **6(8):** 430-438.

Levin, S.A., 2005. Self-organization and the emergence of complexity in ecological systems. *BioScience* **55:**1075-1079.

Liang, S., Liu, X., Chen, F. and Chen, Z., 2004. Current microalgal health food R & D activities in China. *Hydrobiologia* **512:** 45-48.

Lillis, L., Boots, B., Kenny, D.A., Petrie, K., Boland, T.M., Clipson, N. and Doyle, E.M., 2011. The effect of dietary concentrate and soya oil inclusion on microbial diversity in the rumen of cattle. *J Appl Microbiol* **111:** 1426-1435.

Liu, C-Z., Wang, F., Stiles, A.R. and Guo, C., 2012. Ionic liquids for biofuel production: opportunities and challenges. *Appl Energ* **92:** 405-414.

Lobell, D.B., Burke, M.B., Tebaldi, C., Mastrandrea, M.D., Falcon, W.P. and Naylor, R.L., 2008. Prioritizing climate change adaptation needs for food security in 2030. *Science* **319:**607-610

Lugtenberg, B. and Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* **63:** 541-556.

Luo, Y. and Zhou, X., 2006. Soil Respiration and the Environment. Academic Press/ Elsevier, San Diego, CA, USA, ISBN 9780120887828, p.328

Mahdi, S.S., Hassan, G.I., Samoon, S.A., Rather, H.A., Dar, S.A. and Zehra, B., 2010. Biofertilizers in organic agriculture. *J Phytol* **2:** 42-54.

Malusa, E., Sas-Paszt, L. and Ciesielska, J., 2012. Technologies for beneficial microorganisms inocula used as biofertilizers. *ScientificWorldJournal* **2012:** 1-12.

Mann, J., Markham, J.L., Peiris, P., Nair, N., Spooner-Hart, R.N. & Holford, P. (2010). Screening and selection of fungi for bioremediation of olive mill wastewater. *World J. Microbiol. Biotechnol.* **26**: 567-571.

Maron, P.A., Ranjard, L., Mougel, C. and Lemanceau, P., 2007. Metaproteomics: a new approach for studying functional microbial ecology. *Microb Ecol* **53**: 486-93.

McMahon, S.M., Harrison, S.P., Armbruster, W.S., Bartlein, P.J., Beale, C.M., Edwards, M.E., Kattge, J., Midgley, G., Morin, X. and Prentice, I.C. 2011. Improving assessment and modelling of climate change impacts on global terrestrial biodiversity. *Trends Ecol. Evol.* **26**: 249-259.

McNamara, C.J., Anastasiou, C.C., O' Flaherty, V. and Mitchell, R., 2008. Bioremediation of olive mill wastewater. *Int Biodeter Biodegr* **61:** 127-134.

McSweeney C. and Mackie R., 2012. Microorganisms and ruminant digestion: state of knowledge, trends and future prospects *Background Study Paper* **No.61** FAO p. 1-62.

Metzker, M.L., 2010. Sequencing technologies the next generation. *Nat Rev Genet* **11**: 31-46.

Miller, S.A., Beed, F. and Harmon, C.L. 2009. Plant disease diagnostic capabilities and networks. *Annu Rev Phytopathol* **47:**15–38.

Miller, S.R, Strong, A.L., Jones, K.L. and Ungerer, M.C., 2009. Bar-coded pyrosequencing reveals shared bacterial community properties along the temperature gradients of two alkaline hot springs in Yellowstone National Park. *Appl Environ Microb* **75**: 4565-4572.

Mohammed, R., Zhou, M., Koenig, K.M., Beauchemin, K.A. and Guan, L.L., 2011. Evaluation of rumen methanogendiversity in cattle fed diets containing dry corn distiller's grains and condensed tannins using PCR-DGGE and qRT-PCR analyses. *Anim Feed Sci Tech* **166:** 122-131.

Monson, R.K., 2006. Winter forest soil respiration controlled by climate and microbial community composition. *Nature* **439**: 711–714.

Moreno, R., Haro, A., Castellanos, A., Berenguer, J., 2005. High-level overproduction of His-tagged Tth DNA polymerase in *Thermus thermophillus*. *AppL. Environ. Microb.* **71:**591-593.

Mtui G.Y.S., 2011. Involvement of biotechnology in climate change adaptation and mitigation: Improving agricultural yield and food security. *Int.l J. Biotechnol. Mol.r Biol. Res.* **13:** 222-231

Muck, R.E., Filya, I., and Contreras-Govea, F.E. (2007). Inoculant effects on alfalfa silage: in vitro gas and volatile fatty acid production. *J Dairy Sci* **90:** 5115-5125.

Mussatto, S.I. and Teixeira, J.A. 2010. Lignocellulose as raw material in fermentation processes. In: Mendez-Vilas, A. (Ed.), Current research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. FORMATEX microbiology Series no. 2, vol. 2, Spain, ISBN 978-84-614-6195-0, pp. 897-907. http://www.formatex.info/microbiology2/897-907.pdf.

Mussatto, S.I., Ballesteros, L.F., Martins, S. and Texeira, J.A., 2012. Use of agroindustrial wastes in solid state fermentation processes. http://cdn.intechopen.com/pdfs/30860/InTech-Use_of_agro_industrial_wastes_in_solid_state_fermentation_processes.pdf.

Nakhro, N. and Dkhar, M.S., 2010. Impact of organic and inorganic fertilizers on microbial populations and biomass carbon in paddy field soil. *J Agron* **9:** 102-110.

Nakkeeran, S., Fernando, W.G.D. and Siddiqui, Z.A., 2005. Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and dideases. In: Siddiqui, Z.A. (Ed.), PGPR: Biocontrol and Biofertilization, Springer, Dordrecht, The Netherlands. ISBN 978-1-4020-4152-5, pp. 257-296.

Natarajan, B., 2002. Conservation of biological diversity and indigenous traditional knowledge as exemplified by the cases of Asia and Africa. *IAJIKS* **1:** 37-48.

Nesatyy, V.J. and Suter, M.J., 2007. Proteomics for the analysis of environmental stress responses in organisms. *Environ Sci Technol* **41**: 6891-6900.

Neue, H.U., 2007. Fluxes of methane from rice fields and potential for mitigation. *Soil Use Manag* **13**: 258–267.

Nielsen, M.N. and Winding, A., 2002: Microorganisms as Indicators of Soil Health. National Environmental Research Institute, Denmark. Technical Report No. 388

Nithya, G. and Lekeshmanaswamy, M. 2010. Production and utilization of vermicompost in agroindustry. *J Phytol* **2:** 68-72.

Nitschke, M., Ferraz C. and Pastore, G.M., 2004. Selection of microorganisms for biosurfactant production using agroindustrial wastes. *Braz J Microbiol* **35:**81-85.

Padmavathiamma, P.K., Li, L.Y. and Kumari U.R., 2008. An experimental study of vermi-biowaste composting for agricultural soil improvement. *Bioresource Technol* **99:** 1672-1681.

Paull, J., 2011. The uptake of organic agriculture: a decade of worldwide development. *J. Soc. Dev, Sci.* **2(3):** 111-120.

Peng, C.H., Apps, M.J. 2000. Simulating global soil-CO_2 flux and its response to climate change.

Environm Sci (China) **12**:257-265

Pereira, H.M., Leadley, P.W., Proenca, V., Alkemade, R., Scharlemann, J.P.W., Fernandez-Manjarres, J.F., Araujo, M,B., Balvanera, P., Biggs, R., Cheung, W.W.L., Chin,i L., Cooper, H.D., Gilman, E.L., Guenette, S., Hurtt, G.C., Huntington, H.P., Mace, G.M., Oberdorff, T., Revenga, C., Rodrigues, P., Scholes, R.J., Sumaila, U.R. and Walpole M. 2010. Scenarios for global biodiversity in the 21st Century. *Science* **330**: 1496-1501.

Perez-Piqueres, A., Edel-Hermann, V., Alabouvette, C. and Steinberg, C. 2006. Response of soil microbial communities to compost amendments. *Soil Biol Biochem* **38:** 460-470.

Perfecto, I. and Badgley, C. 2007. Can organic agriculture feed the world? *Pesticides News* **78:** 17-19.

Philippousis, A.N. 2009. Production of mushrooms using agroindustrial residues as substrates. In: Singh nee' Nigam, P. and Pandey, A. (Eds.), Biotechnology for Agroindustrial Residues Utilisation. Springer & Business Media B.V., ISBN 978-1-4020-9942-7, pp. 163-198.

Pindi P.K., 2012. Liquid Microbial Consortium- A Potential Tool for Sustainable Soil Health. *J Biofertil Biopestici* **3:**124. doi:10.4172/2155-6202.1000124

Podile, A.R. and Kishore, G.K. 2006. Plant growth-promoting rhizobacteria. In: Gnanamanickam, S.S. (Ed.), Plant-Associated Bacteria. Springer, The Netherlands, ISBN 978-1-4020-4538-7, pp. 195-230.

Poretsky, R.S., Bano, N., Buchan, A., LeCleir, G., Kleikemper, J., Pickering, M., Pate, W.M., Moran, M.A. and Hollibaugh, J.T., 2005. Analysis of microbial gene transcripts in environmental samples. *Appl Environ Microb* **71**: 4121-4126.

Popova, M., Martin, C., Eugène, M., Mialon, M.M., Doreau, M. and Morgavi, D.P., 2011. Effect of fibre- and starch-rich finishing diets on methanogenic Archaea diversity and activity in the rumen of feedlot bulls. *Anim Feed Sci Tech* **166:** 113-121.

Powlson, D.S., Whitmore, A.P. and Goulding, K.W.T., 2011. Soil carbon sequestration to mitigate climate change: A critical re-examination to identify the true and false. *Eur J Soil Sci* **62**: 42-55.

Pritchard, S.G. and Rogers, H.H., 2000. Spatial and temporal deployment of crop roots in CO_2 - enriched environments. *New Phytol* **147**: 55–71.

Pritchard, S.G. and Strand, A.E., 2008. Can you believe what you see? Reconciling minirhizotron and isotopic estimates of fine root longevity. *New Phytol* **177**: 285–287.

Pritchard, S.G., 2011. Soil organisms and global climate change. *Plant Pathol* **60**: 82–99.

Raes, J. and Bork, P., 2008. Molecular eco-systems biology: Towards an understanding of community function. *Nat Rev Microbiol* **6**: 693-699.

Raich, J. W., and Schlesinger, W. H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* **44B:** *81-99.*

Ram, R.J., Verberkmoes, N.C., Thelen, M.P., Tyson, G.W., Baker, B.J., Blake, R.C., Shah, M., Hettich, R.L. and Banfield, J.F., 2005. Community proteomics of a natural microbial biofilm. *Science* **308**: 1915-1920.

Rao, K. P. C., Verchot, L.V., Laarman, J. 2007. Adaptation to Climate Change through Sustainable Management and Development of Agroforestry Systems. *Journal of SAT agricultural research*. **4**: 1-30

Raposo, S., Pardao, J.M., Diaz, I. and Emilia Lima-Costa M. 2009. Kinetic medelling of bioethanol production using agro-industrial by-products *Int. J. Energy Environ.* **1(3):**1-8

Ravindra, A.P., 2000. Value-added food: single cell protein. *Biotechnol Adv* **18:** 459-479.

Reay. D.S., Dentener, F., Smith, P., Grace, J. and Feely, R., 2008. Global nitrogen deposition and carbon sinks. *Nat Geosci* **1**: 430–437.

Robles-Gonzalez, I.V., Fava, F. and Poggi-Varaldo, H.M. 2008. A review on slurry bioreactors for bioremediation of soils and sediments. *Microb Cell Fact* **7:** 5-17.

Roesch, L.F.W., Fulthorpe, R.R. and Riva, A., 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J* **1**: 283–290.

Ronaghi, M., 2001. Pyrosequencing sheds light on DNA sequencing. *Genome Res* **11**: 3-11.

Roy-Bolduc, A. and Hijri M., 2010. The use of mycorrhizae to enhance phosphorus uptake: a way out the phosphorus crisis. *J Biofertilizers & Biopesticides* **2:** 1-5.

Saharan, B.S. and Nehra, V., 2011. Plant growth promoting rhizobacteria: a critical review. *Life Sci Med Res* **2011:** 1-30.

Sangar S., 2011. Enabling poverty relevant biofertilizer innovation systems – Lessons from India. *Innovation and Sustainability Transitions in Asia, Kuala Lumpur, Malaysia* http://www.bioinnovationpolicies.ait.asia/sites/default/files/file/2_BIS_paperF_SunitaSangar_KL%20Paper.pdf

Sarma, S.J., Brar, S.K., Sydney, E.B., Le Bihari, Y., Buelna, G. and Soccol, C.R., 2012. Microbial hydrogen production by bioconversion of crude glycerol: a review. *Int J Hydrogen Energ* **37:** 6473-6490.

Schimel, J., Balser, T. C. and Wallenstein M. 2007. Microbial stress- response physiology and its implications for ecosystem function, *Ecology*. **88**: 1386–1394.

Schimel, D. S., 1995. Terrestrial ecosystems and the carbon cycle. *Glob. Change Biol.* **1:** 77-91.

Schlesinger, W. H. 1997. *Biogeochemistry*, 2nd ed., Academic Press, San Diego, Calif., ISBN 0-12-625155-X.

Searchinger, T., 2008. Use of US croplands for biofuels increases greenhouse gases through emissions from land-use change. *Science* **319**: 1238–1240.

Shen, K.P. and Harte, J., 2000. Ecosystem climate manipulations. In: Sala, O.E., Jackson, R.B., Mooney, H.A. and Howarth, R.W. (Eds.), Methods in Ecosystem Science. Springer, New York, ISBN 978-0-387-98734-7, pp. 353–369.

Shiferaw, B. A., Okello, J. and Reddy, R.V. 2007. Adoption and adaptation of natural resource management innovations in smallholder agriculture: reflections on key lessons and best practices. *Environ. Dev. Sustain* (2009) **11**:601–619.

Sidkey N.M., Al-Rahman Abo-Shadi, M.A., Al-Mutrafy, A.M., Sefergy, F. and Al-Reheily, N., 2010. Screening of microorganisms isolated from some enviro-agro-industial wastes in Saudi Arabia for amylase production. *J Am Sci* **6:** 926-939.

Simon, O., 2005. Microorganisms as feed additives-probiotics. *Adv Pork Proc* **16:** 161-167.

Singh nee' Nigam, P., 2009. Production of bioactive secondary metabolites. In: Singh nee' Nigam, P and Pandey, A. (Eds.), Biotechnology for Agroindustrial Residues Utilisation, Springer & Business Media B.V., pp. 129-145.

Singh nee' Nigam, P., 2009. Production of organic acids from agro-industial residues In: Singh nee' Nigam, P and Pandey, A. (Eds.), Biotechnology for Agroindustrial Residues Utilisation, Springer & Business Media B.V., ISBN 978-1-4020-9942-7, pp. 37-60.

Singh, B.K, Richard, D., Bardgett, R.D, Smith, P. and Reay D.S, 2010. Microorganisms and climatechange: terrestrial feedbacks and mitigation options. *Nat. Rev. Microb.* **8:** 779-790

Singh, J.S., Pandey, V.C. and Singh, D.P., 2011. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. *Agric Ecosyst Environ* **140:** 339-353.

Skoufias, E., Rabassa, M. and Olivieri, S., 2011. The Peverty Impacts of Climate Change, The

World Bank http://www-wds.worldbank.org/servlet/WDSContentServer/WDSP/IB/2011/04/04/000158349_20110404100435/Rendered/PDF/WPS5622.pdf.

Smith, P., 2004. Soils as carbon sinks: the global context. *Soil Use Manag*. **20**: 212–218.

Smith, P., 2008a. Greenhouse gas mitigation in agriculture. *Phil Trans R Soc B Biol Sci* **363**: 789–813.

Smith, P., 2008b. Land use change and soil organic carbon dynamics. *Nutr Cycl Agroecosyst* **81**: 169–178.

Solomon, S., 2007. Intergovernmental Panel on Climate Change. Climate Change 2007: the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (eds Solomon, S. et al.) Cambridge Univ. Press, Cambridge, UK, ISBN 978-0-521-70596-7.

Soomro, A.H., Me Sud, T. and Anwaar, K., 2002. Role of lactic acid bacteria (LAB) in food proservation and human health- a review. *Pakistan J Nutr* **1:** 20-24.

Staudinger, M. D., Grimm, N. B., Staudt, A., Carter, S L., Stuart Chapin III, F., Kareiva, P., Ruckelshaus, M. and Stein, B A., 2012. Impacts of Climate Change on Biodiversity, Ecosystems, and Ecosystem Services: Technical Input to the 2013 National Climate Assessment. *Cooperative Report to the 2013 National Climate Assessment*. 296 p.

Stephanopoulos, G., 2007. Challenges in engineering microbes for biofuels production. *Science* **315***:* 801–804.

Strobel, G.A., 2008. The production of myco-diesel hydrocarbons and their derivatives by the endophytic fungus *Gliocladium roseum* (NRRL 50072). *Microbiology* **154**: 3319–3328.

Suthar, S. and Singh, S., 2008. Vermicomposting of domestic waste by using two epigeic earthworms (*Perionyx excavatus* and *Perionyx sansibaricus*). *Int J Environ Sci Te* **5:** 99-106.

Tabacco, E., Piano, S., Revello-Chion, A., and Borreani, G. 2011. Effect of *Lactobacillus buchneri* ln4637 and *Lactobacillus buchneri* LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. *J. Dairy Sci.* **94:** 5589-5598.

Tafdrup, S., 1995, Viable energy production and waste recycling from anaerobic digestion of manure and other biomass materials. *Biomass Bioenerg* **9**: 303–314.

Tate, K.R., Ross, D.J., O'Brien, B.J. and Kelliher, F.M., 1993. Carbon storage and turnover, and respiratory activity, in the litter and soil of an old-growth southern beech (nothofagus) forest. *Soil Biol. Biochem.* vol. **25:** 1601-1612.

Themelis, N.J. and Villoa, P.A., 2007. Methane generation in landfills. *Ren Energy* **32**: 1243–1257.

Toberman, H., Evans, C,D., Freeman, C., Fenner, N., White, M., Emmett, B.A. and Artz, R.R.E., 2008. Summer drought effects upon soil and litter extracellular phenol oxidase activity and soluble carbon release in an upland Calluna heathland. *Soil Biol and Bioch* **40(6):** 1519-1532.

Townsend, A.R., Vitousek, P.M. and Holland, E.A., 1992 Tropical soils could dominate the shortterm carbon-cycle feedbacks to increased global temperatures. *Clim Change* **22:** 293–303.

Treasury, H.M., 2009. Green biotechnology and climate change. Euro Bio., p.12. Available online at http://www.docstoc.com/docs/15021072/Green-Biotechnology-and-Climate-Change

Tripathi, C.K.M., Tripathi, D., Praveen, N. and Bihari V., 2007. Microbial diversity-Bitoechnological and industrial perspectives. *Indian J Exp Biol* **45:** 326-332.

Tylianakis, J.M., Didham, R.K., Bascompte, J. and Wardle, D.A., 2008. Global change and species interactions in terrestrial ecosystems. *Ecol Lett* **11**: 1351–1363.

Uemura, S., Aitken, S.E., Korlach, J., Flusberg, B.A., Turner, S.W., Puglis, J.D., 2010. Real-time tRNA transit on single translating ribosomes at codon resolution. *Nature* **464**: 1012-1017.

Ugwuanyi, J.O., McNeil, B. and Harvey, L.M., 2009. Production of protein-enriched feed using agroindustrial residues as substrates. In: Singh-nee'-Nigam, P. and Pandey, A. (Eds.), Biotechnology for Agroindustrial Residues Utilisation, Springer & Business Media B.V., ISBN 978-1-4020-9942-7, pp. 77-103.

Vandamme, E.J., 2003. Bioflavours and fragrances via fungi and their enzymes. *Fungal Divers* **13:** 153-166.

Van-der-Heijden, M.G.A., Bardgett, R.D. and van-Straalen, N.M., 2008. The unseen mafority: soil microbes as diverse of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* **11:** 296-310.

Venkateswarlu, B. and Srinivasarao, Ch., 2005. Soil microbial diversity and the impact of agricultural practices ENVIS Newsletter 3(1) http://www.envismadrasuniv.org/oct_news.htm

Waldrop, M.P. and Firestone, M.K., 2006. Response of microbial community composition and function to soil climate change. *Microbial Ecology* **52:** 716–24.

Wall, M.E., Dyck, P.A. and Brettin, T.S., 2001. SVDMAN -- singular value decomposition analysis of microarray data. *Bioinformatics* **17:**566-68.

Wang, H.B., Zhang, Z.X., Li, H., He, H.B., Fang, C.X., Zhang, A.J., Li, Q.S., Chen, R.S., Guo, X.K., Lin, H.F., Wu, L.K., Lin, S., Chen, T., Lin, R.Y., Peng, X.X. and Lin, W.X., 2011. Characterization of metaproteomics in crop rhizospheric soil. *J Proteome Res* **10:**932-940.

Ward, M. and Singh-nee'-Nigam, P., 2009. Anaerobic treatment of solid agro-industial residues. In: Singh-nee'-Nigam, P. and Pandey, A. (Eds.), Biotechnology for Agroindustrial Residues Utilisation, Springer & Business Media B.V., ISBN 978-1-4020-9942-7, pp.417-430.

Wieczorek, A. (2003). Use of biotechnology in agriculture-Benefits and risks. *Biotechnol.* p.1-6. http://www.ctahr.hawaii.edu/oc/freepubs/pdf/BIO-3.pdf

Wilke, A., Ruckert, C., Bartels, D., Dondrup, M., Goesmann, A., Huser, A.T., Kespohl, S., Linke, B., Mahne, M., McHardy, A., Puhler, A. and Meyer, F., 2003. Bioinformatics support for high-throughput proteomics. *J Biotechnol* **106**: 147-156.

Wilmes, P. and Bond P.L., 2004. The application of two-dimensional polyacrylamide gel electrophoresis and downstream analyses to a mixed community of prokaryotic microorganisms. *Environ Microbiol* **6**: 911-920.

Wilmes, P. and Bond, P.L., 2006. Towards exposure of elusive metabolic mixed-culture processes: the application of metaproteomic analyses to activated sludge. *Water Sci Technol* **54**: 217-226.

Williams, M.L., 2007. UK air quality in 2050 – synergies with climate change policies. *Environmental Science Policy* **10**, 2: 169–175.

Wood, T.K., 2008. Molecular approaches in bioremediation. *Cur. Opin. Biotechnol.* **19(6):** 572-578.

WRI. World Resources 2008: Roots of Resilience-Growing the Wealth of the Poor. 2008. Washington, DC: WRI.

Wu, L., Wang, H., Zhang, Z., Lin, R., Zhang, Z. and Lin, W., 2011. Comparative metaproteomic analysis on consecutively Rehmannia glutinosa-monocultured rhizosphere soil. *PLoS One* **6:** e20611.

Xie, J., Hu, L., Tang, J., Wu, X., Li, N., Yuan, Y., Yang, H., Zhang, J., Luo, S., and Chen, X. 2011. Ecological mechanisms underlying the sustainability of the agricultural heritage rice-fish coculture system. *PNAS* **108(50):** 1381-1387.

Xinhua Agencies 2012. Cuba plans to increase biofertilizer production. Global Times http://www.globaltimes.cn/NEWS/tabid/99/ID/736059/Cuba-plans-to-increase-biofertilizer-production.aspx

Yagi, K., 1996. Effect of water management on methane emission from a Japanese rice paddy field: automated methane monitoring. *Glob Biogeochem Cy* **10**: 255–267.

Yokoyama, T., and Ohyama, T., 2009. Current status and future direction of commercial production and use of biofertilizers in Japan http://www.agnet.org/library.php?func=view&id=20110804151728&type_id=4.

Yoon, S., Hong, E., Kim, S., Lee, P., Kim, M., Yang, H., and Ryu, Y. (2012). Optimization of culture medium for enhanced production of exopolysaccharide from *Aureobasidium pullulans*. *Bioprocess Biosystems Engi.* **35:** 167-172.

GLOSSARY

Basidiomycetes: a large group of fungi including puffballs, shelf fungi, rusts, smuts, and mushrooms that bear sexually produced spores on a basidium.

Bioaugmentation is the process that is applied when microorganisms are imported to a contaminated site to enhance degradation.

Biofertilizers are preparations containing live or latent cells of efficient microbial strains used for application to seed or plant surfaces. The objective is to accelerate those microbial processes that augment the availability of nutrients that can be easily assimilated by plants.

Biological control is defined as the reduction of a pest population by natural enemies.

Biological diversity or "biodiversity" has been defined as the variability among living organisms from all sources including *inter alia,* terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part. This includes diversity within species, between species and of ecosystems.

Biopesticides are living organisms (viruses, bacteria and fungi) as well as more complex organisms such as protozoa, nematodes and beneficial insects able to protect crops against insect pests, fungal and bacterial diseases and weeds. Types of pesticides are insecticides (for insects), fungicides (for fungus or moulds), bactericides (for bacteria), nematocides (for nematodes), herbicides (for weeds or herbs), rodenticides (for rodents or rats) and acaricides (for mites).

Bioremediation can be defined as the natural or managed biological degradation of environmental pollution into less toxic forms, using organisms ranging from bacteria to plants, or their derivatives.

Biotechnology is any technique involving the application of living organisms or their components, systems or processes by manufacturing and service industries to make or modify products, to improve plants or animals or to develop microorganisms for special uses.

Compost is a soil amendment produced by a controlled decomposition process in which aerobic microorganisms degrade and transform organic material into a range of increasingly complex organic substances, some of which are referred to as humus.

Crop rotation is the practice of growing a series of dissimilar/different types of crops in the same area in sequential seasons.

Effective Microorganisms (EM) are natural microorganisms existing freely in nature, and, when applied as single strains or mixtures, may serve to improve the quality and fertility of soil as well as the growth and quality of crops.

Green manure crop is grown for a specific period and then ploughed under and incorporated into the soil.

Integrated pest management (IPM) is a broad based approach that integrates a range of practices for economic control of pests. IPM aims to suppress pest populations below the economic injury level (EIL).

Interplanting is the practice of planting a fast-growing crop between a slower-growing one.

Metagenomics is the analysis of genomic DNA obtained directly from a whole community of organisms inhabiting an environment.

Metatranscriptomics (environmental) retrieves and sequences environmental mRNAs from a microbial ecosystem to assess which genes may be expressed in that community.

Monocropping is the high-yield agricultural practice of growing a single crop year after year on the same land, in the absence of rotation through other crops.

Mycorrhiza is a symbiotic association between host plants and certain groups of fungi at the root system. The fungus benefits by obtaining carbon from the photosynthetic products of the host and the host in turn benefits by obtaining required but otherwise inaccessible nutrients, especially phosphorus, calcium, copper and zinc, with the help of the fungus. These fungi are associated with the majority of agricultural crops. Among the genera producing Arbuscular Mycorrhizal Fungi (AMF) with plants are *Glomus Sclerocystis*, *Gigaspora, Scutellospora, Acaulospora* and *Entrophospora.*

Nanotechnology is the engineering or modulating of matter at the nanometer (atomic, molecular and macromolecular) scale. By nanotechnology a functional device, system or structure with a novel characteristic is produced with at least one dimension sized between 1 and 100 nanometers.

Nitrogen fixers are microorganisms that convert atmospheric nitrogen into ammonia, using a complex enzyme system known as nitrogenase.

Organic agriculture is a production system that sustains agricultural production by avoiding or largely excluding synthetic fertilizers and pesticides.

Phytohormones participate in the control of many important physiological processes of plants such as cell enlargement, cell division, root initiation and growth rate. The effects on the plant could be direct, through plant growth promotion, or indirect, through improving plant nutrition via better development of the roots.

Plant Growth Promoting Fungi are rhizosphere fungi able to promote plant growth when colonising the plant root.

Plant Growth Promoting Rhizobacteria are free-living bacteria that colonise roots or rhizosphere soil some of which invade the tissues of plants and cause latent and asymptomatic infection, and affect plant growth and development directly or indirectly. Direct mechanisms include the production of stimulatory bacterial volatiles and phytohormones, lowering of the ethylene level in the plant (phytostimulators), improvement of the plant nutrient status (mobilise phosphates and micronutrients from insoluble sources, non-symbiotic nitrogen fixation), and stimulation of disease-resistance mechanisms (induced systemic resistance). Indirect mechanisms comprise the action of PGPRs like biocontrol agents, reducing diseases by the stimulation of other beneficial symbioses, or controlling diseases mainly by the production of antibiotics and antifungal metabolites (biopesticides), and, moreover, protecting the plants by degrading xenobiotics like organic pollutants in contaminated soils (rhizoremediators).

Pyrosequencing is a method of DNA sequencing based on the "sequencing by synthesis" principle. This method relies on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxynucleotides

Regional Climate Outlook Forums, active in several parts of the world, routinely provide real-time regional climate outlook products.

Solid State Fermentation (SSF) consists of the microbial growth and product formation on solid particles in the absence (or near absence) of free water.

Submerged fermentation (SmF) is characterised by the cultivation of microorganisms in a liquid medium.

Sustainability is the adoption of practices that allow for the longterm maintenance of the productive capacity, the viability and quality of life, and conservation of the environment and resource base.

Tillage refers to the turning of the soil to bury crop residues, manure and weeds.

Xenobiotics are chemical compounds with a structure or substituent on their structure that is not found in natural compounds.

ANNEX I

Uses of microorganisms in agro-industrial processes

Table I. Plant responses to inoculation with PGPR. Effect of PGPR on plant growth under various abiotic and biotic stress conditions. (Podile and Kishore, 2006; Khalid, *et al.*, 2009; Kaewchai, *et al.*, 2009; Boraste, *et al.*, 2009; Ahemad and Khan, 2011).

PGPR	Host-plant	Effect
***Azospirillum brasilense* Az1 and Az2, *Pseudomonas fluorescens* Pf**	*Oryza sativa*	The inoculation increased aerial and root biomass and grain yield
A. Brasilense, Pantoea dispersa	*Capsicum annuum*	Inoculation increased the concentration of citric, ascorbic and succinic acids in green fruit of sweet pepper
***Bacillus* sp.**	*Solanum tuberosum*	Inoculation caused increment in the growth of the plants
***P. fluorescens, B. subtilis, Sinorhizobium meliloti, Bradyrhizobium* sp.**	*Origanum majorana*	Only *P. fluorescens* and *Bradyrhizobium* sp. showed significant increases in shoot length, shoot weight, number of leaves and node, root dry weight, and essential oil yield
Phyllobacterium brassicacearum* STM196, *P. putida* UW4, *R. leguminosarum* bv. *viciae* 128C53K, *Mesorhizobium loti	*Arabidopsis thaliana*	Inoculated seedlings had significantly longer root hair
***Rhizobium tropici* CIAT899, *Paenibacillus polymyxa* DSM 36, *Rhizobium, P. polymyxa* strain Loutit, *Paenibacillus, Bacillus* sp.**	*Phaseolus vulgaris*	Coinoculation with *Rhizobium tropici* CIAT899 and *P. polymyxa* DSM 36 had higher leghemoglobin concentrations, nitrogenase activity and nitrogen fixation efficiency. Inoculation with *Rhizobium, P. polymyxa* strain Loutit, stimulated nodulation. PGPR also stimulated specific nodulation and increased accumulated N.
***Bacillus* strains**	*Capsicum annuum*	Stem diameter, root elongation, root dry weight, shoot dry weight and yield were increased in response to inoculation
***Pseudomonas* spp.**	*Triticum aestivum*	Inoculation increased growth, yield and nutrient use efficiency of wheat
***Serratia oderifera* J118, *Pantoea dispersa* J112, *Enterobacter gergoviae* J107**	*Cicer arietinum*	The PGPR in the presence of P-enriched compost resulted in a highly significant increase in fresh biomass, number of pods/plant, grain yield and number of nodules/plant
PGPR strains OSU-142, OSU-7, BA-8, and M-3	*Malus domestica*	Inoculation increased average shoot length and fruit yield and also shoot diameter
P. fluorescens, P. fluorescens* subgroup G strain 2, *P. marginalis, P. putida* subgroup B strain 1 and *P. syringae	*Lycopersicon esculentum*	*P. putida* was shown to improve fruit yields in rockwool and in organic medium. Roots of tomato seedlings grown in the presence of

strain 1		increasing concentrations of IAA were significantly longer when seeds were treated with *P. putida*
B. megaterium, B. subtilis, Pseudomonas corrugate	*Zea mays*	Inoculation resulted in an increment in grain yield
***B. subtilis* BEB-1Sbs (BS 13)**	*Lycopersicon esculentum*	Yield per plant, fruit weight and length were increased
Pseudomonas* sp., *Burkholderia caryophylli	*Triticum aestivum*	Positively influenced growth and yield of wheat
***B. pumilus* 8 N-4**	*Triticum aestivum*	Inoculation of wheat resulted in maximum increase in plant biomass, root length. And total N and P contents in plants
Cyanobacterial strains	*Oryza sativa*	Significant increases in grain and straw yield
***Pseudomonas* sp.**	*Zea mays, Vigna radiata*	Significant increases in plant height, root weight and total biomass. Similarly, inoculation significantly improved grain yield of maize in the presence of nitrogenous fertilizers, and positively affected nodulation of mung bean
Pseudomonas, Azotobacter, Azospirillum	*Triticum aestivum*	Significant positive effect of inoculation on germination and growth of wheat
A. Chroococcum, B. megaterium, B. mucilaginous	*Zea mays*	Inoculation significantly increased the plant growth, resulted in the highest biomass and seedling height, increased the nutritional assimilation of plant and improved soil properties
***Pseudomonas* spp.**	*Arachis hypogaea*	Significant high pod yield
***B. licheniformis* CECT 5160, *B. pumilus* CECT 5105**	*Quercus ilex* spp. *ballota*	Only *B. licheniformis* promoted the growth of *Quercus ilex* seedlings and inhibited fungal growth
PGPR isolates	*Triticum aestivum*	Stimulatory effects on grain yields
PGPR	*Quercus ilex* spp. *ballota*, *Pinus pinea*	Inoculated plants had increased stem length, neck diameter and shoot dry weight
Enterobacter cloacae, P. putida, P. fluorescens	*Brassica rapa*	Inoculation significantly enhanced root elongation of canola
Rhizobacteria	*Brassica juncea*	Increase in growth in the inoculated seedlings
***P. putida* Am2, *P. putida* Bm3, *Alcaligenes xylosoxidans* cm4, *Pseudomonas* sp. Dp2**	*Brasica juncea*	Significant increase in root elongation in response to inoculation
***P. putida* GR12-2 and an IAA-deficient mutant**	*Brassica rapa, Vigna radiata*	Primary roots of canola seeds treated were longer
***Arthrobacter mysorens* 7, *Flavobacterium* sp. L30, *Klebsiella mobilis* CIAM880**	*Hordeum vulgare*	Significantly stimulated root elongation
***B. licheniformis* CECT 5106, *B. pumilus* CECT 5105**	*Pinus pinea*	Promoted the growth of seedlings

Rhizobium, Azospirillum	*Oryza sativa*	Significant growth promoting effects on rice seedlings
***R. leguminosarum* E11**	*Oryza sativa*	Growth promoting effects of inoculation
Azotobacter	*Zea mays*	Significant growth promoting effects on maize seedlings
***P. putida* GR 12-2, GR12-2/acd36**	*Vigna radiata*	The wild type strain produced longer roots
***Pseudomonas mendocina* Palleroni**	*Lactuca sativa* cv. Tafalla	The inoculated plants had significantly greater shoot biomass, at low and high salinity levels
***Pseudomonas* spp.**	*Sorghum bicolor, Zea mays*	Inoculation improved fresh biomass under water deficient field conditions
***Pseudomonas* spp.**	*Pisum sativum*	The inoculation partially eliminated the effects of water stress on growth, yield and ripening
***P. putida* UW4**	*Brassica rapa*	Inoculation promoted the growth of canola in a saline environment
***P. fluorescens* TDK1**	*Apios americana*	Inoculation enhanced the saline resistance in the plants and increased yield
Unidentified PGPR	*Zea mays*	Significantly increased plant growth under salinity stress conditions
***Burkholderia phytofirmans* PsJN**	*Vitis vinifera*	Inoculation increased root growth and plantlet biomass and significantly improved plantlet cold tolerance
PGPR	*Solanum tuberosum*	PGPR were capable of antagonizing potato pathogens
***Pseudomonas* sp.**	*Pisum sativum*	Inoculation counteracted the Cd inhibition
PGPR	*Brassica napus*	Increases in root elongation, root dry weight, shoot dry weight in cadmium amended soil
***Variovorax paradoxus, Rhodococcus* sp.**	*Brassica juncea*	Plant growth was improved in Cd^{2+} supplemented media
***Variovorax paradoxus* 5C-2**	*Pisum sativum*	Inoculated plants gave more seed yield, seed number and seed nitrogen accumulation under moisture stress
P. fluorescens	*Chamaecytisus proliferus*	Positive effect in antagonizing of *fusarium oxysporum* and *Fusarium proliferatum*
***Burkholderia* sp.**	*Mimosa pudica*	Antagonistic activity against *Rhizoctonia solani* and *Sclerotinia sclerotiorum*
***Pseudomonas asplenii* AC**	*Phragmites austalis*	Normal plant growth under high levels of Cu^{2+} and creosote
Achromobacter piechaudii	*Lycopersicon esculentum*	Increased fresh and dry weights in the presence of NaCl and transient water stress
B. subtilis* BEB4, NEB5, *B. thuringiensis* NEB17, *Bradyrhizobium japonicum	*Glycine max*	Consistent and significant increase in nodule number, nodule weight, shoot weight, root weight, total nitrogen and grain yield at low root

		zone temperatures
Pseudomonas* sp., *Alcaligenes* sp., *Variovorax paradoxus, b. pumilus, Rhodococcus sp.	*Brassica juncea*	Stimulation of root elongation in the presence of Cd^{2+}
P. putida* UW4, *Enterobacter cloacae* CAL2, *P. putida	*Lycopersicon esculentum*	Inoculated plants showed substantial tolerance to flooding stress
Kluyvera ascorbata	*Brassica juncea*, *Lycopersicum esculentum*	Toxic effects of heavy metals (Ni^{2+}, Pb^{2+}, and Zn^{2+}) were not pronounced in inoculated plants
***P. putida* UW4**	*Cucumis sativus*	Effective biocontrol of *Pythium ultimum*
***Burkholderia phytofirmans* PsJN**	*Solanum tuberosum*	Potato plants maintained normal growth under heat stress
***Rhizobium* strains**	Pulses, groundnut, soybean, soil treatment for non legume crops including dry land crops	Yield increase
***Azotobacter* strains**	Non legumes like maize, barley, oats, sorghum, millet, sugarcane, rice etc.	Yield increase
***Azospirillum* strains**	Non legumes like maize, barley, oats, sorghum, millet, sugarcane, rice etc.	Yield increase
Phosphate solubilizers	Soil application for all crops	Yield increase
Cyanobacteria and *Azolla*	Rice/wet lands	Yield increase
Mycorrhizae (VAM)	Many trees, some crops and some ornamental plants	Yield increase
***Pseudomonas* sp.**	*Vigna radiate* (greengram)	Increased plant dry weight, nodule numbers, seed yield, seed protein, etc
Rhizobium	*Pisum sativum* (pea)	Inoculation increased the growth parameters at all tested concentrations of herbicides
***Rhizobium leguminosarum* MRP1**	*Pisum sativum* (pea)	Significant increase in the growth, symbiotic properties, amount of N and P in plant and organs, yield and seed protein
***Meshorhizobium* MRC4**	*Cicer arietinum* (chickpea)	Significantly increased symbiotic properties, root and shoot P and N, yield, seed protein
***Pseudomonas putida* R-168 & DSM 291, *Pseudomonas fluorescens* R-93 & DSM 50090, *Azospirillum lipoferum* DSM 1691 & DSM 1690**	*Zea mays* (maize)	Plant height, seed weight, number of seed per ear and leaf area, shoot dry weight significantly increased
Azotobacter chroococcum, Azospirillum	*Gossypium hirsutum*	Seed yield, plant height and microbial population

lipoferum	(cotton)	in soil increased
***Pseudomonas putida* CC-R2-4, *Bacillus subtilis* CC-pg104**	*Lactuca sativa*	Shoot and root length significantly increased
Azospirillum amazonense	*Oryza sativa* (rice)	Grain dry matter, number of panicles, grain N increased
***Pseudomonas* species**	*Oryza sativa* (rice), *Zea mays* (maize)	High ability to control bacterial and fungal root pathogens
***Azospirillum brasilense* Sp245**	*Phaseolus vulgaris* (common bean)	Root growth increased
***Pseudomonas fluorescens* PGPR1, PGPR2, PGPR4**	*Arachis hypogaea* (peanut)	Significantly enhanced pod yield, haulm yield and nodule dry weight
Azospirillum brasilense	*Oryza sativa* (rice)	Increased rice grain yield
***Bacillus pantothenticus, Pseudomonas pieketti, Bacillus* spp.**	*Hordeum vulgare* (barley)	Increased root and shoot weight
Bacillus subtilis	*Solanum lycopersicum* (tomato), *Abelmoschus esculentus* (okra), *Amaranthus* sp.(African spinach)	Dry biomass increased
Pseudomonas aeruginosa	*Solanum lycopersicum* (tomato), *Abelmoschus esculentus* (okra), *Amaranthus* sp. (African spinach)	Dry biomass increased
Unidentified PGPR, isolate	*Triticum aestivum* (wheat)	Increased root and shoot elongation, and root and shoot dry weight
***Bradyrhizobium* sp. (vigna) RM8**	*Vigna radiate* (greengram)	Enhanced the nodule number, leghaemoglobin, seed yield, grain protein, root and shoot N at Ni soil
***Mesorhizobium* sp. RC3**	*Cicer aerietinum* (chickpea)	Increased dry matter, number of nodules, seed yield, grain protein, N in roots and shoots at Cr soil
***Rhizobium* sp. RP5**	*Pisum sativum* (pea)	Enhanced dry matter, nodule numbers, root and shoot N, leghaemoglobin, seed yield, grain protein, at Ni soil
Delfia acidovorans	Canola	Commercially available, worldwide, promote plant growth
***Azotobacter* spp.**	Sunflower, tomato and other vegetable crops	Commercially available, worldwide, promote plant growth
***Bacillus* spp.**	Tomato, tobacco, cucumber and pepper	Commercially available, worldwide, promote plant growth
***Bacillus, Pseudomonas* and *Streptomyces* spp.**	Turfgrass, nursery and greenhouse planttions	Commercially available, worldwide, promote plant growth

***B. subtilis* GB03**	Fruits and vegetables	Commercially available, worldwide, promote plant growth
***Pseudomonas fluorescens* 92rk with AM *Glomus mosseae* BEG 12**	Tomato	Increased growth of plants
Pseudomonas striata*, with *Bradyrhizobium* sp. and AM *Glomus fasciculatum	Green gram	Increased N and P uptake
***Azospirillum* sp., *Pseudomonas striata* with *Rhizobium* sp.**	Pigeon pea	Increased nodule number and dry weight, plant height, dry weight and yield
Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, with *Bradyrhizobium japonicum	tomato	Increase growth when applied as soil drench
Bacillus* sp. CECT450 with *Rhizobium tropici	bean	Increased growth
Pseudomonas fluorescens* with *bradyrhizobium japonicum	soybean	Increased nodule number and nitrogen fixation in roots
Pseudomonas* spp. with *Rhizobium leguminosarum* biovar *viceae	pea	Increased shoot height, root length and dry weight
Pseudomonas fluorescens* DF57 with AM *Glomus intaradices* and *Glomus caledonium	cucumber	Increased P uptake
***Pseudomonas* sp. MRS 13 and MRS 16 with *Bradyrhizobium* sp. S24**	greengram	Increased nodule weight, plant dry weight and total N content
***Bacillus circulans* with *Glomus* sp.88**	wheat	Increased N and P uptake and grain and straw yields
Bacillus circulans* with *Glomus fasciculatum	mungbean	Increased N and P uptake
Settaria liquefaciens, Serratia proteamaculans* with *Bradyrhizobium japonicum	soybean	Increased grain yield and grain protein yield
Aeromonas hydrophila, Settaria liquefaciens, Serratia proteamaculans* with *Bradyrhizobium japonicum	soybean	Increased number of nodules and amount of nitrogen fixed in the rhizosphere
***Bacillus megaterium* var. *phosphaticum*, *Pseudomonas striata*, *Bacillus polymyxa*., *Penicillium* sp., *Aspergillus* sp. (phosphate solubilising bacteria PSB)**	Paddy, wheat, other cereals, vegetables, cotton, groundnut, mustard, sunflower, soybean, potato, onion, ginger, turmeric, sugarcane, mango, grapes, citrus, fruits, banana, coffee, cardamom and tea	Commercially available, worldwide, promote plant growth, increase uptake of phosphate by plants
***Azospirillum* sp.**	Flowers, various non-leguminous crops,	Commercially available, worldwide, promote plant growth

	cereals, paddy, sugarcane, plantation crops, vegetables	
***Frateuria aurantia* (Potash mobilizing bacteria)**	Various crops, rice, wheat, barley, oat, jowar, sugarcane, sugarbit, tobacco, cotton, jute, potato, brinjal, onion, tomato, cabbage, cauliflower, bhendi, sunflower, mustard, sesame, grapes, linseed, coffee, tea and other flowers and horticulture plants.	Commercially available, worldwide, promote plant growth. Helps in flowering and fruiting of crop plants, improves the quality of product
Rhizobium	Legumes, pulses (black, green, red gram, ground nut and soybean), groundnut, chickpea, pigeon pea, cowpea, peas, etc	Commercially available, worldwide, promote plant growth
Azotobacter chroococcum	Various non-leguminous crops, cereals, sugarcane and vegetables	Commercially available, worldwide, promote plant growth, better germination, root and shoot growth, increase productivity. Produce substances which check the growth of harmful plant pathogens such as *Alternaria, Fusarium, Rhizoctonia, Sclerotium* and *Helminthosporium*
Zinc activator	Various crops	Commercially available, worldwide, promote plant growth, increases crop yield, improve soil health
Acetobacter diazotrophicus	Sugarcane	Commercially available, worldwide, promote plant growth, nitrogen fixation
Pseudomonas fluorescens	All crops	Commercially available, worldwide, promote plant growth
Acetobacter,* phosphobacter and *Pseudomonas	Sugarcane	Commercially available, worldwide, promote plant growth
Azospirillum,* phosphobacter and *Pseudomonas	Paddy	Commercially available, worldwide, promote plant growth
Vesicular Arbuscular Mycrrhizae (VAM)	For all crops	Commercially available, worldwide, promote plant growth
Mycorrhizae spores	Grapes, citrus, melons, oaks and pines	Commercially available, worldwide, promote plant growth and nutrients uptake
Atmospheric nitrogen fixing microbes, phosphate solubilizing, iron, zinc and magnesium solubilizing microbes	Cereals, millets, forage crops, vegetables, wheat, paddy, sugarcane, cotton, banana, vine, mango, coffee, tea, cadramon,	Commercially available, worldwide, promote plant growth, improves growth, yield and quality

	rose, hibiscus, chrysanthemum, gerbera, petunia, daffodia, jasmine, sunflower, dahlia etc	
Azospirillum, Rhizobium, Azotobacter	Various crops, rice, sugarcane, orchards, vegetables, medicinal and aromatic plants	Commercially available, worldwide, promote plant growth
***Glomus fasciculatum* 81961, *G. mosseae, G. intraradices, G. dussii, G. clarum, G. deserticola, G. microaggregatum* Vesicular Arbuscular Mycorrhizae (VAM)**	Various plants, flowers, trees and shrubs	Commercially available, worldwide, promote plant growth. Efficient mobilization and uptake of fertilizers and other nutrients
***Bacillus coagulans* 81964 phosphate solubilizing bacteria**	Various plants	Commercially available, worldwide, promote plant growth
Ectomycorrhizal fungi (*Pisolithus tinctorius, Rhizopogon, Scleroderma, Laconia*)	Alder, arborvitae, arctostaphylos, aspen, basswood, beech, birch, chesnut, chinquapin, eucalyptus, fir, hazelnut, hemlock, hickory, larch, linden, madrone, oak, pecan, pine, poplar, spruce	Commercially available, worldwide, promote plant growth
***Bacillus subtilis* GB03**	Various plants	Commercially available, worldwide, promote plant growth, iron solubilization
Bacillus subtilis* GB03, *Bacillus subtilis, bacillus licheniformis, Bacillus megaterium, Azotobacter	Various plants	Commercially available, worldwide, promote plant growth
***Rhizobium* sp., *Azotobacter* sp.**	Legumes (bean, pea, lentil, chickpea), soybean, peanut, clover, alfalfa, carrot, beet, potato	Commercially available, worldwide, promote plant growth
***Azospirillum* sp., *Azotobacter* sp.**	Cereals, rice, vegetables, trees, vine, flowers and bushes	Commercially available, worldwide, promote plant growth
***Bacillus megaterium* vP, *Pseudomonas* sp.**	Cereals, rice, pasture (clover, alfalfa), legumes (bean, pea, lentil, chickpea),vegetables, trees, vine, flowers and bushes	Commercially available, worldwide, promote plant growth, phosphorus solubilization
Ectomycorrhizal fungi		Various commercial products, genera are not mentioned
Mycorrhizal fungi		Various commercial products, genera are not

		mentioned
Endomycorrhizal fungi		Various commercial products, genera are not mentioned
Trichoderma harzianum, Trichoderma hamatum		Commercial product
Mycorrhizal fungi, *Trichoderma* spp.		Commercial product
AM fungi (*Glomus intraradices*)		Commercial product
***Trichoderma* spp.**		Commercial product
Ectomycorrhizal fungi (*Pisolithus tinctorius*)		Commercial product

- ❖ Commercial products are produced by different companies under various names. The manufacturers often do not mention the scientific names of the microorganisms they use.
- ❖ The table has been completed with the help of commercial products from companies' websites and brochures.

Table II. Biocontrol agents for the control of plant pathogens (Rodgers, 1993; Gohel, *et al.*, 2006; Nakkeeran, *et al.*, 2005; El-Husseini, 2006; Berg, 2009; Kaewchai, *et al.*, 2009; Jee, 2009; Pandya and Saraf, 2010; Chandler, *et al.*, 2011).

Biological control agent	Target pathogens/disease	Plant
***Candida oleophila* I-182**	*Botrytis* spp., *Penicillium* spp.	Citrus, pome fruit (commercial product)
***Trichoderma* spp.**	Pathogenic fungi that cause wilt, take-all, root rot and internal decay of wood products and decay in tree wounds, wood infecting fungal pathogens of vineyard, orchard, ormanemtal trees and vines	Flowers, fruit, ornamentals, turf and vegetables (commercial product)
Fusarium oxysporum	*Fusarium oxysporum, Fusarium moniliforme*	Basil, carnation, cyclamen, tomato (commercial product)
***Trichoderma* spp.**	*Sclerotinia, Phytophthora, Rhizoctonia solani, Pythium* spp., *Fusarium, Verticillium, Botrytis cinerea*	Flowers, strawberries, trees, vegetables, horticulture, forestry (commercial product)
Trichoderma polysporum	Wood decay	Commercial product
Trichoderma viride	*Sclerotinia, Rhizoctonia*	Commercial product
Trichoderma sperellum	Many soil borne pathogens	Ornamentals, fruiting vegetables, leafy vegetables, cole crops, legumes, aromatic herbs, cucurbits, berries, small fruits and turf
Trichoderma gamsii	Many soil borne pathogens	Ornamentals, fruiting vegetables, leafy

		vegetables, cole crops, legumes, aromatic herbs, cucurbits, berries, small fruits and turf
Pseudomonas aureofaciens	Dollar spot, Anthracnose, *Pythium aphanidermatum*, Michrochium, patch (pink snow mold)	Turf and other crops (commercial product)
Pseudomonas syringae	*Botrytis cinerea*, *Penicillium* spp., *Mucor pyroformis*, *Goetrichum candidum*	Fruit, citrus, cherries, and potatoes (commercial product)
Pseudomonas fluorescens **A506**	Frost damage, *Erwinia amylovora* and russet inducing bacteria	Almond, apple, apricot, blueberry, cherry, peach, pear, potato, strawberry, tomato (commercial product)
Pseudomonas chlororaphis	Leaf stripe, net blotch, *Fusarium* sp., spot blotch, leaf spot, and others	Barley and oats, potential for wheat and other cereals (commercial product)
Pseudomona fluorescens	*Pseudomonas tolassii*	mushrooms
Bacillus subtilis **GB03, other** ***B. subtilis, B. licheniformis, B. megaterium***	*Rhizoctonia*, *Pythium*, *Fussarium* and *Phytophthora*	Greenhouse and nursery (commercial product)
Burkholderia cepacia **type Wisconsin**	*Rhizoctonia*, *Pythium*, *Fusarium* and disease caused by lesion, spiral, lance and sting nematodes	Alfalfa, barley, beans, clover, cotton, peas, grain sorghum, vegetable crops, and wheat (commercial product)
Ulcocladium oudemansii	*Botrytis cinerea*, *Sclerotinia sclerotiorum*	Fruit, vegetables, ornamentals
Myrothecium verrucaria	Parasitic nematodes	Cole crops, grape, ornamentals, turf, trees (commercial product)
Bacillus licheniformis **SB3086**	Dollar spot, low and moderate disease pressure	Turf (commercial product)
Fusarium oxysporum	*Fusarium oxysporum*	Asparagus, basil, carnation, cyclamen, gerbera, tomato (commercial product)
Agrobacterium radiobacter **K84**	Crown gall disease caused by *Agrobacterium tumefaciens*	Fruit, nut, and ornamental nursery stock (commercial product)
Bacillus subtilis **MBI600, rhizobia**	*Fusarium* spp., *Rhizoctonia* spp., *Aspergillus*	Soybean, alfalfa, dry/snap beans, peanuts (commercial product)
Burkholderia cepacia	*Rhizoctonia solani*, *Fusarium* spp., *Pythium* spp. *Phytophthora*, by lesion, spiral, lance, and sting nematodes	Vegetables, cotton, alfalfa, barley, beans, clover, peas, sorghum, wheat, maize (commercial product)
Bacillus subtilis **GB03**	*Rhizoctonia solani*, *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. that attack roots, *Pythium*, *Phytophthora*	Cotton, legumes, horticultural crops and turf (commercial product)
Coniothyrium minitans	*Sclerotinia sclerotiorum* and *S.*	Cucumber, lettuce, capsicum, tomato,

	minor	and ornamental flowers in greenhouse production (commercial product)
***Streptomyces griseoviridis* K61**	*Fusarium* spp., *Alternaria brassicola*, *Phomopsis* spp., *Pythium* spp., and *Phytophthora* spp. that cause seed, root and stem rot and wilt disease	Field, ornamental and vegetable crops, protected horticulture (commercial product)
***Agrobacterium radiobacter* K 1026**	*Agrobacterium tumefaciens*	Fruit and nut trees, caneberries, roses, and other ornamental nursery stock (commercial product)
Pythium oligandrum	*Pythium* spp., *Pythium ultimum Fusarium* spp., *Botrytis* spp., *Phytophthora* spp., *Aphanomyces* spp., *Alternaria* spp., *Tilletia caries*, *Pseudocercosporella herpotrichoides*, *Gaeumannomyces graminis*, *Rhizoctonia solani*, *Sclerotium cepivorum* soilborne pathogens that cause seed, root and stem rot, and wilt disease	Vegetables (tomatoes, potatoes, pepper, cucumbers, Brassicaceae vegetables), fruits (grapes, strawberries, citrus), legumes, cereals, canola, forest nurseries and ornamental plants, ornamental, vegetable, and tree crops (commercial product)
***Pseudomonas fluorescens* VO61**	*Pythium ultimum*	*Lotus corniculatus* (commercial product)
Pichia anomala	*Penicillium roquefortii*	Wheat, rye, barley and oats (commercial product)
***Bacillus subtilis* BACT-D**	*Pythium aphanidermatum*	Tomato, peanuts, cotton (commercial product)
***Paenibacillus* sp. 300**	*Fusarium oxysporum*	Cucumber (commercial product)
Chaetomium cupreum	*Fusarium oxysporum* f. sp. *lycopersici*, *Phytophthora parasitica*, *Colletotrichum gloeosporoides*, *Fusarium moniliform*, *Pyricularia oryzae*, *Sclerotium rolfsii*, *Drechsiera maydis*	Commercial product
Chaetomium globosum	*Phytophthora palmivora*	Commercial product
***Pseudomonas aureofaciences* AB254**	*Pythium* spp.	Sweet corn (commercial product)
***Pseudomonas aureofaciences* AB244**	*Pythium* spp.	Tomato (commercial product)
***Pseudomonas fluorescens* VO61**	*Rhizoctonia solani*	Rice (commercial product)
***Pseudomonas fluorescens* WCS358**	*Fusarium oxysporum* f. sp. *lini*	Flax (commercial product)
***Pseudomonas putida* BTP1**	*Pythium aphanidermatum*	Cucumber (commercial product)
Serratia plymuthica	*Pythium ultimum*	Cucumber (commercial product)
Bacillus brevis	*Fusarium udum*	Pigeonpea (commercial product)
Bacillus subtilis* and *Bacillus	Greenhouse pathogens	Tomato, cucumber, pepper, tobacco

amyloliquefaciens		(commercial product)
***Bacillus subtilis* FZB24**	*Rhizoctonia solani*, *Fusarium* spp., *Alternaria* spp., *Sclerotinia* and *Verticillium*	Greenhouses grown crops, forest tree seedlings, ornamentals, and shrubs (commercial product)
***Bacillus subtilis* QWT713**	Powdery mildew, downy mildew, cercospora leaf spot, early blight, late blight, brown rot, fire blight and others	Cucurbits, grapes, hops, vegetables, peanuts, pome fruits, stone fruits and others (commercial product)
***Bacillus subtilis* QST713**	Powdery mildew, sour rot, downy mildew, and early leaf spot, early blight, late blight, bacterial spot and walnut blight diseases	Cherries, cucurbits, grapes, leafy vegetables, peppers, potatoes, tomatoes and walnuts (commercial product)
***Bacillus subtilis* MB 1600**	*Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp.	Ornamental and vegetable crops (commercial product)
***Bacillus subtilis* Y 1336**	Powdery mildew	Strawberry, pepper, cucumber (registered)
***Bacillus subtilis* QST 713**	Gray mold, powdery mildew, bacterial leaf spot	Strawberry, cucumber, peach, tomato (registered)
***Bacillus subtilis* JKK 238**	Powdery mildew	Strawberry (registered)
***Bacillus subtilis* GB-0365**	*Phytophthora* blight, *Pythium* blight, gray mold	Turf grass, fig, tomato (registered)
***Bacillus subtilis* KBC 1010**	Gray mold	Cucumber (registered)
***Bacillus subtilis* DB 1501**	Brown leaf blight	Turf grass (registered)
Bacillus pumilus	*Rhizoctonia* and *Fusarium* which attack developing soybean roots	Soybean (commercial product)
***Bacillus pumilus* QST 2808**	Fungal pests such as molds, mildews, blights, rusts and to control Oak death	In nurseries, landscapes, oak trees and green house crops (commercial product)
***Bacillus subtilis* GB03 and chemical pesticides**	Seedling pathogen	Barley, beans, cotton, peanut, pea, rice, soybean (commercial product)
***Bacillus cereus* BPO1-*Bacillus cereus* UW85**	Used as growth regulator	Cotton (commercial product)
***Streptomyces colombiensis* WYE 20**	Gray mold, brown leaf blight, powdery mildew	Strawberry, turf grass, cucumber (registered)
***Streptomyces goshikiensis* WYE 325**	Sheath blight, large patch	Rice, turf grass (registered)
***Ampelomyces quisqualis* AG 94013**	Powdery mildew	Strawberry, cucumber (registered)
***Paenibacillus polymyxa* AC-1**	*Phytophthora* blight, powdery mildew	Pepper, cucumber (registered)
***Beauveria bassiana* 1**	Spider mite, white fly	Strawberry, tomato (registered)
***Pseudomonas putida* B E2**	*Verticillium dahliae* Kleb	Strawberry (commercial product)
***Pseudomonas syringae* ESC-100**	*Botrytis cinerea*, *Penicillium*	Pome fruit, citrus, cherries and potatoes

	spp., *Mucor pyroformin, Geotrichum candidum*	(commercial product)
Pseudomonas* sp. + *Azospirillum	Brown batch and dollar spot disease	Turf and other crops (commercial product)
Alcaligenes xylosoxydans	*Fusarium urdum*	Pigeonpea (commercial product)
Pseudomonas dispersa	*Fusarium urdum*	Pigeonpea (commercial product)
***Ampelomyces quisqualis* M-10**	Powdery mildew	Apples, cucurbits, grapes, ornamentals, strawberries and tomatoes (commercial product)
Phlebiopsis* (*Peniophora*) *gigantea	*Heterobasidium annosum*	Forestry (commercial product)
Pseudomonas chlororaphis	Leaf stripe, net blotch, *Fusarium* sp., sot blotch, leaf spot, etc	Barley and oats
***Pseudomonas chlororaphis* 63-28**	*Pythium* spp., *Rhizoctonia solani, Fusarium oxysporum*	Ornamentals and vegetables (commercial product)
***Trichoderma harzianum* T22**	*Pythium* spp., *Rhizoctonia solani, Fusarium* spp., fungi causing wilt, root rot	Corn, (field, sweet, silage), soybeans, potatoes, tomatoes, beans (green and dry), cabbage, cucumbers, cotton, peanuts, turf, trees, shrubs, other transplants and ornamental crops Commercial product
Trichoderma harzianum	*Phytophthora, Fusarium, Pythium* sp., *Cercospora, Colletotrichum, Alternaria, Ascochyta, Macrophomina, Myrothecium, Ralstonia, Rhizoctonia,Sclerotinia*	Commercial product
***Trichoderma harzianum* T-39**	fungal diseases e.g. *Botrytis cinerea, Colletotrichum, Monilinia laxa, Plasmospara viticola, Rhizopus stolonifer*	Commercial product
Trichoderma viride, Trichoderma harzianum	Seed and soil borne and foliar diseases	Many crops (rice, sugarcane, tobacco, groundnut, soybean, pepper, cardamom, turmeric ginger, coffee, tea, rubber, vegetables, and fruit crops. Commercial product
***Bacillus subtilis* QST713**	*Botrytis* spp.	Vegetables, soft fruit, herbs and ornamentals (commercial product)
Coniothyrium minitans	*Sclerotinia* spp.	Outdoor edible and non-edible crops and protected crops (commercial product)
***Gliocladium catenulatum* JI446**	*Pythium* spp., *Rhizoctonia solani, Botrytis* spp., *Didymella* spp.	Commercial product
***Gliocladium virens* GL21**	Several plant diseases damping-off and root pathogens, *Pythium* spp., *Rhizoctonia solani,*	Greenhouse, ornamental and food crops, snapbeans, zinnia, cabbage, tomato, cotton, corn (commercial product)

	Sclerotium rolfsii	
***Reynoutria sachalinensis* extract**	Powdery mildew, downy mildew, *Botrytis*, late blight, citrus canker	Protected ornamental and edible crops (commercial product)
***Candida oleophila* O**	Grey mold (*Botrytis cinerea*), blue mold (*Penicillium expansum*)	Apples, pears, post-harvest on apples
***Verticillium* WCS 850**	Dutch Elm disease	Elm trees
***Pantoea agglomerans* C9-1**	Fire blight (*Erwinia amylovora*)	Apples pears
***Pantoea agglomerans* E325**	Fire blight (*Erwinia amylovora*)	Apples pears
***Chonrostereum purpureum* HQ1**	herbicide	
Colletotrichum gloeosporoides	Weeds, Northern Jointvetch	Arable crops (rice, wheat, soya) (commercial product)
***Phytophthora palmivora* (herbicide)**	weeds, *Morenia orderata*	Citrus trees (commercial product)
***Pseudomonas* sp./chemical herbicides**	weeds	Arable crops (commercial product)
***Xanthomonas* sp.**	weeds	Turf (commercial product)
***Alternaria destruens* 059**	Weeds (herbicide) Dodder (*Cuscutta* spp.),	Agricultural fields, dry bogs and ornamental nurseries
***Chondrostereum purpureum* (herbicide)**	Cut stumps of hardwood trees and shrubs	Forestry (commercial product)
Virus type: GV	Pest insect: *Adoxophyes orana*	Commercial product
Virus type: GV	Pest insect: *Agrotis segetum*	Commercial product
Virus type: GV	Pest insect: *Cydia pomonella*	Commercial product
Virus type:NPV	Pest insect: *Mamestra brassicae*	Commercial product
Virus type:NPV	Pest insect: *Neodiprion sertifer*	Commercial product
Virus type:NPV	Pest insect: *Spodoptera exigua*	Commercial product
Virus type:NPV	Pest insect: *Spodoptera littoralis*	Commercial product
***Cydia pomonella* GV**	Codling moth	Apples and pears (commercial product)
***Bacillus popilliae* Dutky, *Bacillus lentomorbus* Dutky**	Japanese beetle larvae	
Bacillus thurigiensis* subsp. *kurstaki	Lepidopteran larvae, caterpillars	Vegetables, forestry, soft fruit, ornamentals and amenity vegetation (commercial product)
Bacillus thurigiensis* subsp. *israelensis	Dipteran larvae	Vegetables (commercial product)
Bacillus thurigiensis* subsp. *tenebrionis	Coleopteran larvae	Vegetables, forestry (commercial product)
Bacillus thuringiensis* subsp. *san diego	Coleopteran larvae	
***Bacillus thuringiensis* subsp. *kurstaki* strain EG 2348**	Lepidopteran larvae	Vegetables, forestry (commercial product)

***Bacillus thuringiensis* subsp. *kurstaki* strain EG 2424**	Lepidopteran/coleopteran larvae	Vegetables, forestry (commercial product)
***Bacillus thuringiensis* subsp. *kurstaki* strain EG 2371**	Lepidopteran larvae	Vegetables, forestry (commercial product)
***Bacillus thuringiensis* subsp. *aizawa* strain GC-91**	Lepidopteran larvae	Apiculture (commercial product)
Bacillus thuringiensis* subsp. *aizawa	Lepidopteran larvae	Apiculture (commercial product)
***Bacillus thuringiensis* Vip3Aa20**	Lepidopteran, armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), western bean cutworm (*Striacosta albicosta*), black cutworm (*Agrotis ipsilon*)	Event MIR162 maize.
***Bacillus thuringiensis* Cry2Ab2**	Lepidopteran, European corn borer, corn earworm, southwestern corn borer, fall armyworm, sugarcane borer	
***Bacillus thuringiensis* var. *aizawai* PS811, NB 200**	Lepidopteran larvae, European corn borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), fall armyworm (*Spodoptera frugiperda*), black cutworm (*Agrostis ipsilon*)	corn
***Bacillus thuringiensis* subsp. *aizawai* GB 413**	Diamond back moth, cabbage armyworm, cutworm, armyworm, common cabbage worm etc	Chinese cabbage, cucumber, leek, (10 vegetables) (registered)
Bacillus thuringiensis* var. *kurstaki	Diamond-back moth, common Chinese worm, armyworm, *Pyrausta panopealis*	Perilla, Chinese cabbage, wild vegetable (registered)
***Bacillus thuringiensis* subsp. *aizawai* GB 413**	Tobacco cutworm, diamond back moth, rice leaf roller, armyworm, etc.	Pepper, perilla, Chinese cabbage, cucumber, rice (registered)
Bacillus thuringiensis	Cabbage armyworm, diamond back moth, cutworm, armyworm common cabbage worm etc	Pumpkin, pear, Chinese cabbage, apple, leek, pine, onion, kale (registered)
***Paecilomyces fumosoroseus* DBB 2032**	Mite, white fly	Cucumber, strawberry (registered)
***Bacillus sphaericus* Neide**	Dipteran larvae	
***Clavibacter xyli* (Bt toxin)**		Maize (commercial product)
***Metarhizium anisopliae* (formerly *Entomophthora anisopliae*)**	Wide range of insects (200 species of insects and other arthropods), termites (*Reticulitermes* sp.), wheat grain beetle, *Anisoplia austriaca*,	Horticulture, sugar cane, coffee (commercial product)

	sugarbeet curculio *Cleonus punctiventris*, Japanese beetle, black vine weevil, mosquitoes, meadow spittlebug, leafminer, froghopper, *Tomaspis saccharina*, ticks, may also infect beneficial organisms	
***Pseudomonas fluorescens* (Bt toxin)**		Vegetables (commercial product)
Bacillus popilliae		Turf (commercial product)
Serratia entomophila		Turf (commercial product)
Verticillium lecanii		Protected horticulture (commercial product)
Verticillium lecani, Beauveria bassiana, Metarhizium anisopliae	Soft bodied insects pests like aphids, mealy bugs, whiteflies, thrips, scaleinsects, bollworms, (*Helicoverpa*, or *Spothptera*) of cotton, fruit bores of vegetable crops, white grub/root grubs and mango hoppers	Commercial product
***Paecilomyces fumosoroseus* PFR**	Insects in over 25 families, diamondback moth (*Plutella xyllostella*), Russian wheat aphid (*Diuraphis noxia*), silverleaf whitefly (*bemisia argentifolii*)	Greenhouses plants (commercial product)
***Heliothis zea* NPV**	caterpillars	Commercial product
***Cydia pomonella* GV (virus)**		Fruit trees (commercial product)
***Limantria dispar* NPV (virus)**		Forestry (commercial product)
***Neodiprion sertifer* NPV (virus)**		Forestry (commercial product)
Beauveria bassiana	Whitefly, silkworm, aphids, grasshoppers, termites, Colorado potato beetle, Mexican bean beetle, Japanese beetle, boll weevil, cereal leaf beetle, bark beetles, lygus bugs, chinch bug, fire ants, European corn borer, codling moth and Douglas fir tussock moth, natural enemies such as lady beetles are susceptible too	Protected edible and ornamental plant production (commercial product)
***Verticillium lecanii* (nematode pest)**		Soy (commercial product)
***Bacillus thuringiensis* (nematode pest)**		Arable crops (commercial product)
***Pasteuria usage* (nematicide)**	Sting nematode	Turf (commercial product)
***Paecilomyces lilacinus* (nematicide)**	Plant parasitic nematodes in soil	Vegetables, soft fruit, citrus, ornamentals, tobacco and turf

		(commercial product)
***Quillaja saponaria* (nematicide)**	Plant parasitic nematodes	Vineyards, orchard, field crops, ornamentals and turf (commercial product)
***Pasteuria usgae* BL1**	Sting nematode (*Belonolaimus longicaudatus*)	Variety of crops, turf
Bacillus firmus	nematodes	Fruit, vegetable, field crops, turf, ornamentals
***Paecilomyces lilacinus* 251**	nematodes	
A family of natural products derived from fermentation of actinomycetes *Saccharopolyspora spinosa*	Insect of *Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, Hymenoptera,* examples: *Lobesia botrana, Frankiniella occidentallis, Liriomyza* spp., *Spondoptera* spp., *Cydia pomonela, Lithocolletis blancardella, Cacopsylla pyri, Anarsia lineatella, Leptinotarsa decemlineata, Helicoverpa almigera, Bactrocera (Dacus) oleae, Ceratitis capitata, Anastrepha ludens, Rhagoletis pomonella, Rhagoletis cerasi, Anastrepha suspense, Bactrocera cucurbitae, Bactrocera dorsalis, Bactrocera tryoni, Anastrepha fraterculus, Rhagoletis completa*	Vine, vegetables (field, glasshouse), orchard, olive oil, potato, cotton (commercial product)
AMF (*Glomus fasciculatum, Gigaspora margarita, Glomus clarum, Glomus mosseae*)	Control soil borne pathogens such as species of *Aphanomyces, Cylindrocladium, Fusarium, Marophomina, Pythium, Rhizoctonia, Sclerotinium, Verticillium, Fusarium oxysporum* f.sp. *asparagi, Rhizoctonia solani, Gaeumannomyces graminis* var. *tritici*	Asparagus, cowpea, barley, and other crops
***Piriformospora indica* (endophyte)**	Reduced symptoms from *Blumeria graminis* f. sp. *tritici, Pseudocercosporella herpotrichoides, Fusarium culmorum*	Winter wheat, greenhouse cultures
***Erwinia amylovora* (produce the protein harpin)**	Elicits resistance to pathogens and insects and enhances plant growth	Wide range of plants (vegetables, flowers, turf, trees, landscape plants)

- ❖ Commercial products are manufactured by different companies with various names. The manufacturers often do not mention the scientific names of the microorganisms they use.
- ❖ The table has been completed wigth the help of commercial products from companies' websites and brochures.

Table III. Use of agro-industrial residues and by-products for the production of microbial metabolites (Singh nee' Nigam, 2009; Ugwuanyi *et al.,* 2009; Dastager, 2009; Babitha, 2009; Kosseva M.R., 2009; Janssens *et al.,* 1992; Mussatto *et. al.,* 2012; Raposo *et. al.,*2009; Jang *et. al.,* 2011).

Microorganism	Product	Agro-industrial waste
Rhizopus oryzae	Lactic acid	Oat cereal, sugar cane bagasse, sugar cane press-mud
Aspergillus niger GCMC-7	Citric acid	Molasses
Aspergillus niger	Citric acid	Sweet potato, pineapple waste, carrot-processing waste, okara, soy-residues, carob-pods, corn-cobs, cassava, cassava bagasse, sugarcane press mud, coffee husks, starch containing root kumara, amberlite (inert solid support), polyurethane (inert solid support)
Aspergillus foetidus	Citric acid	Pineapple waste
Mixed lactobacilli	Lactic acid	Wheat bran
Immobilised *Rhizopus oryzae*	Lactic acid	Starch
Aspergillus foetidus	Citric acid	Pineapple waste
Aspergillus niger	Lactic acid	Cassava,
Aspergillus niger, Aspergillus oryzae	Gallic acid	Gallo seeds-cover powder
Rhizopus sp.	Oxalic acid	Sweet potato
Rhizopus oryzae	Gallic acid	Myroballan seeds, gallo seeds cover
Aspergillus niger NRRL 567	Citric acid	Kiwifruit peel
Streptococcus thermophilus	Fumaric acid	Cassava
Lactobacillus helveticus	Lactic acid	Sweet sorghum
Lactobacillus casei	Lactic acid	Sugar cane press-mud
Lactobacillus paracasei	Lactic acid	Sugar cane press-mud
Lactobacillus delbrueckii	Lactic acid	Cassava bagasse, sugarcane bagasse
Rhizopus oryzae	Lactic acid	Carrot-processing waste
Aspergillus niger UV60, CFTR130, NRRL2001, NRRL2270, NRRL 328, NRRL 567, NRRL599, NRRL567, ATCC942, CFTRI30, ATCC1015, ACM4942, CBS733.88, LPB-21	Citric acid	Food wastes, wheat bran, apple-pomace, cassava residue, rice bran, de-oiled rice bran, sugar cane press-mud+wheat bran, grape pomace, kiwifruit peel, sugar cane, orange waste, beet molasses, sugar cane

		bagasse, coffee husk, carrot waste, okara (soy residue), pineapple waste, glucose (sugar cane bagasse), kumara (starch containing), cassava bagasse
Aspergillus oryzae	Oxalic acid	Wheat kernels
Aspergillus niger	Gluconic acid	Tea waste with sugarcane molasses
Saccharomyces cerevisiae, Lactobacillus spp., *Rhizopus oryzae, Rhizopus* spp., *Aspergillus niger, Aspergillus* spp., *Cephalosporium eichhorniae, Pleurotus* spp., *Lentinus* spp. *Brevibacterium divaricatum, Geotrichum fragrans*	Animal feed and food, protein enriched biomass, edible mushroom, protein enriched flour, glutamic acid, citric acid, volatile compounds	Cassava wastes (peels, slurry, bagasse, waste water,) cassava tubers, cassava starch, wastewater
Streptomyces, Pleurotus spp.	Protein enrichment, anti-nutrient removal, protein rich biomass	Coffee pulp, coffee husk, other coffee wastes
Microsphaeropsis sp., *Streptomyces cyaneus,* various Basidiomycetes fungi, *Coprinus fimetarius, Micromycetes, Phanerochaete chrysosporium, Pleurotus ostreatus, Thamnidium elegans,* cellulolytic bacteria, *Neurospora sitophila, Rhodotorula gracilis, Trametes* spp., *Ganoderma* spp., *Coriolus versicolor, Trichoderma* spp., *Lentinus edodes, Cellulomonas biazoteain*	Single cell oil, protein enriched straw/feed, single cell protein, mushroom, gamma linoleic acid, citric acid, vitamins, essential amino acids Medicinal fungus, feed	Wheat bran, straw, corn, stover, buckwheat, millet, sugar beet pulp, citrus waste, water hyacinth, mustard straw, bean straw, agave bagasse, agro residues, perennial grass
Rhizopus oligosporus, Candida utilis, Pleurotus ostreatus, Kloeckera apiculata, Penicillium funiculosum, Myrothecium verrucaria, Aspergillus niger, Saccharomyces spp.	Protein rich fungi and feed, single cell protein	Apple pomace, apple waste, apple pulp, grape waste, carob pod, pineapple waste
Saccharomyces cerevisiae, Aspergillus niger	Protein enriched feed	Cactus pear, cactus waste fibre
Candida utilis, Aspergillus niger, Trichoderma viride, Pleurotus sajor-caju, Pleurotus ostreatus, Trichoderma reesei, Saccharomyces cerevisiae, Aspergillus oryzae	Protein rich biomass/feed, protein rich mushrooms	Rice polishing, rice bran, straw, chaff, sago fibre, saw dust, paddy straw, lignocellulosic waste
Pleurotus spp.	Protein rich food/feed	Viticulture waste
Silage population	Protein enriched silage	Corn straw
Ceratocystis fimbriata	Fruit aroma	Various agro waste
Trichoderma reesei and *Trichoderma viride, Aspergillus niger,* white rot fungi, *Pleurotus* spp	Protein rich feed	Cane bagasse and residues, other cane wastes in solid and slurry
Pleurotus spp.	Protein enriched waste feed	Saw dust
Pleurotus spp.	Protein rich waste/feed/ single	Mango waste, date industry waste

	cell protein	
Trichoderma reesi, Trichoderma aureoviride	Protein enrichment	Sugar beet pulp
Various yeasts	Protein enrichment	Cashew waste
Sclerotium rolfsii, Trichoderma harzianum, Trichoderma longiobrachiatum, Trichoderma koninggi and *Aspergillus niger*	Protein enrichment, cellulose degration	Palm kernel cake
Candida utilis, Pichia stipitis, Kluyveromyces marxianus, Saccharomyces cerevisiae, indigenous microbes	Protein enriched waste, hydrolytic enzymes, single cell protein	Cabbage waste, Chinese cabbage
Pediococcus pentosaceus, Lactobacillus acidophilus	Butter flavour	Semisolid maize
Kluyveromyces marxianus	Fruity aroma	Cassava bagasse and giant palm bran
Ceratocystis fimbriata	Fruity aroma	Cassava bagasse, apple pomace, amaranth and soybean
Neurospora sp.	Fruity aroma	Pre-gelatinized rice
Zygosaccharomyces rouxii	HEMF (aroma of red salty rice miso, strong, sweet cake like)	Miso (fermented soybean paste)
Ceratocystis fimbriata	Pineapple aroma	Coffee husk
Bacillus subtilis	Pyrazine (green pepper/ green bean aroma)	Soybeans
Aspergillus oryzae	Volatile compounds (cheese aroma)	Rice koji
Rhizopus oryzae	Volatile compounds	Tropical agro-industrial substrates
Aspergillus oryzae, Aspergillus panasitus	Aflatoxin	Wheat, oat, rice, maize, peanuts
Bacillus subtilis	Antifungal volatiles	Impregnated loam based compost
Bacillus thuringiensis,	Bacterial endotoxins (insecticide)	Coconut waste
Cephalosporium	Cephalosporin	Barley
Streptomyces clavuligerus	Cephalosporin C	Wheat straw with cotton seed cake and sunflower cake
Streptomyces clavuligerus	Clavulanic acid	Wheat straw with cotton seed cake and sunflower cake
Tolypocladium infautum	Cyclosporin A	Wheat bran
Metarhizium anisopliae	Destrucxins A and B	Rice, rice bran, rice husk
Claviceps purpurea, Claviceps fusiformis	Ergot alkaloids	Sugarcane bagasse

Gibberella fujikuroi, Fusarium moniliforme,	Gibberellic acid	Wheat bran, corn cob, cassava flour, sugarcane baggase
Bacillus subtilis	iturin	Okara, wheat bran
Penicillium brevicompactum	mycophenolic	Wheat bran
Aspergillus oryzae, Aspergillus panasitus	ochratoxin	Wheat, oat, rice, maize, peanuts
Streptomyces rimosus	Oxytetracycline	Corn cob
Penicillium chrysogenum	Penicillin	Sugarcane bagasse
Bacillus subtilis	Surfactin	Soybean residue
Streptomyces viridifaciens	Tetracycline	Sweet potato residue
Ustilaginoidea virens	Ustiloxins	Rice panicles
Fusarium moniliforme	Zeralenone	corn
Janthinobacterium lividum (bacterium)	Bluish purple	
Achromobacter (bacterium)	Creamy	
Bacillus sp. (bacterium)	Brown	
Brevibacterium sp. (bacterium)	Orange, yellow	
Corynebacterium michigannise (bacteria)	Greyish to creamish	
Pseudomonas sp. (bacterium)	Yellow	
Rhodococcus maris (bacterium)	Bluish red	
Streptomyces sp. (bacterium)	Yellow, red, blue	
Serratia sp. (bacterium)	Red	
Aspergillus sp. (fungi)	Orange, red	
Blakeslea trispora (fungi)	Cream	
Monascus purpureus (fungi)	Yellow, orange, red	
Helminthosporium catenarium (fungi)	Red	
Helminthosporium gramineum (fungi)	Red	
Helminthosporium cynodontis (fungi)	Bronze	
Helminthosporium avenae (fungi)	Bronze	
Penicillium cyclopium (fungi)	Orange	
Penicillium nalgeovensis (fungi)	Yellow	
Rhodotorula sp. (yeast)	Red	
Yarrowia lipolytica (yeast)	Brown	
Cryptococcus sp. (yeast)	Red	
Phaffi rhodozyma (yeast)	Red	

Dunaliella salina (algae)	Red	
Aspergillus niger	Cellulose, β-glucosidase, cellulose, glucoamylase	Bagasse, sawdust, corn cobs, wheat bran
Aspergillus phoenicis	β- glucosidase	Sugarbeet pulp
Aspirgillus niger, Aspergillus oryzae,	α-galactosidase	Wheat bran, soybean cake waste
Candida sp., *Neurospora sitophila, Penicillium candidum, Mucor* sp., *Kluyveromyces lactis*	b-galactosidase	Wheat bran, soybean cake waste
Aspergillus flavus	Protease	Wheat bran
Bacillus licheniformin	Proteases	Rice straw
Penicillium sp.	Proteases	Defatted soybean cake
Aspergillus carbonarius	Pectinase	Wheat bran
Pleurotus sp., *Phanerochaete chrysosporium*	Ligninase	Wheat straw and bagasse
Aspergillus niger	Tannase	Palm kernel cake
Aspergillus ficuum, Aspergillus carbonarius	Phytase	Wheat bran
Aspergillus niveus	Catalase	Wheat bran
Trichoderma viride and *Aspergillus niger*	Cellulose and amylase	Sugarbeet pulp
Trichoderma spp.	Cellusose, β-glucosidase	Wheat bran and rice straw
Aspergillus ustus, Botrytis spp., *Sporotrichum pulverulentum*	Xylanase	Wheat bran and rice straw
Thermoascus aurantiacus, Penicillium decumbens	Xylanase	Corn silage, Corn straw
Penicillium spp., *Geotrichum candidum, Mucor meihei, Rhizopus* spp.	Lipase	Wheat bran
Candida rugosa,	Lipases	Rice bran, wheat bran, peanut
Candida sp., *Monascus fulginosus, Neurospora sitophila, Aspergillus niger*	Lipases	Press cake and coconut oil cake
Penicillium capsulatum	Enzymes	Sugarbeet pulp
Penicillium charlesii, Talaromyces flavus, Tubercularia vulgaris	Pectic enzymes	Citrus pulp-pellets
Tubercularia vulgaris	Pectic enzymes	Citrus pulp
Polyporous spp.	Cellulose and ligninase	Bagasse
Bacillus subtilis	Cellulases	Banana fruit stalk
Aspergillus ustus, Sporotrichum pulvenulentum, Trichoderma sp.,	Cellulases	Wheat bran, rice straw

Botrytis sp.		
Trichoderma aureoviride, Trichoderma reesi, Trichoderma viride	Cellulases	Leached beet pulp, wheat sorghum sillage, coconut pith
Penicillium citrinum	Celllulases	Rice husks
Bacillus subtilis	Cellulases	Banana fruit stalk
Lentinula edodus	Enzymes	Lignocellulosic
Bacillus licheniformis	α- amylase	Wheat bran
Aspergillus sp., *Rhizopus* sp., *Mucor* sp., *Bacillus* sp., *Saccharomyces* sp., *Bacillus subtilis, Aeromonas caviae*	α-amylase, β-amylase	Rice husk, coconut cake, tea waste, cassava, cassava bagasse, sugarcane bagasse, banana waste, corn flour
Bacillus subtilis	Protease	Wheat bran
Neurospora crasse	Carbomethyl cellulose, β-glucosidase	straw
Thamnidium elegans	γ-linolenic acid	Spent malt grains, apple pomace
Rhizopus (4 strains)	Volatile carbons as flavors	Cassava bagasse, apple pomace
Ceratocystis fimbriata	Banana flavour and fruity complex flavors	Cassava bagasse, wheat bran and sugarcane bagasse
Microbial consortium	Pectin substrate, liquid biofuel	Citrus, apple, sugar beet pomace
Trichoderma viride, Rhizopus	Extracellular enzymes	Cranberry pomace
Moniliella suaveolens, Trichoderma harzianum, Pityrosporum ovale, Ceratocystis moniliformis	d- and c-decalactone	Linseed cake, Castrol oil cake, olive press cake, sunflower cake
Trichoderma sp., *Candida utilis, Saccharomyces cerevisiae*	Crude protein for animal fodder	Olive pomace
Aspergillus foetidus	Pharmaceuticals, food industry, preserving agent	Pineapple waste
Two microorganisms	Flavour vanillin	Sugar beet pulp, cereal bran
Trichoderma reesei, Sporotrichum sp.	Enhance lignin and protein content	Tomato pomace
Aspergillus foetidus	β-glucosidase production	Apple pomace
Candida utilis	Lignocellulolytic enzymes	Apple pomace
Aspergillus niger	Pectin methylesterase	Apple pomace
Polyporus squamosus	Pectinases	Apple pomace
Aspergillus niger	Pectolytic enzymes	Apple pomace
Lentinus edodes	Polygalacturonase	Apple pomace
Rhizopus sp., *Rhizopus oryzae*	Aroma compounds	Apple pomace
Kluyveromycesmarxianus	Aroma compounds	Apple pomace
Ceratocystis fimbriata	Fruity aroma	Apple pomace

Trichoderma viride, T. harzianum, T. pseudokoningii	Phenolic compounds	Apple pomace
Gongronella butleri	Animal feed	Apple pomace
Candida utilis, Kloeckera sp.	Nutritional enrichment	Apple pomace
Rhizopus oligosporus	Protein enrichment	Apple pomace
Gongronella butleri	Chitosan	Apple pomace
Beijerinckia indica	Heteropolysaccharide	Apple pomace
Xanthomonas campestris	Xanthan	Apple pomace
Aspergillus niger	Citric acid	Apple pomace
Saccharomyces cerevisiae	Ethanol	Apple pomace
Thamnidium elegans, Mortierella isabelina, Cunninghamella elegans	γ-linolenic acid	Apple pomace
Gibberella fujikuroi, Fusarium moniliforme	Gibberellic acid / plant growth hormone	Corn cob, sugarcane bagasse, cassava flour,
Bacillus subtilis	Antifungal compounds	Impregnated loam based compost
Bacillus thuringiensis	Bacterial endotoxins/insecticide	Coconut waste
Penicillium chrysogenum	Penicillin	Sugarcane bagasse
Streptomyces rimosus	oxytetracycline	Corn cob
Streptomyces viridifaciens	tetracycline	Sweet potato waste
Monascus purfureus	Pigments	Sugarcane bagasse
Rhizopus oligosporus	Phenolic antioxidant compound	Pineapple waste, cranberry pomace, guava, soy flour
Streptomyces sp.	Polyphenols, tannins, chlorogenic acids	Coffee pulp waste
Bacillus subtilis	Surfactin/antibiotic	Soybean waste Okara
Agaricus bisporus	Mushroom like odour	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Ascoidea hylecoeti	Fruity, rose-like odour	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Aspergillus oryzae	Mushroom aroma	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Bjerkandera adusta	Vanilla like odour	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Boletus edulis	Dried mushroom	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Ceratocystis coerulescens, C. fimbriata, C. populina	Fruity odour	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Ceratocystis moniliformis	Banana, pear, rose-like, peach	*de novo* produced flavour (fermentation of

	odour	simple nutrients) by **fungi**
Ceratocystis variospora	Geranium like	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Ceratocystis variospora	Rose like, fruity	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Chaetomium globosum	Earthy	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Cladosporium cladosporoides	Fruity	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Cystostereum murraii	Vanilla, coconut flakes	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Fusarium poae	Fruity, peach	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Geotrichum sp.	Apple	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Geotrichum candidum	Fruity, melon	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Geotrichum penicillatum, Trichosporon penicillatum, Geophyllum odoratum	Fruity	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Hypomyces odoratus	Camphor like	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Inocybe coridalina, Inocybe pyriodora	Fruity, rose like	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Lentinellus cochleatus	Anise like	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Lentinus lepideus	Fruity, aromatic, anise, cedar wood	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Lepista irina	Iris oil, orange blossoms,	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Leptographium lundbergii	Fruity, sweet	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Monilia fruticola	Peach	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Mycoacia uda	Fruity	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Neurospora sp.	Fruity	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Oospora suaveolens	Fruity	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Penicillium decumbens	Soap perfume, pine like, rose like, apple, mushroom	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Phellinus igniarius, P. laevigatus, P.	Sweet, fruity, green, rose like	*de novo* produced flavour (fermentation of

tremulae		simple nutrients) by **fungi**
Pleurotus euosmus	Sweet, floral	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Polyporus durus	Coconut, pine apple	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Poria aurea	Sweet	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Trametes odorat, Gloeophyllum odoratum,	Rose like, anise like	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Trametes suaveolens	Anise like	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Trichoderma koningii, T. reesei, T. viride	Coconut like	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Tyromyces sumbuceus	Peach, passion fruit	*de novo* produced flavour (fermentation of simple nutrients) by **fungi**
Dipodascus magnusii , Pityrosporum sp.	Apple, fruity	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Dipodascus sp.	Apple, pine apple	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Hansenula anomala	Fruity flora	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Hansenula mrakii	Fruity banana	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Hansenula saturnus, Pichia farinosa	Rose like	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Kluyveromyces lactis	Fruity, rose like, faintly floral	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Pityrosporum sp.	Fruity, peach	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Saccharomyces cerevisiae	Sake yeast	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Sporobolomyces odorus	Peach flavour	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Zygosaccharomyces rouxii, Saccharomyces cerevisiae, Candida sp.	Intense sweet flavour	*de novo* produced flavour (fermentation of simple nutrients) by **yeasts**
Corynebacterium sp., *Bacillus*	Roasted flavours	*de novo* produced flavour (fermentation of simple nutrients) by **bacteria**
Erwinia carotovora	Banana	*de novo* produced flavour (fermentation of simple nutrients) by **bacteria**
Lactobacillus maltaromicus	Malty	*de novo* produced flavour (fermentation of

		simple nutrients) by **bacteria**
Pseudomonas aeruginosa	Grape like, sweet, aromatic, jasmine like	*de novo* produced flavour (fermentation of simple nutrients) by **bacteria**
Pseudomonas fragi	Pine apple flavour, fruity, strawberry like	*de novo* produced flavour (fermentation of simple nutrients) by **bacteria**
Pseudomonas perolens, Pseudomonas taetrolens	Musty, potato like	*de novo* produced flavour (fermentation of simple nutrients) by **bacteria**
Streptomyces odorifer	Earthy, musty	*de novo* produced flavour (fermentation of simple nutrients) by **bacteria**
Saccharomyces cerevisiae	Ethanol (biofuel)	Beet molasses, carob pod extract, citrus waste pulp, mahula, water hyacinth, water lettuce,
Saccharomyces cerevisiae, Aspergillus niger	Ethanol (biofuel)	Potato starch
Zymomonas mobilis, Candida tropicalis	Ethanol (biofuel)	Fruit and vegetable residues, Agro industrial waste (thippi)
Zymomonas mobilis	Ethanol (biofuel)	Agro industrial waste (thippi), sugar cane molasses
Candida tropicalis	Ethanol (biofuel)	Agro industrial waste (thippi)
Kluyveromyces marxianus	Ethanol (biofuel)	Sugar cane juice, cheese whey powder
Saccharomyces cerevisiae, Escherichia coli, Zymomonas mobilis	Ethanol (biofuel)	Strain development using metabolic engineering strategies
Clostridium acetobutylicum, Clostridium beijerinckii, Escherichia coli	Butanol (biofuel)	Strain development using metabolic engineering strategies
Escherichia coli	Isobutanol (biofuel)	Strain development using metabolic engineering strategies
Escherichia coli	Alternative biofuels (geraniol, geranyl acetate, limonene, farnesyl hexanoate	Strain development using metabolic engineering strategies

ANNEX II

COUNTRY AND REGIONAL EXAMPLES ON POLICIES AND MAIN GAPS IN THE INDUSTRIAL USES OF MICROORGANISMS

Case 1: India

Even if part of the increased demand for fertilizers could be met by biofertilizers, it is likely to result in savings for farmers. This is especially important for developing countries such as India, where farming is in the hands of small farmers who cannot afford high priced fertilizers (as fossil based fuel prices increase), even though there is already a nearly 80% subsidy being given to the fertilizer industry. Biofertilizers have emerged as one of the alternatives for transitions towards more sustainable development approaches in India. Contribution of biofertilizers depends upon the efficacy of microbial strains present in the biofertilizer packet. The Indian Council of Agricultural Research (ICAR) is the main co-ordinating agency for any research work concerning biofertilizers in India. In India the central scheme "National Project on Development and Use of Bioferitlizers" was launched in 1983 and National Biofertilizer Development Centre (NBDC) (1984-85) was established in Ghaziabad with six Regional Biofertilizer Development Centers in Jabalpur, Hisar, Nagpur, Bangalore, Bhubaneswar and Imphal. The objectives of the scheme included production and distribution of biofertilizers, developing standards and quality control, release of grants for setting up biofertilizer units, training and publicity. The Central Government promoted capital investment subsidy schemes for setting up biofertilizer units for various stakeholders ranging from individuals, groups of farmers, proprietary and partnership firms, cooperatives, the fertilizer industry, companies and Civil Society Organisations. An eligible subsidy amount was released to the National Bank for Agricultural and Rural Development (NABARD) by the Centre (Department of Agriculture & Cooperation, Government of India) in advance, as per the requirement. The grants are released to the State Department of Agriculture and Cooperatives through Public sector undertakings of fertilizers, NGO's, and also to private entrepreneurs, provided their proposals are received through respective State Governments. The Government has also been facilitating biofertilizers through schemes of the Department of Biotechnology and Department of Science and Technology. The National Project on Organic Farming (NPOF) has been supporting private/public sector organisations to put up biofertilizer production units.

Overall production of biofertilizers in the country, which stood at less than 500 tons/annum during 1984-85, rose to more than 10,000 tons/annum within two decades. Currently, about 164 firms belonging to the public, cooperative and private sector are engaged in the mass production of different biofertilizers. These firms have the total annual production capacity of about 67,162 tons. The biofertilizers currently produced in India are based on *Azotobacter, Azospirillum* and Phosphorous solubilizing bacteria (PSBs). There is also an increasing variety of locally adapted biofertilizer products available in the market. Much of this is produced and supplied through the state agriculture departments, though private and civil society organisations are also active in the biofertilizer market. Biofertilizer production is not uniform throughout the country. Among all the states, the southern and western states contribute to almost the entire biofertilizers production in the country. Five states, namely Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu, account for 75.5% of the total biofertilizer production. Also, the overall production has always been lower than the production capacity of the biofertilizer units.

The Bureau of India Standards (BIS) has published necessary specifications/standards for different biofertilizers, with a few firms holding BIS certification. The government of India and

various State governments have made efforts to promote usage of biofertilizers involving farmers and producer/investors. This has been pursued through measures such as farm level extension and promotion programs, financial assistance to investors for setting up units, subsidies on sale, direct production in public sector and cooperative organisations, universities and research organisations.

The Indian Government is still the largest producer and distributor of biofertilizers in the country. The biofertilizer sector has been largely pushed through the centrally sponsored schemes of the Government and reaching the farmers through State Governments. The promotion of biofertilizer has been carried out through the extensive network to convince the farmers about the need to adopt biofertilizers for higher productivity. Deliberations focus on issues related to propagation, promotion, marketing and production of biofertilizers and micronutrients in India.

Other schemes include initiatives as part of organic farming, Integrated Nutrient Management (INM), National Food Security Mission (NFSM), Integrated Scheme of Oilseeds, Pulses, Maize and Oilpalm (ISOPOM), Technology Mission on Oilseeds (TMOP), and Agricultural Technology Management Agency (ATMA) (Sangar, 2011; http://www.nistads.res.in/indiasnt201011/T5_Rural/S&T%20on%20biofertilizers.pdf).

Biofertilizer utilisation has been higher in farming systems that are relatively poor consumers of chemical fertilizers, and producers of pulses and oilseeds. There is a need for alternative policy and institutional frameworks that could make biofertilizer based innovations profitable for the small farmers. Measures that should be taken include:

- **Supporting the pulse sector**. Integration of legumes with crop production is important for sustainable agriculture. Prompting the pulses will give a chance of poor participation in agriculture. Biofertilizers can play an important role in this case, as legume/pulse crops need to be inoculated with rhizobia,
- **Supportive policies**. Rural bioinnovations are essential to motivate economic growth and development. Innovation in rural areas is more likely to occur through small-scale joint venture and entrepreneurs than industrial research and development. Policy should be intended to bring more flexibility and appropriate conditions for the entry of other stakeholders and partners beyond the public sector,
- **Supportive institutional mechanisms**. Important factors for enabling biofertilizer innovation systems are maintaining quality and monitoring of bioinoculant materials. Strengthening institutions that serve the interest of poor farmers enhance their capability and improve their participation in adapting and testing research and extension services through organization and exchange of information related to the innovation.

There is a need to rethink this model especially on aspects of agro-ecology, anthropology, economics, environment sector, etc., so as to obtain an integrated view on approaches working at the local level. Maintenance of R & D quality should be the responsibility of the government, while the private players should take over the production part of the system (Sangar, 2011). The Indian government aims not only to encourage the use of biofertilizers in agriculture but also to promote private initiatives and commercial viability of production. The emphasis of any government policy should be in popularising the use at the farmer's level through varietal improvement, information dissemination, risk coverage, and also sales subsidies. The acceptance at the farmers' level would go a long way in providing commercial benefits to producing units and encouraging investment. The government should either support the units financially or otherwise by developing suitable strains and carriers, and in accessing affordable finance for investment, and in working out viable schemes for distribution, especially as smaller and less experienced units are tending to dominate the market.

Case II: Taiwan

Over the years, biofertilizers have been developed in several laboratories in Taiwan. Beneficial microbes such as rhizobia, associative and free living nitrogen fixing bacteria, phosphate solubilising bacteria (PSBs), arbuscular mycorrhiza (AM), fungi, organic materials and decomposing microorganisms, are considered as biofertilizers. They are continuously isolated from various ecosystems, and their performances in field conditions are assessed in laboratories. The extensive research program on beneficial microorganisms has resulted in the development of a wide range of biofertilizers. Many experiments in greenhouses and under field conditions have revealed that different crops responded positively to microbial inoculation. Multifunctional biofertilizers were developed to reduce about 1/3 - 1/2 of chemical fertilizer applications. In the future, enhancement and maintenance of soil fertility through microorganisms will be a higly significant concern. Long-term conservation of the soil's health is the key benefit of biofertilizers, being vital to sustainable agriculture.

Since 1988, the department of Soil and Environmental Sciences at National Chunghsing University in Taiwan actively started the production of efficient inoculants (liquid and solid biofertilizers). During last 20 years, from 1987 to 2006, enough inoculants were produced and over the years, farmer's economic gain also increased significantly. Over the past decades, AM inoculants have been produced by the Agricultural Research Institute of COA, National Chunghsing University, National Pingtung University of Science and Technology in Taiwan. The inoculants were distributed and technologically demonstrated to farmers by several Agricultural Experimental and Improvement District stations for inoculating numerous crops, particularly horticultural and ornamental plants such as muskmelon, citrus, strawberry, lily, tomato, chrysanthemum, gerbera, tea and fruit trees. For the future, major research focus should be on the production of efficient and sustainable biofertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems. It is necessary to undertake short-term, medium and long-term research to highlight the following points:

1. Selection of effective and competitive multi-functional biofertilizers for a variety of crops,
2. Quality control system for the production of inoculants, application in the field to ensure and explore the benefits of plant microorganism symbiosis,
3. Study of microbial persistence of biofertilizers in soil environments under stressful conditions,
4. Agronomic, soil and economic evaluation of biofertilizers for diverse agricultural production systems,
5. Transferring technological know-how on biofertilizer production to the industrial level and for optimum formulation and
6. Establishment of "Biofertilizer Act" and strict regulation for quality control in markets and application (Chien *et. al.,* 2007).

In Taiwan, there are about 36 publicly owned makers and sellers of biofertilizer. Most of these are small size enterprises that lack the talent and capital for R & D. These biofertilizers are used to supply the domestic market. As there are still no laws in place regulating biofertilizer, their use by farmers in high economic crop areas is quite popular. The current state of the bioferilizer industry is to focus on the research and development of new products.

The government should also announce related rules and regulations in order to manage and register biofertilizer products. In addition, technology transfer between industry and academia should be fully implemented in order to enhance Taiwan's biofertilizer production techniques and quality and strengthen the education of farmers as well as the development of overseas markets.

In Taiwan, biofertilizers are accepted by only a few farmers growing high economic value crops such as wax-apple, grafted pear, and grapes. There is still ample room for developing Taiwan's biofertilizer market. Domestic sales and marketing channels for biofertilizers are similar to other fertilizers and pesticides, with manufacturers directly selling to the farming villages, retail stores, farmers' associations and farmers' production and marketing groups. The performance of biofertilizers is affected by the type of crop and soil type. Therefore, the sales are even more regional and linked with the usage habits of the farmers.

The production technology of biofertilizers is very similar around the world, depending mostly on research conducted by colleges or agricultural department institutions developing isolation, purification, verification, production, and application techniques. In Taiwan, R & D among the commercial sector is still insufficient. Domestic biofertilizer manufacturers are mostly small and middle size enterprises, short on capital and without ability for R & D. Most are family businesses, and few have overseas operations, and thus, farmers need to be more educated. Organic agriculture and environmental protection have been heavily promoted worldwide in recent years, with consequent promotion of biofertilizers and biopesticides.

Taiwan's location in the sub-tropical region is favourable to screening microorganisms that can adapt to the Southeast Asian region. Current difficulties for industry are, the lack of research talent within the industry, the inconsistent quality of products, lack of application knowledge from ordinary farmers with the exception of the farmers in high economic value crop areas, the small scale of manufacturers with no R & D ability. Concerning the technical difficulties, the preservation and stability technology for biofertilizers need to be improved, product's multifunction needs to be improved, biopesticides' adaptability toward the environment and crops needs to be enforced. The current industry policy involves, strengthen of high quality R & D for using nitrogen fixing bacteria, phosphorus releasing microorganisms, mycorrhizae and other organic fertilizers, multifunctional and stable products, enhanced development and production of products with high stability, resistance and adaptation to different soil, crops and negative environment, use of molecular biology techniques to research for high efficiency and good acclimatization products. The research for biofertilizers is under the Council of Agriculture Technology. The research results from academia and industry collaboration projects are tested and promoted through the agriculture research and extension stations.

Case III: China

Biofertilizer production started in China in the 1950s mainly working on *Rhizobium* inoculations for legume crops, with a very small total production. From 1960 to the 1970s, during the time of the Cultural Revolution, local low level but low quality production prevailed. Between 1980 and 1990, there were some developments, but no regulations, no good management, and no standardised criteria. Some companies also produced fake biofertilizers. In 1996, the Ministry of Agriculture started working on the management of biofertilizers and their registration. In 1997, the first group was registered with a total of 8 biofertilizer products. In 2006, a total of 511 products have received temporary registration and about half of them received permanent registration. The first biofertilizer standard was released in 1994, and until present date (December 2012), a total of 17 biofertilizer standard criteria have been released including 3 national standards and 14 industrial standards. In 2000 the Ministry of Agriculture released "The Management Methods for Biofertilizers". During this period, there have been some products that were used in crop production and adopted by farmers. But there are still great differences in terms of product quality among the various companies.

China has over 300 biofertilizer producing enterprises with an annual product output of about half million tons. The biofertilizer application area in China has reached 167 million hectares at present.

China is one of the countries with the richest biological resources and diversity, with an estimated 30 thousand species of plants, 200 thousand species of animals and 30 thousand species of microorganisms. China is also a huge potential market because of the large population with large demands for food, health, drugs, energy, environment etc. In addition, China has a great number of talented personnel.

Biotechnological achievements in agriculture include: a two line hybrid rice, disease resistant wheat, insect resistant rice, transgenic cow, fish, lamb, pig and rabbit, biological pesticide (BT insecticide), biofertilizer (recombinant nitrogen fixing bacteria) and biological forage additives (phytase). Regarding biofertilizers production using biotechnology, there is a 10,000-ton production line based on DNA-recombinant nitrogen fixing bacteria. Furthermore, three microbial insecticides (*Bacillus thurigiensis* trans-gene microbial preparations) have already been authorised (Xie, *et. al.*, 2011).

Case IV: The Republic of Korea

The agricultural policy of the Korean government (Ministry of Agriculture and Forestry) has driven the "Environment-friendly Agriculture Supporting Law" since 1997. This policy aims to sustain productivity and conserve environmental quality of soil and water, reduce pollution and other environmentally harmful effects, recycle organic resources, produce safe foods, and reduce 40% of chemical fertilizers and pesticides produced between 2003 and 2013.

At present a total of 138 companies are registered as biofertilizer manufacturers, producing hundreds of commercial products and 23 biopesticides. The most commonly used microbial agents are *Bacillus* spp., *Rhizobium* spp., *Pseudomonas* spp., *Bradyrhizobium* sp., and *Azospirillum* sp. Various research projects to develop biofertilizers have been conducted by using plant growth promoting rhizobacteria (as part of the PGPRs), phosphate solubilization and nitrogen fixation microbes, etc. Among the commercial biofertilizers, EXTN-1 is the most widely accepted in Korea and is identified as *Bacillus vallismortis.* This is a PGPR and ISR agent that originated from pepper roots and is used for more than 20 crops. Futhermore, it shows a broad controlling spectrum to various viral, bacterial and fungal plant diseases.

Among the registered biopesticides, 12 are fungicides and the others are insecticides. The microbial agent used for biopesticides include *Paenibacillus polymyxa, Bacillus subtilis, Bacillus amyloliquifacians, Paecilomyces fumosoroseus, Streptomyces goshikiensis, Bacillus thuringiensis* var. *kurstaki, Bacillus thuringiensis* subsp. *aizawai,* etc. Target diseases of the biofungicides powdery mildew, gray mould, *Pythium* and *Phytophthora* blight, *Rhizoctonia* patch and sheath blight. Nine out of the 11 bioinsecticides are *Bacillus thuringiensis* products for the control of various moths attacking vegetables like Chinese cabbage. The bioinsecticides formulated with *Bauveria bassiana* and *Paecilomyces fumosoroseus* targeted mite and white fly that are troublesome pests in greenhouse cultivation. Biopesticides occupy only 2.8% share in the pesticide market of Korea, comprisingUS$35 million and is expected that the market will steadily increase to reach aboutUS$80 million (Jee, 2009).

Case V: The Asia-Pacific Region (ASPAC)

In the ASPAC region, the major strategies of some advanced countries are promoting the declining of fertilizer demand trends towards increasing fertilizer efficiency and recycling of organic

resources. Although in most developing countries fertilizer consumption is still increasing rapidly, it is an important policy to provide sufficient fertilizer supply to small-scale farmers at reasonable prices.

However, while the biofertilizer industry has a great potential in the region, the products must satisfy the users in terms of quality, versatility, effectiveness, ease of use and cost. In some countries, biofertilizers are recognized by the government as a way to increase productivity and attain environmental sustainability, still remain on infant stage. For some developing countries where small-scale farming still prevails, fertilizer affordability and availability are still the main concerns. Most countries are now adopting agricultural policies aimed to support the eco-friendly, sustainable agriculture and high-quality and safe production. To support this concept, biofertilizers production and adoption and low input chemicals, are some of the practices. Besides, in order to enhance farmers' acceptance and utilisation of biofertilizers, there is need of extension, demonstration and awareness programs to show farmers the benefits of the technology (Fatah, 2007).

Case VI: Japan

A large part of farmers in Japan do not recognise the importance of using biofertilizers. Four million tons of soybean seeds and 16 million tons of corn seeds are imported from other countries such as USA and Brazil. Large part of the imported soybean and corn seeds are used for feeds of domestic animals and a large amount of waste is obtained from animal husbandry. Seventy thousand tons of ammonia and organic nitrogen fertilizers are supplied to fields every year, since a large number of farmers in Japan do not consider biofertilizer use as important.

The Ministry of Agriculture, Forestry and Fishery established the law promoting organic agriculture in 2006. This law increased interests among farmers in organic agriculture involving the use of biofertilizers. The Japanese government does not authorize most biofertilizers, except the VA mycorrhiza, which is an ordinance designated soil conditioner in the country. The Tokachi Federation of Agricultural Cooperatives (TFAC, Japanese abbreviation: Tokachi Nokyoren) in Hokkaido is the major producer and distributor of the rhizobium biofertilizer in Japan. In TFAC, three kinds of biofertilizers are produced and sold. "Memezo" is a normal type of biofertilizer for soybeans, azuki beans and phaseolus beans. "R-Processing Seeds" are leguminous seeds inoculated with rhizobia and "Hyper Coating Seeds". These biofertilizers are used by about 80% of farmers in Hokkaido. However, the remaining regions in Japan, the farmers' income from soybean seeds production is too small. Therefore, to promote biofertilizer usage for increased soybean seed production in the remaining areas in Japan, it must be developed as a new technology adapting to the different agricultural systems in Hokkaido (Yokoyama and Ohyama, 2009).

Case VII: Brazil

Brazil **is** becoming an agricultural superpower and is an important emerging economy at the global level. The country is signatory of relevant legal conventions intended to protect human health and the environment (e.g. the Conventional for Biological Diversity and the Kyoto Protocol). Brazil contains highly diverse biomes, harbouring about 1/5 of the world's biodiversity. It has to find ways to effectively preserve its natural resources, especially its rainforest, but also its soil and water supply. Ambitious public policies have been implemented for conservation and sustainable use of biological resources.

Biotechnology, biofuels and other sectors of the bio-economy are receiving governmental investments through concerted actions strengthening economic growth. Environmental friendly technologies have been applied on a large scale to agriculture and industry. A great number of

programs to develop science, technology and innovation have been implemented. Research projects are mainly being developed by public universities, institutions and companies, with relatively modest contribution from the private sector. However, this trend is changing thanks to the growing economy. The government has recently launched several infrastructure projects to be developed by the private sector, and important progress has been made regarding the use of biotechnology in agriculture. Meanwhile, the public sector has to deal with old issues such as rural labor relations, agrarian reform, and indigenous peoples' issues. Research activities are mostly concentrated in the southeastern and southern states, but recently there is accelerating development in other regions. Brazil spent about 1.5% of its Gross Domestic Product on Science and Technology, representing a 50% increase in one decade. Within 10 years, Brazil's agribusiness is expected to reach a share of 1/3 of soybean production, and 1/4 of sugar production, while 50% of the exports of broilers and beef will come from Brazil. Moreover, it is expected that Brazil will double its ethanol production. Besides the investments in basic infrastructure (transportation, energy, etc.) substantial agribusiness (farm and agroindustry) investments will be needed, much of which is expected to come from the private sector, as well as from abroad. Microbiology has a long tradition in Brazil. Biofertilizers used in Brazil amount to around 60,000 to 70,000 tons yearly and are applied for crops like beans, maize, rice, sugarcane, soybean, eucalypturs, citrus, tomatoes, cotton, forage crops and carrots. The advantages of these products are 1) lower soil acidification, 2) higher longevity of P bioavailability for commercial crops, 3) substitution of soluble P sources at same P rates, 4) better cost/benefit rations, and 5) more environmentally friendly.

Case VIII: Cuba

Cuba has some 350,000 farmers employed in food production for a population of 11.2 million. Cuba imports each year 80% of its food, with a cost fluctuating between 1.5 and 2 billion dollars. Cuba recently approved an investement program to build several plants to produce biofertilizers and biopesticides as part of its efforts to revitalize the depressed agricultural sector. Besides providing farmers with biofertilizers and biopesticides, the project aims to maintain yields, import substitutes and protect the environment. Research seeking nutritional alternatives means to reduce the use of high doses of mineral fertilizers, including nitrogen, phosphorus and potassium has been reported (Xinhua Agencies 2012).

Case IX: Argentina, Paraguay, Bolivia and Uruguay

In these four countries, most of the soybean crops are inoculated with *Bradyrhizobium* inoculants. It is estimated that 70% of the area is being inoculated annually, providing better nitrogen nutrition but also greater yields. This is a large soybean market of more than 30 million hectares. Other inoculants used to a much lesser extent are *Pseudomonas* sp., and *Azospirillum brasilense*. These products are recommended mainly for wheat and maize, with quiet variable responses.

Case X: Russia

In Russia, some efficient and prospective strains (*Pseudomonas fluorescens* P 469, *Bacillus subtilis* IMP 215) have been developed for industrial cultivation, used as bio P fertilizers and plant protectors against plant diseases caused by *Fusarium* (*F. graminearium, F. culmorium, F. avenaceum*). Furthermore, the following biofertilizers, Bamil which contains *Bacillus, Micrococcus* and *Clavibacter,* Ekud containing *Bacillus* and *Staphylococcus hominis,* Pudret which containing *Bacillus* and *Staphylococcus* and Omug which containing *Bacillus, Micrococcus* and *Clavibacter,*

have been tested in several Podzol soils of Central Russia, and resulted in increased activity in the nitrification process. The biofertilizers Azotovit that contains *Azotobacter chroococcum* and Bactophosphin that contains *Bacillus mucilaginosus*, have been tested in field trials with winter and spring wheat, spring barley, potato and sugar beet in different soils in Central Russia, and resulted in increased yields.

Case XI: Africa

Africa has abundant arable land and labour that could be transformed into increased production, incomes and food security. This has not materialised because of lack of consistent policies and effective implementation strategies. Strategies for transforming African agriculture have to address such challenges as low investment and productivity, poor infrastructure, lack of funding for agricultural resesrch, inadequate use of yield-enhanceing technologies, weak linkages between agriculture and other sectors, unfavourable policy and regulatory environments and climate change.

The continental level commodities are rice, legumes, maize, cotton, palm oil, beef, dairy, poultry and fisheries, while regional level commodities include casava, sorghum and millet. African agriculture remains largely traditional and concentrated in the hands of small holders and pastoralists and as agriculture is rain-fed, yields are low and farmers live in a cycle of poverty and food insecurity for decades. Africa holds 733 million hectares of arable land (27.4% of world total), however, increased environmental degradation has been observed on the continent. Africa accounts for 27% of the world's land degradation and has 500 million hectares of moderately or severely degraded land. Degradation affects 65% of cropland and 30% of pastureland. Soil degradation is associated with low land productivity and it is mainly caused by loss of vegetation and land exploitation, especially overgrazing and shifting cultivation.

Insecurity in land ownership has been blamed for accelerated land degradation and lack of longterm investments in sustainable land management and handle of natural resources. Global warming is another factor that affects African agriculture, and it may cause losses of over 25% of agricultural productivity in Southern and West Africa. Countries in East and Central Africa are also projected to experience losses of 5% to 25%. It is necessary to increase research efforts on adaptive agriculture, while closer collaboration between Africa and other developing regions should be promoted in order to address common climatic threats. Challenges to agricultural transformation in Africa are to:

- Improve agricultural research and technology dissemination and adoption. Improving access to education and technical skills development of rural population will enhance labour productivity in agriculture and related activities;
- Increase food supply chains, reduce hunger and improve response to emergencies,
- Extend areas under sustainable land management and reliable water control systems, for the purpose of increasing productivity while protecting the environment. This requires increasing irrigated land from the current 7% to at least 10% of arable land, rehabilitating the estimated 500 million hectares of degraded land through soil and water conservation measures, addressing land policy issues, addressing the causes of biodiversity losses, and implementing the recommendations of various conventions that the governments have signed in this regard. Moreover, this also implies improving of land use by restricting the encroachment of cultivation into fragile ecosystems, urbanization and inappropriate agricultural practices, in addition to improving water management through the protection of water sources and enhancement of water utilisation systems and quality, and improving human capital stock by providing access to health facilities and basic education,

- Improve rural infrastructures and trade related capacities for market access, including better road networks, communication, rural electrification and water supply, improved port-handling facilities.

Development in the biofuel subsector provides both opportunities and challenges to sustainable agricultural development and food security in Africa. To reduce the potential impact of biofuel production it should be promoted bio fuel production only from non-food crop species (e.g., jatropha), or species where biofuel is manufactured from the byproducts (e.g., sugarcane molasses), design and implement strategies to ensure a careful long-term balance between food security and biofuel production. Benefits and potential use of Arbuscular Mycorrhizal Fungi (AMF) in banana (*musa* spp.) systems have bdeen reported for Africa (Economic Report on Africa 2009, UNECA, http://www.uneca.org/era2009/chap4.pdf).

CaseXII: Europe

In the European Union (EU) the Common Agricultural Policy (CAP) and its system of agricultural subsidies and programs require farmland to be maintained in "good agricultural condition" by the application of particular land management activities considered beneficial to the environment. Some countries have included the principles of "humus/organic matter management" (e.g., use of more compost) in these requirements and their checking within the frame of the cross compliance obligations. Features of the Common Agricultural Policy of the EU are:

1. Subsidizing production of basic foodstuffs in the interests of self-sufficiency,
2. Emphasising of direct payments to farmers as the best way of guaranteeing farm income, food safety and quality and environmentally sustainable production,
3. EU enlargement in May 2004, when the 15 became 25 and now 27 countries and the number of farmers in the EU increased by nearly 70%,
4. Actions to prepare farmers in the new member countries for life in the EU by making funding available to modernize farms, food processing and marketing structures and by encouraging environmentally sound farming,
5. Special funding packages in support of early retirement, less favoured areas, environmental protection, afforestation, semi-subsistence farms and producer groups and for compliance with EU food, hygiene and animal welfare standards.

On 28 June 2007 the Council Regulation EC No 834/2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91 were released. Organic production is an overall system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for commodities produced by using natural substances and processes. The share of the organic agricultural sector is on the increase in most Member States.

Growth in consumer demand is particularly remarkable. Recent reforms of the common agricultural policy, with emphasis on market orientation and the supply of quality products to meet consumer demands, are likely to further stimulate the market in organic produce. The legislation on organic production plays an increasingly important role in the agricultural policy framework and is closely related to developments in the agricultural market. The development of organic production should be facilitated further. Until recently, subsidies and legislation in Europe were designed to increase agricultural production, assure farmers a fair income and to keep food prices at a reasonably low level. Today, food production in the Western World is at a sufficient level. Moreover, the excessive use of chemicals has resulted in health hazards. The European Union has adopted its CAP

with price cuts for key products, and incentives for a reduction in chemical input. Farmers are currently faced with environmental taxes and the need to produce lower yields per hectare.

Case XIII: North America

In North America, most of the microbial biofertilizers (rhizobia, mycorrhizae, PGPRs) have been largely discredited, although new materials and ideas keep popping up. There is no current research in the western part of North America on this issue. Though there have been many attempts to use bioinoculants (various rhizobacteria, yeasts, fee-living nitrogen fixing microorganisms such as azospirillum), the results were not consistent. In some regions of North America, crop production is dominated by commodity crops such as wheat, corn (maize), soybean and cotton. Further, forages (hay, silage and pasture) are also an important proportion of the overall production in this region. The use of biofertilizers and microbial inoculants is relatively insignificant in these regions, since most commodity crops are relatively low value and farmers are thus less likely to use them because of prices sensitivity. An exception would be the inoculation of legume crops with rhizobia. In these regions, no real increase in the use of biofertilizers is expected in the foreseeable future. The exception is the use of humic acids, where the growth in the use of these products is growing rapidly and they are widely promoted, although recent research in California revealed no positive effects. Several biofertilizer products are circulating throughout southeastern North America. Many of these are being marketed by small fertilizer dealers, but some also by the big dealers. No one really had an exact sense for how widespread their usage is, because products come and go so rapidly from the market. When they are used, they are applied mainly as a spray adjunct for liquid fertilizer solutions. A widespread commercial use appears to be in the turf and organic markets in Florida, but there is no research in the region, demonstrating the efficacy (IPNI, 2011).