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Microbial responses to salt-induced osmotic stress

V. Effects of salinity on growth and displacement of soil bacteria

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Summary Soil columns were exposed to balanced (low Na^+) or unbalanced (high Na^+) high-salt solutions for a period of 7 days followed by 7 days of stress reflief. Total numbers of bacteria released into the perfusates rose under both types of stress, but the proportion of displaced bacteria that were viable fell significantly. Relief from both types of stress stimulated rapid increases in the number of viable micro-organisms released from soil. Examination of the soils at the end of the relief periods revealed that soils exposed to stress contained more viable bacteria than the non-stressed controls. However, high levels of balanced stress led to a significant decrease in species diversity within the microbial population, but a similar effect was not observed in soils exposed to unbalanced, high Na^+ stress. These results suggest that, while salt stress may cause a significant reduction in the number of microorganisms in a soil, a large portion of the microbial population can rapidly adapt to marked changes in salinity.

Introduction

High levels of salts in agricultural soils can result from adverse environmental conditions or from man's manipulation of the physical and chemical properties of the soils[5, 8, 20]. The problem is becoming increasingly common and is causing significant losses of arable farmland in several parts of the world[9]. To date, investigations of the effects of salinity have emphasized the physical and chemical changes induced in stressed soils[4, 26, 28], and various mathematical models have been developed to predict effects of excessive salts on ion exchange activities[1, 2, 10, 25]. Nevertheless, the effects of salinity on the microbial component of stressed soils have not been examined in detail[6, 16, 31, 32]. While the physiological effects of salinity on plants have been well documented[3, 12, 13, 23], most of the research with microorganisms has concentrated on the halophiles, *i.e.*, on those micro-organisms that thrive in high-salt environments[21, 22], rather than on the species that normally comprise the microbial population in agricultural soils.

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Rates of exudation tend to increase from plant roots exposed to osmotic stress[14, 34], and the increased availability of substrate, particularly monosaccharides[33], to rhizosphere microorganisms should, presumably, increase microbial activity in the root regions of stressed plants. However, previous work in this laboratory has shown that even mild levels of salt stress inhibited microbial activity in the rhizosphere of barley[32], but the effects of stress on bacterial counts were only minor in soils without roots, although distinct shifts in microbial distribution patterns were detected[31]. In the present study, therefore, we have examined the effects of high-salt concentrations on the displacement of microorganisms from the soil matrix and on their viability during exposure to balanced (low Na^+) and unbalanced (high Na^+) osmotic stress.

Materials and methods

A sandy loam soil was collected from Wellington Country, Ontario in September 1981. Analyses of the soil with techniques described by Hesse[18], indicated that the total nitrogen content of the soil prior to treatments was 0.28 mg g^{-1} (including nitrates and nitrites), the organic carbon content was 13.25 mg g^{-1}, the pH was 7.2 and the cation exchange capacity was 14.72 meq 100 g^{-1}. The number of viable bacteria was $3.5 \times 10^3 \pm 0.15$ (S.E.) and the total number of microorganisms was $1.68 \times 10^{11} \pm 0.01$ g^{-1} of dry soil as determined by the dilution-plate method[29] and by direct microscopy[24], respectively.

Glass chromatography columns (38 × 914 mm) containing sintered glass disks at their bases, were used to prepare soil columns for all treatments. They were initially filled to a depth of 45 mm with clean silica sand, then packed with 500 g of sieved soil (2-mm mesh) and perfused with 200 ml of Hoagland and Arnon's solution number 2[19] (HA) containing 1% glucose (w:v). After an initial incubation for 7 days, the assigned treatment solutions were applied.

Two treatment regimes were used to evaluate the effects of high-salt concentrations on the displacement of bacteria from soil and on the ionic composition of the soil perfusates. The first series of treatments involved the use of balanced salt solutions formulated by proportional increases in the concentration of each of the major nutrient elements (KNO_3, $Ca(NO_3)_2$, Mg_2SO_4) in the HA solution[11]. For the second treatment regime, unbalanced salt solutions were prepared by additions of NaCl to the HA standard[31]. HA standard nutrient was used as the control solution in both studies. Since plants were intentionally excluded from the soil columns, glucose was added as the carbon source in all treatment solutions (final concentration was 1% w:v).

The levels of osmotic stress applied were the same in each study: (a) — 70 kPa (control), (b) — 500 kPa, (c) — 1000 kPa, and (d) — 1500 kPa.

After the initial 7-day incubation period, 100-ml volumes of the treatment solutions were added to the soil columns until the osmotic potentials of the displaced perfusates essentially matched those of the perfusing solutions. The soil columns were then incubated for 7 days, after which stress was relieved by additions of the HA solution in 100-ml volumes until the osmotic potentials of the perfusates were comparable to the HA controls. After 7 days of stress relief, each column was perfused with two 100-ml volumes of the HA solution.

The total number of bacteria and number of viable bacteria displaced from the soil columns were counted after each perfusion. The total number of bacteria was determined by filtering an appropriate dilution of the perfusate through a 0.45-μm membrane filter (Millipore HABP black filter followed by staining with 1 ml of a 2.5-mg ml^{-1} solution of the ammonium salt of 8-anilino-1-naphthalene sulfonic acid[30] (NH_4-ANS). The stained filters were placed in a drop

of glycerol on a microscope slide, covered with a cover slip and observed with an epi-illumination optical system on a Nikon Labophot microscope equipped with violet excitation and barrier filters. The number of viable bacteria in the perfusates was determined with the dilution-plate technique using soil-extract agar as the growth medium. Differences between the counts of bacteria were assessed by analysis of variance followed by Duncan's multiple range test. Changes in the species composition of the microorganisms released into the perfusates following each treatment period were determined by their Gram reaction, colony morphology, pigmentation, and growth on various carbohydrates. The total number and number of viable bacteria remaining in the soils at the conclusion of each study were determined as outlined above.

The levels of Na^+, K^+, Ca^{2+} and Mg^{2+} present in the soil solutions after each perfusion were determined with a Perkin-Elmer Model 303 atomic absorption spectrophotometer.

Results

Effects of balanced salt stress

Five 100-ml volumes of the salt solutions were required to stabilize the osmotic potentials of the perfusates from the individual balanced salt treatments. The total number of bacteria displaced from the soil columns increased significantly in the -1000 and -1500 kPa treatments (Table 1). In contrast, even the lowest level of stress (-500 kPa) significantly reduced the number of viable bacteria present in the perfusates and higher stress intensities (-1000 and -1500 kPa) were without additional effect.

After 7 days exposure to balanced salt solutions, relief from stress required the addition of six 100-ml volumes of the control solution before the osmotic potentials of the perfusates approximated those from the controls. Soil structure in the stressed columns had deteriorated and became increasingly compacted, causing a substantial increase in percolation time. Inhibition of microbial activity was still apparent following relief from stress, with the number of viable bacteria remaining depressed in soils that had been stressed at the two highest intensities (Table 1).

After 7 days of stress relief, all columns were perfused with two 100-ml volumes of the control solution. Microbial activity was fully restored in columns that had been stressed, and counts of viable bacteria present in the perfusates were significantly greater than in the controls (Table 1).

Microscopic examination of the treated soils at the conclusion of the study showed higher total numbers of micro-organisms in soils stressed with -500 and -1000 kPa solutions than in the control or -1500 kPa treatments (Table 2a). However, counts of viable microorganisms were higher in soils previously exposed to the two highest intensities of stress.

Table 1. Total numbers of bacteria and numbers of viable bacteria present in soil perfusates during application of balanced salt stress, during relief of stress, and 7 days after stress relief*

Treatment	Number of viable bacteria ml^{-1} perfusate	Total number of bacteria ml^{-1} perfusate
Stress application **		
Control	1.00×10^7 a ± 0.06	4.09×10^7 c ± 0.17
− 500 kPa	2.21×10^5 b ± 0.27	4.57×10^7 c ± 0.17
− 1000 kPa	7.17×10^4 b ± 0.43	6.08×10^7 b ± 0.34
− 1500 kPa	1.05×10^5 b ± 0.06	8.47×10^7 a ± 0.59
Time of stress relief†		
Control	4.35×10^6 a ± 0.36	6.83×10^8 a ± 0.35
− 500 kPa	2.47×10^6 b ± 0.52	5.82×10^8 b ± 0.38
− 1000 kPa	2.15×10^5 c ± 0.09	1.93×10^7 c ± 0.24
− 1500 kPa	1.74×10^5 c ± 0.14	2.60×10^7 c ± 0.32
7 days after stress relief††		
Control	1.36×10^5 d ± 0.13	6.19×10^8 c ± 0.60
− 500 kPa	1.04×10^8 b ± 0.07	3.11×10^9 a ± 0.17
− 1000 kPa	1.65×10^8 a ± 0.05	9.64×10^8 b ± 0.58
− 1500 kPa	1.84×10^7 c ± 0.16	7.36×10^8 bc ± 0.43

*Means in the same treatment column not followed by the same letter are significantly different at the 5% level of probability.
**Means of twenty replicates ± standard errors.
†Means of twenty four replicates ± standard errors, counted immediately after stress relief.
††Means of eight replicates ± standard errors.

Analyses of the diversity of microbial populations in the perfusates from the control soil columns at each treatment period indicated that a variety of genera, including Arthrobacter, Bacillus, Cornebacterium, Listeria, Pseudomonas, and Flavobacter were released from the soil. Although balanced stress reduced the number of viable bacteria present in the perfusates, the diversity of the populations was not affected during application or relief of stress. However, by the end of the 7-day recovery period following stress relief, population diversity had been drastically reduced and only two distinct groups of micro-organisms, Bacillus and Listeria, were found in perfusates from columns previously exposed to − 1000 and − 1500 kPa of osmotic stress.

Effects of unbalanced salt stress

Four 100-ml volumes of the unbalanced salt solutions (high Na^+) were required to stabilize the osmotic potentials of the perfusates from the stressed soils. The total number of bacteria displaced from the columns during stress increased at the two highest Na^+ concentrations, but the number of viable bacteria in the perfusates declined in all three stress treatments (Table 3).

Table 2. Total numbers of bacteria and numbers of viable bacteria isolated from soil 7 days after stress relief*†

Treatment	Number of viable bacteria g^{-1} dry wt soil	Total number of bacteria g^{-1} dry wt soil
(a) Counts in soils stressed with balanced salt solutions		
Control	5.52×10^6 c ± 0.56	1.55×10^{13} b ± 0.39
− 500 kPa	1.23×10^7 c ± 0.05	1.64×10^{13} ab ± 0.33
− 1000 kPa	1.23×10^8 a ± 0.42	1.97×10^{13} a ± 0.48
− 1500 kPa	1.36×10^7 b ± 0.28	1.46×10^{13} b ± 0.07
(b) Counts in soils stressed with unbalanced salt solutions		
Control	7.90×10^6 b ± 0.79	1.48×10^{13} a ± 0.07
− 500 kPa	3.45×10^6 c ± 0.19	1.01×10^{13} b ± 0.06
− 1000 kPa	9.83×10^7 a ± 0.38	1.12×10^{13} b ± 0.06
− 1500 kPa	1.58×10^7 a ± 0.31	9.32×10^{12} b ± 0.11

*Means of four replicates ± standard errors.
†Means in the same treatment column not followed by the same letter are significantly different at the 5% level of probability.

Following relief from stress, the total number of bacteria released from the soil columns decreased, whereas the number of viable bacteria increased significantly in the perfusates from all previously-stressed soils (Table 3).

Table 3. Total numbers of bacteria and numbers of viable bacteria present in soil perfusates during application of unbalanced salt stress, during relief of stress, and 7 days after stress relief*

Treatment	Number of viable bacteria ml^{-1} perfusate	Total number of bacteria ml^{-1} perfusate
*Stress application***		
Control	1.10×10^7 a ± 0.08	4.51×10^7 b ± 0.36
− 500 kPa	1.32×10^6 b ± 0.34	4.19×10^7 b ± 0.14
− 1000 kPa	3.22×10^5 b ± 0.21	1.62×10^8 a ± 0.14
− 1500 kPa	9.29×10^4 b ± 0.61	1.67×10^8 a ± 0.16
*Time of stress relief***		
Control	6.35×10^4 d ± 0.81	4.86×10^8 a ± 0.42
− 500 kPa	2.30×10^5 c ± 0.30	1.33×10^7 b ± 0.10
− 1000 kPa	7.68×10^5 a ± 0.26	5.56×10^6 b ± 0.47
− 1500 kPa	5.41×10^5 b ± 0.17	4.23×10^6 b ± 0.28
7 days after stress relief†		
Control	3.47×10^5 b ± 0.20	1.07×10^9 a ± 0.53
− 500 kPa	1.44×10^5 b ± 0.07	8.94×10^8 b ± 0.74
− 1000 kPa	3.45×10^6 a ± 0.48	6.34×10^8 c ± 0.36
− 1500 kPa	4.11×10^6 a ± 0.25	4.09×10^8 d ± 0.40

*Means in the same treatment columns not followed by the same letter are significantly different at the 5% level of probability.
**Means of sixteen replicates ± standard errors.
†Means of eight replicates ± standard errors.

After 7 days of stress relief, all columns were perfused with two 100-ml volumes of the HA control solution. The total number of bacteria released into the perfusates from previously-stressed columns was lower than in the controls, but counts of viable bacteria from the −1000 and −1500 kPa treatments rose and exceeded those from the lowest level of stress (−500 kPa) and from the controls (Table 3).

Counts of bacteria remaining in the soils at the end of the relief period showed that osmotic stress had reduced the total number of bacteria present, but the two highest stress treatments contained larger numbers of viable organisms than the controls or than soils that had been exposed to the −500 kPa treatment (Table 2b).

Examination of microbial isolates obtained from the perfusates during the course of the study indicated that exposure to unbalanced salt stress did not affect the diversity of the microbial population in the soils.

Changes in the ionic composition of the perfusates during balanced stress indicated that the soils had a net loss of K^+ which increased with stress intensity. During unbalanced stress, losses of K^+ from the soil were smaller, but losses of Ca^{2+} increased and there was a large net transfer of Na^+ to the soil matrix (Table 4).

Table 4. Net effects of osmotic stress and relief from stress on ion exchange between the soil and perfusates*

	μg cations ml^{-1} perfusate			
Treatment	K^+	Ca^{2+}	Mg^{2+}	Na^+
Balanced stress				
Control	− 145	+ 2783	+ 81	+ 473
− 500 kPa	+ 3703	+ 2984	− 30	+ 267
− 1000 kPa	+ 8332	− 702	− 394	+ 542
− 1500 kPa	+ 12900	− 1213	− 84	+ 454
Unbalanced stress				
Control	− 46	+ 2279	+ 54	+ 523
− 500 kPa	− 287	+ 1445	+ 84	+ 1142
− 1000 kPa	+ 411	+ 1949	+ 144	+ 2469
− 1500 kPa	+ 573	+ 1859	+ 128	+ 8983

*Negative signs preceding values indicate net movement (and retention) of cations from the stress solutions to the soil. Positive signs preceding values indicate net movement (and loss) of cations from the soil to the perfusates.

Discussion

Soils are generally considered to be harsh environments where microbial activity is restricted to localized microsites within aggregates and where the primary objective appears to be the simple maintenance

of viability[17]. The effects of salt stress on microbial activity in a given soil are likely to be complex and unpredictable because of the many possible interactions that may occur among the ions, bacteria, soil particles and other parameters that control the soil environment[15, 27]. Changes in the physical or chemical components of a soil ecosystem will destabilize the microbial population and will result in a period of adjustment during which the individual species will either die or adapt to the new stress conditions and complete with other surviving organisms to colonize microsites that became available as a direct result of the stress[7, 35].

During the current study, it was evident that both balanced and unbalanced salt stress had significant effects on the displacement of bacteria from soil. In general terms, higher levels of salinity displaced higher total numbers of bacteria from soil but the numbers of viable bacteria in the perfusates decreased (Tables 1 and 3). This implies that salt stress either displaced nonviable bacteria, preferentially, from the soil or that salinity was lethal to certain components of the population.

The rapid recovery of the soil microbial populations during the priods of stress relief showed that any direct perturbation of microbial numbers in soil by salt stress is probably of a transient nature. It was apparent that balanced stress caused a distinct change in the composition of the microbial population after stress had been removed and after recovery had occurred. Why a similar change was not observed in soils previously exposed to unbalanced stress is not clear, but high levels of specific nutrient ions present in the balanced solutions may have favored certain bacterial species and accounted for their domination at soil microsites during the 7-day relief period.

The presence of significantly higher numbers of viable bacteria in previously-stressed, as compared to control, soils by the end of the stress relief period may have resulted from changes in the composition of the microbial population, from marked changes that occurred in soil structure, and/or from changes in the nutrient-ion composition and concentration in stressed soils (Table 4). Although the precise explanation remain unclear, our results do indicate that, while a significant part of the microbial population in soils is sensitive to salt stress, a major portion of that population can readily adapt to large shifts in soil salinity.

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