APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1992, p. 1284–1291 Vol. 58, No. 4
0099-2240/92/041284-08$02.00/0

Relationship between Desiccation and Exopolysaccharide Production in a Soil *Pseudomonas* sp.

EMILY B. ROBERSON[1] AND MARY K. FIRESTONE[2]*

Abacus Concepts, Inc., 1984 Bonita Avenue, Berkeley, California 94704,[1] and Department of Soil Science, University of California, Berkeley, California 94720[2]

Received 15 July 1991/Accepted 2 February 1992

The relationship between desiccation and the production of extracellular polysaccharides (EPS) by soil bacteria was investigated by using a *Pseudomonas* species isolated from soil. Cultures subjected to desiccation while growing in a sand matrix contained more EPS and less protein than those growing at high water potential, suggesting that resources were allocated to EPS production in response to desiccation. Desiccation did not have a significant effect on activity as measured by reduction of iodonitrotetrazolium. Purified EPS produced by the *Pseudomonas* culture contained several times its weight in water at low water potential. Sand amended with EPS held significantly more water and dried significantly more slowly than unamended sand, implying that an EPS matrix may buffer bacterial colonies from some effects of desiccation. We conclude that bacteria may use EPS production to alter their microenvironment to enhance survival of desiccation.

Bacteria in soil generally live in colonies within a matrix largely composed of extracellular polysaccharides (EPS) (10, 11, 16, 26). Although there is general agreement that bacteria in many environments, including soils and lungs, live within an EPS matrix, relatively little is known about the function(s) of this EPS matrix. One possibility that has often been discussed but that has been the subject of few studies is that an EPS envelope may protect bacteria from drying and from fluctuations in water potential (15, 39).

Water potential is the potential energy of water. Because water moves freely across microbial cell membranes, thermodynamic laws require that the internal water potential of unicellular organisms be in equilibrium with the external water potential. When the external water potential decreases in a drying soil, soil microorganisms may retain water by increasing their internal solute concentration, or they may lose water to their surroundings (plasmolyze), which can result in cell death. Soil microorganisms have been reported to synthesize organic compatible solutes (14, 24) such as betaines or amino acids and to selectively take in inorganic solutes such as K^+ (21) to increase their internal solute concentrations during periods of low external water potential. Bacteria may also change the structure of their membranes (9) or make other physiological changes in response to desiccation. An EPS matrix may slow the rate at which a bacterial colony equilibrates with the surrounding soil. Slowing the rate of drying within the colony microenvironment could increase bacterial survival by increasing the time available for metabolic adjustment. Clays, which slow the rate of drying of soil (29), have been shown to increase the ability of bacteria to survive desiccation in soil (6, 15).

An EPS matrix may provide another advantage to bacteria living within it. Decreasing the water content of soil restricts diffusion of nutrients to microorganisms (7, 14, 23, 34a). Polysaccharides are hygroscopic (25, 38) and therefore may maintain a higher water content in the colony microenvironment than in the bulk soil as water potential declines. This increase in water content could increase nutrient availability within the bacterial colony.

* Corresponding author.

There is some evidence in plants that polysaccharides increase survival and activity during drying. Morse (25) studied two varieties of the composite *Hemizonia luzulifolia*, whose leaf intercellular spaces contain very different amounts of EPS. Plants with high levels of EPS showed higher transpiration rates than low-level-EPS plants during midday water stress. Morse found that water potential within the leaves of high-level-EPS plants at midday was significantly higher than in the leaves of low-level-EPS plants, allowing the increased activity. She described the polysaccharides as "buffers" which held water and protected leaves from drying.

Several studies (5, 6, 15, 30) have investigated the relationship between bacterial EPS production on agar plates or in liquid medium and ability to survive desiccation in soil. These studies have generally found no relationship or an inverse correlation between EPS production and ability to survive desiccation. However, to our knowledge, no one has examined whether bacteria respond to desiccation by increasing production of EPS.

In the work reported here, we determined (i) whether desiccation stimulates polysaccharide production by a soil bacterial isolate and (ii) whether the water retention characteristics of the EPS produced by the isolate might help bacteria living within an EPS matrix to survive desiccation.

MATERIALS AND METHODS

Bacteria. The strain used was isolated from an agricultural soil in the Sacramento Valley of California. The Mediterranean climate in this area exposes the soil microbial community to frequent and severe desiccation. The strain was selected for mucoid colony appearance. It is a motile, yellow-pigmented, gram-negative, oxidase-positive rod and has been identified as a *Pseudomonas* species by its fatty acid profile (MIDI, Inc., Newark, Del.). The EPS produced by this strain contains both neutral monosaccharide and uronic acid subunits. The ratio of neutral to uronic acid subunits in the EPS varies depending on the medium composition (data not shown).

Two series of experiments were performed. The first group monitored bacterial protein, activity, and EPS produc-

tion during 1 cycle of wetting and drying. The second group examined the water retention characteristics of the EPS produced by the bacteria and the effect of EPS on the rate of drying.

Metabolic response of bacteria to the wet-dry cycle. A sand matrix and mineral salts growth medium was chosen for this experiment so that nutrient supply could be tightly controlled and monitored and so that bacterial protein and EPS production could be precisely measured. Bacteria were grown to mid-log phase (about 2×10^8 cells per ml) in a mineral salts medium consisting of 4 g of NH_4Cl, 6.98 g of $Na_2HPO_4 \cdot 7H_2O$, 5.58 g of KH_2PO_4, 20 g of glucose, and 40 ml of Huntner's mineral supplement (13) per liter. The initial pH was adjusted to 6.8. Sand cultures were grown in 99-mm-diameter Pyrex petri dishes. Fifty grams of sterile quartz sand which had been acid washed, rinsed with deionized water, and equilibrated with growth medium was inoculated with sufficient unwashed culture to bring the total water potential to −0.025 MPa (130 μl of culture per g of sand). All petri plates were placed in sterile desiccators containing solid LiCl to produce a relatively constant low relative humidity and to dry the plates reproducibly. The cultures were allowed to dry to −1.5 MPa and then readjusted to −0.025 MPa with 0.25× growth medium. Three plates were collected for measurements on day 0, after this wetting. The bacteria were exposed to this drying and wetting cycle before the experiment in order to acclimatize them to growing in the presence of surfaces.

On day 0, after wetting, half of the remaining plates were replaced in the LiCl-containing desiccators and half were placed in desiccators containing sterile distilled water. The distilled water maintained a high relative humidity in the desiccators. The water potential in the cultures in the water-containing desiccators remained roughly constant during the experiment, and this was used as a control treatment group.

Three petri plates from the LiCl chambers and three petri plates from the control chambers were collected each day, weighed to determine water content, and sampled for viable counts, protein, electron transport activity, total carbohydrates, glucose, and ammonium. A moisture release curve was used to convert water content to water potential. The experiment was terminated when the average water potential in the desiccated treatment group reached −1.5 MPa, a water potential which previously has been reported to limit bacterial activity (14, 32, 34a).

CFU were counted by spreading dilutions of the sand cultures on agar plates containing growth medium. One drop of the surfactant Tween 80 was added to the initial sand-buffer mixture, and the mixture was gently mixed in a vortex mixer to facilitate detachment of bacteria from sand surfaces (33). The protein content of the cultures was determined by the Coomassie blue method (Bio-Rad Laboratories, Richmond, Calif.). The cultures were heated to 100°C for 10 min in 1 N NaOH to release cell contents and were filtered, and the amount of protein was measured in the filtrate. Lysozyme in 1 N NaOH was used as the standard. The results are reported as micrograms of lysozyme equivalents per gram of sand.

Respiratory activity was estimated by the reduction of iodonitrotetrazolium (INT) to INT-formazan (36). The INT solution was sterilized by passage through a sterile 0.2-μm-pore-size Acrodisc filter (Gelman Sciences, Ann Arbor, Mich.). Sand culture was weighed into sterile glass vials, and a 0.4% (wt/vol) solution of INT (grade 1; Sigma Chemical Co., St. Louis, Mo.) in distilled water was added. The suspension was well mixed, incubated at 30°C for 2 h in the sealed vial, and stored at −20°C until it was extracted and analyzed. The amount of INT-formazan in a dimethylformamide extract of the sand cultures was measured. Sand was extracted twice with dimethylformamide (12), the extracts were combined, and the A_{485} was read with a spectrophotometer. The A_{485} was converted to concentration by using a standard curve of the absorbance of INT-formazan in dimethylformamide.

Polysaccharides were extracted from the sand by a method similar to that of Oades et al. (27). Cultures were heated to 120°C with 5 N H_2SO_4 for 30 min and filtered through a glass fiber filter. Samples were further extracted once with boiling water. The extracts were pooled, and the amounts of neutral and uronic acid carbohydrates were determined with anthrone and *m*-hydroxydiphenyl, respectively (3, 4). The results are reported as micrograms of glucose and glucuronic acid equivalents per gram of sand, respectively.

Residual amounts of glucose and NH_4^+ and NO_3^- in the culture solution were measured in distilled water extracts. The amount of glucose was determined by the glucose oxidase assay (19), and NH_4^+ and NO_3^- amounts were measured colorimetrically (18) with a Lachat autoanalyzer. Since only trace concentrations of nitrate were found in the cultures, only NH_4^+ is reported. The amount of glucose was subtracted from that of total neutral carbohydrates to calculate the amount of neutral polysaccharides.

Microscopy. Microscopic observations were made at the Station de Science du Sol, Institute National de la Recherche Agronomique, Versailles, France. A separate but identical experiment was performed to produce samples for examination by scanning electron microscopy (SEM). We used a cryoscan device (Oxford Cryotrans Temperature and Preparation Controllers), which makes it unnecessary to dry samples before they are placed in the microscope. Conventional SEM preparation techniques such as alcohol dehydration and critical point drying have been found to severely affect the structure and apparent quantity of bacterial EPS (37).

Small samples were collected from both desiccated and control treatment groups each day, frozen to −210°C in an N_2 slush, and transferred into a liquid N_2-cooled Phillips 525 M SEM. Ice was removed from the surface of the samples by 20 min of sublimation at −80°C, and the samples were coated with gold and observed at −180°C. By using cryoscan SEM, we were able to observe qualitative changes in both the amount and the hydrated structure of the EPS in the cultures as water potential changed during desiccation.

EPS water retention characteristics. (i) Polysaccharide moisture release curve. Bacteria were grown to late log phase in mineral salts medium and separated from EPS in solution by two 14-min centrifugations at 10,000 × *g* at 4°C. The supernatant from the first centrifugation was decanted, and the cells were resuspended in phosphate buffer and centrifuged again. The two supernatants were combined and extensively dialyzed against distilled water by using Spectra/Por 1,000-Da-cutoff dialysis membranes (Spectrum Inc., Los Angeles, Calif.) to remove residual growth medium. Polysaccharides were then concentrated approximately fivefold by further dialysis against solid polyethylene glycol (average molecular weight, 8,000) and finally evaporated to dryness with a Speedvac concentrator (Savant Medical Industries, Farmingdale, N.Y.). The absence of protein contamination in the concentrated solution was confirmed by the Coomassie blue method (Bio-Rad Laboratories). A

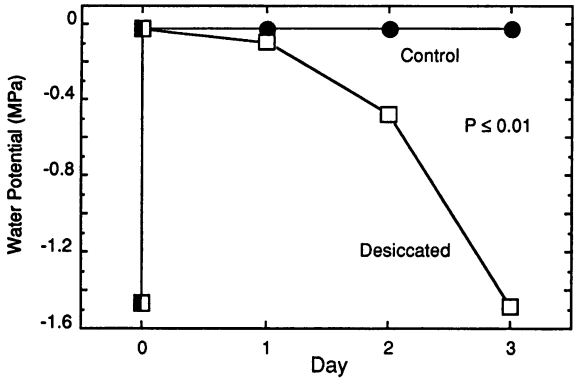

FIG. 1. Water potential changes in the two treatment groups. *P* value is the level of significance for difference between values for desiccated and control treatment groups from days 1 to 3. ◧ indicates that the treatment groups were not separated until after day 0 measurements.

range of volumes (30 to 400 μl) of distilled water was added to approximately 40 mg of dry polysaccharide in gas-tight 1.5-ml microcentrifuge tubes. The mixtures were mixed in a vortex mixer and then centrifuged at 14,000 × *g* for 2 min to thoroughly mix the water with the polysaccharide, and they were allowed to equilibrate overnight at 4°C. The water potential at each water content was determined.

(ii) Effect of EPS on drying rate. EPS were purified and concentrated by dialysis as described above but were not lyophilized. Sufficient concentrated EPS solution (100 mg of EPS per ml) was added to three replicate petri plates containing 50 g of sterile acid-washed sand to bring the water potential to −0.2 MPa. Concentrated EPS solution was used in order to approximate the immediate bacterial microenvironment, which is essentially pure polysaccharide. Three control plates were brought to −0.2 MPa with sterile deionized water. Both solutions contained 0.2% sodium azide to maintain sterility. Pairs of sand-filled petri plates, one containing EPS solution and one containing water, were placed in desiccators containing LiCl and allowed to evaporate. Samples were taken from each dish at each hour, and the water content and water potential were measured.

(iii) Water potential. In all experiments, water potential values of < -0.2 MPa were determined with an HR33T microvoltimeter with a C32 thermocouple psychrometer sample chamber (Wescor Co., Logan, Utah) by using an equilibration time of 50 min before measurement. Water content at water potential values of > -0.2 MPa were measured with a pressure plate apparatus (Soil Moisture Equipment Corporation, Santa Barbara, Calif.). Sand and medium were sterilized with an autoclave.

Statistical analyses. All statistical analyses were performed and graphs were created with StatView and SuperANOVA software (1, 2).

RESULTS

Metabolic response of bacteria to the wet-dry cycle. The change in water potential in the two treatment groups is shown in Fig. 1. All points in the figures represent the means of three replicates. Drying in the desiccated treatment group began immediately, and by day 2, the water potentials in the cultures averaged −0.47 MPa. On day 3, the desiccated cultures had reached a water potential of −1.46 MPa, which

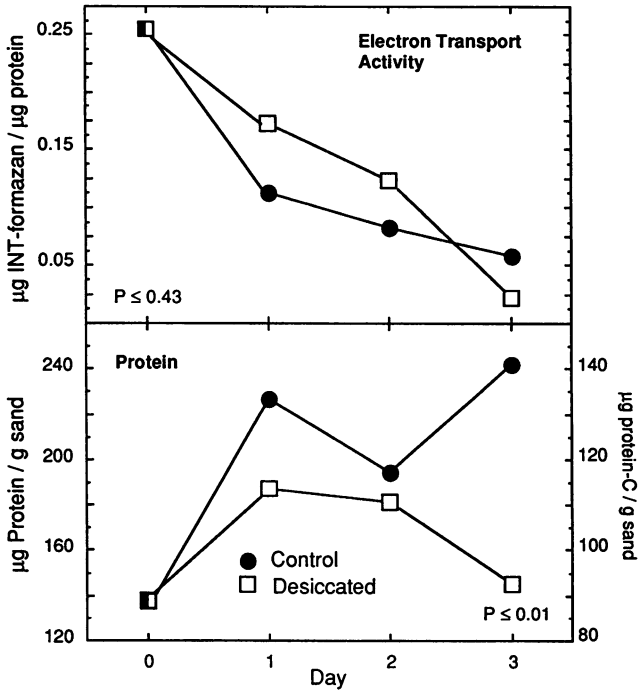

FIG. 2. Changes in the amount of protein and electron transport activity as measured by the reduction of INT. *P* values are the levels of significance for differences between desiccated and control treatment groups from days 1 to 3. ◧ indicates that treatment groups were not separated until after day 0.

was close to their water potential after the preexperiment drying cycle on day 0. The water potentials in the control treatment group remained relatively constant after initial wetting.

Figure 2 shows the change in activity and protein amount during the experiment. Electron transport activity decreased in both treatment groups over the course of the experiment. Although the treatments did not significantly affect activity, the level of activity appeared slightly higher in the desiccated treatment than in the control group on days 1 and 2 but fell below that of the control group by day 3. Desiccation significantly reduced the amount of protein in the cultures. The amount of protein in the control cultures increased between days 0 and 3, but in the desiccated treatment group, protein concentration increased for only 1 day after wetting and then declined. The number of CFU was also significantly lower in the desiccated treatment group than in the control group (data not shown).

The changes in glucose and ammonium concentrations in the cultures are shown in Fig. 3. These are expressed as the percentage of the total amount supplied (initial culture medium plus 0.25× medium added on day 0) that was found in the cultures each day. Almost no glucose was detected in the cultures during the experiment, and the amount of glucose was not affected by the treatments. Significantly more residual ammonium was present in the control cultures than in the desiccated treatment group. After the ammonium added in the diluted medium on day 0 was used by the cultures, the amount of ammonium changed little during the experiment in either treatment group.

Figure 4 shows the amounts of neutral, uronic acid, and total polysaccharide in the cultures each day. There was a

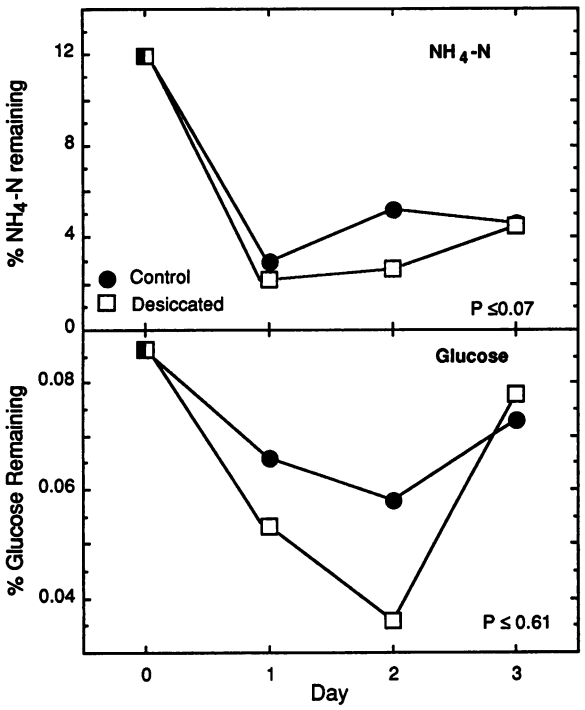

FIG. 3. Availability of glucose and ammonium, expressed as the percentage of the total amount supplied, that was present in the cultures each day. *P* values are the levels of significance for differences between desiccated and control treatment groups from days 1 to 3. ◧ indicates that treatment groups were not separated until after day 0 measurements.

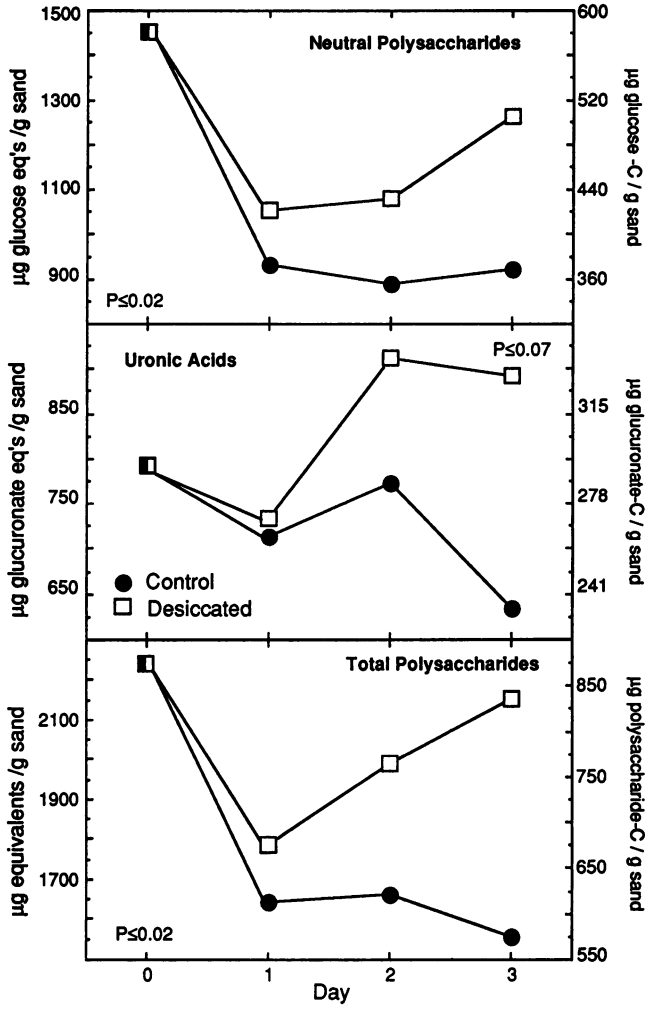

FIG. 4. Neutral, uronic acid, and total polysaccharides in the cultures. Uronic acids and neutral polysaccharides are expressed as glucuronic acid and glucose equivalents, respectively. *P* values are the levels of significance for differences between desiccated and control treatment groups from days 1 to 3. ◧ indicates that treatment groups were not separated until after day 0 measurements.

sharp decrease in these amounts after wetting on day 0 for both treatment groups, possibly the result of EPS consumption by the growing cultures. The amount of polysaccharides in the control cultures remained low throughout the experiment. The amount in the desiccated treatment group, however, began to increase immediately as the water potential decreased and continued to increase until day 3. As was found for protein, the quantity of polysaccharides in the desiccated cultures at the end of the experiment was similar to that on day 0, at the end of the preexperiment desiccation. There was no significant difference between the proportion of uronic acid in the EPS in the desiccated and control treatment groups (data not shown).

Micrographs. The micrographs (Fig. 5) show bacteria and EPS on sand after wetting on day 1, when the water potential in the cultures was −0.025 MPa (Fig. 5A through C), and after the cultures had dried to approximately −1.0 MPa (D through F). Fibers of EPS are visible in Fig. 5B and C, but bacteria without obvious EPS can also be seen in Fig. 5A. Panels D through F show that the amount of EPS in the cultures substantially increased after exposure to desiccation. Thick layers of EPS covered bacteria and sand surfaces. Before desiccation, the edges of the bacteria appear sharp (Fig. 5A through C), while after desiccation, the bacteria appear to be partially embedded in EPS.

EPS water retention characteristics. (i) Polysaccharide moisture release curve. The moisture release curve (Fig. 6) shows the relationship between water potential and water content for the purified EPS. The EPS showed a high affinity for water at all water potentials. At −1.5 MPa, the EPS held approximately five times its weight in water. At −0.5 MPa, it contained 10 times its weight in water. These values are similar to those reported for the neutral fungal polysaccharide scleroglucan (8). For comparison, a medium-textured soil holds between 0.04 and 0.1 g of water per g of soil at −1.0 MPa.

Figure 6 also shows the effect of EPS on the moisture release curve of quartz sand. The addition of a small amount of EPS greatly increased the amount of water held by the sand at all water potentials. The points fit a curvilinear function overall, but to further analyze the effect of EPS, they can be split at −0.9 MPa into two linear portions. Each linear portion can be analyzed separately by linear regression. This approach has the advantage of allowing a test of the statistical significance of the effect of EPS by using an analysis of covariance (Table 1) (34). Table 1 shows the slopes of the regressions between water potential and water content for the control and EPS-amended sand. Table 1 also shows the significance level (*P* value) for the hypothesis that the slopes differ between the control and EPS-amended

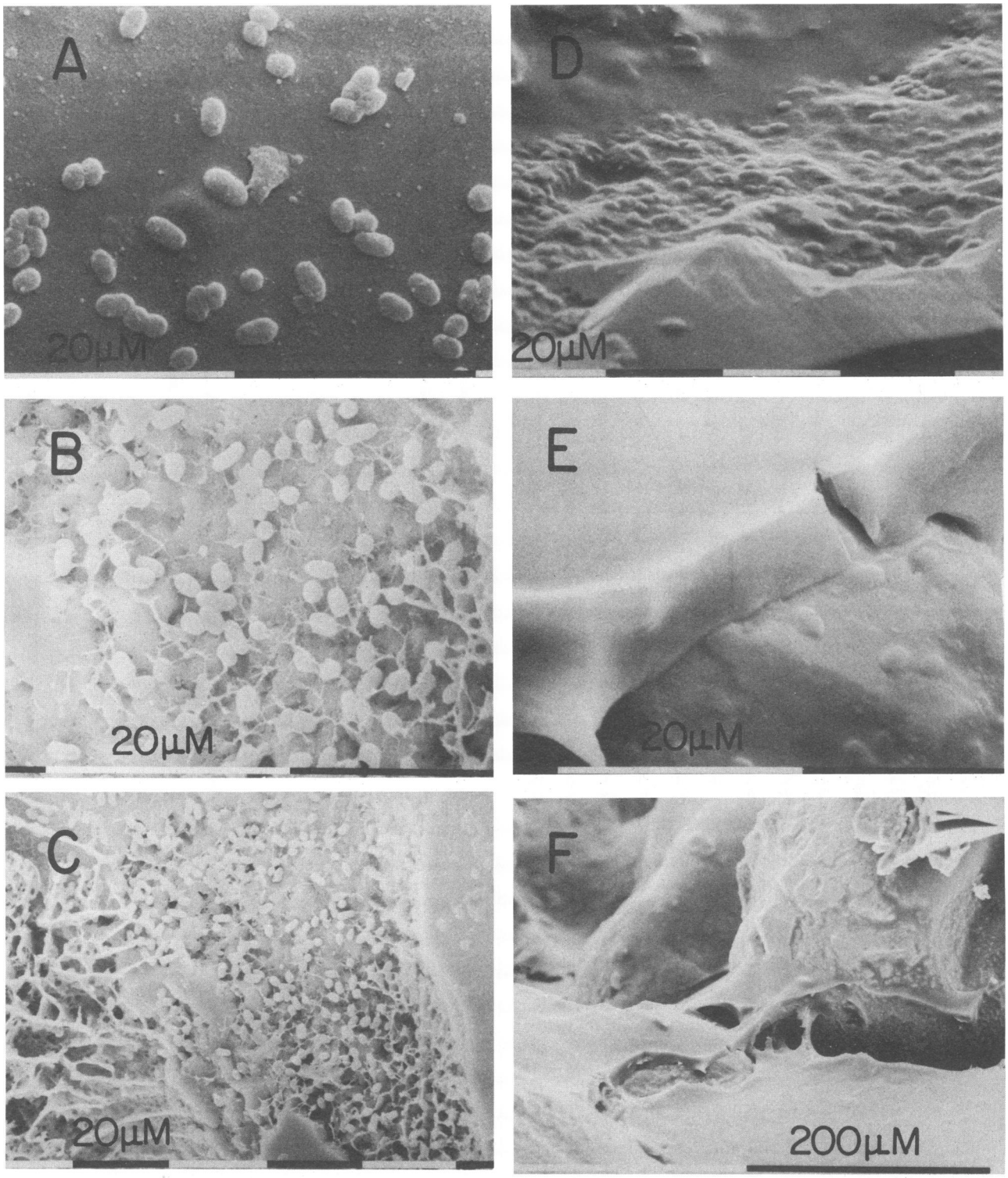

FIG. 5. SEM micrographs showing changes in the amount and hydrated structure of EPS in *Pseudomonas* cultures growing on quartz sand at −0.025 MPa (A through C) and after desiccation to −1.0 MPa (D through F).

sand. Over both water potential ranges, the slope of the regression line was significantly ($P < 0.1$) greater for the control sand than for the EPS-amended sand. This means that water potential decreased more with decreasing water content in the control sand. In other words, an identical decrease in water content caused a smaller decrease in water potential in the EPS-amended sand than in the control sand.

(ii) Effect of EPS on drying rate. The effect of EPS on the

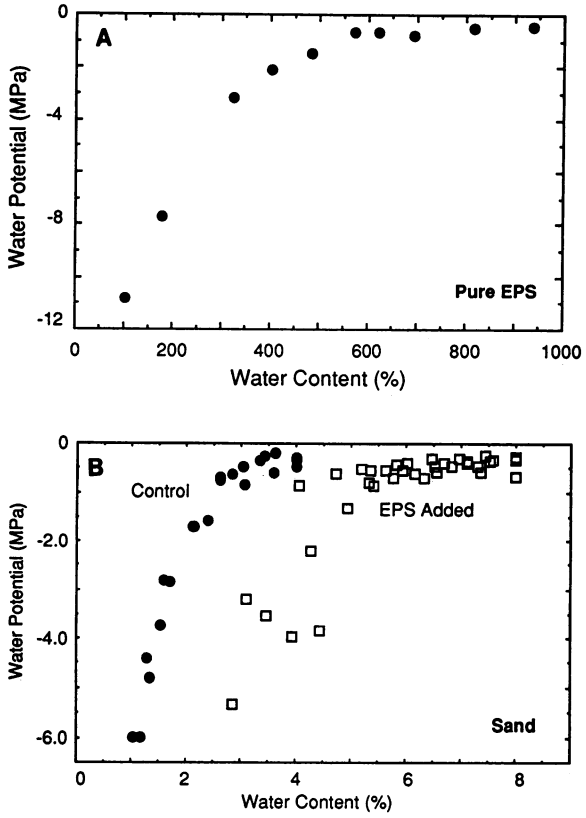

FIG. 6. Relationship between water potential and water content in purified EPS (A) and in sand (B) with (□) and without (●) EPS added.

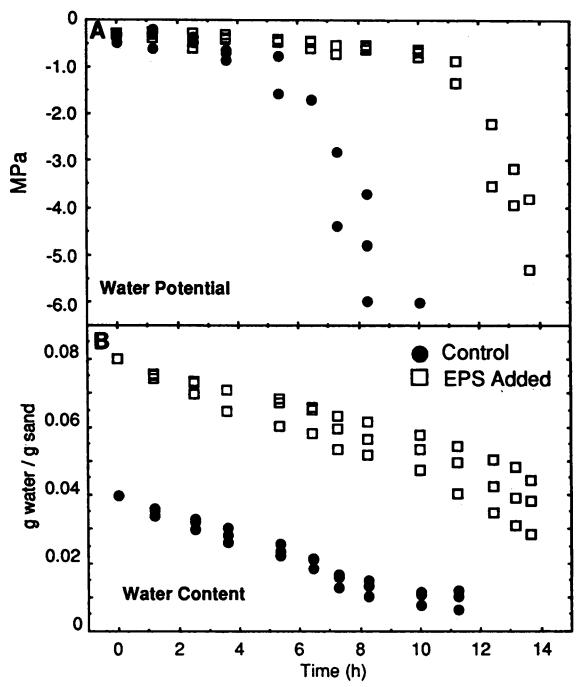

FIG. 7. Changes in water potential (A) and water content (B) during 14 h of drying in sand with and without EPS added.

decrease of water content and water potential with time of drying is shown in Fig. 7. EPS had no effect on the rate of decrease of water content in the sand. This rate was constant throughout the experiment in both the EPS-amended and the control sand. However, EPS did have a significant effect on the rate of decrease of water potential values of >-0.9 MPa (Table 1). The slope of the regression of water potential against time was significantly smaller in the EPS-amended sand. The rate of water potential decrease in the sand was therefore significantly slowed by the addition of EPS at values of >-0.9 MPa, although the rate of water content decrease was not significantly slowed (Fig. 7). The effect of this is that the control sand dried to a water potential of -0.9 MPa in approximately 5 h, whereas roughly double that length of time was required for EPS-amended sand. At values of <-0.9 MPa, the slopes for the two treatments were not significantly different.

TABLE 1. Linear relationship of water potential with time and water content in drying sand, with and without EPS added, over two water potential ranges

Variable	Water potential (MPa)	Slope[a]		
		Control	EPS added	*P* value[b]
Water content	>-0.9	0.28	0.11	0.07
	<-0.9	3.55	1.25	<0.01
Time	>-0.9	−0.09	−0.04	0.03
	<-0.9	−1.15	−1.30	0.74

[a] The slopes of the regression of water potential against time (measured in hours) or water content (measured as percentage) in the given water potential range using the data in Fig. 6 and 7.

[b] Significance levels for the differences between the slopes of the control and those of the EPS-amended sand.

DISCUSSION

Metabolic response of bacteria to the wet-dry cycle. Water availability strongly controlled the production and consumption of protein and polysaccharide by the bacteria. Wetting caused an initial decrease in the amount of polysaccharide in all cultures between days 0 and 1 (Fig. 4). This may have been the result of consumption of polysaccharides by bacteria growing in response to the increase in water availability. The concurrent increase in protein concentration (Fig. 2) suggests that some polysaccharide carbon may have been used for protein production. Conversely, in the desiccated treatment group, the amount of polysaccharide after day 1 increased while that of protein decreased, implying that protein and possibly other cellular components as well are used for polysaccharide production in response to desiccation.

It should be noted that some bacterial carbohydrate other than EPS may have been measured as EPS because of the technique used to extract the sand cultures. The most likely contaminating carbohydrates are compatible solutes. Bacteria have been found to accumulate the sugars trehalose and sucrose as compatible solutes in response to low water potential (17, 22). The amounts reported to be synthesized are too low to affect the results of the present study, however. It is also possible, although less likely, that intracellular storage polysaccharides were measured in the cultures. *Pseudomonas* species do not generally accumulate intracellular polysaccharides (28, 35), but the amounts synthesized by organisms that do, such as *Escherichia coli*, are

also too small to significantly affect the present experiment (31).

Almost no glucose was detectable in the cultures during the experiment (Fig. 3). The major sources of carbon, therefore, were cell contents and EPS. This supports the conclusion that C was shuttled between protein and polysaccharides as the water status of the cultures changed. This lack of an external source of available C may be similar to the situation in soil, in which the pool of available C is often small and microbial biomass released after wetting of dry soil can be an important portion of the C available to the microbial community (20, 40). The ammonium concentration did not change significantly after day 1 for either treatment group, although more ammonium was present in the control cultures than in the desiccated cultures until day 3. This may reflect elevated use of ammonium by the bacteria in response to desiccation stress.

Electron transport activity does not appear to have been as strongly controlled by water potential as were protein and EPS. Activity was not significantly affected by the treatments and showed a pattern of steady decrease in both treatment groups throughout the experiment (Fig. 2). This pattern may reflect the development of a limitation of a nutrient, most likely C, in the cultures. Nutrients, in the form of dead cells and EPS, were relatively abundant in the cultures on day 0 after the preexperiment desiccation and day 0 wetting with nutrient solution. This finite supply was used by the bacteria, particularly between days 0 and 1, as shown by the increase in protein amount (Fig. 2), and limitation developed. Additional limitation may have developed in the desiccated treatment group as low water content reduced solute diffusion to the bacteria. This additional nutrient limitation in the desiccated treatment may partially explain the lower viable counts and protein contents measured for those cultures.

Different time periods are represented by each of the measurement techniques used in this study. The tests for carbohydrates, glucose, and protein measure the cumulative synthesis and consumption of these compounds before each measurement. The activity assay, on the other hand, indicates the physiological state of the cultures at the time of measurement. The tests must therefore be interpreted somewhat differently. On day 0, for example, large amounts of polysaccharide and low levels of protein (Fig. 2 and 4) were measured. These results reflect the drying cycle prior to the experiment. A high level of water availability was not shown in these measurements because the cultures had not yet responded to it. By day 1, however, the increase in protein and decrease in polysaccharides showed the effects of the wetting. On the other hand, on day 0, the level of activity was high, reflecting the high nutrient and moisture availability after wetting the dry cultures with nutrient solution (Fig. 2).

EPS water retention characteristics. (i) Polysaccharide moisture release curve. The EPS produced by this bacterium held several times its weight in water at low water potential (Fig. 6). The EPS was an effective competitor, relative to the sand, for the limited supply of water at low water potential. The EPS therefore has the ability to retain water and possibly to concentrate dissolved nutrients in the bacterial microenvironment during desiccation. By maintaining a high water content, the EPS may also increase diffusional availability of nutrients to the bacterium.

The effects of the water-holding capacity of the EPS can be seen in the effect of EPS on the moisture release curve of sand (Fig. 6). The EPS-amended sand held more water at all water potentials than the unamended sand. The analysis of covariance results in Table 1 show that in addition to quantitatively holding more water, the EPS-amended sand also lost significantly more water than the control over the same decrease in water potential. EPS, therefore, can protect bacteria against drying in two ways. It holds a reservoir of water in the microenvironment surrounding bacteria, and it can lose substantial amounts of water from this reservoir during desiccation with relatively little change to the internal water potential in the microenvironment. An EPS-rich microenvironment surrounds bacteria like a protective sponge, buffering them against external changes in water potential.

(ii) Effect of EPS on the drying rate. The presence of EPS substantially slowed the rate of water potential decrease in the sand (Fig. 7; Table 1). The drying rate within a matrix of pure polysaccharide, such as that in a bacterial colony in soil, would be lower than that measured in the EPS-sand mixture shown in Fig. 7. Bacteria must maintain equilibrium with the water potential of their surroundings. The production of EPS in response to desiccation may provide significant additional time in which to make metabolic adjustments that allow bacteria to survive this environmental stress.

In this experiment, we did not test the effect of EPS on the rate of wetting. However, EPS may slow the rate of water potential change during wetting as well as drying within the microenvironment surrounding bacteria. Wetting occurs more rapidly than drying in soil, and sudden increase in water potential can be an important stress for soil microorganisms (20). The production of EPS may be as important to bacterial survival during wetting as during the desiccation that precedes it.

Conclusions. Many researchers have hypothesized that microbial EPS protect microorganisms from desiccation. Although this study does not confirm that hypothesis, it does provide evidence supporting it. EPS can provide a microenvironment that holds water and dries more slowly than its surroundings. This property may at least partially explain our observation that bacteria respond to desiccation by channeling energy and nutrients into polysaccharide production. Soil is an extremely heterogeneous environment, and wetting and drying do not proceed uniformly throughout it. Many microbial processes in soil depend on this heterogeneity. This work shows that in addition to passively taking advantage of the heterogeneity in soil, bacteria may also intervene during desiccation to create a controlled microenvironment that enhances their survival.

ACKNOWLEDGMENTS

We thank Claire Chenu of the Station de Science du Sol, Institut National de la Recherche Agronomique, Versailles, France, for the use of the cryoscan SEM and for her help and advice during this project. We also thank Anne-Marie Jaunet, also of the Station de Science du Sol, who took the electron micrographs and whose expertise on the SEM was invaluable.

This research was supported by the Kearney Foundation of Soil Science and by a Hatch project of the University of California Experiment Station.

REFERENCES

1. **Abacus Concepts.** 1987. StatView II. Abacus Concepts, Inc., Berkeley, Calif.
2. **Abacus Concepts.** 1989. SuperANOVA. Abacus Concepts, Inc., Berkeley, Calif.
3. **Blumenkrantz, H., and G. Asboe-Hansen.** 1973. New method for quantitative determination of uronic acids. Anal. Biochem. **54**:484–489.
4. **Brink, R. H., P. Dubach, and D. L. Lynch.** 1960. Measurement

of carbohydrates in soil hydrolysates with anthrone. Soil Sci. **89:**157–166.
5. **Bushby, H. V. A., and K. C. Marshall.** 1977. Some factors affecting the survival of root-nodule bacteria on desiccation. Soil Biol. Biochem. **9:**143–147.
6. **Bushby, H. V. A., and K. C. Marshall.** 1977. Water status of rhizobia in relation to their susceptibility to desiccation and to their protection by montmorillonite. J. Gen. Microbiol. **99:**19–27.
7. **Campbell, G. S.** 1977. An introduction to environmental biophysics. Springer-Verlag, New York.
8. **Chenu, C.** 1989. Influence of a fungal polysaccharide, scleroglucan, on clay microstructures. Soil Biol. Biochem. **21:**299–305.
9. **Crowe, J. H., L. M. Crowe, J. F. Carpenter, and C. A. Wistrom.** 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem. J. **242:**1–10.
10. **Foster, R. C.** 1981. Polysaccharides in soil fabrics. Science **214:**665–667.
11. **Foster, R. C., A. D. Rovira, and T. W. Cook.** 1983. Ultrastructure of the root-soil interface. American Phytopathological Society, St. Paul, Minn.
12. **Griffiths, B. S.** 1989. Improved extraction of iodonitrotetrazolium formazan from soil with dimethylformamide. Soil Biol. Biochem. **21:**179–180.
13. **Guirard, B. M., and E. E. Snell.** 1981. Biochemical factors in growth, p. 79–111. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
14. **Harris, R. F.** 1981. Effect of water potential on microbial growth and activity, p. 23–97. *In* J. F. Parr, W. R. Gardner, and L. F. Elliott (ed.), Water potential relations in soil microbiology. Soil Science Society of America, Madison, Wis.
15. **Hartel, P. G., and M. Alexander.** 1986. Role of extracellular polysaccharide production and clays in the desiccation tolerance of cowpea bradyrhizobia. Soil Sci. Soc. Am. J. **50:**1193–1198.
16. **Hepper, C. M.** 1975. Extracellular polysaccharides of soil bacteria, p. 93–111. *In* N. Walker (ed.), Soil microbiology, a critical review. John Wiley & Sons, New York.
17. **Hershkovitz, N., A. Oren, and Y. Cohen.** 1991. Accumulation of trehalose and sucrose in cyanobacteria exposed to matric water stress. Appl. Environ. Microbiol. **57:**645–648.
18. **Keeney, D. R., and D. W. Nelson.** 1982. Nitrogen—inorganic forms, p. 643–699. *In* A. L. Page, R. H. Miller, and D. R. Keeney (ed.), Methods of soil analysis, part 2, 2nd ed. American Society of Agronomy, Madison, Wis.
19. **Keston, A. S.** 1956. Specific colorimetric enzymatic analytical reagents for glucose, abstr. 129, p. 310. Abstr. Amer. Soc. Chem. Soc. Meet.
20. **Kieft, T. L., E. Soroker, and M. K. Firestone.** 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol. Biochem. **19:**119–126.
21. **Killham, K., and M. K. Firestone.** 1984. Salt stress control of intracellular solutes in streptomycetes indigenous to saline soils. Appl. Environ. Microbiol. **47:**301–306.
22. **Larsen, P. I., L. K. Sydnes, B. Landfald, and A. R. Strom.** 1987. Osmoregulation in *Escherichia coli* by accumulation of organic osmolytes: betaines, glutamic acid, and trehalose.
23. **McAneney, K. J., R. F. Harris, and W. R. Gardner.** 1982. Bacterial water relations using polyethylene glycol 4000. Soil Sci. Soc. Am. J. **46:**542–547.
24. **Measures, J. C.** 1975. Role of amino acids in osmoregulation of nonhalophilic baceria. Nature (London) **257:**398–400.
25. **Morse, S. R.** 1990. Water balance in *Hemizonia luzulifolia*: the role of extracellular polysaccharides. Plant Cell Environ. **13:**39–48.
26. **Oades, J. M.** 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil **76:** 319–337.
27. **Oades, J. M., M. A. Kirkman, and G. H. Wagner.** 1970. The use of gas-liquid chromatography for the determination of sugars extracted from soils by sulfuric acid. Soil Sci. Soc. Am. Proc. **34:**230–235.
28. **O'Leary, W. M.** 1989. Practical handbook of microbiology. CRC Press, Boca Raton, Fla.
29. **Osa-Afiana, L. O., and M. Alexander.** 1982. Clays and the survival of *Rhizobium* in soil during desiccation. Soil Sci. Soc. Am. J. **46:**285–288.
30. **Osa-Afiana, L. O., and M. Alexander.** 1982. Differences among cowpea rhizobia in tolerance to high temperature and desiccation in soil. Appl. Environ. Microbiol. **43:**435–439.
31. **Preiss, J., and D. A. Walsh.** 1981. The comparative biochemistry of glycogen and starch, p. 25–60. *In* V. Ginsburg and P. Robbins (ed.), Biology of carbohydrates, vol. 1. John Wiley & Sons, New York.
32. **Rosacker, L. L., and T. L. Kieft.** 1990. Biomass and adenylate energy charge of a grassland soil during desiccation. Soil Biol. Biochem. **22:**1121–1127.
33. **Schmidt, E. L.** 1974. Quantitative autecological study of microorganisms in soil by immunofluorescence. Soil Sci. **118:**141–149.
34. **Snedecor, W. G., and G. W. Cochran.** 1980. Statistical methods, 7th ed. Iowa State University Press, Ames.

34a. **Soroker, E. F.** 1990. Ph.D. thesis. University of California, Berkeley.

35. **Stanier, R. Y., J. L. Ingraham, M. L. Wheelis, and P. R. Painter.** 1986. The microbial world, 5th ed. Prentice-Hall, Englewood Cliffs, N.J.
36. **Trevors, J. T., C. I. Mayfield, and W. E. Innis.** 1982. Measurement of electron transport system (ETS) activity in soil. Microb. Ecol. **8:**163–169.
37. **van Doorn, W. G., F. Thiel, and A. Boekestein.** 1990. Cryoscanning electron microscopy of a layer of extracellular polysaccharides produced by bacterial colonies. Scanning **12:**297–299.
38. **Wiebe, H. H.** 1966. Matric potential of several plant tissues and biocolloids. Plant Physiol. (Bethesda) **41:**1439–1442.
39. **Wilkinson, J. F.** 1958. The extracellular polysaccharides of bacteria. Bacteriol. Rev. **22:**46–73.
40. **Williams, S. T.** 1985. Oligotrophy in soil: fact or fiction?, p. 81–111. *In* M. M. Fletcher and G. D. Floodgate (ed.), Bacteria in their natural environments. Academic Press, London.