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BACTERIAL POPULATION DYNAMICS IN A SOIL–PLANT SYSTEM

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The dynamics of *Rhizobium leguminosarum* and *Arthrobacter crystallopoietes* populations introduced into soil at different densities were studied in the barley rhizosphere. The region of microbial life with a high multiplication rate is represented only by the root surface (rhizoplane). A dependence was established for *A. crystallopoietes* between the final abundance of bacteria in the rhizoplane and the initial population density. A tendency was noted in nodule bacteria toward abundance stabilization at the same level regardless of the initial population density. It is suggested that the single level of stabilization in nodule bacteria and the pronounced dependence of the final abundance of *Arthrobacter* on the level of introduction reflect the ecological strategy of these microorganisms. Introduction of *A. crystallopoietes* and *R. leguminosarum* did not affect the dynamics of soil bacteria assayed by plating.

The interest in studying the behavior of microorganisms in the soil–plant system is traditional, but the abundant factual material available can be analyzed only with difficulty.

Works had appeared recently (Bowen and Rovira, 1976; Newman and Watson, 1977), in which attempts were made to correlate the accumulation of empirical data and at the same time to construct a theoretical model for a microbial system in the rhizosphere. A broad range of significant conclusions arise from the Newman–Watson model. Specifically, the model predicts growth of microorganisms primarily only on the root surface, which almost eliminates the traditional concept of the rhizosphere.

Our work is devoted to the experimental verification of the major conclusions of the Newman–Watson model for populations of *Arthrobacter crystallopoietes* and *Rhizobium leguminosarum* growing in the rhizoplane, rhizosphere, and edaphosphere of barley.

MATERIALS AND METHODS

Barley (*Hordeum vulgare*) was grown in a greenhouse in pots filled with sod-weakly podzolic highly cultivated top soil. Experiments were performed from June to September 1979 without artificial light, and from October to December with additional light (more than 18 h per day). Soil moisture was maintained at 60% of total moisture capacity.

Streptomycin-resistant populations of *R. leguminosarum* ZK (the original strain was graciously presented by Prof. V. P. Klasen) and *A. crystallopoietes* 655 (from the collection of the Institute of Microbiology, Academy of Sciences of the USSR) were selected as the research subjects. Spontaneous mutants resistant to a high streptomycin concentration were selected by conventional procedure (Klasen, 1974).

Nodule bacteria were grown on a bean infusion for 2 days at 28°C in batch culture. *A. crystallopoietes* was grown on meat–peptone agar. Bacterial cultures were washed three times with sterile water, after which they were introduced into soil at several different population densities. Barley seeds (30 per 5-liter pot) were sown in soil immediately thereafter.

To enumerate microorganisms in relation to barley growth, seven-eight plants were placed in sterile Petri dishes at specified times. The selection of samples is related to the arbitrary division of the space around roots into three zones according to the goals set in this work. The furthest zone from the roots (edaphosphere) was represented by soil collected when the roots were lightly shaken. Soil firmly held on the roots was removed with a sterile scalpel so that the remaining layer was not thicker than 0.3 cm.; this remaining layer was then scraped off and regarded as the rhizosphere, and microorganisms remaining on roots as the rhizoplane. Since roots were not washed, rhizoplane microflora was not mixed with that from the rhizosphere. Soil samples from the rhizosphere and edaphosphere (1 g) and roots (0.1-0.2 g) were placed in three replications in flasks with 100 ml of sterile water and treated with a low-frequency UZDN-1-type pulverizer (22 kHz, 0.4 A, 2 min). Inoculations were carried out by the dish method (five parallel dishes) with different dilutions of the suspension on the following media: bean agar with streptomycin (1.39 mg/ml) to enumerate nodule bacteria; meat–peptone agar diluted 10-fold to enumerate the soil bacteria complex; and meat–peptone agar with streptomycin (0.6 mg/ml) to enumerate *A. crystallopoietes*.

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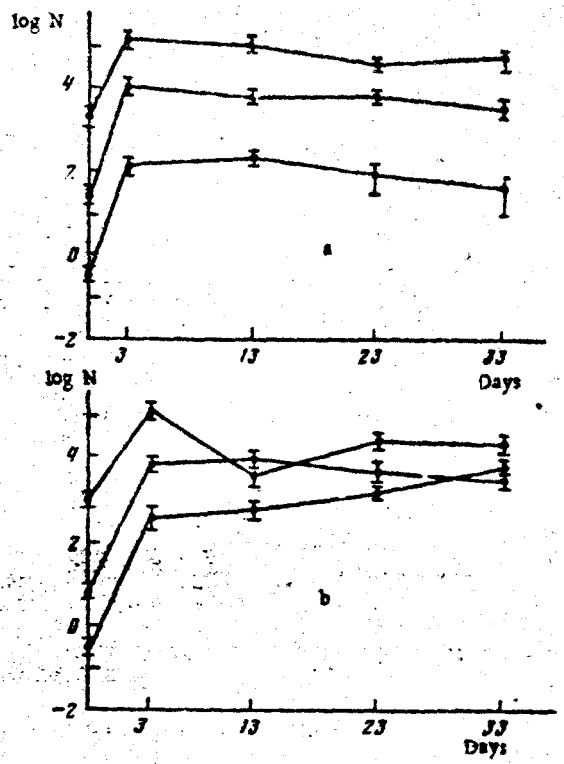

Fig. 1. Population dynamics of bacteria introduced at different population densities into the rhizoplane. a) *A. crystallopoietes*; b) *R. leguminosarum*. N = bacterial cell number /cm² of root surface. The vertical lines in Figs. 1-3 indicate the confidence interval at a 0.05 significance level.

The procedure we used for differentiating zones near the root makes it possible to select samples according to zone with a high reliability in comparison with the root-washing method. In this case, however, when rhizoplane microorganisms are counted, soil cannot be totally removed from the roots, which is particularly noticeable in work with young seedlings. Therefore, it is necessary to verify the relative completeness of the bacterial count in the rhizoplane. For this purpose, young barley seedlings (3 and 5 days) were analyzed by two procedures: that described above and a traditional procedure (Rovira et al., 1974). In the second case, roots were washed in flasks with 100 ml of sterile water on a shaker for 3 min, then sterilely transferred to 250-ml flasks with 100 ml of sterile water and 5 g of washed coarse sand, and shaken on a shaker for 15 min. This procedure did not significantly increase the completeness of the microbial count in the rhizoplane in comparison with the basic method we used, and observed differences between corresponding abundance parameters did not exceed 20-30%.

The microbial count was calculated per unit of root and soil surface. We assumed that the specific surface of barley roots was 0.11 m^2/g of soil (Guzeva, 1978). Contact between bacteria and seeds occurred in soil, since the bacterial population was added directly to soil and not to the seeds. Therefore, population abundance at time zero on the root during its formation was evaluated on the basis of the surface bacterial concentration. Thus, the corresponding parameter of soil population density was used as the initial value for counting bacteria on the root surface.

Statistical analysis of results was performed following standard procedure (Ashmarin et al., 1975).

RESULTS AND DISCUSSION

Population Dynamics of *R. leguminosarum* and *A. crystallopoietes* in the Rhizoplane. The abundance of introduced bacterial populations in all tests increased in the rhizoplane; this was particularly intense during the first test periods (3-5 days) (Fig. 1). Thus, if the initial density for *A. crystallopoietes* on barley roots was $1.65 \cdot 10^3$, $3.49 \cdot 10^1$, and 0.34 cells/cm² of root, by the third day abundance increased, respectively, to $1.5 \cdot 10^5$, $1.25 \cdot 10^4$, and $1.6 \cdot 10^2$ cells/cm² of root. Generation time from these data was approximately 10 h. We used the following formula in the calculations (Pirt, 1975):

$$t_d = \frac{(t_2 - t_1) \log 2}{\log N_2 - \log N_1}$$

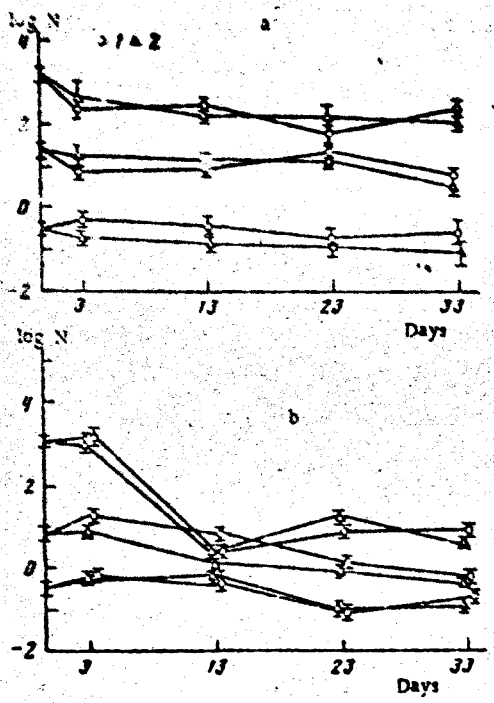

Fig. 2. Population dynamics of bacteria introduced into the rhizosphere and edaphosphere at different population densities. a) *A. crystallopoietes*; b) *R. leguminosarum*; 1) rhizosphere; 2) edaphosphere; N = bacterial cell number/cm² of soil.

where t_g is the generation time; and N_1 and N_2, the number of microorganisms at times t_1 and t_2, respectively. A similar pattern was observed also for nodule bacteria at the start of the experiment. If the initial population density was $1 \cdot 10^2$, $0.77 \cdot 10^1$, and 0.31 cells/cm² of root, by day 5 bacterial abundance increased, respectively, to $1.6 \cdot 10^3$, $6.3 \cdot 10^2$, and $5.4 \cdot 10^2$ cells/cm² of root. Generation time was approximately 13 h, if counts were made with the use of the specific soil and root surfaces proposed (Guzeva, 1978). The legitimacy of this assumption requires verification; we should point out, however, the coincidence of results on population dynamics and bacterial multiplication rate we obtained with the corresponding conclusions from the Newman–Watson model. In subsequent periods of the experiment, the abundance of *A. crystallopoietes* hardly varied and remained at the level reached by day 3.

It is noteworthy that there is a clear correlation between stabilization level and initial density of the *Arthrobacter* population: A more abundant introduction corresponds to a higher final abundance. This particular aspect of the population dynamics of this population in the rhizoplane is significant and corresponds to the dynamics of nodule bacteria in soil (Kozhevin et al., 1977). This means that there is no single value for the amount of medium specified in the experiment for *Arthrobacter*. Moreover, the Newman–Watson model indicates that, in contrast to soil, the rhizoplane is characterized by the same amount of medium, and the stabilization level essentially does not depend on the level of introduction. In our experiment a more abundant *Arthrobacter* population is maintained in the final count with a more abundant introduction into the rhizoplane, but this high level only represents a potential and is not realized at a lower population introduction density. One possible reason for this is the disparity in the ecological strategy of *Arthrobacter* and conditions in the rhizoplane in the presence of other organisms.

The population of nodule bacteria introduced at a high level decreased slightly by day 33 after a short multiplication period. At an average introduction density, the *R. leguminosarum* population hardly varied after the fifth day. Moreover, the nodule bacteria population in the variant with the lowest introduction level increased by the end of the experiment. In this case, a tendency was observed for stabilization at the same level regardless of the dependence on the level of introduction of nodule bacteria, as indicated for the general case by the model. This indicates that the ecological strategy of nodule bacteria, in comparison with that of *Arthrobacter*, makes possible a more complete realization of potential possibilities for growth at a low initial abundance. The realization of these possibilities in the rhizoplane may be significantly determined by the capacity for migration (transition into a nearby favorable microzone and its assimilation) and other characteristics (growth rate, expenditures for maintenance, half-life during starvation, etc.). If the strategy characteristics are considered (May, 1976), it may be concluded that *Arthrobacter* is inclined toward a K-strategy and nodule bacteria on the same background are microorganisms with a more pronounced r-strategy.

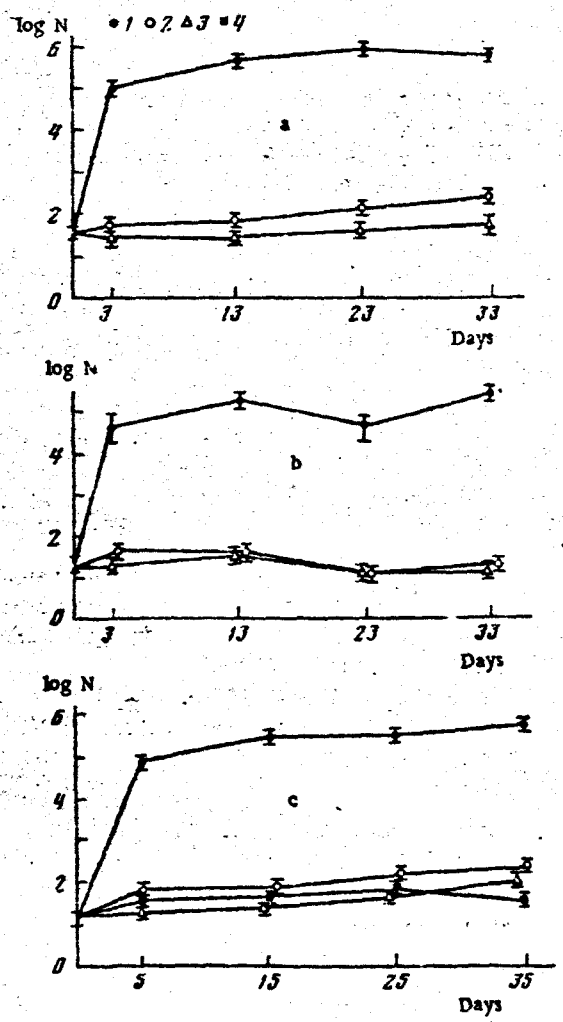

Fig. 3. Population dynamics for the soil bacteria complex. a) For introduction of *A. crystallopoietes* at all levels of initial population density; b) for introduction of *R. leguminosarum* at all levels of initial population density; c) without introduction of a population; 1) rhizoplane; 2) rhizosphere; 3) edaphosphere; 4) soil without plants; N = cell number/cm² of surface of roots for the rhizoplane and of soils for others.

Population Dynamics of *R. leguminosarum* and *A. crystallopoietes* in the Rhizosphere and Edaphosphere. No significant differences in bacterial abundance in the rhizosphere and edaphosphere were observed in all tests (Fig. 2). The *Arthrobacter* population density generally decreased during the first 3 days, and then remained approximately stable; the level of stabilization was higher if the introduced population density was higher. The abundance of nodule bacteria began to increase slightly in all variants, and then decreased. The decrease was more extensive at a higher population introduction level. Considering the theory on soil microzonality, we can suggest that most zones that nodule bacteria entered were unfavorable for them, which resulted in bacterial death. On the whole, population dynamics for the two populations in zones defined as the rhizosphere and edaphosphere differed essentially from the dynamics in the rhizoplane. The starting level was exceeded 10-, 100-, and even 1000-fold only in the rhizoplane.

Thus, our results do not contradict the conclusion that rhizoplane microflora is a powerful filter through which barely transmits root excretions into the rhizosphere and soil (Newman and Watson, 1977). The zone of microbial life with a high multiplication rate (about 10 h) is represented almost only by the root surface, and as one proceeds even a few millimeters from it the so-called rhizosphere effect is almost not observed.

Population Dynamics of Soil Bacteria in the Barley Root Zone after Introduction of *R. leguminosarum* and *A. crystallopoietes* to Soil. The population dynamics of soil bacteria, counted on meat–peptone agar, was the same in all

experiments and did not depend either or the species of bacteria introduced or on their population density (Fig. 3). As in the control experiment (without introduction of populations, Fig. 3c), the bacterial abundance in the rhizoplane increased considerably during the first 3-5 days (a generation time of about 10 h), and then increased slowly, reaching 10^6 cells/cm² of root by the end of the experiment (initial bacterial count was 13 cells/cm² of root). The bacterial number increased by not more than an order of magnitude in the rhizosphere and edaphosphere. This increase is evidently not related to the inflow of root secretions, since the bacterial population dynamics did not differ significantly from that in the control variant (moistened soil without plants). Consequently, a distinct difference between the behavior of bacteria in the rhizoplane, on the one hand, and in the rhizosphere and edaphosphere, on the other, was observed as in previous cases. It is noteworthy that even abundant introduction of *Arthrobacter* and nodule bacteria populations did not significantly alter the dynamics of the soil bacteria complex on meat–peptone agar; however, the effect of introduced microorganisms on rhizoplane and soil microflora deserves comprehensive analysis.

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MICROBIOLOGICAL PROCESSES IN THE MEROMICTIC LAKE SAKOVO

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The freshwater meromictic Lake Sakovo, located in the Vologda Oblast, was analyzed. Its maximum depth is 16 m. The lake contained unusually high concentrations of sulfate up to 816 mg/liter, but sulfate reduction intensity in the water was low, not more than 16 μg of H_2S/liter per day, whereas in the sediment 4.5 mg/liter of H_2S was produced per day. Bacterial sulfate reduction was limited by an organic substrate deficiency. The nonmixing layers of the monimolimnion contained not more than 11 mg of H_2S. The boundary of the hydrogen sulfide zone occurred at 3.5-4.5 m, which coincided with the thermocline and chemocline. In this area the water was green, which was determined by the growth of two species of green sulfur bacteria: *Pelodictyon luteolum* (maximum of $7.35 \cdot 10^6$ cells/ml) and *Chlorobium linicola*, which participates in a symbiotic complex with *Chlorochromatium aggregatum* (maximum of $0.42 \cdot 10^6$ aggregates/ml). Phototrophic bacteria synthesized 320 μg of C/liter per day at 4.25 m. Assimilation of carbon dioxide in the dark at the interface of hydrogen sulfide and oxygenated water comprised 100 μg of C/liter per day, and was evidently determined by the chemosynthesis of thionic bacteria (maximum count of 1000 cells/ml). In the water column, 9 mg of H_2S/m^2 per day and in the silt 500 mg of H_2S/m^2 per day were formed. Green bacteria oxidized about 268 mg of H_2S/m^2 and thionic bacteria 250 mg of H_2S/m^2 per day. Sulfur bacteria may obtain sulfide by syntrophism with sulfate-reducing and sulfate bacteria and from diffusion of H_2S from silt deposits.

Meromictic lakes, the water column of which is always separated into aerobic and anaerobic zones, are a particularly suitable subject for studying microbiological processes involved in the sulfur and carbon cycles.

The specifics of physicochemical conditions in these water bodies are determined by the uniqueness of microbiological processes occurring in water and silt deposits and also by the microflora composition. Results from research performed on

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