ISSN 0026-2617, Microbiology, 2006, Vol. 75, No. 2, pp. 226–230. © Pleiades Publishing, Inc., 2006.
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EXPERIMENTAL ARTICLES

Colonization of Plant Rhizosphere by Actinomycetes of Different Genera

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Received May 26, 2005

Abstract—The survival of environmental isolates of actinomycetes introduced with the seeds of agricultural plants in root-free soil and in the rhizosphere and rhizoplane was studied. Different strategies of colonization of the rhizosphere were revealed for the representatives of the genera *Streptomyces, Micromonospora*, and *Streptosporangium*, organisms typical for the moderate climate rhizosphere. The plants of winter rye (*Secale cereale* L.) inoculated with actinomycetes were shown to have growth advantages, while the cow clover plants (*Trifolium pratense* L.) had no growth advantages compared to uninoculated plants. The role of the plant component in the interaction with mycelial prokaryotes is discussed.

DOI: 10.1134/S0026261706020184

Key words: actinomycetes, colonization, introduction, rhizosphere, winter rye, cow clover.

Since mycelial prokaryotes participate in the protection of the host plant from root infection, rhizosphere colonization by actinomycetes is of interest. According to direct microscopic studies, actinomycete mycelium constitutes 12 to 20% of the total bacterial biomass in the rhizosphere [1, 2]. Although they represent a minor component of the rhizosphere microbiota, many representatives of the order *Actinomycetales* exhibit pronounced lytic activity and are able to produce antibiotics and other biologically active compounds. The content of actinomycete mycelium is significantly higher in the root system of healthy plants than in the rhizosphere of diseased ones [1]. The authors maintain that increased numbers of mycelial prokaryotes on the roots is beneficial for the physiological condition of plants. A positive experience exists of using actinomycetes as agents for the biological control of phytopathogens [3–7].

The evaluation of the ability of actinomycetes of different taxonomic affiliation to colonize the root system of plants is essential for the development of efficient biopreparations using these organisms. In this respect, the field study of the process of root colonization by mycelial prokaryotes is certainly of interest. Simulation of this process under laboratory conditions of controlled temperature, humidity, and illumination is, however, also useful due to the possibility to eliminate external interference and to monitor the internal logics of the process. Investigation of the process of colonization of the roots of different plants by mycelial prokaryotes of different genera is important for understanding the role of the host plant in the associative interaction with actinomycetes.

The goal of the present work was a comparative investigation of the survival of actinomycetes of three typical rhizosphere genera in sterile sand, on the roots, and in the rhizosphere of winter rye and cow clover and the estimation of their effect on plant growth.

MATERIALS AND METHODS

The strains *Streptomyces globisporus* 1-K-4, *Micromonospora* sp. M-1k, and *Streptosporangium* sp. Stm-F, previously isolated from the rhizosphere of winter rye grown on soddy-podzolic soil, were the objects of the present study. These are representatives of the mycelial actinobacteria predominant in this environment [8].

The plants of Krona and Vyatka 2 winter rye (*Secale cereale* L.) and of Trio cow clover (*Trifolium pratense* L.) were used to investigate the population dynamics of the rhizosphere actinomycetes and the efficiency of seed inoculation. Rye seeds were sterilized with a mixture of 3% hydrogen peroxide and 96% ethanol (1 : 1), and clover seeds, with concentrated sulfuric acid. After a 15 min treatment, the seeds were washed thrice with sterile distilled water and germinated on agarized water in the dark at 25–27°C for two days.

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The germinated seeds were planted individually in test tubes (20 mm diameter, 200 mm height) and filled with moistured (10% v/w) sterile sand; the sand was sterilized by tyndallization as recommended by Segi [9]. Actinomycete suspensions were added to the sand together with the seeds; sand without plant seeds was used as a control.

Actinomycete cultures used for inoculation were grown in the liquid Gauze medium [10]. Since the population structure of actinomycetes with complex growth cycles can substantially affect the degree of substrate colonization, the spores and mycelia of each inoculum were assayed independently before and after 10-min heating at 55°C. A comparison of the number of colonies obtained from heated and unheated cultures demonstrated that the *Micromonospora* sp. and *Streptosporangium* sp. populations consisted of 100% mycelium; the majority (90%) of the *Streptomyces globisporus* population also consisted of mycelium; the spores constituted only 10% of the population.

The number of mycelial fragments per 1 g sandy substrate for *Streptomyces globisporus* 1-K-4 was $1.1–9.0 \times 10^6$ ($1.2 \times 10^6–1.0 \times 10^7$ including spores); for *Micromonospora* sp. M-1 and *Streptosporangium* sp. Stm-F, it was 2.0×10^3 and 0.2×10^2, respectively. Uninoculated plants were used as controls for the estimate of the effect of actinomycetes on plant growth from the height of the aerial portion.

The plants were grown in a light chamber (16-h day, 8-h night) at 18–22°C. The samples of the rhizosphere, rhizoplane, and of the root-free sandy substrates were taken on the 2nd, 6th, 9th, 14th, 21st, 27th, and 30th day after inoculation. Three to six plants (depending on their species and age) were removed aseptically from the test tubes; the root system was separated, and the excessive sand was removed from the roots. The roots with the remaining sand particles were washed for 5 min with shaking in a known volume of sterile water (rhizosphere samples), and then homogenized in a mortar (rhizoplane samples). Dilution series were prepared and inoculated onto a Gauze 1 medium for the enumeration of streptomycetes and onto sodium propionate medium [11] for the enumeration of *Streptosporangium* and *Micromonospora.* The mass of roots and sand was determined gravimetrically after filtration and drying of the filters.

The radial growth rate of actinomycetes was determined in an independent experiment, by measuring the daily increase in colony diameters in two perpendicular directions. The cultures were grown at 28°C on Czapek medium with 1.0, 10.0, 20.0, and 30.0 g/l sucrose. The radial growth rate of the colonies was calculated using the equation:

$$K_r = d_2 - d_1/t_2 - t_1,$$

where d_1 and d_2 (mm) are the colony diameters in the initial and final time, respectively; t_1 and t_2 (days) are the time of the initial and final measurements. The measurements were performed for 14 days in 10 repetitions.

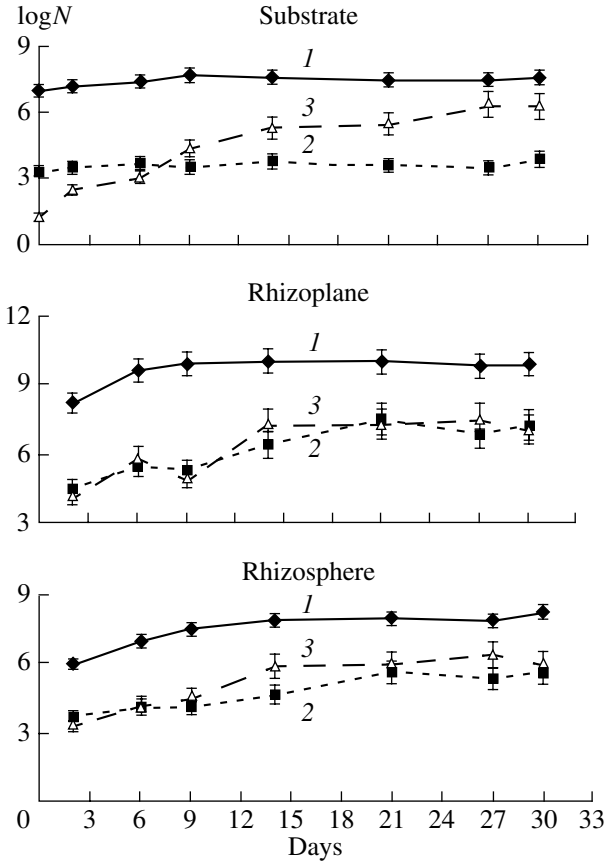

Fig. 1. Population dynamics (*N*, CFU/g) of the representatives of different genera of actinomycetes in the substrate and in the rhizosphere of Krona winter rye: (*1*) *Streptomyces globisporus* 1-K-4; (*2*) *Micromonospora* sp. M-1k; (*3*) *Streptosporangium* sp. Stm-F.

Standard procedures of dispersion and correlation analyses were applied to the data. Excel and STATGRAFICS software packages were used.

RESULTS AND DISCUSSION

The dynamics of the colonization of the sandy substrate and of the winter rye rhizosphere and rhizoplane by actinomycetes of different genera (*Streptomyces, Micromonospora*, and *Streptosporangium*) was the first issue investigated. Colonization of the favorable environments by actinomycetes with mycelial growth is less dependent on the initial population density than the colonization by nonmycelial bacteria. The existence of an initial "medium capacity" level independent of the initial number of actinomycetes was suggested [12]. The initial densities of inocula, compared in the experiments, were therefore not equalized.

The results are presented on Fig. 1. Inoculation of 0.2×10^2 mycelial fragments of *Streptosporangium* sp. Stm-F per 1 g substrate into sterile sand resulted in its increase by 4 orders of magnitude at the end of the experiment. The numbers of *Streptomyces globisporus*

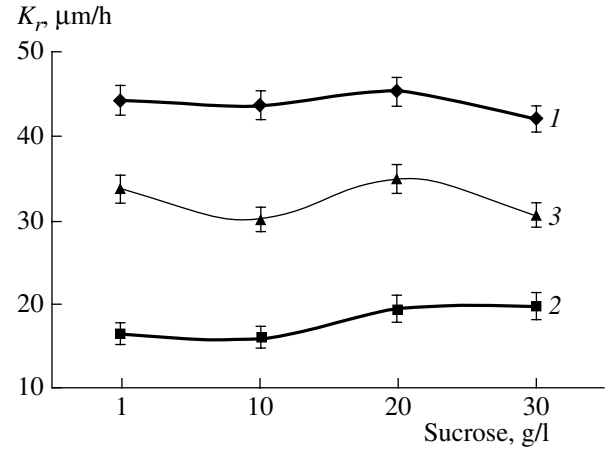

Fig. 2. Effect of sucrose concentration in the medium of the colony radial growth rate (K_r). The designations are the same as in Fig. 1.

1-K-4 and *Micromonospora* sp. M-1k did not differ significantly from the initial values (10^6 and 10^3 mycelial fragments, respectively) throughout the experiment. The stable population density for a prolonged period indicated a dynamic equilibrium between the resting and actively growing forms within the population. This notion was experimentally confirmed in a number of works [13–15], in which the development of *Streptomyces* cells introduced into nonsterile soil was monitored by plating and microscopy and by the differential immunofluorescence enumeration of spores and vegetative cells. The prolonged maintenance of the quantity of *Streptomyces* and *Micromonospora* in the root-free substrate was possibly due to the specific intrapopulational mechanism of autoregulatory inhibition revealed on poor media [16].

The rhizosphere is enriched with nutrients from plant root excretions. In this environment, the quantity of *Micromonospora* sp. M-1k increased by an order of magnitude on the second day of incubation; the numbers of *Streptosporangium* sp. Stm-F increased by 2 orders of magnitude. On the 30th day of incubation, the quantity of these strains reached 3.6×10^5 and 10^6 CFU/g, respectively. The quantity of *Streptomyces globisporus* 1-K-4 in the rhizosphere increased by more than 2 orders of magnitude during the experiment, form 10^6 to 2.14×10^8 CFU/g.

The dynamics of the actinomycete numbers on washed roots revealed that the colonization of the rhizoplane of the germinating seeds commenced immediately after inoculation; on the second day of the experiment, the numbers of *Streptosporangium, Micromonospora*, and *Streptomyces* reached 1.5×10^4, 1.7×10^4, and 10^8 per g roots, respectively. The subsequent dynamics of *Streptomyces globisporus* 1-K-4 was different from the dynamics observed for the two other genera. The density of the streptomycete population reached 10^9 CFU/g on the sixth day and remained at this level till the termination of the experiment. The dynamics of root colonization by the representatives of the genera *Micromonospora* and *Streptosporangium* exhibited fluctuations, caused possibly by the unbalance between the active and resting stages of their life cycle. In spite of the differences in the initial density, the quantities of both populations were practically identical by the end of the experiment, 10^7 CFU per 1 g roots.

Thus, the differences in the colonization of both the sandy substrate and the plant roots were determined by the taxonomic position of the strains investigated. This may be caused by the different ranges of growth rates in different genera of actinomycetes. The measurements of the radial growth rate (K_r) of the studied strains on agarized media with different sucrose concentrations revealed that the range for *Streptosporangium* was 28 to 37 μm/h, and for the *Micromonospora* strain, only 14 to 21 μm/h (Fig. 2). Although the representative of the genus *Streptomyces* exhibited the highest values of radial growth rate (40 to 47 μm/h), this value decreased at the highest sucrose concentration (3% sucrose). On the other hand, the values of radial growth rate for *Micromonospora* increased with increased sucrose concentration. The representative of the genus *Streptomyces* combined relative oligotrophy with rapid spreading; it was using the so-called "search strategy" [18]. The representative of the genus *Micromonospora* combined a preference for high substrate concentrations with the relatively low growth rates; it followed the "utilization of a rich microzone" strategy [18]. Therefore, the ecological niches differing in nutrients abundance were beneficial for these species. Thus, the population density of *Micromonospora* increased the most pronouncedly in the rhizosphere where the carbon of plant root excretions was present. The dynamics of the *Streptomyces* and *Streptosporangium* populations in the rhizosphere and in the root-free substrate were the same.

Apart from the growth rates of the vegetative cells, the different behavior of *Micromonospora* and the representatives of two other genera in the rhizosphere zone may be caused by the specific features of their reproductive structures. The efficiency of spreading by spores is certainly higher for streptomycetes and sporangial actinomycetes, than for the monosporous ones; this can explain the lower population density of the latter in the rhizosphere.

A direct effect of the carbon content of the medium on K_r was not found for the representative of the genus *Streptosporangium.* The number of *Streptosporangium* CFU increased equally in the substrates with and without root excreta; compared to the initial level, this increase was higher for *Streptosporangium* than for the representatives of two other genera.

The different genus-dependent character of actinomycetes growth dynamics in their colonization of the rhizosphere, the rhizoplane, and the root-free substrate is in accordance with the properties of their ecological niches as determined in [17] and for the representatives of

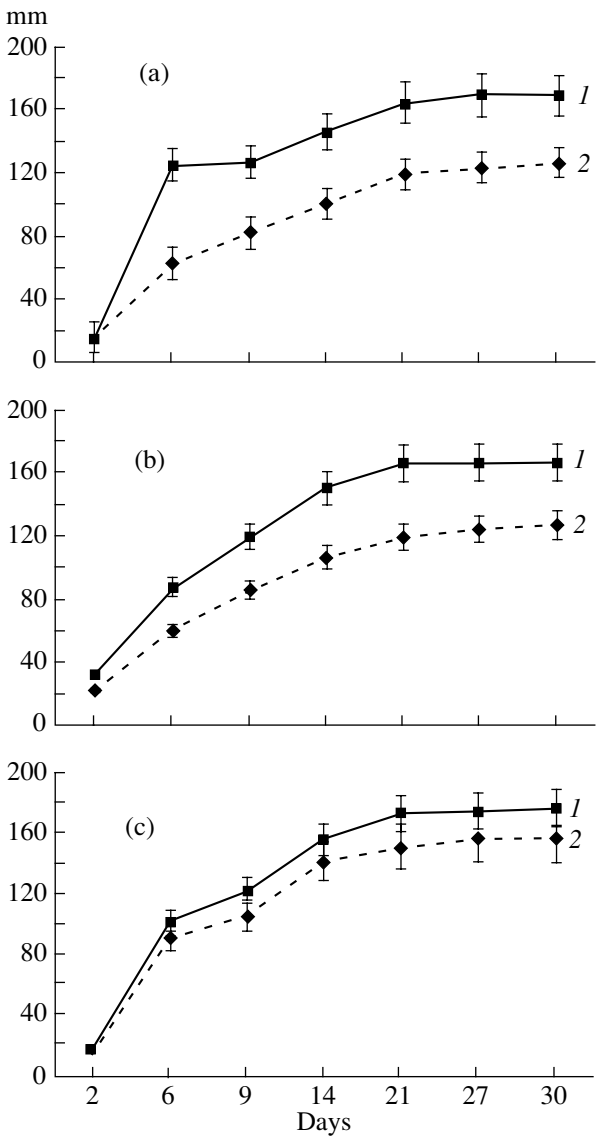

Fig. 3. Height of the Krona winter rye plants grown from the seeds treated with the natural isolates of actinomycetes: (a) *Streptomyces globisporus* 1-K-4; (b) *Micromonospora* sp. M-1k; (c) *Streptosporangium* sp. Stm-F. *1*, inoculation; *2*, control.

the genera *Streptomyces, Streptosporangium*, and *Micromonospora*, on the basis of their kinetic characteristics and of the results of the multisubstrate testing. In the reported multidimensional space of the studied characteristics, the ecological niches for the genera *Streptosporangium* and *Micromonospora* were closer to each other than each of them, to the niche of *Streptomyces.*

Since the strains in the present work were selected as predominant in the winter rye rhizosphere complex, it was important to determine their effect on plant growth. The tests have demonstrated that the winter rye plants grown from the seeds inoculated with the cultures of *Streptomyces globisporus, Micromonospora* sp., and *Streptosporangium* sp. exhibited better initial

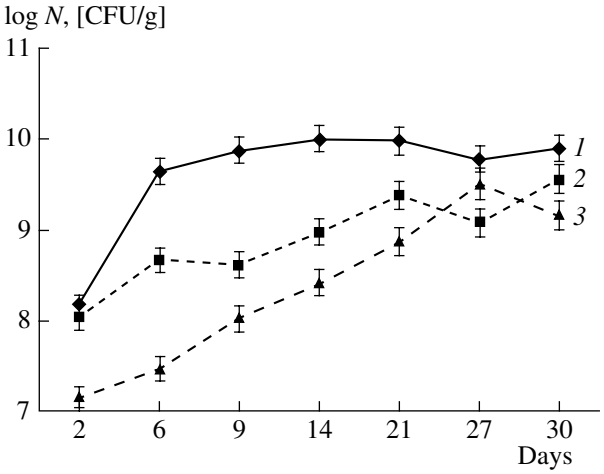

Fig. 4. Dynamics of the colonization of the roots of (*1*) Krona winter rye; (*2*) Vyatka 2 winter rye; (*3*) Trio cow clover by strain *Streptomyces globisporus* 1-K-4.

growth compared to uninoculated plants (Fig. 3). A close correlation was found between the density of root colonization by actinomycetes and the height of winter rye plants (r = 0.79–0.83 at $P \geq 0.95$). The stimulatory effect of the natural isolates on plant linear growth may be the result of the ability of actinomycetes to produce the substances of a hormonal nature. Actinomycetes in pure cultures are known to produce almost all of the known phytohormones [19].

The study of the dynamics of *Streptomyces globisporus* 1-K-4 colonization of the roots of different species and varieties of plants was the next goal of our work. The interaction between the streptomycete and the germinating seeds of three plant partners (Krona and Vyatka 2 winter rye, Trio cow clover) was investigated. The density of inoculum (10^6 CFU/g) was the same for all the plants. The results demonstrated that the curves of the actinomycetes population density on the roots of both varieties of winter rye were mostly similar; the numbers gradually increased (Fig. 4). The numbers of actinomycete mycelial fragments in the rhizoplane of Krona winter rye, the host plant from which the strain was isolated, was always significantly higher than in the rhizoplane of the Vyatka 2 winter rye. On the roots of Trio cow clover, the density of *Streptomyces globisporus* 1-K-4 was the lowest. During the first three weeks, the density of actinomycete population was much lower on clover roots (10^7 CFU/g) than on winter rye roots (10^8–10^9 CFU/g); later, the densities of actinomycete populations were of the same order of magnitude (10^9 CFU/g).

The Vyatka 2 plants inoculated with the strain *Streptomyces globisporus* 1-K-4, which was isolated from the Krona variety rhizosphere, did not have any growth advantages compared to the uninoculated plants (Fig. 5). The clover plants inoculated with the same strain had a lower height than the control plants after 14 days of cultivation. These results suggest a certain selectivity of colonization of living roots by actinomycetes; it possibly depends on the characteristics of a

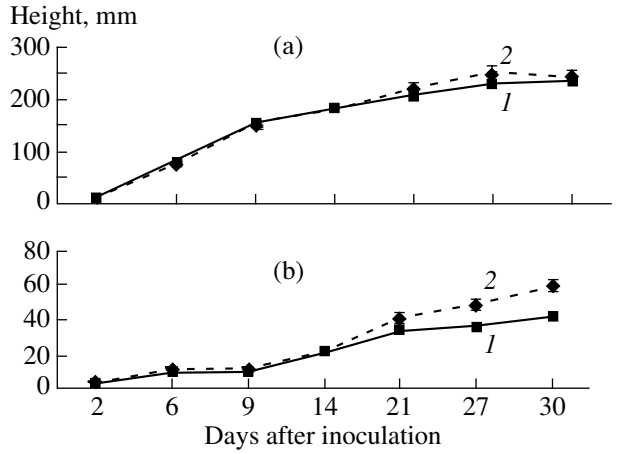

Fig. 5. Effect of the strain *Streptomyces globisporus* 1-K-4, introduced with the seeds on the height of the aerial parts of the Vyatka 2 winter rye (a) and Trio cow clover (b) introduced with the seeds (*1*) compared to control plants (*2*).

plant species and even varieties. Although the mechanism of this selectivity is complex and not yet understood, our results have demonstrated that selective compatibility of the partners can be observed at the early stages of plant–actinomycete association.

Colonization of plant roots and rhizosphere by actinomycetes depends, therefore, on the type of ecological strategy used by the representatives of the different genera of mycelial prokaryotes, as well as on the characteristics of the host plant species and variety. These data must be considered during the development of the principles and procedures of actinomycetes-based biopreparation production, for the enhancement of plant growth and for protection against phytopathogens.

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