Microbiology, Vol. 69, No. 1, 2000, pp. 109–112. Translated from Mikrobiologiya, Vol. 69, No. 1, 2000, pp. 127–131.

EXPERIMENTAL ARTICLES

Dynamics of an Oligosporous Actinomycete Population in Chernozem Soil

G. M. Zenova, N. V. Mikhailova, and D. G. Zvyagintsev

Department of Soil Biology, Soil Science Faculty, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia

Received March 3, 1999; in final form, June 9, 1999

Abstract—Investigation of the dynamics of an oligosporous actinomycete population in chernozem soil in the course of succession induced by soil wetting allowed us to reveal the time intervals and conditions optimal for the isolation of particular oligosporous actinomycetes. Saccharopolysporas and microbisporas proved to be best isolated in the early and late stages of succession, whereas actinomycetes of the subgroup *Actinomadura* and saccharomonosporas could be best isolated in the early and intermediate stages of succession.

Key words: oligosporous actinomycetes, succession, population dynamics.

At present, the group of actinomycetes and related microorganisms comprises more than 100 genera [1], among which only few have been ecologically studied. In particular, this group includes actinomycetes that produce spores on the aerial or aerial/substrate mycelium, which can be fragmenting or not. Spores, whose diameter is sometimes greater than that of hyphae, occur singly or in short chains composed of no more than ten spores. Oligosporous actinomycetes of the genera *Saccharopolyspora*, *Saccharomonospora*, and *Thermomonospora* are predominant in self-heating substrates (hay, grain, straw, compost) [2], of the genus *Actinomadura*, in chernozem and chestnut soils [3], of the genus *Microbispora*, on plant leaves [4]. Many oligosporous actinomycetes produce biologically active substances, such as antibiotics, vitamins, and enzymes [5, 6]. Actinomycetes that produce extracellular hydrolases are involved in the degradation of cellulose, chitin, and humic substances [7].

The aim of the present work was to study the dynamics of an oligosporous actinomycete population in chernozem soil in the course of succession induced by wetting and to determine the time periods and succession conditions optimal for the isolation of particular actinomycetes from soils.

MATERIALS AND METHODS

Samples of chernozem soil (horizon Ad) were collected at the Kursk State Reservation. To study the dynamics of a soil actinomycete population in the course of induced succession, air-dried soil (40 g) was freed of rootlets and foreign inclusions, ground in a porcelain mortar, and sieved through a screen with 1-mm mesh sizes. The soil was placed in petri dishes, thoroughly mixed, and wetted to 30% of the field moisture content [8]. The dishes with soil were incubated at 20–22°C in desiccators (to maintain the aforementioned moisture content of soil), which were opened each two days for 30 min. Samples of soil were taken at the time intervals indicated below. The experiment was carried out in duplicate.

The population of actinomycetes was assessed by plating soil suspension dilutions onto agar media of the following composition (g/l): KH_2PO_4, 0.46; Na_2HPO_4, 0.73; KNO_3, 0.1; NaCl, 0.29; $MgSO_4$, 0.1; $CaCO_3$, 0.02; sodium propionate, 0.2; and agar, 20. The medium was supplemented with trace elements (mg/l) $FeSO_4$, 0.2; $ZnSO_4$, 0.18; and $MnSO_4$, 0.02, as well as 1.5 μg/ml nalidixic acid, 1.0 μg/ml rubomycin, 50 μg/ml nystatin, and 4 mg/l thiamine. Soil samples were plated at the moment of the initiation of succession (zero time) and 7, 14, 21, 28, and 42 days afterwards [9]. Prior to plating, soil samples were heated for 1 h at 120°C. The plates were incubated for 3–4 weeks at 28°C and analyzed under a light microscope (400×) for the total number of actinomycete colonies and for the number of their particular morphotypes differing in the type of mycelium (aerial or substrate) and sporophore branching, the presence or absence of sporangia, and in the arrangement of spores occurring either singly, or in pairs, or in chains.

Each actinomycete morphotype was isolated in pure culture and provisionally identified according to *Bergey's Manual* [10] based on morphological characteristics (the presence of the septate or branched mycelium, the arrangement of spores on the aerial and/or substrate mycelium, the number of spores in chains, the diameter and motility of spores, and the presence of sporangia) and chemotaxonomic characteristics (the presence of L- or *meso*-isomer of diaminopimelic acid (DAPA) and characteristic sugars in whole-organism hydrolysates [11]).

0026-2617/00/6901-0109$25.00

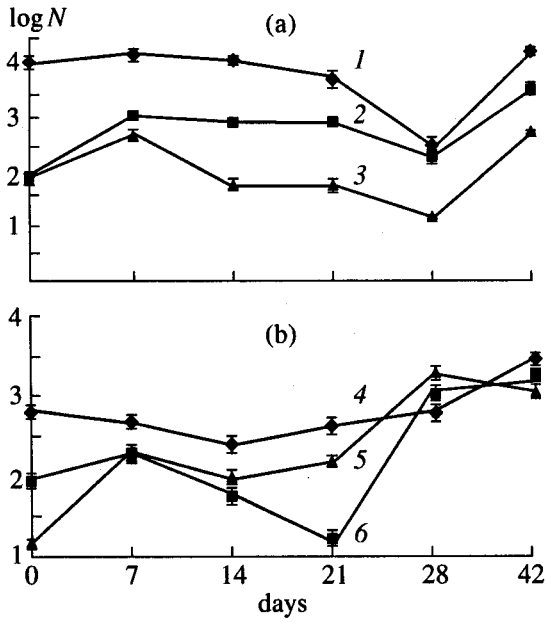

Fig. 1. Dynamics of the population of actinomycetes in chernozem soil in the course of succession induced by soil wetting: (*1*) *Streptomyces*, (*2*) *Saccharopolyspora*, (*3*) *Microbispora*, (*4*) *Micromonospora*, (*5*) *Actinomadura*, (*6*) *Saccharomonospora*.

RESULTS

The succession induced in chernozem soil involved streptomycetes, micromonosporas, and oligosporous actinomycetes. Among the actinomycetes, the following four phenetic groups were distinguished: (1) Actinomycetes producing paired spores on the aerial mycelium; the substrate mycelium is nonfragmenting; the cell wall of chemotype IIIB. (2) Actinomycetes producing short chains of spores positioned perpendicular to the central hypha of the aerial mycelium; the aged substrate mycelium is fragmenting; the cell wall of chemotype IV. (3) Actinomycetes producing monospores on the aerial mycelium; the fragmentation of mycelium is indistinct; the cell wall of chemotype IV. (4) Actinomycetes producing short chains of spores on the hooked or irregularly spiral (1–4 turns) aerial mycelium; substrate mycelium is nonfragmenting; the cell wall of chemotype IIIB.

The prevalent member of succession was the genus *Streptomyces*. The population of streptomycetes was maximum on the 7th and 42nd day after the initiation of succession in soil (see Fig. 1a), and minimum on the 28th day of succession.

The genus *Micromonospora* ranked second in the abundance in chernozem soil. The maximum population density of micromonosporas in chernozem soil was observed on the 42nd day after the initiation of succession (see Fig. 1b).

In early terms of succession, oligosporous actinomycetes ranked third in their abundance in chernozem soil (see Fig. 1). The population of the 1st and 2nd phenetic groups of oligosporous actinomycetes was maximum on the 7th and 42nd day after the initiation of succession and minimum on the 28th day of succession (see Fig. 1a).

The population of members of the 3rd phenetic group of oligosporous actinomycetes peaked on the 7th and on the 28th to 42nd day after the initiation of succession and was minimum on the 21st day of succession (see Fig. 1b).

At the moment of the initiation of succession, the 4th phenetic group was the least abundant. The population of this group peaked on the 7th and 28th day of succession and showed a slight decrease on the 14th day of succession (see Fig. 1b).

It should be noted that the population dynamics of streptomycetes and actinomycetes of the 1st and 2nd phenetic groups were similar: their population density peaked in the early and late terms of succession and was minimum on the 28th day of succession.

Unlike actinomycetes of the 1st and 2nd phenetic groups, oligosporous actinomycetes of the 3rd and 4th groups resembled micromonosporas in population dynamics. Thus, the population of all of these actinomycetes peaked on the 7th and on the 28th to 42nd day of succession and was minimum on the 14th to 21st day of succession.

Oligosporous actinomycetes of the 1st phenetic group (strains 4, 8, 9) were characterized by the presence of the nonfragmenting branched aerial mycelium with longitudinally positioned paired spores on short sporophores; the diameter of spores was greater than that of hyphae; the substrate mycelium contained no spores. Whole-organism hydrolysates were found to contain *meso*-DAPA and madurose. When grown on nutrient agar, these actinomycetes produced leathery colonies with the colorless substrate mycelium and thin dirty-white aerial mycelium. Based on these data, oligosporous actinomycetes of the 1st phenetic group were provisionally classified into maduromycetes of the genus *Microbispora*.

Oligosporous actinomycetes of the 2nd phenetic group (strains 16, 17, 18) were characterized by well-developed thin, branched, septate vegetative mycelium, which separated into rod-like elements in the aged regions of colonies. The aerial hyphae bore short chains of 6 to 8 spores that resembled beads separated by sections of "empty" hypha; the diameter of the spores was greater than that of the hyphae; spore chains occurred perpendicular to the central hypha; whole-organism hydrolysates contained *meso*-DAPA, arabinose, and galactose. Colonies were thin, colorless, and slightly wrinkled; the dirty-white aerial mycelium was undeveloped. Actinomycetes of this phenetic group were provisionally identified as nocardioforms

of the genus *Saccharopolyspora* of the subgroup *Pseudonocardia* and related genera.

Oligosporous actinomycetes of the 3rd phenetic group (strains 1, 2, 3, 5) produced solitary spores on the aerial mycelium; whole-organism hydrolysates contained *meso*-DAPA, arabinose, and galactose. Colonies were leathery and colorless; the thin and powdery aerial mycelium was white, then turned white-greenish. Actinomycetes of this phenetic group were provisionally identified as nocardioforms of the genus *Saccharomonospora* of the subgroup *Pseudonocardia* and related genera.

Oligosporous actinomycetes of the 4th phenetic group (strains 12, 14, 19) were characterized by the nonfragmenting substrate mycelium; the short chains of arthrospores (up to 8 in number) on the aerial mycelium were straight, hooked, and sometimes irregularly spiral; whole-organism hydrolysates contained *meso*-DAPA and madurose. Leathery colonies were cream-colored; the aerial mycelium was white–pink. Actinomycetes of this group were provisionally classified into maduromycetes of the subgroup *Actinomadura.*

DISCUSSION

Earlier, the population of actinomycetes of the genera *Streptomyces* and *Micromonospora* was investigated in various soils [12–15].

Analysis of succession and the selective isolation of oligosporous actinomycetes allowed us to gain a better insight into the diversity of this group of microorganisms in chernozem soil. Investigation of the dynamics of the rare genera of actinomycetes in the course of succession induced by soil wetting made it possible to determine the time intervals and conditions optimal for the isolation of particular oligosporous actinomycetes. Actinomycetes that were provisionally identified as saccharopolysporas and microbisporas proved to be best isolated in the early (7 days after the initiation of succession) and late (42 days of succession) stages of succession, whereas actinomycetes of the subgroup *Actinomadura* and saccharomonosporas could be best isolated on the 7th and 28th day of succession.

The rare genera of actinomycetes, including those which we placed into the group of oligosporous actinomycetes, have been isolated by different methods, namely, by plating the suspensions of air-dried soil on agar media with propionate or pea meal [16], by phage typing [16], and by succession analysis. It should be noted that actinomycetes of the genus *Saccharomonospora* were revealed in chernozem soil only in the course of succession induced by soil wetting. Actinomycetes of the genus *Saccharopolyspora* were found in chernozem through phage typing and in the course of succession induced by wetting. In the late stages of succession (42 days), the population density of saccharopolysporas in the soil reached thousands of CFU/g soil, which is comparable with the population density of streptomycetes (tens of thousands of CFU/g soil). At the same time, the population of saccharopolysporas determined by plating the suspensions of air-dried soil on solid nutrient media did not exceed hundreds of CFU/g soil [16].

To conclude, succession analysis combined with the selective isolation of microorganisms made it possible to reveal the presence of actinomycetes in soil and to determine the time intervals and conditions under which the population of actinomycetes is maximum.

It should be noted that the enumeration of actinomycetes in soils by plating them on agar media with propionate is inadequate. However, a comparison of the results of the enumeration of poorly studied oligosporous actinomycetes in soil with the respective data for well-studied streptomycetes and micromonosporas can be useful.

REFERENCES

1. Stackebrandt, E., Rainey, F.A., and Ward-Rainey, N.L., Proposal for a New Hierarchic Classification System *Actinobacteria* classis nov., *Int. J. Syst. Bacteriol.*, 1997, vol. 47, no. 2, pp. 479–491.
2. Agre, N.S., *Sistematika termofil'nykh aktinomitsetov* (Systematics of Thermophilic Actinomycetes), Pushchino, 1986.
3. Galatenko, O.A., Preobrazhenskaya, T.P., and Terekhova, L.P., Isolation of Actinomaduras from Various Soils, *Poisk produtsentov antibiotikov sredi aktinomitsetov redkikh rodov* (Search for Antibiotic Producers among Actinomycetes Belonging to Rare Genera), Alma-Ata: Gylym, 1990, pp. 13–19.
4. Kizuka, M., Enokita, R., *et al.*, Studies on Actinomycetes in Plant Leaves, *Theses 9th Int. Symp. Biol. Actinomycetes, 1994*, Moscow, 1994, p. 232.
5. Kawamura, N., Kinoshita, N., Sawa, R., Takahashi, Y., Sawa, T., Naganawa, H., Hamada, M., and Takeuchi, T., Pyralomicins, Novel Antibiotics from *Microtetraspora spiralis*: 1. Taxonomy and Production, *J. Antibiot.*, 1996, vol. 49, pp. 706–709.
6. Hessler, P.E., Larsen, P.E., Constantion, A.I., Schram, K.H., and Weber, J.M., Isolation of Isoflavones from Soy-Based Fermentations of the Erythromycin-producing Bacterium *Saccharopolyspora erythraea, Appl. Microbiol., Biotechnol.*, 1997, vol. 47, no. 4, pp. 398–404.
7. *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecology, Isolation, and Applications*, Ballows A. *et al.*, Eds., Springer-Verlag, 1991.
8. *Metody pochvennoi mikrobiologii i biokhimii* (Methods in Soil Microbiology and Biochemistry), Moscow: Mos. Gos. Univ., 1991.
9. Kozhevin, P.A., *Mikrobnye populyatsii v pochve* (Microbial Populations in Soil), Moscow: Mos. Gos. Univ., 1989.
10. *Bergey's Manual of Determinative Bacteriology*, Holt, J.G., Krief, N.R., *et al.*, Eds., Williams & Wilkins, 1994.

11. Hasegawa, T., Takizawa, M., and Takida, S., A Rapid Analysis for Chemical Grouping of Aerobic Actinomycetes, *J. Gen. Appl. Microbiol.*, 1983, vol. 29, pp. 319–322.

12. Sorokina, L.E., Zenova, G.M., and Kozhevin, P.A., Successional Changes in the Taxonomic Composition of Actinomycetes, *Mikrobiologiya,* 1991, vol. 60, no. 6, pp. 165–171.

13. Babich, T.L., Zenova, G.M., and Kozhevin, P.A., Successional Changes and Overlapping of Ecological Niches in the Actinomycete Complex in Chernozem soil, *Mikrobiologiya*, 1994, vol. 63, no. 2, pp. 294–297.

14. Babich, T.L., Zenova, G.M., Sudnitsyn, I.I., and Kozhevin, P.A., Successional Changes in the Actinomycete Complex of Peaty Soil, *Mikrobiologiya,* 1996, vol. 65, no. 1, pp. 117–127.

15. Polyanskaya, L.M., Microbial Succession in Soil, *Doctoral (Biol.) Dissertation*, Moscow: Moscow State University, 1996.

16. Likhacheva, A.A., Mikhailova, N.V., and Zenova, G.M., Methods to Reveal in Soil Actinomycetes of Rare Genera, *Vestn. Mosk. Univ. Ser. 17: Pochvoved.*, 1998, no. 1, pp. 44–48.