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EXPERIMENTAL ARTICLES

A Chitinolytic Actinomycete Complex in Chernozem Soil

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Abstract—A chitinolytic actinomycete complex in chernozem soil has a specific taxonomic composition, which differs from that of the actinomycete complex typically isolated on standard nutrient media containing sugars and organic acids as carbon sources. The actinomycete complex that was isolated by using nutrient media with chitin as the source of carbon and nitrogen was dominated by representatives of the genus *Streptosporangium*, and the actinomycete complex that was isolated by using nutrient media with sugars and organic acids as the carbon sources was dominated by representatives of the genus *Streptomyces*. The confirmation of the ability of actinomycetes to utilize chitin as a sole source of carbon and nitrogen came from the augmented length and biomass of the mycelium, the increased number and biomass of the actinomycete spores, the production of carbon dioxide, and the accumulation of NH_4^+ ions in the culture liquid of the actinomycetes grown in the nutrient media with chitin.

Key words: chitinolytic actinomycetes, streptomycetes, actinomycetes of rare genera.

The ability to degrade chitin is widespread among soil streptomycetes, many of which have been found to contain extracellular chitinases [1–3]. The enrichment of soil with chitin or the chitin-containing cell walls of fungi augments the number of streptomycetes [4]. In acidic soils and forest floors with a high productivity of fungi, streptomycetes play an important role in the degradation of the fungal mycelium. With the degradation of chitin in these media, the soil pH increases because of the liberation of alkaline metabolites (such as NH_4^+ ions), thereby inducing a successional substitution of neutrophilic actinomycetes for acidophilic actinomycetes [5]. Due to their high chitinase activity, soil actinomycetes can utilize recalcitrant chitin as a source of nitrogen.

The ability of the actinomycetes of rare genera to utilize chitin, the taxonomic composition of the chitinolytic actinomycete complex, and its significance among other chitin-degrading soil microorganisms are as yet poorly studied [6]. This prompted us to investigate the ability of soil actinomycetes to utilize chitin as a sole source of carbon and nitrogen and to study the taxonomic composition of the chitinolytic soil actinomycete complex.

MATERIALS AND METHODS

Samples of ordinary chernozem soil were taken from horizon A1 on the territory of the Kamennaya Step (Stony Steppe) Reserve in the Voronezh oblast.

Chitin-degrading microorganisms were isolated from the soil samples by using a nutrient medium with chitin [7]. Microbiological analysis included the determination of the relative abundances of mycelial prokaryotes and total soil bacteria, the total number of actinomycetes (expressed in colony-forming units (CFU) per g soil), and the relative abundances of different bacterial taxa.

Actinomycetes were differentiated microscopically at a magnification of ×400 by determining the type of mycelium produced (either aerial or substrate), the presence of single, double, or multiple spores on the mycelium, and the occurrence of sporangia. The colonies assigned to particular colonial morphotypes were enumerated separately, and some of the colonies of each morphotype were isolated in pure cultures.

Actinomycetes were cultivated by using oat extract agar and Gauze 2 medium [8]. To study the morphological properties of the isolated strains, they were grown by the groove method [9], and the specimen slides with the grown mycelium were examined under an optical microscope. To study the chemotaxonomic properties of the isolated strains, their whole-cell homogenates were analyzed for the presence of the LL- and *meso*-isomers of diaminopimelic acid (DAPA) and for certain (the so-called differentiating) sugars, which were identified by a modified version of ascending thin-layer chromatography in a cellulose layer [10]. The actinomycete isolates were preliminarily identified to a generic level according to the morphological and chemotaxonomic criteria presented in Bergey's Manuals [11, 12]. The conclusive identification of the actinomycete isolates requires the determination of such chemotaxonomic parameters as the cellular contents of menaquinones, fatty acids, and phospholipids.

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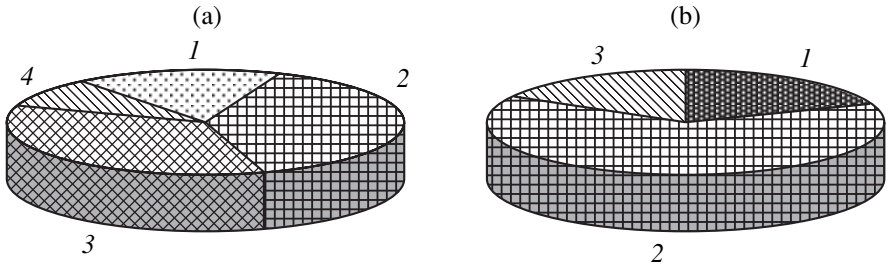

Fig. 1. The taxonomic composition of the actinomycete complex that was isolated from chernozem soil by using the nutrient media with (a) chitin and (b) sodium propionate: (*1*) *Micromonospora*, (*2*) *Streptomyces*, (*3*) *Streptosporangium*, and (*4*) the group of the allied genera *Actinomadura*, *Microtetraspora*, and *Nonomuraea*.

The isolates were preliminarily assigned to the genus *Streptomyces* if they showed the following characteristics: (1) the presence of vegetative hyphae from 0.5 to 2.0 μm in diameter, which formed an occasionally fragmented and heavily branched aerial mycelium with chains composed of 3 to 50 nonmotile spores; (2) the presence of straight or spiral sporophores, which were either monopodial or verticillate; and (3) the presence of LL-DAPA and the absence of differentiating sugars in the whole-cell homogenates.

The isolates were preliminarily assigned to the genus *Micromonospora* if they exhibited the following characteristics: (1) the presence of a well developed, branched substrate mycelium with hyphae about 0.5 μm in diameter and single sessile spores or spores that occurred on sporophores, which were often arranged in bundles; (2) the presence (if present at all) of a sterile and relatively undeveloped aerial mycelium; and (3) the presence of *meso*-DAPA, xylose, and arabinose in the whole-cell homogenates.

The isolates were preliminarily assigned to the genus *Streptosporangium* if they displayed the following characteristics: (1) the presence of a nonfragmented substrate mycelium with spheric sporangia up to 10 μm in diameter and of an aerial mycelium with spiral sporophores, which contained sessile spores and (2) the presence of *meso*-DAPA and madurose in the whole-cell homogenates.

The isolates were preliminarily assigned to the group of the allied genera *Actinomadura*, *Microtetraspora*, and *Nonomuraea* if they manifested the following characteristics: (1) the presence of a nonfragmented substrate mycelium and a relatively undeveloped aerial mycelium with the short chains of spores in the form of hooks or irregular spirals, which had one to four turns; (2) the diameter of spores exceeded the diameter of hyphae; and (3) the whole-cell homogenates contained *meso*-DAPA, madurose, galactose, glucose, mannose, and ribose.

The streptomycete isolates were identified to the species level according to the criteria presented in the manual of actinomycetes [8].

The degradation rate of chitin by the actinomycetes that were grown in the presence of this substance as the sole source of carbon and nitrogen was determined from the increase in the length and biomass of the mycelium and in the number and biomass of the actinomycete spores and by measuring the production rate of

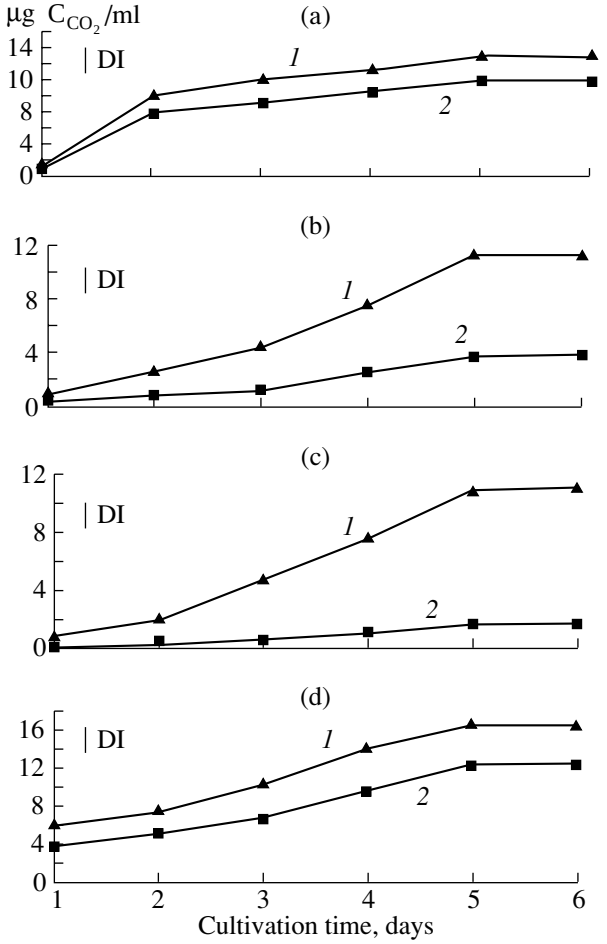

Fig. 2. The dynamics of the carbon dioxide production by the pure cultures of (a) *Micromonospora* sp., (b) *S. albolongus*, (c) *Streptosporangium* sp., and (d) the isolate belonging to the group of the allied genera *Actinomadura*, *Microtetraspora*, and *Nonomuraea* that were grown (*1*) in the medium with chitin and (*2*) on non-nutrient agar. DI is dispersion index.

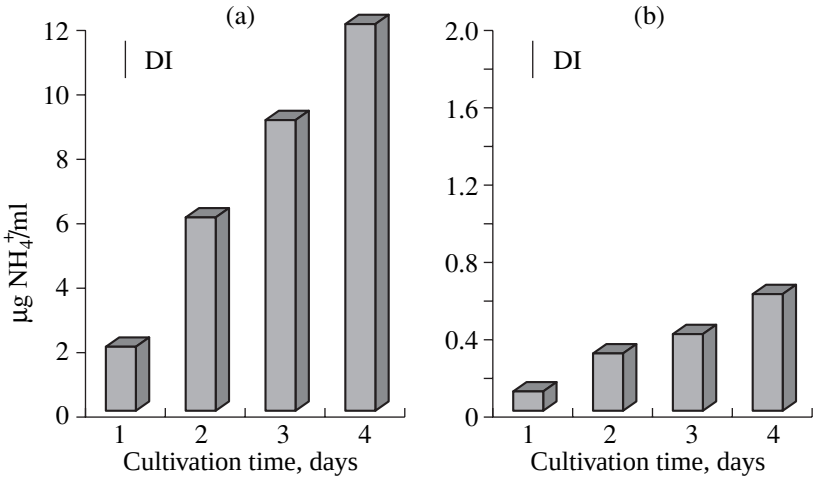

Fig. 3. The accumulation of NH_4^+ ions in the culture liquid of (a) *Micromonospora* sp. and (b) *Streptosporangium* sp. that were grown in the medium with chitin.

carbon dioxide and the accumulation rate of NH_4^+ ions in the culture liquid of the actinomycetes. In these experiments, the actinomycetes were grown in penicillin flasks with liquid media, which were inoculated with the suspensions of monosporous actinomycetes containing 10^6 spores per milliliter [13].

The biomass and length of the mycelium and the number of actinomycete spores were determined by using a Lyumam-I3 luminescence microscope as described by Polyanskaya [14].

The accumulation of carbon dioxide in the headspace gas of the chitin-grown actinomycetes was determined by using a gas chromatograph equipped with a thermal conductivity detector and a 3-m column packed with Polysorb-1. The carrier gas was helium at a flow rate of 25 ml/min. The production of carbon dioxide by pure actinomycete cultures was studied by the method described in the handbook [15], using non-nutrient agar as the control medium.

The accumulation of NH_4^+ ions in the culture liquid of actinomycetes grown with chitin was studied colorimetrically by using a medium with sucrose as the control [16].

RESULTS AND DISCUSSION

The number of actinomycetes grown on the chitin-containing nutrient agar inoculated with the appropriate soil suspension dilutions was found to be 7.55×10^3 CFU/g soil, which comprised 64% of the total number of soil bacteria grown on the same medium. For comparison, the relative number of mycelial prokaryotes amounts to 40–50% of the total bacteria of chernozem soil grown on standard actinomycete media with sucrose, starch, or casein as the sole source of carbon [7].

The commonly isolated actinomycete genera were *Streptomyces*, *Micromonospora*, *Streptosporangium*, and a group of the allied genera *Actinomadura*, *Microtetraspora*, and *Nonomuraea* (Fig. 1a), with the genus *Streptosporangium* being dominant (47% of all the isolates). The fraction of the genera *Streptomyces*, *Micromonospora*, and the group of the allied genera *Actinomadura*, *Microtetraspora*, and *Nonomuraea* was 30, 15, and 8%, respectively.

The chitinolytic actinomycete complex had a specific taxonomic composition, which differed from that of the conventional actinomycete complex isolated by using the standard medium with sodium propionate [7] (Fig. 1b). Specifically, the conventional actinomycete complex was represented by three genera (*Streptomyces*, *Micromonospora*, and *Saccharomonospora*) with occurrence rates of 64, 19, and 17%, respectively, whereas representatives of *Streptosporangium* and the group of the allied genera *Actinomadura*, *Microtetraspora*, and *Nonomuraea* were not detected at all.

The chitinolytic streptomycete complex was dominated by the species *S. candidus*, *S. albolongus*, and *S. sindenensis* from the series *Albus* of the section *Albus* and by the species *S. bikiniensis* from the series *Achromogenes* of the section *Cinereus.* It should be noted that the use of the standard casein–glycerol medium typically fails to detect the section *Albus* in the streptomycete complex of ordinary chernozem soil [7].

The mass of the mycelia of the *Streptosporangium*, *Streptomyces*, and *Micromonospora* actinomycetes that were cultivated in the medium with chitin for 7 days was 23.4, 23.4, and 15.6 μg, respectively. In this case, the actinomycetes of the genus *Streptosporangium* produced spores in the greatest amount (104 μg), which can be explained by the specific life cycle of these actinomycetes. The total mass of the mycelium and spores

of the *Streptosporangium* actinomycetes was also the greatest among the chitinolytic actinomycetes.

In general, the amount of carbon dioxide produced by the *Streptosporangium*, *Streptomyces*, and *Micromonospora* actinomycetes cultivated in the medium with chitin was greater than when they were incubated in the control medium (Fig. 2). For instance, the content of C_{CO_2} in the headspace gas of the *Micromonospora* actinomycete that was incubated in the medium with chitin for 4 days reached 15 μg C_{CO_2}/ml as compared with 10 μg C_{CO_2}/ml accumulated during the incubation of the same actinomycete in the control medium.

The most intense accumulation of NH_4^+ ions was observed for the *Micromonospora* actinomycetes that were grown in the nutrient medium with chitin (Fig. 3). For instance, by the 4th day of cultivation, the concentration of NH_4^+ ions in the culture liquid of *Micromonospora* sp. reached 20 μg/ml, while the concentration of these ions in the case of *Streptosporangium* spp. was only 1 μg/ml. The actinomycetes of the genus *Streptomyces* that were cultivated in the medium with chitin did not produce NH_4^+ ions. Nor were these ions detected in the culture liquids of the actinomycetes that were cultivated in the control medium with sucrose.

Thus, the chitinolytic actinomycete complex of chernozem soil has a specific taxonomic composition, which differs from that of the actinomycete complex which is typically isolated on the standard nutrient medium with sodium propionate as the carbon source. The actinomycete complex that was isolated by using the nutrient medium with chitin as the source of carbon and nitrogen was dominated by representatives of the genus *Streptosporangium*, and the actinomycete complex that was isolated by using the nutrient medium with sugars and organic acids as the carbon sources was dominated by representatives of the genus *Streptomyces*. The confirmation of the ability of actinomycetes to utilize chitin as the sole source of carbon and nitrogen came from the augmented length and biomass of the mycelium, the increased number and biomass of the actinomycete spores, the production of carbon dioxide, and the accumulation of NH_4^+ ions in the culture liquid of the actinomycetes that were grown in the nutrient media with chitin.

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REFERENCES

1. Skujns, I., Pukite, A., and McLaren, A.D., Chitinase of *Streptomyces* sp.: Purification and Properties, *Enzymologia*, 1970, vol. 39, pp. 353–370.
2. Tiunova, N.A., Pirieva, L.A., Feniksova, R.V., and Kuznetsov, V.D., The Production of Chitinase by Actinomycetes Grown in Submerged Cultures, *Mikrobiologiya*, 1976, vol. 45, no. 1, pp. 246–248.
3. Tiunova, N.A., Pirieva, L.A., and Feniksova, R.V., The Production and Properties of Chitinase in *Actinomyces kursanovii*, *Mikrobiologiya*, 1976, vol. 45, no. 4, pp. 43–646.
4. Williams, S.T., Hatfield, M.L., and Mayfield, C.Y., New Methods for the Observation of Actinomycetes Growing on Artificial and Natural Substrates, *The Actinomycetes*, Prauser, M., Ed., Jena: Gustav Fisher, 1970, pp. 379–399.
5. Williams, S.T. and Robinson, C.S., The Role of Streptomycetes in Decomposition of Chitin in Acid Soils, *J. Gen. Microbiol.*, 1981, vol. 127, pp. 55–63.
6. *The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd ed., Balows, A. *et al.*, Eds., New York: Springer, 1991, pp. 921–1157.
7. Zvyagintsev, D.G. and Zenova, G.M., *Ekologiya aktinomitsetov* (The Ecology of Actinomycetes), Moscow: GEOS, 2001.
8. Gauze, G.F., Preobrazhenskaya, T.P., Sveshnikova, M.A., *et al., Opredelitel' aktinomitsetov* (Manual of Actinomycetes), Moscow: Nauka, 1983.
9. Prokof'eva-Bel'govskaya, A.A., *Stroenie i razvitie aktinomitsetov* (The Structure and Development of Actinomycetes), Moscow: Akad. Nauk SSSR, 1963.
10. Hasegawa, T., Takizawa, M., and Tanida, S., A Rapid Analysis for Chemical Grouping of Aerobic Actinomycetes, *J. Gen. Appl. Microbiol.*, 1983, vol. 29, pp. 319–322.
11. *Bergey's Manual of Systematic Bacteriology*, Williams, S.T. *et al.*, Eds., Williams & Wilkins, 1989, vol. 4.
12. *Bergey's Manual of Determinative Bacteriology*, 9th ed., Holt, J.G. *et al.*, Eds., Baltimore: Williams & Wilkins, 1994. Translated under the title *Opredelitel' bakterii Berdzhi*, Moscow: Mir, 1997.
13. Kuznetsov, V.D., A Study of the Variability of Actinomycetes Producing Antibiotics and Other Biologically Active Substances, *Antibiotiki*, 1972, no. 17, pp. 666–671.
14. Polyanskaya, L.M., Microbial Succession in Soil, *Doctoral (Biol.) Dissertation*, Moscow: Moscow State Univ., 1996.
15. *Metody pochvennoi mikrobiologii i biokhimii* (Methods of Soil Microbiology and Biochemistry), Moscow: Mosk. Gos. Univ., 1991.
16. Durynina, E.P. and Egorov, V.S., *Agrokhimicheskii analiz pochv, rastenii, udobrenii* (The Agrochemical Analysis of Soils, Plants, and Fertilizers), Moscow: Mosk. Gos. Univ., 1998, pp. 18–19.