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EXPERIMENTAL ARTICLES

Acidophilic Soil Actinomycetes

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Abstract—A clear-cut dependence of the distribution of acidophilic actinomycetes on the pH value of soil was established. Acidophilic actinomycetes were found to be present in soils whose pH does not exceed 6.8 (acid forest soils, lowland peaty soil, and ordinary chernozem) and not in slightly alkaline soils (chestnut sodic and alluvial meadow soils). In acid lowland peaty soil, the species diversity of acidophilic streptomycetes was lesser than the species diversity of streptomycetes revealed in the same soil by using neutral medium.

Key words: acidophilic actinomycetes, acidotolerant actinomycetes, pH optimum for growth.

Until the investigations of Corke and Chase [1] and Khan and Williams [2, 3] had been published, all soil actinomycetes were believed to be neutrophilic. Even now, the ecology of acidophilic actinomycetes and their role in the functioning of the soil microbial complex are far from being well studied.

The most frequently encountered acidophilic actinomycetes belong to the genus *Streptomyces* [4, 5], which, in general, dominates fungal complexes in all types of soil [6]. The actinomycete complex of an acid tea field soil was found to contain acidotolerant streptomycetes and acidophilic nocardioforms [7]. Investigations also showed that acidotolerant actinomycetes, dominated by micromonosporas and streptomycetes, are invariably present in acid forest soils and chernozem [8].

This work was aimed at studying the distribution of acidophilic actinomycetes in acid, neutral, and alkaline soils.

MATERIALS AND METHODS

Investigations were carried out using samples of acid soils (gray and brown forest soils and lowland peaty soil), neutral soil (ordinary chernozem), and alkaline soils (chestnut sodic and alluvial meadow soils).

Actinomycetes were enumerated by plating soil suspension dilutions onto agar Gauze 1 medium [9] supplemented with nalidixic acid (1.5 μg/ml) to inhibit the growth of bacteria. The pH of the medium was adjusted to either 7.0 or 5.3 with phosphate buffer. The inoculated plates were incubated at 28–30°C for 2–3 weeks.

Grown colonies were enumerated differentially under an optical microscope using the following morphological criteria: the type of mycelium (aerial or substrate); the type of sporophore branching; the type of spore arrangement (single, in pairs, or in chains); and the presence of sporangia. Colonies of each morphotype were enumerated separately and used for the isolation of actinomycetes in pure cultures on oat agar [9]. This agar was also used for the cultivation of actinomycete isolates. The results of actinomycete count were expressed in colony-forming units (CFU).

The isolates were identified according to the identification criteria of Bergey's Manuals [10, 11] on the basis of their morphological and chemotaxonomic characteristics, such as the presence of L- or meso-diaminopimelic acid and differentiating sugars in cell hydrolysates [12].

Optimal and limiting values of pH for the growth of actinomycetes were determined from the radial growth rate of colonies cultivated on agar Gauze 1 medium whose pH was varied from 3 to 8 [10]. The buffer system of the medium included phosphate and citrate salts. The radial growth rate of a colony was calculated by the formula:

$$K_r = \frac{d_2 - d_1}{t_2 - t_1},$$

where d_1 and d_2 are the diameters of the colony at times t_1 and t_2 expressed in days.

RESULTS AND DISCUSSION

Microbiological analysis with the use of neutral media (pH 7.0) revealed the maximum number of actinomycetes (hundreds of thousands of CFU/g soil) in ordinary chernozem and alluvial meadow soil and the minimum number of actinomycetes (thousands of CFU/g soil) in peaty podzolic and brown forest soils. The smaller number (by two orders) of actinomycetes in the last two types of soil as compared with the first two soils can be explained by their low humus content (3–4%) and reductive conditions typical of peaty podzolic soils. Analysis with the use of acid media (pH 5.3) revealed the maximum number of actinomycetes (hun-

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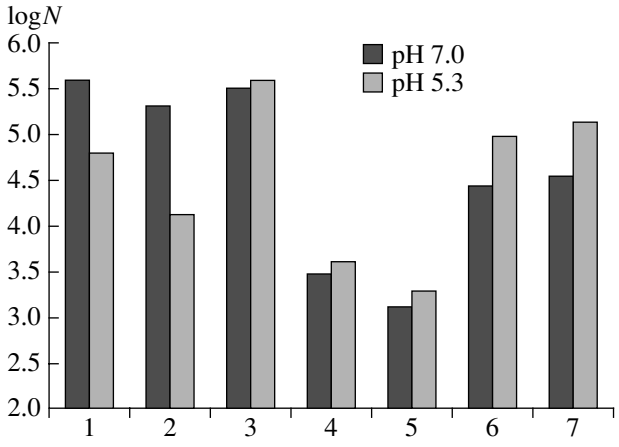

Fig. 1. The number of actinomycetes isolated at pH 7.0 and 5.3 from (1) alluvial meadow soil, (2) chestnut sodic soil, (3) ordinary chernozem; (4) peaty podzolic soil, (5) brown forest soil, (6) gray forest soil, and (7) lowland peaty soil. *N* is expressed in CFU/g soil.

dreds of thousands of CFU/g soil) in the ordinary chernozem and lowland peaty soil and the minimum number of actinomycetes (hundreds of CFU/g soil) in the peaty podzolic and brown forest soils (Fig. 1).

To express the proportion between the numbers of actinomycetes detected with the use of neutral and acid media, we used the so-called coefficient of acidophilicity (K_{ac}) defined as the ratio of the number of actinomycetes detected at pH 5.3 to their number detected at pH 7.0 (see table). The number of actinomycetes in the gray forest and lowland peaty soils detected at pH 5.3 considerably exceeded their number detected at pH 7.0 (Fig. 1). Correspondingly, the coefficient K_{ac} of these soils (3.5) was greater than of any other soil (see table). The number of actinomycetes in the brown forest and peaty podzolic soils detected at pH 5.3 was also larger than their number detected at pH 7.0 (Fig. 1), so that the coefficient K_{ac} of these soils was more than 1 (see table). In the case of ordinary chernozem, the numbers of actinomycetes detected at pH 7.0 and 5.3 were almost the same (Fig. 1), and the coefficient K_{ac} of this soil was close to 1 (see table). The number of actinomycetes in the slightly alkaline alluvial meadow and chestnut sodic soils detected at pH 5.3 was an order of magnitude smaller than their number detected at pH 7.0 (Fig. 1). Correspondingly, the coefficient K_{ac} of these soils was less than 1 (see table).

Thus, the acid and neutral soils under study are characterized by the coefficient $K_{ac} > 1$, whereas the slightly alkaline soils are characterized by $K_{ac} < 1$.

Depth-related changes in the coefficient K_{ac} were different for different types of soils. For chestnut sodic soil, whose acidity increases with depth, the coefficient K_{ac} tended to increase in this direction (see table). Conversely, the pH of the A1 horizon of the alluvial meadow soil is higher than that of the Ad horizon, and the coefficient K_{ac} tended to decrease with depth (see table).

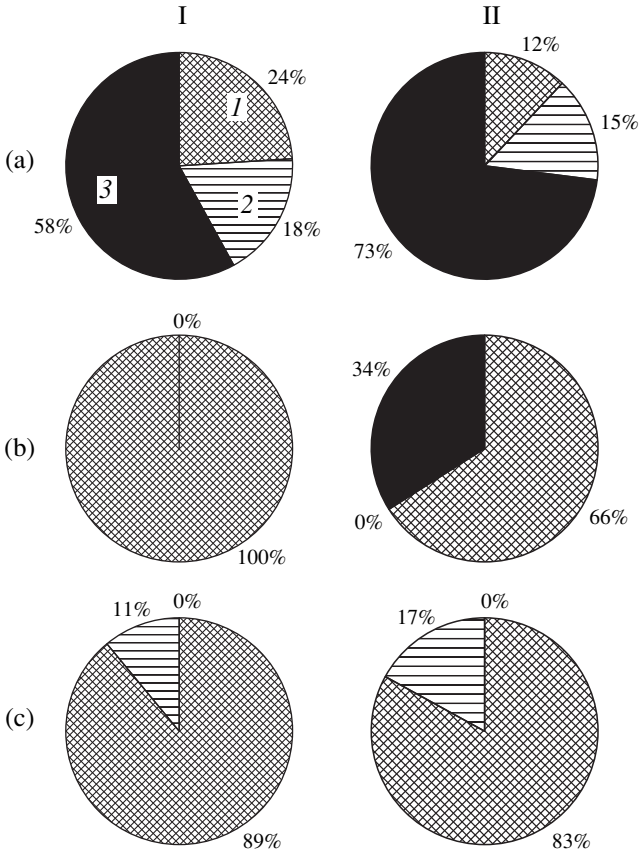

Fig. 2. The fraction of various actinomycete genera isolated at (I) pH 7.0 and (II) pH 5.3 from (a) lowland peaty soil, (b) chestnut sodic soil, and (c) ordinary chernozem: (*1*) *Streptomyces*, (*2*) rare genera, and (*3*) *Micromonospora*.

The data presented suggest that acid and neutral soils contain acidophilic actinomycetes, which show good growth when soil suspensions are plated onto acid media. Slightly alkaline soils lack acidophilic actinomycetes.

The taxonomic structure of actinomycete complexes is specific with respect to soil acidity (Fig. 2). The actinomycetes of the acid lowland peaty soil were found to be dominated by micromonosporas, and the chestnut sodic soil and ordinary chernozem were dominated by streptomycetes. The fraction of micromonosporas isolated from the lowland peaty soil at pH 5.3 was larger than at pH 7.0 (Fig. 2), indicating the predominance of micromonosporas among the acidophilic mycelial prokaryotes of this soil. The actinomycete complexes isolated from the ordinary chernozem at pH 5.3 and pH 7.0 were similar, except that the former complex contained a little more rare genera than the latter. The actinomycete complex isolated from the chestnut sodic soil at pH 5.3 contained micromonosporas, which could not be detected at pH 7.0 (Fig. 2).

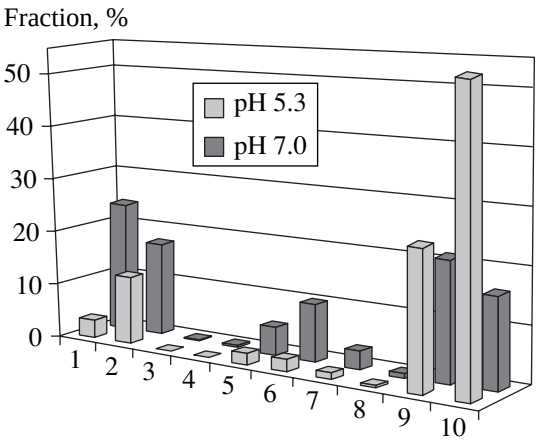

Fig. 3. The fraction of various species in the streptomycete complex of lowland peaty soil: (1) *Cinereus chromogenes*, (2) *Cinereus achromogenes*, (3) *Cinereus violaceus*, (4) *Cinereus chrysomallus*, (5) *Albus albus*, (6) *Albus albocoloratus*, (7) *Helvolo-flavus helvolus*, (8) *Roseus fradiae*, (9) *Roseus ruber*, and (10) *Imperfectus.*

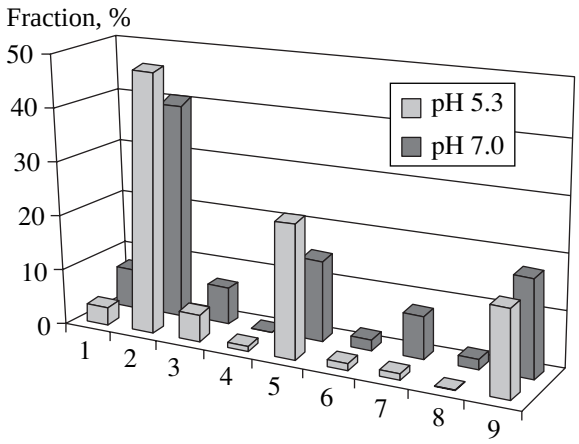

Fig. 4. The fraction of various species in the streptomycete complex of ordinary chernozem: (1) *Cinereus chromogenes*, (2) *Cinereus achromogenes*, (3) *Cinereus violaceus*, (4) *Cinereus aureus*, (5) *Albus albus*, (6) *Albus albocoloratus*, (7) *Helvolo-flavus helvolus*, (8) *Roseus ruber*, and (9) *Imperfectus.*

Streptomycetes are the most widespread actinomycetes in all terrestrial ecosystems nearly throughout their horizons [6]. Bearing this in mind, we analyzed the acidophilic streptomycete complexes of two contrasting (with respect to their pH) soils, namely, the lowland peaty soil and ordinary chernozem, and found that they were considerably different (Fig. 3). The Shannon diversity indices (*H*) of the streptomycete complexes isolated from the lowland peaty soil at pH 5.3 and 7.0 were 1.37 and 2.23, respectively. The former complex contained 6 times fewer species of the section *Cinereus* of the series *Chromogenes* than the latter. Acidophilic streptomycetes were dominated by species of the section *Imperfectus*.

The composition of the streptomycete complex of the ordinary chernozem, which is characterized by neutral pH, high humus content, and considerable buffer capacity, showed a weak dependence on the pH of the medium used for its isolation. Specifically, the number and the species diversity of streptomycetes isolated from this soil at pH 5.3 and 7.0 were the same (Fig. 4) (the Shannon diversity index of streptomycetes in these two cases was equal to 1.44 and 1.47, respectively).

Analysis showed that the streptomycetes that were isolated from various soils at pH 5.3 could grow at pH 4.0 to 8.0, with optimum growth at pH 5.0. As for the streptomycetes isolated at pH 7.0, they could grow at pH 5.0 to 9.0, with optimum growth at pH 6.0–7.0. It is evident that there is a correlation between the pH value at which streptomycetes were isolated and their pH optima for growth.

The boundary between acidophilic and acidotolerant actinomycetes is rather arbitrary. Both have identical lower limits of pH for growth (ca. 3.5) and identical

The number and acidophilicity of actinomycetes isolated from different soils through media with pH 5.3 and 7.0

Soil	Soil horizon and its pH		Number of actinomycetes isolated at pH 5.3, 10^3 CFU/g soil	Number of actinomycetes isolated at pH 7.0, 10^3 CFU/g soil	Acidophilicity coefficient (K_{ac})
Lowland peaty soil	T	5.3	120	34	3.5
Gray forest soil	A	4.5	88	25	3.5
Brown forest soil	A	3.7	1.8	1.2	1.5
Peaty podzolic soil	$A2_{white}$	5.1	4.0	2.7	1.5
Ordinary chernozem	A	6.8	360	300	1.2
Chestnut sodic soil	A1	8.0	13.3	193.3	0.07
	AB	7.5	6.6	53.3	0.12
	B_{cca}	7.6	6.6	13.3	0.5
Alluvial meadow soil	Ad	7.6	480	672	0.71
	A	8.1	66	360	0.18

optimum pH (ca. 5.5). The only difference between these groups of actinomycetes is the upper limit of pH for growth: true acidophilic actinomycetes cannot grow at pH > 6.5, whereas acidotolerant actinomycetes are able to grow at a pH up to 7.5 [2, 3]. It should, however, be noted that Williams *et al.* analyzed the actinomycetes that were isolated from wastes of coal and ore mines, which are more acidic substrates than soils. We believe that it makes no sense to distinguish acidophilic and acidotolerant soil actinomycetes, since soils represent heterogeneous systems with multiple microzones where pH may considerably differ from the average value. Soil actinomycetes adapt more easily to a given soil the wider the range of pH values suitable for this actinomycete. In light of this, it would be reasonable to suggest that the optimum growth pH of soil organisms, including actinomycetes, characterizes their acidophilicity better than the upper limit of pH for their growth. We propose to use the term acidophilic with respect to such soil actinomycetes which show optimal growth at pH 5.0, while the upper limit of pH for their growth may be close to pH 7.0. In fact, all soil actinomycetes that are isolated through media with acidic pH and are able to grow at pH 5.0 or lower can be considered to be acidophilic.

To conclude, acidophilic actinomycetes were found to be present in soils whose actual pH does not exceed 6.8 (acid forest soils, lowland peaty soil, and ordinary chernozem) and not in slightly alkaline soils (chestnut sodic and alluvial meadow soils).

ACKNOWLEDGMENT

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