Microbiology, Vol. 74, No. 4, 2005, pp. 471–476. Translated from Mikrobiologiya, Vol. 74, No. 4, 2005, pp. 545–551.

EXPERIMENTAL ARTICLES

The Saprotrophic Bacterial Complex in the Raised Peat Bogs of Western Siberia

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Received May 30, 2004

Abstract—The population density of bacteria in peat deposits along the landscape profile of the Vasyugan Marsh has been found to be as high as tens of millions of CFU/g peat. The abundance and diversity of bacteria increased with depth within the peat deposit, correlating with an increasing level of peat degradation. Variations in these parameters with depth and season were greater in peat deposits located in transaccumulative and transitional positions than in the sedge–sphagnum bogs located at the eluvial region of the profile. In the upper 1-m-thick layer of the peat deposits studied, bacilli, represented by five species, dominated, whereas, in the deeper layers, spirilla and myxobacteria prevailed. These bacteria are major degraders of plant polymers. Unlike the bacterial communities found in the peat deposits of European Russia, the dominant taxa in the studied peat deposits of western Siberia are represented by bacteria resistant to extreme conditions.

Key words: western Siberia, raised peat bogs, bacteria, plating method, cell number, diversity.

Earlier, we evaluated the microbial biomass pool in peat deposits along the landscape profile in the southern taiga subzone of western Siberia [1] and studied the structure of the micromycete complex found there [2]. The present work, in which we studied the taxonomic composition of the saprotrophic bacterial complex in this peat deposit, is a continuation of a series of microbiological investigations included in a plan of integrated research aimed at elaborating the scientific basis for regional monitoring of the Vasyugan Marsh [3].

Only a few studies have encompassed the bacterial complexes of the peat deposits of western Siberia [4, 5]; moreover, the data reported mainly concern the abundance of bacteria and the occurrence of various physiological groups in the upper layers of the peat deposits, the biological activity in soils, and the effect of ecological factors on these parameters. No complex studies of the bacterial community of the western Siberian peat deposits has been carried out that includes determination of the taxonomic structure on the basis of indices of bacterial abundance and diversity and investigation of the factors responsible for the dynamics of these parameters. Taking the above into consideration, we undertook the present investigation.

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MATERIALS AND METHODS

The investigations were carried out in the natural swampy ecosystem of the southern taiga subzone of western Siberia. The landscape profile crossed, in the direction of periphery to center, the following three types of peat bog biogeocenoses (BGCs): tall pine–shrub–sphagnum BGC (transaccumulative region, station 2), low pine–shrub–sphagnum BGC (transitional region, station 3), and sedge–sphagnum-bogged BGC (eluvial region, station 5) [3]. The profile, with geochemically conjugated landscape biogeocenoses, is the standard ecosystem of the Bakchar marshy region.

Samples for analyses were taken in spring, summer, and autumn (2002–2003). The thickness of the peat deposits varied from 0.9 m (st. 2) to 3 m (st. 3 and st. 5). Suspended peat samples (1 g in 100 ml of sterile water) were treated in a UZDN-1 ultrasonic disintegrator (22 kHz, 0.44 A, 2 min). Bacteria were enumerated by plating dilutions of the peat suspensions onto a modified glucose–peptone–yeast extract agar [6] in 5 replicates. The dishes were incubated at 20°C for 2–3 weeks. The total number of bacteria was expressed in colony-forming units (CFUs) per g of peat. Different macromorphological types of colonies were counted, and 3–5 colonies of each type were isolated in pure cultures. The isolates were identified to a generic level on the basis of morphological, cultural, and chemotaxonomic properties using *The Procaryotes* manual [7]. The group of

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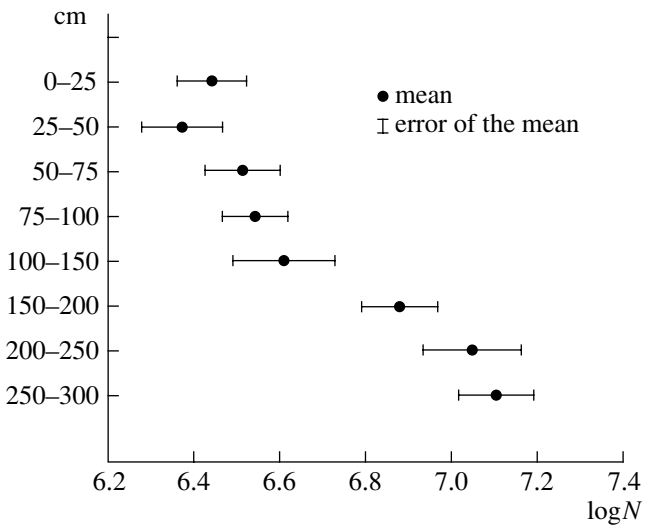

Fig. 1. Mean values of the bacterial numbers (*N*, CFU/g peat) along the landscape profile of the peat deposits studied.

saprotrophic aerobic and facultatively anaerobic bacteria capable of growth on glucose–peptone–yeast extract agar was chosen as a model.

The occurrence rate of the bacteria was defined as the ratio of the number of samples in which a given taxonomic group was detected to the total number of samples analyzed. The dominance rate was defined as the ratio of the number of samples in which a given taxonomic group was dominant (namely, when the number of colonies belonging to a given taxonomic group exceeded 30% of the total number of grown colonies) to the total number of analyzed samples.

The number and taxonomic composition of polymer-utilizing bacteria were determined by the replica plating method [8] with the use of media containing starch (to reveal amylolytic activity) or yolk (to reveal lecithinase, lipase, and protease activity). Aliquots (0.1 ml) of different dilutions of soil suspensions (from $1 : 10^1$ to $1 : 10^6$) were placed into the replicator wells in six replicates and then plated onto the above-mentioned polymer-containing media. Taking into account the volume of the drop plated onto the medium, the dilution, and the number of replicates, the most probable number of bacteria was calculated. The capacity of the bacteria for starch hydrolysis and the activity of lipase, protease, and lecithinase were determined by routine methods [9]. Specimens for microscopic examination were prepared from colonies grown after the replica plating. Pure cultures isolated by the replica plating method were identified using the same procedures as for the other isolates (see above).

RESULTS AND DISCUSSION

In the peat deposits studied, the numbers of bacteria determined by the plating method varied from 0.3 to 60 million CFU/g of peat, with the mean values ranging from 2 to 13 million CFU/g (Fig. 1). The population density of the bacterial groups increased gradually from the upper layers of raised peat deposits, characterized by a low level of peat degradation, through medium layers consisting of transitional peats, to the lower layers of lowland peats exhibiting a high level of degradation (Fig. 1).

The analysis of a large number of samples allowed us to compile two complete schemes of three-factor variance analysis (Table 1). The effect of the following three factors on the abundance of bacteria was evaluated: depth (the upper 1-m-thick layer or deeper peat layers); peat position in the landscape profile (transaccumulative, st. 2; transitional, st. 3; or eluvial, st. 5); and the season in which the samples were taken (spring, summer, or autumn).

The abundance of bacteria in the upper 1-m-thick layer depended on all three factors; however, the season in which the samples were taken had the strongest influence. The mean values of bacterial numbers were higher in the spring–summer period than in autumn; specifically, 2-fold higher in the sedge–sphagnum bog and 10- to 15-fold higher in the peat deposits located in the transaccumulative and transitional parts of the landscape profile (st. 2 and st. 3, respectively).

In the deeper peat layers, the number of bacteria was determined mainly by the peat position in the landscape profile. At depths of 1–3 m, the population densities of the bacterial groups were higher in the peat deposits located at st. 3 (transitional part of the profile) than in those located at st. 5 (eluvial part of the watershed slope, along which surplus surface-soil waters are discharged). A reliable correlation was also found between the cell number of bacteria in the deep layers and the season in which the samples were taken: in summer, the titer of the bacteria was fivefold to eightfold higher than in the other seasons; this seasonal dynamics suggests multiplication of bacteria at depths of 1–3 m.

Eight taxonomic groups were revealed in the bacterial communities: spirilla, myxobacteria, cytophagas–flavobacteria, sporocytophagas, bacilli, streptomycetes, and arthrobacters, which were represented by nonpigmented and yellow-pigmented species. The mean values of the Shannon diversity index varied from 0.1 to 2 bits in the peat deposits along the landscape profile.

In the upper 1-m-thick layer, bacterial diversity was mainly determined by the peat position in the landscape profile (Table 2). The highest values of the Shannon index were recorded in the peat deposits of the transaccumulative part of the profile (st. 2), which plays the role of a geochemical barrier. The lowest values of the Shannon index were typical of the sedge–sphagnum bog (st. 5), where, at a depth of 1 m, only one or two out of the eight taxonomic bacterial groups monitored were revealed. The peat deposit located in the transitional part of the profile (st. 3) represented, in its bacterial diversity, an intermediate point between the peat depo-

sits at stations 2 and 5. The depth within the peat deposit ranked second in influence on the bacterial diversity. In the shallow peat deposit at st. 2, the Shannon index increased with depth, whereas, in the thick peat deposits at st. 3 and st. 5, it remained at a minimum level. The seasonal dynamics of the bacterial diversity was revealed only in the peat deposit at st. 2 (the Shannon index was minimal in autumn). It is noteworthy that, in the deep peat layers (1–3 m), the bacterial diversity was more season-dependent than in the upper 1-m-thick layer (the Shannon index in the deep layers was fivefold to sixfold higher in autumn than in the other seasons). Next, in order of decreasing influence on bacterial diversity, were the peat position in the landscape profile and the depth within the peat deposit (Table 2).

Thus, the abundance and diversity of saprotrophic bacteria in the peat deposits along the landscape profile exhibited a statistically significant dependence on the depth within the peat deposit (the abundance and diversity of the bacteria increased with depth, i.e., with the extent of peat degradation); the peat position in the landscape profile (the parameters studied were more variable in the peat deposits occupying transaccumulative and transitional positions than in the sedge–sphagnum bog located in the eluvial part of the profile); and the season (the abundance of bacteria was higher in spring and summer, with the bacterial diversity reaching its maximum in spring in the upper 1-m-thick layer and in autumn in the deeper layers).

Three dominant taxonomic groups were revealed in the bacterial complex: spirilla, myxobacteria, and bacilli (Table 3). In the upper 1-m-thick layer of the peat deposits, bacilli usually dominated (their relative abundance was over 80%). Only in some seasons and at some depths did they give way to myxobacteria and spirilla. In the deeper layers, spirilla and myxobacteria dominated (Table 3). The dominant taxonomic groups were characterized by high occurrence rates (72–74%), whereas the occurrence rates of the other taxonomic groups varied from 10 to 28% (Fig. 2).

Bacilli were detected in all the peat horizons and evidently play the key role in the saprotrophic bacterial complex of the upper peat layers; their population density varied from 0.1 to 10 million CFU/g peat. The abundance of bacilli mainly depended on the depth within the peat deposit (the Fisher criterion (F) was equal to 2529.88 at a significance level $p < 0.0001$), with their primary occurrence being in the upper layers of the peat deposits. In all the peat samples studied, the population density of bacilli, as well as the magnitude of its variations, reached its maximum in spring.

The bacilli isolated from the peat samples belonged to five species: *Bacillus subtilis, B. licheniformis, B. pumilus, B. sphaericus*, and *B. polymyxa.* The occurrence rates of *B. subtilis, B. licheniformis*, and *B. sphaericus* amounted to 67, 52, and 23%, respectively, and that of *B. polymyxa* was 26%; however, the occurrence rate of the rose-colored *B. pumilus* did not exceed 17%. It should be noted that two of these species, *B. polymyxa* and *B. licheniformis*, were facultatively anaerobic. The species diversity of bacilli was highest in the upper 1-m-thick peat layer and ranged from 0.6 to 1.7 bits. The dominant species were *B. subtilis* and *B. licheniformis* (their dominance rates comprised 53 and 46%, respectively); the total level of the other species did not exceed 20%. In the deeper peat layers, bacilli were represented by only one species: *B. subtilis.* It should be noted that *B. subtilis* and

Table 1. Evaluation of the effect of three factors* on the number of bacteria in peat deposits along the landscape profile (on the basis of a three-factor variance analysis)

0–100 cm (st. 2, st. 3, and st. 5)			
Factors*	The number of degrees of freedom**	Variance	Fisher criterion
1	3**	0.26	71.84
2	2**	2.72	751.36
3	**2****	**13.55**	**3743.26**
12	6**	0.28	77.93
13	6**	0.45	125.37
23	4**	3.97	1096.76
123	12**	0.30	81.54
Residual	144	0.036	
100–300 cm (st. 3 and st. 5)			
Factors*	The number of degrees of freedom**	Variance	Fisher criterion
1a	3**	1.47	720.89
2	**1****	**8.71**	**4274.97**
3	2**	7.44	3651.43
1a2	3**	0.56	273.04
1a3	6**	0.34	166.45
23	2**	3.86	1894.68
1a23	6**	0.37	181.01
Residual	96	0.002	

* Factors and their gradation: 1 indicates depth within the peat deposit (0–25, 25–50, 50–75, or 75–100 cm); 1a, depth within the peat deposit (100–150, 150–200, 200–250, or 250–300 cm); 2, peat layer position in the landscape profile (transaccumulative at st. 2, transitional at st. 3, or eluvial at st. 5); 3, season (spring, summer, or autumn); 1a2, combined effect of factors 1 and 2, etc.

** Significant series of values (at a significance level <0.001).

Table 2. Evaluation of the effect of three factors* on the bacterial diversity (the Shannon index) in peat deposits along the landscape profile (on the basis of a three-factor variance analysis)

0–100 cm (st. 2, st. 3, and st. 5)			
Factors*	The number of degrees of freedom**	Variance	Fisher criterion
1	3**	1.78	100.99
2	**2****	**10.88**	**618.65**
3	2**	0.45	25.60
12	6**	1.38	78.77
13	6**	0.42	24.14
23	4**	1.62	92.17
123	12**	0.24	13.51
Residual	144	0.017	
100–300 cm (st. 3 and st. 5)			
Factors*	The number of degrees of freedom**	Variance	Fisher criterion
1a	3**	0.69	35.45
2	1**	1.92	98.73
3	**2****	**8.07**	**414.01**
1a2	3**	0.15	7.56
1a3	6**	0.51	26.13
23	2**	0.82	41.93
1a23	6**	0.25	12.99
Residual	96	0.019	

* Factors and their gradation are designated as in Table 1.
** Significant series of values (at a significance level <0.001).

B. licheniformis are typical inhabitants of the rhizosphere and plant residues. Since intense degradation of mosses and grasses occurs in various peat layers, the dominance of the aforementioned species of bacilli there is not surprising.

Variations in the number and diversity of bacilli depending on the season and depth within the peat thickness were typical of the peat deposits located in the transaccumulative and transitional parts of the landscape profile (st. 2 and st. 3), whereas the sedge–sphagnum bog was characterized by a stable high level of these parameters.

The replica plating method revealed the following regularities in the distribution of physiological groups of bacteria. Bacteria that exhibited protease, lipase, lecithinase, and amylase activity were revealed in all the seasons and at all the depths studied; their cell number varied from 2 to 600 thousand CFU/g peat. The maximum titer of these bacteria was detected in lowland peat deposits at depths of 2–3 m, where the peat was characterized by a high level of degradation. The proteolytic and lipolytic species belonged to spirilla, myxobacteria, and bacilli. Amylolytic activity was detected in myxobacteria, cytophagas, and streptomycetes. Myxobacteria occurred at all of the depths studied, streptomycetes were revealed in the upper 1-m-thick peat layer, and cytophagas were found in the deep layers, which represented lowland peat deposits. Thus, the dominant bacterial groups in the microbial communities of the peat deposits studied possessed a potential ability to degrade various polymers (starch, proteins, and lipids).

Thus, our study allowed us to reveal features of the spatial distribution, composition, and seasonal dynamics of the saprotrophic bacterial complex in the geochemically conjugated biogeocenoses of peat deposits occurring along the landscape profile. In our study, we followed the convention of distinguishing between active and inert layers of peat deposits and, therefore, considered the upper 1-m-thick layer and deeper peat layers (1–3 m) separately. However, we found that the seasonal variations in bacterial abundance and diversity took place throughout the whole peat massive, which casts doubt on the existence of a clear borderline between the active and inert layers. Moreover, in the thick peat deposits, the abundance and diversity of the bacterial complex were higher in the deep layers than in the upper 1-m-thick layer. This fact is due to the stratigraphic structure of the peat deposits studied, in which the deeper layers were lowland peats with a high level of degradation and the upper layers consisted of raised peats with a low degradation level.

The abundance and diversity of bacteria of the saprotrophic complex also depended on the peat position in the landscape profile. Variations in the number and diversity of bacteria depending the season and depth within the peat thickness were higher in peat deposits located at transaccumulative and transitional positions than in the sedge–sphagnum bog located in the eluvial part of the profile.

No significant difference was found between the Shannon diversity indices determined for the raised peat deposits of European Russia [11] and western Siberia. The bacterial diversity in peat deposits of different regions depended, to a greater extent, on the composition of the peats. The maximum and minimum values of the Shannon index were attained in lowland and raised peat deposits, respectively; in the transitional peat deposits, it exhibited an intermediate value.

However, the bacterial communities of the peat deposits of European Russia and western Siberia differed considerably in their taxonomic structure, namely, in relation to parameters such as the spectrum

Table 3. Dominants (making up over 30% of the total number of bacteria grown on the medium used) in the saprotrophic bacterial complexes in the peat deposits studied

Depth, cm \ Station	St. 2			St. 3			St. 5		
	spring	summer	autumn	spring	summer	autumn	spring	summer	autumn
0–25									
25–50									
50–75									
75–100									
100–150									
150–200									
200–250									
250–280									

Note: , bacilli; , myxobacteria and spirilla.

of potential dominants, occurrence of certain bacterial genera, and ratios between the component taxa. In the peat deposits of western Siberia, peat layers occurring at different depths were dominated by different bacterial taxa: bacilli played the key role in the upper peat layers, whereas spirilla and myxobacteria were abundant in the lower layers. In the peat deposits of Tver oblast, the domination of spirilla and facultatively anaerobic bacteria of the genera *Klebsiella, Plesiomonas, Aeromonas, Vibrio*, and *Proteus* was independent of the season and depth within the peat deposit; coryneform bacteria were represented by the genera *Rhodococcus, Cellulomonas*, and *Micrococcus* [11]; and no representatives of the genus *Arthrobacter* (typical pedobiont) were found in these biotopes, whereas, in peat deposits of western Siberia, arthrobacters occurred persistently as the single representative of coryneforms. The species compositions of bacilli were also different in these regions. In the peat deposits of Tver oblast, pigmented bacilli were represented by the *B. lentus–firmis* group, whereas, in western Siberia, they were represented by the *B. licheniformis–B. pumilus* group.

The above-mentioned differences in taxonomic composition of the bacterial communities of the peat deposits of European Russia and western Siberia are associated with the climatic features of these regions. Thus, the abruptly continental climate of Siberia is responsible for the domination, in peat deposits, of bacterial forms resistant to extreme impact, such as drying and freezing: these are bacilli and myxobacteria, which are able to form specialized resting structures (spores and cysts). Under the milder climate conditions of European Russia, in all seasons, the bacterial communities of peat deposits are dominated by facultatively anaerobic proteobacteria, represented by various genera of the families *Enterobacteriaceae* and *Vibrionaceae* [11]. These bacteria are able to survive only under more or less stable temperature, humidity, and redox potential values and have not been revealed in the peat deposits of Siberia, which were also characterized by a lower diversity of other bacterial species.

However, it should be noted that bacterial communities located in the peat deposits of different regions were characterized by certain common features, such as the abundance of spirilla and facultatively anaerobic bacilli (as a consequence of the high humidity) and the occurrence of pigmented forms of bacilli, which are usually associated with plants, particularly, mosses, and do not occur in soil.

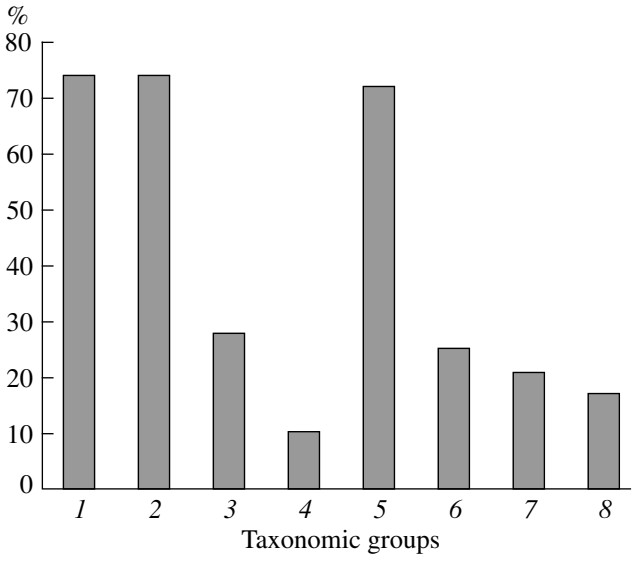

Fig. 2. Occurrence rates of taxonomic groups of bacteria in the peat deposits studied: (*1*) spirilla; (*2*) myxobacteria; (*3*) cytophaga–flavobacterium group; (*4*) sporocytophagas; (*5*) bacilli; (*6*) streptomycetes; (*7*) white arthrobacters; (*8*) yellow arthrobacters.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, grant nos. 01-05-64163 and 01-04-48249.

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