

# Biodegradation of Polyhydroxyalkanoates by Soil Microbial Communities of Different Structures and Detection of PHA Degrading Microorganisms

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**Abstract**—Biodegradation of microbial linear polymers of hydroxyalkanoic acids (polyhydroxyalkanoates, PHAs) by soil microbial communities of different structures has been studied during two field seasons in different weather conditions. This process was shown to be influenced by the polymer chemical composition, temperature, humidity, and the microbial soil component. The PHA degradation was accompanied by a decrease in the polymer molecular weight and an increase in the degree of crystallinity, indicating the preferential destruction of the amorphous phase compared to the crystalline one. The quantity of the true PHA destructors developing at the surface of the polymer samples was lower than the quantity of accompanying bacteria. The dominant PHA degrading microorganisms under the test conditions were identified as bacteria of the genera *Variovorax*, *Stenotrophomonas*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, and *Xanthomonas* and as micromycetes from *Penicillium*, *Paecilomyces*, *Acremonium*, *Verticillium*, and *Zygosporium*.

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The scale of production and application of synthetic polymers, which do not degrade in the environment, has reached 200 million tons per year, becoming a global ecological problem. Biodegradable polyesters of hydroxyalkanoic acids (polyhydroxyalkanoates, PHAs) synthesized by microorganisms are real candidates for the gradual substitution of synthetic plastics, together with polylactides. At present, many countries have pilot and small-capacity PHA plants producing the polymer under the following labels: Biopol™, Nodax™, DegraPol/btc®, and Mirel® [1, 2]. Due to the decline in the cost, PHA application is more and more directed toward the production of degradable containers, packages, and disposable dishware and household goods. As the volume of production increases and the fields of PHA application are extended, it becomes relevant to study the mechanisms of their destruction under various natural conditions.

Soil is an important natural reservoir for PHA degradation. However, most studies of PHA degradation in soil have been performed under laboratory conditions [3–10], whereas field studies are few. In one of the first works on PHA biodegradation under natural

conditions [11], it was noted that a golf peg made of this polymer was almost completely destroyed in soil during a period of four weeks; unfortunately, neither the exact PHA composition nor the soil characteristics were specified in the work. It is known that the process of PHA degradation depends on the type of soil. Thus, in mangrove and forest soils, the rate of polymer degradation differs by several times [12–14]. The influence of the quantity of soil microorganisms on the process of poly-3-hydroxybutyrate degradation in garden soil has been demonstrated [15]. However, works disclosing the mechanisms of PHA degradation in different types of soils with different microbial components are extremely few.

Microorganisms degrading the polymer of 3-hydroxybutyric acid (poly-3-hydroxybutyrate), which is the first and most studied representative of this family of polymers, were isolated more than 40 years ago [16]. Later, microorganisms degrading polymers with different carbon chain lengths of monomers were identified and characterized [4, 17–19]. However, it is still discussed which PHAs are degraded more actively and under which environmental conditions.

The goal of this work was to isolate PHA-degrading microorganisms and to study the degradation of polymers with different chemical structures by soil microorganisms in the root zones of coniferous and deciduous trees under changing temperature conditions of the environment.

## MATERIALS AND METHODS

**PHA characterization.** PHA samples of two types were investigated. One of them was a homopolymer of 3-hydroxybutyric acid (poly-3-hydroxybutyrate **PHB**) with a degree of crystallinity (DC) of 61% and a weighted average molecular weight ( $M_w$ ) of  $710 \pm 1.5\%$  kDa. The other one was a copolymer of 3-hydroxybutyric and 3-hydroxyvaleric acids (poly-3-hydroxybutyrate-3-hydroxyvalerate, **PHBV**) with the inclusion of 3-hydroxyvalerate 10 mol%, DC 50%,  $M_w$   $799 \pm 3.1\%$  (in the experiment of 2007), and  $680 \pm 1.1\%$  kDa (2010). Polymer samples were synthesized in a culture of *Wautersia eutropha* B5786. The strain is deposited at the Russian National Collection of Industrial Microorganisms [20, 21].

PHB and PHBV were sampled as film discs obtained by pouring chloroform PHA solutions onto polished surface followed by solvent evaporation (30 mm in diameter, 0.1 mm in thickness, and  $73 \pm 5$  mg in weight). The chemical structure of the PHAs was analyzed after methanolysis of the polymer samples in a 5975 Inert chromatograph mass spectrometer (Agilent, United States).

**PHA biodegradation.** Studies were carried out on the territory of the arboretum of the Sukachev Institute of Forest, Siberian Branch, Russian Academy of Sciences (Krasnoyarsk). Preweighted sterile PHA samples were placed in synthetic fine mesh fabric (mill gauze) covers at a depth of 5 cm in the root zones of a Siberian larch (*Larix sibirica* L.) and a drooping birch (*Betula pendula* L.). The sod-carbonate soil of the arboretum consisted of a humus layer of 10–15 to 30–40 cm and an underlying carbonate rock.

The studies were performed during two field seasons differing from each other in temperature conditions. The first season covered the period from July 2 to October 19, 2007; the second one was from June 7 to September 7, 2010. The second season was preceded by a severe winter with a mean winter air temperature of  $-22.1^\circ\text{C}$  (temperatures of up to  $-40^\circ\text{C}$  are typical of half of January and part of February), which was by  $13.5^\circ\text{C}$  less than the mean air temperature of the winter period before the first season ( $-8.6^\circ\text{C}$ ). The second season was notable for its late spring warming; the snow cover persisted until the end of April.

During the experiment, the covers with polymer samples ( $n = 3$  of each type) were pulled out of the soil; the samples were taken out, purified from soil residues by mechanical and enzymatic methods, washed with distilled water, and dried at  $40^\circ\text{C}$  for 24 h. The soil temperature, pH, and humidity were simultaneously

recorded using standard physicochemical methods [22]. The samples were weighed on an analytical balance of accuracy class IV (Mettler, United States). The molecular weight and the molecular weight distribution of PHA were studied using a Breeze System chromatograph for gel-penetrating chromatography (Waters, United States) relative to polystyrene standards (Fluka, Switzerland, Germany). The weighted average ( $M_w$ ) and average ( $M_n$ ) molecular weights and the polydispersity ( $PD = M_w/M_n$ ) were obtained. The degree of crystallinity of the polymers (DC, %) was determined by a D8 ADVANCE X-ray spectrometer (Bruker, Germany).

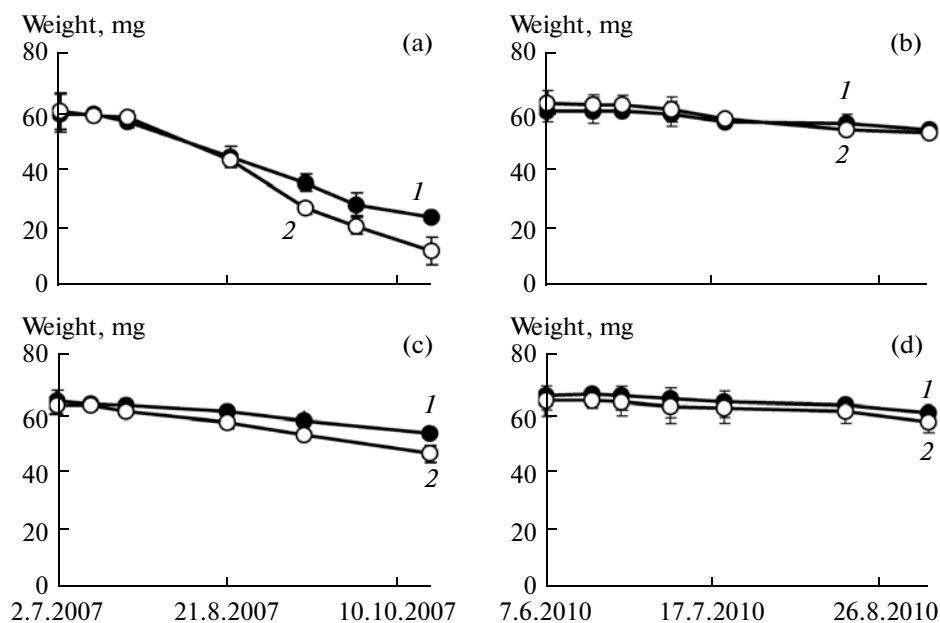
**Microbiological studies.** The soil was exposed to microbiological analysis at the moment when the samples were placed and three months later; in the latter case, control soil samples from the root zones of the birch and the larch at the sites of location of the PHA samples and washouts from their surface were taken for investigation. It was previously shown that the qualitative composition of the microflora isolated from the surface of PHB films did not differ from that isolated from the surface of PHBV copolymer films [4, 23]; therefore, the soil samples from the surface of both PHA types were not separated.

The soil microbial communities were characterized by the generally accepted methods of soil microbiology [22]. The total quantity of bacteria was determined on fish-peptone agar (**FPA**); the quantity of prototrophs and oligotrophs was determined on starch and ammonia agar (**SAA**) and soil extract agar (**SA**), respectively. Micromycetes were isolated on wort agar (**WA**). Soil suspension ( $10^4$ – $10^7$  dilution) was used for inoculation in three repeats. The plates were incubated for 3–7 days at  $30^\circ\text{C}$  in order to count the bacteria and for 7–10 days at  $25^\circ\text{C}$  to count the micromycetes. The dominant microorganisms were isolated and identified based on the cultural and morphological characters, using standard biochemical tests [24, 25]. During the two field seasons, 400 isolates were obtained and analyzed.

The soil micromycetes were identified by morphological characters (the structure and color of the colonies and the structure of the mycelium and fruiting organs) extensively used for identifying the systematic affiliation of these microorganisms [26–29].

The true PHA destructors were revealed by inoculation of the samples taken from the polymer surface onto a diagnostic medium containing 0.25% powder-like PHB as the only carbon source [30]. The growth of microorganisms possessing PHA-depolymerase activity was accompanied by the formation of typical transparent zones around the colonies. PHA degrading bacteria, in addition to the generally accepted morphological and biochemical studies, were selected for identification by 16S rRNA gene sequencing.

**DNA analysis.** DNA was isolated with an AquaPure Genomic DNA Isolation kit (Bio-Rad, United States). The 16S rRNA gene was amplified using the



**Fig. 1.** Dynamics of the decrease in the weight (mg) of polymer samples in the soil of the root zones of (a, b) the larch and (c, d) birch: (a, c) 2007, (b, d) 2010, (1) PHB, (2) PHBV.

universal primers 27F (5'-AGAGTTTGATCCTG-GCTCAG-3') and 1492R (5'-GGTTACCTTGT-TACGACTT-3') corresponding to positions 8–27 and 1510–1492 of the *Escherichia coli* gene. PCR was performed in a Mastercycler Gradient DNA amplifier (Eppendorf, Germany); a Doc Print transilluminator (Vilber Lourmat, France) was used for visualization and documentation. For the determination of the nucleotide sequence, the samples were sequenced by the Sanger method in an ABI PRISM 3100 genetic analyzer (Applied Biosystems, United States).

**Phylogenetic analysis.** The determined nucleotide sequences were compared with homologous sequences of strains from the GenBank and the EMBL and DDBJ databases using the NCBI BLAST software (<http://www.ncbi.nlm.nih.gov/BLAST/>), aligning them with ClustalX v. 2.08. A phylogenetic analysis was carried out by the Jukes–Cantor model in TREE-CON v. 1.3b [31].

The obtained nucleotide sequences (16 PHA-degrading microorganisms) were deposited in the GenBank database (nos. HQ689679–HQ689694).

## RESULTS AND DISCUSSION

The dynamics of the changes in the polymer sample weight presented in Fig. 1 shows that the process of PHA degradation depends on the chemical composition of the polymer and the location of the samples in soil.

The first field season was characterized by a significant increase in the soil temperature from 18 to 28°C in the initial dry period (July 2007) followed by a gradual decrease to 8–10°C at the end of the studies

(October 2007). The active reaction of soil was neutral or weakly alkaline under the larch (pH 7.1–8.1, with a short-term decrease to 6.7 in late July) and weakly acid (pH 6.1–7.1, with an analogous drop to 4.5) under the birch. The soil humidity from late July to late September was higher under the larch (18–28%) than under the birch (11–23%). The field season of 2010 was characterized by a soil temperature of 14–18°C in the first month of observations and exhibited a gradual increase to 26°C followed by a decrease to 13–18°C at the end of observations. pH of the soil under the birch (6.5–7.2) and larch (7.2–8.0) demonstrated insignificant fluctuations during the field season. The soil humidity was as a whole higher than in 2007, but also noticeably lower in the soil under the birch.

The initial soil microbial communities in the places of growth of the two tree species under study substantially differed in the total quantity (Table 1) and species composition. The total titer of aerobic microflora in early June 2007 under the larch was higher than under the birch, making  $(1.47 \pm 0.08) \times 10^9$  CFU/g soil. In three months, the total quantity of bacteria in the soil was  $(5.11 \pm 0.42) \times 10^9$  CFU/g soil under the larch and  $(2.21 \pm 0.24) \times 10^9$  CFU/g soil under the birch. The quantity of prototrophs and oligotrophs was also higher in the soil samples taken under the larch, where the coefficient of oligotrophicity ( $SA/FPA$ ) was 0.75, demonstrating the active assimilation and utilization of the nutrient elements from the dispersal zone.

The bacterial component of the soil microbiocenosis in the root zone of the larch was represented by the dominant bacteria *Alcaligenes* (25.0%), *Aureo-*

**Table 1.** Total quantity of bacteria in soil samples in the root zones of a larch and birch

| Soil sample                              | Total microbial number, CFU in 1 g of soil |                               |
|--|--|-------------------------------|
|  | 2007                                       | 2010                          |
| <i>L. sibirica</i>                       |  |                               |
| Root zone at the moment of PHA placement | $(1.47 \pm 0.08) \times 10^9$              | $(3.16 \pm 0.24) \times 10^6$ |
| Root zone at the end of the season       | $(5.11 \pm 0.42) \times 10^9$              | $(5.28 \pm 1.76) \times 10^7$ |
| PHA surfaces                             | $(1.60 \pm 0.04) \times 10^{11}$           | $(1.35 \pm 0.11) \times 10^8$ |
| <i>B. pendula</i>                        |  |                               |
| Root zone at the moment of PHA placement | $(1.33 \pm 0.47) \times 10^8$              | $(2.67 \pm 0.16) \times 10^5$ |
| Root zone at the end of the season       | $(2.21 \pm 0.24) \times 10^9$              | $(6.77 \pm 4.51) \times 10^7$ |
| PHA surfaces                             | $(1.29 \pm 0.08) \times 10^9$              | $(1.96 \pm 0.05) \times 10^8$ |

*bacterium* (15.9%), *Pseudomonas* (4.5%), *Cellulomonas* (52.3%), and *Acinetobacter* (2.3%). The soil microflora under the birch contained a more diverse microbial community. *Pimelobacter* (48.4%), *Actinomyces* (16.1%), and *Micrococcus* (9.7%) were identified as dominant species; *Flavimonas*, *Mycobacterium*, *Corynebacterium* and *Arthrobacter* were present in minor quantities. The *Acremonium* (1.8% of the total quantity), *Mucor* (5.5%), *Verticillium* (18.2%), and dominating *Penicillium* (74.5%) micromycetes were identified in the soil under the larch. *Cladosporium* (1.7%), *Hyphoderma* (1.7%), *Pytium* (3.3%), *Cephalosporium* (8.3%), and *Beltrania* (31.7%) were identified under the birch in addition to the representatives of *Penicillium* and *Verticillium* typical of the soil under the larch; two species (*Beltrania* and *Penicillium*) were defined as dominating. The total titer of fungi was similar under the larch and the birch.

In the colder year of 2010, the qualitative picture of microbial communities was as a whole analogous to that of 2007, but the total quantity of microorganisms in the initial soil was lower (Table 1). The total titer of aerobic microflora in early June 2010 under the larch and the birch was  $(3.16 \pm 0.24) \times 10^6$  and  $(2.67 \pm 0.16) \times 10^5$  CFU/g soil, respectively. At the end of the experiment, the total quantity of bacteria was lower by two orders of magnitude compared to 2007:  $(5.28 \pm 1.76) \times 10^7$  CFU/g soil under the larch and  $(6.77 \pm 4.51) \times 10^7$  CFU/g soil under the birch.

The difference between the soil parameters in the places of tree growth and the microbiocoenosis characteristics influenced the process of PHA degradation (Fig. 1a). In 2007, both types of PHAs were more actively degraded in the more humid soil under the larch, which was more abundantly inhabited by microorganisms, than under the birch. At the end of the observation, the residual masses of the PHB samples and the copolymer were 45 and 22% of the initial values, respectively; the half-life period was 85 and

68.5 days with an average weight loss of 0.325 and 0.44 mg/day per field season, respectively. In the soil of the root zone of the birch, in spite of the greater fungal diversity, both types of PHAs were degraded slower. In 109 days, the residual weights of PHB and PHBV were 84 and 74% of the initial values (Fig. 1b) with average homopolymer and copolymer weight losses of 0.097 and 0.15 mg/day, respectively.

The copolymer was degraded more actively than the high-crystalline PHB. It is in agreement with the results of some works, including those of our team, showing that the copolymer PHA samples are degraded in biological environments more rapidly than the homopolymer PHB [4, 6, 19, 32]. However, another work [33] demonstrated a higher rate of homogenous PHB biodegradation compared to PHBV; the authors explained it by the peculiarities of the structure and surface properties of the samples.

In 2010, the PHA degradation was less active, and at the end of observations, the residual mass of the exposed PHB and PHBV samples was 89.9 and 74% under the larch and 91.4 and 89% under the birch, respectively. Because of the insignificant decrease in the weight of the polymer samples in 2010, it was impossible to reliably detect the differences in the degradability of the PHA types under study.

The factors that influence PHA biodegradability, in addition to the chemical composition and environmental temperature, are stereoconfiguration, degree of crystallinity, and the molecular weight of the polymer [10, 34, 35]. PHA samples with different degrees of crystallinity (Table 2) have been studied in this work (Table 2). An X-ray structure analysis of the samples at the end of the observation showed an increased degree of crystallinity for both types of polymers, indicating the preferable degradation (washing out) of the amorphous phase of the polymers compared to the crystalline phase in the course of PHA biodegradation in the soil under the test conditions. It is in agreement with a

**Table 2.** Values of the degree of crystallinity and molecular weight of PHA samples in the course of biodegradation

| Conditions                          | 2007       |             |                           |             |                           |             | 2010                      |             |                           |             |
|-------------------------------------|------------|-------------|---------------------------|-------------|---------------------------|-------------|---------------------------|-------------|---------------------------|-------------|
|                                     | DC, %, PHB | DC, %, PHBV | M <sub>w</sub> , kDa, PHB | PD, PHB     | M <sub>w</sub> , kDa, PHB | PD, PHB     | M <sub>w</sub> , kDa, PHB | PD, PHB     | M <sub>w</sub> , kDa, PHB | PD, PHB     |
| Initial value                       | 61         | 50          | 710 ± 1.5%                | 2.05 ± 2.1% | 799 ± 3.1%                | 1.77 ± 4.4% | 710 ± 1.5%                | 2.05 ± 2.1% | 680 ± 1.1%                | 2.32 ± 0.3% |
| 90 days of exposure under the birch | 69         | 54          | 691 ± 3%                  | 2.22 ± 6.5% | 633 ± 5.4%                | 2.23 ± 8.6% | 704 ± 2.3%                | 2.32 ± 3.2% | 628 ± 5.0%                | 2.35 ± 2.5% |
| 90 days of exposure under the larch | 65         | 68          | 577 ± 4.6%                | 2.39 ± 4.1% | 660 ± 2.6%                | 2.41 ± 2.6% | 692 ± 2.9%                | 2.25 ± 3.4% | 660 ± 2.6%                | 2.41 ± 2.6% |

number of works [13, 35] but differs from the results that we obtained when studying PHA degradation in seawater under tropical conditions, when the crystallinity of the polymer samples practically did not change [36].

Since the properties of polymers considerably depend on molecular weight values, the variations of the latter during PHA degradation in soil have been investigated (Table 2). The maximally degraded samples of 2007 showed an insignificant, but reliable, decrease in the M<sub>w</sub> value; in 2010, no significant M<sub>w</sub> variation was registered. The value of the polymer polydispersity increased in all variants, indicating the PHA chain destruction and the changed ratio of polymer fragments with different degrees of polymerization. It is in agreement with the data obtained in the studies of the PHA destruction in seawater in the tropics [36], as well as with the data of other authors demonstrating the existence of a relationship between PHA degradation and changing the M<sub>w</sub> value [3, 37].

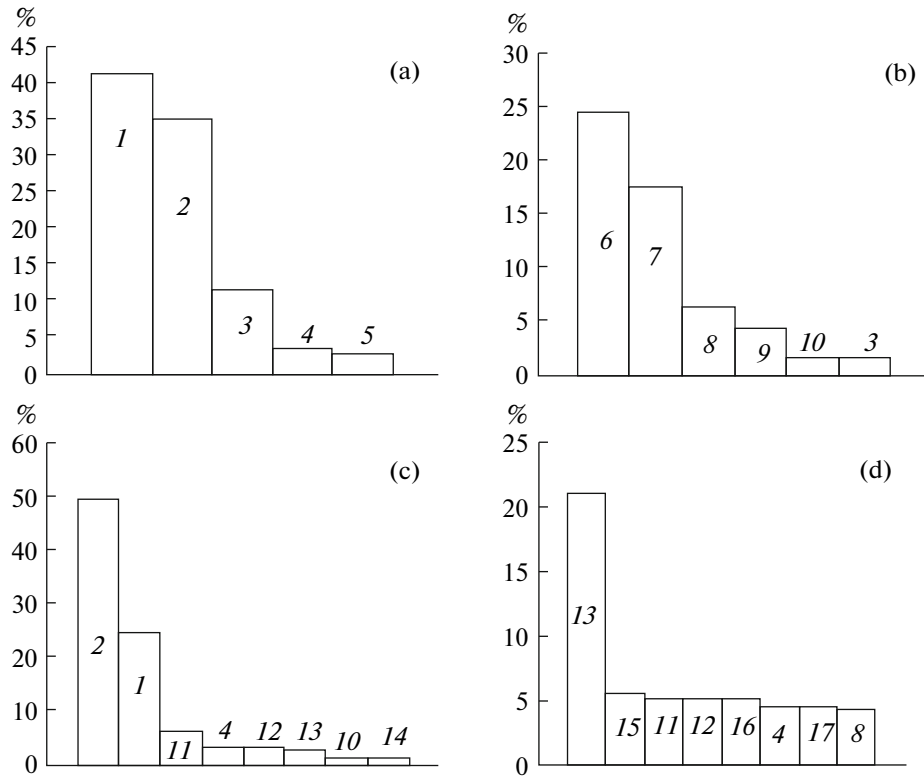
PHA, in contrast to other degradable polymers (polysaccharides and polylactides), undergo true biological degradation via cellular and humoral pathways under the influence of phagocytosing cells and PHA-depolymerizing enzymes excreted by the microflora [2].

Comparison of the data in respect of the total quantity and composition of the control soil microbiocenes and the samples taken from the surface of the polymer samples at the end of the observation period revealed significant differences. The soil under both tree species showed an approximately threefold increase in the quantity of prototrophs compared to the initial soil. The quantity of oligotrophs under the birch increased to  $(3.81 \pm 0.71) \times 10^8$  CFU/g soil, demonstrating the higher activity of this group of microorganisms. On the contrary, the quantity of oligotrophs and the coefficient of oligotrophicity were shown to decrease in the soil under the larch. It is probable that the higher quantity of hydrolytics (the primary degraders of organic substances) in the soil

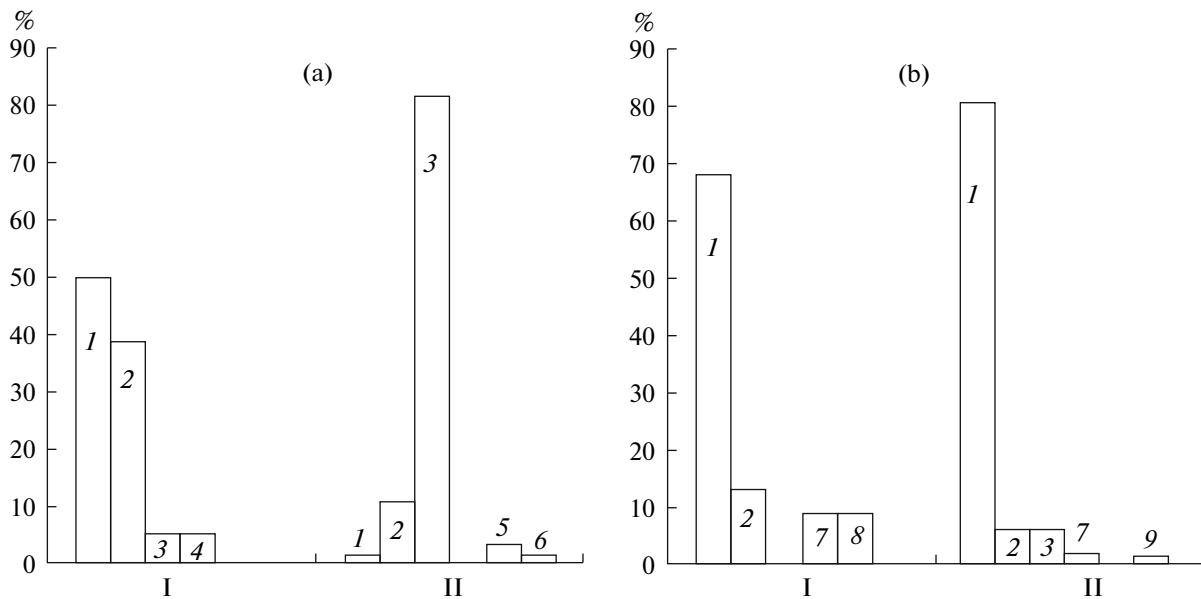
under the larch suppressed the development of oligotrophs ("microflora of dispersion").

The quantitative parameters (Table 1) and qualitative composition of the microorganisms (Fig. 2) isolated directly from the PHA surface differed significantly from those of the control soil samples. The total quantity of microorganisms at the surface of the polymer samples was by two orders of magnitude higher in 2007 and by an order of magnitude in 2010 compared to the titer in the control soil. In the control soil samples under the larch, representatives of the genus *Micrococcus* were identified as dominant (Fig. 2a); spore-forming rods of the genus *Vacillus* and arthro-bacteria were isolated as well. The isolated representatives of the Gram-negative microflora were bacteria of the genera *Acinetobacter*, *Flavobacterium*, and *Pseudomonas*. Representatives of the genera *Agrobacterium* and *Cellulomonas* were predominant in the samples taken from the polymer surface (Fig. 2b). The genera *Alcaligenes*, *Aureobacterium*, *Acinetobacter*, *Pseudomonas*, and *Arthrobacter* were present in minor quantities. An analogous result was obtained during the analysis of the control and experimental soil samples in the root zone of the birch (Figs. 2c, 2d). Bacteria of the genus *Micrococcus* dominated in the control sample (Fig. 2c); bacteria of the genus *Bacillus* dominated at the surface of the polymer films. *Arthrobacter*, *Micrococcus*, *Nocardia*, *Actinomyces*, *Pimelobacter*, and *Alcaligenes* were also isolated (Fig. 2d).

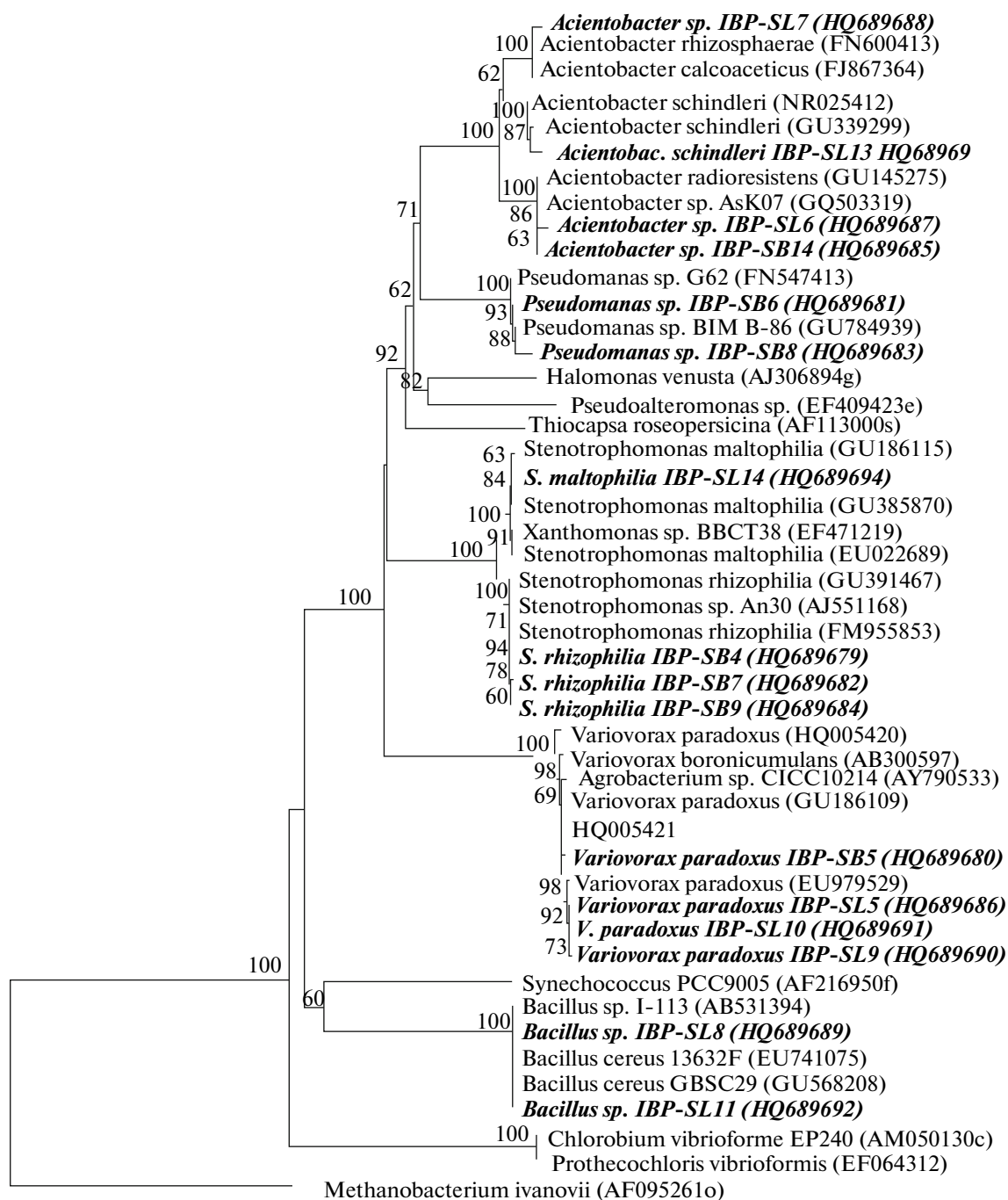
At the end of the vegetative period, the micromycetes in the control samples included representatives of the genera *Penicillium*, *Paecilomyces*, *Aureobasidium*, and *Verticillium* (Fig. 3) with a predominance of *Penicillium* fungi. This is in agreement with the data on the dominance of *Penicillium* fungi among the micromycetes of northern soils [26]. In the soil samples from the surface of the polymer films, the total quantity of micromycetes was shown to increase by a factor of 36.5 in the larch rhizosphere and by a factor of 4 in the birch rhizosphere compared to the control.



**Fig. 2.** Dominant representatives of bacteria isolated from the soil of the root zone of the larch (a) and from the polymer surface (b), as well as from the root zone of the birch (c) and from the polymer surface (d) on September 20, 2007. (1) *Micrococcus varians*; (2) *Micrococcus roseus*; (3) *Acinetobacter* sp.; (4) *Arthrobacter* sp.; (5) *Bacillus alvei*; (6) *Agrobacterium* sp.; (7) *Cellulomonas* sp.; (8) *Alcaligenes* sp.; (9) *Aureobacterium* sp.; (10) *Pseudomonas* sp.; (11) *Actinomyces* sp.; (12) *Micrococcus luteus*, (13) *Bacillus fastidiosus*; (14) *Flavobacterium* sp.; (15) *Bacillus brevis*; (16) *Pimelobacter*; and (17) *Nocardia* sp.



**Fig. 3.** Main representatives of micromycetes isolated from the soil of the root zones of the larch (a) and birch (b): (1) *Penicillium*\*, (2) *Aureobasidium*; (3) *Paecilomyces*\*, (4) *Mycelia sterilia*, (5) *Acremonium*\*, (6) *Zygosporium*\*, (7) *Verticillium*\*, (8) *Trichoderma*, (9) *Nigrospora* (true PHA degrading fungi are marked with an asterisk), (I) control and (II) PHA surface.



**Fig. 4.** Phylogenetic position of the tested strains of PHA-degrading bacteria (in italic) based on the comparison of the nucleotide sequences of the 16S rRNA gene by the neighbor-joining method. The scale corresponds to 1 nucleotide substitution per every 10 sequences. The numerals show bootstrap index values of 60% and higher.

This fact demonstrates the active involvement of fungi in PHA degradation, which is confirmed by literature data [4, 38, 39].

The active PHA degrading microorganisms described by now are bacteria of different species [5, 30, 34] and fungi [3, 7, 28, 34]. The detection of such microorganisms often includes the analysis of microorganisms

isolated from fouling films at the surface of polymers exposed to soil (compost, water, etc.). However, among these microorganisms, there may be accompanying species that utilize monomers and other products of high-molecular-weight PHA destruction appearing in the medium as a result of the vital activity of true PHA destructors. For the isolation of the latter,

it is necessary to use a method of transparent zones [4], which envisages the inoculation of the samples onto a mineral agar containing PHA as a sole carbon source.

The study of isolated fungi showed that only five out of the eight identified taxa possessed PHA-depolymerase activity. Transparent zones were formed around the colonies of organisms from the genera *Penicillium*, *Paecilomyces*, *Acremonium*, *Verticillium*, and *Zygosporium*. The main PHA destructors in the soil of the root zone of the larch were representatives of the species *Paecilomyces lilacinus* (81.5%), which is in agreement with the works of other authors [38]. Among the fungi localized at the PHA surface, *Penicillium* sp. BP-1 and BP-2 were dominant in the birch rhizosphere: their total quantity reached 81%.

Based on similar morphotypes, 16 strains of PHA-degrading bacteria were selected and identified by an array of morphological, cultural, biochemical, and molecular genetic characters. The IBP-SB5, IBP-SL5, IBP-SL9, and IBP-SL10 strains were referred to the genus *Variovorax*; the IBP-SB4, IBP-SB7, IBP-SB9, and IBP-SL14 strains were referred to the genus *Stenotrophomonas*; the IBP-SB14, IBP-SL6, IBP-SL7, and IBP-SL13 strains were referred to the genus *Acinetobacter*; the IBP-SB6 and IBP-SB8 strains were referred to the genus *Pseudomonas*; and the IBP-SL8 and IBP-SL11 strains were referred to the genus *Vacillus* (Fig. 4). An analysis of the nucleotide sequences of the 16s rRNA gene of the isolated strains showed a high degree of their homology with the sequences of some previously identified strains from the GenBank database. The strains form five separate clusters on the tree (Fig. 4); one of them is composed of  $\beta$ -proteobacteria of the genus *Variovorax* (the strains of *Variovorax paradoxus*), and the other three are composed of  $\gamma$ -proteobacteria from the genera *Stenotrophomonas*, *Pseudomonas*, and *Acinetobacter*. One more cluster corresponds to the Gram-positive bacteria of the genus *Bacillus*.

The complex studies of the biodegradation of the two types of PHAs by soil microbial communities of different structures during the two field seasons with different weather conditions showed that this process was influenced by both the chemical composition of the polymer and the soil characteristics: temperature, humidity, and the microbial component. The PHA degradation was accompanied by a decrease in the polymer molecular weight and an increase in the degree of crystallinity, demonstrating the preferential destruction of the amorphous phase compared to the crystalline phase. It has been established that a microbiocenosis qualitatively different from the control soil samples with dominant representatives of *Penicillium* fungi and *Micrococcus* bacteria is formed at the surface of the polymer samples. It has been shown that the microorganisms developing directly at the polymer surface, in addition to the true destructors with PHA-depolymerase activity, also include accompanying bacteria, which actively develop at the expense of the PHA hydrolysis products. Among the true PHA

destructors, 20 bacterial and 5 fungal species have been identified. The dominant PHA destructors under the test conditions were identified as bacteria from the genera *Variovorax*, *Stenotrophomonas*, *Acinetobacter*, *Pseudomonas*, and *Bacillus*. The dominant species in the population of soil micromycetes developing on the polymer samples are PHA destructors *Penicillium* sp. BP-1, *Penicillium* sp. BP-2, and *Paecilomyces lilacinus* reaching 80%.

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## REFERENCES

1. Chen, G.-Q., *Chem. Soc. Rev.*, 2009, vol. 38, no. 8, pp. 2434–2446.
2. Kim, D.Y., Kim, H.W., Chung, M.G., and Rhee, Y.H., *J. Microbiol.*, 2007, vol. 45, no. 2, pp. 87–97.
3. Bonartseva, G.A., Myshkina, V.L., Nikolaeva, D.A., Kevbrina, M.V., Kallistova, A.Y., Gerasin, V.A., Iordanskii, A.L., and Nozhevnikova, A.N., *Appl. Biochem. Biotechnol.*, 2003, vol. 109, nos. 1–3, pp. 285–301.
4. Mergaert, J., Webb, A., Anderson, C., Wouters, A., and Swings, J., *Appl. Environ. Microbiol.*, 1993, vol. 59, no. 10, pp. 3233–3238.
5. Suyama, T., Tokiwa, Y., Ouichanpagdee, P., Kanagawa, T., and Kamagata, Y., *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 12, pp. 5008–5011.
6. Woolnough, C.A., Charlton, T., Yee, L.H., Sarris, M., and Foster, J.R., *Polym. Int.*, 2008, vol. 57, no. 9, pp. 1042–1051.
7. Mokeeva, V.L., Chekunova, L.N., Myshkina, V.L., Nikolaeva, D.A., Gerasin, V.A., and Bonartseva, G.A., *Mikol. Fitopatol.*, 2002, vol. 36, no. 5, pp. 59–63.
8. Bhatt, R., Shah, D., Patel, K.C., and Trivedi, U., *Biores. Technol.*, 2008, vol. 99, no. 11, pp. 4615–4620.
9. Colak, A. and Guner, S., *Int. Biodeterior. Biodegrad.*, 2004, vol. 53, pp. 103–109.
10. Nishida, H. and Tokiwa, Y., *J. Environ. Polym. Degrad.*, 1993, vol. 1, no. 1, pp. 65–80.
11. Mukai, K. and Doi, Y., *RIKEN Review*, 1993, no. 3, pp. 21–22.
12. Lim, S.-P., Gan, S.-N., and Tan, I., *Appl. Biochem. Biotechnol.*, 2005, vol. 126, no. 1, pp. 23–32.
13. Sridewi, N., Bhubalan, K., and Sudesh, K., *Polym. Degrad. Stab.*, 2006, vol. 91, no. 12, pp. 2931–2940.
14. Lopez-Llorca, L.V., Colom Valiente M.F., Gascon A, *Micron*, 1993, vol. 24, no. 1, pp. 23–29.
15. Yew, S.-P., Tang, H.-Y., and Sudesh, K., *Polym. Degrad. Stab.*, 2006, vol. 91, no. 8, pp. 1800–1807.



16. Chowdhury, A.A., *Arch. Mikrobiol.*, 1963, vol. 47, pp. 167–200.
17. Brandl, H. and Puchner, P., *Biodegradation*, 1991, vol. 2, no. 4, pp. 237–243.
18. Briese, B.-H., Jendrossek, D., and Schlegel, H.G., *FEMS Microbiol. Letts.*, 1994, vol. 117, no. 1, pp. 107–112.
19. Mergaert, J., Anderson, C., Wouters, A., and Swings, J., *J. Environ. Polym. Degrad.*, 1994, vol. 2, no. 3, pp. 177–183.
20. RF Patent No. 2053292, 1996.
21. Volova, T.G., Kalacheva, G.S., and Steinbuchel, A., *J. Sib. Fed. Univ. Biol.*, 2008, vol. 1, no. 1, pp. 91–101.
22. Zvyagintsev, D.G., *Metody pochvennoi mikrobiologii i biokhimii* (Methods of Soil Microbiology and Biochemistry), Moscow: Mosk. Gos. Univ., 1990.
23. Voinova, O.N., Kalacheva, G.S., Grodnitskaya, I.D., and Volova, T.G., *Appl. Biochem. Microbiol.*, 2009, vol. 45, no. 4, pp. 427–431.
24. *Bergey's Manual of Determinative Bacteriology*, Holt, J., Krieg, N., Staley, J., and Williams, S., Eds., Baltimore: Williams and Wilkins, 1994. Translated under the title *Opredelitel' bakterii Berdzhii*, Moscow: Mir, 1997.
25. Weyant, S.R., Moss, W., Weaver, R., Hollis, D., Jordan, J., Cook, F., and Daneshvar, M., *Opredelitel' netrivial'nykh patogennykh gramotritsatel'nykh bakterii* (Identification of Unusual Pathogenic Gram-Negative Aerobic Bacteria), Moscow: Mir, 1999.
26. Egorova, L.N., *Pochvennye griby Dal'nego Vostoka* (Soil Fungi of the Far East), Leningrad: Nauka, 1986.
27. Sutton, D., *Opredelitel' patogennykh i uslovno patogennykh gribov* (Determinant of Pathogenic and Conditionally Pathogenic Fungi), Sutton, D., Fotergill, A., and Rinaldi, M., Eds., Moscow: Mir, 2001.
28. Kurakov, A.V., Novikova, N.D., Ozerskaya, S.M., Deshevaya, E.A., Gevorkyan, S.A., and Goginyan, V.B., *Kosm. Biol. Aviakosm. Med.*, 2007, vol. 41, no. 5, pp. 49–56.
29. Kondratyuk, T.A., *Immunopatol. Allergol. Infektol.*, 2010, no. 1, pp. 65–66.
30. Delafield, F.P., Doudoroff, M., Palleroni, N.J., and Lusty, C.J., *J. Bacteriol.*, 1965, vol. 90, no. 5, pp. 1455–1466.
31. Van de Peer, Y. and De Wachter, R., *Comput. Applic. Biosci.*, 1993, vol. 9, no. 2, pp. 177–182.
32. Volova, T.G., Lukovenko, S.G., and Vasil'ev, A.D., *Biotehnologiya*, 1992, no. 1, pp. 19–22.
33. Rosa, D.S., Filho, R.P., Chui, Q.S.H., and Guedes, C.G.F., *Eur. Polym J*, 2003, vol. 39, no. 2, pp. 233–237.
34. Jendrossek, D. and Handrick, R., *Annu. Rev. Microbiol.*, 2002, vol. 56, pp. 403–432.
35. Abe, H., Doi, Y., Aoki, H., and Akehata, T., *Macromolecules*, 1998, vol. 31, no. 7, pp. 1791–1797.
36. Volova, T.G., Boyandin, A.N., Vasiliev, A.D., Karpov, V.A., Prudnikova, S.V., Mishukova, O.V., Boyarskikh, U.A., Filipenko, M.L., Rudnev, V.P., Bui Ba Xuan, Vũ Viêt Dũng, and Gitelson, I.I., *Polym. Degrad. Stab.*, 2010, vol. 95, no. 12, pp. 2350–2359.
37. Quinteros, R., Goodwin, S., Lenz, R.W., and Park, W.H., *Int. J. Biol. Macromol.*, 1999, vol. 25, nos. 1–3, pp. 135–143.
38. Sang, B.-I., Hori, K., Tanji, Y., and Unno, H., *Appl. Microbiol. Biotechnol.*, 2002, vol. 58, no. 2, pp. 241–247.
39. Lee, K.-M., Gimore, D.F., and Huss, M.J., *J. Polym. Environ.*, 2005, vol. 13, no. 3, pp. 213–219.