Specific Character of Bacterial Biodegradation of Polyhydroxyalkanoates with Different Chemical Structure in Soil

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Abstract—The study addresses the influence of the physicochemical properties of the reserve cellular macromolecules (polyhydroxyalkanoates, PHA) with different chemical composition on their biodegradation in the agro-transformed field soil of the Siberian region (Krasnoyarsk Territory, Russia). It was shown that the degradation of the PHA samples depends on the degree of polymer crystallinity (Cx). For the first time, it was shown that the range of PHA-degrading microorganisms differs for each of PHA types. The study defines the primary degraders specific to each PHA type and common to all types of examined polymers.

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Natural polyesters polyhydroxyalkanoates (PHAs), reserve intracellular compounds of prokaryotes, are promising for application in various fields of natural sciences [1, 2]. The most important property of PHAs is their ability to undergo degradation in biological fluids, which is realized in vivo through humoral and cellular pathways to form CO₂ and H₂O and makes them promising for medicine [3, 4], as well as in nature under the influence of microorganisms [5, 6]. The increasing scope of biosynthesis of PHAs [1] necessitates the investigation of the consequences of their use, primarily degradation in the environment. In natural ecosystems, PHAs are degraded by microorganisms possessing extracellular PHA depolymerases [5, 6]. The microorganisms degrading PHAs have been isolated from different natural ecosystems (soil, compost, sewage sludge, fresh and sea water, and estuarine sediments). Most studies describe the degradation of the homopolymer of 3-hydroxybutyrate and, to a lesser extent, the copolymers of 3-hydroxybutyr-ate and 3-hydroxyvalerate [7–10]. At the same time, PHAs are a family of polymers of different chemical structure, substantially differing in the composition and ratio of monomers and the physicochemical properties [11]. However, publications on comparative study of degradability of PHAs of different chemical composition, including the particularly promising copolymeric PHAs, are scanty [12]. These works provide data primarily on the kinetics of degradation of some copolymeric PHAs irrespective of their physicochemical properties and without consideration of degrading microorganisms. The study of the characteristics of biodegradation of PHAs of different structures was the subject of this communication.

The chemical composition and properties of PHA samples (crystallinity Cₓ, molecular weight, and temperature characteristics), which were synthesized by the Cupriavidus eutrophus strain B10646 by the technology of the Institute of Biophysics, Russian Academy of Sciences [13], were studied using gas chromatography–mass spectrometry, X-ray diffraction, differential thermal analysis, and HPLC. Films formed from the homopolymer P(3HB) and copolymers with 3-hydroxyvalerate [P(3HB/3HV, 12 mol %)], 3-hydroxyhexanoate [P(3HB/3HHX, 12 mol %)], and 4-hydroxybutyrate [P(3HB/4HB, 10 mol %)] were obtained. Biodegradation of polymer samples was studied in laboratory soil microecosystems. Preliminarily weighed samples of films formed of the four types of PHAs were placed in soil (agro-transformed field soil, village Minino, Krasnoyarsk Territory). The samples were exposed for 35 days at a temperature of 28°C and soil humidity of 50%. After exposure, the decrease in the weight of polymeric films was determined, and the microorganisms from film surface scrapings were seeded.

The microorganisms that were primary PHA degraders were identified using the method of transparent zones [14]. For this purpose, soil samples were seeded on mineral agar containing powdered respective PHA—homopolymer P(3HB) or a copolymer of 3HB with 4HB, 3HV, or 3HHX—as the only source of carbon. The polymer-degrading microorganisms were
identified using morphophysiological and molecular genetic characteristics. The obtained nucleotide sequences of the 16S rRNA gene of the isolated degraders were deposited in GenBank (accessions KT321679-KT321704 and KU052942-KU052950). For the phylogenetic analysis, the nucleotide sequences were compared with the homologous reference sequences of the NCBIRefSeq database (http://www.ncbi.nlm.nih.gov/refseq/) using the Muscle package of the MEGA software version 6 [15]. Phylogenetic analysis was performed using a three-parameter Tamura model with the use of the neighbor-joining method in the MEGA software package version 6. Statistical estimation of the phylogenetic tree significance was performed by bootstrap analysis using 1000 random samples.

We have found that the PHA degradation rate depends on crystallinity ($C_X$): the lower is the degree of crystallinity (the higher the volume of unordered (amorphous) phase in the polymer), the more actively the polymer is degraded. The results of a comparative study of degradation of four types of PHAs in soil are shown in Fig. 1. The chemical composition and properties of polymers with different degree of crystallinity affected the process of degradation. By the activity of degradation in soil, the studied PHAs can be arranged in the following series: $P(3HB/4HB) > P(3HB/3HHX) > P(3HB/3HV) > P(3HB)$. For 21 days, the films of the copolymer $P(3HB/4HB)$ were degraded by 97% of the original weight, whereas the films of the homopolymer $P(3HB)$ were degraded by 60%. The degrees of degradation of copolymers $P(3HB/3HHX)$ and $P(3HB/3HV)$ were similar, and, by this index, these copolymers occupied an intermediate position.

The degree of crystallinity of the least destroyed samples $P(3HB)$ after 35 days of exposure practically did not change (Table 1). This fact suggests that, in the course of degradation of this homopolymer, both phases (crystalline and amorphous) were destroyed evenly. The degree of crystallinity of samples of copolymers $P(3HB/3HV)$ and $P(3HB/3HHX)$ increased significantly compared to the initial values. In other words, in this case the amorphous phase was degraded more actively. This effect was even more pronounced in copolymers $P(3HB/4HB)$, which had the lowest degree of crystallinity.

In the study of microbial biofilms formed on the surface of the polymer samples, we have isolated 128 isolates belonging to the genera *Achromobacter, Acidovorax, Alcaligenes, Arthrobacter, Bacillus, Cellulomonas, Chitinophaga, Corynebacterium, Cupriavidus, Delftia, Ensifer, Flavobacterium, Lysobacter, Microbacterium, Mitsuaria, Mycobacterium, Nocardia, Pseudomonas, Pseudonocardia, Pseudoxanthomonas, Roseateles, Roseomonas, Streptomyces, and Variovorax*. However, seeding the microorganisms on a mineral agar medium containing different types of PHAs as a source of carbon showed that the number of primary degraders exhibiting depolymerizing activity is considerably smaller (only 35 isolates). Therefore, the biofilms contained both the primary PHA degraders, which are able to metabolize the high-molecular-weight original polymer, and the commensal microorganisms that utilize the products of degradation of the high-molecular-weight PHAs, which appear in the environment as a result of life activity of the primary and true PHA degraders.

We have first shown that the spectrum of degraders is different for different types of PHAs (Fig. 2). Specific degraders for $P(3HB)$ are representatives of the genera *Mitsuaria, Chitinophaga*, and *Acidovorax*; for $P(3HB/4HB)$, *Roseateles* and *Cupriavidus*; for