IDENTIFICATION OF PHA-PRODUCING BACILLI AND OPTIMIZATION OF PHA BIOSYNTHESIS

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Abstract
Polyhydroxyalkanoates present a promising alternative to traditional plastics. This study focused on characterizing two PHA-producing Bacillaceae family strains. The bacteria's ability to produce PHA granules was confirmed by microscopy and GCMS. Identification was based on chemotaxonomic analysis. B. weihenstephanensis 5w2 demonstrated the highest PHA yields when cultivated with glucose.
Keywords: *Polyhydroxyalkanoates, bioplastic, Bacillaceae bacteria*

Introduction. Because of their poisonous and nonbiodegradable qualities, products created using petroleum-based compounds generate a variety of environmental hazards, including soil infertility, and contamination of waterways and soils. This can be minimized by using biopolymers, such as polyhydroxyalkanoates (PHAs) like polyhydroxybutyrate (PHB), which exist as intracellular granules created by bacteria and are stored by the cell to function as both carbon and energy reservoirs. Several bacteria have been shown to synthesize PHA in settings with high levels of carbon as well as reduced nitrogen or phosphorus concentrations. However, PHA manufacturing costs are a serious issue, with carbon alone accounting for forty-five percent of overall production costs, impeding market expansion for PHA-based plastics [1]. Because of their prevalence in nature, *Bacillaceae* bacteria are an important resource for the commercial manufacturing of PHA. It has been established that various *Bacillaceae* bacteria create PHA from a range of substrates such as fructose, glucose, sucrose, and starch [2, 3]. However, only a few number of bacilli can thrive on low-cost biowaste substrates and produce PHA [4, 5]. Therefore, it is highly relevant to isolate and identify novel PHA-producing strains among *Bacillaceae* family bacteria and determine the most suitable cultivation parameters for the maximal PHA yield.

Materials and methods. Two PHA-producing strains, *Bacillus sp.* UCM B-5715 and *Bacillus sp.* 5w2, were isolated from the cotton plant phyllosphere and benthal deposits of the Chornobyl NPP cooling pond, respectively. To confirm PHA synthesis the strains were grown on the culture medium of the following composition (g/l): peptone – 3.25, NaCl – 2.5, yeast extract – 0.75, glucose – 20, agar-agar – 14, pH – 7.4. PHA granules were visualized via light and luminescence microscopy. To identify the selected isolates, the Analytical Profile Index tests for *Bacillus* species (API 50 CHB), and fatty acid methyl esters (FAMEs) analysis via gas-chromatography-mass spectrometry (GCMS) were conducted. To analyze the best cultivation conditions for PHA biosynthesis, the influence of different Carbon sources on PHA production was studied. The strains were grown on the culture medium described earlier, with glucose substituted with equivalent masses of arabinose, fumarate, glycerol, inositol, lactose,

malate, maltodextrin, maltose, mannitol, molasse, propionate, raffinose, saccharose, succinate, and xylose. The PHA content was determined by GCMS analysis.

Results. The strains *Bacillus sp*. UCM B-5715 and *Bacillus sp*. 5w2 isolated from the cotton plant phyllosphere and benthal deposits of the Chornobyl NPP cooling pond were confirmed to synthesize PHA. After the Nile blue staining, the intracellular PHA granules fluoresced yellow under the UV light, which indicates that the granules are formed by compounds of a lipid nature (fig. 1).

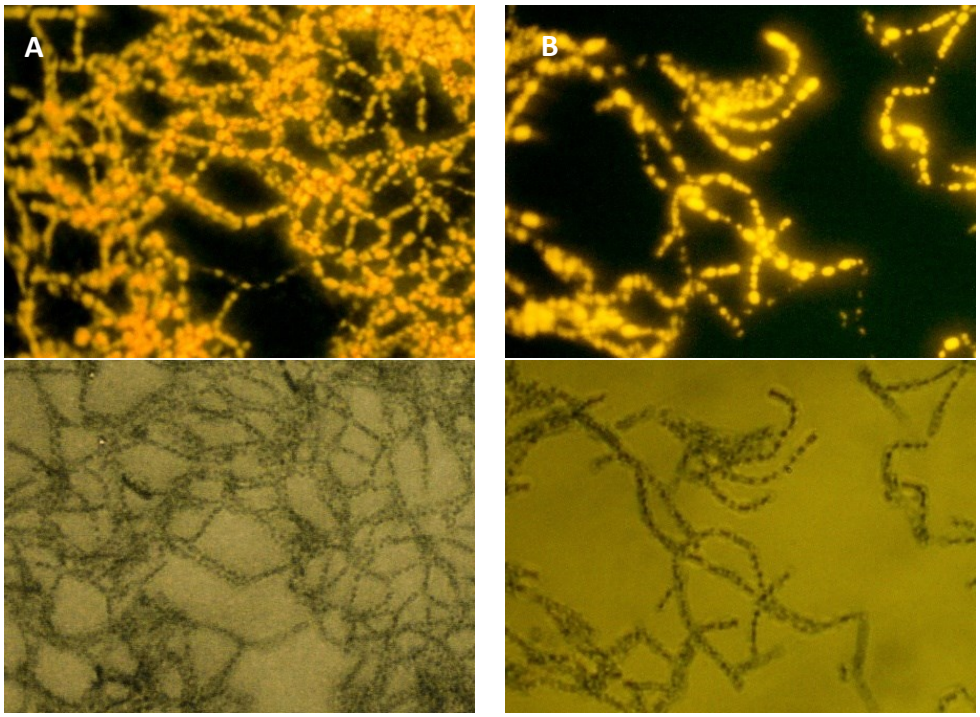

Fig. 1. Micrographs of *Bacillus sp.* UCM B-5715 (A) and *Bacillus sp.* 5w2 (B): fluorescence microscopy with Nile blue stain to detect lipid inclusions - top row, light microscopy with Nile blue stain to detect lipid inclusions – bottom row.

The PHA content of the granules was confirmed by GCMS analysis, which showed that the granules are made of a polymeric substance consisting of 3-hydroxybutyrate monomers.

The biochemical characteristics of the studied strains are presented in Table 1. Based on the results, both strains belong to the *Bacillaceae* family.

Table 1. Biochemical characteristics of the PHA-producing strains.

Taxonomic feature	*Bacillus sp.* UCM B-5715	*Bacillus sp*. 5w2
Gram staining	G+	G+
The presence of spores	+	+
Form of spores	elliptical	elliptical
Location of spores	terminal, subterminal	terminal, subterminal
Formation of granules	+	+
Formation of acid from glycerol	-	-
Erythrol	-	-
D-arabinose	-	-
L-arabinose	-	-
D-ribose	+	+

Continued Table 1

D-xylose	+	w
L-xylose	-	-
Aldonitol	-	-
Methyl-beta-D-xylopyranoside	-	-
D-galactose	-	-
D-glucose	-	+
D-fructose	-	+
D-mannose	-	w
L-sorbose	-	-
L-rhamnose	-	-
Dulcitol	w	-
Inositol	-	-
D-mannitol	-	w
D-sorbitol	w	-
Methyl-aD-mannopyranoside	w	-
Methyl-aD-glucopyranoside	-	w
N-acetylglucosamine	-	+
Amygdalin	+	-
Arbutin	-	+
Esculin	+	+
Salicin	+	+
D-cellobiose	+	w
D-maltose	w	+
D-lactose	w	-
D-melibiose	w	w
D-sucrose	-	+
D-trehalose	+	+
Inulin	+	w
D-raffinose	w	+
Starch	-	+
Glycogen	+	+
Xylitol	w	+
Gentobiose	-	w
D-turanose	+	-
D-luxose	w	-
D-tagatose	-	-
D-fucose	-	-
L-fucose	-	-
D-arabitol	-	-
L-arabitol	-	-
Calcium gluconate	-	-
Calcium 2-ketogluconate	-	-
Calcium 5-ketogluconate	-	-

To further confirm the taxonomic status of the strains, the analysis of the whole-cell lipid content was conducted. The main fatty acids of the *Bacillus sp.* UCM B-5715 strain were iC15:0 and aiC15:0, 17.27% and 43.87%, respectively, the amount of C16:0 was quite high, amounting to 6.10%. The total content of branched fatty acids reached 85% of the total amount. The FAMEs profile of the *Bacillus sp.* 5w2 strain

was characterized by equally high amounts of iC15:0 and aiC15:0, 32.07% and 32.27%, respectively, and high amounts of C16:0 (10.16%), and low amounts of branched fatty acids.

Based on the data obtained, the strain *Bacillus sp.* UCM B-5715 was identified as *Priestia endophytica*, and the strain *Bacillus sp.* 5w2 was closely related to *Bacillus weihenstephanensis*.

The cultivation on different Carbon sources revealed different substrate preferences of the PHA-producing strains studied. Thus, *P. endophytica* UCM B-5715 was able to synthesize PHA on various Carbon sources, namely, arabinose, glucose, glycerol, inositol, malate, maltose, mannitol, molasse, raffinose, saccharose, and xylose. It failed to produce PHA when growing on the media containing fumarate, lactose, propionate, and succinate. The highest level of PHA synthesis by *P. endophytica* UCM B-5715 was obtained on the arabinose-containing medium and reached 1.28% per gram of wet biomass. On the contrary, *B. weihenstephanensis* 5w2 did not produce PHA on culture media other than the one containing glucose. Its PHA synthesis levels reached a maximum of 1.82% per gram of wet biomass.

Conclusions. Two PHA-producing strains were identified as *P. endophytica* UCM B-5715 and *B. weihenstephanensis* 5w2 based on the chemotaxonomic analysis. *P. endophytica* UCM B-5715 was able to produce PHA on a larger range of Carbon sources; however, the maximum PHA yield was obtained from *B. weihenstephanensis* 5w2 on the glucose medium. Optimizing cultivation conditions, such as carbon sources, provides a means to increase PHA yields and lower production costs.

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