AEROBIC SPORE-FORMING MICROORGANISMS AS PROMISING PRODUCERS OF BIODEGRADABLE BIOPLASTICS

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INTRODUCTION

Plastic manufacturing has increased from almost no production in the 1950s to over 335 million tons in 2017, with an annual growth of 4% expected in the next years. It accumulates in environment and causes prolonged inflammation and allergic reactions in humans, which can lead to tumor formation. Presently, there is a concerted effort to develop butyrate-derived plastics that exhibit analogous properties and application domains to their chemically synthesized counterparts. Bacteria belonging to the *Bacillaceae* family emerge as a significant source for industrial PHA production due to their prevalence in the natural environment and their amenable characteristics for use in biotechnology. This study aimed to screen these bacteria and identify PHA-producing strains with potential applications in both agriculture and medicine.

METHODS

The screening was performed utilizing 300 *Bacillaceae* family bacteria strains collected from soil, plant leaves, and waters of the Black Sea. Nile blue stain was added following the cultivation to visualize PHA-producing microorganisms. The presence of PHA granules was confirmed by light microscopy utilizing Sudan black staining. GC-MS analysis was performed to further evaluate the qualitative and quantitative composition of PHAs produced. To identify the PHA-producing isolates, GS-MS FAMEs analysis was conducted. The data obtained served to construct ANN trained to identify FAMEs profiles of aerobic spore-forming bacteria of the order *Bacillales*.

RESULTS

The research showed that 43 of 300 bacterial strains were proficient producers of PHA. Light microscopy further affirmed their ability to form intracellular PHA granules (Fig. 1).

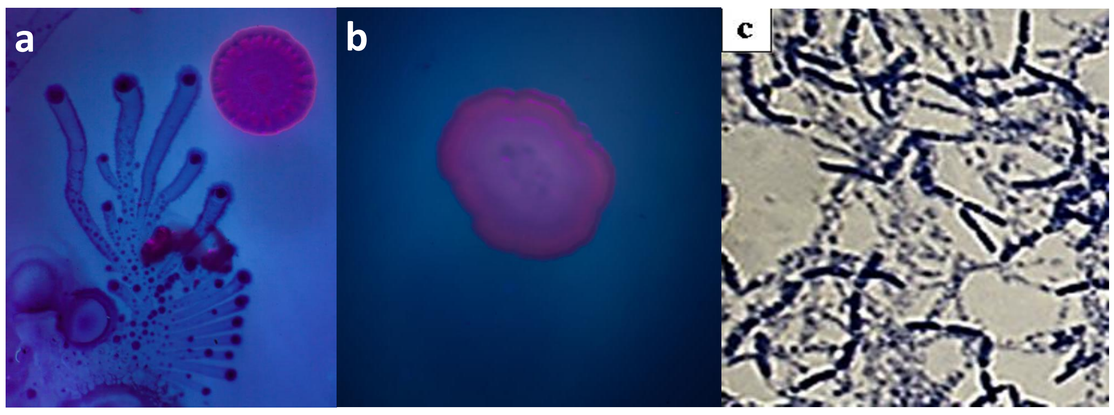


Fig. 1. Fluorescence of the PHA-producing colonies under the UV light (a, b); Light microscopy with Nile blue stain to detect lipid inclusions (c)

Gas chromatography showed that the vast majority of strains synthesized PHA homopolymers, but some strains demonstrated the ability to synthesize bioplastics of a heteropolymeric nature (Fig. 2.).

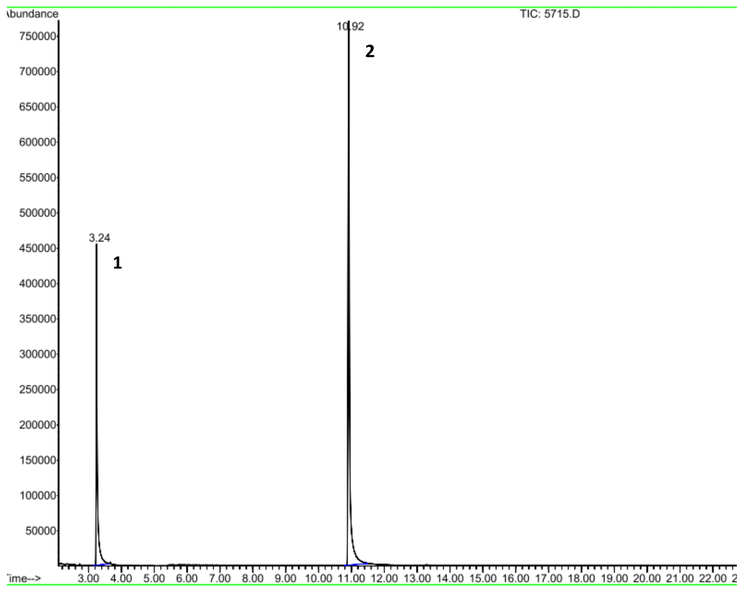


Fig. 2. TIC-chromatogram of polyhydroxyalkanoates produced by *Bacillus sp.* UCM B-5715 (1 – Butanoic acid, 3-hydroxy-, methyl ester, 2 – Benzoic acid, methyl ester).

GC-MS analysis indicated that biosynthetic levels significantly diverged based on strain, culture medium, and biomass type. Based on the obtained data, the 10 most productive strains were selected, and the level of PHA synthesis was from 1.92% to 2.73% per gram of wet biomass (Fig. 3).

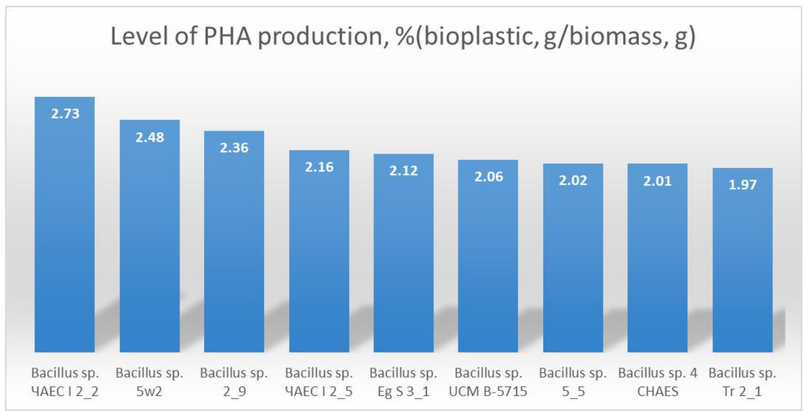
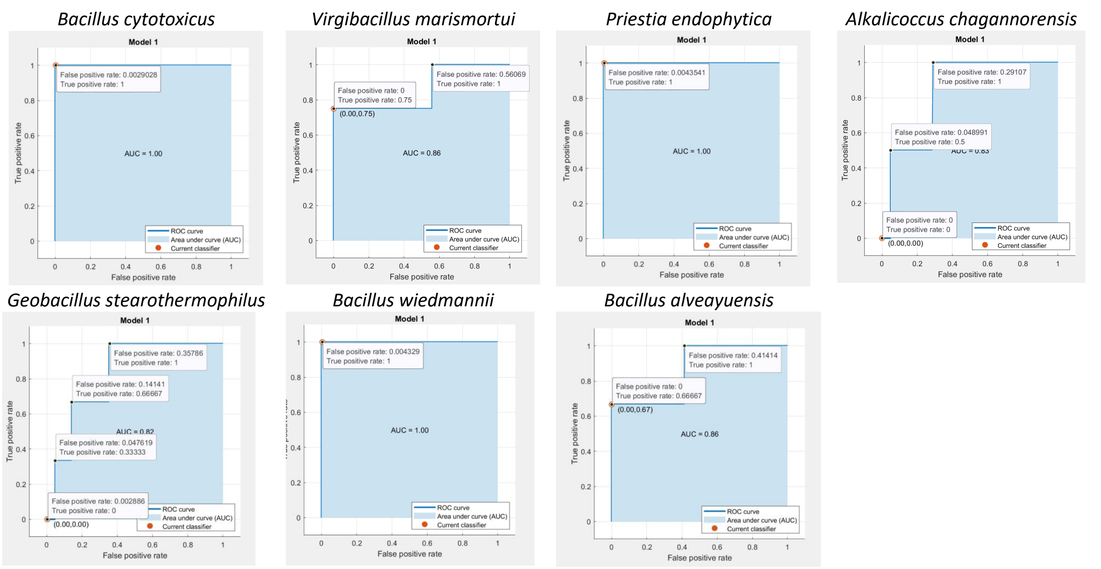


Fig. 3. Level of PHA production, %(bioplastic, g/biomass, g) by the top-producing strains

To identify the isolates based on their FAMEs profiles, an Artificial Neural Network (ANN) was constructed. To train and validate classification model for multiclass problem, Optimizable Ensemble model type with Decision tree learning type and Bayesian optimizer with 50 iterations was selected. The training set included open data for 696 FAMEs profiles of the *Bacillaceae* family bacteria. ROC curve for species identification is depicted in Fig. 4.



FAMEs analysis revealed that PHA-producing bacteria belonged to the *Bacillus* sensu stricto: rRNA group 1, *Virgibacillus*, and *Geobacillus* genera. The top ten producers classification is presented in Table 1.

Table 1. PHA-producing strains according to ANN identification

Strain	ANN identification
Bacillus sp. ЧАЕС I 2 $_2$	*Bacillus cytotoxicus*
Bacillus sp. ЧАЕС I 2 $_5$	*Bacillus cytotoxicus*
Bacillus sp. 5w2	*Virgibacillus marismortui*
Bacillus sp. UCM B-5715	*Priestia endophytica*
Bacillus sp. Eg S 3 $_1$	*Alkalicoccus chagannorensis*
Bacillus sp. Tr 2 $_1$	*Geobacillus stearothermophilus*
Bacillus sp. 4 ЧАЕС	*Geobacillus stearothermophilus*
Bacillus sp. 5 $_5$	*Bacillus wiedmannii*
Bacillus sp. 2_9	*Bacillus alveayuensis*

A method for isolating pure bioplastic was selected, which consisted of the destruction of cells with chlorine-containing agents and purification in a number of organic solvents. Employing this method, we obtained a platelet of pure bioplastic, depicted in Fig. 5.

Fig. 5. Biodegradable bioplastic produced by the *Bacillaceae* family bacteria

CONCLUSIONS

Consequently, our study has yielded a collection of aerobic, spore-forming bacilli, specialized in the synthesis of PHA. Based on the inherent characteristics and the extent of bioplastic synthesis, we have identified five preeminent strains originating from the genera *Bacillus*, *Virgibacillus*, *Priestia*, and *Geobacillus*, which have been earmarked for subsequent investigations.