MICROBIAL PRODUCTION OF POLYHYDROXYBUTYRATE BY NATIVE *Bacillus* STRAINS USING A BIODIESEL BY-PRODUCT AS CARBON SOURCE

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Introduction. Polyhydroxyalkanoates (PHA), 100% biodegradable polymers, are synthesized by many kinds of bacteria. Nowadays, PHAs are attractive substitutes for petroleum-derived plastics (PDP). Among PHAs, polyhydroxybutyrate (PHB) is the most sought after due to its similarity to polypropylene (1). PHB is intracellularly accumulated and may amount up to 70% of cell dry weight (CDW) in *Bacillus* species. However, bacterial PHB is up to 10 times more expensive that PDP because of the substrates used for its production (2).
The aim of this research was to evaluate different *Bacillus* strains according with PHA accumulation ability, using raw glycerol from biodiesel production, as sole carbon source.

Methods. Strains were isolated from different sources by heat-shock and screened for PHB production by optical microscopy using the Sudan black staining method. Promising strains were adapted to glycerol by successive culturing. PHB biosynthesis was carried out in two stages: bacterial growth and PHB accumulation. PHB granules were extracted from cells using a modified SDS method (3). PHB quantitation was done by UV-Vis measurements at 235 nm. In addition, *Bacillus megaterium* ATCC 14581 was used as the reference microorganism for comparison (4).

Results. Two native strains (B2 and B8) and the reference (*B. megaterium* ATCC 14581) accumulated PHB granules (dark blue spots) after the adaptation process. The ATCC strain had never been described as a PHB-producer strain from raw glycerol (**Fig.1**).

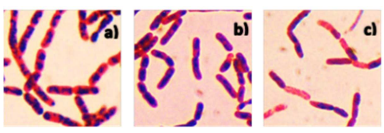

Fig.1 PHA granules stained with Sudan black. B2 (a), B8 (b) and *B. megaterium* ATCC 14581(c)

All *Bacillus* species consume raw glycerol to produce PHB **(Fig.2)**. In the time interval under evaluation, B2 produced more PHB than B8 and *B. megaterium* ATCC 14581, 0,3927 g/L; 0,2632 g/L and 0,1564 g/L respectively. The best productivity for B2 was reached at 4 h (0,0281 g/L*h). A comparable behavior has been observed with other native strains using pure glycerol as carbon source: *Bacillus* CL1 (0,0588 g/L*h) (4) and *Bacillus thuringiensis* R1 (0,0543 g/L*h).

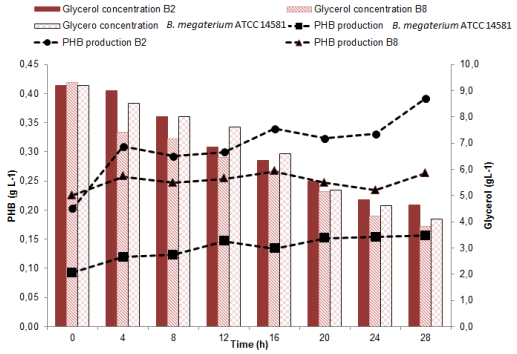

Fig.2 Glycerol concentration and PHB production of B2.B8and *B. megaterium* ATCC 14581.

Conclusions. Two native *Bacillus* strains, B2 and B8 were able to adapt, thrive and accumulate PHB using raw glycerol as sole carbon source. These strains produced more PHB than the reference microorganism.

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