# SYNTHESIS OF POLY-β-HYDROXYBUTYRATE WITH *phb*A and *phb*C GENE CLONED IN EXPRESSION VECTOR IN *Azospirillum brasilense*.

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Key Word: *Poly-ß-hydroxybutyrate, Azospirillum, PHB.*

**Introduction.** *Azospirillum brasilense* is a Gram negative α-proteobacteria cosmopolitan living in the rhizosphere, diazotrophs and produces siderophores, contains a polar flagellum and lateral. Features useful in identifying are the way vibroid, pleomorphism and mobility spiral, *Azospirillum brasilense* is plant growth promoting root (PGPR). Produces poly-β-hydroxybutyrate known as PHB, the PHB is polyester of microbial origin, intracellular synthesized in unbalanced growth conditions, with an excess of carbon source and a limitation on the nitrogen source. The PHB accumulates in the cytoplasm as a carbon and energy reserve (electron acceptor). This polymer is a thermoplastic, biodegradable, biocompatible and is considered good substitute for petrochemical plastics. In bacterial PHB cycle there are tree biosynthetic genes, *phb*A, *phb*B and *phb*C, (β-ketotiolasa, acetoacetyl-CoA reductase y PHB sintase, enzymes respectively)

**Methods.** In this study, the *phb*A gene was PCR-amplified by using the primers AB7AD 5'-ATGACCTCGGCGCCTTTGACCA-3' and AB7AR 5'-TCAGACCGCTTCCAGGACCGTG-3'. Similarly, the *phb*C gene was amplified by using the primers GCSp7F 5'-ATGGTCGGAGATCTGGGTGTGG-3' and GCSp7R 5'-TCAGACGATGCGGACCTTGGC-3'. The amplicons were digested with blunting enzyme and cloning in CloneJet PCR Cloning kit (Fermentas®) and TOPO TA Cloning Kit (Invitrogen®) respectively, *E. coli* DH5α was transformed with the ligation mix and transformants were selected on Luria agar containing 100 µg ampicillin $ml^{-1}$. The nucleotide sequence of the clones of *phb*A and *phb*C were determined and showed the expected size of inserts. On the other hand, the amplicons were digested with *Sma* I and ligated with the similarly digested expression vector pMMB206. *E. coli* DH5α was transformed with the ligation mix and transformants were selected by blue/white selection on Luria agar plates containing 1 mM IPTG, 80 µg X-Gal $ml^{-1}$ and 20 µg chloramphenicol $ml^{-1}$. The expected size was determined by restriction enzymes. The clones harbouring *phb*A and *phb*C genes from *A. brasilense* Sp7 were transferred into *E. coli* S17.1, and then conjugatively movilized into *A. brasilense* Sp7 (1). The clones were grown overnight in LB medium with 25 µg chloramphenicol $ml^{-1}$, and inoculated into fresh medium. Cultures were then incubated with shaking at 30°C until they attained $OD_{600}$ 0.3. At this stage, IPTG was added to give a final concentration of 1 mM to induce the expression of the cloned genes. The cultures were incubated for 72 hours stationary-phase cells of *A. brasilense* and then were collected by centrifugation and lysed by sonication. The PHB was determined by $H_2SO_4$ method (2).

**Results.** The *phb*A and *phb*C genes were cloned each in tree plasmid, pJET1.2/blunt and pCR®2.1-TOPO® for sequence and pMMB206 for expression. The PHB was determined and showed an altered production in the clones *A. brasilense* harbouring pMMB206::*phb*A and pMMB206::*phb*C genes compared with the wild type strain, the *A. brasilense* harbouring the pMMB206 expression vector only was internal control, and details were analyzed.

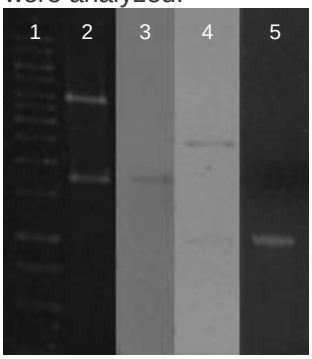


**Fig. 1.** PCR amplification of *phb*C and *phb*A and cloning.
1. MW marker.
2. *phb*C cloned in pCR® 2.1-TOPO®.
3. *phb*C amplified.
4. *phb*A cloned in pJET1.2/blunt.
5. *phb*A amplified.

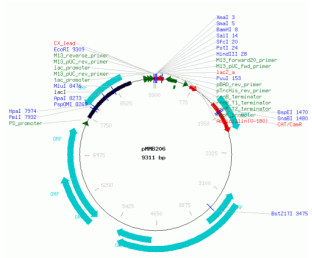

**Fig.2.** Expression vector pMMB206 used for *phb*A and *phb*C genes cloning in *Sma* I site.

**Conclusions**. The PHB production was altered with the *phb*A and *phb*C cloned in expression vector.

**Acknowledgements.** This work was partly supported by a grant from the VIEP-Benemerita Universidad Autonoma de Puebla.

**References.**

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