BIOPROSPECTING IN A METAGENOMIC LIBRARY FROM ATLANTIC FOREST SOIL FOR GENES INVOLVED ON THE BIOSYNTHESIS OF BIODEGRADABLE POLYMERS

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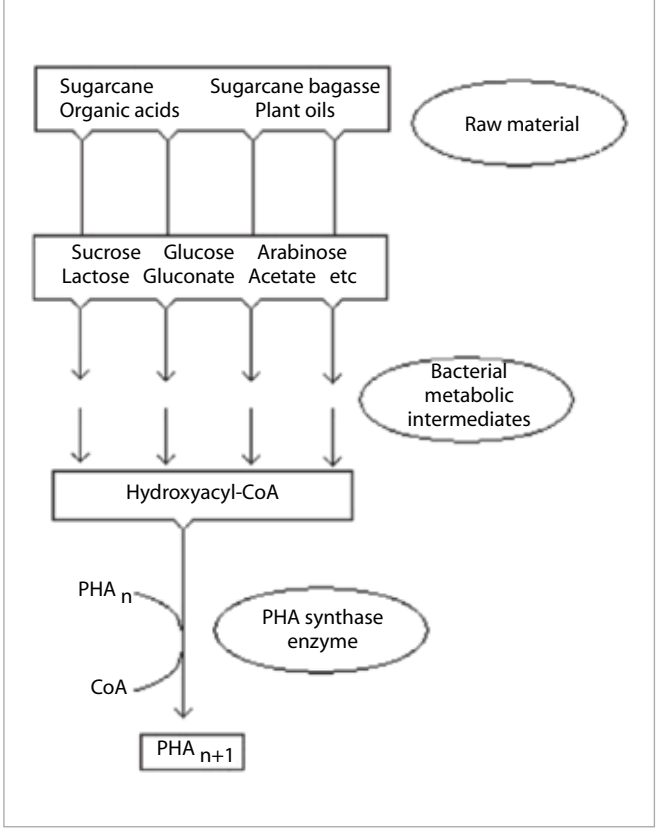

Figure 1. Factors affecting polyhydroxyalkanoate (PHA) production in bacteria

Polyhydroxyalkanoates (PHA) are polyesters accumulated by a wide variety of bacteria from renewable carbon sources and have industrial interest since they are thermoplastics, biodegradable and biocompatible. PHA monomer composition determines its mechanical properties allowing different applications. PHA are composed either by short-chain-lenght (HA_{SCL}) or medium-chain-length monomers (HA_{MCL}) or even HA_{SCL}-co-HA_{MCL} with intermediate properties, more rare due to PHA synthase specificity either to HA_{SCL} or to HA_{MCL}. Three factors are essential to produce PHA: carbon source, bacterial metabolic pathways and the PHA synthase key-enzyme that catalyses R-hydroxyacyl-CoA polymerization to form PHA. Four types of PHA synthase are known depending on their substrate specificity and subunit composition. Types I & II have one peptide unit and types III & IV have two. The project is bioprospecting an Atlantic Forest soil metagenomic library for putative new genes, using phenotypic detection and PCR. Expression of selected genes will be tested on PHA-accumulating *Pseudomonas sp* and *Burkholderia sacchari* mutants impaired on their native PHA synthase. Thus the genes will be expressed on hosts providing diverse metabolic backgrounds to verify if new PHA can be produced. The focus will be HA_{SCL}-co-HA_{MCL} copolymers due their promising properties.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Plasmid DNA from all clones from the metagenomic library was extracted and tested with the six primers described in the literature for the detection of PHA synthase genes. Genomic DNA of *Bacillus megaterium* M.A3.3, *Ralstonia eutropha* and *Pseudomonas aeruginosa* were used as representative controls of each type of PHA synthase. Evaluation of the PCR sensitivity for gene detection in DNA mixtures revealed a detection level of 10-3 ng/µL of the target gene (*Figure 2*). From the metagenomic library, PCR products of the four classes of PHA synthases were found distributed among 80 positive clones. These results are relevant because they suggest that PCR screening, in metagenomic libraries, is more effective than Southern hybridization screening used in reported works found in the literature and also phenotypic detection which fail on finding positive clones. Some randomly selected positive clones were sequenced and confirmed the confidence of the results; presenting a high similarity with PHA synthases of different classes. Next step will evaluate the potential of some of those putative PHA synthases in producing PHA in different hosts. Selected genes will be expressed on two hosts: one with a natural background to accumulate HA_{SCL} and other HA_{MCL}, both affected on their natural PHA synthase. The production of PHA with the target composition will be evaluated in the recombinant strains produced.

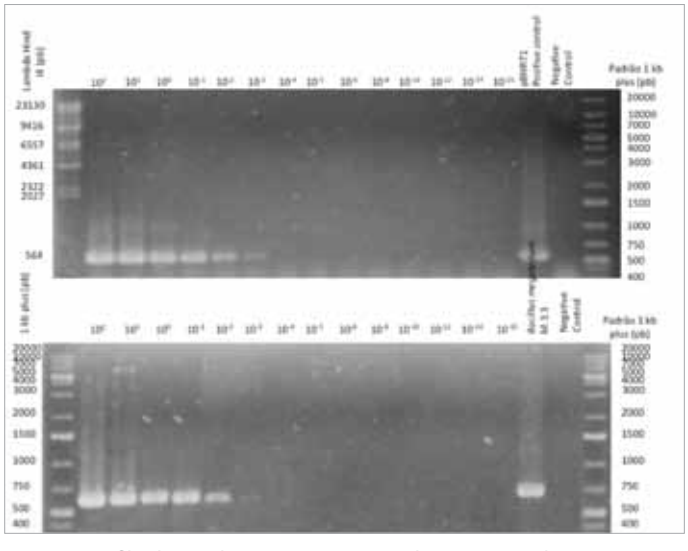

Figure 2. Profile electrophoretic on agarose gel 0.8% (w / v), showing the sensitivity of PCR to detect the minimum concentration (in ng /ul) of the type IV PHA synthase gene of Bacillus megaterium *3.3 MA using the primers in B1F/B1R PCR reaction. The minimum concentration detected by the technique is 0001 ng / ul. At the extremes of the gel can be visualized molecular weight marker 1 kb DNA plus (Fermentas)*

MAIN PUBLICATIONS

Galindo YP. 2011. Bioprospecting in a metagenomic library from Atlantic forest soil for genes involved on the biosynthesis of biodegradable polymers [Master dissertation (Biotechnology)]. São Paulo: Instituto de Ciências Biomédicas da Universidade de São Paulo, 2011.

Flora AB, Galindo YPR, Kawai LA, Contiero J, Silva LF, Gomez JGC. 2011. Validation of PCR sensitivity for gene detection in genomics and metagenomics libraries . 26th Brazilian congresso of Microbiology, Foz do Iguaçu, Paraná, Brazil, October, 2011.

Galindo Rozo YP, Massini KC, Padilla G, Gomez JGC, Silva LF. 2010. PCR screening of PHA synthase genes in Brazilian soil samples of Atlantic Forest. International Symposium on Biopolymers ISBP 2010, Stuttgart, Germany , October 2010.

Rozo YPG, Pinzon DCT, Flora A, Gomez JGC, Silva LF. 2010. Bioprospección de genes de la PHA sintasa en metagenoma de suelo brasileño de Selva Atlântica. XX Latinamerican congress on Microbiology, Montevideo, Uruguay, Septiembre 2010.

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