Production of polyhydroxyalkanoates on waste cooking oil employing *Pseudomonas rhizophila* S211

Khouloud Hammami, Amal Souii, Habib Chouchane, Ameur Cherif, Mohamed Neifar

Univ. Manouba, BVBGR-LR11ES31, Higher Institute of Biotechnology, Biotechpole Sidi Thabet, Ariana, Tunisia

Background and aim: Polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible polymers which are considered as competitive alternative to petroleum-based polymers. Waste cooking oil (WCO) is a major pollutant, primarily managed through incineration. The high cell density bioprocess developed here allows for better use of this valuable resource since it allows the conversion of WCO into biodegradable polymer polyhydroxyalkanoate (PHA). The aim of this work was to study the potential of *Pseudomonas rhizophila* S211 for conversion of waste cooking oil into PHA.

Methods: The accumulation of intracellular PHA granules in *P. rhizophila* was detected by Nile blue A staining of the colonies. To achieve maximum PHA yield by the strain S211, the culture conditions were optimized through response surface methodology (RSM) employing a doehlert with three factors namely, substrate concentration (5.7-14.3%), inoculum size (2-10%), and incubation time (1.2-2.8 days). The produced polymer was assessed using Fourier transform infrared spectroscopy.

Results: The statistical and graphical analysis results of this study confirm the adaptability of the strain and its ability to exploit waste cooking oil as a sole substrate for PHA production. *P. rhizophila* achieved a 1.8 g/l of PHA using waste cooking oil (Table1). The Doehlert design was suitable for identifying the optimum production conditions and that the new PHA copolymer is a promising biodegradable and biocompatible bioplastic for medical and food packaging applications. These results were validated by genome analysis, which revealed the presence of typical structural genes involved in PHBV metabolism including *phaC1, phaZ, phaC2, phD, phaF, and phaI.* The class II operon is well conserved in *Pseudomonas* and consists of two PHA synthase genes (*phaC1* and *phaC2*) flanking a PHA depolymerase gene (*phaZ*) and *phaD* encoding a transcriptional activator (Figure1). The *phaF* and *phaI* genes encoding phasin are divergently transcribed into other genes. Multiple sequence alignment showed that the putative PHA synthase gene contains all the highly conserved amino acid residues.

Conclusion: The results of this study confirm the ability of the S211 strain to exploit waste oil as the sole carbon source for PHAs production. This use of waste oil for PHA production has paved the way for recycling of waste oil for the benefit of the environment and PHA production. Therefore, *P. rhizophila* can be considered being an auspicious candidate for PHA production from waste cooking oil.

Keywords: *Pseudomonas rhizophila* S211, Polyhydroxyalkanoates, waste cooking oil, Doehlert design, Genome annotation