Título: Prospecting of PHA (polyhydroxyalkanoates)-producing bacteria in Araçá River – AM.

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Abstract

Biopolymers (bioplastics) are an environmentally friendly alternative to fossil fuel-based materials. They are produced from renewable materials such as plants, animals and also from bacteria. Several bacteria accumulate polyhydroxyalkanoates – PHA, linear polyesters as intracellular granules to store carbon and energy. It presents outstanding material characteristics and exhibits a large variety of applications. In order to reduce the production costs to meet the competitiveness of bioplastics market, it is necessary to prospect a large number of microorganisms producing PHA efficiently. The traditional colorimetric procedure using Sudan Black, for its detection, presents several limitations. However, the recently introduced methodology using Nile Red dye has resulted in: (1) greater specificity for targeting PHA, increasing the accuracy of the screening, and also (2) the possibility of real time tracking of PHA accumulation, concomitant to the cellular growth, due to the fact of having the dye present in the culture medium. The aim of the present work was to improve the screening a large number of isolates by using the microvolumes of dye-containing broth, instead of traditional solid culture system. This new methodology was tested with 8 bacteria isolated from Rio Araçá. All tests were carried out with positive and negative controls. Pre-inoculum was prepared in 3 ml of Luria-Bertani broth and grown overnight at 30^{o} C, under agitation. Twenty microliters of the pre-inoculum were added to 200 µl of TGP broth containing 0.5 µg/ml of Nile Red dye. After 48 hours of growth at 30^{o}C, under agitation at 200 rpm, samples were centrifuged at 2,250 *g* for 20 minutes. The supernatant was discarded and the pellet was observed under UV light at 312 nm. Under these conditions, the samples showing orange-pink brightness are expected to contain biopolymer granules. Among 8 samples, 6 were positive for the dye accumulation, which were confirmed by the plate method using Nile Red dye. In order to improve the methodology, several additional tests are simultaneously being run, such as (1) elimination of the pre-inoculum phase, and (2) reading the plates at an early phase on a microplate reader. Our preliminary results indicate the new methodology to be an efficient and robust screening alternative for PHA-accumulating microorganisms from a large number of isolates, reducing time of processing and cost.

Key words: Bacteria, polyhydroxyalkanoates, Nile Red, biopolymers.

Agência de Fomento: CNPq, FAPEAM