

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024

Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.



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SYNTHESIS AND STRUCTURAL ANALYSIS OF AZIDO-FUNCTIONALIZED POLYHYDROXYALKANOATES PRODUCED BY *Pseudomonas putida* KT2440 STRAIN

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Polyhydroxyalkanoates (PHA) are biopolymers produced by several bacterial species from the genus *Pseudomonas*, which exhibit different properties according to the composition of their monomeric units. Cofeeding is a useful approach to achieve the modifications on the chemical structure of PHA, to spread the application range of the polymers. This technique implies the inclusion of an additional carbon source together with the main one. As a result, the functional groups present in the supplementary source are introduced into the PHA chain during the biosynthesis. Functionalization allows further chemical reactions on the installed chemical groups, enhancing the structural variability. Herein, a non-pathogenic strain of *Pseudomonas putida*, KT2440, was used to generate an azido-functionalized PHA (PHA N3) by cofeeding with 6-azido-hexanoic acid. Hence, PHA and PHA-N3 were obtained in 0.5 NE2 medium supplemented with caprylate or a mixture of caprylate/azido-hexanoic acid, respectively. PHA synthesis was first confirmed by Nile blue staining and FTIR spectroscopy. The polymers were then isolated and purified, after which structural analysis was performed using methanolysis-gas chromatography and 1D and 2D NMR spectroscopy. Native PHA was mainly constituted by the 3-OH C8 monomer, together with 11% of 3-OH C6 as a β -oxidation product. On the other hand, PHA-N3 was composed by a 3-OH C8 : 3-OH C6 : 3-OH C6 N3 : 3-OH C4 N3 molar ratio of 47 : 5 : 34 : 14. This indicated a molar ratio of unsubstituted : azidated units of 52 : 48, very close to the molar ratio of the acids used in the cofeeding. The molecular weight of the polymers was estimated by gel permeation chromatography, giving Mn 67,1 and 55,0 kDa for PHA and PHA-N3 respectively. Finally, the thermal analysis showed a two-step decomposition for PHA-N3, in agreement with the composition in substituted residues. Additionally, PHA-N3 was an amorphous polymer with a random distribution of the different monomers on the backbone (Tg -30 °C). This temperature was slightly higher than the Tg of PHA (-35 °C), suggesting that intermolecular

interactions derived from the azide groups on the polymeric chain may be relevant. The functionalized PHA-N₃ polymer enables the possibility of achieving polymerization click CuAAC reactions on the azide function, thus yielding a grafted biopolymer with bio-based side chains composed of poly(amide-triazole)s derived from D-galactose further enhancing structural and functional diversity.

Palabras clave: Pseudomonas - polyhydroxyalkanoates - cofeeding - click - structural analysis