

TÍTULO: MOLECULAR PHYLOGENY AND IMMOBILIZATION OF CYCLODEXTRIN GLICOSYLTRANSFERASE FROM *BACILLUS sp.* SM-02 IN CALCIUM ALGINATE

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ABSTRACT:

The *Bacillus sp.* SM-02 is a bacterium phylogenetically related to *Bacillus tryplexicola* and produces cyclodextrin glycosyltransferase (CGTase, E.C 2.4.1.19), an enzyme of industrial importance produced by prokaryotic microorganisms, mainly species of the genus *Bacillus*. CGTase converts starch into cyclodextrins (CD), cyclic oligosaccharides capable of forming inclusion complexes with different molecules, and are used industrially in the solubilization, stabilization and distribution of different substances. Previous studies indicate that the immobilization of enzymes in matrixes such as calcium alginate and chitosan contributes to the increase of its stability. Therefore, the objectives of this work were to immobilize CGTase from *Bacillus sp.* SM-02, in calcium alginate and to analyze the evolutionary relations between CGTases characterized experimentally. For this, the crude enzyme extract was produced in submerged fermentation using cassava flour and cornflower as sources of carbon and nitrogen, respectively. Thereafter, the supernatant was immobilized on calcium alginate 3%. The stability of the *pellets* were tested in two buffers: Na₂HPO₄ pH 7,0 and Tris-HCl + CaCl₂ pH 8,0. To know the taxonomic distribution, a search was done for genes that encode the CGTase in the prokaryotic genomes available in the database of the National Center for Biotechnology Information (NCBI). The results showed that the cassava flour presented enzymatic activity of 370.88 U / μmol. It was observed a greater stability of the pellets in Tris-HCl buffer with CaCl₂. The taxonomic distribution demonstrated that CGTase is an enzyme that appears in different prokaryotic groups such as Gram-positive bacteria, Proteobacteria and Archaea and the phylogeny shows that *Bacillus tryplexicola* is related to a group of bacteria that produces β-CGTase. With these results, it is concluded that, the CGTase production occurs mainly in Gram positive bacteria, especially of the genus *Bacillus*, it is also suggested that *Bacillus sp.* SM-02 produces CGTase type β-. The immobilization was performed successfully, and the use of Tris-HCl buffer with CaCl₂ proved to be efficient for pellet storage.

Keywords: Enzymes, prokaryotes, biotechnology.

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