ENZYMATIC CHARACTERIZATION OF AMYLASES OF BACTERIA ISOLATED FROM SOIL CULTIVATED WITH SUGARCANE

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Introduction. Amylases hydrolyze glycosidic bond α-(1-4) and α-(1-6) of starch, glycogen and other polysaccharides are primarily derived from microbial sources, and have several applications in industry, mainly in food industry, hence the high demand. In the global market at year 2010, amylases sales were estimated at $480 million of dollars (cienciacierta.uadec.mx). The Cuenca of the Papaloapan (in Oaxaca, Mexico) is a megadiverse region, which has not been used to search for strains with enzymatic activities.
The objective of this work were the isolation of amylase producing bacteria from soil cultivated with sugarcane in the Cuenca of the Papaloapan, the taxonomic identification of the selected bacteria, the approach of a process for the production of amylases, and partial characterization part of the amylases produced by evaluating the effect of temperature and pH on enzyme activity.

Methods. A scrutiny of amylase activity to bacteria isolated from soil cultivated with sugarcane was done, the bacteria growing them on nutrient agar supplemented with starch 1% (w/v). The enzymatic activity detected on the substrate was determined by the presence of hydrolysis halos around the colonies after adding lugol solution. Identification of the isolates was carried out using Gram stain, biochemical profile was evaluated using API 20A and API 20EA and 16S DNA sequencing. The ratio hydrolysis halo: colony diameter, was employed for isolates selection using various culture media. The enzyme was recovered from the liquid medium by precipitation with $NH_4(SO_4)_2$ to 60%, after cells remotion by centrifugation. The molecular weight was determinated after SDS-PAGE electrophoresis. Finally, we evaluated the effect of pH and temperature on enzyme activity.

Results. Four isolates were obtained with amylase activity, named JJC31, JJC32, JJC33M and JJC33N. Gram staining and biochemical galleries revealed that strains JJC31, JJC33M and JJC33N are Gram positive belonging to the genus *Bacillus* whilst JJC32 resulted Gram negative. The phylogenetic analysis of the 16S DNA gene from the isolated JJC31, JJC33N JJC33M and showed 99% identity related to the 16S gene sequence reported for *Bacillus amyloliquefaciens* NBRC 15535 (Nishiwaki et al., 2006), and to the 16S DNA gene from the isolated JJC32, 99% identity to the sequence reported for *Enterobacter asburiae* 16S JCM6051 (Harada et al., 1996). With the preliminary screening, using the ratio of hydrolysis halo: colony diameter, strain *B. amyloliquefaciens* JJC33M highest value obtained using as a culture medium $CaCl_2$ (0.1g / l), yeast extract (5g/l) and medium Mandel enriched with starch 1% (w/v), thus selecting the enzyme of this strain for the enzymatic characterization. Amylase *B. amyloliquefaciens* JJC33M (AmiJ33) was recovered by precipitation with $NH_4(SO_4)_2$, 60% saturated. The molecular weight of AmiJJ33 was determined as 55KDa. The amylase activity was evaluated at pH 4, 5, 6, 7 and 8, with the highest activity at pH 6, also evaluated the activity of the enzyme at 40, 50, 60, 70, 80, 90 and 100°C, showing the highest activity at 80°C. Currently its thermostability is being evaluated.

Conclusions. Various strains were obtained with amylolytic activity from soil cultivated with sugar cane in the Cuenca of the Papaloapan. The enzymatic characteristics of AmiJ33 from *B. amyloliquefaciens* JJC33M, enable their employment in the industry, probably cleaning industry.

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