AMYLOLYTIC POSITIVE CLONES DERIVED FROM A SUGAR CANE SOIL METAGENOME

Adolfo Pérez Muñoz[1], Rina Gonzalez[1], Esteban Barranco[1] and Adelfo Escalante[2].[1]Departamento de Sistemas Biológicos, UAM-Xochimilco, C.P. 04960, D.F. México. [2]Departamento de Ingeniería Celular y Biocatálisis. Instituto de Biotecnología, UNAM, Cuernavaca, Mor. 62210, México. adpemu1@hotmail.com.

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Introduction. Metagenomics is a new field of research developed in the last decade, which has as one of its main objectives to identify the enzymatic potential of microorganisms. (1) Amylases were the first enzymes to be discovered and described, they have been among the most important enzymes in many industrial fields, and they have a special place in the food industry. (2)

The main objective of this research was to construct a metagenomic library from sugarcane soil and screen for amylolytic enzyme activities.

Methods. For the metagenomic library construction, soil samples were taken from a sugarcane soil in Tlalquitenango, Morelos state of Mexico. Extraction of total DNA was made using Power Soil DNA Isolation Kit (MO-BIO). *Escherichia coli.* Top 10 was used as a host and pCC1FOS (Epicentre), was employed as a vector. *E. coli* top 10 was then transformed and chloramphenicol resistant strains were tested for amylolytic activity. Transformed cells were grown in liquid M9 medium supplemented with 1% of starch. From this culture, serial dilutions were plated on Luria–Bertani (LB) agar plates supplemented with 0.1% starch, 12.5 µg/ml of chloramphenicol and incubated for 24 h at 37°C. Plates were flooded with iodine solution and colonies detected by the formation of clear halos against a dark violet background were later tested for quantitative amylolyitic activity assay in liquid M9 medium as before. The cells were removed by centrifugation at 10,000 rpm for 10 min. Supernatant was used as the extracellular enzyme source. *Bacillus sp* strain was used as a positive control, negative controls were the *E. coli* Top 10 strain and *E. coli* / pCC1FOS without insert. Amylase activity assay of the enzymes was performed by using soluble starch followed by determination of reducing sugars by a DNS assay.(3) One unit of hydrolyzing activity (UA / min l) was defined as the amount of enzyme required to produce 1 mol of glucose equivalent reducing sugar in 1 min.

Results. A sugarcane soil metagenomic library was obtained. The average inserts size was between 20-30 kb. Extracellular amylase activity of three isolates (a,b,c) in soluble starch was observed by hydrolysis of starch, presenting a clear zone formation in LB starch Agar medium (Fig. 1)

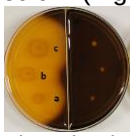

Fig.1 LB starch Agar plate showing 3 clones expressing clear zone formation (left side) and no amylase activity with no clear zone formation by negative controls (right side)

Out of 3 selected clones in agar plates, clone c presented the highest activity (0.5130 UA / min l) in liquid M9 medium, compared with negative controls and *Bacillus sp.*as well. The other 2 strains showed very low activity

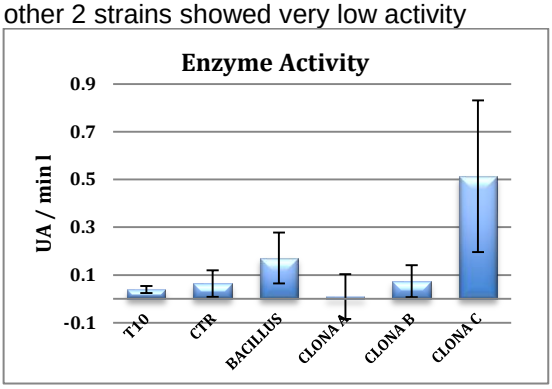

Fig.2 Enzyme activity (UA / min l)

Conclusions. Using a strategy of, first, culturing the metagenomic pool in minimal liquid medium with starch and then plate in LB agar/starch medium; from about 300 clones, we found one positive clone for amylase enzyme activity.

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