CARBOXYLESTERASES PRODUCED BY *Aspergillus nidulans* AND ITS APPLICATION IN BIOCATALYSIS.

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Introduction. Fatty Acid Methyl Esters (F.A.M.E.) are among the most common substitutes for petrodiesel (1). These are usually produced by reacting lime and methanol with oil, usually from a vegetable source (2). While this is a cheap and useful system, the main drawback lies in toxic waste produced during the reaction which requires extra effort to neutralize (due its high pH) and the catalyst is lost. Enzymatic catalysis offers advantages, because products do not require such complex cleaning and, if properly immobilized, they can be used for several cycles of reaction (3).The purpose of this work is to evaluate several esterases produced by *Aspergillus nidulans*(*ANCUT1, ANCUT2, PrtA and NStcI*) (4,5) immobilized on different supports for the production of methyl esters.

Methods. Culture media designed specifically for the production of each enzyme were used (4,5). After cell harvest, the supernatant was filtered, concentrated and SDS PAGE gels were run in order to identify the presence of the enzymes of interest. Celite, nylon and Accurel were used as immobilization supports. The selected biocatalyst was employed in a 24 hours esterification reaction along methanol and oil (molar rate 6:1) and a 3 Å molecular sieve.

Results.

Figure 1 shows the carboxylesterases obtained in different production conditions

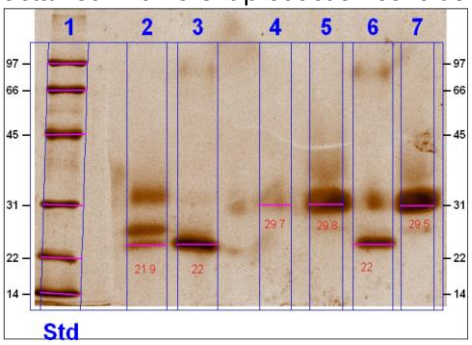

Fig.1 SDS PAGE gel. Bands of interest are identified through Biorad's Image lab software. Each lane depicts different production time and an approximated molecular weight similar to that of the enzyme of interest

100 mg of lyophilized enzyme were added to 10 ml of sodium phosphate buffer 50 mM pH 7. The lyophilized enzyme powder had 90.82 µg/mg of powder and 757.77 activity units per mg of protein. Table 1 shows the results for the enzyme that showed the highest activity towards long chain fatty acids and its behavior in different immobilization supports.

Table 1.

Enzyme	Support	Bound proteína	Bound enzyme (Units)
ANCUT2	Accurel MP1000	28.48%	15.51%
	Nylon 6	27.68%	28.91%
	Celite 545	66.28%	27.31%

Thin layer chromatography was used to detect possible transesterification products using ANCUT2 immobilized on Celite 545 on sesame oil. Figure 2 shows the presence of methyl esters.

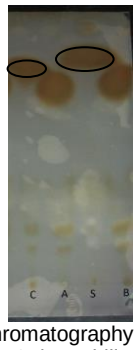

Fig.2 Thin layer chromatography was used to detect possible products. C immobilized ANCUT2 , A for sesame oil, S for biodiesel from sesame oil, B as a blank.

Conclusions. Among the caboxylesterases produced by A. nidulans, ANCUT2 was the one that showed highest affinity towards long chain fatty acids. When Celite was used as immobilization support, bound protein was higher. This biocatalyst yielded methyl esters in sesame oil.

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