FUNCTIONAL METAGENOMICS IN THE AQUIFER OF YUCATAN: SEARCH FOR NOVEL PROTEASES

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Introduction. Proteases are enzymes that break down protein into amino acids so the cells can use them. The inability of current commercial proteases to meet the catalytic specifications that the world demands in several industries has led to an increased interest in microbial proteases. Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity, so proteases from microbial sources possess almost all the characteristics desired for specific biotechnological uses. They have a large variety of applications, mainly in the detergent and food industries, and more recently exist a trend to develop environment-friendly technologies. Proteases are envisaged to have extensive applications in leather treatment and in several bioremediation processes (Mala, et al., 1998).
Some estimates suggest that more than 99% of bacteria present within any ecosystem cannot currently be cultured using conventional approaches (Amann and Schleifer, 1995), To grantfully access to exploit the potentially unique biochemistry present within this unculturable fraction, metagenomic approaches need to be employed.

Methods. 250 L of underground water were sequentially filtered through a 5 µm pore size polycarbonate filter and the filtrate passed through 0.22 µm pore size Sterivex filters (Durapore, Millipore) using a peristaltic pumping system. eDNA was isolated using the "Metagenomic DNA Isolation kit for water (epicentre, No. Cat. MGD08420), and cloned in pCC2FOS™ vectors (Epicentre Cat.No. CCFOS059) according to the manufacturer directions. The screening media was LB containing chloramphenicol and 1% (w/v) fat free milk.

Results. As shown in figure 1, the inserts in the fosmids metagenomic DNA library were all different from each other and had a mean of 40 kb. Currently, we are doing the screening.

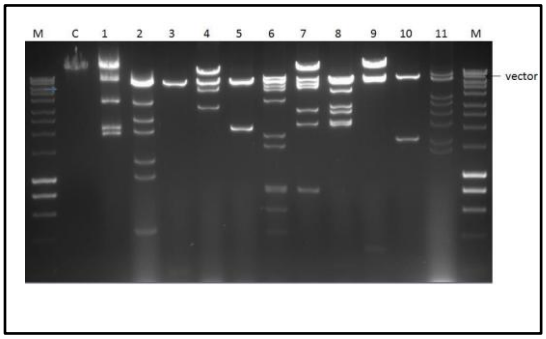

Fig.1 *BamHI* digestión of eleven randomly selected clones de clones of the fosmids library. M: 1 Kb molecular marker; C, 40 kb DNA control; 1-11 clones digested witn *BamHI*.

Conclusions. We have been cloned successfully fragments of metagenomic DNA (~ 40kb); so far we have a library with 5,000 clones and we will isolate more in order to obtain a total count of 20,000 whose we will screen functionally on milk agar.

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