



MICROBIAL LIPASES IN A METAGENOMIC LIBRARY

Sergio Ramos-Jiménez¹, Bartolomé Chi-Manzanero¹, Miguel Canseco-Pérez¹, Max M. Apolinar-Hernández¹, Miguel Marfil-Santana¹, César De los Santos-Briones¹, Rafael Rojas-Herrera³, Alejandra Prieto-Davó², Aileen O'connor-Sánchez¹, Blondy Canto-Canché¹.

1 Centro de Investigación Científica de Yucatán, A.C. Calle 43 No. 130, Colonia Chuburná de Hidalgo, Mérida, Yucatán. México. C.P. 97200. 2 Facultad de Química, Unidad Sisal. Universidad Nacional Autónoma de México. Puerto de Abrigo s/n Sisal municipio de Hunucmá, Yucatán México C.P. 97356. 3 Universidad Autónoma de Yucatán. Facultad de Ingeniería Química. Campus de Ingenierías y Ciencias Exactas. Periférico Norte Kilómetro 33.5, Tablaje Catastral 13615, Col. Chuburná de Hidalgo Inn. Mérida, Yucatán, México. C.P. 97203. E-mail: cantocanche@cicy.mx

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Introduction. Lipases are an important group of enzymes for the food, chemical, textile, pharmaceutical and biofuels mainly (Hardeman and Sjoling, 2007). Moreover, microorganisms are an important potential source of new products, but production is limited by difficulties to cultivate them by traditional methods (Zheng et al, 2010). An alternative is the development of metagenomic libraries for further screening in the search for new products of biotechnological interest.

In this work, we are looking for microbial lipases in a metagenomic library through functional and bioinformatic Approaches.

Methods. We present here the progress in the screening of the metagenomic library in the search of lipases. This metagenomic library was constructed from DNA isolated from the underground aquifer of Yucatan, yielding about 20,000 fosmids clones. Seeking for lipases, we have developed two strategies: one is by functional screening with 1% olive oil and rhodamine B as an indication of lipase activity, and the other strategy is by *in silico* analysis of the pyrosequencing library data.

Results. To date the *in silico* analysis allowed us find 6 sequences, three of them corresponding to a putative lipase (corresponding to a same fragment of 548 base pairs) (Table 1). Blastx analysis revealed the consensus sequence has a domain corresponding to the superfamily of esterases, lipases, with 59% identity with a lysophospholipase from *Brevibacillus sp.*. The others sequences have homologies corresponding to hypothetical proteins and carboxypeptidases.

The preliminary analysis of 1000 fosmid clones has not yielded any identification of any clone with lipase activity, but this is a very limited screening so far, reason why this cannot be a conclusive result. Functional screening is continuing on another 5000 clones.

Table 1. Sequences founded by *in silico* analysis in the metagenomic library.

Sequence	Length (bp)	Identity and accesión Number
HRLWF2101D5Y8 G	548	<u>Lysophospholipase [Brevibacillus sp. BC25] ZP_10574753.1</u>
HRLWF2101BAKB 1	328	<u>Lipase [Brevibacillus brevis NBRC 100599] YP_002773383.1</u>
HRLWF2101AWX CW	495	Lysophospholipase [Brevibacillus sp. CF112] ZP_10741650.1
HRLWF2101BCXY J	528	<u>Hypothetical protein PelgB_06151 [Paenibacillus elgii B69] ZP_09074036.1</u>
HRLWF2101ETUW 4	623	<u>Hypothetical protein PelgB_06151 [Paenibacillus elgii B69] ZP_09073119.1</u>
HRLWF2101DJVJ D	434	Membrane carboxypeptidase (penicillin-binding protein) [Brevibacillus sp. CF112] ZP_10741249.1

Conclusions. To date we have found three sequences in the metagenomic library data and we are screening for another 5000 clones.

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