Characterization and identification of hydrolytic enzymes of potential interest in biofuel production by using metagenomics approaches

Cecilia Rodriguez, Daniela Senatore, Vanesa Amarelle, Adriana Peri, Uriel Koziol, Elena Fabiano, and Francisco Noya
Laboratorio de Bioquímica y Genómica Microbiana, IIBCE, Montevideo, 11600, Uruguay

Nowadays, there is a strong trend towards the use of alternative energy sources that replace the fossil ones, both for economical and environmental reasons. Biofuels, such as biodiesel, constitute an alternative renewable source of energy that has a small carbon footprint. Biodiesel is produced by transesterification of vegetable or animal fats. The transesterification reaction is catalyzed by strong acids or bases that require massive quantities of alcohols. These processes are both very expensive and unfriendly to the environment.

An alternative to the chemical transesterification is the use of enzymes like esterases and lipases that can catalyze the conversion of triglycerides into esters of fatty acids. In addition, these enzymes have various applications in the food, dairy, detergent and pharmaceutical industries.

The aim of this work is to characterize and identify hydrolytic enzymes of potential interest for the production of biodiesel by using metagenomic approaches. Previously, this approach resulted in the generation of large datasets derived from various environments, such as soil, ocean water and animal rumen. In this work, we built a metagenomic library from 300 intestines of native termites. Fragments of 40kb were selected and cloned into fosmids, packaged into phages and used to infect E.coli strain EPI300T1R.

We were able to select six positive lipases and/or esterases on a screening medium based on glyceryl tributyrate. We are currently determining the activity of these enzymes on other substrates. To identify the genes responsible for these activities we are carrying out a Tn5-based generalized mutagenesis into the fosmids looking for loss-of-function phenotypes. The fosmids in which Tn5 insertions abolished enzyme activities will be sequenced outwards from the transposon flanking repeats.