Identification of new microbial enzymes from forest and marine ecosystems by a metagenomic approach.

While, less than one percent of the micro-organisms living in an environment are known and cultivable, metagenomics, studying the entire genome of an environment sample, is a new approach that enhances our knowledge about environmental microbial life and the "unknowns and unculturables", bypassing any cultivation step.

The aim of our study is to identify new microbial enzymes with an industrial interest and new enzymes families and structures from unknown and unculturables micro-organisms, by a metagenomic approach, in two kinds of environment (a terrestrial and a marine one).

Firstly, a metagenomic DNA library was constructed from a forest soil sample and screened for different enzymatic activities (lipolytic enzymes, xylanases, cellulases, alpha-amylases, arabinanases, caseinases and beta-glucosidases) in Eschericihia coli.

Three new lipolytic enzymes (LipMM1a.1, LipMM1a.4 and LipMM1a.6) were identified by plate test on tributyrin agar. A sequence analysis revealed that LipMM1a.1 (299 amino acids residues) and LipMM1a.4 (308 aa) should be classified in family IV of the lipase and esterase classification by Arpigny and Jaeger (Arpigny & Jaeger, 1999) and LipMM1a.6 (241 aa) in the family V from the same classification.

Two beta-glucosidase activities (B-gluMM1a.1 and B-gluMM1a.2) were also identified by plate test on a medium with esculin and ammonium ferric citrate. After sequence analysis, none of the two candidates seemed to encode for a known beta-glucosidase, but one (B-gluMM1a.1) encoded for a new glycosyltransferase (256 aa) of the group 4 from the CAZy classification (Coutinho et al, 2003).

Finally, one alpha-amylase activity was also identified by a plate test on agar containing 0.5% of starch. This candidate plasmid (A-amMM1a.2) contained an ORF (832 aa) with more than 30% of identity with a peptidase (probably responsible for the alpha-amylase activity) from a Plantomycetes.

Those five new microbial enzymes will then be purified aiming to characterize their enzymatic activities and to identify their structures.

In the second part of this study, aiming to compare our results with a complete different environment, a metagenomic DNA library from marine samples (coastal seawater and algae microflora) was constructed. This library will be screened for the same enzymatic activities and the obtained candidates will be compared with the terrestrial's ones: phylogeny, substrate specificity, enzymatic characterization, etcetera.

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- Coutinho P.M., Deleury E., Davies G.J., Henrissat B, 2003, An Evolving Hierarchical Family Classification for Glycosyltransferases, J. Mol. Biol.,328, 307–317