Enrichment and High Throughput Screening of POME Metagenomic Libraries for Bioprospecting Novel Cellulose-degrading Enzymes

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INTRODUCTION

Fig. 1: Production of cellulosic ethanol by **enzymatic degradation of cellulose** and microbial fermentation.

Cellulose degradation

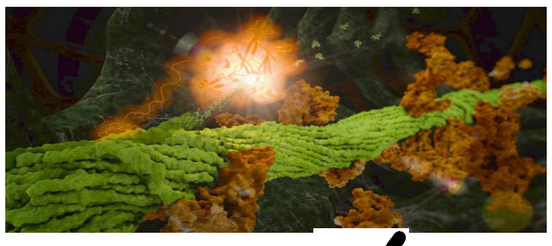
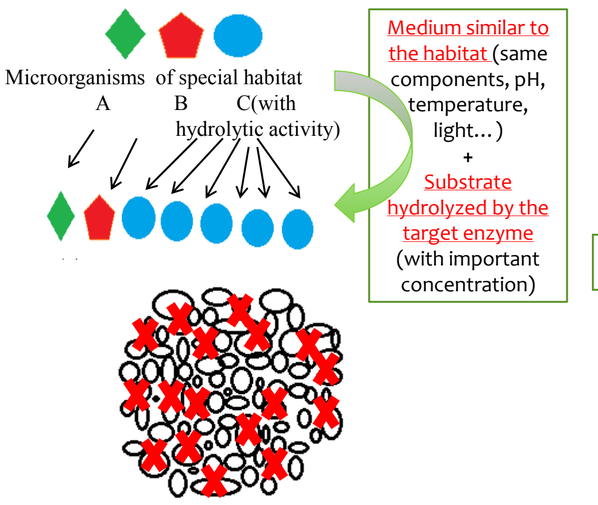


Fig. 2: Deconstruction of polysacharides to oligo- & monosacharides by the cellulose-degrading enzymes

Natural enzymes discovery

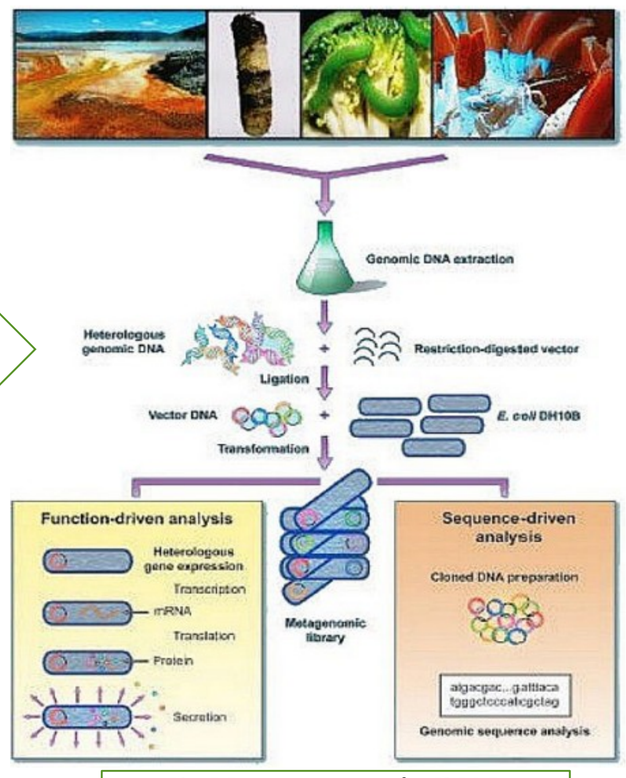
Fig. 3: Only a fraction (~1%) of microorganisms is culturable as potential resource of enzymes.

METHODOLOGY

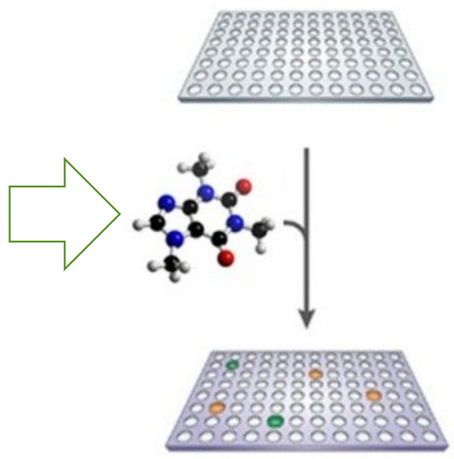


Medium similar to the habitat (same components, pH, temperature, light…)
+
Substrate hydrolyzed by the target enzyme
(with important concentration)

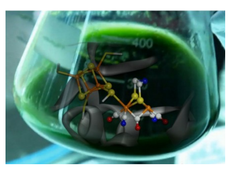
A. Enrichment strategy by natural selection



B. Metagenomic DNA library



C. High throughput screening



D. Production of specific, efficient and highly available cellulose-degrading enzymes

Fig. 4: Overview of bioprospecting novel cellulose-degrading enzymes from POME microorganisms

RESULTS AND DISCUSSION

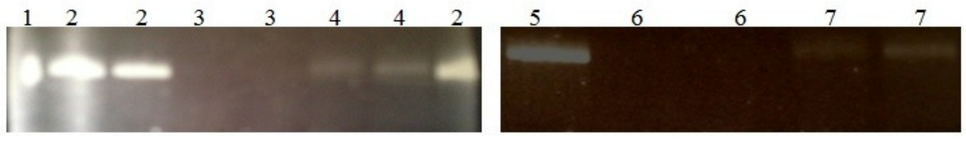


Fig. 5: LMP agarose gel electrophoresis of different enriched and non-enriched metagenomic DNA of POME. 1: fosmid control, 2: enriched-anaerobic, 3: enriched cooled, 4: anaerobic, 5: enriched- fresh, 6: cooled, 7: fresh. The enriched-anaerobic metagenomic DNA presents the highest quantity of DNA; it is 5 to 7 times more higher than non enriched metagenomic DNA, while the quantity of enriched-fresh is 5 times more important than non-enriched fresh

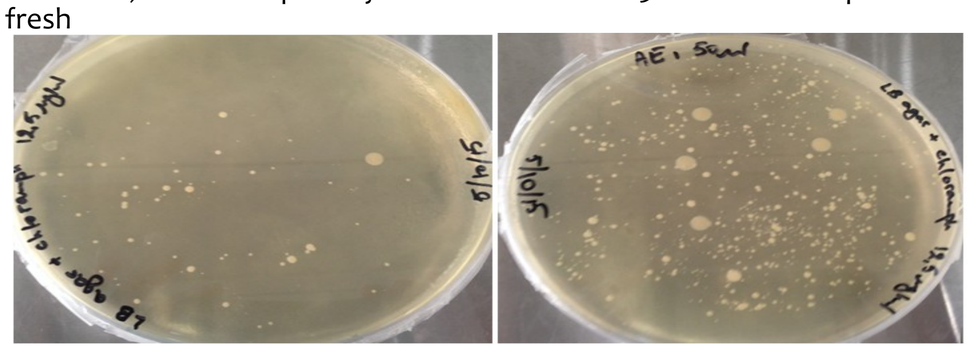
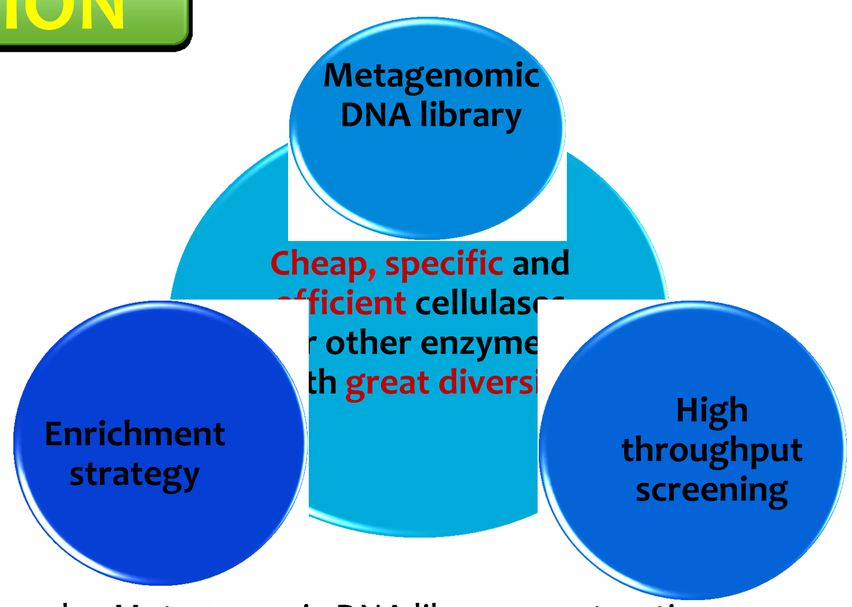


Fig. 6: Large Petri-dishes of transformed EPI300T1 plated on LB agar with 12.5 µg/ml antibiotic. (right) 400 to 600 colonies per Petri-dish from enriched cultures; (left) 70 and 100 colonies per Petri-dish from non-enriched cultures.



- Strategies namely «Metagenomic DNA library construction » combined with «high-throughput screening» and enhanced with « enrichment strategy » can improve the bioprospecting of novel catalysts from Nature.
- This study shows the potential of **bioprospecting** natural enzymes for green industry; cheaper, specific and efficient and highly available.

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