A strategy to isolate extended protein open ready frames from metagenomic DNA

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The increased use of enzymes in industry requires technological innovation that to improve catalytic performance and reduce costs. Cellulases catalyze the hydrolysis of cellulose, and can be applied in numerous processes including the production of second generation bioethanol. With a view to applications in biomass biorefinery, the objective is to identify new cellulases from environmental DNA samples that present high catalytic activity at acidic pHs, and wich are thermostable. The bioprospection strategy uses metagenomic analysis through tracking the promoter region of prokaryote genes to build ORF libraries from a microbial community in samples. And as innovative strategy was used to create libraries containing extended proteins coding regions. Key steps for screening of extended ORFs were standardized using *Bacillus subtillis*, and the feasibility of the methodology was confirmed by DNA sequencing with identified a 16S pseudoridylate synthase complete gene of 720nt . The strategy for cellulose screening was validated using the endo-β-1-4 glucanase from *Bacillus subtilis*, in which detection of the enzyme is performed on Petri plates containing solid LB medium with carboxymethyl cellulose as substrate. The use of this innovative technique was able to identify a complete gene in the correct frame, and shows promise for screening metagenomic libraries to identify enzymes of biotechnological interest.

Keywords: metagenomic, cellulase, industrial enzymes, biotechnology, bioprospection

Supported by: FAPESP, CAPES, CNPq e PRP-USP

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