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Oral / Poster

1497-2 Two Glycosyl Hydrolase Clones Isolated from a Small-insert Metagenomic Library from Amazon Soil Environmental DNA

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Resumo

Bacterial enzymes are promising biocatalysts for the production of second generation bioethanol. Since about only 1% of bacteria can be readily cultured, the metagenomic approach was employed in this study to explore the biotechnological prospects from unknown microorganisms present in the Brazilian Amazon soil. A small-insert metagenomic library of approximately 70,000 clones was constructed with environmental DNA from the Brazilian Amazon soil. The library was screened for several enzymatic activities, where 34 clones with positive activities were isolated. Two of these, which presented glycosyl hydrolase activity, named AmEndo02 and AmEndo03, were chosen to be further studied. These clones were isolated by enzymatic screening with pre-swollen Carboxymethyl cellulose (CMC) and, after confirming their phenotype stability by re-transforming their plasmidial DNA, they were sequenced by primer-walking. The sequences obtained were analyzed by the ORF Finder and Blast tools. Clone AmEndo03's structure for the protein coded by its ORF of interest was predicted by comparative modelling using the software Modeller 9v8. Also, this structure was validated through analyses by the PROCHECK and ProSA-web tools. Clones AmEndo02 and AmEndo03, with insert sizes of 3.3 and 7kb respectively, both presented activity on different types of CMC, birchwood Xylan and colloidal chitin as early as 3 days after they were streaked on culture media containing these substrates. Analysis of clone AmEndo02's sequence did not reveal open reading frames with similarity to known glycosyl hydrolases, while AmEndo03 has an open reading frame with similarity to a bacterial β-mannanase. Also, AmEndo02's sequence Blastn analysis presents similarity to microorganisms from the phylum Planctomycetes, whereas AmEndo03's insert has similarity to the phylum Acidobacteria. Finally, a predicted structure was generated for the protein coded by clone AmEndo03's ORF of interest with four domains, presenting 91.5% of its residues in most favored regions and a Z-score of -7.99. Clones AmEndo02 and AmEndo03 are currently undergoing further studies and, in the future, their enzymes will be expressed in heterologous expression systems.