https://doi.org/10.31436/iiumej.v25i2.2024

FUNCTIONAL METAGENOMICS APPROACH FOR DISCOVERY OF NOVEL COLD-ACTIVE PROTEASE FROM ANTARCTIC REGION

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(Received: 5 November 2023; Accepted: 12 March 2024; Published online: 15 July 2024)

ABSTRACT: The structural complexity of bacterial life makes most of it impossible to culture. Functional metagenomics approaches overcome the limitations of a culture-based approach in exploring and assessing the genetic materials of uncultured microbes. The objective of this study was to identify clones with cold-active proteases through functional metagenomics. In this work, the environmental DNA (eDNA) isolated directly from Antarctic soils was ligated into the pCC1FOS fosmid vector, transformed into EPI300-T1R *E. coli* host cells, and screened for proteolytic enzymes. Positive protease-producing clones were identified and isolated on skim milk agar supplemented with chloramphenicol and arabinose. This clone harbored a fosmid, pCC1FOS, which has a 48.5 kb insert that has been completely sequenced in both directions. Further analysis of the insert showed 70 NODEs. The NODE_42 encoded hypothetical protein of 297 amino acids showed a significant match to Peptidase M23 and PG-binding 1 proteins families. A three-dimensional model of the predicted protease was generated based on the known mesophilic protease of *Neisseria meningitides* (PDB: 3SLU). The structural alignment showed 27.07 % similarity with RMSD value of 0.402 Å based on 58 aligned residues. The active site residues were identical, but major deletions were observed in the predicted proteases. This predicted protease showed higher activity at -20 °C and 20 °C than the positive control (protease from bovine pancreas). Functional metagenomics is a promising approach in the discovery of cold-active protease with low homology to the known sequences and expressed in the host cell that has the potential for bioprospecting in low-temperature applications.

ABSTRAK: Kesukaran struktur kehidupan bakteria menyebabkan kebanyakan bakteria sukar dikultur. Saringan fungsi metagenomik dapat mengatasi kekangan saringan berasaskan kultur dalam meneroka dan menilai bahan genetik mikrob tidak kultur. Objektif kajian ini adalah