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

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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 meningitidis* (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. © (2024), (International Islamic University Malaysia-IIUM). All rights reserved.

Author keywords

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