UNIVERSITI TEKNOLOGI MARA

CHARACTERIZATION OF PROTEASE FROM THERMOPHILES ISOLATED FROM LOCAL HOT SPRINGS

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ABSTRACT

To obtain enzymes with improved thermostability, many have resort to isolate enzymes from naturally occuring thermophilic organisms. However, the disadvantage is that it is often difficult to get good yield. Hence this study was undertaken to investigate the thermophilic protease production in eight isolated thermophiles (A1, A3, A4, A5, A6, A8, A13 and A14) by both biochemical PCR. All the eight thermophiles were subjected to skim milk agar assay to detect protease enzyme and further amplified by PCR for protease gene. All the eight thermophiles were positive for protease gene but in skim milk assay, only A1, A3, A4, A6 and A8 demonstrated hydrolysis. Comparative analysis amongst these eight indicated that A8 had the highest proteolytic activity and therefore was further examined its potentials. A8 was found to be motile, Gram positive rod with endospore, catalase positive, oxidase negative and is a glucose, sucrose, fructose fermenter except maltose. The protease enzyme was stable at 55°C to 75°C and was most active at pH 7. Furthermore, A8 protease activity was inhibited by 5 mM ethyldiaminetetraacetyl acid and 5 mM phenysulphonylmethylfluoride thus indication that it produced two types of proteases; metalloproteases and serine proteases. Further indentification by 16S rRNA gene sequencing revealed that it is closely related to *Geobacillus thermocatenulatus* strain BGSC 93A1 with 70% similarity to peptidase of *Geobacillus* sp. strain C56-T3 (Accession No. CP002050.1), *Geobacillus* sp. strain Y412MC61 (Accession No. CP001794.1) and *Geobacillus kaustophilus* strain HTA426 (Accession No. BA000043.1). The protease fragment sequence *of Geobacillus* sp. strain A8 was submitted to GenBank (Accession No. JF960945) where it codes for S8 peptidase group (a group for serine-type protease). Molecular weight determination by SDS-PAGE for A8 was found to be 20-27 kDa. The total protease activity by fermentation study was 185 U/ml. In conclusion this study had successfully isolated a thermostable protease producer identified as *Geobacillus* sp. A8 based on 16S rRNA and biochemical analysis.

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