(ID 038)

Industrial Perspective of Thermo-Stable Proteinase Enzyme Producing Bacteria Isolated from Maha Oya Hot Spring, Sri Lanka

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Abstract

The microbial enzymes are eco-friendly and cost-effective alternatives for chemical catalysts that adversely affect the ecosystem. Among the commercially available enzymes, proteases are the most demanded enzyme and contributes nearly 60-65 % of the world enzyme market. Therefore, this study aimed on isolation and characterization of thermo-stable proteinase producing hot spring bacteria. For the present study, water samples were collected from surface and bottom of Maha Oya hot springs. Temperature, Electrical Conductivity (EC), pH, and Dissolved Oxygen (DO) were measured at the site. The standard pour plate method was performed to isolate bacteria and primarily screened for proteinase production by Skimmed Milk Agar (SMA) plate assay. Secondary screening was performed using the Folin-Ciocalteu's Phenol reagent method. Optimum temperatures and pH for enzyme activity were measured for crude enzyme extracts of most potential candidates. Molecular-level identification of bacterial isolates was performed using the 16S rRNA gene sequencing method. Briefly, genomic DNA was extracted from pure bacterial isolates and PCR amplification of V3 and V4 regions of 16S rRNA gene was done using 27F and 1492R bacterial domain specific primers. Then, the DNA sequencing was done at Macrogen, Korea. The resulted sequences were aligned with available sequences at NCBI using BLASTn tool and GenBank accession numbers were obtained. Temperature, EC, pH, and DO of the surface and bottom of the springs varied from 51.7-52.4º C, 1487-1,507 µS/cm, 8.05-8.07, and 2.01-2.05 mg/L respectively. Three morphologically different, proteinase producing bacterial isolates were observed ($Mh_1$1, $Mh_1$2 and $Mh_2$7) at the primary screenings and bacterial isolate: Mh11 which was identified as Bacillus cereus strain VBE03 (Accession number: ON819722) showed the highest enzyme activity of 209.205 U/mL at optimum temperature of 60º C, and optimum pH of 9. Hence, the bacterial isolate: $Mh_1$1 would be successfully used for industrial settings operate at temperatures around 60º C and alkaline conditions.

***Keywords**:* Hot springs, Enzymes, Proteinase, Biotechnology, Thermophiles