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Geobacillus* sp. SH-1 Isolated from Thermal Springs of Mahaoya with Industrially Feasible Thermostable Alkaline Protease Activity*Nandanee G.G.W.* , Wijeyaratne S.C., Dasanayaka P.N.***Department of Botany, University of Sri Jayewardenepura, Nugegoda, Sri Lanka***nandanee@sjp.ac.lk***Abstract**

Sri Lanka embraces ten thermal springs running along a narrow belt from Hambanthota to Trincomalee. Thermal springs are rich sources of thermostable bacteria that can have high industrial potential. Mahaoya thermal springs; one of the main springs recorded in Sri Lanka situated at Latitudes 7.55° North and Longitudes 81.359° East in the Ampara District, Eastern Province of Sri Lanka. Bacteria were isolated from Mahaoya thermal springs with the view of isolating bacteria producing thermostable protease. Water samples were collected from the thermal springs and inoculated into pre-warmed Luria Bertani Broth medium at the sampling site itself. Purified bacterial cultures were further identified based on morphological, biochemical and physiological parameters. Isolates were screened for protease production at 45° C in skimmed milk agar plates and crude proteases were extracted from positive isolates. The activity of protease was investigated over a range of temperatures; 35-75° C using a medium with an initial pH of pH 10 in the medium with 1% tyrosine as the substrate. The thermal stability of the enzyme extracts and the effect of pH on protease activity were studied. The preliminary screening tests for protease activity, revealed that one of the cultures isolated had high enzyme activity. The respective culture had cream coloured, dry, elevated colonies having transparent out growths with irregular margin were morphological characters observed on cultures in Luria Bertani agar. It was a Gram positive long rod with an optimum growth temperature of 45° C at pH 7. The culture was catalase positive, fermented glucose and sucrose and reduced nitrate. The crude protease extracted from the culture had its maximum activity of 3.84 U/ml at 70° C at pH 10. To investigate the effect of temperature on the stability of crude protease extracts, enzyme extracts were incubated at 50° C, 60° C and 70° C. and enzyme samples were drawn out at 15 minutes intervals up to 60 minutes and were tested for their residual enzyme activity. Extracellular protease of the culture was found to retain 75% and 65% of its activity at 50° C and 60° C respectively but lost its activity by 50% at 70° C once incubated for 60 minutes. The bacterium was identified as *Geobacillus* sp. SH-1 by 16S RNA sequencing using 27F;5' AGAGTTTGATCCTGGCTCAG3' and 1492R; 5'TACGGYTACCTTGTTACGACTT3' universal primers. The activity assay experiments reveal that the *Geobacillus* sp. SH-1 isolated from natural thermal springs of Mahaoya has the potential of producing thermostable alkaline protease with an industrial potential.

Keywords: Thermal springs, Protease, *Geobacillus***Acknowledgement:** Sri Jayewardenepura University grant ASP/06/RE/SCI/2012/02