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Screening and Characterisation of Thermophilic Bacteria from Hot Springs of Sri Lanka for Potential Industrial Application

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Abstract

Hot springs are the natural springs that contain water warmer than 37^0 C. They are rich sources of thermophilic or thermo-stable microorganisms; algae, cyanobacteria, archaea and bacteria. In Sri Lanka ten hot water springs are found along a narrow belt running from Hambanthota to Trincomalee with temperatures ranging from 35°C to 61° C. However, these hot water springs have mainly been used for recreation and only limited microbiological studies have been conducted. The objective of this study was to screen thermophilic bacteria from Madunagala, Mahaoya, Wahawa and Nelumwewa hot springs in the southern and eastern Provinces of Sri Lanka with the view of exploring the possibility of using them in industrial applications.

Water samples (10 ml) were inoculated aseptically at the sites to Lauria Bertani (LB) broth (1% tryptone, 1% NaCl, 0.5% extract) medium (100 ml), pre-warmed to the temperature of the spring, in sterile flasks (three×250 ml flasks for each well) aseptically and brought to the laboratory in thermo-stable containers. Flasks were incubated at 45° C for 24 h. Subsequently isolations were done using standard techniques on LB agar medium and incubated at 45° C. Sixteen strains of thermophilic bacteria were isolated, based on colony morphology, cell morphology and gram's stain. Temperature ranges for growth of isolated pure cultures were determined by incubating them at temperature range between 30°-60° C for 24 h. The pH range for growth was studied by growing the cultures overnight (16 h) on an orbit shaker at 150 rpm at 45° C in LB broth medium adjusted to different pH values (5-9). Growth was measured by turbidity measurement at 600 nm on a double beam UV/VIS scanning spectrophotometer (Nair and Surendran, 2004). Biochemical characteristics (gram's stain, 3% KOH, IMViC tests, catalase and secretion of hydrolase enzymes; amylase, cellulase, lipase, protease and gelatinase) were studied following standard procedures.

The isolates had growth temperatures ranging from 35^0 C to 65^0 C and the optimum temperatures of these ranged between 38^0 C to 50^0 C and the highest optimum temperature was 48^0 C for the culture, N4. The optimum pH of all the cultures was 7.0. Out of the 16 isolates only one strain was gram negative while the rest were gram positive. Only seven cultures showed good growth and clear halo zones in skimmed milk agar medium confirming their ability to produce protease enzyme. Out of the 15 positive cultures for amylase and 13 for cellulase production; only 9 and 6 cultures were selected for the respective enzyme activity tests using DNS (3,5-dinitrosalicylic acid) method. The effect of temperature on the activities of amylase and cellulase enzymes was determined at various temperatures ranging from 42^0 C to 65^0 C at pH 7. Cultures; N4, N5 and M3 showed considerable amylase activities (1.75 U/ml, 0.71 U/ml, and 0.97 U/ml respectively) at 60^0 C; culture N5 gave the highest amylase activity (0.71 U/ml) at 60 ^{0}C. Further, when the temperature increased from 60^0 C to 65^0 C, N4 culture showed an increasing tendency of the activity (0.75 U/ml) whereas the other two cultures showed a reduction activity (1.71 U/ml and 1.12 U/ml). N4 and N5 cultures demonstrated high cellulase activity at 60^0 C (3.75 U/ml and 4.3 U/ml) and the activity reduced when temperature increased up to 65^0 C (5.5 U/ml and 4.9 U/ml respectively in N4 and N5). Results of the present study indicate that there is a high potential in indigenous thermophilic bacterial strains isolated from hot springs of Sri Lanka to be utilised for industrial ventures which operate at high temperature.

Keywords: Hot springs, Thermophilic microorganisms, Hydrolase enzymes