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ISOLATION AND CHARACTERIZATION OF THERMO-STABLE CELLULASE ENZYME PRODUCING BACTERIA FROM WAHAVA HOT SPRING, SRI LANKA

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Microbial enzymes are used in multifold applications in industrial processes as they are. Eco-friendly and cost-effective alternatives for chemical catalysts. This study aimed at the isolation and characterization of thermo-stable, cellulase-producing hot spring bacteria. Triplicate samples were collected from two sites of the Wahava hot spring. Temperature, Electrical Conductivity (EC), pH, and Dissolved Oxygen (DO) were measured. The standard pour plate method was performed to isolate bacteria and primarily screened for cellulase production by Congo red assay on Carboxymethyl Cellulose (CMC) agar plates. Secondary screening of cellulase-producing bacteria was performed using the Di-nitro salicylic acid (DNS) assay. The most potential bacterial candidates for enzyme production were selected, and optimum temperatures, pH and effect of cations on enzyme activity were measured using a temperature range of 30-80 ℃, pH range of 4-10 and cation concentration of 1-5 mM. Molecular-level identification of bacterial isolates was performed using the 16S rRNA gene sequencing method. Temperature, EC, pH, and DO of the Wahava artesian tube well and dug well springs varied from 42.0-42.8 ℃, 1,378 - 1,474 µS/cm, 7.91-7.97 and 3.50-1.05 mg/L, respectively. Three morphologically different cellulase-producing bacterial isolates were observed (W2, W3 and W8) at the primary screening. Bacterial isolate W3, identified as *Aeromonas veronii*, showed the highest cellulase enzyme activity of 3.63 U/ml at an optimum temperature of 60 ℃ and optimum pH 7 for cellulase enzyme. The enzyme activity of W3 was accelerated by Ca^{2+} ions. The highest enzyme activity of 18.36 U/ml was recorded at 5 mM concentration of Ca^{2+}. Hence, the bacterial isolate W3 isolated from Wahava hot spring would be successfully used for industrial settings operating at temperatures around 60 ℃ and neutral pH conditions. Calcium (Ca^{2+}) solutions can be used as cofactors to enhance the cellulase enzyme activity. Thus, the bacterium *A. veronii* is a promising candidate for industrial applications.

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