

ABSTRACT

Muchammad Basyori Alwi. 24020220140024. **Isolation and Characterization of Thermotolerant Cellulolytic Bacteria from Kaliulo Hot Spring Sediment, Semarang Regency, Central Java.** Supervised by Sri Pujiyanto and Arina Tri Lunggani.

Hot spring ecosystems are known to harbor thermotolerant microorganisms that produce thermostable enzymes with important applications in biotechnology, particularly in biomass degradation and bioenergy production. Kaliulo Hot Spring in Semarang Regency, Central Java, is a geothermal environment with water temperatures of approximately 42 °C, which may serve as a potential habitat for thermotolerant cellulolytic bacteria. This study aimed to isolate and characterize thermotolerant cellulolytic bacteria from sediment samples collected from Kaliulo Hot Spring. Bacterial isolation was performed using the serial dilution method followed by cultivation on Nutrient Agar (NA). Screening of cellulolytic bacteria was conducted using NA medium supplemented with Carboxymethyl Cellulose (CMC). Cellulolytic activity was detected using the Congo Red staining method, while further characterization included Gram staining and cellulase enzyme activity assays using the 3,5-dinitrosalicylic acid (DNS) method under different temperature conditions (40, 45, 50, and 55 °C) and pH levels (5, 6, 7, and 8). The results showed that twelve bacterial isolates were able to produce clear zones on CMC medium, indicating cellulolytic activity. Among them, isolates K1 and K2 exhibited the highest hydrolysis index values of 7.70 and 7.59, respectively. Enzyme activity assays revealed that the highest cellulase activity reached 4.799 U/mL at an optimal temperature of 50 °C and pH 7. Gram staining results indicated that the cellulolytic bacterial isolates were Gram-positive bacilli. These findings suggest that sediments from Kaliulo Hot Spring represent a promising source of thermotolerant cellulolytic bacteria that may be further developed as producers of thermostable cellulase enzymes for various biotechnological applications, including bioenergy production, industrial bioprocesses, and lignocellulosic waste conversion.

Keywords: cellulolytic bacteria, thermotolerant, cellulase, hot spring,, Kaliulo