

ABSTRACT

Jauharah Shabrina. 24020218140059. **Bioprospection and Molecular Identification of Amylase-Producing Thermotolerant Bacteria from Sediment Samples of Nglimit Hot Springs, Gonoharjo, Kendal Regency** with the guidance of Wijanarka and Anto Budiharjo.

α -amylase (EC.3.2.11) is one of the commercial enzymes that supplies 25% of the world's enzyme market. Amylase derived from thermotolerant microorganisms has high thermostability, making it valuable in industry and biotechnology. This study aims to determine the best isolates producing amylase at different temperatures and pH treatments, as well as to identify the isolate species based on molecular identification of 16S rRNA. The research process included rejuvenating thermotolerant bacterial isolates isolated from sediment samples from Nglimit hot springs, Gonoharjo, Kendal Regency (TS 1-23 and NS 1-21), screening for isolates of amylase-producing bacteria, testing the optimal temperature and pH of the best isolates, and identifying the best isolates molecular using 16S rRNA. This study employed a completely randomized design (CRD) with a factorial pattern of two factors, namely temperature and pH, with various treatments at temperatures of 40 °C, 45 °C, 50 °C, and pH 5, 7, and 9. The data were analyzed using the *two-way* ANOVA test and Duncan's follow-up test. The results revealed that 32 of the 44 bacterial isolates were positive amylase-producing bacteria, with the highest amylolytic index of 3.65 produced by TS 14 bacterial isolates. Furthermore, ANOVA test results on the treatment of TS 14 bacterial isolates showed that each treatment, namely temperature and pH, as well as the interactions between treatments, had a significant effect on the amylolytic index that resulted. Meanwhile, Duncan's follow-up test yielded the highest average value of 1,758 for the 40°C temperature treatment, the highest average value of 1,267 for the pH 7 treatment, and the highest average value of 2,379 for the interaction of the 40°C and pH 7 treatments. This showed that the optimal TS 14 isolate produced amylase at a temperature of 40 °C and a pH of 7. Lastly, based on molecular analysis with 16S rRNA and interpretation of phylogenetic characters using the *Neighbor-Joining Method*, bacterial isolate TS 14 was identified as *Bacillus amyloliquefaciens*.

Keywords: amylase, thermotolerant bacteria, molecular identification of 16S rRNA