Production, Purification and Characterization of Thermostable Xylanase from Thermophilic *Bacillus licheniformis* 3CA

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Xylanase is generally found in the structure of plant cell walls in nature and takes part in the hydrolysis of xylan. This enzyme, with many areas of use and functions, has important effects, application areas, and great value in terms of both industry and biotechnology. Bioprocesses in the production of xylanase, considered in industrial manufacturing, are carried out microbially. In this study, optimum conditions such as temperature (25–80 °C), pH (4.0–11.0), and time (4–60 hours) were determined for the production of xylanase from thermophilic *Bacillus licheniformis* 3CA obtained from Çermik hot water spring in Diyarbakir/Türkiye. Xylanase activity was determined by the DNS method. Chromosomal DNA was isolated for the cloning of the xyl gene using recombinant techniques for the desired amount of xylanase and amplified with the designed primers. PCR products were visualized on the gel, purified from the gel and sequencing was performed. The obtained enzyme was purified using precipitation and various chromatographic techniques, such as gel permeation and ion exchange. It was determined that the purified enzyme was purified 5–fold and its specific activity was 9561 U/mg. Biochemical characterization of the purified enzyme (such as temperature and pH stability, effect of inhibitors, molecular weight) was performed. It was determined that the activity of the enzyme was stable at pH 9.0, 75% of the activity was preserved at pH 7.0, and 40–50% was preserved in the pH 6.0–11.0 range. For thermal stability, it was observed that the activity was around 90% in the first hour at 55°C. Later, it was determined that the activity was 80% at the 90th minute. It was determined that the enzyme activity was preserved at 80% at 60 and 65°C.

Index Terms—Xylanase, thermophilic, Bacillus