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DETERMINATION OF ENDO-B-GLUCANASE MOLECULAR WEIGHT AND CMCASE ACTIVITY OF CELLULASE PRODUCING ACTYNOMYCETES USING SDS-PAGE AND ZYMOGRAM ELECTROPHORESIS

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Abstract

This research aimed to determine of endo-β-glucanase molecular weight and CMCase activity of cellulase producing actinomycetes using SDS–page and Zymogram electrophoresis with the utilize of oil palm empty fruit bunch (OPEFB) as medium for the production of cellulase. A potential of using OPEFB to accelerate cellulase production by cellulolytic microorganism was tested. The high cellulase producing actinomycetes isolate 12.3.A that was isolated from the Microbiology Department of King Mongkut's University of Technology Thonburi, Thailand was used in this study. The isolate 12.3.A was preliminary appointed to be Streptomyces hirsutus from the result of 16s rRNA gene analysis. The optimal conditions for cellulase production of *S. hirsutus* isolate 12.3.A were determined. The best yields were derived from culturing the cells at pH 7, 30°C, and substrate concentration at 1% for 5 days. The highest cellulase activity from OPEFB as a medium was 0.71 U/ml respectively. The suitable nitrogen source for the culture medium made of OPEFB was ammonium sulfate, respectively. The partial purification of cellulase was shown specific activity from ammonium sulfate precipitation was raised as 3.74 Units/mg and for cellulase purified by dialysis was 7.38 Units/mg. The zymogram assay with polyacrylamide amended with Carboxy methyl cellulose (CMC) demonstrated that the isolate 12.3.A produced a kind of CMCase (endo-β-glucanase) which had shown a clear zone area on

gel. Meanwhile the molecular weight of endo-β- was 47.64 kD with a SDS-page as tested method.

Keywords: Endo β glucanase, molecular weight, CMCase, oil palm empty fruit bunch, Streptomyces hirsutus isolate 12.3A, cellulase activity