Antika Boondaeng 2011: Phylogenetic Study and Characterization of Xylanase Produced by New Species of Actinomycetes Isolated from Thai Soil. Doctor of Philosophy (Microbiology), Major Field: Microbiology, Department of Microbiology. Thesis Advisor: Associate Professor Vichien Kitpreechavanich, Dr.Eng. 172 pages.

A total of 13 isolates of actinomycete strains belonging to the family *Streptosporangiaceae*, isolated from Thai soil were tested for xylan degrading on xylan agar plate. It was found that 11 isolates belonged to the genera *Herbidospora*, *Microbispora*, *Microtetraspora* and *Nonomuraea*, showed the xylan degrading activity. Phylogenetic position, characteristic of the strains and their ability to degrade xylan had paid attention to strain DMKUA 205 and 245. Polyphasic taxonomy indicated that strain DMKUA 205 and 245 were proposed to be *H. sakaeratensis* sp. nov. and *M. siamensis* sp. nov., respectively. In addition, DNA-DNA relatedness values between *Streptosporangium claviforme* NBRC 15623^T and *H. cretacea* JCM 8553^T were higher than 70%, indicating that *S. claviforme* is related as *H. cretacea*. Therefore, the name *S. claviforme* should be treated as a synonym of *H. cretacea.* On the other hand, DNA-DNA relatedness values also showed that *M. amethystogenes* is a separate genomic species from *M. rosea* subsp. *rosea*. Therefore, use a combination of genotypic and phenotypic data *M. amethystogenes* was considered to merit species status. The thermotolerant strain DMKUA 245^T showed the highest xylanase activity when grown at 40°C. To improve the productivity of this strain, a three step-strategy was followed: screening of five factors (xylan, casein, $MgSO_4.7H_2O$, K_2HPO_4 and temperature), using a Plackett-Burman desing for the selection of the most critical variables, localization of the optima of the three most important quantitative factors, casein, $MgSO_4.7H_2O$ and temperature by response surface methodology with a Central composite design (CCD) and confirmation of the conditions determined with the quadratic model by comparison of the optimized conditions with the initial ones. Temperature was the main factor influencing the production of xylanase by the new thermotolerant *M. siamensis* DMKUA 245^T. The optimized medium consisted of (g/L): xylan, 10; casein, 0.16; $MgSO_4.7H_2O$, 0.05; K_2HPO_4, 0.1 and temperature of 45°C yielded 44 U/ml of xylanase activity in shaking flask experiments. Low casein concentration increased the xylanase activity and decreased proteolytic degradation of the xylanase. The maximum activity of 292 U/ml was achieved within 72 h cultivation with uncontrolled pH and an aeration rate of 0.5 vvm in the 3-L airlift fermenter, which increased by 49 folds compared to the un-optimized medium. The purified xylanase has specific activity of 219.4 U mg^{-1} proteins. SDS-PAGE demonstrated molecular weight of purified xylanase from the strain DMKUA 245 at about 65.8 kDa. The optimal pH and temperature for xylanase activity were 5.5 and 60ºC, respectively. The xylanase retained its activity over wide ranges of pH (4-11) and temperature up to 60ºC. The purified xylanase was stimulated by 1 mM Co^{2+}, K^+ and Mg^{2+}, whereas 1 mM Mn^{2+} and 5 mM EDTA inhibited enzyme activity. The purified xylanase was highly specific towards xylan, indicating that is a true xylanase. The K_m value for beechwood xylan was 3.3 mg mL^{-1}. According to hydrolysis production resulting in series of short-chain xylooligosaccharide, the purified enzyme was indicated to be an endo β-1,4-xylanase.

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