Khwanchai Khucharoenphaisan 2009: Purification, Characterization and Induction of Xylanase from *Thermomyces lanuginosus* Isolated in Thailand. Doctor of Philosophy (Microbiology), Major Field: Microbiology, Department of Microbiology. Thesis Advisor: Associate Professor Vichien Kitpreechavanich, Dr.Eng. 148 pages.

The characterizations on thermostability of the pure enzymes of low and high thermostable xylanase produced by new isolates of *T. lanuginosus* THKU-9 and THKU-49 were elucidated. Half-life at 70°C of the pure xylanases from *T. lanuginosus* THKU-9 and THKU-49, in 50 mM phosphate buffer (pH 6.0) was 178 and 336 min, respectively. These enzymes were unstable at pH 5.0 and completely lost their activity after incubation at 70°C for 30 min. The xylanase produced by THKU-9 retained 87% and 30% activity in 50 mM sodium phosphate buffer (pH 7.0) after 1080 min incubation at 60°C and 70°C, respectively whereas xylanase produced by THKU-49 retained full activity and 41% activity, respectively. The types and concentrations of buffer had the effect on thermostability of the pure enzymes. The enzymes in phosphate buffer were more stable than those in citrate buffer. When buffer concentration increased, the half-life of the enzymes decreased significantly. Amino acid sequence analysis of low thermostable *T. lanuginosus* THKU-9 xylanase and high thermostable *T. lanuginosus* THKU-49 xylanase showed that high thermostable xylanase had a single substitution (V96G), which is a small hydrophobic amino acid, of β sheet (B5) of the protein locating on the outer surface of the enzyme structure.

A central composite design (CCD) was performed in order to find the best conditions of pH and temperature for β-xylanase activity and to maintain its activity for prolonged periods of time of pure xylanase produced by *T. lanuginosus*THKU-49. The CCD used for the analysis of treatment combinations showed that a regression models of optimization of xylanase activity and xylanase stability were good agreements to experimental results with R^2 = 0.98 and 0.99, respectively. The maximum activity of xylanase was obtained at 66°C and pH 6.3. The maximum enzyme stability was 70°C and pH 7.3. Under this condition xylanase having half-life of 825 min indicated the highest thermostable xylanase.

The strains produced high xylanase either in the xylan or xylose medium having ratio of xylanase activity in a range of 1.1-1.5. Addition of xylose to the xylan medium did not decrease xylanase production by *T. lanuginosus* THKU-11, THKU-25 and ATCC 44008 that were members of this group. In contrast, there was another group that produced high xylanase only in the xylan medium. Addition of xylose to the xylan medium resulted decreasing of xylanase formation in *T. lanuginosus* TISTR 3465, THKU-85 and ATCC 46882 that were belonged to this group. Though, xylose caused xylanase induction by resting cell of tested strains in both groups but the low xylanase formation by growing cell in the xylose medium may cause by catabolic repression of xylose in the particular strains. In addition, RAPD analysis allowed us to distinguish between the high and low xylanase producing strains using xylose as a carbon source.

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