Thesis Title	Study on xylanase from alkaliphilic thermophilic microorganisms for application in pulp and paper industry
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Candidate	Mr. Chakrit Tachaapaikoon
Supervisors	Assoc. Prof. Dr. Khanok Ratanakhanokchai
	Dr. Khin Lay Kyu
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Abstract

Thermostable alkaline xylanase producing thermophilic alkaliphilic xylanolytic microorganisms were isolated from a wastewater treatment plant and soil sample from Siam Cellulose Co. Ltd., in Thailand. All fifteen isolated strains produced xylanases but did not produce cellulase when grown in alkaline xylan medium. The optimum pHs of the xylanase activity of ten strains C 1-2, C 4-1, C 4-2, C 5-1, C 5-3, C 9-2, C 9-3, C 10-1, C 10-2, C 10-3 and C 11-1 were 6.0, while those of the other three strains C 6-1, C 9-1 and C 11-2 had optimum pHs of 8.0. Only one strain C 5-2 had two optimum pHs of 6.0 and 8.0. All strains showed the stability in the broad range of pH 7.0 to 11.0 for the xylanase activity and optimum temperature of 70 °C. Binding of xylanases produced from all strains to insoluble cellulose and insoluble xylan were examined. Xylanases from the five strains C 9-2, C 9-3, C 10-1, C 10-3 and C 11-1 were found to be capable of binding to both insoluble polysaccharides whereas those from the two strains, C 5-1 and C 11-2 were able to bind slightly. Xylanase from the rest of the strains, C 1-2, C 4-1, C 4-2, C 5-2, C 5-3, C 6-1, C 9-1 and C 10-2 were able to bind to only insoluble xylan. The hydrolysis of kraft pulps in alkaline conditions by the xylanases from all strains was also investigated and showed that the hydrolysis rates of eucalyptus pulp were greater than those of sugarcane bagasse and pine pulps by xylanases from four strains C 6-1, C 9-1, C 10-1 and C 10-3. However, the hydrolysis rates of sugarcane bagasse pulp were greater than those of eucalyptus and pine pulps by xylanases from the rest of the strains. One of the strains, C 9-1 was

found to produce high level of thermostable alkaline active cellulase-free xylanase with xylan-binding ability. Therefore, xylanase from the strain C 9-1 had a great potential for application in prebleaching of kraft pulps. On the basis of physiological and biochemical studies, the strain C 9-1 was identified as belonging to the genus *Bacillus*. *Bacillus* sp. C 9-1 produced extracellular xylanolytic enzymes such as xylanase and arabinofuranosidase. The SDS-PAGE and active-PAGE showed that the culture supernatant contained three active bands with molecular masses of 23.5, 44.5 and 63.5 kDa. One of the bands (23.5 kDa) was found to be xylan-binding xylanase (XBX). It was purified to homogeneity by affinity chromatography. The optimum pH and temperature of the purified xylan-binding xylanase were 6.0 and 70°C, respectively. Its stability was in the range of pH 4.0 to 10.0 and temperature up to 50°C. Metal ions did not affect the xylanase activity and the purified XBX showed hydrolytic activity toward xylan only.

Keywords : thermostable alkaline xylanase / xylan-binding xylanase / adsorption-desorption on insoluble xylan (affinity chromatography) / prebleaching of kraft pulp / *Bacillus* sp. C 9-1