| | |
|---|---|
| Dissertation Title | Study on Multienzyme Complex (Cellulosome/ Xylanosome) from Bacterium under Anaerobic and High Temperature Conditions |
| Dissertation credits | 48 |
| Candidate | Miss Suphavadee Chimtong |
| Dissertation Advisors | Assoc. Prof. Dr. Khanok Ratanakhanokchai<br>Asst. Prof. Dr. Chakrit Tachaapaikoon |
| Program | Doctor of Philosophy |
| Field of Study | Biochemical Technology |
| Department | Biochemical Technology |
| Faculty | School of Bioresources and Technology |
| B.E. | 2556 |

# ABSTRACT

This study described the screening and isolation of anaerobic thermophilic biomass-degrading bacteria species and elucidated the enzymatic complex produced from high potential biomass-degrading enrichment culture. Soil, decomposed wastes, and agricultural residue samples were collected from several places in Thailand. One hundred and fifty samples were cultivated in basal medium containing corn hull as the sole carbon source. One of enrichment cultures (namely NKP) showed the highest degrading corn hull. The stable existence NKP enrichment culture contained two different bacterial strains. Two bacteria strains, NKP-2 and NOI-1 were successfully isolated from NKP by utilized carbon sources of properties. Strain NKP-2 grew well on cellulose and xylan as sole carbon sources, but at a slower pace (2-5 weeks) on xylan whereas strain NOI-1 grew well on xylan as a carbon source but not cellulose. Therefore, the isolate strains can be separated in this study. Purified culture of both strains (namely strain NKP-2 and NOI-1) were identified by using 16S rRNA gene analysis. Strain NKP-2 showed high similarity (99%) with *Clostridium thermocellum*, whereas strain NOI-1 was identified as *Thermoanaerobacterium*

*thermosaccharolyticum* (99% similarity). Thus, pure isolated strains were named as *C. thermocellum* NKP-2 and *T. thermosaccharolyticum* NOI-1, respectively. Furthermore, the purity of the strain NOI-1 and NKP-2 were performed by using specific primer for each strain and indicated that each strains were pure culture. Both strains were anaerobic, thermophilic, Gram-positive, rod-shaped and spore-forming. The optimum temperature for growth of both strains was 60°C and optimum pH was 6.0. The enzymatic system of each isolated strains when cultivated individually on corn hulls demonstrated different cellulolytic and xylanolytic enzyme activities. *C. thermocellum* NKP-2 produced cellulose- and xylan-main chain cleaving enzymes such as carboxymethylcellulase (CMCase), avicelase and xylanase as major enzymes, whereas strain NOI-1 produced dominantly short- and side-chain cleaving enzymes such as cellobiohydrolase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase and acetyl esterase. The determination of plant cell wall polysaccharide degradation by combining cellulolytic enzymes from *C. thermocellum* NKP-2 and endocellulase-free xylanolytic enzymes from *T. thermosaccharolyticum* NOI-1 showed that the maximum of synergistic effect between endocellulase-free xylanolytic enzymes and cellulolytic enzymes was 2.8 at 3 hours for corn hull degradation. Moreover, corn hull utilization, cell growth, and fermentation products (ethanol, butanol, acetic acid, butyric acid, $H_2$ and $CO_2$) were found to be highly increased in the co-culture as compared with individual cultivation of each strain. The symbiotic behavior of co-culturing between both strains is one form of mutualism. In this case, synergistic biomass-degrading enzyme system helps them obtain sugar needed for living in their natural environment.

Interestingly, enzymatic system in term of multienzyme complex in *Thermoanaerobacterium* species has not been reported. Therefore, *T. thermosaccharolyticum* strain NOI-1 was characterized and elucidated. After cultivation of strain NOI-1 in basal medium containing oat spelt xylan as the sole carbon source, the culture supernatant was used as crude enzyme. The culture could produce crude enzyme that comprises xylanase, β-xylosidase, α-L-arabinofuranosidase, acetyl esterase, cellobiohydrolase and β-glucosidase, but could not produce endocellulase. The crude enzyme was active in broad ranges of pH and temperature, however the optimum condition was pH 6.0 and 60°C. Scanning electron microscopy (SEM) analysis revealed that the bacterial cells adhere to insoluble xylan and Avicel. The endocellulase-free

multienzyme complex was isolated from crude enzyme of the strain NOI-1 by affinity purification on cellulose and subsequently with Sephacryl S-300 gel filtration chromatography. The molecular mass of the multienzyme complex was estimated to be about 1,200 kDa. The multienzyme complex showed one protein on native-polyacrylamide gel electrophoresis (native-PAGE), one xylanase on native-zymogram, 21 proteins on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and 5 xylanases on SDS-zymogram. It was found that the multienzyme complex, which consists of xylanase, β-xylosidase, α-L-arabinofuranosidase, β-glucosidase and cellobiohydrolase effectively hydrolyzes oat spelt xylan and corn hull. This is the first report of an endocellulase-free multienzyme complex produced by *Thermoanaerobacterium* species.

Key words: Anaerobic thermophilic bacterium/ *Clostridium thermocellum*/ Cellulolytic-xylanolytic enzymes/ Co-culturing/ Endocellulase-free multienzyme complex/ Symbiotic behavior/ *Thermoanaerobacterium thermosaccharolyticum*