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Cellulolytic Enzymes from *Acrophialophora nainiana*

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Abstract

A cellulolytic fungus isolated from Agave plantation in northeastern Thailand was identified as *Acrophialophora nainiana*. The fungus was capable of growing at pH between 3 -7 and 25 - 45 ℃, with the optimum conditions at pH 5.0 and 40 ℃. The wild isolate produced cellulases, comprising of exoglucanase (0.019 U/mg protein), endoglucanase (0.366 U/mg protein), and β-glucosidase (0.001 U/mg protein). Mutations with UV and NTG produced the UV 10-2 mutant with cellulases activities including exoglucanase (0.093 U/mg protein), endoglucanase (0.585 U/mg protein), and β-glucosidase (0.013 U/mg protein). Purification of the enzymes with ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography yielded the maximal cellulase specific activities of 2.736 U/mg protein (exoglucanase), 0.235 U/mg protein (endoglucanase), and 0.008 U/mg protein (β-glucosidase). The mutant's cellulases were the most active at pH 5.0 and 60 ℃. Ion-exchange chromatography revealed that *A. nainiana* UV 10-2 cellulases were comprised of two peaks with one peak showing the single endoglucanase activity while the other peak showed a mixture of the three enzyme activities. Production of *A. nainiana* UV 10-2 cellulases using banana leaf stalk as the sole carbon source gave comparable yields to that of the pure α-cellulose. The enzymes were used in the simultaneous saccharification and fermentation (SSF) of plant residue (*Coix aquatica*) along with *Kluveromyces marxianus* to produce ethanol. Moreover, when the enzymes were used in the bioscouring process of fabric, the desirable traits of textile processing including immediate water absorbency, increased in whiteness and reduction of yellowness of the treated fabric were observed.

Keywords : cellulase, *Acrophialophora nainiana*

Methodology

Acrophialophora nainiana was isolated from Agave plantation soil in Nakornratchasrima Province, Thailand, using Czapex'doc medium with filter paper strip as the sole carbon source as described by Punnapayak et al (1999). The identification was based on the microscopic examination and carbon source utilization. Growth study was performed in liquid medium at pH between 3 to 7 and 25 ℃ to 45 ℃. The fungus was mutated with UV and NTG to produce mutants expressing superior cellulase activities. The

enzyme was produced in liquid production medium (Punnapayak et al., 1999) and was purified with ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography. Enzymes activities were analyzed for exoglucanase (Ghose, 1987), endoglucanase (Ghose, 1987), and β-glucosidase (Sternberg, 1988). The enzyme was used in the bioconversion process of plant residue (*Coix aquatica*) into ethanol using the Simultaneous Saccharification and Fermentation (SSF) process as described by Punnapayak and Hoffman (1994). The bioscouring process of cotton fabric with the prepared enzyme was accomplished (Sangwatanaroj et al., 2003).

Results and Discussion

Acrophialophora sp. positively identified as *Acrophialophora nainiana* due to its distinct characteristic spiral spores and the ability to produce cellulolytic enzymes including exoglucanase, endoglucanase, and β -glucosidase (Figure 1). The fungus was considered as thermotolerant for its ability to grow up to 45 ^{o}C (Punnapayak et al., 1999). *A. nainiana* UV 10-2 mutant produced cellulases with superior activities for exoglucanase, endoglucanase, and β-glucosidase than those of the wild type (Figure 2). The purified cellulases revealed that they were comprised of 1 peak of β-glucosidase, 1 peak of exoglucanase, and 2 peaks of endoglucanase (Figure 3). Thus, suggesting the possibility of having two forms of endoglucanase I and endoglucanase II.

Production of *A. nainiana* cellulases using banana leaf stalk as the sole carbon source gave cellulases with activities comparable to those produced from pure α-cellulose (Figure 4). It was notable that the endoglucanase activity seemed enhanced with banana leaf stalk substrate.

In an attempt to demonstrate the use of the cellulases produced from *A. nainiana* for biotechnological applications, the crude enzyme was used in two processes involving fermentation and bioscouring. The simultaneous saccharification and fermentation of tropical weed (*Coix aquatica*) mixed with *A. nainiana* cellulases and *Kluveromyces marzianus* in batch fermentation, yielded 0.18 g ethanol/ g substrate

The treatment of woven cotton fabric revealed that the cellulase-treated fabric increased its whiteness, while decreasing its yellowness and absorbed water immediately. These were the desirable traits in the bioscouring process of textile.

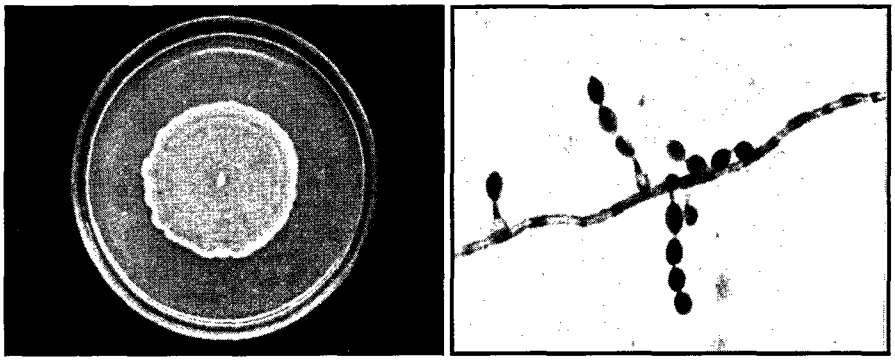

Fig. 1 *Acrophialophora nainiana* UV 10-2

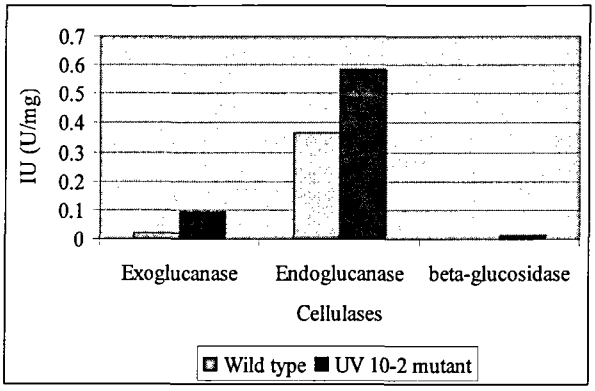

Fig. 2 Cellulase activities of *A. nainiana* wild type and UV 10-2 mutant

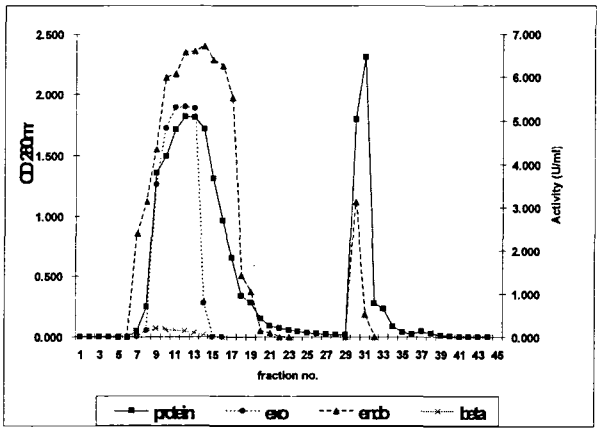

Fig. 3 Components of purified cellulases from *A. nainiana* UV 10-2

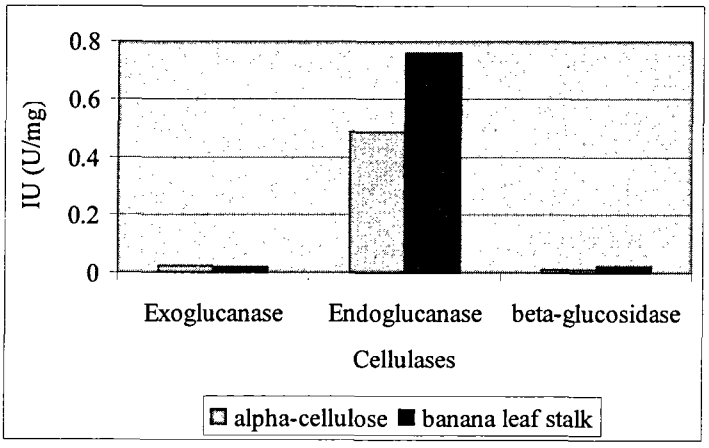

Fig. 4 Cellulase activities of *A. nainiana* UV 10-2 produce from two different substrates.

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References

1. Ghose,T.K. 1987. Measurement of cellulase activities. Inter Union Pure and App Chem. 59 : 257-268.
2. Punnapayak, H., M.Kuhirun,and P.Thanonkeo.1999. Cellulolytic fungi and the bioconversion of fiber from *Agave sisalina*. Science Asia, 25 : 133 - 136.
3. Punnapayak, H., and Hoffman, J.J. 1994. *Amsonia* spp. As potential fuel crops for arid lands. World Journal of Microbiology and Biotechnology. 10 : 290 - 292.
4. Sternberg, D., Vijayakumar, P. and Reese, E. T. 1977. β-glucosidase : microbial production and effect on enzymatic hydrolysis of cellulose. Canadian Journal of Microbiology. 23 : 139 - 147.
5. Sangwatanaroj, U., Choonukulpong, K. and Mitsuo, U. 2003 AATCC REVIEW. 3, 17