THESIS

CHARACTERIZATION OF β-1,3-1,4-GLUCANASE FROM *Bacillus subtilis* GN156 FOR SILAGE FERMENTATION

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GRADUATE SCHOOL, KASETSART UNIVERSITY

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THESIS APPROVAL

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To solve the problem of lacking of sufficient amount of WSC in grass ensiling process, addition of enzyme to release structural carbohydrate is the aim of this study. Therefore, the enzymatic system of *B. subtilis* GN156 was studied and it exhibited the activities of β-1,3-1,4-glucanase, xylanase, CMCase, dextrinase, cellobiase, polymethylgalacturonase and polygalacturonase but no activity of laminarinase could be detected. Among several enzymes, β-1,3-1,4-glucanase provided the highest activity against barley β-glucan which was a major hemicellulose in grass. The profile of growth and enzyme production indicated that β-1,3-1,4-glucanase produced by *B. subtilis* GN156 was growth associated. Induction of β-1,3-1,4-glucanase production by CM-cellulose, pectin and xylan revealed an increment of activities of 47, 41 and 11-folds, respectively. The maximum β-1,3-1,4-glucanase production was obtained when 0.8% (w/v) CMC was used as an inducer. In addition, the β-1,3-1,4-glucanase showed an optimal pH and temperature of 7.0 and 60°C, respectively, while the stability did wide pH and temperature range of 3.0-11.0 and 20-50° C, respectively.

To purify β-1,3-1,4-glucanase from cell-free culture fluid of *B. subtilis* GN156, the purified J1 and the partial purified pJ2 were obtained from affinity chromatography of Epoxy-activated Sepharose 6B and ultrafiltration. Homogeneity of the enzymes were determined by 10 % SDS – PAGE and 7.5 % native – PAGE, the purified J1 was a single protein approximately 25 kDa with pI of 3.5, while the partial purified pJ2 was mixed proteins of the purified J1 and J2 with pI of 3.5 and 3.6, respectively. This is the first report of bacterial β-1,3-1,4-glucanases, which showed the lowest pI of 3.5 and 3.6. In addition, it also elucidated that purified J2 contained three protein subunits of 40, 32 and 18 kDa. The study of the purified J1 and the partial purified pJ2 properties indicated that those were similar. The optimum pH was around neutral and the optimum temperature was the same at 60°C. Both purified J1 and partial purified pJ2 β-1,3-1,4-glucanase were significantly inhibited by Mn^{2+} ions, and were reduced in the presence of Cu^{2+} ions. The purified J1 and the partial purified pJ2 were highly specific to barley β-glucan, no activities were found against CMC, xylan, laminarin, and dextrin. Lineweaver – Burk plots for K_m and V_{max} determination against β-glucan were 1.53 mg/ml and 8,511 μU/ml.min, respectively for the purified J1, and were 4.36 mg/ml and 7,397 μU/ml.min, respectively for the partial purified J2.

The degradation patterns from the crude enzyme and J1 action on β-glucan were similar, while differ pattern from pJ2 would be due to thermal unstability of pJ2. The degradation products detected were the oligosaccharides with mobility intermediate between cellobiose - cellotriose, cellotriose – cellotetraose, cellotetraose – cellopentaose and the oligosaccharides larger than cellopentaose, which were considered to be triose, tetraose, pentaose and the oligosaccharides larger than cellopentaose, respectively.

Degradation activity of β-glucan by both alone and combination of crude enzyme and J1 or pJ2 were studied. The activity obtained from the crude enzyme and the combination ones were not significant different but the ones from J1 and pJ2 alone were lower significant. Thus, there was not synergism of crude enzyme and J1 or pJ2 in β-glucan degradation. Corresponding to the grass degradations, there was not synergism of crude enzyme and J1 or pJ2 either.

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Jirawan Apiraksakorn

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