Mannose production by beta-mannosidase from *Micromonospora* sp. TISTR 1553

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Abstract

Beta-mannosidase (EC 3.2.1.25) is the most important enzyme for producing of mannose because it cleaves β-1,4 linkage of between mannose at non-reducing end of mannan. Mannose can be used in medical and cosmetic. The β-mannosidase enzyme is produced by plant, animal, human and microorganism. This study focused on screening of β-mannosidase from the three *Micromonospora* species, *Micromonospora aurantionigra* TISTR 1532, *Micromonospora* sp. TISTR 1553 and *Micromonospora siamensis* TISTR 1554. The screening was performed by culture the three strains in minimum medium supplemented with 0.5% locus bean gum. The enzyme activity was assayed by using 4-nitrophenyl-β -D-mannopyranoside as substrate and the absorbance 420 nm was measured. The result revealed that *Micromonospora* sp. TISTR 1553 produced the highest β-mannosidase activity. The optimum culture condition was at 30°C for 5 days. Thin layer chromatography (TLC) analysis showed that the degradation of manno-oligosaccharide by crude β-mannosidase yielded high amount of mannose. Further purification procedure of this enzyme in a large scale will be developed.

Keywords: Beta-mannosidase, Mannose, *Micromonospora* sp., Manno-oligosaccharide

Introduction

Mannans polysaccharides are a component of hemicellulose in plant cell wall. The mannan is found about 15-20 % in softwood and 5% in hardwoods [1]. The backbone of mannan consists of β-1,4 -linked of mannose or mannose in combination with glucose residues. In addition, some mannans contain branched side chains with α-1,6-linked galactosyl residue. A variety of enzymes are needed for complete conversion of mannan polysaccharide to monosaccharides including β-mannanase, β-mannosidase, β-glucosidase, and α-galactosidase acetyl mannan esterase [2,3]. Interestingly, the β-mannosidase (EC 3.2.1.25, exo-mannosidase) is an enzyme which acts by digestion β-1,4-linkage of manno-oligosaccharide from the non-reducing ends to produce mannose[4,5]. Mannose is a hexose sugar and has a potential applications in biomedical such as anti-adhesion of *Escherichia coli* [6] and cosmetic. The actinomyces strains, *Micromonospora aurantionigra* TISTR 1532, *Micromonospora* sp. TISTR 1553 and *Micromonospora siamensis* TISTR 15547 was isolated from soil sample in Thailand. This strain has been no report about hemicellulase enzyme production. Therefore, this study was focused on β-mannosidase produced from *Micromonospora* species as selection strain.

Materials and Methods

Selection of bacterial strain producing beta-mannosidase

The actinomyces strains including *Micromonospora aurantionigra* TISTR 1532, *Micromonospora* sp. TISTR 1553 and *Micromonospora siamensis* TISTR 1554[7] obtained from the Thailand Institute of Scientific and Technological Research (TISTR) were selected for studying β-mannosidase production. *Micromonospora* species has been reported to contain β-mannosidase gene deposited in GenBank database. However, there has been no report on isolation, expression and characterization of the β-mannosidase from this bacterial strain.

Assay for beta-mannosidase activity

Eighty-microliters of supernatant was incubated with 120 µl of 0.02% 4-nitrophenyl β-D-mannopyranoside in 20 mM KPB pH 6.0 at 37°C. The reactions were stopped by adding 800 µl of 0.2 M Na_2CO_3 for 15 min at room temperature. The absorbance of the product was measured at the 420 nm.

Screening of beta-mannosidase producing bacteria

The bacteria were activated by culturing in 10 ml ISP2 medium for 2 days before inoculating into 1 L of minimum medium containing 0.5% locust bean gum (ratio of mannose: galactose = 4:1). β-mannosidase activity was measured every 24 h. For measuring enzyme activity, 1 ml of culture was centrifuged at 12,000 rpm for 5 min and the cell pellet was washed with 20 mM KPB, pH 6.0 and resuspended in 200 µl of the same buffer. The cell pellet was disrupted by sonication. Cell lysate (80 µl) was incubated with 120 µl of 0.02% 4-nitrophenyl- β-D-mannopyranoside at 45°C for 15 min and measured the absorbance at 420 nm.

Analysis of degradation product by Thin layer chromatography (TLC)

To examine the products after digestion of manno-oligasaccharide by crude β-mannosidase, 100 µl of 10 mg/ml of mannotetraose in 20 mM KPB pH 6.0 was mixed with 20 µl of crude enzyme, and incubated at 45°C for 24 h. The reaction was terminated by heating for 2 min. The digested products (2 µl) were applied onto TLC plate which was soaked in n-butanal- acetic acid- water (2:1:1). The mannose (**M1**), mannobiose **(M2**), mannotriose **(M3**) and mannotetraose (**M4**) were applied as standards. The products after enzymatic digestion and standards appeared on the TLC plate were visualized by dipping in copper sulfate solution and heating.

Results and discussion

Bata-mannosidase producing bacteria

Three actinomyces strains comprising *Micromonospora aurantionigra* TISTR 1532, *Micromonospora siamensis* TISTR 1553 and *Micromonospora* sp. TISTR 1554 were screened for β-mannosidase activity following culturing in minimum medium supplement with 0.5 % locust bean gum. The result showed that *Micromonospora* sp. TISTR 1553 and *Micromonospora aurantionigra* TISTR 1532 contained β-mannosidase activity. *Micromonospora* sp. TISTR 1553 produced the highest yield of β-mannosidase.

For enzyme production curve, β-mannosidase activity was examined every 24 h. The result showed that the enzyme activity was highest at 5 days of culture (Figure 1). A 5 day of culture was selected for enzyme production.

Assay for beta-mannosidase activity

The beta-mannosidase activities of the three *Micromonospora* sp. were detected in cells extract, but not in the culture medium, indicating that the enzymes are localized intracellularly. The released *p*-nitrophenol (*p*-NP) produced a light-yellow solution (Figure 2) and the amount was measured at a wavelength 420 nm.

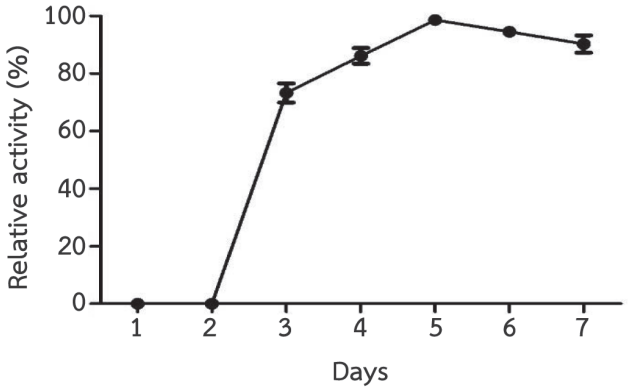

Figure 1: Beta-mannosidase producing curve of *Micromonospora* sp. TISTR 1553.

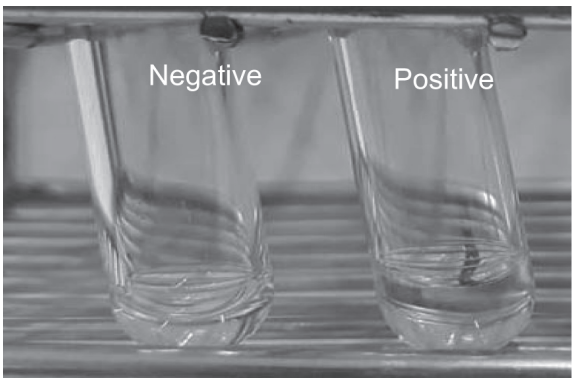

Figure 2: Rapid assay for beta-mannosidase activity using 4-nitrophenyl- β-D-mannopyranoside as substrate. The present of β-mannosidase convert the substrate to p-nitrophenol which produced a light-yellow solution reading as positive control), whereas the colorless solution was lack of β-mannosidase activity reading as negative control.

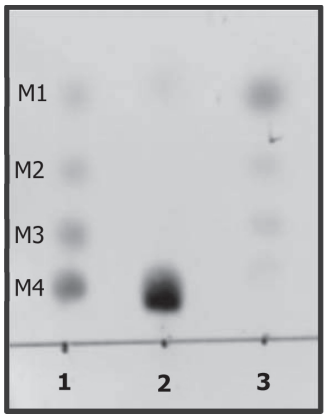

Figure 3: Analysis of the digested products by TLC. Lane 1, Mixed standard including mannose (M1), mannobiose (M2), mannotriose (M3) and mannotetraose (M4); Lane 2, product from substrate and buffer without crude enzyme; Lane 3, products from digestion of mannotetraose by crude enzyme at 45°C for 24 h.

Thin layer chromatography analysis of degradation product of mannooligosaccharide

Figure 3 showed the TLC analysis of the products from degradation of manno-oligasaccharide by crude β-mannosidase. It was found that mannose was the main product of enzymatic digestion. This result indicated that the enzyme is an exo-acting enzyme, which is the property of β-mannosidase.

Conclusions

The three strains of *Micromonospora* species were screened for their ability to produce β-mannosidase enzyme. The *Micromonospora* sp. TISTR 1553 was found to produce the highest yield of β-mannosidase and can degrade manno-oligosaccharides to produce free mannose.

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