Enhanced mannanase production by a novel mannanase producing bacterium *Acinetobacter* sp. KUB-ST1-1

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Abstract

A novel mannanase producing bacterium *Acinetobacter* sp. KUB-ST1-1 isolated from soil of coconut waste in Thailand was found to produce the highest level of mannanase activity as 4.80 and 3.87 U/ml when grown in the enzyme production medium (PM) and nutrient broth medium (NB), respectively with 0.5% locust bean gum (LBG) as the substrate at 37°C for 24 hrs of cultivation time. On the other hand, the NB medium showed the highest mannanase specific activity as 2.28 U/mg of proteins. The mannanase activity reached 7.27 U/ml, increased by 2.86–fold when the optimized culture medium was added 1.5% glucose concentration on NB medium. Maximum mannanase activity was achieved when the optimized culture medium was initially adjusted to pH 7.0.

Keywords: mannanase, *Acinetobacter*, locust bean gum

Introduction

β-mannanase (mannan endo-1,4-β-mannohydrolase, E.C. 3.2.1.78) are hydrolytic enzymes which hydrolyze randomly β-1,4 mannosidic linkages within the backbones of mannan, galactomannan, glucomannan and galactoglucomannan (McCleary, 1988). In recent years, mannanases have gained increasing attention because of their various biotechnological applications in the food, feed, coffee extraction, detergent, as well as pulp and paper industries (Dhawan and Kaur, 2007; Naganagouda *et al*, 2009 and Akinyele *et al*, 2013). They can also be used in the production of manno-oligosaccharides (MOS) including mannobiose and mannotriose which were reported to be excellent prebiotics stimulating growth of beneficial intestinal microorganisms (Titapoka et al. 2008). Mannanases can be produced from a wide range of microorganisms such as bacteria, fungi, yeasts, and marine algae, as well as from germinating seeds of terrestrial plants (Ferreira and Filho, 2004; Jiang *et al*, 2006; Lin *et al*, 2007 and Meenakshi *et al*, 2010). Mannanases production from microorganisms is more promising due to its low cost, high production, and readily controlled conditions (Mabrouk *et al*, 2008). This time, a novel mannanase producing bacterium *Acinetobacter* sp. KUB-ST1-1 isolated from soil of coconut waste in Thailand was exhibited mannanase activity of 0.185 U/mg against copra meal as substrate (Titapoka *et al*, 2008). Nevertheless, the efficiency on mannanase production was low for industrial application which necessary to develop. The aim of the present study was improved and enhanced mannanase production by a novel mannanase producing bacterium *Acinetobacter* sp. KUB-ST1-1.

Methodology

Bacterial strain and enzyme preparation

The bacterial isolate used in the study was *Acinetobacter* sp. KUB-ST1-1, which was obtained from the Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Thailand (Titapoka *et al*, 2008). The inoculum was prepared by transferring a loop of cells to 5 ml sterile NB broth and incubated at 37 ^{o}C 150 rpm for 18-24 hrs for seed culture. 5% inoculum of seed culture of *Acinetobacter* sp. KUB-ST1-1 was grown in 50 ml of medium production in a 250 ml Erlenmeyer flask and incubated at 37 °C 150 rpm for 24 hrs, After 24 hrs cells was removed by centrifugation at 4 °C 10,000 rpm for 10 min. The supernatant was analyzed for mannanase activity.

Mannanase productions

Time course of fermentation for mannanase production

Time course of fermentation is an important parameter for mannanase production by *Acinetobacter* sp. KUB-ST1-1. In this study, the fermentation experiment was carried out up to 48 hrs and production rate was measured at 12 hrs interval and incubated at 37 °C 150 rpm. Cell was removed by centrifugation at 4 °C 10,000 rpm for 10 min. The supernatant was analyzed for mannanase activity.

Effect of fermentation media for mannanase production

The Fermentation media depended on media for growth of *Acinetobacter* sp. KUB-ST1-1 including; Brain Heart Infusion broth (BHI) (Nemec *et al*, 2010) consisted of (w/v) 1% Gelatin peptone, 1% Beef Heart Infusion, 0.75% Calf Brain Infusion, 0.5% NaCl, 0.25% Na_2PO_4, 0.2% Dextrose and 0.5% Locust bean gum (LBG) as a substrate, Luria Bertani broth (LB) (Holst *et al*, 2007) consisted of (w/v) 1.0% tryptone, 0.5% yeast extract, 1.0% NaCl and 0.5% Locust bean gum (LBG) as a substrate, Enzyme production medium (PM) (modified from Mohammad *et al*, 1996) consisted of (w/v) 3% polypeptone, 2.5% corn steep liquor, 1.5% KH_2PO_4, 0.06% $MgSO_4$ and 0.5% Locust bean gum (LBG) as a substrate, Trypicase soy broth (TSB) (Chang *et al*, 2005) consisted of (w/v) 1.7% tryptone, 0.3% soytone, 0.25% glucose, 0.5% NaCl, 0.25% K_2HPO_4 and 0.5% Locust bean gum (LBG) as a substrate and Nutrient broth (NB) (Hrenovic and Ivankovic, 2009) consisted of (w/v) 0.3% beef extract, 0.5% polypeptone and 0.5% Locust bean gum (LBG) as a substrate. 5% inoculum of a preculture of *Acinetobacter* sp. KUB-ST1-1 was grown in 50 ml of media production in a 250 ml Erlenmeyer flask and incubated at 37 °C 150 rpm for 24 hrs, After 24 hrs cells was removed by centrifugation at 4 °C 10,000 rpm for 10 min. The supernatant was analyzed for mannanase activity.

Effect of initial pH of medium for mannanase production

The Initial pH of medium was adjusted to 3-12 into NB medium production by 1N HCl and 1N NaOH, respectively and incubated at 37 °C 150 rpm for 24 hrs, After 24 hrs cells was removed by centrifugation at 4 °C 10,000 rpm for 10 min. The supernatant was analyzed for mannanase activity.

Effect of glucose concentrations for mannanase production

The effect of glucose concentrations for mannanase production by *Acinetobacter* sp. KUB-ST1-1, the present study was studied by varying the glucose concentration on NB medium from 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0% in the replacement experiments. Enzyme production was studied in 250 ml Erlenmeyer flasks containing 50 ml of NB medium with 0.5% LBG as a substrate. The culture was incubated at 37 °C 150 rpm for 24 hrs, After 24 hrs cells was removed by centrifugation at 4 °C 10,000 rpm for 10 min. The supernatant was analyzed for mannanase activity.

Determination of mannanase activity

Mannanase activity was determined by mixing 0.1 ml of 0.5% Locust bean gum (LBG) in 50 mM phosphate buffer pH 7.0 with 0.1 ml of enzyme sample for 15 min at 50 °C otherwise mentioned elsewhere. The reducing sugars release was measured by the dinitrosalicylic acid; DNS method (modified from Miller, 1959) against a standard curve of mannose.

One unit of enzyme activity was defined as the amount of enzyme that gave rise to reducing sugar end groups corresponding to 1 µmol of mannose per minute under the experimental conditions.

Determination of protein concentration

Protein concentration was determined by the method of Lowry *et al*. (1951) Bovine serum albumin will use as a protein standard.

Statistical analysis

Data presented on the average of three replicates are obtained from there independent experiments.

Results and discussions

Time course of fermentation for mannanase production

The optimization of the time course is of prime importance for mannanase biosynthesis by bacteria. Since fermentation duration is crucial, it is also important to find out the optimum period for mannanase production. Some organisms are reported to produce maximally in the log phase of growth, whereas some at their stationary phase (Ray *et al*, 2007). Data presented in Figure 1 showed the effect of different incubation periods on mannanase production by *Acinetobacter* sp. KUB-ST1-1. From the results, it was found that mannanase revealed its best production at 24 hrs of incubation (0.24 U/ml and 0.58 U/mg). At longer incubation periods beyond 24 hrs, the activity decreased sharply. However, the decrease in the production of mannanase by *Acinetobacter* sp. KUB-ST1-1 after 24 hrs of incubation period might be due to the depletion of nutrients (Olaniyi *et al*, 2013).

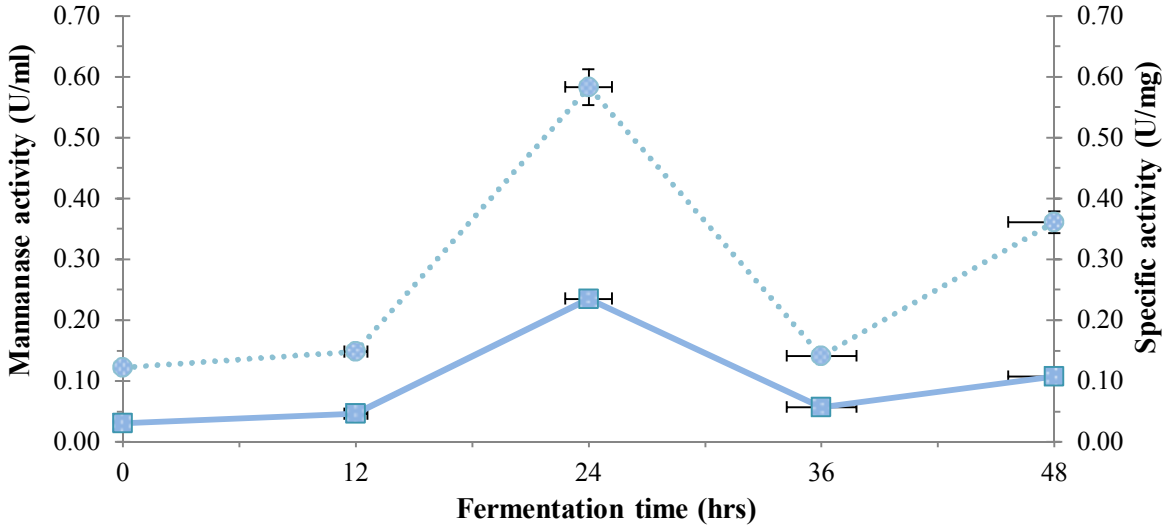

Figure 1 Time course of fermentation for mannanase production produced by *Acinetobacter* sp. KUB-ST1-1: ( ) Mannanase activity (U/ml); ( ) Specific activity (U/mg)

Effect of initial pH of medium for mannanase production

The initial pH of medium is important for mannanase production (Dhawan and Kaur, 2007). In this study, the mannanase activity on various Initial pH of NB medium (adjusted to 3-12) was produced by *Acinetobacter* sp. KUB-ST1-1, were shown in Figure 2. The mannanase exhibited highest mannanase activity of 1.65 U/ml and specific activity of 1.65 U/mg of protein against initial pH 7.0 in NB medium. From the results was similar to the initial pH of medium from *Bacillus* sp. and *Clostidium* sp. (Helow and Khattab, 1997; Talbot and Sygusch, 1990). In addition, the result can be explained this enzyme can be activated in acidic pH range than alkaline pH range and could be applied to various industries (Willem *et al*, 2010).

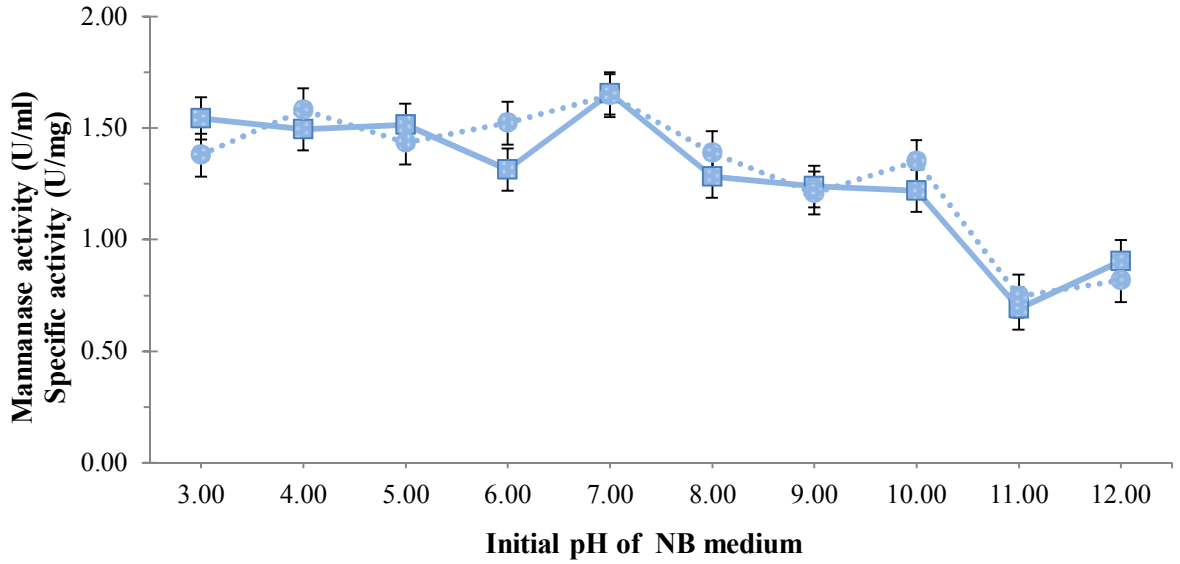

Figure 2 The initial pH of NB medium for mannanase production produced by *Acinetobacter* sp. KUB-ST1-: ( ) Mannanase activity (U/ml); ( ) Specific activity (U/mg)

Effect of fermentation media for mannanase production

The mannanase activity on various medium produced by *Acinetobacter* sp. KUB-ST1-1 were shown in Figure 3. The mannanase showed highest mannanase activity of 4.80 and 3.87 U/ml against PM and NB medium, respectively. Because of in PM medium contained high nitrogen and carbon source (Mohammad *et al*, 1996). However, the mannanase showed highest specific activity of 2.28 U/mg of protein against NB medium compared with the others medium. In my research focus on the high specific activity and we selected the NB as the medium for mannanase production in further study.

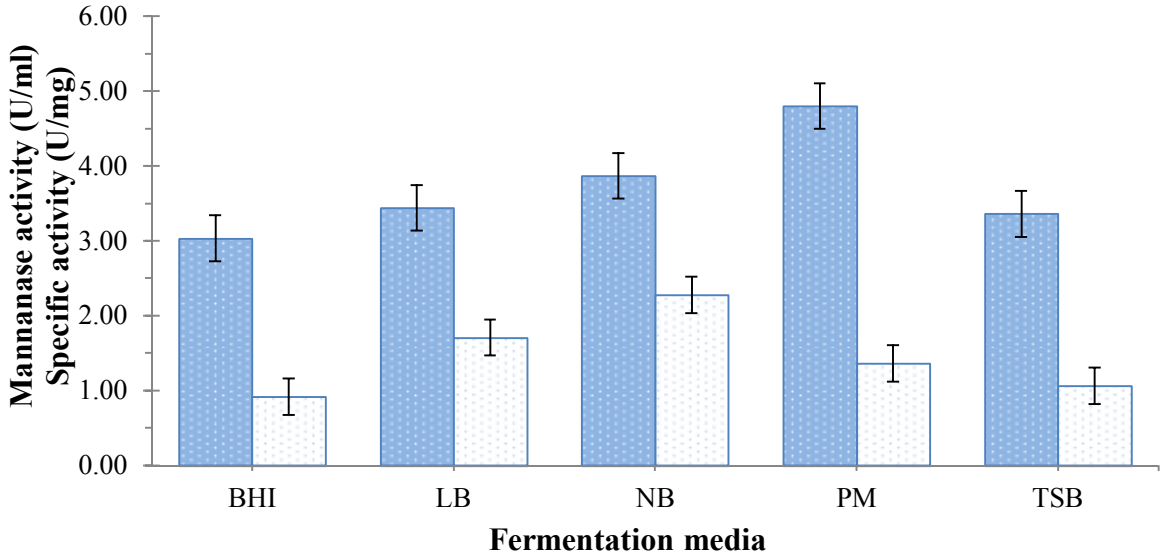

Figure 3 The mannanase activity on different fermentation media produced by *Acinetobacter* sp. KUB-ST1-1: ( ) Mannanase activity (U/ml); ( ) Specific activity (U/mg)

Effect of glucose concentrations for mannanase production

The effect of various glucose concentrations from 0-5% supplemented to the enzyme production medium containing 0.5 % LBG in order to evaluate its induction or repression effect on mannanase production were showed in Figure 4. The highest mannanase activity was exhibited by NB medium at 1.5% glucose concentration of 7.27 U/ml and 1.94 U/mg of protein, respectively. However, the association of additional sugars more than 2% glucose cocentration was accompanied by severe inhibitory effects on enzyme production. Such results may be due to the catabolic repression processes when easily assimilated carbon sources were added (Moussa and Thawat, 2007).

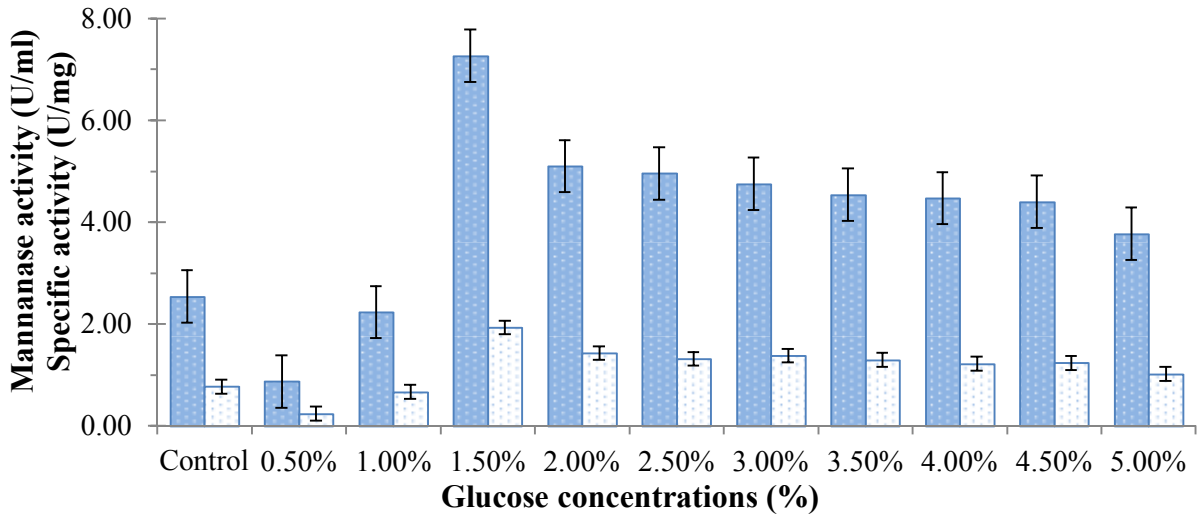

Figure 4 The mannanase activity on glucose concentrations (%) on NB medium produced by *Acinetobacter* sp. KUB-ST1-1: (▩) Mannanase activity (U/ml); (□) Specific activity (U/mg)

Conclusion

Enhanced mannanase production by a novel mannanase producing bacterium *Acinetobacter* sp. KUB-ST1-1 was achieved successfully. Its can produce the highest mannanase activity and specific activity of 7.27 U/ml and 1.94 U/mg, respectively when incubated in NB medium at 1.5% glucose concentration with 0.5% LBG as substrate (pH 7) for 24 hrs.

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