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Identification of Suitable Condition for Mannanase Production by *Bacillus* sp. GA2(1)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SC designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SC, SN and KP managed the analyses of the study. Authors SC and JA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The effects of 1% (w/v) supplementation of additional 5 agricultural wastes, corn cob, bagasses, coffee residues, soybean meal, and copra meal for mannanase production by *Bacillus* sp.GA2(1) were studied. Hence, partial characterization of mannanase was determined.
Methodology: The 1%(v/v) overnight cultured of *Bacillus* sp. GA2(1) was transferred into the basal medium and shaken at 150 rpm for 18 h at 37ºC. The additional of 5 AWs, corn cob, bagasses, coffee residues, soybean meal, and copra meal for the mannanase production were investigated. The cell suspension was centrifuged, and the crude mannanases were collected and stored at –20ºC for enzyme assay. The mannanase activities were measured by the dinitrosalicylic acid method. The optimal pH of mannanase were studied by measuring enzyme activity at pH 3-10 using 50 mM of following buffers; citrate (pH 3.0-6.0), phosphate (pH 6.0-8.0), and glycine-NaOH

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(pH 8.0-10.0). The optimal temperature was measured at 30-80ºC. Under standard assay conditions, locust bean gum was used as substrate to determine the optimal pH and temperature of the reaction. Thermostability was determined by preincubating the enzyme at different temperatures (30-80ºC) for 1 h. The residual mannanase activities were measured under standard condition.

Results: Among bagasses, coffee residues, soybean meal, corn cob and copra meal, the coffee residues was the most effective carbon source, the maximum yield of mannanase activity was 0.26 U/ml. The optimal temperature and pH for mannanase activity was pH 6.0 and 50ºC of 0.44 and 0.35 U/ml, respectively. The stability of enzyme was determined at 30-80ºC for 60 min. The results revealed that mannanase retained more than 96% of remaining activity after incubation of 60 min at 50ºC.

Conclusion: The maximum mannanase production was found when the medium was supplemented with coffee residues. Crude mannanase showed the highest activities of 0.44 U/ml at pH 6.0 and of 0.35 U/ml at 50ºC. The mannanase from *Bacillus* sp. GA2(1) retained more than 90% of theirs activities at 30-60ºC after preincubated for 60 min and then rapidly decreased.

Keywords: Mannanase; Bacillus; agricultural waste.

1. INTRODUCTION

Beta-D-mannanase (mannan endo-1,4-β-mannohydrolase, EC 3.2.1.78) are hydrolytic enzymes which hydrolyze randomly β-1,4 mannosidic linkages within the backbones of mannan, galactomannan, glucomannan and galactoglucomannan. Mannans, which have main component as D-mannose are important for several industries including food, feed, and feed stocks. It can be broken down into simple sugars or oligosaccharides by a synergistic action of endo-mannanases (EC number 3.2.1.78, mannan endo-1,4-β-mannosidase) and exo-acting β-mannosidase (EC number 3.2.1.25).

Beta-mannanase is widely distributed in prokaryotes, eukaryotes and higher eukaryotes, including protozoa, insects, snails and germinating plant seeds. Beta-mannanase from various bacterial and fungal sources have been studied. In this study, *Bacillus* sp. GA2(1) was isolated from soil around soybean field in Khon Kaen province of Thailand. Extracellular mannanase was produced by using 5 agricultural wastes, corn cob, bagasses, coffee residues, soybean meal, and copra meal as the carbon source. Hence, partial characterization of mannanase was determined.

2. MATERIALS AND METHODS

2.1 Materials

All chemicals, media and media components were analytical grade, obtained from Sigma-Aldrich Chemical Ltd., USA; Carlo Erba reagent, France; and HiMedia Laboratories Ltd.

2.2 Preparation of Agricultural Wastes

Five of agricultural wastes (AWs) including coffee residues, soybean meal, copra meal, baggasse, and corn cob were used as carbon sources for mannanase production. AWs were dried at 60ºC for 48 h, blended, milled by a hammer mill (IKA Labortechnik; Janke & Kunkel, Germany), and then sieved to obtain a product with an average particle size of 30 mesh. All samples were kept in a desicator until used.

2.3 Microorganisms and Cultivation

Bacillus sp. GA2(1) was isolated from soil in a soybean field in Khon Kaen province and cultured by using xylan agar medium (pH 7.0) at 37ºC. Stock cultures were maintained and stored at -20ºC on nutrient broth at culture collection of the Department of Biotechnology, Khon Kaen University, Thailand.

2.4 Enzyme Production

The enzyme was produced in an 250 ml Erlenmeyer flask containing 50 ml of basal medium (control medium) [(% w/v): 0.20 $NaNO_3$, 0.05 K_2HPO_4, 0.02 $MgSO_4•7H_2O$, 0.02 $MnSO_4•2H_2O$, 0.02 $CaCl_2•H_2O$, 0.02 $FeSO_4•7H_2O$, and 1 peptone]. The overnight grown *Bacillus* sp. GA2(1) with the OD 600 of 0.5 was inoculated with 1%(v/v) and shaken at 150 rpm for 18 h at 37ºC. The cell suspension was centrifuged at 7,000X*g* for 20 min at 4ºC, and the crude enzymes were collected and stored at –20ºC for further study.

2.5 Enzyme Assay

The activity of mannanase was determined at 37ºC for 15 min. To determine mannanase activities, the reaction mixtures contained 0.5 ml of crude enzyme and 0.5 ml of 50 mM phosphate buffer pH 7.0 with 0.5%(w/v) locust bean gum were used as substrate. The amounts of reducing sugar were measured by the dinitrosalicylic acid (DNS) method [1]. All experiments were investigated in triplicate and results represented mean values of the activities.

One unit of mannanase activity was defined as the amount of enzyme producing 1 micromole of mannose per minute under experimental conditions.

2.6 Effect of Carbon Source in Medium Composition

Effect of 1% (w/v) supplementation of additional carbons to control medium were investigated. The AWs as carbon sources such as coffee residues, soybean meal, copra meal, baggasse, and corn cob for the mannanase production were studied.

2.7 Partial Characterization of Mannanase

2.7.1 Effect of pH on enzyme activity

The optimum pH of mannanase activity was evaluated at pH 3.0 to 10.0 using 50 mM of citrate (pH 3.0-6.0), phosphate (pH 6.0-8.0) and glycine-NaOH (pH 8.0-10.0).

2.7.2 Effect of temperature on enzyme activity

The optimum temperature was determined by incubating the enzyme in 50 mM of appropriate buffers at different temperatures between 30ºC and 80ºC. The thermostability was performed by incubation at different temperatures for 60 min. The remaining enzyme activities were measured.

3. RESULTS AND DISCUSSION

3.1 Effect of Carbon Sources for Mannanase Production

Lignocellulose is the major component of plant cell wall. It consists of three majors components, cellulose, hemicellulose, and lignin [2]. Among five AWs, as shown in Fig. 1, coffee residues (0.26 U/ml) was the best carbon sources for mannanase production by *Bacillus* sp. GA2(1). AWs in this experiment are containing of hemicellulose. Mannans and heteromannans are a part of the hemicellulose fraction in AWs. Moreover, carbon sources usually contain high content of polysaccharides which substrates-like or substrates of the enzyme. Meanwhile, it could be proposed that extracellular mannanase from *Bacillus* sp. GA2(1) was specific for the mannose molecule structure. However, to confirm this results, further studied should be performed employing purified mannanase.

Coffee is one of the world's most widely consumed beverages. The major polysaccharides, arabinogalactan and mannan, have been reported. The arabinogalactan on hydrolysis provides arabinose (19.8% w/w), galactose (48.2% w/w), mannose (0.8% w/w) and rhamnose (1.1% w/w). In addition, the mannan on hydrolysis provides mannose (94% w/w) galactose (3.3% w/w) and glucose (1.7% w/w) [3] Interestingly, *Bacillus* sp. GA2(1) coffee residues could be used as carbon sources for the production of extracellular mannanase. The result was similar to Chantorn et al. [4]. It were reported that the best carbon source for mannanase production from *Penicillium oxalicum* KUB-SN2-1 is robusta coffee residues.

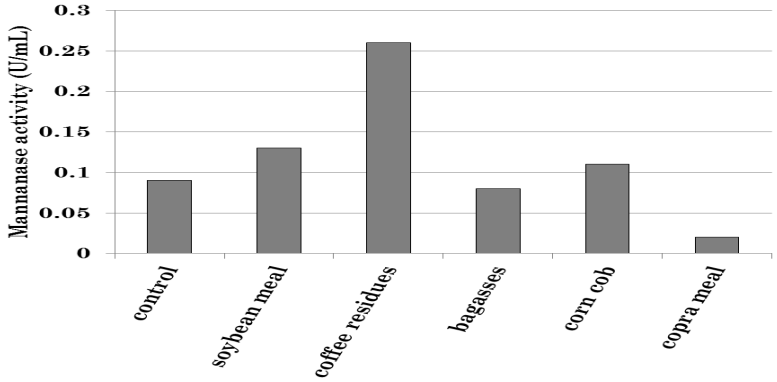

Fig. 1. Effect of carbon sources for mannanase production by *Bacillus* sp. GA2(1)

3.2 Effect of Temperature on Mannanase

The optimum temperature for mannanase was investigated by conducting the activity assay on LBG in phosphate buffer, pH 6.0 at various temperatures from 30-80ºC for 15 min. The maximum activity of mannanase was at 50ºC as shown in Fig. 2A. Generally, the bacterial mannanase was optimally active at 50-60ºC [5-8]. However, thermostable mannanase from *Bacillus subtilis* WY34 and *Thermotoga neapolitana* 5068, showed optimum temperature of 65ºC and 90-92ºC, respectively [9-10].

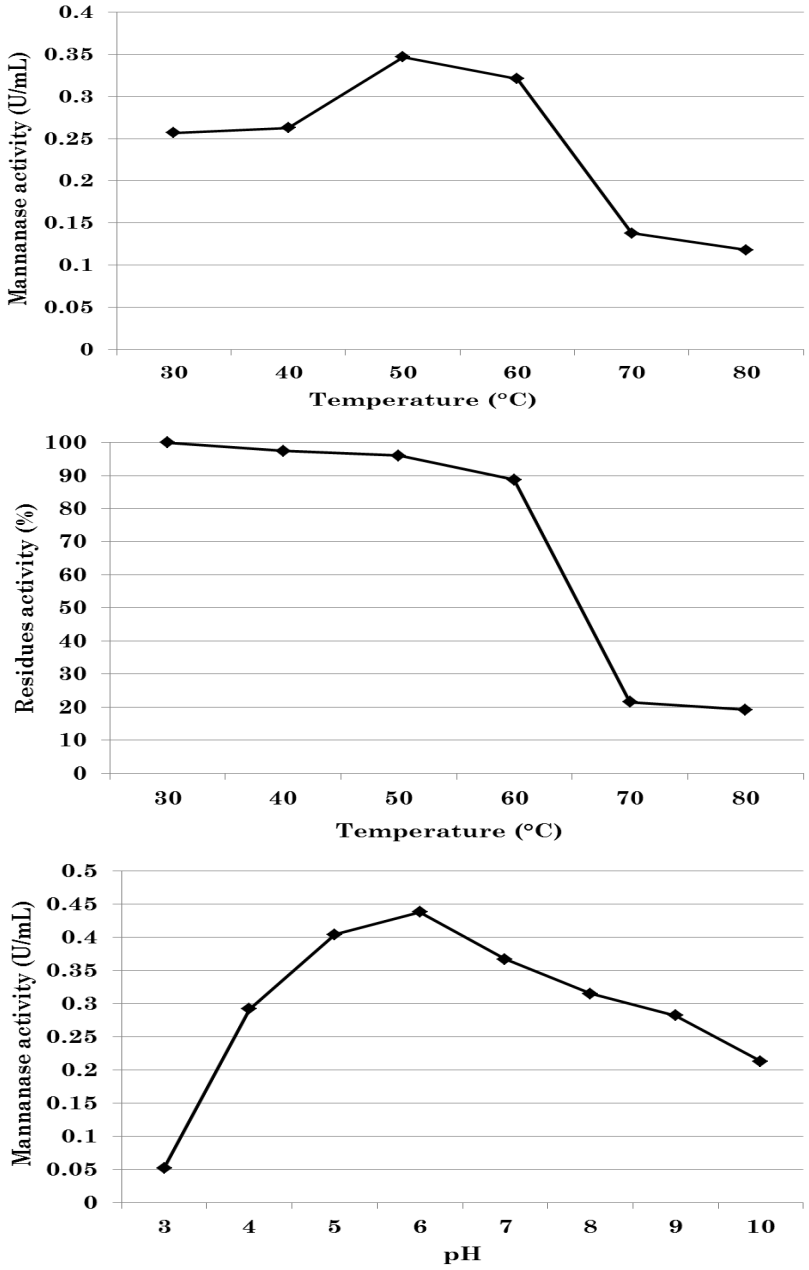

Fig. 2. Effects of (A) optimum temperature (B) thermostability and (C) pH on the activity of mannanase from *Bacillus* sp. GA2 (1)

Considering stability of mannanase at various temperatures, mannanase from *Bacillus* sp. GA2(1) retained more than 90% of their activities at 30-60ºC and then rapidly decreased (Fig. 2B). These results showed that mannanase was quite stable at high temperature corresponding to mannanase from *Klebsiella oxytoca* KUB-CW2-3 which showed stability at 30-70ºC with half life times of several hours [11].

3.3 Effect of pH on Mannanase

The effect of pH on mannanase activity are shown in Fig. 2C. At pH 6.0 mannanase exhibited the maximum activity of 0.44 U/ml. The acitivity was slightly decreased to 0.21 U/ml at pH 10.0. Corresponding to the pH optimum of both bacterial and fungal mannanase, they were in the range of 3.0-6.0 [12-15]. However, some of mannanases from *Bacillus subtilis* KU-1, *Bacillus* sp. KK01, *Bacillus* sp. strain JAMB-750, *Thermotoga neapolitana* 5068 and *Streptomyces ipomoea* showed higher optimum pH in the range of 7.0–10.0 [16-18].

4. CONCLUSION

Bacillus sp. GA2(1) was isolated from soybean field in Khon Kaen Province of Thailand. The maximum mannanase production was found when the medium was supplemented with coffee residues. Crude mannanase showed the highest activities of 0.44 U/ml at pH 6.0 and of 0.35 U/ml at 50ºC. Hence, the mannanase from *Bacillus* sp.GA2(1) retained more than 90% of their activities at 30-60ºC after preincubated for 60 min and then rapidly decreased. The capability action at acidic pH and stability at high temperature, this enzyme can be a good candidate for applying used in several industrial processes.

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