Factors Affecting the Production of Extracellular Xylanase by *Bacillus* sp. GA2 (1) and Application for Oligosaccharides Production

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Abstract

Endo-1,4-β-Xylanases (1,4-β-D-xylanohydrolase, EC.3.2.1.8) depolymerised Xylan, a major component of hemicellulose, by the random hydrolysis of xylan backbone. The concentrations of enzyme production medium (EPM) and conditions for xylanase production from *Bacillus* sp. GA2(1) were investigated. *Bacillus* sp. GA2(1) was cultured in nutrient broth at 37°C for 16-18 h. After that, the culture was transferred to EPM containing 5 g/l yeast extract, 0.2 g/l $MgSO_4.7H_2O$, 1 g/l KH_2PO_4, 5 g/l peptone and 5 g/l corn cobs, called controlled EPM. The concentrations of EPM in the range of 0-5 g/l for yeast extract, 0-0.4 g/l for $MgSO_4.7H_2O$, 0-2 g/l for KH_2PO_4, 0-5 g/l for peptone and 0-5 g/l for corn cobs were evaluated by comparing xylanase activities to that of the controlled EPM. The experiments showed the highest xylanase activity of 0.23 U/ml with EPM composing of 2 g/l yeast extract, 0.3 g/l $MgSO_4.7H_2O$ 1 g/l, KH_2PO_4, 2 g/l peptone, and 3 g/l corn cobs. The initial pH and the optimum temperature of EPM for xylanase production were studied. The maximum xylanase activity of 0.45U/ml was found at the initial pH and EPM temperature of 7.0 and 37°C, respectively. The agricultural wastes, corn cobs, sugarcane bagasses, coffee residues, soybean meal, potato peels and copra meal were selected for oligosaccharide production. The reducing sugar amounts represented the amounts of oligosaccharides that were analyzed by dinitrosalicylic acid methods. The highest reducing sugar of 412.49 µg/ml was obtained from sugarcane bagasses hydrolysate.

Keywords: xylanase; Bacillus; agricultural wastes.

1. Introduction

Xylan is the most common hemicellulose and it is the second most abundant biopolymer found in plant cell walls, in both hardwood and annual plants [1]. Xylan is recognized as the major hemicellulose component of agricultural residues and is composed of a linear backbone of β-1,4-linked-D-xylopyranosyl units, which are differently substituted

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branch chains [2]. Many agricultural and agro-industrial wastes contain hemicelluloses mainly xylan, which could be changed into more valuable products by microorganisms. The endo- β-1,4-xylanases (1,4-β-D-xylanohydrolase; EC 3.2.1.8) are the principle enzymes for hydrolyzing xylan polymer. The xylan polymer is completely hydrolyzed to xylooligosaccharides and xylose by a synergism action of endo-xylanases and the other enzyme such as β-xylosidase, α-L-arabinofuranosidase, acetylxylan, feruloyl, and α-glucuronidase [3].

Therefore, ways to optimize various physiological and nutrition parameters were explored in order to increase the level of enzyme production.

2. Materials and Methods

2.1 Materials

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

2.2 Microorganism and growth conditions

Bacillus sp. GA2(1) was obtained from the culture collection of the Department of Biotechnology, Faculty of Technology, Khon Kaen University, Thailand. The stock culture was maintained and stored at -20°C on nutrient broth (NB). The microorganism was grown in NB at 37°C for 18 h with shaking at 150 rpm and used as starting inoculum.

2.3 Enzyme production

One percent of starting inoculum, adjusted to the absorbance of 0.5 at 600 nanometers, was transferred into enzyme production medium modified from Battan *et al* [4]. The enzyme production medium consisted of 2 g/l yeast extract, 0.3 g/l $MgSO_4.7H_2O$, 1 g/l KH_2PO_4, 2 g/l peptone, and 3 g/l corn cobs. After 24 h of shaking at 150 rpm at 37°C, cells were removed by centrifugation at 4°C, 10000g for 20 min. The supernatant was collected and stored at – 20°C for further studies.

2.4 Enzyme assay

The activities of the extracellular xylanase were determined at 50°C for 10 min. To determine xylanase activities, we made reaction mixtures containing 0.5 ml of crude enzyme and 0.5 ml of 50 mM citrate-phosphate buffer pH 6.0 with 1%(w/v) of birch wood xylan. The amounts of reducing sugar were measured using the dinitrosalicylic acid (DNS) method [5]. All experiments were conducted in triplicate, and the results represented the mean values of the activities.

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

2.5 Growth and xylanase production profiles

The growth and xylanase production were investigated in enzyme production medium in 1 l Erlenmeyer flask. Samples were collected at 2 h intervals for 0 to 48 h for xylanase production and biomass evaluation. Biomass was determined spectrophotometrically at the optical density of 600 nm while the production of xylanase was determined by xylanase activity as described above.

2.6 Temperature and initial pH in the culture medium on xylanase production

The growth and the xylanase activity were determined at different temperatures ranging from 30-45°C. The effects of initial pH values of culture medium from 4 to 10 on growth and the xylanase production were assayed. The initial pH of the culture medium was adjusted by using 1M of hydrochloric acid or sodium hydroxide solution.

2.7 Optimization of culture medium for xylanase production

The nutrient concentrations of $MgSO_4.7H_2O$ (0–0.4 g/l), peptone(0–5 g/l), yeast extract (0–5 g/l) potassium hydrogen phosphate (0.2 g/ml), and corn cobs (0–5 g/l) in the enzyme production medium were varied to optimize the xylanase production.

2.8 Oligosaccharide production from xylanase hydrolysis

2.8.1 Agricultural waste preparation

Six agricultural wastes (AWs): copra meal, coffee residues, soybean meal, potato peels, sugarcane bagasse, and corn cobs were used as substrates for this study. All agricultural wastes were dried at 60°C for 48 h, blended, milled by a hammer mill (IKA Labortechnik; Janke & Kunkel, Germany) and then sieved to obtain products with an average particle size of 30 mesh. All samples were kept dessicated until use.

2.8.2 Oligosaccharide production

Crude extracellular xylanase of 0.33 U/ml was used in the reaction mixture of 15 ml of crude extracellular enzyme sample and 15 ml of 0.5% (w/v) AWs in 50 mM phosphate buffer pH7.0 and carried out at 37°C for 60 min. The reaction was stopped by placing the samples in boiling water for 10 min. The amounts of reducing sugar from hydrolysis were analyzed with DNS methods.

3. Results and Discussion

3.1 Growth and xylanase production profiles

The time course of growth and the production of extracellular xylanase were studied using corn cobs as a carbon source (Fig1). After 16 h of incubation time, the maximum xylanase production of 0.24 U/ml was observed. Further incubation did not show any increase in xylanase production probably due to the increase of the toxic unwanted wastes and the depletion of nutrients in the media, which in turn led to the decrease in growth and enzyme production [6]. This study has been expanded recently to include a variety of nutritional and environmental changes with the aim of improving growth in natural applications and industrial applications.

3.2 Effects of temperature and initial medium pH on xylanase production

The effect of growth temperature on xylanase production in the range of 30-45°C was investigated. The highest xylanase activity of 0.23 U/ml was observed when the strain grew at 37°C (Fig2A). At the high temperature, microorganisms may synthesize only substances essential for growth and other metabolisms for survival, so they did not secrete the enzyme [7]. Many reports showed the optimum temperature for xylanase production from *Bacillus* sp of 37°C [8-9]. The strain showed a greater xylanase production at neutral and slightly acidic pH (5 and 6), and alkaline pH (8 and 9) than at acidic pH (Fig 2B).

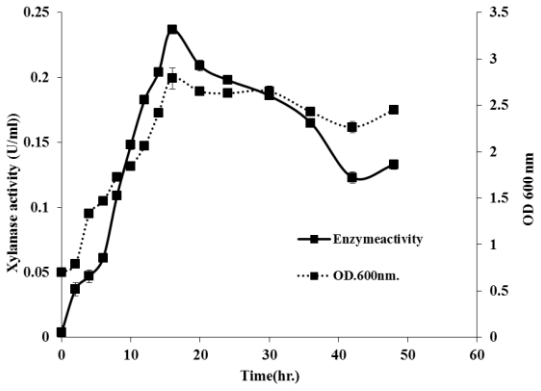


Fig.1. Effect of carbon sources on xylanase production by *Bacillus* sp GA2(1) after incubation at 37 ◦C for 48 h under shaking at 200 rpm.

Xylanase production by various bacteria and

fungi markedly depended on pH. The acidic pH (4.0–6.0) was generally favoured for fungal xylanases while the higher pH was favoured for bacterial xylanase [10]. Hence, the results differed from that of *Streptomyces olivaceoviridis* E-86 xylanase, which showed the highest activities at pH 5.0 and 5.5 [11].

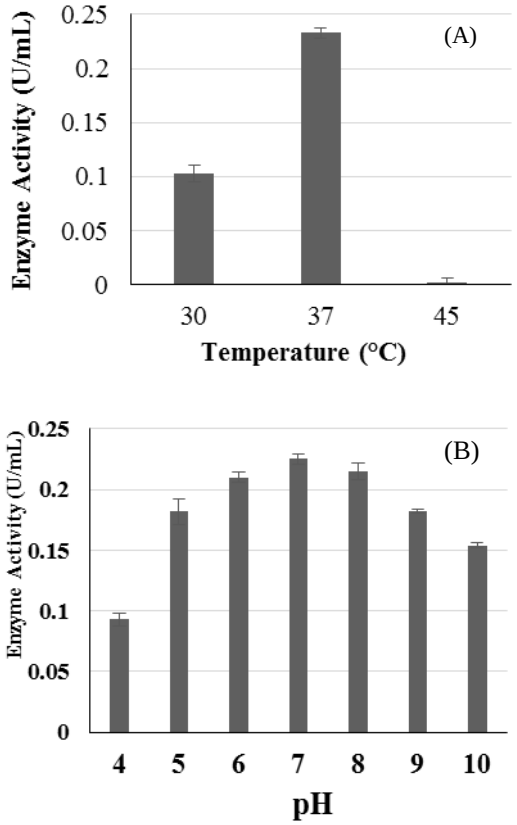
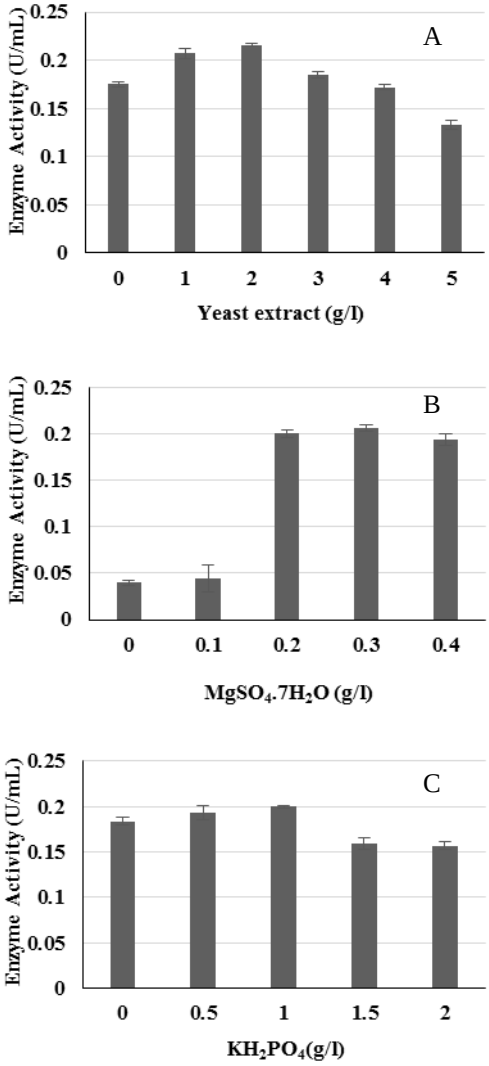


Fig.2. Effects of temperature(A) and initial pH (B) on xylanase production.

3.3 Effect of culture medium for xylanase production

In the first optimization trial, *Bacillus* sp.GA2(1) was cultivated in enzyme production medium modified from Battan *et al* [4] consisting of (g/l) 2 g yeast extract, 0.3 g $MgSO_4.7H_2O$ 1 g, KH_2PO_4, 2 g peptone, and 3 g corn cobs. The highest xylanase activity of 0.13 U/ml was reported. Thus, the nutrient concentrations in the enzyme production medium were determined. *Bacillus* sp. GA2(1) showed the highest xylanase production when the medium was supplemented with yeast extract (2 g/l), $MgSO_4.7H_2O$ (0.3 g/l), KH_2PO_4 (1 g/l), peptone (2 g/l), and corn cobs (3 g/l).



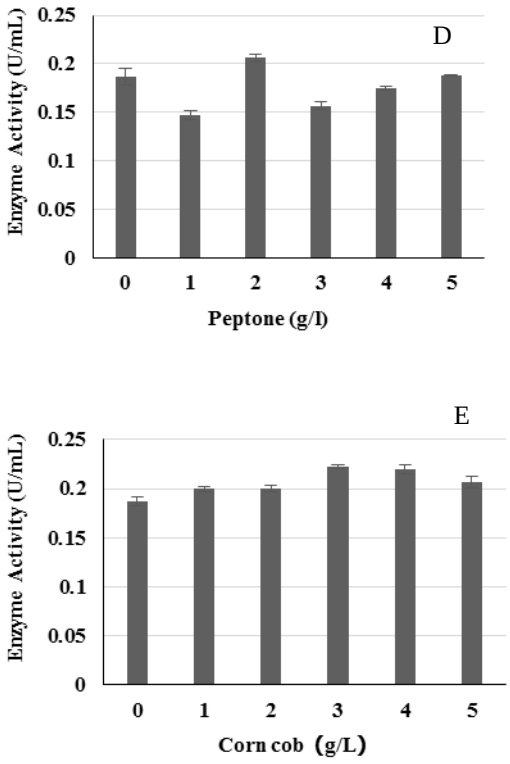


Fig.3. Effects of concentrations in the various concentrations of: (A) yeast extract; (B) ammonium sulphate ($MgSO_4$.$7H_2O$); (C) potassium hydrogen phosphate (KH_2PO_4); (D) peptone; and (E) corn cobs.

Table 1. Reducing sugar contents (µg/ml) from AWs hydrolysates by crude xylanase from *Bacillus* sp. GA2(1).

Agricultural wastes (AWs)	Reducing sugar (µg/ml)
Corn cob	142.49 ± 0.02
Bagassess	412.49 ± 0.10
Coffee residues	208.33 ± 0.09
Soybean meal	134.99 ± 0.02
Potato peel	138.33 ± 0.03
Copra meal	119.99 ± 0.01

(Fig3A-3E). In addition, for the optimized condition (37°C, pH 7.0) and the enzyme production medium consisting of yeast extract (2 g/l), $MgSO_4.7H_2O$ (0.3 g/l), KH_2PO_4 (1 g/l), peptone (2 g/l), and corn cobs (3 g/l), *Bacillus* sp. GA2(1) secreted the maximum xylanase activity of 0.45 U/ml.

3.4 Oligosaccharide production

Among tested AWs, the results showed that the crude xylanase from *Bacillus* sp. GA2(1) could hydrolyze various AWs as shown in Table1. The maximum reducing sugar level of 412.49 ± 0.10 µg/ml was obtained from sugarcane bagassess hydrolysates. Moreover, it also hydrolysed coffee residues, corn cobs, potato peels, soybean meal, and copra meal to obtain 208.33 ± 0.09, 142.49 ± 0.02, 138.33 ± 0.03, 134.99 ± 0.02, and 119.99 ± 0.01 µg/ml reducing sugar, respectively. All 6 AWs in this study were different in hemicellulose components [12]. Several enzymes were required to complete hydrolysis. Thus, crude enzymes which might contain various enzymes such as cellulase, mannanase, and xylanase were nessessary to hydrolyse AWs. The results had implications and future applications regarding the production of ethanol fuel from AWs.

4. Conclusion

Bacillus sp. GA2(1) was isolated from a soybean field in Khon Kean Province of Thailand. *Bacillus* sp. GA2(1) was cultured in the optimized enzyme production medium which was composed of yeast extract (2 g/l), $MgSO_4.7H_2O$ (0.3 g/l), KH_2PO_4 (1 g/l), peptone (2 g/l), and corn cobs (3 g/l) at 37°C and pH 7.0. At the optimum condition, *Bacillus* sp.GA2(1) showed the highest xylanase activity of 0.45 U/ml. The sugarcane bagassess hydrolysate showed the maximum reducing sugar content of 412.49 µg/ml. Thus, the enzymes from *Bacillus* sp. GA2(1) could hydrolyze the AWs to produce sugars for further applications.

5. Acknowledgement

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6. References

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