ORIGINAL ARTICLE

Partial Purification and Characterization of Xylanase from *Bacillus weihenstephanensis* Strain ANR1 using Watermelon Rind

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Abstract

Aim: The aim of this study is to partially purify and characterize xylanase from *Bacillus weihenstephanensis* strain ANR1 using watermelon rind as a substrate. **Materials and Methods:** The soil bacterium, *B.weihenstephanensis* strain ANR1 was grown at 37°C for 72 h on rotary incubator shaker at 150 rpm in a flask containing 250 ml of medium. The xylanase enzyme was partially purified using ammonium sulfate precipitation and dialysis. It was further purified using DEAE-sepharose chromatography. Molecular weight of the xylanase enzyme was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. **Results and Discussion:** The xylanase enzyme was partially purified to a fold of 8.15 and a yield of 83.56%. Molecular weight of the protein was found to be 45 kDa. The purified enzyme was found to be optimally active at pH of 7.0 and at a temperature of 37°C. **Conclusion:** Based on the results obtained, the purified xylanase enzyme from *B. weihenstephanensis* strain ANR1 using watermelon rind as a substrate can be effectively used in various food industrial applications.

Key words: Purification, watermelon rind, xylanase

INTRODUCTION

Xylan is the major component of the hemicellulose fraction present in cell walls of higher plants.[1] This component is present in the cell wall and in the middle lamella of plant cells. Around 20-30% of the total dry weight of plant biomass contains xylan. It is of great interest in the enzymatic degradation of xylan, the major constituent of hemicelluloses which has various industrial applications. Xylanases are an extracellular enzyme required for the hydrolysis of y-1, 4-xylans. Microbial xylanases have a wide range of commercial applications in food, feed, textile, and paper processing.[2] To reduce the cost of xylanase enzyme production various lignocellulosic residues are used to replace xylan. Therefore, this study was designed for partial purification and characterization of xylanase enzyme obtained from *Bacillus weihenstephanensis* strain ANR1 using watermelon rind as a substrate. To the best of our knowledge, there are no reports on the partial purification and characterization of the xylanase from *B. weihenstephanensis* strain ANR1 using watermelon rind.

MATERIALS AND METHODS

Cultivation of bacteria

The soil bacterium, *B. weihenstephanensis* strain ANR1 was grown at 37°C for 72 h on rotary incubator shaker at 150 rpm in a flask containing 250 ml of medium. The medium composition as follows: 2% watermelon rind, 0.7% KH_2PO_4, 0.2% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.1% $(NH_4)_2SO_4$, and

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0.06% yeast extract. The fermentation broth was filtered and centrifuged at 5000 rpm for 15 min at 4°C, and the supernatant was used as a crude enzyme for xylanase enzyme activity.

Xylanase assay

Xylanase activity was determined by measuring the reducing sugar by the dinitrosalicylic acid (DNS) method (Miller, 1959) using D-xylose as the standard. The enzyme assay was carried out at 40°C using 0.5% (w/v) birchwood xylan (sigma) as substrate (0.05 M sodium acetate buffer, pH 7). Then, 0.5 ml DNS reagent was added and heated in a boiling water bath for 10 min. After cooling to room temperature, the absorbance was measured at 560 nm. Enzyme activity was expressed as IU/mL. One unit of xylanase enzyme activity was defined as 1 μ mole of xylose liberated min^{-1} ml^{-1} of the enzyme under assay conditions.

Partial purification of xylanase enzyme

Ammonium sulfate precipitation

The crude xylanase enzyme (produced in a previous study)[3] was partially purified from the supernatant using ammonium sulfate in 0.05M sodium acetate buffer of pH of 6.8. Various ammonium sulfate concentrations, such as 30%, 40%, 50%, 60%, 70%, and 80%, were used for precipitating the enzyme. These concentrations were mixed with 500 ml of crude enzyme filtrate and continuously stirred at 4°C and was left overnight. The precipitate was recovered by centrifugation at 10,000 g for 20 min at 4°C and analyzed for xylanase activity.

Dialysis

After precipitation, the ammonium sulfate present in the enzyme solution was removed by dialysis. The precipitate was dissolved in 50 mM sodium acetate buffer of pH 7.0 at 4°C for 24 h.[4] The dialyzed fraction of the crude enzyme extract was used for further purification.

DEAE-sepharose chromatography

The dialyzed crude enzyme extract was further purified using DEAE-sepharose column chromatography. The column was packed with activated DEAE-sepharose (2.5 cm × 80 cm) equilibrated with 50 mM sodium acetate buffer (pH 7.0). Fractions of 10 ml were collected at a flow rate of 2 ml/min. The fractions with xylanase enzyme activity constituting a single peak were pooled and analyzed for xylanase activity.

Molecular weight determination by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed to determine the molecular mass of the protein. The partially purified xylanase enzyme was performed in a 10% acrylamide gel.[5] Coomassie brilliant blue R-250 was used to stain the gel. The protein molecular marker ranging from 14.5 to 94 kDa was used as a reference.

Thermal stability of enzyme

The xylanase enzyme was incubated at various temperatures ranging from 30, 40, 50, 60, 70 and 80°C for 15 min was performed.[6]

Effect of metal ions and additives

The enzyme was incubated with 2 mM solution of Cu^{2+}, Na^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+} and ethylenediaminetetraacetic acid (EDTA) for 15 min before substrate addition.

RESULTS AND DISCUSSION

The summary of purification of xylanase enzyme from *B. weihenstephanensis* strain ANR1 has been shown in Table 1. The crude enzyme was found to have total xylanase activity of 7500 IU, total protein as 180 mg, and the specific activity as 40.85 IU/mg protein. The crude enzyme filtrate was precipitated with different concentrations of ammonium sulfate such as 30%, 40%, 50%, 60%, 70%, and 80%. It was observed that 70% concentration of ammonium sulfate showed enhanced performance of enzyme precipitation resulting 6050 IU activity, 77.18 IU/mg specific activity, 78 mg of total protein, 78.48% of purification yield, and 1.88 of purification fold. The xylanase enzyme was further purified by DEAE-sepharose ion exchange column with 8.15 of purification fold and specific activity of 615.07 IU/mg. The enzyme yield was reduced to 78.48% after ammonium sulfate precipitation which reached to 49.56% after ion exchange chromatography.

After purification, xylanase enzyme was characterized for thermal stability, effect of metal ions, and molecular

Table 1: Partial purification and characterization of xylanase

Step	Total protein (mg)	Total activity (IU/ml)	Specific activity (IU/mg)	Yield %	Purification fold
Crude	180	7500	40.85	100	1
$(NH_4)_2SO_4$	78	6050	77.18	78.48	1.88
DEAE-sepharose	20.84	2845	615.07	49.56	8.15

Table 2: Effect of metal ions on xylanase activity

Metal ions	Enzymes activity %
Control	100
Cu^{2+}	80
Na^{2+}	254
Mn^{2+}	124
Zn^{2+}	64.5
Ca^{2+}	60.1
Mg^{2+}	312
EDTA	65.5

EDTA: Ethylenediaminetetraacetic acid

mass of the protein. Thermal stability of an enzyme was calculated at different temperatures. The enzyme showed maximum activity at 40°C [Figure 1]. Increase in the temperature showed a negative effect on xylanase activity. When the temperature was increased to 70°C, the enzyme activity was decreased due to denaturation of enzyme at higher temperatures. Wamalwa *et al*.[7] also reported the optimum temperature of 40°C for xylanase from the strain of *Bacillus halodurans*. Blanco *et al*.[8] also reported an optimum temperature of 50°C for the enzyme produced from a *Bacillus* sp. strain BP-23. Araki *et al*.[9] reported the optimum temperature of 40°C for xylanase from *Alcaligenes* sp. XY-234. Hence, it is evident that there is a wide difference in the optimum temperature of xylanase obtained from different microbes. The influence of metal ions and additives on xylanase enzyme activity was determined. The xylanase activity was stimulated in the presence of Na^{2+} and Mg^{2+} ions with the residual activity of 254% and 312%, respectively, and it was inhibited by Cu^{2+}, Mn^{2+} and EDTA [Table 2]. There was almost no effect by Zn^{2+}, Ca^{2+} ions. However, there are reports of similar results such as inhibition activity by Cu^{2+} and Mn^{2+}.[10-12]

The purified xylanase enzyme obtained from *B. weihenstephanensis* strain ANR1 was subjected to SDS-PAGE as shown in Figure 2, which results into a single protein band. It is compared with the standard protein molecular markers; the purified xylanase had a molecular weight of 45 kDa. Min-Jen *et al*.[13] reported xylanase enzyme from *Bacillus firmus* with a molecular weight of 45 kDa.

CONCLUSION

In conclusion, the results obtained from the present work gives an insight of purification and characterization of xylanase enzyme. Purification methods effectively purified xylanase enzyme from *B. weihenstephanensis* strain ANR1 using watermelon rind as a substrate. The purification process resulted in increased yield of enzyme activity. The purified xylanase enzyme has a potential for its cost-effective application in food industry.

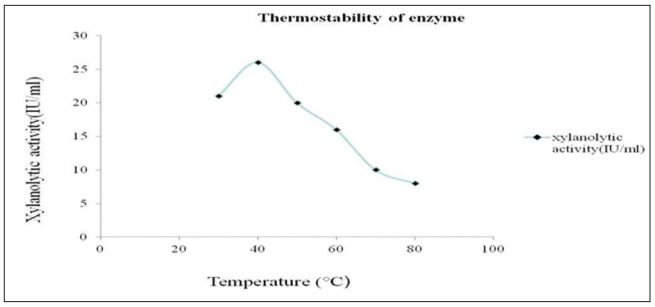

Figure 1: Thermostability effect of xylanase enzyme

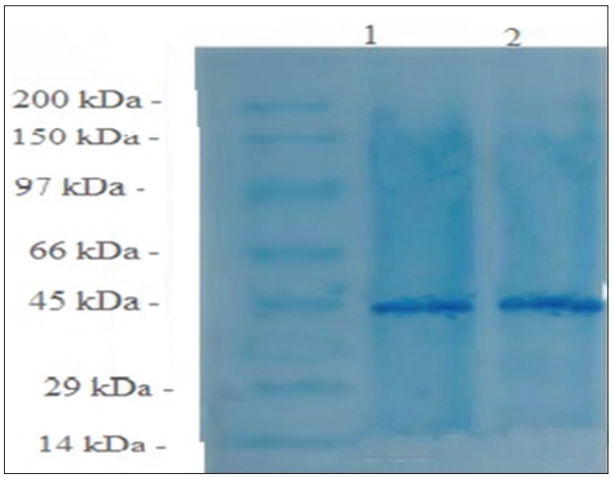

Figure 2: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified xylanase from *Bacillus weihenstephanensis* strain ANR1. Lane 1: Protein standard marker, Lane 2: Purified xylanase

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