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Research Paper

Production and Purification of Xylanase Enzyme through some Bacteria and Investigation of its Hydrolysis Effects

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Abstract

The Xylans are non-heterogeneous poly saccharides forming the major part of hemi cellulose in plant cell walls. Extracted from different species of microorganisms, xylanases (EC:3.2.1.8) are the enzymes capable of degrading the xylosidic bonds in the xylan backbone, producing xylose and other mono saccharides.

In the present research, the Xylanase enzyme was first purified from basiculls thermophilus (ATCC12980) through sediment by ammonium solphate, gel filtration chromatography by sephadex G-100 and ion exchange chromatography by di ethyl amino ethyl-cellulose. Then, the enzyme effectiveness was studied in hydrolysis of two kinds of xylan (obtained from Betula and Avena sativa). Di ethyl amino ethyl-cellulose column chromatography was observed in three individual picks with only one pick showing Xylanase activity. The degree of purity obtained from the pick fractions was determined as 63.09.

The specific activity value of the purified enzyme was calculated as 87.7 I.U in/per milligram and its purification yield was determined as 17.45%. Through the enzyme, the hydrolysis levels of xylans from Betula and Avena sativa were 100 and 56.8 percent in 24 hours, respectively. Based on the results, it turned out that using the purified xylanase enzyme will be helpful for the industries exploiting the Betula woods.

Keywords: Basiculls stearothermophilus; Xylanase; Purification; Xylan.

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1. Introduction

Xylanase (EC:3.2.1.8) are produced by plant thermophile bacteria and fungi [1]. The enzyme can hydrolyze the hemi cellulose contained in non-starch carbohydrates [2]. In the past years, the industrial applications of the enzyme have gotten the researchers' attentions all over the world. The xylanase enzymes have been used in various industries such as paper manufacturing/making (paper decolorization), producing clear edible oils and converting the solid fuels to liquid ones [3,4]. The researchers isolated the enzyme from different bacterial and fungal resources and showed its different amounts and levels of activity between them [5]. Due to highly specific function and more by-products, the xylanases extracted from the bacteria as xylan-hydrolyzing catalyzers are more important than the other kinds of the enzyme. Basiculls stearothermophilus is a thermophile bacterium and an agent of food materials deterioration [6,7]. The standard strain of this bacterium (ATCC 12980n) is considered the industrial recourse to produce the endonuclease enzyme BstPI [8]. It is a biologic indicator used in paper bands for sterilization and also a proper resource to produce the thermo/tolerant/resistant enzymes in industrial applications [9].

The tree woods and brans have various applications in the industries such as papermaking and livestock food production. Xylanase enzymes

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are used to degrade the xylan content of the mentioned materials [1]. The purpose of the present study was to investigate the effectiveness of the purified Xylanase enzymes on two types/kinds of xylana contained in betula woods and Avena sativa/oat brans in order to determine the industry for/in which the enzyme has the most effectiveness (papermaking industry or livestock food productions). In this project, the enzyme was first isolated from Basiculls stearothermophilus (ATCC 12980). Then its degradability regarding the two xylan types mentioned was studied and compared.

2. Materials and Methods

2.1 Preparing basiculls stearothermophilus (ATCC 12980) and culturing it

The bacterium basiculls stearothermophilus (ATCC 12980) was purchased from The USA Rockville Company. It was cultured in the specific liquid growth medium for optimal production of xylanase enzyme. In order to prepare 1 liter of this growth medium in an Erlenmeyer flask, 10 gr of xylan, 20 gr of polypeptone, 2.5 gr of m Monopotasic phosphate, 1 gr hydrated Magnesium solphate and 0.05 gram of Manganese(II) sulfate were added and diluted to 1 liter by adding distilled water. The pH was set on 7 [10].

2.2 Extracting and purifying the xylanase enzyme from the growth medium

In this method, the bacterium containing medium was incubated for 48 hours in 60 ° C and mixed by the shaker. The medium was centrifuged at 10000g for 10 minutes at 4 ° C. Then the supernatant was added by saturated ammonium sulfate and centrifuged at 10000g for 20 minutes. Finally, the resulted/obtained pellet containing xylanase enzyme was dissolved in 100 ml of potassium phosphate buffer (0.05 M with pH=7) and dialyzed per 5 liter of the same buffer for 5-10 hours at 4 ° C [11].

2.3 Purifying the xylanase enzyme

The enzyme was purified through two individual steps of gel-filtration column chromatography and ion exchange chromatography [11].

Gel-filtration column chromatography: The enzyme containing dialyzed solution was added to the chromatography column (2.7 X 100cm) containing sephadex G-100 in equilibrium with the potassium phosphate buffer (0.05 M with pH=7). Then, the column was passed through by the mentioned buffer at the flow rate of 120 ml/h and the output solution was collected by the automatic fraction collector. Absorbance values of the samples were read in/at wavelength of 280 nm by the visible-UV spectrophotometer for protein detection. The fractions having/with xylanase activity were mixed and dialyzed against the potassium phosphate buffer (0.05 M with pH=7). The mixture was concentrated to 10 ml using the rotary evaporator in the vacuum conditions [11].

2.4 Ion exchange column chromatography

The concentrated enzyme solution from the gel-filtration step was added to the chromatography column (1.5 X 40cm) containing DEAE-cellulose in equilibrium with the potassium phosphate buffer (0.05 M with pH=7). Then, the column was passed through by 1500 ml of the same buffer at the flow rate of 25 ml/h and the gradients of 0-0.25 M of NaCl were passed through the column by the NaCl-containing potassium phosphate buffer (0.05 M with pH=7). The isolated fractions were collected by the automatic fraction collector and their absorbance values were read in the wavelength of 280 nm. In the next step, the absorbance-containing fractions were studied/tested for enzyme activities [11].

2.5 Calculating the xylanase enzyme activity

The Miller's method was used in order to measure the activity of the xylanase enzyme in different samples obtained from different steps of extraction and purification [12]. 0.5 ml of the sample solution was poured into a test tube and added by 0.5 ml of the potassium phosphate buffer (0.05 M with pH=7). Then the content was mixed by an electric shaker device and incubated for 30 minutes at 60 ° C. The mixture was immediately placed in the ice containing cold water and centrifuged at 10000g for 3 minutes. In this way, not degraded xylans were deposited. In a tube test, then, 0.5 ml of the supernatant was added by 0.5 ml of the %0.5 3,5-dinitro salicylic acid solution. Afterwards, the tube was placed in hot water bath for 5 minutes and its content was cooled by the water flow. Using the spectrophotometer, finally, the absorbance value was read at the wavelength of 535 nm and the sample enzyme activity was calculated based on the standard chart/diagram. By definition, one unit of xylanase enzyme is determined as the amount of enzyme that liberates 1micromole of D-xylose per minute from the xylan substrate under the standard conditions (the temperature of 60° C and pH of 7).

2.6 Measuring the protein content

In all steps of the present research, protein content of the samples was measured based on the method by Lawery and colleagues [13].

2.7 Poly acrylamide gel electrophoresis (SDS-PAGE)

Different steps of enzyme purification were assessed by poly acrylamide gel electrophoresis. The percentage of the resolving gel was selected as %12.5 [14,15].

2.8 Measuring the hydrolysis percentages of two xylan types by the purified xylanases

In this step, the method of Khasin and colleagues were used [16]. A solution containing one international unit of the xylanase enzyme in 0.5 ml of the potassium phosphate buffer (0.05 M with pH=7) was prepared in a test tube, added by 25 ml of xylan obtained from the birch wood. The content was stirred and the tube was placed in 60 ° C for 2 hours. Then, the amount of D-xylose produced was calculated in micromole according to the standard chart. Using the following equation, finally, the hydrolysis percentage of the studied type of xylan by the xylanase enzyme was measured in 1 hour.

2.9 The percentage of xylan Hydrolysis

D-xylose produced in 1 hour/ the early xylan mass (mg)) X100X0.9, where 0.9 is the factor for adding water molecules to the substrate in the hydrolysis reaction. The same calculation was repeated for the times of 0 to 24 hours. In the present study, the above mentioned test was performed for oat xylan and the charts were plotted for changes in hydrolysis percentages of both xylan types in the noted hours.

Table 1. the results of different steps of xylanase extraction and purification.

Steps	Final volume (ml)	Total protein content (mg/ml)	Specific activity (u/mg)	Total Activity (u/mg)	Purification yield (percent)	Purification order
Isolating the bacterial cells from the culture medium	932.8	470	1.39	653	100	1
Depositing with ammonium solphate	5	125	2.88	360	56.69	2.07
Gel filtration chromatography with Sphadex G-100	42	8	38.75	310	47.47	27.87
Chromatography with DEAE- Cellulose	16	1.3	87.7	114	17.45	63.09

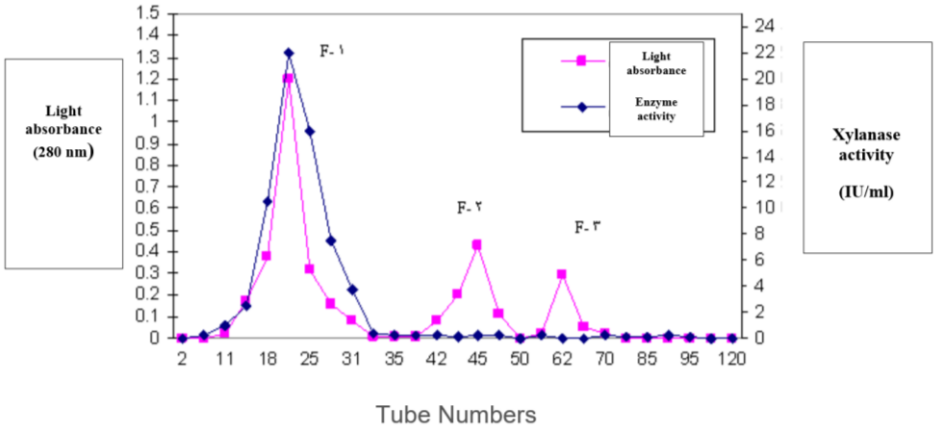

Chart 1. Gel filtration chromatography of 125 mg of protein obtained from depositing step with ammonium solphate on the sephadex G-100 column (2.5X102cm) in equilibrium with the potassium phosphate buffer (0.05 M with pH=7) and the flow rate of 120 ml/h.

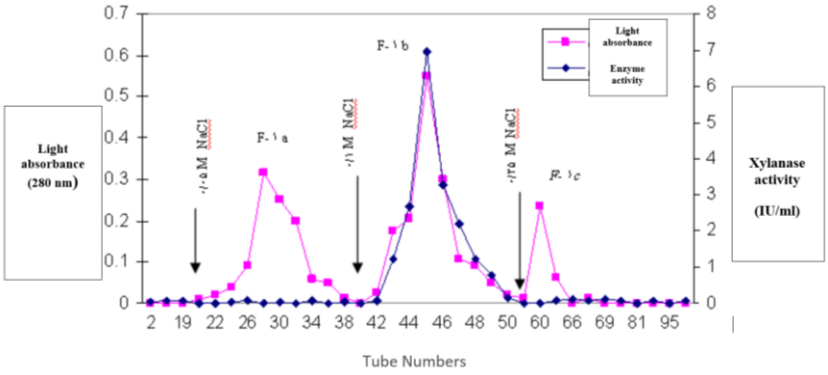

Chart 2. Ion exchange chromatography of F-1 pick resulted from the gel filtration step on the DEAE-cellulose column (1.5X140cm) in equilibrium with the potassium phosphate buffer with the salt gradient of 0-0.25 M and the flow rate of 30 ml/h.

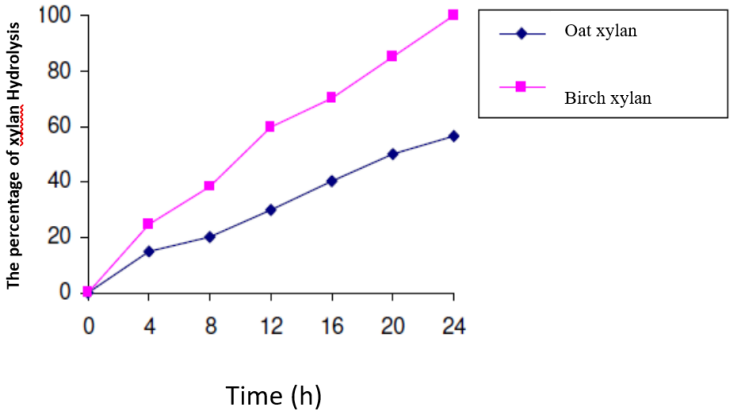

Chart 3. Changes in the Hydrolysis percentages of two type of xylan by the xylanase enzyme within 24 hours

3. Results and Discussion

3.1 The results of ion exchange chromatography

The chart 2 has indicated the results of ion exchange DEAE-cellulose gel chromatography. As shown, 3 individual picks were created which had absorbance in 280 nm but only fractions of the F-1b pick showed xylanase activity. The pick included 8 fractions with the final volume of 16 ml. Once concentrated, these 8 fractions were assessed by SDS-PAGE electrophoresis. The protein band shown on the gel implied the high enzyme purification (Figure 1). The values of the specific activity and the total activity of the purified enzyme are presented in table 1 for all the purification steps.

3.2 Measurement results for the hydrolysis percentages of two xylan types from birch woods and oat plants

Through the purified enzyme acting on two types mentioned in different hours, their hydrolysis percentages were determined. The chart 3 shows changes in the hydrolysis percentages of two type of xylan by the xylanase enzyme within 24 hours. As shown, the highest percentage of hydrolysis (%100) corresponds to xylan obtained from the birch wood within 24 hours after incubation. During the same time frame, the hydrolysis percentage for the oat xylan was %56.8.

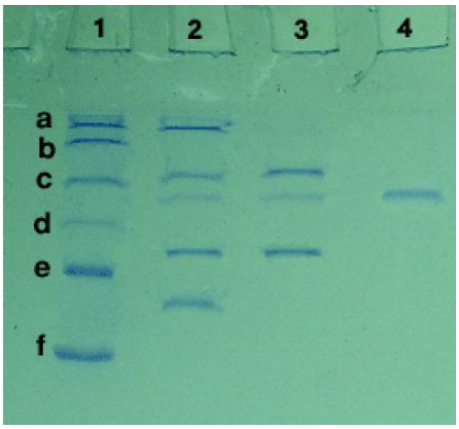

Figure 1. The slab SDS-PAGE electrophoresis from different steps of the enzyme extraction and purification. The percentages of the stacking and resolving gels were 12.5 and 4 and the voltage used was 120 V. The column 2 indicates the protein content of the sediment obtained by ammonium solphate. The column 3 presents the protein content of the pick F-1b resulted from gel filtration chromatography. The column 4 shows the concentrated purified xylanase enzyme (pick F-1b) resulted from the ion exchange chromatography on the DEAE-cellulose column. There are molecular weight markers in the column 1(a= phosphorylase with MW of 94 KD; b=bovine serum albumin with MW of 66 KD; c=Ovoalbumin with MW of 45 KD; d=carbonic anhydrase with MW of 29 KD; e= soy trypsin inhibitor with MW of 20 KD and f=lactalbumin with MW of 29 KD). The molecular weight of the purified enzyme was determined around 40 KD according to the above mentioned values.

4. Discussion

In the present study, the specific growth medium was prepared based on the method by Nanmori and colleagues [10]. Containing materials such as xylan, yeast extract, polypeptone etc., the medium was used by Khasin and Mandeva and their colleagues to optimally produce the xylanase enzyme in 1993 and 1998, respectively [16,17]. Using this specific growth medium allows the highest levels of xylanase production by the bacterium basiculls stearothermophilus [1,18,19].

In the present study, extraction and purification of the xylanase enzyme was carried out based on the method of Roy and colleagues [11]. The method yield was around %17.45, higher than the value reported by Nanmori et al (%11.3). They extracted the enzyme from a new strain of basiculls stearothermophilus [10]. The mentioned yield (%17.45) was also higher than the value reported by Gupta et al (%5). They had isolated the enzyme from a specific strain of the bacterium staphylococcus (SG-B) [20]. This explains the

higher applications of basiculls stearothermophilus than the other bacteria in order to produce xylanase enzyme. The molecular weight of the purified enzyme was determined as 40 KD using the acrylamide gel electrophoresis method and the weight determinant markers. The value was roughly consistent with the value obtained for the purified enzyme by Nanmori (39.5 KD) as well as Khasin et al (43 KD) [10,16]. Therefore, it can be concluded that the values of molecular weight for xylanase enzymes isolated from the different strains of basiculls stearothermophilus are nearly the same; however, the strains of the other bacteria such as *Aeromonas caviae* and basiculls (Sp.) exhibit different values of 22 and 36 KD, respectively [20,21]. As the substrates of the xylanase enzymes, xylans have different types like the ones from the birch woods and oat plants [1]. It turned out that there are different percentages of xylose in these two types; for example, more than % 90 of the birch xylan and around %70 of the oat xylan consist of xylose. Based on the research results, hydrolysis percentage of the birch xylan is higher than the oat xylan within 24 hours. According to the other researchers' findings, this can be due to differences in biochemical structure of these two types and their xylose content [1,22].

5. Conclusion

Given the purified xylanase enzyme could hydrolyze %100 of the birch xylan within 24 hours, it is proposed that the enzyme be exploited to enhance the product yield and optimization in various industries using the birch woods such as papermaking.

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