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Production of xylanase from watermelon rind by *Bacillus weihenstephanesis* strain ANR1

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Abstract : Xylanases are a group of hemicellulolytic extracellular enzyme required for the hydrolysis of β-1, 4-xylans present in lignocellulosic materials. In this study, xylanase producing bacteria *Bacillus weihenstephanensis* strain ANR1 was isolated from the soil. The production of xylanase enzyme was carried out by submerged fermentation utilizing various lignocellulosic substrates such as corn cobs, orange peel, rice bran, water melon rind, grape pomace , pomegranate peel, musk melon rind and pineapple peel at 1% (w/v). Among the substrate used watermelon rind produced the maximum of 426 U/ml of xylanase enzyme. The cellulose free xylanase enzyme was active and stable at the pH of 7 and the enzyme retained its activity at 37°C. Based on the results obtained that this superior property of xylanase enzyme produced from *Bacillus weihenstephanensis* strain ANR1 makes it ideal for the application in food industry.

Keywords: Watermelon rind, Xylanase, Food industry

1. Introduction:

Xylanases are the xylan degrading enzymes produced by various microorganisms like bacteria [1-3], fungi [2], actinomycetes [4] and yeast [5, 6]. Xylanases has a wide range of applications in various industries such as pre-bleaching of pulp, improving the digestibility of animal feed stocks, modification of cereal-based stuffs, bioconversion of lignocellulosic material and agro-wastes to fermentable products, clarification of fruit juices and degumming of plant fibers [7-11]. The high cost of this enzyme production has hindered the industrial application of xylan bioconversion. Hence in this study, cost effective natural substrates like corn cobs, orange peel, rice bran, water melon rind, grape pomace, pomegranate peel, musk melon rind and pineapple peel will be used in order to replace xylan which is a costly substrate for xylanase production. Xylanase producing bacteria, Bacillus *weihenstephanensis* strain ANR1 was isolated from the soil and found to be efficient in enzyme production. Among the natural lignocellulosic substrate used watermelon rind showed enhanced production of enzyme under submerged cultural conditions. To the best of our knowledge, this is the first report on watermelon rind specific xylanase enzyme production by *Bacillus weihenstephanensis* strain ANR1.

2. Materials and Methods:

2.1 Microorganism and culture conditions:

The bacterial strain, *Bacillus weihenstephanensis* strain ANR1 was isolated from the soil samples collected from VIT University, Vellore using birch wood xylan (1%) based agar medium by serial dilution method. The purified strain was maintained in the Horikoshi-xylan agar plates [12] (Table 1) and incubated at 37°C. After 24h of growth, the plates were stained with 0.5% (w/v) congo red for 15 min and destained with 1.0M NaCl. Xylanase producing bacteria showed a yellow zone around the colonies against the red background [13].

Table 1: Composition of Horikoshi-xylan agar media

Medium components	Quantity (%w/v)
Xylan	0.5
Peptone	0.5
Yeast extract	0.5
KH_2PO_4	0.1
$Mgso_4$	0.01
Agar	2.0
pH	7

2.2 Xylanase production studies:

Xylanase enzyme production fermentation experiments were performed in 250ml Erlenmeyer flasks containing 100ml of fermentation medium supplemented with watermelon rind as carbon source and moistened with mineral salt solution containing (g/l): KH_2PO_4 1.0,NaCl 2.5,$MgSO_4.7H_2O$ 0.1,$(NH_4)_2SO_4$ 1.0,$CaCl_2$ 0.1 at pH 6.0. The substrate was inoculated with 10.0% (v/w) of 14 h old (O.D600nm ≈2.5) seed culture and incubated for 5 days at 37°C in rotary shaker at 150 rpm. The enzyme production was monitored periodically after every 24 h for 5 days. The xylanase was harvested aseptically by adding 5 ml of pH 6.0 citrate-phosphate buffer (100.0 mM), vortexed at 300 rpm for 1 min at room temperature and incubated under static conditions for 15 mins. Thereafter, centrifugation at 10,000 rpm for 5 min at RT was carried out and the collected supernatant was used to estimate xylanase activity.

2.3 Xylanase assay:

Xylanase activity was determined by measuring the reducing sugar by the dinitrosalicylic acid (DNS) method [14] using D-xylose as the standard. The enzyme assay was carried out at 40°C using 0.5% (w/v) birch wood xylan (50mM Acetate buffer, pH 6.0) as substrate. One unit of xylanase activity was defined as 1 µ mole of xylose liberated min^{-1} $ml^{-1}$of enzyme under assay conditions.

2.4 Optimization of xylanase production and activity:

Bacillus weihenstephanensis strain ANR1 was cultivated in 50ml of production medium with 1% (v/v) inoculum at different pH (4.0-10.0) and temperatures (30°C-70°C) for 48h under standard shaking conditions to assay the produced xylanase enzyme. Effect of carbon and nitrogen sources on production was studied by adding xylose (2%) in the production medium with various synthetic and natural carbon sources (1% w/v) and yeast extract with organic and inorganic nitrogen sources (1% w/v) to assess the production of xylanase. Xylanase production was studied up to 7 days in production medium supplemented with 0.5% birch wood xylan using 1% (v/v) inoculum of 24h old culture (OD at 540 nm = 1.0) at 40°C in an incubator shaker at 150 rpm. Xylanase activity was assayed at different pH of buffer (50mM) ranging from 4.0 to 10.0 at various temperatures ranging from 30° to 70°C to determine the optimal xylanase activity.

3. Results and Discussion:

3.1 Isolation and screening of cellulose free xylanase producing microorganism:

The screening process of xylanase producing microorganism was carried out to select the capable bacteria. In total 10 bacterial isolates were isolated from 5 diverse soil samples and it was subsequently purified on Horikoshi agar medium. Among the isolates one of the bacterial isolate showed best xylanase producing ability and was selected for further studies (Fig. 1). The bacterial isolate produced cream colored, mucoid

colonies on xylan agar plates was further identified as *Bacillus weihenstephanensis* strain ANR1 by 16srRNA. The nucleotide sequence was submitted in Gen bank under the accession number JX307112.

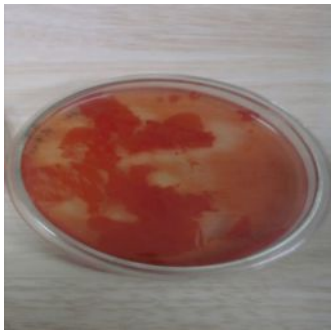

Fig. 1. Xylan -agar plate showing zone of hydrolysis by *Bacillus weihenstephanensis* strain ANR1

3.2 Optimization of xylanase production:

3.2.1 Optimization of physical parameters in xylanase production:

Xylanase production depends on various physical factors and nutritional parameters. The major physical parameters involve in the enzyme production are time of incubation, temperature and pH [15]. In order to study the effect of pH of the medium on xylanase production different initial pH values ranging from 4.0 to 10.0 was used and incubated for 72 h. Results showed that the pH has profound effect on xylanase and showed maximum production at pH 7.0 (Fig.2). The effect of temperature on xylanase production medium was studied with different temperature ranging from 30° to 70°C and incubated for 72 h. The isolate showed maximum growth and production of 426 U/ml of xylanase enzyme at 37°C (Fig.3). The xylanase activity was assayed and determined by measuring the reducing sugar by the dinitrosalicylic acid (DNS) method using D-xylose as the standard (Fig.4).

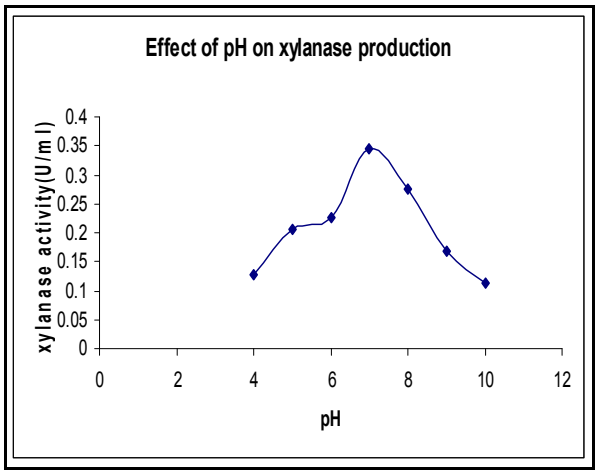

Fig. 2. Effect of pH on xylanase production

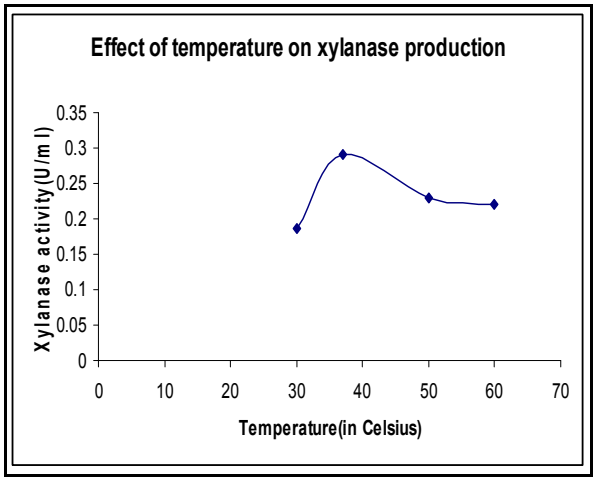

Fig.3. Effect of temperature on xylanase production

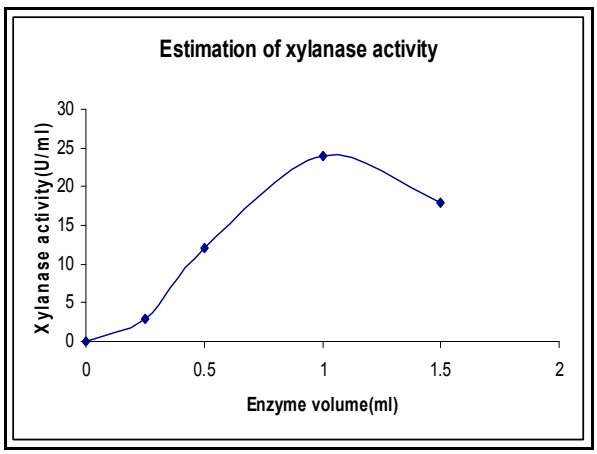

Fig.4. Estimation of xylanase activity

3.2.2 Optimization of the nutritional parameters:

Nutritional parameters also play a very major role in maximizing the yield of the xylanase production [16]. The role of different agro industrial waste materials impact on xylanase production by *Bacillus weihenstephanensis* strain ANR1 was evaluated. Natural lignocellulosic cheap substrates such as corn cobs, orange peel, rice bran, water melon rind, grape pomace, pomegranate peel, musk melon rind and pineapple peel at 1% (w/v) was supplemented to the fermentation medium. Among the substrate used watermelon rind showed enhanced production of xylanase enzyme of 426U/ml (Fig.5). Various carbon sources like maltose, glucose, fructose, xylose and lactose along with natural substrates was studied. Xylose showed maximum of 189.27 IU/ml of xylanase enzyme (Fig.6). Organic and inorganic nitrogen sources like yeast extract, beef extract, ammonium sulphate, ammonium chloride and sodium nitrate were studied. Ammonium chloride was found to be the best nitrogen source for xylanase production and showed maximum of 167.23 IU/ml (Fig.7).

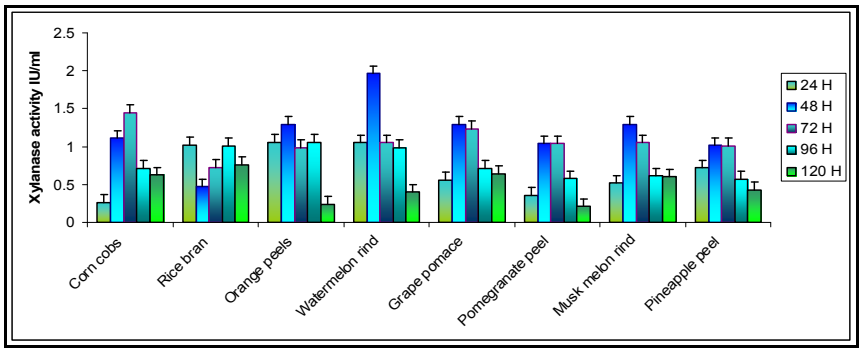

Fig.5. Effect of various lignocellulosic substrates on xylanase production by *B.weihenstephensis* strain ANR1

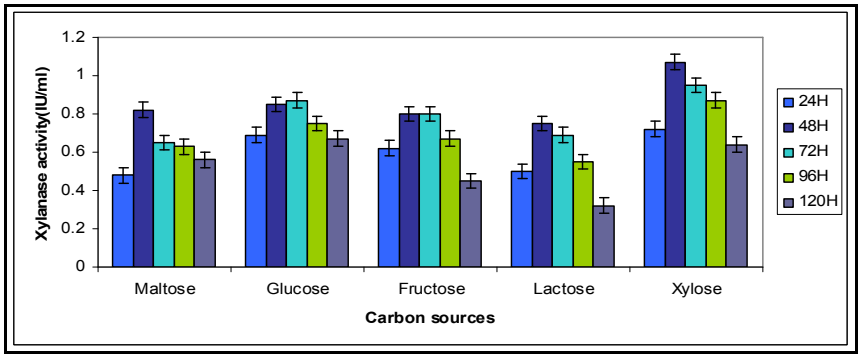

Fig .6. Effect of carbon source on xylanase production

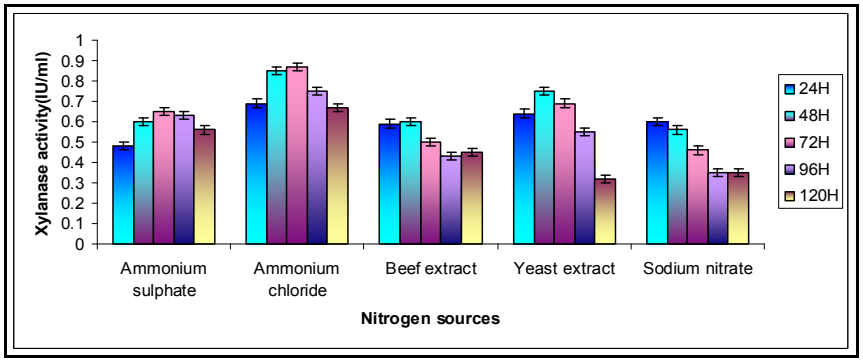

Fig .7. Effect of nitrogen source on xylanase production

4. Conclusion:

The present investigation of xylanase enzyme production by *Bacillus weihenstephanensis* strain ANR1 under submerged fermentation revealed that watermelon rind is the best preferred carbon source. The high cost of this enzyme production has hindered the industrial application of xylan bioconversion. The results obtained from the present work revealed that cost effective natural lignocellulosic substrate watermelon rind can replace

xylan which is a costly substrate for xylanase production. Application of xylanase enzyme obtained from watermelon rind, owing to its multidimensional role indicate potential for its economical application in baking industry to improve dough handling characteristics and baking quality traits. It can also be used as biobleaching agent and also in clarification of juices and wine. The detailed characterization of the application studies of the xylanase enzyme is in process.

5. References

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