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ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF XYLANASE PRODUCING *STREPTOMYCES SP* FROM MARINE ECOSYSTEMS

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ABSTRACT

Xylanase produced by microorganisms has many applications in the pulp and paper industry. Xylanase are used in bleaching process where the chlorine and hypochlorine were used which acts as a major pollutant in this industry. Xylanase are also used in food and feed industry for the clarification of juice, to improve the coffee and tea extraction. The present study was carried out to isolate the actinomycetes that produce xylanase. Out of the 50 isolates, 10 were found to produce xylanase. Out of these, one isolate showed maximum enzyme activity that was identified as S*treptomyces* sp. using 16s rDNA sequencing. Among the different carbon source, organism grown in wheat bran showed maximum enzyme activity. Urea and peptone were the best nitrogen source for maximum enzyme activity. The effect of different temperature and pH on xylanase production was studied. Maximum level of xylanase activity was observed at temperature 30-60ºC, pH 6.0-7.0.

KEYWORDS: **Actinomycetes, wheat bran, xylanase, 16s rDNA sequencing.**

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INTRODUCTION

Xylan, a natural noncellulosic polysaccharide abundantly present in plant cell walls [1]. Since xylan is present abundantly in nature, its enzymatic degradation is one of the important industrial applications. To degrade this xylan, xylanolytic enzyme is necessary and this enzyme has various applications in paper and pulp industry. The important applications of xylanase are bioconversion of lignocellulosic material and agro-wastes into fermentative products, clarification of juices, improvement in consistency of beer, surfactants production and bioconversion of lignocellulosic materials into fuel [2]. One of the most important biotechnological applications of xylanase is its use in pulp bleaching [3]. Identifying an enzyme that is stable at high temperature and alkaline pH is need of the hour in paper and pulp industry. Nowadays, xylanase derived from bacteria, actinomycetes and fungi have obtained much attention because these enzymes may be stable at different temperature and pH. Xylanase produced from bacteria are stable in high temperature and active at alkaline pH [4]. Apart from temperature and pH, industries are focused on cellulase-free xylanase which can be produced by microorganisms [5]. Actinomycetes are filamentous gram positive bacteria widely distributed in terrestrial and aquatic ecosystem. This species is very important for the production of industrial enzymes. Different species of actinomycetes are used to produce xylanase using solid state and submerged fermentation. Actinomytes such as *Streptomyces sp.*, *Saccharomonospora sp.* and *Streptomyces roseiscleroticus* are capable of producing xylanase free of substantial cellulase activity ([6, 7, 8]). In the present study, actinomycetes were isolated from marine sediments for the production of xylanase. The best carbon and nitrogen source were screened for the production of enzymes and the properties of the enzyme were studied.

MATERIALS AND METHODS

Culture and Growth Conditions

The marine sediments were collected from east coast of India and cultured in an actinomycetes broth contains 0.5% xylan to isolate xylan utilizing bacteria. Xylanase producing actinomycetes were screened based on the production of extracellular xylanase present in the medium. The culture was purified, and stored at 4◦C in actinomycetes agar. Isolated cultures were streaked in actinomycetes agar plates supplemented with 0.5% Xylan and were incubated for 3-5 days at room temperature. The plates were flooded with congo red solution. Orange color was observed around the colonies. 10 isolates with xylanase activity has been identified and the organism which showed more activity was cultured for further study.

PCR Amplification

DNA was isolated directly from colonies of purified isolates [9]. PCR Amplification of 16S rRNA gene was performed using universal primer (FD1-AGAGTTTGATCCTGGCTCAG and RP2-ACGGCTACCTTGTTACGACTT). The reaction mixture comprised of 25µl mixture containing both forward and reverse primers (25ng, 2µl each), PCR reaction buffer (10X, 2.5 µl), dNTPs (10mM 0.5 µl), Taq DNA polymerase (1U/µl, 1.0 µl), Template DNA (~100ng/µl) and nuclease free water (18.0 µl). The PCR profile is as follows: Initial denaturation at 95ºC for 5 min followed by 30 cycles of denaturation at 95ºC for 1 min, annealing at 57ºC for 1 min, extension at 72ºC for 2 min and final extension at 72ºC for 5 min. The amplified product of 16s rRNA was confirmed by running in 1% agarose gel in 1x TAE buffer. The amplicons were then purified using HiYield PCR Clean-up kit (RBC, Taiwan) and sequenced using automated DNA sequencer (*1st Base*, Malaysia).

Xylanase activity assay

Actinomycetes spores were inoculated in the production medium and was incubated at room temperature on a rotary shaker for 7 days. After one week the fermented broth was centrifuged at 10,000 rpm at 4°C for 15 min. The supernatant was used as crude enzyme and assayed for xylanase activity. Xylanase activity was determined by the 3, 5 dinitrosalicyclic acid (DNSA) method [10], which measures the release of reducing sugar oat spelt xylan (HIMEDIA, India) at 575nm. Reaction mixture containing 1ml 1% xylan in citrate buffer 50mM, pH 6.0 plus one ml diluted crude enzyme was incubated at 60°C for 30 min. one unit of xylanase was defined as the amount of enzyme required to release 1µ mol of xylose from xylan in 1min under the assay condition.

Effect of different carbon and nitrogen sources on xylanase production

Effect of various carbon and nitrogen sources on the xylanase production was assessed by culturing the isolates in Actinomycetes broth at room temperature (28±2°C) either of xylan, xylose, glucose, starch, wheat bran, Rice bran, various organic nitrogen and inorganic nitrogen sources including $(NH_4)_2$ SO_4, NH_4Cl, $NaNo_3$, Yeast extract, peptone and urea were added to the medium at fixed level (0.1 % w/v) to test the effect of nitrogen sources on xylanase production.

Effect of temperature and pH on partially purified xylanase activity

To evaluate the effect of different temperature on the enzyme activity the enzymes were incubated at different temperatures ranging from 20 to 90◦C in a preheated water bath. The optimum pH was determined using buffer ranging from 3.0 to 9 citrate buffer, phosphate buffer, and tris buffer were used for 3.0, 4.0-5.0, 6.0-7.0, 8.0,9.0 respectively.

RESULTS AND DISCUSSION

Fifty isolates were isolated from the east coast of India among these 10 isolates showed xylanse activity. However one isolate showed higher enzymatic activity and hence was chosen for further study. Cultures grown

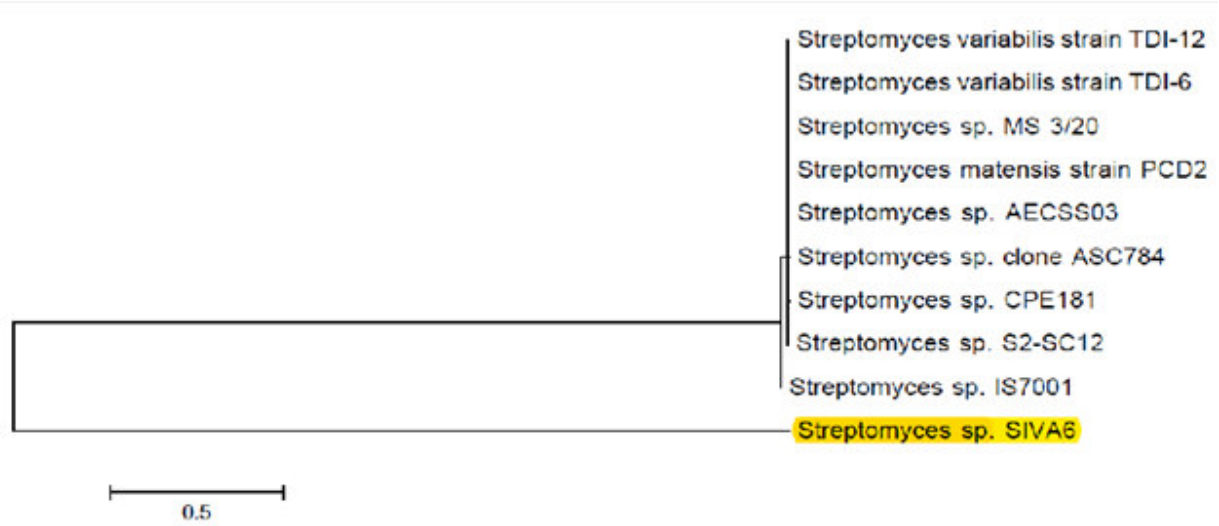
in Actinomycetes agar medium supplemented with 0.5% birch wood xylan at room temperature showed luxuriant growth pattern.

Bacterial identification by 16s rRNA gene sequence

The 16s rRNA sequence was amplified and the product was sequenced. The sequenced product was matched with the sequence available in NCBI database to identify the species. The isolate which showed highest enzyme activity was closely related to *Streptomyces sp* (Fig 1). The phylogenetic analysis of the strain isolated from the east coast of India showed 90% similarity with *Streptomyces sp.* The 16s rRNA sequence of the isolate with the highest enzyme activity were submitted to NCBI GenBank with the assigned accession number FJ775008. The phylogenetic tree was constructed using mega 6 software.

Figure 1

Phylogenetic tree based on 16s rRNA gene comparison of the bacterial strains



Neighbour joining phylogenetic tree from 16s rRNA gene sequence analysis

Effect of different carbon sources on xylanase production

Pure xylan is very expensive and hence an effective method for its production is required. Hence it is necessary to use different carbon and nitrogen source from agricultural wastes which can be used as a potential raw material for economic production of the enzyme [13]. Effect of various carbon sources on the xylanase production was assessed by culturing the isolates in actinomycetes medium containing various carbon sources (Fig 2). The results indicated xylanase production was highest when 1% wheat bran (65.68± 0.03) was added as the carbon source. Previous studies also indicate that enzymatic activity was very high when 0.5% wheat bran was used. Wheat bran contains non starch polysaccharides such as Glucan (10.5 %), Xylan (18.3 %), and Arabinan (10.1 %) so these constituents might be the reason for the high enzyme activity [12]. Many studies showed that rather than using pure xylan as substrate for xylanase production, wheat bran can be substituted as an effective carbon source. The reason for high enzyme activity when using wheat bran as a carbon source is attributed to its structural complexity[14].

Effect of different nitrogen sources on xylanase production

Nitrogen source is also required for the production of xylanase. Apart from growth, nitrogen source is required for maintaining the pH which is essential for enzyme activity [15]. The maximum xylanase activity (45.60) was evident when 0.1% urea was added (Fig 3). Also peptone supplemented media showed highest enzyme activity.

Figure 2
Effect of different carbon source on xylanase production

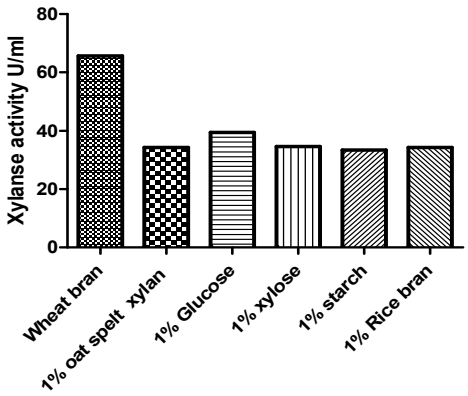
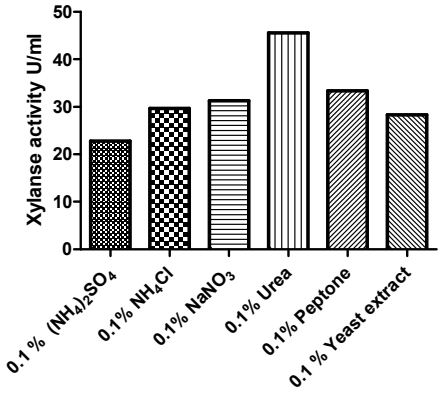


Figure 3
Effect of different nitrogen source on xylanase production



Effect of pH on xylanase activity

The stability of the enzyme in varying pH was optimized. It was found that the favorable pH for xylanase activity was in the range 6.0 and 7.0. However a decrease in enzyme activity was observed below pH 6 and also above pH 7 (fig: 4). Xylanase isolated from *B. subtilis* cultured in solid state fermentation showed maximum activity at pH 6.0 [16]. Similarly *B. licheniformis* A99 and *B. coagulans BL69* culture showed enzyme activity at pH 7.0 ([17 & 18]).

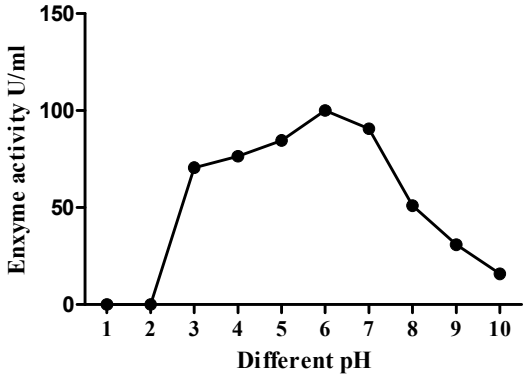


Figure 4
Effect of different pH on enzyme activity

Effect of temperature on xylanase activity and stability

The effect of temperature on the xylanase activity of isolate was shown in (fig: 5) for 30 min reaction, the optimum temperature for xylanase was 60^0C. Enzyme activity was rapidly reduced when the temperature was at 70^0C and there was negligible activity above 80^0C. When the temperature increases the enzyme activity decreases. Xylanase from *B. subtilis* was also found to be stable at 60◦C and stability decreased at 65^0C and at 90^0C there was no enzyme activity reported[19].

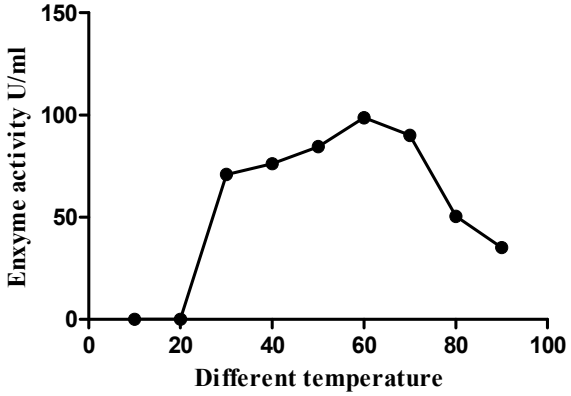


Figure 5
Effect of different temperature on enzyme activity

CONCLUSION

Among the different isolates from the marine samples *Streptomyces sp* showed maximum enzymatic activity. Further this S*treptomyces sp* was able to produce xylanase enzyme using economic means of both carbon (wheat bran) and nitrogen source (urea and peptone). The enzyme produced by S*treptomyces sp* showed their maximum enzyme activity at 50 – 60^0C and stable at pH 6.0 -7.0.

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