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Studies On Xylanase Producing Thermophilic Streptomyces sp From Compost Soil

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Abstract: Several samples of compost were taken from different localities in and around Tamilnadu, India. Forty one isolates of thermophilic actinomycetes were obtained. The morphological, cultural, and physiological properties as well as temperature relation of these isolates were carried out to classify and identify them. They were then screened for xylanolytic activity. We observed nineteen isolates out of forty one (46.3%) to produce xylanase. The most active isolate was identified as S*treptomyces* sp S R^J. The xylanase enzyme production has been carried out by submerged fermentation. Xylanase activity was detected using 3,5 dinitrosalicyclic acid (DNSA) assay method. The crude enzyme showed maximal activity at pH 7 and 55 °C. The maximum xylanase production was 925 U/ml. The aim of this study was to characterize xylanolytic thermophilic S*treptomyces* sp from compost soil.
Key words: Compost, Pulp bleaching, Thermostable enzyme, *Streptomyces*, xylanase.

Introduction

Streptomyces species are heterotrophic feeders, and they utilize both simple and complex molecules as nutrients. About three fourths of the *Streptomyces* sp produce antibiotics. In addition to antibiotics, *Streptomyces* sp liberates extracellular enzymes[3]. In this paper we investigate the screening of xylanase from thermophilic *Streptomyces* sp isolated from compost soil. Xylan is a major component of hemicellulose in plant cell walls. It is covalently and noncovalently attached to cellulose, lignin, pectin, and other polysaccharides to maintain cell wall integrity[8]. Enzymes that degrade xylan have many useful industrial applications, including the conversion of lignocellulosic material to fuels and chemicals, animal feed[5] and the processing of hemicellulose to paper[2]. During the process of pulp bleaching, for example, xylanases have been used instead of chlorine to increase the extractability of lignin for the production of high-quality paper[6]. The use of xylanase to either replace or reduce the amount of chlorine used in pulp bleaching would have a strong positive effect on the environmental impact of the process. The widespread use of xylanase for pulp bleaching, however, has been limited by the high temperature and alkaline pH of pulp-bleaching processes, since most available xylanases are not active under these conditions. Furthermore, the use of xylanase for commercial pulp bleaching requires low cost, high volume production of the enzyme. In addition, it is desirable that xylanases used for bioleaching are stable and active under alkaline conditions at high temperatures. There has been a considerable amount of research devoted

to identifying thermostable xylanases and improving the properties of wild types of xylanases to meet the requirements of the biotechnological applications.

The present study was undertaken to isolate the thermophilic xylanolytic actinomycetes from compost, to identify the most active isolate, and to determine production of the enzyme.

Materials and Methods

Isolation of Actinomycetes strains from compost

Many strains of the genus *Streptomyces* sp were isolated from compost soil samples collected from more than five areas of Tamil Nadu, India. Five grams of soil were incubated at 40 minutes for 60°C and re-suspended in 5 ml of saline. The suspension was diluted with 45ml of saline containing 1.5% phenol (w/v) and kept at 55° C in a pre-warmed water bath for 30 minutes. After filtration through sterile glass wool, the samples were serially diluted; 0.1ml of aliquots was spread on to starch-casein nitrate agar[11] containing fungicide 0.4mg/L. The plates were incubated 14 days at 55°C. The resulting colonies were transferred to starch-casein nitrate agar and ISP2 agar International streptomyces project [19].

Screening for Xylanase producing strains

Purified isolates of S R^J were cultured on oat spelt xylan agar medium[15] and incubated at 55°C for 4 days. The plates were then flooded with absolute ethanol and kept for 16 hours at room temperature to precipitate xylan. Colonies producing xylanase enzymes were surrounded with a clear zone against an opaque background of non-hydrolyzed media. The *Streptomyces* sp SR^J Showed the largest clear zone, was selected for further investigations and characterization according to the guidelines of International Streptomyces Project.

Characterization of the isolate

I. Cultural and morphological characterization

The medium used for microbiology and morphological studies was yeast extract malt extract agar (ISP2)[19]. The Morphological observation of spores was made with a light microscope at 100X magnification. Spore morphology was studied by examining gold-coated dehydrated specimens with a model with EDS-JSM 5610LV, JEOL. The type of diaminopimelic acid in the cell wall was determined by the method of Becker *et al*. Utilization of carbon sources was examined by the method of Pridham & Gottlieb[17].

II. Biochemical Tests

The media & tests used to evaluate the physiological properties of strain S R^J were Tryptone- yeast extract agar (ISP1), Yeast extract-malt extract agar (ISP2), Oatmeal agar (ISP3),Inorganic salts-starch agar(ISP4)**,**Glycerol asparagine agar (ISP5),Tyrosine agar (ISP7)**,** and tests of the International Streptomyces project (ISP) namely, optimum temperature for growth, determination of soluble pigments, formation of melanoid pigment, gelatin liquefaction, coagulation of milk, Peptonization of milk, starch hydrolysis, utilization of various carbon sources respectively as recommended previously[10,17,19]

Xylanase production and enxyme assay

The inoculum for Submerged studies was prepared using *Streptomyces* sp. The spore suspension was prepared form a freshly raised 14 day old- ISP 2 agar slant by dispersing the spores in 2ml of sterile distilled water. 2ml of this inoculum was used for inoculation.

A 350 ml conical flask containing 100 ml of the production medium (Starch 2%, glucose 1.5%, Yeast extract 0.5%, CaCo3 0.3%, MgSO4 0.25%) was inoculated. The flask was incubated at 55°C on a rotary shaker for 7 days. After suitable periods of growth time, the fermented broth was centrifuged at 10,000 rpm at 4°C for 15 min. And the clarified supernatant was used as crude enzyme was assayed for xylanase activity.

Assays for crude Xylanase were performed using 0.5% soluble oat spelt xylan in 50 mM sodium phosphate buffer, pH 7.0.The reaction mixture was composed of 1.8ml substrate and 0.2 ml crude enzyme. The

mixture was incubated in a water bath at 60°C for 15 min. The released reducing sugar was measured by the 3,5 dinitrosalicyclic acid (DNSA)method of Miller[13] , in which the reaction was stopped by adding 3ml of DNSA acid reaction. A reddish brown colour developed after placing the reaction tubes in boiling water bath for 5 min. After cooling the reaction tubes to room temperature, the O.D was measured at 575 nm against a reagent blank, where One unit of xylanase activity is defined as the amount of enzyme that releases 1 µmol of reducing sugar formed (measured as xylose) in 1mL medium in 1 min under the above-mentioned assay conditions.

Results

Actinomycete strains

A total of forty one thermophilic actinomycetes isolates were isolated from compost soil collected from various areas. They were then screened for xylanase activity. We observed that nineteen isolates produced xylanase. The most active isolate, i.e. the isolate showing the maximum zone on spelt xylan agar medium was selected for taxonomic investigations and production of enzyme.

Taxonomic characterization of the xylanase producing strain, **SR^J**

The phenotype and taxonomic characterization of strain SR^J is summarized in Tables 1 & 2 respectively. The substrate mycelia were branched and well developed. Mature aerial mycelia corresponded to the grey color series, characteristics feature for *Streptomyces* sp. Thick masses of spores with rectiflexibles spore chains were formed in ISP media No 2&3 (Fig.1). Scanning electron microscopic observations reveals the presence of oval shaped spores with smooth surface (Fig 2). Reverse mycelial pigment was not pH sensitive. Melanoid and other soluble pigments were not produced. Permissible temperature for growth ranged from 20-55°C**.** Strain S R^J was positive for liquefaction of gelatin, peptonisation of milk, and hydrolysis of starch**.** However, milk was not coagulated. Xylose, glucose, galactose, fructose, sucrose, raffinose, inositol, manitol, mannose, but not arabinose and rhamnose, were utilized individually as sole carbon sources. Analysis of whole cell hydrolysate of strain S R^J showed the presence of LL-diaminopimelic acid, indicating that the cell wall belongs to type I.

Based on the taxonomic properties described above, we consider strain S R^J to belong to the genus *Streptomyces*. On analysis of ISP descriptions of known *Streptomyces* species in the report of Shiriling & Gottileb[19]. We learned that strain S R^J was related to *Streptomyces* although it could not be identified with any of the type strains. Further taxonomic study will be undertaken in order to further classify strain SR^J, although we do suggest, based on biochemical and taxonomic tests above, that the strain be classified as *Streptomyces* sp.

Table 1: Cultural characteristics of strain S R^J

Medium	Growth	Aerial mycelium	Substrate mycelium	Reverse color	Soluble pigment
Yeast-extract malt extract agar (ISP No.2)	Good	Thick yellowish white	Dull yellow Green	Dark yellow	None
Oat meal agar (ISP No.3)	Moderate	Abundant reddish gray	Dark orange	Dark orange	None
Inorganic salt starch agar (ISP No.4)	Good	Abundant, light orangish gray	Dark purple	Dark purple	None
Glycerol asparaginine agar (ISP No.5)	Poor	Thin, White	Pale yellow	Pale yellow	None
Peptone-Yeast extract iron agar(ISP N0.6)	Poor	Scant, pale reddish yellow	Dull yellow	Dull yellow	None
Tyrosine agar(ISP No.7)	poor	Thin, White	Pale yellow	Pale yellow	None

Table 2: Taxonomic characteristics of strain SR[J]

Spore chain	Rectiflexibles
Spore surface	Smooth
Soluble pigment	Negative
Formation of melanoid pigment	Negative
Liquefaction of gelatin	Positive
Coagulation of milk	Negative
Peptonization of milk	Positive
Hydrolysis of starch	Positive
Temperature for growth(°C)	55°C
Utilization of sugars	
L- Arabinose	Negative
D- Xylose	Positive
L- Rhamnose	Positive
D-glucose	Negative
D-galactose	Positive
D-fructose	Positive
Sucrose	Positive
Raffinose	Positive
Inositol	Positive
D-mannitol	Positive
Mannose	Positive

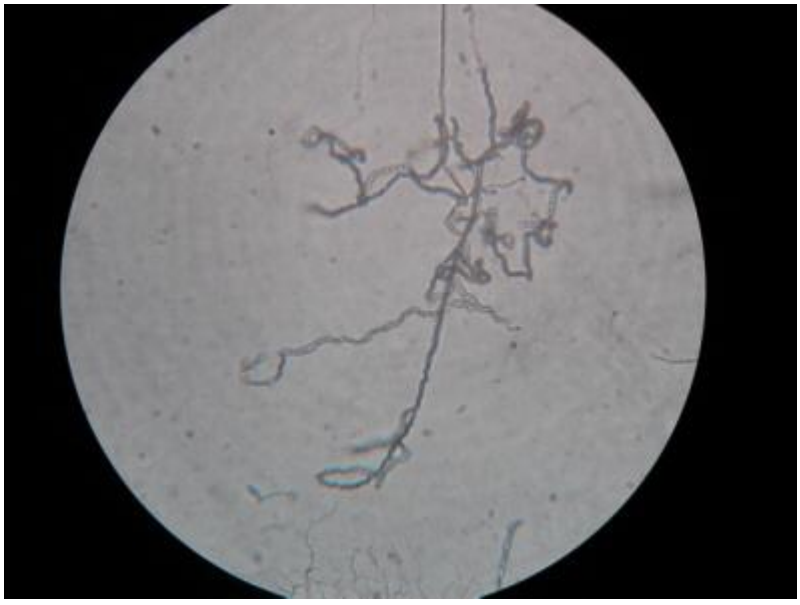

Fig 1: SR[J] showing rectiflexibles spore chain

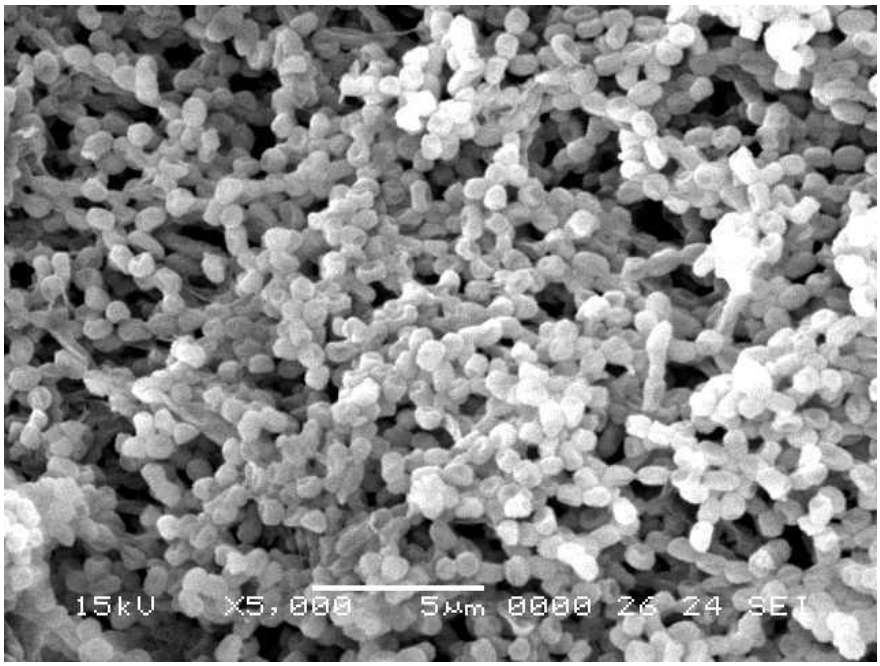

Fig :2 Scanning electron micrographs of *Streptomyces* sp. SR[J] strain grown on ISP-2 agar at 27ºC for 14 days.

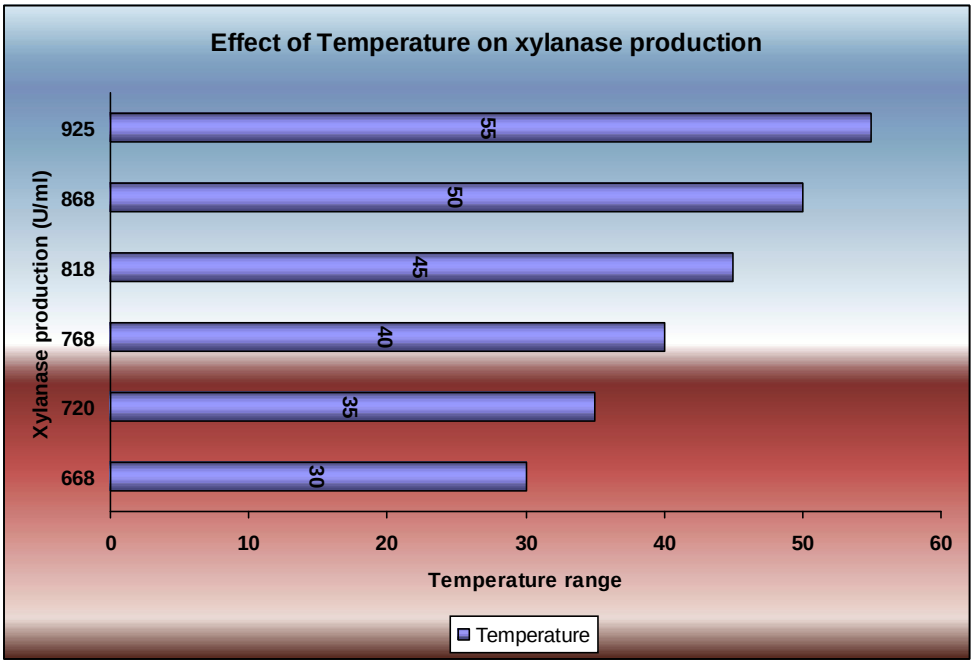

Fig 3: Effect of Temperature on Xylanase production

Xylanase production

To examine the effect of temperature on xylanase production growth of *Streptomyces* sp between 30°C and 55°C was studied. Maximum xylanase production occurred at 55°C. At 30°C, a significant decline (27.5%) in xylanase activity was evident in Fig 3. The results clearly indicated the thermophilic nature of the *Streptomyces*. The xylanase maximal production was 925U/ml (Fig 3).

Discussions

Microorganisms are rich sources of xylanase enzymes, which are produced by diverse genera and species. Members of the *Bacillus* sp, *Streptomyces* sp, *Thermoascus aurantiacus*, *Fusarium proliferatum* have been reported to produce Xylanases which are active at temperature between 50°C and 80°C[16]. Thermostable Xylanase are highly specific and thus have considerable potential for many industrial applications.

Xylan, which is the dominating component of hemicelluloses, is one of the most abundant organic substances on earth. It has a great application in the pulp and paper industry[7, 4, 21]. The wood used for the production of the pulp is treated at high temperature and basic pH, which implies that the enzymatic procedures require proteins exhibiting a high thermostability and activity in a broad pH range[9]. Treatment with xylanase at elevated temperatures disrupts the cell wall structure. According to these authors all commercially available xylanases can only partially fulfill these requirements, and the optimum temperature for the activity of most xylanases is reported to be 50–60°C with a half-life of about 1 h at 55°C [9]. However, some xylanases have been reported to exhibit higher thermal stability and optimal activity ranging from 80 to 100°C [18, 14, 20].

Since thermal stability of xylanase is a very important property due to its potential application in several industrial processes, the strain isolated by us would be a good candidate for biotechnological industry. Further work is recommended to purify and characterize the xylanase from *Streptomyces* sp., (SR^J) and study the effect of this enzyme on various carbon sources. Assessment of the properties of this enzyme in bioleaching of pulp and paper is also recommended. The industrial application of enzymes that can withstand harsh conditions has greatly increased over the past decade. From the present study it has been concluded that compost soil serve as a valuable source for such specific thermostable enzymes and to indicate potential opportunities for useful applications derived from these features. Classical mutation and (or) selection techniques, together with advanced cloning and protein engineering strategies, will be exploited to develop the product. Thus, with the availability of thermostable xylanase a number of new applications in the future are likely.

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