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Exploitation of novel Actinomycetes isolated from Wetern ghats of Tamilnadu and production of Xylanase by *Streptomyces* spp.pds1

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ABSTRACT

Western ghat is very rich in flora , fauna and microorganisms and it extent from Kerala, Karnataka and Tamilnadu. In the present totally 22 different soil actinomycets were isolated using Starch Casein Nitrate Agar plate method. After morphological identification one starain was identified by 16sRNA typing and identified as *Streptomyces* spp. pds1. And submitted to NCBI. The strain was used to produce Xylanase in production medium. The Xylanase activity was maximum on 96th hr of incubation (470U/mL). The optimum pH(474 Um/L) and temperature were7.5 (468U/mL) and 40oC respectively. Further the Xylanase was purified by 70% acetone followed by dialysis and DEAE - Sephadex - 100 . The molecular weight was determined using SDS - PAGE and was found to be 60kDa.

KEYWORDS: Western Ghats, *Streptomyces*, 16sRNA, Xylanase , SDS -PAGE

INTRODUCTION

Actinomycetes are representative of terrestrial microorganisms and are usually isolated from soils and marine water. Actinomycetes are the most economically and important valuable prokaryotes able to produce wide range of bioactive compounds and enzymes (**1**) . Xylan is the second largest recyclable natural resource after the cellulose made up of 15-35% of dry weight of plant cell. The enzyme based degradation of xylan received more attention by the researchers recently (**2**) . Many of the microbes produce Xylanase and mostly produced by bacteria . Among bacteria Actinomycetes strains secreting high activity of Xylanase. (**3 & 4**). Nowadays , the interest in xylanases has increased due to many potential applications (**5**). However only few reports have been reported on the xylanases of actinomycetes. The objective of the present work is to produce Xylanase from *Streptomyces* spp. pds 1 isolated form western ghats and then focusing on the optimization of enzyme production.

MATERIALS AND METHODS

Microorganism

Soil samples were collected from various locations in the western ghats and processed as described by (**6**). The already identified strain *Streptomyces* spp. pds1 (NCBI Accession Number: **HM598125 238)** was used for the xylanase production.

Xylanase production

One mL of heavy spore suspensions of the studied microorganisms was used to inoculate Erlenmeyer flasks of 250 mL capacity, each containing 50 mL of the following ingredients (g/L): yeast extract, 6; NaHPO4, 5.03; KH2PO4, 1.98; NaCl, 0.2; MgSO4.7H2O, 0.2; CaCl2.2H2O, 0.05; trace element solution-1ml (g/L - FeSO4.7H2O, 1.0; MnSO4.7H2O, 0.2; ZnSO4.7H2O, 0.9) and oat spent xylan 10.0, pH - 7.2. The inoculated flasks and uninoculated control were incubated at 30ºC for 7 days at 120 rpm. At the end of the fermentation period, the content of each flask was centrifuged at 10000 rpm for 5 min at 4ºC. The supernatant was used as source of enzyme and analyzed for enzyme activity.

Xylanase Assay

The xylanase activity of the crude enzyme was determined by the method of (**7** Bailey et al., 1992). The substrate solution contained 1% oat spelts xylan, solubilised in sodium phosphate buffer (pH 8). The reaction mixture consisted of 1.8 ml of substrate solution and 0.2 ml properly diluted enzyme. After 5 minute of incubation at 50ºC, the liberating reducing sugars (Xylose equivalent) were estimated by the dinitrosalysilic acid method (**8**). The reaction mixture was filtered, from that 1 ml was taken and to that 1 ml of DNS reagent was added and boiled for 5 minutes at 50ºC, then diluted with distilled water to avoid color transmission and was read at 540 nm. The blank was also prepared adding distilled water in place of enzyme. The xylanase assay procedure was followed for all the crudes of fermented agro-residues by both bacteria and fungi and the OD values were tabulated using xylose as standard.

Effect of optimum day, pH and temperature on Xylanase production.

The optimum day was (1 to 7days) pH (4.0 to 8.0) and temperature (30 to 45 0C) were studied .

Enzyme purification

After the fermentation the medium was centrifuged at 40C

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and the clear supernatant was collected and used for purification studies. Prechilled acetone (70%v/v) was added to the crude enzyme extract , stored overnight at 40C. The precipitate was collected and dialyzed against the same buffer and loaded on the column (2.5 X14cm) of DEAE-Sephadex-50 and the various fractions were collected (already equilibrated with the buffer).

Determination of Molecular weight (9)

The purified Xylanase was examined by using SDS- PAGE to determine the molecular weight.

RESULTS AND DISCUSSION

In the present investigation out of22 isolated one isolate exhibited maximum activity and identified as *Streptomyces* spp. pds1 .

Growth of Streptomyces spp. psd1 on SCA medium

Table1. Colony Morphology on SCA Medium.

Test	CT1 (pds1)	ACT2	ACT3	ACT4
Gram Stain	.+	. +	. +	. +
Colony pigmentation	Red	White	Gray	Gray
Aerial Mycelium	White	White	White	White
Spores	Fine oval	No	Rough	No

Xylanase production

The optimum day or the enzyme production was observed o 96th hrs. of incubation with enzyme activity of 470U/mL. Optimum pH was determined as 7.5 (474U/mL). The optimum temperature was 400C (468U/mL). (Fig : 1, 2 &3)

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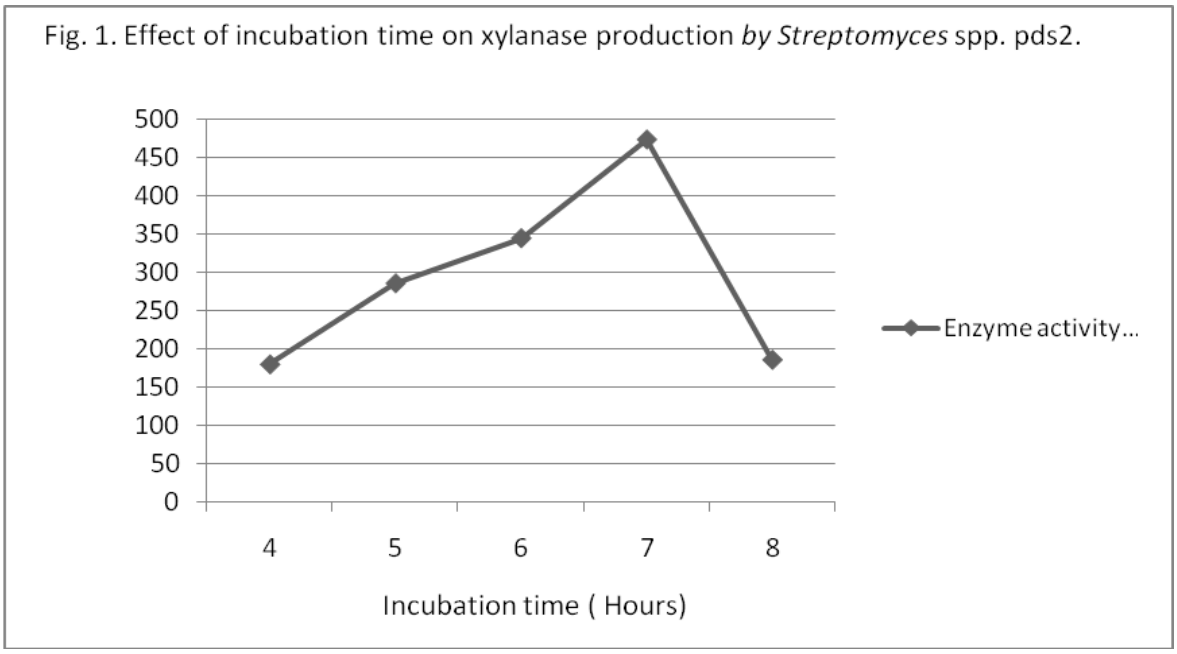


Fig 2: Effect of pH on Xylanase activity

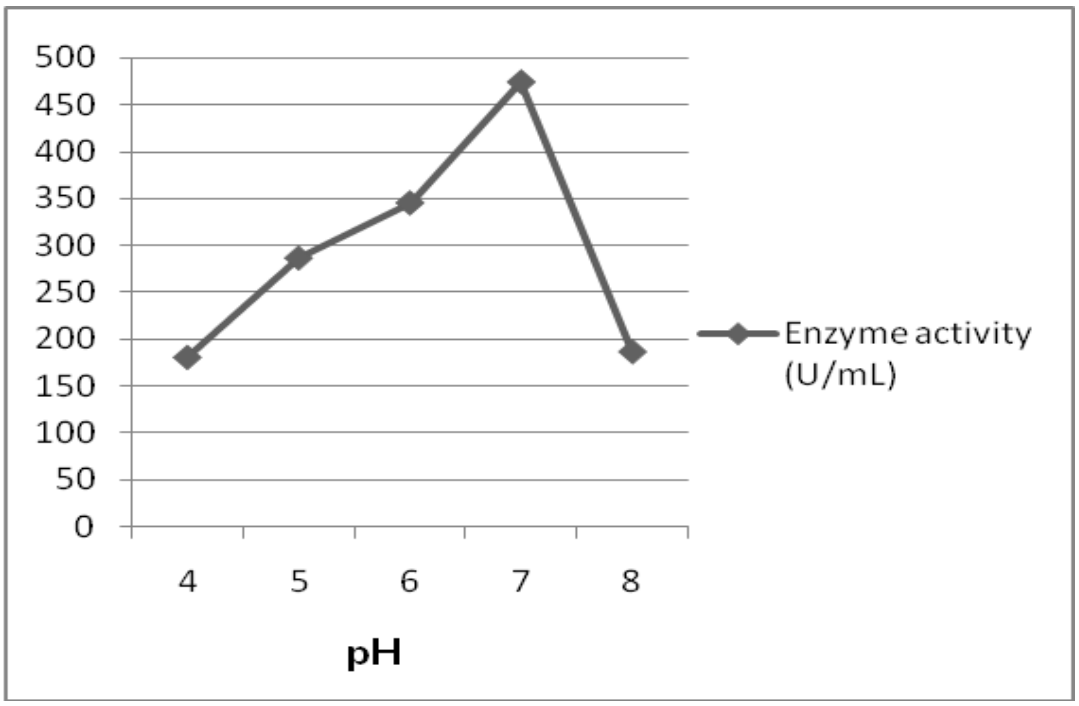
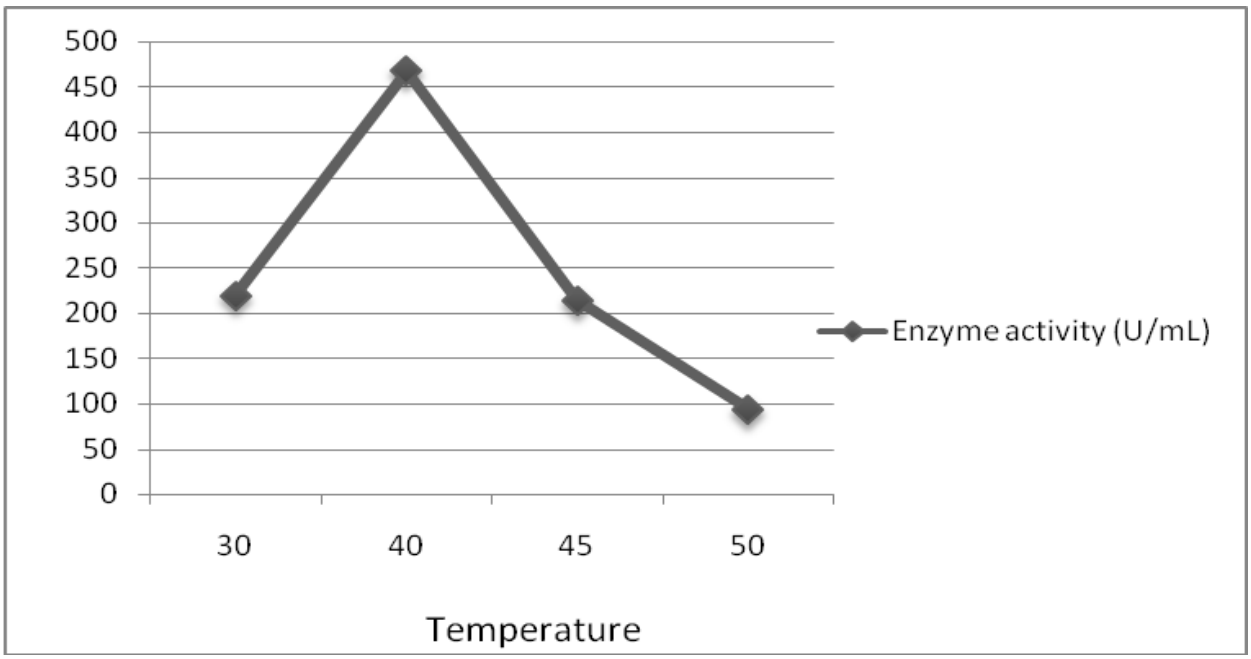


Fig 3: Effect of temperature of Xylanase activity



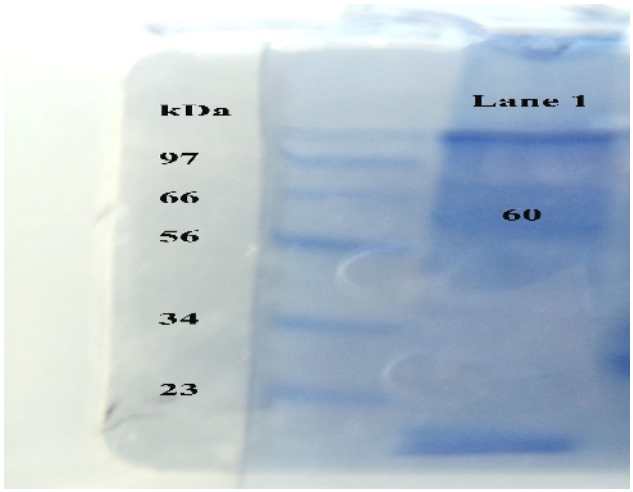
Purification of Enzyme

The Xylanase was partially purified by 70% acetone (v/v) and subjected to dialysis and gel filtration. The results were summarized on table 2.

Table 2. Purification of amylase

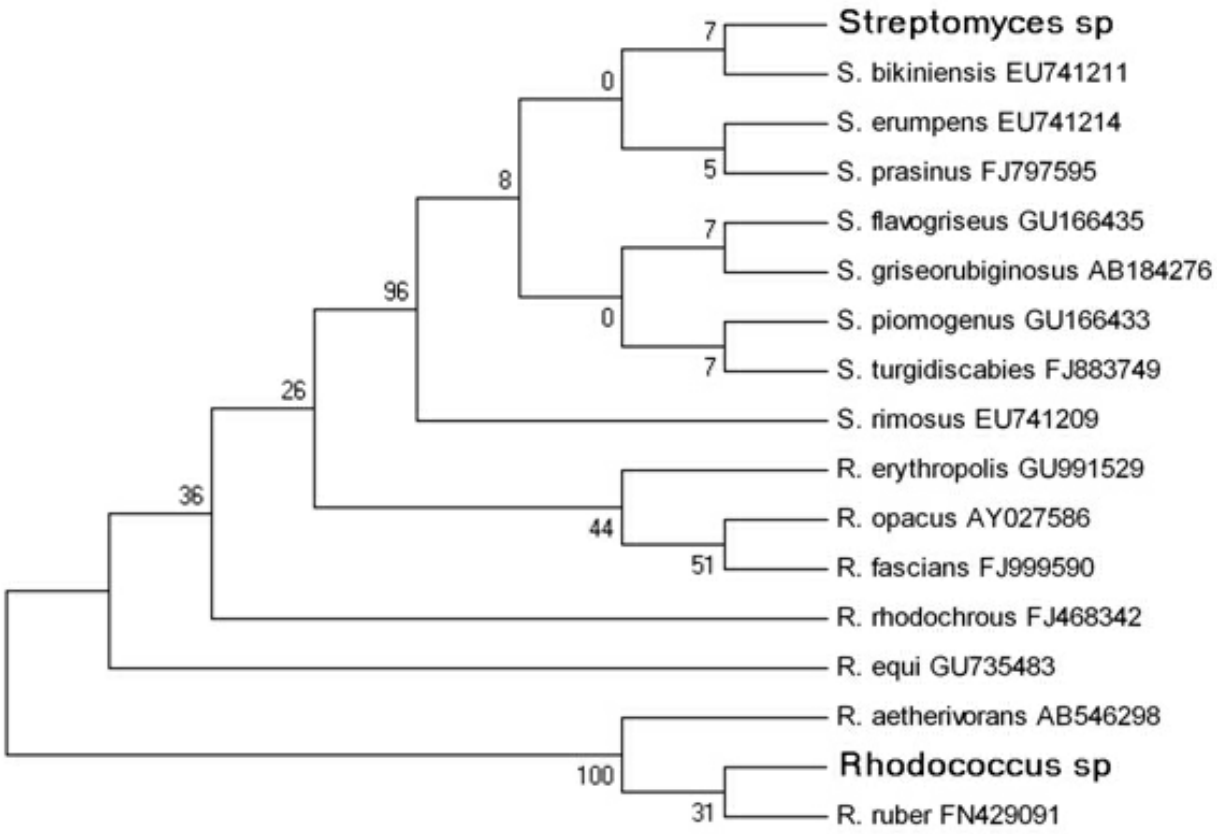
Steps	Volume (ml)	Protein (mg)	Activity (mmol./min)	Recovery (%)	Specific activity (mmol /min/mg)	Purification (fold)
Culture	20	196	560	100	2·86	1
Acetone ppt.	4	106	426	76	4·01	1·40
DEAE – Sephadex – 50.	6	19	136	22·14	7·16	56·00

Fig. 4. SDS- PAGE The molecular weight was determined using SDS-and it was found to be 60kDa.



Evolutionary relationships of 17 taxa

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.07480987 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [2]. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Non coding. All positions containing gaps and missing data were eliminated from the data set (Complete deletion option). There were a total of 235 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [4].



DISCUSSION

Actinomycetes are known to be rich source of novel compounds. Actinomycetes account of the 70% of the earth's surface and represent attractive source for isolation of novel microorganisms and production of potent bioactive metabolities (**10**). In the present study a novel actinomycete *Stroptomyces* spp. pds1 was used for the production and characterization of xylanase production. It was reported that soil microorganisms are rich in novel compounds to date about 100 products were derived from soil microbes (**11).** Identification of actinomycetes through molecular typing especially through PCR plays more dominant role than culture method .Recently (**12, 13**) used the 16sRNA studies for the identification of actinomycetes .

Xylanases have potential applications in food, feed, chemical, pharmaceutical and paper industries. These enzymes produced by several bacterial and fungal species. In the present study *Streptomyces* spp. pds1 was used to produce the xylanase and it was found that the maximum production was obtained on 96^{th} hrs. of incubation which is identical with the earlier reports (**14).** (**15**)Li et al (2012) isolated 102 xylanase producing actinomycetes strain and found that strain L2001 has produced 815U/mL on 4^{th} day of incubation.

Usually xylanases exhibits greatest activity in the pH range of 5 to 8 (5). In the present study we found that the optimum pH of 7.5 (Fig. 2) (474U/mL) which is agree with the results of (15, 16).From the fig3 it was observed that the optimum temperature of 40^0C. which is similar to the past finding. According to (**16**) the optimum temperature is 400C (200 U/mL). (**17, 18**) who obtained the optimum temperature of 45^0C . In the present study enzyme is partially purified and subjected to SDS – PAGE to identify the molecular weight of the xylanase . (**19**) reported the molecular weight of 60kDa. In our study also it was reported the same (60kDa) Fig. 4.

CONCLUSION

In the present study a novel Actinomycete *Streptomyces* spp.. pds1 was used for the production of xylanase . The optimum day, pH and temperature were found to be 96^{th} hrs; 7.5 and 40^0 C respectively. The enzyme was purified and the molecular weight was calculated and found to be 60kDa.

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