Research Article GU J Sci 38(2): 560-574 (2025) DOI: 10.35378/gujs.1489525

Gazi University

Journal of Science

http://dergipark.gov.tr/gujs

Isolation and Molecular Identification of *Streptomyces tendae* and its Xylanase Production Using Cost-Effective Agro-Residues

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Highlights

- The SHB_02 isolate has been identified as *Streptomyces tendae*.
- Wheat straw (3%) represents an attractive agricultural substrate.
- Maximum activity of xylanase, equivalent to 9.4 U/ml.
- After optimising fermentation parameters, xylanase yield was increased by 74%.
- The xylanase production by *Streptomyces tendae* was reported for the first time.

Article Info

Received: 24 May 2024
Accepted: 10 Dec 2024

Keywords

Agro-residues
Fermentation
Production
Streptomyces tendae
Xylanase activity

Abstract

Due to their biotechnological importance in different industrial fields, xylanases from microorganisms have been the focus of great attention. In this regard, bacterial strain, SHB_02, one of the best xylanase producer isolated during a screening study from soil, was identified as *Streptomyces tendae* (GenBank accession no. OP893637) based on phenotypic characters and 16S rRNA gene sequencing. Experiments were carried out with three different agro-residues, such as corncob, wheat bran and wheat straw, for xylanase production by fermentation. The optimum parameters for producing the xylanase from *Streptomyces tendae* were temperature 30 °C, pH 7.5, incubation period 72 h, inoculum concentration 2%, and shaking speed 140 rpm. Among the agricultural by-products, wheat straw, 3% (w/v), has the highest xylanase as the sole carbon source. On the other hand, the best nitrogen source was determined to be yeast extract (0.25%). The xylanase was stable at pH 8 and 50 °C for at least two h. These findings suggest that a novel strain of *Streptomyces tendae* may produce xylanase that can be obtained inexpensively by fermentation using agro-residues.

1. INTRODUCTION

Microbial enzymes are widely used in many industries, such as cheese and bread making, pharmaceutical production, detergent additives, alcoholic beverages, fruit juice, leather, and textiles [1, 2]. Among microbial enzymes, xylanases have recently become of great interest due to their versatile potential applications. The enzymes called xylanases are β-1.4-endoxylanases and β-xylosidases. Other xylanolytic enzymes required for the degradation of side branches are considered hemicellulolytic enzymes [3, 4]. Xylanases are β-1.4-endoxylanases which hydrolysed the β-1.4 glycosidic bonds between xylan monomers in the xylan backbone [4–6].

Xylanases can be used in many industrial areas such as bakery, animal feed, fruit and vegetable processing, wine and beer making, xylitol production, starch hydrolyses [7], and textiles [8], especially in the paper industry. They have found use in removing residual lignin from the sulfite-dissolving pulp [9] and in cellulose purification to produce artificial silk [4, 5]. The use of xylanases is not only limited to the paper industry but also has special importance in the fields of transformation of lignocellulosic substrates, decomposition of agro-industrial waste into essential products, improvement of the beer consistency and improvement of the digestion of animal foods [4]. In broiler farming, xylanase applications to rye-based feeds reduced intestinal viscosity, thus providing effective feed utilisation and weight gain. The

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effectiveness of xylanases was also observed in improving bread quality by increasing bread volume. This situation increased even more with the use of amylases and xylanases [10, 11]. One of the most promising applications of xylanases is the reduction of chlorine used as a whitening agent in pulp preparation and their ability to separate lignin from pulp. Enzyme application increases pulp fibrillation and water retention and provides selective removal of xylan from the dissolved pulp. Some xylanases also soften the cell wall to produce protoplasts from plant cells [12].

Many living groups, such as bacteria, yeasts, fungi, algae, protozoa, arthropods, and gastropods, can produce the majority of known xylanases. These organisms that produce xylanase have characteristics ranging from thermophilic and alkalophilic to acidophilic [4, 7, 13]. Many bacteria and fungi can break down xylan, cellulose, and hemicellulose. In general, *Bacillus subtilis*, *B. circulans*, *B. pumilus*, *Geobacillus galactosidasius* and *B. polymyxa* break down xylan aerobically or facultatively anaerobically [2, 14, 15]. Some well-known xylanase producers are *Aspergillus kawachii*, *Thermomyces lanuginosus*, *Aspergillus niger*, *Trichoderma harzianum*, *Pediococcus acidilactici* and *Trichoderma reesei* [7, 10]. In addition to their ecological importance, such as biodegradation, actinobacteria produce industrially valuable metabolites such as antibiotics and enzymes [14, 16, 17]. Among actinobacteria, members of the *Streptomyces* are the most isolated in soil and the most studied because they produce many essential metabolites such as antibiotics, immunosuppressants, hydrolytic enzymes (proteases, xylanases, amylases, and pectinases etc.), anticancer drugs and other active substances [3, 18-21].

The current study aimed to report the isolation and identification of the strain SHB_02, which showed the highest xylanase activity on cheap agro-residues as a carbon source by submerged fermentation (SmF) processes. It also aimed to investigate some characteristics of xylanase, first reported from *Streptomyces tendae*.

2. MATERIALS AND METHODS

2.1. Isolation and Identification of SHB_02

The soil sample was collected near Spil Mountain, Manisa Province, Turkiye (38°34'08.0" N-27°27'18.5" E). The SHB_02 was isolated by soil dilution method using yeast malt extract agar (ISP 2) and added nystatin (50 µg/ml) to prevent fungal contamination [18, 19]. Spore suspensions containing pure culture were stored in 15% glycerol deposited at –80 °C.

The SHB_02 selected due to its high xylanase activity was identified. For this purpose, the morphological, biochemical and physiological characteristics were compared based on the literature [22-25]. Species identification of SHB_02 was performed by partially sequencing 16S rRNA genes using the oligonucleotides primers 27F: AGAGTTTGATCCTGGCTCAG and 1525R: AAGGAGGTGWTCCARCC [26]. The DNA was isolated using the commercial genomic DNA Purification Kit (Promega, A1120) according to the conditions specified by the Promega Company. Polymerase chain reaction (PCR) mixture (50 µl) was prepared, composed of 1 µl dNTPs (10 mM from each), 4 µl $MgCl_2$ (25 mM), 1.5 µl forward primer (20 µM), 1.5 µl reverse primer (20 µM), 0.40 µl (5 U) Ultra-Pure *Taq* DNA polymerase (Geneaid, UT051), 0.5 µl template DNA (about 50 ng), 5 µl 10× PCR buffer with KCI and 36.10 µl PCR grade water. The sample was placed in an Applied Biosystems Veriti Thermal Cycler. PCR protocol was carried out by initial DNA denaturing at 96 °C for 2 min, and then 36 cycles (denaturation at 96 °C for 45 sec, annealing at 51 °C for 30 sec, and extension at 72 °C for 2 min) and a final extension at 72 °C for 10 min. The PCR product and also with a molecular size marker (100 bp, Geneaid DL007) were subjected to electrophoresis in 0.8% agarose gel with TBE buffer, stained with DNA dye (Safe DNA Gel Stain, Invitrogen) and examined under UV trans-illuminator (gLite gel scanner). The resulting single pure PCR amplicon was sent to GATC Company (Germany) for sequencing. The raw sequences were edited and assembled using the BioEdit program (V.7). The 16S rRNA reference gene sequences for related *Streptomyces* were collected from the GenBank database (NCBI) and aligned with the SHB_02 sequence using the MEGA XI (V, 11.0.13) program [27]. The phylogenetic tree was created using neighbour-joining [28] and Jukes-Cantor algorithms [29] with a bootstrap test (1000 replicates) [30].

2.2. Agricultural By-Products and Substrate Preparation

The agricultural by-products, namely corncob, wheat straw, and wheat bran, analyzed for xylanase production, were collected locally. Pre-treatment of the waste was carried out as stated in the literature [31-33]. The treated agro-residues were added at a volume of 1-5% (w/v) into the production medium as a carbon source. The best concentration of agricultural wastes was determined when optimum xylanase production was achieved.

2.3. Optimization of Fermentation Parameters for Xylanase Production

Fermentation of SHB_02 was optimised using the traditional method in SmF. The isolate was streaked onto ISP2 medium for the complete sporulation and incubated at 30 ºC for 14 days. The inoculum was then prepared by adding a loopful of the active isolate to 250 ml vials containing 50 ml of medium [(g/l) Glycerol 10, yeast extract 1, $MgSO_4.7H_2O$ 0.5, K_2HPO_4 1 and NaCl 1] incubated at 150 rpm at 30 °C for 48 h. 2 ml inoculum containing 1.0 x 10^6 spores/ml of this culture was added into a 50 ml fermentation broth and SmF was carried out in xylanase production medium that consisted of nitrogen sources (g/l) 0.25, $MgSO_4.7H_2O$ 0.5, K_2HPO_4 1 and NaCl 1, and birchwood xylan (1%) or agricultural wastes as carbon sources (1-5%, w/v). The fermentation was started at 30 ºC at 150 rpm and continued for 120 h. The effect of various cultural parameters such as initial pH values (7–10), temperatures (25–45°C), aeration rate (100-160 rpm), inoculum concentration and nitrogen sources (ammonium sulphate, asparagine, potassium nitrate, peptone, tryptone and yeast extract) were tested for xylanase production. The culture broth was centrifuged at 12000 rpm for 20 min at 4 ºC and the supernatant was used for enzyme activities by the method of dinitrosalicylic acid (DNS) [34]. The pH, temperature, inoculum concentration, fermentation time, agitation rate, carbon, and nitrogen source supporting the optimum production of xylanase were used for experiments.

2.4. Assay of Xylanase Activity

The modified DNS method [34] was used for xylanase activity. Shortly, the xylanase solution (200 µl) for xylanase activity was added to 500 µl of 1% Birchwood xylan (BW-X) as a substrate in 50 mM Tris-Cl buffer (pH 8.0) and incubated at 50 ºC for 10 min. The reaction was terminated using DNS reagent (1300 µl) and then kept at 100 °C for 10 min for colour development. After cooling, the absorbance at 540 nm was measured using a UV-visible spectrophotometer. One unit (U) of xylanase was defined as the enzyme producing one µmol of xylose per minute under the assay conditions. The reducing sugar reference (*D*-Xylose) prepared the standard curve.

2.5. Enzymatic Characteristics

The optimum temperature of xylanase activity was investigated by incubating the enzyme at a temperature ranging from 20 to 90 °C at pH 8. The effect of pH (4-10) on xylanase activity at 50 °C was measured with standard assay conditions, and residual enzyme activity was measured. The thermal stability of the xylanase was assayed by first incubating the enzyme at 50 ºC for intervals of 60-180 min at pH 8, and the residual enzyme activity was determined. The effect of different chemicals on the xylanase activity was investigated by incubating the enzymes in the presence of metal ions ($CaCl_2$, $CuCl_2$, $ZnCl_2$, $MgCl_2$, $CoCl_2$ and $FeSO_4.7H_2O$) at a final concentration of 1 mM and Tween 80, Triton x-100, Sodium dodecyl sulphate (SDS) and Ethylenediaminetetraacetic acid (EDTA) (0.1% w/v). The residual activity was recorded under the optimized conditions and compared with control without any additive.

2.6. Statistical Analysis

All the experimental data represented the average of triplicate measurements and were expressed as mean ± standard deviation of the mean (SD). A one-way analysis of variance (ANOVA) was performed, and comparisons of means were made using the Dunnett Method, with 95% confidence using the program (Minitab® 19).

3. RESULTS

3.1. Identification of Isolate SHB_02

Many biochemical characteristics of isolate SHB_02 were determined to identify the bacterium. SHB_02 is an aerobic, mesophilic, Gram-positive bacterium with widely branched aerial mycelia and grows readily on most of the nutrient media. The colour of the colony is generally grey and whitish, wrinkled on ISP2 agar, revealing the presence of the aerial and vegetative hyphae (Figure 1).

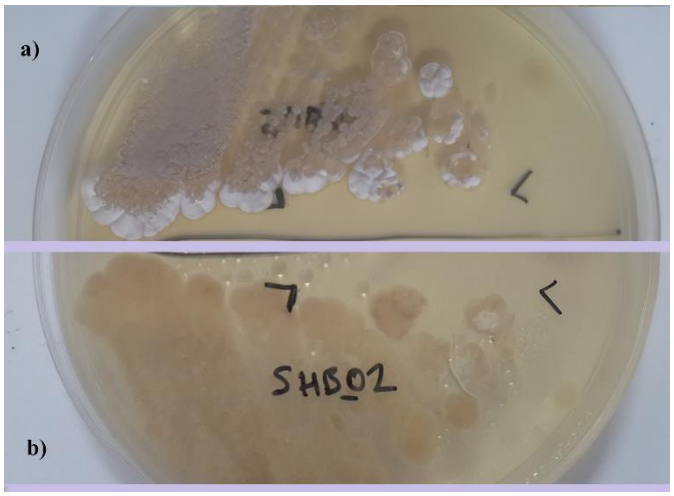

Figure 1. *Colony morphology of SHB_02 on ISP 2 medium after 14 days incubation. a) Aerial mycelium colour, b) Reverse colour*

The formation of soluble pigments was not observed in tyrosine agar and peptone yeast iron agar after 14 days. The isolate does not produce melanin on both ISP 6 and ISP 7. SHB_02 did not utilise D-melesitose, adonitol, dextran and xylitol as carbon sources. Other nitrogen sources were tested and used for growth except for the L-arginine and L-hydroxyproline. It degrades xanthine and arbutin, reduces nitrates and produces H_2S. Although the growth temperature ranged from 15 to 37 °C, the optimal temperature was 30 °C, but there was no growth at 4 and 45 °C. It grew at different concentrations of NaCI (3-10%). Other biochemical characteristics of SHB_02 are summarized in Table 1.

Table 1. *Some of the physiological and biochemical characteristics of SHB_02*

Characteristics	SHB_02	Characteristics	SHB_02
Aerial mycelium	+	**Growth with (%, w/v)**	
Spore morphology[a]		NaCI (3)	+
Rectinaculiaperti	V	NaCI (5)	+
Rectiflexibiles	-	NaCI (7)	+
Spirals	V	NaCI (10)	+
Verticillate	-	Sodium azide (0.01)	-
Spore colour		Phenol (0.01)	+
Red	-	Potassium telluride (0.001)	-
Grey	+	**Growth pH range**	6-8
Melanin production on		**Utilization of nitrogen sources:**	
Peptone yeast iron agar	-	L-Asparagine	+
Tyrosine agar	-	L-Arginine	-
Antibiosis against to		L-Cysteine	+
B. subtilis	-	L-Valine	+
A. niger	-	L-Phenylalanine	+
Enzyme activity		L-Histidine	+
Lecithinase[b]	-	L-Hydroxyproline	-
Lipolysis[b]	-	**Utilization of carbon sources:**	

Nitrate reduction	+	D-Glucose	+
H_2S production	+	Arabinose	+
Degradation ability		Xylose	+
Hippurate	-	Sucrose	V
Elastin	+	*Meso*-Inositol	+
Xanthine	+	Mannitol	+
Arbutin	+	D-Fructose	+
Resistance to		L-Rhamnose	+
Neomycin (30 µg/mL)	S	Raffinose	V
Rifampicin (5 µg/mL)	R	D-Melesitose	-
Penicillin G (10 IU)	S	Adonitol	-
Growth at (ºC)		D-Mellibiose	+
45	-	Dextran	-
37	+	Xylitol	-
15	+		
4	-		

[b]*Result on ISP 2 medium after 14 days incubation,* [b]*egg-yolk medium results, IU; international units, +; positive, -; negative, V; variable, S; sensitive, R; resistant.*

According to the results of the nearly complete 16S rRNA sequence (1.482 bp) BLAST analysis and the neighbour-joining phylogenetic tree shown in Figure 2, the SHB_02 belongs to the genus *Streptomyces*, showed high similarity to the *Streptomyces tendae* (99.78%). The DNA sequence of the *Streptomyces tendae* strain SHB_02 was obtained and submitted to GenBank, and accession number OP893637 was assigned.

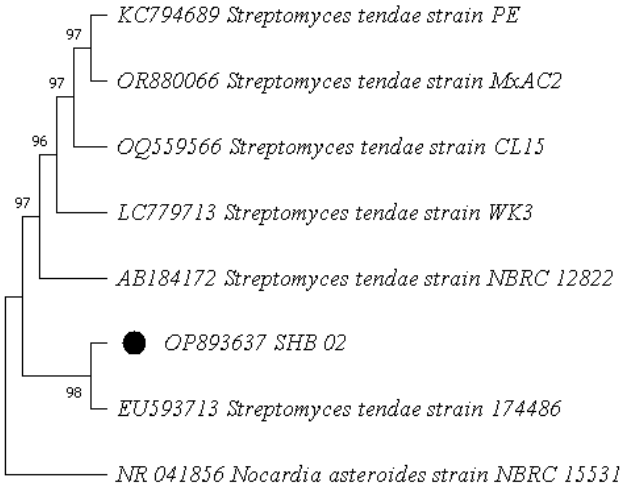

Figure 2. *Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence of S. tendae SHB_02*

3.2. Influence of Different Fermentation Parameters on Xylanase Production

Streptomyces tendae SHB_02, which produced high amounts of xylanase enzyme in screening studies (Figure 3), was therefore selected for further investigation. This strain was optimized for xylanase production by changing different nutritional and environmental conditions using the SmF process.

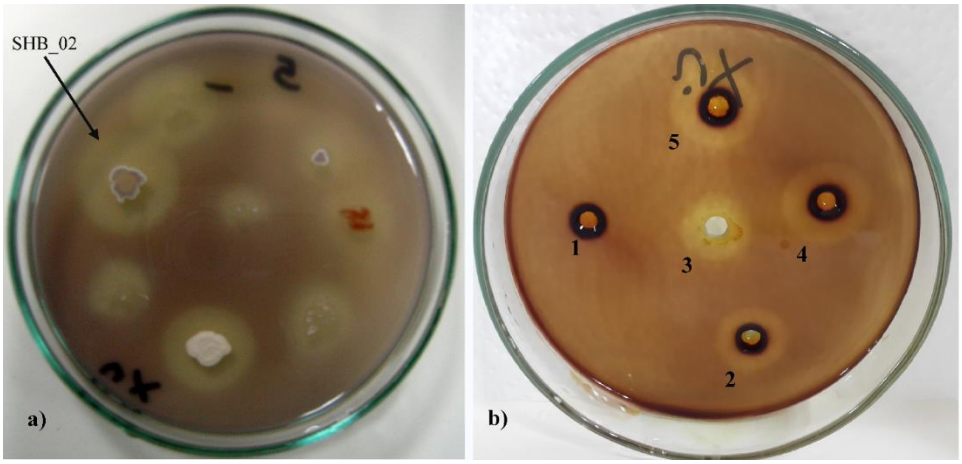

Figure 3. *Xylanase activity of S. tendae SHB_02 a) On BW-X agar medium, b) Activity of enzyme extract obtained from the wheat straw medium. The plates were treated with an iodide solution. Well diameter 5 mm, transferred volume 1) Free of enzyme extract (negative control), 2) 20 µl, 3) 30 µl, 4) 40 µl and 5) 50 µl/well*

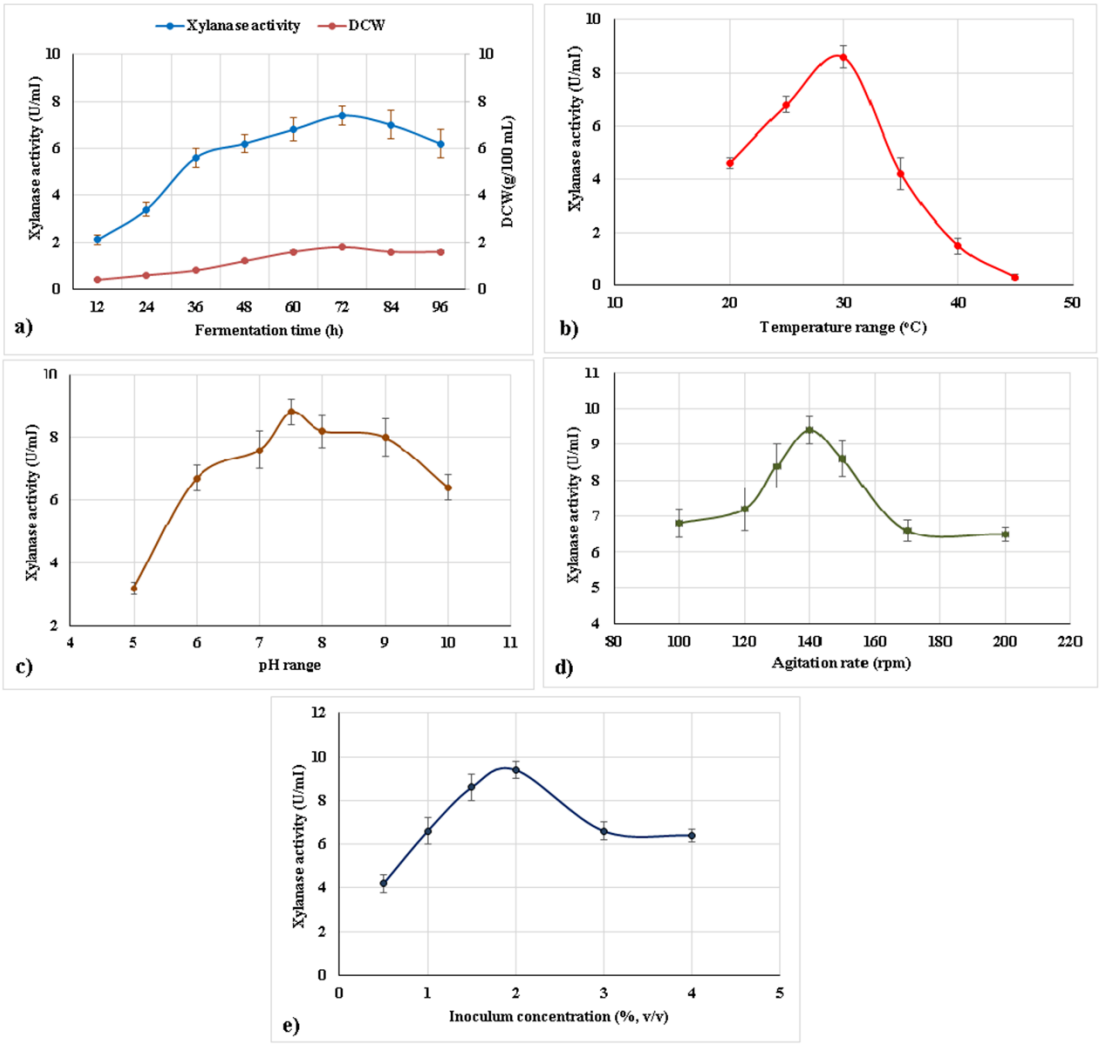

Figure 4. *Time course profile of various factors affecting the growth and production of xylanase before optimization of the production of SmF process. a) Fermentation time, b) Temperature range, c) pH range, d) Agitation rate and e) Inoculum concentration. DCW; Dry cell weight, n = 3 ± SD*

The results of the influence of incubation time, temperature, pH, aeration rate and inoculum dosage on xylanase production are shown in Figure 4. As indicated in Figure 4a, xylanase production of *S. tendae* SHB_02 showed a lower xylanase activity in the early stages and increased its activity continuously to a high level of 8.4 U/ml at 72 h of fermentation. The maximum cell growth yield of the strain (DCW) was 1.8 g/100 ml in a medium containing BW-X, obtained after 72 h at 30 °C. The influence of pH on xylanase production revealed that the optimum pH was 7.5, with an activity of 8.8 U/ml. The enzyme activity decreased at temperatures above 30 ºC and a pH above 9. For the optimum xylanase activity (9.4 U/ml), the fermentation with agitation rate and inoculum concentration was 140 rpm and 2%, respectively. By optimising five different parameters with pre-fermentation experiments, xylanase activity increased from 8.4 to 9.4 U/ml (Figure 4).

3.3. Effect of Agro-Residues and Nitrogen Sources on Xylanase Production

Under the optimised fermentation conditions, the effect of several lignocellulosic raw substrates as sole carbon sources on xylanase production was determined using BW-X as a positive control. The agro-residues at 1-5% ratio were used in the fermentation process. The best agro-residue for maximum xylanase production was found as wheat straw at the concentration of 3% (w/v). The highest (9.8 U/ml) of xylanase was produced on commercial BW-X following wheat straw, whereas the activity obtained with corncob (5.4 U/ml) was the lowest amount (Figure 5).

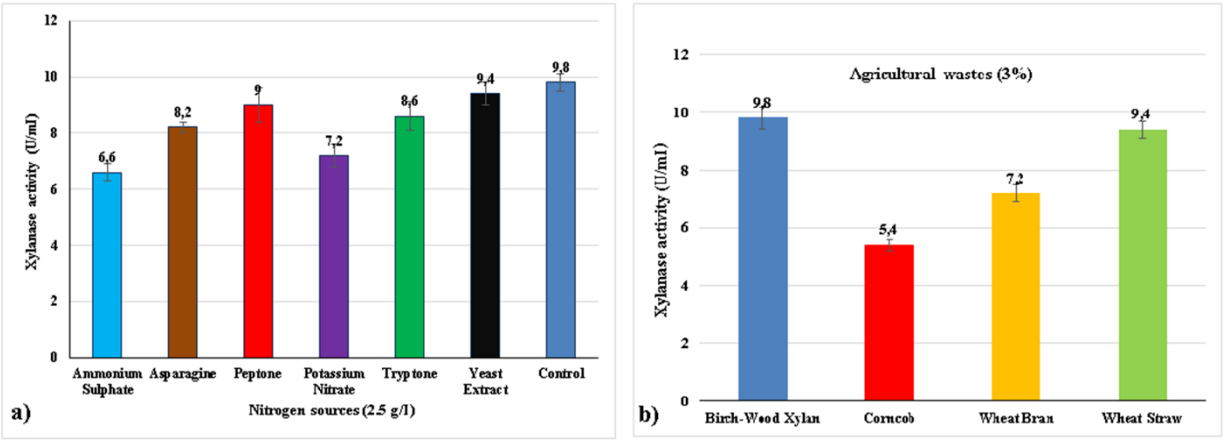

Figure 5. *Influence of nitrogen sources (a) and agricultural wastes (b) on xylanase production by S. tendae SHB_02. Culture conditions: Inoculum concentration 2%; temperature 30 ºC; initial pH 7.5; agitation rate 140 rpm; and incubation time 72 h; other components of production medium composition are given in the materials and methods section. Mean values, n = 3 ± SD*

According to the results obtained from all preliminary experiments, the production medium defined by optimizing the effects of fermentation conditions, carbon, nitrogen and other ingredients on xylanase production was as follows: inoculum concentration 2% (v/v), temperature 30 ºC, pH 7.5, aeration rate 140 rpm, incubation time 72 hours, the best nitrogen source is yeast extract 2.5 (g/l), the best carbon source is wheat straw 3% (w/v), $MgSO_4.7H_2O$ 0.5, K_2HPO_4 1 and NaCl 1, g/l. The enzyme activity in the non-optimized production medium with wheat straw was 5.4 U/ml, but after optimization, it reached 9.4 U/ml and an increase of approximately 74% was determined (Figure 6). In the optimized commercial BW-X medium, xylanase production increased from 7.6 to 9.8 U/ml and showed an increase of 29%.

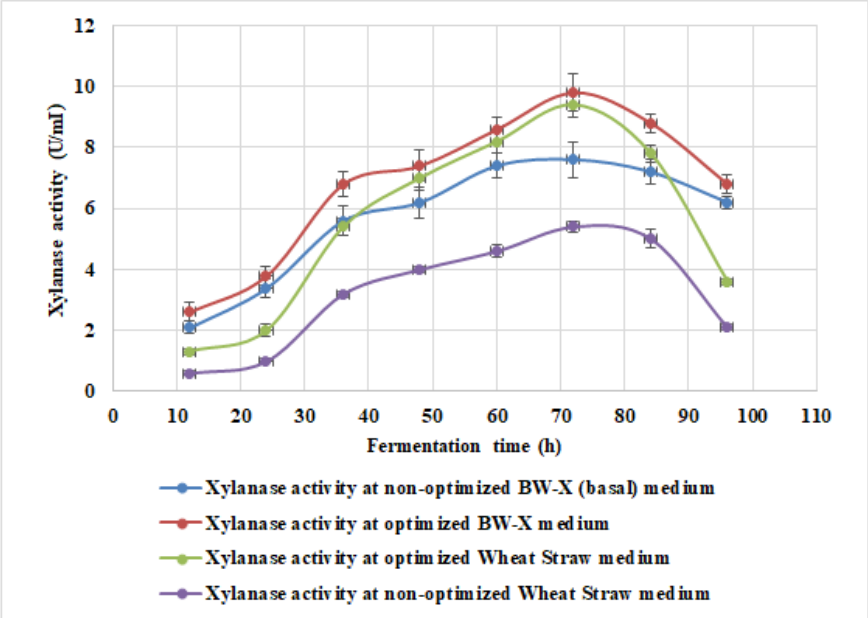

Figure 6. *Xylanase production by S. tendae SHB_02 before and after optimization of fermentation parameters. For optimum fermentation conditions for xylanase production: Inoculum concentration 2%; temperature 30 °C; pH 7.5; aeration rate 140 rpm; and incubation time 72 h, nitrogen source yeast extract; other components of production medium composition are as given in materials and methods section. BW-X; Birchwood xylan, mean values, n = 3 ± SD*

3.4. Characterization of Xylanase

The optimum reaction temperature and pH of maximum xylanase activity were determined as 50 °C and 8, respectively. The activity decreased sharply at a temperature above 50 °C. Xylanase was observed to be stable over the slight pH 8 for 80 min at 50 °C. Thermal stability was found to be stable at 50 °C for 120 min at pH 8. The xylanase was less stable at a reaction temperature of 50 °C below pH 6 and above pH 8.5. The residual activities of xylanase at different temperatures and pH values are shown in Figure 7.

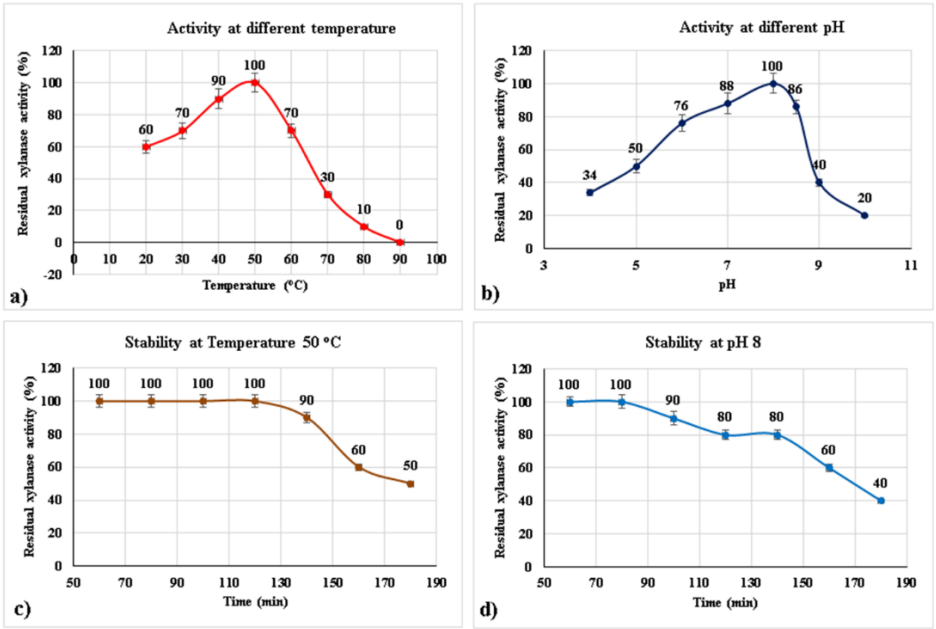

Figure 7. *Influence of pH and temperature on activity of xylanase from S. tendae SHB_02 and pH and temperature stability of xylanase. Reaction conditions are as given in the materials and methods. Mean values, n = 3 ± SD*

In the effect of metal ions addition of 1 mM, $MgCI_2$ increased the remaining enzyme activity by 150%, followed by CaCI2 and ZnCI2 by 130% and 110%, respectively, compared to the control. Other metal ions, especially $CuCI_2$, showed an inhibitory effect on xylanase activity (50%). It was determined that the EDTA (1 mM) and SDS (0.1% w/v) strongly inhibit the xylanase activity at 30% and 40%, respectively. Triton x-100 and Tween 80 (0.1% w/v) partly reduced xylanase activity, as reported in Table 2.

Table 2. *Influence of some metal ions and surfactants on xylanase activity of S. tendae SHB_02*

Chemicals[a]	**Relative enzyme activity (%)**
$CaCI_2$	130 ± 6
$CuCI_2$	50 ± 2
$MgCI_2$	150 ± 8
$FeSO_4$	82 ± 4
$CoCI_2$	90 ± 4
$ZnCI_2$	110 ± 6
EDTA	30 ± 2
SDS	40 ± 2
Triton x-100	94 ± 6
Tween 80	97 ± 6
Control	100 ± 4

[a]*Metal ions and EDTA concentrations are one mM and the concentration of surfactants is 0.1% (w/v). Xylanase activity in the absence of metal ions was considered 100% (control). Reaction conditions are as given in the materials and methods section. Mean values, n = 3 ± SD.*

4. DISCUSSION

The present study determined the isolation and identification of soil actinobacterium, namely *Streptomyces tendae* SHB_02 and its xylanase activity using agro residues as a substrate. The effect of different nutritional and fermentation parameters on the xylanase activity was investigated to optimize the enzyme production in SmF. Further, the influence of temperature, pH, metal ions and some chemicals on xylanase activity was determined for the enzyme characterisation. This research is the first study on xylanase production from the *Streptomyces tendae*.

In the fermentation experiments conducted with *Streptomyces tendae*, xylanase production in a medium supplemented with BW-X started at the 12th h and gradually increased until the 72nd h. The maximum xylanase production was observed temperature of 30 ^{o}C and pH of 7.5 in the fermentation medium. The inoculum rate was 2% for optimum xylanase production, and the shaking rate was 140 rpm. At the end of the optimization studies, xylan was used as a substrate, and the yeast extract (%0.25) as a nitrogen source supported the highest xylanase yield, which was 9.8 U/ml. A study conducted by Kim and Chi [35], obtained the highest xylanase enzyme yield in an LBX medium containing xylan as a substrate during a fermentation period of 24 h at 40 ^{o}C. The fermentation conditions in this research differed from the current study, and enzyme production was relatively high (131.8 U/ml). Adıgüzel and Tunçer [36], explained that the *Streptomyces* sp. AOA40 produced the highest xylanase activity at pH 7.0, temperature at 30 °C, shaking rate at 120 rpm and incubation period of 3 d. Xylanase production conditions by fermentation were similar to those in this study and supported the present results. A similar result was obtained for *Streptomyces olivaceus* (MSU3) [37], *Streptomyces variabilis* [38] and *Streptomyces thermocerradoensis* I3 [39].

The production of xylanases from microbial sources through fermentation is limited by the high cost of commercial xylan, which can be overcome using agro-residues in the culture medium. On the other hand, using the microorganisms in enzyme production, pre-treatment of lignocellulosic wastes has shown that exposure to an alkali solution is the most preferable and potentially toxic components are removed from the substrate. In this study, pre-treatment of agricultural wastes was carried out according to Azeri et al.

[31], Lan Pham et al. [32] and Dyshlyuk et al. [33]. Xylanase production of *Streptomyces tendae* SHB_02 was carried out by SmF with agro-residues such as wheat straw, wheat bran and corncob. The strain produced the maximum level of xylanase (9.4 U/ml) by adding 3% (w/v) wheat straw to the production medium. However, when the same amount of wheat bran was used, the production was around 7.2 U/ml, and when corncob was used, it was at least 5.4 U/ml. Many studies are showing that lignocellulosic raw materials such as rice bran, sawdust, wheat germ, wheat straw, rice straw, brewers spent grain, corn hull, corncob, bagasse, soybean meal, wheat bran, ragi bran and empty fruit bunch etc., are used to reduce costs in enzyme production [39-49]. Research on microbial xylanase production, especially with *Streptomyces* species, indicates that wheat straw was the most effective low-cost agricultural waste. In this study, the xylanase yield from wheat straw was significantly higher than wheat bran and corncob, and the results support previous studies. There are different results in xylanase production because the enzyme production medium, fermentation conditions and source bacteria are different. However, production medium optimization is very important. Based on the current results, wheat straw is expected to be a good xylanase inducer. Danso et al. [41] reported that the *Streptomyces* sp. MS-S2 produced the highest xylanase (6.56 U/ml) in a production medium supplemented with wheat straw (15 g/l) and efficiently utilised it as the carbon source. However, in the present study, the maximum xylanase production (9.4 U/ml) obtained with wheat straw is higher and differs from the results obtained in this study. On the other hand, previous studies on xylanase production using wheat bran as agricultural waste reveal that it stimulated the production of significant amounts of xylanase by the *Streptomyces* species [39, 40, 43, 44].

In the preparation of microbial fermentation media for using some low-cost agricultural wastes, nitrogen deficiency is a key consideration. These substrates are supplemented with organic or inorganic nitrogen sources, such as asparagine, peptone, urea, tryptone, yeast extract, corn steep liquor, ammonium sulphate, KNO_3 and soybean, to support nutritional balance for bacterial growth and enzyme synthesis. In the current research, the effects of six nitrogen sources on xylanase production were analysed at different concentrations in the production medium supplemented with wheat straw. Although the highest xylanase production was obtained with yeast extract (9.4 U/ml), enzyme values obtained with peptone (9 U/ml), tryptone (8.6 U/ml) and asparagine (8.2 U/ml) were also found to be significant. The highest xylanase efficiency was determined at the concentration of nitrogen source used at 2.5 g/l (0.25%), and a decrease in xylanase production was observed at higher rates. The xylanase activity of several *Streptomyces* has been determined using yeast extract by different researchers. *Streptomyces* sp. strain MS-S2 at a rate of 1.5% [41], *Streptomyces olivaceus* (MSU3) at a rate of 3% [37], and *Streptomyces thermocoprophilus* TC13W at a rate of 0.5% [50], which were not consistent with the results of this research.

Some metal ions increased the xylanase activity, especially $MgCI_2$, $CaCI_2$ and $ZnCI_2$, at 150%, 130% and 110%, respectively. $CuCI_2$ showed an inhibitory effect on xylanase activity (50%). According to Poornima et al. [49]**,** $CuCI_2$ inhibited the activity of xylanase from *Streptomyces geysiriensis*. As in other studies, EDTA and SDS inhibited the xylanase enzyme [35, 36]. Triton x-100 and Tween 80 caused a slight decrease in xylanase activity and showed different results from another report [44].

Reactions of the xylanase from *Streptomyces tendae* SHB_02 were carried out in buffers adjusted to different pH levels and different temperatures were determined. The optimum reaction temperature and pH for optimum xylanase activity were 50 ºC and 8, respectively. On the other hand, the thermal stability of xylanase at pH 8 was stable at 50 ºC even after 120 min. Incubation time on xylanase activity has shown that it retained its activity at pH 8 and 50 ºC for 80 min. Several recent reports that xylanases from *Streptomyces* members agreed on these results. Xylanase from *S. viridodiastaticus* MS9 showed high activity at optimal pH 7, and its optimum activity temperature was determined as 60-65 ºC and significantly decreased at 70 ºC [35]. The examination of the influence of pH, temperature and incubation time on the xylanase of *S. olivaceus* showed that the maintained its activity in the pH range of 6.5-8.5 and had thermal stability from 20 ºC to 60 ºC for up to 180 min [37]. In another study, the maximum activity of purified xylanase was determined at 40 ºC and pH 8 [35]. Gama et al. [39] observed that the optimum activity of xylanase was at 70 ºC and pH 6.0**.** Wu et al. [51] examined the xylanase of *Streptomyces griseorubens* LH-3. It showed optimum activity of xylanase at pH 5.0 and was stable at temperatures from 30 ºC to 50 ºC and showed optimum activity at 60 ºC. A similar study determined xylanase's pH and temperature profile as pH 6, 70 °C [40]. Mander et al. [43] reported a xylanase (EX624) from *Streptomyces* sp. CS624 was

cultured in a medium containing wheat bran as an agricultural substrate. Biochemical characterization of purified xylanase-EX624 was performed and this enzyme showed its highest activity at pH 6.0 and 60 ºC. Different results were observed by Li et al. [52] and Rahman et al. [44]. Li et al. [52] explained that xylanase purified from *Streptomyces rameus* L2001 was stable at a pH ranging from 4.3 to 6.7, with an optimum pH of 5.3. The optimum stability of xylanase was shown at 70 ºC. The xylanase of *Streptomyces* sp. CS628 was found stable over a wide pH range (5.0-13.0) and up to 60 °C, with optimum temperature and pH of 60 °C and 11.0, respectively [44].

5. CONCLUSIONS

The production of microbial xylanases is limited by the high cost of commercial xylan, which can be overcome by using agro-wastes as a xylan source in the fermentation medium. In the current research, xylanase production of a newly isolated and identified actinobacterium, *S. tendae* SHB_02, was optimized using low-cost agricultural wastes, specifically wheat straw. This finding suggests that although *S. tendae* provides a significant xylanase activity using the alternative lignocellulosic biomass and has potential in various biotechnological applications before it becomes further studies will need to be conducted to evaluate the effectiveness of xylanase in industrial applications.

ACKNOWLEDGEMENTS

This research was partially supported by the Manisa Celal Bayar University Scientific Research Projects Coordination Unit under project number 2015-075.

CONFLICTS OF INTEREST

No conflict of interest was declared by the author.

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