Journal of Genetic Engineering and Biotechnology 16 (2018) 693–701

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Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Original Article

Rice straw fermentation by *Schizophyllum commune* ARC-11 to produce high level of xylanase for its application in pre-bleaching

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ARTICLE INFO

Article history:
Received 8 December 2017
Received in revised form 9 January 2018
Accepted 15 February 2018
Available online 23 February 2018

Keywords:
Schizophyllum commune
Xylanase
Pre-bleaching
Rice straw
Eulaliopsis binata

ABSTRACT

Rice straw is valuable resource that has been used as substrate for cost effective production of xylanase under solid-state fermentation by a newly isolated white rot fungi, *S. commune* ARC-11. Out of eleven carbon sources tested, rice straw was found most effective for the induction of xylanase that produced 4288.3 IU/gds of xylanase by *S. commune* ARC-11. Maximum xylanase production (6721.9 IU/gds) was observed on 8th day of incubation at temperature (30 °C), initial pH (7.0) and initial moisture content (70.0%). The supplementation of ammonium sulphate (0.08% N, as available nitrogen) enhanced the xylanase production up to 8591.4 IU/gds. The xylanase production by *S. commune* ARC-11 was further improved by the addition of 0.10%, (w/v) of Tween-20 as surfactant. The maximum xylanase activities were found at pH 5.0 and temperature 55 °C with a longer stability (180 min) at temperature 45, 50 and 55 °C. This xylanase preparation was also evaluated for the pre-bleaching of ethanol-soda pulp from *Eulaliopsis binata*. An enzyme dosage of 10 IU/g of xylanase resulted maximum decrease in kappa number (14.51%) with a maximum improvement 2.9% in ISO brightness compared to control.

1. Introduction

Rice (*Oryzae sativa*) is the main staple food in most of the Asian countries and rice crops generate a huge amount of rice straw in the fields. Globally, about 744.995 million metric tonnes of rice was produced during the year 2013–14. In India, about 106.54 million tonnes of rice is produced that generates approximately 160 million tonnes of rice straw with the ratio of 1: 1.5 for rice grain to rice straw. India is the second largest producer of rice all over the world [1–3]. In India, some of the produced rice straw is used as substrate for mushroom cultivation, cardboard making and as fuel for modern biomass power plants, brick kilns and domestic biomass cookstoves in rural areas. But, almost two-third of rice straw is being burned openly to clear the field for sowing of next crop due to uneconomical price (approximately 500 INR per metric tonne) [1,4]. The open burning of rice straw in the fields not only produces large amount of greenhouse gases, but also destroy farmer's valuable by-product. It is a potential resource that has been used as substrate for the generation of several industrial products such as bioethanol [5], biomethane [6], cellulases and xylanases [7]. In the current study rice straw has been used as substrate for xylanase production under solid-state fermentation (SSF). Xylanases have gained the focus of intense research during recent years due wide biotechnological applications such as pre-bleaching of pulp, refining of waste paper, deinking of waste paper, hydrolysis of lignocellulosic biomass, extraction and clarification of fruit juices, improving digestibility of animal feed, desizing of cotton fabrics in textile and improving bakery products [8–10].

The aim of current study was to utilize rice straw for xylanase production by a newly isolated fungal strain of *Schizophyllum commune*. Not much literature has been reported on xylanase production by white rot fungi, *Schizophyllum commune* and its application in prebleaching. Xylanase production was maximized by optimizing cultural parameters. High level production of a cellulase-poor xylanase using agro-residues was carried out under SSF from *Schizophyllum commune*. The xylanase from *Schizophyllum commune* was applied for selective hydrolysis of the xylan component in ethanol-soda pulp from *Eulaliopsis binata*.

Peer review under responsibility of National Research Center, Egypt.
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https://doi.org/10.1016/j.jgeb.2018.02.006
1687-157X/

2. Materials and methods

2.1. Microorganism and identification

The fungal isolate ARC-11 was isolated from the soil samples collected from Paonta Sahib, Himachal Pradesh, India. The fungal isolates ARC-11 was identified as *Schizophyllum commune* based on morphological and molecular characteristics (ITS1-5.8S-ITS2 gene sequences) at National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune. The identified fungal strain was designated as *Schizophyllum commune* ARC-11 and deposited with accession numbers NFCCI 3029 at NFCCI, Agharkar Research Institute, Pune. The fungal isolate was maintained over potato dextrose agar slants at 4 °C. The ITS sequences of *S. commune* ARC-11 were submitted to GenBank with accession numbers KY864394.

2.2. Xylanase production using rice straw under SSF

Xylanase production was carried out by under solid-state fermentation (SSF) using different agro-residues as the carbon source. Different agro-residues such as corn cob, corn stover, congress grass, maize bran, pearl millet stover, rice straw, rice husk, sabai grass, sugarcane bagasse, sugarcane tops, sun hemp residue, wheat bran and wheat straw were tested for xylanase production. The agro-residues were washed with tap water to remove dirt particles before drying in sunlight. The agro-residues were chopped small pieces by a chopper, then ground in a Wiley mill. The particles ranging of 250–1400 μm were utilized as the substrates (carbon source) for xylanase production. Each of agro-residue (5 g) was moistened with Mandel Weber medium (77.5% initial moisture content) with following composition expressed as g/l: 1.4 $(NH_4)_2SO_4$, 2.0 KH_2PO_4, 0.3 $CaCl_2$, 0.3 $MgSO_4.7H_2O$, 0.02 Tween-80 and trace elements: 0.005 $FeSO_4.7H_2O$, 0.0016 $MnSO_4.7H_2O$, 0.0014 $ZnSO_4.7H_2O$, 0.002 $CoCl_2.6H_2O$. The initial pH of Mandel Weber medium for enzyme production was maintained to 5.5 with 1.0 N HCl or 1.0 N NaOH. Erlenmeyer flasks (250 ml) containing 5 g of substrate were sterilized by autoclaving at 121 °C for 30 min and inoculated with five discs of actively growing fungi. Inoculated flasks were incubated at 30 °C for 6 days. 50 ml of distilled water was added to fermented substrates and crushed with glass rod before shaking at 150 rpm for 60 min in an incubator shaker. The enzyme was harvested by squeezing fermented substrate and centrifuged at 8000 rpm to obtained clear supernatant that was used as enzyme source.

2.3. Optimization of physiochemical parameters for xylanase production

Xylanase production was optimized by employing one variable at a time (OVAT) approach. The effect of various factors including incubation time (2–12 days), incubation temperature (26–42 °C), initial pH (4–10), and initial moisture content (55–85%) was analyzed. The effect of supplementation of various nitrogen sources and surfactants at different concentrations was also analyzed.

2.4. Partial purification of xylanase

Partial purification of xylanase was carried out by fractional ammonium sulphate precipitation method. Ammonium sulphate at saturation level of 10–100% w/v was added slowly to 200 ml of crude enzyme and stirred continuously at 4 °C to precipitate the protein. The precipitate was collected by centrifugation at 9,000x g for 30 min after 10–12 h incubation at 4 °C. The pellets were dissolved in minimal amount of citrate buffer (pH 5.5, 50 mM) and subjected to xylanase activities and protein contents estimation. The fractions having significantly higher xylanase activities were pooled together before dialysis. Xylanase enzyme solution was dialyzed against the same buffer at 6 °C for 24 h and centrifuged to remove the undissolved dialysate.

2.5. Effect of pH and temperature and thermo-stability of xylanase

To determine the effect of pH on xylanase activity, following three buffers were used to maintain the desired pH range: Sodium citrate buffer (pH 3.0–6.0), sodium phosphate buffer (pH 6.5–8.0), and glycine–NaOH buffer (pH–9.0). Temperature profile for xylanase activity was determined by performing assay at different reaction temperatures (40–75 °C with difference of 5 °C) at optimum pH. To determine the thermo-stability, xylanase was pre-incubated at 45, 50, 55, and 60 °C for 0–180 min at optimum pH and the residual xylanase activity was measured.

2.6. Effect of cations on partially purified xylanases

The effect of different metal ions on the xylanase activity was determined under optimum assay condition with the reaction mixtures containing 1 and 10 mM additional reagents. Different metal ions were incubated with xylanase for a period of hour at room temperature and then analyzed for xylanase activities. The xylanase activity assayed in the absence of metal ions (control) was taken as 100%.

2.7. Enzyme assays and protein content estimation

Xylanase activity was estimated by using 1% (w/v) of birch wood xylan (Sigma Chemical Co. St Louis, MO, USA) in 50 mM citrate buffer at pH 5.5 according to Bailey method [8,11]. One unit of xylanase activity is defined as the amount of enzyme that librates 1 μmole of xylose per min per ml under assay conditions. Xylanase activity was expressed as activity units per mass of initial dry solid substrates (IU/gds). Cellulase activity was determined by using carboxymethyl cellulose (Sigma Chemical Co. St Louis, MO, USA) of medium viscosity as substrate according to International Union of Pure and Applied Chemistry (IUPAC) standard method [8,12]. The reducing sugars were quantified at 540 nm using UV–Vis spectrophotometer (SHIMADZU, UV-1800) by dinitrosalicylic acid (DNS) method [13]. Protein content of enzyme preparation was estimated according to the Lowry method [14].

2.8. Xylanase pre-bleaching of ethanol-soda pulp of E. Binata

The unbleached ethanol-soda pulp of *E. binata* was used for bleaching studies. The efficiency of xylanase pre-bleaching was estimated by the analysis of kappa number and viscosity of the pulp, releases of chromophores and reducing sugars in filtrate. The ethanol-soda pulp from *E. binata* was treated with different doses of xylanase (0–14 IU/g of OD pulp) at 8% consistency and reaction time of 120 min. Xylanase treated pulp was extracted with 1.2% NaOH (as such). Brightness, viscosity, and kappa number of pulp were measured by using standard TAPPI test methods. For brightness determination pads of 4 ± 0.2 g were prepared according to TAPPI T 218 sp-02 (Forming handsheets for reflectance testing of pulp). Brightness was determined according to TAPPI T 452 om-02 (Brightness of pulp, paper, and paperboard (directional reflectance at 457 nm)). Enzyme treated and untreated pulp samples were subjected to viscosity determination according to TAPPI T 230 om-04 (Viscosity of pulp). Kappa number of all pulp samples was also estimated according to TAPPI T 236 cm-85 (Kappa number of pulp) [15]. The release of chromophores during xylanase treatment was quantified the absorbance of 237, 280 and 465 nm

using UV–Vis spectrophotometer (SHIMADZU, UV-1800) [16]. Pulp filtrates from enzyme treated and untreated samples were analyzed for reducing sugars according to 3, 5-dinitrosalicylic acid (DNS) method [13].

2.9. Statistical analysis

All the experiments were carried out in triplicate independently and experimental results were represented as the mean ± standard deviation of three identical values.

3. Results and discussion

3.1. Xylanase production under SSF

Various agro-residues were tested as carbon source for biosynthesis of xylanase. *S. commune* ARC-11 utilized all the agro-residues effectively to produce xylanase. Maximum xylanase production from *S. commune* ARC-11 was found with rice straw (4288.36 IU/gds) as the carbon source under SSF conditions. Among agro-residues tested maize bran, wheat straw, Sabai grass and sugarcane bagasse, wheat bran, sugarcane leaves, and Congress grass gave xylanase yield by 92.33, 87.95, 85.85 and 84.93, 83.64, 80.70, and 75.44% respectively compared to rice straw (Fig. 1). *S. commune* ARC-11 was able to utilize several agro-residues to produce fairly good amount of xylanase. The results indicated that along besides rice straw several other agro-residues like maize bran, wheat straw, Sabai grass and sugarcane bagasse, wheat bran can also be exploit for xylanase production. In the present study, rice straw was selected for xylanase production during further studies.

During SSF, xylanase production is dependent on the nature of carbon source, favorable degradability, bare chemical composition, physical associations, accessibility of substrate, and presence of some nutrients [8,10,17]. Rice straw is rich in hemicelluloses (20–40%) and hence found to be the suitable carbon source for xylanase production. A substrate with higher content of xylan induces the higher xylanase production [5,18]. Few reports were available on xylanase production by the white-rot fungus, *Schizophyllum commune* [19,20]. Kolenova et al. [19] observed maximum xylanase activity (71.3 U/ml) by *Schizophyllum commune* under submerged fermentation after 11th day of cultivation on cellulose containing medium. Rahnama et al. [7] found higher enzyme production by *Trichoderma harzianum* SNRS3 using untreated rice straw compared to alkali-pretreated rice straw and reported maximum endoglucanase, FPase and xylanase by 111.31, 6.25 and 433.75 U/g of substrate respectively under SSF. Rice has been used as substrates for the production of various value-added products such as cellulases, xylanases, bioethanol and biomethane (Table 1).

3.2. Effect of physiochemical parameters on xylanase production

The xylanase production enhanced gradually with increasing incubation period and reached at the highest level on 8th day of incubation at 30 °C i.e. 5199.02 IU/gds (Table 2). Further increase in the incubation time i.e. after 8th day, xylanase activity started to decrease. Protein content increased up to 9th day of incubation and beyond that it became almost constant. Enzyme production reaches to maximum level in stationary phase and declines during death phase due to depletion of nutrients and cellular fragmentation. Accumulation of toxic waste materials decreases the growth and causes the inactivation of secretary machinery of enzymes [21,22]. Maximal xylanase production was observed after an incubation period of 7th day for *Coprinellus disseminates* [23], 8th day for *Volvariella diplasia* [24], and 11th day for *Schizophyllum commune* [19].

S. commune ARC-11 produced maximal xylanase (5358.93 IU/gds) at an incubation temperature of 30 °C. A deviation from optimum temperature (30 °C) affected the xylanase production adversely. The fungal strain *S. commune* ARC-11 was not able to grow beyond a temperature of 42 °C. Similarly, crude enzyme protein concentration also followed the same pattern as observed in of xylanase production. Higher temperature may lead to poor fungal growth due to denaturation of enzymes which results reduction in

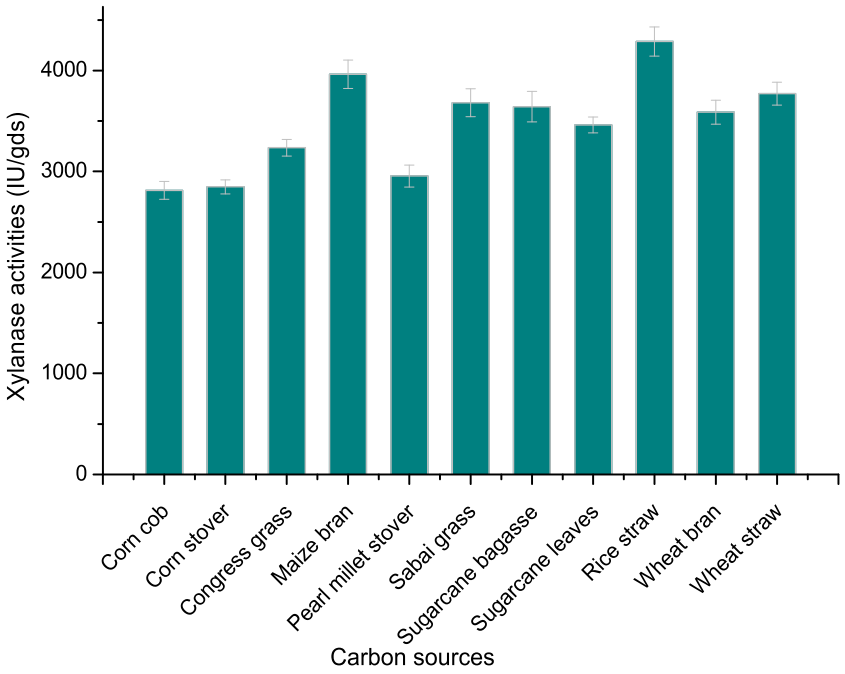

Fig. 1. Effect of carbon sources on xylanase production by *S. commune* ARC-11 (5 g of each agro-residue moistened with Mandel Weber medium, SSF cultural conditions: Incubation time-6 days, temperature-30 °C, initial moisture level-77.5%, and initial pH-5.0; error bars show standard deviation).

Table 1
Biotechnological conversion of rice straw into value-added products.

Microorganism involved	Pretreatment	Value-added product obtained	Reference
Anaerobic digestion	Mechanical treatment	Biomethane & bioethanol	[1]
Trametes hirsuta	Biological pretreatment	Bioethanol	[5]
Anaerobic digestion	Alkaline pretreatment	Biomethane	[6]
Trichoderma harzianum SNRS3	Untreated	Cellulase & xylanase	[7]
Trichoderma reesei & *Humicola insolens*	Untreated & Alkali pretreated	Cellulase & xylanase	[25]
Aspergillus niger BK01	Alkali-assisted acid pretreatment	Fermentable sugars	[26]
Clostridium acetobutylicum	Organosolv pretreatment	Butanol, acetone & ethanol	[27]
Candida guilliermondii FTI20037	Acid hydrolysis	Xylitol	[28]
Schizophyllum commune	Untreated	Xylanase	This study

enzyme biosynthesis [22]. At lower temperature, xylanase production decreases due to lower transport of substrates through the cell membrane [29]. Saleem et al. [30] reported maximal xylanase production at 30 °C by various white-rot fungi such as *Phanerochaete sordida* MRL3 (272.74 IU/ml), *Lentinus pigrinus* MRL6 (278.52 IU/ml) and *Poliporus caliatus* MRL7 (292.86 IU/ml).pH affects the microbial enzyme secretary machinery and therefore, enzyme production. Xylanase production increased with increasing initial pH from 4.0–7.0 and maximal xylanase production (6340.71 IU/gds) was attained at initial pH of 7.0. Beyond a pH of 7.0, xylanase activity started to decrease (Table 2). Xylanase production was lowered by an increase or decrease in initial pH from the optimal value. In view of the fact that enzymes are proteins that are polymers of amino acids and the ionic characters of the amino and carboxylic acid groups of amino acids are influenced by the change in pH. The catalytic properties of the enzymes are strikingly affected by the change in ionic characters of amino acids [23]. Agnihotri et al. [23] observed 6.4 as the optimum pH for xylanase production by a white-rot fungi *Coprinellus disseminatus* under SSF conditions.

Xylanase production increased with increasing moisture content from 55 to 70%, and reached to its maximum value (6721.96 IU/gds) at 70% moisture contents. Further increase in moisture content (beyond 70%) resulted into poor xylanase production. The xylanase activity of 93.16% was retained at 75% moisture level. During SSF, the swelling of substrate is controlled by moisture level and makes the substrate suitable for microbial growth and utilization. The optimal moisture content results the faster microbial growth and early initiation of enzyme biosynthesis [31–32].

Among simple nitrogen sources, ammonium sulphate (0.08% as available N) followed by urea (0.08% as available N) were found most effective nitrogen sources for xylanase production by *S. commune* ARC-11. Ammonium sulphate showed maximum xylanase activity of 8591.38 IU/gds and followed by urea (8123.20 IU/gds) (Fig. 2a). Among complex nitrogen sources, maximum xylanase production was found with beef extract (8221.60 IU/gds) and followed by peptone (7787.82 IU/gds) at a concentration of 0.8%

Table 2
Effect of incubation time, temperature, initial pH, and moisture content on xylanase production by *S. commune* ARC-11.

Cultural Parameter		Xylanase activity (IU/gds)	Protein content (mg/ml)	SSF conditions
Incubation time (days)	2	256.00 ± 7.99	0.85 ± 0.03	1. Incubation time-varied,
	3	338.93 ± 10.85	0.97 ± 0.04	2. Temperature-30 °C,
	4	1389.07 ± 35.42	1.11 ± 0.03	3. Initial moisture level-77.5%,
	5	1490.93 ± 40.85	1.44 ± 0.05	4. Initial pH-5.0
	6	4262.76 ± 153.46	1.69 ± 0.06	
	7	4556.98 ± 212.36	1.73 ± 0.06	
	8	5199.02 ± 132.06	1.86 ± 0.08	
	9	4039.91 ± 148.67	1.99 ± 0.06	
	10	2898.93 ± 93.93	1.96 ± 0.08	
	11	1747.73 ± 44.39	1.95 ± 0.07	
	12	1472.80 ± 33.87	1.90 ± 0.06	
Temperature (°C)	26	4001.96 ± 158.08	1.53 ± 0.09	1. Incubation time-8 days,
	30	5358.93 ± 253.48	1.88 ± 0.09	2. Temperature-varied,
	34	3689.87 ± 143.17	1.58 ± 0.07	3. Initial moisture level-77.5%,
	38	1829.07 ± 88.89	1.50 ± 0.08	4. Initial pH-5.0
	42	ND	ND	
Initial pH	4.0	5242.40 ± 144.69	1.65 ± 0.09	1. Incubation time-8 days,
	5.0	5299.73 ± 171.18	1.92 ± 0.09	2. Temperature-30 °C,
	5.5	5459.73 ± 191.09	1.98 ± 0.12	3. Initial moisture level-77.5%,
	6.0	5661.16 ± 157.38	2.17 ± 0.11	4. Initial pH-Varied
	6.5	5973.33 ± 221.01	2.24 ± 0.10	
	7.0	6340.71 ± 207.34	2.25 ± 0.12	
	8.0	5424.18 ± 227.82	2.12 ± 0.11	
	9.0	4934.31 ± 188.49	1.94 ± 0.10	
	10.0	4811.82 ± 188.62	1.90 ± 0.12	
Initial moisture content (%)	55.0	3026.13 ± 86.24	1.32 ± 0.07	1. Incubation time-8 days,
	60.0	4118.49 ± 130.14	1.56 ± 0.08	2. Temperature-30 °C,
	65.0	5459.73 ± 177.44	1.83 ± 0.10	3. Initial moisture level- 70.0%,
	70.0	6721.96 ± 235.27	2.14 ± 0.10	4. Initial pH-7.0
	75.0	6262.67 ± 180.36	2.30 ± 0.12	
	77.5	4428.62 ± 217.00	2.21 ± 0.12	
	80.0	2054.31 ± 87.51	2.15 ± 0.11	
	82.5	1619.73 ± 51.83	1.92 ± 0.10	
	85.0	1303.64 ± 49.80	1.87 ± 0.09	

± Refers standard deviation.

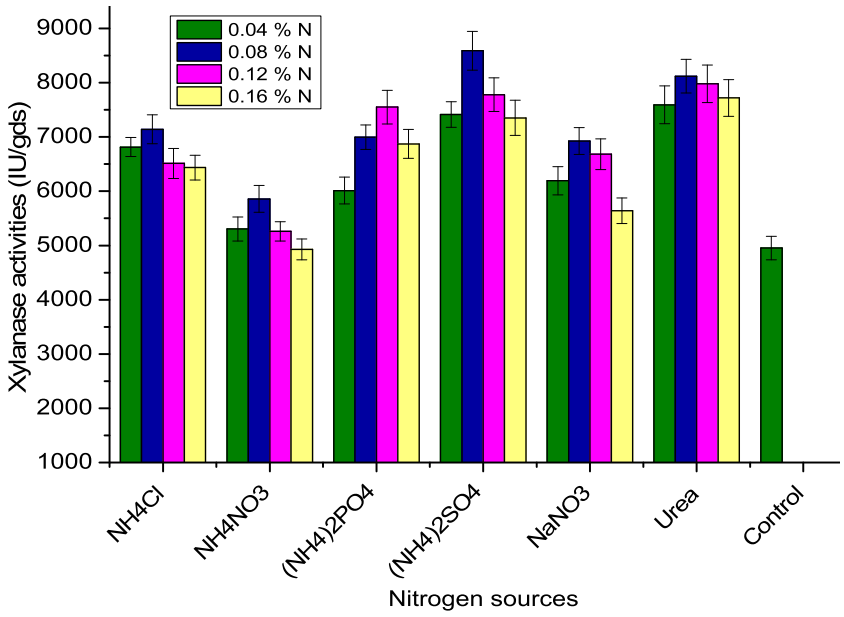

Fig. 2a. Effect of simple nitrogen sources on xylanase production by *S. commune* ARC-11 (Each of nitrogen source tested at 0.04, 0.08. 0.12 and 0.16% as available Nitrogen, SSF cultural conditions: Incubation time-8 days, temperature-30 °C, initial moisture level-70.0%, and initial pH-7.0; error bars show standard deviation).

(w/v) (Fig. 2b). Ammonium sulphate was used as the best and cost effective nitrogen source for xylanase production by *S. commune* ARC-11 [32]. Production of xylanases is known to be sensitive to nature of nitrogen source used and the percentage of available nitrogen used in production medium [33]. Ammonium sulphate was also reported to produce maximum xylanase production by some other fungi such as *Aspergillus terreus* [34], and *Thermoascus aurantiacus* [32].

S. commune ARC-11 produced maximum xylanase (10196.53 IU/gds) with Tween-20 (0.10%, w/v) followed by other surfactants in descending order i.e. Tween-80 (0.10%, w/v), Tween-60 (0.10%, w/v), Tween-40 (0.10%, w/v) and Triton-x-100 (0.05%, w/v) (Table 3). All the concentrations of EDTA and SDS were found inhibitory for xylanase production. Surfactants enhance the permeability of microbial cell membrane which affects the secretion of certain proteins. Some amount of the hemicellulolytic enzymes is bound to fungal hypha which is released by the action of surfactants [35].

S. commune ARC-11 produced maximum xylanase (1147.11 IU/ml) and cellulase (1.47 IU/ml) activities at optimum cultural conditions such as incubation time 8th day, temperature 30 °C, pH 7.0, moisture content 70.0%, nitrogen source (ammonium sulphate, 0.08% as available nitrogen), and surfactant (Tween-20, 0.10% (w/v) using rice straw as the carbon source under SSF conditions.

3.3. Partial purification of xylanases

Xylanase produced by *S. commune* ARC-11 was partially purified by ammonium sulphate precipitation. Maximum xylanase activity was recorded at 50–70% ammonium sulphate fraction with 41.86% yield, specific activity of 632.18 IU/mg and 2.75 purification fold (Table 4). The xylanases have previously been purified by using ammonium sulphate. Deshmukh et al. [36] studied the purification of xylanase from *Aspergillus fumigatus* R1 and observed maximum xylanase activity with 30–55% fraction of ammonium salt precipitation with 24.6% yield and 9.04 purification fold.

3.4. Effect of pH and temperature on xylanase activities

Maximum xylanase activity was observed at pH 5.0 and the xylanase was active at a broad pH range of 4.0–7.0. The xylanase activities of 84.17 and 55.24% were retained at pH 6.0 and 7.0 respectively while at pH 8.0 only 26.66% of xylanase activity was retained (Fig. 3a). Similar observations were reported by Kolenova et al. [19] who studied the effect of pH on xylanase from *Schizophyllum commune* and reported xylanase stability at a pH range of 4.0–7.0 with optimum activity at pH 5.5.

Xylanase from *S. commune* ARC-11 showed maximal activity at 55 °C and retained 47.06, 47.25, and 24.78% of maximum xylanase activity at 30, 65, and 70 °C respectively (Fig. 3b). Kolenová et al. reported maximum xylanase activity at 50 °C *Schizophyllum commune* [19]. The maximum activity of xylanase at 55 °C was reported for fungal strains like *Aspergillus versicolor* [37] and *Acrophialophora nainiana* [38].

3.5. Thermo-stability of xylanase and effect of metal ions

Xylanase from *S. commune* ARC-11 was stable at temperature 45 and 50 °C for 180 min while at 55 °C (optimum temperature) xylanase activity decreased slightly after 120 min. At temperature 60 °C xylanase activity decreased drastically and only 24.29% of activity was retained after 180 min of holding time (Fig. 3c). Xylanases from *S. commune* ARC-11 showed stability for a longer time at temperature 45–55 °C that makes this enzyme suitable for prebleaching of unbleached pulp.

Two concentrations i.e. 1 and 10 mM of metal ions were used to study the effect of metal ions on xylanase activity. Metal ions including Na^+ and K^+ showed stimulatory effect on xylanase activity whereas other metal ions had inhibitory effect on xylanase activity (Fig. 4). Xylanase activity was increased by 66.03, 62.19, and 39.69% by the addition of Na^+, K^+ and Zn^{++} respectively at 10 mM concentration. Hg^{++} (1 and 10 mM) strongly inhibited the xylanase activity.

3.6. Xylanase pre-bleaching

Prebleaching of *E. binata* ethanol-soda pulp was performed with *S. commune* ARC-11 xylanase at enzyme doses varying from 0–14 IU/g, keeping other variables constant. The extraction with 1.5% NaOH was performed after pretreatment of ethanol-soda pulp with xylanases and its effect on kappa number, brightness and viscosity

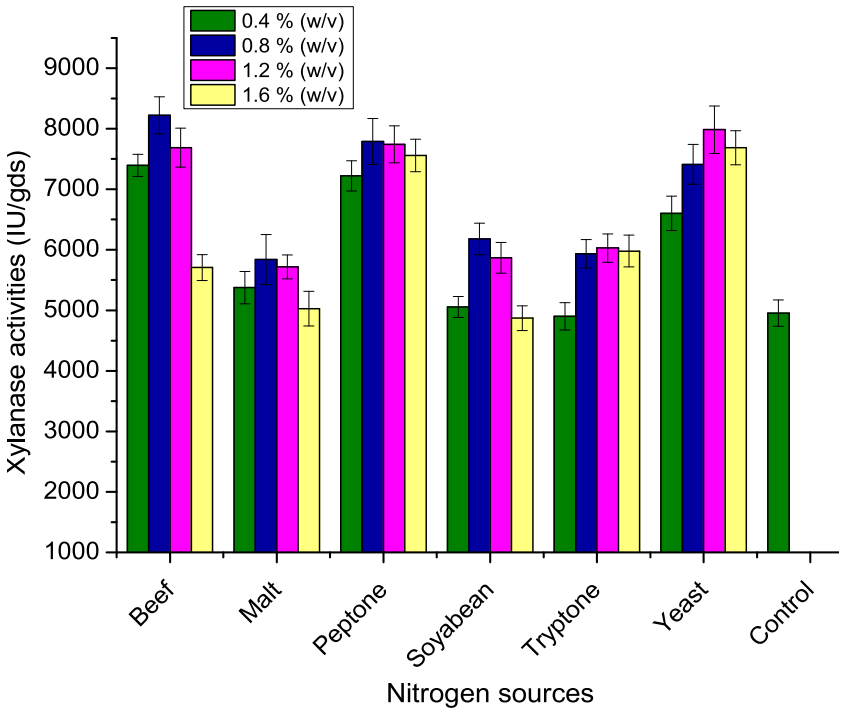

Fig. 2b. Effect of complex nitrogen sources on xylanase production by *S commune* ARC-11 (Each of nitrogen source tested at 0.40, 0.80, 1.2 and 1.6% (w/v) SSF cultural conditions: Incubation time-8 days, temperature-30 °C, initial moisture level-70.0%, and initial pH-7.0; error bars show standard deviation).

Table 3
Effect of surfactants on xylanase production by *S. commune* ARC-11.

Surfactants	Surfactants at different doses (%, w/v)			
	0.05	0.10	0.20	0.30
S. commune ARC-11				
Tween-20	9163.38 ± 286.81	10196.53 ± 444.57	10131.29 ± 33.28	9430.04 ± 308.36
Tween-40	8714.04 ± 318.06	8755.556 ± 302.07	8015.733 ± 19.38	7670.04 ± 243.91
Tween-60	8697.24 ± 367.02	9033.067 ± 351.39	8356.533 ± 28.45	8072.17 ± 305.13
Tween-80	8971.82 ± 281.72	9272.089 ± 358.83	8999.467 ± 27.88	8568.88 ± 349.61
Triton-x-100	8643.91 ± 376.01	8481.956 ± 371.51	8173.778 ± 31.69	8039.46 ± 253.24
SDS	5795.56 ± 199.37	5718.489 ± 205.87	5673.067 ± 22.65	5566.40 ± 181.46
EDTA	6032.53 ± 183.99	5825.156 ± 186.40	5056.711 ± 19.17	4389.06 ± 170.30
Control	6680.44 ± 225.80			

SSF conditions: Incubation time-8 days, temperature-30 °C, initial moisture level-70.0%, and initial pH-7.0, Nitrogen source- ammonium sulphate (0.08% as available N). ± Refers standard deviation.

Table 4
Partial purification of xylanases from *S. commune* ARC-11.

Steps	Volume (ml)	Activity (IU/ml)	Total activity (IU)	Protein (mg/ml)	Total protein (mg)	Specific activity (IU/mg)	Yield (%)	Fold purification
Crude enzyme	200.0	1169.60	233920.0	5.09	1018.00	229.78	100	1
$(NH_4)_2SO_4$ (50–70%) dialyzed fraction	17.0	5760.50	97928.50	9.11	154.90	632.18	41.86	2.75

was analyzed. During prebleaching, release of chromophores was estimated by spectrophotometric analysis at 237, 280 and 465 nm wavelengths. The absorbances at wavelength 237, 280 and 465 nm found to increase up to a xylanase dose of 10 IU/g of pulp, beyond that increase in enzyme dose did not show any significant increase in absorbances. The absorbance pattern of 237 and 465 nm indicated that maximum phenolic compounds and hydrophobic compounds respectively were released with an enzyme dose of 10 IU/gds and further increase in enzyme doses did not increase the release of phenolic compounds, hydrophobic compounds [39]. The release of reducing sugars was observed 2.52 mg/g of pulp at enzyme dose (10 IU/g of pulp) and increased up to 3.56 mg/g of pulp at xylanase dose 14 IU/g of pulp. The maximum decrease (14.51%) in kappa number of ethanol-soda pulp of *E. binata* was observed at an enzyme dosage of 10 IU/g of xylanase compared to control. Further increase in enzyme dose did not affect kappa number significantly. Similarly, pulp brightness improved by 2.9% (ISO) compared to control, at an enzyme dosage of 10 IU/g of xylanase and there were no major changes in brightness on increasing enzyme dose above 10 IU/g. Pulp viscosity was improved by 3.90% with xylanase from *S. commune* ARC-11 at an enzyme dose of 10 IU/g (Table 5). The reduction in Kappa number and improvement

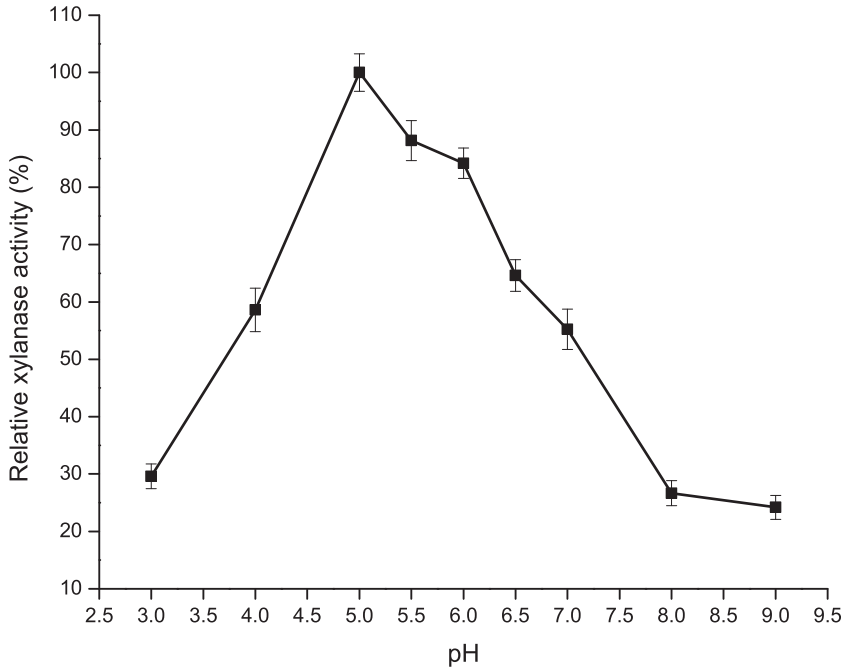

Fig. 3a. Effect of pH on xylanase activity from *S. commune* ARC-11 (pH was maintained 3.0–6.0 by sodium citrate buffer, 6.5–8.0 by sodium phosphate buffer and the pH 9.0 by glycine–NaOH buffer; error bars show standard deviation).

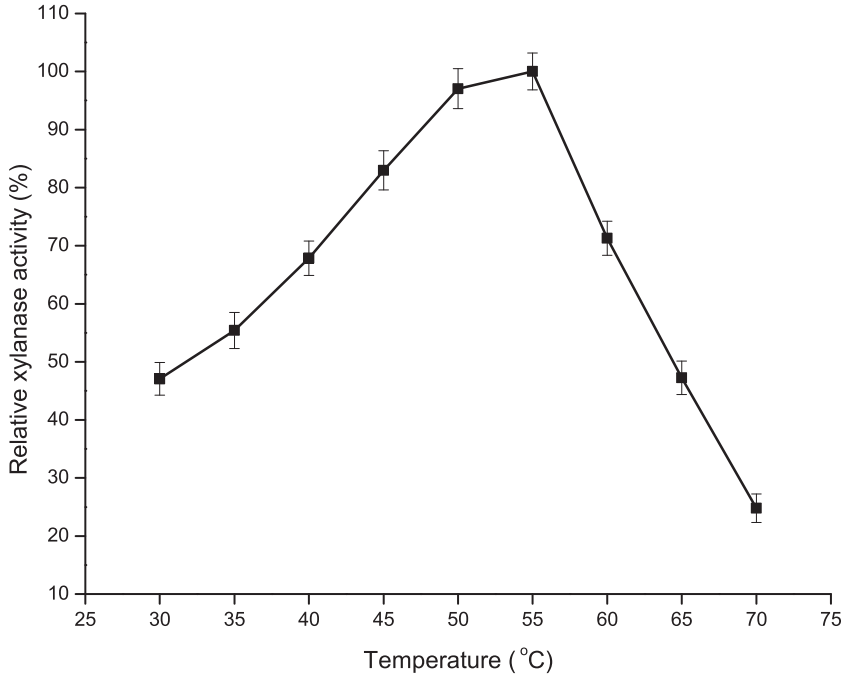

Fig. 3b. Effect of temperature on xylanase activity from *S. commune* ARC-11 (Assay were performed at reaction temperatures 40–75 °C with difference of 5 °C; error bars show standard deviation).

in brightness as well as pulp viscosity coupled with release of chromophores, hydrophobic compounds and reducing sugars, indicated the dissociation of LCC (lignin carbohydrate complex) from the pulp fibers. The release of reducing sugars and chromophores is an interdependent phenomenon. When the pulp samples were treated with xylanase, it hydrolyzes the hemicellulose layer of pulp fiber and releases the reducing sugars. Xylan is sandwiched between lignin and cellulose layer and degradation of xylan by the xylanase, results in the release of lignin and phenolic compounds from the pulp fibers. The removal of LCC enhances the porosity of the pulp that subsequently allows the easy diffusion of bleaching chemicals [39–42]. Xylan is a polymer with low degree of polymerization and its degradation by xylanase resulted increased the pulp viscosity due to improvement in average molecular weight of polymer system [43]. Earlier studies on bleaching with xylanases showed a decrease in kappa number after treatment [42,44]. Kumar et al. [42] also correlated the release of reducing sugars with xylanase mediated xylan degradation and showed

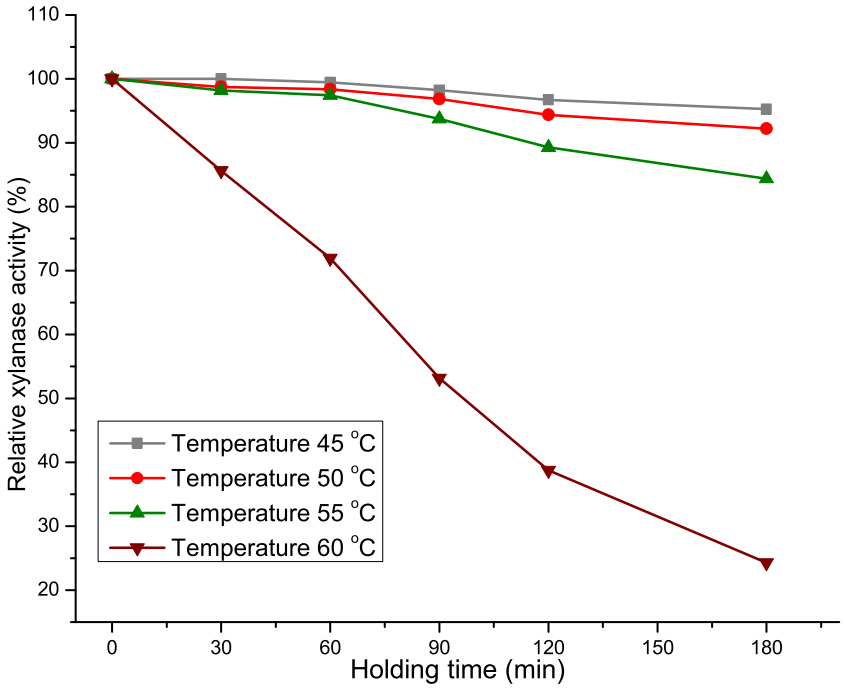

Fig. 3c. Thermo-stability of xylanases from *S. commune* ARC-11 at optimum pH (Enzyme pre-incubated at 45, 50, 55, and 60 °C for 0–180 min at pH 5.0 and assay was performed at pH 5.0 & temperature 55 °C).

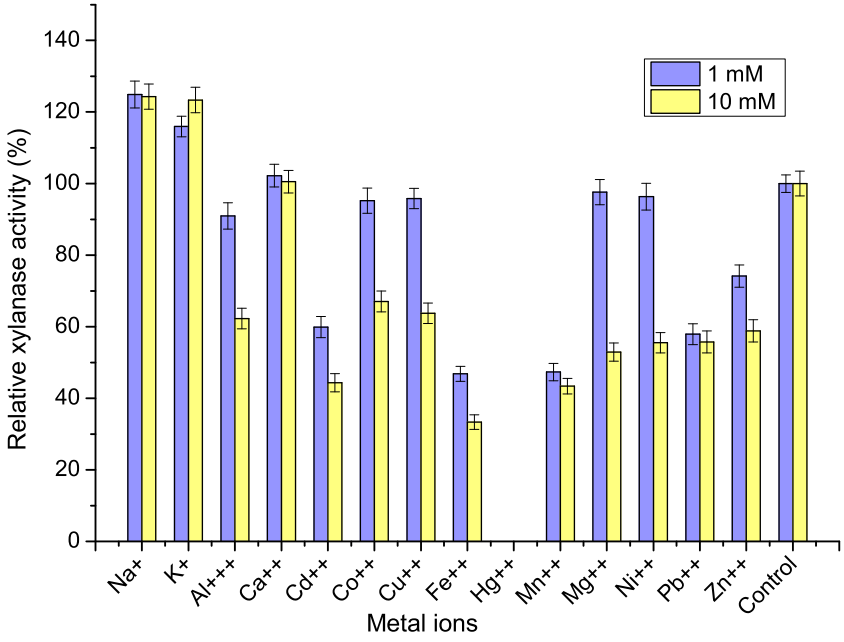

Fig. 4. Effect of metal ions on xylanase activity from *S. commune* ARC-11 (Reaction mixtures contained 1 and 10 mM of metal ions; error bars show standard deviation).

18% reduction in kappa number of hardwood unbleached kraft pulp after 2 h treatment by *Bacillus amyloliquefaciens* xylanase. Sridevi et al. [44] studied the prebleaching of recycled pulp with xylanase from *Aspergillus niger* and found the reduction of 3.5 points in Kappa number and improvement in brightness by 3.1 points.

4. Conclusion

White rot fungus *S. commune* ARC-11 produced higher yield of xylanase. Rice straw was utilized as carbon source for xylanase production that caused maximum induction of xylanase by *S. commune* ARC-11. Xylanase production was maximized by optimizing cultural parameters through one variable at one time approach. During optimization, xylanase production was improved by 2.38 times compared to initial fermentation conditions. Partially purified xylanase was found stable for longer time at temperature 45–55 °C that indicated its significance for prebleaching of pulp. *S. commune* ARC-11 xylanase showed its potential in prebleaching of ethanol-soda pulp of *E. binata*. Xylanase pretreatment of ethanol-soda pulp of *E. binata* caused a decrease of 14.51% in kappa number while pulp brightness and viscosity were improved by 2.90% and 3.90% respectively.

Table 5
Effect of xylanase dose for prebleaching of *E. binata* ethanol-soda pulp.

Xylanase dose, IU/g	*Kappa number	Reducing sugars released, mg/g	*Viscosity, cps	Brightness,% (ISO)	Chromophores released, Optical density		
					237 nm	280 nm	465 nm
0	16.2 ± 0.40	-	28.2 ± 0.14	43.9 ± 0.5	–	–	–
4	15.2 ± 0.32	0.52 ± 0.04	28.4 ± 0.15	44.4 ± 0.4	0.243 ± 0.014	0.202 ± 0.006	0.141 ± 0.009
6	14.8 ± 0.31	0.95 ± 0.05	28.7 ± 0.10	44.9 ± 0.3	0.207 ± 0.015	0.243 ± 0.008	0.167 ± 0.010
8	14.3 ± 0.21	1. 58 ± 0.08	29.2 ± 0.16	45.6 ± 0.4	0.372 ± 0.018	0.283 ± 0.015	0.198 ± 0.008
10	13.8 ± 0.20	2.52 ± 0.10	29.3 ± 0.13	46.8 ± 0.5	0.414 ± 0.019	0.332 ± 0.016	0.225 ± 0.010
12	13.1 ± 0.25	3.02 ± 0.16	28.9 ± 0.14	47.1 ± 0.4	0.422 ± 0.015	0.341 ± 0.009	0.230 ± 0.012
14	13.1 ± 0.23	3.56 ± 0.23	28.4 ± 0.12	47.6 ± 0.3	0.432 ± 0.016	0.346 ± 0.010	0.236 ± 0.015

± Refers to standard deviation.
Operational conditions: Extraction stage = 1.2% NaOH at 70 °C temperature for 90 min, X stage = enzyme dose varied, reaction time 120 min, pH 5.0, temperature 55 ± 2 °C, consistency 8%.

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