

## RESEARCH ARTICLE

## Xylanolytic *Bacillus flexus* PSX1 isolated from earthworm casts for xylanase production and biomass conversion

Daphy Meurial Chandra and Sivakumar Uthandi\*

\*Biocatalysts Laboratory, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore - 641 003.

### ABSTRACT

Lignocellulosic biomass is the major agricultural waste in countries like India, which has a significant income source is agriculture. There is a need to study the conversion of cellulose, hemicellulose, and lignin, which are the major components of lignocellulosic biomass. This study mainly focuses on bioconversion of hemicellulose by screening and identifying bacteria from vermicasts using different natural lignocellulosic materials such as paddy straw, coir pith, dried leaves, saw-dust, and leaf residue as a substrate that can produce xylanase. Selective enrichment of cultures was done in xylan enriched minimal media. Bacterial isolates capable of hydrolyzing xylan were screened. Selected and purified bacterial isolates grown in xylan media were activated and transferred into the xylan broth for further enzymatic assay. The bacterial isolates along with the standard culture *Bacillus pumilus* are taken for enzymatic assay. Among the 7 isolates, PSX1 recorded the maximum activity of 11.55 IU mL<sup>-1</sup> followed by SDX3 (10.71 IU mL<sup>-1</sup>) at 48 h of growth and declined thereafter. The standard culture *Bacillus pumilus* recorded 11.27 IU mL<sup>-1</sup>. Hence, the potential strain, *Bacillus flexus* PSX1, is selected for further studies to produce xylanase.

**Key words:** Vermicast; *Bacillus sp.*; Paddy straw; xylan; Bioconversion

### INTRODUCTION

The effective and economical utilization of lignocellulosic materials could be necessary means to overcome the shortage of food, feed and fuel, which the world may face in the near future. The development of efficient routes to fuels and chemicals from lignocellulosic biomass is an area of significant research interest. Earthworm casts have been shown to have enhanced microbial and enzyme activities and micro and macro-nutrients. Such enhancement of microbial population in the casts was due to: (1) rich nutrient concentration, (2) multiplication of microbes while passing through the gut of worms, (3) optimal moisture, and (4) large surface area of casts ideally suited for better feeding and multiplication of microbes.

Cellulose is the major component of lignocellulose, making up between 40 and 50% of lignocellulosic biomass. Hemicellulose and lignin are present at approximately 25 to 35% and 15 to 25%, respectively, depending on the source. Therefore, after cellulose, hemicellulose is the next most abundant polymer. Hemicellulose is a polymer similar to cellulose, consisting of chains of sugar molecules. However, unlike cellulose, which consists only of glucose monomers, hemicellulose is heterogeneous,

containing varying compositions of pentose sugars depending on the biological source. Usually, a chain of D-xylose molecules (xylan) forms the backbone of hemicellulose with side chains containing mannose, arabinose, galactose, glucuronic acid and other sugars. Xylan is, therefore, the predominant component in hemicellulose. Xylans are linear homopolymers that contain D-xylose monomers linked through  $\beta$ -1, 4-glycosyl bonds. Xylanase (E.C 3.2.1.8) degrades xylan by cleaving  $\beta$ -1, 4 glycosidic linkages randomly, and the resultant products such as xylose and xylo-oligosaccharides like xylobiose are industrially useful in various applications spanning from biofuels to various environmental applications (Miyamoto, 1997).

Several species of *Bacillus* secrete high amounts of extracellular xylanases. These microbes are the key players in the vermicomposting process, which is the best microbial composting of organic wastes through earthworm activity. Besides, vermicast, nature's perfect plant food, a product of vermicomposting becomes not only a source of plant and soil booster but also an inexpensive source of microbes and enzymes such as proteases, amylases, lipase, cellulase, and chitinase, which bring about a rapid biochemical conversion of the cellulosic, hemicellulosic and the proteinaceous

\*Corresponding author's e-mail: usiva@tnau.ac.in

materials in the variety of organic wastes. Hence, the present study was undertaken to develop effective xylan bioconversion using potential microbes to properly manage crop residues/waste to sustain environmental quality by utilizing waste for productive purposes.

## MATERIALS AND METHODS

### Isolation of bacteria from earthworm casts

About 1g of earthworm casts was taken and added to the minimal media that is enriched with xylan. After 15 days of incubation, the sample was serially diluted with sterile distilled water and plated on a nutrient agar medium to isolate bacteria. The plates were incubated at 28°C, and the colonies were counted from 2<sup>nd</sup> day onwards till 10 days. The isolated bacteria were purified by a streak plate technique. A single colony was transferred to nutrient agar slopes and maintained under refrigerated conditions.

### Screening of isolates based xylanolytic activity

The xylan plates with bacterial isolates were tested for hemicellulose hydrolysis using congo red solution, and the plates were flooded with 10 mL of congo red solution for 15 mins. Then the plates were destained with 1mL of 1M NaCl by washing the plates. A clear halo zone around the bacterial colony indicated hemicellulose hydrolysis. After screening, the isolates are used for measuring xylanase activity. The method involving dinitrosalicylic acid was followed for estimating xylanase enzyme activity by measuring the production of reducing sugar. Morphological, biochemical, and molecular (16S rRNA) characterization of these potential isolates were carried out.

### Xylanase production under submerged fermentation

Enzyme production by *Bacillus flexus* PSX1 using optimized conditions under SmF was carried out with 2 per cent of paddy straw as substrate (carbon source). The paddy straw substrate was milled and sieved to a particle size of 200 µ. After incubation, culture supernatants obtained by centrifugation was used as extracellular enzyme source.

### Simultaneous xylanase production and biomass deconstruction under solid-state fermentation

Different natural lignocellulosic materials such as paddy straw, corn cobs, sugarcane trash, sawdust, and crop residue (sapota leaves) were sun-dried to reduce the moisture content and then milled and sieved to 200 µ. About 10 g of substrates were taken moistened with the minimal salt medium in a ratio of 1:2, and autoclaved at 121 °C for 15 min. The untreated substrates were inoculated with the overnight grown inoculum in nutrient broth and incubated at 37°C for 48 h. The contents were extracted by suspending 50 mM phosphate buffer (pH 5.0), centrifuged at 10,000 rpm for 10 min, and the clear cell-free supernatant was used for the enzymatic assay.

## RESULTS AND DISCUSSION

Microorganisms produce extracellular enzymes during the decomposition of crop residues. These enzymes include amylase, cellulase, xylanase, ligninase and lipase involved in hydrolyzing, oxidizing or reducing crop residues, and converting them into the metabolic substrate.

**Table 1. Screening of bacterial isolates from different vermicasts for xylanase activity**

Culture	Xylanase activity (IU mL <sup>-1</sup> )							
	24h		48h		72h		96h	
PSX1	7.60	0.44	11.55	0.66	10.74	0.61	9.87	0.57
SDX3	6.46	0.37	10.72	0.68	9.92	0.57	9.75	0.56
LRX3	4.91	0.28	8.09	0.46	6.54	0.37	6.03	0.34
DLX1	4.57	0.26	5.45	0.31	4.94	0.28	3.84	0.22
PSX5	3.71	0.21	9.79	0.56	5.59	0.32	4.42	0.25
SDX1	3.55	0.20	5.95	0.34	3.16	0.18	2.88	0.16
<i>Bacillus pumilus</i> (MTCC 9861)	6.32	0.36	11.26	0.65	10.78	0.62	9.53	0.055

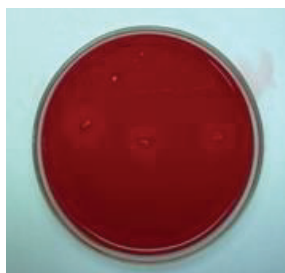
IU - One enzyme unit is expressed as 1 µmol of glucose released mL<sup>-1</sup> of enzyme min<sup>-1</sup> under standard conditions

In this context, the predominant polymers hemicelluloses have to be effectively converted into corresponding simple sugar hemicellulase for biobased product development. In search of potential microbes producing these enzymes, vermicasts obtained from various agricultural wastes served as sources for selective enrichment and isolation of xylanolytic microorganisms. In the present study, many xylanolytic bacteria were isolated from vermicasts of different agricultural residues by

following enrichment and isolation. Of the eleven isolates screened based on hydrolysis capacity on the congo red plates enriched with xylan, two potential isolates; PSX1 (5.75 cm) followed by SDX3 (5.67 cm) (Figure 1), were further characterized based on the activity of enzyme xylanase. Nagar *et al.* (2012) screened the xylanolytic bacteria *Bacillus subtilis* and *Bacillus pumilus* and qualitatively identified them with the congo red test.

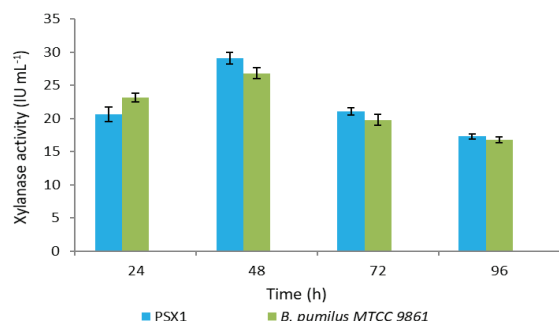
Xylanase activity of bacterial isolates measured

using xylan as a substrate is presented in Table 1. While measuring enzyme production during the growth of the isolates, 48 to 54 h of fermentation was found maximal activity and declined thereafter.



**Figure 1. Hemicellulose hydrolyzing ability of bacterial isolate *B. flexus* PSX1**

Growth and xylanase production by *B. flexus* PSX1 ( $11.63 \text{ IU mL}^{-1}$ ) was assayed and found that the isolates reached their maximum growth at 42 h of incubation. Xylanolytic enzymes could be produced by several bacteria and fungi and have been extensively studied for their enzyme production as well as their ability to grow on waste cellulosic material (Domingues *et al.*, 2000). *Bacillus sp.* are the major agents for decomposition and decay and thus possess the capacity to produce a broad range of enzymes. Based on the hydrolytic capacity of the isolates for xylanolysis and xylanase activity, further studies on the characterization of the isolates and their ability to produce xylanase were restricted to the above isolate PSX1.

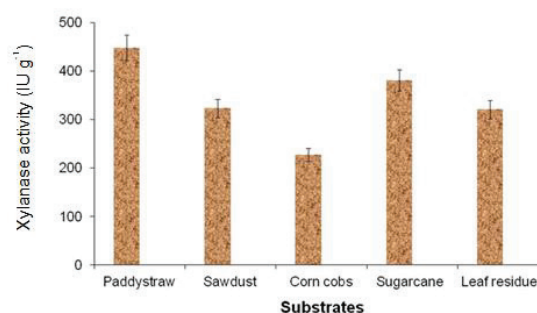


**Figure 2. Xylanase enzyme production by *B. flexus* PSX1 under submerged fermentation using paddy straw as substrate**

The isolated xylanolytic bacteria were analyzed at the molecular level using the 16S rRNA gene sequencing, and the results revealed that the xylanolytic isolate PSX1 showed maximum similarity with *Bacillus flexus* to about 98 per cent. In addition to molecular characterization, biochemical and morphological characteristics of the above isolates also confirmed the identity of the xylanolytic isolate PSX1 as *Bacillus flexus* PSX1. Similarly, xylanolytic *Bacillus pumilus* and *Bacillus sp.* were also identified based on their physiological and morphological characteristics (Nagar *et al.*, 2012).

Many microorganisms are stimulated to increase

enzyme synthesis by adding an inducer to the culture media. This inducer may be the substrate for the enzyme or the modification of the substrate. Most of the hydrolytic enzymes are inducible enzymes and hence substrate rich in cellulose and hemicellulose content must therefore be added to the minimal salt medium to stimulate enzyme production. In the present study, xylanase production by *B. flexus* PSX1 using paddy straw in submerged fermentation yielded a maximum of  $29.08 \text{ IU mL}^{-1}$  (Figure 2). At the same time, monitoring the time course of xylanase production during the growth of the strain, PSX1 revealed that the xylanase synthesis started along with growth and reached a maximum at 48 h and declined thereafter. Result of present study was supported by Saleem *et al.* (2002) who reported the production of xylanase in submerged culture in the minimal salt medium using *Bacillus subtilis*.



**Figure 3. Xylanase production by *B. flexus* PSX1 under solid-state fermentation using different lignocellulosic substrates**

Agro-industrial residues are generally considered the best substrates for solid-state fermentation and enzyme production by microorganisms (Virupakshi *et al.*, 2005). Among the different substrates tested for xylanase production by *B. flexus* PSX1 under solid-state fermentation, maximal xylanase activity was obtained with paddy straw ( $436.52 \text{ IU g}^{-1}$ ) as substrate (Figure 3). Similarly, Banu and Ingale (2012) reported a xylanase yield of  $489.4 \text{ IU g}^{-1}$  by *Bacillus pumilus* AB-1 using wheat bran as a substrate under SSF. A considerable reduction in the hemicellulose content and increased recovery of reducing sugar as xylose due to the growth of *B. flexus* PSX1 on paddy straw suggest that xylan conversion by the inoculated culture due to secretion of xylanase. The reported xylanase activity for *B. flexus* PSX1 both under submerged fermentation and solid-state fermentation was comparable and higher than some reported yield.

## CONCLUSION

From the study, it was concluded that isolated bacterium *Bacillus flexus* PSX1 had the efficiently converted hemicellulose component of paddy straw by the production of xylanase. Hence, further

improvement of xylanase yield would be possible by both strain improvement and fermentation strategies.

## REFERENCES

- Banu, A. and S. Ingale. 2012. Xylanase production by *Bacillus pumilus* AB-1 under solid state fermentation and its application. *Bullet. Environ. Sci. Res.*, **1(1)**: 35-43.
- Domingues, F.C., Queiroz, J. A., Cabral, J.M. and L. P. Fonseca. 2000. The influence of culture conditions on mycelial structure and cellulase production by *Trichoderma reesei* Rut C-30. *Enzyme Microb. Technol.*, **26**: 394-401.
- Miller, G. L. 1972. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. *Anal. Chem.*, **31**: 426-428
- Miyamoto, K. 1997. Renewable biological systems for alternative sustainable energy production. *FAO Agricultural Services Bulletin*. Italy.128.
- Nagar, S., Mittal, A. and V.K. Gupta. 2012. A cost-effective method for screening and isolation of xylan degrading bacteria using agro-waste material. *Asian J. Biol. Sci.*, **5(8)**: 384-394.
- Saleem, M., Akhtar, M.S. and S. Jamil. 2002. Production of xylanase on natural substrates by *Bacillus subtilis*. *Int. J. Agri. Biol.*, **4(2)**: 211-213.
- Shanker, T., Mariappan, V. and L. Isaiarasu. 2011. Screening Cellulolytic Bacteria from the Mid-Gut of the Popular Composting Earthworm, *Eudrilus eugeniae* (Kinberg). *World J. Zool.*, **6(2)**: 142-148.
- Sushil, N., Kumar, V., Kumar, D., Kumar, L. and R. Chander Kuhad. 2010. Production and optimization of cellulase-free, alkali-stable xylanase by *Bacillus pumilus* SV-85S in submerged fermentation. *J. Ind. Microbiol. Biotechnol.*, **37**: 71-83
- Virupakshi, S., Babu, K.G., Gaikwad, S.R. and G.R. Naik. 2005. Production of a xylanolytic enzyme by a thermoalkaliphilic *Bacillus* sp. JB-99 in solid-state fermentation. *Process Biochem.*, **40**: 431-435.