



## RESEARCH ARTICLE

# Xylanolytic bacteria isolated from earthworm casts and its potentiality for biomass conversion

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## ABSTRACT

Lignocellulosic biomass is the major agricultural waste in countries like India which have major source of income as agriculture. There is a need to study the conversion of cellulose, hemicellulose and lignin which are the major components of lignocellulosic biomass. Being a recalcitrant material microbial conversion of lignin is of minor important. This study mainly focuses on bioconversion of hemicellulose and the study encompasses screening and identification of bacteria that can produce xylanase enzyme from vermicasts using different natural lignocellulosic materials such as paddy straw, coir pith, dried leaves, saw dust and leaf residue as substrate. Bacterial cultures from vermicasts, capable of hydrolyzing xylanase are screened. Enrichment of cultures is done in xylan enriched minimal media. Selected and purified bacterial cultures are grown in xylan media were activated and transferred into the xylan broth for further enzymatic assay. The bacterial cultures along with the standard culture *Bacillus pumilus* are taken for enzymatic assay. Among the 7 cultures PSX1 recorded the maximum activity of  $11.55 \text{ IU ml}^{-1}$  followed by SDX3 ( $10.71 \text{ IU ml}^{-1}$ ) at 48 h of growth and declined further. The standard culture *Bacillus pumilus* recorded  $11.27 \text{ IU ml}^{-1}$ . PSX1 culture is selected for further studies to produce xylanase.

Key words: vermicast, *Bacillus* sp., paddy straw, xylan, bioconversion

## Introduction

The effective and economic utilization of ligno-cellulosic materials will be important 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 ligno-cellulosic 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, glu- curonic acid and other sugars. Xylan is therefore the predominant component in hemicellulose.

#### *Materials and methods*

##### **Isolation of bacteria from earthworm casts**

About 1g of earthworm casts was taken and add- ed 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 nutri- ent agar medium for isolation of bacteria. The plates are incubated at 28° C and the colonies are counted from 2<sup>nd</sup> day onwards till 10 days. The isolated bac- teria were purified by streak plate technique. Single colony was transferred to nutrient agar slopes and maintained under refrigerated condition.

##### *Screening of isolates based xylanolytic activity*

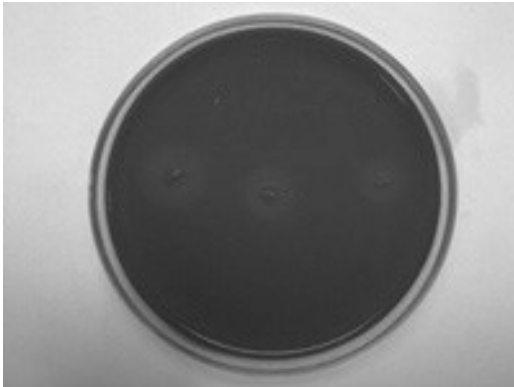
The xylan plates with bacterial isolates were tested for hemicellulose hydrolysis using congo red solution and the plates were flooded with 10 ml of congo red solution for 15 mins. Then the plates were destained with 1ml of 1M NaCl by washing the plates. Clear halo zone around the bacteria colony indicated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

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

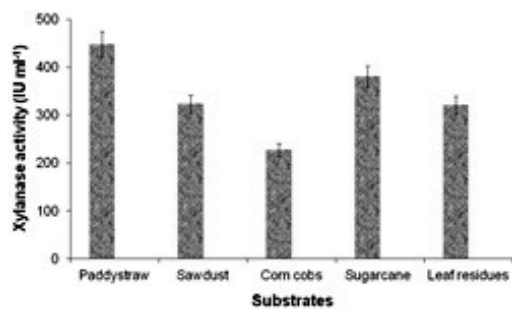
Different natural lignocellulosic materials such as paddy straw, corn cobs, sugarcane trash, saw dust and crop residue (sapota leaves) were sun dried to reduce the moisture content and then milled and sieved to 200 micron size. 10 g of substrates were tak- en moistened with minimal salt medium in a ratio 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 in 50 mM phosphate buffer (pH 5.0), centrifuged at 10,000 rpm for 10 min and the clear cell free superna- tant was used for the enzymatic assay.

##### *Results and discussion*

In this context, the predominant polymers hemi- celluloses have to be effectively converted into corre- sponding simple sugar hemicellulase respectively for biobased product development. In search of poten- tial microbes producing these enzymes, vermicasts obtained from various agricultural wastes served as a source for selective enrichment and isolation of xylanolytic microorganisms. Of the eleven isolates screened based on hydrolysis capacity on the congo



Hemicellulose hydrolysing ability of bacterial isolate *B. flexus* PSX1



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

Xylanase activity (IU ml <sup>-1</sup> )				
Culture	24hrs	48hrs	72hrs	96hrs
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				

red plates enriched with xylan, two potential isolates PSX1 (5.75 cm) followed by SDX3 (5.67) were further characterized based on the activity of respective enzyme (xylanase). Morphological, biochemical and molecular (16S rRNA) characterization of these potential isolates identified as *Bacillus flexus* PSX1 and *Bacillus subtilis* SDX3.

Xylanase production by *B. flexus* PSX1 using paddy straw in submerged fermentation yielded a maximum of 29.08 IU ml<sup>-1</sup>. While monitoring time course of xylanase production during growth of the culture revealed that the xylanase synthesis started along with growth and reached maximum at 48 h and declined thereafter. Among the different substrates tested for xylanase production by *B. flexus* PSX1 under solid state fermentation, a maximal xylanase activity obtained with paddy straw (436.52 U g<sup>-1</sup>) as substrate. 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.

### Conclusions

From the study it was concluded that isolated bacterium *Bacillus flexus* PSX1 had the efficiency of converting 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

Shanker, T., V. Mariappan 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.

Miller, G. L. 1972. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem.,31: 426-428

Sushil, N., V. Kumar, D. Kumar, L. Kumar 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

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