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# Production of Xylanase by Litter Degrading Fungal Species Using Agro-industrial Wastes as Substrates by Solid State Fermentation

**Muthusamy Palaniswamy[1*], Thenmozhi V Arulanandham[1], Jayaraman Angayarkanni[2]**

*[1]Department of Microbiology, School of Life Science, Karpagam University, Coimbatore 641 021, Tamil Nadu, India.
[2] Department of Microbial Biotechnology, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India.

## ABSTRACT

Xylan is a heterogeneous polysaccharide which are mainly constituted by β-1,4-linked-D-xylopyranose. Xylan forms a main constituent of hemicellulose, a plant polysaccharide. The most important enzyme in the xylan biodegradation is the endo-1, 4-β-xylanase (EC 3.2.1.8) that releases xylopyranose units. Agricultural wastes are found in staggering amounts in our country. These residues represent one of the most energy-rich resources available and when not properly discharged or used, add to environmental pollution. In the present study, thirty fungal species of various genera were isolated from litter soil. The objective of the study is to find the fungus that produces high titres of endo-β-1, 4-xylanase in solid state fermentation using various agro-industrial wastes as substrate. Three fungal species were selected and solid state fermentation was carried out. Aspergillus nidulans and Trichoderma viride produced higher enzyme production of 561.75 U/g and 446.25 U/g respectively when grown on rice bran. Penicillium frequentans produced the highest among the three producers when grown on paddy straw to an extent of 735 U/g. These results indicate that agro-industrial wastes can be used to produce xylanase and thus reduce the risk of environmental pollution.

**Keywords:** Xylanase, solid state fermentation (SSF), submerged fermentation (SmF), agro-industrial wastes

***Corresponding author***
Email: m.palaniswamy@gmail.com

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## INTRODUCTION

Hemicellulose is one of the most abundant naturally occurring polymers, next to cellulose. They are present in plant cell walls in association with lignin and cellulose. A major component of hemicellulose is xylan, a polymer consisting of β-(1,4)-D-Xylopyranoside residues as the backbone. Xylans from different origins have different molecular weight, composition, number of side chains and level of aceylation [6]. Upto 35% of the dry weight of monocot plants is comprised of xylan. Xylanase (endo-1,4-β-xylanase) and β-xylosidase (β-d-xyloside xylohydrolase) are the main constituents of microbial xylanolytic enzyme systems. The xylanolytic enzymes include endo-β-1,4-xylanase (1,4-β-D-xylan xylanohydrolase, E.C. 3.2.1.8), β-D-xylosidase (1,4-β-xylan xylanohydrolase, E.C. 3.2.1.37), and debranching enzymes (esterases) [4]. Many organisms produce xylanase, the key enzyme in degrading xylan into xylooligosaccharides. Fungi are the major producers of different types of xylanases that degrade the different polymeric xylan at various linkages [7]. Filamentous fungi are more attractive xylanase producers than bacteria or yeast, because they excrete the enzymes into medium at a high level [9].

Xylanases and their mechanism of degradation are of particular interest in the recent past because they have a wide variety of industrial application. They can be used in biobleaching of paper and kraft pulp, food industry, animal feed industry, waste treatment, bioethanol and textile industry [1, 10].

Production of xylanase by solid-state fermentation (SSF) using various lignocellulosic substrates has been reported previously and was found to have several advantages, such as higher productivity as well as lower operational and capital costs. Because the cost of the enzyme is the major factor for broad application, approaches that either decrease the medium cost or increase production efficiency should be investigated. Since SSF can be performed on a variety of lignocellulosic materials, such as wheat straw, wheat bran and corncob, the cost of xylanase production can be reduced greatly [5, 15,16].

In the present study, we have isolated thirty fungal species of various genera from Karpagam University campus by litterbag technique [12]. The objective of the study is to find the fungus that produces high titres of endo-β-1, 4-xylanase (E.C. 3.2.1.8) in solid state fermentation using various agro-industrial wastes as substrate.

## MATERIALS AND METHODS

### Study site

Mycoflora were isolated from litter in and around the campus of Karpagam University, Coimabtore, Tamil nadu, India.

**Isolation of mycoflora**

Litter mycoflora were isolated by nylon net litterbag technique [12]. Paddy straw, coir pith, Eucalyptus globules and Tectona grandis leaves were air dried and used as baits. Each of the air dried material (10 g) was placed in a nylon net bag (1 mm mesh; 20 x 20 cm size) with colored beads (to identify the baits) and buried in the litter soil at 10 cm depth. For each sample, three replicates were maintained. Samples were collected at regular monthly intervals in pre-sterilised polyethylene bags. The litter samples were powdered and suspended in sterile distilled water (0.1 g/50 ml). This suspension (1.0 ml) was plated onto the Petri plate containing rose-bengal-streptomycin-agar medium.

All thirty fungal species were identified and deposited in the Karpagam Microbiology Culture Collection Center (KMCCC), Karpagam University, India. The fungal species were sub cultured on potato dextrose agar slants and maintained in a refrigerated condition. The fungal species were identified by morphological and physiological analysis.

**Preparation of spore inocula**

Spores were harvested from 4 to 6 day old agar slants by suspending spores in sterile distilled water and filtering through gauze into Erlenmeyer flask [11].

**Screening for xylanase production by submerged fermentation (SmF)**

One percent spore suspension ($10^6$ spores/ml) were inoculated into 100 ml Erlenmeyer flask containing 10 ml of minimal salt medium [3] composed (g/l) of urea 1.4, magnesium sulphate 0.25, calcium chloride 0.05, zinc sulphate 0.02, manganese sulphate 0.02, copper sulphate 0.005, ferrous sulphate 0.1, sodium sulphate 1.0, ethylene diamine tetra acetate 0.6, sodium dihydrogen orthophosphate 1.56, peptone 7.5, yeast extract 2.5, glucose 5.0 and xylan 10.0. Flasks were incubated for 7 days at 30$^{o}$C in an orbitory shaker at 150 rpm. After the incubation period, the cultures were centrifuged at 10,000 rpm for 20 min at 4$^{o}$C and the clear supernatant was used as enzyme source [11].

**Production of xylanase by solid state fermentation (SSF)**

Various agro-industrial wastes like paddy straw, corn cob, wheat bran, rice bran, rice husk, wood chips, sugarcane baggase, cotton seed and maize stover were used as solid substrate for xylanase production. The agro-industrial wastes were collected from the local market and agricultural farms in and around Coimbatore, Tamil Nadu. Sterilized solid substrate (10 g) moistened with 15 ml of mineral salts solution (containing sodium nitrate, di-potassium hydrogen phosphate, magnesium sulphate, ferrous sulphate, potassium chloride, peptone and yeast extract) was inoculated using 1ml spore suspension ($10^6$ spores/ml). It was incubated at room temperature for 4 days. After completion of fermentation, 50 ml of sodium phosphate buffer (pH 6) was added, mixed thoroughly and kept for shaking at 150 rpm for 1hr. The

enzyme was then extracted by filtration using Whatman No.1 filter paper. The enzyme solution was centrifuged at 10,000 rpm for 15min and xylanase activity of the supernatant was measured.

**Xylanase assay**

Xylanase activity was determined by measuring the increase in reducing sugars formed by enzymatic hydrolysis of oat spelt xylan. An appropriately diluted enzyme sample (1 ml) was mixed with 0.1 ml of 1% (w/v) oat spelt xylan in 0.05M sodium citrate buffer (pH 5.0), and the mixture was incubated at 50°C for 30min. The reducing sugar generated was quantified by the Somogyi method [16]. D-Xylose was used as a standard. One unit of enzyme activity was defined as the amount of enzyme which catalyzed the liberation of 1µmol of reducing sugar per min.

## RESULTS

Litterbag technique was adopted for isolating xylanolytic fungal species using the various litter samples, paddy straw, coir pith, Tectona grandis and Eucalyptus globulus leaf litter collected in and around study area as described in materials and methods. In order to select new potential fungal species, thirty fungal species were screened for the production of xylanase (E.C. 3.2.1.8) activity when grown by shake flask batch cultivation on oat spelt xylan as the carbon sources. The isolates were screened by measuring xylanolytic activity in shake flask fermentation method. All the fungi exhibited fairly good enzyme activities in shake flask cultivation, with production ranging from 5.60 to 182 U/ml. Three organisms (Aspergillus nidulans, Penicillium frequentans and Trichoderma viride) that produced the highest level of xylanase were chosen for solid state fermentation and they have showed promising results with few solid substrates. Table 1 shows the results of screening of xylanase production in submerged fermentation (SmF) by the thirty fungal species. Fig. 1 shows the profile of xylanase production by the three chosen organisms while using various agro-industrial wastes as substrates.

**Table 1: Screening of litter degrading fungi for xylanolytic enzyme production by SmF**

| Name of the fungal species | Xylanase activity (U/ml) |
|---|---|
| Acremonium murorum | 29.50 |
| Aspergillus alutaceus | 105.80 |
| Aspergillus flavipes | 91.23 |
| Aspergillus flavus | 76.33 |
| Aspergillus fumigatus | 62.67 |
| Aspergillus galucus | 77.91 |
| Aspergillus nidulans | 170.23 |
| Aspergillus niger | 43.01 |
| Aspergillus ornatus | 5.60 |
| Aspergillus oryzae | 25.34 |
| Aspergillus sydowii | 68.11 |
| Aspergillus terreus | 78.90 |

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|---|---|
| Aspergillus ustus | 43.55 |
| Aspergillus versicolor | 45.01 |
| Aspergillus wentii | 16.89 |
| Fusarium culmorum | 58.45 |
| Humicola insolens | 23.10 |
| Mucor circinelloids | 78.20 |
| Penicillium chrysogenum | 16.30 |
| Penicillium frequentans | 182.00 |
| Penicillium nigricans | 9.11 |
| Penicillium restrictum | 39.09 |
| Penicillium citrinum | 34.30 |
| Penicillium brevicompactum | 21.99 |
| Rhizopus oryzae | 60.95 |
| Rhizopus stolonifer | 75.66 |
| Trichoderma koningii | 129.55 |
| Trichoderma reesei | 23.01 |
| Trichoderma viride | 176.40 |
| Trichoderma harizanum | 70.93 |

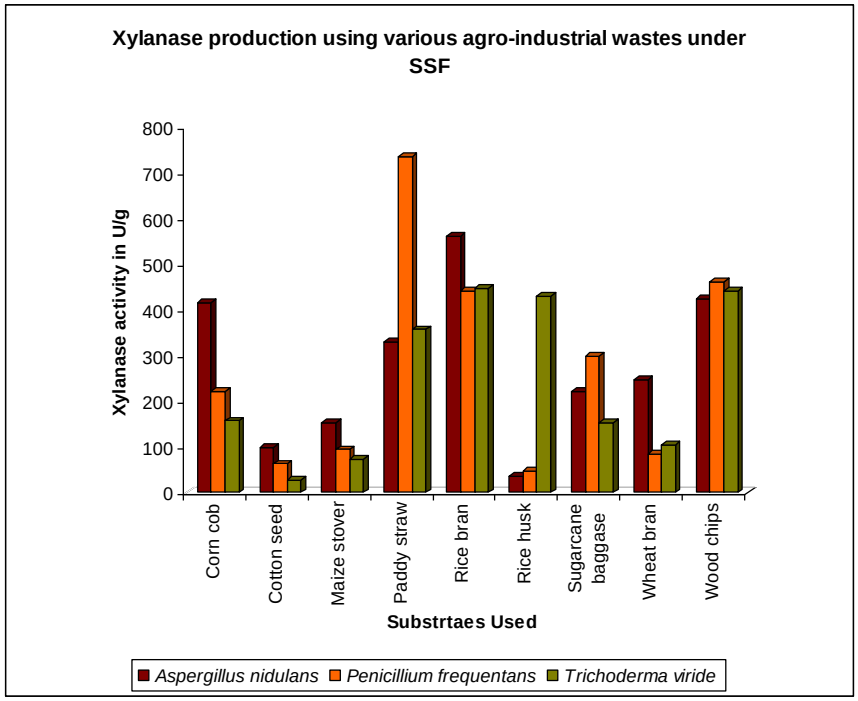


**Fig 1: Xylanase production using various agro-industrial wastes under SSF**

## DISCUSSION

Xylanolytic enzyme production is dependent on various parameters like temperature, pH, carbon, nitrogen source etc. Many organisms from the isolates showed promising xylanase production when grown in shake flask cultivation using xylan as the sole carbon source. Out of

the thirty fungal species the following like Aspergillus alutaceus, A. nidulans, Penicillium frequentans, Trichoderma koningii and T. viride showed high level of enzyme production.

In the present study, out of the five high level producers of xylanase we have chosen one organism from each genera for further studies. Aspergillus nidulans and Trichoderma viride produced higher enzyme production of 561.75 U/g and 446.25 U/g respectively when grown on rice bran. Penicillium frequentans produced the highest among the three producers when grown on paddy straw to an extent of 735 U/g. Aspergillus nidulans showed a 3 fold increases and Trichoderma viride showed an increase of 2.5 fold enzyme production in SSF compared to SmF. Penicilliun frequentans showed a 4 fold increase in the enzyme production in SSF. Camassola et al. used different ratios of sugarcane bagasse and wheat bran as a solid substrate and found 37.87±2.26 U/gdm of xylanase activity using a Penicillium echinulatum. Jeya et al. used wheat bran as substrate for optimization of solid state fermentation using Aspergillus versicolor and found the xylanase activity to be 3249.9 U/g. Reis et al. isolated a wild strain of Aspergillus nidulans grown on xylan and lignocellulosic residues like corn cob, wheat bran, wheat straw and sugarcane bagasse as substrates for xylanase production. The highest activity was found when 5% wheat bran was used as substrate and it was found to be 269±31 U/ml. Pang et al. produced xylanase from Trichoderma sp. isolated from soil on various agro-industrial wastes like sugarcane bagasse, palm kernel cake and rice husks. Highest xylanase activity was found to be around 61.11 U/g when grown on sugarcane bagasse [17].

## CONCLUSION

In conclusion, this is the first report of the production of xylanolytic enzyme by litter degrading fungal isolates using solid state fermentation. These results indicate that agro-industrial wastes can be used to produce xylanase for use in various industries like food, textile and kraft pulp bleaching.

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