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RESEARCH ARTICLE

AGROWASTE UTILIZATION AND PRODUCTION OF EXTRA CELLULAR ENDO XYLANASE BY *Penicillium janthinellum* MTCC 10889 IN SOLID STATE FERMENTATION

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ABSTRACT

For cost effective production of endo xylanase, an enzyme of immense industrial importance, a fungal strain was isolated and identified as *Penicillium janthinellum.* The strain was found to ferment a number of agricultural residues in the solid state fermentation media, of which dried grass and rice straw showed promising results. The best concentration of these substrates was found to be 0.5% (w/v) and 1% (w/v) respectively at a moisture content of only 5-6%. The kinetics of endoxylanase production showed that highest enzyme production could be achieved within 72nd hours of cultivation at pH 6.0 and at 27°C. Amongst the nitrogen sources, peptone was found to be the best organic source for boosting up of the endo xylanase production. Among the metal ions tested, Mn^{2+} brought about a 1.15 fold increase in enzyme production in both dried grass and rice straw supplemented culture medium probably acting as a cofactor of the enzyme

INTRODUCTION

Large quantities of lignocellulosic wastes are generated through forestry, agricultural practices and industrial processes, particularly from agro-allied industries (Okafor *et al*., 2007) which generally accumulate in the environment and thereby cause pollution problem (Abu *et al*., 2000). Most of the wastes are disposed by burning; a practice considered as major factor in global warming (Levine, 1996) and is not successfully utilized as renewable source for enzyme production. But since last decade, there has been increasing research interest in the value of bio-sourced materials recovered from residual biomass (FitzPatrick *et al*., 2010) and agro wastes. The agro wastes being the enriched source of ligno cellulosic residues can be effectively used as the raw material for the commercial production of various value added materials by bringing about a significant reduction in the cost of production (Hahn-Hägerdal *et al*., 2006). Agricultural wastes include a diversity of grasses, rice and wheat straw and are hydrolyzed to fermentable sugars by ligno cellulolytic enzymes, of which endoxylanase (EC 3.2.1.8) attacks the main chain of xylans, a hemicellulosic fraction of higher plant cell walls (Burke *et al.*, 1974). Hence endo xylanase becomes one of the important enzymes for their wide range of potent applications in different industries, like bio-pulping of wood (Eriksson, 1985; Eriksson and Kirk, 1985), pulp bleaching (Jurasek and Paice, 1988; Kantelinen *et al*., 1988.), treating animal feed to increase digestibility (Wong *et al.,* 1988), processing food to increase clarification (Biely, 1985), and converting ligno cellulosic substances into feed stocks and fuels (Eriksson, 1985; Jeffries, 1985; Kim *et al.,* 2000). Although a number of micro organisms were reported to synthesize extra cellular endoxylanase (Goyal *et al.,* 2008), filamentous fungi are attracting greater attention than bacteria as potential sources of plant cell wall hydrolyzing enzymes such as xylanases because they secrete high levels of the enzymes into the culture medium (Bakri *et al,* 2003; Berry and Paterson, 1990). Moreover the product titers produced in SSF of these fungi were reported to be many-fold higher than that from submerged culture. SSF also appears to possess several biotechnological advantages such as, higher product stability, operation simplicity, high productivity fermentation, less favorable for growth of contaminants and concentrated product formation (Gupta *et al*., 2001), lower catabolic repression, cultivation of microorganisms specialized for water-insoluble substrates or mixed cultivation of various fungi, and lower demand on sterility owing to the low water activity used in solid state fermentation (Camassola and Dillon, 2010). The present study was designed to investigate the potential use of two agro-wastes namely dried grass and rice straw as carbon sources for endo xylanase production by the strain of *P.janthinellum PU 10* and to optimize various parameters for cost effective production of the enzyme.

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MATERIALS AND METHODS

Microorganism: The working strain, *Penicillium janthinellum* MTCC 10889 was isolated from the decaying vegetation enriched soil of West Bengal, India. The strain was identified and deposited to Microbial Type Culture Collection, India. The strain was maintained at 4° C on xylan (oat spelt)-agar medium.

Chemicals: All chemicals used were of analytical grade. All the substrates used were collected from agricultural wastes, dried, pulverized as 40 mesh particle size and were supplemented in the culture media in place of pure xylan.

Cultivation of the strain in solid state fermentation medium (SSF): The strain was cultivated in 50 mL Erlenmeyer flasks each containing 10 mL Basal Medium (BM)composed of (g L-1): peptone 0.9; (NH4)2HPO4 0.4; KCl 0.1; MgSO4.7H2O 0.1 and pure xylan from beechwood (Sigma) 0.5. (pH: 6) at 27°C.For SSF, the strain was cultured in 100 ml Erlenmeyer flasks containing totally dried substrates and salts (based on 10ml medium) moistened with 0.5 ml of distilled water at 27°C and the beech wood xylan (Sigma) was replaced by agro waste residues.

Enzyme extraction and assay: Cultures were picked up at different time intervals, sterile water was added to make up its final volume to make it equivalent to that of 10 ml LSF media, followed by a thorough cyclomixing and centrifugation at 10,000 rpm for 5 min at 4°C. The supernatant was used as the crude enzyme. To measure the activity of endoxylanase, the assay mixture (1ml) containing an equal volume of enzyme and 1 %(w/v) oat spelt xylan (Sigma, U.S.A) in 0.1(M) phosphate buffer (pH-6) was incubated at 60°C for 10 minutes. The reducing sugar released was measured by the dinitro salicylic acid method (Bernfeld, 1955) taking xylose as standard. Blanks were prepared with inactivated enzymes. One unit of endo xylanase was defined as the amount of enzyme that liberated 1µ mole of xylose per ml per minute of reaction.

Optimization of different parameters for enzyme production: Fermentation period is important parameter for enzyme production by *Penicillium janthinellum*. With a view to replace beech wood xylan (Sigma), a costly substrate for xylanase production, various cheap and abundantly available agro wastes, namely dried grass and rice husk, master seed husk, orange beggase were supplemented as carbon source. The concentration of the agro wastes acting as the inducers was tested by varying their concentration (0.25-1.50% w/v). The effect of different temperature was studied by incubating the culture containing flasks at various temperatures (7-57°C). The strain was grown in different flasks containing media with various pH (4-9) to check the optimum pH for enzyme production. Similarly, the effect of nitrogen source and metal ions were studied by supplementing various organic and inorganic nitrogen sources (0.09% w/v) and different salts of metal ions (10 mg) in the culture medium. The effect of different moisture content was studied by incubating the culture containing flasks with initial moisture content of the substrate [1- 7% (v/w)]. The effect of cultivation time was determined by picking up the culture containing flasks with optimized media at various time intervals (24-120 hours) , followed by an assay of the enzyme activity.

RESULTS AND DISCUSSION

Bio potential of agrowastes for endo xylanase production: Xylan being costly for large-scale production of xylanases, lignocellulosic materials can be used as cost-effective substrates for xylanase production (Haltrich *et al*., 1996; Beg *et al*., 2000). *Penicillium janthinellum* PU 10 MTCC 10889 was found to degrade various ligno cellulosic agro wastes. Biopotential of six agricultural wastes were evaluated in solid state fermentation for xylanolytic enzyme production by *Penicillium janthinellum* namely dried grass , rice straw , orange bagasse , sugar cane bagasse, mustard seed husk and jute fiber, among these wastes rice straw followed by dried grass was found to be the best substrate (Table 1) and hence rice straw and dried grass were used as the carbon source in subsequent experiments in place of expensive xylan.

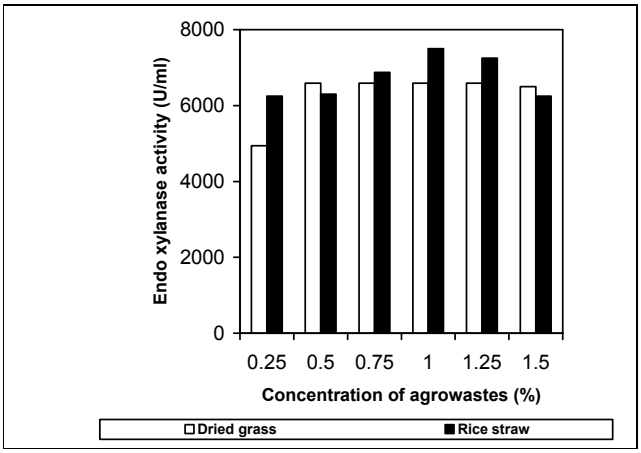

Fig 1. Effect of substrate concentration on endoxylanase production by *P. janthinellum* PU 10

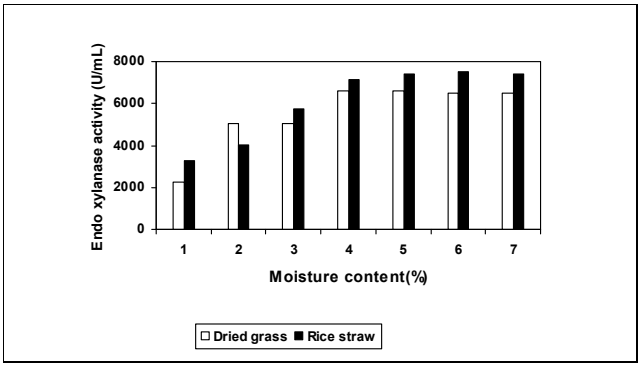

Fig 2. Effect of moisture concentration on endoxylanase production by *P. janthinellum* PU 10

Effect of concentration of agrowastes on endo xylanase production: The optimum concentration of rice straw and dried grass for endoxylanase production were found to be 1% (w/v) and 0.5%(w/v) respectively (Fig 1), further increase of which in the fermentation media reduced the enzyme production probably due to the adverse effect of higher load of nutrient supplements present in these substrates (Omojasola *et al*., 2008) or as a result of hindrance of mass transfer of oxygen by higher amount of solid substrate.

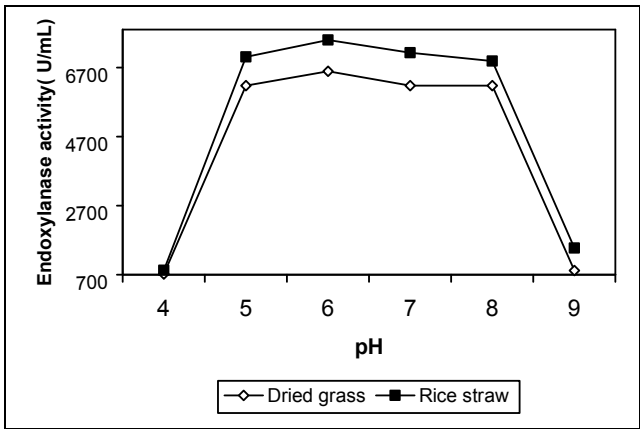

Fig 3. Effect of initial pH on the production of endoxylanase by *P. janthinellum* PU 10

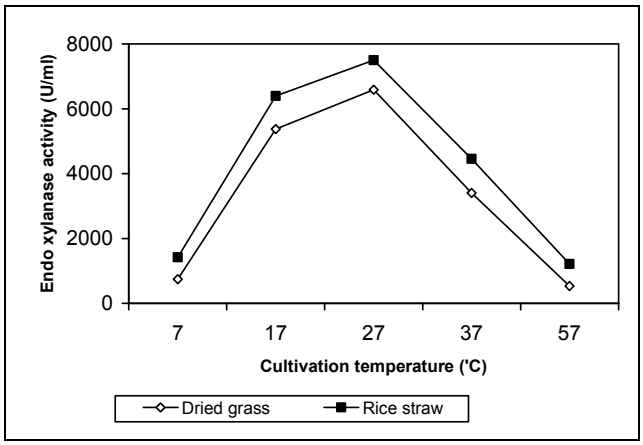

Fig 4. Effect of temperature on the production of endoxylanase by *P janthinellum* PU 10

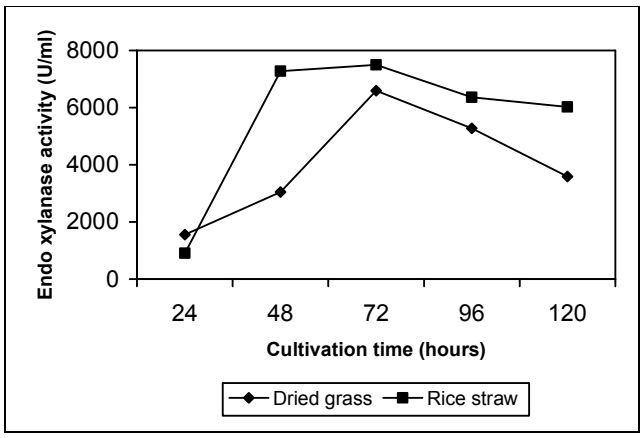

Fig 5. Effect of cultivation time on the production of endoxylanase by *P janthinellum* PU 10

Effect of moisture content on endo xylanase production: Initial moisture content is one of the key factors influencing xylanase production (Yang *et al,* 2006) and the endoxylanase production by the present strain sharply increased with increasing moisture content. The best production was found if the SSF media was moistened with 5% water in case of dried grass and 6 % in case of rice straw (Fig 3). But further increase in moisture content could no longer increase the enzyme yield.

Table 1. Inducing effect of various agro wastes on endoxylanase production from the SSF of *P. janthinellum* PU 10

Carbon source	Xylanase activity (U/ml)
Pure xylan (sigma)	4545
Dried grass	6590
Rice straw	7499
Jute fiber	1931
Master seed husk	4886
Orange begasse	1590
Sugar cane begasse	2386

Cultivation time: 72 hrs.

Table 2.Effect of nitrogen sources on endo xylanase production production from the SSF of *P. janthinellum* PU 10

Nitrogen source (0.09%)	Xylanase activity (U/ml)	
	Dried grass	Rice straw
Peptone	6590	7499
Gelatin	5649	6933
Tryptone	4472	5333
Urea	6002	8133
Yeast extract	5884	7466
Ammonium chloride	6825	6800

Cultivation time: 72 hrs.

Table 3. Effect of metal ions on endo xylanase production production from the SSF of *P. janthinellum* PU 10

Metal ions (0.1 % w/v)	Endoxylanase activity (U/ml)	
	Dried grass	Rice straw
None	6590	7499
Na^{+}	5474	7195
K^{+}	5981	6275
Mg^{2+}	5373	6823
Mn^{2+}	7604	8312
Ca^{2+}	6083	7817
Li^{2+}	507	993
Cu^{2+}	709	2233
Pb^{2+}	3548	5459
Hg^{2+}	405	1240
Sn^{2+}	ND	868
Sr^{2+}	6286	7444
Fe^{2+}	5373	5210
EDTA	4258	4590

Cultivation time: 72 hrs.

Effect of initial pH on endo xylanase production: To optimize the endoxylanase production, the SSF was carried out for 72 h at various pH ranging from 4.0 to 9.0. Although the enzyme production was stable at a pH range of 5-8, the best production was obtained both from dried grass and rice straw, when the initial pH was set at 6.0. Other xylanase producing strains of *P. janthinellum* showed varied pH preference for endoxylanase production (Rodrigues *et al*, 1999; Oliveira *et al*, 2004; Curotto *et al*, 1994) ranging from 4 to 7.

Effect of incubation temperature on endo xylanase production: To examine the effect of temperature on endo xylanase production by *Penicillium janthinellum* PU 10, the fungus was grown at various temperatures (7°C to 57°C). The maximum endoxylanase production from both rice husk and dried grass supplemented media was achieved at 27-28°C; a significant decline in endoxylanase activity was evident with further increase in temperature (Fig 4). Higher temperature severely affected the enzyme production, which may be due to the fact that with increase in temperature, sporulation is induced that hampers the mycelial growth in fungus. Although

some thermophilic fungi showed higher optimum temperature for growth and enzyme production (Yang *et al,* 2006) similar temperature preference for xylanase production was found in *Penicillium janthinellum* (Rahman *et al*., 2003).

Effect of incubation time on xylanase production: The enzyme production kinetics showed that the maximal endo-xylanase production by *Penicillium janthinellum* was acieved at 72nd hour of incubation. After that it rapidly decreased, probably due to the depletion of nutrients in the medium which stressed the fungal physiology resulting in the inactivation of secretary machinery of the enzymes (Nochure *et al.,* 1993). Goyal *et al.,* 2008 reported maize straw and jowar straw induced xylanase production took 14 to 17 days for maximum production, whereas *Streptomyces* sp OM 09 could produce maximum level of endoxylanase from dried grass within only 48th hour of growth (Ray, 2010).

Effect of nitrogen source on xylanase production: Various organic and inorganic nitrogen sources were used to test their effect on the productivity of endo xylanase. These included peptone, gelatin, tryptone , urea , yeast extract and NH_4Cl at a concentration of 0.09% w/v. Table 2 indicates that among organic sources, peptone was the best source for enzyme production both from grass and rice straw supplemented media. On the other hand, urea enhanced enzyme production strongly in cultivation medium supplemented with rice straw than that of dried grass. But enzyme production was remarkably decreased in presence of urea, a report contrary to that of by *Aspergillus niger* (Acharya *et al*., 2008).Unlike *Penicillium canescens,* (Bakri *et al*., 2003) yeast extract could not enhance the enzyme production.

Effect of metal ions on endo xylanase production: Among the metal ions tested, Mn^{2+} brought about a 1.15 fold increase in enzyme production in both dried grass and rice straw supplemented culture medium probably acting as a cofactor of the enzyme. On the other hand, the addition of Sn^{2+}, Hg^{2+}, Li^{2+}, Cu^{2+} reduced the enzyme activity probably through the destruction of the active site by denaturing the thiol groups present there (Table 3).

CONCLUSION

The present work has established the potential of the newly isolated *Penicillium janthinellum* MTCC 10889 for endo xylanase production in SSF. High-level endo xylanase production by this strain in SSF than the other already reported strain of *Penicillium janthinellum* (Milagres *et al*, 1993**;** Milagres and Rolf**,** 1994; Curotto *et al.,*1994; Rodrigues *et al*., 1999; Oliveira *et al.,* 2004) and the recently reported other strains of *Penicillium* sp (Camassola and Dillon, 2010; Bakri *et al*., 2003) suggested that the working strain might be considered as a promising source for commercial production of endo xylanase. Further the ability of the strain to utilize agricultural residues like dried grass and rice straw would not only curtail the cost of enzyme production but help in successful disposition of agricultural wastes.

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