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Extra Cellular Endoxylanase Production from Solid State Fermentation of Dried Grass by *Streptomyces* sp OM 09

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ABSTRACT: The present study deals with the cost effective production of endoxylanase by a strain of *Streptomyces* sp, isolated from soil of Kanha National park and Tiger Reserve, India. Among various agricultural residues used in the solid state fermentation media, grass followed by rice straw proved to be the best carbon source for the growth and production of extracellular endoxylanase by the strain. Drying of the grass improved the enzyme production and best enzyme production was obtained at a concentration of 1%. Highest endoxylanase production was found at pH 6.0 and at 37°C. The kinetics of growth and enzyme production showed that highest enzyme production could be achieved within 48 hours of cultivation. The endoxylanase produced, after partial purification showed highest activity at 60°C and at pH 6.0.The enzyme showed moderate thermostability and stability at a pH range of 5.0- 8.5 and a substrate specificity towards xylan only. The improvement of enzyme activity in presence of thiol compounds indicated the presence of thiol group at the active site of the enzyme.

Key words: Endoxylanase, solid state fermentation, Streptomyces, Dried grass

Introduction

Xylan is a complex branched heteropolysaccharide found in plant cell wall , which can be randomly cleaved by endoxylanase (E.C.3.2.1.8) into xylose and xylo oligosaccharides (Rawasdeh et al, 2005).Apart from its use in pulp and papr industry, xylanase are also used for improving bread quality, for juice clarification and for liquefying the fruits and vegetables (Gupta and Kar, 2009).In spite of the enormous industrial importance, the production of the enzyme was hindered by the high cost of production (Goyal *et al*, 2008).

In order to curtail the production cost, one should use inexpensive substrates and follow an efficient fermentation process. It was established that the solid state fermentation (SSF) has several advantages over submerged fermentation (SmF) , due to higher productivity, convenience in product recovery, lower contamination and foaming problems and better exploitation of agro residues as substrates(Nigam and Singh, 1994, Gupta and Kar, 2009).Secondly, in the recent years utilization of agro wastes as a substitute for expensive xylan in the cultivation medium has gained interests among the researchers to make the production process cost effective. (Alberton *et al,* 2009, da Silva *et al,* 2005 , Ninawe and Kuhad, 2005).

A number of micro organisms like bacteria, fungi and yeasts have been reported to produce extra cellular endoxylanase (Rezende *et al*, 2002), but actinomycetes have been explored to the lesser extent, especially regarding xylanase production (Ninawe *et al,* 2006).

The present paper deals with the production of extra cellular endoxylanase by solid state fermentation of dried grass by a strain of *Streptomyces sp* .and characterization of the partially purified enzyme.

Materials and Methods

Micro organism and inoculum preparation: The working strain *Streptomyces sp* OM 09, isolated from forest buffer zone of Kanha National Park and Tiger Reserve, M.P. India (22° 20' 85" N and 80° 53' 95" E) at an altitude of 600-900 m (Bhor and Ray, 2009) was used for further studies and was maintained at 4° C on xylan (oat spelt)-agar medium.

The strain was initially grown in 100 ml Erlenmeyer flasks each containing 20 ml Basal Medium (BM) composed of (gl^{-1}): peptone 0.9;$(NH_4)_2HPO_4$ 0.4; KCl 0.1; $MgSO_4.7H_2O$ 0.1 and oat spelt xylan (Sigma, U.S.A) 0.5. (pH: 6) at 37°C.

Chemicals: All chemicals used, are of analytical grade. The agrowastes used were collected from local dumps, dried, pulverized, autoclaved and added directly in the medium.

Pretreatment of grass: The collected grass was treated with 0.5-1(N) HCl, 0.5-1(N) NaOH and 0.5% urea for two hours followed by washing. The grass was also used after boiling with deionized water for one hour or was simply dried at oven (80°C) for two hours.

Cultivation in solid state fermentation medium: The strain was cultured in 100 ml Erlenmeyer flasks containing totally dried substrates and salts ,moistened with 0.5 ml of distilled water at 37°C .But for subsequent experiments, common grass was collected, dried , pulverized to 40 mesh particle size and was supplemented in the medium replacing oat spelt xylan (Sigma, U.S.A).

Enzyme extraction and assay: Cultures were picked up at different time intervals, sterile water was added to make up it's 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. Enzyme was partially purified by salting out by 80% ammonium sulfate and dialysis against 0.1(M) phosphate buffer (pH-6) at 4°C.

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.

Measurement of growth: The growth of the pellet form was measured by turbidometric method at 650nm (Noisommit-Rizzi et al, 1996).

Optimization of various parameters: Cultural conditions with respect to inducing effect of various agro wastes, their pretreatment and concentration, variable temperature range (20°-42°C)and pH range(4-9) and cultivation time (24-120 hours) on productivity of the enzyme were optimized.

Characterization of enzyme: The temperature and pH optima were detected by incubating the assay mixture at different temperatures (20°-90°C) at pH 6 and at various pH ranges (4-9) at 33°C for 10 minutes respectively. Thermostability of the enzyme was determined by exposing the enzyme at 60°C for 0-60 minutes followed by the measurement of their residual activities. The pH stability was determined by keeping the enzyme in presence of various buffers 0.1M acetate buffer (pH 4-6), 0.1M phosphate buffer (pH 5-8) and 0.1m Tris glycine buffer (pH 8-9) at 33°C for 120 minutes followed by the estimation of their residual activities.

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Substrate specificity of the enzyme was determined by using different substrates in the assay mixture and incubating the same under optimized conditions. Effect of additives on enzyme activity were measured by adding 10mM of various metallic salts and thiol compounds and inhibitors, followed by the measurement of the respective residual activities.Each experiment was carried out in triplicate and their values were averaged.

Results and discussion

Effect of agro wastes as inducer of endoxylanase: With a view to replace expensive xylan, various agro wastes were tested as sole carbon sources. *Streptomyces sp* was found to degrade various hemicellulosic agro wastes in solid state fermentation conditions (Table 1), of which grass showed the most promising result. Therefore further experiments were continued with grass instead of pure and expensive oat spelt xylan (Sigma). Although pretreatment of the grass with acid or alkali reduced the enzyme production, drying and pulverization of grass brought about a remarkable increase in growth and enzyme production by the present strain (Fig 1), probably due to the conversion of hemicellulosic residues of grass in a more accessible form. Highest endoxylanase production was observed in culture broth supplemented with 1% (w/v) dried grass dust as substrate, above which the enzyme synthesis became reduced (Fig 2). This might be due to the higher concentration of nutrient supplements present in these substrates that might have adversely affected enzyme production or as a result of hindrance of mass transfer of oxygen by higher amount of solid substrate.

Table 1.Role of hemicellulosic wastes as inducer of endoxylanase synthesis in SSF

Carbon Source	Growth	Endoxylanase activity(U/ml)
Xylan(Oat spelt)	0.45	1091
Grass	1.22	1341
Rice husk	0.16	773
Saw dust	0.37	318
Jute fiber	0.11	773
Sugar cane bagasse	0.14	636
Rice straw	0.16	1272

Fig 1. Effect of pretreatment of grass on endoxylanase production by *Streptomyces* sp OM 09

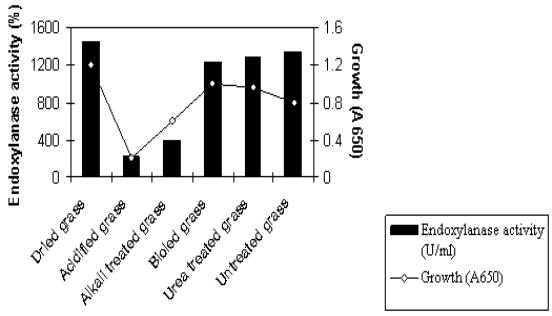
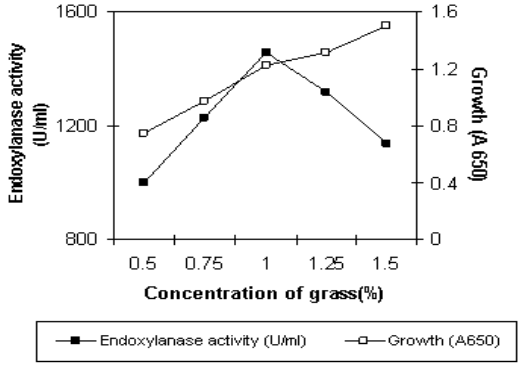


Fig 2.Effect of concentration of dried grass for growth and production of endoxylanase by *Streptomyces* sp OM 09



Effect of temperature: Both growth and production of extra cellular endoxylanase by *Streptomyces sp* was preferred at 37°C in SSF (Fig 3), similar temperature preference was found in submerged fermentation also (Bhor and Ray, 2009).But it was quite different from temperatures reported from other *Streptomyces* strains (Rawasdeh *et al*, 2005,Techapun *et al,* 2002 and Nascimento *et al,* 2003).

Fig 3.Effect of temperature on SSF of grass for growth and production of endoxylanase by *Streptomyces* sp OM 09

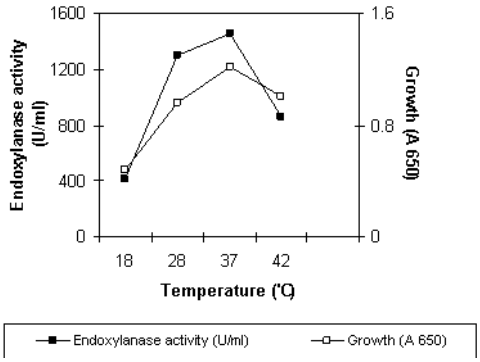
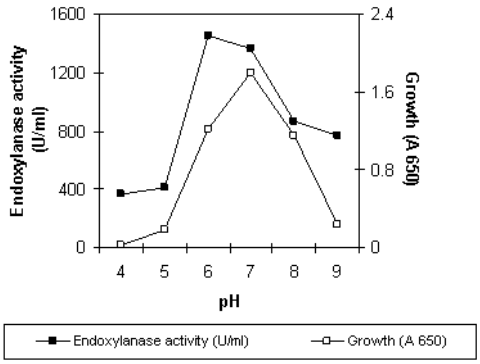
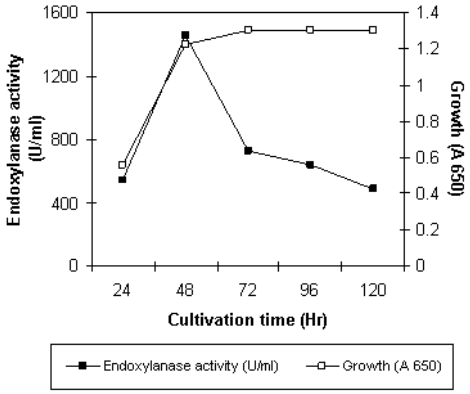


Fig 4. Effect of pH on SSF of grass for growth and production of endoxylanase by *Streptomyces* sp OM 09



Effect of pH: *Streptomyces sp* OM 09 showed highest growth with highest endo xylanase synthesizing ability at pH 6.0 (Fig 4), which was similar to that reported from *Streptomyces sp* 7b (Bajaj and Singh, 2010) but higher than *Thermoascus aurantiacus* miehe (da Silva *et al,* 2005).

Fig 5.Effect of time on SSF of grass for growth and production of endoxylanase by *Streptomyces* sp OM 09



Effect of cultivation time: The kinetics of growth and enzyme production of *Streptomyces sp* OM 09 in SSF of dried grass showed that highest enzyme production could be achieved within 48 hours of cultivation (Fig 5), at the early stationary phase of growth. This was a much rapid rate than the other *Streptomyces* strains reported by Rawashdeh *et al,* 2005 , Rifaat *et al*, 2005**,** da Silva *et al* 2005,Ding *et al,* 2004, even the time taken by the present strain in submerged fermentation of dried grass (Bhor and Ray, 2009).

Characterization of the enzyme

Optimum temperature: The optimum temperature for endoxylanase activity was 60°C (Fig. 6), similar to that of thermophilic *Streptomyces* sp. K37 (Mansour *et al*, 2003), *Streptomyces sp.*Ib 24D (Rawashdeh *et al,* 2005) but different from that of other endoxylanases having temperature optima at 50°C *(* Bajaj and Singh, 2010) and 75°C (da Silva *et al,* 2005).

Thermostablity Thermal stability is an interesting enzyme property due to the great industrial importance. The half life of the present enzyme at 60°C was found to be 15 minutes (Fig. 7). Therefore the present endoxylanase could be called moderately thermostable.

Optimum pH This study revealed that the best pH for this xylanase activity was 6.0 (Fig. 8) similar to that from other strains of xylanase producing *Streptomyces* sp (Bajaj and Singh, 2010; Mansour *et al,* 2003).Studies carried out with other strains of *Streptomyces* as well with others fungal species also concluded that the most suitable pH value for xylanase activity was within the acid region.

Fig 6.Effect of temperature on the activity of the endoxylanase from *Streptomyces* sp OM 09
[100% activity: 1400U/ml]

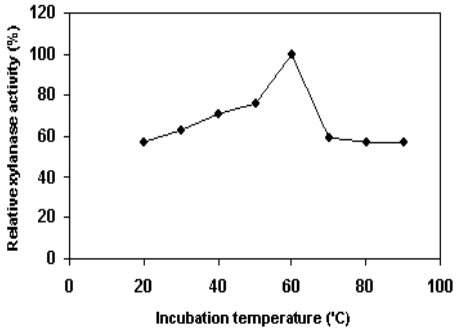
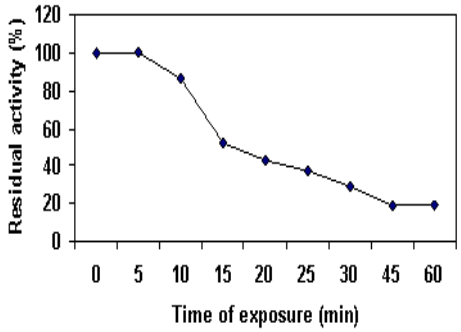


Fig 7. Effect of temperature on the stability of the endoxylanase from *Streptomyces* sp OM 09
[100% activity: 1400U/ml]



pH stability The xylanase produced by *Streptomyces sp* OM 09 maintained its stability over a broad of pH as 80% of the activity was retained at a range of 5-8.5 (Fig.8) which might be significant in industrial application of the enzyme.

Fig 8.Effect of pH on the activity and stability of the endoxylanase from *Streptomyces* sp OM 09
[100% activity: 1400U/ml]

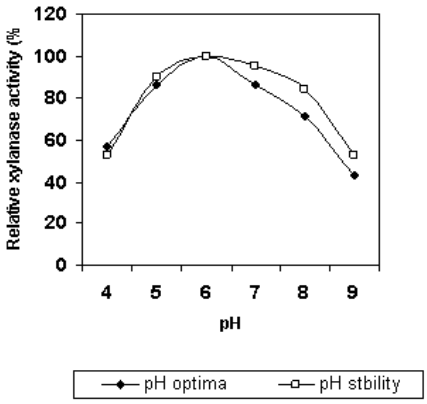
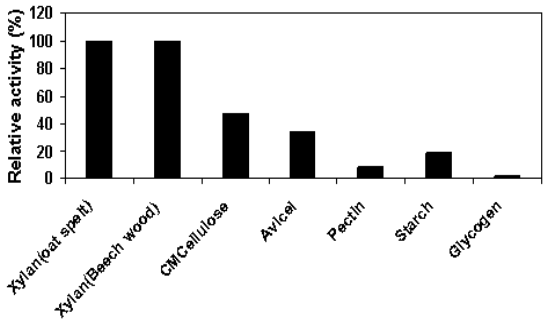


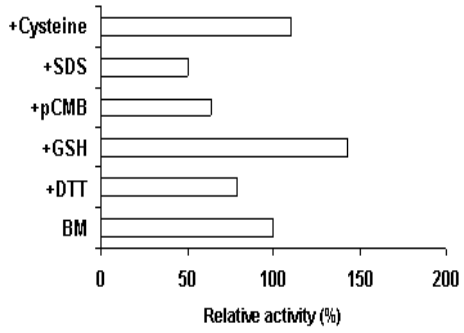
Fig 9. Substrate affinity of the endoxylanase from *Streptomyces* OM 09 [100% activity: 1400U/ml]



Affinity towards substrates: The endoxylanase showed highest affinity towards Oat spelt (sigma) Beech wood (Sigma) xylan (Fig 9). It showed only 50% affinity towards carboxymethyl cellulose and almost no specificity towards pectin, avicel or starch.

Effect of various additives: The enzyme activity was not much influenced by the common metal salts, except the heavy metals like Cu, Ag and Hg (data not shown) but was enhanced by the addition of external thiol compounds (Fig 10), indicating the presence of thiol groups at the active site.

Fig 10. Effect of different additives on the endoxylanase activity of *Streptomyces* OM 09
[100% activity: 1400U/ml]



Conclusion

Dried grass could provide an economical advantage as a carbon source for the production of extra cellular endoxylanase by *Streptomyces* OM 09 as it not only opens up an avenue for the appropriate utilization of renewable resources but also it makes the production process cost effective. The rapid rate of production and higher productivity of the strain made it important for bulk production of endoxylanase. Further, the enzyme with moderate thermo stability and broad range of pH tolerance could be used for sugar and alcohol production industries.

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