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Production of Xylanase from Various Lignocellulosic Waste Materials by *Streptomyces* sp. and its Potential Role in Deinking of Newsprint

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ABSTRACT

In this study, the agricultural waste was used to screen for an organism that is capable of producing enzymes for degrading xylan. *Streptomyces* sp. are the important source of enzyme involved in lignocellulosic degradation. *Streptomyces* sp. was isolated from different soil samples. Out of 10 different soil samples, 6 samples gave the best result in starch casein agar plates. Screening was mainly carried out to detect the enzyme and a clear zone surrounding the growth was seen if enzyme xylanase was present. Substrates being cheap and readily available, have recently gained considerable interest because of their possible use in fermentation process. *Streptomyces* sp., produces xylanase on various feed stuffs like sugarcane molasses, oat spelt xylan, Tomato pomace, Rice bran, wheat bran and saw dust under submerged fermentation condition. The attempts have been made to replace a xylan, costly substrate for xylanase to make xylanase production cost effective. The xylanase activity in each production medium was confirmed by measuring the amount of reducing sugars liberated from the medium by the DNS method using crude extract. The application of the crude enzyme in deinking of newsprint was also studied.

Key words: Radio diffusion assay, dinitrosalicylic acid method, deinking of newsprint

INTRODUCTION

Xylanase is the name given to a class of enzymes, which degrade the linear polysaccharide-1,4 xylan into xylose thus breaking down hemicellulose, which is a major component of the cell wall of plants (Singh *et al*., 1995). As such, it plays a major role in the digestive system of herbivorous microorganisms (mammals, conversely, do not produce xylanase). Xylanases are generally single chain glycoproteins, ranging from 6-80kDa, active between pH 4.5-6.5 and at temperature between 40 and 60°C (Lopez *et al*., 1998).

Xylanase is used in industrial applications such as the pulp-prebleaching process to remove hemicelluloses, utilization of hemicellulosic biomass for production of biofuels, food and feed additives, bakery processing and xylitol production. Xylo-oligosaccharides, the hydrolysis products of xylan, exhibit various biological activities including prebiotic, antioxidative and antibacterial activities. Thus, xylo-oligosaccharides can also be used in the pharmaceutical and cosmetic industries (Zhang *et al*., 2011). Xylanases are produced by many microorganisms, such as; bacteria,

fungi and actinomycetes (Beg *et al*., 2000; Dutta *et al*., 2007). Microbial xylanases have attracted considerable research interest in recent years, because of their potential application in the food, animal feed, paper and pulp industries (Collins *et al*., 2005; Beg *et al*., 2000; Ayyachamy and Vatsala, 2007).

Actinomycetes enzymes which are thermostable are of particular interest in industrial use. Many species of *Streptomyces* are reported to produce multiple xylanases when cultivated in xylan containing medium (Nascimento *et al*., 2003). Each actinomycete strain has probably genetic potential for producing 10-20 secondary metabolites. It is well known that actinomycetes produce 70-80% of bioactive secondary metabolites, where approximately 60% of antibiotics are isolated from *Streptomyces* spp. (Ilic *et al*., 2007). *Streptomyces* species are widely recognized as industrially important microorganisms because of their ability to produce different kinds of novel secondary metabolites. It has an enormous biosynthetic potential that remains unchallenged, without a potential competitor among other microbial groups (Solanki *et al*., 2008).

However, the cost of xylan dependent xylanase production limits its use in industrial applications. Alternatively, agricultural byproducts containing cellulose, hemicelluloses and lignin could serve as, effective and inexpensive sources for xylanase production (Nascimento *et al*., 2002; Techapun *et al*., 2003). The production of xylanases is strongly influenced by their culture conditions and medium constituents (Kuhad and Singh, 1993). The present study was to evaluate the capacity of the Agro waste to serve as low cost substrate for xylanase production by *Streptomyces* sp. via submerged fermentation.

MATERIALS AND METHODS

Collection of sample and processing: Ten soil samples were collected from the different area containing decomposed rice straw pulp. Each soil samples were crushed, mixed thoroughly and sieved through a 2 mm sieve to get rid of large debris and the sieved soil was used for the isolation of *Streptomyces* (Williams *et al*., 1972). The identification of *Streptomyces* was confirmed by various biochemical test and Staining methods. Natural lignocellulosics (Agricultural biomass) namely tomato pomace, rice bran, wheat bran, sugarcane molasses, oat spelt xylan and saw dust were collected from local market in Vellore, were used as substrates for the production of xylanase under submerged fermentation. One percent natural substrate in distilled water is mixed with 0.1 mL of trace salt solution/mineral salt solution and the pH is adjusted to 7.5 and autoclaved at 121°C for 15 min at 15 lbs. Each was inoculated with 0.5 mL spore suspension (10^{-7} CFU mL^{-1}). Incubated at room temperature for 15 days with continuous shaking. The production medium is monitored daily for enzyme production.

Radial diffusion: Radial diffusion assays are sensitive methods for detecting xylanase enzyme. It involves incorporating a polymer (xylan) into an agarose gel cutting a well in the gel introducing enzyme into the well and then detecting the zone of clearing produced by polymer hydrolysis (Mostow *et al*., 1975).

Dinitrosalicylic acid method: One percent oat spelt xylan was incorporated into a 0.5% agarose gels. Mixtures were autoclaved and after partial cooling, gels were poured into RID slides. After the gel solidified, wells were cut with a gel puncher. Ten microliter of Enzyme, produced from different substrates were inoculated into the wells. Incubated at room temperature for overnight

and zone of clearing was observed. Dinitrosalicylic acid test is carried out to estimate the xylanase activity and to determine the concentration of xylanase produced as per the method of Mohun and Cook (1962).

Deinking of newsprint by xylanase: An ink jet printed-paper is soaked into the xylanase enzyme and incubated for 3-4 days and monitored daily for the absence of ink in newsprint.

RESULTS AND DISCUSSION

Screening for xylanase producing *Streptomyces* sp.: Xylanase is a good example of an industrial enzyme that needs to be stable in high temperature and active in physiological temperatures and pH when used as feed additive and in alkaline conditions when it is used for bleaching in the pulp and paper industry. Only a few bacterial and actinomycete xylanases have been reported earlier with pH optima in the neutral or alkaline ranges (Nakamura *et al*., 1994; Duarte *et al*., 1999; Ratanakhanokchai *et al*., 1999). Purified isolates of *Streptomyces* sp. were cultured on oat spelt xylan medium (Nanmori *et al*., 1990) and incubated at 28°C for 24 days. The plates were then flooded with absolute ethanol and left for few seconds at room temperature to precipitate xylan at room temperature to precipitate xylan. Treatment of soil samples with calcium carbonate was reported to be the most efficient technique for the preferential isolation of actinomycetes (Alferova *et al*., 1989). Colonies producing xylanase enzymes were surrounded by clear zones against an opaque background of non-hydrolyzed media (Fig. 1). Out of six isolates one of the isolate, which showed the largest clear zone (approx 1 cm) was selected for the production of xylanase (Shirling and Gottlieb, 1996). The *Streptomyces* isolate that showed the largest clear zone (approx 1 cm) was selected for the production of xylanase (Shirling and Gottlieb, 1996). Similarly, crude extracts of five day old cultures of *S. purpeofuscus* and *S. albidoflavus* were active against gram positive as well as gram negative bacteria and fungi (Anupama *et al*., 2007). While, extracts of four day old cultures of *S. griseus* and *S. psammoticus* exhibited good antimicrobial activity (Toshio *et al*., 2000; Sujatha *et al*., 2005).

Production of xylanase under submerged fermentation: Natural lignocellulosics (Agricultural biomass) namely tomato pomace, rice bran, wheat bran, sugarcane molasses, oat spelt

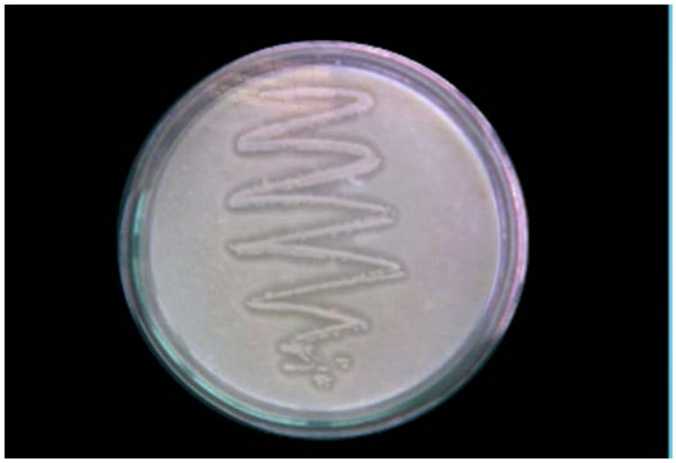

Fig. 1: Screening for xylanase producing *Streptomyces*

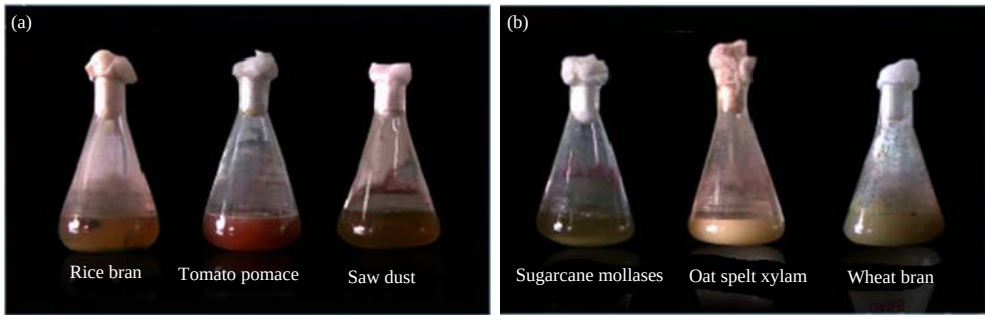

Fig. 2(a-b): Production medium before fermentation

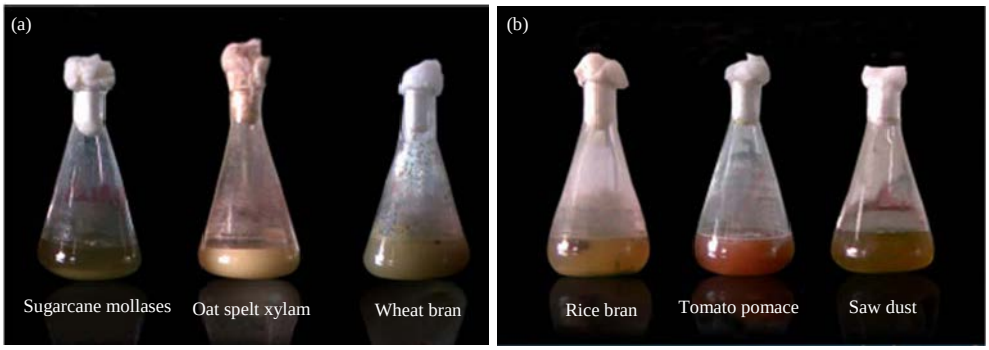

Fig. 3(a-b): Production medium after fermentation

xylan and saw dust were used as substrates for the production of xylanase under submerged fermentation. Xylanases in the family retain glycosidase activity, which could be attributed to a common catalytic mechanism (MacLeod *et al*., 1994). The cloning of cellulase and xylanase genes into non-cellulolytic/xylanolytic backgrounds confirmed that they were also capable hydrolyzing both polymers i.e., cellulases hydrolyzing xylan and vice versa (Wakarchuk *et al*., 1994) (Fig. 2 and 3).

Radial diffusion assay: Radial diffusion assay are mainly done for detecting the hydrolytic enzymes. Digestion produces a clear zone around enzyme containing wells. The diameter of the zone is proportional to the amount of enzyme in the well and length of digestion. Diameter of zone of inhibition of oat spelt xylan was found to be maximum at 7 mm, which is followed by Sugarcane molasses 6 mm, wheat bran 6mm,saw dust 4 mm, rice bran 3 mm and tomato pomace 1 mm, which is shown in the (Fig. 4a).

Similar reports regarding higher production of xylanases from lignocellulosics have appeared earlier in many organisms like *Bacillus thermoalkalophilus* utilising bagasse (Rajaram and Varma, 1990), *Streptomyces chattanoogensis* UAH 23 and *Streptomyces* sp. (Vyas *et al*., 1990) utilising wheat bran, *Thermomonospora curvata* using bagasse, *Trichoderma reesei* using wheat bran (Bailey and Poutanen, 1989) and *Melanocarpus albomyces* IIS-68 consuming wheat bran (Saraswat and Bisaria, 1997). By comparing the fermentation profile in xylan, xylose and wheat bran media it was evident that the decrease in the reducing sugar concentration is the key factor for enzyme induction.

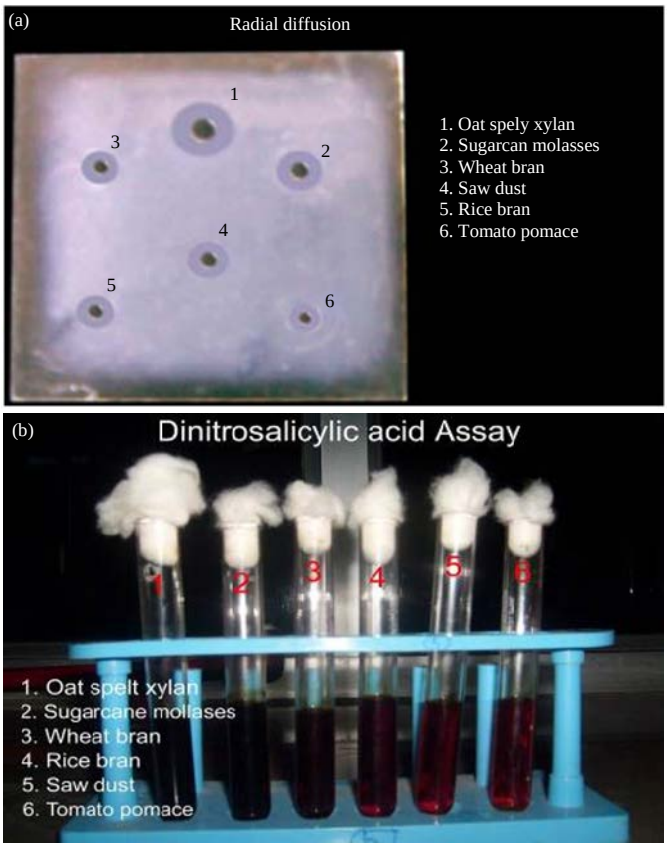

Fig. 4(a-b): (a) Radial diffusion assay and (b) Dinitrosalicylic acid assay

Table 1: Production of xylanase on 15th day of incubation

Substrate	Temperature (°C)	pH	Xylanase (IU mL^{-1})
Oat spelt xylan	25	7.5	7.250
Sugarcane molasses	25	7.5	5.225
Wheat bran	25	7.5	5.000
Rice bran	25	7.5	4.000
Saw dust	25	7.5	3.250
Tomato pomace	25	7.5	2.250

Dinitrosalicylic acid assay: This test is mainly done to detect the concentration of xylanase produced. The absorbance of each liquid is measured at 575 nm using calorimeter. Xylanase production was detected daily in all the production medium. All the production medium showed gradual increase in xylanase production daily (Fig. 4b).

Out of all lignocellulosic waste, the highest amount of xylanase enzyme was produced in oat spelt xylan medium of about 7.250 IU mL^{-1} of enzyme. Xylanase enzyme was also produced in other substrates. Next to oat spelt xylan, the highest amount of sugarcane molasses was 5.225 IU mL^{-1}, which is followed by wheat bran produced 5.000 IU mL^{-1}, Rice bran produced 4.000 IU mL^{-1}, saw dust produced 3.250 IU mL^{-1}. The lowest amount of xylanase enzyme was produced in tomato pomace of about 2.250 IU mL^{-1}. This was represented in Table 1.

Deinking of newsprint by xylanase: Ink jet printed paper soaked in xylanase enzyme showed deinking of printed-paper which was absent in control (Fig. 5).

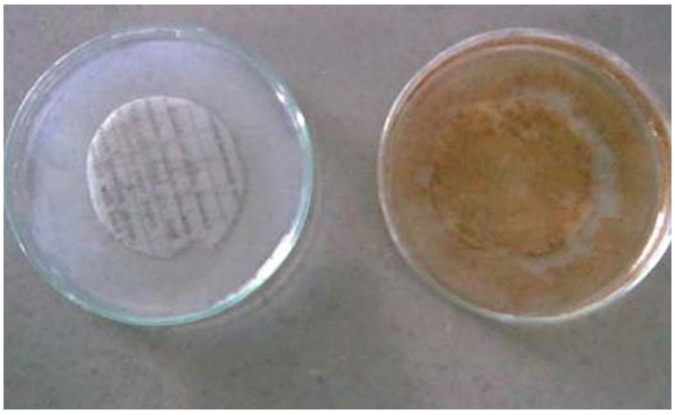

Fig. 5: Deinking of ink-jet printed paper by xylanase

CONCLUSION

Xylanase have a wide range of potential biotechnological application. The potential application of microbial xylanase in the pulp and paper industry is gradually increasing. The increasing cost of recycling of waste paper is challenging. It uses large amounts of expensive, potentially and environmentally damaging chemicals. A process based on xylanase was not hazardous. It was found that xylanase could effectively deink old newsprint. Xylanase may induce the formation of low molecular weight free radicals in the paper, which might be responsible for deinking of newsprint. Thus it can be concluded from the present study that the production of xylanase can be made cost effective by using various agro residues. Further work is recommended to purify and characterize the xylanase from *Streptomyces* sp. and study the effect of this enzyme on other agricultural waste. Assessment of the properties of this enzyme in biobleaching of pulp and paper is also recommended.

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