

Isolation and Screening of Xylanolytic Fungi from Textile Sizing Site

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Abstract: Filamentous fungi play a significant role due to their capacity for high enzyme production. Numerous enzymes generated by fungi are linked to biotechnological uses across various industries. This research investigates the isolation and characterization of fungi that produce xylanase from environmental samples collected from a textile sizing facility, emphasizing their biotechnological capabilities. The objective of this study was to isolate and assess the xylanolytic fungi found in textile sizing areas in Bhiwandi, India. Soil samples were gathered, and fungal colonies were separated using serial dilution methods. From a total of 120 fungal isolates, 90 were tested for their xylanase production ability on minimal agar medium with 0.5% Beechwood Xylan as the carbon source. The screening process identified that 46 isolates displayed xylanolytic activity, demonstrated by the formation of halo zones surrounding the colonies, suggesting their potential for xylanase production. Microscopic examination revealed that the majority of the isolates were from the genera *Aspergillus*, *Trichoderma*, *Penicillium*, *Fusarium*, and *Rhizopus*. The research underscores the significance of xylanase enzymes and their extensive applications across various industries, such as food, feed, baking, pulp, and paper industries. It emphasizes the need for efficient and economical xylanase synthesis as well as the ability of microorganisms particularly fungi to fulfil this requirement. The textile sizing sites are favourable habitats for xylanolytic microorganisms. This study advances the knowledge of microbial diversity in waste environments and highlights the possibility of using these organisms to produce sustainable enzymes, which will help advance environmental cleanup and bioprocessing initiatives

Keywords: Xylanolytic, Textile Sizing Site, *Aspergillus* sp., *Trichoderma* sp., Hydrolytic enzymes, Bioremediation.

I. INTRODUCTION

Life's vast array of biochemical processes is predominantly facilitated by enzymes, which are biological catalysts. These enzymes exhibit high substrate specificity and operate effectively in aqueous environments under mild conditions. Primarily produced by plants, animals, and microorganisms, enzymes accelerate a wide range of chemical reactions crucial for life. The industrial demand for enzymes, especially those of microbial origin, is increasing due to their cost-effective production. Microbial enzymes offer a diverse spectrum of characteristics, making them suitable for specific applications. Various microbial enzymes, including protease, amylase, xylanase, cellulase, invertase, and lipase, are utilized in food, agriculture, pharmaceuticals, cosmetics, and other biotechnology industries both domestically and internationally (Mandal, 2015).

The plant cell wall consists of polysaccharides, with cellulose, hemicelluloses, and lignin as its primary components (Anand et al., 2018). Xylan, the most prevalent hemicellulose, is considered the second most abundant biopolymer in plants (Ebringerová and Heinze, 2000; Li et al., 2021b). It features a linear backbone of β -1,4-linked xyloses (Ju et al., 2013; Collins et al., 2005; Shallom and Shoham, 2003). Xylan degradation is primarily accomplished by xylanases, including endo-1,4- β -xylanase (EC 3.2.1.8), which cleaves glycosidic bonds in the xylan backbone, and β -D-xylosidases (EC 3.2.1.37), which act on xylooligomers to release xylose (Juturu and Wu, 2014; Knob et al., 2010). Xylanases have numerous industrial applications, such as lignocellulosic bioconversion, juice clarification, bioethanol



production, animal feed, paper and pulp industries, and the production of fuels and chemicals (Ali et al., 2017; Shi et al., 2013; Singh et al., 2013; Goluguri et al., 2012; Li et al., 2000; Hatanaka., 2012; Anand et al., 2018).

A wide range of organisms produce xylanases, including bacteria, algae, fungi, protozoa, gastropods, and arthropods (Collins et al., 2005; Shabeena et al., 2017). Bacteria, fungi, and actinomycetes are notable sources of xylanases (Walia et al., 2017; Anand et al., 2018). Despite the extensive diversity of fungal species known to secrete xylanase, industrial-scale production primarily relies on terrestrial isolates of *Aspergillus* and *Trichoderma species* (Lu et al., 2008). Nevertheless, researchers continue to investigate organisms, particularly filamentous fungi, capable of secreting high levels of xylanase enzymes to enhance the diversity and abundance of xylanase-producing cultures in their collection (Polizeli et al., 2005).

Microorganisms' xylanolytic enzymes have garnered interest in recent decades, especially due to their biotechnological potential in a variety of industrial processes, including those in the food, feed, baking, pulp, and paper industries (Arulanandham and Muthusamy, 2014). One of the best biological uses in the pulp and paper sector is the biobleaching and bioprocessing of pulps with xylanases. Xylanases serve as physical barriers to the entry of bleaching chemicals and are mostly employed to remove the lignin-carbohydrate complex produced during the kraft process (Betini et al., 2009). To guarantee little to no harm to the pulp fibers, xylanases in the pulp and paper sector must be cellulase-free., xylanases must increase pulp's drainage and fibrillation qualities, paper strength, and process efficiency (Singh et al., 2013). Xylanases must have applications in generating rayon grade or superior quality pulps. By using xylanases in biobleaching, hazardous discharges and chlorine use are decreased. Furthermore, by making the pulping process easier, xylanases in biomechanical pulping lessen the need for the mechanical pulping approach, which in turn uses less energy. In order to improve juice clarity and consequently lower viscosity, xylanases are used in fruit and vegetable juices (Arulanandham and Muthusamy, 2014). They increase the dough's strength and elasticity during baking, which results in larger loaves and a more varied texture (Jiang et al., 2008; Azad et al., 2013).

According to the feed industry, adding xylanase to broiler chickens' rye-based diet lowers intestinal viscosity, which enhances the chicks' weight increase and feed conversion efficiency. Furthermore, bio-refining also makes use of xylanases (Sharma et al., 2015). The current work focused on the isolation and screening of fungi for the synthesis of xylanase in light of biotechnological and industrial applications (Ramanjaneyulu et al., 2015).

The goal of this study was to collect samples, isolate them, examine them under a microscope, and assess the variety of native fungi that could hydrolyse xylan from a textile sizing location. The objective was to comprehensively explore and assess the hydrolytic capabilities of fungi for potential widespread applications in the future, driven by the need for xylanase in food, feed, baking, pulp and paper and pharmaceutical sectors.

II. MATERIALS AND METHOD

Selection of sample sites

Different textile sizing sites of Bhiwandi city were selected for the sample collection.

Collection of soil samples

The samples were collected from different spots in each site in zip lock polythene bags. Almost 5-10 soil samples were taken from each sizing industries. The soil sample was mixed well and processed next day.

Isolation Of fungi

Fungal colonies were isolated from soil samples by serial dilution method where SDA (Sabouraud dextrose agar) media was prepared, autoclaved and poured in sterile petri plates. Soil samples (1 gm) diluted up to 10^{-5} dilution was spread on respective solidified SDA plates with the help of sterile spreader. The inoculated petri plates were incubated at 28°C for 48 h. About 120 different fungal isolates differentiated based on physical characteristics obtained after incubation wereselected for the further processes. The isolates were further inoculated on SDA plates by point inoculation and incubated at 28°C for 73 hours to obtain pure fungal cultures (Khan and Kumar, 2011).

Screening of Fungal Isolates for Xylanase Production

The fungal isolates were subjected to screening for their xylanase activity using the plate screening method on minimal agar medium with 0.5% Beechwoodxylan as the only carbon source. From 120 fungal isolates about ninety fungal isolates were screened for xylanase production efficiency. The medium contained the following constituents (g/L):



0.05g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.005g CaCl_2 , 0.005g NaNO_3 , 0.009g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002g ZnSO_4 , 0.012g MnSO_4 , 0.23g KCl , 0.23g KH_2PO_4 , 2g peptone, 19g agar (Adesina and Onilude, 2013). The entire ninety fungal discs were centrally inoculated on sterile solidified agar plates with the help of cork borer. The diameter of disc was 1cm. Plates were incubated at $28 \pm 2^\circ\text{C}$ for 72 to 96 hours, depending on the strain and analysed at every 24 hours for the occurrence and evaluation of the halo diameter. To visualize the clear zone the Petri dishes were flooded with 0.4% Congo red dye and after 10 minutes washed with 1M NaCl (Adesina and Onilude, 2013; Burlacu et al., 2016), Figure 1.

Microscopy of Xylanolytic Isolates

Microscopy of all the positive isolate was done by lactophenol cotton blue staining method. In aseptic condition, a loop full of fungal cultures was placed on a clean glass slide, a drop of lactophenol cotton blue stain was mixed with the culture. A clean coverslip was placed over the culture and viewed under the microscope (10X and 45X). Morphological characteristics including colour of the colony and growth pattern studies, as well as their vegetative and reproductive structures were carefully observed under the microscope (Devi and Kumar, 2012), Figure 2.



Figure 1: Screening Plates



Figure 2: Microscopic Images

III. RESULTS AND DISCUSSION

A wide variety of microorganisms can be found in soil, making it an essential habitat. Potent isolates are frequently obtained in natural environments, and the choice of soil samples can affect the effectiveness of enzymes. Fungi were isolated utilizing serial dilution techniques. The isolated fungi were subsequently evaluated for their xylanase production capabilities. A screening process involving 90 fungal isolates was conducted on Minimal Agar Media plates, which contained Beechwoodxylan as a substrate for the xylanase enzyme. These plates were incubated for 72 to 96 hours. Following the incubation period, varying levels of xylan utilization were observed subsequent to the application of 0.4% Congo red followed by 1 M NaCl. The Xylanolytic activities of these isolates were assessed based on the formation of halo zones on minimal media containing Beechwoodxylan (Figure 1). The screening results revealed that 46 isolates exhibited xylanolytic activities, evidenced by halo zone formation around the colonies, indicating potential



for xylanase production. Microscopic analysis identified most isolates as belonging to the genera *Aspergillus*, *Trichoderma*, *Penicillium*, *Fusarium* and *Rhizopus*.

Numerous sources, including bacteria, fungi, yeast, algae, seeds, snails, and crustaceans, have been shown to produce xylanases (Mandal, 2015; Polizeli et al., 2005). However, bacteria and fungus are the main manufacturers of these enzymes. However, because of their large yields, extracellular release of the enzymes (Nair et al., 2008), and enhanced xylanase activity (in comparison to bacteria and yeast) (Mandal, 2015; Motta et al., 2013), fungi continue to be important producers. The presence of cellulase is another factor that distinguishes bacterial and fungal xylanases; few research has reported fungal xylanase that does not exhibit cellulase activity (Subramaniyan and Prema, 2002; Burlacu et al., 2016).

Filamentous fungus are appropriate species for the commercial synthesis of enzymes due to their capacity to synthesize huge quantities of extracellular enzyme. Filamentous fungi used for the production of xylanase include *Penicillium sp.*, *Trichoderma viride*, *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus sp.*, etc. The most attention has been paid to certain *Aspergillus* species, including *A. niger*, *A. tamarii*, *A. awamori*, and *A. oryzae*, in order to extract several hydrolytic enzymes, including xylanase, α -amylase, and protease. However, due to its widespread distribution, low nutritional needs, and high xylanase productivity, *A. niger* is thought to be more suitable (Kang et al., 2004; Kheng and Omar, 2005; Al-Widyan et al., 2008; Shahi et al., 2011). The need to increase the production of native enzymes and look for faster techniques has increased due to the growing use and consumption of xylanase in many industries. A country's commercial development can benefit greatly from the proper utilization of agro-industrial wastes. It is meaningful to use such abandoned materials in the production of xylanase. Researchers' main objective is to use agro-industrial waste to lower the economic cost of producing enzymes (Abdullah et al., 2015).

The spectrum of distinct xylanases and the capacity of microorganisms to manufacture these enzymes vary greatly in terms of their activity (Hinz et al., 2009). However, fungi are the only source of xylanase that are widely used in industry. Nowadays, genetically engineered strains of *Aspergillus* or *Trichoderma* create the majority of commercial xylanolytic preparations (Mussatto and Teixeira, 2010).

Filamentous fungi are the most distinguished producers of enzymes involved in the decomposition of lignocellulosic material. Since fungi produce a wide variety of enzymes with varying biochemical characteristics and great potential for biotechnological applications, their usage in bioprocesses has become more significant in recent decades. Filamentous fungi are commonly used to create enzymes and are usually thought to be more effective at producing xylanase than either bacteria or yeast (Polizeli et al., 2005; Knob et al., 2014).

Several mesophilic fungal species have been evaluated in relation to xylanase production, including members of *Aspergillus*, *Trichoderma*, and *Penicillium*. *Aspergillus niger* has been one of the most investigated microorganisms as a xylanase producer, regardless of carbon source or system used (submerged or solid-state fermentations). On the other hand, thermophilic microorganisms such as *Thermomyces lanuginosus*, *Thermoascus aurantiacus*, *Talaromyces thermophiles*, and *Myceliophthora thermophila* have been widely investigated, due to the increased biotechnological importance of thermostable xylanases (Maalej et al., 2009; Milagres et al., 2004; Moretti et al., 2012; Yang et al., 2006; Knob et al., 2014).

The focus of present study was directed to isolate fungal from environmentally exposed textile sizing site soil. Furthermore, because the fungi can effectively employ complex agro-industrial wastes as substrates for enzyme production, they are more resilient and better suited to changes in the ambient variables for growth (Sudan and Bajaj, 2007; Bajaj et al., 2011; Sharma et al., 2015).

Worldwide, fermentation processes are used to manufacture xylanase, which has a wide range of industrial uses. The generation of enzymes from lignocellulosic biomass solves waste management problems and lessens the harm that waste disposal causes to the environment. In conclusion, textile sizing sites offer appropriate homes for microorganisms that produce xylanase, and they merit additional investigation to ascertain whether these isolates can aid in the bioremediation of waste environments associated with sizing processing. Future studies should focus on employing possible xylanolytic isolates to produce and purify xylanase enzymes on a pilot scale.



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