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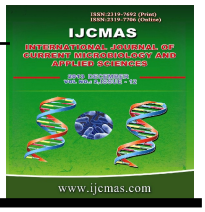


**Original Research Article**

# Screening for xylanase producing microorganisms from marine sources

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**A B S T R A C T**

The marine microbial world has evolved over a period of time as the repository of several novel biocatayst metabolites and compounds of commercial value. Lignocellulosic biomass is very abundant in organic source which can be utilized as a renewable source of obtaining bioenergy. It is a polymer made up of cellulose, hemicellulose and lignin. Several organisms have been explored for their abilities to degrade this polymer. The present study focused on screening the organisms from marine sources for selective hemicellulase activity. Hemicelluloses include xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan. Xylans are polysaccharides made from units of xylose (a pentose sugar). Xylans are almost as ubiquitous as cellulose in plant cell walls and contain predominantly β-D-xylose units linked as in cellulose.In the present study microorganisms were isolated from the coastal areas of Tamil nadu and Karnataka using standard microbiological techniques, the isolates were subjected to xylan degradation assay to check for the hemicellulose activity. Nine bacteria, four fungi and two actinomycetes showed the ability to degrade xylan. These organisms may have a great potential in the lignocellulosic biomass degradation which could be of great commercial value.

**Keywords**

Hemicellulose; Marine sediment; Xylan; Xylanolysis Basal Medium (XBM); Lignocellulose.

## Introduction

The world ocean with a coastline of 312,000 km (193,000 miles) and a volume of 137 × 106 km3, is the largest ecosystem on earth, and has been used for a variety of purposes by man for millennia because of its large volume and vast area, influence of the world ocean on world climate is profound. Microorganisms occur nearly everywhere in nature and occupy an important place in human view of life.

Marine microbes represent a potential source for commercially important bioactive compounds and their bioremediation capabilities are also remarkable. They also play a crucial role in decomposition of organic matter and cycling of nutrients. Microbes also serve as food for some bottom-living organisms. Our knowledge of marine microbial diversity has, however, been severely

limited by relying on microorganisms that have been cultured (Surajit Das *et al*., 2006).

The marine environment is characterized by the hostile parameters such as high pressure, salinity, low temperature, absence of light, etc. and marine heterotrophic bacteria have adapted themselves to survive in this environment – they require Na+ for growth because it is essential to maintain the osmotic environment for protection of cellular integrity.

Oligotrophy is also one more adaptation because of the small amount of available nutrient. However, heterotrophic bacterial action promotes organic degradation, decomposition and mineralization processes in sediments and in the overlying water, and releases dissolved organic and inorganic substances (Purushothaman, 1998).

Marine microorganisms which are salt-tolerant provide an interesting alternative for therapeutic purposes. Marine microorganisms have a diverse range of enzymatic activity and are capable of catalysing various biochemical reactions with novel enzymes. Especially, halophilic microorganisms possess many hydrolytic enzymes and are capable of functioning under conditions that lead to precipitation of denaturation of most proteins. Further, it is believed that sea water, which is saline in nature and chemically closer to the human blood plasma, could provide microbial products, in particular the enzymes, that could be safer having no or less toxicity or side effects when used for therapeutic applications to humans (Sabu, 2003).

Lignocellulose consists of lignin, hemicellulose and cellulose. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value (Malherbe and Cloete, 2003). Large amounts of lignocellulosic "waste" are generated through forestry and agricultural practices, paper-pulp industries, timber industries and many agro industries and they pose an environmental pollution problem. Sadly, much of the lignocellulose waste is often disposed by burning (Levine, 1996).

Hemicellulose, a branched polymer composed of pentose (5-carbon) and hexose (6-carbon) sugars, can be hydrolyzed by hemicellulases or acids to release its component sugars, including xylose, arabinose, galactose, glucose and/or mannose. Hexoses such as glucose, galactose, and mannose are readily fermented to ethanol by many naturally occurring organisms, but the pentoses including xylose and arabinose are fermented to ethanol by only a few microorganisms and with relatively low yields. Since xylose and arabinose generally comprise a significant fraction of lignocellulosic biomass, especially hardwoods, agricultural residues and grasses, it must be utilized to make economics of biomass ethanol processing feasible.

Cellulases and hemicellulases have numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture (Wong and John N.Saddler, 1992; Bhat, 2000; Sun and Cheng, 2002).

Plate assays are efficient methods used in screening organisms for hemicellulose degrading enzyme production. Such tests give a positive or negative indication of

enzyme production. They are particularly useful in screening large numbers of isolates for several classes of enzyme. The reagents required are all commonly available and relatively inexpensive.

## Materials and Methods

### Sampling and Isolation

Marine samples were collected from coastal areas of Tamil Nadu and Karnataka during the monsoon season. The samples were collected in sterile polythene bags and bottles which were preserved in refrigerator until further investigation. Standard microbiological methods were followed for the purpose of isolation (Brown, 1985). One ml of the desired dilution was transferred aseptically into Potato Dextrose Agar (PDA) and Marine agar plates for fungi and bacteria respectively. Plates were incubated for 24-48 hrs for bacterial and 5-7 days for fungal growth. The isolates thus obtained were characterized.

### Hemicellulolytic (Xylanolytic) enzyme assay

Relatively little attention has been given to qualitative assays for xylan utilization and only a few assay procedures have been described. Actively growing culture was used to inoculate each assay medium, for bacteria 24-48hrs culture was used and for fungi 5-7 days old culture was used to inoculate each assay medium individually (Jørgensen *et al*., 2003).

### Dye staining of Xylan agar (XBM)

Xylanolysis Basal Medium (XBM) was prepared and incorporated with 4% w/v xylan and 1.6% w/v of agar and autoclaved. The sterile media was aseptically transferred to the sterile petri dishes and inoculated with the test organism individually. The plates were then incubated at room temperature for 48 h for bacteria and 5-7 days for fungi. The media after growth was flooded with iodine stain (0.25% w/v aqueous $I_2$ and KI) for 5 min, the stain was removed and the agar surface washed with distilled water. Xylan degradation around the colonies appeared as yellow-opaque area against a blue/reddish purple color for un-degraded (Biely, 1985; Cai *et al*., 1995).

## Results and Discussion

All of the samples plated showed good growth, plates with a cfu between 30-300 per plate were selected for documentation, as overcrowding of the plate would lead to erroneous result. It was seen that the sediment and soil samples yield the maximum number of isolates followed by the degraded wood and the least number of bacteria was isolated from the sea water samples, which clearly indicates the presence of a large number of organisms occurred at the bottom of the sea or the backwaters and not in sea water itself.

All the isolated organisms (Bacteria, Fungi and Actinomycetes) were subjected to screening for the production of hemicellulases on pure substrate such as Xylan to check for their degradation by methods detailed in "Materials and Methods".

All isolates showing lignocelluloytic activity grew well on the media containing the respective substrates with varying colony diameters. The results for the zone of hydrolysis on dye staining was in concurrence with the findings of (Kausar *et al*., 2011).

Nine bacterial isolates, four fungi and two actinomycetes showed hemicellulolytic activity, this is on par with the bacteria reported by (König *et al*., 2005) i.e., the genera *Bacillus*, *Escherichia*, *Enterococcus*, and *Citrobacter* belonged to the intermediate stage of microorganisms, involved in oxidation and fermentation of cellulose and hemicellulosic substrates.

Hemicellulolytic activity is found to be in lesser number of organisms when compared to those which are cellulolytic, our assay in the present in the present study the marine isolates have exhibited a relatively higher ability to degrade xylan indicating that these organisms may also have cellulolytic ability or can be utilized to break down lignocellulosic biomasses along with the identified cellulose and lignin degraders and thus can be used for lignocellulosic biomass degradation which has many applications in various industries.

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