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RESEARCH ARTICLE

STUDY OF THE POTENTIAL OF MARINE FUNGAL ISOLATES FOR LIGNOCELLULOSIC BIOMASS UTILISATION

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Abstract

Agrowastes are abundantly destroyed by burning**.** The present study investigates the potential of the microorganism isolated from the marine source to degrade cellulose, lignin and hemicelluloses, they may even have high potential to produce secondary metabolites which are commercially important for the industries along with the lignocelluloyltic enzymes. In this study, marine samples were collected from the coastal areas of Mangalore, cuddalore and puducherry. The isolation of fungi was carried and the fungal isolates were screened for lignocellulose degradation. The fungi showing maximum degradation were identified as *Trichoderma viride* and *Aspergillus niger* and these were chosen for testing the degradation capacity on eighteen different substrates. These substrates were chosen from rural Bangalore based on their abundance and economic feasibility. The biochemical conversion to simpler sugars for each substrate by the fungal isolates was determined by DNS and lignin oxidation assay. *Aspergillus niger* and *Trichoderma viride* exhibited maximum degradation of Cellulose in 8 substrates within the 1st 5 weeks. Whereas lignin oxidation was maximum during the 3rd week, *Aspergillus niger* oxidized 13 substrate and *Trichoderma viride* oxidized 12 substrate. Lignin is a phenolic polymer which gives structural stability and resistance to enzymatic hydrolysis to the cellulosic biomass. Very few organisms have been found to have an extensive lignin oxidation ability which liberates the cellulose content which can be used by cellulose degraders to yield sugar and energy, this potential was found in the isolates on the tested substrates, making them industrially important strains. In addition, the wastes have also proved to generate sugars and energy which can be utilised by the several other organisms for applications in various industries.

Introduction

Bio-Ethanol from renewable resources has been of interest in recent decades as an alternative to the current fossil fuels. Lignocellulosic materials are cheap renewable resources, available in large quantities throughout the world (Millati et al., 2002). Cellulose, the major fraction of lignocellulosic biomass, can be hydrolyzed to glucose by cellulase enzymes. This hydrolysis can be affected by porosity (accessible surface area) of lignocellulosic biomass, cellulose fiber, crystallinity, and lignin and hemicellulose content (Keikhosro Karimi et al., 2006). Depending on the substrate and the conditions used, up to 95% of the hemicellulosic sugars can be recovered by from the lignocellulosic feedstock (Jeffries et al., 2000).

Plant biomass offers an alternative for fossil resources and balancing the time constants of feedstock production and carbon dioxide fixation. Transport fuels are a major product of the petrochemical industry, thus plant biomass can be extremely attractive to have cost effective, sustainable means of producing transport fuels. One of the most promising processes in this respect is the production of fuel ethanol. Ethanol can be blended with conventional fuels or used as such. Depending on the mixture used, modifications to conventional car motors are either limited or not required (Iogen Corporation 2005).

There are three major habitats of the biosphere where in the marine realm covers 70% of the earth's surface and provides the largest inhabitable space for living organisms, particularly microorganisms. Marine microbes thrive not only in the surface waters of the sea, but also in the lower and abyssal depths from coastal to the offshore regions and from the general oceanic to the specialized niches like blue waters of coral reefs to black smokers of hot thermal vents at the sea floor (Surajit Das et al., 2006).

Many microorganisms that produce various enzymes have been studied for many decades; *Trichoderma* genus has been especially famous for producing cellulolytic enzymes with high activity. However, it is also known that the *Trichoderma* enzymes do not effectively hydrolyze cellulose biomass alone because of their enzyme composition. The saccharification activity of enzymes is specifically important to produce reducing sugars, especially glucose, from cellulolytic biomass (Yamanobe et al., 1987).

The aim of this research is to identify the fungi from marine source having the ability to degrade cellulosic biomass, thus to realize efficient production of cellulolytic enzymes which can be useful on an industrial scale. The present work particularly focuses on the optimization of the 18 agro waste and industrial waste, which acts as a substrate and can be maximally degraded by the identified microorganism from the aquatic source.

MATERIALS AND METHODOLOGY:

Isolation and Identification of Microorganism: Samples were collected from different parts of Tamilnadu (Cuddalore) and Karnataka (Mangalore) sea coast. The samples included sea water, wood pieces in the process of degradation, rock scrap, soil from vegetation in backwaters and sediment samples. The samples were collected in Sterile Polyethene zip bags and Borosil sampling flasks which were then transported to the laboratory in thermocol boxes packed in ice and were preserved in refrigerator until further studies.

Isolation of organisms from marine sources has always been a challenge due to the various physical and chemical conditions of sea water thus, the isolation in the present study was carried out in media prepared in sea water. The sea water sample was filtered using muslin cloth and stored in sterile cans under room temperature till further use. Sea water was autoclaved before use where ever required.

All the standard Media and Chemicals used for the present investigation were procured from Hi-Media (Mumbai) and Sigma Chemicals. Standard microbiological methods were followed for the purpose of isolation of Fungi from the marine samples (Brown, 1985).

Serial dilution of the sample was carried out and one millilitre of the desired dilution (10^{-1}, 10^{-3} and 10^{-5}) was transferred aseptically into media for isolation of Fungi (Potato Dextrose Agar in marine water), by spread plate method. The inoculated media plates were incubated for 5-7 days at room temperature for Fungi. Plates which showed colony counts between 30-300 were selected for identification and the colonies thus obtained on isolation were counted using a colony counter and the number and types of organisms were recorded for each dilution. Simple staining using Lacto phenol cotton blue was conducted on each isolate to check the characteristic feature of the hyphae, spores etc and the purity of the organism. Individual fungal isolate was then subcultured in PDA slants prepared in marine water and maintained as pure culture.

Screening the organisms for production of cellulase, hemicellulase and ligninases under culture conditions: All of the isolated organisms were subjected to screening for the production of cellulases (Pointing *et al.* 1999a.), hemicellulases (Jorgensen *et al*, 2003) and ligninases (Buswell *et al.*, 1996.) on chemically purified substrates CMC, Xylan and Lignin to check for degradation.

Substrate optimization: Eighteen different agro, domestic, industrial waste samples were collected and were dried for 3-5 days depending on the moisture content and then powdered. The substrate was mixed with distilled water containing 0.8% Ammonium nitrate and sterilized (Chundakkadu, 1999; Keikhosro et al., 2006). The substrates were inoculated with fungal cultures. The inoculated substrates were incubated at room temperature. DNS (Gail Lorenz Miller, 1959) and Lignin assay (Acharya *et al,*. 2008, NutawanYoswathana *et al,*. 2009), was carried out at an interval of 7 days for 8 weeks to check for cellulose degradation. The amount of reducing sugar –glucose, released indicated the ability of the organism to degrade the complex lignocellulosic polymer of the waste.

RESULTS AND DISCUSSION:

A total of 43 fungal species (Figure 1) were isolated from different samples. Different colony morphology was observed like round, smooth, cottony, leathery, pigmented, powdery surface, etc. The colonies isolated exhibited different colors due to the spores formed which varied from grey, green, olive green, white, black, brown, pinkish, white with black pins, green with white margins.

Mangalore sediment sample from the backwaters showed the highest number of isolates followed by Cuddalore sediment sample and Cuddalore backwater soil sample. In the present study two fungal isolates CF19 and MF5 showed the maximum degradation of all the three substrates. All the organisms which showed this level of degradative capacity were isolated from the sediment samples and sediment from the backwaters from Mangalore and Cuddalore which indicates the flow of vegetation and soil from the terrestrial areas and in turn adaptation of these organisms to the marine environment with abundant organic matter for degradation and colonization. Based on these characteristic features the isolate CF19 was identified as *Trichoderma viride* and isolate MF5 was identified as *Aspergillus niger*.

A diverse spectrum of lignocellulolytic microorganisms, mainly fungi (Baldrian and Gabriel, 2003; Falcón *et al*. 1995) have been isolated and identified over the years and the list continues to grow.

Aspergillus niger exhibited maximum degradation of Cellulose (Figure 2 and 3) in 8 substrates, they are: Eucalyptus, Maize, Saw dust, Rice straw, Sugar cane, Paper, Ragi straw, Nerium within the 1st 5 weeks. *Aspergillus niger* exhibited maximum oxidation of lignin (Figure 2 and 3) in 13 substrates, they are: Eucalyptus, Maize, Rice straw, Ragi straw, Maize leaves, Teak big leaves, Castor oil leaf, Nerium, Champak, Ficus, Jamum, Hongge, Mango leaves within the 1st 3 weeks.

Trichoderma viride exhibited maximal degradation of Cellulose (Figure 4 and 5) in 8 substrates, they are: Eucalyptus, Maize, Saw dust, Rice straw, Sugar cane, Paper, Ragi straw, Nerium within the 1st 5 weeks. *Trichoderma viride* exhibited maximal oxidation of lignin (Figure 4 and 5) in 12 substrates, they are: Eucalyptus, Rice straw, Sugar cane, Ragi straw, Maize leaves, Teak big leaves, Castor oil leaf, Nerium, Champak, Jamun, Hongge, Mango leaves within the 1st 3 weeks.

Akin *et al*., 1995; Gold and Alic, 1993 suggested that *T. reesei* might be a good producer of hemicellulolytic and cellulolytic enzymes but is unable to degrade lignin, whereas the *Trichoderma viride* isolated in the present study had the ability to degrade lignin as well.

One of the fungal isolates identified as *Trichoderma sp*. exhibited a tri-phasic lignocellulytic activity, i.e., lignolytic (on lignin), hemicellulolytic (xylan) and cellulolytic (CMC) activities on respective plate assays as per the findings of Rubeena *et al.* (2013) and Jun Xie *et al.* (2010).

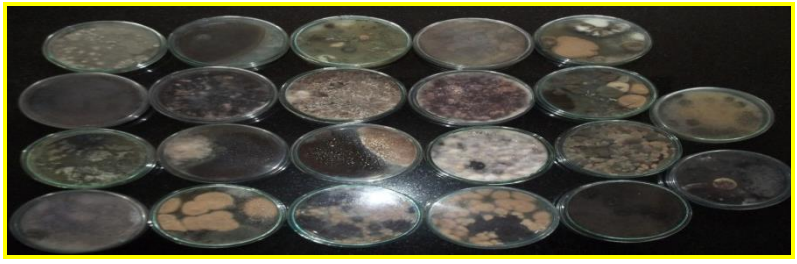

Figure1: Fungal isolates

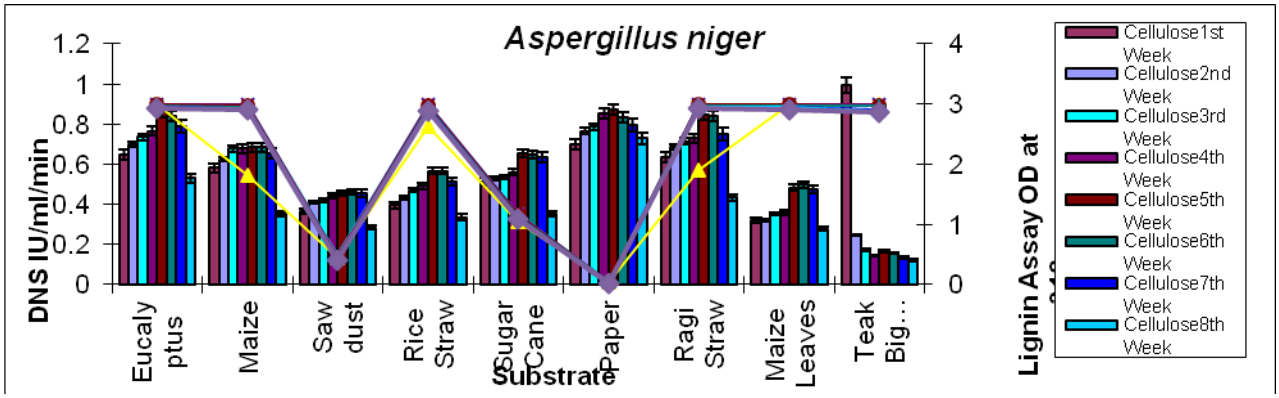

Figure 2: Substrate optimization by *Aspergillus niger*.

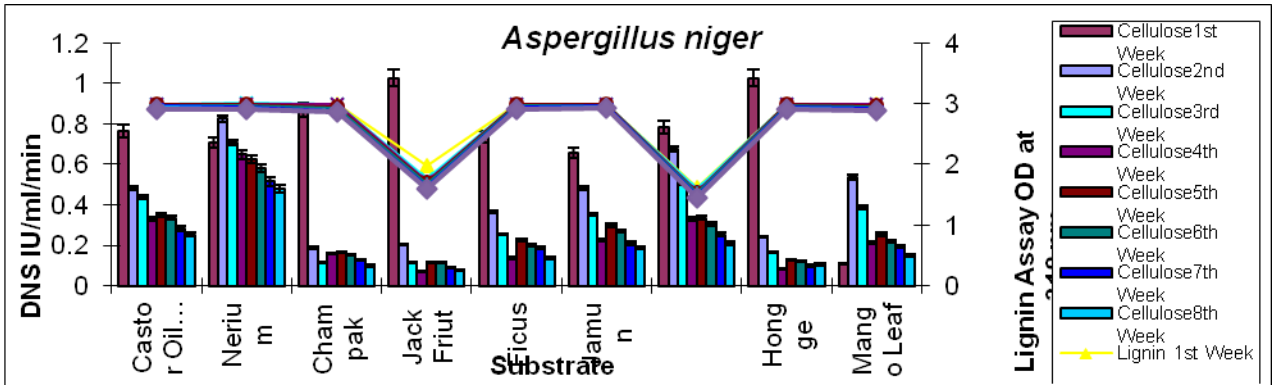

Figure 3: Substrate optimization by *Aspergillus niger*

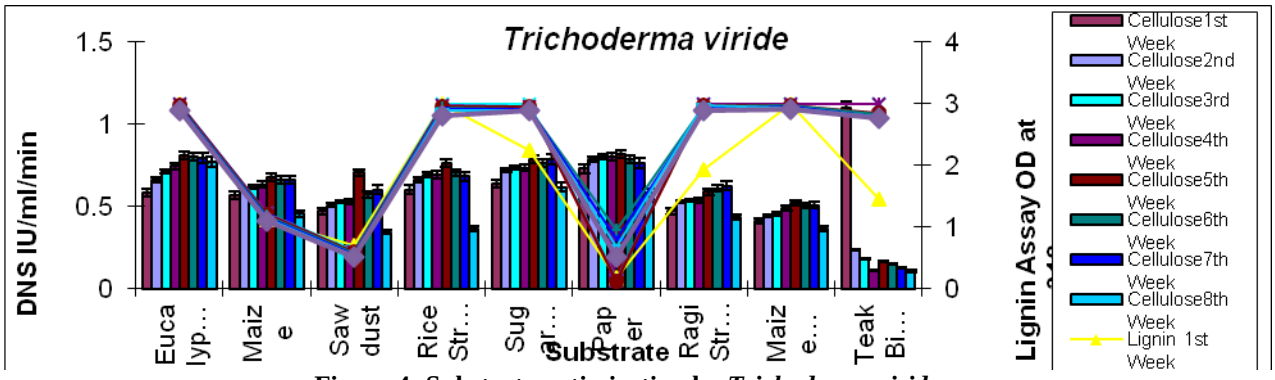

Figure 4: Substrate optimization by *Trichoderma viride*

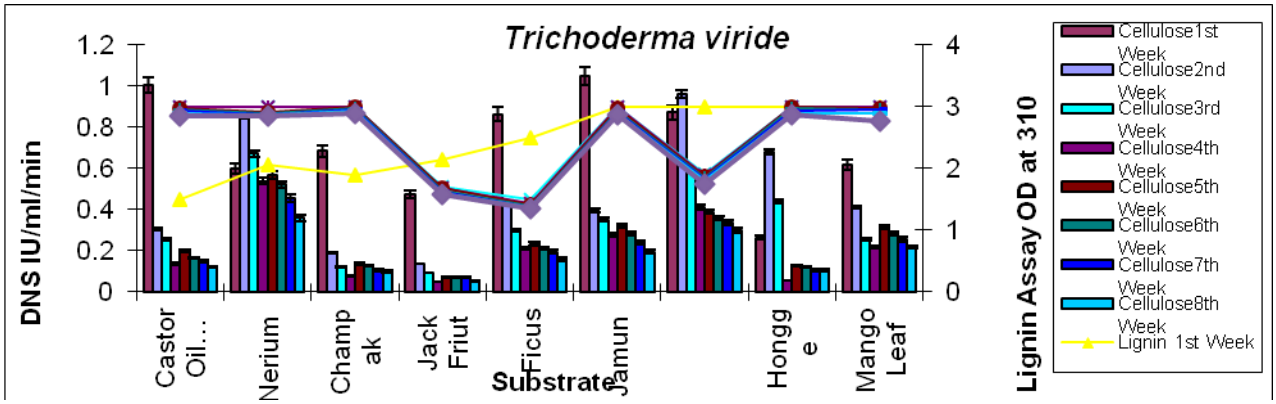

Figure 5: Substrate optimization by *Trichoderma viride*

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