Evaluation of marine fungal isolates for degradation of lignocellulosic biomass

Prasad.M1* and Rekha Sethi2

1Department of Microbiology/Biotechnology, Sangenomics Research Lab, Domlur Layout, Bangalore 560071, India.
2Department of Microbiology, Jain University, Bangalore, India.
*Corresponding Author: E Mail- drprasadm@gmail.com; Ph.: 9844357929

ABSTRACT

The present study investigates the potential of the microorganism isolated from the marine source to degrade cellulose, lignin and hemicelluloses. 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. 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. The potential to break lignin 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.

Key words: Lignocellulose, Agrowaste, Lignin, DNS, Lignin Oxidation, Marine Microbes.

INTRODUCTION

Bio-Ethanol from renewable resources has been of interest in recent decades as an alternative to the current fossil fuels. Cellulose, the major fraction of lignocellulosic biomass, can be hydrolyzed to glucose by cellulase enzymes. Plant biomass offers an alternative for fossil resources and balancing the time constants of feedstock production and carbon dioxide fixation. 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. 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. 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.

MATERIALS AND METHODOLOGY

Isolation and Identification of Microorganism: Samples were collected from different parts of Tamilnadu (Cuddalore) and Karnataka (Mangalore) sea coast. Standard microbiological methods were followed for the purpose of isolation of Fungi from the marine samples on 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. 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, hemicellulases (Jorgensen et al, 2003) and ligninases (Buswell et al., 1996.) on chemically purified substrates CMC, Xylan and Lignin to check for degradation. The substrate was mixed with distilled water containing 0.8% Ammonium nitrate and sterilized. 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.) 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 were isolated from different samples. A diverse spectrum of lignocellulolytic microorganisms, mainly fungi (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 and Lignin (Figure 1 and 2) in 8 and 15 substrates respectively, they are; Eucalyptus, Maize, Saw dust, Rice straw, Sugar cane, Paper, Ragi straw, Nerium Champak, Ficus, Jamum, Hongge and Mango leaves within the 1st 5 weeks. Trichoderma viride exhibited maximal degradation of Cellulose and Lignin (Figure 3 and 4) in 8 and 12 substrates respectively, they are; Eucalyptus, Maize, Saw dust, Rice straw, Sugar cane, Paper, Ragi straw, Nerium within the 1st 5 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 lignocellulolytic 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).
Figure 1. Substrate optimization by *Aspergillus niger*.

Figure 2. Substrate optimization by *Aspergillus niger*.

Figure 3. Substrate optimization by *Trichoderma viride*.

Figure 4. Substrate optimization by *Trichoderma viride*. 
REFERENCES


