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Lignocellulolytic Conversion of Agrowaste by Marine Bacterial Isolates

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Abstract: ***Industrially important strains of Bacterial isolates which posses lignocellulolytic ability have not been extensively found. In the present study, the ability of the marine microorganisms to degrade cellulose, lignin and hemicelluloses is carried out, which acts as the preliminary analysis to identify the potential of the microorganisms. The microorganisms were subjected to degrade 18 different agrowaste which contain less amount of starch and carbohydrate and high amount of lignocellulosic complex. The biochemical conversion to simpler sugars for each substrate by the bacterial isolates was determined by DNS and lignin assay. Bacillus pumilus and Mesorhizobium sp. showed maximum degradation in Rice straw, Ragi straw, Paper and Eucalyptus. Cellulose degradation was seen maximum in the 5th week and lignin assay was maximum in 3rd week. Among the two isolates Bacillus pumilus exhibited a more efficient enzymatic hydrolysis of the substrates when compared to Mesorhizobium sp. These isolates can be used for commercial scale enzymatic hydrolysis of lignocellulosic biomass.***

Keywords: Marine Bacteria, Agrowaste, Lignin, Cellulose, Cellulase enzyme.

1. Introduction

Ocean is the largest ecosystem on earth with a coastline of 193,000 miles and a volume of 137 ´ 10^6 km^3, and has been used for a variety of purposes by human beings for millions of years, because of its large volume and vast area. Microorganisms are ubiquitous in nature and occupy an important place in human view of life. Marine microbes are a potential source for commercially important bioactive compounds and their bioremediation capabilities. They also play a crucial role in decomposition of organic matter and cycling of nutrients. Microorganisms also serve as food for some bottom-living organisms in the ocean. Our knowledge of marine microbial diversity has, however, been severely limited by relying on microorganisms that have been cultured (Surajit Das et al., 2006).

All woody and non-woody plants such as grass have lignocellulose as their major chemical constituent, plants can be cultivated or even otherwise grown under natural conditions therby making lignocellulose a renewable organic matter resource. Great biotechnological value has constantly been discussed because of the properties which make lignocellulose, consisting of cellulose, hemicellulose and lignin (Malherbe and Cloete., 2003). forestry and agricultural practices, paper-pulp industries, timber industries and many agro industries lead to generation of a huge amount of lignocellulosic waste through which pollution in the environment can be increased. Burning is the common method which is used to reduce the lignocellulosic waste which can otherwise contribute to environmental pollutions (Levine, 1996). Many useful products like biofuels, chemicals, improved animal feeds and human nutrients can be produced making use of these residues of plant biomass which is actually treated as waste.

Cellulose is one of the major constitute of plant biomass and highly available in agricultural wastes, industrial effluents and food industry. Energy production potential from microcrystalline cellulose at mesophilic conditions with heat-digested sludge was investigated by Lay et al., 2001.

2. Materials and Methodology

2.1. Isolation of the Microorganisms

Marine samples were collected from different parts of Cuddalore, and Mangalore sea coast. The samples included rock scrap, sea water, sea algae, wood pieces in the process of degradation, soil from vegetation in backwaters and sediment samples. The samples were collected in sterile containers and transported to the laboratory in thermocol boxes packed in ice and were preserved in refrigerator until further studies. Isolation of Bacteria from the marine samples was carried out using standard microbiological methods (Brown, 1985).

2.2. Screening the organisms for production of cellulase, hemicellulase and ligninases under culture conditions:

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

2.3. Identification of the microorganisms

The isolates which exhibited maximal zone of degradation on all the three substrates were chosen as the test organisms for further studies. Two bacteria isolates were identified to species level based on their morphological, biochemical and molecular characterization. These two organisms were used to check for the degradation of 18 different agrowastes as substrates for the present study.

2.4. Substrate Optimization

The substrates used for the present investigation were collected from different parts of rural Bangalore and were selected based on the ready availability, economic value and abundance; eighteen substrates were used in the present study to check out for the degradation of cellulose and lignin

using the isolated test organisms. The substrates were: Rice straw (*Oryza sativa* L.), Saw dust (*Dustaphobek* L.), Paper, Ragi straw (*Eleusine coracana* L.), Maize cobs (*Zea mays* L.), Maize leaves (*Zea mays* L.),Eucalyptus (*Eucalyptus camaldulensis* L.),Sugarcane waste (*Saccharum officinarum* L.), Teak leaf (*Tectona grandis* L.), Castor oil leaf (*Ricinus communis* L.), Nerium (*Nerium oleander* L.), Champak (*Magnolia champaca* L.),Jack fruit waste (*Artocarpus heterophyllus* L.), Ficus leaves (*Opuntiaficus-indica* L.), Jamun leaves (*Jambulina* L.), Crotalaria leaves (*Crotalaria* L.),Honge leaves (*Pongamia pinnata* L.) and Mango Leaves (*Mangifera indica* L.).

Each of the substrate was individually inoculated with the test organisms which were identified as *Bacillus pumilus and Mesorhizobium sp.*, The DNS assay was carried out for estimation the degradation of cellulose and release of simple sugars (Gail Lorenz Miller, 1959) and Lignin oxidation assay, was carried out at an interval of 7 days to check for lignin degradation. Enzyme activity was calculated based on the oxidation of veratryl alcohol to veratryl aldehyde (Acharya *et al*,. 2008, NutawanYoswathana *et al*,. 2009). The results were documented individually for each organism and all the substrates.

3. Results and Discussion

The results recorded for isolation from different samples showed the bacteria in the range of 14 to 83 CFU/gm or ml for different samples. Total of 92 bacterial species were isolated from different samples which varied in their colony morphology and Gram's characters.

All the bacterial isolates were subjected to screening for the production of cellulases, hemicellulases and ligninases on pure substrates such as Carboxy Methyl Cellulose (CMC), Xylan and Lignin to check for their degradation. The test result for the degradation of Cellulose, Lignin and Hemicellulose showed that most of the organisms were positive for cellulose and hemicelluloses where as lignin degradation capacity was limited to very few isolates. Two bacterial isolates showed degradation for all the substrates and these were identified as *Bacillus pumilus* and *Mesorhizobium* sp.

These isolates were inoculated onto+ 18 different substrate and their enzymatic activity was determined. *Bacillus pumilus* exhibited maximum degradation of Cellulose in 8 substrates: Eucalyptus, Maize, Saw dust, Rice straw, Paper, Ragi straw, Maize leaves, Nerium within the 1st 5 weeks. *Bacillus pumilus* exhibited maximum oxidation of lignin in 14 substrates: Eucalyptus, Maize, Rice straw, Sugar cane, Ragi straw, Maize leaves, Teak big leaves, Castor oil leaf, Nerium, Champak, Ficus, Crotoliria, Hongge, Mango leaves within the 1st 3 weeks (Figure 1 and 2).

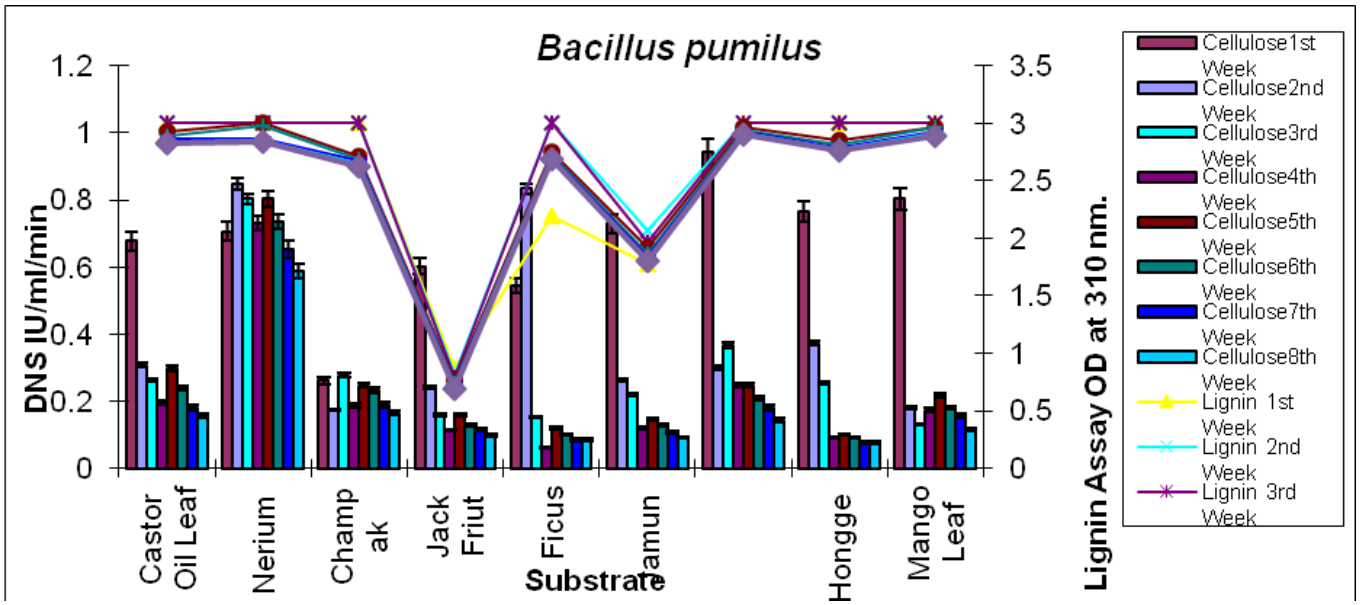

Figure 1: lignocellulase production by *Bacillus pumilus*

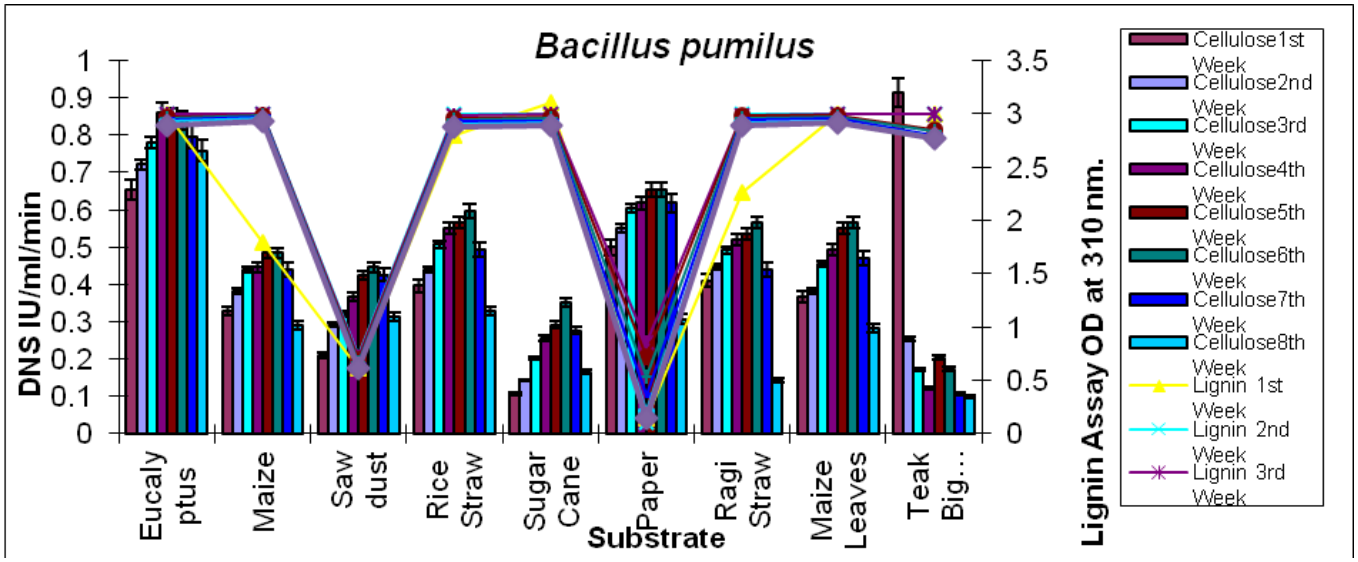

Figure 2: lignocellulase production by *Bacillus pumilus*

Mesorhizobium sp. exhibited maximum degradation of Cellulose in 5 substrates, they are: Eucalyptus, Rice straw, Paper, Ragi straw, Nerium, within the 1st 5 weeks. *Mesorhizobium sp.* exhibited maximum oxidation of lignin in 14 substrates, they are: Eucalyptus, Maize, Rice straw, Sugar cane, Maize leaves,Teak big leaves, Castor oil leaf, Nerium, Champak, Ficus, Jamun, Crotoliria, Hongge, Mango leaves within the 1st 3 weeks (Figure 3 and 4).

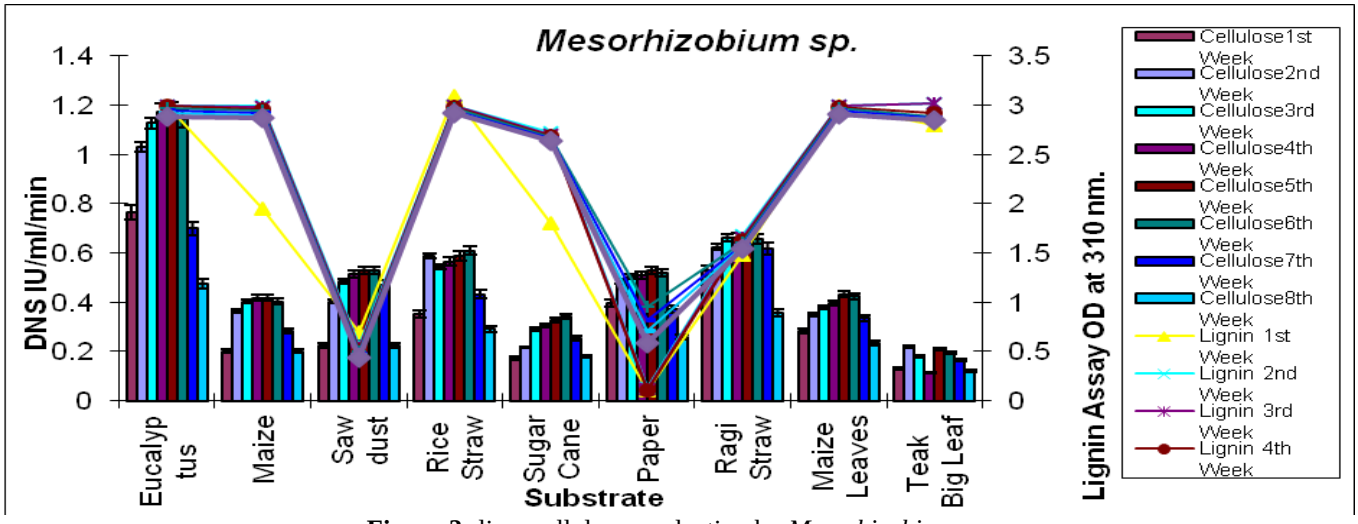

Figure 3: lignocellulase production by *Mesorhizobium sp.*

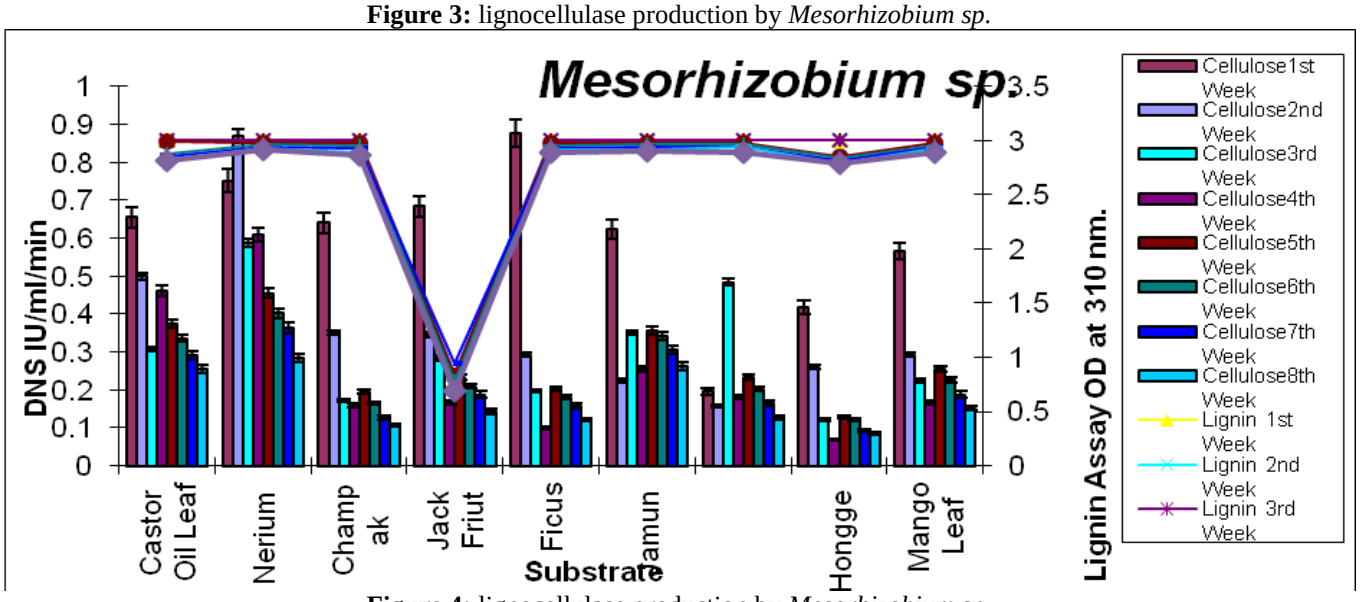

Figure 4: lignocellulase production by *Mesorhizobium sp.*

Similarly Brewer's Spent Grain (BSG) is a material that posses sugars polymerised into cellulose and hemicellulose. The fractionation process under adequate conditions of BSG may produce a liquor rich in xylose, a sugar that can be used as a carbon source for xylitol or ethanol production (Nigam et al., 2002 and Mussatto et al., 2004).

Lignocellulosic biomasses have been reduced to fermentable sugars utilisable by other isolates for ethanol production and thus proved to have a high energy value which is in comparison to statement of Scheper, (2007).

In the present study, bacterial isolates have broken down a complex polysaccharide with the enzymes produced by them on par with the known fungal isolate extensively studied *T.ressei (*Akin *et al*., 1995; Gold and Alic, 1993) and also the ability of bacteria to carry out efficient enzymatic hydrolysis is comparable to the observations of Rheinheimer (1980), where he observed that the matter can be broken down by many Bacteria. Thus, contributing to the recycling of nutrients, matter in the sea and thus maintain an equilibrium..

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