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ISOLATION, IDENTIFICATION AND FUNCTIONAL DIVERSITY OF LIGNOCELLULOSE DEGRADING BACTERIA

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ABSTRACT A study has been performed for Screening, isolation and identification of Lignocellulose degrading bacteria from Raigad District soil. The main objective of this study was to perform diversity analysis of Lignocellulose degrading bacteria with respect to their functional ability to show various enzyme activities. The screening and isolation results showed that the isolates are having capability to enzyme activities for Lignocellulose degradation, Cellulose degradation, Xylanase enzyme and Laccase enzyme production. As an outcome 16s RNA Identification, 06 isolates were isolated and identified as *Achromobacter xylosoxidans, Leclercia adecarboxylata, Ochrobacterium anthropic, Shigella flexneri, Bacillus cerus and Bacillus albus.* The findings show that these microorganisms have significant potential for use in applications for the treatment of Lignocellulose degradation and lignin related environment pollutants.

KEYWORDS : Lignocellulose degrading bacteria, Laccase, Cellulose degradation, Bacillus.

1. INTRODUCTION:

Lignocellulosic biomass is abundant in nature. This is the organic compounds which comprise about 95 % of land built biomass produced by plants. Lignin is having taut association with cellulose and hemicelluloses to form lignocelluloses as a rigid recalcitrant material in woody plants (Hannah L. Woo et al, 2014) [2]. Lignocelluloses are the renewable biomass resources in the world (Wong et al., 1988) [3]. Microorganisms play an important role in bioconversion of Lignocellulosic biomass. The degradation of Lignocellulosic biomass is characterized by the biosynthesis of multicomponent enzymes. The lignocellulosic enzymes are found free or associated with cells (Hwang et al., 2008) [4].

Lignocellulose is made up of three types of polymer compounds, which includes lignin, cellulose and hemicellulose which is forming complex compound with strong bonding (Perez et al., 2002 and Howard et al., 2003) [5, 6]. Complete degradation of these polymers can be achieved by some microorganisms such as Lignolytic bacteria. Hence it is important to isolate and identify the bacteria which are capable of degradation of Lignocellulosic biomass.

Recently several developments have helped to make bioremediation feasible. Varieties of bacteria are capable of caring out degradation of lignocellulosic biomass in the polluted soil and water. To undergo fully metabolized like other polymer must be broken down extracellularly into fragments that are small enough to enter the cells.
The goal of this research study is to isolate, identify and study functional diversity of novel lignin degrading bacteria present in Raigad district soil.

2. MATERIALS AND METHODS:

Source of Soil samples for Isolation:

The Lignocellulose degrading bacteria were isolated from Raigad district soil. Soil samples were collected from various regions of Raigad district based on type and region of the soil. These soil samples were used for screening and isolation of Lignocellulose degrading bacteria. Figure 1, Figure 2, Figure 3 and Figure 4 shows different soil sample.

Figure 1: Soil sample A

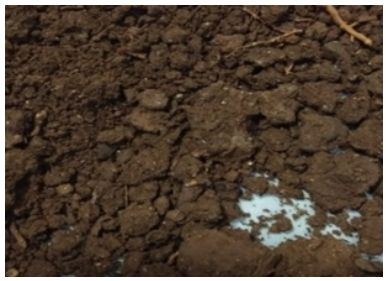

Figure 2: Soil sample B

Figure 3: Soil sample C

Figure 4: Soil sample D

Isolation of Lignocellulose degrading bacteria:

Soil samples were collected from different locations based on region and soil types of Raigad district in Maharashtra India. These samples were used for screening and isolation of Lignocellulose degrading bacteria.

The enrichment of soil samples is achieved by using minimal salt medium containing sole source of carbon source like alkali lignin (Lignin source), Xylan (hemicellulose source), Carboxy methyl cellulose (CMC) (Cellulose source). These mediums are used for

enrichment and isolation of Lignocellulose degrading bacteria. The inoculated flasks were incubated at room temperature for 5-7 days. For isolation of Lignocellulose degrading bacteria agar medium of minimal salt medium with alkali lignin (Lignin source), Xylan (hemicellulose source), Carboxy methyl cellulose (CMC) (Cellulose source) were used.

Confirmation of Lignocellulose degrading bacteria:
Enriched sample of 1ml was transferred to 99 ml of sterile 0.9% NaCl. The solution were stirred vigorously and allowed to settle down. By using 1 ml of liquid mixture serial dilution were prepared in 0.9% NaCl. About 100 µl of diluted samples were spread on minimal salt agar medium containing 1% Lignin. The growth of lignocellulose degrading bacteria observed after incubation at room temperature for 5-7 days.

The colony morphology was checked for bacterial isolates. These Lignocellulose degrading bacteria checked for biochemical characterization. After biochemical characterization the 08 isolates of Lignocellulose degrading bacteria were undergone molecular identification by using 16S rRNA sequencing method.

Functional diversity confirmation of Lignocellulose degrading bacteria:
The isolated Lignocellulose degrading bacteria were screened for their capability to produce various enzymes. Total 08 bacterial isolates were selected for evaluation of functional diversity.

The functional diversity of 08 isolated bacteria was checked for capability of cellulose degradation, Xylanase enzyme and Laccase enzyme activity.

3. RESULTS AND DISCUSSION:

The colony morphology and Gram's nature of selected Lignocellulose degrading bacteria was checked. The morphological characterization is tabulated in Table 1.

Table 1: Colony characters

Sr. No.	Name of Isolate	Colony characters						Gram's nature
		Size	Shape	opacity	color	margin	elevation	
1	SD-01	1-2 mm	round	opaque	Off white	entire	raised	Gram -ve rod
2	SD-02	2 mm	round	opaque	Off white	entire	raised	Gram +ve short rod
3	SD-03	1-2 mm	round	opaque	Off white	entire	raised	Gram -ve short rod
4	SD-04	1-2 mm	round	opaque	Off white	entire	raised	Gram -ve long rod
5	SD-05	1 mm	round	opaque	Off white	entire	raised	Gram -ve short rod
6	SD-06	1-2 mm	round	opaque	Off white	entire	raised	Gram +ve short rod
7	SD-07	1-2 mm	round	opaque	Off white	entire	raised	Gram -ve short rod
8	SD-08	1-2 mm	round	opaque	Off white	entire	raised	Gram +ve short rod

To identify the selected strains biochemical characterization was done. Different biochemical tests are performed they include Indole test, citrate test, Methyl red and Voges-Proskauer test of selected Lignocellulose degrading bacteria were performed and following observations were drawn,

Table 2: Biochemical characterization of Lignocellulose degrading bacteria isolated from Raigad District soil

Sr. No.	Name of Isolate	Formation of Endospore	Motility	Biochemical Tests					
				Catalase	Oxidase	IMViC Tests			
						Indole	MR	VP	Citrate
1	SD-01	-	+	+	+	-	-	-	+
2	SD-02	+	+	+	+	-	-	+	-
3	SD-03	-	+	+	-	-	-	-	-
4	SD-04	-	+	+	+	-	-	+	-
5	SD-05	-	+	+	-	-	-	+	-
6	SD-06	+	+	+	-	-	-	+	+
7	SD-07	-	+	+	-	-	-	-	-
8	SD-08	+	+	+	-	-	-	+	+

Confirmation of Lignocellulose degrading activity:
The growth of isolated colonies on minimal salt medium containing 1% lignin confirms the ability of Lignocellulose degradation as the carbon source present in the agar medium was Lignin. Based on Gram's nature and colony morphology, total 08 bacterial isolates (SD1 to SD8) were selected from the plates of minimal salt medium containing 1% lignin.

Functional diversity confirmation of Lignocellulose degrading bacteria:
The selected Lignocellulose degrading bacteria have shown functional diversity with respect to Lignin degradation, cellulose degradation, Xylanase enzyme and Laccase enzyme production. 08 Lignocellulose degrading bacteria have shown diversity with respect to enzyme activity.

Nucleotide Sequence Accession Numbers:
All the DNA sequences of the partial 16S rRNA genes of the 08 strains reported in this study have been deposited into the GenBank database under the accession numbers from SUB6861401 SD1 MN960398 to SUB6861401 SD8 Mn960405.

DISCUSSION:

The enrichment of Lignocellulose degrading bacteria was achieved by using minimal salt medium containing 1% alkali lignin. Lignolytic activity was confirmed by using methylene blue dye indicator. Lignin degradation activity was also studied by Bondounas et al., 2011 [8].

Cellulose degrading ability of bacterial isolates was performed by streaking the culture on the cellulose congo-red agar media. The cellulose degradation activity was confirmed by decolorization of congo-red indicator. Similar study was performed by Pratima et al., 2012 [9].

Laccase producing bacteria were also isolated from forest by Hemaraju S. and Narasegowda P. N. [1]. Laccase producing bacteria was studied by M. Kuddus et al [8]

Different types of Lignocellulose degrading bacterial isolates were selected which are isolated from Raigad district soil, which is showing diversity and capability to utilize and produce various biochemical and enzymes.

These Lignocellulose degrading bacteria were identified by using molecular biology techniques like 16S rRNA sequencing and confirmed as Achromobacter xylosoxidans (GenBank Accession No. SUB6861401 SD1 Mn960398), Leclercia adecarboxylata (GenBank Accession No. SUB6861401 SD3 MN960400), Ochrobacterium anthropic (GenBank Accession No. SUB6861401 SD4 MN960401), Shigella flexneri (GenBank Accession No. SUB6861401 SD5 MN960402), Bacillus cerus (GenBank Accession No. SUB6861401 SD8 MN960405) and Bacillus albus (GenBank Accession No. SUB6861401 SD2 MN960399). Cellulose degrading bacteria were also isolated and identified and studied by Yan-Ling et al. 2014 [7].

4. CONCLUSION:

Total 08 Lignocellulose degrading bacterial strains were selected from the isolates obtained from Raigad district soil. The diversity of these selected strains was confirmed with respect to various enzyme activities like Cellulose degradation, Xylanase, Laccase etc. The Lignocellulose degrading bacterial strains have shown the capability

to degrade lignocellulosic biomass. The predominant isolates identified by using 16S rRNA sequencing and further the 16S rRNA gene sequences were compared with other 16S rRNA gene sequences available in GenBank by using (https://www.ncbi.nlm.nih.gov/nuccore/) and were identified as Achromobacter xylosoxidans, Leclercia adecarboxylata, Ochrobacterium anthropic, Shigella flexneri, Bacillus cerus and Bacillus albus. The isolates were then maintained on nutrient agar plates for further studies.

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