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Research Paper

Screening and Characterization of Lignin Degrading Bacteria from Decayed Sawdust

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Abstract: *In this study an attempt was made to isolate and characterize the lignolytic bacteria. The decayed sawdust sample was collected and screened for lignolytic bacteria using Crawford medium containing of tannic acid as lignin equivalent. Seven bacterial isolates such as Bacillus spp1, Streptococcus* spp*, Pseudomonas spp2, Acinetobacter spp, Serratia spp, Escherichia spp, and Proteus spp2 were selected based on their solubilization index against tannic acid. All these isolates were further characterized for their activity to degrade lignin analogue i.e., tannic acid in various growth conditions such as various concentration of tannic acid viz.,0.25, 0.50, 0.75, 1.0 and 1.25 different temperature viz.,28°C, 37°C and 45°C and various pH viz., acidic neutral and alkaline conditions. The bacterial isolates such as Bacillus spp1, Pseudomonas spp2, Acinetobacter spp, and Serratia spp were showed the better lignolytic activity at a concentration of 1.0 percent tannic acid in the acidic (pH 6.0) and neutral (pH 7.0) conditions at 37°C.*

Keywords: Decayed sawdust, Lignolytic bacteria, Crawford medium, Tannic acid.

Introduction

Ligno-cellulose is the predominant component of woody plants and the most abundant biomass on earth. Ligno-cellulose is a renewable organic material and is the major structural component of woody plants and non-woody plants such as grasses. It is composed of three major components: cellulose (35-50%), hemicelluloses (20-35%) and lignin (10-25%) (Saha, 2003). It is a major renewable natural resource of the world and represents a major source of renewable organic matter. Worldwide approximately 3480 Trillion grams/year of lignocellulose in the form of agricultural waste is

accumulated every year (Kim *et al.,* 2004). The wastes produced from industries and agricultural farm lands constitute a major problem to our environment. Such wastes include cereal straws, corn cobs, wood pulp, sawdust, cotton wastes and many others. Usually they are burnt in heaps there by releasing offensive odor and gases to the atmosphere. Some are thrown into rivers and streams thereby endangering aquatic life. These wastes could be put into appropriate use in order to reduce environmental hazard and pollution (Jonathan, 2008). The plant biomass regarded as wastes and can be converted into valuable products such as biofuels, chemicals, cheap sources for fermentation, improved animal feeds and human nutrients. In nature, many diverse group of microorganisms are capable to degrade lignin rich biomass. The ability of filamentous and non-filamentous bacterial species of *Acinetobacter* (Vasudevan and Mahadevan, 1991), *Arthrobacter* (Kerr *et al.*, 1983), *Bacillus* (Gurujeyalakshmi and Mahadevan, 1987-a), *Branhamella*, *Brochothrix* (Gurujeyalakshmi and Mahadevan, 1987a-b *; Kumar et al.,* 2001*) Micrococcus* (Kumar *et al., 2001), Nocardia* (Trojanowski *et al*., 1977), *Pseudomonas* (Kaplan and Hartenstein, 1980), *Serratia* (Perestelo *et al.,* 1994), *Streptomyces* (McCarthy and Broda, 1984) and *Xanthomonas* (Kern, 1984) to degrade lignin has been established. Certain bacteria such as *Bacillus pumillus* (ATCC12905), *Bacillus stearothermophilus, Rhodobacter sphaeroides, Rhodomonas palustri, Steptococccus lactis, P. purrocinna* ATCC 15958, *P. flourescens* NRRL B-11, *P. ovalis, P. putida FK-1,* and *FK-2* etc., are able to produce polyphenol peroxidases, which can mineralize lignin and lignin-containing compounds **(**Kawakami, 1975). Hence in the present study, an attempt was made to screen and characterize lignolytic microbes isolated from decayed sawdust.

Materials and Methods

The decayed sawdust sample was collected from the dumping yard near sawdust industry, Thenkarai Periyakulam, Theni (District), Tamil Nadu, India. The samples were collected at four different spots randomly and blended uniformly. All the samples were transported aseptically to the laboratory, Department of Biology, Gandhigram Rural Institute – Deemed University, Gandhigram for further analysis.

Enumeration of Microbial Population

The standard plate count method (Subbarao, 1995; Kannan, 1996) was used for enumerating the total Colony Forming Units(CFU) of bacteria, fungi and actinomycetes in decayed sawdust. One gram of decayed sawdust sample was taken in a 250 ml sterile conical flask containing 100 ml of Normal Saline (0.85% NaCl) and shaken in a Vortex mixture for 30 minutes. From this stock, various dilutions were prepared from 10^{-2} to 10^{-6} with Normal Saline (0.85% NaCl) and the diluted samples representing 10^{-2}(for fungi), 10^{-3} (for actinomycetes) and 10^{-6} for bacteria) were plated on Martin's Rose Bengal Agar , Kenknights Agar and Nutrient Agra respectively for 5 days at 28^0C (for fungi), 5 days at 37^0C (for Actinomycetes) and 24hrs at 37^0C (for bacteria). After incubation the total Colony Forming Units of bacteria, fungi and actinomycetes on the respective growth medium were counted and statistically analysed for microbial load in the sawdust sample.

Screening for Lignolytic Activity

Seven bacterial isolates were further screened for lignolytic activity by standard procedures (Bavendamm, 1927 and Aggelis *et al*., 2002). All the seven bacterial strains were separately inoculated in the Crawford medium supplemented with tannic acid as source of lignin and as selective agent to check the organisms secreat polyphenol peroxidase. Inoculated plates were incubated at 30°C ± 2°C for 5 d and observed for zone of clearance with brown color development around the colony as a positive indication for polyphenol peroxidase activity. Based on the results exhibited by the isolates on the selective medium, only seven bacterial isolates were selected for further characterization.

Identification of Predominant Bacterial Isolates

Seven bacterial isolates were selected based on their abundance growth on the respective culture media for further study. All the isolates were pure cultured by streak plate method and then identified through morphological and biochemical characteristics viz., Colony morphology, Gram's reactions, motility, indole production, methyl red reaction, Voges-Proskauer reaction, citrate utilization, catalase reaction, oxidase reaction, urease production, gelatin hydrolysis and nitrate reaction (Apun *et al.*, 2000). The morphological and biochemical test results were compared with Bergey's Manual of Determinative Systematic Bacteriology (Holt *et al.,* 1994) and thus identified all seven bacterial isolates.

Characterization of Selected Lignolytic Bacteria

The seven selected bacterial isolates were characterized in different environmental conditions by the standard procedure (Mahalingam and Daniel, 2007). Crawford medium was prepared with various buffered solutions, using acetate buffer (pH 6.0) and phosphate buffer (pH 7.0) and tris-Hcl buffer (pH 8.0). The medium of each pH was supplemented with increasing concentrations of tannic acid (an analogue to lignin) i.e., 0.25, 0.50, 0.75, 1.0 and 1.25 percent. The media were sterilized and the seven selected bacteria were separately inoculated and incubated at 28°C, 37°C and 45^{o}C. The growth performances of the seven bacterial strains were observed in all the growth conditions on 7d and the results were recorded and tabulated.

Results

Enumeration of Microbial Population

The results of total population of the different groups of microorganisms such as bacteria, fungi and actinomycetes present in decayed sawdust are given in Table 1. The total number of Colony Forming Units (CFU) of bacteria, fungi and actinomycetes varied in sawdust sample. The actinomycetes population was found to be highest when compared to the bacterial and fungal population.

Table 1: Total colony forming units of bacteria, fungi and actinomycetes observed in decayed sawdust

Samples	Microbial population		
	Bacteria ($x\ 10^{6}\ g^{-1}$)	Fungi ($x\ 10^{4}\ g^{-1}$)	Actinomycetes ($x\ 10^{5} g^{-1}$)
Decayed Sawdust	16.46±0.5	14.54±0.47	17.25±0.52

Values of mean ± Standard deviation

Screening of Lignolytic Activity

Seven different bacterial isolates were selected from the culture medium based on their abundance growth and followed by all these isolates were screened for better lignolytic activity using Crawford medium supplemented with tannic acid. The seven bacterial isolates shown better lignolytic activity through the formation of brown color around the colonies as positive indication of lignolytic activity and the solubilization index was calculated and the results were recorded (Table2).

Table 2: List of lignolytic bacterial isolates and their Solubilization Index (SI)

Isolate code	Name of the bacterial Isolates	Type	Solubilization index (SI) (%)
LBIS-1	*Bacillus* spp1	Gram +ve	28.37 ± 0.03
LBIS-2	*Streptococcus* spp	Gram +ve	22.33 ± 0.07
LBIS-3	*Pseudomonas* spp2	Gram-ve	25.30 ± 0.05
LBIS-4	*Acinetobacter* spp	Gram-ve	27.85 ± 0.01
LBIS-5	*Serratia* spp	Gram-ve	24.55 ± 0.09
LBIS-6	*Escheritia* spp	Gram-ve	21.22 ± 0.02
LBIS-7	*Proteus* spp2	Gram-ve	19.90 ± 0.01

LBSI- Lignolytic Bacterial Isolates

Identification of Efficient Lignolytic Bacteria

Seven selected lignolytic bacterial isolates were identified based on their morphological and biochemical characteristics. The results for the morphological and biochemical tests of the seven lignolytic bacterial isolates were recorded in Table 3 and 4.

Table 3: The Morphological characteristics of seven lignolytic bacterial isolates in decayed sawdust

Isolate code	Colony morphology	Grams Reaction	Cell shape	Motility
LBIS-1	White glossy membranous colonies	Positive	Rod	-

LBIS-2	Mucoid colonies	Positive	Cocci	-
LBIS-3	Pale yellow pigmented colonies	Negative	Rod	+
LBIS-4	Opaque white	Negative	Coccobacilli	-
LBIS-5	Orange colonies	Negative	Rod	+
LBIS-6	White Irregular	Negative	Rod	+
LBIS-7	Watery colonies	Negative	Rod	+

LBSI- Lignolytic Bacterial Isolates

Table 4: Biochemical characteristics of seven lignolytic bacterial isolates in decayed Sawdust

Isolate code	Biochemical characteristics									Identification result (Name of the isolates)
	Indole production	Methyl red reaction	Voges-Proskauer reaction	Citrate utilization	Catalase reaction	Oxidase reaction	Urease production	Gelatin hydrolysis	Nitrate reduction	
LBIS-1	+	+	+	+	+	+	-	+	+	*Bacillus* spp1
LBIS-2	+	-	-	+	-	-	-	-	-	*Streptococcus* spp
LBIS-3	+	-	+	+	+	+	+	-	+	*Pseudomonas* spp2
LBIS-4	-	-	-	+	+	-	-	-	-	*Acinetobacter* spp
LBIS-5	-	+	+	+	+	+	-	+	+	*Serratia* spp
LBIS-6	+	-	-	+	+	-	+	+	+	*Escheritia* spp
LBIS-7	-	+	-	-	+	-	+	+	+	*Proteus* spp2

LBIS- Lignolytic Bacterial Isolates

Characterization of Selected Lignolytic Bacteria

The observations on the growth performance of the seven selected lignolytic bacteria in the acidic condition (pH 6.0), in the neutral condition (pH 7.0) and in the alkaline condition (pH 8.0) with various concentrations of tannic acid at three different temperatures are given in Tables 5, 6 and 7 respectively. The growth performance varied for various bacteria in different concentrations of tannic acid, temperatures and pH. The bacteria, *Bacillus* spp1, *Pseudomonas* spp2, *Acinetobacter* spp and

Serratia spp showed good growth performance at 37^{o}C with 1.0 percent tannic acid in the acidic (pH 6.0) and in the neutral (pH 7.0) conditions (Tables 5 and 6).

Table 5: Growth performance of the seven selected lignolytic bacteria grown in Crawford medium containing various concentrations of tannic acid at three different temperatures in acidic Condition (pH 6.0) on 7d

Growth temperature (^{0}C)	Tannic acid concentration (%)	*Bacillus* spp1	*Streptococcus* spp	*Pseudomonas* spp2	*Acinetobacter* spp	*Serratia* spp	*Escherichia* spp	*Proteus* spp2
28	0.25	-	-	-	-	-	-	-
	0.50	+	-	+	+	+	-	-
	0.75	++	+	+	++	+	+	+
	1.0	++	+	++	++	+	+	+
	1.25	++	+	+	+	++	+	+
37	0.25	+	-	+	+	+	-	+
	0.50	++	+	++	++	++	+	+
	0.75	++	++	++	++	++	++	++
	1.0	+++	++	+++	+++	+++	++	++
	1.25	++	+	++	++	++	+	+
45	0.25	-	-	-	-	-	-	-
	0.50	-	-	-	-	-	-	-
	0.75	-	-	-	-	-	-	-
	1.0	-	-	-	-	-	-	-
	1.25	-	-	-	-	-	-	-

- = Poor Growth, + = Poor Growth, ++ = Moderate Growth, +++ = Good Growth

Table 6: Growth performance of the seven selected lignolytic bacteria grown in Crawford medium containing various concentrations of tannic acid at three different temperatures in neutral condition (pH 7.0) on 7d

Growth temperature (^{0}C)	Tannic acid concentration (%)	*Bacillus* spp2	*Streptococcus* spp	*Pseudomonas* spp2	*Acinetobacter* spp	*Serratia* spp	*Escherichia* spp	*Proteus* spp2
28	0.25	+	-	+	-	-	-	-
	0.50	+	+	+	+	+	+	+
	0.75	+	+	+	+	+	+	+
	1.0	++	+	++	++	++	+	+
	1.25	++	+	++	++	++	+	+
37	0.25	+	-	+	-	+	-	-
	0.50	+	+	+	+	+	+	+
	0.75	+++	++	+++	+++	+++	+++	++
	1.0	+++	++	+++	+++	+++	+++	++
	1.25	++	++	++	++	++	++	++
45	0.25	-	-	-	-	-	-	-
	0.50	-	-	-	-	-	-	-
	0.75	-	-	-	-	-	-	-
	1.0	-	-	-	-	-	-	-
	1.25	-	-	-	-	-	-	-

- = Poor Growth, **+** = Poor Growth, **++** = Moderate Growth, **+++** = Good Growth

Table 7: Growth performance of the seven selected lignolytic bacteria grown in Crawford medium containing various concentrations of tannic acid at three different temperatures in alkaline condition (pH 8.0) on 7d

Growth temperature (^{0}C)	Tannic acid concentration (%)	*Bacillus* spp1	*Streptococcus* spp	*Pseudomonas* spp2	*Acinetobacter* spp	*Serratia* spp	*Escherichia* spp	*Proteus* spp2
28	0.25	-	-	-	-	-	-	-
	0.50	+	-	+	+	+	-	-

	0.75	+	+	+	+	+	+	+
	1.0	++	+	++	++	++	+	+
	1.25	+	+	+	+	+	+	+
37	0.25	-	-	-	-	-	-	-
	0.50	+	+	+	+	+	+	+
	0.75	+	+	+	+	+	+	+
	1.0	++	++	++	++	++	++	+
	1.25	+	+	++	++	+	+	-
45	0.25	-	-	-	-	-	-	-
	0.50	-	-	-	-	-	-	-
	0.75	-	-	-	-	-	-	-
	1.0	-	-	-	-	-	-	-
	1.25	-	-	-	-	-	-	-

- = Poor Growth, **+** = Poor Growth, **++** = Moderate Growth, **+++** = Good Growth

Discussion

In general diverse microbial groups in the environment are capable of degrading lingo-cellulosic rich organic substrate into a simplest nutrient form. Hence an attempt was made to screen and characterize lignolytic microbes isolated from decayed sawdust. Fifteen bacterial strains were isolated from decayed sawdust and all of them were screened for lignolytic activity using Crawford medium supplemented with tannic acid. Among fifteen bacterial strains screened, only seven bacterial isolates showed lignolytic activity and their solubilization index was calculated (Table 2). Followed by, all seven lignolytic bacterial isolates were identified based on morphological and biochemical characteristics (Table 3 and 4). In the present study, various growth conditions such as pH, substrate concentration and temperature for the growth of lignin degrading microorganisms were optimized and the results are shown in Tables 5, 6 and 7. Mahalingam and Daniel (2007) Isolated lignin degrading *Pseudomonas aeruginosa* along with three fungi such as *Penicillium* spp, *Fusarium* spp and *Aspergillus* spp from termite gut and partially characterized their growth parameter against different pH condition such as pH 4,7 and 8. The results in the present study revealed that the growth performance differ from among the different isolates. The bacteria such as *Bacillus* spp1, *Pseudomonas* spp2, *Acinetobacter* spp and *Serratia* spp showed good growth performance at 37^{o}C with 1.0 percent tannic acid in the acidic (pH 5.0) and neutral (pH 7.0) conditions (Tables 5 and 6).Thus, they may play some role in final mineralization of lignin. Similar results were observed by Vicuna (1988) and Zimmermann (1990) and they have reported that *Pseudomonas* spp are the most efficient degraders among may eubacterium were screened for lignolytic activity. Mahalingam (2007) reported that *Pseudomonas* spp also has good potential for industrial degradation of lingo-celluolytic biomass. In addition, several able to degrade various lignin preparations such *Pseudomonas spp* are as milled wood lignin, dioxane lignin and lignin from poplar wood (Odier *et al.*, 1981). A novel process of lignin degradation using a consortium of bacteria containing three lignolytic bacteria such as *Serratia marcescens* (MTCC 5094*), P. aeruginosa* (MTCC 5095) and *P. aeruginosa* (MTCC 5098) were isolated from a mixture of sawdust and soil has been reported by Rita and Anil (2006). *Mycobacterium tuberculosis* var. grows well but slowly (2-6 weeks) on a solid media such as coagulated egg, serum or blood or on glycerin agar at 37°C. *Vibrio*, *Streptococcus faecalis* and *Escherichia coli* also tolerate an alkaline reaction (pH 8-9). *Streptococcus lactis* can be cultivated in sterile milk or on agar containing milk or whey or tomato juice at about 25°C. It grows best in the presence of glucose or lactose. It grows better at about 35°C than at 25°C Frobisher (1961).

Conclusion

Seven bacterial strains such as *Bacillus* spp1, *Streptococcus* spp, *Pseudomonas* spp2, *Acinetobacter* spp, *Serratia* spp, *Escheritia* spp and *Proteus* spp2 isolated from decayed sawdust showed and better lignolytic activity against different growth conditions such as temperature, pH and substrate concentration. Hence these organisms could be used commercially in the rapid degradation of lignocellulose rich sawdust material into nutrient rich biomanure for Agricultural practice in Rural India

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