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Isolation and Identification of Bacterial Strains From Decayed Sawdust

KEYWORDS: Decayed sawdust, Gram positive bacteria and Gram negative bacteria

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ABSTRACT *Six gram positive and nine gram negative bacterial strains were isolated from decayed sawdust using nutrient agar and identified based on morphological and biochemical characteristics. All the, gram positive bacterial isolates Bacillus spp1, Bacillus spp2, Streptococcus spp, Micrococcus spp, Staphylococcus spp and Clostridium spp and gram negative bacterial isolates were identified as Pseudomonas spp1, Pseudomonas spp2, Acinetobacter spp, Serratia spp, Escherichia spp, Klebsiella spp , Proteus spp1, Proteus spp2 and Enterobacter spp .*

Sawdust is a material produced from cutting wood with a saw. It contains 40%–50% cellulose, 25%–35% hemicellulose, and 20%–30% lignin (Sınag *et al*., 2009). It is the main by-product of wood processing in sawmills and can be processed into particles board, burnt in sawdust burner, used on poultry floors or used to make heat for other milling operations (Eze *et al*., 2011). When not used as these, it is required as a waste which is disposed of and eventually collected in piles and harmful leachates into water system and block water ways which create environmental hazards. If burnt, they produce very thick smoke with high environmental consequences (Lennox *et al*., 2010). Several studies have been carried out to identify microorganism responsible for degradation of wide range of organic substrates (Maheshkumar and Mahalingam, 2011; Mahalingam and Daniel, 2008; Angaleshwari and Mahalingam, 2014). Several author were isolated bacterial strains belongs various genera from sawdust based waste materials (Parthasarathi and Renganathan, 1998; Eze *et al*., 2011). This study is focused on isolation of bacterial strains from decayed sawdust.

Materials and methods

Decayed sawdust sample was collected from the dumping yard located near Thenkarai Sawmill, Periyakulam, Theni District, Tamil Nadu, India and transported to the laboratory for microbial analysis. Sawdust sample was serially diluted and plated on Nutriant agar. The predominant colonies of fifteen bacterial isolates were selected identified based on various parameters viz., colony morphology, gram's reactions, motility, and various biochemical properties such as indole production, methyl redreaction, Voges – Proskauer reaction, citrate utilization, catalase reaction, oxidase reaction, urease production, gelatin hydrolysis and nitrate reduction. The results of cultural and biochemical characteristics for various bacterial isolates were compared with Bergey's Manual of Determinative Systematic Bacteriology (Holt *et al.,* 1994) and thus identified all the fifteen bacterial isolates.

Results

Isolation and identification of predominant bacterial and fungal groups

The identification results of 15 bacterial which showed predominant growth on the culture medium are given in Tables 1 and 2. From among the total bacterial isolates**,** 6 strains are found to be Gram positive and the other 9 strains are of Gram negative. The morphological and biochemical characteristics of all the six gram positive bacterial isolates are given in Table 1. The results are compared with Bergey's manual of determinative bacteriology and thus identified all the six gram positive bacterial isolates as *Bacillus* spp1, *Bacillus* spp2, *Streptococcus* spp, *Micrococcus* spp, *Staphylococcus* spp and *Clostridium* spp. While the morphological and biochemical characteristics for all the 9 gram negative bacterial isolates are given Table 2. Based on the comparison of these results with Bergey's manual of determinative bacteriology, all the gram negative bacterial isolates are identified as *Pseudomonas* spp1, *Pseudomonas* spp2, *Acinetobacter* spp, *Serratia* spp, *Escherichia* spp, *Klebsiella* spp , *Proteus* spp1, *Proteus* spp2 and *Enterobacter* spp .

Table 3: Morphological and biochemical characteristics of Gram positive bacterial isolates from decayed sawdust

Isolate code	Colony morphology	Gram's reaction and Cell shape	Biochemical Characteristics											Identification result (Name of the isolate)
			Motility test	Indole production	Methyl red reaction	Voges - Proskauer reaction	Citrate utilization	Catalase reaction	Oxidase reaction	Urease production	Gelatin hydrolysis	Nitrate reduction	Casein hydrolysis	
GPBIS – 1	White glossy membranous colonies	+ve, Rod	-	+	+	+	+	+	+	-	+	+	-	*Bacillus* spp1
GPBIS – 2	Large, irregular, entire, creamy, opaque colonies.	+ve, Rod	-	+	-	-	+	+	+	-	-	+	-	*Bacillus* spp2

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GPBIS – 3	Mucoid colonies	+ve, Cocci	-	+	-	-	+	-	-	-	-	-	-	*Streptococcus* spp
GPBIS – 4	White round colonies	+ve, Cocci	-	+	-	-	+	+	-	+	+	+	-	*Micrococcus* spp
GPBIS – 5	Tin, milky large creamy colonies	+ve,Cocci	-	-	-	+	-	+	-	+	-	-	-	*Staphylococcus* spp
GPBIS – 6	Circular, smooth and creamy colonies	+ve, Rod	-	-	+	-	+	-	-	-	-	-	-	*Clostridium* spp

GPBIS = Gram Positive Bacterial Isolates + = Positive reaction, - = Negative reaction

Table 5: Morphological and biochemical characteristics of Gram negative bacterial isolates from decayed sawdust

Isolate code	Colony morphology	Gram's reaction and Cell shape	Biochemical Characteristics											Identification result (Name of the isolate)
			Motility test	Indole production	Methyl red reaction	Voges - Proskauer reaction	Citrate utilization	Catalase reaction	Oxidase reaction	Urease production	Gelatin hydrolysis	Nitrate reduction	Casein hydrolysis	
GNBIS – 1	Green color pigmented colonies	-ve, Rod	+	+	-	+	+	+	+	-	-	+	-	Pseudomonas spp1
GNBIS – 2	Pale yellow pigmented colonies	-ve, Rod	+	+	-	+	+	+	+	+	-	+	-	Pseudomonas spp2
GNBIS – 3	Opaque white colonies	-ve, Coccobacilli	-	-	-	-	+	+	-	-	-	-	-	Acinetobacter spp
GNBIS – 4	Orange colonies	-ve, Rod	+	-	+	+	+	+	+	-	+	+	-	Serratia spp
GNBIS – 5	White Irregular colonies	-ve, Rod	+	+	-	-	+	+	-	+	+	+	-	Escherichia spp
GNBIS -- 6	Large mucoid colloid colonies	-ve, Rod	-	-	-	+	+	+	-	+	-	+	-	Klebsiella spp
GNBIS – 7	Yellow colonies	-ve, Rod	-	-	+	-	-	-	-	+	+	+	-	Proteus spp1
GNBIS – 8	Watery colonies	-ve, Rod	+	-	+	-	-	+	-	+	+	+	-	Proteus spp2
GNBIS – 9	Yellow circular colonies	-ve, Rod	+	-	-	+	+	+	-	-	+	+	-	Enterobacter spp

GNBIS = Gram Negative Bacterial isolates + = Positive reaction - = Negative reaction

Discussion

The study on identification of predominant bacteria and fungi showed the presence of six Gram positive bacteria (Table 1) and nine Gram negative bacteria (Table 2) were isolated from decayed sawdust. These organisms play an important role in the biodegradation of organic wastes. Parthasarathi and Ranganathan (1988) have isolate ten type of gram negative and four type of gram positive bacteria from various substrates such as sawdust and press mud. A wide variety of Gram- positive and Gram-negative bacterial species are reported to produce cellulose, these results clearly reveals that the decaying of sawdust was coordinated by several group of bacterial strains.

Conclusion

The presence of wide variety of bacterial strain in decayed sawdust would infers that these organisms are closely associated with bioconversion of sawdust in to nutrient rich organic manure. Hence, these bacterial isolates were taken further for ligno-cellulolytic characterization.

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