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Screening and Partial Characterization of Cellulose Degrading Bacteria from Decayed Sawdust

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Abstract: ***In this study, sixteen bacterial strains were isolated from decayed sawdust and they were further screened for cellulolytic activity using Czapek's medium containing carboxy methyl cellulose (CMC) as selective agent. Based on solubilization index, ten bacterial isolates were selected and were further characterized by grown in Czapek's medium containing various concentration of carboxy methyl cellulose at 28°C, 37°C and 45°C in different pH condition such as acidic (6.0), neutral (7.0) and alkaline (8.0) conditions. All the isolates were able to degrade the sawdust to varying extent. But the cellulolytic bacterial isolates showed a good growth in medium with 1.5 percent carboxy methyl cellulose at neutral (pH 7.0) conditions. The effect of temperature also showed that bacterial isolates were able to grow well at 37°C. Among, five isolates Bacillus spp1, Bacillus spp2, Micrococcus spp, Pseudomonas spp1 and Acinetobacter spp, showed the better cellulose activity.***

Keywords: Cellulolytic activity, sawdust, CMC, Czapek's medium

1. Introduction

Cellulose is the major constituent of plant matter and thus represents the most abundant organic polymer on Earth. About 1015 kg of cellulose is synthesized and degraded on Earth each year. Cellulose makes a large fraction of the plant dry weight, being typically in the range of 35-50%. It gives stability to the plants even in the absence of water and makes them more resistant. Ligno-cellulosic biomass makes about 50% of the total plant biomass in the world with an estimated annual production of 10-50 billion tons (Sanchez and Cardona, 2008). The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of intense research and industrial interest (Bhat, 2000). Cellulose is the primary product of photosynthesis in terrestrial environments, and the most abundant renewable bio resource produced in the biosphere (100 billion dry tons / year) (Holtzapple,1993 ; Jarvis, 2003 ; Zhang and Lynd, 2004-b). Sawdust a by- product of wood processing and are regarded as a waste. It is often heaped near carpenters shades, burnt or dumped in to rivers. Consequently, they block the water ways and if burnt, produce very thick smoke with high environmental consequences. Wastes and their disposal is a subject of environmental concern worldwide especially when they are non-biodegradable to useful goods and services (Banjo and Kubuoye, 2000). The production of saw softwood, *i.e.* timber has varied between 9.80 and 13.65 million solid m^3. This means that the forest industry has created 2.94–4.09 million solid m^3 sawdust. The quality of sawdust is mainly dependent on the particle size of sawdust and it is not uniform and the distribution is usually concentrated on the smallest size fractions (Isotalo *et al*., 1964-a ; Surewicz, 1974 ; Bublitz and Yang, 1975 ; Taylor, 1977 ; Joshi *et al.,* 1982 ; MacLeod and Kingsland, 1990 ; Korpinen and Fardim, 2006 ; Bergström *et al*., 2008). The complete degradation of these compounds requires enzymes that act synergistically. Microbes have been found to play important roles in degradation of cellulose (Haite *et al*., 2010 and Makeshkumar and Mahalingam, 2011). Bacteria has high growth rate as compared to fungi has good potential to be used in cellulose production. Celluloytic property of some bacterial genera such as *Cellulomonas species*, *Pseudomonas species*, *Bacillus species and Micrococcus species,* were reported by Nakamura and Kappamura, (1982). Here Screening for efficient cellulolytic bacteria is very much needed for recycling of cellulosic biomass.

2. Materials and methods

2.1 Collection of Sample

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

2.2 Screening of cellulolytic bacteria

The standard plate count method was used for enumerating the total Colony Forming Units (CFU) of bacteria in decayed sawdust using Nutrient agar (Subbarao, 1996). About sixteen bacterial strains were isolated from the culture medium and were screened for cellulolytic activity using Czapek's medium supplemented with carboxy methyl cellulose (CMC) as selective agent (Apun *et al.,* 2000 and Hart *et al*., 2002).

The results were expressed as Solubilization Index (SI) and they were measured using the following formula (Edi-Premono, *et al.,* 1996):

SI = Colony diameter + Halozone diameter / Colony diameter

Based on the highest cellulolytic activity, ten bacteria were selected for further characterization.

2.3 Identification of predominant bacterial isolates

All the ten cellulolytic bacteria isolates were pure cultured by streak plate method and then identified through

morphological and biochemical characteristics viz the parameters such as 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 reduction (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 ten bacterial isolates.

2.4 Characterization of selected cellulolytic bacteria and fungi

Growth conditions such as pH (at 6.0 , 7.0 and 8.0), substrate concentration (0.5, 1.0, 1.5, 2.0 and 2.5 percent of carboxy methyl cellulose) and temperature (at 28°C , 37°C and 45°C) were optimized for the ten selected cellulolytic bacterial isolates using standard methods (Kavian *et al.,* 1999; Al-Tai *et al.,* 1989; Sharma *et al.,* 1997).

Czapek's medium (Minimal medium) was prepared with various buffered solutions using acetate buffer (pH 6.0), phosphate buffer (pH 7.0) and tris-Hcl buffer (pH 8.0). The medium of each pH was supplemented with increasing concentration of carboxy methyl cellulose i.e., 0.5, 1.0, 1.5, 2.0 and 2.5 percent .Ten different bacterial isolates were inoculated and the plates were incubated separately at 28°C, 37°C, and 45°C. After incubation the growth performance of the ten bacterial isolates were observed in different growth conditions on the 7d and the results were recorded.

3. Result

Table 1: Solubilization activity of different cellulolytic bacterial isolates in medium containing carboxy methyl cellulose at 37°C on 5d

Isolate code	Solubilization index
CBIS-1	37.55 ± 0.10
CBIS-2	36.50 ± 0.05
CBIS-3	33.37 ± 0.06
CBIS-4	23.15 ± 0.30
CBIS-5	25.33 ± 0.08
CBIS-6	34.55 ± 0.20
CBIS-7	35.22 ± 0.09
CBIS-8	26.30 ± 0.02
CBIS-9	30.66 ± 0.04
CBIS-10	24.75 ± 0.07

CBIS-Cellulolytic Bacterial Isolates

3.1 Identification of most efficient cellulolytic bacteria

The morphological and biochemical characteristics of cellulolytic bacteria isolate from decayed sawdust are given in Table 2 and 3

Table 2: The Morphological characteristics of cellulolytic bacterial isolates in decayed sawdust

Isolate code	Colony morphology	Grams Reaction	Cell shape	Motility
CBIS-1	White glossy membranous colonies	Positive	Rod	-
CBIS-2	Large, irregular, entire, creamy, Opaque colonies.	Positive	Rod	-
CBIS-3	White round	Positive	Cocci	-
CBIS-4	Tiny ,milky large creamy colonies	Positive	Cocci	-
CBIS-5	Circular,smooth and cream	Positive	Rod	-
CBIS-6	Green color pigmented colonies	Negative	Rod	+
CBIS-7	Opaque white	Negative	Coccob acilli	-
CBIS-8	Large mucoid colloid colonies	Negative	Rod	-
CBIS-9	Yellow colonies	Negative	Rod	-
CBIS-10	Yellow circular	Negative	Rod	+

CBIS - Cellulolytic Bacterial Isolate

Table 3: Biochemical characteristics of the cellulolytic bacterial isolates

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*	
CBIS-1	+	+	+	+	+	+	-	+	+	*Bacillus spp1*
CBIS-2	+	-	-	+	+	+	-	-	+	*Bacillus spp2*
CBIS-3	+	-	-	+	+	-	+	+	+	*Micrococcus spp*
CBIS-4	-	-	+	-	+	-	+	-	-	*Staphylococcus spp*
CBIS-5	-	+	-	+	-	-	-	-	-	*Clostridium spp*
CBIS-6	+	-	+	+	+	+	-	-	+	*Pseudomonas spp1*
CBIS-7	-	-	-	+	+	-	-	-	-	*Acinetobacter spp*
CBIS-8	-	-	+	+	+	-	+	-	+	*Klebsiella spp*
CBIS-9	-	+	-	-	-	-	+	+	+	*Proteus Spp1*
CBIS-10	-	-	+	+	+	-	-	+	+	*Enterobacter spp*

+represents Positive result; - represents Negative result

3.2 Characterization of selected cellulolytic bacteria

The observations on the growth performance of the ten selected cellulolytic bacteria in a pH of 6.0 (acidic condition), at a pH of 7.0 (neutral condition) and at a pH of 8.0 (alkaline condition) with various concentration of carboxy methyl cellulose at three different temperatures are given in Tables 4, 5 and 6 respectively. The growth performance varied for various bacteria in different concentrations of CMC, temperature and pH. The bacterial isolates such as *Bacillus spp1, Bacillus spp2, Micrococcus spp*, *Pseudomonas spp1* and *Acinetobacter spp* showed better growth performance at 37°C with 1.5 percent CMC in the medium of acidic and neutral pH conditions than the alkaline pH condition (Tables 4 and 5).

Table 4: Growth performance of the ten selected cellulolytic bacteria grown in Czapek's medium containing various concentrations of carboxy methyl cellulose (CMC) at three different temperatures in acidic condition (7 d)

Growth Temper-ature (^{0}C)	*CMC Concentr-ation (%)*	*Bacillus spp1*	*Bacillus spp2*	*Micrococcus spp*	*Staphylococcus spp*	*Clostridium spp*	*Pseudomonas spp1*	*Acinetobacter spp*	*Klebsiella spp*	*Proteus spp1*	*Enterobacter spp*
28	0.5	+	+	+	+	+	+	+	+	+	+
	1.0	+	+	+	+	+	+	+	+	+	+
	1.5	+	+	+	+	+	+	+	+	+	+
	2.0	+	+	+	+	+	+	+	+	+	+
	2.5	+	+	+	+	+	+	+	+	+	+
37	0.5	+	+	+	+	+	+	+	+	+	+
	1.0	+++	+++	+++	++	++	+++	+++	++	++	++
	1.5	+++	+++	+++	++	+++	+++	+++	++	++	++
	2.0	++	++	++	++	++	++	++	++	++	++
	2.5	++	++	++	++	++	++	++	++	++	++
45	0.5	-	-	-	-	-	-	-	-	-	-
	1.0	-	-	-	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-	-	-	-
	2.0	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-

- = No Growth , + = Poor Growth , ++ = Moderate Growth , +++ = Good growth

Table 5: Growth performance of the ten selected cellulolytic bacteria grown in Czapek's medium containing various concentrations of carboxy methyl cellulose at three different temperatures in neutral condition (7d)

Growth Temper-ature (0C)	*CMC Concentr-ation (%)*	Bacillus spp1	Bacillus spp2	Micrococcus spp	Staphylococcus spp	Clostridium spp	Pseudomonas spp1	Acinetobacter spp	Klebsiella spp	Proteus spp1	Enterobacter spp
28	0.5	+	+	+	+	+	+	+	+	+	+
	1.0	++	++	+	+	+	++	++	+	++	+
	1.5	++	++	++	+	+	++	++	+	++	+
	2.0	+	+	+	+	+	+	+	+	+	+
	2.5	+	+	+	+	+	+	+	+	+	+
37	0.5	++	++	+	+	+	++	++	+	++	+
	1.0	+++	+++	+++	++	++	+++	+++	+++	++	++
	1.5	+++	+++	+++	++	++	+++	+++	++	+++	+++
	2.0	+++	+++	+++	++	++	+++	+++	++	++	++
	2.5	++	++	++	++	++	++	++	++	++	++
45	0.5	-	-	-	-	-	-	-	-	-	-
	1.0	-	-	-	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-	-	-	-
	2.0	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-

- = No Growth, + = Poor Growth , ++ = Moderate Growth , +++ = Good Growth

Table 6: Growth performance of the ten selected cellulolytic bacteria grown in Czapek's medium containing various concentrations of carboxy methyl cellulose at three different temperatures in alkaline condition (7 d)

Growth Temperature (^{0}C)	*CMC Concentr-ation (%)*	*Bacillus spp1*	*Bacillus spp2*	*Micrococcus spp*	*Staphylococcus spp*	*Clostridium spp*	*Pseudomonas spp1*	*Acinetobacter spp*	*Klebsiella spp*	*Proteus spp1*	*Enterobacter spp*
28	0.5	-	-	-	-	-	-	-	-	-	-
	1.0	++	++	++	+	+	++	++	++	++	+
	1.5	++	++	+	+	+	+	+	+	++	+
	2.0	++	++	+	+	+	++	++	+	+	+
	2.5	++	+	+	+	+	+	+	+	++	+
37	0.5	+	+	+	+	+	+	+	+	+	+
	1.0	++	++	++	+	+	++	+	+	++	+
	1.5	+++	+++	+++	+	++	+++	+++	+	++	++
	2.0	++	++	++	+	+	++	++	+	+	+
	2.5	-	-	-	-	-	-	-	-	-	-
45	0.5	-	-	-	-	-	-	-	-	-	-
	1.0	-	-	-	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-	-	-	-
	2.0	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-

- = No Growth, + = Poor Growth, ++ = Moderate Growth , +++ = Good Growth

4. Discussion

Disposal and management of lingo-cellulosic waste is became major environmental concern worldwide. Hence this study was focused on Screening and partial characterization of cellulolytic bacteria from decayed sawdust. Sixteen bacterial strains were isolated from decayed sawdust and screened for cellulolytic activity. Among different isolates only *Bacillus spp1, Bacillus spp2, Micrococcus spp, Pseudomonas spp1,* and *Acinetobacter spp* showed better solubilization index (Table 1). On the Basis of solubilization index ten potential bacterial isolates were selected and all of them were identified through culture and biochemical characterization (Table 2 and 3). The cellulolytic gram positive bacterial isolates includes *Bacillus spp1, Bacillus spp2, Micrococcus spp, Staphylococcus spp* and *Clostridium spp,* and the gram negative bacterial isolates include *Pseudomonas spp1, Acinetobacter spp, Klebsiella spp, Proteus spp1* and *Enterobacter spp*. The effect of various concentration of CMC on the growth of cellulolytic isolates also observed and the results showed a good growth of bacterial isolates at a concentration of 1.5 percent carboxy methyl cellulose as shown in Table 4, 5 and 6. Similar kind of results were recorded by Fagade and Bamigboye, (2012) they showed the highest cellulase activity for *B. licheniforms* I and II at 40°C with a value of 0.52 mg/mL and 0.44 mg/mL reducing sugar. *B. thuringiensis* produced maximum relative cellulase activity of 110 U/mL at 1% CMC at 40°C (Lin *et al.*, 2012) . In the present study it was observed that isolates such as *Bacillus spp1, Bacillus spp2, Micrococcus spp, Pseudomonas spp1,* and *Acinetobacter spp* were able to grow in the neutral (pH 7) conditions better than acidic conditions (pH 6) as shown in the Table 5, 6 and 7. Observation made on the effect of temperature also showed

that all the isolates were able to grow well at. The Effect of pH and temperature on the cellulolytic activity of microbial isolates have been well studied by researchers at different across the world (Al-Tai *et al.,* 1989; Sharma *et al.,* 1997 and Kavian *et al.,* 1999).

Immanuel *et al*., (2006) also recorded maximum endoglucanase activity in *Cellulomonas*, *Bacillus,* and *Micrococcus* sp. at 40°C in neutral pH. Similarly Otajevwo *et al., (*2011) reported that isolates such as *Bacillus*, *Clostridium*, *Pseudomonas* and *Erwinia* showed optimum cellulase production at 40°C and pH 6.

5. Conclusion

Ten bacterial strains such as *Bacillus spp1, Bacillus spp2, Micrococcus spp, Staphylococcus spp, Clostridium spp, Acinetobacter spp, Pseudomonas spp1, Klebsiella spp, Proteus spp1 and Enterobacter spp,* were isolated from decayed sawdust showed better cellulolytic activity against different growth conditions such as temperature, pH and substrate concentration. Hence these organisms could be used in the rapid degradation of lignocellulose rich sawdust material for making nutrient rich organic manure.

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