Cellulolytic Microorganisms of Soil under Deciduous and Evergreen Forest at Chittagong University Campus

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Abstract

An attempt was made to isolate and study the cellulolytic microorganisms from the soil under two deciduous (*Tectona grandis* L. f. and *Lagerstroemia speciosa* L.) and two evergreen (*Eucalyptus camaldulensis* Dehnh. and *Acacia auriculiformis* A. Cunn. ex Benth.) forest plantations, and the relation of these organisms with the soil nutrient of the forest (N, P, K and carbon) has been observed. Among the isolates, finally selected 11 isolates comprised of one bacterial strain (*Cellulomonas* sp.), five strains of *Streptomyces* and five fungal strains (*Aspergillus flavus, A. niger, A. ochraceous, Trichoderma lignorum, T. glaucum).* Cellulolytic activity of these strains has been reported.

সারসংক্ষেপ

দু'টি পত্রঝরা (সেগুন ও জারুল) ও দু'টি চিরসবুজ (ইউক্যালিপটাস ও আকাশমনি) বন বাগান হতে সেলুলোলাইটিক অণুজীব পৃথক করে তাদের পরীক্ষা করা হয়েছে এবং মাটিস্থ পুষ্টি উপাদানের সাথে তাদের সম্পর্ক প্রত্যক্ষ করা হয়েছে। পৃথককৃত স্ট্রেইনগুলোর ১১টিকে নির্বাচন করে তাদের একটি ব্যাকটেরিয়া (*Cellulomonas* sp.) পাঁচটি *Streptomyces* এবং পাঁচটি ছত্রাকের (*Aspergillus flavus, A. niger, A. ochraceous, Trichoderma lignorum, T. glaucum)* অন্তর্ভুক্ত পাওয়া গেছে। এই স্ট্রেইনগুলোর সেলুলোলাইটিক কার্যাবলীর বিবরণ দেওয়া হয়েছে।

Key words : Cellulolytic microorganisms, forest soil

Introduction

The biomass production is an important factor in the forest floor. In a forest ecosystem, plant leaves are periodically or continuously dropped on the ground. The leaf litter decomposes releasing the nutrient in the soil for recirculation. Degradation of forest litter by soil microorganisms provides nutrients to growing plants. The release of nutrients from the litter depends upon the soil micro-ecosystem, growth of microorganisms, amount of extracellular enzyme released by microbes to the soil and ultimately on the rate of decomposition of organic materials and release of nutrients to the plants. The decomposition leads to chemical simplification of various complex compounds to simple monomeric sugars, alcohol, protein, etc. (Mee-Young *et al.* 1978). Concentration of

nitrogen and lignin are important factors which in large part regulate the decomposition rate (Berg and Staaf 1980, Melillo *et al.* 1982). According to Kirk *et al.* (1976) microbes cannot use pure lignin as an energy source. Degradation of lignin and growth of microbes occur after the addition of carbohydrate (cellulose) that could be used as energy source. So, cellulose provides the microbes with energy for the degradation of lignin-rich materials. Thus cellulolytic microbes play an important role in forest litter decomposition as well as in nutrient recycle process.

The present work was undertaken to study the soil microflora, specially the cellulolytic microorganisms under deciduous and evergreen forest plantations at Chittagong University campus.

Materials and methods

In the present study, soil samples were collected from two evergreen (*Eucalyptus camaldulensis* Dehnh. and *Acacia auriculiformis* A. Cunn. ex Benth.) and two deciduous (*Tectona grandis* L. f. and *Lagerstroemia speciosa* L.) forest plantations of Chittagong University campus. Samples were collected in four seasons (January-March, 1990 ; April-June, 1990 ; July-September, 1990 and October-December, 1990). Each forest plantation was divided into three topographical levels such as hill top, mid-slope of the hill and hill base. Soil samples were collected from 2.54 to 15.24 cm depth by an auger. During collection of soil 95% ethanol sterilized polyethylene bags were used. Twenty samples were collected randomly from each topographical level of the hill, and separate polythene bags were used in each case. Later 20 samples of each level were mixed together to form a composite sample. After collection the samples were brought to the laboratory and carefully preserved in the refrigerator at 10^0C for microbial analysis. Another set of soil samples were oven-dried at 105^0C for 24 hours for chemical analysis. Nutrient Agar (NA), Potato Dextrose Agar (PDA) and Czapek's medium with 2% Carboxy Methyl Cellulose (CMC) were used for the detection of bacteria and fungi.

Morphological, physiological and biochemical studies of the selected strains were performed. Field moisture, pH, total carbon, total nitrogen, available phosphorus and potassium of the soil samples were determined following the methods described in soil chemical analysis by Jackson (1973).

Results and discussion

Microbial population, pH, percent moisture, and C, N, P and K content in the samples from different topography are shown in Table 1. It was found that the soil under mid-slope of the hill generally showed maximum microbial population in CMC medium. But the field moisture, pH, total carbon, total nitrogen, available phosphorus and available potassium in the soil of mid-slope did not show any clear indication which may explain their role on the above findings. The highest fungal population (5.08×10^6/g soil) and bacterial population (50.75×10^6/g) were recorded from the soil under *L. speciosa* plantation in PDA and NA media respectively. In CMC medium, soil under *T. grandis* plantation showed maximum population of cellulolytic fungi (9.25×10^6/g) and also of actinomycetes (18.50×10^6/g), whereas the cellulolytic bacterial population (5.26×10^6/g) was found highest in soil under *L. speciosa* plantation (Table 1). In the present study, it was also found that, in general, percentage of moisture (20.77%) and pH value (6.34) of the soil under *T. grandis* plantation were higher than those under other forest plantations and these higher values may have role on the population of cellulolytic fungi and actinomycetes. On the other hand, low pH (5.66) was recorded in the soil under *L. speciosa* plantation which may have role on cellulolytic bacterial population. Total percentage of nitrogen, available phosphorus and available potassium were recorded highest in the soil under *E. camaldulensis* and *A. auriculiformis* plantations.

Table 1. Total mean fungal, actinomycetes and bacterial population, percentage of field moisture, pH percentage of total carbon, percentage of total nitrogen available phosphorus, available potassium of soil under *T. grandis*, *L. speciosa*, *E. camaldulensis* and *A. auriculiformis* plantations. (Average data of all the seasons on topography basis).

Samples of different species and topography	Total mean population (x 10^6/g soil) in different media					% field moisture	pH	% total carbon	% total nitrogen	Available phosphorus (ppm)	Available potassium (ppm)
	PDA	NA	Czapek's medium with 2% CMC								
	Fungi	Bacteria	Fungi	Bacteria	Actinomycetes						
T_1	3.00	13.75	6.75	3.25	12.25	21.48	5.29	1.32	0.15	8.99	87.60
T_2	2.75	5.50	13.25	4.25	26.00	19.28	6.48	1.18	0.16	8.99	63.92
T_3	3.50	10.00	7.75	4.25	17.25	21.57	6.27	2.30	0.14	8.94	77.20
	(3.08)	(9.75)	*(9.25)	(3.91)	*(18.50)	*(20.77)	*(6.34)	(1.26)	(0.15)	(8.97)	(76.24)
J_1	2.75	71.25	5.00	1.50	3.75	15.78	5.70	0.17	0.17	12.32	96.58
J_2	4.25	62.25	6.25	7.75	18.50	19.98	5.60	1.41	0.16	13.43	74.30
J_3	8.25	18.75	7.00	6.50	12.25	20.97	5.70	1.44	0.15	12.68	66.15
	*(5.08)	*(50.75)	(6.06)	*(5.26)	(11.15)	(20.64)	(5.66)	(1.00)	(0.16)	*(12.81)	(79.00)
E_1	4.75	4.00	3.75	4.75	9.75	14.12	5.88	2.49	0.25	11.06	64.10
E_2	4.25	23.00	5.25	2.75	9.00	17.47	5.76	2.20	0.23	14.06	79.55
E_3	3.75	25.75	2.25	1.50	3.75	19.17	5.77	1.76	0.17	13.31	78.50
	(4.25)	(17.58)	(3.75)	(3.00)	(7.50)	(16.92)	(5.80)	*(2.15)	*(0.21)	*(12.81)	(74.04)
A_1	4.25	19.75	3.78	1.75	2.25	11.66	5.82	1.43	0.14	11.93	93.50
A_2	3.25	13.00	4.00	3.75	4.50	10.77	5.75	1.48	0.14	10.75	86.20
A_3	4.50	14.50	3.50	2.25	6.50	10.11	15.86	1.22	0.12	9.84	98.26
	(4.00)	(15.75)	(3.75)	(2.58)	(4.41)	(10.84)	(5.81)	(1.37)	(0.13)	(10.84)	*(83.58)

Note : T = *Tectona grandis* 1= Hill top PDA = Potato dextrose agar ( ) Parenthesis = Grand total mean
J = *Lagerstroemia speciosa* 2 = Mid-slope of the hill NA = Nutrient agar * = Highest number
E = *Eucalyptus camaldulensis* 3 = Hill base CMC = Carboxy methly cellulose
A = *Acacia auriculiformis*

Table 2. Total mean fungal, actinomycetes and bacterial population, percentage of total carbon, percentage of total nitrogen, available phosphorus, available potassium, % field moisture, pH of soil under *T. grandis*, *L. speciosa*, *E. camaldulensis* and *A. auriculiformis* plantations of four different period of study (composite samples of three topography).

Seasons (1990)	Sample	Total mean population (x 10⁶/g soil) in different media					% field moisture	pH	% total carbon	% total nitrogen	Available phosphorus (ppm)	Available potassium (ppm)
		PDA Fungi	NA Bacteria	Czapek's medium with 2% CMC Fungi	Bacteria	Actinomycetes						
January	T	3.33	20.66	*14.66	6.66	*25.00	22.47	*7.32	1.23	0.125	8.626	100.00
to	J	4.33	*166.33	5.00	6.33	3.66	10.19	6.44	1.86	0.124	8.626	*112.33
March	E	3.33	56.00	2.66	1.33	6.66	14.12	5.89	1.80	0.170	11.830	70.13
	A	3.33	49.33	4.66	5.00	18.66	11.11	6.45	1.13	0.121	7.560	37.53
April	T	2.33	10.00	4.33	0.33	6.00	21.26	6.67	1.20	0.12	7.50	44.86
to	J	5.00	20.33	4.00	5.00	8.66	17.16	6.08	1.47	0.14	13.25	61.93
June	E	4.33	5.66	2.00	3.00	0	16.78	6.03	*2.70	0.28	8.58	16.80
	A	4.00	7.33	4.33	4.00	1.66	5.85	6.33	1.07	0.10	7.21	59.06
July	T	4.33	5.33	8.66	0.33	20.00	24.55	5.26	1.46	0.26	6.11	82.63
to	J	*7.66	8.66	11.00	7.66	20.66	*24.62	4.60	1.31	*1.17	6.76	70.66
September	E	4.00	5.00	6.33	6.66	14.66	22.86	5.17	2.17	0.19	17.20	108.60
	A	3.00	2.66	2.66	0	11.33	14.28	4.78	1.95	0.19	13.84	68.00
October	T	3.00	3.00	9.33	*8.66	23.00	14.84	6.13	1.17	0.11	13.66	77.13
to	J	3.33	7.66	4.33	2.00	13.00	12.98	5.63	1.08	0.11	*15.16	71.06
December	E	5.33	3.66	4.00	1.00	8.66	13.92	6.11	2.22	0.22	13.84	76.40
	A	6.00	3.66	3.00	1.33	2.66	12.16	5.66	1.35	0.13	14.75	98.26

Note : * (Star mark) = highest number

Microbial population, percentage of total carbon and nitrogen, percentage of field moisture, available phosphorus, available potassium, pH in composite samples of three topographies are shown in Table 2. The highest fungal population was recorded in PDA from the soil under deciduous forest (*T. grandis*, 4.33×10^6/g and *L. speciosa*, 7.66×10^6/g) plantations during the period of July-September, 1990. Whereas, from the soil under evergreen forest plantation the highest fungal population was recorded during the period of October-December, 1990. In NA medium the highest bacterial population was recorded from the soil that was collected during January-March, 1990 in all the forest plantations studied. Among the forest plantations studied, the soil under *L. speciosa* forest showed maximum number of bacterial colonies (166.33×10^6/g) during the period of January-March, 1990. It was found that during July-September, 1990 the soil under *L. speciosa* forest showed lower soil pH (4.6) and the highest field moisture (24.62%) which may induce general fungal population. On the other hand, *L. speciosa* forest also showed maximum percentage of total nitrogen, available phosphorus and potassium which may have role on common soil bacterial population.

Total number of microbial colonies from the collected samples in different media is shown in Table 3. In PDA medium, a total of 199 fungal individual colonies were isolated from composite soil under *T. grandis*, *L. speciosa*, *E. camaldulensis* and *A. auriculiformis* plantations. In CMC medium 177 fungal, 522actinomycetes colonies and in NA medium 1126 bacterial colonies were isolated. All the isolates were grouped into 35 groups on the basis of their morphological, physiological and cultural characters. Among the 35 selected colonies 15 were selected on the basis of production of zone of clearance around the colonies of CMC (Czapek's with 2% CMC).

Table 3. Total number of microbial colonies detected from the collected samples in different media.

Microbes	Name of the species	Number of microbial colony in different media				
		PDA		GMC		NA
		Individual	Total	Individual	Total	Total
Fungi	*Aspergillus niger*	44		72		
	A. flavus	28		48		
	A. ochraceous	16		16		
	Cephalosporium sp.	21	199	-	272	
	Penicillium sp.	12		17		
	Rhizopus sp.	27		40		
	Trichoderma sp.	19		26		
	Mycelia sterilia	32		53		
Bacteria	ND	-		ND	177	1126
Actinomycetes	ND	-		ND	522	-

Note : Tetracycline 250 mg/litre PDA was used to prevent bacterial growth. Bacterial population was recorded after 48 hours of incubation in NA medium to avoid fungal growth. ND = Not done, Minus (-) = Not recorded.

These 15 strains were again screened for their higher cellulolytic activity. Among them only eleven (11) were found to show comparatively higher cellulolytic activity which were finally selected for detailed study (Table 4). These finally selected strains comprised of one bacterial strain, five strains of *Streptomyces* and five fungal strains. All the selected strains were compared on the basis of their morphological, cultural, biochemical and pigment characters with the standard description contained in Breed *et al.* (1957), Buchanan and Gibsons (1974) and Sneath *et al.* (1986). The characters of the genera *Trichoderma* and *Aspergillus* were compared with the descriptions given by Gilman (1975) and Raper and Fennell (1977) respectively.

The strain B_1 was found to be closely related to the genus *Cellulomonas*. The strains A_1, A_2, A_3, A_6 and A_7 were found to belong to the genus *Streptomyces* (Walksman and Henrici 1984). These 5 strains of *Streptomyces* differed among themselves in a number of morphological, cultural and biochemical characters such as growth in different media (citrate, glucose, asparagine broth), in the production of hydrogen sulphide, variability in starch hydrolysis, proteolysis of egg albumin, fermentation of sugar, etc. The fungal strains F_1, F_2, F_3 and F4 were identified as *Aspergillus ochraceous*, *A. niger*, *A. flavus*, *Trichoderma lignorum* and *T. glaucum* respectively (Raper and Fennell 1977, Gilman 1975).

The fungal strains belonging to the genus *Aspergillus* and *Trichoderma* were interesting in their ability to utilize leaf litter powder (*T. grandis* *L. speciosa*, *E. camaldulensis* and *A. auriculiformis*) CMC as the source of cellulose substrates. Whereas, the actinomycetes strains did not grow better in the medium containing the leaf litter powder (Table 4).

Table 4. Growth of various strains of fungi, actinomycetes and bacteria in different cellulose sources (2% CMC, *T. grandis*, *L. speciosa*, *E. camaldulensis* and *A. auriculiformis* leaf litter powder) in Czapek's medium.

Strain no.	Growth in different cellulose sources				
	2% CMC	2% *T. grandis* leaf litter powder	2% *L. speciosa.* leaf litter powder	2% *E. camaldulensis* leaf litter powder	2% *A. auriculiformis* leaf litter powder
Fungi					
F_1	+++	++	++	+++	+++
F_2	++	++	++	++	+++
F_3	++	+++	+++	+++	+++
F_4	++++	++++	++++	++++	++++
F_5	++++	++	+++	+++	+++
F_6	++	++	+	+	+
Actinomycetes					
A_1	++	+++	-	+++	+++
A_2	++	+	-	+++	+++
A_3	+	-	-	-	-
A_4	+	-	-	-	-
A_5	++	+	++	++	+++
A_6	++	_	++	-	+++
A_7	++	+	++	++	+++
Bacteria					
B_1	++	++	+	+	++
B_2	++	-	+	-	-

Note : - =No growth, + = Scanty growth, ++ = Moderate growth, +++ = Good growth, ++++ = Heavy growth.

All the finally selected actinomycetes strains were isolated from the soil under *L. speciosa* and *E. camaldulensis*, plantations, whereas the selected five fungal and one bacterial strains were isolated from the soil under *E. camaldulensis* and *T. grandis* plantations. Among the selected microbes, the strain F_5 (*Trichoderma* sp.) was found to utilize all the cellulose sources studied herein. Comparatively the fungal strains were better in their ability to utilize cellulose sources (CMC as well as leaf litter powder). Among the actinomycetes the strain A_1 was found to be better than other strains in utilizing *T. grandis*, *E. camaldulensis* and *A. auriculiformis* leaf litter powder. On the other hand, the strain A_2 was found to grow well only in *A. auriculiformis* leaf litter powder containing medium. Beside these, all the selected actinomycetes strains were able to grow well CMC containing Czapek's medium. The bacterial strain B_1 was found to utilize the cellulose source of CMC, *T. grandis* and *A. auriculiformis* leaf litter moderately.

Degradation of cellulosic materials is a complex process which requires participation of a number of enzymes and many environmental and cultural factors. Further studies with different cultural conditions, and mutation to increase the production of cellulase activity, protein and biomass yield are most interesting topics to study. The successful exploitation of these strains with proper biotechnology for conversion of wastes to useful products will be beneficial.

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