Asian Journal of Science and Applied Technology
ISSN: 2249-0698 Vol. 4 No. 1, 2015, pp.5-9

Cellulolytic Efficiency of Forest Litter Microflora Isolated from Sirumalai Hills of Dindigul District

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Abstract - Soil samples having decomposed forest litter of tree species viz., *Bischofia javanica*, *Phoebe paniculata*, *Dimocarpus longan* and *Terminalia chebula* were collected from Sirumalai Hills of Dindigul District and three bacterial colonies were isolated. Extracellular protein production, reducing sugar level, saccharification values and cellulase activity of three bacterial cultures were tested in pure carboxy methyl cellulose (CMC) and holocellulose from selected leaf litter powder. From this study it is inferred that all the three bacteria have high potential to produce extra cellular protein. *Mycobacterium lavaneformes* has the ability to produce enzymes for saccharification. *Cellulomonas fimi* can be effectively used for the production of microbial biomass protein (MBP) from the CMC and also for biodegradation of the cellulose present in the leaf litter of forest plantations.
Keywords: Carboxy methyl cellulose, cellulolytic bacteria, *Mycobacterium lavaneformes*, Sirumalai Hills

I.INTRODUCTION

In forest ecosystem, plant leaves are periodically or continuously shed on the ground. The leaf litter decomposes releasing the nutrient into the soil for re-circulation. Degradation of forest litter by the action of soil microorganisms provides nutrient to growing plants. The release of nutrients from the litter depends upon the soil micro-ecosystem, growth of microorganisms, amount of extra-cellular enzymes released by microbes to the soil and ultimately on the rate of decomposition of organic materials.

Decomposition of plant litter and humus is a fundamental ecosystem process, which maintains a continuous supply of essential nutrients to plants (Satchell, 1974; Swift *et al*., 1979). This process is controlled by three different main factors: the physico-chemical environment, substrate quality and the decomposer populatio n (Swift *et al*., 1979). Cellulose and lignin are the major components in agricultural wastes and wood chips (Reese *et al*., 1972). Cellulose being the most abundant biological compound as well as the important renewable source of energy on earth, its hydrolysis to soluble sugar is a process of considerable significance.

The cellulolytic enzymes play an important role in natural biodegradation process and it efficiently degrade plant lignocellulosic materials.The biological degradation of cellulose has been of paramount importance in the activities of living system. A number of microorganisms have the ability to use cellulose for their growth. In optimum conditions, these organisms can convert cellulose into single cell protein (SCP) or microbial biomass protein (MBP). Hence microbiologists are trying to grow such microbes on cellulose waste material for their use as a source of protein (Ghose and Ghose, 1978).

In this regard the present study has been undertaken to isolate cellulolytic bacteria from forest soil and to screen for growth and cellulase activity on pure cellulose substrates.

II.MATERIALS AND METHODS

Collection of Soil Samples

Soil samples were collected from the forest tree plantations of Sirumalai Hills, Dindigul District. Soil samples containing decomposed forest litter of *Bischofia javanica*, *Phoebe paniculata*, *Dimocarpus longan* and *Terminalia chebula* were collected in 95% ethanol sterilized polyethylene bags. Ten samples from each plant were mixed together to form a composite sample.

Isolation of Microorganisms

The refrigerated samples were studied for cellulolytic bacteria within a week of collection. The samples were plated using nutrient agar (NA) medium and Czapek's medium with 2% carboxy methyl cellulose (CMC) for primary selection of organisms. Cellulolytic bacteria use cellulose for their nutrition. The primary selected isolates were again screened, for their higher cellulolytic activity based on their growth, vigor and zone of clearance around the colony, on Czapek's solid medium with CMC powder at 2% w/v as carbon source used as overlay on the basal medium. These inoculated plates were incubated at 37 ± 2°C for 2 to 3 days. The strains, which showed comparatively higher cellulolytic activity, were finally selected for detail study. Three bacterial colonies were isolated by repeated plating.

Identification of Microorganisms

The isolated bacteria were grown on different media for identification based on cultural characteristic, microscopic studies, physiological and biochemical analysis. Based on these criteria the bacteria were compared with standard descriptions given in the Bergey's Manual (Buchanan and Gibsons, 1974; Krieg and Holt, 1984).

Alkaline Treatment of Cellulosic Substrates

For natural cellulose source, holocellulose of leaf litter powders of *Bischofia javanica, Phoebe paniculata, Dimocarpu slongan* and *Terminalia chebula* were extracted separately by following Pushalkar and Rao (2002).

Leaves were ground and sieved through standard 20 mm mesh size sieve. Delignification was carried out by treating 5 g substrate with 1.0% w/v NaOH at 121°C for 1 hr. The substrates were subsequently neutralized by washing with water and dried at 80°C in a hot air oven to get constant weight.

Growth Measurement

Holocellulose from selected leaf litter powder and CMC were used with Czapek's solid medium at the rate of 1.5% for the measurement of growth in petriplates. The bacteria inoculated plates were incubated at 30±2°C for three days. The colony diameter was measured for all the bacteria.

Determination of Celluloytic Activity

The cellulose extracted from leaves and CMC was separately used as carbon source in Czapek's broth (pH 7.5) at a rate of 1.5% for testing the degradation capacity of the three selected bacterial isolates. The 24hr old culture suspensions of the selected strains were inoculated in 100 ml Czapek's medium (pH 7.5) in 250 ml flask, transferred to rotary shakers (180 rpm) incubated at 30±2°C for two days, centrifuged at 2500 rpm for 15 minutes and the supernatants, which are the crude enzyme source were collected separately and analyzed for pH, extracellular protein (Lowery *et al*., 1951), reducing sugar (Nelson, 1944), saccharification values (Hossain and Anwar, 1996a, b) and carboxyl methyl cellulase activity (Miller, 1959).

Measurement of Biomass

Bacterial biomass was determined by measuring its absorbance at 600 nm (Henriette *et al*., 1993).

Saccharification of Different Cellulose Sources

The percentage of saccharification was calculated by applying the following equation.

$$\text{Saccharification (\%)} = \frac{\text{mg of reducing sugar/ml}}{\text{mg of substrate/ml}} \times 1000$$

To estimate CMCase activity, the crude enzyme preparations of various dilutions (0.2, 0.4, 0.6, 0.8, and 1.0 ml) were mixed with 4.5 ml of 1% CMC prepared in 0.1M citrate buffer (pH 4.6) and incubated at 50°C for 60 minutes and immediately tested for reducing sugars. The saccharification values were calculated for these different concentrations of crude enzymes. Enzyme activity was expressed by the amount of reducing sugar released/ml of extract/unit time.

III. RESULTS AND DISCUSSION

The bacterial colonies were subjected to plating and three bacterial colonies which grew well in Czapek's solid medium with 1.5% CMC as carbon source were identified.Of these two belong to *Cellulomonas* species (i.e., *C. uda* and *C.fimi*) and one to from *Mycobacterium* species (i.e., *M. lavaneformes*).

Growth of isolated bacteria in 1.5% of CMC and holocellulose of various leaf litter powder containing Czapek's solid medium was assessed and the results are given in Table 1. All the bacteria were efficient in utilizing the pure as well as the native cellulose source. The bacterium *M. lavaneformes* has the ability to utilize pure CMC and all the four leaf litter powders. Earlier workers had used different concentrations of CMC (Mogal and Dube, 1994; Hossain and Anwar, 1996a, b). Changes in the pH of the medium having 1.5% CMC and holocellulose, brought by various bacteria are shown in Table 1. It was found that the pH increased in all the bacterial isolates tested. pH of the culture medium varied from 8.45 to 9.17. The highest increase in the pH was observed by *M. lavaneformes* in the holocellulose of *B. javanica* followed by *T. chebula.*

The results of the rate of production of extra cellular protein, reducing sugar level and the percentage of saccharification of the isolated bacteria grown in Czapek's medium having 1.5% of CMC and holocellulose of various leaf litter powders as carbon sources are given in Table 2. The maximum extra-cellular protein production was recorded in the medium having CMC as carbon source. Among the bacterial cultures the rate of production of extra-cellular protein was highest in *M. lavaneformes* i.e., 875 µg/ml (CMC) and the least in *C. uda* i.e., 595 µg/ml (holocellulose of *D. longan*). The results of extra-cellular protein production indicate that all the three bacteria have high potential to produce extra cellular protein from CMC and holocellulose of leaf litter. Hence, these isolates can be employed for large-scale production of microbial biomass protein. The maximum reducing sugar level and the percentage of saccharification was observed in the medium having CMC as carbon source. *M. lavaneformes* showed maximum ability to release the reducing sugar and percentage of saccharification in all the carbon sources. Similar results were found in *A. niger* (Hossain and Anwar, 1976b), *T. viride* (Mandels and Stenberg, 1976) and *Trichoderma* spp. and *Aspergillus* spp. (Shewale and Sadana, 1981). Saccharification results indicate that holocellulose of leaf litter has a major role in cellulosic bioconversion.

.TABLE I. GROWTH OF VARIOUS BACTERIA IN DIFFERENT CELLULOSE SOURCES IN CZPEAK'S SOLID MEDIUM AND CHANGES IN THE PH OF THE LIQUID MEDIUM

Organisms	Cellulose source (1.5%)				
	CMC	*B. javanica*	*P. paniculata*	*D. longan*	*T. chebula*
Growth (cm)					
C. uda	0.3	0.2	0.4	0.1	0.3
C. fimi	0.5	0.4	0.1	0.3	0.4
M. lavaneformes	0.8	0.7	0.4	0.3	0.5
pH*					
C. uda	8.62	8.54	8.51	8.67	8.45
C. fimi	8.94	8.70	8.82	8.50	8.61
M. lavaneformes	9.00	9.17	8.73	8.94	9.08

* Initial pH of the medium is 7.5

TABLE II EXTRACELLULAR PROTEIN PRODUCTION (MG/ML), REDUCING SUGAR LEVEL (MG/ML) AND SACCHARIFICATION (%) OF THE BACTERIA IN DIFFERENT CELLULOSE SOURCES IN CZAPEK'S BROTH.

Organisms	Source of cellulose				
	CMC	*B. javanica*	*P. paniculata*	*D. longan*	*T. chebula*
Extracellular protein production (µg/ml)					
C. uda	805	715	705	595	765
C. fimi	860	740	755	610	765
M. lavaneformes	875	725	840	845	750
Reducing sugar level (µg/ml)					
C. uda	165	105	070	045	085
C. fimi	285	095	065	030	125
M. lavaneformes	375	145	110	125	140
Saccharification (%)					
C. uda	1.10	0.70	0.47	0.30	0.59
C. fimi	1.90	0.63	0.43	0.20	0.83
M. lavaneformes	2.50	0.97	0.73	0.83	0.93

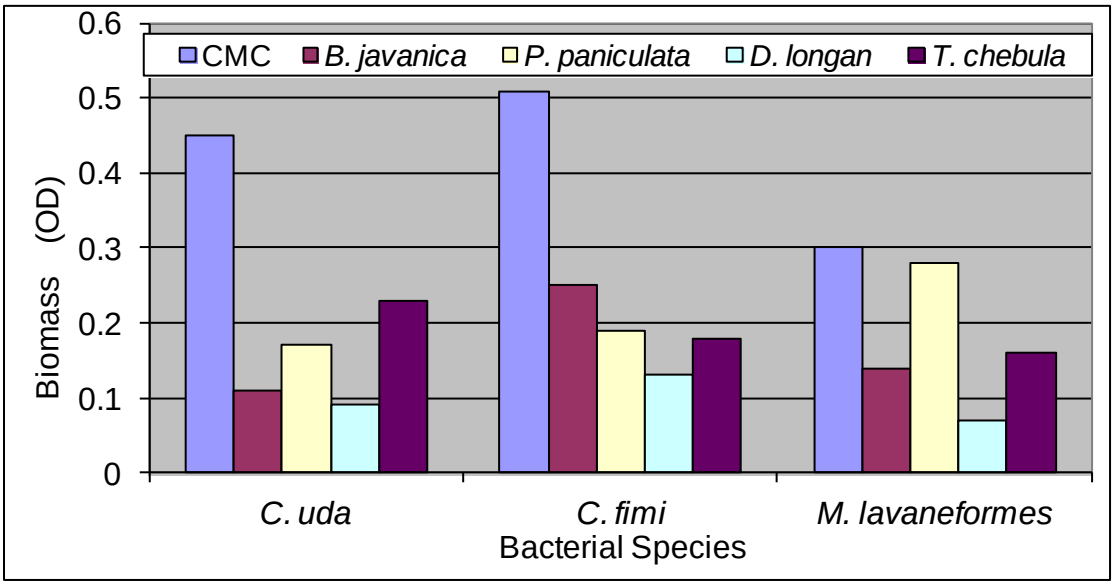

Fig. 1.Biomass yield of the bacteria in different cellulose sources in Czapek's broth.

Biomass yield of the bacteria cultured in different cellulose source in Czapek's broth are shown in Fig.1. Maximum bacterial biomass was observed based on the turbidity of the medium. The bacterial biomass concentration of the medium was ranged from the absorbance 0.07 (*M. lavaneformes* in holocellulose of *D. longan*) to 0.51 (*C. fimi* in CMC). This suggests that *C. fimi* has highest ability to produce maximum biomass and CMC is the best source of carbon for these bacteria.

The cellullolytic activity (saccharification) of the crude enzymes extracted from three bacterial cultures was separately tested at five different concentrations in Czapek's medium and is given in Fig.2. The results showed that an increase in cellulolytic activity is directly related to an increase in concentration of the crude enzymes. The saccharification rate was comparatively higher in enzymes extracted from *M. lavaneformes* when compared to the rest of the isolates. The concentration of substrate required for enzyme production varied but 1-2% of CMC proved to be optimal (Anandapandian *et al*., 2002). The optimum concentration of CMC for CMCase activity of the crude enzymes extracted from the selected three bacteria was found to be 1.5%. Further increase in the substrate concentration reduced the enzyme activity, probably due to increased levels of reducing sugar accumulated through saccharification, which is responsible for the repression of cellulose synthesis (Anandapandian *et al*., 2002). The rate of celluloltytic activity was high at the minimum enzyme concentration.

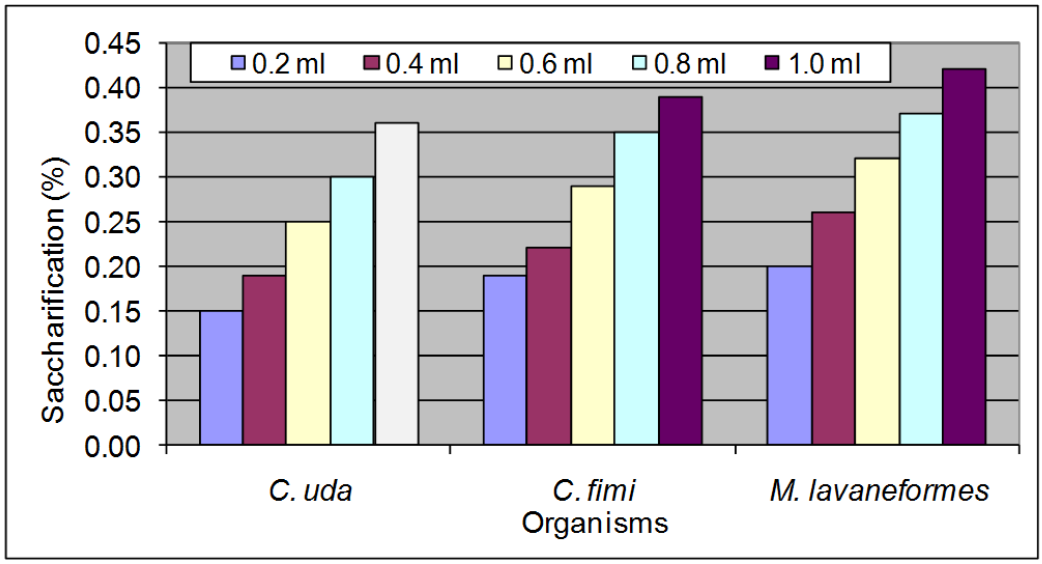

Fig. 2.Effect of enzyme concentration on saccharification of CMC

CMCase activity of 0.2 ml concentration of crude enzyme extracted from bacteria is shown in Table 3. Maximum cellulolytic activity was found in *M. lavaneformes* among the bacterial isolates i.e., 91 µg/ml/hr. In general, biomass yield and cellulose activity were found to have no direct relationship as is evident in similar observations made earlier workers (Singh *et al*., 1986; Anwar and Zaman, 1994). Besides these holocellulose was found to be nearly similar to CMC as a degrading carbon source for microbial degradation.

TABLE III. CELLULOLYTIC ACTIVITY OF 0.2 ML CONCENTRATION OF CRUDE ENZYME EXTRACT OF MICROORGANISMS

Organisms	CMCase activity (µg/ml/hr)
C. uda	66
C. fimi	85
M. lavaneformes	91

From this study it is concluded that all the three bacteria have high potential to produce extra cellular protein and *M. Lavaneformes* has the highest ability to produce enzymes for saccharification.

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