CELLULOLYTIC BACTERIA OF SOIL OF GUNUNG HALIMUN NATIONAL PARK

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ABSTRACT

The population of aerobic cellulolyitic bacteria (ACB) of soil Gunung Halimun National park and its celulolytic capacity were studied. The soil samples were collected from various altitude (500-1500) m asl. Microbial isolation was performed by culture enrichment technique with CMC (carboxymethyl cellulose) as the major carbon sources. The quantitative determination of ACB was performed by growing the microbes on CMC containing media, and utilizing congored as an indicator. ACB was indicated by formation of clearing zone surrounding growing colony. Cellulytic capacity of each isolates was determined by analysing the ratio of colony and clear zone formation. ACB were quite heterogenous include *Bacillus* sp., *Clostridium* sp., *Chromobacterlum* sp., *Enterobacter* sp., *Moraxella* sp. and *Pseudomonas* sp.

Key words: Culturable aerobic cellulolytic bacteria, Gunung Halimun National Park.

INTRODUCTION

Plant materials are the major component of organic materials in forest soil. The importance role of soil microflora on the decomposition of organic materials is well recognized (Anderson and Domsch, 1978). Though ecologically soil microflora play significant contribution on nutrient cycling and also maintain nutrient and energy flow in forest ecosystem, yet our understanding on diversity of soil microflora, particularly bacteria (Chandler *et al,* 1997), and its physiological nature is still limited to enable to manage and to conserve soil microflora. Recently there is a growing interest in understanding the role of aerobic ACB (Coughlan and Meyer, 1992; Bossio *et al,* 1998), as an important microbial group that has significant contribution on decomposition and hydrolyses of celulolytic materials in forest soil. Vegetation is major component of forest ecosystem, and may significantly affect diversity of soil microflora (Alexander, 1961; Doran and Parkin, 1996). The presence of 3 dominating plant *Schima wallichii, Altingia excelsa* and *Castanopsis javanica* were studied in a permanent plot at 1100 m asl at Cikaniki study site is also a focus of our present study. Surface water and ground water flow bring nutrition for plant growth as well as soil microflora growth from geographically higher to lower altitude may also affect microbial distribution at each site. Each plant may has structurally various lignocelulose components, and bio-chemically they may have diverse decomposition patterns (Rose, 1980; Eriksson *et al,* 1992). Each microbes produce hydrolytic enzymes to breakdown those complex substances into its monomer (Eriksson *et al.,* 1992; Heinemeyer *et al,* 1989). These all indicate that understanding the nature of soil microflora in biodegradation of organic substances in forest soil is important aspect to implement forest conservation measures (Hiroki and Watanabe, 1996).

MATERIAL AND METHODS

Sampling plot establishment

To study the culturable aerobic cellulolytic bacteria, the soil samples were collected from several study sites located at about 500 m, 1000 m and 1500 m above sea level at Ciptarasa, Cikaniki and Gunung Boto of Gunung Halimun National Park.

RESULT

Soil sample collection

Forest soil

From each study site (Cikaniki, Gunung Boto and Ciptarasa) 3 sub sampling points were made at each study site. Totally about 1 kg soil sample was collected randomly from soil surface to a depth of 0-15 cm. The soil samples were transported in polybag, and keept in 4 °C prior to analyses. Biological analyses were performed before 1 week.

Rhizosphere soil

To investigate the interaction between plant root and microbes (ACB), a study was conducted at Cikaniki site, located at 1100 m asl. There were three dominating plants *(Schima wallichii, Castanopsis javanica* and *Altingia excelsa).* Soil samples were collected from each rhizosphere plants at a depth of 0-15 cm.

Aerobic cellulolytic bacteria (ACB) isolation

The total number of culturables of soil bacteria was determined using a soil dilution plate count technique. Soil samples (1 g dry weight basis) were suspended in 100 ml sterile distilled water by magnetic stirring (500 rpm, 5 minutes) in other to establish dilution series. Replica aliquots (1 ml) were over poured and dispersed by swirling with soil extract agar (SEA). Soil extract was prepared from the top soil layer (0-30 cm) of the study site by autoclaving (1 kg in 1.5 litre tap water) and filtering (Waksman 42). Additionally, SEA contained bacto agar (20 g/liter), and cycloheximide (50 mg/liter) to suppress fungal growth. The medium was adjusted to pH 7.0 prior to autoclaving. Agar plates were incubated for 28°C for 3 to 7 days. The density of culturable cellulolytic bacteria was assayed on SEA containing 0.2 % carboxymethyl cellulose (CN-cellulose). After suitable incubation time 5 d, 28°C, Congo red was used as an indicator for the detection and enumeration of cellulolytic colonies, as described by Mullings and Parish (1984). For the isolation of cellulolytic bacteria, randomly chosen colonies were transferred to CM-cellulose, SEA, subsequently incubated (3-5d, 28°C) and finally tested for cellulolytic activity as described above.

The characteristic of ACB in forest floor soil at 500 m asl is listed in Table 1. Isolate AI3 *(Bacillus* sp) qualitatively appeared to have the highest cellulolytic ability. Most of the isolated strain belonged to gram negative bacteria. *Pseudomonas* sp is dominant bacteria in soil at 500 m altitude. *Cellulomonas* sp reported by Hiroki and Watanabe, 1996 to have high cellulolytic capacity, however isolated *cellulomonas* sp from soil in 500 m altitude has lower cellulolytic ability (Table 1).

At higher altitude (1000 m) encountered three dominating genera namely *Bacillus, Pseudomonas* and *Clostridium* (Table 2). Alexander *et al.* (1961) also reported that the three genera were common bacteria encountered in soil.

Similarly to 1000 m altitude *Bacillus, Pseudomonas* and *Clostridium* were dominant genera isolated from 1500 m altitude (Table 3).

The characteristic of ACB at the soil rhizosphere of three dominating plants is shown in Table 4. Isolated microbe also shown heterogenous morphological characteristic. The highest cellulolytic activity was observed in CJl isolated from the soil rhizosphere of *Castanopsis javanica.*

The abundance population of aerobic cellulolytic bacteria (ACB) isolated from three biodiversity research sites was about 1.2×10^5 to 9.5×10^5 (Table 4). The total number of ACB observed is higher than that of encountered in peat soil (Hiroki and Watanabe, 1996).

Table 1. ACB isolated from altitude 500 m

No.	Isolat No.	Name	(CZ/C)
1.	AIl	*Pseudomonas* sp.	3.2
2.	AI2	*Pseudomonas* sp.	2.6
3.	AI3	*Bacillus* sp.	5.1
4.	AI4	*Chromobacterium* sp.	2.7
5.	AI5	*Pseudomonas* sp.	2.8
6.	AI6	*Bacillus* sp.	1.5
7.	AI7	*Chromobacterium* sp.	1.4
8.	AI8	*Pseudomonas* sp.	3.4
9.	AI9	*Bacillus* sp.	3.2
10.	AI10	*Bacillus* sp.	2.1
11.	AI11	*Chromobacterium* sp.	2.5
12.	AI12	*Pseudomonas* sp.	1.2
13.	AI13	*Pseudomonas* sp.	2.2
14.	AI14	*Clostridium* sp.	1.2
15.	AI15	*Bacillus* sp.	2.1
16.	AI16	*Pseudomonas* sp.	2.4
17.	AI17	*Moraxella* sp.	2.1
18.	AI18	*Pseudomonas* sp.	2.4
19.	AI19	*Clostrdium* sp.	2.4
20.	AI20	*Pseudomonas* sp.	1.1
21.	AII3	*Pseudomonas* sp.	1.1
22.	AIM	*Pseudomonas* sp.	1.2
23.	AII6	*Pseudomonas* sp.	1.2
24.	AII21	*Pseudomonas* sp.	2.5
25.	AII22	*Pseudomonas* sp.	1.2
26.	AII23	*Clostridium* sp.	1.3
27.	AII24	*Bacillus* sp.	1.1
28.	AIII1	*Pseudomonas* sp.	1.7
29.	AIII2	*Acinetobacter* sp.	1.4
30.	AIII6	*Bacillus* sp.	1.4
31.	AIII7	*Cellulomonas* sp.	2.2

Table 2. ACB isolated from 1000 m

No.	Isolat No.	Name	(CZ/C)
1.	BIl	*Bacillus* sp.	1.5
2.	BI2	*Bacillus* sp.	2.1
3.	BI6	*Pseudomonas* sp.	2.2
4.	BI8	*Clostridium* sp.	2.3
5.	Bill	*Enterobacter* sp.	2.1
6.	BIl 2	*Chromobacterium* sp.	1.1
7.	BII4	*Bacillus* sp.	1.1
8.	BII5	*Pseudomonas* sp.	1.1
9.	BII6	Moraxella sp.	1.3
10.	BIT7	*Pseudomonas* sp.	1.4
11.	BII9	*Clostridium* sp.	1.9
12.	BIl 10	*Clostridium* sp.	1.3
13.	BII11	*Bacillus* sp.	1.5
14.	BII12	*Pseudomonas* sp.	1.8
15.	BII13	*Bacillus* sp.	1.6
16.	BIII5	*Enterobacter* sp.	1.3
17.	BIII6	*Enterobacter* sp.	1.2
18.	BIII7	*Chromobacterium* sp.	1.5
19.	BIII8	*Pseudomonas* sp.	1.7
20.	BIII9	*Clostridium* sp.	1.2
21.	Bill 10	*Clostridium* sp.	1.5
22.	Bill 11	*Bacillus* sp.	1.3
23.	Bill 12	*Pseudomonas* sp.	1.5

Table 3. ACB isolated from 1500 m

No.	Isolat No.	Name	(CZ/C)
1.	CI1	*Chromobacterium* sp.	1.4
2.	CI2	*Bacillus* sp.	1.9
3.	CI3	*Chromobacterium* sp.	1.8
4.	CI5	*Bacillus* sp.	1.5
5.	CI6	*Enterobacter* sp.	1.8
6.	CI7	*Pseudomonas* sp.	1.4
7.	CHI	*Clostridium* sp.	**t.5**
8.	CIO	*Pseudomonas* sp.	1.2
9.	CH3	*Pseudomonas* sp.	1.5
10.	CII4	*Pseudomonas* sp.	1.6
11.	CII6	*Clostridium* sp.	1.5
12.	CII7	*Pseudomonas* sp.	1.8
13.	CII8	*Pseudomonas* sp.	1.3
14.	CII9	*Pseudomonas* sp.	1.2
15.	CII10	*Clostridium* sp.	1.1
16.	CII11	*Pseudomonas* sp.	1.5
17.	**cm 2**	*Bacillus* sp.	1.4
18	CIII1	*Pseudomonas* sp.	1.5
19	**cm 2**	*Clostridium* sp.	1.2

Table 4. The abundance population of aerobic cellulolytic bacteria (ACB) isolated from three biodiversity research sites

Plot location	Research Site	ACB (mean value) CFU/g.soil
Elevation (500 masl)	Cikaniki	2.1 x10^5
	Ciptarasa	6.5 x10^5
Elevation (1000 masl)	Cikaniki	1.8 x10^5
	Gunung Boto	2.5 x10^4
	Gunung boto	5.6 x10^5
	Ciptarasa	2.0 x10^5
Elevation (1500 masl)	Cikaniki	1.2 x10^5
	Gunung Boto	9.5x10^5
	Gunung Boto	2.5x10^5
	Ciptarasa	1.3x 10^5

Table 6. The abundance population of aerobic cellulolytic bacteria (ACB) isolated from plants rhizosphere

Study site	Dominant plant	Number of ACB (CFU/g.soil)
SSIC	Rhizosphere of *Castanopsis javanica*	1.2 x10^7
SSIS	Rhizosphere of *Schima wallichii*	5.5 x 10^7
SSI A	Rhizosphere *ofAltingia excelsa*	1.8 x10^7
SSIL	Litter	1.7 x10^7
SSI (control)	Forest floor	1.1 x10^5

The abundance population of aerobic cellulolytic bacteria (ACB) is about 1.2 x 10^7 to 5.5 x 10^7 is higher in plant rhizosphere than that of in forest floor (Table 6).

DISCUSSION

Recently, there is a growing interest in the need of verification of the role of aerobic cellulolytic bacteria in the soil and its taxonomic position (Coughlan and Meyer, 1992; Chandler *et al.,* 1992 and Bossio *et al,* 1998). In line with this objective ACB was isolated from several ecosystem type as defined by altitude. The microorganisms that were isolated from several ecosystems type have heterogeneous morphological and physiological characteristic as shown in Table 1,2,3. Aerobic cellulolytic bacteria (ACB) that grow in CMC-containing produce clear zone after addition of congo red (1 mg/ml) and washed with sodium chloride (0.1N). The morphological characteristic and their ability to form clear zone are listed in Table 1,2,3 and 4.

The number of isolated ACB from the forest floor of 1500 m is less than that of lower altitude (Table 1, 2 and 3). The lower soil acidity (data not shown) at higher altitude may suppress the growth of ACB or the characteristic of cellulose containing materials are less varied at higher altitude. Alexander (1961) has noted the effect of cellulose species on diversity of ACB. He also noted that many organisms grow poorly in media containing purified cellulose as the sole carbon, yet, on sterile plant material, the same organism vigorously utilise polysaccharide. The presence of xylans in media containing cellulose may stimulate the bacterial growth. Addition of other readily metabolizable substances accelerates cellulose decomposition. The nutrient flow from higher to lower altitude may also affect the bacterial diversity as shown in Table 1, 2 and 3. Lower altitude receives higher organic materials input from the higher altitude and thus favoured growth of ACB at the lower altitude.

Isolate AI3, AI8, and All were isolates that produced highest clear zone respectively. Morphologically and physiologically ACB were divided into A, B and C group and composed of 31, 23 and 20 isolates respectively. Group A, B, C was determined based on areas at which isolates were originated, namely 500 m, 1000 m and 1500 m respectively. Ultimate explanation of highest diversity of ACB in the lower areas is unclear. It is supposed that variability of lignocelulose materials of plant origin was highest at the lowest altitude, and this will select the most adapted species will dominant. Other physical and chemical nature of soil and microclimates at which microbes adapted for such a long time such intensity and variability of light penetration, profile of soil humidity, pH, redox potential and fluctuation of soil temperature, impact of human activities may also affect microbial population dynamic of ACB

Population density of ACB seems to be high in Gunung Boto research site. This was due to the larger input of plant materials into soil, and the lowest population density was in Ciptarasa especially at 1500 m asl. The cellulose input into soil seems to be the most important factor determining the population density. Table 5 also indicated that moderate number of ACB was found in Cikaniki site.

Ecologically, in-situ activities of ACB in soil forest ecosystem could be studied by in-situ analyses of biomass, soil respiration rate, soil enzymes activities and nutrient turn over (Heinemeyer *et al,* 1989; Eriksson *et al.,* 1992; Hiroki and Watanabe, 1996; Doran and Parkin, 1996; Kennedy and Gewin, 1997 and Bossio *et al,* 1998). Complete understanding of ecology and physiology of microbial involved in organic substances turn over is important forest ecosystem management.

Interaction between plant and ACB

Microorganisms play important role on the acceleration of nutrient cycling. Microbe produce extracellular enzymes that will hydrolyse the organic substances and also play role on the mediation of transformation of microelement that are needed for plant growth. The objective of this study was to investigate the relation between ACB and plant. This study was performed in Suzuki plot

1 at which three were three dominating plants *(Schimct wallichii, Castanopsis javanica* and *Altingia excelsa).* Soil samples were collected from the rhizosphere, and the population of ACB is presented in Table 6.

Population ACB was higher at the soil rhizosphere especially in *Schima wallichii* rhizosphere, whereas forest floors containing less ACB. Though a remarkable population different of ACB in rhizosphere and forest floor but it seem there was significant different among plant rhizosphere observed, indicating that rhizosphere and litter provide better environment for ACB growth than that of forest floor. Population density of ACB is more affected by organic material composition than by type of plants. Ulrich and Wirth (1999) also indicate that ACB density was mainly influenced by organic materials content of soil. Quantitative assessment of ACB is then important to predict the rate of carbon turnover,

CONCLUSION

High diversity of ACB was observed in both forest floor and soil rhizosphere indicating that ACB play role in decomposition of organic materials in forest ecosystem.

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