CHARACTERISTIC OF CMCASE OF *Bacillus* sp. ISOLATED FROM SOIL OF GUNUNG HALIMUN NATIONAL PARK

[Karakteristik CMCase *Bacillus* sp. Yang Diisolasi dari Tanah Taman Nasional Gunung Halimun]]

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ABSTRACT

Dclapun isolat baktcn diisolasi dari lanah Taman Nasional Gunung Halimun dipelajari posisi laksonomi dan karaktcr fisiuloginyu. Kemanipuan sclululilik dari 8 isolat Icrscbut diuji dengan membandingkan luas koloni dan zona bening yang terbentuk pada koloni yang ditumbuhkan pada CMC. Selanjutnya Karakteristik CMC-ase dari satu isolat yang mempunyai kemampuan sclulolitik yang paling tinggi diuji. Isolat dengan cepal menggunakan CMC scbagai sumber carbon utama pada suhu 30° C dengan keeepatan pertumbuhan 1.71''. 1,58'", dan 1.50'' pada pH ft. 7 dan 8. Tidak ada pertumbuhan yang bcrarti pada kultur yang nienggunakan Avicel sebagai sumbcr karbon utama. Aklivitas CMCase maksiinum sebcsar6 Unit terjadi pada pH 7 setclah 4 hari kultivasi. Maksimum pertumbuhan scl dicapai pada hari ke 4. Penambahan gliikosa incnstimulusi pertumbuhan binmassa tctatpi menghambat **aklivitas** enzim. Km dan Vmuk dari CMC-ase adalah IDmM dan2.(,8mM.d'.

Kata kunci: Baklcri selulolilik. *Bacillus* sp., Taman Nasional Ouming Halimun. . . . L1.

INTRODUCTION

Bacteria are the most second abundant of soil microflora after fungi. Their population is mostly governed by the soil organic content. The sources from which an organism derives its ccll-C and energy are useful for describing basic physiological differences among bacteria. The soil organic substances composed dominantly by plant materials of which cellulose materials are the major component. Those substances should be hydrolyzed by complex of microorganism, which perform various metabolic patterns (Hiroki and Watanabe, 1996).

Aerobic cellulolytic bacteria (ACB) in soil and other environment are quite heterogeneous (Eberson *el al.,* 200; Coughlan and Meyer, 1992; Hiroki and Watanabe, 1996; Gall *et al.,* 1997; Bélaich *et al.,* 1997) and play important role in nutrient cycle (Hiroki and Watanabe, 1996; Verhoven *et al.,* 1983). Other soil microflora like yeast also contribute to the decomposition of organic material in soil (Hatano *et al.,* 1991; Nakase *et al.,* 1994). Forest in Indonesia are of special significance because they form one of the main natural resources a.id contribute substantially to the national economy through wood, medicinal plant and spot for ecological tourism.

Present studies have indicated that cultivable ACB in forest soil are quite heterogeneous both physiologically and morphologically. It has been reported that soil microbes phylogenetically consist of quite diverse Eubacteria belonging to sub class of gram negative *Proteobacteria,* gram positive low GC, and high GC DNA content, *Cytopluiga* group and many others. A few studies have devoted on verification of phylogenetic affiliation of cultivable ACB (Coughlan and Meyer, 1992; Hiroki and Watanabe, 1996).

Ecologically the ACB play central key on the organic carbon turned over in soil which turn have a crucial impact on nutrient availability to plants (Hiroki and Watanabe, 1996). In addition to the later case, conservation of forest ecosystem in relation with green house gas management is becoming a global issue. Characteristic of microbial biomass of ACB and its activity must be further investigated to fully understand soil C

turned over and nutrient dynamic of several ecosystems type of whole forest ecosystem. It is also worth to explore the economic potential use of tropical bio-resources. Collecting, studying and depositing of cultivable ACB are worth effort since natural destruction is occurring at alarming stage. Though quantification of the rate of extinct of microbes have not been investigated intensively, but many microbiologist believe that measures on ex-situ conservation should be conducted as soon.

Our present study are trying to quantify the CMCase activity, and determine the characteristic of biomass growth and profile of pH during bacterial growth.

MATERIALS AND METHODS

Media and strains bacteria isolation

Soil samples (! 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 modified cytophage medium containing (NH^SC^ lg, $MgSO_4$ 0. lg, MnSO4 O.lg, yeast extract lg, FeCl, 0.01 g). The medium was adjusted to pH 4.8 prior to autoclaving. Agar plates were incubated for 28°C for 3 to 7 day. The density of culturable cellulolytic bacteria was assayed on modified cytophage medium containing 1 % 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 modified cytophage, subsequently incubated (3-5 d, 28°C) and finally tested for cellulolytic activity as described above.

Identification.

Bacteria identification was done follow Madigan *el* a/., 2000.

Effect of glucose on CMC-ase activity

To study the effect of glucose augmentation on cell and enzymes synthesis, 0.1 g glucose was augmented to 1 liter medium, and for control no glucose was added.

Growth on *Avicell*

The bacteria was cultured on cytophage modified medium namely substituting 1% CMC with an equal amount of *Avicell* with addition or without addition of glucose.

Quantitative determination of CMCase The strain was cultured in liquid modified cytophage medium at 30° C for 4 days, the enzyme activity was determined 0 h, 24 h, 4 days incubation, and its CMCase activity was determined follow Hatano *et al.y* 1991, expressed in unit i.e. umol glucose equivalent produced.minute^{-1}. ml enzyme^{-1}.

pH determination

Profile of pH during culture growth was determined using pH meter.

CMCase determination

A twenty fourth aged culture (5 ml) grown on modified cytophage medium, was inoculated into 100 ml CMC containing media with and without addition of 0.1 g/1 glucose, and the culture was observed at interval of 2 h, and its CMC-ase activity was determined follow Joson and Coronel, (1986) expressed in unit i.e. umol glucose equivalent produced.minute^{-1}. ml enzyme^{-1}.

RESULT

Isolation and identification

Out of 8 strains isolated only 5 strain formed clear zone (Table 1) of which *Bacillus* sp. was has the highest ratio colony to clear zone. Kim (1995) recognized that *Bacillus circulan* has high cellulolytic capacity. Whereas Blackal (1985) noted that several member of genus *Cellvibrio* are cellulolytic.

Table 1. Cellulolytic ability of ACB after 4 days incubation in cytophage media,

No	Name of species	Ability	Ratio of clear zone vs colony
1	*Bacillus* sp	+	3.1
2	*Bacillus* sp	+	1.8
3	*Bacillus* sp	+	1.6
4	*Bacillus* sp	+	1,4
5	*Bacillus* sp	+	1.2
6	*Pseudomonas* sp	-	nd
7	*Bacillus* sp	-	nd
8	*Clostridium* sp	-	nd

Biomass growth

Culture growth.

Glucose rapidly, and they easily converted into biomass as indicated by a rapid increase of cell density (Figure 1). Appear less cell synthesis was observed in media with CMC as the sole carbon source. Denis (1971) observed that culture growth is concomitant with enzymes synthesis 1.71 d^{-1}, 1.58 d^{-1} and 1.50 d^{-1} at pH 6, 7 and 8 respectively (Figure 2).

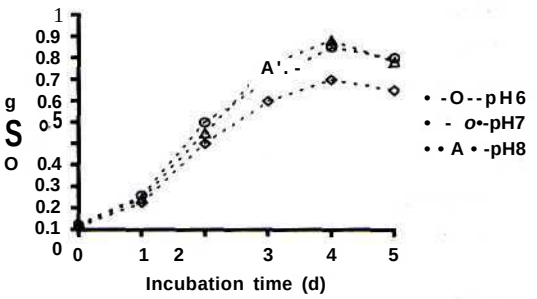

Figure 1. Profile of cell growth of *Bacillus* sp. in cytophage medium at various pH.

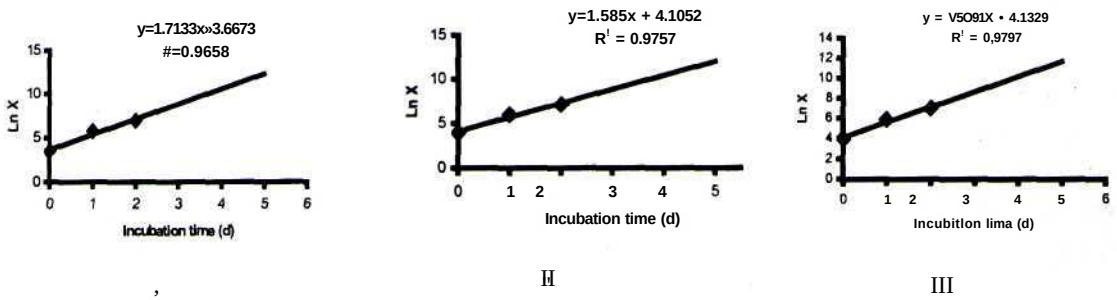

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Figure 2. Specific growth rate of *Bacillus* sp. in cytophage medium at pH 6 (I), pH 7 (II) and pH 8 (III).

Activity of endo-l,4-|3-D-glucanase (CMC-ase)

A complex of cellulase enzymes act upon organic substrate in soil leading on degradation and mineralization of organic materials. Most of organic materials in soil is of plant origin, and its biodegradation rate is affected by cellulose type. Our present study have indicate that *Bacillus* sp. has capacity to hydrolize carboxymethyl cellulose. The later substance is used to determined endoglucanase activity (Enari, 1983). The degradation rate of carboxymethyl cellulose is affected by glucose addition (Figure 4). In most cases, during cell cultivation, addition of glucose seems to have lower CMCase activity. Maximum enzymes activity was 6.0 unit for CMC as the sole carbon source observe after 96 hours incubation at pH 7, whereas at pH 6 and 8 CMCase activity was less than that of pH 7 (Figure 3). Those indicate that enzymes synthesis was pH dependent.

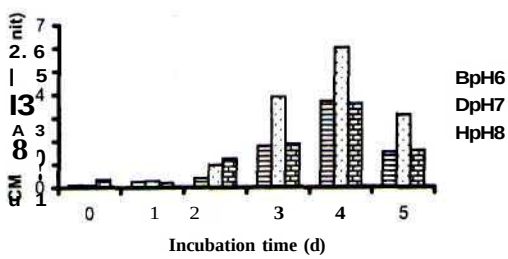

Figure 3. Profile of CMCase activity *of Bacillus* sp. in cytophage medium with or at various pH.

Km and Vmax

Substrate concentration affect the enzyme activity rate. Km was calculated by analyzing the enzyme activity at various substrate concentrations, and plotted follow the Michaelis Menten equation and further analyzed by Lineweaver-Burk confronting the value of l/S and l/V (Figure 4). Km value was 6.25 mM and Vmax was 15.64 mM.h^{-1}

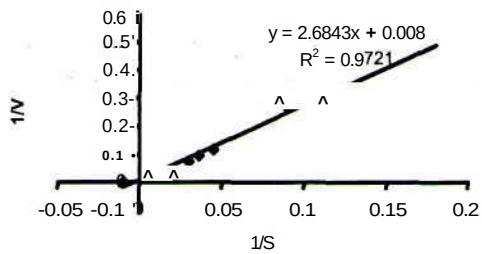

Figure 4. Kurva Lineweaver-Burk, as a substitution of Michaelis Menten for determination of Km and Vmax.

Growth on *Avicell*

Ability cell to grow on avicell as a sole carbon sources and to produce avicellase of was observed every . Compared to CMC, avicell is less suitable for culture growth than that of CMC (Figure 5) indicating that the strain produce less of exo-l,4-P-D-glucanase, Cl (Enari, 1983). Addition of glucose has positive effect on the bacterial growth indicating that the bacteria preferentially utilize glucose as C-source than *avicell.*

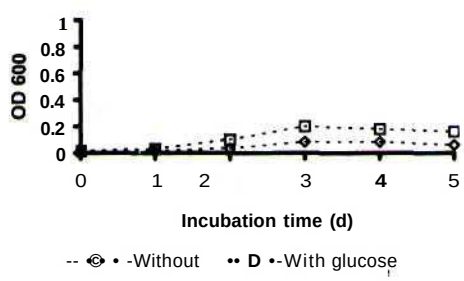

Figure 5. Profile of cell growth of *Bacillus* sp. in cytophage medium with or without glucose.

Profile of pH

Decreased of pH was observed at the beginning of incubation but then increased after 1 d incubation (Figure 6). The reason for this is unclear, it supposed that degradation of avicell (crystaline cellulose) occur slowly, and glucose

was converted into organic acid, as s reported by Holt *et al.* (1994). Many factors affect pH of culture such as degradation of protein may have result in basic amino acid release into bulk solution, complex enzyme and substrate, the presence of inhibitor and culture age (Dees *et al.*, 1995).

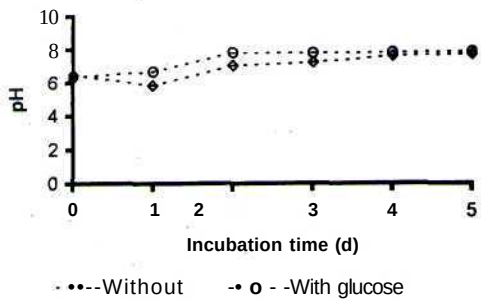

Figure 6. Profile of pH of *Bacillus* sp. in avicell augmented cytophage medium with or without glucose.

DISCUSSION

Aerobic cellulolytic bacteria play significant role in transformation of organic substances in forest soil. From Cikaniki site isolated 4 strain belonged to the genera *Bacillus* able to utilize organic substrate (carboxymethyl cellulose). Phylogenetically, *Bacillus* sp. belonged to sub class of gram positive low GC DNA content. Hydrolyses rate is strain pH dependent. Many other microflora such as *Achromobacter, Angiococus, Celfcicula, Cellumonas, Cytophaga, Polyangium* and *Pseudomonas* reported by Alexander (1961) and Elberson *et al.* (2000) to be cellulolytic bacteria.

The isolates that form highest clear zone also has highest CMCase activity. Formation of clear zone is therefore could be used as a rapid method to detect cellulolytic bacteria. Aerobic transformation of cellulose in soil contribute mainly on the transformation of organic substances. However anaerobic conversion by anaerobic microflora also play significant effect on the transformation of organic substrate in anaerobic environment. Among divers anaerobic soil microflora, *Clostridium* is a common genera observed capable of anaerobic transformation. These bacteria are also found in compost, manure, river mud, and sewage.

The ability of utilizing various species of cellulose materials in nature are they important feature of microflora to sustain its life stage in environment. Though take place slowly, isolated bacteria also able to utilize avicell suggesting that they hydrolyse crystaline cellulose, and therefore they may play significant role on the transformation of organic material in soil, and thus ecologically important to keep their population high in forest ecosystem.

CONCLUSION

Bacillus sp. is common bacteria encountered in soil. They play important role in bio-transformation of organic substrate in soil.

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