Cellulolitic Activities of Actinomycetes Isolated from Soil Rhizosphere of Waigeo, Raja Ampat, West Papua

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ABSTRACT

Cellulolitic Activities of Actinomycetes Isolated From Soil Rhizosphere of Waigeo, Raja Ampat, West Papua (A. Nurkanto): Seven actinomycetes isolated from Waigeo's soil rhizosphere have been characterized in growth, cellulase activities and pH. These actinomycetes were chosen based on their capabilities in cellulose degradation which were recognized by previous study. Those isolates are belonging to *Streptomyces* and *Actinoplanes* genera based on morphological and molecular analyses. The growths of actinomycetes were investigated based on spectrophotometric approach and cellulase activities were conducted based on reduction sugar. Cellulase activities, cell growth, and pH had significant correlation based on statistical analysis. The highest enzyme activities was acquired from *Streptomyces bobili* LIPIMC-A-283 (0,519 μmol mL^{-1} $min.^{-1}$) at 96 h of incubation. This study provided important basic information on potential cellulolitic alternative from actinomycetes group, as consideration on choosing low-cost technique using microbial agent in cellulose treatment.

Keywords: Actinomycetes, cellulose, enzyme activities, growth, rhizosphere, Waigeo

INTRODUCTION

Cellulose is the most abundance compound in the world. Cellulose, the most abundant component of plant biomass, is found in nature almost exclusively in plant cell walls, although it is produced by some animals (e.g., tunicates) and few bacteria. Despite great differences in composition and anatomical structure of cell walls across plant taxa, high cellulose content, typically in the range of approximately 35 to 50% of plant dry weight (Lynd *et al.*, 2002). Cellulose from plant biomass is the only foreseeable sustainable source of fuels and materials available to humanity. Cellulose materials are particularly attractive in this context because of their relatively low cost and plentiful supply. In addition, cellulose has central role on nutrition cycle in a first step (Lynd *et al.*, 1999).

The recently problem was knew that cellulose is stable material in nature and need complex degradation before used, as well as microbes, plants or human. Cellulose decomposition can be conducted in chemistry process or using microbe agent. Microbes are the potential agent for cellulose decomposition. Some of microbes i.e. fungi, yeast, bacteria, and actinomycetes group have cellulolitic ability and convert them in to similar sugar (glucose). Cellulose decomposition process requires an enzyme complex called cellulase. There have three types of enzyme activities in microbe. Components of cellulase systems were first classified based on their mode of catalytic action and have more recently been classified based on structural properties. Three major types of enzymatic activities are found: (i) endoglucanases or 1,4-β-D-glucan-4-glucanohydrolases (EC 3.2.1.4), (ii) exoglucanases, including 1,4- β-D-glucan glucanohydrolases (also known as cellodextrinases) (EC 3.2.1.74) and 1,4- β-D-glucan cellobiohydrolases (cellobiohydrolases) (EC 3.2.1.91), and (iii) β-glucosidases or β-glucoside glucohydrolases (EC 3.2.1.21). Endoglucanases cut at random at internal amorphous sites in the cellulose polysaccharide chain, generating oligosaccharides of various lengths and consequently new chain ends. Exoglucanases act in a processive manner on the

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reducing or nonreducing ends of cellulose polysaccharide chains, liberating either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as major products. Exoglucanases can also act on microcrystalline cellulose, presumably peeling cellulose chains from the microcrystalline structure β-Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose (Persson *et al.*, 1991; Lynd *et al.*, 2002).

Actinomycetes is group of bacteria which dominant in soil, leaf litter, and organic material. They can growth and proliferate in broad spectrum of ecosystem (Elberson *et al.*, 2000) with main role to protect global ecosystem health. Actinomycetes are saprophytic and active to decomposing organic material. In soil, it increases soil fertility (Nonomura and Ohara, 1969). One of actinomycetes important role is the ability in cellulose decomposition, although exclusive for some isolates (Nakase *et al.*, 1994; Xu *et al.*, 1994).

Exploration was conducted in Waigeo Island, Raja Ampat West Papua in July 2007. From this exploration, we have been isolated actinomycetes (139 isolates) from soil rhizosphere. Cellulolitic actinomycetes screening had been accomplished and obtained cellulolitic actinomycetes up to 82.7% (Nurkanto, 2008[a]). This research focusing in seven highest cellulolitic abilities isolates.

The objectives of this research are profiling growth and measuring of cellulase activities as of chosen cellulolitic actinomycetes from Waigeo, Raja Ampat, West Papua. This research was expected to give information about potential cellulolitic alternative from actinomycetes, in order to be developed such as biofertilizer, manure or sugar and biofuel industries based on microbes using Indonesian local isolates.

MATERIALS AND METHODS

Isolates Source

Seven actinomycetes, isolated from various soil rhizosphere in Waigeo, Raja Ampat, were used in this research. Those isolates were identified based on morphological character, microscopic and molecular analyses using 16S rDNA. These isolates had the highest ability on cellulolitic activities, derived from screening which had been conducted in previous research (Nurkanto, 2008a).

Cellulolitic Activities Test

Cellulolitic activities test were investigated in CMC broth medium ($(NH_4)_2SO_4$, 1 g L^{-1} $MgSO_4.7H_2O$, 1 g L^{-1} $MnSO_4$, 1 g L^{-1} $FeCl_3$, 0,1 g L^{-1} glucose, 1 g L^{-1} yeast extract and 10 g L^{-1} Carboxyl Methyl Cellulose). Actinomycetes isolates (in starter form at 20 h incubation, 1% v/v) were transferred into CMC broth medium. Parameters of investigation in this research are: cell growth, medium acidity, and cellulase activities test. It were measured every 24 h during 168 h incubation.

Spectrophotometric method was used to detect the cell growth. Two mL sample were centrifuged at 6000 rpm for 10 minutes. Supernatant was removed and cell pellet washed with aquadest and then re-centrifuged. After this process, supernatant had been removed and added with aquadest (2 mL) before homogenized in vortex for 5 minutes. Cell biomass measured using spectrophotometer (UV mini 1240, Shimadzu, Japan) at 600 nm wave length. Medium acidity was detected using pH meter (Corning Pinache 530) in supernatant. Cell growth measurement were also seen from specific growth rate (μ) and doubling time (dt). Specific growth rate and doubling time counted by using equation:

$$\mu = \ln (N_1-N_0) / \ln (T_1-T_0)$$
$$dt = 0{,}693/\mu.$$

μ, specific growth rate (h^{-1})
dt, doubling time (h)
N_0, biomass at initial growth (g)
N_1, biomass at final growth (g)
T_0, time at initial growth (h)
T_1, time at final growth (h)

Enzyme activities were measured by means of comparation among reduction sugar standard. It was prepared based on the gradient of glucose concentration (0 – 500 ppm). Enzyme activities were calculated from reduction of sugar remains in supernatant. Medium (2 mL) were centrifuged (6.000 rpm, 30 minutes, 4°C) and supernatant was separated to be analyzed. Pre reduction sugar (G0) was determinated with the way of added 1 mL CMC 1% subtract and 1 mL DNS reagent to 1 mL supernatant afterwards boiled at 100°C for 7 minutes. After this, reduction sugar detected with spectrophotometer (540 nm in wave length). Final reduction sugar (G1) was quantified with the equal way (G0), but supernatant and CMC 1% had been incubated at 37 °C for 2 h

Table 1. Characterization and source of isolates from cellulololitic actinomycetes.

Isolales number	Name of species	Ecosystem type	Host of vegetation	Soil pH	Altitude (m)
LIPIMC-A-278	*Streptomyces* sp.	Lowland primery forest	*Pometia pinnata*	6.5	60
LIPIMC-A-283	*Streptomyces bobili*	Savana	Poaceae	6.0	9
LIPIMC-A-251	*Streptomyces* sp.	Open forest palm dominated	*Pigafetta filaris*	6.6	9
LIPIMC-A-194	*Streptomyces* sp.	Karst forest	*Agathis* sp.	6.8	35
LIPIMC-A-279	*Actinoplanes minutisporangius*	Karst forest	*Agathis* sp.	6.9	35
LIPIMC-A-269	*Actinoplanes minutisporangius*	Savana	*Pometia pinnata*	6.0	9
LIPIMC-A-247	*Streptomyces olivochromogenes*	Lowland primery forest	*Pandanus* sp.	7.4	3

before boiled. Enzyme activities every unit are amount of micromole glucose resulted by 1 mL enzyme each minute (Kanti 2005; Dwiati *et al.*, 1999). To determinate enzyme activities, we used the formula:

$$\text{Enzyme activities} = \frac{(Gt\text{-}Go) \times \text{Volume (mL)} \times 10^3\ \mu\text{mol}}{\text{Glucose MR (180)} \times \text{enzyme volume} \times \text{incubation time (120)}}$$

RESULT AND DISCUSSION

Morphological character, microscopic and molecular analyses based on 16 S rDNA to seven isolates which used in this research indicating two groups of species (*Streptomyces* and *Actinoplanes*). It isolated from soil rhizosphere in various ecosystem type, host of vegetation, soil acidity and altitude (Table 1).

Seven actinomycets from 139 isolates which had the highest cellulolitic activities had been found based on screening which was done in previous study. It was conducted in CMC agar (Nurkanto, 2008[a]). However, the biggest of clear zone ratio in screening process weren't followed by the highest cellulose activities while next investigation conducted in broth medium with batch fermentation system. The highest enzyme activities acquired from *Streptomyces bobili* LIPIMC-A-283. Generally in my research, we obtained that the higher enzyme activities, the higher specific growth rate and the lower doubling time period (Table 2).

Table 2. The comparison of clear zone ratio in cellulolitic screening with the highest cellulase, its specific growth rate, and doubling time from seven isolates during fermentation.

Isolate number	Clear zone ratio	The highest of cellulase ($\mu mol^{-1}mL^{-1}$minute)	Incubatin time (h)	Specific growth rate (h^{-1})	Doubling time (h)
LIPIMC-A-278	7.30	0.200	96	0.437	1.586
LIPIMC-A-283	3.70	0.519	96	0.567	1.222
LIPIMC-A-251	3.01	0.318	96	0.329	2.108
LIPIMC-A-194	7.00	0.290	72	0.365	1.899
LIPIMC-A-279	6.00	0.251	96	0.468	1.479
LIPIMC-A-269	5.20	0.179	96	0.496	1.398
LIPIMC-A-247	3.35	0.275	120	0.527	1.315

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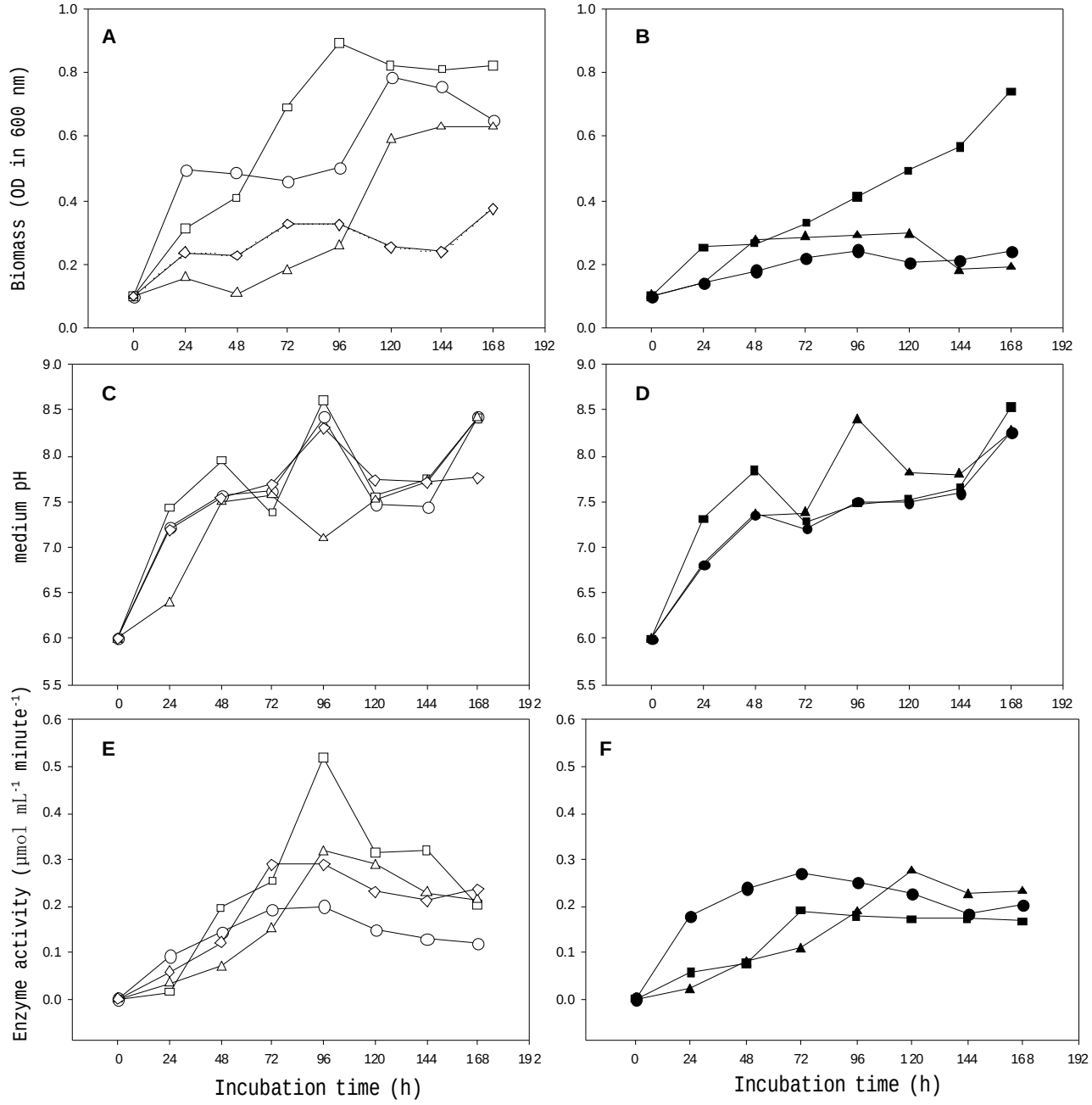

Figure 1. Cell growth (A,B), medium pH (C,D) and cellulase activities (E,F) in CMC medium (○ = LIPIMC-A-278, □ = LIPIMC-A-283, Δ = LIPIMC-A-251, ◊ = LIPIMC-A-194, ● = LIPIMC-A-279, ■ = LIPIMC-A-269, ▲ = LIPIMC-A-247).

The profile of growth, acidity and enzyme activities during 168 h incubation seen dissimilar and tends to have fluctuation (Figure 1). Commonly, the highest enzyme activities achieved at 96 h incubation. This condition was long term period, on the other hand, it could be excusable because actinomycetes are *slow growth bacteria* group compared with others bacteria or fungi and yeast (Nurkanto, 2008[b]; Lee and Hwang, 2002). However, enzyme activities in this research were higher than previous one (in wild type isolates). *Clostridium cellulolyticum* was obtained up to 0.11 µmol mL^{-1} $minute^{-1}$ (Fierobe *et al*, 1991), 0.014 – 0.027 µmol mL^{-1} $minute^{-1}$ from *Trichoderma reesei* (Tomme *et al.*, 1988), 0.083 µmol mL^{-1} $minute^{-1}$ by

Clostridium thermocellum (Schwarz *et al*., 1986). The result higher compared with other actinomycetes isolated from soil in Bukit Duabelas Jambi (0.007 and 0.134 μmol mL^{-1} $minute^{-1}$) (Kanti, 2005). Cellulase production linked with the cell growth, because they need sugar as carbon sources. Sugar could be supplied from cellulose through decomposition process using cellulase. Using this way, cells have to create cellulase secreted to medium. Using this enzyme, one unit cellulose could be decomposed and converted to 800 unit glucose. Some of them were used by cell as main nutrition in their metabolism system.

Based on statistic analyses using SPSS ver. 13, there showed very significant correlation ($p < 0.05$) among cellulase activities, cell biomass and medium acidity (pH), which can be explained in followed linier regression.

Enzyme activities = 0,096 A + 0,086 B – 0,507
A: cell biomass and B: medium acidity (pH)

From this investigation we found that enzyme activities had linear comparison with cell growth and medium acidity. It implied that the higher of atinomycetes growth, the higher of enzyme activities ($R = 0.701$). There also seen that increased of enzyme activities followed by medium acidity, wherever medium more alkali than before fermentation.

Actinomycetes cellulase was produced in neutral to alkalis medium. There were two isolates which had interesting profile. *Streptomyces bobili* LIPIMC-A-283 produced the highest enzyme when maximum pH was reached (8.72). *Streptomyces* sp. LIPIMC-A-251 produced the highest enzyme when medium pH lower (7.00). Enzyme of *Streptomyces bobili* LIPIMC-A-283 could be classified as alkali cellulase. It was also seen in decreasing of enzyme activities while pH decreased after 96 h incubation (Figure 1C and 1E).

Cellulase activities profile from 7 tested isolates were increased reach maximum value, and then gradually decreased. These cases assumed by some effect. The first was enzyme activities measurement. It was conducted based on glucose remaining in medium, not amount of enzyme production. In batch culture fermentation in this research, there had medium limitation factor. Glucose, a molecule had been produced, were used by cell for metabolism process, with no measure detected in the result. This hypothesis was supported by data obtained, where maximum cellulase activities acquired in growth logarithmic final phase. When cell growth reached stationer phase, enzyme production has been diminished. The second factor was pH stimulated. In alkalis environment condition, there had negative feed back mechanism which could block the growth and cellulase production (except for alkali cellulase).

CONCLUSION

Generally, cell growth and medium acidity had effect in cellulase activities from actinomycetes. Some actinomycetes isolated from Waigeo, Raja Ampat had the high of cellulolitic activities. The highest enzyme activity was obtained by *Streptomyces bobili* LIPIMC-A-283. This result can expanding research opportunity and the usage in organic and microorganism fertilizer and cellulose bio-conversion to valuable compound like glucose and bio-ethanol.

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REFFERENCES

Dwiati, M.P., P. Lestari and L. Prayogo. 1999. Uji aktivitas ekstrak selulase dari *Cassytha filiformis* yang berparasit pada tanaman *Duranta repens*. Majalah Ilmiah Universitas Jendral Soedirman 25(2): 105.

Elberson, M.A., F. Malekzadeh, M.T. Yazdi, N. Kameranpour, M.R. Nooridloii, M.H. Matte, M. Shahamat, R.R. Colwell and K.R. Sowers. 2000. cellulomonas persica sp. nov. and *Cellulomonas irensis* sp. nov.mesophilic cellulose degradating bacteria isolated from forest soil. Inter. J. Syst. Evol. Microbiol. 50: 993-996.

Fierobe, H.P., C. Gaudin, A. Belai, M. Loutû, E. Faure, C. Bagnara, D. Baty and J.P. Be laich. 1991. Characterization of endoglucanase A from *Clostridium cellulolyticum*. J. Bacteriol 173: 7956-7962.

Kanti, A. 2005. Actinomycetes Selulolitik dari Tanah Hutan Taman Nasional Bukit Duabelas, Jambi. J. Biodiversitas 6(2): 85- 89.

Lee, Y.J. and B.K. Hwang. 2002. Diversity of Antifungal Actinomycetes in Varios Vegetative soils of Korea. J. Microbiol 48: 407-417. .

Lynd, L.R., P.J. Weimer, W.H. van Zyl, and I.S. Pretorius. 2002. Microbial Cellulose Utilization: Fundamentals and Biotechnology. Microbiology and Molecular Biology Reviews. Am. Soc. Microbiol. 66 (3): 506-577.

Lynd, L. R., C. E. Wyman, and T. U. Gerngross. 1999. Biocommodity engineering. Biotechnol. Prog. 15: 777-793.

Nakesu, T., S. Motofumi, T. Masako, H. Makiko, H. Takhusi and F. Sukuzo. 1994. A Taxonomy studies in cellulolitic yeast and yeast-like microorganisms isolated in Japan I. Ascomycetous yeasts genera *Candida* and *Williopsis* and a yeast-like genus *Prototheca*. J. Gen. Appl. Microbiol. 40: 519-531.

Nanomura, H. and Y. Ohara. 1969. distribution of soil actinomycetes. VII. A culture mehod effective for both preferential isolation and enumeration of Microbiospora and Streptosporangium strains in soil (part II). J. Ferment. Tech. 47: 701-709.

Nurkanto, A. 2008a. Keragaman Aktinomisetes Dari Kepulauan Waigeo, Kabupaten Raja Ampat, Papua dan Potensinya Sebagai Pendegradasi Selulosa dan Pelarut Fosfat. J. Berita Biol. 9 (1): 9-18.

Nurkanto, A. 2008b. Studi Kelimpahan Aktinomisetes Tanah dan Hubungannya Terhadap Enzim Amilase, Total Karbon dan Nitrogen Hutan Pasca Kebakaran Bukit Bangkirai Kalimantan Timur. J. Biol. Indon. 5(1): 81-89.

Persson, I., F. Tjerneld and B. H. Hagerdal. 1991. Fungal Cellulolytic Enzyme Production: A Review. Process Biochem. 26: 65-74.

Schwarz, W. H., F. GrÓbnitz, and W. L. Staudenbauer. 1986. Properties of *Clostridium thermocellum* endoglucanase produced in Escherichia coli. Appl. Environ. Microbiol. 51: 1293-1299.

Tomme, P., H. Van Tilbeurgh, G. Pettersson, J. Van Damme, J. Vanderker-ckhove, J. Knowles, T. Teeri, and M. Claeyssens. 1988. Studies of thecellulolytic system of *Trichoderma reesei* QM 9414. Analysis of domainfunction in two cellobiohydrolases by limited proteolysis. Eur. J. Biochem. 170: 575-581.

Xu, L.H., Q.R. Li, and C.L. Jiang. 1996. Diversity of soil actinomycetes in Yunnan, China. Appl. Environ. Microbiol. 62 (1): 244-248.