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Isolation and screening of actinomycetes producing cellulase and xylanase from Mamasa soil, West Sulawesi

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Isolation and screening of actinomycetes producing cellulase and xylanase from Mamasa soil, West Sulawesi

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Abstract. Actinomycetes are Gram-positive bacteria with high G+C content that important for nutrient recycling of natural substrates and degradation of soil organic material. Actinomycetes can secrete enzymes to degrade organic material such as lignocellulose. Some enzymes produced by actinomycetes for degradation of lignocellulose including cellulase and xylanase. The aim of this study was to isolate actinomycetes from soil originated from Mamasa, West Sulawesi, Indonesia, and screen their cellulase and xylanase activity. A total of 57 isolates of actinomycetes have been isolated using SDS-YE method. Those isolates were screened for their cellulase and xylanase activity. The abilities of actinomycetes to degrade cellulose and xylan were observed by clear zone on CMC agar medium and xylan agar medium. Out of 57 isolates, 17 isolates produced cellulase; five isolates produced xylanase and three isolates produced both cellulase and xylanase. After the identification of potential isolates, the cellulolytic actinomycetes were identified belong to 6 genera (*Asanoa*, *Dactylosporangium*, *Kitasatospora*, *Nonomurae*, *Streptomyces*, and *Streptosporangium*). Meanwhile, the xylanolytic actinomycetes were identified belong to 3 genera (*Asanoa*, *Kribella*, and *Streptomyces*). The result showed that the ability of actinomycetes to produce cellulase and xylanase were very low. Therefore isolation of actinomycetes from the specific substrate is necessary to be conducted.

1. Introduction

Lignocellulose is a most abundant renewable biomass from plants that comprised of cellulose, hemicellulose, and lignin [1]. Cellulose is the most abundant component of lignocellulose for about 35-50% followed by hemicellulose for about 20-35%. Xylan is the major component of hemicellulose and it is the second most abundant component after cellulose [2]. The hydrolysis of these lignocellulosic materials to monosaccharides can catalyze by hydrolytic enzymes which include cellulase, hemicellulase, and ligninolytic enzymes. The hydrolysis of cellulose is catalyzing by cellulase. They are generally divided into endoglucanases (EC 3.2.1.4), β -glucosidase (EC 3.2.1.21), cellobiohydrolase (EC. 3.2.1.91) and exoglycanases (EC 3.2.1.74) [3], while the xylan hydrolysis by xylanase involves two main enzymes endo-1,4- β -xylanase (EC 3.2.1.8) and β -D-xylosidase [4].

The benefit of enzyme application in the industrial sector is reducing chemical pollution and require the mild condition of the process [5]. In the pulp and paper industry, cellulase and xylanase were used in de-inking and bleaching process. Enzymes produced by microbe have become attention in the industry in recent years and replace the chemical catalyst in the industry such as pharmaceuticals, textiles, paper, food industries [6]. Some groups of bacteria have been reported to



produce cellulase and xylanase enzyme including Gram-positive and Gram-negative bacteria [7];[8]. Actinomycetes are a group of Gram-positive bacteria with high Guanine and Cytosine (GC) content that report can produce those enzymes, especially *Streptomyces* group [9]. Actinomycetes are primarily aerobic and form mycelia and spores. Actinomycetes can live in soil, freshwater sediment, and sea sediment or saprophyte with a wide range of organic substrate such as leaf litter and wood [10];[11]. Some actinomycetes groups important for nutrient recycling of natural substrate, degradation of soil organic material and decomposition of same materials, produces antibiotic and enzymes [12]. The present study was aimed for isolation, screening, and identification of potential cellulolytic and xylanolytic enzymes producing by actinomycetes from soil samples in Mamasa, West Sulawesi.

2. Materials and Methods

2.1. Sample collection and pretreatment of samples

Soil samples were taken from different soil (near the waterfall, forest, and cultivated land) located in Mamasa, West Sulawesi, Indonesia. Soil samples were air-dried for three days and incubated at 65°C for 15 minutes. The samples were then ground with a mortar and sieved. About one gram of each sample was used for the isolation of actinomycetes.

2.2. Isolation of actinomycetes

Actinomycetes were isolated using Sodium Dodecyl Sulphate - Yeast Extract (SDS-YE) method [13]. About one gram of each samples was dissolved with 10 ml of sterile distilled water. About 0.5 mL of soil suspension then diluted with 4.5 mL. The samples then incubated at 40°C for 20 minutes on water bath shaker. Soil suspension was then diluted with sterile water up to 10^{-5} . The soil suspension was then pipetted and was spread onto the plates of Humic Acid Vitamin Agar (HVA) medium supplemented with cycloheximide (50 mg/l) and nalidixic acid (20 mg/l) [14]. Furthermore, the plates were incubated at 30°C for 7-14 days. Isolates of actinomycetes that have grown well on HVA medium selected based on their morphological. The selected isolates were purified on Yeast Starch Agar (YSA). A pure colony of actinomycetes was then stored in 10% glycerol at -80°C for further screening.

2.3. Enzymatic screening and Identification of potential isolates

Isolates of actinomycetes have been collected, then screened for their abilities to produce extracellular enzymes including cellulase and xylanase. Screening of isolates producing extracellular enzymes was done by growing actinomycetes both on Carboxyl Methyl Cellulose (CMC) medium plates [15] and SCA medium supplemented with 0.5% xylan [16] to observe the cellulase and xylanase activity. The plates were incubated for 14 days at 30°C. The clearing zones around of isolates were observed after the plates flooded with 0.1% Congo red solution. The plates incubated for 30 minutes and furthermore, washed with 1 M NaCl. The formation of clearing zones indicates positive results that the isolate potential for producing either cellulase or xylanase.

2.4. Identification of the potential isolates

Isolates that shows cellulase and xylanase activities were identified based on morphological and 16S rRNA gene sequence. DNA of actinomycetes was extracted following a modified method [17]. PCR amplification of the 16S rRNA gene was carried out with universal primers of prokaryotic, forward primer 27F (5' AGAGTTTGATCCTGGCTCAG 3') and reserve primer 1492R (5' GGTTACCTTGTTACGACTT 3'). The PCR program was used as following: an initial denaturation (94°C for 1 min), 30 cycles of denaturation (95°C for 30 s), annealing (50°C for 30 s), extension (72°C for 1 min, 30 s). Purified PCR product was sequenced by Macrogen®. The raw sequencing data were trimmed and assembled using CromasPro program version 1.6. Furthermore, the assembling data was compared by taxonomy browser using the EZ-Taxon server (<https://www.ezbiocloud.net/identify>) [18]

3. Results and Discussion

A total of 57 isolates of actinomycetes were obtained from soil samples collected from Mamasa, West Sulawesi, Indonesia using SDS-YE method. Khama & Yokota (2009) reported that the number of actinomycetes isolated from soil pre-treated with 0.05% SDS was higher than with 1.5% phenol [19]. SDS -YE method is a method for isolation of general actinomycetes from soil. Humic Acid Agar (HVA) medium contain soil humic acid as a source of carbon and nitrogen and suitable for actinomycetes recovery from soil [19]. All the isolates grew well on YSA medium with the variation of colony morphology. From the 57 isolates, 17 of isolates (29.9 %) produced soluble pigment into the medium. Most of the isolates belonged to genus *Streptomyces* sp. Those isolates have fungi-like branching, form aerial and substrate mycelia. The diversity of colonies of actinomycetes showed in Figure 1. Some actinomycetes isolates had the ability to produce extracellular cellulase and xylanase enzymes. The positive results of extracellular cellulase and xylanase activity on CMC agar medium and xylan agar medium suggested by the formation of the clear zone after Congo red staining (Figure 2).

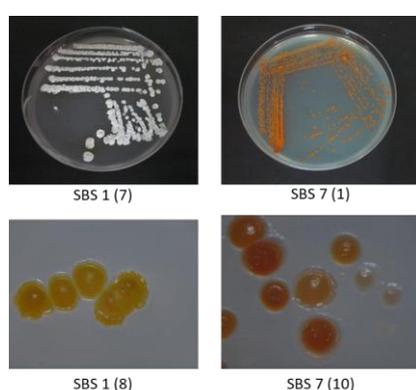


Figure 1. Variation of colony morphology of actinomycetes isolated from soil in Mamasa, West Sulawesi grew in YSA medium for 14 -21day.

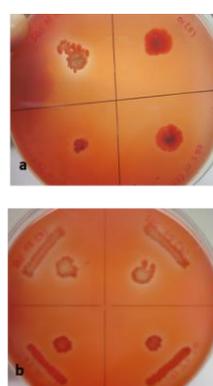


Figure 2. A clear zone of substrate hydrolysis shown on a) carboxy methyl cellulose agar medium; b) xylan agar medium.

From the 57 of actinomycetes isolates, 17 isolates produced extracellular cellulase enzyme with the ratio of clear zones diameter to colony diameter between 1.2 and 3.1 mm (Table 1). The isolates that potentially produce cellulase enzymes identified as six genera (*Streptomyces*, *Kitasatospora*, *Nonomurae*, *Asanoa*, *Streptosporangium*, and *Dactylosporangium*) (Table 1). Majority of the cellulase enzymes was produced by genus *Streptomyces*. Many studied have been reports that *Streptomyces* hydrolyzed of cellulose by produce cellulase enzymes [9];[20];[21];[22]. The diversity of actinomycetes have been reported produce cellulase enzymes such as *Streptomyces viridobrunnes* SCPE-09 produced endoglucanase [23], and *Streptomyces longispororuber* produce carboxymethylcellulase [24]. A new species of *Streptomyces* also reported can hydrolyzed cellulose such as *Streptomyces abietis* isolated from soil of pine forest [25] and *Streptomyces matensis* isolated from the soil of agricultural field of Patna area that produced endoglucanase and exoglucanase enzymes [24]. Not only *Streptomyces* but also *Kitasatospora* sp. and *Dactylosporangium* have been reported produce either cellulase or xylanase enzymes [26];[27]. Four isolates with the highest clear zones were SBS 07(11), SBS 07(8), SBS 07(10), and SBS 01(6). Two isolates have similar sequences with *Asanoa iriomotensis*, one isolate has a similar sequence with *Dactylosporangium tropicum*, and one isolates has a similar sequence with *Streptomyces* sp. (Table 1).

Table 1. The clear zone ratio produced by some actinomycetes isolates on CMC media.

No	Isolate code	Genus / species	Clear zone ratio (mm)
1	SBS 01 (5)	<i>Streptomyces sp</i>	1,7
2	SBS 01 (6)	<i>Streptomyces sp</i>	2,7
3	SBS 03 (5)	<i>Streptomyces sp</i>	1,8
4	SBS 03 (6)	<i>Streptomyces sp</i>	2,3
5	SBS 05 (3)	<i>Streptomyces yanii</i>	1,3
6	SBS 05 (5)	<i>Streptomyces abietis</i>	1,2
7	SBS 05 (6)	<i>Kitasatospora nipponensis</i>	2,0
8	SBS 05 (11)	<i>Nonomuraea dietziae</i>	1,8
9	SBS 05 (13)	<i>Asanoa iriomotensis</i>	2,7
10	SBS 06 (4)	<i>Streptomyces sp</i>	2,3
11	SBS 06 (5)	<i>Streptomyces sp</i>	1,7
12	SBS 07 (4)	<i>Streptosporangium subroseum</i>	1,7
13	SBS 07 (8)	<i>Asanoa iriomotensis</i>	2,9
14	SBS 07 (10)	<i>Asanoa iriomotensis</i>	2,7
15	SBS 07 (11)	<i>Dactylosporangium tropicum</i>	3,1
16	SBS 09 (5)	<i>Kitasatospora kifunensis</i>	1,7
17	SBS 09 (6)	<i>Kitasatospora kifunensis</i>	2,1

Besides having cellulase enzymes, actinomycetes isolates have the ability to produce extracellular xylanase enzymes. Of the 57 isolates of actinomycetes screened for the xylanase activity, only five isolates utilized xylan and showed xylanase activity with ratio clear zones to colony diameter between 0.45 and 0.9 mm (Table 2). The ratio clear zone was very low. Five isolates able to hydrolyze xylan belong to three genera. They are *Asanoa*, *Kribbella*, and *Streptomyces*. *Streptomyces sp.* has been reported potential produce xylanase enzymes [28];[29]. Meanwhile, the isolates that potential produce cellulase and xylanase were SBS 3(5), SBS 06(4), and SBS 07(10). The isolates belonged to two genera (*Asanoa*, and *Streptomyces*). Genus of *Streptomyces sp.* have been reported can hydrolyze either cellulose or xylan and produce both cellulase and xylanase enzymes [30],[31]. SBS 7(10) has a similar sequence with *A. iriomotensis* based on 16S rRNA gene. This isolate showed produced both cellulase and xylanase enzymes. *Asanoa iriomotensis* was novel actinomycetes isolated from mangrove soil and has not been reported its potential for hydrolyzing cellulose or xylan [32].

Table 2. The clear zone ratio produced by some actinomycetes isolates on xylan media

No	Isolate code	Genus / species	Clear zone ratio (mm)
1	SBS 03 (5)	<i>Streptomyces sp</i>	0,85
2	SBS 05 (7)	<i>Kribbella sindirgiensis</i>	0,6
3	SBS 06 (2)	<i>Streptomyces sp</i>	0,9
4	SBS 06 (4)	<i>Streptomyces sp</i>	0,45
5	SBS 07 (10)	<i>Asanoa iriomotensis</i>	0,5

4. Conclusion

This research is the preliminary study to get information about potential actinomycetes isolated from Soil in Mamasa, West Sulawesi producing cellulase and xylanase enzymes. Data showed that 17 isolates could potentially produce extracellular cellulase enzymes, and five isolates potentially produce extracellular xylanase enzymes. But the ability of actinomycetes to produce cellulase and xylanase were very low. Therefore the isolation of actinomycetes from the specific substrate is necessary to be conducted, such as a collected sample from rhizosphere soil, agriculture waste, and wood.

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