Screening the capabilities of Indonesian indigenous mold in producing cellulase enzyme

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Abstract. This study aims to get the isolates of mold that can potentially produce cellulase enzyme. This research was conducted in December 2017 until January 2018 in Microbiology Laboratory of Universitas Negeri Jakarta. The screening test of potential isolates producing cellulase enzymes was performed on 31 isolates of UNJCC in CDA medium with addition of *Carboxymethyl Cellulose* (CMC) substrate and testing of clear zone formation using 0.1% congo red reagent. From the result of the research, there are 18 potential mold isolates which can produce cellulase enzyme. 3 isolates which have the highest Cellulolytic index are isolate A17, K4, and 1 which have cellulolytic index value of 1.03, 0.32, 0.29. From macroscopic and microscopic identification it can be assumed that the coded isolates of K4 and A17 belong to the genus *Aspergillus*, while, isolates code 1 belong to the genus *Penicillium*. Forming the clear zone on CMC subtrate with dropped congo red reagent it because congo red reagent can bind strongly with polysaccharides containing β (1-4) D-glucopyranosyl bonds. And the ability of clear zone formation on CMC substrates shows the presence of enzyme endo-β-1,4 glucanase (CMCase) that can breaks off the β -1,4-glycosidic bond linking D-glucose monomer to CMC.

1. Introduction

Indonesia is a country with high biodiversity and high fungal diversity. Indonesia has a diversity of fungi around 180000-240000 species (6-8% of the total estimated 1.5 million species) [1]. Mold can be found from plant [2]; chicken feed [3]; fruit [4] and many other substrate [5].

Such high biodiversity has a potential in promoting domestic industry, for, such as the production of cellulase enzymes. The cellulase enzyme has wide array of potential and application in the industry. Cellulase enzymes is commonly used in various industries such as food biotechnology, textiles, paper, agriculture as well as in research development. Generally, the enzyme currently in use is imported and expensive [6].

Cellulase Enzyme can produce from Microorganism like fungi, bacteria, protozoa and can produce from plant and animal [7] but, mold is a common microorganism found in cellulose degradation [8].

This study aims to test the capabilities of Indonesian indigenous mold in producing cellulase enzyme**.** Isolation and screening of Indonesian indigenous mold is expected to obtain mold that has the potential to produce cellulase.

2. Method

2.1. Time and place
This study was conducted in December 2017 until January 2018 at Universitas Negeri Jakarta Culture Collection UNJCC; and Microbiology Laboratory of Universitas Negeri Jakarta.

2.2. Tools and materials
Laminar air flow, erlenmeyer tube, petridisc, cotton, yellow page paper, heat resistant plastic, spiritus, reaction tube shelf, stirring rod, scales, measuring cup, skewer as needle plant, beaker, tube reactions, stoves, autoclaves, and other research support tools.

The materials used during the study were potato dextrose agar (PDA) medium, CDA-CMC media. While the solution used among others is congo red reagent 0.1%, aquades, and alcohol 70%.

2.3. Methodology

2.3.1. Sample Determination. This study used thirty-one (31) collections of pure mold culture from UNJCC Microbiology Laboratory (Universitas Negeri Jakarta Culture Collection). Some Isolates molds were isolated from livestock feed, Rotten Malang Apple and Rotten Orange by previous researchers, mold isolates were cultured in PDA slant media on test tubes.

2.3.2. Manufacture of medium. Cellulase enzymes qualitative test was done by making a CDA medium with the addition of 1% CMC substrates mixture into medium. Then the medium was heated while stirring until homogenous. After that, the medium was sterilized using an autoclave at 121ºC at 2 atm for 15 minutes. Furthermore, the medium is poured into a petri dish sterile as much as approximately 20 ml.

2.3.3. Screening of mold based on the clear zone. Quantitative test of cellulase enzymes was done by first, the petri dish was devide into four quadrants using permanent markers. After that, the petri dish was devide into four quadrants using permanent markers. And then the medium was incubated for 48 hours at room temperature. After 48 hours, Each petri was dropped congo red reagent until covering the entire surface of the medium. And the formation of clear zone around the mold colony is the positive indicator of potential mold that can produce cellulase enzyme. It indicates that CMC substrate has been hydrolyzed.

2.3.4. Cellulolytic index measurements.
The measurements of the cellulolytic index were performed by measuring the diameter of the colony and the diameter of the cleared zone, they were calculated by 3 repetitions on several sides and then the average diameter was taken. Measurements were made using a caliper. While to calculated the cellulolytic index used the formula : [9].

$$\text{Index of Cellulolytic} : \frac{\text{Diameter of the clear zone} - \text{Diameter of the colony}}{\text{Diameter of the colony}}$$

2.3.5. Characteristics identification. Identification of fungal cultures is based on macroscopic characteristics such as color of colony, texture of colony, zonation, exudate drops, radial furrow and microscopic characteristics such as type of vesicle, type of conidiofor, type of hypha [10].

3. Results and discussion

3.1. Screening of mold based on the clear zone
Selection of potential isolates in producing cellulolytic enzymes can be determined by observing the presence or absence of the clear zone formed around the colony (Figure 1). From the result of

screening 31 isolates potentials to producing cellulase enzyme, there was 18 isolates were founded can formed the clear zone. Isolate mold with code A17, K4, K5, 1, and 49 forming the highest clear zone and the cellulolytic index value of the five isolate with the highest cleared zone will be calculated.

Forming the clear zone on CMC subtrate with dropped congo red reagent it because congo red reagent can bind strongly with polysaccharides containing β (1-4) D-glucopyranosyl bonds. And the ability of clear zone formation on CMC substrates shows the presence of enzyme endo-β-1,4 glucanase (CMCase) [11], that can breaks off the β -1,4-glycosidic bond linking D-glucose monomer to CMC [12].

Cellulase enzyme is an extracellular enzyme produced in cells that work to degrade cellulose polymer compound that can be easily absorbed through the cell walls [13]. Cellulase enzyme consists of three types of enzymes namely endo-β-1,4-glucanase complex, ekso- β-1,4-glucanase, and β-1,4-glucosidase or selobiase [14].

Mold is a common microorganism found in cellulose degradation [15]. Molds are microorganisms of the kingdom of fungi can form hyphae and included into the type of multicellular fungi who can break down complex organic materials into simpler materials [16].

3.2. Cellulolytic index
There's a difference of cellulolytic index value on the five isolates (Table 1), It indicates that there are differences of each isolate in producing cellulase enzymes to hydrolyze cellulose on CMC medium [17].

The ability of fungi to degrade CMC can support the growth mycelia of fungal due to form a simpler of cellulose so easily hydrolyzed by mold [5].

Based on the cellulolytic index value, 3 isolates with the highest cellulolytic index values were identified for macroscopic characteristics and microscopic characteristics.
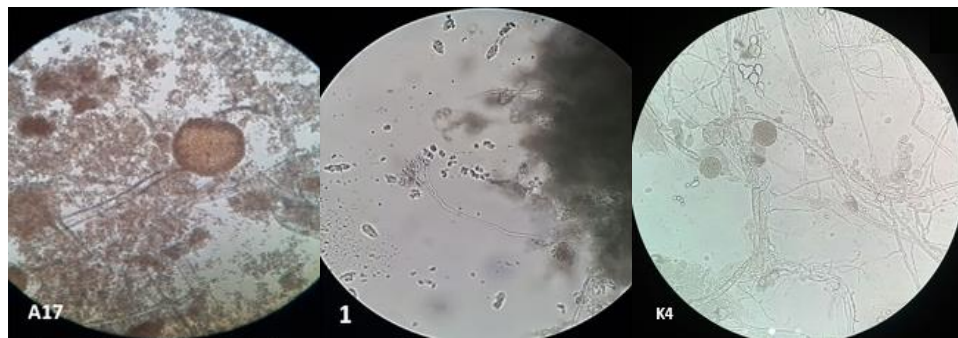
Table 1. Cellulolytic index value.

Number	Code of Isolate	Diameter (mm)		Indeks Selulolitik (mm)
		Clear Zone	Colony	
1	A17	14.73	7.24	1.03
2	K4	28.10	21.30	0.31
3	1	15.56	12.055	0.29
4	K5	24.34	18.99	0.28
5	49	25.01	20.61	0.21

3.3. Macroscopic and microscopic identification
From macroscopic and microscopic identification on all three of the highest cellulolytic index (Table 2 and Table 3) (Figure 1), it can be assumed that the coded isolates of K4 and A17 belong to the genus *Aspergillus*, while code 1 isolates belong to the genus *Penicillium*.

Table 2. Microscopic identification.

Number	Code of Isolate	Vesicle	Conidiofor	Hypha
1.	A17	Biseriate	Radiate	Aseptat
2.	K4	Uniseriate	Radiate	Aseptat
3.	1	Biseriate	Branched	Septat

Figure 1. Microscopic identification.

M : 400X, incubation on 1% CDA-CMC medium, 48 hours at room temperature.
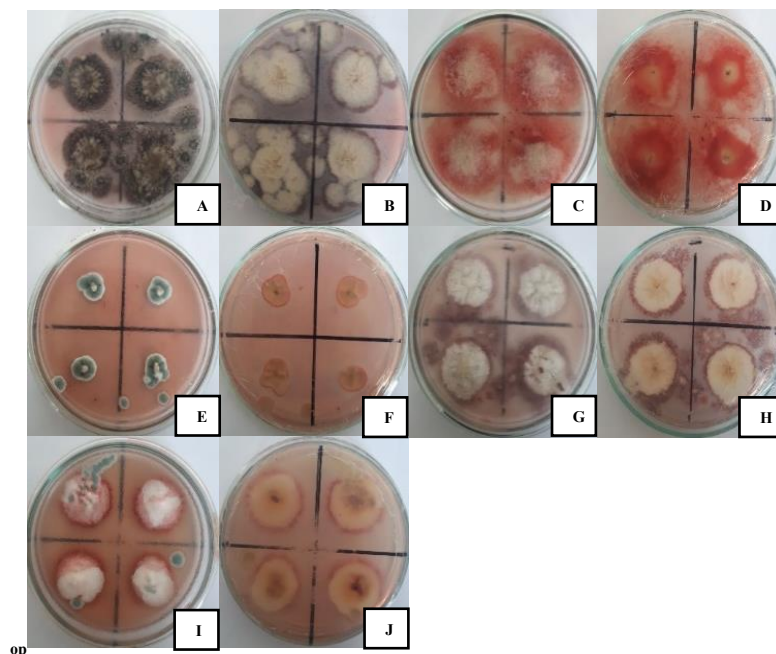
Table 3 . Macroscopic identification.

Number	Code Isolate	Color of Colony	Texture of Colony	Zonation	Exudate drops	Radial furrow
1.	A17	Soft Black	Granula	+	-	-
2.	K4	Cedar Green	Vavelty	-	-	+
3.	1	Hooker's Green	Granula	+	+	+

Table 4. Screening of Mold Based on The Clear Zone Incubation on 1% CDA-CMC Medium, 48 hours, at room temperature.

Number	Code of Isolates	Clear Zone	Diameter Of the Clear Zone (mm)	Source
1	A1	-	-	Rotten Malang Appel, Jakarta Timur
2	A17	+	10.7	Rotten Malang Appel, Jakarta Timur
3	A20	-	-	Rotten Malang Appel, Jakarta Timur
4	K1	+	0.5	Rotten Orange, Jakarta Timur
5	K2	+	3.6	Rotten Orange, Jakarta Timur
6	K3	+	4.3	Rotten Orange, Jakarta Timur
7	K4	+	26.3	Rotten Orange, Jakarta Timur
8	K5	+	6.7	Rotten Orange, Jakarta Timur
9	K9	+	2.3	Rotten Orange, Jakarta Timur
10	1	+	10.7	Chicken Feed
11	2	-	-	Chicken Feed
12	4	+	4.6	Chicken Feed
13	7	-	-	Chicken Feed
14	11	-	-	Chicken Feed

Tabel 4.Cont

15	12	-	-	Chicken Feed
16	13	-	-	Chicken Feed
17	17	+	3.9	Chicken Feed
18	18	+	4.5	Chicken Feed
19	20	+	4.8	Chicken Feed,
20	26	-	-	Chicken Feed
21	40	-	-	Chicken Feed
22	41	+	3.2	Chicken Feed
23	42	-	-	Chicken Feed
24	45	+	4.3	Chicken Feed
25	46	-	-	Chicken Feed
26	47	-	-	Chicken Feed
27	49	+	5.5	Chicken Feed
28	50	-	-	Chicken Feed
29	51	+	3.7	Chicken Feed
30	54	+	4.5	Chicken Feed
31	56	+	3.7	Chicken Feed

Figure 2. Screening of mold based on the clear zone.

A : isolate A17 Observe, B : isolate A17 Reverse, C : isolate K4 Observe, D : isolate K4 Reverse, E : isolate 1 Observe, F : isolate 1 Reverse, G : isolate K5 Observe, H : isolate K5 Reverse, I : isolate 49 Observe, J : isolate 49 Reverse
(Inoculation On 1% CDA-CMC Medium, 48 hour incubation at room temperature, testing with 0.1% congored reagent)

4. Conclusion
From the result of the research, there are 18 potential mold isolates which can produce cellulase enzyme. 3 isolates which have the highest Cellulolytic index are isolate A17, K4, and 1 which have cellulolytic index value of 1.03, 0.32, 0.29. From macroscopic and microscopic identification it can be

assumed that the coded isolates of K4 and A17 belong to the genus *Aspergillus*, while code 1 isolates belong to the genus *Penicillium.*

Acknowledgement

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