Screening and identification of cellulolytic fungi at rhizosphere of safira taro plant

E Wisdawati1,2, T Kuswinanti3, A Rosmana3, A Nasruddin3

1 Agricultural Science, Graduate School, Hasanuddin University, Makassar 90245 Indonesia
2 Plantation Department, Pangkep State Polytechnic of Agriculture, Pangkep, Indonesia
3 Department of Plant Pest and Disease, Faculty of Agriculture, Hasanuddin University, Makassar 90245 Indonesia

E-mail: ekawisdawati@gmail.com

Abstract. Agriculture waste and pathogen cell wall of plant contain a lot of cellulose which are very difficult to degrade. One of the ways to hydrolyse them is through utilizing cellulase, an enzyme produced from secondary metabolite of cellulolytic fungi. This research was aimed at isolating fungi from rhizosphere of safira taro plant and testing the isolate capability to produce cellulase enzyme to be able to act as decomposer and biocontrol of the plant. The research methods are isolating rhizosphere fungi, testing isolate capability in producing cellulase enzyme and identifying potential isolate producing cellulolytic fungi. It obtained 10 fungi isolates, 4 were negative and 6 positive in producing cellulase enzyme with cellulolytic index 1.16 – 1.66. The highest index was RTB14 isolate which belongs to Aspergillus flavus.

1. Introduction

Indonesia as agrarian country produces abundant agriculture waste every year. Most of them are lignocellulose, a main component of plant compiler consisting of cellulase, hemicellulose and lignin [1]. There are 15-40% in the agriculture waste and difficult to be naturally degraded due to the presence of lignocellulose in the secondary cell wall of plant [2, 3] hence the decomposition time takes about 4–5 months [4, 5].

Cellulose is glucose linear polymer with β-1, 4-glicosidic bond [6]. Cellulase hydrolysis into glucose is a complex process, it is carried out through conversion both physically, chemically and biologically. The most studied conversion process is enzymatic biological conversion. Enzyme which can degrade cellulose is cellulase, an enzyme created by cellulolytic microbial, capable of hydrolyzing β-1,4 glycoside bound at cellulose molecules thus produces glucose [7].

Cellulase enzyme that might be produced from various decomposer microbes such as bacteria, fungi, actinomycetes and protozoa takes important role in lignocellulolytic biodegradation process [8, 9]. Fungi are kind of most capable microbes in decomposing organic materials especially cellulose. Several fungi can decompose cellulose in the died plant tissue, for instance changing agriculture waste into simpler compound that is beneficial for another organism. Generally, cellulose can be decomposed by Alternaria, Aspergillus, Fomes, Fusarium, Myrothecium, Penicillium, Polysporus, Rhizopus and Verticillium [8, 10]. Popular fungi producing cellulase enzyme are Trichoderma reseei [11], Aspergillus niger [12,13], Ganoderma lucidum [14], Trichoderma viride [15], Penicillium nalgiovense [16], Fusarium oxysporum [12], Chaetomium and Myrothesium [17].

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Beside of decomposing organic material from plant, cellulolytic fungi act as biocontrol agent in controlling disease caused by fungi as well. They are able to degrade pathogen cell wall of plants since one of their cell walls contains complex cellulose. The cell wall of omycetes mostly consists of 1,3-β-D-glucan, 1,6-β-D-glucan and cellulose [18]. Therefore, fungi which producing cellulase enzyme can be used as biocontrol agent against pathogen fungi. Complete decomposition of cellulose is a process including some enzyme activities like endo-β-1,4-glucanase, exo-β-1,4-glucanase and glucosidase. 

Rhizosphere is a habitat dominated by microorganisms; bacteria, fungi, alga, and protozoa [19]. There are root exudates in the rhizosphere such as glucose, amino acid, vitamins and organic material providing environment condition for microorganism like cellulolytic fungi to grow and multiply [20]. Therefore, this research was purposed to isolate fungi from rhizosphere of Safira taro plant and characterizing isolate capability in producing cellulolytic enzyme as decomposer and biocontrol for plant.

2. Methods

2.1. Isolation of rhizosphere fungi

10 grams of soil sample of rhizosphere were taken then suspended into 100 ml sterile aquades, mixed for 20 minutes. 1 ml of soil suspension was moved into 9 ml sterile aquades (dilution 10⁻¹), this process was repeated until dilution 10⁻⁶. 1 ml of dilution was put respectively into petri dish which contained PDA media. For the next step, they got incubated at room temperature for 3 days. The growing fungal colonies were brought to new PDA media for purifying.

2.2. Capability to Produce Cellulose Enzym

Purified fungi isolate was taken into CMC media for screening to find out the isolate capability in producing cellulase enzyme. Fungi isolates were grown at CMC media agar (2 gr NaNO₃, 1 g K₂HPO₄, 0,5 gr MgSO₄, 0,5 gr KCl, 2 gr CMC, 0,2 gr pepton and 17 gr agar). After growing for 3 days, the media was dripped with Iodin solution (2.0 g KI and 1.0 g dissolved Iodin in 300 ml aquades) and left for 3-5 minutes. It would form clear zone around the colony of isolate that is capable of producing cellulase enzyme after the incubation [22]. Ratio of cellulolytic activity is determined by comparison formula between clear zone and colony diameter [23]. Ratio calculation was performed at 72 hours incubation

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\text{Activity Ratio} = \frac{\text{Diameter of clear zone}}{\text{Diameter of colony}}
\]

2.3. Identification

Isolate producing highest cellulolytic index was identified both macroscopically and microscopically. Identification of morphological character of fungi in macroscopic way is a kind of observation about colony surface color, reverse colony and surface texture. Microscopic fungi identification was started by making preparat from pure culture of rejuvenation results. Preparat was observed at magnification 400 x. It was performed by matching fungi characteristic obtained from observation result through book identification, Watanabe [24].

3. Results and Discussion

Formatting the text Fungi isolate producing cellulose testing was carried out by growing it on CMC media 1%. CMC substrate (Carboxy Methyl Cellulose) is pure cellulose substrate to be applied as cellulase enzyme inductor. CMC is cellulose derivative, dissolved in the water and a kind of substrate to detect cellulose production as it is dedraged fast by microorganism [23]. Fungi isolate producing cellulose was marked by the presence of clear zone around the colony after iodin solution addition (figure 1A). It is seen from table 1 that out of 10 isolates, there are 6 positive isolates, capable of producing cellulase enzyme with cellulolytic index (CI) around 1,16 – 1,66, with categorized medium [25], while 4 other isolates showed negative or produced no cellulase enzyme (figure 1B).
Table 1. Isolate capability testing in producing cellulase enzyme.

| No | Isolates | Producing of Cellulase Enzyme |
|----|----------|-------------------------------|
|    |          | Cl               | Reaction    |
| 1  | RTB5     | 1.45             | Medium      |
| 2  | RTB8     | 0                | No reaction |
| 3  | RTB14    | 1.66             | Medium      |
| 4  | RTB18    | 1.47             | Medium      |
| 5  | RTB19    | 1.2              | Medium      |
| 6  | RTB24    | 0                | No reaction |
| 7  | RTB27    | 0                | No reaction |
| 8  | RTB30    | 0                | No reaction |
| 9  | RTB31    | 1.31             | Medium      |
| 10 | RTB35    | 1.16             | Medium      |

Note: Strong : ≥2, medium : 1 ≥ x ≤2, weak : ≤1, no reaction : 0 [25]

Cellulolytic activity can be detected by the presence of clear zone around the colony as the result from CMC degradation by cellulase enzyme produced by microbes [26]. It indicated that isolate with clear zone is the isolate which has ability to degrade cellulose. Complete cellulose degradation is a process involving several enzyme activities namely endo-β-1,4-glucanase, exo-β-1,4-glucanase, and β-glucosidase. They work in the CMC chain, creating oligosacaride or shorter cellulose chain [27].

Figure 1. A. Isolate with clear zone (positive), B. Isolate without clear zone (negative)

The size of clear zone is an initial indication about the amount of produced cellulase enzyme, the biggest the clear zone the highest cellulase enzyme obtained [28]. Degradation testing through clear zone method is categorized semi quantitative test since found data is a comparison between formed clear zone diameter and colony diameter [9, 29, 30]. Cellulase enzyme is an enzyme which is able to catalyze the hydrolysis of β-1,4-glicosidic bound at cellulose molecule, thus can produce glucose[27].

10 tested isolates demonstrated that RTB14 isolate had the highest cellulolytic index, 1.66. In the morphological identification based on macroscopic and microscopic character showed that RTB14 isolate was Aspergillus flavus (figure 2). On the first day of post inoculation, the isolate had white mycelium. Within 3 days, isolate generated green conidia, dominating the colony surface. Colony was surrounded by white mycelium with pale reverse colony. The observation showed conidiophore had no color and had vesicle. Metule covered almost all the vesicula surface and spreaded to many directions. Conidia is round and has thin wall [31, 32]. Cellulase enzyme can be produced by Aspergillus, Chaetomium Penicillium, Fusarium, Myrothesium and Trichoderma [17]. It is also able to degrade cellulose through changing macromolecule into simple nitrogen form such as ammonium-N thus make easier to be absorbed by root to enhance plant growth [33].
Figure 2. Macroscopic (A) and Microscopic (B) Character of RTB14 isolate.

4. Conclusion
There are 10 rhizosphere fungi and 6 of them are potential isolates producing cellulase enzyme with cellulolytic index 1.16-1.66. The highest value is at RTB14 isolate, and is Aspergillus flavus.

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References
[1] Perez J, Munoz, Rubia T and Martinez J 2002 Int Microbiol 5 53-63
[2] Beguin P and Aubert J P 1992 Cellulaces. In: Encyclopedia of Microbiology I New York: Academic Press
[3] Fengel D and Wegener G 1995 Kayu: Kimia, Ultrastruktur, Reaksi-reaksi, ed Sastrohamidjojo Yogyakarta: UGM Press
[4] Klemm D, Schuman D, Kramer F, Hebler N, Hornung M, Schmauder H P and Marsch S 2006 Adv Polym Sci 205 49-96
[5] Koesnandar H and Nurhayati N 2008 Microbiology Indonesia 2 101-2
[6] Kim K H, Jeong J S, Ham C B, Yang I B, Chung M K and Kim K N 2004 J. Compost Sci 12 242-8
[7] Crueger W and Crueger A 1984 Biotechnology: A Textbook of Industrial Microbiology, ed T D Brock Sunderland : Minuera Associates p. 267-76
[8] Lynd L R, Weimer P J, van Zyl W H, Pretorius I S 2002 Microbiol Mol Biol. Rev 66 506–77
[9] Peculiyte D 2007 Ekologija 53 11–8
[10] Alexander M 1977 Introduction Soil Microbiology USA: John Willey and Sons
[11] Kodri, Dwi B, and Yulianingsih R. 2013 Bioproses Komoditas Tropis 1(1)
[12] Kadarmoidheen M, Saranraj P and Stella D 2012 Intl J Appl Microbil Sci 1 13-23
[13] Julfana R, Anita T and Idiawati N 2013 JKK 2 52-7
[14] Basuni Y 2008 Aktivitas Selulase dari Ganoderma lucidum yang diinkubasikan dalam Media Jerami Padi Bogor: Institut Pertanian Bogor
[15] Tridasma E 2006 Produksi Selo-Oligosakarida dari Fraksi Selulosa Tongkol Jagung oleh Selulase Trichoderma Viride Bogor : Institut Pertanian Bogor
[16] Nugraha R 2006 *Produksi Enzim Selulase oleh Penicillium nalgiovense SS240 pada Substrat Tandan Sawit* Bogor: Institut Pertanian Bogor
[17] Akinyele J B and Olaniyi O O 2013 *Innov Romanian Food Biotechnol* 13 71-9
[18] Bartnick G S 1968 *Annu Rev Microbiol* 22 87–108
[19] Rao S 1995 *Soil Microorganisms and Plant Growth, 3rd ed* New Hampshire: Science Publisher Inc p.12-50
[20] Prashar P, Kapoor N and Sachdeva S 2014 *Rev Environ Sci Biotechnol* 13 63-77
[21] Gams W, Van der Aa H A, Van Der PlaatsNiterink A J, Samson R A, Stalpers J A 1987 *CBS Course of Mycology* Holland: Centraalbureau voor Schimmel Cultures
[22] Kasana R C, Salwan R, Dhar H, Dutt S and Gulati A 2008 *Curr. Microbiol* 57 503-7
[23] Hankin L and Anagnostakis S 1977 *J Gen Microbiol* 98 109–15
[24] Watanabe T 2002 *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species 2nd Edition* Boca Raton: CRC Press
[25] Choi YW, Hodgkiss I J and Hyde K D 2005 *Journal of Agricultural Technology*
[26] Kanti A 2005 *J Biodiversitas* 2 85-9
[27] Meryandini A, Widosari W, Maranatha B, Sumarti T C, Rachmania N and Satria H 2009 *J Makara Sains* 1 33-8
[28] Khokhar I, Haider M S, Mushtaq S and Mukhtar I 2012 *Scholarly Journal of Agricultural Science* 2 126-9
[29] Teather R N and Wood P J 1982 *Applied Environmental Microbiology* 43 777-80
[30] Bradner J R, Gillings M, Nevalainen K M H 1999 *World J. Microbiol. Biotechnol* 15 131–2
[31] Thathana M G, Murage H, Abia A L K, Pillay M 2017 *Agriculture* 7 1-14
[32] Nithiyaa P, Izzati N A, Kalsom U, Salleh 2012 *Trop Agric Sci* 35 103 - 16
[33] Shivanna M B, Meera M S and Hyakumachi M 1994 *Canadian Journal of Microbiology* 40 637-44