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Molecular identification of endophytic fungi isolated from the tuber of *Dahlia variabilis* and exploration of their ability in producing ß-galactosidase

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Abstract. *Saryono, Rakhmana S, Rahayu F, Ardhi A, Rusli, Pratiwi NW, Nugroho TT. 2017. Molecular identification of endophytic fungi isolated from the tuber of* Dahlia variabilis *and exploration of their ability in producing β-galactosidase. Biodiversitas 18: 145-152.* LBKURCC67 and LBKURCC69 were endophytic fungi isolated from the tubers of dahlia (*Dahlia variabilis*) in Padang Panjang, West Sumatra. This study aimed to identify the microscopic and molecular characteristics of endophytic fungi LBKURCC67 and LBKURCC69 based on ITS-rDNA region sequences and determine the ability of the isolates to produce *β*-galactosidase. Microscopic characteristic of LBKURCC67 and LBKURCC69 indicated that both isolates belonged to *Fusarium* sp. Furthermore, DNA sequence analysis based on mega BLAST program showed that LBKURCC67 was identified to be *Fusarium solani* with 99% homology and LBKURCC69 showed the perfect homology similarity with *Fusarium oxysporum*. Meanwhile, *β*-galactosidase activity was determined based on the amount of *o*-nitrophenol produced from the substrate *o*-nitrophenil-*β*-D-galactopiranoside, whilst the specific activity was indicated by activity per unit of proteins. The result showed that both fungi could produce *β*-galactosidase. However, the enzyme activity did not increase significantly at various production times of 2, 4, 6, 8, and 10 days. The highest enzyme activity of LBKURCC67 and LBKURCC69 were 0.163 ± 0.064 U/mL (at day 8) and 0.126 ± 0.039 U/mL (at day 2) respectively. LBKURCC69 showed higher specific *β*-galactosidase activity (1.886 ± 0.277 U/mg) than LBKURCC67 (1.179 ± 0.081 U/mg).

Keywords: ß-galactosidase, dahlia tuber, endophytic fungi, *Fusarium*, ITS-rDNA

INTRODUCTION

Microorganisms are important and potential sources of bioactive natural products with for the discovery of new molecules for drug discovery, industrial use and agricultural applications (Strobel 2006; Porras-Alfaro and Bayman 2011). Many plants contain endophytic organisms defined as fungi or bacteria which, for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues but cause no symptoms of disease (Wilson 1995). In previous reports, endophytic fungi are known to produce antibacterial substances (Saryono et al. 2015a) and have been shown to improve the tolerance of host plants to a variety of biotic and abiotic stresses (Malinowski and Belesky 2000; Rodriguez et al. 2004). It has been observed that the wealth of microbial biodiversity with novel biochemistry and secondary metabolite production resides in plant tissues (Strobel 2006; Porras-Alfaro and Bayman 2011; Saryono et al. 2015a). Numerous bioactive molecules have been obtained from endophytic fungi since this ground breaking discovery (Strobel 2006; Wang et al. 2008; Zhang et al. 2006).

Dahlia tubers are known to contain compounds which have high bioactivity. In some regions in Sumatra with the mountainous topography, for instance, in the province of West Sumatra, such as in Solok, Padang Panjang, Payakumbuh, and Batu Sangkar; Brastagi (North Sumatra), Curup (Bengkulu), and Lubuk Linggau (South Sumatra) dahlia can naturally grow. While in Kalimantan, dahlia are relatively difficult to find due to environmental factors affecting the growth of dahlia. The leaves, stems, and tubers of dahlia contain a number of potential bioactive compounds that can be functioned as anti-microbial compounds (Lorenita et al. 2013; Sikumbang and Hindersah 2009).

However, massive production of antimicrobial compounds from dahlia requires a very large number of dahlia plants, and it takes large area and long growing-type plants as well. On the other hand, plants generally contain endophytic microbes that can produce bioactive compounds as what can be found in its host plant (Strobel 2006). Some microbes such as fungi, yeast, and bacteria can be associated with the plant because they help the host plant metabolism and produce potential secondary metabolites (Kumala et al. 2006). Some studies also showed that the endophytes provided benefits for the host plant, such as protection against insects, pathogens, and

herbivores (Ding et al. 2010). Endophytes are metabolically more active than their free counterparts due to their specific functions in nature and activation of various metabolic pathways to survive in the host tissues (Strobel 2006; Ul-Hassan et al. 2012).

β-Galactosidase (EC 3.2.1.23) is a class of enzymes that can hydrolyze *β*-galactoside bond of various compounds and typically used to hydrolyze lactose into glucose and galactose. Enzymatic hydrolysis of lactose is a very important process in the food industry, such as hydrolyzing lactose from milk and its derivative products (Kumar et al. 2012). This enzyme has been isolated from animals, plants, and microorganisms. However, *β*-galactosidase enzyme derived from microorganisms will generate the enzyme more potential and more effective in the aspect of technology than the enzyme from animals and plants (Grosova et al. 2008). Commercially, *β*-galactosidase is produced from microorganisms including yeast from the genus *Kluyveromyces* and fungal filaments from genus *Aspergillus* (Choonia and Lele 2013). Until now, the price of *β*-galactosidase is still relatively expensive and economically problematic (Grosova et al. 2008).

In the previous study, endophytic fungi LBKURCC67 and LBKURCC69 had been isolated from the tuber of yellow-flowered and red-flowered dahlia, respectively, from Padang Panjang, West Sumatra (Lorenita et al. 2013). In this study, morphological characterization was performed for initial identification of species. Furthermore, the results of this characterization needed to be verified by genetic characterization which is known to be relatively more consistent than morphological characters that can be used for identification to species level. A proper identification will be carried out based on ribosomal DNA sequences of the ITS region equipped with a phylogenetic analysis of the fungi. Afterward, initial assessment of potential *β*-galactosidase production by endophytic fungi LBKURCC67 and LBKURCC69 will contribute a novel alternative source for commercial *β*-galactosidase production. Overall, this result will have a great benefit for further research in order to find novel bioactives from the endophytic fungi and its optimum condition for production as well.

MATERIALS AND METHODS

Maintenance of endophytic fungi strains and morphological characterization

Fungi LBKURCC67 and LBKURCC69 used in this study were endophytic fungi that had been isolated from the tuber of yellow-flowered and red-flowered dahlia (*Dahlia variabilis* (Willd.) Desf.), respectively, from Padang Panjang, West Sumatra, Indonesia. Both isolates were collected in the Laboratory of Enzymes, Fermentation, and Biomolecular Research, Department of Chemistry, Universitas Riau, Pekanbaru, Riau, Indonesia. The fungi were subcultured into Potato Dextrose Agar (PDA). LBKURCC67 inoculation was conducted using a spread plate, which isolate on slant agar was rinsed with 3 mL sterile distilled water. Fungi suspension was taken as much as 100 mL and then inoculated in petridishes containing PDA. The isolates were incubated for five days to undertake morphological characterization. On the other hand, the isolate was incubated until the mycelia grew but with no spores to continue to step of DNA extraction.

Genomic DNA extraction

DNA was extracted from cultures of endophytic fungi mycelia using the Wizard Genomic DNA Purification kits ex Promega Corp. (Madison, USA). Mycelia was scraped off and put into a micro tube, and added 293 µL 50 mM EDTA (pH 8) and lyticase. Furthermore, the mycelia was incubated for 60 min at 37°C and centrifuged at 13.000 g for 2 minutes. The 300 µL nucleus lysis solution and 100 µL protein precipitation solution were added into the sediment. Samples were centrifuged at 13.000 g for 3 minutes and the supernatant containing DNA was transferred into a microtube containing 300 µL isopropanol. Samples were homogenized by centrifugation at 13.000 g for 5 min, the supernatant then was discarded. After the DNA pellet was dry, 300 µL of 70% ethanol was added. The addition of 25 µL DNA rehydrating solution and 25 µL RNAse solution was conducted and followed with incubation. The DNA isolated was stored at 2-8°C. The integrity of the DNA was verified by agarose gel electrophoresis and stained with ethidium bromide.

PCR amplification

PCR amplification in ITS rDNA region of isolates was conducted using primers ITS5 and ITS4 in accordance with the position map designed by White et al. (1990). Amplification was carried out with a total volume of 50 mL consisting of 2 mL of DNA sample, 5 mL of dNTP, 10 mL of Colourless GoTaq PCR buffer, 10 mL of each primer, 3 mL of $MgCl_2$, 0.25 mL GoTaq DNA polymerase, and the rest of sterile distilled water. The reaction began with a hot start at a temperature of 95°C for 5 minutes, denaturation at a temperature of 94°C for 1.5 minutes, annealing at a temperature of 41°C (LBKURCC67) or 45°C (LBKURCC69) for 1 min, and extension at a temperature of 72°C for 3 minutes. PCR process lasted a total of 35 cycles and ending with the extension at 72°C for 5 minutes.

Electrophoreses

The result of DNA extraction and PCR were analyzed by electrophoresis on a 0.8% agarose gel for DNA extraction, and 1.2% agarose gel for PCR amplification results. The process of gel electrophoresis was immersed in a solution of Tris Acetate EDTA (TAE) 1x and electrified at 110 V. After electrophoresis was completed, the gel was soaked with ethidium bromide and analyzed by UV-rays transilluminator. The weight of molecular fragment was determined using DNA Ladder 1 Kb (Promega Corp., Madison, USA).

DNA sequencing of PCR amplification products

PCR amplification products of endophytic fungi LBKURCC67 was sent to Eijkman Institute for Molecular Biology, Jakarta (GenNeka Foundation) for DNA sequence determination. Determination of DNA sequences was conducted in both directions of the double chain of DNA

PCR products by using primers ITS2, ITS3, ITS4, and ITS5 (Table 1) using Bioedit program (White et al. 1990). The results were then analyzed their sequence homology using mega BLAST program that could be accessed at the NCBI web site (http://blast.ncbi.nlm.nih.gov/blast.cgi). Sequence homology analysis was carried out by comparing the sample sequences of isolates of endophytic fungi LBKURCC67 and LBKURCC69 in the region of ITS rDNA sequences were aligned with that of the NCBI GenBank Database.

Production and measurement of *β*-galactosidase

Fermentation was carried out in 250 mL flasks using 50 mL of fermentation medium (1% peptone, 0.5% yeast extract and 0.01% chloramphenicol), sterilized at 121ºC for 15 min. The flasks were inoculated with the pre-inoculum to give an initial cell count 10^7. Fungal inoculum which has reached OD600nm~0.1 is equivalent to 10^7 CFU/mL (Martins et al. 2011). Production of *β*-galactosidase was undertaken by inoculating 5% inoculum (OD 600nm~0.1) in 150 mL fermentation medium in a rotary shaker incubator with agitation speed of 150 rpm. The time variations of production used were 2, 4, 6, 8, and 10 days. On each day of production time variation, crude extract enzyme was isolated. *β*-galactosidase contained in media production from cell isolates was separated by cold centrifugation with a speed of 10.000 rpm for 10 minutes. Supernatant was obtained through filtration using Whatman GF/C in vacuum to remove the mycelium. If the enzyme was not directly used for analysis of enzyme activity, 1mM NaN_3 was added into each supernatant solution. Activity of crude extract *β*-galactosidase was analyzed using *o*-nitrophenyl-*β*-D-galactopiranoside (*o*NPG) substrate concentration of 4 mg/mL at 60°C at pH 4,4. Measurement of *β*-galactosidase activity was according to Moeini et al. (2004) with some modifications, the OD 600 nm was recorded and then 1 mL of fungal culture was spun out. A total of 1.000 mL of 0.1 M acetate buffer pH 4.4 and 100 mL of the enzyme were inserted into a test tube and then incubated at 60°C for 5 minutes. The solution was added with 200 mL of (*o*NPG) as an enzyme substrate. Furthermore, the mixture was incubated at 60°C for 30 minutes and at the last minute was added with 1.000 mL of 1 M Na_2CO_3. After that, the solution was analyzed using UV-Vis spectrophotometer at a wavelength of 420 nm. The enzyme activity was defined as the number of *o*-nitrophenol (*o*NP) formed in experimental conditions. The standard curve was prepared by making the stock of *o*NP at various concentrations of 0.01 to 0.15 mg/mL with the use of 0.01 M acetate buffer pH 4.4. The cells were spun out, OD420 nm of supernatant was read and Miller units were calculated (activity = micromole *o*NP/volume of enzyme x incubation time). Protein determination followed Lowry method.

RESULTS AND DISCUSSION

Results

Both isolates showed different apparent morphological characteristics, mainly in the color and the form of the culture medium. Nelson et al. (1983) performed a morphological characterization using the coloration on PDA as indicating that each species has a specific color. Based on the color obtained, a preliminary classification was conducted as shown in Figures 1 and 2.

Table 1. The primers used to amplify rDNA of endophytic fungi (White et al. 1990)

rDNA	Primers sequence	Molecular weight of PCR product (bp)	Melting point (°C)
ITS1	5’-TCCGTAGGTGAACCTGCGG-3’	290	65
ITS2	5’-GCTGCGTTCTTCATCGATGC-3’	290	62
ITS3	5’-GCATCGATGAAGAACGCAGC-3’	330	62
ITS4	5’-TCCTCCGCTTATTGATATGC-3’	-	58
ITS5	5’-GGAAGTAAAAGTCGTAACAAGG-3’	315	63

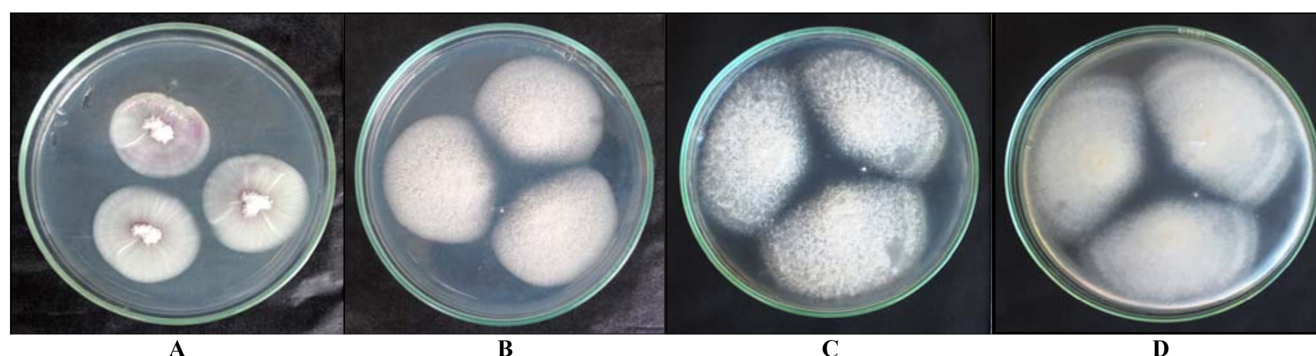

Figure 1. Colonial morphology of endophytic fungi isolates used in this research; A. LBKURCC67, B. Reverse view of LBKURCC67, C. LBKURCC 69, D. Reverse view of LBKURCC69

Figure 2. Microscopic appearance of endophytic fungi LBKURCC67 (A) and LBKURCC69 (B) with 100x magnification

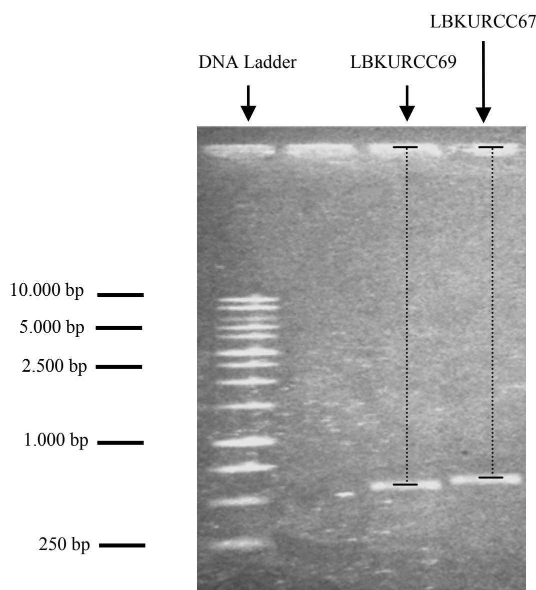

Figure 3. Electrophoreses product of amplificated DNA of LBKURCC67 and LBKURCC69 at annealing temperature of 41°C using primers ITS5 and ITS4

Mycelial growth rate was assessed by transferring 6-mm-diameter PDA medium discs derived from 7-day-old colonies into other PDA medium plates, and assessing colony growth daily until the day 7 of incubation by averaging the colony's diameter in diametrically opposite directions. Conidial length and width were assessed from a sample from a 2-mL fungal conidia suspension in sterile distilled water, which was pipetted onto a microscope slide. Conidia were measured using a light microscope with a micrometer at 100x magnification. Morphological characterization of endophytic fungi LBKURCC67 and LBKURCC69 was shown in Table 2.

DNA isolates of endophytic fungi LBKURCC67 and LBKURCC69 were isolated from mycelium with the age of 4 days. If older than 4 days, DNA isolation could not be performed because the fungi had already produced spores. Spores produced led lyticase to unlikely break down the cell walls of fungi that would affect on the success in obtaining DNA isolates. Condition for PCR annealing temperature was set 41°C and 45°C for LBKURCC67 and LBKURCC69 respectively. The result of electrophoreses analysis of PCR products indicated a single band of each amplificated DNA (Figure 3).

Basically, DNA was arranged by four nitrogen bases; thymine (T), adenine (A), guanine (G), and cytosine (C), which generated the specificity and uniqueness of each DNA. DNA protein sequence alignment result in the region of ITS-1 and ITS-2 rDNA using Bioedit program was shown in Figure 4.

Species identification was carried out by comparing DNA sequences obtained to the DNA sequences contained in NCBI GenBank Database (Table 3 and Table 4).

ITS-1 rDNA region was verified by aligning the DNA sequences obtained using primers ITS-5 and ITS 2 reverse, while ITS-2 rDNA region used primers ITS-4 and ITS-3 reverse. DNA sequence verification result in the region of ITS-1 rDNA was then aligned with the reverse of DNA sequence in the region of ITS-2 rDNA in order to obtain complete DNA sequences of LBKURCC67 and LBKURCC69.DNA sequencing of LBKURCC67 in the region of ITS which was matched to the entire database in NCBI GenBank Database showed that the DNA sequences of LBKURCC67 had the highest similarities with *Fusarium solani* while LBKURCC69 indicated the highest similarities with *Fusarium oxysporum* (Table 4).

This research also carried out the exploration of benefits which could be taken when utilizing these endophytic fungi, especially in β-galactosidase-producing ability. This enzyme is an important part in food industries, ruling to hydrolyze lactose into glucose and galactose. The β-galactosidase activity produced by *F. solani* LBKURCC67 and *F. oxysporum* LBKURCC69 can be seen in Table 5.

The highest activity of β-galactosidase was specifically obtained at day 8 for *F. solani* LBKURCC67 and day 2 for *F. oxysporum* LBKURCC69 and these production times of each isolate were used as a basis time to determine specific enzyme activity (Table 6).

Table 2. Morphological characterization of LBKURCC67 and LBKURCC69

Isolates	Color		Macroconidia				Chlamidio-spore	Morphological identification
	Anverse	Reverse	Apical	Basal	Size (µm)	#septa		
LBKURCC67	White-violet	White-violet	Papillate	Barely notched	35-55	4-5	No	*Fusarium* sp.
LBKURCC69	White	White-violet	Blunt	Barely notched	40-60	3-4	No	*Fusarium* sp.

DNA sequence of LBKURCC67 (5'→3')

T**GGAAGTAAAAGTCGTAACAAGG**TCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTCTAAACAACTCATCAACCCTGTG
AACATACCTAAAACGTTGCTTCGGCGGGAACAGACGGCCCCGTGAAACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTGCTA
TATGTATCTTCTGAGTAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCTG**GCATCGATGAAGAACGCAGC**GAA
ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATG
CCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCCGGGCCTGGCGTTGGGGATCGGCGAGGCGCCCCCCTGCGGGCACGCGCCGTCC
CCCAAATACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAGCTAACACCTCGCAACTGGAGAGCGGCGCGGCCATGCCGTAAA
ACACCCAACTTCTGAATGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAA**GCATATCAATAAGCGGAGGA**A

DNA sequence of LBKURCC69 (5'→3')

T**GGAAGTAAAAGTCGTAACAAGG**TCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGA
ACATACCAATTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATAT
GTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTG**GCATCGATGAAGAACGCAGC**AAAATG
CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCT
GTTCGAGCGTCATTTCAACCCTCAAGCCCAGCTTGGTGTTGGGACTCGCGAGTCAAATCGCGTTCCCCAAATTGATTGGCGGTCACG
TCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACC
TCGGATCAGGTAGGAATACCCGCTGAACTTAA**GCATATCAATAAGCGGAGGA**A

Notes: **GGAAGTAAAAGTCGTAACAAGG** = primer ITS5
GCATATCAATAAGCGGAGGA = primer ITS4 r*everse*
GCATCGATGAAGAACGGAGC = primer ITS3 = primer ITS2 reverse

Figure 4. Alignments of DNA sequence of LBKURCC67 and LBKURCC69 verificated with pairwise alignment using Bioedit program

Table 3. The strains on NCBI GenBank Database having close kinship to LBKURCC67

Species	Strain	Genbank access code	Reference	*Score*	Query cover	Max identity
Fusarium solani	MAFF 240346	AB498984.1	Nakayama and Aoki (2010)	1046	96%	99%
Fusarium solani	MAFF 240345	AB498983.1	Nakayama and Aoki (2010)	1046	96%	99%
Fusarium solani	Clone BK2-1	JN882256.1	Gutierrez-Acosta et al. (2012)	1031	99%	98%
Fusarium solani	Clone BK1-1	JN882255.1	Gutierrez-Acosta et al. (2012)	1031	99%	98%
Fusarium solani	UOA/HCPF	KC254048.1	-	1029	99%	98%
Nectria ipomoeae	MAFF 238974	AB513849.1	Nakayama and Aoki (2010)	1027	96%	99%
Nectria ipomoeae	MAFF 237668	AB513848.1	Nakayama and Aoki (2010)	1027	96%	99%
Nectria ipomoeae	MAFF 237667	AB513847.1	Nakayama and Aoki (2010)	1027	96%	99%
Nectria ipomoeae	MAFF 240022	AB513846.1	Nakayama and Aoki (2010)	1027	96%	99%

Table 4. The strains on NCBI GenBank Database having close kinship to LBKURCC69

Species	Strain	Genbank Access Code	Reference	*Score*	Query cover	Max identity
Fusarium oxysporum	LCF32	FJ867936.1	-	1055	99%	100%
Fusarium sp.	PRE4b	JN254793.1	-	1053	99%	100%
Fusarium sp.	4 BRO-2013	KF367548.1	Oliveira et al. (2013)	1050	99%	99%
Fusarium verticillioides	LCF1	FJ867932.1	-	1050	99%	99%
Fusarium sp.	3 TMS-2011	HQ630965.1	Shrestha et al. (2011)	1050	99%	99%
Fusarium proliferatum	BLE1	FN868470.1	Botella and Diez (2011)	1048	99%	99%
Fusarium proliferatum	-	EF577235.1	-	1048	99%	99%
Gibberella moniliformis	-	AY533376.1	Dornbusch et al. (2005)	1048	99%	99%
Fusarium andiyazi	CBS 134430	KC954400.1	-	1044	99%	99%

Table 5. *β*-galactosidase activity of *F. solani* LBKURCC67 and *F. oxysporum* LBKURCC69 in a varied production times

Production time (days)	Activity of *β*-galacosidase (Unit/mL)	
	F. solani LBKURCC67	*F. oxysporum* LBKURCC69
2	0.132 ± 0.037[a]	0.126 ± 0.039[a]
4	0.147 ± 0.059[a]	0.118 ± 0.032[a]
6	0.143 ± 0.069[a]	0.121 ± 0.034[a]
8	0.163 ± 0.064[a]	0.120 ± 0.041[a]
10	0.145 ± 0.057[a]	0.110 ± 0.029[a]

Note: Statistical analysis at 95% confidence level with same letters indicating no significant difference

Table 6. Specific enzyme activity of *β*-galactosidase produced by *F. solani* LBKURCC67 and *F. oxysporum* LBKURCC69

Isolate	Specific enzyme activity (unit/mg protein)
LBKURCC 67	1.179 ± 0.081[a]
LBKURCC 69	1.886 ± 0.277[b]

Statistical analysis at 95% confidence level with same letters indicating no significant difference

Discussion

LBKURCC67 and LBKURCC69 were endophytic fungi isolated from dahlia tuber. Both isolates were indicated to have different morphological features but both exhibited almost the same color with white as the dominant color and light violet in reverse view of the colonies. The isolate of LBKURCC67 had a thick colony, approximately 25 mm in diameter, and a white peak in the center of the colony, while the isolate of LBKURCC69 had a wider colony with approximately 50 mm in diameter. LBKURCC67 produced papillate and barely notched-type of apical and basal macroconidia with 4-5 septa, whilst LBKURCC69 produced blunt and barely notched-type of apical and basal macroconidia with 3-4 septa (Table 2). From these characteristics, both isolates could be identified as *Fusarium* sp. González-Pérez et al. (2009) reported that species causing rot in Mexico, including *Fusarium*, differed in terms of colony color and morphological characteristics, agreeing with the results obtained in this work for descriptions of macroconidia and chlamydospores. At the other parts, Hafizi et al. (2013) described morphological characteristics of *F. solani* and *F. oxysporum*, and supported with Montiel-Gonzalez et al. (2005) which reported about morphological characteristics of *Fusarium* species such as *F. oxysporum*, *F. solani*, *F. lateritium*, *F. reticulatum*, *F. equiseti*, *F. verticillioides*, *F. culmorum*, *F. crookwellense*, *F. proliferatum*, and *F. sporotrichioides* presented in bean roots in five states of central Mexico. The descriptions made for the species identified in this study also agreed with those reported by these authors. Microscopically, *F. oxysporum* produced three forms of asexual spores, which were microconidia, macroconidia, and chlamydospores (Kumar et al. 2013). *F. oxysporum* also generated floccose mycelia with purple in color. This fungus had round or oval microconidia, a short form of monophialide, and slightly curved or crescent-shaped macroconidia with septa (Zakaria and Ning 2013).

Molecular identification of these endophytic fungi was conducted by sequencing DNA in the region of ITS-1 and ITS-2 rDNA. DNA of each species contained genetic information that could be inherited to the offspring, so that by sequencing DNA isolates of endophytic fungi LBKURCC67 and LBKURCC69, proper species identification results would be obtained. DNA isolates of LBKURCC69 and LBKURCC67 were amplified in accordance with the primer position map stated by White et al. (1990), that the region of ITS-1 and ITS-2 use ITS-5 and ITS-4 as pair primers. Among the methods which researchers had used to analyze the phylogenetics of *Fusarium* species are rDNA-IGS, rDNA-ITS regions, large submit RNA gene, and translation elongation factor-alpha (Zhang et al. 2006). Internal transcribed spacer (ITS) region was probably the most widely sequenced region of DNA in fungi. rDNA-ITS and rDNA-IGS (intergenic spacer) regions showed a higher degree of diversity than other ribosomal regions (Brasileiro et al. 2004). Molecular weight of the PCR amplification products was determined using 1 kb DNA ladder Promega standard, by measuring the migration distance reached by standard DNA and DNA amplification products. The relationship between DNA migration distance and the logarithm of standard base pairs of DNA produced the standard regression equation y =- 0.36x + 4,782. According to this equation, molecular weight obtained of PCR amplification product of LBKURCC67 and LBKURCC69 were 583 and 537 bp, or equivalent to 3,8 x 10^5 and 3,5 x 10^5 g/mol respectively (1 bp DNA ≈ 660 g / mol).

DNA amplification product fragments were sequenced in the region of ITS-1 and ITS-2 rDNA using primers ITS-2, ITS-3, ITS-4, and ITS-5. ITS primer consisted of the region of ITS-1 and ITS-2 rDNA, which ITS-1 was located between 18S and 5,8S while ITS-2 was between 5,8S and 28S. ITS region was a part of the DNA with a high level of evolution which led to a change in the nucleotide sequences of the DNA of fungi so that the region of ITS could be used to differentiate between one species and another. Fungi with a high degree of homology that had similarities at the nucleotide base sequences were most likely the same species or have a close kinship (White et al. 1990). Furthermore, verification process was completed using DNA sequencing Bioedit program to generate complete DNA sequences of endophytic fungi LBKURCC67 and LBKURCC69 (Figure 4). DNA sequencing of LBKURCC67 in the region of ITS matched to the entire database in NCBI GenBank Database showed that the DNA sequences of LBKURCC67 had similarities with *Fusarium solani* and *Nectria ipomoeae*. Degree of homology similarity of samples to the DNA sequences in the NCBI Genbank Database referred to as a maximum identity, in which the highest value of 99% was found in *F. solani* strain MAFF 240346 and MAFF 240345, *N. ipomoeae* strain MAFF 238974, MAFF 237668, MAFF 237667, and MAFF 240022. *F. solani* strain Clone BK2-1, Clone BK1-1, and UOA/HCPF had coverage values query as high as 99%, with the identity of its maximum value was

98% so that these strains showed the lower similarity of homology. However, *F. solani* strain MAFF 240346 and MAFF 240345 produced the highest score 1046 indicating that isolate of LBKURCC67 on ITS region had the highest degree of similarity with species of *F. solani*. DNA sequence analysis showed that LBKURCC69 had reached 99% similarity with *Fusarium* sp., *F. verticillioides*, *F. proliferatum*, *Gibberella moniliformis*, and *F. andiyazi*. However, LBKURCC69 had the perfect homology similarity, reaching 100%, with *F. oxysporum* indicating that LBKURCC69 was *F. oxysporum*.

Genus of *Fusarium* was commonly known as pathogens that caused various diseases in plants. Nevertheless, there were several strains of *Fusarium*, such as *F. oxysporum* isolated from soil which was non-pathogenic and utilized as a biocontrol agent for treating diseases caused by fungi from the genus of *Fusarium* itself (Moretti 2009). The existence of the genus of *Fusarium* was not always associated with losses. For instance, infection of *F. cylindriscorpum* and *F. oxysporum* on agarwood trees (*Aquilaria malaccensis*) led the tree to scent widely and generated a high economic value (Mega and Phabiola 2010). Saryono et al. (2015a) managed to isolate some inulinase-producing fungi that grow in the rhizosphere of dahlia tubers that had rotted. One of the fungi was of the genus *Fusarium* which was likely to be endophytic fungi. Another research conducted by Saryono et al. (2015b) stated that some endophytic fungi isolated from the rhizosphere of dahlia showed ability in producing inulinase. In line with that, this research explored the potency of *F. solani* LBKURCC67 and *F. oxysporum* LBKURCC69 to produce another important enzyme namely *β*-galactosidase. *β*-galactosidase was obtained from microorganisms such as fungi, bacteria and yeasts; plants, animals cells, and from recombinant sources. The enzyme had two main applications; the removal of lactose from milk products for lactose intolerant people and the production of galactosylated products (Husain 2010).

Production of *β*-galactosidase enzyme by both fungi was carried out by varying fermentation period into 2, 4, 6, 8, and 10 days. Based on Table 5, there was no significant difference among all fermentation periods on both isolates and optimal production time could not be determined with variations of up to 10 days of production time. The enzyme activity obtained in this study was much lower compared to the activity of the enzyme *β*-galactosidase derived from *Aspergillus niger* and *Kluyveromyces lactis* (Oliveira et al. 2011). Meanwhile, the specific activity of the enzyme could be used as a measurement of the purity of the isolated enzyme. The higher the specific activity of the enzyme, the higher the purity levels of the enzyme. This was due to non-enzyme protein loss at some stages of separation passed in the enzyme purification. From the result, the specific activity of *β*-galactosidase produced by *F. oxysporum* LBKURCC69 was higher than *F. solani* LBKURCC67.

To summarize, microscopic characteristic of LBKURCC67 and LBKURCC69 indicated that both isolates belonged to the genus of *Fusarium* sp. Furthermore, molecular identification according to NCBI GenBank affirmed the previous identification which LBKURCC67 was identified to be *Fusarium solani* with 99% homology similarity and LBKURCC69 showed perfect homology similarity (100%) with *Fusarium oxysporum*. On the other hand, both fungi were able to produce *β*-galactosidase, however, the activity did not change significantly at various production times of 2, 4, 6, 8, and 10 days. LBKURCC69 *β*-galactosidase showed higher specific activity (1.886 ± 0.277 U/mg) than LBKURCC67 (1.179 ± 0.081 U/mg). This initial report could be new information for the next study to explore more about the potency of endophytic fungi isolated from dahlia tubers as *β*-galactosidase producers and to determine the optimum fermentation condition in order to obtain the highest yield of *β*-galactosidase as well.

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