Bioproduction of cytochalasin D by endophytic fungus *Xylaria* sp. DAP KRI-5

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ABSTRACT

Recently, endophytic fungi have attracted attention due to their ability to produce a variety of bioactive metabolites. In the course of our study on endophytic fungi associated with *Albertisia papuana* Becc, it has been found the endophytic fungus *Xylaria* sp. DAP KRI-5 produce cytochalasin D. Isolation of cytochalasin D was conducted by using an open column chromatography method. The chemical structure of cytochalasin D was deduced from 1 dimension, 2 dimension nuclear magnetic resonance, and ultra-performance liquid chromatography quadrupole time of flight mass spectrometry analyses and also compared with the published data. The bioproduction capacity of cytochalasin D by the fungus *Xylaria* sp. DAP KRI-5 was 0.05763 g/l.

INTRODUCTION

Endophytic fungi are microorganisms that live inside plant tissues without causing negative effects to its host plant (Petrini, 1991). Endophytic fungi can produce diverse bioactive secondary metabolites (Zhang *et al.*, 2006). Various bioactive compounds produced by endophytic fungi have many kinds of biological activity such as anticancer, antibiotic, antifungal, and antidyslipidemic. Some of those bioactive substances are taxol, an anticancer compound produced by the fungus *Taxomyces andreanae* (Stierle *et al.*, 1993), (+)-1,1′-bislunatin and (+)-2,2′-epicytoskirin A, antibacterial, and anticancer compounds produced by the fungus *Diaporthe* sp. GNBP-10 (Agusta *et al.*, 2006; Praptiwi *et al.*, 2013), micafungin, an antifungal compound from *Coleophoma empetri* (Frattarelli *et al.*, 2004), rosuvastatin from *Penicillium citrinum* and *P. brevicompactum*, which is used for treating dyslipidemias (Scott *et al.*, 2004).

In previous work, screening of antibacterial activity of 15 endophytic fungi from leaves and young stems of a medicinal plant, *Albertisia papuana* Becc.: Menispermaceae, resulted in one selected fungus, *Xylaria* sp. DAP-KRI-5. This fungus produced some bioactive metabolites with strong antibacterial activity against *Staphylococcus aureus*. The lead compounds as the bioactive metabolites from this fungus have been characterized as phloroglucinol (Fathoni *et al.*, 2013), and cytochalasin D (unpublished data). Isolation, characterization, and bioproduction of cytochalasin D from *Xylaria* DAP KRI-5 have not yet been studied.

Cytochalasin D was first isolated by Aldridge *et al.* (1967) and was also known as zigosporin A (Hayakawa *et al.*, 1968). The bioproduction capacity of cytochalasin D has been reported from *Xylaria* sp. SCSIO 156 in liquid medium (2% mannitol, 2% malt extract, 1% glucose, 1% sodium glutamate, 0.05% KH_2PO_4, 0.03% $MgSO_4 \cdot 7H_2O$, 0.1% corn meal, 0.3% yeast extract, and 3% sea salt in distilled water and pH 6.5), *Xylaria* sp. BT05 in a medium of 1,000 g/l of rice in seawater and *Xylaria arbuscula* in a medium of 1,333 g/l of wheat in distilled water (Amarala *et al.*, 2014; Chen *et al.*, 2011; De Felício *et al.*, 2015).

To the best of our knowledge, there are no reports on the bioproduction capacity and antibacterial activity of cytochalasin D that have been produced by *Xylaria sp.* DAP KRI-5 in a

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medium of potato dextrose broth. In this study, we evaluated the bioproductive capacity and antibacterial activity of cytochalasin D from *Xylaria* sp. DAP KRI-5 associated with the *A. papuana* Becc plant.

MATERIALS AND METHODS

Fungal growth

Endophytic fungus DAP-KRI-5 was isolated from *A. papuana* Becc. plant collected from the Bogor Botanical Garden, West Java. This fungus was identified as *Xylaria* sp. in a previous study (Fathoni *et al.*, 2013) and were preserved at −80°C in the culture collection of InaCC, Indonesia. This fungus was cultured in 2.7 l (12 × 200 ml in a 500-ml culture flask and 1 × 300 ml in a 1,000-ml culture flask) of potato dextrose broth (PDB) at 25°C for 14 days in the dark. The fungal culture was harvested and extracted three times with ethyl acetate (EtOAc). The extracts were filtered and concentrated using a rotary evaporator.

Extraction and isolation of cytochalasin D

Isolation of cytochalasin D from an EtOAc extract of *Xylaria* sp. DAP KRI-5 was conducted by using column chromatography. The EtOAc extract was applied on Sephadex™ LH-20 (GE Healthcare) (200 g) and eluted with methanol. The dominant fraction was then fractionated by using a SiO_2 gel 60 (Merck) (70-230 mesh ASTM, 100 × weigh of samples), using n-hexane-EtOAc eluent in the ranges of 3:1 to 1:3, followed by $CHCl_3$-MeOH in the ranges of 5:1 to 1:7. The biggest weight fraction F2.3 (113.0 mg) was further applied on a SiO_2 gel 60 (Merck) (70-230 mesh ASTM, 100 × weigh of samples eluted with n-hexane-EtOAc in ranges of 6:1 to 0.5:6) resulting a pure compound (**1**), which was then analyzed for its chemical structure by high-resolution mass spectroscopy (HRMS), ^{1}H-, and ^{13}C-nuclear magnetic resonance (^{13}C-NMR), and antibacterial activity by microdilution methods.

Chemical structure analysis

High-resolution mass spectroscopy

Analysis of HRMS of **1** was performed using an ultra-performance liquid chromatography quadrupole time of flight mass spectrometer (UPLC–qTOF-MS/MS) System (Waters). The experimental conditions were column Acquity UPLC BEH (C18 1.7 μm, 2.1 × 50 mm); flow rate of 0.3 ml/minute; Temperature 40°C, mobile phase using a linear gradient of 5% ACN in milliQ water going to 100% ACN for 7 minutes. Both solvents were buffered with 1% formic acid, at an injection volume of 5 μl. The analyte infusion experiments were performed using an in-built syringe pump. A UPLC–qTOF-MS with an electrospray ionization (ESI) interface was used for MS/MS analysis. ESI parameters were the capillary voltage of 3 kV for the positive mode; a source temperature of 110°C; a desolvation temperature of 300°C; a cone gas flow of 16 l/hour and a desolvation gas flow of 550 l/h.

^{1}H- and ^{13}C-NMR

^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a JEOL JNM-Lambda 500 spectrometer at 500 and 125 MHz, respectively. Chemical shift was expressed in δ values relative to an internal standard of trimethyl silyl.

Antibacterial assays

The tested bacteria were cultured in Mueller Hinton Broth (Difco) in an incubator at 37°C overnight. The compound was tested against a panel of two Gram-positive bacteria (*Staphylococcus aureus*-InaCC B4 and *Bacillus subtilis*-InaCC B1) and two Gram-negative bacteria (*Escherichia coli*-InaCC B5 and *Pseudomonas aeruginosa*-InaCC B3). The inoculum was prepared as described previously. The cytochalasin D and the commercial antibiotic were dissolved in 2.5% sterile filtered dimethyl sulfoxide. Bacteria were cultured in the same manner and the compound was serially diluted two-fold across the wells of 96-well plates (Nunc™) with concentrations ranging from 0.25 to 32 μg/ml (final concentration), and plated in triplicate (*n* = 3). The resultant mid-log phase cultures were diluted to a concentration of 5.10^5 CFU/ml and included a sterile control (containing broth culture) and a growth control (containing broth culture and bacteria, without antimicrobial substance). MIC is defined as the lowest concentration showing no visible growth (clear) and was determined visually after the addition of 20 μl of Iodonitrotetrazolium chloride (INT, Sigma-Aldrich) water solution (4 mg/ml). The color in the wells changes to purple after the addition of INT if there is a bacterial growth. The commercial antibiotics, vancomycin hydrochloride (Sigma-Aldrich), streptomycin sulfate (Sigma-Aldrich), and chloramphenicol (Sigma) were used as positive controls. The results were expressed in μg/ml.

RESULTS

Endophytic fungus, *Xylaria* sp. DAP-KRI-5, isolated from *A. papuana* Becc plant that was collected from Bogor Botanical Garden, West Java, was cultured in 2.7 l of PDB at 25°C for 14 days in the dark. The culture of this fungus was harvested and extracted with EtOAc. The extraction process yielded 2.7887 g of EtOAc extract.

Extract of *Xylaria* sp. DAP KRI-5 was fractionated by using column chromatography. First, the extract of EtOAc (600 mg) was fractionated by Sephadex™ LH-20 (GE Healthcare) (200 g, eluent metanol) and resulted in F1–F5 (weights were 34.2, 300.4, 4.0, 244.4, and 1.1 mg, respectively). Then, the dominant fraction F2 (208.7 mg) was applied on a SiO_2 gel 60 (Merck) (70-230 mesh ASTM, 100 × weigh of samples, eluent n-hexane-EtOAc in the ranges of 3:1 to 1:3, $CHCl_3$-MeOH in the range of 5:1 to 1:7). This resulted in F2.1–F2.9 (respective weights were 12.9, 19.8, 113.5, 11.3, 18.2, 4.9, 17.7, 5.8, and 3.6 mg). Next, the biggest weight fraction, F2.3 (113.0 mg) was further fractionated by using SiO_2 gel 60 (Merck) (70-230 mesh ASTM, 100 × weight of samples in n-hexane-EtOAc in the range of 6:1 to 0.5:6) and resulted in F2.3.1–F2.3.10 (respective weights were 0.7, 0.2, 0.6, 1.6, 0.6, 1.0, 1.9, 3.5, 17.3, and 1.3 mg). One fraction, F2.3.9, was pure compound **1** which was analyzed by HRMS, 1D- and 2D-NMR, and antibacterial activity was tested using microdilution methods.

Data of HRMS of pure compound **1** were obtained using UPLC–qTOF-MS/MS: m/z value M^+ of 507.2615 (calc. 507.2611, Δ +0.4 mmu) with the molecular formula $C_{30}H_{37}NO_6$.

The ^{1}H-NMR and distortionless enhancement by polarization transfer (DEPT) data of **1** showed the presence of benzyl with aromatic proton at $δ_H$ 7.12 (2H, d);

7.31 (2H, t); 7.25 (1H, t) and two diastereotopic methylene protons of H-10 were shifted upfield at δ_H 2.67 (1H, dd) and 2.82 (1H, dd), one exocyclic olefinic proton of H-12 was shifted downfield at 5.09 (1H, s) and 5.29 (1H, s), two *trans*-disubtituted of H-13/H-14 and H-19/H-20 [δ_H 5.14 (1H, dd), 5.69 (1H, dd), 5.34 (1H, ddd), and 6.11 (1H, dd), respectively], and one amido proton was shifted at δ_H 5.43; 1H, s (Table 1).

The ^{13}C-NMR data of 1 (Table 2) showed 30 carbon signals including four methyls (δ_C 13.8, 19.5, 24.3, and 21.0), three carbonyls including two ketones and one ester which were shifted at δ_C 173.8, 210.4, and 169.9, respectively, and two couples of identical aromatic carbon signals C-2′/C6′ and C-3′/C-5′ which were shifted at δ_C 129.3 and 129.1, respectively. Using DEPT, it can be inferred that signals at δ_C 173.8, 147.7, 53.7, 210.4, 77.8, 169.9, and 137.4 are quarternary carbon atoms.

The heteronuclear multiple bond correlation (HMBC) correlation also showed a correlation from H-10a (δ_H 2.67) to C-3 (δ_C 53.4), C-1' (δ_C 137.4), C-2'/C-6' (δ_C 129.3), from H-10b (δ_H 2.82) to C3 (δ_C53.4), from H-21 (δ_H5.63) to C4 (δ_C50.2), C20 (δ_C 132.5), and C13 (δ_C 127.8), and from H-8 (2.85) to C7 (δ_C 70.0) and C-1(δ_C 173.8), which supported the 3-benzyl-substituted isoindolone skeleton and the HMBC correlations from an exocyclic proton H-12a/H12b to quarternary carbons C-5 and C7, and from methyl proton H-11 to quarternary carbons C-5, and C-6 which indicated that the structure of compound **1** was analogous to cytochalasins (Fujii *et al*., 2000; Feng *et al*., 2002; Lin *et al*., 2009; Liu *et al*., 2006; 2008; Xue *et al*., 2012; Zhang *et al*., 2008). Other studies have reported that most cytochalasin skeletons share the same stereochemistry (viz., *cis*-configuration*s* atC-8/C-9 and C-4/C-9 ring junctions), and it has been proposed that the macrocyclic skeleton is stable and relatively rigid despite the large ring size and different functionalities (Buchanan *et al*., 1996; Feng *et al*., 2002; Liu *et al*., 2006; 2008).

The ^{1}H- and ^{13}C-NMR data of isolated compound was identic with cytochalasin D as shown in Tables 1 and 2 with a slightly different in measured of chemical shift of proton of NH moiety for isolated [5.43 (s, 1H)] and reported by Luo *et al.* (2007) (5.28, brs, 1H). However, the chemical shift of NH moiety of cytochalasin D reported by Xu *et al.* (2001) was appeared in more downfield area (5.53, brs, 1H), with no hydroxy signals (7-OH and 18-OH). The difference of this chemical shift is probably caused by availability of small amount of H_2O molecules in the NMR sample that affect to the chemical shift of proton on NH moiety. Based on the above data, it was concluded that the isolated compound from *Xylaria* sp. DAP-KRI-5 is cytochalasin D and indicated the molecular formula of $C_{30}H_{37}NO_6$ that agree with molecular formula of cytochalasin D published on http://dnp.chemnetbase.com (Buckingham *et al*., 2010).

Antibacterial assays for compound **1** compared with commercial antibiotics (streptomycin, vancomycin, and chloramphenicol) appeared in Table 3. Chemical structure of cytochalasin D is shown in Figure 1.

DISCUSSION

In this study, cytochalasin D, a fungal metabolite was successfully isolated from *Xylaria* sp. DAP KRI-5. *Xylaria* sp. DAP KRI-5 is an endophytic fungus that was isolated from the

Table 1. ^{13}C-NMR data of compound **1** and Cytochalasin D in CDCl$_3$ (125 MHz).

C no.	1	Luo *et al*., 2007	Xu *et al*., 2001
	δC	δC	δC
1	173.8	173.8	173.6
3	53.4	53.3	53.2
4	50.2	49.8	50.0
5	32.8	32.6	32.6
6	147.7	147.5	147.5
7	70.0	69.8	69.8
8	47.1	46.9	47.0
9	53.7	53.5	53.5
10	45.5	45.2	45.3
11	13.8	13.6	13.6
12	114.7	114.4	114.5
13	127.8	127.5	127.6
14	130.8	130.6	130.6
15	37.9	37.7	37.7
16	42.5	42.3	42.3
17	210.4	210.2	210.2
18	77.8	77.6	77.7
19	134.3	134.0	134.1
20	132.5	132.3	132.3
21	77.2	77.0	77.3
22	19.5	19.3	19.4
23	24.3	24.1	24.2
24	169.9	169.6	169.7
25	21.0	20.8	20.8
1′	137.4	137.2	137.2
2′, 6′	129.3	129.1	129.1
3′, 5′	129.1	128.8	128.9
4′	127.3	127.0	127.1

A. papuana Becc plant. This compound was characterized by spectrometry (1D- and 2D-NMR, and HRMS) and was confirmed as cytochalasin D.

Cytochalasin D was first isolated from *Metarrhizium manisopliae* by Aldridge *et al.* (1967) and it was also known as zigosporin A because it was produced by the fungus *Zygosporium mansonii* (Hayakawa *et al*., 1968), and was also isolated from *Engleromyces goetzii* (Jikai *et al*., 2002). However, there are no data citing the bioproductive capacity of cytochalasin D by *Z. mansonii*, *M. manisopliae*, or *E. goetzii*.

In other studies, cytochalasin D was also produced by *Xylaria* sp. SCSIO 156 and *Daldinia concentrica* (Chen *et al*., 2011; Luo *et al*., 2007). The bioproductive capacity of cytochalasin D was reported from *Xylaria* sp. SCSIO 156, and *Xylaria* sp. BT05: 0.00126 and 0.00085 g/l of its liquid culture medium, respectively (Chen *et al*., 2011; De Felício *et al*., 2015). It has been produced by *X. arbuscula* with a bioproductive capacity around 0.52733g/l (Amarala *et al*., 2014). In this present study, *Xylaria* sp. DAP KRI-5 produced cytochalasin

Table 2. ^{1}H-NMR data of compound 1 and cytochalasin D in CDCl$_3$(500 MHz).

C. no.	1 δH	Luo *et al.,* 2007 δH	Xu *et al.,* 2001 δH
1	-	-	-
3	3.23 (m, 1H)	3.23 (m, 1H)	3.22 (m, 1H *J* = 4.5, 4.0)
4	2.14 (dd, 1H, *J* = 4.4, 3.9)	2.14 (dd, 1H, *J* = 4.4, 3.8)	2.14 (t, 1H, *J* = 5.0)
5	2.72 (m, 1H)	2.72 (m, 1H)	2.83 (m, 1H, *J* = 10.5, 5.0, 2.0)
6	-	-	-
7	3.81 (d, 1H, *J* = 10.4)	3.80 (d, 1H, *J* = 10.6)	3.80 (1H, d, *J* = 10.5)
8	2.85 (t, 1H, *J* = 10.4, 9.1)	2.84 (dd, 1H, *J* = 10.1, 9.8)	2.84 (m, 1H, *J* = 5.0, 2.0)
9	-	-	-
10	2.67 (dd, 1H, *J* = 13.3, 9.1)	2.68 (dd,1H,*J* = 13.4, 9.2)	2.65 (dd, 1H, *J* = 13.5, 9.5)
10	2.82 (dd, 1H, *J* = 13.6, 5.2)	2.81 (dd,1H,*J* = 13.4, 5.3)	2.83 (m, 1H, *J* = 13.5, 10.0, 2.5)
11	0.96 (d, 3H, *J* = 6.5, CH$_3$)	0.91 (d, 3H, *J* = 6.7, CH$_3$)	0.95 (d, 3H, 7.0, CH$_3$)
12	5.09 (s, 1H)	5.07 (brs, 1H)	5.09 (s, 1H);
12	5.29 (s, 1H)	5.28 (brs, 1H)	5.29 (s, 1H)
13	5.14 (dd, 1H, *J* = 15.6, 2.6)	5.13 (dd, 1H, *J* = 15.7, 2.3)	5.35 (m, 1H, *J* = 15.5, 10.0, 5.0)
14	5.69 (dd, 1H, *J* = 15,6, 9.8)	5.67 (dd, 1H, *J* = 15.4, 9.8)	5.65 (dd, 1H, *J* = 15.5, 10.0)
15	2.02 (dd,1H, *J* = 12.9, 5.2)	2.01 (dd,1H,*J* = 12.7, 4.8)	2.02 (dd, 1H, *J* = 13.5, 11.0)
15	2.51 (dd, 1H, *J* = 12.95,11.05)	2.50 (ddd, 1H, *J* = 12.7,11.1,11.1)	2.51 (dd, 1H, *J* = 13.5, 11.0)
16	2.74 (m, 1H)	2. 72 (m, 1H)	2.73 (m, 1H, *J* = 11.0, 4.5)
17	-	-	-
18	-	-	-
19	5.34 (ddd, 1H, *J* = 15.5, 10.5, 5.2)	5.32 (ddd, 1H, *J* = 15.7, 10.7, 5.2)	5.35 (m, 1H, *J* = 15.5, 10.0,5.0)
20	6.11 (dd, 1H, *J* = 15.6, 2.6)	6.10 (dd, 1H, *J* = 15.7, 2.6)	6.11 (dd, 1H, *J* = 16.0, 2.5)
21	5.63 (t, 1H, *J* = 2.6)	5.61(dd, 1H, *J* = 2.6, 2.3)	5.63 (t,1H, *J*=2.5)
22	1.19 (d, 3H, *J* = 7.2, CH$_3$)	1.19 (d, 3H, *J* = 6.8, CH$_3$)	1.20 (d, 3H, *J* = 7.0, CH$_3$)
23	1.51 (s, 3H, CH$_3$)	1.50 (s, 3H, CH$_3$)	1.51 (s, 3H, CH$_3$)
24	-	-	-
25	2.26 (s, 3H, CH$_3$)	2.25 (s, 3H, CH$_3$)	2.26 (s, 3H, CH$_3$)
1'	-	-	-
2', 6'	7.12 (2H, d, *J* = 7.1)	7.13 (d,2H, *J* = 7.2)	7.25 (m, 2H, *J* = 15.0, 7.0, 1.5)
3', 5'	7.31 (t, 2H, *J* = 7.1)	7.31 (t, 2H, *J* = 7.3)	7.25 (m, 2H, *J* = 15.0, 7.0, 1.5)
4'	7.25 (t, 1H, *J* = 7.8)	7.23 (t, 1H, *J* = 7.3)	7.25 (m, 1H, *J* = 15.0, 7.0, 1.5)
NH	5.43 (s, 1H)	5.28 (brs, 1H)	5.53 (brs, 1H)
7-OH	1.91 (s, 1H)	2.03 (s, 1H)	nd
18-OH	4.65 (s, 1H)	4.64 (brs, 1H)	nd

nd = no data.

Table 3. Antibacterial activity of cytochalasin D and some commercial antibiotics.

Tested bacteria	MIC value (ug/ml) Commercial antibiotics			
	Chloramphenicol	Streptomycin	Vancomycin	Cytochalasin D
Gram-positive bacteria				
S. aureus-InaCC* B4	4	2	0.5	>32
B. subtilis-InaCC B1	2	16	0.5	>32
Gram-negative bacteria				
E. coli-InaCC B5	8	32	>32	>32
P. aeruginosa-InaCC B3	32	> 32	>32	>32

*InaCC = Indonesia culture collection.
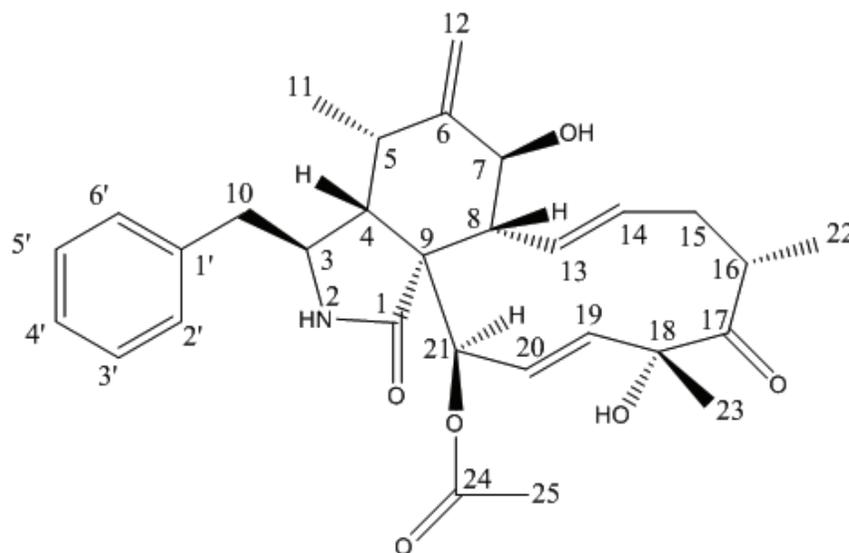
Figure 1. The structure of cytochalasin D.

Table 4. Bioproduction capacity of cytochalasin D isolated from some fungi.

No	Source	Medium	Bioproduction Capacity (g/l)	Literatures
1.	*Zygosporium mansonii*	3% glucose, 2% peptone, and 0.5% sodium, chloride in sterilized water (pH 6.8)	nd	Hayakawa *et al.*, 1968
2.	*Metarrhizium anisopliae*	nd	nd	Aldridge *et al.*, 1967
3.	*Daldinia concentrica*	nd	nd	Luo *et al.*, 2007
4.	*Engleromyces goetzii*	nd	nd	Jikai *et al.*, 2002
5.	*Xylaria* sp. SCSIO 156	2% mannitol, 2% malt extract, 1% glucose, 1% sodium glutamate, 0.05% KH_2PO_4, 0.03% $MgSO_4 \cdot 7H_2O$, 0.1% corn meal, 0.3% yeast extract, and 3% sea salt in distilled water (pH 6.5)	0.00126	Chen *et al.*, 2011
6.	*Xylaria* sp. BT05	Rice in seawater (1,000 g/l)	0.00085	De Felício *et al.*, 2015
7.	*Xylaria arbuscula*	Wheat in distilled water (1,333 g/l)	0.52733	Amaral *et al.*, 2014
8.	*Xylaria* sp. DAP KRI-5	PDB in sterilized water (24 g/l)	0.05763	The present study

nd = no data.

D with a bioproductive capacity of 0.05763 g/l (Table 4). The bioproductive capacity of *Xylaria* sp. DAP KRI-5 is less than that of *X. arbuscula* because the *X. arbuscula* (wheat: 1,333 g/l) culture medium contains more free amino acids and proteins than the *Xylaria* sp. DAP KRI-5 (PDB: 24g/l) culture medium. The level of free amino acid and proteins in the culture medium are essential factors of the production of cytochalasin D (Amarala *et al.*, 2014).

In this study, cytochalasin D was tested for antibacterial agent against Gram-positive bacteria (Table 3) with a MIC value of > 32 µg/ml. Cytochalasin D was less active than the commercial antibiotics chloramphenicol, streptomycin, and vancomycin. Meanwhile, the MIC value of cytochalasin D was similar to vancomycin against Gram-negative bacteria (> 32 µg/ml). In other studies, cytochalasin D has been reported to have activity as an antitumor, antineoplastic, antiviral, antianaphylactic, antifungal, and disruptor of actin polymerization agents (Betina *et al.*, 1972; Cohen *et al.*, 1999; Oppenheimer *et al.*, 1995; Shin *et al.*, 1997; Trendowski, 2015; Wang *et al.*, 2003). This compound is effective as a disruptor of microfilament formation so it could act as a chemotherapeutic agent (Trendowski, 2015).

CONCLUSION

The endophytic fungus *Xylaria* sp. DAP-KRI-5 that is associated with the *A. papuana* Becc. plant can produce cytochalasin D with a bioproductive capacity of 0.05763 g/l in PDB medium. Cytochalasin D was less potent as an antibacterial agent against Gram-positive and negative bacteria than several commercial antibiotics.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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