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ISOLATION AND ANTIBACTERIAL ACTIVITY OF ENDOPHYTIC MOLD FROM DAHLIA TUBER PLANTS (Dahlia variabilis) AGAINST Stapylococcus aureus and Stenotrophomonasmaltophilia strainW1-2

Muhammad Diki Juliandi[1)], Rita Permatasari[2)],Dewi Yudiana Shinta[3)],Mohd Fadly Md Ahid[4)], Evana Kamarudin[5)]*

[1,2,3]Faculty of health sciences, Universitas Perintis Indonesia, Padang,27251,West Sumatera Indonesia
[4,5]UniversitiTeknologiMARA,CawanganSelagorKampusPuncak Alam,42300,Selagor Malaysia

*Email: Dikijulianda@gmail.com

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Umbi Dahlia
Kapang Endofit
Anti Bakteri
GC-MS

Penulis Korespondensi

Name : Muahmmad Diki Juliandi
Affiliation : Universitas Perintis Indonesia
E-mail : Dikijuliandi@gmail.com

A B S T R A C T

Antibiotic resistance to pathogenic bacteria has developed in a short period of time and faster than has been thought. Therefore, it is necessary to find new antibiotic alternatives that are sourced from natural materials such as endophytic molds of dahlia tubers. This study was conducted to test the antibacterial activity of endophytic molds isolated from dahlia bulbs against Staphylococcus aureus and Stenotrophomonasmaltophilia strainW1-2. The results of the selection of antimicrobial activity of endophytic mold isolated from the dahlia tuber were fermented using the shaking method on Potato Dextrose Broth medium. The results of fermentation are tested for antibacterial activity using the disc diffusion method. Of the 10 isolated endophytic molds, two isolates with antibacterial activity were obtained, namely A-1 and A-7 isolates. The results of the antibacterial activity test of A-1 isolate against Staphylococcus aureus bacteria had the highest inhibition zone of 32.75 mm and for Stenotrophomonasmaltophilia strain W1-2 had the highest inhibition zone of 19.25 mm. In A-7 isolate against Staphylococcus aureus bacteria had the highest inhibition zone of 29.5 mm and against Stenotrophomonasmaltophilia strain W1-2 had the highest inhibition zone of 15.25 mm. Based on the results of the antibacterial activity test carried out by the isolate, the isolate mold isolates with codes A-1 and A-7 have the highest antibacterial activity against Staphylococcus aureus and have a strong inhibitory power against

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Stenotrophomonasmaltophilia strain W1-2.The results showed that molds A-1 and A-7 had the potential to be antibacterial agents and GC-MS analysis of antibiotic compounds detected by hexanorlabdane. Molecular identification using the ITS gene observed that the A-1 mold isolate was 100% similar to the genomic2011f6MT558940.1 strain of Aspergillus Fumigatus

INTRODUCTION

Is one of the plants that is widely used as an ornamental plant because it has characteristics in the form of attractive flower colors. In addition to having beautiful flowers, dahlias also have bulbs that are rarely used, especially in the field of medicine and nutrition (Marlinda.S, et.al, 2019) Dahlias flowers are reported to contain minerals, vitamin C, *phenolic* compounds, anthocyanins, carotenoids and other antioxidant compounds (Ohno,S.*et al*., 2018). The stems and leaves of dahlias are rich in flavonoids such as butein and flavonol derivatives (Shinta D.Y, *et al,* 2019). In addition, dahlia tubers are a good source of inulin because of their characteristics that utilize endophytic microbes in producing active compounds have several advantages, including (can be produced on a large scale and the possibility of obtaining new bioactive components by applying different conditions (Wulandari, D.*et.al,* 2020).

Research by Shinta, *et.al* (2023) stated that endophytic fungi isolated from dahlia bulbs (*Dahlia variabilis*) are able to inhibit the growth of E. coli, Candida albicans and S. aureus bacteria. This is also supported by research conducted (Shinta, *et.al*, 2018), endophytic fungi isolated from dahlia bulbs (*Dahlia variabilis*) are able to inhibit the growth of Streptococcus aureus bacteria. Endophytic fungi protect plants against a wide variety of pathogens such as bacteria, fungi, and insects which allows the antimicrobial properties to be commonly found in several genera of fungi such as Aspergillus, Alternaria, Colletotrichum, Fusarium, Penicillium, and Pestalotiopsis. Some potential fungal types such as Fusarium tricinctum isolated from Rhododendron tomentosum, produce antibacterial and antifungal compounds against *Staphylococcus carnosus* and *Candida albicans and C. utilis.* Meanwhile, the helgan extract of the fungus Colletotrichum gloeosporioides isolated from the medicinal plant Vitex negundo has antibacterial activity against S. aureus bacteria that are resistant to methicillin, penicillin and vancomycin (Amalia 2016).

Methods

Instruments and Materials

The tools used are vortex, centrifuge, microscope, autoclave, laminar air flow, micropipettes and tips, incubators, analytical balances, microwaves, bunsen, beakers, measuring cups, stirring rods, droppers, test tubes, ose needles, object glass and cover glass, spatula, paper punchers, other glassware used in microbiology laboratories.

The materials used are tubers from sterile aquatic dahlia plants, cotton, tissues, aluminum foil, rope, gauze, warb plastic, sodium hypochlorite 5.3%, ethanol 70%, methylene

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blue, Potato Dextrosc Agar (PDA) media, Potato DextrosBroth (PDB) media, and Nutrient Agar (NA) media, the standard antibiotic comparison Ciprofloxacin 20 ng. test microbes used in this study are gram-positive and negative Staphylococcus aureus bacteria and bacteria Stenotrophomonasmaltophilia strainW1-2 obtained from bacterial cultures at RSUP. Dr.M Djamil Padang

Procedure

The stages of the implementation of the research consist of several main stages,namely: Preparation Stage

Endophytic Mold Insulation

Samples of Dahlia plant tubers are washed with running water and then left to dry. Next, the sample was cut according to the cooled size, then the sample was then sterilized on the surface using 70% ethanol and 5.25% NaOCl alternately and then rinsed using a sterile aqueduct. Furthermore, the rinse water is inoculated into the PDA medium as a control (Mahardhikact al., 2021). Sterilized dahlia tuber pieces are aseptically inoculated into petri dishes that have been filled with PDA media and incubated at 28 °C until endophytic mold growth is visible, Transfer the pure culture to PDA media and incubate for 14 days at 28 °C (Radji, 2011).

Endophytic Mold Purification

Purification of endophytic mold is carried out to separate mold columns that have different morphological characteristics. The isolation process is carried out in stages and each visible column is taken which is then noculated in a petri dish containing PDA media and incubated during the day at room temperature This purification is carried out to obtain pure isolate from each mold colony formed (Devi et al., 2021)

Characterization of Endophytic Mold Isolate

Characterization of pure isolate of endophytic mold is carried out through macroscopic and microscopic observations. Macroscopic observations were made by observing the shape and growth of colonies which included the color and surface of the colony (granular, such as flour, mountainous, slippery), texture, concentric circle circles (concentric or non-concentms), reverse color, exudate droplets, and growth diameter of mold colonies (Ilyas, 2007). Microscopic characterization is carried out by observing septums in hyphae (partitioned or undivided), hyphae growth (branched or unbranched), spore shape and ornamentation (Setiawan &Musdalipah, 2018).

Manufacture of Test Bacteria Suspension

The test microbial suspension is made by taking 1 bacterial ose and putting it into a test tube containing 10 ml of NaCl 0.9%Making a Bacterial Suspension Test The test microbial suspension is made by taking 1 bacterial ose and putting it into a test tube containing 10 ml of NaCl 0.9%

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Selection of Endophytic Molds with Potential as Antibacterial

Selection of endophytic molds that have the potential to be anti-bacterial is carried out by inoculating pieces of endophytic mold colonies into petri dishes that have been inoculated with test bacteria Furthermore, the entire culture is incubated for 5 days at a temperature of 37°C. Endophytic mold isolates that have antibacterial potential can be seen from the presence or absence of inhibitory zones around endophytic mold isolates (Abna*et al* , 2021).

Production of Secondary Metabolites of Endophytic Mold

The production of secondary metabolites is carried out by inoculating endophytic mold isolates that have the highest bacten-inhibiting activity into the streil GDP medium Incubation of endophytic mold cultures is carried out at room temperature and given aeration with the help of a shaker at a speed of 150 rpm. The sampling process is carried out every 6 hours and then centrifuged at a speed of 3000 rpm for 20 minutes. The results of centrifugation are then separated from the biomass and stored in the refrigerator for use in the next test (Abna et al., 2021).

Antibacterial Activity Test

The antibacterial activity test was carried out using the well diffusion method. The suspension of Staphylococcus aureus bacteria was taken as much as 1 ml and inoculated in a pour plate into a different petri dish and then 15 ml of NA media was inserted. After the NA medium solidifies, a well hole is made as many samples as used using a protractor. Each hole is then added with a test sample of 50 µl and then marked (sample, positive control; negative control) Then incubated in an incubator at a temperature of 37 °C for 24 hours, the inhibition zone formed is then measured using a caliper (Ngajow et al., 2013, Rahayu et al., 2021).

Data AnalysisThe data obtained were analyzed descriptively.By comparing the data obtained with the literature related to the results of this study.

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Results And Discussion

Isolation of Dahlia Bulb Mold

Isolation of dahlia bulb mold on PDA medium and incubation at 28 °C for 14 days obtained 10 endophytic mold isolates that can be observed Macroscopic and Microscopically in Figure 1.

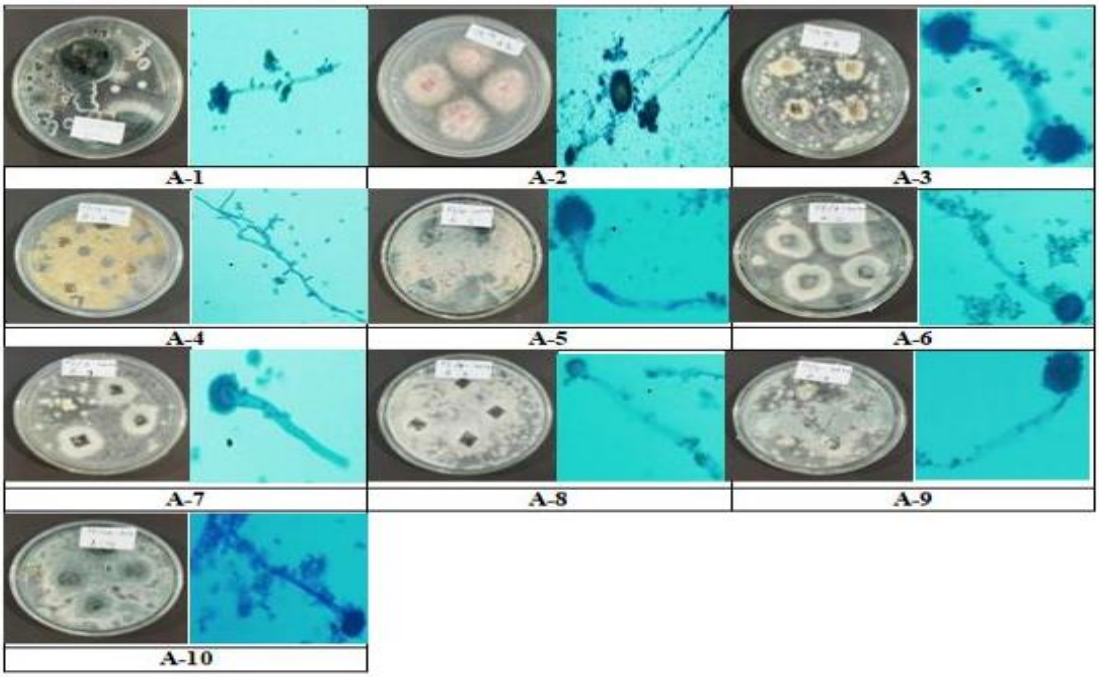

Table 1.Selection of Endophytic Mold Isolate Against Test Bacteria

No	Inhibition Zone Diameter	Inhibition Zone Diameter	
		Staphylococcus aureus	***Stenotrophomonas maltophiliastarin W1-2***
1	A-1	32,75 mm	19,25 mm
2	A-2	0 mm	0 mm
3	A-3	28,5 mm	0 mm
4	A-4	27 mm	15 mm
5	A-5	21,5 mm	17,25 mm
6	A-6	25,5 mm	16,15 mm
7	A-7	29,5 mm	15,25 mm
8	A-8	23,25 mm	14,75 mm
9	A-9	24,5 mm	10,25mm
10	A-10	24,75 mm	15,75 mm

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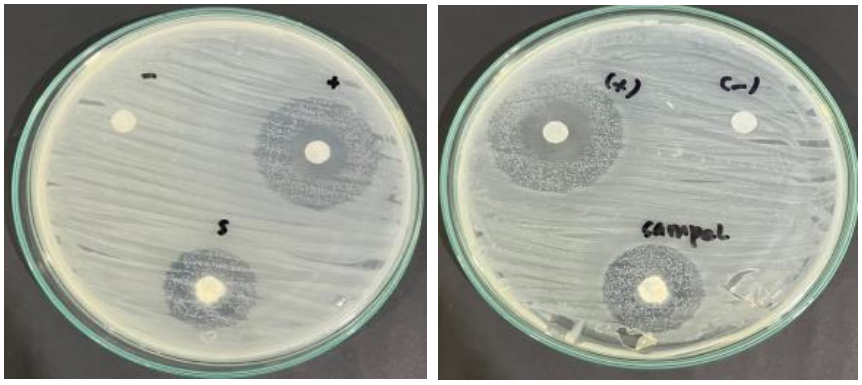
Potential endophytic mold isolates are able to provide an inhibitory effect on test microbes. The two isolates are A-1 and A-7 isolates which are able to inhibit *Staphylococcus aureus* and *Stenotrophomonas maltophiliastarin W1-2 bacteria*, respectively. The cause of this is the difference in bioactive compounds produced by each mold differently.

Antibacterial Activity of Potential Endophytic Mold Supernatants

The antibacterial activity test of endophytic mold supernatant on MHA (*Mueller Hiton Agar*) medium shows endophytic mold with codes A-1 and A-7 with *Staphyloccus aureus test* bacteria can be seen in Figure 2.



(A-1) (A-7)

The antibacterial activity test of endophytic mold supernatant on MHA (*Mueller Hiton Agar*) media showed endophytic mold with codes A-1 and A-7 with *Stenotrophomonasmaltophilia strain W1-2* test bacteria can be seen in Figure 3.

(A-1) (A-7)

Antibacterial activity of potential secondary metabolite supernatants of dahlia tuber plants with *Peper Agar Diffusion Technique*, which aims to determine the strength of antibacterial activity or test the inhibition of potential endophytic mold secondary metabolite supernatants that are able to inhibit the growth *of Staphylococcus aureus* and

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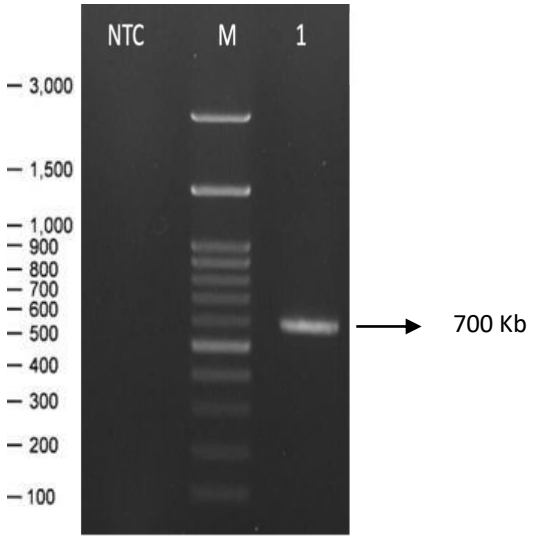
Stenotrophomonas maltophiliastarin W1-2. The magnitude of antibacterial activity is determined by the size of the inhibition zone or clear zone around the *paper disk.*

The results of ITS gene amplification from electrophoresis showed that PCR activity had successfully applied the ITS gene region of dahlia bulb endophytic mold with code A-1 Figure 4.



Analysis of ITS gene sequencing of endophytic mold The results of sequencing the isolate of endophytic mold of dahlia tubers were compared with Gene Bank data using the BLAST program conducted online on the NCBI hhtp://blast.ncbi.nih.gov/Blast.cgi website. The sequencing data and results of BLAST analysis of dahlia tuber endophytic mold can be seen in Table 2.ITS BLAST results from A-1

Table 2.The ITS BLAST Result of A-1 KapangEndofit Umbi Dahlia

No	Kapang	% Smilarity	No.Acession
1	*AspergillusFumigatus AU_S_MS_B2a*	100%	OQ726780
2	*Aspergillus Fumigatus Isolate MA-3*	100%	MW335143
3	*Aspergillus Fumigatus Isolate SM-730*	100%	PP725513
4	*Aspergillus Fumigatus genomic DNA*	100%	OW983688
5	*Aspergillus Fumigatus Isolate NIANP*	100%	MK640673
6	*Aspergillus Fumigatus strain CCPMBF003*	100%	OP730546
7	*Aspergillus Fumigatus genimic DNA*	100%	OW983894
8	*Picia Fermentans genomic DNA*	100%	OW988289
9	*Aspergillus Fumigatus genomic DNA*	100%	OW983559
10	*Aspergillus Fumigatus strain NAUCP3*	100%	MK334211

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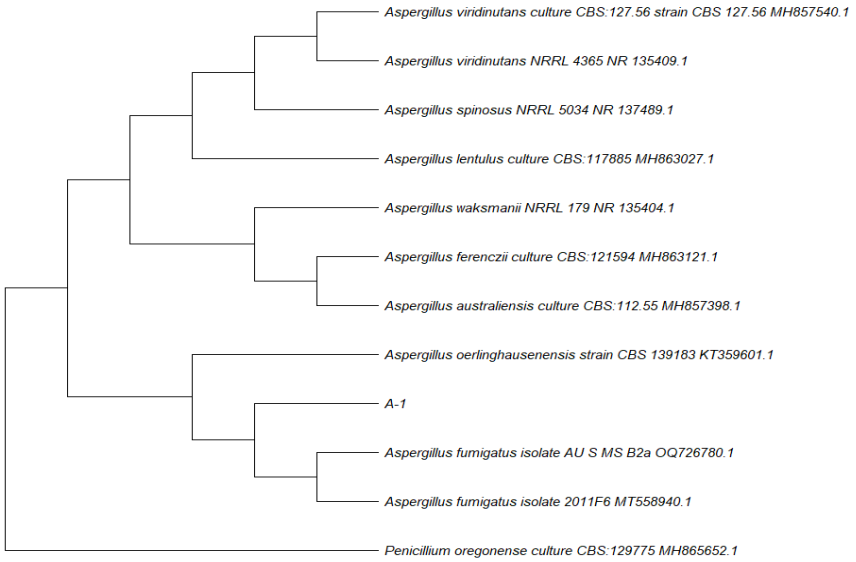
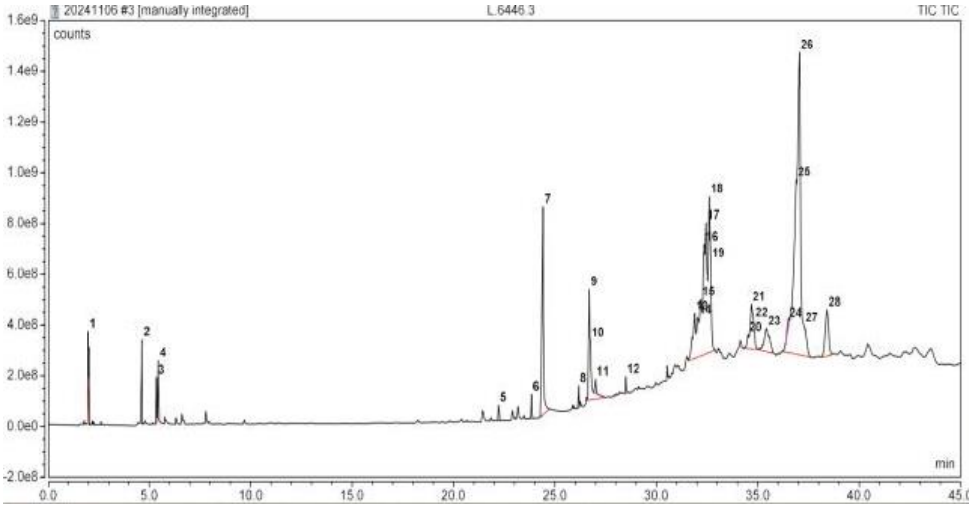
Phylogenetic endophytic mold A-1 Dahlia tuber in Figure 5.





GC-MS Analysis of A-1 Endophytic Mold Extract from Dahlia Tubers in Figure 6.

Results And Discussion

The process begins isolation by sterilizing the dahlia bulbs that will be used. Sterilization was carried out to ensure that the mold growing around the stem sample was indeed endophytic mold and not due to contamination of epitite microbes present on the surface of the plant (Daud *et al., 2012; Maheshwari, 2017).*

The results of macroscopic and microscopic characteristics in Figure 1 found that a pure endafit mold has different characteristics in terms of color, colony shape, and colony growth rate, microscopic differences were found in terms of whether or not there is a partition on the

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hyphae, the presence or absence of branches on the hyphae and the ornamentation of the hyphae. Mold isolates that have the potential to be antibacterial can be extended to the fermentation stage. The selection of endophytic molds is carried out using the agar diffusion method which is carried out by placing mold pieces on a medium that has been inoculated with test bacteria, and then antibacterial activity can be seen from the visible inhibition zone around the mold pieces (Pratama*et al*., 2018).

The results of the selection of endophytic molds shown in Table 1 show that out of ten endophytic molds, there are only two potential molds that are able to provide an inhibitory effect on the test microbes. The two isolates are A1 and A7 isolates which are able to inhibit *Staphylococcus aureus* bacteria and *Stenotrophomonas maltophiliastarin W1-2 bacteria*, respectively. The cause of this is the difference in bioactive compounds produced by each mold differently. This is in accordance with what was revealed by Siswandono (2017) that bioactive compounds have their own specifications and effectiveness.

Antibacterial activity tests were carried out on *Staphylococcus aureus* bacteria and *Stenotrophomonas maltophiliastarin W1-2* bacteria were selected as bacteria representing the group of gram-positive bacteria and gram-negative bacteria where these bacteria have been declared resistant to a number of antibiotics (Radji*et al*.., 2011). In this test, ciprofloxacin antibiotics were used as a positive control because ciprofloxacin is a broad-spectrum antibiotic (Wahyudi*et al*., 2019). Ciprofloxacin is a quinolone class of antibiotics that works by stopping the growth of bacteria or bacteriotoxins. Ciprofloxacin plays a role in inhibiting the mechanism of action of the DNA girase enzyme in the process of bacterial cell division (Rame&Dewangga, 2022).

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Based on the results of 6 GC-MS analysis, the extractable A-1 ultracellular isolate is known to have butyl glyoxylate, ethylbenzene, cyclohexane, 1,2-dimethyl-(cis/trans), 2,3-butanediol, retina, methyl stearate, n-hexadecanoic acid, 2' HydroxyCyclohexyl) Propanol, 2H-Pyrrol-2-one, 1,5-dihydro-1-methyl-, and indole. Compound 8 beta.,12-Epoxy-13,14,15,16,17,19-hexanorlabdane is a compound that can be extracted by using ethyl acetate solvents. This compound is also known to have functions as an antineoplastic (anticancer), a protector of the musculoscule membrane, and is able to prevent scalp inflammation (antiseborrheic) (Sayuti et al., 2017). Other compounds that are read as solvents are butane, ethyl propionate, toluene, butyl acetate, benzene.

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Conclusion

The isolated mold is one of the highest potential antibiotic isolates found from one dahlia bulb mold isolate in Kerinci Regency, Indonesia. Based on the molecular identification of isolated mold A-1 using the ITS gene, it was observed that A-1 is 100% similar to the *genomic strain of Aspergillus Fumigatus2011f6MT558940.1*

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