

Isolation and Evaluation of Antifungal Activity of Sand Sea Cucumber Symbiont Bacteria (*Holothuria scabra*) from Wakatobi National Park against *Malassezia furfur*

Isolasi dan Evaluasi Aktivitas Antifungi Bakteri Simbion Teripang Pasir (*Holothuria scabra*) dari Taman Nasional Wakatobi terhadap *Malassezia furfur*

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Abstract. *Malassezia furfur* is a fungus that causes tinea versicolor which requires antifungal compounds for treatment. Antifungal compounds can be obtained from bacteria that are in symbiosis with marine organisms. One candidate for bacteria producing antifungal compounds is the sand sea cucumber symbiont bacteria (*Holothuria scabra*). This research aims to obtain bacterial isolates that are able to inhibit the growth of the *Malassezia furfur* fungus that causes tinea versicolor (*Pityriasis versicolor*). Isolation of sea cucumber symbiont bacteria was carried out using the pour plate method. The antifungal activity of symbiont bacteria was carried out using the well diffusion method. The results of the research obtained 5 isolates of the sand sea cucumber symbiont bacteria which had *M. furfur* antifungal activity. Three bacterial isolates, namely isolates DT1, DT4 and isolate DT5, produced antifungal compounds which were able to inhibit the growth of *M. furfur* with an inhibition zone ranging from 8.60 mm-11.30 mm within 24 hours. Based on the identification results using the profile matching method, it is known that the bacterial isolates DT1 and DT5 belong to the genus *Pseudomonas*, while the bacterial isolate DT4 belongs to the genus *Bacillus*. Therefore, the three isolates of sand sea cucumber symbiont bacteria can be developed and applied to produce medicinal ingredients for tinea versicolor skin disease.

Kata kunci: Symbiont Bacteria; *Holothuria scabra*; Well-diffusion method; *Pityriasis vesicolor*

Abstrak. *Malassezia furfur* merupakan fungi penyebab penyakit panu yang membutuhkan senyawa antifungi untuk pengobatannya. Senyawa antifungi dapat diperoleh dari bakteri yang bersimbiosis dengan organisme laut. Salah satu kandidat bakteri penghasil senyawa antifungi adalah bakteri Simbion Teripang pasir (*Holothuria scabra*). Penelitian ini bertujuan untuk memperoleh isolat bakteri yang mampu menghambat pertumbuhan fungi *Malassezia furfur* penyebab penyakit panu (*Pityriasis versicolor*). Isolasi bakteri simbion teripang dilakukan menggunakan metode tuang (pour plate). Aktivitas antifungi bakteri simbion teripang pasir yang memiliki aktivitas antifungi *M.furfur*. Tiga isolat bakteri yaitu isolat DT1, DT4 dan isolat DT5, menghasilkan senyawa antifungi yang mampu menghambat pertumbuhan *M. furfur* dengan zona hambat berkisar 8,60 mm-11,30 mm dalam waktu 24 jam. Berdasarkan hasil identifikasi dengan metode profile matching diketahui bahwa Isolat bakteri DT1 dan DT5 termasuk genus *Pseudomonas* sedangkan isolat bakteri DT4 termasuk genus *Bacillus*. Dengan demikian, Ketiga isolat bakteri simbion teripang pasir dapat dikembangkan dan diaplikasikan sebagai penghasil bahan obat untuk penyakit kulit panu.

Kata kunci: Bakteri simbion; *Holothuria scabra*; Metode Sumuran; *Pityriasis vesicolor*

INTRODUCTION

Indonesia as a tropical country, is fertile and ideal land for fungal growth. People in tropical countries often experience skin diseases caused by fungi. The presence of the fungus can infect infants and elderly people (Hayati dan Zivenzi, 2014). One of the skin diseases caused by fungi is tinea versicolor (*Pityriasis versicolor*). Tinea versicolor is a disease caused by fungi from the yeast group, namely *Malassezia furfur* (Labeledz et al., 2023). According to Zahra et al. (2019), Tinea versicolor is a skin disease that often occurs in children and adults. This disease can be transmitted through skin

contact or clothing contaminated with fungal spores (Labeledz et al., 2023). To treat this infection, most people use antifungal as medicine.

The necessity for antifungal medicinal ingredients is often met from natural sources, including the ocean. Indonesia's marine waters, which cover more than 60% of its territory, offer a diversity of marine biota which has the potential to be a source of active pharmaceutical ingredients, especially antibiotics (Rijai, 2019). These active compounds from marine biota can act as antifungals (Alawiyah et al. 2016; Maskur et al., 2024). Sea cucumbers are a member of invertebrate animals that are widely used as a source of new biopharmaceuticals (Hanif et al. 2019; Maskur et al. 2024). Alawiyah et al. (2016), reported that sea cucumber extract contains alkaloids, triterpenoids and saponins which are able to inhibit the growth of the fungus *M. furfur*, which causes tinea versicolor. Sand sea cucumbers (*Holothuria scabra*) are known to be rich in secondary metabolites, including sapogenins, saponins, steroids, triterpenoids, phenols, flavonoids, glucosaminoglycans, lectins and alkaloids (Akerina and Sangaji., 2019; Maskur et al. 2024). However, mass extraction of bioactive compounds from sea cucumbers has the potential to threaten wild sea cucumber populations and defy conservation efforts. Therefore, it is important to look for other alternatives to obtain bioactive compounds from sand sea cucumbers without disturbing their sustainability, such as using symbiotic bacteria.

The sand sea cucumber symbiont bacteria shows significant potential as a producer of antibiotic compounds (Pringgenies et al. 2019; Sugireng and Suwarni, 2021; Chen et al., 2021). These symbiotic bacteria tend to produce bioactive compounds similar to their hosts, which is caused by biochemical interactions between the bacteria and the host organism. (Pastra and Surbakti, 2012; Samirudin et al., 2018; Chen et al., 2021). Several symbiont bacteria that interact with sand sea cucumbers are able to produce bioactive compounds that have the potential to act as antifungal agents (Chen et al., 2021; Ereguero et al., 2022). These compounds, produced by sea cucumber symbiont bacteria, could potentially be used to treat *M. furfur*, the fungus that causes tinea versicolor in humans. (Omran & Allam, 2013).

Isolation of sand sea cucumber symbiont bacteria from the waters of Wakatobi National Park which has potential as a *Malassezia* antifungal was carried out in this research. The results of the literature study show that there has been no previous exploration of sea cucumber symbiont bacteria from Wakatobi waters. Therefore, in this research, sand sea cucumber symbiont bacteria were screened which are capable of inhibiting the growth of the fungus *M. furfur* which causes tinea versicolor disease.

MATERIALS AND METHODS

This research was conducted in August-October 2022. The sampling location for sand sea cucumbers was a source of isolates of symbiont bacteria in the waters of Wakatobi National Park, Numana village beach, South Wangi-wangi District, Wakatobi Regency, Southeast Sulawesi. The materials used in this research were sand sea cucumber (*H. Scabra*), *Malassezia furfur* ATCC 14521 as test fungus, Potato Dextrose Agar (PDA) media, semi-solid PDA media, Zobell Marine Agar media, Nutrient Broth (NB) media, alcohol 70%, NaCl 0.9%, distilled water, safranin reagent, crystal violet, H₂O₂ 3%, and olive oil. The tools that will be used include autoclave, bunsen, petri dish, 100-1000 μ L micropipette, hot plate, incubator, glass slide, laminar air flow, microscope (Leica DM 750), analytical balance, digital caliper (MTE), shaker incubator (Stuart S1500), centrifuge (Boeco, Germany).

Adult sand sea cucumbers with a size of 20-30 cm were used as a source of bacterial isolates in this study. Sea cucumbers taken from Wakatobi waters were put in sterile plastic samples and stored in a cool box to be taken to the laboratory. The fungal culture of *M. furfur* ATCC 14521 is the fungus that causes tinea versicolor (Far et al., 2018) obtained from Nano Biolaboratory. The fungal culture was rejuvenated by growing the fungal culture on slanted PDA media in a test tube using the scratch method and incubating at 30°C for 48 hours.

Samples of sand sea cucumbers from which bacteria will be isolated are washed using sterile sea water. Sea water was sterilized using an autoclave at 121°C, 1 atm pressure for 15 minutes. The flesh, skin and intestines of the sand sea cucumber samples were cut and crushed separately then placed in a diluent solution (0.9% NaCl) and a sample dilution series was made from 10⁻¹ to 10⁻⁴. Sample suspensions from dilutions 10⁻², 10⁻³ and 10⁻⁴ were taken as much as 1 mL each and inoculated into three different petri dishes using Zobell Marine Agar media using the pour plate method and then incubated for 24 hours (Arie et al. , 2020).

Screening of the *H. scabra* symbiont bacteria as an antifungal for *Malassezia* was carried out in two stages, namely the first screening to obtain bacteria that are antagonistic to the *M. furfur* fungus

through a bacterial cell antagonist test using the cross streak method and the second screening to obtain bacteria that produce bioactive compounds which has *Malassezia* antifungal activity. In the second stage of screening, bacterial liquid culture supernatant (free cell) was used as a test sample for antifungal activity using the well-diffusion method.

The fungus *M.furfur* is used as a test microbe in testing antifungal activity. The test fungus were prepared in suspension form in 0.85% NaCl solution. The suspension of the test fungus was made by using 1-2 inoculation needles of the *M.furfur* fungus which had been grown on slanted PDA media for 24 hours and suspended it in a sterile 0.85% NaCl solution. The density was equalized to the Mc Farland standard 0.5 (Yanti et al., 2020). This fungal suspension was used in testing antifungal activity using the well diffusion method.

Antifungal activity testing using the well diffusion method uses two layers of media, namely solid PDA media as the base layer and semi-solid PDA media as the seed layer. The well diffusion method is carried out by pouring 10 mL of PDA media into a petri dish until it covers the bottom of the petri dish and leaving it to solidify. The well mold with a diameter of 6 mm is placed on the solidified base medium. After that, 9 mL of semi-solid PDA media which had been mixed with 1 mL of the *M.furfur* fungus suspension and homogenized using a vortex, was poured onto the base media which had a mold. After the semi-solid PDA media solidifies, the mold is removed to form a well. The next step is to insert 50 µL of sample (bacterial supernatant) into the well. The positive control in the antibacterial activity test used the antifungal ketoconazole 100 ppm. The negative control used in antimicrobial testing uses sterile distilled water. After all the wells are filled, the petri dish is then kept in the refrigerator for ± 30 minutes to give the compound a chance to diffuse before incubation (Yanti et al., 2020). After that, the petri dish was incubated at 37°C for 24-48 hours. Bacteria that have *Malassezia* antifungal activity are characterized by the formation of a clear zone around the well. The diameter of the clear zone formed in the test is measured using a caliper.

The bacterial isolates used in the optimization stage of bioactive compound production time are isolates obtained from the second stage of screening, namely screening using bacterial liquid culture supernatant. Optimization of the production time for bioactive compounds was carried out by means of five isolates of symbiont bacteria, each was grown using NB medium which was dissolved using 9 mL of sterile sea water in a test tube, then incubated at a temperature of 34°C for 48 hours with intervals for harvesting the bacterial culture every 6 hours. One mL of bacterial culture was sampled every 6 hours, then the bacterial culture was centrifuged at 7,000 rpm for 15 minutes to separate the pellet and supernatant (Xie et al., 2021). The supernatant is a cell-free metabolite compound used to test *Malassezia* antifungal activity.

Supernatant testing for optimization of the production time of antifungal bioactive compounds by the symbiont bacteria *H. scabra* was carried out using the well diffusion method. In the media that had been inoculated with the *M. furfur* fungus, 50 µL of symbiont bacterial supernatant according to the growth period (6, 12, 18, 24, 30, 36, 42 and 48 hours) was added into the wells. The diameter of the clear zone formed, was measured with a caliper.

The data obtained in the form of the diameter of the clear zone (inhibition zone) were analyzed using the zone measurement formula to determine the antifungal activity of bioactive compounds from sea cucumber isolates against the fungus *M. furfur* (*Malassezia* antifungi). The diameter of the inhibition zone is measured using the following formula (Yanti et al., 2020):

$$Z = \frac{(D1-Ds)+(D2-DS)+(D3-DS)}{3} \quad (1)$$

- D1 : vertical diameter
- D2 : horizontal diameter
- D3 : diagonal diameter
- Ds : well diameter

Characterization of symbiont bacterial isolates that have *Malassezia* antifungal activity was carried out phenotypically. The phenotypic characters observed include cell morphology, biochemical and physiological characters. Morphological characters include cell shape and Gram reaction observed by Gram staining, endospore formation observed by endospore staining and motility observed by growing cultures on semi-solid media using the puncture method. Biochemical characteristics include catalase test, citrate test, indole test and gelatin hydrolysis test. Physiological characteristics include temperature and NaCl tolerance tests. The temperature tolerance test was

carried out by growing bacterial isolates using NB media and incubating them at different temperatures, namely 4°C, 37°C and 45°C. NaCl tolerance testing was carried out by growing cultures on Nutrient Agar (NA) media to which NaCl had been added at concentrations of 0%, 5% and 7%. Identification of symbiont bacterial isolates was carried out using the profile matching method (Yanti et al., 2019). The profile matching method is carried out by matching the characters of symbiont bacterial isolates compared to the characters of the reference genus referring to Bergey's Determinative Bacteriology (Holt et al., 1994).

RESULTS

The symbiont bacteria isolate came from three sources in the sand sea cucumber, namely skin, flesh and intestines. There were 11 bacterial symbiont isolates isolated from sea cucumbers, namely 2 isolates from the skin, 6 isolates from flesh and 3 isolates from the intestines of sand sea cucumbers (Table 1).

Table 1. Number of isolates of sand sea cucumber (*H. Scabra*) symbiont bacteria

No	Isolate source	Number of bacterial isolate	Isolates code
1	Skin sand sea cucumber (KT)	2	KT1
			KT2
2	Flesh sand sea cucumber (DT)	6	DT1
			DT2
			DT3
			DT4
			DT5
			DT6
3	Intestines sand sea cucumber (UT)	3	UT1
			UT2
			UT3

Eleven isolates of sand sea cucumber symbiont bacteria were screened based on their ability to inhibit the growth of the fungus *M. furfur* using an antagonist test. The ability to inhibit the growth of the *M. furfur* fungus was indicated by the formation of a clear zone that was not covered by the fungus around the streaks of the bacterial isolate (Figure 1). The results of the antagonist test, as shown in Table 2, showed that nine out of eleven isolates of sand sea cucumber symbiont bacteria were positive for inhibiting the growth of the test fungus *M. furfur*. Bacterial isolates that were able to inhibit the growth of *M. furfur* were bacterial isolates DT1, DT2, DT3, DT4, DT5, DT6, KT1, UT1 and UT2 (Table 2). The results of screening for the supernatant of the *H. Scabra* symbiont bacteria which had the ability to inhibit the growth of *M. furfur*, after incubation for 48 hours, five of the nine bacterial isolates were found to produce bioactive compounds with *Malassezia* antifungal activity (Table 2).

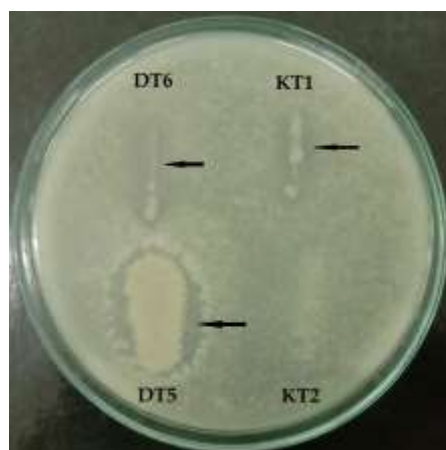


Figure 1. Antagonistic activity of sand sea cucumber symbiont bacterial isolates against the fungus *M. Furfur*. Arrows indicate zones of inhibition (clear zones)

Table 2. Screening results of *H. Scabra* symbiont bacterial isolates based on their ability to inhibit the *M. furfur* fungus

No	Isolate source	Isolate code	Antagonist test result	Antifungal activity of <i>Malassezia</i> supernatant (mm)
1	Flesh sand sea cucumber (DT)	DT1	+	3.6
2		DT2	+	-
3		DT3	+	-
4		DT4	+	0.6
5		DT5	+	2
6	Skin sand sea cucumber (KT)	DT6	+	0.3
7		KT1	+	-
8		KT2	-	nt
9	Intestines sand sea cucumber (UT)	UT1	+	0.2
10		UT2	+	-
11		UT3	-	nt

Description: +: Inhibits, -: Does not inhibit, nt: not tested

The results of optimizing the production time of bioactive compounds that have antifungal activity from the five symbiont bacterial isolates shown in Figure 2, show that the bioactive compounds from DT1 and DT5 bacterial isolates began to show antifungal activity after 12 hours of incubation, DT4 bacterial isolate at 18 hours, UT1 isolate at 42 hours and DT6 isolate at 48 hours of incubation. Bacterial isolates DT1 and DT5 had a quicker production time for bioactive compounds with the highest *Malassezia* antifungal activity, namely 24 hours, then isolate DT4 had the highest antifungal activity at an incubation time of 36 hours. Isolates DT6 and UT1 required a longer incubation time than the three isolates, namely 48 hours.

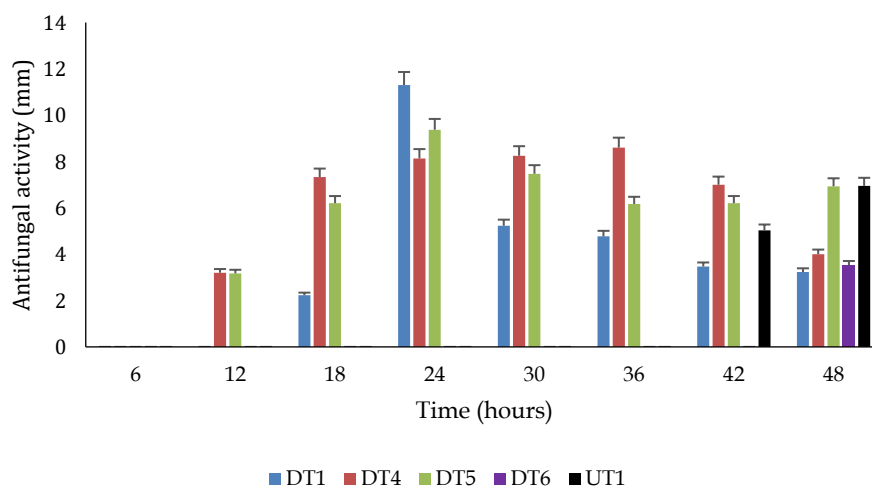


Figure 2. Antifungal activity of *Malassezia* symbiont bacterial isolates based on the time of production of bioactive compounds

The antifungal activity of *Malassezia* of bioactive compounds produced by the three selected isolates of the sand sea cucumber symbiont bacteria which were tested using the well method, showed the formation of a clear zone around the bioactive compounds of the symbiont bacteria isolates, as shown in Figure 3. The results of measuring the antifungal activity of *Malassezia* based on the diameter of the clear zone are presented in Table 3.

Table 3. Antifungal activity of *Malassezia* bioactive compounds isolated from sand sea cucumber symbiont bacteria

No	Isolate code	Antifungal activity of <i>Malassezia</i> (mm)	Time optimization (hours)	Interpretation
1	DT1	11.3±0.47	24	S
2	DT4	8.60±0.55	24	I
3	DT5	9.37±0.38	36	I

Note: S= Strong (inhibition zone diameter 11-20 mm), I = Intermediate/medium (inhibition zone diameter 5-10 mm) (Sirri et al., 2022).



Figure 3. Visualization of the antifungal activity of bioactive compounds from isolates DT1, DT4 and DT5.

Based on the results of profile matching of phenotypic characters between the selected bacterial isolates and the reference bacterial genus, it is known that these bacteria are similar to two genera, namely *Bacillus* and *Pseudomonas*. The results of profile matching of three bacterial isolates with 2 reference genera are shown in Table 4.

Table 4. Identification of sand sea cucumber symbiont bacterial isolates using the profile matching method using phenotypic characters

Characters	Isolate code			Reference genera	
	DT1	DT4	DT5	<i>Pseudomonas</i> ¹	<i>Bacillus</i> ¹
Cell Morphology					
Cell shape	Bacil	Bacil	Bacil	Bacil	Bacil
Endospore	-	+	-	-	+
Gram	Negative	Positive	Negative	Negative	Positive
Motility	+	+	+	+	+
Biochemical Characters					
Catalase	+	+	+	+	+
Citrate test	-	+	+	+/-	+/-
Indole test	-	-	-	-	-
Gelatine hydrolysis	+	+	+	+	+/-
O ₂ requirement	Aerobes	Aerobes	Aerobes	Aerobes	Aerobes/ facultative anaerobes
Physiological Characters					
Temp. 4°C	-	-	-	-	-
Temp. 37°C	+	+	+	+	+
Temp. 45°C	+	+	+	+	+
NaCl 0%	+	+	+	+	+
NaCl 5%	+	+	+	+	+
NaCl 7%	+	+	+	+	+
Identification result	<i>Pseudomonas</i>	<i>Bacillus</i>	<i>Pseudomonas</i>		

¹⁾Bergey's Determinative Bacteriology 9th ed.

DISCUSSION

The results of the isolation of the sea cucumber symbiont bacteria *H. scabra* showed that symbiont bacterial isolates were more commonly found in sea cucumber flesh (6 bacterial isolates) than in the intestines (3 bacterial isolates) and sea cucumber skin (2 bacterial isolates). Flesh is the main part of the sea cucumber's body and its weight reaches 50% of the total body weight of the sea cucumber (Karnila et al., 2011). Bioactive compounds including saponins, which are antifungal compounds, are often produced in the flesh of sea cucumbers (Mewengkang et al., 2022; Maskur et al., 2024). This indicates that the abundance of bioactive compounds can be influenced by the abundance of symbiont bacteria in the flesh of sea cucumbers. Wibowo et al. (2019), also stated that high metabolic activity cannot be separated from the role of symbiont bacteria. The abundance of symbiont bacteria in meat is influenced by the nutritional content of sea cucumber flesh. Sea cucumber flesh contains a lot of protein and carbohydrates which can be utilized by microorganisms as a source of

nutrition. Sea cucumber skin does not contain essential nutrients needed for the growth of microorganisms, because its skin only consists of a layer of calcium carbonate. Likewise, the intestines contain a lot of water and sand (Karnila et al., 2011; Ahmed et al., 2023), so the number of bacterial isolates obtained from the skin and intestines of sea cucumbers tends to be less than the flesh.

The screening results of symbiont bacterial isolates presented in Table 2 show that 9 out of 11 symbiont bacterial isolates were able to inhibit the growth of the *M. furfur* fungus. However, the results of testing the supernatant (cell-free compound) of the nine bacterial isolates, it was found that only 5 bacterial isolates had *Malassezia* antifungal activity, namely isolates DT1, DT4, DT5, DT6 and UT1, while the other 4 isolates were isolates DT2, DT3, KT1 and UT2 did not have *Malassezia* antifungal activity (Table 2). The results of the supernatant screening showed that five isolates of sand sea cucumber symbiont bacteria could produce bioactive compounds to inhibit the growth of *M. furfur*. This indicates that the five isolates have the ability to antagonize antibiosis, while the other four bacterial isolates have the ability to antagonize nutrient competition against *M. furfur*. The mechanism of action of antagonistic microbes against pathogenic microbes can occur through parasitism, antibiotics and competition for space and nutrients (Rochmawati & Trimulyono, 2020; Peterson et al., 2020). According to Mani-Lopez et al. (2022), the supernatant from the fermentation of bacterial liquid culture has antimicrobial activity because in this medium there are many compounds resulting from secondary metabolites that are excreted by bacteria during bacterial growth and have antibiosis against other microbes.

The results of optimizing the production time of bioactive compounds from 5 isolates of sand sea cucumber symbiont bacteria were found to have different optimum times (Figure 2). The optimum time for production of bioactive compounds that have *Malassezia* antifungal activity for DT1 and DT5 is 24 hours, DT4 isolates at 36 hours, DT6 and UT1 isolates at 48 hours. According to Mani-Lopez et al. (2022), differences in growth or incubation time between one bacteria and another can be influenced by the enzymes each bacteria has and this affects the metabolic process in producing bioactive compounds. Based on the results of optimizing the production time for bioactive compounds, it is known that isolates DT1, DT4 and DT5 have the most potential to be developed as medicinal ingredients for tinea versicolor, because the production time is fast.

The bioactive compounds from the symbiont bacterial isolate DT1 succeeded in forming the largest zone of inhibition against the *M. furfur* fungus, with an average diameter of 11.30 mm and was categorized as strong inhibitory (Table 3). This shows that the antifungal activity of *Malassezia* from the bioactive compound of bacterial isolate DT1 is higher compared to bacterial isolates DT4 and DT5, both of which show antifungal activity in the medium category. The diameter of the inhibition zone formed around the well describes the antimicrobial activity of a compound; the greater the diameter of the inhibition zone, the higher the antimicrobial activity of the compound (Yanti et al., 2020; Yanti et al., 2021; Oktaviani et al., 2024).

Based on the results of profile matching of phenotypic characters between the selected symbiont bacterial isolates and the reference bacterial genus, it is known that these bacteria are similar to the two genera. Table 4 shows that the bacterial isolates DT1 and DT5 are identical to the key characters of the genus *Pseudomonas*, namely bacil/rod cell shape, Gram negative, motile, catalase positive and aerobic (Holt et al., 1994) while the DT4 isolate is identical to the key characters of the genus *Bacillus*, namely the cells form of bacil/rod, Gram positive, forms endospores, motile and catalase positive (Holt et al., 1994; Yanti et al., 2019). This indicates that DT1 and DT5 belong to the genus *Pseudomonas* while DT4 belongs to the genus *Bacillus*. Several studies report that bacteria belonging to the genus *Pseudomonas* and *Bacillus* have antimicrobial activity (Pringgenies et al., 2021; Khan et al., 2022; Wang et al., 2022). Khan et al. (2022) reported that bacteria from the genus *Pseudomonas* can produce antifungal compounds such as epiandrosterone, oxolinic acid, Nocodazole rhodotulic acid, pyochelin 9,12-octadecadienoic acid, di-peptide, tri-peptide, pinolenic acid methyl ester, venlafaxine and ursodiol. Bacteria from the genus *Bacillus* are also known as antifungal-producing bacteria (Wang et al., 2022). According to Rochmawati & Trimulyono (2020), bacteria of the genus *Bacillus* can produce bioactive compounds as antifungals such as surfactin, iturin and fengisin or plipastatine.

CONCLUSION

Three isolates of sand sea cucumber symbiont bacteria (bacterial isolates DT1, DT4 and DT5) can produce bioactive compounds that have antifungal *Malassezia furfur* activity. Bacterial isolates DT1 and DT5 belong to the *Pseudomonas* genus, while DT4 bacterial isolates belong to the *Bacillus* genus.

The three isolates of sand sea cucumber symbiont bacteria have the potential to be used as medicine for tinea versicolor.

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