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Metabolite from supernatant of soil and plantassociated bacteria control biofilm of fish pathogens

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Abstract

Objectives This research aimed to identify and quantify the antibiofilm activity of bioactive compounds from bacteria isolated from rhizosphere and nodule butterfly pea (*Clitoria ternatea*), rhizosphere clove afo 3 (*Syzygium aromaticum*), nodule mimosa (*Mimosa pudica* L.), and soil from gold mining land which were recovered from Ternate, Tidore, Obi Island, and Marotai Island, Eastern part of Indonesia.

Results Eight supernatants from soil and plant-associated bacteria were found to have quorum quenching activity against *Chromobacterium violaceum*. All supernatants exhibited antibiofilm activity against biofilm formed by *Aeromonas hydrophila* and *Vibrio harveyi*. The supernatant of FT5 showed the highest activity in disrupting (66.59%) and inhibiting (85.63%) the biofilm of *A. hydrophila*. For *V. harveyi*, the supernatant of PTM3 showed the highest disruption activity (72.61%), whileRCA7 showed the highest inhibition activity(75.68%). The Gas Chromatography-Mass Spectrometry (GC-MS) identified fatty acids, ester, and diketopiperazine as the compounds related to the antibiofilm activity. Molecular identification revealed that the isolates belong to the genera *Bacillus*, *Priestia*, and *Chryseobacterium*.

Keywords Antibiofilm, Fish pathogens, Plant-associated bacteria, Soil bacteria, Supernatant, Quorum sensing

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Introduction

Consumption of seafood products in Indonesia has increased due to public awareness of health, which has also led to a rise in the demand for seafood. Indonesia ranks third after China and Japan as the highest seafood consumer. As a maritime country, Indonesia also exports seafood products, especially shrimp, making it the fourth-highest exporting country after India, Ecuador, and Argentina. To continue meeting the domestic demand and increase exports of fishery products, the Indonesian Ministry of Marine Affairs and Fisheries aims for an 8.5% annual increase in aquaculture production [1]. Therefore, aquaculture is expected to become the leading food supplier by 2026 [2]. However, there are



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some challenges related to infectious diseases and antibiotics have been used to overcome this problem, but some pathogenic bacteria, such as *A. hydrophila* and *V. harveyi*, can form biofilms; antibiotics cannot eradicate cells inside the biofilm matrix.

Biofilm is a layer formed by some bacteria that contains an Extracellular Polymeric Substance (EPS). This EPS consists of polysaccharides, proteins, lipids, and extracellular DNA (eDNA) [3]. Examples of fish pathogen bacteria that can form biofilms are Vibrio harveyi and Aeromonas hydrophila [4]. Before some bacteria form a biofilm, they will first communicate with each other through the quorum sensing (QS) mechanism using specific signal molecules called autoinducers [5]. This communication process between bacteria regulates biofilm formation and other important processes, including the production of bioluminescence and expression of some virulence factors. Hence, bacteria in biofilm, even in extreme conditions or environments, can still adapt and survive and make it more difficult to control [5, 6]. Therefore, it is important to prevent or destroy the biofilms formed by aquaculture pathogens using antibiofilm agent. Metabolites from various bacteria in nature have the potential to be explored with various bioactive compounds. Hence, in this research uses metabolites from soil and plant-associated bacteria as natural sources. Bioactive compounds of phyllosphere bacteria that were isolated from guava (Psidium guajava) leaves reported had anti-quorum sensing activity or quorum quenching (QQ) against C. violaceum and had antibiofilm activity against (A) hydrophila, V. harveyi, and Streptococcus agalactiae [7]. Other study reported, bacteria from soil had inhibiting biofilm formation of Escherichia coli strain M4, P. aeruginosa strain M19, (B) subtilis strain M18, and Klebsiella pneumoniae strain M19 [8]. Therefore, bioactive compounds of soil and plant-associated bacteria have potential as an antibiofilm agent.

Methods

Bacterial cultivation

Thirteen soil and plant-associated bacteria (FT5, F3A.2, RC2.2, RC3.1, M6.2, RCA8, RCA4, RCA7, PTM3, DHG3, DWR1, TE1, TE2) obtained from the Department of Biology Education, Khairun Ternate University, *A. hydrophila* and *V. harveyi*, obtained from Health Aquatic Organism Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University; and *C. violaceum* obtained from Atma Jaya Catholic University of Indonesia culture collections were cultured on Luria-Bertani Agar (LA) that contains 10 g of tryptone (Tryptone; Oxoid), 5 g of yeast extract (Yeast Extract; Oxoid), 10 g of Natrium chloride (NaCl; HIME-DIA), 20 g of agar bacteriological (Agar Bacteriological; Oxoid) in 1000mL of aquadest, except *V. harveyi* cultured

on LA with 2% of NaCl—all bacteria were incubated at 28 °C for 24 h, except *C. violaceum* for 48 h.

Production of supernatant

A single colony of each soil and plant-associated bacteria was cultured on 100 mL of Luria-Bertani Broth (LB) medium and incubated at 28 °C for 24 h in a water bath shaker at 120 rpm. Then, each culture was centrifuged at $5752 \times \text{g}$ for 20 min. The supernatant of each culture was freeze-dried for 48 h to obtain 5× concentration, thereby increasing its activity [9].

Antibacterial activity assay

This assay used the agar well diffusion method. A. hydrophila was cultured on LB medium, and V. harveyi was cultured on LB medium supplemented with 2% of NaCl (w/v). Both cultures were incubated in a water bath shaker at 28 °C, 120 rpm for 24 h. Then, 100 μ L of A. hydrophila ($OD_{600} = 0.132$) was streaked using the continuos streak method in three different directions on Muller Hinton Agar (MHA; Oxoid) medium. Similarly, V. harveyi (OD₆₀₀=0.132) was streaked on MHA supplemented with 2% of NaCl. Each well was filled with 100 µL of supernatant of soil and plant-associated bacteria. For negative control, we used 1% of Dimethyl Sulfoxide (DMSO) while the positive control consisted of 10 mg/ mL of streptomycin. This assay was carried out in triplicate. The plates were incubated at 28 °C for 24 h).Then a clear zone around each well was observed [10].

Qualification of anti-quorum sensing activity

C. violaceum wild type was grown in LB medium for 24 h using a water bath shaker (120 rpm; 28 °C). Then, 100 μ L of *C. violaceum* wild type (OD₆₀₀=0.132) was inoculated into LA. Wells were made using a sterilized cork borer, and each well was filled with 100 μ L of the supernatant. In this assay, we used 1% of DMSO as negative control, while 10 mg/mL of streptomycin was used as positive control. This assay was carried out in triplicate. The plates were incubated at 28 °C for 24 h. After incubation, an opaque zone around each well was observed [10].

Destruction and inhibition of biofilm formation

A. hydrophila was cultured on Brain Heart Infusion Broth (BHIB; Merck) medium, while *V. harveyi* was cultured on BHIB supplemented with 2% of NaCl (w/v). this culture were incubated in water bath shaker at 28 °C for 24 h. For the biofilm destruction assay, 100 μ L of each culture (OD₆₀₀=0.132) was added into a polystyrene 96-well microplate and incubated at 28 °C for 24 h. Then, 100 μ L of the supernatant of soil and plant-associated bacteria was put into the same microplate and re-incubated. For the biofilm inhibition assay, 100 μ L of each culture (OD600=0.132) and 100 μ L of the supernatant of soil and plant-associated bacteria were added together into a microplate and incubated (28 °C; 24 h). Cultures of fish pathogenic bacteria and sterile BHIB were used as untreated control. After incubation, the media and the planktonic cells were discarded using aquadest and air-dried for 5–10 min. The biofilm in each well was stained using 200 μ L of 0.4% (w/v) of crystal violet (Sigma-Aldrich) for 30 min. The crystal violet was rinsed with aquadest and air-dried for 5–10 min. The crystal violet that bound to the biofilm structure was dissolved using200 μ L of 96% ethanol. The absorbance in each well was determined using a microplate reader (TECAN M200 PRO) at 595 nm. This assay was carried out in triplicate and the percentage of destruction and inhibition was determined using this formula [11].

%Destruction & Inhibition =
$$\frac{Abs \text{ fish pathogen} - Abs \text{ sample}}{Abs \text{ fish pathogen}} \times 100\%$$

Biofilm observation

We selected the highest biofilm destruction assay and continued to be assessed using light microscopy and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDS) [12]. For observation of biofilm, 100 μ L of each suspension of fish pathogen bacteria (OD₆₀₀=0.132) was added into a cover glass and incubated at 28 °C for 24 h. Then, 100 μ L of the supernatant of soil and plant-associated bacteria was put into the same cover glass and re-incubated [12].

For light microscopy observation, the cover glass containing the sample was rinsed with aquadest. The biofilm was stained with 0.4% (w/v) of crystal violet for 10 min, rinsed again with aquadest and observed with a microscope at 10×40 [12].

For observation using SEM, the cover glass that contains the sample was rinsed with aquadest. The biofilm was fixated with 2% (w/v) glutaraldehyde (Sigma-Aldrich) and incubated at 4 °C for 24 h. The biofilm was dehydrated with alcohol 30% (v/v), 50% (v/v), 70% (v/v), 96% (v/v), and 99.7% (v/v) for 15 min and let dry using an incubator for 5 min at 37 °C. The biofilm samples were observed under SEM-EDS [13].

Toxicity assay

The toxicity assay used the Brine Shrimp Lethality Assay (BSLA) method [14]. For this assay, concentrations of supernatants used are 100, 500, and 1000 ppm. A total of 3.2 g of brine shrimp eggs (*Artemia salina*) were hatched 24 h in a 3.8% (w/v) salt solution (artificial seawater) with aeration and an LED lamp. Ten *A. salina* at the nauplii stage was added to the 4.5 mL of new artificial water and 0.5 mL of the supernatant of soil and plant-associated bacteria, incubated for 24 h and illuminated with an LED

lamp. This assay used 10 mg/mL of $K_2Cr_2O_7$ as the positive control, while negative control consisted of artificial seawater. Then, the mortality rate of nauplii was determined by using this formula [15].

 $\frac{\text{death nauplii in sample-death nauplii in control}}{\text{alive nauplii in control}} \times 100\%$

Identification of bioactive compounds

Bioactive compounds produced by soil and plant-associated bacteria were identified using Gas Chromatography and Mass Spectrometry (GC-MS) using GC Trace 1310 and MS ISQ LT. The column type that was used is TG5MS. The Carrier gas used was helium (99.999%) with a flow rate of 1 μ L/min. The mode of injection that was used is the split mode, using 250 °C for inlet temperature, split flow 10 μ L /min. The data was read after 2 min (start time) [16].

Molecular identification of soil and plant-associated bacteria

The genome from each soil and plant-associated bacteria was isolated using Wizard® Genomic DNA Purification Kit A1120 (Promega). Amplification of the gene encoding 16S rRNA used five components, such as GoTaq[®] Green Master Mix, primer 63F (5'-CAGGCCTAACACATGCA AGTC-3') for forward primer, primer 1387R (5'-GGGC-GGWGTGTACAAGGC-3') for reverse primer, DNA template, and nuclease-free water [17]. PCR conditions that were used were one cycle of pre-denaturation (95 °C; 2 min), 30 cycles of denaturation (95 °C; 30 s), annealing (55 °C; 30 s), extension (72 °C; 2 min), and one cycle post-extension (72 °C; 10 min). PCR results were visualized using gel electrophoresis 1% of agarose (Agarose; Vivantis) for 60 min at 90 V. Then, the PCR products were sequenced by Genetika Science. The sequence DNA results were processed using SeqTrace software and each data set was compared to data at GenBank using BLASTN.

Statistical analysis

Destruction and Inhibition of biofilm formation assays were carried out in triplicate and analyzed statistically using IBM SPSS Statistics 24. The data tested the normality using Shapiro-Wilk. Independent Sample T-Test was used, if α > 0.05.

Results

Antibacterial activity

The results showed negative for all of the supernatants, showing no clear zone around well. Therefore, antibacterial activity was absent in supernatants of soil and plant-associated bacteria against *A. hydrophila* and *V. harveyi* (Supplementary Table 1).

Qualification of anti-quorum sensing activity

Supernatants of FT5, RC3.1, RCA4, RCA7, PTM3, DWR1, TE1, and TE2 had anti-quorum sensing activity, showing an opaque zone (Supplementary Table 2). The positive result was shown for several supernatants, which showed the presence of an opaque zone that indicated anti-quorum sensing activity (quorum quenching).

Destruction and inhibition of biofilm formation

The destruction and inhibition assay showed that all supernatants had antibiofilm activity against *A. hydrophila* and *V. harveyi* (Fig. 1). In the destruction assay, the supernatant of PTM3 showed the highest activity in disrupting the biofilm of *V. harveyi*, while FT5 showed the highest activity in disrupting the biofilm of *A. hydrophila*. In the inhibition assay, the supernatant of RCA7 performed the highest in inhibiting the biofilm formation of *V. harveyi*, while FT5 showed the highest in inhibiting the biofilm formation.



Fig. 1 The absorbance of (A) destruction (B) inhibition biofilm of A. hydrophila and V. harveyi

the biofilm formation of *A. hydrophila* (Supplementary Table 5).

Biofilm structure

We selected several supernatants with high destruction of biofilm and further continued with light microscopy determination (Fig. 2A-D) and SEM-EDS (Fig. 2E-H). Both light microscopy and SEM showed a decrease in biofilm biomass after the biofilm was treated. The elements of each biofilm were identified using SEM-EDS (Supplementary Table 3).

Toxicity assay

Several supernatants, such as FT5, PTM3, RCA7, TE2, RCA4, and DHG3 were selected for toxicity assay. These supernatants were considered non-toxic because each concentration (100, 500, and 1000 ppm) performed 0% of mortality percentage against nauplii.

Bioactive compounds of supernatant

The bioactive compounds of all the supernatants were identified. The results of the GC-MS analysis are shown in (Table 1). Most compounds had fatty acids, diketopiperazines, esters, and dihydroergotamine.

Species of soil and plant-associated bacteria

Soil and Plant-associated bacteria isolates were identified by sequencing of 16 S-rRNA gene. The sequence DNA results were processed using SeqTrace software, and each data set was compared to data at GenBank using BLASTN, with the result shown in (Supplementary Table 4).

Statistical analysis

Based on SPSS, in the destruction and inhibition assay, biofilm treated with each supernatant had significant differences from the control (*V. harveyi* or *A. hydrophila* biofilm) (p < 0.05).

Discussion

Gold mining soil and plant-associated bacteria have the potential to be explored for their biocontrol abilities, particularly as anti-biofilm agents. In addition to exploring and utilizing natural resources in Indonesia, the discovery of this anti-biofilm agent will help overcome the challenges of infectious diseases caused by pathogenic bacteria that form biofilms in the aquaculture industry. As an initial step, it is essential to evaluate the antibacterial properties of these anti-biofilm agents to ensure they do not interfere with the inhibition of biofilm formation. Based on the Supplementary Table 1, thirteen supernatants of soil and plant-associated bacteria were tested and none showed antibacterial activity against either *V. harveyi* or *A. hydrophila*. It was showed the absence of clear

zones around wells, indicating that the supernatant did not inhibit the fish pathogens [18].

The quorum quenching activity was also analyzed to determine the potential of the supernatant of soil and plant-associated bacteria to inhibit the communication process between bacterial cells. This assay used *C. violaceum* wild type, which produces a dark purple pigment called violacein through QS. The absence of this pigment indicated that the QS of *C. violaceum* wild type was inhibited by various possible mechanisms [19]. There are several mechanisms to inhibit quorum, such as inhibiting the autoinducer synthase, using an autoinducer antagonist, or using an enzyme or other component to inactivate or degrade the autoinducer [20]. Several supernatants of soil and plant-associated bacteria have QQ activity, such as FT5, RC3.1, RCA4, RCA7, PTM3, DWR1, TE1, and TE2 (Supplementary Table 2).

The destruction and inhibition of biofilm formation showed that all supernatants from soil and plant-associated bacteria had antibiofilm activity. The antibiofilm activity in this assay was measured by quantitatively assessing crystal violet binding to the biofilm. Since crystal violet is positively charged, it is bonded with negatively charged biofilm [21]. The results of antibiofilm activity varied between pathogenic bacteria, with FT5 showed the highest activity in disrupting (66.59%) and inhibiting (85.63%) A. hydrophila biofilm, while the supernatant of PTM3 and RCA7 showed the highest activity in disrupting (72.61%) and inhibiting (75.68%) the biofilm of V. harveyi, respectively (Supplementary Table 5). The variability in antibiofilm activity between pathogenic bacteria, potentially due to difference in the biofilm components produced by each bacterium. The biofilm matrix of A. hydrophila includes protein, mannose, glucose, fucose, N-acetylgalactosamine, uronic acid, hydroxyl group, carboxyl group, amine and amide [22]. In contrast, V. harveyi biofilm consists of galactose, glucose, uronic acids, proteins, sulfates, other deoxyhexoses, such as fucose and rhamnose, and pentoses, such as mannose, ribose, arabinose, and xylose [23]. Potential mechanisms for antibiofilm agents include enzymatic degradation of biofilm structure, the use of compounds that can prevent or disrupt biofilm formation, and the inhibition of bacterial QS activity [24].

The quantitative results for destruction assay were further validated through visual observation. Observation of the destroyed biofilm structure under a light microscope and SEM produced results consistent with the quantitative testing (Fig. 2). In Fig. 2B, the biofilm of *A. hydrophila* treated with the FT5 supernatant shows a clear reduction in biomass compared to the control in Fig. 2A. This decrease is further supported by SEM images (Fig. 2E-F). Similarly, for *V. harveyi*, Fig. 2D showed a reduction in



Fig. 2 Biofilm structure of (A) *A. hydrophila* (B) *A. hydrophila* treated with supernatant of FT5 (C) *V. harveyi* (D) *V. harveyi* treated with supernatant of PTM3 using light microscopy and biofilm structure of (E) *A. hydrophila* (F) *A. hydrophila* treated with supernatant of FT5 (G) *V. harveyi* (H) *V. harveyi* treated with supernatant of PTM3 using SEM

Isolate Code	Bioactive Compounds Name	Retention time (min)	% Area
FT5	trans-13-octadecenoic acid	20.10	23.41
	n-Hexadecanoic acid	15.49	14.41
	Hexadecanoic acid,2-hydroxy-1 (hydroxymethyl)ethyl ester	27.54	3.80
F3A.2	trans-13-Octadecenoic acid	20.09	26.98
	n-Hexadecanoic acid	15.49	17.55
	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	27.55	9.10
RC2.2	trans-13-Octadecenoic acid	20.07	23.16
	n-Hexadecanoic acid	15.51	16.21
	Hexadecanoic acid,2-hydroxy-1 (hydroxymethyl)ethyl ester	27.57	3.86
RC3.1	cis-Vaccenic acid	20.04	17.64
	n-Hexadecanoic acid	15.49	12.11
	Ergotaman-3',6',18-trione,9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl), (5'à,10à)-	25.18	2.63
M6.2	trans-13-Octadecenoic acid	20.09	22.53
	n-Hexadecanoic acid	15.53	15.65
	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	27.59	3.88
RCA8	n-Hexadecanoic acid	15.55	6.04
	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	25.26	4.54
	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	13.10	3.61
RCA4	n-Hexadecanoic acid	15.53	6.04
	trans-13-Octadecenoic acid	20.05	5.34
	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	25.25	4.00
RCA7	trans-13-Octadecenoic acid	20.07	11.40
	n-Hexadecanoic acid	15.54	10.86
	Ergotaman-3',6',18-trione,9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl), (5'à,10à)-	25.25	3.54
PTM3	trans-13-Octadecenoic acid	20.06	8.48
	n-Hexadecanoic acid	15.53	8.19
	Ergotaman-3',6',18-trione,9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl), (5'à,10à)-	25.24	3.68
DHG3	n-Hexadecanoic acid	15.57	7.23
	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	25.30	5.80
	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	13.12	4.77
DWR1	n-Hexadecanoic acid	15.56	6.45
	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	25.28	5.05
	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	13.11	4.33
TE1	9-Octadecenoic acid (Z)-,	22.20	14.93
	2-hydroxy-1-(hydroxymethyl)ethyl ester		
	n-Hexadecanoic acid	15.56	4.90
	Ergotaman-3',6',18-trione,9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl), (5'à,10à)-	25.29	4.88
TE2	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	25.31	5.53
	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	14.80	5.03
	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	13.12	4.84

Table 1	Bioactive com	pounds of su	pernatant of	soil and i	plant-associa	ated bacteria
		0001103 01 30	permana or	5011 al 10		

biofilm biomass relative to the control in Fig. 2C, with SEM results confirming this finding (Fig. 2G-H).

In addition to the visual analysis using SEM, EDS analysis revealed additional details about the elemental composition of the biofilms. As shown in Supplementary Table 3, the mass percentages of Carbon (C) and Nitrogen (N) were higher in untreated compared to treated biofilm. This difference may be because carbon and nitrogen are elements contained in biofilm, with carbon as the main element in polysaccharides and nitrogen as the main element in proteins. Additionally, phosphate (P), plays a role as the main elements of extracellular DNA (eDNA).Silicon (Si) was also detected, likely because the biofilm was grown on cover glass that may contain silicon [9, 15].

Based on the GC-MS result, several supernatants had similar compounds; we identified eight major compounds (Table 1). Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester and n-Hexadecenoic acid had quorum quenching activity and antibiofilm activity, particularly against *C. violaceum*. The mechanism of Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, in inhibiting the QS of *C. violaceum* involves binding to CViR, its AHL receptor, through hydrogen

bonding. Specifically, the Gly 70 of the compound binds to the active site of the receptor [19]. Similarly, n-hexadecenoic acid inhibits the QS of C. violaceum through a binding mechanisms with CViR [25]. Cis-vaccenic acid could also exhibit anti-QS against C. violaceum and has the potential as an anti-biofilm agent [26]. Additionally, trans-13-octadecenoic acid has been reported as an antibiofilm against the biofilm of *P. aeruginosa* [27]. We also identified rgotaman-3,6,18-trione,9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl), (5'à,10à)- as one of the primary compounds in the supernatant. However, reports related to this compound as antibiofilm are still limited, although it has been reported for its anti-fungal [28]. Another compound, 9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester might play a role in quorum quenching in inhibiting communication of A. hydrophila and Streptococcus agalactiae [9]. Two diketopiperazines, such as Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) and Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- have been reported to have anti-quorum sensing activity [29, 30]. According to other research, Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) has been proven to inhibit and disrupt the formation of P. aeruginosa, while Pyrroloi[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2methylpropyl)- can inhibit biofilm of P. mirabilis strain BDUMS-2 and E. coli strain BDUMS-3 [30-32].

The brine shrimp lethality assay, which utilizes A. salina, is simple, inexpensive, and highly accurate method for testing toxicity. In the untreated control of this test, there is a possibility of natural deaths occurring, typically no more than 25%. The natural death of (A) salina nauplii is primarily caused by several factors, including the duration of the test, the age of the nauplii, the composition, pH, and salinity of the artificial seawater, as well as the environmental temperature. Typically, aeration is not used during the assay, which reduced the available oxygen for the nauplii. As a result, the nauplii usually cannot survive for more than 48 h [33]. According to the results, toxicity assay of supernatant FT5, PTM3, RCA7, TE2, RCA4, and DHG3 showed that all tested concentrations resulted in 0% mortality against nauplii, whereas the positive control resulted in 100% mortality. Due to the absence of mortality in S. salina, the LC_{50} was not determined. In BSLA, 1000 ppm is typically the highest concentration for determining the LC_{50} value. The absence of mortality across all concentrations indicated that the supernatants do not show toxic effects on nauplii [34]. Based on other study an oral acute toxicity test was conducted on the hydro-methanol extract of Anacardium occidentale, resulting in a 0% mortality rate. This finding indicates that the extract is non-toxic and safe, as the subject used in the study, did not cause mortality upon treatment [35]. Molecular identification of soil and plant-associated bacteria performs three genera: Bacillus, Priestia, and Chryseobacterium (Supplementary Table 4). There are Bacillus thuringiensis, Bacillus sp, Priestia megaterium, Bacillus cereus, Priestia aryabhattai, Chryseobacterium sp., and Bacillus tropicus, It has been reported (B) thuringiensis produces Aiia lactonase. Enzyme from this family is one of the reasons quorum sensing and bacterial biofilm formation can be inhibited [36]. Bacillus sp. and P. megaterium also has been found to produce protease which can degrade protein in biofilm matrix of Staphylococcus aureus [37]. Meanwhile, B. cereus and P. aryabhattai produce metabolite that has anti-QS activity by degrading C6-HSL, a signaling molecule of Gram-negative bacteria. This occurs because these bacteria can produce the enzyme N-acyl homoserine lactonase, which breaks down N-acyl-L-homoserine lactone (AHL). Additionally, these bacteria also can inhibit the formation of biofilms by Vibrio cholerae, P. aeruginosa, and S. aureus [38]. Similarly, Chryseobacterium sp. produces the AHL-lactonase enzyme, thereby inhibiting the quorum sensing process [39]. On the other hand, studies related to the use of B. tropicus metabolites as antibiofilm agents are still limited. However, it is possible that this bacterium has potential as an antibiofilm agent. B. tropicus can produce keratinase, a type of protease that can degrade keratin [40]. Based on other study, keratinase has been shown to reduce biofilm formation of E. coli and S. aureus [41].

Limitations

This research conducted only for two fish pathogens, *A. hydrophila* and *V. harveyi*, which require the use of other fish pathogen bacteria. The anti-quorum sensing activity in this research was identified as a qualitative; therefore, it must be assessed quantitatively.

Abbreviations

- BHIB Brain Heart Infusion Broth
- LB Luria-Bertani Broth
- SEM Scanning Electron Microscope
- EDS Energy Disperse X-ray Spectroscopy
- EPS Extra Polymeric Substance
- QS Quorum Sensing
- QQ Quorum Quenching

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-024-06974-7.

Supplementary Material 1

Author contributions

VE: Conduct the research, analyze the data, and prepare the manuscript under DEW's advisory. DEW: design the research, advise the research, and personal investigator. NP and N: conducted bacteria isolation. PGSJ: data analysis. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Henriksson PJG, Banks LK, Suri SK, Pratiwi TY, Fatan NA, Troell M. Indonesian aquaculture futures-identifying interventions for reducing environmental impacts. Environ Res Lett. 2019;14(12):124062. https://doi. org/10.1088/1748-9326/ab4b79.
- Farradia Y, Sunarno MTD. Consumer purchase decision on fresh fish in the New Norm: preliminary Case Study in Indonesia. J Soc Transform Reg Dev. 2020;2(1). https://doi.org/10.30880/jstard.2020.02.01.006.
- Di Martino P. Extracellular polymeric substances, a key element in understanding biofilm phenotype. AIMS Microbiol; 2018.
- Lukman G, Waturangi DE, Julyantoro PGS, Papuangan N. Phyllosphere bacteria with antiquorum sensing and antibiofilm activities against fish pathogenic bacteria. BMC Res Notes. 2024;17:1.
- Hawver LA, Jung SA, Ng WL. Specificity and complexity in bacterial quorumsensing systems. FEMS Microbiol Rev. 2016;40(5):738–52.
- 6. Rutherford ST, Bassler BL. Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med. 2012;2(11):1–26.
- Nathalia O, Waturangi DE. Extract from phyllosphere bacteria with antibiofilm and quorum quenching activity to control several fish pathogenic bacteria. BMC Res Notes. 2021;14:1.
- Prastya ME, Simbolon S, Priyanto J, Hasidu LOAF, Permatasari V, Primahana G et al. Antibacterial and antibiofilm activities from soil *Streptomyces* spp. isolated from Muna Island, Indonesia against multidrug-resistant clinical isolates. Res Sq. 2024.
- Kurniawan J, Waturangi DE, Julyantoro PGS, Papuangan N. Ice nucleation active bacteria metabolites as antibiofilm agent to control *Aeromonas hydrophila* and *Streptococcus agalactiae* infections in aquaculture. BMC Res Notes. 2024;17:1.
- Mulya E, Waturangi DE. Screening and quantification of anti-quorum sensing and antibiofilm activity of *Actinomycetes* isolates against food spoilage biofilm-forming bacteria. BMC Microbiol. 2021;21:1.
- 11. Theodora NA, Dominika V, Waturangi DE. Screening and quantification of anti-quorum sensing and antibiofilm activities of phyllosphere bacteria against biofilm forming bacteria. BMC Res Notes. 2019;12:1.
- Anne G, Waturangi DE. Supernatant of Actinomycetes isolates with antiquorum sensing and antibiofilm activity against food spoilage bacteria. Biodiversitas. 2023;24:9.
- 13. Sun B, Luo H, Jiang H, Wang Z, Jia A. Inhibition of quorum sensing and biofilm formation of esculetin on *Aeromonas hydrophila*. Front Microbiol. 2021;12.
- 14. Raissa G, Waturangi DE, Wahjuningrum D. Screening of antibiofilm and anti-quorum sensing activty of *Actinomycetes* isolates extracts against aquaculture pathogenic bacteria. BMC Microbiol. 2020;20:1.
- Ajibola OO, Lihan S, Hussaini A, Saat R, Ahmed IA, Abideen W, et al. Toxicity assessment of lactococcus lactis IO-1 used in coconut beverages against *Artemia salina* using brine shrimp lethality test. Appl Food Biotechnol. 2020;7:3.

- Miller T, Waturangi DE, Yogiara. Antibiofilm properties of bioactive compounds from *Actinomycetes* against foodborne and fish pathogens. Sci Rep. 2022;12:1. https://doi.org/10.1038/s41598-022-23455-8.
- 17. Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, et al. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl Environ Microbiol. 1998;64:1.
- Nalawade TM, Bhat KG, Sogi S. Antimicrobial activity of endodontic medicaments and vehicles using agar well diffusion method on facultative and obligate anaerobes. Int J Clin Pediatr Dent. 2016;9:1.
- Venkatramanan M, Sankar Ganesh P, Senthil R, Akshay J, Veera Ravi A, Langeswaran K, et al. Inhibition of quorum sensing and biofilm formation in *Chromobacterium violaceum* by fruit extracts of *Passiflora edulis*. ACS Omega. 2020;5:40.
- Paluch E, Rewak-Soroczyńska J, Jędrusik I, Mazurkiewicz E, Jermakow K. Prevention of biofilm formation by quorum quenching. Appl Microbiol Biotechnol. 2020;104:5.
- 21. Ebert C, Tuchscherr L, Unger N, Pöllath C, Gladigau F, Popp J, et al. Correlation of crystal violet biofilm test results of *Staphylococcus aureus* clinical isolates with Raman spectroscopic read-out. J Raman Spectrosc. 2021;52:12.
- 22. Castro L, Zhang R, Muñoz JA, González F, Blázquez ML, Sand W, et al. Characterization of exopolymeric substances (EPS) produced by *Aeromonas hydrophila* under reducing conditions. Biofouling. 2014;30:4.
- Bramhachari PV, Dubey SK. Isolation and characterization of exopolysaccharide produced by Vibrio harveyi strain VB23. Lett Appl Microbiol. 2006;43:5.
- Algburi A, Comito N, Kashtanov D, Dicks LMT, Chikindas ML. Control of biofilm formation: antibiotics and beyond. Appl Environ Microbiol. 2017;83:3.
- Patel M, Siddiqui AJ, Ashraf SA, Surti M, Awadelkareem AM, Snoussi M, et al. Lactiplantibacillus plantarum-derived biosurfactant attenuates quorum sensing-mediated virulence and biofilm formation in *Pseudomonas aeruginosa* and *Chromobacterium violaceum*. Microorganisms. 2022;10:5.
- 26. Tsilo PH, Maliehe ST, Shandu JS, Khan R. Chemical composition and some biological activities of the methanolic encephalartos ferox fruit extract. Pharmacogn J. 2020;12:5.
- El-Sapagh S, El-Shenody R, Pereira L, Elshobary M. Unveiling the potential of algal extracts as promising antibacterial and antibiofilm agents against multidrug-resistant *Pseudomonas aeruginosa*: in vitro and in silico studies including molecular docking. Plants. 2023;12:18.
- Lulamba TE, Green E, Serepa-Dlamini MH. Photorhabdus sp. ETL antimicrobial properties and characterization of its secondary metabolites by gas chromatography–mass spectrometry. Life. 2021;11:8.
- Pooja S, Aditi T, Naine SJ, Subathra Devi C. Bioactive compounds from marine Streptomyces sp. VITPSA as therapeutics. Front Biol. 2017;12:4.
- Díaz MA, González SN, Alberto MR, Arena ME. Human probiotic bacteria attenuate *Pseudomonas aeruginosa* biofilm and virulence by quorum-sensing inhibition. Biofouling. 2020. https://doi.org/10.1080/08927014.2020.1783253.
- Parasuraman P, Devadatha B, Sarma VV, Ranganathan S, Ampasala DR, Siddhardha B. Anti-quorum sensing and antibiofilm activities of *blastobotrys Parvus* PPR3 against *Pseudomonas aeruginosa* PAO1. Microb Pathog. 2020;138.
- Rajivgandhi G, Vijayan R, Maruthupandy M, Vaseeharan B, Manoharan N. Antibiofilm effect of *Nocardiopsis* sp. GRG 1 (KT235640) compound against biofilm forming Gram negative bacteria on UTIs. Microb Pathog. 2018;118.
- Salleh SF, Ajibola OO, Nolasco-Hipolito C, Husaini A, Zarrabal-Octavio C, Lihan S, et al. Fatty acid profile and antioxidant capacity of dabai (*Canarium Odon-tophyllum* L.): effect of origin and fruit component. Molecules. 2022;27:12.
- El-Fadaly HAA, El-Kadi SM, El-Moghazy MM, Soliman AAS, El-Haysha MSM. Correlation between active components of rocket (*Eruca sativa*) as cytotoxicity (brine shrimp lethality assay). Am J Biomed Sci Eng. 2017;3:20–4.
- Omeke JN, Anaga AO, Okoye JA. Brine shrimp lethality and acute toxicity tests of different hydro-methanol extracts of *Anacardium occidentale* using in vitro and in vivo models: a preliminary study. Comp Clin Path. 2018;27:1717–21.
- Anandan K, Vittal RR. Quorum quenching activity of AiiA lactonase KMMI17 from endophytic *Bacillus thuringiensis* KMCL07 on AHL- mediated pathogenic phenotype in *Pseudomonas aeruginosa*. Microb Pathog. 2019;132.
- Mantaring S, Almazan DJDJ, Arcan SK, Noval N, Palanca A, Jose JP, et al. Locally-isolated protease-producing *Bacillus* spp. from soil inhibits biofilm formation of *Staphylococcus aureus*. Pharm Sci Asia. 2023;50:138–46.
- Shevate SN, Shinde SS, Bankar AV, Patil NP. Identification of quorum quenching N-Acyl Homoserine Lactonases from *Priestia Aryabhattai* J1D and *Bacillus cereus* G isolated from the rhizosphere. Curr Microbiol. 2023. https://doi. org/10.1007/s00284-023-03186-3.

- Rashid R, Morohoshi T, Someya N, Ikeda T. Degradation of N-acylhomoserine lactone quorum sensing signaling molecules by potato root surface-associated chryseobacterium strains. Microbes Environ. 2011;26:144–8.
- Shen N, Yang M, Xie C, Pan J, Pang K, Zhang H, et al. Isolation and identification of a feather degrading *Bacillus tropicus* strain Gxun-17 from marine environment and its enzyme characteristics. BMC Biotechnol. 2022. https:// doi.org/10.1186/s12896-022-00742-w.
- Bhange K, Chaturvedi V, Bhatt R. Potential biofilm dispersal by a partially purified keratinase produced by *Stenotrophomonas maltophilia* strain Kb2. Biocatal Agric Biotechnol. 2015. https://doi.org/10.1016/j.bcab.2015.10.010.

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