BMC Research Notes

RESEARCH NOTE

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Assessment of antibiofilm and quorum quenching potencies of environmental bacteria in controlling biofilm of food spoilage bacteria

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Abstract

Objectives This research aims to investigate anti-quorum sensing and antibiofilm activity of supernatants from environmental bacteria against the biofilm formed by food spoilage bacteria such as *Bacillus cereus*, *Bacillus subtilis*, and *Shewanella putrefaciens*.

Results Supernatants were generated from ten environmental bacteria isolates (A19, A30, A32, A40, B10, B212, C1, J70, J73, and T152), with four isolates (A19, A32, A40, B212) showed anti-quorum sensing activity against *Chromobacterium violaceum* wild type as indicator bacteria. In inhibition and destruction assays, the highest percentage inhibition of 81.42% and 81.33% by B10 and B212, respectively, against *B. cereus* and J73 against *B. subtilis* was recorded at 87.45%. While A32, T152, and C1 performed the highest destruction against *B. cereus*, *B. subtilis*, and *S. putrefaciens* with percentages of 45.4%, 83.81%, 74.81%, respectively. Observation using light microscopy and Scanning Electron Microscopy (SEM) revealed C, O, Na, Mg, Al, Si, K, and Ca elements were detected which might play role in biofilm formation. Based on 16s rRNA sequencing, the environmental bacteria isolates were identified as *Enterobacter*, *Acinetobacter*, *Acinetobacter*, *Pantoea* genera, C1, and T152. These results imply that these bacteria have destructing and inhibiting potential against *Bacillus cereus*, *Bacillus subtillis*, *Shewanella putrefaciens*.

Keywords Antibiofilm, Environmental bacteria, Food spoilage, Quorum quenching

Introduction

Food spoilage bacteria cause substantial economic losses in the food industry by reducing the quality and shelf life of products. While these bacteria primarily pose economic challenges, they can also lead to occasional safety concerns when their metabolites, such as toxins, are involved. Common food spoilage bacteria include *Bacillus cereus*, *Bacillus subtilis*, and *Shewanella putrefaciens*. These microorganisms not only degrade food products but are also capable of forming biofilms that are highly resistant to conventional cleaning and sanitization methods, creating persistent challenges in food processing environments [1, 2].

Biofilm formation is initiated through quorum sensing, a bacterial communication mechanism that regulates gene expression in response to population density. This adaptation allows biofilms to become more resilient to environmental stresses, making their removal difficult and contributing to the persistence of spoilage bacteria in food production facilities. Furthermore, biofilms act as protective structures for bacterial cells, shielding them from antimicrobial agents and cleaning processes [3]. Recent studies have highlighted the significant role of biofilms in harboring spoilage bacteria in dairy and other food products, emphasizing the importance of effective biofilm control strategies [4].

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In light of these challenges, alternative strategies have been developed to combat biofilm formation. The use of natural antibiofilm agents derived from microbial metabolites has emerged as a promising approach [5]. This study aims to explore bioactive compounds produced by environmental bacteria and evaluate their potential as antibiofilm agents, contributing to the development of sustainable solutions for managing food spoilage bacteria.

Methods

Bacterial cultivation

Ten environmental bacteria isolates (A19, A30, A32, A40, B10, B212, C1, J70, J73, and T152), previously recovered by Stephanie and Waturangi [6], were cultured on Luria Agar (LA) (Oxoid, Basingstoke, United Kingdom) and incubated at 30 ℃ for 48 h. *Chromobacterium violaceum* wild type and 026 (mutant) as indicator bacteria were cultured on Luria Agar (Oxoid, Basingstoke, United Kingdom) at 28 ℃ for 48 h. Additionally, food spoilage bacteria whereas *Bacillus cereus*, *B. subtilis*, and *S. putrefaciens* were cultured on Luria Agar (Oxoid, Basingstoke, United Kingdom) at 37 ℃ and 28 ℃ for 24 h, respectively.

Production of supernatant

Ten environmental bacteria isolates (A19, A30, A32, A40, B10, B212, C1, J70, J73, and T152) were cultured in 250 mL Erlenmeyer flask with 100 mL of Luria Broth (LB, Oxoid, UK) and incubated at 30 ℃ with 120 rpm agitation for 48 h. These ten environmental bacteria suspensions were subsequently centrifuged at 5752×*g* for 20 min. The resulting cell-free metabolites were concentrated fivefold using a freeze-dryer [7].

Antibacterial activity

This assay utilized the agar well diffusion method on Mueller-Hilton Agar (MHA). *B. cereus*, *B. subtilis*, and *S. putrefaciens* were cultured in LB medium and incubated at optimal temperatures with shaking at 150 rpm for 24 h. The food spoilage suspensions were diluted in sterile LB medium until reached an absorbance value of 0.132 at 600 nm. Subsequently, 100 µL of these food spoilage diluted suspension cultures were spread onto MHA plates. Wells were then created in the agar using a sterile cork borer, and each well was filled with 100 µL of environmental bacteria metabolites. The plates were then incubated at optimal temperatures. Negative control (sterile LB medium) and positive control (streptomycin 10 mg/mL) were included, and the presence or absence of clear zones around the wells determined the antibacterial activity [8].

Detection of quorum quenching activity

Ten environmental bacteria isolates (A19, A30, A32, A40, B10, B212, C1, J70, J73, and T152) metabolites were assessed for quorum quenching activity using the agar-well diffusion method, following Mulya and Waturangi [8] protocol. Wild type *Chromatobacterium violaceum* ATCC 12,472 was cultured in 5 mL Luria Broth at 28 ℃ with 120 rpm agitation for 24 h. The resulting suspension was adjusted to OD600 = 0.132 and spread onto Luria Agar (LA) medium. Wells were created and filled with 100 µL of environmental bacteria metabolites independently, followed by incubation at 28 ℃ for 24 h. Negative controls (Luria Broth) and a positive control (streptomycin 10 mg/mL) were included. Quorum quenching activity, indicated by transparent zones around the wells, was observed in triplicate.

Validation of Quorum sensing inhibition

Selected environmental bacteria isolates (A19, A32, A40, and B212) were analyzed using a modified version of Rajivgandhi et al. [9] study. *C. violaceum* 026 was cultured in 10 mL sterile LB medium for 24 h at 28 ℃ with 120 rpm agitation. Then, CV026 suspension was adjusted to 0.1 at 540 nm. As much as 500 µL CV026 and 500 µL environmental bacteria metabolite were added at ratio 1:1 into microtubes. Then, 1 µmol/mL of Hexanoyl Homoserine Lactone (HHL) (Sigma) was added into the mixture and incubated for 24 h at 28 ℃.

After centrifugation at 5214×*g* for 15 min, the supernatant was discarded, and the culture was resuspended in 1 mL of Dimethyl Sulfoxide (DMSO) 1% (v/v) and centrifuged again. The violacein activity in the supernatants was measured at 540 nm. Positive and negative controls were included, where the positive control consisted of a mixture of bacterial culture and HHL, and the negative control consisted of *C. violaceum* 026. This was done in triplicate.

Quantification of antibiofilm

This modified method was based on assays described by Behzadi et al. and Misra et al. [10, 11] and comprised inhibition and destruction assays. Food spoilage bacteria were cultured in Brain Heart Infusion Broth (BHIB) (Oxoid, Basingstoke, United Kingdom) with 2% glucose overnight at optimal temperature and with optimal density adjusted to 0.132 at 600 nm. In inhibition test, as much as 100 µL of both food spoilage bacteria suspensions and environmental bacteria metabolites were added to microplate wells and incubated at optimum temperature. Meanwhile, for the destruction test, food spoilage bacteria suspension was added to wells, followed by incubation at optimum temperature. The next

day, environmental bacteria suspensions were added and incubated again. The positive control used was food spoilage cultures and the negative control was sterile BHIB + 2% glucose.

After incubation, planktonic cells and media were discarded and each well was rinsed with aquadest, air-dried, and stained with 200 µL of 0.4% (w/v) crystal violet. Then, the crystal violet was discarded and rinsed with aquadest and being air-dried for 30 min. As much as 200 µL of ethanol was added to each well, and absorbance was measured at 595 nm. This assay was performed in triplicate, and the percentage of biofilm inhibition and destruction was calculated using the formula:

$$\%\ \text{inhibition/destruction} = \frac{\text{OD Control} - \text{OD Sample}}{\text{OD Control}} \times 100\%$$

Biofilm morphology analysis

To analyze the cell morphology of the biofilms formed by the selected isolates (A32, C1, and T152), light microscopy and Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDS) techniques were employed. Food spoilage bacteria were grown in BHIB + 2% glucose overnight at optimal temperature until reaching OD600 = 0.132. Bacterial suspensions were added to sterile cover glass and incubated overnight. Then, 100 µL of selected bacterial metabolite was added and incubated for 24 h. For light microscopy, cover glass was rinsed with sterile aquades, stained with crystal violet, and incubated for 15 min before observation at 40× magnification. For SEM-EDS, cover glass was fixed in 2% glutaraldehyde at 4 ℃ for 24 h, dehydrated with alcohol (30%, 50%, 70%, 96%, and 100% concentrations) for 15 min each, and coated with gold (Au). Observations were conducted at Badan Riset dan Inovasi Nasional (BRIN) at magnifications of 65×, 1000×, 2000×, and 5000× [12, 13].

Identification of environmental bacteria isolates

Ten environmental isolates were identified using a modified version of the method described by Jahnke and Bahnweg [14]. Genomic DNA was isolated using the Promega Wizard Genomic DNA Extraction Kit. PCR amplification of the 16 S rRNA gene was performed using primers 63 F (forward) and 1387R (reverse). PCR conditions included an initial denaturation step at 94 ℃ for 2 min, followed by 2 cycles of denaturation at 94 ℃ for 30 s, annealing at 55 ℃ for 30 s, and extension at 72 ℃ for 1 min. The final elongation step was at 72 ℃ for 20 min. PCR products were separated on a 1% agarose gel at 70 V for 90 min and visualized. DNA Sequencing was performed at Genetika Science, and the results were submitted to GenBank. Phylogenetic tree of molecular identification of environmental bacteria was generated using MegaX.

GC-MS analysis

Bacterial metabolites from various bacteria (A32, B10, B212, C1, J73, and T152) were analyzed using GC-MS [15]. A 1 µL injection of the filtered bacterial metabolite was made into a column measuring 30 m in length, 250 µm in diameter, and 0.25 µm in width. Helium gas served as the carrier gas at a total flow rate of 24 mL/min, with an oven temperature set at 325 ℃ for 36 min. Compound identification was based on mass spectrum data of each peak.

Statistical analysis

The datas were analyzed using statistical software called IBM SPSS Statistics 24. This program used non-parametric Kruskal-Wallis on the data. If the probability obtaining observed differences by chance is less than 0.05.

Results

Antibacterial activity

Among all tested environmental isolates, only T152 showed antibacterial activity against *S. putrefaciens*, as evidenced by the clear zone around the well (supplementary Table 1).

Detection of quorum quenching activity

We found that A19, A32, A40, and B212 demonstrated positive results, as evidenced by the formation of opaque zone around the wells (supplementary Table 2).

Validation of quorum sensing inhibition

The four environmental isolates exhibited anti-quorum sensing activity, as indicated by reduced absorbance compared to the control. Among them, A19 displayed the highest quorum sensing inhibition against *C. violaceum* 026, with a notable absorbance difference of 0.0385 (Supplementary Fig. 1).

Quantification of antibiofilm activity

All environmental bacterial metabolites displayed varied antibiofilm activities against food spoilage bacteria biofilms, encompassing both inhibition and destruction. Statistical analysis (Kruskal-Wallis test, $p < 0.05$) revealed significant differences between untreated control groups and all treatment groups, indicating their potential activity compared to controls. Notably, B10 metabolite exhibited the highest inhibition activity against *B. cereus* (81.42%), J73 metabolite against *B. subtilis* (87.45%), and B212 metabolite against *S. putrefaciens* (70.21%). Moreover, the highest destruction activity against biofilm formation was observed with

A32 (45.4%) against *B. cereus*, T152 (83.81%) against *B. subtilis*, and C1 (74.81%) against *S. putrefaciens* (Fig. 1).

Microscopic examination of biofilm

Biofilm morphology was analyzed using light microscopy and SEM with EDS examination. Selected metabolites, chosen based on their destruction activity, were observed at 40× magnification (Supplementary Figs. 2–4; Fig. 2; supplementary Table 3).

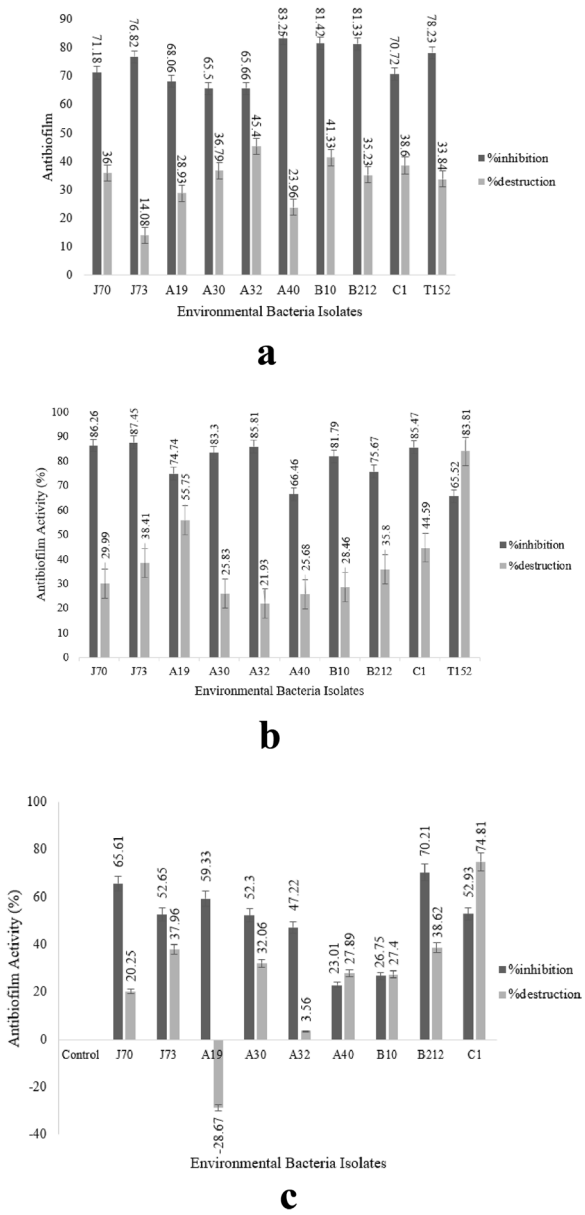

Fig. 1 Antibiofilm activity of environmental bacteria metabolites against **a** *B. cereus*, **b** *B. subtilis*, and **c** *S. putrefaciens*

Identification of environmental bacteria isolates and phylogenetic construction

DNA sequencing of the 16 S rRNA gene was used to identify eight INA bacterial isolates, and the results were submitted to GenBank. All eight isolates showed similarity levels above 85%. Specifically, isolates A19, A30, and A32 were found to be closely related to *Pantoea* with similarity percentages of 99.59%, 100%, and 99.92%, respectively. Isolates A40, B212, and J73 were identified as closely related to *Acinetobacter* with similarity percentages of 100%, 100%, and 99.92%, respectively. Lastly, isolates B10 and J70 were identified as similar to *Enterobacter*, with percentages of 100% and 99.66%, respectively (Table 1). We constructed a phylogenetic tree and the analysis clearly clustered the species sequences in separate groups. The phylogenetic tree grouped A32 and A19 with 99% identitiy. It also grouped isolates A40, B212, and J73 together, indicating they were closely related. Moreover, the genetic distance matrix showed a value of 0.002 between isolate A32 and A19 (Supplementary Fig. 14).

GC-MS analysis

GC-MS analysis revealed major compounds in A32, B10, B212, C1, J73, and T152 metabolites. A32 contained n-Hexadecanoic acid and trans-13-Octadecenoic acid (retention time: 16.21, 17.99), while B10 had trans-13-Octadecenoic acid and 9-Octadecenoic acid (retention time: 18.00, 28.34). B212 consisted of cis-Vaccenic acid and 9-Octadecenoic acid (retention time: 17.97, 28.28), and C1 contained trans-13-Octadecenoic acid and 9-Octadecenoic acid (retention time: 17.98, 28.3). Similarly, J73 had trans-13-Octadecenoic acid and 9-Octadecenoic acid (retention time: 17.99, 28.34), while T152 contained n-Hexadecanoic acid and 9,12-Octadecadienoic acid (retention time: 16.3, 17.99) (Table 2).

Statistical analysis

Using SPSS statistical analysis, it was found that both the inhibition and destruction assays against *B. cereus*, *B. subtilis*, *S. putrefaciens* produced a p-value of 0.003, 0.019, and 0.001 respectively (Supplementary Figs. 11–13).

Discussion

B. cereus, *B. subtilis*, *S. putrefaciens* are prevalent and common problematic in food processing environments due to their ability to form biofilm and toxins under suitable conditions [16]. The capacity of these bacteria embedded in biofilms to withstand traditional treatments highlights the need for ongoing research to discover antibiofilm agents from metabolite of environmental bacteria.

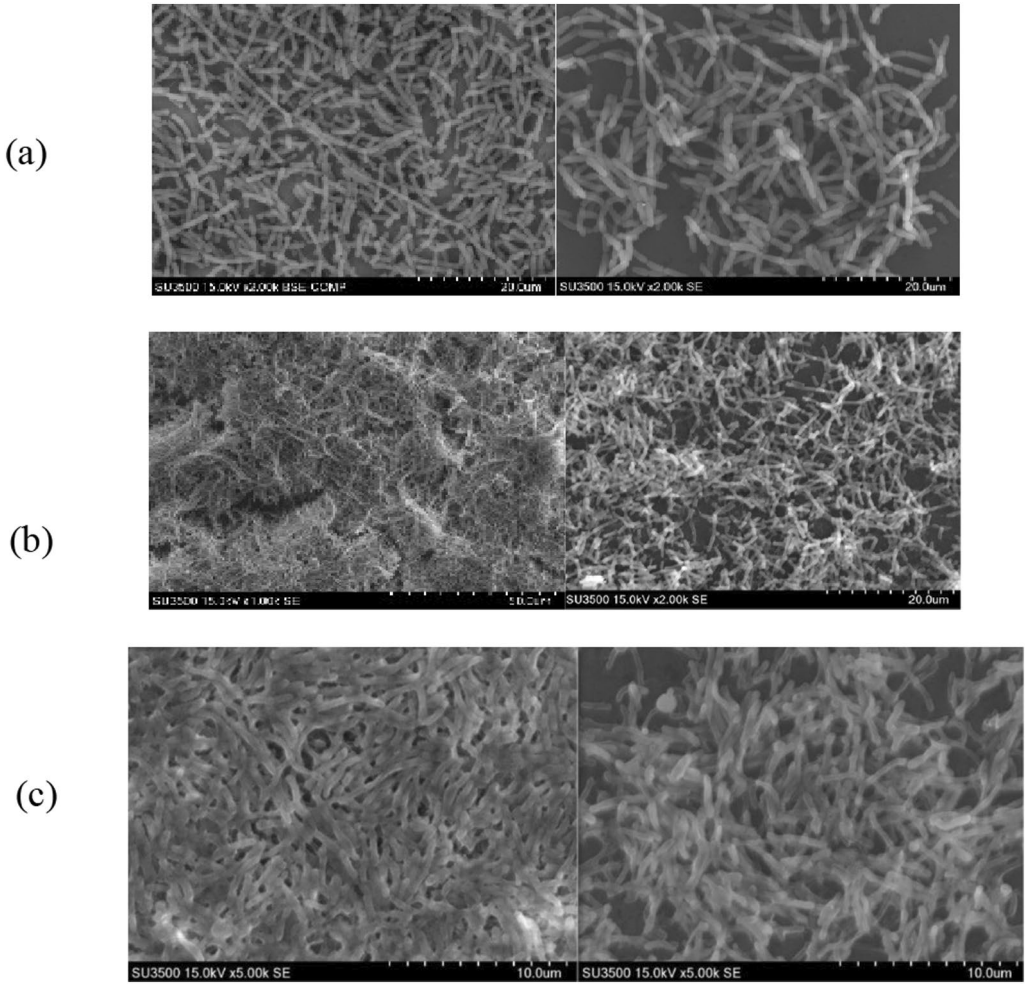

Fig. 2 Biofilm on SEM. **a** *B. cereus* biofilms (left to right: untreated biofilm, treated with A32 extracts); **b** *B. subtilis* biofilms (left to right: untreated biofilm, treated with T152 extracts); **c** *S. putrefaciens* biofilms (left to right: untreated biofilm, treated with C1 extracts)

Table 1 Molecular identification of environmental bacteria

Isolates	Bacterial Strain	% Similarity	GeneBank Accession Number
A19	*P. stewartii* HR3-48	99.59%	OR128363
A30	*P. stewartii* HR3-48	100%	OR131584
A32	*P. stewartii* HR3-48	99.92%	OR131589
A40	*Acinetobacter pittii* LT504	100%	OR131746
B10	*Enterobacter sp.* P6-14	100%	OR131747
B212	*Acinetobacter baumannii* A32	100%	OR133538
J70	*Enterobacter* sp. M204	99.66%	OR133539
J73	*Acinetobacter junii* QLN201710OPB4	99.92%	OR133540

As a preliminary step, an antibacterial assay was conducted. According to Supplementary Table 1, none of the metabolites from environmental bacteria demonstrated antibacterial activity against *B. cereus*, *B. subtilis*, and *S. putrafaciens*, except for isolate T152, which showed antibacterial activity against *S.*

Table 2 GC-MS analysis of environmental bacteria metabolite

Isolates	Component Name	RT	%Area
A32	n-Hexadecanoic acid	16.31	5.13
	trans-13-Octadecenoic acid	17.99	9.7
B10	trans-13-Octadecenoic acid	16.31	11.01
	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	18.00	12.09
	cis-Vaccenic acid	16.28	6.63
B212	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	17.97	15.33
	trans-13-Octadecenoic acid	17.98	8.11
C1	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	28.3	15.7
J73	n-Hexadecanoic acid	16.30	12.81
	trans-13-Octadecenoic acid	17.99	11.21
T152	n-Hexadecanoic acid	16.33	8.84
	9,12-Octadecadienoic acid (Z,Z)-	17.99	15.39

putrefaciens. This variation may be due to the effect of concentration of the agent and bioactive components [17]. To prevent potential false positive results in susbsequent antibiofilm analyses, the bioactive compounds from isolates exhibiting antibacterial activity were not pursued further [18].

In screening anti-quorum sensing (QS) bacteria against *Choromobacterium violaceum* wild type, four out of ten environmental bacteria metabolites (A19, A32, A40, and B212) displayed positive quorum quenching activity without affecting bacterial growth. These isolates contain natural compounds capable of inhibiting quorum sensing, thereby reducing violacein production [19]. *C. violaceum* wild type, which produces the purple pigment violacein regulated by quorum sensing, serves as an indicator bacterium. The QS mechanism involves the vio operon, responsible for violacein production using tryptophan and regulated by AHLs through the Cvil protein [20].

For further investigation of the selected bacterial metabolites (A19, A32, A40, and B212), we conducted validation using *C. violaceum* mutant 026. This mutant strain lacks AHL production due to a double transposon Tn5 insertion in the *CviI* gene but can still produce violacein pigment [21]. The tested metabolites showed quorum quenching activity against *C. violaceum* mutant 026, as indicated by decreased absorbance at 540 nm (Fig. 1). Absorbance levels reflect violacein pigment production, thus indicating quorum sensing activity [22]. This suggests that the observed quorum sensing inhibition in the supernatant may involve enzymatic degradation, competition, synthesis inhibition, or signal transduction blocking of signal molecules [23].

In investigating ten environmental bacteria metabolites for their antibiofilm activity against three food spoilage bacteria, we assessed both inhibition and destruction activities. Results showed that all

metabolites inhibited and destroyed *B. cereus*, *B. subtilis*, and *S. putrefaciens* biofilms to varying degrees (Fig. 1).

Biofilm composition and architecture vary between bacterial species and strains due to gene tic regulation. For instance, *B. cereus* forms biofilms through PlcR, PapR, and NprB proteins, with EPS containing polysaccharides, proteins (TasA), and extracellular DNA [2, 24, 25]. *B. subtilis*, a Gram-positive bacterium, requires ComQXPA for biofilm formation, with EPS composed of polysaccharides and proteins (TasA, TapA, BslA) [26, 27]. *S. putrefaciens*, a Gram-negative bacterium, uses AI-2 and the *bpfA* operon for biofilm formation, with EPS mainly consisting of proteins rather than polysaccharides [28, 29].

From Fig. 1 the highest inhibition activity of bacterial metabolite against biofilm of *B. cereus*, *B. subtilis*, and *S. putrefaciens* were B10 (81.42%), J73 (87.45%), and B212 (70.21%). In terms of destruction activity, the highest destruction activity against biofilm formation of *B. cereus*, *B. subtilis*, and *S. putrefaciens* were A32 (45.4%), T152 (83.81%), and C1 (74.81%), respectively. In terms of antibiofilm, the specific mechanisms of biofilm inhibition are inhibiting the attachment of planktonic cells, disrupt of cell signaling system, supress the expression of related genes. Meanwhile, the mechanism of destruction biofilm, by interfere the bacterial membrane and destroy formed biofilm [30].

The selected metabolite, which exhibited destruction activity against those spoilage bacteria, were further analyzed using Scanning Electron Microscopy (SEM) (Fig. 2) along with the utilization of Energy-Dispersive X-Ray Spectroscopy (EDS) for additional characterization (supplementary Table 3). This approach used to see biofilm morphology and elements. The results of SEM anlaysis revealed a significant difference between the untreated biofilm and the biofilm treated with the environmental bacteria metabolite. The untreated biofilm appeared compactness, while the addition of the bacterial metabolite resulted in the disruption of the biofilm.

The element compositions were determined by EDS analysis (Supplementary Table 3). It showed that there were different weight% between untreated and treated biofilm surface. Several elements displayed, consisting of carbon (C), oxygen (O), sodium (Na), magnesium (Mg), alumininium (Al), pottasium (K), and calcium (Ca). We found in untreated and treated biofilm assay, weight% of carbon and oxygen were increased while others decreased. Colwell and Grimes) [31] stated that various elements can play a role in biofilm formation, by which carbon and oxygen are an element that play crucial roles in primary building blocks of EPS structures, such as polysaccharides [32]. These polysaccharides can be present both within the biofilm matrix and inside the biofilm structure. The presence of bioactive compounds disrupt the biofilm, leading to decay of polysaccharides in matrix and increase the carbon and oxygen mass [33]. Meanwhile, sodium in the form of sodium chloride can enhance bacterial attachment and aggregation of cell [34, 35]. Magnesium can affect cell adhesion, biofilm stability, and structure integrity [32]. Aluminium plays a significant role in biofilm formation, primarily during the attachment phase. It achieves this by neutralizing surface charges, promoting precipitation, and facilitating the production of hydrophobic proteins [36]. Potassium plays an important role in coordinating bacterial metabolic processes and potassium ion channels as carrying signal among bacterial communities in biofilms [37]. Finally, calcium is responsible for initial attachment, maintain biofilm structures [38].

Based on molecular identification results (Tables 1), three isolates, A19 (OR128363), A30 (OR131584), and A32 (OR131589) belong to the genus *Pantoea* [39]. Meanwhile, three other isolates, A40 (OR131746), B212 (OR133538), and J73 (OR133540) belong to the genus of *Acinetobacter* [40]. B10 (OR131747) and J70 (OR133539) belong to the genus of *Enterobacter* [41].

While there have been no previous reports of antibiofilm activity for *Pantoea stewartii strain* HR3-48, *Acinetobacter baumannii strain* 2022CK-00772, *Acinetobacter junii* strain QLN201710OPB4, *Enterobacter sp.* strain HSTU-Sh65, and *Acinetobacter pittii*, our research results have shown that these isolates do possess antibiofilm activities.

Gas chromatography-mass spectrometry (GC-MS) analysis revealed that the major detected bioactive components of isolate A32, B10, B212, C1, and J73 were n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z, Z)-, and 9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexad ecyl)oxy]propyl ester. However, bioactive compounds from isolate B212 were the same as others, only in addition to cis-Vaccenic acid (Table 2; Supplementary Figs. 5–10; Supplementary Figs. 11–13). N-hexadecanoic acid or palmitic acid (PA) is a saturated fatty acid that has a strong inhibitory effect on biofilm formation, as well as on exopolysaccharide. Palmitic acid may decrease in the transcription of signal molecules production, disrupt the cell membrane by leading to membrane destabilization and disruption, and interfere biofilm matrix [42]. Besides that, palmitic acid has potential as antioxidant [43]. On the previous study, Prasath et al. [44], these compounds have demonstrated antibiofilm activity against *Candida tropicalis*. Meanwhile, 9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexad ecyl)oxy]propyl ester is a specific ester compound that can disrupt EPS, interfere microbial adhesion, impaired bacterial motility,

antifungal, disperse existing biofilm. Trans-13-Octadecenoic acid is an unsaturated acid that can disrupt mature biofilm, prevent attachment, interfere QS, and antimicrobial. Cis-Vaccenic acid might interfere composition of EPS, weakening the matrix, and promote dispersion of cells within the biofilm, disrupt the membrane integrity by affecting fluidity and permeability of bacterial cell membranes, and interfere QS signaling by targeting *las* gene [45]. According to a previous study, this compound had been produced by Marine *Alcaligenes faecalis* as antibiofilm, anti-biocorrosion, and antimicrobial effect against *Desulfovibrio sp.* from crude oil [46, 47].

Conclusion

All of environmental bacteria metabolite examined in this study exhibited antibiofilm activity, either inhibition or destruction biofilms against biofilm of food spoilage bacteria. In addition, some of them performed anti-quorum sensing activity. Further identification of the compound showed it contains various fatty acids that contribute to antibiofilm activities.

Limitations

Further study is needed to explore the molecular antibiofilm mechanism of the compounds contained in environmental supernatants.

Abbreviations

EPS Extracellular polymeric substances
AHL Acyl homoserine lactone

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-025-07141-2.

Supplementary material 1.

Acknowledgements

The authors acknowledge research funding support by Indonesian Ministry of education and culture through competitive national research grant 2019-Fundamental research.

Author contributions

C.C.Y conducted research, performed data analysis, and prepared the manuscript under the guidance of DEW. D.E.W personal investigator, conception and design research project, data analysis and interpretation, revised overall manuscript and advisory the research. All authors read and approved the final manuscript.

Funding

The study received funding from Kemdikbud 2021. However, the funder had no involvement in the study's design, data collection, analysis, interpretation, and writing of the manuscript.

Data availability

The GenBank accession number grants public access to all data that has been deposited in GenBank, and can be access these datasets using this corresponding links OR128363 (https://www.ncbi.nlm.nih.gov/nuccore/OR128363), OR131584 (https://www.ncbi.nlm.nih.gov/nuccore/OR131584), OR131589 (https://www.ncbi.nlm.nih.gov/nuccore/OR131589), OR131746 (https://www.ncbi.nlm.nih.gov/nuccore/OR131746), OR131747 (https://www.ncbi.nlm.nih.gov/nuccore/OR131747), OR133538 (https://www.ncbi.nlm.nih.gov/nuccore/OR133538),OR133539 (https://ncbi.nlm.nih.gov/nuccore/OR133539), and OR133540 (https://www.ncbi.nlm.nih.gov/nuccore/OR133540) .

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 24 September 2024 Accepted: 6 February 2025
Published online: 17 February 2025

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