

Antibiofilm Activity and Chemical Profiling of Biomolecule Extracts from Marine Sediment Bacteria

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Abstract

Some of the secondary metabolites in the marine ecosystem control the adhesion of microfouling microorganisms to surfaces, thereby exhibiting antibiofilm properties. The main objective of this research was to understand the antibiofilm and antibacterial activity of biomolecule extracts of bacteria from marine sediments. Each complex and pure biomolecule was evaluated for inhibition effects against two marine biofilm bacteria using the antibiofilm activity assay. The maximum activity of the biomolecules in preventing bacterial adhesion was determined to range between 68.59 percent and 91.84 percent for *Pseudoalteromonas agarivorans* and between 15 percent and 65.68 percent for *Exiguobacterium homiense*. Additionally, the antibacterial activity of biomolecule extracts against four marine biofilm bacteria was tested by the minimum inhibitory concentration method. The strongest minimum inhibitory activity of pure extract (0.78 mg/mL) from *Bacillus simplex* was recorded against *Alteromonas genoviensis*. Research has also focused on the determination of compounds such as alkaloids, phenolics and flavonoids in the structure of biomolecular extracts using spectrophotometric analysis. It was concluded that the pure biomolecules isolated from sediment bacteria are predominantly composed of alkaloids. These novel microbial biomolecule extracts could be used as sources to produce antibiofilm and antifouling products.

Introduction

Biofouling in marine environments is a progressive process initiated by microfouling and subsequently progressing to macrofouling (Adnan et al., 2018). The initial phase of microfouling is defined as the development of microbial biofilm

(Caruso, 2020). The microfouling stage is developed by the rapid production of extracellular polymeric substances (EPS) after biofilm bacteria attach to underwater surfaces (Abdulrahman et al., 2022). α -, γ -Proteobacteria, and Cytophaga-Flavobacterium-Bacteroides

bacteria are dominant groups in marine environments. Members of γ -proteobacteria such as *Pseudoalteromonas* and *Alteromonas* are most frequently isolated from seawater, and there are also studies focused on their EPS production and biofilm formation mechanisms (Nichols et al., 2004; Nandakumar et al., 2004).

Bacterial EPS attach to underwater structures and living organism surfaces, colonize their environment, and provide a background for the main fouling marine organisms (Patil and Anil, 2005; Khandeparker et al., 2006; Cavalcanti et al., 2020). These extracellular polymeric structures from bacteria facilitate their access to underwater surfaces and preserve them from unfavorable conditions and detrimental risks (Zeng et al., 2020). The physical properties and morphology of bacteria as colloidal particles, such as their dimensions, range of growth ratios, and common negative charge, simplify their incursion into diverse habitats (De Carvalho, 2018; Patra et al., 2022).

The negative impacts of marine fouling and its economic penalties on marine transport and industries are notable (Bekiari et al., 2015; Dang and Lovell, 2016). Additionally, it is well-known that biofouling is the reason for the destruction of metallic structures by significant corrosion. Many attempts have been made to reduce fouling with the use of physical, chemical, and biological substances. However, the greatest success has been achieved with the use of marine coatings (Adnan et al., 2018; Cao et al., 2011; Iorhemen et al., 2016; Lade et al., 2014). Replacing prior surface colonizers of marine biofilm bacteria is an effective strategy for controlling biofouling on underwater surfaces (Abdulrahman et al., 2022). The application of damaging antifoulants and their relations with environmental situations have increased the progress of environmentally friendly choices (Callow and Callow, 2011; Nurioglu et al., 2015). From this point of view, research concerning marine bacteria's chemical defenses in the prevention of biofouling has significant expectations. A great deal of research has been carried out to shed light on antifoulants derived from bioactive molecules as replacements for environmentally unfriendly marine coatings (Ciriminna et al., 2015; Omae, 2003; Qian et al., 2009; Wang et al., 2017). The application of

ecologically friendly biocides as replacements for synthetic chemicals has recently appeared because the only aim of chemistry is to produce or discover novel and effective chemical products that are reliable for use with expanded prolificacy (Adnan et al., 2018). Therefore, recently developed technologies with alternative resolutions are being discovered to inhibit this marine biofilm (Patra et al., 2022).

The extreme conditions of marine environments have caused microorganisms to synthesize different biomolecules to adjust to the conditions for survival (De Carvalho and Fernandes, 2010). Over the past five decades, researchers have identified over 20,000 naturally occurring marine biomolecules with potential biotechnological significance (Gallimore, 2017). New biomolecules capable of preventing biofouling were also explored by extraction from marine bacteria because of their secondary metabolism (antibiofilm, antibacterial, antilarval, and antialgal) (Aguila-Ramírez et al., 2014; Vimala, 2016). The secondary metabolites in the marine environment control the attachment of microfouling organisms to any surface, which is the cause of antifouling surfaces (Engel et al., 2002; Sjogren et al., 2004). The various molecules with notable antibiofilm activity may prohibit biofouling formation based on the role of the biofilm bacteria (Abdulrahman et al., 2022). As a matter of course, biomolecules produced by marine bacteria can induce deterioration in biofilm formation (Ganapiriya et al., 2012), so they can be functional in improving ecologically friendly metabolites to prevent biofouling (Holmström and Kjelleberg, 1999). When compared with the bioactive antibiofilm molecules from macro-organisms (polychetes, tunicates, bryozoans, molluscs, sponges, etc.), scarce data are present from marine bacteria (Fusetani, 2004; Adnan et al., 2018).

After years of intense research on terrestrial bacteria, the focus has shifted to marine ecosystems (WHO, 2015; Böhringer et al., 2017; Choudhary et al., 2017). Antibiofilm contents that are secondary metabolites of marine bacteria fascinate many researchers due to their superior antibacterial potential. These metabolites are exposed to broad research conducted on the chemistry of marine microbial biomolecules,

which has been intensively developing recently. Due to their unique possessions, they have become one of the precedents for marine biotechnology (Andryukov et al., 2019). Marine bacteria are a plentiful source of various categories of protein derivatives of secondary metabolites (Andryukov et al., 2019). For example, hydrolase enzymes (Chen et al., 2013; Aykin et al., 2019) and biomolecules such as alkaloids, terpenoids, polyketides, peptides, etc. (Satheesh et al., 2016; Chen et al., 2018) from marine bacteria exhibit important antibiofilm activity. Many of the isolated antimicrobial-based secondary metabolites are capable of rapidly killing biofilm bacteria (Andryukov et al., 2019). With the increasing interest in discovering marine-derived biomolecules, the biotechnological potential of marine alkaloids has arisen as a rising class of bioactive metabolites. The number of these bioactive metabolites that can be isolated is very limited. Researchers should consider studying the pure and complex chemical structures of these biomolecules and the various biological activities of marine alkaloid molecules. These alkaloids are natural biomolecules that contain nitrogen and have important activities (Rodrigues et al., 2016; Zhou and Huang, 2020).

The main objective of present research was to understand the antibiofilm and antibacterial activity of marine sediment bacteria. It is hypothesized that the biomolecule extracts of bacteria isolated from marine sediments were a feature in antibiofilm activity. Based on experimental analysis and our previous research, the study was designed to understand the differences in antibiofilm activity of pure and complex biomolecule extracts obtained from sediment bacteria.

Materials and Methods

Pure and Complex Biomolecule Extracts from Marine Sediment Bacteria

The seven complex extracts were obtained from Turkish marine sediment bacteria, including *Bacillus simplex* KJ161411, *Alkalihalobacillus macyae* MW559742, *Kocuria rosea* MW559735, *Lacisediminihabitans profunda* MW559737, *Bacillus safensis* MW559602, *Bacillus vietnamensis* MW559607, and *Bacillus baekryungensis* KJ161399, using methanol with

ion exchange chromatography (Diaion-HP20) (Omuzbuken et al., 2022). On the other hand, Silica gel SiliaFlash column resin was used for the purification process of complex extracts of *Kocuria rosea*, *Alkalihalobacillus macyae*, and *Bacillus simplex*. 61 : 32 : 7 chloroform : methanol : water, 64 : 50 : 10 chloroform : methanol : water, methanol, 40 : 10 : 50 butanol : acetic acid : water and water were passed through the columns, respectively. After the purification process, three pure extracts were obtained.

The Biofilm Bacteria Tested

The biofilm bacteria *Pseudoalteromonas agarivorans* FJ040188, *Vibrio lentus* FJ200649, *Alteromonas genoviensis* FJ040186, and *Exiguobacterium homiense* FJ200653 were isolated from Izmir Bay (Eastern Aegean Sea, Turkey) (Kacar et al., 2009). The biofilm bacteria were grown overnight on Zobell Marine Broth (HiMedia) at 26 °C (OD600 value of 2).

Testing Biomolecule Extracts for Antibacterial Activity: Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined by the microdilution method described by Zgoda and Porter (2001), with some modifications against *V. lentus*, *P. agarivorans*, *A. genoviensis* and *E. homiense* (Kacar et al., 2018). Briefly, a series of dilutions of the pure and complex biomolecule extracts was provided, ranging from 0.78 mg/mL to 25 mg/mL. This was followed by a series of dilutions of biomolecule extracts transferred to the broth in transparent 96-well microtiter plates. The transparent 96-well microtiter plates were incubated for 24 h at 26 °C. The results were obtained using 1% triphenyl tetrazolium chloride (Sigma USA), which may be bacterial growth positive when a red color, indicating the formation of triphenyl formazan, was recorded. The biofilm bacteria without biomolecule extracts were tested as a positive control in the analyses. The minimum inhibitory concentration experiments on the antibacterial activity of biomolecule extracts were performed in duplicate.

Screening of Biomolecule Extracts for Antibiofilm Activity

The prevention of bacterial attachment testing for a change in the percentage of bacterial adhesion

(intense biofilm producer isolates; *P. agarivorans* and *E. homiense*) on the surface of black polystyrene microplates (Greiner Bio-One, Austria) in seawater was performed with sterile conditions at 20 °C, as described and modified by Aykin et al. 2019 and Leroy et al. (2008). Briefly, the biomolecule extracts were analyzed for inhibition of bacterial attachment. The biomolecule extracts were diluted at different concentrations (0.15 to 25 mg/ml) and spotted 1 h before introducing the bacterial suspension. After the incubation at 20 °C for 24 h using orbital shaking (120 rpm), three washes, and fixing for 1.5 h at 4 °C with 2.5% formaldehyde, the samples were stained with 200 µL DAPI (4 g/ml) for 20 min. Microplates were measured using a Synergy HTX multimode reader (Biotek, USA) at 350 nm light excitation and 510 nm light emission wavelengths. Sterile seawater was used as a control with the bacterial suspension. All experiments on the antibiofilm activity of biomolecule extracts were performed in duplicate.

The change in bacterial attachment was measured as the percentage reduction (CR) by comparing the fluorescence of the blank (FB: without bacteria, negative control), the fluorescence of the control (FC: bacteria without biomolecule extracts, positive control), and the fluorescence of the sample (FS: bacteria with biomolecule extracts).

$$CR = \{[(FC-FB) - (FS-FB)] / (FC-FB)\} \times 100$$

Chemical Profiles of Biomolecule Extracts

The chemical profiles of biomolecule extracts were determined using spectrophotometric methods (Omuzbuken et al., 2022). The total phenolic (as gallic acid equivalents), alkaloid (as boldine equivalents), and flavonoid substances (as quercetin equivalents) were determined as follows.

Total phenolics: The total phenolics of the bacterial biomolecule extracts were determined with the spectrophotometric method (Rohaeti et al., 2017). Gallic acid (0.9 mL) was used as a standard solution. Folin-Ciocalteu solution (4.5 mL) was used as the reagent. The sample solution was spiked with 3.6 mL of 7.5% Na₂CO₃ and incubated for 1 h. The samples were measured at 765 nm. The total phenolic concentration was calculated based on three replicates.

Total alkaloids: The total alkaloid content of the bacterial biomolecule extracts was detected with the spectrophotometric method (Shamsa et al., 2008; Patel et al., 2015). The boldine standard solution was used as a standard solution. The bromocresol green solution was prepared with 2 N NaOH. The samples were measured at 470 nm. The total alkaloid concentration was calculated based on three replicates.

Total flavonoids: The total flavonoids of the bacterial biomolecule extracts were measured with the spectrophotometric method (Rohaeti et al., 2017). The quercetin equivalent solution was used as a standard solution. Samples were spiked with 1 M potassium acetate, ethanol, and 10% aluminium chloride and incubated for 30 min at room temperature. Absorbance was measured at 415 nm, and the total flavonoid concentration was calculated based on three replicates.

Statistical Analysis

The statistical data analysis was performed using STATISTICA (v11.0 software). Pearson's correlation test was applied to detect correlations between variables (chemical profiles of extract).

Results

Minimum Inhibition Concentrations of the Biomolecule Extracts as Antibacterial Activity

Results of the antibacterial activity of the complex and their pure biomolecule extracts against the four marine biofilm bacteria are presented in tables 1 and 2, which show that all biomolecule extracts from sediment bacteria have minimum inhibitory activity against the tested biofilm bacteria (*V. lentus*, *A. genoviensis*, *P. alteromonas*, *E. homiense*). The range of activity varies from 0.78 mg/ml to 25 mg/ml, whereas some are without activity. The strongest minimum inhibitory activity of pure extract (0.78 mg/mL) from *Bacillus simplex* was recorded against *Alteromonas genoviensis*, followed by the pure extract of *Kocuria rosea* with 6.25 mg/ml inhibiting *Alteromonas genoviensis*. Also, complex extracts of *Kocuria rosea* and *Bacillus simplex* were effective in inhibiting *Alteromonas genoviensis* at 6.25 mg/ml. The complex extracts of *Kocuria rosea*, *Alkalihalobacillus macyae*, and the complex and pure extracts of *Bacillus simplex* were determined to be ineffective at the minimum

inhibition concentration against the tested strain, *Exiguobacterium homiense*. Dimethylsulfoxide

(> 99.5%) (Carl Roth) was used as a negative control.

Table 1. The minimum inhibition concentrations (mg/ml) of pure biomolecule extracts against marine biofilm bacteria

Isolates of Biofilm Bacteria	Minimum Inhibition Concentration of Pure Biomolecule Extracts from Sediment Bacteria (mg/mL)		
	<i>Kocuria rosea</i>	<i>Alkalihalobacillus macyae</i>	<i>Bacillus simplex</i>
<i>Vibrio lentus</i>	12.5	25	25
<i>Alteromonas genoviensis</i>	6.25	12.5	0.78
<i>Pseudoalteromonas agarivorans</i>	12.5	25	25
<i>Exiguobacterium homiense</i>	25	25	(-)

(-) not determined MIC values

Antibiofilm Activity of Biomolecule Extracts

Antibiofilm analyses of the biomolecule extracts, whose antibacterial activities were determined by the MIC test, evaluated each of the extracts for antibiofilm activity against *Pseudoalteromonas agarivorans* and *Exiguobacterium homiense* from marine biofilm bacteria. During the experiments, these two biofilm-forming bacterial species demonstrated more notable biofilm production activity, so they were selected for further analysis. The range of maximum activities of pure and complex molecule extracts in the prevention of bacterial attachment varied from 68.59% to 91.84% for *P. agarivorans* and from 15% to 65.68% for *E. homiense*, as shown in Figure 1. The pure molecule extract of *Kocuria rosea* showed the highest prevention of bacterial attachment activity at 85.19% against *P. agarivorans*. In contrast, the pure biomolecule

extract of *B. simplex* didn't prevent bacterial attachment activity against *E. homiense*. The complex biomolecule extract of *B. vietnamensis* showed the highest prevention activity of 91.83% against *P. agarivorans*, followed by the complex biomolecule extract of *B. baekryungensis*. For the prevention of attachment by *E. homiense*, the most effective complex biomolecule extract was determined to be *K. rosea*. Some of the biomolecule extracts demonstrated strong activity against both antibacterial activity and biofilm formation, while others were better against antibiofilm activity and vice versa (Figures 2-3). It can be stated that the decrease in EPS components has the potential to disrupt the structure of biofilms (Viju et al. 2020). Consequently, it is postulated that the antibiofilm effect of the extracts disrupts the structure-activity against biofilm formation. It can result from the antibiofilm activity.

Table 2. The minimum inhibition concentrations (mg/ml) of complex biomolecule extracts against marine biofilm bacteria

Isolates of Biofilm Bacteria	Minimum Inhibition Concentration of Complex Biomolecule Extracts from Sediment Bacteria (mg/mL)						
	<i>Kocuria rosea</i>	<i>Bacillus safensis</i>	<i>Lacisediminihabitans profunda</i>	<i>Alkalihalobacillus macyae</i>	<i>Bacillus vietnamensis</i>	<i>Bacillus simplex</i>	<i>Bacillus baekryungensis</i>
<i>Vibrio lentus</i>	25	25	25	25	25	25	25
<i>Alteromonas genoviensis</i>	6.25	12.5	12.5	12.5	12.5	6.25	12.5
<i>Pseudoalteromonas agarivorans</i>	25	12.5	25	25	25	25	25
<i>Exiguobacterium homiense</i>	(-)	25	25	(-)	25	(-)	25

(-) not determined MIC values

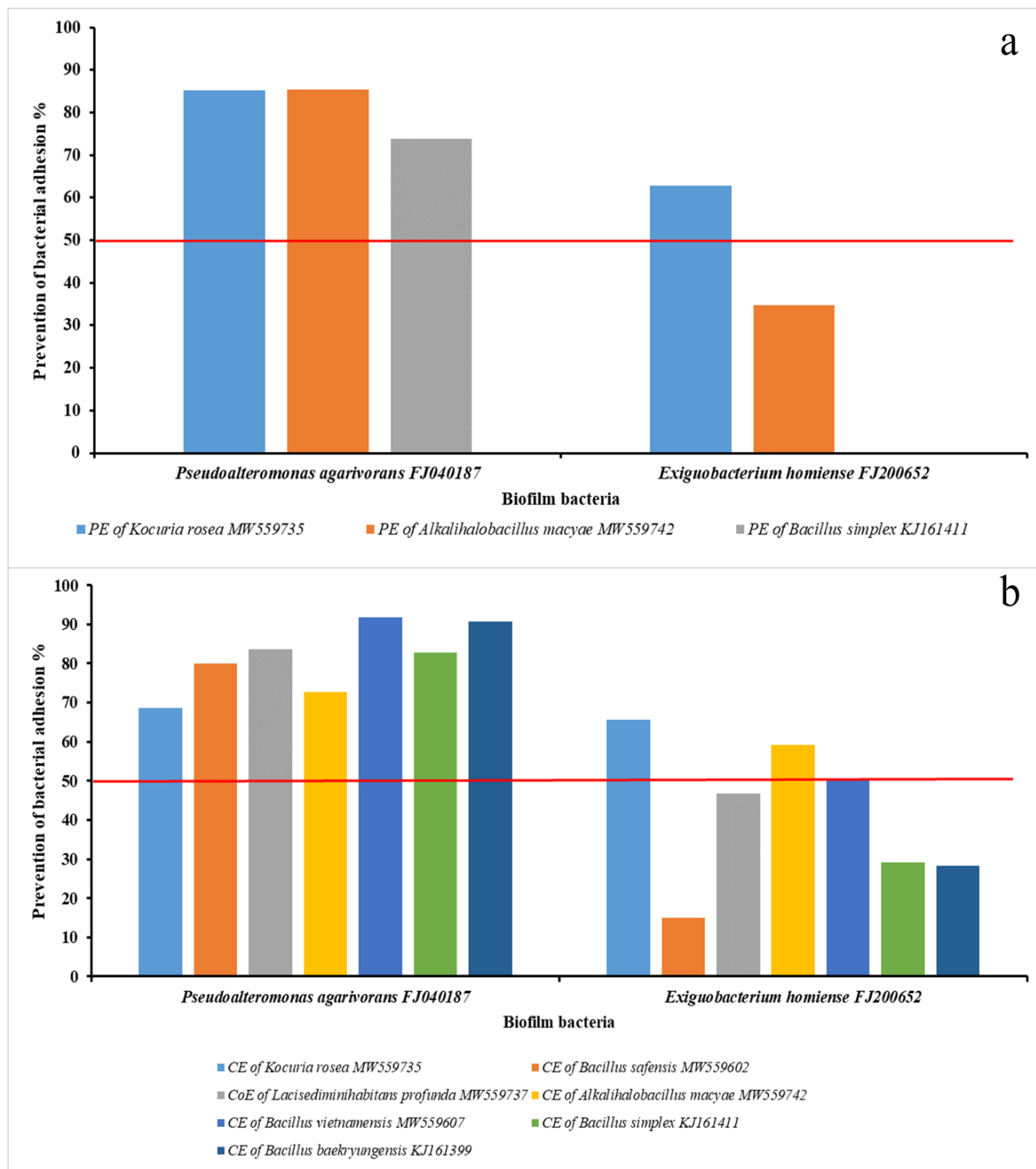


Figure 1. The results of reduction in biofilm bacteria by more than 50% according to prevention tests (this is shown in the figure as a red line).

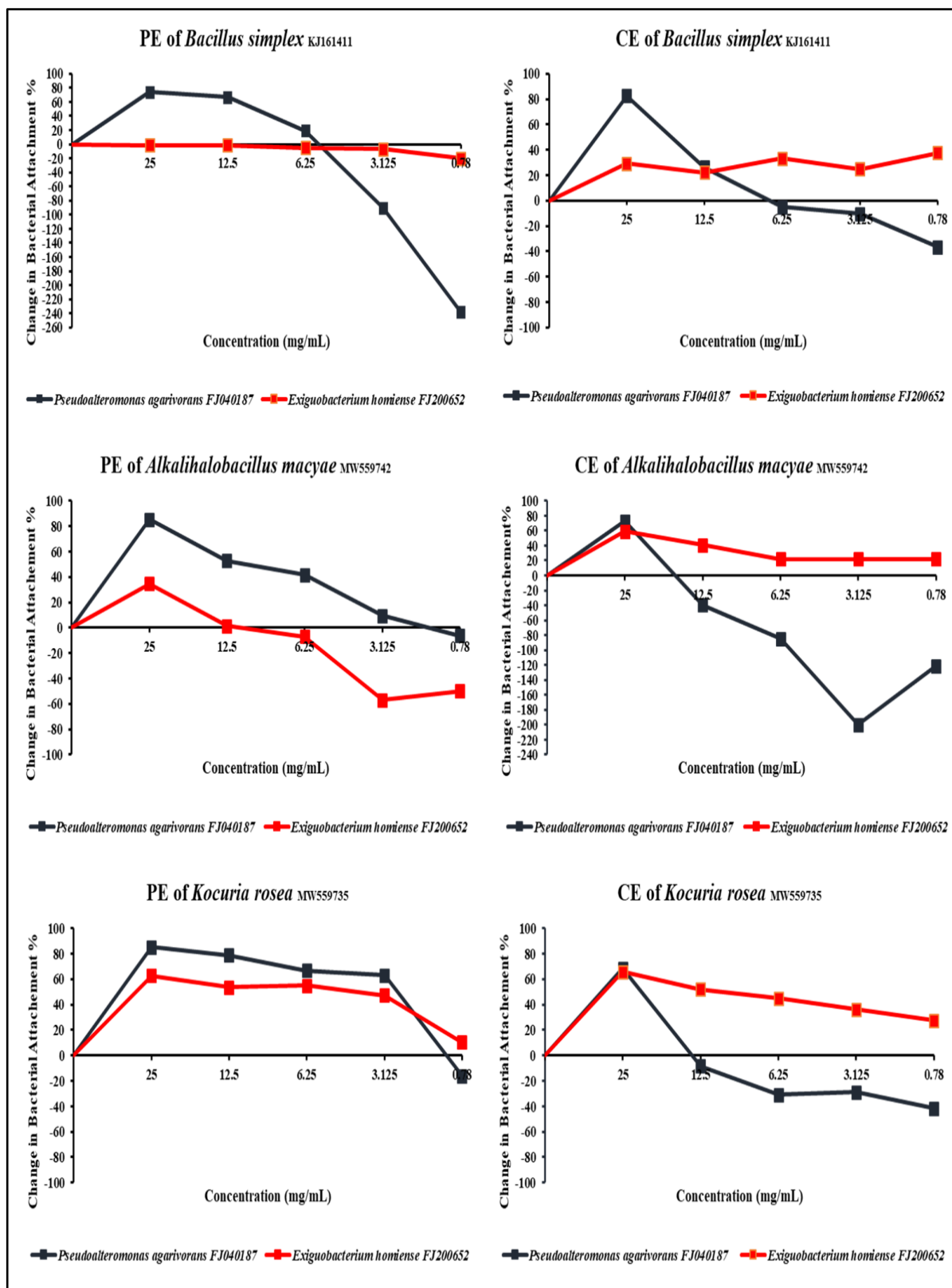


Figure 2. Percentage reduction in bacterial attachment of prevention tests according to biomolecule extracts concentrations of *B. simplex*, *A. macyae* and *K. rosea* (CE: Complex biomolecule extract, PE: Pure biomolecule extract)

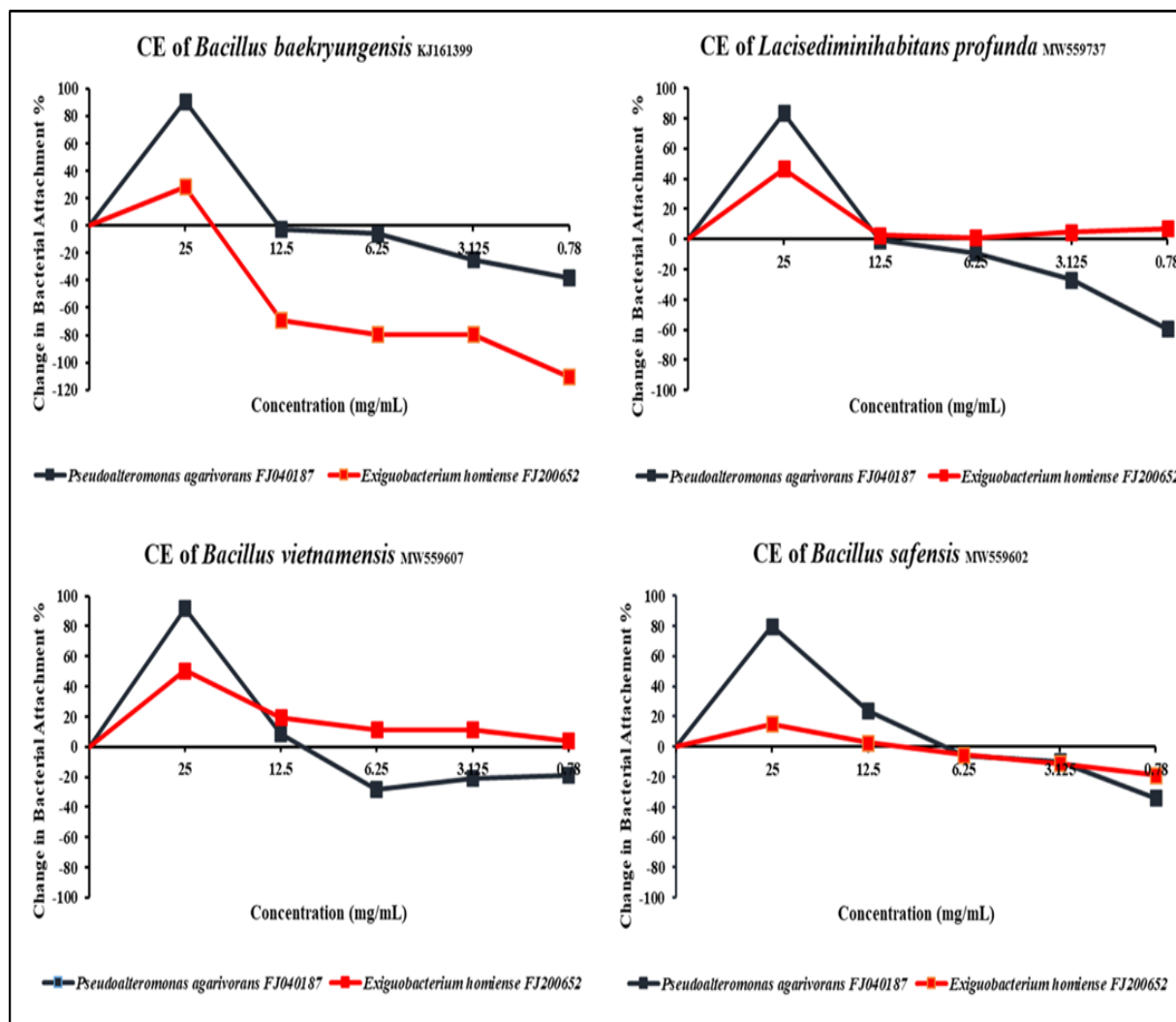


Figure 3. Percentage reduction in bacterial attachment of prevention tests according to biomolecule extracts concentrations of *B. baekryungensis*, *L. profunda*, *B. vietnamensis* and *B. safensis* (CE: Complex biomolecule extract)

Table 3. The results of chemical profile analyses of bacterial biomolecule extracts (PE: Pure biomolecule extract, CE: Complex biomolecule extract)

Bacterial biomolecule extracts	Total Alkaloid concentration ¹	Total Phenolic concentration ¹	Total Flavonoid concentration ¹
	(mg equivalent boldin / g biomolecule extract)	(mg equivalent quercetin / g biomolecule extract)	(mg equivalent gallic acid / g biomolecule extract)
PE of <i>Bacillus simplex</i>	21.57 ± 1.08	Nd ²	Nd ²
PE of <i>Kocuria rosea</i>	56.33 ± 0.54	Nd ²	Nd ²
PE of <i>Alkalihalobacillus macyae</i>	36.83 ± 1.19	Nd ²	Nd ²
CE of <i>Bacillus vietnamensis</i>	14.06 ± 0.51	101.57 ± 0.10	5.65 ± 0.18
CE of <i>Bacillus safensis</i>	12.77 ± 0.16	73.91 ± 0.77	6.65 ± 0.40
CE of <i>Lacisediminihabitans profunda</i>	16.29 ± 0.29	84.92 ± 0	4.12 ± 0
CE of <i>Bacillus baekryungensis</i>	3.61 ± 0.17	51.39 ± 0.16	4.51 ± 0.08
CE of <i>Alkalihalobacillus macyae</i>	123.51 ± 26.01	71.37 ± 9.46	11.74 ± 0.29
CE of <i>Bacillus simplex</i>	22.59 ± 14.48	86.28 ± 18.57	20.08 ± 3.41
CE of <i>Kocuria rosea</i>	142.16 ± 10.38	92.58 ± 22.52	12.03 ± 0.57

mean of 3 replicates ± standard deviation, ² Nd: not detected

Chemical Profiles of Biomolecule Extracts

The chemical profiles of pure and complex biomolecule extracts were analyzed by spectrophotometric methods. Analyzing biomolecule extracts can provide important data about the chemical profiles of sediment bacteria. The research also focused on the chemical evaluation of types of biomolecule extracts, alkaloids, phenolics, and flavonoids, whose antibiofilm activities were determined (Figure 4). As seen in Table 3, all of the pure molecule extracts were found to be alkaloids. The highest number of alkaloids (142.16 ± 10.38 mg equivalent boldine/g biomolecule extract) was observed in *K. rosea* and the lowest one ($3.61 \pm$

0.17 mg equivalent boldine/g biomolecule extract) in *B. baekryungensis*. The phenolic substance ranged from 51.39 ± 0.16 to 101.57 ± 0.10 mg equivalent gallic acid/g biomolecule extract and was highest in *B. vietnamensis*. The highest flavonoid substance (20.08 ± 3.41 mg equivalent quercetin/g biomolecule extract) was noticed for *B. simplex*, whereas the lowest flavonoid substance was found for *Lacisediminihabitans profunda*, with 4.12 ± 0 mg equivalent quercetin/g biomolecule extract. Additionally, Pearson's correlation test was not detected significant positive or negative relationship between the chemical compounds (alkaloids, phenolics, flavonoids).

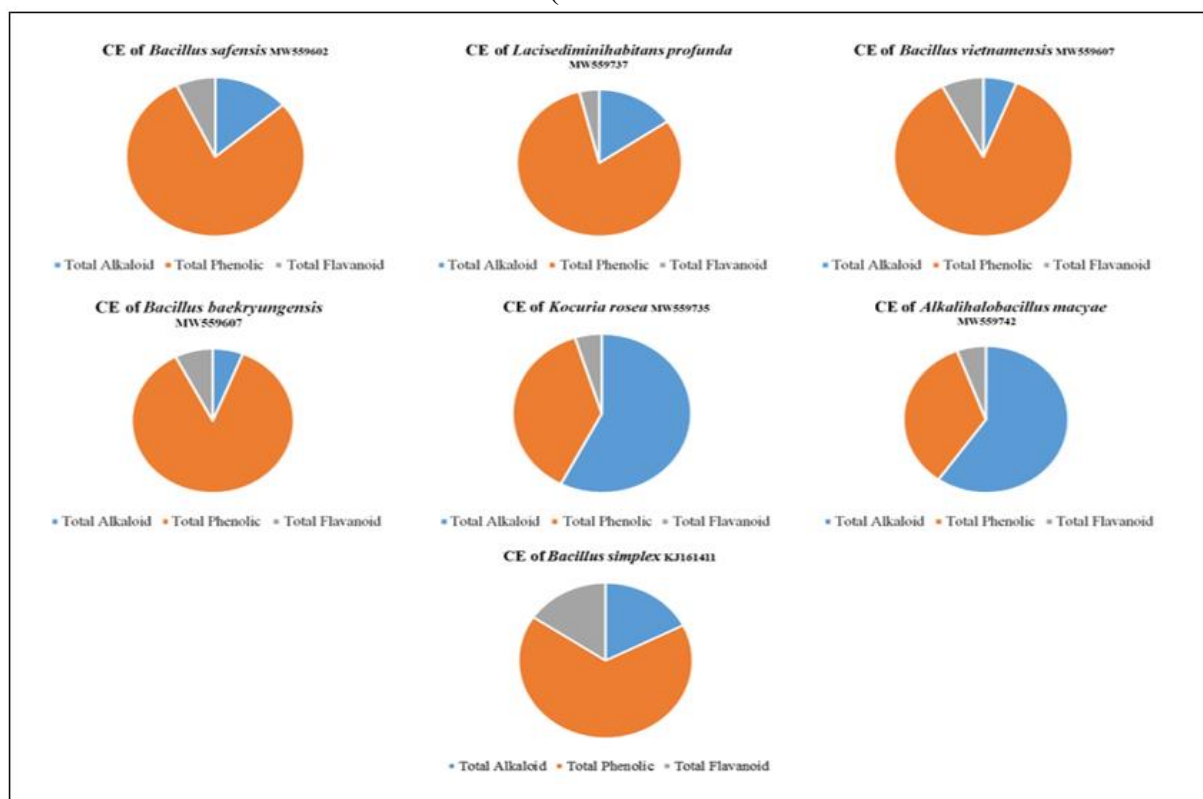


Figure 4. Total alkaloid (mg equivalent boldine/ g biomolecule extract), total phenolic (mg equivalent gallic acid/ g biomolecule extract), total flavonoid (mg equivalent quercetin/ g biomolecule extract) profiles of bacterial complex biomolecule extract (CE: Complex biomolecule extract)

Discussion

In recent years, researchers investigating marine biomolecules have expanded the scope of their studies from macro-organisms, such as ascidians, sponges, soft corals, and algae, to marine microorganisms (Habbu et al., 2016). According to our results, the complex and pure biomolecules were obtained from marine sediment bacteria widely prevented marine biofilm bacteria. It is well known that bacterial initial colonization is the

most important stage in the microfouling progression of the fouling process. Prevention of bacterial formation on a surface is an inhibition strategy in controlling the whole fouling formation. Primarily, biomolecules exhibiting both antibiofilm and antibacterial activities have been identified to be more effective in reducing biofouling activity (Viju et al., 2020; Abdulrahman et al., 2022).

The results of the MIC tests illustrate the antimicrobial activity of properties of biomolecule extracts on marine biofilm bacteria. The strong antibacterial activity based on MIC results was attained by the complex biomolecule extract of *Bacillus simplex*. In previously similar studies from other researchers, antibacterial and antibiofilm activity was also declared strong for different *Bacillus* species against marine biofilm bacteria (Sanchez-Rodríguez et al., 2018; Viju et al., 2020; Abdulrahman et al., 2022). Also, Abdulrahman et al. (2022) demonstrated a wide spectrum of activity by endophytic marine bacterial biomolecules from similar *Bacillus* species; *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, *B. cereus*, *B. laterosporus*, and *B. silvestris* (Mondol et al., 2013; Santhi et al., 2017). Presently, biomolecules, including peptides, have exhibited antibiofilm, antifouling, antialgal, insecticidal, and anticancer activities (Ben Khedher et al., 2011; Hamdache et al., 2011; Baruzzi et al., 2011). *Bacillus* species have also been discovered to generate promising compounds that exhibit noteworthy effects against drug-resistant pathogens (Wibowo et al., 2023). In other research, Coasta et al. (2018) isolated *Bacillus* sp. P34, which produced a peptide that showed strong antibiofilm activity against *Staphylococcus* sp. (Viju et al., 2020).

On the other hand, bioactive metabolites derived from brominated alkaloids have indicated antagonistic effects on biofilm formation (Peters et al., 2003). Le-Norcy et al. (2017) studied a group of alkaloids, and their study has shown activity on biofilm formation in the marine bacterial strain *Paraccocus* sp. Furthermore, another study documented that the marine-derived phenethylamine and tyramine alkaloids obtained from *Shewanella aquimarina* exhibit antibiofilm activity in the initial stage of *Staphylococcus aureus* biofilm formation (Giugliano et al., 2023). Our results revealed that pure biomolecules have chemical profiles consisting predominantly of alkaloids, while complex biomolecules predominantly of phenolic compounds. Almost all concentrations of pure extracts were found to exhibit significant antibiofilm activity, against marine biofilm bacteria. Especially, pyrrolo pyrimidine alkaloids and their synthetic analogs produced by marine bacteria were effective

against various biofilm-forming bacteria (Muzychka et al. 2024). Various alkaloids inhibit communication system between biofilm forming bacteria known as quorum sensing and this process causes deterioration of the biofilm structure. Thereby, enabling the facile removal of microbial cells from surfaces (Khalid et al., 2022).

The biomolecules are products of bacterial secondary metabolism in response to different marine environmental signals because of the extreme conditions (De Carvalho and Fernandes, 2010). Besides, the biomolecules are deliberately extricated from the bacterial cell to preserve it from defects compared with the other compounds that are discharged within the bacterial cells (Pinu et al., 2017). Therefore, it is highly significant to know the bacterial biomolecules involved and reveal their bioactive action and knowledge of metabolites from pure and complex biomolecules is highly significant (Abdulrahman et al., 2022).

The studies are focused on experimental approaches where active components were analyzed in relation to novel biomolecule extracts from marine bacteria. As mentioned by De Rop et al. (2022), between the years of 2017 and 2021, 77 novel marine Actinobacteria alkaloid derivatives were represented, mainly pyrroles, indoles, glutarimides, indolizidines, and diketopiperazines. The search for these alkaloids' antimicrobial activity supports the importance of biomolecule extracts from marine bacterial resources. According to Wibowo et al. (2022), the marine-derived indole alkaloids reported from various marine organisms including bacteria, fungi, sponges, algae, and bryozoans were determined. Although the search for the bioactivities of these biomolecules has been revealed, there should be a great amount of assessment, including their mechanisms, to obtain lead substances for developing new chemically active compounds (Wibowo et al., 2022).

Conclusions

The marine environments have a rich microbial diversity, and these organisms could generate various biologically active molecules. These biomolecules, including antibiofilm properties, have been isolated from marine microorganisms and could be used in marine coatings as antifoulant. The present study describes the

application of bacterial extracts derived from Turkish marine sediments as an antibiofilm strategy against marine biofilms. Our findings suggest that marine bacterial extracts have the potential to produce chemical compounds since they could perform antibacterial and strong antibiofilm activity. So, these novel microbial bioactive molecules could be used as sources to produce antibiofilm and antifouling products.

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Ethical statement

The authors declare that this study complies with research and publication ethics.

Informed consent

Not available.

Conflicts of interest

There is no conflict of interests for publishing our study.

Data availability statement

The authors declare that data are available from authors upon reasonable request.

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Author contribution

Authors are encouraged to submit an "Author statement" providing individual contributions of authors such as:

Ayse Kazan: Methodology, Formal analysis, Investigation, Resources, Validation, Writing original draft,

Asli Kacar: Supervision, Visualization, Conceptualization, Review, Editing, Funding acquisition,

Burcu Omuzbukan: Investigation, Resources, Methodology, Formal analysis, Validation, Writing original draft.

References

- Abdulrahman, I., Jamal, M. T., Pugazhendi, A., Dhavamani, J., & Satheesh, S. (2022). Antibiofilm activity of secondary metabolites from bacterial endophytes of Red Sea soft corals. *International Biodeterioration & Biodegradation*, 173, 105462. <https://doi.org/10.1016/j.ibiod.2022.105462>
- Adnan, M., Alshammari, E., Patel, M., Ashraf, S. A., Khan, S., & Hadi, S. (2018). Significance and potential of marine microbial natural bioactive compounds against biofilms/biofouling: necessity for green chemistry. *Peer J- Life and Environment*, 6, e5049. <https://doi.org/10.7717/peerj.5049>
- Aguila-Ramírez, R. N., Hernández-Guerrero, C. J., González-Acosta, B., Id-Daoud, G., Hewitt, S., Pope, J., & Hellio, C. (2014). Antifouling activity of symbiotic bacteria from sponge *Aplysina gerardogreeni*. *International Biodeterioration & Biodegradation*, 90, 64–70. <https://doi.org/10.1016/j.ibiod.2014.02.003>
- Andryukov, B., Mikhailov, V. & Besednova, N. (2019). The biotechnological potential of secondary metabolites from marine bacteria. *Journal of Marine Science and Engineering*, 7(6), 176. <https://doi.org/10.3390/jmse7060176>
- Aykin, E., Omuzbukan, B. & Kacar, A. (2019). Microfouling bacteria and the use of enzymes in eco-friendly antifouling technology. *Journal of Coating Technology and Research*, 16(3), 847–856. <https://doi.org/10.1007/s11998-018-00161-7>
- Baruzzi, F., Quintieri, L., Morea, M. & Caputo, L. (2011). Antimicrobial compounds produced by *Bacillus* sp. and applications in food science against microbial pathogens. *Communication Current Research in Technology Advance*, 2, 1102–1111.
- Bekiari, V., Nikolaou, K., Koromilas, N., Lainioti, G., Avramidis, P., Hotos, G., Kallitsis, J. K. & Bokias, G. (2015). Release of polymeric biocides from synthetic matrices for marine biofouling applications. *Agriculture and Agricultural Science Procedia*, 4, 445–450. <https://doi.org/10.1016/j.aaspro.2015.03.051>
- Ben Khedher, S., Kamoun, A., Aaoua, S. & Zouari, N. (2011). Improvement of *Bacillus*

- thuringiensis bioinsecticide production by sporeless and sporulating strains using response surface methodology. *New Biotechnology*, 28(6), 705–712.
<https://doi.org/10.1016/j.nbt.2011.01.008>
- Böhringer, N., Fisch, K. M., Schillo, D., Bara, R., Hertzner, C., Grein, F., Eisenbarth, J.-H., Kaligis, F., Schneider, T., Wagele, H., König, G. M. & Schäberle, T. F. (2017). Antimicrobial potential of bacteria associated with marine sea slugs from North Sulawesi, Indonesia. *Frontiers in Microbiology*, 8, 1092.
<https://doi.org/10.3389/fmicb.2017.01092>
- Cao, S., Wang, J., Chen, H., & Chen, D. (2011). Progress of marine biofouling and antifouling technologies. *Chinese Science Bulletin*, 56(7), 598–612. <https://doi.org/10.1007/s11434-010-4158-4>
- Callow, J. A., & Callow, M. E. (2011). Trends in the development of environmentally friendly fouling-resistant marine coatings. *Nature Communications*, 2(1), 244.
<https://doi.org/10.1038/ncomms1251>
- Caruso, G. (2020). Microbial colonization in marine environments: overview of current knowledge and emerging research topics. *Journal of Marine Science and Engineering*, 8(2), 78.
<https://doi.org/10.3390/jmse8020078>
- Cavalcanti, G. S., Alker, A. T., Delherbe, N., Malter, K. E., & Shikuma, N. J. (2020). The influence of bacteria on animal metamorphosis. *Annual Review of Microbiology*, 74, 137–158.
<https://doi.org/10.1146/annurev-micro-011320-012753>
- Chen, F., Gao, Y., Chen, X., Yu, Z. & Li, X. (2013). Quorum quenching enzymes and their application in degrading signal molecules to block quorum sensing-dependent infection. *International Journal of Molecular Sciences*, 14(9), 17477–17500.
<https://doi.org/10.3390/ijms140917477>
- Chen, L., Hu, J. S., Xu, J. L., Shao, C. L. & Wang, G. Y. (2018). Biological and chemical diversity of ascidian-associated microorganisms. *Marine Drugs*, 16(10), 362.
<https://doi.org/10.3390/md16100362>
- Coasta, G. A., Rossatto, F. C. P., Medeiros, A. W., Correa, A. P. F., Brandelli, A., Frazzon, A. P. G. & Motta, A. D. S. (2018). Evaluation antimicrobial and antibiofilm activity of the antimicrobial peptide P34 against *Staphylococcus aureus* and *Enterococcus faecalis*. *The Annals of the Brazilian Academy of Sciences*. 90(1), 73–84.
<https://doi.org/10.1590/0001-3765201820160131>
- Choudhary, A., Naughton, L. M., Montánchez, I., Dobson, A. D. W. & Rai, D. K. (2017). Current status and future prospects of marine natural products (MNPs) as antimicrobials. *Marine Drugs*, 15, 272.
<https://doi.org/10.3390/md15090272>
- Ciriminna, R., Bright, F. V., & Pagliaro, M. (2015). Ecofriendly antifouling marine coatings. *ACS Sustainable Chemistry & Engineering*, 3(4), 559–565. <https://doi.org/10.1021/sc500845n>
- Dang, H., & Lovell, C. R. (2016). Microbial surface colonization and biofilm development in marine environments. *Microbiology and Molecular Biology Reviews*, 80(1), 91–138.
<https://doi.org/10.1128/MMBR.00037-15>
- De Carvalho, C. C. C. R. (2018). Marine Biofilms: A successful microbial strategy with economic implications. *Frontiers in Marine Science*, 5, 126.
<https://doi.org/10.3389/fmars.2018.00126>
- De Carvalho, C. C. C. R., & Fernandes, P. (2010). Production of metabolites as bacterial responses to the marine environment. *Marine Drugs*, 8, 705–727. <https://doi.org/10.3390/md8030705>
- De Rop, A.-S.; Rombaut, J.; Willems, T.; De Graeve, M.; Vanhaecke, L.; Hulpiaw, P.; de Maeseneire, S. L., de Mol, M. L. & Soetaert, W. K. (2022). Novel alkaloids from marine Actinobacteria: discovery and characterization. *Marine Drugs*, 20, 6.
<https://doi.org/10.3390/md20010006>
- Engel, S., Jensen, P. R., & Fenical, W. (2002). Chemical ecology of marine microbial defense. *Journal of Chemical Ecology*, 28, 1971–1985.
<https://doi.org/10.1023/a:1020793726898>
- Fusetani N. (2004). Biofouling and antifouling. *Natural Product Reports*, 21(1), 94–104.
<https://doi.org/10.1039/b302231p>

- Gallimore, W. (2017). Marine metabolites: oceans of opportunity. In *Pharmacognosy* (pp. 377-400). Academic Press.
- Ganapiriya, V., Maharajan, A., & Kumarasamy, P. (2012). Antifouling effect of bioactive compounds from marine sponge *Acanthella elongata* and different species of bacterial film on larval attachment of *Balanus amphitrite* (Cirripedia, Crustacea). *Brazilian Archives of Biology and Technology*, 55(3), 395–402. <https://doi.org/10.1590/s1516-89132012000300010>
- Giugliano, R., Della Sala, G., Buonocore, C., Zannella, C., Tedesco, P., Palma Esposito, F., Ragozzino, C., Chianese, A., Morone, M. V., Mazzella, V., Nunez-Pons, L., Folliero V., Franci, G., De Filippis, A., Galdiero, M. & de Pascale, D. (2023). New Imidazolium Alkaloids with Broad Spectrum of Action from the Marine Bacterium *Shewanella aquimarina*. *Pharmaceutics*, 15(8), 2139. <https://doi.org/10.3390/pharmaceutics15082139>
- Habbu, P., Warad, V., Shastri, R., Madagundi, S. & Kulkarni, V. H. (2016). Antimicrobial metabolites from marine microorganisms. *Chinese Journal of Natural Medicines*, 14(2), 0101-0116. [https://doi.org/10.1016/S1875-5364\(16\)60003-1](https://doi.org/10.1016/S1875-5364(16)60003-1)
- Hamdache, A., Azarken, R., Lamarti, A., Aleu, J. & Collado, I. G. (2011). Non-peptide metabolites from the genus *Bacillus*. *Journal of Natural Products*, 74(4), 893–899. <https://doi.org/10.1021/np100853e>
- Holmström, C., & Kjelleberg, S. (1999). Marine Pseudoalteromonas species are associated with higher organisms and produce biologically active extracellular agents. *FEMS Microbiology Ecology*, 30(4), 285–293. <https://doi.org/10.1111/j.1574-6941.1999.tb00656.x>
- Iorhemen, O. T., Hamza, R. A., & Tay, J. H. (2016). Membrane bioreactor (MBR) technology for wastewater treatment and reclamation: membrane fouling. *Membranes*, 6(2), 33. <https://doi.org/10.3390/membranes6020033>
- Kacar, A., Kocyigit, A., Ozdemir, G. & Cihangir, B. (2009). The development of biofilm bacteria on panels coated by different antifouling paints in the marinas. *Fresenius Environmental Bulletin*, 18, 2004-2012.
- Kacar, A., Avunduk, S., Omuzbuken, B. & Aykin, E. (2018). Biocidal activities of a triterpenoid saponin and flavonoid extracts from the *Erica manipuliflora* salisb. against microfouling bacteria. *International Journal of Agriculture, Forestry and Life Science*, 2(2), 40-46.
- Khandeparker, L., Chandrashekar Anil, A., & Raghukumar, S. (2006). Relevance of biofilm bacteria in modulating the larval metamorphosis of *Balanus amphitrite*. *FEMS Microbiology Ecology*, 58(3), 425–438. <https://doi.org/10.1111/j.1574-6941.2006.00177.x>
- Khalid, S. J., Ain, Q., Khan, S. J., Jalil, A., Siddiqui, M. F., Ahmad, T., & Adnan, F. (2022). Targeting Acyl Homoserine Lactones (AHLs) by the quorum quenching bacterial strains to control biofilm formation in *Pseudomonas aeruginosa*. *Saudi Journal of Biological Sciences*, 29(3), 1673-1682. <https://doi.org/10.1016/j.sjbs.2021.10.064>
- Lade, H., Paul, D., & Kweon, J. H. (2014). Quorum quenching mediated approaches for control of membrane biofouling. *International Journal of Biological Sciences*, 10(5), 550–565. <https://doi.org/10.7150/ijbs.9028>
- Le Norcy, T., Niemann, H., Proksch, P., Linossier, I., Vallée-Réhel, K., Hellio, C. & Fay F. (2017). Anti-Biofilm effect of biodegradable coatings based on hemibastadin derivative in marine environment. *International Journal of Molecular Sciences*, 18(7), pp.1520. <https://doi.org/10.3390/ijms18071520>
- Leroy, C., Delbarre, C., Ghillebaert, F., Compere, C. & Combes, D. (2008) Effects of commercial enzymes on the adhesion of a marine biofilm-forming bacterium. *Biofouling*, 24(1) 11–22. <https://doi.org/10.1080/08927010701784912>
- Mondol, M. A. M., Shin, H. J. & Islam, M. T. (2013). Diversity of secondary metabolites from marine *Bacillus* species: chemistry and biological activity. *Marine Drugs*, 11, 2846–2872.
- Muzychka, L. V., Humeniuk, N. I., Boiko, I. O., Vrynchanu, N. O., & Smolii, O. B. (2024). Synthesis and antibiofilm activity of novel 1, 4-dihydropyrido [1, 2- α] pyrrolo [2, 3-d]

- pyrimidine-2-carboxamides. *Biopolymers and Cell*, 40(1).
- Nandakumar, K., Obika, H., Utsumi, A., Ooje T., & Yano, T. (2004). In vitro laser ablation of natural marine biofilms. *Applied Environmental Microbiology*, 70, 6905–6908.
- Nichols, C. A. M., Garon, S., Bowman, J. P., Raguene, G., & Guezennec, J. (2004). Production of exopolysaccharides by Antarctic marine bacterial isolates. *Journal of Applied Microbiology*, 96, 1057–1066.
- Nurioglu, A. G., Esteves, A. C., & de With, G. (2015). Non-toxic, non-biocide-release antifouling coatings based on molecular structure design for marine applications. *Journal of Materials Chemistry B*, 3(32), 6547–6570. <https://doi.org/10.1039/c5tb00232j>
- Omae, I. (2003). Organotin antifouling paints and their alternatives. *Applied Organometallic Chemistry*, 17(2), 81–105. <https://doi.org/10.1002/aoc.396>
- Omuzbukun, B., Kacar, A., Avunduk, S. & Erden Pazi, İ. (2022). Screening of beta-glucosidase inhibitors and their chemical Profiles from marine sediment bacteria. *Thalassas: An International Journal of Marine Sciences*, 38, 1057-1065. <https://doi.org/10.1007/s41208-022-00451-3>
- Patel, R. K., Patel, J. B. & Trivedi, P. D. (2015). Spectrophotometric method for the estimation of total alkaloids in the *Tinospora cordifolia* M. and its herbal formulations. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(10):249–251.
- Patil, J. S., & Anil, A. C. (2005). Influence of diatom exopolymers and biofilms on metamorphosis in the barnacle *Balanus amphitrite*. *Marine Ecology Progress Series*, 301, 231–245. <https://doi.org/10.3354/meps301231>
- Patra, A., Das, J., Agrawal, N. R., Kushwaha, G. S., Ghosh, M., & Son, Y. O. (2022). Marine antimicrobial peptides-based strategies for tackling bacterial biofilm and biofouling challenges. *Molecules*, 27(21), 7546. <https://doi.org/10.3390/molecules27217546>
- Peters, L., König, G. M., Wright, A. D., Pukall, R., Stackebrandt, E., Eberl, L. & Riedel, K. (2003). Secondary metabolites of *Flustra foliacea* and their influence on bacteria. *Applied and Environmental Microbiology*, 69(6), 3469–3475. <https://doi.org/10.1128/AEM.69.6.3469-3475.2003>
- Pinu, F. R. & Villas-Boas, S. G. (2017). Extracellular microbial metabolomics, the state of the art. *Metabolites*, 7(3), 43. <https://doi.org/10.3390/metabo7030043>
- Qian, P. Y., Xu, Y., & Fusetani, N. (2009). Natural products as antifouling compounds: recent progress and future perspectives. *Biofouling*, 26(2), 223–234. <https://doi.org/10.1080/08927010903470815>
- Rodrigues, T., Reker D., Schneider P. & Schneider G. (2016). Counting on natural products for drug design. *Nature Chemistry*, 8, 531–541. <https://doi.org/10.1038/NCHEM.2479>
- Rohaeti, E., Fauzi, M. R. & Batubara, I. (2017). Inhibition of α -glucosidase, total phenolic content and flavonoid content on skin fruit and flesh extracts of some varieties of snake fruits. *IOP Conf. Series: Earth and Environmental Science*, 58, 012066. <https://doi.org/10.1088/1755-1315/58/1/012066>
- Sanchez-Rodríguez, D. E., Ortiz-Aguirre, I., Aguila-Ramírez, R. N., Rico-Virgen, E. G., Gonzalez-Acosta, B. & Hellio, C. (2018). Marine bacteria from the Gulf of California with antimicrofouling activity against colonizing bacteria and microalgae. *Revista de Biología Tropical*, 66(4), 1649–1663. <https://doi.org/10.15517/rbt.v66i4.31963f>
- Santhi, L. S., Talluri, V. S. S. L. P., Nagendra, S. Y. & Krishna, E. (2017). Bioactive compounds from marine sponge associates: antibiotics from *Bacillus* sp., *Natural Product Chemistry Research*, 5, 4.
- Satheesh, S., Ba-akdah, M. A. & Al-Sofyani, A. A. (2016). Natural antifouling compound production by microbes associated with marine macroorganisms, A review. *Electronic Journal of Biotechnology*, 19(3), 26–35. <https://dx.doi.org/10.1016/j.ejbt.02.002>
- Shamsa, F., Monsef, H., Ghamooshi, R. & Verdianrizi, M. (2008). Spectrophotometric determination of total alkaloids in some Iranian

medicinal plant. *ThaiScience Journal of Pharmaceutical Sciences*, 32, 17–20.

Sjögren, M., Göransson, U., Johnson, A. L., Dahlström, M., Andersson, R., Bergman, J., Jonsson, P. R. & Bohlin, L., (2004). Antifouling activity of brominated cyclopeptides from the marine sponge *Geodia barretti*. *Journal of Natural Products*, 67(3), 368–372. <https://doi.org/10.1021/np0302403>

Viju, N., Punitha, S. M. J. & Satheesh, S. (2020). Antibiofilm activity of endophytic *Bacillus* species associated with marine gastropods. *Annals of Microbiology*, 701, 1–12. <https://doi.org/10.1186/s13213-020-01554-z>

Wang, K. L., Wu, Z. H., Wang, Y., Hao, Y. Y., & Wang, Y. (2017). Mini-review: antifouling natural products from marine microorganisms and their synthetic analogs. *Marine Drugs*, 15(9), 266. <https://doi.org/10.3390/md15090266>

Vimala, R., (2016). Marine organisms: a potential source of natural antifouling metabolites. *International Journal of ChemTech Research*, 9, 208–217.

Wibowo, J. T., Bayu, A., Aryati, W. D., Fernandes, C., Yanuar, A., Kijjoa, A., & Putra, M. Y. (2023). Secondary Metabolites from Marine-Derived Bacteria with Antibiotic and Antibiofilm Activities against Drug-Resistant Pathogens. *Marine Drugs*, 21(1), 50. <https://doi.org/10.3390/md21010050>

Wibowo, J. T., Ahmadi, P., Rahmawati, S. I., Bayu, A., Putra, M. Y. & Kijjoa, A. (2022). Marine-derived indole alkaloids and their biological and pharmacological activities. *Marine Drugs*, 20, 3. <https://doi.org/10.3390/md20010003>

World Health Organization (WHO). (2015). *Antimicrobial Resistance*. Available online: <http://www.who.int> (accessed on 23 February 2019).

Zeng, W., Li, F., Wu, C., Yu, R., Wu, X., Shen, L., Yuandong, L., Guanzhou, Q. & Li, J. (2020). Role of extracellular polymeric substance (EPS) in toxicity response of soil bacteria *Bacillus* sp. S3 to multiple heavy metals. *Bioprocess and Biosystems Engineering*, 43(1), 153–167. <https://doi.org/10.1007/s00449-019-02213-7>

Zhou, S., & Huang, G. (2020). The synthesis and biological activity of marine alkaloid derivatives and analogues. *RSC Advances*, 10, 31909–31935. <https://doi.org/10.1039/D0RA05856D>

Zgoda, J. R. & Porter, J. R. (2001). A convenient microdilution method for screening natural products against bacteria and fungi. *Pharmaceutical Biology*, 39, 221–225.