Electronic Journal of Biotechnology 66 (2023) 75–83

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage: www.elsevier.com/locate/ejbt

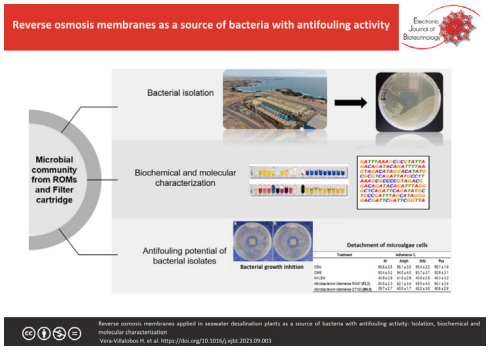
Research article

Reverse osmosis membranes applied in seawater desalination plants as a source of bacteria with antifouling activity: Isolation, biochemical and molecular characterization

Hernán Vera-Villalobos [a,*], Alejandro Cortes-Martinez [a], Álvaro Gonzalez-Gutierrez [a], Vinko Zadjelovic [b,c], Carlos Riquelme [b], Fernando Silva-Aciares [d,e,*]

[a] Laboratorio de Ecología Microbiana, Centro de Bioinnovación, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile
[b] Centro de Bioinnovación, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile
[c] Centre for Biotechnology and Bioengineering, Beauchef 851, Santiago, Chile
[d] Unidad de Microbiología Aplicada, Centro de Bioinnovación, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile
[e] Departamento de Biotecnología, Universidad de Antofagasta, Antofagasta, Chile

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 June 2023
Accepted 28 September 2023
Available online 5 October 2023

Keywords:
Antibacterial supernatant
Antifouling
Bioactive molecules
Biofilms
Biofouling
Exoenzimatic activity
Exoenzymes

ABSTRACT

Background: During lifespan of reverse osmosis systems applied in seawater desalination plants, a common phenomenon of undesirable growth and accumulation of biological material known as biofouling occurs. Microbial cells are first settlers, attaching and enclosing themselves in extracellular polymeric substances –biomass accumulation usually referred to as microfouling or biofilms, which becomes a serious issue when having a detrimental effect on materials or hampering industrial processes. Thus, control of microfouling on reverse osmosis membranes is crucial since chemical and physical treatments are usually costly and ineffective. In this context, even though microorganisms are the source of the problem, they can also provide possible solutions due to their capacity to produce secondary metabolites, being several of these competition mediators secreted compounds (i.e., bioactive molecules) produced during the stationary phase (e.g., lipases, proteases, DNases and other enzymes with alginate lyase and xanthine oxidases). Such bioactive substances can be utilized against the development of biofilms. Considering biofouled surfaces a niche for complex microbial interactions, here we have used reverse osmosis

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso
* Corresponding authors.
E-mail addresses: hernan.vera@uantof.cl (H. Vera-Villalobos), fernando.silva@uantof.cl (F. Silva-Aciares).

https://doi.org/10.1016/j.ejbt.2023.09.003
0717-3458/© 2023 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Microfouling
Reverse Osmosis Membrane
Seawater desalination plants

membranes and cartridge filters applied in a seawater desalination plant as a source for the isolation of bacteria with anti-fouling potential, among other biotechnological applications.
Results: We obtained 27 bacterial isolates able to secrete exoenzymes displaying a wide array of bioactivity such as lipases, alginases, and glucosidases, among others. Additionally, we showed two main candidates able to inhibit the growth of common microbial colonizers of ROM and cartridge filter.
Conclusions: These results highlight the potential biotechnological application of bioactive molecules against biofouling formation.
How to cite: Vera-Villalobos H, Cortes-Martinez A, Gonzalez-Gutierrez Á, et al. Reverse osmosis membranes applied in seawater desalination plants as a source of bacteria with antifouling activity: Isolation, biochemical and molecular characterization. Electron J Biotechnol 2023;66. https://doi.org/10.1016/j.ejbt.2023.09.003.
© 2023 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

According to recent reports, 40% of the world's population is currently experiencing severe water scarcity, and this proportion is expected to increase to nearly 6 billion people by 2050 [1]. These statistics are alarming, especially considering that as of 2013, an estimated 4 billion people –approximately 66% of the global population– experienced inadequate access to water for at least one month [2]. In the present context of Climate Change, where extensive desertification and droughts are ever more common concepts for society, seawater desalination plants have become a popular alternative to mitigate water scarcity issues stemming from conventional sources such as freshwater reservoirs and lakes. In the past two decades, desalination plants developed into increasingly more cost-effective technologies, where reverse osmosis (RO) systems stand out as a promising solution due to their continuous seawater supply [3].

During the lifespan of RO systems applied in seawater desalination plants, a common phenomenon known as biofouling takes place. This phenomenon is defined as, the undesirable growth and accumulation of biological material on surfaces that have been submerged in water. In the initial stadium of biofouling development, microbial cells are the first settlers, attaching and enclosing themselves in extracellular polymeric substances (EPS) –biomass accumulation usually referred to as microfouling or biofilms. It has been established that biofilm formations are not possible in the absence of EPS [4]. These substances consist mainly of water, polysaccharides, exDNA, proteins, glycoproteins and phospholipids. The build-up of such biological structures (from micro to macrofouling) is quite stable, which becomes a severe issue when having a detrimental effect on materials or hampering industrial processes [5].

In seawater desalination plants, the control of microfouling on RO membranes (ROM) is crucial since chemical and physical treatments are usually costly and ineffective, as well as techniques that do not completely remove EPS in which microbes are embedded. Microfouling has been shown to damage ROM by mechanical obstruction and deformation, which ultimately impact the permeability of these membranes to seawater. Even though microorganisms are the source of the problem, they can also provide possible solutions. Many microbes (*e.g.,* bacteria, fungi and phytoplankton) produce bioactive compounds, which, in some cases involve the production of secondary metabolites able to mediate competitive interactions between microbes [6]. Interestingly, several of these substances that mediate competition are secreted compounds produced during the stationary phase (*e.g.,* lipases, proteases, DNases and other enzymes with alginate lyase and xanthine oxidases), bioactive molecules that can be utilized against the initial development of biofilms [7,8].

To develop biotechnological applications using bioactive compounds, it is crucial to obtain and characterize microbes that can prevent the settling of biofilm-forming microbes –a process that still heavily relies on conventional techniques for growing microorganisms. While classical microbiology methods are being increasingly replaced by high-throughput molecular approaches, the advantages of working with culturable microorganisms are still valuable when testing the effectiveness and applicability of a candidate compound. Once candidate compounds are obtained, it is essential to determine what array of species have settling preferences for the surface to be subjected to treatments for a successful application of inhibitory compounds [9], as microbial communities can react differently depending on the treatment [10]. An example of such isolation and characterization efforts is a previous study in which extracellular bioactive compounds from *Alteromonas* spp. were found to be effective in degrading and avoiding biofouling growth even when applied under operating conditions of desalination processes, such as high pressure and water flux [11].

Considering biofouled surfaces a niche for complex microbial interactions, here we have used ROM and cartridge filters applied in a seawater desalination plant as a source for the isolation of bacteria with anti-fouling potential, among other biotechnological applications. We obtained 27 bacterial isolates able to secrete exoenzymes displaying a wide array of bioactivity such as lipases, alginases, and glucosidases, among others.

2. Materials and methods

2.1. Sampling procedure

ROM and sections of cartridge filters were obtained from the local seawater desalination plant "La Chimba" (Aguas Antofagasta S.A.), situated on the coast of Antofagasta, Chile (lat. –23.55, long.-70.40). Sections of 10 × 10 of either ROM or cartridge filters (with at least 3 years of continuous usage) were subjected to sonication (Sonicator water bath - Elmasonic E60H) applying a frequency of 37 kHz *per* 5 min. Then, 10 mL of the resulting resuspended supernatants were used to perform serial dilutions. Next, 100 µL of each dilution were inoculated to Zobell Agar and incubated at 20°C for a week. Isolates were obtained and morphologically characterized based on standard parameters such as shape, margin, elevation, and color.

2.2. Bacterial culture for exoenzymatic tests

The exoenzymatic activity was assessed by bacterial growth onto specific culture media (commercial kits for detecting enzymes from gastrointestinal enterobacteria, API20, API zym and xanthine oxidase assay). For this evaluation, each strain was sub-cultured on M9 minimal medium (M9; distilled water 960 mL, casamino acids 1 g/L, Na_2HPO_4 6 g/L, KH_2PO_4 3 g/L, NH_4Cl 1 g/L, NaCl 21 g/L, filter-sterilized glucose 0,1M $MgSO_4{}^*7H_2O$ 10 mL, $CaCl_2{}^*2H_2O$ 10 mL, Vitamin B1 (1%) 0.2 mL y glucose (20%) 20 mL) incubated at 20°C, with continuous shaking for 3 d [11].

2.3. Enzymatic characterization of bacterial isolation by culturing on plates

Lipase, DNase, cellulase, protease, carrageenase and alginase were assessed by culturing on plates of the bacterial isolates and performed as follows:

- Lipase activity was determined by the method described by Sierra [12]. The plates were incubated for 3 d at 20°C. A positive reaction can be assigned when calcium crystal precipitation halos are formed around bacterial colonies, indicating lipids degradation.
- DNase activity was determined using Agar ADNse (Difco) according to the method described by Jeffries et al. [13]. Agar plates were incubated between 3 and 5 d at 20°C. The DNase activity was revealed by flooding the plates with 5 mL of hydrogen chloride (HCl) 1 N. A positive reaction can be assigned when clear-zone halos are observed around the colonies.
- Cellulase activity was determined using TSA NaCl (2% w/v) plates supplemented with carboxymethylcellulose (0.2% w/v - Sigma). Plates were incubated between 3 and 5 d at 20°C. Afterward, the plates were stained using Lugol (Merck) for 1 min. A positive reaction can be assigned when yellow halos are observed around the colonies [14].
- Protease activity was determined by the casein hydrolysis in TSA NaCl (2% w/v) plates supplemented with hydrolyzed casein (1% w/v - Oxoid). Plates were incubated between 3 and 5 d at 20°C. A positive reaction can be assigned when clear-zone halos are observed around the colonies [15].
- Carrageenase activity was determined using TSA NaCl (2% w/v) plates supplemented with carrageenan (0.2% w/v - Sigma). Plates were incubated between 3 and 5 d at 20°C. Plates were revealed by Lugol staining for 1 min. As in the case of cellulase activity, a positive reaction can be assigned when yellow halos are observed around the colonies [16].
- Alginase activity was determined following the method presented by Gacesa and Wusteman [17] –with modifications. Bacterial isolates were cultured on TSA NaCl (2% w/v) plates supplemented with sodium alginate (1.5% w/v) and bromothymol blue (0.003% w/v) and incubated between 3 and 5 d at 20°C. A positive reaction can be assigned when clear-zone halos are observed around the colonies.

2.4. Biochemical characterization of bacterial isolated using API20 and APIzym kits

The marine bacterial isolates were cultured on M9 [18] for 3 d at 20°C using constant shaking of 120 rpm. Then, APY 20 y APIzym kits (bioMerieux) were applied for the exoenzymatic characterization of the strains following the supplier's recommendations. Then, Hierarchical Cluster Analysis (HCL) and Network analysis were performed using Pearson correlation followed by a complete linkage algorithm using MeV: MultiExperiment Viewer [19].

2.5. Antimicrobial activity of isolated strains against bacterial colonizers of ROM

To assess the potential antifouling activity of our isolated against microbes colonizing the ROMs (further referred to as the mix of native biofouling-producing bacteria), we conducted the following experiment. First, we aimed to obtain resuspended cells from the ROM by sonication (using an Elmasonic model E60H water bath) for 5 min at a frequency of 37 kHz in 100 mL of sterile seawater. Then, 100 µL of the resulting resuspended cells were plated using a spreading technique onto Müller Hinton Agar NaCl (2% w/v). In parallel, the previously isolated candidates with antifouling activity obtained in Zobell Marine Broth (reaching exponential phase) were further cultured onto Müller Hinton Agar NaCl (2% w/v) by plating 100 µL of inoculum and incubating 72 h at 20°C. Then, a section of 1 cm^2 was cut off from the agar matrix, placed on top of the growing cultures from the ROM, and left for incubation for 72 h at 20°C. The results were collected by determining the radius of inhibitory clear zone halos as follows: a radius of 1 cm was assigned a clear red; between 1 and 2 cm a middle intensity red; and a radius > 2 cm was assigned a high intensity red.

2.6. Inhibitory effect of bacterial extracts on the adherence of common intertidal and ROM-associated microalgae

Bacterial isolates with potential antifouling activity were grown to reach the stationary phase using M9 medium (room temperature and constant shaking). Then, cultures were centrifuged at 11,000 rpm for 15 min at 4°C. The supernatants were retrieved and filtered sterile twice through 0.2 µm membranes (Millipore). The inhibition adherence bioassays were carried out using *Nitzchia Ovalis*, *Navicula incerta*, *Psammodictyon* sp. and *Amphora* sp. –common intertidal and ROM-associated microalgae. The microalgae were cultured in seawater supplemented with F/2 medium. The cells were harvested, and the cellular pellets were washed with seawater only in order to minimize a possible background effect of the additional nutrients provided by F=/2 [20]. The concentration of resuspended microalgae was set to $\sim 7.6 \times 10^6$ cells•mL (1×10^6 cells•cm^{-2}).

For the bioassay, 12-well polystyrene plates were loaded with 2 mL of each resuspended and washed microalga at the pre-stationary phase growth and 1 mL of the bacterial extract. Here, the suppressant of *Alteromonas* sp. Ni1LEM (with already described antifouling activity) was applied as the positive control [10]. The 12-well plates were kept at 20°C with a photoperiodic cycle of 12 h (light:dark) with a light intensity of 100 µmol $\times$ m^{-2} $\times$ s^{-1}. After the period of incubation (24 h attachment), 12-well plates were emptied, and the wells were washed 5 times using sterile seawater in order to remove the remaining loosely attached cells. Next, cell counts of microalgae were taken by microscopic visualization using a $\times$ 100 objective lens (inverted microscope Olympus

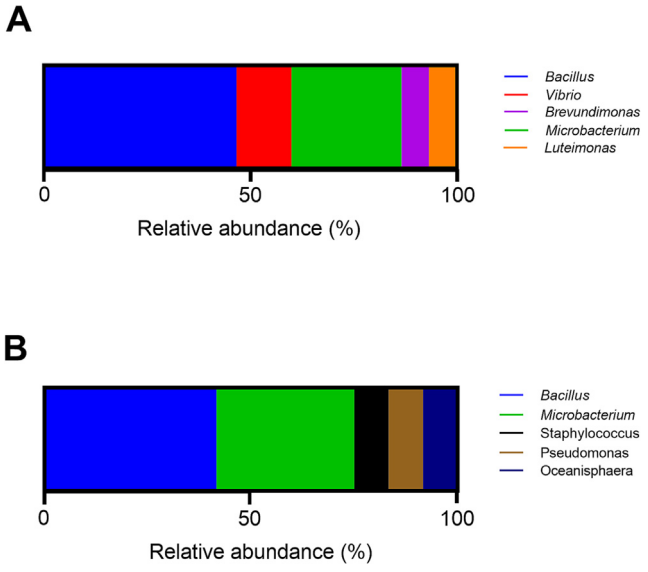


Fig. 1. Relative abundance of bacterial isolates from (A) Cartridge Filter and (B) Reverse osmosis membranes. It is possible to observe that the predominant bacterial genera are *Bacillus* (blue color) and *Microbacterium* (green color).

IX 50) and expressing the values as cells *per* cm^2. Additional negative controls included bioassay using sterile M9 and seawater only. All experimental conditions were performed in triplicate.

2.7. 16S rRNA molecular characterization

DNA from bacterial candidates was extracted using Wizard Genomic DNA Purification (Promega). PCR amplification was carried out using the universal primers 27F (5′-AGAGTTT GATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR amplification products were further purified and sequenced by Macrogen Inc. (Seul, Korea).

3. Results

3.1. Molecular identification of bacterial isolates obtained from ROM and cartridge filters

A total of 27 isolates were obtained from cartridge filters and ROMs used in desalination processes. These isolates possessed different morphotypes, and the bioinformatic analyses showed that curated 16S rRNA sequences obtained high identity and query coverage scores. In detail, we were able to obtain a diversity of bacterial genera from our samples, being the genus *Bacillus* the most dominant isolate (7 isolates from cartridge filters and 5 from ROM), followed by *Microbacterium*. Overall, we found a different profile of isolates obtained from either ROM or filters. For instance, the isolates *Staphylococcus* and *Pseudomonas* were exclusively observed from ROM samples and *Brevundimonas,* and *Vibrio* appeared only in cartridge filters (Fig. 1). Additionally, isolates related to the *Bacillus* genus showed a predominance of *B. cereus* species; meanwhile, *Microbacterium* isolates showed that *M. kitamiense* species were observed in greater occurrence (Table S1).

3.2. Molecular characterization of exoenzymatic activity assessed by plating methods

Using this method, it was possible to screen for different exoenzymatic activities; Lipase, DNase, Cellulase, Protease, Carragenase, and Alginase. We were capable of detecting positive exoenzymatic

A

Sample	Lipase	Carragenase	Protease	DNAse	Celulase	Alginase
Bacillus pacificus (F1.1)						
Bacillus thuringiensis (F1.4)						
Microbacterium maritypicum (F1.6)						
Vibrio alginolyticus (F1.7)						
Microbacterium kitamiense RW47 (F2.3)						
Bacillus cereus st MD152 (F2.4)						
Bacillus cereus st BC1 (F2.5)						
Bacillus cereus st A (F.2.7)						
Vibrio sp. MNW3.1 (F3.1)						
Brevundimonas sp. (F3.2)						
Microbacterium oxidans (F3.3)						
Luteimonas sp. (F3.4)						
Microbacterium oxidans (F3.5)						
Bacillus cereus (F3.6)						
Bacillus wiedmannii (F3.7)						

B

Sample	Lipase	Carragenase	Protease	DNAse	Celulase	Alginase
Bacillus cereus HYM82 (M1.3)						
Bacillus cereus MIR2 (M1.4)						
Microbacterium kitamiense (M1.5)						
Staphylococcus lugdunensis (M1.6)						
Pseudomonas sp. DF5 (M1.7)						
Microbacterium oxidans (M2.1)						
Oceanisphaera donghaeinsis (M2.3)						
Bacillus safensis (M2.4)						
Microbacterium oxidans (M3.6)						
Bacillus tropicus (M4.2)						
Microbacterium kitamiense QT183 (M4.8)						
Bacillus thuringiensis (M4.9)						

Fig. 2. Exoenzymatic activity of bacterial isolates. A) Isolates obtained from cartridge filters. B) Isolates obtained from ROM; red color intensity showed high semiquantitative enzymatic activity depending on the radium of the halo formation.

activity out of all substrates tested, with all isolates obtained from cartridge filters showing to be active against the substrates assessed (Fig. 2A). Conversely, ROM isolates were overall less enzymatically active, with 3 isolates belonging to the genera *Pseudomonas* (M1.7), *Microbacterium* (M2.1) and *Bacillus* (M2.4) showing no positive exoenzymatic activity at all (Fig. 2B). Our

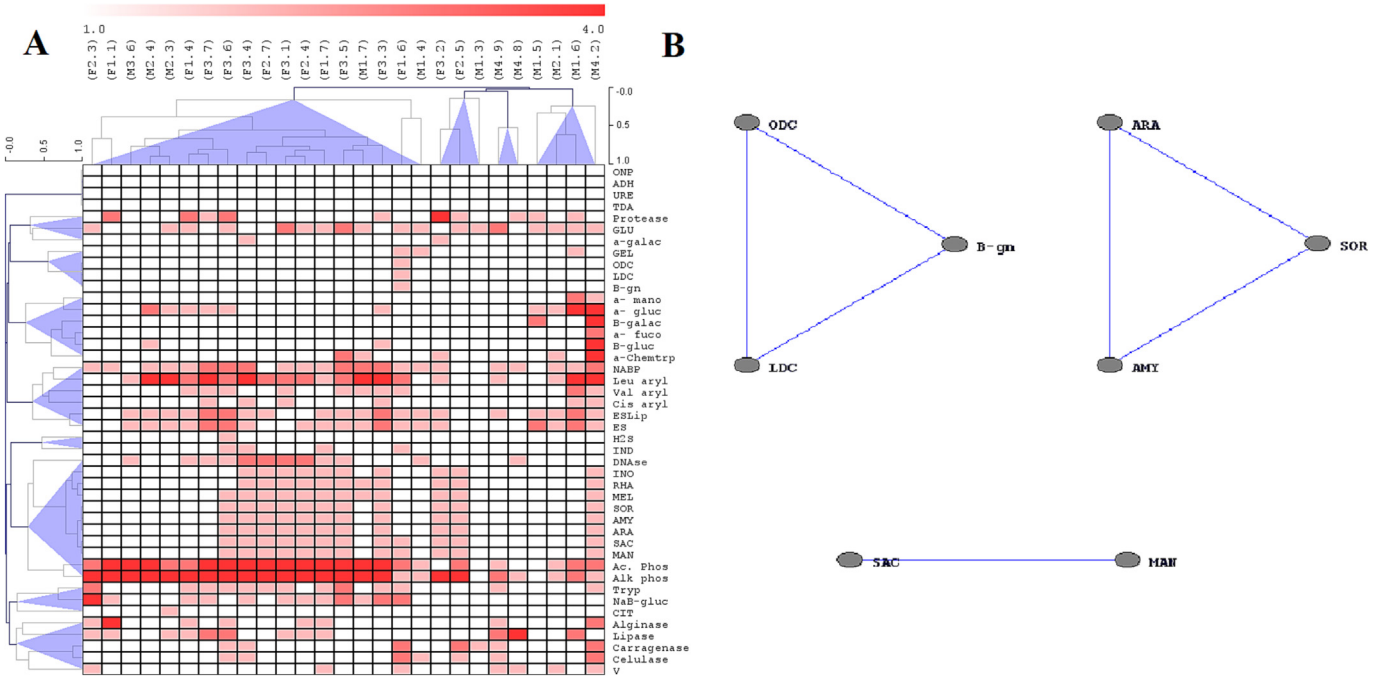


Fig. 3. (A) Hierarchical Clustering Analysis (HCA) of bacterial isolates and their respective exoenzymatic activities from plating, Api20e and Api ZYM methods; (B) Network analysis of enzymatic activities relationships. Alkaline phosphatase (Alkphos), esterase (ES), esterase-lipase (ESlip), leucine (Leu aryl), valine (Val aryl), cysteine (Cis aryl), arilamidases, trypsin (Tryp),α-chemotrypsin (α-chemotryp), acid phosphatase (Ac.phos), naftol-fosfohydrolase (NABP), α y 6-Br-2-naphthyl-glucopyranoside β galactosidase (α-galac y β-galac), β-glucuronidase (β-gn), α y β glucosidase (α-gluc y β-gluc), glycosaminidase (Naβ-gluc), α-mannosidase (α-mano) y α-fucosidase (α-fuco), 2-nitrophenyl-glucopyranoside β-galactosidase (ONP), (Arginine di-hydrolase (ADH), lysine (LDH), ornithine (ODC) decarboxylase, citrate (CIT) usage, H2S production (H2S), Urease (URE), Tryptophan deaminase (TDA), indole (IND), acetoin (V) production, Gelatinase (GEL) and glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), sacarose (SAC), melibiose (MEL), amygdaline (AMY) and arabinose (ARA) degradation were evaluated in each bacterial isolate.

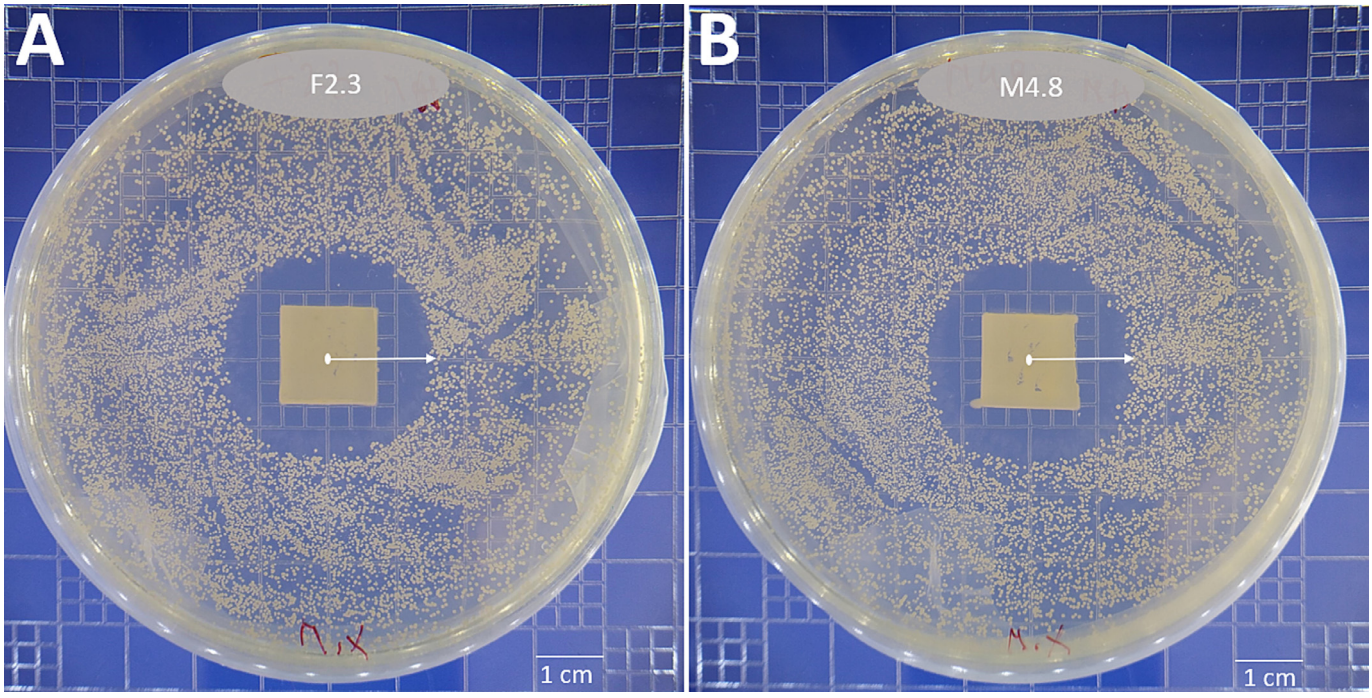


Fig. 4. Antimicrobial activity of *M. kitamiense* strains against bacterial colonizers of ROM. (A) Antimicrobial activity of strain F2.3 obtained from cartridge filter. (B) Antimicrobial activity of strain M4.8 obtained from ROM.

Table 1
Microalgae adherence inhibition exposed to bacterial supernatant from isolated strains F2.3 and M4.8. Two common diatoms of biofouling from Antofagasta coastal intertidal *Navicula incerta (Ni); Amphora* sp. *(Amph)* and 2 diatoms commonly found in ROMs *Nitzschia* sp. *(Nitz) y Psammodictyon* sp. *(Psa).* As the negative control, seawater (CtSW) and M9 culture medium were used (CtM9), and *Alteromonas* sp. supernatant as a positive control (Ni1LEM).

Treatment	Adherence %			
	Ni	Amph	Nitz	Psa
CtSw	95.6 ± 2.5	96.1 ± 3.0	95.4 ± 2.2	95.7 ± 1.9
CtM9	93.4 ± 3.2	94.0 ± 4.0	93.7 ± 3.7	93.9 ± 3.1
Ni1LEM	40.9 ± 2.9	41.5 ± 2.8	40.9 ± 2.5	40.3 ± 3.3
Microbacterium kitamiense RW47 **(F2.3)**	60.5 ± 2.3	62.1 ± 3.4	59.5 ± 4.3	60.1 ± 2.4
Microbacterium kitamiense QT183 **(M4.8)**	39.7 ± 2.7	40.0 ± 1.7	42.2 ± 3.0	40.6 ± 2.9

results showed that the most represented exoenzymatic activity was Lipase (12 isolates in total), followed by DNase and Alginase activities (Fig. 2).

3.3. Biochemical characterization of bacterial isolates using API20 and APIzym kits

To enhance our characterization of the bacterial isolates and better understand their exoenzymatic activity, we employed a set of standardized biochemical tests commercially contained in *API20* and *APIzym* kits. Regardless of molecular similarities at the phylogenetical identity level (*i.e.,* 16S rRNA identification), the results collected from *API* galleries provide a distinctive pattern of enzymatic activity for each of our bacterial isolates. However, we also found that common enzymatic activities are also observable; for instance, lipase activity seems widely dominant across the isolate tested. When collecting results using *APIzym*, isolates from cartridge filter samples displaying positive activities were more abundant and dominated by acid and alkaline phosphatases. In contrast, the enzymatic activities of isolates from ROM samples were dominated by lipases and esterases. Additionally, a similar pattern emerged using *API20*, with most enzymatically active isolates obtained from filters displaying oxidative and fermentative reactions (Fig. S1 and Fig. S2). Even though isolates from ROM samples exhibited lower enzymatic activity, overall, *Bacillus tropicus* (M4.2) stood out due to its exceptional oxidative and fermentative capacity.

To gain a broader view of the enzymatic activity of the bacterial isolates, we conducted a hierarchical clustering analysis (HCA) to visualize correlations between isolated and enzymatic activities detected by both API and plating methods. Our results showed the formation of a dominant cluster mainly composed of microbial isolates obtained from cartridge filters and a minor representation of 3 isolates from ROM samples; however, high correlations (M1.7, M2.3 and M2.4) (Fig. 3A). Conversely, ROM isolates were showed to produce 2 main clusters, which is indicative of a relatively higher enzymatic activity diversity. These 2 clusters were found (M4.8, M4.9 and M1.5, M1.6, M2.1 and M4.2) to be dominated by more than 15 enzymatic activities where phosphatases (acid and alkaline) appear in booth clusters. Additionally, the enzymatic network revealed that the cluster formation was influenced by mainly 2 prevailing metabolic triad functions, one consisting of ODC, LDC and β-gn, and a second ARA, SOR and AMY (Fig. 3B).

3.4. Antimicrobial activity of bacterial isolates against microbial colonizers of ROMs

The antimicrobial capacity of our bacterial isolates was tested against a mix of native biofouling-producing bacteria. Our results show that only 2 out of the 27 testes strains were capable of forming clear-zones halos –an indication of growth inhibition or antimicrobial activity. Interestingly, both isolated strains were identified as *Microbacterium kitamiense* and obtained from either cartridge filter or ROM samples (Fig. 4). However, we could see that *M. kitamiense* M4.8 displayed wider inhibition halo –radium of clear-zone halo.

3.5. Inhibitory effect of bacterial extracts on the adherence of common intertidal and ROM-associated microalgae

Here, we show that most of the extracts do not exhibit adherence inhibition. However, consistent with the results obtained from our assays using live cultures, *M. kitamiense* strains (F2.3 and M4.8) do display an inhibitory effect over typical biofouling-forming microalgae, resulting in a reduction of approximately 40% of the fouling capacity (Table 1). This effect is similar to that observed with Ni1-LEM supernatant used as a positive control.

4. Discussion

4.1. Bacterial isolates obtained from different stages of the RO systems

While molecular and further bioinformatics approaches to describe complex microbial communities have attracted most of the attention, it is well known that only a fraction of those microbes (less than 2% - Wade [21]) is susceptible to being grown by classic microbiological methods. Therefore, this approach, despite its limitations, allows for potential biotechnological application. More specifically, we targeted the discovery of novel bacteria with the capacity to inhibit biofouling in RO membranes and cartridge filters. For example, *Altermonas* sp. Ni1-LEM [22], a strain that we previously isolated and showed its high cleaning potential of ROMs used in seawater desalination plant conditions such as high pressure and water flux [11]. Aiming to obtain bacterial strains highly adapted to marine systems and not mere opportunistic bacteria, our enzymatic assessments were conducted by growing microbes in a minimal medium (M9 – 2% of NaCl). This strategy ultimately allows us to have a better approach to the actual functionality under environmentally relevant conditions, where potential biotechnological solutions would be applied [22].

At an operational level, cartridge filters perform the initial and more substantial reduction of the total microbial load of the seawater that enters the desalination process. This initial filtering step helps to increase the lifespan of the secondary ROM. Unsurprisingly, relatively higher diversity was detected from the cartridge filters compared to ROM samples. Overall, our results show a variety of taxa isolated from ROM and cartridge filter, for instance, *Bacillus, Microbacterium, Vibrio, Pseudomonas, Brevundimonas*, *Luteimonas, Oceanisphaera* and *Staphylococcus.* Interestingly, the genus *Bacillus* and *Microbacterium* were found in both cartridge and ROM samples, which could be due to a high relative abundance in seawater or morphological features that allows them to go through these physical barriers and ensure their persistence along this process [23,24]. Consistently, a substantial number of our

isolates obtained in our investigation have been previously identified from such RO systems [10,25,26].

4.2. Variety of exoenzymatic activity of bacterial isolates obtained from the RO systems

Bacteria are prime examples of organisms with outstanding potential as sources of bioactive compounds and enzymes with a wide range of activities applicable to industrial purposes [27,28,29,30]. In the literature, there are examples of antifouling compounds, such as quorum-quenching molecules [31], enzymes capable of degrading EPS matrix with polysaccharides lyase or hydrolase, peptidase and DNAse activities [32,33]and compounds that inhibit the growth of bacteria [34]. In this study, we observed several enzymatic activities related to biofilm control. Our plating approach targeting several substrates displayed different types of exoenzymatic activities, from which, alginases were among the most efficient enzymes –using the extension of the clear-zone halos to indicate enzymatic efficiency *e.g.,* degradation halo from *B. pacificus* (isolate F1.1). Additionally, Lipases and DNases and hydrolases –enzymes with the potential to control biofilm formation [35,36]– were also over-represented, as indicated by the enzymatic activities characterized in this investigation.

Our biochemical approach –based on the application of *API* galleries– showed that alkaline and acid phosphatases were the top exoenzymatic activity displayed by the bacterial isolates assayed. Although phosphatases have not been previously characterized as antifouling agents, these properties should not be dismissed since these types of enzymes have shown to be effective in other bioremediation strategies as well as in phosphate solubilization (*e.g.* Abdelgalil et al. [37]; Kour et al. [38]). Moreover, we were able to characterize bacterial isolates such as *M. oxidans* (M3.6) with glycolytic enzymes (*e.g.,* α and β glucosidase, α mannosidase and α fucosidase), capable of degrading specific polysaccharides, which suggest a potential as inhibition molecules due to the disassembling of the glycosidic structures within biofouling [39]. As expected, we found an overall higher number of positive biochemical reactions out of the isolates obtained from the cartridge filter, with many of these micros capable of metabolizing of variety of carbohydrates *e.g.,* glucose, mannitol, inositol, sorbitol, rhamnose, saccharose, melibiose, amygdalin, arabinose). Considering that biofilms are composed of an ample array of biomolecules–including a high number of polysaccharides [40]– enzymes displaying such lytic activity become of importance.

4.3. Antifouling activity of bacterial isolates obtained from a seawater desalination plant

Several authors have described the capacity of microbes to produce an array of compounds able to dimmish the fouling formation of surfaces exposed to seawater. These compounds can exert their action at different levels; for instance, some peptides are efficient at interrupting quorum-sensing mechanisms by attaching to surfaces, which ultimately hampers the settling of microbes [10,41,42]. Biomolecules with such properties have attracted increasing attention due to their eco-friendly nature compared with chemical alternatives. In this context, the application of antifouling molecules could become of utter importance, especially in seawater purification systems where fouling can cause serious detrimental effects to the process, such as in the city of Antofagasta, where nearly 90% of tap water production has seawater origin, impacting the continuous tap water supply and endangering living conditions. We have previously worked on similar applications involving bacterial supernatants [43], which can be a potentially more efficient cleaning method than traditional chemical treatments. Here, our targeted approach using filtration systems from a seawater desalination plant as the source for bacteria with antifouling activity showed high potential as a replacement or complementary strategy for cleaning protocols. Typically, conventional chemical cleaning routines entail treating filtration systems with different acid and alkaline solutions for around 10 h and 58 psi of flow. In our experience, the application of raw supernatants as a cleaning solution on ROMs can effectively reduce biofouling; however, the application of specific active antifouling molecules needs to be further explored. Although applying bacterial supernatant can serve as a replacement for industrial chemicals, several variables must be considered, such as treatment time, cleaning routines (*i.e.,* cleaning cycles), and efficiency over time.

As demonstrated throughout this investigation, we successfully obtained a variety of strains with antifouling capacity. Notably, isolate F3.6, which exhibited a high genetic identity to *Bacillus cereus*, showed a diverse range of enzymatic activity, yielding positive results across all targeted substrates assessed in this study. Unfortunately, further exploration of this potential was hindered as *B. cereus* showed poor growth in M9 cultures. Considering future biotechnological applications, the utilization of a minimal medium such as M9 provides both cost-related advantages and the capacity, in some cases, to stimulate the production of secondary metabolites of interest due to nutrient-restrictive growing conditions [44,45]. Under the culture condition considered in our approach, we showed that 2 out of 27 bacterial isolates were capable of hampering biofilm formations –as mentioned previously, a key initial step in the build-up of more complex structures characteristic of biofouling– of either bacterial or microalgal nature previously described affecting RO and cartridge filter in seawater desalination systems. For instance, *Nitzchia Ovalis, Navicula incerta, Psammodictyon* sp., and *Amphora* sp. have been shown to contribute to biofouling [10,22]. Here, the two main candidates screened (*i.e., Microbacterium kitamiense*) belong to a genus that has been reported to produce antifouling compounds [8,46].

We previously mentioned that both *Microbacterium kitamiense* isolates were capable of reducing the growth and attachment of microalgae. Nevertheless, the isolates obtained from ROM samples demonstrated greater efficiency compared to the ones retrieved from filter cartridges. We further hypothesize that the variation in performance is attributed to microenvironmental differences presented by the materials and industrial conditions where these microbes settle. In this context, the selective abiotic pressure on ROMs could be increased due to high-pressure flows (58–800 psi) [43], which may influence the enrichment of microorganisms equipped with versatile survival mechanisms and intensify competition for resources. For instance, *Microbacterium* has been found to actively secrete enzymes with antifouling properties such as proteases, DNases and amylases [46], which aligns with our results. While no further description of the chemical nature of the antifouling molecules produced by our isolates is presented, this investigation provides of wide characterization of the antifouling potential of *M. kitamiense* by classical microbial isolation methods and biochemical characterizations –still valuable approaches in order to produce biotechnological solutions. Even though a full molecular and structural description of antifouling compounds is desirable and currently underway, such isolation efforts should not be dismissed since many other potential microbial compounds might bear answers for several industrial and environmental issues.

5. Conclusions

In this study, we obtained 27 bacterial isolates obtained from desalination plant processes such as filter cartridges and ROMs. These isolates were able to secrete exoenzymes displaying a wide array of bioactivity such as lipases, alginases, and glucosidases,

among others. Additionally, we showed two main candidates able to inhibit the growth of common microbial colonizers of ROM and cartridge filter. These results highlight the potential biotechnological application of bioactive molecules against biofouling formation.

Author contribution

- Study conception and design: H Vera-Villalobos, F Silva Aciares.
- Data collection: H Vera-Villalobos, A Gonzalez-Gutierrez, A Cortes-Martinez.
- Analysis and interpretation of results: H Vera-Villalobos, A Cortes-Martinez, C Riquelme, F Silva Aciares.
- Draft manuscript preparation: H Vera-Villalobos, V Zadjelovic.
- Revision of the results and approval of the final version of the manuscript: H Vera-Villalobos, A Gonzalez-Gutierrez, A Cortes-Martinez, V Zadjelovic, C Riquelme, F Silva Aciares.

Financial support

This study was financed by the Fund for Promotion of Scientific and Technological Development of the National Research and Development Agency (FONDEF, ANID) under Grant N° ID21I10044. V.Z. was supported by ANID - Subvención a la Instalación en la Academia convocatoria año 2022, Folio 85220034.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgments

Filter cartridge and ROMs were kindly provided by Aguas Antofagasta SA, Antofagasta, Chile.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejbt.2023.09.003.

References

[1] Boretti A, Rosa L. Reassessing the projections of the World Water Development Report. NPJ Clean Water 2019;2:15. https://doi.org/10.1038/s41545-019-0039-9.
[2] Schewe J, Heinke J, Gerten D, et al. Multimodel assessment of water scarcity under climate change. PNAS 2014;111(9):3245–50. https://doi.org/10.1073/pnas.1222460110. PMid: 24344289.
[3] Jones E, Qadir M, van Vliet MTH, et al. The state of desalination and brine production: A global outlook. Sci Total Environ 2019;657:1343–56. https://doi.org/10.1016/j.scitotenv.2018.12.076. PMid: 30677901.
[4] Wang Y, Zhang R, Duan J, et al. Extracellular polymeric substances and biocorrosion/biofouling: Recent advances and future perspectives. Int J Mol Sci 2022;23(10):5566. https://doi.org/10.3390/ijms23105566. PMid: 35628373.
[5] Qian PY, Cheng A, Wang R, et al. Marine biofilms: Diversity, interactions and biofouling. Nat Rev Microbiol 2022;20:671–84. https://doi.org/10.1038/s41579-022-00744-7. PMid: 35614346.
[6] Hibbing ME, Fuqua C, Parsek MR, et al. Bacterial competition: Surviving and thriving in the microbial jungle. Nat Rev Microbiol 2010;8:15–25. https://doi.org/10.1038/nrmicro2259. PMid: 19946288.
[7] Dobretsov S, Abed RMM, Teplitski M. Mini-review: Inhibition of biofouling by marine microorganisms. Biofouling 2013;29(4):423–41. https://doi.org/10.1080/08927014.2013.776042. PMid: 23574279.
[8] Nagaraj V, Skillman L, Li D, et al. Control of biofouling by xanthine oxidase on seawater reverse osmosis membranes from a desalination plant: Enzyme production and screening of bacterial isolates from the full-scale plant. Lett Appl Microbiol 2017;65(1):73–81. https://doi.org/10.1111/lam.12747. PMid: 28418590.
[9] Dobretsov S, Dahms HU, Qian PY. Inhibition of biofouling by marine microorganisms and their metabolites. Biofouling 2006;22(1):43–54. https://doi.org/10.1080/08927010500504784. PMid: 16551560.
[10] Vera-Villalobos H, Pérez V, Contreras F, et al. Characterization and removal of biofouling from reverse osmosis membranes (ROMs) from a desalination plant in Northern Chile, using *Alteromonas* sp. Ni1-LEM supernatant. Biofouling 2020;36(5):505–15. https://doi.org/10.1080/08927014.2020.1776268. PMid: 32545993.
[11] Avendaño-Herrera RE, Riquelme CE, Silva F. Utilización de biopelículas bacterianas en el asentamiento de larvas de *Argopecten purpuratus* (Lamarck 1819) en un hatchery comercial. Rev Biol Mar Oceanogr 2002;37(1):35–41. https://doi.org/10.4067/S0718-19572002000100006.
[12] Sierra G. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie Van Leeuwenhoek 1957;23:15–22. https://doi.org/10.1007/BF02545855. PMid: 13425509.
[13] Jeffries CD, Holtman DF, Guse DG. Rapid method for determining the activity of microorganisms on nucleic acids. J Bacteriol 1957;73:590–1. https://doi.org/10.1128/jb.73.4.590-591.1957. PMid: 13428699.
[14] Kasana RC, Salwan R, Dhar H, et al. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. Curr Microbiol 2008;57:503–7. https://doi.org/10.1007/s00284-008-9276-8. PMid: 18810533.
[15] Zhang X, Shuai Y, Tao H, et al. Novel method for the quantitative analysis of protease activity: The casein plate method and its applications. ACS Omega 2021;6(5):3675–80. https://doi.org/10.1021/acsomega.0c05192. PMid: 33585747.
[16] Chauhan PS, Saxena A. Bacterial carrageenases: An overview of production and biotechnological applications. Biotech 2016;6:146. https://doi.org/10.1007/s13205-016-0461-3. PMid: 28330218.
[17] Gacesa P, Wusteman FS. Plate assay for simultaneous detection of alginate lyases and determination of substrate specificity. Appl Environ Microbiol 1990;56(7):2265–7. https://doi.org/10.1128/aem.56.7.2265-2267.1990. PMid: 16348243.
[18] Rédei GP. M9 Bacterial Minimal Medium. In: Encyclopedia of Genetics, Genomics, Proteomics and Informatics, Springer, Dordrecht. 2008, p. 1135. https://doi.org/10.1007/978-1-4020-6754-9_9692.
[19] Howe E, Holton K, Nair S, et al. MeV: MultiExperiment viewer. In: Ochs M, Casagrande J, Davuluri R, editors. Biomedical Informatics for Cancer Research. Boston, MA: Springer; 2010. p. 267–77. https://doi.org/10.1007/978-1-4419-5714-6_15.
[20] Guillard RRL. Culture of phytoplankton for feeding marine invertebrates. In: Smith W.L., Chanley M.H., editors. Culture of Marine Invertebrate Animals. Boston, MA: Springer. https://doi.org/10.1007/978-1-4615-8714-9_3
[21] Wade W. Unculturable Bacteria—The Uncharacterized organisms that cause oral infections. J R Soc Med 2002;95(2):81–3. https://doi.org/10.1177/014107680209500207. PMid: 11823550.
[22] Infante CD, Castillo F, Pérez V, et al. Inhibition of *Nitzschia ovalis* biofilm settlement by a bacterial bioactive compound through alteration of EPS and epiphytic bacteria. Electron J Biotechnol 2018;33:1–10. https://doi.org/10.1016/j.ejbt.2018.03.002.
[23] Al-Abri M, Kyaw HH, Al-Ghafri B, et al. Autopsy of used reverse osmosis membranes from the largest seawater desalination plant in Oman. Membranes 2022;12(7):671. https://doi.org/10.3390/membranes12070671. PMid: 35877874.
[24] Nagaraj V, Skillman L, Li D, et al. Culturable bacteria from a full-scale desalination plant: Identification methods, bacterial diversity and selection of models based on membrane-biofilm community. Desalination 2019;457:103–14. https://doi.org/10.1016/j.desal.2019.01.028.
[25] Bereschenko LA, Stams AJM, Heilig GHJ, et al. Investigation of microbial communities on reverse osmosis membranes used for process water production. Water Sci Technol 2007;55(8–9):181–90. https://doi.org/10.2166/wst.2007.257 PMid: 17546985.
[26] Vitzilaiou E, Stoica IM, Knøchel S. Microbial biofilm communities on Reverse Osmosis membranes in whey water processing before and after cleaning. J Memb Sci 2019;587:. https://doi.org/10.1016/j.memsci.2019.117174117174.
[27] Kafarski P, Lejczak B. Application of bacteria and fungi as biocatalysts for the preparation of optically active hydroxyphosphonates. J Mol Catal B Enzym 2004;29(1–6):99–104. https://doi.org/10.1016/j.molcatb.2003.12.013.
[28] Jonkers HM, Thijssen A, Muyzer G, et al. Application of bacteria as self-healing agent for the development of sustainable concrete. Ecol Eng 2010;36(2):230–5. https://doi.org/10.1016/j.ecoleng.2008.12.036.
[29] Oh HS, Tan CH, Low JH, et al. Quorum quenching bacteria can be used to inhibit the biofouling of reverse osmosis membranes. Water Res 2017;112:29–37. https://doi.org/10.1016/j.watres.2017.01.028. PMid: 28129553.
[30] Abdulrahman I, Jamal MT, Pugazhendi A, et al. Antibiofilm activity of secondary metabolites from bacterial endophytes of Red Sea soft corals. Int Biodeter Biodegr 2022;173:. https://doi.org/10.1016/j.ibiod.2022.105462105462.
[31] Huang S, Xu B, Ng TCA, et al. Feasibility of implementing quorum quenching technology to mitigate membrane fouling in MBRs treating phenol-rich pharmaceutical wastewater: Application of *Rhodococcus* sp. BH4 and quorum quenching consortium. Bioresour Technol 2022;358:. https://doi.org/10.1016/j.biortech.2022.127389. PMid: 35636678127389.
[32] Bachosz K, Vu MT, Nghiem LD, et al. Enzyme-based control of membrane biofouling for water and wastewater purification: A comprehensive review. Environ Technol Innov 2022;25:. https://doi.org/10.1016/j.eti.2021.102106102106.

[33] Barzkar N, Sheng R, Sohail M, et al. Alginate lyases from marine bacteria: An enzyme ocean for sustainable future. Molecules 2022;27(11):3375. https://doi.org/10.3390/molecules27113375. PMid: 35684316.
[34] Srinivasan R, Santhakumari S, Poonguzhali P, et al. Bacterial biofilm inhibition: A focused review on recent therapeutic strategies for combating the biofilm mediated infections. Front Microbiol 2021;12:. https://doi.org/10.3389/fmicb.2021.676458. PMid: 34054785676458.
[35] Kiran GS, Lipton AN, Kennedy J, et al. A halotolerant thermostable lipase from the marine bacterium *Oceanobacillus* sp. PUMB02 with an ability to disrupt bacterial biofilms. Bioeng Bugs 2014;5(5):305–18. https://doi.org/10.4161/bioe.29898. PMid: 25482232.
[36] Sharma K, Singh AP. Antibiofilm effect of DNase against single and mixed species biofilm. Foods 2018;7(3):42. https://doi.org/10.3390/foods7030042. PMid: 29562719.
[37] Abdelgalil SA, Soliman NA, Abo-Zaid GA, et al. Biovalorization of raw agro-industrial waste through a bioprocess development platform for boosting alkaline phosphatase production by *Lysinibacillus* sp. strain APSO. Sci Rep 2021;11:17564. https://doi.org/10.1038/s41598-021-96563-6. PMid: 34475429.
[38] Kour D, Kl R, Kaur T, et al. Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and -mobilizing microbes: A review. Pedosphere 2021;31(1):43–75. https://doi.org/10.1016/S1002-0160(20)60057-1.
[39] Aykin E, Omuzbuken B, Kacar A. Microfouling bacteria and the use of enzymes in eco-friendly antifouling technology. J Coat Technol Res 2019;16:847–56. https://doi.org/10.1007/s11998-018-00161-7.
[40] Barnes RJ, Bandi RR, Chua F, et al. The roles of *Pseudomonas aeruginosa* extracellular polysaccharides in biofouling of reverse osmosis membranes and nitric oxide induced dispersal. J Memb Sci 2014;466:161–72. https://doi.org/10.1016/j.memsci.2014.04.046.
[41] Cloete WJ, Adriaanse C, Swart P, et al. Facile immobilization of enzymes on electrospun poly(styrene-alt-maleic anhydride) nanofibres. Polym Chem 2011;2(7):1479–81. https://doi.org/10.1039/c1py00069a.
[42] Gule NP, Begum NM, Klumperman B. Advances in biofouling mitigation: A review. Crit Rev Environ Sci Technol 2016;46(6):535–55. https://doi.org/10.1080/10643389.2015.1114444.
[43] Vera-Villalobos H, Riquelme C, Silva-Aciares F. Use of *Alteromonas* sp. Ni1-LEM supernatant as a cleaning agent for Reverse-Osmosis Membranes (ROMs) from a desalination plant in Northern Chile affected by biofouling. Membranes 2023;13(5):454. https://doi.org/10.3390/membranes13050454. PMid: 37233515.
[44] Kim J, Kim KH. Effects of minimal media vs. complex media on the metabolite profiles of *Escherichia coli* and *Saccharomyces cerevisiae*. Process Biochem 2017;57:64–71. https://doi.org/10.1016/j.procbio.2017.04.003.
[45] Rugbjerg P, Feist AM, Sommer MOA. Enhanced metabolite productivity of *Escherichia coli* adapted to glucose M9 minimal medium. Front Bioeng Biotechnol 2018;6:166. https://doi.org/10.3389/fbioe.2018.00166. PMid: 30483499.
[46] Saggu SK, Jha G, Mishra PC. Enzymatic degradation of biofilm by metalloprotease from *Microbacterium* sp. SKS10. Front Bioeng Biotechnol 2019;7:192. https://doi.org/10.3389/fbioe.2019.00192. PMid: 31448272.