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Adhesion potential of bacteria retrieved from intake seawater and membrane biofilms on full-scale reverse osmosis desalination process

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ABSTRACT

Micro-organisms were isolated from intake seawater and reverse osmosis (RO) membrane biofilms collected from a full-scale membrane-based desalination process. The results from a culture-dependent approach using 12 media were combined with the microbial community structure on fouled RO membranes as analyzed by a 16S rRNA clone library construction in our previous study. This was followed by selection of 11 target bacteria for further analysis, which were suspected to be responsible for biofilm formation on membrane surfaces. The adhesion of potential biofoulants differing in surface hydrophobicity and charge was examined. Cell wall hydrophobicity was measured as the contact angle of a lawn of bacteria, and by adhesion to hexadecane. The cell surface charge was investigated by measuring electrophoretic mobility. The data obtained from these methodologies were compared. According to the cell surface charge measurements, *Pseudomonas aeruginosa*, *Acinetobacter venetianus*, *Cellvibrio mixtus* subsp. Mixtus, *Bacillus* sp. Eur1 9.5, and *Escherichia coli* K12 could mediate initial adhesion to negatively charged RO membranes through electrostatic attraction. *Limnobacter* sp. KNF002, *A. venetianus*, and *Simiduia agarivorans* showed higher affinity to hexadecane than other bacterial strains tested, and *Bacillus* sp. Eur1 9.5, *C. mixtus* subsp. Mixtus, and *P. aeruginosa* were determined to have greater hydrophobic interactions with hydrophobic RO membranes.

Keywords: Membrane biofouling; Biofilm bacteria; Bacterial cell surface characterization; Bacterial adhesion

1. Introduction

A major drawback in a membrane system is fouling of the membrane surface, which can be caused by a variety of contaminants in the feed water, including inorganic compounds, colloidal or particulate matter, dissolved organics, and micro-organisms and their microbial products [1–3]. In a membrane system, biofouling is thought to be one of the biggest technical challenges for reverse osmosis (RO) membranes in

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particular, since all other foulants are controllable using various inhibitors (inorganic scaling) and pretreatments (particulate fouling) [4]. Micro-organisms are commonly found in all aquatic systems. They can survive, and even multiply, in oligotrophic environments [5]. Biofouling was proven to be more complex than other types of membrane fouling since it is influenced by membrane surface properties (roughness, hydrophobicity, electrokinetic charge, and pore size), feed water chemistry (pH, ionic strength, and osmotic pressure), and also microbial properties (size, cell surface hydrophobicity, and charge) [6].

When a clean membrane is exposed to an aqueous environment containing micro-organisms and dissolved organics, the surface is initially covered with a layer of organic molecules, generally called a "conditioning layer" [3]. This process occurs more rapidly than microbial attachment. Next, suspended microbes migrate and attach to the membrane surface. During this stage, attachment is influenced by hydrophobic and electrostatic interactions between microbial cells and the membrane surface. Alternatively, micro-organisms may migrate toward each other, forming microbial co-aggregates. Lastly, attached micro-organisms start growing on the membrane, leading to biofilm formation [7,8]. It has been known that extracellular polymeric substance (EPS) and soluble microbial product are the main fouling factors in membrane systems [9]. It is now recognized that transparent exopolymer particles (TEPs) play an important role in the process of aquatic biofim formation [10], particularly in the early stage of biofilm development. Bar-Zeev et al. [10] introduced the new term "proto-biofilms" to refer to TEPs with microbial outgrowth and colonization. The authors have found that TEPs and proto-biofilms were the main sources of the early biofilm formation, particularly under the seawater condition.

Micro-organisms have a strong tendency to become associated with substratum surfaces in aquatic environments [11]. Cell surface hydrophobicity is one of the most important properties involved in adhesion phenomena. Of long-range, non-covalent interactions in biological systems, hydrophobic interactions are normally the strongest. They are defined as the attraction between apolar, or slightly polar, cells and other molecules when immersed in an aqueous solution [12,13]. Short-range interactions become significant only when cells and the substratum surface are in close contact [14]. Bacterial cell surface hydrophobicity can be determined by the contact angle measurement of the liquid cell on the solid surface, by partitioning bacteria between two aqueous phases, or by quantifying the number of bacteria adhering to organic solvent droplets [15]. In other words, hydrophobicity is the tendency of water to exclude non-polar molecules and atoms, and it is based on the simple rationale that the hydrocarbon interface and the interface of other hydrophobic ligands against an aqueous solution would be uncharged. Hydrophilic organisms would remain in the aqueous phase and hydrophobic organisms would adhere to the hydrophobic hydrocarbon phase. While the process of bacterial adhesion on medical device substrata has been extensively investigated, the possible mechanisms involved in bacterial transport and attachment in water purification systems, such as RO membrane systems, have not been thoroughly explored. The relative importance of different adhesion mechanisms between bacterial cells and the membrane surface is also not well understood [16]. Previous studies revealed that hydrophobic bacterial strains could mediate bacterial adhesion onto commercially available hydrophobic RO membrane surfaces through hydrophobic interactions [17,18]. For example, adhesion of a hydrophobic strain of *Pseudomonas aeruginosa* to glass, copper, stainless steel, and silicon surfaces was more significant than the less hydrophobic *Pseudomonas fluorescens* strain, suggesting that hydrophobic bacterial cells have more effective adhesive properties in real systems [19].

Bacterial cell surfaces have a net negative charge owing to ionized phosphoryl and carboxylate functional groups on outer cell envelope macromolecules that are exposed to the extracellular environment. The overall cell surface charge can be assessed by the zeta potential, which is determined by electrophoretic mobility of the cells [20]. When a bacterial cell exists in an aqueous phase, ions with an opposite charge to that of the bacterial cell surround the cell, close to the cell surface. This forms an electrical double layer around each cell. The liquid layer surrounding the bacterial cell consists of two regions; the Stern layer, where ions are strongly bound, and the diffuse layer, where ions are loosely attached. When a bacteria move in the aqueous phase, ions on the surface of bacteria make a stable boundary in the diffused layer. Even though ions within this boundary were transferred with the cell, ions beyond this boundary keep remained in the aqueous phase [21]. This boundary is called the slipping plane, and its potential is known as the zeta potential [20]. Not only hydrophobic, but also electrophoretic interactions mediate adhesive contacts between bacterial cells and membranes in the aqueous phase [13]. Commercialized RO membranes and bacterial cells in their physiological pHs tend to be negatively charged [14], indicating that electrostatic repulsion may occur between bacterial cells and the membrane surface when membranes are exposed to aqueous environments containing micro-organisms

[22]. Thus, strains having a greater negative charge would experience greater repulsion than less negatively charged cells, thus allowing lesser charged cells to adhere more readily to membrane surfaces [13]. A micro-organism's cell surface charge can be used to determine its adhesive properties, together with other factors such as cell surface hydrophobicity.

In this study, cell surface properties of 11 bacteria were evaluated to determine cell surface hydrophobicity and charge. These bacteria were selected based on our previous clone library construction and, in the present study, by culture-dependent isolation using 12 different media. Two widely used bacterial model strains were also used for analysis. To determine the importance of hydrophobic and electrostatic interactions between potential biofilm bacterial cells and RO membranes, we investigated cell surface properties, such as hydrophobicity using microbial adhesion to hydrocarbon (MATH), contact angle measurements (CAM), and surface charge by particle zeta potential, under three different ionic strengths in conjunction with the surface characteristics of RO membranes.

2. Materials and methods

2.1. Intake seawater and fouled membrane samples

Intake seawater and fouled RO membrane samples (SWC 4+, Hydranautics, CA, USA) were collected from a RO system in a commercial desalination plant in Fujairah, United Arab Emirates in November 2008. Brief details of the RO system were described in previous studies [23,24].

2.2. Isolation and identification of biofilm bacteria

Bacterial biomass (0.13 g) of the fouling layer of fouled RO membranes was scraped off using a sterilized blade soaked in 30 mL of sonication buffer solution (50-mM Tris-HCl and 10-mM EDTA in 1.5-M NaCl; pH 8.0). The bacterial biomass and intake seawater samples were used as inoculum sources for bacterial isolation. The pH of the intake seawater was 8.1. Various nutrient media were selected by literature review and listed in Table 1 [25–28]. Oligotrophic media (diluted marine broth, ZoBell, and R2A) and copiotrophic media (named super ZoBell) were also used. Dehydrated culture media and formulas were obtained from Difco (MI, USA). Anaerobic media were prepared in serum vials (Wheton Scientific Co., NJ, USA) and pressurized by oxygen free nitrogen gas (Sinilgas Co., Gwangju, Korea), and all cultivation and isolation steps were conducted in an anaerobic glove box (Coy Lab. Products, Inc., MI, USA). In order to achieve cultivation conditions that were as natural as possible, 3.5% (w/v) of sodium chloride (NaCl) and 5% (v/v) of 0.2 μm filter-sterilized intake seawater were added, and the pH was adjusted to 8.0 before autoclaving. Serial dilutions (100 μL each) of foulants/buffer solution and seawater samples were spread onto each nutrient agar plate and incubated for 1–14 d with and without light source, depending on their growth rate. For subculture, colonies were screened for morphological differences, picked using a sterilized toothpick, and transferred into culture tubes containing the same composition of liquid media. After cell growth, cultures were streak plated to obtain purified single colonies.

A colony polymerase chain reaction (PCR) technique was employed for identification of isolates as described in our previous study [29]. In short, colony PCR was performed using one pair of vector primers; M13f (5′-GTT TTC CCA GTC ACG AC-3′), and M13r (5′-TCA CAC AGG AAA CAG CTA TGA C-3′). All colony PCR products were then sequenced using a DNA Analyzer (ABI 3730XL, Applied Biosystems, USA), and the results were compared with 16S rRNA sequences available in the GenBank database (January 2010) using a BLAST search.

2.3. Analytical methods

2.3.1. Contact angle measurements (CAM) of bacterial cell surfaces

The hydrophobicity of biofilm bacteria was determined by CAM. Cell cultures grown overnight were harvested, washed twice using 0.85% (w/v) NaCl solution to remove traces of medium components, and resuspended in 15 mL 0.85% (0.15 M), 3.5% (0.60 M) and 4.5% (0.77 M) NaCl solution to obtain an absorbance value (OD_{600}) of 1.0. To investigate the influence of ionic strength on the hydrophobicity and zeta potential measurements, three different salt concentrations were chosen: 0.15 M, as representative bacterial physiological conditions; 0.6 M, natural seawater; and 0.77 M, the increased total dissolved solids (TDS) concentration on the RO membrane surface when salt rejection occurred (based on the operational data from the desalination plant). These bacterial cell mixtures were deposited on 0.2-μm polycarbonate filters (PC, Whatman, UK) using negative pressure (vacuum filtration). Filters with a lawn of bacteria were mounted on glass slides and air-dried for 30–60 min to remove moisture. Then, deionized (DI) water was introduced onto the PC filter surfaces. The contact angle of each bacterium was measured using the commercialized

Table 1
Composition of media collection for isolation

Medium	Formula per liter
Marine Broth	Marine Broth 2,216 37.4 g (Peptone 5.0 g; Yeast extract 1.0 g; Ferric citrate 0.1 g; Sodium chloride 19.45 g; Magnesium chloride 5.9 g; Magnesium sulfate 3.24 g; Calcium chloride 1.8 g; Potassium chloride 0.55 g; Sodium bicarbonate 0.16 g; Potassium bromide 0.08 g; Strontium chloride 34.0 mg; Boric acid 22.0 mg; Sodium silicate 4.0 mg; Sodium fluoride 2.4 mg; Ammonium nitrate 1.6 mg; Disodium phosphate 8.0 mg)
ZoBell	Peptone 5 g; Yeast extract 1 g; Ferric citrate 0.1 g
R2A	Yeast extract 0.5 g; Proteose peptone No. 3 0.5 g; Casamino acids 0.5 g; Dextrose 0.5 g; Soluble starch 0.5 g; Sodium pyruvate 0.3 g; Dipotassium phosphate 0.3 g; Magnesium sulfate 0.05 g
mPlate Count Broth	Tryptone 10 g; Yeast extract 5 g; Dextrose 2 g
Tryptic Soy Broth	Pancreatic digest of casein 17.0 g; Enzymatic digest of soybean meal 3.0 g; Sodium chloride 5.0 g; Dipotassium phosphate 2.5 g; Dextrose 2.5 g
LB	Tryptone 10 g; Yeast extract 5 g; Sodium chloride 0.5 g
Diluted Marine Broth (Yeast extract and peptone only)	Peptone 0.5 g; Yeast extract 0.1 g; Ferric citrate 0.1 g; Sodium chloride 19.45 g; Magnesium chloride 5.9 g; Magnesium sulfate 3.24 g; Calcium chloride 1.8 g; Potassium chloride 0.55 g; Sodium bicarbonate 0.16 g; Potassium bromide 0.08 g; Strontium chloride 34.0 mg; Boric acid 22.0 mg; Sodium silicate 4.0 mg; Sodium fluoride 2.4 mg; Ammonium nitrate 1.6 mg; Disodium phosphate 8.0 mg
Diluted ZoBell	Peptone 0.5 g, Yeast extract 0.1 g, Ferric citrate 0.01 g
Super ZoBell	Peptone 5 g; Yeast extract 1 g; Ferric citrate 0.1 g; Glucose 20 g
Diluted R2A	Yeast extract 0.05 g; Proteose peptone No.3 0.05 g; Casamino acids 0.05 g; Dextrose 0.05 g; Soluble Starch 0.05 g; Sodium Pyruvate 0.03 g; Dipotassium phosphate 0.03 g; Magnesium sulfate 0.005 g
PTYG	Peptone 5 g; Tryptone 5 g; Yeast extract 10 g; Magnesium sulfate heptahydrate 0.6 g; Calcium chloride 0.06 g
TYGPN	Tryptone 20 g; Yeast extract 10 g; Glycerol 10 g; Disodium Phosphate 5 g; Potassium nitrate 10 g

Note: In case of anaerobic cultivation, strict anaerobic technique was employed.

sessile drop method and contact angle goniometer (Model 100, Ramé-Hart Inc., NJ, USA). Ten measurements were taken for each sample; the maximum and minimum values were discarded, and the results were averaged.

2.3.2. Microbial adhesion to hydrocarbon (MATH)

In addition to CAM, cell surface hydrophobicity was evaluated by microbial adhesion to hydrocarbon, as previously described with minor modifications [13]. Cells cultured overnight were harvested, washed twice using 0.85% NaCl solution, and resuspended in 0.15, 0.60, and 0.77 M NaCl solutions to obtain an OD_{600} value of 0.6. Then, 1.5 mL of the diluted cell mixture was transferred into test tubes and mixed with different volumes of hexadecane at 0.2 mL increments, from 0.4 to 1.4 mL. This mixture was pre-incubated for 10 min, vortexed vigorously for 2 min, and then hydrocarbon and aqueous phase separation was allowed to proceed for 30 min. The loss in absorbance in the aqueous phase relative to the initial absorbance value was taken to represent the amount of cells adhering to hexadecane by the formula: $\%\ \text{Adherence} = \left[1 - \left(\frac{A}{A_0}\right)\right] \times 100$ where A_0 is the initial OD_{600} value and A is the OD_{600} value after its separation from hexadecane. The highest value was used to compute hydrophobicity due to the sensitivity of the methods during vortexing and interference by the formation of small droplets of hexadecane in the aqueous phase [30].

2.3.3. Zeta potential measurements

Since direct methods to measure microbial cell surface charge does not exist [20], cell surface charge was indirectly measured and expressed as zeta potential in this study. Cells cultured overnight were harvested, washed twice using 0.85% NaCl solution, and then resuspended in 18-mL 10-mM KCl solution (pH ~7.5) spiked with different NaCl concentrations (0.15, 0.60, and 0.77 M). The OD_{600} values of these cell mixtures were adjusted to 1.0. The electrophoretic mobilities of bacterial cells were then measured using a Photal ELS-8000 (Otsuka Electronics, Japan) and converted into zeta potential values using the Smoluchowski equation.

3. Results and discussion

3.1. Bacterial isolates

Thirty-eight different bacterial isolates (84 in totals) were identified from the RO membrane samples, while 13 different isolates (32 in totals) were identified from intake seawater samples, based on their morphological patterns. In both sample types, bacterial isolates were obtained from Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and uncultured groups (environmental samples). Two phyla were common in both sample types, namely Proteobacteria and Bacteroidetes (see Table 2). Isolates in RO and intake seawater samples primarily belonged to the γ-Proteobacteria class (78.1% and 63.9% of total isolates, respectively) as shown in Fig. 1. In the seawater sample, isolates were highly similar to γ-Proteobacteria, α-Proteobacteria, Actinobacteridae, and Flavobacteriia bacterial classes, whereas isolates were closely related γ-Proteobacteria, α-Proteobacteria, β-Proteobacteria, Bacilli, Clostridia, and Cytophagia classes in the RO samples. The proportion of unknown group grew from 3.1% in the seawater sample to 10.9% in the RO sample (refer to Table 2). This result supports the complexity of the microbial community of RO sample as presented in our previous study [29]. In the seawater samples, 9 and 4 isolates were closely related to *Alcanivorax* sp. Mho1 and *Vibrio* sp. PM6A, respectively, which are dominant in γ-subclass. These isolates were different from that of γ-Proteobacteria found in the RO samples, where *Acinetobacter venetianus*, *Marinobacter hydrocarbonoclasticus*, and *Vibrio* sp. PM6A were dominant. Intake seawater and RO samples contained three common isolates within this subclass: *Gammaproteobacterium* B32, *M. hydrocarbonoclasticus*, and *Vibrio* sp. PM6A. Among these, *Vibrio* sp. PM6A was prevalent in both samples. Since these dominant isolates in γ-subclass from RO were commonly found in marine environments [31–34], it is likely that they were initially present in the intake seawater until the pre-treatment stage (microfiltration pre-treatment), and they were later deposited on the RO membrane surfaces. In the RO samples, bacterial isolates of the Bacilli class were related to *Bacillus* sp. CNJ733 PL04, *Clostridium* sp. enrichment culture clone MB3_7, *Halobacillus* spp., *Exiguobacterium* sp. TL, and *Caldanaerocella colombiensis*.

3.2. Selection of target bacteria for cell surface characterization

A group of 11 target bacteria were selected and listed in Table 3. This group includes *A. venetianus* and *M. hydrocarbonoclasticus* which were isolated from fouled RO membranes, *Alcanivorax* sp. Mho1 and *Vibrio* PM6A from intake seawater, as well as *Simiduia agarivorans* (Accession Number; JCM 13881), *Limnobacter* sp. KNF002 (DSM 13612), *Cellvibrio mixtus* subsp. Mixtus (NCIMB 8633), and *Escherichia coli* K12, which were identified in our previous study as potential biofoulants by microbial community analysis [29]. Note that in our previous study, 16S rRNA sequence results were compared with the EZ TAXON database, while same sequence results were compared with 16S rRNA sequences available in the GenBank database using a BLAST search in the present study. Thus, the differences in major strains by the culture independent technique between two studies attributed to the different 16S rRNA sequence database. The major strains obtained by the culture independent technique were then purchased from the culture collections based on the results from the GenBank database. Additionally we used, *Bacillus* sp. EUR1 9.5 (DSMZ 18773; deposited as *Tumebacillus permanentifrigoris*), which was analyzed in our previous study as a dominant bacterium from the same sampling site [35]. *Bacillus* sp. (KCTC 3872) and *P. aeruginosa* (KCTC 1636), which are widely used as model biofoulants in membrane systems, were also selected for this study.

3.3. Cell surface characterizations of bacterial isolates

3.3.1. Cell surface hydrophobicity by CAM

The CAM results for each bacterium are summarized in Table 4. The contact angle values ranged from most hydrophilic (12.3° for *S. agarivorans*) to the most hydrophobic (72° for *P. aeruginosa*). Overall, the strains were relatively hydrophobic, ranging between 20° and 50°. *Bacillus* sp. Eur1 9.5, *C. mixtus* subsp. Mixtus, and *P. aeruginosa* were more hydrophobic, with higher cell surface hydrophobicity than other bacterial strains. This indicated that short- and long-range hydrophobic interactions are greater in these micro-organisms. Changes

Table 2
Isolates as revealed by conventional isolation from intake seawater and fouled RO membrane

Bacterial group		Closest related bacteria	No. of Isolates	Closest related bacteria	No. of Isolates
Phylum	Class	Intake seawater	32	RO membrane	84
Actinobacter	Actinobacteridae	*Microbacterium trichothecenolyticum*	2	ND	
Bacteroidetes	Cytophagia	ND[a]		*Cytophaga* sp. SA1	1
	Flavobacteriia	*Tamlana crocina*	2	ND	
	α-Proteobacteria	*Shimia marina*	2	*Alphaproteobacterium* C06	2
				Martelella mediterranea	2
				Alpha proteobacterium IMCC1702	3
				Novosphingobium capsulatum	1
	β-Proteobacteria	ND		*Alcaligenes* sp. S-SL-5	1
Proteobacteria	γ-Proteobacteria			*Acinetobacter venetianus*	16
				Acinetobacter venetianus Strain ACI555	4
				Acinetobacter sp. TS IW 07	2
				Balneatrix alpaca[b]	1
				Enterobacter cloacae	1
				Enterobacter sp. AAJ3	1
				Gamma proteobacterium 12IX/A01/168	1
				Gamma proteobacterium B32	1
		Alcanivorax sp. Mho1	9	*Klebsiella pneumoniae* subsp.	1
		Alteromonas sp. NJSS41	2	*Pneumonia*	
		Gammaproteobacterium B32	3	*Marinobacter hydrocarbonoclasticus*	5
		Microbacterium sp. 2761	1	*hydrocarbonoclasticus* strain	
		Pseudoalteromonas sp. UST981101-030	2	S6-02	
				Marinobacterium litorale	1
		Vibrio sp. PM6A	4	*Microbacterium* sp. S15-C	1
		Vibrio campbellii	2	*Oceanimonas denitrificans*	1
		Vibrio harveyi	1	*Oceanimonas denitrificans* strain F13-1	2
				Pseudomonas sp. D6-6	1
				Pseudomonas pseudoalcaligenes	2
				Pseudomonas sp. BWDY-42	2
				Pseudomonas sp. FSB	1
				Rheinheimera aquimaris	2
				Rheinheimera aquimaris strain SW-369	2
				Vibrio sp. PM6A	4
Firmicutes	Bacilli	ND		*Bacillus* sp. CNJ733 PL04	2
				Uncultured *Virgibacillus* sp.	1
				Halobacillus sp. B298Ydz-ds	2
				Halobacillus sp. NT N163	1
				Exiguobacterium sp. TL	2
	Clostridia	ND		*Caldanaerocella colombiensis*[b]	3
				Clostridium sp. enrichment culture clone MB3_7	1
Unknown Group		Uncultured bacterium clone S25_969	1	*Bacterium* K2-53A	2
				Endophytic bacterium S02	1
				Uncultured bacterium	6

[a]Not detected.
[b]Similarity with the GenBank database is lower than 97%. All other isolates showed similarity ranged from 97 to 100%.

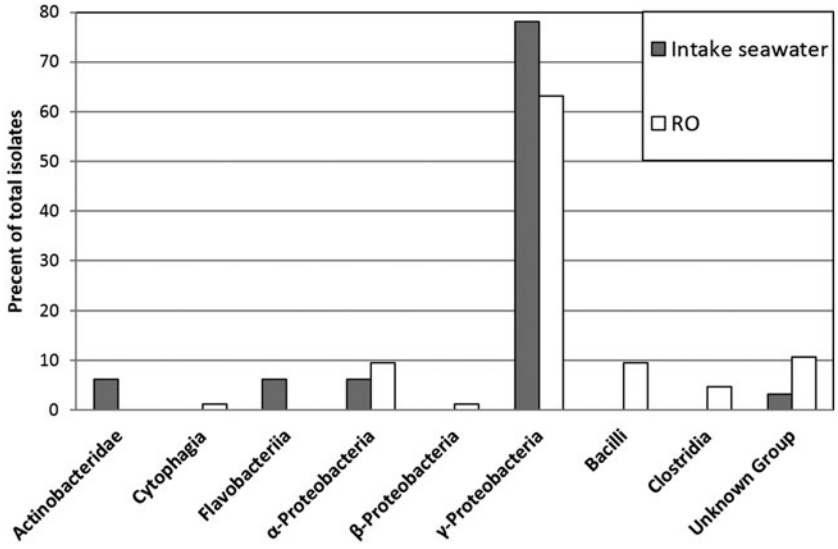

Fig. 1. Summary of bacterial groups isolated from intake seawater and RO membrane.

Table 3
Target bacteria for cell surface characterizations

Group	Target bacteria	Source
β-proteobacteria	*Limnobacter* sp. KNF002 (DSM 13612)	RO clone library [29]
γ-proteobacteria	*Alcanivorax* sp. Mho1	Isolates from intake seawater
	Vibrio sp. PM6A	
	Acinetobacter venetianus	Isolates from RO
	Marinobacter hydrocarbonoclasticus	
	Cellvibrio mixtus subsp. Mixtus (NCIMB 8633)	RO clone library [29]
	Escherichia coli K12	
	Simiduia agarivorans (JCM 13881)	
	Pseudomdnas aeruginosa (KCTC 1636)	Control
Firmicutes	*Bacillus* sp. Eur1 9.5 (DSM 18773)	[35]
	Bacillus sp. (KCTC 3872)	Control

in ionic strength showed insignificant effects on contact angle results. Although bacterial contact angles were not changed significantly under different ionic strength conditions, most bacterial strains, except for *Bacillus* sp. and *M. hydrocarbonoclasticus*, showed decreased contact angles as ionic strength increased. The contact angle value for *E. coli* K12 and *P. aeruginosa* decreased from 47.5 to 29.8 and 72.0 to 41.3, respectively, as ionic strength increased, yet only minor changes were observed for all other bacterial strains tested. The contact angle of *S. agarivorans* and *Vibrio* sp. PM 6A could not be measured from the microbial lawn on the membrane filter due to irregular surface structures formed after removing moisture during preparation process.

3.3.2. Cell surface hydrophobicity by MATH

Large variations were observed in cell surface hydrophobicity (see Table 4). *Limnobacter* sp. KNF002 (RO clone library), *A. venetianus* (Isolate from RO), and *S. agarivorans* (RO clone library) had higher cell surface hydrophobicity (70.4, 61.1, and 56.0%, respectively) than other bacteria tested. As ionic strength increased, the relative hydrophobicity increased, except in *Bacillus* sp. Eur1 9.5, *Bacillus* sp. and *Vibrio* sp. PM 6A strains. The hydrophobicity of *Bacillus* sp. Eur1 9.5 and *Vibrio* sp. PM 6A decreased as ionic strength increased, indicating that their hydrophobic interactions weakened in seawater or in a membrane system where salt concentration increased due to salt

Table 4
Cell surface hydrophobicity of target bacteria by CAM and microbial adhesion to hydrocarbon

	Contact angle (°)			% Cell adhesion in MATH		
Ionic strength	0.15 M	0.60 M	0.77 M	0.15 M	0.60 M	0.77 M
Simiduia agarivorans	12.3 ± 1.6	ND[a]	ND	46.2	52.5	56.0
Escherichia coli K12	47.5 ± 1.7	28.3 ± 1.0	29.8 ± 2.0	10.6	15.5	51.1
Limnobacter sp. KNF002	36.7 ± 3.3	34.1 ± 1.8	27.2 ± 2.7	67.3	70.4	70.4
Bacillus sp. Eur1 9.5	50.9 ± 2.7	54.8 ± 1.3	44.0 ± 2.0	15.8	13.0	4.8
Cellvibrio mixtus subsp. Mixtus	52.7 ± 1.6	52.3 ± 1.8	49.4 ± 1.5	ND	ND	ND
Bacillus sp.	23.2 ± 1.5	29.6 ± 0.1	34.3 ± 3.1	32.4	ND	ND
Pseudomdnas aeruginosa	72.0 ± 6.6	41.7 ± 1.5	41.3 ± 0.1	0.10	18.9	26.5
Acinetobacter venetianus	26.9 ± 2.2	26.8 ± 3.0	28.5 ± 1.1	48.3	49.6	61.1
Marinobacter hydrocarbonoclasticus	18.0 ± 0.6	23.2 ± 1.7	34.0 ± 0.7	15.0	22.4	31.6
Vibrio sp. PM 6A	ND	ND	ND	55.4	26.1	27.9
Alcanivorax sp. Mho1	43.2 ± 2.3	34.6 ± 0.2	37.5 ± 3.4	ND	8.2	7.3
Virgin RO membrane	65.6 ± 0.8	66.9 ± 2.6	69.9 ± 3.9	–	–	–

[a]Not detected.

rejection. *C. mixtus* subsp. Mixtus, *Bacillus* sp. and *Alcanivorax* sp. Mho1 formed stable emulsions in the aqueous phase after incubation with hexadecane, and the optical density was not changed due to their extremely high affinity to water.

3.3.3. Zeta potential measurements

Cell surface charge was measured by zeta potential (in mV) in each bacterial strain (Fig. 2). All bacterial strains possessed a negative surface charge at pH 7.5 under low ionic strength (0.17 M) conditions, ranging from −25.7 to −5.8 mV. *P. aeruginosa*, which is known to produce large amounts of EPSs [36], was the most positively charged in the 0.60 M salt solution, followed by *A. venetianus* and *Vibrio* sp. PM 6A. At higher ionic strength, *C. mixtus* subsp. Mixtus showed the highest net positive surface charge, followed by *A. venetianus* and *Bacillus* sp. Eur1 9.5.

The zeta potential became less negative as the ionic strength increased. This was caused by counter-ion charge screening and compression of the diffuse double layer [37]; however, this was not the case in *S. agarivorans* and *Alcanivorax* sp. Mho1 strains. The zeta potential of *S. agarivorans* and *Alcanivorax* sp. Mho1 decreased by 27 and 58%, respectively. *S. agarivorans*, *Alcanivorax* sp. Mho1 and *Bacillus* sp. were more negatively charged than other bacteria tested at an ionic

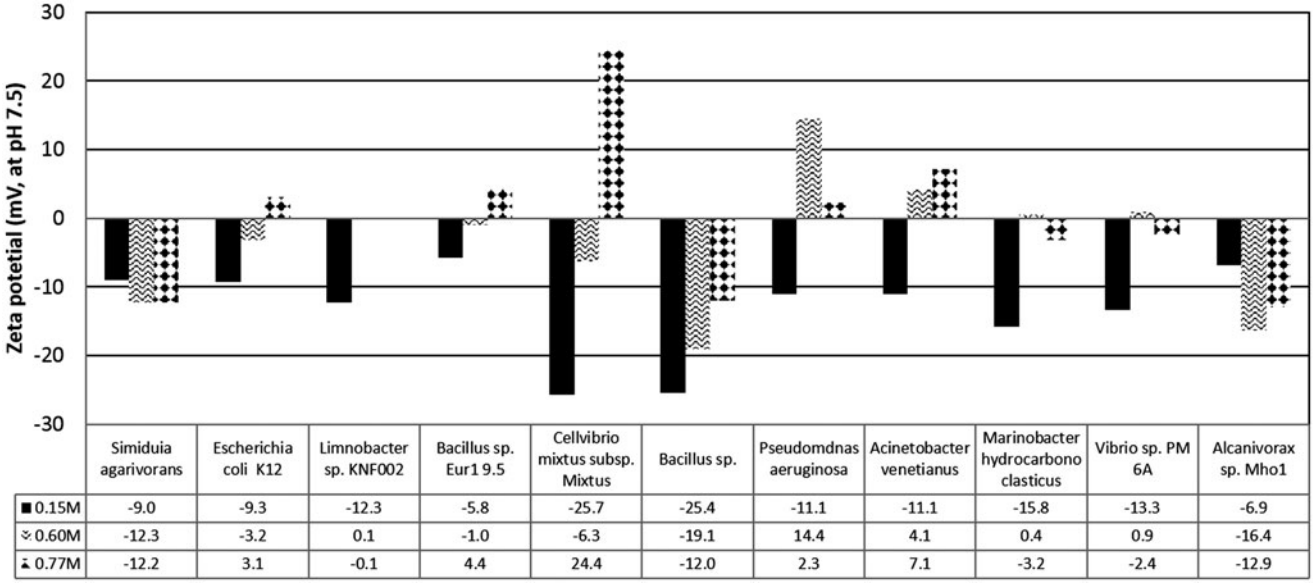

Fig. 2. Cell surface charge of target bacteria, as expressed by zeta potential.

strength of 0.77 M (Fig. 2). The zeta potential of RO membrane samples was −23.47 mV at pH 7.5. This result suggested that when both bacterial cells and membrane surfaces are negatively charged, electrokinetic potential is diminished. The zeta potential of bacterial cells was highly affected by the addition of NaCl. Therefore, electrostatic attraction may be favored in seawater or high salinity conditions. When microbial cells are positively charged, as in case of *C. mixtus* subsp. Mixtus (RO clone library), *A. venetianus* (isolate from RO), *Bacillus* sp. Eur1 9.5 (RO clone library from previous study [35]), *E. coli* K12 (RO clone library), and *P. aeruginosa* (control) at an ionic strength of 0.77 M, adhesion through electrostatic double layer repulsion is minor and attraction or initial adhesion could be mediated on the negatively charged membrane surface. Busscher et al. [38] showed that the initial deposition rate of dairy strains possessing a positive charge of +20 mV to negatively charged glass was two times higher than strains having a zeta potential of +10 mV. Moreover, compared to negatively charged strains, positively charged strains had strong adhesive tendencies to negatively charged surfaces.

However, it should be noted that although electrostatic interactions play a role in bacterial adhesion, they do so to a lesser extent [39], especially in RO systems where high pressure and shear stress are involved. Electrostatic interactions are not only repulsive because the zeta potential of the interacting surfaces in the membrane and bacterial cells are negative [11]. Attractive hydrophobic interactions were found to play a dominant role in biological attraction, and, presumably, the total attraction force is sufficient to overcome the steric repulsive component [40]. Subramani et al. found that after microbial deposition on nanofiltration (NF) or RO membranes, the electrostatic free energy is negligible at membrane contact sites compared to acid–base and van der Waals free energies [22]. In addition, the surface charge of *E. coli* and *P. aeruginosa* strains appeared to have a less direct influence on bacterial adhesion in a previous study [36].

3.3.4. Correlation between cell surface characteristics

To examine to what extent the preparation of bacterial strains under different ionic strength conditions influenced cell surface properties; a comparison was made between hydrophobicity measurements and electrostatic behavior. Based on the Derjaguin and Landau, Verwey, and Overbeek (DLVO) theory, adhesion should decrease with decreasing ionic strength, as previously reported [41]. Our results showed no significant correlation among the three analyses. Only *A. venetianus* and *M. hydrocarbonoclasticus* had closely correlated CAM and MATH measurements, with *R*-squared values of 0.9792 and 0.9814, respectively. These were both isolates from fouled RO membrane surfaces. When the data were compared between MATH and CAM analyses for other strains, the results were contradictory. In MATH, cells were suspended in aqueous phase, while CAM measures dried cells. This could, in part, explain the disparity in results between the two methods [42]. CAM is the only direct method for measuring cell surface hydrophobicity. Other indirect methods, such as hydrophobic partitioning in aqueous two phase systems, do not generally correlate, and correlation between direct and indirect measurements was observed in only a few cases [15]. Previous reports revealed that MATH does not measure cell surface hydrophobicity, but instead, it detects a complicated interplay of hydrophobicity (van der Waals and Lewis acid–base forces) and electrostatic interactions. It was reported that low water contact angle correlates with poor adhesion to hexadecane [41,43]. However, none of these general rules were observed in our study. Based on a previous study, irreversible adhesion is observed when the surface and bacterium were both hydrophobic and a high electrolyte strength or di- or trivalent cations were present [41]. The target bacterial strains in this study showed relatively hydrophobic cell surface characteristics in seawater, or under high ionic strength conditions. Together with hydrophobic nature of pristine RO membranes (66.9° at pH 7.5), these target bacterial strains may potentially facilitate irreversible biofouling.

It is known that zeta potential is sensitive to electrolyte concentration. Overall, cell surface charge becomes less negative as ionic strength increases. This result is in agreement with previous studies [14,22,44]. Microbial cells suspended in high ionic strength solutions have a reduced electrical double layer, causing cell aggregation and enhanced adhesion. Whereas in low ionic strength solutions, the electrical double layer surrounding suspended cells increases to such an extent that it causes electrostatic repulsion. Previous study has shown that electrokinetic potential strengthens between hydrophilic surfaces and hydrophobic particles [39]. Thus, we compared the zeta potential results to the hydrophobicity results. *Bacillus* sp. (control) and *Alcanivorax* sp. Mho1, which are isolates from intake seawater, showed a strong correlation between CAM and zeta potential measurements, with *R*-squared values of 0.9854 and 0.999, respectively. *E. coli* K12 and *Limnobacter* sp. KNF002, which were identified from the RO clone library, showed a significant correlation between MATH and zeta potential measurements, with *R*-squared values of 0.8467 and 0.9998, respectively. At electrolyte strengths of 0.15

and 0.60 M, the correlation between analyses was reduced because of inconsistent CAM and MATH results. It must be noted that a decrease in ionic strength not only decreases the surface charge, but it also changes the length and conformation of polymers on a bacterial surface [40]. High ionic strength conditions, such as 0.60 and 0.77 M, may affect the surface conductivity of bacterial cells by altering the specific activity of certain membrane proteins under extreme conditions [21].

It has been documented that within several hours, most of the bacteria attach irreversibly to surfaces via strong dipole–dipole forces, hydrogen and covalent ionic bonding, and hydrophobic interactions [10]. A recent study also reported that bacterial adhesion is influenced by more than electrostatic and hydrophobic interactions when there is a flux [45]. Model predictions indicated that the drag force overcome all other colloid-surface interaction forces when the flux increases to a certain level (7.2 L/h m^2) on NF membranes. Further studies need to be considered such factors together with membrane surface characteristics (surface morphology, roughness, and chemical composition) to provide insight into the adhesion potential of the biofilm bacteria and inhibit irreversible biofouling in early stage.

4. Conclusions

Currently, there is a lack of understanding surrounding microbial adhesion properties of micro-organisms in engineered environments, such as seawater desalinization plants that utilize RO membrane systems. Thus, we investigated the bacterial diversity in natural seawater and on RO membranes taken from currently operating desalination systems and explored the cell surface characteristics of potential biofoulants. Eleven bacterial strains were selected to determine their adhesion properties and potential as biofoulants. MATH was measured, coupled with bacterial CAM, and expressed as cell surface hydrophobicity. *Limnobacter* sp. KNF002, *A. venetianus*, *S. agarivorans*, and *E. coli* K12 adhered effectively to hydrophobic hexadecane. CAM suggested that most of the bacterial cell surface was relatively hydrophobic. *P. aeruginosa*, *C. mixtus* subsp. Mixtus, and *Bacillus* sp. Eur1 9.5 had relatively higher hydrophobic potential compared to other bacteria. The zeta potential of both the membranes and biofoulants were negative in natural seawater. Initial adhesion of *P. aeruginosa*, *A. venetianus*, *C. mixtus* subsp. Mixtus, *Bacillus* sp. Eur1 9.5, and *E. coli* K12 can be facilitated by their cell surface charge when exposed to RO membrane surfaces, and the concentration of TDS on the membrane increased.

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