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Characterisation of extracellular polysaccharides from bacteria isolated from a full-scale desalination plant

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ABSTRACT

Bacterial extracellular polymeric substances (EPS), particularly slime consisting of high-molecular weight soluble exopolysaccharides contribute significantly to seawater reverse osmosis (SWRO) membrane fouling. In this study, we characterized the soluble exopolysaccharides of 25 model bacterial strains isolated from different locations of a full-scale desalination plant in Western Australia. Biofilms of individual strains were initially quantified using crystal violet assay; significant biofilm production was detected in 21/25 isolates. Enzyme-linked lectin sorbent assay using lectins, Conacavalin A (ConA) and Ulex identified glucose and/or mannose in 11/25 isolates, and fucose in 24/25 isolates to significant levels. Exopolysaccharides were extracted and purified from bacterial cultures using centrifugation, TCA precipitation, cold acetone precipitation, dialysis and vacuum drying. Yield of exopolysaccharides varied between 90 mg to 480 mg/L of broth culture. Purified exopolysaccharides of 14 strains were analysed by High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) and ATR-FTIR. Fucose, rhamnose, glucuronic and galacturonic acids were present in majority of the isolates. These sugars are common to polysaccharides of glycosphingolipid-producing bacteria, a predominant subset of RO membrane biofilm community of the full-scale plant. They are known to impart better physical integrity to EPS and form strong, sticky recalcitrant biofilms. Mannose, glucose, galactose, xylose and ribose were also present.

1. Introduction

Membrane biofouling is a major concern to the desalination industry around the world and microbial biofilms contribute hugely to the process of fouling. It has been established that bacteria are unable to form biofilms without the most crucial component, extracellular polymeric substances (EPS) that comprise of polysaccharides, extracellular DNA, proteins, glycoproteins and phospholipids. Of these, polysaccharides are the major components of the matrix [1,2]. The collective term exopolysaccharides was first used by Sutherland to describe high molecular weight carbohydrate polymers produced by marine bacteria [3]. EPS plays a critical role in reducing permeate flux in SWRO systems, leading to requirement of higher pressure to drive the water through, thus increasing energy costs [4]. EPS are present as either capsular EPS, or loosely associated soluble EPS known as slime. Soluble EPS have greater binding capacity for organic matter than bound EPS [5] and are among the most recalcitrant naturally occurring organic foulants of RO membranes [6]. In aqueous environments, slime may be released onto surfaces that are not conducive for bacterial attachment directly [7,8]. The loose slime exopolysaccharides may then condition surfaces such as internal pipes, prefilters and RO membranes, facilitating bacterial attachment and biofilm formation.

In membrane fouling, polysaccharides and proteins are the main polymers in biofilm EPS, with polysaccharides being the dominant component [9]. Due to their higher viscosity, the polysaccharides are easily accumulated on the membrane surface, leading to an increase in hydraulic resistance and decreased permeate flux. Compact and dense biofilms formed under the influence of high shear force, such as those in spirally wound SWRO membranes, are known to be high in their polysaccharide content. It has been documented that polysaccharides, as opposed to extracellular proteins, are crucial for maintenance of the EPS matrix attached to the RO membrane surface, under shear force [10].

Apart from polysaccharides, biofoulants on filtration membranes are also comprised of proteins and other organic macromolecules [11]. The composition of EPS is diverse, with respect to the proportions of the

Abbreviations: EPS, extracellular polymeric substances; SWRO, seawater reverse osmosis; HPAEC-PAD, high performance anion exchange chromatography with pulsed amperometric detection; ATR-FTIR, attenuated total reflectance-Fourier transformed infrared spectroscopy; PSDP, perth seawater desalination plant; RSW, raw seawater; S, sand/dual media prefilters; C, cartridge filters; FSW, filtered seawater; RO, reverse osmosis; FO, forward osmosis; CSLM, confocal laser scanning microscopy

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major components, polysaccharides and proteins. In some circumstances, such as particular bacterial species e.g. *Staphylococcus* or in particular environments such as differing C/N ratios, EPS are mainly and sometimes entirely composed of proteins [12–14]. In this study, we focus on the characterization of exopolysaccharides, particularly the slime polysaccharides from model bacterial isolates of a full-scale SWRO plant.

Most studies on biofouling in SWRO plants have focused on investigation of bacteria themselves that cause biofouling, but the role of exopolysaccharides they produce has been overlooked. While researchers have investigated specific types of EPS of newly described bacterial strains [15,16], no reports have been published describing EPS from individual species from RO desalination plants. There are significant gaps in knowledge about the nature of the polysaccharides, their physical properties and chemical composition that enable bacteria to survive under harsh conditions within the plant. Although one recent study found some interesting facts on marine bacterial polysaccharides of extreme habitats [17], to the best of our knowledge, there is no information on exopolysaccharides of full-scale SWRO systems. We have previously demonstrated that free radical generating compounds effectively disperse biofilms and improve flux by targeting polysaccharides on industrially fouled membranes [18]. It has also been established in our laboratory that purified bacterial polysaccharides derived from *Pseudomonas* isolates of the full-scale plant, fouled membranes more severely in RO systems compared to FO [19].

To better understand the chemical composition of naturally occurring EPS, the objective of our study was to characterize high-molecular weight (> 12 kDa), slime exopolysaccharides purified from twenty-five model bacterial isolates of a full-scale desalination plant in Western Australia (PSDP). Particular emphasis was given to the detection of specific sugars like uronic acids, fucose and rhamnose that may impart better structural strength to the EPS. Within the desalination plant, environmental stressors such as high pressure, salinity, fluid dynamic forces and temperature fluctuations may have selected novel or resistant organisms capable of producing unusual polysaccharides that are better adapted to harsh conditions. Some polysaccharides containing rare monomers like L-fucose, L-rhamnose and uronic acids have superior biofilm forming abilities due to stronger chemical structures [20]. Fucose containing polysaccharides of marine bacteria are known to have viscoelastic properties and also good emulsion stabilizing ability [21]. Rhamnose is predominant in sphingans and gellans, polysachharides produced by sphingomonads. Sphingans are characterized by strong physical integrity and irreversible attachment abilities owing to their glycosphingolipids and uronic acids. In our preliminary study, the predominance of sphingomonads and other glycosphingolipid-producing bacteria in the membrane biofilm community strongly suggested their EPS play a prominent role in membrane biofouling [22]. However, they could not be isolated in culture. This study investigates the presence of unique or rare components in the chemical structure of bacterial exopolysachharides from our model strains, to find out if there were similarities between the model polysaccharides and sphingans.

This paper presents information on the chemical composition of key high-molecular weight slime exopolysaccharides fouling on SWRO component systems, which is a valuable contribution to scientific knowledge on biofouling mechanisms. It would be ideal to determine the chemical structure of individual EPS components, but due to difficulty and time involved in EPS extraction and purification, characterization was done by analysing component monosaccharides, which form the backbone structure of the EPS molecules.

2. Materials and methods

Bacterial strains isolated from the Perth Seawater Desalination Plant (PSDP) in Western Australia were selected as models for the evaluation of polysaccharides. The design of the plant consists of a i) pretreatment; ii) prefiltration stage consisting of two banks of 12 pressure dual media filters (anthracite + sand) and two banks of 7 cartridge filters, each fitted with 360 cartridges (5 μM pore size); and a iii) reverse osmosis stage consisting of a double pass; 1st pass consists of 12 trains with 162 pressure vessels each and 2nd pass consists of 6 trains with 124 pressure vessels each. Materials used for the study included bacterial isolates from samples of raw seawater (RSW), sand/dual media prefilters (S), cartridge filters (C), filtered seawater (FSW), and RO membranes (RO) of PSDP. The RO membranes used in the present study were autopsied after their lifespan of 7 years in the full-scale plant. The membranes were also analysed in our related studies involving a) next-generation sequencing analysis of biofilm bacterial communities [22], b) isolation of bacteria and selection of model strains for characterization of their polysaccharides and biofouling studies [23], and c) confocal laser scanning microscopy (CLSM) imaging studies of biofilms stained with FITC-conjugated ConA, for the investigation of the effect of free radical generating compounds that alleviated membrane biofouling by degradation of biofilm polysaccharides [18].

Preliminary work involved selection of representative biofouling bacterial model strains as described in our recent study [23]. Triplicates of 10 mL aliquots were sampled from each of 2 L volumes of raw, filtered and polished seawater samples collected. Triplicates of 2 g aliquots were sampled from each of sand, cartridge and membrane samples. All samples were grown in 30 mL each of enrichment media; Marine Broth (BD Difco) and Tryptone Soy Broth (Sigma-Aldrich), and incubated at 25 °C for 4–5 days with shaking. Liquid cultures were streaked onto following solid media; R2A agar (Thermo Scientific), Tryptone Soy Agar (Sigma-Aldrich) and Zobell's Marine Agar (BD Difco) and incubated at 25 °C for 3–4 days. Resulting bacterial growth was subcultured up to three times under the same incubation conditions until single pure colonies were isolated. Over 60 distinct types of colonies were isolated in pure culture [23].

Identification of strains was done either to genus or species level by using more than two methods, namely phenotypic bacterial Identification based on the biochemical test kit Biolog Gen III, Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) and 16S rRNA gene sequencing. Due to restricted genetic and protein profile database of environmental bacteria, especially those of the desalination process, one method was not sufficient to identify all isolates. A combination of three methods was used and after careful comparisons and evaluation of results, the most accurate identifications were achieved. The 16S rRNA gene sequencing method successfully identified most isolates at a genus level and a few at species level. Accurate species level identifications were achieved by MALDI-TOF. Biolog Gen III provided a comprehensive biochemical profile of all isolates; however identifications were not as efficient as the previous two methods [23].

Isolates were then compared to the biofilm community on RO membranes autopsied after reaching their complete life span of 7 years in the full-scale plant. The bacterial community profile was generated by next-generation sequencing using Illumina MiSeq (Illumina) [22]. Based on comparative evaluation of culture library and next-generation sequencing data, suitable models were selected from a collection of 64 isolates. All the model isolates characterized in the present study, regardless of where they were isolated, have been identified on the membrane biofilm community and they are likely to play an important direct or indirect role in RO membrane biofouling. Strains were selected on the basis of phylogenetic diversity across the culture collection to best represent the prevalent biofilm bacterial community found on RO membranes, ensuring that bacteria were also selected from every possible location of the full-scale desalination plant that polysaccharides could arise from. Twenty five isolates were tested for biofilm and lectin assays while only 14 strains were characterized by FTIR and HPAEC-PAD due to practical difficulties in producing sufficient quantities of dried purified exopolysaccharides for analysis. Standard methods used for exopolysaccharide characterization are listed in