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Marine bacterial transparent exopolymer particles (TEP) and TEP precursors: Characterization and RO fouling potential

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HIGHLIGHTS

- Different bacteria showed different TEP/TEP precursor production rates.
- Composition of isolated bacterial TEP/TEP precursors is similar to reported RO membrane foulants.
- Bacterial TEP precursors caused an obvious RO membrane fouling.
- Particulate TEP formed on RO membranes via TEP precursors self-assembly and/or accumulation.

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ABSTRACT

This paper investigated the characteristics and membrane fouling potential of bacterial transparent exopolymer particles (TEP)/TEP precursors released from two marine bacteria, *Pseudidiomarina homiensis* (*P. homiensis*) and *Pseudoalteromonas atlantica* (*P. atlantica*), isolated from the Red Sea. Results showed that both bacteria grew at the similar rate, but the production of TEP/TEP precursors from *P. atlantica* was higher than that from *P. homiensis*. During the 168 h of incubation time, production rates of TEP/TEP precursors from *P. atlantica* and *P. homiensis* were 0.30 and 0.08 xanthan gum eq. mg/L-h, respectively. Isolated bacterial TEP precursors were mainly biopolymer, and *P. atlantica* produced a significantly higher concentration of biopolymer than that produced by *P. homiensis*. TEP/TEP precursors from both marine bacteria possessed protein-like material and were very similar in composition to previously reported foulants isolated from a fouled reverse osmosis (RO) membrane. Bacterial TEP/TEP precursors mostly consisted of aliphatic hydrocarbon from amino acids and amide group carbon of proteins (around 55%). Bacterial TEP precursors caused obvious fouling on RO membranes, which may create an ideal environment for bacteria attachment and promote to biofouling.

1. Introduction

Reverse osmosis (RO) membrane filtration has been widely applied in seawater desalination for decades due to its relatively low cost compared to traditional distillation. However, biofouling of RO membranes continues to be a problem in seawater desalination [1,2]. Biofouling of RO membranes is known to increase the hydraulic resistance of filtration and affect membrane flux [2]. In order to control the RO membrane biofouling, a better understanding of the biofoulants is necessary.

Many aquatic organisms, including phytoplankton and bacteria produce large amounts of exopolymeric substances (EPS). One type of EPS, the transparent exopolymer particles (TEP), has been recently reported as a major component of biofilms because the TEP exist as individual particles rather than as cell coatings or dissolved slimes. Their characteristics in aquatic systems differ from other forms of EPS. They can be presented as individual particles as well as aggregated ones so they can be collected by filtration [3–5]. In the Mediterranean Sea, TEP are frequently formed and often cause mucilage events. Consequently, these substances can form a sticky biofilm on RO membranes if the seawater containing TEP is used as feed water for desalination plants. For this reason, TEP may enhance the biofouling on RO membranes.

Particulate TEP is ubiquitous in the ocean, and defined as sticky particles larger than 0.4 μm, mainly composed of acidic polysaccharides and stainable with alcian blue. Spontaneous self-assembly of dissolved TEP precursors is a major process of TEP formation [6,7]. Algae have been

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generally considered to be the major source of TEP in marine ecosystems [8,9], either releasing dissolved TEP precursors during exponential growth [9,10] or excreting TEP directly via sloughing and lysis of senescent cells [11]. A previous study has reported that algal TEP caused UF/RO membranes fouling [12]. In the Red Sea and Arabian Gulf, some desalination plants have been forced to shut down due to serious fouling during an algae bloom period, which may have been due to the presence of TEP [13].

Most TEP related studies on RO membrane biofouling have been focused on algal TEP/TEP precursors. However, TEP/TEP precursors can also be produced from bacteria in sea or surface water [9,14]. It has been reported that bacteria contributed to the formation of TEP in coastal waters of Agami Bay, Japan [15]. The production and molecular weight distribution of bacterial TEP/TEP precursors at different bacterial growth phases has not been investigated. Moreover, the fouling potential of bacterial TEP substances has not been addressed as well.

The molecular weight distribution of bacterial TEP precursors may be crucial in the pretreatment selection and RO operations. TEP precursors are smaller than 0.4 μm and could be biopolymers, humics, building blocks and low molecular weight (LMW) organics. If TEP precursors exist, they may pass through conventional and low pressure membrane pretreatment processes, and possibly re-assemble under increased shearing conditions [6,7], created by cross-flow velocities at the RO membrane surface. Since particulate TEP is likely to be removed by the pretreatments, the fouling potential of TEP precursors is probably a more problematic issue.

In this study, two marine bacteria were isolated from the Red Sea; *P. homiensis* and *P. altantica*. The productions of TEP/TEP precursors during bacteria growth in marine broth were studied. The compositional structure of isolated TEP/TEP precursors from both bacteria was characterized using liquid chromatography with organic carbon detection (LC–OCD) analysis and solid-state ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectrometry. The isolated bacterial TEP precursors were also tested in a RO filtration system to investigate their fouling potential on RO membranes.

2. Materials and methods

2.1. Marine bacteria preparation

Seawater collected from the Red Sea was streaked onto a marine agar plate (Difco Marine agar 2216, Becton, Dickinson and Company) and incubated at room temperature overnight. Two marine bacteria, *P. homiensis* and *P. atlantica*, were isolated from the incubated plate and sub-cultured with marine broth (Difco Marine broth 2216, Becton, Dickinson and Company) solution.

The isolated bacteria were identified via Sanger sequencing. First of all, the sub-cultured bacteria were gathered using inoculating loops to extract genomic DNA for use in a polymerase chain reaction (PCR). Extracted DNA was amplified using PCR with forward 27F (5′-AGAGTTTG ATCATGGCTCAG-3′) and reverse 1492R (5′-GGTTACCTTGTTACGACTT-3′) primers for 16S-rRNA gene. The PCR conditions were employed as follows: 95 °C for 5 min followed by 30 cycles of 94 °C for 0.5 min, 57 °C for 0.5 min, 72 °C for 0.5 min, followed by 72 °C for 5 min. Every amplified 16S-rRNA gene was then sequenced and aligned. For bacteria identification, the partial 16S-rRNA gene sequences were compared with the full sequences available in the GenBank database using a BLAST search and these then were registered in GenBank with accession numbers of KF019205 and KF019206 for SW1 and SW2 bacteria, respectively.

2.2. Production of TEP/TEP precursors from marine bacteria

After sub-culturing, the two purified bacteria stocks (7.5×10^5 CFU/mL) were inoculated into 500 mL marine broth solution and incubated for 10 days at 200 rpm at room temperature on a shaker. Fifty (50) mL of the marine broth solution was collected daily for cell number determination and TEP/TEP precursors' measurement over a 10 days period of incubation. Daily 50 mL marine broth solution was centrifuged at 9000 rpm for 5 min and the precipitates of settled bacteria were discarded. Since TEP/TEP precursors can be glycoprotein, the ammonium sulfate precipitation for proteins in biochemistry was applied in this study for TEP/TEP precursor isolation [16]. The supernatant after discarding bacteria was mixed with ammonium sulfate (697 g/L, Fisher Scientific) to reach a 100% precipitation, assuming the initial concentration of ammonium sulfate was zero. The precipitates were allowed to settle on an ice trail for 1 h. The precipitates were recovered by centrifugation at 5000 rpm for 15 min. The recovered precipitates (extracellular material, including TEP/TEP precursors) were re-dissolved in ultrapure water and analyzed using LC–OCD for molecular weight distribution of dissolved extracellular material (Section 2.5). Since salt content could make the alcian blue dye form insoluble pigments, and thus influence the TEP/TEP precursor analyses. The isolated extracellular material was desalted using dialysis. A 0.1% (*w*/*v*) sodium azide (Sigma Aldrich) solution was prepared and added to the extracellular material solution to limit the bioactivity during the dialysis. The extracellular material was placed into a 3500 Da molecular weight cut-off (MWCO) membrane dialysis bag (Spectrum Laboratories, Inc) and dialyzed for 4 days against ultrapure water. The concentration of TEP/TEP precursors in desalted extracellular material was then quantified with the alcian blue spectrophotometric method described in Section 2.4. Furthermore, the desalted extracellular material before and after reaction with alcian blue dye was characterized using LC–OCD (Section 2.5) to investigate whether all the desalted extracellular material was TEP/TEP precursors. All desalted extracellular materials after 10 days of incubation were lyophilized for solid state ^{13}C NMR spectrometry analysis to investigate their composition (Section 2.7).

2.3. Monitoring of cell growth

Plate counts were carried out using marine agar. The measurement of each sample was conducted at least in duplicates. Cell growth for both bacteria observed in three divided phases (phase I: 0–48 h; phase II: 48–120 h; and phase III: 120–240 h) based on their growth pattern (i.e. an exponential increase and a decline in cell numbers). In other words, each phase was identified the incubation time reached at the maximum population.

2.4. Determination of total TEP/TEP precursors

An alcian blue assay was modified for TEP/TEP precursors measurement based on a previous study [17]. The modification in this study was that the samples were not filtered to eliminate colloids, which could be part of interest: colloidal TEP precursors. A volume of 5 mL of isolated extracellular material after dialysis was added to a 10 mL volumetric flask. Afterwards, 0.5 mL of 0.06% (m/v) alcian blue solution was added and the volume was made up to 10 mL with ultrapure water. The volumetric flask was stirred for 1 min. The resulting suspension was then centrifuged at 3000 rpm ($2160 \times g$) for 30 min and the absorbance of the supernatant (representing alcian blue solution in excess) was measured in a UV spectrometer (UV-2550, SHIMADZU) at a wavelength of 610 nm. The lower absorbance of supernatant presents the less excessed alcian blue. This means that the higher amount of alcian blue reacted with TEP in sample and TEP concentration of sample is higher. This method was selected because it probably could measure part of colloidal TEP precursors in the samples. The calibration curve was linear in the range of 0–10 μg/mL xanthan gum equivalents, with an R^2 of 0.977. The calibration curve tests were duplicated and reproducible. The corresponding concentrations of total TEP/TEP precursors for unknown samples were determined using the established xanthan gum calibration