Supplementary Information

Influence on the bacterial production of transparent exopolymer particles during cross-flow seawater reverse osmosis.

Tamar Jamieson[1], Amanda V. Ellis[2], Dmitriy A. Khodakov[2], Sergio Balzano[1], Deevesh A. Hemraj[1], Sophie C. Leterme[1*]

[1] School of Biological Sciences, Flinders University, GPO BOX 2100, Adelaide SA 5001, Australia

[2] Flinders Centre for NanoScale Science and Technology, School of Chemistry and Physical Sciences, Flinders University, GPO BOX 2100, Adelaide SA 5001, Australia

Experimental procedure

DNA extraction

A modified protocol from Real Genomics HiYield™ DNA extraction kit (Real Biotech Corporation, Taiwan) was used to extract DNA. Samples (10 mL) were centrifuged (4000 rpm/10 min/RT) and the supernatant removed. The pellet was resuspended in 200 µL Lysozyme buffer (20 mg/mL lysozyme), transferred to a 1.5 mL microfuge tube and incubated at RT for 15 min. The cells were lysed by the addition of 200 µL of GB Buffer from the kit by vortexing and the subsequent incubation for 15 min at 70 °C. Ethanol (200 µL) was then added to precipitate DNA and samples were transferred to a GB column. The columns were then centrifuged (13,500 rpm/5 min/RT) and the filtrate containing the lysate mixture discarded. The DNA bound to the membrane of the column was then purified by adding 400 µL W1 buffer, centrifuging as above, then 600 µL wash buffer was added and centrifuged again. An additional centrifugation was carried out to remove residual ethanol, which may otherwise interfere with the following elution step. DNA was then eluted from the column by addition of 100 µL of preheated elution buffer, 20 min incubation at RT and centrifugation as above. DNA concentration was estimated using a Thermo Scientific NanoDropTM 1000 spectrophotometer (Biolab)

Preparation of seawater from RO feed tank

To remove all bacteria and viruses from the water collected from Penneshaw desalination, the RO feed water was filtered. It was first filtered through a 100 KDa hydrosart cartridge (Sartorius Stedim Biotech) using Vivaflow 200 tangential flow filtration (TFF) (Sartorius Stedim Biotech) in combination with a Masterflex L/S peristaltic tubing pump (Cole Parmer, Chatswood, Australia) to remove bacteria. Additional filtration to remove viruses was conducted using Vivaflow TFF (Sartorius Stedim Biotech) with a Masterflex L/S peristaltic tubing pump (Cole Parmer, Chatswood, Australia) through a 10 KDa polyethersulfone (PES) cartridge (Sartorius Stedim Biotech).

Cleaning of the laboratory-based cross-flow system prior to and after experiments

Prior to the to the insertion of the RO membrane the entire cross-flow system was disinfected and cleaned to remove any trace organic matter by the following steps: [1] Circulation of 0.5% hypochlorite at 400 psi for 15 min, [2] circulation of deionized water at 400 psi for 15 min, [3] circulation of 0.5% hypochlorite at 400 psi for 1 h, [4] circulation of deionized water at 400 psi for 1 h, [5] addition of 5 mM EDTA inside the cross-flow cell to incubate overnight, and [6] repetition of step 1 followed by repetition of step 2 (Modified from Herzberg and Elimelech, 2007).

References

M. Herzber, and M. Elimelech, *J Membe Sci,* 2007, **295,** 11-20

Table S1. Time periods (days) of removal and replacement of each SWRO TFC membrane used in static experiments and the analysis to be carried out on each. The periods of renewal of water is also indicated.

Periods (days)	14	28	56
membrane 1 – isolation of bacteria	x		
membrane 2 – TEP	x		
membrane 3 – isolation of bacteria		x	
membrane 4 – TEP		x	
membrane 5 – isolation of bacteria			x
membrane 6 – TEP			x
Renewal of water	x	x	x

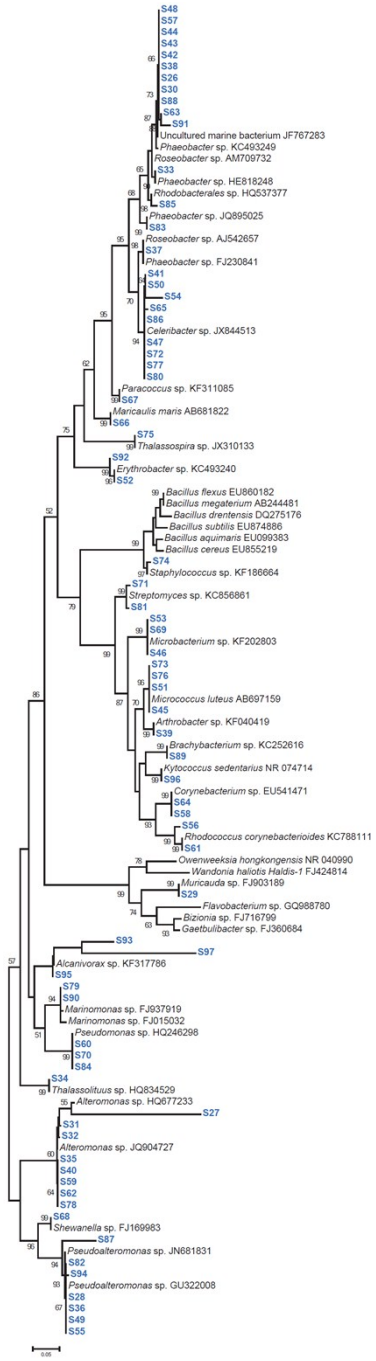

Figure S1: Maximum likelihood 16S rDNA phylogenetic tree showing the phylogenetic relationships between the bacteria isolated in the present study. The branch length corresponds to the number of substitutions per site and the percentage of likelihood for the taxa to be clustered together is shown next to the branches. The analysis involved 116 nucleotide sequences for a total of 489 positions.

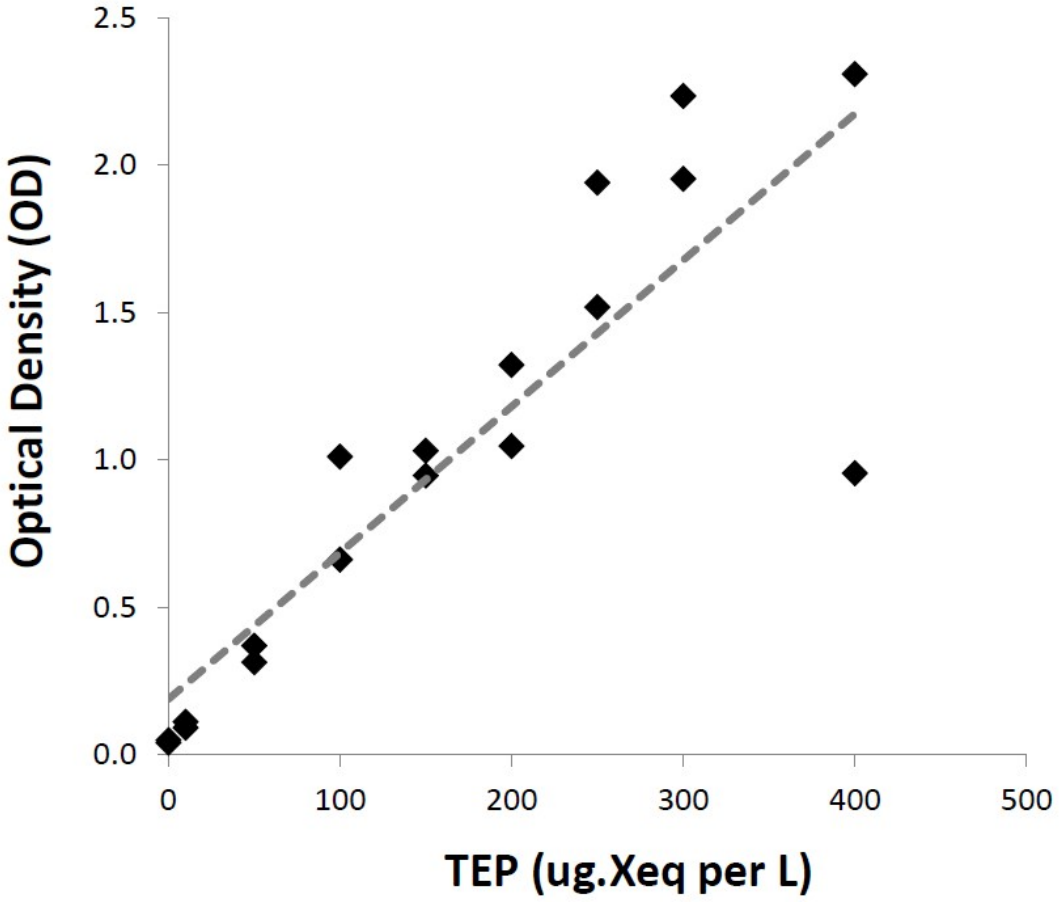

Figure S2: Xanthan gum standard curve used for calculating the TEP concentrations. The linear regression line fitted to the scatter plot is represented by the equation $y = 0.005x + 0.1896$ with a R^2 value of 0.7547.