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Characterization of microbial colonization and diversity in reverse osmosis membrane autopsy

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ABSTRACT

Biofouling can cause serious problems in reverse osmosis membranes (RO membranes) reducing module performance and their useful life. The main goal of this study was to gain insight into microbial colonization of used RO membranes with different feed water and inorganic fouling. We studied three RO membranes. Two were collected from the same desalination plant, fed with brackish water. These membranes belonged to two consecutive phases of the desalination process. The third one was from a seawater desalination plant. A three-tiered approach was proposed: The first-tiered approach was the use of SEM to detect fouling and presence of adhered microorganisms on the RO membranes. The second-tiered approach was to use specific stains, which indicated viable cells and the presence of extracellular biofilm matrix due to microbial colonization; ATR-FTIR was used to better determine the chemical nature of the matrix. The third-tiered approach was Illumina sequencing to study microbial composition and diversity. The study helped identifying key microorganisms (bacteria and fungi) as biofilm formers and the extent of the biofilm matrix; this knowledge may be useful for new antifouling treatments.

Keywords: Biofouling; Desalination; Microbial diversity; Reverse osmosis membrane

1. Introduction

Reverse Osmosis (RO) membranes are the most used technology for water desalination [1]. Despite their widespread use, RO membranes have some important problems like the high energetic cost [2] or an easy deterioration by oxidizing agents [3]. However, the biggest problem of RO membranes is fouling. Fouling is the accumulation of unwanted material on the membrane. Fouling produce a decrease of obtained permeated and a reduction of ionic rejection [4]. There are four types of fouling: inorganic (produced by precipitation of salts), organic (composed by humic acid), colloidal (suspended particles) and biofouling (generated by microorganism such as bacteria, fungi, algae that usually form biofilms on the membrane) [5]. Feed water chemistry or intrinsic membrane properties may greatly affect membrane fouling [6].

Biofouling affects more than one-third of RO membranes [7] and, normally, it is only detected but not fully characterized. For this reason, only general strategies exist to eliminate or prevent biofouling like chlorination, changes in membrane surface properties (hydrophobicity and roughness) or a chemical cleaning [7–11]. However, these techniques are not entirely efficient, for example, chlorination cannot eliminate initial biofilm formed because bacteria can be resistant to chemical stress or can grow after the treatment [8]. Thus, strategies for microbial antifouling have to

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rely on knowledge of the potential causes and monitoring of biofilm formation should be implemented.

Studies on biofilm development in RO membranes have evolved from culture-dependent methods, genetic clone libraries, fluorescence in situ hybridization to – omics (for a comprehensive review, see Sanchez [12]). Many of these studies involved advanced wastewater treatments, effluents from industrial or water purification plants or laboratory –scale RO systems and only a few addressed RO membranes from desalination plants [11,13–15]; – omics studies overcome the limitation of biased-selectivity of culture-dependent methods and facilitates a deeper knowledge of the real microbial composition of RO membranes biofilms. Mostly, pyrosequencing platforms have been used [9,13,16–19] and in a few cases Illumina sequencing has been used [20,21]. Studies have dealt with bacterial identification and diversity, paying less or no attention to fungal diversity [13,22]. Within bacteria, the phylum Proteobacteria has been found to be dominant in all studies; within Proteobacteria, family Sphingomonadaceae, particularly genus *Sphingomonas*, seems to be involved in biofilm initiation while family Rhodobacteracea seems to be associated with mature biofilms [13,22–25]. With regards to fungi, Al Ashab et al. [13,22] found that phyla Ascomycota and Basidiomycota were dominant in RO membranes from filtered treated wastewaters.

The objective of this study was to gain insight into microbial colonization of used RO membranes with different feed water and inorganic fouling. We studied three RO membranes, two were collected from the same desalination plant, fed with brackish water and the third was from a seawater desalination plant. A three-tiered approach was performed: Firstly, the presence of inorganic fouling and biofouling was detected by Scanning Electron Microscopy (SEM). Secondly, we determined cell viability of microorganisms of the biofilm using the stain Filmtracer™ LIVE/DEAD® biofilm viability kit. The presence and extension of biofilm matrix was evaluated by the stain Filmtracer™ SYPRO® Ruby biofilm matrix Stain; ATR-FTIR was used to better determine the chemical nature of the matrix. Finally, microbial composition and diversity (bacteria and fungi) was studied by Illumina sequencing. Our results give information about biofouling development in different RO membranes and allows identifying key microorganisms that might be useful to understand better this fouling process.

2. Material and methods

2.1. Sampling RO membranes

Genesys Membrane Products, S.L., provided membrane samples used for this study. Table 1 includes some characteristics of the three membranes (A, B and C) used for the study. Selected membranes were mainly chosen considering two main factors: Nature of feed water: brackish water (membranes A and B) *vs* seawater (Membrane C) and nature of the fouling: mainly inorganic *vs* mainly organic. It should be noted that the three membranes corresponded to polyamide-polysulphone commercial models although brands were different. Besides, on the samples with inorganic fouling (brackish water membranes), samples showed also different inorganic components: colloidal matter vs scaling.

Sampling of the membranes was carried out during conventional autopsies and membranes coupons were obtained from the middle length of each module.

No data about operation time is available. By the way the three membranes were autopsied due to a significant presence of fouling which was producing failures in plant.

Fouling detected on each sample is very common for the kind of water and membrane position. Besides the samples described in Table 1, a conventional polyamide-polysulphone membrane was used as reference for some of the analyses carried out during the study.

A fourth RO membrane unused (named D) was used as control membrane in all experiments.

All the samples were delivered in fragments of 20×20 cm and conserved in sealed bags to avoid air exposure and reduce environmental contamination.

2.2. SEM

The morphological characterization of RO membrane surface was performed using SEM. All samples were dissected in the different layers that composed the RO mem-

Table 1
Membrane samples details

	Brackish water membranes (BW)		Sea water membrane (SW)
	Membrane A	Membrane B	Membrane C
Membrane model	TORAY TM720-400	TORAY TM720-400	DOW FILMTEC SW30XHR-440 i
Membrane position	1st membrane – 1st stage	Last membrane – 2nd stage	1st membrane
Feedwater	Coastal well water (Ibiza, Balearic Islands, Spain)		Sea wáter (Muscat, Oman)
Organic content	13%	12%	87%
Inorganic content	87%	88%	13%
Inorganic component	Aluminosilicates-colloidal matter and particles of iron-chromium as main components	Calcium carbonate as main component	Aluminosilicates-colloidal matter, magnesium, calcium, phosphorus, sulphur

brane (polyester layer, polyamide layer, mesh spacer and permeate carrier) checking biofouling formation in each layer.

Samples were fixed with a solution of glutaraldehyde 5% (v/v) in sodic cacodylate 0.2 M (pH 7.2) for 1 h. Afterwards, the fixer was removed with two washes with sodium cacodylate 0.2 M (pH 7.2). Subsequently, samples were dehydrated by immersing them in solutions with increasing concentrations of ethanol in periods up to 10 min to a concentration of 100 % (v/v). At this moment, a solution of acetone (100%) was used for the immersion of samples for 10 min. With this, critical drying point was achieved in samples using a sample dryer by critical point Polaron model CPD7501.

When the samples were dry, they were metallized with a gold layer of 30 mm using a metallizer Polaron model SC7640. Then, the RO membrane layers were observed with a scanning electron microscope Zeiss DSM 950, using Quartz PCI software for analysis and image capture. The images obtained were coloured using GIMP v. 2.8.22.

2.3. Attenuated total reflection–Fourier transform infrared (ATR–FTIR) spectral analysis

ATR–FTIR spectra were recorded on a Thermo Nicolet IS10 spectrometer (Thermo Fisher Scientific Inc., Massachusetts, USA) using an ATR-FTIR accessory (smart iTR) and the OMNIC software version 9.1.26 (Thermo Fisher Scientifc Inc., Massachussets, USA). Spectra were collected in absorbance mode (log 1/R). For each measure, 16 scans were accumulated. The resolution was 4, the window aperture was at medium resolution, the gain was 2 and the optical velocity was 0.4747. At these parameters, good quality spectra with less spectral noise were obtained. 0.5 cm^2 of the RO membrane were measured between the range 1800–800 cm^{-1}. Between samples, the ATR-crystal was cleaned with isopropanol and the background was updated. For each RO membrane, 3 random spots were analysed. Data were saved as. spa and .csv files.

The analysis of results and their graphical plots were performed with the software Sigmaplot 12.0 (Systat Software, San Jose, CA).

2.4. Application of specific stains to study biofilm cellular viability and biofilm matrix

Cellular viability and the presence of biofilm matrix were checked using stains applied to the polyamide layer. Bacterial viability assays were performed using Filmtracer™ LIVE/DEAD® biofilm viability kit (Thermofisher Scientific). This kit allows discrimination between live and dead cells; it is based on a cell permeable dye for staining live cells (green fluorescence; SYTO 9) and a cell impermeable dye (red fluorescence, propidium iodide, PI) for staining dead and dying cells which are characterized by compromised cell membranes. For the staining of the polyamide layer, samples were cut in fragments of 0.5 cm^2 under sterile conditions and 50 µL of Filmtracer stain (a mixture of SYTO 9 and PI in DMSO, following the manufacturer's recommendations) were used. The incubation was performed in the dark for 15 min at room temperature. Then, samples were observed using confocal microscope (Confocal SP5 Leica Microsystems). For green fluorescence (SYTO 9), excitation was performed at 480 nm and emission at 500 nm. For red fluorescence (PI, dead cells), the excitation/emission wavelengths were 490 nm and 635 nm, respectively.

For the visualization of the extracellular polymeric matrix, samples were cut in fragments of 0.5 cm^2 under sterile conditions. 200 µL were stained with FilmTracer SYPRO Ruby (Molecular Probes, Invitrogen) per sample, incubated in the dark for 30 min at room temperature, and rinsed with distilled water. Filmtracer SYPRO Ruby stained most classes of proteins, including glycoproteins, phosphoproteins, lipoproteins, calcium binding proteins, fibrillar proteins and other proteins that constituted the biofilm matrix. Then, they were observed using confocal microscope (Confocal SP5, Leica Microsystems) with excitation/emission wavelengths of 450 nm and 610 nm, respectively.

In addition, several controls were included to check the performance of the stain in the presence of salt and also of a true bacterial biofilm. For all these controls, membrane D was initially taken and sterilized in the autoclave at 120°C in a short and dry cycle of 20 min. For the first control, a layer of crystals of NaCl salt was allowed to be formed on membrane D to check if the salts could interfere with the fluorochromes. For this, membrane was bathed in a solution of 1 M NaCl and then allowed to dry in an oven at 50°C until the salt crystal layer was formed. In a second control, *Pseudomonas putida*, which is a reference bacteria for biofilm formation, was cultured in a liquid medium and afterwards put into contact with membrane for 24 h, time enough for biofilm formation.

2.5. Microbial diversity analysis

2.5.1. DNA extraction

A square of 1 cm^2 was cut from every RO membrane, including all layers. The feed layer was separated and crushed with a mortar using liquid nitrogen to reduce the layer to powder while the rest of the layers were cut in smaller fragments. The DNA of the entire sample was extracted using the FastDNA® Spin Kit for Soil (MP Biomedicals) and subsequent stored at –80°C until sequencing.

The procedure was the same for all samples. Three independents replicates were done for each RO membrane for reproducibility.

2.5.2. DNA sequencing

PCR amplifications of the regions V3-V4 of the 16S rDNA and the ITS2 regions were carried out by the Genomics service of the Parque Científico de Madrid (Madrid, Spain) using the primers described in Table 2.

PCR products were purified and Miseq (Illumina) were prepared according to manufactureer's instructions. DNA libraries were checked for size, concentration and integrity using a Bioanalyzer (Agilent). Amplicon sequencing was performed using an Illumina Miseq sequencer. Paired -end reads (2×300) were generated according to manufacturer's instructions obtaining at least 100000 reads per replicate.

Table 2
Description of the primers used to perform DNA amplification. The regions which were amplified and the sequences of the primers are indicated. The primer tail is indicated in bold

Region	Reference number	Sequence
16S	16SV3-V4-CS1	**ACACTGACGACATGGTTCTACA**CCTACGGGNGGCWGCAG
	16SV3-V4-CS2	**TACGGTAGCAGAGACTTGGTCT**GACTACHVGGGTATCTAATCC
ITS	ITS4-CS1	**ACACTGACGACATGGTTCTACAT**CCTCCGCTTATTGATATGC
	ITS86F-CS2	**TACGGTAGCAGAGACTTGGTCT**GTGAATCATCGAATCTTTGAA

2.5.3. Data analysis

16S rDNA (bacterial) and ITS (fungi) profiling was determined using QIIME v. 1.8.0 [26] following the protocols [27,28] described in de Brazilian Microbiome Project (http://www.brmicrobiome.org).

Briefly, reads were quality filtered and trimmed by Trimmomatic v. 0.32 [29]. First reads were paired and filtered to remove low quality pairs and singletons. In the case of ITS reads, an additional step using ITSx [30] as carried out to remove non-fungal sequences. USEARCH v7 [31] was employed to calculate operational taxonomic units (OTUs) at a 97% similarity level using the UPARSE v. 9 [32] algorithm and to remove chimeric OTUs using UCHIME algorithm [33]. Taxonomic assignation was performed by the Uclust method [31] using Greengenes v13_8 [34] for 16S sequences and UNITE v12_11 [35] for ITS sequences.

Diversity metrics as CHAO1 [36] and Unifrac [37] were calculated to determine alpha and beta diversity respectively. Unweighted Unifrac values were used to represent sample variability by PCoA. Shannon – Weaver Index [38] was calculated as an estimate of the fungal and bacteria diversity.

2.5.4. Accession numbers

Sequences used in this study were submitted to the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) under Sequence Read Archive (SRA) accession number: SRP131637.

3. Results and discussion

3.1. Visual RO membrane observations, SEM

Visual examination of the polyamide membrane in all used RO membranes (membranes A, B and C) showed fouling on the membranes. In general, this accumulation was produced in the valley areas and located in bands of deposits (not shown). These bands were established in the contact area between the spacer and the membrane [39,40]. As shown below by using scanning electron microscopy (SEM), it was possible to appreciate the presence of microorganisms and differentiate between fouling and biofouling because of the properties of the RO membrane made the perfect environment for the growth of microorganisms on the polyamide surface [41].

In membrane A (shown in Fig. 1a), the polyamide surface was covered by a series of crystalline and round morphology particles. These structures have a heterogeneous distribution, so there are areas where large compacted crystals of 10 μm are formed and in other areas smaller crystals up to 2 μm can be observed. Although inorganic *fouling* is the main one in this sample, there were also microorganisms which could be seen between the compact crystals of the fouling. This distribution was somewhat irregular, and microorganism were not very abundant.

In membrane B (Fig. 1b), fouling was composed of a thick layer of highly compacted crystals which were homogeneously distributed throughout the membrane. The size of each of these crystals was much greater than in the case of membrane A. This could be due to the fact that the concentrated water leaving membrane A was used as feed water for this membrane to increase the efficiency [9]. The water was more concentrated in salts and that facilitated the formation of a fouling layer of greater thickness with larger crystals. In the case of microorganisms, the few microorganisms that could be visualized were settled on the salt crystals and not in the matrix holes. Also, some microorganisms grew in the spacer and not only in the polyamide layer. Two types of microorganisms based on their shapes (coccoid and bacillar) were seen over the salt crystals.

Inorganic fouling in membrane C was significantly smaller than that of A and B membranes (Fig. 1c). It was only seen in the form of small incrustations in the surface of the polyamide layer. Conversely, biofouling was homogeneously distributed throughout this layer. It was appreciated throughout the whole membrane that there was a mucilaginous substance that covered the crystals and that surrounded the microorganisms. These microorganisms were visible both below this mucilage layer and above, when this occurred, the microorganisms appeared embedded in this layer. The morphology of these microorganisms was more varied than those found in the A and B membranes, appearing structures with coccoid shape of small size (0.2 μm) along with bacilli of heterogenous sizes.

Visualization of control membrane (membrane D) in Fig. 1d shows the normal appearance of an unused RO membrane surface. The surface had a morphology of ridge-and-valley structures due the two monomers constituting the layer of polyamide 1-4-benzenediamine bound to terephthaloyl chloride [40].

To summarize, in membranes A and B, inorganic fouling predominated, corroborating data in Table 1. The thicker layer of crystals was found in membrane B that operates at the last position from the second stage of the brackish water desalination plant. Microorganisms were not very abundant

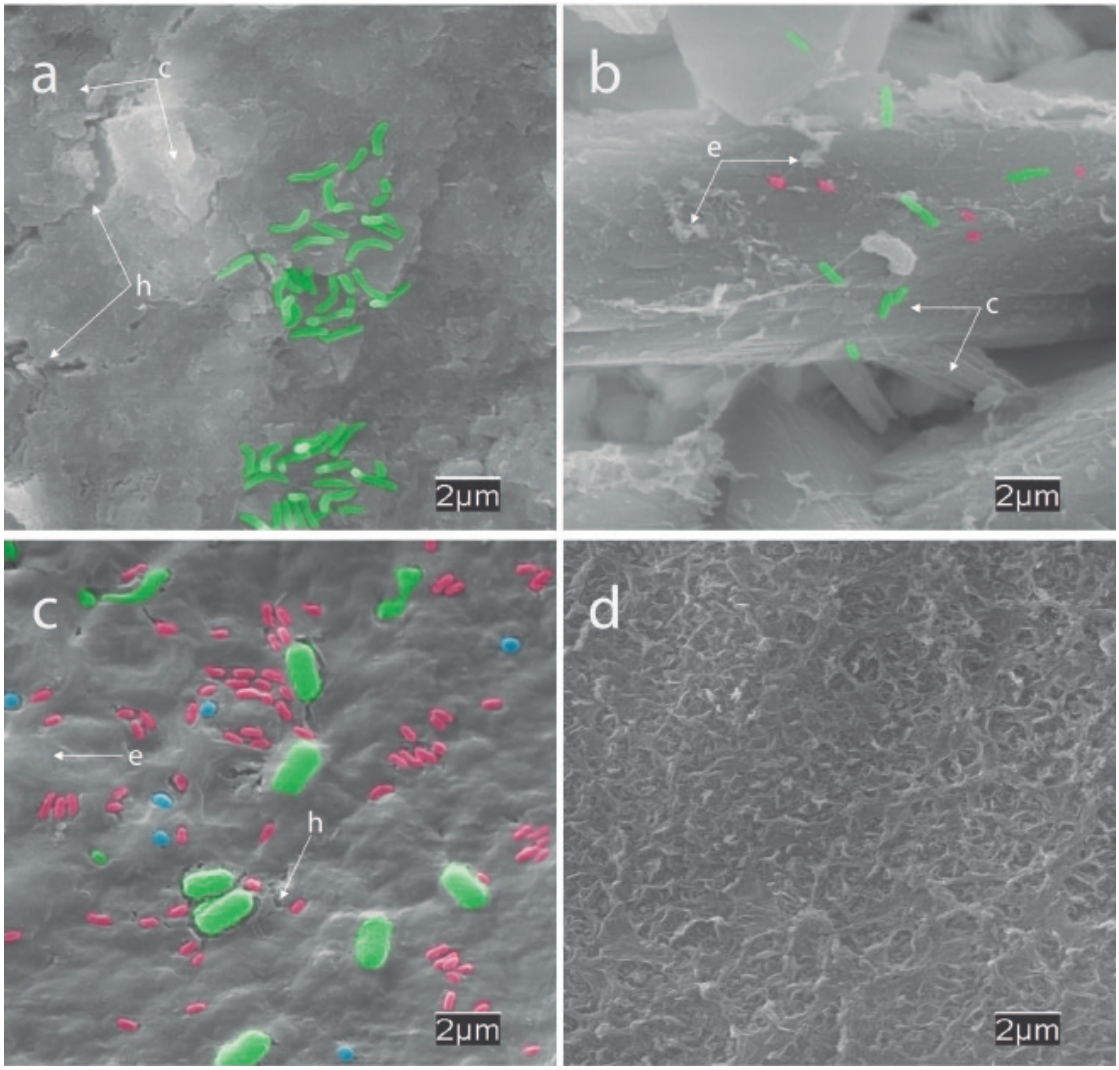

Fig. 1. Distribution of fouling / biofouling in polyamide layer in each membrane sample. The microorganisms are marked in colour with GIMP v. 2.8.22 for better visualization. Each colour indicated different sizes and morphological shapes. a) Distribution of bacilli-shaped microorganisms between the fouling crystals in membrane A. b) Distribution of microorganisms on the fouling crystals in Sample B. c) Microorganisms embedded in membrane C. d) Control membrane D. Legend of acronyms: c: crystals, e: EPS, h: holes.

in any of the two membranes, being less abundant in membrane B; no clear extracellular polymeric substance (EPS) matrix could be visualized by SEM in these membranes. Membrane C was characterized by mostly organic fouling; the biofouling layer was much more evident than that of the RO membranes from brackish water. Differences in fouling between membranes A, B and membrane C are probably related to the different feed water, brackish *vs.* seawater.

SEM allowed to visualize fouling and biofouling in all three membranes. This is a technique commonly used in RO membranes studies and autopsies. Depending on feed water, pre-treatment and chemical structures of the membranes, fouling and biofouling has been visualized in many RO membranes [13,22,23,39]. Nevertheless, to study in detail biofouling confocal laser scanning microscopy (CLSM) which specific stains and FTIR analyses were performed.

3.2. Viability of microorganisms and presence of biofilm matrix by using CLSM and ATR-FTIR

The visualization of the membranes using CLSM allowed checking cell viability and their distribution on the membrane surface using the Filmtracer™ LIVE/DEAD® biofilm viability kit. Bacterial colonization does not consist only in the adhesion of free bacteria onto the membrane. The microorganisms, once adhered, are embedded in an extracellular polymeric substance (EPS) forming a biofilm. The EPS provides stability to the biofilm [42]. The biofilm matrix was visualized using the FilmTracer SYPRO Ruby biofilm matrix stain that stains mostly EPS proteins.

Membrane A (Fig. 2a) had few cells distributed throughout the membrane. Although there was a high percentage of dead cells (red fluorescence), many microorganisms remained alive. The biofilm matrix in membrane A can be

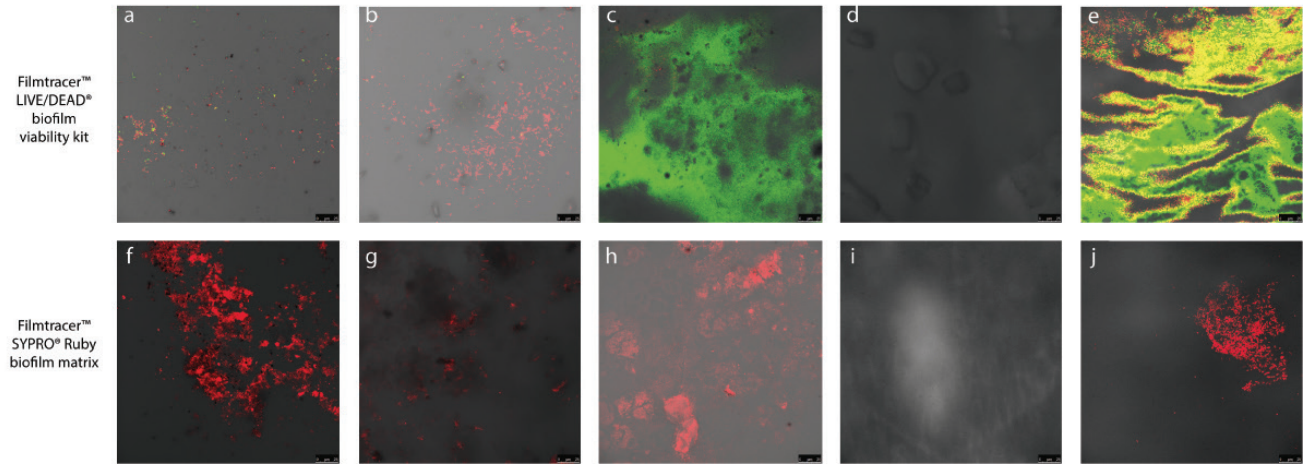

Fig. 2. CLSM images. Staining with Filmtracer ™ LIVE / DEAD® biofilm viability kit was used in the first row (a–e) and Staining with Filmtracer ™ SYPRO® Ruby biofilm matrix Stain is shown in the second row (f–j). a and f corresponded to sample A, b and g are taken of sample B and sample C was shown in c and h. The other four photographs corresponded to the controls performed in membrane D. The stains made on the sterile membrane with a layer of NaCl are shown in d and i. Images e and j correspond to the biofilm formed with *Pseudomonas putida*. Legend: membrane A (a, f), membrane B (b, g), membrane C (c, h), control with a layer of NaCl (d, i) and control with *Pseudomonas putida* biofilm (e, j).

seen in Fig. 2f. The matrix was much more distributed on the membrane than could be initially observed with the Filmtracer™ LIVE/DEAD® biofilm viability kit.

In membrane B, as can be seen in Fig. 2b, a larger number of microorganisms with a clearly defined shape could be seen, although mostly dead. Fig. 2g shows that biofilm matrix accumulated in small clusters. This situation was very different from that of membrane A despite being part of the same desalination plant. This is because plants that treat brackish water use two parallel RO membranes. In this way, the water rejected in membrane A serves as feed water for the RO membrane B to increase process throughput [3].

Microorganisms adhered on membrane C (Fig. 2c) were very abundant and were spread evenly throughout the membrane. No red fluorescence was observable meaning that microorganisms were alive probably due to the fact that it barely had salt deposits on the membrane that could affect the biofilm. Fig. 2h shows that the biofilm matrix was spread throughout the sample, although there were areas in which a larger fluorescence was observed due to a higher concentration of extracellular proteins. This result confirmed the SEM images previously shown for this membrane.

To demonstrate the validity of the results, two controls were performed. The first control consisted of arranging a layer of salts (composed of NaCl) on membrane D to check if the salts interacted with the performance of the stains. As shown in Fig. 2d and Fig. 2i, no fluorescence was observed meaning that the stains do not interact with NaCl crystals so that no false positives can be attributed to sample staining. In the second control, a *Pseudomonas putida* culture was grown for 24 h on the polyamide layer of membrane D because of its great ability to rapidly form biofilms. In Fig. 2e most of the cells are stained green because most of the bacteria were viable. The cells that showed a yellow colour may be slightly damaged [43,44]. For this reason, yellow cells were generally considered viable, while orange cells could be considered severely damaged [45]. Fig. 2j shows the matrix of the *Pseudomonas putida* biofilm.

The control experiment with the *Pseudomonas putida* biofilm indicates that both stains were valid for biofilm visualization and can be used regularly in RO membranes.

These stains had certain advantages over SEM because they gave clearer results and did not interfere with the inorganic incrustations that existed in fouled membranes.

ATR-FTIR was applied to further analyse fouling/biofouling of the three membranes (Fig. 3).

The black trace in the figure corresponds to a characteristic polyamide-polysulphone membrane surface spectrum. This spectrum was used as reference to verify the presence of fouling/biofouling on the membranes sample surface.

Thus, IR spectra from membrane A (red trace in Fig. 3) and membrane B surface (blue trace in Fig. 3) do not show any of the characteristic bands from membrane composition, which demonstrates the significant presence of fouling on their surface [46]. Fouling bands obtained from these membranes are characteristic of the components previously identified (Table 1): Aluminosilicates on membrane A (peak at around 1000 cm^{-1}) [47] and calcium carbonate on membrane B (peak around 1400 cm^{-1}) [48]. Peaks indicating chemical bonds related to EPS matrix such as those around 1650 cm^{-1} and 1540 cm^{-1} assigned to C=O and N-H [22,48–50], respectively, indicative of proteins were not identified; also those assigned to polysaccharides (peaks around 1000 cm^{-1}) [48,50] are masked by inorganic fouling.

On the other side, membrane C spectrum (green trace in Fig. 3) shows bands from fouling/biofouling, but also many bands from membrane composition (thinner fouling than previous samples). Fouling bands appear at wavelengths characteristic of aluminosilicates (Fig. 3 - 1.000 cm^{-1}) but there is a distinctive peak at 1038 cm^{-1} m which may be assigned to P=O, COO and C-O-C stretching vibrations present in phosphodiesters and rings in polysaccharides [49]. The peak at 1628 cm^{-1} was assigned to C=O (amide bond) [49] that could be related to protein derivatives commonly related to the presence of biofilm.

CSLM stains and ATR-FTIR corroborated that biofouling was present mostly in seawater membrane C, where adhered microorganisms were highly abundant and viable; the biofilm matrix was well developed as indicated by the FilmTracer SYPRO Ruby biofilm matrix stain and ATR-FTIR clearly indicated the presence of proteins in the matrix. Regarding membranes A and B from brackish water, mostly inorganic fouling was found and adhered microorganisms were in lesser abundance and many were dead.

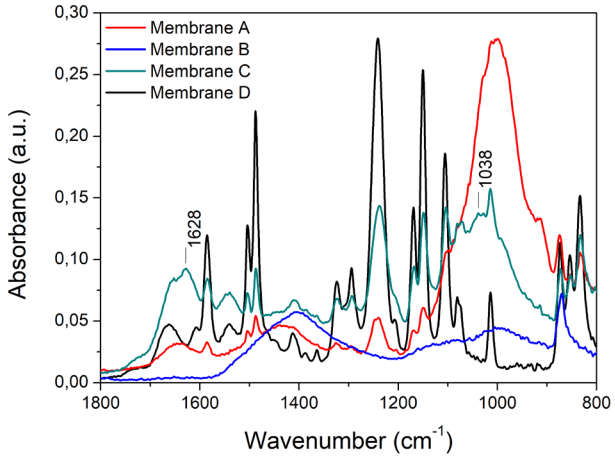

Fig. 3. FTIR spectra of the control membrane D (black trace), membrane A (red trace), membrane B (blue trace) and membrane C (green trace).

3.3. Analysis of microbial composition and diversity

A metagenomic approach using next generation sequencing techniques (Illumina platform) was carried out to determine microbial composition and diversity on RO membranes.

3.3.1 Bacterial composition and diversity

The most representative phylum was Proteobacteria that was present in all samples in a range between 64.3% and 53.1% (Fig. 4; see also Supplementary table S1 that shows the relative abundance at the genus level for all three membranes). This result fits with previous studies demonstrating the dominance of this phylum in the Mediterranean Sea [51] and in the Arabic sea [52]. Other studies have also demonstrated the dominance of this phylum over the microbial communities adhered to RO membranes [13–15,22,25,53].

In membrane C, the main phylum after Proteobacteria was Firmicutes, with a representation of 23.5%; within this

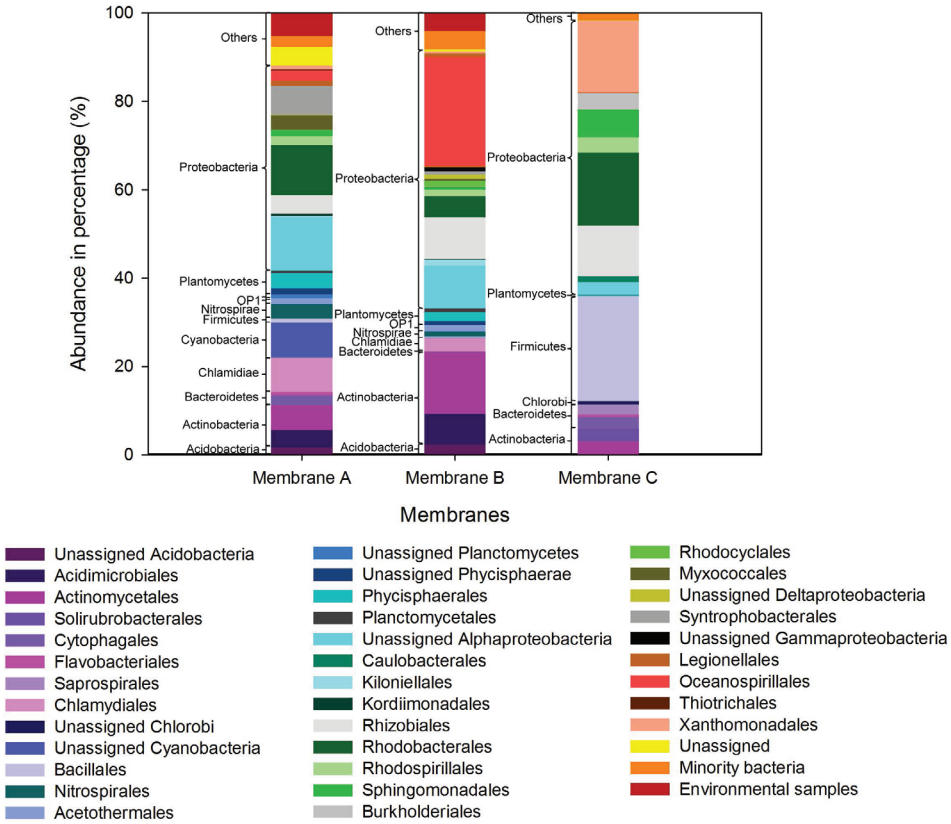

Fig. 4. Relative abundance of prokaryotic communities at the order level in used membranes. To the left of the bars the orders are grouped in phyla. Minorities are OTUs whose representation is less than 0.5%; unassigned are those sequences that have only been identified as bacteria and lastly the Environmental Sample refer to those sequences that have not been recognized at any taxonomic level.

phylum, family Paenibacillaceae and genus *Brevibacillus* were the most abundant. This phylum has been observed in other systems of RO membranes as one of the most important biofilm formers [54] and was the main group in biofilms from milk processing membranes [21].

Phylum Bacteroidetes abundance was higher in membrane C (5.63%) than in membranes A 3.13%) or B (0.20%). The most representative families were Cytophagaceae and Flavobacteriaceae. Phylum Bacteroidetes has also been found to be abundant in RO membranes from seawater like membrane C or secondary effluents from WWTPs [9,13,18,55].

The rest of the phyla and their relative percentages varied greatly among the samples, although no phylum reached the importance of Proteobacteria. Membrane A presented also the phyla Actinobacteria (9.5%), Chlamydiae (7.8%) and Cyanobacteria (7.8%). In membrane B the phylum Actinobacteria (20.9%) was more abundant with respect to membrane A (9.6%). Within this phylum, genus *Mycobacterium* was the one that increased its relative abundance the most (in membrane A 2.60% and in membrane B 6.37%) although *Mycobacterium* grows slowly, it is capable of tolerating saline environment [24,56]. All these phyla have also been reported in RO membrane biofilms although at low abundance [21,23–25].

Within Proteobacteria, the Alphaproteobacteria class was dominant in membranes A and C (32.1% in A and 42.23% in C); The relative abundance of Alphaproteobacteria was 27.7% in membrane B. Gammaproteobacteria abundance was higher in membrane B (27.6%) as compared to membrane A (5.1%) and C (16.6%). The differences of Gammaproteobacteria abundance between RO membrane A and RO membrane B might mainly be due to the salinity changes that occurred in the feed water, as Gammaproteobacteria can increase their population in biofilms under saline conditions during the late stages of biofilm maturation [57]. Delta- and Betaproteobacteria were in significant lower proportions: 9.9% in membrane A, 2% in B and absent in C for Deltaproteobacteria; absent in membrane A, 0.7% in B and 3.5% in C for Betaproteobacteria.

Alphaproteobacteria and Betaproteobacteria have been usually found in RO membrane biofilms [13,22]. Regarding Gammaproteobacteria, Al Ashhab et al. [13] found that this class predominated in RO membranes after a cleaning cycle while Betaproteobacteria were almost completely excluded after cleaning. Deltaproteobacteria was found at very low abundance in RO membranes, in agreement with the results reported here [13,24]. Alpha-, Beta- and Gammaproteobacteria have been suggested to be involved in initial colonization and biofilm development [13,58–60]; in fact, Alphaproteobacteria have been claimed as responsible for the biofouling in RO membranes [24].

Within Alphaproteobacteria, the order Rhizobiales predominated in membrane B (9.4%), while in sample C it represented 11.5% and in membrane A only represented 5.6%. Family Hyphomicrobiaceae with genera *Devosia* and *Hyphomicrobium* was dominant particularly in membranes A and B (brackish water). This order has been found as dominant in biofilms from RO membranes [16,25,58]. Pang and Liu [58] found that Rhizobiales were metabolically versatile under aerobic conditions which might be an important advantage in environments with limited nutrients input like RO membranes. Some members of this order have been found to degrade organic contaminants and to secrete glycosphingolipids which have been suggested to play a relevant role in the initial colonization of RO membranes as well as in the production of EPS during biofilm maturation [61]. In addition, within Alphaproteobacteria, order Rhodobacterales predominated in membranes A and C (11.3% in A and 16.5% in C), which was represented mainly by the family Rhodobacteraceae (10.9% in A and in C 15.5%); the members of this family such as *Rhodobacter* have been found to be associated with mature biofilms [19]. Family Sphingomonadaceae is also frequently found in RO membranes and in particular, genus *Sphingomonas*, also known to produce sphingolipids [62,63], has been reported as initial colonizers of biofilms [59,64]. Bereschenko et al. [59] reported that the unique capability of *Sphingomonas* for spreading and producing a layer of EPS may outcompete other microorganisms such as *Pseudomonas* that may exist as floating aggregates in feed water. This family was present in all three membranes although it was less abundant than family Rhodobacteraceae. Rhizobiales may replace family Sphingomonadaceae during the process of biofilm [59].

Within Betaproteobacteria, order Burkholderiales was the most abundant with families Comamonadaceae, Rhodocydaceae and Alcaligenaceae as majoritarian. These families have been found as abundant in the biofilms of membrane bioreactors (MBRs) used for wastewater treatment [11,65,66]. Family Comamonadaceae was also found to participate in denitrification processes within the biofilm [67].

In the case of the Gammaproteobacteria, the order Oceanospirillales was predominant in membrane A (2.4%) and membrane B (24.6%), whose main family, Oceanospirillaceae, was also predominant in both samples, although in different percentages (membrane A was 1.8% and membrane B was 18%). The family Oceanospirillaceae is characterized for being marine microorganisms [68].

In membrane C the most abundant order was Xanthomonadales (16.2%), whose only representative in this case was the family Xanthomonadaceae (16.2%). The most abundant genus of this family, *Pseudoxanthomonas* (8.4%), is remarkable for its ability to metabolize recalcitrant metabolite substances, so they are often used in biofilters [69]. Their great abundance might imply that these microorganisms can metabolize unconventional carbon sources that reach RO membranes, serving their products as substrates for other microorganisms in the biofouling community, facilitating their development.

The absence of the Pseudomonaceae in all membranes (representing less than 0.1% of the community in C and absent from the rest of membranes) is a relevant fact. This family encompasses the genus *Pseudomonas*, a genus widely investigated and used in trials for its great ability to form biofilms as it is able to produce large amounts of EPS [70–72]. Many studies have reported the presence of this genus in RO membranes [9,16–18,20,25,59,73,74]. Although this is not the first time that the absence of the genus *Pseudomonas* in biofouling of RO membranes has been observed [51], this genus seems to be more frequent in RO membranes from wastewater treatments [22].

The rest of the phyla and their relative percentages varied greatly among the samples, although no phylum reached the importance of Proteobacteria. Membrane A presented also the phyla Actinobacteria (9.5%), Chlamyd-

iae (7.8%) and Cyanobacteria (7.8%). In membrane B the phylum Actinobacteria (20.9%) was more abundant with respect to membrane A (9.6%). Within this phylum, genus *Mycobacterium* was the one that increased its relative abundance the most (in membrane A 2.60% and inmembrane B 6.37%) although *Mycobacterium* grows slowly, it is capable of tolerating saline environment [56].

The Shannon-Weaver index was calculated to evaluate α – diversity (Table 3). The diversity was high in the three samples, but membrane A and membrane B presented a high value in comparison with that of membrane C. This could be very relevant, since a greater microbial diversity implies a greater resistance to diverse factors of stress and the development of diverse metabolic pathways among the microorganisms that make up the community [75].

The results obtained with β - diversity allowed to statistically differentiate between the three samples. Distances were represented through Principal Coordinates Analysis 2D-Plots that are shown in supplementary Fig. S1. Significant differences were found between membrane A, membrane C (with a *P*-value of 9.95×10^{-15}), membrane B, and membrane C (*p*-value: 1.57×10^{-15}). Results showed less significant differences between membrane A and membrane B (*p*-value of 6.32×10^{-7}). This statistically significant differences might be explained by the facts that membranes A and B had different feed water than membrane C (brackish *vs.* seawater); that membranes A and B showed mostly inorganic fouling while that of membrane C was mainly organic; and also, although the three studied membranes corresponded to polyamine-polysulphone commercial models, they were from different companies. Although biofouling is a problem that develops in all RO membranes independently of their origin [76], the composition of the community of microorganisms seems to vary depending on the location, inorganic fouling, salinity and even membrane brand. Thus, this kind of analysis is important to prepare site-specific treatments to diminish or delay biofouling.

3.3.2. Fungal composition and diversity

Unlike prokaryotes, in the case of fungi, there was a large percentage of OTUs that could not be identified (the average of the three membranes was 21.3%) or only were identified as fungi (22.6%) because the generation of unintentional chimeras during PCR amplification is frequent. These chimeras have been detected even in the UNITE database (included 1825 chimeras) because detecting chimeras was a challenge [77].

The fungal communities identified in the three membranes were classified mainly in the Ascomycota and Basidiomycota phyla (shown in Fig. 5; Supplementary Table S2 shows the relative abundance at the genus level for all three membranes).

Table 3
α – diversity Shannon-Weaver Index. The index was calculated using the relative abundance of the detected genera in each DNA region

Region	Membrane A	Membrane B	Membrane C
16S RNA	3.44	3.28	2.89
ITS	2.7	2.33	2.11

The Ascomycota phylum was more abundant (in membrane A it represented 36.6%, membrane B 35.3% and membrane C 51.6%) than the phylum Basiodiomycota (membrane A: 16.13%, membrane B: 13.4% and membrane C: 15.3%).

In membrane A and B, the classes Sordariomycetes (membrane A: 19.6% and membrane B: 7.4%) and Eurotiomycetes (membrane A: 9.5% and membrane B: 11.6%) were predominant in Ascomycota. Within Eurotiomycetes there was a divergence between families depending on the membrane. In membrane A, the family Trichocomaceae (7.4%) was most abundant while in membrane B it was Chaetothyriaceae with a representation of 9.3%.

In membrane C, the Ascomycota phylum was mainly represented by the genus *Candida* (55%), the rest being microorganisms of the class Saccharomycetes (1.2%). *Candida* constituted by unicellular fungi, had already been identified previously in other RO membranes [13]. This fungus is also able to form biofilms as a way to develop resistance to antifungal products [78], which, together with other microorganisms that constitute biofouling, causes a greater difficulty in elimination and must be considered for the development of more effective cleaning of RO membrane.

Fungal diversity in RO membranes was low. The values obtained with the Shannon-Weaver index (Table 3) were all below three for RO membranes. As with prokaryotes, the fungal diversity was higher in membrane A and B than in membrane C. This could be due to the apparent low diversity of fungi in saline environments [79].

Contrary to what happens with prokaryotic communities, fungi have hardly been studied in RO membranes. The only study that considered them analysed a water treatment system in which RO membranes functioned as a tertiary treatment system, concluding that most fungi were Ascomycota, as found in our study [13]. Within Ascomycota, family Capnodoaceae, has been reported to form biofilms in hard substrates such as rocks [80]; in our study, order Capnodiales was present at percentages ranging from 0.7% (Membrane C) to 3.3% in membrane A; but within this order, family Capnodaceae was not found. In a more recent study, Al Ashhab et al. [22] also found that Ascomycota and Basidiomycota were dominant, although Ascomycota was found at higher abundance, but after a cleaning procedure there was a significant shift with Ascomycota predominating in cleaned RO membranes and Basidiomycota dominating control biofilms. Authors also reported that the community composition of Ascomycota at the beginning and at the end of the cleaning procedure changed but considered that there is remarkable lack of information regarding fungal community members and further research is needed. Thus, the lack of studies about the presence of fungi in biofilms developed in RO membranes must be considered as an important limitation for biofilm prevention and elimination.

The results obtained with β - diversity allowed to statistically differentiate between the three membranes. Distances were represented through Principal Coordinates Analysis 2D-Plots, which are shown in supplementary Fig. S2. The fungal communities established in the three RO membranes were significantly different between them: membrane A and membrane B with a *p*-value of 6.6×10^{-3}, membrane B and C with *p*-value of 3×10^{-3} and finally membrane A and membrane C with a *p*-value of 8.88×10^{-6}. As stated

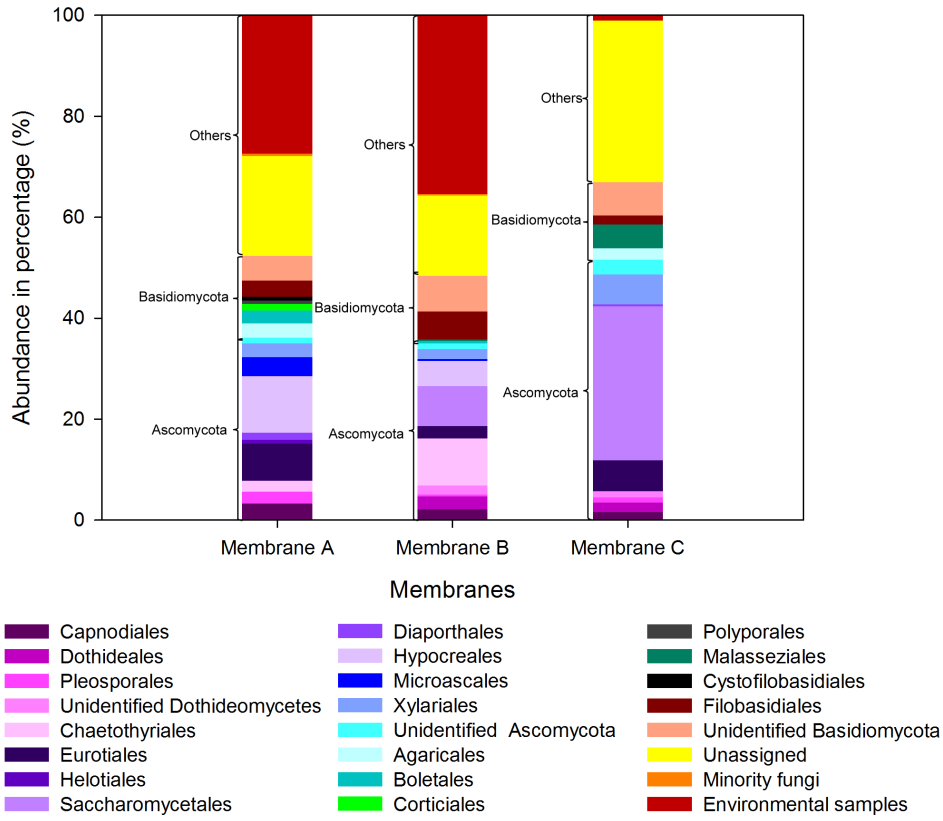

Fig. 5. Relative abundance of fungi at the order level in used membranes. To the left of the bars the orders are grouped in phyla. Minorities are OTUs whose representation is less than 0.5%; unassigned are those sequences that have only been identified as fungi and lastly the Environmental Sample refer to those sequences that have not been recognized at any taxonomic level.

above for bacterial diversity, these statistically significant differences might be explained by the different feed water, inorganic fouling and even membrane brand and location.

4. Conclusions

A three-tiered approach that might be useful for RO membranes autopsies was proposed in the study that included the determination of inorganic fouling and biofouling by SEM; biofilm cell viability and biofilm matrix presence by specific stains for CLSM and FTIR analysis and Illumina sequencing to study microbial composition and diversity.

SEM may be used as a first-tiered approach as it provides clear information about inorganic fouling and may detect microorganisms attached to the membrane surfaces but it cannot give information about the viability of these organisms or the extension and nature of the biofilm matrix. The second-tiered approach should be the use of specific stains like the Filmtracer™ LIVE/DEAD® biofilm viability kit and the FilmTracer SYPRO Ruby biofilm matrix to detect viable cells and matrix extension by CSLM, respectively. ATR-FTIR analysis might be useful to provide information about the chemical nature of the biofilm matrix; this is relevant because cleaning procedures such as conventional chemical treatments have been found to fail in removing developed biofilms in RO membranes. Once biofouling has been detected, the third-tiered approach is the study of microbial composition and diversity with the objective of identifying key microorganisms in the process of biofouling; this information may be useful for the development of advanced antibiofouling treatments for the desalination industries. This approach may take advantage of techniques of massive DNA sequencing like the Illumina platform used in this study.

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Supplementary

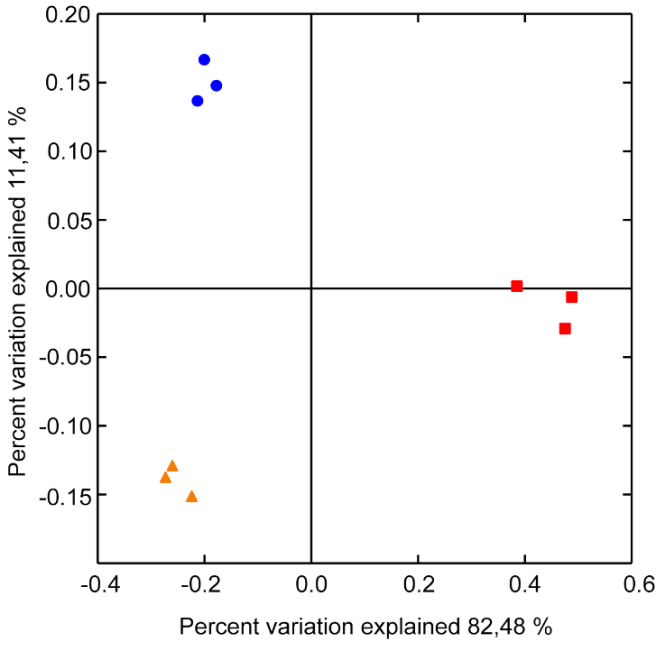

Fig. S1. Principal component analysis of the bacterial diversity in: membrane A (blue circles), membrane B (orange triangles) and membrane C (red squares). Each sample was represented three times, one for replicate. The Y axis explained 82.48 % and Y axis 11.41 % of the variability in the data for the bacterial groups.

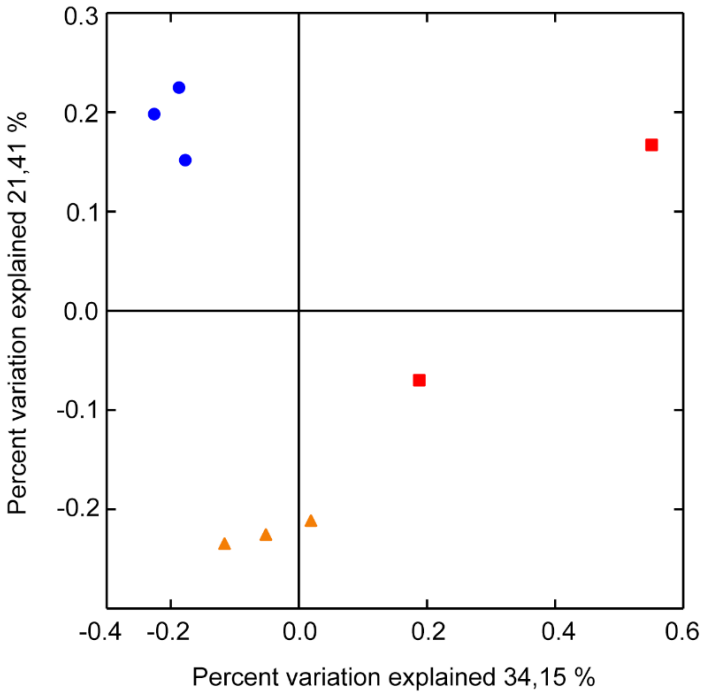

Fig. S2. Principal component analysis of the fungal diversity in: membrane A (blue circles), membrane B (orange triangles) and membrane C (red squares). Three replicates were made per membrane except for membrane C which only two replicates were considered due to insufficient reads for the third replicate. The Y axis explained 21.41 % and Y axis 34.15 % of the variability in the data for the fungal groups.

Table S1
Relative abundance of microorganisms identified up to genus level by region 16S

Kingdom	Phylum	Class	Order	Famlily	Genus	Membrane A	Membrane B	Membrane C
Unassigned	Other	Other	Other	Other	Other	5.83%	4.87%	0.83%
Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	0.30%	0.47%	0.00%
Bacteria	Acidobacteria	AT-s2-57	Unassigned	Unassigned	Unassigned	0.00%	0.33%	0.00%
Bacteria	Acidobacteria	Holophagae	Holophagales	Unassigned	Unassigned	0.07%	0.10%	0.00%
Bacteria	Acidobacteria	Solibacteres	Solibacterales	PAUC26f	Unassigned	0.03%	0.00%	0.00%
Bacteria	Acidobacteria	Sva0725	Sva0725	Unassigned	Unassigned	1.67%	2.33%	0.00%
Bacteria	Acidobacteria	[Chloracidobacteria]	RB41	Ellin6075	Unassigned	0.00%	0.00%	0.00%
Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Unassigned	Unassigned	3.30%	6.20%	0.17%
Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	Unassigned	0.07%	0.10%	0.00%
Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Iamiaceae	Iamia	0.47%	0.63%	0.00%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Cellulomonas	0.00%	0.00%	0.00%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Gordoniaceae	Gordonia	0.03%	0.03%	0.33%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Unassigned	0.00%	0.00%	0.00%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agromyces	0.00%	0.00%	0.07%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Leucobacter	0.00%	0.00%	0.10%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Microbacterium	0.00%	0.00%	0.10%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	2.60%	6.37%	1.57%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Nocardia	0.10%	0.00%	0.00%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Unassigned	1.87%	7.07%	0.63%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Pimelobacter	0.20%	0.03%	0.00%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudonocardia	0.77%	0.33%	0.00%
Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	Unassigned	Unassigned	0.00%	0.00%	0.03%
Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Unassigned	Unassigned	0.00%	0.00%	2.80%
Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Conexibacteraceae	Conexibacter	0.00%	0.00%	0.00%
Bacteria	BHI80-139	Unassigned	Unassigned	Unassigned	Unassigned	0.07%	0.00%	0.00%
Bacteria	BRC1	NPL-UPA2	Unassigned	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	BRC1	PRR-11	Unassigned	Unassigned	Unassigned	0.03%	0.00%	0.00%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	SB-1	Unassigned	0.00%	0.00%	0.00%
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Other	0.00%	0.00%	0.00%
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Unassigned	1.73%	0.03%	0.00%
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Leadbetterella	0.00%	0.00%	2.67%
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	Unassigned	0.40%	0.07%	0.00%
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	Fulvivirga	0.00%	0.00%	0.00%
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	Reichenbachiella	0.00%	0.00%	0.00%
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Unassigned	Unassigned	0.07%	0.10%	0.10%

(*Continued*)

Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Unassigned	1.80%	17.97%	0.00%
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Unassigned	0.47%	0.00%	0.00%
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Arenibacter	0.03%	0.00%	0.00%
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	0.00%	0.00%	0.43%
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Muricauda	0.23%	0.00%	0.00%
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Robiginitalea	0.03%	0.00%	0.00%
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Unassigned	0.10%	0.00%	0.07%
Bacteria	Bacteroidetes	VC2_1_Bac22	Unassigned	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	Bacteroidetes	[Rhodothermi]	[Rhodothermales]	Rhodothermaceae	Unassigned	0.07%	0.00%	0.00%
Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Unassigned	0.00%	0.00%	2.30%
Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	Unassigned	0.00%	0.00%	0.00%
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Other	Other	5.00%	1.03%	0.00%
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Unassigned	Unassigned	1.87%	0.23%	0.00%
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Parachlamydiaceae	Other	0.27%	1.37%	0.00%
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Parachlamydiaceae	Candidatus Protochlamydia	0.03%	0.00%	0.00%
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Rhabdochlamydiaceae	Candidatus Rhabdochlamydia	0.07%	0.10%	0.00%
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Simkaniaceae	Other	0.40%	0.03%	0.00%
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Waddliaceae	Other	0.00%	0.00%	0.00%
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Waddliaceae	Unassigned	0.20%	0.00%	0.00%
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Waddliaceae	Waddlia	0.00%	0.10%	0.00%
Bacteria	Chlorobi	OPB56	Unassigned	Unassigned	Unassigned	0.00%	0.00%	0.67%
Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	Unassigned	0.00%	0.03%	0.00%
Bacteria	Chloroflexi	TK17	Unassigned	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	Chloroflexi	Thermomicrobia	JG30-KF-CM45	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	Cyanobacteria	4C0d-2	MLE1-12	Unassigned	Unassigned	7.87%	0.10%	0.00%
Bacteria	Cyanobacteria	ML635J-21	Unassigned	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	Cyanobacteria	Nostocophycideae	Nostocales	Nostocaceae	Anabaena	0.00%	0.03%	0.00%
Bacteria	Cyanobacteria	Nostocophycideae	Nostocales	Nostocaceae	Nostoc	0.00%	0.00%	0.03%
Bacteria	Firmicutes	Bacilli	Bacillales	Unassigned	Unassigned	0.40%	0.03%	0.03%
Bacteria	Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	Alicyclobacillus	0.00%	0.00%	0.87%
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Unassigned	0.03%	0.00%	0.00%
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0.43%	0.10%	0.07%
Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Brevibacillus	0.07%	0.07%	22.60%
Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Cohnella	0.00%	0.00%	0.07%
Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	0.00%	0.00%	0.17%
Bacteria	Firmicutes	Bacilli	Bacillales	Thermoactinomycetaceae	Unassigned	0.00%	0.00%	0.00%
Bacteria	Gemmatimonadetes	Gemm-2	Unassigned	Unassigned	Unassigned	0.00%	0.20%	0.00%

(*Continued*)

Table S1 (*Continued*)

Bacteria	Gemmatimonadetes	Gemm-3	Unassigned	Unassigned	Unassigned	0.00%	0.00%	0.13%
Bacteria	Gemmatimonadetes	Gemm-4	Unassigned	Unassigned	Unassigned	0.00%	0.13%	0.00%
Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	0.00%	0.00%	0.33%
Bacteria	NKB19	Unassigned	Unassigned	Unassigned	Unassigned	0.00%	0.07%	0.00%
Bacteria	NKB19	noFP_H4	Unassigned	Unassigned	Unassigned	0.47%	0.27%	0.00%
Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Unassigned	1.23%	1.10%	0.00%
Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	2.03%	0.03%	0.00%
Bacteria	OD1	ZB2	Unassigned	Unassigned	Unassigned	0.00%	0.07%	0.00%
Bacteria	OP1	[Acetothermia]	[Acetothermales]	Unassigned	Unassigned	1.30%	1.40%	0.00%
Bacteria	Planctomycetes	C6	MVS-107	Unassigned	Unassigned	0.00%	0.37%	0.00%
Bacteria	Planctomycetes	OM190	CL500-15	Unassigned	Unassigned	0.20%	0.10%	0.00%
Bacteria	Planctomycetes	OM190	agg27	Unassigned	Unassigned	0.83%	0.00%	0.00%
Bacteria	Planctomycetes	Phycisphaerae	CCM11a	Unassigned	Unassigned	1.47%	0.93%	0.00%
Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales	Unassigned	Unassigned	2.67%	0.83%	0.17%
Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	Unassigned	0.83%	1.23%	0.00%
Bacteria	Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae	Unassigned	0.00%	0.00%	0.13%
Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	Unassigned	0.00%	0.10%	0.00%
Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	A17	0.00%	0.00%	0.07%
Bacteria	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces	0.40%	0.77%	0.17%
Bacteria	Proteobacteria	Unassigned	Unassigned	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Other	Other	Other	0.00%	0.00%	0.13%
Bacteria	Proteobacteria	Alphaproteobacteria	Unassigned	Unassigned	Unassigned	12.20%	9.73%	2.90%
Bacteria	Proteobacteria	Alphaproteobacteria	BD7-3	Unassigned	Unassigned	0.23%	0.13%	0.17%
Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Other	0.00%	0.00%	0.73%
Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Unassigned	0.00%	0.00%	0.27%
Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Mycoplana	0.00%	0.00%	0.03%
Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0.00%	0.00%	0.27%
Bacteria	Proteobacteria	Alphaproteobacteria	Kiloniellales	Unassigned	Unassigned	0.00%	0.83%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Kiloniellales	Kiloniellaceae	Unassigned	0.17%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Kiloniellales	Kiloniellaceae	Thalassospira	0.00%	0.53%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Kordiimonadales	Kordiimonadaceae	Unassigned	0.50%	0.20%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Other	Other	0.10%	0.10%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Unassigned	Unassigned	0.03%	0.00%	1.53%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Chelatococcus	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	0.00%	0.00%	0.03%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Unassigned	0.10%	0.67%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	0.43%	2.27%	0.50%

(*Continued*)

Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Unassigned	1.80%	17.97%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Parvibaculum	0.10%	4.50%	0.10%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Other	0.00%	0.00%	6.80%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Unassigned	0.07%	0.03%	1.37%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Nitratireductor	0.00%	0.10%	0.87%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	0.00%	0.03%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Kaistia	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Xanthobacter	0.00%	0.00%	0.13%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Unassigned	0.00%	0.00%	0.93%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Hyphomonas	0.03%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Maricaulis	0.43%	1.10%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Other	0.00%	0.07%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Unassigned	10.90%	3.67%	15.50%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	0.00%	0.00%	0.10%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Unassigned	0.00%	0.03%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Unassigned	1.97%	1.17%	3.23%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	0.03%	0.07%	0.10%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Rhodovibrio	0.00%	0.03%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Other	Other	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Unassigned	Unassigned	0.10%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Other	0.33%	0.13%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Unassigned	0.80%	0.13%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Other	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Unassigned	0.03%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0.00%	0.00%	0.17%
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	0.10%	0.40%	6.10%
Bacteria	Proteobacteria	Betaproteobacteria	Unassigned	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Other	Other	0.00%	0.00%	0.07%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Unassigned	0.00%	0.00%	1.03%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter	0.00%	0.00%	2.07%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Pigmentiphaga	0.00%	0.00%	0.03%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Unassigned	0.03%	0.00%	0.23%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Delftia	0.00%	0.00%	0.03%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Hydrogenophaga	0.00%	0.00%	0.10%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnobacter	0.00%	0.13%	0.00%

(*Continued*)

Table S1 (*Continued*)

Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Ralstonia	0.00%	0.03%	0.00%
Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	Unassigned	0.00%	0.00%	0.10%
Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	Nitrosovibrio	0.00%	0.00%	0.03%
Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Unassigned	0.07%	1.23%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	Bacteriovorax	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	0.00%	0.07%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Unassigned	Unassigned	2.13%	0.20%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	Unassigned	0.00%	0.17%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	Plesiocystis	1.13%	0.00%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	NB1-j	JTB38	Unassigned	0.10%	0.00%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	NB1-j	MND4	Unassigned	0.00%	0.03%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	NB1-j	NB1-i	Unassigned	0.00%	0.93%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	Unassigned	6.53%	0.77%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	34P16	Unassigned	Unassigned	0.00%	0.07%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Unassigned	0.00%	0.03%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	HB2-32-21	0.10%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	HTCC2188	HTCC	0.03%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Unassigned	0.03%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	J115	Other	0.00%	0.03%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	J115	Unassigned	0.03%	0.07%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Other	Other	0.17%	0.13%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Unassigned	Unassigned	0.00%	0.20%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Unassigned	0.07%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unassigned	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	Unassigned	0.07%	0.93%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Unassigned	Unassigned	0.33%	0.10%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	Unassigned	0.23%	0.13%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Other	0.13%	0.00%	0.27%
Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Unassigned	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella	0.33%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Unassigned	Unassigned	0.47%	0.20%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Alcanivoracaceae	Alcanivorax	0.17%	6.47%	0.00%

(*Continued*)

Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Unassigned	1.80%	17.97%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Unassigned	0.00%	0.00%	0.07%
Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	Unassigned	0.23%	0.90%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Unassigned	0.67%	0.37%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Other	0.00%	0.00%	0.97%
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Arenimonas	0.10%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Dokdonella	0.00%	0.00%	0.00%
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	0.00%	0.00%	8.40%
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	0.00%	0.00%	5.70%
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Thermomonas	0.00%	0.00%	1.10%
Bacteria	Proteobacteria	Gammaproteobacteria	[Marinicellales]	[Marinicellaceae]	Unassigned	0.00%	0.03%	0.00%
Bacteria	Proteobacteria	TA18	CV90	Unassigned	Unassigned	0.00%	0.23%	0.00%
Bacteria	SBR1093	EC214	Unassigned	Unassigned	Unassigned	0.10%	0.37%	0.00%
Bacteria	SBR1093	VHS-B5-50	Unassigned	Unassigned	Unassigned	0.00%	0.10%	0.00%
Bacteria	Spirochaetes	[Leptospirae]	[Leptospirales]	Leptospiraceae	Turneriella	0.00%	0.00%	0.00%
Bacteria	TM6	SJA-4	Unassigned	Unassigned	Unassigned	4.27%	0.43%	0.07%
Bacteria	TM6	SJA-4	YJF2-48	Unassigned	Unassigned	0.00%	0.00%	0.00%
Bacteria	Verrucomicrobia	Opitutae	Puniceicoccales	Puniceicoccaceae	Other	0.00%	0.00%	0.00%
Bacteria	Verrucomicrobia	Opitutae	Puniceicoccales	Puniceicoccaceae	Unassigned	0.00%	0.00%	0.00%
Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prosthecobacter	0.00%	0.00%	0.07%
Bacteria	WPS-2	Unassigned	Unassigned	Unassigned	Unassigned	0.00%	0.17%	0.00%

Table S2
Relative abundance of fungi identified up to genus level by region ITS

Kingdom	Phylum	Class	Order	Family	Genus	Membrane A	Membrane B	Membrane C
No blast hit	Other	Other	Other	Other	Other	27.40%	35.37%	1.00%
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Cladosporium	3.30%	1.03%	0.77%
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Septoria	0.00%	1.10%	0.80%
Fungi	Ascomycota	Dothideomycetes	Dothideales	Dothideaceae	Endoconidioma	0.00%	2.53%	0.00%
Fungi	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	unidentified	0.00%	0.00%	1.90%
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Incertae_sedis	Phoma	0.00%	0.33%	0.00%
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	unidentified	0.00%	0.00%	0.97%
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Chalastospora	0.87%	0.00%	0.00%
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Lewia	1.47%	0.00%	0.00%
Fungi	Ascomycota	Dothideomycetes	unidentified	unidentified	unidentified	0.00%	1.87%	1.30%
Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Chaetothyriaceae	Cyphellophora	2.17%	6.40%	0.00%
Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Exophiala	0.00%	2.87%	0.00%
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus	1.87%	0.00%	1.87%
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Eupenicillium	0.00%	0.00%	0.00%
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Paecilomyces	4.30%	1.77%	0.00%
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	1.23%	0.63%	4.27%
Fungi	Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	unidentified	0.70%	0.00%	0.00%
Fungi	Ascomycota	Leotiomycetes	unidentified	unidentified	unidentified	0.00%	0.40%	0.00%
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae_sedis	Candida	0.00%	2.30%	28.87%
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Pichiaceae	Pichia	0.00%	5.20%	1.60%
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	Saccharomyces	0.00%	0.40%	0.00%
Fungi	Ascomycota	Sordariomycetes	Diaporthales	Gnomoniaceae	Gnomonia	0.00%	0.00%	0.37%
Fungi	Ascomycota	Sordariomycetes	Diaporthales	Valsaceae	Valsa	1.43%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	Engyodontium	1.47%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	2.10%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	1.07%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Incertae_sedis	Acremonium	4.17%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Cosmospora	0.63%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	0.80%	2.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Haematonectria	1.03%	2.97%	0.00%
Fungi	Ascomycota	Sordariomycetes	Melanosporales	Ceratostomataceae	Sphaerodes	0.27%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Microascales	Halosphaeriaceae	Sigmoidea	1.10%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Microascales	Microascaceae	Pseudallescheria	2.70%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Microascales	Microascaceae	Wardomycopsis	0.00%	0.47%	0.00%
Fungi	Ascomycota	Sordariomycetes	Xylariales	Amphisphaeriaceae	Truncatella	0.00%	0.00%	1.60%

(*Continued*)

Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	Eutypella	0.53%	0.00%	0.00%
Fungi	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	Xylaria	2.27%	1.97%	4.33%
Fungi	Ascomycota	unidentified	unidentified	unidentified	unidentified	1.13%	1.10%	2.93%
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Lyophyllaceae	Lyophyllum	0.00%	0.00%	0.83%
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	Hypholoma	2.77%	0.00%	0.00%
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Clitocybe	0.00%	0.00%	1.40%
Fungi	Basidiomycota	Agaricomycetes	Boletales	Rhizopogonaceae	Rhizopogon	1.27%	0.00%	0.00%
Fungi	Basidiomycota	Agaricomycetes	Boletales	Sclerodermataceae	Astraeus	0.00%	0.43%	0.00%
Fungi	Basidiomycota	Agaricomycetes	Boletales	Suillaceae	Suillus	1.30%	0.00%	0.00%
Fungi	Basidiomycota	Agaricomycetes	Corticiales	Corticiaceae	unidentified	1.27%	0.00%	0.00%
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Steccherinaceae	Irpex	0.70%	0.00%	0.00%
Fungi	Basidiomycota	Incertae_sedis	Malasseziales	Incertae_sedis	Malassezia	0.00%	0.27%	4.77%
Fungi	Basidiomycota	Tremellomycetes	Cystofilobasidiales	unidentified	unidentified	0.80%	0.00%	0.00%
Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Cryptococcus	3.13%	5.57%	1.83%
Fungi	Basidiomycota	unidentified	unidentified	unidentified	unidentified	4.90%	7.10%	6.50%
Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	19.86%	15.93%	32.10%
						100.00%	100.00%	100.00%