**Drinking Water
Engineering and Science**

Technical Note: Community of bacteria attached on the PVDF MF membrane surface fouled from drinking water treatment, in Seoul, Korea

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Abstract. Alpha, beta, and gamma proteobacteria comprise approximately 68, 16, and 7% of all identified bacteria. In this study, bacterial communities that had fouled polyvinylidene fluoride microfiltration membranes, which are used for drinking water treatment, over an 18 month period were analyzed using the 16s rRNA gene clone library method. The alpha, beta, and gamma proteobacteria were composed of mainly *Bradyrhizobium* and *Rhodopseudomonas*, *Ralstonia*, and *Legionella*, respectively. The presence of a relatively high amount of alpha proteobacteria was due to the oligotrophic condition of the drinking water source, the Han River, tested in this specific case study. The second most prominent bacteria community was the beta proteobacteria, which are typically found in a freshwater environment. This finding supports the notion that the drinking water source was relatively clean. Analyses of the organic foulants indicated that they were most likely from extra cellular polymers and/or cell fractured chemicals from bacteria or micro-organisms, as identified using organic characterizing tools, including 3-D fluorescence excitation-emission matrix and Fourier transform IR analyses.

1 Introduction

Relatively new attempts in the characterization of fouling have been conducted in the areas of biofouling and microbial extra-cellular polymeric substances (EPS). The development of such characterization techniques are especially important for the identification of microbial communities in fouled membrane, which may provide a better understanding of biofouling formation mechanisms including microorganisms and EPS. Bacterial community structure analyses have recently been performed for membranes that were fouled during wastewater and effluent treatment (Ivnitsky et al., 2007; Miura et al., 2007; Pang and Liu, 2007; Chen et al., 2004). The bacterial community was identified using both polymerase chain reaction (PCR)-denaturing gradient

gel electrophoresis (DGGE) and 16S rDNA gene clone library methods, in conjunction with providing phylogenetic tree information. Specific functional genes, such as the denitrifying nirK gene, were also detected in biofilms formed on biofouled membrane (Pang and Liu, 2007). Even with different trends, α-, β-, and γ-proteobacteria were most dominant in fouled membranes that were used to treat both wastewater and advanced treated effluent, with other classes and orders also being identified to lesser extents. Compared to studies that have examined membranes fouled during wastewater treatment, bacterial community structural analysis relating to drinking water treatment has rarely been studied; bacterial community identification was conducted with a drinking water supply system and biofilms formed in a drinking water distribution system (Eichler et al., 2006; Kalmbach et al., 2000). In this case study, *Aquabacterium commune*, which is in the subclass of β-proteobacteria, was found to be the dominant bacteria using the rRNA targeted probe method.

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Foulants from membranes fouled during drinking water treatment have been extensively characterized, mostly using chemical characterizing tools. In these studies, micro-organisms originated macromolecules, including colloidal organic matters (cell-wall fractured and relatively hydrophilic N-acetyl amino sugars), were found to be the major source of biofouling (Leenheer et al., 2000; Kwon et al., 2005). However, the direct identification of the type of bacteria that accumulated on the fouled membranes used for drinking water treatment have been very rare; thus, this was examined in this study.

2 Methods and materials

2.1 Bacteria community analysis

Microbial biomass was collected from the polyvinylidene fluoride (PVDF) MF membrane surface using mechanical beating with a bead beater. Genomic DNA was extracted using the AccuPrep Genomic DNA extraction kit (k-3032, Bioneer, Korea), according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification for 16S rRNA was performed using universal primers 9F (5'-GAG TTT GAT CCT GGC TCA G-3') and 1512R (5'-ACG GCT ACC TTG TTA CGA CTT-3') (Weisburg et al., 1991). The PCR mixture included PCR premix (AccuPower PCR PreMix kit, Bioneer, Korea), $1 \mu L$ of 10 pmole of each primer, and 30 ng of template DNA. The reaction conditions were $94°$ for 10 min, followed by 34 cycles at $94°$ for 30 s, $55°$ for 30 s, and $72°$ for 1 min, and a final extension step at $72°$ for 10 min. The amplicons were purified using the AccuPrep PCR purification kit (k-3034, Bioneer, Korea). The purified PCR products were ligated into the yT&A cloning vector (RBC, Twain) and transformed into HIT-DH5α (RH617, RBC) cells. Transformants were selected on Luria-Bertani agar plates containing ampicilin (70μg/ml), 20μL of X-gal (40 mg/ml), and 50μL of IPTG (200 mM). A single white colony having the recombinant plasmids was inoculated into 1 ml LB broth with ampicilin (70μg/ml), and incubated at $37°$ for 12–14 h. The plasmid DNA was then extracted and purified using the AccuPrep plasmid extraction kit (Bioneer, Korea), according to the manufacturer's instructions. Clones were then sequenced using an ABI 3730xl automated DNA analyzer (Applied Biosystems, Foster City, CA) at Xenotech (Daejeon, Korea). Sequences were analyzed using the BLAST database (www.ncbi.nlm.nih.gov). Sequence alignments of those selected clones and closely related bacterial species were performed using the Clustal W version 1.7 program (Thomson et al., 1997) available in the BioEdit software package (Hall, 1999). Phylogenetic analyses of cloned the nucleotide sequence were conducted using MEGA version 3.1 (Kumar et al., 2004). The tree was constructed from a matrix of pair-wise genetic distances by the neighbor-joining method. The 16S rDNA sequences reported
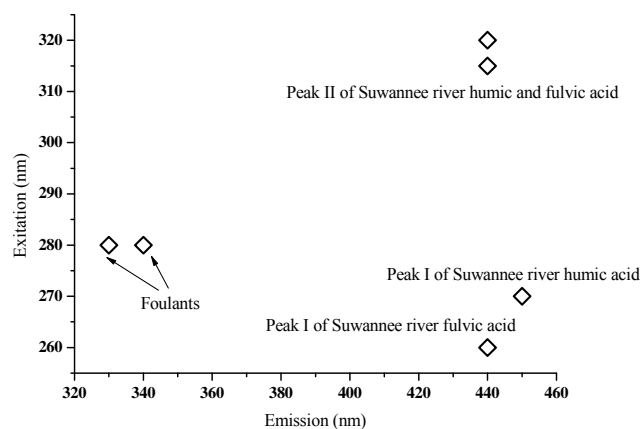
**(a)** Depicts of excitation and emission of peaks from 3-D fluorescence spectrophotometer.
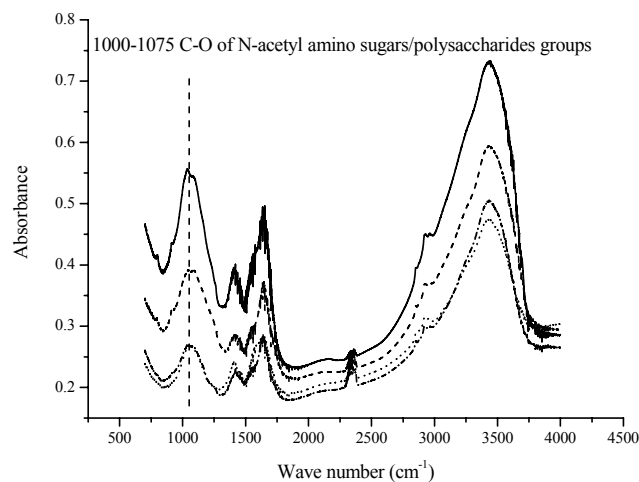
(b) FTIR spectra of the membrane foulants.

Figure 1. Foulants characterization.

here have been deposited in GenBank under the accession numbers FJ572662 to FJ572674.

2.2 Organic foulants characterization

The major components and complexity of organic foulants were identified using fluorescence spectrophotometer (F-2500, Hitachi, Japan), which provided 3-D fluorescence excitation emission spectra. Specific functional groups of various samples were confirmed using Fourier transform infrared (FTIR) spectrometer (FT/IR-460 plus, Jasco, Japan). A freeze dryer (Ilshin, Korea) was used to dessicate the water samples, and the IR spectrum was acquired with the KBr pellet (Pike, USA). Images of fouled membranes were obtained using field emission-scanning electron microscopy (FE-SEM) (Hitachi S-4700, Japan), after pretreatment with 5~6 nm Pt layering, using an Ion Sputter (Hitachi E-1030, Japan).
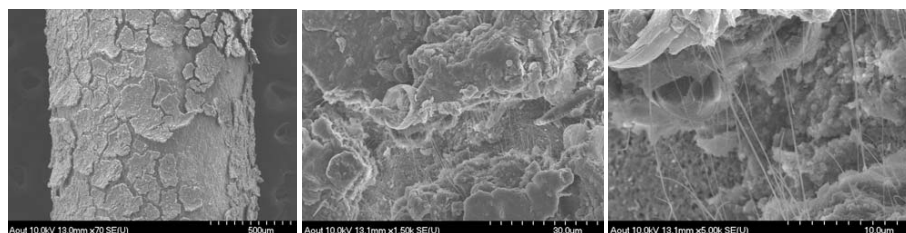
Figure 2. SEM images of fouled MF membranes (×70, ×1500, ×5000).

Table 1. Summary of major bacteria from 120 colonies identified for the fouled MF membrane, as measured using the clone library method.

Phyogentic group	Major found bacteria	Occupying percentage for 120 colonies (%)
α-proteobacteria	*Bradyrhizobium* sp., *Rhodopseudomonas* sp. and *Afipia* sp.	68
β-proteobacteria	*Ralstoina* sp.	16
γ-proteobacteria	*Legionella* sp.	7
Planctomycetes	*Planctomyces* sp.	7

Table 2. Phylogentic distribution of identified bacteria from the fouled MF membrane.

Sequence type (and percentage) of total clones	Phylogentic group	Most closely related bacteria	Similarity (%)
Clone 2 (35.6%)	α-proteobacteria	*Bradyrhizobium* sp.	98–99
Clone 148 (17.8%)	α-proteobacteria	*Rhodopseudomonas* sp.	99
Clones 37 (11.9%), and 142 (0.8%)	α-proteobacteria	*Afipia* sp.	98–99
Clone 147 (0.8%)	α-proteobacteria	*Xanthobacter* sp.	99
Clone 75 (0.8%)	α-proteobacteria	*Agrobacterium* sp.	99
Clones 87 (11.9%)	β-proteobacteria	*Ralstoina* sp.	98–99
Clone 133 (1.7%)	β-proteobacteria	*Alcaligenes* sp.	99
Clones 117 (1.7%)	β-proteobacteria	*Curvibacter* sp.	96
Clone 135 (0.8%)	β-proteobacteria	*Janthinobacterium* sp.	98
Clone 40 (6.8%)	γ-proteobacteria	*Legionella* sp.	94–96
Clone 38 (0.8%)	Bacteroidetes	*Marinicola seohaensis*	88
Clone 21 (0.8%)	High G+C	*Arthrobacter* sp.	99
Clone 23 (0.8%)	High G+C	Environmental clones	96
Clone 26 (6.8%)	*Planctomycetes*	*Planctomyces* sp.	94

3　Results and discussion

Excitation and emission peaks measured from 3-D fluorescence spectra with chemically desorbed foulants using both 0.1 N NaOH and 0.1 N HCl provided information on the peaks that were different from those of humic substances obtained from the Suwannee river humic and fulvic acids (from International Humic Substance Society), and similar to those of protein-like substances (Fig. 1a) (Lee et al., 2006). FTIR peaks around $\sim$1000 cm^{-1}, which corresponded to polysaccharides/cell fractured N-acetyl amino sugar groups, were identified, as shown in Fig. 1b (Leenheer et al., 2000; Kwon et al., 2005). Along with these peaks, sticky materials, presumably EPS, were found through FE-SEM imaging (Fig. 2). Based on these three observations, it was hypothesized that the biofouling on the fouled membrane and related foulants originated from the presence of a substantial amount of micro-organisms on the membrane.

A 16S rDNA clone library was constructed for the fouled MF membrane. Phylogenetic analyses of 120 randomly selected clones revealed 15 operational taxonomic units (OTUs) assigned to various bacterial divisions, as listed in Tables 1 and 2. Phylogenetic analysis (Table 1 and Fig. 3) indicated that the Proteobacteria dominated on the membrane surface, where the α-proteobacteria subdivision was the largest bacterial group (68% of total clones) and the β-proteobacteria subdivision was the second largest bacte-

rial group (16% of total clones) found in the samples. The majority of the α-proteobacterial OTUs were primarily affiliated with *Bradyrhizobium* sp., *Rhodopseudomonas* sp. and *Afipia* sp. The remaining clones in this group were closely related to *Xanthobacter* sp. and *Agrobacterium* sp. In the β-proteobacteria, the most dominant OTU (11.8% of total clones) was closely related to *Ralstoina* sp., while the other 3 OTUs were closely related to *Alcaligenes* sp., *Curvibacter* sp. and *Janthinobacterium* sp.

The MF sample also contained 5 OTUs from the γ-proteobacteria, the *Planctomycetes* group, the gram-positive high G+C group and *Bacteroidetes* group. In the γ-proteobacteria group, clone 40 (6.8% of total clones) was closely related to *Legionella* sp. In the *Planctomycetes* group, clone 26 (6.8% of total clones) was closely related to *Planctomycetes* sp. In the high G+C group, clones 21 and 23 were closely related to *Arthrobacter* sp. and environmental (uncultured and unidentified) clones. One OTU from the *Bacteroidetes* group was related to *Marinicola seohaensis* (<90% similarity).

The α-proteobacteria was found to be the most predominant group on the MF membrane surface, which was in agreement with a previous study (Chen et al., 2004).
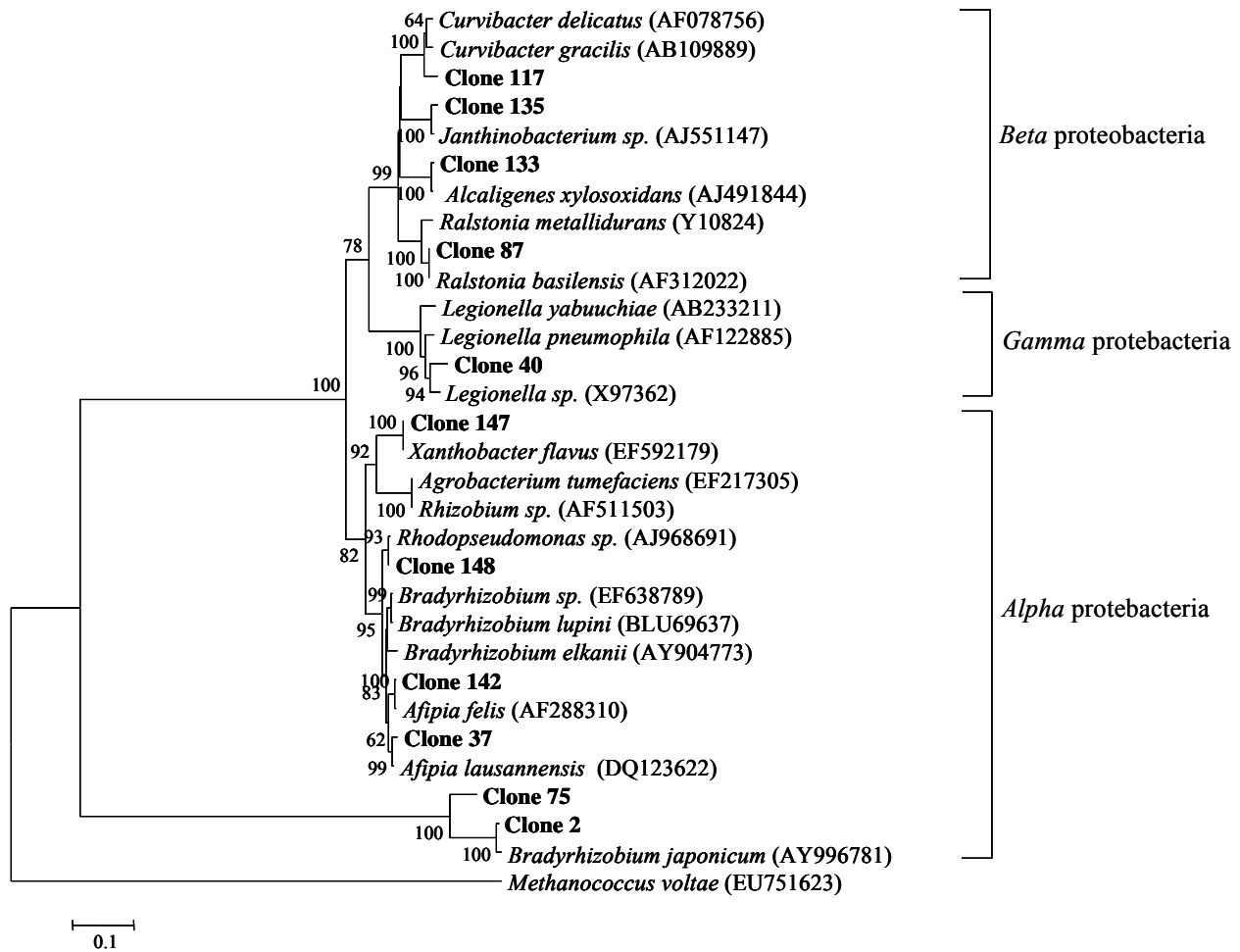
Figure 3. Phylogenetic tree of the proteobacteria based on 16S rDNA sequences obtained from cloning analyses of fouled MF membrane. Clones obtained from this study are shown in bold type. The tree was derived by performing the neighbor-joining method with a Jukes-Cantor parameter in the MEGA version 3.1 programme. Bootstrap values greater than 50% (1000 replicates) are shown. *Methanococcus voltae* EU751623 was used as the out-group to root the phylogram. The scale bar represents one substitution per ten nucleotides.

The α-proteobacteria groups became the most dominant biofoulant under pressurized oligotrophic conditions. This result was expected since they are known to be well adapted to oligotrophic environments (Pinhassi and Hagström, 2000). The second largest β-proteobacteria group on the membrane surface is typically found in a freshwater environment, which is consistent with drinking water sources, like the Han River.

4 Conclusions

In this study, different classes and specific genes/species of bacteria existing in foulants on membranes used for drinking water treatment process were identified using 16S rRNA gene clone library method, in addition to separated chemical foulants characterization, including FTIR and 3-D fluorescence spectra analyses. α-, β-, and γ-proteobacteria were found to be the dominant bacteria; however, specific bacteria found through genetic identification could not be directly compared to similar published results with membranes that were used for drinking water plants. Despite this, the finding that α- and β-proteobacteria were determined to be the most dominant bacteria in the fouled membrane is consistent with the fact that they are known to be mainly found in pressurized and oligotrophic conditions and relatively fresh water environments, respectively. The results provided in this study are expected to initiate future studies in the field of membrane biofouling.

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