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Original Article

Isolation and identification of culturable bacteria, capable of heterotrophic growth, from rapid sand filters of drinking water treatment plants

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Abstract

The microbial community in sand filters (SFs) of drinking water treatment plants (DWTPs) likely contributes to SF functionalities, such as organic carbon removal through heterotrophic metabolism. However, the dynamics and functionality of the SF microbiome and microbial communities in oligotrophic environments, in general, are poorly understood. Therefore, the availability of bacterial cultures from these oligotrophic environments is of great interest, yet it is underrepresented in culture collections. Focusing on heterotrophic carbon metabolism, bacteria were isolated from SFs using conventional media and media that contained SF extracts to mimic the SF environment. The majority of isolates belonged to *Betaproteobacteria*, more specifically to the families *Comamonadaceae* (genera *Acidovorax*, *Curvibacter*, *Hydrogenophaga*, *Simplicispira*, *Paucibacter, Pelomonas*, *Piscinibacter* and *Rhodoferax*) and *Oxalobacteraceae* (*Undibacterium*). Additionally, members of *Alphaproteobacteria* (*Mesorhizobium*), *Gammaproteobacteria* (*Aeromonas* and *Perlucidibaca*) and *Actinobacteria* (*Rhodococcus* and *Brachybacterium*) were isolated. Several of those genera have only rarely been described, but appear typical inhabitants of oligotrophic freshwater environments. In this regard, the *Comamonadaceae* isolates are of particular interest. Our study shows that bacteria representative of oligotrophic environments can be isolated using simple isolation procedures. The isolates provide a microbial framework for extending our knowledge of the taxonomy, physiology and functionality of oligotrophic freshwater microbiomes and their interactions with possible invaders.

Keywords: Drinking water treatment; Rapid sand filters; Bacterial isolates; Culturable bacteria; Bacterial community structure; *Comamonadaceae*

1. Introduction

Rapid and slow sand filter (SF) units are used in drinking water treatment plants (DWTPs) for the removal of iron and manganese. Recent studies indicate that the microbiota in SFs of DWTPs not only contribute to iron/manganese removal, but also remove other unwanted compounds such as ammonium, dissolved organic carbon, methane and various recalcitrant organic micropollutants [1–8]. Furthermore, microbial communities in drinking water treatment units were shown to seed and hence affect downstream microbiomes, including those of drinking water distribution networks [6,9]. Therefore, management of the SF microbiome is of interest, for instance, to establish removal of specific compounds (e.g. through bioaugmentation of SFs with bacteria specialized in micropollutant degradation [10]) and to seed the distribution network with beneficial bacteria [6]. To attain those objectives, a thorough understanding of the composition and functionality of the resident SF microbiome is needed.

Little is known, however, about SF microbial communities, their dynamics and functionality. Rapid SFs in DWTPs impose an interesting ecosystem for microbiota due to a combination of short retention times of less than 30 min [1],

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low assimilable organic carbon (AOC) concentrations within the range of 20–100 μgC/L [11,12] and low temperatures of about 8–13 °C [1,7]. Recent studies based on 16S rRNA gene profiling by next-generation sequencing (NGS) revealed that the bacterial community structure in SFs of DWTPs is complex, but, in terms of identity and distribution of phyla, highly similar for different DWTPs [1,3] and stable in time [9,13], with *Proteobacteria* being most dominant. Other frequently detected phyla are *Acidobacteria, Nitrospirae* and *Actinobacteria* [1,3,9,14–16]. Higher variability, however, emerges when considering lower taxonomic classes and rare taxa [3,6]. Albers et al. [1] pointed out *Gallionella*, *Hyphomicrobium* and *Pedomicrobium*, *Nitrospira* and *Methylococcaceae* as being involved in iron, manganese, ammonia and methane oxidation, respectively, by correlating their presence with the geochemistry of the inlet water. Yet, which and how community members contribute to heterotrophic organic carbon metabolism, appointed as a primary function of the SF biomass [15], remain poorly understood. This is important, since organisms that can perform a heterotrophic or mixotrophic lifestyle (i) will contribute to AOC reduction and hence affect the risk of bacterial regrowth in drinking water distribution systems [17], (ii) are likely responsible for organic xenobiotic degradation [14,15,18] and (iii) might affect the survival and activity of accidentally or deliberately introduced organisms [19].

While NGS-based studies have increased our knowledge of SF microbial ecology, pure cultures remain of interest. Although functional traits can be linked to community scale phylogenetic data, caution is warranted and bacterial isolates can be used to test hypotheses on functionality. Furthermore, isolates are of interest for unravelling physiological and adaptive responses and the corresponding molecular pathways that oligotrophic organisms use to survive in SF systems. In addition, they can be used in standard synthetic communities to examine ecological questions about invasions of SF communities by pathogens or deliberately introduced bacteria, as in the case of bioaugmentation for bioremediation purposes [20]. However, bacterial isolates from drinking water systems and, in particular, from SFs of DWTPs are highly underrepresented in culture collections due to their recalcitrance to cultivation [21]. It is generally known that only a small fraction of all bacteria can be cultivated using conventional isolation techniques. Yet, recent advances have been made in isolating and cultivating bacteria, e.g. by the use of sophisticated techniques or growth media [22]. One of the most important challenges in isolating environmental bacterial strains is the fact that oligotrophic bacteria grow slowly or not at all on rich media, generally used in isolation procedures, and are outcompeted by organisms with a higher growth rate [22]. This can be accounted for by using appropriate low nutrient media and extended incubation times [22–24]. Furthermore, by diluting the sample, organisms that are numerically abundant but whose growth is inhibited at lower dilutions due to competition with superior growers can be isolated [22,25]. Moreover, when cultivating microorganisms, growth media and conditions should simulate the natural environment [22]. For instance, George et al. [24] showed that a higher diversity of relevant soil species could be isolated by adding a soil extract to the growth medium.

We studied whether bacterial cultures, capable of heterotrophic growth and representative of the SF environment, can be isolated using the aforementioned cultivation strategies. A selection of pure cultures was identified based on their 16S ribosomal RNA (rRNA) gene sequence, and the extent to which they represent dominant members of the SF community was assessed based on their abundance in the total bacterial 16S rRNA gene pool, as determined by 454 pyrosequencing.

2. Materials and methods

2.1. Sand filter samples

SF samples were taken from operational rapid SFs at two Belgian DWTPs, one located in Kluizen (K) and one in Klein-Sinaai (S). Physicochemical and biological characteristics of the SFs and DWTP intake water are provided in Table S1. The implemented treatment steps and operational conditions at both DWTPs were described previously [7]. When considering the most abundant taxa, previous studies showed that microbial communities sampled at different depths in regularly backwashed SFs are highly similar [1,3,14]. Highest microbial biomass and activity was found at the top of the SF, attributed to a decreasing nutrient availability with filter depth [14]. Therefore, in this study, we focused on the top layer to isolate bacteria and took SF samples from the top 20 cm. 16S rRNA gene denaturing gradient gel electrophoresis (DGGE) profiling revealed a high degree of bacterial community homogeneity (at least for the most abundant populations) within the examined SFs, for samples taken from different SF units and at different time points (Fig. S1). Therefore, only one sample per DWTP (samples Si1 and K1 SF24) was used for strain isolation and 16S rRNA gene pool sequencing. The sampling procedure was described previously [7]. The bacterial 16S rRNA gene copy number of both SF samples was determined using real-time quantitative PCR (qPCR) as described previously [7].

2.2. Isolation of sand filter bacteria and DNA extraction from pure cultures

SF suspensions were obtained by dispersing 5 g SF material in 100 mL 0.01 M $MgSO_4$, stirring for 3 h at 150 rpm on an orbital shaker and settling of coarse material for 1 h. A tenfold dilution series (from 1× to 1,000×) was made from this suspension and 100 μL of each dilution was plated on different media, including Luria–Bertani (LB), Reasoner and Geldreich (R2A) [26], MMO [27] (composition of the media provided in Table S2) and a number of media based on 10-fold diluted LB. No carbon source was added to the MMO medium, allowing organisms to grow on background AOC in the mQ-H_2O or agar. Five different isolation media based on diluted LB medium were prepared. The first was 10-times diluted LB (LB 1/10). The second and third medium were LB 1/10

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