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**Investigating comammox Nitrospira in rapid sand filters via metagenomics and single-cell genomics**

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**Introduction**

Throughout the last century, nitrification was assumed to be a two-step process executed by two different functional groups, ammonia oxidizing prokaryotes (AOP) and nitrite oxidizing bacteria (NOB). Recently, several articles have shown the capability of a single microorganism (belonging to the *Nitrospira* genus) to carry out the complete oxidation of ammonia to nitrate (comammox) (Daims et al., 2015; van Kessel et al., 2015; Pinto et al., 2015, Palomo et al., 2016). Despite the detection and enrichment of comammox *Nitrospira* from different nitrifying environments, the ecological relevance of comammox remains unknown. Nitrification is often the dominant microbial process in rapid gravity sand filters (RSF), which are engineered microbial systems used for the production of drinking water. High abundance of *Nitrospira* and unusual *Nitrospira*/AOP ratios have been observed in these systems (Albers et al., 2015; LaPara et al., 2015, Gülay et al., 2016). In this study, we applied a combination of metagenomics and single-cell genomics to microbial communities from various locations within an aerated groundwater-fed RSF, where *Nitrospira* are present at high relative abundance (Gülay et al., 2016).

**Material and Methods**

Genomic DNA was extracted from six different locations within a groundwater-fed rapid sand filter. DNA was sequenced using Illumina HiSeq 2000 giving 100 bp paired-end reads. High quality reads were assembled using IDBA-UD. Contigs larger than 1,000 nucleotides were clustered into putative taxonomic groups based on pentanucleotide signatures using VizBin. *Nitrospira* single cell sorting was achieved using an in-house developed cell extraction strategy that enabled the disruption of *Nitrospira* cell clusters attached to the mineral coating of the sand. Extracted cells were identified via fluorescence in situ hybridization (FISH) with *Nitrospira*-specific 16S rRNA probes and were sorted via fluorescence-activated cell sorting (FACS). Genomic DNA of sorted cells was amplified with multiple displacement amplification (MDA), and screened using *Nitrospira*-specific 16S rRNA gene primers. Selected *Nitrospira* genomes were subject to whole-genome sequencing.

**Results and Conclusions**

From the metagenomic data set, fourteen draft genomes were reconstructed and functionally characterized. The organisms represented by draft genomes had the capability to oxidize ammonium, nitrite, hydrogen sulfide, methane, potentially iron and manganese as well as organic compounds. Interestingly, a highly abundant microdiverse composite genome of the *Nitrospira* genus was recovered harboring the genetic potential for complete ammonia oxidation. To evaluate the potential relevance of comammox *Nitrospira* in the removal of ammonium within the studied RSF, we investigated the phylogenetic diversity and abundance of *amoA* as a marker gene of ammonium oxidation. The RSF metagenome *amoA* sequences separated into 4 different clusters. The majority of the *amoA* genes were associated with comammox *Nitrospira* (81% and 52% in top and bulk). Seven and 1% (top and bulk filter, respectively) of *amoA* genes were related to a cluster of canonical beta-proteobacterial AOB.
AOA-associated amoA were rarely detected, making up less than 1% in top and bulk samples. amoA associated with heterotrophic ammonia oxidizers made up 12% and 46% of all amoA (top and bulk filter, respectively). (Figure 1).

Cell extraction from sand samples followed by FACS and single-cell sequencing enabled assembly of Nitrospira genomes from the RSF (Figure 2). Single cell genomes confirmed the presence of a complete ammonia oxidation pathway in Nitrospira and revealed clear taxonomic differences between comammox Nitrospira in the investigated RSF and other recently described comammox Nitrospira genomes.

The high abundance of comammox Nitrospira spp. and the dominance of Nitrospira-associated amoA sequences in the metagenome suggest an essential role in ammonia oxidation of this novel comammox Nitrospira in the investigated RSF.

References

Figure 1. Phylogenetic reconstruction and abundance of amoA sequences recovered from the metagenomes (bold).

Figure 2. FIHIS images showing Nitrospira clusters (a) and single cells (b) (in pink).