Species-level diversity of the filamentous Candidatus Microthrix in Danish full-scale WWTPs and their influence on sludge settling properties

Nierychlo, Marta Anna; Thomsen, Lisette; Andersen, Kasper Skytte; Dueholm, Morten Simonsen; Petriglieri, Francesca; Kristensen, Jannie Munk; Nielsen, Per Halkjær

Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

Species-level diversity of the filamentous *Candidatus* Microthrix in Danish full-scale WWTPs and their influence on sludge settling properties

M. Nierychlo*, L. Thomsen, K.S. Andersen, M. S. Dueholm, F. Petriglieri, J.M. Kristensen, P.H. Nielsen**

Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark
*mni@bio.aau.dk; **phn@bio.aau.dk

Abstract: *Candidatus* Microthrix is a widespread filamentous bacterium frequently causing activated sludge settling problems, making it an important member of microbial community in WWTPs. Recent developments of bioinformatic tools allow for the first time to use amplicon sequencing results to study members of activated sludge community with high phylogenetic resolution and to design species-specific FISH probes. Based on 12 years of data from 22 Danish WWTPs we demonstrate low species-level diversity of *Candidatus* Microthrix, widespread occurrence of the two major species in WWTPs and their unequal impact on sludge settling. We also visualize their morphology using novel FISH probes.

Keywords: *Candidatus* Microthrix; FISH; activated sludge bulking

Introduction

Candidatus Microthrix frequently causes severe bulking and foaming in activated sludge wastewater treatment plants (WWTPs) (Rosetti et al., 2005; Nierychlo & Nielsen, 2017) making it one of the most important filamentous bacteria in this ecosystem. Retrieval of species-level information using popular high throughput amplicon sequencing techniques and bioinformatics have been hampered due to a limited phylogenetic resolution of amplicons and lack of low taxonomic ranks information in public reference databases. The recent availability of activated sludge ecosystem-specific MiDAS 3.1 taxonomy and reference database (Nierychlo *et al*., in prep.) has enabled us to study WWTP microbiome with species-level resolution and to develop novel FISH probes for the genus *Ca*. Microthrix. Here we investigate the diversity of *Ca*. Microthrix in full-scale WWTPs, correlate the abundance of specific species with sludge settling properties and visualize individual species *in situ* using FISH.

Materials and Methods

V1-3 region of 16S rRNA gene was sequenced to retrieve abundance information for *Ca*. Microthrix in samples from 22 full-scale Danish BNR WWTPs collected from 2006 to 2018. Diluted Sludge Volume Index (DSVI) was provided for the biomass samples by the WWTPs and used as a measure for sludge settleability. FISH probes were designed in ARB based on 21,000 high-quality, full-length 16S rRNA gene sequences derived from activated sludge (MiDAS 3.1).

Results

The overview of *Ca*. Microthrix species occurrence in Danish WWTPs (**Fig. 1**) indicates its low diversity in full-scale activated sludge, with only two species widespread across the country; including a hitherto unknown species with the provisional name midas_s_3. The well-known *Ca*. M. parvicella was found to be dominant in the majority of WWTPs, however, midas_s_3 was most abundant in 8 out of 22 WWTPs. Interestingly, the other known species isolated from activated sludge - *Ca*. M. calida, was not found in any of the investigated plants.

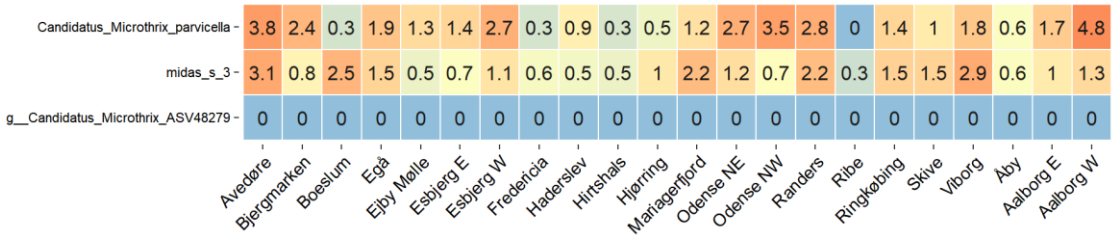
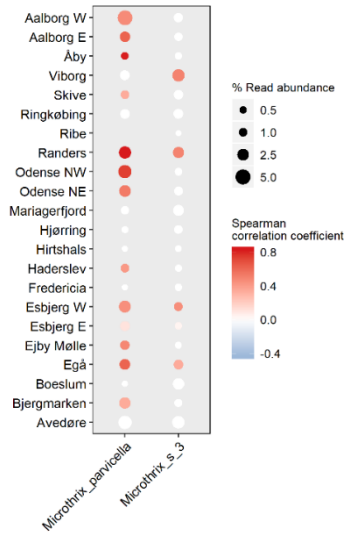
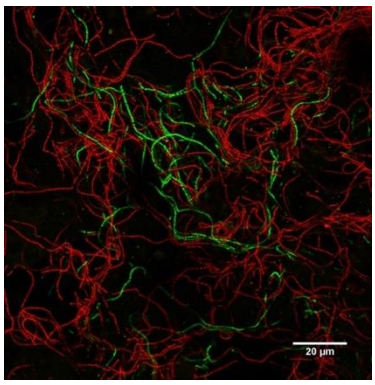


Figure 1 Abundance of *Ca*. Microthrix species in full-scale activated sludge Danish WWTP (values represent an average from samples collected from 2006 to 2018).



The effect of both species on sludge settling properties was examined by calculating Spearman correlation coefficient between DSVI measurements and *Ca*. Microthrix species abundance (**Fig. 2**). The results indicate that *Ca.* M. parvicella has more deteriorating influence on settling properties, compared to the novel S3.

Figure 2 Influence of *Ca*. Microthrix species on settling properties (DSVI) of activated sludge. Spearman correlation coefficient (shown in gradient color) was calculated between abundance of the two species (shown by size) and corresponding DSVI measurement. Coefficient for each plant was calculated using all samples collected from 2006 to 2018.



Novel FISH probes specific for *Ca*. M. parvicella (Mpa177) and midas_s_3 (MCX-S3-181) were applied to visualize them in the full-scale WWTP (**Fig. 3**). Both filaments exhibited similar morphology, however, *Ca*. M. parvicella appeared to be thinner (0.7 vs 1.0 µm) and longer (120 vs 90 µm) than midas_s_3.

Figure 3 FISH micrograph of *Ca*. Microthrix species in activated sludge sample. *Ca*. M. parvicella (targeted by probe Mpa177) and midas_s_3 filaments (targeted by probe MCX-S3-181) appear red and green, respectively.

This study provides for the first time an insight into species-level diversity of *Ca*. Microthrix – an important filamentous member of full-scale activated sludge ecosystem. Species-level identification of microbial communities is a big step forward towards understanding the function of activated sludge ecosystem, as functional traits are often conserved at low taxonomic ranks.

REFERENCES

Rossetti, S., Tomei, M.C., Nielsen, P.H., Tandoi, V. 2005 "*Microthrix parvicella* ", a filamentous bacterium causing bulking and foaming in activated sludge systems: a review of current knowledge. *FEMS Microbiology Reviews* **29**, 49–64.

Nierychlo, M. & Nielsen, P.H. 2017 Denmark – Status on activated sludge settling properties and filamentous microorganisms in: Rossetti, S., Tandoi, V., Wanner, Jiri (Eds.) *Activated Sludge Separation Problems*. International Water Association, pp. 53–66.

Nierychlo M. *et al*., (in preparation) Species-level microbiome composition of activated sludge and anaerobic digesters - introducing MiDAS 3.1 ecosystem-specific taxonomy.