

MICROBIAL ECOLOGY

Do it yourself nitrification

“
Nitrospira
 spp. (...) are
 capable of
 complete
 nitrification
 ”

Microbial oxidation of ammonia (or ammonium) via nitrite to nitrate is a central step in the nitrogen cycle. This process, termed nitrification, was thought to involve two separate steps mediated by distinct microorganisms: the initial oxidation of ammonia to nitrite, which is catalysed by ammonia-oxidizing microorganisms (AOMs; including bacteria (AOB) or archaea (AOA)); followed by the oxidation of nitrite to nitrate, which is catalysed by nitrite-oxidizing bacteria (NOB). Two recent studies challenged this notion by describing *Nitrospira* spp. that can carry out both reactions and therefore are capable of complete nitrification.

The functional separation of ammonia and nitrite oxidation has puzzled microbiologists for decades, particularly as it has been predicted that microorganisms that are capable of complete nitrification (termed complete ammonia oxidizers, or comammox) may have an advantage over AOMs and NOB under substrate-limiting conditions, such as in microbial aggregates and biofilms. Therefore, van Kessel *et al.* and Daims *et al.* enriched cultures for comammox by growing biofilm samples in the presence of low concentrations of ammonium.

van Kessel *et al.* sampled the anaerobic compartment of a trickling filter connected to a recirculation aquaculture system and enriched this community during 1 year, after which the culture was mostly composed of microorganisms belonging to the genus *Brocadia* (which are known anaerobic ammonium oxidizers) and *Nitrospira* (which are known NOB). By sequencing the total DNA from the enrichment culture, the authors were able to

assemble draft genomes of two *Nitrospira* species. Surprisingly, both genomes contained not only the genes involved in nitrite oxidation, but also all the genes necessary for ammonia oxidation (including genes encoding the different subunits of ammonia monooxygenase (AMO) and hydroxylamine dehydrogenase). Furthermore, both species lacked some of the genes necessary for assimilatory nitrite reduction, suggesting that these species have the potential for complete nitrification and may have adapted to ammonium-containing environments; these species were named *Candidatus Nitrospira nitrosa* and *Candidatus Nitrospira nitrificans*.

Daims *et al.* sampled a biofilm from the walls of a pipe in a deep oil exploration well and enriched this community for comammox during 4 years, after which the culture was capable of complete nitrification and composed of a single *Nitrospira* species and a non-nitrifying betaproteobacterium affiliated with the family Hydrogenophilaceae. DNA sequencing and reconstruction of the complete genome of the *Nitrospira* species identified the presence of genes involved in nitrite oxidation and ammonia oxidation, and the absence of some of the genes necessary for assimilatory nitrite reduction; this species was named *Candidatus Nitrospira inopinata*.

To further investigate the ability of *Ca. N. nitrosa* and *Ca. N. nitrificans* to carry out complete nitrification, van Kessel *et al.* combined the use of a fluorescently labelled ammonia analogue that binds to AMO with *Nitrospira*-specific fluorescence *in situ* hybridization (FISH) probes, which revealed that these bacteria express a functional

AMO. Furthermore, combining FISH and ¹⁴C-labelling, the authors demonstrated that the two *Nitrospira* species are capable of inorganic carbon fixation during ammonia oxidation. Similarly, Daims *et al.* demonstrated full nitrification by *Ca. N. inopinata* by growing their enriched culture in media containing ammonium as the sole energy source and CO₂ as the sole carbon source; *Ca. N. inopinata* growth was coupled with the oxidation of ammonium to nitrate. Furthermore, metaproteomic analysis revealed that all of the *Ca. N. inopinata* enzymes involved in both ammonia and nitrite oxidation were expressed during incubation with ammonium.

Finally, both studies investigated the distribution of comammox *Nitrospira* spp. in public databases and by carrying out metagenomic screening of environmental samples, based on the presence of *amoA* (which encodes the A subunit of AMO), which is a functional and phylogenetic marker for AOMs. Notably, *amoA* from these newly discovered *Nitrospira* spp. that are capable of complete nitrification are distinct from *amoA* present in AOB. Furthermore, several highly similar *amoA* sequences were found in *Nitrospira* genomes present in different environments, including agricultural soils, freshwater environments, wastewater treatment plants and drinking water systems, suggesting that comammox *Nitrospira* bacteria are widely distributed.

Cláudio Nunes-Alves

ORIGINAL ARTICLES van Kessel, M. A. H. J. *et al.* Complete nitrification by a single microorganism. *Nature* <http://dx.doi.org/10.1038/nature16459> (2015) | Daims, H. *et al.* Complete nitrification by *Nitrospira* bacteria. *Nature* <http://dx.doi.org/10.1038/nature16461> (2015)



Getty / Photodisc