Supplemental Text: Discussion

Many of the accompanying organisms detected in the *Ca*. N. defluvii enrichment were closely related to cultured bacteria or to 16S rRNA gene sequences retrieved in other studies from wastewater treatment facilities such as nitrifying or denitrifying bioreactors (Table S2). As *Ca*. N. defluvii has been enriched from activated sludge (1), these results suggest that most members of the accompanying community also stem from the original inoculum and were not introduced by later contamination of the enrichment in the laboratory or by us via contaminated reagents used for the various steps in the protocol prior to the final RT-PCR (2). The enrichment of *Ca*. N. defluvii was started in the year 1997 (1), so that most accompanying populations may have been co-cultured with *Nitrospira* for more than a decade. The amounts of non-*Nitrospira* cells in the enrichment varied during this period (1; and unpublished data of the authors), but despite extended efforts, these organisms could not be completely removed to obtain a pure culture of *Ca*. N. defluvii. Thus, it is tempting to speculate that they are persistent commensals or even mutualistic symbionts of *Nitrospira*. The closest cultured relatives of some accompanying organisms are heterotrophs growing on a broad range of organic substrates including carbohydrates, which may be components of the extracellular matrix formed by *Ca*. N. defluvii (1). Members of the *Bacteroidetes,* for example, are known to degrade biopolymers and high-molecular-weight dissolved organic matter (3), and were present in the *Ca.* N. defluvii enrichment (Table S2). Heterotrophic commensals may also feed on lysed *Nitrospira* biomass or may use soluble organic compounds released by the autotrophic nitrifiers (4, 5). Two of the cultured heterotrophic relatives, *Afipia birgiae* and *Terrimonas lutea* (Table S2), are known to reduce nitrate (6, 7). Nitrate reducers would benefit from the nitrate produced by *Nitrospira* from nitrite, especially if they grow in the possibly oxygen-depleted central regions of the large flocs formed by *Nitrospira* in this culture. In addition, nitrate reduction by such organisms could attenuate

end-product inhibition by nitrate of *Nitrospira*, which has been observed for *N. moscoviensis* (8) and also for *Ca.* N. defluvii whose microcolonies disintegrate after nitrate accumulation (1).

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