

**Table S1.** Kinetic constants of nitrite oxidation of NOB isolates and “*Ca. Nitrotoga*” enrichments.

Organism	$K_{m(\text{app})}$ ( $\mu\text{M NO}_2^-$ )	$V_{\text{max}}$ ( $\mu\text{mol NO}_2^- \text{ mg protein}^{-1} \text{ h}^{-1}$ )	$V_{\text{max}}$ ( $\text{fmol NO}_2^- \text{ cell}^{-1} \text{ h}^{-1}$ )	Reference
“ <i>Ca. Nitrotoga fabula</i> KNB” (isolate, WWTP)	89	28	ND <sup>b</sup>	This study
“ <i>Ca. Nitrotoga arctica</i> ” (enrichment, arctic soil)	58	26	ND	(1)
“ <i>Ca. Nitrotoga</i> sp. AM1” (enrichment, eelgrass sediment)	25	ND	6.1	(2)
<i>Nitrospira defluvii</i> (isolate, WWTP)	9	48	ND	(1)
<i>Nitrospira lenta</i> BS10 (isolate, WWTP)	27	20	ND	(1)
<i>Nitrospira</i> sp. ND1 (isolate, WWTP)	6	45	ND	(3)
<i>Nitrospira japonica</i> NJ1 (isolate, WWTP)	10	31	ND	(3)
<i>Nitrospira moscoviensis</i> (isolate, corroded iron pipe)	9	18	ND	(1)
<i>Nitrospira marina</i> Ecomares 2.1 (isolate, marine aquaculture biofilter)	54	21	ND	(4)
<i>Nitrospira inopinata</i> (isolate, hot groundwater) <sup>a</sup>	449	17	ND	(5)
<i>Nitrobacter hamburgensis</i> (isolate, soil)	540 - 1,370	ND	1 - 3.3	(6)
<i>Nitrobacter hamburgensis</i> (isolate, soil)	544	64	ND	(1)
<i>Nitrobacter winogradskyi</i> (isolate, soil)	36 - 260	ND	1.9 - 3.7	(6)
<i>Nitrobacter winogradskyi</i> (isolate, soil)	309	78	ND	(1)
<i>Nitrobacter vulgaris</i> (isolate, sewage)	49	164	ND	(1)
<i>Nitrobacter</i> sp. 311 (isolate, ocean surface water)	28	95	ND	(4)
<i>Nitrolancea hollandica</i> Lb (isolate, WWTP)	1,000	ND	ND	(7)
<i>Nitrococcus mobilis</i> 231 (isolate, ocean surface water)	120	141	ND	(4)
<i>Nitrospina watsonii</i> 347 (isolate, Black Sea)	19	37	ND	(4)

<sup>a</sup>*N. inopinata* is a complete ammonia oxidizer (comammox organism).

<sup>b</sup>ND=not determined.

## References for Table S1

1. Nowka B, Daims H, Spieck E. 2015. Comparison of oxidation kinetics of nitrite-oxidizing bacteria: nitrite availability as a key factor in niche differentiation. *Appl Environ Microbiol* 81:745-753.
2. Ishii K, Fujitani H, Soh K, Nakagawa T, Takahashi R, Tsuneda S. 2017. Enrichment and physiological characterization of a cold-adapted nitrite-oxidizing *Nitrotoga* sp from an eelgrass sediment. *Appl Environ Microbiol* 83:e00549-17.
3. Ushiki N, Jinno M, Fujitani H, Suenaga T, Terada A, Tsuneda S. 2017. Nitrite oxidation kinetics of two *Nitrospira* strains: The quest for competition and ecological niche differentiation. *J Biosci Bioeng* 123:581-589.
4. Jacob J, Nowka B, Merten V, Sanders T, Spieck E, Dahnke K. 2017. Oxidation kinetics and inverse isotope effect of marine nitrite-oxidizing isolates. *Aquat Microb Ecol* 80:289-300.
5. Kits KD, Sedlacek CJ, Lebedeva EV, Han P, Bulaev A, Pjevac P, Daebeler A, Romano S, Albertsen M, Stein LY, Daims H, Wagner M. 2017. Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. *Nature* 549:269-272.
6. Both GJ, Gerards S, Laanbroek HJ. 1992. Kinetics of nitrite oxidation in two *Nitrobacter* species grown in nitrite-limited chemostats. *Arch Microbiol* 157:436-441.
7. Sorokin D, Lücker S, Vejmekova D, Kleerbezem R, Muyzer G, Sinninghe-Damsté J, Wagner M, Le Paslier D, van Loosdrecht M, Daims H. 2012. Nitrification expanded: Discovery, physiology, and genomics of a nitrite-oxidizing bacterium from the phylum *Chloroflexi*. *ISME J* 6:2245-2256.