**Supporting information**

# Growth of nitrite-oxidizing *Nitrospira* and ammonia-oxidizing *Nitrosomonas* in marine recirculating trickling biofilter reactors

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This manuscript contains 2, 8, and 2 supplementary figures, table and text.

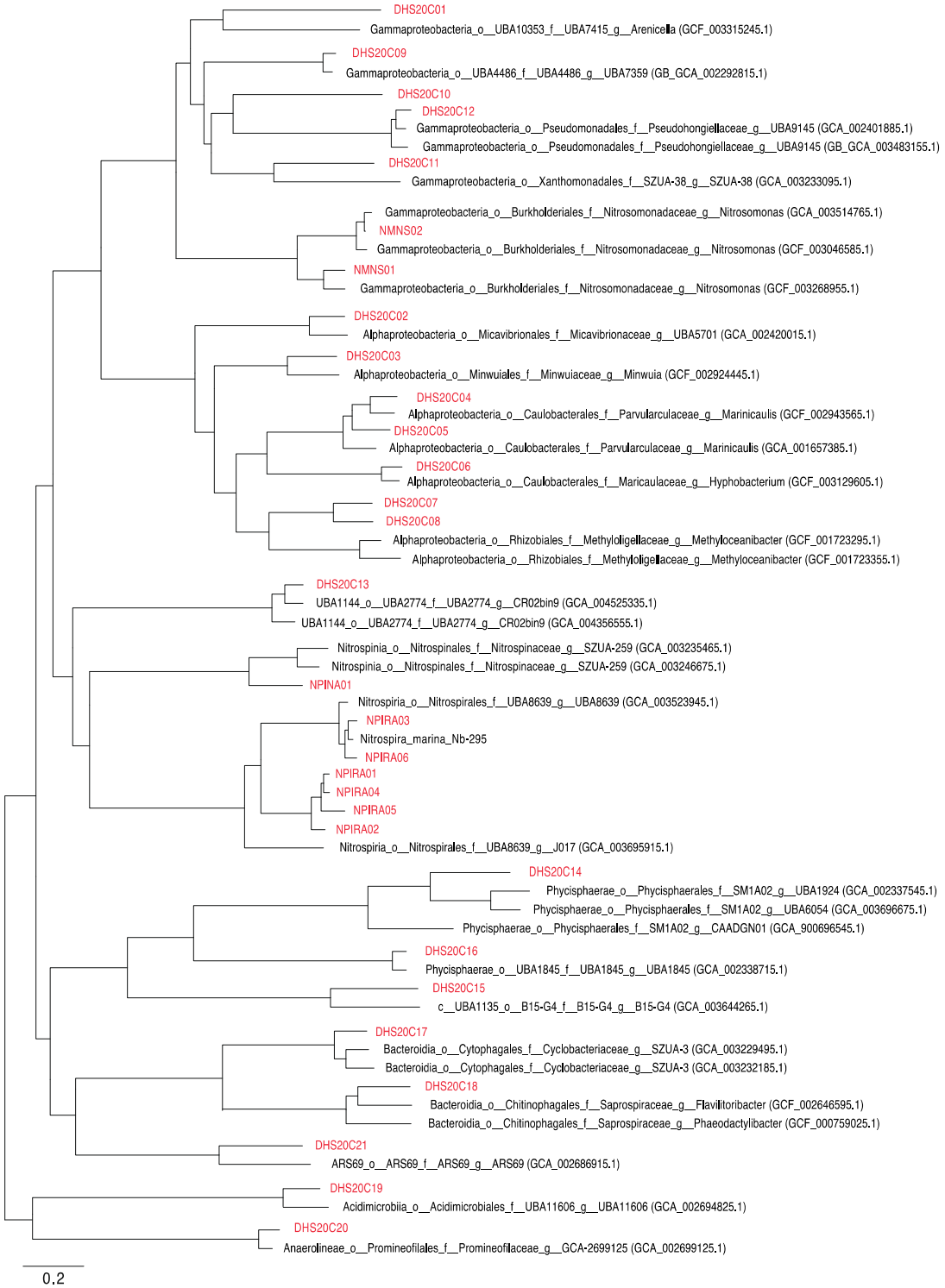


**Figure S1. Phylogeny of the 30 metagenomic bins obtained in the present study.** The phylogenetic position of the obtained metagenomic bins were shown with red color. The scale bar represents 20% sequence divergence.

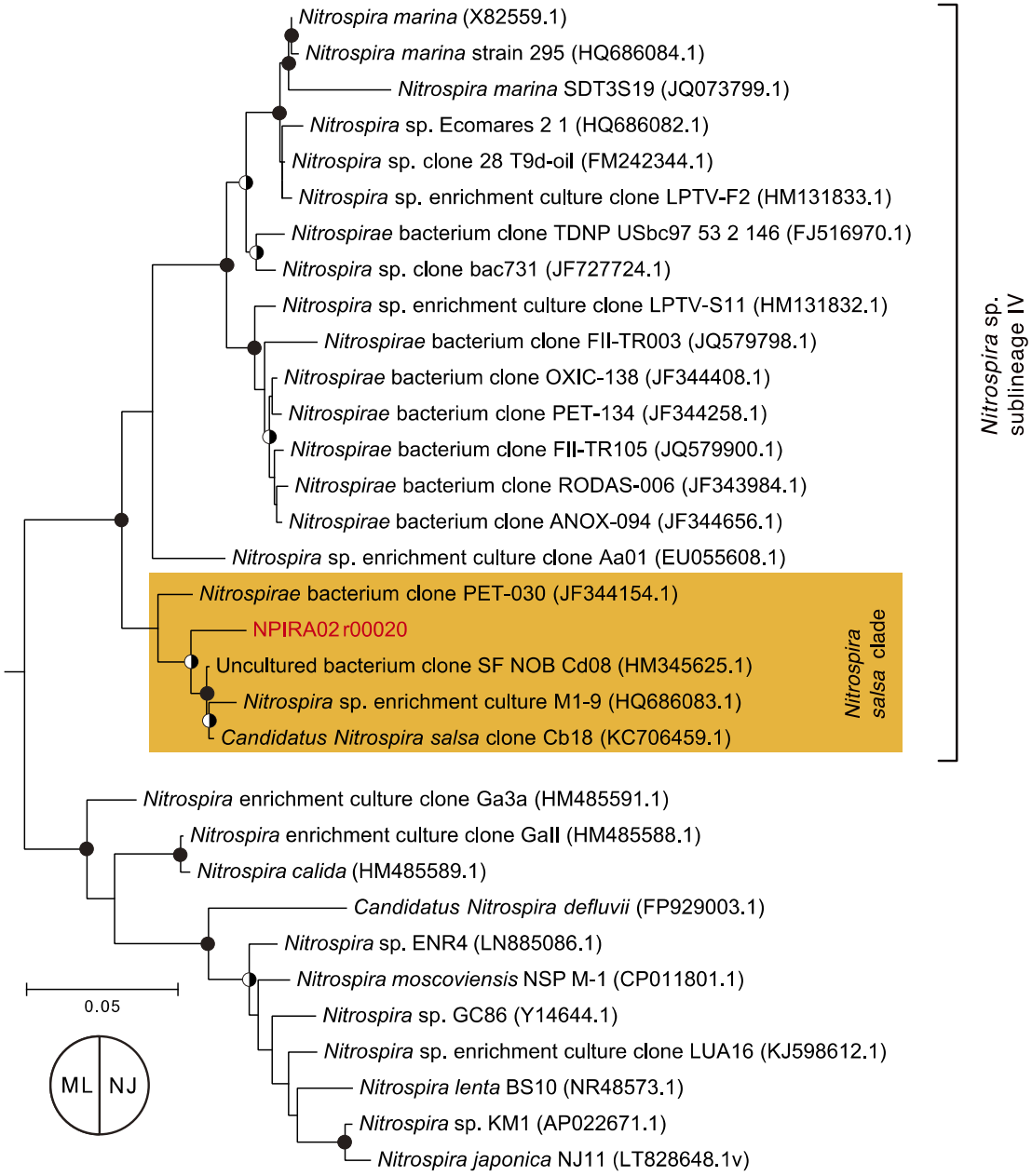


**Figure S2. 16S-rRNA gene based maximum likelihood (ML) tree showing phylogenetic position of the NPIRA02 bin.** Branching points that support probability >80% in the bootstrap analysis (based on 500 replicates), estimated using the ML method and the neighbor joining (NJ) method are shown as filled symbol. The 16S rRNA gene sequence located on the

NPIRA02 bin was affiliated into the clade containing *Nitrospira salsa* 16S rRNA gene sequence (KC706459.1). The scale bar represents 5% sequence divergence.

**Table S1 Average nucleotide identity (ANI) values of the obtained *Nitrospira* bins.** 1; NPIRA01, 2; *Nitrospirae* bacterium SPGG5 (GCA_001643555.1), 3; *Nitrospirae* bacterium (GCA_003228495.1), 4; MAG-cas150m-170 (PRJNA548657), 5; NPIRA02, 6; *Nitrospiraceae* bacterium (GCA_003523945.1), 7; NPIRA03, 8; *Nitrospirae* bacterium (GCA_003233615.1), 9; NPIRA06, 10; NPIRA04, 11; NPIRA05, 12; *Nitrospirae* bacterium (GCA_003696975.1), 13; *Nitrospira* sp. bin75 (GCA_002238765.1), 14; *Nitrospirales*_bacterium_isolate_MH-Pat-all_autometa_1-10 (WLXC01000001.1), 15; *Nitrospirae* bacterium (GCA_003235785.1), 16; *Nitrospira marina* Nb-295 (PRJNA262287), and 17; MAG-cas50m-175 (PRJNA548657).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 100% | 69.9% | 71.0% | 70.6% | 78.8% | 71.0% | 71.6% | 70.7% | 70.8% | 89.3% | 85.4% | 69.7% | 69.9% | 70.9% | 70.7% | 70.7% | 70.6% |
| 2 | | 100% | 71.7% | 77.4% | 70.2% | 70.1% | 70.3% | 71.4% | 70.2% | 70.1% | 70.2% | 70.1% | 72.0% | 70.5% | 70.4% | 70.2% | 77.4% |
| 3 | | | 100% | 70.9% | 71.4% | 73.4% | 73.1% | 79.9% | 73.1% | 71.7% | 70.6% | 69.9% | 70.7% | 71.2% | 78.2% | 73.1% | 71.1% |
| 4 | | | | 100% | 70.6% | 70.3% | 70.3% | 70.9% | 70.1% | 70.6% | 70.3% | 69.9% | 71.5% | 71.1% | 70.4% | 70.7% | 99.9% |
| 5 | | | | | 100% | 71.4% | 71.5% | 70.8% | 70.9% | 78.3% | 77.6% | 69.8% | 70.3% | 71.6% | 70.9% | 71.0% | 70.7% |
| 6 | | | | | | 100% | 83.3% | 72.7% | 84.2% | 72.5% | 75.3% | 70.0% | 70.5% | 70.6% | 74.0% | 84.1% | 70.7% |
| 7 | | | | | | | 100% | 72.5% | 85.1% | 71.8% | 74.7% | 69.5% | 70.1% | 70.6% | 73.5% | 88.2% | 70.7% |
| 8 | | | | | | | | 100% | 72.6% | 71.4% | 70.7% | 69.9% | 70.1% | 71.1% | 76.8% | 72.7% | 71.0% |
| 9 | | | | | | | | | 100% | 71.4% | 77.3% | 69.7% | 70.3% | 70.6% | 73.7% | 86.1% | 70.6% |
| 10 | | | | | | | | | | 100% | 84.6% | 69.9% | 70.4% | 72.1% | 71.1% | 71.6% | 71.1% |
| 11 | | | | | | | | | | | 100% | 69.5% | 70.0% | 70.6% | 71.1% | 74.9% | 70.5% |
| 12 | | | | | | | | | | | | 100% | 70.7% | 70.2% | 69.9% | 69.9% | 70.1% |
| 13 | | | | | | | | | | | | | 100% | 70.4% | 69.9% | 70.4% | 71.6% |
| 14 | | | | | | | | | | | | | | 100% | 70.6% | 70.9% | 71.2% |
| 15 | | | | | | | | | | | | | | | 100% | 73.8% | 70.8% |
| 16 | | | | | | | | | | | | | | | | 100% | 71.0% |
| 17 | | | | | | | | | | | | | | | | | 100% |

**Table S2 Gene sets involved in central metabolism found in *Nitrospira* (i.e., NPIRA) bins.**

(**Table S2** is available as the separated excel file.)

**Table S3 The blastP analysis of Hao protein found in the obtained *Nitrospira* bin.** The blastP analysis was performed using the NPIRA02_01710 protein as the query sequence and *nr* database. *: canonical nitrite-oxidizing *Nitrospira*, †: complete ammonia oxidation (commamox) *Nitrospira*.

| Organisms | Product | Accession No. | Identity | *e*-value |
|---|---|---|---|---|
| *Nitrospirales* bacterium | cytochrome $C_{552}$ | NKB80548.1 | 82.6 | 0 |
| *Nitrospiraceae* bacterium | TPA: cytochrome $C_{552}$ partial | HBP89707.1 | 85.7 | 0 |
| *Nitrospira moscoviensis** | putative octaheme cytochrome *c* | ALA57709.1 | 45.7 | 2.1E-139 |
| *Nitrospira japonica** | conserved protein of unknown function | SLM48584.1 | 42.0 | 9.0E-137 |
| *Nitrospira nitrosa*† | Hydroxylamine dehydrogenase Candidatus | CUS31385.1 | 25.6 | 1.4E-17 |
| *Nitrospira nitrificans*† | Hydroxylamine dehydrogenase Candidatus | CUS32689.1 | 24.9 | 1.6E-17 |
| *Nitrospira inopinata*† | Hydroxylamine dehydrogenase Candidatus | CUQ65042.1 | 23.8 | 2.0E-17 |

**Table S4 Gene sets involved in central metabolism found in *Nitrosomonas* (i.e., NMNS) bins**

(**Table S4** is available as the separated excel file.)

**Table S5 Operational conditions of the marine recirculating bioreactors where *Nitrospira* sublineage IV population outnumbered AOB/AOA population.**

| Reactor | Salinity | Temp. | pH | DO mg/L | $NH_4^{+*}$ µM | $NO_2^-$ µM | $NO_3^-$ µM | Reference |
|---|---|---|---|---|---|---|---|---|
| down-hanging sponge (DHS) reactor | 33‰ | 20ºC | 7.3 | 8 | 7.9 | 9.3 | 4,900 | This study |
| Moving-bed biofilter | 24–30‰ | 15–20ºC | 7 | 9–10 | 5–60 | 10–40 | 6,000 | [1] |
| Bio ball filter | 26.3 psu | *n.a.* | 8.8 | 6.5 | 18 | *n.d.* | 1,800 | [2] |
| Trickling filter | 20‰ | 19–30ºC | 7.7 | >6 | 35 | 15.7 | 2,000 | [3], [4] |

*n.a.*; not available, *n.d.*; not detected

* total ammonia concentration, TAN

**References**


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**Table S6. Oligonucleotide primers used for quantitative PCR (qPCR) assays.** Annealing temperature and extension time are 60ºC and 20 s, respectively.

| Target | Primers | Sequence (5'-3') | Reference |
|---|---|---|---|
| Prokaryotic 16S rRNA gene | 515F<br>806r | GTGCCAGCMGCCGCGGTAA<br>GGACTACHVGGGTWTCTAAT | Caporaso *et al.* (2011) |
| AOB *amoA* | amoA1F<br>amoA2Rv1 | GGGGTTTCTACTGGTGGT<br>CCCCTSKGSRAAKCCTTCTTC | Rotthauwe *et al.,* 1997<br>This study |
| *Nitrospira nxrB* | nxrB169f<br>nxrB638r | TACATGTGGTGGAACA<br>CGGTTCTGGTCRATCA | Pester *et al.,* 2014 |
| *Nitrospira* 16S rRNA gene | Nspra675F<br>Nspra746R | GCGGTGAAATGCGTAGAKATCG<br>TCAGCGTCAGRWAYGTTCCAGAG | Graham *et al.,* 2007 |

## References


Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., *et al*. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. U. S. A.* **108**: 4516–4522.

Graham, D.W., Knapp C.W., Van Vleck, E.S., Bloor, K., Lane, T.B., and Graham, C.E. (2007) Experimental demonstration of chaotic instability in biological nitrification. *ISME J* **1**: 385–393.

63 Pester, M., Maixner, F., Berry, D., Rattei, T., Koch, H., Lücker, S., *et al.* (2014) NxrB
64 encoding the beta subunit of nitrite oxidoreductase as functional and phylogenetic
65 marker for nitrite-oxidizing *Nitrospira*. *Environ Microbiol* **16**: 3055–3071.
66 Rotthauwe, J-H., Witzel, K-P., and Liesack, A.W. (1997) The ammonia monooxygenase
67 structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural
68 ammonia-oxidizing populations. *Appl Environ Microbiol* **63**: 4704–4712.

**Table S7 Accession numbers of the obtained bins.**

| Bin | Accession No. |
|---|---|
| NPIRA01 | BQIU01000001-BQIU01000203 |
| NPIRA02 | BQIV01000001-BQIV01000092 |
| NPIRA03 | BQIW01000001-BQIW01000218 |
| NPIRA04 | BQIX01000001-BQIX01000633 |
| NPIRA05 | BQIY01000001-BQIY01000595 |
| NPIRA06 | BQIZ01000001-BQIZ01000303 |
| NMNS01 | BQJA01000001-BQJA01000199 |
| NMNS02 | BQJB01000001-BQJB01000117 |
| NPINA01 | BQJC01000001-BQJC01000014 |
| DHS20C01 | BQJD01000001-BQJD01000119 |
| DHS20C02 | BQJE01000001-BQJE01000012 |
| DHS20C03 | BQJF01000001-BQJF01000234 |
| DHS20C04 | BQJG01000001-BQJG01000133 |
| DHS20C05 | BQJH01000001-BQJH01000386 |
| DHS20C06 | BQJI01000001-BQJI01000271 |
| DHS20C07 | BQJJ01000001-BQJJ01000027 |
| DHS20C08 | BQJK01000001-BQJK01000006 |
| DHS20C09 | BQJL01000001-BQJL01000420 |
| DHS20C10 | BQJM01000001-BQJM01000065 |
| DHS20C11 | BQJN01000001-BQJN01000210 |
| DHS20C12 | BQJO01000001-BQJO01000282 |
| DHS20C13 | BQJP01000001-BQJP01000173 |
| DHS20C14 | BQJQ01000001-BQJQ01000273 |
| DHS20C15 | BQJR01000001-BQJR01000011 |
| DHS20C16 | BQJS01000001-BQJS01000478 |
| DHS20C17 | BQJT01000001-BQJT01000462 |
| DHS20C18 | BQJU01000001-BQJU01000161 |
| DHS20C19 | BQJV01000001-BQJV01000589 |
| DHS20C20 | BQJW01000001-BQJW01001044 |

**Table S8 Calculation of $\Delta G_r$.** The calculations were performed under 1) the standard condition at pH7, 2) the condition found in $NH_4^+$-feeding DHS reactor, 3) the initial condition of batch incubation, and 4) the condition where the concentrations of reactant and products are one millimolar at pH 7. Additional explanations are available as **Supplementary text 1.**

(**Table S8** is available as the separated Excel file.)

**Supplementary text 1**

**Gibbs free-energy change ($\Delta G_r$) of aerobic ammonia and nitrite oxidation in the operated DHS reactors**

The DHS reactors fed with $NH_4^+$ or $NO_2^-$ have been operated at 20ºC for > 1 y continuously without the change of operational conditions and disturbance (See the section Experimental procedures for details of operational conditions). Typical concentrations of $NH_4^+$, $NO_2^-$, $NO_3^-$ and dissolved oxygen (DO) and pH values were as following [2]; 7.9 μM of $NH_4^+$, 9.3 μM of $NO_2^-$, 4.9 mM of $NO_3^-$, 250 μM of DO, and pH 7.3.

The $\Delta G_r$ of aerobic ammonia (Eq. 1) and nitrite oxidation reaction (Eq. 2) were calculated by using the standard equation ($\Delta G_r = \Delta G_r^{\circ} + RT\ lnk$), where, $\Delta G_r^{o}$; Standard free-energy changes, $R$; gas constant (0.008314 kJ $mol^{-1}$ $K^{-1}$), $T$; temperature (K), $k$; equilibrium constant calculated from Eq.1 and Eq.2. The $\Delta G_f^{o}$ were as following; –26.57 kJ $mol^{-1}$ for $NH_3$, 16.4 kJ $mol^{-1}$ for dissolved oxygen (*i.e.*, $O_{2\ aq}$), 0 kJ $mol^{-1}$ for $H^+$, –37.2 kJ $mol^{-1}$ for $NO_2^-$, –111.34 kJ $mol^{-1}$ for $NO_3^-$, –23.4 kJ $mol^{-1}$ for $NH_2OH$ [3], and –237.17 kJ $mol^{-1}$ for $H_2O$. $NH_3$ concentration was calculated from the $NH_4^+$ concentration by considering pH equilibrium ($pKa$ = 9.25).

$$1\ NH_3 + 1.5\ O_2 = 1\ NO_2^- + 1\ H_2O + 1\ H^+ \quad (1)$$

$$2\ NO_2^- + O_2 = 2\ NO_3^- \quad (2)$$

95 The $\Delta G_r$ were calculated under 1) the standard condition at pH7, 2) the condition
96 found in $NH_4^+$-feeding DHS reactor (described above), 3) the initial condition of the batch
97 incubations with the addition of 0.5 mM $NH_4^+$ or 0.5 mM $NO_2^-$ and $^{14}CO_2$, and 4) the
98 condition where the concentrations of reactant and products are one millimolar. As for the
99 condition 3), initial concentration of oxidized products ($NO_2^-$ or $NO_3^-$) was assumed to be
100 100-folds lower than the initial $NH_4^+$ or $NO_2^-$ concentration.
101 The $\Delta G_r$ of aerobic ammonia oxidation in the operated DHS reactor and at the batch
102 incubation were –271.7 and –283.3 kJ mol-$NH_3^{-1}$, respectively (**Table S8**). The $\Delta G_r$ of
103 aerobic nitrite oxidation in the operated DHS reactor and at the batch incubation were –57.0
104 and –83.5 kJ mol-$NO_2^{-1}$, respectively. Therefore, aerobic ammonia oxidation yields 4.8- and
105 3.4-folds higher free energies than aerobic nitrite oxidation in the DHS reactor and at the
106 batch incubation, respectively.
107 The oxidation reaction of ammonia to $NH_2OH$ is described as following [1], and the
108 $\Delta G_r'^o$ of the reaction is –170.5 kJ mol-$NH_3^{-1}$;
109 $1\ NH_3 + 1\ O_2 + 2\ H^+ + 2\ e^- = 1\ NH_2OH + 1\ H_2O$ (3)
110 Calculations are available as **Table S8**.
111 **Reference**
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**Supplementary text 2**

***2-1) Metabolic potentials of the NPIRA bins***

Metabolic potentials of the obtained NPIRA bins were examined based on presence/absence of functional genes required. In the following description, the NPIRA05 bin was not included because the completeness of the NPIRA05 bin (59%) was lower than others (81 to 97%) (**Table 1**). Commamox *Nitrospira* oxidizes ammonia to $NH_2OH$ by ammonia monooxygenase (Amo), and the formed $NH_2OH$ is subsequently oxidized to NO by hydroxylamine dehydrogenase (Hao) (Daims *et al.*, 2015; van Kessel *et al.*, 2015). The formed NO is finally oxidized to $NO_2^-$ by the as-yet unidentified enzymes and/or abiotic reaction (Carantoa and Lancaster, 2017). Orthologues of commamox *Nitrospira amoCAB* (threshold *e*-value of blastp search $10^{-15}$) were not found in the NPIRA bins and also in the other known *Nitrospira* sublineage IV genomes (**Fig. 3**). As for the *hao*, the NPIRA2 and NPIRA3 bins had the orthologues of *Nitrospira* octaheme cytochrome *c* (OCC) instead of commamox *Nitrospira hao*, which showed high similarities with the OCC found in the canonical $NO_2^-$-oxidizing *Nitrospira* genomes (*Nitrospira moscoviensis* and *Nitrospira japonica* genomes) (**Table S3**). In the *Nitrospira moscoviensis* genome, the OCC consisted of a gene cluster containing a Rieske/cytochrome *b* complex and other genes involved in nitrogen acquisition, whereas such a gene cluster was not located in the NPIRA bins. The

138 *Nitrospira* OCC is currently believed to contribute to assimilatory nitrite reduction and/or the
139 detoxification of hydroxylamine (Koch *et al.*, 2015; Ushiki *et al*., 2018).

140 As for aerobic nitrite oxidation, orthologue of *nxrABC* was found in the NPIRA4 bin,
141 whereas one or two genes among the *nxrABC* were often missing in other NPIRA bins (the
142 NPIRA01, NPIRA02, NPIRA03, and NPIRA06 bins). This is likely due to the incomplete
143 nature of the NPIRA bins (**Table 1**). As shown in **Table 1**, the obtained NPIRA bins showed
144 high strain heterogeneity, which became a bottleneck at the assembly and binning of contig
145 sequences, and the genes highly conserved among *Nitrospira* genomes (*i.e*., *nxr*) were often
146 not assigned into specific NPIRA bins. Indeed, the *nxrA* of the NPIRA03 bin, the *nxrB* of the
147 NPIRA01 and NPIRA04 bins, and the *nxrC* of the NPIRA03 and NPIRA04 bins were
148 annotated as the fragmented genes located at the end of the contigs.

149 As a component of respiratory chain, *nuo*-operons (*nuoNMLKJIHGFECDBA* and
150 *nuoNMMLKJIGDCBA*) encoding NADH:ubiquinone oxidoreductase (complex I) were found
151 from the NPIRA bins except for NPIRA06 bin in which the *nuoNMLKJIHGFECDBA* operon
152 was missing. The NuoNMMLKJIGDCBA is a 2M-type complex I (Chadwick *et al.*, 2018)
153 and likely involved in the reverse electron flow from quinol to ferredoxin which is required
154 for $CO_2$ fixation via rTCA cycle (Lawson *et al.*, 2021). Two copies of *qcr*-operon encoding
155 quinol-cytochrome *c* reductase (complex III) were found from the NPIRA01, NPIRA02,
156 NPIRA03, and NPIRA04 bins, and alternative complex III module (ACIII) previously found

157 in the *Nitrospira marina* genome was located in the NPIRA01, NPIRA03, and NPIRA06
158 bins. *Nitrospira* use a novel cytochrome *bd*-like heme-copper oxidase as a terminal oxidase
159 (Lücker *et al.*, 2010; Simon and Klotz, 2013). Multiple copies of the genes encoding putative
160 cytochrome *bd*-like oxidase were found in the NPIRA bins, and the heme *b* and copper-
161 binding sites conserved in canonical cytochrome *bd* oxidase were found in the
162 NPIRA01_31930, NPIRA02_18030, NPIRA03_03640, and NPIRA04_28020 proteins. The
163 recently determined *Nitrospira marina* genome showed that this *Nitrospira* has an additional
164 putative terminal oxidase with high $O_2$ affinities, *cbb*$_3$-type terminal oxidase (Bayer *et al.*,
165 2021). The gene encoding *cbb*$_3$-type terminal oxidase was found from the NPIRA01,
166 NPIRA03, and NPIRA04 bins. In *Nitrobacter*, soluble and membrane bound monoheme
167 cytochrome *c*$_{550}$ transfer the electrons released from Nxr to terminal oxidase (Nomoto *et al.*,
168 1993). The orthologue of the genes encoding *Nitrobacter winogradskyi* soluble and
169 membrane-bound *c*$_{550}$ (the Nwi_2582 and Nwi_0712 genes) (Starkenburg *et al.*, 2006) was
170 not found in the NPIRA bins. The NPIRA bins had the 57 to 72 genes encoding monoheme
171 cytochrome proteins as well as the *Nitrospira defluvii* genome (Lücker *et al.*, 2010), and those
172 cytochrome proteins supposedly participate the electron transferring from *Nitrospira* Nxr to
173 the above terminal oxidase(s). The $H^+$-translocating $F_1F_0$-ATPase was found in the NPIRA
174 bins, and putative $Na^+$-translocating ATPase previously found in the *Nitrospira marina*
175 genome was located in the NPIRA03, and NPIRA06 bins.

176 The NPIRA bins had the genes encoding 2-oxoglutarate:ferredoxin oxidoreductase
177 (OGOR), five- and/or four-subunit pyruvate:ferredoxin oxidoreductase (POR), and ATP-
178 citrate lyase (ACL) that are key enzymes in the rTCA cycle (Lücker *et al.*, 2010; Bayer *et al.*,
179 2021) (**Table S2**). Four-subunit POR is thought to be more oxygen sensitive but with higher
180 specific activity (Bayer *et al.*, 2021). The gene encoding four-subunit POR was only found
181 from the NPIRA03, NPIRA04 and NPIRA06 bins, whereas the genes encoding the five-
182 subunit POR were well conserved in the NPIRA bins.
183 *Nitrospira marina* (Bayer *et al*., 2021) and *Nitrospira moscoviensis* (Koch *et al*.,
184 2015) are capable of the growth utilizing formate, and the gene encoding formate
185 dehydrogenase (Fdh) was located in the NPIRA03 and NPIRA06 bins affiliated into the
186 *Nitrospira marina* clade (**Fig. 2**). On the other hand, the NPIRA bins affiliated into the
187 *Nitrospira salsa* clade were commonly lacking the gene encoding formate dehydrogenase
188 (**Table S2**), suggesting those *Nitrospira* are incapable of utilizing formate or just reflecting
189 incomplete nature of the NPIRA bins. It would be of interest to examine formate utilization of
190 *Nitrospira salsa* because the formate utilization would be a niche determinant for *Nitrospira*
191 *salsa* and *Nitrospira marina* who are capable of formate utilization.
192 As an oxidative stress defense system, the NPIRA bins carried the genes encoding
193 superoxide dismutase (SOD), catalase, glutathione peroxidase, and cytochrome *c* peroxidase.
194 Sulfite oxidation coupled with the reduction of cytochrome *c* by *Nitrospira* remains

195 controversial due to lack of experimental demonstration, whereas the *sorAB* encoding
196 sulfite:cytochrome *c* oxidoreductase was conserved among the NPIRA bins.

197 ***2-2) Metabolic potentials of the NMNS bins***

198 The *amoCAB* gene cluster was found in the NMNS01 bin (**Table S4**). The *amoCAB* gene
199 cluster (NMNS01_40000 to NMNS01_40020 genes) was located in the short contig (2.6 kb),
200 and no neighbor gene was located in the contig. In the NMNS02 bin, *amoCAB* gene cluster
201 was not found, and only *amoC* was found (NMNS02_32030 gene). Hydroxylamine oxidation
202 to NO and electron transferring to ubiquinone molecules are carried out by a hydroxylamine
203 ubiquinone redox module (Simon and Klotz, 2013) composed of hydroxylamine
204 dehydrogenase (Hao), cytochrome $c_{554}$, and cytochrome $c_{552}$. The *haoAB*, $c_{554}$, and $c_{552}$ genes
205 are composed of a gene cluster in *Nitrosomonas* sp. Nm143 and *Nitrosomonas aestuarii*
206 genomes, and the *haoAB*, $c_{554}$, and $c_{552}$ gene cluster was found in the NMNS02 bin but not in
207 the NMNS01 bin. Hydroxylamine oxidation reaction of *Nitrosomonas europaea* Hao
208 produces nitric oxide (NO), which is subsequently oxidized to $NO_2^-$. Nitrosocyanin (Arciero
209 *et al.*, 2002) is a candidate of NO oxidase (Lancaster *et al.*, 2018), and the gene encoding
210 nitrosocyanin was found from both the NMNS01 and NMNS02 bins. Apart from the above
211 genes, the NMNS01 and NMNS02 bins had the genes encoding copper-containing NO-
212 forming nitrite reductase (NirK), cytochrome-containing nitric oxide reductase (cNor),

cytochrome *c'* beta (Cyt b'), and cytochrome P460 ($Cyt_{P460}$), and those enzymes may contribute to nitrogen oxide transformation other than aerobic ammonia oxidation.

The electrons released from hydroxylamine (and NO) oxidation enter into a respiratory chain in the form of ubiquinol for the generation of proton motive force across a membrane and also of reducing power (*i.e.*, NAD(P)H) required for $CO_2$ fixation. The *pet*-operon (*petABC*) encoding cytochrome $bc_1$ complex (complex III) was found in both the NMNS01 and NMNS02 bins. The electrons released from ubiquinol oxidation are transferred from the complex III to terminal oxidase using a soluble cytochrome *c* ($c_{552}$) (Simon and Klotz, 2013), and the $c_{552}$ gene was located in both the NMNS01 and NMNS02 bins. The NMNS01 and NMNS02 bins had the genes encoding an $aa_3$-type terminal oxidase with low $O_2$ affinity but not a $cbb_3$-type terminal oxidase with high $O_2$ affinity and putative high-$O_2$-affinity terminal oxidase sNor (Sedlacek *et al.*, 2020). The NMNS01 and NMNS02 bins had the two copies of *nuo*-operon (*nuoABCDEFGHIJKLMN* and *nuoABB/C/DEFGHIJKLMN*) encoding NADH:quinone oxidoreductase (complex I) as well as the *Nitrosomonas aestuarii* genome.

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