The effect of bacterial community members on the proteome of the ammonia-oxidizing bacterium *Nitrosomonas* sp. Is79

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Abstract

Microorganisms in the environment do not exist as the often-studied pure cultures but as members of complex microbial communities. Characterizing the interactions within microbial communities is essential to understand their function in both natural and engineered environments. In this study we investigated how the presence of a nitrite-oxidizing bacterium (NOB) and heterotrophic bacteria affect the growth and proteome of the chemolithoautotrophic ammonia-oxidizing bacterium (AOB) *Nitrosomonas* sp. Is79. We investigated *Nitrosomonas* sp. Is79, in co-culture with *Nitrobacter winogradskyi*, in co-cultures with selected heterotrophic bacteria and as a member of the nitrifying enrichment culture G5-7. In batch culture, *N. winogradskyi* and heterotrophic bacteria had positive effects on the growth of *Nitrosomonas* sp. Is79. An isobaric tag for a relative quantification (iTRAQ) LC MS/MS proteomics approach was used to investigate the effect of *N. winogradskyi* and the co-cultured heterotrophic bacteria from G5-7 on the proteome of *Nitrosomonas* sp. Is79. In co-culture with *N. winogradskyi*, several *Nitrosomonas* sp. Is79 oxidative stress response proteins changed in abundance with periplasmic proteins increasing and cytoplasmic proteins decreasing in abundance. In the presence of heterotrophic bacteria, the abundance of proteins directly related to the ammonia oxidation pathway increased, while the abundance of proteins related to amino acid synthesis and metabolism decreased. In summary, the proteome of *Nitrosomonas* sp. Is79 was differentially influenced by the presence of either *N. winogradskyi* or heterotrophic bacteria. Together, *N. winogradskyi* and heterotrophic bacteria reduced the oxidative stress for *Nitrosomonas* sp. Is79, which resulted in a more efficient metabolism.

Importance

Aerobic ammonia oxidizing microorganisms play an important role in the global nitrogen cycle, converting ammonia to nitrite. In their natural environment they coexist and interact with nitrite oxidizers, which convert nitrite to nitrate, and with heterotrophic microorganisms. The presence of nitrite oxidizers and heterotrophic bacteria has a positive influence on the growth of the ammonia oxidizers. Here we present a study investigating the effect of nitrite oxidizers and heterotrophic bacteria on the proteome of a selected ammonia oxidizer in a defined culture to elucidate how these two groups improve the performance of the ammonia oxidizer. The results show that the presence of nitrite oxidizers and heterotrophic bacteria reduced stress for the ammonia oxidizers and resulted in a more efficient energy generation. This study contributes to our understanding of microbe-microbe interactions, in particular between ammonia oxidizers and their neighboring microbial community.

Introduction

Nitrification, the oxidation of ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-), is a microbially driven two-step process within the global nitrogen cycle (1, 2). The first step, the oxidation of NH_3 to NO_2^- is carried out aerobically by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (3-6). AOB are chemolithoautotrophic bacteria that generate energy through ammonia oxidation and fix carbon through the Calvin-Benson-Bassham cycle (3). The second step of nitrification is performed by nitrite-oxidizing bacteria (NOB), which generate energy from the oxidation of NO_2^- to NO_3^- (7-9). Recently it has been discovered that members

77 of the phylum *Nitrospira* are able to perform complete ammonia oxidation (Commamox),
78 oxidizing NH_3 via NO_2^- to NO_3^- (10, 11).

79 Betaproteobacterial AOB are comprised of the genera *Nitrosomonas* and *Nitrosospira*. Within
80 the genus *Nitrosomonas*, AOB form several clusters that can be distinguished by
81 ecophysiological characteristics such as affinity for NH_4^+ as well as tolerance to increasing salt
82 or NO_2^- concentrations (1, 6, 12). *Nitrosomonas* cluster 6a is comprised of AOB adapted to low
83 ammonium (NH_4^+) concentrations that can be found in a range of environments (13-17).
84 *Nitrosomonas* sp. Is79, a member of *Nitrosomonas* cluster 6a, was isolated from the freshwater
85 sediment nitrifying enrichment culture G5-7 (18-20).

86 In the environment, microorganisms do not exist in isolation, but live as members of complex
87 communities of interacting microbial species. Within microbial communities negative
88 interactions such as competition or predation can be detected along with mutually beneficial or
89 synergistic interactions such as cross-feeding, co-metabolism or cell-to-cell communication (21-
90 23). Photo- as well as chemolithoautotrophic microbes interact with heterotrophic microbes in
91 many different environments (24, 25). For example, macro- and microalgae live with specific
92 heterotrophic microbial sub-communities. The exchange of nutrients, enzymes and bioactive
93 compounds in these communities results in increased community productivity (24, 25). AOB
94 have been shown to interact positively with NOB and heterotrophic bacteria (26-30). The
95 positive effect of NOB on the growth of AOB is in large part due to the removal of NO_2^- by
96 NOB, as it inhibits AOB growth (8, 29, 31). In the transcriptome of *Nitrosomonas europaea* an
97 increase in transcripts abundance for electron transport proteins (NADH dehydrogenase
98 complex, ATP synthase complex and several cytochromes including cytochrome c-552) were
99 identified as possible positive effects of co-cultivation with the NOB *Nitrobacter winogradskyi*

100 (29). In addition, AOB provide organic carbon in the form of soluble microbial products to co-
101 cultured heterotrophic bacteria (26, 27, 32). The positive growth response of AOB to the
102 presence of heterotrophic bacteria could be due to a variety of compounds that may be secreted
103 by the heterotrophic bacteria such as organic compounds (33-36), acyl-homoserine lactones (37,
104 38) or siderophores (28). Studies of AOB-heterotroph interactions have mainly focused on the
105 members, structure and functionality of the heterotrophic bacterial communities (26, 27). It has
106 not been elucidated how the proteome of AOB respond to the presence of co-cultured NOB or
107 heterotrophic bacteria.
108 To our knowledge, only a few studies have been conducted focusing on the transcriptome or
109 proteome of AOB. These studies have mainly utilized *N. europaea* or *Nitrosomonas eutropha*,
110 two relatively fast growing AOB often found in nutrient-rich environments (29, 39-43). The
111 AOB proteome analyses have focused on the effect of variable environmental or physiologic
112 growth conditions such as starvation (42), heavy-metal contamination (41), biofilm formation
113 (43) and the effect of nitrogen dioxide (NO_2) or anoxia (40).
114 Here we present a study investigating the effects of *N. winogradskyi* as well as co-cultured
115 heterotrophic bacteria isolated from the enrichment culture G5-7 on the growth and physiology
116 of the AOB *Nitrosomonas* sp. Is79. We employed a combination of growth experiments and
117 isobaric tag for relative quantification (iTRAQ) LC MS/MS proteomics to determine how the
118 proteome of *Nitrosomonas* sp. Is79 is influenced by the presence of *N. winogradskyi* and
119 heterotrophic bacteria.
120

121 **Material and Methods**

122 **Cultures and media**

123 *Cultures*: We utilized the following cultures derived from the original AOB enrichment culture
124 G5-7 (18, 19): (1) AOB isolate *Nitrosomonas* sp. Is79 (20), (2) the AOB enrichment culture G5-
125 7 itself, which contains *Nitrosomonas* sp. Is79, *N. winogradskyi*, and co-cultured heterotrophic
126 bacteria, and (3) heterotrophic bacteria isolated from the enrichment culture G5-7. G5-7 was
127 originally enriched in a chemostat with an initial NH_4^+ concentration of 0.05mM using *Glyceria*
128 *maxima* rootzone sediment (18). The chemostat culture was transferred to batch culture using an
129 initial NH_4^+ concentration of 0.25mM first and later 5mM. The enrichment culture was
130 transferred every 4-6 weeks to fresh medium. *Nitrosomonas* sp. Is79 was isolated from the
131 enrichment culture G5-7 using serial dilution and the complete genome has been sequenced (20).
132 The NOB *N. winogradskyi* (ATCC 25391) was also used in the experiments.
133
134 *Medium to cultivate AOB and NOB:* The AOB mineral salts (AOB-MS) medium used for AOB
135 growth experiments contained variable amounts of $(NH_4)_2SO_4$, 10mM NaCl, 1mM KCl, 1mM
136 $CaCl_2 \bullet 2H_2O$, 0.2mM $MgSO_4 \bullet 7H_2O$ and 1mL $liter^{-1}$ trace elements solution (44, 45). Batch
137 cultures were buffered with HEPES, which was added in a 4-fold molar ratio to the NH_4^+
138 concentration and the pH was adjusted to 7.8 before autoclaving (44). After autoclaving, sterile
139 KH_2PO_4 was added to a final concentration of 0.4mM. *N. winogradskyi* was cultivated in NOB-
140 MS medium that contained 14.5mM $NaNO_2$, 8.6mM NaCl, 1.1mM KH_2PO_4, 0.2mM
141 $MgSO_4 \bullet 7H_2O$, 0.03mM $CaCO_3$ and 1mL $liter^{-1}$ trace elements solution with pH 7.8 (45, 46).
142
143 *Media to cultivate the heterotrophic bacteria:* LB medium (Sigma Aldrich, St Louis, MO, USA),
144 R2A medium (47) and spent AOB-MS medium were used to isolate and cultivate heterotrophic
145 bacteria. Spent AOB-MS medium consisted of 25% sterile filtered medium from an outgrown

146 *Nitrosomonas* sp. Is79 culture, 75% AOB-MS medium and either 0.01g/L casamino acids
147 (Difco, Becton, Dickinson and Company, Sparks, MD, USA) or 0.01g/L yeast extract (Difco). If
148 necessary, media was solidified with 1.5% Bacto agar (Difco).
149
150 *Isolation of heterotrophic bacteria from the enrichment culture G5-7*: Late log phase G5-7
151 culture was serially diluted with AOB-MS medium without NH_4^+ and inoculated onto agar plates
152 containing either LB, 0.1xLB, R2A or spent AOB-MS medium. Individual colonies were
153 selected based on colony morphology, sub-cultured and further maintained on R2A agar plates.
154 Colony PCR and 16S rDNA sequencing were used to identify the isolated colonies.
155
156 *Contamination tests:* Axenic cultures of *Nitrosomonas* sp. Is79 and *N. winogradskyi* were
157 inoculated into LB medium (0.1x) and incubated at 27°C for two weeks to test for purity.
158 Cultures exhibiting growth of heterotrophic bacteria were discarded.
159
160
161 **Kinetic studies**
162 *Apparent Michaelis constant* ($K_{m(app)}$)*:* Batch cultures of *Nitrosomonas* sp. Is79 grown as a pure
163 culture, in co-culture with *N. winogradskyi* and in the enrichment culture G5-7 were cultivated
164 with continuous stirring and bubbling at 25°C in the dark. Late logarithmic growth phase cells (1
165 L) were harvested by centrifugation (20 min, 30,000 x g, 4°C), washed, and re-suspended in
166 NH_4^+-free AOB-MS medium (10 mL). NH_4^+-free AOB-MS medium (3.6 mL) was mixed with
167 the concentrated cell suspension (0.4 mL). Concentrated NH_4^+ solution was added to obtain final
168 NH_4^+ concentrations between 20 and 1000µM. NO_2^-/NO_3^- production was followed by a NO_2^-

169 /NO_3^- biosensor and used to calculate NO_2^-/NO_3^- production rates (48). Michaelis-Menten
170 kinetics were applied to calculate the apparent half saturation constant of ammonia oxidation
171 ($K_{m(app)}$) (49).
172
173 **Growth experiments**
174 *Half saturation constant of growth (K_s):* The growth rate of *Nitrosomonas* sp. Is79 as a pure
175 culture, in co-culture with *N. winogradskyi* and in the enrichment culture G5-7 was determined
176 across a range of initial NH_4^+ concentrations (0.25 – 5mM). The cultures were inoculated with
177 10% (v/v) of a late logarithmic phase culture, incubated at 25°C in the dark, and shaken at 120
178 rpm. Samples were taken regularly to determine NH_4^+, NO_2^- and NO_3^- concentrations. Growth
179 rates were determined by calculating the slope of the log-transformed NO_2^- or NO_2^-/ NO_3^-
180 concentrations against time, assuming a correlation between NO_2^-/NO_3^- production and growth
181 (44, 50). Monod kinetics were applied to determine the half saturation constant of growth (K_s) of
182 *Nitrosomonas* sp. Is79 in the different cultures (49).
183
184 *Growth experiments of Nitrosomonas sp. Is79 with heterotrophic bacteria and N. winogradskyi:*
185 All growth experiments were conducted in AOB-MS medium with 1mM NH_4^+. Cultures were
186 inoculated with 1% (v/v) late logarithmic phase *Nitrosomonas* sp. Is79 and one or more of the
187 following: 0.2% (v/v) R2A media, 0.2% (v/v) culture of heterotrophic bacteria cultivated in R2A
188 or 1% (v/v) *N. winogradskyi* culture. In addition we used the enrichment culture G5-7.
189 Heterotrophic bacterial cultures were pre-cultured in liquid R2A for 2 days prior to inoculation.
190 All 10 heterotrophic isolates were combined into a mixed culture, which was added to
191 *Nitrosomonas* sp. Is79 to resemble as well as possible the original G5-7 heterotrophic

192 population. When the cultures had consumed the NH_4^+, they were transferred to fresh AOB-MS
193 medium (1% v/v). This transfer was repeated once more resulting in three successive growth
194 cycles. Samples were taken regularly during the third growth cycle, stored at -20°C and were
195 later used to determine NO_2^-/NO_3^- concentrations. Following the third growth cycle, co-cultures
196 were plated on R2A agar. Colony PCR and sequencing were used to confirm the identity of the
197 heterotrophic bacteria.
198
199 *Chemostat setup:* Chemostats with a working volume of 5 L were assembled and autoclaved
200 with 4.5 L of unbuffered AOB-MS medium with 5mM NH_4^+. Once cooled the temperature was
201 adjusted to 27°C with a temperature blanket, continuous stirring (300 rpm) and bubbling (500
202 mL min^{-1}) with 0.2µm sterile atmospheric air were started. Sterile KH_2PO_4 was added to a final
203 concentration of 0.4mM and the pH was adjusted to 7.8 with sterile 3% (w/v) $NaCO_3$.
204 Chemostats were incubated in the dark and inoculated with late logarithmic phase batch cultures
205 (0.5 L) of *Nitrosomonas* sp. Is79, *Nitrosomonas* sp. Is79 co-cultured with *N. winogradskyi* or the
206 enrichment culture G5-7. Samples (20 mL) were taken at regular intervals, filtered through a
207 polycarbonate filter (0.2µm, Whatman Nuclepore) and stored at -20°C for chemical analysis.
208 Contamination tests were conducted each time a sample was taken. When the initial NH_4^+ in the
209 chemostat was consumed, unbuffered AOB-MS medium containing 5mM NH_4^+ was added at a
210 dilution rate of approximately 1.25 L day^{-1} (growth rate = 0.25 day^{-1}). Chemostats were run for
211 12 days, which corresponds to 3 volume changes (= 3 generations). Total cell biomass was
212 harvested by centrifugation (20 min, 22,000 x g, 4°C). The cell pellets were washed in phosphate
213 buffer (20mM KH_2PO_4, pH=8), centrifuged (20 min, 28,000 x g, 4°C) and stored at -80°C for
214 proteomic analysis.

Chemical analysis: Colorimetric assays were used to determine NH_4^+, NO_2^- and NO_3^- concentrations in cell-free supernatants (44, 51-53).

Molecular analysis

Identification of the heterotrophic isolates: Cell material of the isolated heterotrophic bacteria was used as template for 16S rRNA PCR with the eubacterial primers 27F and 1492R (Table S1) and GoTaq Green Master Mix (Promega, Madison, WI) (54). PCR products were purified using the Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI) and sequenced with the two different sequencing primers 357F and 518R (Table S1) (55) using a BigDye Terminator cycle sequencing kit on an Applied Biosystems 3730 DNA analyzer (Life Technology Corporation, Carlsbad, CA, USA) at the Center for Bioinformatics and Functional Genomics (CBFG) at Miami University. The sequences were edited in 4Peaks (A. Griekspoor and T. Groothuis, The Netherlands Cancer Institute). ARB was used to align the sequences and to determine the closest cultured relatives (56).

Molecular analysis of the heterotrophic community in the enrichment culture G5-7 using next generation sequencing: The enrichment culture G5-7 (50 ml) was filtered onto a 0.2µm nucleopore filter (Whatman Nuclepore). DNA was extracted with the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH) according to the manufacturer's recommendations with the following modifications. Filters were homogenized using a bead beater (Biospec Products, Bartlett, OK) three times for 30s at 4800rpm. Samples were stored on ice for 10 min between bead-beating steps and centrifuged (15 min, 28,000 x g, 4°C).

238 Enrichment culture G5-7 DNA was amplified in triplicate with Illumina compatible indexed
239 primers designed to amplify the V4 region of the 16S rRNA gene (515F-806R) (57). Since the
240 sample was part of a larger sequencing project a 12 base barcode was incorporated in the reverse
241 primers (57, 58). The PCR products were quantified using a Sybr Green I dsDNA assay and the
242 concentration was measured on a NanoDropTM 3300 Fluorospectrometer (Thermo Fisher
243 Scientific, Wilmington, DE, USA). All PCR products were mixed in equal ratio and sequenced
244 in a MiSeq (Illumina, San Diego, CA, USA) at the CBFG at Miami University.
245 The sequences were processed by the software package MiSeq Reporter into two files: sequences
246 and barcodes. The sequences were split based on the barcodes, quality filtered with a minimum
247 quality score of 25, truncated to 150bp and exported as sequence files (fasta) using the software
248 package QIIME (59). The operational taxonomic units (OTUs) were picked against the
249 Greengenes database "gg_13_8_otus" with 97% similarity. Since there might be an increased
250 diversity in pyrosequencing libraries due to sequencing errors and to focus on the dominant
251 sequences all OTUs with an abundance below 10 were discarded. The taxonomic affiliation of
252 the sequences was determined and representative sequences of each OTU were aligned to the
253 ARB database to determine the similarity to the isolated heterotrophic bacteria. The analysis of
254 the community focused on the heterotrophic bacteria.
255
256 **Protein quantification and analysis**
257 *Total cell protein extraction:* Wet cell pellets (0.033-0.08 g) were suspended in phosphate buffer
258 (500 µL, 20mM KH_2PO_4, pH 8.0), combined with 1 g of 0.1 mm zirconia/silica beads and bead-
259 beated three times for 30 s at 4800 rpm. Samples were stored on ice for 10 min between each

round. Samples were centrifuged (20 min, 28,000 x g, 4°C) and the supernatant was stored at -80°C.

Solubilization and isolation of peptides: Cell lysates from *Nitrosomonas* sp. Is79, *Nitrosomonas* sp. Is79 co-cultured with *N. winogradskyi*, and from the enrichment culture G5-7 were solubilized in Laemmli gel buffer with heating to 110°C for 10 min. 50 ug of each sample (5 samples total) were loaded onto separated lanes of two 1D, 4-12% Bis-Tris acrylamide 1.0 mm mini gel and electrophoresed for 15 min. The gel region containing the proteins (~1.5 cm x 2.5 cm) was cut from the gel, subjected to in-gel trypsin digestion and the peptides were recovered as described previously (60).

Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) labeling: The *Nitrosomonas* sp. Is79 sample was used as control versus each of the treatments. Twenty five micrograms (25µg) of each sample for the two comparative groups were tagged using iTRAQ reagents following the vendor's (Sciex) instructions and as described previously (61). The 116 and 117 reporter tags were used for the isolated peptides of *Nitrosomonas* sp. Is79 co-cultured with *N. winogradskyi* samples (Run1) and the enrichment culture G5-7 (Run2) while the 115 reporter tag was used for the *Nitrosomonas* sp. Is79 control sample in both runs. In each case, labeling reagents sufficient to uniformly tag up to 100µg of digested protein was used thus ensuring complete labeling of all peptides when using only 25µg of protein in each labeling reaction. After labeling, samples were mixed in equal quantities for subsequent separation, identification and quantitative analysis.

Nano-electrospray liquid chromatography couple tandem mass spectrometry (nLC-ESI-MS/MS):

nLC-ESI-MS/MS analyses were performed on a TripleTOF 5600+ (Sciex, Toronto, ON, Canada) coupled to an Eksigent (Dublin, CA) nanoLC.ultra nanoflow system. Two (2) µg of total protein from each mixture was loaded and analyzed as described by Huang et al. (2015) (62) with the following modifications. The time for desalting and concentration was increased to 15 min and 2916 cycles of TOF-MS scans were performed in 80 min. The data were recorded using Analyst®-TF (v.1.6) software.

Data Analyses: Searches from the nLC-MS/MS were accomplished using ProteinPilot software (version 4.5, revision 1656) that utilizes a Paragon algorithm (ver 4.5.0.0, 1654) against a database consisting of *Nitrosomonas* sp. Is79 and *N. winogradskyi* protein sequences retrieved from the NCBI whole genome database supplemented with common contaminating proteins (human keratins, porcine trypsin, etc) for a total of 12,988 proteins searched. ProteinPilot search parameters included iTRAQ tagging of all primary amines as a variable modification, all biological modifications as variable modifications and carboxyamidomethyl cysteine used as a fixed modification as a result of the reduction and alkylation of any disulfide bond with iodoacetamide prior to trypsin digestion. "Gel-based ID" was selected as a special factor in the software to account for any modifications associated with proteins that have been exposed to polyacrylamide gels. The precursor mass tolerance was set to "quadrupole-tof instrument" in the ProteinPilot software. Data normalization across all iTRAQ tag channels was accomplished in the ProteinPilot software using the bias correction function. This function evaluated the relative ratio of the iTRAQ tags across all the peptides identified and normalizes the ratios such that the collective ratio of the used tags is 1:1:1 (i.e. 115:116:117 tag), prior to calculating relative differences among individual peptides/proteins. The output files from the ProteinPilot database

search contained a summary statistics page, the peptide identification tables, protein identification tables and relative quantitation data from the iTRAQ reporter ions from each peptide. Further statistical analysis was conducted using the ProteinPilot Descriptive Statistics Template (PDST, ver 3.005pB) (http://www.absciex.com/Documents/Downloads/Literature/ProteinPilot-Descriptive-Stats-Template-MassSpec-1910211-01.pdf). This analysis includes processing the relative quantitation data among the sample sets and provides statistical probabilities related to the confidence of the protein identification in relationship to an inverse (decoy) protein database, which consisted of hypothetical proteins based on the inverse amino acid primary sequence of all 12,988 proteins in the database. Only those peptides at 99% or greater confidences (<1% false discovery rate) are presented. P-values for the quantitative difference between proteins were calculated from the collective reporter ion ratios of all the peptides identified and quantified for a given protein with a minimum of 2 peptide measurements per protein. A significant change in relative protein abundance between the treatment and control was only presented if the abundance of at least two peptides of the same protein changed at least 1.5 times with $p<0.01$ (Table 3).

Statistical analysis: Statistical analysis (one-way ANOVA) was conducted with SSPS (version 19).

Nucleotide sequence accession numbers: The sequences of the heterotrophic community of the enrichment culture G5-7 determined by NGS were deposited in the NCBI SRA database under the BioProjectID PRJNA289396. The individual 16S rDNA sequences of the isolates were deposited in Genbank under the accession numbers: KT267186-KT267195.

329

330 **Results**

331 **Phylogenetic analysis of the heterotrophic bacteria in the enrichment culture G5-7**. Next
332 generation sequencing (NGS) of the heterotrophic sub-community of the enrichment culture G5-
333 7 revealed a total of 19 unique OTU's (Table S2). Based on reads, the community was comprised
334 of *Alpha-* (70.3%), *Gamma-* (24.4%), *Beta-* (2.5%) *proteobacteria* and *Bacteriodetes* (2.8%).
335 Ten unique heterotrophic *Proteobacteria* were isolated from the enrichment culture G5-7 (Table
336 1). All 10 isolates were detected in the NGS library and comprised 87.7% of the non-nitrifyer
337 sequences (Tables 1 and S2).

338

339 **Ammonia-oxidizing activity and growth characteristics of *Nitrosomonas* sp. Is79**.
340 *Nitrosomonas* sp. Is79 exhibited a longer lag phase and a slower rate of NO_2^- production in pure
341 culture compared to when grown in co-culture with *N. winogradskyi* or as part of the enrichment
342 culture G5-7 (Fig. 1). The growth rate of *Nitrosomonas* sp. Is79 significantly increased when co-
343 cultured with *N. winogradskyi* and increased even more when grown as part of the enrichment
344 culture G5-7 (Table 2). The half saturation constant of growth (K_s) decreased significantly when
345 *Nitrosomonas* sp. Is79 was co-cultured with *N. winogradskyi* or grown as part of the enrichment
346 culture G5-7 (Table 2). However, the apparent Michaelis constant ($K_{m(app)}$) of NH_4^+ consumption
347 remained constant in all three cultures (Table 2).

348

349 **The effect of *N. winogradskyi* and heterotrophic bacteria on the growth rate of**
350 ***Nitrosomonas* sp. Is79**. The growth rate of *Nitrosomonas* sp. Is79 was determined in artificially
351 designed co-cultures containing combinations of the isolated heterotrophic bacteria or *N.*

352 *winogradskyi* over the course of 3 growth cycles (*i.e.* 2 culture transfers). The growth rate of
353 *Nitrosomonas* sp. Is79 significantly increased in the presence of *N. winogradskyi* or
354 heterotrophic isolates (Fig. 2). The growth rate of *Nitrosomonas* sp. Is79 co-cultured with all 10
355 heterotrophic isolates plus *N. winogradskyi* was comparable to the growth rate of *Nitrosomonas*
356 sp. Is79 in the enrichment culture G5-7 (Fig. 2). In addition, the growth rate of *Nitrosomonas* sp.
357 Is79 as part of the enrichment culture G5-7 was significantly higher than the growth rate of
358 *Nitrosomonas* sp. Is79 co-cultured with *N. winogradskyi* or any of the heterotrophic isolates (Fig.
359 2). Growth of *Nitrosomonas* sp. Is79 in the carbon containing control medium with R2A was not
360 significantly different from its growth in AOB-MS medium (Fig. 2).
361
362 **Chemostat Growth.** *Nitrosomonas* sp. Is 79 was grown with NH_4^+ as the sole energy source and
363 growth-limiting substrate as (1) a pure culture, (2) in co-culture with *N. winogradskyi* and (3) as
364 part of the enrichment culture G5-7 in continuous cultures. Consumption of NH_4^+ started 2-4
365 days after inoculation, the pumps for media addition and waste removal were started 5-10 days
366 after inoculation and all chemostats were run as continuous cultures for 3 volume changes (12
367 days) after reaching steady state (Fig. S1). A temporary accumulation of NO_2^- was observed in
368 the enrichment culture G5-7 and in the co-culture with *N. winogradskyi* (Fig. S1). During steady
369 state growth the concentration of the growth-limiting substrate, NH_4^+, in the chemostats was
370 higher in the pure culture of *Nitrosomonas* sp. Is79 (115.94 µM) than in the co-culture of
371 *Nitrosomonas* sp. Is79 with *N. winogradskyi* (2.54 – 6.35 µM) or in the enrichment culture G5-7
372 (0.92 – 1.88 µM) (Table S3).
373
374 **Proteomics**. SDS-PAGE of whole cell extracts from the chemostat cultures showed different

375 protein-banding patterns (Fig. S2). However, direct inferences about the *Nitrosomonas* sp. Is79
376 proteome cannot be made because each culture contained a different bacterial community.
377 Isobaric tagging using iTRAQ reagents followed by LC MS/MS was used to determine the
378 proteome of *Nitrosomonas* sp. Is79 in the chemostat cultures. Relative protein abundance ratios
379 were based on the abundance of detected iTRAQ tags for each protein in each sample. The
380 proteomic analysis presented here focuses solely on *Nitrosomonas* sp. Is79 proteins that
381 significantly changed in abundance (>±1.5 times, ($p<0.01$)) when co-cultured with *N.*
382 *winogradskyi* or as part of the enrichment culture G5-7, relative to the pure culture. A full list of
383 detected proteins, their change in abundance and their calculated significance can be found in the
384 supplemental material (Tables S4 and S5). Due to technical problems only one *Nitrosomonas* sp.
385 Is79 chemostat culture was analyzed and used for comparison to the biological duplicates of
386 *Nitrosomonas* sp. Is79 co-cultured with *N. winogradskyi* (Set1) and G5-7 chemostat cultures
387 (Set2). Since accurate relative quantitation is predicated on uniform labeling of the primary
388 amines with the iTRAQ reagents, the overall iTRAQ label efficiency was captured for all
389 peptides detected in each comparative set. For Set1, 100% of 10,317 peptides terminating in
390 lysine and 95.3% of 16,523 peptides at the amino terminus were labeled with an iTRAQ tag. For
391 Set2, 100% of 9,684 peptides terminating in lysine and 95.6% of 15,318 peptides at the amino
392 terminus were labeled with an iTRAQ tag. Thus collectively, the labeling efficiency above 95%
393 allow for excellent relative quantitation measurement.
394
395 **The influence of *N. winogradskyi* on the proteome of *Nitrosomonas* sp. Is79**. The levels of 55
396 *Nitrosomonas* sp. Is79 proteins significantly changed in abundance by more than 1.5 times
397 ($p<0.01$) when co-cultured with *N. winogradskyi* (Tables 3 and S4). Of these 55 proteins 42 have

398 an assigned function, while 13 were hypothetical proteins (Tables 3 and S4). In total, 24 proteins
399 increased and 31 decreased in abundance. Many of these proteins had cellular functions related
400 to energy generation, oxidative stress response, nutrient-binding, macromolecule degradation or
401 carbon metabolism (Table 3). The ATP synthase F_1 complex proteins (subunits α, β, γ and δ)
402 significantly decreased in abundance, while the F_1 subunit ε and the F_o subunit b also decreased
403 but had p-values above 0.01 (Tables 3 and S4). Proteins related to the cellular oxidative stress
404 response changed in a site-specific manner. The cytoplasmic proteins superoxide dismutase,
405 rubrerythrin, thiol peroxidase and two chaperonins decreased in abundance while several
406 periplasmic di-heme cytochrome peroxidases increased in abundance (Table 3). The abundance
407 of proteins involved in macromolecule degradation also changed in a site-specific manner. The
408 cytoplasmic proteins aminopeptidase A and polyribonucleotide nucleotidyltransferase decreased
409 in abundance while a periplasmic lipase and ribonuclease increased in abundance (Table 3).
410 Nutrient-binding proteins for sulfate (periplasmic sulfate-binding protein), copper (CopC) and
411 iron (extracellular solute-binding protein family 1) all increased in abundance (Table 3). Proteins
412 related to central carbon metabolism such as phosphoglycerate kinase, ribulose-phosphate 3-
413 epiperase and the flavoprotein subunit of the succinate dehydrogenase complex decreased in
414 abundance in the presence of *N. winogradskyi* (Table 3). Interestingly the relative abundance of
415 the copper containing nitrite reductase NirK increased when *Nitrosomonas* sp. Is79 was co-
416 cultured with *N. winogradskyi* (Table 3).
417
418 **The influence of the G5-7 community on the proteome of *Nitrosomonas* sp. Is79**. Seventy
419 *Nitrosomonas* sp. Is79 proteins significantly changed in abundance more than 1.5 times ($p<0.01$)
420 in the enrichment culture G5-7 relative to the pure culture of *Nitrosomonas* sp. Is79 (Tables 3

421 and S5). The abundance of 23 proteins increased and 47 decreased, 55 of which had an annotated
422 function. Most changes in the *Nitrosomonas* sp. Is79 proteome observed when *Nitrosomonas* sp.
423 Is79 was co-cultured with *N. winogradskyi* were also observed in the enrichment culture G5-7.
424 Similar decreases of ATP synthase F_1 complex proteins and increases in nutrient-binding
425 proteins were detected. In addition, proteins involved in the cellular oxidative stress response and
426 macromolecule degradation decreased in abundance in the cytoplasm, but increased in the
427 periplasm. In contrast to being co-cultured with only *N. winogradskyi*, several *Nitrosomonas* sp.
428 Is79 proteins involved in ammonia oxidation increased in abundance in the enrichment culture
429 G5-7: ammonia monooxygenase subunits A and B, ORF5, hydroxylamine dehydrogenase,
430 cytochrome P460 and cytochrome c-554 (Table 3; Table S5).
431
432 **The influence of heterotrophic bacteria on the proteome of *Nitrosomonas* sp. Is79**. The
433 effect of the heterotrophic community in the enrichment culture G5-7 was determined indirectly.
434 If significant changes (>±1.5 times ($p<0.01$)) were observed in the presence of *N. winogradskyi*
435 and in the enrichment culture G5-7, these changes were assumed to be due to the presence of the
436 NOB *N. winogradskyi*. Significant changes (>±1.5 times ($p<0.01$)) only detected in the presence
437 of the heterotrophic bacteria (G5-7) were assigned to be the effect of the heterotrophic bacteria
438 (Table 3). One major effect of the presence of the heterotrophic community was the increase of
439 proteins related to the ammonia oxidation pathway. In the presence of the heterotrophic
440 community the abundance of proteins related to carbon metabolism decreased, *i.e.*
441 triosephosphate isomerase, enolase, phosphofructokinase, succinyl-CoA ligase subunit β,
442 transketolase and the ribulose bisphosphate carboxylase oxygenase (RuBisCO) large and small
443 chain subunits (Table 3). In addition several proteins involved in amino acid synthesis and

444 metabolism such as glycine dehydrogenase subunits 1 and 2, threonine synthase, glycine
445 hydroxymethyltransferase and ATP phosphoribosyltransferase were less abundant in the
446 presence of heterotrophic bacteria (Table 3).
447
448 **Discussion**
449 Individually as well as together, *N. winogradskyi* and the heterotrophic community of the
450 enrichment culture G5-7 positively affected the growth of *Nitrosomonas* sp. Is79 (Fig. 1, 2 and
451 S1; Tables 2 and S3). When co-cultured with *N. winogradskyi* or as part of the enrichment
452 culture G5-7, *Nitrosomonas* sp. Is79 grew at a lower steady state NH_4^+ concentration in
453 continuous culture and at a significantly higher growth rate in batch culture (Fig. 1, 2 and S1;
454 Tables 2 and S3). We used this collection of cultures to investigate the mechanisms underlying
455 the positive influence of NOB and heterotrophic bacteria on the growth of *Nitrosomonas* sp.
456 Is79. iTRAQ proteomics was used to determine the effect of *N. winogradskyi* and the
457 heterotrophic community of G5-7 on the proteome of *Nitrosomonas* sp. Is79 when grown in
458 continuous cultures (Table 3; Fig. 3).
459
460 **Effects of *N. winogradskyi***. In AOB-NOB co-cultures NO_2^- is removed by NOB, resulting in a
461 relief of NO_2^--induced toxic effects and stress on AOB (3, 29, 63). Direct comparison of the
462 proteome of *Nitrosomonas* sp. Is79 when grown in pure culture and when co-cultured with *N.*
463 *winogradskyi* was used to determine possible mechanisms contributing to the positive influence
464 of *N. winogradskyi* on the growth *Nitrosomonas* sp. Is79. Site-specific changes of several
465 proteins related to oxidative stress were observed in the presence of *N. winogradskyi* (Table 3).
466 The abundance of proteins responsible for the neutralization of reactive oxygen species

467 significantly decreased in the cytoplasm and increased in the periplasm, while intracellular
468 chaperonins also decreased (Table 3; Fig. 3). These proteome shifts indicate that the removal of
469 NO_2^- from the growth environment of *Nitrosomonas* sp. Is79 resulted in a reduction of
470 intracellular oxidative stress for *Nitrosomonas* sp. Is79. The reduced intracellular stress response
471 very likely freed-up energy in the form of proton motor force (PMF), ATP or NADPH, as
472 *Nitrosomona*s sp. Is79 would have less intracellular damage to repair. More energy could then be
473 used for biomass production and growth. Oxidative stress relief has been shown to be the
474 underlying mechanism for other microbial autotroph-heterotroph positive interactions, *i.e.*
475 between the photoautotroph *Prochlorococcus* and the marine heterotroph *Alteromonas* sp. (64).
476 *Nitrosomonas* sp. Is79, like *N. europaea*, utilizes PMF to produce both ATP and NADPH (65).
477 NADPH is generated through reverse electron flow, which makes the production of NADPH
478 energetically more expensive for AOB than the production of ATP (65). The abundance of
479 several protein components of the F1-ATP synthase were significantly lower when
480 *Nitrosomonas* sp. Is79 was co-cultured with *N. winogradskyi* (Table 3; Fig. 3) indicating that
481 *Nitrosomonas* sp. Is79 reduced ATP production in the presence of *N. winogradskyi*. This
482 suggests that *Nitrosomonas* sp. Is79 co-cultured with *N. winogradskyi* could divert a larger
483 portion of PMF produced by ammonia oxidation towards NADPH production. The increased
484 generation of reducing power at the expense of lower ATP production would favor biosynthetic
485 pathways such as carbon fixation. This contrasts a study focusing on the transcriptome of *N.*
486 *europaea* in the presence of *N. winogradskyi*, which found *N. europaea* ATP synthase subunit
487 transcripts up-regulated in co-cultures with *N. winogradskyi* (29). These differences could be due
488 to AOB exhibiting species-specific responses to NOB, different tolerances to oxidative and

489 nitrosative stress, or dynamic transcriptional, translational, and post-translational levels of
490 control (40).

491 The abundance of the nitrite reductase NirK increased when *Nitrosomonas* sp. Is79 was co-
492 cultured with *N. winogradskyi* (Table 3; Fig. 3). However, *nirK* in *N. europaea* has been shown
493 to be induced by NO_2^- accumulation and down-regulated in the presence of *N. winogradskyi* (29,
494 63). *Nitrosomonas* sp. Is79 lacks most of the nitrogen oxide metabolism genetic inventory
495 present in *N. europaea* including the rest of the *nir* operon, therefore it is possible that NirK
496 plays different roles in *Nitrosomonas* sp. Is79 and *N. europaea* (20, 66). During ammonia
497 oxidation NirK is hypothesized to act as an electron sink and prevent the buildup of the toxic
498 intermediate hydroxylamine in AOB (67, 68). NirK has been shown to be essential for efficient
499 ammonia oxidation in *N. europaea* with ammonia oxidation rates decreasing in *nirK*-mutants
500 compared to the wild type (68). Since the NO_2^- concentration in the chemostats decreased from
501 4653 µM in the absence of *N. winogradskyi* to below 15 µM when *N. winogradskyi* was present
502 (Table S3), our results suggest that NirK in *Nitrosomonas* sp. Is79 might be associated with more
503 efficient ammonia oxidation rather than acting in response to NO_2^-. Future work is required to
504 decipher the exact and possibly multiple roles of NirK in different AOB.

505 In summary, the shifts in the proteome of *Nitrosomonas* sp. Is79 in the presence of *N.*
506 *winogradskyi* were very likely caused by the reduction of the NO_2^- stress and resulted in a
507 decrease of energy spent in stress response, more energy efficient ammonia oxidation and a
508 subsequently higher growth rate (Fig. 2 and 3; Table 2). This conclusion is supported by the
509 lower K_s and the faster growth rate of *Nitrosomonas* sp. Is79 when co-cultured with *N.*
510 *winogradskyi* in batch culture (Fig. 2; Table 2).

511

512 **Effects of heterotrophic bacteria**. The heterotrophic community in the enrichment culture G5-7
513 consists of *Alpha*-, *Beta*-, *Gammaproteobacteria* and *Bacteriodetes* (Table S2). Members of
514 these phylogenetic groups have previously been found in AOB and cyanobacterial enrichment
515 cultures (26, 27, 69, 70). The addition of heterotrophic isolates as single isolates or together as a
516 community resulted in enhanced growth rates of *Nitrosomonas* sp. Is79 (Fig. 1 and 2). Positive
517 effects of heterotrophic bacteria on the growth and metabolism of autotrophic bacteria have been
518 previously observed in methanotrophic bacteria (71) and cyanobacteria (64, 69, 70, 72). The
519 underlying mechanisms of these interactions appear broad and are often not limited to distinct
520 phylogenetic groups. Heterotrophic bacteria are hypothesized to provide a common cellular
521 metabolite, eliminate toxic compounds or reduce oxidative stress on autotrophs (70-74).
522 When *Nitrosomonas* sp. Is79 was grown as part of the enrichment culture G5-7, several proteins
523 directly related to the ammonia oxidation pathway were significantly more abundant relative to
524 their presence in the *Nitrosomonas* sp. Is79 and *N. winogradskyi* co-culture (Table 3).
525 *Nitrosomonas* sp. Is79 relies on ammonia oxidation for energy generation and a higher
526 abundance of proteins involved in ammonia oxidation could result in more energy production
527 and faster growth (Fig. 3). The increased growth rate and ability of *Nitrosomonas* sp. Is79 in the
528 enrichment culture G5-7 to grow at a lower steady state NH_4^+ concentration (Fig. 2 and S1;
529 Table S3) support this conclusion.
530 In addition, 6 proteins related to amino acid synthesis and metabolism significantly decreased in
531 abundance when *Nitrosomonas* sp. Is79 was grown as part of the enrichment culture G5-7 (Table
532 3; Fig. 3). This suggests that when grown in co-culture with other heterotrophic bacteria,
533 *Nitrosomonas* sp. Is79 might down-regulate specific amino acid biosynthetic pathways, because
534 they receive exogenous amino acid(s) or amino acid metabolite(s) from the heterotrophic

535 community (Fig. 3). In support, certain amino acids have previously been shown to enhance the
536 growth rate of *N. europaea* (34, 35, 75) and *Nitrosomonas* sp. Is79 has two annotated amino acid
537 transporters in its genome (20). The positive interaction between *Nitrosomonas* sp. Is79 and the
538 diverse group of heterotrophic bacteria (Tables 1 and S2) could therefore involve the exchange
539 of amino acids and/or amino acid metabolites. Amino acid exchange has been shown to facilitate
540 bacterial interactions between *Porphyromonas gingivalis* and *Treponema denticola* (76).
541
542 **The effect of N. winogradskyi and heterotrophic bacteria combined**. The shifts in the
543 proteome of *Nitrosomonas* sp. Is79 grown as a part of G5-7 are a combination of the effects of
544 both *N. winogradskyi* and heterotrophic bacteria (Table 3; Fig. 3). The combination of positive
545 interactions in the enrichment culture G5-7 resulted in the lowest K_s (Table 2), the lowest steady
546 state NH_4^+ concentration in continuous culture (0.75-1.22μM) (Table S2) and the fastest growth
547 rate in batch culture (Fig. 2) of *Nitrosomonas* sp. Is79. The increased abundance of proteins
548 involved in energy generation and the decreased abundance of ATP synthase proteins provides
549 insight into how interactions with both *N. winogradskyi* and heterotrophic bacteria had a positive
550 effect on growth of *Nitrosomonas* sp. Is79 (Fig. 3). In summary, *Nitrosomonas* sp. Is79
551 positively interacted with both *N. winogradskyi* and heterotrophic bacteria individually and in
552 defined mixed cultures resulting in less stress and more energy for the AOB (Fig. 3). This type of
553 controlled community culture experiment begins to bridge the gap between pure culture
554 physiologic experiments and natural environmental microbial community studies.
555
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References:

1. **Kowalchuk G**, **Stephen J**. 2001. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. Annu Rev Microbiol **55**:485–529.
2. **Sayavedra-Soto LA, Arp DJ.** 2011. Ammonia-oxidizing bacteria: Their biochemistry and Molecular Biology, p.11-38. In Ward BB, Arp DJ, Klotz MG (ed) Nitrification. ASM Press, Washington DC, USA.
3. **Arp D**, **Sayavedra-Soto L**, **Hommes N**. 2002. Molecular biology and biochemistry of ammonia oxidation by *Nitrosomonas europaea*. Arch Microbiol **178**:250–255.
4. **Könneke M**, **Bernhard AE**, **la Torré de JR**, **Walker CB**, **Waterbury JB**, **Stahl DA**. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature **437**:543–546.
5. **Martens-Habbena W**, **Berube PM**, **Urakawa H**, **la Torré de JR**, **Stahl DA**. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature **461**:976–979.
6. **Koops H**, **Purkhold U**, **Pommerening-Röser A**, **Timmermann G**, **Wagner M**. 2006. The lithoautotrophic Ammonia-oxidizing bacteria. The Prokaryotes **5**:778–811.
7. **Bock E**. 1976. Growth of *Nitrobacter* in presence of organic matter. 2. Chemo-organotrophic growth of *Nitrobacter agilis*. Arch Microbiol **108**:305–312.
8. **Bock E**, **Wagner M**. 2006. Oxidation of inorganic nitrogen compounds as an energy source. The Prokaryotes **2**:457–495.
9. **Chain PSG**, **Xie G**, **Starkenburg SR**, **Scholz MB**, **Beckloff N**, **Lo C-C**, **Davenport KW**, **Reitenga KG**, **Daligault HE**, **Detter JC**, **Freitas TAK**, **Gleasner CD**, **Green LD**, **Han CS**, **McMurry KK**, **Meincke LJ**, **Shen X**, **Zeytun A**. 2011. Genomics for key players in the N cycle from guinea pigs to the next frontier. Meth Enzymol **496**:289–318.

10. **van Kessel MAHJ**, **Speth DR**, **Albertsen M**, **Nielsen PH**, **Op den Camp HJM**, **Kartal B**, **Jetten MSM**, **Lücker S**. 2015. Complete nitrification by a single microorganism. Nature **528**:555–559.
11. **Daims H**, **Lebedeva EV**, **Pjevac P**, **Han P**, **Herbold C**, **Albertsen M**, **Jehmlich N**, **Palatinszky M**, **Vierheilig J**, **Bulaev A**, **Kirkegaard RH**, **Bergen von M**, **Rattei T**, **Bendinger B**, **Nielsen PH**, **Wagner M**. 2015. Complete nitrification by *Nitrospira* bacteria. Nature **528**:504–509.
12. **Koops H**, **Pommerening-Röser A**. 2001. Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. FEMS Microbiol Ecol **37**:1–9.
13. **Bollmann A**, **Laanbroek H**. 2002. Influence of oxygen partial pressure and salinity on the community composition of ammonia-oxidizing bacteria in the Schelde estuary. Aquatic Microbial Ecology **28**:239–247.
14. **de Bie M**, **Speksnijder A**, **Kowalchuk G**, **Schuurman T**, **Zwart G**, **Stephen J**, **Diekmann O**, **Laanbroek HJ**. 2001. Shifts in the dominant populations of ammonia-oxidizing beta-subclass Proteobacteria along the eutrophic Schelde estuary. Aquat Microb Ecol **23**:225–236.
15. **Freitag T**, **Chang L**, **Prosser J**. 2006. Changes in the community structure and activity of betaproteobacterial ammonia-oxidizing sediment bacteria along a freshwater-marine gradient. Environ Microbiol **8**:684–696.
16. **Hayden CJ**, **Beman JM**. 2014. High abundances of potentially active ammonia-oxidizing bacteria and archaea in oligotrophic, high-altitude lakes of the Sierra Nevada, California, USA. Plos One **9**:e111560.
17. **Speksnijder A**, **Kowalchuk G**, **Roest K**, **Laanbroek HJ**. 1998. Recovery of a *Nitrosomonas*-like 16S rDNA sequence group from freshwater habitats. Syst Appl Microbiol **21**:321–330.
18. **Bollmann A**, **Laanbroek HJ**. 2001. Continuous culture enrichments of ammonia-oxidizing bacteria at low ammonium concentrations. FEMS Microbiol Ecol **37**:211–221.
19. **Bollmann A**, **Bar-Gilissen M**, **Laanbroek HJ**. 2002. Growth at low ammonium concentrations and starvation response as potential factors involved in niche differentiation among ammonia-oxidizing bacteria. Appl Environ Microbiol **68**:4751–4757.
20. **Bollmann A**, **Sedlacek CJ**, **Norton J**, **Laanbroek HJ**, **Suwa Y**, **Stein LY**, **Klotz MG**, **Arp D**, **Sayavedra-Soto L**, **Lu M**, **Bruce D**, **Detter C**, **Tapia R**, **Han J**, **Woyke T**, **Lucas SM**,

Pitluck S, **Pennacchio L**, **Nolan M**, **Land ML**, **Huntemann M**, **Deshpande S**, **Han C**, **Chen A**, **Kyrpides N**, **Mavromatis K**, **Markowitz V**, **Szeto E**, **Ivanova N**, **Mikhailova N**, **Pagani I**, **Pati A**, **Peters L**, **Ovchinnikova G**, **Goodwin LA**. 2013. Complete genome sequence of *Nitrosomonas* sp. Is79, an ammonia oxidizing bacterium adapted to low ammonium concentrations. Stand Genomic Sci **7**:469–482.

21. **Keller L**, **Surette MG**. 2006. Communication in bacteria: an ecological and evolutionary perspective. Nat Rev Microbiol **4**:249–258.
22. **Little AEF**, **Robinson CJ**, **Peterson SB**, **Raffa KE**, **Handelsman J**. 2008. Rules of Engagement: Interspecies Interactions that Regulate Microbial Communities. Annu Rev Microbiol **62**:375–401.
23. **Williams P**, **Winzer K**, **Chan WC**, **Cámara M**. 2007. Look who's talking: communication and quorum sensing in the bacterial world. Philos Trans R Soc Lond, B, Biol Sci **362**:1119–1134.
24. **Martin M**, **Portetelle D**, **Michel G**, **Vandenbol M**. 2014. Microorganisms living on macroalgae: diversity, interactions, and biotechnological applications. Appl Microbiol Biot **98**:2917–2935.
25. **Santos CA**, **Reis A**. 2014. Microalgal symbiosis in biotechnology. Appl Microbiol Biot **98**:5839–5846.
26. **Kindaichi T**, **Ito T**, **Okabe S**. 2004. Ecophysiological interaction between nitrifying bacteria and heterotrophic bacteria in autotrophic nitrifying biofilms as determined by microautoradiography-fluorescence in situ hybridization. Appl Environ Microbiol **70**:1641–1650.
27. **Okabe S**, **Kindaichi T**, **Ito T**. 2005. Fate of C-14-labeled microbial products derived from nitrifying bacteria in autotrophic nitrifying biofilms. Appl Environ Microbiol **71**:3987–3994.
28. **Keluskar R**, **Nerurkar A**, **Desai A**. 2013. Mutualism between autotrophic ammonia-oxidizing bacteria (AOB) and heterotrophs present in an ammonia-oxidizing colony. Arch Microbiol **195**:737–747.
29. **Pérez J**, **Buchanan A**, **Mellbye B**, **Ferrell R**, **Chang JH**, **Chaplen F**, **Bottomley PJ**, **Arp DJ**, **Sayavedra-Soto LA**. 2015. Interactions of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* grown in co-culture. Arch Microbiol **197**:79–89.
30. **Schramm A**, **Larsen LH**, **Revsbech NP**, **Ramsing NB**, **Amann R**, **Schleifer KH**. 1996.

649 Structure and function of a nitrifying biofilm as determined by in situ hybridization and the
650 use of microelectrodes. Appl Environ Microbiol **62**:4641–4647.
651 31. **Bae W**, **Baek S**, **Chung J**, **Lee Y**. 2001. Optimal operational factors for nitrite accumulation
652 in batch reactors. Biodegradation **12**:359–366.
653 32. **Noguera DR**, **Araki N**, **Rittmann BE**. 1994. Soluble microbial products (SMP) in anaerobic
654 chemostats. Biotechnol Bioeng **44**:1040–1047.
655 33. **Clark C**, **Schmidt E**. 1966. Effect of mixed culture on *Nitrosomonas europaea* simulated by
656 uptake and utilization of pyruvate. J Bacteriol **91**:367–373.
657 34. **Clark C**, **Schmidt E**. 1967. Growth response of *Nitrosomonas europaea* to amino acids. J
658 Bacteriol **93**:1302–1308.
659 35. **Clark C**, **Schmidt E**. 1967. Uptake and utilization of ammonia acids by resting cells of
660 *Nitrosomonas europaea*. J Bacteriol **93**:1309–1315.
661 36. **Pan P**, **Umbreit WW**. 1972. Growth of obligate autotrophic bacteria on glucose in a
662 continuous flow-through apparatus. J Bacteriol **109**:1149–1155.
663 37. **Batchelor S**, **Cooper M**, **Chhabra S**, **Glover L**, **Stewart G**, **Williams P**, **Prosser J**. 1997.
664 Cell density-regulated recovery of starved biofilm populations of ammonia-oxidizing
665 bacteria. Appl Environ Microbiol **63**:2281–2286.
666 38. **Burton E**, **Read H**, **Pellitteri M**, **Hickey W**. 2005. Identification of acyl-homoserine lactone
667 signal molecules produced by *Nitrosomonas europaea* strain Schmidt. Appl Environ
668 Microbiol **71**:4906–4909.
669 39. **Cho C**, **Yan T**, **Liu X**, **Wu L**, **Zhou J**, **Stein L**. 2006. Transcriptome of a *Nitrosomonas*
670 *europaea* mutant with a disrupted nitrite reductase gene (*nirK*). Appl Environ Microbiol
671 **72**:4450–4454.
672 40. **Kartal B**, **Wessels HJCT**, **van der Biezen E**, **Francoijs K-J**, **Jetten MSM**, **Klotz MG**,
673 **Stein LY**. 2012. Effects of nitrogen dioxide and anoxia on global gene and protein
674 expression in long-term continuous cultures of *Nitrosomonas eutropha* C91. Appl Environ
675 Microbiol **78**:4788–4794.
676 41. **Park S**, **Ely RL**. 2008. Candidate stress genes of *Nitrosomonas europaea* for monitoring
677 inhibition of nitrification by heavy metals. Appl Environ Microbiol **74**:5475–5482.
678 42. **Pellitteri-Hahn MC**, **Halligan BD**, **Scalf M**, **Smith L**, **Hickey WJ**. 2011. Quantitative
679 proteomic analysis of the chemolithoautotrophic bacterium *Nitrosomonas europaea*:

680 comparison of growing- and energy-starved cells. J Proteomics **74**:411–419.
681 43. **Schmidt I**, **Steenbakkers P**, **Op den Camp H**, **Schmidt K**, **Jetten M**. 2004. Physiologic
682 and proteomic evidence for a role of nitric oxide in biofilm formation by *Nitrosomonas*
683 *europaea* and other ammonia oxidizers. J Bacteriol **186**:2781–2788.
684 44. **Bollmann A**, **French E**, **Laanbroek HJ**. 2011. Isolation, cultivation, and characterization of
685 ammonia-oxidizing bacteria and archaea adapted to low ammonium concentrations. Meth
686 Enzymol **486**:55–88.
687 45. **Verhagen F**, **Laanbroek HJ**. 1991. Competition for ammonium between nitrifying and
688 heterotrophic bacteria in dual energy-limited chemostats. Appl Environ Microbiol **57**:3255–
689 3263.
690 46. **Bock E**, **Sundermeyer-Klinger H**, **Stackebrandt E**. 1983. New Facultative
691 Lithoautotrophic Nitrite-Oxidizing Bacteria. Arch Microbiol **136**:281–284.
692 47. **Reasoner D**, **Geldreich E**. 1985. A new medium for the enumeration and subculture of
693 bacteria from potable water. Appl Environ Microbiol **49**:1–7.
694 48. **Bollmann A**, **Schmidt I**, **Saunders A**, **Nicolaisen M**. 2005. Influence of starvation on
695 potential ammonia-oxidizing activity and *amoA* mRNA levels of *Nitrosospira briensis*. Appl
696 Environ Microbiol **71**:1276–1282.
697 49. **Kovarova-Kovar K**, **Egli T**. 1998. Growth kinetics of suspended microbial cells: From
698 single-substrate-controlled growth to mixed-substrate kinetics. Microbiol Mol Biol R
699 **62**:646–666.
700 50. **Belser L**, **Schmidt E**. 1980. Growth and oxidation-kinetics of 3 genera of ammonia-
701 oxidizing nitrifiers. Fems Microbiol Lett **7**:213–216.
702 51. **Kandeler E, Gerber H**. 1988. Short-Term Assay of Soil Urease Activity Using Colorimetric
703 Determination of Ammonium. Biol Fert Soils **6**:68–72.
704 52. **Keeney DR**, **Nelson DW**. 1982. Nitrogen - Inorganic forms, p 643-698. *In* Page AL (ed)
705 Methods of Soil Analysis – Part 2. American Society of Agronomy, Madison, WI, USA.
706 53. **Shand CA**, **Williams BL**, **Coutts G**. 2008. Determination of N-species in soil extracts using
707 microplate techniques. Talanta **74**:648–654.
708 54. **Weisburg WG**, **Barns SM**, **Pelletier DA**, **Lane DJ**. 1991. 16S ribosomal DNA
709 amplification for phylogenetic study. J Bacteriol **173**:697–703.
710 55. **Muyzer G**, **de Waal EC**, **Uitterlinden AG**. 1993. Profiling of complex microbial

populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol **59**:695–700.

56. **Ludwig W**, **Strunk O**, **Westram R**, **Richter L**, **Meier H**, **Yadhukumar**, **Buchner A**, **Lai T**, **Steppi S**, **Jobb G**, **Förster W**, **Brettske I**, **Gerber S**, **Ginhart AW**, **Gross O**, **Grumann S**, **Hermann S**, **Jost R**, **König A**, **Liss T**, **Lüssmann R**, **May M**, **Nonhoff B**, **Reichel B**, **Strehlow R**, **Stamatakis A**, **Stuckmann N**, **Vilbig A**, **Lenke M**, **Ludwig T**, **Bode A**, **Schleifer K-H**. 2004. ARB: a software environment for sequence data. Nucleic Acids Res **32**:1363–1371.
57. **Caporaso JG**, **Lauber CL**, **Walters WA**, **Berg-Lyons D**, **Lozupone CA**, **Turnbaugh PJ**, **Fierer N**, **Knight R**. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA **108 Suppl 1**:4516–4522.
58. **Caporaso JG**, **Lauber CL**, **Walters WA**, **Berg-Lyons D**, **Huntley J**, **Fierer N**, **Owens SM**, **Betley J**, **Fraser L**, **Bauer M**, **Gormley N**, **Gilbert JA**, **Smith G**, **Knight R**. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. Isme J **6**:1621-1624.
59. **Caporaso JG**, **Kuczynski J**, **Stombaugh J**, **Bittinger K**, **Bushman FD**, **Costello EK**, **Fierer N**, **Peña AG**, **Goodrich JK**, **Gordon JI**, **Huttley GA**, **Kelley ST**, **Knights D**, **Koenig JE**, **Ley RE**, **Lozupone CA**, **McDonald D**, **Muegge BD**, **Pirrung M**, **Reeder J**, **Sevinsky JR**, **Turnbaugh PJ**, **Walters WA**, **Widmann J**, **Yatsunenko T**, **Zaneveld J**, **Knight R**. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Methods **7**:335–336.
60. **Eismann T**, **Huber N**, **Shin T**, **Kuboki S**, **Galloway E**, **Wyder M**, **Edwards MJ**, **Greis KD**, **Shertzer HG**, **Fisher AB**, **Lentsch AB**. 2009. Peroxiredoxin-6 protects against mitochondrial dysfunction and liver injury during ischemia-reperfusion in mice. Am J Physiol Gastrointest Liver Physiol **296**:G266–74.
61. **Ross PL**, **Huang YN**, **Marchese JN**, **Williamson B**, **Parker K**, **Hattan S**, **Khainovski N**, **Pillai S**, **Dey S**, **Daniels S**, **Purkayastha S**, **Juhasz P**, **Martin S**, **Bartlet-Jones M**, **He F**, **Jacobson A**, **Pappin DJ**. 2004. Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. Mol Cell Proteomics **3**:1154–1169.
62. **Huang Y**, **Powers C**, **Madala SK**, **Greis KD**, **Haffey WD**, **Towbin JA**, **Purevjav E**, **Javadov S**, **Strauss AW**, **Khuchua Z**. 2015. Cardiac metabolic pathways affected in the

mouse model of barth syndrome. Plos One **10**:e0128561.

63. **Stein L**, **Arp D**. 1998. Loss of ammonia monooxygenase activity in *Nitrosomonas europaea* upon exposure to nitrite. Appl Environ Microbiol **64**:4098–4102.
64. **Morris JJ**, **Johnson ZI**, **Szul MJ**, **Keller M**, **Zinser ER**. 2011. Dependence of the cyanobacterium *Prochlorococcus* on hydrogen peroxide scavenging microbes for growth at the ocean's surface. Plos One **6**:e16805.
65. **Whittaker M**, **Bergmann D**, **Arciero D**, **Hooper A**. 2000. Electron transfer during the oxidation of ammonia by the chemolithotrophic bacterium *Nitrosomonas europaea*. BBA-Bioenergetics **1459**:346–355.
66. **Chain P**, **Lamerdin J**, **Larimer F**, **Regala W**, **Lao V**, **Land M**, **Hauser L**, **Hooper A**, **Klotz M**, **Norton J**, **Sayavedra-Soto L**, **Arciero D**, **Hommes N**, **Whittaker M**, **Arp D**. 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. J Bacteriol **185**:2759–2773.
67. **Cantera JJL**, **Stein LY**. 2007. Role of nitrite reductase in the ammonia-oxidizing pathway of *Nitrosomonas europaea*. Arch Microbiol **188**:349–354.
68. **Kozlowski JA**, **Price J**, **Stein LY**. 2014. Revision of N_2O-producing pathways in the ammonia-oxidizing bacterium *Nitrosomonas europaea* ATCC 19718. Appl Environ Microbiol **80**:4930–4935.
69. **Cole JK**, **Hutchison JR**, **Renslow RS**, **Kim Y-M**, **Chrisler WB**, **Engelmann HE**, **Dohnalkova AC**, **Hu D**, **Metz TO**, **Fredrickson JK**, **Lindemann SR**. 2014. Phototrophic biofilm assembly in microbial-mat-derived unicyanobacterial consortia: model systems for the study of autotroph-heterotroph interactions. Front Microbiol **5**:109.
70. **Sher D**, **Thompson JW**, **Kashtan N**, **Croal L**, **Chisholm SW**. 2011. Response of *Prochlorococcus* ecotypes to co-culture with diverse marine bacteria. Isme J **5**:1125–1132.
71. **Ho A**, **De Roy K**, **Thas O**, **De Neve J**, **Hoefman S**, **Vandamme P**, **Heylen K**, **Boon N**. 2014. The more, the merrier: heterotroph richness stimulates methanotrophic activity. Isme J **8**:1945–1948.
72. **Morris JJ**, **Kirkegaard R**, **Szul MJ**, **Johnson ZI**, **Zinser ER**. 2008. Facilitation of robust growth of *Prochlorococcus* colonies and dilute liquid cultures by "helper" heterotrophic bacteria. Appl Environ Microbiol **74**:4530–4534.
73. **Paerl H**, **Pinckney J**. 1996. A Mini-review of Microbial Consortia: Their Roles in Aquatic

773 Production and Biogeochemical Cycling. Microb Ecol **31**:225–247.
774 74. **Paerl HW**, **Pinckney JL**, **Steppe TF**. 2000. Cyanobacterial-bacterial mat consortia:
775 examining the functional unit of microbial survival and growth in extreme environments.
776 Environ Microbiol **2**:11–26.
777 75. **Frijlink M, AbeeT, Laanbroek HJ, DeBoer W, Konings W.** 1992 Secondary transport of
778 amino-acids in *Nitrosomonas europaea*. Arch Microbiol **157**:389-393.
779 76. **Tan KH**, **Seers CA**, **Dashper SG**, **Mitchell HL**, **Pyke JS**, **Meuric V**, **Slakeski N**, **Cleal**
780 **SM**, **Chambers JL**, **McConville MJ**, **Reynolds EC**. 2014. *Porphyromonas gingivalis* and
781 *Treponema denticola* exhibit metabolic symbioses. PLoS Pathog **10**:e1003955.
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784 **Figure legends:**

785 Figure 1: Nitrite or nitrite/nitrate production [µM] by *Nitrosomonas* sp. Is79 (Is79),
786 *Nitrosomonas* sp. Is79 co-cultured with *N. winogradskyi* (Is79+NOB), and by the
787 enrichment culture G5-7 (G5-7) (mean ± SD, n=3).

788

789 Figure 2: Growth [h-1] of *Nitrosomonas* sp. Is79 in co-culture with different combinations of *N.*
790 *winogradskyi* and heterotrophic bacteria isolated from the enrichment culture G5-7
791 during the third growth cycle (mean ± SD, n=3). *Nitrosomonas* sp. Is79 was combined
792 with all the heterotrophic isolates and *N. winogradskyi* individually in co-cultures and
793 with a mixed culture containing all heterotrophs with and without *N. winogradskyi*
794 (All isolates and *N. winogradskyi* + all isolates). *Nitrosomonas* sp. Is79 was also tested
795 as part of the enrichment culture G5-7. R2A medium was added in place of
796 heterotrophic bacteria as a control. Letters indicate significant differences between
797 Nitrosomonas sp. Is 79, the enrichment culture G5-7 and each particular treatment
798 determined by one-way ANOVA followed by Tukey test ($p<0.05$).

799

800 Figure 3: Model of the effect of heterotrophic bacteria (left) and *N. winogradskyi* (right) on the
801 proteome of *Nitrosomonas* sp. Is79. Select proteins that increased (white) or decreased
802 (grey) in abundance are shown. Proteins with dashed lines are shown for illustrative
803 purposes only and were not significantly differentially regulated. Abbreviations:
804 AMO, ammonia monooxygenase; HAO, hydroxylamine dehydrogenase; c554,
805 cytochrome c554; c_m552, cytochrome c_m552; Q/QH_2, ubiquinone-ubiquinol pool; bc_1,
806 cytochrome bc_1 (complex III); c552, cytochrome c552; HCO (c)aa_3, cytochrome

807 (c)aa_3; ATP Syn, ATP synthase; NADH DH, NADH dehydrogenase; PMF, proton-
808 motive force; P460, Cytochrome P460; GDH1, glycine dehydrogenase subunit 1;
809 GDH2, glycine dehydrogenase subunit2; ThrS, threonine synthase; GlyT, glycine
810 hydroxymethyltransferase; GroEL, 60 kDa chaperonin; GroES 10 kDa chaperonin;
811 TDST, 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase; AADH,
812 Glu/Leu/Phe/Val dehydrogenase; PRT, ATP phosphoribosyltransferase; GluA,
813 Glutamate-1-semialdehyde 2,1-aminomutase; SOD, superoxide dismutase; Rr,
814 rubrerythrin; NirK, nitrite reductase; TPX, thiol peroxidase; Ahp, alkyl hydroperoxide
815 reductase; CcP, di-heme cytochrome c peroxidase; Clp, Clp protease subunit; αGP, α-
816 glucan phosphorylase; PNPT, polyribonucleotide nucleotidyltransferase; CA, cytosol
817 aminopeptidase; CopC, copper resistance protein; ESBP, extracellular solute-binding
818 protein family 1; PSBP, sulfate ABC transporter periplasmic binding protein; HCBR,
819 hemolysin-type calcium-binding region; Lipase, lipase class 3; T2, ribonuclease T2.
820

Table 1: Phylogenetic affiliation and abundance of the cultivated bacterial isolates from the enrichment culture G5-7. Abundance [%] reflects the sequence abundance in the 16S rRNA NGS library.

G5-7	Closest cultured relative	Similarity [%]	Sequence length [bp]	Abundance in G5-7 [%] [1]
Alphaproteobacteria				
Is42	*Afipia broomeae* (U87759)	99.7	1099	44.8
Is3	*Ancylobacter aquaticus* (M62790)	99.0	625	8.7
Is2	*Afipia* genospecies 7 (U87773)	99.4	1089	4.4 (=Is28) [2]
Is28	*Mesorhizobium huakii* (D12797)	99.8	1372	4.4 (=Is2) [2]
Is32	*Caulobacter fusiformis* (AJ007803)	98.5	1082	1.3
Is17	*Rhizobium radiobacter* (M11223)	96.6	1071	1.1
Is23	*Sphingomonas adhaesiva* (D13722)	98.8	1100	0.5
Betaproteobacteria				
Is22	*Pandoraea pnomenusa* (AF139174)	99.8	1076	2.3
Gammaproteobacteria				
Is19	*Pseudomonas putida* (AF094743)	99.6	1137	23.9
Is39	*Lysobacter antibioticus* (AB019582)	95.7	1091	2.3

[1] Abundance [%] in the enrichment culture G5-7 is based on the total reads assigned to OTU's representing heterotrophic bacteria.

[2] Based on the short read length in NGS, Is2 and Is28 cluster together as OTU1 (Table S2).

828 Table 2: Half saturation constant of ammonia-oxidizing activity ($K_{m(app)}$) and growth (K_s) and
829 growth rate at 1mM ammonium of *Nitrosomonas* sp. Is79 in pure culture (Is79), co-
830 cultured with *N. winogradskyi* (Is79+NOB) and as part of the enrichment culture G5-7
831 (G5-7).
832

	Is79	Is79+NOB	G5-7
$K_{m(app)}$ [µM NH_3] [1]	3.32/3.56	3.10/3.56	3.37/3.53
K_s [µM NH_3] [2]	17.9 ± 3.1 [a]	9.4 ± 1.9 [b]	8.2 ± 2.9 [b]
Growth rate [h^{-1}] [2,3]	0.023 ± 0.003 [a]	0.037 ± 0.002 [b]	0.055 ± 0.002 [c]

833 [1] Measurement of biological replicates
834 [2] Mean ± SD, (n=3). Letters indicate significant differences determined by one-way ANOVA
835 followed by Tukey test ($p<0.05$).
836 [3] Growth rate calculated based on the nitrite/nitrate production over time (Fig. 1).
837

838 Table 3: Significant changes (±>1.5 fold; p<0.01) in relative abundance to *Nitrosomonas* sp. Is79 proteins when in co-culture with *N.*
839 *winogradskyi* (Is79+NOB) or as part of the enrichment culture G5-7 (G5-7) compared to when grown as a pure culture. All
840 co-cultures (*N. winogradskyi* – Rep 1 and 2 and G5-7 – Rep 1 and 2) were compared to one control of *Nitrosomonas* sp.
841 Is79. Positive (+) or negative (-) notations for the *N. winogradskyi* and G5-7 replicates (Rep1/Rep2) indicated increased or
842 decreased absolute abundance, respectively. Indirect measurements on the effect of the heterotrophic community are
843 indicated by +/- in the last column (see text for details). Relative protein abundance changes reported that are less than ±>1.5
844 fold are shown in parentheses.
845

			The influence of		
Accession #	Locus Tag: Nit79A3	Description	*N. winogradskyi* (Rep1/Rep2)	G5-7 (Rep1/Rep2)	Heterotrophic community of G5-7 (Rep1/Rep2)
Energy Generation and Nitrogen Metabolism					
gi|338804015	370	ATP synthase F_1 complex subunit delta	-1.50 / -1.73		-
gi|338804016	371	ATP synthase F_1 complex subunit alpha	-2.55 / -3.20	-5.35 / -5.01	-
gi|338804017	372	ATP synthase F_1 complex subunit gamma	-1.65 / -1.91	-2.05 / -2.17	-
gi|338804018	373	ATP synthase F_1 complex subunit beta	-2.47 / -2.84	-4.12 / -3.98	-
gi|338805865	2335	Nitrite reductase, copper-containing (NirK)	+2.40 / +3.54	+4.34 / +3.00	-
gi|338806391	2882	Hypothetical protein: ORF5	-1.91 / -2.04	-2.78 / -2.64	-
gi|338806393	2884	Ammonia monooxygenase, subunit B		+1.99 / +1.70	+
gi|338806394	2885	Ammonia monooxygenase, subunit A		+2.04 / +1.93	+
gi|338806449	2940	Cytochrome c-554		+7.13 / +3.58	+
gi|338806451	2942	Hydroxylamine dehydrogenase		+1.74 /(+1.28)	+
gi|338805203	1628	Cytochrome P460		+2.96 / +2.46	+
Oxidative Stress Response					
gi|338803966	317	Alkyl hydroperoxide reductase/ Thiol specific antioxidant	(-1.42) / -1.50		-
gi|338804387	754	Di-heme cytochrome c peroxidase	(+1.35)/ +1.89		-
gi|338804428	795	Di-heme cytochrome c peroxidase	+1.99 / +2.99	+3.28 / +2.32	-
gi|338805421	1860	Di-heme cytochrome c peroxidase	+1.55 / +1.87		-
gi|338805484	1928	ATP-dependent Clp protease proteolytic subunit		-1.73 / -1.69	+
gi|338805487	1931	60 kDa chaperonin (GroEL)	(-1.42) / -1.79	-1.82 / -2.15	-
gi|338805488	1932	10 kDa chaperonin (GroES)	-1.59 / -1.57	-1.85 / -2.11	-

gi|338804827	1225	Thiol peroxidase	-1.75 / -1.52	-2.19 / -1.95	-
gi|338805541	1988	Manganese/iron superoxide dismutase	-1.74 / -1.73	-2.29 / -2.73	-
gi|338805570	2021	Cysteine desulfurase, SufS subfamily	(-1.49) / -1.80	-2.21 / -2.18	-
gi|338806009	2479	Rubrerythrin	-1.80 / -1.51	-2.00 / -2.33	-
gi|338806646	3150	Ankyrin	+1.97 / +1.75		-
gi|338805517	1961	Sigma E regulatory protein, MucB/RseB		+1.72 / +1.85	+
Carbon Metabolism					
gi|338804098	457	Phosphoglycerate kinase	-1.64 /-1.78	-3.14 / -2.89	-
gi|338805124	1544	Glyoxalase/bleomycin resistance protein/dioxygenase	(-1.47) / -1.74		-
gi|338805269	1697	Ribulose-phosphate 3-epimerase	-1.52 /-1.77	-2.97 / -2.98	-
gi|338806036	2508	6-phosphogluconate dehydrogenase	-2.36 /-2.18		-
gi|338806059	2533	Succinate dehydrogenase, flavoprotein subunit	-1.59 /-1.48		-
gi|338803769	104	Succinyl-CoA ligase (ADP-forming) subunit beta		-1.66 / -1.67	+
gi|338803900	235	Phosphofructokinase		(-1.48) / -1.70	+
gi|338804096	455	Transketolase		-1.57 / -1.83	+
gi|338805641	2094	Enolase		(-1.39) / -1.65	+
gi|338806444	2935	Triosephosphate isomerase		-1.61 / -1.79	+
gi|338806728	3236	Ribulose bisphosphate carboxylase small chain		(-1.17) / -1.54	+
gi|338806727	3235	Ribulose bisphosphate carboxylase large chain		(-1.15) / -1.58	+
Nutrient Binding					
gi|338804077	436	Hemolysin-type calcium-binding region	+3.29 / +2.63	(+1.43)/ +1.91	-
gi|338804618	997	Sulfate ABC transporter, periplasmic sulfate-binding protein	+1.50 / +1.72	+2.09 / +1.78	-
gi|338804785	1174	Extracellular solute-binding protein family 1	+2.28 / +2.17	+2.22 / +1.85	-
gi|338806154	2629	OmpA/MotB domain protein	-1.54 / -1.51	-1.64 / -1.63	-
gi|338807030	3552	Copper resistance protein CopC	+2.10 / +1.91		-
Macromolecule Degradation					
gi|338805167	1591	Polyribonucleotide nucleotidyltransferase	(-1.32) / -1.64	-1.52 / -1.57	-
gi|338805200	1625	Ribonuclease T2	+5.15 / +3.58	+2.93 / +2.83	-
gi|338805583	2034	Cytosol aminopeptidase	-1.63 / -1.57		-
gi|338806285	2776	Lipase class 3	+2.18 / +2.13	+3.12 / +2.53	-
gi|338806518	3014	Alpha-glucan phosphorylase		-2.03 / -1.79	+
gi|338806969	3490	Peptidase M4 thermolysin		(-1.29) / -1.73	+

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Nitrogen Assimilation and Amino Acid Synthesis / Metabolism					
gi|338804310	673	Aminotransferase class V	-1.69 / -2.03	-2.57 / -2.67	-
gi|338805895	2365	Glutamine synthetase, type I	(-1.40) / -1.54	-2.09 / -2.21	-
gi|338806043	2515	Nitrogen regulatory protein P-II	-2.38 / -2.11	-1.52 / -1.52	-
gi|338806861	3379	Alanine dehydrogenase/PNT domain protein	(-1.35) / -1.51	-2.15 / -2.01	-
gi|338803826	161	Glycine hydroxymethyltransferase		-1.60 / -1.69	+
gi|338804474	841	Threonine synthase		-1.65 / (-1.48)	+
gi|338804826	1224	Glycine dehydrogenase (decarboxylating) subunit 1		-1.99 / -2.26	+
gi|338804828	1226	Glycine dehydrogenase (decarboxylating) subunit 2		-1.75 / -1.93	+
gi|338805276	1704	Glutamate-1-semialdehyde 2,1-aminomutase		-1.50 / -1.57	+
gi|338805542	1989	ATP phosphoribosyltransferase		-1.53 / (-1.42)	+
gi|338806404	2895	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase		(-1.10) / -1.55	+
gi|338806806	3317	Glu/Leu/Phe/Val dehydrogenase		-1.52 / -1.50	+
Replication, transcription, translation and nucleotide biosynthesis					
gi|338804081	440	Integration host factor subunit beta	-1.83 / (-1.25)		-
gi|338804209	572	Dihydroorotase, multifunctional complex type	-1.53 / (-1.23)		-
gi|338804216	579	PpiC-type peptidyl-prolyl cis-trans isomerase	(+1.45)/ +1.67	+1.72 / +1.94	-
gi|338804452	819	50S ribosomal protein L7/L12	-1.93 / -1.79	-2.32 / -1.86	-
gi|338804691	1073	Peptidyl-prolyl cis-trans isomerase cyclophilin type	+2.34 / +1.56		-
gi|338805468	1912	S-adenosylmethionine synthase	(-1.43) / -1.66	-2.19 / -2.42	-
gi|338803669	2	DNA polymerase III, beta subunit		-1.62 / -1.52	+
gi|338806332	2823	Translation elongation factor Tu		+2.14 / +1.63	+
gi|338806665	3172	Single-strand binding protein		-1.67 / -1.87	+
Miscellaneous					
gi|338806370	2861	Tetratricopeptide TPR_2 repeat-containing protein	+1.56 /(+1.45)		-
gi|338804646	1025	Peptidoglycan-binding domain-containing protein		+2.79 / +2.73	+
Bacteriophage related					
gi|338804625	1004	Phage tail sheath protein, putative		+2.07 / +1.56	+
Hypothetical and unknown function					
gi|338804162	524	Hypothetical protein	+3.26 / +2.88		-
gi|338804471	838	Hypothetical protein	+2.57 / +3.81	+3.59 / +2.96	-
gi|338804597	974	Hypothetical protein	-2.70 / -1.67	(-1.25) / -1.68	-

gi|338804892	1299	Hypothetical protein	-1.72 / -1.58	-2.25 / -1.91	-
gi|338805151	1573	Protein of unknown function	+1.87 / +2.02		-
gi|338805427	1867	Hypothetical protein	+1.75 / +2.01	+3.14 / +2.13	-
gi|338805428	1868	Hypothetical protein	+1.64 / +1.70	+2.17 / +1.54	-
gi|338805889	2359	Hypothetical protein	+1.85 / +2.49		-
gi|338805974	2444	Hypothetical protein	+2.21 / +1.84	+2.10 / +1.59	-
gi|338806490	2984	Hypothetical protein	+1.57 /(+1.44)		-
gi|338806603	3107	Hypothetical protein	+1.96 / +1.53		-
gi|338806604	3108	Hypothetical protein	+2.26 /(+1.38)		-
gi|338806650	3154	Hypothetical protein	-1.52 / -1.65	-2.00 / -2.54	-
gi|338804639	1018	Hypothetical protein		+1.72 / +1.61	+
gi|338805185	1609	Hypothetical protein		(-1.49) / -1.89	+
gi|338805805	2265	Hypothetical protein		+1.79 /(+1.41)	+
gi|338806101	2576	Hypothetical protein		(-1.47) / -1.77	+
gi|338806350	2841	Hypothetical protein		-1.69 / -1.79	+
gi|338806966	3487	Hypothetical protein		-2.31 / -2.69	+

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