

1 **Enrichment and Physiological Characterization of a Cold-Adapted Nitrite Oxidizer**

2 ***Nitrotoga* sp. from Eelgrass Sediments**

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11 Running title: Enrichment and physiological analysis of *Nitrotoga*

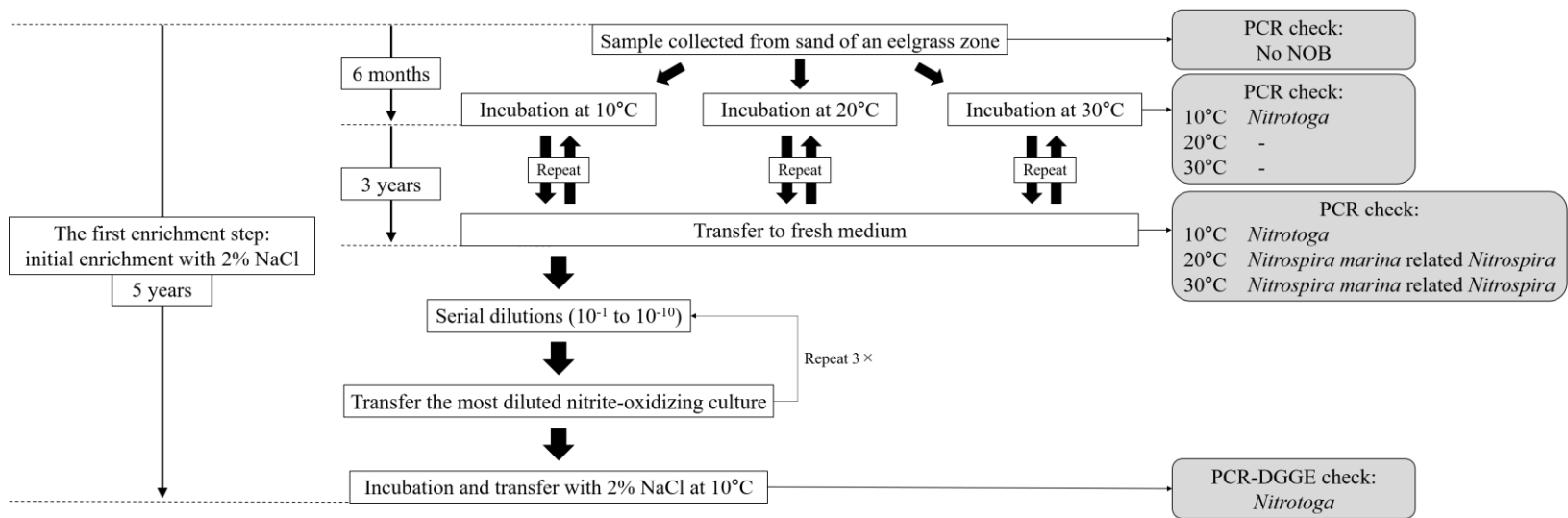
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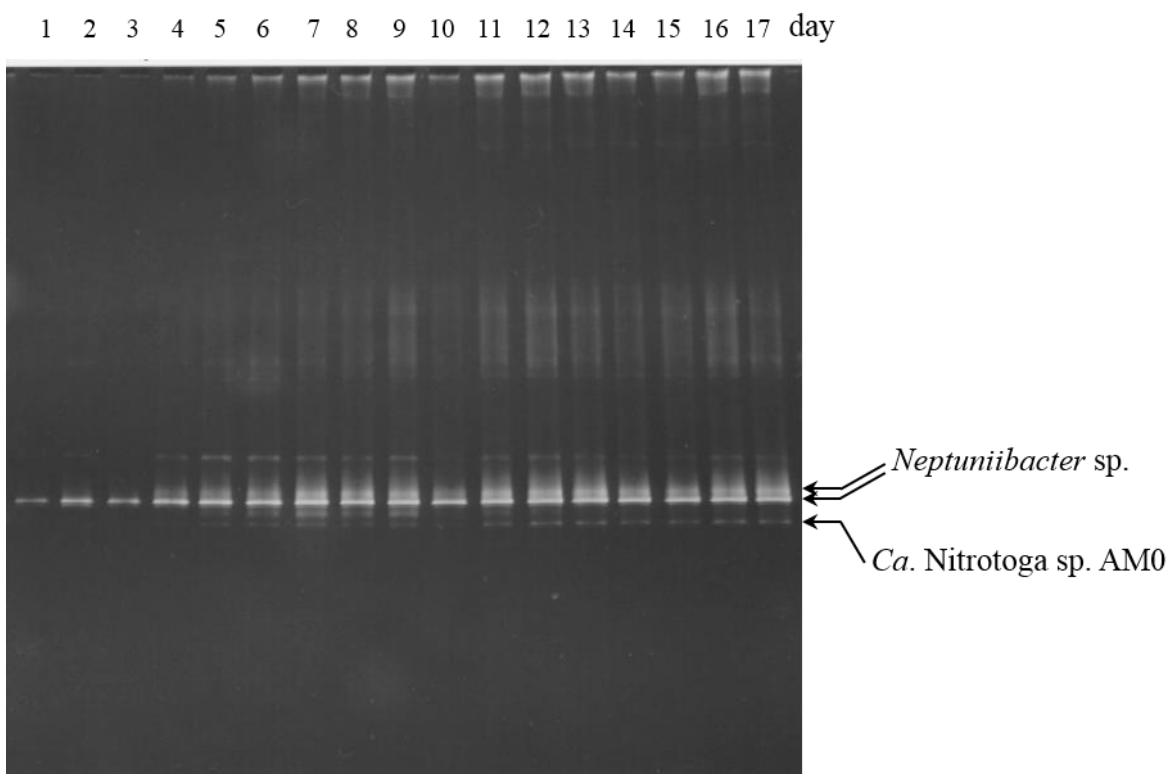
14 **Supplemental data**

15 TABLE S1 Identified clones in the *Nitrotoga*-enriched culture at 16°C. Operational taxonomic units (OTUs) were compared
 16 to the sequences of the most closely related clones or isolates.

17	Clone library	<i>n</i> clone	BLAST hit organism	Accession	Similarity (%)	Silva taxonomy
	OTU1 (LC190436)	8	“ <i>Candidatus Nitrotoga arctica</i> ” clone 6680	DQ839562	99.6	<i>Candidatus Nitrotoga</i>
	OTU2 (LC190437)	4	Bacterium IRO1	AY928215	98.2	<i>Pseudorhodiferax</i>
	OTU3 (LC190438)	2	<i>Acidobacteria bacterium</i> KF4-15/2	JF707406	97.9	<i>Blastocatellaceae</i>
	OTU4 (LC190439)	2	<i>Nevskia ramosa</i> strain OL1	AJ001011	99.7	<i>Nevskia</i>
	OTU5 (LC190440)	2	<i>Tuber borchii</i> symbiont b-17BO	AF070444	93.4	<i>Sphingobacteriales</i>
	OTU6 (LC190441)	1	<i>Tuber borchii</i> symbiont b-17BO	AF070444	91.0	<i>Sphingobacteriales</i>
	OTU7 (LC190442)	1	Bacterium TG152	AB308362	98.8	<i>β-Proteobacteria</i>
	OTU8 (LC190443)	1	<i>Thauera</i> sp. MDS5B	JX420814	93.6	<i>Nitrosomonadaceae</i>
	OTU9 (LC190444)	1	<i>Flavobacterium</i> sp. strain R-12	KU379662	94.3	<i>Flavobacterium</i>
	OTU10 (LC190445)	1	Alpha proteobacterium P-20	AM411930	91.2	<i>Rickettsiales</i>
	OTU11 (LC190446)	1	marine proteobacterium MS-8	AM423076	99.7	<i>Pseudomonas</i>
	OTU12 (LC190447)	1	Bacterium Ellin6502	HM748650	91.5	<i>Haliangium</i>
	OTU13 (LC190448)	1	<i>Acidobacteria bacterium</i> IGE-010	GU187031	92.2	<i>Acidobacteria</i>
	OTU14 (LC190449)	1	<i>Marinilabiliaceae</i> bacterium Q15	KR809872	88.1	<i>Sphingobacteriales</i>
	OTU15 (LC190450)	1	<i>Burkholderiales</i> bacterium RCPCd10	DQ922760	99.7	<i>Comamonadaceae</i>
	OTU16 (LC190451)	1	<i>Sediminibacterium goheungense</i> strain HME7863	JN674641	99.3	<i>Sediminibacterium</i>
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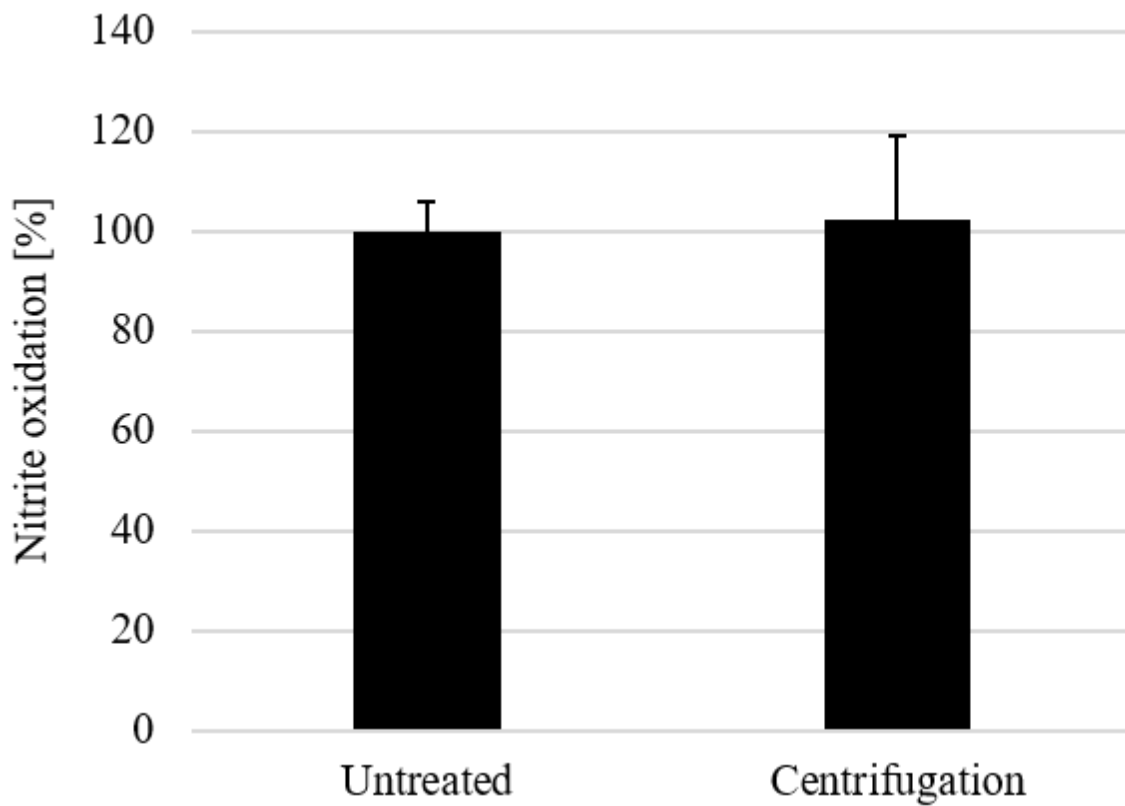


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 19 FIG S1 Flow chart of the detailed procedure to enrich *Nitrotoga*-like bacteria in the first enrichment step. The artificial
 20 seawater medium contained 0.5 mM NaNO₂. Incubation was performed at 10, 20 and 30°C in the dark without agitation. The
 21 16S rDNA of *Nitrotoga* amplified by NTG200F/840R (1) in the early-stage culture of the first enrichment step was directly
 22 sequenced. The cultures were transferred (10% inoculum) to fresh medium when the absence of nitrite was confirmed. The
 23 16S rDNA of *Nitrospira marina* related *Nitrospira* was conformed using Ntspmar62F (2) and Ntspa662R (3) in the cultures
 24 incubated at 20 and 30°C. As for *Nitrospina*, NitSSU_130F and 282R (4) did not amplify the target 16S rDNA gene regardless
 25 of incubation temperature. Subsequent serial dilutions (10⁻¹ to 10⁻¹⁰) were performed in test tubes. The incubated medium in
 26 which nitrite consumption was observed at the highest dilution level was serially diluted until the 10⁻¹⁰ dilution level and then
 27 incubated. After the end point dilution step was repeated three times, nitrite-oxidizing cultures were inoculated in a 100 mL
 28 flask including 30 mL of the modified ASW medium with 2% NaCl and then incubated at 10°C in the dark without agitation.



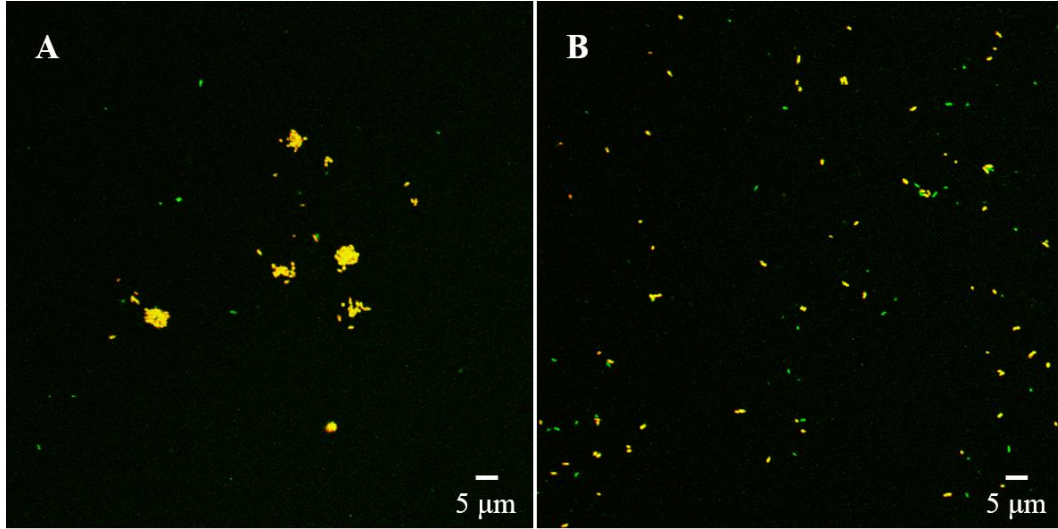
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30 FIG S2 Denaturing gradient gel electrophoresis of bacterial communities in the first
31 enrichment culture incubated at 10°C for 17 days after serial dilution step.

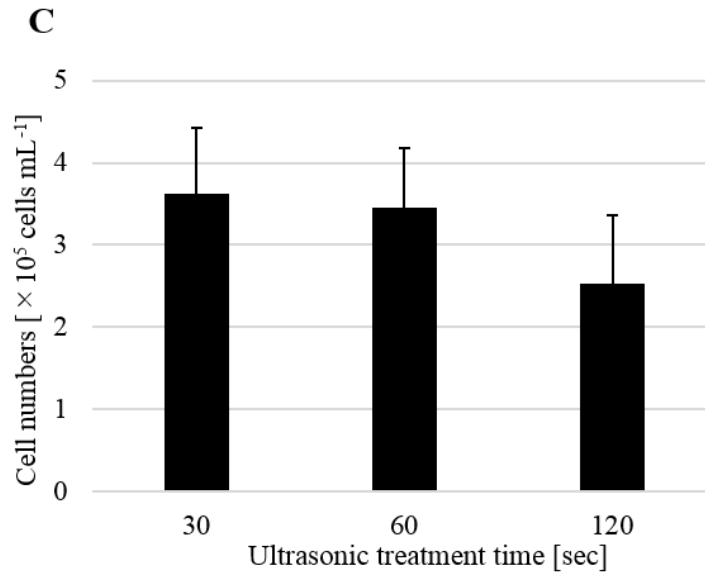


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33 FIG S3 Comparison of nitrite oxidation of untreated and centrifuged (2,900 ×g, 30 min)
34 AM1 cells incubated for 2 days at 16°C. The relative value of centrifuged AM1 cells were
35 shown on the basis of untreated cells. Error bars show the standard deviation of biological
36 triplicate measurements.



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39 FIG S4 *Nitrotoga* aggregates were completely disrupted to single planktonic cells without
 40 too much cell loss by appropriate sonication. Confocal FISH image of the *Nitrotoga*
 41 enrichment (A) before and (B) after sonication for 30 sec. *Nitrotoga* cells hybridized to
 42 probes Cy3-labeled NTG840 (red) and FITC-labeled EUB338mix (green). (C) Sonication
 43 enabled disruption of cell aggregates without loss of too many cells, although excessive
 44 treatment lysed the cells. Error bars show the standard deviation of biological triplicate
 45 measurements.

46 **Reference**

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