

Supplementary material

Journal:

Applied Microbiology and Biotechnology

Title:

**Complete nitrification: insights into the ecophysiology of comammox
Nitrospira**

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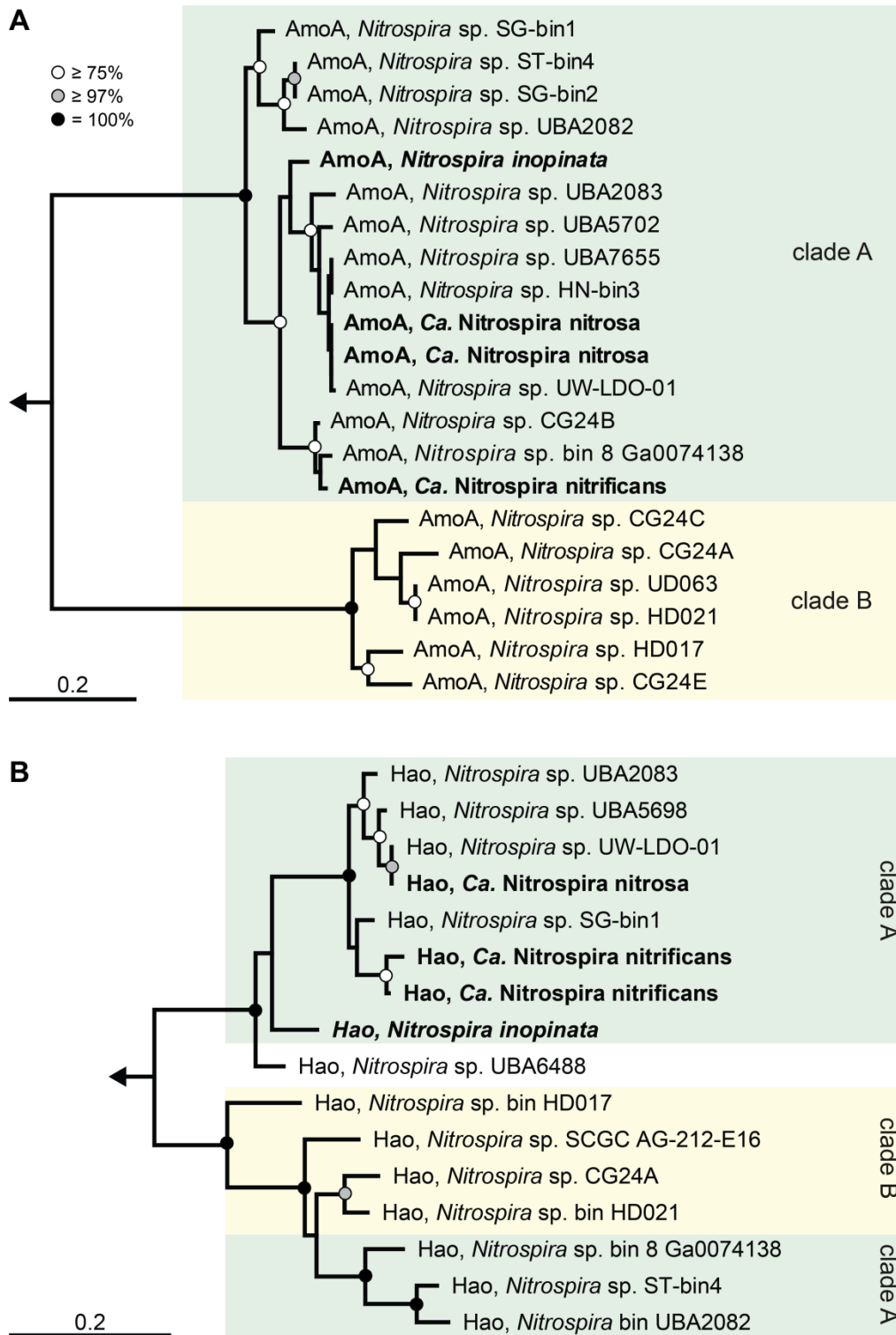


Figure S1: Comparison of (A) AmoA and (B) HaoA-based phylogeny of comammox *Nitrospira*. Protein sequences were aligned using Muscle (Edgar 2004) and maximum likelihood trees were calculated in Geneious (version 11.1.3) using PhyML (Guindon et al. 2009) with the WAG substitution model and 100 bootstrap iterations. Betaproteobacterial protein sequences were used to root the trees. The arrow indicates the position of the outgroup.

Table S1: Summary of key features of publicly available *Nitrospira* genomes in the NCBI database.

Strain	ccmABCDEF ^a (N.inopinata) ^b	HAO (N.inopinata)	CycAB (N.inopinata)	AmoABC (N.inopinata)	NxrABC (N.inopinata)	NrFAH (N.inopinata)	NapA (N.inopinata)	NirK (N.inopinata)	Rh-type AMT (N.inopinata)	AmtB-type AMT (N.moscoviensis)	UreABCDFG (N.inopinata)	UrtABCDE (N.inopinata)	NirA (N.lenta)	OCC (N.moscoviensis)	NirC (N.lenta)	NrtABD (N.moscoviensis)	Cyanase (N.moscoviensis)	Formate dehydrogenase (N.moscoviensis)	3b [NiFe] hydrogenase large subunit (N.inopinata)	Comammox clade	Nitrospira lineage	Scaffolds ^c	Environmental source ^e	CheckM - completeness	Reference	
N. sp. CG24A	ABCDEF ^a	AB	AB	ABC	BC			yes		yes		ABCDE					AB focA ^d		B	2	58	DWTP	87,22	Palomo et al. 2018		
N. sp. CG24B	ABCDEF ^a	AB	AB	ABC	BC			yes	yes		ABCDFG	AB							A	2	36	DWTP	92,22	Palomo et al. 2018		
N. sp. CG24C	ABCDEF ^a			ABC	BC			yes		yes	ABCDFG	A					AB focA		B	2	28	DWTP	93,05	Palomo et al. 2018		
N. sp. CG24D	BCDEF ^a				ABC			yes		yes			yes		ABD	yes	ABG focA		B	2	135	DWTP	92,17	Palomo et al. 2018		
N. sp. CG24E	ABFGHI			AB	ABC					yes	ABCDFG	BCDE					ABG focA		B	2	63	DWTP	93,05	Palomo et al. 2018		
N. sp. HD017	ABCDEF ^a	AB	AB	ABC	C					yes	ABCDFG	ABD							B	2	589	soil	84,04	Orellana et al. 2018		
N. sp. HD021	ABCDEF ^a	AB	AB	ABC	ABC			yes		yes	ABCDFG	ABCD					A		B	2	627	soil	89,56	Orellana et al. 2018		
N. sp. HN-bin3	CDEFGHI	B	AB	AB	BC				yes		ABCG							yes	A	2	357	tap water China	78,22	Wang et al. 2017		
N. defluviil					ABC			yes		yes			yes				AB focA		A	2	1	closed	WWTP	97,67	Lücker et al. 2010	
N. inopinata	ABCDEF ^a	AB	AB	ABC	ABC	AH	yes	yes	yes		ABCDFG	ABCDE						yes	A	2	2	closed	pipe biofilm	96,82	Dalms et al. 2015	
N. moscoviensis					ABC	H		yes		yes	ABCDFG	A		yes		ABD	yes	ABG focA		A	2	2	closed	pipe heating system	95,91	Koch et al. 2015
Ca. N. nitrificans	ABCDEF ^a	AB	AB	ABC	ABC			yes	yes		ABCDFG	ABCDE						yes	A	2	36	trickling filter aquaculture	96,76	van Kessel et al. 2015		
Ca. N. nitrosa	ABCDEF ^a	AB	AB	ABC	ABC			yes	yes		ABCDFG	ABCDE						yes	A	2	15	trickling filter aquaculture	96,76	van Kessel et al. 2015		
N. japonica					ABC			yes		yes	ABCG	ABCDE		yes		ABD	yes		A	2	2	closed	WWTP	96,82	Ushiki et al. 2018	
N. sp. ND1					ABC	AH		yes		yes	ABCDFG	ABCDE	yes				AB focA		A	2	6	WWTP	97,67	Ushiki et al. 2018		
N. sp. OLB3					ABC			yes		yes	ABCDFG	ABCDE	yes	yes			ABG		A	2	79	WWTP	91	Speth et al. 2016		
N. sp. SCGC	ABCEFGHI	AB	AB	BC	ABC			yes									A focA		A	2	162	single cell; soil	52,67	GenBank: LSTC01000001.1		
N. sp. bin75					ABC					yes									A	2	115	marine sponge	94,09	Slaby et al. 2017		
N. sp. SCN59-13					C			yes		yes	DFG	ABCDE	yes				AB focA		A	2	212	bioreactor	35,96	Kantor et al. 2015		
N. sp. bin8	ABCDEF ^a	AB	AB	AB	C			yes	yes		ABCDFG	ABCDE						yes	A	2	61	DWTP	87,67	Pinto et al. 2015		
N. sp. SG-bin1	ABCDEF ^a	AB	AB	ABC	ABC			yes	yes		ABCDFG							yes	A	2	48	tap water Singapore	95,85	Wang et al. 2017		
N. sp. SG-bin2	ABCEFGHI			AB	ABC			yes	yes		ABCDFG	ABDE						yes	A	2	63	tap water Singapore	95,85	Wang et al. 2017		
N. sp. ST-bin4	ABCDEF ^a	A	AB	AB	AC			yes	yes		ABCDFG	DE						yes	A	2	117	tap water USA	92,06	Wang et al. 2017		
N. sp. ST-bin5					ABC	A		yes		yes			yes				AB focA		A	2	29	tap water USA	91,82	Wang et al. 2017		
N. sp. UBA2082	ABCDEF ^a	A	AB	ABC	ABC			yes	yes		ABCDFG	ABCD	yes					yes	A	2	37	residential sediments after rain	93,12	Parks et al. 2017		
N. sp. UBA2083	AFGHI	AB	AB	AB	BC			yes	yes		ABCDFG								A	2	137	residential sediments after rain	87,32	Parks et al. 2017		
N. sp. UBA4129					AC			yes		yes							A focA		A	2	376	WWTP	80,75	Parks et al. 2017		
N. sp. UBA5698	ABCDEF ^a	AB	AB		ABC			yes	yes	yes	ABCDFG	ABCD						yes	A	2	73	industrial sediments after rain	95	Parks et al. 2017		
N. sp. UBA5699	BCDEF ^a				C			yes		yes	ABCDFG	ABCDE	yes	yes		ABD	yes		A	2	124	industrial sediments after rain	90,3	Parks et al. 2017		
N. sp. UBA5702	ABCDEF ^a		AB	AB	ABC			yes	yes		ABCDFG	ABCDE						yes	A	2	109	industrial sediments after rain	94,09	Parks et al. 2017		
N. sp. UBA6488	BCDEF ^a	A			BC			yes		yes		A	yes					yes	A	2	95	river estuary	82,23	Parks et al. 2017		
N. sp. UBA6493	BCDEF ^a				C			yes		yes			yes					yes	A	2	52	river estuary	81,58	Parks et al. 2017		
N. sp. UBA667					BC	AH		yes		yes							B focA		A	2	376	WWTP	76,94	Parks et al. 2017		
N. sp. UBA6909	ABCDEF ^a	AB	AB		ABC	yes		yes	yes		ABCDFG	A							A	2	275	river estuary	86,53	Parks et al. 2017		
N. sp. UBA7240					C	AH		yes					yes				AB focA		A	2	412	WWTP (anoxic tank)	69,12	Parks et al. 2017		
N. sp. UBA7655	ABCDEF ^a		B	AB	ABC			yes	yes		ABCDFG							yes	A	2	132	DWTP	78,07	Parks et al. 2017		
N. sp. UD063	ABEH		AB	ABC	BC			yes		yes	ABCDG	ABCD					AB focA		B	2	1453	soil	91,16	Orellana et al. 2018		
N. sp. UW-LDO-01	ABCDEF ^a	AB	AB	AB	AC			yes	yes		ABCDFG	ABCDE						yes	A	2	230	lab-scale bioreactor	95,8	Camejo et al. 2017		
N. sp. UW-LDO-02					AC	AH		yes		yes			yes				AB focA		A	2	522	lab-scale bioreactor	94,36	Camejo et al. 2017		

^a AmoABC, ammonia monooxygenase; AMT, ammonium transporter; CCM, cytochrome c-biosynthesis proteins; CycAB, cytochromes c554 and cM552; HAO, hydroxylamine dehydrogenase; Nap, periplasmic nitrate reductase; NirA, ferredoxin-dependent nitrite reductase; NirC, nitrite transporter;

NirK, copper-dependent nitrite reductase; NrFAH, pentaheme nitrite reductase; NrtABC, nitrate ABC-type transporter; Nxr, nitrite oxidoreductase; OCC, octaheme cytochrome c; UreABCDFG, urease and urease accessory proteins; UrtABCDE, urea ABC-type transporter.

^b Homologous proteins were identified using BLASTp (Altschul et al. 1990). Only hits with >50% identity and >50% query coverage were considered as homologs. *Nitrospira* species used as query are indicated in brackets.

^c number of scaffolds according to NCBI

^d focA: Formate transporter

^e DWTP, drinking water treatment plant; WWTP, wastewater treatment plant

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