Supplementary information

Evolutionary inactivation of a sialidase in group B *Streptococcus*

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Supplementary Figure legends

Figure S1. Maximum likelihood tree of *nanA* and *nonA* genes. Bootstrap values are shown near the nodes. Strains with identical DNA sequences are listed on the same branch. The scale bar indicates nucleotide substitutions per site.

Figure S2. Schematic illustration of domain structures in streptococcal NanA, NanB, and NanC proteins. Strains with identical sequences are listed on the same branch. *S. iniae* NanA, *S. pneumoniae* NanB and NanC lack an LPXTG motif.

Figure S3. Sialidase activities of bacterial cells and culture supernatants. A. The sialidase activities of GBS A909 and streptococcal type strains. B. The sialidase activities of GBS clinical strains and *S. pneumoniae* D39. C. The sialidase activities of clinically isolated *S. iniae* strains. The strains were isolates from brain of a diseased hybrid-stripedbass or tilapia at the Kent SeaTech aquaculture facility in Mecca, CA. After 2 h incubation at 37°C, fluorescence of degraded sialidase substrate was measured with excitation and emission wavelengths of 350 and 460 nm, respectively. Data are presented as the mean of triplicate or sextuplet samples. S.E.

values are represented by vertical lines. The limit of sensitivity of the assay is 0.3 mU/mL.

Figure S4. Maximum likelihood tree of *nanA, nanB, nanC,* and *nonA* genes. Bootstrap values are shown near nodes. The scale bar indicates nucleotide substitutions per site.

Figure S5. Bayesian phylogenetic tree of *nanA* in *S. pneumoniae.* Percentage of posterior probabilities is shown near the nodes. The scale bar indicates nucleotide substitutions per site. FEL and FUBAR analyses were performed on *nanA* multiple alignment data and this tree.

Figure S6. Bayesian phylogenetic tree of *nonA* in *S. agalactiae.* Percentage of posterior probabilities is shown near the nodes. The scale bar indicates nucleotide substitutions per site. FEL and FUBAR analyses were performed on *nonA* multiple alignment data and this tree.

Figure S7. Bayesian phylogenetic tree of *nanB* in *S. pneumoniae.* Percentage of posterior probabilities is shown near the nodes. The scale bar indicates nucleotide substitutions per site. FEL and FUBAR analyses were performed on *nanB* multiple alignment data and this tree.

Figure S8. Bayesian phylogenetic tree of *nanC* in *S. pneumoniae.* Percentage of posterior probabilities is shown near the nodes. The scale bar indicates nucleotide substitutions per site. FEL and FUBAR analyses were performed on *nanC* multiple alignment data and this tree.

Figure S9. Bayesian phylogenetic tree of *bgaA* in *S. pneumoniae.* Percentage of posterior probabilities is shown near the nodes. The scale bar indicates nucleotide substitutions per site. FEL and FUBAR analyses were performed on *bgaA* multiple alignment data and this tree.

Figure S10. Bayesian phylogenetic tree of *strH* in *S. pneumoniae.* Percentage of posterior probabilities is shown near the nodes. The scale bar indicates nucleotide substitutions per site. FEL and FUBAR analyses were performed on *strH* multiple alignment data and this tree.

Figure S11. Expression of *nonA* in GBS A909. The graph shows fold transcript level of *cylE* and *nonA* in GBS A909 wild type (filled bars) and Δ*nonA* mutant strain (open bars). RNA extractions were performed from mid-log phase cultures. The level of DNA gyrase subunit A gene (*gyrA*) transcription was used as an internal standard. The data represent the mean values of triplicate samples. S.E. values are represented by vertical lines.

Table S1. Homology between NanA, NanB, and NanC of *S. pneumoniae* D39 and NonA of GBS A909

	NanA	NonA	NanB	NanC
NanA	-	49/58/75/1	63/25/43/17	53/27/44/16
NonA	70/58/75/1	-	62/28/45/16	60/27/44/15
NanB	88/25/43/17	62/28/45/16	-	94/51/69/0
NanC	70/27/44/16	57/27/44/15	88/51/69/0	-

Cover/Indentities/Positives/Gaps (%)

Table S2. Bacterial strains and the accession number of the genome sequences used for phylogenetic tree

Bacteria	Strain	Accession number
Streptococcus pneumoniae	D39	CP000410.1
	JJA	CP000919.1
	CGSP14	CP001033.1
	Hungary19A-6	CP000936.1
	ST556	CP003357.1
	Taiwan19F-14	CP000921.1
	SPNA45	CP000936.1
	AP200	CP002121.1
	G54	CP001015.1
	gamPNI0373	CP001845.1
	TCH8431/19A	CP001993.1
	ATCC700669	FM211187.1
	P1031	CP000920.1
	70585	CP000918.1
	TIGR4	AE005672.3
	670-6B	CP002176.1
	NT_110_58	CP007593.1
Streptococcus agalactiae	A909	CP000114.1
	ILRI112	HF952106.1
	GBS2-NM	CP007571.1
	GBS6	CP007572.1
	GBS1-NY	CP007570.1
	SA20-06	CP003919.1
	138spar	CP007565.1
	138P	CP007482.1
	2-22	FO393392.1
	GD201008-001	CP003810.1
	NEM316	AL766854.1
	NGBS572	CP007632.1
	2603V/R	NC_004116.1
	ILRI005	HF952105.1
	CNCTC 10/84	CP006910.1
	09mas018883	HF952104.1
	NGBS061	CP007631.2
	COH1	HG939456.1
Streptococcus iniae	ISNO	CP007587.1
	ISET0901	CP007586.1
	YSFST01-82	CP010783.1
	SF1	CP005941.1
Streptococcus intermedius	B196	CP003857.1
	C270	CP003858.1
	JTH08	AP010969.1
Streptococcus mitis	B6	FN568063.1
	KCOM 1350	CP012646.1
Streptococcus oralis	Uo5	FR720602.1
Streptococcus pseudopneumoniae	IS7493	CP002925.1
Streptococcus sp.	VT 162	CP007628.2
Erysipelothrix rhusiopathiae	SY1027	AP012027.1
	Fujisawa	CP005079.1

Table S3. The frameshift mutation in GBS *nonA* genes.

GBS strain	DNA sequence (5’–3’)
A909	T GAT TGG GGA AAC ATA GGA ATG GTT ATT CGC CGT AGT GA
SA20-06	T GAT TGG GG$\underline{\textbf{G}}$ AAA CAT AGG AAT GGT TAT TCG CCG $\boxed{\text{TAG}}$ $\boxed{\text{TGA}}$
GX026	T GAT TGG GG$\underline{\textbf{G}}$ AAA CAT AGG AAT GGT TAT TCG CCG $\boxed{\text{TAG}}$ $\boxed{\text{TGA}}$
2-22	T GAT TGG GG$\underline{\textbf{G}}$ AAA CAT AGG AAT GGT TAT TCG CCG $\boxed{\text{TAG}}$ $\boxed{\text{TGA}}$
138spar	T GAT TGG GG$\underline{\textbf{G}}$ AAA CAT AGG AAT GGT TAT TCG CCG $\boxed{\text{TAG}}$ $\boxed{\text{TGA}}$
138P	T GAT TGG GG$\underline{\textbf{G}}$ AAA CAT AGG AAT GGT TAT TCG CCG $\boxed{\text{TAG}}$ $\boxed{\text{TGA}}$

Bold and underlined character is the inserted nucleotide. Box shows stop codons generated by the framshift.

Table S4. Evolutionary analyses of *nanA*, *nonA, nanB, nanC, bgaA,* and *strH* genes

Gene	Species	Number of Strains	dN/dS	Codons evolving under positive selection	Codons evolving under negative selection
nanA	*S. pneumoniae*	16	0.29	0.940% (7/745)	15.302% (114/745)
nonA	*S. agalactiae*	16	0.35	0.885% (4/452)	1.770% (8/452)
nanB	*S. pneumoniae*	16	0.35	0.441% (2/454)	0.661% (3/454)
nanC	*S. pneumoniae*	6	0.29	0% (0/740)	2.703% (20/740)
bgaA	*S. pneumoniae*	14	0.29	0.806% (18/2233)	4.881% (109/2233)
strH	*S. pneumoniae*	17	0.50	0.758% (10/1319)	0.607% (8/1319)

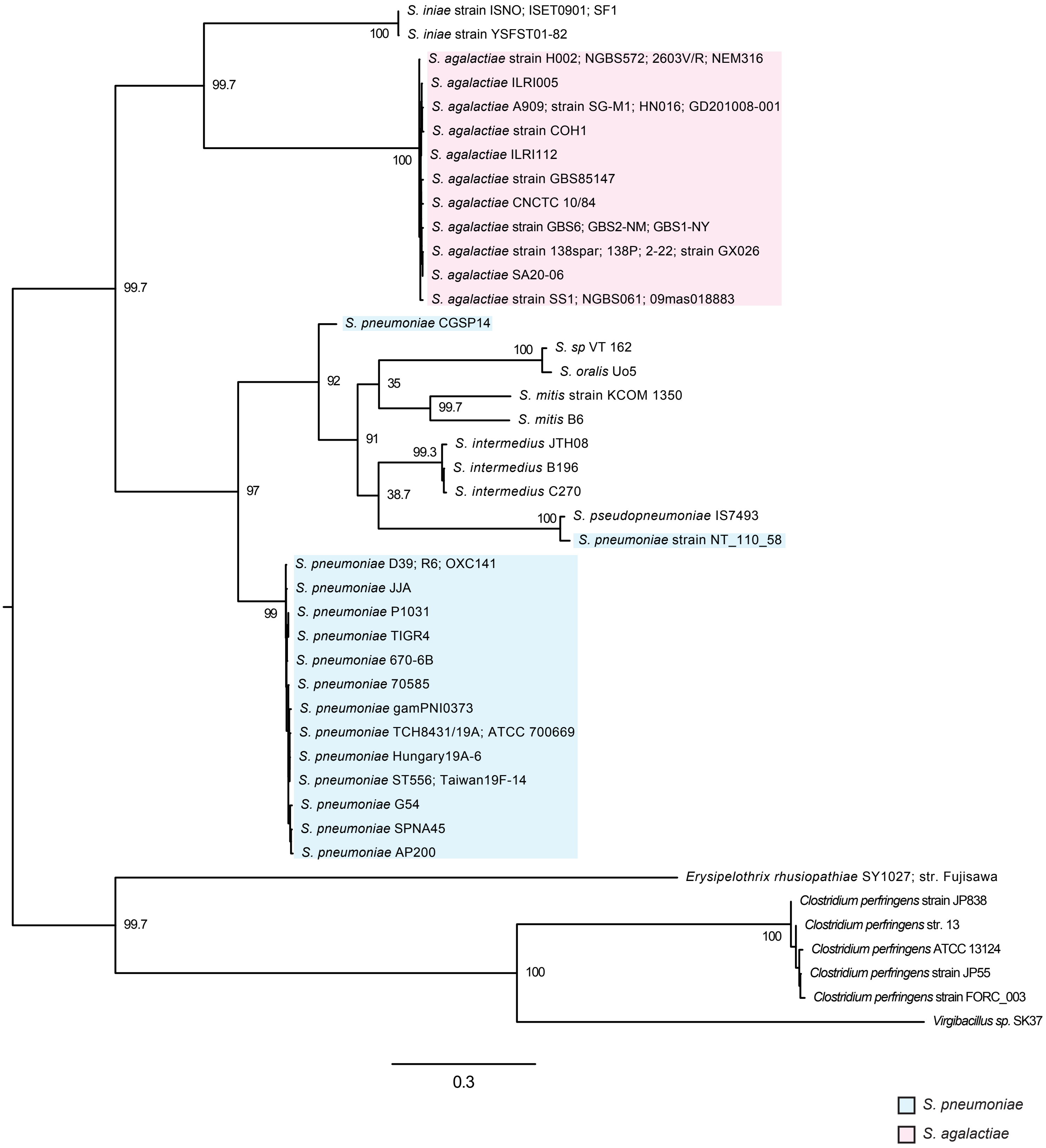
Evolutionary analysis was performed using Baysian inference of aligned *nanA, nonA, nanB, nanC, bgaA,* and *strH* sequences from *S. pneumoniae* or *S. agalactiae*, with FUBAR in the HyPhy software package. The dN/dS means ratio of non-synonymous changes to synonymous changes in overall analyzed genes. Individual codons with a statistically significant signature were also calculated and are expressed as a percentage of the total number of codons used in the analysis.

Table S5. Bacterial strains used in sialidase activity assay

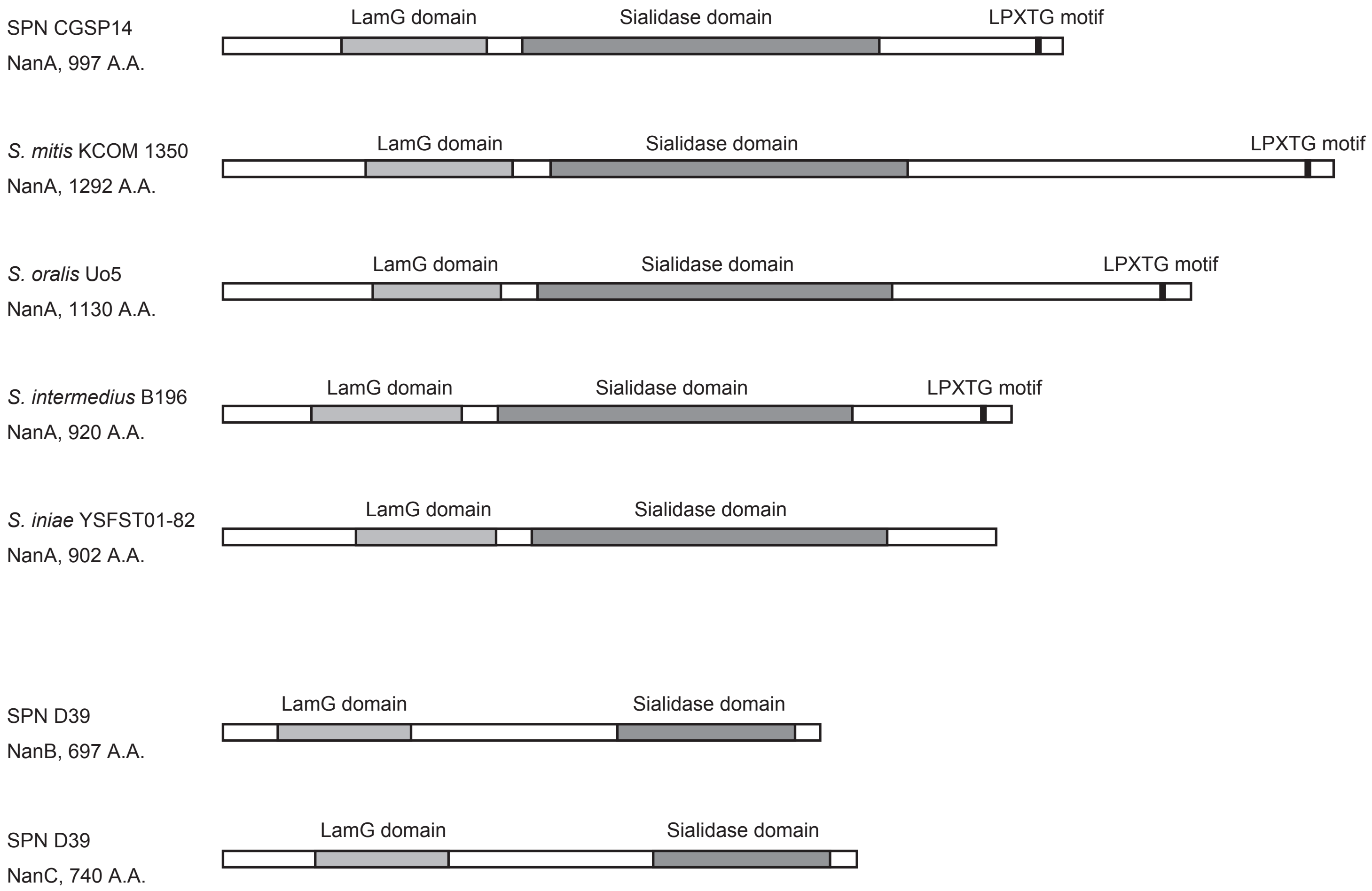
Bacteria	Strain	Remarks
Streptococcus pneumoniae	D39	Serotype 2
Streptococcus agalactiae	A909	Serotype Ia
	M709	Serotype Ib
	GBS2-NM	Serotype II
	GBS6	Serotype III
	GBS1-NY	Serotype V
	SA20-06	Serotype VI
Streptococcus mitis	ATCC 49456/NCTC 12261/SK142	Type strain
Streptococcus oralis	ATCC 35037/NCTC 11427	Type strain
Streptococcus pseudopneumoniae	ATCC BAA-960/CCUG 49455/SK1069	Type strain
Streptococcus intermedius	ATCC 27335/SK54	Type strain
Streptococcus iniae	K122	Clinical strains
	K139	Clinical strains
	K277	Clinical strains
	K288	Clinical strains
	K375	Clinical strains
	K436	Clinical strains
	K540	Clinical strains
	94-426	Clinical strains

Table S6. Real-time RT-PCR Primers used in this study

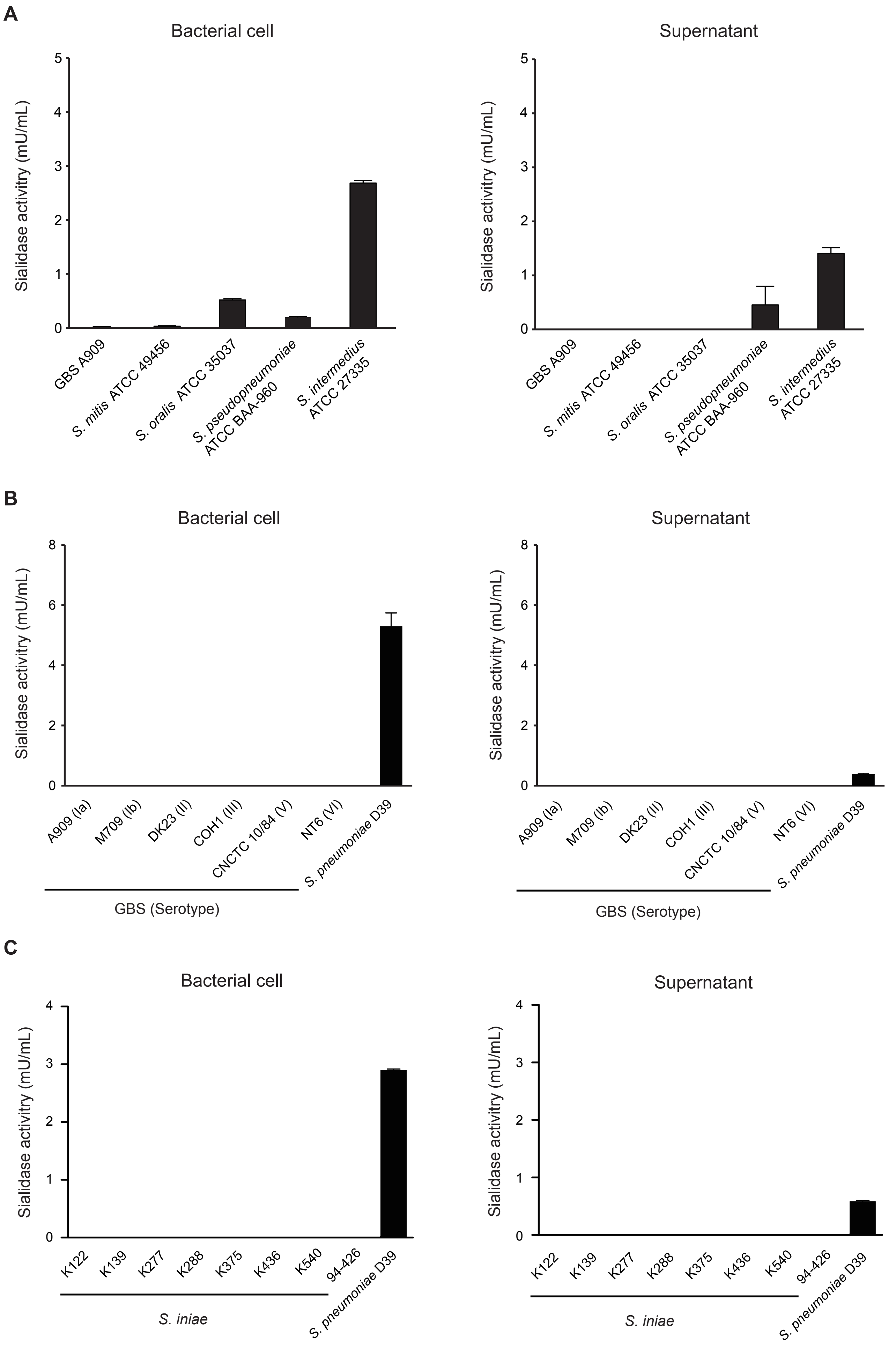
Primers	Sequence (5’ to 3’)
A909nonAF	GTGATTGGGGAAACATAGGAATGG
A909nonAR	CGATTTGGGTATATTCTTTTTCAG
A909cylEF	ATTGAAAATCCCTTGGAAGAGGAGACAG
A909cylER	GTTAGGCTAGGGTGAGCCCTCG
A909gyrAF	CCACATGGTGATTCATCTATTTAC
A909gyrAR	CGCTGGTAAAACAAGAGGTTCACG



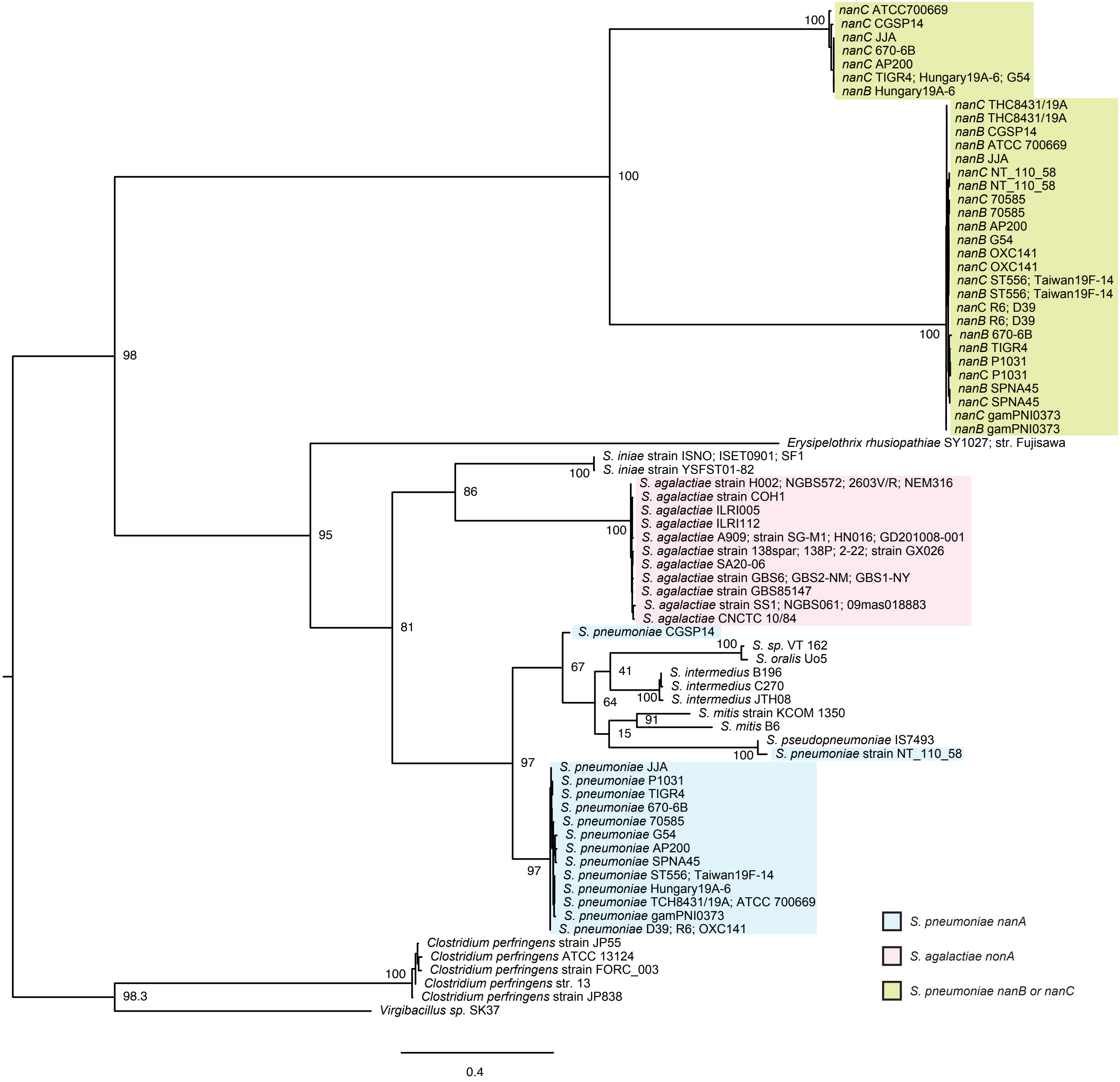
Supplementary Figure 1. Yamaguchi *et al*.



Supplementary Figure 2. Yamaguchi *et al*.



Supplementary Figure 3. Yamaguchi *et al*.



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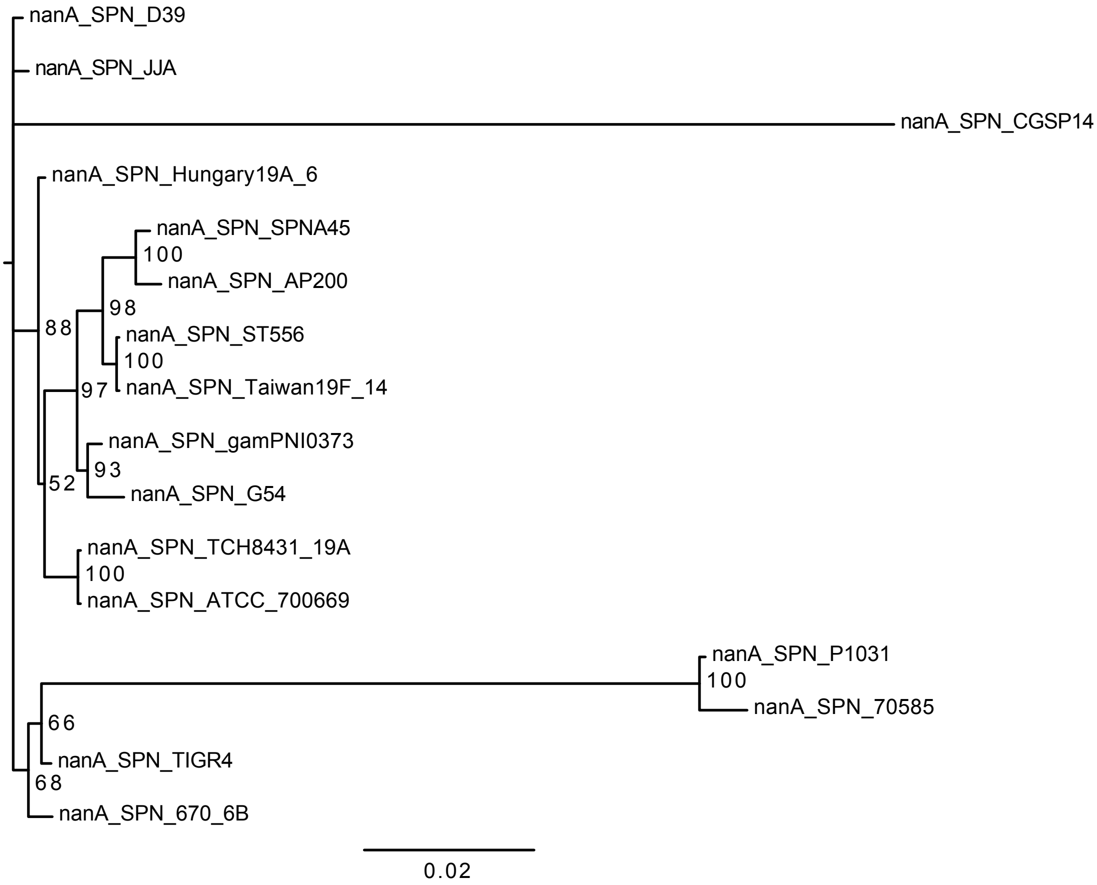
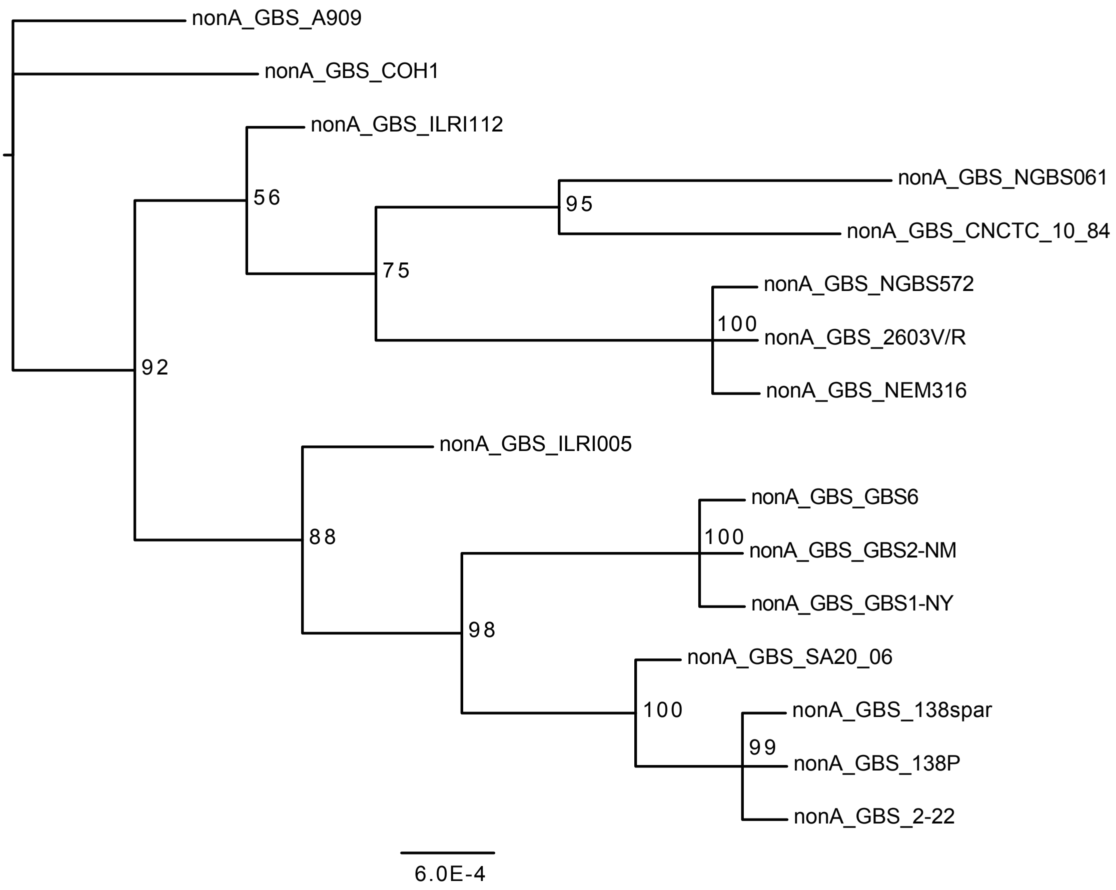
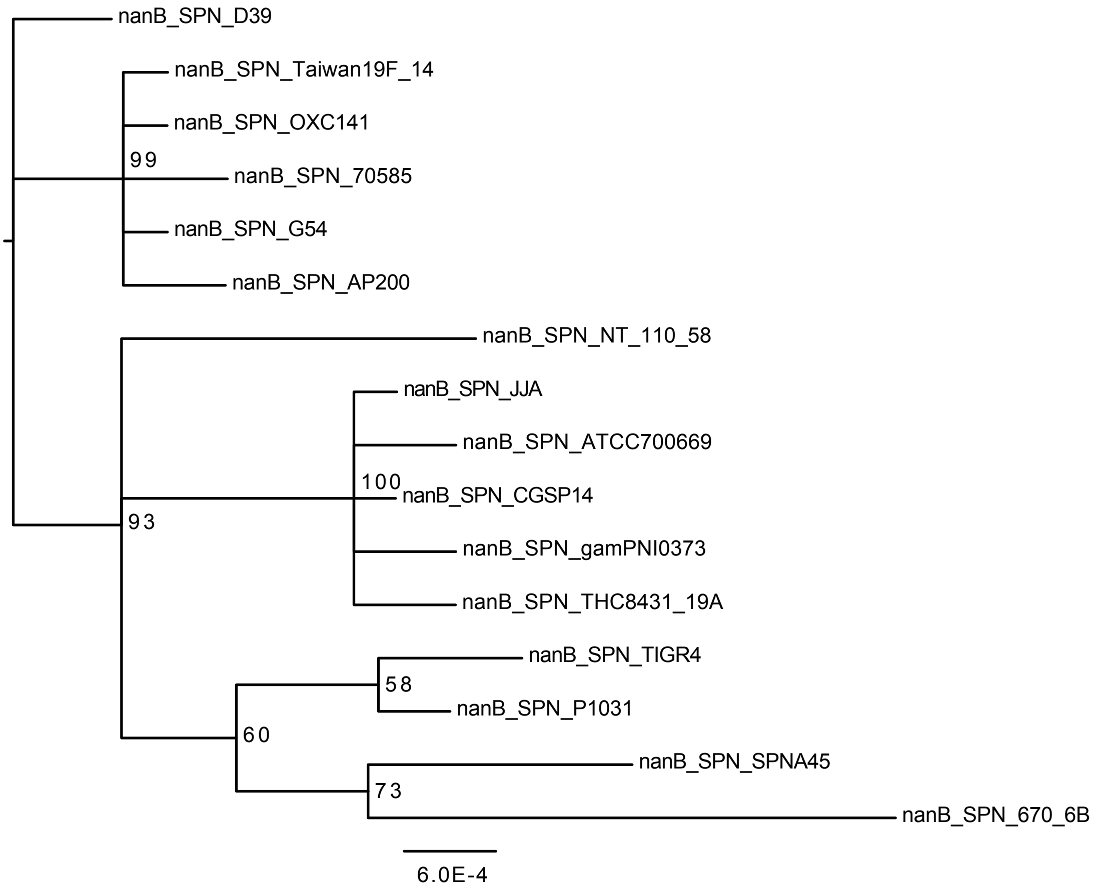


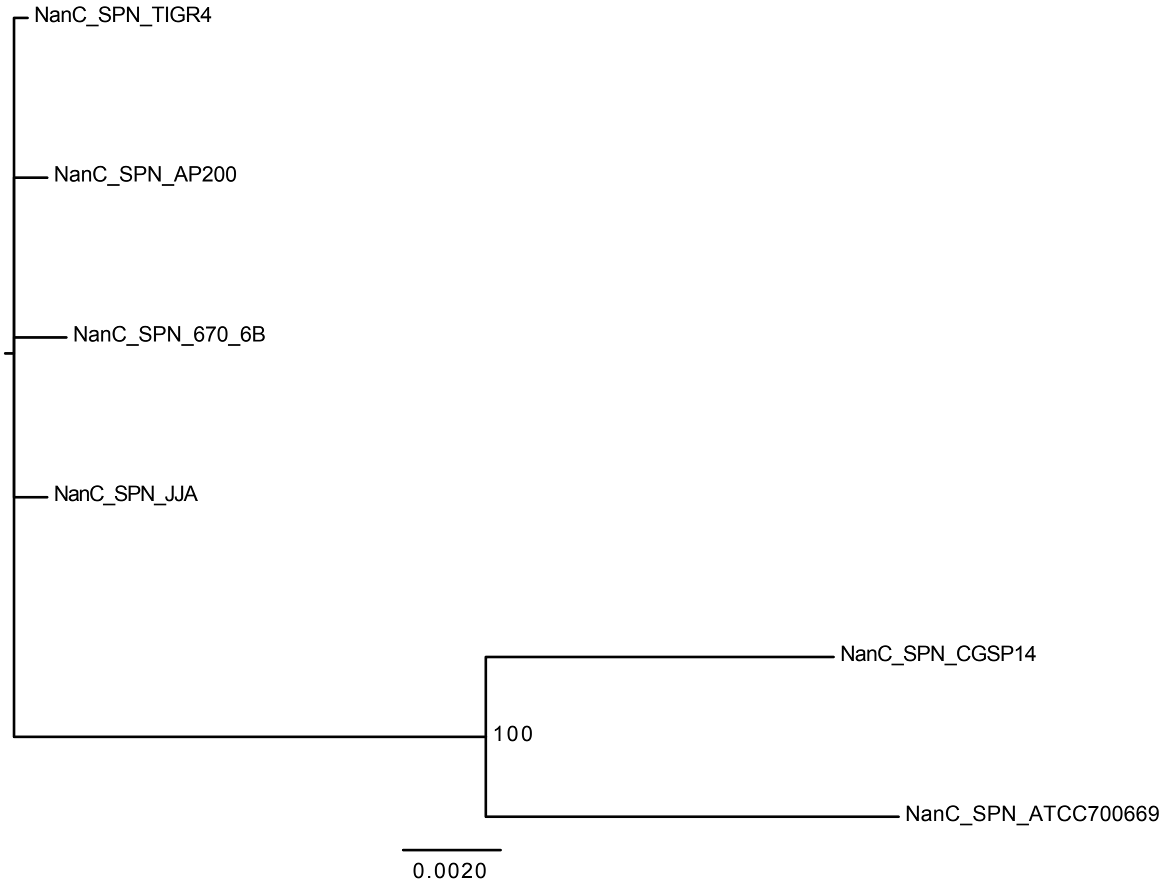
Supplementary Figure 5. Yamaguchi *et al*.



Supplementary Figure 6. Yamaguchi *et al*.



Supplementary Figure 7. Yamaguchi *et al*.



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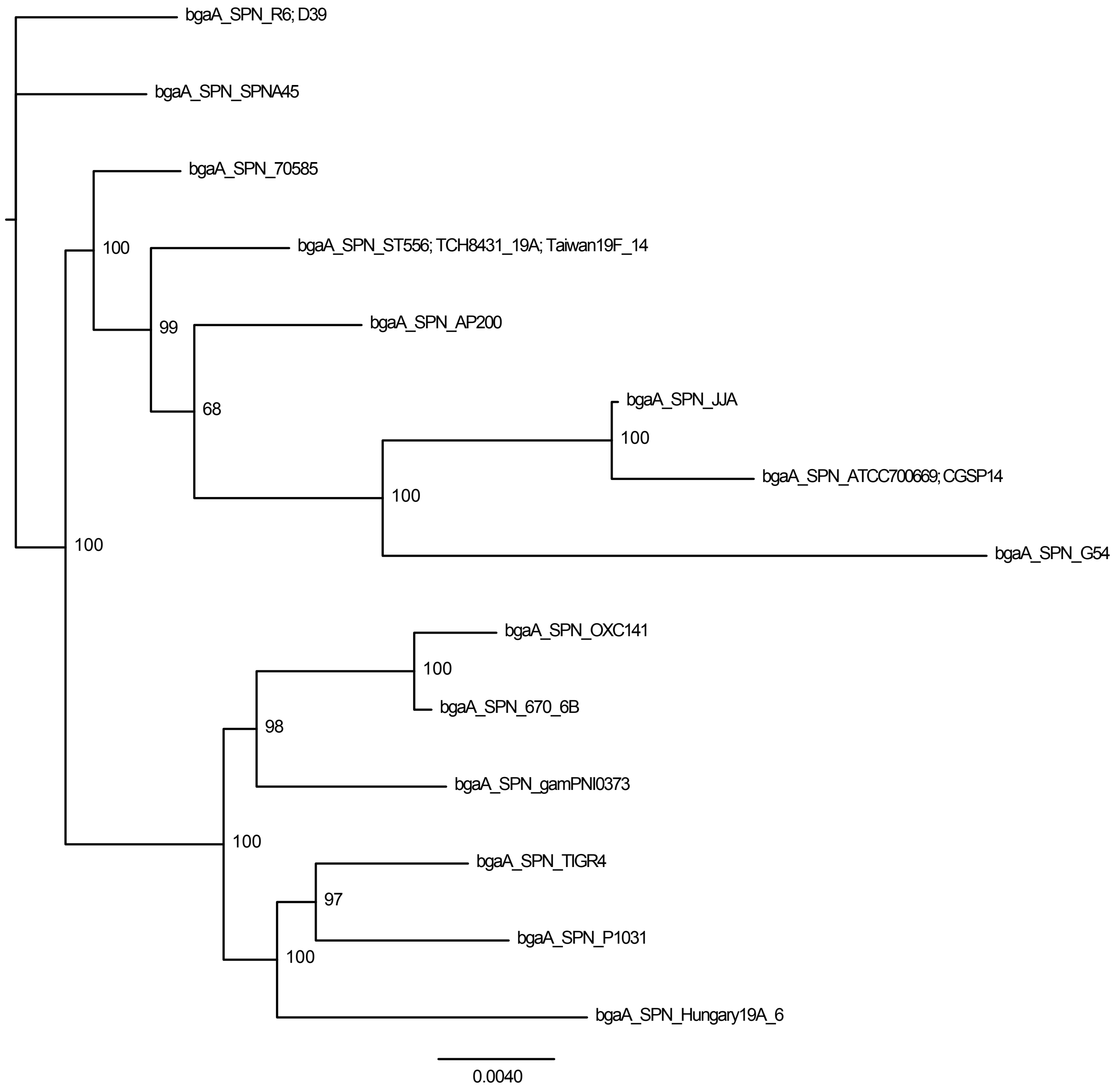

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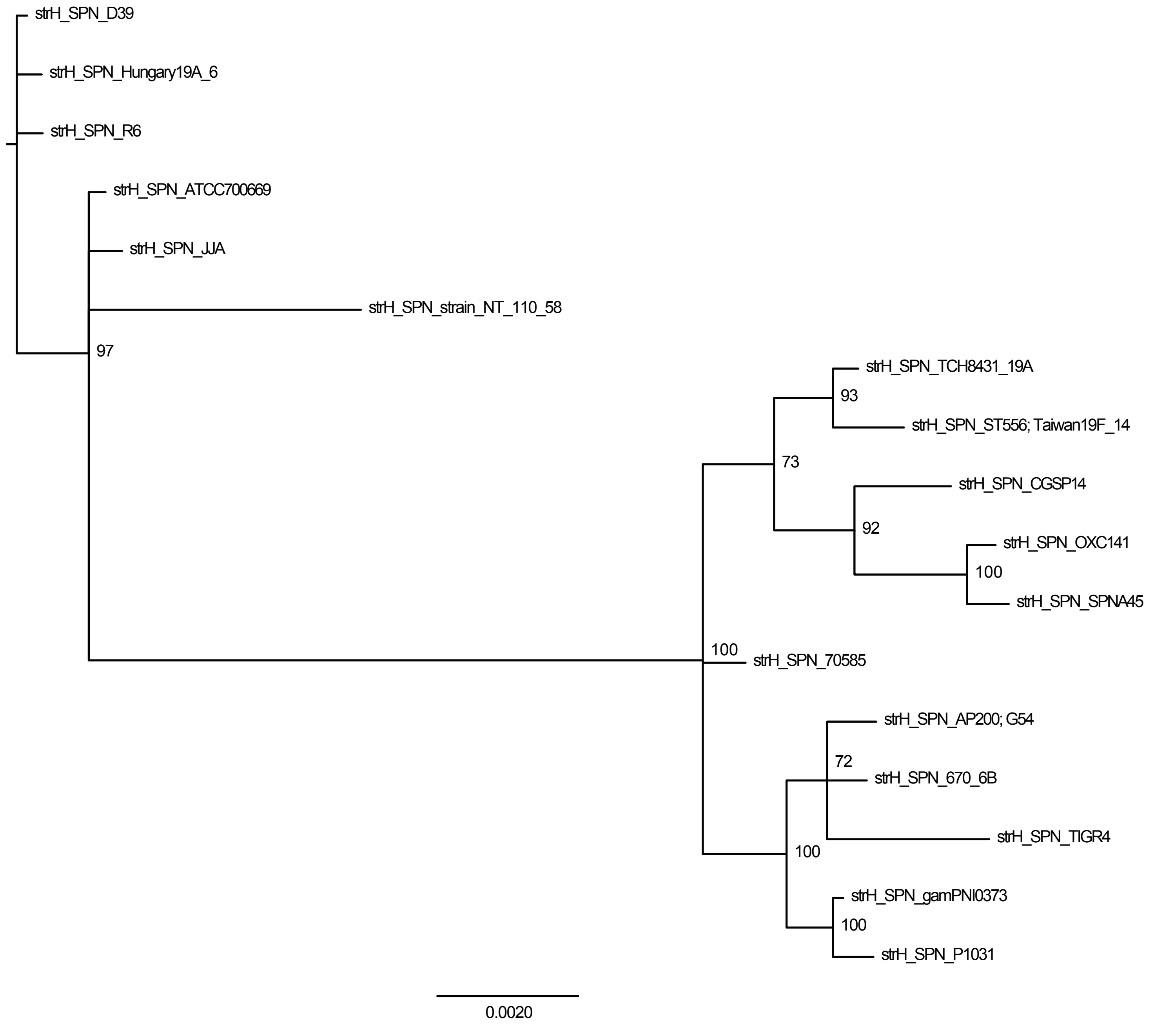
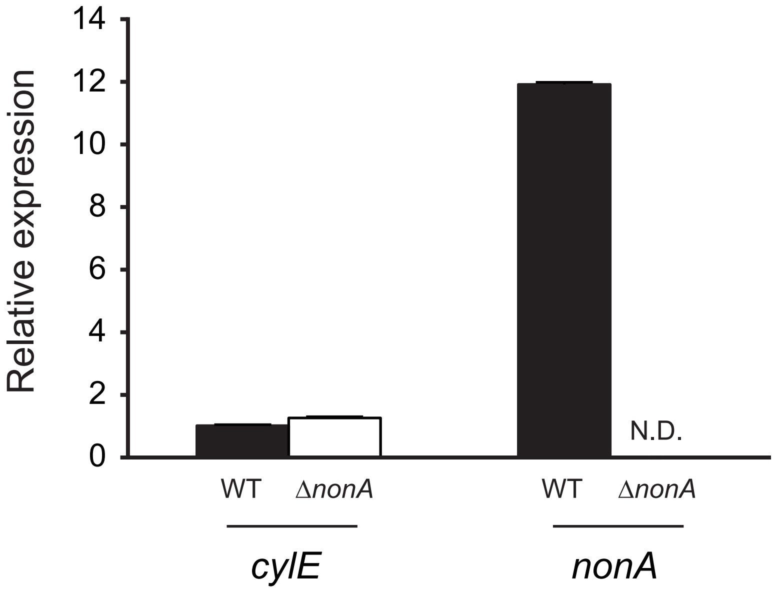


Supplementary Figure 10. Yamaguchi *et al*.



Supplementary Figure 11. Yamaguchi *et al*.