

## ***Nitrosomonas europaea* glycogen synthase: Molecular cloning, expression and purification**

The gene *glgA* (NE2264), coding for glycogen synthase (GSase; ADP-glucose:1,4- $\alpha$ -D-glucan 4- $\alpha$ -D-glucosyl-transferase, EC 2.4.2.21) in *Nitrosomonas europaea* was amplified by PCR. Primers specifically designed from the gene sequence were: *NeuGSFow*, GCTAGCATGTCTTCATCTCCTCCCGC and *NeuGSRev*, TTACTCGAGTCGCTGCATAACAGCG. The PCR reaction contained: 25 ng of *N. europaea* ATCC 19817 genomic DNA; 2 mM MgCl<sub>2</sub>; 2  $\mu$ M of each primer; 200  $\mu$ M dNTPs and 0,1 U/ $\mu$ l *Taq* DNA polymerase (Fermentas). After an initial denaturalization of 30 s at 95 °C, we performed 30 cycles of 95 °C for 60 s, 60 °C for 40 s, and 72 °C for 1:30 min, followed by a 10 min extension at 72 °C. The PCR product was purified in an agarose gel electrophoresis and cloned into pGEM-Teasy vector (Promega). Then, it was subcloned in the pET24a vector (Novagen), using the *NheI* and *XhoI* restriction enzymes. This construction was used to transform *E. coli* BL21 (DE3) + [pG-KJE8] strain. The [pG-KJE8] vector corresponds to the Takara chaperones plasmids series (4, 5).

Transformed cells were grown overnight at 37 °C with shaking at 200 rpm in LB supplemented with 20  $\mu$ g/ml chloramphenicol and 50  $\mu$ g/ml kanamycin. The overnight culture was diluted 1/100 in fresh media supplemented with 20  $\mu$ g/ml chloramphenicol, 50  $\mu$ g/m kanamycin and 2 ng/ml tetracycline (for the induction of GroEL-GroES chaperones expression) and grown under identical conditions to exponential phase (OD<sub>600</sub> of 0.6) at 37 °C. The expression of recombinant GSase was induced by adding 0.2 mM IPTG followed by growing at 23 °C during 18 h. After harvesting by centrifugation, the cell paste was resuspended in binding buffer H [25 mM TrisHCl pH 8,0; 300 mM

NaCl; 10 mM imidazol] and disrupted by sonication. The lysate was centrifuged (36,000 g, 15 min) to remove cell debris. The resultant crude extract was loaded onto a Ni<sup>2+</sup>-NTA resin column that had been equilibrated with binding buffer H. The column was washed with two column volume each of buffer H plus 20 mM and then 40 mM imidazole. Finally, using buffer H plus 300 mM imidazole, the recombinant GSase was eluted with purity higher than 90%, as analyzed in SDS-PAGE. After adding glycerol 10% (v/v) the purified enzyme was stored at -80 °C, under which conditions it remained fully active for at least 3 months.

### Glycogen synthase assay

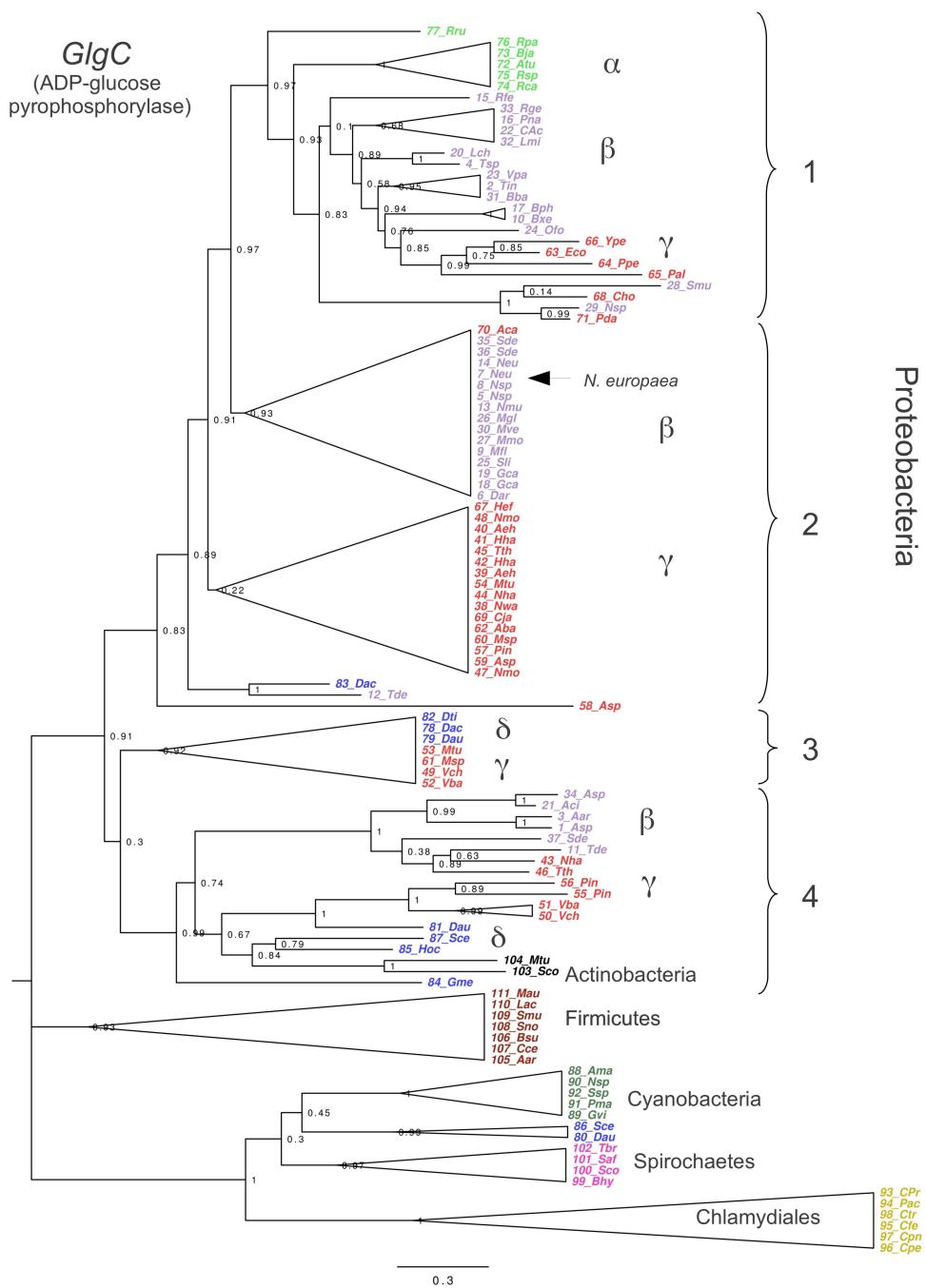
Activity of GSase was assayed with the method described by Wayllace (7). The standard assay medium contained (unless otherwise indicated) 100 mM MOPS pH 8.0; 2 mg/ml rabbit liver glycogen; 2 mM ADP-Glc; 1 mM PEP; 0.3 mM NADH; 10 mM MgCl<sub>2</sub>; 0.2 mg/ml BSA; 0.02 U/μl muscle rabbit pyruvate kinase and lactate dehydrogenase from *Lactobacillus* in a final volume of 50 μl. Enzyme activity was calculated following NADH consumption at 340 nm, using a Multiskan Ascent (Thermo). One unit (U) of enzymatic activity is defined as the amount of enzyme catalyzing the formation of 1 μmol ADP/min under the above specified conditions.

### **Phylogenetic analysis of ADP-Glc PPases and glycogen synthases from bacteria.**

Sequences coding for bacterial ADP-Glc PPases and glycogen synthases were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov/>). They were filtered to remove duplicates and near duplicates (i.e. mutants and strains from same species). After a preliminary alignment, constructed using the ClustalW multiple sequence alignment server (<http://www.genome.jp/tools/clustalw/>) (3), sequences with a wrong annotation or truncated were also eliminated manually. Sequences that had lower than 30% identity to any other and did not have all the characterized critical motifs and catalytic residues were discarded. After this, sequences were chosen to represent most of the taxonomic groups from bacteria. We ensure we had a substantial representation from proteobacteria, which is the group *Nitrosomonas europaea* belong to. Sequences were classified into different groups using taxonomic data provided by the NCBI as depicted in Table S3 and Table S4. Once we settle on the sequences to use, they were aligned using the program T-Coffee (6). A manual inspection was performed to guarantee that all known conserved regions (catalytic residues etc.) were properly aligned. Trees were constructed by maximum likelihood with the program PhyML (2) incorporated into Seaview (1). Confidence coefficients for the tree branches were obtained and plot. Finally, the tree was prepared with the FigTree 1.3 program (<http://tree.bio.ed.ac.uk/>).

## References

1. **Gouy M, Guindon S, Gascuel O.** 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol.* **27**:221-224.
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5. **Nishihara K, Kanemori M, Yanagi H, Yura T.** 2000. Overexpression of trigger factor prevents aggregation of recombinant proteins in Escherichia coli. *Appl Environ Microbiol.* **66**:884-889.
6. **Notredame C, Higgins DG, Heringa J.** 2000. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol.* **302**:205-217.
7. **Wayllace NZ, Valdez HA, Meras A, Ugalde RA, Busi MV, Gomez-Casati DF.** 2011. An enzyme-coupled continuous spectrophotometric assay for glycogen synthases. *Mol Biol Rep.* **39**:585-591.



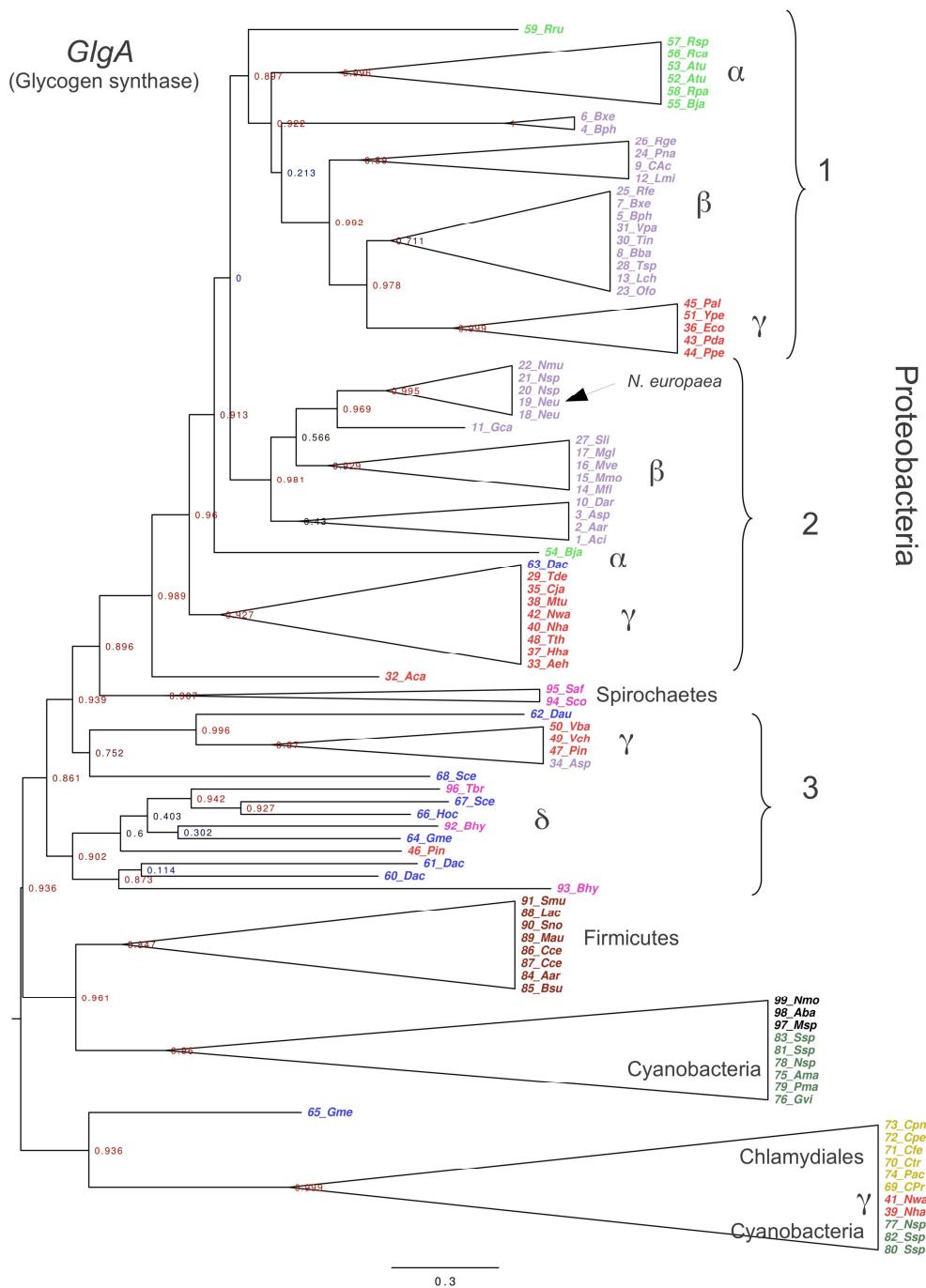
**Fig. S1. Phylogenetic tree of ADP-Glc PPases from bacteria.** Sequences of the *glgC* gene products were collected and the tree was built as described above. In the tree, it is indicated the confidence coefficients and the taxonomy data. Sequences are depicted with codes indexed in Table S3. Different colors represent different taxonomy. Sequences from proteobacteria ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) are indicated. Numbers indicate a particular cluster (1 to 4).

**Table S1. Number of *glgC* gene products located in a given cluster in the phylogenetic tree for bacteria with gene duplications.**

Species <sup>a</sup>	Taxonomy	Cluster <sup>b</sup>			
		2	3	4	Cyano
<i>Thiobacillus denitrificans</i> ATCC 25259	β-proteobacteria	1		1	
<i>Sulfuricella denitrificans</i> skB26	β-proteobacteria	2		1	
<i>Alkalilimnicola ehrlichii</i> MLHE-1	γ-proteobacteria	2			
<i>Halorhodospira halophila</i> SL1	γ-proteobacteria	2			
<i>Nitrosococcus halophilus</i> Nc4	γ-proteobacteria	1		1	
<i>Thioalkalivibrio thiocyanoxidans</i> ARh 4	γ-proteobacteria	1		1	
<i>Vibrio cholerae</i> 12129(1)	γ-proteobacteria		1	1	
<i>Vibrionales bacterium</i> SWAT-3	γ-proteobacteria		1	1	
<i>Methylobacter tundripaludum</i> SV96	γ-proteobacteria	1	1		
<i>Psychromonas ingrahamii</i> 37	γ-proteobacteria	1		1	
<i>Alteromonas</i> sp. SN2	γ-proteobacteria	2			
<i>Moritella</i> sp. PE36	γ-proteobacteria	1	1		
<i>Desulfobacterium autotrophicum</i> HRM2	δ-proteobacteria		1	1	1
<i>Sorangium cellulosum</i> 'So ce 56'	δ-proteobacteria			1	1

<sup>a</sup> These are the bacteria that have more than one homologous *glgC* gene in their genome.

<sup>b</sup> Clusters are the one depicted in the phylogenetic analysis, 2, 3, 4, and Cyanobacteria (Fig. S1). The number in the respective column is the amount of genes per cluster.



**Fig. S2. Phylogenetic tree of glycogen synthases from bacteria.** Sequences of the *glgA* gene products were collected and the tree was built as described. In the tree, it is indicated the confidence coefficients and the taxonomy data. Sequences are depicted with codes indexed in Table S4. Different colors represent different taxonomy. Sequences from proteobacteria ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) are indicated. Numbers indicate a particular cluster (1 to 3).

**Table S2. Number of *glgA* gene products located in a given cluster in the phylogenetic tree for bacteria with gene duplications.**

<i>Species<sup>a</sup></i>	Taxonomy	Cluster <sup>b</sup>				
		1	2	3	Cya	Chla
<i>Burkholderia phymatum STM815</i>	β-proteobacteria	2				
<i>Burkholderia xenovorans LB400</i>	β-proteobacteria	2				
<i>Nitrosococcus halophilus Nc4</i>	γ-proteobacteria		1			
<i>Nitrosococcus watsonii C-113</i>	γ-proteobacteria		1			
<i>Psychromonas ingrahamii 37</i>	γ-proteobacteria			2		
<i>Agrobacterium tumefaciens str. C58</i>	α-proteobacteria	2				
<i>Bradyrhizobium japonicum USDA 110</i>	α-proteobacteria	1	1			
<i>Desulfobacca acetoxidans DSM 11109</i>	δ-proteobacteria		2			
<i>Geobacter metallireducens GS-15</i>	δ-proteobacteria		1			
<i>Sorangium cellulosum 'So ce 56'</i>	δ-proteobacteria		2			
<i>Nostoc sp. PCC 7120</i>	Cyanobacteria		1	1		
<i>Synechocystis sp. PCC 6803</i>	Cyanobacteria		2	2		
<i>Brachyspira hyodysenteriae WA1</i>	Spirochaetes		2			

<sup>a</sup> These are the bacteria that have more than one homologous *glgA* gene in their genome.

<sup>b</sup> Clusters are the ones depicted in the phylogenetic analysis, 1, 2, 3, Cyanobacteria, and Chlamydiales (Fig. S2). The number in the respective column is the amount of genes per cluster.

**Table S3. ADP-Glc PPase sequences (GlgC) used for phylogenetic analysis.**

GI Accession	Species	Code	Taxon
119898088	<i>Azoarcus sp. BH72</i>	1_Asp	Beta-proteobacteria
296135572	<i>Thiomonas intermedia K12</i>	2_Tin	Beta-proteobacteria
56479382	<i>Aromatoleum aromaticum EbNI</i>	3_Aar	Beta-proteobacteria
217970454	<i>Thauera sp. MZIT</i>	4_Tsp	Beta-proteobacteria
325983317	<i>Nitrosomonas sp. AL212</i>	5_Nsp	Beta-proteobacteria
71906225	<i>Dechloromonas aromatica RCB</i>	6_Dar	Beta-proteobacteria
30249970	<i>Nitrosomonas europaea ATCC 19718</i>	7_Neu	Beta-proteobacteria
339484394	<i>Nitrosomonas sp. Is79A3</i>	8_Nsp	Beta-proteobacteria
91775721	<i>Methylobacillus flagellatus KT</i>	9_Mfl	Beta-proteobacteria
91782889	<i>Burkholderia xenovorans LB400</i>	10_Bxe	Beta-proteobacteria
74317193	<i>Thiobacillus denitrificans ATCC 25259</i>	11_Tde	Beta-proteobacteria
74318079	<i>Thiobacillus denitrificans ATCC 25259</i>	12_Tde	Beta-proteobacteria
82701851	<i>Nitrosospira multiformis ATCC 25196</i>	13_Nmu	Beta-proteobacteria
114331286	<i>Nitrosomonas eutropha C91</i>	14_Neu	Beta-proteobacteria
89899325	<i>Rhodoferax ferrireducens T118</i>	15_Rfe	Beta-proteobacteria
121604015	<i>Polaromonas naphthalenivorans CJ2</i>	16_Pna	Beta-proteobacteria
186476555	<i>Burkholderia phymatum STM815</i>	17_Bph	Beta-proteobacteria
302877866	<i>Gallionella capsiferriformans ES-2</i>	18_Gca	Beta-proteobacteria
302878592	<i>Gallionella capsiferriformans ES-2</i>	19_Gca	Beta-proteobacteria
171058572	<i>Leptothrix cholodnii SP-6</i>	20_Lch	Beta-proteobacteria
120611645	<i>Acidovorax citrulli AAC00-1</i>	21_Aci	Beta-proteobacteria
257093709	<i>Candidatus Accumulibacter phosphatis clade IIA str. UW-1</i>	22_CAc	Beta-proteobacteria
239813432	<i>Variovorax paradoxus S110</i>	23_Vpa	Beta-proteobacteria
237748940	<i>Oxalobacter formigenes OXCC13</i>	24_Ofo	Beta-proteobacteria
291614267	<i>Sideroxydans lithotrophicus ES-1</i>	25_Sli	Beta-proteobacteria
253998981	<i>Methylovorus glucosetrophus SIP3-4</i>	26_Mgl	Beta-proteobacteria
253996202	<i>Methylotenera mobilis JLW8</i>	27_Mmo	Beta-proteobacteria
294788710	<i>Simonsiella muelleri ATCC 29453</i>	28_Smu	Beta-proteobacteria
298369492	<i>Neisseria sp. oral taxon 014 str. F0314</i>	29_Nsp	Beta-proteobacteria
297538687	<i>Methylotenera versatilis 301</i>	30_Mve	Beta-proteobacteria
375105315	<i>Burkholderiales bacterium JOSHI_001</i>	31_Bba	Beta-proteobacteria
319944837	<i>Lautropia mirabilis ATCC 51599</i>	32_Lmi	Beta-proteobacteria
383758760	<i>Rubrivivax gelatinosus IL144</i>	33_Rge	Beta-proteobacteria
395008322	<i>Acidovorax sp. CF316</i>	34_Asp	Beta-proteobacteria
394987804	<i>Sulfuricella denitrificans skB26</i>	35_Sde	Beta-proteobacteria
394987872	<i>Sulfuricella denitrificans skB26</i>	36_Sde	Beta-proteobacteria
394988308	<i>Sulfuricella denitrificans skB26</i>	37_Sde	Beta-proteobacteria
300113472	<i>Nitrosococcus watsonii C-113</i>	38_Nwa	Gamma-proteobacteria
114320119	<i>Alkalilimnicola ehrlichii MLHE-1</i>	39_Aeh	Gamma-proteobacteria
114319816	<i>Alkalilimnicola ehrlichii MLHE-1</i>	40_Aeh	Gamma-proteobacteria
121998643	<i>Halorhodospira halophila SL1</i>	41_Hha	Gamma-proteobacteria
121997897	<i>Halorhodospira halophila SL1</i>	42_Hha	Gamma-proteobacteria
292491590	<i>Nitrosococcus halophilus Nc4</i>	43_Nha	Gamma-proteobacteria
292491218	<i>Nitrosococcus halophilus Nc4</i>	44_Nha	Gamma-proteobacteria
350561821	<i>Thioalkalivibrio thiocyanoxidans ARh 4</i>	45_Tth	Gamma-proteobacteria
350561659	<i>Thioalkalivibrio thiocyanoxidans ARh 4</i>	46_Tth	Gamma-proteobacteria
88813574	<i>Nitrococcus mobilis Nb-231</i>	47_Nmo	Gamma-proteobacteria

88811612	<i>Nitrococcus mobilis</i> Nb-231	48_Nmo	Gamma-proteobacteria
229528475	<i>Vibrio cholerae</i> 12129(1)	49_Vch	Gamma-proteobacteria
229529245	<i>Vibrio cholerae</i> 12129(1)	50_Vch	Gamma-proteobacteria
148977548	<i>Vibrionales bacterium</i> SWAT-3	51_Vba	Gamma-proteobacteria
148975147	<i>Vibrionales bacterium</i> SWAT-3	52_Vba	Gamma-proteobacteria
344943819	<i>Methylobacter tundripaludum</i> SV96	53_Mtu	Gamma-proteobacteria
344940183	<i>Methylobacter tundripaludum</i> SV96	54_Mtu	Gamma-proteobacteria
119946654	<i>Psychromonas ingrahamii</i> 37	55_Pin	Gamma-proteobacteria
119946655	<i>Psychromonas ingrahamii</i> 37	56_Pin	Gamma-proteobacteria
119945042	<i>Psychromonas ingrahamii</i> 37	57_Pin	Gamma-proteobacteria
333892129	<i>Alteromonas</i> sp. SN2	58_Asp	Gamma-proteobacteria
333893518	<i>Alteromonas</i> sp. SN2	59_Asp	Gamma-proteobacteria
149910892	<i>Moritella</i> sp. PE36	60_Msp	Gamma-proteobacteria
149911154	<i>Moritella</i> sp. PE36	61_Msp	Gamma-proteobacteria
119468777	<i>Alteromonadales bacterium</i> TW-7	62_Aba	Gamma-proteobacteria
16131304	<i>Escherichia coli</i> str. K-12 substr. MG1655	63_Eco	Gamma-proteobacteria
226330079	<i>Proteus penneri</i> ATCC 35198	64_Ppe	Gamma-proteobacteria
212710453	<i>Providencia alcalifaciens</i> DSM 30120	65_Pal	Gamma-proteobacteria
384138208	<i>Yersinia pestis</i> A1122	66_Ype	Gamma-proteobacteria
392953280	<i>Hydrocarboniphaga effusa</i> AP103	67_Hef	Gamma-proteobacteria
258543877	<i>Cardiobacterium hominis</i> ATCC 15826	68.Cho	Gamma-proteobacteria
192360471	<i>Cellvibrio japonicus</i> Ueda107	69_Cja	Gamma-proteobacteria
255020713	<i>Acidithiobacillus caldus</i> ATCC 51756	70_Aca	Gamma-proteobacteria
260914094	<i>Pasteurella dagmatis</i> ATCC 43325	71_Pda	Gamma-proteobacteria
15890896	<i>Agrobacterium tumefaciens</i> str. C58	72_Atu	Alpha-proteobacteria
27381569	<i>Bradyrhizobium japonicum</i> USDA 110	73_Bja	Alpha-proteobacteria
294677377	<i>Rhodobacter capsulatus</i> SB 1003	74_Rca	Alpha-proteobacteria
146277181	<i>Rhodobacter sphaeroides</i> ATCC 17025	75_Rsp	Alpha-proteobacteria
115522826	<i>Rhodopseudomonas palustris</i> BisA53	76_Rpa	Alpha-proteobacteria
83593581	<i>Rhodospirillum rubrum</i> ATCC 11170	77_Rru	Alpha-proteobacteria
328953288	<i>Desulfobacca acetoxidans</i> DSM 11109	78_Dac	Delta-proteobacteria
224367273	<i>Desulfobacterium autotrophicum</i> HRM2	79_Dau	Delta-proteobacteria
224367976	<i>Desulfobacterium autotrophicum</i> HRM2	80_Dau	Delta-proteobacteria
224369989	<i>Desulfobacterium autotrophicum</i> HRM2	81_Dau	Delta-proteobacteria
392410016	<i>Desulfomonile tiedjei</i> DSM 6799	82_Dti	Delta-proteobacteria
95930373	<i>Desulfuromonas acetoxidans</i> DSM 684	83_Dac	Delta-proteobacteria
78223964	<i>Geobacter metallireducens</i> GS-15	84_Gme	Delta-proteobacteria
262198960	<i>Haliangium ochraceum</i> DSM 14365	85_Hoc	Delta-proteobacteria
162453622	<i>Sorangium cellulosum</i> 'So ce 56'	86_Sce	Delta-proteobacteria
162455376	<i>Sorangium cellulosum</i> 'So ce 56'	87_Sce	Delta-proteobacteria
209527099	<i>Arthrosira maxima</i> CS-328	88_Ama	Cyanobacteria
37523829	<i>Gloeobacter violaceus</i> PCC 7421	89_Gvi	Cyanobacteria
17232137	<i>Nostoc</i> sp. PCC 7120	90_Nsp	Cyanobacteria
123968364	<i>Prochlorococcus marinus</i> str. AS9601	91_Pma	Cyanobacteria
16332282	<i>Synechocystis</i> sp. PCC 6803	92_Ssp	Cyanobacteria
46445743	<i>Candidatus Protochlamydia amoebophila</i> UWE25	93_CPr	Chlamydiales
338174863	<i>Parachlamydia acanthamoebiae</i> UV-7	94_Pac	Chlamydiales
89898680	<i>Chlamydophila felis</i> Fe/C-56	95_Cfe	Chlamydiales
330444153	<i>Chlamydophila pecorum</i> E58	96_Cpe	Chlamydiales
16752433	<i>Chlamydophila pneumoniae</i> AR39	97_Cpn	Chlamydiales

301335976	<i>Chlamydia trachomatis L2tet1</i>	98_Ctr	Chlamydiales
225619033	<i>Brachyspira hyodysenteriae WA1</i>	99_Bhy	Spirochaetes
330836901	<i>Sphaerochaeta coccoides DSM 17374</i>	100_Sco	Spirochaetes
383790014	<i>Spirochaeta africana DSM 8902</i>	101_Saf	Spirochaetes
332298391	<i>Treponema brennaborense DSM 12168</i>	102_Tbr	Spirochaetes
161353683	<i>Streptomyces coelicolor A3(2)</i>	103_Sco	Actinobacteria
289757310	<i>Mycobacterium tuberculosis T85</i>	104_Mtu	Actinobacteria
302391855	<i>Acetohalobium arabaticum DSM 5501</i>	105_Aar	Firmicutes
296330046	<i>Bacillus subtilis subsp. <i>spizizenii</i> ATCC 6633</i>	106_Bsu	Firmicutes
238054288	<i>Clostridium cellulolyticum H10</i>	107_Cce	Firmicutes
292670340	<i>Selenomonas noxia ATCC 43541</i>	108_Sno	Firmicutes
397650148	<i>Streptococcus mutans GS-5</i>	109_Smu	Firmicutes
325956380	<i>Lactobacillus acidophilus 30SC</i>	110_Lac	Firmicutes
332982541	<i>Mahella australiensis 50-1 BON</i>	111_Mau	Firmicutes

**Table S4. Glycogen synthase sequences (GlgA) used for phylogenetic analysis.**

GI Accesion	Species	Code	Taxon
120611649	<i>Acidovorax citrulli AAC00-1</i>	1_Aci	Beta-proteobacteria
56479379	<i>Aromatoleum aromaticum EbN1</i>	2_Aar	Beta-proteobacteria
119898092	<i>Azoarcus sp. BH72</i>	3_Asp	Beta-proteobacteria
186474118	<i>Burkholderia phymatum STM815</i>	4_Bph	Beta-proteobacteria
186476556	<i>Burkholderia phymatum STM815</i>	5_Bph	Beta-proteobacteria
91778822	<i>Burkholderia xenovorans LB400</i>	6_Bxe	Beta-proteobacteria
91782888	<i>Burkholderia xenovorans LB400</i>	7_Bxe	Beta-proteobacteria
375105316	<i>Burkholderiales bacterium JOSHI_001</i>	8_Bba	Beta-proteobacteria
257093710	<i>Candidatus Accumulibacter phosphatis clade IIA str. UW-1</i>	9_CAc	Beta-proteobacteria
71906220	<i>Dechloromonas aromatica RCB</i>	10_Dar	Beta-proteobacteria
302878554	<i>Gallionella capsiferriformans ES-2</i>	11_Gca	Beta-proteobacteria
319944836	<i>Lautropia mirabilis ATCC 51599</i>	12_Lmi	Beta-proteobacteria
171058573	<i>Leptothrix cholodnii SP-6</i>	13_Lch	Beta-proteobacteria
91775825	<i>Methylobacillus flagellatus KT</i>	14_Mfl	Beta-proteobacteria
253996721	<i>Methylotenera mobilis JLW8</i>	15_Mmo	Beta-proteobacteria
297538699	<i>Methylotenera versatilis 301</i>	16_Mve	Beta-proteobacteria
253999146	<i>Methylovorus glucosetrophus SIP3-4</i>	17_Mgl	Beta-proteobacteria
30250196	<i>Nitrosomonas europaea ATCC 19718</i>	18_Neu	Beta-proteobacteria
114330623	<i>Nitrosomonas eutropha C91</i>	19_Neu	Beta-proteobacteria
325983313	<i>Nitrosomonas sp. AL212</i>	20_Nsp	Beta-proteobacteria
339484398	<i>Nitrosomonas sp. Is79A3</i>	21_Nsp	Beta-proteobacteria
82701849	<i>Nitrosospira multiformis ATCC 25196</i>	22_Nmu	Beta-proteobacteria
237748939	<i>Oxalobacter formigenes OXCC13</i>	23_Ofo	Beta-proteobacteria
121604016	<i>Polaromonas naphthalenivorans CJ2</i>	24_Pna	Beta-proteobacteria
89899324	<i>Rhodoferax ferrireducens T118</i>	25_Rfe	Beta-proteobacteria
383758759	<i>Rubrivivax gelatinosus IL144</i>	26_Rge	Beta-proteobacteria
291614268	<i>Sideroxydans lithotrophicus ES-1</i>	27_Sli	Beta-proteobacteria
217970453	<i>Thauera sp. MZ1T</i>	28_Tsp	Beta-proteobacteria
74318075	<i>Thiobacillus denitrificans ATCC 25259</i>	29_Tde	Beta-proteobacteria
296135571	<i>Thiomonas intermedia K12</i>	30_Tin	Beta-proteobacteria
239813431	<i>Variovorax paradoxus S110</i>	31_Vpa	Beta-proteobacteria
255020599	<i>Acidithiobacillus caldus ATCC 51756</i>	32_Aca	Gamma-proteobacteria
114320117	<i>Alkalilimnicola ehrlichii MLHE-1</i>	33_Aeh	Gamma-proteobacteria
333894848	<i>Alteromonas sp. SN2</i>	34_Asp	Gamma-proteobacteria
192361131	<i>Cellvibrio japonicus Ueda107</i>	35_Cja	Gamma-proteobacteria
16131303	<i>Escherichia coli str. K-12 substr. MG1655</i>	36_Eco	Gamma-proteobacteria
121997895	<i>Halorhodospira halophila SL1</i>	37_Hha	Gamma-proteobacteria
344940181	<i>Methylobacter tundripaludum SV96</i>	38_Mtu	Gamma-proteobacteria
292490263	<i>Nitrosococcus halophilus Nc4</i>	39_Nha	Gamma-proteobacteria
292492253	<i>Nitrosococcus halophilus Nc4</i>	40_Nha	Gamma-proteobacteria
300112811	<i>Nitrosococcus watsonii C-113</i>	41_Nwa	Gamma-proteobacteria
300114912	<i>Nitrosococcus watsonii C-113</i>	42_Nwa	Gamma-proteobacteria
260914093	<i>Pasteurella dagmatis ATCC 43325</i>	43_Pda	Gamma-proteobacteria
226330078	<i>Proteus penneri ATCC 35198</i>	44_Ppe	Gamma-proteobacteria
212710452	<i>Providencia alcalifaciens DSM 30120</i>	45_Pal	Gamma-proteobacteria
119946006	<i>Psychromonas ingrahamii 37</i>	46_Pin	Gamma-proteobacteria
119946656	<i>Psychromonas ingrahamii 37</i>	47_Pin	Gamma-proteobacteria

350562434	<i>Thioalkalivibrio thiocyanoxidans</i> ARh 4	48_Tth	Gamma-proteobacteria
229529246	<i>Vibrio cholerae</i> 12129(1)	49_Vch	Gamma-proteobacteria
148977547	<i>Vibrionales bacterium</i> SWAT-3	50_Vba	Gamma-proteobacteria
384138209	<i>Yersinia pestis</i> A1122	51_Ype	Gamma-proteobacteria
15890897	<i>Agrobacterium tumefaciens</i> str. C58	52_Atu	Alpha-proteobacteria
16119514	<i>Agrobacterium tumefaciens</i> str. C58	53_Atu	Alpha-proteobacteria
27377889	<i>Bradyrhizobium japonicum</i> USDA 110	54_Bja	Alpha-proteobacteria
27381570	<i>Bradyrhizobium japonicum</i> USDA 110	55_Bja	Alpha-proteobacteria
294677376	<i>Rhodobacter capsulatus</i> SB 1003	56_Rca	Alpha-proteobacteria
146277182	<i>Rhodobacter sphaeroides</i> ATCC 17025	57_Rsp	Alpha-proteobacteria
115522825	<i>Rhodopseudomonas palustris</i> BisA53	58_Rpa	Alpha-proteobacteria
83593580	<i>Rhodospirillum rubrum</i> ATCC 11170	59_Rru	Alpha-proteobacteria
328952818	<i>Desulfobacca acetoxidans</i> DSM 11109	60_Dac	Delta-proteobacteria
328954080	<i>Desulfobacca acetoxidans</i> DSM 11109	61_Dac	Delta-proteobacteria
224369988	<i>Desulfobacterium autotrophicum</i> HRM2	62_Dau	Delta-proteobacteria
95930377	<i>Desulfuromonas acetoxidans</i> DSM 684	63_Dac	Delta-proteobacteria
78221927	<i>Geobacter metallireducens</i> GS-15	64_Gme	Delta-proteobacteria
78224366	<i>Geobacter metallireducens</i> GS-15	65_Gme	Delta-proteobacteria
262199344	<i>Haliangium ochraceum</i> DSM 14365	66_Hoc	Delta-proteobacteria
162449333	<i>Sorangium cellulosum</i> 'So ce 56'	67_Sce	Delta-proteobacteria
162451433	<i>Sorangium cellulosum</i> 'So ce 56'	68_Sce	Delta-proteobacteria
46447230	<i>Candidatus Protochlamydia amoebophila</i> UWE25	69_CPr	Chlamydiales
301335380	<i>Chlamydia trachomatis</i> L2tet1	70_Ctr	Chlamydiales
89898000	<i>Chlamydophila felis</i> Fe/C-56	71_Cfe	Chlamydiales
330443835	<i>Chlamydophila pecorum</i> E58	72_Cpe	Chlamydiales
16752082	<i>Chlamydophila pneumoniae</i> AR39	73_Cpn	Chlamydiales
338175893	<i>Parachlamydia acanthamoebae</i> UV-7	74_Pac	Chlamydiales
209525093	<i>Arthrosira maxima</i> CS-328	75_Ama	Cyanobacteria
37520501	<i>Gloeobacter violaceus</i> PCC 7421	76_Gvi	Cyanobacteria
17227527	<i>Nostoc</i> sp. PCC 7120	77_Nsp	Cyanobacteria
17229371	<i>Nostoc</i> sp. PCC 7120	78_Nsp	Cyanobacteria
123968200	<i>Prochlorococcus marinus</i> str. AS9601	79_Pma	Cyanobacteria
16329217	<i>Synechocystis</i> sp. PCC 6803	80_Ssp	Cyanobacteria
16331219	<i>Synechocystis</i> sp. PCC 6803	81_Ssp	Cyanobacteria
384435276	<i>Synechocystis</i> sp. PCC 6803	82_Ssp	Cyanobacteria
384437283	<i>Synechocystis</i> sp. PCC 6803	83_Ssp	Cyanobacteria
302391856	<i>Acetohalobium arabaticum</i> DSM 5501	84_Aar	Firmicutes
296330048	<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> ATCC 6633	85_Bsu	Firmicutes
220928759	<i>Clostridium cellulolyticum</i> H10	86_Cce	Firmicutes
220929307	<i>Clostridium cellulolyticum</i> H10	87_Cce	Firmicutes
325956382	<i>Lactobacillus acidophilus</i> 30SC	88_Lac	Firmicutes
332982544	<i>Mahella australiensis</i> 50-I BON	89_Mau	Firmicutes
292670336	<i>Selenomonas noxia</i> ATCC 43541	90_Sno	Firmicutes
397650146	<i>Streptococcus mutans</i> GS-5	91_Smu	Firmicutes
225619029	<i>Brachyspira hyodysenteriae</i> WA1	92_Bhy	Spirochaetes
225619571	<i>Brachyspira hyodysenteriae</i> WA1	93_Bhy	Spirochaetes
330836900	<i>Sphaerochaeta coccoides</i> DSM 17374	94_Sco	Spirochaetes
383790017	<i>Spirochaeta africana</i> DSM 8902	95_Saf	Spirochaetes
332298143	<i>Treponema brennaborense</i> DSM 12168	96_Tbr	Spirochaetes