Bacterial community changes in an industrial algae production system

Fulbright et al.

Supplemental Information

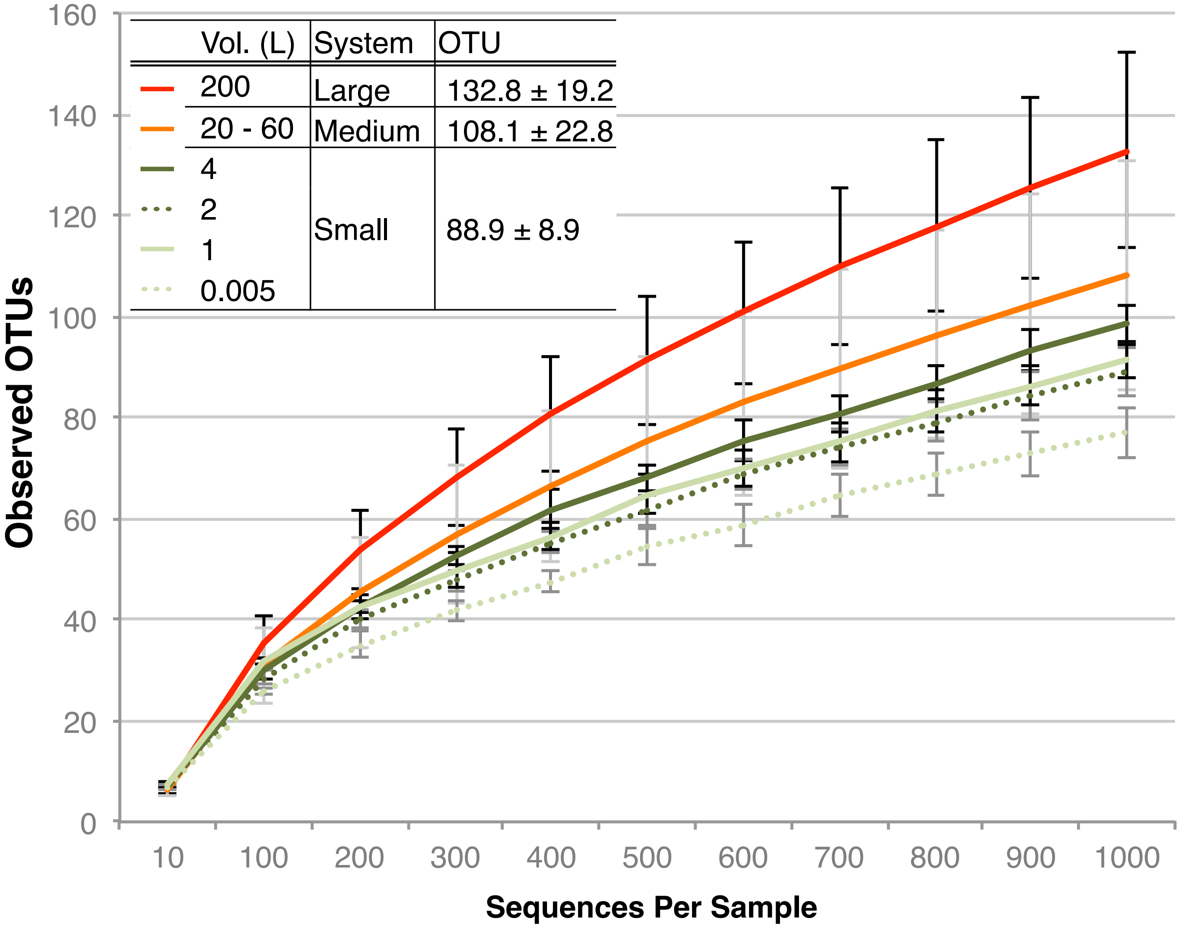
Figure S1. Observed OTUs increase with system scale. Rarefaction curve showing, on average, that there are increased OTUs at larger system scales than smaller ones. Small systems include volumes ranging from 5 mL – 4 L, plotted separately. Average observed OTUs for each system are listed in the sample legend. Error bars (plot) and ranges (±) represent ± 1 standard deviation.

Figure S2. Phylogenetic distance increases among communities in medium and large systems. Rarefaction curve showing phylogenetic distance (PD) increases on average at larger system scales. Small systems include volumes ranging from 5 mL – 4 L, plotted separately. Average PD for each system is listed in the sample legend. Error bars (plot) and ranges (±) represent ± 1 standard deviation.

Table S1. Abundance of bacterial orders across *N. salina* growth systems. Relative abundances of bacterial orders identified in *N. salina* growth systems are represented. Data are formatted as in Table 1. Ph, Phylum (for phylum abbreviations, see Table 1).

Table S2. Bacteria predominant in medium *N. salina* cultivation systems. The ten most abundant bacterial orders identified in medium growth systems are shown. Zero values represent relative abundances less than 0.05%. Ph, Phylum (for phylum abbreviations, see Table 1). Heat maps are formatted separately for each growth system as in Table 1. Σ, total abundance of these ten orders in each system.

Table S3. Comparison of prevalent bacteria in two studies of *Nannochloropsis* cultivations. Comparison of bacteria identified in the cultivations of *N. salina* reported here with those reported by Wang, et al. (2012) in cultivations of *N. oceanica* IMET1.



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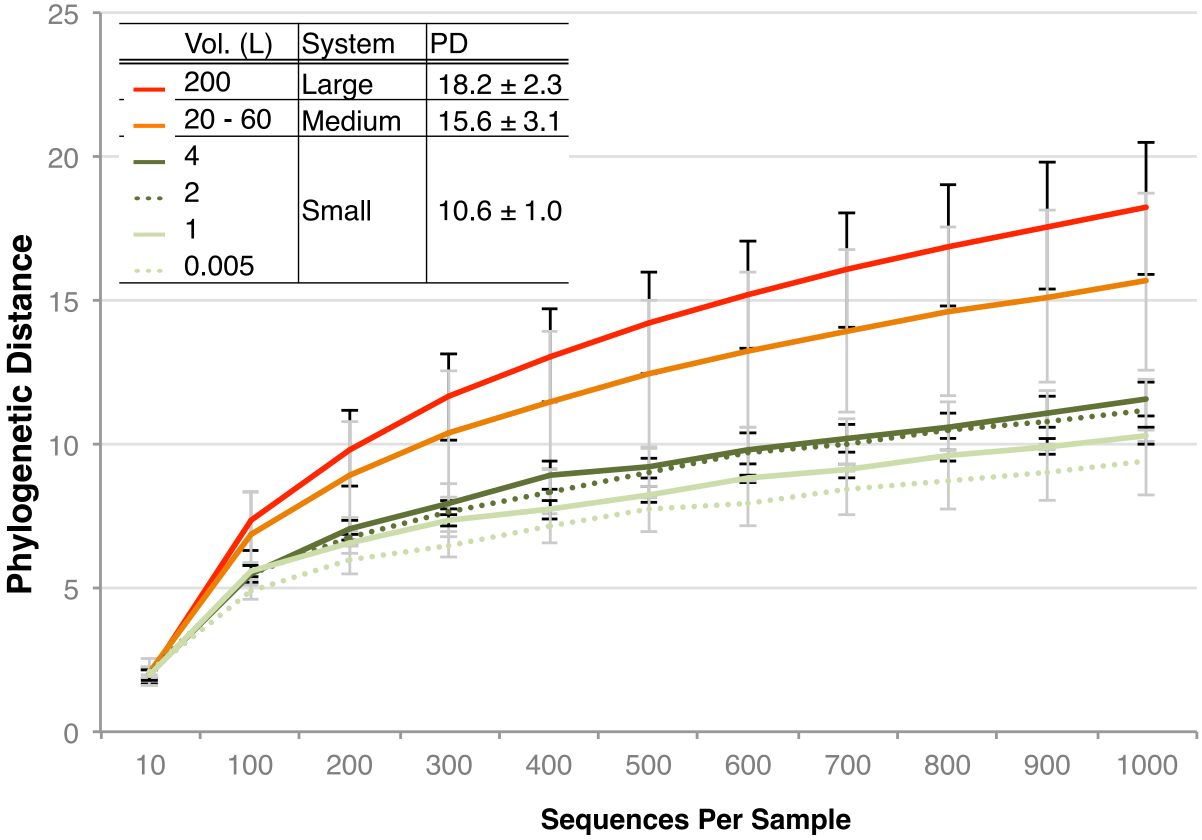

Figure S2. Phylogenetic distance increases among communities in medium and large systems. Rarefaction curve showing phylogenetic distance (PD) increases on average at larger system scales. Small systems include volumes ranging from 5 mL – 4 L, plotted separately. Average PD for each system is listed in the sample legend. Error bars (plot) and ranges (±) represent ± 1 standard deviation.

Ph	Class	Order	Abundance		
			Sml	Med	Lrg
B	Bacteroidia	Bacteroidales	0.0	0.0	0.1
	Cytophagia	Cytophagales	5.0	18.9	22.3
	Flavobacteria	Flavobacteriales	20.1	4.6	3.4
	Sphingobacteria	Sphingobacteriales	0.0	5.6	3.0
	[Rhodothermi]	[Rhodothermales]	0.0	0.2	0.1
	[Saprospirae]	[Saprospirales]	23.2	32.0	37.3
Pr	Alphaproteobacteria		0.1	0.4	0.2
		BD7-3	0.0	1.8	1.5
		Caulobacterales	0.0	0.1	0.1
		Kiloniellales	0.0	1.1	7.1
		Rhizobiales	19.7	8.0	6.2
		Rhodobacterales	8.7	3.6	2.9
		Rhodospirillales	1.1	1.3	2.0
		Rickettsiales	0.0	0.2	0.3
		Sphingomonadales	5.5	2.1	1.4
	Betaproteobacteria	Burkholderiales	0.0	0.0	0.1
		Rhodocyclales	0.0	1.3	0.3
	Deltaproteobacteria	Desulfovibrionales	0.0	0.0	0.4
		Myxococcales	0.0	0.0	0.2
		Spirobacillales	0.0	5.0	1.5
	Epsilonproteobacteria	Campylobacterales	0.0	0.0	0.1
	Gammaproteobacteria	Alteromonadales	6.9	2.2	1.0
		Chromatiales	0.0	0.2	0.1
		Legionellales	0.0	0.1	0.0
		Oceanospirillales	1.6	0.8	0.1
		Vibrionales	0.0	0.0	0.1
		Pseudomonadales	0.0	0.1	0.0
		Thiotrichales	0.0	0.1	0.0
		Vibrionales	0.0	0.0	0.1
		Other	0.1	0.0	0.0
A	Solibacteres	Solibacterales	0.0	0.1	0.1
Ac	Acidimicrobidae	Acidimicrobiales	0.2	0.1	0.0
	Actinobacteria	Actinomycetales	0.2	0.1	0.0
Ar	[Fimbriimonadia]	[Fimbriimonadales]	0.0	0.1	0.0
Ch	Anaerolineae	SBR1031	0.0	0.1	0.1
Cy	4C0d-2	SM1D11	0.0	0.2	0.0
	ML635J-21		2.8	0.3	0.1
	Synechococcophycideae	Pseudanabaenales	0.0	2.2	0.0
F	Clostridia	Clostridiales	0.0	0.0	0.2
P	Planctomycetia	Pirellulales	1.4	1.5	0.7
		Planctomycetales	0.0	0.3	0.1
T	SC3		0.0	0.3	0.0
V	Opitutae	Opitutales	1.1	0.5	0.7
		Puniceicoccales	0.0	0.1	0.2
	Verrucomicrobiae	Verrucomicrobiales	0.0	0.3	1.2
cd	PRR-11 (BRC1)		0.0	0.0	0.1
	ZB2 (OD1)		0.0	0.2	0.2
Unassigned			2.4	3.4	4.4
		$\Sigma =$	100.1	99.5	100.0
		$\Sigma_{(B+Pr)} =$	92.0	89.7	91.9

Table S1. Abundance of bacterial orders across *N. salina* growth systems. Relative abundances of bacterial orders identified in *N. salina* growth systems are represented. Data are formatted as in Table 1. Ph, Phylum (for phylum abbreviations, see Table 1).

			Abundance		
Ph	Class	Order	Sml	Med	Lrg
B	[Saprospirae]	[Saprospirales]	23.2	32.0	37.3
B	Cytophagia	Cytophagales	5.0	18.9	22.3
Pr	Alphaproteobacteria	Rhizobiales	19.7	8.0	6.2
B	Sphingobacteria	Sphingobacteriales	0.0	5.6	3.0
Pr	Deltaproteobacteria	Spirobacillales	0.0	5.0	1.5
B	Flavobacteria	Flavobacteriales	20.1	4.6	3.4
Pr	Alphaproteobacteria	Rhodobacterales	8.7	3.6	2.9
Pr	Gammaproteobacteria	Alteromonadales	6.9	2.2	1.0
C	Synechococcophycideae	Pseudanabaenales	0.0	2.2	0.0
Pr	Alphaproteobacteria	Sphingomonadales	5.5	2.1	1.4
		Σ =	89.1	84.2	79.0

Table S2. Bacteria predominant in medium *N. salina* cultivation systems. The ten most abundant bacterial orders identified in medium growth systems are shown. Zero values represent relative abundances less than 0.05%. Ph, Phylum (for phylum abbreviations, see Table 1). Heat maps are formatted separately for each growth system as in Table 1. Σ, total abundance of these ten orders in each system.

Bacteria isolated from *Nannochloropsis oceanica* IMET1 cultures*									Organism Taxonomy** / Relationship to OTUs Identified in Industrial *N. salina* Cultivations***				
		Temp:	15˚C		25˚C		30˚C						
Identity****	**GenBank nt**	**GenBank Source Organism** Day:	0	9	0	9	0	9	**Phylum**	**Class**	**Order**	**Family**	**Genus**
98%	EU073067.1	Rhodococcus sp. TS1		🟩					Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus
99%	EU440992.1	Microbacterium schleiferi					🟩	🟩	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium
95%	AB073576.2	Cytophaga sp. MBIC04669	🟩	🟩	🟩		🟩		Bacteroidetes	Cytophagia	Cytophagales	**Cytophagaceae**	Cytophaga
98%	DQ080996.1	Roseivirga spongicola			🟩		🟩	🟩	Bacteroidetes	Cytophagia	**Cytophagales**	Flammeovirgaceae	Roseivirga
98%	EU443204.1	Jejuia pallidilutea	🟩	🟩	🟩	🟩		🟩	Bacteroidetes	Flavobacteriia	**Flavobacteriales**	Flavobacteriaceae	Jejuia
95%	AB526333.1	Maribacter sp. JAM-BA06				🟩			Bacteroidetes	Flavobacteriia	**Flavobacteriales**	Flavobacteriaceae	Maribacter
95%	FJ229467.1	Maribacter sp. KLE1063		🟩					Bacteroidetes	Flavobacteriia	**Flavobacteriales**	Flavobacteriaceae	Maribacter
100%	HM355805.1	Muricauda beolgyonensis				🟩		🟩	Bacteroidetes	Flavobacteriia	**Flavobacteriales**	Flavobacteriaceae	Muricauda
98%	AJ231180.1	Planctomycete str. 391						🟩	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	
99%	AB257592.1	Hoeflea sp. KF7	🟩						Proteobacteria	Alphaproteobacteria	Rhizobiales	**Phyllobacteriaceae**	Hoeflea
99%	AY332116.1	Mesorhizobium sp. GWS-BW-H238			🟩	🟩			Proteobacteria	Alphaproteobacteria	Rhizobiales	**Phyllobacteriaceae**	Mesorhizobium
97%	FJ230835.1	Henriciella litoralis					🟩		Proteobacteria	Alphaproteobacteria	Rhodobacterales	**Hyphomonadaceae**	Henriciella
94%	AB377216.1	Hyphomonadaceae bacterium NAMAF001						🟩	Proteobacteria	Alphaproteobacteria	Rhodobacterales	**Hyphomonadaceae**	
96%	AJ605746.2	Roseibaca ekhonensis						🟩	Proteobacteria	Alphaproteobacteria	**Rhodobacterales**	Rhodobacteraceae	Roseibaca
99%	FJ868588.1	Roseobacter sp. RAS3					🟩		Proteobacteria	Alphaproteobacteria	**Rhodobacterales**	Rhodobacteraceae	Roseobacter
99%	AY332663.1	Roseobacter sp. TM1042	🟩	🟩		🟩	🟩	🟩	Proteobacteria	Alphaproteobacteria	**Rhodobacterales**	Rhodobacteraceae	Roseobacter
99%	AY682197.1	Sulfitobacter sp. KMM 3457		🟩	🟩				Proteobacteria	Alphaproteobacteria	**Rhodobacterales**	Rhodobacteraceae	Sulfitobacter
96%	GU936120.1	Thalassobacter sp. W-2-2				🟩			Proteobacteria	Alphaproteobacteria	**Rhodobacterales**	Rhodobacteraceae	Thalassobacter
97%	AB302370.1	Tropicibacter naphthalenivorans			🟩	🟩	🟩		Proteobacteria	Alphaproteobacteria	**Rhodobacterales**	Rhodobacteraceae	Tropicibacter
98%	EF512131.1	Rhodobacteraceae bacterium CSQ-8	🟩		🟩		🟩		Proteobacteria	Alphaproteobacteria	**Rhodobacterales**	Rhodobacteraceae	
99%	AB062106.1	Porphyrobacter sanguineus	🟩		🟩	🟩	🟩	🟩	Proteobacteria	Alphaproteobacteria	Sphingomonadales	**Erythrobacteraceae**	Porphyrobacter
99%	DQ412064.1	Marinobacter sp. LOB-4						🟩	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	**Marinobacter**
98%	DQ985041.1	Halomonas sp. JL1044						🟩	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas
89%	GQ867230.1	Pseudomonas sp. BRAZ6-1						🟩	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas

* **Data adapted from Wang, et al. (2012) Supplemental Table S2: "16S rRNA gene sequence identities of isolates from *N. oceanica* IMET1 culture"**; bacteria from *N. oceanica* cultures grown at 15˚C, 25˚C or 30˚C were isolated on Marine Agar at Day 0 and Day 9; *16S rRNA* genes were amplified, sequenced, and used to identify the closest cultured organism in the GenBank nucleotide database; heat map shows bacteria isolated from culltures maintained at the indicated temperature at Day 0 (light green) or Day 9 (dark green)

** Taxonomy based on indicated GenBank Nucleotide Records

*** Highlighting indicates taxonomic classifications shared with OTUs identified in *N. salina* cultivations in the present study. For additional information, see "Color coding of bacterial types" key (below) and "Comments" (far right), plus Tables 1, 2, 3, S1 and S2.

**** Percent identity of *16S rRNA* gene sequences of bacteria isolated in Wang, et al. (2012) compared with respective "Genbank Source Organism." Identities ≤ 97% are shown in red.

Color coding of bacterial types:

Isolated from ***N. oceanica*** cultures at **Day 0***	Isolated from ***N. oceanica*** cultures at **Day 9***	Associated with **90%** of ***N. salina*** cultivations in this study	Associated with **95%** of ***N. salina*** cultivations in this study	Other notable bacteria; see Comments

Comments

An OTU of the Family **Cytophagaceae** was associated with **95%** of *N. salina* cultivations

A second OTU of the Order **Cytophagales** was associated with **90%** of cultivations

Though **not** conserved in 90% or more of *N. salina* cultivations, members of the Order **Flavobacteriales** were, on average, the 2nd most abundant OTUs in Small cultivation systems (20.1%), the 5th most abundant in Large systems (3.4%), and the 6th most abundant in Medium systems (4.6%)

OTUs of the Order **Planctomycetales** averaged 0.3% and 0.1% relative abundance in Medium and Large systems, respectively

3 OTUs of the Order **Rhizobiales** were associated with **95%** of *N. salina* cultivations, including the Family **Phyllobacteriaceae**, plus 2 OTUs of Family Hyphomicrobiaceae

2 OTUs of the Family **Hyphomonadaceae** were each associated with **90%** of *N. salina* cultivations; there were 7 additional members of the Order **Rhodobacterales** associated with *N. oceanica* IMET1

An OTU of the Family **Erythrobacteraceae** was associated with **90%** of *N. salina* cultivations

An OTU of the Genus **Marinobacter** was associated with **90%** of *N. salina* cultivations

OTUs of the Order **Oceanospirillales** were, on average, the 9th most abundant OTUs in Small cultivation systems (1.6%) and were also identified in Medium and Large systems

OTUs of the Order **Pseudomonadales** averaged 0.1% relative abundance in Medium systems

Table S3. Comparison of prevalent bacteria in two studies of *Nannochloropsis* cultivations. Comparison of bacteria identified in the cultivations of *N. salina* reported here with those reported by Wang, et al. (2012) in cultivations of *N. oceanica* IMET1.