Electronic Supplementary Material

ESM_4. Average relative abundance of major groups of plankton operational taxonomic units (OTUs) in Lake Nakuru, Kenya, based on 454 pyrosequencing

to the article published in “Hydrobiologia”, Springer ©

Food algae for Lesser Flamingos: a stocktaking

by
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Phylotypes Group	Closest match taxon (Acc. No. NCBI)	Relative abundance (%) (representative OTU) [a]	Identity	Origin	Reference
Cyanobacteria					
Synechococcales	*Cyanobium* sp. JJ27STR (**AM710383**)	18.69 (OTU2*)	99%	freshwater reservoir, Ceske Budejovice, Czech Republic	Unpublished
Synechococcales	Uncultured *Synechococcus* clone SP-B1-29 (**JF428826)**	20.09 (OTU49*)	99%	shrimp pond sediment, India	Unpublished
Synechococcales	Uncultured *Synechococcus* clone SP-B1-29 (**JF428826**)	4.37 (OTU6*)	99%	shrimp pond sediment, India	Unpublished
Synechococcales	Uncultured *Synechococcus* sp. clone YCC98 (**EF205493**)	2.81(OTU35*)	99%	geothermal spring mat, Central Tibet, China	Lau et al. (2009)
Synechococcales	*Synechococcu*s sp. BE08071 (**FJ763789**)	1.25 (OTU 47*)	97%	eutrophic freshwater, Great Mazurian, Poland	Jasser et al. (2010)
Synechococcales	*Synechocystis minuscula* SAG 258.80 (**KM019989**)	6.93 (OTU41*)	99%	Lake Nakuru, Kenya	Unpublished
Synechococcales	*Synechocystis* sp. SAG 37.92 (**KM020010**)	0.30 (OTU40*)	93%	tropical marine aquarium Scripps Institution, USA	Unpublished
Synechococcales	*Leptolyngbya* sp. BTA356 (**KF953499**)	0.36 (OTU25*)	99%	unknown	Unpublished
Synechococcales	*Haloleptolyngbya alcalis* KR2005/106 (**JN712770**)	0.30 (OTU8*)	99%	Lake Nakuru, Kenya	Dadheech et al. (2012)
Chroococcales	*Geminocystis* sp. 1.1 (**KJ654307**)	2.13 (OTU18*)	99%	fresh wastewater canal, India	Unpublished
Oscillatoriales	*Phormidium* cf. *aerugineo-coeruleum* R-aq (**EU196641**)	4.61(OTU12*)	98%	unpublished, Czech Republic	Unpublished
Oscillatoriales	*Planktothricoides raciborskii* strain NIES-207 (**NR_040858**)	0.95 (OTU24*)	99%	Lake Kasumigaura, Ibaraki, Japan	Suda et al. (2002)
Spirulinales	*Spirulina laxissima* SAG 256.80 (**KM019976**)	3.04 (OTU28*)	99%	Lake Nakuru, Kenya	Unpublished
Nostocales	Uncultured *Anabaenopsis* sp. clone LNK (**JX462679**)	1.46 (OTU19*)	99%	Lake Nakuru. Kenya	Krienitz et al. (2013)
Nostocales	*Nodularia spumigena* GSL023 (**FJ546713**)	8.58(OTU10*)	98%	Great Salt Lake, South Arm, USA	Unpublished
Nostocales	*Nodularia spumigena* strain HEM (**AF268005**)	2.61(OTU26*)	98%	Baltic Sea, brackish	Moffitt et al. (2001)
Cyanobacteria	Uncultured *cyanobacterium* clone MEsu06cnp11D8 (**FJ828385**)	0.66 (OTU27*)	99%	Lake Mendota, freshwater, USA	Newton et al. (2011)
Cyanobacteria	Uncultured *cyanobacterium* clone Alchichica_AQ1_2_1B_148 (**JN825308**)	0.38 (OTU37*)	96%	Lake Alchichica, alkaline, Mexico	Couradeau et al. (2011)
Cyanobacteria	Uncultured *cyanobacterium* clone: 91251007 (**AB935900**)	0.41 (OTU31*)	98%	Lake Hachiro-ko, Akita, Japan	Okano et al. (2015)
Cyanobacteria	Uncultured *cyanobacteriaum* clone 5-31 EH 287 (**HQ724806**)	0.37 (OTU46*)	98%	Erhai Lake, China	Unpublished

Algae					
Haptophyta	Plastid *Isochrysis* sp. SAG 927-2 (**X75518**)	11.39 (OTU44*)	99%	Strain SAG 927-2	Unpublished
Eustigmatophyceae	Plastid *Nannochloropsis limnetica* (**KC598089**)	11.30 (OTU50*)	99%	Strain CCMP505	Wei et al. (2013)
Chlorophyta	Plastid *Picochlorum* sp. UMPCCC 1108 (**KM218890**)	0.21 (OTU43*)	99%	commercial evaporated sea salts	Unpublished
Bacillariophyceae	*Anomoeoneis sphaerophora* strain FD160 (**KJ011612**)	27.37 (OTU29)	96%	Strain FD160	Nakov et al. (2014)
Dinophyta	Uncultured alveolate clone NKS106 (**JX296581**)	16.10 (OTU57)	98%	Lake Nakuru, Kenya	Luo et al. (2013)
(Alveolate)?	Uncultured eukaryote clone KRL01E37 (**JN090897**)	0.31 (OTU80)	98%	Lake Karla, Greece	Oikonomou et al. (2012)
Metazoa					
Rotifera	*Brachionus plicatilis* (**U49911**)	25.76 (OTU124)	99%	-	Aguinaldo et al. (1997)
Rotifera	*Ptygura libera* (**DQ297689**)	18.75 (OTU99)	97%	-	Sorensen & Giribet (2006)
Rotifera	*Notommata allantois* (**DQ297710**)	6.84 (OTU52)	98%	-	Sorensen & Giribet (2006)
Rotifera	*Filinia longiseta* (**DQ079914**)	1.14 (OTU68)	97%	-	Sorensen et al. (2006)
Rotifera	*Euchlanis alata* (**DQ079915**)	0.89 (OTU1)	98%	-	Sorensen et al. (2006)
Rotifera	Uncultured bdelloid rotifer clone G07_Rot_T3T6 (**GQ922334**)	3.51 (OTU22)	98%	Niwot Ridge Talus soil from T3T6, Colorado,USA	Robeson et al. (2009)
Chaetonotidae	*Chaetonotus polyspinosus voucher* TK215 (**JQ798586**)	5.88 (OTU97)	98%	Brazil	Kanneby et al. (2013)
Cercozoa	Uncultured eukaryote clone KRL03E11 (**KC315810**)	0.74 (OTU125)	98%	Lake Karla, Greece	Nikouli et al. (2013)
Cercozoa	Uncultured isolate DB-2703-5 (**EU567262**)	0.67 (OTU123)	93%	Rhizobium	Bass et al. (2009)
Nematoda	Nematoda environmental clone NEMAG25 (KF147667)	3.14 (OTU43)	88%	Agarbadi, west coast, India	Unpublished
Crustacea	*Cancrincola plumipes* (**L81938**)	0.52 (OTU13)	97%	-	Spears et al. (1997)

(Total reads of Cyanobacteria = 42641, total reads of microbial eukaryotes = 19656)

[a] The relative abundance of each representative OTU was determined from Cyanobacteria and Eukaryotes respectively. In order to distinguish two libraries, we marked the OTU from Cyanobacteria library with '*' in the table.

Materials and Methods

PCR and pyrosequencing

The filters were mechanically sliced into small pieces and rinsed with cell lyses-buffer AP1 (Qiagen GmbH, Hilden, Germany). Cells were disrupted with the help of glass beads (Carl Roth GmbH, Karlsruhe, Germany; 0.7 mm) in the Tissuelyser II (Qiagen GmbH, Hilden, Germany). Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) following the instructions given by the manufacturer.

Polymerase chain reactions (PCR) were performed using primers with barcode flanking the hypervariable V4 region of the 18S rRNA gene: 3NDf (Cavalier-Smith et al. 2009) with the reverse primer V4_euk_R2 (Bråte et al. 2010). PCRs were conducted in 20-µL reactions with 0.2 µM each of the primers, ~10 ng of template DNA, 1 × PCR buffer, and 2.5 U of Pfu DNA Polymerase (Promega, USA). The amplification program consisted of an initial denaturation step at 95 °C for 2 min s, followed by 30 cycles of 95 °C for 30 s, 55°C for 30 s, and 72 °C for 30 s, and a final extension of 72 °C for 5 min.

A nested PCR approach was used for the amplification of 16S rRNA gene fragments from the cyanobacterial genomic DNA present in samples. This technique has greater specificity than regular PCR (Zwart et al. 2005). A low number of PCR cycles (20) were used to avoid biased amplification of the 16S rRNA gene fragment. For the first PCR, the primers CYA106F (Nübel et al. 1997; Li et al. 2001) and R4R (Li et al. 2001) were used to selectively amplify long fragments of cyanobacterial 16S rRNA genes from the samples as described previously (Ballot et al. 2004). For the second PCR, tagged 454-pyrosequencing fusion primers, forward CYA359F and reverse primer CYA781R (Nübel et al. 1997) were used. The following PCR protocol was employed for amplification of the V3-V4 region of the 16S rRNA gene: an initial denaturation at 94 °C for 3 min, followed by 19 cycles at 94 °C for 1 min, 55 °C for 45s and 72 °C for 1 min, followed by a final extension of 5 min at 72 °C.

All PCR products were pooled and purified using the DNA gel extraction kit (Axygen, Hangzhou, China). The DNA concentration of each PCR product was determined using a QuantiFluor™ -ST PicoGreen double-stranded DNA assay (Promega, USA) and was quality controlled on a TBS-380 Mini-Fluorometer (Turner Biosystems, Sunnyvale, CA, USA). Finally, amplicons of all samples were pooled in equimolar concentrations. Amplification and sequencing on the Roche Genome Sequencer FLX + were done according to the Roche GS FLX+ Sequencing Method Manual_XLR70 kit (Roche Diagnostics GmbH).

Diversity and community structure analyses

Analysis of sequences were operated by Usearch (version 7.1 http://drive5.com/uparse/) in this study. Previous studies described sources of errors in 454 sequencing runs. Hence, barcodes and primer sites were removed, and the valid reads should comply with the following rules: each pyrosequencing read containing a primer sequence should be ≥200 bp in length, must have no ambiguous bases, must match the primer and one of the used barcode sequences, and should present at least an 80 % match to a previously determined SSU rRNA gene sequence. Poor-quality sequences and suspected chimeras were checked by using BLAST with sequence segments separately, and then using the Chimera check program UCHIME (http://drive5.com/usearch/manual/uchime_algo.html). Singletons were deleted. Tag sequences found in this study were deposited at the NCBI under the accession number SRP064229.

In order to determine OTUs, SILVA database (Release 119: http://www.arb-silva.de) which contained high quality SSU rRNA genes was chosen as templates to cyanobacteria, and Unite (Release 6.0 http://unite.ut.ee/index.php) was chosen to microbial eukaryotes. Sequences were clustered into operational taxonomic units (OTUs) defined by 97 % similarity. We randomly picked 19,656 eukaryotic sequencing reads and 42,641 cyanobacteria sequencing reads from Lake Nakuru. Sequence reads with an average of 409 bps (eukaryotes) and 426 bps (cyanobacteria) were generated after trimming of the primer sequences from the beginning and end of the raw data. A total 91 OTUs of eukaryotes and 44 OTUs of cyanobacteria were determined according to following estimates:

Comparison of the estimated OTU richness and diversity indices of the 16S rRNA and 18S rRNA gene libraries for clustering at 97% (3%) identity, as obtained from the pyrosequencing analysis in Lake Nakuru.

Lake Nakuru	**Reads**	**OTU**	**ace**	**chao**	**coverage**	**shannon**	**simpson**
Cyanobacteria	42641	44	44 (44,44)	44 (44,44)	1.000000	2.52 (2.51,2.53)	0.1195 (0.1181,0.1209)
Eukaryotes	19656	91	93 (91,99)	93 (91,103)	0.999746	2.51 (2.49,2.53)	0.1405 (0.1379,0.1431)

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