Is there a succession in the autotrophic picoplankton of temperate zone lakes?

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ABSTRACT

The seasonal periodicity in phytoplankton species composition as well as its causes were extensively studied in Lake Constance located on the northern fringe of the European Alps. Algal species and their predators turn up and disappear in specific phases of the season. However, in the microbial loop small coccoid cyanobacteria of the *Synechococcus* type dominate the autotrophic picoplankton throughout the year. In this size class, a recurrent pattern of seasonal differences in abundance is observed. *Synechococcus* spp. isolated from this environment were genetically diverse and genotypes were shown to differ in growth rates with variances enhanced by exposure to stress. Furthermore, small predators from the same environment exhibited differential feeding preferences for single *Synechococcus* strains. These results indicated that genotypes may have adapted to highly specific niches. Phylogenetic analyses of the 16S rDNA and the 16S-23S ribosomal spacer region (ITS-1) showed that this adaptation could have occurred during a recent radiation. Further analysis of the population was impeded by a low plating efficiency, scarcity of morphological characteristics and lack of divergence in 16S rRNA sequences. For discrimination and quantification of genotypes on basis of ITS-1 target sequences the application of fluorescent TaqMan probes in *Taq* nuclease assays (TNA) was examined.

Ecology of photoautotrophs in temperate zone lakes

In 1986 the plankton ecology group (PEG) headed by U. Sommer proposed a model that describes the seasonal events which occur in the phytoplankton and zooplankton of temperate zone lakes [12]. Briefly, the most important factor for phytoplankton growth is the seasonal pattern of stratification and overturn. In deep lakes such as Lake Constance (surface area 500 km^2, maximum depth 254 m, mean depth 100 m) winter overturn resets the community close to zero each year. Algal mass growth starts with the onset of stratification allowing algae to stay permanently in the euphotic zone. Under continuously stratified conditions, small rapidly-growing algae will soon decrease the water transparency, deplete nutrients, and allow the build-up of grazer populations. The spring bloom is terminated when grazing exceeds algal reproduction leading to a rapid decline of algal biomass. After this clear-water phase, different species will rise and constitute the highly diverse summer phytoplankton. Changes in the summer population are controlled by successive depletion of different nutrients and a replacement of more edible algae by poorly edible species.

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The PEG model does not include the autotrophic picoplankton. This size fraction, mainly consisting of small (0.2 - 2 μm) chroococcoid cyanobacteria, contributes little (<5%) to phytoplankton biomass but was estimated to account for 25% of the primary production of Lake Constance [14]. The ecology of the picocyanobacteria was studied in this lake from 1987 to 1997, a period of declining winterly phosphorus concentration [6]. The small autotrophs showed a recurrent pattern of seasonal differences in abundance with peaks in spring and late summer, interspersed by a pronounced minimum during and after the clear-water phase. As with higher algae, the growth period of picocyanobacteria predictably starts with the decrease in vertical mixing intensity in the upper 20 m of the water column. However, neither an unequivocal response to changes in nutrient concentrations was observed nor a decline of the population in autumn correlated with the decrease in phyto-plankton biovolume or with vertical mixing. Furthermore, no consistent trends in control of picocyanobacteria by daphnids, the most important grazers of algal phytoplankton, were observed. However, the predator/prey relations may be complex because some daphnids may not be able to filter small picocyanobacteria but may thrive on their predators, small ciliates and flagellates, thereby reducing the grazing pressure on picocyanobacteria.

Synechococcus diversity

Fluorescence characteristics indicated that red-pigmented, phycoerythrin (PE)-rich *Synechococcus* spp. dominate over bluegreen, phycocyanin (PC)-rich strains in the pelagic zone of Lake Constance. The average biovolume ranges from 0.3 to 1.2 μm^3, with slightly bigger cells occurring in summer than in winter [14]. Structural differences in the outer membrane of picocyanobacteria were disclosed by electron microscopic examination of water samples (Fig. 1) that were collected from the pelagic zone of the deep north-western basin of Lake Constance (Lake Überlingen). From the same sampling site, 25 unialgal cultures of *Synechococcus* spp. were established between 1988 and 1994 [2,3,10]. The isolates will be referred to as 'pelagic *Synechococcus* spp.' in order to distinguish them from *Synechococcus* spp. thriving in other ecosystems. Restriction fragment length polymorphism (RFLP) of DNA fragments that carry *psbA* genes coding for D1-proteins of photosystem II showed that about half of all cultured isolates represented unique genotypes [10]. The outer membrane of most isolates exhibited no remarkable structure when examined by TEM but one genotype (two isolates, BO8807 and BO8810) was completely covered by a highly glycosylated protein [4] whose structure differed from those depicted in Fig. 1. Thus the picocyanobacteria population of this lake appears genetically and structurally diverse. Although PE-rich *Synechococcus* strains dominate the autotrophic picoplankton of Lake Constance, PC-rich isolates were also obtained. Both pigment types lack complementary chromatic adaptation of phycobiliproteins but, under similar nutrient and light conditions (BG11, 10 $\mu E\ m^{-2}\ s^{-1}$), ratios of phycobiliproteins and carotenoids varied from strain to strain [5] and N-deprivation as well as light stress affected pigmentation of individual strains differently [9,11]. The differences in stress response also affect growth and, as expected, resulted in a strong competitive advantage of the better adapted strain when cultivated together with a strain for which conditions were less favourable [11]. These results

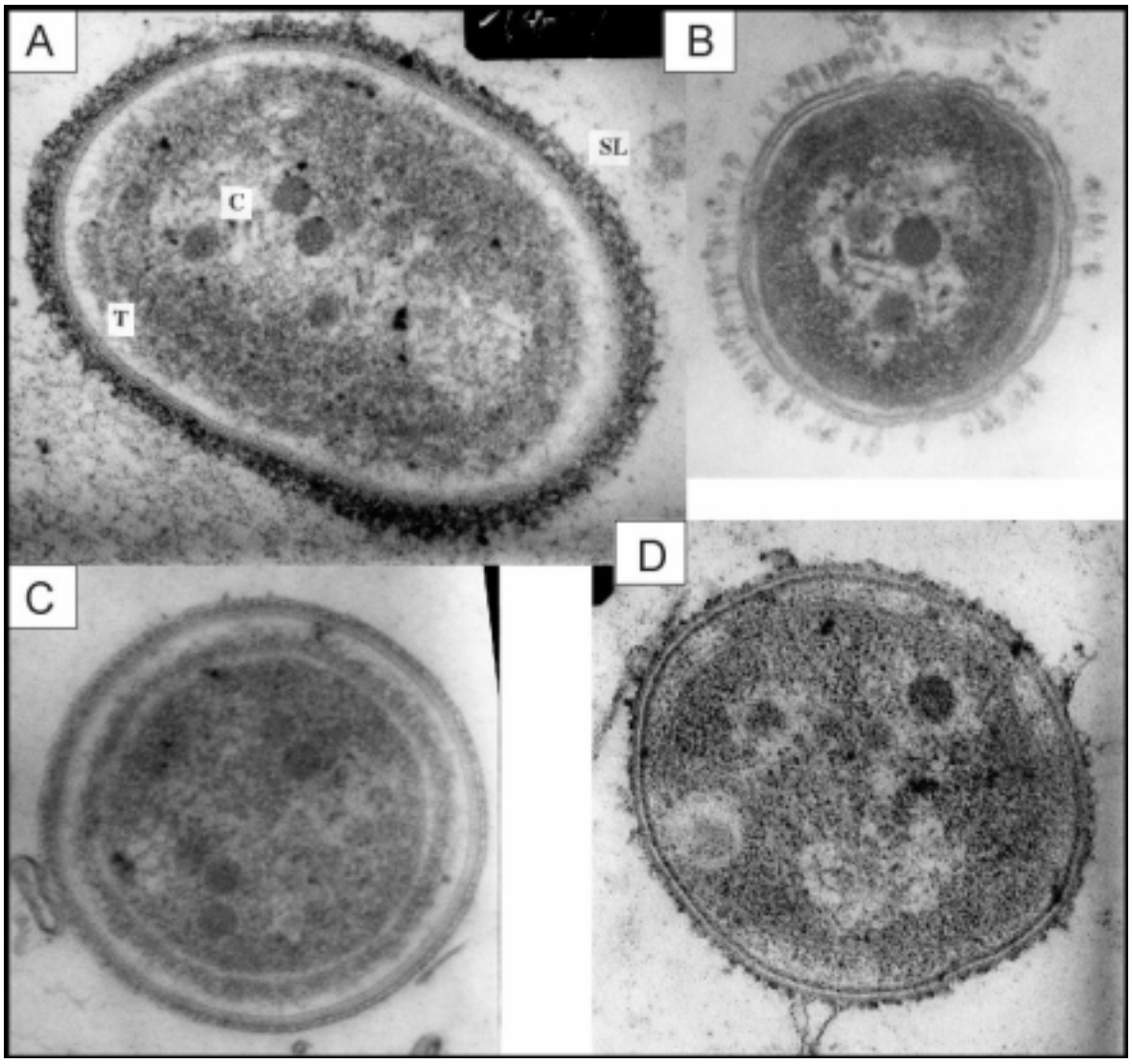

Fig. 1: Structural diversity of the outer membrane revealed by TEM of four non-cultured autotrophic prokaryotes from the pelagic zone of Lake Constance. Cell morphology, presence of carboxysomes and thylakoid spacing characterize the organisms as PE-rich *Synechococcus* spp.; T, thylakoids; C, carboxysomes; SL, surface layer.

indicated that light conditions but also nutrients and temperature may allow different genotypes to explore specific niches during the growth season.

In addition, genetic factors may influence loss rates of picocyanobacteria. In the microbial loop the proliferation of the picoplankton is closely controlled by the heterotrophic nanoplankton [14]. Using cultured *Synechococcus* spp. as feed, a genotype-specific prey selection of *Paraphysomonas* sp. and *Bodo saltans* was demonstrated [1,8]. In particular, *B. saltans* was unable to proliferate on a diet of BO8801 which was the preferred prey of *Paraphysomonas* sp.. When feeding on a coccoid PE-rich strain, BO8809, both predators exhibited higher clearance and growth rates than on the phylogenetically closely related isolate BO8810, one of the strains covered by a unique highly glycosylated S-layer protein [4]. Thus, under laboratory conditions basic requirements for a succession of

species, namely differences in growth and loss rates, were demonstrated. However, to prove succession of species and, hence, niche diversification we have to develop detection tools that allow strain discrimination on a genetic level.

Phylogenetic analysis of pelagic *Synechococcus* spp.

The highly conserved 16S rDNA and a less conserved internal transcribed spacer sequence located between 16S rDNA and 23S rDNA (ITS-1) of the ribosomal operon were amplified from DNA of unialgal *Synechococcus* cultures and sequenced directly. A monophyletic tree with three short terminal branches was inferred from analysis of 1483 nucleotides coding for 16S rDNA of eight pelagic *Synechococcus* spp.. Irrespective of the phylogenetic method used (maximum parsimony, neighbour-joining, or maximum likelihood) PC-rich isolates sorted into two terminal branches while PE-rich *Synechococcus* spp. clustered to a single terminal branch. As the divergence in 16S rDNA was insufficient to discriminate among genotypes assigned to single pigment type we examined the less conserved ITS-1. Non-coding sections of the ITS-1 exhibited multiple mutations including insertions/deletions that allowed us to distinguish all isolates previously characterized as unique genotypes by RFLP of *psbA* genes [3,10]. Phylogenetic analysis of ITS-1 sequences revealed three clades corresponding to the three terminal branches of the 16S rDNA derived tree (Fig. 2). The results indicate that pelagic *Synechococcus* spp. underwent a recent genetic diversification that is hardly seen in a divergence of 16S rDNA but can be traced in the ITS-1. Hence, analysis of a population dominated by closely related PE-rich *Synechococcus* spp. cannot be based on techniques targeting 16S rRNA.

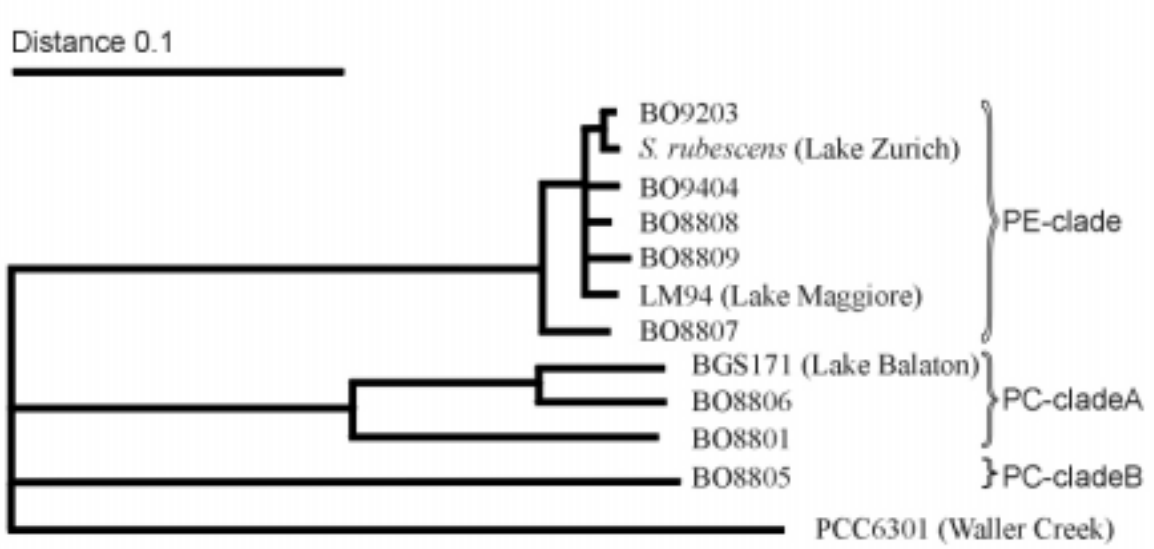

Fig. 2: Phylogenetic tree inferred from analysis of ITS-1 sequences of the ribosomal operon of cultured *Synechococcus* spp.. Tree topology was calculated by the neighbour-joining method as provided in the software package TREECON [13]; insertions/deletions were taken into account. The non-pelagic *Synechococcus* sp. PCC6301 was used as outgroup. The three clades shown correspond to three terminal branches in a tree inferred from 16S rDNA analysis (data not shown).

Development of probes for *Taq* nuclease assays

The low plating efficiency of PE-rich *Synechococcus* spp. not only forced us to develop tools for a cultivation-independent detection of genotypes but also to focus on those strains that were already in culture. For quantification of individual genotypes the feasibility of the *Taq* nuclease assay (TNA), a quantitative PCR assay based on the 5'→3' nuclease activity of *Taq* polymerase [7] was evaluated. The TNA is based on a conventional PCR to which a 3' phosphorylated oligonucleotide probe labelled with a reporter dye and a quencher dye, the TaqMan probe, is added. In the intact probe, the fluorescence of the reporter dye is quenched by the second dye. If the probe hybridizes to a sequence amplified by *Taq* polymerase, the 5'→3' nuclease activity of the enzyme will digest the probe thereby releasing the reporter dye. This results in an increase of the fluorescence signal, measured as Δ RQ. Thus, Δ RQ directly reports the amplification of a specific template. Using PCR primers complementary to conserved parts of the 16S rDNA and 23S rDNA of pelagic *Synechococcus* spp., the ITS-1 of *Synechococcus* isolates was amplified and the specificity of two fluorescent probes, S8807 and S8807A, targeting sequences unique to strain *Synechococcus* sp. BO8807, was examined. PCR conditions were optimized to obtain efficient PCR amplification of ITS-1 with all *Synechococcus* spp. (Table 1, rows 3,4). In the TNA assay, probe S8807 showed a high Δ RQ when ITS-1 of strain BO8807 was amplified but significant fluorescence was also observed with two other strains, BO8808 and BO9404 (Table 1A). On the other hand, probe S8807A was highly specific for BO8807 only (Table 1B). With respect to target sequences in strains BO8808 and BO9404 probe S8807 exhibited mismatching bases at 5' end while probe S8807A had a central mismatch. This result indicates that the specificity of a TNA probe depends on the relative position of a mismatch in target sequences. To avoid ambiguities that may arise from so far unknown target sequences in natural samples, TNA should be complemented by a mutation sensitive analysis of PCR products generated in the TNA, for example by DGGE.

In Lake Constance, abiotic and biotic factors that affect proliferation of isolated *Synechococcus* spp. exhibit seasonal periodicity. Therefore, this temperate zone lake may provide niches with a seasonal component for differentially adapted picocyanobacteria. In order to examine whether the differences among cultivated *Synechococcus* spp. are products of niche diversification and whether the *Synechococcus* population is formed by succession, the use of a quantitative PCR method, TNA, was examined. Preliminary experiments showed that TNA is suitable for identification and quantitative analysis of *Synechococcus* spp. but signal interference is possible among closely related strains. Thus, for an *in situ* analysis of a mixed *Synechococcus* population additional analyses of PCR products are necessary and succession in the microbial loop is still an open question.

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Table 1. Semiquantitative analysis of *Taq* nuclease assays: amount of amplified DNA (PCR product) and normalized fluorescence signal Δ RQ of the reporter in probes S8807 and S8807A.

Template[a]		Amplified DNA[b]		Probe[c]	Δ RQ[d]	
Conc[n] (ng)	Source	relative. amount of DNA	%		relative fluorescence	%
10.0	no template	0	0	S8807	-0.01	0
10.0	BO8807	7.1	92.2	S8807	1.26	100
10.0	BO8808	7.3	94.8	S8807	0.3	23.8
10.0	BO8805	7.1	92.2	S8807	-0.02	0
10.0	BO9404	7.3	94.8	S8807	0.15	11.9
10.0	*A. variabilis*	0	0	S8807	0.01	0.8
10.0	BO8402	4.6	59.7	S8807	-0.02	0
10.0	*Microcystis* sp.	7.7	100	S8807	-0.04	0
10.0	*E. coli*	0	0	S8807	-0.12	0
10.0	A. PCC 7120	n.d.[e]	n.d.	S8807	-0.04	0
1.0	BO8807	6.2	88.6	S8807A	0.83	100
1.0	BO8808	5.9	84.3	S8807A	0.02	2.4
1.0	BO8805	5.4	77.1	S8807A	0.02	2.4
1.0	BO9404	7.0	100	S8807A	0.05	6.0

[a] Template DNA was obtained from *Synechococcus* spp.: BO8805, BO8807, BO8808, BO9404; from a *Synechocystis* sp. BO8402; from two *Anabaena* spp. (*A.*) and other organisms.

[b] The relative amount of amplified DNA was estimated from the area covered by an ethidium bromide stained PCR product after separation in a 1% agarose gel. For gel electrophoresis, 2 µl of a 25 µl PCR assay were applied per lane. The gel was recorded with gel reader and analyzed using the program ImageQuant from Molecular Dynamics.

[c] Probe concentration 20 nM (S8807) and 50 nM (S8807A).

[d] Δ RQ is the normalized fluorescence signal of the probes measured after 30 PCR cycles. The fluorescence was recorded with a Hitachi F-2000 fluorescence spectrophotometer.

[e] Three PCR products, areas not determined.

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