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SHORT COMMUNICATION

Genetic Relationships among Cyanobacterial Strains Originally Designated as '*Anacystis nidulans*' and Some Other *Synechococcus* Strains

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Determinations of the genetic relationships between six *Synechococcus* strains demonstrated that three strains, originally designated as '*Anacystis nidulans*', belong to one and the same species. The other three strains belong to other species and/or genera. This conclusion is discussed with regard to a recently proposed revised classification of the genus *Synechococcus*.

INTRODUCTION

Rippka *et al.* (1979) provisionally recognized only two genera of unicellular cyanobacteria (blue-green algae) that possess thylakoids and divide by equal binary fission in one plane: *Gloeothece* and *Synechococcus*. On the basis of the DNA base compositions of the strains belonging to the latter genus, three compositional subgroups, with spans of 39–43, 47–56 and 66–71 mol% GC, were recognized (Herdman *et al.*, 1979; Rippka *et al.*, 1979), which already indicated that generic separations could be justified.

In a revised classification, based mainly on the differences in GC content, Rippka & Cohen-Bazire (1983) proposed that the genus *Synechococcus* should be split into four genera: *Cyanobacterium*, with a relatively low GC content (39–41 mol%) and a relatively low cell width (about 2 μm); *Cyanothece*, with a mol% GC of 42 and relatively wider cells (4–6 μm); *Synechococcus*, with 47–56 mol% GC; and *Cyanobium* with 66–71 mol% GC. The recognition of these four genera is more or less supported by mutual differences in a number of phenetic properties. However, Rippka & Cohen-Bazire (1983) did not exclude the possibility of further generic subdivisions of the newly defined genus *Synechococcus*.

The differences in GC contents used for the revised classification indicate considerable genetic differences both between the proposed genera *Cyanobacterium*, *Cyanobium* and *Synechococcus* and within the latter genus. This paper describes results obtained from DNA–DNA reassociation experiments with DNA from six strains, one representative of *Cyanobium*, one of *Cyanobacterium* and four of *Synechococcus*, including those strains originally, but erroneously, designated '*Anacystis nidulans*', viz. strains Tx20 (UTEX 625), R2 and 602.

METHODS

Strains. All strains (Table 1) were obtained from the Pasteur Culture Collection (PCC), Paris, France. Table 1 also includes the original designation of the strains and their generic identity as proposed by Rippka & Cohen-Bazire (1983). Growth conditions were described by Stulp & Stam (1984), except that strains were mass-cultured in BG-11 medium (Rippka *et al.*, 1979).

Extraction of DNA and determination of base composition. DNA was extracted as described by Stulp & Stam (1984), except that the cells were disrupted in a French press only. The DNA base composition of strain 7942 was determined from the UV absorbance–temperature profile (Mandel & Marmur, 1968; de Bont *et al.*, 1981).

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Table 1. *Synechococcus strains used*

For more detailed information on strain histories and properties see Rippka *et al.* (1979) and Rippka & Cohen-Bazire (1983). PCC, Pasteur Culture Collection, Paris, France; UTEX, University of Texas Culture Collection, Austin, Tx., USA.

PCC strain no.	Original designation	Proposed generic identity*
7502	*Synechococcus* sp.	'*Cyanobacterium*'
6301	*Anacystis nidulans* Tx20; UTEX 625 and 1550	'*Synechococcus*'
6312	*Synechococcus* sp.	'*Synechococcus*'
7942	*Anacystis nidulans* R2	'*Synechococcus*'
7943	*Anacystis nidulans* 602	'*Synechococcus*'
6307	*Coccochloris peniocystis* 120; UTEX 1548	'*Cyanobium*'

* Rippka & Cohen-Bazire (1983).

Labelling of DNA and DNA–DNA hybridizations. Nick-labelled [^{3}H]DNA samples (Stulp & Stam, 1984) were purified by passage through a Sephadex G100 (Pharmacia) column eluted with 0·03 M-sodium phosphate buffer (NaPB) containing 0·135 M-NaCl. After heat denaturation (100 °C, 10 min) the [^{3}H]DNA was loaded onto a hydroxyapatite micro-column. After prewashing with 0·03 M-NaPB + 0·135 M-NaCl at 50 °C and 0·03 M-NaPB at 60 °C, the single-stranded (ss) [^{3}H]DNA was eluted with 0·15 M-NaPB at 60 °C.

Hybridizations were performed, in duplicate, at 64 °C. Unreassociated DNA (ss fraction) was collected by hydroxyapatite chromatography at 60 °C with 0·15 M-NaPB. The reassociated (double-stranded) DNA (ds fraction) was subjected to thermal elution with 0·15 M-NaPB (Stulp & Stam, 1984) including a final 0·3 M-NaPB elution at 95 °C. Total radioactivity in the ds fraction relative to the total radioactivity (ss fraction + ds fraction) gave the renaturation rate for each hybridization. The renaturation rate in a heteroduplex relative to that in the homoduplex gave the relative binding (RB, %) of a heteroduplex. The difference in thermal stability ($\Delta T_{m(e)}$) between hetero- and homoduplexes was derived from computer-plotted thermal elution curves as the difference in temperature at 50% elution (Stulp & Stam, 1984).

RESULTS AND DISCUSSION

Table 2 presents the RB and $\Delta T_{m(e)}$ values of strains 7502 (*Cyanobacterium*); 6312, 7942 and 7943 (*Synechococcus*); and 6307 (*Cyanobium*) determined from reassociations of DNA from these strains with DNA from the reference strain 6301 (*Synechococcus* = '*Anacystis nidulans*' 625). Strains 7942 ('*Anacystis nidulans*' R2) and 7943 ('*Anacystis nidulans*' 602) show high RB values (94 and 97%, respectively) and low $\Delta T_{m(e)}$ values (1·3 and 2·2 °C, respectively), which means that these strains are genotypically almost identical with strain 6301. Strain 6312 shows some genotypic relationship with strain 6301 (RB = 31%; $\Delta T_{m(e)}$ = 5·6 °C), while strains 7502 and 6307 are genotypically only slightly related to strain 6301 (RB = 13% for both). For strains 7502 and 6307 $\Delta T_{m(e)}$ was not calculated since the amount of heteroduplexes in the reassociation mixture was too low to obtain accurate thermal elution patterns.

The known GC contents of the strains (Table 2) are in agreement with the results discussed above, including the intermediate RB and $\Delta T_{m(e)}$ values for strain 6312 and its 6 mol% GC content difference from reference strain 6301.

These data permit the following taxonomic conclusions. Strains 6301, 7942 and 7943 belong to one and the same species. Strain 6312 is a member of the same genus as strain 6301 and strains 7502 and 6307 probably belong to other and mutually different genera. These conclusions are in agreement with the revised classification proposed by Rippka & Cohen-Bazire (1983) and confirm their doubt about the taxonomic homogeneity of the newly defined genus *Synechococcus*, although in the case of strain 6312 only specific, rather than generic, subdivision seems appropriate.

Following Stanier *et al.* (1971), Rippka & Cohen-Bazire (1983) proposed that strain 6301 be recognized as the neotype of *Synechococcus elongatus*. If this proposal is validated under the rules of the International Code of Nomenclature of Bacteria, strains 7942 and 7943 should be named

Table 2. *DNA base compositions of the Synechococcus strains and relative binding (RB) and $\Delta T_{m(e)}$ values determined from DNA–DNA reassociations using DNA from strain 6301 as reference*

PCC strain no.	GC content* (mol%)	RB (%)	$\Delta T_{m(e)}$ (°C)
7502	41	13 ± 2·3	—
6301	56	(100)	(0)
6312	50	31 ± 2·4	5·6 ± 0·5
7942	55	94 ± 0·1	1·3 ± 0·1
7943	—	97 ± 3·1	2·2 ± 0·5
6307	70	13 ± 0·2	—

* From Herdman *et al.* (1979), except the GC content for strain 7942, which was determined by the present authors.

accordingly. It is most likely that strains PCC 6311 and PCC 6908 also belong to this species. These strains do not differ from strain 6301 with regard to the phenetic and genetic properties listed by Rippka & Cohen-Bazire (1983) and also contain two plasmids (almost) identical to those found in strains 6301, 7942 and 7943 (Herdman, 1982).

Padjama & Desikachary (1968) and Stanier *et al.* (1971) concluded that the '*Anacystis nidulans*' strain 625 (= PCC 6301) is a representative of *S. elongatus*, despite the fact that the cells of this strain are slightly narrower than given by Geitler (1932) in the description of this *Synechococcus* species, the smallest he recognized. In contrast, Komárek (1970) regarded strain UTEX 625 as *S. leopoliensis*, an even smaller species, until then described as *Romeria leopoliensis* and included in the *Oscillatoriaceae* (Geitler, 1932). However, Stam & Holleman (1979) concluded that strain UTEX 563 (= PCC 6907) fits even better the description Komárek (1976) gave for *Synechococcus leopoliensis*. Strain UTEX 563/PCC 6907 belongs to strain cluster 1 of Stanier *et al.* (1971), now proposed as *Cyanobium* (Rippka & Cohen-Bazire, 1983), including strains which are genetically quite distinct from strain UTEX 625/PCC 6301. In his taxonomic review of the genus *Synechococcus*, Komárek (1976) also concluded that *S. leopoliensis* corresponds with the strains of this cluster 1 and that *S. elongatus* resembles clusters 4 and 5 of Stanier *et al.* (1971). Since strain UTEX 625/PCC 6301 belongs to cluster 4, the conclusions of Komárek (1976) are in contradiction with his earlier conclusion (Komárek, 1970) concerning the specific identity of this strain. All this illustrates that the nomenclatural problems with strain UTEX 625/PCC 6301 are not yet resolved.

The genetic difference between strains 7502 and 6301 (RB = 13%) is in agreement with 16S RNA cataloguing data, which revealed that these strains are only distantly related (Doolittle, 1982). The taxonomic position of strain 7502 with regard to the newly proposed type strain *Cyanobacterium stanierii* PCC 7202 (Rippka & Cohen-Bazire, 1983) is still unclear and should be revealed by further DNA–DNA reassociation experiments.

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