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CULTURES OF PHORMIDIUM, PLECTONEMA, LYNGBYA AND SYNECHOCOCCUS (CYANOPHYCEAE) UNDER DIFFERENT CONDITIONS: THEIR GROWTH AND MORPHOLOGICAL VARIABILITY

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SUMMARY

Phormidium, *Plectonema*, *Lyngbya* and *Synechococcus* strains from freshwater- and marine habitats were incubated in media with different concentrations of seawater, under different light intensities and temperatures, submitted to different desiccation procedures, tested for endolithic growth in shells and grown on two different media solidified with agar.

On the basis of the observed differences in halotolerance, cell dimensions and the reaction pattern with regard to the other parameters used, three taxa, existing at about species level, could be recognized within those strains which were identified as *Schizothrix calcicola* sensu Drouet. Characters as false branching and sheath morphology could not be used for the distinction between these taxa. The results also led to the conclusions that two other strains, which belong to the Drouet species *Microcoleus lyngbyaceus*, are no ecophenes of each other. The two coccoid *Synechococcus* strains could be ranked under two different species.

The results are discussed with regard to Drouet's species concept within the Cyanophyceae. It is suggested to determine the genotypic relationships between the strains used, as a verification of the usefulness of the morphological and ecological characters involved in the delimitation of the different taxa recognized.

1. INTRODUCTION

As already stated in a previous paper (STAM & HOLLEMAN 1975) Drouet's species concept within the Cyanophyceae (DROUET 1962, 1963, 1964, 1968, 1973, 1978 and DROUET & DAILY 1956) implies that each cyanophycean species has only one genotype and many phenotypes. All such so-called ecophenes (ecological growth-forms) of one species should show the same phenotype when growing under identical conditions.

In his paper on the ecophenes of *Schizothrix calcicola* and in his revision of the Oscillatoriaceae, DROUET (1963, 1968) claims that this species encloses ecophenes without a sheath (traditionally known as *Oscillatoria* species), ecophenes with a sheath (traditionally known as *Phormidium* and *Lyngbya* species), ecophenes with more than one trichome within one sheath (traditionally known as *Schizothrix* species), ecophenes forming layers in an amorphous jelly (for instance known as *Phormidium crosbyanum* Tilden), endolithic ecophenes (traditionally known as

Plectonema terebrans Bornet & Flahault), and ecophenes which incrustate carbonate (for instance known as *Schizothrix lacustris* A. Braun). Moreover, *S. calcicola* is said to show such phenotypic polymorphism both in freshwater and marine habitats (salt water ecophenes).

In addition Drouet considers cultures of blue-green algae, for example identified as *S. calcicola*, a true ecophene of this species, living in a very restricted environment. This implicates that such cultures can also show the phenotypic polymorphism typical for *S. calcicola*.

Drouet also states that morphological and physiological variations appear when trichomes are exposed to more or less catastrophic changes in the environment and regenerate after it, such as abrupt changes in the chemical content of the medium (salinity), desiccation, and sudden inundation. The last two of these conditions should lead to appearance or disappearance of a sheath, respectively.

We have set up a number of culture experiments with strains belonging to *Schizothrix calcicola* sensu Drouet, primarily to give an answer to the following questions:

1. Do these strains show identical growth and morphology when cultured in media with different salinities? The results of previous experiments (STAM & HOLLEMAN 1975) with a number of *Phormidium* strains from freshwater and marine habitats, all to be considered as belonging to *S. calcicola* sensu Drouet, have provided a negative answer to this question. We suggested a genetic basis for the differences found in halotolerance.
2. Can all strains survive desiccation and does their morphology, especially their sheath-morphology, undergo changes both when they are dried up and when they are rewetted with medium?
3. Are these strains capable to show boring activity when brought into contact with a calcareous substrate?
4. Do these strains show identical optima (temperature and light intensity) for growth and identical morphological variability when cultured under different temperatures and light intensities?

Another goal of these culture experiments was to determine and examine the variability of morphological (and ecological) characters of the strains and subsequent to evaluate the usefulness of these characters for the taxonomy of blue-green algae.

In their taxonomic study on Oscillatoriaceae, BAKER & BOLD (1970) distinguish a number of varieties of *S. calcicola* sensu Drouet, on the basis of the morphology of the strains studied, in particular their growth patterns on agarized media. To test the usefulness of such growth patterns as a taxonomic criterium, we determined these patterns of our strains, most of which were also used by Baker and Bold.

The above listed questions of course also pertain to other Drouet-species. Therefore we introduced two other strains into our experiments, which belong, according to GEITLER (1932), to two different genera (*Lyngbya* and *Phormidium*) and to *Microcoleus lyngbyaceus* according to DROUET (1968).

Two coccoid strains (both belonging to *Synechococcus elongatus* sensu Geitler)

were also submitted to the culture experiments because DROUET (1963) claims that these two strains also belong to *S. calcicola*. STAM & VENEMA (1977), with the aid of DNA-DNA hybridization, already showed that one of these strains (strain 625) differs genotypically from a number of other *S. calcicola* strains.

2. MATERIAL AND METHODS

2.1 Strains

Table 1 enumerates the blue-green algal strains used. Strains 426, 427, 482, 485, 487, 488, 581, 594, 595, 596, 597, 598 and 790 belong to one and the same species (STAM & HOLLEMAN 1975, STAM & VENEMA 1977) and are hereafter indicated as the 'LPP strains'. Strains 71/12.1, 71/13, 71/14.1 and 71/16.4, which are isolates from brackish to marine habitats and which show little mutual differences in morphology and halotolerance (STAM & HOLLEMAN 1975), are indicated as the 'seawater strains'.

2.2 Growth conditions

All strains, except the strains 563 and 625, were grown in modified Chu-10 medium (STAM & HOLLEMAN 1975). For the strains 71/12.1, 71/13, 71/14.1, 71/16.4 and 71/5.1 the medium was prepared in filtered seawater instead of demineralized water.

Strain 563 and 625 were grown in BG-11 medium according to STANIER et al. (1971). For the agar-experiment also the 3N BBM medium (BAKER & BOLD 1970) was used.

Media were solidified with 1.5% Difco Bacto-Agar. For the salinity experiment the desired salinites were obtained by preparing the media in demineralized water and/or filtered seawater (whether or not concentrated by evaporation).

Unless stated otherwise, all incubations were carried out at 25°C, 800 lux (cool white fluorescent tube, Philips TL 20 W/34 de luxe) and a 12 hr light-12 hr dark period.

2.3 Test procedure

Growth rates were determined qualitatively, including determination of the morphology, by macro- and microscopic observation and quantitatively by measuring the chlorophyll-*a* concentrations (expressed as extinctions at 665 nm and 431 nm) in cell extracts (STAM & HOLLEMAN 1975). Growth rates after recovery periods were only determined qualitatively.

2.4 Experimental procedure

2.4.1 Effect of salinity on growth

Of all strains, except the LPP strains and the seawater strains (whose halotolerance has been subject of a previous paper, see STAM & HOLLEMAN 1975), 0.2 ml from a 3–4 weeks old stock culture were transferred into cottonwool-plugged culture tubes containing 10 ml of 0% (= freshwater), 50%, 100% and 2-, 4- and

Table 1. Blue-green algal strains used.

strain number	name attached to the strain (1)	name if identified with DROUET (1968)	name if identified with GEITLER (1932)	culture origin or sampling locality	figure
426	*Phormidium luridum* var. *olivacea* Boresch	*Schizothrixcalcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
427	*Phormidium foveolarum* Gomont	*Schizothrix calcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
482	*Plectonema notatum* Schmidle	*Schizothrix calcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
485	*Plectonema* sp.	*Schizothrix calcicola* (Ag.) Gomont	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
487	*Lyngbya* sp.	*Schizothrix calcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
488	*Lyngbya* sp.	*Schizothrix calcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
581	*Plectonema boryanum* Gomont	*Schizothrix calcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
594	*Plectonema boryanum* Gomont	*Schizothrix calcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
595	*Plectonema boryanum* Gomont	*Schizothrix calcicola* (Ag.) Gomont	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
596	*Plectonema boryanum* Gomont	*Schizothrix calcicola* (Ag.) Gomont	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
597	*Plectonema boryanum* Gomont	*Schizothrix calcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
598	*Plectonema calothrichoides* Gomont	*Schizothrix calcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
790	*Plectonema boryanum* Gomont	*Schizothrix calcicola* (Ag.) Gomont (2)	*Phormidium* cf. *foveolarum* Gomont	UTEX (1)	(3)
71/12.1		*Schizothrix calcicola* (Ag.) Gomont	*Phormidium* cf. *ectocarpi* Gomont	Waddenzee, land-reclamation sectors	(3)

71/13		*Schizothrix calcicola* (Ag.) Gomont	Phormidium cf. *ectocarpi* Gomont	Waddenzee, on shells	(3)
71/14.1		*Schizothrix calcicola* (Ag.) Gomont	*Phormidium* cf. *ectocarpi* Gomont	Waddenzee, on barnacles	(3)
71/16.4		*Schizothrix calcicola* (Ag.) Gomont	*Phormidium* cf. *ectocarpi* Gomont	Waddenzee, land reclamation sectors	(3)
1817	*Schizothrix calcicola* (Ag.) Gomont (4)	*Schizothrix calcicola* (Ag.) Gomont	*Plectonema* cf. *gloeophilum* Borzi	UTEX (1)	*1,5*
1818	*Schizothrix calcicola* var. *vermiformis* Baker et Bold (4)	*Schizothrix calcicola* (Ag.) Gomont	*Plectonema* cf. *gloeophilum* Borzi	UTEX (1)	*1,5*
1819	*Schizothrix calcicola* var. *radiata* Baker et Bold (4)	*Schizothrix calcicola* (Ag.) Gomont	*Plectonema* cf. *gloeophilum* Borzi	UTEX (1)	*1,5*
1815	*Microcoleus vaginatus* var. *cyano-viridis* Baker et Bold (4)	*Microcoleus lyngbyaceus* (Ag.) Crouan	*Lyngbya aerugineo-coerulea* (Kütz.) Gomont	UTEX (1)	*2,6*
1566	*Oscillatoria tenuis* Agardh	*Microcoleus lyngbyaceus* (Ag.) Crouan	*Phormidium ambiguum* Gomont	UTEX (1)	*3,7*
71/5.1		*Schizothrix calcicola* (Ag.) Gomont	*Phormidium molle* Gomont	Waddenzee, land reclamation sectors	*4,8*
563	*Synechococcus elongatus* Nägeli	*Schizothrix calcicola* (Ag.) Gomont (5)	*Synechococcus elongatus* Nägeli	UTEX (1)	*9*
625	*Anacystis nidulans*	*Schizothrix calcicola* (Ag.) Gomont (5)	*Synechococcus elongatus* Nägeli	UTEX (1)	*10*

(1) STARR (1964, 1971), University of Texas Culture Collection (UTEX, formerly the Indiana University Culture Collection)
(2) Also according to DROUET (1963, p. 270, footnote 10)
(3) See figures in STAM & HOLLEMAN (1975)
(4) Strains 1817, 1818, 1819 and 1815 are strains K44, 1, 10 and K27 from BAKER & BOLD (1970), respectively.
(5) According to DROUET (1963, p. 270, footnote 10).

10-fold concentrated seawater medium.

After 7 and 28 days of incubation, the cells from 0‰, 100‰, 2-, 4- and 10-fold concentrated seawater medium cultures were centrifuged and resuspended into their appropriate original media and incubated during another 14 days (recovery).

The growth rates of 0‰, 50‰ and 100‰ seawater medium cultures were determined after 42 days of incubation (tolerance).

2.4.2 Effect of light on growth

Of all strains 0.2 ml from a 3–4 weeks old stock culture were transferred into culture tubes with 10 ml of their appropriate media and incubated under light intensities of 450, 850, 1700 and 3800 lux. The growth rates were determined after 21 and 70 days of incubation.

2.4.3 Effect of temperature on growth

Of all strains 0.2 ml from a 3–4 weeks old stock culture were transferred into culture tubes with 10 ml of their appropriate media and incubated at 12, 20, 30 and 37°C. The growth rates were determined after 32 days of incubation.

After 7 and 28 days cultures were transferred to 25°C and incubated during another 14 days.

2.4.4 Survival from drying 1

Cells from 2 ml of a 3–4 weeks old stock culture were collected on a filter (Schleicher and Schull, Selecta 0, nr. 595, ϕ 9 cm) by suction filtration through a Büchner-funnel. The filters were dried at room temperature, placed in a petri-dish and stored at incubation conditions. After 7, 28 and 140 days storage the filters were placed in 10 ml of the original medium and incubated for 14 days (recovery).

Moreover, the morphology was studied at the third and seventh day of the drying period of 7 days and immediately after this period when the filter had been rewetted with medium.

2.4.5 Survival from drying 2

One ml of a 3–4 weeks old stock culture was centrifuged, the cells washed twice with sterile aqua bidest., resuspended in 1 ml sterile aqua bidest. and placed at incubation conditions in a small non-sealed vessel. When the culture was completely dried up (after about 5–7 days), 10 ml of the appropriate medium was added, and the culture was incubated for 14 days (recovery).

Another vessel with cells in aqua bidest. was sealed off and also incubated for about 5–7 days (control).

Morphology was also studied when the cultures had nearly dried up and immediately after adding the medium.

2.4.6 Boring activity in calcareous substrate

Of the LPP strains, the seawater strains and the strains 1817, 1818 and 1819, 0.2 ml from 3–4 weeks old stock cultures were inoculated into culture tubes with appropriate media, containing cleaned and sterilized oyster shell fragments (NIELSEN

1973). After 3 weeks of incubation the fragments were decalcified with EDTA (PRUD HOMME VAN REINE & VAN DEN HOEK 1966b) and examined microscopically.

2.4.7 Growth on agar containing media

Of all strains, except strains 1815, 1566, 625 and 563, a drop of a concentrated cell suspension was put onto the centre of both a 3N BBM agar plate and plates with agar-solidified media currently used for the strains.

After incubation of 28 days at 22°C, 1900 lux and 12 hr light-12 hr dark period, the macroscopic growth forms were studied.

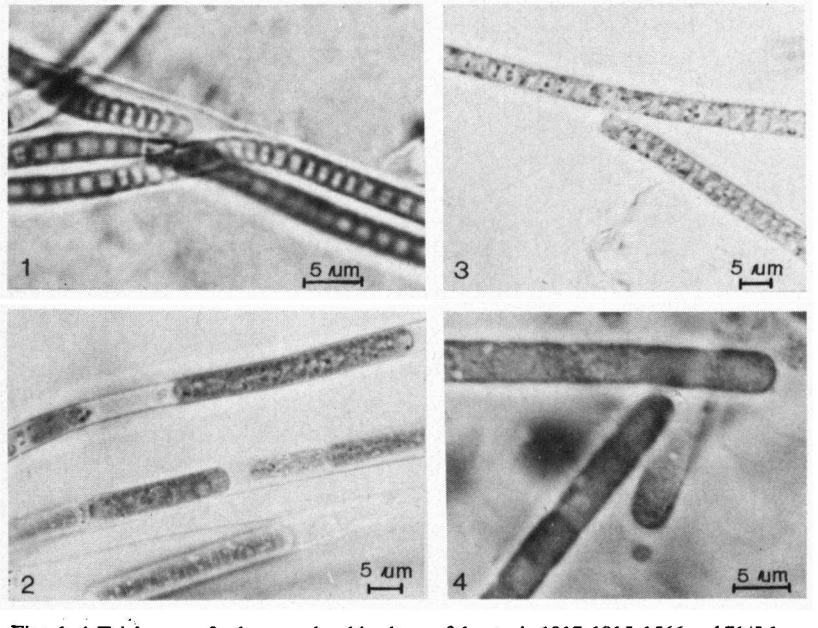
3. DESCRIPTION OF THE STRAINS

For the description of both the morphologically identical strains 426, 427, 482, 485, 487, 488, 581, 594, 595, 596, 597, 598 and 790 (LPP strains) and the strains 71/12.1, 71/13, 71/14.1 and 71/16.4 (seawater strains) one is referred to STAM & HOLLEMAN (1975).

3.1 Strains 1817, 1818 and 1819

These strains are morphologically identical and can be described as follows:

Trichomes green, forming a loosely packed dark-green plant-mass, straight to curved, constricted at the crosswalls, not tapered at the end, without or with a thin



Figs. 1–4. Trichomes of a three weeks old culture of the strain 1817, 1815, 1566 and 71/5.1, respectively.

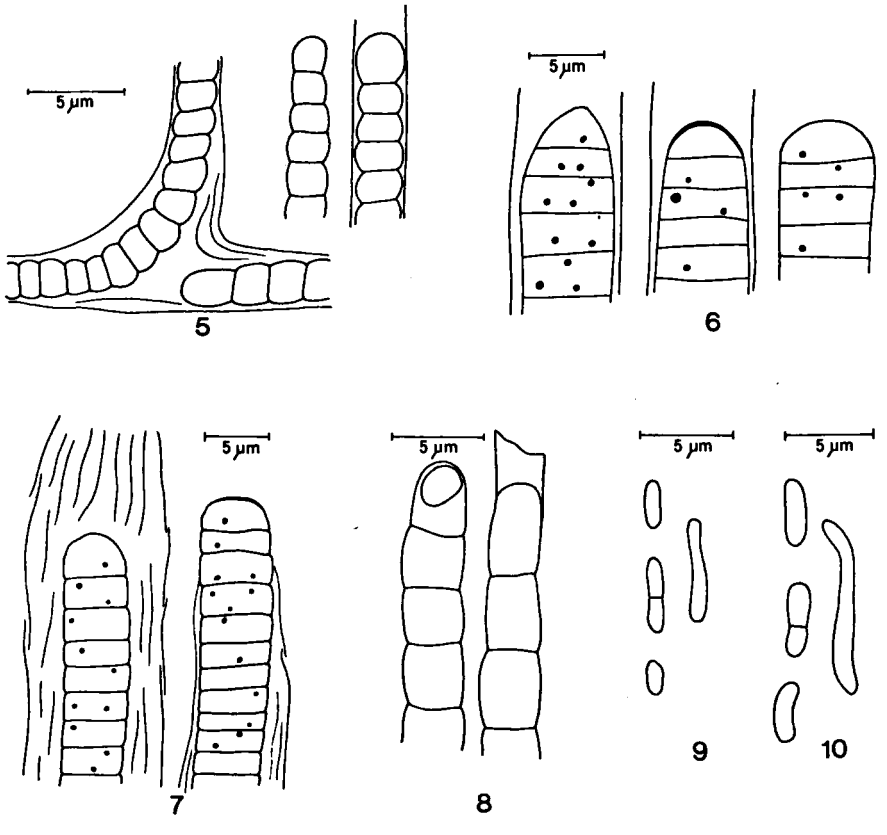
(up to 0.5 μm wide) colourless sheath, rarely showing false branching. Cells 2.4–3.0 μm wide and 1.1–2.4 μm long, not granulated. Terminal cell rotund to spherical (*figs. 1* and *5*).

3.2 Strain 1815

Trichomes green, forming curved bundles, not constricted at the cross-walls, sometimes tapering at the end, without or with a firm colourless sheath, 0.5–0.8 μm wide, without false branching. Cells 4.8–6.4 μm wide and 1.6–3.2 μm long, granulated at the crosswalls. Terminal cell rotund, sometimes slightly tapered or thickened (*figs. 2* and 6).

3.3 Strain 1566

Trichomes green, forming a dark green pellicle, straight to curved, not or only slightly constricted at the crosswalls, with a colourless sheath (0.8–3.2 μm wide).



Figs. 5–10. Trichomes from strain 1817, 1815, 1566 and 71/5.1 and cells from strain 563 and 625 respectively.

Thick sheaths lamellated and mostly frayed at the outside. Trichomes rarely without a sheath, without false branching. cells 4.2–5.8 μm wide and 1.6–2.4 μm long, granulated (occasionally situated at each side of the crosswall, mostly scattered through the whole cell). Terminal cell rotund with a thickened outer cell wall (*figs. 3* and *7*).

3.4 Strain 71/5.1

Trichomes green, forming a gelatinous green pellicle, straight to curved, not or only slightly constricted at the crosswalls, not tapered at the end, without or with a colourless sheath, up to 1 μm wide, without false branching. Cells 3.3–3.6 μm wide and 3.3–5.2 μm long, not granulated. Terminal cell rotund (occasionally spherical), equally long as, or longer than the other cells and occasionally containing a 'vacuole' (*figs. 4* and *8*).

3.5 Strain 563

Cells pale green, solitary or arranged in loosely packed cell-masses, filamentous formations up to 5 cells, rod-like, sometimes curved, 0.7–0.9 μm wide and 1.3–3.3 (–15.8) μm long. Plane of cell division perpendicular to the long axis of the cell *(fig. 9)*.

3.6 Strain 625

Cells green, solitary or arranged in loosely packed cell-masses, filamentous formations up to 6 cells, rod-like, straight to curved, 1.1–1.3 μm wide and 2.0–3.0(–26) μm long. Plane of cell division perpendicular to the long axis of the cell *(fig. 10)*.

4. RESULTS

4.1 Effect of salinity on growth

As shown in *table 2* all filamentous strains derived from freshwater habitats do not grow in 100% seawater medium and only show moderate growth in a 50% seawater medium. The seawater strains and strain 71/5.1, all derived from a brackish to salt habitat, grow in 2-fold concentrated seawater medium.

Strain 563 grows in freshwater medium only while strain 625 also grows in 50% and also, although rather poorly, in 100% seawater medium. Strains 1817, 1818 and 1819 show, when incubated in 50% and 100% seawatermedium, the same kind of morphological change as observed in the LPP strains (table 4 in STAM & HOLLEMAN 1975). In 50% seawater medium the average length decreases to 1.3 μm and in 100% seawater medium the cells seem to be inflated and their number per trichome is reduced. This reduction is also observed by the other filamentous freshwater forms. Occasionally cells from strain 625 show an increase in length (up to 10 times as long as normal) when growing in 50% and 100% seawater medium *(fig. 10)*.

As far as the filamentous freshwater strains are concerned, their capacity to survive high salinities not only depends on the degree of salinity but also on the

Table 2. Effect of salinity on growth. Growth rates of all strains after 42 days of incubation in media with different seawater concentrations.

strain number /group	concentration seawater in the medium			
	0%	50%	100%	2-fold concentrated
LPP strains (1)	+	±	−	n.t.
seawater strains (1)	++	+	+	+
1817–1818–1819	+	±	−	n.t.
1815	+	±	−	n.t.
1566	+	±	−	n.t.
71/5.I	+	+	+	±
563	+	−	−	n.t.
625	+	±	(±)	−

++ = good growth; + = normal growth; ± = moderate growth; (±) = poor growth; − = no growth; n.t. = not tested
(1) Results borrowed from STAM & HOLLEMAN (1975).

Table 3. Effect of salinity on growth. Recovery of all strains after 7 and 28 days of incubation in media containing various concentrations of seawater (Period of recovery 14 days in strain specific medium).

strain number/group	pre-recovery period of incubation (days)	concentration seawater in the medium				
		0%	100%	2-fold concentrated	4-fold concentrated	10-fold concentrated
LPP strains (1)	7	+	+	±	−	−
	28	+	±	−	n.t.	n.t.
seawater strains (1)	7	+	+	+	±	−
	28	+	+	+	±	−
1817–1818–1819	7	+	+	+	±	−
	28	+	±	−	−	n.t.
1815	7	+	−	−	−	n.t.
	28	+	−	−	−	n.t.
1566	7	+	+	+	±	−
	28	+	+	−	−	n.t.
71/5.1	7	+	+	±	±	−
	28	+	+	±	±	−
563	7	+	+	−	−	−
	28	+	+	−	−	−
625	7	+	+	±	−	−
	28	+	+	±	−	−

+ = normal growth; ± = moderate growth; − = no growth n.t. = not tested
(1) Results borrowed from STAM & HOLLEMAN (1975)

duration of the incubation *(table 3)*, except for strain 1815 which cannot endure any salinity tested at all. After 28 days of incubation *(table 3)* none of the non-saline strains can endure salinities higher than that of 100% seawater medium. The strains from marine habitats (the seawater strains and strain 71/5.1) can survive salinities up to four times that of seawater.

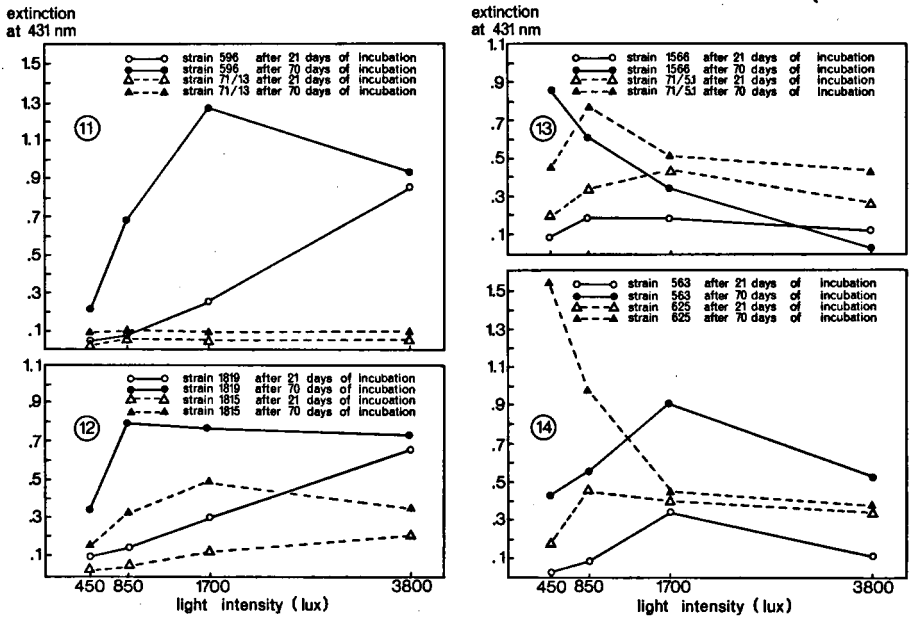
Table 3 also shows that the capability of the cocoid-strains to survive does not depend on the incubation times used, and that strain 625 can survive higher salinities (twice that of seawater) than strain 563.

All strains growing after 14 days of recovery showed normal morphology (cf. section 3).

4.2 Effect of light on growth

Table 4 and *figs. 11–14* show the effect of light on growth of the various strains. From this it can be concluded that the LPP strains, strains 1817, 1818 and 1819 and strain 71/5.1 gave the highest harvest after incubation under light intensities of 850–1700 lux. Strain 1815 gave the highest harvest at 1700–3800 lux, strain 1566 at 450 lux, strain 563 at 1700 lux and strain 625 at 450–850 lux. The seawater strains gave equal harvests under all light conditions used.

Morphological changes, with regard to their morphology as described in sec-



Figs. 11–14. The effect of light on growth. Relation between light intensity and size of harvest, expressed as the extinction at 431 mm of an acetone extract. Cultures of strain 596 and 71/13 *(fig. 11)*, strain 1819 and 1815 *(fig. 12)*, strain 1566 and 71/5.1 *(fig. 13)*, and strain 563 and 625 *(fig. 14)* were incubated under light intensities of 450, 800, 1700 and 3800 lux, during 21 and 70 days.

Table 4. The effect of light on growth. Relative size of harvests of all strains after 21 and 70 days of incubation under light intensities of 450, 850, 1700 and 3800 lux, as compared to growth at 850 lux.

strain number/ group	growth period (days)	light intensities (lux)			
		450	850 (reference)	1700	3800
LPP strains	21	±	+	++	+++
	70	±	+	++	+
seawater strains	21	±	+	+	+
	70	+	+	+	+
1817–1818–1819	21	±	+	++	+++
	70	±	+	+	+
1815	21	±	+	++	+++
	70	±	+	++	+
1566	21	±	+	+	+
	70	++	+	±	−
71/5.1	21	±	+	++	+
	70	±	+	±	±
563	21	±	+	++	+
	70	±	+	++	+
625	21	±	+	+	+
	70	++	+	±	±

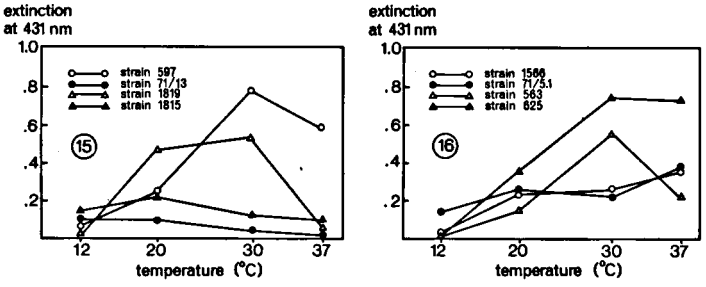
+++ = very large harvest; ++ = large harvest; + = normal harvest; ± = moderate harvest; − = negligible harvest

tion 3 were observed for the LPP strains, strains 1817, 1818 and 1819 and the coccoid strains. After growth for 21 days at 3800 lux the cells of the LPP strains were shorter (1.3 μm) and broader (2.6 μm) and the cells of strains 1817, 1818 and 1819 were slightly inflated and their number per trichome was reduced, which was also the case for the cells of the LPP strains and strains 1817, 1818 and 1819 after 70 days of incubation at 1700 and 3800 lux. The cells of strain 563 formed short chains (up to 4 cells per chain) after 21 days of incubation at 450 and 850 lux, and the cells of strain 625 formed such chains after 70 days of incubation at all light intensities.

4.3 Effect of temperature on growth

Table 5 and *figs. 15* and *16* show the effect of temperature on growth of the various strains. From this it can be concluded that the LPP strains and strain 563 gave the highest harvest at 30°C. The seawater strains gave the highest harvest at 12–20°C, strains 1817, 1818 and 1819 at 20–30°C, strain 1815 at 20°C, strain 625 at 30–37°C and strains 1566 and 71/5.1 at 37°C.

After incubation at 12°C the LPP strains showed changes in cell morphology when related to their morphology as described in section 3. Most cells were shorter (1.3 μm) and broader (2.6 μm). The cultures of the seawater strains bleached when incubated at 30°C.



Figs 15 and 16. The effect of temperature on growth. Relation between incubation temperature and size of harvests, expressed as the extinction at 431 nm of an acetone extract. Cultures of strain 597, 71/13, 1819 and 1815 *(figs. 15)* and strain 1566, 71/5.1, 563 and 625 *(fig. 16)* were incubated during 32 days at 12, 20, 30 and 37°C.

Table 5. Effect of temperature on growth. Relative size of harvests of all strains after 32 days of incubation at 12, 20, 30 and 37°C, as compared to growth at 20°C.

strain number/group	temperature (°C)			
	12	20	30	37
LPP strains	±	+	+++	++
seawater strains	+	+	±	−
1817–1818–1819	±	+	+	±
1815	±	+	±	±
1566	±	+	+	++
71/5.1	±	+	+	++
568	−	+	++	+
625	−	+	++	++

+++ = very large harvest; ++ = large harvest; + = normal harvest; ± = moderate harvest; − = negligible harvest

Table 6 shows that the seawater strains were unable to survive incubation at 30°C. All the other strains survived incubation at all temperatures, also the strains 563 and 625 at 12°C, although being unable to growth at that temperature. All strains which grew after 14 days recovery were morphologically normal (cf. section 3).

4.4 Survival from drying 1

The seawater strains and the coccoid strains did not survive drying on a filter *(table 7)*. Strain 71/5.1 hardly survived a dry period of 7 days. All other strains tolerated at least a dry period of 30 days.

The LPP strains and, in a less pronounced way, strains 1817, 1818 and 1819

Table 6. Effect of temperature on growth. Recovery of all strains after 7 and 30 days of incubation at 12, 20, 30 and 37°C (Period of recovery 14 days at 25°C).

strain number/group	pre recovery period of incubation (days)	temperature (°C)			
		12	20	30	37
LPP strains	7	+	+	+	+
	30	+	+	+	+
seawater strains	7	+	+	+	−
	30	+	+	±	−
1817–1818–1819	7	+	+	+	+
	30	±	+	+	±
1815	7	±	+	+	+
	30	+	+	+	±
1566	7	+	+	+	+
	30	±	+	+	+
71/5.1	7	+	+	+	+
	30	+	+	+	+
563	7	+	+	+	+
	30	±	+	+	±
625	7	+	+	+	+
	30	±	+	+	+

+ = normal growth; ± = moderate growth; − = no growth

show inflated cells and short trichomes during their stay of 7 days on the filter, but regain their normal morphology (cf. section 3) after 14 days of recovery. The occurrence of a sheath in some of the LPP strains could be detected as frequently as when grown under normal conditions. Strains 1817, 1818 and 1819 continued to have a sheath at all stages of the experiment. All the other strains which survived a stay of 7 days on the filter showed normal morphology (cf. section 3), both during their stay on the filter, after rewetting and after recovery. Therefore no changes in sheath morphology could be detected.

4.5 Survival from drying 2

All strains survived incubation during about 7 days in aqua bidest. (control). The filamentous strains survived desiccation except two of the seawater strains (71/12.1 and 71/16.4). The coccoid-strains died off *(table 8)*.

Here too the LPP strains and strains 1817, 1818 and 1819 showed a small increase of cell size when the culture had almost completely dried up. Shortly after the addition of medium the strains showed again their normal morphology (cf. section 3), just like all other surviving strains did during the whole experimental procedure. Unspecific changes in sheath morphology were not detected.

Table 7. Survival from drying 1. Relative growth rates of all strains after storage on a dried filter at 25°C during 7, 30 and 140 days, and 14 days recovery in their appropriate media.

strain number/group	dry period		
	7 days	30 days	140 days
LPP strains	+	±	(±)
seawater strains	–	–	–
1817–1818–1819	+	+	(±)
1815	+	+	(±)
1566	+	+	(±)
71/5.1	(±)	–	–
563	–	–	–
625	–	–	–

+ = normal growth; ± = moderate growth; (±) = poor growth; – = no growth

4.6 Boring experiment

TheLPP strains, seawater strains and strains 1817, 1818 and 1819 did grow on the oyster shells added to the medium, but no boring activity was registered. Although NIELSEN (1973) and PRUD'HOMME VAN REINE & VAN DEN HOEK (1966a) showed that endolithic growth of blue-green algae in culture is possible, we were not able to test if our culture conditions were optimal for shell boring since no strain was available which showed endolithic growth in nature.

The results of the experiments presented in sections 4.1–4.6 are summarized in *table 9*. For the estimation of the optimal growth conditions in the salinity-, light- and temperature experiments, deviating morphology, as an indicator for sub-optimal culture conditions, was also taken into consideration.

4.7 Growth on agar containing media

Table 10 shows that for the LPP strains and strain 1817 our results are in agreement

Table 8. Survival from drying 2. Effect of drying after incubation in aqua bidest. on the survival of all strains. Growth was evaluated after 14 days of recovery in appropriate medium. All strains survived incubation of about 7 days in aqua bidest. without desiccation (control).

strain number/group	test
LPP strains	+
seawater strains	(1)
1817–1818–1819	+
1815	+
1566	+
71/5.1	+
563	–
625	–

+ = growth; – = no growth

(1) Strains 71/13 and 71/14.1 survived desiccation, strains 71/12.1 and 71/16.4 did not.

Table 9. Table summarizing the strains' reactions to the culture conditions tested.
Column A: Salinity optimum for growth; column B: Maximum salinity survived; column C: Light intensity giving highest harvest; column D: Temperature giving highest harvest; column E: Maximum dry period survived; column F: Survival from desiccation; column G: Endolithic growth in shells.

strain number/group	A (concentration seawater)	B (concentration seawater)	C (lux)	D (°C)	E (days)	F	G
LPP strains	0(–50)	100%	850–1700	30	30(–140)	yes	no
seawater strains	0–200	4-fold concentrated	850–3800	12–20	0	yes(1)	no
1817–1818–1819	0(–50)	100%	850–1700	20–30	30(–140)	yes	no
1815	0(–50)	0%	1700–3800	20	30(–140)	yes	n.t.
1566	0(–50)	100%	450	(20–)37	30(–140)	yes	n.t.
71/5.1	0–200	4-fold concentrated	850–1700	(20–)37	0(–7)	yes	n.t.
563	0	100%	1700	30	0	no	n.t.
625	0(–100)	2-fold concentrated	450–850	30–37	0	no	n.t.

(1) Except strains 71/12.1 and 71/16.4
n.t. = not tested

Table 10. Growth on agar containing media. Macroscopic growth forms of the LPP- and seawater strains and strains 1817, 1818, 1819, 71/5.1 and 1815 on agarized medium, both modified Chu-10 and 3N BBM (column A), and the results obtained by BAKER & BOLD (1970) with agarized 3N BBM-medium (column B). Names of the macroscopic growth forms according to BAKER & BOLD (1970)

strain number/group	macroscopic growth forms	
	A	B
LPP strains (except 485)	*S. calcicola* v. *glomerulata*	v. *glomerulata*
485	*S. calcicola* v. *amorpha*	v. *amorpha*
71/12, 71/16.4	*S. calcicola* v. *discreta*	n.t.
71/13	no resemblance	n.t.
71/14, 71/5.1	*S. calcicola* v. *circinalis*	n.t.
1817	*S. calcicola*	*S. calcicola*
1818	*S. calcicola*	v. *vermiformis*
1819	*S. calcicola*	v. *radiata*

n.t. = not tested

with those of BAKER & BOLD (1970). In contrast to these authors, we could not distinguish between the growth patterns of strains 1818, 1819 and 1817. Three of the seawater strains and strain 71/5.1 fitted into the growth patterns described by Baker and Bold, strain 71/13 did not.

DISCUSSION

The LPP strains belong to one and the same species (STAM & HOLLEMAN 1975, STAM & VENEMA 1977) within the genus *Phormidium*. The seawater strains also belong to this genus.

Strains 1817, 1818 and 1819 were identified (GEITLER 1932) as *Plectonema* cf. *gloeophilum* Borzi. The generic identity of these strains is determined by their morphological character of false-branching.

Strain 71/5.1 was identified as *Phormidium molle* Gomont according to GEITLER (1932), DESIKACHARY (1959) and FREMY (1934).

Identification with DROUET (1968) leads to the conclusion that all strains mentioned above should be ecophenes of *Schizothrix calcicola* (Ag.) Gomont.

For strain 1815 – *Microcoleus lyngbyaceus* (Ag.) Crouan within the Drouet classification – the most appropriate species name found was *Lyngbya aerugineo-coerulea* (Kütz.) Gomont.

According to STARR (1971) strain 1566 should belong to the genus *Oscillatoria*. Since the trichomes of these strains clearly show a sheath, this name is incorrect. We identified this strain as *Phormidium ambiguum* Gomont, and as *Microcoleus lyngbyaceus* (Ag.) Crouan sensu Drouet.

The coccoid strains 563 and 625 both belong to the genus *Synechococcus*. Below their identity will be discussed in more detail.

For all identifications mentioned above one is also referred to *table 1.*

Based on their morphology and their reaction patterns towards the parameters tested *(table 9)* we conclude that within the *S. calcicola* sensu Drouet strains (LPP-, seawater strains and strains 1817, 1818, 1819 and 71/5.1) three taxa can be distinguished. The first taxon includes the LPP strains and strains 1817, 1818 and 1819, the second taxon the seawater strains and the third taxon strain 71/5.1.

Both the LPP strains and strains 1817, 1818 and 1819 show the same reaction patterns, including morphological deviations under suboptimal to sublethal conditions. These results suggest a close relationship between all strains belonging to both strain groups. Still morphological differences are present. In addition to slight differences in cell size and presence of a sheath – although the sheath of the LPP strains can be considered as ephemeral (STAM & HOLLEMAN 1975) – the false branching of strains 1817, 1818 and 1819 is the most striking difference. Without overlooking the possibility that the LPP strains showed false branching when taken into culture, we conclude that the use of this character for the distinction of the genus *Plectonema* should be taken into reconsideration. In this context it should be noted that BAKER & BOLD (1970) reject the possibility for a number of LPP strains to be 'laboratory species', since these authors have isolated another strain which showed the same characteristics as these strains both at the time of isolation and after prolonged cultivation.

The two other taxa include strains which are of marine origin. We already showed (STAM & HOLLEMAN 1975) that the seawater strains are more halotolerant than the LPP strains. The present results obtained with marine strain 71/5.1 and freshwater strains 1817, 1818 and 1819 show the same difference in halotolerance. This confirms our conclusion that marine blue-green algae are more halotolerant than freshwater forms and it supports our suggestion that this difference in halotolerance has a genetic basis (see also BATTERTON & VAN BAALEN 1971).

Strain 71/5.1 is separated from the seawater strains (71/12.1, 71/13, 71/14.1 and 71/16.4) because of differences in reaction pattern, but mainly because of different cell dimensions.

We think that these three taxa can be differentiated at about species level, which implicates that the characters used for the separation of the taxa (marine origin, different cell dimensions and different optima for growth) can be used for distinction between the strains at this taxonomic level, while other characters (false branching, sheath morphology and small differences in cell dimensions) can not. This can be verified by determination of the genotypic relationships (DNA base composition and/or DNA-DNA hybridization) of the strains involved. Determination of these relationships has already led to the conclusion that the LPP strains belong to one species and that DROUET's opinion (1963) that '*A. nidulans* strain 625' is conspecific with a number of the LPP strains is incorrect (STAM & VENEMA 1977).

The results of the culture experiments and the conclusions drawn conflict with Drouet's concept of *S. calcicola* as a genetically homogeneous species, comprising a multitude of ecological growth forms (ecophenes). The strains investigated do not show equal growth under all conditions tested and do not show the same

morphology under identical conditions. This raises the question whether or not *S. calcicola* sensu Drouet could be a valid species if conceived as a genetically variable species (which is normally the case with species). If this would be the case, the 'ecophenes' would be varieties with a genetic background. However, in view of the extreme morphological differences between, for instance, strains 563 and 625 on the one hand and strain 1817 on the other hand (compare *figs. 9* and *10* with *fig. 5*) this would seem hardly likely at all. Therefore the determination of the genotypic relationship between the strains can give more definite evidence about the taxonomic status of these strains.

It should be stressed here again that Drouet's culture experiments with samples he identified as *S. calcicola* (DROUET 1968) only prove the considerable polymorphism of these samples, but not that the samples which produce forms resembling forms of other blue-green algal species, do have the same, or even similar genotypes.

Both morphology (plant mass and sheath) and the different reaction patterns toward salinity, temperature and light lead to the conclusion that strains 1566 and 1815 do not belong to the same taxon, which is in agreement with their identification described above. The growth patterns of strain 1566 under different temperatures (*table 5* and *fig. 16*) agree with the results found by KOMAREK (1972) for *Ph. ambiguum*.

Evidently these strains are not ecophenes of one and the same species. However, both strains do form granules. KANN & KOMAREK (1970), KOMAREK (1972) and BAKER & BOLD (1970)found that the formation of such granules mainly depends on the physiological status of the culture. By determination of genotypic characters possibly a genetic basis of the ability of some species to build up granules can be traced. The use of this character as a prime criterium for generic distinction, as has been done by DROUET (1968), is possibly premature.

In agreement with PADMAJA & DESIKACHARY (1969), KOMAREK (1970, 1976) and STANIER et al. (1971) we identified both strain 562 and 625 as *Synechococcus*. According to our cell measurements, cells of strain 563 are a little narrower than those of strain 625 and the lower limit of their length is 0.7 μm less than of strain 625. Moreover the results of our culture experiments lead to the conclusion that we are dealing with two different species of this genus. PADMAJA & DESIKACHARY (1969) also found small differences in cell-length between their strain *S. elongatus* 1479/1 (Cambridge culture collection), which is identical with strain 563, and their *A. nidulans* strains 1, 2 and 3, which are all derived from strain 625. However, they decided to classify both strains as *S. elongatus* Nägeli.

STANIER et al. (1971) showed that we are dealing with two different species by the determination of the DNA base composition. Strain 625 (their strain 6301) has a DNA base composition, expressed as moles % of guanine plus cytosine (% GC) of 55, and strain 563 (their strain 6907) has a % GC of 71. They divided their *Synechococcus* strains studied into several clusters, without attaching specific names to them, although they expressed a great preference to fix the name *S. elongatus* to cluster 4. This cluster includes strain 625 and strain 563 was put into

cluster 1.

In his taxonomic review of the genus *Synechococcus* KOMAREK (1976) has compared the species he distinguished with the strain clusters of Stanier et al. The species group *S. gracilis, plancticus* and *leopoliensis* should correspond with cluster 1 and *S. elongatus* should correspond with cluster 4.

So far these conclusions agree with our results. Indeed the cell dimensions of strain 563 are smaller than those of strain 625, and fit well in Komarek's description of *S. leopoliensis* and strain 625 may very well be a representative of *S. elongatus*. However, in the same review Komarek has concluded that the *A. nidulans* strain 625 should belong to *S. leopoliensis*, a conclusion based on his earlier study concerning the generic identity of this strain (KOMAREK 1970), and not to *S. elongatus*. So, strain 625 should not belong to cluster 4 but to cluster 1, which is in complete contradiction with the results of Stanier et al.

In agreement with Stanier et al. we also prefer to fix the name *S. elongatus* Nägeli to their cluster 4. This implies that strain 625 has to be referred to accordingly. Strain 563 can be considered as a representative of *S. leopoliensis* (Racib) Kom.

The present results conflict with Drouet's statement that morphological and physiological variations appear after regeneration of the trichomes from a more or less catastrophic change in the environment. All strains which survived a number of adverse culture conditions, showed normal morphology when incubated for some time at normal conditions. Only the LPP strains, strains 1817, 1818 and 1819 and both coccoid strains 625 and 563 can show deviating growth forms during incubation at conditions which are suboptimal to sublethal (see also STAM & HOLLEMAN 1975 and KOMAREK 1970).

The results of the drying experiments show that no qualitative or quantitative changes in the sheath occur. The morphology of all surviving strains remained rather normal. So Drouet's conclusion that sheaths appear during desiccation and disappear after immersion is not valid for the strains we have tested.

In our opinion the varieties described for *S. calcicola* by BAKER & BOLD (1970) cannot be accepted since they are based on the incorrect classification made by DROUET (1968). Baker & Bold omitted to make a comparison with the classical classification of the Oscillatoriaceae (GOMONT 1892 and GEITLER 1932).

Still a macroscopic growth form on agarized medium, as a recognizable character of a certain strain is present and is highly reproducible *(table 10)*. From previous results (STAM & HOLLEMAN 1975 and STAM & VENEMA 1977) and the results in the present paper, we conclude that as far as the tested strains are concerned the use of this character for taxonomic purposes is doubtful. Within the morphologically and genotypically very closely related LPP strains, strain 485 shows a deviating pattern and strains 71/1.4 and 71/5.1 show the same pattern, although they were put into different taxa.

In general we may conclude:

– Drouet's species concept, in particular for his *Schizothrix calcicola*, is not

correct. Although it is possible to identify all strains investigated as *S. calcicola* sensu Drouet, these strains are no ecophenes of one and the same species.
– Blue-green algae can show deviating morphologies when growing under adverse conditions. (So when identifying cultured blue-green algae by morphological criteria, one firstly has to be sure that optimum growth conditions are present, and secondly one has to know the morphology and ecology of the natural population from which was isolated.)
– Culture experiments with blue-green algae can provide evidence about the usefulness of certain ecological and morphological characters for taxonomic purposes. Determination of the genotypic relationships should verify this evidence.

We agree with GOLUBIC (1969) that the results of this kind of research has to feed back to a taxonomy of blue-green algae mainly based on morphology and simple testing methods. The system given by GEITLER (1932) is to be preferred over that of Drouet, despite its shortcomings (KOSTER 1961 and VAN DEN HOEK 1967), as a point of departure for this taxonomic work.

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