Gas vesicle genes in *Planktothrix* spp. from Nordic lakes: strains with weak gas vesicles possess a longer variant of *gvpC*

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In cyanobacteria of the genus *Planktothrix*, there are three length variants of *gvpC*, the gene that encodes the outer protein of the gas vesicle. Sequence analyses indicated that the three allelic variants of *gvpC* differ principally in the presence or absence of a 99 nt and a 213 nt section. Strains with the new variant, *gvpC*²⁸, which encodes a 28 kDa form of GvpC, produce gas vesicles that collapse at the relatively low critical pressure (p_c) of 0.61–0.75 MPa. The authors have identified 12 classes of *gvp* genotypes that differ in the number and arrangement of alternating *gvpA*–*gvpC* genes and in the presence of ΩC , a fragment of *gvpC*. The *gvpC*²⁸ gene was found to be the most common variant of *gvpC* amongst 71 strains of *Planktothrix* isolated from Nordic lakes: 34 strains contained only *gvpC*²⁸; 22 strains, which possessed only the shorter *gvpC*²⁰ gene, produced gas vesicles with a higher p_c of 0.76–0.91 MPa; and 15 strains, which possessed both *gvpC*²⁰ and *gvpC*²⁸, also produced the stronger gas vesicles. Genotypes with only the *gvpC*²⁸ genes were more common amongst green *Planktothrix* strains (33 out of 38) than red strains (one out of 33). It is suggested that there is competition between the strains producing the two types of gas vesicles, with the stronger forms favoured in lakes deeper than 60 m, in which the combination of cell turgor pressure and hydrostatic pressure can collapse the weaker gas vesicles. The fact that none of the Nordic lakes are deeper than 67 m would explain the absence of the *gvpC*¹⁶-containing strains that produce even narrower gas vesicles of p_c 1.0–1.2 MPa, which are common in the much deeper Lake Zürich.

Keywords: cyanobacterial genetics, gas vesicles, *Planktothrix*

INTRODUCTION

The possession of gas vesicles is the principal character that distinguishes cyanobacteria in the genus *Planktothrix* from those that were once classified in the genus *Oscillatoria* (Anagnostides & Komárek, 1988). Gas vesicles provide the buoyancy that enables these organisms to regulate their position in the metalimnion, or float in the epilimnion of lakes. However, during autumnal and vernal lake mixing, cyanobacteria may be

circulated to depths at which the hydrostatic pressure causes collapse of their gas vesicles, destroying their buoyancy.

Gas vesicles are constructed from two principal proteins: GvpA is a small, highly conserved protein that forms the ribs of the cylindrical structure (Walker & Walsby, 1983; Hayes *et al.*, 1986); GvpC is a larger, less conserved protein, which is attached to the outer surface of the structure (Walsby & Hayes, 1988) and stabilizes it (Hayes *et al.*, 1992). In *Calothrix* spp. there are two copies of *gvpA*, the gene that encodes the rib protein, located upstream of a single copy of *gvpC*, the gene that encodes the outer protein (Tandeau de Marsac *et al.*, 1985; Damerval *et al.*, 1987). The latter contains four partially conserved repeats of 99 nt, encoding four 33-

Abbreviations: 33RR, 33-residue repeat; p_c , critical pressure; p_t , turgor pressure; p_h , hydrostatic pressure.

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residue repeats (33RRs) in the protein sequence. In *Anabaena flos-aquae* there are at least five copies of *gvpA* in tandem repeat (Hayes & Powell, 1995) and one copy of *gvpC*, which contains five 99 nt elements (Hayes *et al.*, 1988). It has been suggested that the length of GvpC may influence the width of the gas vesicle as it assembles (Walsby, 1994). In halobacteria, gas vesicle morphology is affected by mutations in *gvpC* (DasSarma *et al.*, 1994; Offner *et al.*, 1996). Changes in width will affect the critical pressure (p_c) at which the gas vesicle collapses, and this has consequences for gas vesicles of organisms that occur in lakes that mix to different depths (Walsby, 1994).

In *Planktothrix* spp., two forms of GvpC have been described, one of 16 kDa, encoded by *gvpC*¹⁶, and one of 20 kDa, encoded by *gvpC*²⁰ (Beard *et al.*, 1999). The larger GvpC possesses an additional sequence of 33 amino acids that shows similarity to three other 33-residue sections in the molecule. In strains of *Planktothrix rubescens* isolated from Lake Zürich, Switzerland, the *gvpA* and *gvpC* genes alternate in one of three arrangements: genotype GV1 contains *gvpC*²⁰ only; GV2 contains *gvpC*²⁰ and Ω C, an untranslated 72 bp fragment from the 3'-end of *gvpC*²⁰; GV3 contains *gvpC*¹⁶, *gvpC*²⁰ and Ω C (Beard *et al.*, 1999). Trichomes with genotype GV1 or GV2 produce gas vesicles that are wider and weaker ($p_c = 0.86\text{--}1.0$ MPa) than those with GV3 ($p_c = 1.0\text{--}1.17$ MPa) (Bright & Walsby, 1999). These GV-genotypes can be distinguished by diagnostic PCR using primers complementary to sequences within *gvpA* or *gvpC*. This technique can be used to identify the genotype, and hence the p_c phenotype, of single trichomes taken from a lake (Beard *et al.*, 1999).

Walsby *et al.* (1998) showed that during the winter months some *Planktothrix* trichomes in Lake Zürich are circulated to depths at which the hydrostatic pressure causes collapse of their gas vesicles. After cold winters, when the lake mixes to greater depths, this might lead to natural selection of strains with narrower and stronger gas vesicles, more of which remain buoyant and float upwards to form the spring inoculum. The proportions of strains with stronger gas vesicles can be investigated by PCR analysis of GV-genotypes.

It has been suggested that red strains of *Planktothrix* may be adapted to the conditions in deeper lakes. An investigation of seven strains revealed that four red-coloured strains possessed gas vesicles that collapsed at 0.9–1.0 MPa, while those in three green-coloured strains collapsed at a pressure of 0.6–0.7 MPa (Utkilen *et al.*, 1985). We report here a survey of the gas-vesicle genotype of 71 strains of *Planktothrix*, isolated from 21 Nordic lakes ranging in depth from 8 to 67 m. In recent years these strains have been catalogued using the groupings suggested by Skulberg & Skulberg (1985), on the basis of pigmentation and trichome width: *P. rubescens* group, red forms that contain phycoerythrin (trichomes < 6 μ m, *P. prolifica*; > 6 μ m, *P. rubescens*); and *P. agardhii* group, green forms that lack phycoerythrin (trichomes < 6 μ m, *P. agardhii*; > 6 μ m, *P.*

mougeotii). In this paper we retain the two group names for the different red and green strains, but do not distinguish them further. We report here the discovery, in many *Planktothrix* strains from Nordic lakes, of a longer variant of the *gvpC* gene that is correlated with the production of even wider and weaker gas vesicles. We investigated its association with the pigmentation of the strains, and its selection in relation to the depth of lakes in which they occur.

METHODS

Cultures of *Planktothrix* spp. The cultures of *Planktothrix* spp. investigated were those held in the NIVA Collection of Algae (Skulberg & Skulberg, 1990) that were isolated over the period 1964–1997 from inland waters of Nordic countries (Norway, Sweden, Denmark and Finland) and from one location in the brackish water of the Gulf of Finland. Cultures were established by Skulberg & Skulberg (1990) from single trichomes using the technique of Rippka (1988), with subsequent purification on agar plates, although the genetic uniformity of the cultures had not been confirmed. Stock cultures were maintained in Z8 culture medium at 17 ± 2 °C under illumination from fluorescent lamps (Philips T1 65 W/33) (Skulberg & Skulberg, 1990). During subculturing, no special precautions were taken to select the floating trichomes and, as a consequence (Walsby, 1981), some of the cultures contained mainly non-buoyant trichomes. Experimental cultures were grown in the medium of Bright & Walsby (2000).

Isolation of genomic DNA. Genomic DNA was isolated from *Planktothrix* cultures as described previously (Beard *et al.*, 1999). Alternatively, the following procedure was used to prepare cell lysates that were also suitable templates for diagnostic PCR. A 1 ml sample from a dense culture (pressurized at 1.4 MPa to collapse the gas vesicles) was mixed with proteinase K (0.2 mg ml⁻¹) and incubated for 15 min at 55 °C, followed by 15 min at 80 °C. After a brief centrifugation, the supernatant was collected, and 5–10 μ l used in subsequent PCR amplifications. The cell lysates were stored at 4 °C for up to one year.

PCR amplification and sequencing. Purified genomic DNA preparations or cell lysates were used as templates in PCR amplifications with the *Planktothrix*-specific *gvp* primers described in Table 1 and by Beard *et al.* (1999). All reactions were subjected to an initial denaturation step of 94 °C for 4 min, followed by 30 cycles of amplification (94 °C, 45 s; primer-specific annealing temperature, 1 min; 72 °C, 1 min), and a final extension step of 72 °C for 5 min. The annealing temperatures used for the diagnostic PCR amplifications were as follows: 65 °C (4 cycles) followed by 70 °C (26 cycles) for GVPA1 and GVPA2; 65 °C for GVPC1B, GVPC9 and GVPC11; 53 °C for GVP Ω 4, GVP Ω 5 and GVPC10. PCR products were purified and sequenced as described previously (Beard *et al.*, 1999).

Measurement of gas vesicle critical pressures. The p_c distributions of gas vesicles in *Planktothrix* trichomes were determined with a pressure nephelometer (Walsby, 1973). Measurements were made on 2 ml samples of cultures mixed with 2 ml 1.0 M sucrose solution, buffered with 5 mM K₂HPO₄, to remove cell turgor pressure. The means and standard deviations of the p_c distributions were calculated from triplicate measurements using the procedure of Walsby & Bleything (1988).

Table 1. New oligonucleotide primers used for PCR amplification and sequence analysis of the *gvp* genes in *Planktothrix* spp.

Primer	Sequence (5' to 3')*	Direction†	Target site‡	
			Gene or spacer	Position
GVPC1B	TGGGAAGTCCCCAACTGTCTG	R	<i>gvpC</i>	443–421 (AJ253125)
GVPC9	ACGCCAACAGGGAGTTCAAGAACG	F	<i>gvpC</i>	42–65 (AJ253125)
GVPC10	GCTGTTTCTGATAATTGCTA	R	<i>gvpC</i>	187–167 (AJ253125)
GVPC11	GGCTACCCACAAACGCTCGGTG	R	<i>gvpC</i> ²⁸	234–213 (AJ253131)
GVPC12	TTATCCCTGGGGTTGATCCAG	R	<i>gvpC</i>	522–502 (AJ253125)
GVPC13	TAGATGATCAAGAATCTCGACAGG	F	<i>gvpC</i> ²⁸	137–160 (AJ253131)
GVPΩ4	CAAGCCATTCCCCATTCTAT	F	<i>gvpA</i> –ΩC spacer	217–236 (AJ253126)
GVPΩ5	GCTTGGGATCGGCTTTGG	R	<i>gvpA</i> –ΩC spacer	221–204 (AJ253126)

* R = A or G.

† Direction indicates the primer orientation, forward (F) or reverse (R).

‡ Position of target site is given with respect to the relevant sequence deposited in the GenBank database (accession numbers indicated in parentheses).

RESULTS

Identification of a new variant of *gvpC*

In previous studies on *Planktothrix* strains isolated from Lake Zürich we identified three gas vesicle genotypes based upon different combinations of the three amplification products (1–3 in Fig. 1a) obtained using the

gvpA-specific primers GVPA1 and GVPA2 (Beard *et al.*, 1999; Fig. 1a). A product of length 595 bp indicated the presence of ΩC, the 965 bp product indicated *gvpC*¹⁶, and the 1064 bp product indicated *gvpC*²⁰, each located between adjacent copies of *gvpA*. Many of the *Planktothrix* strains in the NIVA collection gave a new product, of 1.3 kbp, when genomic DNA was used as a template

Fig. 1. Diagnostic PCR amplifications used to distinguish gas vesicle genotypes of *Planktothrix* spp. (a) The four different *gvpA*–*gvpC*–*gvpA* gene clusters (1–4) and the PCR products generated by amplification with *Planktothrix*-specific *gvpA* and *gvpC* primers. The boxed regions indicate the *gvpA* and *gvpC* ORFs; the box drawn with broken lines represents the 72 bp region (ΩC) identical to the 3' end of *gvpC*. The hatched area is the 99 nt region (in both *gvpC*²⁰ and *gvpC*²⁸) and the black area is the 219 nt region (in *gvpC*²⁸). The locations of the 99 nt and 219 nt regions are indicated by filled and open triangles, respectively. The lines under each gene cluster indicate the PCR products (in bp) obtained using the *gvpA*-specific primers GVPA1 and GVPA2, and the *gvpC*-specific primers GVPC1B, GVPC9 and GVPC11 (the prefix GVP is omitted). (b) Electrophoretic separation on an agarose gel (1.5%, w/v) of the products obtained by amplification of *gvpC* using primers GVPC1B, GVPC9 and GVPC11. Lanes: 1, 100 bp ladder (Promega); 2, *P. rubescens* Pla 9303 (GV1); 3, *P. rubescens* Pla 9316 (GV2); 4, *P. rubescens* Pla 9401 (GV3); 5, *P. rubescens* PCC 7821 (GV4); 6, *P. agardhii* CYA 29 (GV5); 7, *P. agardhii* CYA 137 (GV6); 8, no-template control.

conserved sequences at the 5'- and 3'-ends, respectively, of the three different *gvpC* ORFs. Amplification between GVPC9 and GVPC1B generates products of the following lengths: 303 bp from *gvpC*¹⁶, 402 bp from *gvpC*²⁰ and 623 bp from *gvpC*²⁸ (Fig. 1). Due to the preferential amplification of the 402 bp product in strains containing both *gvpC*²⁰ and *gvpC*²⁸ (genotype GV4), the 623 bp product was sometimes very faint (Fig. 1b, lane 5). For this reason, a second reverse primer, GVPC11 (Table 1), which is complementary to a sequence within the 219 bp region that is unique to *gvpC*²⁸, was included in the PCR assays: amplification between GVPC9 and GVPC11 generated a product of 181 bp from *gvpC*²⁸ (Fig. 1). The higher molecular mass products (> 623 bp) observed in Fig. 1(b) (lanes 2–7) represent either the products generated by amplification between subadjacent copies of *gvpC* or heteroduplexes formed by hybridization between variants of *gvpC* (Beard *et al.*, 1999).

Arrangement and sequence of gas vesicle gene clusters

The arrangement and sequence of the *gvpA–C* gene clusters in type strains of the three new GV genotypes were determined by the analysis of various PCR products (Fig. 2).

Genotype GV4. The arrangement of the *gvpA–gvpC*²⁰ repeats in *P. rubescens* PCC 7821 is similar to that found in *P. rubescens* strain Pla 9316 (GV2) from Lake Zürich (Beard *et al.*, 1999). Indeed, the sequences of two *gvpA* ORFs, two *gvpC*²⁰ ORFs (one complete, one partial), the Ω C region, and all intergenic regions were identical to the corresponding regions in strain Pla 9316. *P. rubescens* PCC 7821 was also found to contain *gvpC*²⁸ although we were unable to amplify the complete ORF. We were, however, able to amplify the first 709 nt of the 735 nt *gvpC*²⁸ ORF as two overlapping PCR products using *gvpC*²⁸-specific forward and reverse primers GVPC13 and GVPC11 (Fig. 2). On either side of the additional 219 bp segment, the sequence of *gvpC*²⁸ is identical to that of *gvpC*²⁰ in PCC 7821, although ambiguities at two positions indicate that this strain contains at least two allelic variants of *gvpC*²⁸. Four subclasses of genotype GV4 that differed in the arrangement of *gvpC*²⁰, *gvpC*²⁸ and Ω C were subsequently identified in strains of the NIVA Collection (Table 2).

Genotype GV5. The *gvpA–C* gene cluster in *P. agardhii* CYA 29 consists of two consecutive copies of a *gvpA– Ω C* repeat followed by two copies of a *gvpA–gvpC*²⁸ repeat (Fig. 2). The sequence of *gvpA* is identical to that in *P. rubescens* PCC 7821, but there are three substitutions in the *gvpA– Ω C* spacer and one substitution in the Ω C–*gvpA* spacer. The sequence of *gvpC*²⁸ shows three differences from that of *gvpC*²⁸ in *P. rubescens* PCC 7821. Two subclasses of genotype GV5 were identified in the NIVA strains: one with a single copy of Ω C (GV5a) and one with two copies (GV5b) (Table 2).

Griffiths (1992) isolated from the gas vesicles of strain CYA 29 an SDS-soluble protein whose mobility in SDS-

PAGE indicated a molecular mass of 28.8 kDa, close to the size calculated for the product of the *gvpC*²⁸ gene. [The 21.7 kDa size for the product given by Griffiths *et al.* (1992) was in error.]

Genotype GV6. *P. agardhii* CYA 137 was found to contain two consecutive copies of the *gvpA–gvpC*²⁸ repeat (Fig. 2). The sequence of the complete *gvpC*²⁸ ORF shows two differences from that in *P. rubescens* PCC 7821. The sequences of two *gvpAs* and intergenic regions are identical to those in PCC 7821, except for one substitution in the *gvpC–gvpA* spacer. The partial sequence of the downstream copy of *gvpC*²⁸ differs from the upstream copy in three positions (see below).

Sequence differences in the *gvpC* variants

The 99 nt section that distinguishes *gvpC*²⁰ from *gvpC*¹⁶ encodes a 33 amino acid residue section that can be considered to be a cryptic 33RR as it aligns with three other 33-residue sections with which it shows some sequence similarity (Beard *et al.*, 1999). The additional 219 nt section that distinguishes *gvpC*²⁸ from *gvpC*²⁰ encodes an additional section of 73 amino acid residues. We have looked for evidence of 33RRs within this sequence: the sequence ER--VA-Q---QL occurs both at the C-terminal end of this segment and 33 residues before the end. Elsewhere, there is little sequence similarity.

Apart from the additional 99 nt and 219 nt sections that distinguish the variants *gvpC*¹⁶, *gvpC*²⁰ and *gvpC*²⁸, these genes show only a few single-nucleotide differences. These differences are restricted to six positions, which occur in regions common to all three of the variants. Three of these differences affect the encoded amino acid, as follows: Arg or Thr at residue 125, and Pro or Ser at residues 223 and 228 (positions given with respect to the sequence of *GvpC*²⁸).

Survey of GV-genotypes of other *Planktothrix* strains in the NIVA collection

In 69 of the 72 *Planktothrix* strains in the NIVA collection isolated from lakes in Nordic countries, the GV-genotypes were determined, using three sets of PCR amplifications.

(1) The diagnostic PCR for *gvpC* (Fig. 1) was used to distinguish between genotypes GV1/2, GV3, GV4 and GV5/6.

(2) Amplification with primers GVPA1 and GVPA2 was used to screen for the presence of the Ω C region between adjacent copies of *gvpA* (Beard *et al.*, 1999). This PCR was used to distinguish between genotypes GV1 and GV2, and between GV5 and GV6.

(3) Subclasses of genotypes GV2, GV4 and GV5, which differ according to the location and number of copies of Ω C, were identified by amplification with primers GVP Ω 4, GVP Ω 5 and GVPC10 (Table 1). The forward and reverse primers, GVP Ω 4 and GVP Ω 5, respectively, are specific to the unique sequence immediately upstream of Ω C, and are complementary to one another at

Table 2. Arrangement of *gvp* genes in the different GV-genotypes of *Planktothrix* spp. and the products of diagnostic PCRs

The gene arrangements were determined by sequencing PCR products or by comparing the observed and predicted sizes of PCR products. The *gvp* gene orders shown in parentheses may in some cases overlap (e.g. GV4b, GV4c), but in other cases (e.g. GV4a) they cannot. BC, Bristol Collection.

Genotype	Type strain	<i>gvp</i> gene order	Products of diagnostic PCRs/bp							NIVA <i>Planktothrix</i> strains	
			C1B/C9/C11			A1/A2	Ω4/Ω5/C10				
			181 <i>C</i> ²⁸	303 <i>C</i> ¹⁶	402 <i>C</i> ²⁰		584 <i>ΩC–A–ΩC</i>	701 <i>ΩC–A–C</i> ¹⁶	800 <i>ΩC–A–C</i> ²⁰		1019 <i>ΩC–A–C</i> ²⁸
GV1	<i>Pla</i> 9303	<i>A–C</i> ²⁰ <i>–A–C</i> ²⁰			+						
GV2a	<i>Pla</i> 9316	<i>A–C</i> ²⁰ <i>–A–ΩC–A–C</i> ²⁰ <i>–A–C</i> ²⁰			+	+			+	Red: CYA 13, 24, 37/1, 37/2, 37/4, 55, 73, 89/3, 90, 97/3, 97/4, 108, 151. Green: CYA 88/1, 88/2, 88/3, 91, 127	
GV2b	<i>Pla</i> 9731	<i>A–C</i> ²⁰ <i>–A–ΩC–A–ΩC–A–C</i> ²⁰ <i>–A–C</i> ²⁰			+	+	+		+	Red: CYA 89/1, 89/4, 97/1, 97/5	
GV3a	<i>Pla</i> 9401	<i>A–C</i> ¹⁶ <i>–A–ΩC–A–C</i> ¹⁶ <i>–A–C</i> ¹⁶ <i>–A–C</i> ²⁰		+	+	+		+			
GV3b	<i>Pla</i> 9736	<i>A–C</i> ¹⁶ <i>–A–ΩC–A–ΩC–A–C</i> ¹⁶ <i>–A–C</i> ¹⁶ <i>–A–C</i> ²⁰		+	+	+	+	+			
GV4a	PCC 7821	(<i>C</i> ²⁰ <i>–A–ΩC–A–C</i> ²⁰ <i>–A–C</i> ²⁰) (<i>ΩC–A–C</i> ²⁸)	+		+	+			+	+	Red: PCC 7821, CYA 85, 89/5
GV4b	CYA 18	(<i>A–C</i> ²⁰ <i>–A</i>) (<i>A–ΩC–A–ΩC–A–C</i> ²⁸)	+		+	+	+			+	Red: CYA 18
GV4c	CYA 35	(<i>A–ΩC–A–C</i> ²⁰ <i>–A</i>) (<i>A–C</i> ²⁸)	+		+	+			+		Red: CYA 35
GV4d	CYA 129	(<i>ΩC–A–ΩC</i>) (<i>ΩC–A–C</i> ²⁰ <i>–A</i>) (<i>ΩC–A–C</i> ²⁸)	+		+	+	+		+	+	Red: CYA 98, 119, 128, 129, 391, 392, 395, 396, 401, 402
GV5a	CYA 126	<i>A–ΩC–A–C</i> ²⁸ <i>–A</i>	+			+				+	Red: CYA 87. Green: CYA 12, 15, 21, 30, 34, 64/4, 64/6, 86, 56/1, 56/3, 117/3, 117/4, 126, 263/2
GV5b	CYA 29	<i>ΩC–A–ΩC–A–C</i> ²⁸ <i>–A–C</i> ²⁸	+			+	+			+	Green: CYA 11, 29 (BC), 64/3, 64/5, 116, 278, 393, 394, 397
GV6	CYA 137	<i>A–C</i> ²⁸ <i>–A–C</i> ²⁸	+								Green: CYA 9, 10, 29 (NIVA), 59, 64/1, 65, 133, 137, 229, 299

Table 3. Mean p_c values of gas vesicles in *Planktothrix* strains with different GV-genotypes

The p_c is related to the combination of turgor pressure ($p_t = 0.2$ MPa) and hydrostatic pressure (p_h) at the maximum depth of the lake from which the strain was isolated. BC, Bristol Collection.

GV- genotype	CYA strain	Lake or locality	Geographical position		Max. depth/m	Max. p /MPa	Mean p_c (SD)/MPa
			N	E			
<i>P. rubescens</i> group							
2a	24	Levrasjön	56° 04′	14° 28′	15	0.35	0.79 (0.13)
	73	Kolbotnvatnet	59° 48′	10° 34′	18	0.38	0.90 (0.18)
	151	Almend Sø	56° 12′	9° 29′	20	0.40	0.88 (0.15)
	108	Mälaren	59° 28′	17° 28′	64	0.83	0.91 (0.18)
2b	97/1	Steinsfjorden	60° 04′	10° 19′	24	0.44	0.81 (0.20)
	89/1	Ören	58° 25′	16° 01′	25	0.45	0.82 (0.15)
4a	89/5	Ören	58° 25′	16° 01′	25	0.45	0.78 (0.19)
4b	18	Gjersjøen	59° 47′	10° 47′	64	0.83	0.88 (0.21)
4c	35	Kolbotnvatnet	59° 48′	10° 34′	18	0.38	0.85 (0.23)
4d	128	Långsjön	60° 15′	19° 55′	15	0.35	0.76 (0.14)
	395	Stovivatnet	59° 55′	10° 27′	15	0.35	0.86 (0.21)
	402	Steinsfjorden	60° 04′	10° 19′	24	0.44	0.82 (0.22)
	129	Vesijärvi	61° 19′	25° 00′	40	0.59	0.82 (0.17)
5a	87	Levrasjön	56° 04′	14° 28′	15	0.35	0.71 (0.15)
<i>P. agardhii</i> group							
2a	88/1	Ören	58° 25′	16° 01′	25	0.45	0.81 (0.21)
	127	Vesijärvi	61° 19′	25° 00′	40	0.59	0.85 (0.18)
	91	Mälaren	59° 28′	17° 28′	64	0.83	0.92 (0.19)
5a	64/4	Helgetjernet	59° 29′	11° 40′	8	0.28	0.62 (0.13)
	126	Långsjön	60° 15′	19° 55′	15	0.35	0.75 (0.10)
	15	Kolbotnvatnet	59° 48′	10° 34′	18	0.38	0.68 (0.11)
	56/1	Steinsfjorden	60° 04′	10° 19′	24	0.44	0.70 (0.09)
	263/2	Vestvatnet	59° 21′	11° 03′	28	0.47	0.66 (0.08)
	30	Frøylandsvatnet	58° 45′	5° 40′	29	0.48	0.68 (0.13)
	117/4	Gjersjøen	59° 47′	10° 47′	64	0.83	0.69 (0.10)
	21	Gulf of Finland	59° 35′	23° 11′	67	0.86	0.68 (0.15)
5b	116	Årungen	59° 41′	10° 45′	13	0.33	0.66 (0.09)
	11	Akersvatnet	59° 15′	10° 20′	14	0.34	0.61 (0.15)
	397	Stovivatnet	59° 55′	10° 27′	15	0.35	0.67 (0.14)
	29 (BC)	Gjersjøen	59° 47′	10° 47′	64	0.83	0.68 (0.10)
6	64/1	Helgetjernet	59° 29′	11° 40′	8	0.28	0.68 (0.15)
	229	Östra Kyrksundet	60° 16′	20° 10′	10	0.30	0.72 (0.11)
	137	Steinsfjorden	60° 04′	10° 19′	24	0.44	0.68 (0.09)
	299	Kalvsjøtjernet	60° 17′	10° 33′	26	0.46	0.66 (0.16)
	9	Frøylandsvatnet	58° 45′	5° 40′	29	0.48	0.63 (0.18)
	133	Øgderen	58° 46′	11° 25′	35	0.54	0.68 (0.11)
	59	Jarenvatnet	60° 23′	10° 33′	38	0.57	0.63 (0.17)
	65	Vansjø	59° 27′	10° 45′	41	0.60	0.66 (0.13)
	10	Gjersjøen	59° 47′	10° 47′	64	0.83	0.69 (0.08)
	29 (NIVA)	Gjersjøen	59° 47′	10° 47′	64	0.83	0.72 (0.14)

their 5' ends. These primers generated a 584 bp product by amplification between two copies of ΩC located either side of a copy of *gvpA*. A second reverse primer GVPC10 (complementary to a region within *gvpC*) was included in this PCR to amplify the region between ΩC (GVP Ω 4) and the next *gvpC* downstream: an 800 bp product indicated the gene arrangement ΩC -*gvpA*-*gvpC*²⁰, whereas a 1019 bp product indicated the arrangement ΩC -*gvpA*-*gvpC*²⁸.

***P. rubescens* group.** Of 33 red-coloured strains of *Planktothrix*, 17 were of genotype GV2, 15 were of genotype GV4 and one (strain CYA 87 from Lake Levrasjön) was of genotype GV5a (Table 2). In five of seven cases where there was more than one isolate from a lake, the genotypes differed: both GV2 and GV5 occurred in Lake Levrasjön; GV2 and GV4 occurred in Lakes Kolbotnvatnet, Ören and Steinsfjorden; and three subclasses of GV4 occurred in Lake Gjersjøen. There was a

Fig. 3. Histogram of the frequency of mean p_c values of gas vesicles in *Planktothrix* strains from the NIVA Culture Collection of Algae identified by GV-genotype: ▨, GV2; □, GV4; ■, GV5; □, GV6. Also indicated is the p_c distribution in strains of genotype GV3 isolated from Lake Zürich (▩). Frequencies are given as percentages of the total number of strains in each genotype.

discrepancy in the genotype of different cultures of *P. rubescens* CYA 18 (deposited as strain PCC 7821 at the Institut Pasteur): strain PCC 7821 was of genotype GV4a, whereas strain CYA 18 (NIVA Collection) was of genotype GV4b.

***P. agardhii* group.** Of 38 green-coloured strains of *Planktothrix*, 5 were of genotype GV2a, 23 were of genotype GV5 and 10 were of genotype GV6. Again, the genotypes differed in five of the seven cases where there was more than one isolate from a lake: both GV5 and GV6 occurred in Lakes Fröylandsvatnet, Gjersjøen and Steinsfjorden, and both subclasses of GV5 occurred in Lakes Årungen, Helgetjernet, Gjersjøen and Steinsfjorden. There was a discrepancy in the genotype of *P. agardhii* CYA 29: the culture maintained at Bristol since 1981 was of genotype GV5b (and represented the type strain of genotype GV5), but a culture sampled recently from the NIVA Collection was of genotype GV6. These discrepancies (and those observed in different cultures of *P. rubescens* CYA 18) may indicate that genetic rearrangements had occurred, or that the original isolates contained trichomes of different genotype.

p_c values of gas vesicles

p_c values were determined for 39 strains, representing most of the species and GV-genotype combinations available from each of the different lakes (Table 3). The principal finding was that the mean p_c was primarily associated with GV-genotype. The range of mean p_c for the different genotypes was: GV2, 0.79–0.91 MPa; GV4, 0.76–0.88 MPa; GV5, 0.61–0.75 MPa; GV6, 0.63–0.72 MPa. The frequency distribution of mean p_c in GV-genotypes revealed no significant difference between GV2 and GV4, or between GV5 and GV6 (Fig. 3).

However, the difference between the two groups (GV2/4 with a mean p_c of 0.85 MPa and GV5/6 with a mean of 0.65 MPa) is highly significant ($t = 12.9$, $P < 0.001$).

The correlation between p_c and GV-genotype overrides other correlations with species nomenclature or pigmentation. Thus, the only strain of *P. rubescens* with the GV5 genotype has the weaker gas vesicles (p_c 0.71 MPa), whereas all others, of GV2 and GV4, have stronger gas vesicles. Within the green-pigmented strains, three of the *P. agardhii* group are of genotype GV2 and have stronger gas vesicles (p_c 0.81–0.92 MPa) whereas the rest, of GV5 or GV6, have weaker gas vesicles (Table 3).

DISCUSSION

Correlations between p_c and GV-genotype

It has previously been suggested that the length of *gvpC* may influence the width of gas vesicles and hence their p_c ; strains of *Planktothrix* of genotype GV3, with several copies of the shorter *gvpC*¹⁶, produce narrower gas vesicles of higher p_c than those of GV1 and GV2, which have several copies of *gvpC*²⁰ only (Bright & Walsby, 1999). The results from the Nordic *Planktothrix* strains extend these correlations: strains of genotype GV4, which have several copies of *gvpC*²⁰ in addition to *gvpC*²⁸, produce gas vesicles of the same range of p_c as GV2; those of genotypes GV5 and GV6, which have several copies of *gvpC*²⁸ only, have gas vesicles with the lowest range of p_c . In *Planktothrix* strains that contain two variants of *gvpC* (genotypes GV3 and GV4) these correlations with p_c hold only for the shorter form. An explanation for this may be that more of the shorter *gvpC* is expressed. In strain *Pla* 9401 (GV3) it is expected that more *GvpC*¹⁶ than *GvpC*²⁰ will be produced because there are three copies of *gvpC*¹⁶ and only one of *gvpC*²⁰ (Beard *et al.*, 1999).

Correlations between p_c and lake depth

Many of the lakes from which the *Planktothrix* strains had been isolated were formed in glacial basins. The smaller lakes occupy basins of the kettle type or those formed by stream action. The maximum depths are given in Table 3.

We have previously suggested that there has been natural selection in planktonic cyanobacteria for gas vesicles of sufficiently high p_c to withstand the maximum combination of cell turgor pressure (p_t) and hydrostatic pressure (p_h) in different lakes (Walsby, 1994; Walsby *et al.*, 1999). The simplistic expectation is that the p_c should be sufficient to withstand the maximum combination of pressures ($p_t + p_h$) but should not exceed this combination. During the summer *P. agardhii* tends to occur in the epilimnion and *P. rubescens* in the metalimnion of lakes, at depths not usually exceeding 20 m (where $p_h = 0.20$ MPa); during active photosynthesis the p_t may reach 0.4 MPa, giving a combined pressure ($p_h + p_t$) of 0.6 MPa. The mean p_c exceeds this value in all of the strains investigated (Table 3). The first expectation is therefore fulfilled for this period.

During the autumn and spring, however, Nordic lakes are mixed to their greatest depth (most are dimictic and turn over before and after formation of ice cover); a proportion of the *Planktothrix* population is then circulated to the lake bottom. During this period p_t is unlikely to exceed 0.2 MPa. Table 3 lists the depths of the lakes from which the different strains were collected and the maximum combination of $(p_h + p_t)$, where p_h is calculated from the greatest depth (9810 Pa m⁻¹ at 4 °C) and p_t is assumed to be 0.2 MPa. Again, the mean p_c exceeds $(p_t + p_h)$ for strains from all of the lakes except the *P. agardhii* strains in the 64 m deep Lake Gjørsjøen and the 67 m deep Gulf of Finland. For these strains, trichomes circulating below a depth of 50 m will lose buoyancy; they will be lost to the bottom sediment and will not contribute to the spring inoculum. The selection of strains with stronger gas vesicles depends on the relative costs of their production and the benefits they provide in decreasing these losses (see Walsby *et al.*, 1999, for a more detailed discussion). Of particular interest here is the occurrence of strains whose gas vesicle genotype is atypical for their species but is correlated with lake depth: *P. agardhii* CYA 91 has genotype GV2, which confers the medium p_c of 0.92 MPa, sufficient to withstand the $(p_h + p_t)$ of 0.84 MPa at the greatest depth, 64 m, in Lake Mälaren. Of the other two strains of *P. agardhii* with genotype GV2, strain 127 ($p_c = 0.85$ MPa) occurs in the moderately deep Vesijärvi (40 m) and strain 88/1 ($p_c = 0.81$ MPa) in the shallower Lake Ören (25 m).

Concerning the second expectation, provision of p_c that does not exceed requirements, it is first noted that *Planktothrix* strains of genotype GV3 with the highest p_c values (the dominant form in the 136 m deep Lake Zürich) are absent from all of the Nordic lakes investigated. We suggest that the strains of genotype GV3 are restricted in occurrence to the deepest lakes, like Lake Zürich. Six of the Nordic lakes contained strains of *P. rubescens* with genotype GV2/4 that have gas vesicles whose p_c exceeds that required during winter mixing by 0.4 MPa or more (Table 3). There is, though, one exception: *P. rubescens* CYA 87 has the atypical genotype GV5 and gas vesicles of lower p_c ; it occurs in the shallow Lake Levrassjøen (15 m). This exception shows that the association between the GV2/4 genotype and the red strains, although strong, is not obligatory. Possible reasons why red strains with the GV5/6 genotype have not been selected in other lakes include the following: (1) insufficient time has occurred for selection since development of the population in the lake; (2) the benefit in reducing costs is insufficient; (3) the GV5/6 genotype is linked with other characters that are counterselective in these lakes; (4) the turgor pressures might be higher than 0.2 MPa in the red forms during periods of deep circulation; (5) the strains in culture are not all representative of the population in the lakes (*P. rubescens* of genotypes GV2 and GV5 occur in Levrassjøen).

Several lakes of different depths were shown to contain *Planktothrix* strains of both the GV2/4 and GV5/6

genotypes. This suggests that there is competition between strains producing the two types of gas vesicles: there should be selection for strains of genotype GV2/4, producing the stronger gas vesicles, following mixing events beyond 60 m; and there may be enrichment for strains of genotype GV5/6, producing the more efficient weaker gas vesicles, during summer growth in the metalimnion or epilimnion. This can now be investigated quantitatively by using the diagnostic PCR described in this paper to identify the GV-genotype of individual *Planktothrix* trichomes in lakewater samples.

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