An *rpoB* signature sequence provides unique resolution for the molecular typing of cyanobacteria

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The use of morphological characters for the classification of cyanobacteria has often led to ambiguous strain assignment. In the past two decades, the availability of sequences, such as those of the 16S rRNA, *nif*, *cpc* and *rpoC1* genes, and the use of metagenomics, has steadily increased and has made the reconstruction of evolutionary relationships of some cyanobacterial groups possible in addition to improving strain assignment. Conserved indels (insertions/deletions) are present in all cyanobacterial RpoB (β subunit of RNA polymerase) sequences presently available in public databases. These indels are located in the Rpb2_6 domain of RpoB, which is involved in DNA binding and DNA-directed RNA polymerase activity. They are variable in length (6–44 aa) and sequence, and form part of what appears to be a longer signature sequence (43–81 aa). Indeed, a number of these sequences turn out to be distinctive among several strains of a given genus and even among strains of a given species. These signature sequences can thus be used to identify cyanobacteria at a subgenus level and can be useful molecular markers to establish the taxonomic positions of cyanobacterial isolates in laboratory cultures, and/or to assess cyanobacterial biodiversity in space and time in natural ecosystems.

INTRODUCTION

Cyanobacteria represent a monophyletic group of oxygenic photosynthetic bacteria within the Eubacteria. Since their appearance between 2.45 and 2.32 billion years ago (Rasmussen *et al.*, 2008), the cyanobacteria have developed a number of adaptive strategies and thus form a very diverse group of prokaryotes. Their classification has been established based on morphological and physiological criteria under the International Code of Botanical Nomenclature (ICBN) (Greuter *et al.*, 2000). In 1978, having recognized their truly prokaryotic nature, Stanier *et al.* (1978) proposed that their nomenclature be governed by the provisions of the International Code of Nomenclature of Bacteria (ICNB) (Lapage *et al.*, 1992). Although efforts to harmonize the use of both Codes and to achieve a consensus nomenclature have been made over the past 25 years to further integrate cyanobacteria into the Bacteriological code, the names of only a very limited number of taxa have been validly published under the

Bacteriological Code (Trüper, 1986; Oren, 2004). This dual nomenclature has resulted in the use of multiple names for the same organism, causing considerable confusion in the literature. Therefore, cyanobacterial taxonomy and strain assignment still remains an important issue in the scientific community.

Since the development of molecular biology methods, the taxonomy of many bacterial taxa has been challenged, and the morphological traits employed for the description of cyanobacteria are not sufficient to unambiguously identify or describe a new isolate, resulting in a classification that is not always phylogenetically coherent (Honda *et al.*, 1999; Ishida *et al.*, 2001; Wilmotte & Herdman, 2001). On the other hand, sequence analysis of the 16S rRNA gene, largely used to establish the phylogenetic relationships among cyanobacteria, has only in some cases led to the description of phylogenetically coherent taxa (Giovannoni *et al.*, 1988; Turner *et al.*, 1999; Wilmotte & Herdman, 2001). Therefore, additional genetic markers, used alone or in combination with morphological criteria, are often necessary to resolve some specific nodes or taxa (Rajaniemi *et al.*, 2005; Suda *et al.*, 2002; Tanabe *et al.*, 2007).

The gene encoding the RNA polymerase β subunit (*rpoB*) has been shown to be a reliable genetic marker for phylogenetic analyses for many bacterial phyla (Case *et al.*, 2007; Hong *et al.*, 2004; Salerno *et al.*, 2007; Volokhov *et al.*, 2007). Interestingly, an indel (insertion/

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Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

Supplementary data are available with the online version of this paper.

deletion) was noticed in the *rpoB* sequences of Nostocaceae, but was not discussed further (Rajaniemi *et al.*, 2005). Indels have been used as phylogenetic characters to assess evolutionary relationships among many bacterial and archaeal phyla (Gupta, 1997, 1998) and recently among cyanobacterial taxa (Gupta, 2003, 2009). However, as indels often correspond to protein loops, they can easily be lost independently in evolutionarily unrelated sequences and thus give rise to misleading convergences. This phenomenon of convergence limits the use of indels for the investigation of large-scale evolutionary relationships. However, in combination with molecular phylogenies, indels may resolve some specific nodes, and also may be useful for sequence comparisons at a genus or species level.

In the present study, we sequenced part of the *rpoB* gene from different strains of cyanobacteria belonging to the genus *Oscillatoria* and investigated the presence and distribution of a specific signature (including an indel) in their RpoB sequences, as well as in publicly available RpoB sequences from 118 additional cyanobacterial strains. Comparative analyses of the amino acid sequences of these signatures reveal that they might be a very useful tool for cyanobacterial screening and assignment at a subgenus level.

METHODS

Strains and culture conditions. The 16 *Planktothrix* and *Oscillatoria* strains used in this study (indicated in bold in Supplementary Table S1, available with the online version of this paper) were supplied by the Pasteur Culture Collection of Cyanobacteria (PCC). All strains were incubated at 25 °C under white light (Osram Universal White) with a photosynthetic photon flux density (PPFD) of 30 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (measured with a LICOR LI-185B quantum/radiometer/photometer equipped with a LI-190SB quantum sensor). All strains were maintained in liquid BG-11 medium (Rippka, 1988) containing 0.4 mM Na_2CO_3 , except strains PCC 10106 and PCC 10110, for which the concentration of NaNO_3 in the BG-11 medium was reduced to 2 mM.

DNA extraction, amplification and sequencing. Aliquots (2 ml) of liquid cultures were centrifuged at 12 000 g for 10 min at 20 °C and washed twice with sterile MilliQ water. The final pellets were resuspended in 200 μl sterile MilliQ water and frozen in liquid nitrogen. Lysates of the frozen samples were prepared by a minimum of ten alternating cycles of thawing at 100 °C for 5 min and refreezing in liquid nitrogen. For *Planktothrix* and *Oscillatoria* strains, specific primers RPObF1 (5'-AGGAATTCACCACCACAACT-3') and RPObR1 (5'-ACCATCGGCTAATACCTG-3') were designed to amplify a 600 bp region. For the 16S rRNA total amplification, the forward primer A2 (5'-AGAGTTTGATCCTGGCTCAG-3'; Iteman *et al.*, 2002) and the 16SR1 primer (5'-GGTCTCCCTAAAAGGAG-GTG-3') located respectively at the beginning of the 16S rRNA and in the internal transcribed spacer (ITS) were used. The DNA sequences used for primer design were collected from the NCBI (National Center for Biotechnology Information) database (www.ncbi.nlm.nih.gov). New primers were designed with Primer 3 v.0.4.0 (<http://frodo.wi.mit.edu/>) using the sequences of *Planktothrix* sp. NIVA-CYA 126 and NIVA-CYA 127 (AJ628133, AJ783334). The PCR mixtures, in a final volume of 50 μl , contained 5 μl lysate, 5 μl 10 $\times$ PCR buffer, 1.5 μl 50 mM MgCl_2 , 1.5 μl 2.5 mM deoxyribonucleotides mix,

1.25 μl each primer diluted to 0.1 $\mu\text{g } \mu\text{l}^{-1}$ (MWG Biotech) and 5 μl 5 U *Taq* DNA polymerase μl^{-1} (Invitrogen). After an initial step consisting of 5 min at 95 °C, 35 cycles of amplification were performed; each amplification cycle consisting of 30 s at 95 °C, 30 s at 52 °C and 1 min at 72 °C. A final elongation step was carried out for 5 min at 72 °C. All DNA amplifications were performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems). A total of 10 μl of each sample was analysed by electrophoresis on 2% (w/v) agarose (Litex FMC) gels (200 ml) in 1 $\times$ Tris-borate/EDTA buffer (pH 8.0). For visualization of the products, the gels were stained with 200 μl ethidium bromide (0.7 $\mu\text{g ml}^{-1}$). After amplification, fragments of interest were sent to MWG Biotech for sequencing using cycle sequencing technology (dideoxy chain termination/cycle sequencing) on ABI 3730XL sequencing machines.

Sequence retrieval and alignment. Except for the sequences of the 16 strains established in the present study, the *rpoB* and 16S rRNA gene sequences of 118 other cyanobacterial strains were retrieved from the NCBI database (Table S1). Both the nucleotide sequences and their corresponding amino acid sequences were aligned with CLUSTAL_X v.1.83 (Jeanmougin *et al.*, 1998). Multiple alignments were edited with GeneDoc v.2.6.002 (Nicholas *et al.*, 1997) and ambiguously aligned positions were removed using Gblocks v.0.91b with default parameters (Castresana, 2000).

Phylogenetic analyses. Phylogenetic trees were calculated on nucleotide alignments by using three methods: neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML). NJ (Jukes–Cantor model) and MP trees were calculated by using MEGA4 (Tamura *et al.*, 2007). ML trees were computed with Phyml v.2.4.1 (Guindon & Gascuel, 2003) and the HKY (Hasegawa–Kishino–Yano) model of nucleotide substitution (Hasegawa *et al.*, 1985) using one category of substitution rate. For each method, the robustness of each branch was estimated by non-parametric bootstrap analysis (100 replicates) using the two programs cited above. Sequences from strains of each monophyletic group were extracted from the general alignment, and sequences of strains among this group were independently realigned, giving a new tree. This operation was repeated until there was no further resolution in the resulting tree. These consecutive trees are called tier trees in this study.

Signature sequence localization. The position of the indel within the *rpoB* gene was visualized using SMART (<http://smart.embl-heidelberg.de/>). The position of the indel and its impact on the three-dimensional structure of the β subunit of RNA polymerase were evaluated by aligning the *rpoB* sequences of *Synechocystis* sp. strain PCC 6803 (NC000911) and *Anabaena/Nostoc* sp. strain PCC 7120 (BA000019) with the sequence of *Thermus thermophilus* (YP_145079.1), for which the RNA polymerase structure has been crystallized (2GHO) (Kuznedelov *et al.*, 2002), by using RASWIN Molecular Graphics v.2.6 (Sayle & Milner-White, 1995).

RESULTS AND DISCUSSION

Characterization of the indel within the *rpoB* gene

As described in Table S1, the PCR amplicons obtained for 12 of the 16 strains tested were 453 bp long, while shorter products, 351 bp long, were obtained for the four remaining strains: *Planktothrix agardhii* PCC 9214 and *Oscillatoria* sp. strains PCC 8926, PCC 8954 and PCC 9631. A multiple alignment showed that the longer *rpoB* sequences display an indel of 120 nt and the shorter an indel of 18 nt. The sequence of the inserts in the longer

Fig. 1. Alignment of amino acid sequences corresponding to the *rpoB* gene region that contains an indel. The *rpoB* gene sequences available in public databases were selected and their corresponding amino acid sequences were aligned with the sequences from 16 *Planktothrix* and *Oscillatoria* strains obtained in this study, and the *Escherichia coli* K-12 sequence. The different cyanobacterial genera and strain identifiers are indicated on the left. The darker the highlighting of the amino acid, the more conserved it is in all the aligned sequences.

(b)

		Indel location	
	ANA281AR_Anabaena_circinalis	: DFFVFKTGNFCN-----	-----KEMKRLSKYC : 23
	FCC7108_Anabaena_sp	: DFFVREFLT-----	-----ETNTQHF-----PKEMRVLSFYC : 29
	BCHINCAK198443_Anabaena_oscill	: DFFVVRGCLSGV-----	-----TGNLIAGKCHTNEGGQCVT-----CREMNVLSFYC : 43
	SJ4a_Anabaena_augustana	: DFFVREQTTC-----	-----KTLTQSPASSKESFTGKFCQVITLYLSFYC : 40
	Otu37s7_Aphanizomenon_issatsch	: DFFVSSQNSIVSGQYS-----	-----FTKQCTTNEQCTTCKFQDPRNLSFYC : 45
	ANA227_Anabaena_flos_aquae	: DFFVSSQNSIVSGQYS-----	-----FTKQCTTNEQCTTCKFQDPRNLSFYC : 45
	277_Anabaena_sp	: DFFVSSQNSIVSGQYS-----	-----FTKQCTTNEQCTTCKFQDPRNLSFYC : 45
	ltu36s8_Anabaena_planctonica	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu35s5_Anabaena_rucosa	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu39s17_Anabaena_spiroides	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	Otu38s4_Anabaena_sigmoidea	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	Otu36s7_Anabaena_sigmoidea	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ANA118A_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ANA150A_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ANA175A_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ANA049A_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ANA301AR_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ANA349H_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ANA337A_Anabaena_solitaria	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ANA148_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ANA059A_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu33s8_Anabaena_planctonica	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu28s8_Anabaena_planctonica	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu33s10_Anabaena_planctonica	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu27s7_Anabaena_crassa	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu33s12_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu30s11_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu30s13_Anabaena_planctonica	: DFFVSSQNSIVSGQYS-----	-----LAEKRTTNEQLATCKFQDPRNLSFYC : 45
	ltu34s7_Anabaena_sp	: DFFVSSQNSIVSGQYS-----	-----LANCCTTCKEHAFAKFCQDPRNLSFYC : 38
	ltu26s2_Aphanizomenon_flos_aqu	: DFFVVGGRIVVGC-----	-----EETGDCREQLRTCKFQDPRNLSFYC : 40
	ltu29s19_Aphanizomenon_flos_aq	: DFFVVGGRIVVGC-----	-----EETGDCREQLRTCKFQDPRNLSFYC : 40
	ltu37s13_Aphanizomenon_flos_aq	: DFFVVGGRIVVGC-----	-----EETGDCREQLRTCKFQDPRNLSFYC : 40
	ltu39s8_Anabaena_smithii	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu35s12_Anabaena_flos_aquae	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	90_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu32s11_Anabaena_lemmermannii	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu30s4_Anabaena_flos_aquae	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu31s11_Anabaena_flos_aquae	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	66A_Anabaena_lemmermannii	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ANACCMCR_Anabaena_compacta	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu26s10_Anabaena_circinalis_r	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu27s5_Anabaena_circinalis_ra	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu28s13_Anabaena_circinalis_r	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	14_Anabaena_flos_aquae	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	TR183_Aphanizomenon_flos_aquae	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	FCC7905_Aphanizomenon_flos_aqu	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	202_Aphanizomenon_sp	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	Otu25s6_Anabaena_circinalis_ra	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu26s16_Aphanizomenon_gracile	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	HEANEYCwb198614011_Aphanizome	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu23s3_Anabaena_circinalis_ra	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	Otu39s7_Anabaena_sp	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	Otu37s9_Anabaena_sp	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	Otu33s2a_Anabaena_flos_aquae	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	Otu33s15_Anabaena_flos_aquae	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ltu34s5_Anabaena_circinalis	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	BECIC22_Anabaena_oscillarioides	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	BECIC22_Anabaena_oscillarioides	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	XP6B_Anabaena_cylindrica	: DFFVSSQNSIVSGQYS-----	-----SQVIEKGQDPRNLSFYC : 32
	ATCC29413_Anabaena_variabilis	: DFFVSSQNSIVSGQYS-----	-----KSTINGCLTSCKGQDPRNLSFYC : 38
	FCC7120_Nostoc_sp	: DFFVSSQNSIVSGQYS-----	-----KSTINGCLTSCKGQDPRNLSFYC : 38
	ANA062_Anabaena_sp	: DFFVSSQNSIVSGQYS-----	-----CSEETSSFFVSKEFCQDPRNLSFYC : 41
	HINCAK20014_Trichormus_variabi	: DFFVSSQNSIVSGQYS-----	-----DFFVRECEKECTITRGCGTEKNVKLGCTFTASPRIPITSSSSSPKEDPRNLSFYC : 57
	GREIFSWALC_Trichormus_variabil	: DFFVSSQNSIVSGQYS-----	-----DFFVRECEKEC-HGRGCGTEKNVKLGCTFTSPSPRIPITSSSSSPKEDPRNLSFYC : 56
	Trichormus_dololium	: DFFVSSQNSIVSGQYS-----	-----DFFVSNQSKSGSEHG-----CATNQKFCQDPRNLSFYC : 33
	KowBAI1983_Trichormus_azollae	: DFFVSSQNSIVSGQYS-----	-----DFFVFKFNPF-----DPRNLSFYC : 20
	V_Nostoc_ellipsosporum	: DFFVSSQNSIVSGQYS-----	-----DFFVFKFNPF-----DPRNLSFYC : 20
	ltu14s8_Nostoc_sp	: DFFVSSQNSIVSGQYS-----	-----DFFVFKFNPF-----DPRNLSFYC : 20
	X_Nostoc_edaphicum	: DFFVSSQNSIVSGQYS-----	-----DFFVFKFNPF-----DPRNLSFYC : 20
	VI_Nostoc_calicicola	: DFFVSSQNSIVSGQYS-----	-----DFFVFKFNPF-----DPRNLSFYC : 20
	III_Nostoc_calicicola	: DFFVSSQNSIVSGQYS-----	-----DFFVFKFNPF-----DPRNLSFYC : 20
	II_Nostoc_ruscorum	: DFFVSSQNSIVSGQYS-----	-----DFFVFKFNPF-----DPRNLSFYC : 20
	I_Nostoc_ruscorum	: DFFVSSQNSIVSGQYS-----	-----DFFVFKFNPF-----DPRNLSFYC : 20
	152_Nostoc_sp	: DFFVSSQNSIVSGQYS-----	-----DFFVFKFNPF-----DPRNLSFYC : 20
	FCC6803_Synechocystis_sp	: DFFVSSQNSIVSGQYS-----	-----DFFVCPHSPCNFAEK-----GEDEVHICFYC : 26
	WH8501_Crocosphaera_watsonii	: DFFVSSQNSIVSGQYS-----	-----DFFVVGPEGHEKV-----GQDQICILCFYC : 26
	130_Microcystis_sp	: DFFVSSQNSIVSGQYS-----	-----DFFVADCECKEIFG-----KSEDEVHICFYC : 26
	NIES843_Microcystis_aeruginosa	: DFFVSSQNSIVSGQYS-----	-----DFFVADCECKEIFG-----KSEDEVHICFYC : 26
	FCC7806_Microcystis_aeruginosa	: DFFVSSQNSIVSGQYS-----	-----DFFVADCECKEIFG-----KSEDEVHICFYC : 26
	269_Microcystis_sp	: DFFVSSQNSIVSGQYS-----	-----DFFVADCECKEIFG-----KSEDEVHICFYC : 26
	K12_Escherichia_coli	: DFFVSSQNSIVSGQYS-----	-----DFFVNECEMYFGE-----AGIDIDNHEFT : 26

Anabaena
Aphanizomenon
Trichormus

Nostoc

Synechocystis

Crocosphaera

Microcystis

Escherichia coli

I 6

y 6 Kyd

amplicons was used for a BLASTN search (Zhang & Madden, 1997; Zhang *et al.*, 2000) against the NCBI non-redundant (nr) sequence database. This insert sequence appeared to be highly specific, appearing only in the *rpoB* gene of the two *Planktothrix* strains NIVA CYA-126 and NIVA CYA-127.

Signature sequences in the *rpoB* genes of different cyanobacterial taxa

To determine if cyanobacterial taxa other than *Planktothrix* have inserts in their *rpoB* sequences, all available cyanobacterial *rpoB* sequence homologues were collected from public databases. A multiple alignment of their corresponding amino acid sequences revealed the existence of a conserved region containing inserts (indels) of lengths varying from 6 to 44 aa (Fig. 1).

The indel is situated in the Rpb2_6 domain of RpoB (determined in the *Anabaena/Nostoc* sp. strain PCC 7120 sequence) that is involved in DNA binding and DNA-directed RNA polymerase activity (<http://smart.embl-heidelberg.de/>). To locate the insert sequence in the RpoB molecule more precisely, the three-dimensional structure of the *Synechocystis* sp. strain PCC 6803 and *Anabaena/Nostoc* sp. strain PCC 7120 RpoB proteins was modelled based on an alignment of their sequences with that of *Thermus thermophilus* using RASMOL v2.6 (Sayle &

Milner-White, 1995) (only the RpoB structure of *Synechocystis* strain PCC 6803 is shown in Supplementary Fig. S1, available with the online version of this paper). This clearly showed that the indel sequence adds an extra loop to a folding region of the *Thermus thermophilus* consisting of β strands. The role of this loop is unknown, but the presence of a few polar amino acids in its sequence indicates that it is unlikely to interact with nucleic acids, thus excluding a direct role in RNA polymerase function. Moreover, no cationic binding sites are apparent. Peptide binding to the loop sequence cannot be excluded but further experiments are required to clarify this assumption.

RpoB signature sequences as a molecular tool for cyanobacterial taxonomy

To assess the utility of these RpoB sequence signatures as a tool for cyanobacterial taxonomy, we examined if the *rpoB* gene followed a vertical inheritance or has been exchanged among cyanobacterial strains by horizontal gene transfer. For this, we compared phylogenies (built using three methods: ML, NJ and MP) based on analysis of 16S rRNA and *rpoB* gene sequence phylogenies of the same set of 134 strains. As the length of the *rpoB* indels is variable depending on the genus (minimum 18 nt; maximum 132 nt), the correction implemented by the Gblocks program removed

Fig. 2. ML tree based on 16S rRNA gene sequences (1207 nt) from 134 cyanobacterial strains for which an *rpoB* gene sequence is available. Trees were rooted with the *Escherichia coli* K-12 *rpoB* sequence as outgroup. Roman numbers indicate clusters of strains. Black triangles represent groups of related strains; the number in each group is indicated in parentheses. Bootstrap values are shown for each node if >50%. Bar, 0.05 substitutions per site.

an important part of the sequence, leaving only 286 nt. Consequently, due to the small size of the aligned sequences, the *rpoB* tree appears less robust than the tree based on 16S rRNA gene sequences (data not shown). However, the *rpoB* tree is largely congruent with the 16S tree presented in Fig. 2 (five of the six clusters fully conserved). Moreover, when aligned with the sequences of this *rpoB* region from other closely related bacterial taxa (data not shown), cyanobacteria form a well-separated group. These two observations show there is no suggestion of obvious horizontal gene transfer, indicating probable vertical inheritance.

Some of the clusters defined in the present study correspond to the clades recently described by Gupta (2009) using distinct indels located in different proteins. Indeed, cluster I identified in this report corresponds to the clade A described by Gupta, while cluster III corresponds to clade C. Group II is an independent node in Gupta (2009) and has not been included in a specific clade.

Strains in clusters IV, V and VI (or on independent nodes for *Synechocystis* sp. PCC 6803 and *Crocospaera watsonii* WH 8501) group differently in clade B (Gupta, 2009), which includes cyanobacteria from various taxa. This inconsistency in the data obtained by the two research groups may be due to a bias introduced by a difference in the number of strains used in the two studies, a higher number being analysed in the present report than in Gupta (2009).

To evaluate the usefulness of the *rpoB* signatures for molecular typing of cyanobacterial strains, successive trees (hereafter designated tier trees) were constructed for the two clusters (V and VI) inferred from the 16S rRNA gene-based tree (Fig. 2) for which the greater numbers of nucleotide sequences (20 and 86, respectively) were available. Starting from the general *rpoB* nucleotide alignment, DNA sequences belonging to the same group within clusters V (groups V-1 and V-2) and VI (groups VI-1 and VI-2) were realigned separately and trees were

Fig. 3. (a) ML tree based on the *rpoB* nucleotide sequences of 86 cyanobacterial strains of cluster VI as defined in Fig. 2; (b) 37 strains of group VI-1; (c) Eight strains of subgroup VI-1-1; (d) 25 strains of subgroup VI-1-2. Groups and subgroups correspond to those defined in Table 1. The number of nucleotides used to reconstruct the trees is indicated in parentheses. Bootstrap values are shown for each node if >50 %. Bars, 0.05 substitutions per site.

constructed (Figs 3a and 4a). This made possible the inclusion of residues that were discarded at the previous level of analysis because of automatic gap removal. The procedure was repeated on each new well-supported subgroup (bootstrap value >50 %) until all the sequences realigned possessed exactly the same signature sequence and thus maximal resolution was achieved. For cluster V, the number of nucleotide differences in the sequences being limited, the maximal resolution was obtained after two tier trees were constructed (data not shown), while for cluster VI more tier trees were required (Figs 3 and 4).

Using this approach, we could deduce from the final nucleotide sequences selected 80 distinct specific patterns of RpoB protein signatures and 56 different indels (Table 1) whose clustering is congruent with the groups established using the 16S rRNA gene and *rpoB* gene sequences. Interestingly, these protein signatures differentiate strains at a subgenus level (Table 1). For example, cluster VI can be divided into two groups (Figs 3a and 4a). Group VI-1 contains members of the genera *Nostoc* and *Nodularia* (Fig. 3b, c and d), while the group VI-2 contains members of the genera *Anabaena*, *Aphanizomenon* and

Fig. 4. (a) ML tree based on the *rpoB* nucleotide sequences of 86 cyanobacterial strains of cluster VI as defined in Fig. 2; 49 strains of group VI-2 (b); 15 strains of subgroup VI-2-1 (c); 24 strains of subgroup VI-2-2 (d). Groups and subgroups correspond to those defined in Table 1. The number of nucleotides used to reconstruct the trees is indicated in parentheses. Bootstrap values are shown for each node if >50 %. Bars indicate 0.05 substitutions per site.

Table 1. Patterns of RpoB signature sequences (including indels) in cyanobacteria

Cluster*/Group†	Subgroup†	Common pattern‡	Species	Strain
I		E(x)QAARDSGMV(x)- VS(25-26x)Y(x)LQ		
I-1	I-1-1	EAQAARDSGMVVVSRTNGVVT- YVSADEIVVR PDDGGDP IVYRLQ	<i>Synechococcus</i> sp.	JA23Ba
	I-1-2	EAQAARDSGMVVVSRTSGVVTY- VSADEIVVR PDDGGDP IVYRLQ	<i>Synechococcus</i> sp.	JA33Ab
I-2		EGQAARDSGMVIVSDIDGAITY- VSGEQIRVR GENGQEF AYPLQ	<i>Gloeobacter violaceus</i>	PCC 7421
II		EPQAARDSGMVITSPVDGTISY- VDATHIEVTADTGEKYGYALQ	<i>Synechococcus elongatus</i>	PCC 6301; PCC 7942
III		E(x)QVARDSGMVPI(2x)- VNG(x)V(2x)VDA(2x)I(x)- V(4x)G(2x)H(x)H(x)LQ		
III-1		<i>E(x)QVARDSGMVPI(2x)- VNG(x)V(x)YVDAN(x)IVV- (4x)G(2x)H(x)H(x)LQ</i>		
III-1-1		<i>E(x)QVARDSGMVPI(2x)VNG- (x)V(x)YVDAN(x)IVV(4x)G- (2x)H(x)H(x)LQ</i>		
	III-1-1-1	ESQVARDSGMVPITKVNIGIVSY- VDANEIVVK DE DGNEHFHYLQ	<i>Prochlorococcus marinus</i>	MIT 9301
	III-1-1-2	ESQVARDSGMVPITKVNIGTVSY- VDANEIVVK DDH GNHFHYLQ	<i>Prochlorococcus marinus</i>	AS 9601
	III-1-1-3	ESQVARDSGMVPITKVNIGIVSY- VDANEIVVK DVD GNHVFHFLQ	<i>Prochlorococcus marinus</i>	CCMP 1986 (=MED4)
	III-1-1-4	ESQVARDSGMVPITKVNIGIVSY- VDANEIVVK DVD GNHVFHYLQ	<i>Prochlorococcus marinus</i>	MIT 9515
	III-1-1-5	ESQVARDSGMVPITKVNIGTVSY- VDANEIVVK GED GNHFHYLQ	<i>Prochlorococcus marinus</i>	MIT 9312
	III-1-1-6	ETQVARDSGMVPISQVNGTVTY- VDANSIVVT DDE GGHEHLHELQ	<i>Prochlorococcus marinus</i>	CCMP 1375 (=SS120)
III-1-2		ETQVARDSGMVPISKVNGTVSY- VDANAIVVT DDE GNHDFHYLQ	<i>Prochlorococcus marinus</i>	NATL1A; NATL2A
III-2		<i>ETQVARDSGMVPI(x)RVNG- (x)V(2x)VDA(x)AI(x)V(x)- DE(x)G(2x)H(x)H(x)LQ</i>		
III-2-1		<i>ETQVARDSGMVPISRVNG- (x)V(2x)VDA(x)AI(x)V(x)- DE(x)G(2x)H(x)H(x)LQ</i>		
	III-2-1-1	<i>ETQVARDSGMVPISRVNG- TV(2x)VDA(x)AIVV(x)DE- (x)G(2x)H(x)H(x)LQ</i>		
	III-2-1-1a	ETQVARDSGMVPISRVNGTVVYV- DANAIVVL DE DGQEHFHYLQ	<i>Synechococcus</i> sp.	CC 9902
	III-2-1-1b	ETQVARDSGMVPISRVNGTVTF- VDATAIVVR DE EGYDHFHYLQ	<i>Synechococcus</i> sp.	CC 9311
	III-2-1-2	<i>ETQVARDSGMVPISRVNG- (x)V(x)FVDATAI(x)V(x)- DE(x)G(2x)H(x)H(x)LQ</i>		
	III-2-1-2a	ETQVARDSGMVPISRVNGTVTF- VDATAIVVR DE EGVDHSHYLQ	<i>Synechococcus</i> sp.	WH 7803
	III-2-1-2b	ETQVARDSGMVPISRVNGMVTF- VDATAIIVR DE DGVDFHYLQ	<i>Prochlorococcus marinus</i>	MIT 9303; MIT 9313
	III-2-1-2c	ETQVARDSGMVPISRVNGTVIFV- DATAIVVQ DE DGQEHFHYLQ	<i>Synechococcus</i> sp.	WH 8102

Table 1. cont.

Cluster*/Group†	Subgroup†	Common pattern‡	Species	Strain
III-2-2		ETQVARDSGMVPISRNGT- VTYVDANAIVVQ <u>DEDGN-</u> <u>DH</u> THFLQ	<i>Synechococcus</i> sp.	CC 9605
III-2-3		ETQVARDSGMVPITRVN- GEVVFVDSTQIIVR <u>DD-</u> <u>QGV</u> DHYHLLQ	<i>Synechococcus</i> sp.	RCC 307
IV		EAQAARDSGMVIVSRTNG- VVS(x)VDANRIR(x/-)KVAD- (x)DK(x)IFGKSEIEYEQ		
IV-1		EAQAARDSGMVIVSRTNG- VVSYVDANRIRIKV <u>ADE</u> DK- <u>DIF</u> GKSEIEYEQ	<i>Microcystis</i> sp.	130
IV-2		EAQAARDSGMVIVSRTNG- VVSYVDANRIRIKV <u>ADE</u> D- <u>KEIF</u> GKSEIEYEQ	<i>Microcystis</i> sp.	NIES 843§; 269
IV-3		EAQAARDSGMVIVSRTNG- VVS ^H VDANRIRIKV <u>ADQ</u> D- <u>KEIF</u> GKSEIEYEQ	<i>Microcystis aeruginosa</i>	PCC 7806
V		EAQAARDSGMVIVS(2x)DGE- (x)SY(x)DG(13-47x)EYELQ		
V-1	V-1-1	EAQAARDSGMVIVSQIDGEVS- YVDGARIRVT <u>SPEGQ</u> EVEYELQ	<i>Lyngbya</i> sp.	PCC 8106
	V-1-2	EAQAARDSGMVIVSRTDGEVSY- IDGSCIRVMD <u>TTGKE</u> HEYELQ	<i>Oscillatoria</i> sp.	PCC 8926; PCC 8954; PCC 9631
V-2	V-2-1	EAQAARDSGMVIVSRMD- GEISYIDGSRIIVK <u>SLTVDA-</u> <u>DSNS</u> SEESFLIREYDDLK- <u>TKQPSVWQKYAGY</u> IEYELQ	<i>Planktothrix agardhii</i>	PCC 9637; PCC 9702; PCC 7805; PCC 9239
	V-2-2	EAQAARDSGMVIVSRMDGEV- SYIDGSRIIVK <u>TLGVDAEPNS-</u> <u>SEESFLIREYDDLKTKQPS-</u> <u>VWKQKYAGY</u> IEYELQ	<i>Oscillatoria</i> sp.	PCC 8927
	V-2-3	EAQAARDSGMVIVSRTDGEVS- YIDGARIIVK <u>SLADAESNFS-</u> <u>EESFLIREYDDLKTKQPS-</u> <u>VWKQKYAAY</u> IEYELQ	<i>Oscillatoria</i> sp.	PCC 9018
	V-2-4	EAQAARDSGMVIVSRTDGEVSYI- DGSCIRVID <u>NNGKE</u> YEYELQ	<i>Planktothrix agardhii</i>	PCC 9214
VI		(x)AQGARDSGMV(x)VSRTD(2x)V- (x)YVDA(2x)IRVR(9-46x)LS		
VI-1		(x)AQGARDSGMV(x)VSRTD- GDVTYDVATEIRVRPK(9-17x)LS		
	VI-1-1	EAQGARDSGMVIVSRTDGDVTYV- DATEIRVRPK(4x)E(2x)Y(x)LS		
	VI-1-1-1	EAQGARDSGMVIVSRTDGDVTYV- DATEIRVRPK(4x)E(x)KY(x)LS		
	VI-1-1-1a	EAQGARDSGMVIVSRTDGDVTY- VDATEIRVR <u>PKPNA</u> SELKYLS	<i>Nostoc ellipsosporum</i>	V
	VI-1-1-1b	EAQGARDSGMVIVSRTDGDVT- YVDATEIRVR <u>PKTSTQ</u> EIKYRLS	<i>Nostoc</i> sp.	152
	VI-1-1-2	EAQGARDSGMVIVSRTDGDVTY- VDATEIRVRPKPNTSEIRY(x)LS		
	VI-1-1-2a	EAQGARDSGMVIVSRTDGDV- TYVDATEIRVR <u>PKPNTSEIRY</u> LLS	<i>Nostoc muscorum</i>	I (=Lukesova 2/91); II
	VI-1-1-2b	EAQGARDSGMVIVSRTDGDVT- YVDATEIRVR <u>PKPNTSEIRY</u> TLS	<i>Nostoc calcicola</i>	III; VI (=VI.5)

Table 1. cont.

Cluster*/Group†	Subgroup†	Common pattern‡	Species	Strain
VI-2	VI-1-2	(x)AQGARDSGMV(x)VSRTDGDV- TYVDATEIRVRPK(6-14x)RYRLS		
	VI-1-2-1	EAQGARDSGMVIVSRTDGDVTY- VDATEIRVRPK(4-12x)EIRYRLS		
	VI-1-2-1a	EAQGARDSGMVIVSRTDGDVT- YVDATEIRVR <u>PKEGNSE</u> IRYRLS	<i>Nodularia harveyana</i>	Huebel 1983/300
	VI-1-2-1b	EAQGARDSGMVIVSRTDGDVT- YVDATEIRVR <u>PKDNNSGESERS-</u> <u>RNE</u> IRYRLS	<i>Nodularia harveyana</i>	BECID 29
	VI-1-2-1c	EAQGARDSGMVIVSRTDGDVT- YVDATEIRVR <u>PKDNNSGDSE-</u> <u>RSRNE</u> IRYRLS	<i>Nodularia harveyana</i>	BECID 27
	VI-1-2-1d	EAQGARDSGMVIVSRTDGDVT- YVDATEIRVR <u>PKDSNSGEPERS-</u> <u>RNE</u> IRYRLS	<i>Nodularia harveyana</i>	Bo53
	VI-1-2-2	(x)AQGARDSGMVVSRTDGDVT- YVDATEIRVRPKGGN(3x)RYRLS		
	VI-1-2-2a	EAQGARDSGMVVSRTDGDVT- YVDATEIRVR <u>PKGGNSE</u> IRYRLS	<i>Nodularia sphaerocarpa</i>	PCC 7804; BECID 35; BECID 36
	VI-1-2-2b	EAQGARDSGMVVSRTDGDVTY- VDATEIRVR <u>PKGGNSE</u> TRYRLS	<i>Nodularia sphaerocarpa</i>	HKVV; Up16a; Up16f
	VI-1-2-2c	KAQGARDSGMVVSRTDGDVT- YVDATEIRVR <u>PKGGNFRI</u> RYRLS	<i>Nodularia spumigena</i>	Fae19
	VI-1-2-3	EAQGARDSGMVIVSRTDGDVT- YVDATEIRVR <u>PKDGNSE</u> IRYRLS	<i>Nodularia spumigena</i>	PCC 9350; HEM; AV63; Huebel 1987/ 311; AV1
	Ungrouped	EAQGARDSGMVIVSRTDGDVV- YVDATEIRVR <u>VSGQLPAASGKS-</u> <u>TDNGQLTSQKGQEI</u> RYTVS	<i>Anabaena variabilis</i>	ATCC 29413
		EAQGARDSGMVIVSRTDGDVV- YVDATEIRVR <u>VSGQLPTASGKS-</u> <u>TDNGQLTSQKGQEI</u> RYTVS	<i>Anabaena</i> sp.	PCC 7120
		EAQGARDSGMVIVSRTDGDVT- YVDATEIRVR <u>PKPNSPEI</u> RYTVS	<i>Trichormus azollae</i>	Kom BAI 1983
		EAQGARDSGMVIVSRTDGDVT- YVDATEIRVR <u>VSNQSKGSEHG-</u> <u>QATNQKPQEIRV</u> YYLS	<i>Trichormus dololium</i>	Dololium 1
		EAQ(x)ARDSGMVIVSRT(x)G(x)- V(x)YVDA(2x)IRVR(21-46x)Y(x)LS		
	VI-2-1	EAQAARDSGMVIVSRTDGEVV- YVDATEIRVRV(20-33x)Y(x)LS		
	VI-2-1-1	EAQAARDSGMVIVSRTDGE- VYVDATEIRVRVSS(x)WSV- (x)SGQSSLAEK(x)TDNEQL- (3x)K(x)Q(x)IRYNLS		
	VI-2-1-1a	EAQAARDSGMVIVSRTD- GEVVYVDATEIRVR <u>VSSQ-</u> <u>WSVISGQSSLAEKRTTDN-</u> <u>EQLATDKPQDI</u> RYNLS	<i>Anabaena planctonica</i>	1tu36s8
	VI-2-1-1b	EAQAARDSGMVIVSRTDGEVVYV- DATEIRVRVSSSEWSVSGQSSLA- <u>EKRSTDNEQLMSEKFQEIRYNLS</u>	<i>Anabaena planctonica</i>	1tu33s8; 1tu28s8; 1tu33s10; 1tu30s13
	VI-2-1-1c	EAQAARDSGMVIVSRTDGEVVYV- DATEIRVRVSSQWSVISGQSSLA- <u>EKRSTDNEQLT</u> DKPQEIRYNLS	<i>Anabaena spiroides</i>	1tu39s17

Table 1. cont.

Cluster*/Group†	Subgroup‡	Common pattern‡	Species	Strain
	VI-2-1-2	EAQAARDSGMVIVSRTDGDVVYV- DATEIRVRVGGQRLVVGDEEK- TGDREQLRTDKPQELKYKLS	<i>Aphanizomenon flos-aquae</i>	1tu26s2; 1tu29s19; 1tu37s13
	VI-2-2	EAQAARDSGMVIVSRTDGDV(x)- YVDATEIRVRASGQLSAASGS(6- 7x)Q(x)IEKGQELKYKLS		
	VI-2-2-1	EAQAARDSGMVIVSRTDGD- VVYVDATEIRVRASGQLSA- ASGSQVIEKGQELKYKLS	<i>Anabaena</i> sp.	90
	VI-2-2-2	EAQAARDSGMVIVSRTDGD- VIYVDATEIRVRASGQLSA- ASGSPQIEKGQELKYKLS	<i>Anabaena circinalis</i>	1tu34s5
	VI-2-3	EAQGARDSGMVIVSRTDGD- VVYVDAAEIRVRVREQEKEDE(2- 3x)RGQDTEKNVKLGDTF(2x)- SPRLPITSSSS(x)PKE(x)RYVLS		
	VI-2-3-1	EAQGARDSGMVIVSRTDGDV- VVYVDAAEIRVRVREQEKEDE- LTRGQDTEKNVKLGDTFT- ASPRLPITSSSSPPKEIRYVLS	<i>Trichormus variabilis</i>	HINDAK 2001/4
	VI-2-3-2	EAQGARDSGMVIVSRTDGDV- VVYVDAAEIRVRVREQEKEDE- GRGQDTEKNVKLGDTFSPSPR- LPITSSSSPPKEIRYVLS	<i>Trichormus variabilis</i>	GREIFSWALD
	VI-2-4	EAQAARDSGMVIVSRTHGDDV- YVDATEIRVRVSGQ(x)SAASGS- QVIEKGQEIRY(x)LS		
	VI-2-4-1	EAQAARDSGMVIVSRTHGDDVY- VDATEIRVRVSGQLSAASGSQ- VIEKGQEIRYNLS	<i>Anabaena oscillarioides</i>	BECID 22; BECID 32
	VI-2-4-2	EAQAARDSGMVIVSRTHGDDVY- VDATEIRVRVSGQISAASGSQVI- EKGQEIRYTLS	<i>Anabaena cylindrica</i>	XP6B
	Ungrouped	EAQGARDSGMVIVSRTDGDVVY- VDAAQIRVRVRGDLSGVTGNLIA- GKQHTNEQGQQVTDREIRYVLS	<i>Anabaena oscillarioides</i>	BO HINDAK 1984/43
		EAQGARDSGMVIVSRTDGDV- VVYVDAAEIRVRVREQTDDKTL- TQSPASSKESPTGKPQEVYVLS	<i>Anabaena augstumalis</i>	SCHMIDKE JAHNKE/4a
		EAQAARDSGMVIVSRTDGEVV- YVDATEIRVRVSGQSLANQQ- TTDKQHAPEKPQEIKYTLS	<i>Anabaena</i> sp.	1tu34S7
		EAQAARDSGMVIVSRTDGEVVYV- DATEIRVRVSSQWSIVSGQQTT- DNEQQTTDKPQEIKYTLS	<i>Aphanizomenon issatschenkoi</i>	0tu37S7
		EAQGARDSGMVIVSRTDGDVTY- VDAAEIRVRVREPLTETNTQHP- PKEVRYVLS	<i>Anabaena</i> sp.	PCC 7108
	Unclustered	EAQAARDSGMVIVSRTEGIVTYA- DANEIRVKVTGPEGHEKVGQE- IQYILQ	<i>Crocospaera watsonii</i>	WH8501
		EAQAARDSGMVIVSRTHGIVTY- VDATEIRVQPHSPDNPAEKGE- EIVYPIQ	<i>Synechocystis</i> sp.	PCC 6803
		EAQAARDSGMVILSQTNGVVS- VDANQIRVKTDNGPEITYTLQ	<i>Thermosynechococcus elongatus</i>	BP1

Table 1. cont.

Cluster*/Group†	Subgroup†	Common pattern‡	Species	Strain
Cyanobacteria		EPQAARDSGMVVVTRTDGEVSYV- DSTKISVID K EGNQADYPLCKYQ (2x)Q(x)ARDSGMV(30-72x)	<i>Trichodesmium erythraeum</i>	IMS 101

*Clusters have been determined according to the 16S rRNA gene sequence-based phylogenetic tree presented in Fig. 2, and specified into groups depending on their organization in *rpoB* trees.

†Groups and subgroups have been determined based on signature sequence identity.

‡Indels are in shown in bold; signature sequences are underlined.

§Strain NIES 843 has been reassigned to the species *Microcystis aeruginosa*.

Trichormus (Fig. 4b, c and d). In group VI-1, the *Nostoc* and *Nodularia* strains are separated into two monophyletic subgroups (VI-1-1 and VI-1-2) (Fig. 3c and d). Moreover, subgroups VI-1-2-1 and VI-1-2-3 include strains with the same species attribute, *Nodularia harveyana* and *Nodularia spumigena*, respectively. This shows that, in some cases, the signature can differentiate strains at the species level. However, some other subgroups contain two different species, e.g. subgroup VI-1-2-2b includes both *Nodularia sphaerocarpa* and *Nodularia spumigena* (Table 1 and Fig. 3d). Although one cannot exclude that the mutation rate of this *rpoB* region differs in some strains, this might result from a misleading species assignment.

Rajaniemi *et al.* (2005) published a partial tree from the same *rpoB* gene sequence portion, but did not distinguish between groups VI-1 and VI-2, although groups A and B defined by these authors appear to perfectly correspond to the subgroups VI-2-1-1 and VI-2-1-2 identified in the present study (Fig. 4b and c; Table 1). In this example, even if the signature sequence comprises a small number of nucleotides, it provides the same resolution as longer sequences. The fact that strains belonging to the genera *Anabaena*, *Aphanizomenon* and *Trichormus* are not separated into discrete subgroups (Fig. 4c and d) supports the hypothesis that these cyanobacterial strains might belong to the same genus, within which several species can be distinguished, as discussed by Rajaniemi *et al.* (2005).

Interestingly, in cluster V (Table 1), RpoB signature sequences can be useful to identify groups first on the basis of pattern length and second on the basis of their specific amino acid sequence. For example, in cluster V, the *Oscillatoria* strains present six and 40 aa indels. Notably, all the *Planktothrix* strains (*P. agardhii* and *P. rubescens*) whose sequences have been determined in the present study fall within group V-2 with a longer indel, with the exception of strain PCC 9214 (Table 1). Thus, knowing that *Planktothrix* strains can be distinguished on the basis of the *rpoB* signature sequence length, a simple PCR might be sufficient, as a primary screen, to identify subgroups to which particular strains belong. In contrast, the five *Oscillatoria* strains analysed in the present study are distributed in three subgroups, V-1-2, V-2-2 and V-2-3, that include either long or short sequences (Table 1). This

observation suggests that a re-examination of the taxonomic position of *Oscillatoria* sp. strains PCC 8926, PCC 8927, PCC 8954, PCC 9018 and PCC 9631, as well as *Planktothrix agardhii* PCC 9214, using a polyphasic approach (Suda *et al.*, 2002) is required to clearly establish if they belong to the genera *Planktothrix* or *Oscillatoria*.

Concluding remarks

The signatures of the *rpoB* sequences of cyanobacteria identified in this study that allow the identification of strains at a subgenus level even permit species separation for some taxa. These signature sequences might thus be particularly useful for the identification and classification of a large number of isolates, as well as for assessment of cyanobacterial biodiversity in natural microbial communities and for following the seasonal abundance of cyanobacteria in a given community over time. Generally, these *rpoB* signatures may be used as a tool to change the current view of the classification of nostocacean strains, and possibly that of other cyanobacterial groups. The contribution of molecular typing provided by *rpoB* and other housekeeping gene indels may well advance the resolution of specific cases in cyanobacterial taxonomy or provide an intermediate level of variability suited to various molecular typing applications, including DGGE and SSCP (Boutte *et al.*, 2006; Dorigo *et al.*, 2005).

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