The *cpcB–cpcA* locus as a tool for the genetic characterization of the genus *Arthrospira* (Cyanobacteria): evidence for horizontal transfer

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To investigate the genetic diversity of the genus *Arthrospira* and to compare it with other cyanobacteria, sequences of 670 nt from the phycocyanin operon were determined for 23 natural, cultivated or commercial strains of *Arthrospira* and compared with sequences from 20 other non-*Arthrospira* cyanobacterial strains. The sequenced DNA fragment comprises the last 255 nt of *cpcB*, the *cpcB–cpcA* spacer and the first 304 nt of *cpcA*. The resulting phylogenetic tree confirms that the genus *Arthrospira* is not related to *Spirulina*. So far, *cpcB–cpcA* data suggest that the closest relative of *Arthrospira* is *Planktothrix*. Based on this locus, the genus *Arthrospira* consists of three genetically clustered lineages. However, the distribution of nucleotide substitutions indicates that these three lineages are not the result of a simple cladogenesis characterized by the accumulation of independent substitutions. Instead, the observed clustering is the result of horizontal transfers of blocks of sequences. Analysis of the distribution of substitutions in the sequenced fragment indicates a point of intragenic recombination close to the stop codon of *cpcB*. The capacity of exchange of genetic material among strains probably explains why morphology and geographical origin do not correlate with the *cpcB–cpcA* clusters. Nevertheless, this study shows for the first time that the genus *Arthrospira*, represented here by cultivated and wild specimens, is clearly monophyletic. Moreover, the *cpcB–cpcA* DNA fragment, comprising both highly and moderately variable regions, allows (1) a strict differentiation of the taxon *Arthrospira* from other cyanobacteria (using the coding regions only) and (2) the study of relationships inside *Arthrospira* (using both the coding and non-coding regions).

Keywords: *Arthrospira*, *cpcB–cpcA*, horizontal transfer, molecular phylogeny

INTRODUCTION

Arthrospira is an inexpensive, high quality nutritional supplement that has an annual industrial production reaching several hundreds tons worldwide (Ciferri & Tiboni, 1985; Belay *et al.*, 1993; Fox, 1996). This organism lives in warm lakes with high carbonate content and high pH. The main purpose of this work is to clarify the diversity of natural, cultivated and

commercial strains of *Arthrospira* using sequence data from a highly variable DNA fragment. To define and delimit the genus *Arthrospira*, this study includes other cyanobacteria. A second goal of this study is to identify specific molecular markers to fingerprint different strains of *Arthrospira* and to develop tools for species and strain identification.

Originating from the taxonomic treatise of cyanobacteria of Geitler (1932), confusion exists between the genera *Spirulina* and *Arthrospira*. In fact, these two genera are only distantly related (Guglielmi *et al.*, 1993; Nelissen *et al.*, 1994). In the so-called ‘*Spirulina*’ biotechnology field, putative species of *Arthrospira*

Micrographs showing the different morphologies observed among *Arthrospira* strains are available as supplementary data in IJSEM Online (<http://ijs.sgmjournals.org>).

Table 1. List of strains analysed

Name	Origin*	Provided by:†	Accession no.‡
<i>Arthrospira platensis</i>	?	?	Y09074
<i>Arthrospira</i> sp. Paracas P2	Peru (w)	G. Planchon, 1995	AJ401166
<i>Arthrospira</i> sp. BU1	Burma (w)	G. Planchon, 1995	AJ401167
<i>Arthrospira</i> sp. Maxima	Mexico (w)	F. Ayala, 1997	AJ401168
<i>Arthrospira</i> sp. M1	Chad/Spain (c)	IMADE SA, 1996	AJ401169
<i>Arthrospira</i> sp. Paracas P0	Peru (w)	A. Prior, 1996	AJ401110
<i>Arthrospira</i> sp. ThD	?/Switzerland (c)	J.-B. Menut, 1998	AJ401111
<i>Arthrospira</i> sp. Titi	Peru (w)	R. Fox, 1996	AJ401112
<i>Arthrospira</i> sp. M2	Chad (w)	M. Tredicci, 1995	AJ401113
<i>Arthrospira</i> sp. Fv-8	?/USA (c)	Earth Raise Co., 1997	AJ401114
<i>Arthrospira</i> sp. Paracas GE98	Peru/Switzerland (c)	J. Falquet, 1998	AJ401115
<i>Arthrospira</i> sp. Dihé	Chad (w)	Louis Bergier Int., 1998	AJ401116
<i>Arthrospira</i> sp. Lonar-LoH7	India/France (c)	J.-P. Jourdan, 1997	AJ401117
<i>Arthrospira platensis</i> PCC 7345 ^T	California, USA (w)	PCC 7345 ^T	AJ401118
<i>Arthrospira</i> sp. PK	?/China (c)	Sea Source Corp., 1997	AJ401119
<i>Arthrospira</i> sp. Mad	India (c)	Antenna Trust, 1998	AJ401120
<i>Arthrospira</i> sp. BU2	Burma (w)	G. Planchon, 1995	AJ401121
<i>Arthrospira</i> sp. Mix	India/France (c)	J.-P. Jourdan, 1997	AJ401122
<i>Arthrospira</i> sp. Sun§	India (c)	N. Sundaram, 1995	AJ401123
<i>Arthrospira</i> sp. Nord Geitl	?/Ukraine (c)	L. Kovechnikov, 1999	AJ401124
<i>Arthrospira</i> sp. Sark	?/Switzerland (c)	Arkogélules spiruline, 1999	AJ310555
<i>Arthrospira</i> sp. Paracas E2	Peru/Ecuador (c)	Biorigine SA, 2000	AJ310553
<i>Arthrospira</i> sp. E1	?/Ecuador (c)	Biorigine SA, 2000	AJ310554
<i>Oscillatoria sancta</i>		PCC 7515 ^T	AJ401125
<i>Oscillatoria acuminata</i>		PCC 6304 ^T	AJ401126
<i>Lyngbya aestuarii</i>		PCC 7419 ^T	AJ401127
<i>Spirulina major</i>		PCC 6313 ^T	AJ401128
<i>Microcystis aeruginosa</i>			AJ003181
<i>Fischerella</i> sp.			M75599
<i>Anabaena</i> sp.		PCC 7120	X05239
<i>Nodularia spumigena</i>			AF101444
<i>Synechococcus elongatus</i>			D13173
<i>Synechocystis</i> sp.		PCC 9413	AF068771
<i>Pseudanabaena</i> sp.		PCC 7409	M99426
<i>Calothrix</i> sp.		PCC 7601	M36276
<i>Fremyella diplosiphon</i>			X07012
<i>Synechocystis</i> sp.		PCC 6803	U34930
<i>Chroococcus dispersus</i>			AJ003184
<i>Synechococcus</i> sp.		PCC 7002	K02660
<i>Synechococcus</i> sp.			X59809
<i>Synechococcus</i> sp.		PCC 7942	AB008546
<i>Synechococcus</i> sp.		PCC 6301	M94218
<i>Planktothrix rubescens</i>			AJ131820
<i>Cyanidium caldarium</i>			S77125
<i>Aglaothamnion neglectum</i>			Z11906
<i>Rhodella violacea</i>			Z48165

* w, Wild; c, cultivated. Where two countries are given, the first indicates the origin of the strain and the second indicates where it was cultivated; ?, unknown.

† PCC, Pasteur Culture Collection.

‡ Sequences determined in this study are indicated in bold.

§ Upon microscopic examination, this sample was found to be morphologically different from other known *Arthrospira* sp. This sequence was recovered from an *Arthrospira* culture whose the medium composition was far from the common standards (see text).

have been recognized (Tomaselli, 1997). By total DNA restriction profile analyses of different strains of two putative species (*Arthrospira platensis* and *Arthrospira maxima*), Viti *et al.* (1997) were able to recognize two clusters corresponding to the putative morphological species. In contrast, using restriction profiles of the ribosomal ITS region, Scheldeman *et al.* (1999) divided 51 strains of *Arthrospira* (comprising several putative species) into two clusters unrelated in their morphology, species denomination or geographical origin.

Preliminary data (randomly amplified polymorphic DNA and total DNA restriction profiles, data not shown) have shown that the genetic background of *Arthrospira* is not correlated to morphological traits. For instance, strains with different morphology (spirals or rods) may have close DNA profiles, whereas strains with identical morphology may have divergent profiles. To determine the phyletic relationships between morphologically different *Arthrospira* strains, a region of the phycocyanin locus, namely the *cpcB–cpcA* spacer, has been sequenced because its nucleotide substitution rate is potentially higher than that of the widely used 16S rDNA sequence (Nelissen *et al.*, 1996; Nübel *et al.*, 1997; Ishida *et al.*, 1997; Lyra *et al.*, 2001; Barker *et al.*, 2000a, b; Tillett *et al.*, 2001; Robertson *et al.*, 2001). Other cyanobacteria strains were used for comparison; the sequences of these were collected from DNA databases or produced during this work. The relationships between *Arthrospira* and *Lyngbya* (Guglielmi *et al.*, 1993) and the lack of relations between *Arthrospira* and *Spirulina* are confirmed. A relationship between *Planktothrix* and *Arthrospira* is suggested. The available *Arthrospira* strains are divided into three groups. However, the *cpcB–cpcA* data indicate exchange of genetic material between strains, making phylogenetic hypotheses impossible to establish. The intragenic recombination observed at this locus (and probably at others) would explain the discrepancies observed between morphological data and sequence data.

METHODS

Strains. Table 1 gives the list of *Arthrospira* strains studied, with their origin, culture history and the corresponding DNA database accession number of the *cpcB–cpcA* locus. Data on the non-*Arthrospira* strains used in this work are also included.

DNA extraction, amplification and sequencing. Fresh or dry bacterial pellets were extracted following the method of Neilan *et al.* (1995) using lysozyme and proteinase K. The *cpcB–cpcA* spacer was amplified using primers located within the coding region of *cpcB* and *cpcA*, respectively. The forward primer was PCβF (Neilan *et al.*, 1995). The reverse primer (5'-CCAGTTCCACCAGCAATCAG) was modified from the primer PCαR (Neilan *et al.*, 1995) to match with the unpublished available sequence of *A. platensis* (accession no. Y09074). The amplified fragments (annealing temperature 50 °C) were directly sequenced using the forward and reverse primers with the BigDye Terminator Cycle Sequencing kit and the 373 ABI sequencer (Perkin-Elmer).

Phylogenetic analysis. An overview of the phylogenetic

position of *Arthrospira* in cyanobacteria was created by comparing the sequences of the *Arthrospira cpcB–cpcA* spacer to corresponding cyanobacterial sequences available in the databases and sequences obtained in this work from *Spirulina major* PCC 6313^T, *Oscillatoria acuminata* PCC 6304^T, *Oscillatoria sancta* PCC 7515^T and *Lyngbya aestuarii* PCC 7419^T. The outgroup comprises the *cpcB–cpcA* locus of three alga plastids: *Cyanidium caldarium* (S77125), *Aglaothamnion neglectum* (Z11906) and *Rhodella violacea* (Z48165). As the spacer was very variable, alignment of this region was impossible for most of these taxa (except *L. aestuarii* PCC 6313^T). Thus, phylogenetic analysis of this matrix was performed with the aligned coding regions only. On the other hand, the analyses of strains of *Arthrospira* were done with both the coding sequences and the spacer. Phylogenetic trees were obtained with PAUP 3.1 (parsimony analysis; Swofford, 1993) using a heuristic search with 10 replications of RANDOM stepwise additions of sequences, the TBR branch swapping and MULTIPARS options in effect. Support of the clades was calculated with bootstrap analyses of 1000 replicates and TBR branch swapping.

Likelihood analysis of recombination. Using the program LARD as described by Holmes *et al.* (1999), the likelihood of the null hypothesis, H0, that there has been no recombination event at the *cpcB–cpcA* locus and the likelihood, H1, that there has been a recombination event, were assessed from a simple unrooted tree of three sequences (Paracas P2, Maxima and Bu1, each belonging to the three putative classes of recombinants). To assess whether H1 is a significantly better fit to the data (i.e. that the likelihood ratio of H1 to H0 is greater than would be expected by chance), 500 sequence datasets were simulated using the program SEQ-GEN (Rambaut & Grassly, 1997) under the maximum-likelihood H0 model and reanalysed using LARD.

RESULTS AND DISCUSSION

1. Structure of the *cpcB–cpcA* locus and phylogenetic implications

Sequences obtained from the amplified fragments of the genus *Arthrospira* and *L. aestuarii* PCC 7319^T comprised the last 255 nt of *cpcB*, a 111 nt spacer and the first 304 nt of *cpcA*. For the other cyanobacteria, the size of the spacer was highly variable. A codon insertion was observed downstream of the start codon of *cpcA* in *Fischerella* sp., *Anabaena* sp. PCC 7120, *Chroococcus dispersus*, *Nodularia spumigena* and two strains of *Synechococcus* sp. (PCC 7942 and PCC 6301). A two-codon insertion was present in *cpcB* of *Microcystis aeruginosa*.

The consensus tree of the four most parsimonious trees obtained (with all cyanobacterial taxa available and with the partial *cpcB* and *cpcA* coding sequences only) is shown in Fig. 1. The four most parsimonious trees are 2079 steps long and have a consistency index of 0.36 (0.34 excluding uninformative sites) and a retention index of 0.57, suggesting that the coding sequence of this locus comprises a large number of homoplasies (reversions, parallelisms). Of the 23 *Arthrospira* strains studied, one strain (Sun), unrelated to the other *Arthrospira* accessions, formed a clade with four of the five *Synechococcus* strains analysed. The data also indicate a close phylogenetic relationship

Fig. 1. Strict consensus tree of the four most parsimonious trees obtained from analysis of parts of the *cpcB* and *cpcA* coding sequences only. The outgroup comprises three algae platid (Pt) sequences. Bootstrap values are indicated on the branches. The geographical origin of the *Arthrospira* strains are indicated by ASI (Asia), NAM (North America), SAM (South America) and AFR (Africa). The morphological aspect of strains is indicated by R (rod), S (spiral) and RS (relaxed spiral).

between *Arthrospira* and *Planktothrix* (*Oscillatoria*) *rubescens* (Anagnostidis & Komarek, 1988), as suggested from 16S rDNA data (Wilmutte, 1994; Nelissen *et al.*, 1996). As already shown from 16S rDNA data (Guglielmi *et al.*, 1993; Nelissen *et al.*, 1996; Ishida *et al.*, 1997), *L. aestuarii* PCC 7419^T is also closely related to *Arthrospira* and is a sister to the clade *Planktothrix*/*Arthrospira*. As it is now widely accepted (Guglielmi *et al.*, 1993), *Spirulina major* PCC 6313^T is well distinct and relatively distant from *Arthrospira*. Results indicate that several genera are para- or polyphyletic: *Synechocystis*, *Synechococcus* and *Oscillatoria*. This is in accordance with 16S rDNA data showing that several genera comprise unrelated strains (Nelissen *et al.*, 1996; Ishida *et al.*, 1997).

Regarding the general phylogenetic implications inferred by this DNA fragment in cyanobacteria, definitive conclusions must not be drawn because sampling in this analysis is low, the analysis is based on only one DNA sequence and high homoplasy is found

Informative characters

	11222222	22223333	4555566
	13318233334	56780112	4004723
Taxon	950919017893	74749131	4483970

Paracas P2	CATTCTGCTGCT	TCCCTTTC	ACTTGTC
Paracas P0	CATTCTGCTGCT	TCCCTTTC	ACTTGTC
Paracas P8	CATTCTGCTGCT	TCCCTTTC	ACTTGTC
Paracas E2	CATTCTGCTGCT	TCCCTTTC	ACTTGTC
FV-8	CATTTTGCTGCA	CAAGGCGG	ATGGACA
M2	CATTTTGCTGCA	CAAGGCGG	ATGGACA
Maxima	CATTTTGCTGCA	CAAGGCGG	ATGGACA
PCC 7345	CATTTTGCTGCA	CAAGGCGG	ATGGACA
PK	CATTTTGCTGCA	CAAGGCGG	ATGGACA
Nord Geitl	CCTTTTGCTGCA	CAAGGCGG	ATGGACA
Y09074	TTCCCCATATGT	CAAGGCGG	GTGGACA
Titi	TTCCCCATATGT	CAAGGCGG	GTGGACA
ThD	TTCCCCATATGT	CAAGGCGG	GTGGACA
M1	TTCCCCATATGT	CAAGGCGG	GTGGACA
Bu1	TTCCCCATATGT	CAAGGCGG	GTGGACA
Dihé	TTCCCCATATGT	CAAGGCGG	GTGGACA
Lonar H7	TTCCCCATATGT	CAAGGCGG	GTGGACA
Mad	TTCCCCATATGT	CAAGGCGG	GTGGACA
Bu2	TTCCCCATATGT	CAAGGCGG	GTGGACA
Mix	TTCCCCATATGT	CAAGGCGG	GTGGACA
E1	TTCCCCATATGT	CAAGGCGG	GTGGACA
Sark	TTCCCCATATGT	CAAGGCGG	GTGGACA

cpcB spacer cpcA

Fig. 2. Mosaic distribution of the variable sites at the *cpcB*–*cpcA* locus of *Arthrospira*. To make the observation easier, the strains are classified according to the clusters of Fig. 1. Numbers above the alignment indicate the nucleotide position of the informative sites. Blocks of orthologous sequences are boxed.

in this DNA fragment. However, 16S rDNA data and *cpcB*–*cpcA* data are often in agreement. As others have suggested from 16S rDNA data, *cpcB*–*cpcA* data also indicate that several taxa are genetically heterogeneous and that the taxonomy of cyanobacteria has to be reconsidered (Ishida *et al.*, 1997; Guglielmi *et al.*, 1993; Nelissen *et al.*, 1996).

The phylogenetic structure of the genus *Arthrospira* was studied using both the coding and non-coding sequences. The matrix of the 22 *Arthrospira* strains (excluding the unrelated strain Sun) comprises 670 sites, 34 of them are variable and 27 are informative. The consistency index of the unrooted most parsimonious trees obtained using this matrix is equal to 1.0, suggesting no homoplasy. Fourteen of the 27 informative sites define an internal branch separating the group of the four strains of Paracas (Peru) from all other strains.

2. Evidence of recombination in the *cpcB*–*cpcA* locus of *Arthrospira*

The informative sites of the *cpcB*–*cpcA* DNA matrix of *Arthrospira* (with the taxa arranged according to the results of Fig. 1) are shown in Fig. 2. The mosaic

Fig. 3. *Arthrospira* phylogenies resulting from positions 1–255 (*cpcB*) and 256–670 (the spacer and *cpcA*). Bootstrap values are indicated on the branches.

distribution of the substitutions suggests recombination events at this phycocyanin locus. A first block comprises positions 1–255 (the 3' end of the *cpcB* translated sequence). A second block comprises positions 256–670 (the *cpcB*–*cpcA* spacer and the 5' end of the *cpcA* translated sequence). Fig. 3 shows the trees obtained with positions 1–255 and 256–670, respectively. The first block clearly differentiates clades 1 and 2 as defined in Fig. 1. On the other hand, the substitutions of the second block do not separate clades 1 and 2, but differentiate the Paracas clade from all other strains. This is reminiscent of the mosaic structure of the distribution of informative sites observed in the *rbcLX* gene of *Nostoc* (Rudi *et al.*, 1998). In *Arthrospira*, the phycocyanin locus also indicates intragenic recombination and suggests exchanges of genetic material between strains. Genetic exchanges have also been postulated in *Nodularia* (Barker *et al.*, 2000a).

The LARD maximum-likelihood method (Holmes *et al.*, 1999) was applied to find the breakpoint in the alignment which gave the highest likelihood under an evolutionary model incorporating recombination. This point was located after position 244, in agreement with Fig. 2. The likelihood ratio of H1 (recombination after position 244) to H0 (no recombination) was found to be 15.69. Monte Carlo simulation using SEQ-GEN (Rambaut & Grassly, 1997) indicates that this ratio was significantly greater than for the 500 simulated datasets (mean of 4.18, maximum of 11.07). Thus, the *Arthrospira* tree topology does not reflect a

true cladogenesis because of horizontal transfers. There is no correlation of the DNA matrix with the geographical origin or the morphology (rod or spirals), except for strains of the Paracas clade, which are characterized by a particular morphology (relaxed spirals; see micrographs in supplementary data in IJSEM, <http://ijs.sgmjournals.org>).

In nature, bacteria are living in local communities continuously exchanging biochemicals, such as nutritional factors, and genetic material, such as replicons, e.g. plasmids and prophages (Mathieu & Sonea, 1996; Rudi *et al.*, 1998). Thus, natural or cultured conditions offer totally different opportunities for evolution to bacteria. This could explain the discrepancies frequently observed between natural and cultivated cyanobacteria (Palinska *et al.*, 1996). Ten years ago, Komarek & Anagnostidis (1989) estimated that the features of more than 50% strains in collections do not correspond to the diagnoses of the taxa to which they are assigned. Are natural exchanges of genetic material between related or more distant taxa responsible for these observed discrepancies?

Several mechanisms have been proposed for the exchange of genetic material between strains by homologous recombinations as a consequence of conjugation, transduction by bacteriophages and occasional natural competence. Ecological, physiological and genetic mechanisms of natural exchanges of genetic material between bacteria are proposed (Lorenz & Wackernagel, 1994; Syvanen, 1994). Some of these mechanisms might involve long-distance exchanges.

Several observations indicate that exchange of genetic material is usual in (cyano)bacteria (Lawrence & Ochman, 1998; Ueda *et al.*, 1999; Rudi & Jakobsen, 1999; Barker *et al.*, 2000a). It is even proposed that such mechanisms are predominant in bacterial evolution and could explain the homogeneity of morphological characters and the observed morphological stasis for more than two billion years (Rudi *et al.*, 1998). These observations make the use of DNA sequences questionable in a phylogenetic perspective, at least for phyletically related strains readily susceptible to homologous recombinations (Syvanen, 1987; Lawrence & Roth, 1996; Lawrence, 1999; Young, 2001). In the case of *Arthrospira*, this would explain the discrepancies observed between morphological data (for instance rods or spirals) and sequence data, because of the comparison of paralogous DNA sequences. As the variations observed in this DNA fragment are not the result of an historical cladogenesis, a correlation between the geographical origin and the genetic clusters is not observed.

3. The *cpcB-cpcA* locus as a tool for identification of *Arthrospira* strains

Based on the *cpcB-cpcA* fragment, a phylogeny reflecting historical cladogenesis of the genus *Arthrospira* is not possible because this locus is subject to intragenic recombination. Consequently, the topology of the *Arthrospira* tree is the result of the comparison of historically unrelated DNA sequences. The fact that substitutions are transmitted in block explains a consistency index of the *cpcB-cpcA*-based tree of *Arthrospira* equal to 1.0.

The *cpcB-cpcA* locus of the phycocyanin operon comprises moderately variable regions (both the *cpcB* and *cpcA* coding regions) and a highly variable region (the non-coding spacer between the coding regions). Thus, the coding regions can be used for comparisons with other genera of cyanobacteria. Excluding one strain (Sun), the *Arthrospira* strains available for this study form a strongly sustained monophyletic group. On the other hand, the spacer region is so variable in size and sequence that it allows strict differentiation of the genus *Arthrospira* from other cyanobacteria. As an illustration, the unrelated strain Sun, from Madurai (India), was readily identified as non-*Arthrospira*. In retrospect, it was observed that this strain does not grow on the standard *Arthrospira* medium (Zarrouk, 1966) and is morphologically distinct. This easily noticed error demonstrates that this DNA fragment is suitable for an easy identification of the genus *Arthrospira* and probably other genera. In fact, the *cpcB-cpcA* spacer is so variable that alignment is impossible between most of the genera and such a DNA fragment isolated from a particular strain is readily identifiable by comparison with data in a DNA bank.

Even if it is suspected of intragenic recombination, this DNA fragment allows clustering in *Arthrospira*. Fig. 3

shows that the genus comprises three clusters, the hierarchy of them being unknown, because of suggested horizontal transfer. The first cluster is represented by the Paracas lineage only and has a specific morphology and a restricted geographical origin. The second lineage comprises Fv-8, M2, Maxima, *A. platensis* PCC 7345^T, PK and Nord Geitl. The third lineage comprises Y09074, Titi, ThD, M1, Bu1, Dihé, Lonar H7, Mad, Bu2, Mix, E1 and Sark. The last two lineages have no correlation with morphology (rods/spirals) or geography. Similarly, in a study based on amplified rDNA restriction analysis (Scheldeman *et al.*, 1999), *Arthrospira* is divided into two clusters that have no clear relationships with geographic origin and morphology. However, a comparison with their work is difficult because most strains used cannot be related with those analysed here. From the few identifiable strains, it is observed that Titi and Lonar cluster together and that *A. platensis* PCC 7345^T is related to the other cluster, as in this study. However, in contradiction with the results reported here, their Maxima strain did not cluster with *A. platensis* PCC 7345^T.

In conclusion, the results demonstrate convincingly several points concerning the genus *Arthrospira*: (1) horizontal genetic transfers are suggested between the *Arthrospira* lineage; (2) the genus *Arthrospira* is monophyletic; (3) *Arthrospira* strains cannot be grouped according to their geographic origin; (4) *Arthrospira* strains cannot be grouped according to morphology, except for the 'relaxed spiral' type, which seems limited to strains originating from the Paracas region (Peru); and (5) because of very specific growing conditions (particularly high pH), dense cultures of *Arthrospira* seems to exclude contamination with other cyanobacteria and an eventual accidental contamination can readily be detected by the analysis of the *cpcB-cpcA* locus.

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