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RESEARCH ARTICLE

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Cyanobacterial diversity in extreme environments in Baja California, Mexico: a polyphasic study

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Abstract Cyanobacterial diversity from two geographical areas of Baja California Sur, Mexico, were studied: Bahia Concepcion, and Ensenada de Aripez. The sites included hypersaline ecosystems, sea bottom, hydrothermal springs, and a shrimp farm. In this report we describe four new morphotypes, two are marine epilithic from Bahia Concepcion, *Dermocarpa* sp. and *Hyella* sp. The third, *Geitlerinema* sp., occurs in thermal springs and in shrimp ponds, and the fourth, *Tychonema* sp., is from a shrimp pond. The partial sequences of the 16S rRNA genes and the phylogenetic relationship of four cyanobacterial strains (*Synechococcus* cf. *elongatus*, *Leptolyngbya* cf. *thermalis*, *Leptolyngbya* sp., and *Geitlerinema* sp.) are also presented. Polyphasic studies that include the combination of light microscopy, cultures and the comparative analysis of 16S rRNA gene sequences provide the most powerful approach currently available to establish the diversity of these oxygenic photosynthetic microorganisms in culture and in nature.

Keywords Cyanobacteria · Phylogeny · Diversity · Extreme environments · Baja California

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Introduction

Cyanobacteria are prominent primary producers in marine and hypersaline environments of Baja California, Mexico, especially in the benthos [22], including shrimp farms [28], marine thermal springs, and sea bottom [27]. They may also be the dominant photosynthetic component in soils in which higher plant vegetation is absent or restricted [12]. The microbial ecology of hypersaline ecosystems of Baja California has been studied extensively [8, 16, 20, 22, 26–29, 33, 35, 40,41]. However, cyanobacteria from other biotopes of Baja California have not yet been systematically studied. Knowledge of marine cyanobacteria from this region is limited to sea-bottom samples from Bahia Concepcion [27] and to cyanobacteria adapted to shallow water and high salinity such as shrimp farms [28] in Ensenada de Aripez, Gulf of California. Table 1 summarizes current knowledge about the diversity of cyanobacteria from extreme environments of Baja California.

State-of-the-art investigations on the phylogeny and systematics of cyanobacteria and the composition of natural cyanobacterial communities involve microscopy, cultivation, and molecular analyses. Among microbiologists, it is now commonly accepted that only a small fraction of all bacteria have been isolated and characterized [43,46]. Comparisons of the percentage of cultivable bacteria with total cell counts frequently show enormous discrepancies [2]. The relatively small size and non-distinctive appearance of microorganisms, especially prokaryotes, is a problem that frustrates attempts to differentiate populations microscopically [44]. This problem was partially solved by the use of transmission electron microscopy, which allows the ultrastructure of cyanobacteria from natural populations to be analyzed [8, 40,41]. Nevertheless, the lack of morphological variation has led to another problem: the reliance upon cultivation of microorganisms for their identification. Culture methods may not provide an accurate description of microorganisms as they occur

Table 1 Diversity, approaches, environments, and relative abundance of cyanobacteria from extreme environments of Baja California, Mexico. LM light microscopy, EM electron microscopy, C cultures, Ph phylogeny, x present, xx common, xxx abundant, xxxx dominant

Morphotypes	Approaches				Structures and environments	Abundance	References
	LM	EM	C	Ph			
1. *Anabaena* sp.	x		x		Desert crusts	x	[11]
2. *Aphanothece bullosa (Menegh.)*	x		x		Mats in thermal springs	xx	[27]
3. *Aphanothece* sp.	x	x			Hypersaline microbial mats	xx	[40]
4. *Calothrix contarenii* (Born. et Flah.)	x				Marine epilithic	xxx	[27]
5. *Calothrix crustaceae* (Thuret)	x				Hypersaline microbial mats	xxxx	[22]
6. *Calothrix elenkinii* (Kossinskaja)	x		x		Desert crusts	x	[30]
7. *Calothrix marchica* (Lemm.)	x		x		Desert crusts	x	[30]
8. *Calothrix* sp.	x				Marine epilithic	xx	[27]
9. *Chroococcidiopsis* sp.	x		x	x	Hypersaline microbial mats	xx	[16]
10. *Chroococcus minutus* (Kütz.)	x				Mats in thermal springs	xx	[27]
11. *Chroococcus turgidus* (Kütz.) Näg.	x				Mats in thermal springs	x	[27]
12. *Chrooccoccus* sp.	x				Mats in shrimp ponds	xx	[28]
13. *Cyanothece* sp.	x				Hypersaline microbial mats	xx	[15]
14. *Dermocarpa* sp.	x				Marine epilithic	xx	This work
15. *Enthophysalis* sp.	x				Hypersaline microbial mats	xxx	[20]
16. *Euhalothece* sp.	x		x	x	Hypersaline microbial mats	xxx	[32]
17. *Geitlerinema* sp.	x		x	x	Mats in shrimp ponds	xx	This work
	x		x	x	Mats in thermal springs	xx	This work
18. *Gloeocapsa* sp.	x				Hypersaline microbial mats	x	[26,29]
	x				Mats in thermal springs	xx	[27]
19. *Halospirulina* sp.	x		x	x	Hypersaline microbial mats	xxx	[23, 32,36]
20. *Halospirulina tapeticola* (Nübel and García-Pichel)	x		x	x	Hypersaline microbial mats	xx	[36]
21. *Halothece* sp.	x		x	x	Hypersaline microbial mats	x	[16]
22. *Hydrocoleum* sp.	x				Marine epilithic	xx	[27]
23. *Hyella* sp.	x				Marine epilithic	xx	This work
24. *Leptolyngbya thermalis* (Anag. and Komárek)	x		x		Filaments in thermal springs	xxxx	[27]
25. *Limnothrix amphigranulata* (Meffert)	x		x		Filaments in thermal springs	xxx	[27]
	x				Mats in thermal springs	xx	[27]
	x				Marine epipelic	x	[27]
26. *Lyngbya aestuarii* (Liebm.)	x		x		Mats in shrimp ponds	xxxx	[28]
	x				Marine epilithic	xx	[27]
	x		x		Hypersaline microbial mats	xxx	[22]
27. *Lyngbya digueti* (Gom.)	x				Desert crusts	x	[11]
28. *Lyngbya majuscula* (Harvey)	x				Mats in shrimp ponds	xx	[28]
29. *Lyngbya putealis* (Mont.)	x				Desert crusts	x	[11]
30. *Lyngbya* sp.	x				Hypersaline microbial mats	xx	[26, 28,29]
31. *Microcoleus chthonoplastes* (Thuret)	x	x	x	x	Hypersaline microbial mats	xxxx	[8, 17, 20, 22, 26–29, 40,41]
32. *Microcolues paludosus* (Kütz.) Gom.	x		x		Desert crusts	xxx	[30]
33. *Microcoleus sociatus* (West)	x				Desert crusts	xx	[30]
34. *Microcoleus steenstrupii* (Boye-Pet.)	x		x		Desert crusts	x	[11]
35. *Microcoleus vaginatus* (Vaucher) Gom.	x		x		Desert crusts	xx	[11]
36. *Myxosarcina burmensis* (Sjuja)	x		x		Desert crusts	x	[11]
37. *Myxosarcina spectabilis* (Geitler)	x		x		Desert crusts	x	[11]
38. *Nodularia* sp.	x				Mats in shrimp ponds	x	[28]
39. *Nostoc commune* (Vaucher)	x		x		Desert crusts	xxxx	[11]
40. *Nostoc microscopicum* (Carm.)	x				Desert crusts	xxx	[30]

41. *Nostoc muscorum* (Ag.)	x		x		Desert crusts	x	[11]
42. *Nostoc piscinale* (Kütz.)	x		x		Desert crusts	xx	[11]
43. *Nostoc punctiforme* (Kütz.) Hariot	x		x		Desert crusts	xx	[11]
44. *Oscillatoria laetevirens*	x				Hypersaline microbial mats	xxx	[23]
45. *Oscillatoria limnetica* (Lemm.)	x	x	x	x	Hypersaline microbial mats	xx	[8, 27, 28,32]
46. *Oscillatoria longearticulata* (Hansg.)	x		x		Hypersaline microbial mats	xx	[22]
47. *Oscillatoria lloydiana* (Gom.)	x		x		Hypersaline microbial mats	xx	[22]
48. *Oscillatoria margaritifera* (Kütz.)	x		x		Mats in shrimp ponds	xxx	[28]
49. *Oscillatoria salina* (Biswas)	x	x			Hypersaline microbial mats	xx	[8]
50. *Oscillatoria subuliformis* (Kütz.)	x				Mats in shrimp ponds	xxx	[28]
51. *Oscillatoria vizagapatensis* (Rao)	x				Marine epilithic	xx	[27]
52. *Oscillatoria* sp.	x	x			Hypersaline microbial mats	xxx	[15, 26, 29,41]
53. *Phormidium hypolimneticum* (Campbell)	x		x	x	Hypersaline microbial mats	xx	[28,32]
54. *Phormidium molle* (Gom.)	x				Marine epipelic	x	[27]
55. *Phormidium okenii* (Anag. and Komárek)	x		x		Marine epilithic	xxx	[27]
56. *Phormidium purpurascens* (Kütz.) Gom.	x				Marine epipelic	xxx	[27]
57. *Phormidium rubrum* (Tilden)	x		x		Marine epilithic	xx	[27]
58. *Phormidium tenue* (Menegh.) Gom.	x				Marine epilithic	xx	[27]
59. *Phormidium* sp.	x		x		Desert crusts	xxx	[30]
	x		x		Hypersaline microbial mats	xx	[22, 28,29]
	x		x		Marine epipelic	xx	[27]
60. *Plectonema tomasinianum* (Hansg.)	x		x		Desert crusts	x	[11]
61. *Pleurocapsa* sp.	x	x			Hypersaline microbial mats	xxx	[40]
62. *Pseudanabaena* sp.	x		x		Hypersaline microbial mats	xx	[26,29]
63. *Schizothrix arenaria* (Berk.) Gom.	x		x		Desert crusts	xx	[11]
64. *Schizothrix calcicola* (Ag.) Gom.	x		x		Desert crusts	xxxx	[11]
65. *Scytonema ocellatum* (Lyngb.)	x		x		Desert crusts	xxxx	[11,30]
66. *Scytonema* sp.	x		x		Desert crusts	xx	[11,30]
67. *Spirulina meneghiniana* (Zanard.)	x				Marine epilithic	xx	[27]
68. *Spirulina subsalsa* (Oerst.)	x				Hypersaline microbial mats	xxx	[15, 23, 26, 27,29]
69. *Spirulina labyrinthiformis* (Gom.)	x				Hypersaline microbial mats	xxx	[15]
70. *Spirulina subtilissima* (Kütz.)	x				Hypersaline microbial mats	xx	[27]
71. *Spirulina* sp.	x	x	x		Hypersaline microbial mats	xxx	[8, 27–29,41]
72. *Synechococcus aeruginosa* (Näg.)	x				Hypersaline microbial mats	xx	[27]
73. *Synechococcus elongatus* (Näg.)	x		x		Nodules in thermal springs	xx	[27]
74. *Synechococcus* sp.	x	x			Hypersaline microbial mats	xxx	[8, 26,29]
75. *Synechocystis pevalekii* (Ercegovic)	x		x		Hypersaline microbial mats	xx	[27]
	x				Nodules in thermal springs	xx	[27]
76. *Trichormus* sp.	x		x		Mats in shrimp ponds	x	[28]
77. *Tychonema* sp.	x		x		Mats in shrimp ponds	xxx	This work

within natural habitats [42,43]. The cultivation approach, however, is essential for the thorough characterization of microorganisms and has been the basis for understanding microbial physiology and genetics. It is desirable to match isolated strains with their counterparts in nature. Only then can physiological data gained from culture studies begin to be confidently extrapolated to natural conditions [7,17]. In many cases, however, inadequate culture conditions leading to the loss of various morphological characteristics [7], the inability to grow certain cyanobacteria in the laboratory [10,45], and misidentification of strains in culture collections [17,48] make it difficult to apply taxonomic assignments based on cultures to field populations. Pure-culture studies have led to new molecular approaches [37] that are revolutionizing microbiology by providing an evolutionary framework and by enabling new ecological approaches. Molecular approaches have made it possible to observe the patterns of occurrence of the individual populations upon which the structures of microbial communities are based [44]. Both classification systems for cyanobacteria – the bacteriological approach [7, 25,39] and the traditional botanical one [3,18] – rely primarily on morphological characteristics of cells and colonies and do not necessarily lead to the identification of phylogenetically coherent taxa [6,49].

The sequence analysis of genes encoding small-subunit ribosomal RNA (16S rRNA) is currently the most promising approach for the phylogenetic classification of cyanobacteria [48]. Furthermore, the comparative analysis of 16S rRNA gene sequences is a new means to investigate discrepancies between strain collections and natural communities [9,47]. Sequences of 16S rRNA genes are independent of cultivation or growth conditions and can be retrieved by PCR from small amounts of DNA extracted either from laboratory cultures or natural environments [19]. The combination of PCR and denaturing gradient gel electrophoresis (DGGE), a technique for the sequence-dependent separation of DNA molecules [24,31], have proved useful in visualizing the diversity of cyanobacterial 16S rRNA genes in environmental samples, to detect the uniqueness of isolated strains, and to assign PCR products derived from cultures to populations in the field. PCR products containing a single homogeneous population of DNA molecules can be recognized as a single band after DGGE and can be directly sequenced. Sequence data, therefore, can be generated without the time-consuming techniques required for molecular cloning procedures from cyanobacterial cultures containing heterotrophic bacteria, thereby allowing the rapid survey of a collection of strains for genetic diversity [35].

Here, we present preliminary results regarding the cyanobacterial diversity of two geographical sites in Baja California Sur, Mexico, using a polyphasic approach that combines morphology and sequencing of the 16S rRNA genes of cyanobacterial cultures.

Materials and methods

Study sites

Hydrothermal and marine environments in Bahia Concepcion

In Santispac at Bahia Concepcion (26° 45' 46'' N; 111° 53' 38'' W), macroscopically visible, filamentous, cylindrical, domal masses, mats, and nodules grow and develop in thermal springs in a coastal evaporitic system (''mini sabkha''), bordered towards the sea by mangrove trees, a small lagoon, and a beach (Fig. 1). Here, environmental conditions are unusual: high temperature (41–58 °C), moderate salinity (2.5–2.8%), high light intensity (1,500 $\mu Em^{-2}s^{-1}$), the presence of elemental sulfur and other toxic chemicals such as arsenic (1650 ppm) and manganese (22.37 ppm) (E. Shumilin, CICIMAR-IPN, personal communication). At the end of the warm period in October, 1995, a green, colorful, filamentous microbial community occurred in a hydrothermal vent (52–58 °C). The filamentous structure was formed in the direction of flowing hot water from the vent, reaching the surfaces (35 cm), and adopting a flat shape. Dominant morphotypes and cultures have been described previously [27].

Marine-hypersaline shrimp ponds ecosystems

Stratified microbial mats dominated by filamentous and unicellular cyanobacteria have been described previously for shrimp production in Ensenada de Arizpez (24° 08' 17'' N; 110° 25' 49'' W), La Paz, Baja California Sur [28] (Fig. 1).

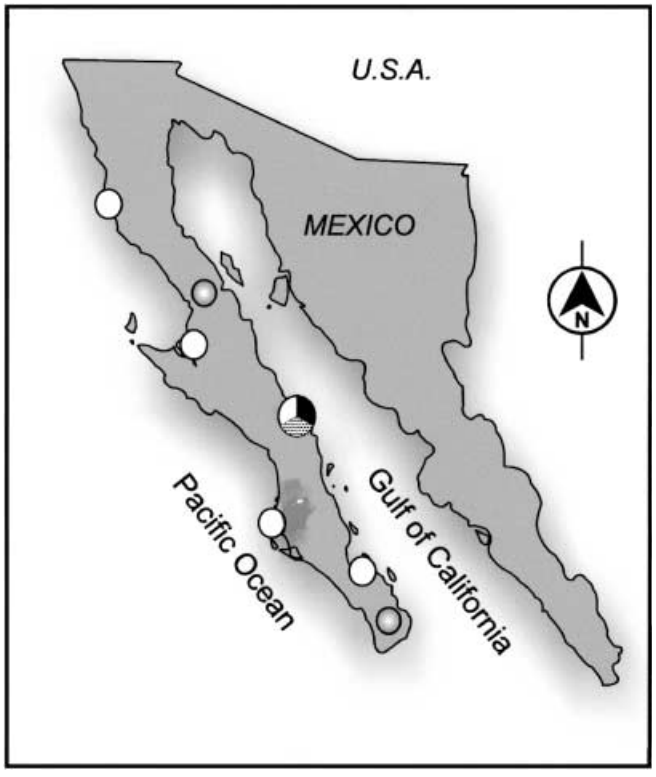

Fig. 1 Map of the study sites, and types of microbial communities. 1 Laguna Figueroa, Baja California Norte (B.C.N.); 2 Cataviña Desert, B.C.N.; 3 Guerrero Negro, Baja California Sur (B.C.S.); 4 Bahia Concepcion, B.C.S.; 5 Bahia Magdalena, B.C.S.; 6 Ensenada de Aripez, B.C.S.; 7 Sierra de La Laguna B.C.S. *White circles* Hypersaline ecosystems, *gray circles* soil microbiotic crusts, *dotted circle hydrothermal springs*, *black circles* sea-bottom environments

Microscopy and culturing

Morphology, morphometry and abundance were determined according to procedures described previously [26–30], and by including fresh samples and fixed samples in a buffered ($MgCO_3$) 3% solution of formaldehyde in water from each site. Botanical taxonomic assignment was according to Geitlerian and bacteriological classification systems [3, 4,18, 7]. The culture media used were ASNIII for marine and hypersaline environments [39], and Z8 for cryptobiotic cyanobacteria from desert crusts [14].

PCR amplification

PCR amplification was performed with a Cyclogene temperature cycler (Techne, Cambridge, United Kingdom) using previously described cyanobacterium- and plastid-specific primers [35]. Primers CYA106F and CYA781R were used to specifically generate amplification products from unicyanobacterial cultures, yielding fragments of approximately 600 bp. To minimize nonspecific annealing of the primers to non-target DNA, 0.5 U of Super*Taq* DNA polymerase (HT Biotechnology, Cambridge, UK) was added to the reaction mixture after the initial denaturation step, at 94 °C for 5 min, and then a step at 80 °C for 1 min. This was followed by 35 incubation cycles, each one consisting of 1 min at 94 °C, 1 min at 60 °C, and 1 min at 72 °C, except the last cycle, 9 min at 72 °C. A 40-nucleotide GC-rich sequence, referred to as a GC clamp, was attached to the 5′-end of primer CYA106F to improve the detection of sequence variation in amplified DNA fragments by subsequent DGGE (see below).

DGGE analyses

DGGE involves the separation of a population of DNA of equal length in a polyacrylamide gel containing a gradient of denaturants. The separation is based on differences in melting characteristics, and in turn upon the sequence differences, of the double-stranded segments. The result is the detection of 16S rRNA molecules as a pattern of bands. The DNA concentration was calculated by comparison to a low-DNA-mass standard (Gibco, Eggenstein, Germany) after gel electrophoresis; 500 ng of DNA was applied to denaturing gradient gels. DGGE was performed as described previously [35]. Briefly, polyacrylamide gels with a denaturing gradient from 20 to 60% were used, and electrophoresis was carried out for 3.5 h at 200 V. Subsequently, the gels were incubated for 30 min in TAE buffer [40 mM Tris-HCl, pH 8.3, 20 mM acetic acid, 1 mM ethylene diaminetretaacetate (EDTA)] containing 20 mg ethidium bromide/ml. Fluorescence of dye bound to DNA was excited by UV irradiation in a transilluminator and was photographed with a Polaroid MP4+ instant camera system.

Sequencing

PCR products were purified with the QIAquick PCR purification kit (Qiagen) and were subsequently used as templates for sequencing reactions with the PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit supplied with Ampli*Taq* DNA polymerase (Applied Biosystems). The primers used were the same as for initial amplification. Products of sequencing reactions were sequenced commercially.

Phylogenetic reconstruction

Cyanobacterial 16S rRNA gene sequences available from GenBank and those determined in this study were aligned using Lasergene software (DNAstart). Phylogenetic relationships were calculated by the maximun-parasimody program of the PAUP 4.05b. database. A phylogenetic tree of isolates and their nearest neighbors was derived from maximum parsimony analysis. The phylogram was calculated from the divergence in the partial sequences of 16S rDNA (600–650 bp from nucleotide position 106–781, corresponding to the *Escherichia coli* numbering [5].

Nucleotide sequence accession numbers

The sequences determined in this study were deposited in GenBank. Their accession numbers are listed in Table 2.

Cyanobacterial culture collection at CIBNOR

The Center for Biological Research of the Northwest, CIBNOR, serves as the depository of a culture collection of cyanobacteria preserved in liquid nitrogen. At the moment 20 strains have been preserved and are recorded in a database available on-line (htpp://www.cibnor.mx/malgas/ealgas.html).1

Results and Discussion

Diversity of morphotypes

Below are described the morphotypes used in this study. In this article, the morphotypes *Dermocarpa, Geitlerinema, Hyella,* and *Tychonema* are reported as specific biotopes of Baja California for the first time (Fig. 2 and Table 2).

Morphological descriptions based on fresh and fixed natural samples

Dermocarpa sp., unicellular cyanobacteria that have apical-basal polarity (i.e., ovoid to pyriform in shape) and that reproduce only by multiple fission. Baeocytes are immotile, possessing an outer fibrous wall layer at the time of release from the parental cell.

Table 2 Cultural rRNA gene sequences determined during this study

GenBank accession no.	Strain no.[a]	Length (bp)	Taxonomic assignment[b]
AF410931	SP2A9606–8	614	*Synechococcus elongatus*
AF410932	SP12A9510–1	614	*Leptolyngbya thermalis*
AF410933	CR109510–2	609	*Geitlerinema* sp.
AF410934	CR109510–1	609	*Oscillatoria limnetica*

[a] CIBNOR, The Center for Biological Research of the Northwest culture collection, La Paz, Baja California Sur, Mexico
[b] Taxonomic assignments are cum forma and do not necessarily imply that a phylogenetically coherent taxon can be defined for the morphology described by the taxon

Hyella sp., endolithic cyanobacteria, cells of varying form, rarely solitary, forming characteristic polarized pseudofilaments at any stage of their life cycle, in which cells divide transversally or irregularly in several planes. Producing facultatively nanocytes. Sheaths mainly firm, usually lamellated.

Tychonema sp., filamentous cyanobacteria, with solitary trichomes, benthic, 5–7 μm in diameter, without sheaths or facultatively with fine mucilaginous sheaths, usually straight or occasionally slightly screw-like coiled or more or less curved, not attenuated at the ends. All cells more or less with the same morphology, cylindrical, more or less isodiametric. Without aerotopes (gas vesicles), but usually with prominent granules inside the cells; sometimes with keratomized chromatoplasma. Changeable content of phycobilins (changes the color from olive to reddish brown), motility reduced or lacking.

Morphological descriptions based on natural samples and cultures

Geitlerinema sp. strains CR109510–2 and SP19606–11, filamentous cyanobacteria which form straight cylindrical trichomes. Trichomes 2.0–2.5 μm in diameter, cells longer than broad, constrictions between adjacent cells are absent, polar gas vesicles are present, apical cells are rounded, active gliding motility and sheath envelope lacking.

Leptolyngbya spp. strains CR109510–1 and SP2A9510–1, cylindrical trichomes 1.0 μm in diameter, cells longer than wide, rarely isodiametric, 1.0–5.0 μm in length; constrictions between cells are shallow, apical cells rounded, produce persistent thin sheaths, trichomes surrounded by a ''mucous-like'' matrix, trichomes nonmotile or inconspicuously motile as seen by light microscopy.

Synechococcus elongatus strain SP2A9606–8. Unicellular cyanobacteria, cells are spherical to rod-shaped, 1.4–2.0 μm in diameter, and 5.0 μm in length. The cells may be united into colonial aggregates by mucilage formation, but never produce well-defined sheath layers.

Based on microscopic observation of the morphology of cells and colonies [3, 4,18], cyanobacterial diversity described up to now in Baja California comprises 35 genera. Of these, 24 genera are found exclusively in hypersaline and marine environments: *Aphanothece*, *Chroococcidiopsis*, *Chroococcus*, *Cyanothece*, *Dermocarpa*, *Enthophysalis*, *Euhalothece*, *Geitlerinema*, *Gloeocapsa*, *Halospirulina*, *Halothece*, *Hydrocoleum*, *Hyella*, *Leptolyngbya*, *Limnothrix*, *Nodularia*, *Oscillatoria*, *Pleurocapsa*, *Pseudanabaena*, *Spirulina*, *Synechococcus*, *Synechocystis*, *Trychormus* and *Tychonema*. Notable is the frequent occurrence of *Microcoleus chthonoplastes* in marine and hypersaline environments. This species has been considered to be a cosmopolitan cyanobacterium [17] and has been extensively studied. Other morphotypes of *Microcoleus* were detected only in the desert soil

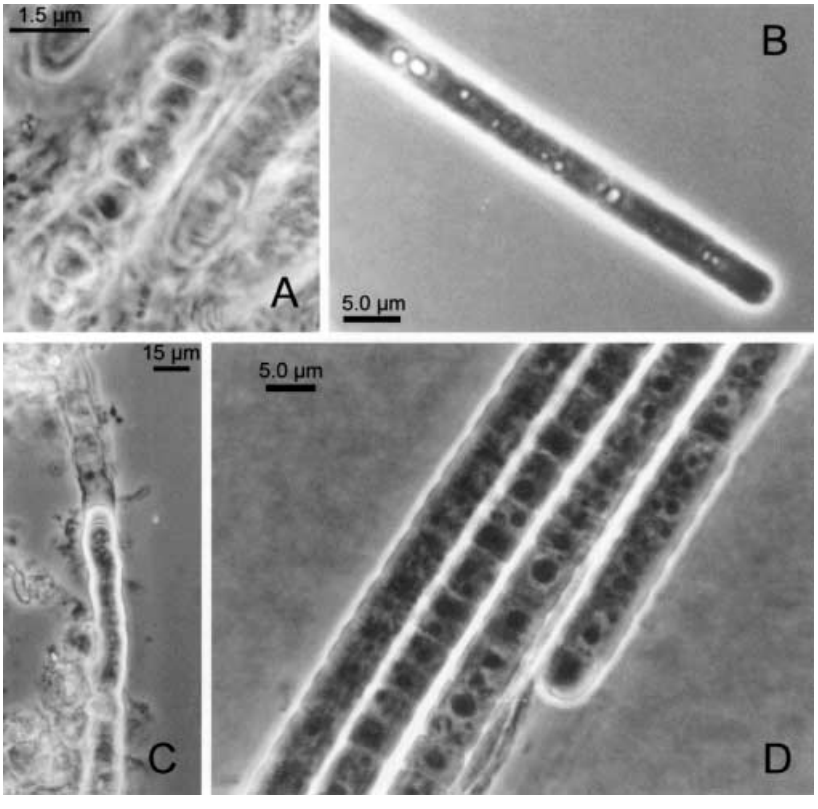

Fig. 2 Phase-contrast photomicrographs of four cyanobacterial morphotypes from the studty sites in Baja California Sur, Mexico. **A** *Dermocarpa* sp., **B** *Geitlerinema* sp., **C** *Hyella* sp., **D** *Tychonema* sp

crust, where *Microcoleus* cf. *paludosus* and *Microcoleus* cf. *sociatus* were distributed only in Sierra de La Laguna, in the southern area of the peninsula [30], whereas *Microcoleus* cf. *steenstrupii*, and *Microcoleus* cf. *vaginatus* occurred only in Cataviña, in the central part of the peninsula [11].

The most extensively studied site, the facilities at Exportadora de Sal, S.A., Guerrero Negro, has been analyzed by using four different approaches (Table 1). Javor and Castenholz (1984) and Kruschel and Castenholz (1998) used phenetic criteria of natural populations and cultures to identify cyanobacteria and they recognized seven genera. By transmission electron microscopy only four genera were identified [8, 40,41].

Molecular diversity of 16S rRNA genes

We studied benthic cyanobacteria from ponds of a hydrothermal spring in Santispac, Bahia Concepcion, and from a shrimp production operation in the Ensenada de Arispez, La Paz, Baja California Sur.

From the hydrothermal springs, two dominant morphotypes, *Leptolyngbya* cf. *thermalis* strain SP12A9510–1 and *Synechococcus* cf. *elongatus* strain SP2A9606–8, were isolated. Partial 16S rRNA gene sequences (nucleotide positions 106–781 corresponding to *E. coli* numbering [5]) were determined and compared with sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) [1] at the National Center for Biotechnology Information (NCBI), Washington, D.C. The sequence of strain SP12A9510–1 showed 93% similarity with *Leptolyngbya ectocarpi* PCC7375 (sequence accession number AF132786), the marine lineage, whereas strain SP2A9606–8 showed 98% similarity with *Synechococcus* sp. PCC 7003 (sequence accession number AB015059), the marine cluster.

From shrimp ponds, partial sequences of the 16S rRNA of two strains were determined. Cyanobacterial strain CR109510–2 was identified as a member of the genus *Geitlerinema* sp., according to the identification key provided by Anagnostidis and Komárek [4]. Note that the 16S rRNA gene sequence from strain CR109510–2 was found to have 99% identity with a partial sequence from the morphologically similar strain *Geitlerinema* PCC9452 (sequence accession number U96442). The closest relative of strain CR109510–1 found in GenBank was strain *Leptolyngbya ectocarpi* PCC7375 (sequence accession number AF132786). However, the sequence similarity here (93%) is less significant. DGGE comparisons of strains CR109510–2 and CR109510–1 with DNA of five standard cyanobacterial strains were done. Strain CR109510–2 showed a discrete band in the gel near to that of the *Geitlerinema* PCC9452 DNA standard (Fig. 3).

A preliminary analysis of the phylogenetic relationships of the 16S rRNA sequences was constructed from the Ribosomal Data Base Project, two cultures from thermal springs and two from shrimp farms (Fig. 4). The phylogenetic tree showed that the sequences of strains CR109510–2 and SP2A9606–8 are related to that of *Geitlerinema* PCC 9452 and *Synechococcus* PCC 7003, respectively, in two coherent taxa. The sequence of strain CR109510–2 is distantly related to that of *Lyngbya* sp. PCC 7419 and with "*Oscillatoria limnetica*" U96443, whereas the sequence of strain SP2A9606–8 forms part of a very well-defined cluster comprising sequences of *Synechococcus* morphotype, marine cluster. A novel cluster composed of the sequences of strain SP2A9510–1 and strain CR109510–1 is distantly related with sequences of filamentous cyanobacteria LPP-group and *Oscillatoria neglecta* M-82. This new group of sequences corresponds to filamentous cyanobacterial strains with the same morphology but different genotypes, with 98.5% similarity in overlapping regions.

This study shows that knowledge of cyanobacterial diversity in Baja California is still quite limited and sometimes confusing. Just a few studies on marine cyanobacteria from the coast and open sea from Baja California have been carried out. Furthermore, much less is known about cyanobacterial diversity from terrestrial environments and freshwater reservoirs of the

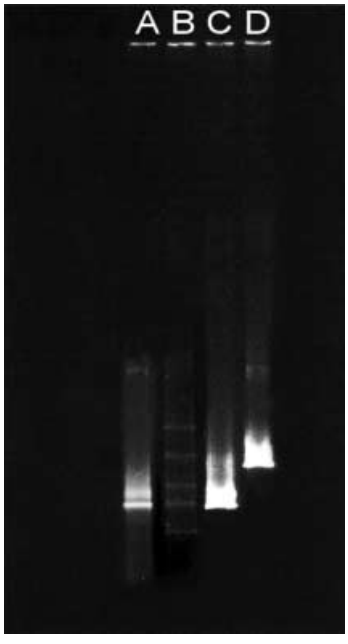

Fig. 3 Composite figure of ethidium bromide-stained denaturing gradient gel electrophoresis (DGGE) separation pattern of PCR-amplified segments of 16S rRNA genes. *Lane A* PCR products derived from SP19606–11 strain, (thermal springs). *Lane B* A mixture of PCR products derived from five cyanobacterial strains was applied on each gel as standard to allow gel-to-gel comparisons [*top to bottom*: *Scytonema* strain B-77 Scy-jav., *Synechococcus leopoliensis* SAG 1402-1, *Microcoleus chthonoplastes* MPI-NDN-1, *Geitlerinema* strain PCC 9452 (strain mfx), *Cyanothece* strain PCC 7418]. *Lane C* PCR products derived from CR109510–2 strain (shrimp farm). *Lane D* PCR products derived from CR109510–1 strain (shrimp farm). Primers CYA106F and CYA781R were used for amplification

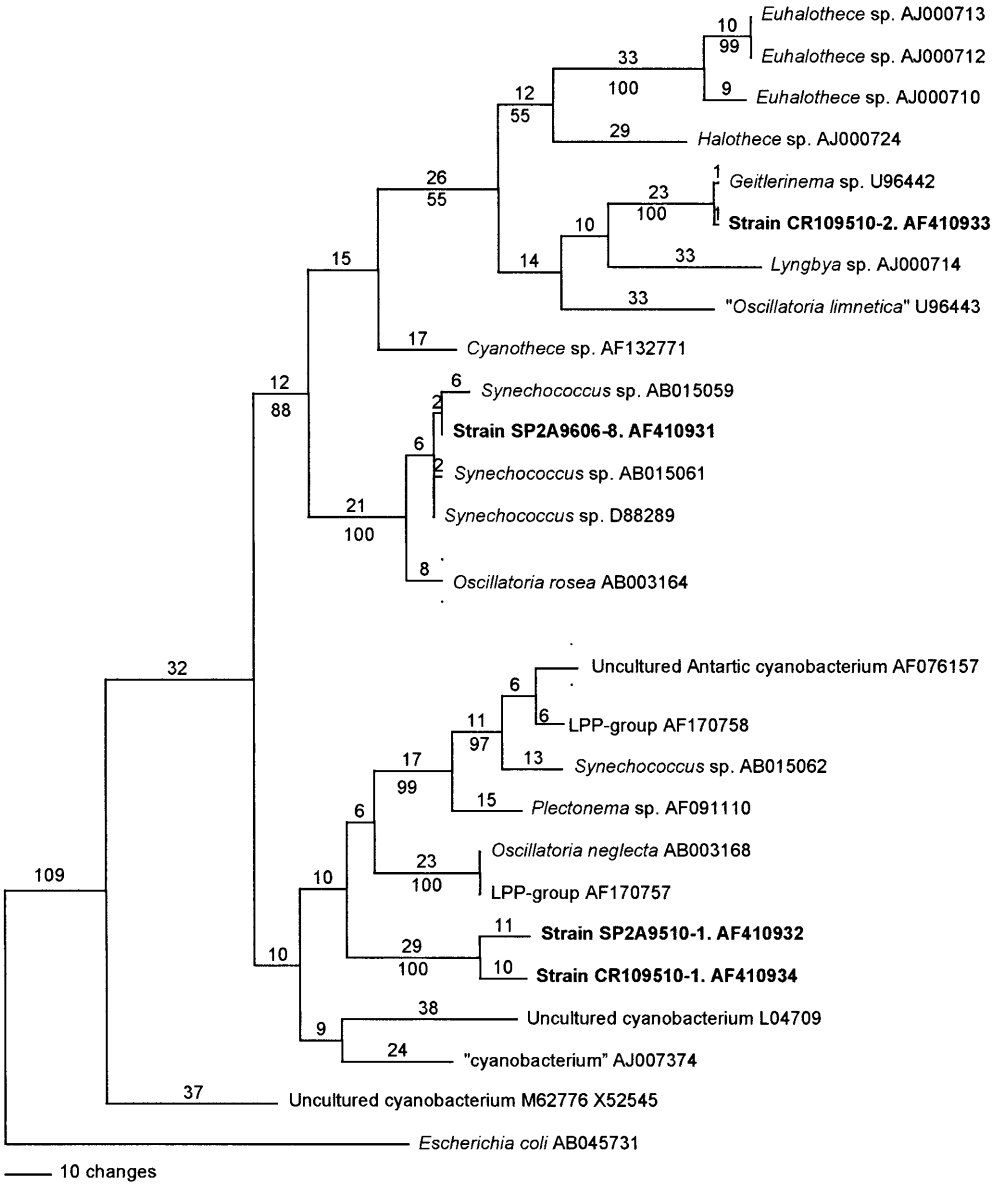

Fig. 4 Phylogenetic tree of isolates and their nearest neighbors derived from maximum parsimony analysis. Accession numbers are given after the taxonomic assignment. The phylogram was calculated from the divergence in partial sequences of 16S rDNA (600- to 650-bp lengths from nucleotide position 106–781 corresponding to *Escherichia coli* numbering). Branch lengths are proportional to nucleotide differences as indicated on the *marker bar* and their values are indicated on branches. *Numbers* given below branches are frequencies (expressed as percentages) with which a branch appeared in 500 bootstrap replicates. Frequencies under 50% are not shown. Tree length = 747, consistency index = 0.544, retention index = 0.678, homeoplasy index = 0.456

peninsula. Nevertheless, the combination of morphological studies with molecular analysis of natural populations and cultures of cyanobacteria offers a powerful approach to understanding the diversity of cyanobacteria. Their interpretation should allow the description of microorganisms as they occur within natural habitats, and the identification of phylogenetically coherent taxa. This polyphasic study will be used for further analyses of cyanobacterial diversity in Baja California, Mexico, to complete previous studies based on phenetic criteria.

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