



Cyanobacterial Microbiotic Crusts in Eroded Soils of a Tropical Dry Forest in the Baja California Peninsula, Mexico

YOLANDA MAYA
ALEJANDRO LÓPEZ-CORTÉS

Centro de Investigaciones Biológicas del Noroeste (CIBNOR)
La Paz, B.C.S., Mexico

AL SOELDNER

Department of Botany and Plant Pathology
Oregon State University
Corvallis, Oregon, USA

This study deals with the cyanobacterial composition, and the nitrogen fixation of four members, of cryptobiotic crusts collected from eroded soils in a transitional area between arid and tropical climatic environments. Identification was based on microscopic analyses. Morphotypes were identified directly from reactivated natural crusts and from cultured strains. The identified morphotypes were Scytonema cf. ocellatum, Scytonema sp., Microcoleus cf. paludosus, M. cf. sociatus, Calothrix cf. elenkinii, C. cf. marchica, Nostoc cf. microscopicum, and Phormidium sp. Results show that the cyanobacterial composition of the microbiotic crusts studied is different from those in warmer and cooler deserts, particularly the absence of Microcoleus cf. vaginatus and Nostoc cf. commune in our samples. Such differences could be caused by the transitional character of the area. The results of the acetylene reduction assay show that the capacity of nitrogen fixation of some morphotypes is limited to the heterocyst-forming morphotypes.

Keywords *Calothrix*, cyanobacteria, *Microcoleus*, nitrogen fixation, *Phormidium*, *Scytonema*, soil crusts, soil erosion

Introduction

Many studies have pointed out the biological importance of microbiotic crusts, also called desert crusts and cryptobiotic crusts (Belnap and Lange 2001). Descriptions of the general biological diversity of microbiotic soil crusts from cold and warm deserts are common in the literature (Johansen 1993). Some authors have described the importance of cyanobacteria as the dominant photosynthetic component in soils where higher plant vegetation

Received 31 August 2000; accepted 14 February 2002.

This work was supported by grant AGEC-3/CIBNOR from SEP-CONACYT to YM, and partially by CONACYT, reference 31670-N to ALC and Fundación Produce, A.C., reference 2414 to ALC. The authors appreciate the technical assistance of Ariel Cruz for gas chromatographic analyses, Franco Cota and Teresa Arteche for collaboration in field and laboratory, and Ira Fogel for editing the English text.

Address correspondence to: Yolanda Maya, CIBNOR Mar Bermejo 195, Col. Playa Palo de Sta. Rita, La Paz, B.C.S. 23090, Mexico, E-mail: ymaya@cibnor.mx

is absent or restricted (Campbell et al. 1989; García-Pichel and Belnap 1996). More recently, new techniques and approaches, such as the use of electron microscopy for ultrastructure (Cameron and Devaney 1970), microelectrodes for monitoring physiological and physicochemical variables (García-Pichel and Belnap 1996), the study of photosynthetic pigment profiles and their role as protective mechanisms against UV irradiance damage (Proteau et al. 1993), and fingerprint DNA markers of cyanobacterial population (Hoffman et al. 1999; García-Pichel et al. 2001) have been used to increase our knowledge of the composition and ecological role of these types of desert microbial organizations.

Research on cyanobacterial crusts has been done on samples from arid environments and forests, but less or nothing in transitional climatic areas. This study describes the structure and cyanobacterial populations of natural microbiotic crusts and isolated cultures from eroded soils of a tropical dry forest in a transitional climate region of the Baja California Peninsula, Mexico

Material and Methods

Study Area

The study area is within the altitudinal belt of tropical dry forest between 500 and 850 meters in a prominent mountain range called Sierra de la Laguna in the southern part of the Baja California Peninsula (Figure 1a, 1b). The range is surrounded by arid plains dominated by xerophyllous scrubs. This mountain range acts as an island supporting the only tropical dry and pine-oak forests of the whole southern region of the peninsula, which testifies to its geological and climatic history (Ortega and Arriaga 1988).

The tropical dry forest of this range represents the most extreme conditions of high temperatures and low precipitation, and is the northwestern distribution border of this kind of vegetation. Its permanence has been possible because of the climatic characteristics caused by the transition between the southwestern limit of the Sonoran Desert in the north and the more humid Cape Region in the south (Brandege 1892; Shreve 1937). Such environmental differences are influenced by the latitudinal nature of the subtropical variations in the belt surrounding the Tropic of Cancer, considered as going across the Peninsula at 23°30'N (Figure 1a, 1b).

In rangeland areas, which represents most of the southern peninsula, vegetation has been severely impacted, including soil-bearing and erosion problems (Figure 1c). Compact microbiotic soil crusts, which seem to stabilize the loose particles, were found growing on the surface of eroded soils.

Sampling and Reactivation of Crusts

Samples of dry microbiotic crusts were collected in October 1997. Samples of crust, about 5 cm × 5 cm, 1 cm deep, were taken from the soil surface and transported in sterile plastic Petri dishes to the laboratory. Based on their surface appearance, either flat or rough, it was possible to differentiate two kinds of crusts.

Samples of the two types of crusts were reactivated by adding fresh water. We noticed that only water saturation led to the complete reactivation of the samples, so that they were kept permanently moist in controlled conditions of temperature and light at 30°C, and 100 $\mu\text{E m}^{-2} \text{sec}^{-1}$. This procedure was limited to a week to avoid fungi development, often observed after this time interval.

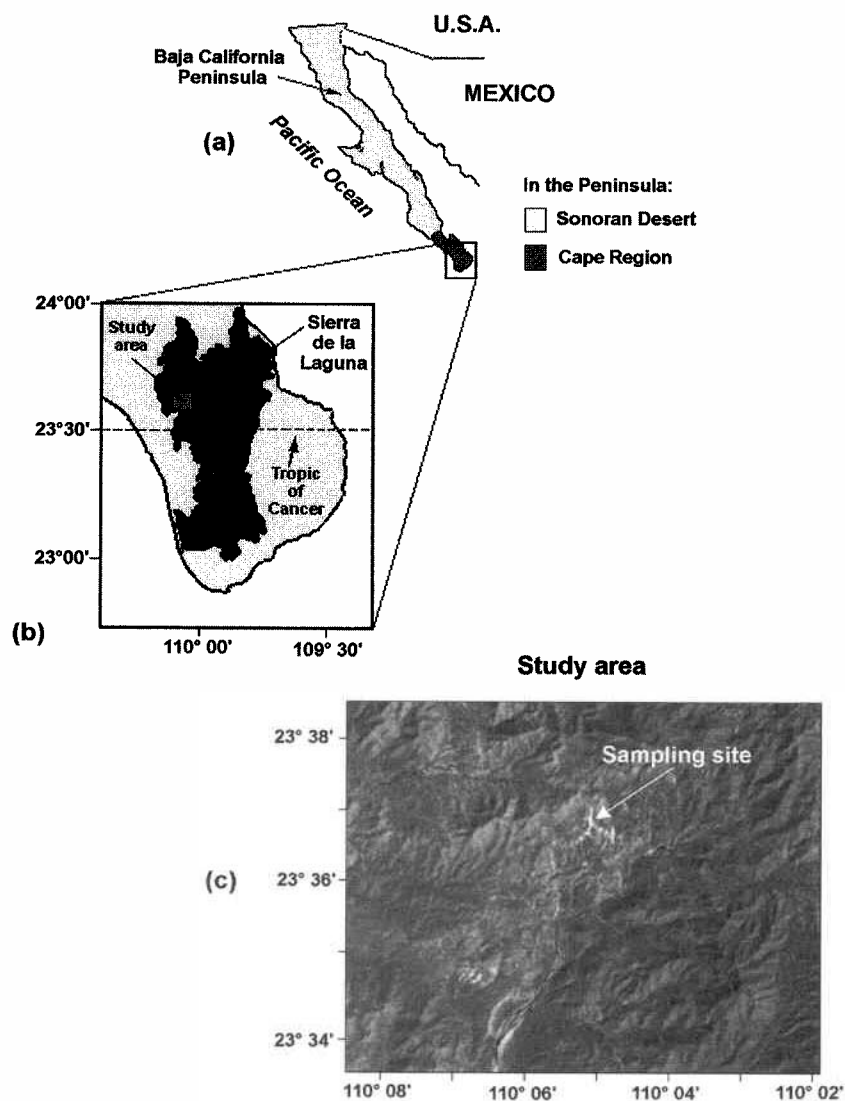


FIGURE 1 (a) Location of the study area in a transitional climate region of the Baja California Peninsula. (b) Location of the study area in the Sierra de la Laguna. (c) LANDSAT TM5 (close infrared) subimage showing the study area (16 September 1996) in the middle of the wet season. Chlorophyllic vegetation (grey) covers most of the image surface. Soil erosion occurs in patches (white). The arrow indicates the sampling site.

Cyanobacterial Morphotypes Identification

Microscopy

Reactivated crusts were analyzed at different times using a stereoscopic microscope (Zeiss). Fresh preparations of filaments and colonies, taken manually from different cyanobacterial thalli by micromanipulation, were observed using a phase contrast microscope (Nikon Labophot). Botanical species assignment was based on Geitler (1932), and compared with *Bergey's Manual of Systematic Bacteriology* (Boone and Castenholz 2001).

Sixteen micrographs of both types of crusts at 0.6X, 1.2X, and 4.0X were taken to estimate the relative abundance of each cyanobacterial morphotype. Surfaces occupied by lichens in the rough crusts were excluded. Estimates were based on the percentage of surface occupied by the thalli of each cyanobacterial morphotype. Five classes were used—0% = not detected, 1–20% = present, 20–50% = common, 50–70% = very common, and 70–100% = dominant.

Isolation and Culturing

To facilitate the manipulation of the small and fragile cyanobacterial thalli, we used microhandles. These were made by heating and pulling the tip of a thin capillary Pasteur pipet with a forceps (Prufert-Bebout and García-Pichel 1994). This provided a tip with a thinner diameter than any dissecting needle. Microhandles were useful to obtain small sample quantities to make fresh preparations. In culture manipulation, it was possible to get a single filament to place into the culture media, reducing the steps needed to obtain a clone strain.

Filaments and microcolonies of the different and more conspicuous morphotype thalli were obtained by manual micromanipulation from reactivated natural crusts. They were incubated in both fresh solid and liquid BG11, BG11° (Rippka et al. 1981) and Z-8 and Z-8° culture media (Carmichael 1986) under controlled conditions of temperature and light (30°C, 100 $\mu\text{E m}^{-2} \text{sec}^{-1}$) to promote the growth and isolation of cyanobacterial strains. In the beginning, cycloheximide (Sigma Chemical Co., St. Louis, MO, USA) was added to the culture media to promote the elimination of eukaryotic cells (Rippka 1988). The cyanobacterial strains obtained were preserved in liquid nitrogen with a cryoprotector (5% dimethyl sulfoxide).

Scanning Electron Microscopy (SEM)

Representative samples of flat and rough microbiotic crusts under dry and wet conditions were prepared for scanning electron microscope observation. These samples were fixed for 2 h with 2.5% glutaraldehyde in 0.1 M Sorensen's sodium-potassium phosphate buffer, pH 7.2. After fixing, the specimens were taken through an ethanol series (concentrations 25–100%, 15 min/change) and then infiltrated with 95% isoamyl acetate anhydrous. Samples were dried in a critical point dryer (SAMDRI-PVT-3P) following the method of Anderson (1956). Specimens were then fastened with carbon adhesive tape to aluminum specimen mounts and coated with ~8 to 10 nm of 60/40 weight% gold/palladium alloy in a sputter-coating system (Edwards S150B). Microscopy was done using a scanning electron microscope (AmRay 3300FE) at Oregon State University, Corvallis, OR, USA. Images were recorded on Polaroid Type 55 positive-negative film.

Nitrogen Fixation

To determine nitrogen-fixing abilities of some cyanobacterial cultures, an acetylene reduction assay was used. Before the experiment, strains were cultured for 8 days in Z-8° liquid medium without a nitrogen source to promote nitrogenase activity. Five treatments (Postgate 1972) were then performed in duplicate: (1) Z-8° with cyanobacteria; (2) Z-8° with cyanobacteria and 2 mL 10% acetylene; (3) Z-8° with 2 mL 10% acetylene; (4) Z-8° with 0.3 mL 1.5% ethylene; and (5) Z-8° alone.

Acetylene reduction was determined with a gas chromatograph (Varian 6000, Varian Instrument Group, USA) equipped with a hydrogen flame ionization detector. Instrument operating conditions include 0.12 cm ID mesh, 60°C isotherm temperature, nitrogen carrier gas and hydrogen at a flow rate of 30 mL s⁻¹, air flow rate at 300 mL s⁻¹, 50°C injector temperature, 200°C detector temperature with an attenuation of 8, graph speed at 5 mm min⁻¹, 1-mV

recorder sensitivity. The flame ionization detector rates used were 10^{-12} and 10^{-11} . Data were recorded every 4 h over 24 h to obtain the kinetics of fixation for each morphotype.

Reduced acetylene was converted to fixed nitrogen using a ratio of 4:1 (Jensen and Cox 1983; Stal 1988; Ortega-Calvo and Stal 1991). From these numbers the rate of nitrogen fixation for each 4 h interval was calculated.

Results

Identification of Morphotypes

Based on the observations done on fresh preparations of natural crusts (MNC) and cultured strains, it was possible to identify the morphotypes and the estimated abundance of each morphotype. Results are shown in Table 1.

According to the *Bergey's Manual of Systematic Bacteriology* (Boone and Castenholz 2001), which is based mainly on the phylogenetic relationships among the groups, the cyanobacteria morphotypes recognized in the crusts belong to Subsections III and IV. The main characteristics of Subsection III (Castenholz et al. 2001) are filamentous organisms dividing by binary fission in a single plane at right angles, producing "vegetative cells" only so that heterocysts or akinetes are not present. Cyanobacteria of Subsection IV (Rippka et al. 2001) are filamentous organisms dividing exclusively by binary fission in one plane only, some with the possibility of "false branching" and having potential to produce heterocysts in the absence of combined nitrogen.

Bergey's classification system permits the identification of each morphotype to genus group level, in contrast to the system of Geitler (1932), which permits the identification of species based mainly on morphological features. A comparison between the results obtained from Bergey's and Geitler's systems for each morphotype is presented in Table 2.

A brief description of each identified morphotype, based on the morphological system of Geitler (1932), is presented next.

Strain SLC1097-3 (Figure 2c) was isolated from a flat crust and identified as *Microcoleus* cf. *paludosus*, a filamentous cyanobacterium with several trichomes densely aggregated within the sheath. Trichomes are 6 μm diameter, not constricted at the joints, cells are nearly as long as broad (5–7 μm); the end cell is more or less conical.

TABLE 1 Cyanobacterial morphotypes and their relative abundance in microbiotic crusts in the Sierra de la Laguna of Baja California Sur, Mexico

Assignment by Geitler (1932)	Relative abundance ^a	
	Flat	Rough
<i>Scytonema ocellatum</i>	++++	++++
<i>Scytonema</i> sp.	+++	++
<i>Microcoleus paludosus</i>	++++	+++
<i>Microcoleus sociatus</i>	++	++
<i>Phormidium</i> sp.	+++	+++
<i>Nostoc microscopicum</i>	+	++
<i>Calothrix marchica</i>	–	+
<i>Calothrix elenkinii</i>	–	+

^a – = not detected; + = present; ++ = common; +++ = very common; ++++ = abundant.

TABLE 2 Comparison of the main characteristics of two classification systems used to identify the morphotypes found in the microbiotic crusts of the Sierra de la Laguna, BCS

Bergey's (2001)		Geitler (1932)	
Genus	Main characteristics	Species	Main characteristics
<i>Microcoleus</i>	Two to several trichomes enclosed by a common homogeneous sheath; cells 3–6 μm diameter, longer than wide; mature end cells conical; gliding motility without rotation.	<i>Microcoleus</i> cf. <i>sociatus</i>	Filaments long, unbranched; 9–13 trichomes in each sheath; trichomes constricted at the cross-walls, 2.7–3 μm broad; end cell conical and pointed, up to 5 times as long as broad.
		<i>M. cf. paludosus</i>	Individual or several trichomes in each sheath; end cell not capitate, conical; cells nearly as long as or twice as long as broad, 5–7 μm broad, not granulated.
<i>Leptolyngbya</i>	Cylindrical trichomes less than 3 μm diameter; cells longer than wide; constrictions between cells shallow; apical cell rounded.	<i>Phormidium</i> sp.	Trichomes cylindrical, 1.9 μm broad, single within a sheath; end sheaths open always; trichomes constricted at the joints; apices often attenuated, straight.
<i>Nostoc</i>	Heterocystous filaments; developmental cycle in which hormogonia are produced.	<i>Nostoc</i> cf. <i>microscopicum</i>	Colony spherical or ellipsoidal, until 1 cm long. Trichomes without firm sheath, 5–8 μm broad; intercalar almost spherical heterocysts; end cells not elongated.
<i>Scytonema</i>	Heterocystous filaments; false branching, single or geminate; akinetes are not produced.	<i>Scytonema</i> sp.	Double pseudobranching, formed by the perforation of the sheath by the trichome; sheaths laminated and homogeneous; filaments 8.5–10 μm broad; in freshwater or soil.
<i>Calothrix</i>	Mature ensheathed trichomes exhibit a pronounced degree of tapering; basal, one-pored heterocyst.	<i>S. cf. ocellatum</i> <i>Calothrix</i> cf. <i>elenkinii</i> <i>C. cf. marchica</i>	In addition to the general features, filaments are 10–18 μm . Filaments 80–250 μm long, united in tufts, bent at the base; 6–9 μm broad at the base and 4.5–6 μm in the middle. Filaments not so swollen at the base; cells almost as long as broad or shorter than broad; trichome 4–4.5 μm broad.

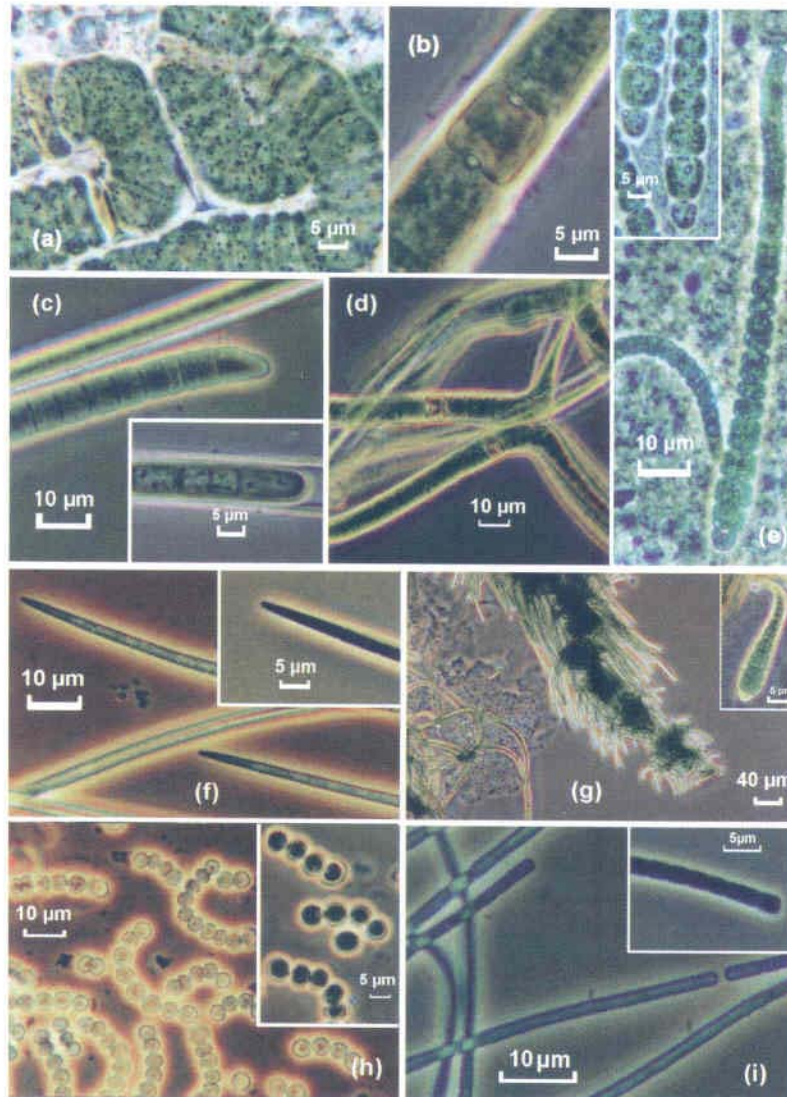


FIGURE 2 Phase contrast micrographs of the morphotypes present in the cyanobacterial microbiotic crusts studied. (a) *Scytonema* cf. *ocellatum*; (b) double-pored heterocyst of *Scytonema*; (c) *Microcoleus* cf. *paludosus*; (d) *Scytonema* sp.; (e) *Calothrix* cf. *marchica*; (f) *Microcoleus* cf. *sociatus*; (g) *Calothrix* cf. *elenkinii*; (h) *Nostoc* cf. *microscopicum*, and (i) *Phormidium* sp.

MNC 1 (Figure 2f) was picked from a flat crust. This morphotype was identified as *Microcoleus* cf. *sociatus*. This filamentous cyanobacterium is narrower than *M. cf. paludosus*. Cell diameter is 2.5 to 3.25 μm and length is 6 to 9 μm . Cells are constricted at the cross-walls, and the end cell is conical and pointed, up to 5 times as long as broad.

Two morphotypes of genus *Scytonema* were observed. A distinctive feature of this genus is the primarily geminate double-pseudobranching filaments, formed by the perforation of the sheath by the trichome, each branch developing a sheath of its own (Figure 2a, 2d). The double-pored heterocysts can be observed (Figure 2b).

Strain SLC1097-8 (Figure 2a) was isolated from a rough crust, and identified as *Scytonema* cf. *ocellatum*, a filamentous cyanobacterium whose filaments are 15 to 18 μm in diameter.

MNC 2 (Figure 2d) was picked from a flat crust and identified as *Scytonema* sp. The main difference from *S.* cf. *ocellatum* was the narrower filament diameter, which is 8.5 to 10 μm .

MNC 3 (Figure 2h) was picked from a rough crust and identified as *Nostoc* cf. *microscopicum*, a filamentous cyanobacterium of spherical cells forming a rounded, gelatinous soft colony <5 mm with a firm outer surface. Heterocysts are intercalary, 7.0 μm diameter, trichomes are 5.0 to 5.5 μm diameter and filaments 7.7 to 8.0 μm diameter.

Two morphotypes of genus *Calothrix* were found. Distinctive features of this genus are filamentous structure, a basal heterocyst, filaments tapering from base to apex, and thalli arranged in penicillate tufts.

MNC 4 (Figure 2e) was picked from a rough crust and identified as *Calothrix* cf. *marchica*. The basal filament diameter is 8 to 10 μm , the middle diameter 6 to 7 μm ; trichomes are 100 to 200 μm long, single or a few together.

Strain SLC1097-9 (Figure 2g) was isolated from a rough crust. This morphotype was identified as *Calothrix* cf. *elenkinii*. The filaments, distinctly swollen at the base are 55 to 88 μm long and free or united in tufts, trichome diameter at the base is 6 to 11 μm and 4.5 μm in the middle.

Strain SLC1097-4 (Figure 2i) was isolated from a flat crust and identified as *Phormidium* sp. This is a filamentous cyanobacterium with cylindrical trichomes constricted at the joints and single within a prominent mucilaginous sheath. Trichome diameter is 1.9 μm ; filament diameter is 3.8 μm ; and apices of trichome are straight.

The diversity of cyanobacterial morphotypes was greater in rough crusts than flat crusts. *Nostoc* cf. *microscopicum* colonies were frequent in rough crusts, whereas few were observed in flat crust samples. *Calothrix* cf. *elenkinii* and *C.* cf. *marchica* were found only in rough crusts.

Besides having a richer composition of cyanobacterial morphotypes, rough crusts also had lichens and mosses, which were responsible for the rough appearance. Lichens and mosses were absent in flat crusts, where *Scytonema* spp. and *Microcoleus* spp. were the main morphotypes. In both types of crusts, *Scytonema* thalli always remained on the surface, even when dry (Figure 3a, 3b), whereas *Microcoleus* thalli appeared some hours after reactivation (Figure 3d). *Microcoleus* was responsible for a “greening” effect in flat crusts after wetting (Figure 3f), whereas in rough crusts, the greening effect was also caused by lichens (Figure 3c, 3e).

The presence of heterocysts in *Scytonema* and *Calothrix*, in contrast to other morphotypes, such as *Microcoleus* and *Phormidium*, suggested the ability of the two former morphotypes to fix nitrogen.

Some morphotypes, such as *Microcoleus* cf. *sociatus*, *Nostoc* sp., and *Calothrix* cf. *marchica* were observed only in fresh preparations obtained from natural crusts. They could not be manipulated so it was not possible to culture them. Therefore, the nitrogen-fixing assay was performed only on four morphotypes: *Microcoleus* cf. *paludosus*, *Phormidium* sp., *Scytonema* cf. *ocellatum*, and *Calothrix* cf. *elenkinii*. Strains of these four morphotypes are in the CIBNOR Cyanobacteria Collection in the Microbial Ecology and Biotechnology Laboratory.

Scanning Electron Microscopy

In dry flat crusts, the prevalence of *Scytonema* on the surface can be seen (Figure 4a), and identified by its typical primarily geminate double pseudobranching filaments (Figure 4b).

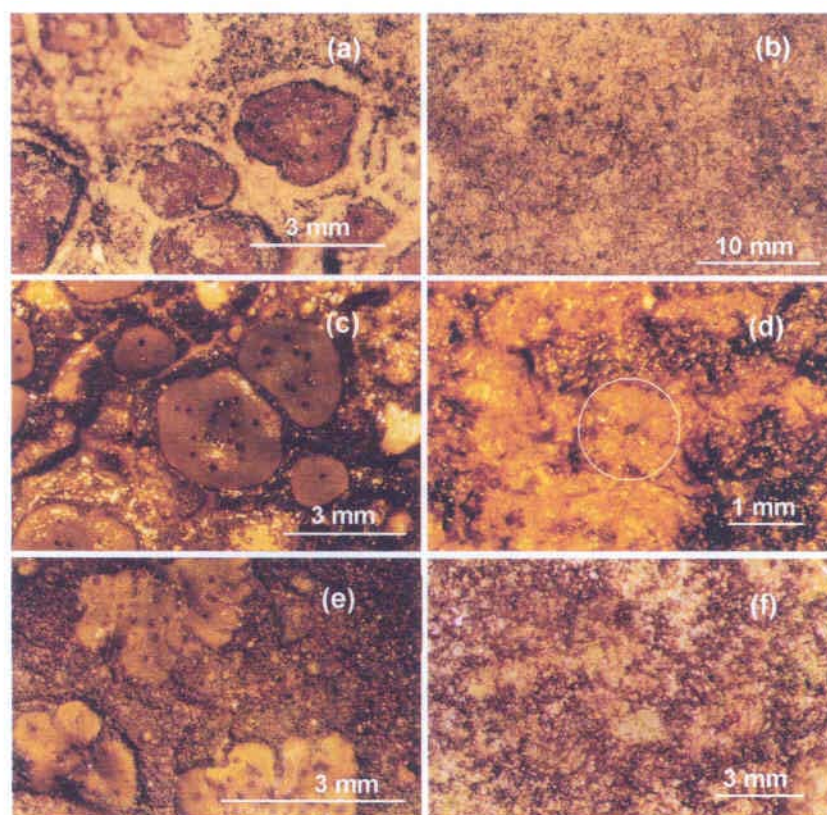


FIGURE 3 Stereoscopic micrographs of natural dry and reactivated microbiotic crusts. (a) dry rough crust; (b) dry flat crust; (c) 20 h reactivated rough crust; (d) 20 h reactivated flat crust. The circle shows *Microcoleus* emerging from a subsurface layer; (e) 47 h reactivated rough crust; (f) 71 h reactivated flat crust.

When wet, filaments of *Microcoleus cf. paludosus* emerge from a deeper layer (Figure 4c) along with some soil particles trapped by the slime produced during cyanobacterial activity (Figure 4d).

The dry rough crusts also show the dominance of *Scytonema* (Figure 4e). The same dry crusts show soil particles that seem to be tied by slimy secretions from microbial components (Figure 4f). In the reactivated rough crusts, filaments of *Microcoleus cf. paludosus* emerge (Figure 4g). Figure 4h shows the intricate sheath mesh produced during cyanobacterial activity.

Nitrogen Fixation

The acetylene reduction assay of the four cyanobacterial morphotypes show that *Scytonema* and *Calothrix* were able to reduce acetylene to ethylene. *Microcoleus* and *Phormidium* were unable to do this. This suggests that only heterocyst-forming filamentous cyanobacteria *Scytonema* and *Calothrix* are nitrogen fixers.

Figure 5 shows the kinetics of acetylene reduction obtained for these two morphotypes in moles of ethylene produced per mg of biomass. Table 3 shows the estimated nitrogen production for each morphotype, expressed in mg of N₂ per mg of biomass.

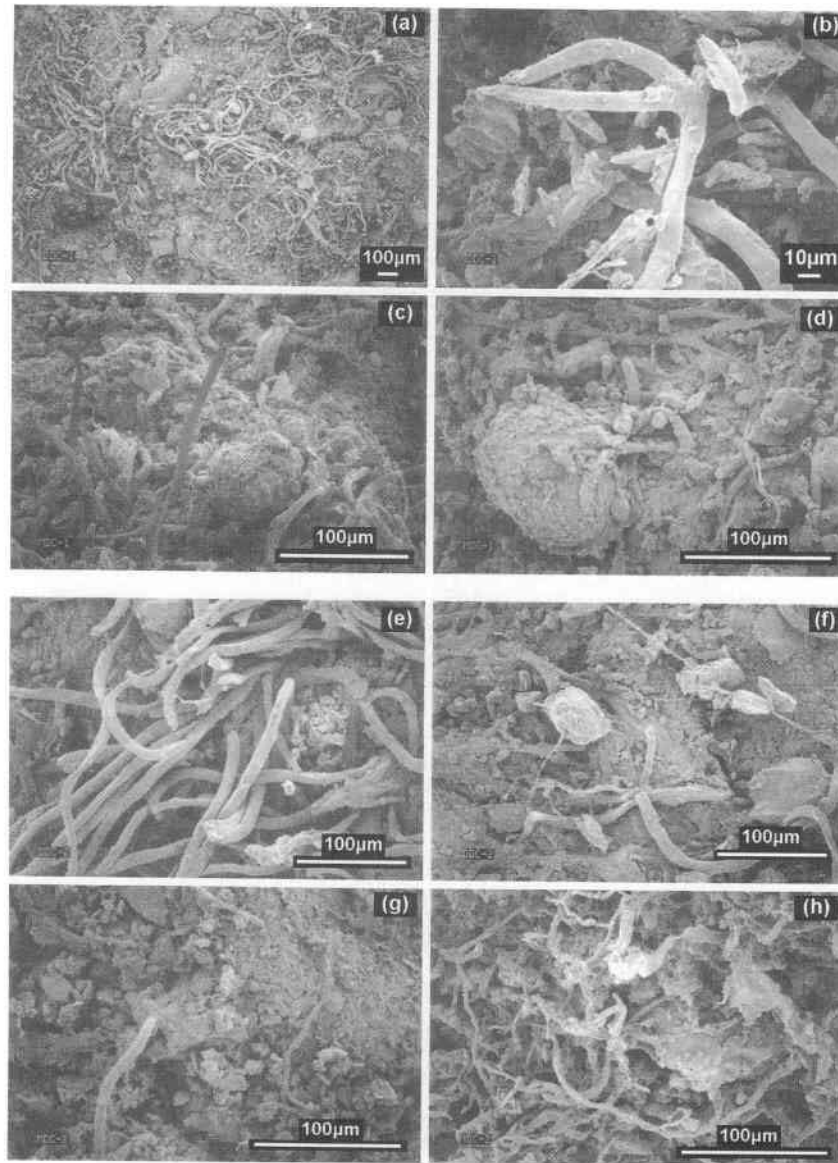


FIGURE 4 SEM micrographs of natural dry and reactivated microbiotic crusts. (a) dry flat crust; (b) pseudobranched filament of *Scytonema*; (c) filaments of *Microcoleus* cf. *paludosus* emerging from a deeper layer; (d) soil particles attached by the cyanobacterial activity; (e) *Scytonema* filaments in a dry rough crust; (f) detail of enmeshed soil particles; (g) filament of *Microcoleus*; (h) sheath mesh on the soil surface.

Discussion

Cyanobacterial Composition of the Crusts

Comparison between *Bergey's Systematic Manual* and Geitler's classification system resulted in new taxonomic assignments for some of our specimen morphotypes. Thin filamentous cyanobacteria described by Geitler as *Phormidium* are described as *Leptolyngbya*

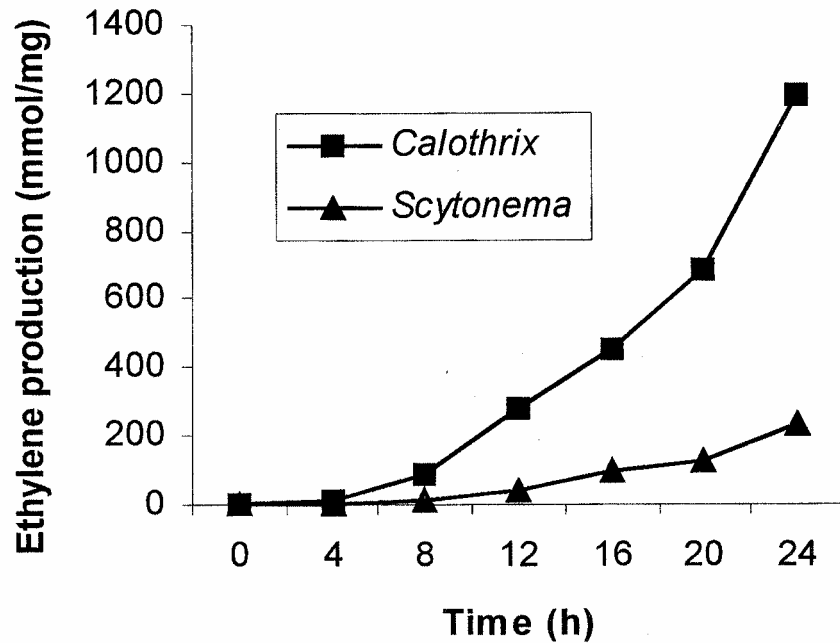


FIGURE 5 Pattern of acetylene reduction in mmoles of ethylene produced per mg of biomass obtained for the two morphotypes that were able to reduce acetylene to ethylene.

by the Bergey's system. Changes of such importance were expected especially in Subsection III (to which *Microcoleus* and *Leptolyngbya* belong) of *Bergey's Manual* because this system is based on multiple 16S rRNA sequence analyses, which is more valid than only morphological features. Geitler discussed the arrangement of the genus *Phormidium* based on features such as constriction at the cross-walls, mentioning that it was undoubtedly artificial. In *Bergey's Manual*, Castenholtz et al. (2001) affirm that Subsection III seems not to be phylogenetically coherent, even if the genetic relationships of this group are still incompletely determined. Recent studies, like the one conducted by García-Pichel et al. (2001), used 16S rRNA gene analyses to characterize cyanobacterial components of microbiotic crusts from the Colorado Plateau, and to help resolve the genetic relationships. This study showed the consistence of a cluster the authors called "Xeronema," which encompasses a variety of taxa of thin filamentous cyanobacteria, usually reported in botanical accounts as *Phormidium* and *Schizothrix*.

Another difference is that Geitler's system considers *Tolypothrix* and *Scytonema* as different genera, the main difference in morphological features is the number of pseudo-branches, one for the former and two for the latter. However, due to the form of development

TABLE 3 Total acetylene reduction during a 24-h cycle and the calculated rate of nitrogen fixation

Heterocystous morphotype	Acetylene reduction [mmol	
	C_2H_4 (mg biomass) $^{-1}$ (24 h $^{-1}$)] \pm sd	Nitrogen fixation [mg nitrogen fixed (g biomass) $^{-1}$ h $^{-1}$ \pm sd
<i>Calothrix</i>	1185.148 \pm 114	0.449 \pm 0.13
<i>Scytonema</i>	228.334 \pm 37	0.083 \pm 0.02

of the pseudobranches, Geitler already discussed the difficulty in differentiating these two groups. In *Bergey's Manual*, *Tolypothrix* is included in the *Scytonema* genus. In contrast to Subsection III, all heterocystous members of Subsection IV form a coherent genetic cluster.

The cyanobacterial morphotypes dominant in the crusts studied were not the more common types cited as dominant in other areas of North America, particularly dry areas, such as *Microcoleus vaginatus* (García-Pichel and Belnap 1996) and *Nostoc commune* (Johansen 1993; Evans and Johansen 1999). We did not find these two species, even after considerable effort to locate them. The main difference between *M. vaginatus* and *M. paludosus* is the caliptrated end cell in the former while between *Nostoc commune* and *Nostoc microscopicum* the main difference is the size of the colony.

In a study made on samples from the Sonoran Desert in Arizona, Cameron (1960) established that *Microcoleus vaginatus* was less prevalent in microbiotic crusts of hot deserts. Cameron found that crusts were dominated by *Schizothrix* species. These differences, together with the isolation of the peninsula, suggest that these cyanobacterial morphotypes have been able to prosper because they are best adapted to the particular conditions of the area. The cyanobacterial composition of the crusts studied can be explained by the transitional climate conditions of the study area and the particular geological history of the Baja California Peninsula. A study of samples collected from the nearby Sonoran Desert plains to the north would support this hypothesis.

Erosion

The scanning electron microscope allowed us to observe fine details, such as soil particles that seem to be tied by the old sheath mesh remaining from the last active period (Figure 4f), in addition to the mesh formed by the filaments (Figure 4h) during activity. This suggests the polysaccharides produced during the short periods of activation (as much as three months per year in the study area) are able to retain soil particles even in dry conditions, contributing to aggregation and stability of the sediment.

Our results support the hypothesis expressed by Johansen (1993) that probably the most important role of microbiotic crusts is stabilization of the soil surface and consequent reduction of soil erosion. Our observations suggest this constructive process is more important in the study area than destructive processes related to microbiotic crusts, such as initial runoff caused by soil hydrophobicity and pore clogging (Kidron et al. 1999). In eroded soils where the loss of particles is faster than soil formation, soil stability related to microbiotic crusts should improve chances for seed germination and seedling establishment of higher plants, a very important link in the dynamics between destructive and constructive soil processes.

Nitrogen Fixation

The results obtained from the nitrogen-fixation assay support our belief that the capacity to do this metabolic process efficiently is limited here to cyanobacteria morphotypes having differentiated cells, such as heterocysts that undertake nitrogen-fixation effectively. This suggests that the growth of nonheterocystic morphotypes, such as *Microcoleus* and *Phormidium*, depend on the presence of those that are able to fix nitrogen, that would explain the delayed emergence of *Microcoleus* filaments after reactivation, if it is related to the activity of the nitrogen fixers.

Based on the kinetics of acetylene reduction shown in Figure 5, *Calothrix* seems to be a more efficient nitrogen fixer than *Scytonema*. However, in our crusts *Scytonema* should be the first contributor of nitrogen in terms of biomass because, as Table 1 shows, it is present

in both kind of crusts and it is better represented than *Calothrix*, even in those crusts where both morphotypes coexist.

The aim of our study was to determine the nitrogen-fixing capacity of the morphotypes studied, and the comparative efficiency among them. Our results were good enough to fulfill this objective, even if they are not enough to compare with other studies of nitrogenase activity based on ARA. Assay designs are so different from one study to another that comparison was not possible. Future studies will offer the possibility of a comparison with other results.

The presence of confirmed nitrogen-fixing morphotypes in the crusts studied suggest their role as bio-improvers of impoverished, eroded soils. However, the study of Harper and Marble (1988) indicates that the balance of nitrogen-fixing after denitrifying bacteria in the same crust could not be enough to sustain higher plants. We think that the presence of efficient nitrogen fixers contribute, at least, to creating adequate conditions for the development and permanence of different microbial populations and even mosses and lichens, which results in the more complex structure of the rough crusts. The presence of at least two types of structures, flat or rough, supports the hypothesis of a successional process toward more complex associations of microorganisms, leading to a climax (Campbell et al. 1989; Belnap and Lange 2001).

References

- Anderson TF. 1956. Electron microscopy of microorganisms. *Phys Tech Biol Res* III:178–181.
- Belnap J, Lange OL (eds.). 2001. Biological soil crusts: Structure, function and management. *Ecological Studies*, Vol. 150. Berlin, Heidelberg: Springer-Verlag, 503p.
- Boone DR, Castenholz RW. 2001. *Bergey's Manual of Systematic Bacteriology*. Vol. 1. The *Archaea* and the Deeply Branching and Phototrophic *Bacteria*. 2nd ed. New York: Springer. p 473–599.
- Brandegees TS. 1892. The distribution of the flora of the Cape Region of Baja California. *Zoe* 3:223–231.
- Cameron RE. 1960. Communities of soil algae occurring in the Sonoran Desert in Arizona. *J Arizona Acad Sci* 1:85–88.
- Cameron RE, Devaney JR. 1970. Antarctic soil algal crust: Scanning electron and optical microscope study. *Trans Amer Microsc Soc* 39:264–273.
- Campbell SE, Seeler JS, Golubic S. 1989. Desert crust formation and soil stabilization. *Arid Soil Res Rehabil* 3:217–228.
- Carmichael WW. 1986. Isolation, culture and toxicity testing of toxic freshwater cyanobacteria (blue-green algae). In: Shilov V, editor. *Fundamental research in homogeneous catalysis*. Vol. 3, New York: Gordon and Breach, p 1249–1262.
- Castenholz RW, Rippka R, Herdman M, Wilmotte A. 2001, Subsection III. (Formerly Oscillatoriales). In: Boone DR, Castenholz RW, editors. *Bergey's Manual of Systematic Bacteriology*. Vol. 1. The *Archaea* and the Deeply Branching and Phototrophic *Bacteria*. 2nd ed. New York: Springer, p 539–562.
- Evans RD, Johansen JR. 1999. Microbiotic crusts and ecosystem processes. *Crit Rev Plant Sci* 18(2):183–225.
- García-Pichel F, Belnap J. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. *J Phycol* 32:774–782.
- García-Pichel F, López-Cortés A, Nüebel U. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from Colorado Plateau. *Appl Environ Microbiol* 67:1902–1910.
- Geitler L. 1932. Cyanophyceae. In: Kolkwitz U, editor. *Rabenhorst's Kryptogamenflora von Deutschland, Österreich und der Schweiz*. Vol. 14. Leipzig: Akademische Verlag, p 594–1146.
- Harper KT, Marble JR. 1988. A role for nonvascular plants in management of arid and semiarid rangelands. In: Tueller PT, editor. *Vegetation science applications for rangeland analysis and management*. Boston: Kluwer Academic Publishers, p 135–169.

- Hoffman L, Asencio AD, Schmit C. 1999. Towards a taxonomic revision of the genus *Scytonema*: Morphological, biochemical and molecular approaches. *Arch Hydrobiol/Algolog Studies* 94:173–174.
- Jensen BB, Cox RP. 1983. Direct measurements of steady-state kinetics of cyanobacterial N₂ uptake by membrane-leak mass spectrometry and comparisons between nitrogen fixation and acetylene reduction. *Appl Environ Microbiol* 45:1331–1337.
- Johansen JR. 1993. Cryptogamic crusts of semiarid and arid lands of North America. *J Phycol* 29:140–147.
- Kidron GJ, Yaalon DH, Vonshak A. 1999. Two causes for runoff initiation on microbiotic crusts: Hydrophobicity and pore clogging. *Soil Sci* 164(1):18–27.
- Ortega A, Arriaga L (eds). 1988. La sierra de la laguna de Baja California sur. Pub. No. 1. Centro de Investigaciones Biológicas de Baja California Sur, A.C. Mexico. 237 p.
- Ortega-Calvo JJ, Stal LJ. 1991. Diazotrophic growth of the unicellular cyanobacterium *Gloethece* sp. PCC 6909 in continuous culture. *J Gen Microbiol* 137:1789–1797.
- Postgate JR. 1972. The acetylene reduction test for nitrogen fixation. In: Norris JR, Ribbons DW, editors. *Methods in microbiology*, Vol. 6B. New York: Academic Press Inc., p 343–356.
- Proteau PJ, Gerwick WH, García-Pichel F, Castenholz R. 1993. The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. *Experientia* 49:825–829.
- Prufert-Bebout L, García-Pichel F. 1994. Field and cultivated *Microcoleus chthonoplastes*: The search for clues to its prevalence in marine microbial mats. *NATO ASI Series, G* 35:111–116.
- Rippka R. 1988. Isolation and purification of Cyanobacteria. *Meth Enzymol* 167:3–27.
- Rippka R, Castenholz RW, Herdman M. 2001. Subsection IV. (Formerly Nostocales). In: Boone DR, Castenholz RW, editors. *Bergey's Manual of Systematic Bacteriology*. Vol. 1. *The Archaea and the Deeply Branching and Phototrophic Bacteria*. 2nd ed. New York: Springer, p 562–589.
- Rippka R, Waterbury JB, Stanier RY. 1981. Isolation and purification of cyanobacteria: Some general principles. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG, editors. *The prokaryotes*, New York: Springer-Verlag, p 212–223.
- Shreve F. 1937. The vegetation of the Cape Region of Baja California. *Madroño* 4:105–113.
- Stal LJ. 1988. Nitrogen fixation in cyanobacterial mats. *Meth Enzymol* 167:474–484.