

# Molecular Characterization of an Oil-Degrading Cyanobacterial Consortium

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## Abstract

Recent studies have shown that the cyanobacterium *Microcoleus chthonoplastes* forms a consortium with heterotrophic bacteria present within the cyanobacterial sheath. These studies also show that this consortium is able to grow in the presence of crude oil, degrading aliphatic heterocyclic organo-sulfur compounds as well as alkylated monocyclic and polycyclic aromatic hydrocarbons. In this work, we characterize this oil-degrading consortium through the analysis of the 16S rRNA gene sequences. We performed the study in cultures of *Microcoleus* grown in mineral medium and in cultures of the cyanobacterium grown in mineral medium supplemented with crude oil. The results indicate that most of the clones found in the polluted culture correspond to well-known oil-degrading and nitrogen-fixing microorganisms, and belong to different phylogenetic groups, such as the Alpha, Beta, and Gamma subclasses of Proteobacteria, and the Cytophaga/Flavobacteria/Bacteroides group. The control is dominated by one predominant organism (88% of the clones) closely affiliated to *Pseudoxanthomonas mexicana* (similarity of 99.8%). The presence of organisms closely related to well-known nitrogen fixers such as *Rhizobium* and *Agrobacterium* suggests that at least some of the cyanobacteria-associated heterotrophic bacteria are responsible for nitrogen fixation and degradation of hydrocarbon compounds inside the polysaccharidic sheath, whereas *Microcoleus* provides a habitat and a source of oxygen and organic matter.

## Introduction

Several research groups [5, 17] attribute to cyanobacteria an important role in the biodegradation of organic pollutants. In fact, there is evidence that microbial communities dominated by cyanobacteria can be actively involved in oil degradation [2]. Observations made after oil spills in the Arabian Gulf showed that cyanobacteria grew, forming heavy thick mats on the top of the sediments [4, 39]. Other studies have focused on the capacity of cyanobacteria isolates to degrade hydrocarbons. Cerniglia *et al.* [9, 10] observed the degradation of naphthalene, a major component of the water-soluble fraction of crude oil, and biphenyl, by the same strain of *Oscillatoria*. It has also been reported that phenanthrene can be metabolized by the unicellular marine cyanobacterium *Agmenellum quadruplicatum* [27]. Also, *Oscillatoria salina*, *Plectonema tenebrans*, and *Aphanocapsa* sp. degraded crude oil when grown in artificial medium and natural seawater [32].

However, it is by no means clear whether oil degradation is carried out by cyanobacteria alone or by heterotrophic bacteria associated to cyanobacteria. Some studies point to heterotrophic bacteria associated to cyanobacteria as coresponsible of hydrocarbon degradation. Al-Hasan *et al.* [5] demonstrated that nonaxenic cyanobacterial samples containing *Microcoleus chthonoplastes* and *Phormidium corium* consumed and oxidized *n*-alkanes. They found that cyanobacterial growth steadily declined with progressive axenicity, and they identified four genera and species of associated heterotrophic bacteria (*Rhodococcus rhodochrous*, *Arthrobacter nicotianae*, *Pseudomonas* sp., and *Bacillus* sp.) able to oxidize *n*-alkanes, although cyanobacteria directly contributed to hydrocarbon uptake and oxidation. On the other hand, Al-Hasan *et al.* [6] demonstrated that picocyanobacteria from the Arabian Gulf accumulated hydrocarbons from the water body, but did not utilize these compounds; the authors assumed that associated bacteria may be carrying

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out the degradation of these contaminants. Furthermore, Abed and Köster [1] confirmed that *Oscillatoria*-associated aerobic heterotrophic bacteria were responsible for the biodegradation of *n*-alkanes.

Recent studies have shown that *M. chthonoplastes* develop by forming a consortium with heterotrophic bacteria capable of biodegrading crude oil [16]. This consortium was able to grow in the presence of sulfur-rich petroleum, although the changes in crude oil composition were small, essentially involving degradation of aliphatic heterocyclic organo-sulfur compounds such as alkylthiolanes and alkylthianes. Also, other groups of compounds, such as the alkylated monocyclic and polycyclic aromatic hydrocarbons, underwent some degree of transformation. Ultrathin sections of this cyanobacterium revealed the presence of different bacterial morphotypes inside the polysaccharidic *Microcoleus* sheath [13].

In this work we used molecular techniques, such as denaturing gradient gel electrophoresis (DGGE) and clone libraries, to characterize the components of this consortium.

## Methods

**Microorganism and Culture Conditions.** *M. chthonoplastes* was isolated from microcosms of Ebro Delta microbial mats (Tarragona, Spain) polluted with Maya crude oil via serial transfers in agar plates containing the mineral medium described by Van Gernerden and Beertink [42], as previously detailed by Diestra *et al.* [13]. Cultures were grown under anoxic conditions in a continuous light regime (15  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) at 27°C. Initially, our aim was to isolate pure cultures of *Microcoleus*, but microscopic observations via transmission electron microscopy (TEM) and scanning electron microscopy (SEM) indicated the existence of other bacteria growing in intimate contact with *Microcoleus* filaments. With TEM, we observed heterotrophic bacteria growing within the polysaccharidic sheath, while SEM allowed the visualization of bacteria adhering to *Microcoleus* filaments [13].

The consortium was grown on agar plates both with mineral medium containing carbonate as the only carbon source and with the same medium lacking carbonate and supplemented with 150  $\mu\text{l}$  of Maya crude oil spread on top of the plate.

**DNA Extraction.** Nucleic acid extraction of cultures was performed as described by Massana *et al.* [24]. Samples of cyanobacterial biomass collected from the surface of the plates were suspended in 2 mL of lysis buffer (50 mM Tris-HCl, pH 8.3; 40 mM EDTA, pH 8.0; 0.75 M sucrose). Sterile glass beads (diameter: 0.5 mm) were added to the cultures, and vortexed to disrupt the filaments. DNA was extracted using the lysis/phenol

extraction method as described below. Lysozyme (final concentration: 1  $\text{mg}\cdot\text{mL}^{-1}$ ) was added and samples were incubated at 37°C for 45 min in slight movement. Next, sodium dodecyl sulfate (final concentration: 1%) and proteinase K (final concentration: 0.2  $\text{mg}\cdot\text{mL}^{-1}$ ) were added and samples were incubated at 55°C for 60 min in slight movement. Nucleic acids were extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1, vol), and the residual phenol was removed once with chloroform/isoamyl alcohol (24:1, vol). Nucleic acids were purified, desalted, and concentrated with a Centricon-100 concentrator (Millipore). DNA integrity was checked by agarose gel electrophoresis, and quantified using a low DNA mass ladder as a standard (Invitrogen). The same extract of the two samples was split for analysis by DGGE and clone library.

**PCR-DGGE Fingerprinting.** Fragments of the 16S rRNA gene suitable for DGGE analysis were obtained by using the bacterial specific primer 358F with a 40-bp GC clamp, and the universal primer 907RM [26, 35]. Polymerase chain reaction (PCR) was carried out with a Biometra thermal cycler using the following program: initial denaturation at 94°C for 5 min; 10 touchdown cycles of denaturation (at 94°C for 1 min), annealing (at 65–55°C for 1 min, decreasing 1°C each cycle), and extension (at 72°C for 3 min); 20 standard cycles (annealing at 55°C, 1 min) and a final extension at 72°C for 5 min.

Primers 344f-GC and 915r were used for archaeal 16S rRNA amplification [33, 37]. The PCR protocol included an initial denaturation step at 94°C for 5 min, followed by 20 touchdown cycles of denaturation (at 94°C for 1 min), annealing (at 71 to 61°C for 1 min, decreasing 1°C each cycle), and extension at 72°C for 3 min. This procedure was followed by 15 additional cycles at an annealing temperature of 61°C. During the last cycle of the program, the length of the extension step was 10 min [8].

PCR mixtures contained 1–10 ng of template DNA, each deoxynucleoside triphosphate at a concentration of 200  $\mu\text{M}$ , 1.5 mM  $\text{MgCl}_2$ , each primer at a concentration of 0.3  $\mu\text{M}$ , 2.5 U *Taq* DNA polymerase (Invitrogen), and PCR buffer supplied by the manufacturer. Bovine serum albumin (BSA; final concentration: 600  $\mu\text{g}\cdot\text{mL}^{-1}$ ) was added to minimize the inhibitory effect of humic substances [18]. The volume of reactions was 50  $\mu\text{l}$ . PCR products were verified and quantified by agarose gel electrophoresis with a low DNA mass ladder standard (Invitrogen).

DGGE was run in a DCode system (Bio-Rad) as described by Muyzer *et al.* [26]. A 6% polyacrylamide gel with a gradient of DNA denaturant agent was cast by mixing solutions of 0 and 80% denaturant agent (100% denaturant agent is 7 M urea and 40% deionized formamide). A 700-ng portion of PCR product was loaded for each sample and the gel was run at 100 V for 18 h at 60°C in 1 $\times$  TAE buffer [40 mM Tris (pH 7.4),

20 mM sodium acetate, 1 mM EDTA]. The gel was stained with SybrGold (Molecular Probes) for 45 min, rinsed with 1×TAE buffer, removed from the glass plate to a UV-transparent gel scoop, and visualized with UV in a Gel Doc EQ (Bio-Rad).

Prominent bands were excised from the gels, resuspended in Milli-Q water overnight, reamplified, and purified using a High Pure PCR Product Purification Kit (Roche) for its sequencing.

**Clone Library and RFLP Analysis.** For cloning, bacterial 16S rRNA gene was amplified between positions 27 and 1492 (*Escherichia coli* 16S rRNA gene sequence numbering), using the primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). PCR mixtures contained 10 ng of template DNA, with each deoxynucleoside triphosphate at a concentration of 200 μM, 1.5 mM MgCl<sub>2</sub>, each primer at a concentration of 0.3 μM, 2.5 U *Taq* DNA polymerase (Invitrogen), and PCR buffer supplied by the manufacturer. Reactions were carried out in an automated thermocycler (Biometra) with the following cycle: an initial denaturation step at 94°C for 5 min, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C and 2 min at 72°C, and a final extension step of 10 min at 72°C.

The PCR product was cloned with the TOPO TA cloning kit (Invitrogen) according to the manufacturer's instructions. Putative positive colonies were picked, transferred to a multiwell plate containing Luria-Bertani medium and 7% glycerol, and stored at -80°C. Recombinant plasmids were extracted using the QIAprep spin miniprep kit (QIAGEN), following the manufacturer's instructions. Purified plasmids were digested at 37°C overnight with *Hae*III (Invitrogen) and the product was run in 2.5% low melting point agarose gel. The different band patterns were chosen for partial sequencing. The coverage of the clone library was calculated according to the following equation:  $C = 1 - (n / N)$ , where  $n$  is the number of unique clones and  $N$  is the total number of clones examined [34].

Our choice of these primers (358F with a GC clamp and 907R for DGGE analysis, and 27F and 1492R for clone libraries) was deliberate because they do not amplify *Microcoleus*. We checked this by using the match probe tool of the ARB program package applying as target strings the primer sequences and also with cultures of *Microcoleus*. This allowed us to eliminate the high background of this dominant microorganism, as it could have interfered with the final results.

**rRNA Sequencing.** Sequencing reactions were performed by Macrogen (South Korea) with the primers 907R for bacterial DGGE bands (approximately 500 bp in length), and 27F for cloned 16S rRNA genes. They utilized the Big Dye Terminator version 3.1 se-

quencing kit and reactions were run in an automatic ABI 3730XL Analyzer-96 capillary type.

Sequences were subjected to a BLAST search [7] to obtain a first indication of the phylogenetic affiliation, and to the CHECK-CHIMERA program from RDP [22] to determine potential chimeric artifacts. Sequences were aligned by using the automatic alignment tool of the ARB program package (<http://www.mikro.biologie.tu-muenchen.de>) [20]. Then partial sequences were inserted into the optimized tree derived from complete sequence data by the *Quick add using parsimony tool*, which does not affect the initial tree topology. The resulting tree was pruned to save space and only the closest relatives were retained.

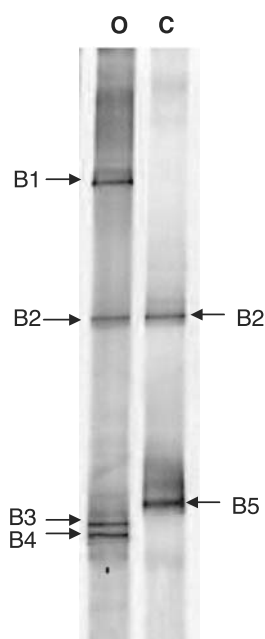
**Accession Numbers.** Forty-four 16S rRNA gene sequences were sent to the EMBL database (<http://www.ebi.ac.uk/embl>) and received the following accession numbers: from AJ871043 to AJ871081 for the clone library and from AJ870388 to AJ870392 for the DGGE bands (the detailed assignment of each sequence appears in Fig. 2).

## Results

*M. chthonoplastes* was grown both on agar plates containing mineral medium with carbonate as the only carbon source, and on agar plates with the same medium composition, except for carbonate, which had been omitted and replaced by a small amount of crude oil. Cyanobacterial biomass was collected from the surface of the plates and after DNA extraction, the diversity of the 16S rRNA genes present in the samples was characterized by DGGE analyses, and also by sequencing the clone libraries built from each, the control (C), and the oil-polluted (O) culture.

**DGGE Analysis of the Consortium.** DGGE analysis of the control and the oil polluted culture using the general bacterial primers 358F-GC and 907R showed the existence of substantial differences between samples (Fig. 1). Comparison of the two cultures demonstrated that in both cases, the number of bands observed in the DGGE gel was low (two bands in the control and four bands in the oil polluted culture), indicating low diversity of microorganisms associated to *Microcoleus*.

Prominent bands were sequenced, and the closest matches (and percentages of similarity) for the sequences retrieved were determined by a BLAST search (Table 1). The number of bases used to calculate each similarity value is also shown in Table 1 as an indication of the quality of the sequence. One of the bands (B2), present in both samples, was related to an uncultured Bacteroidetes microorganism. In the oil-polluted sample (O), one of the most intense bands (band B3) corresponded to a



**Figure 1.** Negative image of DGGE gel with PCR products amplified with bacterial primer sets (O: consortium with oil; C: consortium without oil).

microorganism closely related to *Rhizobium* sp. Two more bands appeared in this lane; they were related to an uncultured Bacteroidetes microorganism (band B1) and to *Thioclava pacifica* (Rhodobacteraceae, band B4). The control had also one prominent band (band B5) closely related to *Pseudoxanthomonas mexicana* (Xanthomonadaceae).

**Clone Library Construction and rRNA Sequencing.** DGGE is a fingerprinting technique that allows easy and fast comparison of related microbial profiles. However, sequences obtained from excised DGGE bands are short and of variable quality, sufficient to determine broad phylogenetic affiliations but inadequate to carry out a precise phylogenetic analysis. Thus, we investigated the phylogenetic composition of the two samples (O and C) by building two independent clone libraries. We obtained 94 and 187 clones for samples O and C, respectively, which resulted in different RFLP patterns. A representative clone of each operational taxonomic unit (OTU) was partially sequenced. Their frequency in the library, the similarity values, and their closest relatives are listed in Table 2.

Coverage of the libraries was 87.2 and 95.7% for samples O and C, respectively. Members of the  $\alpha$ - and  $\gamma$ -subclasses of Proteobacteria were the main components of the two samples.

In general, groups of bacteria that could only be detected in the oil-contaminated sample included members of the Rhizobiaceae (*Rhizobium* and *Agrobacterium tumefaciens*, 26%), members of the Rhodobacteraceae (*T. pacifica* and *Roseobacter*, 29%), as well as  $\beta$ -Proteobacteria closely related to *Hydrogenophaga* (5%). Only one species of  $\alpha$ -Proteobacteria, identified as *Hyphomonas polymorpha*, was unique to the control sample (1.1%). The remaining sequences were found both in the control and in the polluted sample although with different frequencies, and included members of the families Pseudomonadaceae, Xanthomonadaceae, Phyllobacteriaceae, and the Cytophaga/Flavobacteria/Bacteroides (CFB) group. It is remarkable that 88% of the clones in the control sample (C) corresponded to *P. mexicana*. In general, there was agreement between the DGGE and the gene library data. Inclusion of all the sequences in a phylogenetic tree indicates that all the DGGE band sequences corresponded to several of the most abundant clones recovered from the library (see Fig. 2). However, a relatively large number of clones, some of them quite abundant, were not represented in the DGGE gel.

## Discussion

Basically, our results demonstrate that bacteria associated to *M. chthonoplastes* grown in the presence of crude oil belong to the following four families: Rhizobiaceae (*Rhizobium* sp., *A. tumefaciens*, 26.6%). Rhodobacteraceae (*Roseobacter* sp., *T. pacifica*, 28.7%), Phyllobacteriaceae (*Parvibaculum lavamentivorans*, 13.9%), and Xanthomonadaceae (*Pseudoxanthomonas japonensis*, 11.7%). In the control culture grown in the absence of crude oil, the most abundant organism (*P. mexicana*, 87.7% of the clones) belongs to the family Xanthomonadaceae.

Growth of heterotrophic bacteria associated to *Microcoleus* or other cyanobacteria has been reported previously [29]. Steppe *et al.* [38] demonstrated the existence of epiphytic nitrogen-fixing bacteria forming a diazotrophic consortium with *Microcoleus* spp. This observation is particularly interesting because *Microcoleus*,

**Table 1.** Sequence similarities of excised bacterial bands that appear in Fig. 1

Band	Closest match	Percent similarity (no. bases) <sup>a</sup>	Taxonomic group	Accession no.
B1	Uncultured Bacteroidetes	95.8 (525)	CFB	AJ534682
B2	Uncultured bacterium	98.7 (532)	CFB	AF502212
B3	<i>Rhizobium</i> sp.	98.9 (528)	$\alpha$ -Proteobacteria	AY500261
B4	<i>Thioclava pacifica</i>	98.5 (530)	$\alpha$ -Proteobacteria	AY656719
B5	<i>Pseudoxanthomonas mexicana</i>	99.6 (560)	$\gamma$ -Proteobacteria	AF273082

<sup>a</sup>Numbers in parentheses denote the number of bases used to calculate the levels of sequence similarity.

**Table 2.** Phylogenetic affiliation of clone sequences retrieved

Group	Closest match (accession no.)	Similarity (%)	Cultured closest match (accession no.)	Similarity (%)	Percent clones	
					O	C
$\alpha$ -Proteobacteria	<i>Rhizobium</i> sp. (AF364858)	98.1			25.5	–
	<i>Agrobacterium tumefaciens</i> (AF508094)	97.2			1.1	–
	<i>Thioclava pacifica</i> (AY656719)	97.7			25.5	–
	$\alpha$ -Proteobacterium (AY162070)	97.7	<i>Roseobacter</i> sp. (AY332661)	97.7	3.2	–
	<i>Roseomonas</i> sp. (AF533359)	94.1			1.1	–
	<i>Roseomonas gilardii</i> (AY150051)	94.4			–	0.5
	<i>Hyphomonas polymorpha</i> (AJ227813)	99.2			–	1.1
	Uncultured bacterium (AF143824)	97.8	<i>Parvibaculum</i> <i>lavamentivorans</i> (AY387398)	97.1	1.1	–
	<i>Parvibaculum lavamentivorans</i> (AY387398)	99.6			12.8	1.6
$\beta$ -Proteobacteria	Uncultured bacterium (AB186829)	98.9	<i>Hydrogenophaga</i> sp. (AB166889)	98.5	2.1	–
	Uncultured bacterium (AY625151)	99.4	<i>Hydrogenophaga taeniospiralis</i> (AF078768)	99.2	3.2	–
$\gamma$ -Proteobacteria	<i>Pseudomonas stutzeri</i> (U26414)	99.9			8.5	0.5
	<i>Pseudomonas stutzeri</i> (AJ006103)	99.7			–	6.4
	<i>Pseudoxanthomonas mexicana</i> (AF273082)	99.8			–	87.7
	Uncultured $\gamma$ -proteobacterium (AJ619035)	99.9	<i>Pseudoxanthomonas japonensis</i> (AB008507)	99.6	11.7	–
	Uncultured $\gamma$ -proteobacterium (AF445725)	98.4	<i>Aquamonas vora</i> (AY544768)	94.7	1.1	–
	Uncultured $\gamma$ -proteobacterium (AF445726)	96.7	<i>Pseudoxanthomonas</i> <i>daejeonensis</i> (AY550264)	93.7	1.1	–
CFB	Uncultured bacterium (AF502210)	95.5	<i>Cytophaga</i> sp. (AF250407)	96.7	1.1	2.1
	Antarctic bacterium (AJ441001)	94.5	<i>Cytophaga</i> sp. (X85210)	94.1	1.1	–

O: Culture with oil; C: control without oil.

which does not have the capacity to fix nitrogen, is the major constituent of marine microbial mats, well known for their ability to proliferate in nitrogen deficient environments.

In a separate study, Olson *et al.* [28] described a similar phenomenon in cyanobacteria-dominated microbial aggregates embedded in the permanent ice cover of Lake Bonney (Antarctica) and in cyanobacterial mats found in soils adjacent to the ice edge. Their microscopic observations revealed the presence of heterotrophic bacteria associated to cyanobacteria and also growing in their mucilaginous sheath. Based on the presence of *nifH* sequences typical of heterotrophic organisms, the authors

suggested that they were probably contributing to nitrogen fixation.

In our case, several of the 16S rRNA sequences recovered from the gene libraries correspond to nitrogen-fixing microorganisms. Two of these organisms (*Rhizobium* and *Agrobacterium*) were only detected in the cultures of *Microcoleus* that had been grown in the presence of oil. The third organism, *Pseudomonas stutzeri*, found in the culture incubated with oil and also in the control, fixes nitrogen under microaerophilic conditions in the free-living state [12]. It is tempting to speculate about a possible role of these organisms as nitrogen providers for growth of *Microcoleus* under the conditions tested.



**Figure 2.** Phylogenetic tree including sequences from the two clone libraries (O: consortium with oil; C: control without oil), and excised bands from DGGE in Fig. 1. The scale bar indicates 10% sequence similarity. Sequences appearing in both the DGGE gel and the clone libraries are clustered in gray rectangles.

There is evidence that some of the nitrogen-fixing species found (*Rhizobium* and *Agrobacterium*) can also develop in oil-contaminated soils [3, 19, 31, 40]. These studies pointed at the potential use of nodule-forming, symbiotic bacteria for oil bioremediation.

On the other hand, when the consortium was grown in the presence of oil, we identified several clones closely related to *P. stutzeri*. It was also detected in the control, although at a lower percentage (7%). The genus *Pseudomonas* has been extensively associated with the degradation of petroleum compounds [23, 43]. Members of this group are able to degrade S- and N-heterocyclic [15], alkanes, and other polyaromatic hydrocarbons [30]. An algal-bacterial consortium formed by *Chlorella sorokiana* and a *Pseudomonas migulae* strain was able to degrade phenanthrene [25] in two-phase partitioning bioreactors. *Pseudomonas* can also grow associated with cyanobacteria [5]. Furthermore, *P. stutzeri* was isolated from fuel-contaminated Antarctic soils [14]; Eckford *et al.* pointed to a possible selection of diazotrophs, because fuel-contaminated soils have adequate carbon but present nitrogen-limited conditions.

Several species of purple nonsulfur bacteria metabolize aromatic compounds during phototrophic growth, including in particular the species *Rhodopseudomonas palustris*, but also *Rhodomicrobium vannieli*, *Rhodocyclus purpureus*, *Phaeospirillum fulvum*, and *Rhodobacter capsulatus* [21]. We detected several members of the family Rhodobacteraceae in samples O and C, although they seemed more abundant in the oil-polluted sample.

In addition, we identified other minor groups capable of degrading hydrocarbon compounds. Some clones shared a high similarity (99%) with *P. lavamentivorans*, a heterotroph that grows with octane and is able to initiate catabolism of linear alkylbenzenesulfonate [36].

It has also been described that the  $\beta$ -Proteobacterium *Hydrogenophaga* sp., which was detected in our oil-degrading consortium, can metabolize 4-aminobenzenesulfonate when grown in a mixed culture with *Agrobacterium* [11].

*P. mexicana* was first described by Thierry *et al.* [41]. To date, little is known about the physiology of this organism, beyond the fact that it has a strictly respiratory metabolism and that it can reduce nitrite to N<sub>2</sub>O. Exploration of the metabolic capabilities of this microorganism would be essential to understand its ecological function in the consortium, particularly its ability to degrade organic compounds.

In a recent study, Abed and Köster [1] evaluated the role of cyanobacteria and their associated aerobic heterotrophic bacteria in biodegradation of petroleum compounds. They found that five different cyanobacteria (*Aphanothece halophyletica*, *Dactylococcopsis salina*, *Halothece* strain EPUS, *Oscillatoria* strain OSC, and *Synechocystis* strain UNIGA) were able to degrade *n*-

alkanes, while three isolates (*A. halophyletica*, *Oscillatoria* strain OSC, and *Synechocystis* strain UNIGA) degraded aromatic compounds. The authors demonstrate that the heterotrophic bacteria associated with *Oscillatoria* strain OSC are responsible for the observed biodegradation. The molecular analysis of cultures of *Oscillatoria* grown on different petroleum model compounds shows the presence of associated heterotrophic bacteria belonging to the  $\gamma$  subclass of Proteobacteria. The control experiment (*Oscillatoria* culture without model compounds) reveals the presence of a bacterial sequence from the Cytophaga/Flavobacteria/Bacteroides group.

The metabolic capacities displayed by the organisms found in association with *Microcoleus* in our study are in agreement with the type of degradation previously reported for this consortium by Garcia de Oteyza *et al.* [16]. In summary, our findings suggest that the microorganisms associated with the cyanobacterium *M. chthonoplastes* could be carrying out most of the nitrogen fixation and degradation of hydrocarbon compounds inside the polysaccharidic sheath. As discussed by other authors, degradation of petroleum compounds is unlikely to be characteristic for cyanobacteria, although they play an indirect role in mixed populations such as microbial mats by supporting the growth and activity of the actual degraders [1, 2].

In general, as stated by Paerl and Pinckney [29], many prokaryotes develop microbial consortial associations with other prokaryotes and eukaryotes depending on their nutrient necessities. In our consortium, *Microcoleus* would provide a habitat and a readily available source of oxygen and organic matter produced by excretion of photosynthates, cell lysis, and decomposition, whereas the accompanying bacteria would contribute to the consortium by fixing nitrogen. Furthermore, the complete degradation of petroleum compounds to CO<sub>2</sub> can be used by cyanobacteria for photosynthesis.

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