RESEARCH ARTICLE

Bacterial diversity, pigments and nitrogen fixation of biological desert crusts from the Sultanate of Oman

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Abstract

Biological desert crusts are relatively common in the arid deserts of the Sultanate of Oman; however, little is known about their microbial community composition and role in soil fertilization. We compared three crusts from geographically different locations for their soil texture, bacterial community structure, pigment composition and nitrogenase activity. The crusts were growing on alkaline (pH 7.6–8.7) loamy sand and silty loam soils. Microscopically, *Microcoleus vaginatus* was the most abundant cyanobacterium, but *Nostoc* and *Scytonema* types dominated in cultures. The 16S rRNA gene sequences showed close similarities in the crusts' bacterial composition, with 77–81% of the total clones belonging to cyanobacteria and the rest distributed among *Alpha-* and *Deltaproteobacteria*, *Bacteriodetes*, *Gemmatimonas* and *Planctomycetes*. Thirty-seven percent of the cyanobacterial clones were affiliated with heterocystous types such as *Nostoc*, *Scytonema*, *Brasilonema* and *Petalonema*. Chlorophyll *a* concentrations suggest a similar abundance of phototrophs in all crusts. High levels of the UVA sunscreen scytonemin were detected in the exposed crusts. The three crusts exhibited comparable acetylene reduction rates in the light and in the dark, with a maximum rate of $58.5 \pm 2.6 \, \mu mol \, C_2H_2$ reduced $m^{-2} \, h^{-1}$. We conclude that the crusts, regardless of their geographical location, were rich in heterocystous cyanobacteria that can fix nitrogen and could possibly improve soil stability and productivity.

Introduction

Biological soil crusts (referred to as crusts hereafter) are composed of a few millimeters of topsoil layer of highly specialized organisms, including cyanobacteria, green algae, fungi and many heterotrophic bacteria (Belnap, 1990, 2001; Garcia-Pichel *et al.*, 2001; Knudsen & La Doux, 2005; Zancan *et al.*, 2006). While bacteria constituted the major component of crustal organisms, fungi and archaea were minor (Bates & Garcia-Pichel, 2009; Soule *et al.*, 2009) and algae were commonly found in acidic environments (Smith *et al.*, 2004). Crusts are distributed worldwide, particularly in arid and semi-arid landscapes, which constitute approximately 30% of the global land area (Whitford, 2002). In these ecosystems, cyanobacteria play a vital role in fixing carbon and nitrogen (Evans & Johansen, 1999; Belnap, 2002a), stabilizing soil against erosion (Campbell, 1979; Maya *et al.*, 2002), protection against UV radiation by

producing sunscreen pigments (Garcia-Pichel & Castenholz, 1993; Büdel *et al.*, 1997) and altering the hydrological properties of crust-covered soils (Mazor *et al.*, 1996). The diversity of cyanobacteria, their adaptation to desiccation and their contribution to fixed nitrogen in crusts have been widely studied (Garcia-Pichel *et al.*, 2001; Belnap, 2002a; Redfield *et al.*, 2002; Pringault & Garcia-Pichel, 2004; Yeager *et al.*, 2007). Most of this research has been on samples from cold and temperate deserts from the United States, Israel and Australia (Belnap & Lange, 2001). However, very little is known about crusts from hot subtropical deserts of the Arabian Peninsula and adjacent countries (Belnap, 2002b).

Oman's lowland desert climates vary from hyperarid in the central desert to arid in the coastal regions, receiving an annual mean rainfall of about 40 and 85 mm, respectively. Unpredictable rains usually occur from November to March, and the hot summer months are normally dry. Dew occasionally moistens the soil surface at some localities of

the coastal plain and further inland, but the geographic extent and frequency of this moisture has not been studied. Soils of the lowlands are aridosols that are generally shallow (5–20 cm) and lack distinctive organic horizons (Ministry of Agriculture and Fisheries, Oman). Summer air temperatures are normally in the 40s, but can reach 50 °C, and soil surface temperature may exceed 65 °C. The central desert occasionally experiences freezing temperatures while the coastal lowlands are warmer, with low temperatures exceeding 10 °C. Crusts in this region are subjected to several threats including extensive grazing by camels and goats, oil exploration and human activities such as off-road driving and removal of topsoil for horticultural purposes. Such disturbances reduce the activity of soil organisms that provide nitrogen and soil stability and will take years to recover (Belnap & Eldridge, 2001). Therefore, investigations of Oman's unexplored crusts are needed to increase the awareness of the importance of these microbial communities for improving soil stability and fertility and facilitating revegetation.

In this study, we compare the soil characteristics, microbial community composition, pigments and acetylene reduction rates of crusts from different geographical locations in Oman. The bacterial communities were studied using direct microscopy, enrichment cultivation and 16S rRNA gene-based clone libraries. The main goals are to characterize the microbial communities in these crusts and to determine whether bacterial diversity and nitrogen fixation rates change as a function of geographical location.

Materials and methods

Origin of desert crust samples

Crust samples were collected from three sites, two near Muscat (WK and SD) and the third from Adam (AD) in the north central region of Oman (Fig. 1). The distance between samples WK and SD was *c.* 5 km, but both were *c.* 170 km away from the AD sample. At each site, three to six Eppendorf vials (2 mL) of the topmost 2 cm of dry crusts with visible evidence of microorganisms were collected and approximately 1 kg of soil was taken for the analysis of soil characteristics. The crust samples were stored dry at room temperature for 2 months until analysis. The WK site is surrounded by low limestone hills and the surface is covered with small to medium gravels. Random crust samples were collected from open areas without surface gravels and from beneath the canopy of *Acacia tortilis* trees. The SD site is an elevated plain above a large wadi. The surface is covered with various-sized stones and small areas of bare soil. Samples were taken from small depressions. AD is a site located in the northern portion of Oman's central desert. Organisms and soil were collected from a large shallow depression without

stone cover. Vegetation with *A. tortilis* trees was only observed in WK, but not in SD and AD sites. All the crusts are endedaphic and smooth, following the classification of Belnap & Lange (2001).

Soil characteristics

Soil samples (1 kg of the uppermost 2–3 cm) were air-dried and crushed to pass through a 2-mm sieve. A standard soil hydrometer method (Klute, 1986) was used to determine the percent sand, silt and clay, which was used to identify the soil texture class from a soil triangle (Brady, 1984). The sand portion was further fractionated into smaller particle sizes. Ten grams of soil was thoroughly mixed with 50 mL of deionized water and allowed to stand for 20 min. The solution was resuspended and the filtrate was collected. Soil pH and electrical conductivity were measured from the filtrate using calibrated YSI instruments.

Light microscopy and enrichment cultivation

Crust samples were wetted with sterile water and the uppermost layer was torn apart, mounted in water on a microscope glass slide and observed using transmitted light, phase contrast and fluorescence microscopy. Different cyanobacterial morphotypes were identified and photographed. Three crust samples from each location were observed microscopically to ensure a good overall representation of the resident morphotypes. Morphological identification was performed in accordance with traditional phycological (Geitler, 1932; Komárek & Anagnostidis, 1999, 2005) and bacteriological (Castenholz, 2001) systems, while awaiting further confirmation by molecular sequencing.

Small pieces from the crust samples were placed on liquid and solid BG11 medium with and without $1.5 \, \mathrm{g \, L^{-1}}$ $NaNO_3$ as the only source of nitrogen other than N_2 (Rippka *et al.*, 1979). Unicyanobacterial cultures were obtained after several transfers to fresh media. All cyanobacterial cultures were incubated at a temperature of 35 ± 5 °C under natural sun light. Monoclonal cyanobacterial cultures were obtained by separation on an agarose-solidified BG11 medium.

Molecular analysis

The microbial communities within the three crust samples were investigated using 16S rRNA gene clone library construction. The upper 1–2 mm of triplicate samples (*c.* 300–500 mg each) were subjected to nucleic acid extraction as described previously (Abed & Garcia-Pichel, 2001). The 16S rRNA genes were PCR amplified using the primers GM3F and GM4R (Muyzer *et al.*, 1995) at an annealing temperature of 42 °C. The PCR products were purified using the QIA quick PCR purification kit (Qiagen, Hilden, Germany) and were cloned using the TOPO TA Cloning Kit

Fig. 1. A map depicting the location of the Sultanate of Oman in the context of other countries in the Arabian Penninsula and the distance between the crust sampling sites WK, SD and AD.

(Invitrogen, Karlsruhe, Germany) according to the manufacturer's instructions. The obtained clones were screened for the presence of inserts and the positive clones were then sequenced. The sequences were submitted to the GenBank and their accession numbers are GU362206–GU362412.

Phylogenetic analysis and biodiversity indices

The clone sequences were analyzed using the ARB program (Ludwig *et al.*, 1998). Phylogenetic trees were calculated using maximum parsimony based on long 16S rRNA gene sequences (> 1300 bp). The sequences obtained were then inserted into the reconstructed tree by applying parsimony criteria, without allowing changes in the overall topology.

For determining the number of operational taxonomic units (OTUs), similarity matrices among the sequences were calculated using the ARB program. Sequences that have > 97% similarity are defined as one OTU. Rarefaction curves were constructed using the freeware program ARARE-FACTWIN (available at http://www.uga.edu/~strata/software/Software.html). The coverage of the clone libraries, species richness, species evenness and the Shannon–Weaver index of diversity were calculated as described previously in Atlas & Bartha (1998) and Singleton *et al.* (2001).

Pigment analysis

Triplicate subsamples from the top 2–3 mm of the studied crusts were used for pigment extraction. Extraction of the pigments was performed with ice-cooled 90% acetone after sonication and incubation at $-20\,^{\circ}C$ for 24 h. The supernatants were filtered through 0.45 μm Acrodisc® CR 4-mm syringe filters (Pall Gelman Laboratory). The whole procedure was carried out on ice under dim light. The pigments were analyzed using reverse-phase HPLC that consisted of a Waters 996 photo diode array detector and a Waters 2690 separation module (Waters, MA). A 125×4.6 mm vertex column packed with Eurospher-100 C18 of 5 μm particle size was used (Knauer GmbH, Berlin, Germany). The pigments were identified by comparing the retention time and the spectrum with commercially available pigment standards: chlorophyll *a*, lutein, chlorophyllid *a* and β-carotene (DHI Water and Environment, Denmark).

Acetylene reduction rates

Nitrogenase activity was estimated using an acetylene reduction assay (Steward *et al.*, 1967) in the light and in the dark. Triplicate air-dried crust samples ($1\,cm^2$ each) were placed in glass bottles of 10 mL total volume, after wetting with 2 mL distilled water. The samples were not wetted again throughout the experiment. The bottles were sealed with rubber stoppers and aluminum crimps. Ten percent of the headspace volume was replaced by acetylene and the bottles were incubated for 10 h outside under $35\,^{\circ}C$ and a light intensity of 66 900 lx. In the case of the dark incubation, the bottles were also kept outside after wrapping them with an aluminum foil. Ethylene concentration was measured after 10 h by injecting 500 μL from the headspace into a gas chromatograph (Shimadzu, Japan) equipped with a flame

ionization detector. Nitrogen was used as a carrier gas and the following temperatures were set for the GC (injector, 100 °C, detector, 120 °C and column, 100 °C). Calibration was performed using a range of different ethylene gas concentrations prepared by mixing pure ethylene with synthetic air. Acetylene reduction activity was expressed as μmol ethylene $m^{-2} h^{-1}$. The triplicate values of acetylene reduction rates from the three localities were pooled to yield a sample size sufficient for statistical analysis ($n = 9$ for each treatment). Means were compared using an unpaired t-test with Welch's correction for unequal variances.

Results

Soil characteristics, direct microscopy and cultivation of cyanobacteria

The soils at WK were coarser (loamy sand) than those at SD and AD, being composed mainly of medium and finer sand particles. Soil at this locality also had more dissolved salts as indicated by the higher electrical conductivity. Soil at SD and AD was finer grained, having a much larger fraction of silt and less dissolved salts. Alkalinity was greater at AD (pH 8.7; Table 1). At all three sites, the surface layer consisted of a thin (1–2 mm) patina of very fine-grained particles and below this was a vesicular horizon comprised of larger-grained soil aggregates interspersed with large air spaces.

Different filamentous cyanobacteria were observed by direct microscopy of wetted crusts (Fig. 2a–d), with the nonheterocystous *Microcoleus vaginatus* being the most abundant cyanobacterium in all the samples (Fig. 2a). The other cyanobacterial morphotypes could be taxonomically assigned to the botanical genera *Scytonema* (Fig. 2b), *Leptolyngbya* (Fig. 2c) and *Lyngbya* (Fig. 2d). More cyanobacterial types were observed in cultures with and without nitrate (Fig. 2e–m). *Microcoleus vaginatus* cultures (Fig. 2e) grew in BG11 regardless of the presence of nitrate. Filaments and colonies of morphotypes corresponding to the heterocystous genera *Scytonema* (Fig. 2f and g) and *Nostoc* (Fig. 2j and k) were readily cultured from all crusts. While *Scytonema*-like cyanobacteria exclusively grew without nitrate,

Nostoc-like types exhibited growth, regardless of nitrate presence. On BG11 without nitrate, the *Nostoc* strains formed dark green ovoid colonies or pearls. Cultures of thin filamentous cyanobacteria with *Leptolyngbya*-like morphotypes (Fig. 2h and i) and unicellular *Xenococcus*-like (Fig. 2l) and *Chroococcus*-like (Fig. 2m) cyanobacteria could be detected.

Bacterial diversity in the crusts

Triplicate samples displayed a similar denaturing gradient gel electrophoresis pattern (data not shown), implying the presence of a homogenous community of cyanobacteria within each site. Cloning of 16S rRNA genes using universal bacterial primers (i.e. GM3 and GM4) provided detailed insights into the bacterial community structure of the three crusts (Table 2 and Fig. 3). The rarefaction curves showed that our clone libraries could not reveal all different genotypes in our crusts (Fig. 3, bottom) and the homologous coverage was between 48% and 62% (Fig. 3, top). A total of 265 sequences were obtained and were distributed among 112 OTUs (Fig. 3, top). The phylogeny and % distribution of the sequences among the clone libraries of the three crust samples are described below.

Cyanobacteria

Cyanobacterial clones constituted between 77% and 81% of the total clones distributed among 78 OTUs. Around 34% of the total cyanobacterial sequences formed a unique cluster that did not share any similarities to any known sequences in the database (> 8% sequence divergence to the closest sequence of *Scytonema hofmanni*). Sequences belonging to this group were found at all sites (Fig. 4). The remaining sequences were distributed among the groups *Oscillatoriales*, *Scytonematales*, *Nostocales* and *Chroococcales* (Fig. 4). Sequences related to *M. vaginatus* were retrieved from all samples. *Barsilonema*-related sequences constituted around 20% of the cyanobacterial clones in SD and AD crusts, but only 2.7% in the WK sample (Table 2). A large group of sequences, comprising between 10% and 36% of the total clones, was found in all clone libraries and fell next to

Table 1. Geographical location and soil characteristics of crusts from the Sultanate of Oman

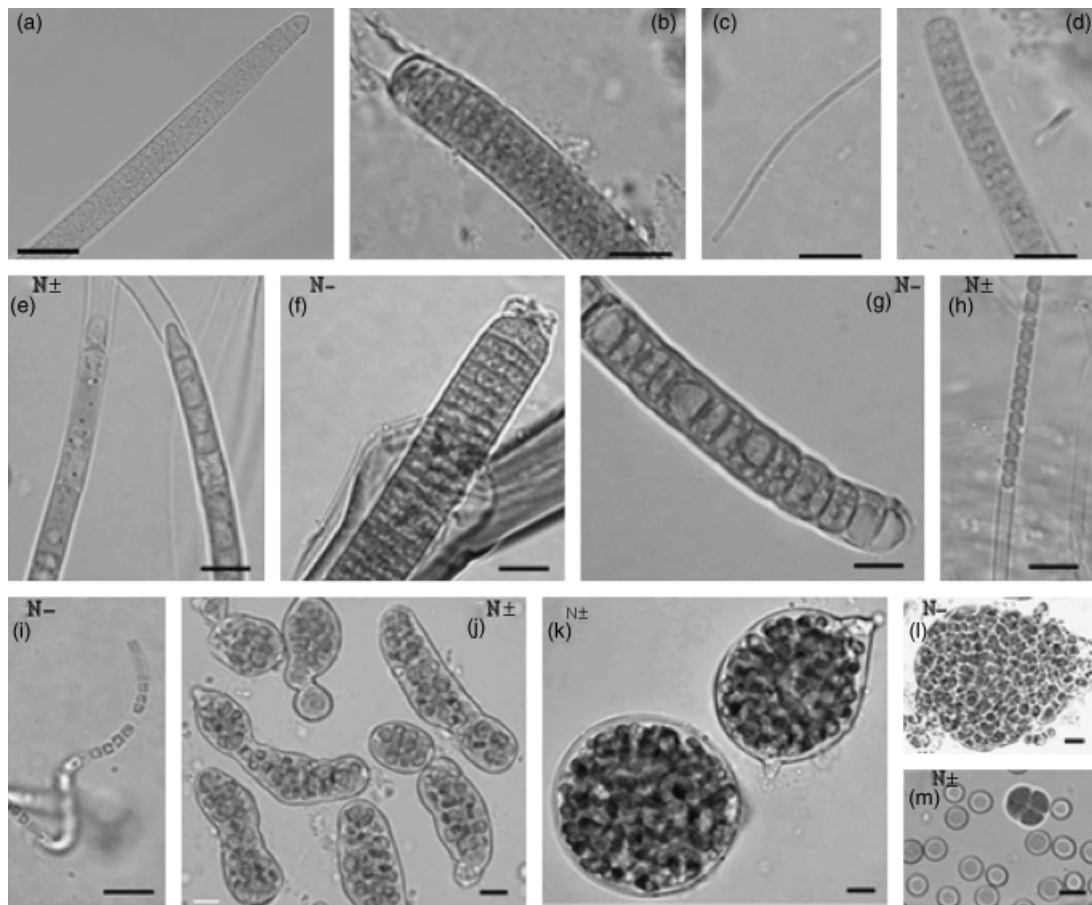| Sampling site | Location | Soil texture (%) | | | | Sand fractions in mm (%) | | | | | | EC (dS m^{-1}) | pH |
		Silt	Clay	Sand	Texture	2	1	0.5	0.25	0.053	< 0.053		
WK	23°4.595′N 58°9.055E	16	10	74	Loamy sand	0.2	24	18.3	15.7	25.4	16.4	2.89	7.6
SD	23°36.115′N 58°5.574′E	50	12	38	Silt loam	0.05	15.5	13.5	9.7	40.85	20.4	1.26	7.9
AD	22°22.842′N 57°30.550′E	44	30	26	Silt loam	0.1	2.8	26.9	16.9	39.5	13.8	1.65	8.7

Fig. 2. Photomicrographs of field (a–d) and cultured (e–m) filamentous and unicellular morphotypes of cyanobacteria. Scale bar = 10 μm. N$^+$ and N$^-$ indicate the growth in the presence and absence of nitrate. Morphotypes correspond to *Microcoleus vaginatus* (a and e), *Scytonema*-like (b, f and g); *Leptolyngbya*-like (c, h and i); *Lyngbya*-like (d); *Nostoc*-like (j, k); *Xenococcus*-like (l); and *Chroococcus*-like (m) cyanobacteria.

sequences of the genus *Scytonema*. Sequences affiliated to the genera *Chroococcidiopsis* and *Crinalium* were retrieved from the three clone libraries with a maximum occurrence of 7% and 9% of the total cyanobacterial clones, respectively. *Nostoc*-related sequences were found in the WK, but not in the SD and AD clone library, whereas sequences related to *Petalonema* were found in the WK and AD, but not in the SD clone library. In the WK and AD crusts, 12 clones fell next to the sequence of *Microcoleus* spp. DAI (Siegesmund *et al.*, 2008). The remaining cyanobacterial clones were distributed among the genera *Phormidium*, *Leptolyngbya* and *Symploca*.

Proteobacteria

The crust samples exhibited slight differences in the composition of the *Proteobacteria* group. While *Alphaproteobacteria* constituted 4–16% of the total clones, *Deltaproteobacteria* formed 2–12% (Table 2). No clones related to the *Gamma*- and *Betaproteobacteria* were found. The alphaproteobacterial

clones in the WK crust were related to the known UV-resistant *Deinococcus* spp., the crust bacterium *Belnapia moabensis* and a *Rhodospirillales* bacterium. In the SD and AD crusts, the alphaproteobacterial sequences belonged to the genera *Methylobacterium*, *Rhodovarius*, *Rubellimicrobium*, *Azospirillum* and *Paracraurococcus*. The deltaproteobacterial sequences closely resembled sequences from the genera *Cystobacter*, *Corallococcus*, *Hyalangium*, *Spirobacillus*, *Polyangium* and *Stigmatella*.

Bacteriodetes (CFB) and other bacterial groups

The remaining 4% of the clones were distributed among the groups *Bacteriodetes*, Candidate division, *Gemmatimonas* and *Planctomycetes* (Table 2). *Bacteriodetes*-related sequences were detected in the three clone libraries and belonged to *Rhodocytophaga*, *Adhaeribacter* and some uncultured environmental clones. The single sequence related to *Gemmatimonas* was found in the AD crust sample, whereas the single sequence of *Planctomycetes* was found in the WK sample.

Table 2. Relative percentage distribution of bacterial taxa and cyano-bacterial genera among the three studied crust samples as revealed by 16S rRNA gene-based clone libraries

Bacterial group	% of the total clones		
	WK	SD	AD
Alphaproteobacteria	4.1	15.9	5.1
Deltaproteobacteria	10.3	1.4	12.1
Bacteriodetes	4.1	1.4	2.0
Gemmatimonas	0.0	0.0	1.0
Diatom	1.0	0.0	0.0
Candidate	1.0	0.0	3.0
Planctomycetes	1.0	0.0	0.0
Cyanobacteria	78.4	81.2	76.8
Cyanobacterial group	% of cyanobacterial clones		
	WK	SD	AD
Microcoleus/LPP group*	14.7	16.1	11.8
Chroococcidiopsis	9.3	1.8	5.3
Crinalium	8.0	16.1	2.6
Brasilonema	2.7	21.4	19.7
Nostoc	2.7	0.0	0.0
Scytonema	10.7	16.1	35.5
Petalonema	1.3	0.0	1.3
Others	50.7	28.6	23.7

*LPP refers to the *Leptolyngbya/Plectonema/Phormidium* group.

	WK	SD	AD
Number of clones	97	69	99
Number of total OTUs	37	36	39
Number of cyanobacterial clones	76	56	76
Number of cyanobacterial OTUs	26	29	17
Number of phyla	7	4	6
Coverage (%)	61.9	47.8	60.6
Species richness	18.2	19	19
Shannon–Weaver index	4.47	4	4.5
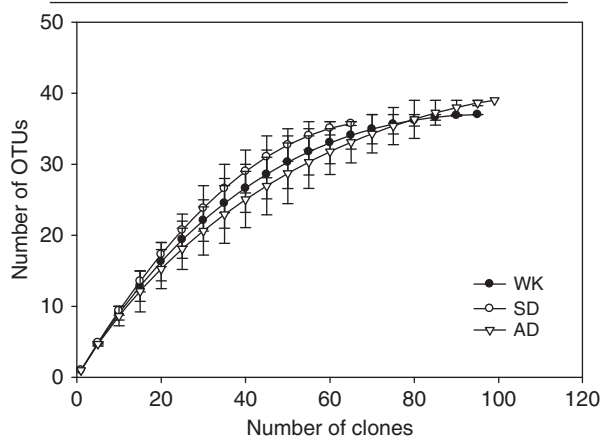
Fig. 3. Diversity-richness measures and features of the crusts' clone libraries (top). Calculated rarefaction curves of observed OTUs (sequences that have > 97% similarity are defined as one OTU) richness among the clone libraries (bottom).

Pigment analysis and acetylene reduction rates

Five pigments (chlorophyll *a*, scytonemin, chlorophyllid *a*, lutein and β-carotene) were detected in all crusts (Fig. 5). Chlorophyllid *a* is a known precursor of chlorophyll *a* and all these pigments originate mainly from oxygenic phototrophs. The concentration of each pigment was comparable and did not vary much among the samples. The concentrations of lutein were higher than the concentrations of chlorophyll *a*; however, this relationship was statistically significant only in the case of the AD sample. The highest concentrations were obtained for the pigment scytonemin and reached two to six times higher than chlorophyll *a*. Chlorophyllid *a* had concentrations comparable to chlorophyll *a*.

The nitrogenase activity of the crusts, assessed by acetylene reduction rates, was measured in the light and in the dark. By monitoring several dry samples, nitrogen fixation was found to start 3 h after wetting, and maximum rates were measured after *c.* 10 h of incubation. Acetylene reduction rates were comparable among the three sites, regardless of their geographical location. The highest rate measured was $58.5 \pm 2.6 \, \mu\text{mol} \, C_2H_2$ reduced $m^{-2} \, h^{-1}$. The rates for light and dark incubations averaged 39.4 ± 13.4 and $55.4 \pm 3.5 \, \mu\text{mol} \, C_2H_2$ reduced $m^{-2} \, h^{-1}$, respectively. The average acetylene reduction rate was significantly greater under dark conditions ($t = 2.835$; $P < 0.05$), although there was no significant difference between the light and the dark rates for individual samples.

Discussion

The crusts studied shared more similarities than differences in their microbial community composition, pigment distribution and nitrogenase activity, although they originate from different geographical locations. The three sites had a comparable abundance of phototrophs, as assessed by chlorophyll *a*, and most of their cyanobacterial clones fell into the same phylogenetic groups. In spite of the crusts' similarities, which could be attributed to similar environmental settings, differences in the alphaproteobacterial sequences, the presence of *Nostoc*-, *Bacteriodetes*-, *Planctomycetes*- and *Gemmatimonas*-related sequences in some crusts, but not in others, and the percentage distribution of *Scytonema*-and *Basilonema*-related sequences were observed. The distribution and abundance of crustal microorganisms is known to vary in relation to the soil chemistry, texture, topography, vegetation and disturbance (Ullmann & Büdel, 2001; Nagy *et al.*, 2005). Therefore, the silty texture of SD and AD samples vs. the loamy sand texture for sample WK, the presence of vegetation in the WK sample, but not in SD and AD, and the differences in pH are all parameters that might speak for such differences in the microbial composition of the crusts.
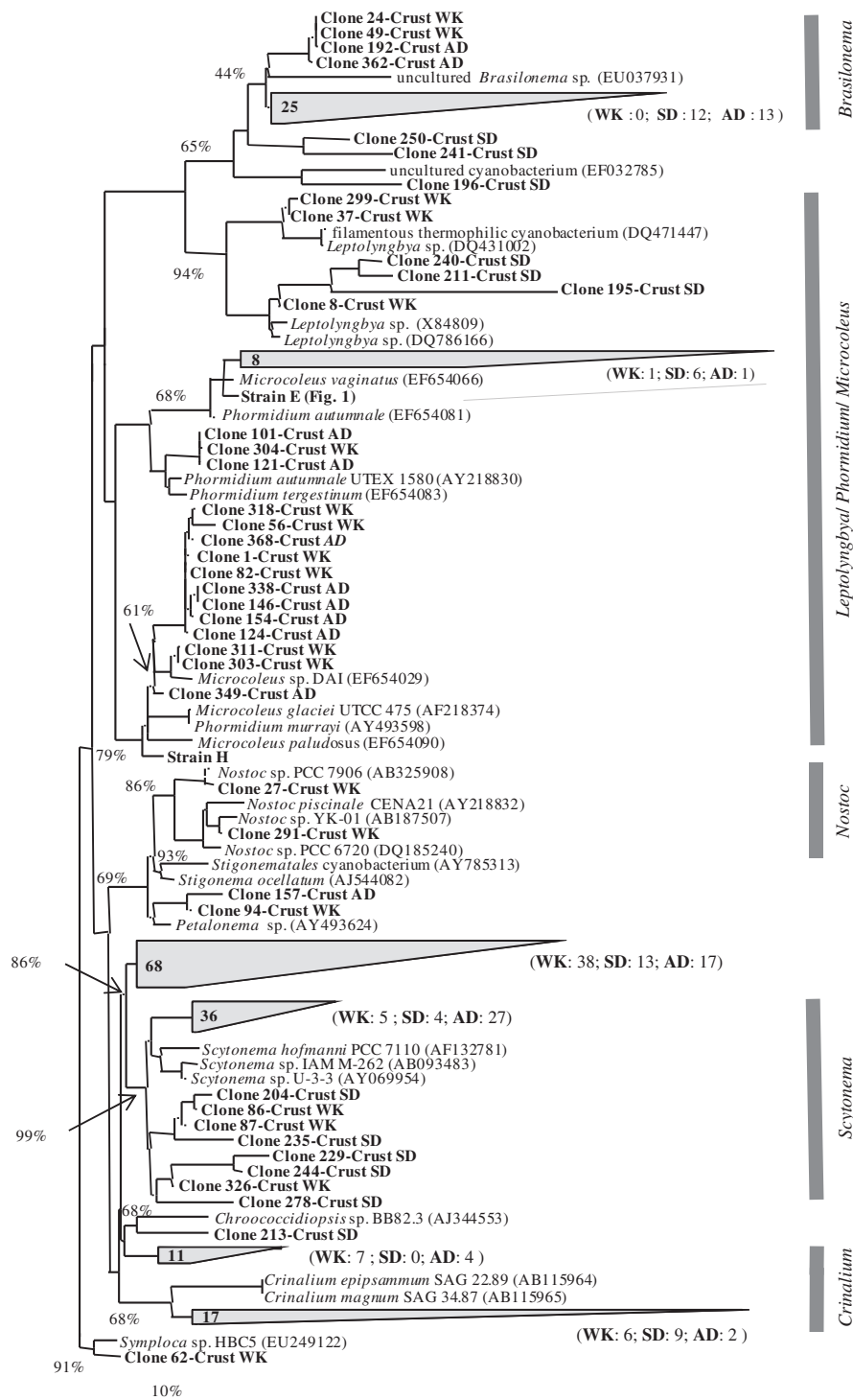
Fig. 4. Maximum likelihood phylogenetic reconstruction of the cyanobacteria based on publicly available, almost complete 16S rRNA genes from members of the cyanobacterial line. Clone sequences were analyzed using parsimony criteria without changing the topology of the pre-established tree. The bar indicates 10% sequence divergence. The bold number within wedges indicates the total number of clones within that cluster and the numbers in brackets indicate the distribution of these clones among the three clone libraries. Bootstrap values from 1000 trees are included and indicated as % at relevant nodes. All sequences have been submitted to GenBank under the accession numbers (GU362206–GU362412).

Cyanobacteria constitute the major component of the prokaryotic community in Oman's crusts. Between 24% and 59% of the cyanobacterial 16S rRNA gene sequences showed < 92% similarity to the closest sequences in the database, suggesting their novelty. Our crusts seemed to be more diverse than those of Colorado Plateau and Sonoran Desert (Nagy *et al.*, 2005; Reddy & Garcia-Pichel, 2006) as indicated by the number of phylotypes (37–39 vs. 10–32 and 12–25 phylotypes per sample, respectively) and Shannon diversity indices (4–4.5 vs. 2.1–3.3 and 2.04–3.02, respectively). Microscopically, *M. vaginatus* was the most abundant cyanobacterium in our crusts. This cyanobacterium,
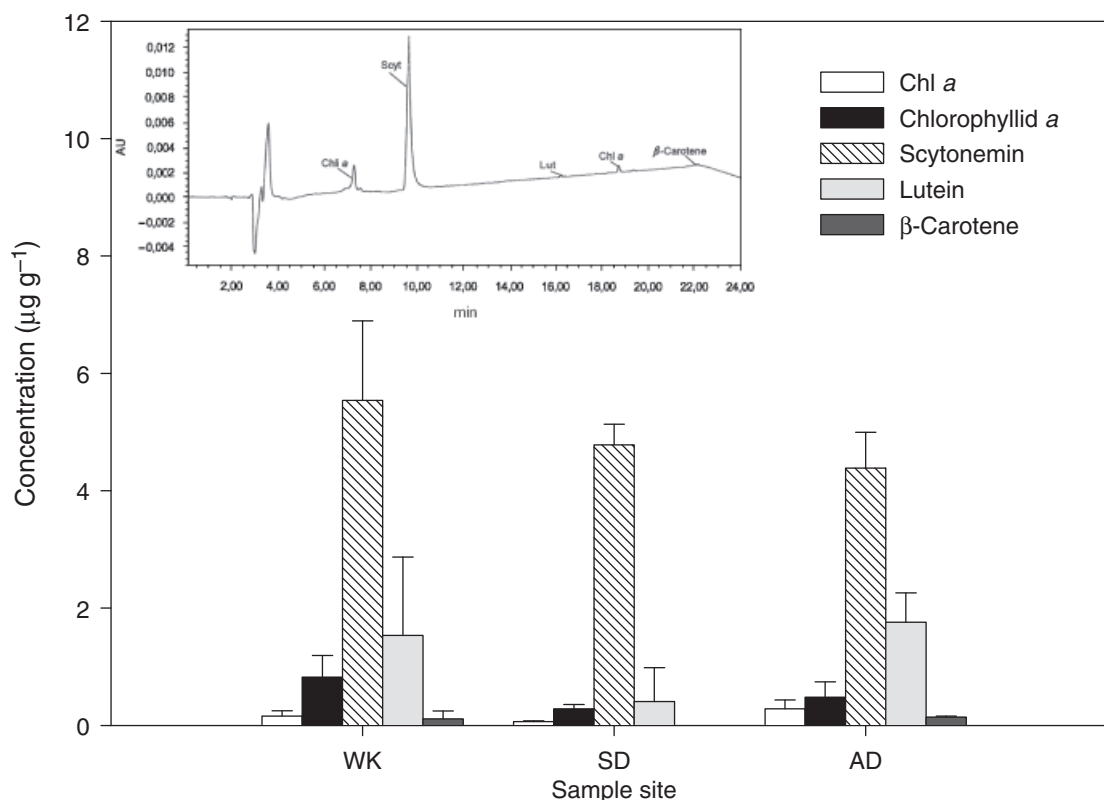
Fig. 5. Distribution of intracellular and extracellular pigments among the three crusts analyzed in triplicate. Note that the concentrations of scytonemin are much higher than those of chlorophyll *a*. The insert shows a representative HPLC chromatogram.

along with *Microcoleus steenstrupii*, also dominated crusts in the Colorado Plateau and the Sonoran Desert (Nagy *et al.*, 2005; Reddy & Garcia-Pichel, 2006). The existence of *M. vaginatus* in many crusts (Belnap & Gardner, 1993; Garcia-Pichel *et al.*, 2001) supports the assumption that this cyanobacterium is likely cosmopolitan in distribution. Many of the detected cyanobacterial clones in our crusts tend to belong to typical known crust types such as *Nostoc*, *Scytonema*, *Crinalium* and *Chroococcidiopsis* (Whitton *et al.*, 1979; Winder *et al.*, 1990; Garcia-Pichel *et al.*, 2001). These cyanobacteria are highly adapted for life in light-intensive arid environments through the production of the UV-sunscreen scytonemin, which protects them and the micro-organisms beneath them from excessive UV irradiation (Garcia-Pichel & Castenholz, 1991; Ehling-Schulz & Scherer, 1999; Dillon *et al.*, 2002). The detection of high concentra-tions of scytonemin by HPLC confirms the finding of these cyanobacteria in our crusts. Exposure to periodic desicca-tion was shown to induce the synthesis of scytonemin in UVA-exposed *Chroococcidiopsis* and *Nostoc* strains (Fleming & Castenholz, 2007).

Oman's crusts are rich in heterocystous cyanobacteria, with around 37% of the total clones related to known heterocystic genera such as *Stigonema*, *Nostoc*, *Basilonema* and *Petalonema*. Species belonging to *Scytonema* and *Nostoc* were detected in nutrient-poor, arid and semi-arid crusts worldwide and contributed significantly to nitrogen fixation (Fritz-Sheridan, 1987; Dodds *et al.*, 1995; Potts, 2000; Yeager *et al.*, 2007). While *Brasilonema* was detected in crusts for the first time here, the heterocystous *Tolypothrix* and *Spirirestis* species, which dominated the Colorado Plateau crusts (Yeager *et al.*, 2007), were absent in our samples. *Brasilonema* is a plant epiphyte from tropical forests (Fiore *et al.*, 2007), but could have settled in crusts by winds carrying it from nearby plants. The considerable tolerance of heterocystous cyanobacteria to desiccation (Stal, 1995) and their possession of heterocysts, which enables them to fix nitrogen even in periods of peak photosynthesis, are competitive traits that allow this group to become the dominant diazotrophs in crusts from arid regions (Yeager *et al.*, 2007).

Most of the noncyanobacterial sequences were affiliated to heterotrophic bacteria known to grow in the presence of oxygen, which is abundant in the top 1–2 mm of crusts. *Alpha-* and *Deltaproteobacteria*, *Bacteriodetes*, Candidate division, the UV-resistant *Deinococcus* and *Gemmatimonas* bacterial groups were all found in other crusts (Rainey *et al.*, 1997; Nagy *et al.*, 2005; Reddy & Garcia-Pichel, 2006). The

crust bacterium *B. moabensis*, found in our samples, was isolated from the crusts of Colorado Plateau (Reddy *et al.*, 2006). Most deltaproteobacterial clones in our crusts belonged to *Myxobacteria* (such as *Myxococcus*, *Cystobacter* and *Stigmatella*), which produce mycospores. Mycospores are resistant to drying, UV radiation and heat, thus enabling these bacteria to survive desiccation (Madigan *et al.*, 2009). *Myxobacteria* were not detected in the crusts of Colorado Plateau and the Sonoran Deserts (Nagy *et al.*, 2005; Reddy & Garcia-Pichel, 2006). In contrast, the *Actinobacteria* group, which dominated these crusts, was surprisingly absent in our crusts. The absence of *Actinobacteria* was also reported in temperate acidic soil crusts from Cape Cod Seashore (Smith *et al.*, 2004). The presence and absence of *Actinobacteria* in different crusts could be due to differences in the deserts' environmental conditions.

Acetylene reduction rates were comparable for the three crusts, regardless of their geographical location and microbial community composition. The occurrence of nitrogen fixation in the light and in the dark at equivalent rates indicates a role of heterocystous cyanobacteria and heterotrophic bacteria, although the contribution of nonheterocystous cyanobacteria to nitrogen fixation cannot be excluded. A large fraction of nitrogen input in soil crusts was attributed to free-living aerobic heterotrophs (Billings *et al.*, 2003) and/or to those associated with cyanobactera such as *M. vaginatus* (Steppe *et al.*, 1996). We detected sequences affiliated to known nitrogen fixers such as *Azospirillum* in our crusts, suggesting that these heterotrophic bacteria may contribute to nitrogen fixation. Higher rates of nitrogen fixation were reported in crusts when heterocystous cyanobacteria were present (Johnson *et al.*, 2005). Studies on several heterocystous soil cyanobacteria including *Nostoc commune* showed that these strains exhibited higher rates of nitrogen fixation in the dark than in the light (Jones, 1989; Hrouzek *et al.*, 2004). Light-independent nitrogen fixation was also shown to occur at significant rates in the lower layers (> 2.5 mm) of crusts from Colorado Plateau (Johnson *et al.*, 2005). The ability of crusts to fix nitrogen in the dark, after rewetting, may be important in hot deserts, where night temperatures can be 10 or more degrees cooler. Temperature is an important factor in controlling nitrogen fixation rates (Miyamoto *et al.*, 1979; Breitbarth *et al.*, 2006).

In our samples, nitrogenase activity (acetylene reduction) started within 3–6 h after rewetting. Similar observations were made in desiccated Sahelian soils in Niger (Issa *et al.*, 2001) and crusts of Nigerian Savanna (Isichei, 1980). Cultures of *Nostoc* spp. that were subjected to 2 years of dryness needed *c.* 15 h to start fixing nitrogen, and maximum rates were reached in 4 days (Scherer *et al.*, 1984). Our acetylene reduction rates were comparable to those measured in other desert ecosystems such as the Gurbantunggut Desert in Northwestern China (10 μmol C_2H_2 reduced $m^{-2} h^{-1}$; Wu *et al.*, 2009), the Colorado Plateau, southeast Utah, USA (20–100 μmol C_2H_2 reduced $m^{-2} h^{-1}$; Belnap, 2002a), and the Mojave Desert, Nevada, USA (76.9 $\pm$ 5.6 μmol C_2H_2 reduced $m^{-2} h^{-1}$; Billings *et al.*, 2003). The average annual nitrogen input of our crusts would be 5.5 $\pm$ 1 kg N_2 ha^{-1} $year^{-1}$ by assuming that nitrogen fixation occurs during 12 h day^{-1} and 100 days $year^{-1}$ (Isichei, 1980; Jeffries *et al.*, 1992) and by considering a conversion factor of 3 mol of ethylene produced for each mol of N_2 fixed (Belnap & Lange, 2001). This rate is twofold higher than those reported for crusts in southern Utah, USA (Jeffries *et al.*, 1992), and for Savannah regions, Nigeria (Isichei, 1980), using the same parameters for calculation.

Although Oman's summer climate is hotter and more arid and the winters are milder than more temperate deserts, similarities exist in their microbial composition and ability to fix nitrogen. However, it remains to be determined how greater temperature, light intensity and desiccation in Oman may constrain the physiological performance of these crust microorganisms.

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