

HYPOLITHIC CYANOBACTERIA COLONIZATION OF QUARTZ AT SOUTH DESERT

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ABSTRACT

Hypolithic microbial communities (found underneath rocks) were encountered in the southern desert of Qatar where quartz rocks are ubiquitous and are a substrate for hypoliths. These hypoliths thrive where extreme temperature, moisture stress and high light intensity limit the occurrence of higher plants and animal life. A small-scale ecological survey indicated that 80% of the quartz rocks in the area were colonized by green hypolithic microbial communities dominated by colonization of cyanobacteria. Using light and electron microscopic analyses; the cyanobacterial community structure of hypoliths was shown to be dominated by cyanobacteria affiliated to the genera *Chroococcidiopsis*, *Aphanothece*, *Pleurocapsa*, *Oscillatoria*, *Lyngbya*, *Leptolyngbya*, *Phormidium* and *Scytonema*).

Keywords: Qatar, Hypolithic, Quartz, Cyanobacteria, *Chroococcidiopsis*

1. INTRODUCTION

The State of Qatar is a peninsula located between 24° 27' and 26° 10' north and 50° 45' and 51° 40' east (Fig. 1). It is 185 km long and 85 km wide, covering an area of 11,437 km². Rocky hills and sand dunes are found in the southern parts of the country. According to its geographical location and climate, Qatar is classified as a hot subtropical desert; very hot and humid between June and August and pleasant and cool between November and February. Average annual rainfall recorded is 81 mm. The average maximum temperature is 31°C and average minimum temperature is 22°C. The morning humidity averages 71% and the afternoon relative humidity 43%. As Qatar is subjected to an arid climate, it mainly possesses an arid soil, with coarse texture, shallow depth and low retention of soluble substances. The level of salinity is extremely high in the soil and in the ground water and that is attributed mainly to its flatness low altitude.

The Qatar peninsula lies on the broadest part of the interior platform of the Arabian shelf. This platform is extended through much of geological time, from southern Iraq, through eastern Saudi Arabia and Qatar. It is typified by a flat-lying relatively thin sequence of

sediments laid down upon gradually subsiding basement (Ziegler, 2001). Qatar lies on an extension of the central Arabian Arch, a basement ridge that extends in an ENE direction through the Summan area. A continuation of this feature is thought to be responsible for the probable extension of the interior platform into parts of Iran north of Qatar. The known stratigraphic section in Qatar is consists of three geological ages: The Paleozoic, the Mesozoic and the Cenozoic (Nairn and Alsharhan, 1997).

Hypolithic microbial communities appear as a layer of growth attached to the underside of various translucent rock types, including flint, limestone, gypsum and sandstone (Azua-Bustos *et al.*, 2011). However, the majority 2 are associated with translucent quartz rocks which allow transmission of sufficient light to support photosynthesis at a depth where other stressors such as moisture availability, UV irradiance and substrate stability are sufficiently minimized to allow the development of a microbial community (Cary *et al.*, 2010; Chan *et al.*, 2012; Pointing and Belnap, 2012). Microbial communities include different types of prokaryotic, bacteria and archaea and eukaryotic, fungi algae and mosses, microorganisms. Archaea have, until recently, been thought to be absent in both hot and cold desert

hypoliths, as repeated attempts to detect archaeal rRNA genes using molecular methods have been unsuccessful (Warren-Rhodes *et al.*, 2007; Pointing *et al.*, 2009). Therefore, very little is known of their ecological role.

As hypolithic communities are primarily photosynthetic, comprising mainly cyanobacteria and unicellular algae, sufficient translucence of the overlying rocks allow for adequate light penetration which is essential (Hu *et al.*, 2012). Cyanobacteria-dominated hypolithic communities have been described in cold deserts (Pointing *et al.*, 2009; Wong *et al.*, 2010; Cary *et al.*, 2010) as well as hot deserts (Warren-Rhodes *et al.*, 2007; Lacap *et al.*, 2011). Morphological studies have indicated that photoautotrophs, cyanobacteria, are dominant in most hypoliths studied to date from the Negev Desert, Antarctic Dry Valleys, Namib Desert, coastal Antarctic, Mojave Desert, Arctic tundra, Atacama Desert and Taklimakan Desert (Chan *et al.*, 2012). Cyanobacteria are oxygenic phototrophic prokaryotes which appeared 3.5-2.5 billion years ago and were responsible for introducing oxygen into the atmosphere of primitive Earth. Cyanobacteria has many strategies to tolerate and avoid environmental factors and stress, even in one organism; these strategies include, stress avoidance by gliding mechanisms, stress defense by synthesis of UV-absorbing compounds, antioxidant enzymes and molecules and DNA repair mechanisms, such as excision repair and photoreactivation (Castenholz and Garcia-Pichel, 2012).

Morphological studies indicated hot arid deserts support an apparent monoculture of *Chroococcidiopsis* sp. (Warren-Rhodes *et al.*, 2007; Pointing *et al.*, 2009) whilst in others, a more diverse range of taxa have been identified (Tracy *et al.*, 2010; Stomeo *et al.*, 2013). While polar regions supported a relatively more diverse cyanobacterial assemblage comprising various *Oscillatoriales*, *Aphanocapsa*, *Aphanothece*, *Chroococcidiopsis* and *Gloeocapsa* morphotypes (Zakhia *et al.*, 2008).

The objectives of this investigation are to survey location in the desert of Qatar for phototrophic hypolithic microbial communities and to obtain information about the nature of these microorganisms.

2. MATERIALS AND METHODS

2.1. Field Sampling

The three field locations investigated were at the Southern of Qatar region. These are characterized as hot and arid sandy deserts. Sampling was carried out in February and November 2013. A small-scale randomized

sampling was carried out by employing triplicate 1 m² quadrates deployed at three separate locations across an area of 100 m². Colonization was visually detected as a green band of microbial growth on the subsurface of white and translucent quartz rocks. For each quadrate, 10 colonized rocks were randomly selected. All rock samples were kept in sterile bags until further analysis.

2.2. Microscopy

Light microscopy and Scanning Electron Microscopy (SEM) was used to determine and identify the hypolithic microbial community, based on standards text books for cyanobacteria identification.

3. RESULTS

Distribution of quartz rocks which colonized with hypolithic microbial community were found at the three sites in South desert, Qatar (**Fig. 1**). Of all quartz rock types counted in these sites about 80% showed hypolithic colonization, as based on visual examination. Hypolithic colonization was only observed on quartz rocks (**Fig. 2**). The size of quartz stones at these sites varied from small (0.5-1 inch), medium (1-2 inches) and large (2-5 inches). As the size of the quartz rock increased, an associated increase in hypolithon colonization was observed. All medium quartz rocks were colonized.

Hypolithic diversity most of the quartz stones buried in the soil showed abundant colonization of hypolithic microorganisms that formed a mat up to 5 mm thick in some places (**Fig. 2c**). In some cases, the biofilm extended outwards from the underside surface of the quartz, with the Exopolysaccharide (EPS) produced by the cyanobacteria agglomerating the soil particles under the rocks and in some cases the soil appear as green color biofilm under the rock.

Figure 3 light microscope images showing coccoid and filamentous cells. **Figure 4** SEM images from hypolithic zone, (a) Overview showing coccoid cells. (b) Showing filamentous cells. (c) Heterotrophic bacteria cell. Observations by light (**Fig. 3**) and SEM (**Fig. 4**) of a well-formed biofilm of Extracellular Polysaccharides (EPS) produced by cyanobacteria were a significant component of these communities and covered the majority of sand particle surfaces.

The microscopy identification of hypolithic microorganisms composed of unicellular and filamentous cyanobacteria genera includes *Chroococcidiopsis* (as the primary component), *Aphanothece*, *Pleurocapsa*, *Lyngbya*, *Leptolyngbya*, *Oscillatoria*, *Phormidium* and *Scytonema*.

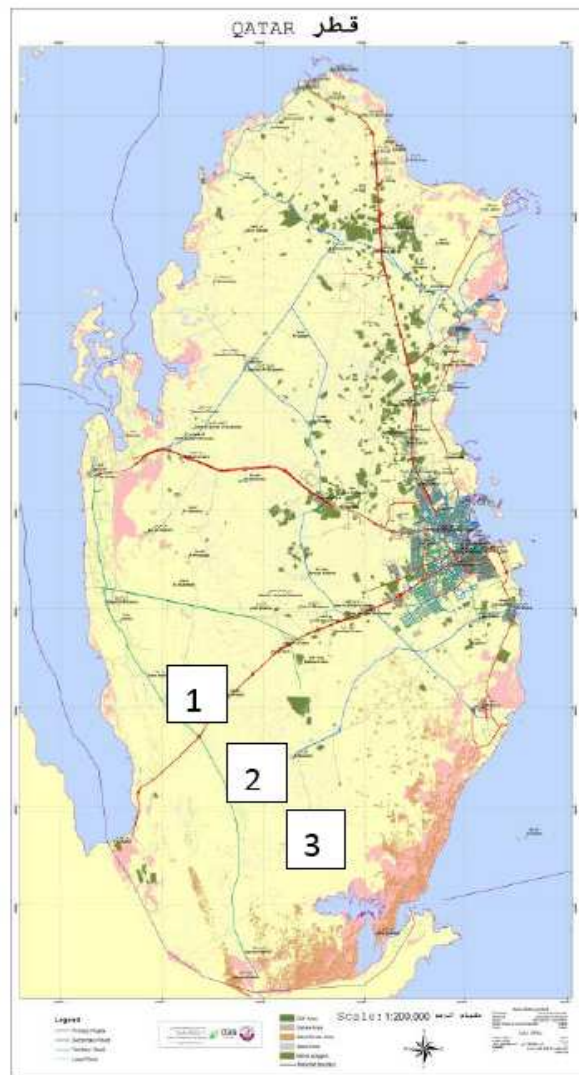


Fig. 1. Qatar map and the 3 location sites of the samples

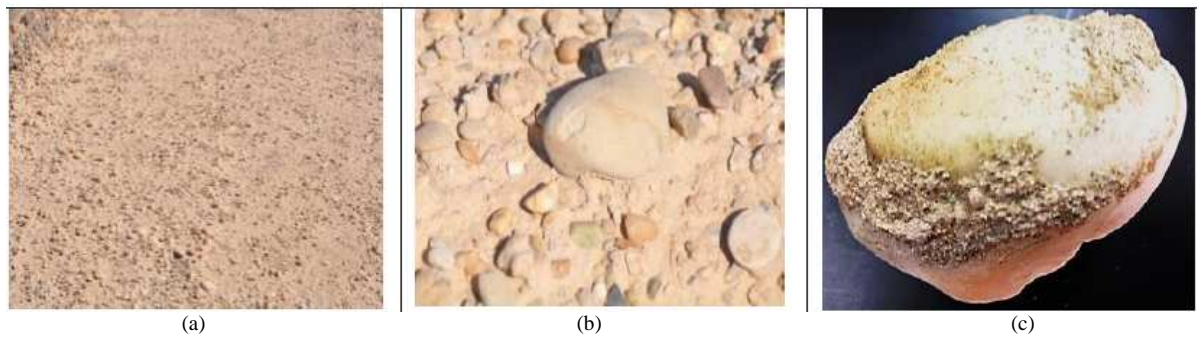


Fig. 2. Field of the quartz rocks a-b scale bar 10 cm and quartz rock colonized with hypolithic microbial community, scale bar 2 cm

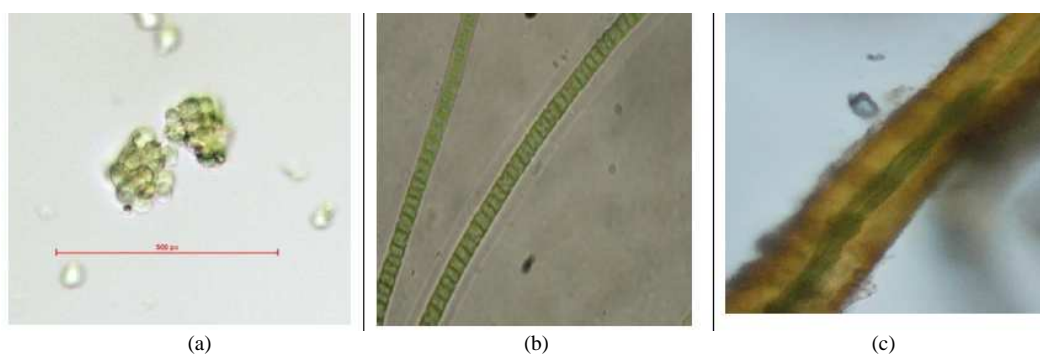


Fig. 3. Different types of cyanobacteria cells unicellular a and filaments b-c determined by light microscope

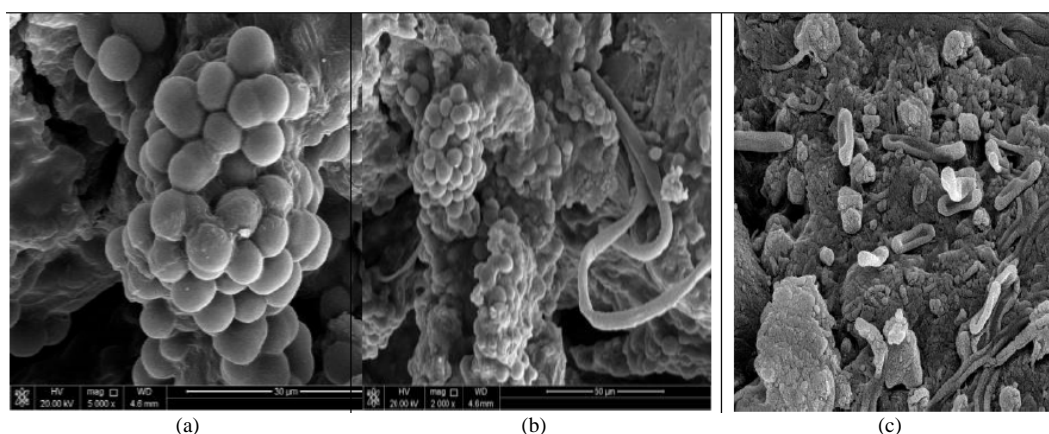


Fig. 4. (a) SEM images for *Chroocodiopsis* with polysaccharide sheath, (b) unicellular and filamentous cyanobacteria and (c) heterotrophic bacteria

4. DISCUSSION

Having surveyed many locations in Qatar, the hypolithic were only encountered in the south desert where translucent quartz rocks are widely dominate and distributed. The South desert of Qatar considered as dry because of the deficiency of rains water and high light intensity with high temperature in summer. A survey of three different sites in the sandy rocky south desert indicated that 80% of quartz rocks were colonized underneath by hypolithic. The frequency of hypolithic has been determined in a variety of hot and cold deserts to range from 0 to 100%, high frequencies of 95 and 94% were documented by Cockell and Stokes (2004) on Devon and Cornwallis Island also Schlesinger *et al.* (2003) reported that 100% of all quartz rocks in the southern Mojave Desert are colonized by photoautotrophs. Those kinds of rocks hold the atmospheric humidity underneath and allow the growth of this microbial community with their unique characteristics to survive.

Many studies (Cary *et al.*, 2010; Cowan *et al.*, 2010; 2011a) suggest that soil atmospheric humidity more sufficient than soil water content as requirement water for the growth of cyanobacteria and therefore influence the diversity, distribution and development of their communities. In cold deserts, large ranges in the colonization rates were observed depending on the impact of fog (Azua-Bustos *et al.*, 2011) and snowmelt (Pointing *et al.*, 2009) in addition to the precipitation influence (Azua-Bustos *et al.*, 2011). With decreasing precipitation and fog, frequency approached zero (Warren-Rhodes *et al.*, 2006). In Qatar desert, temperature could be an important limiting factor for hypolithic growth, as temperatures in excess of 50°C can vaporize all the available water. Cooler early morning or late evening hours may be preferred by such hypolithic communities for their metabolic activities as host rocks could absorb water formed by condensation of dew.

Microscopy studies indicated that cyanobacteria species dominated the hypolithic communities. This is

similar of some hyperarid Antarctic (Cowan *et al.*, 2011b) and the Atacama Desert hypoliths (Azua-Bustos *et al.*, 2011). The most common genera observed from south desert of Qatar were *Chroococcidiopsis*, *Aphanothece*, *Pleurocapsa*, *Oscillatoria*, *Lyngbya*, *Leptolyngbya*, *Phormidium* and *Scytonema* as found by Wong *et al.* (2010) the Tibetan Tundra hypoliths are colonised predominantly by *Chroococcidiopsis* and *Phormidium*-like taxa, followed by *Leptolyngbya*, *Nostoc* and *Oscillatoria* species, while Arctic hypoliths are dominated by *Gloeocapsa* and *Chroococcidiopsis*. The genus *Chroococcidiopsis* was observed to occur exclusively in the hypolithic environment, whereas *Microcoleus* and *Nostoc* were mainly found in soil-inhabiting crust samples in many location of Qatar.

Desert inhabiting *Chroococcidiopsis* species are capable of enter a dormancy state with desiccation onset and, resume metabolic activities when water becomes available (Grilli-Caiola and Billi, 2007). Viable cells of *Chroococcidiopsis* spp. were recovered from rocks from the Negev Desert after storage for 30 years (Grilli-Caiola *et al.*, 1996). Dried aggregates of *Chroococcidiopsis* include live and dead cells must either protect cellular structures from damage and/or repair them upon rewetting. Billi *et al.* (2000) demonstrated that the desiccation surviving cells were avoiding and/or limiting genome fragmentation, preserve intact plasma membranes and phycobiliprotein autofluorescence and exhibit spatially reduced reactive oxygen species accumulation and dehydrogenase activity upon rewetting. Extracellular Polymeric Substances (EPS) may contribute to cyanobacterial desiccation tolerance by providing a repository for water and by stabilizing desiccation-related enzymes and molecules (Flemming and Wingender, 2010). Thick envelopes have been reported to encapsulate *Chroococcidiopsis* cells both in laboratory-dried cultures (Grilli-Caiola *et al.*, 1996) and in natural samples, as in Chilean endoevaporites (Stivaletta *et al.*, 2012). Indeed, the properties, structures and functions of EPS have made bacterial biofilms the most successful form of life on Earth (Flemming and Wingender, 2010). Examination of our samples after 2 years showed a stage of dormancy, very thick polysaccharides sheaths and yellow scytonemin which is known as screen for high UV. *Chroococcus* and *Chroococcidiopsis* were previously shown to produce scytonemin as a UV sunscreen and the production of this pigment was shown to be stimulated by temperature, UV-A irradiation and periodic desiccation (Antony *et al.*, 2012).

The ability of *Chroococcidiopsis* and other filamentous cyanobacteria to resist and survive in arid environments is due in part to the fact that it colonizes

the underside of translucent rocks. The underside of these rocks provides enough condensed moisture for growth while the rock's translucent nature allows just enough light to reach the organism for photosynthesis to occur. In addition, they can withstand environmental stressors not currently met in nature, such as doses of ionizing radiation as high as 15 KGy (Billi, 2012). These features make them proper photosynthetic model organisms to appreciate life's tricks to withstand extreme desiccation on Earth and investigate the survival potential of terrestrial organisms in space or in other planets, such as Mars (Azua-Bustos *et al.*, 2012; Billi, 2012).

5. CONCLUSION

The study using microscopy methods demonstrated a heterogeneous distribution of the cells and the specific pigments as markers of phototrophic organisms in the hypolithic microbial community. Multiple survival strategies of the hypolithic population have been suggested. Besides being buried within the rock, the cells protect themselves from intense light and UV by producing UV-shielding pigments that become deposited in the exopolymers and may be the cause of the green autofluorescent spheres and envelopes surrounding the cyanobacterial microcolonies. The massive extracellular polymeric substances may also protect the cells from dehydration, adsorb water and nutrients and recycle the latter within the living hypolithic microbial community. Members of the genus *Chroococcidiopsis* are not the only photosynthetic prokaryotes in extremely dry deserts such as the Qatar desert. In Qatar desert, the hypolithic biomass is very important especially when the moisture is sufficient to conditioning and stabilization of desert soils for agricultural use and to reduce threats from dust storms. This study suggests that future investigations of hypoliths at Qatar desert need more investigation by culturing and molecular techniques to understand their taxonomy, physiology and ecology.

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