MICROBIAL COMMUNITIES FROM CONTINENTAL SABKHAS OF SOUTHERN TUNISIA: TERRESTRIAL ANALOGUES OF MARS EVAPORITE ENVIRONMENTS.

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Introduction: We are investigating the microbial communities and their biosignatures in sabkha environments and related evaporite deposits of southern Tunisia at the northern border of the Sahara Desert, where ephemeral salt lakes are locally called "chott". These saline environments are characterized by a high level of environmental instability, being under the influence of periodic salinity dilution during the wet season, whereas during the dry season thick salt (halite) crusts precipitate throughout the chott surface (Fig. 1). Artesian springs from the aquifer emerge at the chotts surface, especially in the Chott el Jerid, where they developed in Holocene-Recent time characteristic mounds [1] due to the precipitation of gypsum crusts in loose quartz sands (Fig. 2). In the nearby Chott el Gharsa laminated gypsum deposits of upper Pleistocene age also occur [2] (Fig. 3). In the chotts area, therefore, modern and fossil evaporite deposits allows a proper comparative investigation between living microbial communities and the ones preserved as subfossil and fossil in the mineral record.

Microbes in modern and subfossil evaporite deposits of the Chott el Jerid: Despite the stress caused by high salinity, optical microscope observation of superficial halite crusts, which precipitate in the Chott el Jerid basin during the dry season, have documented halophilic Archaea, cyanobacteria and green algae (Fig. 4). Morphologies related to cells of cyanobacteria (Fig. 5), purple bacteria and green algae were observed in colored millimeter-size bands at the top of the gypsum crusts of the spring mounds. Bacterial colonies also occur on the surface of quartz grains and in the interstial spaces, according to a cryptoendolithic mode of life, where they create biofilms, which bind the quartz grains together. Different degrees of mineralization recognized by SEM and EDX analyses were attributed to the microbial communities. Total environmental DNA from modern halite crusts of the Chott el Jerid and gypsum crusts of the spring mounds was extracted by a specific protocol [3] and resulted to be sufficiently pure to allow PCR (polymerase chain reaction) amplification of eubacterial 16SrDNA. Primer P0 (5' GAGAGTTTGATCCTGGCTCAG) and P6 (5'-CTACGGCTACCTTGTTACGA) were designed [4] on the basis of the conserved bacterial sequences at the 5' and 3' ends of the 16S rRNA gene (positions 27f and 1495r, respectively, on *Escherichia coli* rDNA).

Microbial signatures in fossil evaporite deposits of the Chott el Gharsa: The search of microbial morphologies in Chott el Gharsa deposits was performed by optical and SEM observations in laminated gypsum intervals of upper Pleistocene age. These morphologies are usually parallel to bedding and consist of thinly draping microlaminae a few micrometers thick. Some of these microlaminae consist of coalesced microfibers (diameter: 0.1 μm), arranged as dense bundles interpreted as polysaccharide microfibers as they appear after the partial degradation of bacterial mucilaginous cell walls (Fig. 6). Their mineral composition is a prevalence of gypsum, dolomite and pyrite framboids. The biogenic (microbial) origin of this dolomite is documented by the dumbbell shape in the hollow cores of numerous dolomite crystals. Dumbbells and dumbbell-shaped hollow crystals are bacterial-related morphologies [5,6], and dolomite dumbbells have been described in natural and experimental conditions driven by sulfate-reducing bacteria [7,8]. The presence of iron sulfide (pyrite framboids) also points to microbial processes. In modern evaporite environments the formation of pyrite is commonly driven by bacteria.

Implications: The presumed origin of the Martian sulfate deposits from the evaporation of hypersaline waters points to the astrobiological relevance of the evaporites, including their terrestrial analogues, such as coastal lagoons, sabkhas, and salt lakes [9]. This relevance also depends on the strongly harsh and stressed conditions of hypersaline environments that can mimic alien life conditions. The area of the Tunisian chotts offer the advantage of presenting modern, subfossil and fossil continental sabkha environments, in which live-fossil comparative investigations and evaluations of the preservation potential of the microbial signatures can provide a useful background for an assessment of the astrobiological potential of common evaporite minerals.

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Figure 1 - Halite crusts from a water evaporating pond of the Chott el Jerid. The red color is primarily from carotenoids.

Figure 2 - The top of a spring mound with gypsum precipitation (arrow).

Figure 3 - Gypsum deposits (Pleistocene) of the Chott el Gharsa road-cut section with a regularly layered and cyclic organization.

Figure 4 – Cells of the green algae *Dunaliella* from the halite crusts showed in figure 1.

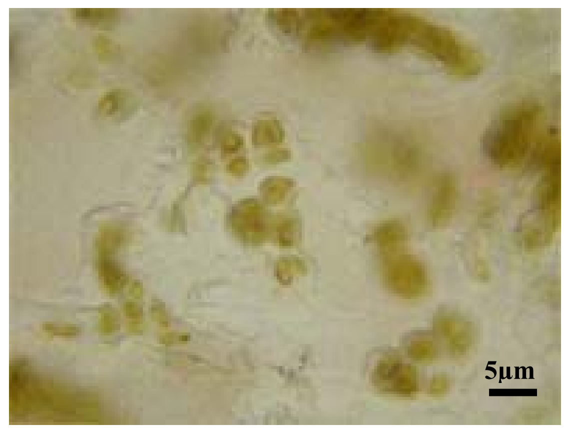

Figure 5 - Morphologies interpreted as cyanobacterial cells embedded in a extracellular polymeric substance (EPS) from gypsum crusts of spring mounds of figure 2.

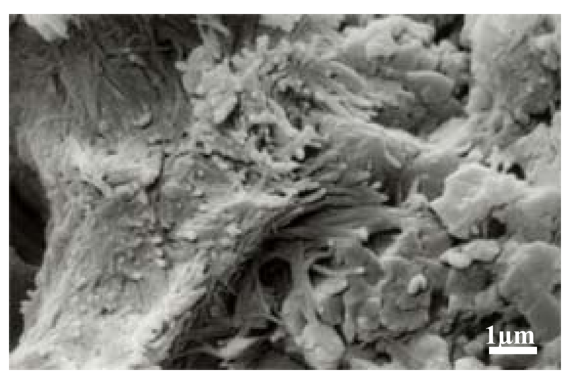

Figure 6 – SEM micrograph from a microcrystalline gypsum lamina showed in figure 3. These structures are interpreted as degraded polysaccaride microfibers from bacterial colonies.