



## Microbial diversity on the Tatahouine meteorite

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**Abstract**—Biological processes can alter the chemistry and mineralogy of meteorites in a very short time, even in cold or hot deserts. It is thus important to assess the diversity of microorganisms that colonize meteorites in order to better understand their physiological capabilities. Microscopy observations of Tatahouine meteorite fragments that were exposed for 70 years in the Sahara desert showed that they were colonized by morphologically diverse biomorphs. A molecular diversity study based on 16S rRNA gene amplification of DNA supported the conclusion that a huge taxonomic diversity of prokaryotes colonized the Tatahouine meteorite in less than 70 years in the Tatahouine sand. Eleven different bacterial divisions were evidenced, among which *Cytophaga-Flexibacter-Bacteroides* (CFB), Cyanobacteria, and Alpha-Proteobacteria were dominantly represented. Crenarcheota were also detected. Most of the Tatahouine meteorite phylotypes were related to sequences identified in the surrounding Tatahouine more generally to sequences detected in soils. Some of them, in particular many of the archaeal phylotypes, were detected in arid regions in association with desert varnish. The results suggest that the diversity of the clone library generated from the meteorite fraction was reduced compared with that of the Tatahouine sand clone library, which can be explained as the result of partial colonization of the meteorite and/or a specific selection of colonizing bacteria by the substrate. We discuss the possibility that several groups detected in this study may play a prominent role in the various alteration processes detected at the surface of the Tatahouine meteorite.

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### INTRODUCTION

Most meteorites are collected days, years, or sometimes thousands of years after their fall. During such periods, the genuine information contained in their mineralogical and chemical features can be overprinted by terrestrial contamination (e.g., Gounelle and Zolensky 2001; Kohout et al. 2004). It is thus of great importance to investigate the terrestrial processes that affect meteorite chemistry in order to restore the pristine message they carry about their petrological history. Many studies have characterized the terrestrial modifications in Antarctic (e.g., Gooding 1986; Jull et al. 1988; Mittlefehldt and Lindstrom 1991; Velbel et al. 1991) and in Saharan meteorites (e.g., Ash and Pillinger 1995; Barrat et al. 1998; Barrat et al. 1999; Bland et al. 1996; Bland et al. 1998). For example, Ash and Pillinger (1995)

have evidenced the loss of organic material in Saharan chondrites. Although they have proposed inorganic processes to explain this loss, microbial activity could have been suggested as well. To date, assessment of the microbial diversity present on “find” meteorites has almost never been performed in studies on meteorite weathering, with the exception of Steele et al. (2000), who carried out microscopy observations, and Cockell et al. (2002), who, with a different perspective, studied the microbial communities colonizing shock-metamorphosed rocks in the Haughton impact structure. Yet, even in so-called extreme environments like Antarctic and Sahara deserts, microorganisms are relatively abundant and diverse (e.g., Wynn-Williams 1996; Steinberger et al. 1999; Garcia-Pichel et al. 2001; Cockell et al. 2001); they can colonize meteorites (Jull et al. 1998; Steele et al. 2000) and modify the chemical and mineralogical

composition of meteorites by mediating diverse weathering and biomineralization reactions (Benzerara et al. 2005a, 2005b). It is thus important to study the diversity of microorganisms that have colonized find meteorites to evidence the taxa that may be involved in alteration processes. Moreover, studying the microbial diversity on meteorites can sometimes help to date the fall of these extraterrestrial objects. Their study can also help to constrain the timing of natural microbial colonization of sterile mineral surfaces in specific environments.

We have characterized the diverse microbes that colonized the Tatahouine diogenite during its 70-year residence on Earth. The fall of the Tatahouine meteorite was observed in 1931 in South Tunisia (Lacroix 1932). Many samples were collected the day after the fall and sent to the Museum d'Histoire Naturelle in Paris. The mineralogy of the pristine meteorite was studied in detail (e.g., Benzerara et al. 2002). Several other fragments were recovered by sifting the first few centimeters of the Tatahouine sand in 1994 and in 2000 for this study. By comparing samples collected just after the fall and those collected in 1994, Barrat et al. (1998) showed that the meteorite fragments experienced incipient orthopyroxene weathering and calcium carbonate precipitation in less than 70 years in the Tatahouine sand. Moreover, it was shown that dissolution and precipitation processes may have been mediated by microorganisms on the Tatahouine meteorite (Gillet et al. 2000; Benzerara et al. 2005a, 2005b). Experimental studies on *Ramlibacter tataouinensis*, a bacterium isolated from the Tatahouine sand, have also demonstrated that this species could potentially mediate dissolution and precipitation processes (Benzerara et al. 2004a, 2004b). As most bacteria found in natural environments are not accessible to cultivation (Amman et al. 1995), we used a DNA-based method to estimate the microbial diversity more accurately. This approach has been used in several Earth science studies (e.g., Birdle et al. 1999; Thorseth et al. 2001; Orphan et al. 2001; Francis et al. 2005), but never so far on a find meteorite.

## MATERIALS AND METHODS

### Field Location and Sampling

Samples were collected in the vicinity of Tatahouine (32°57'N, 10°29'E, elevation: 250 m) located around 100 km west of the border of the Sahara. The climate is arid presaharian with an average annual rainfall of 115 mm. Samples were aseptically collected in May 2000 from a 0–5 cm depth and placed in 50 ml sterile disposable centrifuge tubes. The temperature regimen experienced by the meteorite fragments was likely very sensitive to the depth at which they were buried. However, the depth of the distribution of the meteorite grains was not precisely measured since it was impossible to assess the variations of the thickness of the sand

layer overlying the meteorite fragments, which has been modified throughout the last 70 years by wind movements. The impact of this parameter on the microbial diversity is thus not assessed by this study. Based on the cautious procedures used for sample collection and storage, past experience in molecular diversity studies, the absence of water in the samples when they were collected, and the identity of the sequences retrieved from the samples (see Results section), we can conclude that contamination during collection and storage was negligible.

### Meteorite Sorting

The submillimeter-size meteorite fragments were sorted from the Tatahouine sand using a Frantz-Magnetic Separator. The Tatahouine diogenite is a green orthopyroxenite. The meteorite fragments could thus be easily differentiated from the quartzo-calcitic pyroxene-free surrounding sand. Two hundred ml of the Tatahouine sand (nearly 280 g) were sorted, giving a meteorite fraction of nearly 2 g. Sterile gloves were used and we were careful about the cleanliness of this procedure at each step.

### DNA Processing

Total DNA was extracted directly from the meteorite fraction a few weeks after sample collection, following a protocol described by Porteous et al. (1997) with few modifications. Two grams of meteorite fragments were suspended in 9.25 ml of SDS lysis buffer and 0.75 ml of guanidine isothiocyanate, homogenized 9 min at 60 °C under strong agitation with sterile zirconia beads, then incubated 1 h at 68 °C. The sample was centrifuged at 13,000 g for 15 min at 20 °C, and 5 ml of the supernatant were pipetted. The DNA was precipitated overnight at –20 °C by adding 0.63 ml of 5 M potassium acetate and 2 ml of 40% polyethylene glycol 8000 to the supernatant. The sample was then centrifuged at 13,000 g for 30 min at 4 °C. DNA was purified from the pellet using a Dneasy Tissue kit (Qiagen) as recommended by the manufacturer.

Purified DNA was used as a template for PCR amplification with specific bacterial 16S rRNA gene primers, fD1 (5'-AGAGTTTGATCCTGGCTCAG-3', Position 1492-1509 on the *E. coli* rrs gene) and S17 (5'-GTTACCTTGTTACGACTT-3', position 8-27 on the *E. coli* rrs gene) or S6 (5'-GTATTACCGCGGCTGCTG-3', position 509-526 on the *E. coli* rrs gene) and specific archaeal 16S rRNA gene primers, 21f and 958r (Cytryn et al. 2000). Each amplification reaction mixture (50 µl) contained 1X PCR buffer, 0.2 mM of each dNTP, 0.5 µM of each primer, 2 µl of template DNA, and 1U of HotGoldstar Taq DNA polymerase (Eurogentec). After initial denaturation (94 °C for 5 min), 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min were performed, followed by a final extension (5 min at

72 °C). The PCR products were purified, ligated to pGEM T-easy vector (Promega) and electro-transformed into *E. coli* DH5 cells. Single colonies containing inserts were selected at random, and the inserts were amplified directly from cells using the primers Sp6 (5'-ATTTAGGTGACACTATAGAATAC-3') and T7 (5'-GTAATACGACTCACTATAGGGC-3') specific of the Sp6 and T7 promoters flanking inserts up and downstream in the pGEM T-easy vector. One hundred and nineteen PCR products were purified and sequenced with an ABI automated DNA sequencer by using a Prism dideoxy terminator (Sanger et al. 1977) cycle sequencing kit (protocol recommended by the manufacturer) using the following primers: S6 for bacterial fragments and 21f for archaeal fragments.

In parallel, a reagent-only blank was processed using the same protocol to test for potential contamination. No amplification product was observed using bacterial and archaeal primers showing that reagents were no contaminant sources.

### Phylogenetic Analysis

The closest relatives to our sequences in public databases were identified by BLAST (Altschul et al. 1997). Our sequences and their relatives were incorporated using the program ED from the MUST package (Philippe 1993) within a comprehensive alignment of ~17,000 prokaryotic 16S rDNA sequences. A preliminary phylogenetic analysis of all our phylotypes and a set of sequences representative of the known diversity of bacterial and archaeal groups was carried out using the neighbor-joining (NJ) method with the MUST package (Philippe 1993). This allowed to ascertain the taxonomic affinity of our phylotypes and to divide them into different data sets (e.g., Archaea, Proteobacteria, Cyanobacteria, etc.) for more detailed phylogenetic analyses. These were carried out by maximum likelihood (ML) with the program TREEFINDER (Jobb et al. 2004). The statistical support for the tree nodes was estimated using 1000 bootstrap replicates.

### Scanning Electron Microscopy Observations

Millimeter-size grains of the Tatahouine meteorite were sorted under a binocular with sterile tweezers. They were mounted on aluminium stubs covered with carbon-conductive adhesive tape and then they were carbon coated. Operating conditions of the Philips XL30 S FEG-SEM were 5 kV accelerating voltage with a working distance of 5–15 mm.

## RESULTS

Numerous microbial-like forms could be observed by SEM at the surface of the meteorite fragments collected in the year 2000. Diverse morphologies were detected: e.g., (1) 1 ×

100 µm filaments (Figs. 1b and 1f), sometimes partly degraded. These filaments were sometimes encrusted in mineral precipitates and were interpreted as microorganisms by Lemelle et al. (2004) and Benzerara et al. (2005a, 2005b) (Fig. 1b). (2) Coccoid cells of various sizes (Figs. 1c–e) usually in close association with mineral-shaped objects (Figs. 1c–e). (3) Doughnut-shaped forms associated with nanobacteria-like objects characterized as calcite crystals by Benzerara et al. (2003) (Fig. 1a). Although SEM observations are essential to evidence bacteria/mineral associations, they cannot be generally conclusive on the biogenicity of micrometer-sized objects (see, for example, rods in Fig. 1a). Morphology is not, moreover, indicative of taxonomy and provides a highly biased view of the diversity because long filaments, for example, are much easier to detect than small cocci by microscopy. A molecular approach is thus necessary. SEM observations however supported previous studies showing that some microorganisms had colonized the meteorite fragment surfaces.

Based on agarose gel electrophoresis analysis, extracted DNA yield was roughly estimated to 0.5 µg/g of meteorite with DNA fragments larger than 10 kb. The diversity of bacterial and archaeal populations associated with the Tatahouine meteorite fragments were investigated through phylogenetic comparisons of cloned 16S rRNA gene sequences to related sequences in the NCBI database (Table 1). Partial sequence information was obtained for 89 bacterial 16S rRNA genes covering approximately 450 bp. Several of the bacterial 16S rRNA gene sequences retrieved from the meteorite fragments were closely related to those from the Tatahouine sand (Chanal et al. 2006): 39 sequences over 89 shared more than 97% identity with clones from the Tatahouine sand. The finding of many new bacterial 16S rRNA gene sequences showing only low identity with known cultivated species is usual for this type of study. The analyzed sequences clustered within 11 bacterial divisions (Table 1): *Cytophaga-Flexibacter-Bacteroides* (CFB), Cyanobacteria and Acidobacteria (Fig. 2),  $\alpha$ -,  $\beta$ -, and  $\delta$ -Proteobacteria (Fig. 3), Gram + Actinobacteria, and Gram + Firmicutes (Fig. 4), Gemmatimonadetes, OP10, and Planctomycetes (Fig. 5). However, the clones were not evenly distributed among these divisions:  $\alpha$ -Proteobacteria, CFB, and Cyanobacteria were dominant in the 16S rRNA gene library. Because many biases can exist in the PCR and cloning reactions, there is no direct relation necessarily between the quantitative representation of taxa in a clone library and their actual importance in the environment (von Wintzingerode et al. 1997). For example, the high number of sequences closely related to *Rhizobium* sp. might be artefactual. In any case, the very low diversity among these 16S rRNA gene sequences (identity is almost 100%) suggests that this taxon is represented by a single species. In contrast, the rest of  $\alpha$ -Proteobacteria, CFB, and Cyanobacteria groups (Figs. 2 and 3) are more diverse. This distribution was different from

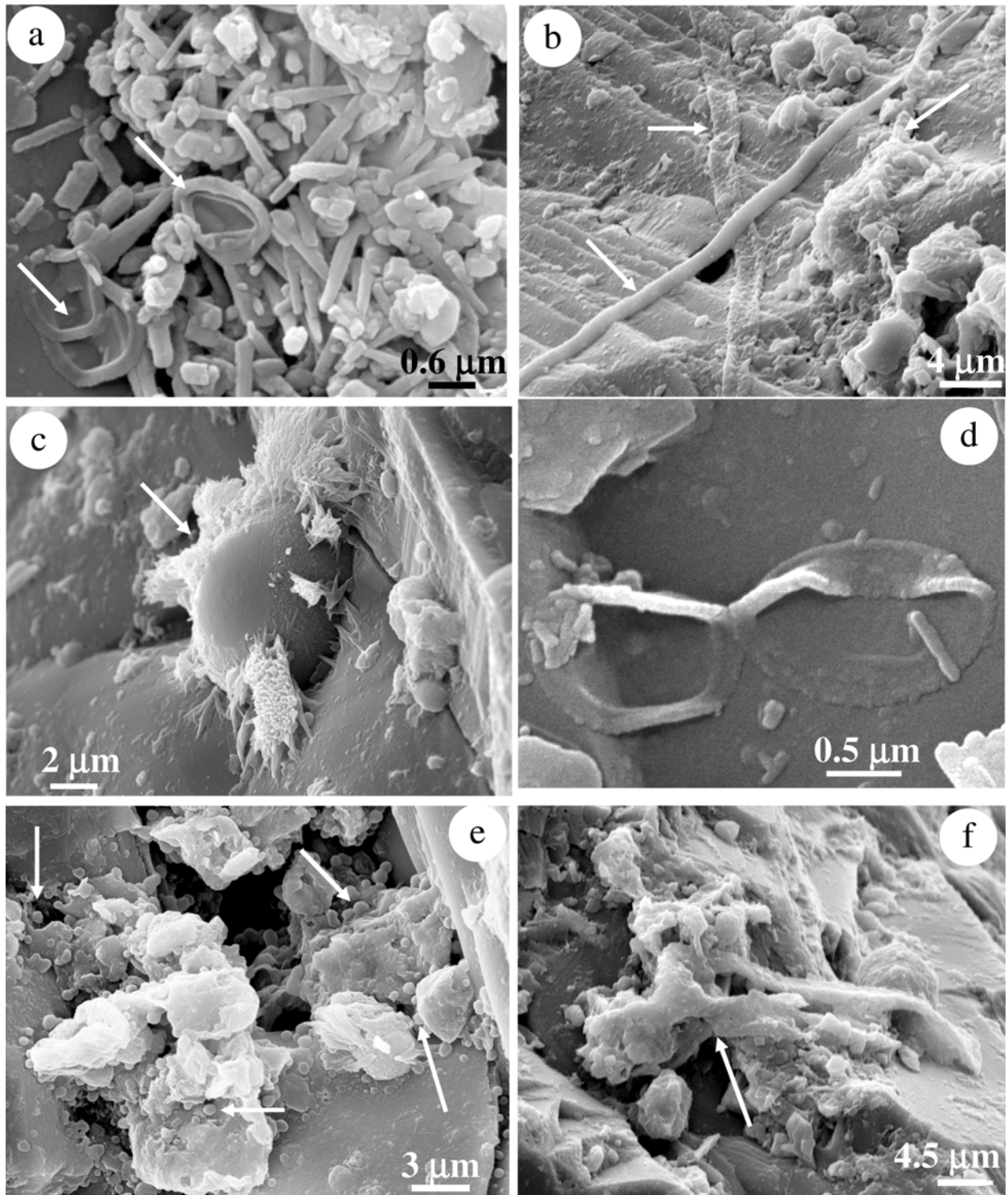


Fig. 1. SEM images of some Tatahouine meteorite fragments collected in 2000, showing microorganism-like forms. (a) Three filamentous microorganisms (arrows) with various textures and preservation states. On the right-hand side, only remnants of a filament can be discerned. In the middle of the picture, the filament oriented SW-NE has a smooth rounded appearance and crosses a flat rough filament (N-S). (b) A curved flat filament (arrow) heavily encrusted with mineral precipitates. (c) A 5  $\mu\text{m}$  large globule (arrow) lying in a shallow depression in the substrate with irradiating needle-shaped crystals on its periphery. (d) A 1  $\mu\text{m}$  large stalk-like sphere (arrow). (e) Coccus-like forms with diameters of 600 nm (arrows) appearing intimately associated with the substrate. (f) Doughnut-shaped forms (arrows) lying in a cluster of bacteria-like rods. These bacteria-like rods, which are around 80 nm wide and 800 nm long, have been characterized by Benzerara et al. (2003) as calcite single crystals.

Table 1. The nearest relatives of 16S gene sequences from the Tatahouine meteorite clone library found by BLAST (<http://www.ncbi.nlm.nih.gov/blast/>). Asterisks refer to Tatahouine clones related to sequences detected in arid environments ( $\geq 95\%$ ).

Clone designation	Abundance	Nearest BLAST match	Identity
<b>Bacteria</b>			
<b><math>\alpha</math>-Proteobacteria</b>			
EP11	10	<i>Rhizobium sp.</i>	99%
EP37	1	Unc. desert bacterium (DQ113828)	99%*
EP21	1	<i>Methylobact. extorquens</i> (AF531770)	94%
EP9	1	Uncultured soil bacterium(AJ233504)	95%
EP19	1	Uncultured soil bacterium (AY360616)	97%
EP33	3	Unc. bacterium from U-contaminated sediments (DQ125646)	97%*
EP32	1	Rhizosphere soil bacterium (AJ252588)	98%
EP41	1	Rhizosphere soil bacterium (AJ252588)	97%
EP27	1	Uncultured deep crust bact (DQ088792)	93%
EP20	1	<i>Sphingomonas asaccharolytica</i> (AJ871435)	96%*
EPII9	1	Uncultured soil bacterium (AY632450)	96%
<b><math>\beta</math>-Proteobacteria</b>			
EPII17	1	Heavy metal contaminated Mine (AY274154)	94%
EPII24	1	Uncultured soil bacterium (AF507408)	92%*
EP65	1	Uncultured soil bacterium (AY039393)	96%
<b><math>\delta</math>-Proteobacteria</b>			
EP42	1	Uncultured Tatahouine bacterium (AY755931)	100%*
EP28	1	Uncultured Nannocystis (AY795719)	97%
EP35	1	Uncultured <i>Geobacter sp.</i> (AY338165)	95%
EP69	1	Hydrocarbon contaminated soil (DQ297965)	97%
<b>Cytophaga-Flexibacter-Bacteroides (CFB)</b>			
EP50	2	Uncultured gold mine bacterium (AF337865)	96%
EPII16	1	Rhizosphere soil bacterium (AJ252599)	92%
EPII18	1	Uncultivated soil bacterium (AF013555)	91%
EP53	1	Uncultured aerosol bacterium (DQ129646)	93%
EP98	1	Bacteroidetes bacterium isolate (AY826623)	91%
EpII7	2	Uncultured soil bacterium (AY274154)	94%
EP103	1	Uncultivated soil bacterium (AJ863256)	92%
EP91	1	Uncultured Tatahouine bacterium	94%*
<b>Acidobacteria</b>			
EP104			
<b>Cyanobacteria</b>			
EP24	3	<i>Oscillatoria sp.</i> (AB074509)	99%*
EPII6	1	<i>Anabaena cylindrica</i> (AF091150)	94%
EPII5	2	<i>Nostoc sp.</i> (AF027653)	
EP43	1	<i>Nostoc sp.</i> (NSP133161)	
EPII20	1	<i>Symploca sp.</i> (AB039021)	
OP10			
EPII19	1	Uncultured soil bacterium (AY192276)	95%
<b>Gram+ Firmicutes</b>			
EP51	1	Uncultured aerosol bacterium (DQ129537)	98%
EP97	1	Uncultured aerosol bacterium (DQ129493)	98%*
EP63	1	Uranium mining waste piles (UBA519636)	99%*
<b>Gram+ Actinobacteria</b>			
EP 14	1	Uncultured Australian arid soil (AF234137)	95%*
EP54	1	Uncultured actinobacterium (AY494641)	98%*
EP30	1	Actinomycetes from soil (D84613)	98%
EP59	1	Uncultured bacterium from human stomach (AY582888)	
EP94	1	Uncultured soil bacterium (AY395393)	94%

Table 1. *Continued.* The nearest relatives of 16S gene sequences from the Tatahouine meteorite clone library found by BLAST (<http://www.ncbi.nlm.nih.gov/blast/>). Asterisks refer to Tatahouine clones related to sequences detected in arid environments ( $\geq 95\%$ ).

Clone designation	Abundance	Nearest BLAST match	Identity
EP101	1	Uncultured soil bacterium	98%*
EP99	1	Uncultured Australian arid soil (AF234137)	97%*
EP92	1	Bacterium from soil with geothermal gradient (AF465644)	
Planctomycetes			
EP87	1	Uncultured soil bacterium (AF507705)	96%*
Gemmatimonadetes			
EP93	1	Uncultured soil bacterium (AF545644)	92%
EP2	1	Uncultured (AF234148)-Australian arid soil	97%*
Archaea			
Crenarcheota			
AP5	1	ODP (AY367310)	
AP3	1	ODP (AY367310)	
AP19	1	ODP (AY367310)	
AP57	3	ODP (AY367310)	
AP7	1	ODP (AY367310)	
AP23	1	Columbia River (AF180712)	97%
AP30	1	Desert varnish, Whipple Mountains (AY923103)	99%*
AP16	1	Desert varnish, Whipple Mountains (AY923103)	99%*
AP14	7	Desert varnish, Whipple Mountains (AY923103)	99%*
AP21	1	Desert varnish, Whipple Mountains (AY923076)	99%*
AP9	1	Desert varnish, Whipple Mountains (AY923076)	99%*
AP34	3	Semiarid soil (AF443589)	98%*
AP8	1	South African gold and diamond mines (AY187899)	99%*
AP35	1	South African gold and diamond mines (AY187899)	99%*
AP22	1	Uncultured soil Crenarcheota (AY016482)	99%
AP31	1	Uncultured soil Crenarcheota (AY278071)	99%
AP25	2	Uncultured soil Crenarcheota (AY601288)	100%
AP37	1	Metal particles in freshwater (AF418930)	98%
AP24	1	Metal particles in freshwater (AF418930)	99%

that of the Tatahouine sand library, obtained with the same protocol and which was dominated by  $\gamma$ -Proteobacteria, Acidobacteria, and Actinobacteria (Chanal et al. 2006).

Rarefaction curves were calculated to assess the quantitative effect of using only a fraction of the total DNA retrieved from the Tatahouine meteorite on the diversity of phylotypes. Given the species abundance distribution in the 16S rRNA gene library, rarefaction curve gives estimates of the species richness of subsamples taken from it (Fig. 6). Two thresholds of sequence identity were considered to discriminate the operational taxon units (OTUs) in the library: (1) In the first case, clones sharing greater than 97% sequence identity were treated as the same OTU. This criterion may be qualitatively considered as a species-level distinction (Stackebrandt and Goebel 1994). Similar to the 16S rRNA gene library from the Tatahouine sand, the rarefaction curve of the meteorite library did not reach a plateau, indicating that the number of clones screened in both libraries was insufficient to reveal the total number of sequence types within the libraries at this phylogenetic level. (2) A phylogenetically broader distinction was made by grouping

clones sharing greater than 90% sequence identity in the same OTU. Contrary to the Tatahouine sand rarefaction curve, the meteorite rarefaction curve flattens out, demonstrating that this library was less diverse than the Tatahouine sand library (Fig. 6).

A separate 16S rRNA gene clone library was generated to determine the diversity of Archaea that colonized the meteorite fragments (Fig. 7). A total of 30 clones were analyzed. The diversity of Archaea was apparently lower than the diversity of Bacteria. They were all affiliated to non-thermophilic members of the *Crenarchaeota* without close affiliation to any cultivated member. Comparison with the diversity of Archaea in the Tatahouine sand supports observations made on the bacterial diversity: all the sequences identified on the meteorite fragments share high identity (sometimes up to 100%) with at least one sequence from the Tatahouine sand (Fig. 7). Interestingly, many of the clones from the meteorite fragments (Table 1) were closely related to sequences identified in desert varnish in Whipple Mountains (Kuhlman et al. 2005), south of Death Valley (California, USA). Other sequences are related to 16S rDNA



CYANOBACTERIA + CFB + ACIDOBACTERIA

Fig. 2. A phylogenetic tree inferred from a maximum likelihood analysis showing the positions of partial SSU rDNA sequences retrieved from the Tatahouine meteorite (full diamond) and the Tatahouine sand (empty diamonds) affiliated to cyanobacteria, *Cytophaga-Flexibacter-Bacteroides* (CFB) and Acidobacteria groups. Numbers at node are bootstrap values. The scale bar represents the number of substitutions per 100 positions per a unit branch length. Accession numbers of bacterial isolates and environmental sequences are given within brackets. The number of additional identical sequences that are not reported in the tree is also given within brackets.



Fig. 3. A phylogenetic tree inferred from a maximum likelihood analysis showing the positions of partial SSU rDNA sequences retrieved from the Tatahouine meteorite (full diamond) and the Tatahouine sand (empty diamonds) and affiliated to Proteobacteria (Alpha-, Beta-, Delta-, and Gamma-).

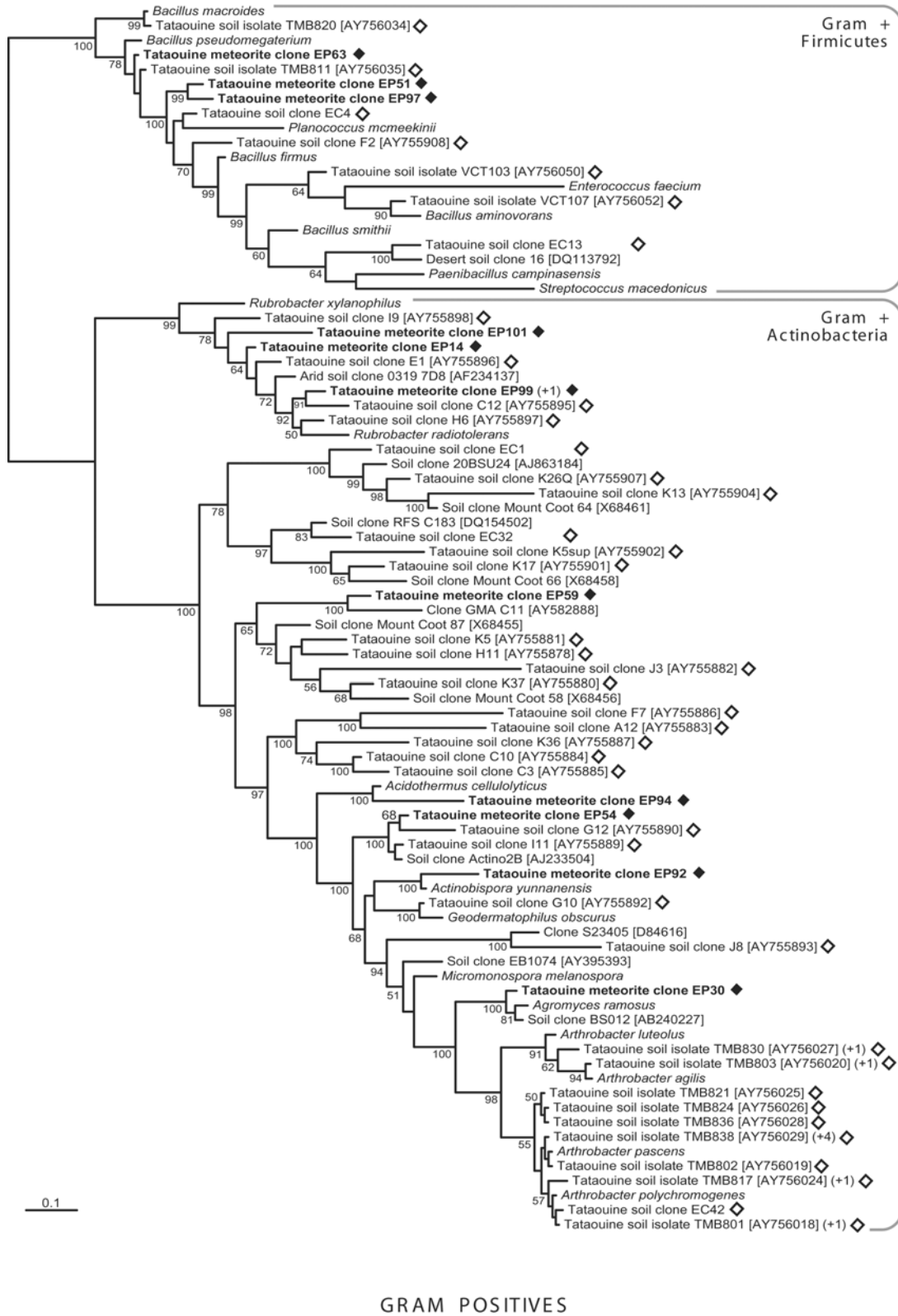


Fig. 4. A phylogenetic tree inferred from a maximum likelihood analysis showing the positions of partial SSU rDNA sequences retrieved from the Tatahouine meteorite (full diamond) and the Tatahouine sand (empty diamonds) and affiliated to Gram positive bacteria.

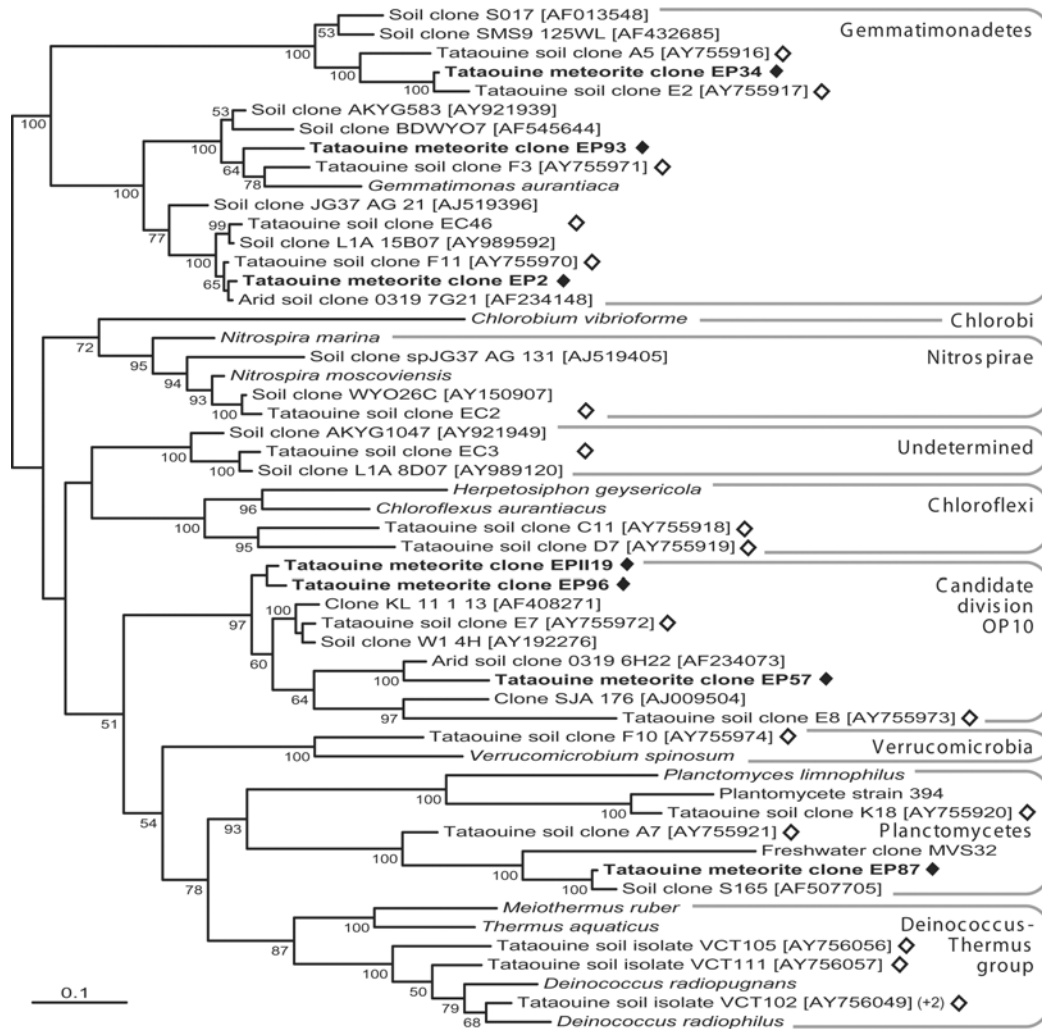


Fig. 5. A phylogenetic tree inferred from a maximum likelihood analysis showing the positions of partial SSU rDNA sequences retrieved from the Tatahouine meteorite (full diamond) and the Tatahouine sand (empty diamonds) and affiliated to Gemmatimonadetes, Nitrospirae, Chloroflexi, ODP10, and Planctomycetes.

gene sequences obtained from the study of dwarf Archaea in a semi-arid soil (Rutz and Kieft 2004) and from soils (Table 1).

## DISCUSSION

### Discussion on the Microbial Colonization of a Meteorite on Earth

The Tatahouine diogenite was likely organic-free at its arrival on Earth 70 years ago (Grady et al. 1997). Despite a relatively short residence time on Earth (less than 70 yr) and the harsh environmental conditions prevailing in the Tatahouine sand (e.g., little organic matter, high daily temperature range, arid conditions), the meteorite fragments

have been extensively colonized by a highly diverse microbial community. Considering an average DNA mass of  $4 \times 10^{-15}$  g per bacteria (Ellenbroek et al. 1991), a 80% efficiency for the DNA extraction procedure, and that all the extracted DNA came from prokaryotes, a density of  $2 \times 10^8$  prokaryotic cells per g of meteorite can be calculated from the extracted DNA yield. Chanal et al. (2005) estimated the number of culturable bacteria in the Tatahouine sand by cultivation on  $0.1 \times$  TSA medium to be  $10^5$  cells per gram of sand. Because of the different assumptions made in our study (some extracted DNA may, for example, come from eukaryotes like fungi), and because extracted DNA can also come from non-culturable and/or dead microorganisms,  $2 \times 10^8$  prokaryotic cells per g of meteorite is clearly overestimated if compared with the number of culturable

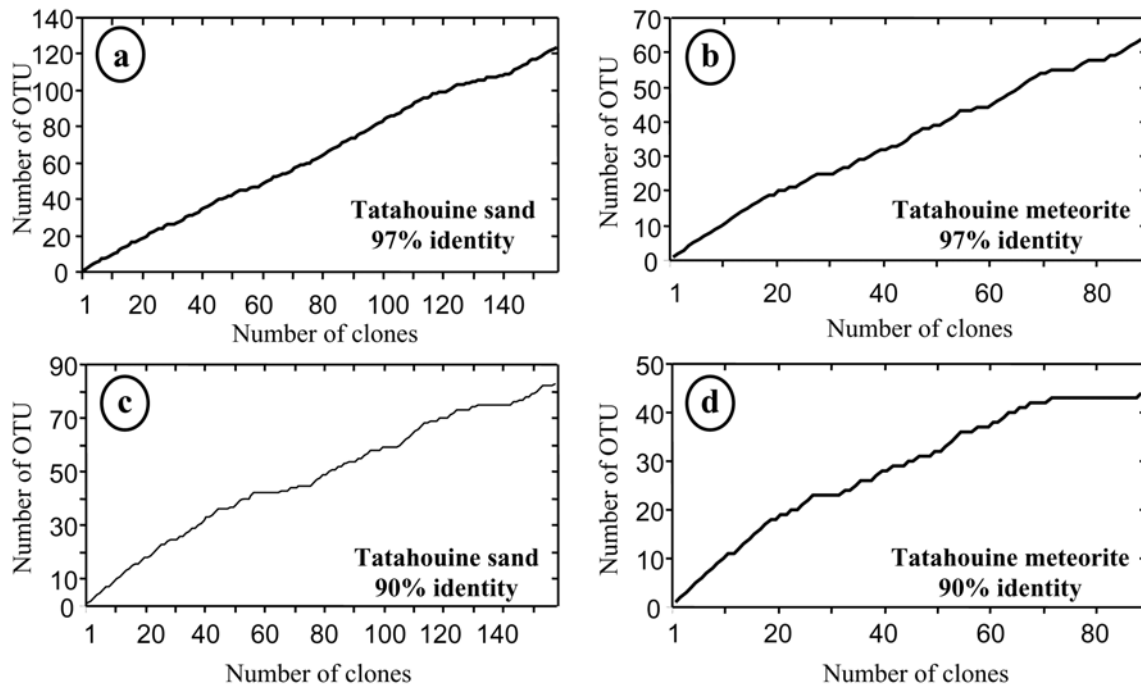


Fig. 6. Rarefaction curves for the Tatahouine meteorite and sand clone libraries. Two different threshold values were used to discriminate OTUs: a species-level threshold (97% sequence identity) for the Tatahouine sand clone library (a) and the Tatahouine meteorite clone library (b); and a 90 % sequence identity threshold for the Tatahouine sand clone library (c) and the Tatahouine meteorite clone library (d).

bacteria. It is, however, interesting to compare that number with estimates for other similar environments. Kuhlman et al. (2005), using direct counting by epifluorescent microscopy and phospholipid fatty acid (PFLA) analyses, reported a very high number of bacteria, between  $10^7$  and  $10^8$  per gram of dry sample, in rock varnish collected in the hot desert of the Whipple Mountains (Death Valley, USA), which is consistent with the number found on the Tatahouine meteorite. Previous studies have evidenced a terrestrial biological “contamination” of meteorites (e.g., Jull et al. 1998; Steele et al. 2000), suggesting inherent difficulties in finding unambiguous evidence for extraterrestrial life remnants in find meteorites like, for example, those proposed in the Martian meteorite ALH 84001 by McKay et al. (1996). Our study emphasizes that idea by showing that a short time delay is compatible with a wide colonization of a carbon-depleted rock even in arid environments. The molecular biology approach used on bulk samples in this study cannot ascertain that all the detected microorganisms have colonized directly the surface of the meteorite fragments. Some, indeed, may have colonized the surface of micron-size sand particles attached to the meteorite surface. SEM observations and the different microbial diversities observed between the meteorite fragments and the sand fraction strongly suggest however that a large proportion of the microorganisms detected have colonized the meteorite surface. Only further analyses using, for example, detection of nucleic probes coupled to gold nanoparticles by SEM (Gerard et al. 2005) will enable a

definite answer to that question for all the microbial groups evidenced here.

Most bacterial and archaeal 16S rRNA genes cloned from the meteorite fragments were highly related to sequences identified in the surrounding sand, which is consistent with a colonization of the meteorite fragments by the microorganisms inhabiting the Tatahouine sand. Moreover, the closest relatives found in the public sequence databases to the meteorite phylotypes were almost all identified in soils, often in arid regions, suggesting that most of the prokaryotes that colonized the meteorite fragments are typical of arid soils, which is consistent with the prevailing conditions in Tatahouine. This strengthens the proposition made by some authors that some prokaryotic taxa may be specialized for arid soils (e.g., Holmes et al. 2000; Chanal et al. 2006).

This phylogenetic study evidences that the Tatahouine meteorite fragments have been colonized by a highly diverse microbial community. There is, in particular, a very diverse cyanobacteria community. A similar high diversity of cyanobacteria in arid environments has been previously abundantly documented (e.g., Garcia-Pichel et al. 2001). Cyanobacteria are potentially able to fix  $N_2$  and  $CO_2$  from the atmosphere and can resist high irradiance and desiccation. Because of their autotrophic metabolism, cyanobacteria are potential pioneer colonizers of the Tatahouine meteorite grains. As proposed by previous studies (e.g., Paerl et al. 2000), the  $CO_2$  and  $N_2$  fixation coupled with the development

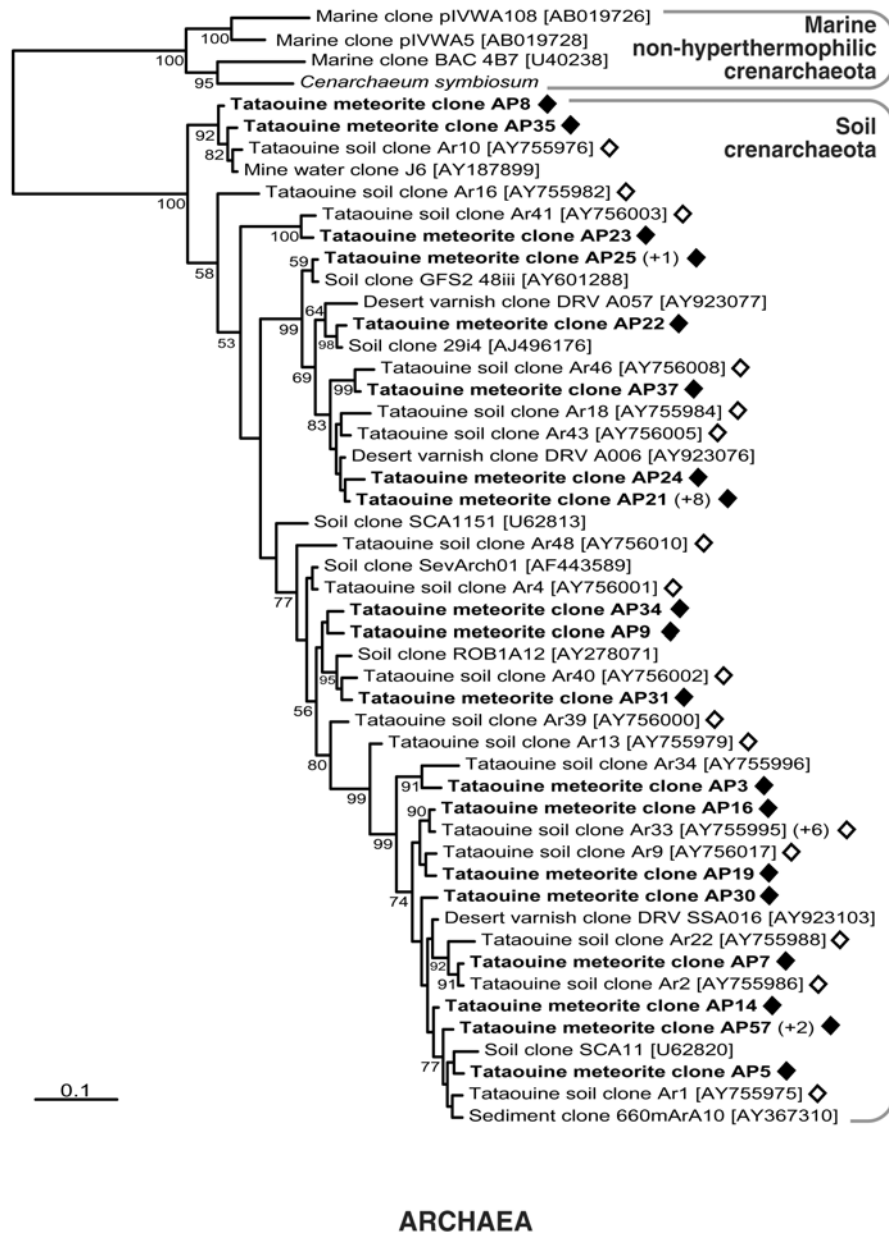


Fig. 7. A phylogenetic tree inferred from a maximum likelihood analysis showing the positions of partial SSU rDNA sequences retrieved from the Tatahouine meteorite (full diamond) and the Tatahouine sand (empty diamonds) and affiliated to Crenarcheota.

of microscale gradients by cyanobacteria can explain the presence of diverse heterotrophs and even anaerobic bacteria communities. Although the presence of anaerobic prokaryotes cannot be excluded (e.g., sequence EP37 was distantly related to *Rhodobacter sphaeroides*, a facultative anaerobic photosynthetic bacterium; EP35 was distantly related to *Geobacter metallidurens*, a strict anaerobe able to couple the oxidation of organic carbon with metal reduction), we do not evidence taxa that are obviously anaerobes on the Tatahouine meteorite, which is consistent with the aerobic conditions existing at the surface of the Tatahouine sand.

### Discussion on the Metabolic Diversity and the Geochemical Reactions Evidenced at the Surface of the Tatahouine Meteorite

The phenotypic properties cannot be determined directly from 16S rRNA gene sequences, but for various groups at least some important ecophysiological traits may be shared by phylogenetically closely related species (e.g., Pace 1996). Hence, although no definitive prediction can be made, especially when Tatahouine members branch far from known cultivated species, comparisons of some sequences with the

closest cultivated species allows making hypotheses on the physiology of bacteria that have colonized the meteorite. The phylogenetic diversity evidenced on the Tatahouine meteorite is likely associated with a metabolic diversity potentially producing various organic molecules as mentioned previously. Cyanobacteria produce by photosynthesis diverse organic polymers on which heterotrophic bacteria, which are numerous in the Tatahouine meteorite clone library, can feed. For example, many Cytophagales, including *Chitinophaga* (Fig. 2), are gliding bacteria that degrade long polymers and may be able to feed on cyanobacterial EPSs, as proposed by Lopez-Garcia et al. (2005). Alpha- and Beta-Proteobacteria (Fig. 3) are metabolically very versatile. Many of them, like the *Sphingomonas* and *Chelatococcus* species, are aerobic and degrade and/or produce complex organic molecules. Most of the *Bacillus* species (Fig. 4), including *B. megaterium*, can also degrade complex biopolymers. Finally, many Actinobacteria are generally chemoorganotrophs which utilize complex mixtures of organic polymers (Blackall et al. 1996; Ludwig et al. 1997; Yoon et al. 1998). The presence of many sequences related to those genera thus suggests that, at least locally on the Tatahouine meteorite fragments, microorganisms recycle diverse complex organic molecules.

Inorganic modifications can also occur on meteorites. A particular modification is rock varnish, also known as desert varnish, which is a natural coating that forms on rock surfaces during exposure in arid regions. Mineralogy of those coatings have been studied for a long time (e.g., Potter and Rossman 1977; Perry and Adams 1978) and is dominated by clay minerals, carbonates, iron and manganese oxides and hydroxides, and silica and aluminium oxides. Some processes involved in the formation of desert varnish are in situ weathering (dissolution and precipitation) and accumulation of airborne dust (Potter and Rossman 1977). Several authors have proposed that microorganisms may sometimes impact those processes (e.g., Dorn and Oberlander 1981; Kuhlman et al. 2005), but more work is needed to understand the exact mechanisms through which they intervene. Previous studies on the Tatahouine meteorite as well as this study (see below) suggest that similar processes and actors may operate at the surface of the meteorite fragments. Carbonates and localized etch pits in the orthopyroxene were indeed observed by SEM at the surface of the Tatahouine meteorite by Barrat et al. (1998), and Benzerara et al. (2003). Benzerara et al. (2005a, 2005b) showed by TEM that dissolution features, together with carbonate, aluminosilicate, and iron oxide precipitates were sometimes closely associated with microbes. Among the microorganisms identified by molecular methods, some may mediate processes forming desert varnish on the Tatahouine meteorite. Cyanobacteria can mediate calcium carbonate precipitation either by photosynthetic-driven uptake of CO<sub>2</sub> and/or by offering nucleation sites in the cell envelopes (e.g., Merz-Preiss 2000).

Actinobacteria, which are diverse on the Tatahouine meteorite and in the soil (Fig. 4), are other potential candidates for the geochemical reactions mentioned above. Actinobacteria are a diverse group of Gram-positive bacteria, which superficially resemble fungi and are adapted to life on solid surfaces (Ensign 1978). Many studies have evidenced their involvement in the weathering of silicates (e.g., Kalinowski et al 2000; Palla et al. 2002). Interestingly, Actinobacteria are the most abundant cultivated microorganisms in desert varnish from the Whipple Mountains (Kuhlman et al. 2005), and also from the Negev, Mojave, and Namibia deserts (Eppard et al. 1996). Moreover, the morphology that Kuhlman et al. (2005) report for *Geodermatophilus obscurus* (well-formed sheath structures) is very similar to some of the filaments observed by Benzerara et al. (2005) and Lemelle et al. (2004). Although no sequence close to this genus has been detected on the meteorite fragments, one has been evidenced in the Tatahouine sand (Fig. 4; Chanal et al. 2006).

Finally, it is interesting to note that the closest relatives to many of the archaeal sequences retrieved from the Tatahouine meteorite (with occasionally 100% identity) (Table 1) were detected in desert varnish from the Whipple Mountains (Kuhlman et al. 2005). Until very recently, no cultivated non-extremophilic Crenarchaeota was available, so there was no understanding of their physiology or their biogeochemical capabilities. Nevertheless, recent reports suggest that these non-extremophilic Crenarchaeota can be very active autotrophic ammonium-oxidizers both in marine (Konneke et al. 2005) and soil ecosystems (Treich et al. 2005). The geochemical cycling of nitrogen in the Tatahouine sand has never been studied, but such metabolisms could be of primary importance for the development of complex microbial communities. Moreover, the striking resemblance between the archaeal communities evidenced in the desert varnish of Whipple Mountain and on the Tatahouine meteorite should motivate further studies to understand whether the spatial distribution of archaeal cells is connected to mineral precipitation features.

### **Discussion on a Potential Selection of the Colonizing Bacteria by the Minerals**

Microbial diversity in natural communities is very difficult to exhaustively and quantitatively describe because of several technical limitations (see, for example, von Wintzingerode et al. 1997). For example, the 16S rRNA gene copy number varies among the different bacterial species and several potential biases at each step of the experimental procedure make a linear relationship between the actual density of each species in the natural community and the amounts of its PCR products unrealistic. Thus, on one hand, some species present in the samples can be not detected, and on the other hand, the proportions of the different

taxonomic units cannot be quantitatively estimated. However, the characterization of the molecular diversity on the Tatahouine meteorite fraction and on the surrounding sand was performed under exactly the same conditions. The direct comparison between the diversities of both clone libraries is thus possible assuming the reproducibility of the biases (Chandler et al. 1997). As the diversity of the Tatahouine sand was not exhaustively sampled with 158 clones (Chanal et al. 2006), it is not possible to deduce whether some bacterial species are only present on the meteorite fraction and not in the surrounding soil. However, the diversity of the clone library generated from the meteorite fraction was reduced compared with that of the Tatahouine sand clone library (Fig. 6). Moreover, both libraries display different phylogenetic compositions. The most remarkable example concerns the cyanobacteria, which are very diverse in the Tatahouine meteorite fragments (Fig. 2), while they were surprisingly almost absent (1 clone) from the Tatahouine sand libraries (Chanal et al. 2006). Other groups showed the opposite. For example, the radiation-resistant Deinococcales (Fig. 5) were abundant in the sand libraries and several strains were also isolated from the Tatahouine sand (Chanal et al. 2006). Another example concerns a group of  $\beta$ -Proteobacteria related to the genera *Oxalobacter* and *Zoogloea* (Fig. 3), also very abundant in the sand clone libraries and among the soil isolated strains (Chanal et al. 2006). Finally, Acidobacteria show a high diversity in the Tatahouine sand clone library but were almost absent from the Tatahouine meteorite fragments. Interestingly, the Deinococcales, the Beta-Proteobacteria related to *Oxalobacter* and *Zoogloea* and Acidobacteria were almost absent from the meteorite clone libraries (Figs. 2, 3, and 5). These observations suggest that the colonization of the meteorite fragments was not completely unspecific. Similar differences in the bacterial community structure of different mineralogical fractions of a soil have been observed by Sessitch et al. (2001). Two non-exclusive possibilities are consistent with these observations:

(1) There was not enough time (70 years) for all the bacteria present in the Tatahouine sand to colonize the meteorite fragments. Experimental data on the kinetics of colonization of solid surfaces have been obtained in aqueous environments (e.g., Mueller 1996; Dang and Lovell 2000), and the succession of pioneers followed by recruited colonists lasts days or weeks. Nusslein and Tiedje (1998) characterized the diversity of young soils formed from volcanic ash deposited 200 years ago on the island of Hawaii under a tropical climate. Because of too high a diversity, they could not however compare it to the diversity existing in adjacent older soils. Kinetics is likely different in an arid terrestrial environment like the Tatahouine sand, as presence of water (i.e., period of microbial activity) is restricted to few days per year. The average generation time of prokaryotes in the Tatahouine sand is not known, but it has been estimated to several years in soils (Whitman et al. 1998). Seventy years in

the Tatahouine sand could thus be equivalent to only a few days in colonization experiments conducted in aqueous environments.

(2) There is a specific selection of colonizing bacteria by the conditions prevailing at the surface of the Tatahouine meteorite fragments. For example, the meteorite grains did not, at least initially, contain organic matter; only autotrophs for carbon and nitrogen could therefore develop at their surface. Rogers et al. (1998) have suggested that the chemistry of the minerals could control the colonization by microorganisms in regard to their nutrient requirements. On the one hand, we did not detect 16S rRNA gene sequences related to cultivated bacteria able to use reduced elements (e.g.,  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ) contained in the meteorite fragments as electron donors (e.g., Fe- or Mn-oxidizing bacteria). On the other hand, the nutrient selection could act on oligo-elements (e.g., elements like P or Mg, which are needed in low concentrations as essential components of various enzymes), which are much more abundant in the pyroxene composing the Tatahouine meteorite than in the quartz grains from the Tatahouine sand; this possibility remains, however, highly difficult if not impossible to assess.

## CONCLUSION

This is the first time that the diversity of microorganisms on a meteorite is described. This study shows the extensive colonization of a rock in less than 70 years despite the aridity of the environment. Calcite precipitation and pyroxene weathering have been observed on the Tatahouine meteorite and a biological mediation has been previously proposed. Potential candidates for these reactions are suggested in this study. We believe that, aside from the mineralogical and the chemical characterization of a find meteorite, the knowledge of the microbial diversity can be valuable in providing a better understanding of the terrestrial processes affecting the sample. Moreover, this type of study will allow the design of nucleic probes specific to some prokaryote taxa and the performance of fluorescent in situ hybridization (FISH). Recent methodological developments (Gerard et al. 2005) permit the coupling of this technique with chemical and mineralogical observations at the sub-micrometer level, opening up the possibility of deciphering in the near future whether specific taxa are involved in the different geochemical reactions evidenced at the surface of the Tatahouine meteorite, how abundant they are, and what their distribution patterns are on the meteorite.

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