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Biologia. — *Microbes in rocks and meteorites*: *a new form of life unaffected by time, temperature, pressure.* Nota di Giuseppe Geraci, Rosanna del Gaudio e Bruno D'Argenio, presentata (*) dal Socio B. D'Argenio.

Abstract. — Crystals, rocks and mineral ores of different origins contain viable microbial life that appears actively swimming under the microscope when the sample is properly fragmented and suspended in a nutrient medium. This form of life in rocks is unaffected by time, since microbes have been found in samples of all geological ages, from about 2.8 Ga to recent rocks, and by pressure and temperature, since it is present in metamorphic and in igneous rocks. From the tests performed, among which those to secure from sample pollution, it emerges that this form of life is not destroyed, as indeed expected, when the rock is heated above 500 °C in a kiln. However, all cloned microbes are sensitive to growth inhibition by specific antibiotics. A similar search, for the presence of microbes in meteorites, shows that also these materials are rich in microorganisms, indicating that these already existed in early Earth formation stages. Some different microbial species, derived from different samples of rocks and meteorites, have been cultured, cloned and classified by 16S rDNA typing and found to be not essentially different from present day organisms. An interesting consequence of these findings, among others, is the support to the hypothesis that life came from outside Earth with the additional indication that it was already present in those materials that accreted to form the solar planetary system.

Key words: Life in rocks; Life in meteorites; Microbes; Bioastronomy.

Riassunto. — *Microbi in rocce e in meteoriti*: *una nuova forma di vita non influenzata da tempo, temperatura, pressione.* Cristalli, rocce e minerali di diversa origine contengono microrganismi vitali che si osservano nuotare attivamente al microscopio quando il campione solido è frammentato in modo appropriato, raccolto su un vetrino portaoggetti e sospeso in un mezzo nutriente. Questa forma di vita, quando è all'interno della roccia, non è influenzata dal tempo, perché sono stati trovati microrganismi vitali e coltivabili in campioni di diverse età, a partire da circa 2.8 Ga a rocce recenti, e dalla temperatura e pressione, perché è presente in rocce metamorfiche e in rocce ignee. In alcune prove, fra le molte fatte per assicurarsi da possibili contaminazioni, è risultato che questa forma di vita non è distrutta, come ci si sarebbe effettivamente aspettato, quando la roccia è riscaldata al di sopra di 500 °C in un forno per ceramica, mentre tutte le specie clonate non crescono in presenza di antibiotici specifici. La ricerca con lo stesso approccio di forme microbiche in meteoriti ha mostrato che esse sono ricche in microrganismi, indicando che questi già esistevano durante i primi stadi di formazione della Terra. Alcune specie microbiche, derivate da campioni di rocce e di meteoriti, sono state ottenute in coltura, clonate e classificate con il metodo della tipizzazione del 16S rDNA e sono risultate non dissimili dai microrganismi attuali. Questi risultati avvalorano l'ipotesi che la vita sia venuta dall'esterno della Terra e suggeriscono che fosse già presente nei materiali che, condensandosi, hanno generato i pianeti del sistema solare.

Introduction

It is common knowledge that well recognizable fossil forms of microbial life are present in ancient sedimentary rocks like Archean stromatolites. Some microfossils are so well preserved that their identification and characterization in terms of structure and composition has been possible, permitting to establish which types of microorganisms populated Earth in early geological times (Golubic and Seong-Joo, 1999; Nisbet, 2000;

(*) Nella seduta dell'11 maggio 2001.

Rosing, 1999). These studies are relevant to gain insight on the origin and evolution of life on Earth. To this aim, different theories, from the first half of the past century, have been formulated considering the peculiar pre-biotic conditions (J.D.L. Bernal, J.B.S. Haldane, A.I. Oparin), and by directly performing laboratory experiments in conditions mimicking those of the supposed pre-biotic environments (S.L. Miller). Recently, geochemical studies on the presence of life in the young Earth have provided evidence that microorganisms existed some 3.2 Ga ago (Rasmussen, 2000) or even earlier, 3.47 Ga ago, based on the product of the enzymology of microbial sulfate reduction (Shen *et al.*, 2001). This has shifted the initial presence of organized life, able to perform complex biochemical functions, to a period immediately following the end of the heavy meteorite bombardment of the Earth (Gogarten-Boekels *et al.*, 1995; Drake, 2000). How much time was effectively necessary to observe life organized in actively metabolizing cells from the initial accretion of the Earth? Apparently, a relatively short period, a few hundred Ma, was sufficient to close the gaps between inorganic, organic and biological worlds (Nisbet, 2000). The possibility that the origin of life might reside outside Earth (panspermia), where it was imported, was considered since Arrhenius, at the beginning of last century. The possibility has been also considered that life might have been originated in deep space. In support of this hypothesis, it has been reported that solid material, produced by irradiating simple chemicals in cold vacuum, when immersed in water, creates spontaneously membranous structures like soap bubbles, containing an inside and outside layer (Dworkin *et al.*, 2001).

The results of the present work show that microorganisms are present within crystals and rocks of different chemical compositions and also in meteorites, in a form that is endowed of very peculiar and unexpected properties that make it an ideal vector to spread throughout the universe. These findings stemmed from a study of microorganisms in different types of sedimentary rocks. It was found that eubacteria, and in few cases archea and in one case a unicellular eucaryote, are not only present as calcified, dead or partially degraded cells, as well established in a multitude of papers on geomicrobiology (Banfield and Nealson, 1997), but are also in a form that may be reactivated by suspending the properly fragmented solid specimen in a nutrient medium.

From the initial observations, about 50 rock and mineral samples of different geological nature and age and of different chemical compositions have been analyzed, including some meteorites, kindly made available by the «Real Museo Mineralogico» of the University of Naples «Federico II».

Materials and methods

The origins of crystals, rocks and mineral ores, together with those of meteorites used here are reported in the legend of fig. 1*a,b* and in table I.

Small samples were obtained from larger specimens by removing the external layer and then sawing into two halves the inner part with a standard cutting equipment for rocks. The newly exposed surface was soaked with ethanol and then put in the flame of a Bunsen burner for 2 min. On the so prepared surface two 5 mm-deep holes

TABLE I. – *Microbial organisms cloned from crystals and rock specimens typed by 16S rDNA analysis.*

	Rock	Age	PCR typing result	Corresponding microbial species	Notes
1.	Banded Iron Fm. colony 1	≈ 2.8 Ga	eubacterium	*subtilis, pumilus*	
2.	Limestone GB-16 colony 1	> 500 Ma	eubacterium	*subtilis*	SEM fig. 5.1
	Idem = colony 2		eubacterium	*pumilus*	SEM fig. 5.3
3.	Limestone GB-6 colony 1	> 100 Ma	eubacterium	*pumilus*	SEM fig. 5.2
	Idem = colony 2		eubacterium	?	SEM fig. 5.4
	Idem = colony 3		eukaryote	*candida*	SEM fig. 7
4.	Iceland spar colony 1	?	eubacterium	*staphylococcus*	
	Idem = colony 2		eubacterium	*staphylococcus*	
5.	MetA colony 1	> 4.5 Ga	eubacterium	*pumilus*	SEM fig. 6.1
	Idem = colony 2		eubacterium	*staphylococcus*	SEM fig. 6.3
6.	MetC colony 1	> 4.5 Ga	eubacterium	*bacillus*	SEM fig. 6.2
	Idem = colony 5		eubacterium	*staphylococcus*	SEM fig. 6.4

1. Laminated (mostly hematite and quartz) ironstone (BIF) from Temagami Lake, Ontario, Canada. Late Archean, Superior type ore (coll. F. Molnar, Budapest).
2. Archaeocyathid limestone, Nebida Formation, Early Cambrian, SW Sardinia.
3. Carbonate platform mudstone, Aptian, Monte Camposauro, Southern Apennines.
4. Iceland spar, single crystal of ≈ 10 cm^3. Origin and age unknown.
5. MetA. Iperstenic chondrite (see fig. 1.2), fallen on Feb. 3, 1882 at Mocs, Transilvania (kindly provided by the «Real Museo Mineralogico», Naples, inv. 18338).
6. MetC. Enstatitic olivinic chondrite, fallen in 1919 at Bur Hacaba, Somalia (kindly provided by the «Real Museo Mineralogico», Naples, inv. 23019).

were made with a drill with sterile bits, a larger hole inside which a smaller hole was produced. From the bottom of this second hole a sample of the rock was obtained by scraping it with the needle of a sterile syringe, previously brought to red heath in the flame of a Bunsen burner. All operations after the cutting into two parts of the sample rock, were carried out under a laminar flow sterile hood. Operators wore latex gloves. Media and all glassware were sterilized by autoclaving for 40 min at 121 °C. Plasticware was bought sterile. Before utilization as culture medium, the autoclaved solution was incubated for a week to check for possible contamination. All experiments were performed with appropriate controls that were negative during the period of growth of the analyzed samples.

Sampling of very hard materials, such as granite, obsidian, basalts, was performed similarly, with the difference that the newly cut surface, after sterilization with ethanol and by flaming for at least 2 min, was directly used to obtain fragments of the rock by scraping with a 0.4 k low value diamond, blocked with a bolt on the tip of a screw. This asset was disassembled in the three constituting parts that were individually brushed, washed with soap, rinsed with sterile distilled water then sterilized with ethanol between one sampling and the other.

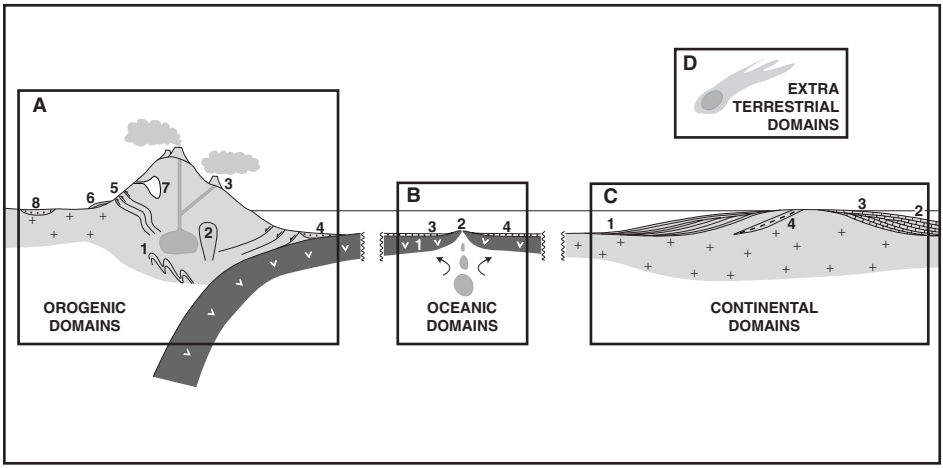

Fig. 1. – Idealized section across Earth's crust to show the locations of the original genetic environments from which the different types of analyzed rocks came. Note that the latter differ from each other in age, spanning from less than 1 to >500 Ma, except for C4 (BIF) which reaches ≈ 2.7-2.8 Ga, while the meteorites, according current literature (Papike, 1998), are clustering around 4.5 Ga.
A. Orogenic domains: 1. Metamorphic rocks; 2. Igneous rocks, intrusive; 3. Igneous rocks, volcanic; 4. Sedimentary rocks, marine: clastics; 5. Mineral ores; 6. Non marine sedimentary (travertine); 7. Speleothems, cave pearls; 8. Other: silicified wood, Iceland spar.
B. Oceanic domains: 1. Igneous rocks; 3. Sedimentary rocks (radiolarite); 4. Other (manganese crust).
C. Passive continental margin domains (including continental interiors and pre-Phanerozoic rock assemblages): 1. Sedimentary rocks: Basinal clastics; 2. Basinal carbonates; 3. Shallow water carbonate rocks and fossils (including corals, mollusks, foraminifera, Archaeocyathids and stromatolites); 4. Banded Iron Formation (laminate ironstone Upper Archean).
D. Extraterrestrial domains: chondrites, pallasites, siderites.

The finely powdered fragments of the analyzed rocks were collected directly on a microscope slide, suspended in sterile LB culture medium, covered with a micro cover glass and immediately observed at 400-1000 enlargements. Powdered samples were also collected in petri dishes, sterile culture medium was added and liquid culture was initiated by gentle agitation on an oscillating plate at room temperature. After appropriate growth, occurring between one day and a week, depending on the sample, the culture was diluted serially and dispersed on solid agar in sterile agar LB plates to isolate individual clones.

PCR amplification of 16S rDNA.

Ribosomal DNA was amplified directly from a single colony grown on agar LB plates using Taq polymerase as recommended by the manufacturer (Sigma, Aldrich).

Reaction mixtures contained 2mM $MgCl_2$, 10mM TrisHCl, pH 8.3, 50mM KCl 200μM deoxynucleoside triphosphates, 2.5 units of *Thermus aquaticus* DNA polymerase, 0.2 μM of each oligonucleotide primer. Thermal cycling was as follows: denaturation at 95 °C for 1.5 min, annealing at 55 °C or 60 °C for 1.5 min and extension at 72 °C for 1.5 min for a total of 30 cycles.

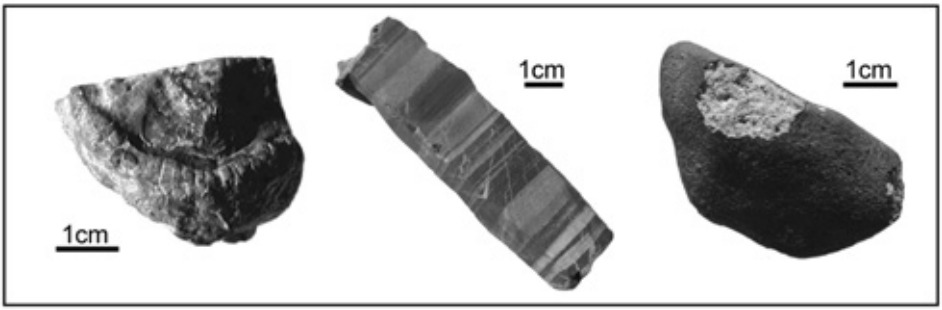

Fig. 1b. – *Left*: Archaecyathid specimen, from Lower Cambrian limestones (Nebida Formation) SW Sardinia (S CB16). *Center*: Laminated ironstone (BIF), Late Archean, Lake Temagami, Ontario, Canada. *Right*: Iperstenic chondrite; encrusted specimen (kindly provided by the «Real Museo Mineralogico», Naples, inv. 18338).

The oligonucleotide primer sequences to identify and amplify rDNA genes were as follows:

EubacF: agagtttgatcctggctcag; EubactR: ggttaccttgttacgactt, for eubacteria;
ArcheaF: ttccggttgatccygccgga; ArcheaR: yccggcgttgamtccaatt, for archea;
EukF: aacctggttgatcctgccagt; EukR: tgatccttctgcaggttcacctac, for eukaryotes.

Ribosomal DNA cloning and sequencing.

PCR amplified DNA, after the purification using the Nucleospin® Extract, a ready to use system for direct and fast purification of PCR products from agarose gels (Macherey-Nagel), was directly sequenced using Thermo Sequenase Cy 5.5 Dye Terminator Cycle Sequencing kit following the protocol suggested by the manufacturer (Amersham Pharmacia Biotech). The products were separated in a gel matrix using the automated sequencing instrument (SeQ 4×4 personal sequencer system). The purified, amplified eubacterial rDNAs were cloned by using the SureClone TM Ligation kit (Amersham Pharmacia Biotech). Insert-containing clones were identified by agarose gel electrophoresis of small-scale plasmid preparation (Maniatis *et al.*, 1982). Plasmid templates were sequenced as reported above.

Phylogenetic analyses.

Determined sequences were aligned to previously determined rDNA sequences using the program Clustal W at http://www.ddbj.nig.ac.jp.

RAPD analyses.

A total of 6 RAPD primers were tested for their ability to provide informative and reproducible RAPD profiles.

RAPD1 (5'ggtttccgccc3'),
RAPD2 (5'ggatcctgac3'),
RAPD3 (5'cggcccctgt3'),
RAPD4 (5'tcccgctgcg3'),

RAPD2T (5'ggttcctgtc3'),
RAPD4C(5'agggcgacgc3').

The results of the experiments with primer 1 and 2 are reported here, because they show clearly that the different clones have different genetic profiles. The PCR reaction mixture (25μl) contained 1X PCR buffer supplemented with 3mM $MgCl_2$ solution (Sigma Aldrich) 200μM each of the four deoxynucleoside triphosphates (dNTPs, Amersham Pharmacia Biotech), 500ng of 10 nucleotide primers (MWG-Biotech AG) 0.5 U of Taq DNA Polymerase (Sigma-Aldrich) and a small part of a single colony from agarose plate. PCR amplification was performed on a Perkin Elmer 2400 Thermal Cycler using the following cycling program: after denaturation at 94 °C for 3 min, the reaction mixtures undergo 45 cycles of denaturation at 94 °C for 0.5 min, annealing at 32 °C for 1min and extension at 72 °C for 2 min, with an additional 10 min extension at 72 °C. 15μl of each amplification products were speared on 1% agarose gel stained with 0.5mg/ml ethidium bromide, examined and photographed under a UV light. A 1.0 kb ladder DNA sample (MBI Fermentas) was used as a molecular weight marker. The RAPD reproducibility was established running all reactions in three independent experiments.

High temperature resistance tests.

These experiments were performed using a kiln for ceramic works Model HC Hexagon, T max 1260 °C equipped with a thermo computer (HC Hobbyceram, Milano, Italy). Rock samples, cut in cubes of approximately 0.5 cm, were tested for different time intervals at the reported temperatures (see below).

Scanning Electron Microscopy.

SEM images were obtained at the «Centro Interdipartimentale di Microscopia Elettronica» of the University of Naples «Federico II».

Results and discussion

In fig. 1*a,b* is reported the geological characterization of the rocks, crystals and mineral ores that have been examined by scraping finely fragmented material from inside the sample directly on an observation microscope slide, where it is suspended in a nutrient medium, covered and observed at 400-1000 enlargements (see methods). Also the chondritic meteorite MetA and the lower Cambrian sample GB-16 are shown as examples of alien and of >500 Ma specimen, respectively.

Samples of ages spanning from recent times to about 2.8 Ga and with different origins, from sedimentary to metamorphic rocks and from igneous rocks to meteorites, have been studied to search for viable microorganisms. In all cases the presence of actively swimming forms, was apparent, with the difference of the time taken to observe beginning of movement and the variety of forms present in the sample. In a number of cases such as minerals and sedimentary rocks, as well as gneiss, granites, gabbros, basalts and other samples (agate, obsidian), movement was apparent as soon as observation

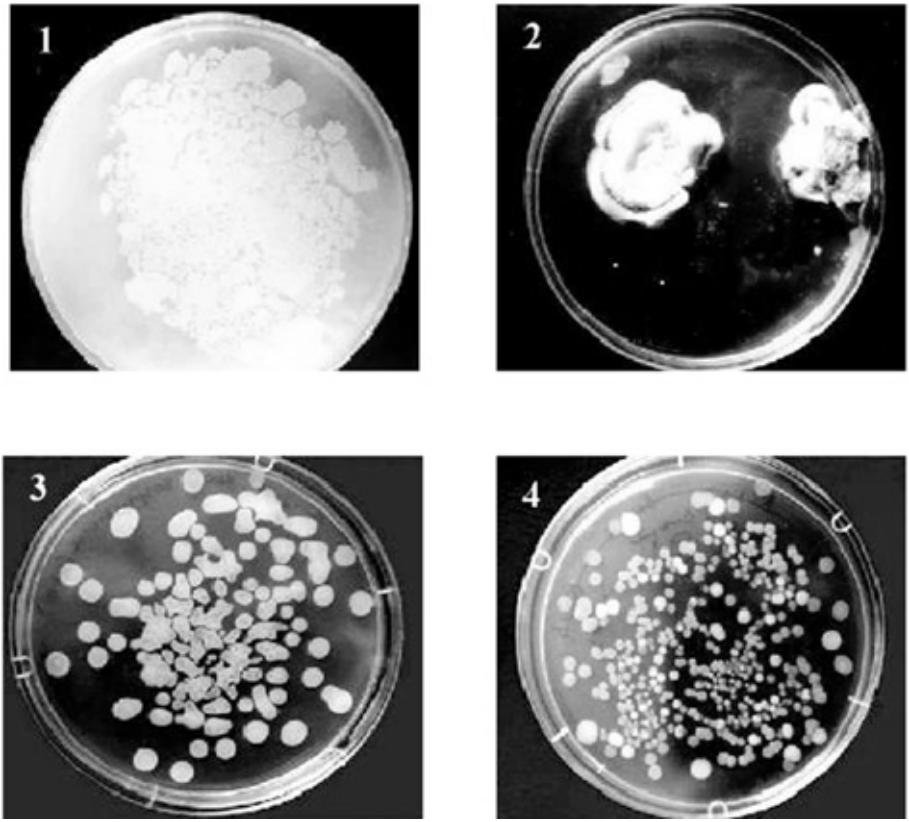

Fig. 2. – Examples of variety of colony forms observed when culturing microorganisms from some rock specimens. 1: Colony from limestone GB-6 producing a lace-like carbonated structure. 2: Structures emerging from two small fragments of the same rock in the nutrient medium. 3-4: Colonies from limestone GB-16. Note the variety of forms, sizes and colors in 4, an intermediate stage of a serial dilution to isolate single colonies.

started, indicating that the transition to the active form was practically immediate upon suspending the fragmented rock in the nutrient medium. The very large number of immediately active microorganisms, together with their assortments of sizes and forms, indicate that they are not a minor part of the samples, and this is a further indirect reason against possible origin due to external contamination.

Suspending fragmented samples in water also produced similar effect but the period of active movement lasted for only few minutes. In other cases observation of initial movement required a longer period of incubation. The longest time was typical of a dolomite sample where active movements were observed at times even one hour after the initial suspension of the powdered sample in the culture medium. The variety of microbial forms is higher in sedimentary rocks, such as limestone samples GB-6 and GB-16, lower Cretaceous and lower Cambrian respectively (fig. 2), where cyanobacteria are frequent, and in the former, together with some Archea and even a Eukaryotic cell (fig. 7).

The ability to resist the challenge of high temperature was tested on limestone samples GB-16 and GB-6, as well as on gabbro and agate samples cut in about 0.5 cm cubes from inside larger specimens. These cubes were heated in a kiln to 550 °C in a cycle lasting about six hours, starting from room temperature. All treated samples appeared not altered in their characteristics except some limited areas of sample GB-6 that showed a white appearance, probably due to initial formation of calcium oxide. Actively swimming microorganisms were still present in all tested samples as determined by the standard procedure, and were able to grow in a liquid medium. When a similar experiment was performed heating the samples at 920 °C, similar results were obtained except for the limestone samples, that were completely altered in their structure, and in which no activity was apparent by any method.

Samples from which microorganisms have been obtained in culture, cloned and characterized genetically by 16S rDNA typing as reported in table I.

The results of these studies show that all isolated clones are eubacteria, except a colony from sample GB-6, that is a eukaryote. Each cloned bacterial sample has sequence correspondence with a modern day bacterium, but each clone is genetically peculiar as demonstrated by RAPD analysis of their DNAs (fig. 3). In fact, different clones show distinctly different patterns of amplified bands although apparently belonging by 16S rDNA typing to the same bacterial species (table I).

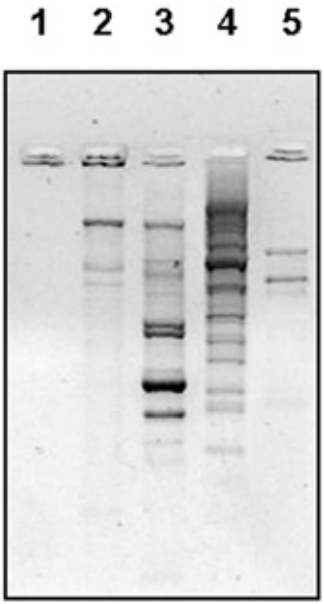

Fig. 3. – Genetic characterization by random polymorphic DNA analysis of the bacteria reported in table I. Lanes 1 to 5, respectively, negative control experiment, GB-6-clone-1; GB-16-clone 2; MetA-clone 1; GB-16-clone 1. Note that different patterns are produced, indicating that bacteria correlated to the same modern day species are indeed genetically different. Two probes were used in order to have patterns with a number of bands sufficient for adequate identification.

All isolated clones were tested for sensitivity to the most common antibiotics (Ampicillin, Tetracycline and Kanamycin), and all were found to be sensitive to their inhibition. This is at a variance with what found on psychrophil microbial isolates from surface soil and Italian spring waters which frequently show antibiotic resistance due to the presence of plasmids (Geraci *et al.*, unpublished data). The eukaryotic cell from limestone GB-6, apparently a *Candida*, was found not sensitive to bacterial antibiotics but to Nocobiotal, as typical of yeasts.

An example of sequence similarity of the isolated clones, reported in fig. 4, shows the sequence composition and the phylogenetic characterization of the 16S rDNA of bacterial clone C1 isolated from the chondritic meteorite MetA. The 16S rDNA of the clone is highly similar to that of three different bacterial species: *Bacillus pumilus, Bacillus subtilis* and *Bacillus* sp VAN 26. The phylogenetic trees, obtained by using Clustal W program for *pumilus* and *subtilis*, clearly show that the bacterium isolated from the meteorite is similarly related to both modern day organisms but appears to be a different strain (panel C, fig. 4).

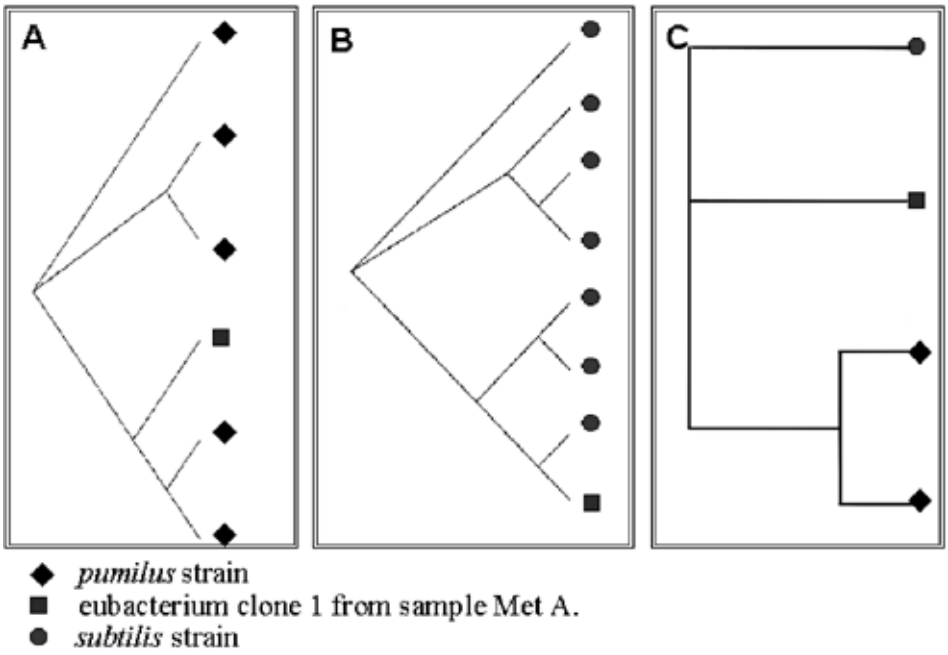

Fig. 4. – Phylogenetic trees of modern day *pumilus* (A) and *subtilis* (B) bacterial species, as derived by Clustal W analysis of sequences deposited in the Data Bank for 16S rDNA of bacteria, and their correlation with the eubacterium clone 1 from sample MetA (C).

Some bacterial strains isolated from samples of different ages have been analyzed by SEM. It is apparent that bacteria from older rocks are smaller in size than those from more recent samples. While the size of the *Bacillus* from lower Cambrian (>500 Ma)

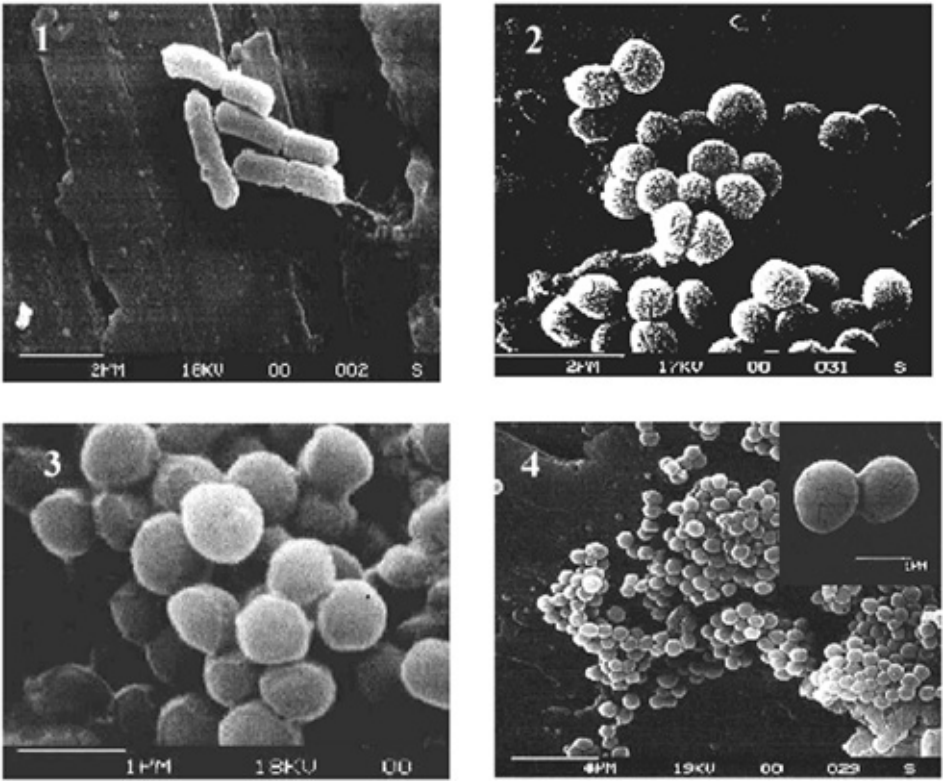

Fig. 5. – SEM of bacterial clones isolated from some rocks. 1-2: Eubacteria isolated from limestone GB-16. 3-4: Eubacteria isolated from limestone GB-6. For details see table I.

limestone GB-16 is about 0.65μm in diameter and about 1.2μm in length (fig. 5, panel 1) and the coccoid bacterium from the same sample is about 0.5μm in diameter (fig. 5, panel 2), the bacterium isolated from the more recent (lower Cretaceous >100 Ma) rock GB-6, is about 1μm in diameter (fig. 5, panels 3 and 4).

Many bacteria show the presence of micro-encrustations that cover them (fig. 5, panels 2 and 4) and all analyzed samples show the tendency to bind each other forming large aggregates and this is clearly evident also in bacterial isolated from meteorites (fig. 6). In this case (fig. 6, panels 1 and 3) the presence of encrustation on the bacterial cells is quite evident, as also the occurrence of structures joining adjacent individuals in a network of thread-like connections (fig. 6, panel 4).

The presence of an *Ascomycetes*, among microbes found in the limestone GB-6, is documented by the characteristic morphology of the cell (fig. 7). Genetic typing indicates that this cell is a *Candida*. However, both the small size of the cell and its growth in serum and in a medium specialized for *Candida*, are different from the corresponding parameters of the modern human pathogen species, to which it is genetically correlated (table I).

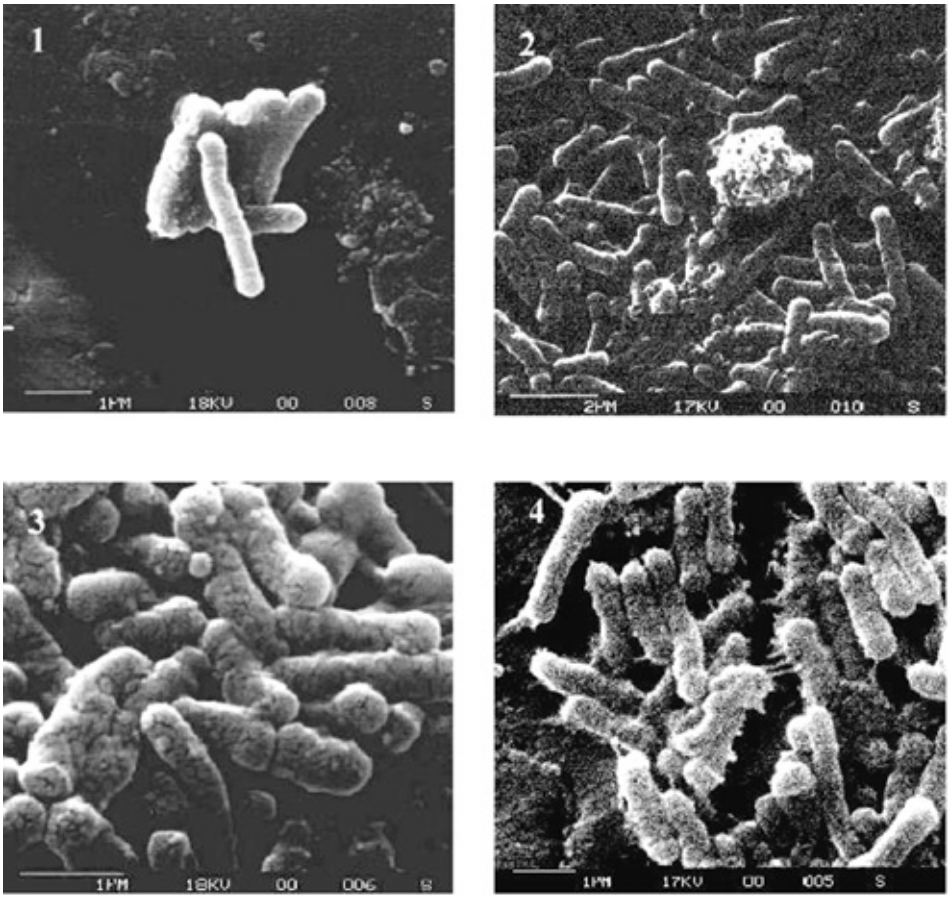

Fig. 6. – SEM of bacterial clones isolated from meteorites. 1 and 3: Eubacteria isolated from sample MetA. 2 and 4: Eubacteria isolated from sample MetC. For details see table I.

Conclusions

Microbial life appears to exist within crystals and solid rocks of different kinds, such as sedimentary rocks even deeply diagenized, metamorphic rocks, down to granulites, igneous rocks, from granite to gabbros and basalts.

The temperature-pressure boundary conditions to which underwent some of the mentioned rocks, suggests that microorganisms, if within rocks, may resist to pressures of 10 kbar, and to temperatures up to 1000 °C. The peculiar, unexpected and shocking ability of microorganisms to resist to destruction at such high temperatures, while in the rocks has been tested directly in the lab by heating different types of rocks in a kiln.

It appears from the genetic typing that eubacteria, similar to the present day organisms, were among those that initiated life on Earth and among the ancestors of modern living beings. The consequences of the possible addition to the modern genetic pools, of genes deriving from microorganisms released by the weathering of ancient rocks and

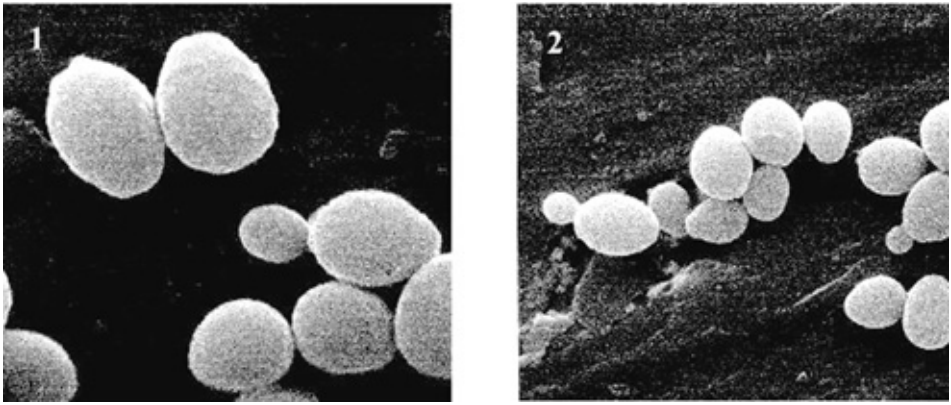

Fig. 7. – SEM of the eukaryote isolated from limestone GB-6, correlated to modern yeast *Candida* by 16S rDNA sequence.

meteorites, that may be a process continuously occurring on Earth, should be taken into consideration when evaluating phylogenetic relations.

The presence of eubacteria in meteorites of different kinds which cluster at about 4.5 Ga (Papike, 1998), speaks in favor of the hypothesis that life came from outside Earth, and, in addition, that it may have been already present in the initial materials by which accretion Earth and planets originated.

The results reported here contribute to the multidisciplinary research approaches that have so far indicated only the presence of non viable microbial forms in ancient rocks (Nisbet, 2000) and in meteorites (Gillet *et al.*, 2000; McKay *et al.*, 2001; Sleep *et al.*, 2001) and the occurrence of some live organisms in fluid inclusions of specimens of hundreds Ma (Vreeland *et al.*, 2000), in amber (Cano and Borucki, 1995) and in samples from deep holes drilled in the ocean floor (Barns and Nierzwicki-Bauer, 1997; Taylor *et al.*, 1999). The new form of life reported here, showing such peculiar properties, suggests the occurrence of parameters, not yet defined, governing interactions between life and energy that might be useful to take into consideration for further studies on the origin, preservation and propagation of life. Indeed the origin of life appears now even more difficult to visualize, if possible, than assuming its formation on Earth, since it seems to involve the outer space.

Also geology of Earth seems to require some reconsideration (*e.g.* for the relevance of microbes in crystal and rock formation) since an unexpected interdependence appears to exist between inorganic world and life.

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APPENDIX

Discussion

Held during the meeting of May 11, 2001 after the presentation by the Lincei fellow Bruno D'Argenio of the Note entitled «Microbes in rocks and meteorites: a new form of life unaffected by time, temperature, pressure» co-authored by Giuseppe Geraci and Rosanna del Gaudio.

Before opening the discussion The President of the Academy, Prof. Edoardo Vesentini, invites the appointed referees, Profs. Giovanni Chieffi and Annibale Mottana, to read their report of the Note.

Report by the referees

The manuscript has been examined by us on three occasions, the first two in the form of drafts, respectively May 3, 2001 and May 5, 2001, and the third as the definitive version, but still lacking illustrations, on May 10, 2001.

The paper reports the discovery of bacterial forms of life inside meteorites, rocks and minerals of various composition and geological age. This life-form seems not to have been affected by time, since it has been found in terrestrial rocks and even in meteorites, whose estimated age is contemporaneous with the formation of the planet Earth. It seems also not to have been affected by the temperature and pressure under which the rocks were formed, as inferred from its presence in metamorphic and igneous rocks, like granulites, gabbros and basalts. The resistance to extreme temperatures has been verified by experiments on various rocks, reduced to small cubes of 0.5 cm per side and subjected to brief cycles of heating up to 550°C in a pottery kiln and to direct exposure to a Bunsen burner flame for two minutes. Finally, the authors of the paper conclude that this is the first evidence of a form of extraterrestrial life, documented in its genetic and morphological properties.

The section on methodology, essential to ascertain the degree of possible contamination of the samples, is relatively lean, especially in the part concerning the preliminary treatment of the rocks (metamorphites, granitoids and granulites, meteorites). No matter how sterile might have been the operating environment of the biological laboratory, the previous history of the individual samples does not seem to have been investigated thoroughly, thus leaving room for doubt about the true representativeness of the samples under study. In particular, the first treatment of the samples with the diamond saw could have introduced extraneous (even organic) materials into the intergranular spaces; the subsequent washing with ethanol, followed by brief heating with the Bunsen burner flame might not have entirely removed such materials. Although not criticizing the procedure followed in the biological laboratory (which appears perfectly suitable for the degree of sterility required for normal terrestrial materials), the undersigned referees believe it appropriate to warn the authors about the need to strengthen their laboratory

controls in relation to the possible contamination in a terrestrial environment of the surfaces and intergranular spaces of the extraterrestrial materials; this also applies to the materials believed to derive from the depths of the Earth's crust and mantle on which the authors base their interpretation of the lack of effect of pressure on the discovered life-forms. Moreover, in this petrographic part, there is an excessive simplification of the petrological terminology, although this does not seem to be very important for the purposes of the study. However, it would be appropriate to describe in more detail the method of «scraping» with diamond, used for the hardest rocks, for which absolute sterility must be certified (or at least declared). Finally, the results of the heat treatment seem to be overrated, given the absence of an appropriate assessment of the thermal conductivity of the rocks: what temperature was actually reached in the internal volume of the sample examined, if its surface was heated to 800-900°C for a few minutes? Without this information, the hypothesis that the life-form is independent of the temperature is not confirmed.

The implications of the discovery, once it has passed the test of further verifications, would seem to have no limits, not only involving fields of Biology (the continuous mixing of ancient gene pools, deriving from the erosion of rocks, with modern ones), but also of Chemistry (the stability of the genomes, still intact after billion of years), of Physical Chemistry (the resistance to temperatures not compatible with the existence of organic materials, indicative of new parameters governing the interaction between energy and living matter), and of Astronomy (the obvious consequence that the life-forms could have arrived on Earth from outer space). Even though the observed characteristics are unusual and do not conform to current scientific knowledge, the procedures for the biological research, the identification of the microbial species present and finally their isolation by cloning were based on minutely described techniques, which make it appear practically impossible that they originated from banal contamination in the laboratory. This assessment is strengthened by the discovery of a multitude of individuals, of different morphologies, immediately active, in milligram fractions of rock fragments. It is also strengthened by the results of the genetic investigations (showing different bacterial types for the various clones isolated), by the SEM analyses (showing cellular morphologies and sizes certainly not typical of modern bacteria), and finally by the abilities of the life-forms in the rocks to resist temperatures that modern forms certainly cannot withstand. In addition, the absence of resistance to antibiotics in all the isolated bacteria is an amazing result, considering its diffusion in current microbial species.

In summary, the results of the biological examination (and their professionalism of supporting experimental details) lead one to conclude that any possible organic contamination is not current; if it ever occurred, it did so at one or more times in the previous geological history of the samples, thus deferring the result obtained to the topic of the initial geological context. We wish to emphasize the appropriateness of comparing the present data with similar previous cautious reports, less advanced in many details (especially the biological ones), such as the recent one by the French group on the Tatahouine meteorite (Gillet *et al.*, 2000).

The undersigned, despite their invitation to conduct further verifications especially concerning the modalities by which the bacteria could have come to be in the structure of the rock (fluid inclusions? intergranular interfaces?), propose that this *Note* be published in the *Rendiconti Lincei*: *Scienze Fisiche e Naturali* of the Academy, in the belief that it might stimulate completely new and fruitful areas of study for the general development of research in Italy.

GIOVANNI CHIEFFI ANNIBALE MOTTANA

The President then opens the discussion in which the following fellows participate: Arrigoni, Bonucci, Boriani, Fornaseri, Furlani, Graniti, and Capanna (*Director of the Editorial Advisory Committee of the* Rendiconti Lincei: Scienze Fisiche e Naturali). *Questions and comments have by necessity been abbreviated but they respect the concepts expressed by the fellow to whom they are ascribed.*

ARRIGONI. – Data presented on the occasion of the debate address a fundamental question in science: the origin of life on Earth. However, it is necessary to use great caution in accepting the interpretations given. The biological forms the authors extracted from meteorites can live in the presence of molecular oxygen. This is unexpected data, since, as far as we know, such organisms «arrived» on Earth much before the development of an oxygen-containing atmosphere. If so, they are supposed to be anaerobic. It would be very interesting to identify in these organisms the presence of «usual» biochemical defences against reactive oxygen species (superoxide dismutase, catalase, peroxidases) or even additional ROS detoxification mechanisms, such as hydrogen peroxide extrusion in the environment. A further concern I have, is the presence, in the specimens shown in the micrographs, of budding cells closely resembling yeasts. In principle, I think that the possibility of contamination by some «local» organisms should be considered.

BONUCCI. – I, too, have some doubts on the true bacterial nature of the structures Dr. Geraci and co-workers have described. The importance of the subject, on the other hand, requires rigorous controls. The demonstration that some RNA is present in *in vitro* cultures does not appear sufficient to confirm the presence of micro-organisms, because laboratory devices can be very easily contaminated by RNA. I would like to suggest some very simple controls, for instance, that total carbon is measured at the beginning and at the end of the cultures, to ascertain whether there is really an increase in organic material, and that the supposed bacteria are examined not only by scanning electron microscopy, but also by transmission electron microscopy, to control the presence of membranes or other organelles.

BORIANI. – I have a question for Bruno D'Argenio: you have been studying samples of igneous and metamorphic rocks. I feel it would be appropriate if you could specify which igneous and which metamorphic rocks you have been dealing with.

FORNASERI. – I am not in a position to express any opinion as far as the techniques used are concerned. My thoughts regard the situation of the announced results in the framework of our knowledge of the origin of the life, in which biology, geology and geochemistry are interconnected. If I correctly remember, different studies were made on the presence of amino acids in meteorites and how much caution was adopted to make sure that such organic compounds were confirmed in carbonaceous chondrites, excluding any suspicion of possible contamination. Something similar occurred regarding the presence of the so called «trace elements» in terrestrial rocks. It turned out that, at least in some cases, their presence was to be connected not only to the mineral chemistry of the rock itself, but to the existence of intergranular discontinuities which allowed the circulation of foreign trace elements dissolved in waters. Many factors have to be taken into account before a new occurrence may be definitely accepted.

FURLANI. – I am astonished at the presentation of a report which, although written by investigators of known capacity and reputation, and with apparently correct methodology, claims independence of investigated phenomena from both time and temperature, contrary to the strict rule that each report must bear clear indications of experimental parameters (temperature, pressure, time evolution), of their change, of their field of stability and of their reproducibility. Furthermore, as a surface chemist I know through my own experience how difficult it is to obtain a really clean surface: mere exposure to air suffices to cover even a freshly produced surface with a very thin but measurable (e.g. by XPS) layer of adsorbed matter including gases, volatile impurities, carbonaceous compounds, and not excluding bacteria. In my opinion there is not yet sufficient evidence that the reported phenomena are not due to contamination from air or other terrestrial sources, and the reported experiments should be carefully checked for reproducibility and absence of possible contaminants, in order to achieve a set of really significant and complete experimental data, upon which to start a serious discussion.

GRANITI. – It would be useful to know the death temperatures for the bacteria and yeasts you have grown in culture. One would expect values higher or at least in the range of those that you used to treat the stones before plating. If not, it would be an apparent discrepancy.

STEFANINI. – In major scientific journals, papers presenting data highly provocative but not yet supported by definitive evidence, are often published together with a commentary finalized to underline problems and perplexities arisen. I propose to adopt a similar procedure by publishing the debate that has followed the presentation by the colleagues D'Argenio and Geraci. Finally, I would like to ask the authors why they have not primarily submitted their manuscript to a scientific journal with anonymous and world-wide peer review; many of us would have preferred to read their data in a major scientific journal such as *Nature* or *Science*.

CAPANNA. – My participation in the discussion fits well at the end of debate since I have been entrusted by the Accademia with the responsibility for the publication of

the *Rendiconti Lincei* and thus of this *Note*. I will not deny my disappointment that the *Note* presented today has not followed the normal procedure for the presentation of *Notes*: Only this morning did I read the text, still lacking the figures, and only a few moments ago the opinions of the referees who, I wish to underline, were not assigned that function by the head of the editorial committee. No matter who designated the referees, their judgement compels the person responsible for the *Rendiconti Lincei* to publish the *Note*. However, it should be stressed that in the conclusion of this judgement several doubts emerge, and the need for further experiments is suggested.

My own doubts are also strong because I know what a crystal lattice is and its size, and I also know the size of a simple micro-organism, even an archeobacterium: the two are not congruent. Obviously the biological material examined cannot fit inside the mineral, but only between the components of the rock, in the intergranular spaces, spaces that can be reached by external factors of contamination.

The other point, already touched on in other comments, concerns the fact that the «resuscitated» colonies' resistance to temperature was not tested.

As the person responsible for the *Rendiconti Lincei*, I am willing to publish this *Note*, which I am sure will stimulate an international debate. However, I want the members of the Accademia Nazionale dei Lincei to be the first to enter this debate. Therefore, accepting the proposal that emerged during the discussion, I will include the text of this discussion in an appendix to the *Note*.

Finally, I am reminded of the controversy between Francesco Redi and Athanasius Kircher on the subject of spontaneous generation more than 300 years ago. We all know that Redi's experiments were conducted correctly, exactly because they avoided contamination of the sample. Kircher, however, could think of no better response than: «[...] *ut proinde falsissimum sit, tale et tale experimentum a Redo factum successum non habuit, ergo a Kircherio factum, falsum est*», which translated from 17th century Latin is: «I don't understand why if an experiment is successful for me but not for Redi, I am wrong and Redi is right».

Authors' reply. – We wish to thank our colleagues for their criticisms and suggestions, that certainly we take into great consideration. We think that it is not necessary to add further specifications, since answers to most questions can be found directly in the paper. Finally, we would like to point out that we are well aware that these are initial results and that further confirmation is required to define more accurately what is reported here.

Note added to proofs. – To answer an observation presented by the Editor concerning our statement about the examined samples, we specify that 12 colonies deriving from 6 samples of rocks and meteorites have been cloned and classified (table I) while about 50 samples from different types of rocks and meteorites (fig. 1) have been inspected under the microscope for microbial activity (G.G., R.d.G., B.D'A.).