

## Research Paper

# Iron Meteorites Can Support the Growth of Acidophilic Chemolithoautotrophic Microorganisms

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### ABSTRACT

Chemolithoautotrophy based on reduced inorganic minerals is considered a primitive energy transduction system. Evidence that a high number of meteorites crashed into the planet during the early period of Earth history led us to test the ability of iron-oxidizing bacteria to grow using iron meteorites as their source of energy. Here we report the growth of two acidophilic iron-oxidizing bacteria, *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans*, on a piece of the Toluca meteorite as the only source of energy. The alteration of the surface of the exposed piece of meteorite, the solubilization of its oxidized metal constituents, mainly ferric iron, and the formation of goethite precipitates all clearly indicate that iron-meteorite-based chemolithotrophic metabolism is viable. Key Words: Iron meteorites—Irons—Toluca meteorite—Iron-oxidizing bacteria—Acidophilic chemolithotrophic microorganisms. *Astrobiology* 5, 406–414.

### INTRODUCTION

**A**CIDOPHILIC STRICT CHEMOLITHOTROPHIC microorganisms that are able to grow with metal sulfides (mainly pyrite) as their only source of energy have been an important subject of research with regard to environmental concerns about acid mine drainage and biotechnological potential in biomining (Ehrlich, 2002). More recently, these organisms have proved important in astrobiological research (Fernández-Remolar *et al.*, 2003, 2004). Because of the redox potential of the different components of pyrite, sulfur-metabolizing microorganisms drew a great deal of atten-

tion. However, the recent application of molecular ecology techniques to study microbial populations involved in the dissolution of sulfidic minerals has shifted research efforts from microbial cycling of sulfur to that of iron (González-Toril *et al.*, 2003).

Iron meteorites (irons) are thought to form at the center of large differentiated planetoids. They are the closest physical analog to the material that forms the outer core of the Earth. Taken together, all irons detected on Earth make up a total known weight of more than 500 tons, and they represent approximately 90% of the entire mass of all known meteorites. Irons rarely fragment upon

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entering the atmosphere and suffer much less from the effects of ablation during their passage through the atmosphere. In fact, the largest meteorites are irons, *e.g.*, the Hoba iron in Namibia, the largest meteorite known on Earth, weighs approximately 60 tons (see <http://www.meteorite.ch/en/classification/ironmain.htm>).

Irons consist primarily of Fe-Ni-Co alloys (around 99%) with minor amounts of carbon, sulfur, and phosphorus. They are made up mostly of kamacite and taenite. Kamacite ( $\text{Fe}^{0+}_{0.9}\text{Ni}_{0.1}$ ; ferrite  $\alpha$ ) has a low nickel content (between 4% and 7%), while the nickel content in taenite ( $\text{Fe}^{0+}_{0.8}\text{Ni}_{0.2}$ ; austenite  $\gamma$ ) can reach as high as 50%. There are three main structural groups of irons: hexahedrites (*e.g.*, Coahuila), octahedrites (*e.g.*, Toluca), and ataxites (*e.g.*, Hoba). The octahedrites, which represent 70% of iron meteorites, are characterized by an intermediate nickel content (6–17% by weight) and contain both kamacite and taenite. The Toluca meteorite is a polycrystalline, coarse octahedrite (Buchwald, 1975). Thousands of meteoritic fragments were found in Xiquipilco, Toluca Valley, Mexico, in 1776 (Chladni, 1819).

Given that chemolithotrophy is considered to have been a valuable metabolic system in the origin and early evolution of life on Earth (Wächtershäuser, 1992) and a vast number of meteorites impacted the planet during this same period of time (3.7–3.8 Gyr) (Schoenberg *et al.*, 2002), we decided to test the hypothesis that iron-oxidizing microorganisms could benefit energetically from the alloy constituents of iron meteorites.

## MATERIALS AND METHODS

### *Microorganisms*

*Leptospirillum ferrooxidans* strain 3.2 and *Acidithiobacillus ferrooxidans* strain Musta isolated from the Tinto River were used (Malki, 2003; Parro and Moreno-Paz, 2003).

### *Meteorite*

The average elemental composition of the Toluca meteorite (Chladni, 1819) consists of 90.5 wt% Fe, 8.14 wt% Ni, 0.49 wt% Co, 0.16 wt% P, and 0.7 wt% S, with minor amounts of C (240 ppm), Ga (70 ppm), Ge (246 ppm), Cu (140 ppm), Zn (33 ppm), Cr (4.9 ppm), and Ir (1.9 ppm)

(Buchwald, 1975). Mineral phases were identified by transmitted and reflected light microscopy, x-ray diffraction, and electron microprobe analyses. Major mineral phases include kamacite and taenite along with troilite-graphite nodules. Ureyite, clinoenstatite, olivine, schreibersite/rhabdite, zircon, and sphalerite were detected as minor mineral phases (El-Goresy, 1965; Frondel and Klein, 1965; Harper-Charles, 1989; Skala and Cisarova, 1999). A 143-g specimen of the Toluca meteorite provided by the Natural History Museum of Madrid (Muñoz-Espadas *et al.*, 2002) was used in this work. Two samples, 0.5 cm wide and 0.5 cm high and weighing approximately 5 g each, were polished under dry conditions.

### *Sterilization*

The polished samples of meteorite were sterilized at 140°C for 12 h (López-Archilla *et al.*, 2001) to eliminate endogenous contaminating microorganisms. The enrichment medium was sterilized as described previously (Mackintosh, 1978).

### *Culture conditions*

In the first experiment, one polished and heat-sterilized piece of the Toluca meteorite was incubated with equal amounts of the acidophilic iron-oxidizing bacteria *L. ferrooxidans* and *A. ferrooxidans* in a 250-ml flask that contained 100 ml of minimal (*i.e.*, without trace elements or energy source) Mackintosh media (Mackintosh, 1978). The initial cell density of each population was  $6 \times 10^6$  cells/ml. Cells were washed with minimal Mackintosh media until complete removal of residual iron prior to inoculation. The initial pH of the media was 1.8. The only source of energy was the reduced inorganic components of the meteorite, which consisted mostly of iron.

In the second experiment another polished and heat-sterilized piece of the Toluca meteorite incubated in the minimal Mackintosh media without bacteria provided a negative control.

In the third experiment the growth capacity of the chemolithotrophic bacteria used in this work was monitored by incubating a mixture of both bacterial populations in Mackintosh media that contained trace elements and ferrous iron as an energy source, using conventional growth conditions for these types of cells (López-Archilla *et al.*, 2001).

In the fourth experiment a negative control for comparison with the growth capacity of the

chemolithotrophic bacteria used in this work consisted of a mixture of both bacterial populations incubated with no energy source in the minimal Mackintosh media. All flasks were incubated at 100 rpm and 30°C in the dark.

#### Iron determination

Ferrous and ferric iron contents in solution were measured using a colorimetric reaction method with 2,2'-dipyridyl ketone benzoyl hydrazone (Nakanishi and Otomo, 1986). Total iron was determined by atomic absorption spectroscopy in a Varian model FS220 instrument (Varian Ibérica S.L., Madrid, Spain).

#### Cell counts and fluorescence *in situ* hybridization

Total and specific cell counts were acquired by visualizing the cells with 4',6'-diamidino-2-phenylindole (DAPI) stain and fluorescence *in situ* hybridization at different times during the incubation. Hybridization and enumeration of hybridized and DAPI-stained cells were performed as described previously (González-Toril *et al.*, 2003). Mean cell counts were calculated by using between 10 and 20 randomly chosen fields for each filter section, which corresponded to 800–1,000 DAPI-stained cells (Amann *et al.*, 1995). Probes used in this work are listed in Table 1. Cy3-labeled probes were synthesized by Qiagen (Barcelona, Spain).

#### Scanning electron microscopy (SEM)

Untreated (unpolished and non-sterilized) and incubated meteorite pieces were analyzed in an FEI Quanta 200 environmental scanning electron microscope (20.0 kV and 10 mm working distance, FEI Co., Hillsboro, OR) coupled to an energy-dispersive x-ray spectroscopy (EDS) probe from Oxford Analytical Instruments-INCA (Concord, MA). The technical characteristics of the SEM used in this work allowed a direct analysis

of the sample, thus retaining all of the components present in the sample after incubation. The meteorite piece was removed from the incubation flask, air-dried, and analyzed.

#### Raman spectroscopy

Micro-Raman spectra were taken from the surface of the incubated meteorite samples and from the precipitates that appeared in the solution in which the meteorite pieces were incubated. The meteorite pieces removed from the incubation solution were air-dried prior to their analysis. Precipitates from the incubation solution were collected by vacuum filtration through a nitrocellulose filter (pore size, 0.22  $\mu\text{m}$ ). Micro-Raman spectra were acquired using a HoloLab 5000 unit (Kaiser Optical, Ann Arbor, MI) (Rull *et al.*, 2004). Illumination was performed with an He-Ne laser operating at 632.8 nm. The power on the samples was approximately 3 mW to avoid thermal degradation and possible phase transformations. The spectral resolution ranged between 3 and 5  $\text{cm}^{-1}$  (Rull *et al.*, 2004). The spectrometer was coupled to a Nikon (Düsseldorf, Germany) microscope equipped with 50 $\times$  and 100 $\times$  objectives through a Kaiser Optical Mark II holographic Raman probe head. The diameter of the laser spot on the sample was 10  $\mu\text{m}$  for the 50 $\times$  objective and 5  $\mu\text{m}$  for the 100 $\times$  objective.

## RESULTS

After 24 h of incubation at 30°C with agitation a reddish color appeared in the flask containing the iron-meteorite and chemolithoautotrophic microorganisms (Fig. 1). The development of color in the flask was followed by an increase in the number of chemolithotrophic microorganisms measured with fluorescent hybridization probes (Fig. 2). After 1 week, the reddish hue of

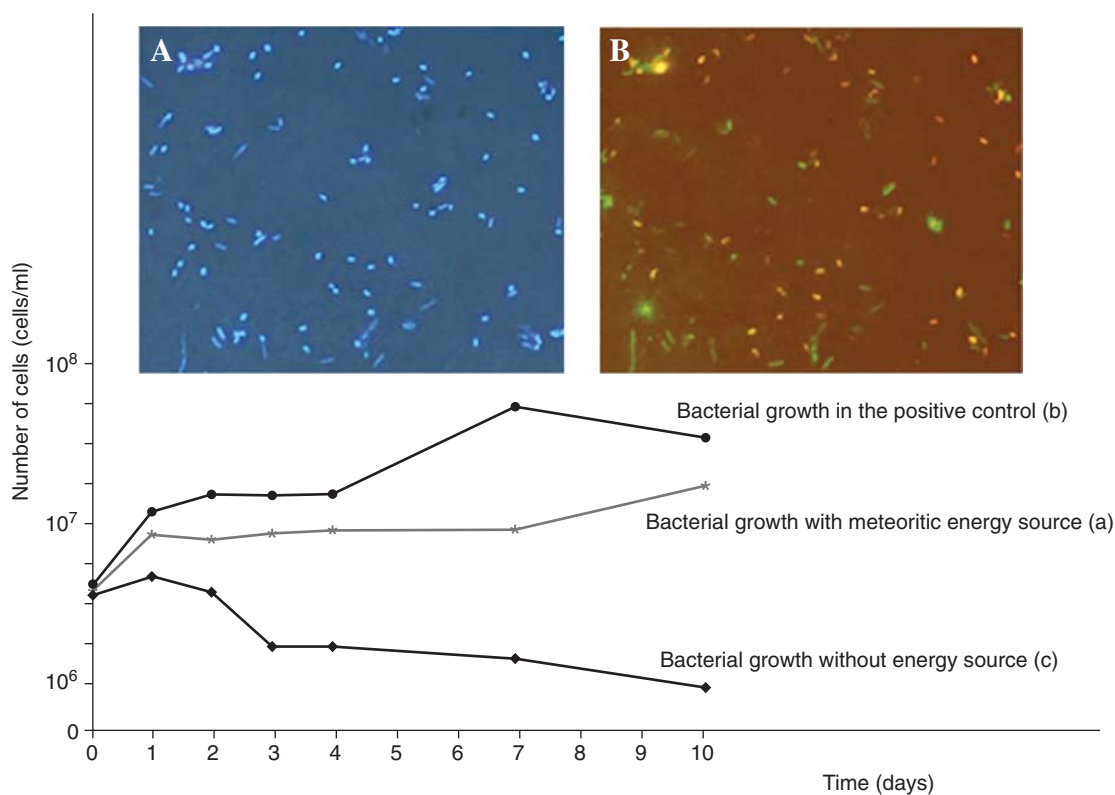
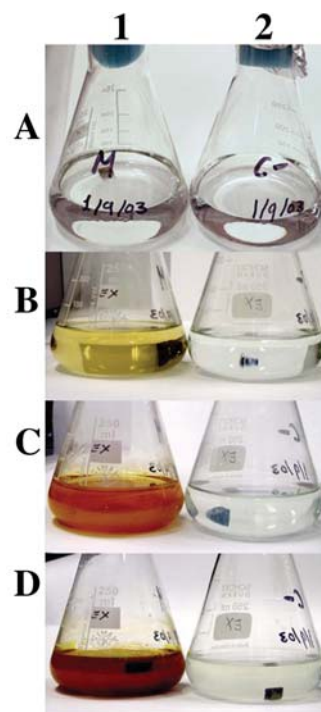
TABLE 1. FLUORESCENCE-LABELED OLIGONUCLEOTIDE PROBES USED FOR *IN SITU* HYBRIDIZATION EXPERIMENTS

Probe	Target	Sequence (5' to 3')	[%] FM <sup>a</sup>	Specificity	Reference
EUB338	16S	GCT GCC TCC CGT AGG AGT	35	Bacteria domain	Amann <i>et al.</i> (1990)
NON338	16S	ACT CCT ACG GGA GGC AGC	35	Negative control	Amann <i>et al.</i> (1990)
THIO1	16S	GCG CTT TCT GGG GTC TGC	35	<i>Acidithiobacillus</i> spp.	Stoffels (unpublished data)
NTR712 <sup>b</sup>	16S	CGC CTT CGC CAC CGG CCT TCC	35	Nitrospira group	Daims <i>et al.</i> (2001)
NON338	—	ACT CCT ACG GGA GGC AGC	35	Negative control	Amann <i>et al.</i> (1990)

<sup>a</sup>Percent (vol/vol) formamide in the hybridization buffer.

<sup>b</sup>The name of this probe in the original publication is S<sup>\*</sup>-Ntspa-0712-a-A-21.

**FIG. 1. Oxidation of Toluca meteorite by iron-oxidizing bacteria.** Flask number 1, mixed culture of *L. ferrooxidans* ( $6 \times 10^6$  cells/ml) and *A. ferrooxidans* ( $6 \times 10^6$  cells/ml) in minimal Mackintosh media with a piece of sterile Toluca iron meteorite as the only source of energy (experiment 1). Flask number 2, meteorite incubated in minimal Mackintosh media in the absence of bacteria (control experiment 2). **A:** Zero-time. **B:** 1 day of incubation. **C:** 3 days of incubation. **D:** 7 days of incubation.



**FIG. 2. Chemolithotrophic cell growth followed by fluorescence *in situ* hybridization.** Increase in the number of cells per milliliter over time is shown for (curve a) mixed culture of *L. ferrooxidans* and *A. ferrooxidans* ( $6 \times 10^6$  cells/ml of each) (experiment 1) minimal Mackintosh media with a meteoritic source of energy, (curve b) positive control (experiment 3), mixed culture of *L. ferrooxidans* and *A. ferrooxidans* in minimal Mackintosh media with ferrous iron as energy source (20 g/L), and (curve c) negative control (experiment 4), mixed culture of *L. ferrooxidans* and *A. ferrooxidans* (same cellular density as in the positive control in minimal Mackintosh media without energy source). **A** and **B:** Epifluorescence micrographs of bacteria from the mixed culture with meteoritic energy source (after 7 days). **A:** DAPI-stained cells. **B:** Same field as **A** showing cells hybridized with GAM42a probe in green (fluorescein-labeled) specific for  $\gamma$ -Proteobacteria (*A. ferrooxidans*) and NTR712 probe in orange (Cy3-labeled) specific for *Nitrospira* (*L. ferrooxidans*). Bar = 5  $\mu$ m.

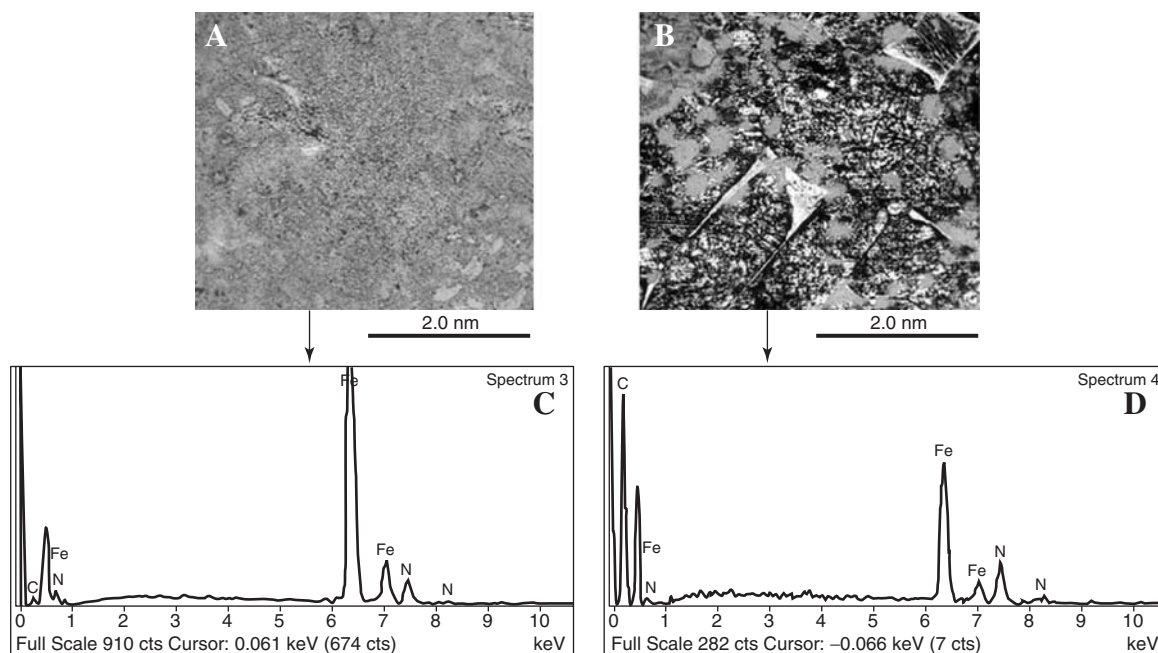
the water intensified, and a massive reddish precipitate appeared in the flask where the bacteria were in contact with the meteorite (Fig. 1). The control experiment without bacteria showed the development of a slightly reddish color (Fig. 1).

After 10 days the measured number of total DAPI-stained cells had increased from  $6 \times 10^6$  cells/ml to  $3 \times 10^7$  cells/ml (Fig. 2). The number of cells detected with the bacterial universal probe (EUB338) was similar to those detected with the specific probes for *L. ferrooxidans* (NTR712) and *A. ferrooxidans* (THIO1). During the first 2 days, the growth of *L. ferrooxidans* was faster, but after the third day the number of *A. ferrooxidans* increased more rapidly (data not shown). The hybridizations with a negative probe (NON338) gave negative results in all samples. The positive control, a mixed culture of *L. ferrooxidans* and *A. ferrooxidans* in standard Mackintosh media with ferrous iron as an energy source, showed a higher yield of growth,  $8.5 \times 10^7$  cells/ml, after 10 days (Fig. 2). The shape of the growth curves was similar in both cases. In the negative control, a mixed culture with no energy source, the number of cells decreased with time, from  $6 \times 10^6$  cells/ml to  $1 \times 10^6$  cells/ml after 10 days of incubation (Fig. 2).

An analysis of the incubation solutions after 1 week at the same ionic conditions showed a considerable concentration (592 ppm) of soluble ferric iron for the first experiment, the iron meteorite exposed to bacteria, when compared with the concentration (3.9 ppm) detected in the control experiment. Beyond 1 week of incubation most of the oxidized iron in the microbial-exposed meteorite appeared in the form of massive precipitates in the solution (Fig. 1).

SEM analysis of the exposed meteorite pieces showed a marked modification of the surface of the meteorite that was in contact with bacteria. Large deposits of low-contrast material were distributed on the surface of the meteorite along with signs of structural modification that were absent in the non-incubated meteorite, and negligible in the control incubated in the absence of bacteria (Fig. 3A and B). Elemental analysis of different sections of the microbial-exposed meteorite showed the presence of carbonaceous material associated with the altered areas, which corresponded mainly to kamacite and taenite (Fig. 3D).

Moreover, a detailed *in situ* micro-Raman analysis was performed on the surfaces and on the red precipitates of the bacterial-incubated meteorites and the control incubated in the absence



**FIG. 3. SEM and EDS analysis of the exposed Toluca meteorite.** **A:** Piece of meteorite incubated in minimal Mackintosh media during 1 month in the absence of bacteria (experiment 2). **B:** Intensively altered meteorite due to bacterial activity after 1 month of incubation (experiment 1). **C** and **D:** EDS analysis of the corresponding areas of the A and B preparations. Note the carbon signal associated with the altered areas of the meteorite exposed to bacteria.

of bacteria. Spectra showed the presence of different iron minerals formed as a consequence of the oxidation of the iron present in the meteorite (Fig. 4). In the case of the control sample, hematite, magnetite, goethite, and lepidocrocite were the main minerals observed on the surface of the meteorite and in the slight precipitate that appeared in the solution after 1 month of incubation in the absence of bacteria (Fig. 4, spectrum A).

The microbial-exposed meteorite showed mainly goethite, located on both the surface of the meteorite and in the massive precipitates collected by filtration, in association with some lepidocrocite (Fig. 4, spectrum B). Comparison between both series of spectra indicated some differences in their band parameters. The microbial-incubated piece showed intensities and full bandwidths higher than in the control piece.

Carbon was also identified mainly in the incubation piece (Fig. 5), and in some cases it was associated with hematite. The observed broad bands at 1,340 and 1,600  $\text{cm}^{-1}$  correspond to a very disordered carbon form that is consistent with a biological origin.

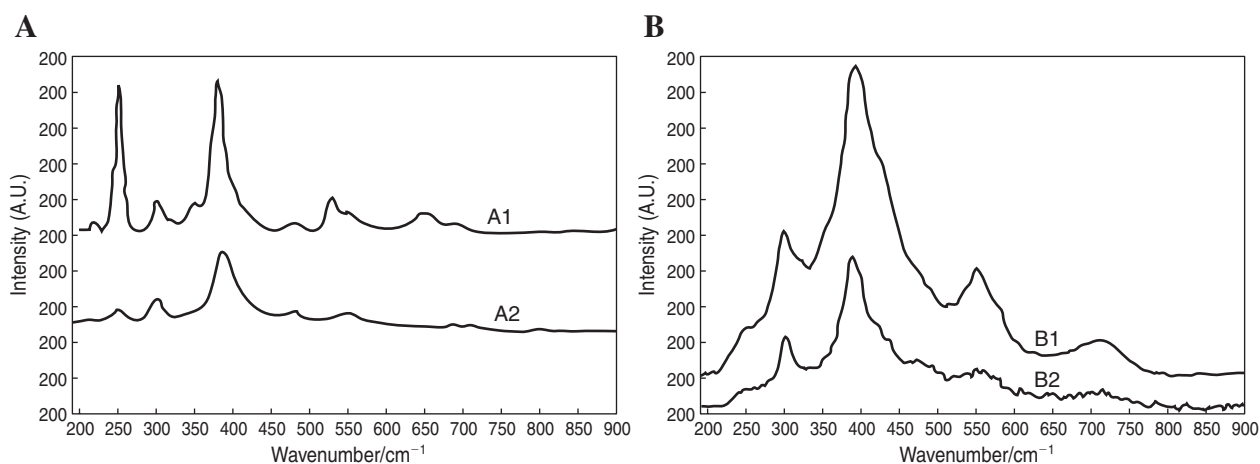
In some cases the  $\nu_1(\text{SO}_4^{2-})$  band of sulfates was observed in association with goethite. This band appears at 982  $\text{cm}^{-1}$  and is associated with a hydrated form of iron sulfate, probably melanterite, although schwertmannite cannot be ruled out. This band was present in the spectra of the precipitates from the bacterial-incubated piece, and was less intense in the spectra from the

precipitates of the control piece incubated in the absence of bacteria (Fig. 6). It was also detected on the surface of the bacterial-incubated piece but was not observed on the surface of the control piece.

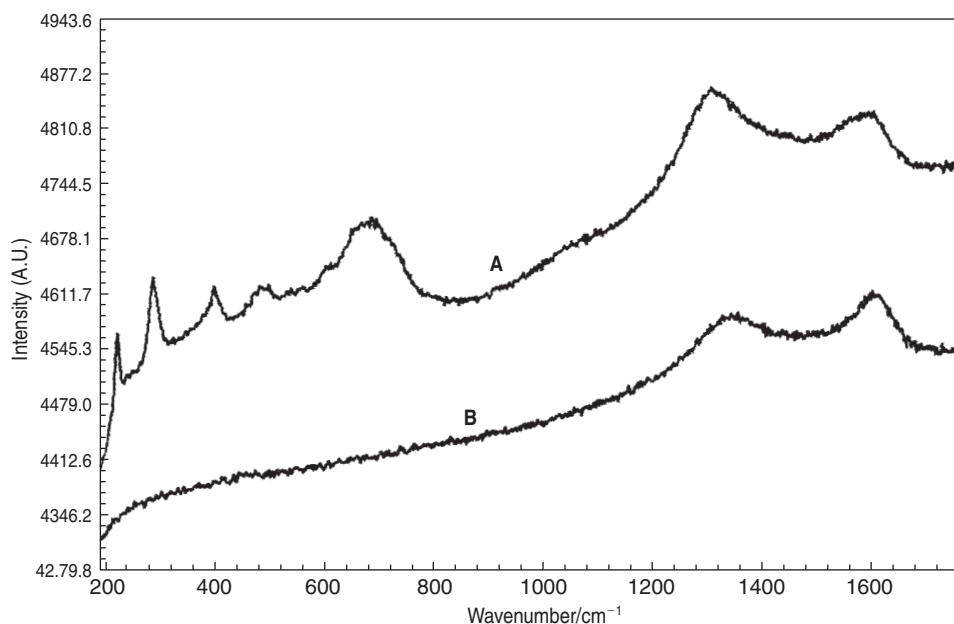
## DISCUSSION

The results obtained after exposure of a piece of Toluca meteorite to acidophilic iron-oxidizing microorganisms, a mixture of *L. ferrooxidans* (a strict iron-oxidizing bacterium) and *A. ferrooxidans* (a strict chemolithotroph able to oxidize iron and reduced sulfur compounds), indicate that the development of color (Fig. 1), due to the solubilization of oxidized iron, was the consequence of active chemolithotrophic metabolism using the reduced metal components, mainly iron, of the meteorite.

Although some residual oxidation was observed in the absence of bacteria, probably because of the chemical attack of the iron meteorite produced by the components of the culture media, the presence of chemolithotrophic microorganisms increased significantly the amount of iron oxidized after 1 week of incubation, to a rate similar to the microbial oxidation of pyrite at acidic pH (González-Toril *et al.*, 2003). The chemolithotrophic microorganisms used the reduced meteoritic iron as a source of energy for active growth. The cell density of both iron-oxi-



**FIG. 4.** Micro-Raman spectra obtained from the surface and precipitates of the non-exposed (A) and bacterial-exposed (B) Toluca meteorite. Spectrum A1, goethite and lepidocrocite on the surface of the meteorite incubated in the absence of bacteria (experiment 2); Spectrum A2, goethite in the slight precipitate generated in the solution after 1 month of incubation in the absence of bacteria (experiment 2); Spectrum B1, goethite from the massive precipitate generated in the solution of the meteorite exposed to bacteria (experiment 1); and Spectrum B2, goethite on the surface of the meteorite exposed to bacteria (experiment 1).

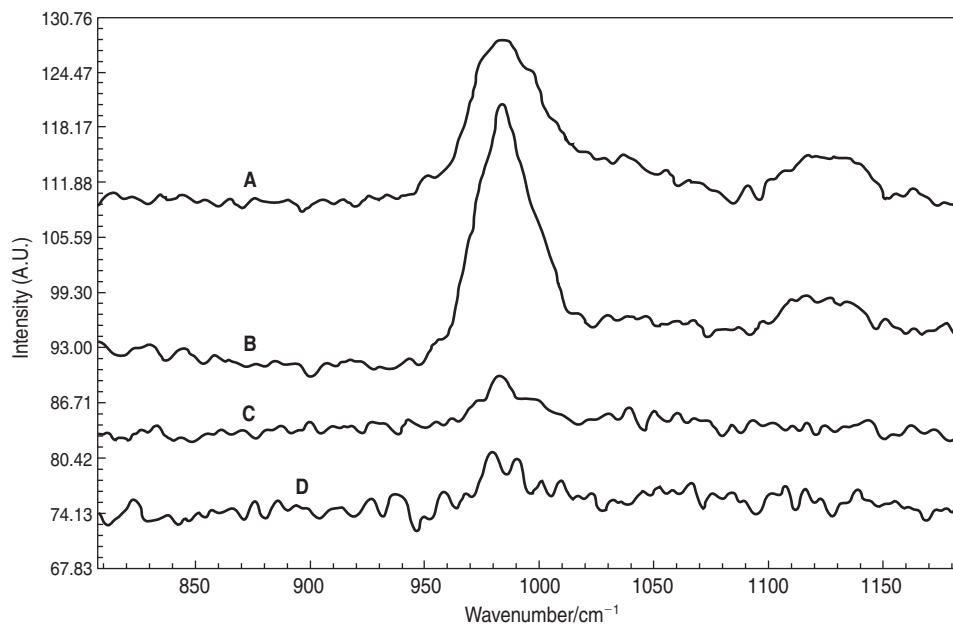


**FIG. 5. Raman spectra of amorphous carbon from the incubated meteorite piece.** Spectrum A, carbon associated with hematite and magnetite; Spectrum B, pure carbon. In A the  $1,340\text{ cm}^{-1}$  carbon band overlaps with the  $1,300\text{ cm}^{-1}$  band of hematite.

dizers, *L. ferrooxidans* and *A. ferrooxidans*, increased after incubation with the meteorite as shown by the hybridization using specific probes for each of the microorganisms (Fig. 2). The decrease in cells in the negative control, mixed culture of chemolithotrophs in the absence of energy

source, was probably due to cell lysis of metabolic inactive microorganisms (Fig. 2).

SEM analysis revealed a distinctive evolution of the surface of the meteorite. The meteorite exposed to iron-oxidizing microorganisms showed the presence of large deposits of low-contrast ma-



**FIG. 6. Raman spectra of sulfate.** Spectra A and B, precipitates from the solution of incubated meteorite (experiment 1); Spectrum C, precipitate from the control piece incubated in the absence of bacteria (experiment 2); and Spectrum D, surface of the bacterial-incubated meteorite (experiment 1).

terial that gave a strong carbon signal using EDS analysis. The alteration was negligible in the control experiments and absent in the untreated meteorite. The organic material detected on the modified meteorite surface appears to be a combination of microbial cell remnants and the extracellular polysaccharides synthesized to facilitate cell adherence to the solid substrate. The formation of iron minerals, mainly goethite, is attributed to the transfer of electrons from the substrate to the electron transfer chain (respiration) in a manner similar to their physiological performance in the habitat from which they were isolated (Fernández-Remolar *et al.*, 2003).

The micro-Raman spectroscopy analysis also showed a clear difference between the microbial-exposed meteorite and the control incubated in the absence of bacteria. In addition to some weak bands corresponding to amorphous forms of carbon, presumably of biological origin, the analysis of both the surface of the meteorite exposed to bacteria and of the massive precipitates showed a spectrum with broad bands corresponding mainly to goethite. Micro-Raman spectra from the surface of the meteorite used in the control experiment consisted of sharp spectra corresponding to small amounts of different iron minerals: hematite, magnetite, goethite, and lepidocrocite. Similar spectra were observed in the precipitates formed after 1 month of incubation in the absence of bacteria. The broader and less defined bands of the microbial-exposed meteorite suggest a less ordered crystalline structure for goethite, a characteristic consistent with a biological genesis (Fig. 4).

The existence of sulfates mainly in the precipitates from the bacterial-incubated piece is consistent with the bacterial oxidation of ferrous to ferric iron ions resulting in the precipitation of Fe oxyhydroxides and oxyhydrosulfates (Buckby *et al.*, 2003). In this case the source of sulfates was mainly due to the components of the incubation media.

It is important to emphasize that this is the first time, to our knowledge, that the behavior of chemolithoautotrophic microorganisms has been positively tested in relation to typically extraterrestrial crystallographic arrangements of Fe, made up of mineral phases (kamacite, taenite) that are practically nonexistent on our planet.

Our findings have important implications with regard to the transfer of life between planets in a solar system. First, chemolithoautotrophic metab-

olism can easily develop on iron meteorites. Only appropriate physical conditions (water and temperature) are needed for microorganisms such as the ones studied here to grow on meteoritic iron. Second, the work supports the hypothesis that microorganisms can survive the harsh conditions of an interplanetary trip when hosted in the interior of an iron-rich asteroid (Horneck *et al.*, 1994; Weiss *et al.*, 2000; Naiper, 2004; Wallis and Wickramasinghe, 2004). Finally, we propose that chemolithoautotrophic microorganisms can use iron meteorites as a source of energy for effective growth when they land in a new, and probably, at least initially, inhospitable planet.

## CONCLUSIONS

Iron-oxidizing bacteria are able to grow on the iron-nickel alloy present in iron meteorites. The oxidation of the iron first promotes its solubilization followed by the generation of ferric oxides, mainly goethite, a pattern similar to the one that occurs when iron in pyrite is oxidized. This experiment proves that iron meteorites are a suitable substrate for some acidophilic chemolithotrophic microorganisms, an observation with important astrobiological implications.

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## ABBREVIATIONS

DAPI, 4',6'-diamidino-2-phenylindole; EDS, energy-dispersive x-ray spectroscopy; SEM, scanning electron microscopy.

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