ISSN 0147-6874, Moscow University Soil Science Bulletin, 2017, Vol. 72, No. 3, pp. 136–141. © Allerton Press, Inc., 2017.
Original Russian Text © A.V. Grigoriev, E.A. Vorobyova, V.S. Cheptsov, 2017, published in Vestnik Moskovskogo Universiteta, Seriya 17: Pochvovedenie, 2017, No. 3, pp. 51–56.

BIOLOGY OF SOILS

Application of ATR Spectroscopy for Astrobiological Investigations aboard Planetary Landers[1,2]

A. V. Grigoriev[a]*, E. A. Vorobyova[a, b], and V. S. Cheptsov[a, b]

[a] *Space Research Institute, Russian Academy of Science, Moscow, Russia*

[b] *Faculty of Soil Science, Moscow State University, Moscow, 119991 Russia*

**e-mail: avgrirn@gmail.com*

Received January 14, 2017

Abstract—We propose the use of infrared attenuated total reflectance (ATR) spectroscopy aboard landers for contact astrobiological soil research on terrestrial planets. The method is based on the absorption bands inherent to biological macromolecules (proteins, DNA/RNA, and carbohydrates). It is also applicable to mineralogical studies of soil, dust, and atmospheric precipitation; the use of balloons (e.g., on Venus) adds aerosols to this list. The optimal spectral range seems to be 2.5–25 μm; the optimal spectral resolution, about 10 cm^{-1}.

Keywords: ATR spectroscopy, soil microorganisms, astrobiology, detection of life

DOI: 10.3103/S0147687417030048

INTRODUCTION

ATR spectroscopy has been used in ground-based laboratories for decades (for both mineralogical and biological applications) but so far it was never used on planetary space missions. This method makes it possible to obtain the absorption spectra of a thin layer (about one wavelength) of a sample in contact with an optical element, which we call here an ATR prism. The typical wavelength range is from 2.5 to 25 μm, and the spectral resolution is about 10 cm^{-1}. The Space Research Institute of the Russian Academy of Sciences (IKI) has experience in creating miniature space Fourier spectroradiometers with such parameters. For astrobiological applications, it would be useful to augment the spectrometer with an infrared (IR) radiation source and a number of disposable cells, each containing a built-in miniature ATR prism. The approximate parameters of such an instrument would be as follows: mass 3 kg, electric power consumption 9 W, single spectrum acquisition 20 s, its data volume 0.4 kB. The simplicity of the sample preparation and the possibility of varying study objects are among the advantages of the ATR method especially important for planetary lander applications. For example, a soil sample can be piled directly onto the ATR prism.

The basic principle of ATR spectroscopy is described, e.g., in [5] and is briefly illustrated in Fig. 1.

IR radiation is directed to the side face of the ATR prism, which is a polished plane-parallel plate made of an IR-transparent material (Ge, ZnSe, KRS-5, etc). The side faces are typically at an angle of 45° to the main faces and are also polished. The radiation reaches the upper face of the plate at an angle of incidence θ large enough to provide total internal reflection (TIR). At the TIR point, an electromagnetic wave penetrates a bit into the neighboring medium, rapidly decaying at a scale of about one wavelength (evanes-

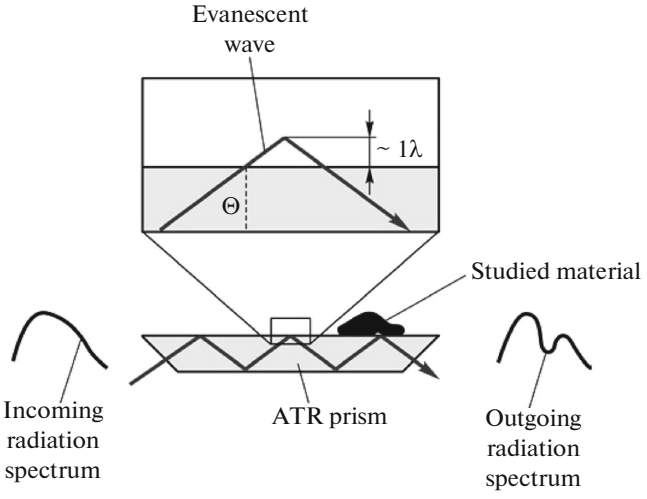

Fig. 1. Explanation of ATR spectroscopy (modified from [17]).

[1] The article was translated by the authors.

[2] This work has been done with the support of Program 1.7P of the Russian Academy of Sciences.

cent wave). If the neighboring medium is a vacuum, there will be no energy flow through the media interface and the spectrum at the prism outlet will have the same shape as at the inlet (to put it simply). However, if the investigated material (having absorption bands) is in contact with the working face of the ATR prism, the corresponding photons of the evanescent wave will be absorbed and the spectrum at the outlet will show these bands.

The possible recording order of the ATR spectrum of a studied material is as follows:

(1) acquisition of the reference spectrum, i.e., the spectrum of a pure ATR prism;

(2) the studied material is put in contact with the working face of the ATR prism;

(3) acquisition of the informative spectrum;

(4) spectrum (3) is divided by spectrum (1), resulting in the relative ATR spectrum in arbitrary units from 0 to 1.

This is a self-calibrating method: the relative ATR spectrum does not depend on the spectrum of incoming radiation, the detector spectral curve, the electronic parameters, etc. It only depends on the absorption spectrum of the studied material.

APPLICATION OF ATR SPECTROSCOPY FOR STUDYING MINERALS

Vibrations of lattice atoms result in characteristic absorption bands in mineral spectra [11, 26]. Their fundamental frequencies and overtones typically correspond to wavelengths >2.5 μm. In particular, in soil studies by IR spectrometry, a range of 2.5–15 μm is commonly used [3]. IR spectrometry makes it possible to study the most important atomic groups and bond types, adsorption and desorption of moisture and gases, minerals with crystallites of any sizes, and amorphous and organic components in mineral media. We have studied a number of minerals with a laboratory Fourier spectrometer with an ATR attachment. To obtain well-pronounced spectra, it suffices to use about 1 mm^3 of a powdered mineral. The smaller the grains, the better the absorption contrast. It is best to use grains smaller than 0.1 mm.

Figure 2 shows spectra with absorption bands of carbonates, borates, sulfates, phosphates, arsenates, and silicates from [4] (a) and some of our results (b): the relative ATR spectra of magnesite and hematite powders, quite similar to the well-known spectra of these minerals. Different libraries of the ATR spectra of minerals are available on the Internet, e.g., [12, 22].

APPLICATION OF ATR SPECTROSCOPY FOR BIOLOGICAL INVESTIGATIONS

ATR spectroscopy is also employed for biological research [9, 14, 27]. In particular, use this method was proposed to differentiate bacterial cells of different physiological status, as well as for studies of the resting state of microbial cells in a pure culture [1].

Our task is to detect microorganisms in a native mineralogical environment by means of ATR spectroscopy and to analyze the possibility of using this technique in astrobiological searches.

Studies of extreme Earth habitats convincingly indicate that once life emerged, it has shown a high ability to adapt to varying environmental factors. The limits of cell adaptation have not yet been elucidated. Microorganisms and/or biological activity have been found in Earth's sediments and rocks to depths of more than 5 km [18, 19], as well as in deep ocean bottom sediments [2]. In ancient permafrost and the ice of polar regions, numerous diverse microbial communities can remain viable for millions of years at negative temperatures (down to –50 to –80°C) under water deficit conditions [7, 13, 15, 25]. In addition, the abilities of microorganisms to metabolize and multiply at temperatures above 100°C, to withstand high doses of radiation [6, 10, 24], etc., are well known. Another important feature of microorganisms is their ability to become resting forms able to survive indefinitely in unfavorable conditions and then reverse into a metabolically active state. Natural environments can provide additional protection for microbial cells closely interacting with the habitat [20]. This resistivity of microorganisms to physical and chemical factors and the level of our knowledge about extraterrestrial environments argue for the possible existence of Earth-like life. Today, astrobiological programs are included into ongoing planetary missions [8, 23]. Spectral methods, including IR spectrometry, are actively used to analyze extraterrestrial soil.

The main biopolymers forming living cells (proteins, nucleic acids, carbohydrates, lipids, etc.) have characteristic absorption bands in the IR part of the spectrum. In Fig. 3b, one can see absorption bands of lipids, carbohydrates, nucleic acids (DNA/RNA), and proteins. The protein absorption band Amide-I coincides with the water band, making it difficult is use. However, the protein absorption band Amide-II is quite visible. The presence of water does not mask the absorption bands of DNA/RNA and carbohydrates. Here we show the relative ATR spectra of bacteria from the genus *Arthrobacter*. Such bacteria are widely distributed in soils and sediments. Representatives of this genus are also found in different extreme habitats. Their resistance to an unfavorable environment is ensured by the proven ability to form cystlike resting forms, making it possible to maintain cells viability in the anabiotic state for a long time [20, 21].

ACTIVATION OF MICROORGANISMS ON THE ATR PRISM FACE

To distinguish the spectral features of microbial cells from those of minerals is a big challenge for spec-

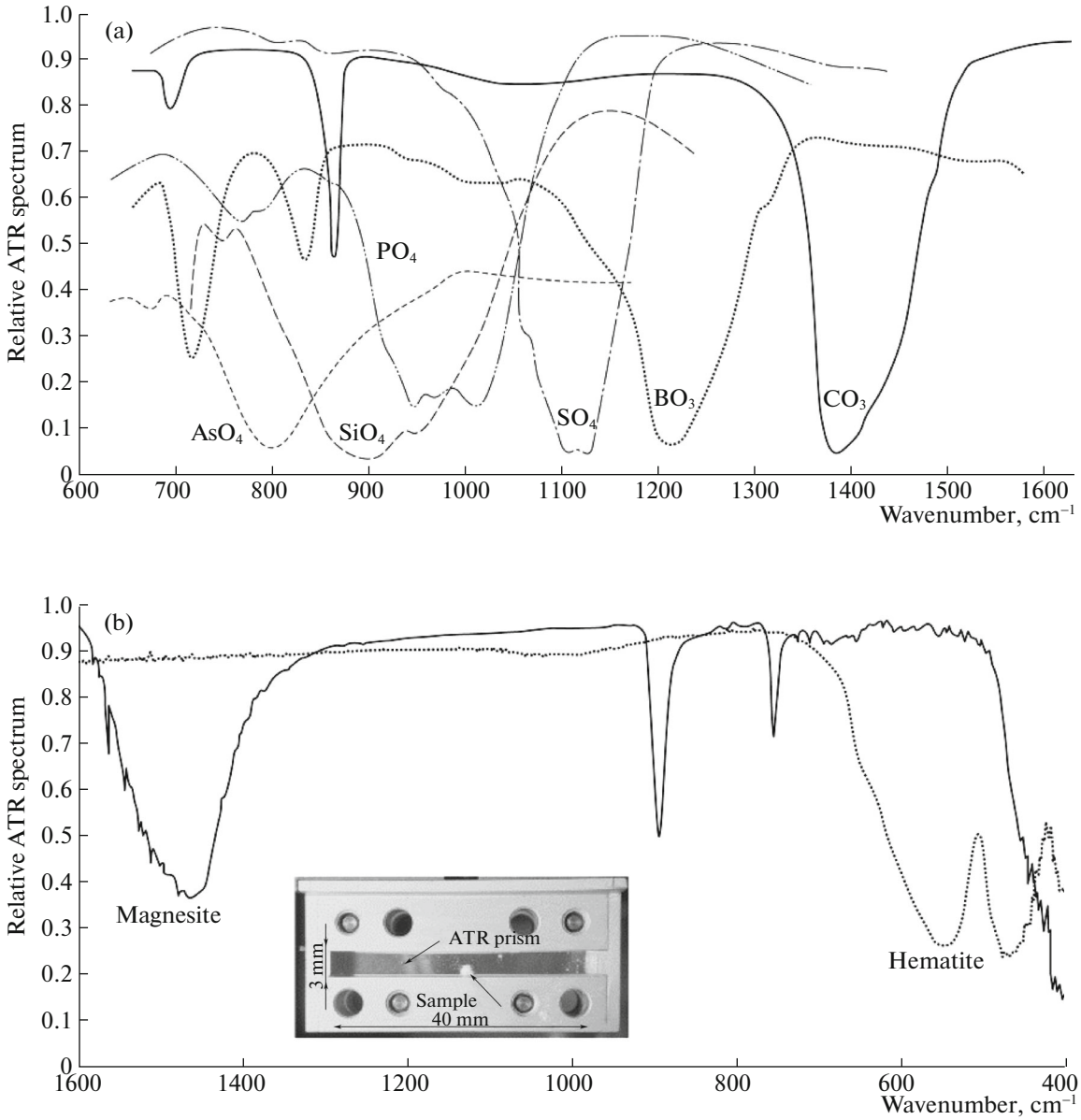

Fig. 2. IR spectra of minerals: (a) main absorption bands location for carbonates, borates, sulfates, phosphates, arsenates, and silicates [4]; (b) relative ATR spectra of magnesite and hematite powders obtained in our laboratory (inset: photo of ATR prism with heaped powder sample, volume of about 1 mm^3).

troscopic soil studies. Direct analysis of native samples makes it possible to see the biological absorption bands, but they can be masked by mineral ones. Moreover, organic matter (proteins, nucleic acids) not related to cells may be present in soils. That is why just observation of absorption bands at the wavelengths of biological absorption bands does not prove the presence of microorganisms. We have to observe the *dynamics*, i.e., *progressive deepening* of biological absorption bands due to the multiplication of microorganisms directly on the working face of the ATR prism (so that they cover a larger and larger area of the working face of the ATR prism).

We performed such experiments first with a laboratory culture of *Rhodococcus* sp., (Fig. 4a), then with a permafrost sample from Antarctica (Fig. 4b). In the latter case, a frozen sedimentary rock taken from the depth of 1.5 m was used (Beacon Valley, 77°50′ S, 160°36′ E, 1270 m above sea level). It was dried to an air-dry state. The total carbon content in the sample was determined as close to zero (0–0.1%), while the content of microorganisms was quite high. The total number of bacterial cells before analysis was 3×10^8 cells/g, of which 1×10^7 CFU/g yielded growth on nutrient media. A detailed description of the sterile selection, delivery, and storage of samples from Ant-

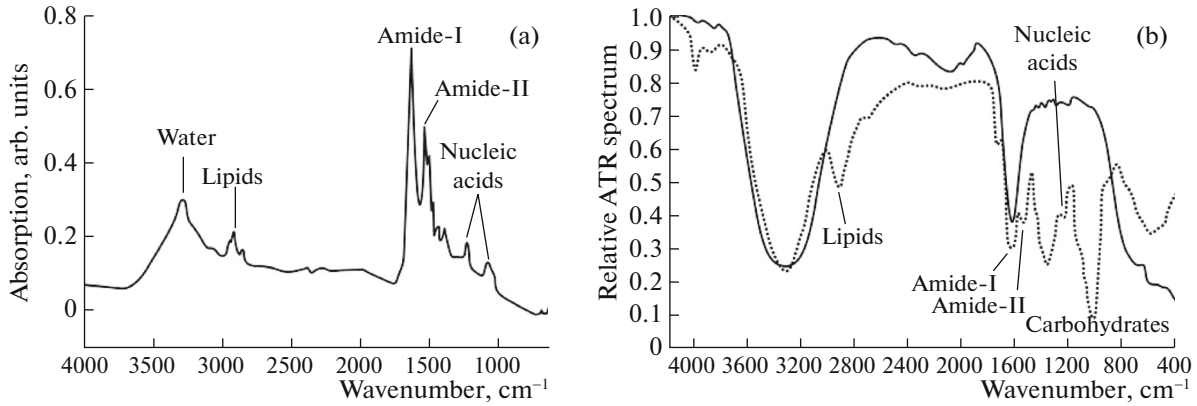

Fig. 3. IR spectra of biological objects: (a) characteristic absorption bands of proteins (Amide-I and Amide-II), nucleic acids, and lipids [16]; (b) relative ATR spectra of liquid water (solid line) and bacterium *Arthrobacter sp.* obtained in our experiments (dashed line).

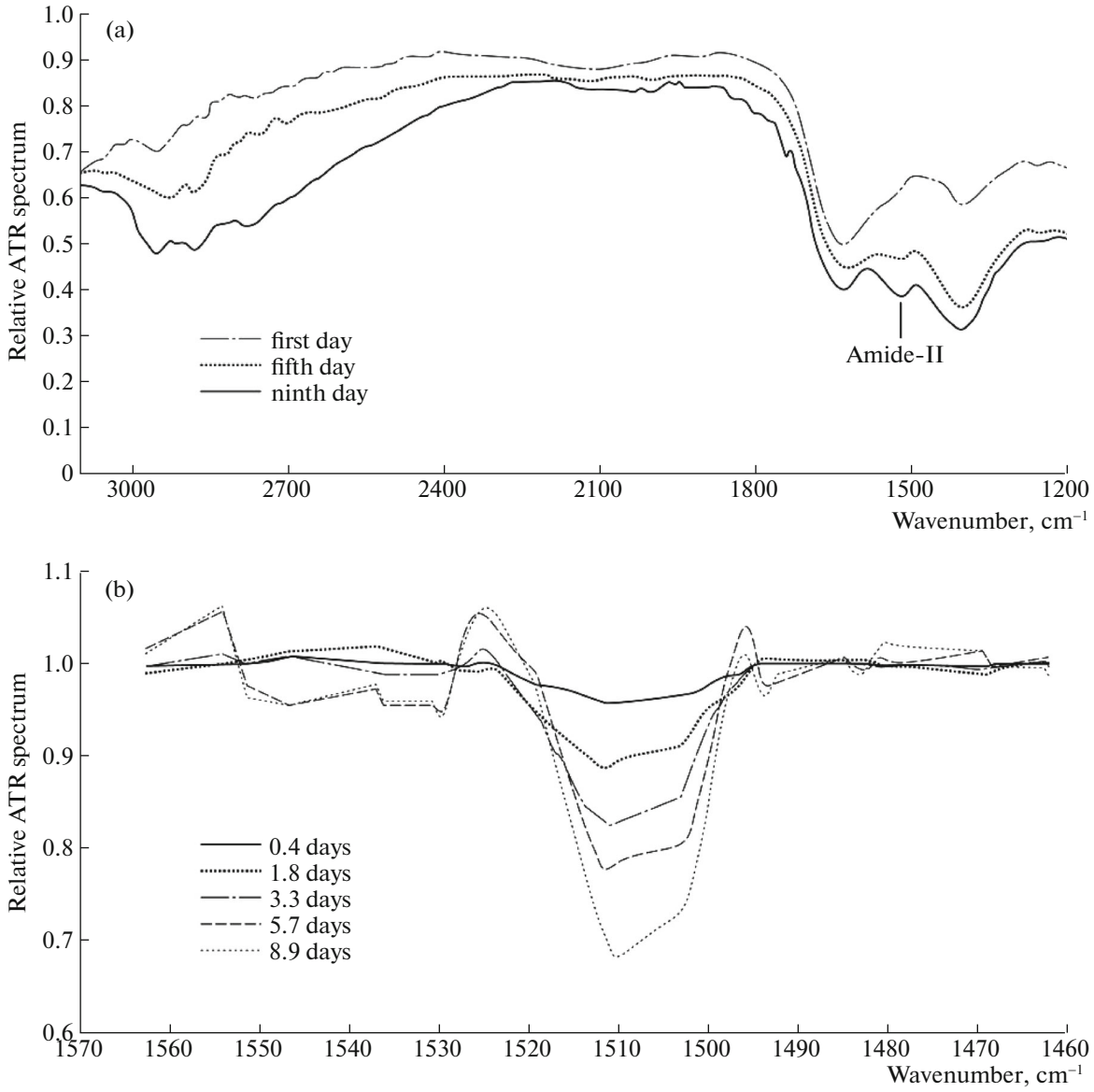

Fig. 4. Multiplication of microorganisms on working face of ATR prism: (a) bacterium *Rhodococcus* sp. (on sterile agarose deposited onto ATR prism); (b) Antarctic permafrost.

arctic permafrost and of the location of their selection are given in [13].

The Antarctic sample (about 0.03 cm^3) was evenly deposited onto the working face of the ATR prism. Microbial succession was initiated by addition of a sterile physiological solution (0.9% NaCl) containing no organic substances. The succession of the microbial community took place over 10 days. Due to this process, the IR absorption bands (characteristic of microorganisms) appeared and deepened with time (Fig. 4b). Similarly, the dynamics of cell activation and growth were studied with pure bacterial cultures. In the spectrum of *Rhodococcus* sp. (Fig. 4a) during the first day of growth, the Amide-II band was absent. On day 5, this band appeared, and on day 9 it became well pronounced. Figure 4b shows the deepening with time of the Amide-II band due to multiplication of microorganisms in the native soil sample. Thus, the possibility of activation of soil sample microorganisms directly on the working face of the ATR prism furnishes additional proof of their presence.

CONCLUSIONS

Application of ATR spectroscopy aboard planetary landers is promising for both mineralogical studies and the search for possible extraterrestrial life (based on proteins and DNA/RNA or just RNA).

The important advantages of this method are the simplicity of sample preparation and the fact that the sample can be located in any part of the ATR prism working face (about 1 cm^2)—it gives a signal regardless the exact location. The larger the portion of the face occupied by the sample, the greater the signal.

The experience and groundwork laid at IKI has made it possible to create a space instrument that can use the ATR method on planetary missions. Its approximate parameters can be as follows: spectral range from 2.5 to 25 μm, spectral resolution 10 cm^{-1}, mass of about 3 kg, electric power consumption 9 W, one spectrum acquisition 20 s, its data volume 0.4 kB.

This instrument can be installed aboard a lander or an atmospheric balloon (e.g., on Venus).

ACKNOWLEDGMENTS

The authors thank Gabriele Arnold (DLR, Germany) and Philip Christensen (ASU, United States) for the mineral samples. The authors express their deepest respect to the late Dr. D.A. Gilichinsky, who provided the ancient Antarctic permafrost sample for this study.

REFERENCES

1. Duda, V.I., Korolev, Yu.N., El'-Registan, G.I., et al., Distribution and spatial ordering of biopolymers molecules in hypnospores, *Mikrobiologiya,* 1978, vol. 47, no. 4.
2. Lein, A.Yu., Glushchenko, N.N., Osipov, G.A., et al., Biomarkers of sulphide ores from ancient and modern "black smokers", *Dokl. Akad. Nauk,* 1998, vol. 359, no. 4.
3. Orlov, D.S. and Osipova, N.N., *Infrakrasnye spektry pochv i pochvennykh komponentov* (Infrared Spectra of Soils and Soil Components), Moscow, 1988.
4. Plyusnina, I.I., *Infrakrasnye spektry mineralov* (Minerals Infrared Spectra), Moscow, 1976.
5. Harrick, N.J., *Internal Reflection Spectroscopy,* New York: Wiley, 1967.
6. Cheptsov, V.S., Vorob'eva, E.A., Gorlenko, M.V., et al., Influence of gamma radiation, low pressure and low temperature on viability of microbial community of the gray soil as analytical model of Martian regolith, *Sovrem. Probl. Nauki Obraz.,* 2015, no. 3.
7. Abyzov, S.S., Microorganisms in the Antarctic ice, in *Antarctic Microbiology,* Friedmann, E.I., Ed., New York, 1993.
8. Board, S.S., *A Science Strategy for the Exploration of Europa,* Washington, 1999.
9. Castro, F.D., Sedman, J., Ismail, A.A., et al., Effect of dissolved oxygen on two bacterial pathogens examined using ATR-FTIR spectroscopy, microelectrophoresis, and potentiometric titration, *Environ. Sci. Technol.,* 2010, vol. 44, no. 11.
10. Dartnell, L.R., Hunter, S.J., Lovell, K.V., et al., Low-temperature ionizing radiation resistance of *Deinococcus radiodurans* and Antarctic dry valley bacteria, *Astrobiology,* 2010, vol. 10, no. 7.
11. Farmer, V.C., *The Infrared Spectra of Minerals,* London, 1974.
12. FDM FTIR and Raman Spectral Libraries. http://www.fdmspectra.com/. Cited 08.12.2016.
13. Gilichinsky, D.A., Wilson, G.S., Friedmann, E.I., et al., Microbial populations in Antarctic permafrost: biodiversity, state, age and implication for astrobiology, *Astrobiology,* 2007, vol. 7, no. 2.
14. Goormaghtigh, E., Cabiaux, V., and Ruysschaert, J.-M., Secondary structure and dosage of soluble and membrane proteins by attenuated total reflection Fourier-transform infrared spectroscopy on hydrated films, *Eur. J. Biochem.,* 1990, vol. 193, no. 2.
15. Kargel, J.S., Kaye, J.Z., Head, J.W., et al., Europa's crust and ocean: origin, composition, and the prospects for life, *Icarus,* 2000, vol. 148, no. 1.
16. *Infrared Spectroscopy of Biomolecules,* Mantsch, H.H. and Chapman, D., Eds., New York, 1996.
17. Martín-Gil, J., Palacios-Leblé, G., Ramos, P.M., and Martín-Gil, F.J., Analysis of a celtiberian protective paste and its possible use by Arevaci warriors, *J. Interdiscipl. Celtic Stud.,* 2007, vol. 5, pp. 63–76.
18. Pedersen, K., Microbial life in deep granitic rock, *FEMS Microbiol. Rev.,* 1997, vol. 20, nos. 3-4.
19. Shekhovtsova, N.V., Osipov, G.A., Verkhovtseva, N.V., and Pevzner, L.A., Analysis of lipid biomarkers in rocks of the Archean crystalline basement, *Proc. SPIE Int. Soc. Opt. Eng.,* 2003, vol. 4939, pp. 160–168.

20. Soina, V.S., Mulyukin, A.L., Demkina, E.V., et al., The structure of resting bacterial populations in soil and subsoil permafrost, *Astrobiology,* 2004, vol. 4, no. 3.

21. Soina, V.S. and Vorobyova, E.A., Role of cell differentiation in high resistance of prokaryotes to cryoconservation in permafrost, *Adv. Space Res.,* 1996, vol. 18, no. 12.

22. S.T. Japan USA, LLC. http://www.stjapan-usa.com. Cited December 8, 2016.

23. Squyres, S., *Vision and Voyages for Planetary Science in the Decade 2013–2022,* Washington, 2011.

24. Vorobyova, E., Cheptcov, V., Pavlov, A., et al., The viability of native microbial communities in Martian environment (model), in *Proc. 40th COSPAR Sci. Assem.,* Moscow, 2014.

25. Vorobyova, E., Soina, V., Gorlenko, M., et al., The deep cold biosphere: facts and hypotheses, *FEMS Microbiol. Rev.,* 1997, vol. 20, nos. 3-4.

26. Wilson, E.B., Jr., Decius, J.C., and Cross, P.C., *Molecular Vibrations: the Theory of Infrared and Raman Vibrational Spectra,* New York, 1955.

27. Yu, C. and Irudayaraj, J., Spectroscopic characterization of microorganisms by FTIR microspectroscopy, *J. Biopolym.,* 2005, vol. 77, no. 6.