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Monitoring of Microbial Degraders in Manned Space Stations

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Abstract—Samples of microorganisms from the surface of constructions of Mir Space Station (Mir SS) were taken and examined after 13 years of operation. The following microorganisms were isolated and identified: 12 fungal species belonging to the genera *Penicillium*, *Aspergillus*, *Cladosporium*, and *Aureobasidium*; 3 yeast species belonging to the genera *Debaryomyces*, *Candida*, and *Rhodotorula*; and 4 bacterial species belonging to the genera *Bacillus*, *Myxococcus*, and *Rhodococcus*. The predominant species in all samples was *Penicillium chrisogenum*. It was shown that the fungi isolated could damage polymers and induce corrosion of aluminum–magnesium alloys. We commenced a study of microbial degraders on constructions of the Russian section of the International Space Station (RS ISS). Twenty-six species of fungi, bacteria, yeasts, and actinomycetes, known as active biodegraders, were identified in three sample sets taken at intervals. We founded a collection of microorganisms surviving throughout space flights. This collection can be used to test spacecraft production materials, in order to determine their resistance to biodegradation.

During long-term flights of manned space stations, a wide range of microorganisms degrading various structural materials can be found on board the spacecraft. Growth of microbial degraders on the surface of a material deteriorates it, eventually causing malfunction of the equipment and danger for space pilots [1–3]. This generates a need for (1) constant monitoring of construction surfaces to investigate microorganisms and microorganism associations (with regard to their eventual effects) and (2) development of efficient methods of their suppression under the conditions of microgravity.

Study of the airborne microflora, inside surfaces, and equipment of Mir space station (Mir SS) revealed numerous species of microscopic fungi, bacteria, and yeasts, a total of 234 species [1]. The bacteria and fungi included opportunistic pathogens, but most species entered the group of so-called technophiles, organisms that inhabit various industrial materials and participate in their biodegradation, including metal corrosion.

Microbial associations inhabiting surfaces of structural materials and their most aggressive members can be used to estimate the biodegradability of materials used in space products. A collection of such microorganisms would allow assessment of the risk of microbial damage.

The objectives of this work are: investigation of the range of microbial species inhabiting the surfaces of structural materials after long-term operation of Mir SS, study of the initial stages of their settlement on these surfaces, isolation of these microorganisms, and their identification.

MATERIALS AND METHODS

The kit *Bioprobes* was designed for the experiment on board the space station, including the delivery of a device for taking samples from inner surfaces of the station, storage of the samples, and their delivery to Earth for microbiological analysis. The kit was used both for taking the samples from the Mir SS and monitoring the microflora on the structural surfaces of the Russian section of the International Space Station (RS ISS).

Two hours after landing, the kit was delivered to a microbiological laboratory. The samples were inoculated into nutrient media. The inoculated plates were transported at about 20°C. After delivery, the plates were incubated at 25 ± 1°C. After the emergence of colonies and at the beginning of fungal growth, the plates were placed into a refrigerator and stored at +4°C. Then, the contents were plated for isolation and identification of pure cultures.

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Sampling in Mir SS was done after 14 years of flight, and in RS ISS, during the 4th, 6th, and 8th missions at 6 and 12-month intervals, starting from November, 2002. Samples were taken sterile and placed into the kit *Bioprobes* for delivery to Earth. Sampling was performed immediately before the return of the crew to Earth. The samples were processed and plated in corresponding media within 2–3 days after the sampling.

Microorganisms were isolated from the samples by conventional methods involving plating on agar nutrient media [4]. Colonies were counted after 4, 7, and 14 days. Microscopic fungi were counted on wort agar. Bacteria and actinomycetes were counted on glucose–peptone–yeast extract medium. Colonies were inoculated onto agar slants and identified according to common keys [5–16].

To test the capacity of the fungi isolated for inducing damage to polymers, the cultures were grown in tubes with solidified Czapek–Dox medium at 29°C for 10 days. Test materials were inoculated with either spore suspensions of individual fungal species or mixtures of spores of several fungal species at equal quantities, so that the suspension density was within $1–2 \times 10^6$/ml. The suspensions were made in water, mineral Czapek–Dox medium, or Czapek–Dox medium supplemented with sucrose as a carbon source. All samples were treated with ethyl alcohol prior to inoculation. The surfaces of materials tested were evenly sprayed with a spore suspension and placed into petri dishes. In experiments with suspensions in complete Czapek–Dox medium, samples of polymers or aluminum–magnesium alloy (AMG-6) were placed into petri dishes on the surface of solidified Czapek–Dox medium. The plates were placed into desiccators (each containing water at the bottom). The desiccators were stored in a temperature-controlled cabinet for 30 days (in experiments with polymer samples) or for one and three months (in experiments with metal biocorrosion). The desiccators were opened once a week to allow air passage.

The biological damage was studied by light and scanning electron microscopy with a CAM SCAN electron microscope (CAMBRIG, Germany) at an accelerating voltage of 20 kV. Secondary electron images were examined. Alloy samples obviously containing no organic remains on their surfaces were examined microscopically without pretreatment, and samples with microbial remains were covered with a gold–palladium alloy by ionic spraying in argon (thickness, 25 nm).

RESULTS AND DISCUSSION

Study of microorganisms growing on the surfaces of construction and equipment of Mir SS during its operation revealed species and associations capable of growing on various substrates and causing metal corrosion and polymer degradation. There is ample evidence that microorganisms, including fungi, can colonize new polymers and induce their damage, particularly under the severe conditions of a space flight, which require resistance to radiation and other adverse factors for a strain to survive. Moreover, microorganisms can cover surfaces with biofilms resistant to antimicrobial drugs [17].

Samples were taken from structural surfaces of space stations using the kit *Bioprobes*, as part of a research program performed in Mir SS in 1997–1998 and as part of the Russian Federal Space Program "ISS–Science" in 2000–2003. Samples were taken with wet or dry wads without preservatives. The experiment was performed by Cosmonaut V. Ryumin during the STS-91 mission in July, 1998. Samples were taken from most contaminated sites of structural surfaces.

The sampling protocol ensured preservation of viable microbial degraders during their transportation from Mir SS to the ground microbiological laboratory.

The experiment (performed on board) and the sampler (designed by the participants) allowed for collection of pure cultures of microorganisms and microorganism associations forming on surfaces of structural materials of space stations during long-term flights.

Plating of samples from Mir SS brought about pure cultures of 68 fungal isolates and 26 isolates of bacteria and yeasts. Further identification revealed 20 microbial species including 12 fungal species, 4 yeast species, and 4 bacterial species. The number of bacterial species on structural surfaces was fewer than that of their fungal counterparts. However, bacteria of the genus *Bacillus* were found in 41% of the samples, probably owing to the fact that these microorganisms are widespread in various habitats on Earth. They are often isolated from associations involved in degradation of various organic compounds. Species of the genus *Rhodococcus*, active degraders of organic compounds [18], including polymers, were found in 18.0% of the samples and those of the genus *Myxococcus*, in 5.6%.

All filamentous fungi isolated belong to anamorphous (imperfect) species related to the order Ascomycota. The strains studied are dominated by species of the genus *Penicillium* (eight species). This genus is the most widespread. It includes many species isolated from various habitats and various substrates (including man-made), as well as from soil. Three species belonged to the genus *Aspergillus*. These fungi are most aggressive and exhibit maximum adaptability to severe conditions. The dark-colored fungus *Cladosporium sphaerospermum* also belongs to technophilic species. These species cause all known varieties of mycoses. When grown on various substrates, they can produce toxins and antibiotics (Table 1) [19, 20].

The isolated cultures differed from species described in the literature in their colony morphology and degree of pigmentation. Some of them, in addition to normal colonies, had fragmentary brushes and malformed phialides [15, 16].

Penicillium chrisogenum was the predominant species both with respect to its presence (in 71% of the

Table 1. Species of filamentous and yeast fungi detected on surfaces of structural materials of Mir SS and RS ISS at the beginning of monitoring

Genus, species	Mir SS	RS ISS		
		2002	2003	2004
Aspergillus flavus Link	+	–	+	+
A. niger van Tieghem	–	–	+	+
A. sydowii (Bainier et Sartory) Thom et Church	+	+	+	–
A. versicolor (Vuillemin) Tiraboschi	+	–	–	+
Cladosporium herbarum (Persoon: Fries) Link	–	–	+	–
C. sphaerospermum Penzig	+	–	–	–
Penicillium aurantiogriseum Dierckx	+	+	+	–
P. brevicompactum Dierckx	+	–	–	–
P. chrisogenum Thom	+	–	+	+
P. commune Thom	+	–	–	–
P. crustosum Thom	–	–	+	–
P. glandicola (Oudemans) Seifert et Samson	+	–	–	–
P. purpurogenum Stoll	–	–	–	+
P. spinulosum Thom	–	–	–	+
P. variabile Sopp	–	–	–	+
P. verrucosum Dierckx	+	–	–	–
P. viridicatum Westling	+	–	–	–
P. waksmanii Zaleski	+	–	–	–
Scopulariopsis brumptii Salvanet-Duval	–	–	+	–
Aureobasidium pullulans (de Bary) Arnaud	+	–	–	–
Candida sp.	+	–	–	–
Debaryomyces hansenii	+	–	–	–
Rhodotorula glutinis (Fresenius) F.C. Harrison var. glutinis	+	+	+	+

Notes: "+", present in samples; "–", absent from samples.

samples examined) and abundance (it accounted for 43% of all fungal isolates in the collections).

It was shown that the fungi isolated as pure cultures could induce damage of polymers and of the aluminum–magnesium alloy used in space products under model conditions on Earth. Some cultures and associations were tested for the ability to grow on and damage polymer surfaces. The following materials were tested: polyamide, polyimide, and polyvinyl alcohol films; products of polyethylene and rubber; and polyester, polyvinyl chloride, and cotton-based fabrics. In other experiments, fungal spores were applied onto surfaces of plates of an aluminum–magnesium alloy (AMG-6). Most materials were accreted, even with the absence of mineral substances and sucrose from the fungal suspension. Figure 1 presents an example of accretion of fungi to cotton and polyethylene terephthalate fabrics tested for fungal resistance after 28 days of incubation in a humid chamber at 29°C. Figures 2 and 3 show light and scanning electron microscopic images, respectively, of samples of polyethylene terephthalate fabric exposed to fungi for 28 days and the respective controls. The damage of the fabric is obvious: fiber breaks caused by fungi are clearly seen.

The fungal associations isolated produced significant changes of the aluminum–magnesium alloy under the same experimental conditions. Biocorrosion caused by a fungal association, revealed by SEM, was reported in [21]. Figures 4a and 4b show specific changes of the metal surface (in comparison with the control) following one- or three-month exposure (in a humid chamber at 29°C). The control sample was exposed under the same conditions for the same time. The images show that the surface of AMG-6 changed more considerably

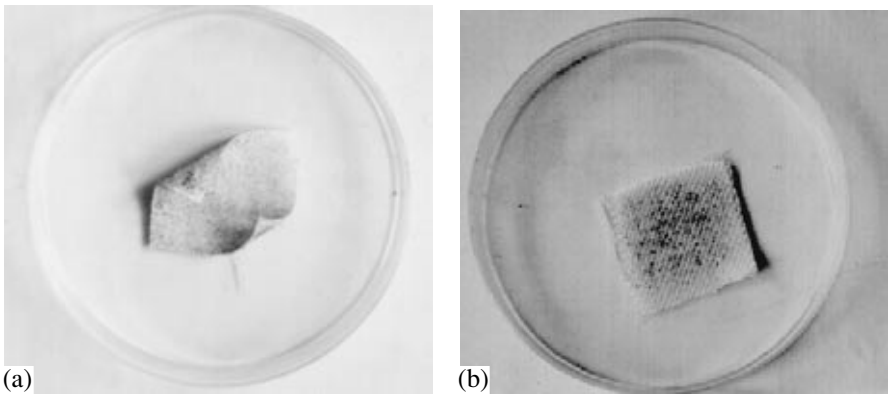

Fig. 1. Growth of a fungal association isolated from structural surfaces of Mir SS on various fabrics: (a) cotton, (b) polyethylene terephthalate.

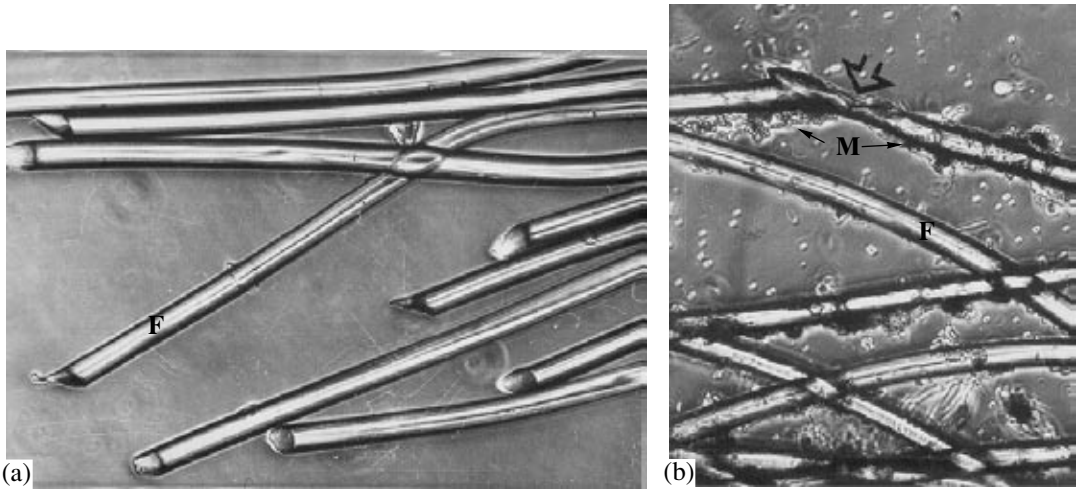

Fig. 2. Sample of polyethylene terephthalate fabric after 28-day exposure to fungi, light microscopical image (×280): (a) control sample; (b) fabric fibers (F) damaged by microorganisms (M).

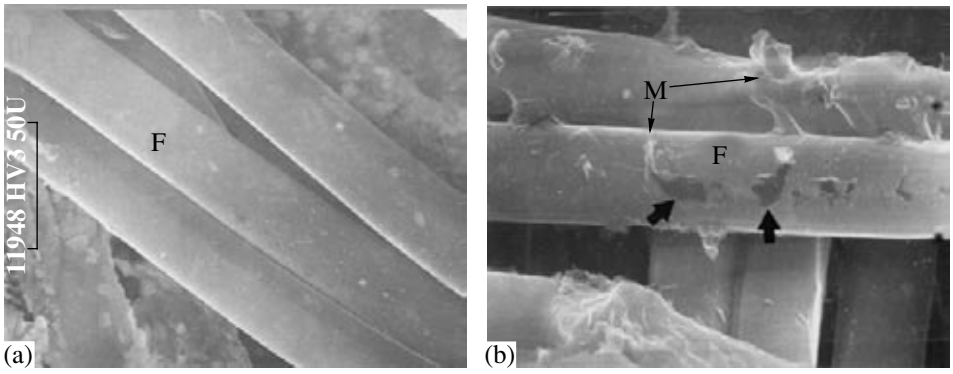

Fig. 3. Sample of polyethylene terephthalate fabric after 28-day exposure to fungi, SEM image (×740): (a) control sample; (b) fabric fibers (F) damaged by microorganisms (M).

when the period of exposure to fungi was increased (Figs. 4g and 4e).

Micromycetes isolated from the surfaces of structural materials in Mir SS accreted on polymers and damaged them. They also induced biocorrosion of aluminum–magnesium alloys.

We conclude that the microflora of the surfaces of structural materials in Mir SS consisted, before its

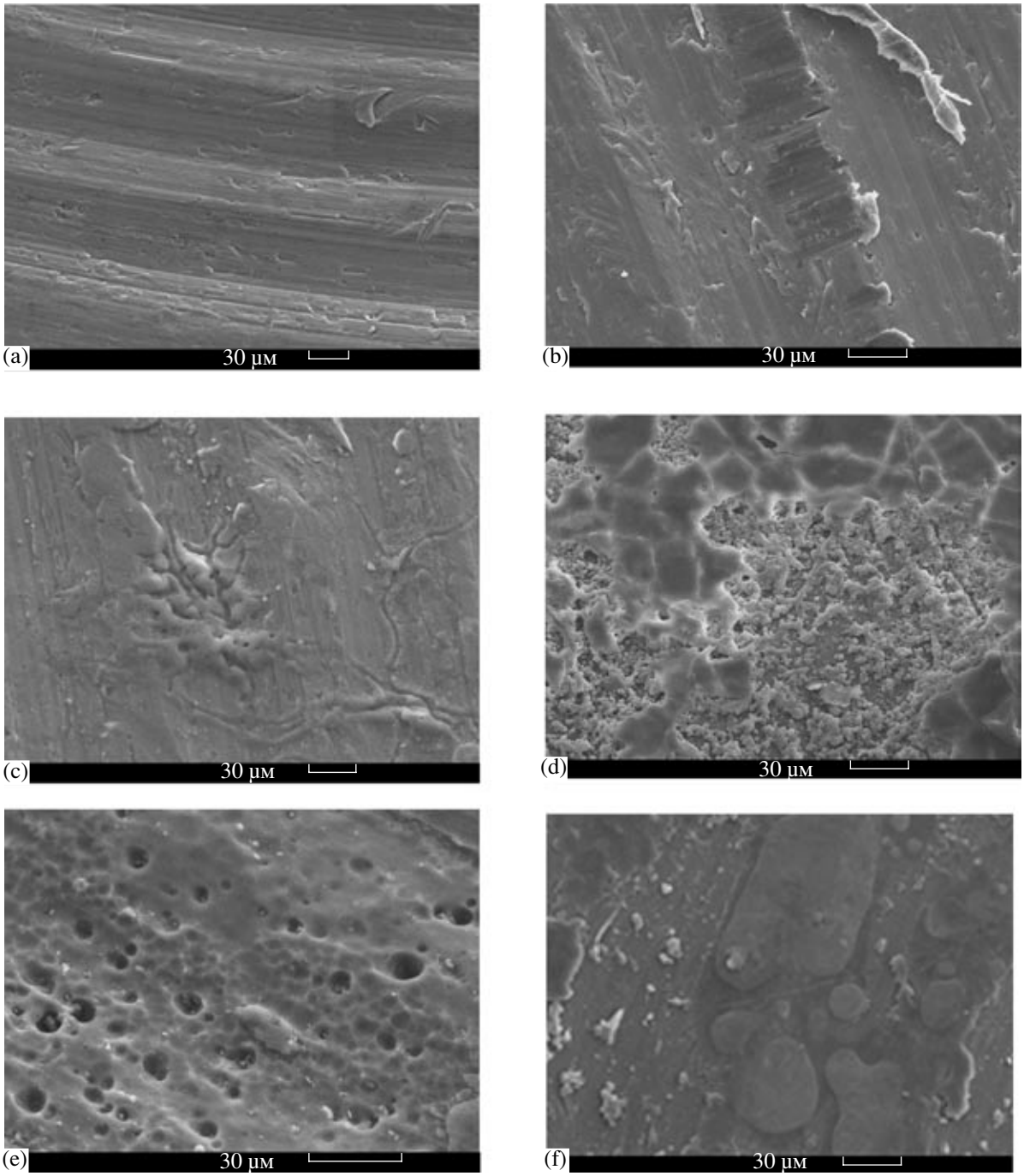

Fig. 4. Surface of aluminum–magnesium alloy exposed to fungi isolated from Mir SS and RS ISS and a control sample (SEM images): (a, c, e) one-month exposure, (b, d, f) three-month exposure; (a, b) control sample, (c, d) exposure to fungi sampled in Mir, (e, f) exposure to fungi sampled in ISS.

drowning, of microorganisms resistant to space flight factors and eventually dangerous for man (by virtue of rendering the equipment operation unreliable).

After the Mir SS drowning, studies of microbial degraders surviving space flight and forming stable associations and biofilms on structural materials continued in RS ISS. Three sets of samples were taken from various structural surfaces of ISS after its launch at intervals of 6 (twice) and 12 months, using the designed kit.

Tables 1 and 2 present species of fungi and bacteria sampled from the surfaces of structural materials of RS ISS during the monitoring. Microorganisms in samples of the second sample set were more abundant than

Table 2. Species of bacteria detected on surfaces of structural materials of Mir SS and RS ISS during monitoring

Genus, species	Mir SS	RS ISS		
		2002	2003	2004
Arthrobacter sp.	–	–	–	+
Bacillus megaterius	+	–	–	–
B. sphericus	–	+	–	–
B. subtilis	+	–	+	+
Bacillus sp.	–	–	+	–
Cytophaga sp.	–	+	–	–
Flavobacterium sp.	–	–	+	–
Geodermatophilus obscurus	–	–	+	–
Myxococcus sp.	+	+	+	+
Micrococcus luteus	–	–	+	–
Nocardia asteroidÂs	–	–	+	–
Streptomyces sp.	–	–	+	–
Rhodococcus luteus	–	–	+	–
Rhodococcus rhodochrous	–	+	–	–

Notes: "+", present in the samples; "–", absent from the samples.

those of the first one, while those in the third set were still more abundant. Surfaces of inner constructions and equipment, clean at the time of launch, were gradually colonized by associations of pro- and eukaryotes.

Eukaryotic and prokaryotic colonies growing on plates (represented by bacteria and actinomycetes) were few. In the first set of samples, there were no more than 10–12 cfu per dish on media used in microbiological analysis. The number of micromycete colonies varied from 1 to 6, depending on sampling site and term, and the number of bacterial colonies varied from 3 to 12 cfu per dish. In addition to monocultures of micromycetes and bacteria, we isolated binary cultures consisting of bacteria and fungi. Their separation is scheduled for subsequent stages of the study.

All filamentous fungi identified belonged to the same group of anamorphous (imperfect) sac fungi (Ascomycota, Ascomycetes) as those sampled in Mir SS (Table 2). At the beginning of the monitoring (first sample set), they included only two species of the genera *Penicillium* and *Aspergillus*. A greater diversity was detected in samples of the second set. The fungi included eight species belonging to four genera of imperfect fungi: *Penicillium* (three species), *Aspergillus* (three species), *Cladosporium* (one species), and *Scopulariopsis* (one species). Five fungal species were isolated from the third set of samples. They were dominated by *Penicillium*. Note that the abundance of microflora in Mir SS was shown to oscillate [1, 2]. This is likely to be true for other closed spaces. The variation in microflora abundance and diversity can also be related to the delivery of various cargoes, accompanied by the appearance of new microorganisms.

Microorganisms were relatively few in samples from outer surfaces of the equipment. They were much more abundant and diverse in sites covered by panels, absorbers, etc. For example, samples taken from under an absorber contained all three *Aspergillus* species and one *Penicillium* species, and the number of colonies exceeded the total number of colonies in all other samples.

All fungal species identified, as in Mir SS, are cosmopolitans. Soil is their natural reservoir, but they also colonize substrates of anthropogenic origin. They are found on plant remains, foodstuff, industrial materials, and other things. They are also present in the air of living rooms. As in Mir SS, all fungi found in ISS belong to technophiles [20], capable of damaging various polymers and enhancing metal corrosion. Figures 4d and 4e present images of AMG-6 surfaces after one- or 3-month exposure to fungi isolated from samples from RS ISS.

The fungal species identified are known to produce active hydrolases, acids, and secondary metabolites, including toxins and antibiotics [1, 19]. Species of the genera *Aspergillus* (particularly *A. flavus*), *Cladosporium*, and *Scopulariopsis* are opportunistic human pathogens. They can cause allergies, mycoses, and lung diseases, particularly in immunocompromised persons [7–9].

Only one yeast species, *Rhodotorula glutinis* (Fresenius) F.C. Harrison var. *glutinis* was identified in RS ISS. It is often detected in soil, dust, foodstuff, and air [10]. This yeast species was also found in Mir SS.

Prokaryotes included gram-negative and gram-positive bacteria (Table 2). A total of nine genera were found: three genera of gram-positive bacteria (*Bacillus, Micrococcus*, and *Rhodococcus*), three genera of actinomycetes (*Nocardia*, *Streptomyces*, and *Geodermatophilus*), and three genera of gram-negative bacteria (*Cytophaga*, *Myxococcus*, and *Flavobacterium*). More genera were detected in the second sample set (May, 2003). The trend was toward an increase in the percentage of fungi in samples from structural surfaces in the course of the flight.

All bacteria and actinomycetes isolated from the surfaces of structural materials belong to genera widely occurring in various soils and related substrates. They colonize diverse habitats. Bacteria of these genera are often detected in air and on the surfaces of plants growing in cities [5].

The prokaryotes isolated formed endospores (bacilli) or exospores (streptomycetes). They also produced pigments: yellow (micrococci and myxococci), orange (rhodococci and cytophages), red (rhodococci), or dark-brown (geodermatophils and bacilli). It is known that microbial pigments protect cells from oxidative stress and various adverse environmental factors [22]. Apparently, this determines the survival of prokaryotic cells under the conditions of space stations. As bacilli produce a wide range of hydrolases, they can, under certain conditions, induce damage of various materials [14]. Of special interest is the fact that the bacilli isolated can produce organic acids. Vapor condensation on surfaces of the equipment and propagation of these microorganisms can induce corrosion. Rhodococci, isolated in both ISS and Mir from surfaces of structural materials, are commonly known to degrade various polymers [18, 23].

Monitoring of microorganisms on structural materials of RS ISS revealed eukaryotes and prokaryotes. Eukaryotes included filamentous fungi (eight species of four genera) and yeasts (one species). Prokaryotes included six genera of bacteria and three genera of actinomycetes. Prokaryotes were isolated not only as pure cultures but also as associations with streptomycetes, yeasts, or micromycetes. This was indicative of the formation of associations and, probably, biofilms on surfaces, much more resistant to ambient than pure cultures. The bacteria isolated included strains capable of inducing corrosion of structural materials. The microorganisms isolated were added to the collection of strains forming the microflora of structural surfaces in space stations.

Microorganisms inhabiting space stations are of interest not only for studies of morphological and biochemical variability (resulting from space flight factors) but also for testing various materials used in space products for corrosion resistance. The fungal association approved by the Russian Federal Standard for testing fungal resistance has been developed under terrestrial conditions. Use of microorganisms and microorganism associations which have experienced a 14-year selection in a space station and survived on materials and equipment allows for a more correct assessment of the risk of biologic damage.

REFERENCES

1. Novikova, N.D., *Kosm. Biol. Aviakosm. Med.*, 2001, vol. 34, no. 4, pp. 32–40.
2. Shnyreva, A.V., Sizova, T.P., Bragina, M.P., Viktorov, A.N., and D'yakov, Yu.T., *Mikol. Fitopatol.*, 2001, vol. 35, no. 3, pp. 37–43.
3. Klintworth, R., Reher, H.J., Viktorov, A.N., and Bohle, D., *Acta Astronaut.*, 1999, vol. 44, nos. 7–12, pp. 569–578.
4. *Metody pochvennoi mikrobiologii i biokhimii* (Methods of Soil Microbiology and Biochemistry), Zvyaguntsev, D.G., Ed., Moscow: Mosk. Gos. Univ., 1991.
5. *Bergey's Manual of Determinative Bacteriology*, 9th ed., Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., and Williams, S.T., Eds., 9th edition, Baltimore: Williams and Wilkins, 1993. Translated under the title *Opredelitel' bakterii Berdzhi*, Moscow: Mir, 1997.
6. Bilai, V.I., Gvosdek, R.I., Skripal', I.G., Kraev, V.G., Ellanskya, M.A., Zirka, G.I., and Mudras, V.A., *Microorganizmy—vozbuditeli boleznei rastenii* (Microorganisms as Causal Agents of Plant Diseases), Kiev: Naukova Dumka, 1988.
7. Satton, D., Fotergill, A., and Rinal'di, M., *Opredelitel' patogennykh i uslovno patogennykh gribov* (A Laboratory Guide to Pathogenic and Conditionally Pathogenic Fungi), Moscow: Mir, 2001.
8. Ellis, M.B., *Dematiaceous Hyphomycetes*, Commonwealth Mycologigical Institute Kew, Kew: Surrey, 1971.
9. Hoog, G.S. and Guarro, J., *Atlas of Clinical Fungi, Baarn: Centraalbureau voor Schimmelcultures*, 1995.
10. Kurtzman, C.P. and Fell, J.W., *The Yeasts, a Taxonomic Study*, Amsterdam: Elsevier Science, B.V., 1998.
11. Pitt, J.I., *A Laboratory Guide to Common Penicillium Species*, North Ryde, N.S.W., Australia: CSIRO, Division of Food Processing, 1991.
12. Ramirez, C., *Manual and Atlas of the Penicillia*, Elsiveier Biomed. Press, 1982.
13. Raper, K.B. and Fennel, D.I., *The Genus Aspergillus*, Baltimore: Williams and Wilkins, 1965.
14. *The Prokaryotes. The Handbook on Habitats, Isolation, and Identification of Bacteria*, vol. 1, 2, Starr, M.P., Stolp, H., *et al.*, Eds., Berlin: Springer, 1981, p. 2284.

15. Thom, C.A. and Raper, K.B., *Manual of the Penicillia*, New York: Hefner Publishing Co., 1968.
16. Domsch, K.H., Gams, W., and Anderson, T.H., *Compendium of Soil Fungi*, 2nd ed., London: Academic, 1993.
17. Gu, J.D., Roman, M., Elsseman, T., and Mitchel, R., *Int. Biodeterior. Biodegrad.*, 1988, vol. 41, no. 1, pp. 25–33.
18. Nallii, S., Cooper, D.G., and Nicell, J.A., *Biodegradation*, 2002, vol. 13, no. 5, pp. 343–352.
19. Kozlovskii, A.G., Zhelifonova, V.P., Antipova, T.V., Adanin, V.M., Novikova, N.D., Deshevaya, E.A., Shlegel', B., Daze, Kh.M., Gollmik, F., and Grefe, U., *Prikl. Biokhim. Mikrobiol.*, 2004, vol. 40, no. 3, pp. 334–349.
20. Lugauskas, A.Yu., Mikul'skene, A.I., and Shlyauzhene, D.Yu., *Katalog mikromitsetov-biodestruktorov poli-mernykh materialov* (Catalogue of Micromycetes–Biodestructors of Polymeric Materials), Gorlenko, M.V, Ed., Moscow: Nauka, 1987.
21. Alekhova, T.A., Aleksandrova, A.A., Novozhilova, T.Yu., Lysak, L.V., Golutvin, I.A., Nasikan, N.S., Zagustina, N.A., Plotnikov, A.D, and Borisov, V.A., *Poverkhnost*, 2005, no. 1, pp. 54–59.
22. Feofilova, E.P., *Pigmenty mikroorganizmov* (Pigments of Microorganisms), Moscow: Nauka, 1974.
23. Nesterenko, O.A., Kvasnikov, E.L., and Nogina, T.M., *Nokardiopodobnye i korinepodobnye bakterii* (Nocardiaform and Coryneform Bacteria), Kiev: Naukova Dumka, 1985.