https://ntrs.nasa.gov/search.jsp?R=20110001427 2019-08-30T13:58:05+00:00Z

Source of Acquisition
NASA Johnson Space Center

Abstract

An evaluation of the microbial flora from air, water, and surface samples provided a baseline of microbial diversity onboard the International Space Station (ISS) to gain insight into bacterial and fungal contamination during the initial stages of construction and habitation. Using 16S genetic sequencing and rep-PCR, 63 bacterial strains were isolated for identification and fingerprinted for microbial tracking. The use of these molecular tools allowed for the identification of bacteria not previously identified using automated biochemical analysis and provided a clear indication of the source of several ISS contaminants. Fungal and bacterial data acquired during monitoring do not suggest there is a current microbial hazard to the spacecraft, nor does any trend indicate a potential health risk. Previous spacecraft environmental analysis indicated that microbial contamination will increase with time and require continued surveillance.

Microbial Diversity aboard Spacecraft: Evaluation of the International Space Station

Victoria A. Castro[1] *, Adrianna N. Thrasher[1], Mimi Healy[2], C. Mark Ott[1], and Duane L. Pierson[3]

[1]EASI/Wyle Laboratories
Microbiology Laboratory
Johnson Space Center
Houston, TX 77058

[2]Bacterial BarCodes, Incorporated
8080 N. Stadium Drive, Suite 1200
Houston, TX 77054

[3]National Aeronautics and Space Administration
Habitability and Environmental Factors Office
Johnson Space Center
Houston, TX 77058

***Corresponding Author**
Victoria A. Castro
EASI/Wyle Laboratories
1290 Hercules Drive
Houston, TX 77058
Telephone: (281) 483-7804
Email: victoria.a.castro1@jsc.nasa.gov

Submitted: February 20, 2003

Running Title: Microbial Diversity of ISS

Introduction

The International Space Station (ISS) was designed as a multifunctional research platform for conducting a wide range of physical and biological science investigations. The microgravity environment provides the opportunity for scientific investigations while presenting unique challenges to those responsible for maintaining the health, safety, and productivity of the crewmembers. Other semi-closed systems, such as submarines (2, 10, 18, 19) environmental chambers (9, 12), and office buildings (3, 5, 14), have been evaluated, however, microbial monitoring of the ISS permitted not only the characterization of the organisms onboard, but also contamination tracking as new components and hardware were introduced into the environment.

At completion, the ISS will consist of more than 10 habitable modules provided by the U.S. and international partners. Thus far, the ISS includes several habitable environments including the Zarya Control Module, the Unity Node, the Zvezda Service Module, the Joint Airlock, and the Destiny Laboratory Module. The projected life of the ISS after completion of construction is about 10 years, during which the station will experience periodic visits from international spacecraft for crew exchanges, resupply of food and other consumables, and many payloads and scientific investigations. The environmental parameters of the ISS are favorable for microbial growth, and the crewmembers will be the predominant sources of bacteria with lesser amounts arriving with ground-supplied materials. Major sources of fungal growth arise from contaminating fungal spores inadvertently accompanying ground-supplied materials. Previous space flight has demonstrated that microorganisms are ubiquitous throughout the habitable modules of spacecraft (11). Data obtained from the Apollo (5), Skylab (17), space shuttle (8, 11), and the Russian space station Mir (6) have demonstrated the capability to provide and maintain space environments compatible with human occupation. However, the ISS presents substantial challenges in limiting microbial contamination to preserve the health and safety of the crews and the integrity of the ISS.

The goal of this study was to develop a baseline of microbial flora during the initial stages of construction and habitation from which to assess the future changes in bacterial and fungal diversity. These changes will form the basis for decisions regarding crew health and systems performance. Fungi were identified using phenotypic analysis while bacteria cultured onboard ISS were characterized using 16S ribosomal sequencing and comparing bacterial genomes using repetitive sequence-based PCR (rep-PCR).

Methods

Sample collection

Preflight samples from surfaces and air were collected from a reusable cargo container [designated as the Multi-Purpose Logistic Module (MPLM)], which is carried aboard the space shuttle to transport flight hardware, and consumables to and from the ISS. These samples and others from flight hardware were collected from 25-cm^2 areas using calcium alginate swabs in phosphate buffer (pH 7.2) as a wetting agent. Surface samples onboard the ISS were collected using contact slides containing tryptic soy agar and Sabouraud dextrose agar with chloramphenicol (Biotest Diagnostics Corporation, Denville, NJ) or by swabbing 25-cm^2 areas with calcium alginate swabs as above and inoculating the contact slides. Air samples (84.9 liters) were collected from the MPLM and on ISS, using a modified Burkard microbial air sampler (Burkard Manufacturing Co. Ltd., Hertfordshire, UK) containing tryptic soy agar plates for bacterial analysis and Sabouraud dextrose agar plates for fungal analysis. Water samples were taken from several sources onboard the ISS. These included ground-supplied Moscow area water and space shuttle fuel cell water delivered through a Russian-built dispenser (designated SVO-ZV). Also evaluated was water collected from the hot and cold ports (designated "SRV-K hot" and "SRV-K cold") of the Russian system used for humidity condensate recovery (13). Water transferred from the space shuttle fuel cells and stored in portable containers (designated CWC for Contingency Water Containers) was also analyzed. Water was collected into sterile Teflon bags (American Fluoroseal, Gaithersburg, MD) and either processed during flight or returned to Earth for analysis. Water samples from the SVO-ZV, "SRV-K hot," and "SRV-K cold" ports were processed during flight using a self-contained system which filtered a 100-ml aliquot through a 0.45-μm cellulose acetate field monitor (Millipore, Bedford, MA) (7). A liquid R3A growth medium was added to an absorbent pad on the downstream side of the filter surface.

Cell culture

Samples processed during flight were incubated at ambient temperature (28 °C to 30 °C) and returned for ground-based analysis on the next available shuttle flight. Because flight samples were received up to 3 months after collection, many of the samples were overgrown or desiccated, and viable cultures could not be recovered from all colony types observed. Colonies were subcultured upon arrival and incubated at 37 °C.

Aliquots of 100 ml from archived water samples were passed through 0.22-μm membrane filters (Millipore, Bedford, MA), cultured on R2A medium (Remel, Lenexa, KS), and then incubated at 37 °C for 48 hours.

Bacterial DNA extraction and identification

Cells from pure cultures were lifted directly from plates, and DNA was extracted using the Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA). Quality and quantity of DNA isolated was verified on 1% agarose gel electrophoresis. DNA extraction was successful in 99% of bacterial cultures.

Microbial genomic DNA was amplified using the PCR module of the Microseq 500 16S rDNA Bacterial Sequencing Kit (Applied Biosystems, Foster City, CA), and PCR products were confirmed by agarose gel electrophoresis. Prior to cycle sequencing, PCR products were purified of excess nucleotides enzymatically using exonuclease I (USB Corporation, Cleveland, OH), and dephosphorylated on the 5′ ends using shrimp alkaline phosphatase (USB Corporation, Cleveland, OH). The sequences were compared by BLAST (Basic Local Alignment Search Tool) analysis to all sequences in the Genbank database (1). Positive identifications were made based on a 98% or better alignment with database entries. In addition, all bacterial isolates were subcultured on blood agar at 37 °C for 24 hours and identified using the VITEK Identification System (bioMérieux, Hazelwood, MO)

Rep-PCR DNA fingerprinting

A master mix was prepared using reagents supplied in the rep_{PRO} Uprime-E rep-PCR Kit (Bacterial Barcodes Inc., Houston, TX), *Taq* DNA polymerase (Applied Biosystems, Foster City, CA), and extracted bacterial genomic DNA. DNA fingerprints were prepared by electrophoresis on a 1.5% agarose gel in 1xTAE buffer and ethidium bromide staining. The images were captured with a Chemi Imager system (Alpha Innotech Corporation, San Leandro, CA) and analyzed using BioNumerics (Applied Maths, Belgium). Using the Pearson's correlation coefficient and the unweighted pair group method with arithmetic mean algorithm (UPGMA), a dendrogram was created. Isolated bacteria were considered indistinguishable if their fingerprints were over 95% similar.

Mycology

Fungal isolates were subcultured on Sabouraud dextrose agar and incubated at 30 °C upon their return. Fungi were identified microscopically by their morphological characteristics.

Results

Bacterial characterization

The use of 16S sequence identification increased the speciation of isolates from 24.6% to 75.4% when compared to the use of only standard biochemical analyses. Of the 63 bacterial strains that were isolated and fingerprinted, 19 displayed similarity to each other. Of those 19 strains, 12 had been isolated from the water system.

Bacterial contamination of internal surfaces of the ISS was below the acceptability limits of 10,000 Colony Forming Units (CFU) / 100 cm^2 more than 75% of the sampling times. Thirty bacterial colony types were isolated from preflight and flight surface samples (Table 1). Isolates were predominantly Gram-positive, with the most common isolates being *Staphylococcus aureus* (4 occasions), *Staphylococcus pasteuri* (3 occasions), and *Micrococcus luteus* (3 occasions). The occurrence of several isolates grouped around certain time points or events, as exemplified by *S. pasteuri*, which was found on surfaces of three different hardware items during the same preflight sampling session. All *S. aureus* were isolated from surfaces during flight, but not on any preflight samples. Rep-PCR analysis showed that two of the *S. aureus* isolates were indistinguishable from those isolated at other sessions (Figure 1a and b). Rep-PCR-based fingerprinting of contaminants confirmed the transfer of isolates from preflight surfaces to the ISS. For example, indistinguishable strains of *Staphylococcus epidermidis* were found both before flight on the surface of the reusable cargo container (MPLM) and during flight on the surface of the ISS treadmill.

Levels of airborne bacteria were consistently below the 10,000 CFU / m^3 acceptability limit. Six bacterial colony types were isolated from preflight and flight air samples (Table 2), with no similar species identified from any session or location. Five of the six isolates were Gram-positive. *S. epidermidis* was isolated from preflight surfaces and in-flight air samples, though the air isolates appeared unrelated to the surface *S. epidermidis* isolates. *M. luteus* was

isolated from preflight surface and air samples. However, all *M. luteus* isolates had distinctly different fingerprints.

The potable water supply generated by reclaiming humidity condensate consistently provided water with bacterial levels below the U.S. acceptability limit of 100 CFU / 100 ml. The ground-supplied and space shuttle-provided potable ISS water bacterial content did occasionally exceed the 100 CFU / 100 ml limit. Twenty-seven bacterial colony types were isolated from flight potable water samples. Water samples were predominantly Gram-negative, and were predominantly made up of the genera *Sphingomonas* (25%) and *Methylobacterium* (18%) (Table 4). Using rep-PCR to track contamination in the water systems indicated the route of contamination for several bacteria. The *Methylobacterium fujisiwansae* strain isolated from the ground water transferred from the space shuttle fuel cells was also isolated from the Russian SVO-ZV water dispenser samples. Another *M. fujisiwansae* strain was also isolated from the SVO-ZV, but it had a distinctly different fingerprint. In a similar fashion, a *Sphingomonas paucimobilis* strain was isolated from both fuel cell water transferred from the space shuttle and the humidity condensate processor "SRV-K cold" water port sample. A genetically similar *Ralstonia eutropha* was found in water samples taken from 3 different sources, including the "SRV-K cold" water port, the SVO-ZV water dispenser, and the filter reactor component of the SRV-K humidity condensate processing system, which leads to the "SRV-K cold" water port.

Fungal characterization

Nineteen fungal isolates were identified from surfaces and air samples consisting mainly of *Aspergillus* and Hyphomycetes species (Table 2). Most of the 8 samples collected during flight were from the genus *Aspergillus*.

Discussion

This study emanated from the environmental monitoring and assessment program which was implemented to provide an environment promoting the health, safety, and productivity of the international crewmembers. Lessons learned from the space shuttle, Skylab, and the Russian space station Mir were implemented into the ISS to ensure an environment capable of supporting human habitation for many years. For example, HEPA filters were incorporated into the air

handling system, which resulted in consistently low levels of airborne bacteria, fungi, and particulates. The bacterial and fungal contaminants on internal surfaces of the ISS are minimized through a robust housekeeping program that includes weekly cleaning and biweekly disinfection. Suspected areas of microbial growth are cleaned and disinfected as soon as practical. The routine cleansing wipe contains a detergent, and the disinfectant wipe contains either a quaternary ammonium amine compound alone or with 1 percent hydrogen peroxide. Acceptable levels of bacteria in potable water are maintained using silver as the disinfectant. Choices of disinfectants are restricted by concerns over usage in the semi-closed environment of the ISS.

The results of other studies (4, 15, 16) led us to expect that 16S sequencing would increase our ability to identify bacteria to species over conventional biochemical analyses. The predominance of *Staphylococcus* species (13 of the 36 colony types isolated) cultured from the ISS samples was also found in the microbial flora isolated from the space shuttle (11), Mir Space Station (6)and from closed environments on Earth (12). All of the staphylococci isolated during flight were *Staphylococcus aureus* and *Staphylococcus epidermidis*, which may be the result of a clean system primarily affected by human occupation.

DNA fingerprinting of bacteria isolated provided insight into the source of contamination of several systems. It also allowed identification of two isolates, *Sphingomonas* species and *Bradyrhizobium japonicum*, that were not identified using either 16S sequencing or the VITEK biochemical analysis. In addition, the use of bacterial fingerprinting provided evidence that the collection of the same bacterial clone during random sampling is not a common event, even in a relatively closed system. Overall, only 30% of isolates displayed fingerprints similar to those of other isolates. Most of these similarities were seen among bacteria isolated from the water system, as only 19% of the air and surface isolates were similar to other air and surface isolates. This infrequency of isolation of the same bacterial clone may be the result of an overall low number of samples when compared to the diverse number of clones throughout the station. This infrequency may also be the result of artifacts associated with sampling protocols, which lead to overgrown or desiccated cultures, or differences in bacterial hardiness that may result in an inability to recover certain organisms. An increase in sampling frequency would provide additional insight, though the optimum sampling frequency to gain an understanding of the baseline microbial flora is not well defined.

High humidity, localized water condensate or water leaks aboard the Mir allowed fungi to proliferate. Crew activities and ventilation resulted in the spread fungal spores throughout the spacecraft. Fungal flora isolated from the Mir Space Station differed from that of the ISS, and the diversity of fungal species aboard the Mir was much greater than seen aboard the ISS. These differences may be explained by the relative ages of the Mir and the ISS when the microbial characterization was conducted. The Mir was approximately 10 years old when the study was conducted. By this time the Mir had seen many different crewmembers, been re-supplied countless times, and conducted many investigations including plants and other investigations which may promote fungal growth. Perhaps more importantly, the Mir had experienced numerous malfunctions leading to elevated temperatures, high humidity, and large amounts of water condensate accumulating on various surfaces. In contrast, the surface, air, and water samples for this study were collected when the ISS was only in the initial stages of its operational life.

These results represent the beginning of ISS habitation and provide a baseline of microbial flora onboard. While additional sampling frequency is beneficial, practical concerns such as use of crew time must be considered in a cost-reward assessment. For this study, the ultimate purpose of determining a microbial baseline was to protect crew health and ensure systems performance. The current data do not suggest there is a potential environmental hazard, nor does any trend indicate a potential health risk. However, microbial evaluations from Mir suggest that the potential for microbial contamination will increase with time and require continued surveillance. As environmental monitoring on the ISS continues, it will be interesting to compare the findings during the initial occupation with results in future years.

Acknowledgements

The authors would like to thank Bacterial BarCodes, Inc for their contribution in the processing and analysis of rep-PCR bacterial fingerprinting. We thank Jane Krauhs of Wyle Laboratories, Inc. for technical editing and James R. Lupski, M.D., Ph.D. for his review of this manuscript. We also thank the crew of the ISS for sample collection and processing, the ISS Program Office, and the Microbiology Laboratory at the Johnson Space Center. This study was supported by NASA contract NAS9-97005 and NASA grant PWC 111-30-40-97.

Table 1. Bacterial Isolates from Surface Samples

Source	Tracking Number	Sample Location	Identifications
Reusable Cargo Container, MPLM (Preflight)	MS-1	A2P Outlet, Ceiling Air Diffuser	*Paenibacillus* species
	MS-2	F3S Inlet, Floor 300μm Filter	*Staphylococcus epidermidis, caprae, or capitis*
	MS-3	F3S Inlet, Floor 300μm Filter	*Micrococcus luteus*
	MS-4	F3S Inlet, Floor 300μm Filter	*Micrococcus luteus*
	MS-5	F2P Outlet, Ceiling Air Diffuser	*Micrococcus luteus*
	MS-6	F2P Outlet, Ceiling Air Diffuser	*Staphylococcus capitis*
	MS-7	Ceiling Locker Bay 2	*Curtobacterium luteum*
	MS-8	Ceiling Locker Bay 2	*Curtobacterium* species
	MS-9	Ceiling Locker Bay 2	*Brevundimonas diminuta*
	MS-10	Ceiling Locker Bay 2	*Acinetobacter radioresistens*
	MS-11	A2S Inlet, Floor 300μm Filter	*Staphylococcus epidermidis*
	MS-12	Hatch Handle, Forward	*Pseudomonas oleovorans*
	MS-13	Hatch Handle, Forward	*Curtobacterium citreum*
	MS-14	Hatch Handle, Forward	Unidentified Gram negative rod
	MS-15	Hatch Handle, Forward	*Brevundimonas diminuta*
Hardware (Preflight)	HS-1	Advanced Video Interface Unit	*Staphylococcus pasteuri*
	HS-2	Buffer Interface Assembly	*Staphylococcus epidermidis*
	HS-3	Printer	*Staphylococcus pasteuri*
	HS-4	Printer	*Bacillus flexus*
	HS-5	Multi Use Tether End Effector	*Staphylococcus pasteuri*
ISS (In Flight)	IS-1	Node 1, Starboard Air Supply Diffuser	*Staphylococcus aureus*
	IS-2	Service Module, Treadmill	*Corynebacterium afermentans*
	IS-3	Service Module, Treadmill	*Staphylococcus epidermidis*
	IS-4	Service Module, Treadmill	*Corynebacterium tuberculostearicum, accolens, or segmentosum*
	IS-5	Service Module, Forward Air Diffuser	*Staphylococcus aureus*
	IS-6	Service Module, Forward Air Diffuser	*Acinetobacter radioresistens*
	IS-7	Service Module, Forward Air Diffuser	*Staphylococcus aureus*
	IS-8	U.S. Laboratory Module, Trace Contaminant Control Subassembly	*Oerskovia xanthineolytica*
	IS-9	U.S. Laboratory Module, Trace Contaminant Control Subassembly	*Bacillus pumilus*
	IS-10	U.S. Laboratory Module, Viewing Window	*Staphylococcus aureus*

Table 2. Bacterial Isolates from Air Samples

Source	Tracking Number	Sample Location	Identifications
Reusable Cargo Container, MPLM (Preflight)	A-1	Aft	*Micrococcus luteus*
	A-2	Forward	*Pseudomonas fulva*
	A-3	Forward	*Bacillus megaterium*
	A-4	Forward	*Micrococcus luteus*
ISS (In Flight)	A-5	Service Module	*Bacillus licheniformis*
	A-6	U.S. Laboratory Module	*Staphylococcus epidermidis*

Table 3. Bacterial Isolates from Water Samples

Source		Tracking Number	Sample Origin	Identifications
Humidity Condensate Processor	SRV-K Cold	W-1	Processed during flight	*Sphingomonas paucimobilis*
		W-2	Processed during flight	*Sphingomonas paucimobilis*
		W-3	Processed during flight	*Sphingomonas stygialis*
		W-4	Processed during flight	Unidentified Gram negative rod
		W-5	Processed during flight	*Bradyrhizobium japonicum* or *Blastobacter denitrificans*
		W-6	Archive	*Sphingomonas paucimobilis*
		W-7	Archive	*Ralstonia eutropha*
	SRV-K Hot	W-8	Archive	*Ralstonia eutropha*
		W-9	Archive	*Sphingomonas stygialis*
	Filter	W-10	Collected at Filter Reactor within system	*Ralstonia eutropha*
Water Dispensing Unit	SVO-ZV	W-11	Processed during flight	*Methylobacterium fujisawaense*
		W-12	Processed during flight	*Ralstonia eutropha*
		W-13	Processed during flight	*Bradyrhizobium japonicum*
		W-14	Processed during flight	*Sphingomonas* species
		W-15	Archive	*Methylobacterium fujisawaense*
		W-16	Archive	*Bradyrhizobium japonicum* or *Blastobacter denitrificans*
		W-17	Archive	Unidentified Gram negative rod
		W-18	Archive	*Bradyrhizobium japonicum* or *Blastobacter denitrificans*
		W-19	Archive	*Methylobacterium fujisawaense*
		W-20	Archive	*Pseudomonas stygialis*
Contingency Water Containers (CWCs)		W-21	S/N 5110	*Methylobacterium fujisawaense*
		W-22	S/N 5031	*Acinetobacter calcoaceticus or baumannii*
		W-23	S/N 5031	Unidentified Gram negative rod
		W-24	S/N 5056	*Sphingomonas paucimobilis*
		W-25	S/N 5055	*Microbacterium liquefaciens, luteolum,* or *oxydans*
		W-26	S/N 5055*	*Enterobacter* species or *Klebsiella* species
		W-27	S/N 5055*	*Delftia acidovorans*

* Not collected under sterile conditions

Table 4. Identifications of Fungal Isolates

Surface Samples	Sample Origin	Identifications
Reusable Cargo Container, MPLM (Preflight)	A3S Inlet Filter	*Penicillium* species
	A3S Inlet Filter	Hyphomycetes
	A3S Inlet Filter	*Aspergillus* species
	A3S Inlet Filter	*Penicillium* species
	Ceiling Locker Bay 2	*Aspergillus* species
	Ceiling Locker Bay 2	*Trichophyton* species
	Ceiling Locker Bay 4	*Streptomyces* species
	Hatch Door, Forward	*Microsporium* species
Hardware (Preflight)	Buffer Interface Assembly	*Curvularia* species
	Buffer Interface Assembly	Hyphomycetes
	Printer	Hyphomycetes
ISS (In-Flight)	Node 1, Starboard Air Supply Diffuser	*Aspergillus* species (two colony types)
	Node 1, Air Return Vent	*Aspergillus* species
	Service Module, Treadmill	Hyphomycetes
	Service Module, Forward Air Diffuser	*Aspergillus* species (three colony types)
	U.S. Laboratory Module, Grill Front of Trace Contaminant Control Subassembly	*Aspergillus* species
Air Samples	**Sample Origin**	**Identifications**
ISS (In-Flight)	Node 1	*Phoma* species
	Service Module	*Aspergillus* species
	U.S. Laboratory Module	*Phoma* species

Figure 1a. Rep-PCR DNA fingerprint analysis

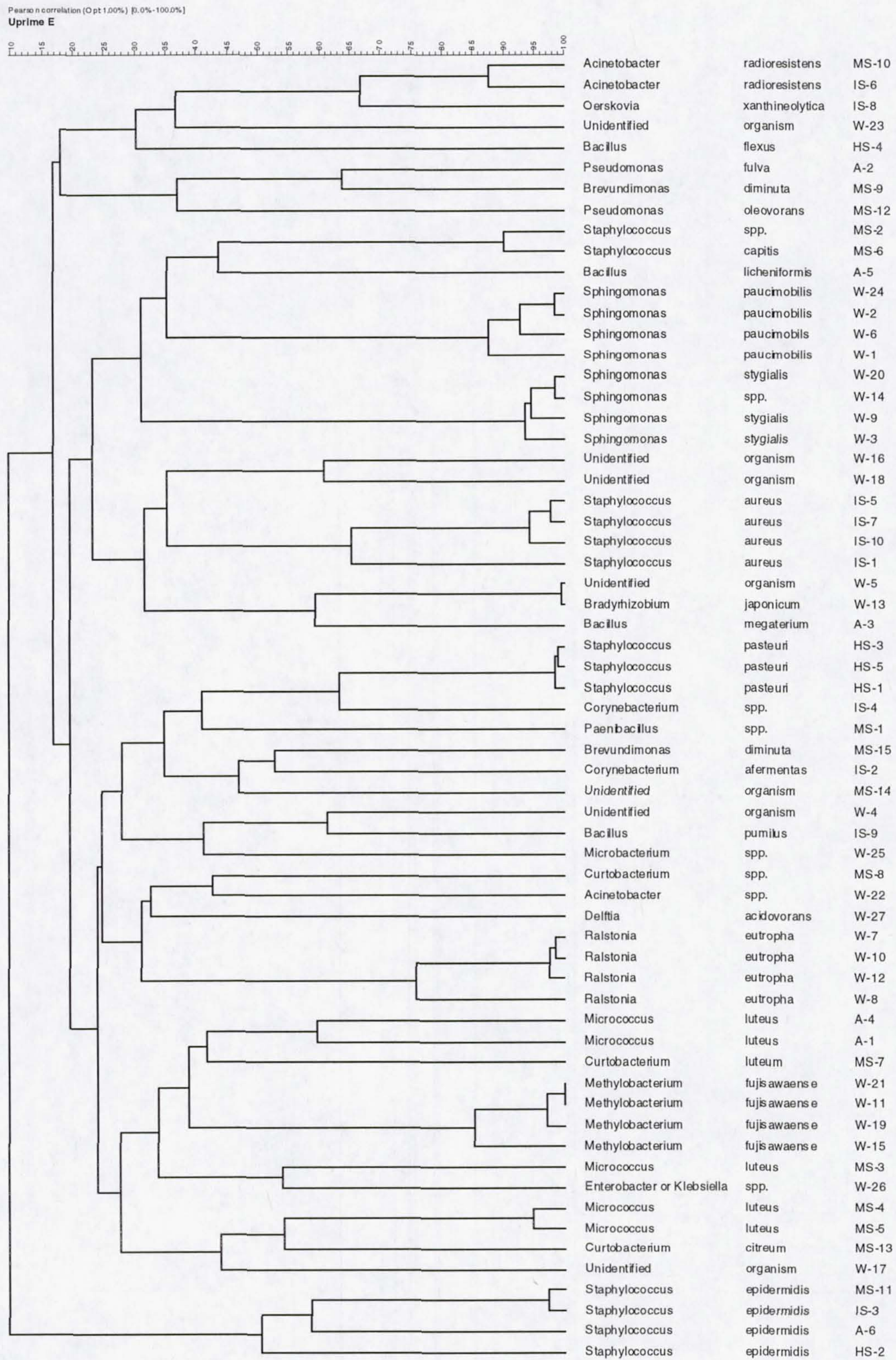
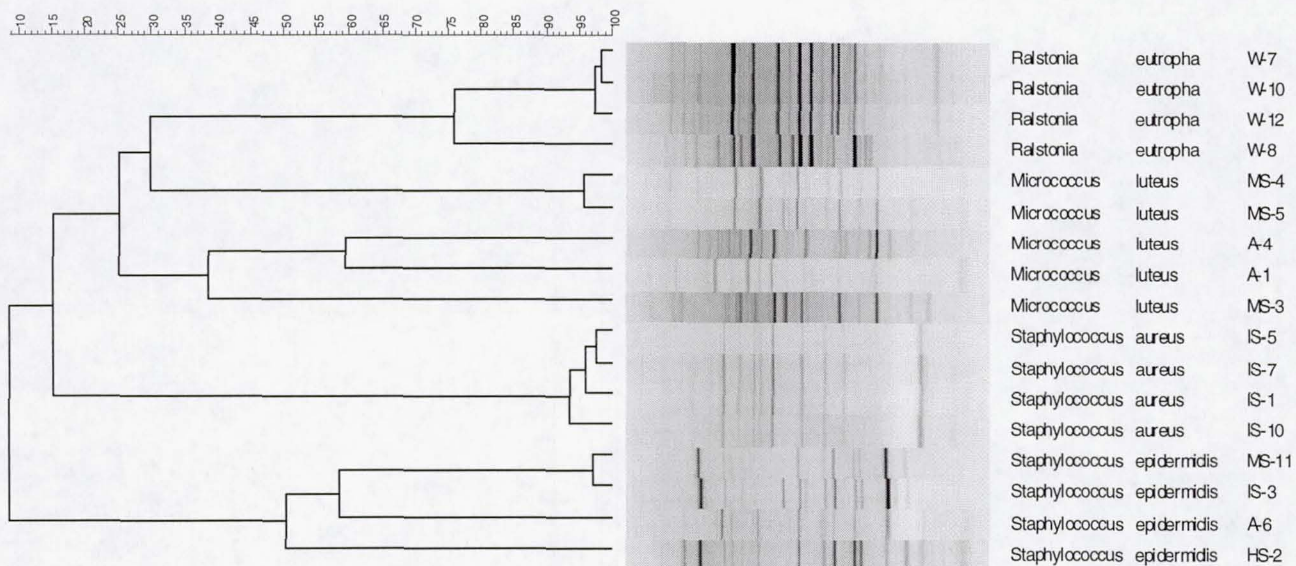


Figure 1b. Rep-PCR fingerprint comparison of selected isolates



References

1. Benson DA, M. Boguski S, Lipman DJ, Ostell J (1997) GenBank. Nucleic Acids Res 25(1):1-6.
2. Boyden DG (1962) The bacterial flora in fleet ballistic missile submarines during prolonged submergence Research Project MR005.14-3002-4.06. U. S. Naval Medical Research Laboratory. Bureau of Medicine and Surgery, Navy Department.
3. Burge HA, Pierson DL, Groves TO, Strawn KF, Mishra SK (2000) Dynamics of airborne fungal populations in a large office building. Curr Microbiol 40:10-16.
4. Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, Raoult D (2000) 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J Clin Microbiol 38(10):3623-30.
5. Ferguson JK, Taylor GR, Mieszkuc BJ (1975) Microbiological investigations. In: Johnston RS, Deitlein LF, and Berry CA (eds.), Biomedical Results of Apollo. Scientific and Technical Information Office, National Aeronautics and Space Administration. pp. 83-103.
6. Kawamura Y, Li Y, Liu H, Huang X, Li Z, Ezaki T (2001) Bacterial population in Russian space station "Mir". Microbiol Immunol 45(12):819-828.
7. Koenig DW, Bell-Robinson DM, Johnson SM, Mishra SK, Sauer RL, Pierson DL (1995) Microbiological analysis in space. Presented at the 25th International Conference on Environmental Systems, San Diego, CA. SAE Technical Paper Series 951683
8. Koenig DW, Pierson DL (1997) Microbiology of the space shuttle water system. Wat. Sci. Tech 35:59-64.
9. Levine HB, Cobb JM, Cobet AB (1970) The Tektite-I dive. Arch Environ Health 20:500-505.
10. Morris JE (1972) Microbiology of the submarine environment. Proc. R. Soc. Med. 65:799-800.
11. Pierson DL (2001) Microbial contamination of spacecraft. Gravitational and Space Biology Bulletin 14(2):1-6.
12. Pierson DL, Ott CM, Groves TO (2002) Characterization of microbial activity in the chamber systems and environment,. In: Lane HW, Sauer RL, Feeback DL (eds.), Isolation: NASA Experiments in Closed-Environment Living. Univelt, Inc, San Diego. pp. 229-259
13. Samsonov NM, Bobe LS, Gavrilov LI, Korolev VP, Novikov VM, Farafonov NS, Soloukhin VA, Romanov SJ, Andrechuk PO, Protasov NN, Rjabkin AM, Telegin AA, Sinjak JE, Skuratov VM (2002) Water recovery and oxygen generation by electrolysisaboard the International Space Station Presented at the 32nd International Conference on Environmental Systems, San Antonio, TX. SAE Technical Paper Series 2002-01-2358
14. Stenberg B, Eriksson N, Hansson Mild K, Höög J, Sandström M, Sundell J, Wall S (1993) The Office Illness Project in Northern Sweden. An Interdisciplinary Study of the "Sick Building-Syndrome" (SBS) Presented at the Indoor Air '93. Proceedings, Helsinki, Finland.
15. Tang YW, Ellis NM, Hopkins MK, Smith DH, Dodge DE, Persing DH (1998) Comparison of phenotypic and genotypic techniques for identification of unusual aerobic pathogenic gram-negative bacilli. J Clin Microbiol 36(12):3674-9.

16. Tang YW, Von Graevenitz A, Waddington MG, Hopkins MK, Smith DH, Li H, Kolbert, CP, Montgomery SO, Persing DH (2000) Identification of coryneform bacterial isolates by ribosomal DNA sequence analysis. J Clin Microbiol 38(4):1676-8.
17. Taylor GR, Graves RC, Brockett RM, Ferguson JK, Mieszkuc BJ (1977) Skylab environmental and crew microbiological studies, In: Johnston RS, Dietlein LF (eds.), Biomedical Results from Skylab. Scientific and Technical Information Office, National Aeronautics and Space Administration. pp. 53-63
18. Thomas TL, Hooper TI, Carmaca M, Murray J, Sack D, Molé D, Spiro RT, Horn WG, Garland FC (2000) A method for monitoring the health of U. S. Navy submarine crewmembers during periods of isolation. Aviat Space Envir Md 71(7):699-705.
19. Upsher JF, Fletcher LE, Upsher CM (1994) Microbiological conditions on Oberon submarines DSTO-RR-0004. Department of Defence, Defence Science and Technology Organisation.