Characterization and Monitoring of Microbes in the International Space Station Drinking Water

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ABSTRACT

This study focuses on the development of procedures to characterize the microbial quality of International Space Station (ISS) and shuttle drinking water at various stages of water treatment. In addition to traditional culture-based techniques, ATP, endotoxin, and DNA targeted microbial enumeration procedures were employed to elucidate the microbial contamination of the ISS drinking water. Drinking water processed at various stages for the STS-113 mission aboard the Endeavor OV-105 shuttle, as well as aboard the ISS, was sampled and examined for microbial contamination using state of the art quantitative and qualitative methodologies. Biomolecule-targeted microbial detection methods revealed the presence of non-cultivable microbes in the drinking water, and confirmed the fact that the measures taken to cultivate microbes from the drinking water samples were estimating only 10% of the microbial contamination. Both culture and DNA-based methodologies reported the presence of *Acidovorax temperans,* a halogen (biocide) reducing bacterium from the ISS-regenerated water sample. Although the water collected from the drinking fountain did not contain any measurable cultivable microbes, the DNA-based procedures retrieved ribosomal sequences of the opportunistic pathogens *Afipia, Delftia, Propionibacterium,* and *Ochrobactrum*. The present study did not confirm the presence of active pathogens in the drinking water, although evidence strongly suggests that implementation of new cultivation approaches to identify the presence of pathogens is essential.

INTRODUCTION

As with any aspect of manned space exploration, the integrity/quality of the drinking water aboard the International Space Station (ISS), and space shuttle orbiters as well, must be meticulously maintained and monitored. Pierson (2001) observed that microorganisms are ubiquitous throughout the habitable modules of the ISS and documented that the crewmembers were the predominant source of contamination. It has been reported that the immune systems of astronauts in space frequently lapse into weaker, compromised states (Stowe et al., 2001). Therefore, it is critical to thoroughly and accurately assess the microbial burdens associated with the water and food to be ingested in space. To date, each and every attempt at describing the microbial species present aboard Apollo missions (Ferguson et al., 1975), Skylab (Taylor et al., 1977), US space shuttles (Koenig and Pierson, 1977; Pierson, 2001), the Russian Mir space station (Kawamura et al., 2001), and the ISS (Castro et al., 2003) has relied on traditional, culture-dependent methods for the isolation of microbes and molecular techniques for species identification. Such techniques are informative, though limited to cultivable microbes, and are very highly biased by cultivation strategies employed. Because of such biases, studies of this nature have proven inadequate in thoroughly reporting the microbial community structure from several environmental samplings, as validated by molecular cloning measures (Pace et al., 1985; Giovannoni et al., 1990).

The presence of viable microorganisms in drinking water is of particular importance to the health of astronauts, but methods for their detection and enumeration, such as culture counting, are fraught with difficulties. Notably, different species require different media or environments for growth, so in current microbial quality verification protocols,

66 not all cells are likely to be detected as colony-forming units (CFU). Adenosine
67 triphosphate (ATP) is used by all living organisms and can be exploited as an indicator
68 for the presence of living organisms, as many earlier studies have suggested (Chappelle
69 and Levine, 1968; Thore et al., 1975; Karl, 1980; Stanley, 1989; Venkateswaran et al.,
70 2002). Likewise, an endotoxin-based commercially available lipopolysaccharide (LPS)
71 detection (LAL) assay was also employed to rapidly analyze microbial contamination in
72 ISS drinking water samples (Chung et al., 2001). It is known that culturing viable
73 microbes from fastidiously processed samples using conventional microbial assay
74 techniques significantly underestimates the viable microbial population because of the
75 non-cultivability of certain microbes (Xu et al., 1982; Barer et al., 1993; Grimes et al.,
76 2000). The ATP-based assay detects all viable cells, as does molecular analyses based on
77 DNA isolated directly from samples (Pace et al., 1985; Giovannoni et al., 1990). These
78 bioassay techniques allow for better evaluations of microbial quality, and when carried
79 out in conjunction can provide a more complete understanding than plate counting of the
80 microbial species most likely to contaminate ISS drinking water samples.

81 This study is the first culture-independent, molecular microbial community analysis
82 of ISS and space shuttle drinking waters. It is of utmost importance to those concerned
83 with space-flight safety to assess inherent risks to astronaut health by continuously
84 monitoring the diversity of microbes that astronauts are most likely to be in contact with
85 while in space. The monitoring scheme employed in this study encompassed several
86 samplings of water intended for use on the STS-113 mission aboard the ISS, from the
87 originating municipal water supply, prior to and following biocide treatment, and directly
88 from fountains and storage cells within the ISS. Of immense concern to all involved with

89 human inhabitation of the ISS is the possibility of pathogenic microorganisms originating
90 in the drinking water adversely affecting the health of astronauts.
91
92 ## MATERIALS AND METHODS
93 **Sample collection.** Water samples processed at the Kennedy Space Center (KSC) for the
94 STS-113 mission aboard the Endeavor OV-105 shuttle and at various sources from the
95 ISS were collected for microbiological examination. Appropriate aliquots of water were
96 collected from the Cocoa municipal water supply, FL, prior to (2 samples) and following
97 (3 samples) biocide treatment (8 ppm Iodine). Water samples were taken from the
98 Russian-built dispenser (designated SVO-ZV), which contained ground-supplied
99 Moscow water and space shuttle fuel cell water. In addition, water samples were obtained
100 from the hot port (designated SRV-K hot) of the Russian system used for humidity
101 condensate recovery (Samsonov et al., 2002). All KSC processed samples were kept at
102 4°C until analyzed. However, samples collected at ISS were transported in ambient
103 temperature and upon shuttle's arrival to Earth, samples were stored at 4°C until
104 examined.
105 **Culture-dependent assay.** Water samples processed at KSC were analyzed immediately
106 after collection. Appropriate aliquots of samples collected from the KSC municipal water
107 prior to, and following, biocidal treatment were placed into petri dishes and total aerobic
108 cultivable bacterial populations were enumerated by the pour plate techniques using R2A
109 agar (Remel, Lenexa, KS) as the growth medium at 25°C. Colony forming units (CFU)
110 were enumerated after 7 days of incubation. Water samples from the SVO-ZV and SRV-

111 K hot port were processed during shuttle flight using a self-contained system which
112 filtered a 100 mL aliquot through a 0.45 μm cellulose acetate field monitor (Millipore,
113 Bedford, MA) as previously reported (Koenig et al., 1995). A liquid R3A growth medium
114 (Remel) was added to an absorbent pad on the downstream side of the filter surface.
115 Samples processed during flight were incubated at ambient temperature (25 to 30°C) and
116 returned for ground-based analysis on the next available shuttle flight. Because flight-
117 grown cultures were received up to 3 months after collection, many of the culture media
118 were overgrown with microorganisms or desiccated, thus viable cultures could not be
119 recovered from all colony types observed. Additionally, aliquots of 100 mL of water
120 samples from ISS were passed through 0.22 μm membrane filters (Millipore) upon
121 shuttle arrival and cultured on R2A medium and incubated at 37°C for 48 hours. Isolates
122 were randomly selected, purified, and stored at –80°C for further processing and analysis.

123 Identification of purified strains was determined based on 16S rDNA sequencing
124 (Castro et a., 2003). Briefly, DNA was extracted using commercial kits (Mo Bio
125 Laboratories, Inc., Solana Beach, CA) and appropriate fragments were amplified and
126 sequenced using Microseq 500 16S rDNA Bacterial Sequencing Kit (Applied
127 Biosystems, Foster City, CA). All bacterial isolates were subcultured on blood agar at
128 37°C for 24 hours and identified using the VITEK Identification System (bioMerieux,
129 Hazelwood, MO).

130 Culture-independent assays. **All samples collected in this study were analyzed**
131 **simultaneously for ATP, endotoxin, and 16S rDNA via molecular cloning analyses.**
132 **The time lag from the collection of the samples to the analysis ranged from 3 to 4**
133 **months and should be considered when results of these analyses are compared with**

conventional culture-dependent assays. It was difficult to directly compare results from the assay techniques employed in this study (colony counts, ATP assay, LAL assay) because these assays measured very different populations.

(i) ATP-based bioluminescent assay. Firefly luciferase catalyzes the reaction of luciferin with ATP to form the intermediate luciferyl adenylate. Its subsequent reaction with oxygen leads to a cyclic peroxide, whose breakdown yields CO_2 and the oxyluciferin product in the electronically excited state, from which a photon is emitted with a quantum yield of about 90% (Wilson and Hastings, 1998). The bioluminescence generated is directly proportional to the amount of ATP in the sample being assayed, and to the number of microorganisms in the sample assayed. The use of firefly luciferase to assay ATP extracted from microorganisms provides a straightforward means to enumerate microbes within minutes, and several companies have developed kits based on this system. For our purposes, these kits allow the detection of total ATP, not only the intracellular ATP from intact microbial cells, but also the extracellular ATP from dead bacteria and non-microbial sources, such as lysed cells or debris. The extracellular ATP can be eliminated enzymatically, so that the remaining ATP is attributable only to intact microbial cells, both cultivable and non-cultivable. A kit for this purpose (CheckLite-HS Set, Kikkoman Corp., San Francisco, CA) was used in the present study.

(ii) LPS-based endotoxin assay. When microorganisms invade an animal, the immune system responds by initiating a highly specific enzyme cascade in its blood cells (i.e. amebocytes). It is known that this cascade is initiated by the presence of LPS in Gram-negative bacteria and beta glucan in yeasts, and results in the formation of a gel-clot that destroys the invading microbes. The LPS-based microbial detection assay exploits this

principle as it occurs in the horse-shoe crab (*Limulus polyphemus*) coupled with a chromogenic substrate. Hereafter, we refer to this as the limulus amoebocyte assay (LAL). This method is most commonly used to quantify endotoxin and is the basis for the American Society for Testing and Materials method E2144-01. Commercially available kits (Charles River Laboratories, Wilmington, MA) were used to perform this rapid and simple assay (2-hour) where appropriate aliquots of samples were mixed with reaction reagents and clotting was measured colorimetrically.

(iii) Molecular Microbial Diversity analysis. DNA was extracted directly from one representative sample each of pre-, post-biocide treatment; SRV-K hot and SVO-ZV water to analyze molecular microbial diversity without cultivation. One hundred mL from each sample was concentrated and subjected to DNA extraction following standard lysozyme/organic solvent extraction protocols. Bacterial small subunit rDNA fragments were PCR-amplified with eubacterially biased primers B27F and B1512R. PCR conditions were as follows: 1 min. 95°C denaturation, 2 min. 55°C annealing, and 3 min. 72°C elongation for 35 cycles using a thermal cycler (MJ Research; Waltham, MA). After 10 minutes incubation at 72°C, the amplification product was purified with a gel excision kit (Qiagen, Chatsworth, CA). Purified amplicons were cloned into the pCR-4 TOPO vector by TA cloning (Invitrogen, Carlsbad, CA), per manufacturers' instruction. Ribosomal DNA inserts were PCR-amplified from plasmid clones with commercially available M13R and T7 primers. Amplification products were digested with the restriction endonuclease *Hha*I (New England Biolabs, Beverly, MA) for 3 hours at 37°C. Banding patterns were grouped according to similarity, and representative members of each distinct pattern group were fully, bi-directionally sequenced (MWG Biotech Inc.,

High Point, NC). Multiple representatives were sequenced for RFLP patterns that had more than five members.

The phylogenetic relationships of organisms covered in this study were determined by comparison of individual rDNA sequences to sequences in the public database (http://www.ncbi.nlm.nih.gov/blast). Evolutionary trees were constructed using PAUP software (Swofford, 1990).

RESULTS

Culture-dependent analysis. Although untreated municipal water samples contained $1.4x10^4$ CFU/100 mL, the biocide agent added into the Cocoa municipal water rendered the entire bacterial population non-cultivable (Table 1). The SRV-K hot water sample collected from the Russian regenerated water system on board the ISS revealed $5.1x10^1$ CFU/100 mL cultivable counts, whereas the water collected from the ISS drinking fountain (SVO-ZV) did not show any CFU. Isolation of *Acidovorax temperans,* a halogen (biocide) reducing bacterium from the SRV-K hot water sample is especially interesting since molecular cloning analyses yielded 7 *A. temperans* clones. The ability of *A. temperans* to withstand varying concentrations of biocide should be studied, as well as the possibility of this microbe entering into a "viable but non-cultivable" state due to the bactericidal agent. Re-analysis of the SVO-ZV water sample upon shuttle arrival yielded colonies of *Methylobacterium fujisawaensae* and *Sphingomonas yanoikuyae* after prolonged incubation. However, such bacterial colonies were not isolated from the in-flight cultivation system. Presumably, under microgravity, *M. fujisawaensae* and *S. yanoikuyae* cells might have undergone changes in cell morphology. Hence, during in-

203 flight filtration, these microcolonies might have passed through the 0.45 μm filters but
204 were trapped in the 0.22 μm filtration system employed on the ground. Further research
205 is warranted to confirm this observation.

206

207 **Culture-independent analyses.**

208 **(i) ATP-based bioluminescent assay.** Samples collected from the Cocoa municipal
209 drinking water storage facility before biocide treatment showed presence of $1.3x10^5$
210 relative luminescence units of ATP per 100 mL, which is one order magnitude higher
211 than cultivable counts, measured (Table 1). The water collected from the SRV-K hot port
212 system contained $4.8x10^4$ relative luminescence units per 100 mL compared to no
213 detectable quantity of ATP from the SVO-ZV drinking water. Further analysis is
214 necessary to confirm this result. The ability of this system to measure relative amounts of
215 ATP in the presence of iodide should also be explored, as partial or total inhibition is
216 possible.

(ii) LPS-based endotoxin assay. The results of the LAL measurements, as measured by endotoxin units, are given in Table 1. As seen in cultivable count analyses and the ATP assay, LAL analysis revealed a lower bio-burden following treatment of the drinking water with iodine. The biological reduction was 2 logs after biocide treatment. However, a considerable amount of measurable endotoxin (equvalent to the Cocoa, FL municipal water) in the water collected from the SRV-K hot port suggests the presence of microbial fragments in this sample. Similarly, microbial endotoxin units were about $2.4x10^1$ pg/100 mL in the sample collected from the SVO-ZV drinking water aboard the ISS where no cultivable counts and measurable ATP were recorded.

(iii) Molecular microbial community analysis. Typical RFLP patterns showing highly diversified microbial populations from the SRV-K hot (Fig. 1A) and SVO-ZV (Fig. 1B) samples are depicted. Representatives of various patterns were selected and sequenced for further community analysis. Among the sequences retrieved directly from the various water samples analyzed in this study were representatives of five clades, the α–, β–, and γ– proteobacteria, the actinomycetales, and CFB-group bacteria (Fig. 2). The phylogenetic tree based on 16S rDNA sequences for the ground-processed Cocoa municipal water, before and after biocide treatment (Fig. 2A) and SRV-K hot as well as SVO-ZV water samples (Fig. 2B) are shown. In total, among 39 clones selected for sequencing, 15 different bacterial species were observed. The clone library representing the Cocoa municipal water was limited in diversity to two bacterial species, *Pseudomonas fragi* (70 of 96 clones; 73%) and *P. stutzeri* (26 of 96 clones; 27%). Likewise, members of *Ralstonia, Herbasprillum* and *Sphingomonas* were seen in the water samples that were treated with biocide. RFLP analyses of cloned bacterial 16S-

240 rDNA fragments recovered from the SVO-ZV drinking water sample yielded 9 distinct
241 species from the 96 individually processed clones, whereas the SRV-K hot water sample
242 yielded 8 species (Table 2). Phylogenetic analyses of the gene sequences showed
243 moderate, overlapping bacterial diversity between the SVO-ZV and SRV-K hot libraries.
244 Sequences related to actinomycetales, and the α-, β-, and γ-proteobacteria lineages, were
245 found in both the drinking water and the regenerated water samples. A sole representative
246 of the CFB group, *Flexibacter japonensis*, was retrieved from the SRV-K hot library. The
247 most dominant genera, based on sequences recovered, were *Afipia* and *Ralstonia*. The
248 *Afipia* genus was representative of 47 of the 96 SRV-K hot clones (49%), but was not
249 represented in the SVO-ZV clone library. It is important to indicate that clones of
250 *Ralstonia* were retrieved from the post-biocide treated Cocoa municipal water, SRV-K
251 hot and SVO-ZV samples. A higher incidence of *Ralstonia* gene sequences on spacecraft
252 (15%) assembled in KSC encapsulation facilities (30%) has been previously documented
253 (unpublished information).
254 Other sequences retrieved from both libraries included *Delftia acidovorans* (~7 to
255 16% of each library), *Caulobacter crescentus* (~7 to 16% of each library), and
256 *Propionibacterium acnes* (~4% of each library). Some sequences were retrieved from one
257 sample or the other, but not both. Gene sequences of *A. temperans*, *Stenotrophomonas*
258 *maltophila*, and *F. japonensis* were retrieved only from the SRV-K hot sample, while
259 *Hyphomicrobium facilis*, *Brevundimonas diminuta*, *Ochrobactrum tritici*, and
260 *Bradyrhizobium japonicum* rDNAs were retrieved only from the SVO-ZV sample.
261

DISCUSSION

Biomolecule-targeted microbial detection methods were extremely sensitive in enumerating the total microbial burden of the ISS potable waters. The use of biocide eliminated the cultivability of certain microbes, however, various sophisticated methodologies employed in this study revealed the presence of "viable but not cultivable" microbes. Perhaps the most significant finding was the retrieval of 16S rDNA sequences having highest similarities with several opportunistic human pathogens (Table 2) directly from ISS potable waters (SVO-ZV and SRV-K). Cultures of *A. broomeae* reportedly cause disease in humans through associations with amoeba. Patients in intensive care units (ICU) often become infected and sometimes gravely ill following exposure to *A. broomeae*. Water samples taken from ICU taps and general hospital reservoir tanks of 4 university hospitals in Marseilles, France were tested for this pathogen by co-culturing with amoeba, and cultures of *A. broomeae* were isolated. In addition, elevated *Afipia*-antibody titers were found in the ICU patient blood (La Scola, et al., 2002). The disease caused by *Afipia* species is commonly referred to as cat scratch disease and in 1994, the Center for Disease Control, USA warned of this bacterium becoming an emerging pathogen. Although we do not know whether these alpha proteobacteria are alive in the ISS water samples collected, it is important to make an attempt to culture these problematic microbes.

In addition to *Afipia*, cultures of *O. anthropi* and *P. acnes* have been reported to cause illness in immuno-compromised patients. The former causes hyperuricemia and the latter microbe damages the livers of healthy individuals. The 16S rDNA sequence of *O. anthropi* is identical to that of certain species of *Brucella*, a highly virulent pathogen. The

opportunistic pathogen, *Ochrobactrum*, produces purine nucleosidases that metabolize purines and pyrimidines. The description of immuno-deficiency syndromes in association with purine enzyme deficiency has led to a novel area of investigation encompassing the biochemical basis for immune function (Edwards and Fox, 1984). Hyperuricemia and gout are the most common disorders arising from this bacterial infection (Ogawa et al., 2001).

Propionibacterium is an anaerobic cocci and is part of the normal microflora of the skin, conjunctiva, mouth, upper respiratory tract, external ear canal and intestine. The culture-dependent assays employed in this study detect only aerobic microbes, thus *P. acnes*, being anaerobic, would not have grown in the methods employed to cultivate microbes in this study. Despite being considered non-pathogenic for humans, there have been several reports of *Propionibacterium* species causing numerous infections (Yoshikawa et al., 1975). Significant infections caused by *Propionibacterium* species are associated with the blood, central nervous system, lymph glands, abscesses, joints, wounds, cysts and sinuses (Brook and Frazier, 1991). It was further observed that *Propionibacterium* causes disease in immuno-deficient patients. Recently, Ullmann et al. (2000) reported hepatic granuloma due to *P. acnes* in a patient with acute myelogenous leukemia. The retrieval of sequences of opportunistic pathogens from ISS potable waters may warrant concern, however, a larger emphasis should be placed on the cultivation, or lack thereof, of these pathogenic microorganisms.

Also of particular interest was the retrieval of halogen-reducing bacterial DNA sequences. Iodide and other halogen-biocides are utilized to minimize the active microbial populations in these water systems. It is interesting that microbes adapted to

these measures, far more – able to degrade such decontaminating agents, are so prevalent when assessed by 16S rDNA community analyses. The presence of *D. acidovorans* is significant since this organism has been reported to reduce bromate compounds. Incidentally, the drinking water for the ISS was processed with iodine as a biocide. Bromium and iodine are of the same molecular weight and the survival of such iodine reducing microbes in the biocide-treated water indicates that biocide treatment was not completely effective in eradicating the total microbial population. Some biocide resistant microbes might have survived the treatment process or have been injured and rendered non-cultivable with the culture techniques employed.

Additionally, microbes that could only survive in low nutrient aquatic environments such as *Hyphomicrobium* and *Caulobacter* species were observed in both ISS potable water samples examined during this study. These stalked bacteria adhere to solid surfaces via a holdfast at the tip of the stalk. From current literature, it can be hypothesized that these oligotrophic microorganisms might have formed biofilms in the pipelines as well as tanks used to store drinking water. Although previous investigations revealed biofilm formation of *Hyphomicrobium* in drinking water treatment plants (Holm et al., 1996), thorough investigation of the ISS drinking water system is necessary to confirm this fact.

The retrieval of only *Pseudomonas* sp. rDNA sequences from the municipal water sample, while a much more diverse population was observed in both of the potable ISS reservoirs, is suggestive of a post-vessel-loading contamination event. Perhaps the bladder(s) of one or more reservoirs in contact with water aboard the shuttle and/or ISS is in some way contaminated. Biofilm attachment to such surfaces is not unusual. Indeed,

studies have shown that surfaces constantly in contact with water are easily colonized and frequently fall victim to biofilm contamination (Soini et al., 2002; Donlan 2002). Further studies must be conducted to validate this claim.

In comparing the results from cultivation-based and molecular approaches, a drastically larger diversity is observed when analyzing by molecular, culture-independent methods. The microbial diversity observed by culturing isolates does not correlate with that seen in clone libraries, as is common in studies of this kind. One possible cause of such disagreement is biases associated with both cultivation and PCR. Molecular cloning-based methods showed more diverse populations from both the SRV-K hot and SVO-ZV potable water samples than did the culture-based methods employed. This is reasonable since recovery of DNA fragments yields information from the total microbial population present, dead or alive, unlike culturing finicky microorganisms that require specific conditions for growth. There were some sequences retrieved from one library or the other, but not both. These non-overlapping sequences could be due to the small sample size (96 clones from each of the water samples).

The physiological state(s) of the opportunistic pathogens indicated by 16S rDNA detection remains to be determined. At this time it is not feasible to offer any hypotheses on the matter. Possible explanations abound, one being that these sequences are arising from non-living, remnants of once active opportunistic pathogens. Another possibility is that the sequences are arising from viable, active microbes. Furthermore, regardless of the physiological state of the pathogen, considerable responses can be elicited by the human immune system to antigens whether associated with a living microbe, or non-living cellular debris. Such immune and/or allergic responses can seriously hinder the

354 comfort, or much worse – general health of astronauts aboard the ISS. Future research
355 into the activity of these contaminating microbes via reverse transcriptase-PCR and
356 related techniques is necessary.
357
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Legends for Figures.

Figure 1. RFLP patterns from *Hha*I digest of the 16S rDNA sequences from several bacterial clones. Genes were amplified and digested with restriction enzymes as described in *Materials and Methods*. Lanes are labeled according to the loading of clone DNA digests. A 100bp DNA ladder is included as a size marker.

Figure 2. Phylogenetic affiliations of microbial clones retrieved from water samples processed for the ISS. (A) KSC-processed water samples, pre-biocide [V4] and post-biocide [M3] treatment. (B) International space station water samples, SRV-K and SVO-ZV designations are given for the regenerative water and drinking water, respectively. Bacterial names of the nearest neighbor followed by GenBank accession numbers are given. Numbers that follow the sample name denote clone number.

Table 1. Microbiological characteristics of drinking water samples processed for the International Space Station

Sample #	Description	Total cultivable counts	Intracellular ATP	Endotoxin
		CFU/100mL	RLU/100mL	pg/100mL
V1	Before biocide treatment	$1.3x10^4$	$1.3x10^5$	$3.0x10^2$
V4	Before biocide treatment	$2.0x10^4$	$1.0x10^5$	$3.0x10^2$
M1	After biocide treatment	0	0	2
M2	After biocide treatment	0	0	0
M3	After biocide treatment	0	0	1
SRV-K hot	Regenerated water to rehydrate foods	$5.1x10^1$	$4.8x10^5$	$2.1x10^2$
SVO-ZV	Potable water	0	0	$2.4x10^1$

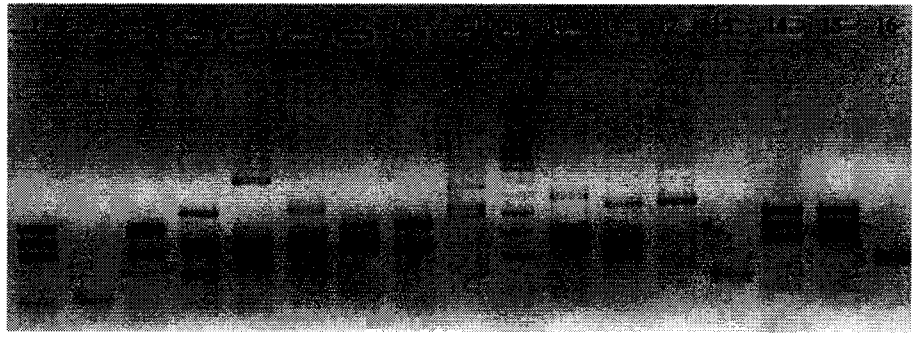
Abbreviations: CFU, Colony forming units; RLU, Relative luminescence unit; pg, picogram

Table 2. Microbial diversity associated with the International Space Station drinking water

Nearest neighboring bacterial species	Percent similarities of 16S rDNA sequences	Number of clones retrieved from: Regenerated water (SRV-K hot)	Potable water (SVO-ZV)	Remarks
Afipia broomeae	>99.3	47		Cat scrach disease; CDC warning as an emerging pathogen
Ralstonia detusculanense	>99.9	14	28	Brackish water microbe; isolated on Mars Odyssesy surfaces
Caulobacter crescentus	>95.0	7	15	Common in drinking water
Delftia acidovorans	>99.7	7	15	Bromate and idodine-reducing bacterium
Propionibacterium acnes	>99.7	3	5	Opportunistic pathogen; liver lesions
Acidovorax temperans	>99.0	7		Denitrification of drinking water
Flexibacter japonensis	>100.0	7		Produce human leukocyte elastase inhibitor
Stenotrophomonas maltophila	>99.8	4		Opportunistic pathogen
Bradyrhizobium japonicum	>97.4		13	Nitogen fixing symbiotic bacterium
Hyphomicrobium facilis	>99.8		10	Common in drinking water
Ochrobactrum anthropi	>97.2		5	Mimics *Brucella* sp. which is a deadly pathogen; *Ochrobactrum* causes hyperuricem
Brevundimonas diminuta	>96.0		3	
Escherichia coli	>99.0		2	Common enteric bacteria

Fig. 1

(A) SRV-K hot (regenerated ISS water)



(B) SVO-ZV (ISS drinking water)

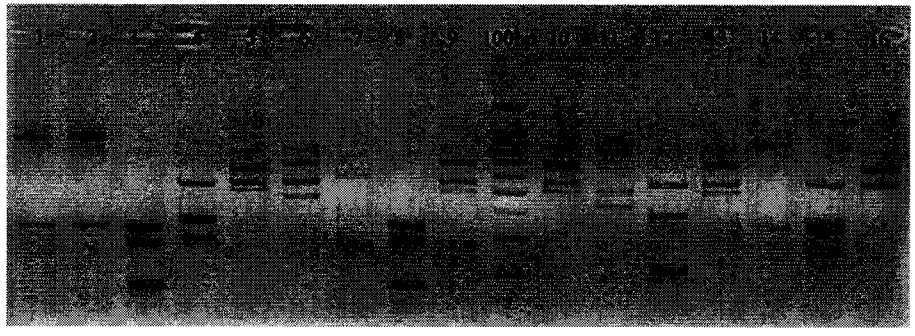
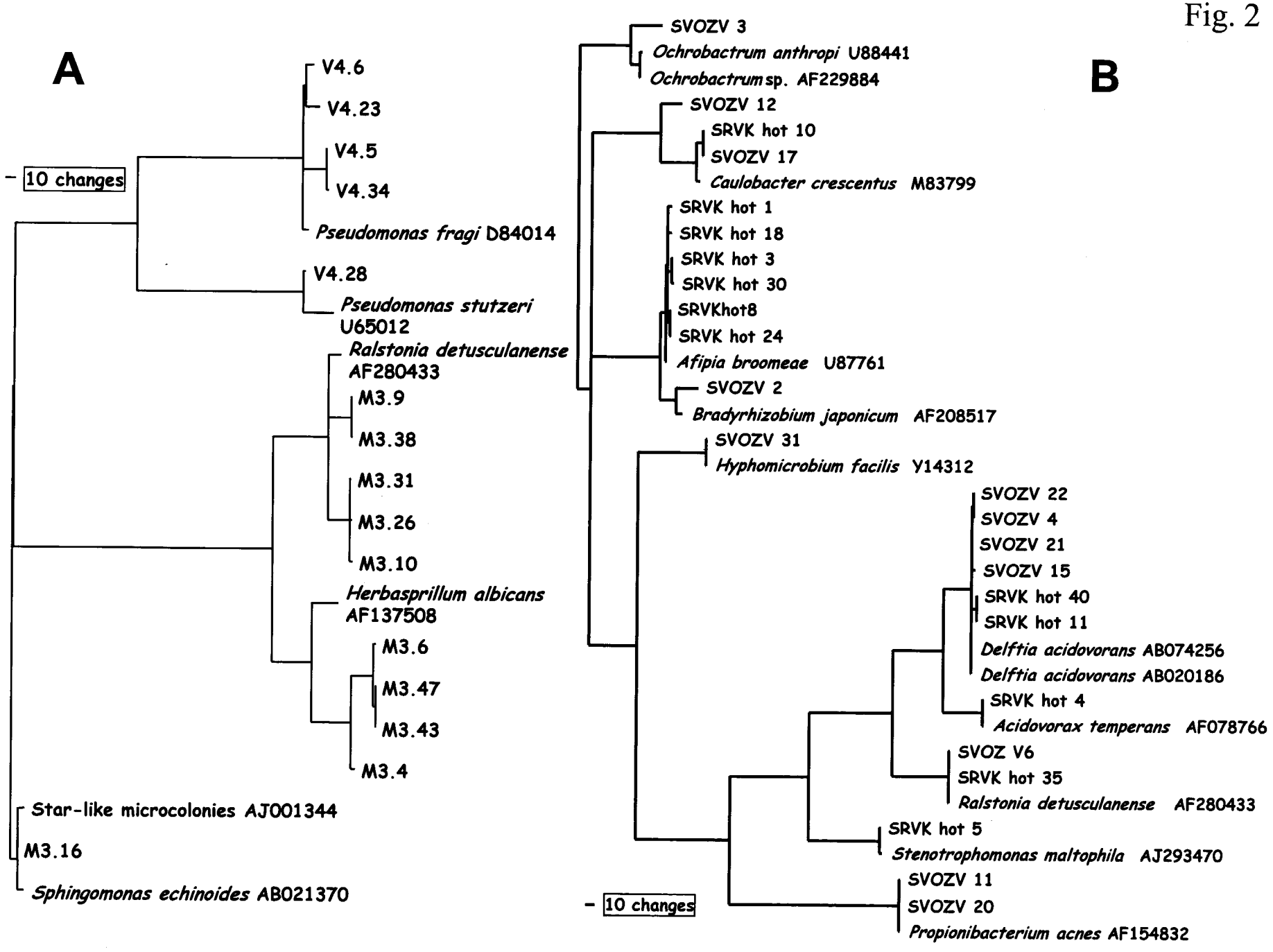


Fig. 2


A
V4.6
V4.23
V4.5
V4.34
10 changes
Pseudomonas fragi D84014
V4.28
Pseudomonas stutzeri U65012
Ralstonia detusculanense AF280433
M3.9
M3.38
M3.31
M3.26
M3.10
Herbasprillum albicans AF137508
M3.6
M3.47
M3.43
M3.4
Star-like microcolonies AJ001344
M3.16
Sphingomonas echinoides AB021370
B
SVOZV 3
Ochrobactrum anthropi U88441
Ochrobactrum sp. AF229884
SVOZV 12
SRVK hot 10
SVOZV 17
Caulobacter crescentus M83799
SRVK hot 1
SRVK hot 18
SRVK hot 3
SRVK hot 30
SRVKhot8
SRVK hot 24
Afipia broomeae U87761
SVOZV 2
Bradyrhizobium japonicum AF208517
SVOZV 31
Hyphomicrobium facilis Y14312
SVOZV 22
SVOZV 4
SVOZV 21
SVOZV 15
SRVK hot 40
SRVK hot 11
Delftia acidovorans AB074256
Delftia acidovorans AB020186
SRVK hot 4
Acidovorax temperans AF078766
SVOZ V6
SRVK hot 35
Ralstonia detusculanense AF280433
SRVK hot 5
Stenotrophomonas maltophila AJ293470
SVOZV 11
SVOZV 20
Propionibacterium acnes AF154832
10 changes