*International Journal of Astrobiology*

**cambridge.org/ija**

# Acid and aluminium-tolerant microbes isolated from China space station assembly cleanroom surfaces and identified by 16S rRNA/ITS sequencing and MALDI-TOF MS

Nino Rcheulishvili[1], Dimitri Papukashvili[1], Yasmeen Shakir[1], Yulin Deng[1,2] and Ying Zhang[1,2]

[1]School of Life Science, Beijing Institute of Technology, Beijing 100081, China and [2]Beijing Key Laboratory for Separation and Analysis in Biomedicine and Pharmaceuticals, Beijing 100081, China

**Research Article**

**Cite this article:** Rcheulishvili N, Papukashvili D, Shakir Y, Deng Y, Zhang Y (2021). Acid and aluminium-tolerant microbes isolated from China space station assembly cleanroom surfaces and identified by 16S rRNA/ITS sequencing and MALDI-TOF MS. *International Journal of Astrobiology* **20**, 133–141. https://doi.org/10.1017/S1473550420000427

Received: 8 June 2020
Revised: 17 December 2020
Accepted: 18 December 2020
First published online: 3 March 2021

**Key words:**
16S rRNA gene sequencing; acid-tolerant microbes; aluminium-corrosion; MALDI-TOF MS; microbiologically influenced corrosion; spacecraft

**Author for correspondence:**
Yulin Deng,
E-mail: deng@bit.edu.cn;
Ying Zhang,
E-mail: zhangying3409@bit.edu.cn

## Abstract

Corrosion of aluminium (Al) is a potential problem for spacecraft as this metal is used for various mechanical parts due to its strength, durability, etc. However, it can be corroded by certain factors including microbes. Studying microbes which can be implicated in microbiologically influenced corrosion (MIC) due to their extremophilic nature is of vital importance. In this current study, Al and acid-tolerant microbes were isolated from the samples of China space station assembly cleanroom surfaces; acidic environments can accelerate the corrosion process on metal surfaces. Nine bacterial and 10 fungal strains were identified with 16S ribosomal RNA gene/internal transcribed spacer region sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The dominant bacteria were of *Bacillus*, fungi of *Penicillium* and *Aspergillus* genera. Knowing the microbes which may be conveyed from the cleanrooms to the space stations with a potential capacity of Al degradation is important for long-term maintenance of station components. This study might aid in designing further researches of the aforementioned microorganisms and, therefore, contribute to the prevention of MIC.

## Introduction

Conducting intensive space-related studies are essential as sooner or later humans will make the first steps towards colonizing space. A large variety of experiments are carried out in space stations (Ichijo *et al.*, 2016; Nadir, 2017; Checinska Sielaff *et al.*, 2019; Voorhies *et al.*, 2019). For successful exploration, the safety of space stations and astronauts play a key role. Except for radiation, stress and medical effects on astronauts' health, microbes play a pivotal role. Microorganisms may cause direct and indirect harm in spaceships. The first corresponds to the diseases-causing infections, and the second implies corrosion of space equipment.

Among the various metals aluminium (Al) is selected for the construction of spacecraft equipment due to its lightweight, durability and strength out of other benefits (Calle *et al.*, 2018). However, it can still be vulnerable to microbial impact. In particular, certain bacteria and fungi can cause corrosion which is called microbiologically influenced (or induced) corrosion (MIC) that refers to electrochemical interaction of metal surface and microorganisms which causes metal degradation (Gu *et al.*, 2015). As the microbiome isolated and identified in spaceships is initially terrestrial, the pre-launch microbial diversity of the space stations during assembly merits attention. Among two types of metal corrosion caused by microbes, type II represents a more speedy process and is defined as metabolite MIC (biocorrosion associated with microbial metabolic products, such as organic/inorganic acids, exopolymers, etc.) which includes acid-producing bacteria and fungi (Gu and Galicia, 2012). Many microorganisms are capable to produce acids. Metal corrosion rate increases in a low pH environment (Gu and Galicia, 2012). Metal surfaces become more susceptible when the environment between the metal and the biofilm is acidic (Videla and Herrera, 2005; Castaneda and Benetton, 2008; Kryachko and Hemmingsen, 2017). Moreover, Al is known to become soluble along with the acidity increment (Martin, 1986; Macdonald and Martin, 1988) that makes it toxic to microorganisms (Pina and Cervantes, 1996; Kunito *et al.*, 2016). Thus, Al-resistant microbes that also tolerate high acidity merit attention with respect to the acceleration of Al corrosion.

Despite the existing preventive operations, the environment in space station assembly cleanrooms cannot be sterile (La Duc *et al.*, 2007; Ghosh *et al.*, 2010; Koskinen *et al.*, 2017). Thus, studying potentially material-damaging extremophilic microbes isolated from space station assembly cleanrooms is of significant importance as it can aid to prevent possible risks of structural failure in space stations and effectively optimize the prevention strategies. The first step of the microbial survey is to identify them to evaluate the risk we are dealing



CAMBRIDGE UNIVERSITY PRESS

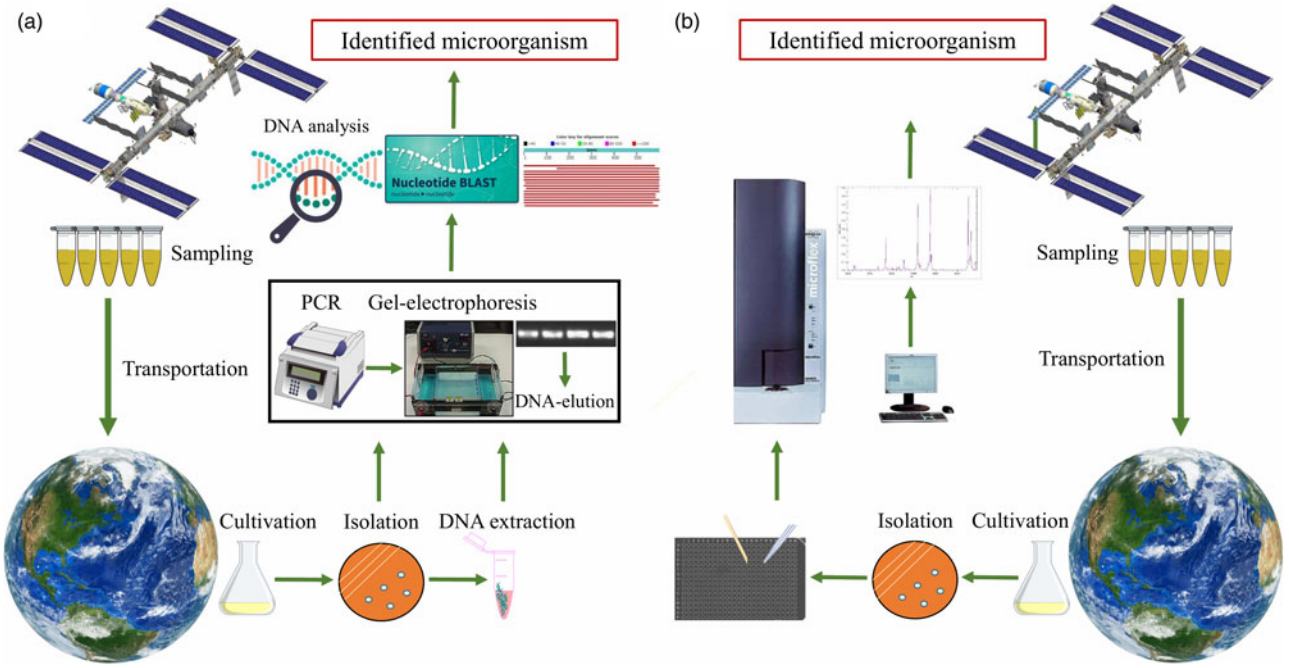


**Fig. 1.** Comparison of two identification methods. (a) The procedures needed to identify microorganisms isolated from the space station using 16S rRNA/ITS sequencing methods. (b) The identification of microorganisms isolated from the space station using the MALDI-TOF MS method.

with. The conventional identification methods, e.g. 16S ribosomal RNA (16S rRNA) gene and internal transcribed spacer (ITS) sequencing, are successfully used. However, the disadvantage still exists as these methods are quite time-consuming and laborious (Sauer *et al.*, 2008). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid and reliable technology used for the identification of microorganisms which is based on the mass detection of molecules, particularly proteins (Sören Schubert and Kostrzewa, 2017). MALDI-TOF MS is a feasible, robust, method while the subsequent steps of conventional method include DNA extraction, agarose gel electrophoresis, DNA elusion, processing the resulted sequences and blasting on the National Center of Biotechnology Information (NCBI). The schematic illustration of the comparison of two identification methods is given in Fig. 1. To sum up, using MALDI-TOF MS significantly shortens the time of identification (Rahi *et al.*, 2016; Sören Schubert and Kostrzewa, 2017). This method is well implemented in clinical laboratories as it effectively replaces conventional biochemical and phenotypic analytical methods (Sören Schubert and Kostrzewa, 2017; Welker *et al.*, 2019). The present study aimed to isolate acid- and Al-tolerant bacteria and fungi from China space station assembly cleanroom (CSSAC) surfaces using Al-containing and acidic media; and to identify microbes with two methods: traditional (16S rRNA/ITS sequencing) and relatively new (MALDI-TOF MS); and to evaluate the feasibility of each method.

## Materials and methods

### *pH measurement*

The pH was checked by pH-meter (Horiba, LAQUAtwin-pH-22) which requires a few drops of a liquid sample to be placed on the flat sensor.

### *Sampling sites and sample processing*

Samples were taken from 39 different surfaces in the Assembly, Integration, and Test (AIT) centre (Tianjin, China). These sampling locations include a logistic channel (LC), clean storage (CS) area, air shower room (AS), dressing room (DR) and encapsulation hall (EH). The sampling was conducted with sterile cotton swabs in accordance with the ECSS-Q-ST-70-55C standard (ECSS 2008). The heads of the swabs were pre-moistened with the sterile phosphate-buffered saline (PBS). Each swab was applied for every 25 $cm^2$ over the entire area horizontally, vertically and diagonally (Kwan *et al.*, 2011); then the swabs, with sticks still attached, were placed in tubes containing 10 ml of sterile PBS. The swabs only moistened with PBS were used as negative controls. For detaching the microbes from the swab heads, sample tubes were ultra-sonicated at 40 kHz for 2 min and vortexed thoroughly before aseptically removing the cotton heads from the solution. In total, 1.5 ml sample suspensions were placed at 4°C for a week while the microbes were isolated and analysed. The rest of the sample solutions were stored at −80°C freezer in a 50% glycerol solution. The samples were named according to the location of the AIT centre which is located in Tianjin (TJ), China. The consistency of sample codes with the identified strains and sampling sites is given in Tables 1 and 2.

### *Isolation of acid- and aluminium-tolerant microbes*

The liquid media was selected for inoculation of acid and Al-tolerant microorganisms as follows: Modified Luria-Bertani (LB) broth was prepared with mixing 10 g of tryptone, 5 g of yeast extract and 10 g of NaCl, and filled with distilled water up to 1 l. After autoclaving it on 121°C for 20 min, $Al_2(SO_4)_3$ water solution and 30 ml citric acid monohydrate (2 M) water solution were added using 0.22 µm filter papers and syringes in order to make the final concentration of Al 100 ppm and pH 3.5 in the liquid media.

https://doi.org/10.1017/S1473550420000427 Published online by Cambridge University Press

**Table 1.** Identified bacteria and their origins

| Identified bacteria | Relevant bacterial strains on NCBI-sequence identity >99% (*E*-value-0.0) | Origin |
|---|---|---|
| TJ-11 (MN932154) | *Bacillus aerius*-NR_118439.1 | Air shower floor |
| TJ-9 (MN932155) | *Bacillus altitudinis*-NR_042337.1 | Hands surface |
| TJ-7-1 (MN932156) | *Bacillus licheniformis*-NR_118996.1 | Storage conveyor belt |
| TJ-35 (MN932157) | *Bacillus pumilus*-NR_043242.1 | Storage conveyor belt |
| TJ-19 (MN932158) | *Bacillus subtilis*-NR_113265.1 | Cabin surface inside |
| TJ-29 (MN932159) | *Bacillus velezensis*-NR_116240.1 | Cabin surface inside, dressing room floor, equipment surface inside |
| TJ-32 (MN932160) | *Brevundimonas diminuta*-NR_113602.1 | Storage conveyor belt |
| TJ-7-2 (MN932161) | *Pseudomonas reidholzensis*-NR_157777.1 | Cabin surface inside |
| TJ-16 (MT027600) | *Bacillus amyloliquefaciens*-NR_117946.1 | Cabin surface inside |

**Table 2.** Identified fungi and their origins

| Identified fungi | Relevant fungal strains on NCBI-sequence identity >99% (*E*-value-0.0) | Origin |
|---|---|---|
| TJ-19-2 (MN945389)* | *Sordariomycetes* sp.-JQ760920.1 | Cabin surface inside |
| TJ-37 (MN945390) | *Aspergillus flavus*-FJ878654.1 | Storage shelves |
| TJ-39-2 (MN945391) | *Aspergillus niger*-FJ878651.1 | Dressing room floor |
| TJ-3 (MN945392)* | *Cladosporium tenuissimum*-MF473304.1 | Logistics channel floor |
| TJ-2-1 (MN945393) | *Fusarium verticillioides*-KF494135.1 | Storage shelves |
| TJ-39-1 (MN945394) | *Penicillium chrysogenum*-MK967560.1 | Cabin surface inside, storage conveyor belt, storage shelves, dressing room closet, dressing room floor, equipment surface inside, desk surface |
| TJ-22 (MN945395) | *Penicillium echinulatum*-MN511335.1 | Equipment surface inside |
| TJ-19-1 (MN945396) | *Penicillium oxalicum*-MH399738.1 | Cabin surface inside |
| TJ-16 (MN945397) | *Rhodotorula mucilaginosa*-MN427959.1 | Cabin surface inside |
| TJ-17 (MN945398)* | *Trichoderma atroviride*-LN713968.1 | Cabin surface inside |

*Note*: (*) indicates the strain that was not included in the MALDI-TOF MS analysis because they were not represented in the MALDI-TOF MS reference database.

In total, 500 µl of the sample was introduced into 20 ml of media in 50 ml tubes. This was followed by incubating all the samples in a shaker (128 rpm) at 28°C for 1 week. Then 100 µl of each sample was inoculated and spread on LB (pH = 6.4) and Potato Dextrose Agar (PDA) (pH = 4.7) solid media using two methods: normal spreading method and facultative anaerobes method (Henning *et al*., 2015) – after another layer of solid media was poured above the microbial lawn to form a facultative anaerobic environment for the microorganisms (see in Fig. 2). The growth of colonies was observed. The media always contained Al with a concentration of 100 ppm in both LB and PDA. After the final isolation of strains, polymerase chain reaction (PCR) and 16S rRNA and ITS sequencing analysis were carried out, respectively, for bacteria and fungi. At the same time, the isolated strains were frozen at −80°C in glycerol solution.

## Identification

### 16S rRNA sequencing

After obtaining isolated colonies, colony PCR (Walch *et al*., 2016) was carried out using P0 forward: 5′-GAGAGTTTGATCCTGGC TCAG-3′ and P6 reverse: 5′-CTACGGCTACCTTGTTACGA-3′ primers specific to the 16S rRNA gene (Popović *et al*., 2013). The PCR reaction was carried out in a total volume of 25 µl containing 2.5 µl of 10X Taq buffer, 1.5 µl of 25 mM $MgCl_2$, 0.5 µl of 2.5 mM dNTPs (Tiangen, Beijing, China), 0.5 µl of 100 nmol P0, 0.5 µl of 100 nmol P6 (Sangon Biotech, Beijing, China), bacterial colony sample, 0.3 µl of 5 U $\mu l^{-1}$ DNA-polymerase (Thermo Scientific, Beijing, China) and 19.2 µl double-distilled (dd) water. The reaction regimen of PCR was as follows: denaturation for 90 s at 95°C was followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 60 s. The reaction was completed with 72°C for 10 min. After confirmation of DNA amplification with agarose gel electrophoresis with fragments of approximately 1500 bp, the samples were sent to Sangon Biotech (Beijing, China) where the amplicons were purified (SanPrep Column PCR Product Purification Kit, Sangon Biotech, Beijing, China) and the sequencing was performed via Sanger sequencing method.

### ITS sequencing

Once isolation of fungi was attained and single colonies were formed, DNA extraction was conducted using the thermolysis method according to Zhang *et al*. (2010).

https://doi.org/10.1017/S1473550420000427 Published online by Cambridge University Press

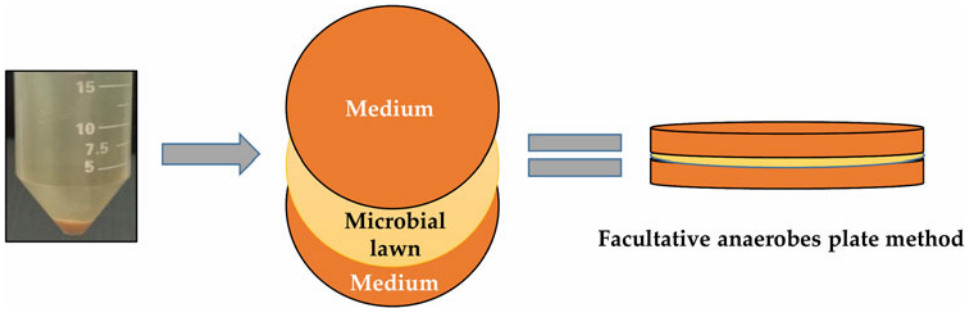


**Fig. 2.** The inoculation method for facultative anaerobes: after spreading the microbial consortia, another layer of media is poured on the microbial lawn.

The primers used for ITS region amplification were: ITS1 forward: 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4 reverse: 5′-TCCTCCGCTTATTGATATGC-3′ (Kumar and Shukla, 2005; Zarrin *et al.*, 2016). The PCR reaction was carried out in a total volume of 25 µl containing 2.5 µl of 10X Taq buffer, 1.5 µl of 25 mM $MgCl_2$, 0.5 µl of 2.5 mM dNTPs (Tiangen), 0.5 µl of 100 nmol ITS1, 0.5 µl of 100 nmol ITS4 (Sangon Biotech, Beijing, China), 1 µl of fungal extracted DNA, 0.3 µl of 5 U $\mu l^{-1}$ DNA-polymerase (Thermo Scientific) and 18.2 µl dd water. The PCR conditions were the following: the initial denaturation by heating to 95°C for 90 s, followed by 25 cycles of 95°C/30 s, 52°C/30 s, 72°C/60 s. The reaction was completed with 72°C for 10 min. After verification of the successful DNA amplification by agarose gel electrophoresis with fragments of approximately 550 bp, the DNA samples were sent to Sangon Biotech (Beijing, China) where the amplification products were purified (SanPrep Column PCR Product Purification Kit, Sangon Biotech, Beijing, China) and the sequencing was performed via Sanger sequencing method.

### *Constructing phylogenetic trees*

The trimmed sequences were aligned and the phylogenetic trees were constructed using the neighbour-joining method available in MEGA version 7.0.26 and the Kimura 2-parameter model (Kumar *et al.*, 2016). The tree topology was evaluated by bootstrapping based on 1000 replicates.

### *MALDI-TOF MS*

Bruker MALDI Biotyper was used for the current experiment. The main procedure of this analysis includes the cultivation of microorganisms (bacteria, fungi) sampling microbial cells from a single colony with the sterile toothpick and smearing it on a metal target plate which is followed by applying formic acid, after drying it α-Cyano-4-hydroxycinnamic acid is overlaid. All this procedure is needed to improve the protein extraction. Once the plate is dry, the laser desorption/ionization time of flight analysis takes place (Jang and Kim, 2018). First, the ionization of sample molecules via laser occurs. Then the detector detects masses of molecules. The calibration was performed using the standard strain of *Escherichia coli* DH5alpha BRL. The standard contained a carefully manufactured extract of this microorganism that shows a characteristic peptide and protein profile in MALDI-TOF MS spectra. The detected spectrum peaks correspond to the mass of the most abundant proteins in a sample which turns out to be specific for the analysed microbial species (Singhal *et al.*, 2015; Seuylemezian *et al.*, 2018). Thus, identification occurs automatically using software Bruker Biotyper MS version 3 which is responsible to compare the spectral profile of tested microorganism with a reference database DB 7311. MN945389, MN945392, MN945398 were not analysed by this method as they were not included in the MALDI-TOF MS library.

## Results

Out of 39 surface samples, nine bacterial and 10 fungal strains were isolated and identified (Tables 1 and 2). All the microbes were found to be aerobic or facultatively anaerobic. After blasting the sequences on NCBI, the accession numbers were assigned (MN932154–MN932161, MT027600; MN945389–MN945398) and the phylogenetic trees of 16S rRNA and ITS genes were constructed for bacteria and fungi, respectively ( Fig. 3).

Concurrently, the isolated microorganisms were identified using the MALDI-TOF MS method. Some of the strains showed identical results to the conventional method while most did not (Tables 3 and 4). Only two of the nine bacterial isolates (Table 3) were identified as the same genus and species between the two methods (TJ-32 and TJ-35), and of the MALDI-TOF MS identifications that had positive reliability levels (five of the nine isolates), one was identified as a different genus (TJ-9). Concerning the fungal isolates that could be identified by MALDI-TOF MS, three out of the seven isolates (Table 4) were identified as the same genus and species between the two methods (TJ-37, TJ-39-2 and TJ-16), and of the MALDI-TOF MS identifications that had positive reliability levels, all identified the same genus. However, only three of the seven MALDI-TOF MS fungal identifications had positive reliability levels. Thus, when MALDI-TOF MS identifications had positive reliability levels, it often identified the same genera of bacteria and fungi, but different species.

## Discussion

Certain inconsistencies were detected in the results of two identification methods. The mismatch may be explained by several reasons. The insufficient spectra in the database are among them. This might result in an incorrect identification or not reliable identification. Mismatches may also take place when part of the analysed microbial species is presented in the MALDI-TOF library while others are absent (Rychert, 2019). One of the limitations of MALDI-TOF is the difficulties to differentiate between certain highly related organisms (Dingle and Butler-Wu, 2013; Rychert, 2019). Another reasonable cause of such diverse results may be the different and unique protein expression as prior to identification they have encountered to environmental stress such as low pH (3.5) of growth media which can cause mutations and changes in their metabolism (Farrell and Finkel, 2003;

https://doi.org/10.1017/S1473550420000427 Published online by Cambridge University Press

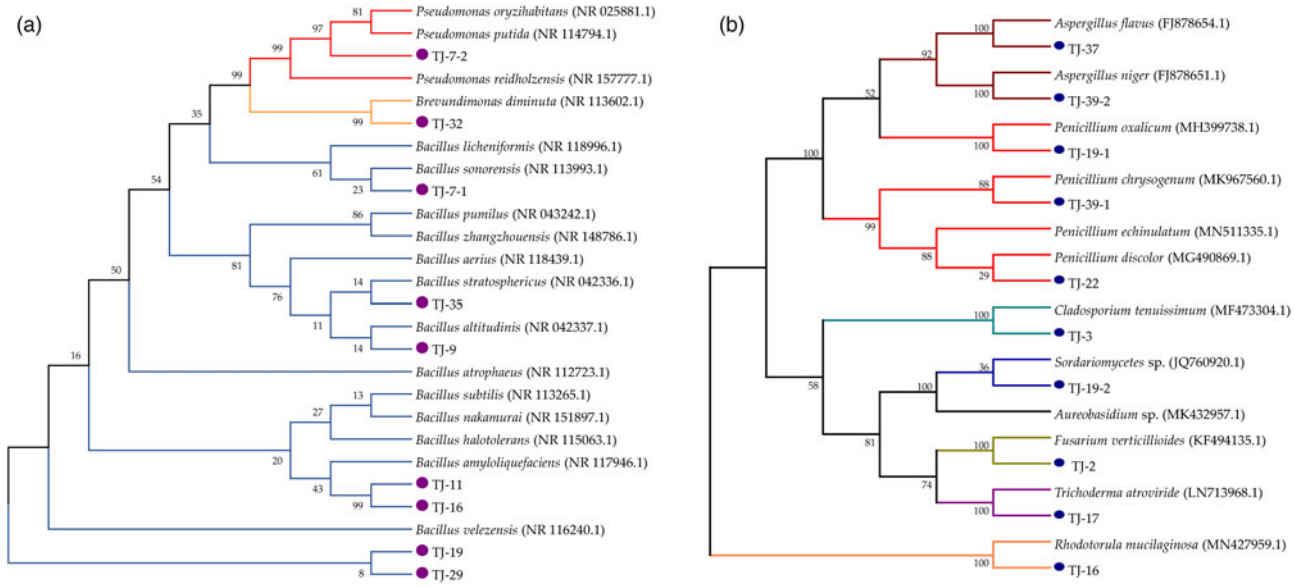


**Fig. 3.** Phylogenetic trees of identified microorganisms. (a) Phylogenetic tree of 16S rRNA genes showing the position of isolated bacterial strains; the predominant identified bacterial genus is *Bacillus*. The rest of the bacteria are of *Pseudomonas* and *Brevundimonas* genera. (b) Phylogenetic tree of ITS genes showing the position of isolated fungal strains. The predominant fungi were of *Penicillium* and *Aspergillus* genera which were followed by *Cladosporium*, *Sordariomycetes*, *Trichoderma* and *Fusarium.*

**Table 3.** The comparison of bacteria identified by two methods: conventional 16S rRNA gene sequencing and MALDI-TOF MS

| Bacterial ID according to 16S rRNA | Sample name/ NCBI accession ID | Bacterial ID according to MALDI-TOF | Reliability level | Biotyper score value |
|---|---|---|---|---|
| *Bacillus aerius*-NR_118439.1 | TJ-11 (MN932154) | *Bacillus pumilus* | (+++) | 2.383 |
| *Brevundimonas diminuta*-NR_113602.1 | TJ-32 (MN932160) | *Brevundimonas diminuta* | (+++) | 2.399 |
| *Bacillus licheniformis*-NR_118996.1 | TJ-7-1 (MN932156) | *Bacillus pumilus* | (++) | 2.231 |
| *Bacillus pumilus*-NR_043242.1 | TJ-35 (MN932157) | *Bacillus pumilus* | (+) | 1.908 |
| *Bacillus altitudinis*-NR_042337.1 | TJ-9 (MN932155) | *Brevundimonas diminuta* | (+) | 1.97 |
| *Pseudomonas reidholzensis*-NR_157777.1 | TJ-7-2 (MN932161) | *Pseudomonas caricapapayae* | (–) | 1.627 |
| *Bacillus subtilis*-NR_113265.1 | TJ-19 (MN932158) | *Staphylococcus saprophyticus* | (–) | 1.66 |
| *Bacillus velezensis*-NR_116240.1 | TJ-29 (MN932159) | *Pseudomonas congelans* | (–) | 1.64 |
| *Bacillus amyloliquefaciens*-NR_117946.1 | TJ-16 (MT027600) | *Moraxella catarrhalis* | (–) | 1.536 |

The sign (+++) indicates highly probable species, (++) indicates secure genus identification and probable species, (+) indicates probable genus identification, and (–) indicates not reliable identification.

Maurer *et al.*, 2005), including altered protein production via upregulation or downregulation of certain genes (Tucker *et al.*, 2002; Broadbent *et al.*, 2010). MALDI-TOF MS bio-typing is already common in clinical microbiology but its reference database still seems to be insufficiently accessible.

In the current study, *Bacillus* was the predominant genus isolated from CSSAC. *Bacillus* spp. use several mechanisms against the adaptation of various stress conditions, such as acidic milieu. The cells survive harsh environments through maintenance of their cell wall integrity (Goswami *et al.*, 2018) as well as developing their stress response which enables their survival in the low pH environment (Browne and Dowds, 2002; Thomassin *et al.*, 2006). *Bacillus* is one of the most dominant genera isolated from MIR (Russian manned spacecraft) (Novikova, 2004) and ISS (Novikova *et al.*, 2006; Lang *et al.*, 2017; Checinska Sielaff *et al.*, 2019). According to the existing data, it is resilient to the harsh cleaning methods (Zhang *et al.*, 2019) as well as space conditions including gravity-associated stress, radiation (Kacena *et al.*, 1999; Gabani *et al.*, 2012; Fajardo-Cavazos *et al.*, 2014), as well as the acidity (Goswami *et al.*, 2018). Cortesao *et al.* have demonstrated that *Bacillus subtilis* spores can survive the simulated Mars surface environment (Cortesão *et al.*, 2019). *Bacillus* is also reported to cause metal corrosion (Rajasekar and Ting, 2010; Xu *et al.*, 2013; Karn *et al.*, 2017; Liu *et al.*, 2019). Another isolated operational taxonomic unit (OTU) *Pseudomonas* is also found to cause metal deterioration (Abdolahi *et al.*, 2014). It represents the slime-forming microbe meaning that after producing the number of enzymes and other extracellular substances it can form biofilms (Kim *et al.*, 2013; Telegdi *et al.*, 2017) on metal surfaces which is also common for *Bacillus* spp. (Rajasekar and Ting, 2010). Biofilm is a microbial consortium with self-produced extracellular polymeric substances on surfaces, e.g., metal surfaces.

https://doi.org/10.1017/S1473550420000427 Published online by Cambridge University Press

**Table 4.** The comparison of fungi identified by two methods: conventional ITS region sequencing and MALDI-TOF MS

| Fungal ID according to ITS | Sample name/NCBI accession ID | Fungal ID according to MALDI-TOF | Reliability level | Biotyper score value |
|---|---|---|---|---|
| *Penicillium echinulatum*-MN511335.1 | TJ-22 (MN945395) | *Penicillium camemberti* | (++) | 2.119 |
| *Aspergillus flavus*-FJ878654.1 | TJ-37 (MN945390) | *Aspergillus flavus* | (++) | 2.086 |
| *Penicillium chrysogenum*-MK967560.1 | TJ-39-1 (MN945394) | *Penicillium* sp. | (++) | 2.174 |
| *Aspergillus niger*-FJ878651.1 | TJ-39-2 (MN945391) | *Aspergillus niger* | (–) | 1.38 |
| *Rhodotorula mucilaginosa*-MN427959.1 | TJ-16 (MN945397) | *Rhodotorula mucilaginosa* | (–) | 1.418 |
| *Penicillium oxalicum*-MH399738.1 | TJ-19-1 (MN945396) | *Aromatoleum terpenicum* | (–) | 1.419 |
| *Fusarium verticillioides*-KF494135.1 | TJ-2-1 (MN945393) | *Lactobacillus sastumensis* | (–) | 1.392 |

The sign (+++) indicates highly probable species, (++) indicates secure genus identification and probable species, (+) indicates probable genus identification, and (–) indicates not reliable identification.

**Table 5.** Dominant bacteria and fungi isolated from MIR, ISS and from the surfaces of CSSAC

| Sampling place | MIR | ISS | CSSAC |
|---|---|---|---|
| Dominant bacteria genera | *Staphylococcus*, *Corynebacterium*, ***Bacillus***, *Micrococcus*, ***Pseudomonas*** (Novikova, 2004) | *Staphylococcus*, ***Bacillus***, *Corynebacterium*, *Propionibacterium*, unclassified *Rikenellaceae/S24-7*, *Streptococcus*, ***Pseudomonas*** (Venkateswaran *et al.*, 2014; Checinska *et al.*, 2015; Ichijo *et al.*, 2016; Lang *et al.*, 2017) *Pantoea* (Checinska Sielaff *et al.*, 2019) | ***Bacillus***, ***Pseudomonas*** |
| Dominant fungi genera | ***Penicillium***, ***Aspergillus***, ***Cladosporium***, ***Fusarium***, *Saccharomyces* (Novikova, 2004) | ***Aspergillus***, ***Penicillium***, *Saccharomyces*, ***Cladosporium***, ***Sordariomycetes***, ***Rhodotorula*** (Novikova *et al.*, 2006; Satoh *et al.*, 2016) | ***Penicillium***, ***Aspergillus***, ***Cladosporium***, ***Fusarium***, ***Sordariomycetes***, ***Rhodotorula***, *Trichoderma* |
| Dominant bacteria species | *Staphylococcus aureus*, ***Bacillus subtilis***, *Bacillus sphaericus* (Novikova, 2004) | *Staphylococcus epidermidis*, *Staphylococcus auricularis*, *Bacillus sphaericus*, ***Bacillus subtilis***, ***Bacillus licheniformis***, ***Bacillus pumilus*** (Novikova *et al.*, 2006) | ***Bacillus licheniformis***, ***Bacillus subtilis***, ***Bacillus pumilus***, *Bacillus aerius*, *Bacillus amyloliquefaciens*, *Bacillus altitudinis*, *Pseudomonas reidholzensis*, *Brevundimonas diminuta* |
| Dominant fungi species | *Penicillium expansum*, ***Penicillium chrysogenum***, **Aspergillus *niger***, *Cladosporium cladosporioides* (Novikova, 2004) ***Rhodotorula mucilaginosa*** (Checinska Sielaff *et al.*, 2019) | *Penicillium aurantiogriseum*, *Aspergillus phoenicis*, ***Aspergillus flavus***, ***Aspergillus niger*** (Novikova *et al.*, 2006; Checinska *et al.*, 2015) | ***Penicillium chrysogenum***, *Penicilliuum echinulatum*, *Penicillium oxalicum*, ***Aspergillus niger***, ***Aspergillus flavus***, ***Rhodotorula mucilaginosa***, *Fusarium verticillioides*, *Cladosporium tenuissimum*, *Trichoderma atroviride*, *Sordariomycetes* sp. |

The boldface indicates the common genera and species for CSSAC isolates and MIR/ISS.

Its development can alter electron transfer and accelerate metal corrosion (Beech and Sunner, 2004; Moura *et al.*, 2013). The biofilms have already appeared and had negative effects on materials used to make space station components (Klintworth *et al.*, 1999; Zea *et al.*, 2018). The impact can be more adverse when the pH beneath the biofilm is low due to organic acid production (Kryachko and Hemmingsen, 2017). In the current study, except for *Bacillus*, and *Pseudomonas*, another genus *Brevundimonas* was also identified. *Brevundimonas* originally classified under the *Pseudomonas* genus is already isolated from the spacecraft assembly cleanroom (Stieglmeier *et al.*, 2012) as well as ISS (Checinska *et al.*, 2015) and owes the biofilm formation capacity (Mori *et al.*, 2013).

Many fungi are known to be resistant to hostile environmental conditions as well as having degradation ability. The identified fungal genera in the current study coincide with the ones isolated from space stations, such as *Penicillium*, *Aspergillus* (Knox *et al.*, 2016; Checinska Sielaff *et al.*, 2019), *Cladosporium* (Novikova, 2004; Novikova *et al.*, 2006), *Rhodotorula* (Reidt *et al.*, 2017; Checinska Sielaff *et al.*, 2019). Furthermore, the studies have revealed their metal deterioration capacity, e.g., *Penicillium* (Yang *et al.*, 1996, 1998; Smirnov *et al.*, 2008), *Aspergillus* (Dai *et al.*, 2017; Jirón-Lazos *et al.*, 2018), *Cladosporium* (Yang *et al.*, 1998), *Fusarium* (Yang *et al.*, 1998; Smirnov *et al.*, 2008). *Penicillium* and *Aspergillus* are also studied to be acid-tolerant genera (Kawai *et al.*, 2000; Glukhova *et al.*, 2018). Interestingly, *Trichoderma* has been evidenced to survive for about 1 year in outer space (Neuberger *et al.*, 2015). According to the abovementioned, up to now, *Bacillus*, *Penicillium*, *Aspergillus* and *Cladosporium* represent predominant and perhaps problematic microbial genera in crewed spacecraft.

The microbes analysed in the present study are acid- and Al-tolerant that are found to be space-associated and involved in metal deterioration processes. The studies referenced above have already revealed acid production and metal degradation ability of the same genera. Thus, the identified microorganisms in this work might have the potential of acid production and Al corrosion capacity. However, the research needs to be continued in this direction to further evaluate the direct impact of isolated microbes on Al. The coexisting microbial consortia, especially in biofilms, usually represent more of a threat than each microbe

https://doi.org/10.1017/S1473550420000427 Published online by Cambridge University Press

separately (Yang *et al*., 1998). Hence, the experiments studying the risks which may arise by the joint impacts of the identified microorganisms are needed to be carried out as well.

Accessing the information on acid- and Al-tolerant microorganisms that are abundant on the surfaces of CSSAC is noteworthy for making the space exploration safer. More information can improve the implementation of preventive and protective methods. As a result, the transportation of microbes into the China Space Station (CSS) can be minimized. This will significantly decrease the chance of Al corrosion. The coincidence of strains isolated in ISS, MIR and CSSAC is given in Table 5. Bacteria and fungi isolated from CSSAC are resistant to the harsh environment and they survive the preventive methods. Besides the environmental stress, in the space station, there is no preferred medium for microbes. These conditions might stimulate the mutation of genes to survive. The protective biofilm formation capacity may also play a certain role in the prevention of Al corrosion in space stations.

Despite all the preventive procedures, e.g. continuous monitoring, frequent cleaning, using biocides, coatings, the environment of spaceships prior to launch is not aseptic (La Duc *et al*., 2007; Schwendner *et al*., 2013). Interestingly, there are some studies regarding the anti-bacterial properties of bacteriophages – bacterial viruses that act very specifically and are already in use in medicine (Parfitt, 2005; Torres-Barceló, 2018). Moreover, phages have already been studied as anti-corrosion agents (Pedramfar *et al*., 2017). Thus, the researches with the direction of phage-therapy development against metal corrosion seem to be promising.

## Conclusions

To conclude, this is the first study where Al- and acid-tolerant bacteria and fungi were isolated from CSSAC surfaces and identified with conventional and MALDI-TOF MS methods. *Bacillus* and *Penicillium* represent the predominantly detected genera. The majority of the analysed microbes in this work match with the previously reported acid-producing and Al-corrosive OTUs from ISS. This stipulates their accidental transportation in spacecraft despite the constant monitoring measures. The provided data might be useful for further evaluation and prevention of Al corrosion. Making the MALDI-TOF database more comprehensive will further facilitate the identification of extremophilic microbes. Consequently, this research may lay the groundwork for studies toward the direction of evaluating Al corrosion by the isolated microbes.

**Acknowledgements.** This study was supported by the National Natural Science Foundation of China, Project No.31600404, and the Pre-research Foundation of Manned Space-Flight, Project No.020301.

**Conflict of interest.** None.

## References

**Abdolahi A, Hamzah E, Ibrahim Z and Hashim S** (2014) Microbially influenced corrosion of steels by *Pseudomonas aeruginosa*. *Corrosion Reviews* **32**, 129–141.

**Beech IB and Sunner J** (2004) Biocorrosion: towards understanding interactions between biofilms and metals. *Current Opinion in Biotechnology* **15**, 181–186.

**Broadbent JR, Larsen RL, Deibel V and Steele JL** (2010) Physiological and transcriptional response of *Lactobacillus casei* ATCC 334 to acid stress. *Journal of Bacteriology* **192**, 2445–2458.

**Browne N and Dowds BCA** (2002) Acid stress in the food pathogen *Bacillus cereus*. *Journal of Applied Microbiology* **92**, 404–414.

**Calle LM, Li W, Buhrow JW, Johansen MR and Calle CI** (2018) Corrosion on Mars: an investigation of corrosion under relevant simulated Martian environments. 48th International Conference on Environmental Systems 8–12 July 2018, Albuquerque, New Mex ICES-2018–125.

**Castaneda H and Benetton XD** (2008) SRB-biofilm influence in active corrosion sites formed at the steel-electrolyte interface when exposed to artificial seawater conditions. *Corrosion Science* **50**, 1169–1183.

**Checinska A, Probst AJ, Vaishampayan P, White JR, Kumar D, Stepanov VG, Fox GE, Nilsson HR, Pierson DL, Perry J and Venkateswaran K** (2015) Microbiomes of the dust particles collected from the International Space Station and Spacecraft Assembly Facilities. *Microbiome* **3**, 50.

**Checinska Sielaff A, Urbaniak C, Mohan GBM, Stepanov VG, Tran Q, Wood JM, Minich J, McDonald D, Mayer T, Knight R, Karouia F, Fox GE and Venkateswaran K** (2019) Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. *Microbiome* **7**, 50.

**Cortesão M, Fuchs FM, Commichau FM, Eichenberger P, Schuerger AC, Nicholson WL, Setlow P and Moeller R** (2019) *Bacillus subtilis* spore resistance to simulated Mars surface conditions. *Frontiers in Microbiology* **10**, 333.

**Dai X, Wang H, Ju LK, Cheng G, Cong H and Newby BMZ** (2017) Corrosion of aluminum alloy 2024 caused by *Aspergillus niger*. *International Biodeterioration and Biodegradation* **115**, 1–10.

**Dingle TC and Butler-Wu SM** (2013) MALDI-TOF mass spectrometry for microorganism identification. *Clinics in Laboratory Medicine* **33**, 589–609.

**ECSS ESA for the members of** (2008) Microbial examination of flight hardware and cleanrooms. ESA Requir Stand Div ESTEC, P.O:ECSS-Q-ST-70-55C.

**Fajardo-Cavazos P, Narvel R and Nicholson WL** (2014) Differing responses in growth and spontaneous mutation to antibiotic resistance in *Bacillus subtilis* and *Staphylococcus epidermidis* cells exposed to simulated microgravity. *Gravitational and Space Research* **2**, 2.

**Farrell MJ and Finkel SE** (2003) The growth advantage in stationary-phase phenotype conferred by rpoS mutations is dependent on the pH and nutrient environment. *Journal of Bacteriology* **185**, 7044–7052.

**Gabani P, Copeland E, Chandel AK and Singh OV** (2012) Ultraviolet-radiation-resistant isolates revealed cellulose-degrading species of *Cellulosimicrobium cellulans* (UVP 1) and *Bacillus pumilus* (UVP 4). *Biotechnology and Applied Biochemistry* **59**, 395–404.

**Ghosh S, Osman S, Vaishampayan P and Venkateswaran K** (2010) From the clean room where Phoenix spacecraft components were assembled. *Astrobiology* **10**, 325–335.

**Glukhova LB, Frank YA, Danilova EV, Avakyan MR, Banks D, Tuovinen OH and Karnachuk OV** (2018) Isolation, characterization, and metal response of novel, acid-tolerant *Penicillium* spp. from extremely metal-rich waters at a mining site in Transbaikal (Siberia, Russia). *Microbial Ecology* **76**, 911–924.

**Goswami G, Panda D, Samanta R, Boro RC, Modi MK, Bujarbaruah KM and Barooah M** (2018) *Bacillus megaterium* adapts to acid stress condition through a network of genes: insight from a genome-wide transcriptome analysis. *Scientific Reports* **8**, 16105.

**Gu T and Galicia B** (2012) Can acid producing bacteria be responsible for very fast MIC pitting? Corros. 2012 NACE Corrosion/2012, No. C2012-0001214, S March 11.

**Gu T, Xu D, Zhang P, Li Y and Lindenberger AL** (2015) Microbiologically influenced corrosion and its impact on metals and other materials. *Microbiology for Minerals, Metals, Materials and the Environment* **25**, 383–408.

**Henning C, Vijayakumar P, Adhikari R, Jagannathan B, Gautam D and Muriana PM** (2015) Isolation and taxonomic identity of bacteriocin-producing lactic acid bacteria from retail foods and animal sources. *Microorganisms* **3**, 80–93.

**Ichijo T, Yamaguchi N, Tanigaki F, Shirakawa M and Nasu M** (2016) Four-year bacterial monitoring in the International Space Station – Japanese experiment module 'Kibo' with culture-independent approach. *NPJ Microgravity* **2**, 16007.

**Jang KS and Kim YH** (2018) Rapid and robust MALDI-TOF MS techniques for microbial identification: a brief overview of their diverse applications. *Journal of Microbiol* **56**, 209–216.

https://doi.org/10.1017/S1473550420000427 Published online by Cambridge University Press

**Jirón-Lazos U, Corvo F, De la Rosa SC, García-Ochoa EM, Bastidas DM and Bastidas JM** (2018) Localized corrosion of aluminum alloy 6061 in the presence of *Aspergillus niger*. *International Biodeterioration & Biodegradation* **133**, 17–25.

**Kacena MA, Merrell GA, Manfredi B, Smith EE, Klaus DM and Todd P** (1999) Bacterial growth in space flight: logistic growth curve parameters for *Escherichia coli* and *Bacillus subtilis*. *Applied Microbiology and Biotechnology* **51**, 229–234.

**Karn SK, Fang G and Duan J** (2017) *Bacillus sp*. acting as dual role for corrosion induction and corrosion inhibition with carbon steel (CS). *Frontiers in Microbiology* **8**, 2038.

**Kawai F, Zhang D and Sugimoto M** (2000) Isolation and characterization of acid- and Al-tolerant microorganisms. *FEMS Microbiology Letters* **189**, 143–147.

**Kim W, Tengra FK, Young Z, Shong J, Marchand N, Chan HK, Pangule RC, Parra M, Dordick JS, Plawsky JL and Collins CH** (2013) Spaceflight promotes biofilm formation by *Pseudomonas aeruginosa*. *PLoS ONE* **8**, e62437.

**Klintworth R, Reher HJ, Viktorov AN and Bohle D** (1999) Biological induced corrosion of materials II: new test methods and experiences from MIR station. *Acta Astronautica* **44**, 569–578.

**Knox BP, Blachowicz A, Palmer JN, Romsdahl J, Huttenlocher A, Wang CCC, Keller NP and Venkateswaran K** (2016) Characterization of *Aspergillus fumigatus* isolates from air and surfaces of the international space station. *mSphere* **1**, e00227–16.

**Koskinen K, Rettberg P, Pukall R, Auerbach A, Wink L, Barczyk S, Perras A, Mahnert A, Margheritis D, Kminek G and Moissl-Eichinger C** (2017) Microbial biodiversity assessment of the European Space Agency's ExoMars 2016 mission. *Microbiome* **5**, 143.

**Kryachko Y and Hemmingsen SM** (2017) The role of localized acidity generation in microbially influenced corrosion. *Current Microbiology* **74**, 870–876.

**Kumar M and Shukla PK** (2005) Use of PCR targeting of internal transcribed spacer regions and single-stranded conformation polymorphism analysis of sequence variation in different regions of rRNA genes in fungi for rapid diagnosis of mycotic keratitis. *Journal of Clinical Microbiology* **43**, 662–668.

**Kumar S, Stecher G and Tamura K** (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

**Kunito T, Isomura I, Sumi H, Park HD, Toda H, Otsuka S, Nagaoka K, Saeki K and Senoo K** (2016) Aluminum and acidity suppress microbial activity and biomass in acidic forest soils. *Soil Biology and Biochemistry* **97**, 23–30.

**Kwan K, Cooper M, La Duc MT, Vaishampayan P, Stam C, Benardini JN, Scalzi G, Moissl-Eichinger C and Venkateswaran K** (2011) Evaluation of procedures for the collection, processing, and analysis of biomolecules from low-biomass surfaces. *Applied and Environmental Microbiology* **77**, 2943–2953.

**La Duc MT, Dekas A, Osman S, Moissl C, Newcombe D and Venkateswaran K** (2007) Isolation and characterization of bacteria capable of tolerating the extreme conditions of clean room environments. *Applied and Environmental Microbiology* **73**, 2600–2611.

**Lang JM, Coil DA, Neches RY, Brown WE, Cavalier D, Severance M, Hampton-Marcell JT, Gilbert JA and Eisen JA** (2017) A microbial survey of the International Space Station (ISS). *PeerJ* **5**, e4029.

**Liu T, Wang Y, Pan S, Zhao Q, Zhan C, Gao S, Guo Z, Guo N, Sand W, Chang X, Dong L and Yin Y** (2019) The addition of copper accelerates the corrosion of steel via impeding biomineralized film formation of *Bacillus subtilis* in seawater. *Corrosion Science* **149**, 153–163.

**Macdonald TL and Martin RB** (1988) Aluminum ion in biological systems. *Trends in Biochemical Sciences* **13**, 15–19.

**Martin RB** (1986) The chemistry of aluminum as related to biology and medicine. *Clinical Chemistry* **32**, 1797–1806.

**Maurer LM, Yohannes E, Bondurant SS, Radmacher M and Slonczewski JL** (2005) Ph regulates genes for flagellar motility, catabolism, and oxidative stress in *Escherichia coli* K-12. *Journal of Bacteriology* **187**, 304–319.

**Mori M, Gomi M, Matsumune N, Niizeki K and Sakagami Y** (2013) Biofilm-forming activity of bacteria isolated from toilet bowl biofilms and the bactericidal activity of disinfectants against the isolates. *Biocontrol Science* **18**, 129–135.

**Moura MC, Pontual EV, Paiva PMG and Coelho LCBB** (2013) An outline to corrosive Bacteria. In Méndez-Vilas A (ed). *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education*. Badajoz, Spain: Formatex Research Centre, pp. 11–22.

**Nadir AJ** (2017) A hitchhiker's guide to an ISS experiment in under 9 months. *NPJ Microgravity* **3**, 1–10.

**Neuberger K, Lux-Endrich A, Panitz C and Horneck G** (2015) Survival of spores of *Trichoderma longibrachiatum* in space: data from the space experiment SPORES on EXPOSE-R. *International Journal of Astrobiology* **14**, 129–113.

**Novikova N** (2004) Review of the knowledge of microbial contamination of the Russian manned spacecraft. *Microbial Ecology* **47**, 127–132.

**Novikova N, De Boever P, Poddubko S, Deshevaya E, Polikarpov N, Rakova N, Coninx I and Mergeay M** (2006) Survey of environmental biocontamination on board the International Space Station. *Research in Microbiology* **157**, 5–12.

**Parfitt T** (2005) Georgia: an unlikely stronghold for bacteriophage therapy. *Lancet (London, England)* **365**, 2166–2167.

**Pedramfar A, Maal KB and Mirdamadian SH** (2017) Phage therapy of corrosion-producing bacterium *Stenotrophomonas maltophilia* using isolated lytic bacteriophages. *Anti-Corrosion Methods and Materials* **64**, 607–612.

**Pina RG and Cervantes C** (1996) Microbial interactions with aluminium. *Biometals* **9**, 311–316.

**Popović T, Ivanović Ž, Živković S, Trkulja N and Ignjatov M** (2013) First report of *Brenneria nigrifluens* as the causal agent of shallow-bark canker on walnut trees (*Juglans regia*) in Serbia. *Plant Disease* **97**, 1504.

**Rahi P, Prakash O and Shouche YS** (2016) Matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS) based microbial identifications: challenges and scopes for microbial ecologists. *Frontiers in Microbiology* **7**, 1359.

**Rajasekar A and Ting YP** (2010) Microbial corrosion of aluminum 2024 aeronautical alloy by hydrocarbon degrading bacteria *Bacillus cereus* ACE4 and *Serratia marcescens* ACE2. *Industrial & Engineering Chemistry Research* **49**, 6054–6061.

**Reidt U, Helwig A, Müller G, Plobner L, Lugmayr V, Kharin S, Smirnov Y, Novikova N, Lenic J, Fetter V and Hummel T** (2017) Detection of microorganisms onboard the international space station using an electronic nose. *Gravitational and Space Research* **5**, 89–111.

**Rychert J** (2019) Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. *Journal of Infection* **2**, 1–5.

**Satoh K, Yamazaki T, Nakayama T, Umeda Y, Alshahni MM, Makimura M and Makimura K** (2016) Characterization of fungi isolated from the equipment used in the International Space Station or Space Shuttle. *Microbiology and Immunology* **60**, 295–302.

**Sauer S, Freiwald A, Maier T, Kube M, Reinhardt R, Kostrzewa M and Geider K** (2008) Classification and identification of bacteria by mass spectrometry and computational analysis. *PLoS ONE* **3**, e2843.

**Schwendner P, Moissl-Eichinger C, Barczyk S, Bohmeier M, Pukall R and Rettberg P** (2013) Insights into the microbial diversity and bioburden in a South American spacecraft assembly clean room. *Astrobiology* **13**, 1140–1154.

**Seuylemezian A, Aronson HS, Tan J, Lin M, Schubert W and Vaishampayan P** (2018) Development of a custom MALDI-TOF MS database for species-level identification of bacterial isolates collected from spacecraft and associated surfaces. *Frontiers in Microbiology* **9**, 780.

**Singhal N, Kumar M, Kanaujia PK and Virdi JS** (2015) MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Frontiers in Microbiology* **6**, 791.

**Smirnov VF, Belov DV, Sokolova TN, Kuzina OV and Kartashov VR** (2008) Microbiological corrosion of aluminum alloys. *Applied Biochemistry and Microbiology* **44**, 192–196.

**Sören Schubert K and Kostrzewa M** (2017) MALDI-TOF MS in microbiology laboratory: current trends. *Current Issues in Molecular Biology* **23**, 17–20.

**Stieglmeier M, Rettberg P, Barczyk S, Bohmeier M, Pukall R, Wirth R and Moissl-Eichinger C** (2012) Abundance and diversity of microbial inhabitants in European spacecraft-associated clean rooms. *Astrobiology* **12**, 572–585.

https://doi.org/10.1017/S1473550420000427 Published online by Cambridge University Press

**Telegdi J, Shaban A and Trif L** (2017) Microbiologically influenced corrosion (MIC). In El-Sherik AM (ed). *Trends Oil and Gas Corrosion Research and Technologies*. Cambridge, England: Elsevier Ltd, pp. 191–214.

**Thomassin S, Jobin MP and Schmitt P** (2006) The acid tolerance response of *Bacillus cereus* ATCC14579 is dependent on culture pH, growth rate and intracellular pH. *Archives of Microbiology* **186**, 229–239.

**Torres-Barceló C** (2018) The disparate effects of bacteriophages on antibiotic-resistant bacteria. *Emerging Microbes & Infections* **7**, 168.

**Tucker DL, Tucker N and Conway T** (2002) Gene expression profiling of the pH response in *Escherichia coli*. *Journal of Bacteriology* **184**, 6551–6558.

**Venkateswaran K, Vaishampayan P, Cisneros J, Pierson DL, Rogers SO and Perry J** (2014) International Space Station environmental microbiome – microbial inventories of ISS filter debris. *Applied Microbiology and Biotechnology* **98**, 6453–6466.

**Videla HA and Herrera LK** (2005) Microbiologically influenced corrosion: looking to the future. *International Microbiology* **8**, 169–180.

**Voorhies AA, Mark Ott C, Mehta S, Pierson DL, Crucian BE, Feiveson A, Oubre CM, Torralba M, Moncera K, Zhang Y, Zurek E and Lorenzi HA** (2019) Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. *Scientific Reports* **9**, 9911.

**Walch G, Knapp M, Rainer G and Peintner U** (2016) Colony-PCR is a rapid method for DNA amplification of hyphomycetes. *Journal of Fungi* **2**, 12.

**Welker M, Van Belkum A, Girard V, Charrier JP and Pincus D** (2019) An update on the routine application of MALDI-TOF MS in clinical microbiology. *Expert Review of Proteomics* **16**, 695–710.

**Xu D, Li Y, Song F and Gu T** (2013) Laboratory investigation of microbiologically influenced corrosion of C1018 carbon steel by nitrate reducing bacterium *Bacillus licheniformis*. *Corrosion Science* **77**, 385–390.

**Yang SS, Chen CY, Wei CB and Lin YT** (1996) Microbial corrosion of aluminum alloy. *Chinese Journal of Microbiology and Immunology* **29**, 185–196.

**Yang SS, Lin JY and Lin YT** (1998) Microbiologically induced corrosion of aluminum alloys in fuel-oil/aqueous system. *Journal of Microbiology, Immunology and Infection* **31**, 151–164.

**Zarrin M, Ganj F and Faramarzi S** (2016) Analysis of the rDNA internal transcribed spacer region of the *Fusarium* species by polymerase chain reaction – restriction fragment length polymorphism. *Biomedical Reports* **4**, 471–474.

**Zea L, Nisar Z, Rubin P, Cortesão M, Luo J, McBride SA, Moeller R, Klaus D, Müller D, Varanasi KK, Muecklich F and Stodieck L** (2018) Design of a spaceflight biofilm experiment. *Acta Astronautica* **148**, 294–300.

**Zhang YJ, Zhang S, Liu XZ, Wen HA and Wang M** (2010) A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. *Letters in Applied Microbiology* **51**, 114–118.

**Zhang Y, Zhang LT, Li ZD, Xin CS, Li XQ, Wang X and Deng YL** (2019) Microbiomes of China's Space Station during assembly, integration, and test operations. *Microbial Ecology* **78**, 631–650.

https://doi.org/10.1017/S1473550420000427 Published online by Cambridge University Press