Microbiome and Metagenome Analyses of a Closed Habitat During Human Occupation

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Abstract

Background: Microbial contamination during the long-term confinements of space exploration present a potential risk for both crew members and spacecraft life support systems. As NASA moves from low Earth orbit further into the solar system, the monitoring of microbial populations within closed human habitation will be necessary to ensure the safety of both the crew and the spacecraft. NASA's Johnson Space Center has recently developed a microbial swab kit specifically for use during astronaut Extravehicular Activity (EVA). The EVA swab kit is designed to be held in an astronauts' bulky gloves and or by a robot's manipulator, and is thus suitable for microbial sample collection in remote and extreme locations. The ability of crew members to successfully use the EVA swab kit to sample the microbial communities of an Analog habitat was tested, resulting in the successful characterization of the microbial communities within this unique habitat.

Results: Several samples (floor, dry wall, glass, and metal surfaces) were collected for estimating cultivable, viable, and metabolically active microbial population using the EVA swab kit. The cultivable microbial population ranged from below the detection limit (BDL) to 106 CFU/sample and their identity was characterized using molecular methods. Next-generation sequencing (NGS; both 16S rRNA amplicon and shotgun) were used to characterize the microbial dynamics, community profiles and functional analysis (metabolic, virulence, and antimicrobial resistance). The 16S rRNA amplicon sequencing revealed abundance of viable Actinobacteria (Brevibacterium, Nesternkonia, Mycobacterium, Pseudonocardia and Corynebacterium), Firmicutes (Virgibacillus, Staphylococcus and Oceanobacillus) and Proteobacteria (esp. Acinetobacter) on floor/wall surfaces, while members of Firmicutes (Leuconostocaceae) and Proteobacteria (Enterobacteriaceae) were high on the glass/metal surfaces. Through non-metric multidimensional scaling (NMDS) determined from both 16S rRNA and metagenomic analyses revealed differential microbial speciation between floor/wall surfaces and glass/metal surfaces.

Conclusion: This study provides the first assessment of monitoring cultivable and viable microorganisms from a closed spacecraft Analog submerged habitat surfaces. Several statistical treatments suggested that the largest selective pressure on the microbial community structure was the surface type since different kinds of microorganisms were observed in the floor/dry wall surfaces when compared to the metal/glass surfaces, these samples also consistently grouped separately. The metal/glass surfaces had less complex community, lower bio-burden, and more closely resembled the controls. These results indicated that material choice is crucial when building closed habitats, even if they are simply analogs. Despite our results indicating the strong role that surfaces play in selecting for the live/viable microbial communities, our study also shows that there is a shared background community of non-viable microorganisms throughout the Analog habitat. Finally, the microbial ecology of the submerged Analog habitat differs greatly from that of previously studied Analog habitats, while a few species were associated with previously cultivated isolates from the International Space Station and MIR spacecraft.

Introduction

Over the next few decades the National Aeronautics and Space Administration (NASA), along with international partners, has planned to begin the expansion of human space exploration beyond low Earth orbit, to the Moon and on to Mars. This endeavor will entail a variety of new space habitats designed for both interplanetary travel and surface habitation [1, 2]. Minimizing and monitoring the number of detrimental microorganisms associated with these habitats will be critical to the safety and success of these missions. Inevitably, the components that will lead to the majority of the contamination of these facilities are the human occupants. Indeed, humans host an intricate microbiome consisting of numerous microorganisms that live on and within us [3]. Most of these microorganisms are either benign and pose no danger to the inhabitant, or are beneficial and perform necessary roles like protection from pathogens or conversion of nutrients into more readily absorbed compounds in our gut. However, humans can also unknowingly harbor, transport, and disperse pathogens and other microorganisms that can lead to microbial induced corrosion (MIC, also known as biocorrosion) [4]). While the presence of microbial contaminants with either pathogenic traits or MIC capabilities will pose a direct threat to the closed habitat and the crew, it is impractical and unrealistic to completely remove all microorganisms. Thus, similar to recent trends in hospital and medical instrument design, engineering mechanisms into habitat design to manage microbial populations will be critical to future space mission success [5, 6].

To aid NASA in developing appropriate closed habitats (a built environment that has minimal atmospheric exchange with the surrounding environment), it will be necessary to characterize the microbial ecology across a variety of current built environments. In this regard, the microbial analysis of several closed systems has been performed, including the spacecraft assembly facilities (SAF) [7], Inflated Lunar/Mars Analogous Habitat (ILMAH) [8, 9], long haul commercial aircraft cabin air [10], and the International Space Station (ISS) [11–13]. However, this is the first report of a pressure capsule (Analog habitat) closed off from the surrounding environment, as that of the space human habitat (Analog to ISS), is characterized for their molecular microbial communities.

In the past, samples from spacecraft and associated environments were collected using swabs made up of cotton, rayon, and polyester swabs [14, 15]. Recently new sampling devices were tested for microbial sampling; this includes upgraded swabs (different materials), polyester wipes, macrofoam sponges, adhesive tapes, Biological Sampling kit (BiSKit; macrofoam), witness coupons, dust, and bulk sampling [14, 16–22]. These novel microbial sampling tools have allowed for much more comprehensive collection of both cultivable and yet to be cultivated microbial populations [15, 21, 23]. Furthermore, these sampling devices were also employed to collect samples from Earth-based Analogs (Mars 500 facility/ILMAH/ Antarctic Concordia Station), and the ISS [11, 13, 24–27]. None of these sampling devices are ideal, with some containing nucleic acids and none being compatible to effectively collect samples from outside ISS which would require handling by astronauts with bulky gloves.

Engineers and scientists from the Human Forward Contamination Assessment team at the NASA's Johnson Space Center (JSC) have recently developed a novel sampling device to address these concerns called the Extravehicular Activity (EVA) microbial swab kit [28]. This swab kit consists of a macrofoam paddle head held in a large tool handle and stored in an eight-canister sample caddy, which is compatible

for handling by astronauts while wearing cumbersome EVA space suit gloves. In addition to this kit being designed for sampling outside surfaces of the ISS and spacesuits, initial evaluations demonstrated that these EVA swabs outperformed standard swabs in their ability to pick up microbial cells [28]. However, these initial evaluations were performed under controlled laboratory conditions and the efficiency of EVA swabs in collecting microbes from an Analog closed habitat is needed.

To more adequately field test EVA swab kits and help determine their readiness for future spaceflight, microbial sampling of a coastal Analog habitat was choosen as a testbed. The Analog habitat tested during this study mimics the ISS in that it is a pressure capsule closed off from the surrounding environment where a crew lives in isolation similar to what they would experience during a three-week long space mission. In this study, the EVA swab kit was employed to obtain samples from various locations (floor, dry wall, glass, and metal surfaces) of the Analog habitat. Traditional microbiological methods and molecular techniques were employed to uncover the microbial diversity of the Analog habitat. In order to understand the viable microorganism using molecular assays, the samples were pretreated with and without propidium monoazide (PMA), a chemical that chelates free DNA within dead cells and prevents its amplification during PCR as previously established [29]. This approach allowed to differentiate the viable/intact microbial community from that of the dead cells. The 16S rRNA gene amplicon and shotgun metagenome sequencing were performed to characterize the microbial diversity as well as functional pathways of the viable microbiome.

Materials And Methods

EVA swab sample kit preparation and sample collection

The EVA swab head is shown in Figure 1Aa and the six EVA swab kits holder (caddy) is depicted in Figure 1Ab. Sample kit sterilization and assembly was performed at JSC. Each sample canister (assembled with filter and ball plungers) and swab end effector assembly was placed into separate autoclave bag. Bagged components were placed into a Steris LV 250 Laboratory Steam Sterilizer and sterilized using a gravity cycle of 45 mins at 121°C at 103.4 kPa (15 psi). Note that neither the sample caddy itself nor the tool handle were autoclaved. Bagged components were allowed up to 1 hour of cool-down time at approximately 22°C for safe handling. Following autoclaving, bagged components were transferred to a Labconco Horizontal Clean Bench (Model # 36100000, ISO Class 5). With the commercial swab inside its sterile packaging, the swab stem was cut to optimal length (approximately 6.0 cm (2.4 in) using sterilized scissors, ensuring that the swab head remained inside its packaging until the final assembly step. The cut end of the swab was then inserted into the end effector slot and set screws were tightened to hold the swab in place. Sterile packaging was removed from the swab head immediately before inserting each swab assembly into its sterile container. Each container/swab assembly was then mounted into the tool caddy, which was placed into storage until sampling. During swab assembly, technicians wore sterile gloves, and both the gloves and assembly tools (Allen wrench, scissors, and forceps) were sprayed with ethanol surface disinfectant. All parts were handled either with sterile forceps or the autoclave bags, with no contact between the gloves and tool areas that must remain sterile. After assembly, the EVA sample

kits were transported to the test site packed inside hard-sided storage cases. Once at the test site, the Analog crew were briefed on tool usage, and were given an opportunity to practice with a spare handle and sample container assembly.

Surface swab samples were collected from 12 locations across the Analog habitat using the EVA microbial swab kit. To serve as controls, two swabs were removed form kits and exposed to the Analog habitat atmosphere before being placed back into the container, and another two were left in the container. Both control swabs and surface swabs were processed in tandem. A schematic representation of the Analog habitat sampling locations, and corresponding images are shown in Figure 1B and Figure 1C. In this study, locations were categorized into three types, control (n=4), wall/floor (n=5), and metal/glass (n=7). Samples 1 through 8 were collected approximately 3 days of crew occupation into the Analog mission; samples 9 through 16 were collected five days later. Both sets of samples were collected in the late afternoon/early evening. Two control samples (1 and 9) remained inside their sample canisters. Two control samples (2 and 10) were removed from their canisters flagged for a few seconds inside the habitat, then replaced without the swab head touching any surface. The Analog habitat sampling locations are illustrated in Figure 1B and Figure 1C, and their associated metadata is summarized in Table 1, describing each sample location.

Test facility and control

NASA's Analog mission involved a crew of six astronaut candidates and trainees inhabiting the habitat for 16 days, with periodic scuba excursions to the surrounding environment to perform simulated EVA spacewalks. The aquatic Analog mission habitat was comprised of three sections (Figure 1B). A 40 m^3 main cabin area which contained the crew living quarters for up to six. The 14 m^3 entry lock that included science and hygiene areas, and the 20 m^3 porch, which allowed crew to ingress/egress between the habitat and external environment (Figure 1B). The main cabin was controlled to a standard atmosphere (21% oxygen) at 101.3 kPa (14.7 psi), provided by an air compressor. Relative humidity ranged from 70 to 100%, and temperatures ranged from 24˚C to 28˚C; crews typically preferred to set the thermostat on the warmer end of the temperature range. Carbon dioxide was chemically scrubbed from the atmosphere inside the cabin.

Sample processing

Sample canisters (with swab assemblies still in place) were removed from the sample caddies and placed into a hard-sided shipping container, with the sample canisters secured in foam packing. Samples were then shipped via air to the Jet Propulsion Laboratory and sample processing took place in a cleanroom. Each EVA swab was aseptically removed from the lock and transferred to a 50 mL sterile falcon tube containing 15 mL of sterile phosphate-buffered saline (PBS; pH 7.4). The falcon tube with the EVA swab was shaken for two minutes followed by the concentration with a Concentrating Pipette (Innova Prep, Drexel, MO) using 0.45 µm Hollow Fiber Polysulfone tips (Cat #: CC08022) and PBS elution fluid. Each sample was concentrated to 1 mL and made up to 5 ml using sterile PBS. A 200 µL aliquot

was combined with 1.8 ml of sterile PBS (up to 10^{-1}) to estimate cultivable population as well as Adenosine triphosphate (ATP) content (Kikkoman Corp., Noda, Japan) as described previously [30]. Three mL of sample was split into two 1.5 mL- aliquots. One aliquot was treated with PMA to assess viability [31], while the second aliquot was handled similarly but without the addition of PMA. The 18.25 μL of 25 μM PMA was added to the PMA treatment samples and then incubated for 5 min at room temperature in the dark, followed by 15 min exposure to the activation system (PMA LED device, Biotium, Hayward, CA). The samples were then split in half again (0.75 mL per tube) and transferred to bead beating tubes containing Lysing Matrix E (MP Biomedicals, Santa Ana, CA). One half of PMA-treated and non-PMA treated samples were individually subjected to bead beating for 60 seconds using a vortex sample holder (MO Bio, Carlsbad, CA). The bead-beaten portion and the unprocessed aliquot were combined, followed by DNA extraction with the Maxwell 16 automated system (Promega, Madison, WI), in accordance with manufacture instructions. Maxwell Control (MC) was an additional cartridge run concurrently with each sample set to account for any contamination during the DNA extraction [32, 33]. The extracted DNA was eluted in 50 μL of water and stored at −20°C until further analysis.

Estimation and identification of cultivable microbial population

For the cultivation experiments, 100 μl of each dilution was plated on Reasoner's 2A agar (R2A), Potato dextrose agar (PDA) with the chloramphenicol (100 μg/mL), and blood agar (BA) (Hardy Diagnostics, Santa Maria, CA). The R2A and PDA plates were incubated at 25°C for seven days and BA plates at 35°C for two days, at which time colony forming units (CFU) were calculated. Whenever possible, a minimum of 5 isolates of distinct morphologies were picked from each plate, from each sampling location. The isolates were then archived in the semisolid R2A or PDA slants (agar media diluted 1:10) and stored at room temperature. Once a culture was confirmed to be pure, DNA was amplified during a colony PCR, or it was either extracted with the UltraClean DNA kit (MO Bio, Carlsbad, CA) or the Maxwell Automated System (Promega, Madison, WI). To identify bacterial isolates we PCR amplified with the 16S rRNA primer pair 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') [34, 35]. The PCR cycle conditions were as follows: denaturation at 95°C for 5 min, followed by 35 cycles consisting of denaturation at 95°C for 50 s, annealing at 55°C for 50 s, and extension at 72°C for 1 min 30 s, followed by a final by extension at 72°C for 10 min. To identify fungal isolates we amplified the fungal variable sized partial internal transcribed spacer (ITS) region with the primer pair ITS1F (5'- TTG GTC ATT TAG AGG AAG TAA-3') [36] and Tw13 (5'-GGT CCG TGT TTC AAG ACG-3') [37]. The PCR conditions were as follows: initial denaturation at 95˚C for 3 min followed by 25 cycles of 95°C for 50 s, annealing at 58°C for 30 s, and extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min. The amplification products were inspected by gel electrophoresis in 1% agarose gel and 1.5 kb molecular-weight size marker. The amplicon sequencing was performed by Macrogen (Rockville, MD, USA) using 27F and 1492R universal primers for bacteria, and ITS1F and Tw13 universal primers for fungi. The sequences were assembled using SeqMan Pro from DNAStar Lasergene Package (DNASTAR Inc., Madison, WI). The bacterial sequences were searched against EzTaxon-e database [38] and the

fungal sequences against the UNITE database [39]. The identification was based on the closest percentage similarity (>97%) to previously identified microbial type strains.

ATP assay

A bioluminescence assay was performed to determine the total ATP and intracellular ATP from all samples using the CheckLite HS kit (Kikkoman, Japan), as described previously [30]. Briefly, to determine total ATP (dead and viable microbes), sample aliquots were combined with an equal volume of a cell lysing detergent (benzalkonium chloride) and incubated at room temperature for 1 min prior to the addition of a luciferin–luciferase reagent. The sample was mixed, and the resulting bioluminescence was measured with a luminometer (Kikkoman). For intracellular ATP measures of intact microbes, a tenth volume of an ATP-eliminating reagent (apyrase, adenosine deaminase) was added to the sample and allowed to incubate for 30 min to remove any extracellular ATP. After extracellular ATP removal, the assay for ATP was performed (as described above) while running sterile PBS in tandem as a negative control. With1 Relative Luminescence Unit (RLU) (the unit of ATP measurement) equates to approximately to 1 CFU.

qPCR assay

Following DNA extraction with the Maxwell Automated system, quantitative polymerase chain reaction (qPCR) targeting both the bacterial 16S rRNA gene and the the fungal internal transcribed spacer (ITS) region was performed with SmartCycler (Cepheid, CA) to quantify the microbial burden. Primers targeting the 16S rRNA gene were 1369F (5'-CGG TGA ATA CGT TCY CGG-3') and modified 1492R (5'-GGW TAC CTTGTT ACG ACT T-3') [40]. Primers targeting the ITS region, were NS91 (5’-GTC CCT GCC CTT TGT ACA CAC-3’) and ITS51 (5’-ACC TTG TTA CGA CTT TTA CTT CCT C-3’) [41]. Each 25-µL reaction consisted of 12.5 µL of 2X iQ SYBR Green Supermix (BioRad, Hercules, CA), 1 µL each of forward and reverse oligonucleotide primers (10 µM each), and 1 µL of template DNA (templates included PMA treated and non-treated samples). Each sample was run in triplicate, the average and standard deviation were calculated based these results. Purified DNA from a model microbial community [42] served as the positive control and DNase/RNase free molecular-grade distilled water (Promega, Madison, WI) was used as the negative control in each run. The reaction conditions were as follows: a 3 min denaturation at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s, and a combined annealing and extension at 55°C for 35 s. The number of gene copies were determined from the standard curve using the rRNA gene as described previously [29].

16S rRNA gene amplicon sequencing

DNA from these samples was amplified using ~100 pg of gDNA in triplicate within 25 µl volume reactions using Platinum Hot Start PCR master mix (Thermo Fisher cat# 13000012) and Earth Microbiome Project standard Golay-barcoded primers of the 16S V4 region, 515fB (5’-GTG YCA GCM GCC GCG GTA A-3’) and 806rB (5’-GGA CTA CNV GGG TWT CTA AT-3’), (with expected amplicon size ~291 bp) as described in the earthmicrobiomeproject.org for 94°C 3 min and 35 cycles denaturing at 94°C for 45s,

annealing at 50°C for 60s, and extension for 72°C for 90s, followed by a final extension step of 72°C for 10 min [43-46]. Triplicate reactions were then pooled into a single tube and quality assessed. The amplicons were run on a 2% agarose gel and quantified using PicoGreen to access quality and relative quantity. All samples were pooled in equal volume into a single tube and then processed through the MoBio PCR cleanup kit to remove excess primers. The final cleaned pooled DNA was then then sequenced on a HiSeq 2500 2x150 bp Rapid Run.

16S rRNA gene amplicon sequence processing

Sequencing reads were demultiplexed with Illumina CASSAVA analysis software. Adapters were clipped and reads with < 20 bp were removed. Corresponding forward and reverse reads were stitched into longer fragments using FLASH (overlap 10 bp, max. mismatch 0.25). Amplicons of samples and controls were further sorted by removing reads without barcodes, single reads (only one barcode) and barcode chimeras (different barcodes on 5 and 3 prime site). Resulting reads were quality filtered for deep diversity analysis with QIIME at phred score q30, 5'-3' orientated, labeled and additional quality filtered using default settings in QIIME [47]. OTUs were checked for chimeric sequences via ChimeraSlayer, clustered at 97% similarity level, taxonomy was assigned with SILVA and determined phylogenetic tree was calculated [48, 49]. The resulting rarefied OTU table served as a basis for alpha and beta diversity analyses. The barplots, pie charts and MDS plots were all created in R (v. 3.3.1) using the Hmisc and vegan packages.

Shotgun metagenome sequencing

DNA libraries from the Analog habitat's surface DNA samples were prepared for shotgun metagenome sequencing using the Nextera DNA Library Preparation Kit from Illumina. The quality and fragment size of each library were assessed on the Bioanalyzer 2100 (Agilent). Separate adapters were added to the DNA from each library, normalized to 2 nM, pooled, denatured, and diluted to 1.8 pM according to the standard recommendations by Illumina. The HiSeq 2500 platform (Illumina) was used for sequencing, resulting in 100-bp paired-end reads.

Metagenome sequence data processing

Paired-end 100 bp reads were processed with Trimmomatic [50] to trim adapter sequences and low-quality ends, with a minimum Phred score of 20 across the entire length of the read used as a quality cutoff. Reads shorter than 80 bp after trimming were discarded. All reads were normalized across samples as recommended by Nayfach and Pollard [51]. All 16 sampling locations and two treatments (PMA and non-PMA), totaling 32 metagenomic samples. High-quality filtered reads were clustered to respective taxonomic levels (domains through species) using the lowest common ancestor (LCA) algorithm provided by MEGAN6 [52] and normalized to do a semi-quantitative comparative analysis. Microbial diversity analyses were carried out on normalized reads (~ 3.1×10^8), and analyses were set to keep at least one unique read to minimize the loss of diversity in low depth samples or for unique reads.

BLAST hits of ≥ 20 amino acids and ≥ 90% similarity were collected and used for taxonomic and functional assignment.

Taxonomic and functional assignment of shotgun metagenome sequences

For lower downstream processing and visualization, the MEGAN6 metagenomics toolkit was used [53]. The NCBI taxonomy database [54], containing over 6.6×10^5 reference sequences, and NCBI-NR protein sequence database, consisting of entries from GenPept, SwissProt, PIR, PDB, and RefSeq, were used to assign taxonomic features to reads by using DIAMOND [55] and the weighted LCA algorithm of MEGAN6 [52]. The identification of the reads to a taxon is not based on the genes only, but it is based on the comparison of the reads with the reference sequences deduced from the genomes of the curated NCBI taxonomy database [56]. Briefly, taxonomic and functional binning of the metagenomic reads is carried out using MEGAN [57], with the following settings: minScore = 50, maxExpected = 0.01, topPercent = 10, and minSupportPercent = 0.01. The resulting taxon assignments are presented in this manuscript. Functional analysis was carried out by mapping filtered DNA sequences against a reference database of all proteins within eggnog [58], SEED [59], and KEGG databases [60]. The search for translated DNA sequences was executed using DIAMOND, and hits that spanned ≥ 20 amino acids with ≥ 90% similarity were retained. In cases where one read matched these criteria against multiple proteins, only the protein or proteins (in the event of a tie) with the maximum bit score were considered. Pathways were analyzed by summing counts of KEGG orthologies for each pathway. Using different databases allowed a detailed view of reads defined by gene function consisting of a collection of biologically defined (i) subsystems, (ii) clusters of orthologous groups, and (iii) collection of metabolic pathways.

Assignment of virulence and antimicrobial resistance

Detected genes were screened for antimicrobial resistance and virulence factors using the Comprehensive Antimicrobial Resistance Database (CARD) and the Virulence Factors Database (VFDB) [61, 62].

Statistical analysis

Hierarchal clustering using the ward2 algorithm, and heatmap2 were conducted in the R programing environment in conjunction with the vegan and compositions package, as was analysis of variance (ANOVA) for univariate analysis of data [63]. Box graphs of CFU and qPCR data were plotted using Prism (version 5.0a). Significance ($p < 0.05$) between groups was tested by a one-way analysis of variance (ANOVA) using Prism.

Results

Estimation of cultivable microbial burden

The microbial populations of various surfaces from within the Analog habitat estimated by the culture-dependent CFU counts and the culture-independent methods of qPCR and ATP assays are given in Table 2. Cultivable population (mesophiles on R2A medium) from the Analog habitat surfaces ranged from 4.0 x 10^0 to 1.8 x 10^5 CFU/25 cm^2 except in samples collected from the location 14 (Wet porch table top) or location 15 (Bottom of top bunk above pillow). Similarly, when Analog habitat surface samples were grown on Blood agar plates the microbial population ranged from 4.0 x 10^0 to 2.3 x 10^4 CFU/25 cm^2 except in samples collected from the locations 14, 15, and 6 (Galley table top). CFUs were higher in the Analog habitat wall/floor samples (4, 7, 12, 13 and 16) than in the metal/glass surface samples (3, 6, 8, and 11). The cultivable fungal population measured on PDA plates ranged from 4.0 x 10^0 to 4.0 x 10^4 CFU/25 cm^2, while location 14 demonstrated no fungal growth (Figure 2A). Notably, locations 7, 13 and 16 harbored higher fungal populations (wall/floor) when compared to the other locations (metal/glass). In general, average cultivable fungi were ~2 log less than the average bacterial population tested in all samples. All the four controls tested (sample #1, #2, #9, and #10) yielded no bacterial (R2A or BA media) or fungal isolates.

Estimation of molecular microbial burden

The total ATP (tATP) value contains both live and dead microbial cells and ranged from below detection limit (BDL) to 2.09 x 10^4 Relative Luminescence Unit (RLU) 25 cm^{-2}, whereas intracellular ATP (iATP) values (from only viable cells) ranged from BDL to 9.40 x 10^3 RLU 25 cm^{-2}. The iATP-based values for viable microbial abundance was higher in three areas: Location #7 (Floor in front of galley sink), #13 (Wall above the WHC) and #16 (trash can storage place) (Figure 2B). The average percentage of viable microbial burden as measured by the iATP content was highest in the wall/floor locations 16 (~45%) and lowest in the metal/glass locations 6 (~1.6 %) as shown in Table 2.

The total bacterial 16S gene copy number of non-PMA treated samples (live and dead cells) range from BDL to 3.25 x 10^6 copies 25 cm^{-2}, whereas PMA treated (live and intact cells) 16S gene copy number range from BDL to 1.88 x 10^5 copies 25 cm^{-2}. The average viable bacterial 16S gene copy number was 5.87 x 10^4 copies 25 cm^{-2}. Notably, the highest percentage of viable bacterial burden was found in wall/floor locations 4, 7, 12 and 16, as compared to the much lower metal/glass locations (Figure 2C-PMA-16S rRNA), and congruent with ATP-based measurement. The total fungal ITS gene copy number of non-PMA treated samples range from BDL to 6.75 x 10^5 copies 25 cm^{-2}, whereas PMA treated ITS gene copy number range from BDL to 4.77 x 10^4 copies 25 cm^{-2}. The average fungal ITS gene copy number was 2.2 x 10^4 copies per sample. The highest viable fungal burden was found at locations 4, 6, 12 and 16, while all other locations were BDL (Figure 2C – PMA-ITS). Overall, both culture dependent and independent method demonstrated highest microbial bioburden at locations 7 and 16, both belonging to the wall/floor category.

Cultivable microbial diversity

The 16S rRNA and ITS amplicons identified via Sanger sequencing and phylogenetic affiliation of the bacterial and fungal strains isolated in this study are shown in Supplemental Figures S1 and S2. A total of 33 bacterial and 47 fungal strains were isolated from the Analog habitat surfaces and identified. The bacterial isolates belonged to phyla: *Actinobacteria, Firmicutes* and *Proteobacteria*. At the genus level the most predominant genera were *Bacillus* and *Staphylococcus*, comprising 72% and 15% of the isolates identified, respectively. At the species level, the most abundant was *Bacillus zhangzhouensis* (comprising 42.4% of total bacterial isolates; Supplemental Figure S1). The fungal isolates were mostly represented by *Aspergillus sydowii* and *Ascomycota* species, comprising 36% and 17% of total fungal isolates, respectively (Supplemental Figure S2).

Bacteriome analysis

A variety of bacteria were detected with 16S rRNA gene amplicon sequencing, belonging to seven phyla, with 5% of the overall reads not able to be assigned to a phylum (**Table 3**). A total of 1,354,846 reads were generated from the non-PMA treated wall/floor samples while 133,843 reads were generated from the PMA treated samples, suggesting that majority of the reads were from dead cells (**Table 3**). Similarly, the reads belong to the PMA treated metal/glass samples were low compared to the PMA untreated samples. A comparison between the two site categories; metal/glass (n=7) and wall/floor (n=5), showed a higher abundance of bacterial reads from the wall/floor samples compared to the metal/glass samples, both from non-PMA and PMA treated samples (**Table 3**).

In total, when classified reads were summarized to the genus level, 52 bacterial genera were identified (**Dataset S1**) with the proportions found in each sample summarized in **Figure 3**. The five most abundant genera detected in the non-PMA group were *Brevibacterium*, *Pseudonocardia*, *Brachybacterium*, *Staphylococcus* and *Acinetobacter* (**Figure 3A**) while the five most abundant genera detected in the PMA treated group were *Brevibacterium*, *Comamonadaceae*, *Oceanobacillus*, *Leuconostocaceae* and *Virgibacillus* (**Figure 3B**).

There were also differences in the bacterial composition amongst the wall/floor and metal/glass samples with wall/floor having a higher proportion of *Brevibacterium*, *Pseudonocardia*, *Brachybacterium* and *Halomonadaceae* compared to metal/glass and metal/glass samples having a higher proportion of *Acinetobacter*, *Streptococcus*, *Endozoicimonaceae*, *Enterobacteriaceae* and *Psychrobacter* compared to wall/floor samples for the non-PMA treated group (**Figure 4A**). For the PMA treated group the wall/floor samples had a higher proportion of *Brevibacterium*, *Virgibacillus*, *Oceanbacillus*, *Brachybacterium* and *Staphylococcus* compared to the metal/glass surfaces, which had a higher abundance of *Leuconostocaceae*, *Comamonadaceae*, *Enterobacteriaceae* and *Novosphingobium* (**Figure 4B**).

Nonmetric multidimensional scaling (NMDS) plots were computed using Bray-Curtis dissimilarity calculations to compare beta diversity (microbial composition and abundance) amongst the different samples. Similar trends in sample groupings were observed between non PMA and PMA treated samples, except for the wall sample which had a distinct microbiome amongst the PMA treated samples (**Figure 5A, right panel**) but which was closely related to the particle board samples in the non PMA treated group

(**Figure 5A, left panel**). The linoleum sample was also similar to the particle board samples in both PMA and non PMA treated groups (**Figure 5A**). **Figure 5B** is the same NMDS plot as Figure 5A, but statistically analyzing the difference between site categories, which clearly show that the microbiome of the metal/glass samples are different than the microbiome of the water/floor samples

Shotgun-Metagenomics sequencing based taxonomic and functional analyses

Metagenomic based taxonomic analysis was performed (in tandem with 16S rRNA amplicon sequence analysis) to characterize the microbial populations across the sample locations. Bacteria were the most dominant domain present in the surface samples from the Analog habitat, accounting for 94% of all characterized sequences. Eukaryotes made up 5.6% of the sequences, with most of these sequences belonging to the fungi and mammals. The remaining sequences were below 0.3% and are thus not discussed in detail. No archaeal signatures were observed in the metagenomic dataset. The key differences in the biome structure were assessed at the single taxon level, they did not exhibit any significant differentiation between communities when PMA untreated samples are compared (Figure 6). However, upon analyzing the community structure from PMA treated samples, all species and bacterial species communities segregate into different groups, specifically between the wall/floor and the metal/glass surface samples (Figure 6). Additionally, the control group related closely with the metal/glass surface samples.

Through shotgun metagenomics 136 genera were observed in the PMA untreated samples, of which, five and 75 genera were not identified in the PMA-treated samples of particleboard and metal surfaces (respectively). Similar to the 16S rRNA amplicon sequencing, shotgun metagenomics sequencing identified similar microbial populations at the genus level with the predominance of *Brevibacterium* (53.6%), *Brachybacterium* (7.8%), *Pseudonocardia* (9.9%), *Mycobacterium* (3.7%), and *Staphylococcus* (2.1%). Analogous to bacterial diversity, the fungal richness of PMA-untreated (total) samples decrease when compared to PMA-treated (viable) samples. Only two fungal phyla were detected that belong to *Ascomycota* and *Basidiomycota* (Supplemental Figure S3A). Frequency of the fungal OTUs at the genus level for various surface samples are presented in Supplemental Figure S3B. The most dominant *Aspergillus* and *Pencillium* in the metagenome analyses also found to be most frequently isolated during cultivation process (Supplemental Figure S2 and S3B).

Functional pathway analysis

Metagenomic sequencing gives the ability to inspect genomic and metabolic capability of the microbial community members. To examine the presence of a functional gene, sequence reads from all samples were mapped to individual microbial genes, which were then assigned to KEGG ortholog pathways. The microbial population within the wall/floor samples exhibited enrichment over other samples for pathways associated with general microbial proliferation, including nucleotide and amino acid metabolism, signal transduction, and cell motility and communication (Supplemental Figure S4). The gene families for carbohydrate utilization, amino acids and derivatives, and protein and RNA metabolism were found across all sampling locations Additionally, presence of antimicrobial resistance genes (AMR) were

annotated in the Comprehensive Antimicrobial Resistance Database (CARD). About 24 antimicrobial resistance gene families were identified throughout the Analog habitat, including resistance to aminoglycosides, beta-lactams, clindamycin, fluoroquinolones, lincosamide, streptomycin, and tetracycline (**Figure 7**). In general, a larger proportion of AMR-associated sequences were observed in the wall/floor samples relative to the metal surfaces. A range of AMR categories were identified, including resistance to aminoglycosides, beta-lactams, clindamycin, fluoroquinolones, lincosamide, streptomycin, and tetracycline. Overall, beta-lactam resistance (21%) and Cationic antimicrobial peptide (CAMP) resistance genes (8%) showed higher abundance across all locations then other AMR genes. In addition to AMR analysis, microbial genes were also screened for virulence factors using the Virulence Factors Database (VFDB). Sequence reads corresponding to virulence genes were binned into functional categories that combine genes contributing to similar mechanisms of virulence including efflux proteins, transposases, methylases, and resistance to a range of antibiotics (**Figure 7**). There is substantial overlap between genes annotated as conferring AMR and those implicated in virulence; thus, a proportion of the microorganisms present that contain virulence associated genes are resistance associated as well.

Discussion

Extensive microbial diversity studies have been conducted in various earth-based Analog environments (e.g. Mars 500, and multiple samplings of ILMAH) as well as aboard the ISS which relied on standard swabs and wipe kits [11, 13, 24, 25, 64]. Even though NASA has used similar cotton, rayon, and polyester swab devices for sampling spacecraft surfaces and associated environments since the 1970s [14, 15], the use of these standard swabs and wipe kits may prove insufficient for valid sampling outside of the ISS and other future spacecraft. Published reports comparing the collection of microbial samples from various surfaces using wipes, sponges, vacuum-based samplers, and a variety of swab head materials for culture-based target organisms has demonstrated large variability in microbial recovery outcomes [16, 17, 19, 20]. Of the devices tested, macrofoam was demonstrated to be the superior material with respect to both collection and recovery efficiency. Macrofoam swabs were demonstrated to collect ≥ 30% more spores as compared to rayon or polyester swabs, even at low biomass testing conditions [19]. Along with having inferior collection rates as compared to macrofoam, these other swabs are also more reactive to temperature and pressure changes that would occur while sampling surfaces outside the ISS, which could lead to sample loss [65]. The inadequacy of the standard swabs is compounded by the difficulty of using swabs or wipes while astronauts are wearing large, bulky EVA gloves. To overcome these hinderances, a team of NASA engineers and scientists have developed a unique microbial swab kit that combines the macrofoam material and a swab holder designed to function both with astronauts wearing EVA gloves and also interface with robotic manipulators [65]. Thus, the use of the new macrofoam swabs during sampling should facilitate a more accurate account of the microorganisms on the surfaces being tested while also allowing for more versatility in sampling locations. The main goal of the proof of concept study presented here was to field test these swab kits ability to collect microbial samples from various surfaces and assess the microbial burden/diversity in a Analog habitat.

Of the 10 cultivatable bacteria identified, species associated with pathogenicity, toxin production, biofouling and biocorrosion were documented. The dominant cultivable bacterial isolates were spore-forming *Bacillus,* with the most common culturable bacterial isolate across the Analog habitat being *Bacillus pumilus* (~48%). *B. pumilus* was isolated from all but the E3 sample site (center of the view port). Strains of *B. pumilus* have been shown to be capable of becoming opportunistic pathogens in immunocompromised patients [66], through the production of toxin [67], while other strains have been implicated in the biocorrosion of galvanized steel [68]. The second most common bacterial isolate was *B. cereus,* making up ~15% of the bacterial CFUs. Strains of this species have previously also been characterized as a pathogen [69], an opportunistic pathogen [69] and toxin producer [70]. Also similar to *B. pumilus,* some *B. cereus* strains have been shown to cause corrosion [71] and water system fouling [71, 72]. As members of the *Bacillus* genus are common inhabitants of soil and dust, they were likely to be cultured from the wall/floors of the Analog habitat. Because *Bacillus* readily forms spores that are difficult to extract DNA from, they have previously escaped detection in some earlier studies when traditional Sanger sequencing, PhyloChip G2 technology, and 454-pyrosequencing analysis of surface samples were employed [73–75]. Likewise, in this study, the sequencing method again did not retrieve sequences of some of the culturable spore formers. However, spore formers, including members of the genera *Bacillus, Paenibacillus, Virgibacillus* and *Oceanobacillus* were detected via cultivation. Also, both cultivation-dependent and sequencing methods confirmed the presence of *Staphylococcus* species in the Analog surface samples. *Staphylococcus* species are normally associated with human skin and may cause infections under certain situations in immunocompromised patients [76]. Similar to the ISS, the closed nature of the Analog habitat makes it likely that the majority of these bacteria originate on the skin of crew members and fall off with the shedding of dead skin cells [11].

Of the nine cultivatable fungal lineages, we found a variety of species with related isolates that have previously been associated with pathogenicity, mycotoxin production, biofouling, and biocorrosion. Members of the *Aspergillus* genera were the dominant cultivable fungi, making up five of the nine species isolated, with the most common culturable fungal isolate across the Analog habitat being *Asperifillus sydowii* (36%). *A. sydowii* was found in all locations but E8 and E16 (entry table and trash can storage, respectively). *A. sydowii* was reported to be a marine pathogen of seafans [77], produce mycotoxin [78], and also associated with biocorrosion damage aboard the ISS [79]. The second most common fungal isolate was *A. tubingensis,* making up ~15% of the fungal CFUs isolated during this study. Although a few strains of *A. tubingensis* have previously been identified as being rare opportunistic pathogen [80] through the production of mycotoxin [81], *A. tubingensis* is more well known for its ability to degrade plastic [82]. Multiple microorganisms and microbial processes have been implicated in the biodegradation and biofouling of human made materials and structures [83]. In this study alone seven of the 19 microbial isolates (fungal and bacterial) have been previously associated with biofouling or biocorrosion, while 13 of the 19 microbial isolates have were previously associated with pathogenicity (at minimum opportunistic) and or toxin/mycotoxin production; however, the previous association of these select isolates with disadvantageous traits does not indicate that these microbes are performig these processes in the Analog habitat, or even that the isolates contain necessary dilatirious genes. There has

been no evidence that any isolate cutured from the Analog habitat has led to harm of the crew or the habitat, or that any of these microbes pose a threat to the crew unless more clinical studies are conducted.

Several studies have been reported on the microbial composition of Analog habitat environments used as proxies for future human exploration using gene-targeted amplicon sequencing of microbial populations. One such study, the ILMAH, exhibited high abundance of *Staphylococcaceae, Corynebacteriaceae, Caulobacteraceae, Pleosporaceae,* and *Sporidiobolaceae [*25]. A similar closed system, Mars 500, showed a high abundance of sequences of *Corynebacteriaceae, Burkholderiaceae,* and *Staphylococcaceae [*24].Based on these cultivable microbial compositions, it is evident that except for the presence of skin-associated members of the family *Staphylococcaceae,* the coastal based Analog habitat has a different bacterial composition than these other Earth-based analogs.

In comparing the OTU assignments generated from 16S rRNA analysis (Figure 3) and OTUs generated from metagenomic analysis (Figure 5), it is clear that when non-viable cells are removed by treatment with PMA, the floor/wall surface biomes and the glass/metal biomes form separate groupings. This is in contrast to when there is no treatment and all cells (viable and non-viable) are compared, and a large poorly defined group is formed. This suggests that there is a shared background of non-viable cells across the habitat and that the different niches of floor/wall and the glass/metal are selecting for different live/intact microbial communities. The dendrogram in Figure 4 closely corroborates this trend, with the exception of location 13 grouping with the floor/wall group. Additionally, the controls in Figures 3, 4, and 5 group closely with the glass/metal surface. It is unclear if this grouping is an artifact due to the low cell numbers in both the control and metal/glass groups, and if so what variables have led to the microbial population of the metal/glass to be so low. These could range from more stringent cleaning regimes, innate anti-microbial properties of the materials, or simply a lack of contact with microbe containing objects and or people.

Metagenome approach revealed metabolic pathways, virulence factors and AMR genes [84, 85]. The AMR gene categories specifically relating to the transformation proteins (PBP), an efflux pump (membrane fusion protein) similar to the ISS metagenomic AMR profile were abundant (Figure 6). Additionally, *Mycobacterium* virulence operon, metal resistance mechanisms such as cobalt-zinc-cadmium resistance and copper homeostasis, were identified which are also similar to the ISS metagenomic profile [64].

Conclusions

The primary objective of this study was to assess the EVA swab kits' ability to adequately sample a spacecraft equivalent habitat outside a laboratory-testing environment and to then use the collected samples to evaluate the microbiome of said habitat. The EVA swab kits were successful in our field tests, collecting viable cells, cultivable microorganisms and sufficient genetic material for 16S rRNA, ITS, and metagenomic shotgun sequencing from various surface materials. The EVA swab kit was effective despite the difficulty of collecting microorganisms from a variety of surfaces that ranged from smooth

glass to rough and irregular materials. These results demonstrate that the EVA swab kit is a viable option for deployment and testing aboard the ISS to determine the microbial load on the outside of ISS surfaces. Based on the results, it is recommended that highly textured and absorbent particleboard should not be used in closed human habitats or in current or future spacecraft, as these harbor more microorganisms. These findings are supported by previous studies that demonstrate rough irregular and spongey surfaces can protect large microbial loads [86]. Finally, numerous microbes isolated from the Analog habitat have also been previously found aboard the ISS and or MIR stations [79, 87, 88]. This similarity, along with the habitat's mix of isolates related to proported opertunistic pathogenic and biocorrosive associated microbes, indicates that the closed Analog habitat maybe the ideal location to test future microbial monitoring and microbial mitigation techniques as NASA begins to build and design new space architecture.

Declarations

Ethics approval and consent to participate

Because the purpose of this test was to characterize microorganisms on the *Analog habitat,* rather than human residing in the closed system, the JSC Institutional Review Board ruled this study as "Exempt Certified." No identifying information about the crew member of the Analog habitat will be published.

Consent for publication

All authors participated in this study and given their consent for publishing the results. All authors read and approved the final manuscript.

Availability of data and material

All raw sequence data used in this study have been uploaded to the NCBI SRA SUB6155724. During review raw reads will be available from NASA GeneLab database. The 16S and ITS sequences of culturable isolates are deposited in GenBank under MN581166 to MN581196.

Competing interests

Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not constitute or imply its endorsement by the U.S. Government or the Jet Propulsion Laboratory, California Institute of Technology. © 2019 California Institute of Technology. Government sponsorship acknowledged.

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Authors' contributions

MR and HA developed EVA tool, collected samples, coordinated and designed the study with input from KV. GBMM contributed to sample processing, traditional microbiology assays, extracted DNA, assayed molecular microbial burden, generated corresponding figures, tables, and text associated with these analyses. GBMM and CU analyzed the 16S amplicon sequencing data and generated the corresponding figures. NKS processed shotgun metagenome sequence data. CP and NKS, compiled data associated with cultivable microorganisms, amplicon sequencing, and metagenome sequencing, respectively. RK and JM generated amplicon, and metagenome sequences and performed preliminary QIIME analyses. KV, GBMM drafted the manuscript and responsible for data analysis and interpretation, CP, CU, RK edited the manuscript.

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References

1.Hoppy P, John B, Firouz N: *A Minimal Architecture for Human Journeys to Mars.* 2015, *3(*2):73–81.

2.Wilhite AW, Chai P: *(2014) Plan B for U.S. Human Space Exploration Program.* In: *AIAA SPACE 2014 Conference and Exposition.*

3.Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI: *The human microbiome project. Nature* 2007, *449(*7164):804–810.

4.Cho I, Blaser MJ: *The human microbiome: at the interface of health and disease. Nat Rev Genet* 2012, *13(*4):260–270.

5.Konieczny J, Rdzawski Z: *Antibacterial properties of copper and its alloys. Archives of Materials Science and Engineering* 2012, *56:*53–60.

6.Maki DG, Tambyah PA: *Engineering out the risk for infection with urinary catheters. Emerg Infect Dis* 2001, *7(*2):342–347.

7.La Duc MT, Venkateswaran K, Conley CA: *A genetic inventory of spacecraft and associated surfaces. Astrobiology* 2014, *14(*1):15–23.

8.Blachowicz A, Mayer T, Bashir M, Pieber TR, De León P, Venkateswaran K: *Human presence impacts fungal diversity of inflated lunar/Mars analog habitat. Microbiome* 2017, *5(*1):62.

9.Mayer T, Blachowicz A, Probst AJ, Vaishampayan P, Checinska A, Swarmer T, de Leon P, Venkateswaran K: *Microbial succession in an inflated lunar/Mars analog habitat during a 30-day human occupation. Microbiome* 2016, *4(*1):1–17.

10.Osman S, LaDuc MT, Dekas A, Newcombe D, Venkateswaran K: *Microbial bioburden and diversity of commercial airline cabin air during short- and long-duration of travel. ISME J* 2008, *2:*482–497.

11.Checinska Sielaff A, Urbaniak C, Mohan GBM, Stepanov VG, Tran Q, Wood JM, Minich J, McDonald D, Mayer T, Knight R *et al: Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. Microbiome* 2019, *7(*1):50.

12.Singh NK, Wood JM, Karouia F, Venkateswaran K: *Succession and persistence of microbial communities and antimicrobial resistance genes associated with International Space Station environmental surfaces. Microbiome* 2018, *6(*1):214.

13.Singh NK, Wood JM, Mhatre SS, Venkateswaran K: *Metagenome to phenome approach enables isolation and genomics characterization of Kalamiella piersonii gen. nov., sp. nov. from the International Space Station. Appl Microbiol Biotechnol* 2019, *103(*11):4483–4497.

14.NASA: *Handbook for the Microbiological Examination of Space Hardware, NASA-HDBK–6022.* In*.:* National Aeronautics and Space Administration, Washington, D.C.; 2010.

15.Puleo JR, Fields ND, Bergstrom SL, Oxborrow GS, Stabekis PD, Koukol R: *Microbiological profiles of the Viking spacecraft. Appl Environ Microbiol* 1977, *33(*2):379–384.

16.Rose L, Jensen B, Peterson A, Banerjee SN, Srduino MJ: *Swab materials and Bacillus anthracis spore recovery from nonporous surfaces. Emerg Infect Dis* 2004, *10(*6):1023–1029.

17.Bruckner JC, Yalamanchili RC, Fields R, Sumner R, Venkateswaran K: *Systematic sampling and processing of nucleic acids for low biomass samples.* In: *105th General meeting of the American Society of Microbiology: 2005; Atlanta, GA.* American Society of Microbiology.

18.Buttner MP, Cruz P, Stetzenbach LD, Klima-Comba AK, Stevens VL, Emanuel PA: *Evaluation of the Biological Sampling Kit (BiSKit) for large-area surface sampling. Appl Environ Microbiol* 2004, *70(*12):7040–7045.

19.Hodges LR, Rose LJ, Peterson A, Noble-Wang J, Arduino MJ: *Evaluation of a macrofoam swab protocol for the recovery of Bacillus anthracis spores from a steel surface. Appl Environ Microbiol* 2006,

*72(*6):4429–4430.

20.Kirschner LE, Puleo JR: *Wipe-rinse technique for quantitating microbial contamination on large surfaces. Appl Environ Microbiol* 1979, *38(*3):466–470.

21.Venkateswaran K, La Duc MT, P. V: *Genetic Inventory Task; final report. JPL Pushlishing Group, Pasadena, CA* 2012.

22.Ganesh Babu Malli Mohan, Benardini N James, Hendrickson. R, Venkateswaran K, Moogega Stricker: *Characterization of Biological Fallout Particles of Cleanrooms to Measure Spacecraft Cleanliness.* In: *47th International Conference on Environmental Systems: 2017; Charleston, SC.*

23.Henrickson R, Urbaniak C, Malli Mohan GB, Heidi Aronson, K V: *A ratio of spore to viable organisms: a case study of the JPL-SAF cleanroom. Jet Propulsion Laboratory, National Aeronautics and Space Administration* 2017.

24.Schwendner P, Mahnert A, Koskinen K, Moissl-Eichinger C, Barczyk S, Wirth R, Berg G, Rettberg P: *Preparing for the crewed Mars journey: microbiota dynamics in the confined Mars500 habitat during simulated Mars flight and landing. Microbiome* 2017, *5(*1):129.

25.Mayer T, Blachowicz A, Probst AJ, Vaishampayan P, Checinska A, Swarmer T, de Leon P, Venkateswaran KJM: *Microbial succession in an inflated lunar/Mars analog habitat during a 30-day human occupation.* 2016, *4(*1):22.

26.Urbaniak C, Sielaff AC, Frey KG, Allen JE, Singh N, Jaing C, Wheeler K, Venkateswaran K: *Detection of antimicrobial resistance genes associated with the International Space Station environmental surfaces. Scientific Reports* 2018, *8(*1):814.

27.Van Houdt R, De Boever P, Coninx I, Le Calvez C, Dicasillati R, Mahillon J, Mergeay M, Leys N: *Evaluation of the airborne bacterial population in the periodically confined Antarctic base Concordia. Microb Ecol* 2009, *57(*4):640–648.

28.Rucker Michelle A, Hood Drew, Walker Mary, Venkatewaran K, C SA: *EVA Swab Tool to Support Planetary Protection and Astrobiology Evaluations.* In: *39th IEEE Aerospace Conference; 3–10 Mar 2018;; Big Sky, MT; United States* 2018.

29.Checinska A, Probst AJ, Vaishampayan P, White JR, Kumar D, Stepanov VG, Fox GE, Nilsson HR, Pierson DL, Perry J *et al: Microbiomes of the dust particles collected from the International Space Station and Spacecraft Assembly Facilities. Microbiome* 2015, *3(*1):50.

30.Venkateswaran K, Hattori N, La Duc MT, Kern R: *ATP as a biomarker of viable microorganisms in clean-room facilities. J Microbiol Methods* 2003, *52(*3):367–377.

31.Vaishampayan P, Probst AJ, La Duc MT, Bargoma E, Benardini JN, Andersen GL, Venkateswaran K: *New perspectives on viable microbial communities in low-biomass cleanroom environments. ISME J* 2013, *7(*2):312–324.

32.Eisenhofer R, Minich JJ, Marotz C, Cooper A, Knight R, Weyrich LS: *Contamination in Low Microbial Biomass Microbiome Studies: Issues and Recommendations. Trends Microbiol* 2019, *27(*2):105–117.

33.Minich JJ, Sanders JG, Amir A, Humphrey G, Gilbert JA, Knight R: *Quantifying and Understanding Well-to-Well Contamination in Microbiome Research. mSystems* 2019, *4(*4).

34.Lane DJ: *Nucleic Acid Techniques in Bacterial Systematics.* In: *Nucleic Acid Techniques in Bacterial Systematics.* Edited by Stackebrandt E, Goodfellow M, vol. 1. New York: Wiley; 1991: 115–175.

35.Turner S, Pryer KM, Miao VP, Palmer JD: *Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. J Eukaryot Microbiol* 1999, *46(*4):327–338.

36.Lai X, Cao,L., Tan,H., Fang,S., Huang,Y., Zhou,S.: *Fungal communities from methane hydrate-bearing deep-sea marine sediments in South China See. ISME Journal* 2007, *1(*8):756–762.

37.Taylor DL, Bruns, T. D.: *Community structure of ectomycorrhizal fungi in a Pinus muricata forest: minimal overlap between the mature forest and resistant propagule communities. Mol Ecol* 1999, *8:*1837–1850.

38.Kim O-S, Cho Y-J, Lee K, Yoon S-H, Kim M, Na H, Park S-C, Jeon YS, Lee J-H, Yi H *et al: Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol* 2012, *62:*716–721.

39.Koljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM *et al: Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol* 2013, *22(*21):5271–5277.

40.Suzuki MT, Taylor LT, DeLong EF: *Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. Appl Environ Microbiol* 2000, *66(*11):4605–4614.

41.Onofri S, de la Torre R, de Vera JP, Ott S, Zucconi L, Selbmann L, Scalzi G, Venkateswaran KJ, Rabbow E, Sanchez Inigo FJ *et al: Survival of rock-colonizing organisms after 1.5 years in outer space. Astrobiology* 2012, *12(*5):508–516.

42.Kwan K, Cooper M, La Duc MT, Vaishampayan P, Stam C, Benardini JN, Scalzi G, Moissl-Eichinger C, Venkateswaran K: *Evaluation of procedures for the collection, processing, and analysis of biomolecules from low-biomass surfaces. Appl Environ Microbiol* 2011, *77(*9):2943–2953.

43.Apprill A, McNally S, Parsons R, Weber L: *Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol* 2015, *75(*2):129–137.

44.Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R: *Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences* 2011, *108(*Supplement 1):4516–4522.

45.Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N: *Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J* 2012, *6.*

46.Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, Gilbert JA, Jansson JK, Caporaso JG, Fuhrman JA *et al: Improved Bacterial 16S rRNA Gene (V4 and V4–5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. mSystems* 2016, *1(*1):e00009–00015.

47.Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI *et al: QIIME allows analysis of high-throughput community sequencing data. Nature Methods* 2010, *7:*335.

48.Wang Q, Garrity GM, Tiedje JM, Cole JR: *Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy.* 2007, *73(*16):5261–5267.

49.Edgar RC: *Search and clustering orders of magnitude faster than BLAST. Bioinformatics* 2010, *26(*19):2460–2461.

50.Bolger AM, Lohse M, Usadel B: *Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics* 2014, *30(*15):2114–2120.

51.Nayfach S, Pollard KS: *Toward Accurate and Quantitative Comparative Metagenomics. Cell* 2016, *166(*5):1103–1116.

52.Huson DH, Auch AF, Qi J, Schuster SC: *MEGAN analysis of metagenomic data. GENOME RES* 2007, *17(*3):377–386.

53.Huson DH, Beier S, Flade I, Gorska A, El-Hadidi M, Mitra S, Ruscheweyh HJ, Tappu R: *MEGAN Community Edition - Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data. PLoS Comput Biol* 2016, *12(*6):e1004957.

54.Sayers EW, Barrett T, Benson DA, Bryant SH, Canese K, Chetvernin V, Church DM, DiCuccio M, Edgar R, Federhen S *et al: Database resources of the National Center for Biotechnology Information. Nucleic Acids Res* 2009, *37(*Database issue):D5–15.

55.Buchfink B, Xie C, Huson DH: *Fast and sensitive protein alignment using DIAMOND. Nature Methods* 2014, *12:*59.

56.Sayers EW, Barrett T, Benson DA, Bolton E, Bryant SH, Canese K, Chetvernin V, Church DM, Dicuccio M, Federhen S *et al: Database resources of the National Center for Biotechnology Information. Nucleic Acids Res* 2010, *38(*Database issue):D5–16.

57.Huson DH, Tappu R, Bazinet AL, Xie C, Cummings MP, Nieselt K, Williams R: *Fast and simple protein-alignment-guided assembly of orthologous gene families from microbiome sequencing reads. Microbiome* 2017, *5(*1):11.

58.Powell S, Szklarczyk D, Trachana K, Roth A, Kuhn M, Muller J, Arnold R, Rattei T, Letunic I, Doerks T *et al: eggNOG v3.0: orthologous groups covering 1133 organisms at 41 different taxonomic ranges. Nucleic Acids Res* 2012, *40(*Database issue):D284–289.

59.Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang HY, Cohoon M, de Crecy-Lagard V, Diaz N, Disz T, Edwards R *et al: The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res* 2005, *33(*17):5691–5702.

60.Kanehisa M, Goto S: *KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res* 2000, *28(*1):27–30.

61.Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN *et al: CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res* 2017, *45(*D1):D566-D573.

62.Chen L, Zheng D, Liu B, Yang J, Jin Q: *VFDB 2016: hierarchical and refined dataset for big data analysis—10 years on. Nucleic Acids Res* 2016, *44(*D1):D694–697.

63.*R: A Language and Envrionment for Statistical Computing [Internet]. [*http://www.r-project.org]

64.Singh NK, Wood JM, Karouia F, Venkateswaran KJM: *Succession and persistence of microbial communities and antimicrobial resistance genes associated with International Space Station environmental surfaces.* 2018, *6(*1):204.

65.Michelle RA, Drew H, Mary W, Venkatewaran K, SC A: *EVA Swab Tool to Support Planetary Protection and Astrobiology Evaluations.* In: *39th IEEE Aerospace Conference; 4–11 March 2018; Big Sky, MT, USA.* 2018.

66.Kimouli M, Vrioni G, Papadopoulou M, Koumaki V, Petropoulou D, Gounaris A, Friedrich AW, Tsakris A: *Two cases of severe sepsis caused by Bacillus pumilus in neonatal infants. J Med Microbiol* 2012, *61(*Pt 4):596–599.

67.Nieminen T, Rintaluoma N, Andersson M, Taimisto AM, Ali-Vehmas T, Seppala A, Priha O, Salkinoja-Salonen M: *Toxinogenic Bacillus pumilus and Bacillus licheniformis from mastitic milk. Vet Microbiol* 2007, *124(*3–4):329–339.

68.Bolton N, Critchley M, Fabien R, Cromar N, Fallowfield H: *Microbially influenced corrosion of galvanized steel pipes in aerobic water systems. J Appl Microbiol* 2010, *109(*1):239–247.

69.Bottone EJ: *Bacillus cereus, a volatile human pathogen. Clin Microbiol Rev* 2010, *23(*2):382–398.

70.Schoeni JL, Wong AC: *Bacillus cereus food poisoning and its toxins. J Food Prot* 2005, *68(*3):636–648.

71.Rajasekar A, Ganesh Babu T, Karutha Pandian S, Maruthamuthu S, Palaniswamy N, Rajendran A: *Biodegradation and corrosion behavior of manganese oxidizer Bacillus cereus ACE4 in diesel transporting pipeline. Corros Sci* 2007, *49(*6):2694–2710.

72.Blanpain-Avet P, Faille C, Delaplace G, Bénézech T: *Cell adhesion and related fouling mechanism on a tubular ceramic microfiltration membrane using Bacillus cereus spores. J Membr Sci* 2011, *385–386:*200–216.

73.Venkateswaran K, Satomi M, Chung S, Kern R, Koukol R, Basic C, White D: *Molecular microbial diversity of a spacecraft assembly facility. Syst Appl Microbiol* 2001, *24(*2):311–320.

74.La Duc MT, Osman S, Vaishampayan P, Piceno Y, Andersen G, Spry JA, Venkateswaran K: *Comprehensive census of bacteria in clean rooms by using DNA microarray and cloning methods. Appl Environ Microbiol* 2009, *75(*20):6559–6567.

75.La Duc MT, Vaishampayan P, Nilsson HR, Torok T, Venkateswaran K: *Pyrosequencing-derived bacterial, archaeal, and fungal diversity of spacecraft hardware destined for Mars. Appl Environ Microbiol* 2012, *78(*16):5912–5922.

76.Otto M: *Staphylococcus colonization of the skin and antimicrobial peptides. Expert Rev Dermatol* 2010, *5(*2):183–195.

77.Soler-Hurtado MM, Sandoval-Sierra JV, Machordom A, Dieguez-Uribeondo J: *Aspergillus sydowii and Other Potential Fungal Pathogens in Gorgonian Octocorals of the Ecuadorian Pacific. PLoS One* 2016, *11(*11):e0165992.

78.Jarvis BB, Miller JD: *Mycotoxins as harmful indoor air contaminants. Appl Microbiol Biotechnol* 2005, *66(*4):367–372.

79.Alekhova TA, Aleksandrova AA, Novozhilova T, Lysak LV, Zagustina NA, Bezborodov AM: *[Monitoring of microbial degraders in manned space stations]. Prikl Biokhim Mikrobiol* 2005, *41(*4):435–443.

80.Bathoorn E, Escobar Salazar N, Sepehrkhouy S, Meijer M, de Cock H, Haas PJ: *Involvement of the opportunistic pathogen Aspergillus tubingensis in osteomyelitis of the maxillary bone: a case report. BMC Infect Dis* 2013, *13:*59.

81.Perrone G, Mule G, Susca A, Battilani P, Pietri A, Logrieco A: *Ochratoxin A production and amplified fragment length polymorphism analysis of Aspergillus carbonarius, Aspergillus tubingensis, and Aspergillus niger strains isolated from grapes in Italy. Appl Environ Microbiol* 2006, *72(*1):680–685.

82.Sheel A, Pant D: *Microbial Depolymerization.* In: *Waste Bioremediation.* Edited by Varjani SJ, Gnansounou E, Gurunathan B, Pant D, Zakaria ZA. Singapore: Springer Singapore; 2018: 61–103.

83.Gaylarde CC, Morton LHG: *Deteriogenic biofilms on buildings and their control: A review. Biofouling* 1999, *14(*1):59–74.

84.Handelsman J: *Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev* 2004, *68(*4):669–685.

85.Vieites JM, Guazzaroni ME, Beloqui A, Golyshin PN, Ferrer M: *Metagenomics approaches in systems microbiology. Fems Microbiol Rev* 2009, *33(*1):236–255.

86.Ismaïl R, Aviat F, Michel V, Le Bayon I, Gay-Perret P, Kutnik M, Fédérighi M: *Methods for recovering microorganisms from solid surfaces used in the food industry: a review of the literature. Int J Env Res Public Health* 2013, *10(*11):6169–6183.

87.Alekhova TA, Aleksandrova AV, Novozhilova TY, Lysak LV, Zagustina NA: *The experiment "initial stages of biological damage and deterioration in space". Moscow Univ Biol Sci Bull* 2008, *63(*4):163–169.

88.Pierson D, Botkin D, Bruce R, Castro V, Smith M, Oubre C, Ott C: *Microbial Monitoring of the International Space Station.* In: *Environmental Monitoring: A Comprehensive Handbook* Edited by Moldenhauer J: DHI Publishing, LLC, River Grove, IL.; 2012.

Tables

Table 1 Description of Analog habitat various sampling location and its description

EVA swab number	Location number used in this study	Sampling Location Description	Surface Material	Site Categorial
EE101	E1	Swab control	Swab	Control
EE102	E2	Air control	Swab	Control
EE103	E3	Center of view port window in bunk area	Glass	Metal/Glass
EE104	E4	Floor between bunk	Particle board	Wall/Floor
EE105	E5	Counter top by phone	Metal	Metal/Glass
EE106	E6	Galley table top	Metal	Metal/Glass
EE107	E7	Floor in front of galley sink	Particle board	Wall/Floor
EE108	E8	Science table top in entry lock	Metal	Metal/Glass
EE109	E9	Swab control	Swab	Control
EE110	E10	Air control	Swab	Control
EE111	E11	Behind the entry lock sink	Metal	Metal/Glass
EE112	E12	Entry lock floor	Particle board	Wall/Floor
EE113	E13	Wall above the Waste/Hygiene Compartment (WHC)	Dry wall	Wall/Floor
EE114	E14	Wet porch table top	Metal	Metal/Glass
EE115	E15	Bottom of top bunk above pillow	Metal	Metal/Glass
EE116	E16	trash can storage place	Linoleum	Wall/Floor

Table 2 Total, viable, and cultivable microbiological characteristics of Analog habitat surface samples

Samples	Cultivable bacterial population (CFU/25 cm^2)		qPCR-based bacterial population (16S rRNA copies 25 cm^{-2})		Viable bacterial population (B/A x 100)	ATP-based microbial population (RLU/25 cm^2)		Viable microbial population (D/C x100)
	Bacteria	Fungi	Untreated (A)	PMA-treated (B)		Total ATP (C)	Intracellular ATP (D)	
E1	NG	NG	BDL	BDL	-	BDL	BDL	-
E2	NG	NG	BDL	BDL	-	BDL	BDL	-
E3	4.0	1.8 x 10^1	1.90 x 10^4	1.77 x 10^4	93.32	4.76 x 10^2	7.00 x 10^1	14.71
E4	4.0 x 10^2	1.0 x 10^1	8.52 x 10^5	5.57 x 10^4	6.54	1.22 x 10^3	3.15 x 10^2	25.92
E5	1.0 x 10^2	2.6 x 10^1	9.39 x 10^4	1.34 x 10^4	14.23	9.40 x 10^2	6.00 x 10^1	6.38
E6	4.0	4.0	1.24 x 10^5	3.99 x 10^4	32.23	1.93 x 10^3	3.00 x 10^1	1.55
E7	1.5 x 10^3	8.8 x 10^1	1.05 x 10^6	1.81 x 10^5	17.34	5.92 x 10^3	7.32 x 10^2	12.36
E8	2.0	1.0 x 10^1	1.32 x 10^5	2.63 x 10^4	19.92	6.20 x 10^2	4.50 x 10^1	7.29
E9	NG	NG	BDL	BDL	-	BDL	BDL	-
E10	NG	NG	BDL	BDL	-	BDL	BDL	-
E11	4.0	4.0	1.99 x 10^5	2.80 x 10^4	14.07	2.80 x 10^2	9.52 x 10^1	34.00
E12	7.6 x 10^2	1.0 x 10^1	1.25 x 10^6	8.11 x 10^4	6.51	1.9 x 10^3	2.85 x 10^2	15.04
E13	1.6 x 10^3	2.22 x 10^2	2.46 x 10^5	2.72 x 10^4	11.09	1.37 x 10^3	4.04 x 10^2	29.45
E14	NG	NG	2.69 x 10^4	2.38 x 10^4	88.54	9.52 x 10^1	1.35 x 10^2	>100
E15	NG	1.0 x 10^1	2.29 x 10^4	2.19 x 10^4	95.47	3.10 x 10^2	1.00 x 10^2	32.26
E16	2.0 x 10^5	4.0 x 10^3	3.25 x 10^6	1.88 x 10^5	5.79	2.09 x 10^4	9.40 x 10^3	44.93

NG – no growth

Table 3 Bacterial Phylum level retrieved from Analog habitat sampled at various surfaces using 16S rRNA amplicon sequencing

Taxon	Number of 16S rRNA amplicon reads from samples:			
	No PMA wall/floor	PMA wall/floor	No PMA metal/ glass	PMA metal/ glass
Actinobacteria	852,891	71,011	16,611	757
Bacteroidetes	2,444	190	58	-
Firmicutes	115,140	21,236	36,816	10,446
Fusobacteria	-	-	3,119	-
Alpha proteobacteria	58,475	4,003	9,467	3,597
Beta proteobacteria	1,240	2,288	3,694	4,753
Gamma proteobacteria	105,666	8,176	76,384	3,259
Unassigned	41,008	4,127	31,833	-
Total Reads	1,176,864	111,031	177,982	22,812

Figures

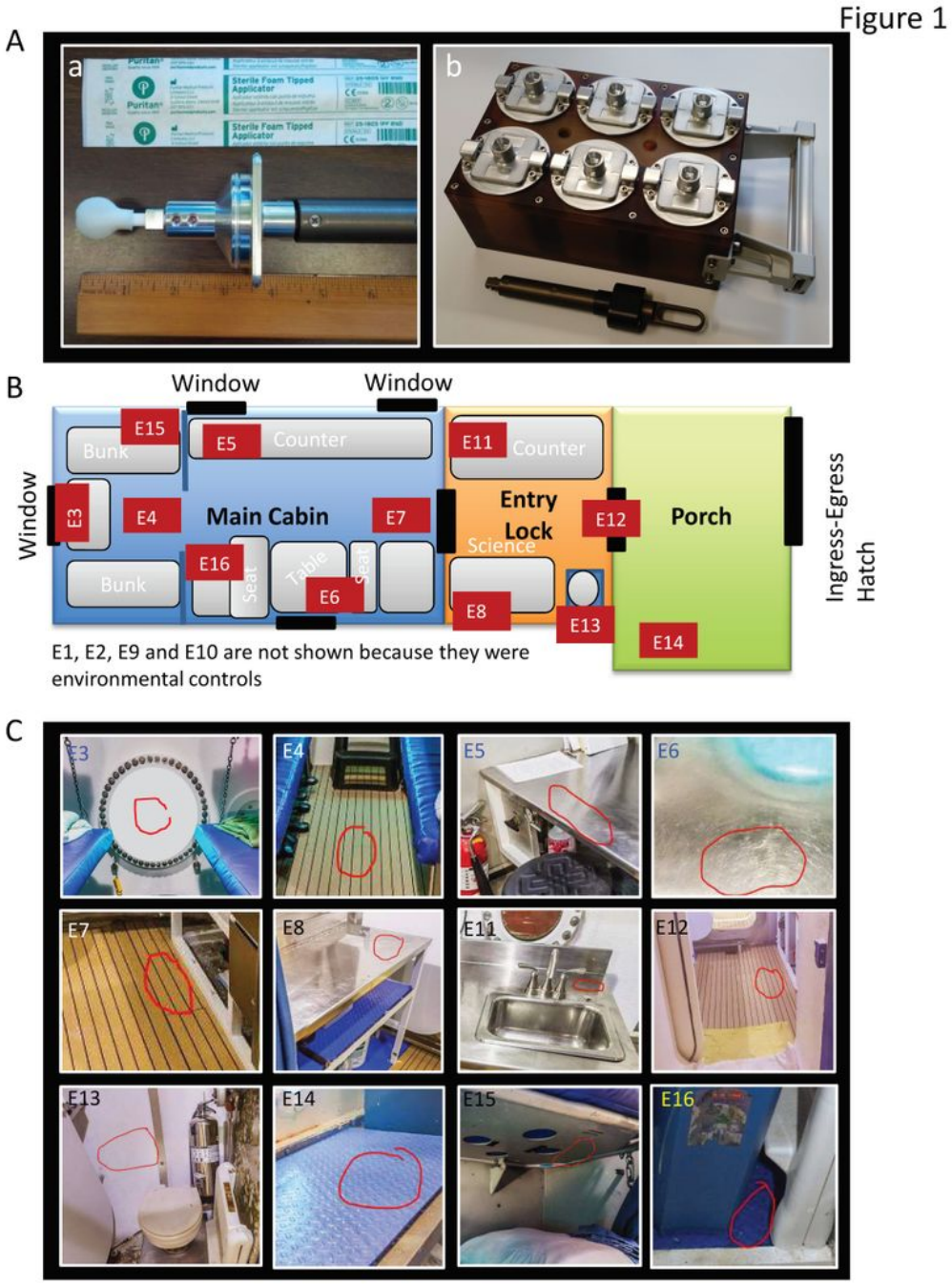

Figure 1

Location and sampling tool kit feature for surface sampling (A) Cantilever swab tool kit storage canister box and b) Swab head attached to the cantilever tool kit. (B) 2D outline of the Analog habitat and sampling location denoted in numbers. (C) Photography of Analog habitat sampling locations and the red circle represents sample-collecting area.

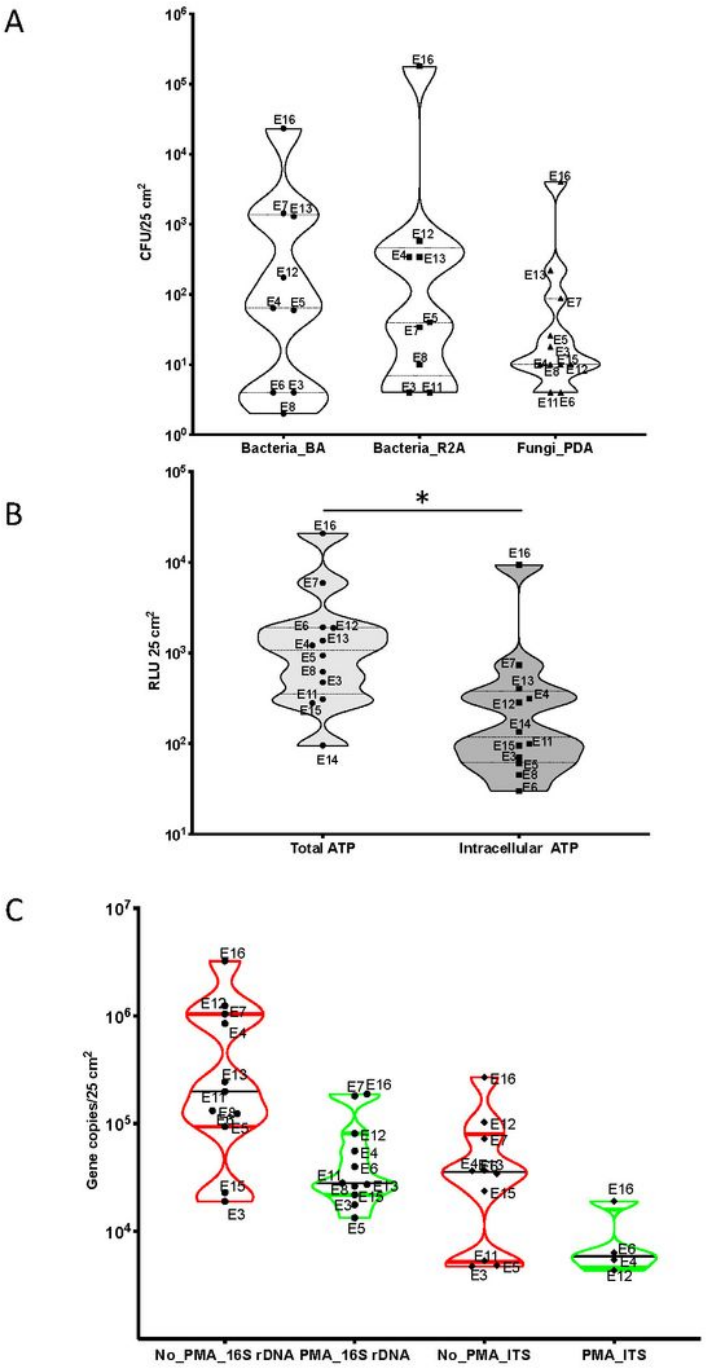

Figure 2

Figure 2

Culture dependent and independent analysis from Analog habitat surface sample (A) Box scatter plot of the abundance of cultivable bacteria and fungi. Each dot in a column represents Analog habitat location sampled. No statistically significant differences in abundances were observed amongst flight missions and between bacteria and fungi (one way- ANOVA, $p>0.01$; ANOVA). (B) Box scatter plot of the relative light unit of ATP counts for total (round dot) and intracellular ATP (square dot). No statistically significant

differences in abundances were observed amongst flight missions and between bacteria and fungi (one way- ANOVA, p>0.01; ANOVA). (C) Box scatter plot shows qPCR (16S rRNA and ITS) gene copies in Analog habitat surface sample. Non-PMA (propidium monoazide) – non-PMA treatment and PMA – PMA treatment

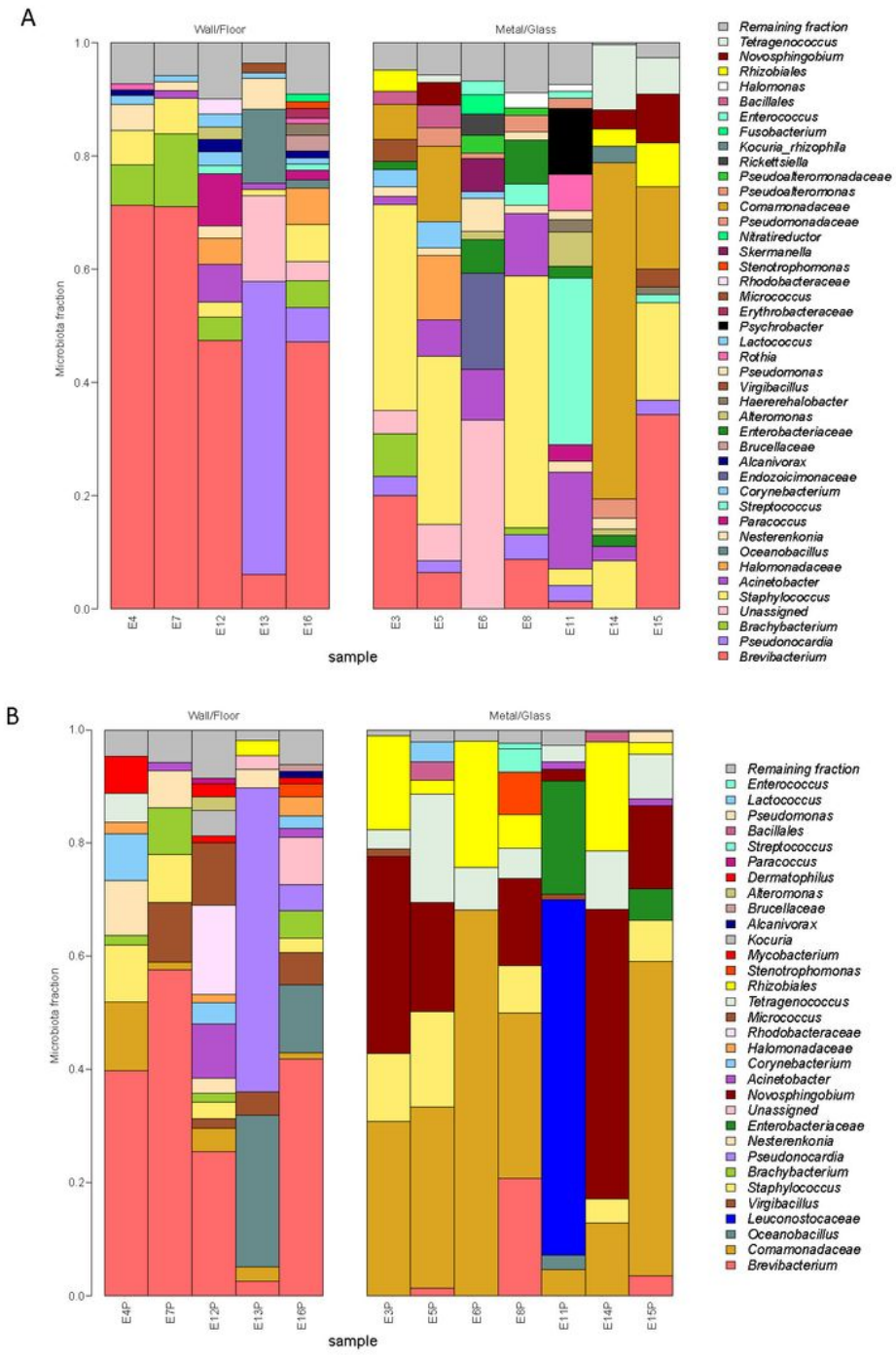

Figure 3

Figure 3

Relative abundaces of bacteria detected by 16S rRNA gene amplicon sequencing The relative abundances of bacterial taxa identified in various samples across the Analog habitat were visualize by bar plots. Each bar represents a specific sample and each colored box a particular taxon. The height of the colored box represents the relative abundance of that particular taxon within the sample. Taxa present in less than 1% abundance in a given sample are displayed in the “remaining fraction” at the top of the graph (gray box). The legend is read from bottom to top, with the bottom taxon in the legend corresponding to the bottom taxon on the graph. Non PMA treated samples are displayed in (A) and the PMA treated samples in (B).

Figure 4

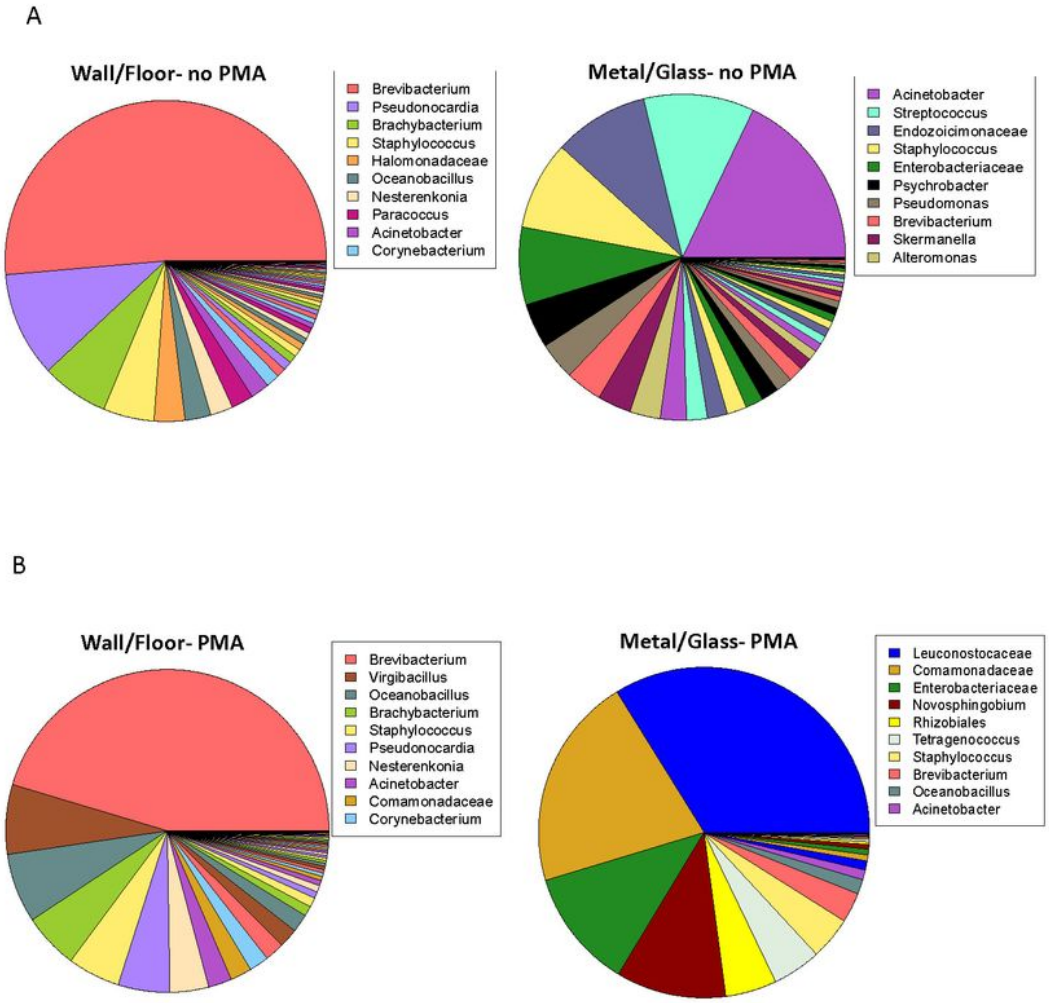

Figure 4

Pie chart of 16S rRNA gene sequencing data Pie chart of the relative abundances of bacteria detected in the Analog habitat. The sequences obtained were summarized to the genus level. In total, 52 taxa were detected but the 10 most abundant ones are just displayed in the legends. The pie graphs are separated based on site categories; wall/floor samples (left panels) and metal/glass (right panels) and treatment group: no PMA (A) and PMA (B).

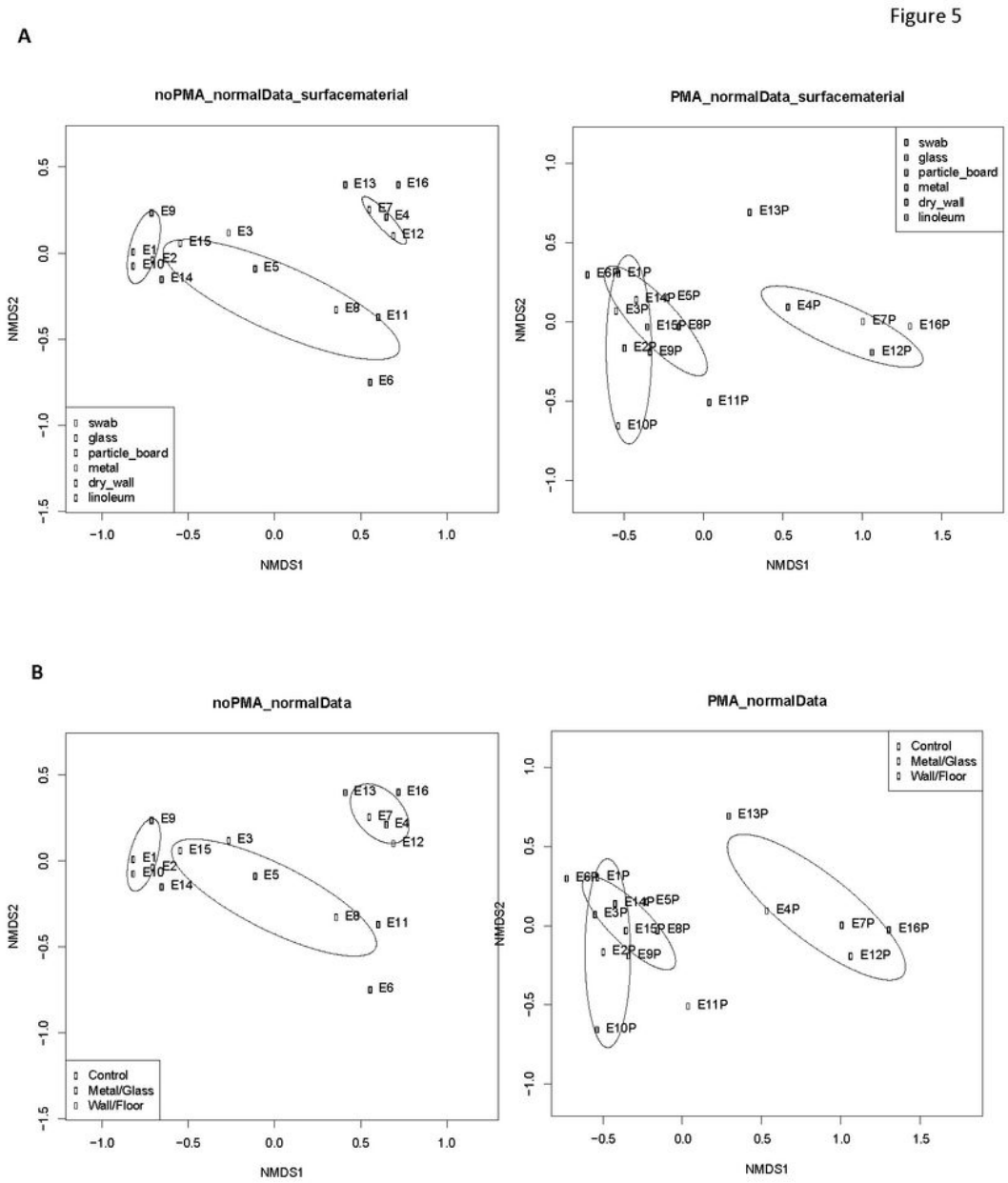

Figure 5

Multi dimensional scaling plots of 16S rRNA gene amplicon sequencing data NMDS ordination showing 99% confidence interval ellipses of non PMA treated (left panels) and PMA treated (right panels) grouped based on surface material (A) and site categories (B). The various samples collected from across the Analog habitat are indicated by a dot and the closer the dots are to each other the more similar their bacterial composition is in terms of types and amount of bacteria.

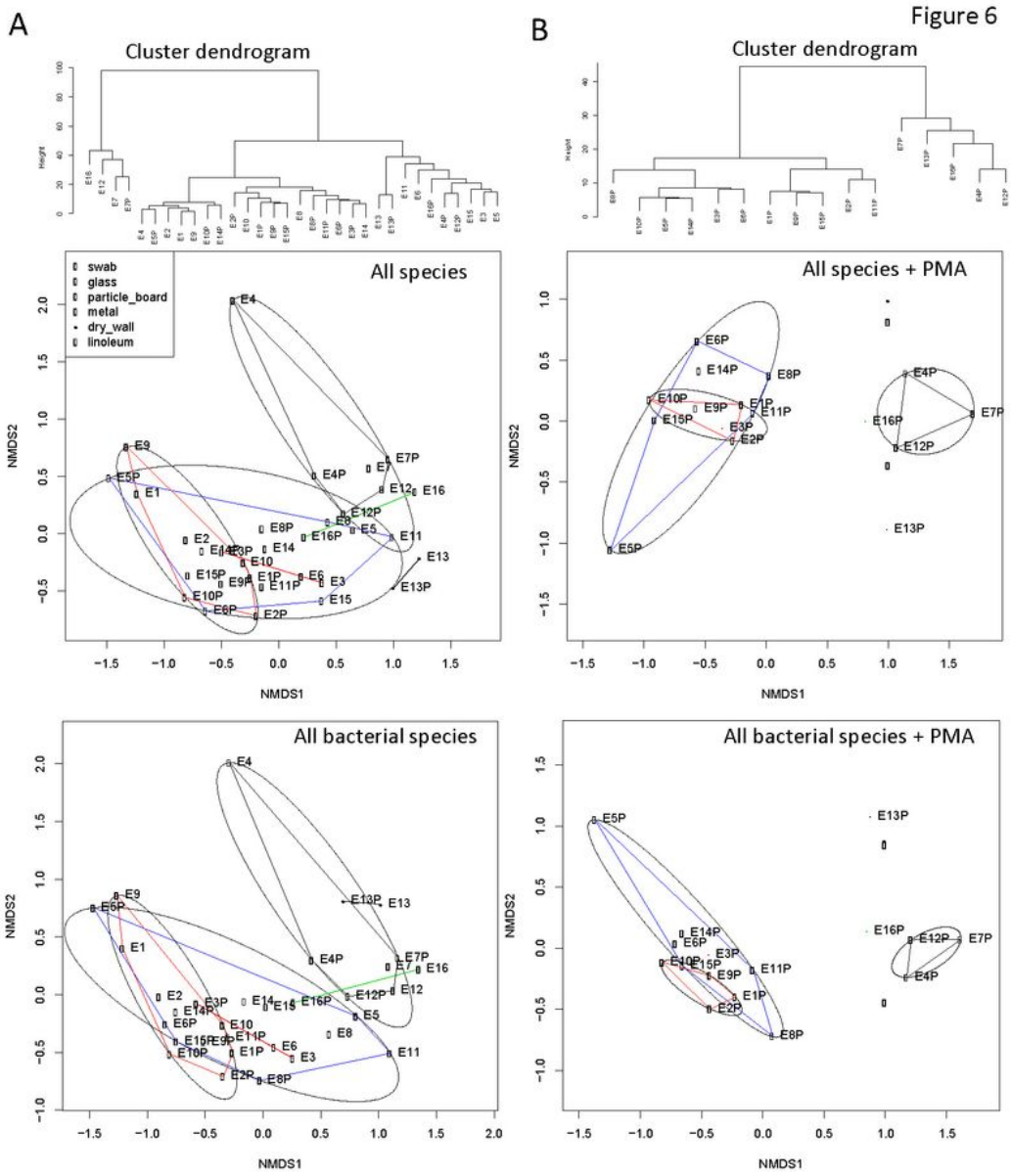

Figure 6

Metagenomic sequencing analysis of bacteria of the Analog habitat (A) Cluster dendrogram of Euclidean distances (Top) was performed on both PMA treated and untreated samples OTUs. NMDS ordination showing the 95% confidence interval ellipse based on (Middle) unifrac distances matrix all species from samples treated from PMA are indicated by “P”. NMDS plot based on (Bottom) unifrac distances matrix of all bacterial OTUs from both PMA treated and untreated samples. The samples collected from various

Analog habitat surfaces was indicated by a color circle such as red – swab; blue – glass; green – particle board; purple – metal; black – dry wall; and yellow – linoleum. and indicated by a color circle such as red – control; blue – metal/glass; green – wall/floor. (B) Cluster dendrogram of Euclidean distances (Top) was performed on both PMA treated and untreated samples OTUs. NMDS ordination showing the 95% confidence interval ellipse based on (middle) unifrac distances matrix of all species from samples treated from PMA are indicated by “P”. NMDS plot based on (Bottom) unifrac distances matrix of all bacterial OTUs from both PMA treated and untreated samples. The samples collected from various Analog habitat surface was indicated by a color circle such as red – swab; blue – glass; green – particle board; purple – metal; black – dry wall; and yellow – linoleum. and indicated by a color circle such as red – control; blue – metal/glass; green – wall/floor.

Figure 7

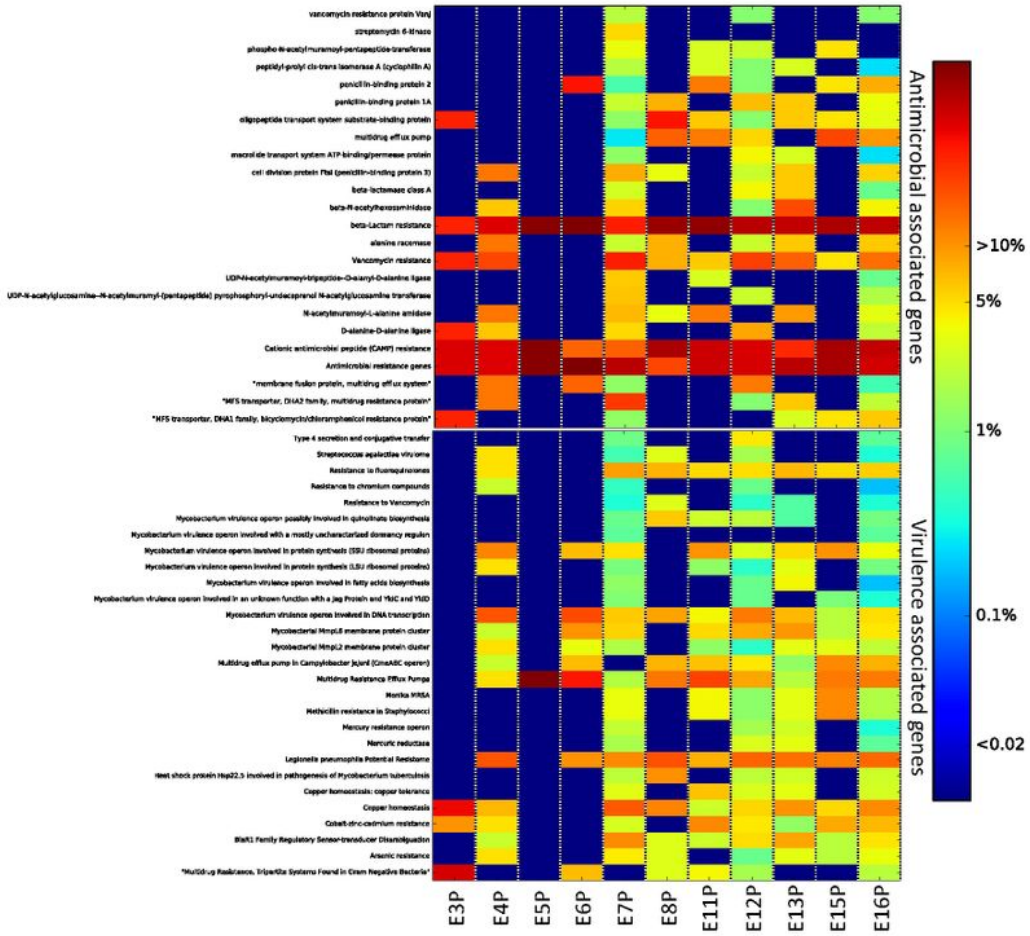

Figure 7

Metagenomic sequencing analysis of bacteria of the Analog habitat Heat map showing the relative abundance of each antimicrobial associated gene (Top) and virulence associated genes (Bottom) detected in each sample collected.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AnalogSupplementalFigures.pptx
- AnalogDatasetS1.xlsx