

SHORT COMMUNICATION

Archaeal diversity analysis of spacecraft assembly clean rooms

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One of the main tasks of NASA's planetary protection program is to prevent the forward contamination of extraterrestrial environments with Earth life, and in turn preserve other planets and the integrity of future life detection missions. Despite information regarding bacterial diversity in NASA's clean rooms, little is known about the presence of Archaea. Archaeal community analysis of spacecraft-associated surfaces is important, as they are considered by some to represent terrestrial life most capable of surviving on Mars. The first insights into the archaeal diversity of clean rooms where spacecraft assembled are attempted. Nucleic acid sequences clustering with uncultivable Archaea within the Eury- and Crenarchaeota were retrieved from 8 of 26 samples collected from several spacecraft assembly clean rooms. Due to their potential capability to survive and proliferate in Martian conditions, screening for Archaea on spacecraft surfaces and instruments that are associated with future life detection missions may be necessary.

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The presence of non-extremophilic Archaea in most biological niches (Olsen, 1994; Bintrim *et al.*, 1997; Schouten *et al.*, 2000) coupled with the varied metabolisms of Archaea as a whole suggests that these microbes play a significant role in Earth's ecology (DeLong *et al.*, 1994). Due to this ubiquity and metabolic diversity, Archaea are considered by some to be capable of surviving, or even thriving, on Mars (Krasnopolsky, 2006). The potential discovery of (ancient) liquid water on Mars (Herkenhoff *et al.*, 2004) and the chemical compositions of the Martian atmosphere (Weiss *et al.*, 2000) and crust (Fisk and Giovannoni, 1999) not only raises the possibility that life may have or may still exist there, but indicates that conditions capable of supporting terrestrial life may also be present. The prevention of contamination of Mars and other extraterrestrial environments with terrestrial biomolecules and/or life (Rummel, 1989) is therefore tremendously important to avoid confounding future life detection

missions and preserve the pristine conditions of other solar bodies.

Current NASA planetary protection protocols for determining microbial burden on spacecraft surfaces were developed using the detection of aerobic spore-forming bacteria (NASA, 2005). The recent isolation of extremotolerant bacteria (La Duc *et al.*, 2007) and the presence of viable but yet to be cultivated bacteria (Moissl *et al.*, 2007) from various spacecraft assembly facility (SAF) clean rooms suggested the need to evaluate other potential microbial communities. Therefore, samples collected from spacecraft-associated clean room facilities at the Jet Propulsion Laboratory (JPL) and Johnson Space Center (JSC) were evaluated for archaeal signatures.

In total, 26 samples were collected (18 from JPL and 8 from JSC; Table 1) and subjected to DNA extraction (Moissl *et al.*, 2007) and Archaea-specific PCR amplification for ~1100bp fragments using appropriate primer sets (methods given in Supplementary Information). Despite strict NASA clean room conditions (NASA-KSC, 1999) and demonstrated low microbial bioburden (La Duc *et al.*, 2007), 6 of 18 JPL-SAF and 2 of 8 JSC-Genesis Curation Laboratory (GCL) samples exhibited positive archaeal 16S rRNA gene amplification (Table 1). Air samples were collected from outside the facility as described elsewhere (Moissl *et al.*, 2007), however the archaeal specific fragments were not obtained. Attempts to cultivate Archaea from clean room samples or to obtain positive archaeal signals

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Table 1 Locations and characteristics of sampling points and samples collected from various spacecraft assembly facilities

| Facility | Location | Sample no. | Description | Area sampled (m ²) | Clean room certification ^a | No. of sampling events | No. of archaea-positive samples | No. of clones analyzed (N) ^b | RFLP patterns | No. of OTU's identified ^c | n1 ^d | Coverage (C) [1-(n1/N)]*100 | |
|--|--------------|------------|---|--------------------------------|---------------------------------------|------------------------|---------------------------------|---|---------------|--------------------------------------|-----------------|-----------------------------|-------|
| Jet Propulsion Laboratory—Spacecraft Assembly Facility | Pasadena, CA | 1 | 3 × 3 tiles, floor in front of the entrance/exit door | 1 | 100 K | 6 | 2 | 95 | 10 | 5 | 0 | 100.0 | |
| | | 2 | 3 × 3 tiles, floor in the middle of the clean room | 1 | 100 K | 6 | 2 | 232 | 13 | 8 | 0 | 100.0 | |
| | | 3 | White cover in the back of the clean room | 1 | 100 K | 6 | 2 | 152 | 4 | 2 | 0 | 100.0 | |
| Johnson Space Center—Genesis Curation Laboratory | Houston, TX | 1 | Lighting fixtures | 0.56 | 10 | 1 | | | | | | | |
| | | 2 | Subfloor | 0.37 | 10 | 1 | | | | | | | |
| | | 3 | Lighting fixtures | 0.56 | 10 | 1 | | | | | | | |
| | | 4 | Subfloor | 0.37 | 10 | 1 | | | | | | | |
| | | 5 | Air duct | 0.37 | 1 K | 1 | | | | | | | |
| | | 6 | Floor | 1 | 1 K | 1 | | | | | | | |
| | | 7 | Threshold; in front of the exit | 0.37 | 1 K | 1 | | 1 | 30 | 1 | 1 | 0 | 100.0 |
| | | 9 | Floor; outside entrance | 0.37 | Uncertified | 1 | | 1 | 33 | 4 | 2 | 0 | 100.0 |

^aCertification is defined by the maximum number of particles of the size >0.5 μm in one cubic foot of air.

^bNumber of archaeal clones picked for RFLP analyses.

^cNumber of operational taxonomic units.

^dNumber of operational taxonomic units occurring once.

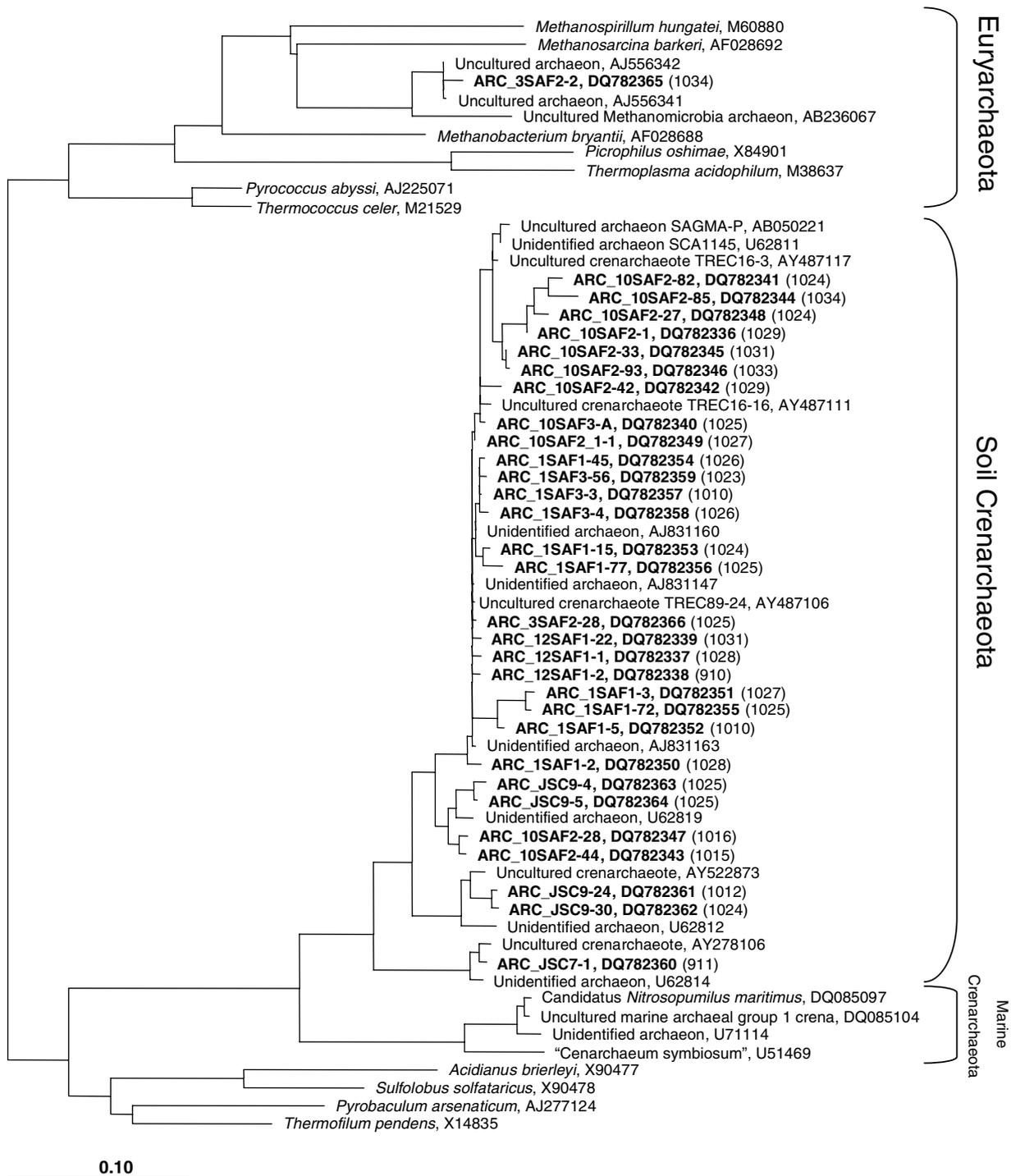


Figure 1 Phylogenetic tree (Maximum Parsimony), showing the archaeal diversity and the phylogenetic affiliation of the clone sequences derived from different sampling locations. The GenBank accession numbers of the clones retrieved from this study (prefix DQ) and reference strains are given. The facility ID are SAF, JPL-SAF; JSC, JSC-GCL. The clones are identified by the facility ID (SAF or JSC) preceded by the month of collection (SAF only) and followed by the location and representative clone number (i.e., 3SAF2-2 represents clone 2 from location 2 collected from the JPL-SAF during March). The numbers in brackets give the sequence length. The scale bar shows a 10% estimated difference in nucleotide sequence positions.

using fluorescent *in situ* hybridization were unsuccessful. Clones selected for sequencing, and the RFLP patterns and operational taxonomic units (OTU) obtained are given in Table 1. Rarefaction analyses indicated the coverage value to be 100%

since none of the OTU's retrieved from any of the tested samples appeared only once.

The results suggested that both facilities contained a wide diversity of Archaea (Figure 1). Among 542 clones analyzed, 31 different archaeal

sequences were obtained: 26 from the JPL-SAF (479 clones) and 5 from JSC-GCL (63 clones) locations. In general, individual archaeal sequences were unique to a given facility or location. The majority of sequences were affiliated with an uncultivable soil Crenarchaeota group (Figure 1). Clones showed sequence differences up to 7.8% with the nearest identified archaeal species, and clustered with sequences obtained from deep South African gold mine waters (Takai *et al.*, 2001), soil (Bintrim *et al.*, 1997), rhizospheres (Simon *et al.*, 2005), permafrost soils (Ochsenreiter *et al.*, 2003) and the leachate of landfill (Laloui-Carpentier *et al.*, 2006). Clones obtained were not closely related to cultivable Archaea; the 16S rRNA gene sequence of the nearest cultured neighbor, the autotrophic ammonia-oxidizing crenarchaeon candidate *Nitrosopumilus maritimus* (Konneke *et al.*, 2005), was up to 16% different.

Additionally, a single clone sequence (ARC_3-SAF2-2, Figure 1) from JPL-SAF was affiliated with methanogens in the Euryarchaeal phylum. The sequence clustered with 16S rRNA gene sequences from uncultivable Archaea present in temperate anoxic soils (Wu *et al.*, 2006) showed at least 18% sequence difference to the closest cultivable neighbor. Although methanogens have generally been regarded as strict anaerobes, it was demonstrated that some methanogens were able to withstand long exposure to high levels of oxygen (Kato *et al.*, 1997) and therefore the aerobic conditions of the clean room may not necessarily be lethal.

The absence of archaeal signatures from two-thirds of the collected samples (and all of the air samples) demonstrated the relative sterility of these clean rooms. A lack of similar sequences between the certified and uncertified rooms (JSC7 and JSC9, respectively) further illustrated the effectiveness of the quality control protocols enforced to maintain these facilities (NASA-KSC, 1999).

All retrieved sequences belonged to the Crenarchaeota and Euryarchaeota phyla. This may be due in part to the primer selection as no truly universal Archaeal primer sets have been established and it was unlikely that any Nanoarchaeota sequences would have been amplified given the primers utilized.

All of the archaeal sequences retrieved during this study belonged to clusters of uncultivable Archaea from cold or moderate environments and their structural properties and physiological capabilities remain unknown. It was unclear whether the detected Archaea were alive at the time of sampling, would be capable of growth in extraterrestrial environments, or able to withstand space travel, but given the ubiquitous nature of Archaea it would be naive to completely dismiss any of these possibilities. The detection of Archaea in these controlled sanitized environments further emphasized the potential of these microbes to exist in the most extreme biotopes. Future studies utilizing quantitative detec-

tion methods as well as the verification of archaeal viability could lead to better characterization and enable the eradication of these microbes.

To date, as per international treaty, it is required to estimate number of spores on spacecraft surfaces using conventional culture-based method (NASA, 2005). While this may be a valid proxy for overall spacecraft cleanliness, this study has clearly demonstrated the presence of Archaea on different surfaces of these spacecraft-associated clean rooms. As Archaea may be considered terrestrial organisms capable of living or surviving on Mars and other extraterrestrial environments, the screening for these microbes on spacecraft surfaces and instruments associated with future life detection missions may be necessary to maintain mission integrity and prevent forward contamination.

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