

Assessment of Nucleic Acid-based Technologies for Detecting and Enumerating Bacterial Spores Embedded Within Spacecraft Materials

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Abstract

Problem: The current assessment of bioburden embedded in solid nonmetallic spacecraft materials is based on work performed in the Viking (1970s) era, and on the ability to culture organisms extracted from such materials.

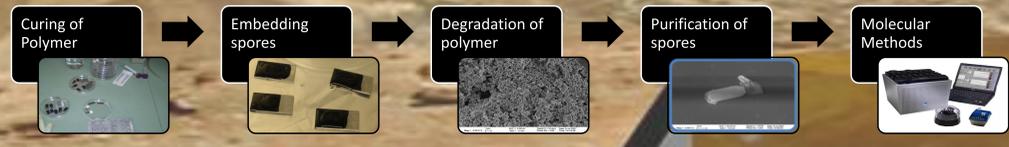
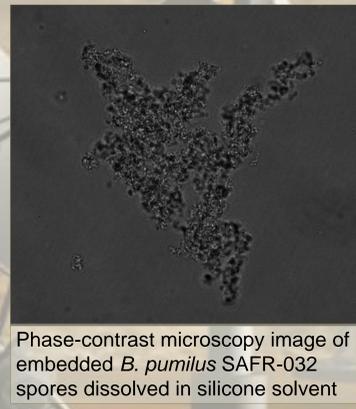
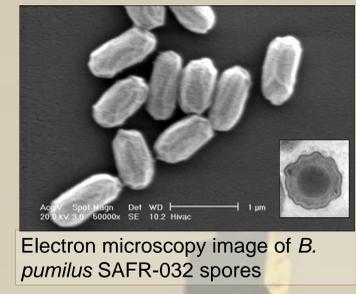
Solution: To circumvent the limitations of culture-based approaches, DNA-based techniques were evaluated alongside culturing techniques in an attempt to determine the occurrence and survival of bacterial endospores embedded in spacecraft-qualified polymer material.

Approach:
Spore embedding: *Bacillus pumilus* SAFR-032 spores were inoculated ($10^2 - 10^7$ spores/mL) onto the surface of the silicone and dried. Uncured silicone was subsequently layered over the dried spores and cured overnight, resulting in the entrapment of spores within the silicone.
Estimation and enumeration of spores:

- The silicone polymer was dissolved in a solvent for the recovery of embedded spores
- Direct plating, MPN assays, and microscopic examinations
- Genomic DNA was isolated and real-time PCR (qPCR) performed.
- Primers targeting the *gyrB* gene sequence specific to *B. pumilus* were used for quantification.

Results:
 Solvent used in dissolving the silicone inhibited germination of *B. pumilus* SAFR-032 spores but the spores used were resistant to the solvent and embedded spores could be recovered with no significant decrease in viability. Detection sensitivities of approximately 10^2 spores/mL were obtained using qPCR. Excellent correlation was obtained between PCR and traditional enumeration methods.

Inference:
 Rapid molecular methods such as PCR based detection and enumeration show promise for use in determining the overall surface and embedded bioburden (cultivable and non-cultivable) associated with spacecraft hardware.



Methods

Strain and sporulation
Bacillus pumilus SAFR-032 was sporulated and harvested according to Nicholson and Setlow (1991).

DNA Isolation

- Spores were decontaminated using the method of Setlow and Setlow (1993) (8 M urea, 150 mM β -mercaptoethanol, 1% SDS, 50 mM Tris, 1 mM EDTA, pH 8)
- Spores were incubated at 60 °C for 60 min
- Spores were re-suspended in 1 mL of 1x PBS with 25% sucrose with 50 μ L of a 100mg/ mL solution of lysozyme
- Spores were incubated at 32 °C for 60 min.
- DNA was isolated using the MoBio Ultra-Clean Microbial DNA isolation kit following manufacturer's instructions (MoBio Laboratories Inc., Carlsbad, CA).

qPCR

- All quantitative PCR reactions were performed using a Cepheid Smart Cycler system (Cepheid; Sunnyvale, CA).
- B. pumilus* specific primers targeting the *gyrB* gene (Moissl et al., 2007).
 - Forward (5' - TGA AGA TGT GCG AGA AGG CT - 3')
 - Reverse primers (5' - AGG ATC TTC CCT CTT AAC GG - 3')
- Amplification was done with the following cycle parameters:
 - 95 °C for 120 sec (1 cycle) followed by
 - 40 cycles of 95 °C for 15 sec, 58 °C for 30 sec and 72 °C for 30 sec.
- The limit of detection of the assay was determined by constructing a standard curve using C_T values obtained from serially diluted DNA initially isolated from the spore crop at 10^8 spores/mL and from serially diluting the spore crop from $10^9 - 10^1$ spores/mL.

Water Vapor Transmission Rate of Silicone
 A 1.0 mm thick silicone layer was sent to Mocon Inc. (Minneapolis, MN) for water vapor transmission rate testing. The silicone was tested using a Mocon Permatran W 3/33 (ASTM F-1249) with 100% relative humidity, 100% nitrogen at ambient pressure and a temperature range of 23 – 170 °C.

Results

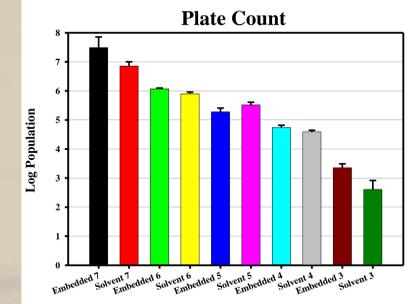
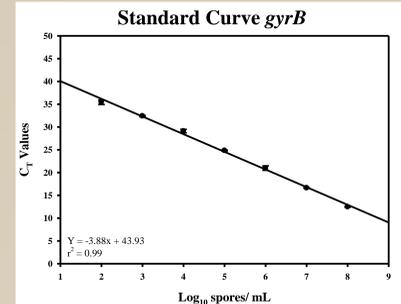
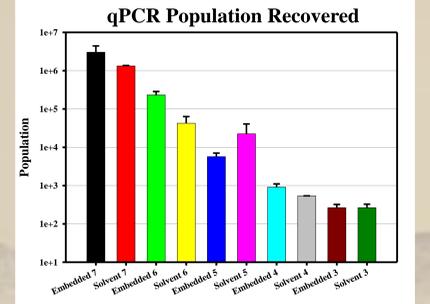


Plate counts results of the recovery of different concentrations of *B. pumilus* SAFR-032 embedded in silicone and recovered using silicone solvent and control spores suspended in silicone solvent without embedding. Recovery of spores from embedding and suspension in silicone solvent resulted in no statistically significant differences. Error bars represent standard error mean of 3 replicates.



Standard curve of diluted *B. pumilus* SAFR-032 spore DNA. Isolation and detecting, using real-time PCR, of *B. pumilus* spores at levels from 10^8 to 10^2 spores/mL. Error bars represent standard error mean of 3 replicates.



qPCR results of the recovery of different concentrations of *B. pumilus* SAFR-032 embedded in silicone and recovered using silicone solvent and control spores suspended in silicone solvent without embedding. Recovery of spores from embedding and suspension in silicone solvent resulted in no statistically significant differences. Error bars represent standard error mean of 3 replicates.

Sample ID	Temperature (°C)	Permeate	Carrier Gas	WVTR (grams/m ² -day)	
				Rep. 1	Rep. 2
Silicone	23	100% RH Moisture	100% Nitrogen, dry	32.3	30.5
	100	100% RH Moisture	100% Nitrogen, dry	959	917
	125	100% RH Moisture	100% Nitrogen, dry	1723	1584
	170	100% RH Moisture	100% Nitrogen, dry	2535	2910

Water Vapor Transmission Rate of silicone. Silicone does not result in true encapsulation of the spores. Instrument: MOCON Permatran W 3/33 (ASTM F-1249).

Conclusions

Forward contamination of Mars or other terrestrial environments by spacecraft remains a concern in future missions searching for extraterrestrial life. Current assessment of bioburden embedded in solid materials is based on work performed in the Viking era. The purpose of this research is to compare current molecular methodologies (i.e. real-time PCR) with traditional culturing to determine the occurrence and survival of bacterial endospores that have become embedded into solid spacecraft materials. Assessing the ability for microorganisms to survive embedding in solid matrices will support improved methods for detecting and quantitating the overall microbial bioburden on and in spacecraft, to prevent forward contamination of extraterrestrial environments. Currently, we have shown that:

- Quantitative nucleic acid amplification resulted in low level detection of spores embedded in silicone
- Excellent correlation was obtained between PCR and traditional enumeration methods
- PCR based detection and enumeration will be beneficial in determining the overall bioburden of spacecraft and for detecting and enumerating hard to culture organisms.
- Silicone resulted in embedding of the spores but not complete encapsulation as determined by water vapor transmission rate
- In future studies, we plan to validate other relevant spacecraft materials.

References

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