Microbial Methods for Testing a Novel Spacecraft Bioburden Reduction Method

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Introduction & Background

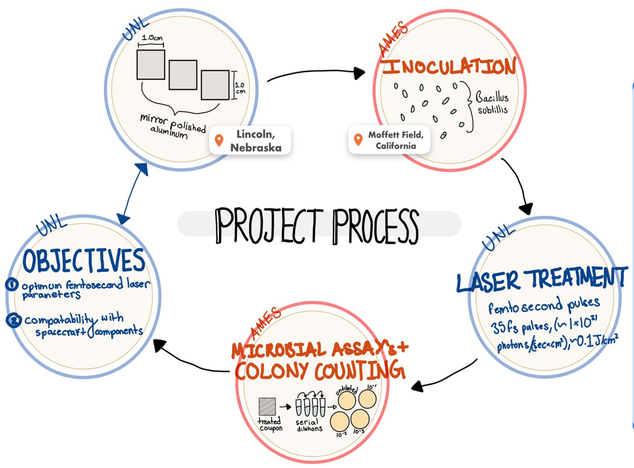
To prevent forward contamination of other planetary bodies. Planetary protection policies often require that spacecraft hardware be sterilized.

Current sterilization practices are:
- time-consuming
- expensive
- incompatible with a variety of sensitive hardware materials (i.e. dry heat sterilization)

Our team is developing a novel laser-based sterilization method and we test it on metal coupons seeded with *Bacillus subtilis* spores.

Our challenge is in developing consistent and high recovery. What is the optimal microbiology assay to quantify the effectiveness of this sterilization technique?
We used the NASA Handbook for the Microbial Examination of Space Hardware (NASA-HDBK-0622) as a starting point.

Our job at Ames:
- How do we get spores on and off coupons?
- How good is our recovery from our spore assays?
- How do we measure the difference in viable spores?



Bacillus subtillis is a spore forming model organism with a moderate stress tolerance. This species has been selected not only for its spore properties but also its ability to be used in comparison to prior Planetary Protection research.

Our standard samples are composed of 1cm² mirror-polished aluminum coupons inoculated with 2.2E5 CFU/ 10uL.

Spore Recovery Method Development

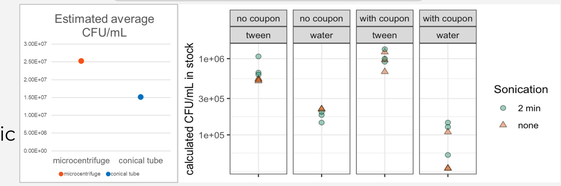
As the first step, coupons seeded with spores are placed in a tube with rinse solution.

Rinse Solutions Tested:
- 0.02% Tween-80
- Millipore water

Tween-80 is a widely used non-ionic surfactant, used in the NASA Handbook.

Rinse Tube Types Tested:
- microcentrifuge
- glass culture tube
- 15-mL polypropylene conical tube

Rinse Solution & Tube Type

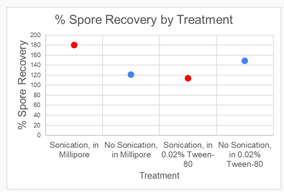


The results are consistent with results found in literature. Not only does Tween-80 help release spores from the metal coupon surface, it also prevents spore adhesion to the to the interior rinse tube walls. (Da Silva et al. 2011). There is stronger interaction between Tween-80 and propylene than between spores and propylene.

Results:
- Regardless if spores are deposited on coupons or directly into the rinse solution, Tween-80 results in a higher spore yield.
- Microcentrifuge tubes had a two-fold higher yield than 15mL conical tubes.
- Glass: No significant difference compared to microcentrifuge tube (p>0.05)

Sonication

Sonication is the use of sound waves are used to agitate particles in solutions. The NASA Handbook uses sonication to dislodge spores from hardware. Is it necessary?



Method: Rinse tubes were sonicated for 5 minutes at 40 kHz

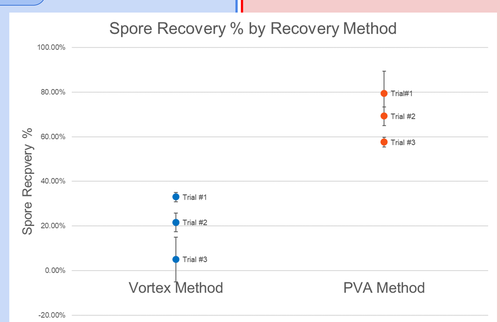
Results: Sonication does not make a significant difference in spore recovery (student's t-Test (p>0.05).

Vortex Method

Vortexing coupons in rinse as a recovery method.

Method: Vortex coupons in 0.02% Tween-80, dilute, and plate.

Results: This method had, high variability and a low average recovery yield of 19.90% (calculated from 9 replicates).



PVA Method

Tested **polyvinyl alcohol (PVA)** peels to strip spores off the coupon as a recovery method.

Method: 100uL of 10% (w/v) PVA is applied to coupons, dried, and peeked. This process is repeated twice to maximise spore recovery. The peels are then rinsed in 0.02% Tween-20, diluted, and then plated,.

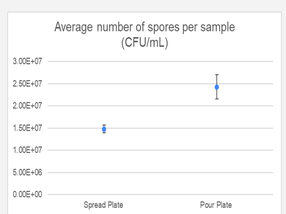
Results: The PVA method yielded a mean recovery of 68.73% percent (calculated from 9 replicates).

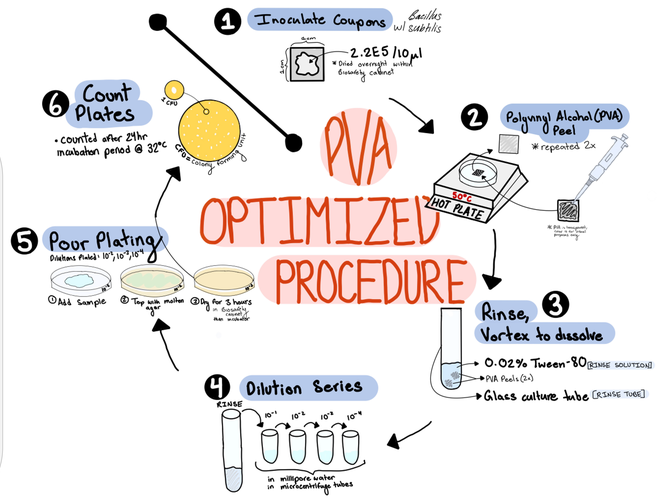
Plating Method

We tested the effect of pour plating vs. spread plating on spore recovery yield.

Method: Serial dilutions of the *B. subtilis* stock were plated.
- Spread plating: spreading the dilution on pre-prepared agar plates.
- Pour plating: swirling the dilution with molten agar and allowing to dry for three hours.

Results: Pour plating yielded more colonies (p=0.06 by Student's T-test)





Calculations

% Spore Recovery is the number of colonies recovered from coupon samples relative to cfu/mL from non-coupon samples.

Calculate CFU/mL
(1) sum the counted cfu per set of plate dilutions
(2) sums the the spore-to-coupon dilution ratios*
(3) divides the sum of the # of colonies by the sum of the spore-to-coupon ratio. This gives us the total # of spores on the orginial coupon (the value for 100% of spores).

*Total Dilution Factor Calculation: The dilution factor value is calculated by dividing the expected CFU/mL in the inoculum dilution by the expected CFU/mL in the stock dilution. This is meant to find what percentage of that dilution makes up what was originally in the stock dilution.

Conclusions & Future Directions

We have adjusted our procedure as a result of the conclusions of these experiments.
Modifications:
- Use PVA as our spore recovery method
- Use glass cultures tubes instead of 15 mL conical tubes
- Continue to use Tween-80 solution to rinse
- Continue to use pour plating

Future Directions:
process laser-treated project samples with the optimised procedure

References

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