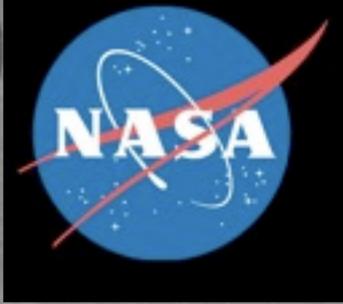


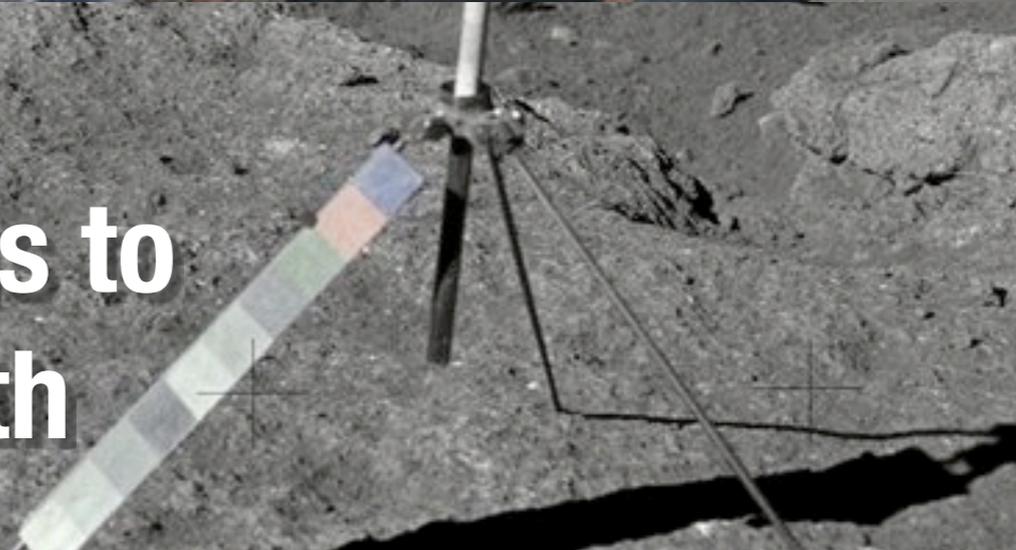
# NASA AMES ACADEMY 2009

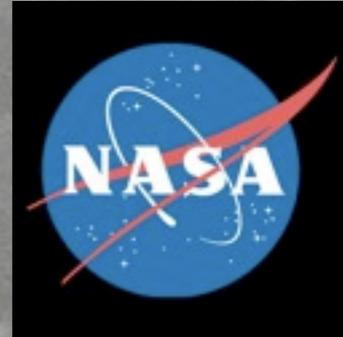
Jake Gamsky and Heather Duckworth  
NASA Lunar Science Forum 2010



## CLUSTER:

Creative Lunar Utilization Systems to  
Transform Extraterrestrial Regolith





# ISRU: IN-SITU RESOURCE UTILIZATION



# GOAL AND HYPOTHESIS



**Goal: To compare the growth of plants in microbial altered lunar regolith simulant (JSC-1A) and determine whether it has created a habitable soil environment.**

**Hypothesis: Adding microbes to lunar regolith simulant will aid in the growth of plants by freeing up the necessary elements.**

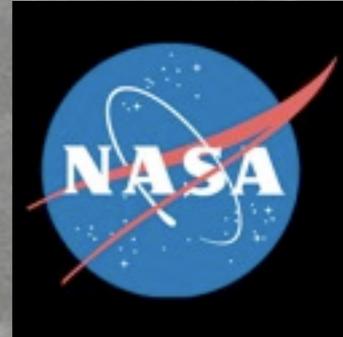
# LUNAR REGOLITH SIMULANT



**Table C.1.** Chemical compositions of JSC-1 and lunar soil 14163.

Oxide	JSC-1 (ash) (wt%)	Lunar soil 14163 (wt%)
SiO <sub>2</sub>	48.77	47.3
TiO <sub>2</sub>	1.49	1.6
Al <sub>2</sub> O <sub>3</sub>	15.65	17.8
Fe <sub>2</sub> O <sub>3</sub>	1.71	0.0
FeO	8.88	10.5
MgO	8.48	9.6
CaO	10.44	11.4
Na <sub>2</sub> O	2.93	0.7
K <sub>2</sub> O	0.81	0.6
MnO	0.19	0.1
Cr <sub>2</sub> O <sub>3</sub>	—	0.2
P <sub>2</sub> O <sub>5</sub>	0.66	—
Total	100.01	99.8

Note the complete absence of Fe<sub>2</sub>O<sub>3</sub> in the lunar soil, whereas JSC-1 contains a percentage of this compound.



# PLANT GROWTH:

MODIFYING REGOLITH  
FOR PLANT CULTIVATION



# PAENIBACILLUS POLYMYXA 9a



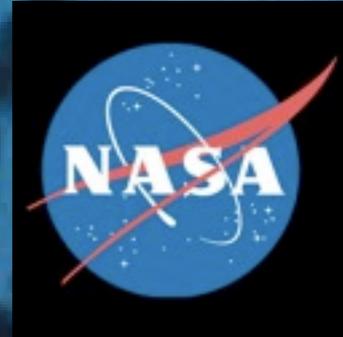
- A siliceous bacteria
- A root-colonizing bacteria that increases plant strength, growth, and yield
- Promotes rhizobacteria = A heterogeneous group of bacteria shown to increase the health or longevity of plants
- Similar microbe: *Paenibacillus* sp. IMBG 156 was shown to free up elements such as iron, silicon, and potassium when inoculated (Kozyrovska et al. 2006)

# INOCULATION

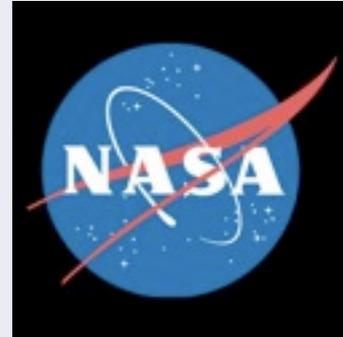


- **Inoculation was shown to make plants more resistant to drought conditions or abiotic stress**
- **Those growing in inoculated soil developed more quickly and flowered earlier than their counterparts**

# NOSTOC



- A genus of cyanobacteria that grows in fresh water and terrestrial environments
- Can survive in a desiccated state for years and recover after re-hydration
- Fixes atmospheric nitrogen in the presence of oxygen
- In the nitrogen-poor lunar regolith, *Nostoc* may provide the *Arabidopsis thaliana* with the nitrogen needed to grow and *P. polymyxa* with the ability to multiply



# ARABIDOPSIS THALIANA

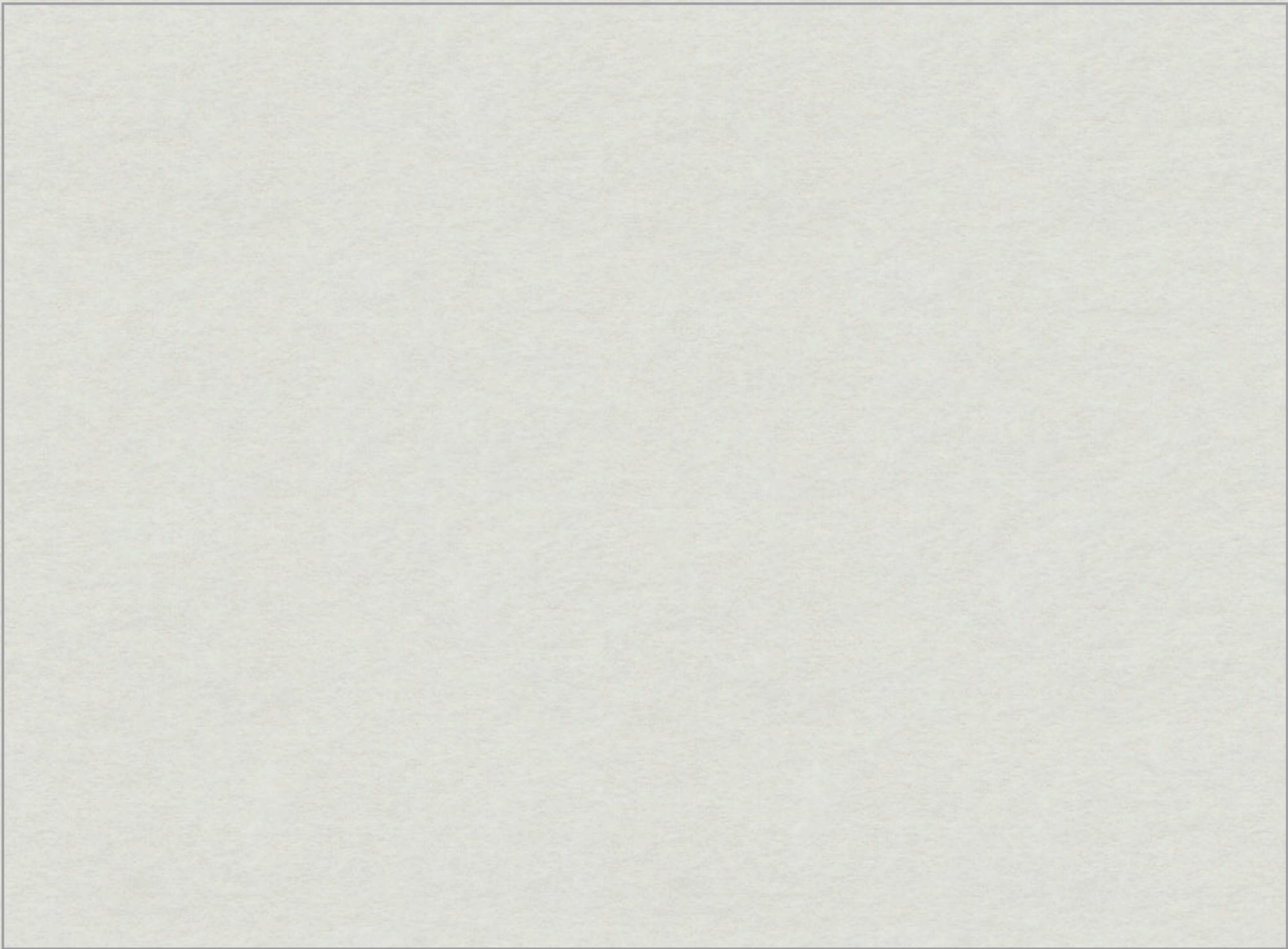
- **Ideal Test Specimen**
- **Rapid life cycle**
  - **Leaves above surface of the soil in 14 days**
  - **Full life cycle in 6 weeks**
- **Small size**
- **Small genome fully characterized**
- **Easily modified**
- **Our strain has been modified with a transgene to signal adverse environmental conditions**

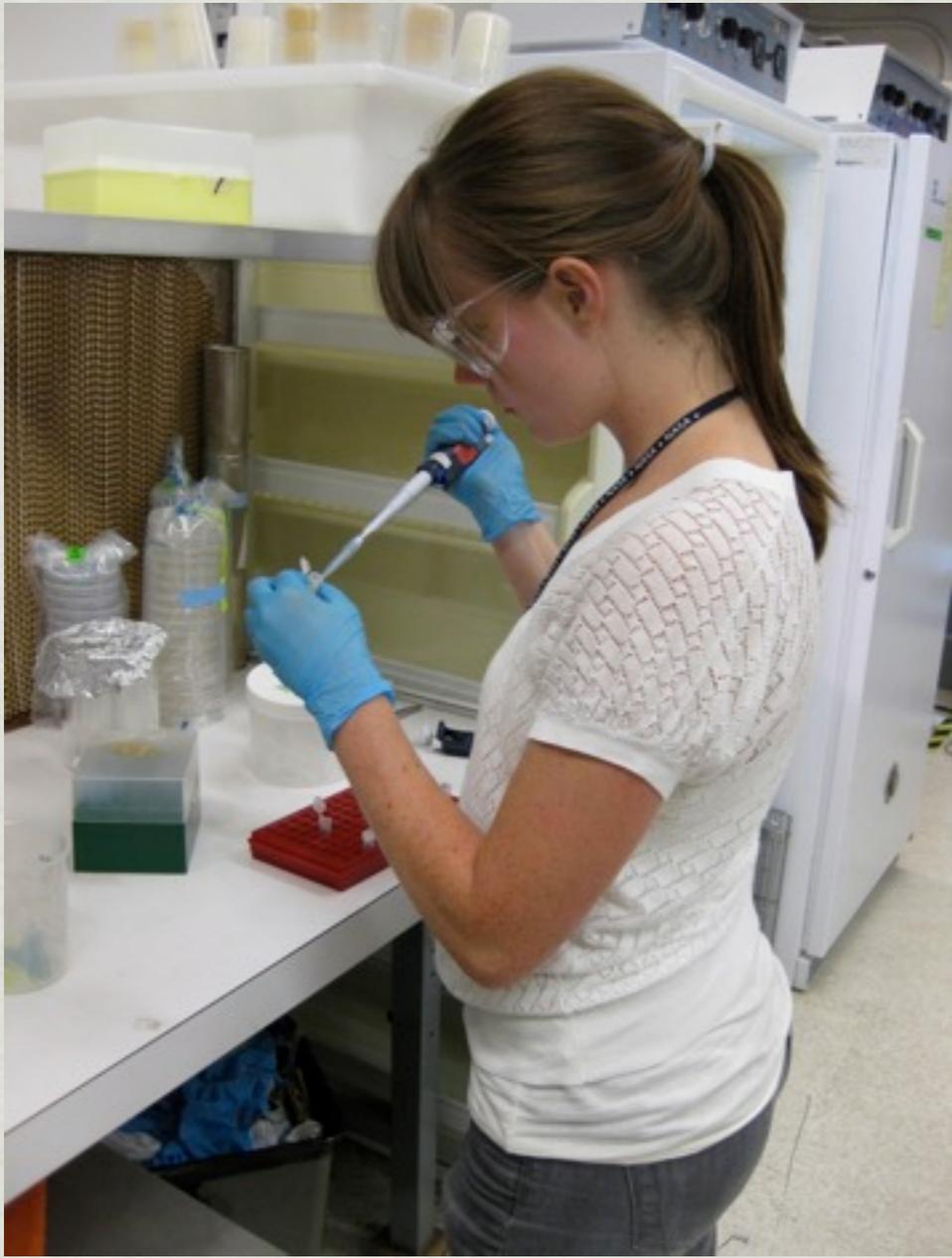
# EXPERIMENTAL SET-UP:



Seeds:	Y	Y	N
Soil:	N	Y	Y
Control (0 microbes)	Growth Media DI water	Growth Media Growth Media	DI water Growth Media
<i>Paenibacillus polymyxa</i> (1 microbe)	<i>P. polymyxa</i> DI water	<i>P. polymyxa</i> <i>P. polymyxa</i>	DI water <i>P. polymyxa</i>
<i>Paenibacillus polymyxa</i> & <i>Nostock</i> (2 microbes)	<i>P. polymyxa</i> DI water	<i>P. polymyxa</i> <i>P. polymyxa</i> & <i>Nostock</i>	DI water <i>P. polymyxa</i> & <i>Nostock</i>

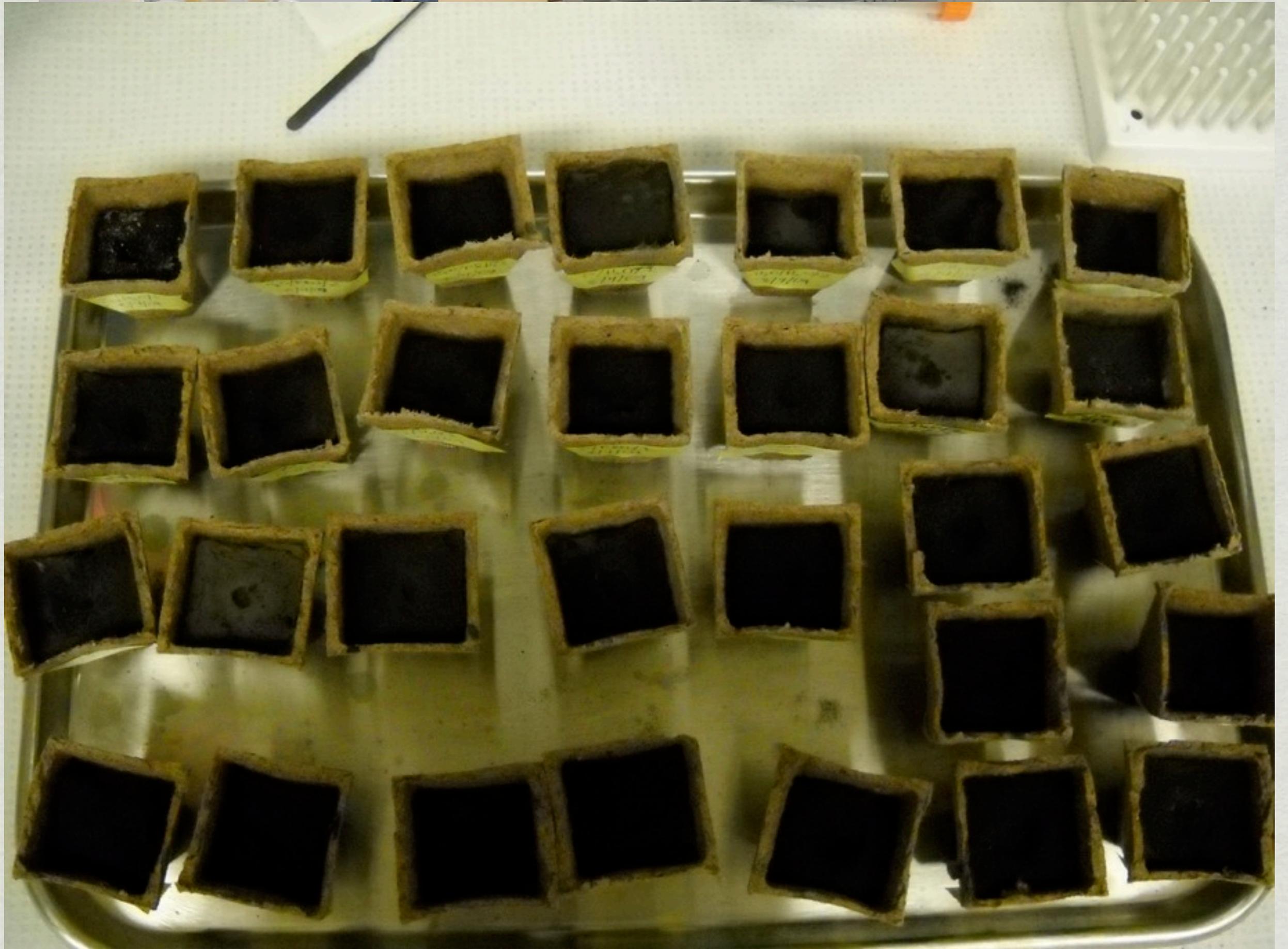
Seeds:	N
Soil:	N
Control (0 microbes)	DI water DI water







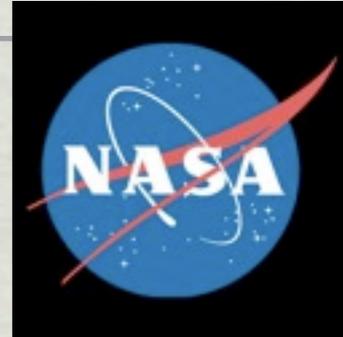






# OBSERVATIONS

- **Two forms of growth not expected:**
  - **White, fibrous growth on three samples**
  - **White flecks on at least 2 samples**
- **Changes in top layer of regolith:**
  - **Lighter, cake-like texture**
  - **Occured SOLELY in experiments NOT inoculated with microbes**



**CAKE-LIKE LAYER**



**WHITE FLAKES**



20 JULY 2010

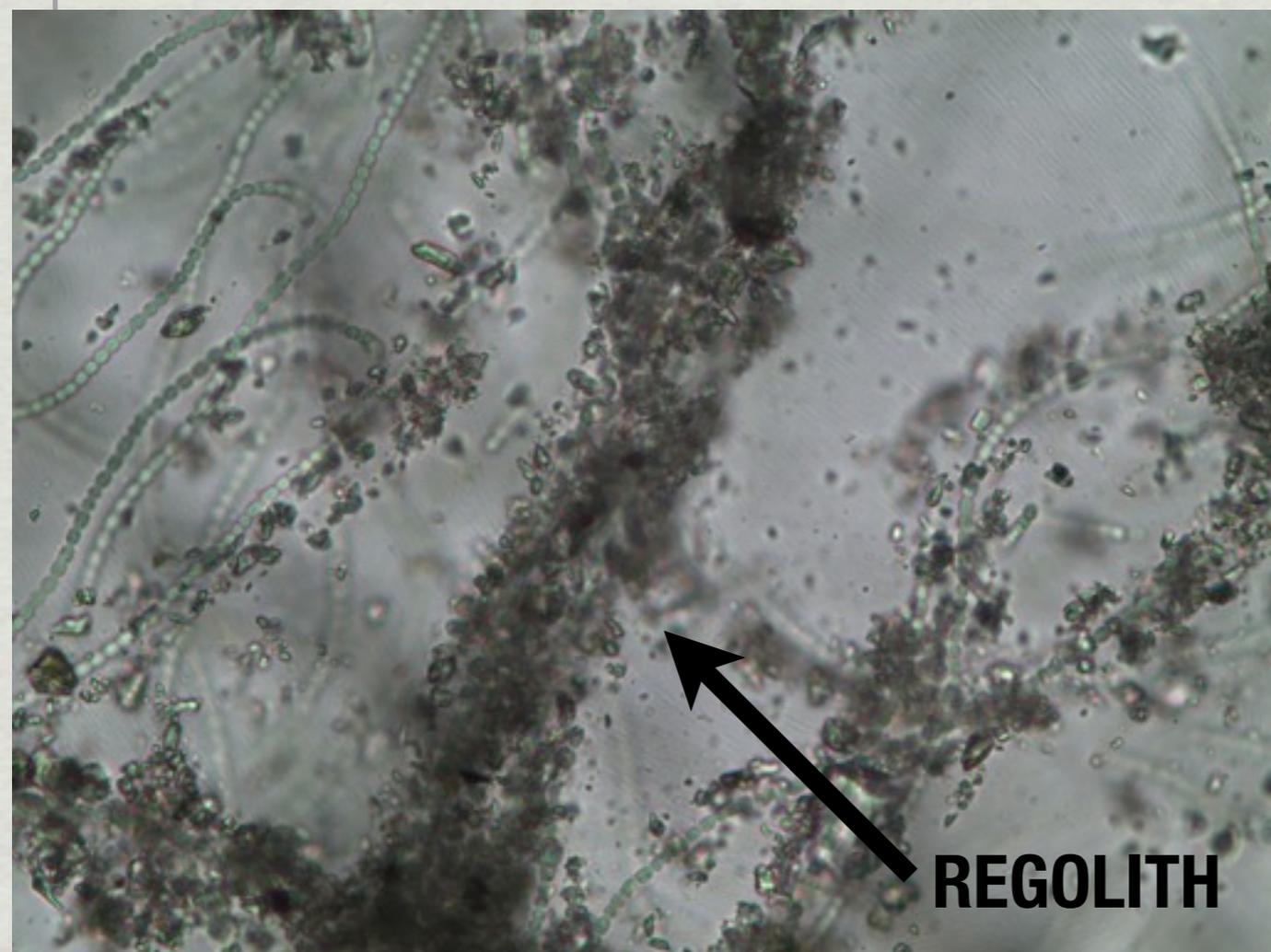
NASA LUNAR SCIENCE FORUM



# EVIDENCE OF GROWTH

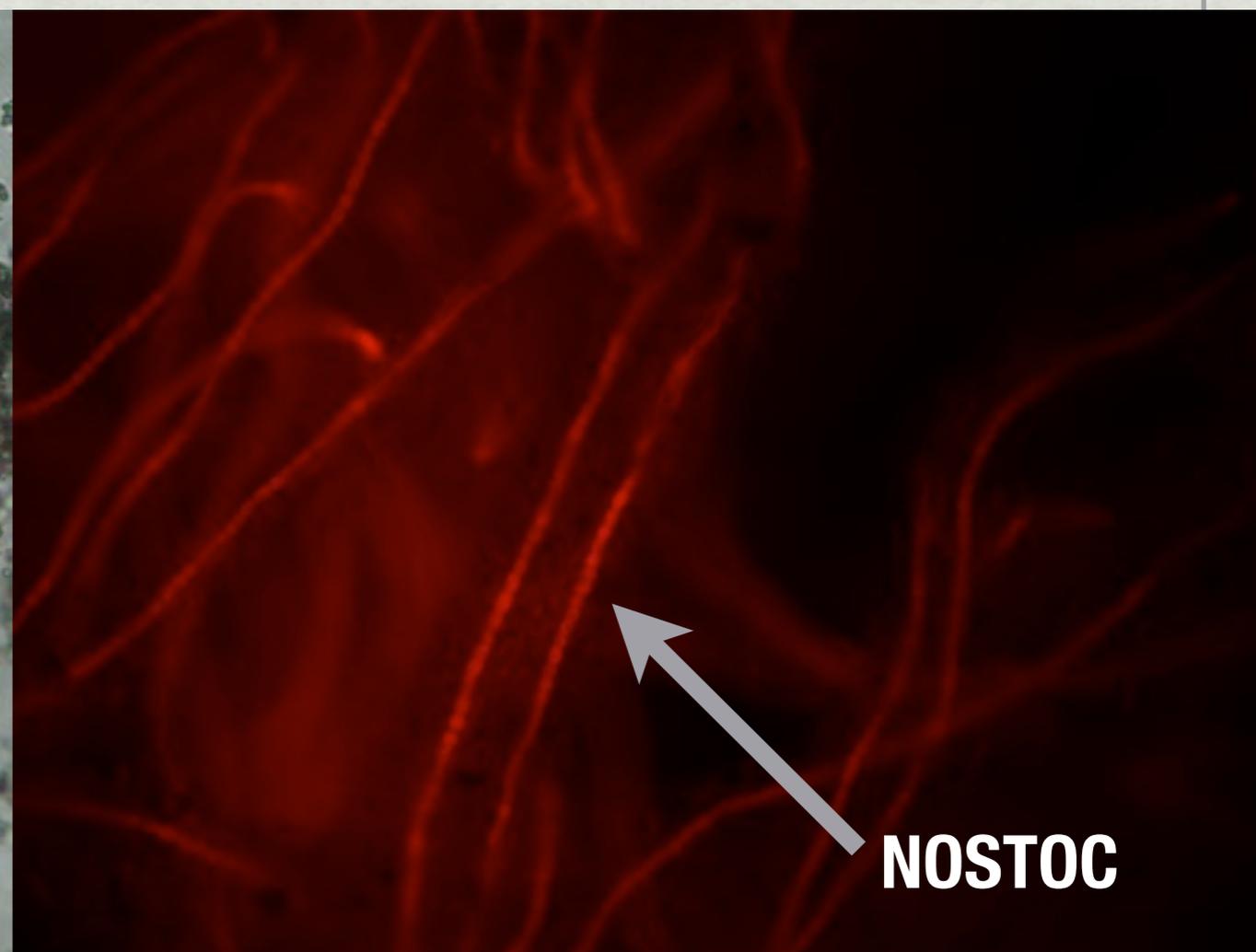
Trial to determine optimal slurry composition

Added microbes and incubated, covered, for 3 weeks



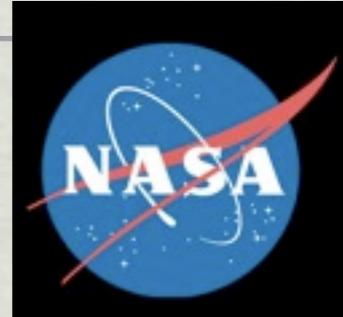
**REGOLITH**

**MICROGRAPH OF TRIAL SLURRY**



**NOSTOC**

**FLUORESCENT MICROGRAPH OF TRIAL SLURRY**



**PLANTS!**

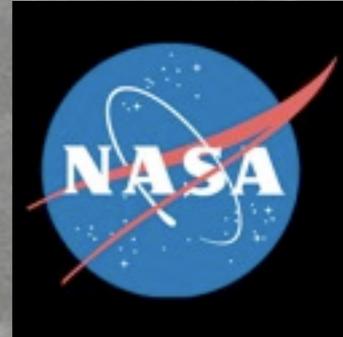


**TOP VIEW**



20 JULY 2010

NASA LUNAR SCIENCE FORUM



**Jon Rask  
Yuri Griko  
Matt Reyes  
Lee Bebout  
Brad Bailey  
Anita Mantri  
Brad Bebout  
Pete Worden  
Chris McKay  
Erin Tranfield  
Erich Fleming  
Kristina Gibbs  
Orlando Santos  
Eduardo Almeida  
Lockheed Martin  
Lunar Science Institute  
California Space Authority**



**THANK YOU:**

# REFERENCES:



- Allen, M. B. "Studies with *Cyanidium caldarium*, an autonomously pigmented chlorophyte." *Archiv fur Mikrobiologia* 32(1959): 270
- Baker, B. J. and J.F. Banfield. "Microbial communities in acid mine drainage." *FEMS Microbial, Ecol.* 44(2003): 139
- Baumler, D.J. et al. "Sulfate requirement for heterotrophic growth of *Ferroplasma acidarmanus* strain fer1." *Research in Microbiology* 156(2005): 492.
- Bent, E. et al. "Alterations in Plant Growth and in Root Hormone Levels of Lodgepole Pines Inoculated with Rhizobacteria." *Canadian Journal of Microbiology* 47,9(2001): 793.
- Connolly, John. United States. NASA. Constellation Program Overview. Constellation Program Office, 2006. Web. <[http://www.nasa.gov/pdf/163092main\\_constellation\\_program\\_overview.pdf](http://www.nasa.gov/pdf/163092main_constellation_program_overview.pdf)>.
- "Cyanidium." Culture Collection of Microorganisms from Extreme Environments. University of Oregon. 26 Jul 2009 <<http://cultures.uoregon.edu/cyanidium.htm>>.
- Dodds, W., D. Gudder, and D. Mollenhauer. "The Ecology of Nostoc." *Journal of Phycology* 31,1(2008):2.
- Doemel, W.N. and T.D. Brock. "The Physiological Ecology of *Cyanidium caldarium*." *Journal of General Microbiology* 67(1971): 17.
- Edwards, K. J. et al. "An Archaeal Iron-Oxidizing Extreme Acidophile Important in Acid Mine Drainage." *Science* 287(2000): 1796.
- Faduka, I. 1958, *Botanical Magazine*, 71, 79
- Golyshina, O. V. and K.N. Timmis. "Ferroplasma and relatives, recently discovered cell wall-lacking archaea making a living in extremely acid, heavy metal-rich environments." *Environmental Microbiology* 7,9(2005): 1277.
- Green, C.T. and K.M. Scow. "Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers." *Hydrogeology Journal* 8,1(2000):126.
- Haggag, W.M. "Colonization of Exopolysaccharide-Producing *Paenibacillus polymyxa* on Peanut Roots for Enhancing Resistance Against Crown Rot Disease." *African Journal of Biotechnology* 6,13(2007): 1568.
- Kado, C.I., W.C. Schnathorst, and H.R. Azad. "Method of using *Bacillus Polymyxa* 9A to Protect Plants Against Verticillium Wilt." United States Patent Number 4,663,162.
- Kozyrovska, N.O. et al. "Growing Pioneer Plants for a Lunar Base." *Advances in Space Research* 37,1(2006): 93.
- McKay, D.S. et al. "JSC-1: A NEW LUNAR SOIL SIMULANT." *Engineering, Construction, and Operations in Space IV* (1994): 857-66. American Society of Civil Engineers. 17 Aug. 2009. <<http://ares.jsc.nasa.gov/HumanExplore/Exploration/EXLibrary/DOCS/EIC050.HTML>>.
- Micallef, S.A., M.P. Shiaris, and A.C. Carmona. "Influence of *Arabidopsis thaliana* Accessions on Rhizobacterial Communities and Natural Variation in Root Exudates." *Journal of Experimental Botany* 60,6(2009): 1729.
- Rosado, A. S. and L. Seldin. "Production of a Potentially Novel Anti-Microbial Substance by *Bacillus polymyxa*." *World Journal of Microbiology and Biotechnology* 9,5(1993): 521.
- Stewart, W.D.P. and H.W. Pearsons. "Effects of aerobic and anaerobic conditions on growth and metabolism of blue-green algae." *Proc. Roy. Soc. Lond. B.* 175(1970): 293.
- Summers, M.L. et al. "Genetic Evidence of a Major Role for Glucose-6-Phosphate Dehydrogenase in Nitrogen Fixation and Dark Growth of the Cyanobacterium *Nostoc* sp. Strain ATCC 29133." *Journal of Bacteriology* 177,21(1995): 6184.
- Timmusk, S., E. Gerhart, and H. Wagner. "The Plant-Growth-Promoting Rhizobacterium *Paenibacillus polymyxa* Induces Changes in *Arabidopsis thaliana* Gene Expression: A Possible Connection Between Biotic and Abiotic Stress Responses." *MPMI* 12,11(1999): 951.
- Timmusk, S. et al. "Cytokinin production by *Paenibacillus polymyxa*." *Soil Biology and Biochemistry* 31,13(1999): 1847.
- Whitton, B.A. "Extracellular Products of Blue-Green Algae." *Journal of General Microbiology* 40(1965): 1.