



# Bacterial, Archaeal, and Fungal Diversity of Spacecraft-Associated Surfaces

**Presentation to the COSPAR-2012,  
Mysore, India**

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# Outline of the presentation



- **Status and Objectives**
- **Molecular microbial community analyses:**
  - Cloning – Sanger Sequencing
  - DNA Microarray (PhyloChip)
  - **454 Tag-encoded Pyrosequencing**
- **Demonstration of the state-of-the-art technologies to catalogue a DNA-based genetic inventory of low biomass surfaces**
  - **Spacecraft assembly facility surfaces**
  - **Spacecraft surfaces**
  - **PhyloChip and 454 Tag-encoded Pyrosequencing**



# Status and Objectives



- Under the auspices of the Mars Program-funded Genetic Inventory task to develop and end-to-end genetic inventory capability for spacecraft, we have:
  - Demonstrated use of the PhyloChip DNA microarray to conduct a comprehensive census of the bacterial communities on the surfaces in three NASA spacecraft assembly cleanrooms (SAC) supporting two distinct missions (Phoenix and MSL).
    - Presented in COSPAR 2010
  - Observed that the maximum diversity of resident bacterial DNA was uncovered by subjecting subsamples from each cleanroom sampling to both PhyloChip and conventional cloning/Sanger sequencing of 16S rRNA genes.
    - La Duc, M. T., S. Osman, P. Vaishampayan, Y. Piceno, G. Andersen, J. A. Spry, and K. Venkateswaran. 2009. Comprehensive census of bacteria in clean rooms by using DNA microarray and cloning methods. *App. Env. Microbiol.* **75**:6559-6567.
  - Demonstrated molecular methods to catalogue genetic inventories of all three microbial domains (bacterial, archaeal, and fungal [*Eukarya*]) associated with the Mars Science Laboratory (MSL) spacecraft and associated environmental samples.
    - PhyloChip (bacteria only, and not discussed here)
    - 454 tag-encoded pyrosequencing (bacteria, archaea, and fungi)

- DNA based technologies detect live and dead cells
- Establishing a link to “PP risk” of terrestrial contamination is future work



# Spacecraft Assembly Environmental Samples



- Over a period of 3 years ('09 to '11), 71 individual surface samples associated with various rooms were collected, including floor samples from spacecraft assembly cleanrooms (JPL-SAF, JPL Building 144), an ordinary room adjacent to JPL-SAF, and a non-NASA cleanroom.
  - Group I (8 sets; 33 samples) samples examined the effect of varying cleanroom certification on resulting DNA data.
    - This group consisted of floor and GSE samples from JPL-SAF, an ordinary room adjacent to JPL-SAF, and a non-NASA cleanroom.
  - Group II samples (4 sets; 38 samples) were collected to assess the impact of cleaning procedures on resulting DNA data.
    - This group comprised floor and GSE samples gathered from the JPL-144 facility that was expected to receive spacecraft hardware both (a) prior to cleaning, and (b) from identical locations one day after cleaning.

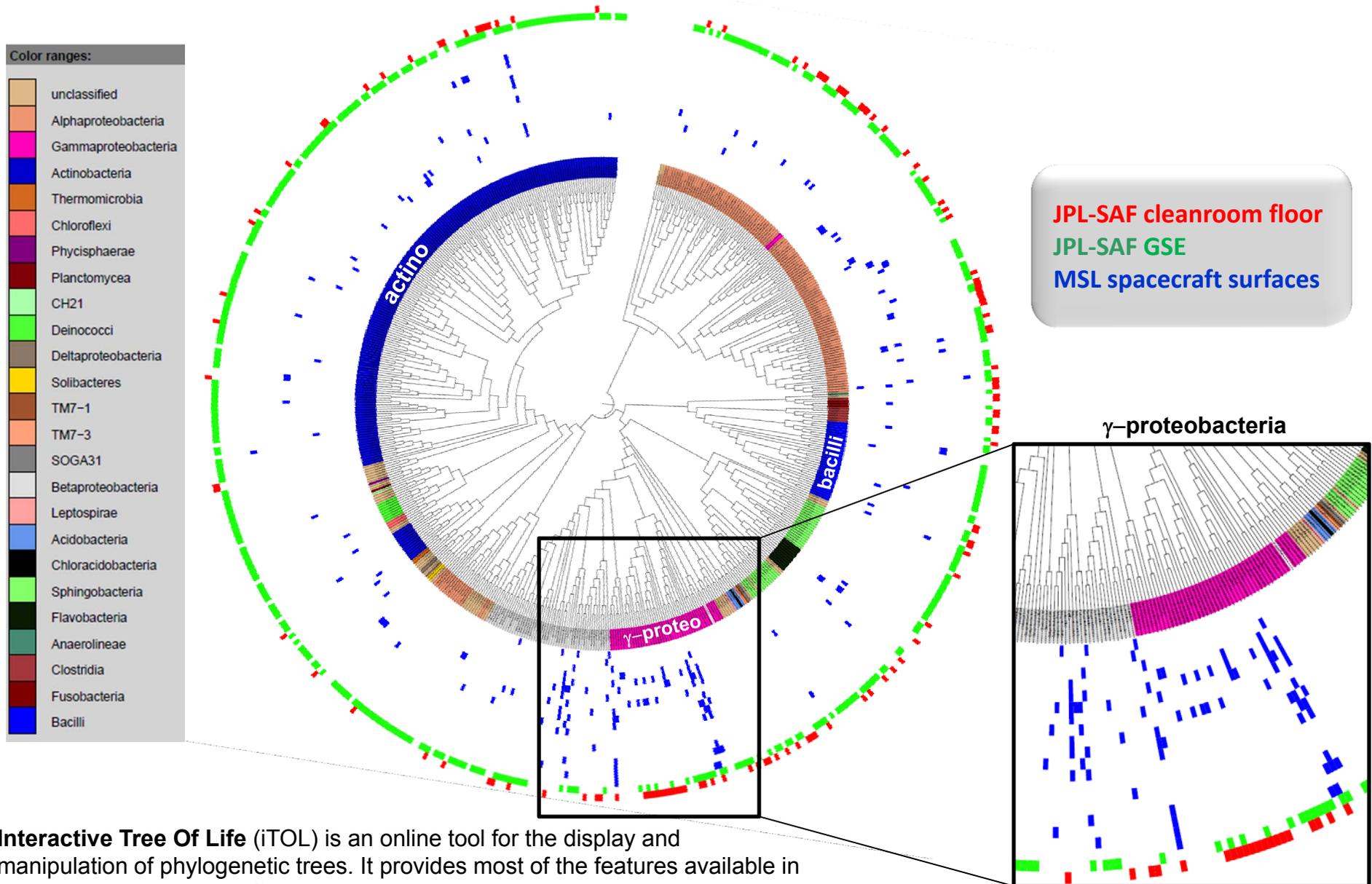


# MSL Spacecraft Surface Samples



- 162 samples collected by the MSL PP implementation team from the MSL spacecraft flight hardware (housed in the JPL–SAF and KSC–PHSF) were analyzed.
  - Group III samples (15 sets; 110 samples) were collected when MSL was being assembled at the JPL-SAF and were grouped together to evaluate the extent of correlation between endospore burden associated with spacecraft hardware surfaces and resulting operational taxonomic unit (OTU) abundance.
    - This group comprised [spacecraft hardware](#) surface samples whose bacterial endospore burden had been previously determined.
  - Group IV samples (4 sets; 52 samples) were collected when MSL was being assembled at the KSC-PHSF and were analyzed together to investigate how DNA data differ across various mission subsystem components (e.g., cruise stage, rover).
    - This group consisted of [spacecraft hardware](#) surface samples collected from various components of the mission subsystems (e.g., cruise stage, descent stage, rover)

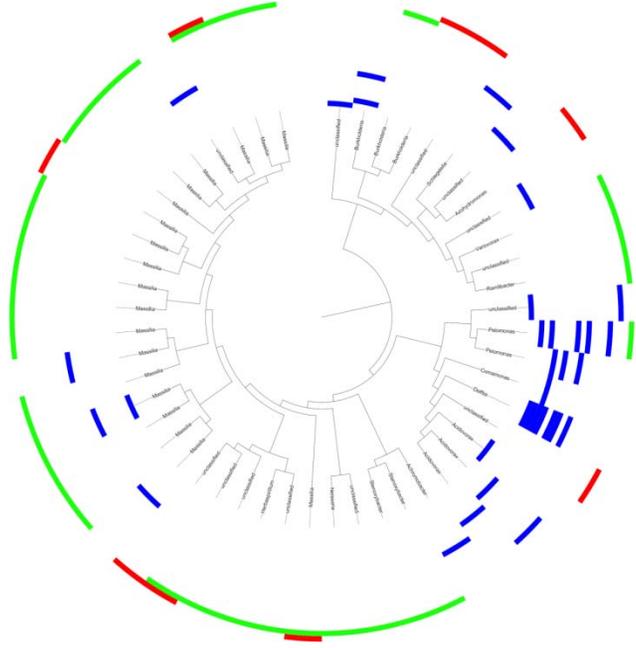
# iTOL tree (Bacterial pyrosequences >350-bp)



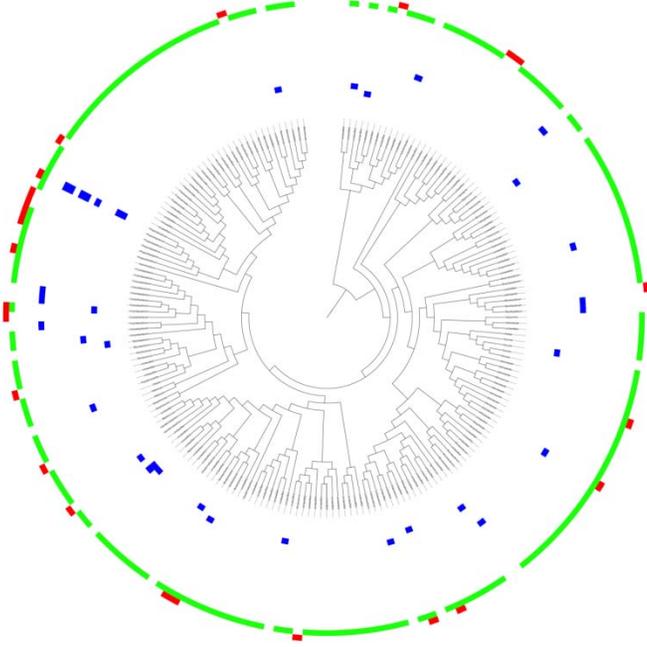
**Interactive Tree Of Life (iTOL)** is an online tool for the display and manipulation of phylogenetic trees. It provides most of the features available in other tree viewers, and offers a novel circular tree layout, which makes it easy to visualize mid-sized trees (up to several thousand leaves).

Pyrosequence diversity of various bacterial genera  
on **floor**, **GSE**, and **MSL spacecraft surfaces**

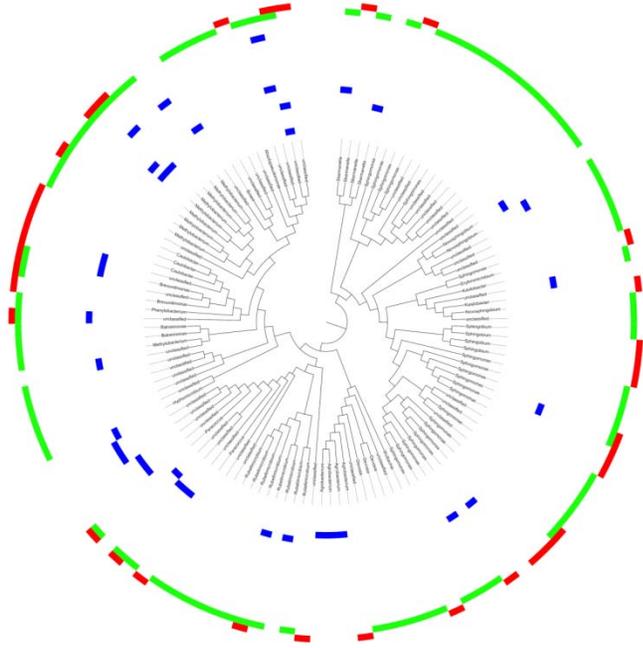
$\beta$ -proteobacteria



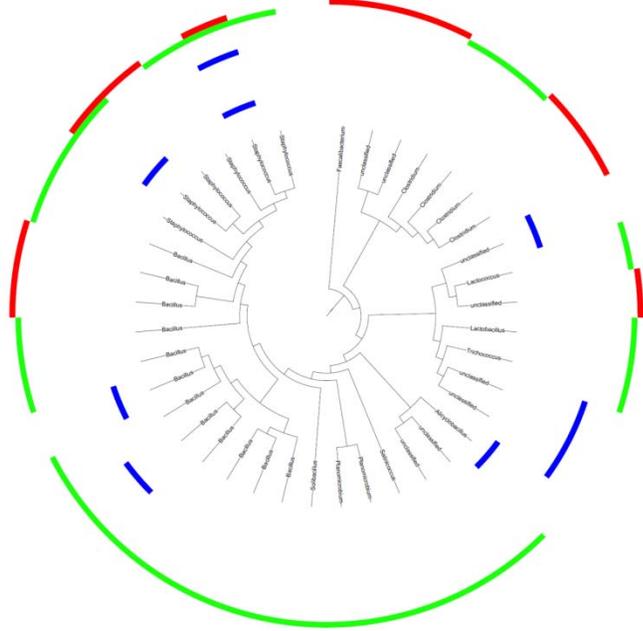
Actinobacteria



$\alpha$ -proteobacteria

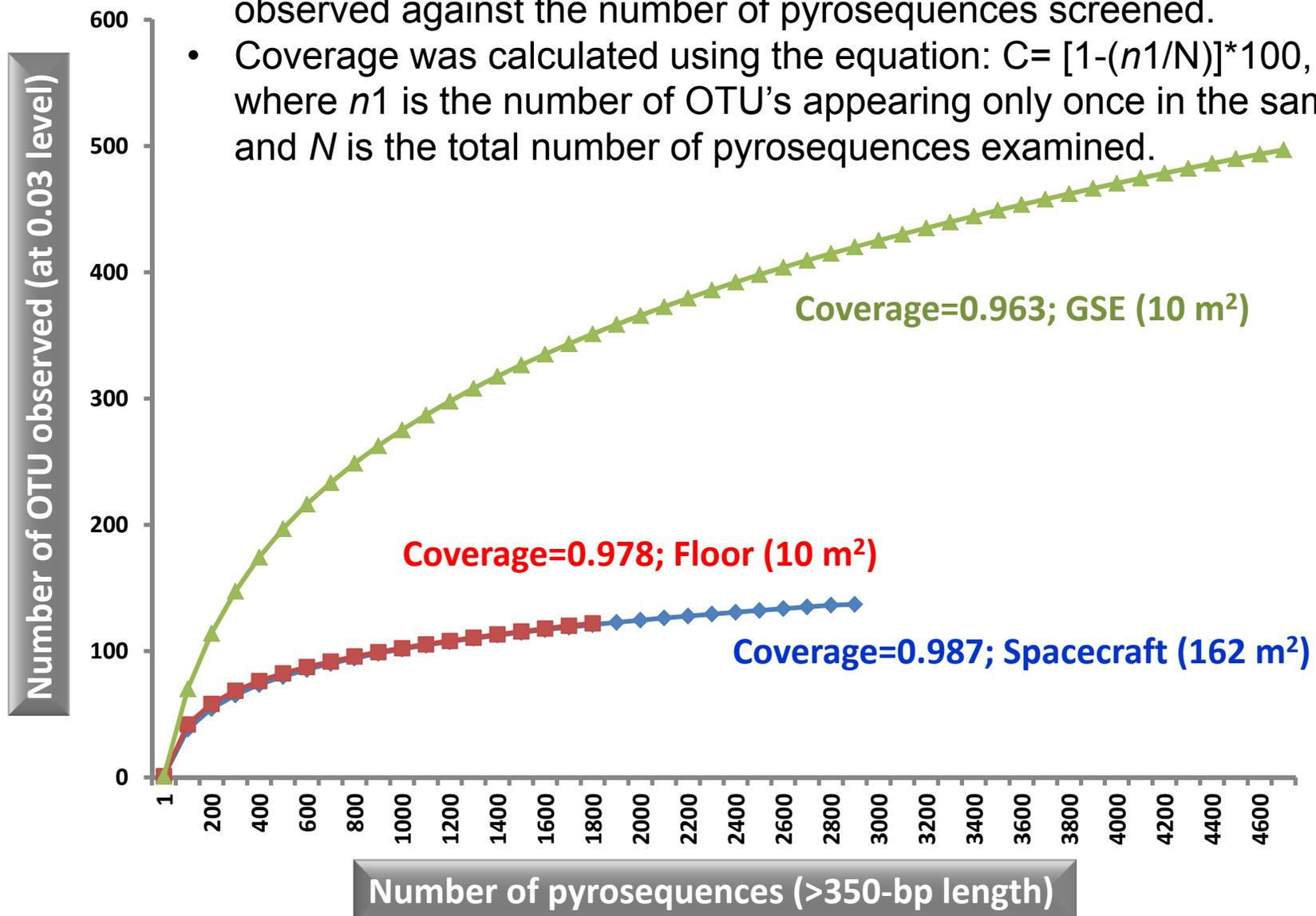


Firmicutes

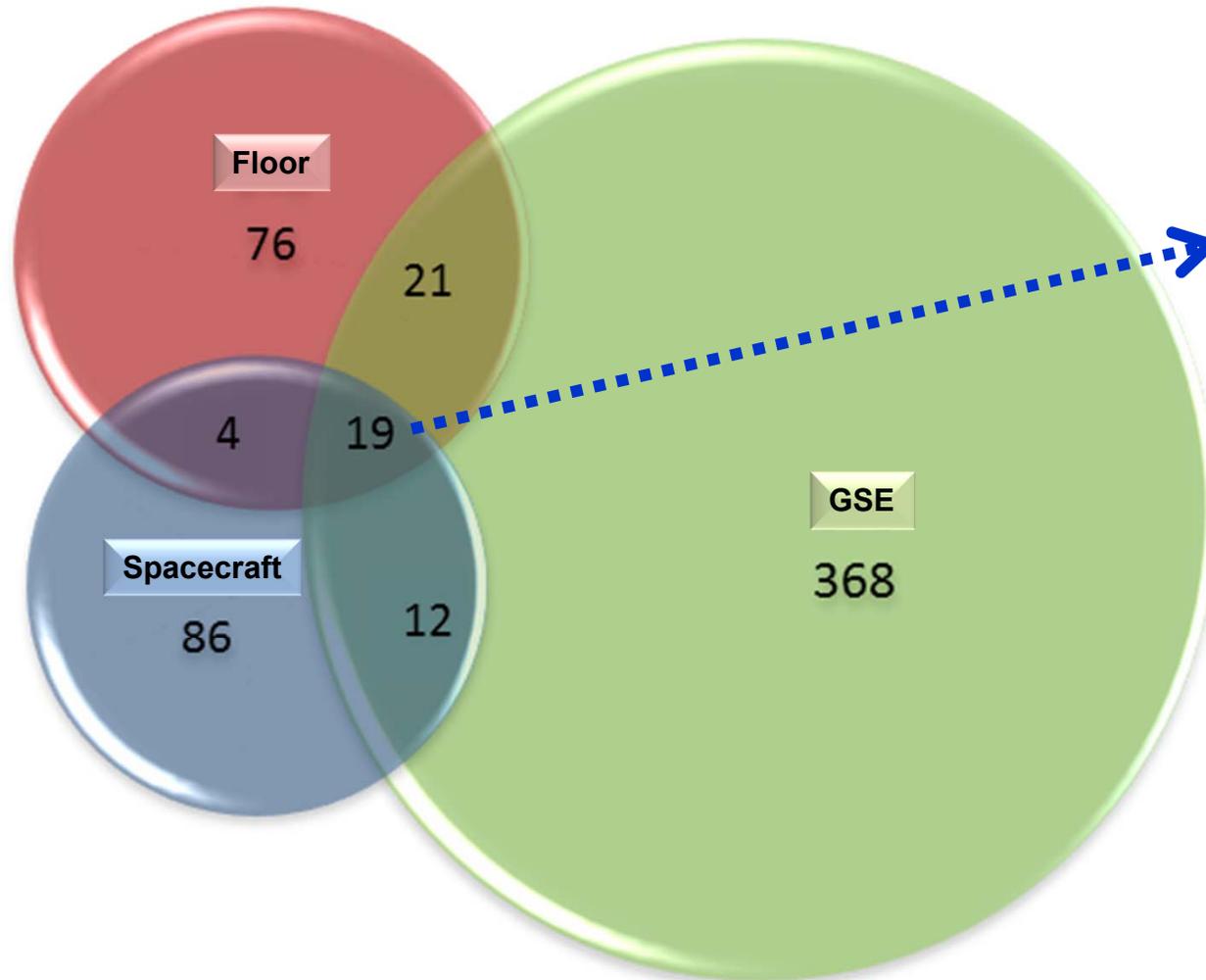


# Rarefaction curves based on bacterial pyrosequences

- Rarefaction curves are produced by plotting the number of OTU observed against the number of pyrosequences screened.
- Coverage was calculated using the equation:  $C = [1 - (n1/N)] * 100$ , where  $n1$  is the number of OTU's appearing only once in the sample, and  $N$  is the total number of pyrosequences examined.



# Overlapping bacterial genera among floor, GSE, and MSL spacecraft surfaces (pyrosequence – based analysis)



Actinobacteria	<i>Corynebacterium</i>
	<i>Modestobacter</i> ✓
α-proteo	<i>Arthrobacter</i> ✓
	<i>Propionibacterium</i>
	<i>Staphylococcus-1</i>
	<i>Staphylococcus-2</i>
	<i>Brevundimonas</i>
	<i>Blastobacter</i> ✓
γ-proteo	<i>Methylobacterium-1</i>
	<i>Methylobacterium-2</i>
	<i>Rubellimicrobium</i> ✓
	<i>Unclassified-1</i>
	<i>Unclassified-2</i>
	<i>Escherichia</i>
	<i>Acinetobacter-1</i> ✓
<i>Acinetobacter-2</i> ✓	
	<i>Acinetobacter-3</i> ✓
	<i>Pseudomonas</i>
	<i>Unclassified</i>

✓ Previously reported to survive in conditions: Desiccation, UV, gamma radiation

## Impact of cleaning procedures on pyrosequencing profiles

Bacterial Taxa	JPL-144 Floor		JPL-144 GSE	
	Before (#155)	After (159)	Before (#157)	After (#161)
<i>Actinobacteria</i>	51	3	186	30
<i>Armatimonadetes</i>	3		1	
<i>Bacteroidetes</i>	39	4	75	22
<i>Chlorobi</i>			1	
<i>Verrucomicrobia</i>	4	2		
<i>Chloroflexi</i>			8	
<i>Deinococcus-Thermus</i>	5		15	3
<i>Acidobacteria</i>	1		5	1
<i>Firmicutes</i>	10		33	2
<i>Fusobacteria</i>			2	
<i>Gemmatimonadetes</i>	3		2	1
<i>Nitrospirae</i>	1		1	
<i>Planctomycetes</i>	2		2	1
<i>Proteobacteria</i>				
Alpha	233	38	188	22
Beta	48	7	61	23
Delta	3		3	
Gamma	76	12	40	16
Unidentified	4		3	
Unidentified division				
SC4			3	
WPS-2	2			
Unclassified bacteria	28		33	15
<b>Total # of OTU</b>	<b>513</b>	<b>66</b>	<b>662</b>	<b>136</b>

The cleaning and maintenance involved in preparing the JPL building 144 cleanroom to receive mission-critical spacecraft hardware successfully reduced and (as made evident by the OTU not detected) even eliminated many bacterial lineages. Prior to cleaning, floor sample 155 gave rise to 513 OTU, whereas after cleaning, the same location (sample 159) showed a complete loss of 447 previously detected OTU. In general, the incidence of all bacterial lineages declined after cleaning of these cleanroom floors. In similar fashion, prior to cleaning procedures, 662 OTU were observed in samples from the JPL building 144 GSE surfaces (sample 157). This was ~5 times more than the number of OTU detected after cleaning (136 OTU; sample 161). Several bacterial groups reported to be capable of surviving desiccation and UV radiation (e.g., actinobacteria, deinococci, firmicutes) were not detected after the cleaning of the floors, whereas the same bacterial types persisted even after cleaning on GSE surfaces. In contrast, the vast majority of purported human-associated bacteria (e.g., proteobacteria) was eliminated from both the cleanroom floors and colocated GSE surfaces after cleaning.



# Archaeal pyrosequences from cleanroom and spacecraft surfaces

TABLE 4 Archaeal pyrosequences and OTU retrieved from various spacecraft and associated surfaces

Archaeal taxon and species	No. of archaeal pyrosequences from indicated sample (no. of archaeal OTU)													
	Group I					Group II		Group III						
	150 (floor) (1)	143 (entrance floor) (1)	141 (shoe cleaner) (2)	142 (air lock) (1)	148 (JPL-SAF GSE)	157 (GSE) (1)	161 (GSE) (1)	124 (spacecraft) (1)	126 (spacecraft) (1)	127 (spacecraft) (1)	128 (spacecraft) (1)	130 (spacecraft) (2)	132 (spacecraft) (1)	
<i>Nitrosphaeraceae</i> SCA114-1	17		35	47		5	2							
<i>Nitrosphaeraceae</i> SCA114-2			1											
<i>Nitrosphaeraceae</i> SCA117		6												
<i>Methanobacteriaceae</i> 1								1	2	7	3	16	1	
<i>Methanobacteriaceae</i> 3												1		

**Pyrosequencing-derived archaeal diversity.** Many fewer high-quality archaeal sequences were generated compared directly to the results of bacterial pyrosequencing for the very same samples. Although ~30,000 sequences spanning >350 bp of the V3 to V5 region of archaeal 16S rRNA gene were generated, bioinformatic quality control measures demonstrated that only 151 sequences were truly of archaeal lineage. The remainder of these sequences were related to bacterial taxa, predominantly *Verrucomicrobiae*. Resulting archaeal sequence and OTU abundances from the various samples examined in this study are given in Table 4. None of the 31 sample sets examined yielded 1.1-kb archaeal 16S rRNA gene amplicons via traditional PCR techniques (35). In most cases, even following nested PCR amplification, there was insufficient DNA to perform successful pyrosequencing analysis.

However, three samples (samples 142 to 144) collected from the ordinary room adjacent to the JPL-SAF cleanroom gave rise to measurable levels of archaeal 16S rRNA gene nested-PCR product

Of the 12 samples composing groups I and II, no long-read (~600-bp) archaeal sequences could be generated from any of the five cleanroom floor (~10-m<sup>2</sup> area) samples tested. In contrast, archaeal sequences were successfully obtained from all five of the ordinary room samples (1-m<sup>2</sup> area) and two of the group II GSE samples (Table 4). In addition, archaeal sequences were generated from 6 of the 15 spacecraft hardware (group III) samples, whereas all four sets of mission subsystem component surface (group IV) samples failed to yield amplifiable archaeal DNA.

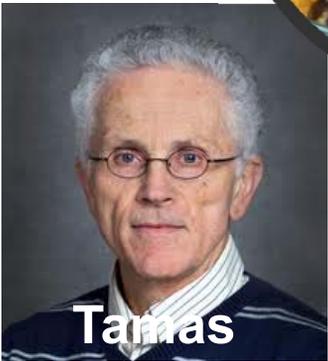
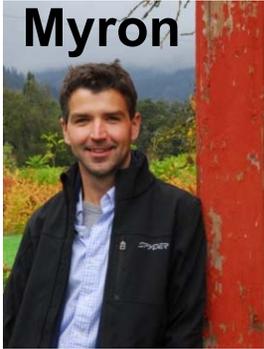
sequences representing members of *Nitrosphaeraceae* (120 sequences; 3 OTU), while group III spacecraft hardware samples generated only *Methanobacteriaceae* OTU (31 sequences; 2 OTU).



## Summary

- **Spacecraft hardware and spacecraft assembly cleanroom (SAC) surfaces (233 m<sup>2</sup> in total) were sampled, total genomic DNA was extracted, hypervariable regions of the 16S rRNA gene (bacteria and archaea) and ribosomal ITS region (fungi) were subjected to 454 tag-encoded pyrosequencing PCR amplified, and 203,852 resulting high quality long read sequences were analyzed.**
- *Bioinformatic analyses revealed correlations between operational taxonomic unit (OTU) abundance and certain sample characteristics, such as source (cleanroom floor, ground support equipment [GSE], or spacecraft hardware), cleaning regimen applied, and location about the facility or spacecraft.*
- **NASA cleanroom floor and GSE surfaces gave rise to more diverse bacterial signatures (619 OTU; 20 m<sup>2</sup>) than co-located spacecraft hardware (187 OTU; 162 m<sup>2</sup>).**
- *In contrast to the results of bacterial pyrosequencing, where sequences were generated from each of the 31 sample sets examined, archaeal and fungal sequences were detected in only 13 and 18 of these sample sets, respectively.*
- **As was the case for bacteria, the occurrence of fungal OTU signatures in the GSE surface samples dramatically diminished (9-times less diversity) once cleaning protocols had been applied.**
- *The presence of OTUs from actinobacteria, deinococci, acidobacteria, firmicutes, and proteobacteria on spacecraft surfaces suggests that certain bacterial lineages persist in clean environments even following rigorous quality control and cleaning practices.*
- **The majority of bacterial OTU observed as being recurrent belonged to actinobacteria and alpha-proteobacteria, supporting the hypothesis that the cleanliness measures applied in SACs may inadvertently provide selective pressure for organisms which may be the most fit to survive interplanetary space.**

# The Team



\$\$\$ : Mars Program Office, JPL  
Dr. Karen Buxbaum – JPL  
Dr. Andy Spry – JPL

Backup slides



# Spacecraft Assembly Environments – 1



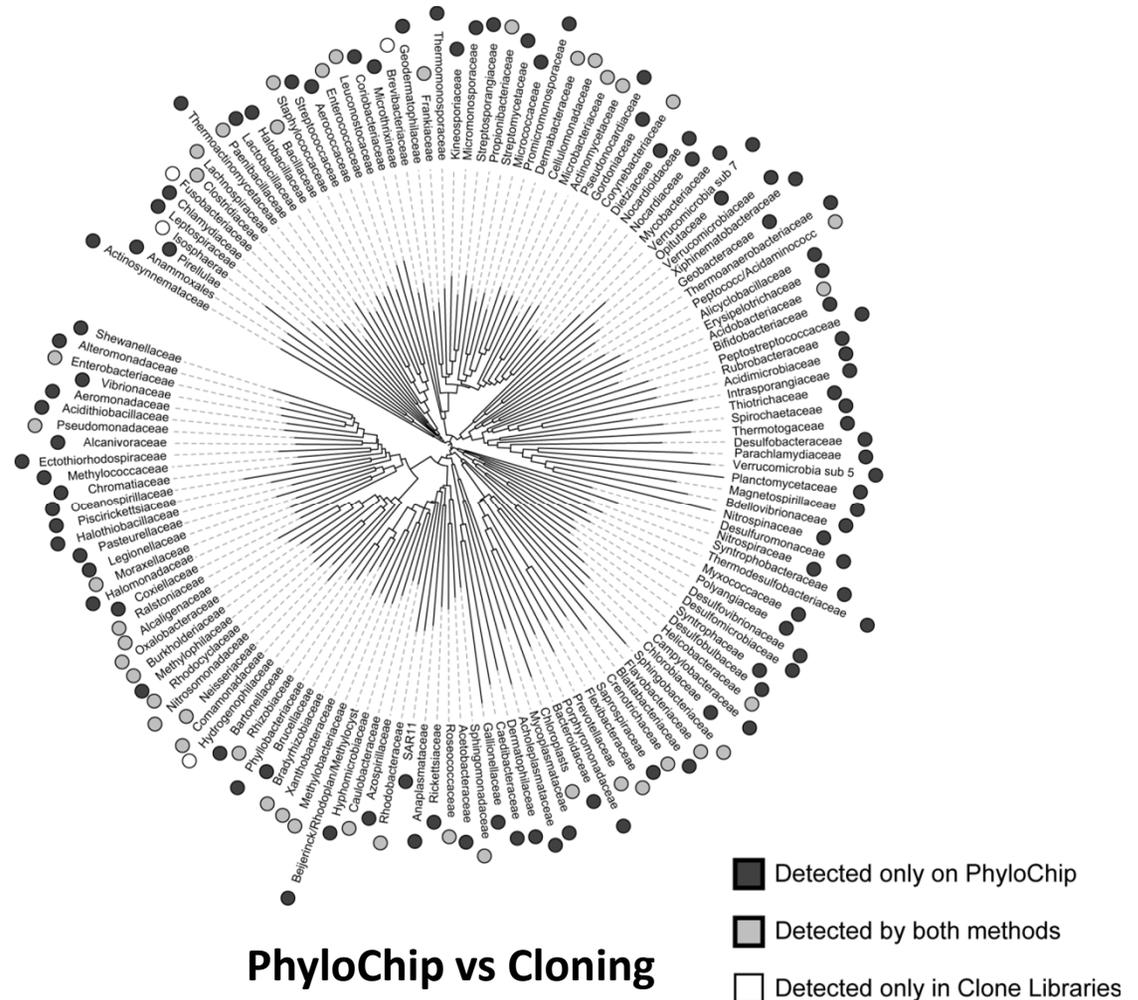
- Over a period of 18 months ('07 to '09), 107 individual surface samples were collected from:
  - Kennedy Space Center Payload Hazardous Servicing Facility (KSC-PHSF)
  - Lockheed Martin Aeronautics Multiple Testing Facility (LMA-MTF)
  - Jet Propulsion Laboratory Spacecraft Assembly Facility (JPL-SAF)
- Categorization of the samples
  - Category A: 37 samples collected in the presence of Phoenix spacecraft hardware.
  - Category B: 30 samples collected within cleanrooms devoid of spacecraft.
  - Category C: 40 samples collected from JPL-SAF during the assembly of the MSL spacecraft.



# PhyloChip vs Clone Libraries



- Phylochips detected OTU from a total of 140 classified bacterial families, 46 of which were also detected by clone libraries.
- Four bacterial families were detected in clones but not chips: **Brevibacteriaceae**, **Isosphaerae**, **Hydrogenophilaceae**, and **Fusobacteriaceae**.
- Majority of clone OTU belonged to the Firmicutes, Proteobacteria and Actinobacteria.

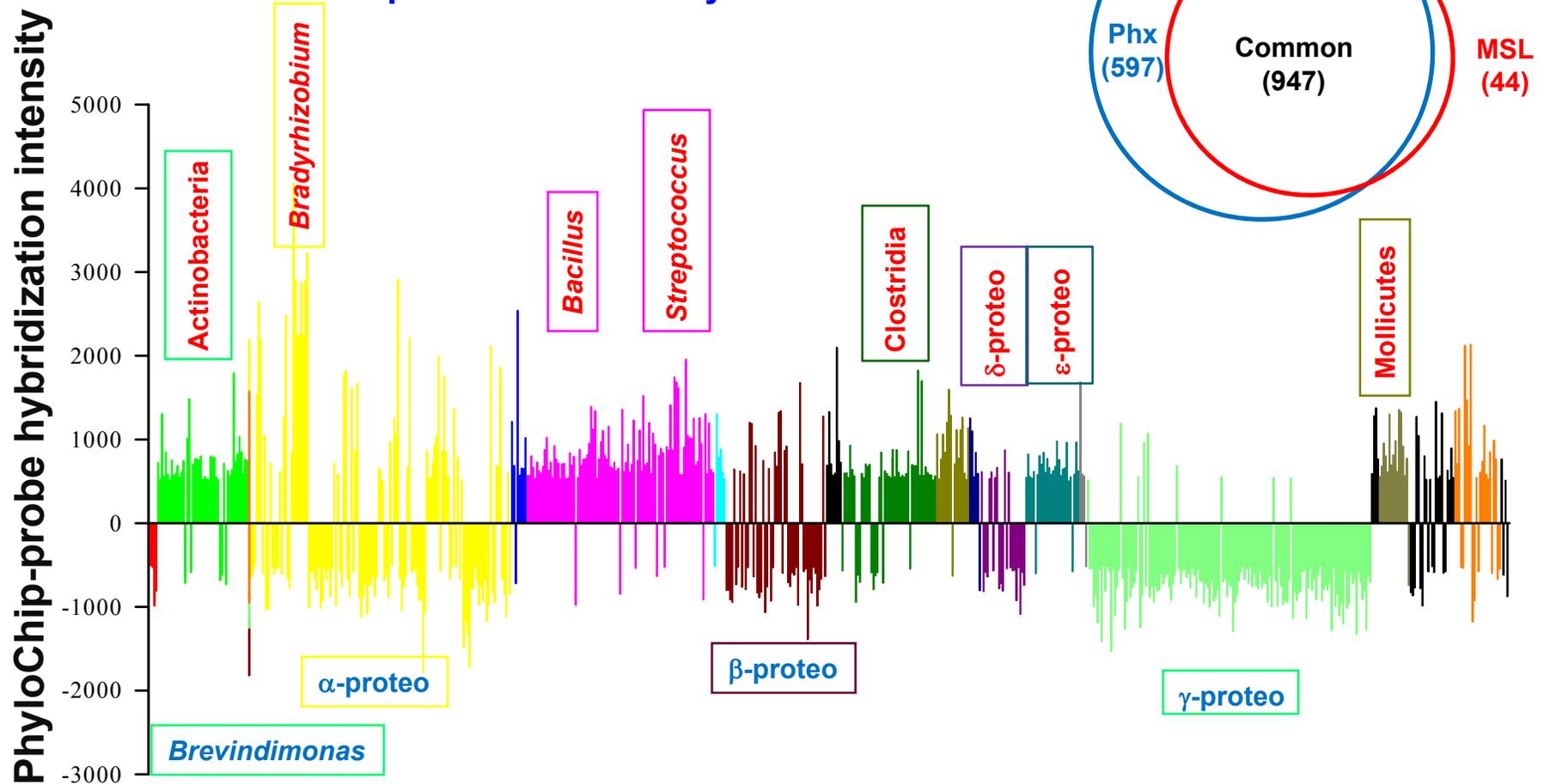




# Identified Differences Between Spacecraft Assembly Facilities



Shift in bacterial (OTU) population when JPL-SAF (MSL) was compared to KSC-PHSF (Phoenix) during the spacecraft assembly



La Duc, M. T., S. Osman, P. Vaishampayan, Y. Piceno, G. Andersen, J. A. Spry, and K. Venkateswaran. 2009. Comprehensive census of bacteria in clean rooms by using DNA microarray and cloning methods. *Applied and environmental microbiology* 75:6559-6567.

Sample (total area sampled)	Sample ID	Sampling devices	Sample type	Area (m2)	Mission	Cleanroom type	Description
<b>Group I: Cleanroom types (33 m2)</b>							
GI-37	150	BisKit	Floor-70A	10	None	Non-NASA Cleanroom	Non-NASA cleanroom (LBNL; #70A)
GI-35-6	143	BisKit	Entrance floor	1	None	Ordinary room	Ordinary room adjacent to JPL-SAF
GI-35-4	141	BisKit	Shoe Cleaner	1	None	Ordinary room	Ordinary room adjacent to JPL-SAF
GI-35-7	144	BisKit	Floor 1	1	None	Ordinary room	Ordinary room adjacent to JPL-SAF
GI-35-8	145	BisKit	Floor 2	1	None	Ordinary room	Ordinary room adjacent to JPL-SAF
GI-35-5	142	BisKit	Air-lock	1	None	Ordinary room	Ordinary room adjacent to JPL-SAF
<b>GI-36-3</b>	<b>146</b>	<b>BisKit</b>	<b>JPL-SAF GSE</b>	<b>9</b>	<b>Mars</b>	<b>Class 100K</b>	<b>During spacecraft assembly (JPL-SAF)</b>
<b>GI-36-4</b>	<b>148</b>	<b>BisKit</b>	<b>JPL-SAF Floor</b>	<b>9</b>	<b>Mars</b>	<b>Class 100K</b>	<b>During spacecraft assembly (JPL-SAF)</b>
<b>Group II: Cleaning vs prior to cleaning (38 m2)</b>							
GI-42-1	155	BisKit	Floor	9	None	Class 100K	JPL-144 cleanroom prior to cleaning
GI-42-2	157	BisKit	GSE	9	None	Class 100K	JPL-144 cleanroom prior to cleaning
GI-43-1	159	Polyester wipe	Floor	10	Mars	Class 100K	JPL-144 cleanroom after cleaning
GI-43-2	161	Polyester wipe	GSE	10	Mars	Class 100K	JPL-144 cleanroom after cleaning
<b>Group III: Spacecraft surfaces [(spore count-based) (110 m2)]</b>							
GI-16	124	Polyester wipe	spacecraft	6	Mars	Class 100K	No spore count
GI-17	125	Polyester wipe	spacecraft	10	Mars	Class 100K	No spore count
GI-25	133	Polyester wipe	spacecraft	8	Mars	Class 100K	No spore count
GI-26	134	Polyester wipe	spacecraft	7	Mars	Class 100K	No spore count
GI-27	135	Polyester wipe	spacecraft	4	Mars	Class 100K	No spore count
GI-28	136	Polyester wipe	spacecraft	6	Mars	Class 100K	No spore count
GI-29	137	Polyester wipe	spacecraft	18	Mars	Class 100K	No spore count
GI-18	126	Polyester wipe	spacecraft	10	Mars	Class 100K	1 to 5 spore per m2
GI-19	127	Polyester wipe	spacecraft	14	Mars	Class 100K	1 to 5 spore per m2
GI-20	128	Polyester wipe	spacecraft	5	Mars	Class 100K	1 to 5 spore per m2
GI-21	129	Polyester wipe	spacecraft	4	Mars	Class 100K	1 to 5 spore per m2
GI-22	130	Polyester wipe	spacecraft	1	Mars	Class 100K	1 to 5 spore per m2
GI-30	138	Polyester wipe	spacecraft	13	Mars	Class 100K	1 to 5 spore per m2
GI-32	140	Polyester wipe	spacecraft	3	Mars	Class 100K	5 to 10 spore per m2
GI-24	132	Polyester wipe	spacecraft	1	Mars	Class 100K	300 spores per m2
<b>Group IV: Spacecraft surfaces [(mission component-based) (52 m2)]</b>							
GI-38	151	Polyester wipe	spacecraft	26	Mars	Class 100K	Cruise stage (0.2 spores per m2)
GI-39	152	Polyester wipe	spacecraft	9	Mars	Class 100K	Descent stage (0.4 spores per m2)
GI-40	153	Polyester wipe	spacecraft	16	Mars	Class 100K	Rover (0.3 spores per m2)
GI-41	154	Polyester wipe	spacecraft	1	None	Class 100K	Non-flight samples (14 spores per m2)

JPL-SAF: Jet Propulsion Laboratory Spacecraft Assembly Facility

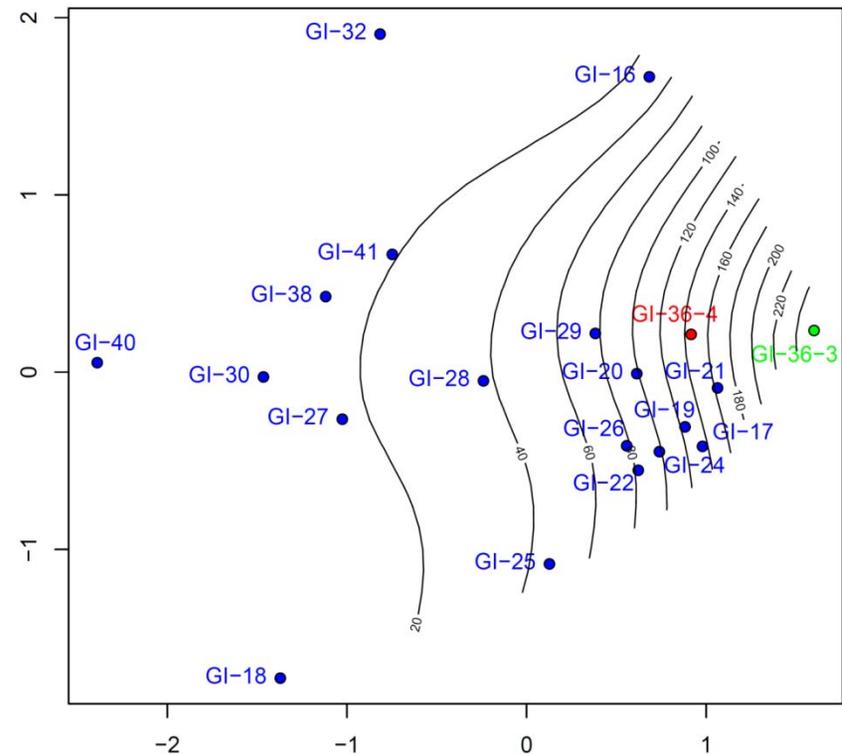
LBNL: Lawrence Berkeley National Laboratory



# Non-metric multidimensional scaling to display the relatedness of various samples based on PhyloChip taxonomy



- The relatedness in presence/absence ordination analysis explains the microbial richness (number of different PTU per sample) and number of common PTU between samples at the same time.
- A curve fitting model was used to display the relation of microbial richness measure on genus level with the environmental clustering method.
  - **GI-36-3 (GSE)** having the highest microbial richness placed somewhat apart from **spacecraft** samples.
  - **GI-36-4 (Floor)** was more related to **spacecraft** samples GI-20, GI-21, and GI-29.
  - Many **spacecraft** samples (7 out of 19) clustered close to sample GI-19, which was the center of these samples.
  - However, 7 of 19 **spacecraft** samples had less than 20 different genera and were spread apart from each other (left side of NMDS), which indicates that they had only few taxa in common.



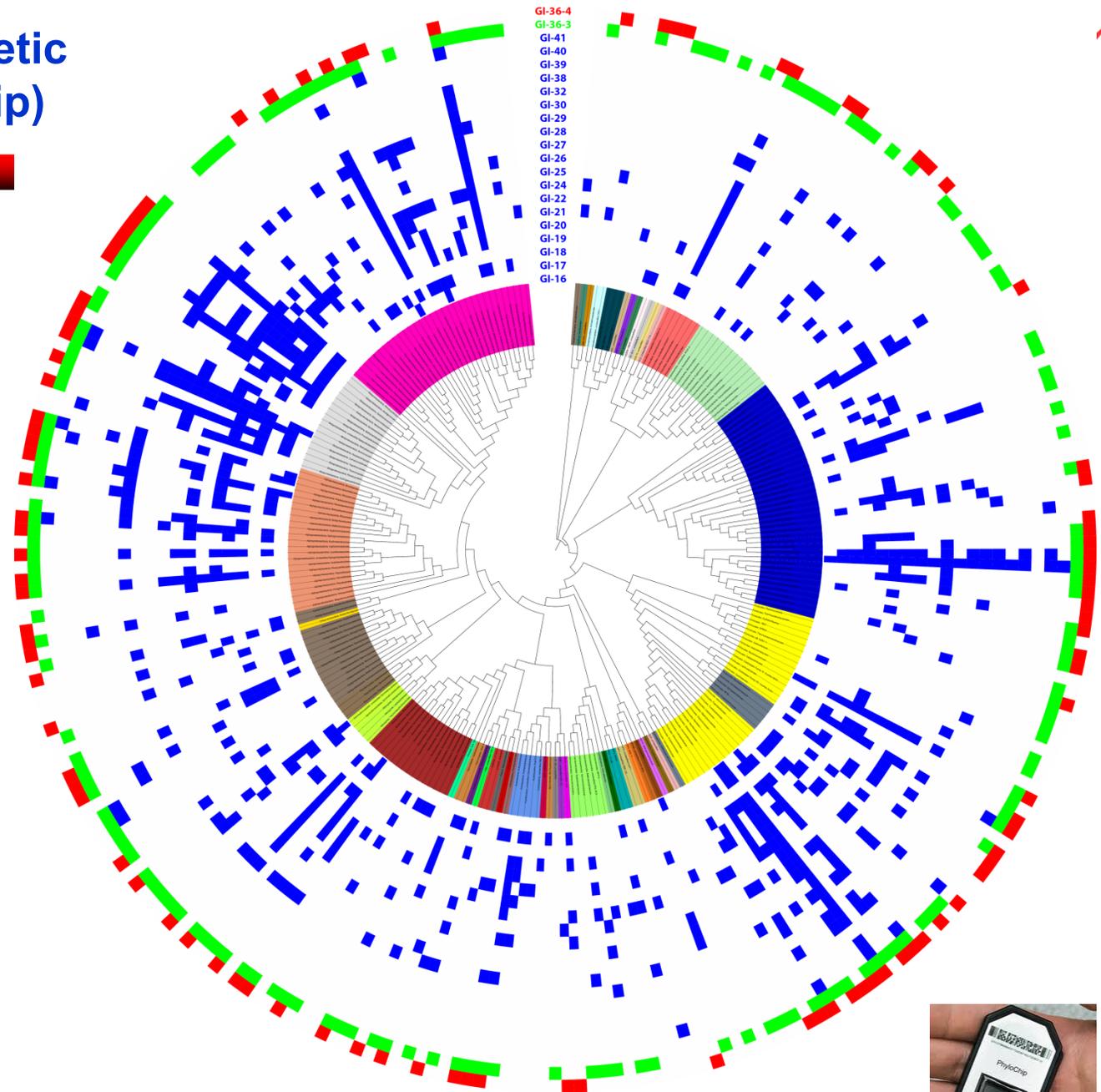
**Red: Floor**  
**Green: GSE**  
**Blue: Spacecraft**





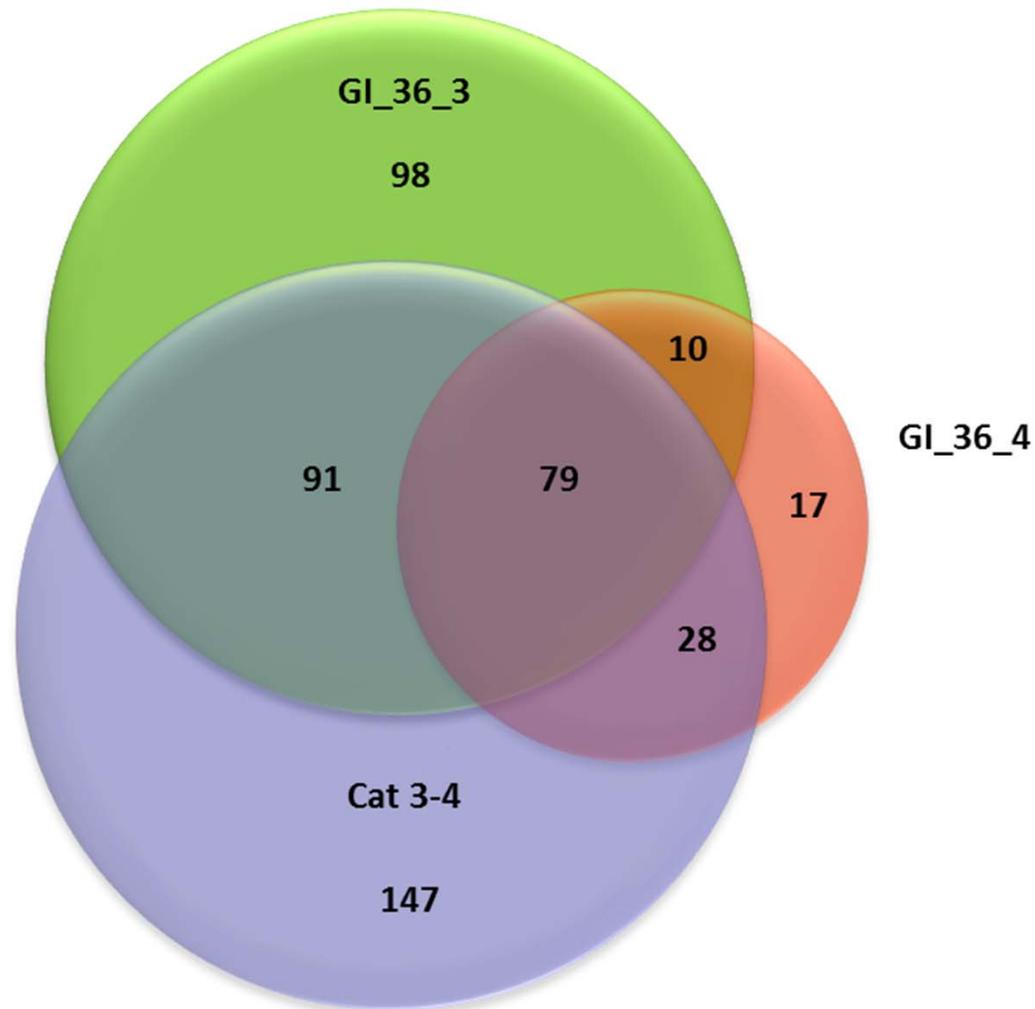
## iTOL phylogenetic tree (PhyloChip)

- 258 different families, which were color-coded at higher taxa level (49 different taxa, mainly phyla or in case of Proteobacteria, class level).
- This way of displaying the presence/absence of families detected in each sample allows an easy, fast visual detection of community patterns.
- For instance *Corynebacteriaceae* (Actinobacteria) were clearly present in all samples (except GI-39 spacecraft sample that did not have possess PTU).





# Persistent bacterial genera **pyrosequences** on **floor**, **GSE**, and MSL **spacecraft** surfaces



The work was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. Copyright 2012 California Institute of Technology. Government sponsorship acknowledged





# Cut set microbiome on genus level – alphabetical order

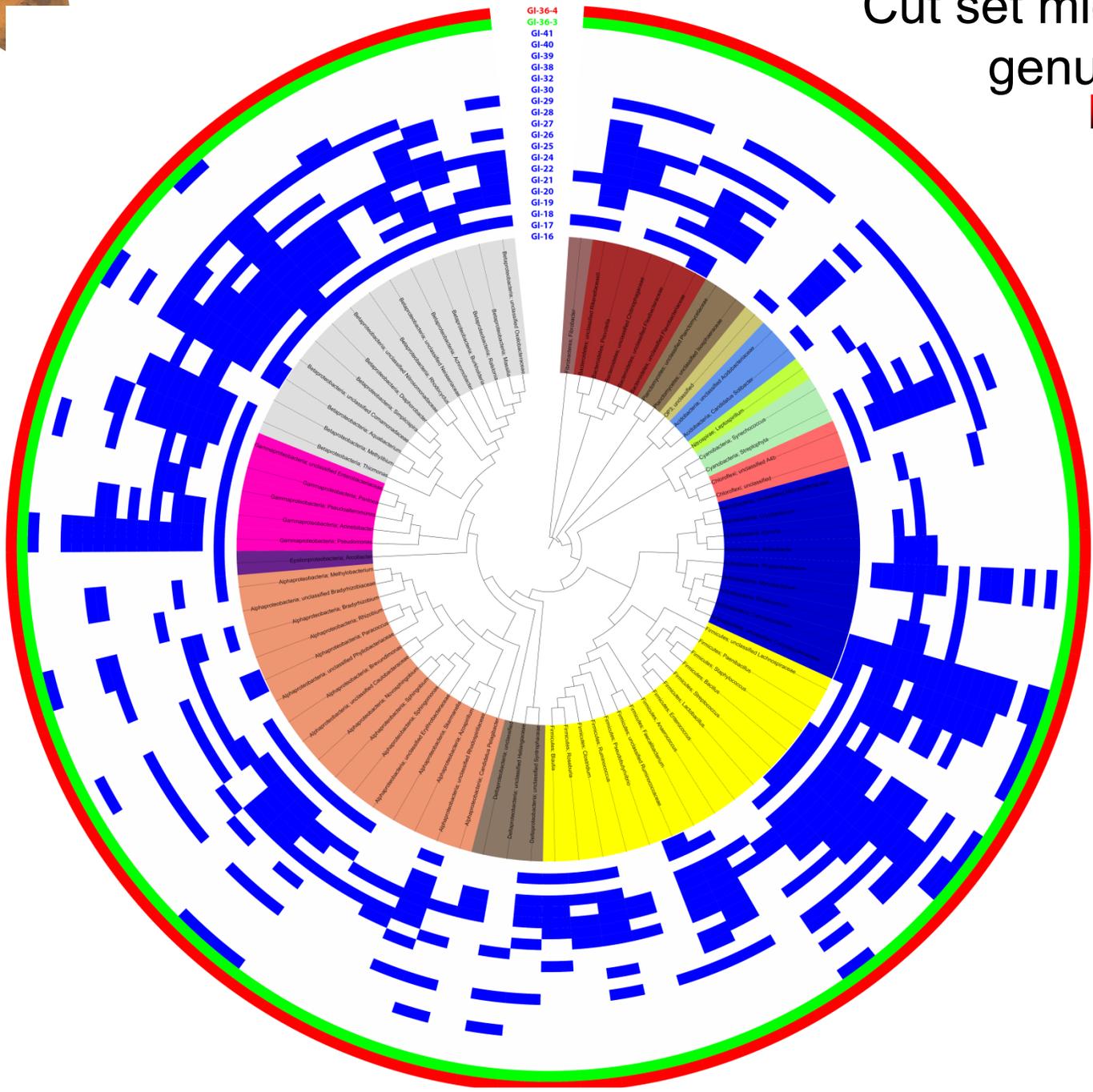


Acidobacteria; Candidatus Solibacter	Bacteroidetes; Prevotella	Fibrobacteres; Fibrobacter
Acidobacteria, unclassified Acidobacteriaceae	Bacteroidetes, unclassified Chitinophagaceae	Firmicutes; Anaerococcus
Actinobacteria; Arthrobacter	Bacteroidetes; unclassified Flavobacteriaceae	Firmicutes; Bacillus
Actinobacteria; Corynebacterium	Bacteroidetes; unclassified Flexibacteraceae	Firmicutes; Blautia
Actinobacteria; Cryobacterium	Bacteroidetes; unclassified Rikenellaceae	Firmicutes; Clostridium
Actinobacteria; Kocuria	Betaproteobacteria; Achromobacter	Firmicutes; Enterococcus
Actinobacteria; Mycobacterium	Betaproteobacteria; Aquabacterium	Firmicutes; Faecalibacterium
Actinobacteria; Propionibacterium	Betaproteobacteria; Burkholderia	Firmicutes; Lactobacillus
Actinobacteria; Rhodococcus	Betaproteobacteria; Diaphorobacter	Firmicutes; Paenibacillus
Actinobacteria; unclassified Corynebacteriaceae	Betaproteobacteria; Massilia	Firmicutes; Pseudobutyrvibrio
Actinobacteria; unclassified Microbacteriaceae	Betaproteobacteria; Methylibium	Firmicutes; Roseburia
Alphaproteobacteria; Azospirillum	Betaproteobacteria; Ralstonia	Firmicutes; Ruminococcus
Alphaproteobacteria; Bradyrhizobium	Betaproteobacteria; Rhodocyclus	Firmicutes; Staphylococcus
Alphaproteobacteria; Brevundimonas	Betaproteobacteria; Simplicispira	Firmicutes; Streptococcus
Alphaproteobacteria; Candidatus Pelagibacter	Betaproteobacteria; Thiomonas	Firmicutes; unclassified Lachnospiraceae
Alphaproteobacteria; Methylobacterium	Betaproteobacteria; unclassified Comamonadaceae	Firmicutes; unclassified Ruminococcaceae
Alphaproteobacteria; Novosphingobium	Betaproteobacteria; unclassified Neisseriaceae	Gammaproteobacteria; Acinetobacter
Alphaproteobacteria; Paracoccus	Betaproteobacteria; unclassified Nitrosomonadaceae	Gammaproteobacteria; Pantoea
Alphaproteobacteria; Rhizobium	Betaproteobacteria; unclassified Oxalobacteraceae	Gammaproteobacteria; Pseudoalteromonas
Alphaproteobacteria; Skermanella	Chloroflexi; unclassified	Gammaproteobacteria; Pseudomonas
Alphaproteobacteria; Sphingobium	Chloroflexi; unclassified A4b	Gammaproteobacteria; unclassified Enterobacteriaceae
Alphaproteobacteria; Sphingomonas	Cyanobacteria; Streptophyta	Nitrospirae; Leptospirillum
Alphaproteobacteria; unclassified Bradyrhizobiaceae	Cyanobacteria; Synechococcus	OP3; unclassified
Alphaproteobacteria; unclassified Caulobacteraceae	Deltaproteobacteria; unclassified	Planctomycetes; unclassified Isosphaera
Alphaproteobacteria; unclassified Erythrobacteraceae	Deltaproteobacteria; unclassified Haliangiaceae	Planctomycetes; unclassified Planctomy
Alphaproteobacteria; unclassified Phyllobacteriaceae	Deltaproteobacteria; unclassified Syntrophaceae	
Alphaproteobacteria; unclassified Rhodospirillaceae	Epsilonproteobacteria; Arcobacter	





# Cut set microbiome on genus level



GI-36-4  
GI-36-3  
GI-41  
GI-40  
GI-39  
GI-38  
GI-32  
GI-30  
GI-29  
GI-28  
GI-27  
GI-26  
GI-25  
GI-24  
GI-22  
GI-21  
GI-20  
GI-19  
GI-18  
GI-17  
GI-16



-  Betaproteobacteria
-  Gammaproteobacteria
-  Epsilonproteobacteria
-  Alphaproteobacteria
-  Deltaproteobacteria
-  Actinobacteria
-  Firmicutes
-  Nitrospirae
-  Cyanobacteria
-  Chloroflexi
-  OP3
-  Acidobacteria
-  Planctomycetes
-  Fibrobacteres
-  Bacteroidetes

