The Face of *Bacillus subtilis*: Genomes & Biofilms

Ashlee M. Earl
Kolter Lab
Harvard Medical School
“Old” World-View of Biodiversity

- Monera (Bacteria)
- Protista
- Plantae
- Fungi
- Animalia
Morphological Trait Comparisons...

...works for finches and other macroorganisms...
...but it doesn’t work for microbes.

WHY?
Microbes Are Hard to See!

There are more microbes in one gram of soil than there are humans alive today.
Microbes don’t have beaks!

Bacillus anthracis  Bradyrhizobium japonicum
Molecular tools: inferring relationships

“Alignment” of 16S/18S rRNA

Homo sapiens ...GTGCCAGCAGCCGCGGTATAATTCCAGCTCCAAATAGGTATATTAAAGTTGCTGCAGTTAAAAAG...
S. cereviceae ...GTGCCAGCAGCCGCGGTATAATTCCAGCTCCAAATAGGTATATTAAAGTTGCTGCAGTTAAAAAG...
Zea maize ...GTGCCAGCAGCCGCGGTATAATTCCAGCTCCAAATAGGTATATTAAAGTTGCTGCAGTTAAAAAG...
Escherichia coli ...GTGCCAGCAGCCGCGGTATAATTCCAGCTCCAAATAGGTATATTAAAGTTGCTGCAGTTAAAAAG...
Anacystis nidulans ...GTGCCAGCAGCCGCGGTATAATTCCAGCTCCAAATAGGTATATTAAAGTTGCTGCAGTTAAAAAG...
Thermotoga maritima ...GTGCCAGCAGCCGCGGTATAATTCCAGCTCCAAATAGGTATATTAAAGTTGCTGCAGTTAAAAAG...
Methanococcus vannielii ...GTGCCAGCAGCCGCGGTATAATTCCAGCTCCAAATAGGTATATTAAAGTTGCTGCAGTTAAAAAG...
Thermococcus celer ...GTGCCAGCAGCCGCGGTATAATTCCAGCTCCAAATAGGTATATTAAAGTTGCTGCAGTTAAAAAG...
Sulfolobus sulfotaricus ...GTGCCAGCAGCCGCGGTATAATTCCAGCTCCAAATAGGTATATTAAAGTTGCTGCAGTTAAAAAG...

Evolutionary “Clock”

QUANTITATIVELY INFER RELATIONSHIPS
A Remarkably Different View of Biodiversity

ALL plants, animals and fungi

Bacteria

Plantae

Fungi

Animalia

Archaea

Protista

Eucarya

Monera
Division/Phyla: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
The Face of *Bacillus subtilis*

Species

>99.8% identical 16S rRNA
What is *Bacillus subtilis*?
I. B. subtilis Genotypic Diversity

II. B. subtilis Phenotypic Diversity
Why *Bacillus subtilis*?
Where In the World Is *Bacillus subtilis*?
**B. subtilis** Isolation

- Heat soil sample (10 µg - 500 mg)
- Plate for colonies, colony purify 3X (all morphotypes)
- Multiplex PCR with “species-specific” 16S rRNA primers
Gene Phylogeny of *Bacillus subtilis*

16S rRNA gene sequence

99.8% nt identity

B. vallismortis

B. subtilis spizizenii

B. subtilis subtilis

**gyrA gene sequence**
Measuring Genotypic Diversity

16S → \textit{gyrA} or other conserved loci

Resolution

Strain A’s genome

\texttt{...ATGTGTCGTTAGCCCTAGCTATATTATCGGG...}

Strain B’s genome

\texttt{...ATGAGTCGTAGGCCAAGCTATATTATCGGG...}
Measuring Genotypic Diversity

16S → gyrA → Genome Sequences

or other conserved loci

Resolution

Strain A’s genome

Strain B’s genome

STRAIN A

STRAIN B
Measuring Genotypic Diversity

16S → gyrA → Genome Sequences

or other conserved loci

Resolution

Time & Money

Microarray-based Comparative Genomic Hybridization
What Is a DNA Microarray?

Chemically-treated glass microscope slides that are arrayed with known pieces of DNA that represent all of the genes from a sequenced representative of your species of interest.
M-CGH Experimental Design
(Microarray-based Comparative Genomic Hybridization)

Isolate Genomic DNA from Control (168) and Test Strain

↓

Sau3AI Digest Genomic DNA Samples

↓

Differentially Label Fragments with Fluorescent Nucleotides

Control

↓

Test

↓

Hybridize, Wash

Measure Fluorescence in 2 channels
red/green

Analyze the data to identify genes that are present/diverged in test strain
M-CGH Reveals Extensive Genome Diversity Among B. subtilis
M-CGH Reveals Extensive Genome Diversity Among *B. subtilis*

Bsu168 deleted for the *eps* operon
M-CGH as a Tool for Phylogeny

Gene phylogeny

Gene order (starting from ori)
Examining Genome Diversity with Microarrays: Comparison to Other Bacterial Species

- **S. epidermidis (42)**: 36%
- **E. coli (22)**: 35%
- **P. aeruginosa (25)**: 4%
- **M. tuberculosis (16)**: 2%

= divergent loci
Examining Genome Diversity with Microarrays: Comparison to Other Bacterial Species

- **S. epidermidis (42)** 36%
- **E. coli (22)** 35%
- **B. subtilis (32)** 40%
- **P. aeruginosa (25)** 4%
- **M. tuberculosis (16)** 2%

= divergent loci
M-CGH Limitations
New *Bacillus subtilis* Genomes!!!

*B. subtilis* subsp. *spizizenii*

*B. subtilis* subsp. *subtilis*
Three *B. subtilis* Strains Only Share 60% of Their Genes!

Strain 168

Strain RO-NN-1

Strain TU-B-10

Total ORFs: 5432

540 74 665

5432 (61.3%)

160 289

372
I. *B. subtilis* Genotypic Diversity

II. *B. subtilis* Phenotypic Diversity
Biofilms: Microbial Communities on Surfaces
Studying Biofilm Formation in *B. subtilis*

3610 Pellicle

3610 Colony
Biofilm Development Requires a Lifestyle Switch

motility

biofilm formation
Regulation of *Bacillus subtilis*’ Lifestyle Switch

motility

SinR

SinI

extracellular matrix production
Extracellular Matrix: Structural Players in 3610

- Exopolysaccharide (EPS)
- SinR
- Protein (TasA)
The Face of *Bacillus subtilis*
Microarray- genes appear to be present

Amino acid identity

**RO-NN-1** (SUB) 90-99%

**TU-B-10** (SPI) 88-98%
EPS Required for Biofilm Formation in Wild Strains

Exopolysaccharide (EPS)  SinR  Protein (TasA)

wild types

mutants
Microarray- genes appear to be present

Amino acid identity

RO-NN-1 (SUB) 95-99%
TU-B-10 (SPI) 88-97%
TasA Not Essential For Biofilm Development in Some Wild Strains

Exopolysaccharide (EPS)  SinR  Protein (TasA)

wild types

mutants
EPS Overexpression Suppresses ΔtasA Phenotype

Wild type

ΔtasA EPS↑

ΔtasA

Δeps TasA↑
Regulators of Biofilm Formation: SinR & SinI

motility

SinR

matrix production
SinR Controls Wild Strain Biofilm Development

motility

matrix production

wild types

mutants
SinI Essential for Biofilm Development in Wild Strains

Wild types

![Wild Type Images]

Mutants

![Mutant Images]
Variation In Regulators of SinI: Likely Culprits

motility

extracellular matrix
Upstream Regulation Very Complex
Summary

M-CGH analysis of *B. subtilis* strains suggested that there was a large amount of genome variation among members of this species- in spite of their sharing nearly 100% 16S rRNA sequence identity.
Thank you!

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