

# The role of culture collections and DNA banks in the Genomic Encyclopedia of *Bacteria* and *Archaea* (GEBA)

*‘how to produce the new wine to fill the old skins’*

Hans-Peter Klenk

WFCC ICC-12 Conference 2010

Florianópolis, Santa Catarina, Brasil, September 29, 2010



## Summary

Introduction to the **G**enomic **E**ncyclopaedia  
of **B**acteria & **A**rchaea

The GEBA production pipeline: Collection & DNA Bank part

Metadata and dissemination of results: *SIGS, Standards in Genomic Sciences* (Genomic Standards Consortium)

First results and status of GEBA: Chapter 1 published

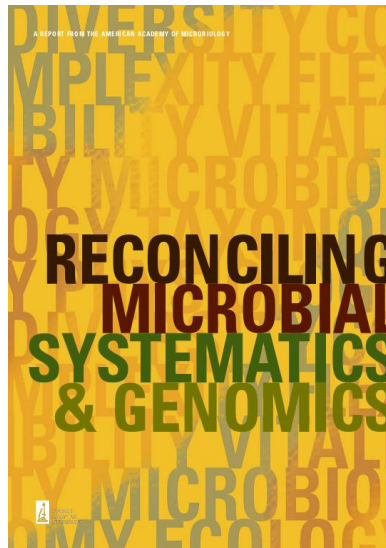
Perspective: The Microbial Earth Project

# Recommendations of the American Academy of Sciences Colloquium on *Reconciling Microbial Systematics & Genomics*

... to coordinate an international effort to construct draft genome sequences of each of the roughly 6500 type strains deposited in public strain collections.

... continue with the collection of genome sequences as new species and type strains are described.

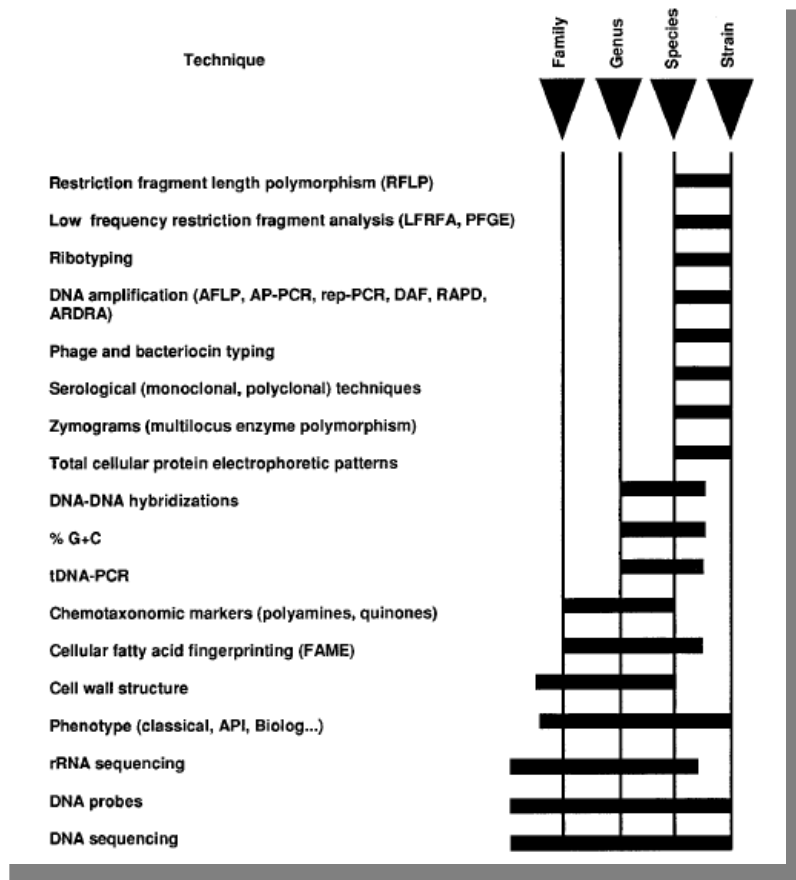
... revise and use phenotypic tests to consider genomics data for an improved species definition.



*Reconciling Microbial Systematics & Genomics*  
Buckley & Roberts, 2006

## Classic Methods for Polyphasic Taxonomy

are based on the analysis of DNA, RNA, proteins, chemotaxonomic markers and expressed features



- laborious
- time consuming
- technically demanding
- standardization required
- not suitable for all organisms

### Chemotaxonomy:

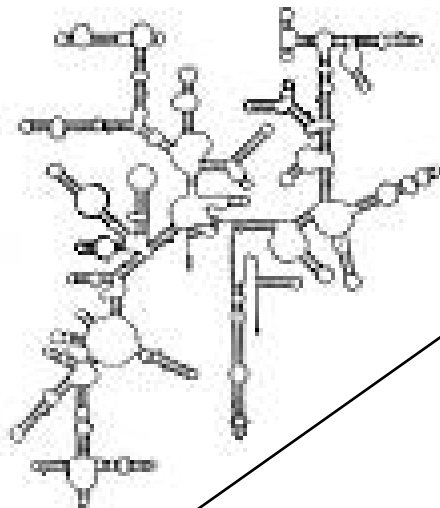
Application of analytical methods to collect information on various chemical constituents of the cells

*Vandamme et al. 1996*

# From Combination of Single Marker Molecules and Physiological Features to the Analysis of Whole Genomes

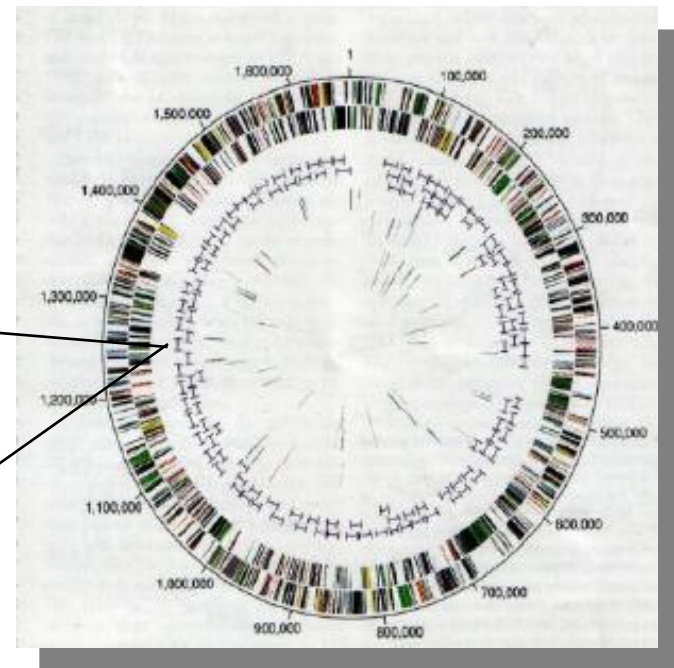
Limitations of 16S rRNA,  
the dominant marker molecule

- limited sequence space
- interoperon differences 0-9%
- interstrain differences 0-16%
- seq. identity // strain identity



16S rRNA

ca. 0,1%

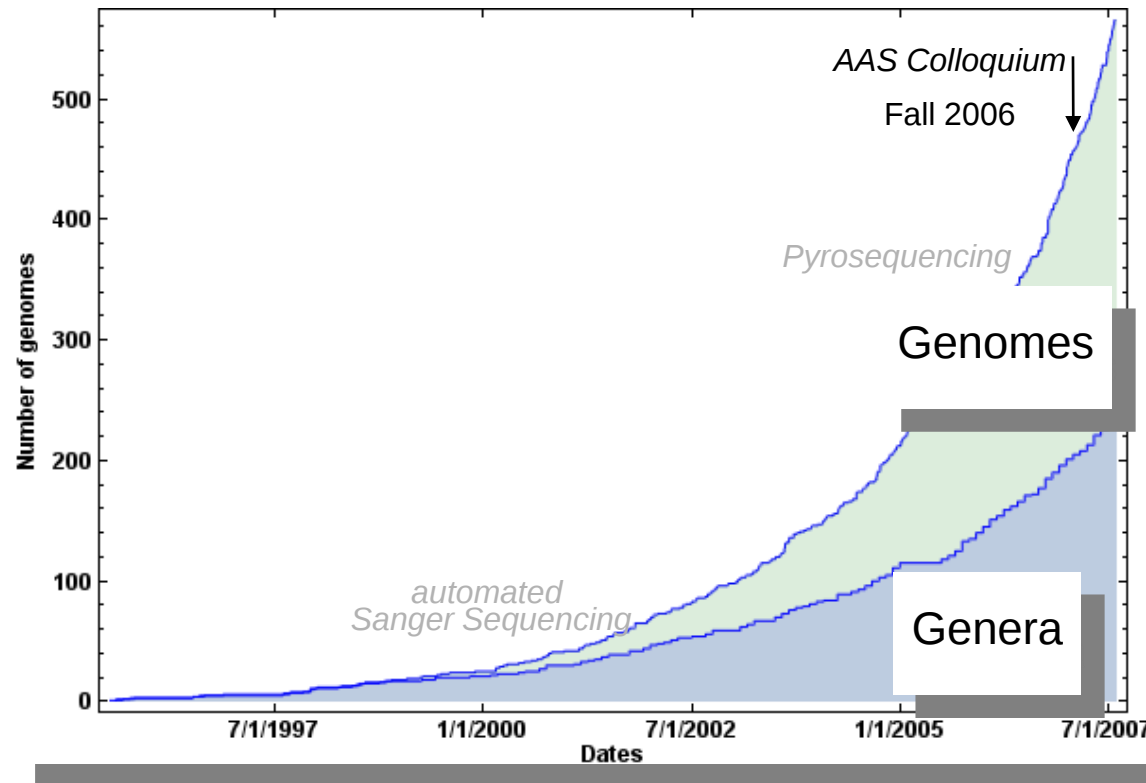


Genome

## Growth of Microbial Genome Sequences

September 26<sup>th</sup> 2010: 1143 bacterial and 94 archaeal completed genome sequences from about 280 genera  
4942 ongoing bacterial genome sequencing projects

*data from [www.genomesonline.org/gold](http://www.genomesonline.org/gold)*



## Our Aim:

**Use of whole-genome sequences to infer the phylogenetic position of organisms in order to support the taxonomic assessment of species**

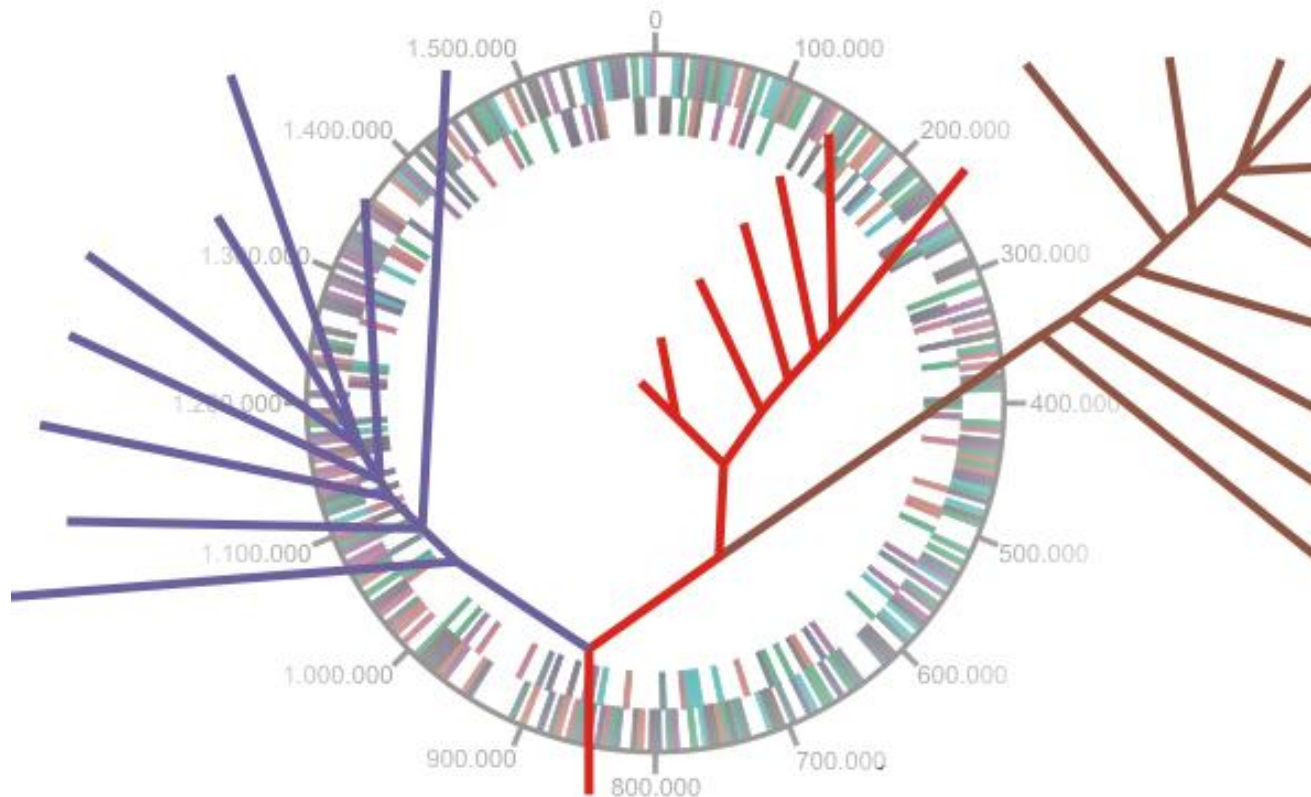


Figure: [molecularbiotechnology.ugent.be](http://molecularbiotechnology.ugent.be)

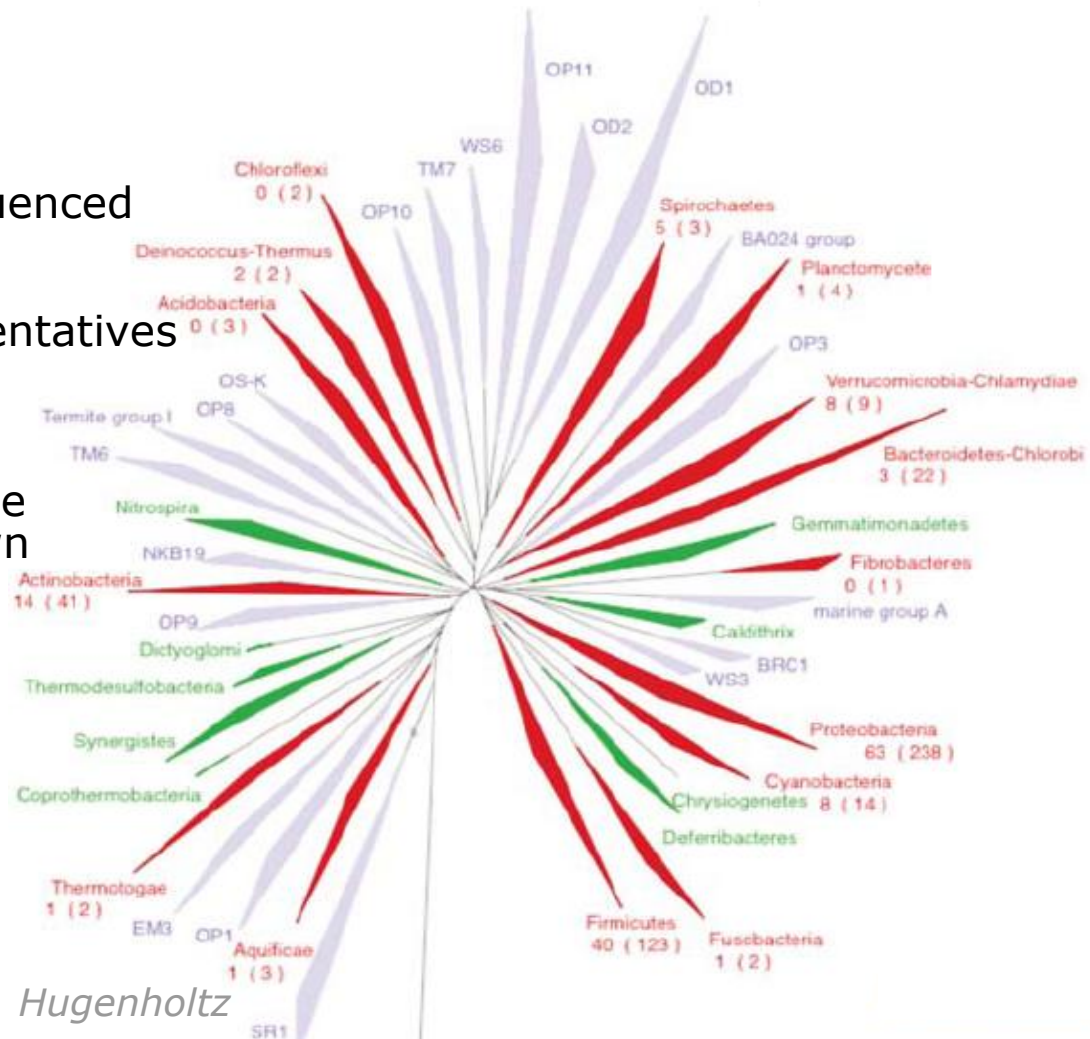
## Phylogenetic Tree of the Bacteria Indicating the Status of Known Genome Sequences

pale blue:  
no genome sequenced

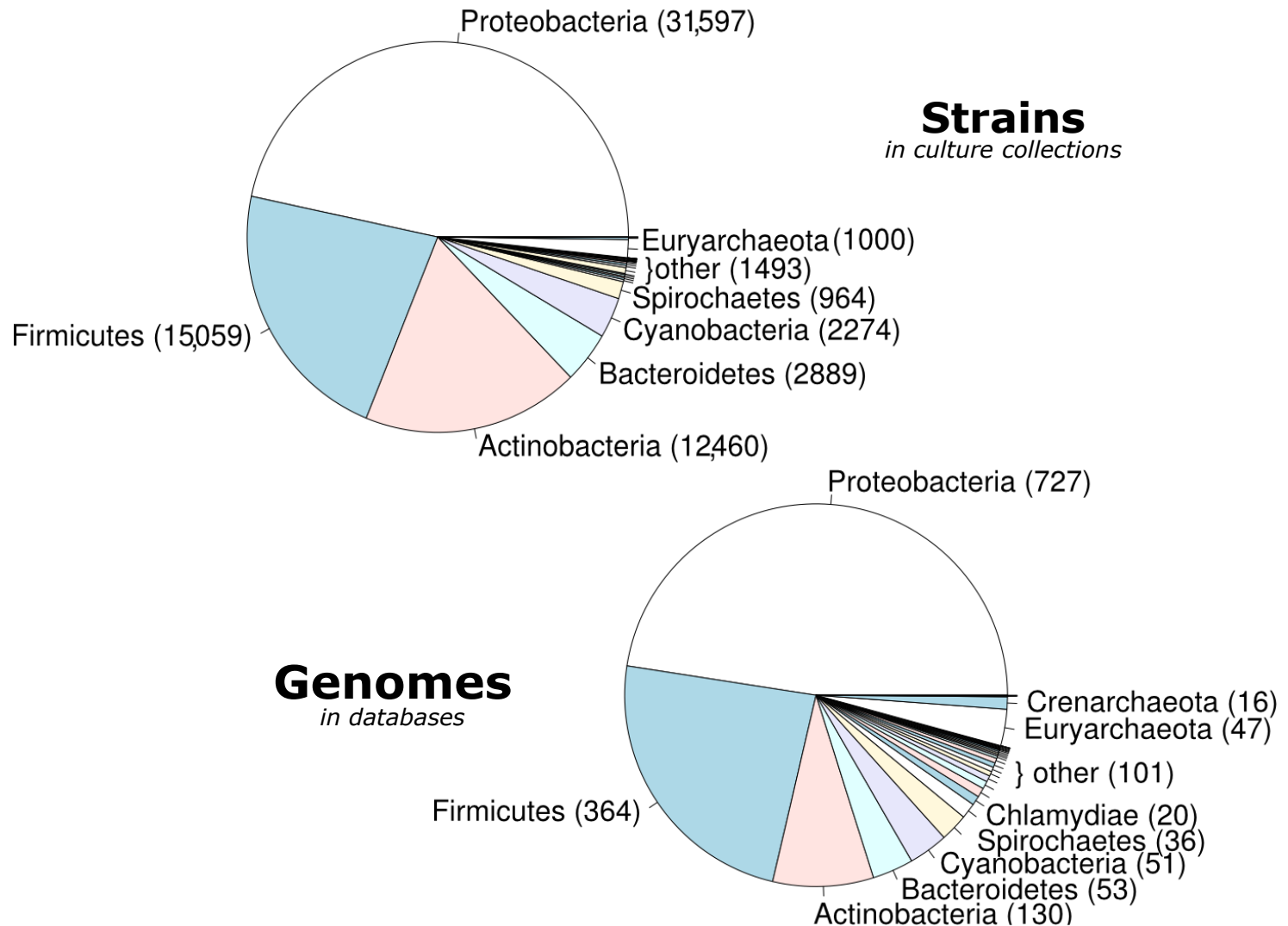
green:  
only few representatives  
sequenced

red:  
plenty of genome  
sequences known

*Proteobacteria*  
*Firmicutes*  
*Actinobacteria*



# Diversity of Cultivated Strains and Publicly Available Genome Sequences (Draft and Finished, 2009)



## Proposed Solution for the Uneven Sampling of Genome Sequences:

Use a phylogenetic tree for the selection of new genomes to be sequenced

### 'Key idea of GEBA'

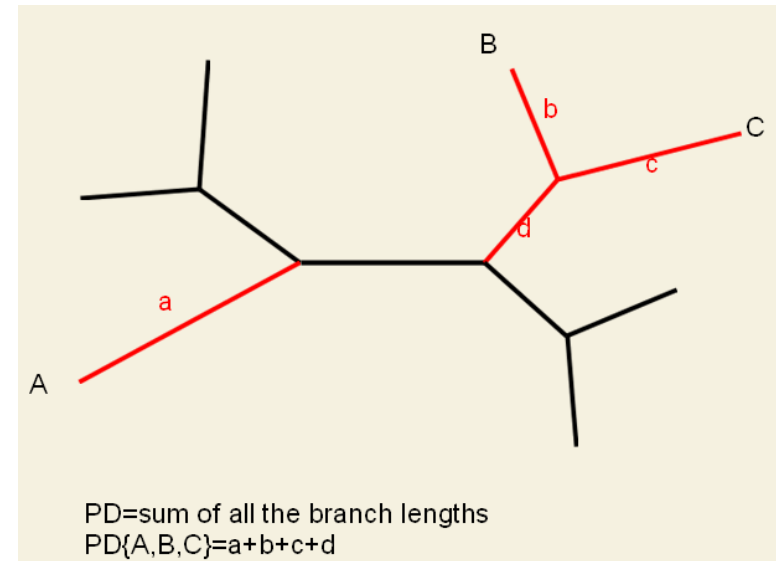
#### Approach

Take rRNA *Tree of Life*

Overlay finished and in progress genome sequencing projects onto it

Rank phylotypes by how they fill into gaps of the tree

Use phylogenetic diversity as measure for strain selection



### Strain selection for GEBA main project

Dongying Wu, JE, PH, NCK, HPK

Phylogenetic Distance (PD) procedure



## The GEBA Genomes Production Pipeline

Selection of target strains

Growth of cell paste

Extraction of high molecular gDNA

QC, confirmation of identity

Construction of sequenc. libraries

Production of draft sequences

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about 20 variations for cell lysis, (ATCC 2%)

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small and large insert size

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Oak Ridge annotation pipeline, manual curation

IMG

after final annotation

after release by GenBank

original description in IJSEM, PubMed

following GSC suggestions

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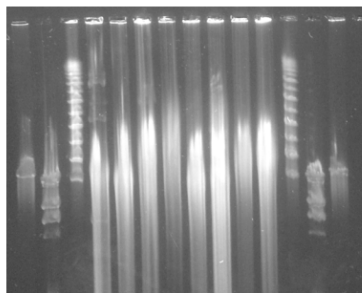
Collection (DSMZ), DNA Bank (DSMZ), Sequencing Center (JGI), jointly JGI-DSMZ

## Input of DSMZ to GEBA

# Quality controlled high molecular DNAs from type strains

### Lengths of Genomic DNA Determined by Pulsed Field Gel Electrophoresis (PFGE)

1 2 3 4 5 6 7 8 9 10 11 12 13 14



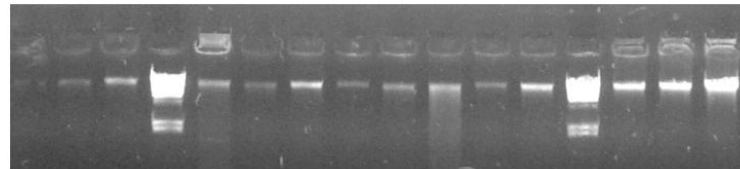
kb

Lane 1: T7 DNA Mass Marker (60 ng)  
Lane 2: DNA Molecular Weight Marker II  
Lane 3: Mid Range Marker II  
Lane 4: DSM 17242, *Alistipes finegoldii*  
Lane 5: DSM 20460, *Megasphaera elsdenii*  
Lane 6: DSM 5511, *Haloterrigena turkmenica*  
Lane 7: DSM 2008, *Veillonella parvula*  
Lane 8: DSM 20758, *Selenomonas sputigena*  
Lane 9: DSM 12940, *Halorhabdus utahensis*  
Lane 10: DSM 1135, *Leptotrichia buccalis*  
Lane 11: DSM 12286, *Halomicrobium mukohataei*  
Lane 12: Mid Range Marker II  
Lane 13: DNA Molecular Weight Marker II  
Lane 14: T7 DNA Mass Marker (60 ng)

PFGE of the genomic DNA of the strains was performed in a contour-clamped homogeneous electric field (CHEF) system on a CHEF-DR III device (Bio-Rad Laboratories, Hercules, Calif.) with 1% agarose gels and modified 0.5 TBE buffer (45 mM Tris, 45 mM boric acid, 0.1 mM EDTA) at 14°C. PFGE times used at 200 V (6 V/cm) were 1 to 15 s for 18 h.

### Quantification gel of the genomic DNA isolated from *Veillonella parvula* (DSM 2008T)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



Lane 1: c(λ-Marker)= 15 ng  
Lane 2: c(λ-Marker)= 30 ng  
Lane 3: c(λ-Marker)= 50 ng  
Lane 4: DNA Molecular Weight Marker II (Roche 236250)  
Lane 5: DSM 17242, *Alistipes finegoldii*; 5 µl, 1:50  
Lane 6: DSM 20460, *Megasphaera elsdenii*; 5 µl, 1:50  
Lane 7: DSM 5511, *Haloterrigena turkmenica*; 5 µl, 1:50  
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Lane 13: DNA Molecular Weight Marker II (Roche 236250)  
Lane 14: c(λ-Marker)= 125 ng  
Lane 15: c(λ-Marker)= 250 ng  
Lane 16: c(λ-Marker)= 500 ng

### Microorganisms

8T) was taken from the German Collection of Microorganisms and Cell Cultures (DSMZ). The genomic DNA was isolated using the Qiagen Genomic 500 DNA Kit (Qiagen 10262). The genomic DNA was 20-150 kb in size as determined by Pulsed Field Gel Electrophoresis (PFGE). The bulk of DNA had a size of 50-100 kb (see attached PFGE image). The DNA concentration was estimated from the gel. Spectrophotometric measurements yielded a DNA concentration of 87.5 µg/µl as estimated from the gel. Spectrophotometric measurements yielded a DNA concentration of 87.5 µg/µl as estimated from the gel. Spectrophotometric measurements yielded a DNA concentration of 87.5 µg/µl as estimated from the gel.

### Microorganisms

## Input of DSMZ to GEBA

# Systematic collection of metadata including classification and phylogentic analysis (rRNA and whole-genome based).

# The DNA Bank Network

<http://www.dnabank-network.org/>

DNA Bank  
Network



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## Banking DNA for Biodiversity Genomics

The main focus of the **DNA Bank Network** is to enhance taxonomic, systematic, genetic, conservation and evolutionary studies by providing:

- at-cost availability of non-human DNA material,
- high quality, long-term storage of DNA material on which molecular studies have been performed, so that results can be verified, extended, and complemented,
- complete on-line documentation of each sample, including the provenance of the original material, the place of voucher deposit, information about DNA quality and extraction methodology, digital images of vouchers and links to published molecular data if available.

## Counts

|                   |       |
|-------------------|-------|
| Total DNA Samples | 36075 |
| Total Taxa        | 11908 |

## News

**September 15, 2010**

Congress "Tools for Identifying Biodiversity: Progress and Problems" in Paris, France [more...](#)

**May 20, 2010**

SPNHC & CBA-ABC Joint Conference in Ottawa, Canada [more...](#)

**May 18, 2010**

GEO Day of Biodiversity 2010 [more...](#)

Funded by

**DFG** Deutsche  
Forschungsgemeinschaft

Initiated by GBIF.de



DNA Bank Network - Main Menu

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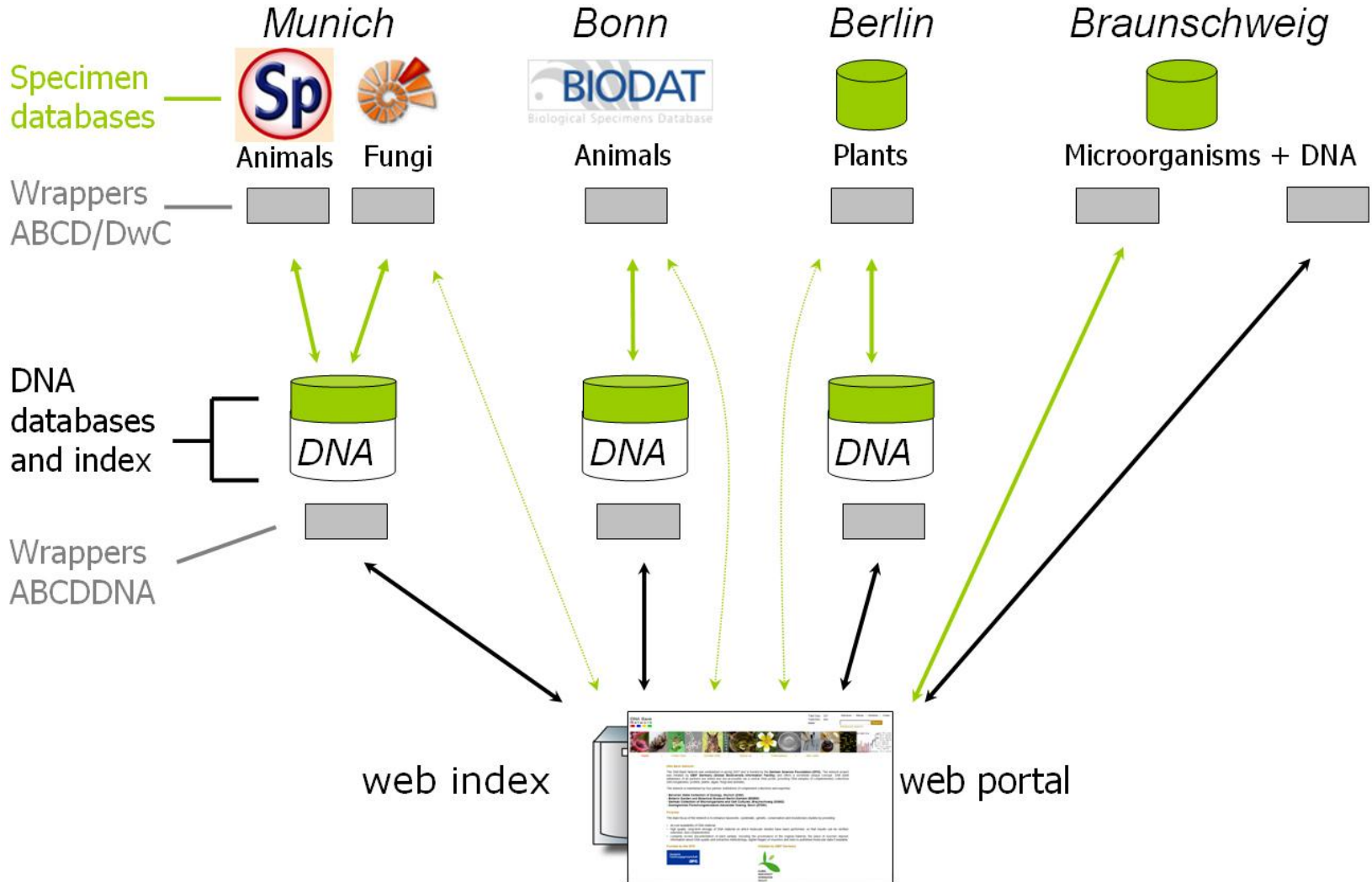









## DNA Bank Data Flow





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Selection of target strains

Growth of cell paste

Extraction of high molecular gDNA

QC, confirmation of identity

Construction of sequenc. libraries

Production of draft sequences

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Oak Ridge annotation pipeline, manual curation

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after final annotation

after release by GenBank

original description in IJSEM, SAM, PubMed

following GSC suggestions, MIGS

cell wall, lipids, quinones, ...

genomic basis of reported phenotypes

truly 'whole genome', protein- and DNA-based

pathway reconstructions and BIOLOG

for SIGS and other journals

about 250 DNAs on stock

Collection (DSMZ), DNA Bank (DSMZ), Sequencing Center (JGI), jointly JGI-DSMZ



# Standardisation and Dissemination



Standards in  
Genomic Sciences

An Open Access Journal of the Genomic Standards Consortium

## FOUNDING MEMBERS

Sam Angiuoli  
Baltimore, MD, USA

Patrick Chain  
Los Alamos, NM, USA

Dawn Field  
Oxford, UK

George M. Garrity  
East Lansing, MI, USA

Frank Oliver Glöckner  
Bremen, DE

Lynette Hirschman  
Bedford, MA, USA

Eugene Kolkar  
Seattle, WA, USA

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## Standards in Genomic Sciences

In the [current issue](#) (published July 28, 2010 - August 31, 2010)

### Short Genome Reports (GEBA)

[Chang et al., Complete genome sequence of \*Acidaminococcus fermentans\* type strain \(VR4<sup>T</sup>\)](#)

[Abt et al., Complete genome sequence of \*Cellulomonas flavigena\* type strain \(134<sup>T</sup>\)](#)

[Tindall et al., Complete genome sequence of \*Meiothermus ruber\* type strain \(21<sup>T</sup>\)](#)

[Sikorski et al., Complete genome sequence of \*Meiothermus silvanus\* type strain \(VI-R2<sup>T</sup>\)](#)

[LaButti et al., Complete genome sequence of \*Planctomyces limnophilus\* type strain \(Mü 290<sup>T</sup>\)](#)

[Sikorski et al., Complete genome sequence of \*Acetohalobium arabaticum\* type strain \(Z-7288<sup>T</sup>\)](#)

[Göker et al., Complete genome sequence of \*Ignisphaera aqreqans\* type strain \(AQ1.S1<sup>T</sup>\)](#)

[Göker et al., Complete genome sequence of \*Olsenella uli\* type strain \(VPI D76D-27C<sup>T</sup>\)](#)

[LaButti et al., Permanent draft genome sequence of \*Dethiosulfovibrio peptidovorans\* type strain \(SEBR 4207<sup>T</sup>\)](#)

### Meeting Reports

[Kyrpides et al., Meeting Report from the Genomic Standards Consortium \(GSC\) Workshop 8](#)

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# The GEBA Report Series in *Stand Genomic Sci*

Standards in Genomic Sciences, Vol 1, No 1 (2009)

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Stand. Genomic Sci. 2009 1:1  
ISSN 1944-3277  
doi:10.4056/sigs.1463

## Complete genome sequence of *Acidimicrobium ferrooxidans* type strain (ICP<sup>T</sup>)

Alicia Clum<sup>1</sup>, Matt Nolan<sup>1</sup>, Elke Lang<sup>2</sup>, Tijana Glavina Del Rio<sup>1</sup>, Hope Tice<sup>1</sup>, Alex Copeland<sup>1</sup>, Jan-Fang Cheng<sup>1</sup>, Susan Lucas<sup>1</sup>, Feng Chen<sup>1</sup>, David Bruce<sup>3</sup>, Lynne Goodwin<sup>3</sup>, Sam Pittluck<sup>1</sup>, Natalia Ivanova<sup>1</sup>, Konstantinos Mavrommatis<sup>1</sup>, Natalia Mikhailova<sup>1</sup>, Anurita Pati<sup>1</sup>, Amy Chen<sup>1</sup>, Krishna Palaniappan<sup>1</sup>, Markus Göker<sup>2</sup>, Stefan Spring<sup>2</sup>, Miriam Land<sup>2</sup>, Loren Hauser<sup>2</sup>, Yun-Juan Chang<sup>2</sup>, Cynthia C. Jeffries<sup>2</sup>, Patrick Chain<sup>1</sup>, Jim Bristow<sup>1</sup>, Jonathan A. Eisen<sup>1,7</sup>, Victor Markowitz<sup>4</sup>, Philip Hugenholtz<sup>1</sup>, Nikos K. Kyrpides<sup>1</sup>, Hans-Peter Klenk<sup>2</sup>, Alla Lapidus<sup>1\*</sup>

<sup>1</sup> DOE Joint Genome Institute, Walnut Creek, California, USA

<sup>2</sup> DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany

<sup>3</sup> Los Alamos National Laboratory, Bioscience Division, Los Alamos, New Mexico USA

<sup>4</sup> Biological Data Management and Technology Center, Lawrence Berkeley National Laboratory, Berkeley, California, USA

<sup>5</sup> Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

<sup>6</sup> Lawrence Livermore National Laboratory, Livermore, California, USA

<sup>7</sup> University of California Davis Genome Center, Davis, California, USA

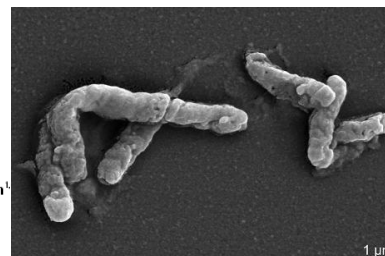
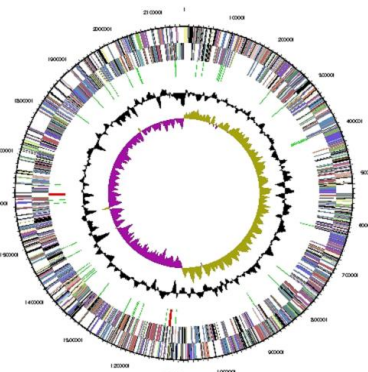
\* Corresponding author: [AllaLapidus](mailto:AllaLapidus@lbl.gov)

Print publication date: July 20, 2009.

### Abstract

*Acidimicrobium ferrooxidans* (Clark and Norris 1996) is the sole and type species of only genus within the actinobacterial family *Acidimicrobiaceae* and in the order *Acidimicrobiales* during autotrophic growth in the absence of an enhanced CO<sub>2</sub> concentration is we describe the features of this organism, together with the complete genome sequi complete genome sequence of the order *Acidimicrobiales*, and the 2,158,157 bp lo protein coding and 54 RNA genes is part of the *Genomic Encyclopedia of Bacteria ar*.

Keywords: Moderate thermophile, ferrous-iron-oxidizing, acidophile, *Acidimicrobiales*.



Current classification

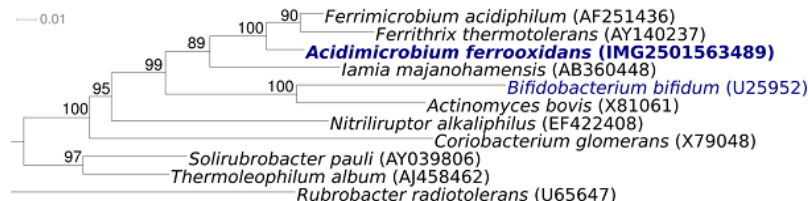
Table 1. Classification and general features of *A. ferrooxidans* ICP<sup>T</sup> based on MIGS recommendations [11]

| MIGS ID | Property               | Term                                                                         | Evidence code <sup>a,b</sup> |
|---------|------------------------|------------------------------------------------------------------------------|------------------------------|
|         |                        | Domain <i>Bacteria</i>                                                       |                              |
|         |                        | Phylum <i>Actinobacteria</i>                                                 | TAS [12]                     |
|         |                        | Class <i>Actinobacteria</i>                                                  | TAS [13]                     |
|         |                        | Order <i>Acidimicrobiales</i>                                                | TAS [13]                     |
|         |                        | Suborder <i>Acidimicrobineae</i>                                             |                              |
|         |                        | Family <i>Acidimicrobiaceae</i>                                              | TAS [13]                     |
|         |                        | Genus <i>Acidimicrobium</i>                                                  | TAS [11]                     |
|         |                        | Species <i>Acidimicrobium ferrooxidans</i>                                   | TAS [11]                     |
|         | Type strain            | ICP                                                                          |                              |
|         | positive               |                                                                              | TAS [11]                     |
|         | rod shaped             |                                                                              | TAS [11]                     |
|         | motile                 |                                                                              | TAS [11]                     |
|         | nonsporulating         |                                                                              | TAS [11]                     |
|         | temperature range      | moderate thermophile, 45–50°C                                                | TAS [11]                     |
|         | temperature            | 48°C                                                                         | TAS [11]                     |
|         | not reported           |                                                                              |                              |
|         | aerobic                |                                                                              | TAS [11]                     |
|         | Carbon source          | CO <sub>2</sub> (autotrophic), yeast extract (heterotrophic)                 | TAS [11]                     |
|         |                        | autotrophic: oxidation of ferrous iron with oxygen as the electron acceptor; |                              |
|         | Energy source          | heterotrophic: yeast extract                                                 | TAS [11]                     |
|         |                        | warm, acidic, iron-, sulfur-, or mineral-sulfide rich environments           |                              |
|         | Habitat                | free living                                                                  | NAS                          |
|         | Biotic relationship    | none                                                                         | NAS                          |
|         | Pathogenicity          | 1                                                                            | TAS [14]                     |
|         | Biosafety level        | hot springs                                                                  | TAS [2]                      |
|         | Isolation              | Krísuvík geothermal area, Iceland                                            | TAS [2]                      |
|         | Geographic location    | before 1993                                                                  | TAS [11]                     |
|         | Sample collection time |                                                                              |                              |
|         | Latitude – Longitude   | 63.93, -22.1                                                                 | TAS [2]                      |
|         | Depth                  | not reported                                                                 |                              |
|         | Altitude               | not reported                                                                 |                              |

Evidence codes - IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the [Gene Ontology](http://www.geneontology.org/) project [15]. If the evidence code is IDA the property was directly observed for a live isolate by one of the authors or an expert mentioned in the acknowledgements.

Table 2. Genome sequencing project information

| MIGS ID   | Property                   | Term                                                           |
|-----------|----------------------------|----------------------------------------------------------------|
| MIGS-31   | Finishing quality          | Finished                                                       |
|           |                            | One Sanger library: 8kb pMCL200                                |
| MIGS-28   | Libraries used             | One 454 pyrosequence standard library and one Illumina library |
| MIGS-29   | Sequencing platforms       | ABI3730, 454 GS FLX, Illumina GA                               |
| MIGS-31.2 | Sequencing coverage        | 6.8 x Sanger; 52.9 x pyrosequence                              |
| MIGS-30   | Assemblers                 | Newbler, Arachne                                               |
| MIGS-32   | Gene calling method        | Prodigal                                                       |
|           | INSDC / Genbank ID         | CP001631                                                       |
|           | Genbank Date of Release    | not available                                                  |
|           | GOLD ID                    | <a href="http://www.gold.org/">G01021</a>                      |
|           | Database: IMG-GEBA         | 2501533204                                                     |
| MIGS-13   | Source material identifier | DSM 10331                                                      |
|           | Project relevance          | Tree of Life, GEBA                                             |



# Novel Approach Towards Research Coordination Genomics Standards Consortium

*towards a richer set of information to describe our complete genome collection*



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## Main Page



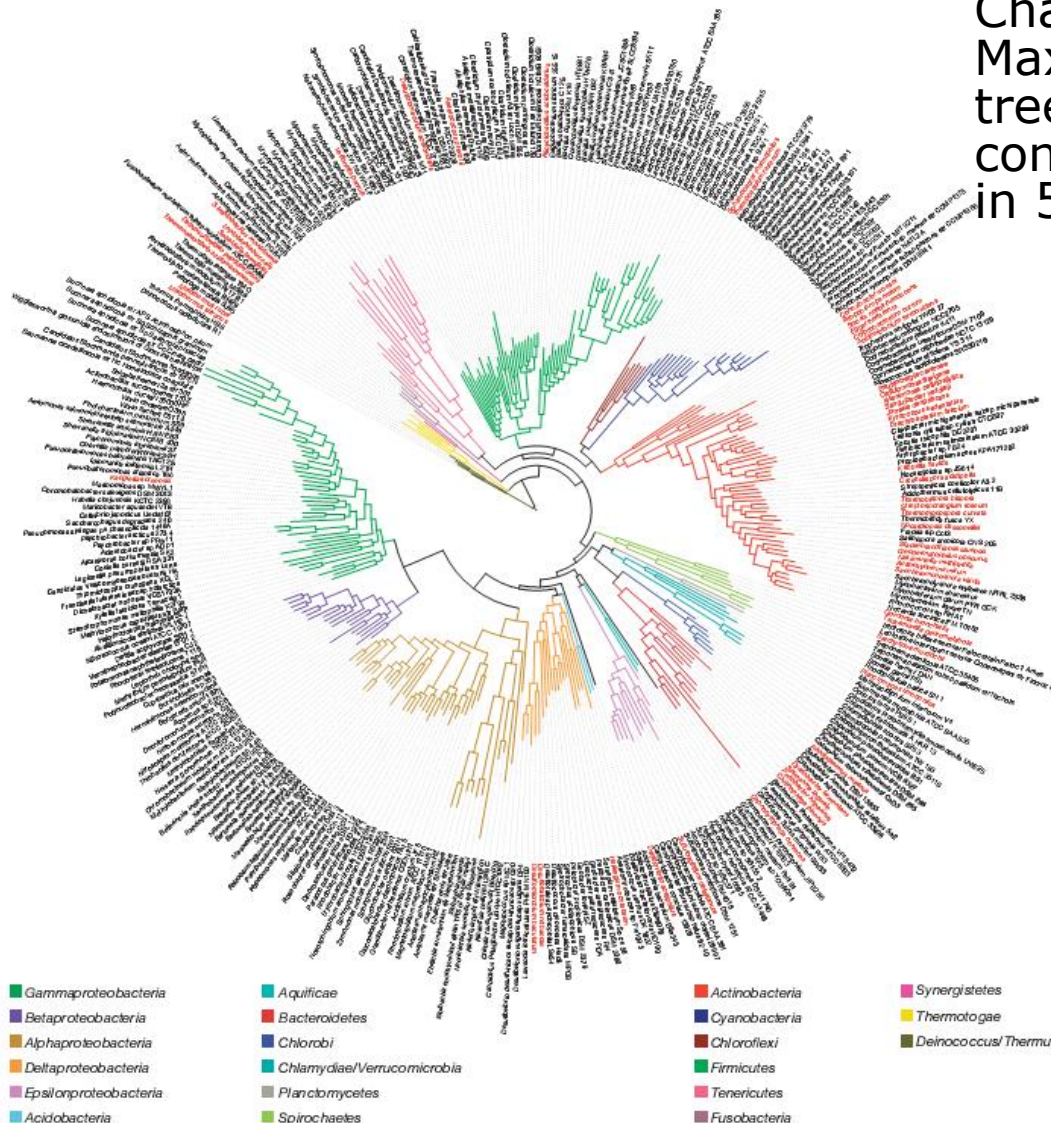
## GSC Mission

Community-driven standards have the best chance of success if developed within the auspices of international working groups. Participants in the GSC include biologists, computer scientists, those building genomic databases and conducting large-scale comparative genomic analyses, and those with experience of building community-based standards.

The mission of the GSC is to work with the wider community towards:

- \* the implementation of new genomic **standards**
- \* methods of capturing and exchanging **metadata**
- \* harmonization of metadata collection and analysis efforts across the wider genomics community

# Chapter I (December 24<sup>th</sup> 2009) Maximum-likelihood phylogenetic tree based on 31 concatenated conserved protein-coding genes in 56 genomes



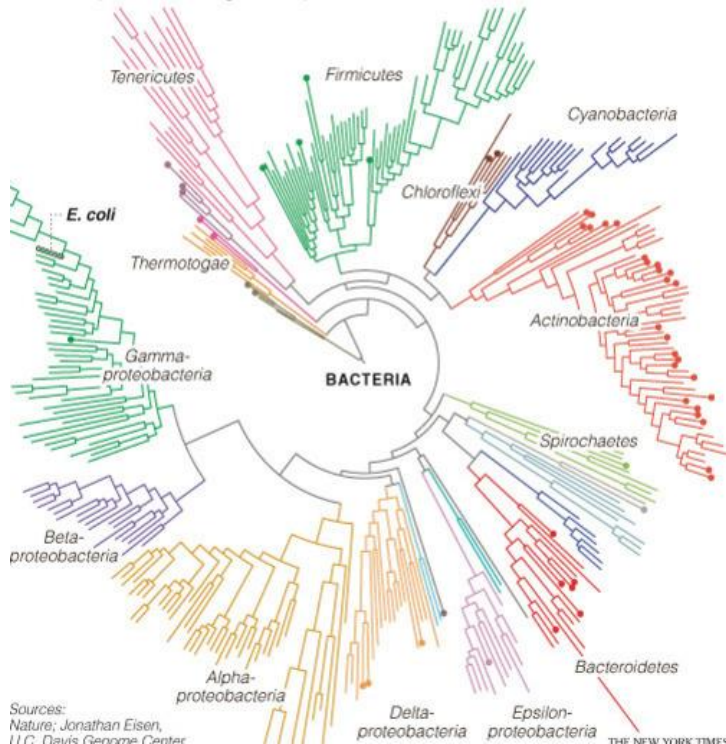
Status September 26<sup>th</sup> 2010

published: 65  
submitted: 12  
finished: 44  
in closure: 46  
in sequencing: ~100

total: ~300

## Filling Out the Branches

This "genome tree" shows relationships among the different species of bacteria that have had their genomes sequenced to date, with major phyla shown in different colors. A new project intended to expand the range and variety of sequenced microbes has completed its first 56 species, including the 53 species of bacteria marked below with dots.



## The New York Times

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December 29, 2009

# Scientists Start a Genomic Catalog of Earth's Abundant Microbes

## First 56 GEBA Genomes Published ...



## naturenews

Published online 23 December 2009 | Nature | doi:10.1038/news.2009.1161

News

## Microbial encyclopaedia guided by evolution

Sequencing project reveals microbial cache of protein families.

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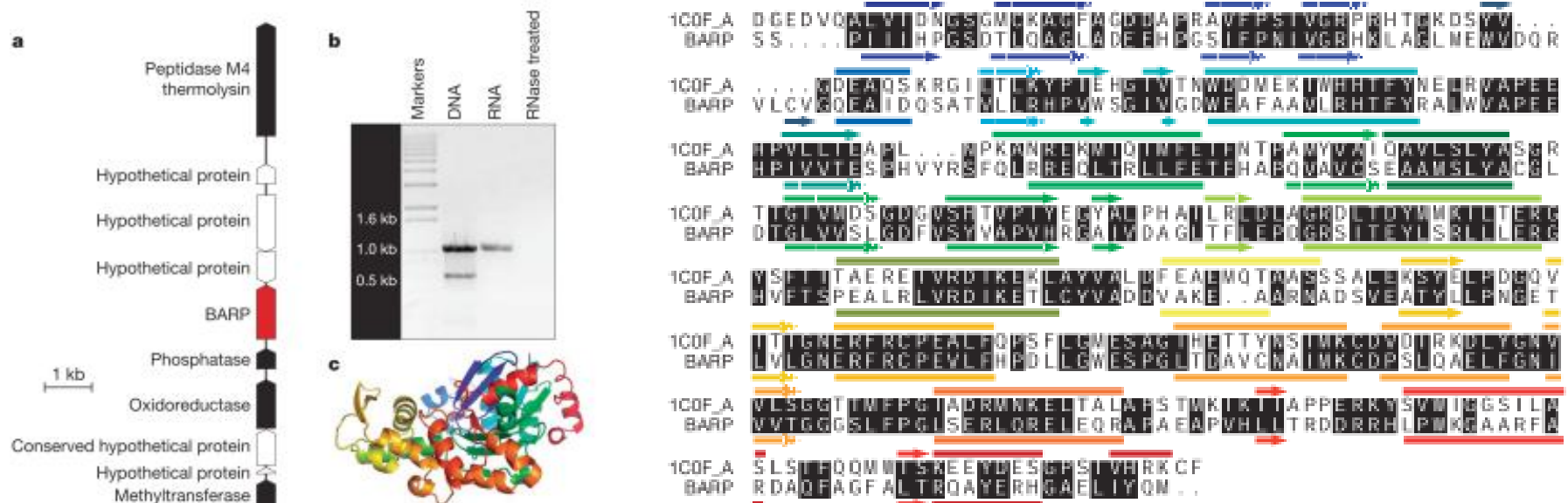


## Results: Effect of 16S rRNA Tree-Based Selection of Organisms on Comparative Genomics Metrics

| Comparative genomic metric                       | GEBA set | Random sets<br>(number of resamplings) | Fold<br>improvement |
|--------------------------------------------------|----------|----------------------------------------|---------------------|
| Genome tree phylogenetic diversity <sup>17</sup> |          |                                        |                     |
| Bacteria (domain)                                | 11.0     | 3.2 ± 0.7 (100)                        | 2.8-4.4             |
| Actinobacteria (phylum)                          | 4.3      | 1.4 ± 0.3 (100)                        | 2.5-3.9             |
| New protein family links                         | 46       | 3 ± 4 (5)                              | 6.6 to >15.3        |
| Genes in new chromosomal cassettes               | 71,579   | 16,579 ± 5,523 (20)                    | 3.2-6.5             |
| New gene fusions                                 | 433      | 65 ± 31 (20)                           | 4.5-12.7            |

GEBA genomes were compared to equivalently sized random sets of reference genomes to quantify the effect of phylogenetic selection.

## Results: A Bacterial Homologue of Actin



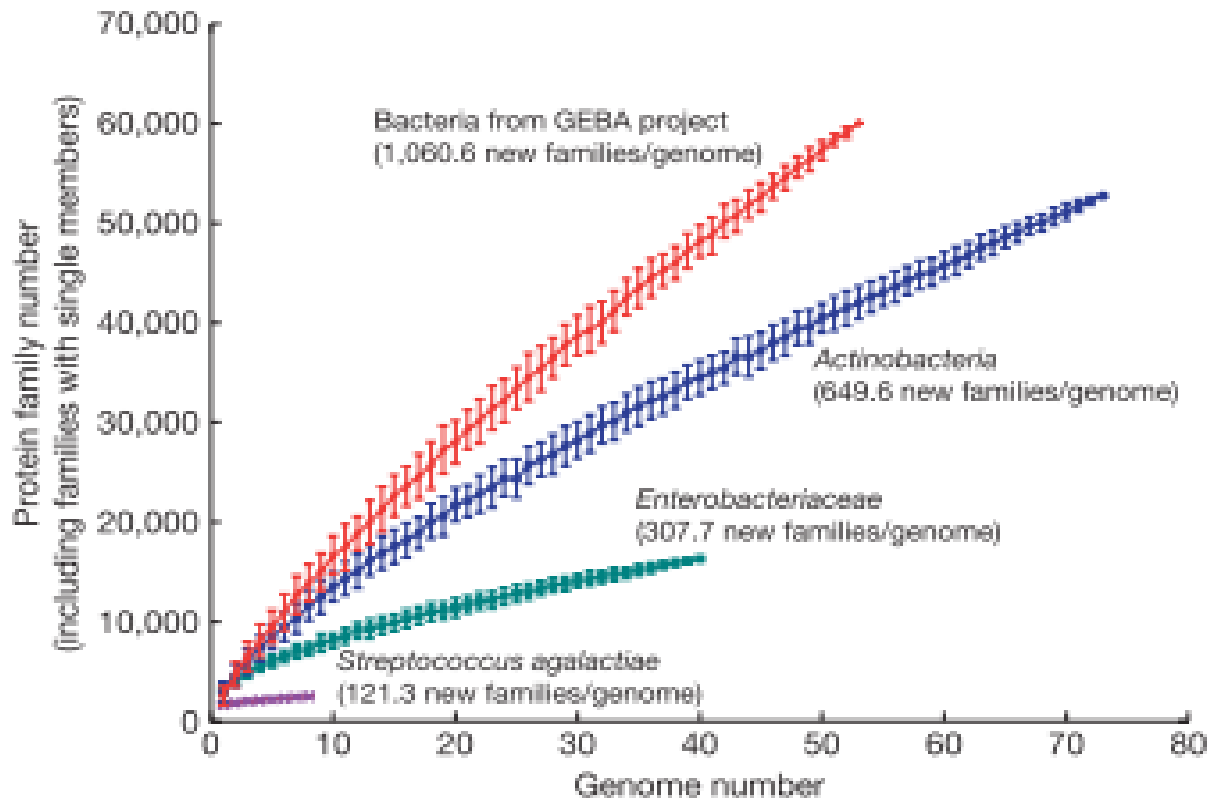
**a)** Genomic context of the bacterial actin-related protein (BARP) gene within the genome of the marine Deltaproteobacterium *H. ochraceum*. Red, gene encoding BARP; white, genes encoding hypothetical proteins; black, genes with functional annotations.

**b)** RT-PCR demonstration of expression of the gene encoding BARP in *H. ochraceum*.

**c)** Ribbon plot of the putative structure of BARP.

**d)** Alignment of BARP with actin from *Dictyostelium discoideum* with similarities in black shaded text. Secondary structure elements (arrows, beta-strands; bars, alpha-helices) are colour-coded as in c

## Results: Rate of Discovery of Protein Families as a Function of Phylogenetic Breadth of Genomes



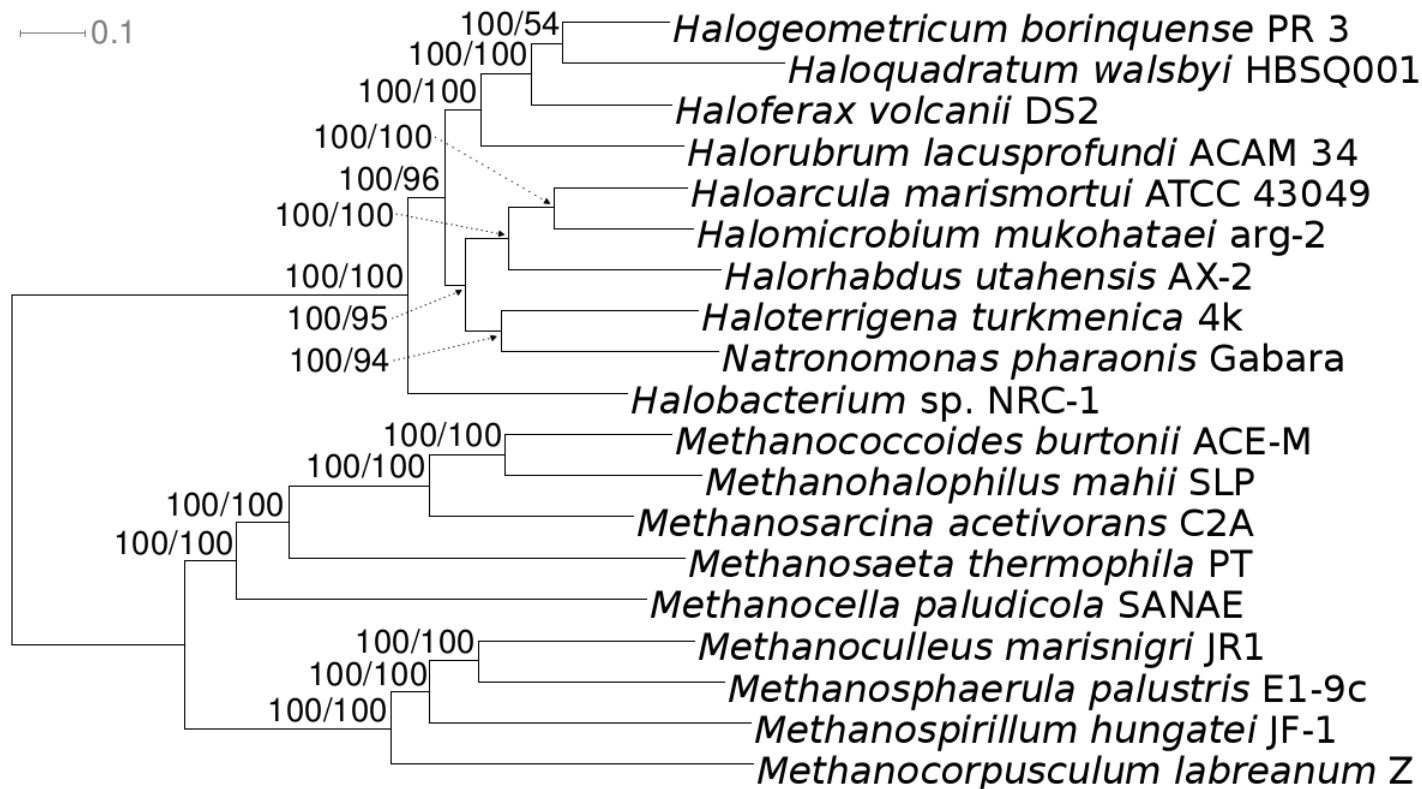
For each of four groupings (species, different strains of ***Streptococcus agalactiae***; family, ***Enterobacteriaceae***; phylum, ***Actinobacteria***; domain, **GEBA-Bacteria**), all proteins from that group were compared to each other to identify protein families. Then the total number of protein families was calculated as genomes were progressively sampled from the group (starting with one genome until all were sampled). This was done multiple times for each of the four groups using random starting seeds; the average and standard deviation were then plotted.

# Whole-genome-sequence analysis software

## Extremely Well Supported Phylogenies

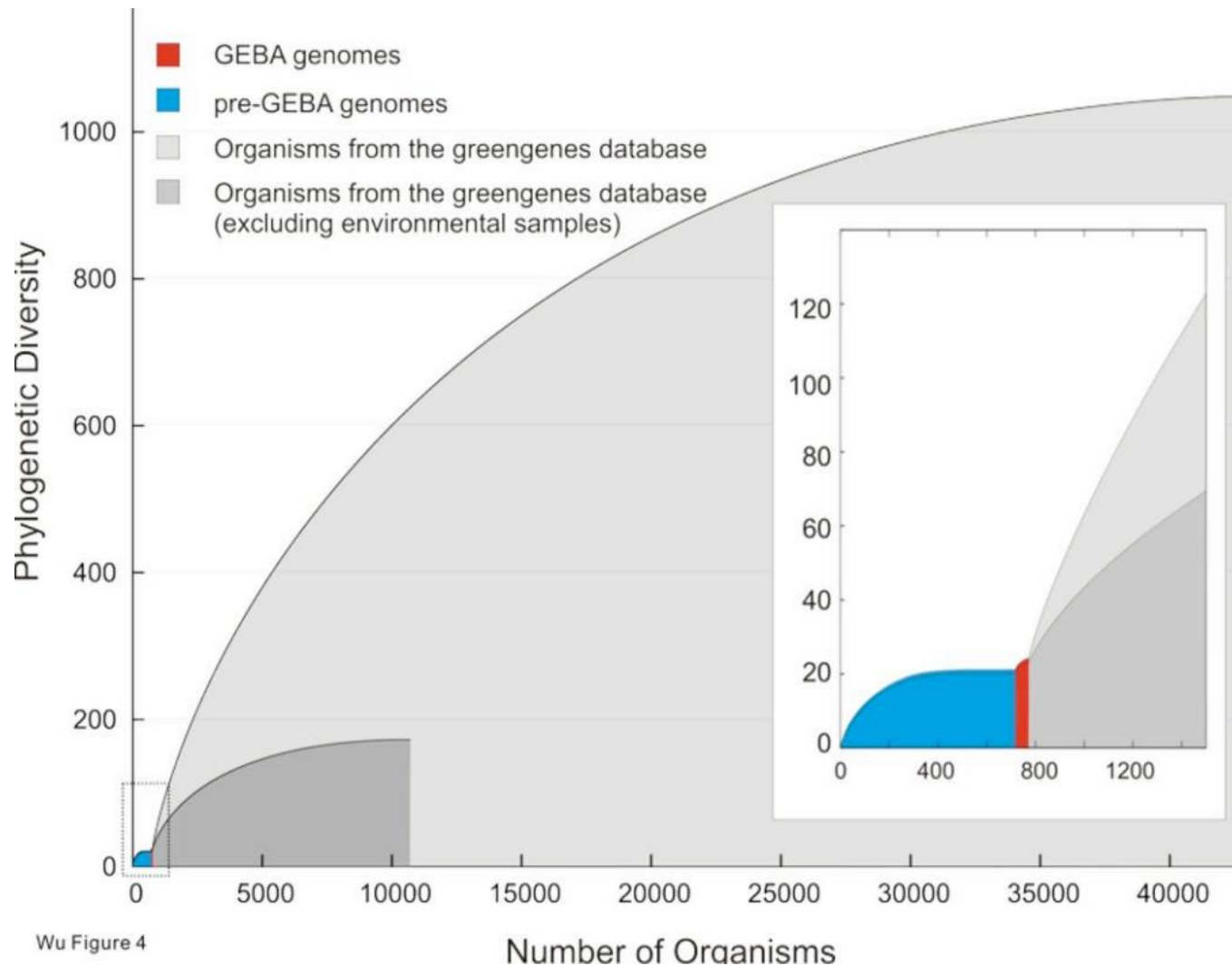
### From Genome Sequences and Proteomes

- generated by automated data handling pipelines



*Halobacteriaceae*

## Results: Estimation of Number of Genomes to be Required for a Overview on Genomic Diversity



## Perspective: The Microbial Earth Project

A systematic, genomic exploration  
of all validly-named species  
of bacteria and archaea.

The ambitious but assuredly tractable\* **goal** of the project, is **to sequence the genome of at least one representative of every bacterial and archaeal species** that has a validly published name in conformance with the Bacteriological Code.

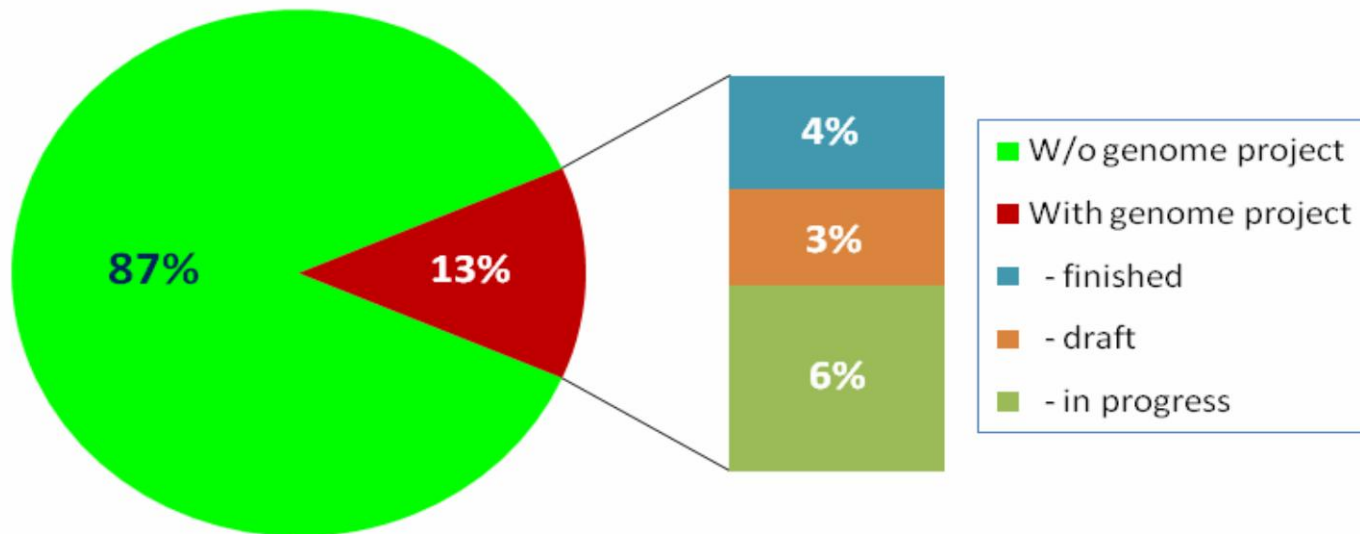
\* given an international effort and current technologies



## Status: Genome Project Coverage of Bacterial and Archaeal Type Strains

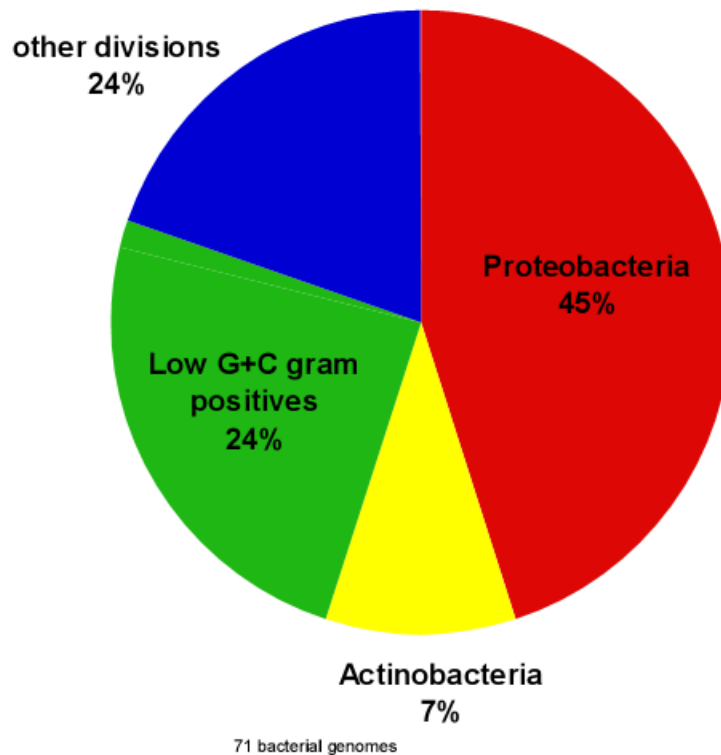
### Phase I:

Sequence one representative from every characterized microbial **type species**

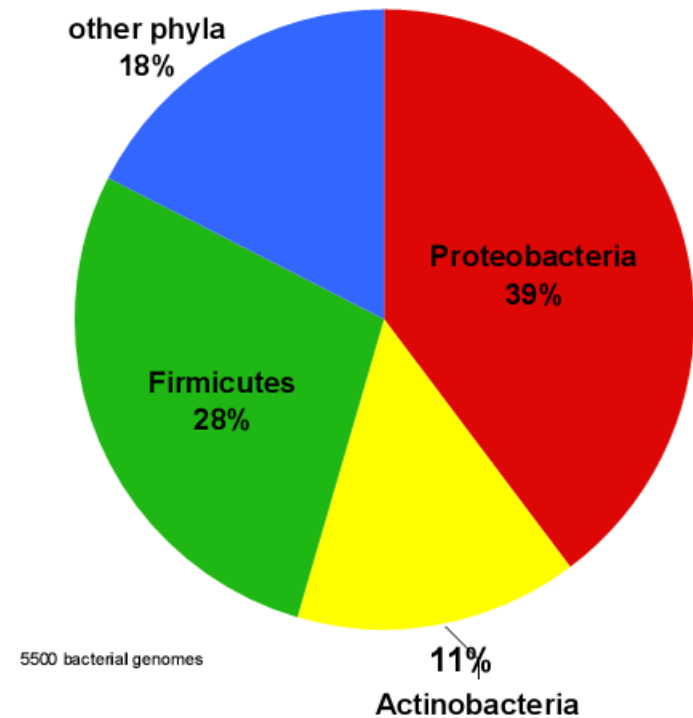


## What we Need to Change ...

Genome projects 2000

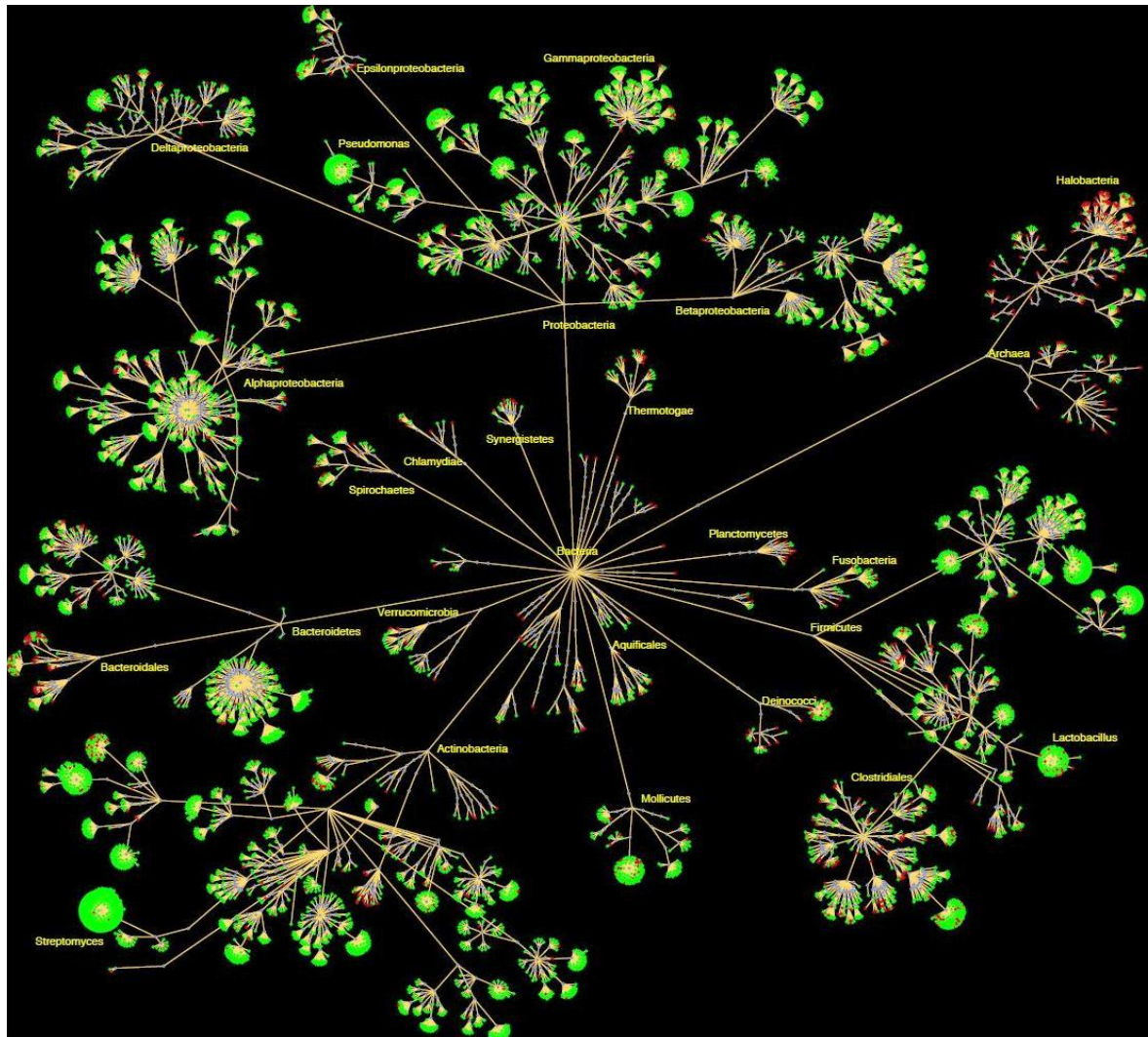


Genome projects 2010



**... with a systematic approach.**

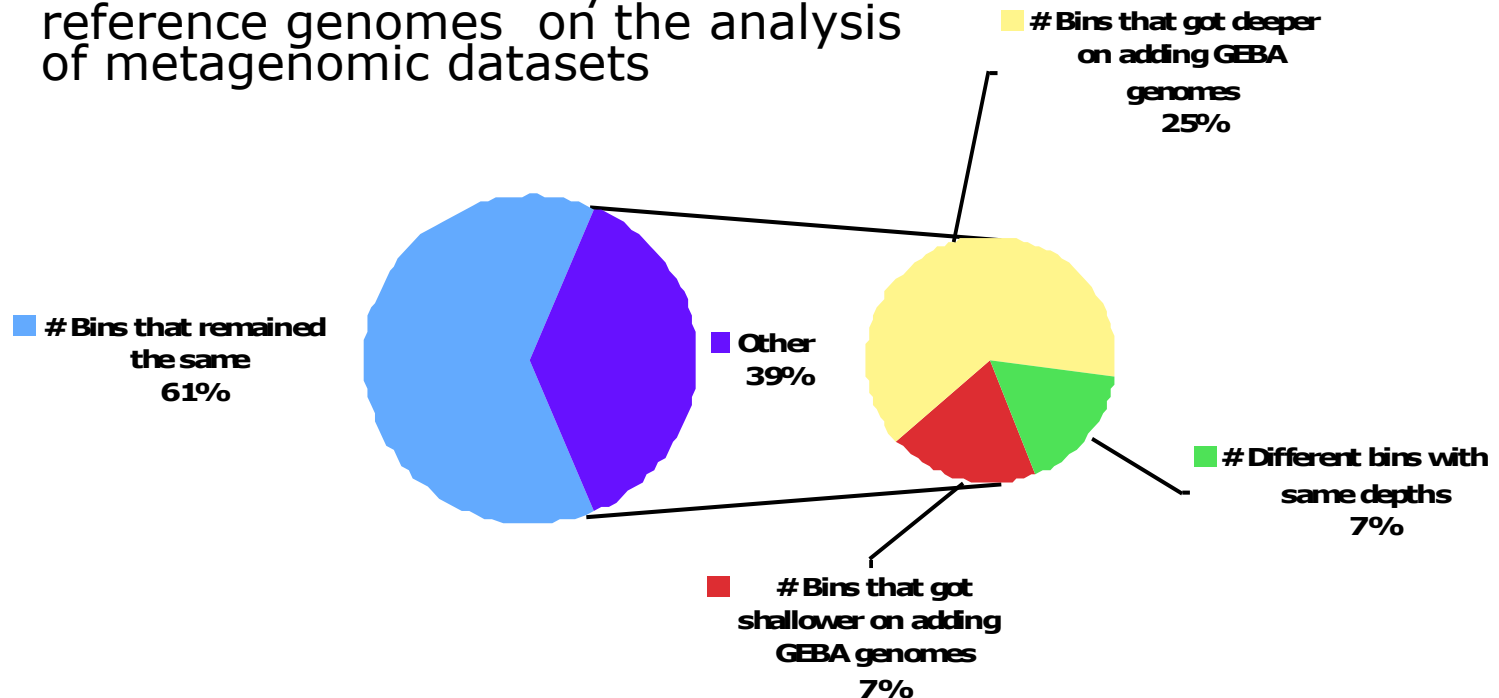
# Graphical Representation of a Tree of DSMZ strains with (red) and without (green) genome sequencing projects



*Victor Kunin  
MEP (JGI)*

## Binning Changes in the Soil Metagenome on Adding GEBA Genomes

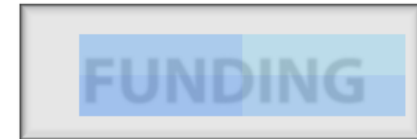
Effect of the availability of reference genomes on the analysis of metagenomic datasets



Without the GEBA/MEP framework,  
the exploration of our microbial planet  
is equivalent to navigation without a compass,  
a map or stars by which one can fix their position.

## The Already Identified Partners For the Microbial Earth Project

- **GSC**
- **CULTURE COLLECTION CENTERS**
  - **DSMZ** [Hans-Peter Klenk]
- **ORGANISM SELECTION AND PRIORITIZATION**
  - **BERGEY's, NAMES4LIFE** [George Garrity, Barny Whitman]
- **REPRESENTATIVES FROM GRAND CHALLENGE PROJECTS**
  - **GEBA** [Phil Hugenholtz, Jonathan Eisen]
  - **TERRAGENOME** [Janet Jansson, Jim Tiedje]
  - **HMP** [George Weinstock, Karen Nelson, Julian Parkhill]
- **CORE PARTICIPANTS**
  - **Large Sequencing Centers** [JGI, BGI, JCVI, Sanger, Broad, WashU, Baylor]
  - **Annotation/Analysis standards** [Owen White, Folker Meyer, & GSC]

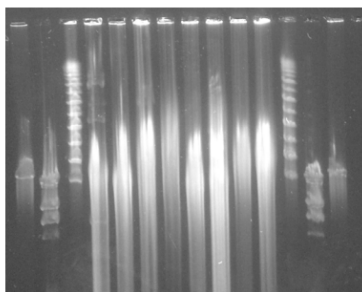


# What the MEP needs from Culture Collections

## Quality controlled high molecular DNAs

### Lengths of Genomic DNA Determined by Pulsed Field Gel Electrophoresis (PFGE)

1 2 3 4 5 6 7 8 9 10 11 12 13 14



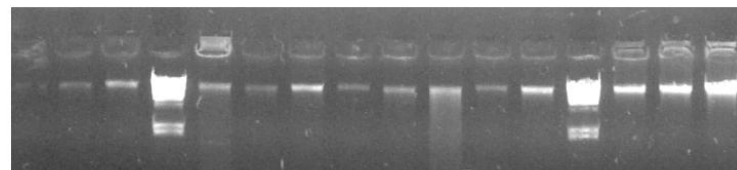
kb

Lane 1: T7 DNA Mass Marker (60 ng)  
Lane 2: DNA Molecular Weight Marker II  
Lane 3: Mid Range Marker II  
Lane 4: DSM 17242, *Alistipes finegoldii*  
Lane 5: DSM 20460, *Megasphaera elsdenii*  
Lane 6: DSM 5511, *Haloterrigena turkmenica*  
Lane 7: DSM 2008, *Veillonella parvula*  
Lane 8: DSM 20758, *Selenomonas sputigena*  
Lane 9: DSM 12940, *Halorhabdus utahensis*  
Lane 10: DSM 1135, *Leptotrichia buccalis*  
Lane 11: DSM 12286, *Halomicrobium mukohataei*  
Lane 12: Mid Range Marker II  
Lane 13: DNA Molecular Weight Marker II  
Lane 14: T7 DNA Mass Marker (60 ng)

PFGE of the genomic DNA of the strains was performed in a contour-clamped homogeneous electric field (CHEF) system on a CHEF-DR III device (Bio-Rad Laboratories, Hercules, Calif.) with 1% agarose gels and modified 0.5 TBE buffer (45 mM Tris, 45 mM boric acid, 0.1 mM EDTA) at 14°C. PFGE times used at 200 V (6 V/cm) were 1 to 15 s for 18 h.

### Quantification gel of the genomic DNA isolated from *Veillonella parvula* (DSM 2008T)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



Lane 1: c(λ-Marker)= 15 ng  
Lane 2: c(λ-Marker)= 30 ng  
Lane 3: c(λ-Marker)= 50 ng  
Lane 4: DNA Molecular Weight Marker II (Roche 236250)  
Lane 5: DSM 17242, *Alistipes finegoldii*; 5 µl, 1:50  
Lane 6: DSM 20460, *Megasphaera elsdenii*; 5 µl, 1:50  
Lane 7: DSM 5511, *Haloterrigena turkmenica*; 5 µl, 1:50  
Lane 8: DSM 2008, *Veillonella parvula*; 5 µl, 1:50

Lane 9: DSM 20758, *Selenomonas sputigena*; 5 µl, 1:50  
Lane 10: DSM 12940, *Halorhabdus utahensis*; 5 µl, 1:50  
Lane 11: DSM 1135, *Leptotrichia buccalis*; 5 µl, 1:50  
Lane 12: DSM 12286, *Halomicrobium mukohataei*; 5 µl, 1:50  
Lane 13: DNA Molecular Weight Marker II (Roche 236250)  
Lane 14: c(λ-Marker)= 125 ng  
Lane 15: c(λ-Marker)= 250 ng  
Lane 16: c(λ-Marker)= 500 ng

### Microorganisms

8T) was taken from the German Collection of Microorganisms and Cell Cultures (DSMZ). The genomic DNA was isolated using the Qiagen Genomic 500 DNA Kit (Qiagen 10262). The genomic DNA was 20-150 kb in size as determined by Pulsed Field Gel Electrophoresis (PFGE). The bulk of DNA had a size of 50-100 kb (see attached PFGE image). The DNA concentration was estimated from the gel. Spectrophotometric measurements yielded a DNA concentration of 87.5 µg/ml. The DNA was shipped (87.5 µg).

### Microorganisms

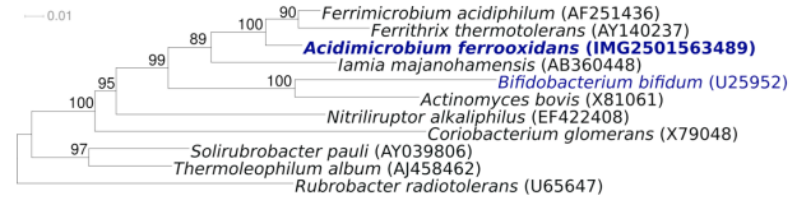
# What the MEP needs from Culture Collections

## Metadata of type strains

**Table 1.** Classification and general features of *A. ferrooxidans* ICP<sup>T</sup> based on MIGS recommendations [11]

| MIGS ID  | Property               | Term                                       | Evidence code <sup>a,b</sup>                                                                              |
|----------|------------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------|
|          | Current classification | Domain <i>Bacteria</i>                     |                                                                                                           |
|          |                        | Phylum <i>Actinobacteria</i>               | TAS [12]                                                                                                  |
|          |                        | Class <i>Actinobacteria</i>                | TAS [13]                                                                                                  |
|          |                        | Order <i>Acidimicrobiales</i>              | TAS [13]                                                                                                  |
|          |                        | Suborder <i>Acidimicrobineae</i>           |                                                                                                           |
|          |                        | Family <i>Acidimicrobiaceae</i>            | TAS [13]                                                                                                  |
|          |                        | Genus <i>Acidimicrobium</i>                | TAS [1]                                                                                                   |
|          |                        | Species <i>Acidimicrobium ferrooxidans</i> | TAS [1]                                                                                                   |
|          |                        | Type strain ICP                            |                                                                                                           |
|          |                        | Gram stain                                 | positive                                                                                                  |
| MIGS-22  | Oxygen requirement     | rod shaped                                 | TAS [1]                                                                                                   |
|          |                        | Motility                                   | motile                                                                                                    |
|          |                        | Sporulation                                | nonsporulating                                                                                            |
|          |                        | Temperature range                          | moderate thermophile, 45-50°C                                                                             |
|          |                        | Optimum temperature                        | 48°C                                                                                                      |
|          |                        | Salinity                                   | not reported                                                                                              |
|          |                        | Carbon source                              | CO <sub>2</sub> (autotrophic), yeast extract (heterotrophic)                                              |
|          |                        | Energy source                              | autotrophic: oxidation of ferrous iron with oxygen as the electron acceptor; heterotrophic: yeast extract |
|          |                        | Habitat                                    | warm, acidic, iron-, sulfur-, or mineral-sulfide rich environments                                        |
|          |                        | Biotic relationship                        | free living                                                                                               |
| MIGS-15  | Pathogenicity          | none                                       | NAS                                                                                                       |
| MIGS-14  | Biosafety level        | 1                                          | TAS [14]                                                                                                  |
| MIGS-4   | Isolation              | hot springs                                | TAS [2]                                                                                                   |
| MIGS-5   | Geographic location    | Krísuvík geothermal area, Iceland          | TAS [2]                                                                                                   |
| MIGS-4.1 | Sample collection time | before 1993                                | TAS [1]                                                                                                   |
| MIGS-4.2 | Latitude – Longitude   | 63.93, -22.1                               | TAS [2]                                                                                                   |
| MIGS-4.3 | Depth                  | not reported                               |                                                                                                           |
| MIGS-4.4 | Altitude               | not reported                               |                                                                                                           |

Evidence codes - IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the [Gene Ontology](#) project [15]. If the evidence code is IDA the property was directly observed for a live isolate by one of the authors or an expert mentioned in the acknowledgements.

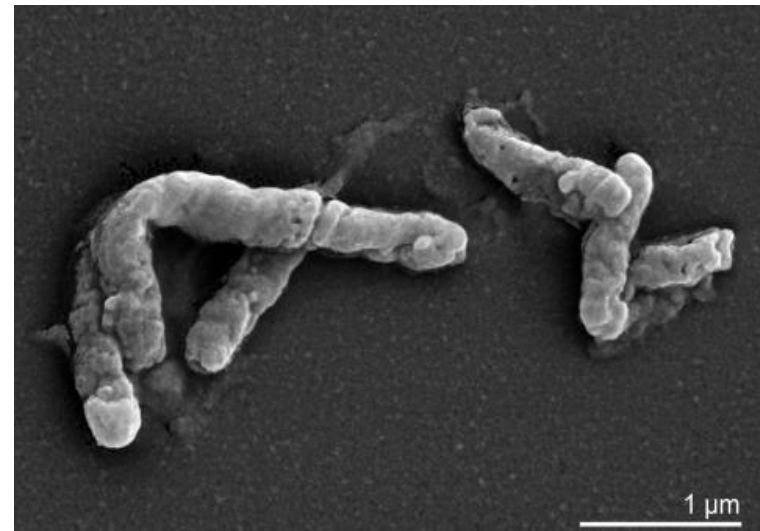


**Figure 1.** Phylogenetic tree highlighting the position of *A. ferrooxidans* strain ICP<sup>T</sup> relative to all other type strains within the *Acidimicrobiales* and the type strains of all other orders within the *Actinobacteria*. The tree was inferred from 1306 aligned characters [7, 8] of the 16S rRNA gene under the maximum likelihood criterion [9] and rooted with *Rubrobacteriales*. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from 1000 bootstrap replicates if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [10] are shown in blue, published genomes in bold.

### Chemotaxonomy

The murein of *A. ferrooxidans* ICP<sup>T</sup> contains meso-DAP, like all other characterized type species from the *Acidimicrobineae* [3, 4]. It differs from the other characterized *Acidimicrobineae* strains in MK-9(H<sub>8</sub>) being the predominant menaquinone, whereas *F. acidiphilum* has MK-8(H<sub>10</sub>) as the predominant menaquinone [4], and *I. majanohamensis* possesses a mixture MK-9(H<sub>8</sub>),

MK-9(H<sub>8</sub>), and MK-9(H<sub>9</sub>) [3]. The major cellular fatty acids of strain ICP<sup>T</sup> are saturated branched acids: iso- (i-) C<sub>16:0</sub> (83%) and anteiso- (ai-) C<sub>17:0</sub> (8%) [3], which is more similar to *F. thermotolerans* (90% i-C<sub>16:0</sub>) and *F. acidiphilum* (64% i-C<sub>16:0</sub> and 11% i-C<sub>14:0</sub>) [4], than to *I. majanohamensis* which predominantly possesses straight chain acids (C<sub>17:0</sub>, C<sub>16:0</sub> and C<sub>15:0</sub>) [3].



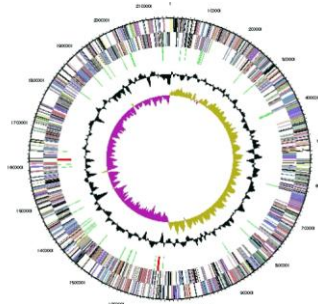
# What the MEP gives back to Culture Collections

## Genomic data of type strains

*Actinosynnema mirum* type strain (101T)

**Table 4.** Number of genes associated with the 21 general COG functional categories

| Code | Value | %    | Description                                                  |
|------|-------|------|--------------------------------------------------------------|
| J    | 182   | 2.6  | Translation, ribosomal structure and biogenesis              |
| A    | 2     | 0.0  | RNA processing and modification                              |
| K    | 607   | 8.5  | Transcription                                                |
| L    | 173   | 2.4  | Replication, recombination and repair                        |
| B    | 2     | 0.0  | Chromatin structure and dynamics                             |
| D    | 34    | 0.5  | Cell cycle control, mitosis and meiosis                      |
| Y    | 0     | 0.0  | Nuclear structure                                            |
| V    | 96    | 1.4  | Defense mechanisms                                           |
| T    | 389   | 5.5  | Signal transduction mechanisms                               |
| M    | 210   | 3.0  | Cell wall/membrane biogenesis                                |
| N    | 45    | 0.6  | Cell motility                                                |
| Z    | 1     | 0.0  | Cytoskeleton                                                 |
| W    | 0     | 0.0  | Extracellular structures                                     |
| U    | 46    | 0.6  | Intracellular trafficking and secretion                      |
| O    | 149   | 2.1  | Posttranslational modification, protein turnover, chaperones |
| C    | 306   | 4.3  | Energy production and conversion                             |
| G    | 441   | 6.2  | Carbohydrate transport and metabolism                        |
| E    | 425   | 6.0  | Amino acid transport and metabolism                          |
| F    | 108   | 1.5  | Nucleotide transport and metabolism                          |
| H    | 223   | 3.1  | Coenzyme transport and metabolism                            |
| I    | 226   | 3.2  | Lipid transport and metabolism                               |
| P    | 241   | 3.4  | Inorganic ion transport and metabolism                       |
| Q    | 265   | 3.7  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 670   | 9.4  | General function prediction only                             |
| S    | 328   | 4.6  | Function unknown                                             |
| -    | 2613  | 36.8 | Not in COGs                                                  |



Graphical circular map of the genome. From outside to the center: Genes on forward (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (red), other RNAs (black), GC content, GC view.

**Table 3.** Genome Statistics

| Attribute                        | Value     | % of Total |
|----------------------------------|-----------|------------|
| Genome size (bp)                 | 2,158,157 | 100.00%    |
| DNA Coding region (bp)           | 1,988,736 | 92.15%     |
| DNA G+C content (bp)             | 1,473,791 | 68.29%     |
| Number of replicons              | 1         |            |
| Extrachromosomal elements        | 0         |            |
| Total genes                      | 2092      | 100.00%    |
| RNA genes                        | 54        | 2.58%      |
| rRNA operons                     | 2         |            |
| Protein-coding genes             | 2038      | 97.42%     |
| Pseudo genes                     | 74        | 3.54%      |
| Genes with function prediction   | 1584      | 75.72%     |
| Genes in paralog clusters        | 1969      | 9.37%      |
| Genes assigned to COGs           | 1526      | 72.94%     |
| Genes assigned Pfam domains      | 1603      | 76.63%     |
| Genes with signal peptides       | 591       | 28.25%     |
| Genes with transmembrane helices | 436       | 20.84%     |
| CRISPR repeats                   | 2         |            |

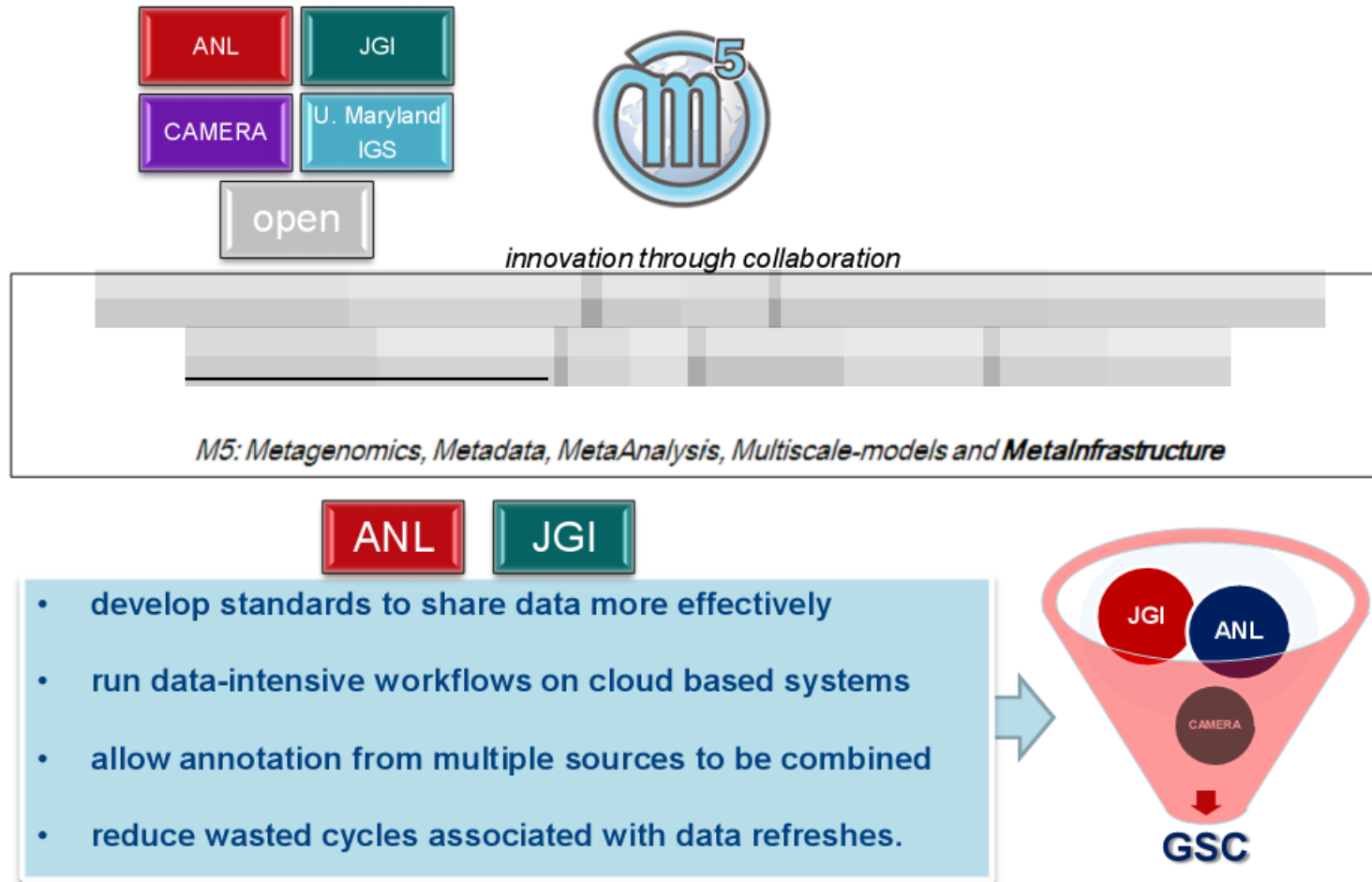


**Figure 4.** Schematic cellular overview of all pathways of the *B. faecium* strain Schefflerle 6-107 metabolism. Nodes represent metabolites, with shape indicating class of metabolite. Lines represent reactions.

→ Genomic data of type strains

Basis for metabolic reconstruction  
phenotyping improved culture conditions

## The GSC Biocomputing Consortium





## **DSMZ GEBA Team** *Braunschweig*

Birte **Abt**  
Evelyne **Brambilla**  
Markus **Göker**  
Sabine **Gronow**  
Elke **Lang**  
Rüdiger **Pukall**  
Johannes **Sikorski**  
Stefan **Spring**  
Brian **Tindall**

## **JGI GEBA Team** *Walnut Creek, CA*

Jim **Bristow**  
Jan-Fang **Chen**  
Jonathan A. **Eisen**  
Lynne **Goodwin**  
Philip **Hugenholtz**  
Nikos C. **Kyrpides**  
Alla **Lapidus**  
Victor **Markowitz**  
Tanja **Woyke**

