

# LECTURE 1. INTRODUCTION TO MICROBIOLOGY

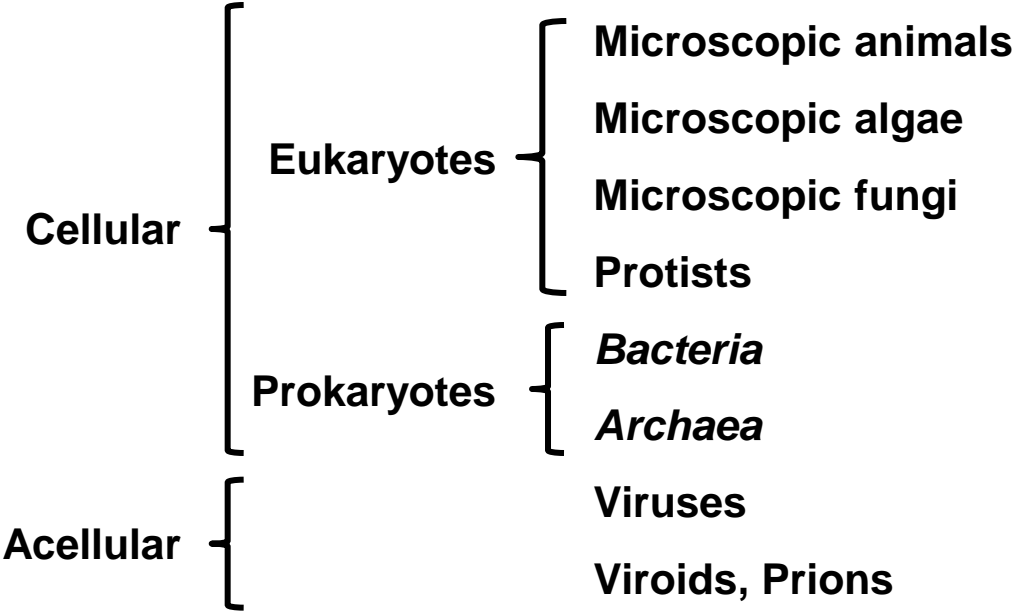
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1. What is microbiology?
2. A short history of microbiology
3. Taxonomy and systematics
4. Classification of microbes: the three domains  
Phylogeny based on molecular clocks
5. Evolution of microorganisms
6. Abundance and relevance of microbes

For copyright reasons, images have been deleted.

# 1. CONCEPT OF MICROBIOLOGY

< 1 mm



*Epulopiscium fishelsoni* (0.2-0.5 mm)

*Thiomargarita namibiensis* (0.1-0.7 mm)

# Why does only one science include the study of so many organisms?

## COMMON METHODOLOGY:

- **Microscopes**
- **Cultures**
- **Sterility techniques**

# 1. CONCEPT OF MICROBIOLOGY

## 2. A SHORT HISTORY OF MICROBIOLOGY

- **PRE-SCIENTIFIC AGE (5.000 B.C. - 1.675)**
- **OBSERVATION AGE (1.675 – 1ST<sup>1/2</sup> XIX)**
- **CULTURE AGE (2ND <sup>1/2</sup> XIX)**
- **PHYSIOLOGICAL STUDY AGE (XX - .....)**
- **MOLECULAR AGE (1.975 – ...)**

### 2.1. PRE-SCIENTIFIC AGE

- **Neolithic:** food conservation and other hygienic measures (drying, use of salt, corps burning)
- **Ancient Egypt:** wine, bread, beer (ferment transference)
- **Roman Empire** (Ciceron) (disease causing “tiny organisms”)

## 2.2. OBSERVATION AGE

**Robert Hooke (1664):** compound microscopes, cell theory, fungi fruiting bodies

**Antonie Van Leeuwenhoek (1632 - 1723):** the discovery of microorganisms

- Better microscopes (50 – 270x)  
“animalcules”
- Basic lab techniques
- Development of histology

# Milestones in Microbiology

1546 to 1940

Translated and Edited by

Thomas D. Brock

Microscopical observations  
about animals in the  
scurf of the teeth

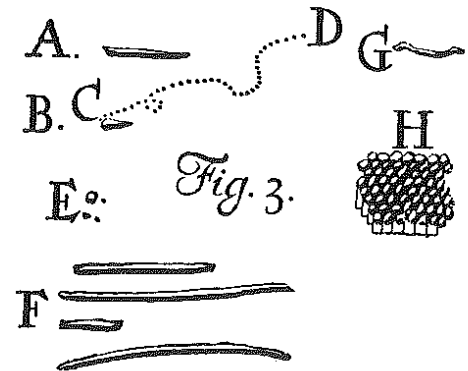
1684 • Antony van Leeuwenhoek

An abstract of a Letter from Mr. Anthony Leewen-hoeck at Delft, dated Sep. 17, 1683. Containing some Microscopical Observations, about Animals in the scurf of the Teeth. . . . *Philosophical Transactions of the Royal Society of London*, Vol. 14, May 20, 1684, no. 159, pages 568-574, 1 pl.

. . . THO MY TEETH ARE KEPT USUALLY very clean, nevertheless when I view them in a Magnifying Glass, I find growing between them a little white matter as thick as wetted flower: in this substance tho I do not perceive any motion, I judged there might probably be living Creatures.

I therefore took some of this flower and mixt it either with pure rain water wherein were no Animals; or else with some of my Spittle (having no Air bubbles to cause a motion in it) and then to my great surprize perceived that the aforesaid matter contained very many small living Animals, which moved themselves very extravagantly. the biggest sort had the shape of A. their motion was strong & nimble, and

they darted themselves thro the water or spittle, as a Jack or Pike does thro the water. These were generally not many in number. The 2d. sort had the shape of B. these spun about like a Top, and took a course sometimes on



one side, as is shown at G. and D. they were more in number than the first. In the 3d. sort I could not well distinguish the Figure, for sometimes it seemed to be an Oval, and other times a Circle. These were so small that they seem'd no bigger than E. and therewithal so swift, that I can compare them to nothing better than a swarm of Flies or Gnats, flying and turning among one another in a small space [Brownian movement?]. Of this sort I believe there might be many thousands in a quantity of water no bigger than a sand, tho the flower were but the 9th part of the water or spittle containing it. Besides these Animals there were a great quantity of streaks or threds of different lengths, but like thickness, lying confusedly together, some bent, and others streight, as at F. These had no motion or life in them, for I well observed them, having formerly seen live Animals in water of the same figure.

I observed the Spittle of two several women of whose Teeth were kept clean, and there were no Animals in the spittle, but the meal between the teeth, being mixt with water (as before) I found the Animals above described, as also the long particles.

The Spittle of a Child of 8 years old had no living Creatures in it, but the meal between the Teeth, had a great many of the Animals above described, together with the streaks.

The Spittle of an old Man that had lived soberly, had no Animals in it; But the substance upon & between his Teeth, had a great many living Crea-

tures, swimming nimbler then I had hitherto seen. . . . The Spittle of another old man and a good fellow was like the former, but the Animals in the scurf of the teeth, were not all killed by the parties continual drinking Brandy, Wine, and Tobacco, for I found a few living Animals of the 3d. sort, and in the scurf between the Teeth I found many more small Animals of the 2 smallest sorts.

I took in my mouth some very strong wine-Vinegar, and closing my Teeth, I gargled and rinsed them very well with the Vinegar, afterwards I washt them very well with fair water, but there were an innumerable quantity of Animals yet remaining in the scurf upon the Teeth, yet most in that between the Teeth, and very few Animals of the first sort A.

I took a very little wine-Vinegar and mixt it with the water in which the scurf was dissolved, whereupon the Animals dyed presently. From hence I conclude, that the Vinegar with which I washt my Teeth, kill'd only those Animals which were on the outside of the scurf, but did not pass thro the whole substance of it. . . .

The number of these Animals in the scurf of a mans Teeth, are so many that I believe they exceed the number of Men in a kingdom. For upon the examination of a small parcel of it, no thicker then a Horse-hair, I found too many living Animals therein, that I guess there might have been 1000 in a quantity of matter no bigger then the 1/100 part of a sand.

#### Comment

These are only a few of the many microscopical observations which van Leeuwenhoek made and reported by letter to the Royal Society of London. The observations presented here are the ones

which seem most likely to represent descriptions of bacteria.

van Leeuwenhoek was a minor city official who built microscopes as a hobby. He became probably the best microscope

builder in Europe, and people traveled long distances to look through his instruments, although he kept his construction methods secret.

It is amazing that van Leeuwenhoek was able to see bacteria, since he built microscopes with a single lens, rather than the compound type used today. It was only because of his great skill as a microscope builder that he was able to achieve the high resolving power needed to see bacteria. He made a large number of observations, painstakingly recorded. His work became widely known through

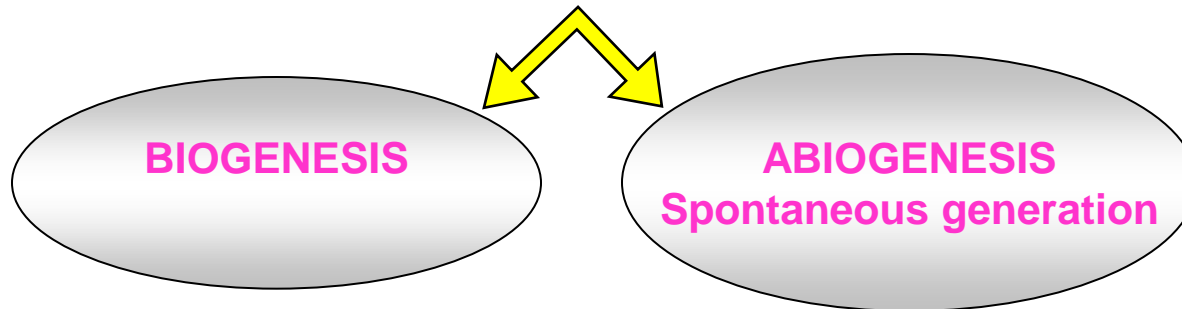
its publication in the Philosophical Transactions of the Royal Society, and was very influential on later workers. Eighteenth and early nineteenth century investigators cited his work frequently.

van Leeuwenhoek himself did not speculate on the origin of microorganisms or on their relationship to disease, although a number of workers felt that these organisms might be implicated in infectious diseases. But it was not until the late nineteenth century, through the work of Koch, that this idea was finally shown to be correct.



## 2.2. OBSERVATION AGE (Spontaneous generation/Origin of infectious diseases)

Which is the origin of microorganisms?




“The spontaneous generation controversy”

## 2.2. OBSERVATION AGE

**Lazzaro Spallanzani**  
(1/2 XVIII)

**Louis Pasteur** (1st 1/2 XIX): swan neck flask experiments



## 2.3. CULTURE AGE

### A) Development of culture media

#### DILUTION METHOD

#### SOLID MEDIA:

POTATO

MEAT EXTRACT + GELATINE (LÍQUID AT 28°C)

MEAT EXTRACT+ AGAR(\*)

### B) The fermentation as a biological process

**Schwann** (1837): alcoholic, “microscopic plants”

**Louis Pasteur** (1/2 XIX): lactic fermentation, “little rods”

### C) The microbes as disease causing agents

**Cicero**

**Joseph Lister** (end XIX): antiseptic surgery

(\*)

# TEXTURAS

*Albert y Ferran Adrià*

Home | Produced by **SOLEGRAELLS**  
Guzmán

INTRO | Spherification | Gelification | Emulsification | Thickeners | Surprises

✉ | ESP | FRA

## Gelification Agar


**GELLAN**  
**KAPPA**  
**IOTA**  
**AGAR**  
**METIL**

**ELABORATIONS**  
**DOSAGES**

Extracted from a type of red algae (of the Gelidium and Gracilaria genera), AGAR is a gelling agent used in Japan since the 15th century. In 1859, it was introduced to Europe as a characteristically Chinese food, and at the start of the 20th century it began to be used in the food industry. It is a source of fiber and can form gels in very small proportions. It can be used to make hot gelatins.

**Characteristics:**

- Presented in a refined powder.
- Mix while cold and bring to a boil.
- Gelification is fast.
- Once gelled, it can withstand temperatures of up to 80 °C (hot gelatin).
- Allow it to rest for correct gelification.
- In acidic mediums, it loses part of its gelling capacity.



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## HISTORY OF THE AGAR PLATE

November 01, 2005 admin 1 Comment

From its humble beginnings as simple meat extract to the advanced science of diagnostic media, the agar plate has always been the workhorse of the microbiologist



The origins of Oxoid's culture media go back to the 19th Century when the science of [bacteriology](#) was just beginning. The original parent company, the Liebig Extract of Meat Company (Lemco) was formed in 1865 and manufactured meat infusion extracts under the trade name Lab Lemco. This could conveniently be used in laboratories to grow bacteria, as well as being a popular food product that started as 'Liebig Company's Extract of Meat' which later became Oxo. Early biologists' generally attempted to grow micro-organisms using the food or sample on which the organism had first been observed – such as Bartolomeo Bizio's 1832 study of 'blood spots' on communion wafers, caused by *Serratia marcescens*, which used bread as a growth medium<sup>1</sup>. However, when dealing with non-pigmented organisms and pathogens, Robert Koch found that broths based on fresh beef serum or meat extracts (bouillions) gave the best growth<sup>2</sup>.

### Solid Media

In 1881 Robert Koch demonstrated a new technique at the International Medical Congress in London. Koch had recognised the difficulties of using broth media for isolation of pure cultures and had looked for solid media alternatives. He evaluated media such as coagulated egg albumen, starch paste and an aseptically cut slice of a potato (as used by the German biologist Schroeter), but then moved to a meat extract with added gelatin. The resulting 'nutrient gelatin' was poured onto flat glass plates which were inoculated and placed under a bell jar. This new plate technique

could be used both to isolate pure cultures of bacteria and to sub-culture them either onto fresh plates or nutrient gelatin slopes in cotton-wool plugged tubes<sup>3</sup>.

Although nutrient gelatin was a major advance, gelatin had two major disadvantages as a gelling agent:

- It turned from a gel to a liquid at 25°C – preventing plates from being incubated at higher temperatures.
- It was hydrolysed by gelatinase – an enzyme produced by most proteolytic organisms.

It was in 1882 that Fannie Hesse suggested replacing gelatin with agar<sup>4</sup>. Fannie, wife of Walther Hesse, was working in Koch's laboratory as her husband's technician and had previously used agar to prepare fruit jellies after hearing about its gelling properties from friends. Agar is a polysaccharide derived from red seaweeds, and proved to be a superior gelling agent. Agar has remarkable physical properties: it melts when heated to around 85°C, and yet when cooled doesn't gel until 34-42°C. Agar is also clearer than gelatin and it resists digestion by bacterial enzymes. The use of agar allows the creation of a medium that can be inoculated at 40°C in its cooled molten state and yet incubated at 60°C without melting.

Although meat extract is a valuable source of many growth factors for bacteria it lacks sufficient amino-nitrogen to allow optimal growth of a range micro-organisms. In 1884 Fredrick Loeffler added peptone and salt to Koch's basic meat extract formulation<sup>5</sup>. The peptone he used was an enzymatic digest of meat, produced in the 19th Century as a pharmaceutical product, usually prescribed for nutritional disorders. This peptone added amino-nitrogen, while the salt raised the osmolarity of the medium.

## COMMENT



Phone hacking - how easy is it?



Talking endogenous retroviruses with Dr Ravinder Kanda



Levitation's what you need

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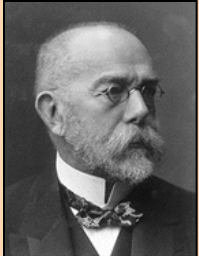
- Cannabis destroys cancer cells
- Hep C vaccine breakthrough
- History of the agar plate
- New state of matter produced by LHC
- Alzheimer's hope in form of wine and tea

## POLLS

Should the UK adopt



## C) The microbes as disease causing agents



Robert Koch (1877): Anthrax

1

3



*Bacillus anthracis*

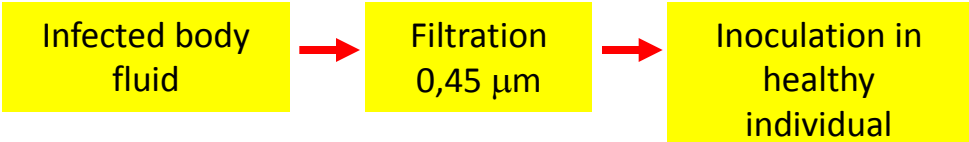
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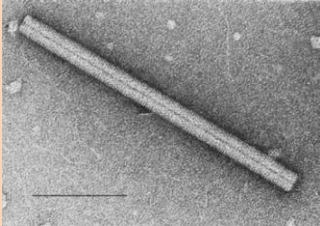
## 2.3. CULTURE AGE

### C) The microbes as disease causing agents

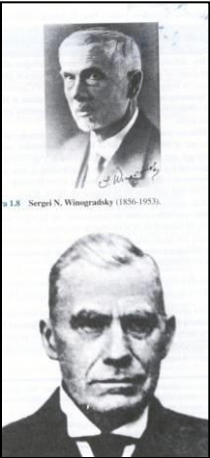
Koch established the foundations of Medical Bacteriology (Koch Institute, Germany)



**Iwanosky (1892)**  
Tobacco mosaic virus  
Birth of Virology



### D) The microbes as biogeochemical agents



**Winogradsky:**  
biochemical agents  
chemolithotrophy  
nitrogen fixation (N<sub>2</sub>)

**Beijerinck:**  
enrichment cultures  
isolation from soil and water



## **2.4. PHYSIOLOGICAL STUDY ERA**

### **INFECTIOUS DISEASE TREATMENT**

**Paul Ehrlich (1910): Chemotherapy**

**Alexander Fleming (1928): Penicillin**



## 2.5. MOLECULAR AGE

### ADVANCES IN MOLECULAR BIOLOGY

Genetic engineering and molecular biology applications in:

**Clinical microbiology** (vaccines, sera, interferon, antibiotics, etc.)

**Food microbiology** (production improvement, transgenic produce, etc.)

**Environmental microbiology** (molecular microbial ecology, biodegradation, water treatment, etc.)

**Industrial microbiology**

...

### 3. TAXONOMY AND SYSTEMATICS

#### **TAXONOMY: the science of biological classification**

- Organizes organisms in groups or taxons (classification)
- Provides names to taxonomic groups (nomenclature)
- Establishes whether a new isolate belongs to a known taxon (identification)

#### **SYSTEMATICS: the study of diversity and relationships between organisms**

Kinds of traits used for the classification and identification of microorganisms:

- ✓ **Morphological**
  - ✓ **Staining**
  - ✓ **Physiological**
  - ✓ **Biochemical**
  - ✓ **Genetic**
- Phenotype**
- Genotype**
- 
- A diagram showing five traits listed vertically: Morphological, Staining, Physiological, Biochemical, and Genetic. A red bracket on the right side groups the first four traits (Morphological, Staining, Physiological, and Biochemical) under the label 'Phenotype'. A red horizontal line under the 'Genetic' trait is connected to the label 'Genotype' on the right.

### 3. TAXONOMY AND SYSTEMATICS

#### UPPER ORDER TAXA

**GENUS:** Taxonomic group defined by one or more species, clearly separated from other genera...



### 3. TAXONOMY AND SYSTEMATICS

#### SYSTEMATIC COMPILATIONS

## Bergey's Manual of Systematic Bacteriology

2nd Edition

George M. Garrity, Editor-in-Chief

Springer, New York

The second edition of *Bergey's Manual of Systematic Bacteriology* will be published in 7 volumes, beginning with Volume 1 in May 2001.



BETA -- Release 3.20 (12/31/2005)

#### Volume 1 (2001)

The *Archaea* and the deeply branching and phototrophic *Bacteria*

ISBN 0-387-98771-1

#### Volume 2 (2005)

The *Proteobacteria* (in three parts)

ISBN 0-387-95040-0

#### Volume 3 (2006)

The low G + C Gram-positive *Bacteria*

ISBN 0-387-95041-9

#### Volume 4 (2007)

The high G + C Gram-positive *Bacteria*

ISBN 0-387-95042-7

#### Volume 5 (2007)

The *Planctomycetes*, *Spiriochaetes*, *Fibrobacteres*, *Bacteroidetes* and *Fusobacteria*

ISBN 0-387-95043-5

### 3. TAXONOMY AND SYSTEMATICS

#### NOMENCLATURE

#### Binomial system

Two names (**ALWAYS** *in italics* or underlined)

**Genus:** Capital letter (abbreviated after first use)

**Species:** lower case

Example:

*Bacillus subtilis* and Bacillus subtilis

*B. subtilis* and B. subtilis

**“INTERNATIONAL CODE OF NOMENCLATURE OF BACTERIA”**

#### COLLECTIONS OF MICROORGANISMS

- ✓ Keep them alive
- ✓ In pure culture
- ✓ Without genetic modifications

**ATCC:** American Type Culture Collection

**DSMZ:** Deutsche Sammlung von Mikroorganismen und Zellkulturen

**CECT:** Colección Española de Cultivos Tipo

### 3. TAXONOMY AND SYSTEMATICS

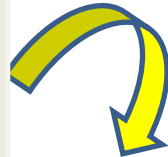
#### CLASSIFICATION SYSTEMS

##### A) Classical and numerical taxonomy

**Classical:** dichotomous keys. Outdated (useful in Clinical Microbiology)

**Numerical:** mathematical analysis applied to Taxonomy. Coefficients. Phenons

Cell morphology  
Cell size  
Ultrastructural characteristics  
Staining  
Cilia and flagella  
Motility  
Endospore traits  
Cellular inclusions  
Color



### 3. TAXONOMY AND SYSTEMATICS

#### CLASSIFICATION SYSTEMS

##### B) Molecular Taxonomy

**Protein comparison**

**Nucleic acid composition**

**RNApol**

**T<sub>m</sub>**

**Proteome**

**G+C**



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## 3. TAXONOMY AND SYSTEMATICS

### CLASSIFICATION SYSTEMS

#### B) Molecular Taxonomy

**Nucleic acid composition**

### 3. TAXONOMY AND SYSTEMATICS

#### CLASSIFICATION SYSTEMS

##### B) Molecular Taxonomy

#### DNA-DNA Hybridization

### 3. TAXONOMY AND SYSTEMATICS

#### CLASSIFICATION SYSTEMS

##### B) Molecular Taxonomy

**Nucleic acid sequencing**

**Discovery of microorganisms**

**Electron microscopy**

**DNA sequencing**



**Domain**

***Archaea***

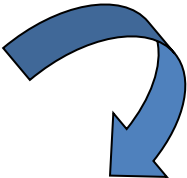
***Bacteria***

***Eukarya***

# 4. CLASSIFICATION: THE THREE DOMAINS

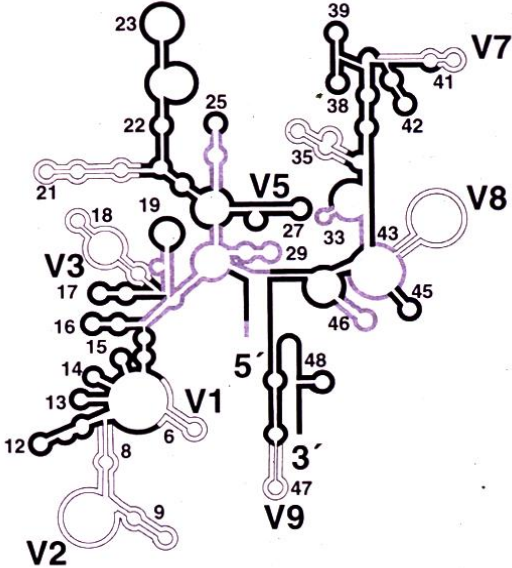


**Karl Woese**  
**Molecular clocks:**  
**Ribosomal RNA 16S/18S**



**Phylogeny of ALL living beings**

Figure 2. Secondary-Structure Model of the 16S rRNA



The degree of conservation is indicated as follows: double lines, variable and hypervariable; grey lines, highly conserved. The major variable regions are numbered V1 to V9.

## 4. PHYLOGENY BASED ON MOLECULAR CLOCKS

### MOLECULAR CLOCKS

#### Ribosomal RNAs

- Ancient
- Constant function
- Universally distributed
- Moderately well conserved  
(conserved and variable regions, signature sequences)

Types:

Prokaryotes : 70S (80S in eukaryotes)

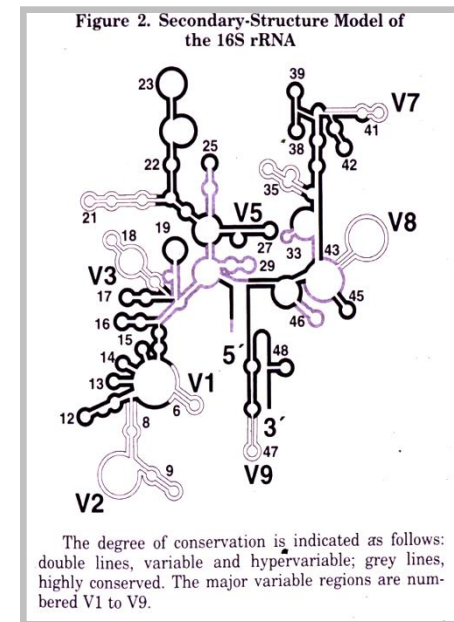
16S, 1500 nucleotides (18S en eu.)

23S, 3000 nt

5S, 120 nt

Other molecular clocks:

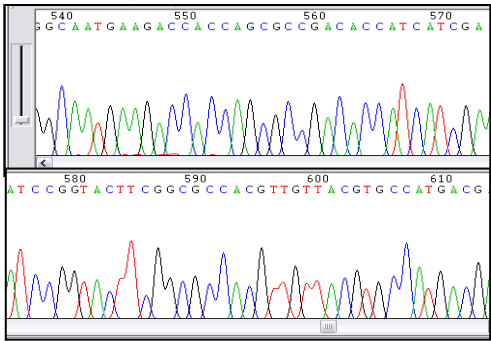
ATPases; TU-elongation factor; gyrases...



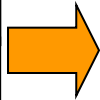
# 4. PHYLOGENY BASED ON MOLECULAR CLOCKS

## MOLECULAR CLOCKS

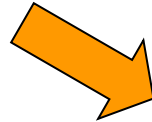
Use of rRNA as a molecular clock: the methodology



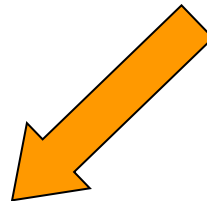
Obtaining the sequence



Alignment with other sequences in databases



Converting differences into evlutive distances



Phlogenetic tree

## 4. MOLECULAR CLOCKS BASED PHYLOGENY

### THE PHYLOGENETIC TREE OF ALL LIVING BEINGS



**LUCA**

**Last Universal Common Ancestor**



# THE THREE DOMAINS

## Main differences between the three Domains

	<i>Bacteria</i>	<i>Archaea</i>	<i>Eukarya</i>
Nuclear membrane	No	No	Yes
Mitochondria and Chloroplasts	No	No	Yes
Peptidoglycane walls*	Yes	No	No
Membrane lipids	ester	ether	ester
Ribosome size	70S	70S	80S
Circular chromosome*	Yes	Yes	No
Initiator tRNA	formil-Met	Met	Met
Genes in operons	Yes	Yes	No
Bacterial protein synthesis inh.*	Yes	No	No
RNA pol/ (subunit.)	1 (4)	(8-12)	3(12-14)
Chemolith./ N <sub>2</sub> Fixation	Yes	Yes	No

## 4. PHYLOGENY BASED ON MOLECULAR CLOCKS

### MOLECULAR CLOCKS

#### The All-Species Living Tree project: A 16S rRNA-based phylogenetic tree of all sequenced type strains

Pablo Yarza<sup>a</sup>, Michael Richter<sup>a</sup>, Jörg Peplies<sup>b</sup>, Jean Euzéby<sup>c</sup>, Rudolf Amann<sup>d</sup>, Karl-Heinz Schleifer<sup>e</sup>, Wolfgang Ludwig<sup>e,\*\*</sup>, Frank Oliver Glöckner<sup>d,f,\*\*</sup>, Ramon Rosselló-Móra<sup>a,\*</sup>

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2008

#### Update of the All-Species Living Tree Project based on 16S and 23S rRNA sequence analyses

Pablo Yarza<sup>a,\*</sup>, Wolfgang Ludwig<sup>b</sup>, Jean Euzéby<sup>c</sup>, Rudolf Amann<sup>d</sup>, Karl-Heinz Schleifer<sup>b</sup>, Frank Oliver Glöckner<sup>d</sup>, Ramon Rosselló-Móra<sup>a,\*\*</sup>

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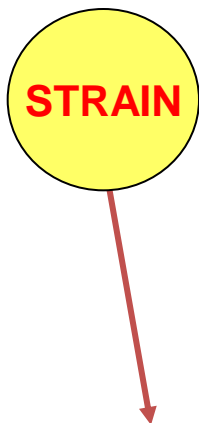
<sup>d</sup> Max Planck Institute for Marine Microbiology, D-28359 Bremen, Germany

2010

# THE PROKARYOTIC SPECIES CONCEPT

Collection of strains with a similar **G+C content** and a similarity at least of **70% in DNA-DNA hybridization** experiments. The similarity in the sequence of **16S rNA gene** of two prokaryotes from the same species is at least **97%**.

**WARNING!** The species definition for “upper” organisms is not valid for prokaryotes



Population of microorganisms descending from a single microorganism or from an isolate in pre culture (clon/clonal population). Types:

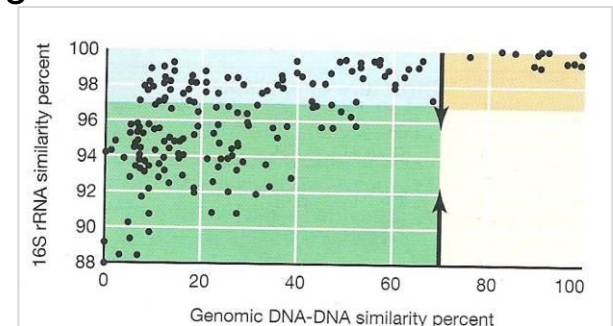
- Biovar
- Serovar
- Morphovar

**Type strain:**

The first one to be studied; gives the name to the species.

Normally, the best characterized

(although not necessarily the best representation of the species)

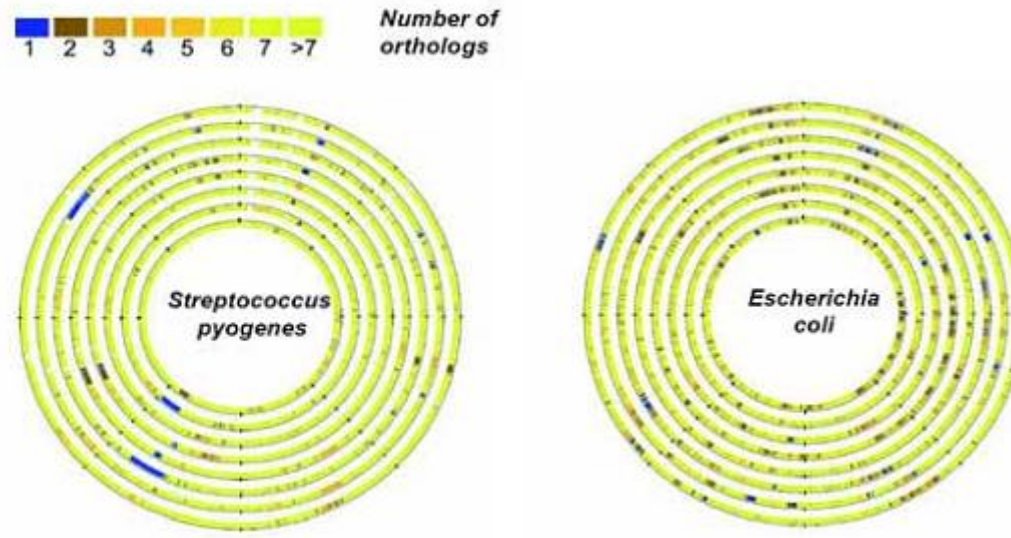


# The bacterial pan-genome: a new paradigm in microbiology

Alex Mira,<sup>1¶</sup> Ana B. Martín-Cuadrado,<sup>2¶</sup> Giuseppe D'Auria,<sup>3,4</sup>  
Francisco Rodríguez-Valera<sup>2</sup>

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<sup>2</sup>Evolutionary Genomics Group, Miguel Hernandez University, San Juan, Alicante, Spain. <sup>3</sup>Joint Unit of Research in  
Genomics and Health, Centre for Public Health Research (CSISP) and Cavanilles Institute for Biodiversity and  
Evolutionary Biology, University of Valencia, Valencia, Spain. <sup>4</sup>CIBER on Epidemiology and Public Health (CIBEResp).

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# Pangenome: *core* and accessory genomes

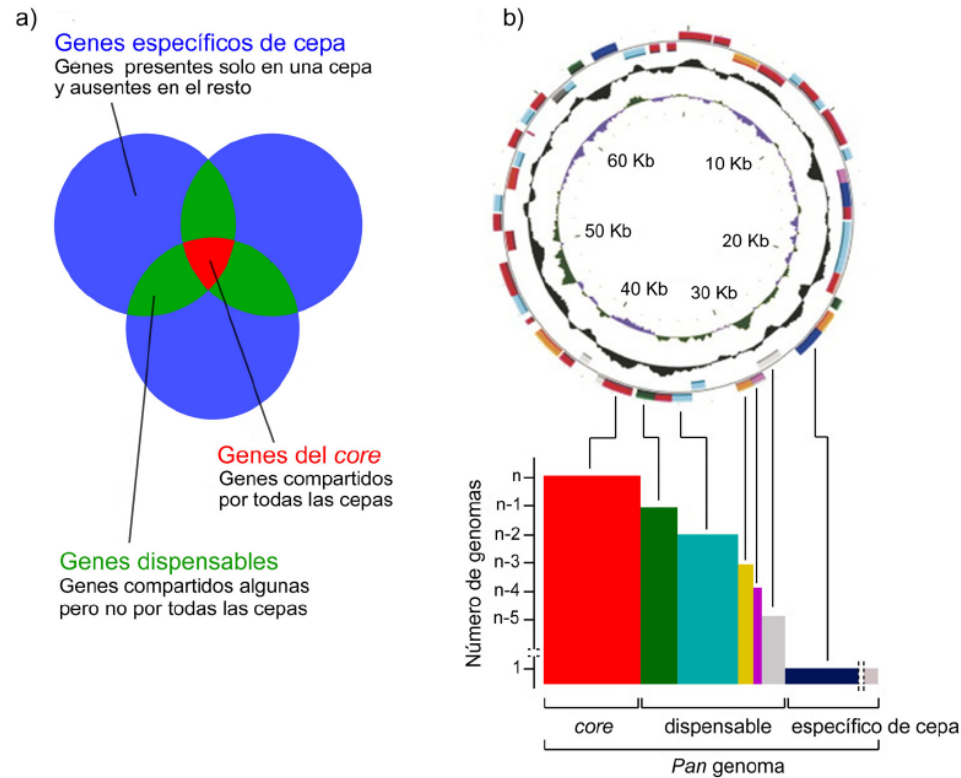
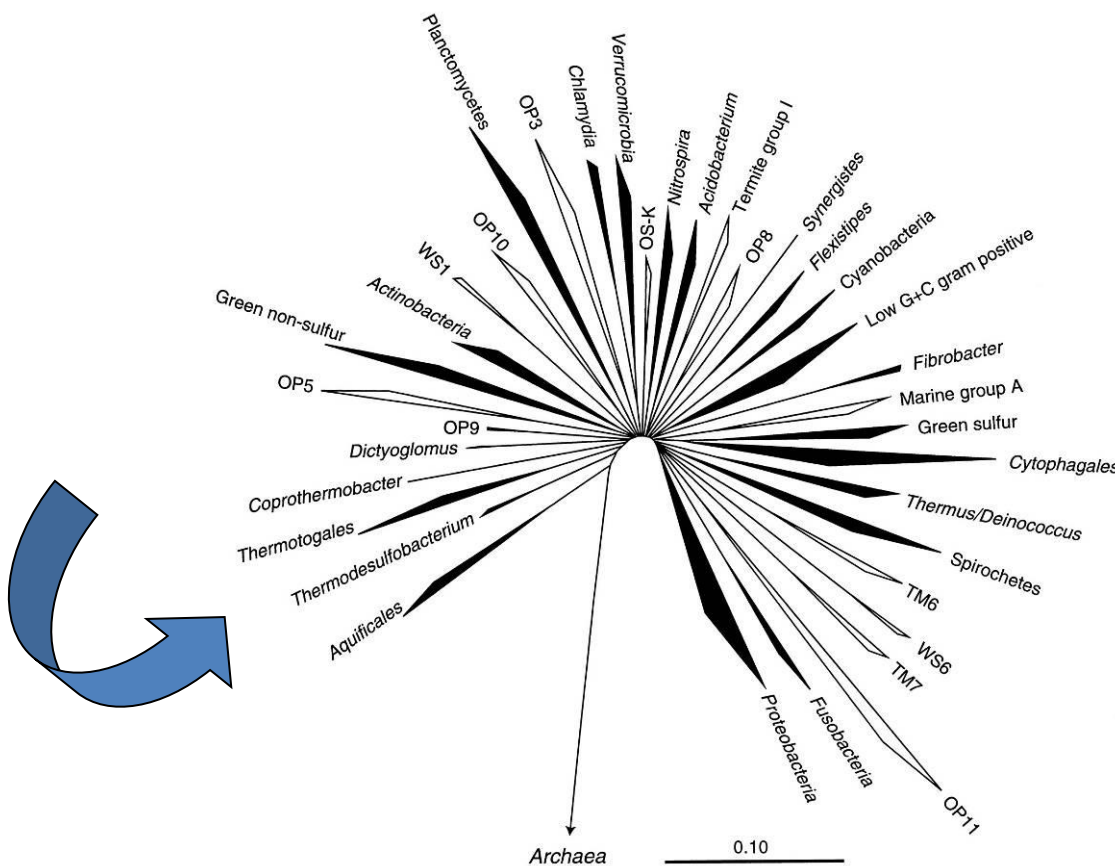


Figura 9. Esquema representativo del pan-genoma. a) Imagen esquemática de la estructura del *pan* genoma. Los genes del *core* genoma, los accesorios y los específicos de cepa están coloreados en rojo, verde y violeta, respectivamente. b) Clasificación según la estructura del *pan* genoma, de los genes de un nuevo genoma secuenciado. Cada barra representa el conjunto de genes conservados en  $n$ ,  $n-1$ ,  $n-2$ , ...,  $1$  genomas (figura adaptada de Muzzi y col., 2007).



# THE PHYLOGENY OF UNCULTURED MICROORGANISMS



# THE PHYLOGENY OF UNCULTURED MICROORGANISMS

Extremophiles (2009) 13:583–594  
DOI 10.1007/s00792-009-0261-3

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REVIEW

## **Cultivating the uncultured: limits, advances and future challenges**

**Karine Alain • Joël Querellou**



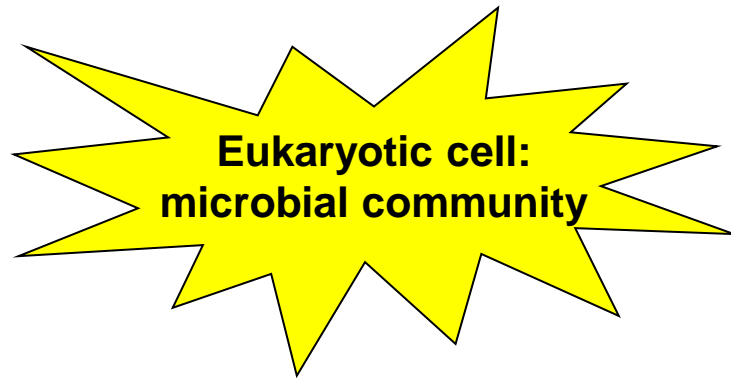
## 5. THE EVOLUTION OF MICROORGANISMS

**stromatolites**

- The planet Earth, in the way it is now, is a product of (micro) biological activity
- During most of the Earth's history (3000 million years) it was inhabited ONLY by microorganisms

## 5. THE EVOLUTION OF MICROORGANISMS

### Lynn Margulis (1981-2011) – The Endosymbiosis Theory

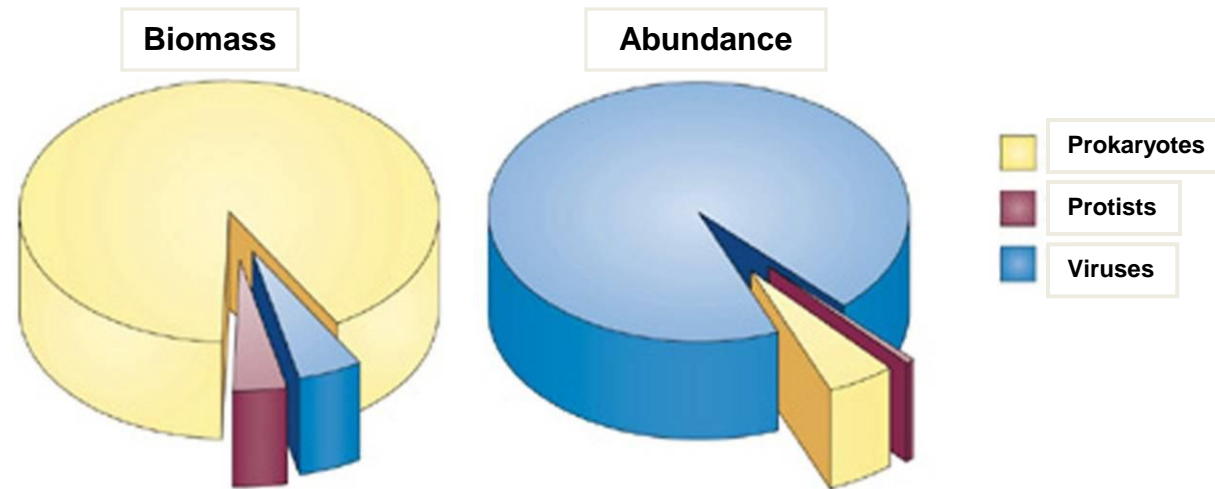


The eukaryotic cell is not “primitive” but miniaturized

The term “prokaryote” lacks phylogenetic meaning. There are two independent lineages of prokaryotes: *Bacteria* and *Archaea*.

## 6. ABUNDANCE AND RELEVANCE OF MICROBES

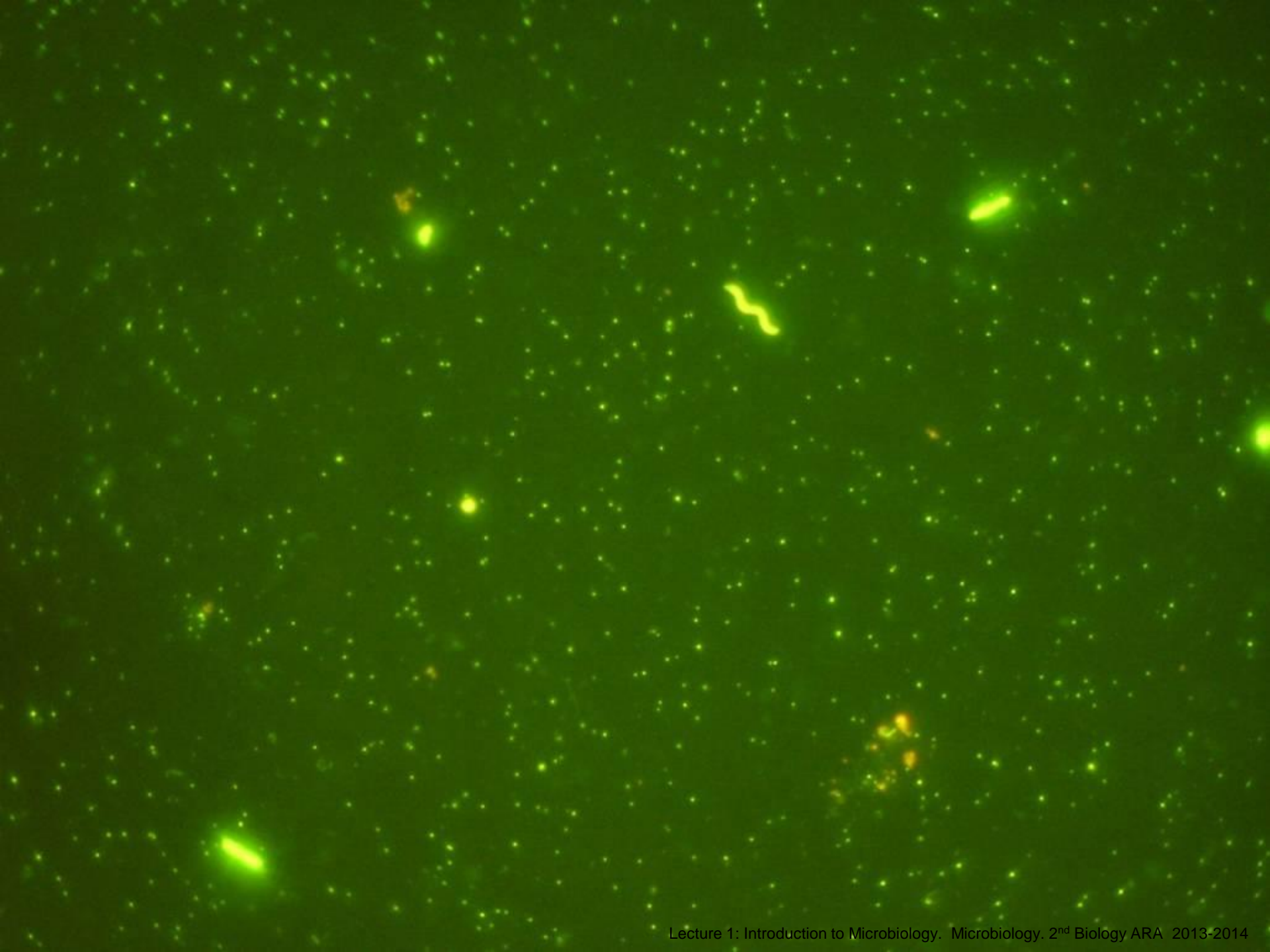
### 6.1. ABUNDANCE



**$4-6 \times 10^{30}$  prokaryotes in the Biosphere**

**$10^{31}$  viruses on Earth**

*“There are more than 5000 viral genotypes per 200 liters of seawater and more than a million per kilogram of sediment.... To put the sheer abundance of viruses in context, we note that they contain more carbon than 75 million blue whales and, if such viruses were joined end-to-end, they would stretch further than 100 times the Milky Way (Suttle, 2005)”.*



# 6. ABUNDANCE AND RELEVANCE OF MICROBES

## 6.1. ABUNDANCE

Table 5. Number and biomass of prokaryotes in the world

Environment	No. of prokaryotic cells, $\times 10^{28}$	Pg of C in prokaryotes*
Aquatic habitats	12	2.2
Oceanic subsurface	355	303
Soil	26	26
Terrestrial subsurface	25–250	22–215
Total	415–640	353–546

\*Calculated as described in the text.

2.9  $10^{29}$  instead of 35.5  $10^{29}$

*Proc. Natl. Acad. Sci. USA*  
Vol. 95, pp. 6578–6583, June 1998

### Prokaryotes: The unseen majority

*William B. Whitman\*†, David C. Coleman‡, and William J. Wiebe§*

### Global distribution of microbial abundance and biomass in seafloor sediment

Jens Kallmeyer<sup>a,b,1,2</sup>, Robert Pockalny<sup>c,1</sup>, Rishi Ram Adhikari<sup>a</sup>, David C. Smith<sup>c</sup>, and Steven D'Hondt<sup>c</sup>

PNAS | October 2, 2012 | vol. 109 | no. 40 | 16213–16216

# 6. ABUNDANCE AND RELEVANCE OF MICROBES

## 6.2. CLINICAL RELEVANCE



## **6. ABUNDANCE AND RELEVANCE OF MICROBES**

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## 6. ABUNDANCE AND RELEVANCE OF MICROBES

### 6.2. CLINICAL RELEVANCE

**Aprox. 50 million deaths each year; more than 20 caused by infectious disease**  
**Different impacts on children and adults**



## **6. ABUNDANCE AND RELEVANCE OF MICROBES**

### **6.2. CLINICAL RELEVANCE**

## **6. ABUNDANCE AND RELEVANCE OF MICROBES**

### **6.2. CLINICAL RELEVANCE**

# 6. ABUNDANCE AND RELEVANCE OF MICROBES

## 6.2. CLINICAL RELEVANCE



## **6. ABUNDANCE AND RELEVANCE OF MICROBES**

### **6.2. INDUSTRIAL RELEVANCE**

## 6. ABUNDANCE AND RELEVANCE OF MICROBES

### 6.3. INDUSTRIAL/ENVIRONMENTAL RELEVANCE

**Wastewater treatment**

## 6. ABUNDANCE AND RELEVANCE OF MICROBES

### 6.4. FOOD

Useful microbes (food production, etc.)... and pathogens

## **6. ABUNDANCE AND RELEVANCE OF MICROBES**

### **6.5. MICROBIAL ECOLOGY AND ENVIRONMENTAL MICROBIOLOGY**

## 6. ABUNDANCE AND RELEVANCE OF MICROBES

### 6.5. MICROBIAL ECOLOGY AND ENVIRONMENTAL MICROBIOLOGY

#### MARINE MICROBIOLOGY

Very abundant prokaryotes, very widely distributed, practically unknown.  
SAR 11, marine *Archaea*, etc...

Most marines microbes  
cannot (so far...) be  
cultured by traditional  
methods



# 6. ABUNDANCE AND RELEVANCE OF MICROBES

## 6.6. MOLECULAR BIOLOGY AND GENETIC ENGINEERING

# Shifting the genomic gold standard for the prokaryotic species definition

Michael Richter and Ramon Rosselló-Móra<sup>1</sup>

19126–19131 | PNAS | November 10, 2009 | vol. 106 | no. 45

Marine Microbiology Group, Institut Mediterrani d'Estudis Avançats (CSIC-UIB), E-07190 Esporles, Spain

PNAS PNAS PNAS

DNA-DNA hybridization (DDH) has been used for nearly 50 years as the gold standard for prokaryotic species circumscriptions at the genomic level. It has been the only taxonomic method that offered a numerical and relatively stable species boundary, and its use has had a paramount influence on how the current classification has been constructed. However, now, in the era of genomics, DDH appears to be an outdated method for classification that needs to be substituted. The average nucleotide identity (ANI) between two genomes seems the most promising method since it mirrors DDH closely. Here we examine the work package JSpecies as a user-friendly, biologist-oriented interface to calculate ANI and the correlation of the tetranucleotide signatures between pairwise genomic comparisons. The results agreed with the use of ANI to substitute DDH, with a narrowed boundary that could be set at  $\approx 95\text{--}96\%$ . In addition, the JSpecies package implemented the tetranucleotide signature correlation index, an alignment-free parameter that generally correlates with ANI and that can be of help in deciding when a given pair of organisms should be classified in the same species. Moreover, for taxonomic purposes, the analyses can be produced by simply randomly sequencing at least 20% of the genome of the query strains rather than obtaining their full sequence.