

**Microbiology: What is it?**

- Study of organisms who are too small to be seen without a microscope.
- Study of small organisms or microorganisms. NOT just Bacteria!
- Study of single celled organisms. The original cell biology!
- Categories & subjects based on the type of organisms:

- (1) Viruses – Virology (acellular)
- (2a) Bacteria – Bacteriology (e.g. Prokaryotes)
- (2b) Archea – Archeaology? (already taken)
- (3) Fungi – Mycology
- (4) Algae – Phycology
- (5) Protozoa – Protozoology

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**WHAT IS A MICROORGANISM?**

“There is no simple answer to this question. The word ‘microorganism’ is not the name of a group of related organisms, as are the words ‘plants’ or ‘invertebrates’ or ‘fish’. The use of the word does, however, indicate that there is something *special* about small organisms; we use no special word to denote large organisms or medium-sized ones.

- Siström (1969)

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**Reasons to study Microbiology:**

- (1) Bacteria are part of us! E. coli lives in our gut and produces essential vitamins (e.g. K).
- (2) Infectivity & Pathogenicity; MO's have the ability to cause disease in compromised &/or healthy hosts.
- (3) MO's in the environment; Bioremediation or use of MO's to breakdown waste compounds like oil, pesticides, etc. Mineral cycling of elements like N, S, Fe, etc.
- (4) Applied Microbiology or use in agriculture and industry.
- (5) Understand basic biological processes: Evolution, Ecology, Genetics, etc.

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**WHY STUDY MICROBIOLOGY?**

“The role of the infinitely small is infinitely large.”

- Louis Pasteur (1862)

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**WE ARE NOT ALONE!**

“We are outnumbered. The average human contains about 10 trillion cells. On that average human are about 10 times as many microorganisms, or 100 trillion cells...As long as they stay in balance and where they belong, [they] do us no harm...In fact, many of them provide some important services to us. [But] most are opportunists, who if given the opportunity of increasing growth or invading new territory, will cause infection.”

- Sullivan (1989)

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**Natural Microbial Populations**

- Typical soil:  $\sim 10^9$  MO's per gram
- Typical fresh water:  $\sim 10^6$  to  $10^7$  MO's per ml
- Open Ocean:  $\sim 10^5$  to  $10^6$  MO's per ml
  
- Complexity (soil):  $10^4$  to  $10^5$  different prokaryote-sized genomes per gram

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## Prokaryotes: The unseen majority

Whitman et al., 1998 PNAS

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.

Pg = Petagram or  $10^{15}$  grams

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## Prokaryotes: The unseen majority

Whitman et al., 1998 PNAS

	Total C (Pg)	Total N (Pg)	Total P (Pg)
Plants:	560	12-20	1-2
Prokaryotes:	350-550	70-120	7-12

**Take Home Message:** Prokaryotes contain 60 to 100% the cellular carbon of all plants along with ~10x the N and P of plants!

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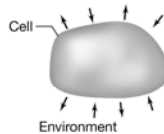
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## Hallmarks of cellular life

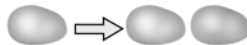
### 1. Metabolism

Uptake of chemicals from the environment, their transformation within the cell, and elimination of wastes into the environment. The cell is thus an *open* system.



### 2. Reproduction (growth)

Chemicals from the environment are turned into new cells under the direction of preexisting cells.




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### Hallmarks of cellular life

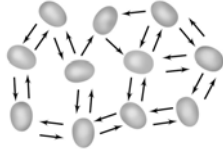
#### 3. Differentiation

Formation of a new cell structure such as a spore, usually as part of a cellular life cycle.



#### 4. Communication

Cells *communicate* or *interact* primarily by means of chemicals that are released or taken up.



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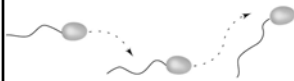
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### Hallmarks of cellular life

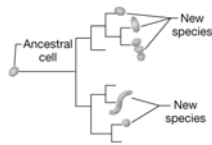
#### 5. Movement

Living organisms are often capable of self-propulsion.



#### 6. Evolution

Cells evolve to display new biological properties. Phylogenetic trees show the evolutionary relationships between cells.



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### Historical Perspective of Microbiology

1. Ancient History: (pre-1660's)
  - The Age of Sanitation
2. Early History: (1660's to 1850's)
  - The Age of Discovery
3. Microbiology's Renaissance: (1850's to 1920's)
  - The Age of Diagnoses
4. Modern History: (1920's to Present)
  - The Age of Biotechnology

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## Ancient History: (pre-1660's) - The Age of Sanitation

- Early civilizations (e.g., Crete, India, Pakistan, and Scotland) showed signs of using toilets and sewers dating back as far as 2800 BC.
- The first cities to use Water Pipes made of clay were in the Indus Valley of India around 2700 BC and in the palace of Knossos on Crete around 2000 BC. Metal pipes were used in Egypt as far back as 2450 BC.
- The Romans in 315 AD had public lavatories where people routinely socialized and conducted business. Rome also built elaborate aqueducts and public fountains and had an appointed water commissioner who was responsible for seeing that the water supply was kept clean. Lead was commonly used for Roman pipes and the subsequent fall of the Roman empire has been related by some to this practice.
- The Chinese as early as 589 AD produced toilet paper, while Europeans were still using moss and hay.

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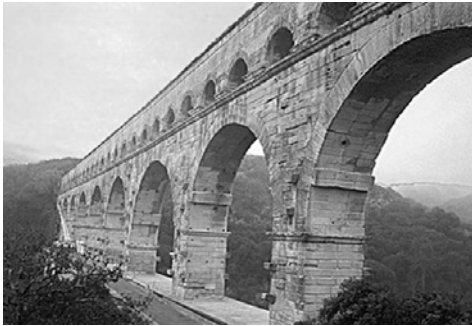
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Roman Aqueduct: Sanitation Age



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## Early History: (1660's to 1850's) - The Age of Discovery

- 1665: **Robert Hooke** – The first person to report seeing MO's under a microscope. He saw the cellular structure of plants and fungi, but his lens were apparently too poor to "see" bacteria.
- 1668: **Francesco Redi** – The first serious attack on the idea of spontaneous generation was made. Redi's experiment proved maggots are not spontaneously produced in rotten meat.
- 1676: **Anton van Leeuwenhoek** – The first to see and describe bacteria and their characteristic morphology. Publishes his drawings of "wee animalcules." Classic example of serendipity, he originally wanting better magnification to judge the quality of cloth.
- Takes 175 years before any major advancements are made:
  1. Technology of producing better microscopes and chemistry.
  2. Leeuwenhoek did not allow others to reproduce and verify his results.
  3. The nature of contagious disease is still paramount.
  4. The question of spontaneous generation lingers.

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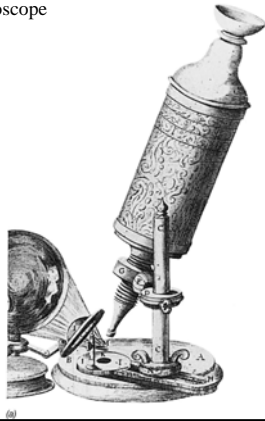
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Robert Hooke's microscope



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Able to see Mold



(b)

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**Francesco Redi**

Showed that maggots came from flies.

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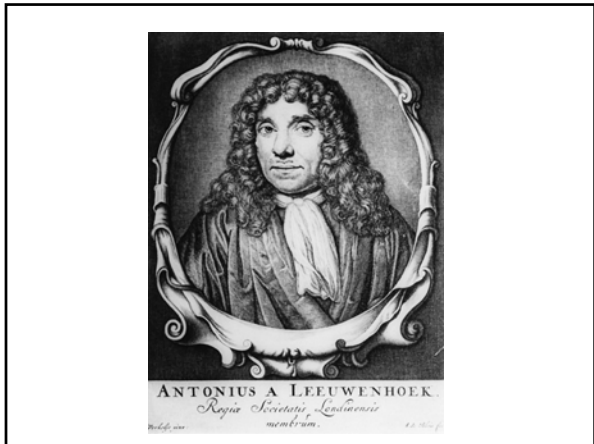
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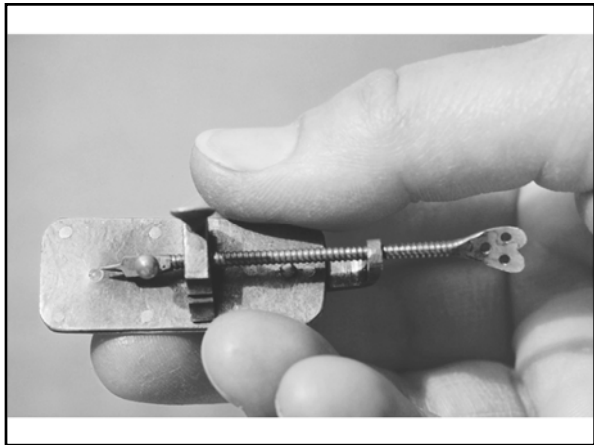
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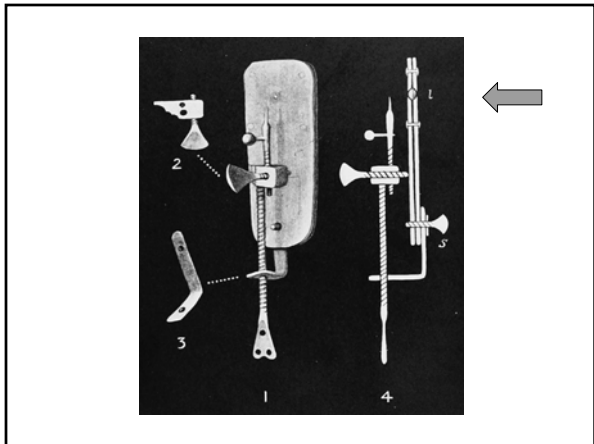
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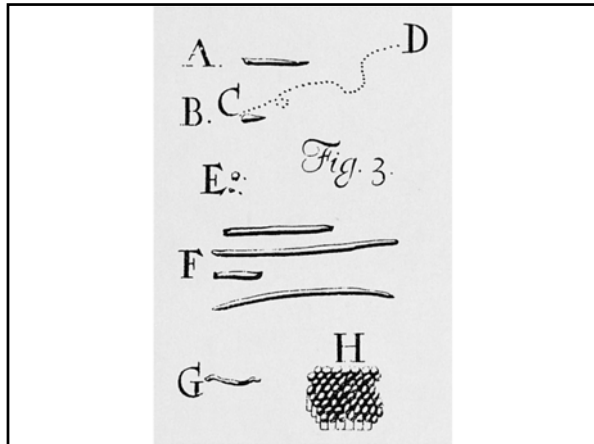
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**Early History: (1660's to 1850's)**  
**- The Age of Discovery**

- 1798: **Edward Jenner** introduces the concepts of vaccination using cowpox material to prevent small pox, unfortunately many questions left unanswered.
- 1804-1806: The Lewis and Clark Expedition.

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Edward Jenner

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## Variolation to Vaccination

- Variolation, immunization against smallpox, was a common practice before vaccination was common. This worked because the patient was exposed to a weak strain of smallpox, which did not kill, yet provided immunity to the disease.
- Edward Jenner discovered that cowpox could protect against smallpox, with a much lower incidence of complications than variolation.
- Pasteur actually coined the term vaccination to describe the technique.

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## Microbiology's Renaissance: (1850's to 1920's) - The Age of Diagnoses

- 1859: **Louis Pasteur** settles the question of spontaneous generation once and for all with "simple" experiments using swan necked flasks. Microbiology becomes a truly scientific discipline as MO's are now known to have the same fundamental properties as other living organisms, aka Germ Theory.
- He contributed to the development of the first vaccines for the immunization against rabies, anthrax, and chicken cholera. He described the scientific basis for fermentation, wine-making, and the brewing of beer. And don't forget Pasteurization, which was originally used to prevent Napoleon's sailors from mutiny and the production of canned food for his armies!
- 1859: Charles Darwin publishes the Origin of the species.
- 1867: **Joseph Lister** – Revolutionized medicine by introducing practices to limit the exposure to infectious MO's during surgery. Developed antiseptic methods for preventing infection using phenol (toxic!) to treat wounds.

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Louis Pasteur

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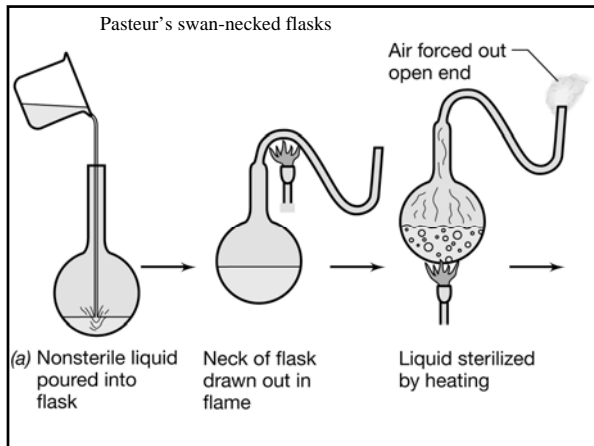
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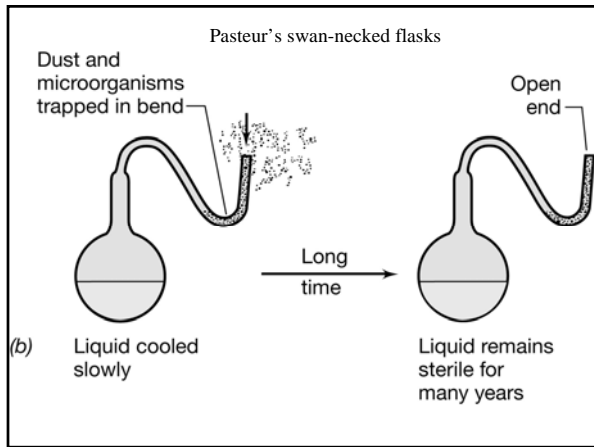
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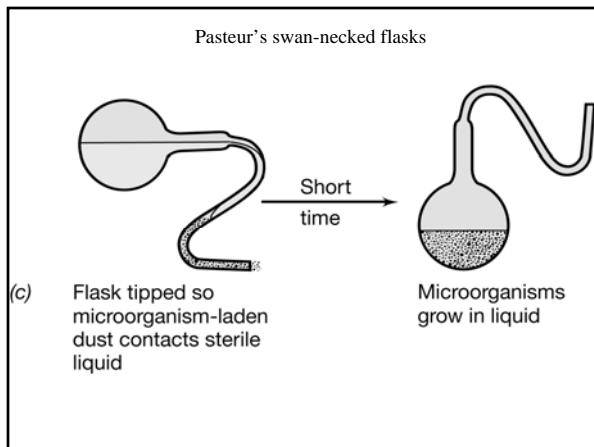
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Joseph Lister is known as the founder of antiseptic surgery.



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### Microbiology's Renaissance: (1850's to 1920's) - The Age of Diagnoses

- 1876: **Robert Koch** – Established the relationship between MO's and infectious disease. Determined Koch's postulates. Discovered causative agent for anthrax, tuberculosis, and cholera. His discoveries, in combination with those of Pasteur, established the **Germ Theory** of disease.
- 1881: **Paul Ehrlich** (working in Koch's lab) introduces vital staining with methylene blue and the visualization of bacteria is greatly improved. Later credited for early work with chemotherapy (chemicals to treat disease).
- 1882: **Walter Hesse (and wife Fannie)** – uses Agar as solid growth medium.
- 1884: **Hans Christian Gram** – develops a differential staining method, which exploits the difference between two basic variations in cell wall structure and is still essential in the classification of bacteria.
- 1884: Mark Twain writes Huckleberry Finn. Koch's Postulates are published.
- 1887: **Richard Petri** – describes the utility of the Petri dish.

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Robert Koch

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**Koch's Postulates:**

- (1) The suspected pathogenic organism should be present in all cases of disease and absent from healthy animals.
- (2) The suspected organism should be grown in pure culture.
- (3) Cells from a pure culture of the suspected organism should cause disease in a healthy animal.
- (4) The organism should be reisolated and shown to be the same as the original.

The diagram illustrates Koch's Postulates in four steps:
 

- A diseased animal is shown with a red blood cell containing a suspected pathogen.
- The pathogen is isolated and grown in a pure culture.
- The pure culture is injected into a healthy animal, which then becomes diseased.
- The pathogen is reisolated from the newly diseased animal and shown to be identical to the original pathogen.

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**Microbiology's Renaissance: (1850's to 1920's) - The Age of Diagnoses**

- 1889-91: **Sergei Winogradsky and Martinus Beijerinck** – cofounders of microbial ecology, each made significant discoveries concerning microbial transformations of inorganic compounds aka **chemolithotrophy**. First to develop ideas in biogeochemical cycling on a global scale.
- Winogradsky isolated and described nitrifying bacteria  $\text{NH}_4^+$  to  $\text{NO}_2^-$  and  $\text{NO}_3^-$  (cations to anions) and other redox reactions including S and Fe and anaerobic N-fixing bacteria.
- Famous for Winogradsky column used to study anaerobic photosynthesis.
- Beijerinck established enrichment culture techniques for the isolation of pure cultures. Isolated anaerobic sulfate reducing bacteria and symbiotic and non-symbiotic aerobic N-fixing bacteria.
- Famous for the statement: "Everything is everywhere, the environment selects."

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Sergei Winogradsky

Martinus Beijerinck

WIN-412 *Chlorococcoides oregonensis*

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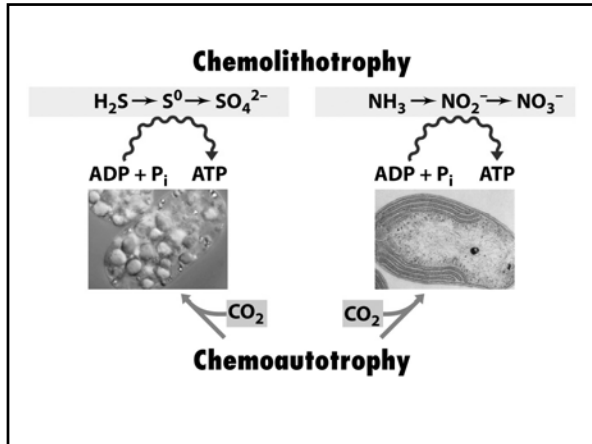
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**Modern History: (1920's to Present)**  
- The Age of Biotechnology

- 1929: **Alexander Fleming** discovers penicillin. An antibiotic produced by a fungus that inhibits bacterial growth.
- 1928: **Fred Griffith** – transforms *Streptococcus pneumoniae*.
- 1933: Invention of the electron microscope.
- 1937: First division of living organisms into prokaryotes and eukaryotes.
- 1940s: **Beadle and Tatum** – One gene-one enzyme hypothesis.
- 1941: **Oswald Avery** – DNA is the genetic material.
- 1953: **Watson and Crick** – DNA structure as double helix.
- 1960s: **Jacob and Monod** – Operon theory, control over enzyme expression.
- 1969: First Man on the Moon – Apollo 11, Neil Armstrong and Buzz Aldrin.
- 1977: **Fred Sanger** – DNA sequencing techniques. Discoveries of both *Archaea* and hydrothermal vents with the submersible Alvin happen this year too.

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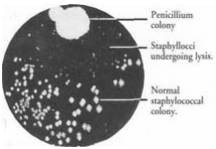
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In 1928, **Alexander Fleming**, a microbiologist working at St. Mary's Hospital in London discovered penicillin. Initially due to purification difficulties and the substance's instability he dismissed the substance as a laboratory curiosity. In 1939, Drs. Howard Florey and Ernst Chain working at Oxford, used freeze drying to stabilize pure penicillin. Using the freeze dried formulation they were able to carry out successful trials, demonstrating the antibiotic's effectiveness. Fleming, Florey and Chain shared the 1945 Nobel prize in medicine for this work.

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## Modern History: (1920's to Present) - The Age of Biotechnology

- **1980's:**  
Genetic manipulation and cloning (PCR).  
AIDS and causative agent HIV.  
Ribozymes or catalytic RNAs.  
RNAs used historical documents – Molecular phylogeny  
reshapes biological systematics to 3 domains of life.
- **1990's:**  
GEMs or genetically engineered microorganisms.  
Prions or specific protein pathogens.  
Dolly the sheep.  
Full genome sequences.
- **2000's:**  
Ed DeLong's contributions to marine microbiology.  
Craig Venter's environmental genome shotgun sequencing.  
What's next???

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### *Archaeoglobus fulgidus* Genome

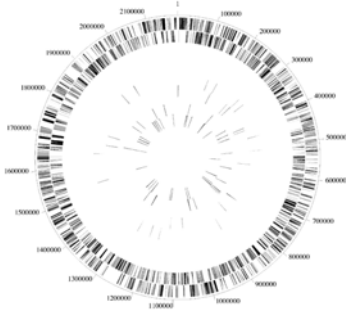


Figure Legend:  
A circular representation of the *A. fulgidus* genome illustrating the location of each predicted coding region as well as selected features of the genome. Outer concentric circle: predicted coding regions on the + strand classified as to role. Second concentric circle: predicted coding regions on the - strand. Third and fourth concentric circles: IS elements (red) and repeats (blue) on the + and - strand, respectively. Fifth and sixth concentric circles: tRNAs (blue), rRNAs (red) and small stable RNAs (green) on the + and - strand, respectively.

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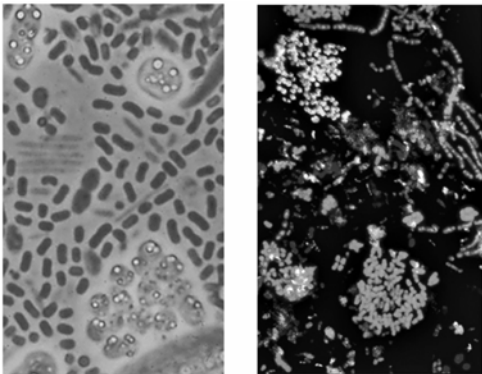
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### Improvements in microscopy



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## Proteorhodopsin phototrophy in the ocean

Oded Béjà<sup>1,†</sup>, Elena N. Spudich<sup>1,†</sup>, John L. Spudich<sup>1</sup>, Marion Leclerc<sup>1</sup> & Edward F. Delong<sup>2</sup>

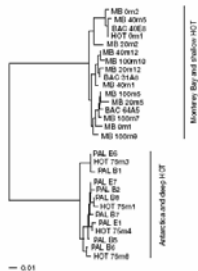
<sup>1</sup> Monterey Bay Aquarium Research Institute, Moss Landing, California 95038, USA

<sup>2</sup> Department of Microbiology and Molecular Genetics, The University of Texas Medical School, Houston, Texas 77030, USA

<sup>†</sup> These authors contributed equally to this work

Proteorhodopsin, a retinal-containing integral membrane protein that functions as a light-driven proton pump, was discovered in the genome of an uncultivated marine bacterium; however, the prevalence, expression and genetic variability of this protein in native marine microbial populations remain unknown. Here we report that photoactive proteorhodopsin is present in oceanic surface waters. We also provide evidence of an extensive family of globally distributed proteorhodopsin variants. The protein pigments comprising this rhodopsin family seem to be spectrally tuned to different habitats—absorbing light at different wavelengths in accordance with light available in the environment. Together, our data suggest that proteorhodopsin-based phototrophy is a globally significant oceanic microbial process.

From Nature, 2001



**Fig. 3** Phylogenetic analysis of the three-dimensional sequences of several proteorhodopsin genes. Distance analysis of 372 positions was used to construct the tree by neighbor joining using the Paup software program of the Wisconsin Package version 3.0 (Genetics Computer Group, Madison, Wisconsin). In addition, bootstrap resampling was used as an analysis, and is not shown. Scale bar represents number of substitutions per site. MB strains indicate the proteorhodopsins that were specifically characterized in this study.

Take Home Messages:

May the real “bacteriorhodopsin” Please stand up!

Major new way to make ATP in the ocean.

Two distinct “flavors” or evolutionary trajectories.

## Environmental Genome Shotgun Sequencing of the Sargasso Sea

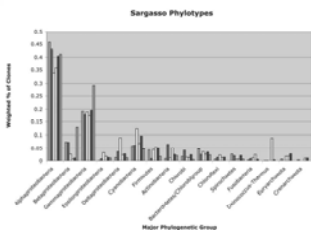
J. Craig Venter,<sup>1,\*</sup> Kevin Robinson,<sup>2</sup> John F. Dickel,<sup>3</sup> Aaron L. Halpern,<sup>4</sup> Dong Ruan,<sup>5</sup> Jonathan A. Eisen,<sup>6</sup> Dongyong Wu,<sup>7</sup> Ian Paulsen,<sup>8</sup> Karen E. Nelson,<sup>9</sup> William Nelson,<sup>9</sup> Derrick E. Fouts,<sup>10</sup> Samuel Levy,<sup>11</sup> Anthony H. Kemp,<sup>12</sup> Michael W. Loms,<sup>13</sup> Ken Nealson,<sup>14</sup> Owen White,<sup>15</sup> Jeremy Peterson,<sup>16</sup> Jeff Hoffman,<sup>17</sup> Rachel Parsons,<sup>18</sup> Holly Broken-Tillson,<sup>19</sup> Cynthia Plankoch,<sup>20</sup> Yu-Hui Rogers,<sup>21</sup> Hamilton O. Smith<sup>1</sup>

<sup>1</sup>The Institute for Biological Energy Alternatives, The Center for the Advancement of Genomics, 9901 Research Boulevard, Rockville, MD 20850, USA, <sup>2</sup>The Institute for Genome Research, 9712 Medical Center Drive, Rockville, MD 20850, USA,

<sup>3</sup>The J. Craig Venter Science Foundation Joint Technology Center, 9 Research Place, Rockville, MD 20850, USA, <sup>4</sup>University of Southern California, 223 Science Hall, Los Angeles, CA 90089-0740, USA, <sup>5</sup>Bermuda Biological Station for Research, Inc., 17 Biological Lane, St George GE 01, Bermuda.

<sup>\*</sup>To whom correspondence should be addressed. E-mail: jventer@tsg.org

We have applied “whole genome shotgun sequencing” to microbial populations collected *en masse* on tangential flow and impact filters from sea water samples collected from the Sargasso Sea near Bermuda. A total of 1.046 billion basepairs of non-redundant sequence was generated, annotated and analyzed to elucidate the gene content, and diversity and relative abundance of the organisms within these environmental samples. These data are estimated to derive from at least 1800 genomic species based on sequence relatedness, including 140 novel bacterial lineages. We have identified over 1.2 million new genes represented in these samples, including more than 782 new rhodopsin-like photoreceptors. Variation in species present and stoichiometry suggests substantial oceanic microbial diversity.



From Nature, 2004

Comparative death rates over the last century  
in terms of top 10 lists

Key:  
Green are non-microbial diseases,  
Red are microbial diseases.

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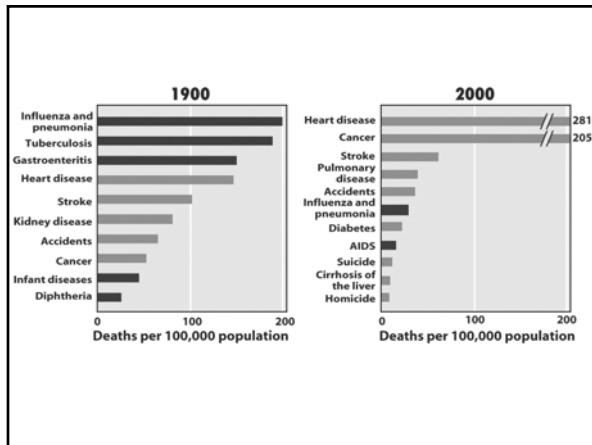
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“The microbes will have the last word.”

- Louis Pasteur

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