

# SISTEMATIKA MIKROBA

Oleh:

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# TAKSONOMI BAKTERI

- Taksonomi mikroba: ilmu yang mempelajari pengelompokan mikroba
- Taksonomi mikroba:
  1. **Klasifikasi** : Pengelompokan organisme ke dalam taksa berdasarkan persamaan maupun hubungan kekerabatan
  2. **Identifikasi**: proses karakterisasi organisme untuk menentukan suatu isolat adalah anggota dari suatu takson atau speies yang telah teridentifikasi sebelumnya
  3. **Nomenklatur**: pemberian nama spesifik berdasarkan aturan Internasional (International Code of Nomenclature of Bacteria[Sneath,1992]).

# TAKSONOMI BAKTERI

- Taksonomi bakteri menggabungkan banyak metode untuk identifikasi dan deskripsi spesies baru
- Menggunakan pendekatan polifasik:
  1. Analisis fenotipik
  2. Analisis genotipik
  3. Analisis filogenetik

# TAKSONOMI BAKTERI

- Karakteristik fenotipik:
  - data morfologi
  - data fisiologis dan biokimiawi
  - karakteristik kemotaksonomi:
    - analisis asam lemak
    - pola lipid polar yang terdapat pada membran
    - komposisi dinding sel
- Karakteristik genotipik
  - Hibridisasi DNA-DNA
  - kandungan G+C
  - Tipe sekuens multilokus
  - profil DNA
- Analisis filogenetik
  - analisis sekuens gen 16S rRNA
  - analisis sekuens multi gen
  - analisis genom

# Konsep Spesies dalam Mikrobiologi

- Eukaryot: spesies adalah dua organisme yang memiliki kemiripan dan hasil hidridisasinya merupakan keturunan yang fertil
- Tidak ada konsep spesies secara universal pada prokaryot
- Definisi spesies pd prokaryot:
  - kumpulan beberapa strain yang memiliki kemiripan yang tinggi pada beberapa sifat yang independen. Sifat yang paling ditekankan : kesamaan > 70% dari hasil Hibridisasi DNA-DNA atau > 98.5% dari analisis sekuens 16S rRNA

**Table 14.3** Some phenotypic characteristics of taxonomic value

**Major category Components**

|            |   |
|------------|---|
| Morphology | Colony morphology; Gram reaction; cell size and shape; pattern of flagellation; presence of spores, inclusion bodies (e.g., PHB <sup>a</sup> granules, gas vesicles, magnetosomes); stalks or appendages; fruiting-body formation |
| Motility   | Nonmotile; gliding motility; swimming (flagellar) motility; swarming; motile by gas vesicles  |
| Metabolism | Mechanism of energy conservation (phototroph, chemoorganotroph, chemolithotroph); utilization of individual carbon, nitrogen, or sulfur compounds; fermentation of sugars; nitrogen fixation; growth factor requirements          |

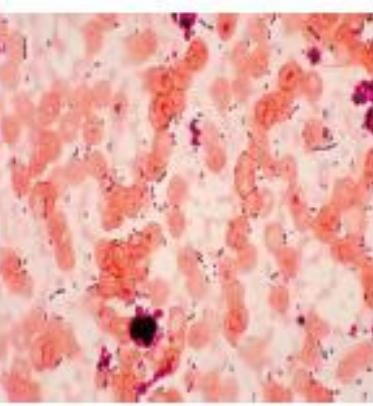
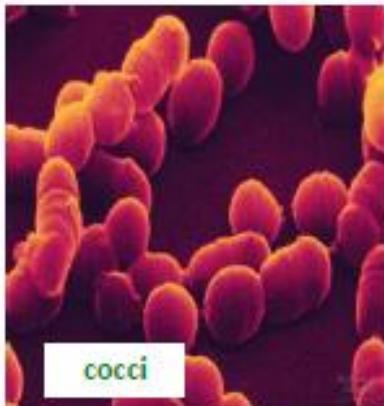
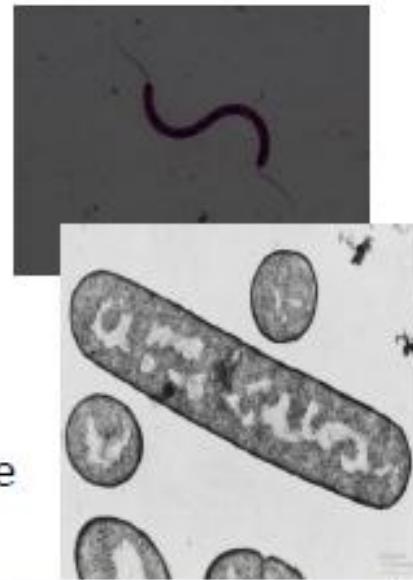
<sup>a</sup>PHB, poly- $\beta$ -hydroxybutyric acid (Section 4.10).

**Table 14.3 Some phenotypic characteristics of taxonomic value**

| <b>Major category</b>   | <b>Components</b>   |
|---|---|
| Physiology  | Temperature, pH, and salt ranges for growth; response to oxygen (aerobic, facultative, anaerobic); presence of catalase or oxidase; production of extracellular enzymes |
| Cell chemistry  | Fatty acids; polar lipids; respiratory quinones   |
| Other traits  | Pigments; luminescence; antibiotic sensitivity; serotype  |
| Other Chemical characterization: Peptidoglycan, Polyamines, techoic acids, mycolic acids, Lipopolysaccharides |   |

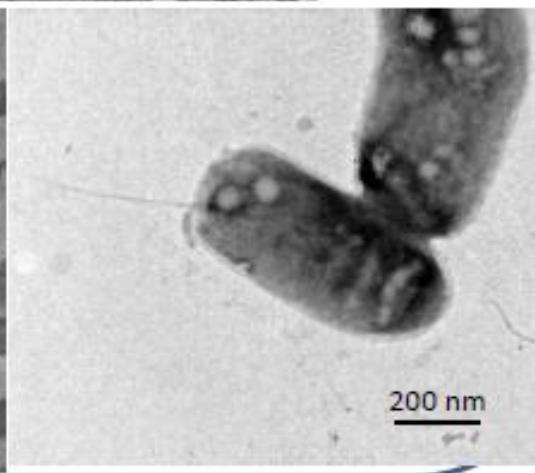
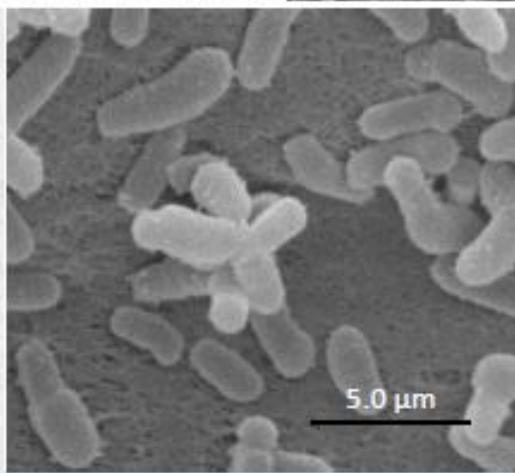
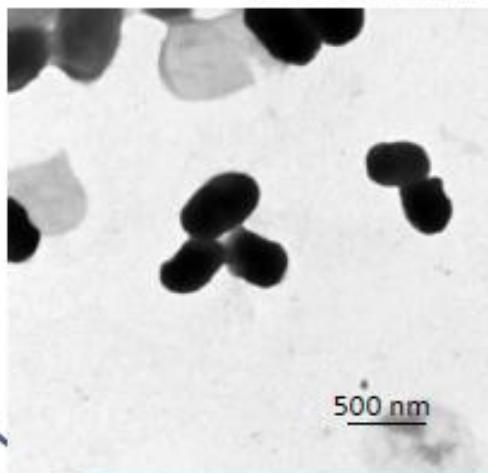
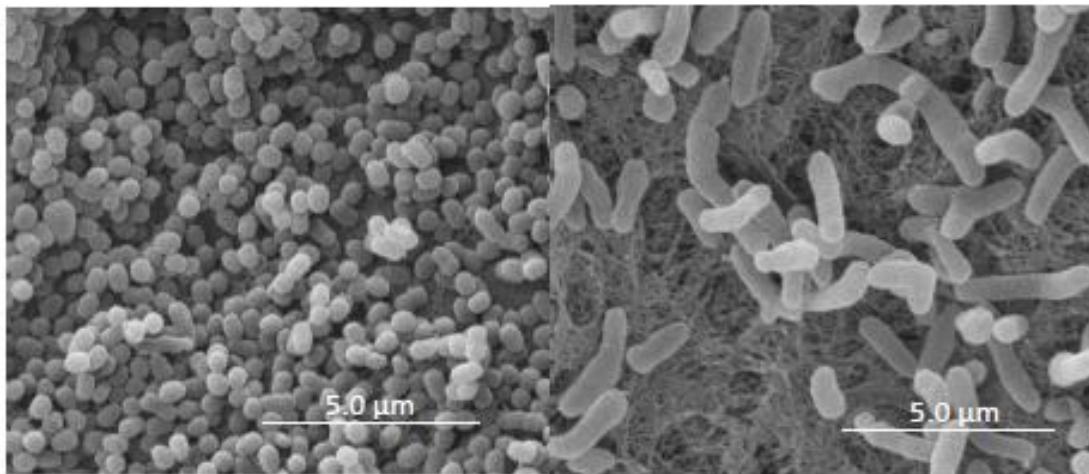
## Microscopic morphology

- Cell morphology: rod, coccus, or spirillum
- Cell arrangement: diplococcus, streptococcus, tetrad, sarcina, irregular clusters (Micrococcus or staphylococcus)
- Special cell structures: flagellum, cilia, spore, capsule



- Morphology

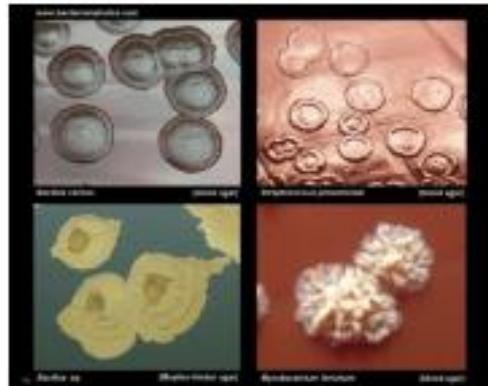
Scanning electron micrograph



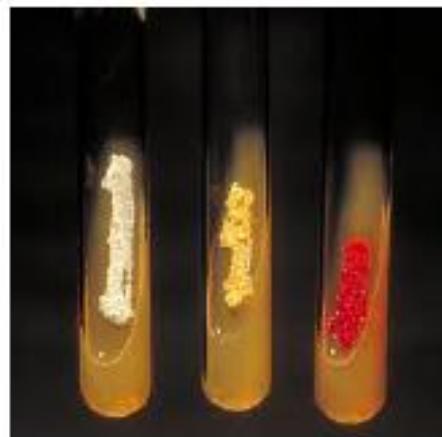
➤ Colony morphology :

Colonies can exhibit macroscopic differences

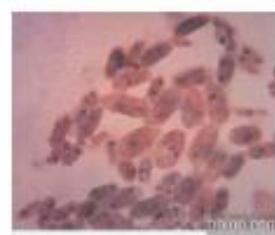
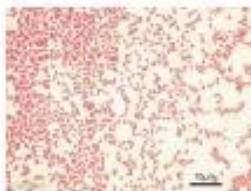
- ◆ colour, size,
- ◆ shape, margin or edge,
- ◆ surface feature etc.



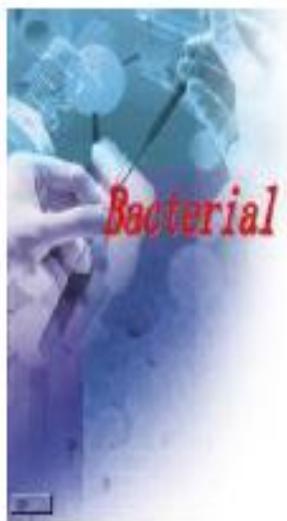
➤ Slant culture morphology



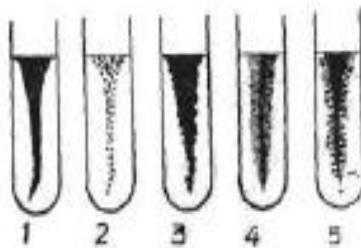
- Morphology
  - Staining
    - Gram stain (the reaction may alter as the cells age)
    - Acid-fast staining (strains containing mycolic acids)
    - Sudan Black staining (stains containing lipophilic cellular inclusions, eg. polyhydroxybutyric acid)
    - Others (eg. spore staining, capsule staining)



# physiological and biochemical data



*Bacterial Physiology*

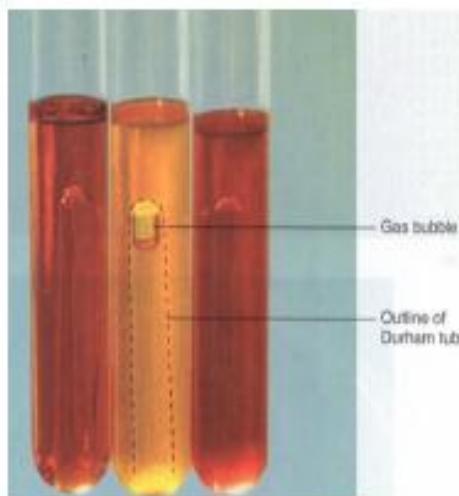


**Table 10.4 Characteristics of Some Important Biochemical Tests**

| Biochemical Test            | Principle of the Test   | Positive Reaction  |
|-----------------------------|---|--|
| Catalase                    | Detects the activity of the enzyme catalase, which causes the breakdown of hydrogen peroxide to produce O <sub>2</sub> and water.                 | Bubbles.   |
| Citrate                     | Determines whether or not citrate can be used as a sole carbon source.  | Growth, which is usually accompanied by the color change of a pH indicator.  |
| Gelatinase                  | Detects enzymatic breakdown of gelatin to polypeptides.   | The solid gelatin is converted to liquid.  |
| Hydrogen Sulfide Production | Detects H <sub>2</sub> S liberated as a result of the degradation of sulfur-containing amino acids.   | A black precipitate forms due to the reaction of H <sub>2</sub> S with iron salts in the medium.                                   |
| Indole                      | Detects the enzymatic removal of the amino group from tryptophan.   | The product, indole, reacts with a chemical reagent that is added, turning the reagent a deep red color.                           |
| Lysine Decarboxylase        | Detects the enzymatic removal of the carboxyl group from lysine.  | The medium becomes more alkaline, causing a pH indicator to change color.  |
| Methyl Red                  | Detects mixed acids, the characteristic end products of a particular fermentation pathway. ■ <b>mixed acids, p. 153</b>                           | The medium becomes acidic (pH < 4.5); a red color develops upon the addition of a pH indicator.                                    |
| Oxidase                     | Detects the activity of cytochrome c oxidase, a component of the electron transport chain of specific organisms.<br>■ <b>cytochrome c, p. 148</b> | A dark color develops upon the addition of a specific reagent.   |
| Phenylalanine Deaminase     | Detects the enzymatic removal of the amino group from phenylalanine.  | The product of the reaction, phenylpyruvic acid, reacts with ferric chloride to give the medium a green color.                     |
| Sugar Fermentation          | Detects the acidity resulting from fermentation of the sugar incorporated into the medium. Also detects gas production.                           | The color of a pH indicator incorporated into the medium changes if acid is produced. An inverted tube traps any gas that is made. |
| Urease                      | Detects the enzymatic degradation of urea to carbon dioxide and ammonia.  | The medium becomes alkaline, causing a pH indicator to change color.   |
| Voges-Proskauer             | Detects acetoin, an intermediate of the fermentation pathway that leads to the production of a 2, 3-butanol.<br>■ <b>2, 3-butanediol, p. 152</b>  | A red color develops upon addition of chemicals that detect acetoin.   |



Nitrate Reduction



Carbohydrate Fermentation



Urease detection



VP test



MR test

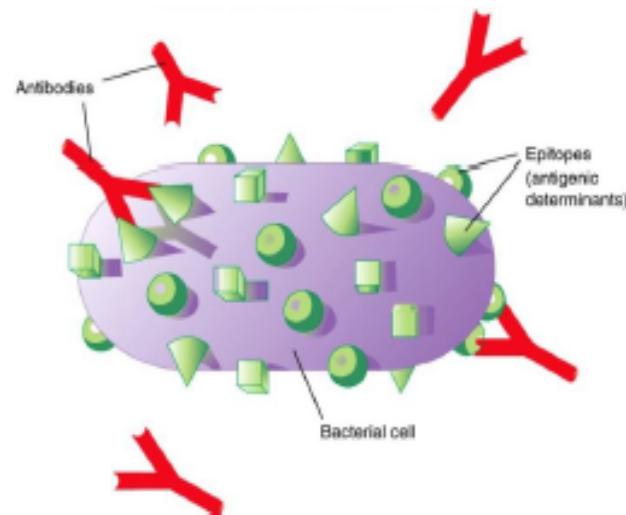
## Antibiotic sensitivity

➤ the ability of a microorganism to withstand the effects of an antibiotic on agar plates (Whether bacteria are susceptible, intermediate, or resistant depends on the amount of antibiotic and the diameter of zone of inhibition).



## Serological analysis

- Proteins and polysaccharides of some bacteria can function as identifying markers
  - Generally molecules on surface structures
    - e.g., Cell wall, glycocalyx, flagella, pili
  - Detection is based upon the specific interaction between antibodies and these antigens
    - e.g., Rapid detection of *Streptococcus pyogenes*



# Kekurangan dari metode konvensional

- Butuh personel yang kompeten
- Proses komplek
- Membutuhkan tenaga
- Membutuhkan waktu

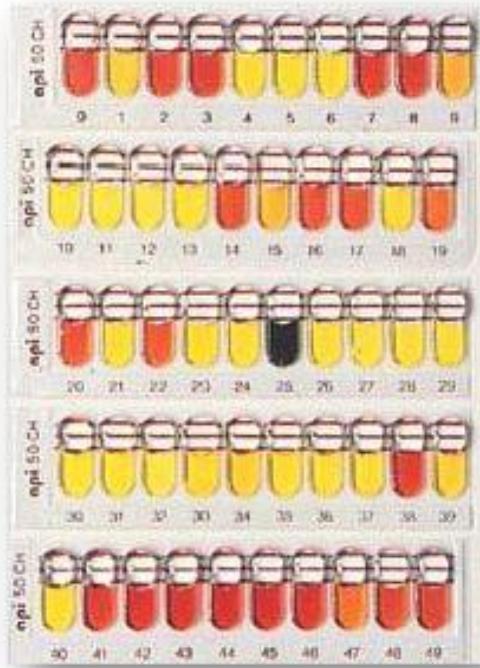
Bagaimana cara yang cepat mengidentifikasi bakteri dengan multi karakteristik??

## Rapid Tests

- Commercial modifications of traditional biochemical tests
  - API™ system
  - Biolog Microbial ID System



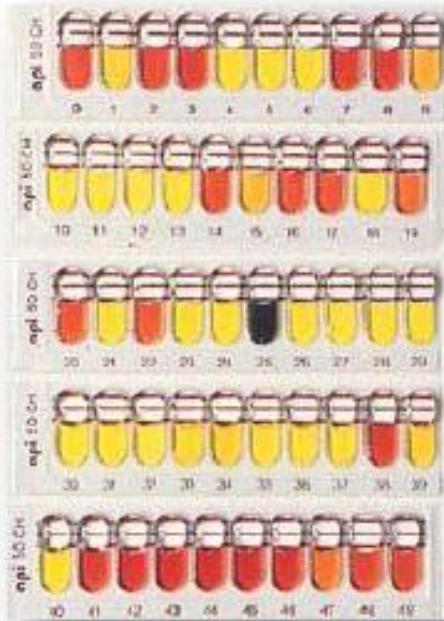
# commercial products for bacteria identification





The API identification system is numerical taxonomy according to the microbial physiological and biochemical characteristics.

- API 50 CH – Performance of carbohydrate metabolism tests
- API ZYM® – Semiquantification of enzymatic activities
- API 20E – 11 biochemical tests and enzymatic activities, 9 Fermentation/Oxidation
- .....

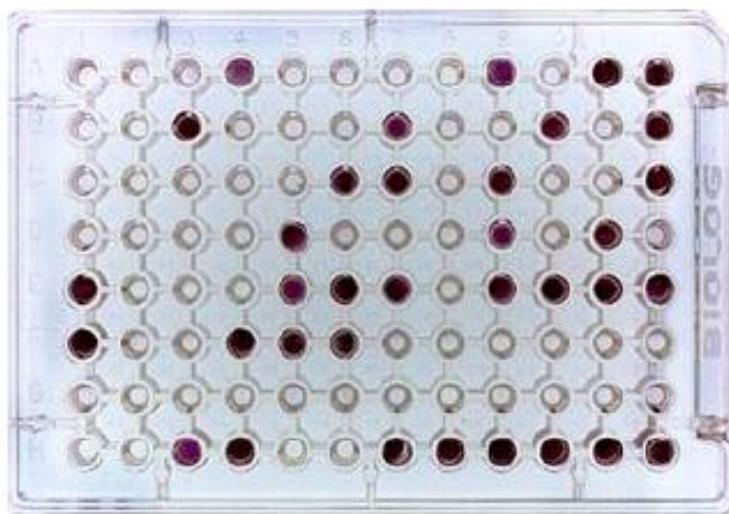
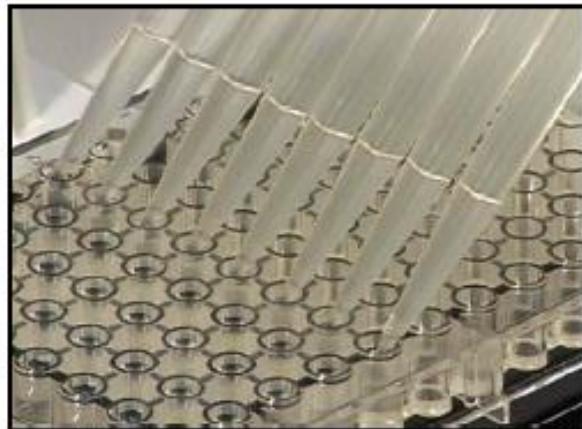
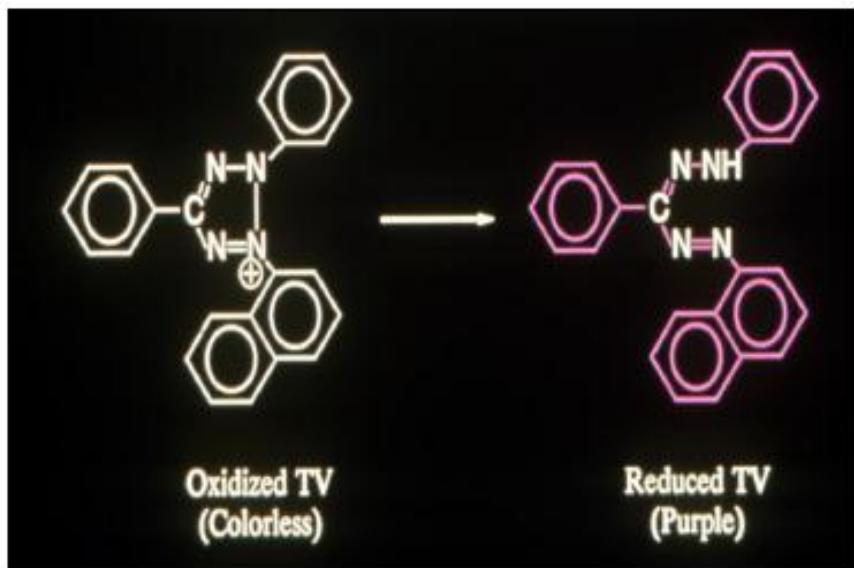




## Bacteria, Yeast and Fungi Identification

The Biolog Microbial ID System can rapidly identify over 2,500 species of aerobic and anaerobic bacteria, yeasts and fungi.

Tetrazolium redox dyes are used to colorimetrically indicate utilization of the carbon sources or resistance to inhibitory chemicals.



# simple, straightforward procedure

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1. Isolate pure culture on agar media
2. Prepare inoculum at specified cell density
3. Inoculate the Biolog MicroPlate
4. Incubate the plate, observe and enter the reaction pattern to obtain ID result



Isolate



Prepare



Inoculate

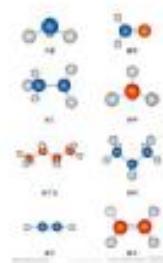
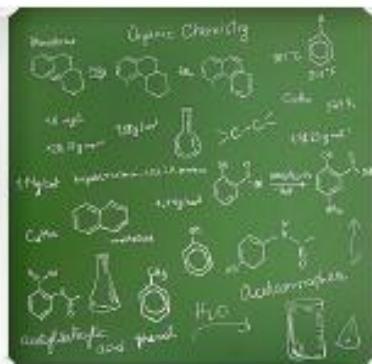


Incubate and Read

- Commercial systems are very accurate for the more common species and provide quick test results in a cost-effective manner.
- The MicroStation System has extensive applications also for microbial community analysis in soil, water and other environments.



## Chemical characterization

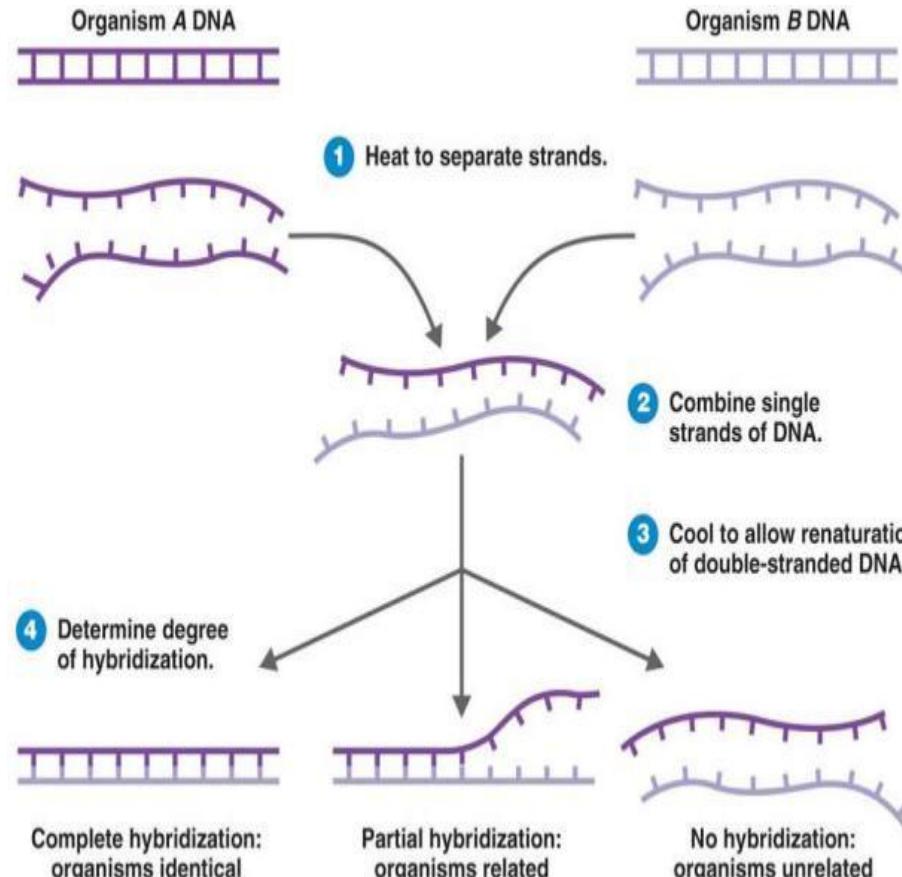


# Karakteristik kimiawi

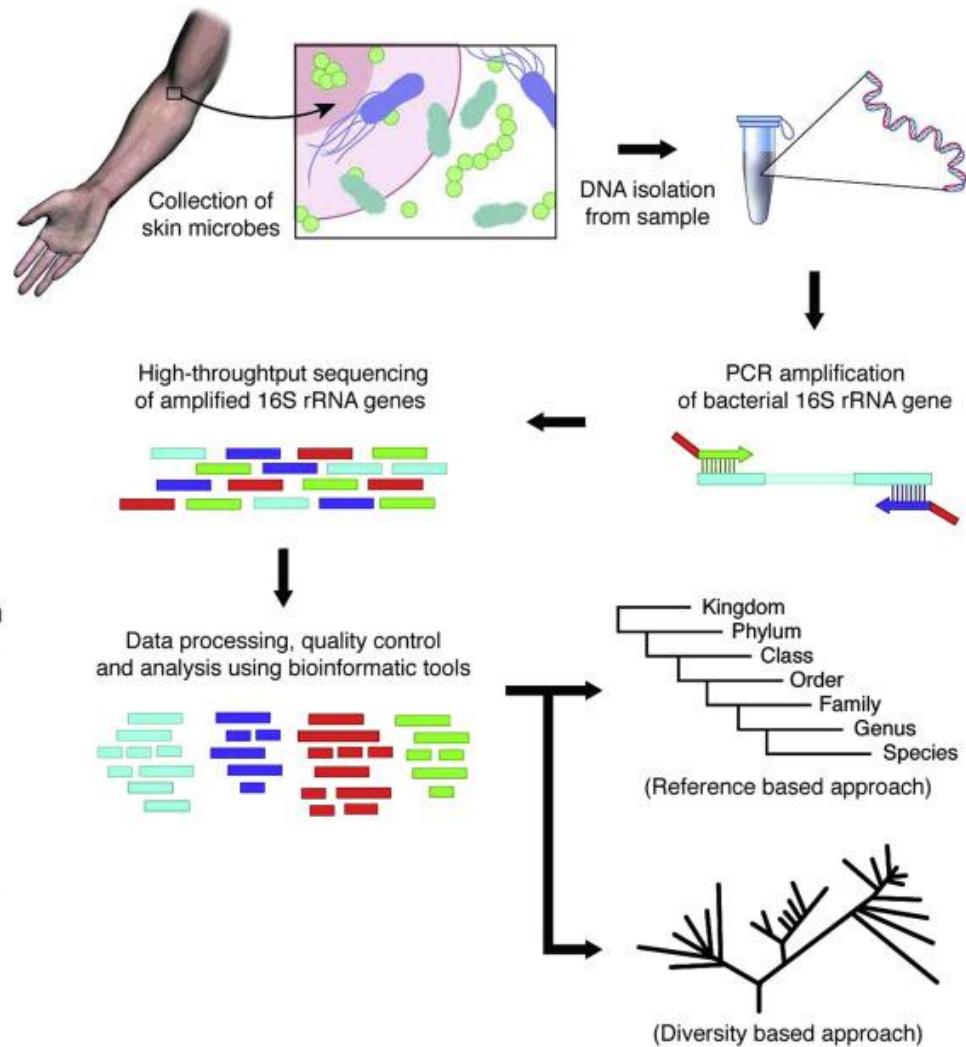
1. Kandungan asam lemak pd membran sel, menggunakan Gass Chomatography-Mass spectrophotometry (GC-MS) dan Microbial Identification (MIDI)
2. Quinon pada sistem respirasi seluler, menggunakan HPLC
3. Analisis phospolipid, menggunakan GC-MS, LC-MS
4. Dinding sel bakteri: peptidoglikan, asam amino, tipe polisakarida (Lipopolisakarida)

# Karakterisasi Genotipik

- Hibridisasi DNA

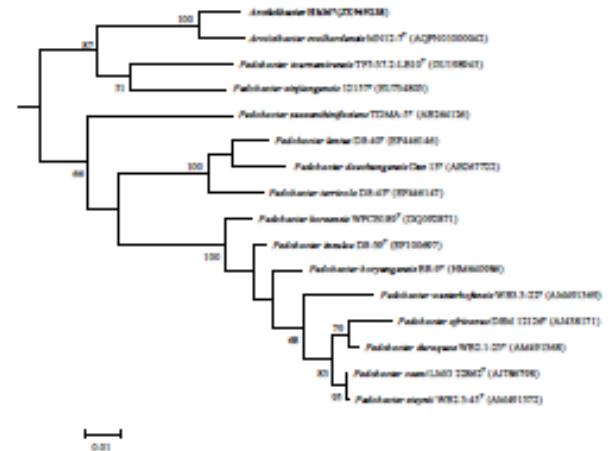
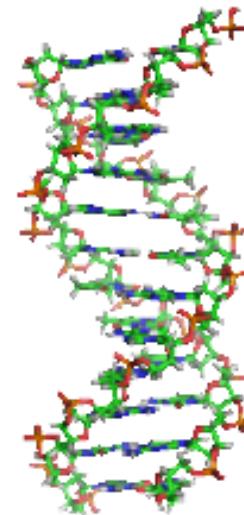


- Sekuens 16S rRNA



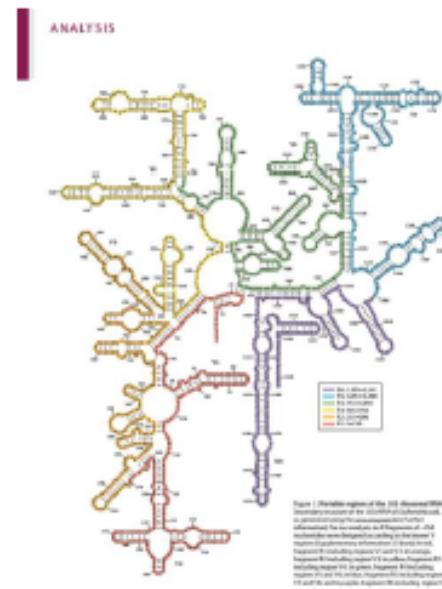
# Phylogenetic Analysis

- 16S rRNA gene sequence analysis
- Multi-gene sequence analysis
- Whole-genome sequence analysis



# 16S rRNA gene sequence analysis

- The most widely used molecular clocks are small subunit ribosomal RNA (SSU rRNA) genes
  - Found in all domains of life
    - 16S rRNA in prokaryotes and 18S rRNA in eukaryotes
  - Functionally constant
  - Sufficiently conserved (change slowly)
  - Sufficient length



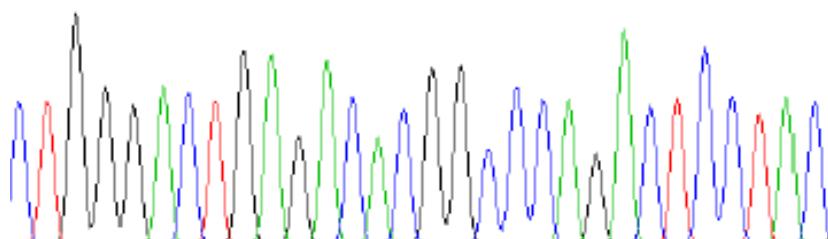
- **Carl Woese**

- Pioneered the use of SSU rRNA for phylogenetic studies in 1970s
- Established the presence of three domains of life:
  - *Bacteria, Archaea, and Eukarya*
- Provided a unified phylogenetic framework for *Bacteria*



## Phylogenetic Analysis—16S rRNA gene

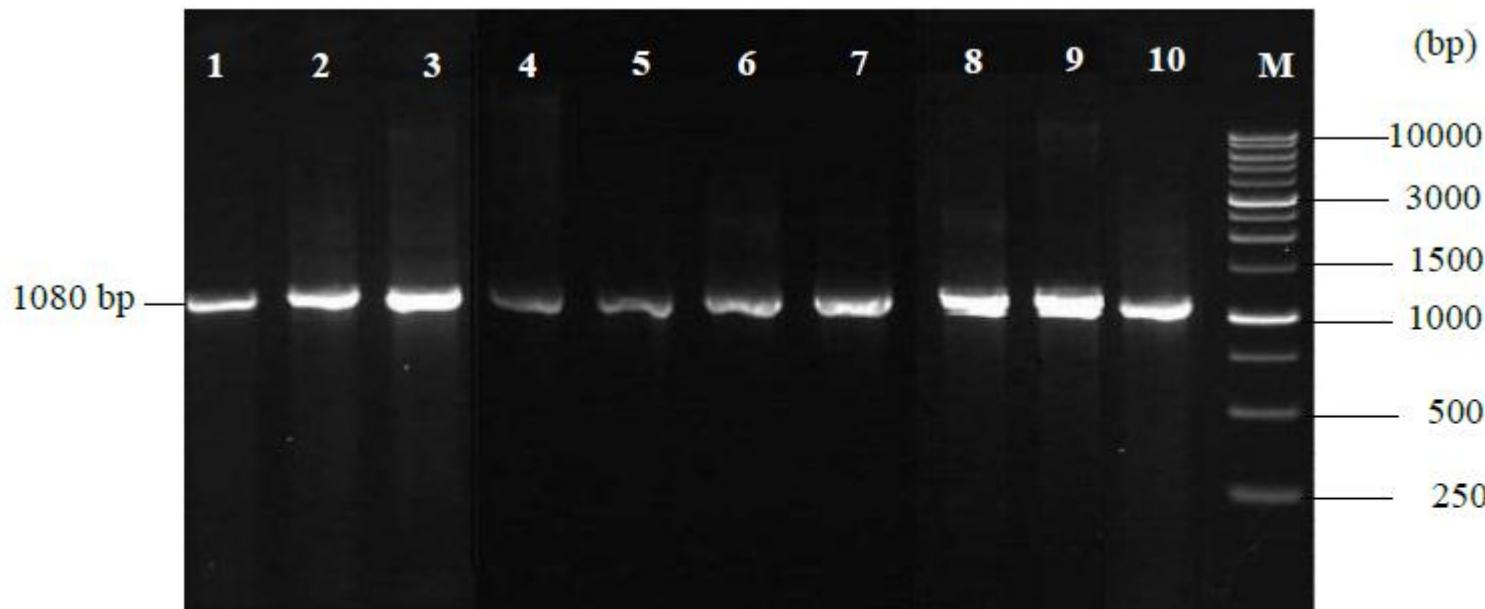
- 16S rRNA gene sequences are useful in taxonomy; serve as “**gold standard**” for the identification and description of new species
  - Proposed that a bacterium should be considered a new species if its 16S rRNA gene sequence differs by more than 3% from any named strain, and a new genus if it differs by more than 5%
  - Less than **98.5%** 16S similarity indicates different species, but greater than 98.5% does not indicate the same species.



Species 1  
Species 2  
Species 3  
Species 4

TACGCAGCCAGATACATGCCAAGATATTG  
TTCGCAACCTGATAACATCCTAACGATATTG  
TTCGCAGCCAGGTACATCCCAGGATATTG  
TTCGCAACCAGGTACATCCTAACGATATTG

# Identifikasi spesies Streptomyces spp. menggunakan sekuen gen 16S rRNA



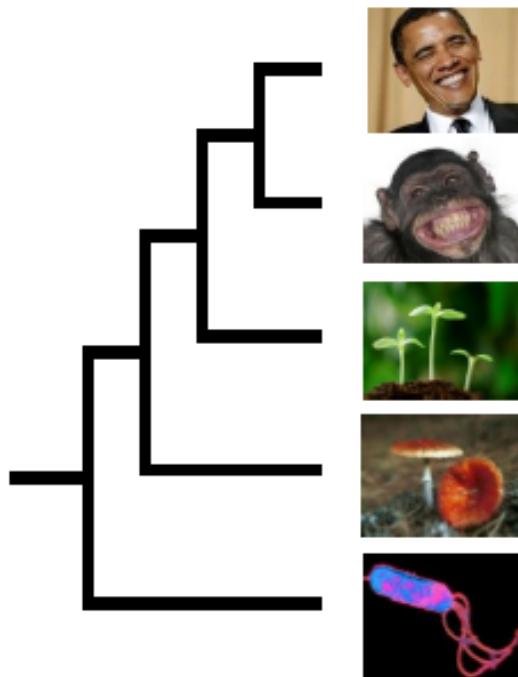
Gambar 17 Visualisasi gen 16S rRNA isolat aktinomiset potensial pada gel agarose 1% menunjukkan pita DNA berukuran 1080 bp. Kode isolat 1: ASR 46, 2: ASR 47, 3: ASR 54, 4: ASR 53, 5: ASR 56, 6: ASR 58, 7: ASR 65; 8: ASR 67, 9: ASR 75 dan 10: ASR 76. M = Marker *DNA ladder* 1 kb.

# Identifikasi spesies Streptomyces spp. menggunakan sekuens gen 16S rRNA

Tabel 7 Penyejajaran sekuens gen 16S rRNA isolat aktinomiset terpilih

| Isolat | Species homolog ( <i>GenBank</i> )              | Query Cover | E-value | Kemiripan (%) | Nomor Aksesi |
|--------|---|-------------|---------|---------------|--------------|
| ASR 46 | <i>Streptomyces belleus</i> NBRC 12844          | 100 %       | 0.0     | 99 %          | NR. 041222.1 |
| ASR 47 | <i>Streptomyces durhamensis</i> CSSP538         | 100 %       | 0.0     | 99 %          | NR. 041083.1 |
| ASR 53 | <i>Streptomyces violaceorubidus</i> LMG 20319   | 100 %       | 0.0     | 98 %          | NR. 042309.1 |
| ASR 54 | <i>Streptomyces thermocarboxydus</i> NBRC 16323 | 99 %        | 0.0     | 99 %          | NR. 112585.1 |
| ASR 56 | <i>Streptomyces griseorubens</i> NBRC 12780     | 100 %       | 0.0     | 99 %          | NR. 041066.1 |
| ASR 58 | <i>Streptomyces tendae</i> ATCC 19812           | 100 %       | 0.0     | 99 %          | NR. 025871.2 |
| ASR 65 | <i>Streptomyces tendae</i> ATCC 19812           | 100 %       | 0.0     | 99 %          | NR. 025871.2 |
| ASR 67 | <i>Streptomyces sesae</i> JR-39                 | 100 %       | 0.0     | 99 %          | NR. 137361.1 |
| ASR 75 | <i>Streptomyces thermocarboxydus</i> NBRC 16323 | 99 %        | 0.0     | 99 %          | NR. 112585.1 |
| ASR 76 | <i>Streptomyces ramulosus</i> NRRL B-2714       | 99 %        | 0.0     | 99 %          | NR. 043503.1 |

# Phylogenetic Analysis——Tree Building methods



- **Phenetic Methods—**

**Distance based**

- UPGMA
- Minimum Evolution
- Neighbor Joining
- Bayesian analyses

- **Cladistic Methods—**

**Character Based**

- Maximum likelihood
- Maximum Parsimony

# Phylogenetic Analysis——Tree Building methods



RAxML

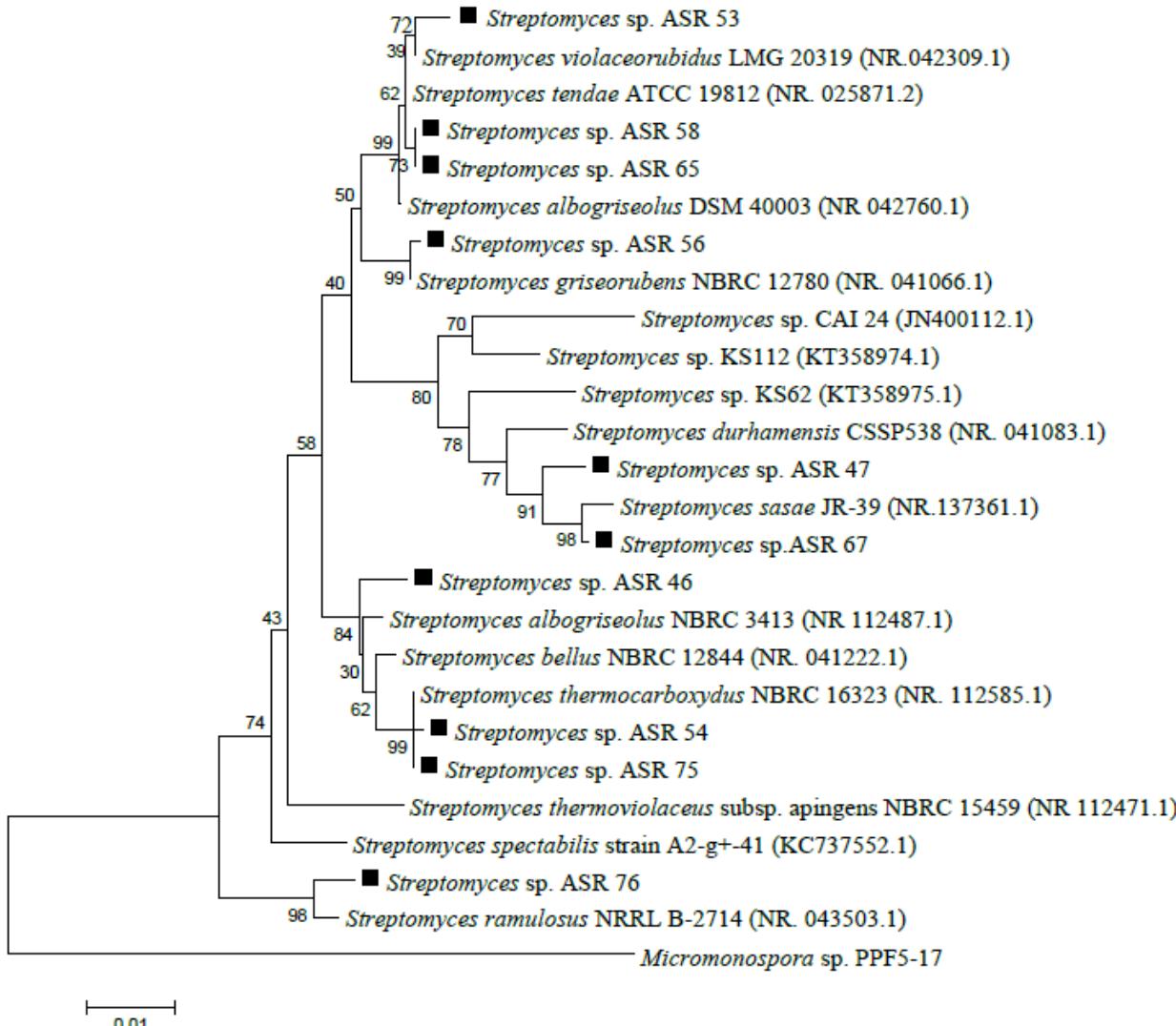


EzTaxon-e



16S rRNA gene database and  
workbench compatible with ARB  
[greengenes.lbl.gov](http://greengenes.lbl.gov)

# Pohon Filogenetik

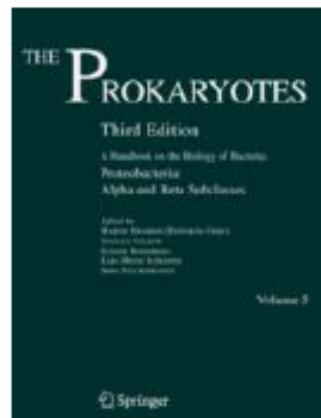
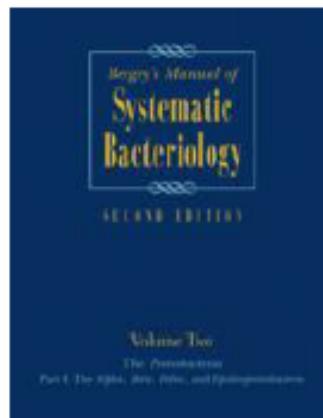


Gambar 18 Pohon filogenetik yang menunjukkan kekerabatan isolat aktinomiset rizosfer kedelai dengan spesies pembanding menggunakan metode *Neighbor-joining* berdasarkan sekvens gen 16S rRNA. Kotak hitam

# Taxonomy References

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- Major references in bacterial diversity
  - *Bergey's Manual of Systematic Bacteriology* (Springer)
    - Bergey's Manual of Determinative Bacteriology
    - Bergey's Manual of Systematic Bacteriology
  - *The Prokaryotes* (Springer)

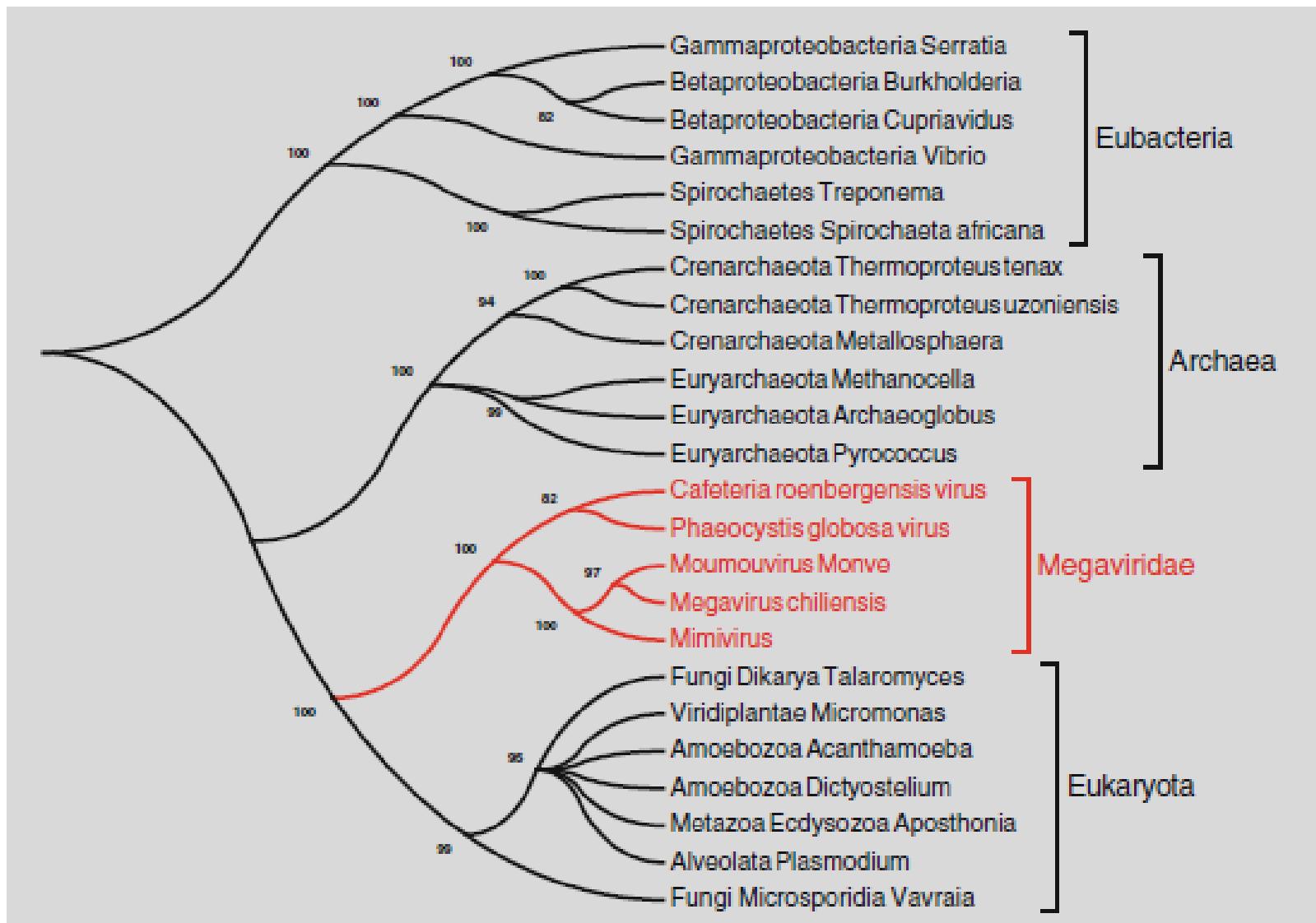


# Taxonomy References

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- NCBI Taxonomy  
<http://www.ncbi.nlm.nih.gov/Taxonomy/>
- TOBA  
<http://www.taxonomicoutline.org/>
- Bergey's Taxonomy  
<http://www.bergeys.org/outlines.html>
- List of Prokaryotic Names with Standing in Nomenclature  
<http://www.bacterio.cict.fr/index.html>
- Bacterial Nomenclature Up-to-Date  
[http://www.dsmz.de/microorganisms/bacterial\\_nomenclature.php](http://www.dsmz.de/microorganisms/bacterial_nomenclature.php)
- The International Code of Nomenclature of Prokaryotes:  
<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=icnb>
- EzTaxon-e Database  
<http://eztaxon-e.ezbiocloud.net/>

# MICROBIAL TAXONOMY



# MICROBIAL TAXONOMY

## Prokaryotes

- Domain Archaea [ancient bacteria]
- Domain Eubacteria [true bacteria]  
make up the old Kingdom Monera

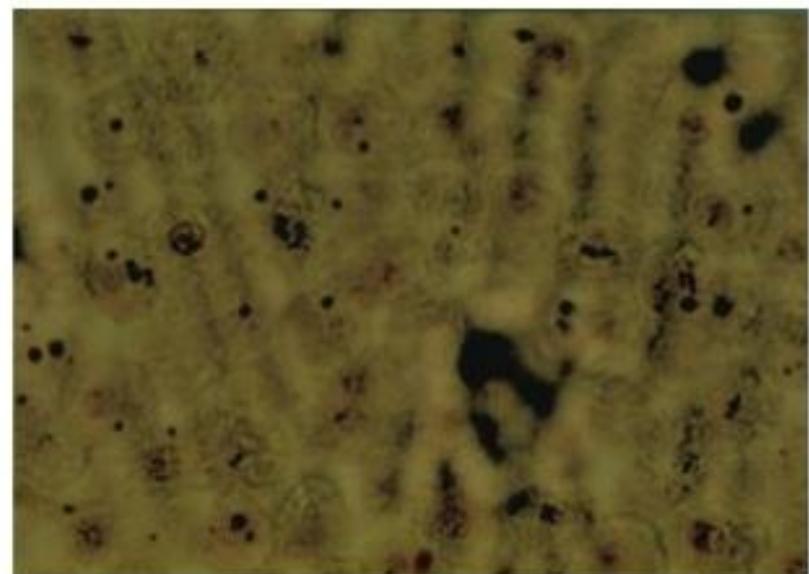
### Prokaryotes

- Most abundant group
- Widely distributed
- Oldest organisms



# Domain Eukarya

- Organelles
- Plasma membrane
- Cell wall, if present,  
made up of  
polysaccharides
  - Fungi : chitin
  - Plants/Algae: cellulose or  
pectins
  - Vertebrates: no cell wall



# Domain Eukarya

- Fungi
  - Molds
  - Yeasts
  - Mushrooms
- Protista
  - Slime Molds
  - Unicellular Algae
  - Protozoa
- Plantae
  - Multicellular Algae
  - Mosses
  - Plants
- Animaliae
  - Insects
  - Worms
  - Sponges
  - Vertebrates

# TAKSONOMI FUNGI

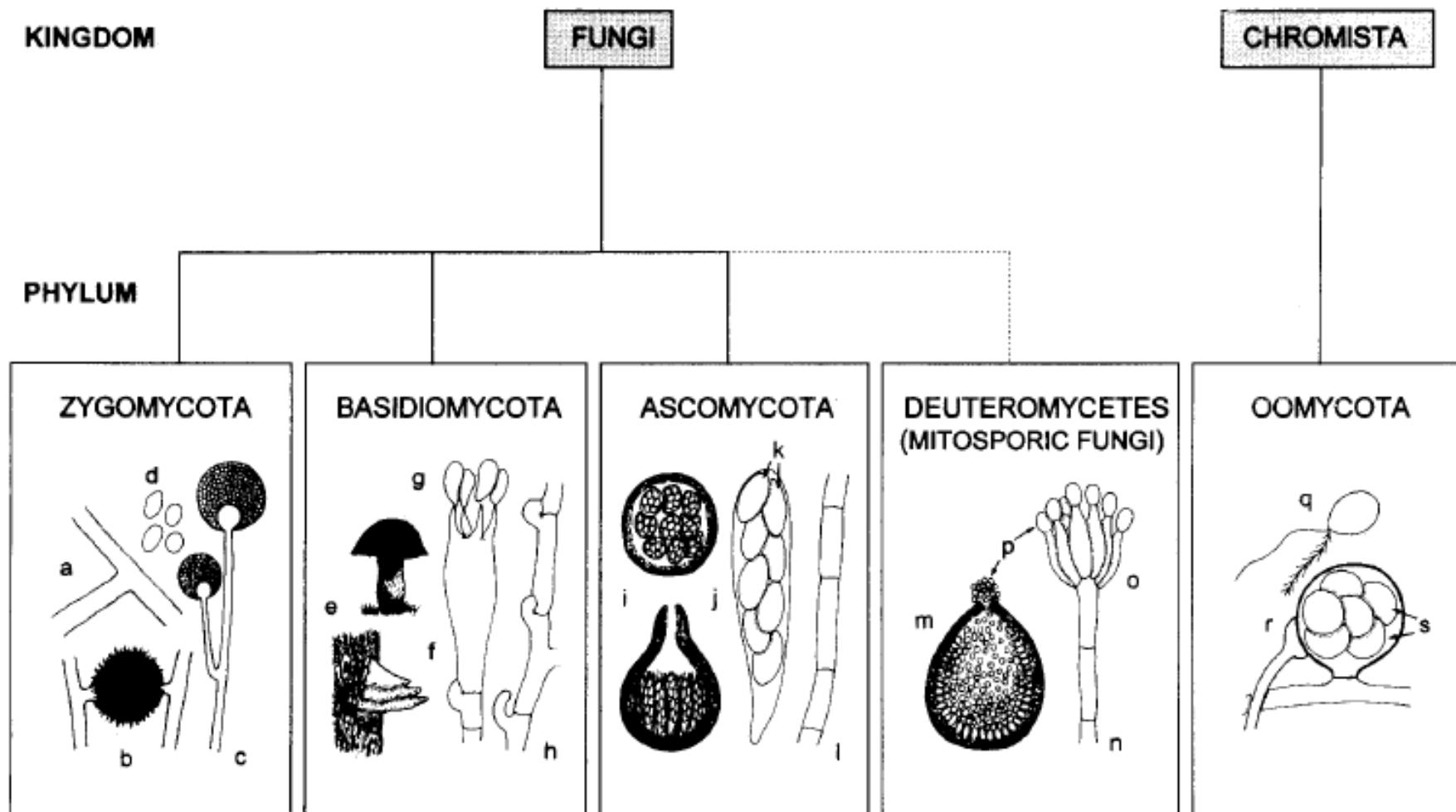
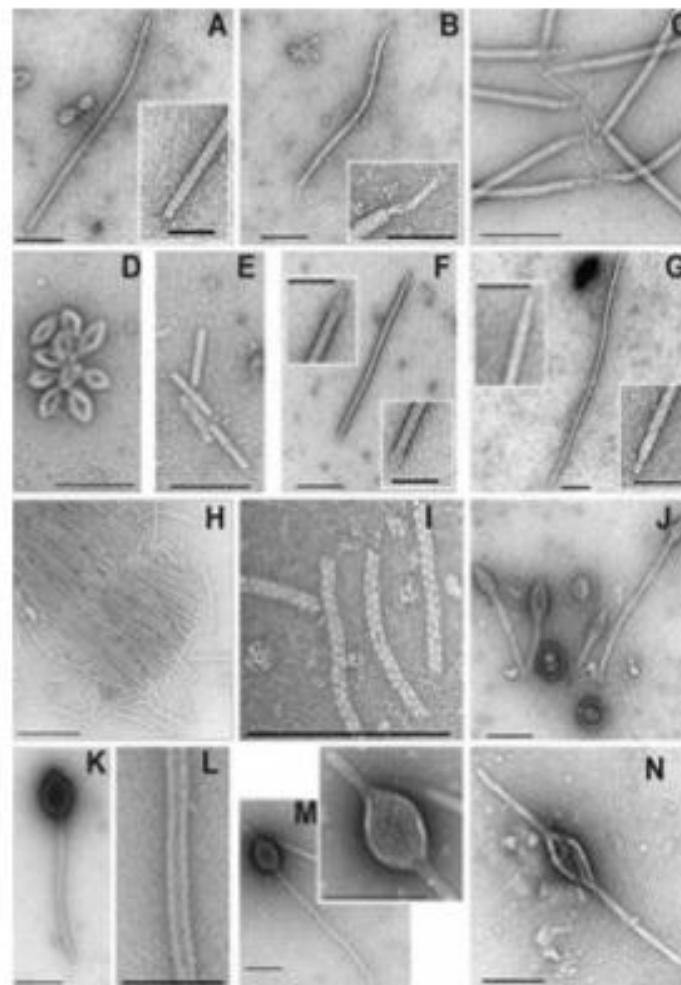


FIG. 2. Diagnostic features of the fungal phyla of clinical relevance. Zygomycota: a, coenocytic hypha; b, zygosporangium; c, sporangiophore; d, sporangiospores. Basidiomycota: e, basidiomata; f, basidium; g, basidiospores; h, hypha with clamp connections. Ascomycota: i, ascocarps; j, ascus; k, ascospores; l, septate hypha. Deuteromycetes: m, pycnidium; n, conidiophore; o, conidiogenous cells; p, conidia. Oomycota: q, zoospore; r, gametangia; s, oospores.

# Domain Archaea

- Evolved from earliest cells
- Methanogens
  - Largest group
  - Use CO<sub>2</sub>
- Extreme environment
  - Halophiles
  - Thermophiles
  - Psychrophiles
  - Acidophiles
  - Alkaliphiles



## **Features of Archaea**

- Prokaryotes (no membrane bound nucleus)
- Lack Peptidoglycan in their cell walls
- Genome is circular DNA
- Histone proteins are present
- Ribosomes are more similar to bacteria than eukaryotes
- Many occupy "extreme" environments.  
**Extremophiles**
- Not known to cause disease in humans or animals

# Extreme Halophiles

Great salt lake



(a)

T. D. Brock

Seawater evaporation ponds



(b)

NASA

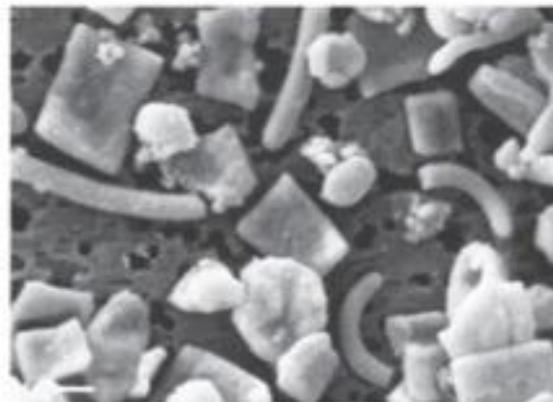
African "soda lake" high alkalinity, high salinity



(c)

Michael T. Madigan

SEM of halophiles



(d)

Francisco Rodriguez-Valera

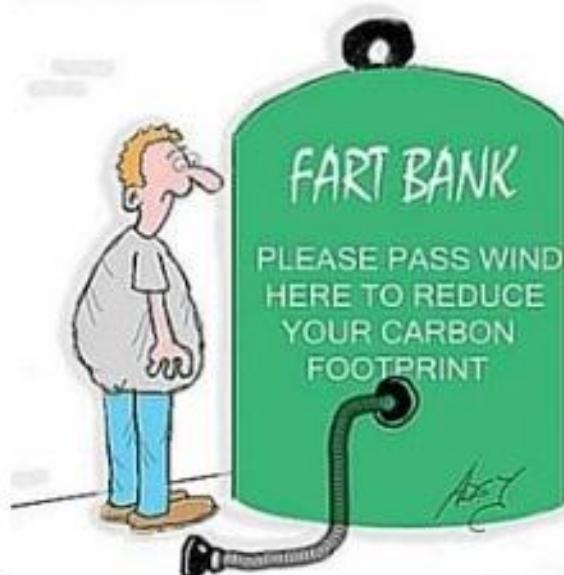
## Methanogens

- Convert carbon dioxide, hydrogen gas, and organic acids to methane gas
- Largest group of archaea
- Convert organic wastes in pond, lake, and ocean sediments to methane
- Some live in colons of animals; are one of primary sources of environmental methane

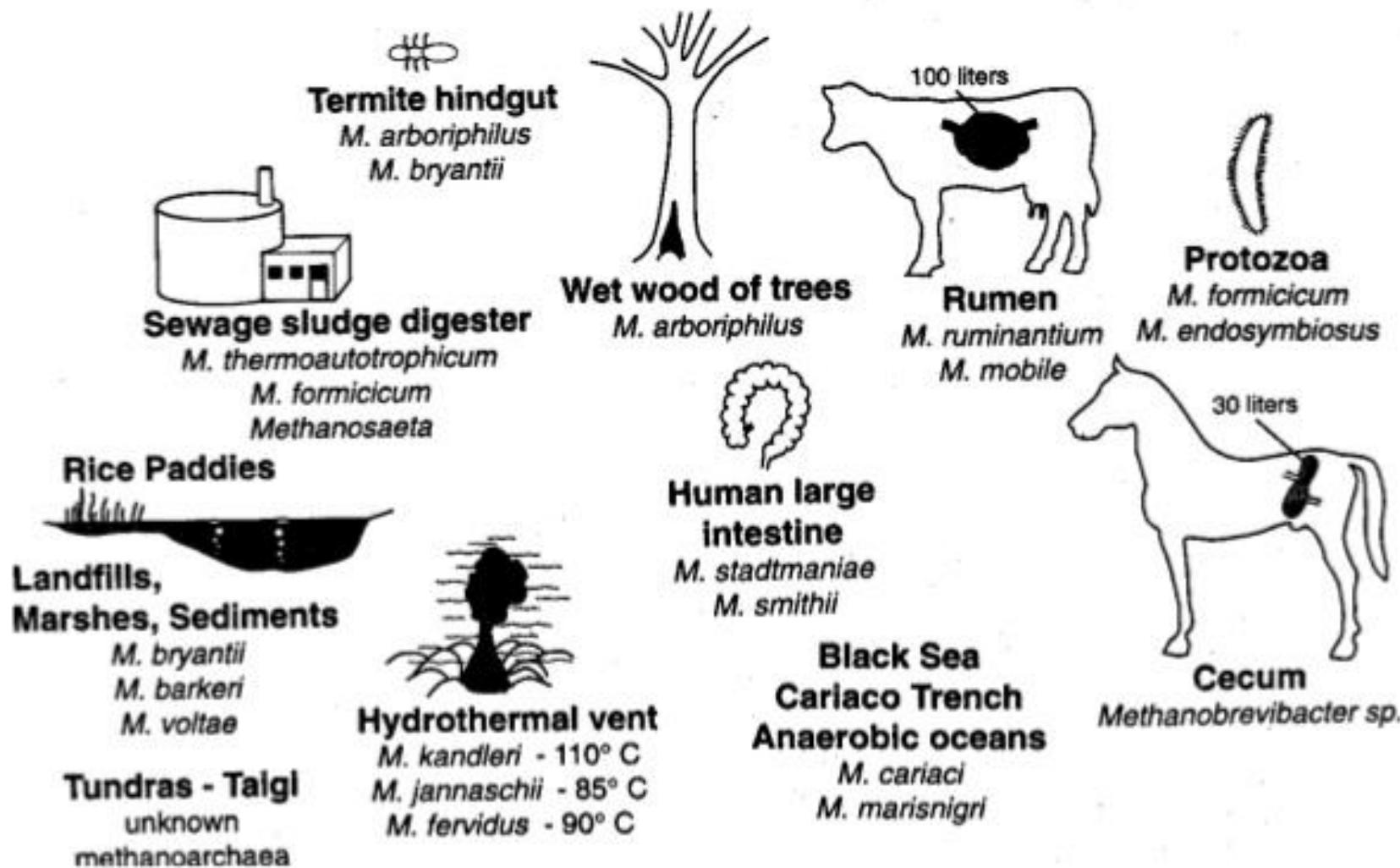
# Methanogens

- CH<sub>4</sub> (methane producers)
- Strict anaerobes
- Example genus: *Methanococcus*

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# Methanogen Habitats



## Hyperthermophiles

- Most are obligate anaerobes
- Most require S° as part of their metabolic scheme
- Example Genera:
  - *Sulfolobus*      *Thermococcus*                  *Pyrolobus*
- Hyperthermophiles – require temperatures over 80°C
- Heat stable biomolecules

# Hyperthermophile Habitats



T. D. Brock

(a)



T. D. Brock

(c)



T. D. Brock

(b)



T. D. Brock

(d)

# DOMAIN EUBACTERIA

1. Bakteri fotosintetik (Phototrophic bacteria)
2. Bakteri Gram-positif (low & high G+C content)
3. Bakteri Gram-negatif proteobacteria
  - a. Alphaproteobacteria
  - b. Betaproteobacteria
  - c. Gammaproteobacteria
  - d. Deltaproteobacteria
  - e. Epsilonproteobacteria

# Phototrophic Bacteria

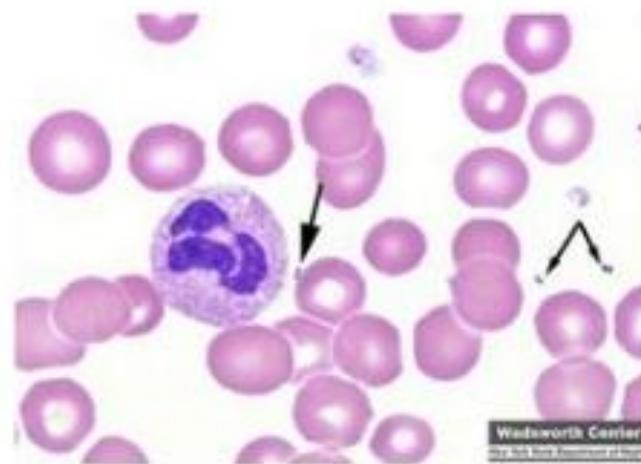
- Photoautotrophs
- Five groups ... (often grouped by color)
  - Blue-green bacteria (cyanobacteria)
    - Chlorophyll a (oxygenic photosynthesis)
  - Green sulfur bacteria bacteriochlorophyll
  - Green nonsulfur bacteria “
  - Purple sulfur bacteria “
  - Purple nonsulfur bacteria “

# Photosynthetic Bacteria

| Selected Characteristics of Photosynthesizing Bacteria |                       |                |  |  |                        |
|--|-----------------------|----------------|--|--|------------------------|
| Common Name  | Example               | Phylum         | Comments   | Electron Donor for CO <sub>2</sub> Reduction | Oxygenic or Anoxygenic |
| Cyanobacteria  | <i>Anabaena</i>       | Cyanobacteria  | Plantlike photosynthesis; some use bacterial photosynthesis under anaerobic conditions | Usually H <sub>2</sub> O                     | Usually oxygenic       |
| Green nonsulfur bacteria                               | <i>Chloroflexus</i>   | Chloroflexi    | Grow chemoheterotrophically in aerobic environments                                    | Organic compounds                            | Anoxygenic             |
| Green sulfur bacteria                                  | <i>Chlorobium</i>     | Chlorobi       | Deposit sulfur granules outside cells  | Usually H <sub>2</sub> S                     | Anoxygenic             |
| Purple nonsulfur bacteria                              | <i>Rhodospirillum</i> | Proteobacteria | Can grow chemoheterotrophically as well  | Organic compounds                            | Anoxygenic             |
| Purple sulfur bacteria                                 | <i>Chromatium</i>     | Proteobacteria | Deposit sulfur granules outside cells  | Usually H <sub>2</sub> S                     | Anoxygenic             |

# Low G+C Gram Positive Organisms

- Rods
  - Clostridia
  - Mycoplasmas
  - Bacillus
  - Listeria
  - Lactobacillus
- Cocci
  - Streptococcus
  - Enterococcus
  - Staphylococcus

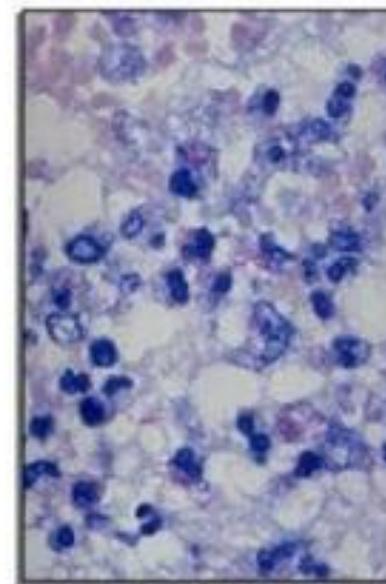
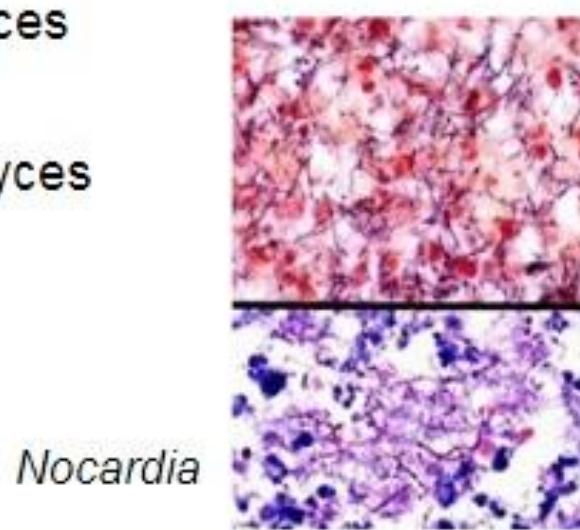
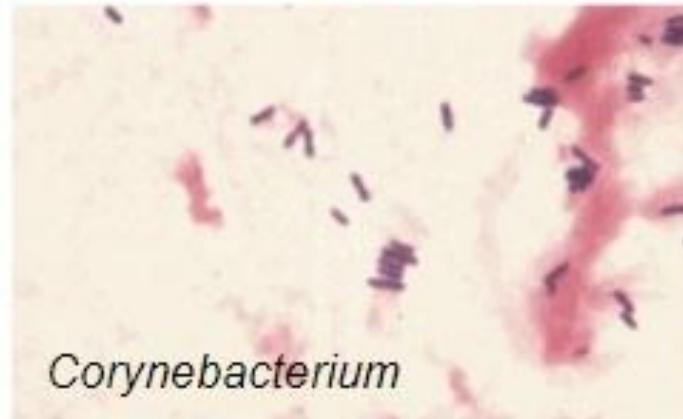


Wadsworth Center  
New York State Department of Health

# High G+C Gram Positives

- Rods

- *Corynebacterium*
- *Mycobacterium*
- *Actinomycetes*
  - *Actinomyces*
  - *Nocardia*
  - *Streptomyces*



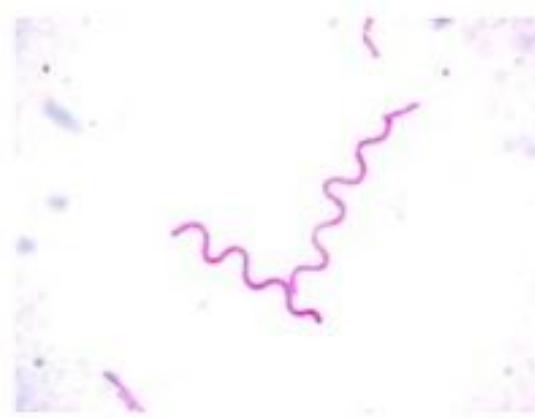
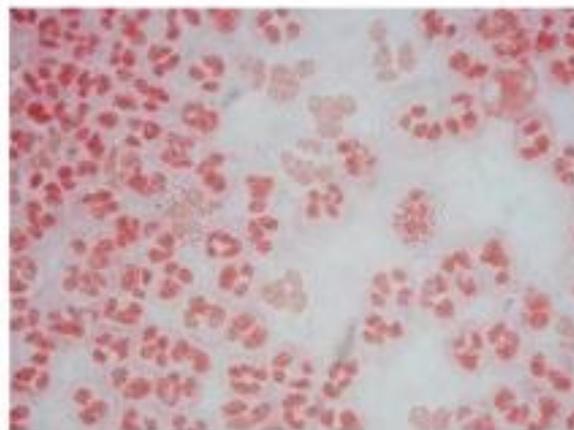
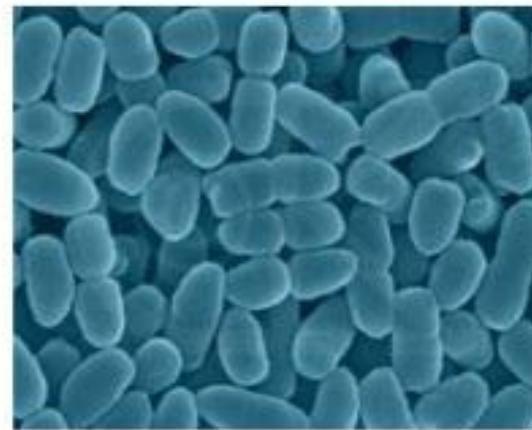
# Alphaproteobacteria

- Nitrogen fixers
  - *Azospirillum*
  - *Rhizobium*
- Nitrifying bacteria
  - *Nitrobacter*
- Purple nonsulfur phototrophs
- Pathogenic alphaproteobacteria
  - *Rickettsia*
  - *Brucella*
  - *Ehrlichia*
- *Caulobacter*



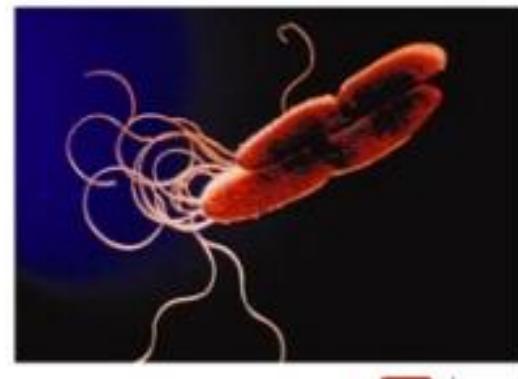
# Gram Negative Beta Proteobacteria

- Pathogenic
  - *Neisseria*
  - *Bordetella*
  - *Spirillum*
  - *Burkholderia*



# Gammaproteobacteria

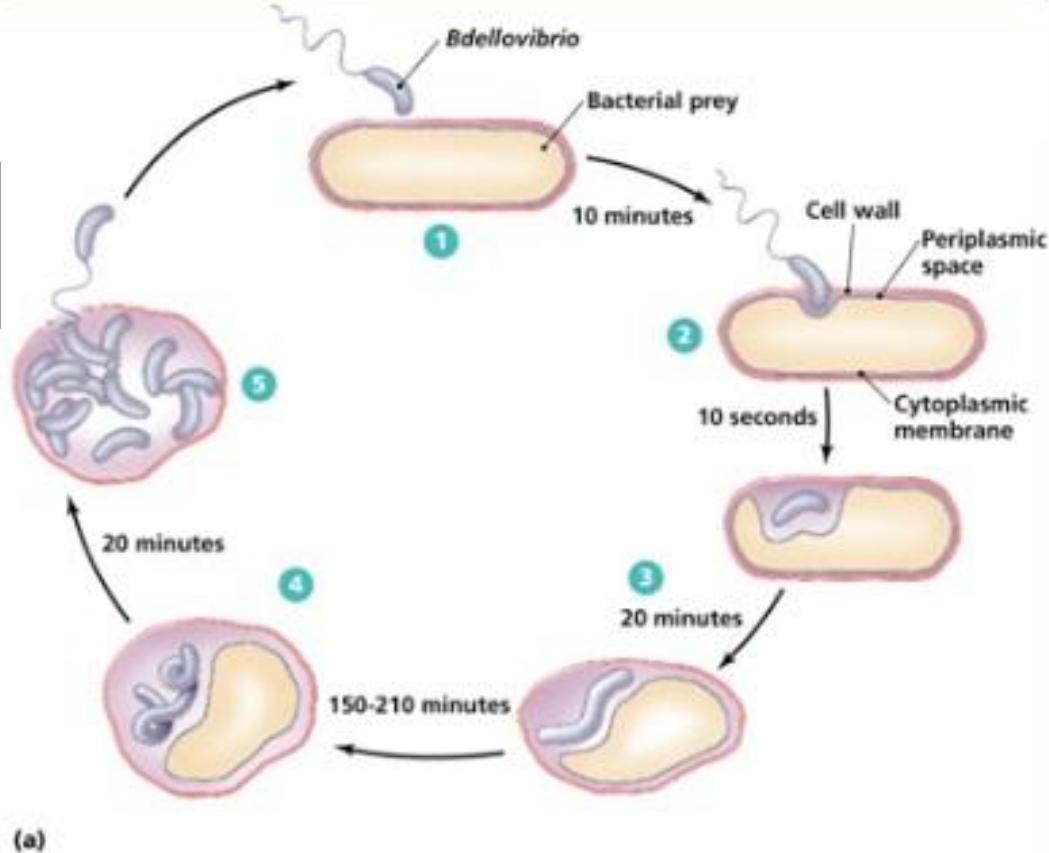
- Purple sulfur bacteria
- Intracellular pathogens
  - *Legionella*
  - *Coxiella*
- Methane oxidizers
- Facultative anaerobes
  - Family *Enterobacteriaceae*
- Pseudomonads
  - *Pseudomonas*
  - *Azotobacter*
  - *Azomonas*



# Deltaproteobacteria

## ■ *Bdellovibrio*

Parizite and kill other gram negative bacteria



(a)

## ■ Myxobacteria

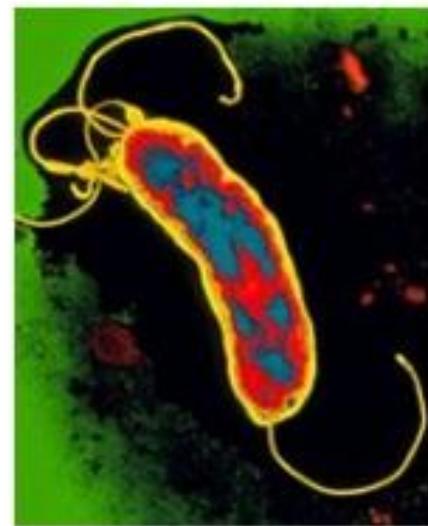
Slime bacteria

# Gram Negative Epsilon Proteobacteria

- Pathogenic

- *Campylobacter*
- *Helicobacter*

Food poisoning bacteria



*Helicobacter*

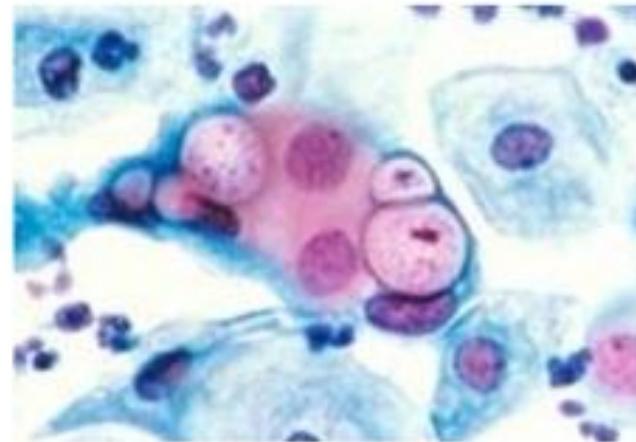


*Campylobacter*

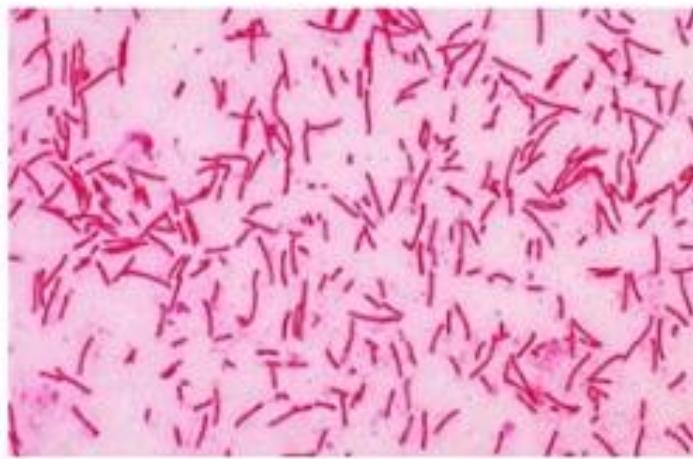
# Other Bacteria

- Pathogenic
  - Chlamydia
  - Spirochetes
    - *Treponema* [syphilis]
    - *Borrelia* [Lyme ds]

Chlamydia



*Borrelia*



*Treponema*

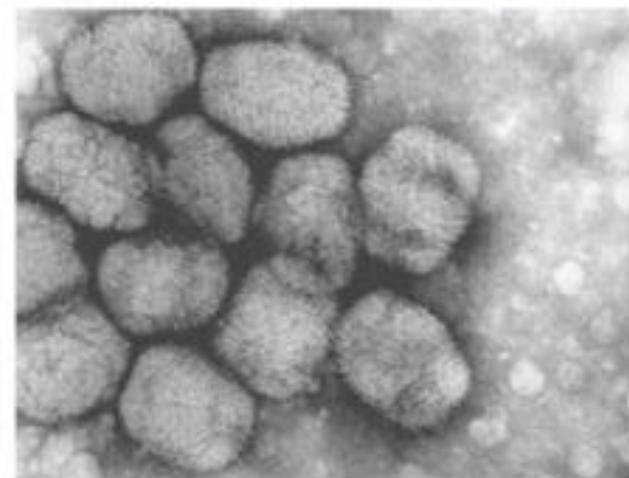


# Virus

- Acellular
- Nucleic acid core
- Protein coat
- Envelope [+/-]



- Modifications
  - Viroids: RNA virus
  - Virions : outside host
  - Prions: proteins



# Mikroba dan respon kekebalan sel

