

IDENTIFICAÇÃO DE BACTÉRIAS

Taxonomia clássica

A stylized, dark teal silhouette of a mountain range is located in the bottom right corner of the slide, adding a decorative element to the background.

Isolamento e características culturais

- ◆ Após escolher ou receber o espécime a ser estudado procede-se ao isolamento em meios sólidos apropriados. Para isolar o microrganismo efetua-se a inoculação ou semeadura, utilizando a técnica do esgotamento do inóculo por estrias; técnicas de semeadura por disseminação com alça de Drigalsky ; ou a técnica de pour-plate quando, além do isolamento, pretende-se quantificar a população microbiana presente originalmente na amostra.

Características da colônia

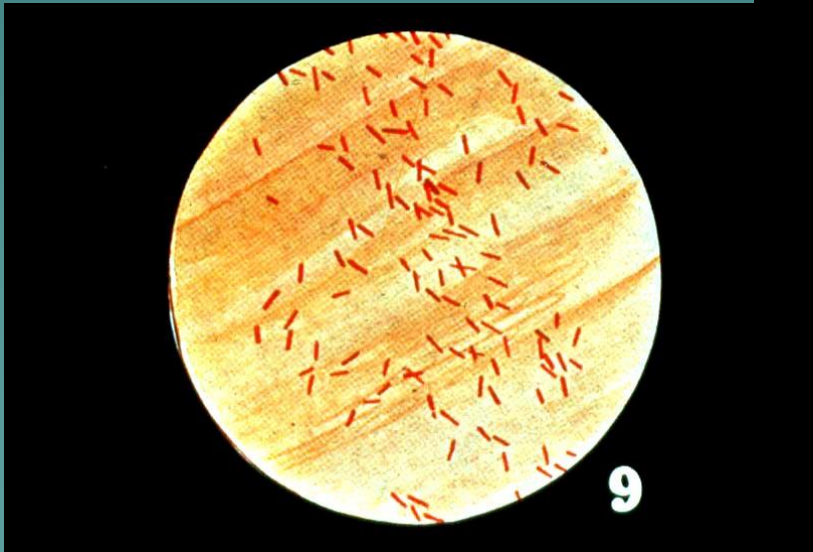
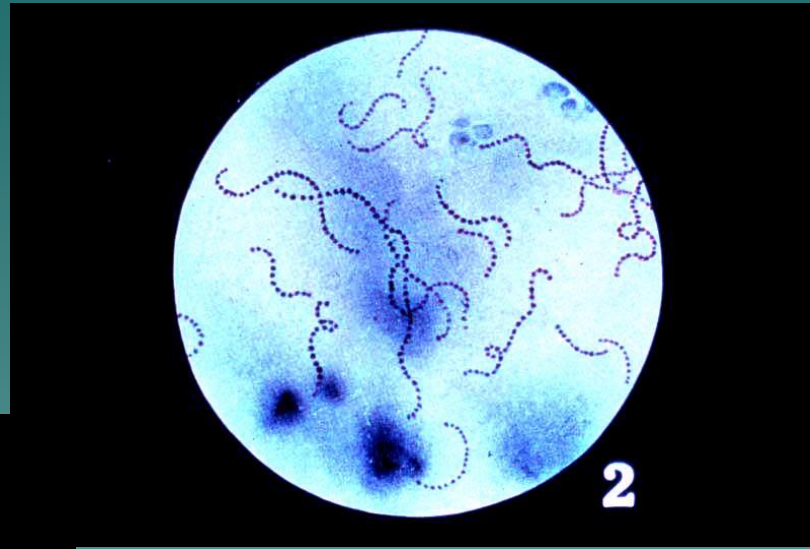
- ◆ 1. **Forma** - punctiforme (até 1 mm de diâmetro); circular, com mais de 1 mm de diâmetro; irregular; filamentosa; radiada, dentre outras;
- ◆ 2. **Tamanho** - ...em milímetros;
- ◆ 3. **Elevação** - achatada (alta, baixa), espraiada, convexa, umbilicada ou umbonada, mamilonada, papilífera, etc.;
- ◆ 4. **Bordos** - inteiros, ondulados, lobados, denticulados, franjados, etc.;
- ◆ 5. **Superfície** - lisa, rugosa;
- ◆ 6. **Estrutura** - amorfa, granulosa, filamentosa;
- ◆ 7. **Caracteres ópticos** - opaca, translúcida e transparente;
- ◆ 8. **Cromogenia ou Pigmentação** - amarela, branca, preta, rósea, etc.;

Características em meio líquido

- ◆ 1. **Tipo de crescimento na superfície** - sem crescimento, película, grumosa, membranosa, anel (circular);
- ◆ 2. **Opacidade ou turvação** - nula, ligeira ou suave, regular, intensa, transitória, persistente;
- ◆ 3. **Cheiro** - nenhum, pronunciado, semelhante a ...;
- ◆ 4. **Sedimento** - compacto, grumoso, viscoso, granular, escamoso, quantidade do sedimento (abundante, escasso, nulo);
- ◆ 5. **Pigmentação.**

Colorações

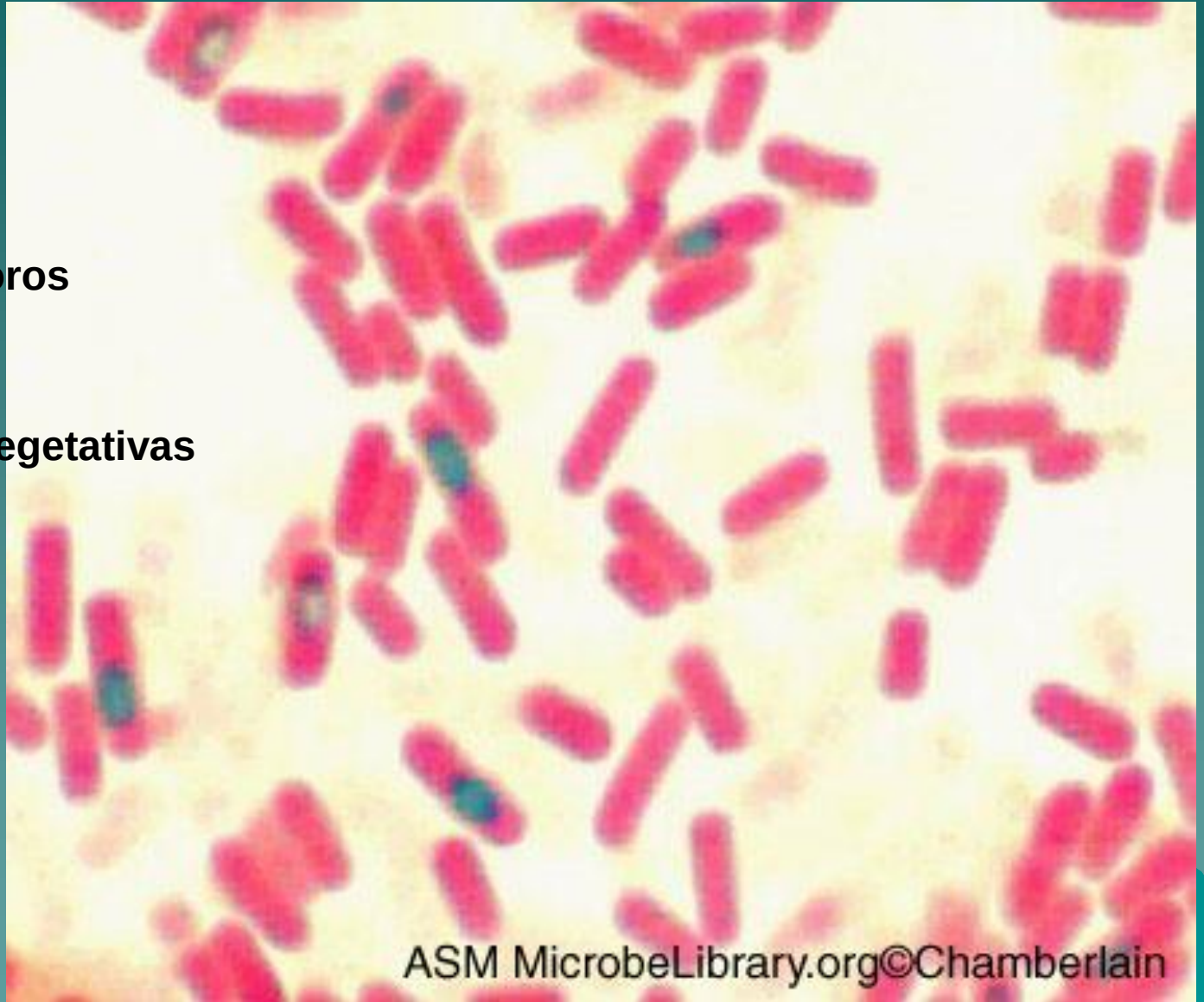
Coloração Gram



Coloração para endósporos

Verde= endósporos

Rosa= células vegetativas

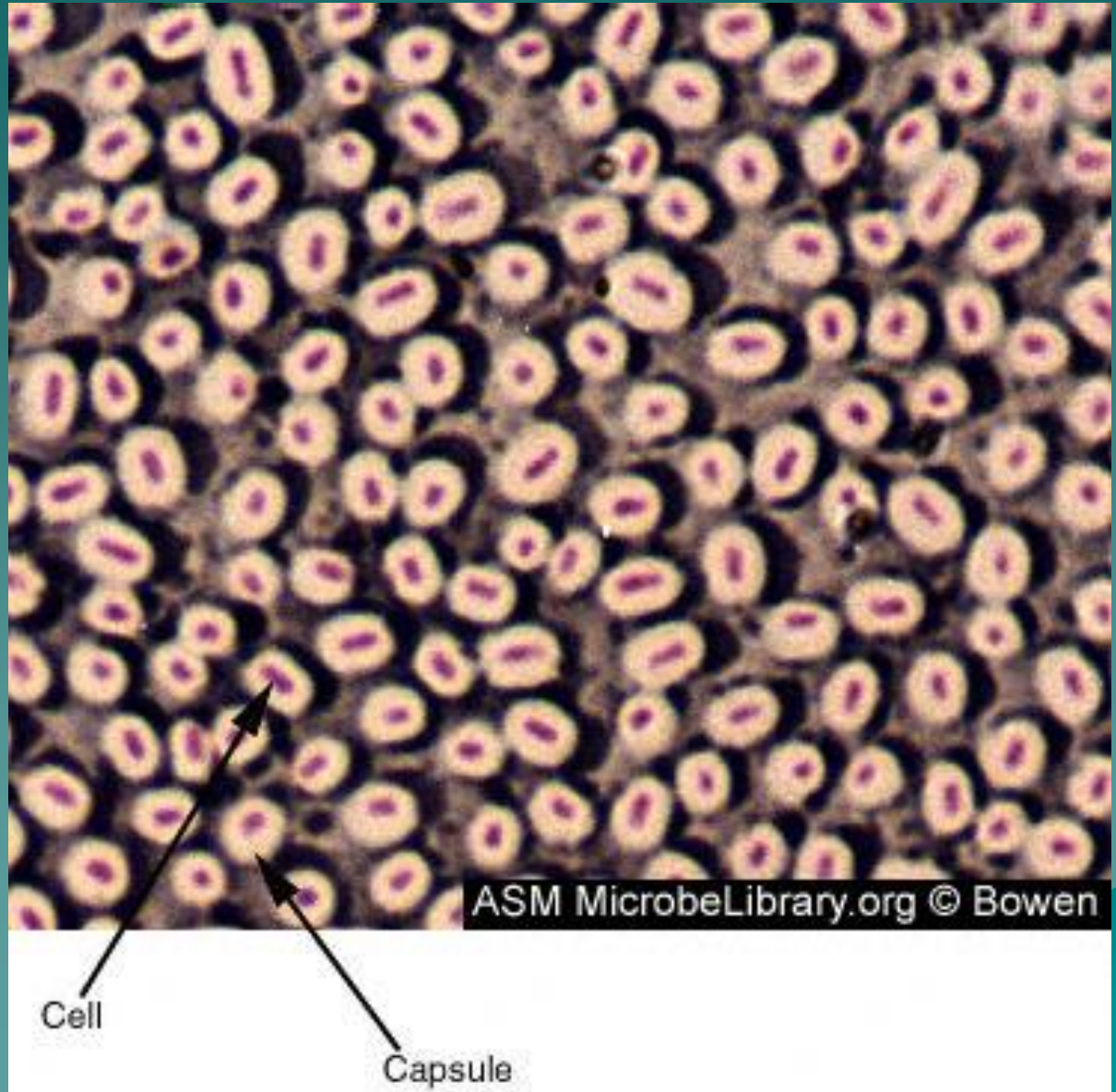


ASM MicrobeLibrary.org©Chamberlain

Técnica de Schaeffer-Fulton

Coloração negativa para cápsulas

Uso de tinta nanquim: não penetra na célula, fornecendo um contraste entre a cápsula e o meio escuro circundante



Testes bioquímicos

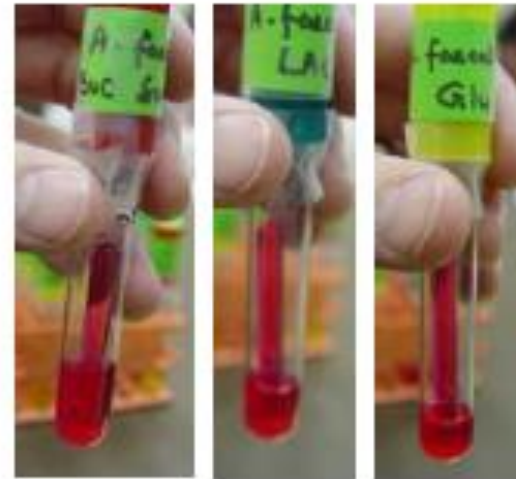
Phenol Red (PR)- Fermentation glucose, sucrose, lactose for *E. coli*

- Lac (left) gas+
 - Glu(middle) gas +
 - Suc (right) no gas –
-
- Phenol red indicator used to see if fermentation has occurred. Durham tubes are red before any fermentation has occurred. Fermentation produces gas and/or acid from the breakdown of carbohydrates



Phenol Red (PR) Fermentation glucose, sucrose, lactose for *Alcaligenes faecalis*

- Suc (left) –
- Lac (middle) –
- Glu (right) –



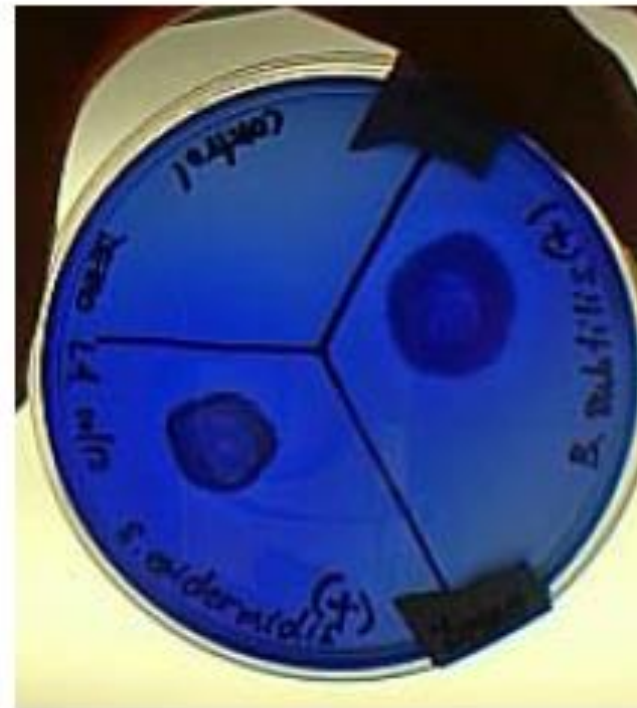
Starch hydrolysis

- Zone of clearing +
- No zone –
- *B. subtilis* +, *A. faecalis* –
- *E. Coli* – (Clockwise)
- Iodine must be on the plate to visualize the zone of clearing surrounding the bacteria. This zone indicates starch was broken down to dextrins, maltose, and glucose/alpha-amylase
- The plate you see has Gram's Iodine



Lipid Hydrolysis

- Dark blue precipitant zone / clearer blue zone +
- No color change –
- *B.subtilis* & *S.epidermidis* + w / clearer blue zone around bacterial growth
- Spirit blue agar w/3%Bacto lipase reagent is used to see if triglycerides are hydrolyzed into glycerol and free fatty acids/lipase



Phospholipid Hydrolysis

- Egg Yolk agar
- Example of two different unknowns
- Positive will show growth surrounded by a white sheen as seen on the top part of the plate
- Negative will just show growth where there was an inoculation



Casein hydrolysis

- Zone of clearing +
- No zone –
- Test used to see if casein is degraded into amino acids for use as a carbon source/proteolytic enzymes
- *E. coli* – , *A. faecalis* –
B. subtilis +



Catalase

- Bubbles +
- No bubbles –
- Reagents 3% H₂O₂

Tests for the ability to break down toxic O₂ products/superoxide dismutase (catalyzes the destruction of superoxide) & catalase peroxidase (catalyzes the destruction of hydrogen peroxide)

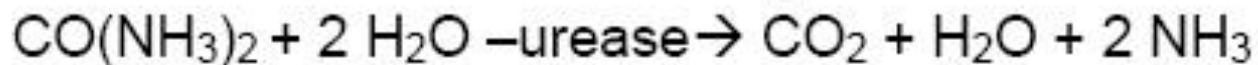


Urease

E. coli – (left)
Proteus vulgaris +



Phenol Red a pH indicator turns tube bright pink
because NH_3 decreases the pH



B-galactosidase

- *E. coli* (yellow) +
- no color change clear – (not shown)
- Reagent ONPG
- Hydrolysis of lactose to glucose and use of lactose as sole carbon source / B-galactosidase
- We use ONPG disks for this test



Nitrate

- Red/pink color after reagents/no color after zinc +
- No color change/red after zinc –
- Nitrate Reagents A & B
- Reduction of nitrate to nitrite to be used as a final electron acceptor/Nitrate reductase
- NO_3^- reductase results:

E. coli +, *S. epidermidis* +, soil -
, *P. fluorescens* -, *M. luteus* -



Coagulase

- Results:
+ clotting in the
bottom of the broth
- Reagents:
Plasma
- Reason/Enzymes
Clots plasma to avoid attack by
host's defenses/Coagulase
Shown above *S. aureus* +;
below *S. epidermidis* –



Hemolysis

- Check which bacteria are capable of lysing red blood cells (RBCs) by using blood agar (sheep blood).
- α = partial lysis of red blood cells blood looks greenish
- β = complete lysis of blood clearing
- γ = no lysing
- Clockwise starting from the left: *S. aureus* β ,
S. epidermidis γ , teeth α



Antibiotic

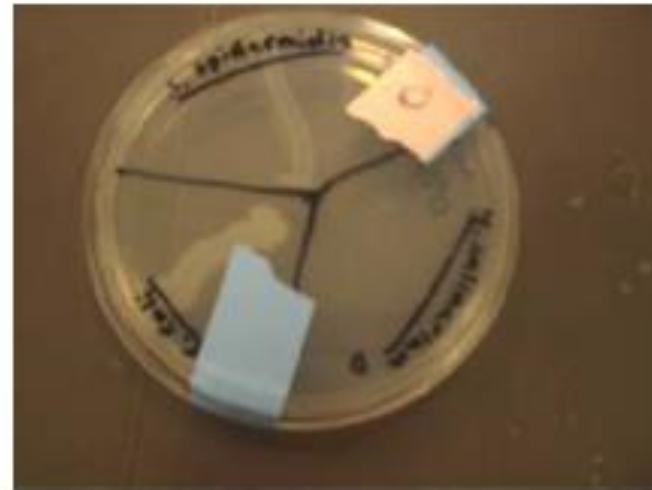
- Ability of antibiotics to inhibit growth on Mueller-Hinton agar plates (Whether bacteria are susceptible, intermediate, or resistant depends on the amount of antibiotic and the diameter of zone of inhibition, check table 43.1 of your lab manual)



Osmotic Pressure

NaCl%= 0 on TSA plate

- Detects ability of an organism's salt tolerance
- *S. epidermidis* +
- *E. coli* +
- *H. salinarium* --



Osmotic Pressure

NaCl%= 20 on TSA plate

- Detects ability of an organism's salt tolerance
- *S. epidermidis* –
- *E. coli* –
- *H. salinarium* +



Bergey's Manual of
**Systematic
Bacteriology**

SECOND EDITION

Volume Two
The Proteobacteria
Part A Introductory Essays

Classificação de Procariotas

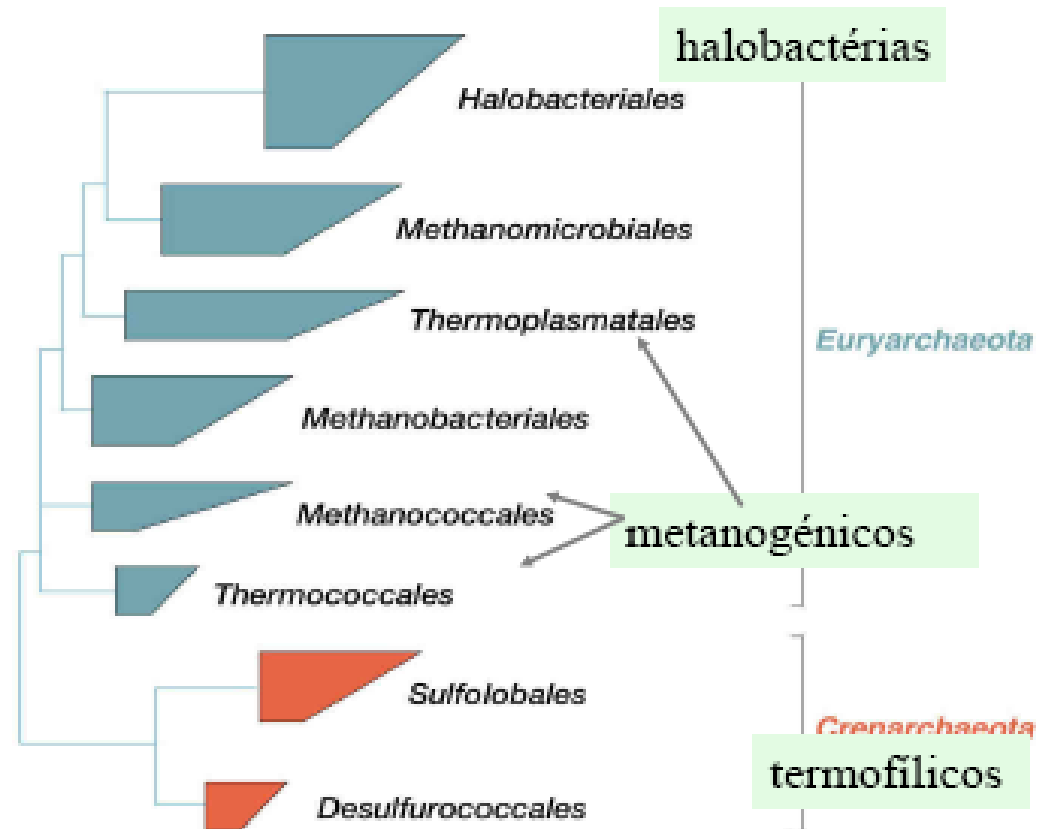
Bergey's Manual of Systematic Bacteriology

Manual que contém descrições de todas as espécies bacterianas identificadas.

- 1ª Edição (1º volume em 1984)
 - Classificação fenética
- 2ª Edição (desde 2001)
 - Essencialmente filogenética

Domínio *Archaea*

2 “phyla”
8 classes
12 ordens



Domínio *Bacteria*

- Metabólica e morfologicamente diversos
- Divididos em 23 “phyla”

