Bacterial taxonomy, classification & labratory diagnosis - lecture 4

Bacterial taxonomy : science of biological classification

Taxon = group Taxa = groups = classification

- nomenclature
- classification
- identification

useful resources

https://youtu.be/UbII3dt-r74?si=P92LRLPA2c4Sah7s

https://youtu.be/IIRU_NNYGe8?si=c-ktO3NRzxjDr_LY

Bacterial Taxonomy rank

Domain or kingdom	Bacteria
Phylum or Division	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*Escherichia coli*
Strains	E. coli K-12

Ultimately, the taxonomic ranks form the basis for the organization of bacteria. Linnaean taxonomy is the system most familiar to biologists. It uses the formal ranks of kingdom, phylum, class, order, family, genus, and species. The lower ranks are approved by a consensus of experts in the scientific community. Of these ranks, the family, genus, and species are the most useful (Table 3-1).

TABLE 3-1 Taxonomic Ranks

Formal Rank	Example
Kingdom	Prokaryotae
Division	Gracilicutes
Class	Scotobacteria
Order	Eubacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*coli*
Subtype	*Escherichia coli* O157:H7

Strain : individual member within a species - individual variant of bacteria that may have genetic or phenotypic characterstics

MRSA (Strain) of the (species) staph. aureus

Species : a collection of strains that share many stable properties - within a species different strains may exsist, but they still retain a core set of characterstics that allow them to be classified under the same species

Steph. aureus [species]

- MRSA
- VRSA (strains)

Species are also identified by Genetic homology (similarity)

DNA homology > 70%

if two strains have 70% or more similarity in their overall DNA sequences, they are considred to belong to same species

16S rRNA > 97% identical

The 16s rRNA a conserved region in bacterial genome must be 97% identical to classify them as the same species

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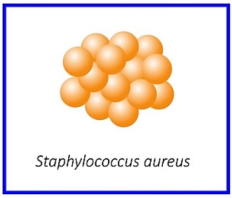
Genus : one or more species that share common properties

- species S. epidermidis
- species S. aureus

if the DNA Similarity between two bacteria is less than 93% → different Genus

Bacterial Nomenclature



Staphylococcus aureus

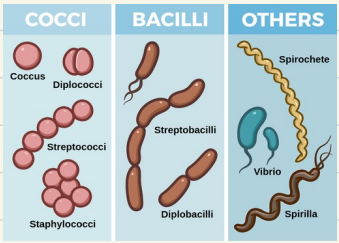
- the first word is the **genus** (capitalized)
- the second word is the **species** (not capitalized)
- both words are **italicized**

for example

Escherichia coli

Scheme of bacteria - Shape

Cocci | Bacilli | Spiral | Miscellaneous



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1 Cocci

- **Gram +ve**
  - **cluster** → Staphylococci
  - **chain or pairs** → Streptococci, Enterococcus
- **Gram -ve**
  - **Pairs** → Neisseria (diplococci) ⇝ only example!!

= Review =

1. Which of the following bacteria is typically arranged in clusters?

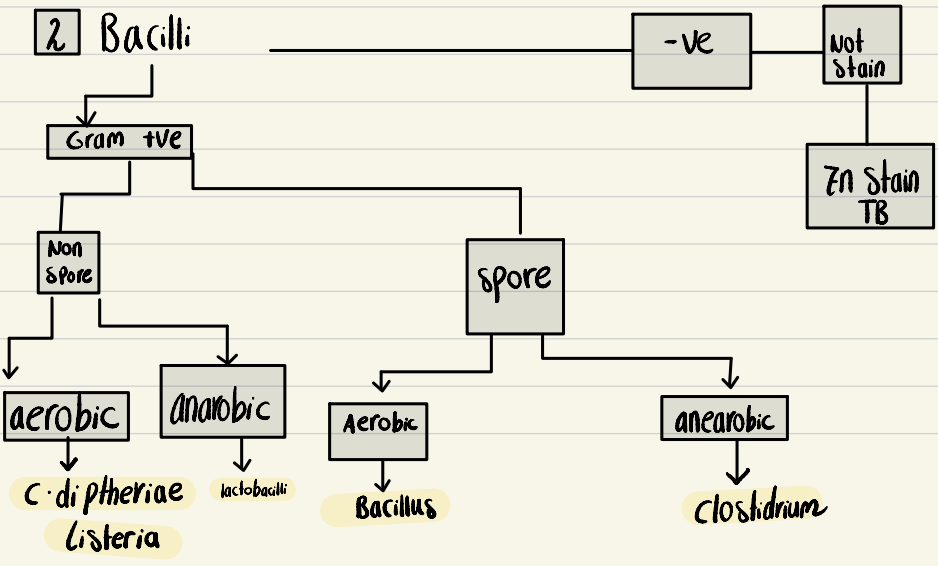
A) Streptococci
B) Staphylococci
C) Neisseria
D) Enterococcus

2. Which bacteria typically appears as pairs (diplococci) in a Gram stain?

A) Neisseria
B) Staphylococci
C) Streptococci
D) Enterococcus

3. Streptococci bacteria are commonly arranged in which pattern?

A) Chains
B) Clusters
C) diplo
D) Irregular groups



=Review=

1. Which of the following bacteria is aerobic but does not stain well with Gram stain and instead requires Ziehl-Neelsen (ZN) staining?

A) Bacillus
B) Clostridium
C) Mycobacterium (TB)
D) Listeria

Answer: C) Mycobacterium (TB)

2. Which of the following is an anaerobic, spore-forming, Gram-positive bacillus?

A) Bacillus
B) Clostridium
C) Listeria
D) Lactobacilli

Answer: B) Clostridium

3. Which of the following is a Gram-positive, non-spore-forming, aerobic bacillus?

A) Bacillus
B) Lactobacilli
C) Listeria
D) Clostridium

Answer: C) Listeria

4. Which of the following bacteria is anaerobic and does not form spores?

A) Lactobacilli
B) Clostridium
C) Bacillus
D) C. diphtheriae

Answer: A) Lactobacilli

5. C. diphtheriae is classified as:

A) Gram-negative, non-spore-forming bacillus
B) Aerobic, Gram-positive, non-spore-forming bacillus
C) Spore-forming, anaerobic bacillus
D) Gram-negative, aerobic bacillus

Answer: B) Aerobic, Gram-positive, non-spore-forming bacillus

6. Which of the following is an aerobic, spore-forming bacillus?

A) Bacillus
B) Clostridium
C) Listeria
D) Lactobacilli

Answer: A) Bacillus

7. Which of the following bacteria is a Gram-positive bacillus that is aerobic and does not form spores?

A) Bacillus
B) Listeria
C) Clostridium
D) Lactobacilli

Answer: B) Listeria

Gram negative bacilli

1- Enterobacteriaceae
2 - Vibrio
3 - Campylobacter
4 -helicobacter
5 - Haemophilus
6 - Bordetella
7 - Psuedomonas
8 - legionella
9 - brucella
10 - Gram -ve anaerobes

Spiral : T B L

- Treponema
- Borrelia
- Leptospira

Miscellaneous group

- no cell wall
- Not stained by gram
- obligate intracellular

1- Mycoplasma
2- chlamidya
3- Rickettsia
4- Coxiella
5- Actinomycetes

=Review=

1.Coxiella

Is it Gram-negative, spiral, or miscellaneous?
Answer: Miscellaneous.

2. Treponema

Is it Gram-negative, spiral, or miscellaneous?
Answer: Spiral.

3. Pseudomonas

Is it Gram-negative, spiral, or miscellaneous?
Answer: Gram-negative.

4. Campylobacter

Is it Gram-negative, spiral, or miscellaneous?
Answer: Spiral.

5. Mycoplasma
Is it Gram-negative, spiral, or miscellaneous?
Answer: Miscellaneous.

6. Legionella
Is it Gram-negative, spiral, or miscellaneous?
Answer: Gram-negative.

7. Haemophilus
Is it Gram-negative, spiral, or miscellaneous?
Answer: Gram-negative.

Review Pt. 2

8. Bordetella
Is it Gram-negative, spiral, or miscellaneous?
Answer: Gram-negative.

9. Enterobacteriaceae
Is it Gram-negative, spiral, or miscellaneous?
Answer: Gram-negative.

10. Rickettsia
Is it Gram-negative, spiral, or miscellaneous?
Answer: Miscellaneous.

11. Brucella
Is it Gram-negative, spiral, or miscellaneous?
Answer: Gram-negative.

12. Vibrio
Is it Gram-negative, spiral, or miscellaneous?
Answer: Gram-negative.

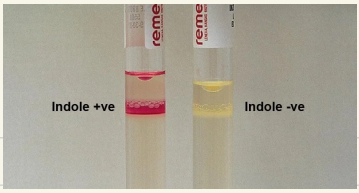
13. Helicobacter
Is it Gram-negative, spiral, or miscellaneous?
Answer: Spiral.

14. Actinomycetes
Is it Gram-negative, spiral, or miscellaneous?
Answer: Miscellaneous.

15. Gram-negative anaerobes
Is it Gram-negative, spiral, or miscellaneous?
Answer: Gram-negative.

16. Chlamydia
Is it Gram-negative, spiral, or miscellaneous?
Answer: Miscellaneous

Biomedical reactions



1 indole test : determines whether a bacterium can breakdown tryptophan into indol using tryptophanase

↓

To detect indole , Kovac's reagent is added to test medium after incubation if indole is present it reacts with Kovac's reagent to form a red layer at the top of the medium

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2 Methyl red test - differentiate bacteria based on their metabolic products from glucose fermentation

↓

organism is incubated in a medium containing glucose

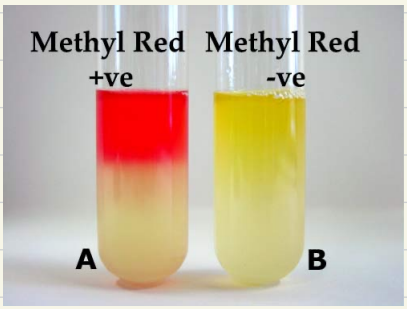
↓

Some bacteria ferment glucose via mixed acid fermentation producing a large amount of acidic by-products → acetic , lactic & succinic acid → lowers pH of medium

↓

Methyl red is added as a pH indicator

Red <4 PH



yellow 6 > PH

3 Voges-Proskauer test VP - detect organisms that ferment glucose leading to production of acetion

"neutral product" ← acetyl methyl carbinol

Test medium contains peptone & glucose → bacteria capable of fermenting glucose produce acetion

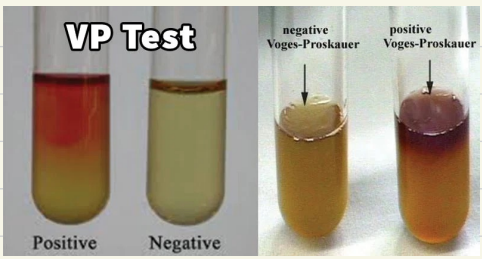
↓

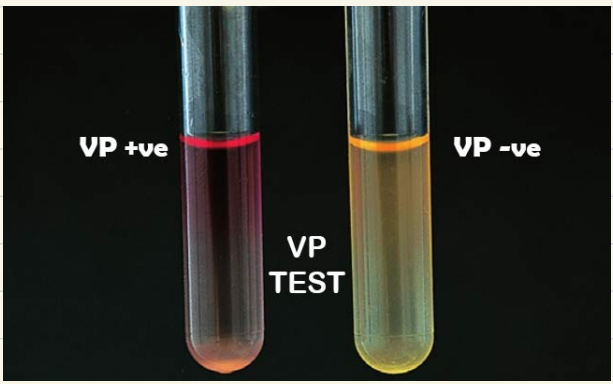
incubation at 37°C for 48h allowing it to ferment

↓

Two reagents are added

- α-napthol → reacts with acetion to oxidize it to diacetyl
- 40% KOH → reacts with diacetyl & peptone to produce a red color if acetoin is present





Methyl Red (MR) & Voges - proskauer (VP)

↓ detects production of stable acids

↓ detects acetion a neutral end-product of glucose

⇩

if MR test is +ve the VP should be -ve & vice versa

Because the test reflect two different fermentation pathways, an organism cannot use both pathway simultaneously

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4 Citrate Utilization test → determine whether an organism can use citrate as its sole source of carbon & energy

Test checks presence of the enzyme citrate lyase, which allows bacteria to utilize citrate. Citrate is the only source of carbon in medium & ammonium salts

↓

if organism can utilize citrate, it converts it into pyruvate & release carbon dioxide. The carbon dioxide reacts with sodium in medium to form sodium carbonate which is alkaline

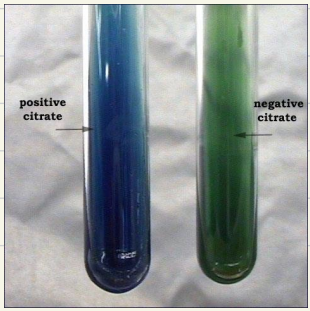
↓

pH indicator → bromothymol blue

if +ve then blue

in alkaline (High pH) enviroment

if -ve then green

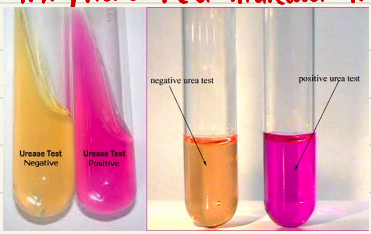


5 Urease Test : detect ability of an organism to produce enzyme urase · Urease hydrolyzes urea into ammonia & carbon dioxide

** Phenol red indicator **

→ +ve result → media turned pink

→ -ve remains yellow or peach



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6 Triple Sugar iron

* Components of TSI test

* 0.1% glucose, 1% lactose, 1% sucrose → Three sugars in the medium to detect sugar fermentation

* Ferrous sulfate : Detects hydrogen sulfide H_2S production forming a black precipitate

* phenol red: PH indicator that turns yellow in acidic conditions & Red in alkaline conditions

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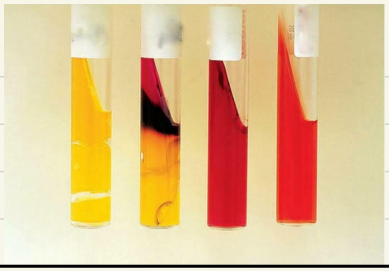
Acid/Acid (A/A) : Fermentation of glucose, lactose or sucrose → Both yellow if gas is produced, it can break down the agar

Alkaline/Acid (K/A) : Glucose fermentation initially turns it yellow but slant turns red as proteins -from medium- are broken. This indicates that bacterium cannot ferment lactose or sucrose

Alkaline/Alkaline (K/K) : No fermentation occurs!! medium remains red, indicating organism does not use any of the sugar

H_2S Production: turns black due to formation of ferrous sulfide

http://www1.udel.edu/mls/dlehman/tsi/lactose-h2s#:~:text=The%20bacteria%20quickly%20metabolized%20the,to%20produce%20ATP%20and%20pyruvate.



7 Phenyl alanine deaminase

Organisms with the enzyme phenyl alanine deaminase can remove amino group(NH_3) from phenyl alanine

Phenyl pyruvic acid & ammonia (NH_3)

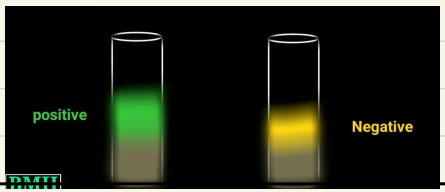
↓

To detect reaction, ferric chloride added to medium after incubation

↓

Phenyl Pyruvic reacts with ferric chloride producing a green color

Application: help distinguish **proteus species** which are +ve from **Salmonella & shigella** -ve



8 Ornithine decarboxylase: if an organism can decarboxylase ornithine

↓

Bacteria with ornithine decarboxylase removes carboxyl group → provides a carbon source for growth

↓

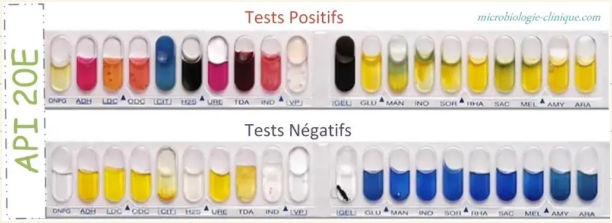
raises pH, turning it alkaline → Purple +ve

-ve → organism only ferments glucose → yellow

9 Analytical profile index (API)

it include a strip with mini test compartments, each containing specific substrates for various biochemical reactions. After inoculating the strip with a bacterial suspension, the biochemical tests are performed simultaneously, after incubation, color changes in the wells indicate results of reactions

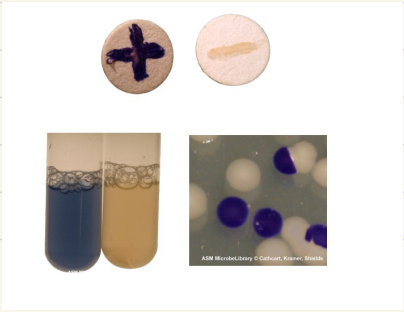
The API system allows for the identification of microorganisms based on their metabolic properties. For example, the API 20E and API 20NE systems are used for Enterobacteriaceae and non-enteric Gram-negative bacteria, while API 20 STREP is used for identifying Streptococci.



Oxidase test

Some bacteria produce oxidase enzyme, when a few drops of colorless oxidase reagent it turns to deep purple

differentiate between Psuedomonas (oxidase +ve) & Enterobacteriaceae (oxidase negative)



Catalase test

an enzyme that breaks down hydrogen peroxide (H_2O_2) into H_2O & O_2 → releases gas in the form of gas bubbles

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

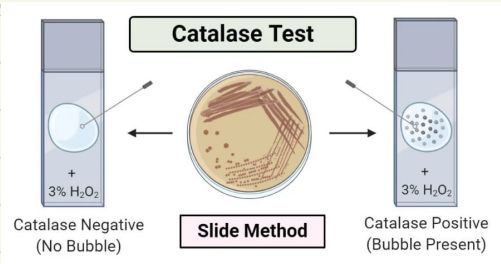
Procedure → 1) a small amount of bacteria colony is smeared onto a glass slide

2) a few drops of hydrogen peroxide (H_2O_2) are added to smear

3) production of bubbles indicates a +ve catalase test

Staphylococcus (+ve)

Streptococcus (-ve)



Coagulase enzyme

Some bacteria like staphylococcus aureus produce coagulase enzyme

Coagulase converts fibronogen into fibrin resulting in clot formation

Used to distinguish coagulase-postive staphylococcus aureus from other staphylcocci that do not produce the enzyme (coagulase -ve staphylococci)

Summary

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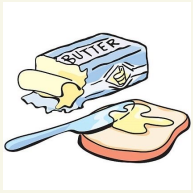

Test Name	Enzyme/Reaction	Positive Result	Purpose
Indole Test	Tryptophanase breaks down tryptophan to indole	Red color after adding Kovac's reagent	Detects the ability to break down tryptophan into indole
Methyl Red Test	Glucose fermentation to mixed acids	Red color (pH < 4)	Determines strong acid production from glucose fermentation
Voges-Proskauer Test	Glucose fermentation to acetoin	Red color after adding reagents	Detects acetoin production from glucose fermentation
Citrate Utilization Test	Citrate utilization as sole carbon source	Blue color (pH > 7)	Identifies organisms that can use citrate as their carbon source
Urease Test	Urease breaks urea into ammonia	Pink color	Detects the production of urease enzyme, leading to ammonia production
TSI (Triple Sugar Iron) Test	Fermentation of glucose, lactose, or sucrose & H_2S production	Yellow (acidic) or red (alkaline), black precipitate for H_2S	Tests sugar fermentation and sulfur reduction
Phenylalanine Deaminase Test	Phenylalanine deaminase converts phenylalanine to phenylpyruvic acid	Green color after adding ferric chloride	Distinguishes *Proteus* from *Salmonella* and *Shigella*
Ornithine Decarboxylase Test	Decarboxylation of ornithine to putrescine	Purple color	Detects ornithine decarboxylase enzyme activity
Oxidase Test	Cytochrome c oxidase present	Deep purple color	Identifies organisms with cytochrome c oxidase
Catalase Test	Catalase breaks H_2O_2 into water and oxygen	Bubbles (O_2 production)	Differentiates between catalase-positive and catalase-negative organisms
Coagulase Test	Coagulase converts fibrinogen to fibrin	Clot formation (visible clumping)	Differentiates *Staphylococcus aureus* from other staphylococci