Microbiology

2025-2024

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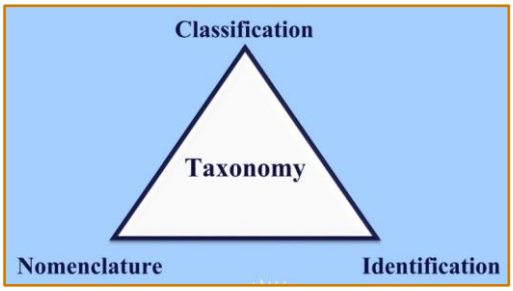
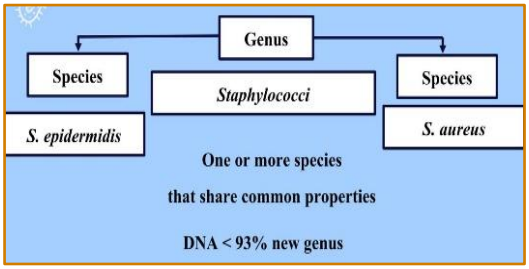
ARKAN
ACADEMY

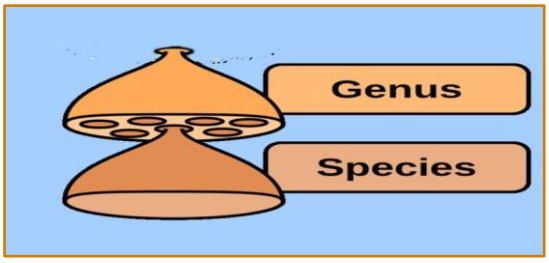
Bacterial taxonomy, Classification, and laboratory diagnosis



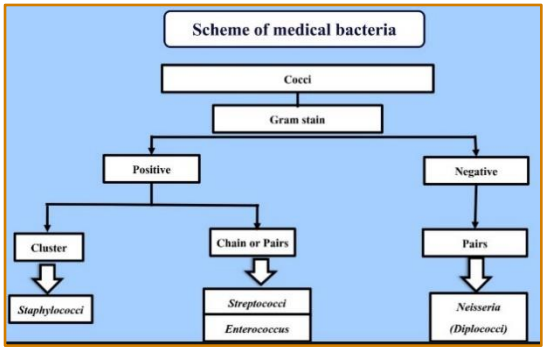
- ## Taxonomy
  - Taxon= group , Taxa= groups= classification
  - The science of biological classification
  - Bacterial Taxonomy Rank:
    - ✓ *Kingdom or Domain*
    - ✓ *Division or Phylum*
    - ✓ *Class*
    - ✓ *Order*
    - ✓ *Family*
    - ✓ *Genus*
    - ✓ *Species:* a collection of strains that share many stable properties
      - Same species, (DNA homology ≥ 70%)
      - (16S rRNA >97% identical)
      - One or more species that share common properties DNA < 93% new genus
      - Example: Staph. Aureus (Species) ⟶ MRSA (Strain) / VRSA (Strain)
    - ✓ *Strains:* individual member within a species

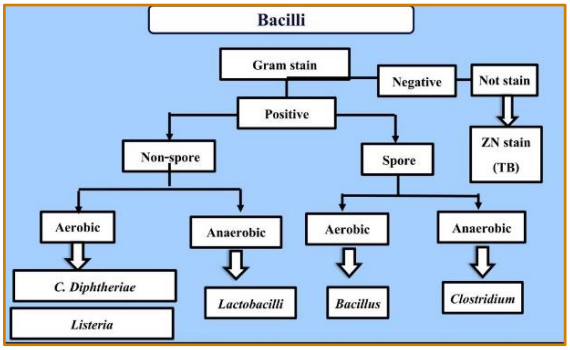


- ## Nomenclature
  - Is a *formal system* of naming species using two Latinized names: genus and species.
  - Example: *Escherichia coli (E. coli)*



- **Scheme of medical bacteria**
  - ***Shape:***
    - ✓ Cocci
    - ✓ Bacilli
    - ✓ Spiral
    - ✓ Miscellaneous





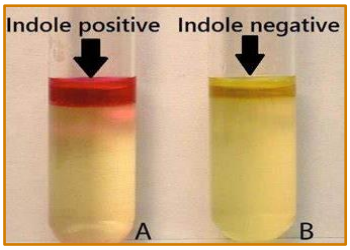
  - **Gram negative bacilli:**
    - ✓ Enterobacteriaceae
    - ✓ Vibrio
    - ✓ Campylobacter
    - ✓ Helicobacter
    - ✓ Pseudomonas
    - ✓ Haemophilus
    - ✓ Bordetella
    - ✓ Brucella
    - ✓ Legionella
    - ✓ Gram –ve anaerobes
  - **Spiral**
    - ✓ Treponema
    - ✓ Borrelia
    - ✓ Leptospira
  - **Miscellaneous group**
    - ✓ *Characteristics:*
      - No cell wall
      - Not stain by gram
      - Obligate intracellular
    - ✓ *Include:*
      - Mycoplasma
      - Chlamydia
      - Rickettsia
      - Coxiella
      - Actinomycetes

- **Biochemical reactions**
  - ***Used for:***
    - ✓ Morphology & Culture
    - ✓ Virulence factor & Pathogenesis
    - ✓ Diseases
    - ✓ Lab. diagnosis
    - ✓ Treatment & Prevention
  - **Biochemical reactions tests:**
    - ✓ ***General equation:***

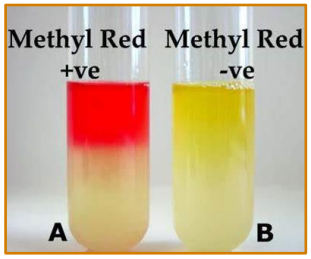
      Bacteria enzyme + Substrate → **Final product**+ Indicator → Positive result → Bacteria identification

	Bacteria enzyme	**Substrate**	**Final product**	**Indicator**	**Positive result**
Indole test	Tryptophanase	Peptone (TRYPTOPHAN)	Indole	Kovac's R	**Red**
Methyl red test	Fermentation	Peptone (Glucose)	Mixed acid: Acetic lactic succinic	MR indicator	Low ph <4 **Red**
Voges-Proskauer test	Fermentation	Peptone (Glucose)	Acetyl methyl carbinol	∝-naphthol + 40% KOH	**Red**
Citrate utilization test	Citrate lyase	Citrate	sodium carbonate	bromothymol blue indicator	**green** to **blue**
Urease test	Urease	Urea	Ammonia	phenol red indicator	**Pink**
Triple Sugar Iron test	Fermentation/ Amino acid metabolism	Glucose/Lactose/ Sucrose/ Ferrous sulfate/ Sodium thiosulfate	Acid Ammonia/ Ferric sulphide/ CO2	Phenol red indicator	Yellow **Red** **Black** Bubbles
Phenylalanine deaminase test	Phenylalanine deaminase	Phenylalanine	Phenylpyruvic acid + NH3	Ferric chloride	**Green**
Ornithine decarboxylase test	Ornithine decarboxylase	Ornithine	Putrescine+CO2	Acidic condition	**Purple**

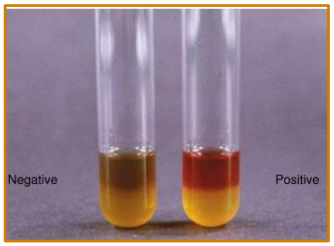
- **Indole test**
  - Bacteria [Tryptophanase] + Peptone (TRYPTOPHAN) → **Indole**+ Kovac's R → Red



- **Methyl red test: (MR)**
  - Bacteria+ Peptone (Glucose) → Incubate at 37°C for 48h → Large amount of Mixed acid (Acetic, lactic, & succinic) Low pH <4 + Methyl red indicator → **Red** (positive) / high ph 6

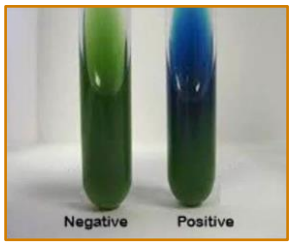


- **Voges-Proskauer test (V.P)**
  - Bacteria +Peptone (Glucose) → Incubate at 37°C for 48h → Ferment glucose→[aceton] Acetyl methyl carbinol + [∝-naphthol + 40% KOH] indicator → **Red**

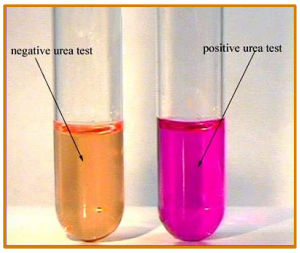


    - ✓ ***MR &VP***
      - If methyl red is positive, the voges-proskauer should be negative and reverse is right.

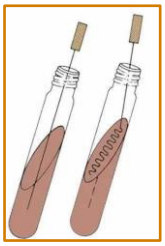
- **Citrate utilization test**
  - Bacteria+ Citrate medium [source of carbon]→ Incubate at 37°C for 48h→ Liberated Co2 + sodium → sodium carbonate (Alkaline product) + bromothymol blue indicator → **green to blue** due to the pH increase (pH > 7.6)



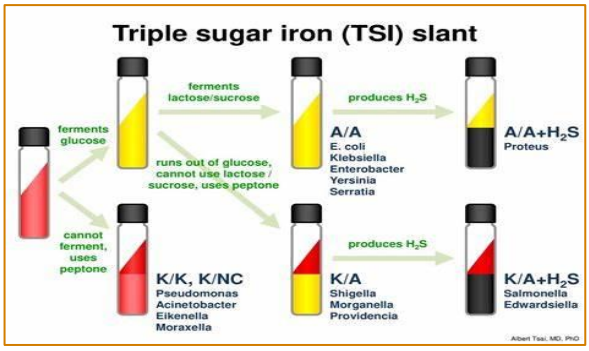
- **Urease test**
  - Bacteria(Urease)+ Urea →Ammonia [alkaline product] + Phenol red indicator → **Pink**



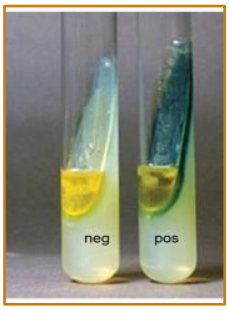
- **TSI (Triple Sugar Iron) test**
  - Bacteria+ TSI (0.1% glucose+1% lactose+1% sucrose+ Ferrous sulfate) → Final product+ Phenol red [pH indicator]
  - ***Components of TSI Medium:***
    - ✓ Glucose, Lactose, Sucrose: The sugars being tested for fermentation.
    - ✓ Sodium thiosulfate: Source for detecting hydrogen sulfide production.
    - ✓ Phenol red: pH indicator (yellow for acid, red for neutral, and pink for alkaline).
    - ✓ Ferrous sulfate: Detects hydrogen sulfide by forming black precipitate with H_2S
      - ***Key Reactions and Equations:***
        1. *Glucose Fermentation:* (slant)
           - Equation:
             Bacteria+ glucose (aerobic conditions) → fermentation → Acidic Products [yellow] +CO2(Gas) [break up the medium or pushed up the tube].
        2. *Lactose and Sucrose Fermentation:* (butt)
           - Equation
             Bacteria+ Lactose and Sucrose (anaerobic condition) → fermentation → Acidic Products [yellow] +CO2(Gas)
        3. *Hydrogen Sulfide (H_2S) Production:*
           - Equation:
             Bacteria+ Sulfur (***sodium thiosulfate*****)** → Hydrogen sulphide (H_2S) +iron → Ferric sulphide (**Black**)
        4. *Amino acid metabolism* **(**slant)
           - Equation:
             Bacteria+ peptones (protein sources) [aerobic condition] → ammonia → **red**
      - ***Test Procedure:***
        - *Inoculation:* The bacterium is inoculated on the slant and stabbed into the butt of the TSI agar.
        - *Incubation:* The tube is incubated for 18–24 hours at 35–37°C.



      - ***Results Interpretation:***
        - Slant/Butt Yellow: Fermentation of glucose and lactose/sucrose (acidic). [Acid over acid (A/A)]
        - Slant Red / Butt Yellow: Glucose fermentation only /alkaline slant due to peptone utilization). [Alkaline over acid (K/A)]
        - Slant Red / Butt red: alkaline slant due to peptone utilization/ The butt also remains red, indicating that there is no fermentation occurring in the anaerobic conditions of the butt [Alkaline over Alkaline (K/K)]
        - Black Precipitate: H_2S production.
        - Cracks or Bubbles: Gas production.



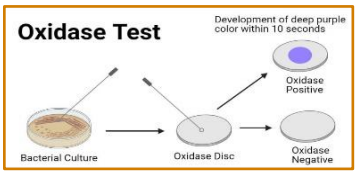
- **Phenylalanine deaminase test**
  - Bacteria [Phenylalanine deaminase]+ Phenylalanine→+ ferric chloride → Phenyl pyruvic acid + NH3 (**Green** color)
  - Distinguishes Proteus from Salmonella & Shigella



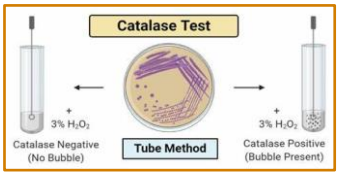
- **Ornithine decarboxylase test**
  - Bacteria [Ornithine decarboxylase] + Ornithine [Source of carbon (energy for growth)] → Putrescine [acidic] +CO2→ **purple/pink**
  - Providencia rettgeri & Morganella morgani (+ve)

- **The analytical profile index (API)**
  - Biochemical tests for identification
  - Several API systems for different groups of organism
  - Example:
    - ✓ API 20E &API 20NE (Enterobacteria)
    - ✓ API 20 STREP (Streptococci) etc.

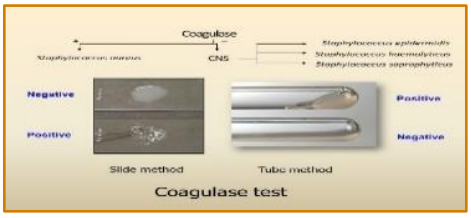
- **Oxidase test**
  - Some bacteria produce Oxidase enzyme
  - Detection by adding few drops of colorless Oxidase reagent Colonies turn **deep purple** in color (positive)



- **Catalase test**
  - Some bacteria produce catalase enzyme (H2O2) lead to production of ***gas bubbles*** (O2 production)



- **Coagulase test**
  - Some bacteria produce coagulase enzyme that converts fibrinogen to fibrin (*plasma clot*)
  - Detected by slide or test tube method



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