Microbiology 1

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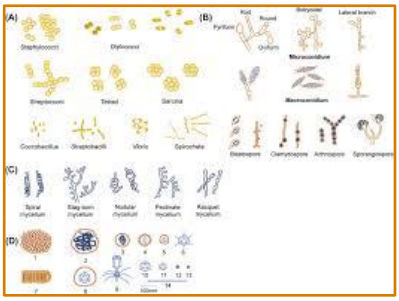
ACADEMY

Identification of bacteria

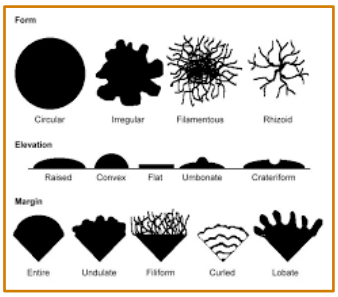
- **The successful identification** of microbiological agent depends on:
  - Proper *aseptic* techniques.
  - *Correctly obtaining* the specimen.
  - *Correctly handling* the specimen
  - *Quickly transporting* the specimen to the lab.
  - *Once reaches* the lab it is *cultured and identified*.
- After the microbe is identified, it is used in ***susceptibility tests*** to find out the effective control measure
- The methods used to identify bacteria fall into **three categories**:
  - Phenotypic- morphology (macro and microscopic)
    - ✓ *Microscopy* (staining)
    - ✓ *Growth on* enrichment, selective, differential *media*
    - ✓ Specimen *biochemical test* (rapid test methods)
  - Immunological (serological) tests
  - Genotypic- Molecular techniques

Phenotypic Methods:

- Microscopic Morphology:
  - ✓ Include *a combination of* cell shape, size, Gram stain, acid fast, special structures e.g. endospores, granule and capsule can be used to give an *initial putative identification.*
  - ✓ Done by using:
    - Simple stain
    - Gram stain
    - Acid-fast stain (Ziehl-Neelsen stain)
    - Special stains



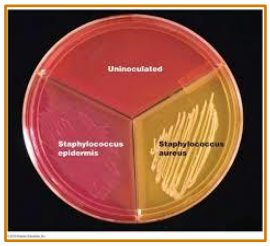
- Macroscopic morphology:
  - ✓ Bacterial *Cultivation* (Isolation of bacteria from specimens)
  - ✓ *Principles of Cultivation:*
    - Nutritional requirements
      - Non-fastidious: simple requirements for growth
      - Fastidious: complex, unusual, or unique requirements for growth
    - Streaking for isolation
    - Streaking for quantitation
  - ✓ *Colony characteristics:*
    - With the *naked eye* e.g. texture, shape, pigment, growth pattern.
      - Colony form: pinpoint, circular, filamentous, irregular
      - Colony elevation: flat, raised, convex
      - Colony margin: smooth, irregular



- ***Types of culture media:***
  - **Basal media**: used for culture of bacteria that *do not need* enrichment of the media.
    - ✓ *Examples:* Nutrient broth, nutrient agar and peptone water.

  - **Enriched media**: by adding blood, serum or egg.
    - ✓ *Examples*: blood agar, Chocolate agar and Lowenstein-Jensen media.

  - **Selective media**: contains *agents that inhibit the growth of all agents except* that being sought (dyes, bile salts, alcohols, acids, antibiotics).
    - ✓ *Examples*: SSA, Mannitol Salt Agar.



  - **Differential media:** An indicator is included in the medium,a particular organism causes change in the indicator, e.g. blood, neutral red.
    - ✓ *Examples*: Blood agar and MacConkey agar

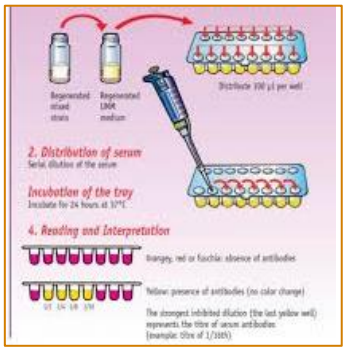
  - **Transport media**: These media are used when specie-men cannot be cultured soon after collection.
    - ✓ *Examples:* Cary-Blair medium, Amies medium, Stuart medium.

  - **Storage media**: Media used for storing the bacteria for a long period of time.

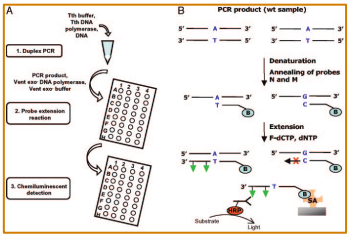
- ***Biochemical Tests:***
  - The microbe is cultured in a media with a special substrate and *tested for an end product*.
  - *Prominent* biochemical tests include enzymes (catalase, oxidase, decarboxylase), fermentation of sugars, acid or gas production and the hydrolysis of gelatine.
  - ***Bacterial enzyme***+ substrate → ***final product***+ indicator → *positive result*→ bacteria identification
  - *Other biochemical tests of interest include:*
    - ✓ Indole test
    - ✓ Methyl Red / Vogues-Proskauer
    - ✓ Citrate utilization
    - ✓ Coagulase test
    - ✓ H2S production (TSA)
    - ✓ Urease test
    - ✓ Phenylalanine deaminase test

  - *Rapid Tests*
    - ✓ Biochemical system for the identification of Enterobacteriaceae
    - ✓ It consist of 20 tests that are converted to digital profile.

- **Immunological (Serological) Tests**
  - *Agglutination tests:*
    - ✓ Direct whole pathogen agglutination assays
    - ✓ Particle agglutination tests
    - ✓ latex beads or RBCs coated with Ag
  - *ELISAs*
  - *IFAs*



- **Genotypic methods**
  - Genotypic methods of microbe identification include the use of:
    - ✓ *Nucleic acid probes*
    - ✓ *PCR* (polymerase chain reaction)
    - ✓ *Nucleic acid sequence analysis*
    - ✓ *RNA analysis*
    - ✓ *RFLP* (restriction fragment length polymorphism)
    - ✓ *Plasmid fingerprinting.*



- **Another test use to bacterial identification**
  - *Vitek*
  - Matrix-assisted laser desorption ionization time of- flight (MALDI-TOF) *MALDI - TOF*
  - *Mass spectrometric method*
  - *Rapid identification:* This offers the analysis of whole bacterial cultures for unique mass spectra from charged macromolecules
  - *Growing database.*

Classification of Bacteria

- Bergey's Manual of Determinative Bacteriology.
  - *Taxonomy* is the science of classification of organisms
    - ✓ Bacterial taxonomy *consists* of three separate, but interrelated areas:
      - *Classification* is the arrangement of organisms into groups (taxa) on the basis of similarities or relationships.
      - *Nomenclature* is the assignment of names to the taxonomic groups according to international rules
      - *Identification* is the practical use of a classification

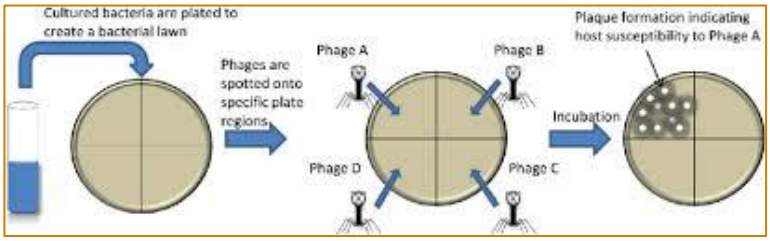
- Scheme to determine the identity of an isolate as a member of an established taxon or as a member of a previously unidentified species.
  - *Taxonomic Rank*
    - ✓ Kingdom or Domain
    - ✓ Division or Phylum
    - ✓ Class
    - ✓ Order
    - ✓ Family
    - ✓ Genus
    - ✓ **Species:**
      - The basic and most important taxonomic group in bacterial systematics.
      - The boundaries of species are rather *difficult to define* precisely however; the boundaries of some genera are *sharply defined*. For example, the genus *Bacillus* and the genus *Escherichia*

- Typing:
  - Means *identification* of the organisms to strain level.
  - The main purpose *to control nosocomial infection* and also can used in different epidemiological purpose (cholera, meningitis ...etc).

  - What Information can Typing Supply
    - ✓ *Similarity between isolates*
      - Wide range of characters that could be examined
      - Significance of differences needs to be understood
      - Significance of differences needs to be clearly expressed
    - ✓ *Relationship to other strains*
      - Comparator strains must
      - Be recognisable by the typing methods
      - Have known characteristics

  - Methods of typing:
    - ✓ Phage typing
    - ✓ Bactericin typing
    - ✓ Resistotyping
    - ✓ Biotyping
    - ✓ Serotyping
    - ✓ Plasmid typing

- **Phage typing:**
  - *Phage* is virus which live in a living cell.
  - *Bacteriophage*: is a virus live in a bacterial cell, and used for typing of bacterial, it is specific and live in certain strain.



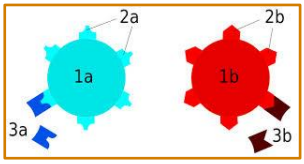
  - There are *two type* of bacteriophage:
    - ✓ Lytic bacteriophage: cause lysis of bacterial cell and used for typing.
    - ✓ Lysogenic bacteriophage: Does not cause lysis of bacterial cell but play important role the gene transferring.
      - There is a bacteriophage for any bacteria but usually used for typing of S. aurus in control of nosocomial infections.
      - Phage typing also used for different epidemiological purpose Mycobacterium, Brucella, and Salmonella...etc.
      - Phage typing of S. aureus: There are 4 main group I, II, Ill and IV. And each group consist of different number of single phage.
      - ***Method:***
        - Reconstitute the phage (activation the virus).
        - Inoculate the NA plate with the tested organism after labelling.
        - Add the phage as drop, incubate and read result
  - *Advantage:* Very specific method.
  - *Disadvantage:* Difficult to perform - need well equipped laboratory (preservation of virus) - time consuming - not used for all type of organisms

- **Bactericin typing:**
  - *Bactericin* is antibiotic-like substance produced by certain organism that can inhibit the growth of some close related bacteria.
    - ✓ Example: Colicin typing used for typing of Shigella sonni

- **Biotyping:**

  - *Depend on* biochemical reaction.
  - Modified from *identification* to typing
  - Most used Analytical profile index (*API*).
  - Example: API 20 E
  - API 20E system for Enterobacteriaceae 2909
    - ✓ *Advantage:* Easy to perform - Standardized.
    - ✓ *Disadvantage:* Cannot used for all organisms.

- **Serotyping:**



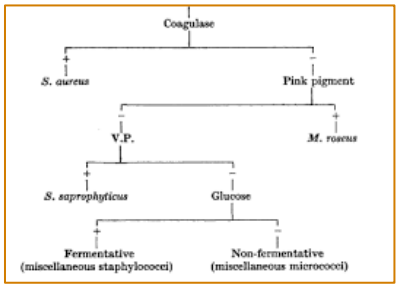
  - *Depend on* serological reactions.
  - *Used for* identification and can be used for typing.
    - ✓ *Advantage:* standardized - specific - easy to perform.
    - ✓ *Disadvantage:* Cannot used for all microorganisms.

- **Resistotyping:**
  - Also called *antibiogram*.
  - It is *sensitivity test* for typing
  - *Used* different type of antimicrobial agents differ from that used for treatment (toxic may be dyes).
    - ✓ *Advantage:* easy to perform-simple-cheap
    - ✓ *Disadvantage*: limited sensitivity (for organism highly sensitive or resistant)

- **Plasmid typing:**
  - Also called *molecular typing*, example: PCR
    - ✓ *Advantage:* very sensitive-very specific.
    - ✓ *Disadvantage:* need special machine-very expensive method.

- **Classification of Bacteria**
  - *Phenotypic* classification
  - *Environmental* reservoirs / Modes of transmission
  - *Genotypic* classification

- **Adansonian classification:**

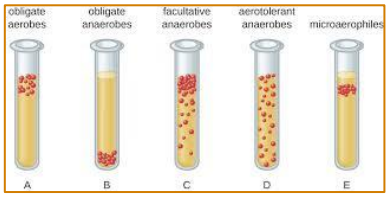


  - In most systems of bacterial classification, the major groups are *distinguished by* fundamental characters such as cell shape, Gram-stain reaction and spore formation
  - Genera and species are usually *distinguished by* properties such as fermentation reactions, nutritional requirements and pathogenicity.
  - *Similarity coefficient* when shared positive characters are considered or a *matching* coefficient when both negative and positive shared characters (matches) are taken into account

- **Phenotypic classification:**
  - *Morphology* and *Gram Staining* characteristics
  - *Growth requirements* and *metabolic behaviour*
  - Morphology:
    - ✓ Cocci
    - ✓ Bacilli
    - ✓ Curved or spiral
    - ✓ Filamentous

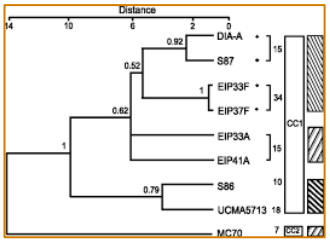
  - Some correlation between *morphology and disease* :
    - ✓ Spiral bacteria---Treponemes, Borrelias, Leptospiras tend to cause systemic diseases
    - ✓ Pathogenic Filamentous bacteria--- Actinomyces, Nocardia, Mycobacteria tend to cause chronic diseases
    - ✓ Gram positive bacteria-- Staphylococcus, Streptococci more likely to cause skin infections

  - Growth requirements and metabolic behaviour
    - ✓ *Nutritional requirements*
      - Organism →Synthesize organic Compounds → autotropic
      - Organism → Require organic Compounds → heterotrophic
    - ✓ *Gaseous Requirements*
      - Aerobic bacteria
      - Anaerobes
      - Facultative anaerobes
    - ✓ *Thermal conditions:*
      - Psychrophiles
      - Mesophiles
      - Thermophiles



Classes	Temp. (°C)
Psychrophiles	20
Mesophilles	35 – 42
Moderate Thermophiles	45 – 50
Extreme Thermophiles	65 – 80
Source: (Backstrom, 2013)	

- **Genotypic classification:**
  - DNA hybridization, used to designate species
  - Genomic 'G+C' content "Guanine +Cytosine ratio"
  - Ribosomal RNA (rRNA) (highly conserved) sequence analysis



- **Naming microorganisms**
  - *Binomial* (scientific) nomenclature
  - Gives each microbe 2 names
    - ✓ *Genus* - noun, always capitalized
    - ✓ *species* - adjective, lowercase
  - *Both italicized or underlined*
    - ✓ Staphylococcus aureus (S. aureus)
    - ✓ Bacillus subtilis (B. subtilis)
    - ✓ Escherichia coli (E. coli)

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