Identification of Bacteria

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* if you have a pateint and suffer from something and you want to take a sample from a ligan and see what is a microorganism coding this symptom → identification of organism, but why? → to see it and know how can I deal with it? and what are the antibiotics that it susceptible → so I can control the problem.

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

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The methods use to identify bacteria fall into three categories:

1. **Phenotypic**: morphology (macro and microscopic)
   - * Microscopy (staining)
   - * Culture: Growth on culture media
   - * Biochemical test.
2. **Immunological**: (serological) tests
3. **Genotypic**: Molecular techniques

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Phenotypic Methods

Microscopic Morphology:

Cell shape, size, Gram stain, acid fast, special structures e.g. endospores, granule and capsule □ initial putative identification.

Done by using:

- Simple stain
- Gram stain
- Acid-fast stain (Ziehl-Neelsen stain)
- Special stains

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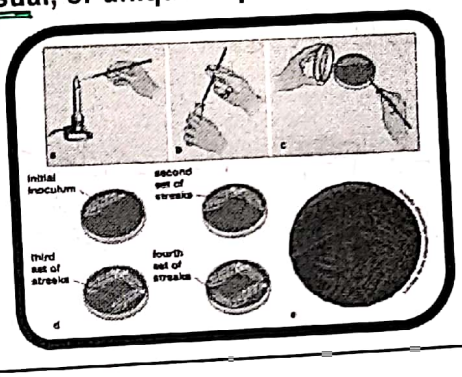
Macroscopic morphology : Bacterial Cultivation.

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



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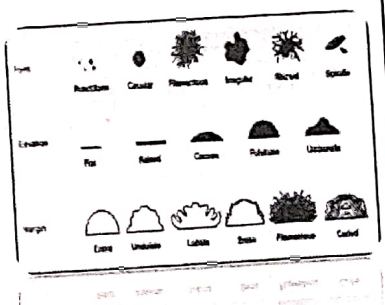
Colony characteristics : with the naked eye e.g. texture, shape, pigment, growth pattern.

- **Colony form**: pinpoint, circular, filamentous, irregular
- **Colony elevation:** flat, raised, convex , Concave , pointed
- **Colony margin:** smooth, irregular



Phases of growth media

Culture media may be found in one of three states:

- liquid (called broth)
- semi-solid
- solid.

Media are solidified by the addition of solidifying agents such as agar. Varying the concentration of agar will yield varying degrees of solidification.

Types of culture media

or basic

1. BASAL MEDIA: used for culture of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water.

* they have least nutrient requirement

- Nutrient broth → liquid
- Nutrient agar → solidified
- Peptone water → liquid
- simplest media

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2. ENRICHED MEDIA: by adding blood, serum or egg. Examples: blood agar, Chocolate agar and Lowenstein-Jensen media.

nutrient (agar) + (by blood or serum) → to give another media which riched to out the microorganism (fastidious)

3. SELECTIVE MEDIA: contains agents that inhibit the growth of all agents except that being sought (dyes, bile salts, alcohols, antibiotics). Examples: SSA, Mannitol Salt Agar. → it is used in [Staphylococci]

Salmonella shigella agar

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* We should know that the media can have more than one character

* إذا اشتغلت عليه البكتيريا تعطي شكل مع بكتيريا تختلف عن شكل آخر مع بكتيريا أخرى

4. 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

بالتالي عملية معرفة ال organells that make hemolysis form not

Blood agar → work as a differential agar

MacConkey agar → it has certain suger (lactose) + indicator → إذا البكتيريا اشتغلت على ال (lactose) → وعملت Fermentation ال indicator يتغير لونه to pink

إذا ما عملت Fermentation تكون لونها Pall yellow

5.TRANSPORT MEDIA: These media are used when specimen cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium. *الاسماء مهمة

تستخدم لنقل ال microorganism after the collection and transfer sample in certain media

6. STORAGE MEDIA: Media used for storing the bacteria for a long period of time.

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Biochemical Tests : تحفظ إنه يفرز enzyme

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- Media with a special substrate and tested for an end product.
- Prominent biochemical tests include (1) enzymes (catalase, oxidase, ...), (2) fermentation of sugars, ...

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* for ex.
- It consist of 20 tests that are converted to digital profile.

Immunological (Serological) Tests

1. **Agglutination tests:**
   Direct whole pathogen agglutination assays
   Particle agglutination tests: latex beads or RBCs coated with Ag.
2. **ELISAs**
3. **IFAs**

Genotypic methods

- **Nucleic acid probes**
- **PCR (polymerase chain reaction)**
- **Nucleic acid sequence analysis**
- **rRNA analysis**
- **RFLP (restriction fragment length polymorphism)**
- **Plasmid fingerprinting.**

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classification of Bacteria

classification of Bacteria can be phenotypic, genotypic, ---

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→ phenotypic classification ليستخدم في * of bacteria

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Bergey's Manual of Determinative Bacteriology.

Taxonomy is the science of classification of organisms

Bacterial taxonomy consists of three separate, but interrelated areas:

→ phenotypic was depend on process called numerical taxonomy → calculating كان في برامج بتعمل للنسبة وحسبة مطابقة المئوية microorganism ← identification نعمل بدينا لـ microorganism

- **Classification**
- **Nomenclature**
- **Identification**

طلع نشوف هل هو متفق بنسبة كام % مع different organism أخرى

* numerical taxonomy → system is used (depend on) morpholog (stain, cell shape, arragment) + physiological character + - - -

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.**

→ * according international role

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.**

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Taxonomic Rank → start with domin and end with specis (genus + Species اسمهما) and maybe family

Taxonomic Ranks	
Example	Formal rank
Bacteria	Domain
Gracilicutes	Phylum
Scotobacteria	Class
Eubacteriales	Order

مهم

Species ناخدها مش نفرق بين ال

Infra-subspecific designations:

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Strains having	Synonym	Preferred name
Special biochemical or physiological properties	Biotype	Biovar
Distinctive antigenic properties	Serotype	Serovar
Pathogenic properties for certain hosts	pathotype	Pathovar
Ability to be lysed by certain bacteriophages (virus)	Phage type	Phagovar
Special morphologic features	Morphotype	Morphovar

* strain = clon (present population of geneticly identical organism arising from single cell)

Classification of Bacteria → depend on morphology

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- Phenotypic classification
- Environmental reservoirs / Modes of transmission
- Genotypic classification

Phenotypic classification:

- Morphology and Gram Staining characteristics
- Growth requirements and metabolic behavior

* هل ممكن شكل البكتيريا يساعدني ال classification ? نعم

* Morphology associated with certain disease

staph bacteria → skin infection
myco bacteria → chronic disease
purnima bacteria → stiffens