Colony Morphology & Identification

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Objectives

Methods used in bacterial identification:

1. Microscopic appearance
2. Cultural characters
   a. Colony morphology
   b. Haemolysis
3. Biochemical test
4. Serological test
5. Polymerase chain reaction (PCR)
Identification

- Accurate and definitive microorganism identification, including bacterial identification and pathogen detection, is essential for correct disease diagnosis, treatment of infection.
- Scientists have devised an array of tests that help to identify bacteria. These tests range in complexity from viewing bacteria under a microscope to sequencing DNA.
1- MICROSCOPIC APPERANCE:

The use of a microscope is a very important skill for a microbiologist.

Microscopic methods used for unstained and stained preparation commonly used in the study of microorganisms.

Unstained preparation
1- Wet Mount
2- Hanging drop

Stained preparation
1- Differential stain
2- Special stain
2- Cultural characters:

The **macroscopic appearance** of colonies of bacteria can also be used to identify bacteria (e.g. **hemolytic** properties on agar containing blood, **pigmentation** of the colonies, **size and shape** of the colonies)
A. Colony Morphology

- Bacteria grow on solid media as colonies. A colony is defined as a visible mass of microorganisms all originating from a single mother cell.

- Each bacteria has a special colony morphology on solid media.

- The commonly observed colonial characteristics are helpful in making a preliminary bacterial identification.

- These cannot be used as the single criteria for identification.
What is the difference between colony morphology and cellular morphology?

**Morphology of colonies** can be defined as their color, shape, edge and elevation. ... However, **cellular morphology** shows the **difference** of the individual cells that is seen under the microscope. **Cellular morphology** of a cell can be cocci, bacilli, spiral etc.
Following are a number of terms used to describe colonial morphology:

<table>
<thead>
<tr>
<th>Shape</th>
<th>Circular</th>
<th>Rhizoid</th>
<th>Irregular</th>
<th>Filamentous</th>
<th>Spindle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margin</td>
<td>Entire</td>
<td>Undulate</td>
<td>Lobate</td>
<td>Curled</td>
<td>Rhizoid</td>
</tr>
<tr>
<td>Elevation</td>
<td>Flat</td>
<td>Raised</td>
<td>Convex</td>
<td>Pulvinate</td>
<td>Umbonate</td>
</tr>
<tr>
<td>Size</td>
<td>Punctiform</td>
<td>Small</td>
<td>Moderate</td>
<td>Large</td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td>Smooth or rough</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appearance</td>
<td>Glistening (shiny) or dull</td>
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<tr>
<td>Pigmentation</td>
<td>Nonpigmented (e.g., cream, tan, white)</td>
<td>Pigmented (e.g., purple, red, yellow)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optical property</td>
<td>Opaque, translucent, transparent</td>
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</tbody>
</table>
Bacillus subtilis
Proteus spp (Swarming)
*Pseudomonas aeruginosa* (Pigmentation)
B. Haemolysis

Is the breakdown of red blood cells and the ability of bacteria colonies to induce hemolysis is used to classify certain microorganisms this is particularly useful in classifying *Streptococcus spp*.

**Alpha:** partial clearing of blood around colonies with green discoloration of the medium.

**Beta:** zone of complete clearing of blood around colonies due to lyses of the RBC.

**Gamma:** no change in the medium around the colony; no lyses of the RBC.
Types of haemolysis

- **Beta Hemolysis**: Complete lysis of RBC
- **Alpha Hemolysis**: The agar is dark & greenish
- **Gamma Hemolysis**: Unchanged no hemolysis
3. Biochemical tests :-

Biochemical tests: Are tests that identify the bacteria on the basis of the presence of certain enzymes and other biochemical properties. Biochemical reactions are used for accurate identification

• Kliger’s Iron Agar (KIA)
• Triple sugar iron
• IMViC test
• Oxidase test
• Catalase test
• Urease test
4. Serological test:

- Identifying and quantifying the specific Ab found in serum of infected patient in early and late stages of infection.

- Detecting and identifying an organisms in a specimens by its surface Ag or by the soluble Ag it produces.

- Some serological tests are not limited to blood serum, but can also be performed on other bodily fluids such as semen and saliva.

Serological techniques:
- Latex agglutination (LA),
- Complement fixation (CF)
- Enzyme-linked immuno-assay (ELISA)
- Fluorescent antibody (FA)
5. Polymerase Chain Reaction (PCR)

PCR is a means to amplify a particular piece of DNA

**Amplify**: making numerous copies of a segment of DNA

PCR can make billions of copies of a target sequence of DNA in a few hours
References
