Oral Bacteriology

Lecture

Gram Stain

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Gram Stain

Gram stain is a differential staining technique used to classify bacteria into:

1. Gram-positive bacteria → appear purple
2. Gram-negative bacteria → appear pink/red

This difference is due to variations in the bacterial cell wall structure.

Gram Stain

Principle of Gram Staining:

1. **Gram-positive bacteria** have a thick peptidoglycan layer, which retains the primary stain.
2. **Gram-negative bacteria** have a thin peptidoglycan layer and an outer membrane, so they lose the primary stain during decolorization.

Stain made through a four-step process: crystal violet staining, iodine mordant, alcohol/acetone decolorization, and safranin counterstaining.

Gram Stain

Step 1: Preparation of Smear

1. Place a drop of distilled water on a clean slide (if using solid culture).
2. Using a sterile loop,
3. transfer a small amount of bacteria.
4. Spread to make a thin smear.
5. Allow to air dry.
6. Heat fix by passing the slide quickly through a flame 2–3 times.

Step 2: Primary Staining

1. Flood the smear with crystal violet.
2. Leave for 1 minute.
3. Rinse gently with water.

All bacteria appear purple at this stage.

Step 3: Mordant Application

1. Add Gram's iodine.
2. Leave for 1 minute.
3. Rinse with water.

Iodine forms a crystal violet–iodine complex inside cells.

Step 4: Decolorization (Critical Step)

1. Apply 95% alcohol or acetone for 10–20 seconds.
2. Rinse immediately with water.

Gram-positive bacteria remain purple. Gram-negative bacteria become colorless

Step 5: Counterstaining

1. Flood with safranin.
2. Leave for 30–60 seconds.
3. Rinse with water.
4. Blot dry gently.

Gram-positive → Purple, Gram-negative → Pink/Red

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Oral Streptococci

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Streptococcus pyogenes

Microbiological Characteristics:

- ✓ Gram-positive: It has a thick peptidoglycan cell wall that retains the purple crystal violet dye during Gram staining.
- ✓ Non-motile: Does not possess flagella.
- ✓ Non-spore forming
- ✓ Facultative anaerobe: Can survive in both aerobic and anaerobic environments.
- ✓ Catalase-negative: Does not produce the enzyme catalase (this helps differentiate it from Staphylococcus species, which are catalase-positive). Staphylococcus is Gram positive, spherical cocci bacteria, arranged in irregular grape- like clusters. It is non- motile, Non-spore forming and Facultative anaerobe. It is Catalase-positive that differentiates from Streptococcus.

Note: The enzyme catalase in bacteria is a critical antioxidant defense mechanism that decomposes toxic hydrogen peroxide (H2O2) into water and oxygen (H2O +O2), protecting cells from oxidative damage. It is primarily found in aerobic and facultative anaerobic bacteria

- **The Reaction:** $2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2(\text{gas bubbles})$
- **The Lab Application:** Scientists use this to differentiate between bacterial groups.
  - **Staphylococcus** (Catalase positive - bubbles)
  - **Streptococcus/Enterococcus** (Catalase negative - no bubbles)

Oral streptococci

- ✓ most abundant in the oral cavity.
- ✓ primary colonizers
- ✓ **Classification:** based on genetic sequencing.

1. **Streptococcus mitis:** commensal, produces H2O2 to inhibit harmful species.
2. **Streptococcus mutans:** dental caries
3. **Streptococcus salivarius.**
4. **Streptococcus anginosus:** abscesses.

Oral streptococci

Role in Health and Disease:

- ✓ In a healthy mouth → "gatekeepers"
- ✓ If balance disrupted in case of:

a. A high-sugar diet → S. mutans → tooth decay.

b. Enter the bloodstream (brushing or invasive dental procedures) →

infective endocarditis (heart valve infection)

Oral streptococci

✓ Oral streptococci are a diverse group of bacteria that represent the most abundant genus in the human oral cavity. As primary colonizers, they are the first bacteria to settle on tooth surfaces and oral tissues in newborns, forming dental plaque or biofilms.

Classification: these bacteria are categorized into several distinct groups based on genetic sequencing.

1. Streptococcus mitis: They are generally commensal (friendly) and help maintain oral health by producing hydrogen peroxide to inhibit harmful species.
2. Streptococcus mutans: the primary cause behind dental caries because of their ability to produce heavy acids from dietary sugars.
3. Streptococcus salivarius.
4. Streptococcus anginosus: which are often associated with purulent infections and abscesses.

Role in Health and Disease: In a healthy mouth, these bacteria exist in a state of homeostasis, acting as "gatekeepers" that defend against pathogens. However, this balance can be disrupted in case of:

a. A high-sugar diet allows acid-tolerant species like S. mutans to thrive, leading to tooth decay.

b. Systemic Disease: If these bacteria enter the bloodstream—through daily activities like brushing or invasive dental procedures—they can travel to distant sites and cause serious infections, most notably infective endocarditis (heart valve infection)

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Culture Media

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Culture media

✓ Culture media are specialized, nutrient-rich liquid, semisolid, or solid formulations designed to support, select, and differentiate microorganism growth in laboratory (in-vitro) settings. They contain essential components like carbon, nitrogen, vitamins, and salts tailored to meet the metabolic needs of specific microbes.

✓ Culture media types Based on Physical State:

1. Liquid
2. Solid
3. Semi-solid

✓ Types of culture media Based on Function/Application:

1. Basal Media (e.g., Nutrient Agar): Supports the growth of non-fastidious, general organisms.
2. Enriched Media (e.g., Blood Agar): Basal media supplemented with nutrients like blood, serum, or egg yolk for fastidious organisms.
3. Selective Media (e.g., Mac Conkey Agar): Contains inhibitors (antibiotics, dyes) that suppress unwanted microbes while encouraging desired ones.

Common Examples in Microbiology

✓ Mac Conkey Agar: Selects for gram-negative bacteria,

✓ Streptococcus Selective Agar (SSA) Contains antibiotics like Neomycin and Polymyxin B to inhibit staphylococci and gram-negative bacilli while allowing most Streptococcus species to grow.

Culture media

any liquid or solid preparation containing basic nutrients like:

C, N2, mineral …..

which can supports the growth of microbial life in-vitro.

Solid



Liquid



semi-solid



Culture media

any liquid or solid preparation containing basic nutrients like carbon source, nitrogen source, mineral salts etc. which can supports the growth of microbial life in-vitro. culture media could be in 3 types based on consistency these are: Solid medium, Semi-solid medium, and liquid medium. Agar is an ideal culture media because it is not digested by most bacteria, remains solid at room temperature, and melts around 85 °C.

A petri dish is a small shallow transparent dish with a lid that is mainly used as a container to grow microbes with culture media in it.

Culture media

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