

Lab :(5) Gram positive Bacilli

Gram positive Spore-forming Bacilli

General Properties:

- Rod shaped gram-positive bacteria (stain purple on a gram-stain test)
- Spores are methods of survival not reproduction.
- Responsible for a number of classical diseases such as **anthrax** and **tetanus**.
- Found mostly in the environment or as part of the normal flora.
- Include genera : **Bacillus** and **Clostridium**

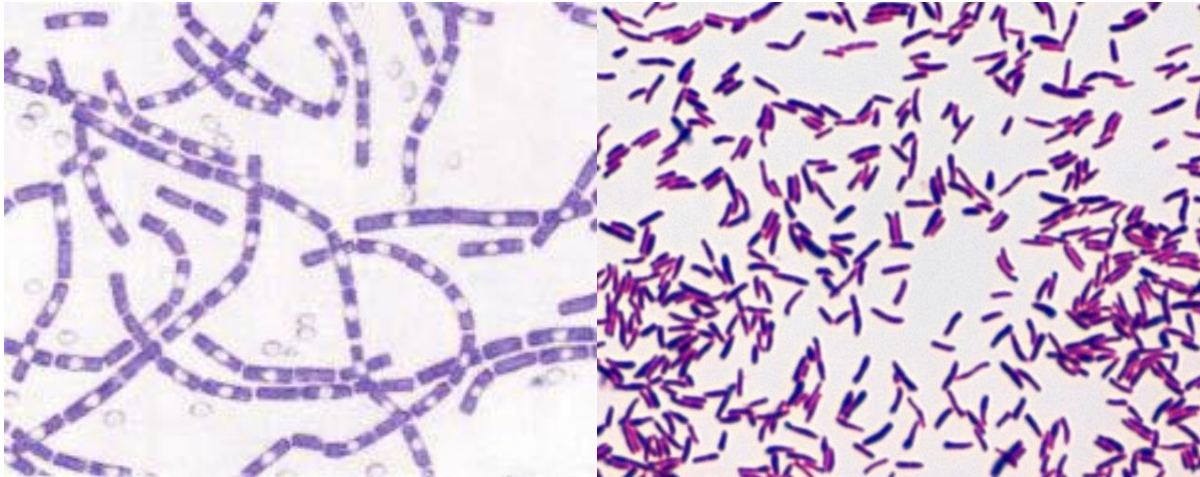


Fig. 1: Gram Positive Bacilli showing spores on the left
And without spores on the right

1. **Bacillus**

- The genus *Bacillus* contains a large group of aerobic and facultative anaerobic species.
- Gram-positive rods.
- Under aerobic conditions, it is able to form spores that release toxins.
- Bacilli aren't pathogenic to humans, but the following two can cause severe medical conditions: *Bacillus anthracis* and *Bacillus cereus*

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Bacillus anthracis

B. anthracis is one of several potential agents of bioterrorism and it can cause anthrax which is a serious infection (also named: malignant pustule or wool sorter's disease) which includes: **pulmonary anthrax** (inhalation) or **cutaneous anthrax** (direct contact).



Fig.2: Cutaneous anthrax

Virulence Factors:

1. Spores.
2. Toxins.
3. Capsule.

Laboratory Identification:

- Specimens include material from cutaneous lesions, blood cultures, and any other potentially infected tissues.
- Intracellular and cell-free spores do not stain by the Gram's technique; therefore, A specific stain (**spore stain**)is done as the following procedure:
 1. A smear of the organism is made on a slide.
 2. The smear is heat-fixed.
 3. The smear is stained with 10% malachite green for 45 minutes.
 4. The smear is washed.
 5. The smear is counterstained with safranin for 30 seconds
 6. The slide is observed under oil immersion.

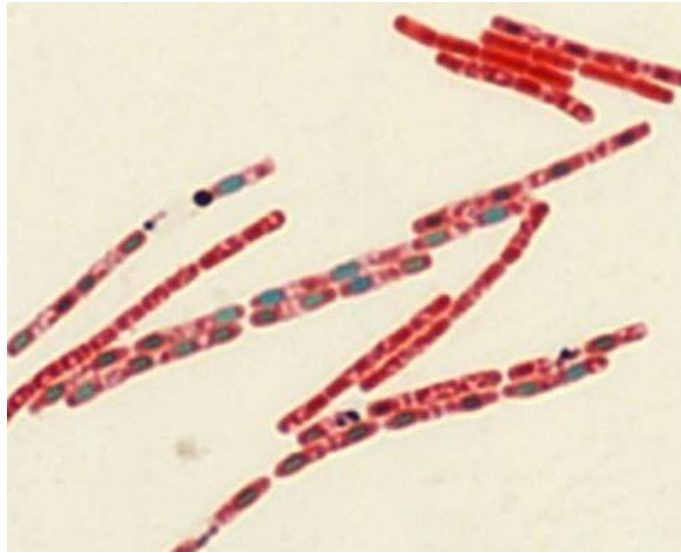


Fig.3: Spore stain using malachite green stain.

2. Clostridium

- This genus is characterized by the following:
 1. Obligate anaerobic
 2. Gram positive
 3. spore-forming
 4. drumstick shape
- About 30 *Clostridia* species cause disease in humans. These bacteria form toxins that lead to serious conditions.

Table (1): The most important Pathogenic Clostridium species:

Species	Disease
• <i>Clostridium tetani</i>	• Tetanus
• <i>Clostridium botulinum</i>	• Botulism
• <i>Clostridium perfringens</i>	• Gas gangrene • Food poisoning

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Laboratory Identification:

Using specific spore stain, the bacterial cells appear as drumstick appearance as the following slide:



Fig.4: *C. tetani* showing terminal spore stained with specific spore stain.

1. The bacteria are isolated on Thio-sulphate agar and broth.
2. Shows double zone of hemolysis when grown on blood agar

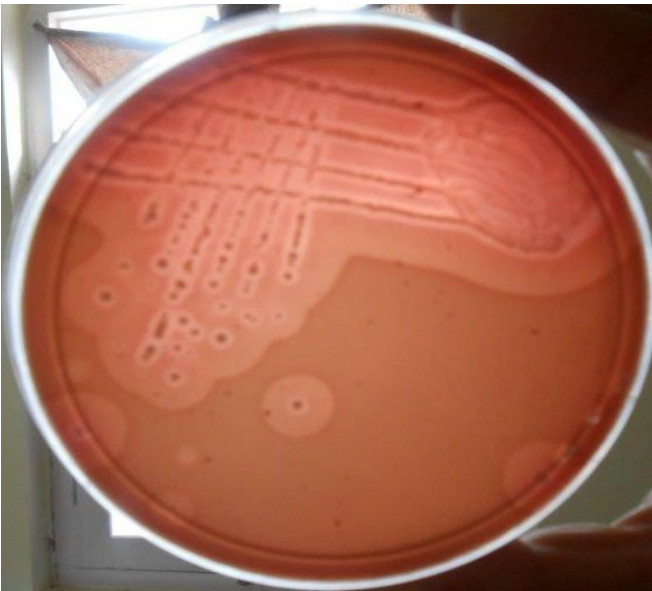


Fig.5: *C. perfringens* showing double zone of hemolysis on sheep blood agar outer zone of incomplete hemolysis and inner zone complete hemolysis.

3. Candle jar cultivation: The candle's flame burns until extinguished by oxygen deprivation, which creates a carbon dioxide-rich, oxygen-poor atmosphere in the jar as well as gas bag system for cultivation.

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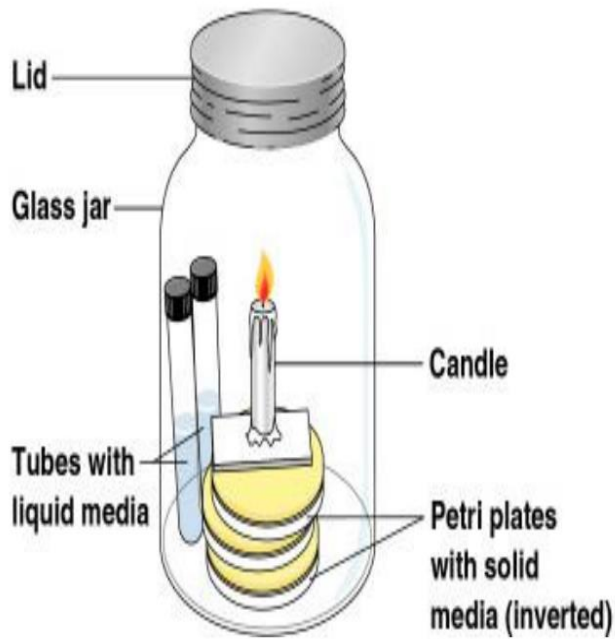
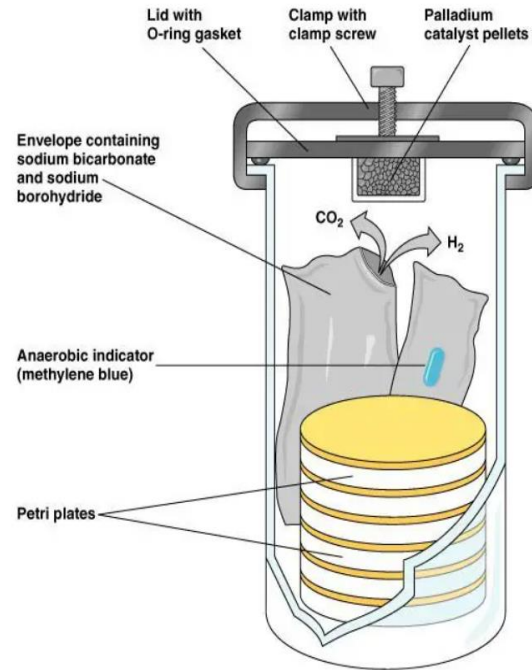


Fig.5: Candle jar cultivation



Gas bag system for cultivation(anaerobic)

Gram-positive non-spore forming Bacilli, *Corynebacterium*

- There are about 30 *Corynebacterium* bacteria associated with human disease.
- *Corynebacterium diphtheriae* is the primary pathogenic organism in this group. Toxigenic strains secrete a potent exotoxin which causes diphtheria and pseudo membrane formation.

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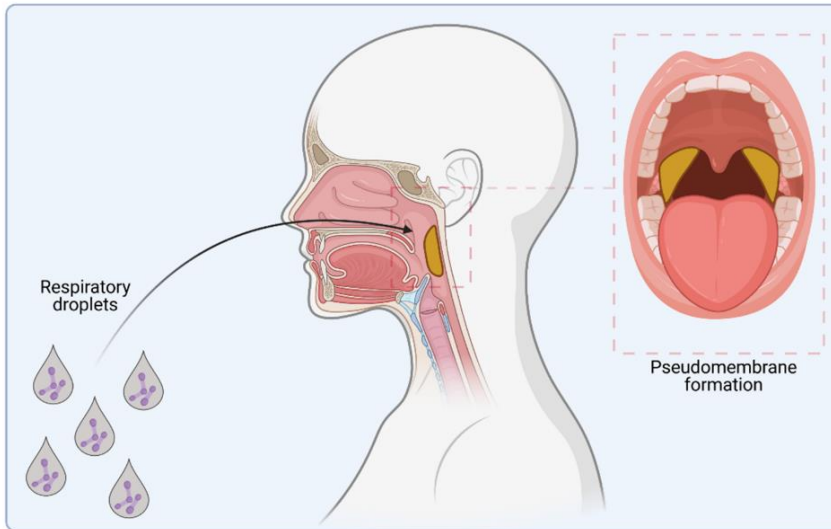


Fig.6: Pseudo membrane formation caused by *C. diphtheriae*

- irregularly shaped ('coryneforms'), with club-shaped swelled ends, and they are arranged as single cells, in pairs, in V forms, or in clusters with a so-called Chinese-letter appearance.

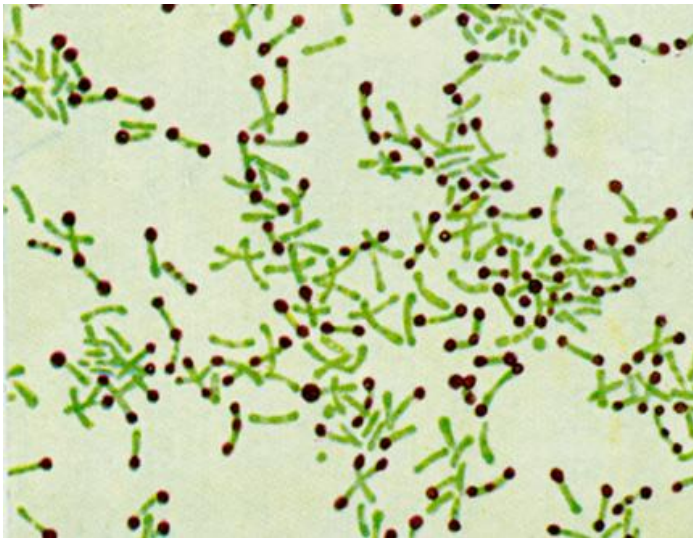


Fig.7: *C. diphtheriae* stained using specific stain