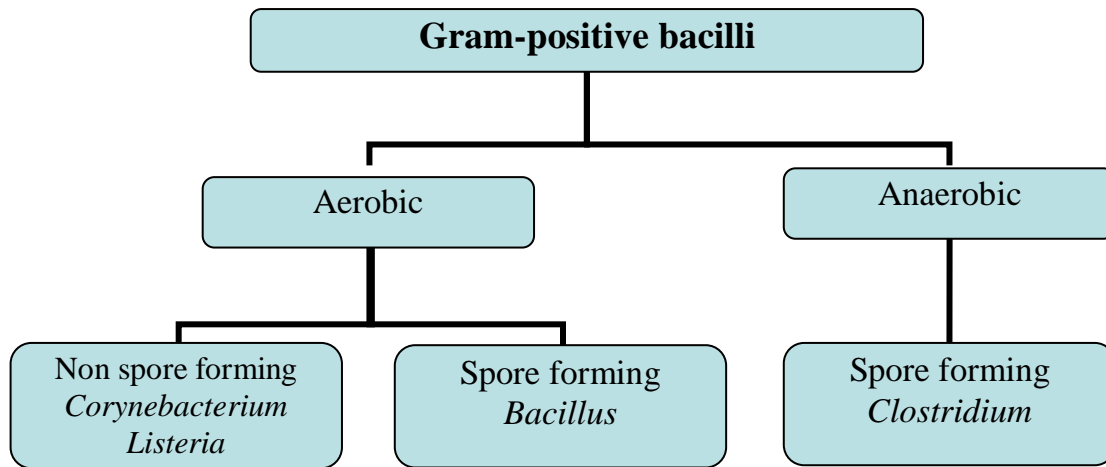
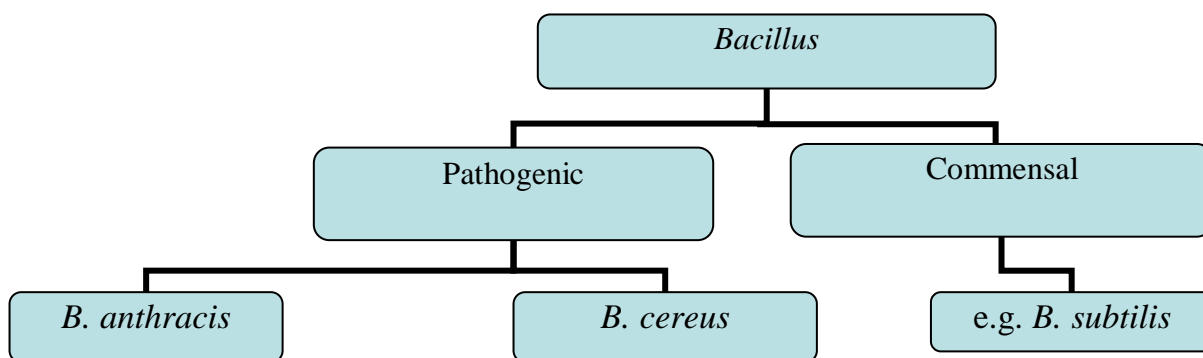


## Practical 4



### A- Aerobic spore forming: *Bacillus*



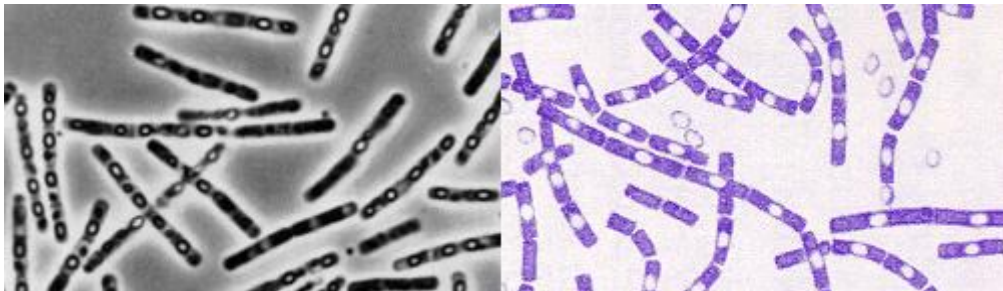
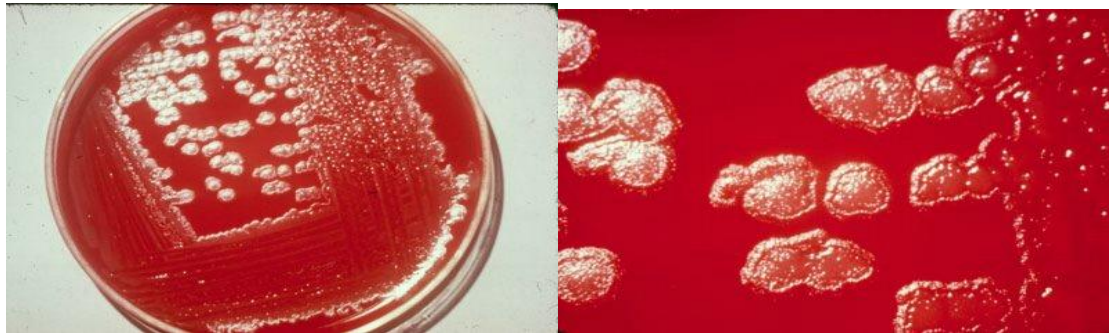
### ➤ General characters:

1. Very large Gram positive bacilli, 1-1.2  $\mu\text{m}$
2. arranged in long chains
3. Motile **except** *B. anthracis*
4. Spore forming (outside the host)
5. Capsulated (inside the host)
6. The bacteria can be cultivated in ordinary nutrient medium i.e. non Fastidious
7. Facultative anaerobic
8. Catalase positive
9. It is found in soil habitats around the world

## ***Bacillus anthracis***

Anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis*.

- Humans infected incidentally when brought into contact with diseased animals
- Direct person-to-person spread of anthrax is extremely unlikely to occur.

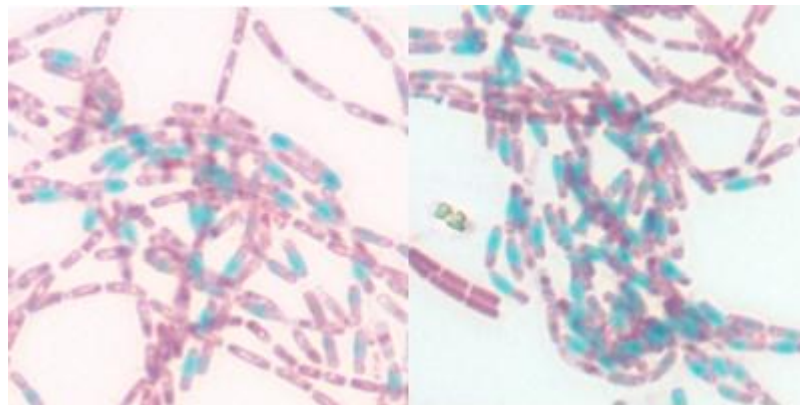
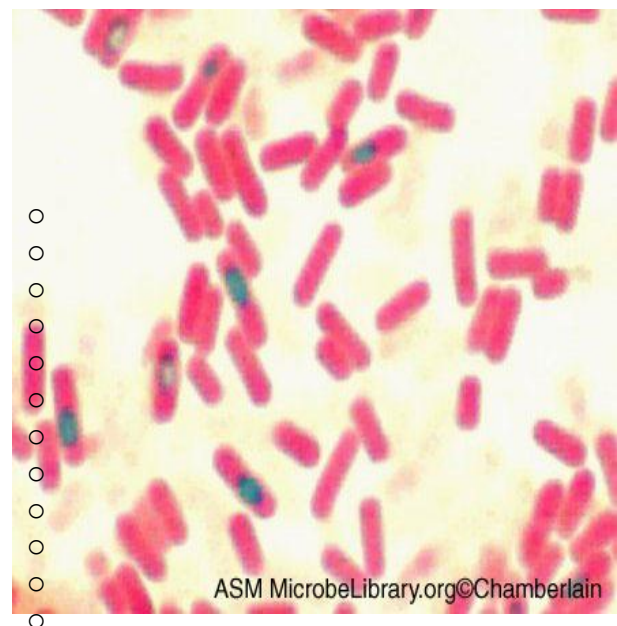
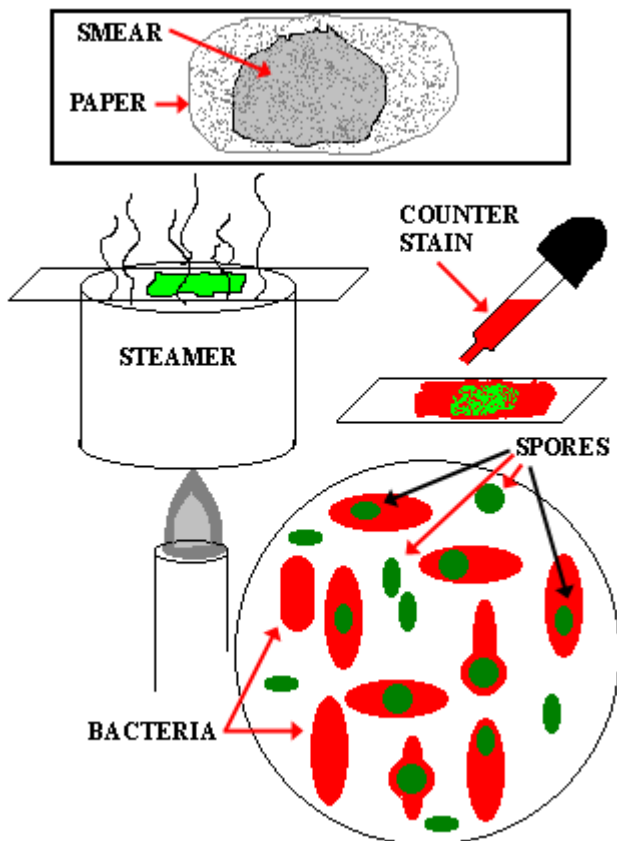


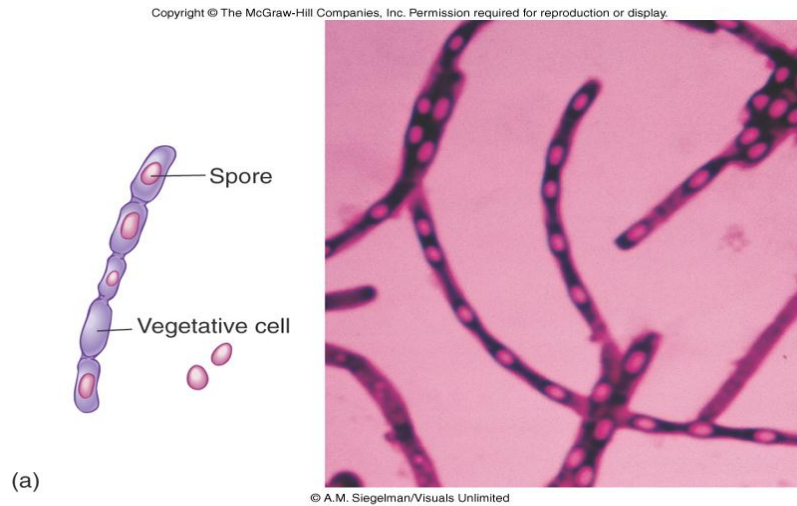
- **Virulence factors:**
  - Poly-D-glutamyl capsule, which mediates the invasive stage of the infection
  - Anthrax toxin, which mediates the toxigenic stage.

The toxin consists of three distinct antigenic components, which is thermolabile protein.

    - Edema Factor (EF): necessary for edema production
    - Protective Antigen (PA): induces protective antitoxic antibodies in guinea pigs
    - Lethal Factor (LF): has a lethal effect of anthrax toxin
- **Diagnosis of anthrax:**
  - Anthrax is diagnosed by isolating *B. anthracis* from the blood, skin lesions, or respiratory secretions or by measuring specific antibodies in the blood of persons with suspected.
    - **Specimen:**
      - Pastular exudates in malignant pustule, sputum in pneumonic anthrax or stool in intestinal anthrax is collected

- Stool specimen is emulsified and heated to 80°C to kill non spore forming microorganism.
- **Stain:**
  - Direct smear is done from specimen and stained by Gram stain
  - The stained smear revealed Gram positive bacilli, found in chains, capsulated inside the host, sporulated outside the host (Spore is central oval and non-bulging) and non motile.
  - By spore stain, the spore appears green and vegetative cell appear red when stained with malachite green and safranin.





### **Culture:**

On ordinary medium, grow aerobically at 37°C with characteristic mucoid or smooth colonies, which indicates the pathogenicity of organism (presence of capsule). Rough colonies are relatively avirulent.

### **Biochemical reactions:**

Ferment glucose, galactose, maltose and dextrin with acid production only.

### **Starch Hydrolysis (Amylase Activity)**

#### **Principle**

Starch + Iodine  $\longrightarrow$  blue color  
 Glucose + Iodine  $\longrightarrow$  No reaction

#### **Procedure**

- Inoculate nutrient agar plate containing 1% Starch
- Incubate the plate at 37 for overnight
- After incubation, flood the plate with Iodine solution

#### **Result**

- Activity of amylase is indicated by a clear zone around the growth while the rest of the plate gives blue color after addition of iodine solution



***Bacillus cereus***

- *B. cereus* is a normal inhabitant of soil, also isolated from food such as grains and spices.
- *B. cereus* causes two types of food poisoning:
- **Emetic form or short incubation:**
  - It is caused by heat stable enterotoxin
  - It is characterized by nausea, vomiting and abdominal cramps and has incubation period of 1-6 hrs.
  - It resembles *S. aureus* food poisoning.
- **Diarrheal form or long incubation:**
  - It is manifested primarily by abdominal cramps and diarrhea with an incubation period of 8-16 hrs.
  - It resembles food poisoning caused by *Clostridium perfringens*
  - It is caused by heat labile enterotoxin.

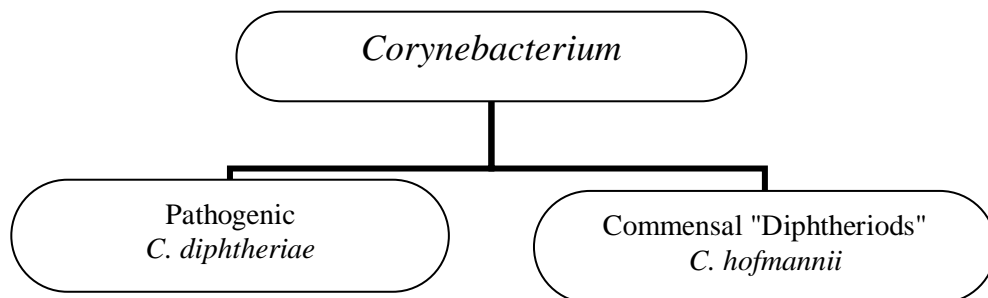
#### Differential characteristics of *B. anthracis* and *B. cereus*

Characteristic	<i>B. anthracis</i>	<i>B. cereus</i>
Hemolysis on blood agar	-	+
Motility	-	+

#### Non spore forming aerobic : *Corynebacterium*

##### General characters:

1. Gram positive bacilli, with characteristic morphology
2. Non motile
3. Non spore forming
4. non capsulated
5. Facultative anaerobic
6. Fastidious
7. Catalase positive
8. Oxidase negative



#### *Corynebacterium diphtheriae*

##### ➤ **Diphtheria:**

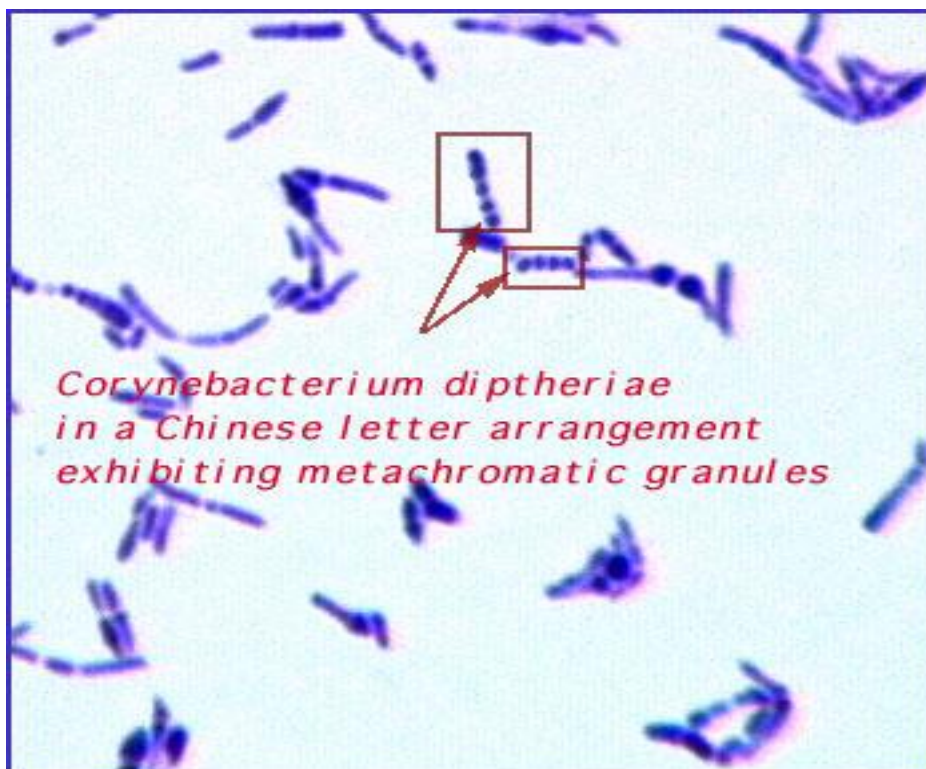
- Diphtheria is an acute, toxin-mediated disease caused by *C. diphtheriae*.

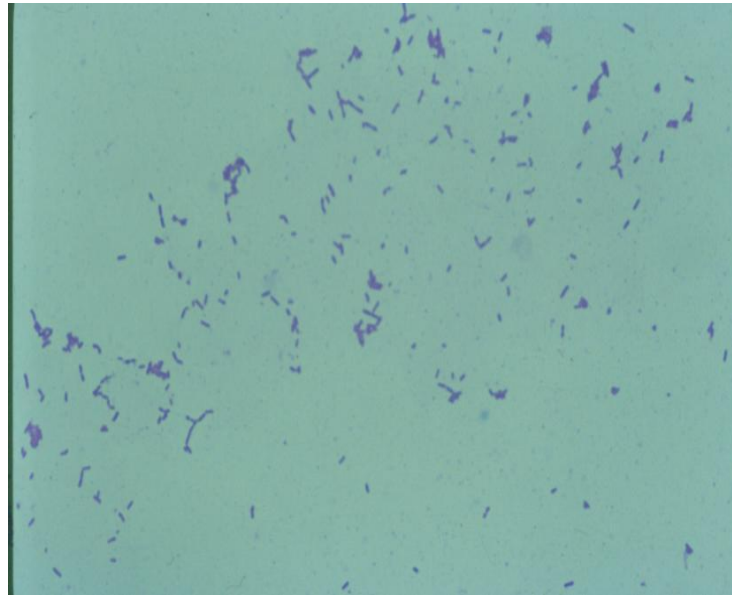


- Diphtheria is a childhood disease affecting the upper respiratory tract and transmitted by droplet infection from case or carrier.
- Early symptoms include malaise, anorexia, headache, **sore throat**, exudative pharyngitis and low grade fever.
- The patient may recover at this point; or if enough toxins are absorbed, develop severe prostration, pallor, rapid pulse, stupor, coma, and may die within 6 to 10 days.
- Complication with severe disease include myocarditis, neuritis and palate perforation.
- Patients with severe disease may develop marked edema of the submandibular areas and the anterior neck along with lymphadenopathy, giving a characteristic "bull neck" appearance.

### ➤ Laboratory diagnosis

- **Specimen:** A throat swap by gentle touching the membrane.
- **Culture:**
  - On Loeffler's serum media: the colonies of *C. diphtheriae* are small, smooth, and creamy.
  - On blood tellurite agar, there is three biotypes of *C. diphtheriae* are characterized.
- **Stain:**
  - Gram stain: *C. diphtheriae* are gram positive bacilli arranged in Chinese letters form often club shaped
  - Polychrome methylene blue stain: *C. diphtheriae* appears beaded due to the presence of intercellular "Metachromatic or volutin" granules. By stain, the granules appear red while the rest of organism appears blue.



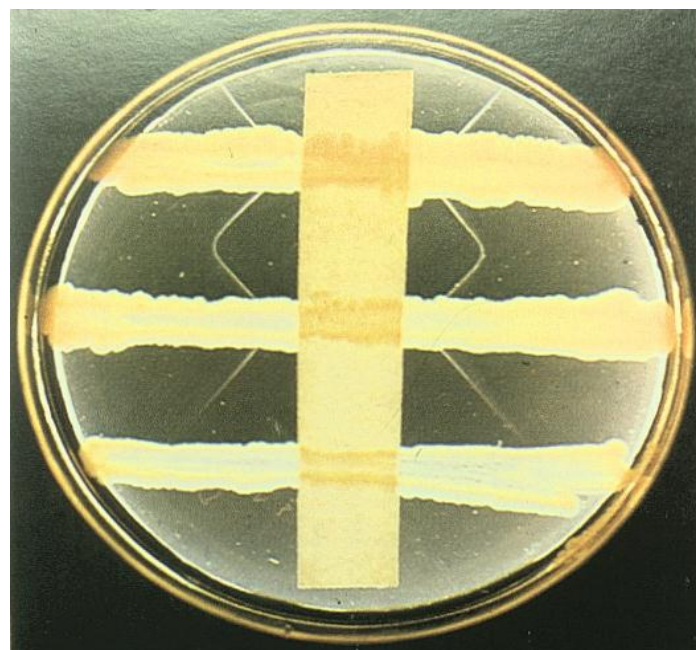
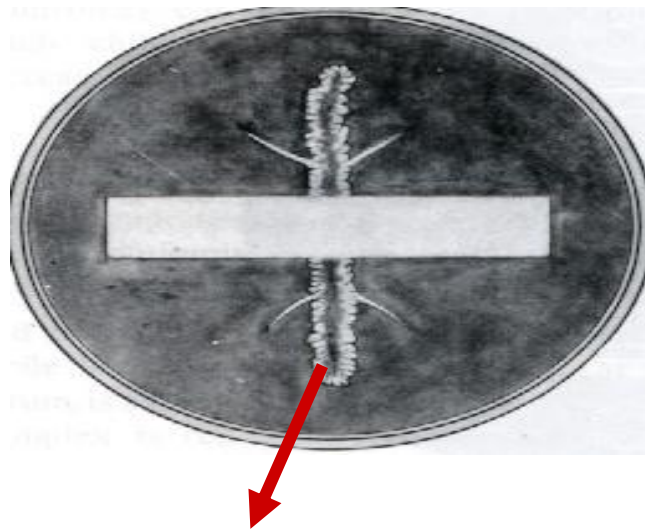


### Diagnosis of carrier:

- Two steps must be done for diagnosis of carrier:
- **Isolation of the microorganism:**
- **Detection of exotoxins produced by isolated microorganism:**
  - A swap are taken from the nose and throat, then inoculated onto Loeffler's serum or blood tellurite agar and incubated aerobically at 37°C for 24 h. The suspected colonies are examined by Gram stain and any diphtheria-like organisms must be submitted to virulence tests.
- **Virulence tests:**
  - **In Vivo:** Two guinea pigs are used; one is the test and the other is the control. The control is injected with diphtheria antitoxin 24 h before the experiment. Then the both guinea pigs, the test and the control, are injected with the suspension of the isolated microorganism
    - If the test animal dies while the control animal survives, it means the isolated organism is virulent *C. diphtheriae*.
    - If both guinea pigs survive, it means the isolated organism is avirulent strain
  - **In Vitro: "Eleks test"**
    - A strip of filter paper impregnated with diphtheria antitoxin is placed on the surface of serum agar. The tested organism is streaked at right angles to the filter paper. After 48 hrs incubation, the antitoxin diffusing from filter paper strip and the toxigenic strains produce exotoxin, which diffuses and resulted in lines four precipitation lines radiating from intersection of the strip and the growth of the organism

Filter paper saturated with diphtheria antitoxin

Lines of precipitations



### Diphtheroids

- Diphtheroids are gram positive bacilli oval short parallel bacilli, not beaded.
- Most of them are commensals in vagina, on the skin and throat.
- Examples of diphtheroids are *C. hofmannii*, *C. xerosis* and *C. acne*.
- *C. acne* may play a role in the pathogenesis of acne vulgaris.
- They can grow on ordinary media as nutrient agar.