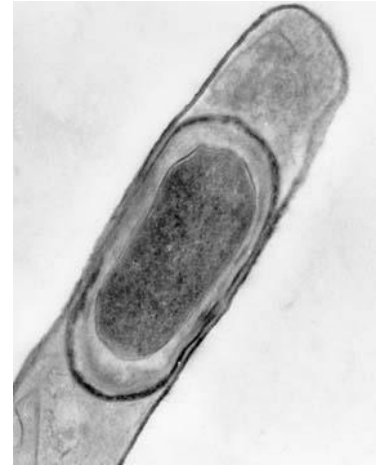
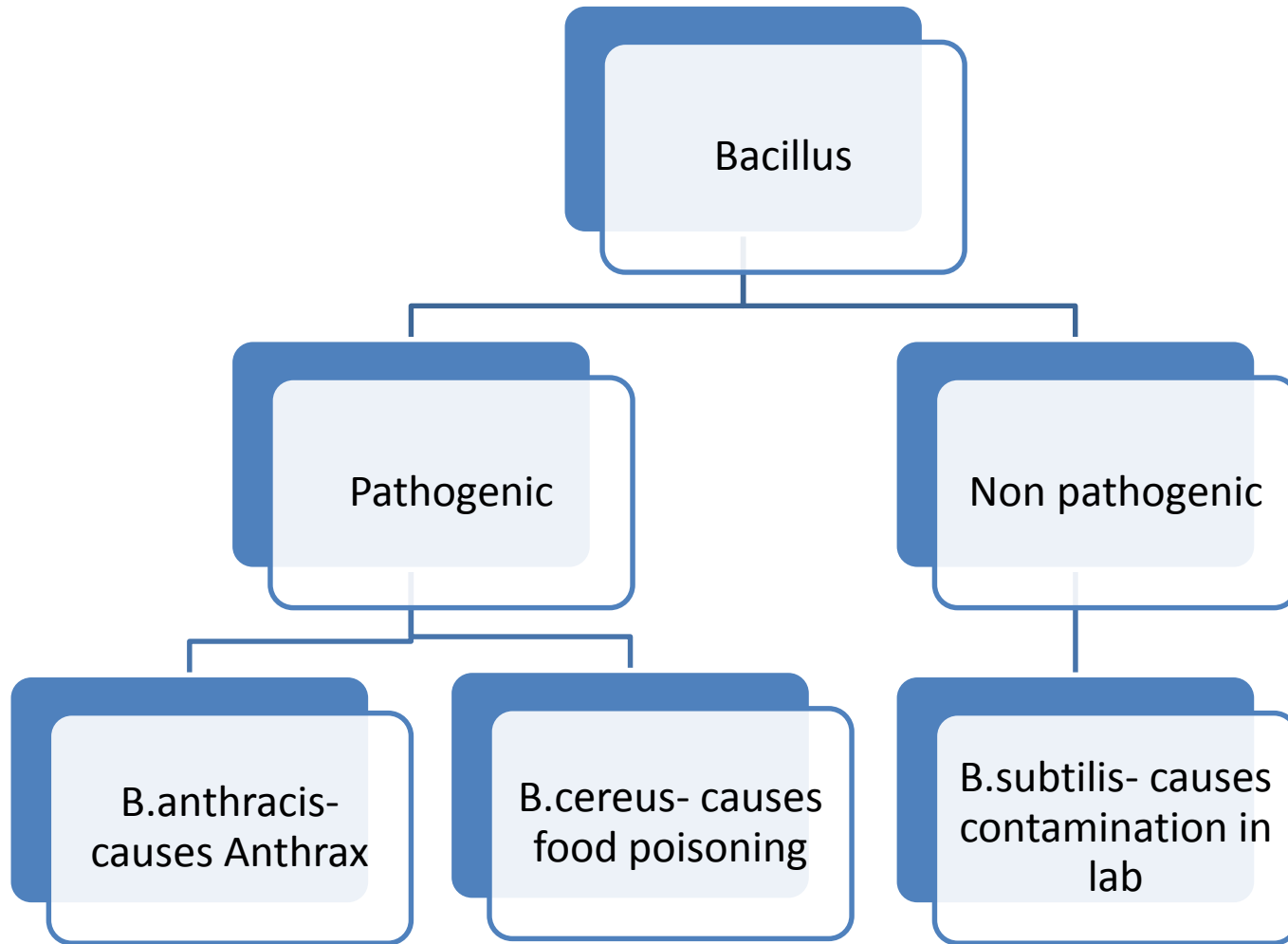


# Bacillus



# Aerobic Spore Forming *Bacillus*



# ***Anthrax***

- **Anthrax** is an acute infectious disease in man & animal caused by the spore-forming *B. anthracis*.
- Animals infected by soil borne spores in food & water or bites from certain insects
- Humans can be infected when in contact with flesh, bones, hides, hair, & excrement
  - nonindustrial or industrial
  - cutaneous & inhalational most common
- Direct person-to-person spread of anthrax is extremely unlikely to occur.

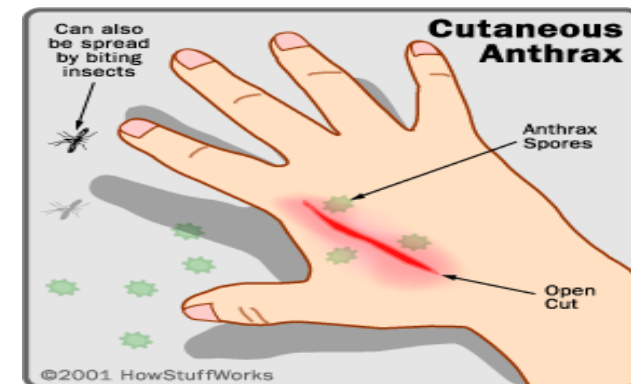
- 3 types of anthrax infection occur in humans:
  - 1) Cutaneous
  - 2) Inhalation
  - 3) GI

All types leading to fatal septicemia or meningitis

# Cutaneous Anthrax

- 95% of anthrax infections occur when the bacterium enters a cut or scratch on the skin due to handling of contaminated animal products or infected animals.(like **shaving brushes** made with animal hair )
- May also be spread by **biting insects** that have fed on infected hosts.
- After the spore germinates in skin tissues, toxin production initially results in itchy bump that develops into a vesicle and then painless black ulcer.(**black eschar**)
- The name anthrax which means coal, comes from the black colour of the eschar.

Ulcers are usually 1-3 cm in diameter.  
Common in dock workers, known as '**hide porter's disease**'.



# Cutaneous Anthrax



CDC, Cutaneous Anthrax—Vesicle Development

# Pulmonary Anthrax

- The infection begins with the **inhalation of the anthrax spore.**
- Death usually results 2-3 days after the onset of symptoms.
- Inhalation Anthrax is the **most lethal type** of Anthrax.
- Incubation period:
  - 1–7 days
  - Possibly ranging up to 42 days (depending on how many spores were inhaled).
- Case fatality after 2 days of infection.
- Common in workers in wool factories, due to inhalation of dust from infected wool. Known as '**wool sorter's disease**'.

# Chest X-ray

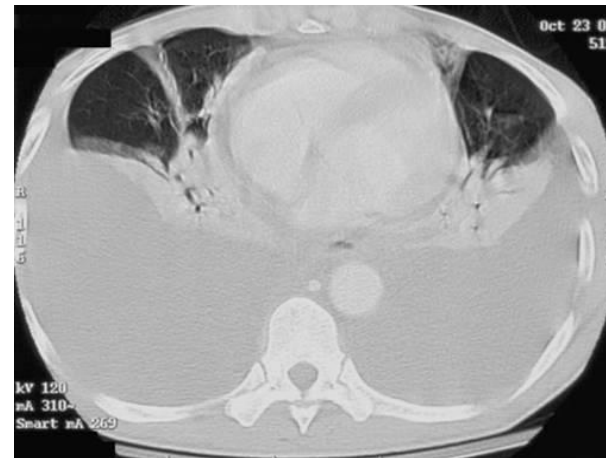
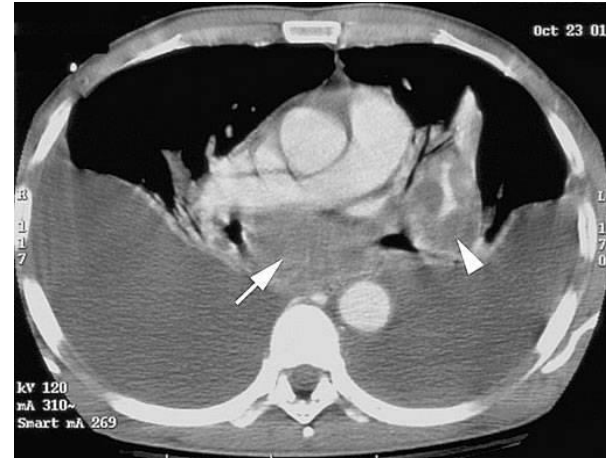


At day 1



At day 3

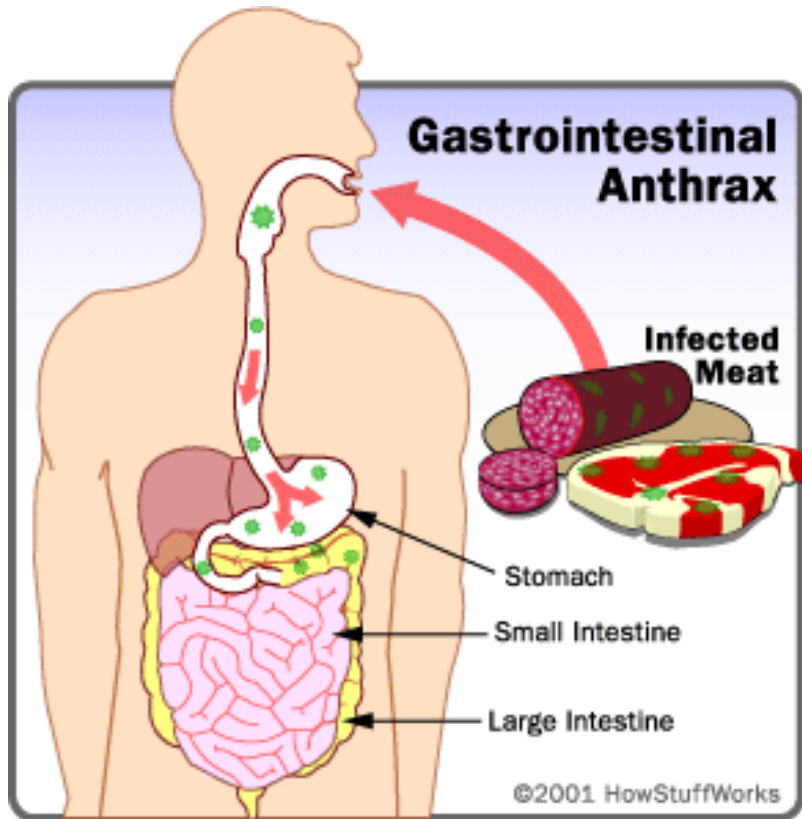
# CT scan



Pleural effusion



# Gastrointestinal Anthrax



- GI anthrax may follow after the consumption of contaminated, poorly cooked meat.
- There are 2 different forms of GI anthrax:
  - 1) Oral-pharyngeal
  - 2) Abdominal
- Abdominal anthrax is more common than the oral-pharyngeal form.

# ***B. cereus***

*B. cereus* is a normal inhabitant of soil, vegetables and other foods like milk, cereals, spices, meat and poultry.

*B. cereus* causes Two Types of food poisoning

**Emetic form /short incubation:** Mostly with contaminated rice

- It is caused by **heat stable enterotoxin**
- **Nausea, vomiting** and **abdominal cramps**
- Incubation period of 1-6 hrs
- It resembles *S. aureus* food poisoning

**Diarrheal form or long incubation:**

- With contaminated meat, vegetables or sauces.
- It is caused by **heat labile enterotoxin**
- **Abdominal cramps** and **diarrhea**
- Incubation period of 8-16 hrs
- Diarrhea may be a small volume or profuse and watery
- It resembles food poisoning caused by *Cl. perfringens*
- In either type, the illness usually lasts < 24 hrs after onset

*Foodborne Diseases of B. cereus*

(Intoxication)

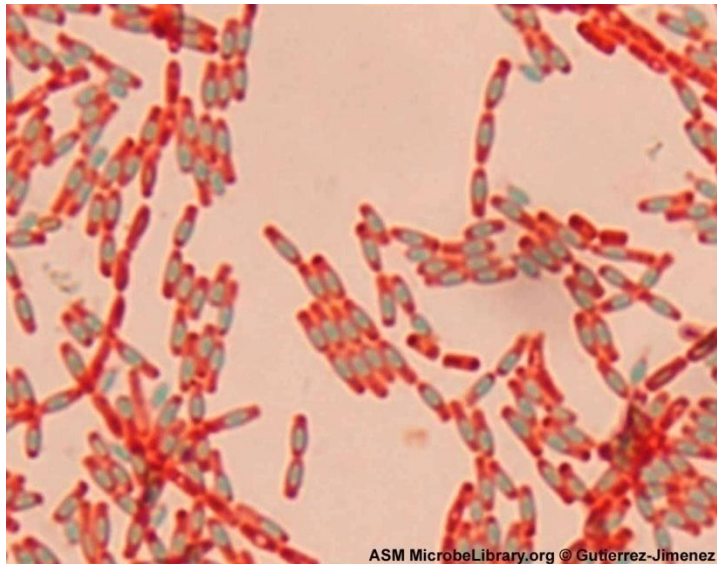
(Foodborne Infection)

	<b>Emetic Form</b>	<b>Diarrheal Form</b>
Implicated food	Rice	Meat, vegetables
Incubation period (hours)	< 6 (mean, 2)	> 6 (mean, 9)
Symptoms	Vomiting, nausea, abdominal cramps	Diarrhea, nausea, abdominal cramps
Duration (hours)	8–10 (mean, 9)	20–36 (mean, 24)
Enterotoxin	Heat-stable	Heat-labile

- Other diseases caused by *B. cereus*
  1. Ocular infection- post traumatic ophthalmitis
  2. Endocarditis
  3. Pneumonia
  4. Bacteremia
  5. Meningitis

- ***Bacillus stearothermophilus***
  - Tolerates very high temperatures
  - Used for quality control of autoclaves

***Bacillus subtilis***, and occasional other species may occasionally cause opportunistic infections



# Identification of *Bacillus Spp.*

## ❖ Specimen

Pastular exudates in malignant pustule

Sputum in pneumonic anthrax

Stool in intestinal anthrax (also in food poisoning by *B. cereus*)

- Stool specimen is emulsified and heated to 80°C to kill non spore forming microorganism

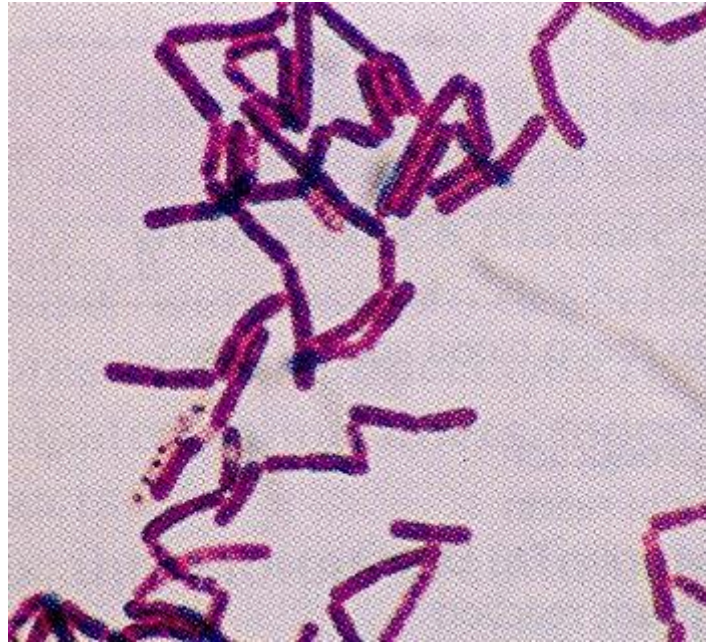
## ■ Morphology

Macroscopical (Cultural characteristics)

Microscopical (Gram Stain, Spore Stain)

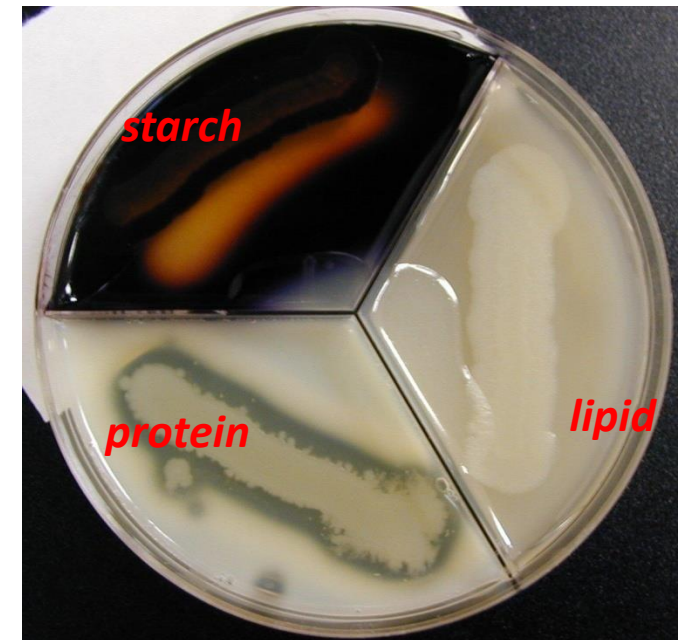
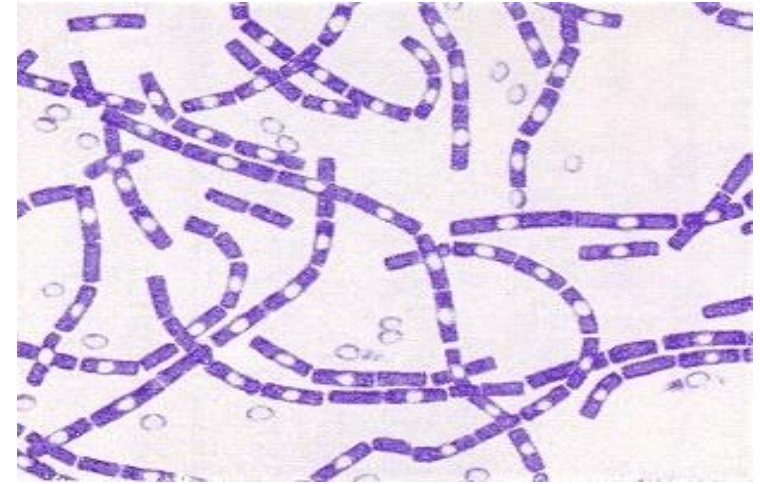


# MICROSCOPY



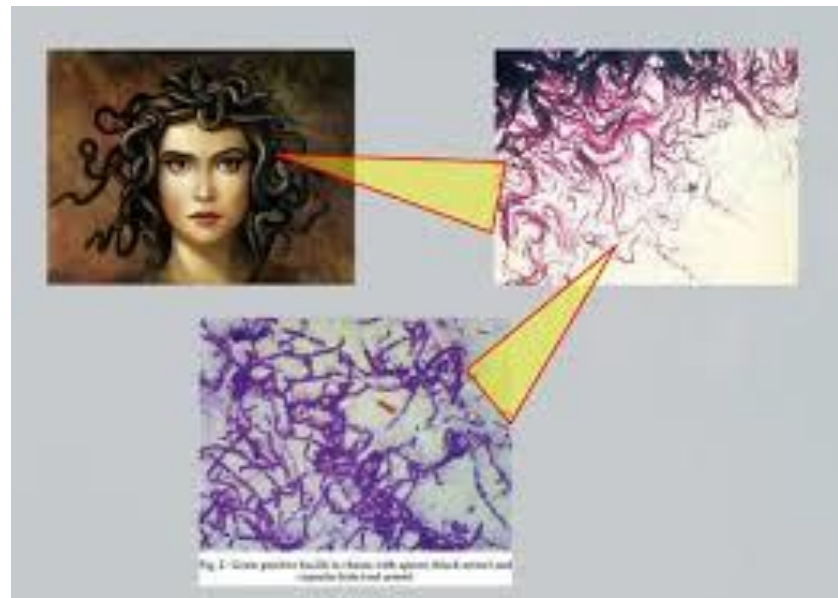
***Bacillus anthracis* in Gram stain**

- Largest of pathogenic bacteria
- 3 x 1.3  $\mu\text{m}$  gram positive , straight , sporing bacilli.
- The bacilli are arranged in end to end in long chains ends are truncated or concave and somewhat swollen known as “**Bamboo stick appearance**”
- **Sudan black stain – fat globules** within the bacilli are seen



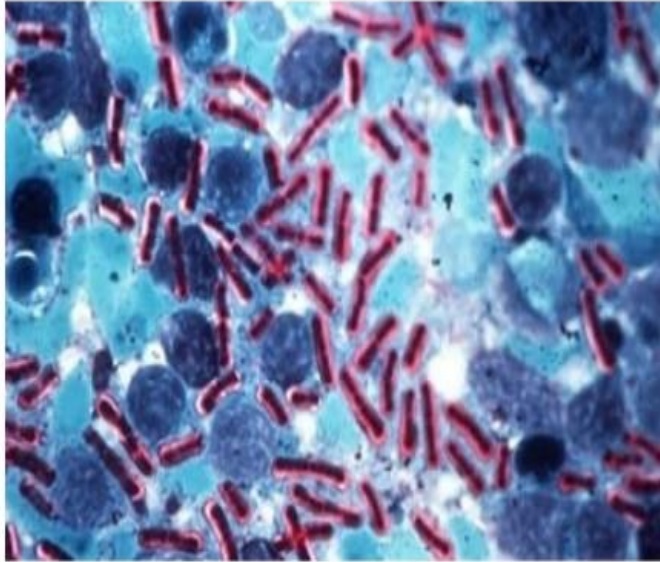


- **Cultural Characteristics**
  - **Grow on nutrient Agar**
    - Grow aerobically at 37C with irregular, raised, dull, opaque, greyish white with a frosted glass appearance. The edges of the colony is composed of long, interlacing chains of bacilli; resembling locks of matted hair under the low power microscope. This is called the '**Medusa head appearance**'.
    - Rough colonies are relatively avirulent
      - Stab culture on gelatin medium results in inverted fir tree appearance.



*B. anthracis*

McFadyean reaction (polychrome MB stain)



- ◉ Smears are stained by Gram's stain and polychrome methylene blue stain. We can see the amorphous purplish material. (**McFadyean's reactions**).

# Cultural Characteristics

## Nutrient Agar

Grow well on ordinary lab media producing large granular colonies with a coarse texture.



# Anaerobiosis

## Principle of anaerobiosis

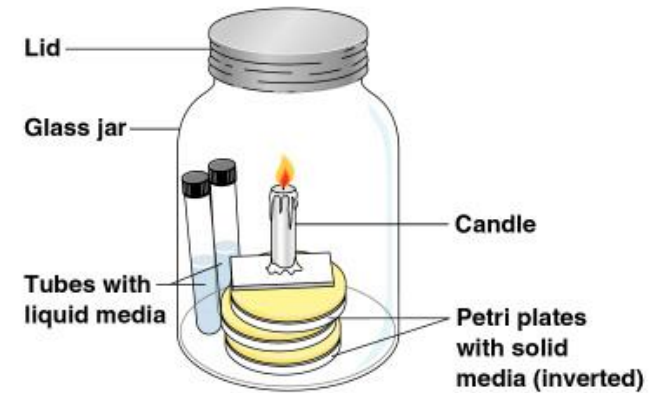
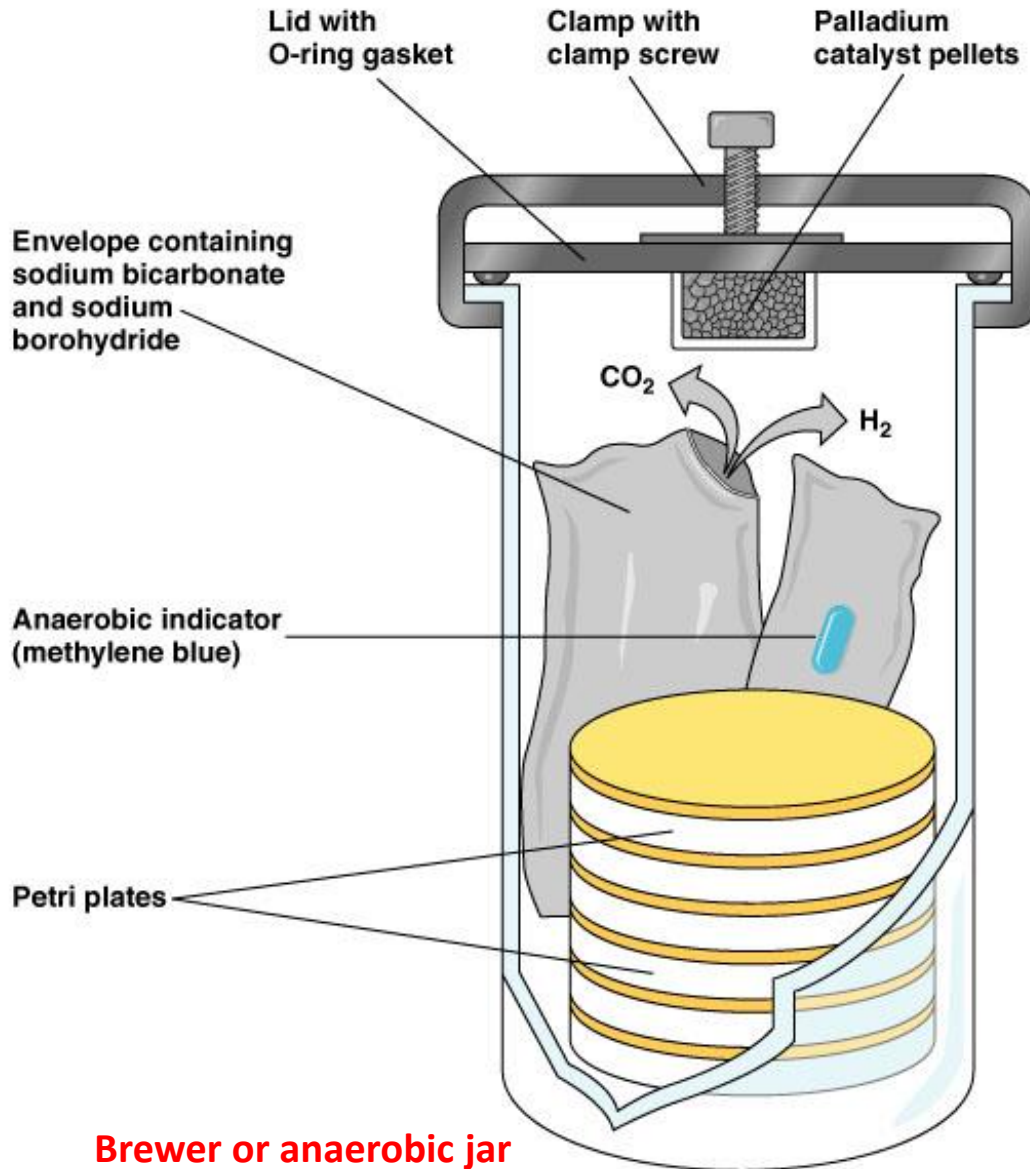
- ❖ Cultivation in **vacuum** by incubating culture in vacuum desiccator (unsatisfactory)
- ❖ Displacement of O<sub>2</sub> with **gases** like H<sub>2</sub>, N<sub>2</sub>, CO<sub>2</sub>, Helium
- ❖ O<sub>2</sub> removal by using **chemical** substances
- ❖ Reduction of redox potential by using **reducing agents** like 1 % glucose- 0.1 % sodium thioglycolate – 0.1 % ascorbic acid – 0.05% cysteine – meat particles in RCM

# Anaerobic techniques for cultivation of anaerobes

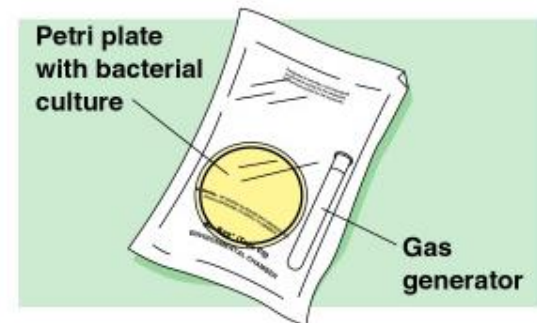
- Anaerobic disposable pouch / Bags technique
- Anaerobic dish technique
- Anaerobic jar technique
  - Mc Intosh Fildes Jar
  - Gas Pack Jar : using gas generating system (water & Catalyst are required)
  - Anaeropack jar: using gas generating system (no need of water & Catalyst)
- Anaerobic chamber technique



# Anaerobic and Low O<sub>2</sub> Culture Methods



**Candle jar**

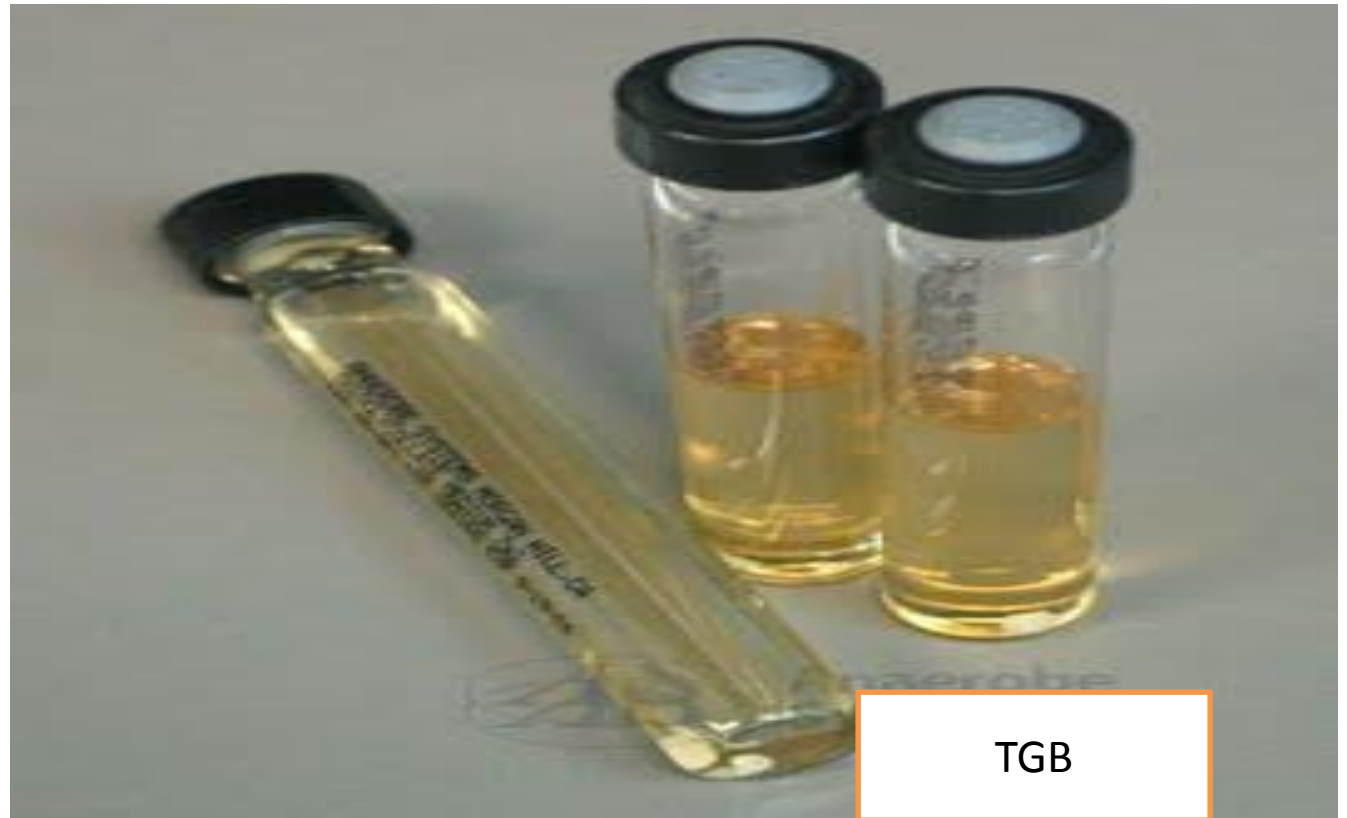


**CO<sub>2</sub> packet**

# *McIntosh-Fieldde's anaerobic jar*



## *Reduction of oxygen in medium*





# *Robertson's cooked meat medium*



**Saccharolytic**



**Proteolytic**

## *Anaerobic chamber (Glove box)*

- *For the fastidious anaerobes pre reduced media and glove box or anaerobic chamber may be used.*
- *Anaerobic chamber is air tight cabinet,*
- *Filled with inert gas,*
- *Glove for the hands.*



THANK YOU























