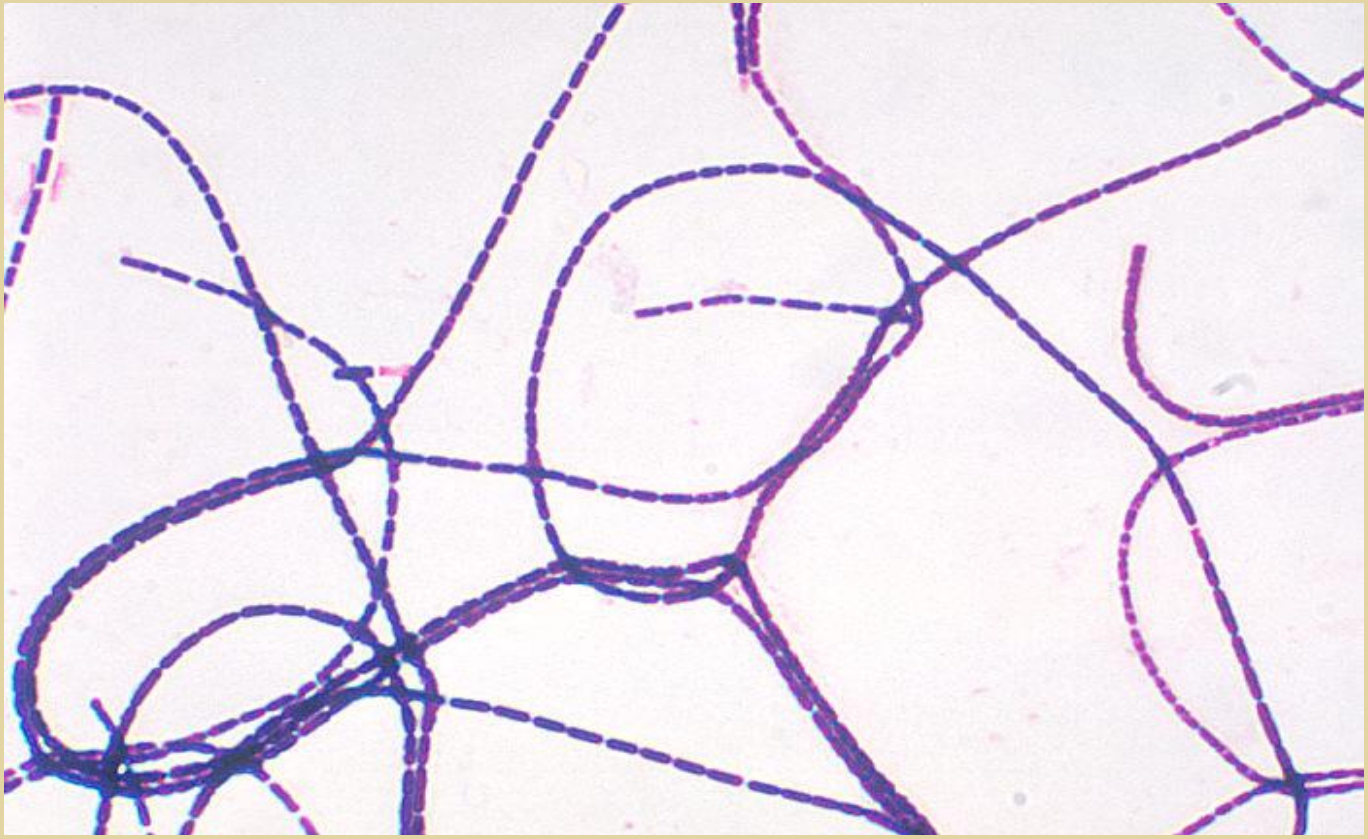
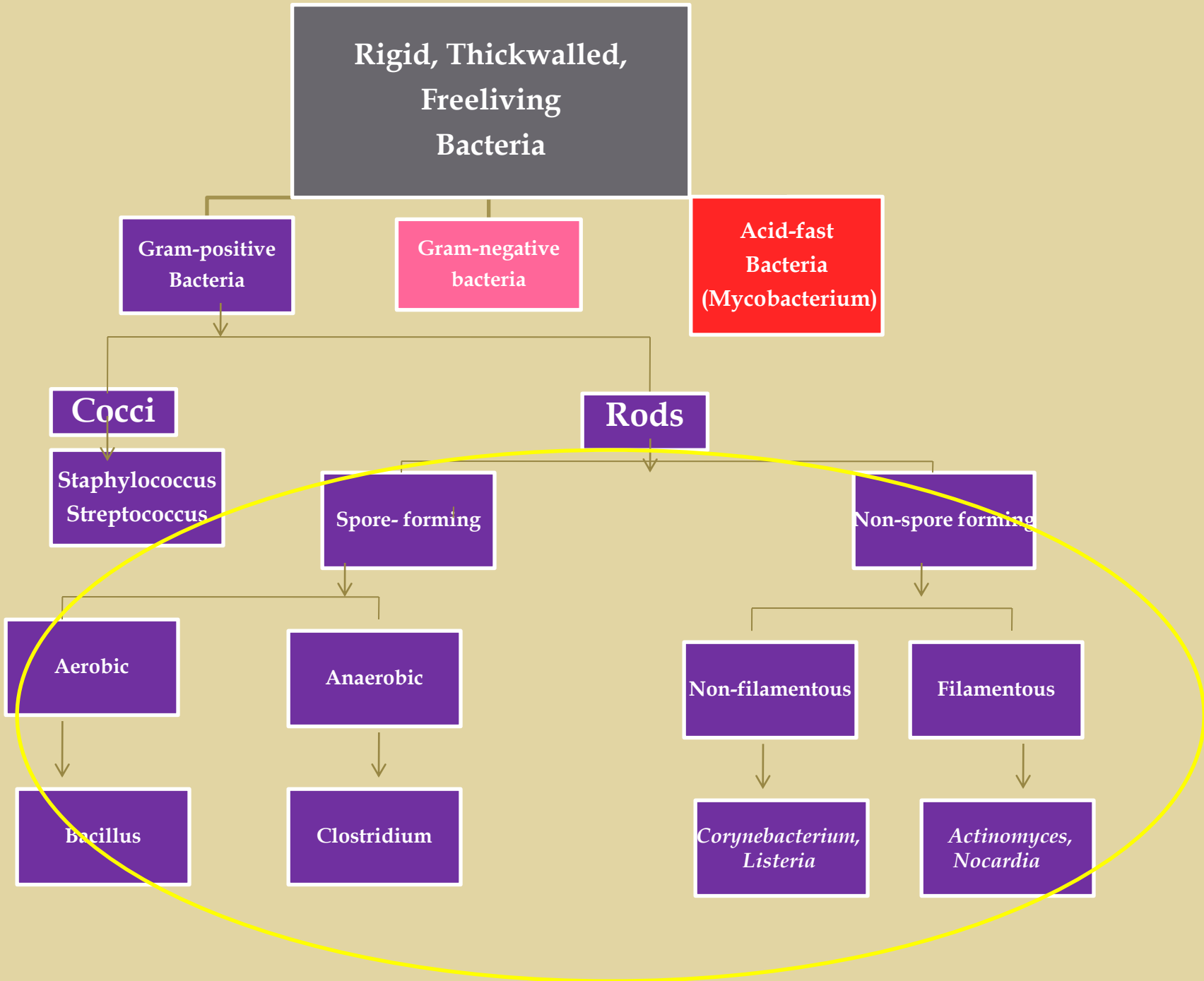
GRAM POSITIVE RODS



Dr Sadia Ikram

Learning Objectives

- To know the Classification of gram positive rods
- To recognize the Modes of transmission and Pathogenesis of diseases caused by spore forming and non-spore forming rods
- To explain the Clinical signs and symptoms of disease caused by *Bacillus cereus* and *Bacillus anthracis*
- To interpret the Lab diagnosis of *Bacillus cereus* and *Bacillus anthracis*


Rigid, Thickwalled, Freeliving Bacteria
Gram-positive Bacteria
Gram-negative bacteria
Acid-fast Bacteria (Mycobacterium)
Cocci
Rods
Staphylococcus Streptococcus
Spore- forming
Non-spore forming
Aerobic
Anaerobic
Non-filamentous
Filamentous
Bacillus
Clostridium
Corynebacterium, Listeria
Actinomyces, Nocardia

Gram Positive Rods

Spore-forming

- ***Bacillus species***
- ***Clostridium species***

Non-spore-forming

- ***Corynebacterium diphtheriae***
- ***Listeria***

Gram Positive Rods

Genus	Anaerobic Growth	Spore Formation	Exotoxins/ Important in Pathogenesis
Bacillus species	–	+	+
Clostridium species	+	+	+
Corynebacterium diphtheriae	–	–	+
Listeria	–	–	–

Spore-forming Gram +ve rods

Two medically important *Bacillus* species

Bacillus anthracis

Bacillus cereus

Bacillus species

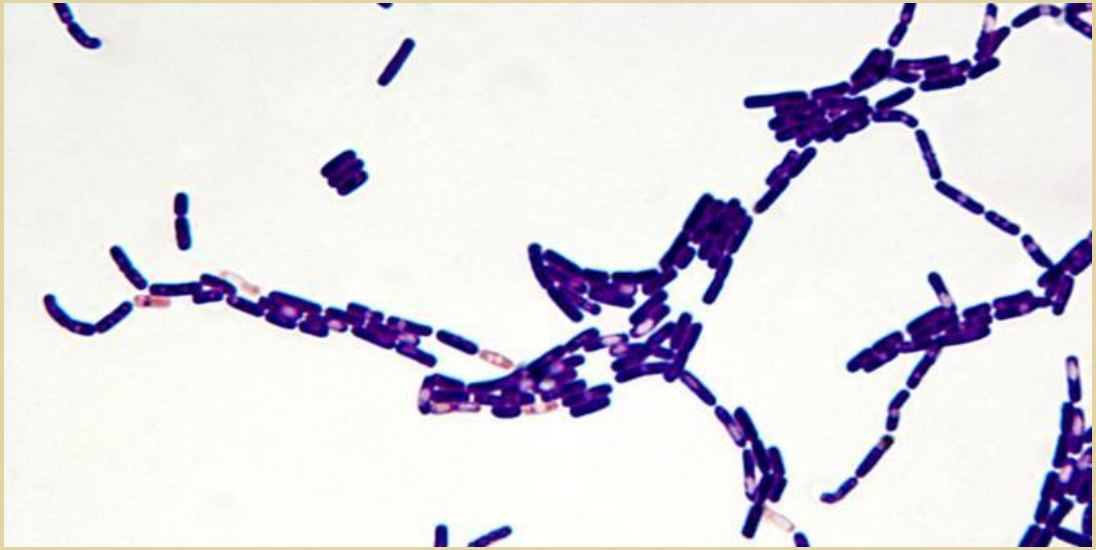
- Large, aerobic Gram positive rods.
- Exist in chains.
- **Growth Characteristics:**
- Saprophytic bacteria, prevalent in soil, water, air & on vegetation.
- Utilize carbon & nitrogen for source of energy.
- Spores resistant: withstand dry heat & chemical disinfectants, persist for years in dry earth.
- Animal products, hair, wool, bone, contaminated with anthrax spores. (sterilized by autoclaving).

1.Bacillus anthracis cause Anthrax

- Primarily a disease of herbivores (goat, sheep, cattle, horses).
- Humans infected incidentally by contact with infected animals, their products.
- **Bioterrorism In 2001**: outbreak of both inhalational & cutaneous anthrax in United States.
- Caused by sending spores of organism through mail.
- 18 cases, **5 died.**

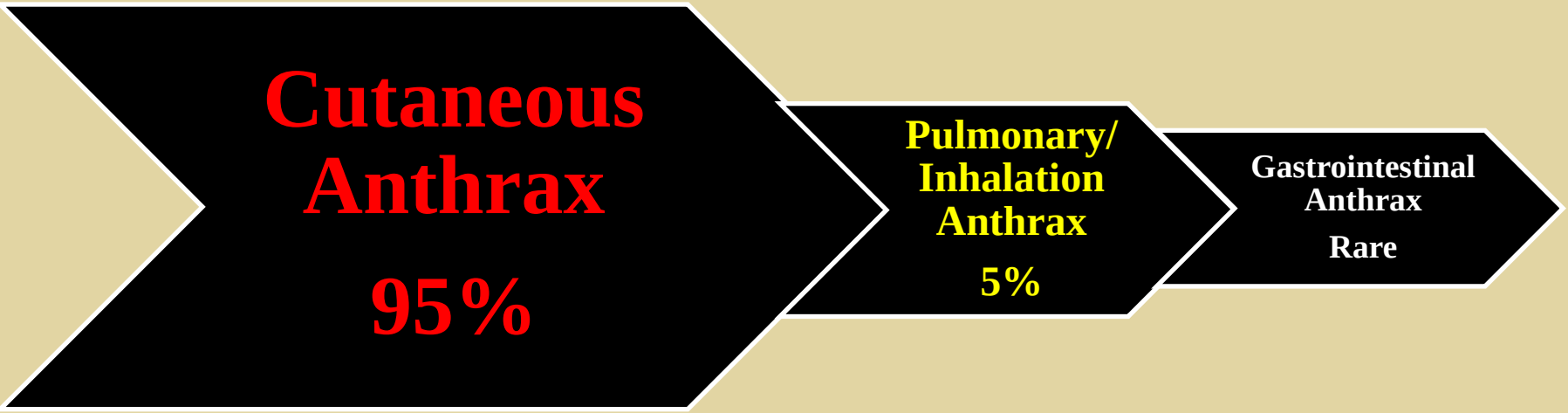
Important Properties

- Large Gram-positive rod with square ends, frequently found in chains.
- Anti-phagocytic capsule (**D-glutamate).**
- Non-motile.



Bacillus anthracis

Three main forms:



Bacillus anthracis

Organism	Disease	Transmission/ Predisposing Factor	Action of Toxin	Prevention
B. anthracis	**Anthrax**	1. **Cutaneous** anthrax: spores in soil enter wound. 2. **Pulmonary** anthrax: spores are inhaled into lung. 3. **Gatrointestinal** anthrax: by eating contaminated meat.	Exotoxin has two components: •**Edema factor:** Adenylate cyclase. •**Lethal factor:** Protease that inhibits cell growth	Vaccine contains protective antigen as immunogen.

Transmission

1. **Cutaneous anthrax (95%)**: Due to trauma to skin, allowing entry of spores on animal products (hides, bristles & wool).
2. **Pulmonary (inhalation)** anthrax (5%): When spores inhaled into lungs.
3. **Gastrointestinal** anthrax (Rare): Ingestion of contaminated meat.

Spores germinate in tissues at site of entry, growth of vegetative organisms leading to edema and congestion.

Bacilli spread via lymphatics to blood stream and multiply in blood and tissues.

Pathogenesis

Bacillus anthracis has two exotoxins

Edema factor

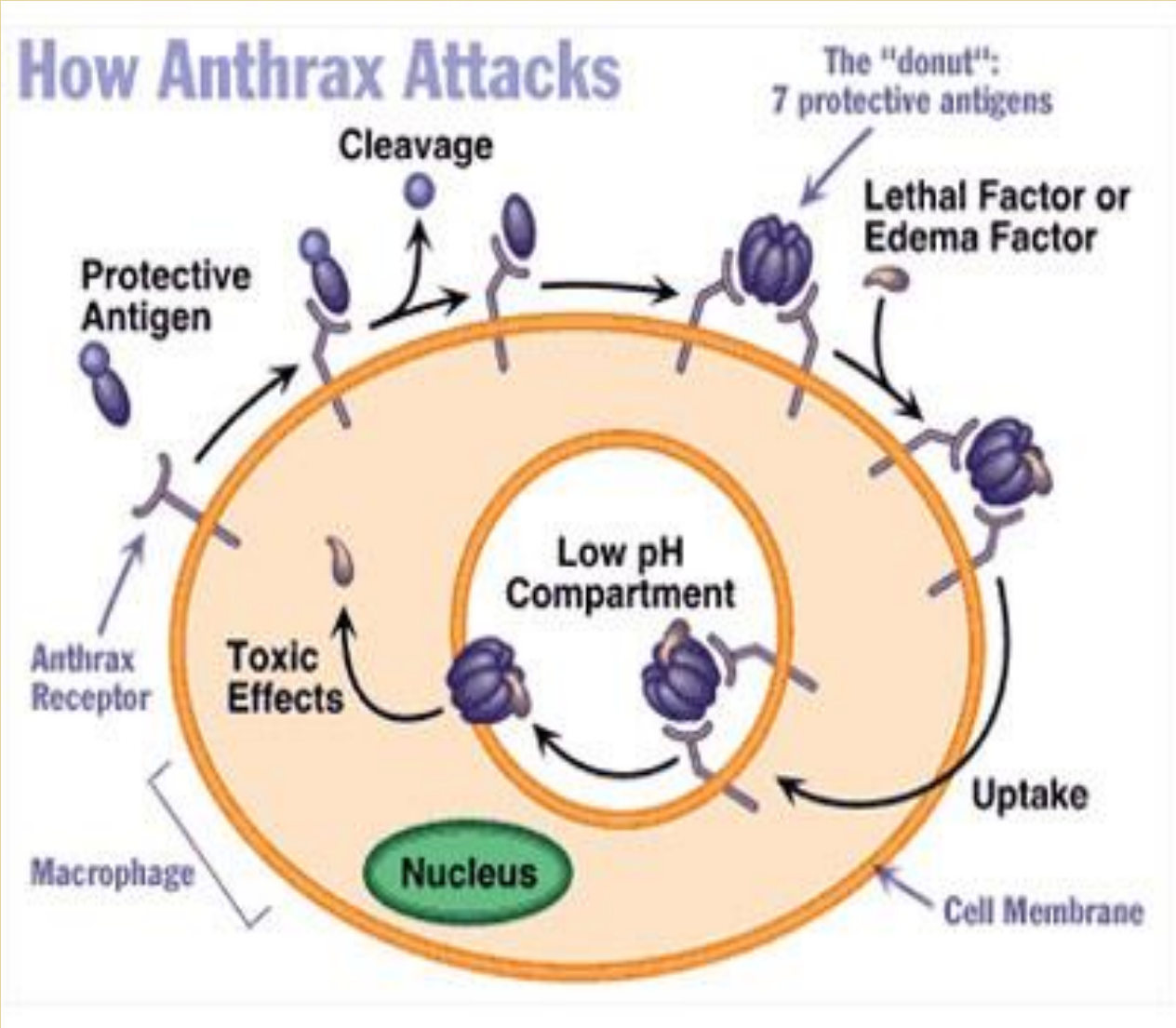
lethal factor

- Each consist of two proteins having A–B subunits.
- B (binding subunit) in each of two exotoxins: **Protective antigen:** forms pores in human cell membrane, allows edema factor and lethal factor to enter cell.
- A (active subunit): enzymatic activity.

Adenylate cyclase (Increases intracellular concentration of cyclic AMP, causing outpouring of fluid from cell into extracellular space causing edema.

Protease (cleaves phosphokinase that activates MAPK signal transduction pathway, controls growth of human cells.

Pathogenesis: Three exotoxins produced by *Bacillus anthracis:*



Protective antigen

- Forms pores in human cell membrane that allows edema factor & lethal factor to enter cell.

- Protective antigen refers to the fact that antibody against this protein protects against disease.

Edema factor

- **Adenylate cyclase: C**auses increase in intracellular concentration of cyclic AMP.

- Causes an outpouring of fluid from cell into extracellular space, manifests as edema.

Lethal factor

- Protease : cleaves phosphokinase that activates **mitogen-activated protein kinase (MAPK)** signal transduction pathway.

- This pathway controls growth of human cells, & cleavage of phosphokinase inhibits cell growth.

Pathology

- **In susceptible individuals:**
- Organims proliferate at site of entry
- Capsule remain intact
- Organisms surrounded by proteineceous material having leukocytes
- They disseminate & reach blood stream
- **In resistant animals:**
- Organims proliferate for few hours
- Massive accumulation of leukocytes
- Capsule break and disappear
- Organisms remain localized

Clinical Findings

- **Cutaneous anthrax:** Painless ulcer with a black eschar (crust, scab), local edema (**malignant pustule).** Untreated cases progress to bacteremia and death.
- **Pulmonary anthrax** (woolsorter's disease): influenza like symptoms, dry cough & substernal pressure, progressing to hemorrhagic mediastinitis, bloody pleural effusions, septic shock & death.
- **Gastrointestinal anthrax: V**omiting, abdominal pain & bloody diarrhea.

Pulmonary Anthrax

- **Inhalation anthrax: N**ot communicable from person-to-person. (Because it leaves lung so rapidly, it is not transmitted by respiratory route to others)

- After inhalation into lung, organism moves rapidly to mediastinal lymph nodes (hemorrhagic mediastinitis).

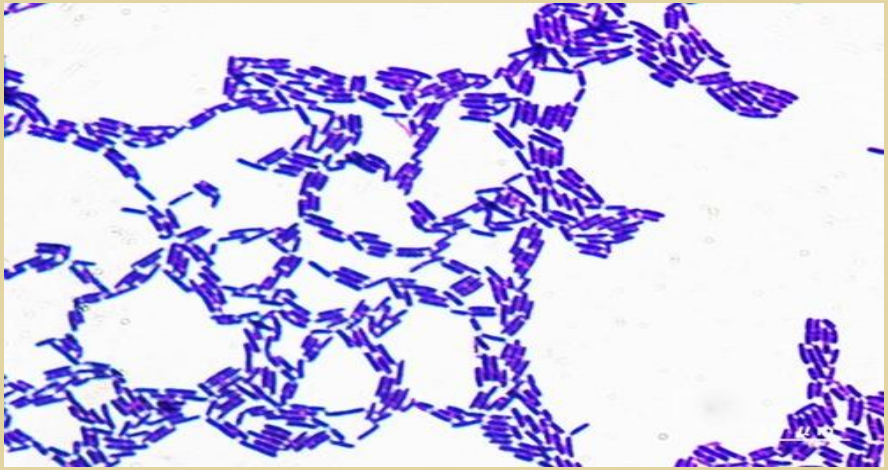
X- ray picture

- Classic features & x-ray picture of pneumonia not present.
- **Mediastinal widening** seen on chest x-ray.
- **Complications:** Hemorrhagic mediastinitis, hemorrhagic meningitis.

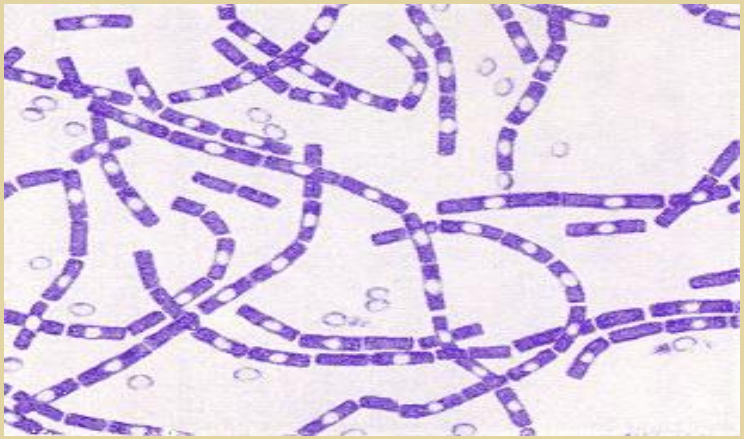
Laboratory Diagnosis

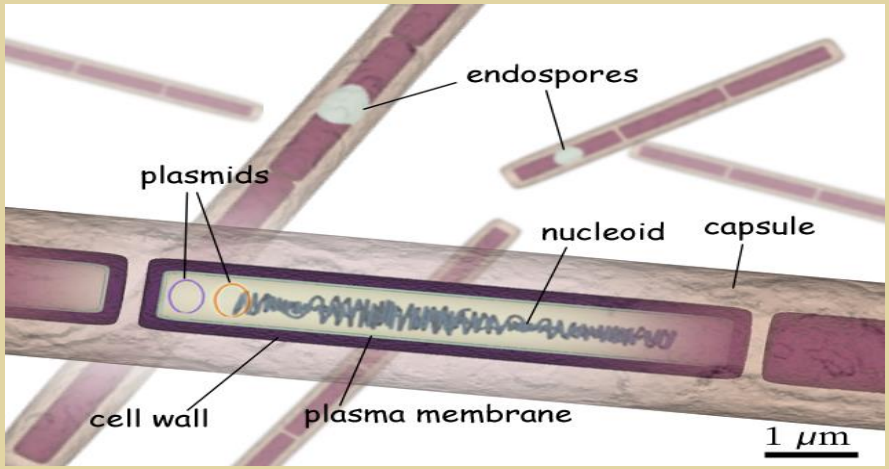
Specimens: Pus or fluid from local lesion, Blood, Sputum

- **Gram Smears:**
- 1x3-4μm in size.
- Square ends.



- Gram positive rods arranged in long chains.





- Spores located in centre of non-motile bacteria.

- **Culture: Blood Agar:** Non-hemolytic gray to white colonies with rough texture and ground glass appearance on blood agar.

- **Motility test:** Non-motile.
- **ELISA** to measure antibodies against edema and lethal factors
- **Polymerase chain reaction (PCR)** based assays.
- **Immunoflorescence techniques:** identifies organisms in dried smears.

Treatment

- Ciprofloxacin.
- Doxycycline.
- No resistant strains isolated clinically.

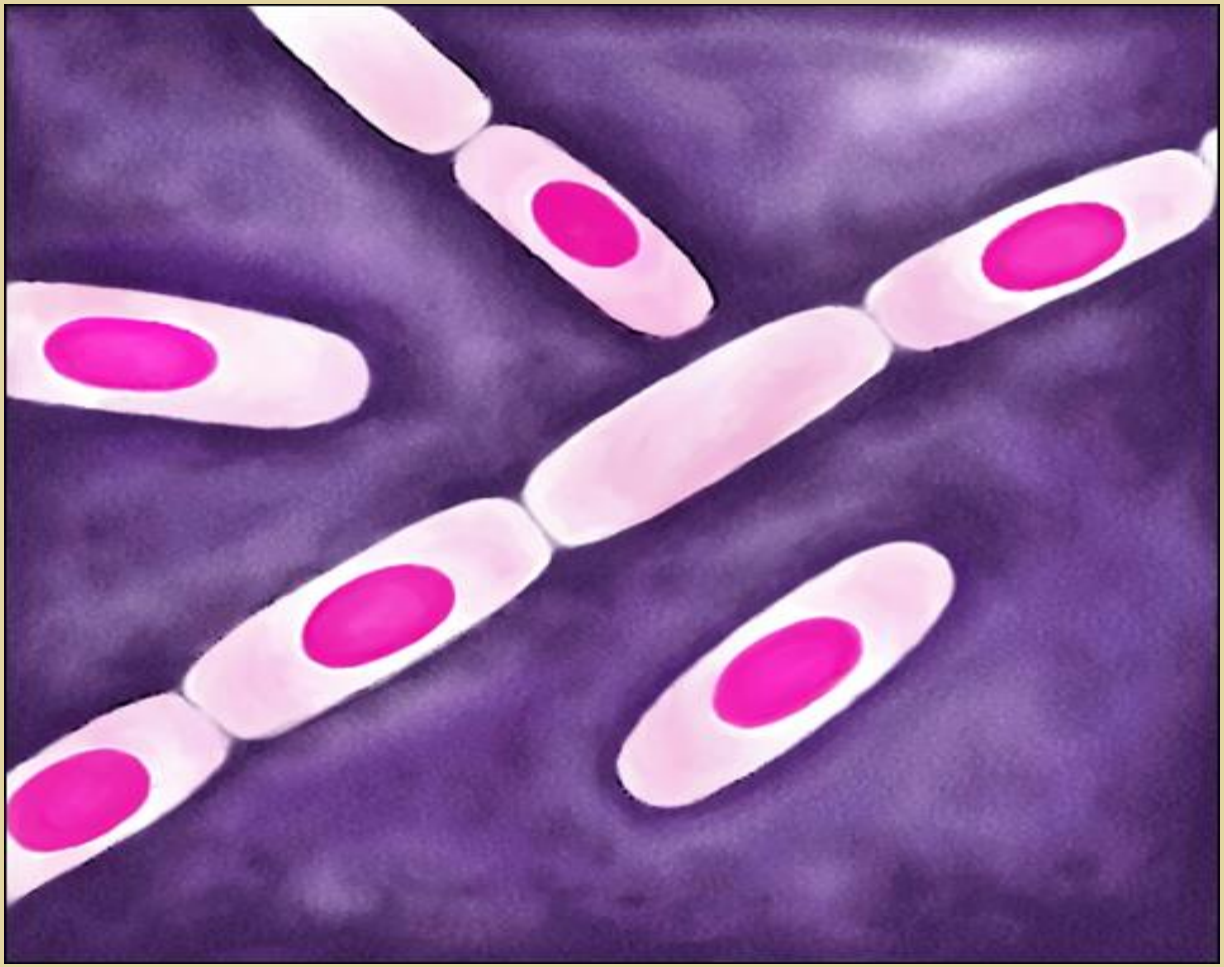
- Ciprofloxacin or doxycycline used as prophylaxis for 4 weeks.

Prevention

- Control measures include:

1. Disposal of animal carcasses by burning or deep burrial.
2. Decontamination of animal products
3. Protective clothing & gloves for handling infected material.
4. Immunization of domestic animals & high risk population with cell-free vaccine containing purified protective antigen as immunogen

Bacillus cereus



Important Properties

- *B.cereus* grows on food & produce enterotoxin causing food poisining.
- **Emetic type:** Spores on grains such as rice survive steaming and rapid frying.
- Spores germinate in **reheated fried rice**.
- **Diarrheal type:** Associated with meat dishes and sauces.

Transmission:

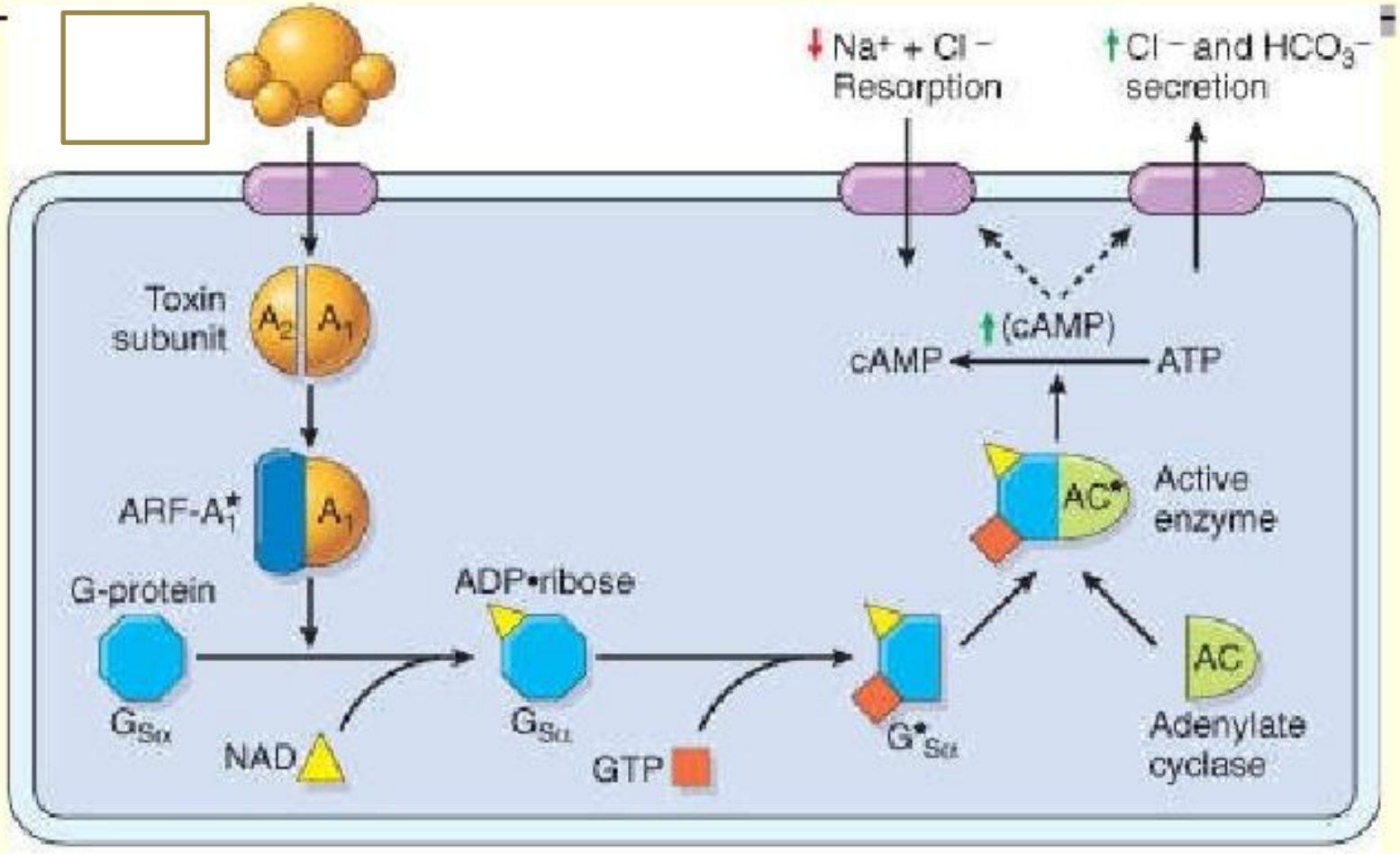
Portal of entry Gastrointestinal tract

Two enterotoxins

Enterotoxins same as cholera toxin; i.e. stimulation of adenylate cyclase, leading to increased concentration of cyclic adenosine monophosphate (AMP) within enterocyte.

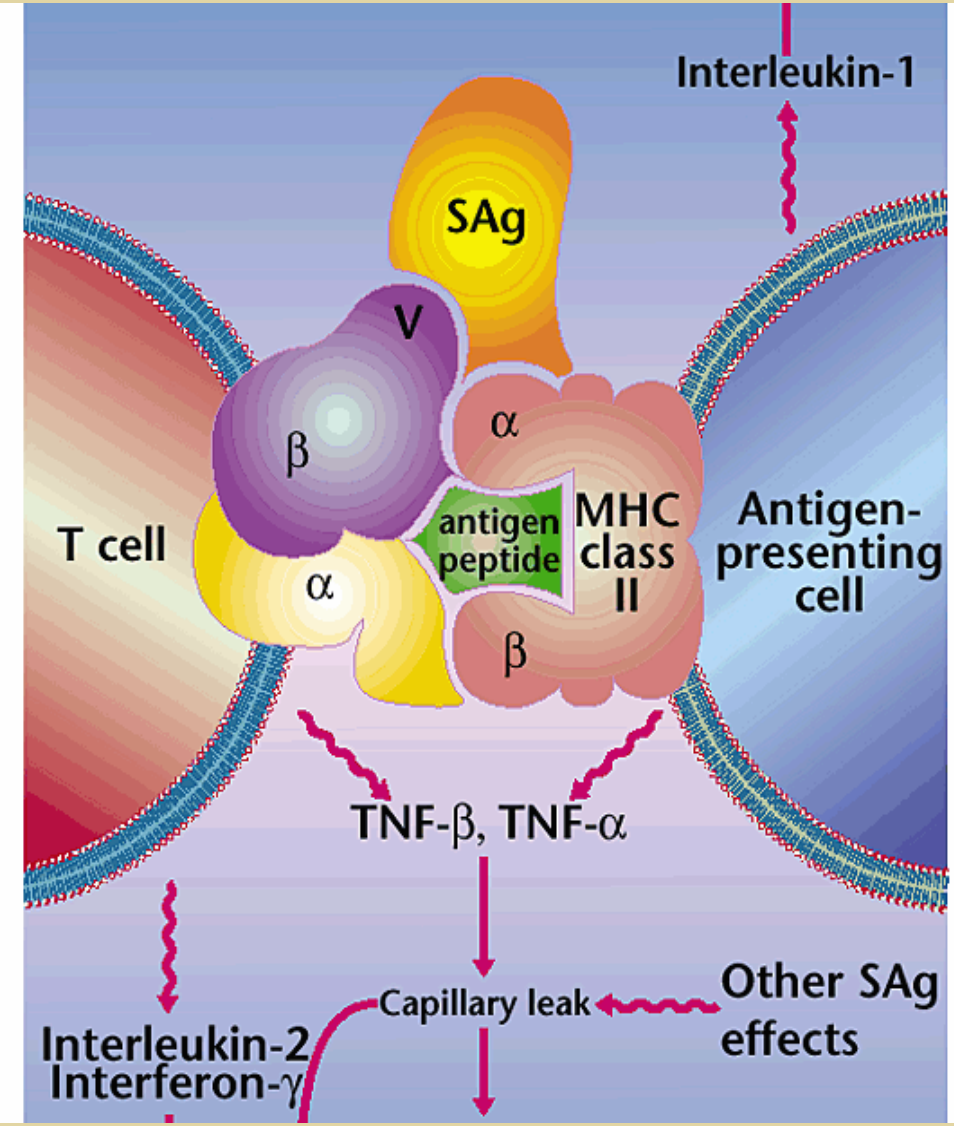
Enterotoxin similar to *Staphylococcal* enterotoxin, i.e. act as superantigen.

Enterotoxins same as cholera toxin



Enterotoxin act as Super antigens

- Binds directly to class II major histocompatibility (MHC) proteins on surface of antigen-presenting cells (macrophages) without intracellular processing.
- This complex interacts with T-cell receptor of many helper T cells



Bacillus cereus

Organism	Disease	Transmission/ Predisposing Factor	Action of Toxin	Prevention
B. cereus	Food poisoning	Spores germinate in reheated rice, then bacteria produce exotoxins, which are ingested.	Two exotoxins (enterotoxins): 1. Similar to cholera toxin, increases cyclic AMP. 2. Similar to staphylococcal enterotoxin (superantigen).	No vaccine

Clinical Features

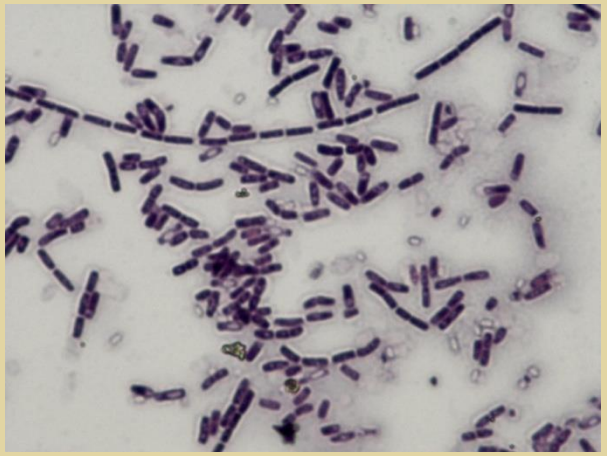
- **Two syndromes:**

- **Emetic type:** Short incubation period (4 hours)
- Nausea, vomiting, abdominal cramps.
- Self limiting, recovery in 24h.

- **Diarrheal type:** long incubation period,18-24h
- Profuse, watery, non-bloody diarrhea, resembling *Clostridial* gastroenteritis.

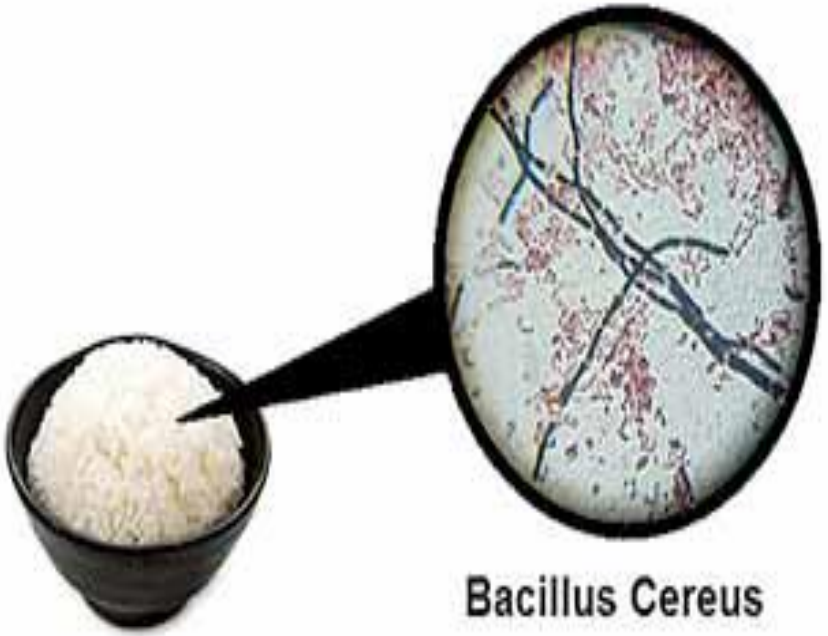
Laboratory Diagnosis, Treatment & Prevention

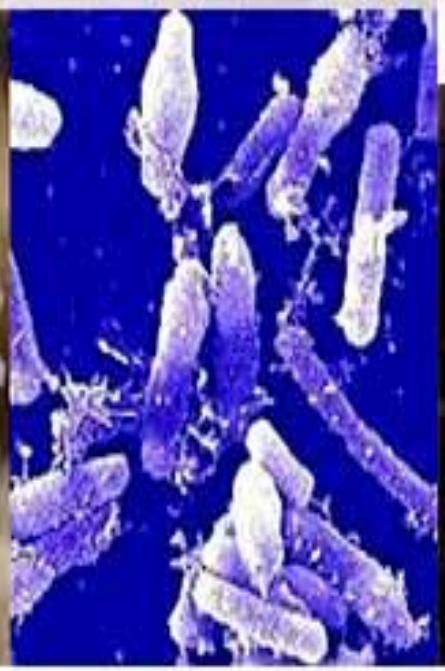
- Gram Staining: Gram positive rods



- No laboratory diagnosis.
- Only symptomatic treatment.
- Rice should not be kept warm for long periods.


Bacillus
cereus


Bacillus Cereus



If you are not willing to learn,
No one can help you!
If you are determined to learn,
No one can stop you!