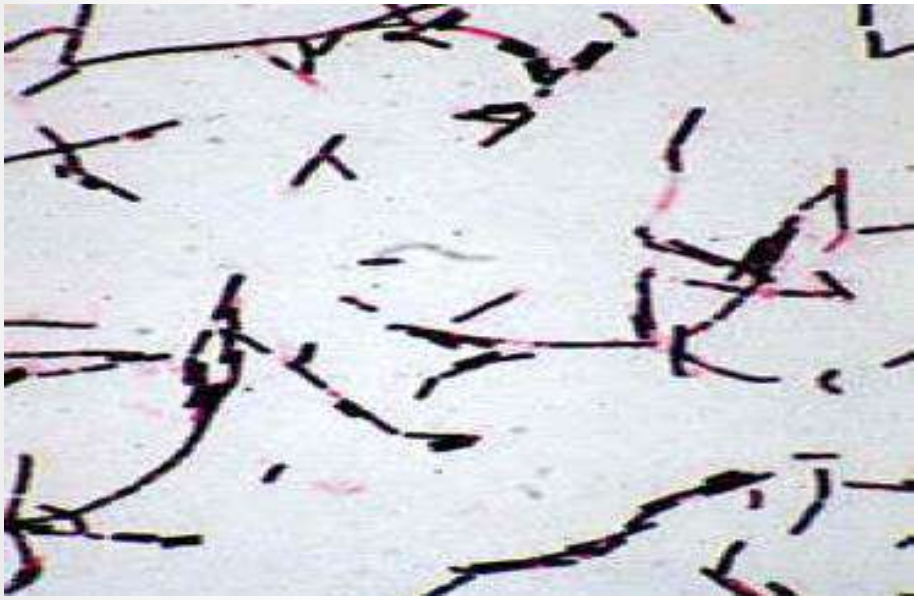
Clostridium

General features

- Large, Gram-positive rods
- Endospores produced
- Anaerobic, catalase- and oxidase-negative
- Motile (except ***C, perfringens)***
- Enriched media required for growth
- Colonies of C. ***perfringens*** surrounded by zones of double haemolysis
- Present in soil, in alimentary tracts of animals and in faeces
- Pathogens can be grouped according to the mode and sites of action of their potent exotoxins:
- -neurotoxic clostridia: Cl.tetani, Cl. botulinum
- -histotoxic clostridia : Cl. Chauvoei, C. septicum, Cl. novyi, A& B, Cl.perfringens A, Cl.hemolytiocum
- Enteropathogenic and enterotoxaemia producing Clostridia: Cl.perfringens type A-E
- Produce diverse forms of disease in many animal species

Genus *Clostridium*



- Consists of large Gram positive rod shaped.
- Arranged in pairs or short chains with rounded pointed ends.
- Have a variable response on gram stain
- All species form endospores and have a fermentative type of metabolism

Genus *Clostridium*

- Live in all of the anaerobic habitats :
  - soil
  - aquatic sediments
  - intestinal tracts of animals
- Ferment a wide variety of organic compounds
- Produce end products: butyric acid
  - acetic acid
  - butanol
  - acetone
- Most clostridia are saprophytes and a few are pathogenic.

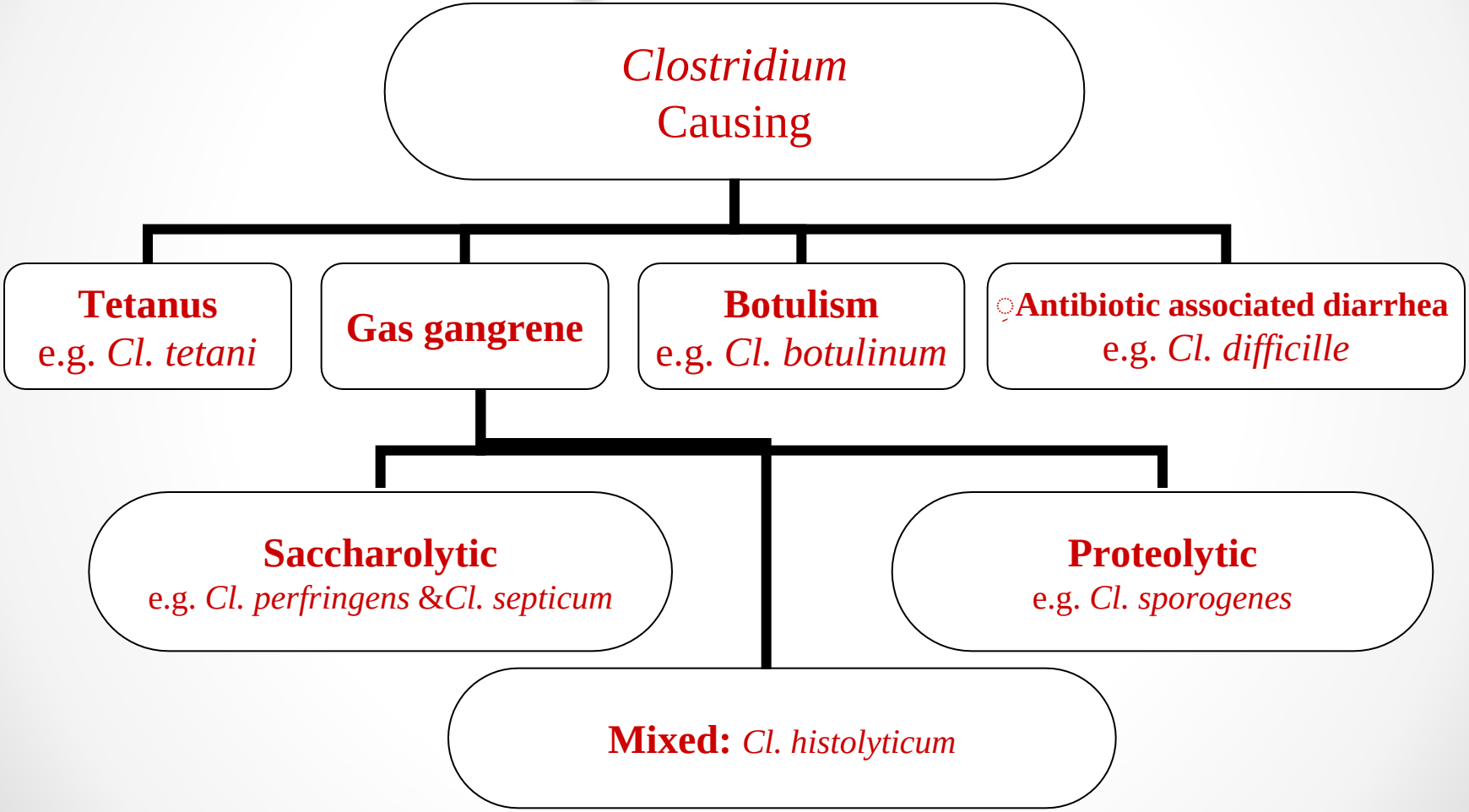
Genus *Clostridium*

- Have three important qualities:
  1. Multiply only in the absence of oxygen
  2. Have the ability to survive adverse conditions
  3. Release potent toxins during process of multiplying

Spores

- *Clostridium* form endospores under **adverse environmental conditions**
- Spores are a **survival** mechanism
- Spores are characterized on the basis of position, size and shape
- Most *Clostridium* spp., including ***C. perfringens*** **and *C. botulinum*, have ovoid subterminal (OST) spores**
- ***C. tetani*** **have round terminal (RT) spores**

Clostridia of medical importance



Nomenclature changes of some ***Clostridium*** **spccics**

Present name	Former name
Clostridium perfringens	*Clostridium welchii*
Ciostridium argentiense	*Clostridium botulinum type G*
Clostridium haemolyticum	*Clostridium novyi ty pe D*
Clostridium novyi	*Clostridium oedematiens*
Clostridium piliforme	*Bacillus piliformis*

Table 48. Summary of the hosts and diseases caused by pathogenic clostridia.

Clostridium species	*Hosts*	*Diseases*
NEUROTOXIC CLOSTRIDIA		
Clostridium tetani	Horses, ruminants, humans and other animals	Tetanus
Clostridium botulinum (types A–F)	Many animal species and man	Botulism
Clostridium argentinense (*C. botulinum* type G)	Humans (Argentina)	Botulism
HISTOTOXIC CLOSTRIDIA		
Clostridium chauvoei	Cattle, sheep, (pigs)	Blackleg (Black quarter)
Clostridium septicum	Cattle, sheep and pigs	Malignant oedema
	Sheep	Braxy
	Chickens	Necrotic dermatitis
Clostridium novyi		
type A	Sheep	Big-head of rams
	Cattle and sheep	Gas gangrene
type B	Sheep, (cattle)	Black disease (necrotic hepatitis)
type C	Water buffalo	Osteomyelitis reported
Clostridium haemolyticum (*C. novyi* type D)	Cattle, (sheep)	Bacillary haemoglobinuria
Clostridium sordellii	Cattle, sheep, horses	Gas-gangrene
Clostridium colinum	Game birds, young chickens and turkey poults	Quail disease (ulcerative enteritis)
ENTEROTOXAEMIAS		
Clostridium perfringens		
type A	Humans	Food poisoning, gas gangrene
	Lambs	Enterotoxaemic jaundice
type B	Lambs (under 3 weeks old)	Lamb dysentery
	Neonatal calves and foals	Enterotoxaemia
type C	Piglets, lambs, calves, foals	Haemorrhagic enterotoxaemia
	Adult sheep	Struck
	Chickens	Necrotic enteritis
type D	Sheep (all ages except neonates) (goats, calves)	Pulpy kidney disease
type E	Calves and lambs (rare)	Enterotoxaemia
CLOSTRIDIA ASSOCIATED WITH ANTIBIOTIC-INDUCED DISEASE		
Clostridium spiroforme	Rabbits	Possible role in mucoid enteritis
	Rabbits and guinea-pigs	Spontaneous and antibiotic-induced diarrhoea
	Foals and pigs	Enterocolitis (natural)
Clostridium difficile	Humans, hamsters, rabbits, guinea-pigs	Antibiotic-induced enterocolitis
	Dogs, foals, pigs, laboratory animals	Naturally occurring diarrhoea

Table 49. Biochemical reactions of the clostridia pathogenic to animals.

Clostridium species		Egg Yolk agar: Lecithinase	Egg Yolk agar: Lipase	Hydrolysis of gelatin	Digestion of casein	Indole production	Acid from: Glucose	Acid from: Lactose	Acid from: Sucrose	Acid from: Maltose	Additional characteristics
C. tetani		–	–	+	–	v	–	–	–	–	Terminal, spherical endospores
C. botulinum	I	–	+	+	+	–	+	–	–	+	Toxin types A, B and F
	II	–	+	+	–	–	+	–	–	+	Toxin types B, E and F
	III	v	+	+	–	v	+	–	–	v	Toxin types C and D
	IV	–	–	+	+	–	–	•	–	•	Toxin type G
C. chauvoei		–	–	+	–	–	+	+	+	+	
C. septicum		–	–	+	+	–	+	+	–	+	
C. novyi	A	+	+	+	–	–	+	–	–	+	
	B	+	–	+	+	v	+	–	–	+	
	C	–	–	+	–	+	+	–	–	•	No toxin produced
C. haemolyticum		+	–	+	+	+	+	–	–	–	
C. sordellii		+	–	+	+	+	+	–	–	+	Urease-positive
C. colinum		–	–	–	–	–	+	–	+	+	
C. perfringens		+	–	+	+	–	+	+	+	+	Non-motile. 'Stormy-clot' in litmus milk
C. spiroforme		–	–	–	–	–	+	•	+	•	Spiral and curved
C. difficile		–	–	+	–	–	+	–	–	–	

Table 50. *Clostridium botulinum:* toxins, susceptible animals, sources of toxin and geographical distribution.

C. botulinum types	Toxin(s) produced	Most susceptible animals	Sources of toxin	Geographical distribution
A	A	Humans, chickens, mink	Vegetables, fruits, meat, fish	Canada, Western USA, and former USSR
B	B	Humans (cattle, horses, chicken)	Meat and meat products (often from pigs), vegetables, fish	Northern and Central Europe Canada, Eastern USA and former USSR
Cα	C_1 (C_2)	Waterfowl	**Limberneck** in long-necked birds. Invertebrate carcases, rotting vegetation and material on refuse dumps	Western USA, Canada, South America, Europe, Australia, New Zealand and Japan
Cβ	C_2, D (C_1)	Cattle, horses, mink, dogs, (humans)	**Forage poisoning** Carcases, baled silage, chicken manure as feed supplement, and spoiled feeds	South Africa, Australia, Europe, USA
D	C_2,D	Cattle, sheep, (horses, humans)	**Lamsiekte** Eating contaminated bones and carcases of small mammals (phosphorus-deficiency)	South Africa, former USSR, southwest USA and France
E	E	Humans, farmed fish	Humans: fish, fish-products and other foods Young fish: sludge in earth-bottomed ponds	Northern Europe, North America, Japan and former USSR
F	F	Humans	Meat (liver paste), fish	Northern Europe, USA and former USSR
C. argentinense G	G	Humans	Soil	Argentina

Table 51. Comparison of the toxins of *Clostridium tetani* and *C. botulinum*.

	Clostridium tetani	*Clostridium botulinum*
Site of toxin production	Wounds	Carcases, decaying vegetation and occasionally wounds and intestine
Mode of action of toxins	Centrally by blocking synaptic inhibition	Peripherally by blocking neuromuscular transmission
Type of paralysis	Spastic paralysis	Flaccid paralysis
Antigenic types of toxin	Tetanospasmin (one antigenic type)	Eight different toxins produced by types A–G

Table 52. Summary of the clostridia commonly causing gas gangrene.

Clostridium species	*Main Hosts*	*Disease*	*Route of entry*	*Clinical and postmortem signs*
C. chauvoei	Calves, 3–24 months old	Blackleg (quarter evil, or black quarter)	Endogenous, from spores in muscles	Usually sudden death, especially if heart muscle is involved. Fever, swelling of muscle masses of hind quarters. Muscles dry and spongy with small gas bubbles. Sweet rancid odour and muscles are dark red to black. Crepitation can be felt
	Sheep		Exogenous, through wounds	
C. septicum	Cattle, sheep, and pigs: all ages affected	Malignant oedema	Exogenous, through wounds	Fever, soft swelling around wound and spreading to muscles. Swelling is oedematous and wet with much exudate and gas. Muscles dark red to black colour
	Sheep	Braxy	Endogenous, from spores in abomasum	Caused by large volume of frozen food in rumen damaging localized area in abomasum. Replication of vegetative cells and toxin produced
***C. novyi* type A**	Young rams	Big-head	Wounds from fighting	Oedematous swelling over head, face and neck
	Cattle, sheep	Gas-gangrene	Wounds	Lesions similar to those of malignant oedema. Sudden death can occur
C. sordellii	Cattle, sheep	Gas-gangrene	Wounds	Similar syndrome to malignant oedema
***C. perfringens* type A**	Humans, dogs	Gas-gangrene	Wounds (road accidents)	Oedema, tissue necrosis and gangrene. Caused by the alpha toxin.

Table 53. Microscopic and colonial appearance of the gas-gangrene clostridia.

Clostridium species	Gram-stained impression smears	Colonial appearance
C. chauvoei	Oval, subterminal or central spores with typical citron (lemon-shaped) forms. Cells 0.6–0.8 x 3–8 μm	Colonies with large zone of clear haemolysis
C. septicum	Characteristic long filamentous forms. Spores oval and subterminal. Individual cells 0.6–0.8 x 3–8 μm, but filamentous forms are much longer	Swarming, spreading, haemolytic growth on normal agar. On 'stiff' agar the colonies are irregular with a rhizoid edge. Some strains produce smooth, round colonies
***C. novyi* type A**	Large Gram-positive rods with oval to cylindrical, subterminal spores. There is little or no swelling of the mother cell. Cells are 0.8–1.0 x 3–10 μm	Large, irregular colonies with a rhizoidal edge and a large zone of clear haemolysis
C. sordellii	Gram-positive rods with cylindrical spores that do not bulge the mother cell	Irregular; translucent colonies on 'stiff' agar, which become white on ageing
C. perfringens	Short, fat, Gram-positive rods that do not commonly produce spores. The spores, if present, are oval, subterminal and bulge the mother cell. Chains of cells can occur. Cells are 0.6–0.8 x 2–4 μm	Smooth, round, glistening colonies surrounded by 'target' or double-haemolysis (theta toxin giving a clear zone and partial haemolysis given by the alpha toxin)

Table 55. The major toxins of *Clostridium perfringens*.

Clostridium perfringens	*Major toxin*			
Type	*Alpha*	*Beta*	*Epsilon*	*Iota*
A	+	−	−	−
B	+	+	(+)	−
C	+	+	−	−
D	+	−	+	−
E	+	−	−	+

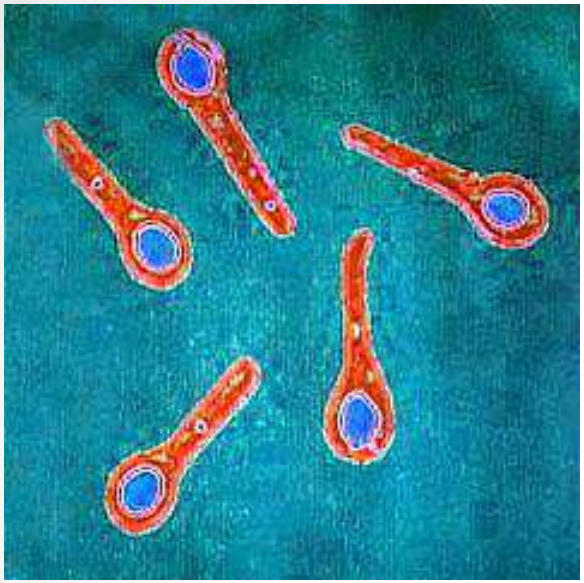
Table 56. The clostridial enterotoxaemias.

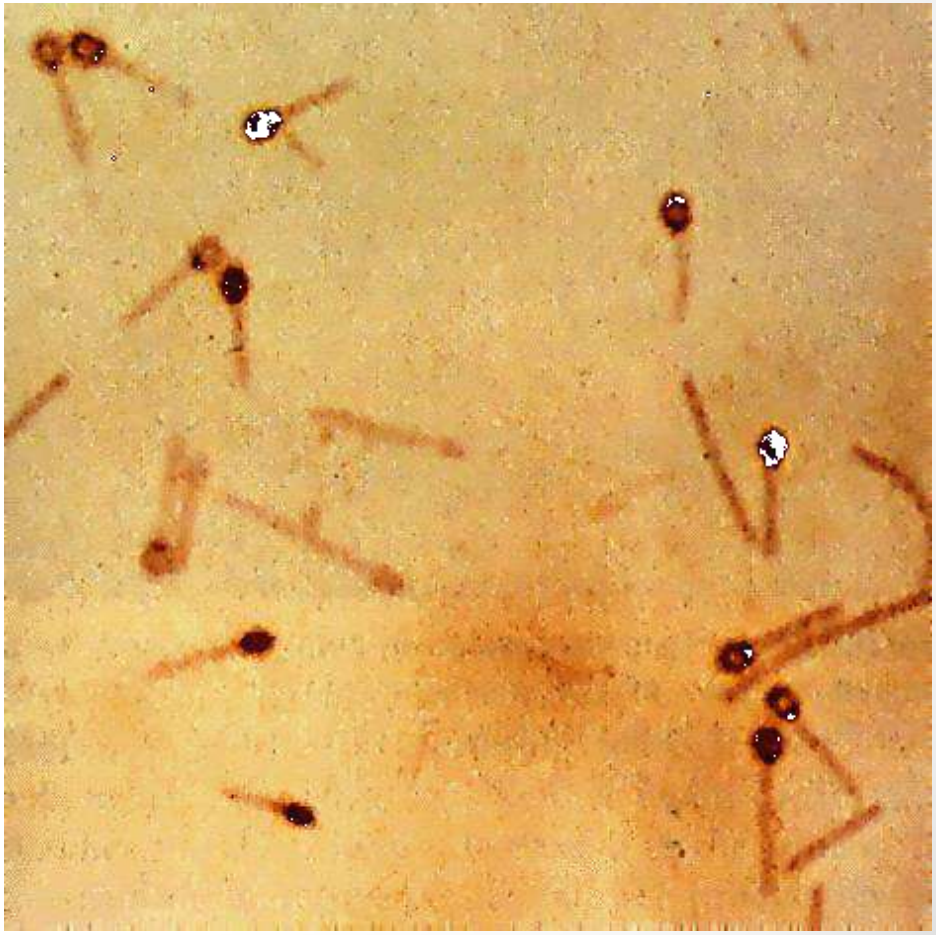
Clostridium perfringens type	Major toxins	Hosts	Disease	Clinical and postmortem signs
A	enterotoxin	Humans	Food poisoning	Sudden onset, diarrhoea, abdominal pain and nausea, but vomiting is uncommon. Short course and rarely fatal
	alpha	Lambs	Enterotoxaemic jaundice	Occurs in California and Oregon in the spring. Depression, anaemia, icterus, haemoglobinuria and lambs die within 6–12 hours of first signs. Known as 'the yellows' or 'yellow lamb disease'
B	beta (epsilon) alpha	Lambs under 3 weeks old	Lamb dysentery	A haemorrhagic and rapidly fatal enterotoxaemia. Lambs are often found dead
		Calves and foals		Enterotoxaemia, not common
C	beta alpha	Piglets 1–3 days old	Haemorrhagic enterotoxaemia (clostridial enteritis)	Dysentery, collapse and death. Small intestine is dark red and has gas bubbles in mucosa. Lumen is full of bloody fluid
		Lambs, foals and calves		
		Broiler chicken 2–12 weeks old	Necrotic enteritis (types A and C)	Depression, diarrhoea, death in a few hours. Mortality 2–50%. Mucosa of small intestine has a brown pseudomembrane. Most common in deep-litter units
		Adult sheep and goats	Struck	Sudden deaths due to an enterotoxaemia
D	epsilon alpha	Sheep all ages except neonates. Rare cases in calves and goats	Pulpy kidney disease	Oedema of brain, glysosuria, sudden deaths. Excess fluid in body cavities, focal symmetrical encephalomalacia in some cases. Occurs in well-grown lambs
E	iota alpha	Calves and lambs	Enterotoxaemia	Pathogenicity unclear

Clostridium tetani

- Causative agent of tetanus
- Found in soil, intestinal tracts, and feces of animals
- Potent neurotoxin (tetanus toxin)
- Result : small puncture wound
  becomes contaminated with C. tetani spores
  migrates along neural paths from local wound

C. tetani





Toxins

- *Cl. tetani* produces two types of toxins:
  - **Tetanolysin**, which causes lysis of RBCs
  - **Tetanospasmin** is neurotoxin and essential pathogenic product
    - Tetanospasmin is toxic to humans and various animals when injected parenterally, but it is not toxic by the oral route
    - Tetanospasmin which causes increasing excitability of spinal cord neurons and muscle spasm

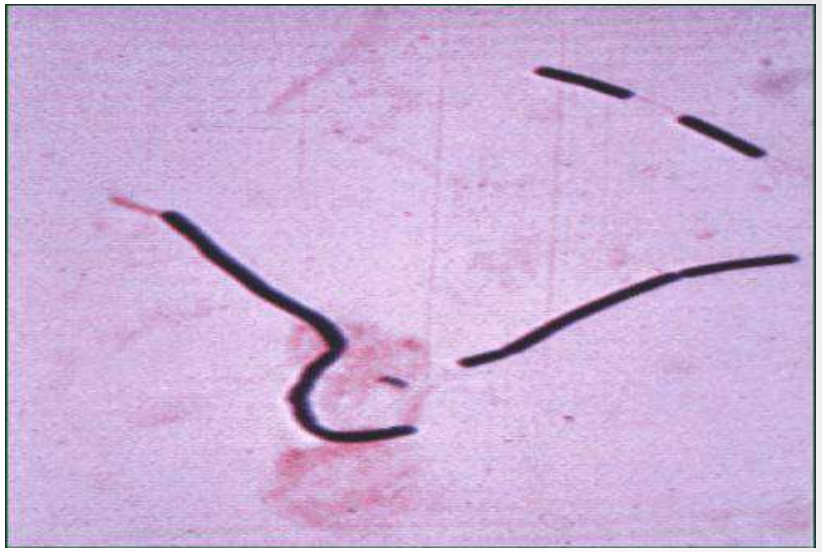
Laboratory Diagnosis of Tetanus

- The diagnosis of tetanus depends primarily upon the clinical manifestation of tetanus including muscle spasm and rigidity.
- **Specimen:** Wound exudates using capillary tube
- **Culture:**
  - On blood agar and incubated anaerobically
  - Growth appears as a fine spreading film.
- Gram stain is a good method for identifying *Clostridium*
  - *Cl. tetani* is Gram positive rod motile with a round terminal spore giving a drumstick appearance

Clostridium botulinum

Used to be a major problem

Spores are not killed by boiling, survive inadequate pressure sterilization



Spores germinate in food, vegetative cells produce toxins.

Toxins are considered the most lethal to man

8 types of botulinum toxin designated by letters:

Humans- A, B , E and F

Cattle and poultry : B,

C. botulinum — **agent of botulism,** a rare, but severe (lethal) neuroparalytic disease

Morphology and Physiology

- heterogeneous group of fastidious, strictly anaerobic bacilli
- **motile by peritrichous flagella**
- heat-resistant **spores (ovoid, subterminal)**
- proteolytic and non-proteolytic

Antigenic Structure

- species divided into **four groups (I-IV)** based on type of toxin produced and proteolytic activity
- **seven antigenically distinct botulinum toxins (types A to G)**
- somatic antigens - heat stable and heat labile; spore antigens - more specific

Pathogenicity Determinants

- **lethal foodbome intoxication with toxin types A,B,E,or F**; shorter incubation period, poor prognosis
- phage-mediated, systemic-acting A-B neurotoxin (botulinum toxin = botulin) released at cell lysis
  - **Mode of Action** - one of most extremely potent neurotoxins known
    (1 ng of purified toxin contains about 200,000 minimal lethal doses (MLDs) for a 20g mouse)
    - **A-B toxin ingested, binds specific receptors on peripheral cholinergic nerve endings (neuromuscular junctions) where it blocks release of presynaptic acetylcholine (excitatory neurotransmitter) blocking muscle stimulation & resulting in flaccid paralysis**
    - **Early**: nausea, vomiting, weakness, lassitude (lack of energy), dizziness, constipation
    - **Later**: double vision, difficulty in swallowing and speaking
    - Final: death due to respiratory paralysis

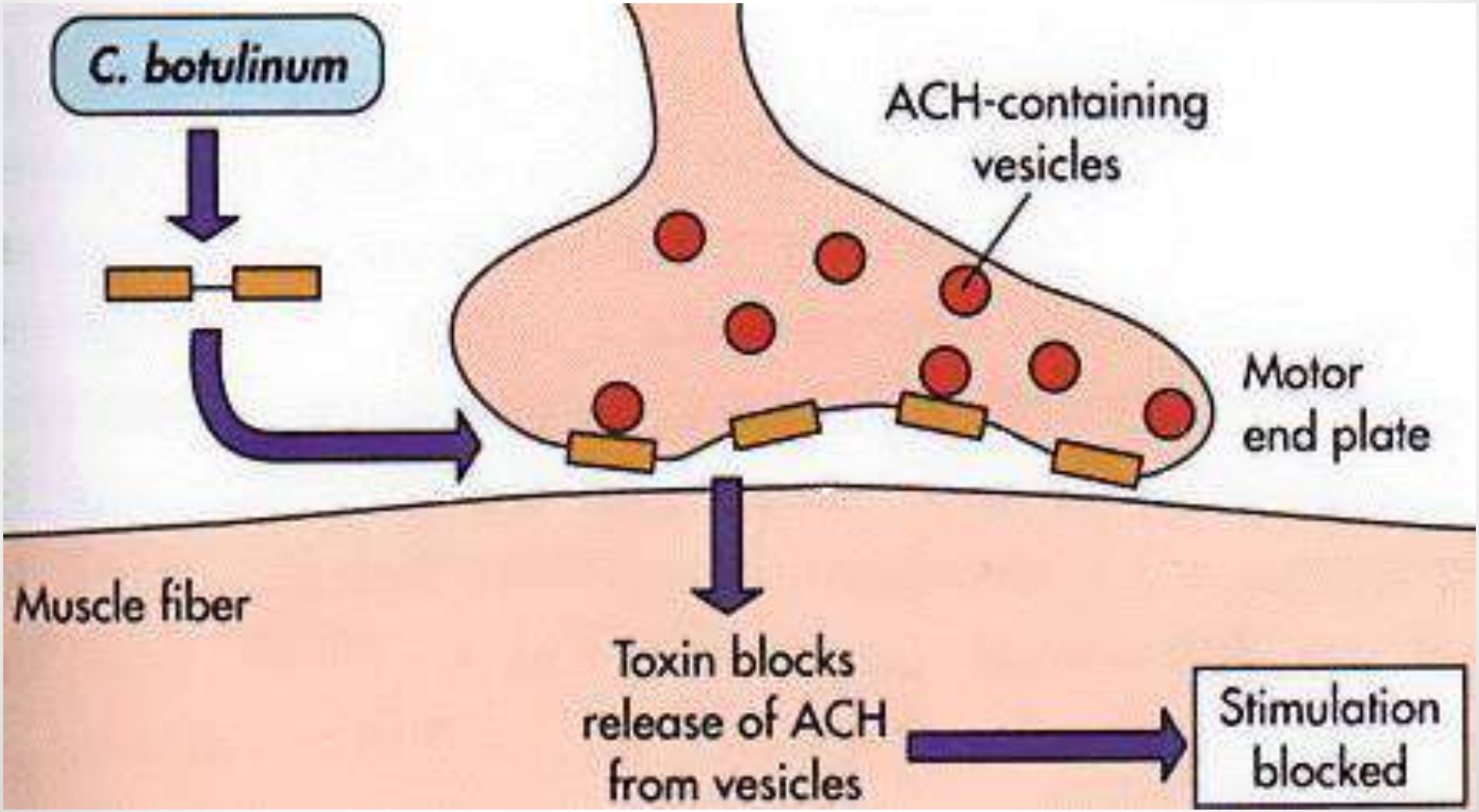
Lab Identification

- microscopic detection or Cx (culture) are often unsuccessful (few organisms and slow growing)
- toxin detected and typed in lab via toxicity and antitoxin neutralization tests in mice or by ELISA

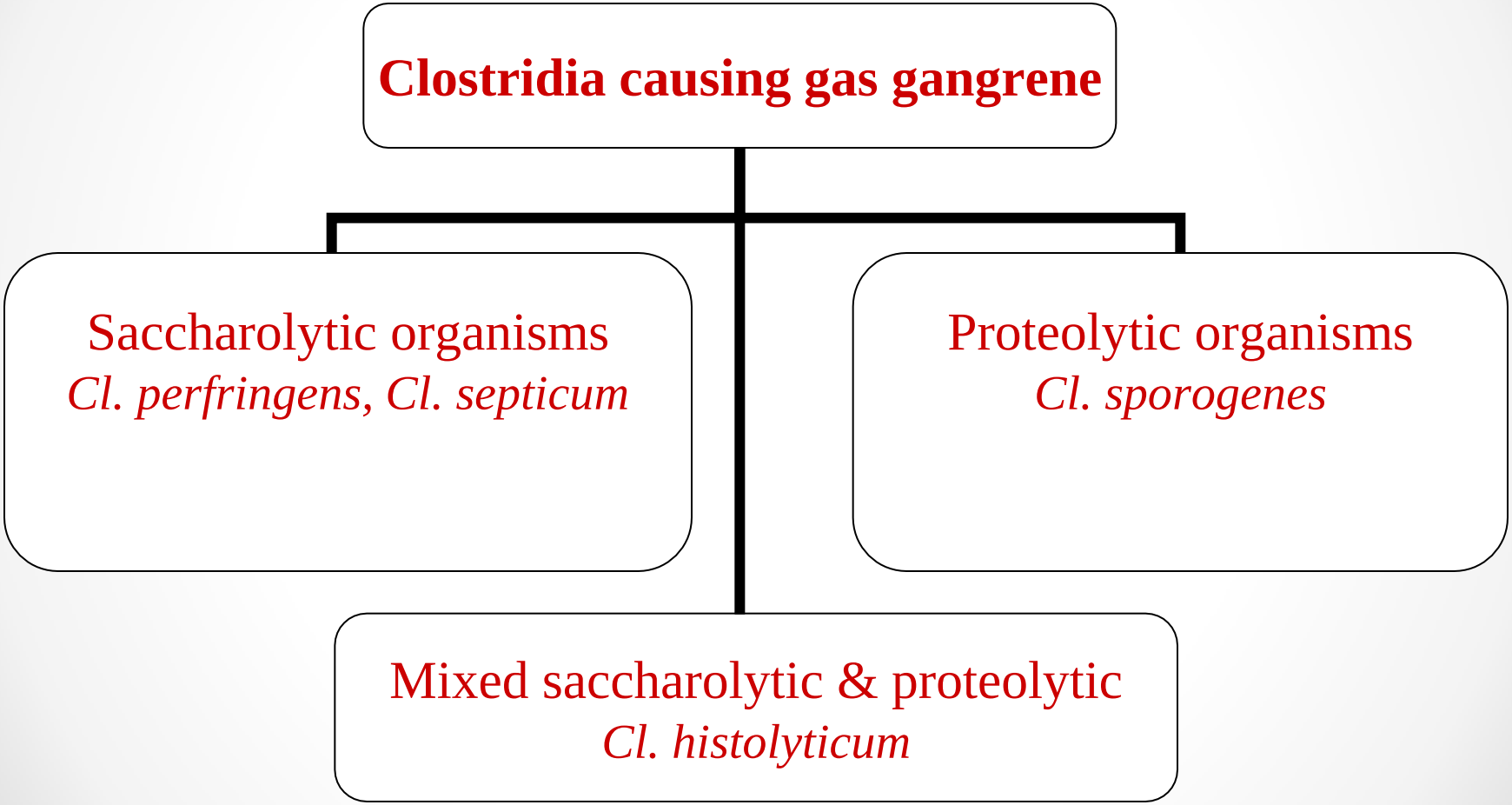
Diagnosis/Treatment/Prevention

- **crucial to rapidly diagnose** (symptoms often confusing); note the type of botulinum toxin involved
- Tx (treatment) should be administered as quickly as possible on basis of clinical Dx (diagnosis)
  - **ventilatory support & trivalent (A, B, E) antitoxin (polyvalent) binds free toxin in bloodstream**
  - administer **gastric lavage & metronidazole or penicillin eliminates organisms from GI tract**
  - **care in home canning** and in heating of home-canned food; toxoid is available

Mechanism of Action of Botulinum Toxin



Clostridium Causing Gas Gangrene



Clostridium perfringens

- Large Gram-positive bacilli with stubby ends
- **Capsulated**
- **Non motile (*Cl. tetani* is motile)**
- Anaerobic
- Grown quickly on selective media
- **Can be identified by** Nagler reaction

Clostridium perfringens — histotoxic or enterotoxigenic infections

Morphology and Physiology

- **large, rectangular bacilli** (rod) staining **gram-positive**
- **spores rarely seen** *in vitro* or in clinical specimens (ovoid, subterminal)
- **non-motile,** but **rapid spreading growth** on blood agar mimics growth of motile organisms
- aerotolerant, especially on media supplemented with blood
- grow at temperature of 20-50°C (optimum 45°C) and pH of 5.5-8.0

Pathogenicity Determinants (note that toxins include both cytolytic enzymes and bipartite exotoxins)

- four **major lethal toxins** (alpha (α), beta (β), epsilon (ε), and iota (ι) toxins) and an **enterotoxin**
- **six minor toxins** (delta(δ), theta(θ), kappa(κ), lambda(λ), mu(μ), nu(η)toxins) **& neuraminadase**
- C. *perfringens* subdivided into five types (A-E) on basis of production of major lethal toxins
- **C. *perfringens* Type A** (only major lethal toxin is alpha toxin) responsible for histotoxic and enterotoxigenic infections in humans; Type C causes necrotizing enteritis (not in U.S.)

Lab Identification

- direct smear and Gram stain, capsules upon direct examination of wound smears
- culture takes advantage of **rapid growth** in chopped meat media at 45° C to enrich and then isolate onto blood agar streak plate after four to six hours
- **gas** from glucose fermentation
- in vivo toxicity testing and identification of the specific toxin types involved
- **double zone of hemolysis** on blood agar (p-hemolytic theta(e) toxin, a-hemolytic alpha(oc) toxin)
- **Nagler rxn;** precipitation in serum or egg yolk media; oc -toxin (phospholipase C) is a lecithinase
- **"stormy" fermentation** (coagulaltion) of milk due to large amounts of acid and gas from lactose

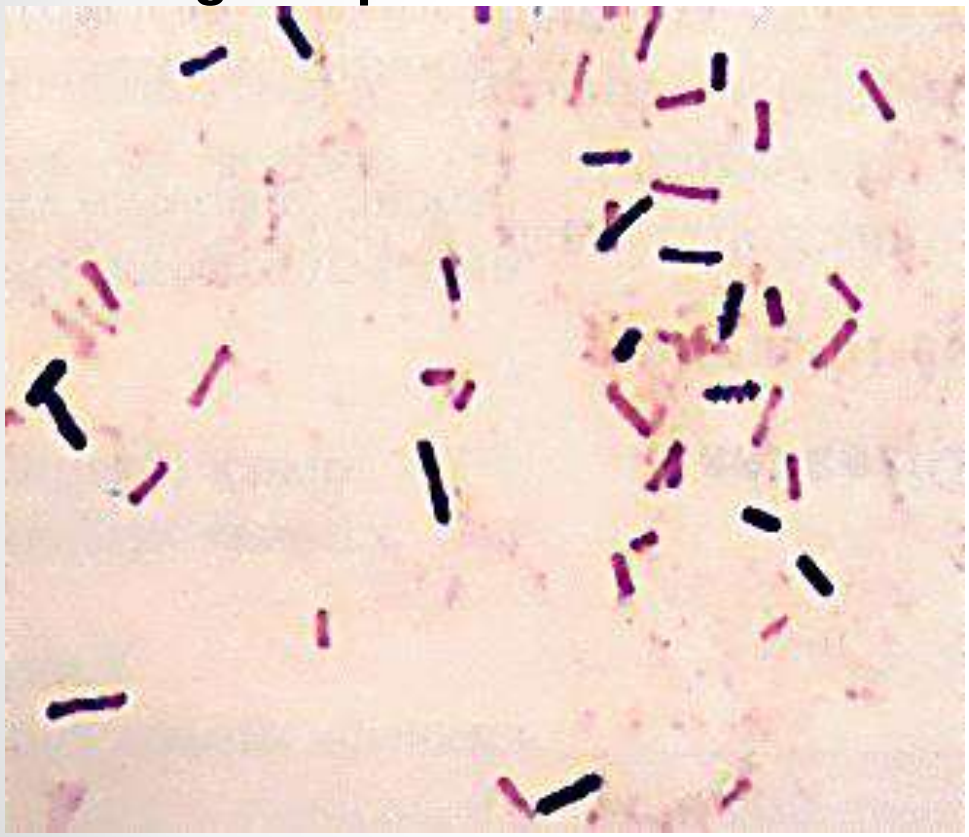
Diagnosis/Treatment of systemic infection — Early diagnosis and aggressive treatment essential

- removal of necrotic tissue **(surgical debridement)**
- **Penicillin G in high doses** if more serious infection

  Of poorly defined clinical value are:
  - administration of antitoxin
  - hyperbaric oxygen (dive chamber) adjunct therapy (??inhibit growth of anaerobe??)

Micro & Macroscopic C. perfringens

NOTE: Large rectangular gram-positive bacilli



NOTE: Double zone of hemolysis

Inner beta-hemolysis = θ toxin
Outer alpha-hemolysis = α toxin

Toxins

- **The toxins of *Cl. perfringens***
  - **α toxin** (phospholipase C, lecithinase) is the most important toxin
    - Lyses of RBCs, platelets, leucocytes and endothelial cells
    - Increased vascular permeability with massive hemolysis and bleeding tissue destruction
    - Hepatic toxicity and myocardial dysfunction
  - **β-toxin** is responsible for necrotic lesions in necrotizing enterocolitis
  - **Enterotoxin** is heat labile toxin produced in colon → food poisoning

C. perfringens Virulence Factors

	Virulence Factors	Biologic Activity
Major	α toxin	Lethal toxin; phospholipase C (lecithinase); increases vascular permeability; hemolysin; produces necrotizing activity
Major	β toxin	Lethal toxin; necrotizing activity
Major	ϵ toxin	Lethal toxin; permease
Major	ι toxin	Lethal binary toxin; necrotizing activity; adenosine diphosphate (ADP) ribosylating
Minor	δ toxin	Hemolysin
Minor	θ toxin	Heat- and oxygen-labile hemolysin; cytolytic
Minor	κ toxin	Collagenase; gelatinase; necrotizing activity
Minor	λ toxin	Protease
Minor	μ toxin	Hyaluronidase
Minor	ν toxin	Deoxyribonuclease; hemolysin; necrotizing activity
	Enterotoxin	Alters membrane permeability (cytotoxic, enterotoxic)
	Neuraminidase	Alters cell surface ganglioside receptors; promotes capillary thrombosis

Laboratory Diagnosis

- **Specimen**: Histological specimen or wound exudates
  - Histological specimen transferred aseptically into a sterile screw-capped bottle & used immediately for microscopical examination & culture
  - Specimens of exudates should be taken from the deeper areas of the wound where the infection seems to be most pronounced
- **Microscopical examination (Gram, Spore stain etc)**
  - Gram-positive bacilli, **non motile**, **capsulated** & sporulated
  - The spore is oval, sub-terminal & non bulging
  - Spores are rarely observed
- **Culture:** Anaerobically at 37C
  - **On Robertson's cooked meat medium** → blackening of meat will observed with the production of H2S and NH3
  - **On blood agar** → β-hemolytic colonies

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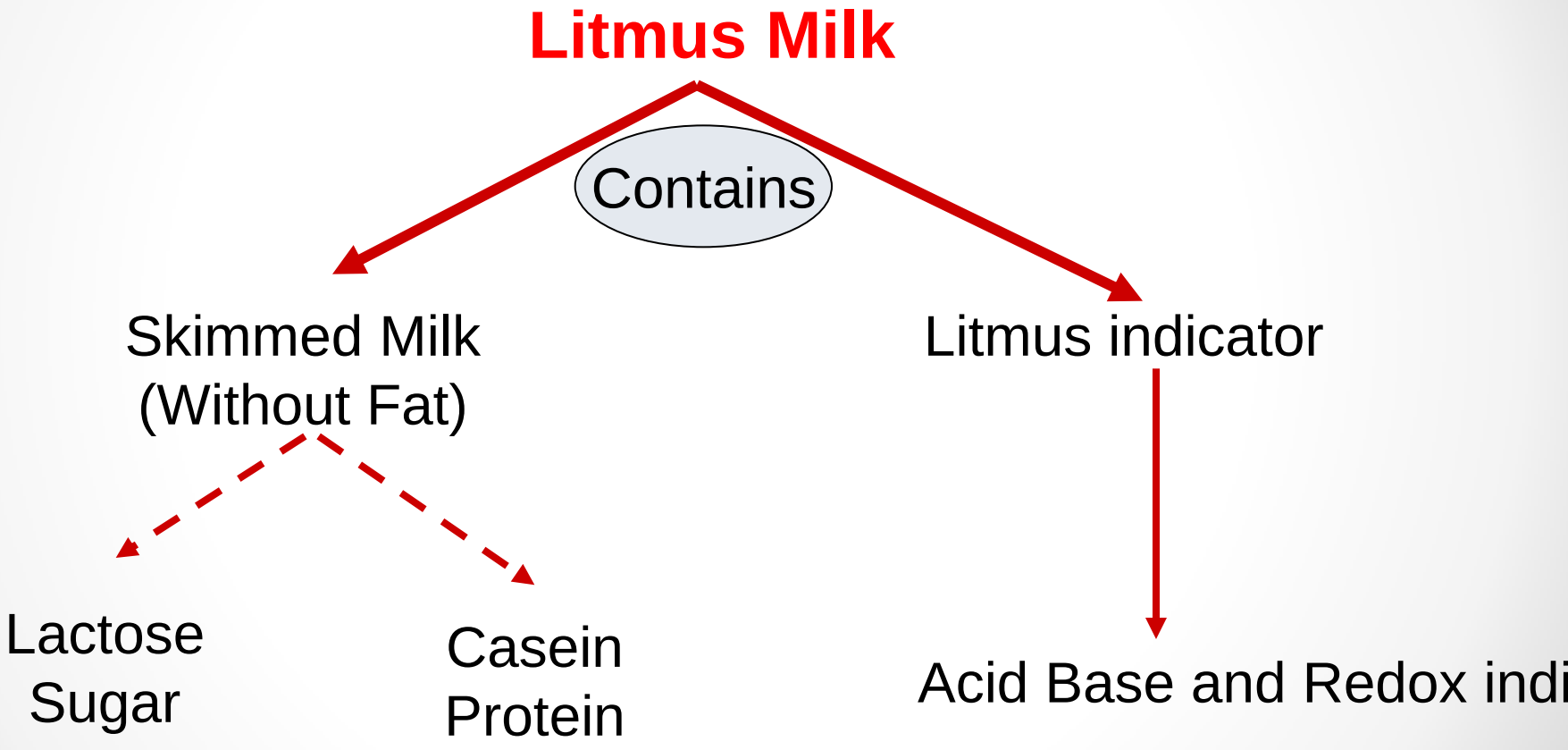
Biochemical Tests

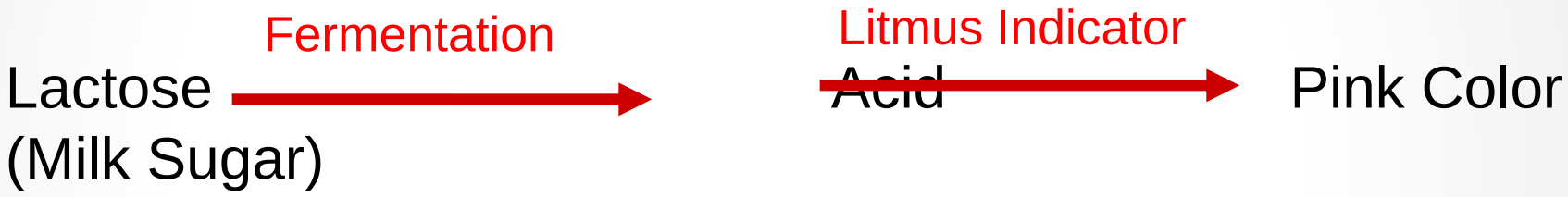
- *Cl. perfringnes* characterized by:
  - It ferments many carbohydrates with acid & gas
  - It acidified litmus milk with stormy clot production
  - Nagler reaction is positive

Reaction on Litmus Milk



Reaction on Litmus Milk

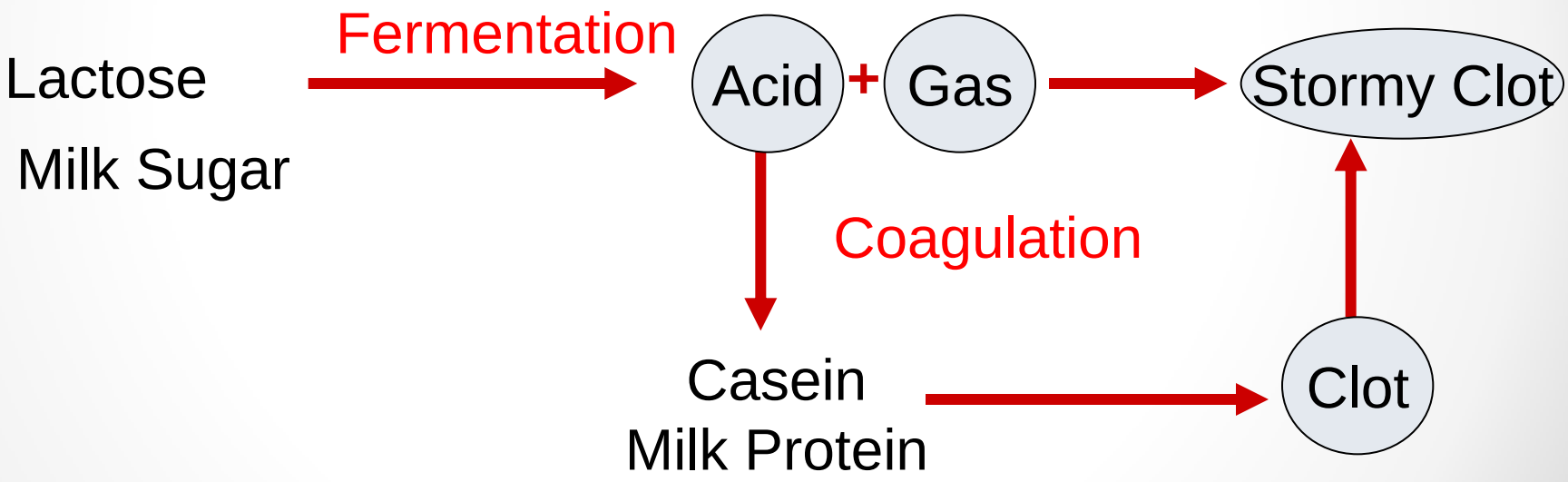
1- Acidic Reaction



2- Basic Reaction

Reaction on Litmus Milk

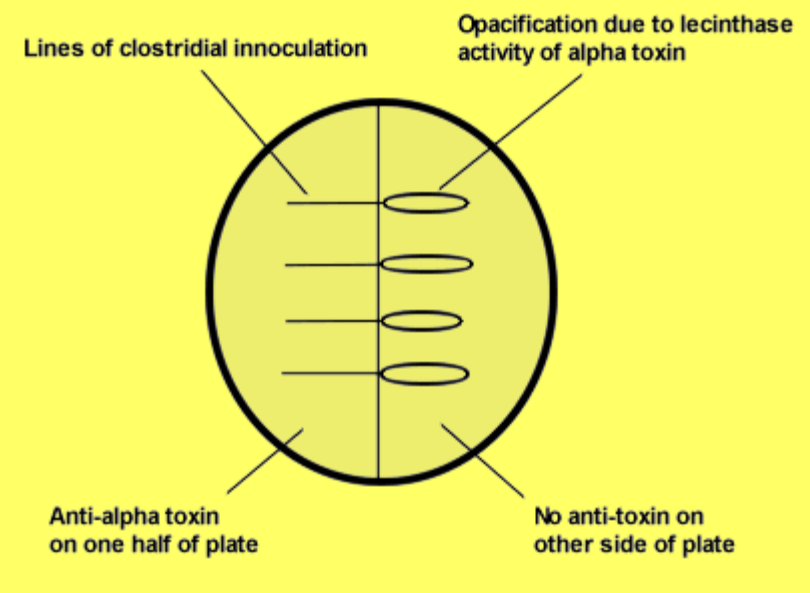
Stormy Clot Formation



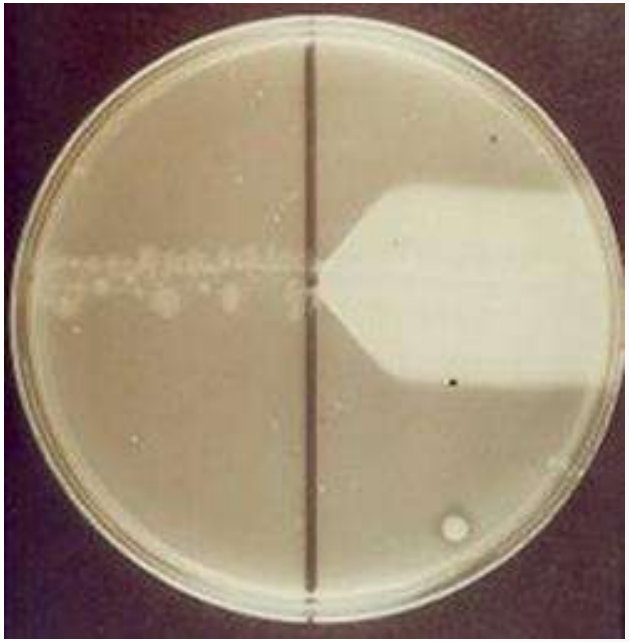
Nagler's Reaction

- This test is done to detect the lecithinase activity
  - The M.O is inoculated on the medium containing human serum or egg yolk (contains lecithin)
  - The plate is incubated anaerobically at 37 C for 24 h
  - Colonies of *Cl. perfringens* are surrounded by zones of turbidity due to lecithinase activity and the effect is specifically inhibited if *Cl. perfringens* antiserum containing α antitoxin is present on the medium

Nagler Reaction



Procedure of Nagler Reaction



Positive Nagler Reaction

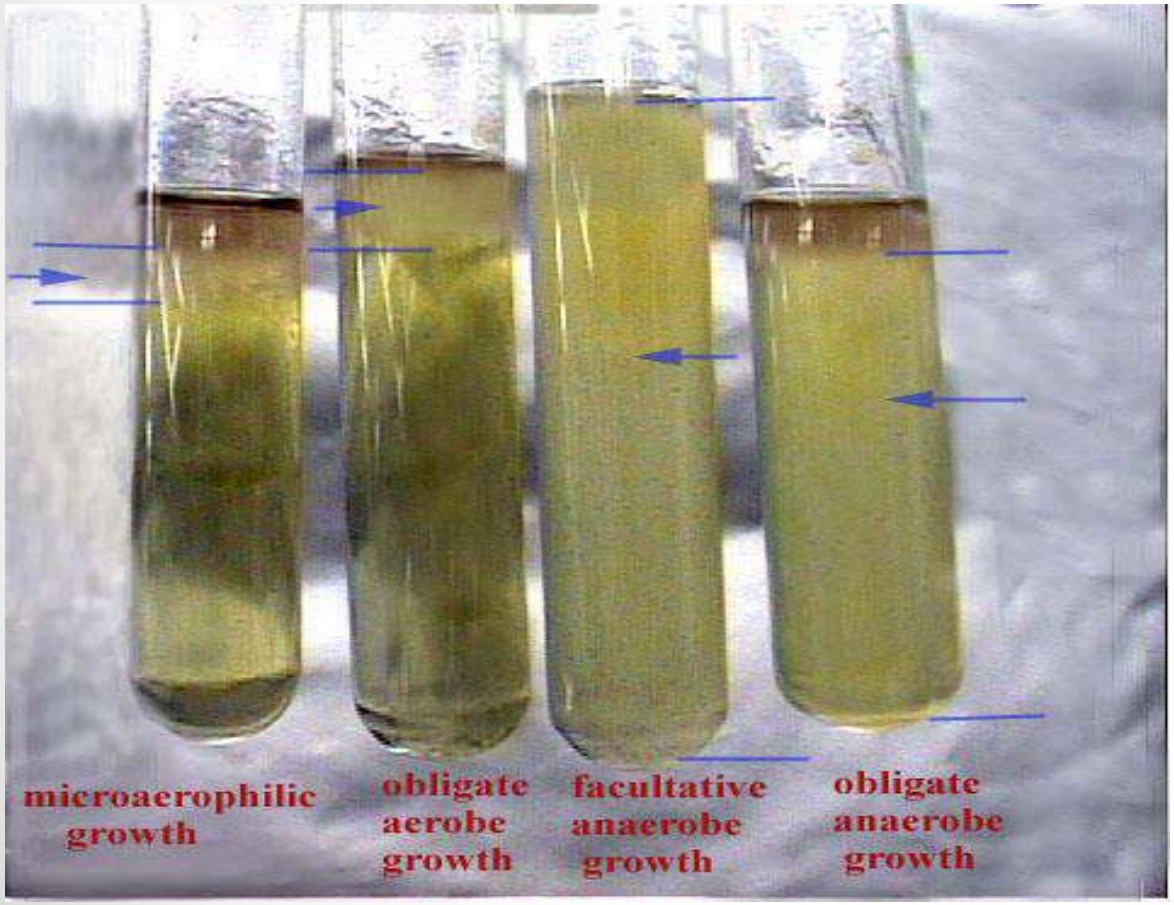
Anaerobic Cultivation

- Removal of oxygen & replacing it with inert gas
  - Anaerobic Jar
    - It is especially plastic jar with a tightly fitted lid
    - Hydrogen is introduced from commercially available hydrogen generators envelop
    - 10 ml of water is added to envelop immediately before placing it in the jar
    - Hydrogen and carbon dioxide will release and react with oxygen in the presence of catalyst to form water droplet
    - Anaerobic indicator (Methylene blue) is placed in the jar
    - Methylene blue is blue in oxidized state (Aerobic condition) while turns colorless in reduced state (Anaerobic condition)

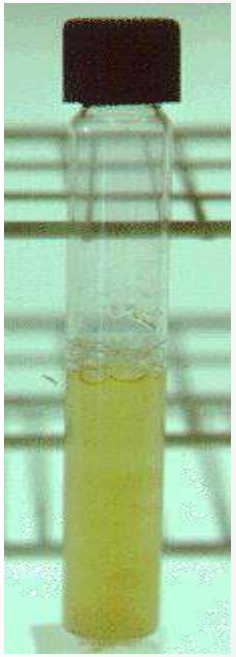
Anaerobic Cultivation

- Culture Media containing reducing agent
  - **Thioglycollate broth**
    - It contains
      - Sodium thioglycollate (Reducing agent)
      - Rezazurin (redox indicator)
      - Low percentage of Agar-Agar to increase viscosity of medium
  - **Cooked Meat Medium**
    - It contains
      - Meat particles (prepared from heart muscles) which contain hematin & glutathione that act as reducing agent

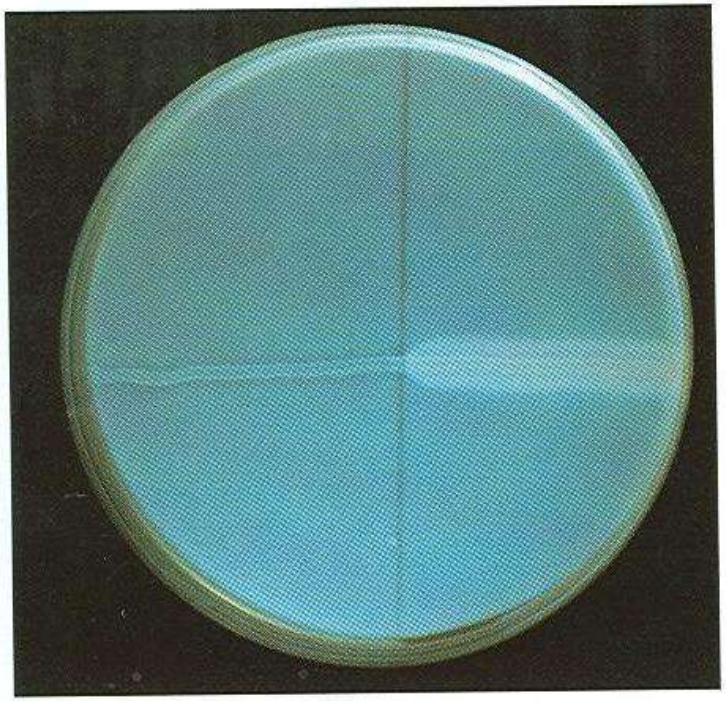
Growth on Fluid Thioglycolate



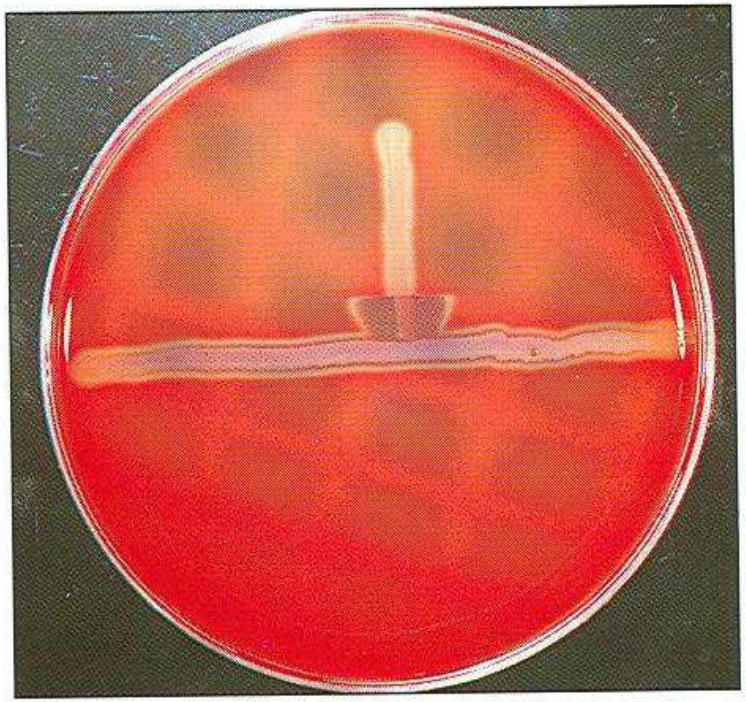
Clostridium sporogenes Growing in Thioglycolate Medium



Reducing agents in the medium absorb oxygen and allow obligate anaerobes to grow



253. Nagler test for *C. perfringens* alpha toxin. The toxin is a lecithinase and attacks the lecithin in egg yolk agar (right). This reaction is neutralised on the left by specific antitoxin.



254 CAMP test with *Streptococcus agalactiae* (vertical streak) enhancing the partial haemolysis produced by the alpha toxin of *C. perfringens*.

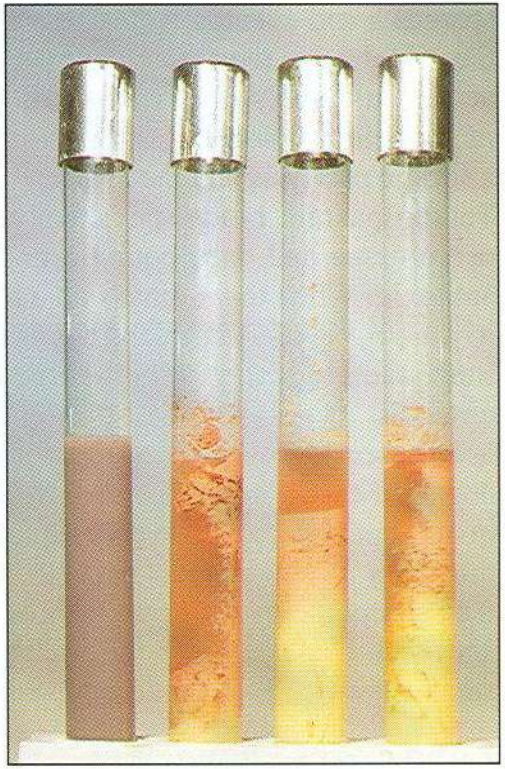
236

236 Growth of *C. perfringens* in thioglycollate medium.

237

237 *C. botulinum* type C on egg yolk medium giving a pearly layer around the colonies due to lipase activity. Lecithinase is not produced by this bacterium.

238

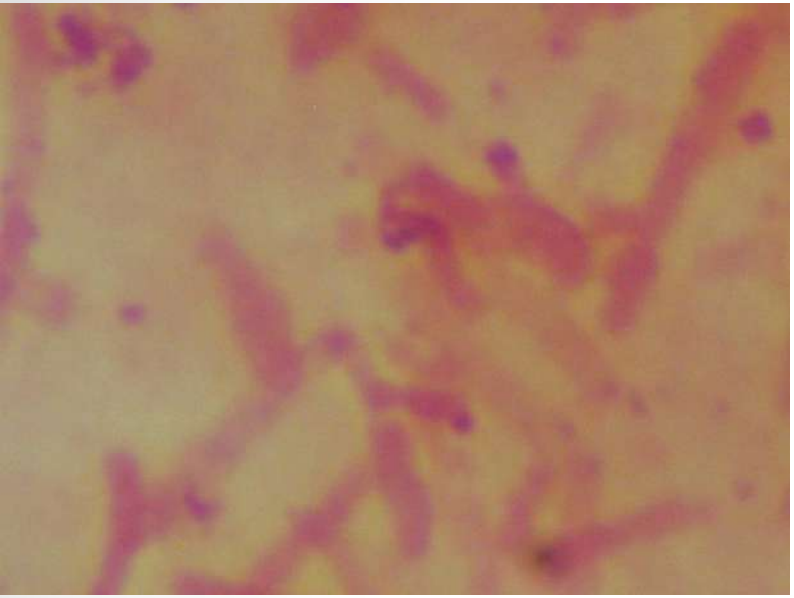


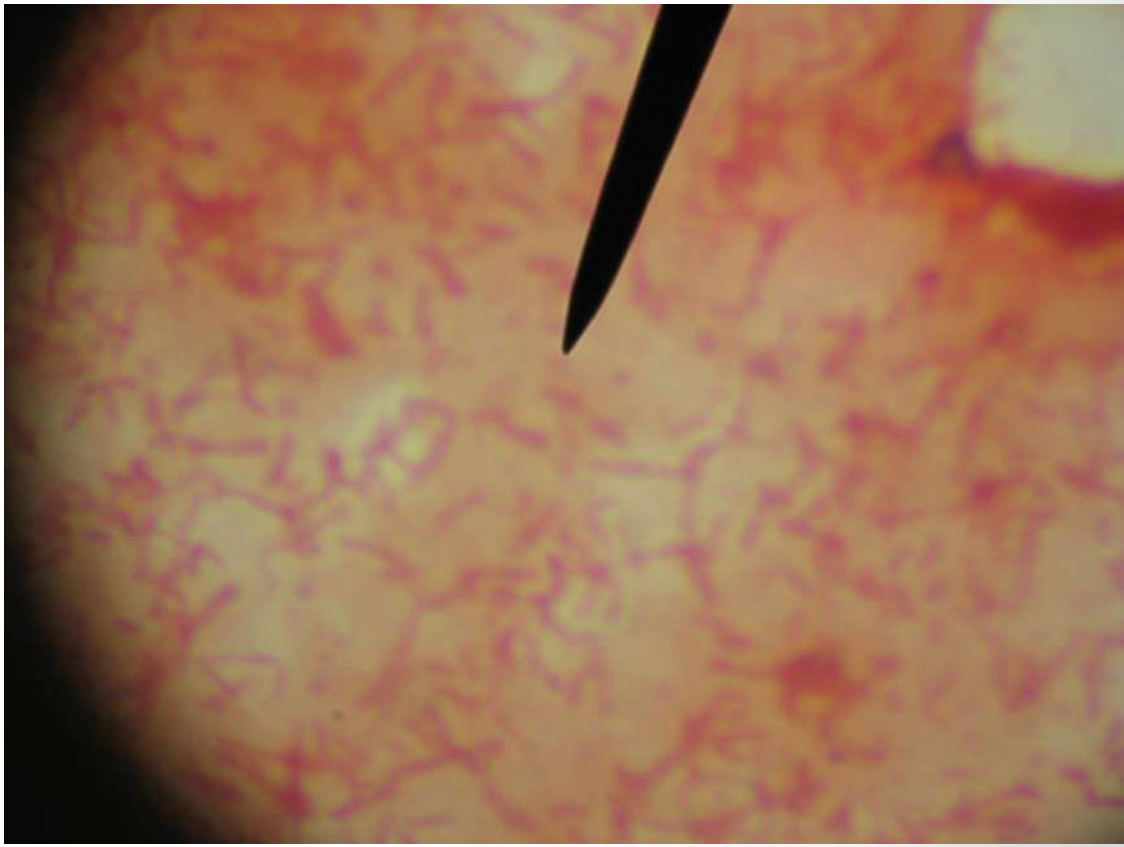
238 The 'stormy clot' reaction of three isolates of *C. perfringens* in litmus milk medium. The tube on the left is uninoculated.

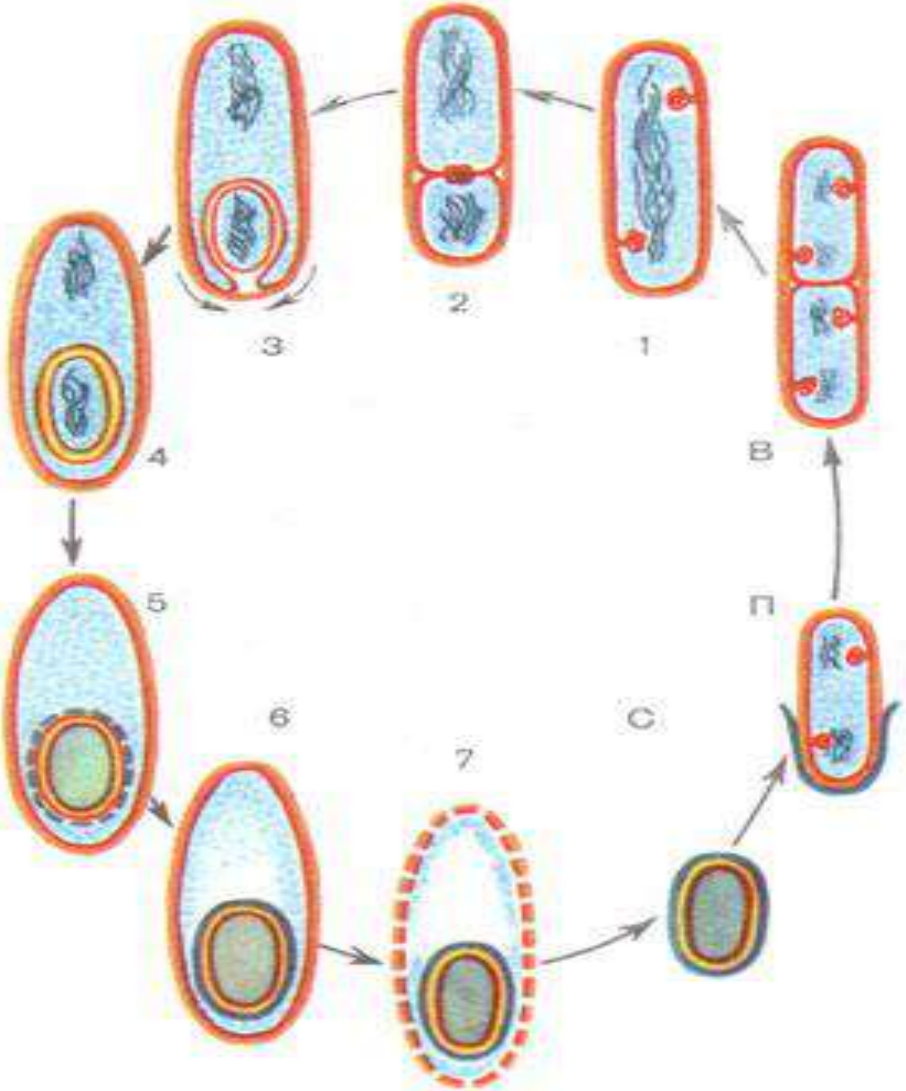
Reaction on Cooked Meat Medium

- Saccharolytic reaction
  - It causes fermentation of glycogen of muscles
  - Production of acid and gas
  - Meat particles remain intact
  - e.g *Cl. perfergines*
- Proteolytic Reaction
  - It causes digestion of meat particles
  - Formation of black, foul smelling due to sulfur compounds

Gram Variability







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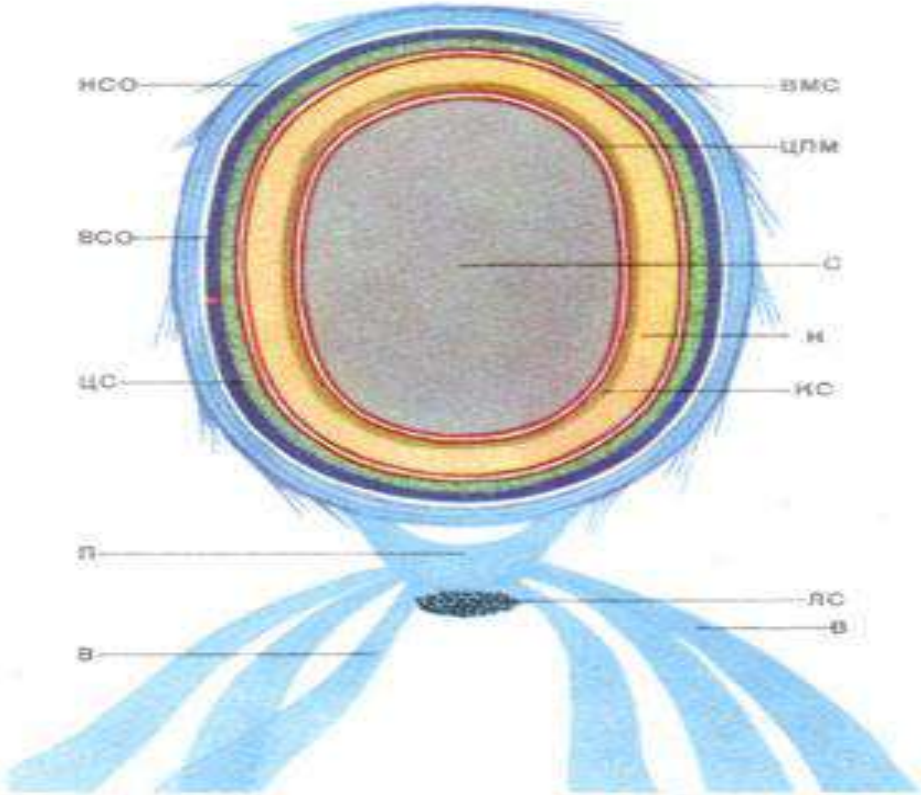

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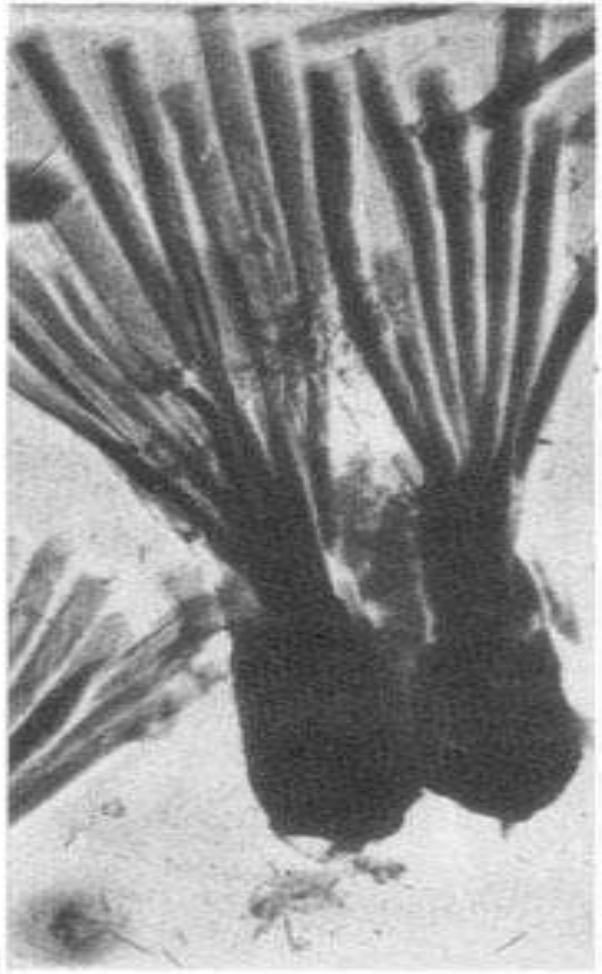

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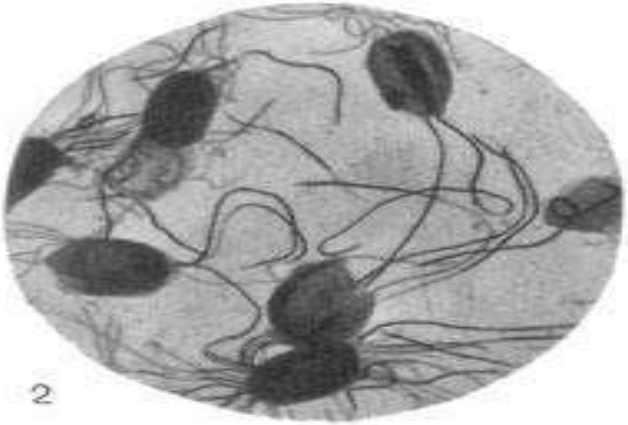

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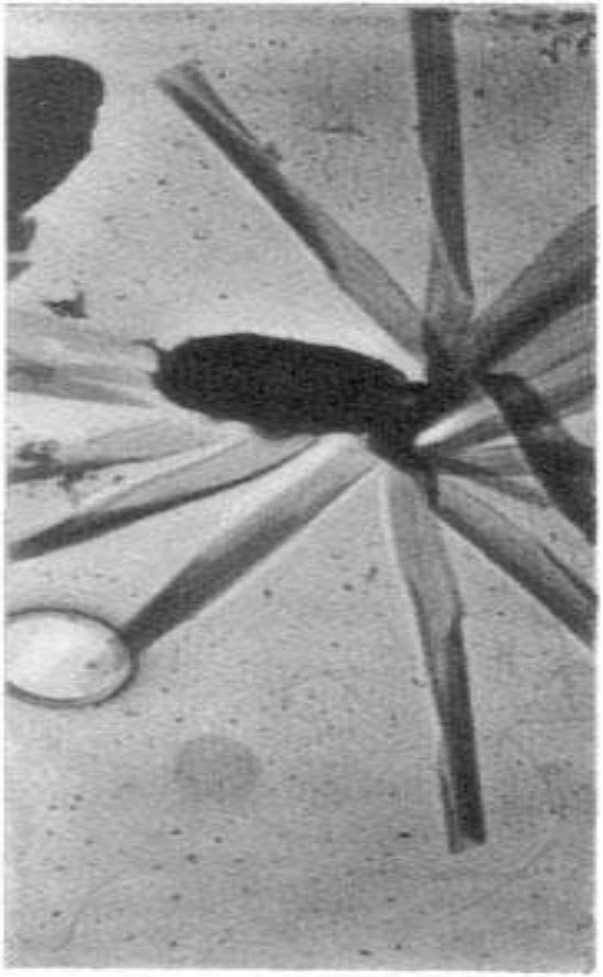

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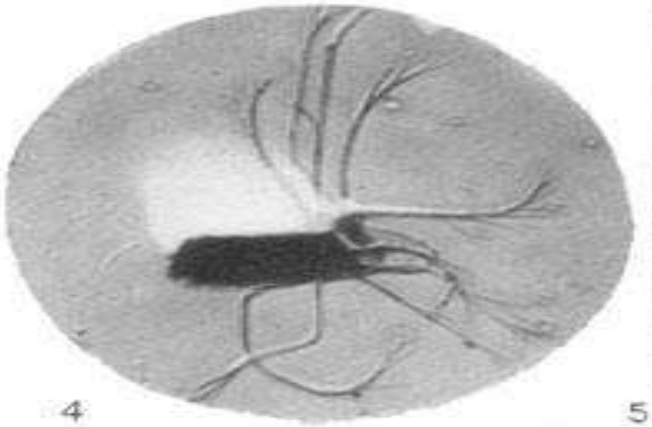

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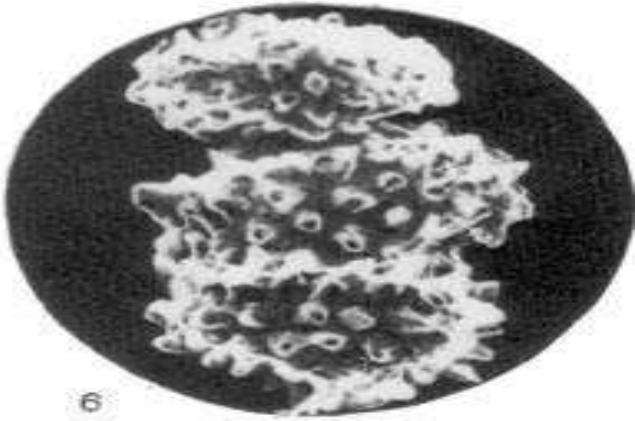

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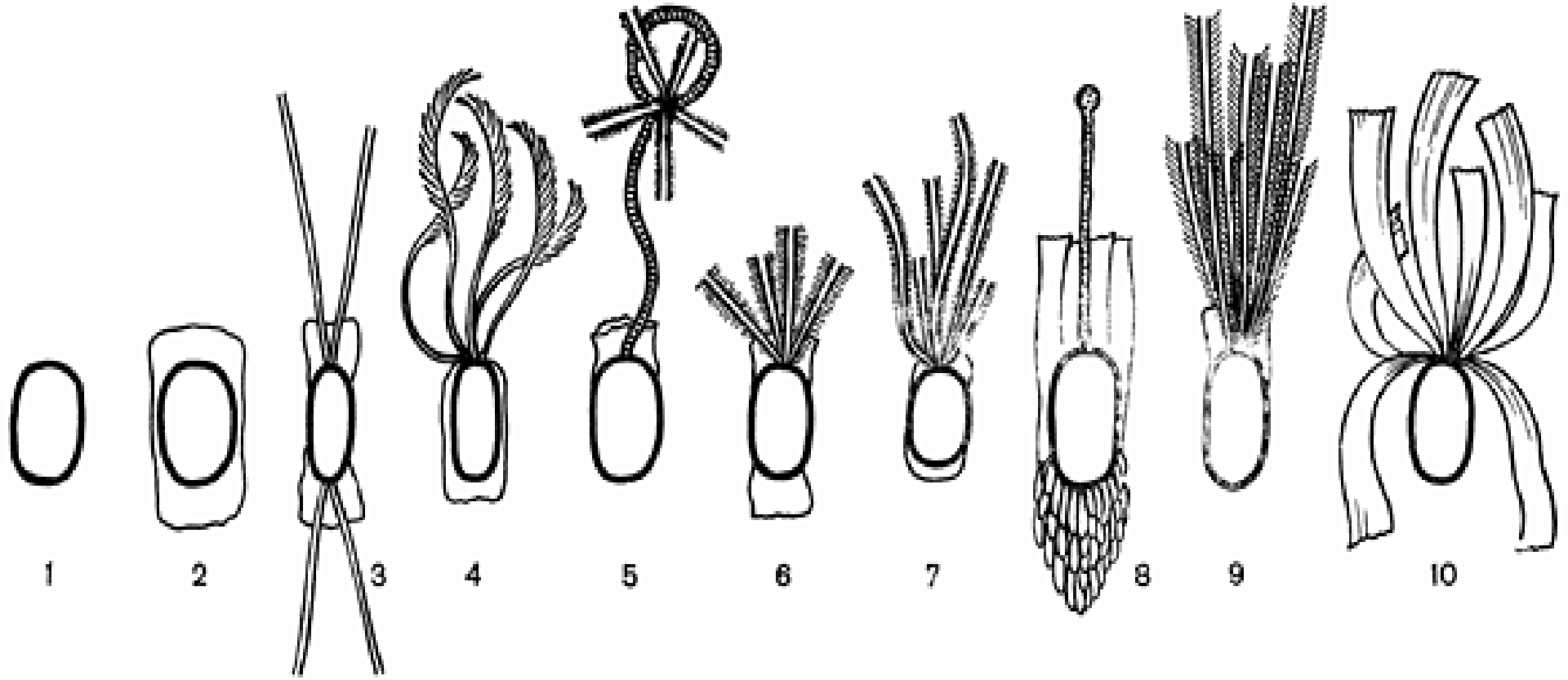
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Clostridial Diseases

- During multiplication toxins are released, they can destroy:

  muscle tissue

  red blood cells

  interrupt nerve impulses

☞ Sites of disease into three groups:

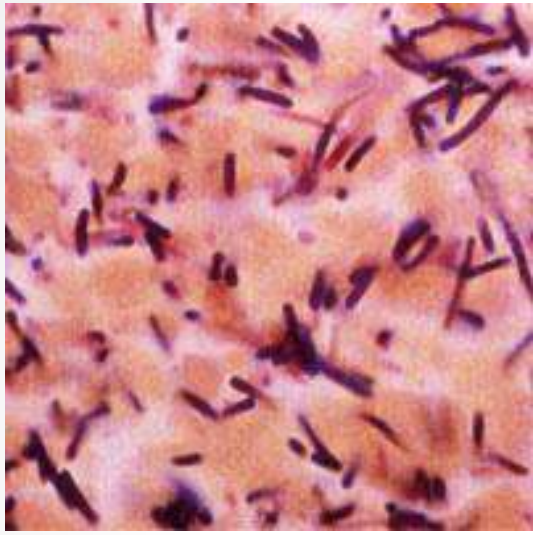
  -Tissue: histotoxic muscle liver group

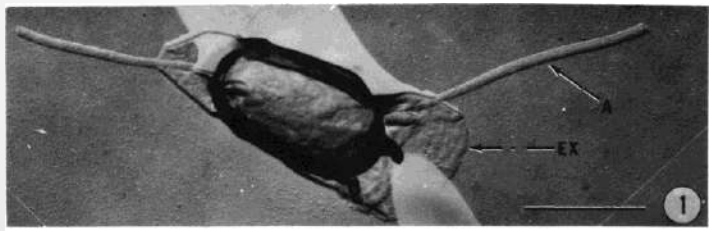
  -Gastrointestinal: enterotoxic gut group

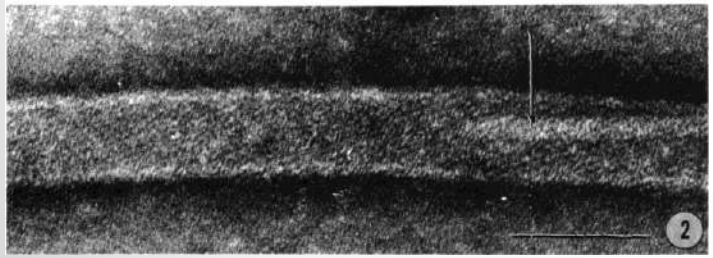
  -Nervous System: Neurotoxic

☞ Clostridia do not cause disease until tissue is damaged .

Clostridium sordelli







- Gram positive
- Anaerobic bacillus, resides on soil
- Formed oval spores with smooth tubular appendages
- Colonizes gastrointestinal or genital tract of healthy humans.
  - -uncommonly found in surveys of stool and vaginal flora
  - -isolated from musculoskeletal tissue of 3% cadaver donors
- Virulence and clinical manifestations determined by two exotoxins:
  - Lethal Toxin
  - Hemorrhagic Toxin

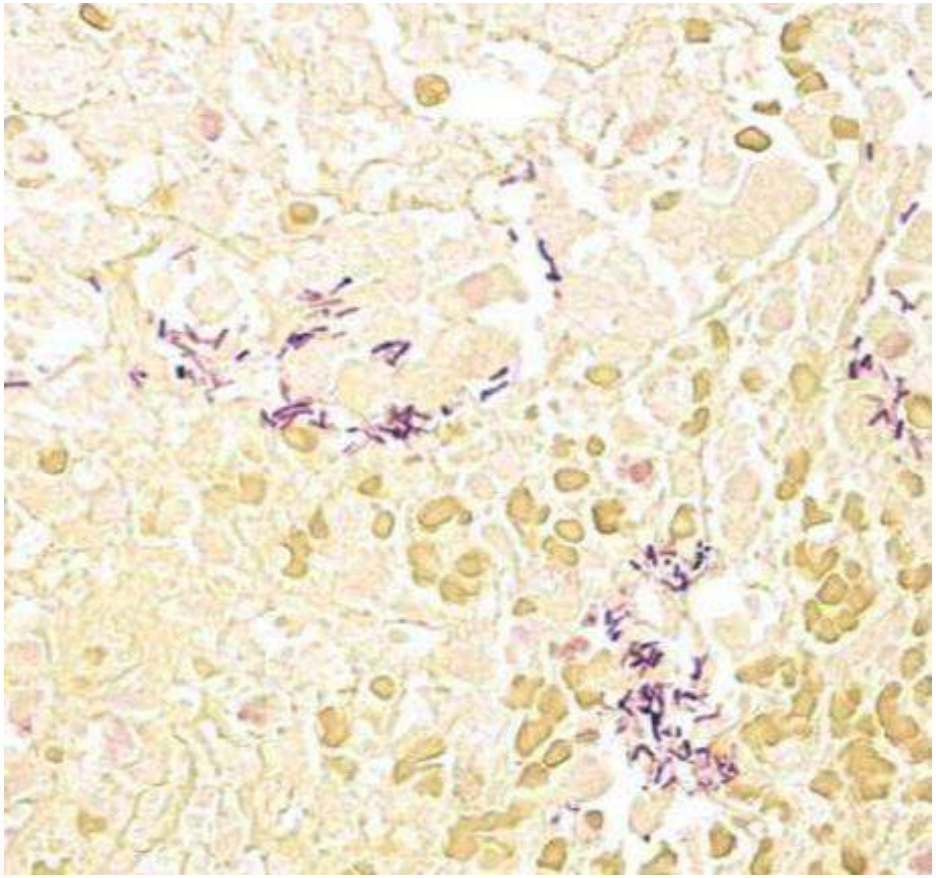
C. sordelli wound infections:

- Myonecrosis
- Tissue allograft infections
- Nenonatal omphalitis
- Postpartum endometritis

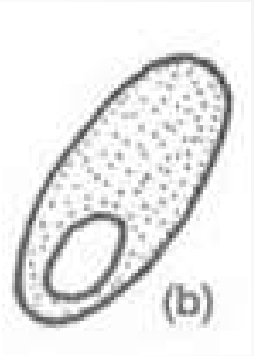
C. sordelli Toxic Shock Syndrome

- Acute onset and rapid progression
- Low grade fever
- Refractory tachycardia and hypotension
- Leukemoid reaction
- Hemoconcentration
- High case fatality

C. sordelli infection



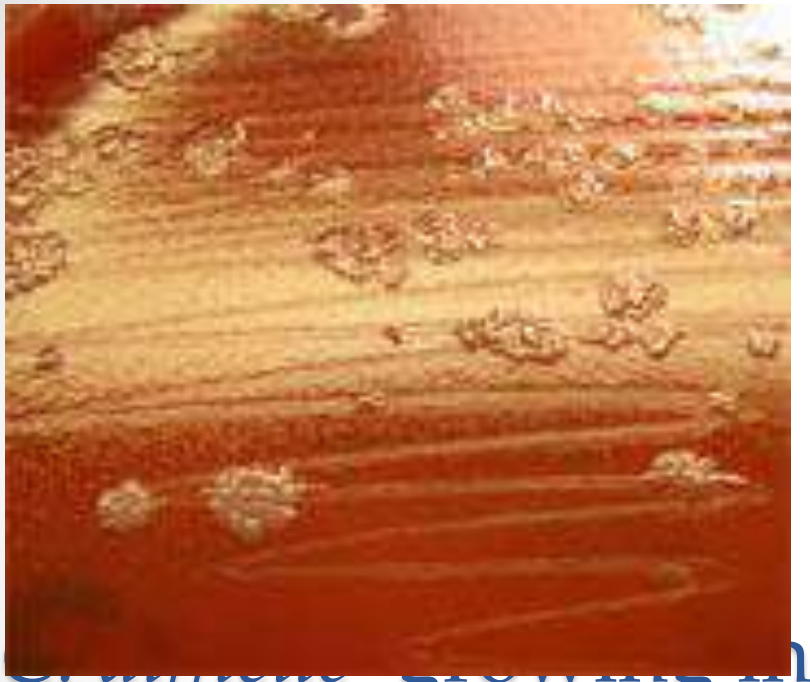
Clostridium septicum

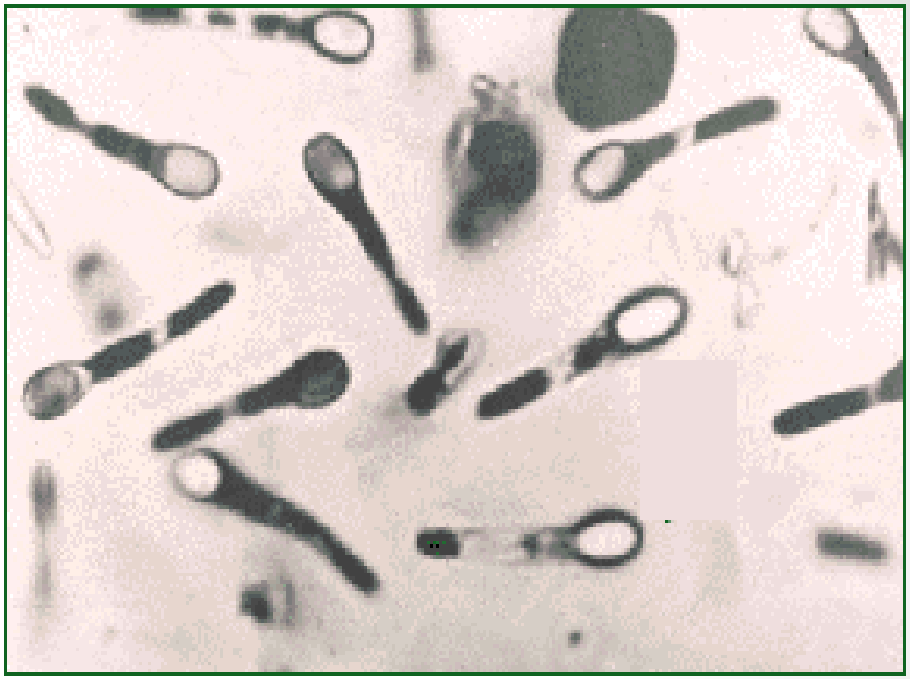


- Gas forming bacillus
- Produces subterminal spores
- Pathogenic
- Occurrence of infection caused by two or more species.
- Source of infection is usually the contaminated wound containing devitalized tissue
- The gas produce by the organism appears to remain limited on the area of metastasis and does not invade healthy tissue

Clostridium difficile

- Produces two toxins:
  - Toxin A (causes fluid accumulation)
  - Toxin B (harmful toxin)
- Causes antibiotic – associated diarrhea (AAD)
- Serious intestinal conditions
- Found on individuals who require prolonged use of antibiotics.
- People who undergo gastrointestinal surgery.

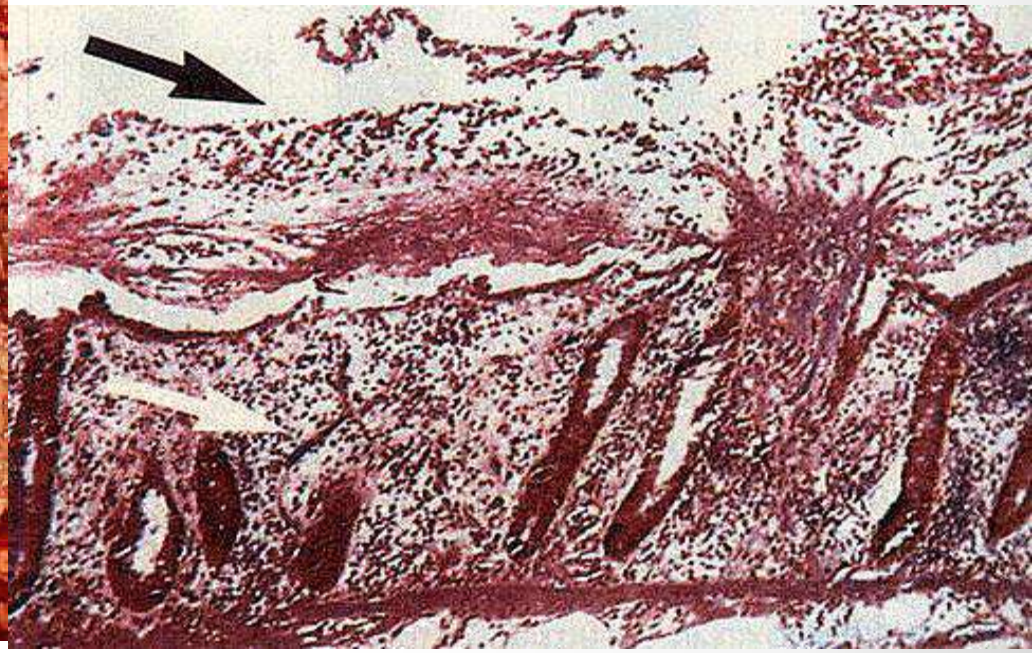




S. pyogenes growing in blood agar

Antibiotic Associates colitis

- Inflamm
  intestines due to antibiotics

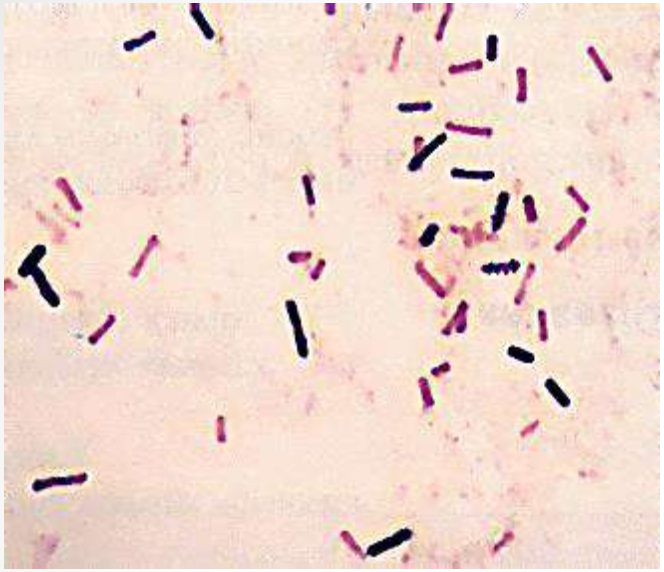


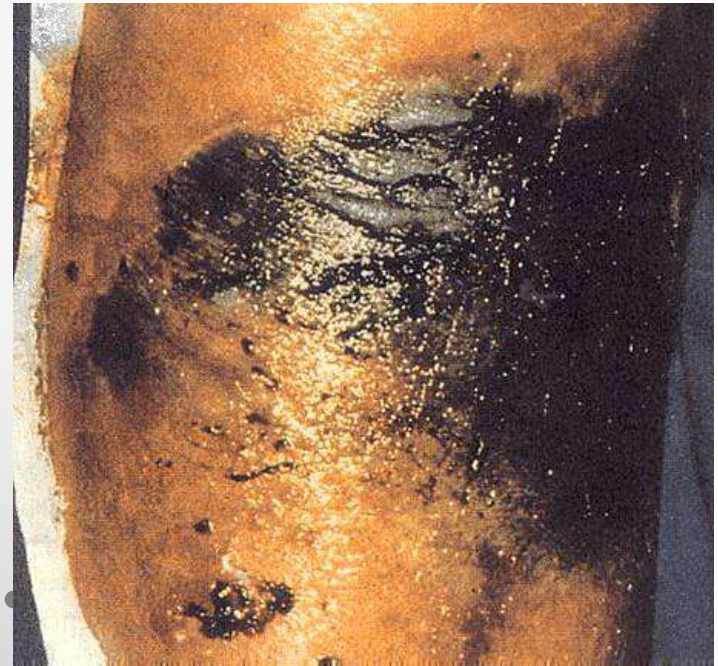
Clostridium perfringes

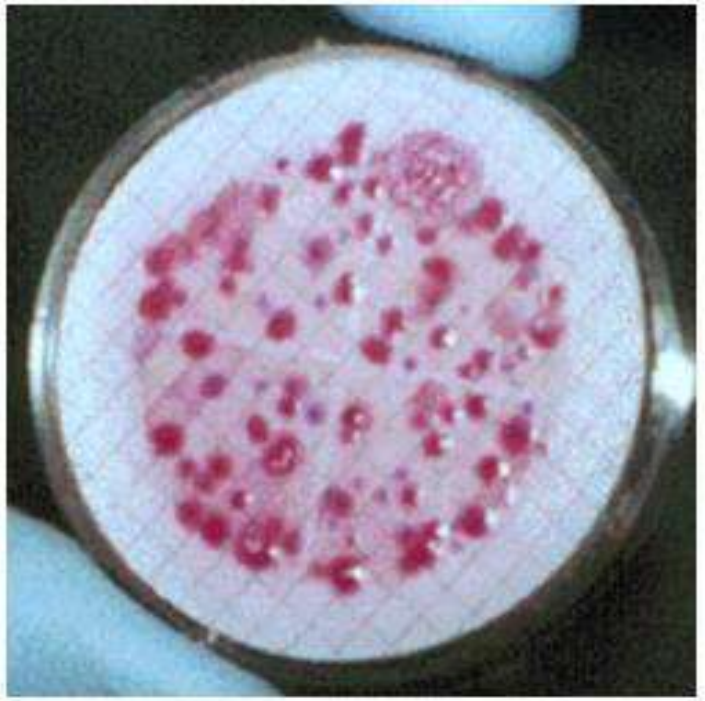
- Produce many different toxins
- Damage to tissues, blood cells, and blood vessels
- Causes wound and surgical infections, and uterine infections.
- Produces enterotoxin, cause food poisoning

Organism encountered improperly sterilized foods in which endospores have germinated

Clostridium perfringens

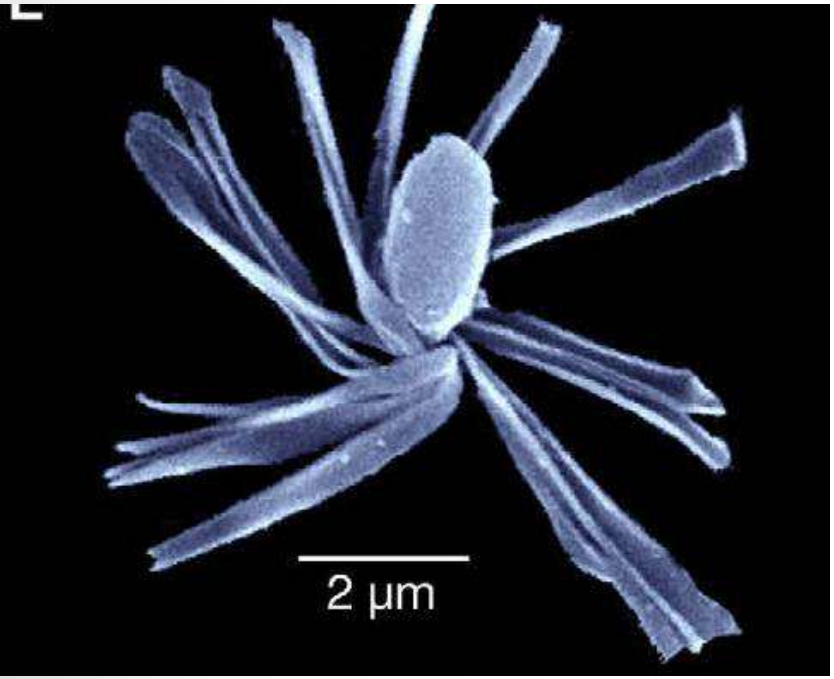






Colonies are pink under ammonium hydroxide (done in fume hood)

Clostridium taeniosporum



- Variable to gram stain
- Is a saccharolytic
- Produces acetic and butyric acid as fermentation products.
- Have 12 large flat ribbon like appendages through a common trunk.

Appendages : composed of fibrils

4.5 um long

0.5 um wide

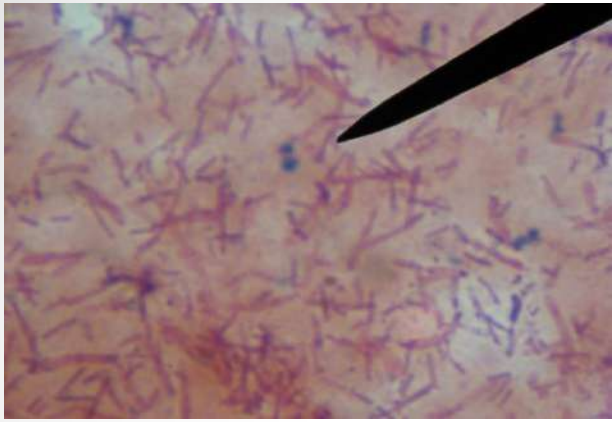
30 nm thick

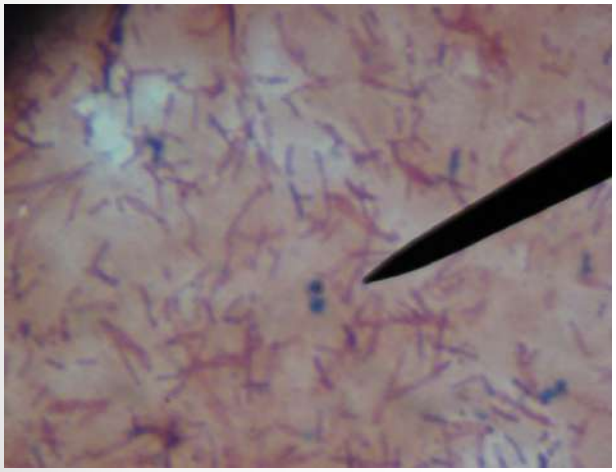
Produces alpha D-glucosidase and N acetyl beta glucoseaminidase

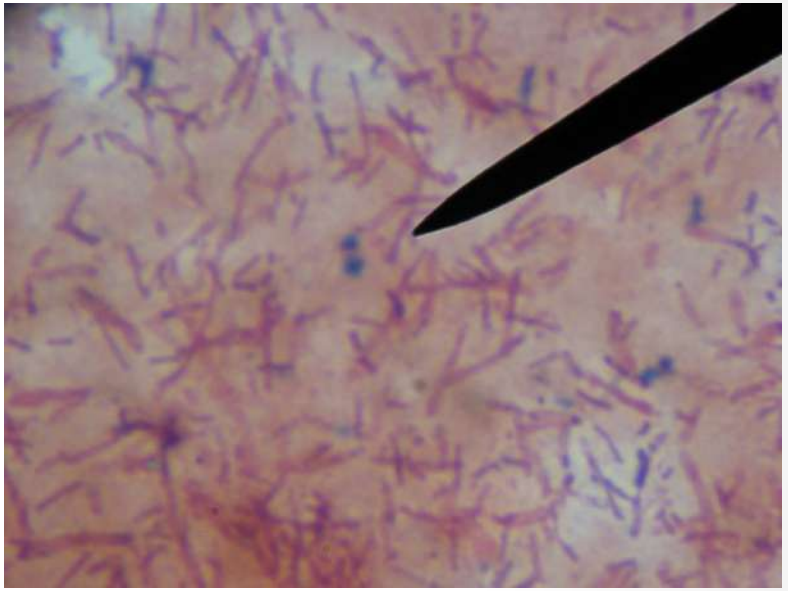
Clostridium taeniosporum

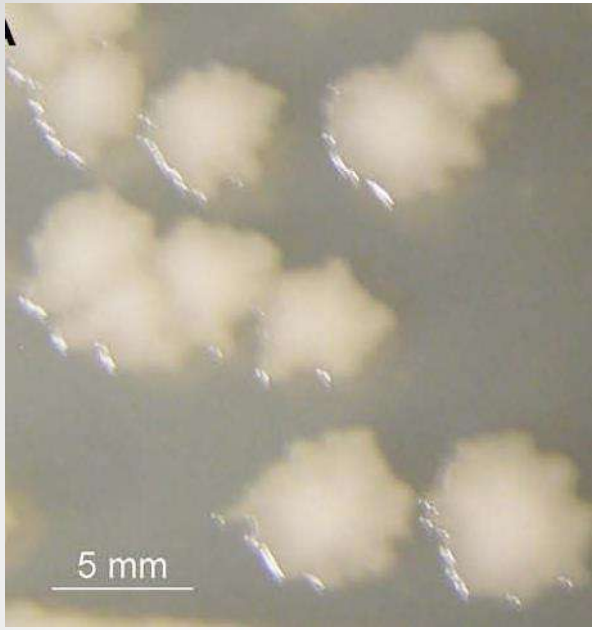
- Appendages consists:
  - 80% protein
  - 15% glucose
    - rhamnose
    - glucosamine
  - 5% phosphorus
- 4 proteins detected:
  - Paralogous pair P29a and P29b
  - GP85 Glycoprotein contains collagen like region
  - SpoVM spore morphogenetic protein

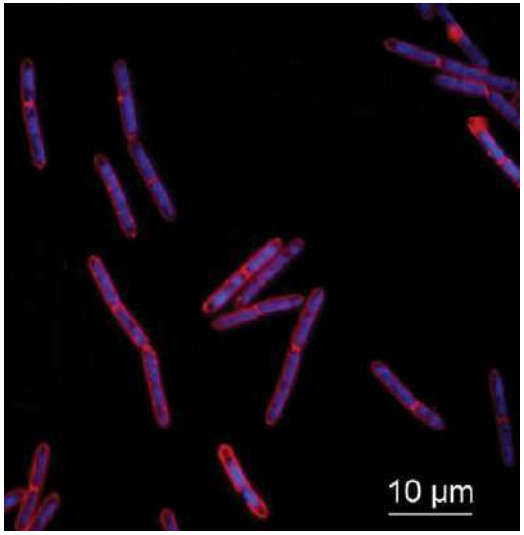
C. taeniosporum endospore stain

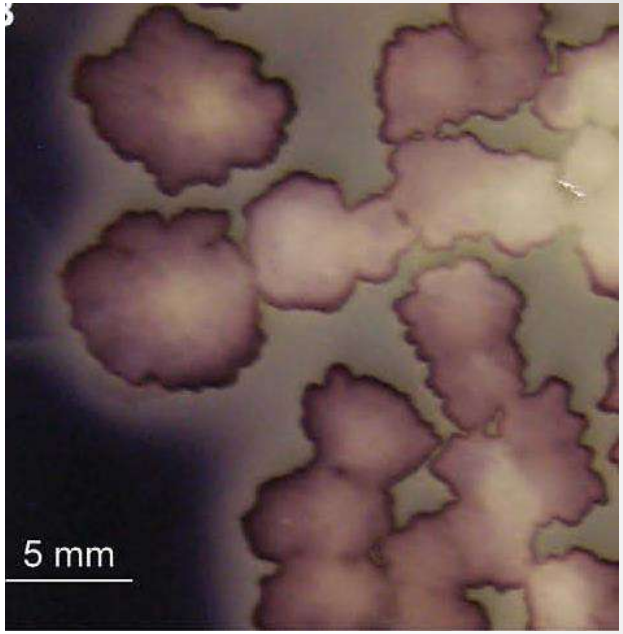


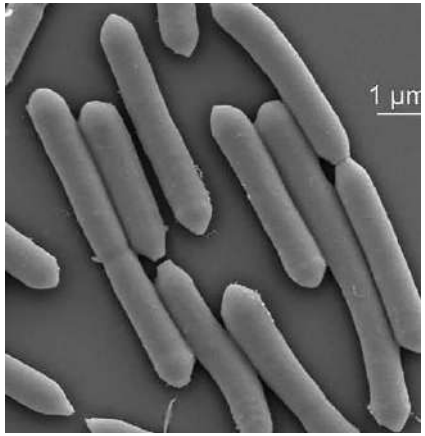


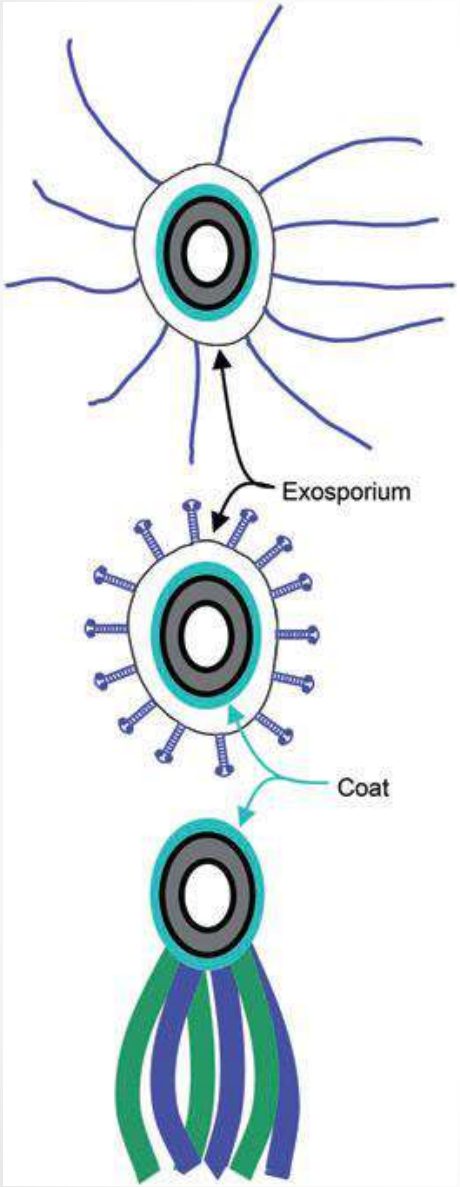



5 mm


10 µm

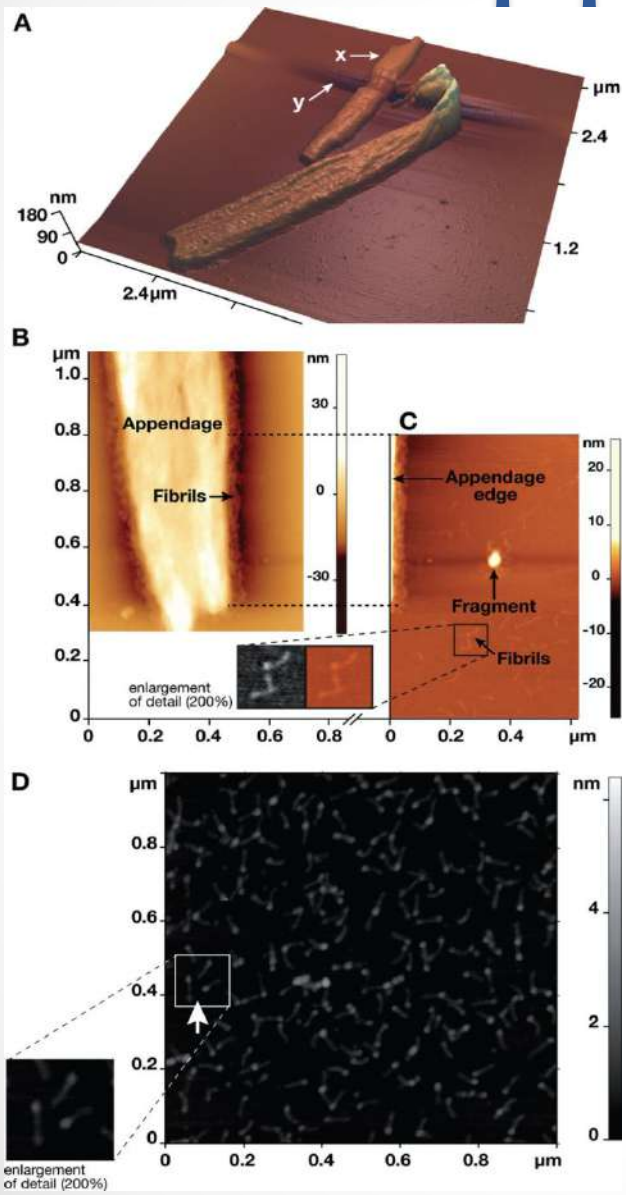

5 mm


1 µm



Cartoons illustrating the diversity of spore appendages. The
coat is drawn as a light blue layer.

Appendages


A
x
y
µm
2.4
1.2
nm
180
90
0
2.4 µm
B
µm
1.0
0.8
0.8
0.6
0.4
0.2
0
Appendage
Fibrils
nm
30
0
-30
C
Appendage edge
Fragment
Fibrils
nm
20
10
0
-10
-20
enlargement of detail (200%)
0
0.2
0.4
0.6
0.8
0
0.2
0.4
µm
D
µm
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
µm
nm
4
2
0
enlargement of detail (200%)