

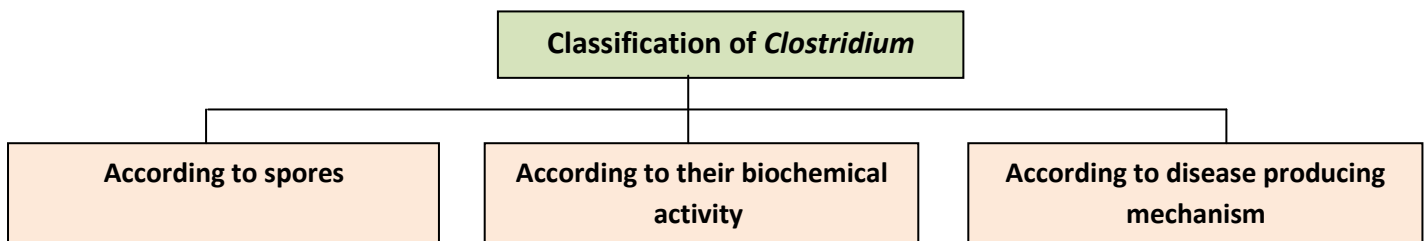
Spore forming bacteria

Family: Bacillaceae

Genus: *Clostridium*

General character of Genus clostridium.

- 1- Consists of Gram +ve spore forming obligatory anaerobic organism.
- 2- Natural inhabitant is the soil special animal dwelling soil or cultivation soil.
- 3- From soil they go into intestinal tract of man and animal.
- 4- The bacilli are typically large bacilli straight or slightly curved and mostly with rounded end.
- 5- Length 3-8 microns x thickness 6-10 microns it larger than other m o.
- 6- Some member tends to lose their Gram +ve reaction in cultures especially on old growth culture and may change to Gram –ve organism.
- 7- All of them produce end spores.



A) Classification according to spores:

- Position & shape of spores is very important in classification of clostridium.
- There are terminal, central, and subterminal.
- Spore in all pathogenic *Clostridium* is normally wider than body of bacilli producing bulging (**bulging spore forming anaerobic organism**).
- In all pathogenic clostridium except *Clostridium tetani* the spore are central or subterminal in position.

*In *Clostridium tetani* terminal spores.

- Almost all member of this genus are motile with numerous long peritrichious flagella except one type which is *Clostridium perfringes* (non motile).
- Also *Clostridium perfringes* & *Clostridium butyricum* are only capsulated members with' well develop capsule.
- *Clostridium* grows only in absence of air or free oxygen and is usually killed in presence of air so it lives deeply in soil.
- In presence of air the *Clostridium* is able to produce toxic hydrogen peroxide w' can't destroyed due to absence of catalase enzyme as catalase enzyme present normally in most aerobic & facultative anaerobic m. o.

B) Classification according to biochemical activity

I. Saccharolytic Clostridium ..

Characterized by their rapid growth on CHO media e' production of acid & hearty amount of gases formation when grown in cooked meat media.

Ferment CHO in meat fragments but not digested its protein. The meat fragment change to dark red color but not reduced its size with formation of high amount of gases & sour smelling odour (acidic)

..e.x.. 1- *Cl. perfrings*.

2 - *Cl. septicum*.

3 - *Cl. chauvae*.

4 - *Cl. fallex*.

5 - *Cl. tetrinum*.

6 - *Cl. bifermentans*.

II. proteolytic Clostridium

Digest meat protein (decompose meat protein) but not fermentation to CHO meat fragment reduce in size become black in color e' formation of foul (bad) smelling odour.

..e.x.. 1- *CL. histolyticum*.

2- *Cl. tetani (mild proteolytic)*.

III. Saccrolytic & proteolytic Clostridium

Digest meat protein & ferment its CHO the meat fragment are completely disappear e' the formation of dark black broth e' very sever putrefactive odour.

..e.x.. 1- *CL. Butyricum*.

2- *CL.sporogens*.

C) classification According To disease producing mechanism.

Non-invasive group.	Invasive group.
<p>Smallest group. Consist of those spp. Which have a little or no power to invade & multiply in the living tissue their pathogenisity due to its power to forming powerful exotoxic w' are</p> <p>1) Produced either in localized deep infected wound such as in <i>Clostridium tetani</i> .</p> <p>2) Outside the body preserved canned processed food such in <i>Clostridium botulinum</i>.</p>	<p>Largest group or gas gangrenous group which have the power to invade & multiply in living tissue & produced its exotoxcin but less potent than 1st gp . as:..</p> <ul style="list-style-type: none"> * <i>Clostridium perfringes</i>. * <i>Clostridium navyi</i>. * <i>Clostridium haemolyticum</i>. * <i>Clostridium chauvai</i>. * <i>Clostridium septicum</i>.

Non-invasive clostridium

Clostridium tetani

It is causative agent of tetanus or lock jaw disease (make spasm to muscle of jaw that the animal can't open its mouth) in different animal especially equine.

Morphology..

- Straight slender and rod shape organism e' rounded end length 2-5 micron & thickness 0.4-0.6 micron.
- Most of serotypes are motile e' long peritrichous flagella.
- Spore is large rounded or spherical 2-4 times diameter of bacilli, terminal in position producing drum stick or bad Milton racket.
- Non capsulated.
- Culture is Gram +ve in young but in old cultures cells usually decolorized from Gram +ve to Gram –ve bacilli.

Culture character.

- * Obligate anaerobic microorganism.
- * Optimum temp 37°C but it can grow at wide range of temperature minimum 14°C – maximum. 43°C.
- * Grow on ordinary nutrient agar media but more easily grow on Cooked meat media.
- * On solid agar media surface colonies of normal motile type of tetanus bacilli are character by long branching part of colony can't grow more than 100 micron in diameter becomes slightly raised and has glass ground in appearance while edges remains showing feathery process.

- * Non motile strain producing similar colonies e' absence of this feathery processes.
- * on blood agar media colonies are surrounding by β .hemolytic zone.

Biochemical reaction.

- * Nutrient gelatin (15%) \longrightarrow slowly liquefaction gelatin.
- * litmus milk media \longrightarrow no change but sometimes (soft clotting).
- * Not ferment CHO but add 1% glucose to the media it increase growth of organism.
- * Cooked meat media \longrightarrow slight digestion of meat protein e' formation of bad smelling odour as *Clostridium tetani* mild proteolytic.

Resistance of *Clostridium tetani*.

- * spore are highly resistance and when protected from heat & light may remain viable 15 years in soil in a' & dwelling soil or cultivated soil.
- * resist streaming 100°C for 40-60 min.
- * Tetanus spore is destroyed by 5% phenol for 10-12 hr. but addition of 1% HCl acid may reduce time to 2 hr.
- * The organism & its spores is usually killed by using 3-5% tincture iodine or 3% H₂O₂.

tetanospasmin	Tetanolysin
W' has a selective action on C.N.S. it may act by inhibiting syntheses liberation of acetyl choline increasing the titanic spasm localized in the muscle.	Another exotoxin oxygen labile & causes lysis of red blood corpuscle.

Diagnosis of tetanus..

1. Symptoms ...

-are similar in all a' it consist of chronic or titanic spasm sometimes from one part of body w' wound is located.

But generally disease extends to other part.

-the titanic spasm reach all body and make difficult to open mouth.

2. Direct smears.

-from infected wound & stain by grams method & examined morphologically.

3. Culture character & biochemical reaction.

-As before.

4. Animal pathogenicity..

-Its most accurate method for diagnosis of tetanus.

-0.2mL. for 5-10 day of cooked meat culture containing suspected samples inoculated s\c in base of mice tail .

After 24 hr . in +ve case may be stiffness of the tail & paralysis of hind limb.

-titanic spasms to all body muscle & death usually occur within 24 hours after appearance of symptoms.

-control of mice are protected by 500-1000 I\V of antitetanic sera injection s\c one hr . before inoculation of culture.

...Immunization against tetanus...

1) passive immunity

* Antitetanic sera.

* Tetanus antitoxin is used 2ml each ml (1500IU\ml) is used as a prophylactic measure till one month.

- * So, immunity lasts 1 month.
- * When A' suffer from disease take single dose not less than 100000-200000 I.U to each ml of antitetanic sera used as therapeutic treatment.

2)Active immunity..

- *tetanus toxoid is used by incubating highly potent toxin for about 14 day e o.2-0.4% formalin until toxicity has been completely destroyed.
- *potassium aluminum sulphate are used to ppt. the toxin if is 2-3 dose at about 3 week interval & so, immunity lasts about 1 year.

3)simaltinuous method..

- *used in endemic area.
- *by injection of antitetanic sera in one side of neck or shoulder & inject toxoid in othe side of neck or shoulder.
- *immunity lates about 18 month.