

The Anaerobes

Bacteroidaceae, Clostridium, and the anaerobic cocci

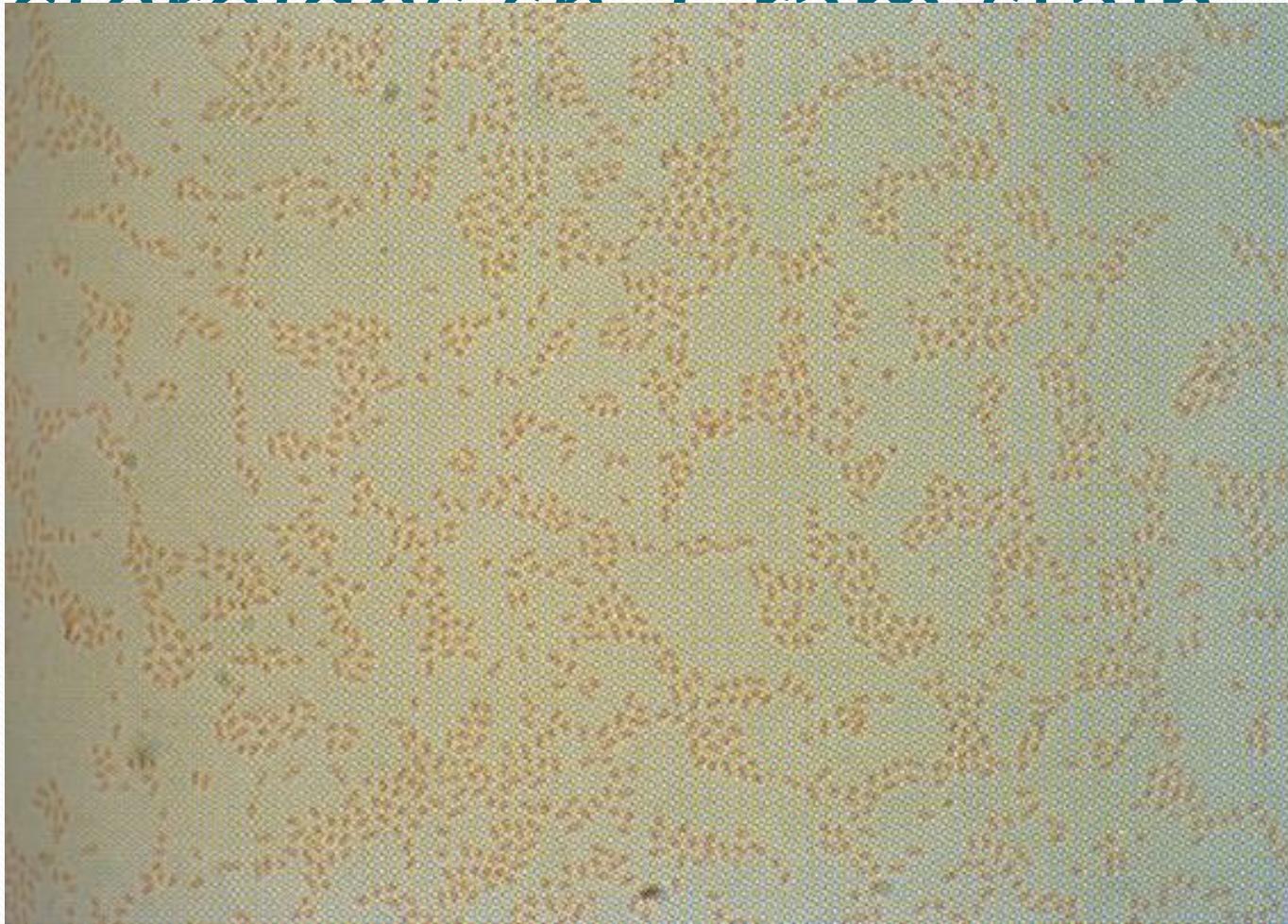
Bacteroidaceae

- Classification
 - Bacteroidaceae family includes the following genera
 - *Bacteroides*
 - *Fusobacterium*
 - *Leptotrichia* (rare in human diseases)
 - *Prevotella*
 - *Porphyromonas*
 - *B. fragilis* is the most commonly isolated anaerobic G-B.
 - All are nonsporing, anaerobic, G-B

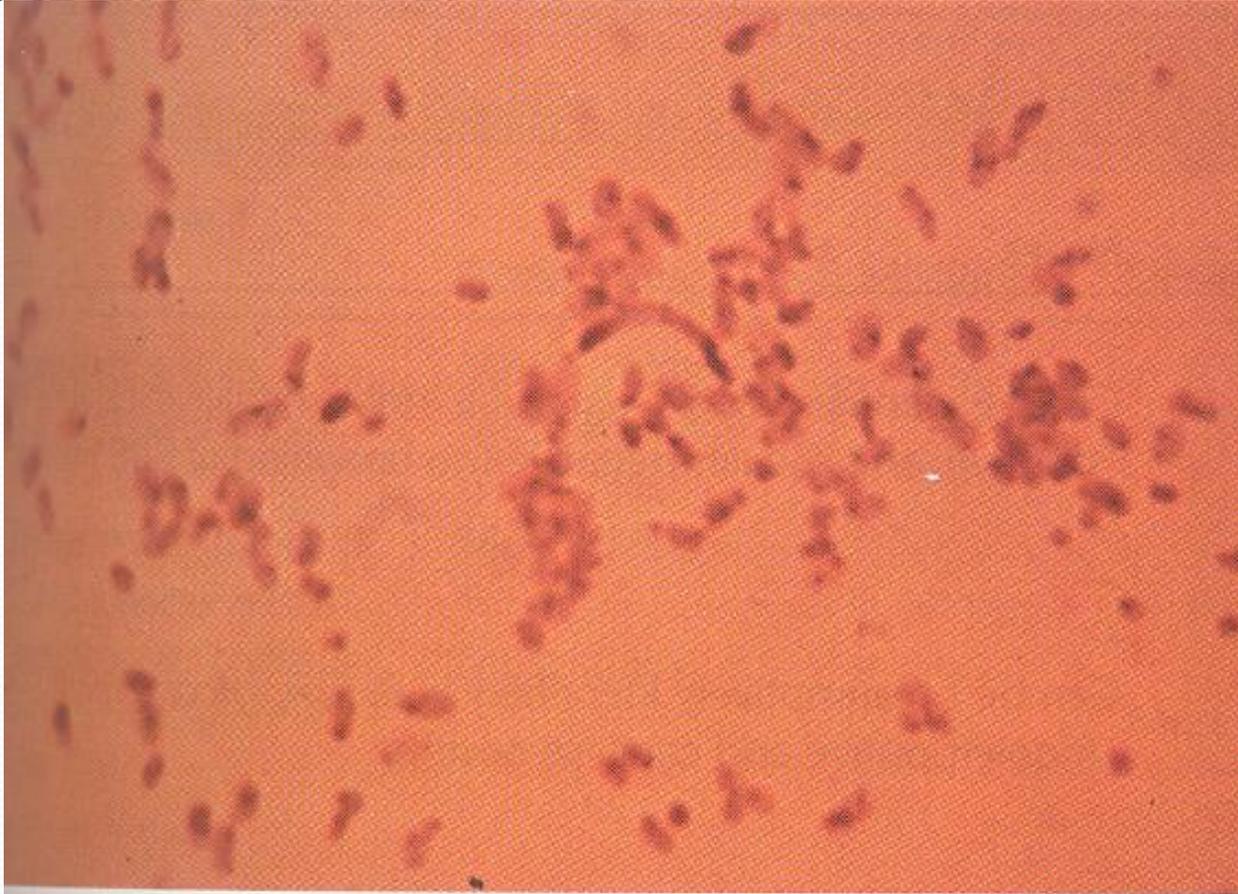
Bacteroidaceae

- They may be NF of the oropharynx, urogenital tract, and colon and are considered opportunistic pathogens
- Morphology/cultural characteristics
 - Pleomorphic G-B showing irregular or bipolar staining.
 - *Fusobacterium nucleatum* characteristically is long and slender with pointed ends

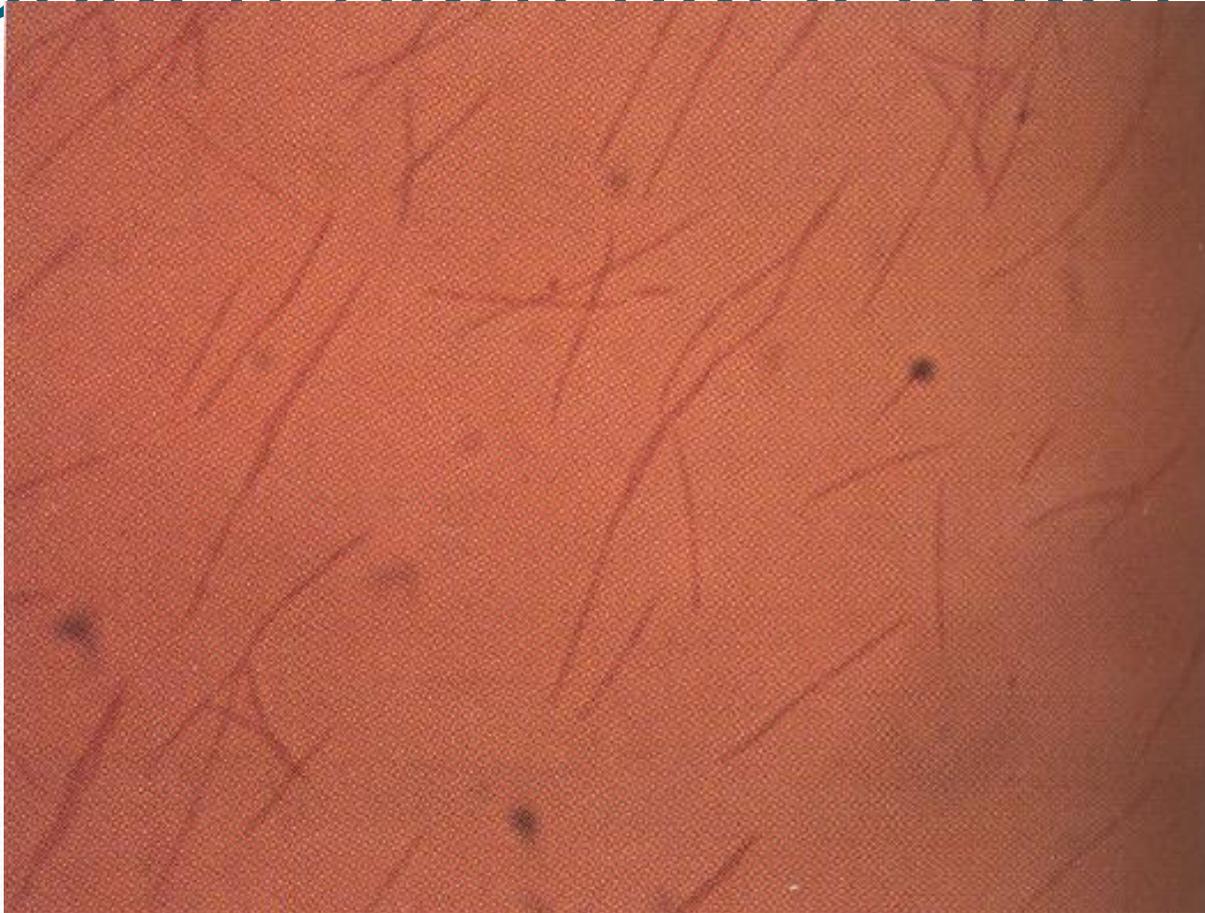
Bacteroides sp. Gram stain



Fusobacterium sp.



Fusobacterium nucleatum



Bacteroidaceae

- To grow these organisms, nonselective anaerobic BA plates, selective anaerobic plates, and liquid should be used for primary isolation.
 - Nonselective anaerobic BA plates= CBA plates plus vitamin K₁, hemin, yeast extract, and L-cystine (supplemented BA)
 - Selective media (all for *Bacteroides species*)
 - Anaerobic PEA BA – suppresses aerobic G-B
 - Kanamycin-Vancomycin BA – inhibits G+ and facultatively anaerobic G -

Bacteriodaceae

- Kanamycin-Vancomycin laked BA (KVLB) prepared by freezing and thawing whole blood
- Bacteroides Bile Esculin (BBE) agar
- Liquid media
 - Thioglycollate
 - Chopped meat glucose
- Must incubate under strict anaerobic conditions
- Incubation at 35-37° C for 48 hours before opening an anaerobic jar.

B. fragilis



Figure 19-26 Appearance of *Bacteroides fragilis* on a KVLB agar (left)/BBE agar biplate (right); browning of the BBE medium is the result of esculin hydrolysis. (Courtesy Anaerobe Systems, San Jose, Calif.)

Bacteroidaceae

- Each colony type that grows should be Gram stained and subcultured to plates grown under both aerobic and anaerobic conditions to confirm that it is an anaerobe.
- Biochemistry
 - The *Bacteroides* group which now includes *Prevotella* and *Porphyromonas* species are divided into groups based on bile tolerance, pigment production, and sensitivity to the antibiotics Vancomycin (V), Kanamycin (K), and Colistin (C)

Bacteriodaceae

	V	K	C	Bile	pigment
<i>Bacteroides fragilis</i>	R	R	R	R	-
<i>Prevotella</i>	R	R	S	S	+/-
<i>Porphyromonas</i>	S	R	R	S	+

- *B. fragilis* is catalase +
- *GLC* – used to differentiate *Fusobacterium* from the others.
 - The major by-product of *Fusobacterium* is butyric acid while the others produce mixtures of acids.
 - *F. nucleatum* and *F. necrophorum* (lipase+) are the major pathogens

GLC



Bacteriodaceae

- Virulence factors
 - *Fusobacterium* – endotoxin; the endotoxin of *Bacteroides* is not highly toxic
 - *B. fragilis* – capsule
 - Some in the *Bacteroides* group produce IgA protease, collagenase, phosphatase, RNAse, or DNAse
- Clinical significance



Bacteroidaceae

- Clinical significance
 - These organisms are NF of the oropharynx, urogenital tract, and colon and cause serious infections when they gain access to tissues and organs.
 - Most commonly they cause intra-abdominal infections
 - The infections are usually polymicrobial infections
 - They can also be found causing:
 - Peridontal disease

Bacteriodaceae

- Chronic otitis media
- Chronic sinusitis
- Wound infections
- Pneumonia
- Female genital tract infections
- Brain abscesses
- Bacterial endocarditis
- Bone infections
- Antimicrobial susceptibility/treatment
 - Incision and drainage
 - Chloramphenicol, clindamycin, cefoxitin, or metronidazole

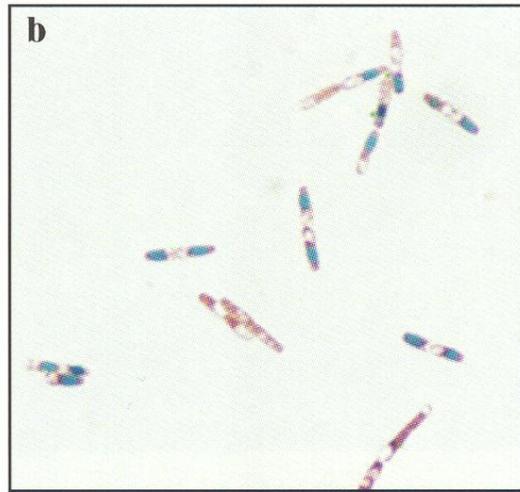
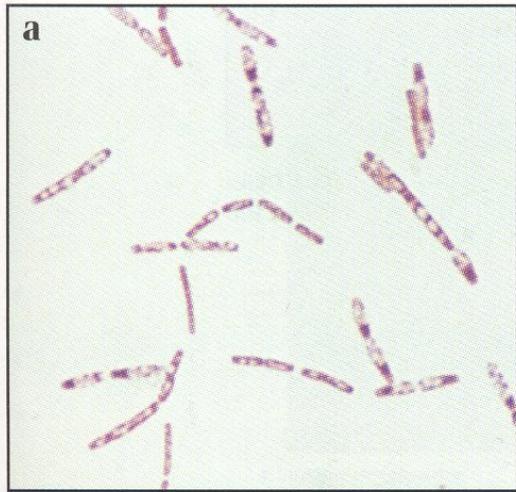
Clostridium

- Classification – no family designation
 - Most are strict anaerobes
 - Are widely distributed in soil and water
 - Some are NF in the GI tract of man and other animals
- Morphology/cultural characteristics
 - Are endospore forming large G+B
 - The site at which the endospore forms in the vegetative cell is characteristic and helps in differentiating *C. tetani* (terminal) from the others (oval and subterminal)

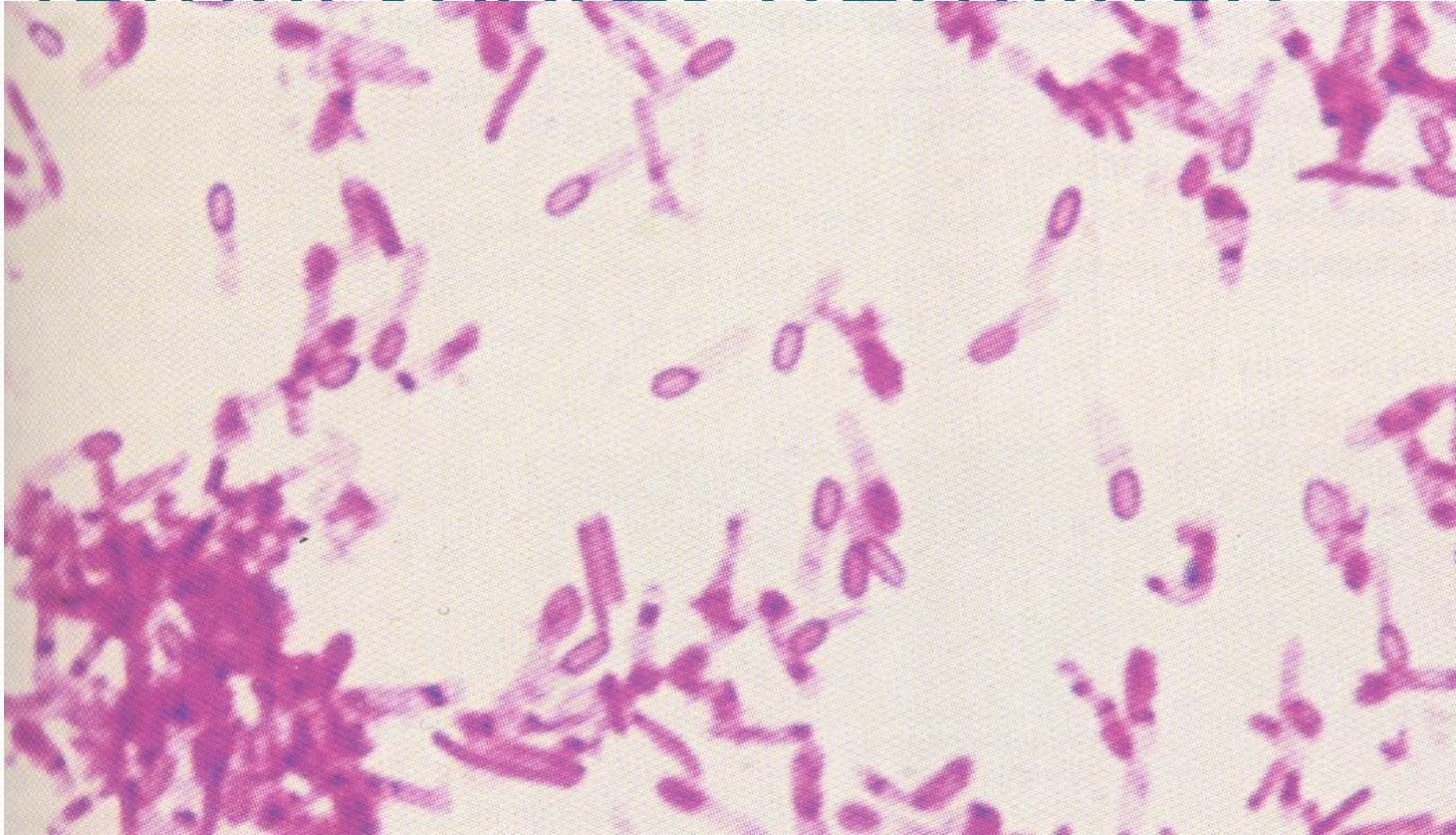
C. perfringens Gram stain



Spore stain with time



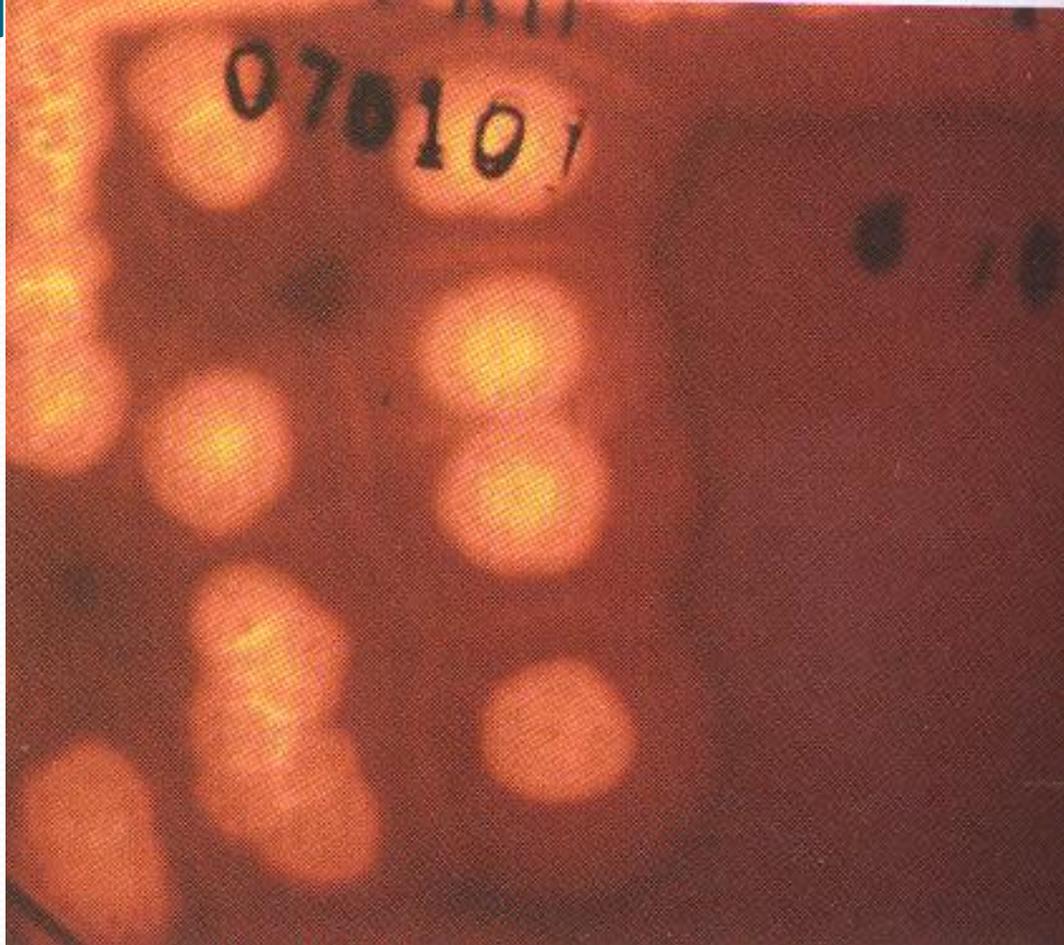
C. tetani spores (terminal)



Clostridium

- All except *C. perfringens* are motile
- Nonselective, selective, and liquid media should be used for primary isolation
 - Nonselective – supplemented anaerobic BA
 - *C. perfringens* produces a classic double zone of hemolysis
 - Nonselective, differential – Egg yolk agar
 - Allows differentiation based on
 - Lecithinase production (white precipitate)
 - Lipase production (sheen around surface of colonies)
 - Protease production (clearing)

C. perfringens double zone hemolysis



Lecithinase production



Lipase production



Clostridium

- Selective – Cycloserine-cefoxitin-egg yolk-fructose agar(CCFA) is selective for *C. difficile*
- Liquid
 - Thioglycollate
 - Chopped meat
- Special isolation procedures – *Clostridia* usually occur in mixed cultures with G-B and nonsporing anaerobes – use heat or alcohol treatment to kill others before plating
- *C. perfringens* grows rapidly at 45° C

CCFA



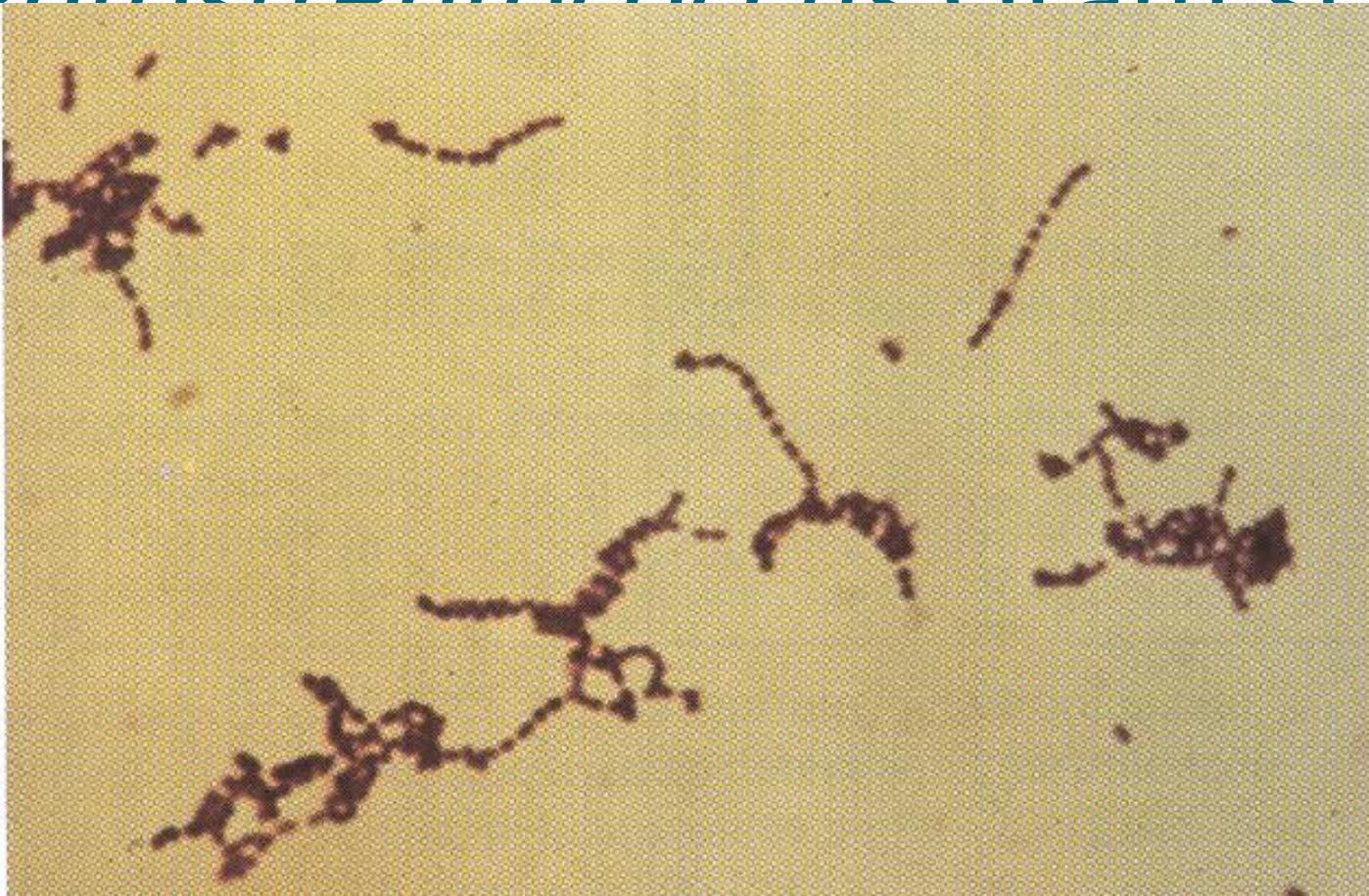
Clostridium

- Biochemistry
 - O₂ tolerance – *C. tertium* and *C. histolyticum* are aerotolerant, but catalase -
 - Lipase vs lecithinase vs protease production on egg yolk agar
 - Naegler reaction - smear ½ of an egg yolk agar plate with type A anti-toxin (anti-lecithinase), streak organism in a single line, and look for inhibition of lecithinase production
 - Sugar fermentations

Anaerobic cocci

- Classification
 - *Peptococcus*
 - *Peptostreptococcus* – anaerobic coccus most often associated with human disease
 - *Veillonella*
 - All can be found as NF of oral cavity, Genital and urinary tracts, G.I. tract , and skin
- Morphology and general characteristics
 - Peptococcus – G+C; 1 species, *P. niger*, produces black colonies
 - Peptostreptococcus G+C; 9 species. *P. anaerobius* is the most commonly isolated species

Pentostreptococcus Gram stain



Anaerobic cocci

- Veillonella – tiny G-C; *V. parvula* is the major isolate of clinical importance.
- All are slow growing and may require prolonged incubation
- Biochemistry
 - I.D. by GLC
 - *P. anaerobius* is presumptively identified based on sensitivity to polyanethol sulfonate (SPS)

Anaerobic cocci

- Clinical significance
 - Usually in polymicrobial infections with aerobic organisms and
 - Caused from spread from a site they normally colonize to an adjacent sterile site
 - Cause pleuropulmonary infections, sinusitis, brain abscesses, intra-abdominal infections, pelvic infections, endocarditis, and osteomyelitis



Anaerobic cocci

- Antimicrobial therapy
 - Cephalosporins, clindamycin, and chloramphenicol