

M.V.SC. (VETERINARY MICROBIOLOGY), MONSOON SEMESTER

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VMC- 602 (BACTERIOLOGY II)
UNIT III

ANAEROBIC NON-SPORE FORMING GRAM NEGATIVE BACILLI- LECTURE 4



(Genus *Bacteroides*)



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Genus *Bacteroides*

- Family: *Bacteroidaceae*
- Gram-negative, non-spore forming, anaerobic, non-motile/motile, pleomorphic rod-shaped
- Non motile except *B. polypragmatus*, *B. xylanolyticus*
- Non capsulated except *B. fragilis*
- May stain irregularly
- Occur singly or in pairs and have rounded ends
- Vacuoles are often present
- Growth on blood agar and *Bacteroides*-bile-esculin (BBE) agar
- Colonies are circular, entire, low convex, translucent to semi-opaque
- Less than 1% of strains are haemolytic



Growth Characteristics:

LKV (Laked Sheep Blood Kanamycin Vancomycin) Agar

Permit to identify Bacteroides by their specific fluorescence activity

Antibiotics inhibit gram-positive and some anaerobic gram-negative bacteria

Hemin and vitamin K are growth factors for Bacteroides

ANAEROBIC PEA (PHENYLETHYL ALCOHOL) AGAR-

Selective medium with addition of phenylethyl alcohol which inhibits facultative anaerobic gram-negative bacilli

For culture:	Brucella Agar with H & K, Chocolate Agar, or Brain Heart Infusion (BHI) Agar
For selective isolation:	LKV Agar, BBE Agar, or Anaerobic PEA Agar
For maintenance:	Cooked Meat Medium, Thioglycollate Broth with Supplements, Brucella Agar with H & K, or BHI Agar



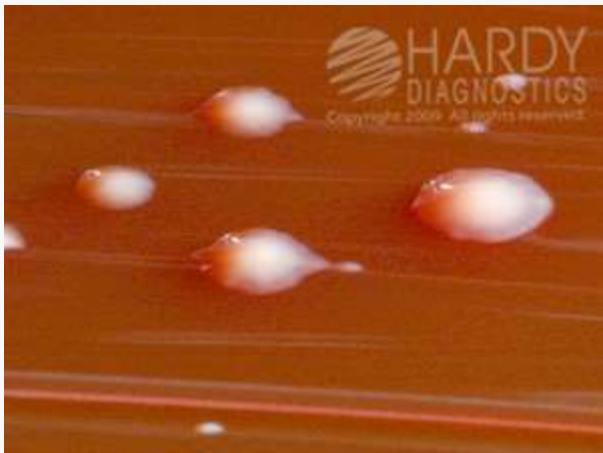
Image source- <https://anaerobesystems.com>

B. fragilis on BBE Agar Plate

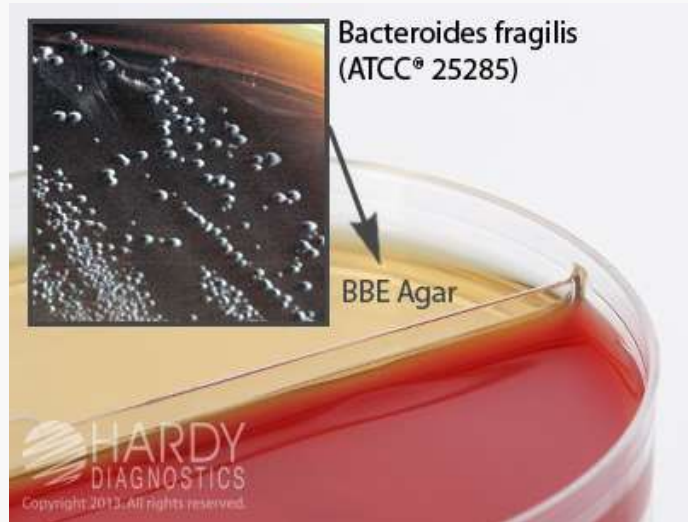


Image source- <https://anaerobesystems.com>

Phenylethyl alcohol blood agar



Bacteroides fragilis colonies on LKV Agar



Bacteroides fragilis
(ATCC® 25285)

BBE Agar

Characteristics:

- Bile-resistant, distinguished from other genera by growth in 20% bile
- Ferments some sugars
- By-products of anaerobic metabolism- acetic acid, isovaleric acid and succinic acid
- *Bacteroides fragilis* group: consists of >20 species
- *B. fragilis* - most clinically important
- Found primarily in intestinal tracts and mucous membranes of humans and animals
- Produce bacteriocin- inhibits other bacteria

Characteristics

(<https://microbenotes.com>)

Basic Characteristics	Properties (<i>Bacteroides fragilis</i>)
Capsule	Positive (+ve)
Catalase	Positive (+ve)
Flagella	Negative (-ve)
Gelatin Hydrolysis	Weakly Positive (+ve)
Gram Staining	Gram-negative (-ve)
Growth in Bile (20%)	Positive (+ve)
Hemolysis	Negative (-ve)
Indole	Variable
Motility	Negative (-ve)
Nitrate Reduction	Negative (-ve)
Oxidase	Variable
Pigment	Negative (-ve)
Urease	Negative (-ve)
Glucose	Positive(+ve)
Glycogen	Positive(+ve)
Inositol	Negative (-ve)
Lactose	Positive(+ve)
Maltose	Positive(+ve)
Sucrose	Positive (+ve)

Enterotoxigenic *B. fragilis*

- Enterotoxin (*B. fragilis* toxin/fragilysin)
 - Heat-labile, approximately 20 kDa protein
 - Causes fluid secretion in intestinal loops
 - Increases bacterial internalization in enterocytes
 - Modulates epithelial permeability
 - Zinc metalloprotease, toxic properties due to proteolytic activity
- The enterotoxigenic strains identified by amplifying the bft gene (by PCR)
- Diagnosis of ETBF infections - detection of bft gene in DNA extracted from feces
- First identified in diarrheic disease of new born lambs
- Diarrhea in lambs, calves, piglets, foals, infant rabbits, and humans, particularly in children

Virulence factors

Three broad categories:

- ❖ Adherence to tissues
- ❖ Protection from host immune response
(oxygen toxicity and phagocytosis)
- ❖ Destruction of tissues

- Fimbriae and agglutinins - adhesins
- Polysaccharide capsule- Antiphagocytic, abscess formation
- Lipopolysaccharide- Stimulates leukocyte chemotaxis and migration
- Superoxide dismutase- Oxygen tolerance
- Wide array of enzymes:
 - Histolytic enzymes (Hyaluronidase, chondroitin sulfatase)- tissue destruction
 - Inactivation of immunoglobulins (IgA, IgM, IgG proteases)
 - Hydrolysis of antibiotics (beta-lactamase)

Disease conditions

- Associated with abscesses, generally intra abdominal, soft tissue infections in humans and animals
- Capsular polysaccharide- major virulence factor for abscesses
- Abscess can be induced in laboratory animals by injecting purified capsular polysaccharide in the absence of bacteria
- **In humans:** intra-abdominal sepsis, peritonitis, liver and brain abscesses, and wound infection
- **Common associated conditions in animals :**
 - ❖ Soft-tissue abscesses, periodontal abscesses, lung and liver abscesses
 - ❖ cellulitis, peritonitis, pyometritis, osteomyelitis
 - ❖ postoperative wound infections
 - ❖ Mastitis
 - ❖ Multiple species - uteri of dairy cows with retained fetal membranes and postparturient endometritis

Bacteroides Species of Veterinary Significance

<i>Bacteroides</i> Species	Associated Disease
<i>B. fragilis</i>	Neonatal diarrhea in foals, calves, piglets, kids, lambs; bovine abortion, mastitis; feline, canine abscesses
<i>B. ovatus</i> , <i>B. thetaiotaomicron</i> , <i>B. vulgatus</i>	Osteomyelitis, soft tissue infections
<i>B. asaccharolyticus</i>	Osteomyelitis in dogs, cats, horses, cattle
<i>B. levii</i>	Mastitis in cows

Laboratory Identification:

- Specimen collection to avoid contamination with normal flora
- Oxygen-free transport medium system
- Bacteroides spp. grow rapidly (within two days) but most other anaerobes are slow growers on selective media
- *B. fragilis* are resistant to 20% bile; kanamycin, vancomycin and colistin
- Animal Inoculation- ETBF strains in mice, produce histopathological alteration in distal ileum-cecum- colon area
- PCR

Treatment, Prevention & Control:

- Carbapenemes, Clindamycin and metronidazole- drug of choice
- Resistant to aminoglycosides.
- Surgical drainage of abscess and removal of necrotic tissue
- Long-term course of antibiotics
- Prophylactic use of antibiotics
- Proper care prior to invasive surgical procedures that disrupt mucosal barriers and immediately following trauma that disrupts mucosal barriers