GRAM POSITIVE BACILLI
Mahon and Manuselis, 2nd edition:
Bacillus sp. pages 390-395
Listeria monocytogenes pages 382-384, Erysipelothrix rhusiopathiae pages 385-386 & Corynebacterium sp. pages 374-380
Gardnerella vaginalis pages 1038-1039, & Lactobacillus sp. pages 386 & 390
Nocardia sp., Actinomyces sp., & Streptomyces sp. pages 395-399

Differentiation of major gram-positive rod genera
- Based upon Gram stain morphology, formation of spores, catalase reaction
  - Spore-forming, catalase positive (aerobic): Bacillus sp.
  - Regularly-shaped, non-spore forming
    - Catalase positive: Listeria monocytogenes
    - Catalase negative: Erysipelothrix rhusiopathiae, Lactobacillus sp., Gardnerella vaginalis
    - Irregularly-shaped, non-spore forming, catalase positive: Corynebacterium sp. ("diphtheroids")
  - Branching: Nocardia sp., Actinomyces sp., & Streptomyces sp.

Spore-forming aerobic or facultative anaerobic bacilli, catalase positive
A. Bacillus species
   1. Epidemiology - Widely distributed in nature (soil, water, airborne dust)
   2. Virulence factors
      a. Production of endospores
         - Spores produced when bacteria is stressed
      b. Some species produce capsules and exotoxins
   3. Clinical Significance
      a. Usually considered environmental contaminants or normal flora
      b. B. anthracis is etiologic agent of anthrax
      c. Opportunistic infections - serious infections can occur in immunocompromised hosts
   4. Laboratory Identification
      a. Gram stain – Large aerobic or facultative anaerobic gram-positive rods that form endospores (may or not exhibit spores)
         - Spores produced when bacteria in stress such as drying conditions, unfavorable temperatures
           - Appear as clear areas with bacterial cell on gram stain
           - Spores do not distended (change shape or swell) the cell wall
           - Aerobic production of spores only
           - Organism can be heat shocked to form spores; heat suspension in 56°C water bath
         - May stain gram variable or negative
         - Perform 3 % KOH test to establish gram reaction if needed
           - Place a drop of 3 % KOH on slide, with a loop emulsify in KOH on slide, gently raise the loop up approximately 0.5 to 1cm:
             - Viscous string (bacterial DNA) follows loop = GNR
             - No viscous string follows loop = GPR
      b. Colony morphology
         - Growth on SBA or CHOC with 24 hours at 35°C in ambient air or 5 % CO2
         - Colonies are usually large, flat with frequent hemolysis
      c. Presumptive Identification – to rule out pathogen B. anthracis
         - Usually catalase positive
         - Most species are:
           - Beta-hemolytic on SBA (B. anthracis is non-hemolytic)
           - Motile (B. anthracis is non-motile)
B. *Bacillus anthracis*

1. **Epidemiology**
   a. Widely distributed in nature
   b. Cause disease in animals and man
      - Animals infected by feeding plants contaminated with spores
      - Human primarily infected as result of contact with animals or animal products
         - Animal hides, fibers, or other animal products
         - Inhalation or traumatic introduction

2. **Virulence factors**
   a. Antiphagocytic capsule
   b. Exotoxins that mediate cell and tissue destruction (edema and lethal toxin, protective factor)

3. **Clinical Significance**
   a. One of the most virulent microorganisms for humans
      - Bioterrorism agent
   b. **Causative agent of anthrax**
      - Cutaneous anthrax – site of spore penetration, ulceration to formation of black eschar, may lead to fatal toxemia (approximately 20 % mortality)
      - Pulmonary anthrax (woolsorter’s disease) – inhalation of spores, respiratory distress, chest edema, cyanosis and death (100 % fatal if not treated very early)
      - Gastrointestinal anthrax – ingestion of spores, most patients die from toxemia and overwhelming sepsis

4. **Laboratory Identification – Lab safety is critical when suspecting *B. anthracis***
   a. Gram stain – large, square-ended, gram-positive rods in singles or chains
      - Subterminal spores (do not distend cell wall)
   b. Colony morphology – non-hemolytic, large, gray flat colonies with irregular margins (filamentous projections – Medusa head) on SBA
      - Bicarbonate agar in 5 % CO2 will induce capsule formation (mucoid colony)
   c. Preliminary Identification
      - Non-hemolytic, non-motile colonies should be sent to reference laboratory of state health laboratory for confirmatory identification (cases reported to state and CDC)
      - Penicillin - Sensitive

5. **Treatment/Antibiotic therapy/Prevention**
   a. Drug of choice is penicillin, supportive therapy may be needed
   b. Animal vaccine is responsible for reducing incidence
   c. Human vaccine is available (military and health care workers)

C. *Bacillus cereus*

1. **Epidemiology**
   a. Widely distributed in nature, human GI tract

2. **Virulence factors**
   a. Toxins (enterotoxin or emetic)

3. **Clinical Significance**
   a. Food poisoning – food contaminated with organism or toxins formed by organism
      - Diarrheal type – abdominal pain and watery diarrhea caused by enterotoxin
         - Associated with fried or boiled rice, symptoms usually 1-6 hours after ingestion, recover 6-24 hours after onset
      - Emetic type – vomiting caused by emetic toxin
         - Associated with poultry, cooked meats, soups and desserts, symptoms usually 10-12 hours after ingestion, recover 12 hours from onset
   b. Serious infections in immunocompromised host
      - Traumatic eye wounds, endocarditis, bacteremia and wounds
4. **Laboratory Identification**
   a. *B. cereus* is normal stool flora, to diagnose food poisoning must culture suspected food NOT stool
   b. Gram stain – large, gram-positive rods w/spores, can stain gram-variable or gram-negative
   c. Colony morphology – beta-hemolytic; large, feathery, spreading on SBA
   d. Preliminary Identification
      - Beta-hemolytic
      - Motile
      - Penicillin – Resistant

**Non-spore-forming bacilli, catalase positive**

A. **Listeria species**
   1. *Listeria monocytogenes*
      a. Pathogenesis
         i. Bacteremia and meningitis in immunosuppressed hosts
         ii. Pregnant females may pass organism onto fetus causing systemic infection and stillbirth
         iii. Ingestion of contaminated food: meat and dairy products
      b. Isolation and identification:
         i. BAP 24-48 hours: **beta-hemolytic (small zone)**; small, grayish-white, translucent, 30-35°C in ambient air or 5% CO₂
            - Facultative
         ii. Gram stain
            - Small gram positive rod (almost coccal), may be in pairs or short chains
            - Non-sporulating
         iii. Identification
            - **Catalase = positive**
            - **Tumbling motility** on wet prep and “**umbrella-shaped**” motility in semi-solid media at room temperature
            - **Esculin hydrolysis** = positive
            - **Sodium hippurate hydrolysis** = positive
            - Ferments glucose
            - Cold enrichment (will grow at 4°C)

B. **Corynebacterium species**
   1. *Corynebacterium* species – “diphtheroids”
      a. General characteristics and morphology
         i. Widely distributed in nature
            - Many species normal flora of skin, mucous membranes
            - Most species are non-pathogenic (referred to collectively as “diphtheroids”)
         ii. Gram stain morphology
            - **Club-shaped and beaded with irregularly staining granules, pleomorphic (many sizes and shapes), palisading (Chinese letters) gram-positive rods**
            - Non-spore forming
iii. Characteristics
  • **Catalase positive**
  • Albert's stain (Loeffler's methylene blue stain) – Babst Ernst granules or metachromatic granules are seen in the organism cells (specific for *Corynebacterium* sp.)
    - Granules stain dark blue/black within greenish rods
  • Glucose fermentation/oxidation: variable
  • Sucrose fermentation/oxidation: variable
  • Urease: variable
  • Nitrate reduction: variable

2. *Corynebacterium diphtheriae* – diphtheria
a. Diphtheria
  • Disease of respiratory tract
  • Pseudomembrane – should be cultured, if not present then culture nose, throat or wound
  • Toxigenic versus non-toxigenic (Exotoxin – toxin)
    - Toxin producing – infected by beta-bacteriophage (virus)
    - Toxin blocks protein synthesis

b. Pathogenesis
  i. Found primarily on the epithelial cells of the respiratory tract of persons with the disease or in carriers
  ii. Infection occurs by droplets or contact to susceptible (no or low antitoxin) individuals
  iii. During infection the organism localizes in upper respiratory tract and produces exotoxin that causes necrosis forming a grayish pseudomembrane (WBCs and organism)
  iv. Toxin is absorbed into the blood and affects the myocardium and peripheral nervous system. Death is usually due to congestive heart failure.

c. Treatment and Prevention
  i. Treatment - antitoxin is given in the form of a toxoid
  ii. Prevention: DPT immunization

d. Isolation & identification
  i. BAP: 24-48 hours at 35°C in ambient or 5% CO₂: small, gray, translucent colonies to medium, white, opaque colonies
  ii. Gram stain morphology
    - Irregularly staining, pleomorphic gram-positive rods
  iii. Identification
    • **Loeffler's media**: used for isolation of *Corynebacterium* species, enhances the granule formation as seen on Albert’s stain and characteristic cellular morphology of *C. diphtheriae*
    • **Tellurite media** (modified Tinsdale agar): tellurite is reduced to metallic tellurium by *Corynebacterium* species causing colonies to appear grayish-black. *Corynebacterium diphtheriae* can be differentiated from other diphtheroids by it having a brown halo around the colony.
    • **Nonlipophilic**
    • **Glucose = “F”**
    • **Sucrose = negative**
    • **Urea = negative**
    • **Nitrate reduction = variable**
3. **Corynebacterium jekeium**
   a. Disease states
   - Immunosuppressed patients – septicemia, meningitis, pulmonary disease
   b. Isolation & Identification
      i. BAP: 48-72 hours at 35°C in ambient air or 5% CO₂ - small, gray to white colony, non-hemolytic
      ii. Gram stain
         - Pleomorphic; occasionally, club-shaped gram-positive rods arranged in V forms or palisades
      iii. Identification
         - Lipophilic (growth is enhanced with lipid added to media such as Tween 80)
         - Glucose = “O”
         - Sucrose = negative
         - Urea = negative
         - Nitrate reduction = negative
   c. Susceptibility testing
      - Exhibits resistance to multiple antibiotics usually used to treat gram-positive infections, to date all isolates have been susceptible to vancomycin

Non-spore-forming bacilli, catalase negative
A. **Gardnerella species**
   1. **Gardnerella vaginalis**
      a. Morphology and characteristics
         i. Colonial morphology
            - Does not grow on SBA
            - Growth on Human Blood agar (V, HBT) shows beta-hemolysis
         ii. Gram stain
            - Pleomorphic, gram variable rod
            - Non-sporulating
            - Specimen gram stain: clue cells: epithelial cells covered with tiny bacilli especially around the edge
         ii. Identification
            - Catalase = negative
            - Sodium hippurate = usually positive
            - SPS = sensitive
   b. Disease states
      i. Bacterial vaginosis (a polymicrobial infection w/Mobiluncus & Bacteroides)
         - Diagnosis – presence of homogeneous, gray discharge, clue cells seen on Gram stain or wet mount, amine or fishy odor when 1 drop of 10%KOH added to discharge on a slide
         - Culture is not recommended for diagnosis
B. Erysipelothrix species
   1. *Erysipelothrix rhusiopathiae*
      a. Morphology and characteristics
         i. Colonial morphology
            - Microaerophilic
            - Non-hemolytic or alpha-hemolytic on blood agar
         ii. Gram stain
            - Both short gram-positive rods and long filamentous rods corresponding to 2 colony types
            - Non-sporulating
      b. Disease states
         i. Erysipelas
            - Zoonotic
            - In swine, it produces an important economic disease called swine erysipelas that is generally fatal
            - Man becomes infected by coming in direct contact with an infected animal
            - Organism enters abraded skin (often finger or hand)
            - A skin disease that is characterized by intense pain and is usually self-limited
            - Rare cases become serious, disseminating to septicemia with arthritis or endocarditis
            - There is no permanent immunity and relapses are common

C. Lactobacilli
   1. *Lactobacillus* species
      a. Morphology and characteristics
         i. Colonial morphology
            - Microaerophilic – incubate in 5-10% CO₂
            - May show alpha-hemolysis on blood agar
         ii. Gram stain
            - Long slender gram positive rods in chains, or short coccobacilli
            - Non-sporulating
      b. Normal flora – mouth, gastrointestinal tract, female genital tract
      c. Disease states
         - Rarely pathogenic – implicated in rare cases of endocarditis and meningitis
Branching Nocardioform Bacilli

A. Nocardia species
   1. *Nocardia asteroides, N. brasiliensis, N. caviae*
      a. Morphology and characteristics
         i. Colonial morphology
            • Aerobic growth appears in 3-30 days
            • Waxy, bumpy or velvety rugose forms, yellow to orange
            • Will grown on SBA, mycology media and LJ media
         ii. Gram stain
            • Pleomorphic, branching, fine, delicate filaments with fragmentation, gram-positive rods that are often beaded in appearance
         iii. Identification
            • Partially acid-fast positive
   b. Habitat
      i. Soil and water
   c. Disease states
      i. Mycetoma (actinomycetoma) – a chronic, localized, painless, subcutaneous infection
         • Tissue swelling
         • Draining sinus tracts
         • Presence of granules
      ii. Lymphocutaneous infections
      iii. Skin abscesses or cellulitis
      iv. Immunocompromised patients – pulmonary and disseminated infections

B. Streptomyces species
   1. *Streptomyces somaliensis, S. anulatus, S. paraguayensis*
      a. Morphology and characteristics
         i. Colonial morphology
            • Aerobic growth appears in 3-30 days
            • Waxy, bumpy or velvety rugose forms, yellow to orange
            • Will grown on SBA, mycology media and LJ media
         ii. Gram stain
            • Gram positive rods with extensive branching, chains and spores; does not fragment easily
         iii. Identification
            • Acid-fast = negative
   b. Habitat
      i. Soil and decaying vegetation
   c. Disease states
      i. Mycetoma (actinomycetoma) – a chronic, localized, painless, subcutaneous infection
Examples:
*Mycobacterium tuberculosis, Mycobacterium avium* complex (“MAC attack” in HIV+ individuals)

**Distinguishing Characteristics**

A. Aerobic, non-spore formers

B. Organism does not stain readily because of high lipid content in the cell wall. With the Gram stain procedure, the organism appears gram-positive and stains irregularly giving it a “beaded” appearance.

C. Acid-fast: the organism retains stain even after attempts to decolorize with acid-alcohol, acids or acid-acetone solutions. This is due to a unique fatty acid in the cell wall – mycolic acid.

   Carbolfuschin stains (Ziehl-Neelsen/Kinyon) – also known as acid-fast stains: acid fast organisms stain red against a blue background
   - Fuchsins = primary stain (red or magenta)
   - Methylene blue = counter stain (blue)

D. Growth requirements
   1. 5-10% CO$_2$
   2. 35-37ºC
   3. 3-8 weeks for growth on solid media, 10-11 days liquid media systems

**Safety – Biosafety Level 3 Procedures**

A. Control aerosols
   1. Biological safety hood – Level II
   2. Centrifuges with self-contained carriers
   3. Wear mask, gloves, and lab coat or gown

B. Use appropriate germicide
   1. Amophyl (phenol-soap mixture)
   2. 10% bleach
   3. 70% ethanol
   4. 5% phenol

C. Ultraviolet (UV) light