CORYNEBACTERIUM SPECIES

- Small Gram-positive pleomorphic (coccoid, club and rod forms) bacteria
- Stained smear reveals cells in palisades of parallel and angular clusters resembling Chinese letters
- Non-motile facultative anaerobes
- Catalase-positive, oxidase-negative
- Fastidious, require enrichment for growth
- Cause pyogenic infection
- Most pathogenic species are host specific
- Type species: C. diptheriae, causes diphtheria in children

Diseases

i. Corynebacterium bovis
   Host (cattle): subclinical mastitis

ii. C. kutscheri
    Host (laboratory rodents): superficial abscesses, causes purulent foci in liver, lungs and lymph nodes

iii. C. pseudotuberculosis (non-nitrate-reducing biotype)
    Host (Sheep and goats): caseous lymphadenitis

iv. C. pseudotuberculosis (nitrate-reducing biotype)
    Host (horses, cattle): ulcerative lymphagitis, abscesses

v. C. renale (type I)
   Cattle: cystitis, pyelonephritis
   Sheep and goats: ulcerative (enzootic) balanoposthitis

vi. C. pilosum (renale type II)
   Cattle: cystitis, pyelonephritis

vii. C. cystitides (renale type III)
    Cattle: severe cystitis, rarely pyelonephritis

viii. C. ulcerans
    Cattle: mastitis
Diagnosis

- Specimen: pus, exudates, tissue, sample, mid-stream urine
- Direct microscopy of Gram-stained smear may reveal coryneform bacteria
- Inoculate sample onto blood agar, selective media (McLeod's blood agar, Loeffler’s medium) containing potassium tellurite, and MacConkey agar
- Incubate aerobically at 37°C for 24 to 48 hours
- Identification: no growth on MacConkey agar
- Colonial Characteristics:
  - *C. bovis*: a lipophilic bacterium. Small white, dry, non-haemolytic colonies
  - *C. kutscheri*: whitish colonies, occasionally haemolytic
  - *C. pseudotuberculosis*: small whitish colonies, surrounded by a narrow zone of complete haemolysis evident after 72 hours of incubation. Colonies become dry, crumbly and cream-coloured with age
  - Members of *C. renale* group produce small, non-haemolytic colonies after 24 hours incubation. Produce pigment after 48 hours of incubation
- Microscopy: Gram’s staining and Albert’s staining techniques
  - Albert’s staining demonstrate metachromatic granule (inclusions)
- Biochemical tests
  - Nitrate reduction: *C. pseudotuberculosis* biotype
  - All pathogenic corynebacteria are urease positive except *C. bovis*

**Differentiation of C. renale group**

<table>
<thead>
<tr>
<th>Feature</th>
<th>C. renale (type I)</th>
<th>C. pilosum (type II)</th>
<th>C. cystidis (type III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of colony</td>
<td>Pale yellow</td>
<td>Yellow</td>
<td>White</td>
</tr>
<tr>
<td>Growth in broth at pH 5.4</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Acid from xylose</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Acid from starch</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Casein digestion</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrolysis of Tween 80</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Enhanced haemolysis by *C. pseudotuberculosis* when inoculated across a streak of *Rhodococcus equi*

**ACTINOMYCES ARCANOBACTERIUM AND ACTINOBACULUM SPECIES**

- Gram-positive bacteria
- Require enriched media for growth
- Non-motile, non-sporing
- Morphologically heterogeneous
- Anaerobic or facultative anaerobic
- Modified Z-N staining negative
- Some members have undergone changes in nomenclature
  - *Corynebacterium pyogenes* = *Actinomyces pyogenes* = *Arcanobacterium pyogenes*
- *Actinomyces species* have long filamentous morphology, although short V, Y, and T configuration also occur
- *Arcanobacterium* and *Actinobacterium* both have a coryneform morphology

**Diseases**

- **Arcanobacterium pyogenes**
  - Host: cattle, sheep, pigs
  - Conditions: Abscessation, mastitis, suppurative pneumonia, endometritis, pyometra, arthritis, umbilical infections
- **Actinomyces hordeovulneris**
  - Host: dogs
  - Conditions: cutaneous and visceral abscessation, pleuritis, peritonitis, arthritis
- **Actinomyces viscosus**
  - Host: dogs
  - Conditions: canine actinomycosis
    - cutaneous pyogranulomas
    - pyothorax and proliferative pyogranulomatous pleural lesions
    - disseminated lesions (rare)
- **Actinomyces bovis**
  Host: cattle
  Conditions: bovine actinomycosus (lumpy jaw)

- **Actinomyces viscosus**
  Horses: cutaneous pustules
  Cattle: abortion

- **Actinomyces spp (unclassified)**
  Pigs: pyogranumatous mastitis
  Horses: poll evil, fistulous withers

- **Actinobaculum suis**
  Pigs: cystitis, pyelonephritis

**Diagnosis**
- Clinical specimens: exudates, aspirates and tissue samples from post-mortem
- Direct Gram staining of smear may reveal morphological forms of aetiological agent
- Inoculate blood and MacConkey agars and incubate at 37°C for up to 5 days. Different species have peculiar atmospheric requirement for culture
- **Identification criteria**
  - *Arcanobacterium pyogenes* produce a characteristics hazy haemolysis along streak lines after 24 hours of aerobic incubation. Pin point colonies are seen after 48 hours. Proteolytic, hydrolyses gelatine
  - *Actinomyces bovis*: adhere to agar media and produces no haemolysis
  - *Actinomyces hordeovulneris*: same as *A. bovis*
  - *Actinomyces viscosus*: produce two colony types
    - Large and smooth: V,Y, and T cell configurations
    - Small and rough: short branching filament
  - *Actinobaculum suis*: poor haemolysis on ruminant blood agar. Colonies have a shiny raised centre and a dull edge. It is urease positive
### SPECIES DIFFERENTIATIONS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Actinomyces bovis</th>
<th>Actinomyces viscosus</th>
<th>Actinomyces hordeovulneris</th>
<th>Arcanobacterium pyogenes</th>
<th>Actinobaculum suis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Filamentous</td>
<td>Filamentous</td>
<td>Filamentous</td>
<td>Coryneforms</td>
<td>Coryneform</td>
</tr>
<tr>
<td></td>
<td>branching, some short forms</td>
<td>branching, short forms</td>
<td>branching, short forms</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Atmospheric requirement</strong></td>
<td>Anaerobic + CO₂</td>
<td>10% CO₂</td>
<td>10% CO₂</td>
<td>Aerobic</td>
<td>Anaerobic</td>
</tr>
<tr>
<td><strong>Haemolysis on sheep blood agar</strong></td>
<td>±</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td><strong>Catalase production</strong></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pitting on Loeffler’s serum slope</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Granules in the pus</strong></td>
<td>Sulphur granules</td>
<td>White granules</td>
<td>No granules</td>
<td>No granules</td>
<td>No granule</td>
</tr>
</tbody>
</table>

- Granules in lesion is caused by A. bovis contains characteristic clubs. Club colonies are also produced by *Actinobacillus ligniersii* and *Staphylococcus aureus* botryomycosis

### RHODOCOCCUS EQUI

- Gram-positive aerobic bacteria
- Non-motile catalase-positive, oxidase-negative
- Weakly acid fart
- Grows on non-enriched media
- Rod or coccibacillus in shape
- Produces pigments, colonies are pink
- It forms capsule. Produces large, moist, viscid/mucoid colonies

**Diseases**
Foals of 1 to 4 months of age: suppurative bronchopneumonia and pulmonary abscessation

Horse: superficial abscessation

Pigs, Cattle: mild cervical lymphadenopathy

Cats: subcutaneous abscesses, mediastinal granulomas

**Diagnosis**

- Specimens: tracheal aspirates, pus from lesion
- Inoculate blood and MacConkey agar
- Incubate aerobically at 37 °C for 24 to 48 hours
- No growth on MacConkey
- Does not ferment carbohydrate
- Does not haemolyse on blood agar. It is cAMP test positive. (enhanced haemolysis) with *S. aureus*
- Most strains are urease and H$_2$S positive

**Tutorial Questions**

1. Describe the type of colouration produced when *Listeria monocytogen* colonies are viewed under oblique illumination

2. What is the significance of Anton’s test in the diagnosis of *Listeria monocytogenes*

3. Describe the cold enrichment procedure for the diagnosis of *Listeria monocytogenes*

4. What is the aetiological agent of diamond skin disease of pigs

5. List two selective media for the isolation of *Corynebacterium spp*

6. What staining technique is employed for the demonstration of *Corynebacterial metachromatic* ranules

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**PSEUDOMONADACEAE**

- Pathogenic members that infect animals include:
  
  *Pseudomonas aeruginosa*

  *Burkholderia mallei*

  *Burkholderia pseudomallei*
- Gram negative rods of medium size
- Obligate aerobes
- Oxidase-positive and catalase-positive
- *Pseudomonas species* and *Burkholderia pseudomallei* are motile by polar flagella
- *Burkholderia mallei* is non-motile and require 1% glycerol for enhanced growth
- *P. aeruginosa* produces pigments which diffuse into culture media
- Pigments of *P. aeruginosa* include:
  - Pyocyanin: blue-green
  - Pyoverdin: greenish-yellow
  - Pyorubin: red
  - Pyomelanin: brownish-black

**Diseases**

- *P. aeruginosa*: causes opportunistic infection in many species of animals
  - Cattle: mastitis, metritis, pneumonia, calve enteritis, dermatitis
  - Pigs: Ear infection, respiratory tract infection
  - Horses: genital tract infection, pneumonia, eye infection
  - Sheep: mastitis, pneumonia, otitis media, fleece rot/ suppurative dermatitis
  - (predisposing factor: heavy rainfall)
  - Dogs and Cats: pneumonia, ulcerative keratitis, cystitis, otitis externa
  - Minks: haemolytic pneumonia, septicaemia, farmed minks very susceptible
  - Rabbits: pneumonia, septicaemia
  - Reptiles: necrotic stomatitis, especially in captive reptile (found in oral cavity of snakes)
- *Burkholderia mallei*: glanders (a contagious disease of equidae characterized by the formation of nodules and ulcers in the respiratory tracts or on the skin)
- *Burkholderia pseudomallei*: causes melioidosis-chronic debilitating disease with disseminated abscesses in many organs of the body
- *Pseudomonas flourescense and P. putida*: pathogens of freshwater fish

**Diagnosis**
- Sample collection: based on observed clinical signs and lesions. Samples may include pus, respiratory aspirates, ear swab, mastitic milk, discharges, blood (for serology) etc.
- Inoculate blood agar and MacConkey agar plates
- Incubate aerobically for 24 to 48 hours at 37°C
- *B. mallei* grows on media containing 1% glycerol and also on MacConkey agar
- Identification criteria:
  - Colonial morphology
  - Microscopy
  - Biochemical reactions
- Serology
  - Compliment fixation test and agglutination technique for *B. mallei* detection
  - Slide agglutination, ELISA, CFT, indirect haemagglutination test used for detection of *B. pseudomallei* serum antibodies
- The mullein test: an efficient field test for screening and confirmation of glanders in animals. Mallein is a glycoprotein extract of *B. mallei*
  - It is injected intradermally just below the lower eyelid
  - A local swelling with mucopurulent ocular discharge is evident after 24 hours in positive cases
- *P. aeruginosa*: produces pigments detectable in media that contains no dye e.g. nutrient agar. It also has a characteristic fruity, grape-like odour

### Comparative features of the Pseudomonadaceae

<table>
<thead>
<tr>
<th>Feature</th>
<th><em>P. aeruginosa</em></th>
<th><em>B. mallei</em></th>
<th><em>B. pseudomallei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonial morphology</td>
<td>Large and flat with serrated edges</td>
<td>White and smooth becoming granular and brown with age</td>
<td>Range from smooth and mucoid to rough and dull becoming yellowish brown with age</td>
</tr>
<tr>
<td>Haemolysis on blood agar</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Diffusible pigment</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Colony odour</td>
<td>Grape-like</td>
<td>None</td>
<td>Musty</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>Growth on MacConkey agar</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 42°C</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase production</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
</tbody>
</table>

| Oxidation of carbohydrate: |   |   |   |
| Glucose      | + | + | + |
| Lactose      | - | - | + |
| Sucrose      | - | - | + |

**ENTEROBACTERIACEAE**

- Members are Gram-negative rods about 3 µm in length
- Oxidase-negative, catalase-positive
- Ferment glucose and a variety of other sugars
- Non-sporing facultative anaerobes
- Reduce nitrates to nitrites
- Mostly enteric organisms
- Motile members possess peritrichous flagella
- Grow well on MacConkey agar because they tolerate bile salts
- Categorised into two broad groups based on lactose fermentation
  - Lactose fermenters e.g. *E. coli*, *Klebsiella spp*
  - Non-lactose fermenters e.g. *Salmonella spp*, *Proteus spp*
- Major animal pathogens (cause both enteric and systemic diseases)
- Examples:
  - *E. coli*
  - *Salmonella* serotype
  - *Yesinia* spp
    - *Y. pestis*
- *Y. enterocolitica*
- *Y. pseudotuberculosis*
- *Y. intermedia*
- *Y. kristensenii*
- *Y. frederiksenii*
- *Y. ruckerii*: pathogen of fish

- Opportunistic pathogens cause disease outside the GIT
- Major pathogens, cause disease in both enteric and non-enteric locations

**Yersinia species:**

- Yesinia stain bipolar on primary isolation
- Yersinia are intracellular organisms localizing in macrophages
  - *Y. pestis:*
    - It is pleomorphic
    - It produces little or no turbidity and small deposit in broth culture
    - Haemin required for aerobic growth on nutrient agar
    - Two forms of colony: smooth and rough
    - Causes plaque: bubonic plaque, (septicemic, pneumonic sylvatic forms). Characterized by lymphadenitis
    - Virulence factor F1 or fraction I (capsular/envelope heat-labile protein), V (protein), W (lipoprotein), F (factor antigens)
    - Probably produces toxin
    - Virulence strains kill mice or guinea pigs following intraperitoneal or subcutaneous injection with as low as 10 viable organisms
    - Transmission: Wild rat (through flea) to town rat (through flea) to humans

**Diagnosis**
- Blood sample, materials from lymph nodes
- Grow on blood agar and selective media
- Fluorescent antibody test on cerebrospinal fluid and in aspirates

Note:
- Colonies of non-lactose fermenting bacteria are alkaline due to utilization of peptone in medium. They are pale
- Colonies of lactose fermenters are pink due to acid production from lactose
- Somatic (O), flagellar (H), and capsular (K) antigens are used for serological identification and classification of the enterobacteriaceae

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### Differentiation and Identification of the Enterobacteriaceae

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>Salmonella serotype</th>
<th>Yersinia species</th>
<th>Proteus species</th>
<th>Enterobacter serotype</th>
<th>Klebsiella pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical importance</strong></td>
<td>Major pathogens</td>
<td>Major pathogen</td>
<td>Major pathogen</td>
<td>Opportunistic pathogen</td>
<td>Opportunistic pathogen</td>
<td>Opportunistic pathogen</td>
</tr>
<tr>
<td><strong>Cultural characteristics</strong></td>
<td>Some strains haemolytic</td>
<td>-</td>
<td>-</td>
<td>Swarming growth</td>
<td>Mucoid</td>
<td>Mucoid</td>
</tr>
<tr>
<td><strong>Motility at 30°C</strong></td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Non-motile</td>
</tr>
<tr>
<td>Lactose fermentation</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IMV,C test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole production</td>
<td>+</td>
<td>-</td>
<td>V</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl red test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Voges ProsKauer test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrate utilization test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H₂S production in TSI agar</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lysine decarboxylate</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Urease production

|       | - | - | + | + | - | + |

**Yersinia pseudotuberculosis**

- Causes infection in many animals including guinea pigs, mice, rats, rabbits, chicken, turkey, pigeons, and canaries
- Sporadic cases reported in horses, cattle, sheep, goats, pigs and cats
- Produced in necrotic nodules in ileum and caecum as well caseous necrosis of mesenteric lymph nodes and omentum
- Grows on blood agar, MacConkey and Salmonella-shigella agar at $37^\circ C$ and at room temperature ($22^\circ C - 28^\circ C$)
- Samples of isolation of organism: liver, spleen, heart blood

**Yersinia enterocolitica**

- Grows on blood agar, Salmonella-shigella agar, desoxycholate citrate agar (DCA) and MacConkey agar
- May require enrichment in phosphate buffered solution (pH 7.6) or peptone broth at $4^\circ C$ for 3 weeks
- Must be differentiated from Pasteurella

*Note the following characteristic of Pasteurella: MR negative, Oxidase positive, no growth on MacConkey except Manhemia haemolytica*

**Proteus**

- *P. vulgaris*
- *P. mirabilis*
- Pathogenic role doubtful
- May cause diarrhoea in young animals
- Otitis media in dogs
- Often causes infection only when found outside the intestinal tract
- Associated with chronic urinary tract infections

**Diagnosis**
- Produces characteristic smell and swarms on solid media

**Klebsiella**

*K. pneumoniae*
- Pneumoniae in humans
- Klebsiella and Enterobacter cause neonatal meningitis in children
- Opportunistic infections in animals
- Pneumonia in fowls, metritis in mare and sow
- Mastitis (chronic) in cow
- Complicate airsac infection and pullorum disease in poultry
- Other species: *K. ozaenae*, *K. rhinoscleromatis*

** Providencia**

*P. stuartii*, *P. rettgeri*, *P. alcalifaciens*
- Involved in urinary tract infection, sepsis, pneumonia and wound infections
- Hospital infection

**Morganella**

*M. morganii*
- Hospital infection
- Implicated in summer diarrhoea in children

**Biochemical differentiation of Proteus species**

<table>
<thead>
<tr>
<th></th>
<th>Proteus vulgaris</th>
<th>Proteus mirabilis</th>
<th>Providencia rettgeri</th>
<th>Morganella morgani</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltose fermentation</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
<td>Delayed</td>
<td>-</td>
</tr>
<tr>
<td>fermentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole production</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H₂S production</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Urease production</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Salmonella**

Selective media:

- Desoxycholate citrate agar: slightly opaque often with central black spot
- Brilliant-green agar: *S. typhi, S. gallirum, S. pullorum, S. cholerae-suis and S. typhi-suis* do not grow on the agar. Colonies are pale-pink usually surrounded by a pink zone. Colonies have a translucent dew-drop appearance
- Wilso and Blair agar: colonies are black
- Salmonella-shigella agar: colonies are pale or colourless
- Hektoen enteric agar: blue-green with black centre
- Motile except *S. galinarium* and *S. pullorum*
- Enrichment media:
  - Selenite F. broth
  - Tetrathionate broth
  - Rappaport broth

### Reactions of Members of Enterobacteriaceae in Triple Sugar Iron (TSI) agar

<table>
<thead>
<tr>
<th>Species</th>
<th>pH change</th>
<th>H₂S production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slant</td>
<td>Butt</td>
<td></td>
</tr>
<tr>
<td>Salmonella serotype</td>
<td>Red (alkaline)</td>
<td>Yellow (acid)</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Y. pseudotuberculosis</em></td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Y. pestis</em></td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Shigella species</em></td>
<td>Yellow</td>
<td>Red</td>
</tr>
</tbody>
</table>

**Shigella**

- Non-motile
- Non-sporing
- Non-capsulated
- Oxidase-negative, catalase-positive
- *Shigella dysenteriae* type I is catalase negative
  - Species
    - *Sh. dysenteriae* (Tropics): dysentery in human and monkey (shigellosis, colitis)
    - *Sh. flevneri* (Tropics): dysentery in human and monkey (shigellosis, colitis)
    - *Sh. boydii* (Tropics): dysentery in human and monkey (shigellosis, colitis)
    - *Sh. sonnei* (temperate): dysentery in human and monkey (shigellosis, colitis)

**Diagnosis**

- Sample: fresh stool
- Small colonies on DCA and MacConkey agar
- *Shigella dysenteriae* type I does not grow on DCA
- No growth on Wilson and Blair medium
- Grow on S-S agar and Hektoen enteric agar producing pale and green colonies respectively
- May be inhibited to a certain extent by selenite F broth

Biochemical reactions:

<table>
<thead>
<tr>
<th>Glucose fermentation</th>
<th>Positive (acid only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose fermentation</td>
<td>Negative</td>
</tr>
<tr>
<td>Sucrose fermentation</td>
<td>Negative</td>
</tr>
<tr>
<td>Mannitol fermentation</td>
<td>Variable</td>
</tr>
<tr>
<td>Indole production</td>
<td>Variable</td>
</tr>
<tr>
<td>MR reaction</td>
<td>Positive</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td>Negative</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>Negative</td>
</tr>
<tr>
<td>H₂S production</td>
<td>Negative</td>
</tr>
<tr>
<td>Urease production</td>
<td>Negative</td>
</tr>
<tr>
<td>Motility</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Biochemical differentiation of Shigellae**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sh. dysenteriae</th>
<th>Sh. Flexneri</th>
<th>Sh. boydii</th>
<th>Sh. Sonnei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Acid (A)</td>
<td>A/A and G (gas)</td>
<td>Acid</td>
<td>Acid</td>
</tr>
<tr>
<td>lactose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Late fermenter</td>
</tr>
<tr>
<td>Mannose</td>
<td>-</td>
<td>Acid</td>
<td>Acid</td>
<td>Acid</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>-</td>
<td>-/A</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ONPG test</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Indole</td>
<td>Variable</td>
<td>Strain variation</td>
<td>Variable</td>
<td>-</td>
</tr>
</tbody>
</table>

ONPG: Orthonitrophenol (β-D-galactopyranoside)