

Module III Gram Positive Rods and Filaments

Lesson 1a Corynebacterium and Eubacterium

Lesson 1b Eubacterium

Lesson 1c Rhodococcus

Lesson 2 The Actinomycetes

Lesson 3a Listeria

Lesson 3b Erysipelothrix

Lesson 3c Bacillus

LESSON 2

THE ACTINOMYCETES

- The actinomycetes are a phylogenetically diverse group of Gram-positive bacteria, which tend to grow slowly and produce branching filaments.
- Because of filament formation and granulomatous responses to tissue invasion, these organisms were originally regarded as fungi.
- However, filaments of the prokaryotic actinomycetes rarely exceed 1 μm in width, whereas hyphae of the eukaryotic fungi are usually more than 5 μm wide.
- The actinomycetes which cause disease in domestic animals belong to the genera *Actinomyces*, *Arcanobacterium*, *Actinobaculum*, *Nocardia* and *Dermatophilus*.
- Some thermophilic actinomycetes, such as *Micropolyspora faeni* found in poor-quality overheated hay, produce spores which can induce allergic pulmonary disease in cattle, horses and man.
- *Streptomyces* species are saprophytic soil actinomycetes and are common contaminants on laboratory media. They elaborate a variety of antimicrobial substances, many with therapeutic activity.

Key points

- ✓ Gram-positive bacteria, many species with branching filaments
- ✓ Relatively slow growth on laboratory media
- ✓ Opportunistic pathogens producing diverse inflammatory responses
- ✓ *Actinomyces*, *Arcanobacterium* and *Actinobaculum* species
 - Anaerobic or facultatively anaerobic
 - Morphologically heterogeneous
 - Non-spore-forming, non-motile
 - MZN-negative
 - Colonize mucous membranes
- ✓ *Nocardia* species
 - Aerobic, non-motile
 - Spores from aerial filaments
 - Growth on Sabouraud dextrose agar
 - MZN-positive
 - Soil saprophytes
- ✓ *Dermatophilus congolensis*
 - Aerobic and capnophilic
 - Motile zoospores
 - No growth on Sabouraud dextrose agar
 - Found in scabs and in foci on skin of carrier animals

Differentiation of the Genera

- Morphology of individual species in stained smears. *Arcanobacterium pyogenes* and *Actinobaculum suis* have coryneform morphology.
- Each species has a defined atmospheric growth requirement.
- Colonial morphology and hemolytic activity:
 - *Arcanobacterium pyogenes* produces a characteristic hazy hemolysis along streak lines after aerobic incubation for 24 hours. Pin-point colonies become visible after 48 hours.
 - *Actinomyces bovis* and *A. hordeovulneris* colonies typically adhere to agar media and are usually non-hemolytic.
 - *Actinomyces viscosus* can produce two colony types, one large and smooth and the other small and rough. The large colony is composed of V, Y and T cell configurations and the smaller colonies form short branching filaments.
 - *Actinobaculum suis* produces colonies which are up to 3 mm in diameter, with a shiny raised center and a dull edge. Poorly defined hemolysis is observed on ruminant blood agar.
- Biochemical reactions
 - *A. pyogenes* = pitting of Loeffler's serum slope within 24 hours, which indicates proteolytic activity. It also hydrolyzes gelatin.
- Granules in pus
 - Granules can be detected when pus is diluted with distilled water in a Petri dish.
 - In infections caused by *A. bovis*, pinhead-sized, yellowish 'sulphur granules' are found.
 - Whitish, soft, grey granules are demonstrable in pus from animals infected with *A. viscosus*.
 - Granules in lesions caused by *A. bovis* contain characteristic clubs.
 - Club colony formation is a feature of other chronic infections such as bovine actinobacillosis caused by *Actinobacillus ligniersii* and botryomycosis usually associated with *Staphylococcus aureus*.
- Urease is usually produced by *A. suis*.

Comparative features of actinomycetes of veterinary importance

Feature	<i>Actinomyces</i> spp.	<i>Arcanobacterium pyogenes</i>	<i>Actinobaculum suis</i>	<i>Nocardia</i> spp.	<i>Dermatophilus congolensis</i>
Atmospheric growth requirements	Anaerobic or facultatively anaerobic and capnophilic	Facultatively anaerobic and capnophilic	Anaerobic	Aerobic	Aerobic and capnophilic
Aerial filament production	-	-	-	+	-
MZN staining	-	-	-	+	-
Growth on Sabouraud dextrose agar	-	-	-	+	-

capnophile - aerobic bact. w/ a req't for CO₂.

Feature	<i>Actinomyces</i> spp.	<i>Arcanobacterium pyogenes</i>	<i>Actinobaculum suis</i>	<i>Nocardia</i> spp.	<i>Dermatophilus congolensis</i>
Usual habitat	Nasopharyngeal and oral mucosae	Nasopharyngeal mucosa of cattle, sheep and pigs	Prepuce and preputial diverticulum of boars	Soil	Skin of carrier animals, scabs from lesions
Site of lesions	Many tissues including bone	Soft tissues	Urinary tract of sows	Thoracic cavity, skin and other tissues	Skin

ACTINOMYCES, ARCANOBACTERIUM AND ACTIBACULUM SPECIES

- The species in these genera are non-motile, non-spore-forming, Gram-positive bacteria which require enriched media for growth.
- *Arcanobacterium pyogenes* has undergone two name changes in recent years; it was formerly called *Actinomyces pyogenes* and before that *Corynebacterium pyogenes*.
- *Actinobaculum suis* has also undergone a number of recent name changes and is closely related to the genus *Arcanobacterium*.
- Both of these organisms have a coryneform morphology whereas the *Actinomyces* species are usually long and filamentous although short V, y and T configurations also occur.
- The species of veterinary importance in the group are *Arcanobacterium pyogenes*, *Actinobaculum suis*, *Actinomyces bovis*, *Actinomyces viscosus* and *Actinomyces hordeovulneris*.

Differentiation of Actinomyces

Characteristic	<i>Actinomyces bovis</i>	<i>Actinomyces viscosus</i>	<i>Actinomyces hordeovulneris</i>	<i>Arcanobacterium pyogenes</i>	<i>Actinobaculum suis</i>
Morphology	Filamentous branching, some short forms	Filamentous branching, short forms	Filamentous branching, short forms	Coryneform	Coryneform
Atmospheric requirements	Anaerobic + CO ₂	10% CO ₂	10% CO ₂	Aerobic	Anaerobic
Hemolysis on sheep blood agar	±	-	±	+	±
Catalase production	-	+	+	-	-
Pitting of Loeffler's serum slope	-	-	-	+	-
Granules in pus	'Sulphur granules'	White granules	No granules	No granules	No granules

Pathogenesis and Pathogenicity

- *Arcanobacterium pyogenes* produces a hemolytic exotoxin which has dermonecrotizing activity and is lethal for laboratory animals. This bacterium also produces a protease and a neuraminidase, neither of which has a defined role in virulence.
- Toxin production by the other *Actinomyces* species has not been established.
- Purulent reactions are typical of infections with *A. pyogenes* whereas *A. bovis* and *A. viscosus* provoke pyogranulomatous reactions.

Diagnostic Procedures

- Clinical presentation, species affected and type and location of lesions may suggest the species involved.
- Specimens suitable for laboratory procedures include exudates, aspirates and tissue samples for culture and histopathology.
- Gram-stained smears may reveal morphological forms typical of the etiological agent. Unlike *Nocardia* species, these bacteria are MZN negative.
- Histopathological examination of specimens from lesions caused by *A. bovis* reveals aggregates of filamentous organisms surrounded by eosinophilic club-shaped structures.
- Blood and MacConkey agars are inoculated with the specimen and incubated at 37°C for up to 5 days.
- Identification criteria for isolates:
 - Colonial characteristics
 - Morphology in stained smears
 - Presence or absence of hemolysis on blood agar
 - Absence of growth on macConkey agar
 - Absence or presence of growth when subcultured onto Sabouraud dextrose agar
 - Pitting of a Loeffler's serum slope (*A. pyogenes*)
 - Urease production (*A. suis*).

Antimicrobial susceptibility

- Penicillin, streptomycin, tetracycline, cephalosporin, lincomycin and sulfonamides
- Sensitive also to iodine

Disease conditions produced by *Actinomyces*, *Arcanobacterium* and *Actinobaculum* species in domestic animals

Species	Hosts	Disease conditions
<i>Arcanobacterium pyogenes</i>	Cattle, sheep, pigs	Abscessation, mastitis, suppurative pneumonia, endometritis, pyometra, arthritis, umbilical infections
<i>Actinomyces hordeovulneris</i>	Dogs	Cutaneous and visceral abscessation, pleuritis, peritonitis, arthritis
<i>Actinomyces bovis</i>	Cattle	Bovine actinomycosis (lumpy jaw)
<i>A. viscosus</i>	Dogs	Canine Actinomycosis - Cutaneous pyogranulomas - Pyothorax and proliferative pyogranulomatous pleural lesions - Disseminated lesions (rare)
	Horses	Cutaneous pustules
	Cattle	Abortion
<i>Actinomyces</i> species (unclassified)	Pigs	Pyogranulomatous mastitis
	Horses	Poll evil and fitulous withers
<i>Actinobaculum suis</i>	Pigs	Cystitis, pyelonephritis

***NOCARDIA ASTEROIDES* AND OTHER *NOCARDIA* SPECIES**

- Members of the *Nocardia* species are Gram-positive, aerobic, saprophytic actinomycetes.
- In smears of exudates from infected tissue, they appear as long, slender branching filaments with a tendency to fragment into rods and cocci.
- When cultured, these organisms produce aerial filaments which may form spores.
- Components of the cell wall, especially mycolic acid, render *Nocardia* species partially acid-fast (MZN-positive).
- In this genus, *Nocardia asteroides* is the pathogen of greatest significance in domestic animals.

Differentiation of *Nocardia* species

- *Nocardia* species are recognized, including *N. asteroides*, *N. facinica* and *N. nova*, which is a human pathogen.. They are closely related.
- Identification of individual species is usually carried out in reference laboratories and is based on specialized biochemical tests, analyses of mycolic acid composition and DNA probes.

Differentiation of *Nocardia asteroides* and *Actinomyces viscosus*

Characteristic	<i>Nocardia asteroides</i>	<i>Actinomyces bovis</i>
MZN-staining of filaments	+	-
Atmospheric requirement	Aerobic	10% CO ₂
Growth on Sabouraud dextrose agar	+	-
Susceptibility to Penicillin G	-	+

Pathogenesis and Pathogenicity

- Infection, which is opportunistic, is usually associated with immunosuppression or, alternatively, may follow a heavy challenge.
- The usual mode of infection is by inhalation but it may also occur through the skin wounds or via the teat canal.
- An intestinal form of nocardiosis may result from ingestion of the organisms.
- Virulent strains of *N. asteroides* survive intracellularly.
- The production of superoxide dismutase and catalase and the presence of thick peptidoglycan layer in the cell wall confer resistance to microbiocidal activity of phagocytes.
- Cell-mediated immunity is essential for protection against infection by this facultative, intracellular bacterium.

Diagnostic Procedures

- Specimens suitable for laboratory examination include exudates, aspirates, mastitic milk, tissue from granulomatous and fixed tissue for histopathology.
- Smears of exudates should be stained by the Gram and MZN methods. *N. asteroides* is MZN-positive while *Actinomyces* species are MZN-negative.
- Histopathological examination of tissue specimens may reveal clusters of nocardial filaments.
- The organism can be cultured on blood agar or on selective growth-enhancing media such as charcoal-yeast extract medium; incubate aerobically at 37°C for up to 10 days.
- Identification criteria for isolates:
 - Colonies on blood agar are usually visible after incubation for about 5 days. They are white, powdery and firmly adherent to the agar. Colonies are variably hemolytic and odorless.
 - Subculture onto Sabouraud dextrose agar yields dry, wrinkled, orange-colored colonies after incubation for up to 5 days.
 - Gram-stained smears from colonies show some filamentous forms with a preponderance of rod and coccil forms.
- *Nocardia asteroides* requires differentiation from *Streptomyces* species which distinguish them from *Nocardia asteroides* include a strong, earthy odor, MZN-negative filaments and colonies on Sabouraud dextrose agar which are powdery-white in appearance.

Treatment

- Sulfadiazine, benzalkonium chloride, cycloserine and sulfonamide in combination

Disease conditions produced by *Nocardia* species in domestic animals

Species	Hosts	Disease Conditions
<i>Nocardia asteroides</i>	Dogs	Canine nocardiosis - cutaneous pyogranulomas - pyogranulomatous pleural lesions and pyothorax - disseminated lesions
	Cattle	Chronic mastitis, abortion
	Pigs	Abortion
	Sheep, goats, horses	Wound infections, mastitis, pneumonia, other pyogranulomatous conditions
	Whales, dolphins and birds	Uncommon infections: respiratory involvement with dissemination to other tissues
<i>Nocardia farcinica</i>	Cattle	Bovine farcy

DERMATOPHILUS CONGOLENSIS

- *Dermatophilus congolensis* is a Gram-positive, filamentous, branching actinomycete with distinctive morphology.
- This actinomycete is unusual because it produces motile coccid zoospores about 1.5 µm in diameter.
- Mature zoospores produce germ tubes which develop into filaments 0.5-1.5 µm in width.
- Within these filaments may be more than 5 µm in width and contain columns of zoospores which impart a 'tram-track' appearance to the filaments.
- Although skin infections caused by *D. congolensis* occur worldwide, dermatophilosis is most prevalent in tropical and subtropical regions.
- Cause of cutaneous streptotrichosis, mycotic dermatitis, lumpy wool, strawberry foot-rot and cutaneous actinomycosis.

Pathogenesis and Pathogenicity

- *D. congolensis* does not usually invade healthy skin. Trauma and persistent wetting predispose to skin invasion.
- Microenvironmental conditions which interfere with normal surface protective mechanisms, such as sebaceous secretions, also lead to activation of dormant zoospores.

- When activated, zoospores produce germ tubes and these develop into filaments which invade the epidermis.
- This ability of individual strains to invade the epidermis is related to their virulence.
- Keratinolytic activity may be a virulence factor.
- Invasion leads to an acute inflammatory response characterized by large numbers of neutrophils which ultimately form microabscesses in the epidermis.
- A cyclical pattern of invasion by the pathogen of regenerating epithelial cells, together with serous exudation and microabscess formation, leads to the development of raised scab-like crusts containing numerous branching filaments.
- Factors which depress specific immune responses, including intercurrent diseases and pregnancy, may increase host susceptibility to dermatophilosis.
- Lumpy wool
 - Entry of zoospores into the skin of a new host involves successful penetration of the hair or fleece, the sebaceous wax layer, and finally the stratum corneum epidermis.
 - The zoospores germinate and the hyphal branch penetrates the epidermis.
 - The hyphae branch laterally and invade the hair or wool follicles. The dermis is not invaded.
 - Neutrophils collect beneath the infected epidermis, and a serous exudate accumulates and leaks to the surface.
 - A new layer of epidermis is formed as the older layer above deteriorates, a process that continues and eventually results in the formation of a thick scab.
 - Infection of newly forming epidermis occurs from organism already in the follicular sheath.
- Streptotrichosis
 - It is seen in cattle, horses, deer and sheep characterized by small, confluent, raised and circumscribed crusts composed of epidermal cells and coagulated serous exudates with embedded hairs on the skin of the back.
 - The lesions can be local or they can be progressive and sometimes fatal.
 - The disease is essentially an exudative dermatitis followed by extensive scab formation.
- Strawberry foot-rot in sheep
 - It begins with the appearance of dry scabs on the legs (at any point between the coronet and the knee or hock).
 - The affected area become denuded by hair or wool.
 - When the areas are large, the exudates mats the hair and forms a hard, dry casing over the region.
 - This usually can be stripped off easily, leaving a mass of granulation tissue that has the appearance of a strawberry.
 - The lesions may remain for long periods but they usually heal within 5-6 weeks.

Diagnostic Procedures

- The clinical picture is usually indicative of the infection, particularly in endemic areas.
- Specimens suitable for laboratory examination include scab material and samples of skin fixed in formalin.
- Smears from the undersurface of scabs or from softened scab material, stained by the Giemsa method, reveal the characteristic branching filaments containing zoospores.
- Scab material softened with water can be cultured on blood agar at 37°C in an atmosphere of 2.5-10% CO₂ for up to 5 days.
- Zoospores, which exhibit chemotaxis for CO₂, can be recovered from heavily contaminated specimens by placing infected scab material in distilled water at room temperature for 3.5 hours, followed by exposure to an atmosphere of CO₂ for 15 minutes. A sample from the surface of the water contains motile zoospores which can be cultured.
- Identification criteria:
 - After incubation for 48 hours, colonies are up to 1 mm in diameter, yellow and hemolytic. When incubated 3-4 days, they become rough, golden-yellow and embedded in the agar. Older colonies may have mucoid appearance.
 - Giemsa-stained smears from colonies reveal solidly-staining filaments.
 - No growth occurs on Sabouraud dextrose agar.
 - Biochemical tests are rarely required for identification. The organism liquefies Loeffler's serum medium, hydrolyzes gelatin and casein, and produces acid from glucose and fructose.

Treatment

- Tetracycline, chloramphenicol, penicillin, streptomycin
- Resistant to Kanamycin, polymyxin B sulfate, bacitracin and sulfonamide.

Review Questions:

1. Give at least five significant morphological, cultural and biochemical characteristics of the Actinomycetes that will differentiate them from other bacteria.
2. Give the comparative features of Actinomycetes of Veterinary importance.
3. Give the comparative features of Actinomyces, Arcanobacter and Actinobaculum.
4. Describe briefly three diseases caused by *Dermatophilus congolensis*.
5. Illustrate a "tram-track" appearance of the filaments of *Dermatophilus congolensis*.