

2nd Professional Year, Veterinary Microbiology (Unit-1)

STREPTOMYCES AND DERMATOPHILUS

Dr. Savita Kumari
Assistant Professor–cum–Jr. Scientist
Department of Veterinary Microbiology
Bihar Veterinary College, BASU, Patna

Classification

Streptomyces

- Phylum: Actinobacteria
- Class: Actinobacteria
- Order: Actinomycetales
- Family: Streptomycetaceae
- Genus: Streptomyces

Dermatophilus

- Phylum: Actinobacteria
- Class: Actinobacteria
- Order: Actinomycetales
- Family: Dermatophilaceae
- Genus: Dermatophilus
- Species: *D. congolensis*

Streptomyces

- In 1944, Selman Waksman and his associates found a new antibiotic, streptomycin, produced by the actinomycete, *Streptomyces griseus*
- majority of antibiotics used in medicine, veterinary practice, and agriculture originate from *Streptomyces bacteria*
- *Streptomyces* species are saprophytic soil actinobacteria
- Elaborate a variety of antimicrobial substances, many with therapeutic activity
- Streptomyces - type genus of the family *Streptomycetaceae* belonging to the order *Actinomycetales*

Characteristics

- Grow as branching hyphal filaments to form a mat of fungus-like mycelium, from which emerge aerial branches that bear chains of spores
- Do not show the usual bacterial bacillary or coccoid forms
- *Streptomyces* species show a Gram-positive reaction
- Have DNA with a GC value of 69–78%
- Distinct "earthy" odor that results from production of a volatile metabolite, geosmin
- common contaminants on laboratory media

Characteristics

- Form white, powdery colonies, embedded in the agar, on blood agar and nutrient agar
- These are very similar in appearance to those of *Nocardia* species
- *Streptomyces* species are also able to grow on Sabouraud dextrose
- *Streptomyces* species have a characteristic and powerful earthy odour



Streptomyces species on nutrient agar showing the white, powdery colonies (Markey *et al.*, 2013)

- Streptomyces species often harbor linear as well as circular plasmids
- Streptomycetes are characterised by a complex secondary metabolism
- Many species important in the decomposition of organic matter in soil
- Produce over two-thirds of the clinically useful antibiotics of natural origin
- Streptomyces is the largest antibiotic-producing genus
- Producing antibacterial, antifungal, and antiparasitic drugs
- Also a wide range of other bioactive compounds, such as immunosuppressants
- Almost all of the bioactive compounds produced by *Streptomyces* are initiated during the time coinciding with the aerial hyphal formation from the substrate mycelium

■ Antifungal compounds of medicinal importance:

- ❑ Nystatin (from *S. noursei*)
- ❑ Amphotericin B (from *S. nodosus*)
- ❑ Natamycin (from *S. natalensis*)

■ Antibacterial pharmaceutical agents:

- ✓ Chloramphenicol (from *S. venezuelae*)
- ✓ Lincomycin (from *S. lincolnensis*)
- ✓ Neomycin (from *S. fradiae*)
- ✓ Streptomycin (from *S. griseus*)
- ✓ Tetracycline (from *S. rimosus* and *S. aureofaciens*)
- ✓ Clavulanic acid (from *S. clavuligerus*)- drug used in combination with some antibiotics

■ Antiparasitic drugs

Ivermectin (*S. avermitilis*)

■ Antineoplastic (anticancer); Antiviral drug

Infections

- Streptomyces are infrequent pathogens
- Infections in humans, streptomycesoma, can be caused by *S. somaliensis* and *S. sudanensis*
- In general, streptomyces cause suppurative granulomatous tissue changes
- The infection starts from the surface skin structures
- If untreated, it proceeds to muscles, bones and may even spread via the lymphatic system or blood and cause a systemic disease
- Certain respiratory diseases (e.g., farmer's lung disease) have been associated with inhalation of spores

- Streptomyces are often mentioned as etiologic agents of inflammatory diseases originated from water-damaged houses
- Other infections of human manifested mainly as:
 - Pulmonary infections
 - Bacteremias
 - Different organs abscesses
- *Streptomyces cyaneus*- pyogranulomatous dermatitis in dogs

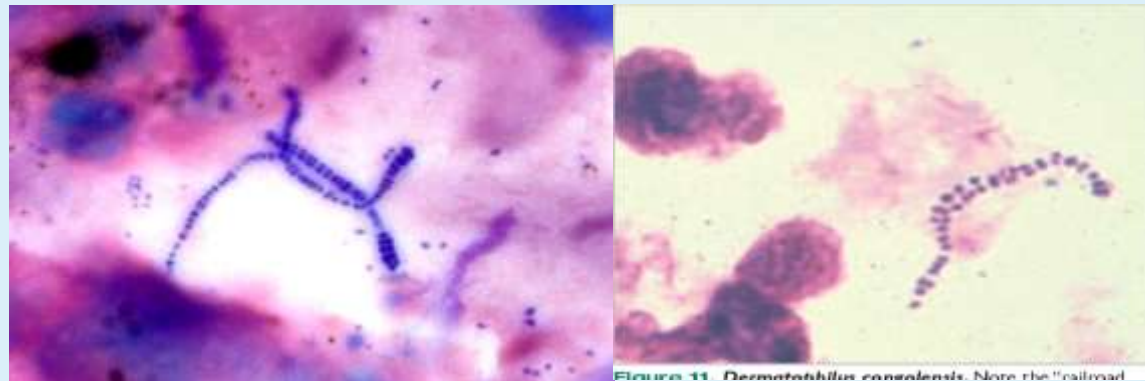
General features of *Streptomyces* and *Dermatophilus* sp.

Characteristics	<i>Streptomyces</i>	<i>Dermatophilus</i>
Atmospheric requirement	Aerobe	Aerobe/capnophilic*
Catalase	+	+
Partially acid-fast (MZN-positive)	-	-
Motility	-	+ (zoospores)
Growth on Sabouraud dextrose agar	+	-
Aerial filaments	+	-
Spores	+ (conidia)	+ (zoospores)
Fragmentation of filaments	+	+
Odour of colonies	Pungent and earthy	-
Metabolism	Oxidative	Weakly fermentative
Reservoir	Soil and common laboratory contaminant	Foci on skin of carrier animal or within scabs in environment
Veterinary importance	Non-pathogenic but similar to <i>Nocardia</i> in cultures. Some species produce antibiotics	Skin disease

(Markey et al., 2013)

Dermatophilus congolensis

- Gram-positive, filamentous, branching actinobacterium with distinctive morphology
- Produces **motile coccal zoospores** about 1.5µ m in diameter
- Mature zoospores produce germ tubes which develop into filaments 0.5 to 1.5 µm in width
- Within these filaments, transverse and longitudinal divisions form segments that ultimately develop into zoospores
- Mature filaments may be more than 5µm in width and contain columns of zoospores which impart a '**tramtrack**' appearance to the filaments
- Skin infections caused by *D. congolensis* occur worldwide, dermatophilosis most prevalent in tropical and subtropical regions



(Image source-Google)

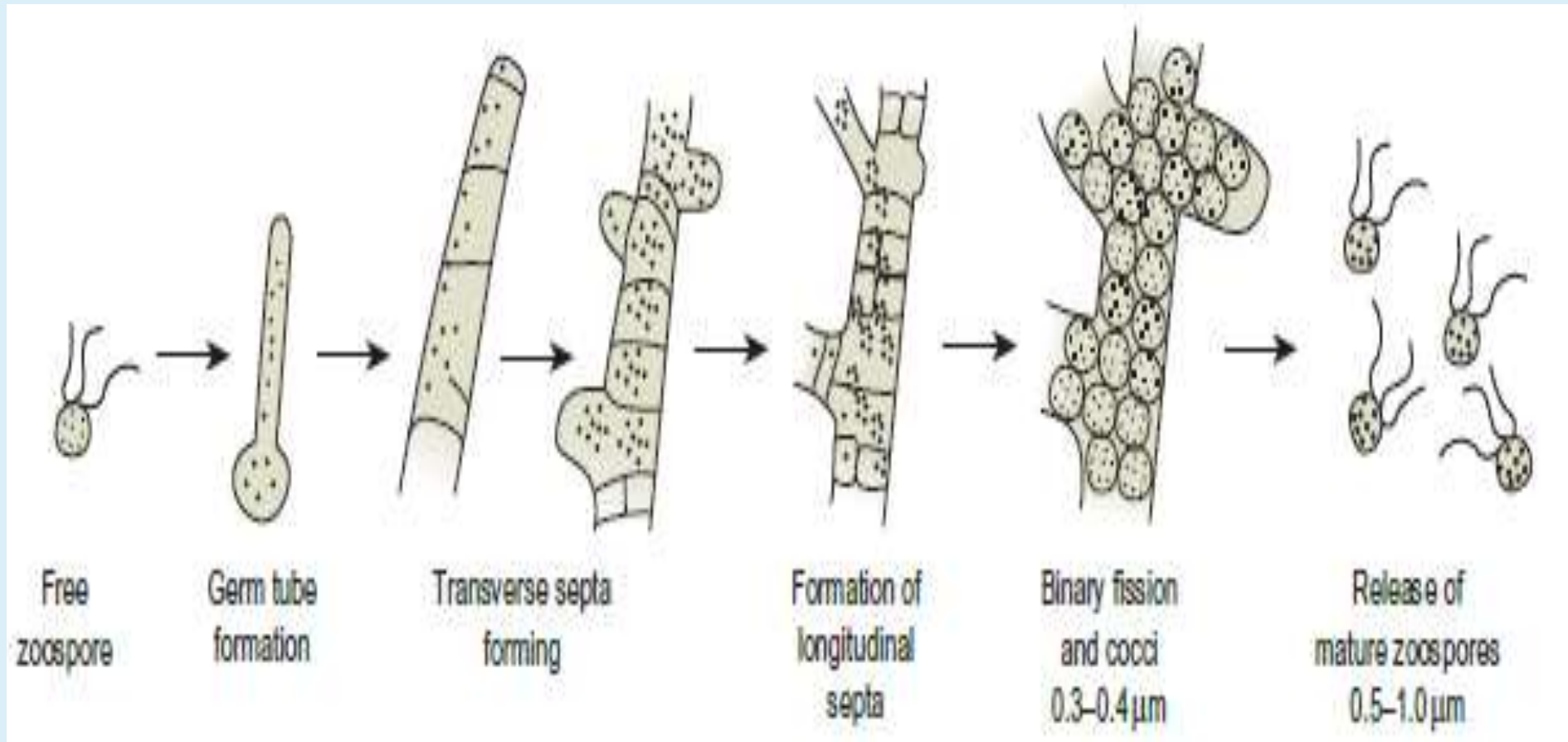


Figure 10.25 Developmental cycle of *Dermatophilus congolensis*.

(Markey *et al.*, 2013)

Cultural Characteristics

- An atmosphere of 5–10% CO₂ enhances the growth of the organism, especially on primary isolation
- The inoculated plates are incubated at 37 °C for upto five days, although colonies may be seen after 24–48 hours' incubation
- Scab material contains many contaminants and Haalstra's method was developed to overcome this problem

Box 10.1 Haalstra's method for the primary isolation of *Dermatophilus congolensis*

- Grind up a small amount of scab material and place a little in 2 ml distilled water in a bijoux bottle for three hours at room temperature.
- Place the container, with lid removed, in a candle jar at room temperature for 15 minutes.
- The motile zoospores are chemotactically attracted to the carbon-dioxide-enhanced atmosphere in the candle jar and move to the surface of the distilled water. Remove a loopful of fluid from the surface and inoculate a blood agar plate. Incubate the inoculated plate at 37°C for 72 hours under 5–10% CO₂.

(Markey *et al.*, 2013)

Colonial appearance

- Small (about 1 mm) greyish-yellow, distinctly haemolytic colonies can be seen after 24–48 hours' incubation
- Firmly adherent to the medium and appear to be embedded in the agar
- After three to four days, isolated colonies can be 3 mm in diameter and are rough, wrinkled with a golden-yellow colour
- Older colonies can become mucoid
- No growth occurs on Sabouraud dextrose agar



Figure 10.26 A culture of *D. congolensis* on sheep blood agar after three days' incubation in 10% CO₂.

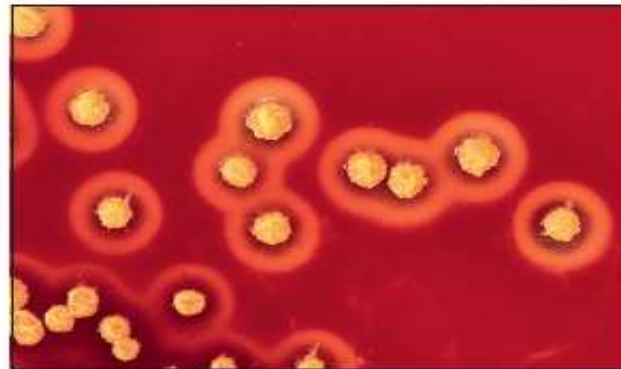


Figure 10.27 A close-up of the three-day-old haemolytic colonies of *D. congolensis* on sheep blood agar showing the rough, dry, golden-yellow appearance. They are firmly embedded in the medium.

(Markey et al., 2013)

Microscopic appearance

- Gram-stained smears from colonies do not show the characteristic 'tramtrack' appearance seen on direct microscopy
- Usually the smears reveal uniformly staining, Gram-positive, branching filaments but sometimes coccal forms predominate

Biochemical reactions

- Catalase-positive, urease-positive, gelatin-positive
- Produces acid from glucose, fructose and maltose
- Indole-negative
- Does not reduce nitrate

Habitat

- The organism seems to persist in foci in the skin of many clinically normal animals, particularly in endemic areas
- Dormant zoospores may become activated when microenvironmental moisture and temperature levels are favourable
- Duration of zoospore survival in the environment is usually limited but may be up to 3 years in dry scabs

Pathogenesis and pathogenicity

- *Dermatophilus congolensis* does not usually invade healthy skin
- Trauma and persistent wetting predispose to skin invasion
- Disruption of sebaceous secretions, also lead to activation of dormant zoospores
- When activated, zoospores produce germ tubes and these develop into filaments which invade the epidermis
- The ability of individual strains to invade the epidermis is related to their virulence

- Strains vary in virulence, which may be related to the ability to produce enzymes such as phospholipases, proteolytic enzymes and an alkaline ceramidase
- 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 of regenerating epithelial cells by the pathogen, together with serous exudation and microabscess formation, leads to the development of raised scab-like crusts containing numerous branching filaments
- Factors that depress specific immune responses, including intercurrent diseases and pregnancy, may increase host susceptibility to dermatophilosis

Clinical infections

- Infections with *D. congolensis* are usually confined to the epidermis
- invasion of subcutaneous tissue has been described in a cat
- Commonly used designations for infection with this organism are dermatophilosis and cutaneous streptothricosis
- **Mycotic dermatitis** (a misnomer) and **lumpy wool**- infection of woolled areas of the skin in sheep
- **Strawberry footrot**- skin of the lower limbs of sheep involved

- Disease affects animals of all ages
- More prevalent and often more severe in young animals
- Damage to the skin predisposes to infection with *D. congolensis*
- Zoospores most often transmitted by direct contact with infected animals
- In endemic tropical regions, the prevalence and severity of dermatophilosis correlates with infestation with *Amblyomma variegatum* (tick)
- A number of blood-sucking insects may also be important in disease transmission in the tropics
- Economic loss derives from damage to hides and fleeces
- In addition, dermatophilosis creates a strong predisposition to fly strike in sheep
- Human skin infections, occasionally acquired through close contact with infected animals -rare

Clinical signs

- Lesion distribution usually correlates with those areas of skin predisposed to infection
- Heavy prolonged rainfall in association with warm environmental temperatures can result in lesions predominantly affecting the dorsum of farm animals
- Trauma to the face and limbs of animals grazing in thorny scrub can predispose to lesions in these sites
- Early lesions present as papules and are often detectable only by palpation
- As lesions progress, serous exudate causes matting of hairs giving them a tufted appearance
- Lesions may coalesce to form irregular elevated crusty scabs

- Tufts of hair can be readily plucked from the lesion along with adherent scab material and underlying exudate
- Scab formation tends to be more pronounced in cattle and sheep than in horses
- Localized infections are usually of little consequence
- Lesions may resolve spontaneously within a few weeks, particularly in dry conditions
- In severe infections, lesions may be extensive and deaths may occasionally occur, particularly in calves and lambs
- Rarely, oral lesions result in depression, difficulty with eating and loss of condition.

Diagnosis

- Based on clinical appearance of lesions and demonstration of *D. congolensis* in scabs
- Isolation of the organism confirmatory
- **Specimens**
 - A tuft of hair that is plucked from the lesion usually detaches with scab material adhering to it
 - Samples of skin fixed in formalin
- **Direct microscopy**
 - Small pieces of material are shaved from the scab with a scalpel and the flakes of scab are softened in a few drops of distilled water on a microscope slide
 - A smear is made, taking care to leave a few flakes of scab material intact
 - Smear can be stained by either Giemsa or Gram stains

- Giemsa - better stain to show the characteristic morphology of the bacterium
- If the conventional Gram stain is used, both the cells of *D. congolensis* and surrounding debris seem to absorb the crystal violet iodine complex avidly and stain too darkly
- A modification of the Gram stain is to leave the crystal violet on the smear for only two to three seconds, after which the morphology of the bacterium is easier to see
- The appearance of *D. congolensis* is so unique that a strong presumptive diagnosis of streptothricosis (dermatophilosis) can be made based on the direct examination of stained smears alone
- Reveal the characteristic branching filaments containing zoospores

- When there is difficulty demonstrating the organism in smears, histopathological or immunofluorescent techniques may be employed

Isolation

- Comparatively easy to culture and grows well on sheep or ox blood agar
- An atmosphere of 5–10% CO₂ enhances the growth of the organism, especially on primary isolation
- Scab material softened with water is cultured on blood agar at 37 °C in an atmosphere of 2.5 to 10% CO₂ for up to 5 days
- The inoculated plates are incubated at 37°C for up to five days, although colonies may be seen after 24–48 hours' incubation

- Scab material contains many contaminants and Haalstra's method was developed to overcome problem
- 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 haemolytic
- When incubated for 3 to 4 days, they become rough, golden-yellow and embedded in the agar. Older colonies may have a mucoid appearance
- Giemsa-stained smears from colonies reveal solidly staining filaments
- Growth does not occur on Sabouraud dextrose agar
- Biochemical tests are rarely required for identification
- The organism liquefies Loeffler's serum medium, hydrolyses gelatin and casein, and produces acid from glucose and fructose
- PCR

Treatment

- Parenterally administered antibiotics such as long-acting oxytetracycline are usually effective
- Alternatively, high doses of penicillin–streptomycin combinations on three consecutive days may be used
- For treatment to be effective, satisfactory epidermal concentrations of the antibiotics are required
- The outcome of treatment is influenced by the severity and extent of lesions
- Topical treatments are ineffective

Control

- Vary with geographical location and climatic factors
- Based on minimizing the effects of predisposing factors and early treatment of clinical cases
- Clinically affected animals should be isolated and treated promptly
- Shelter should be provided during periods of prolonged rainfall
- Grazing areas should be cleared of thorny scrub
- Tick infestation must be reduced by dipping or spraying with acaricides at weekly intervals and by elimination of tick habitats
- Prophylactic use of long-acting tetracyclines may be required in endemic regions
- Control of intercurrent diseases reduces the severity of dermatophilosis