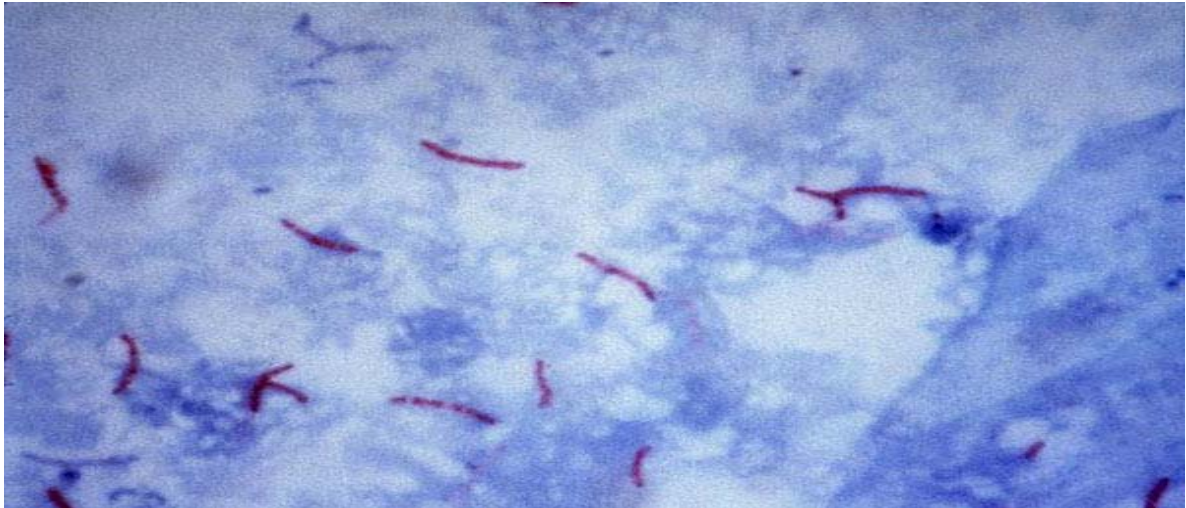


***MYCOBACTERIUM* SPECIES**



SPECIAL MICROBIOLOGY



Instructor

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MYCOBACTERIACEAE

Genus: *Mycobacterium*

GENERAL CHARACTERISTICS

- Mycobacteria genera, are rod shaped acid-fast bacilli.
- Cell walls rich in complex lipids and waxes containing mycolic acids
- Aerobic, non-motile, non-spore-forming
- Complex egg-enriched media required for growth of pathogenic species
- Genus includes obligate pathogens, opportunistic pathogens and saprophytes
- Pathogenic species grow slowly, colonies visible after several weeks
- Some mycobacteria produce carotenoid pigments.

Resistant to chemical disinfectants and environmental influences but susceptible to heat treatment (pasteurization) except certain strains of Johne's bacilli which resist pasteurization & implicated in zoonosis.

- Multiply intracellularly and cause chronic, granulomatous infections
- Major diseases include tuberculosis, Johne's disease and feline leprosy

Usual habitat

- Environmental mycobacteria are found in soil, on vegetation and in water.
- Obligate pathogens, shed by infected animals, can also survive in the environment for extended periods

IMPORTANT PATHOGENS OF MYCOBACTERIA

- ***Mycobacterium tuberculosis* COMPLEX**

- Mycobacterium tuberculosis* (HUMAN & ANIMALS TUBERCULOSIS)
- Mycobacterium bovis* (ANIMALS & HUMAN TUBERCULOSIS)

- ***Mycobacterium avium* COMPLEX**

-*Mycobacterium avium* (Birds & Cattle TUBERCULOSIS)

-*Mycobacterium marinum* (Fish & Human TUBERCULOSIS)

-*Mycobacterium avium* subsp. *paratuberculosis* **MAP**

[Paratuberculosis (Johne's disease) in animals & human (Crohn's disease)]

Growth & Culture Characteristics

- Pathogenic mycobacteria grow slowly on solid media, and colonies are not evident until cultures
- have been incubated for 3 to 6 weeks and longer. In contrast, the colonies of rapidly growing saprophytes are visible within days.
- *Mycobacterium bovis*, *M. tuberculosis* and **MAP** have an optimal incubation temperature of 37°C. Mycobacteria belonging to the *M. avium* complex grow in the temperature range 37 to 43°C.
- Glycerol and sodium pyruvate enhance growth rate & reduce incubation period.
- Mycobactin (Iron-chelating agent) as growth prompter for **MAP**

Pigment production and photoreactivity for opportunistic mycobacteria:

- **Non-chromogens:** produce colonies, devoid of orange, carotenoid pigments.
- **Photochromogens:** when cultured in the dark, produce non-pigmented colonies which become pigmented after a period of exposure to light.
- **Scotochromogens:** produce pigment when cultured in the dark or in light.
- Colonies on solid media are variable:

-*M. tuberculosis* & *M. bovis* are tough, rough & buff (creamy or off-white) or colorless.

-MAP, are translucent to grey, hemispherical & small raised colonies.

Constituents of Tubercle Bacilli

A. Lipids

- Mycobacteria are rich in lipids. These include mycolic acids, waxes and phosphatides. In the cell, the lipids are bound to proteins and polysaccharides.

B. Proteins

- Proteins bound to a wax fraction can upon injection induce:

1-Tuberculin sensitivity.

2-Formation of a variety of antibodies.

C. Polysaccharides

- They can induce the immediate type of hypersensitivity

Tuberculin or Johnin

- Also known as **purified protein derivative (PPD)** is a combination of proteins that are used in the diagnosis of tuberculosis (TUBERCULIN) or paratuberculosis (JOHNIN). Are used as SKIN TEST and is recommended only for those at high risk.
- The results are read at 48-72 hrs after infection which will appear as induration (hard swelling) at the site of injection.

Diagnosis

- **Tuberculin test**

1- Single intradermal (caudal fold) test, 0.1 ml of bovine PPD is injected intradermally into the caudal fold of the tail & check for the induration 72 later.

2- Comparative intradermal test, 0.1 ml of avian PPD and 0.1 ml of bovine PPD are injected intradermally into separate clipped sites on the side of the neck about 12 cm apart.

* **False positive** results as not uncommon.

➤ **Blood-based tests:**

-Gamma interferon assay

-ELISA for detecting circulating antibodies.

➤ **Specimens:** lymph nodes, tissue lesions, aspirates and milk.

➤ **Acid fast staining (Ziehl-Neelsen).**

➤ **Auramine-Rhodamine Fluorescence staining technique.**

➤ **Isolation requires:**

- Decontamination of specimens with NaOH, Oxalic Acid.
- Centrifugation to increase the number of mycobacteria.
- Inoculation on selective media egg-based or even synthetic media.
- Egg-based: Lowenstein-Jensen Media (TB), Stonebrink's (Bovis), Egg Dorest's & Herrold's Egg Yolk Agar (MAP).
- Synthetic: Middlebrook & Dubos's Medium.

➤ **Identification for isolates:**

- Growth rate and colonial appearance
- Positive ZN staining

➤ **Biochemical Profile**

➤ **Molecular typing:** PCR-based techniques used widely recently for detection & genotyping of *Mycobacterium* spp.