



**Bacteriology, Immunology and Mycology  
department**

**Third year (general program)  
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# Family: MYCOPLASMACEAE

Order : MYCOPLASMATALES

family: MYCOPLASMACEAE.

Genus : MYCOPLASMA.

Species:

*Mycoplasma bovis*

*Mycoplasma mycoides.*

*Mycoplasma var Capri.*

*Mycoplasma gallisepticum.*

*Mycoplasma gallinarum.*

*Mycoplasma hominis.*

***Mycoplasma* species are highly fastidious microorganisms characterized by**

Lacking **cell wall** so it's **pleomorphic in shape**.

Low G+C content (23%–40%).

Small size of genome (0.58-1.4 Mbp).

Difficult to culture and slow growing

Special growth requirements are needed for their growth.

Grows on solid media giving the characteristic **“fried egg”** colonies when examined under a plate microscope.

*M. bovis* film and spot formation can also be viewed on the surface of solid media which indicates the presence of lipolytic activity.

## Morphology and staining characters:

- Individual cells of mycoplasma are **extremely pleomorphic** ranging from coccoid to coccobacillary to long branching filaments about (100-150  $\mu$ ).
- **Gram negative**
- *Mycoplasma* is easily stained with **Giemsa** stain

## Growth requirement and Cultural characters:

- *Mycoplasma* needs a rich medium containing **natural animal protein**, a **sterol component** and **moisture** is essential.
- Elevated  $\text{CO}_2$  and low  $\text{O}_2$  are needed and Incubation is best at (33-37°C).
- of the natural animal proteins the most commonly used is **blood serum** in a concentration of (**10-20%**). Serum supplies not only **cholesterol** but also **saturated and unsaturated fatty acids** which the organisms are **unable to synthesize**.
- PPLO media containing 20% horse serum, 10% of a 25% yeast extract, the final concentration of agar is 1%, pH of (7.0-7.8) is advisable.
- (Fluid media, the same constituents as solid media but without agar)

On solid media containing 10% bovine serum,  
*Mycoplasma* produces:



The characteristic microscopic fried egg like colonies

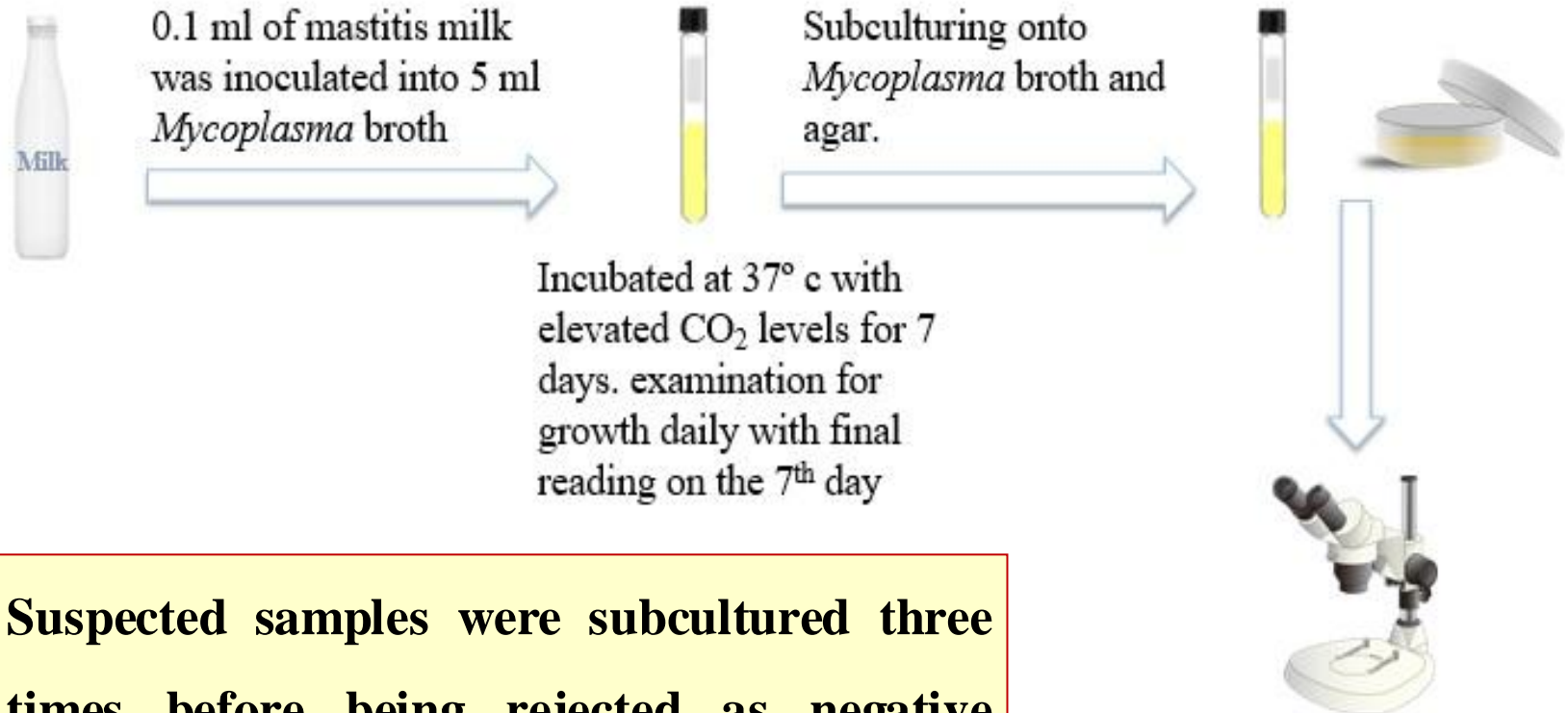
- **microscopic** small, glistening colonies with an entire edge and a **nipple-like darker center** and these colonies are usually **difficult to remove from the surface of the medium** due to **the penetration of the organism below the surface of the medium.**

**For subculturing from agar to broth, **agar block** with colonies was cut using sterile spatula, and dropped into broth.**

In liquid media, *Mycoplasma* produces:

slight turbidity and a finally granular precipitate are formed.

# ➤ Isolation of *Mycoplasma* from milk samples:



- ✓ Suspected samples were subcultured three times before being rejected as negative samples.
- ✓ For subculturing from agar to broth, agar block with colonies was cut using sterile spatula, and dropped into broth.

Plates are examined using stereomicroscope to detect *Mycoplasma* colonies with the characteristic fried egg appearance.



# Biochemical characters:

- Some strains ferment glucose; others do not ferment glucose
- film and spots in egg yolk medium is also helpful.
- *M. pneumoniae* is distinct among human strains in its ability to cause complete lysis of mammalian red corpuscles, although most other strains, human and animal strains produce some degree of haemolysis in blood agar made up with red cells from the guinea pig.
- Arginine hydrolysis test
- Digitonin sensitivity test, Nisin sensitivity tests

**By culturing**

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graph TD; A[By culturing] --> B["Acholeplasma spp.  
are capable of growing on Hayflick medium"]; B --> C["Forming  
Mycoplasma-like-colonies"]; C --> D["Differentiation between  
Mycoplasma spp. and Acholeplasma spp."]; D --> E["By different methods as  
Digitonin, Nisin sensitivity tests and molecular assays"]; E --> F[Accurate diagnosis];
```

*Acholeplasma* spp.  
are capable of growing on Hayflick medium

Forming  
*Mycoplasma*-like-colonies

Differentiation between  
*Mycoplasma* spp. and *Acholeplasma* spp.

By different methods as  
Digitonin, Nisin sensitivity tests and molecular assays

**Accurate diagnosis**

# Differentiation between *Mycoplasma* species.

Species	Inhibition by digitonin	Glucose	Arginine hydrolysis	Phosphatase	Tetrazolium reduction	Film & spot in egg yolk media	Disease
<b>M.mycooides</b>	+	-	-	-	+	-	Contagious bovine Pleuropneumonia
<b>M.bovirhinis</b>	+	+	-	-	variable	+	Pneumonia in calves
<b>M.bovigenitalium</b>	+	-	-	+	Variable	+	Mastitis, vaginitis, Arthritis, seminal vesiculitis
<b>M.bovis</b>	+	-	-	+	+	+	Mastitis, arthritis
<b>M.hominis</b>	-	-	+	-	-	-	Pneumonia. Inflammation of Urogenital tract

# Antigenic characters and Serological behavior:

*Mycoplasmas* are:

- separated into definite number of serotypes.
- Certain species possesses **haemagglutinating, haemadsorbing and cell attaching properties.**
- In being **inhibited** or **neutralized** by specific antiserum.

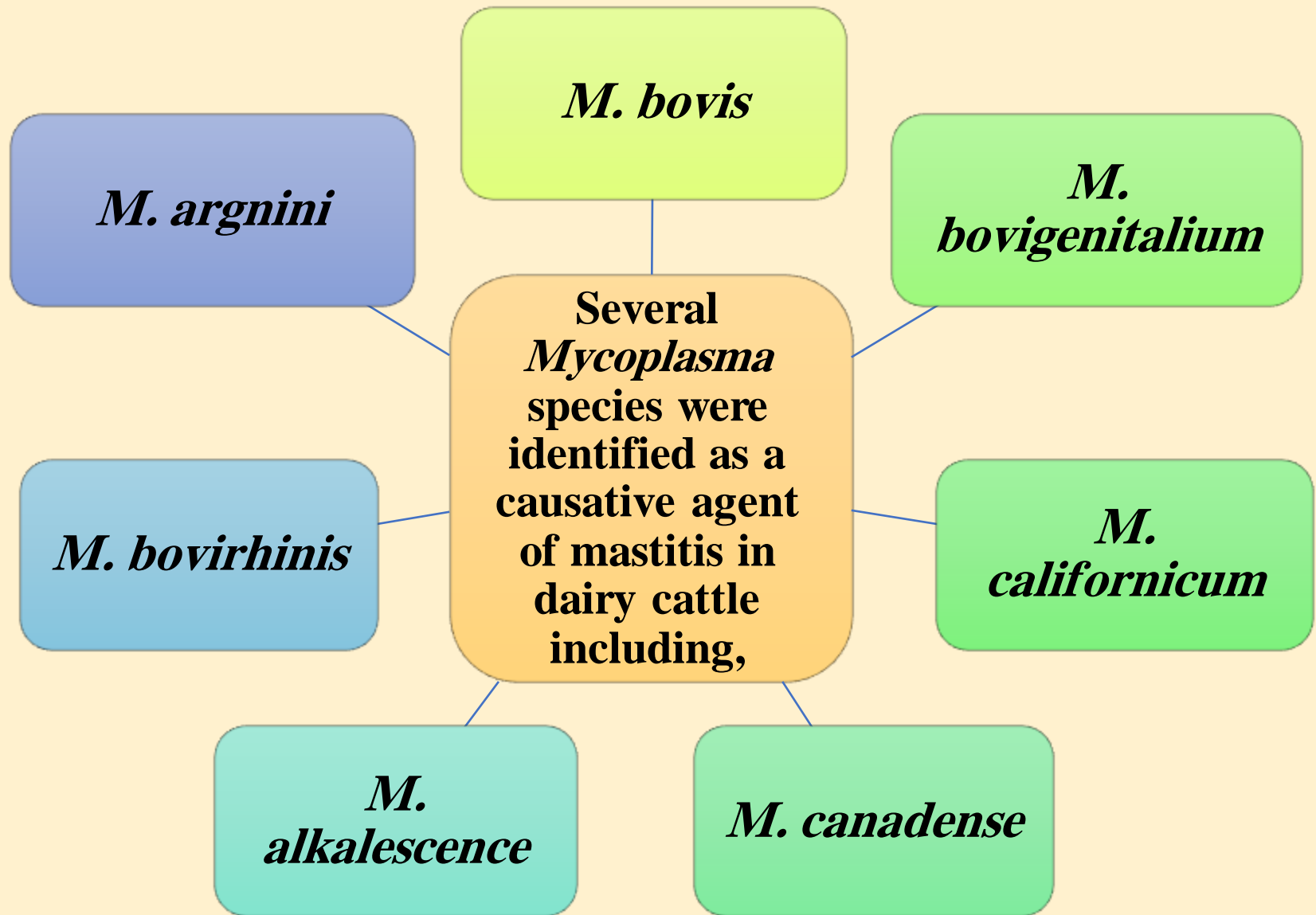


**The common methods used for studying the antigenic structure are:**

- Growth inhibition
- Gel-diffusion test.
- Complement fixation test
- Fluorescent antibody test.
- Indirect haemagghitination test.
- Agglutination tests.

## Pathogenicity of *Mycoplasma*:

- ***M. mycoides*: causes pleuropneumonia in cattle and goats**
- ***M. agalactiae* causes contiguous agalactia (mastitis) in sheep and goats the organism are also pathogenic for dogs, rats, cats and birds.**
- **In human, *M. hominis* causes pleuropneumonia and inflammation of the urogenital tract particularly in female.**



# Laboratory diagnosis:

## **1- Isolation and identification of *Mycoplasma*:**

Isolation and identification of the organism by centrifugation the culture and take the sediment, which was stained with giemsa's stain and observe the typical organism.

(Morphological characters previously discussed )

## **2- Cultural characters and biochemical reactions:**

(previously discussed)

## **3- Serotyping of Mycoplasma isolates.**

## **4- ELISA**

## **5- Molecular identification.**

# Immunization and control:

Experimental vaccines against *Mycoplasma mastitis* were ineffective; even it may worsen the condition. cows that repeatedly suffer from clinical mastitis or become negative upon culturing usually remain subclinical carriers with intermittent shedding of *Mycoplasma* microorganisms, and should be regarded as permanently infected.



Thank you