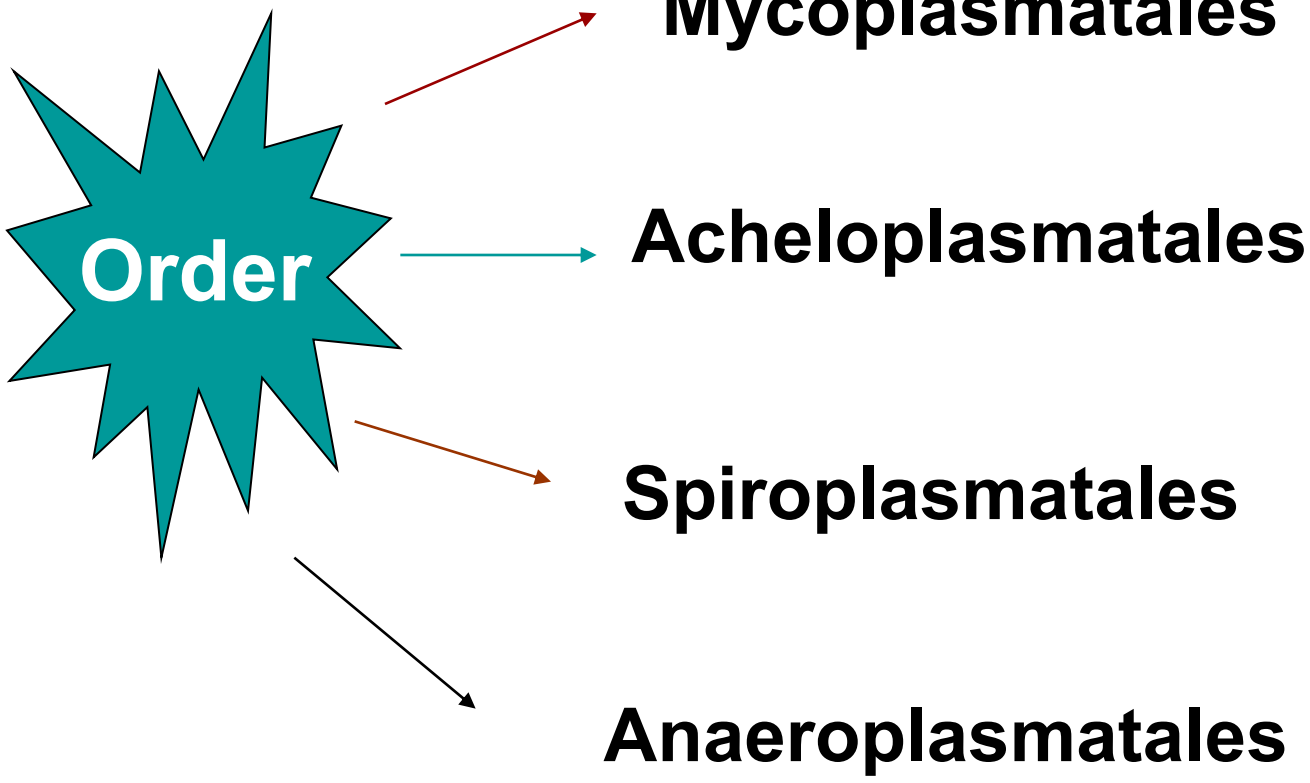
Prepared by

Dr.Hala Sayed Hassan Salam

Assistant prof. of Bacteriology, Mycology and Immunology

Toxonomy of class mollicutes

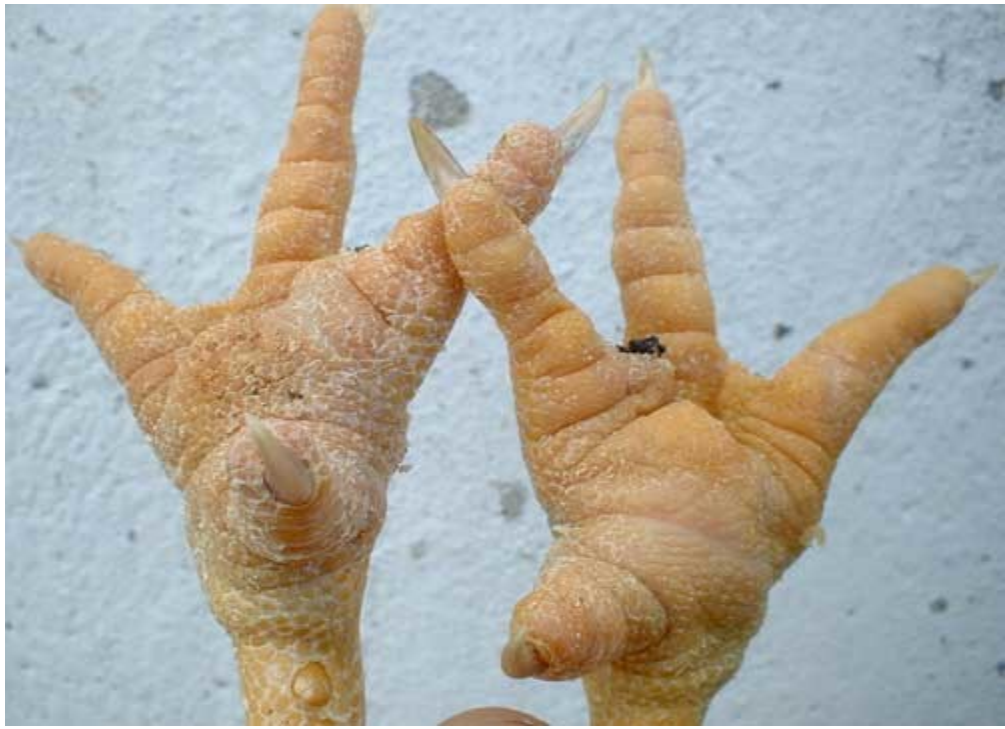


Mycoplasma species of veterinary significance

Species	Hosts	Diseases
Mycoplasma mycoides subsp. Mycoides	Cattle	Contagious bovine pleuropneumonia (CBPP)
M.bovis	Cattle	Mastitis, pneumonia, arthritis
M. agalactiae	Sheep, goat	Contagious mastitis
M. gallisepticum	Chickens, turkeys	Chronic respiratory disease (CRD) Infectious sinusitis
M. synoviae	Poultry	Infectious synovitis
M. meleagridis	turkeys	Airsacculitis, reduced hatchability and growth rate

Chronic Respiratory Disease, Conjunctivitis and breathing problems caused by MG.

Sinusitis in turkey.



Characteristic 'hock sitting' posture of broiler with *Mycoplasma synoviae* arthritis

Tarsal synovitits

- genus Mycoplasma (100 species) contains most of the animal pathogens.
- The first Mycoplasma identified was *Mycoplasma mycoides* subsp. Mycoides, the cause of contagious bovine pleuropneumonia.
- Similar types which were subsequently identified were called pleuropneumonia-like organisms (PPLO).

General characters

1-Minute prokaryotic M.O. (0.1-0.3 μ)

2-Tenericutes (devoid of cell wall)

3- It is the smallest organism capable for self replication (multiply by simple binary fission)

4-It deeply grow in agar due to lack of cell wall

5-Gram –ve, non motile, non sporulated

6-Formed from three original organelles without cell wall which are:

Plasma membrane Ribosomes Nucloid

7-Like virus, it pass through bacterial filter

8-Like bacteria, it grow in culture media

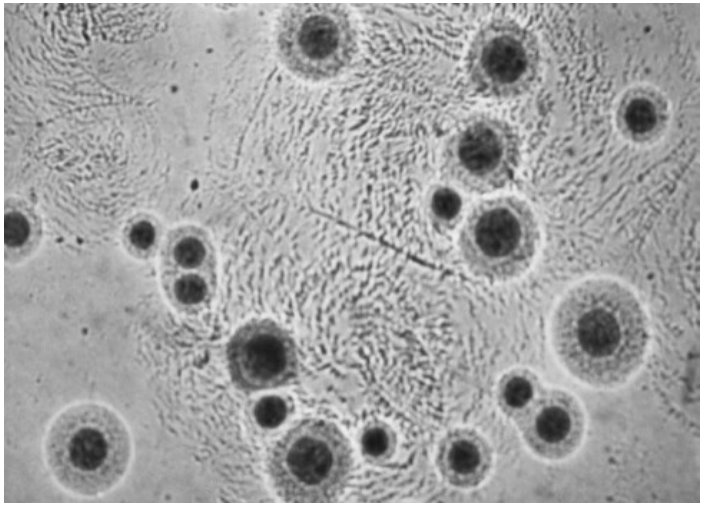
Cultivation and culture characters

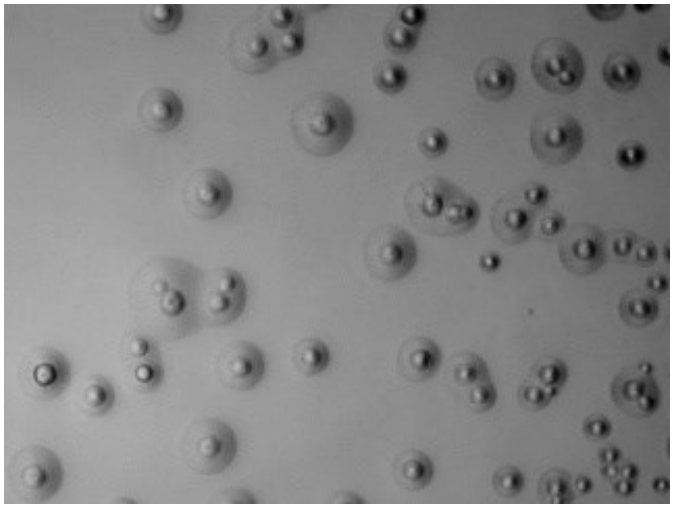
- PPLO broth and agar
- Heart infusion broth and agar
- That contains penicillin G to inhibit G+ve bacteria
- Thallium acetate (inhibit G-ve bacteria and fungi)
- Yeast extract
- Swine or horse serum (provide cholestrol as Mycoplasma not able to synthesize it)
- pH 7.3 to 7.8.
- 5-10% CO2 + humidity.

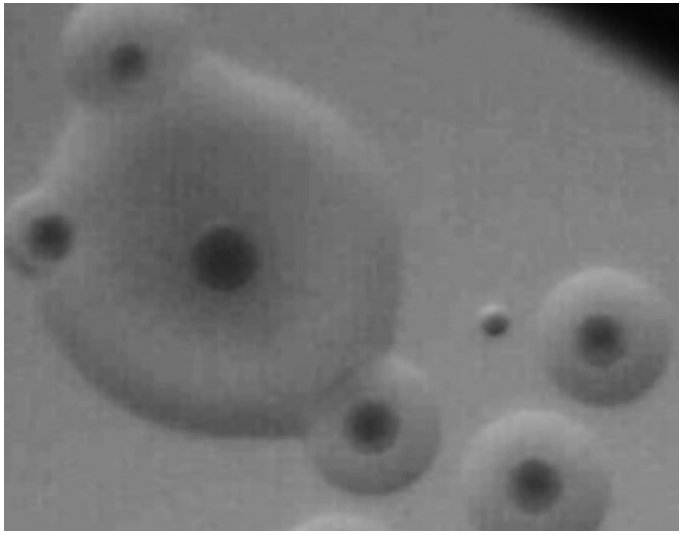
Incubation at 37C for 7-10 days.

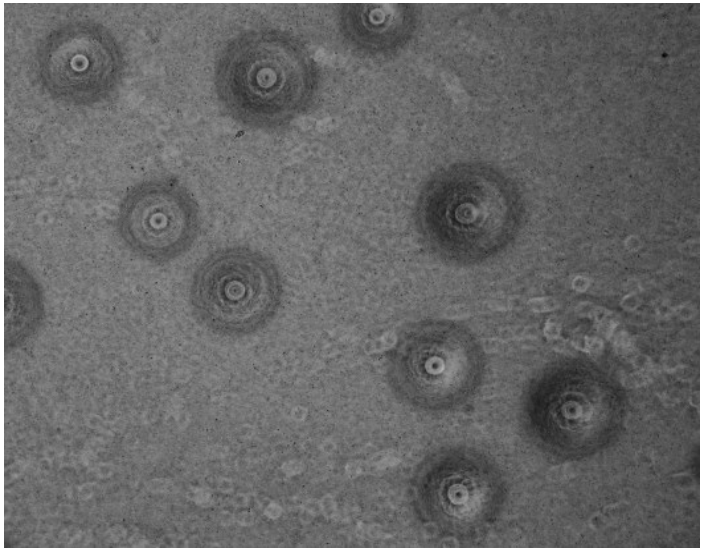
Colonies of Mycoplasma characterized by fried egg appearance or teat of mammary gland (10- 600μ) and examined by dissecting microscope or steriomicroscope

Fried egg appearance









Digitonin test

To differentiate between Mycoplasma and Acheloplasma

(they are identical under micrscope)

Preparation of digitonin solution

1.5 gm digitonin reagent is dissolved into 100ml ethanol then refrigeration

Filter paper disk is saturated by digitonin ethanol solution

Technique

Inoculate agar media with 0.01 ml culture using running drop technique

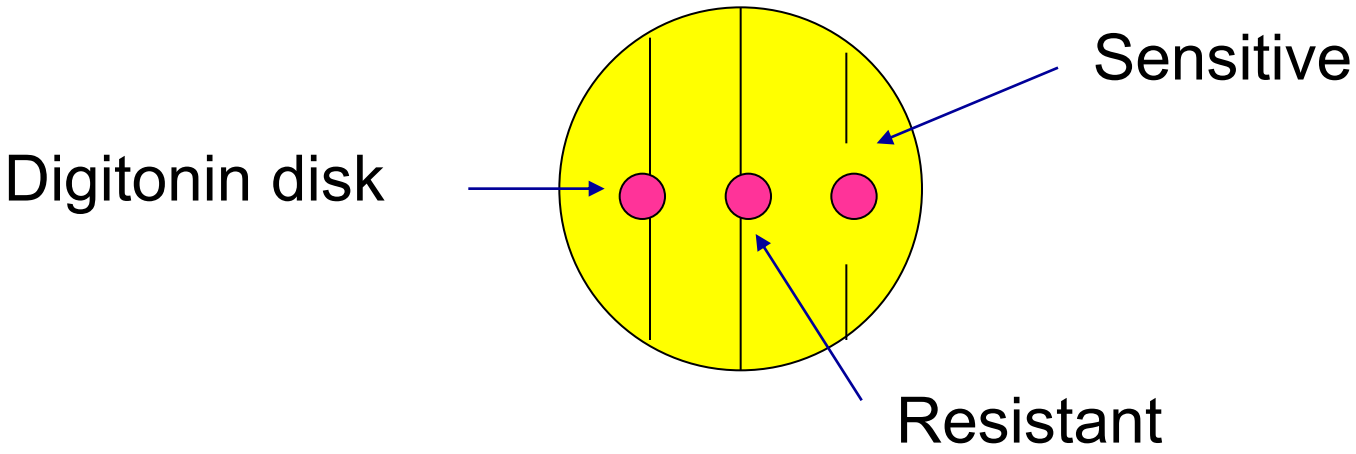
Disks are pressed gently on middle of the streaks

Incubation 3-5 days under optimum condition

Result

Inhibition zone surround disk 5 mm or more (Sensitive to digitonin) ⟶ Mycoplasma

No inhibition zone surround disk ⟶ Not sensitive to digitonin ⟶ Acheloplasma

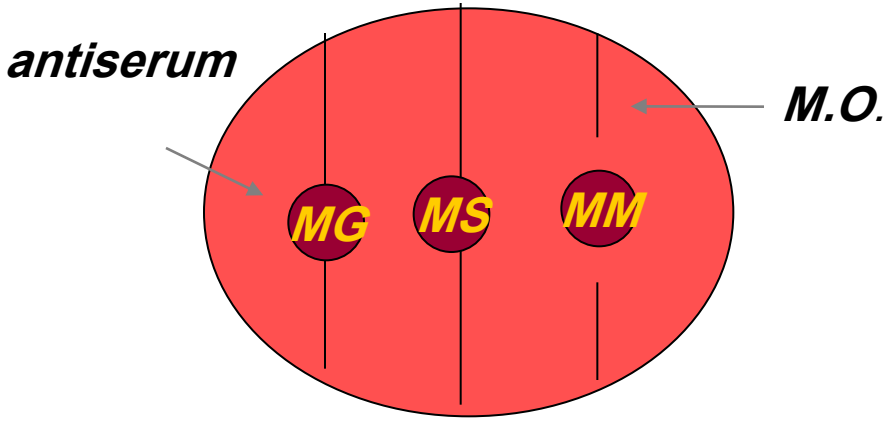


Biochemical identification

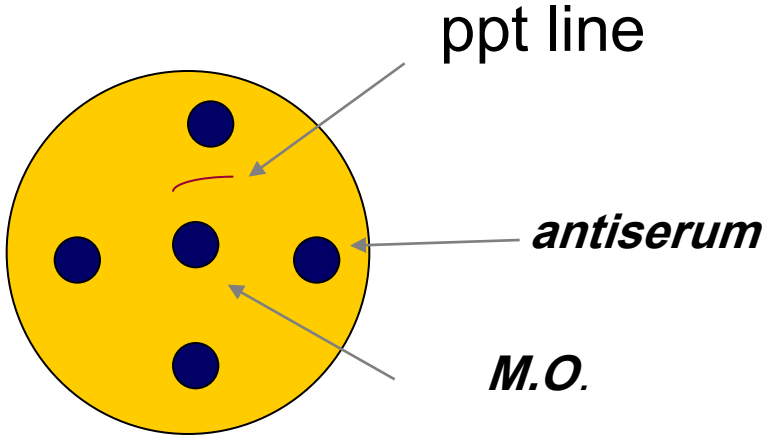
- Glucose fermentation, arginine hydrolysis, casein digestion and phosphatase activity tests are used for differentiation of some pathogenic Mycoplasma species.

immunological identification

- Growth inhibition test
- Growth precipitation test
- Immunoflouresence Ab technique



Growth inhibition



Growth ppt

Serological tests

rapid plate agglutination

haemagglutination inhibition test

- They can be used in avian mycoplasmal diseases.

Molecular diagnosis

Species specific identification using conventional PCR

Detection of Mycoplasma directly in clinical samples using real time PCR.