Mycoplasma

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General Characteristics

- Smallest prokaryotic cells capable of self-replication
- Cannot synthesize Peptidoglycan (resistant to penicillin)
- Do not possess rigid cell walls but have flexible, triple-layed outer membranes
- Lack rigid cell wall and thus definite shape (Pleomorphic organisms)
- Being pleomorphic, they can pass through bacterial filters of 0.22 μm size.
- Require Sterol for growth (except-Acholeplasma)
- Earlier term Pleuropneumonia-like organisms (PPLO) was in use for mycoplasmal organisms.
Media for Mycoplasma

- Smallest prokaryotes that can be grown on artificial media.
- Fastidious in growth requirement.
- Requires sterol and animal protein for growth
- **Penicillin** inhibits growth of Gram positive bacteria
- **Thallium acetate** inhibits Gram negative bacteria and fungi.

<table>
<thead>
<tr>
<th>Media for mycoplasma contains:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Heart infusion broth</td>
</tr>
<tr>
<td>- 20% Horse serum</td>
</tr>
<tr>
<td>- 1% Peptone</td>
</tr>
<tr>
<td>- 1% Albumin</td>
</tr>
<tr>
<td>- Penicillin 1000 IU/ml</td>
</tr>
<tr>
<td>- Thallium acetate</td>
</tr>
<tr>
<td>1: 2000 to 1: 8000 conc.</td>
</tr>
<tr>
<td>- Yeast extract</td>
</tr>
</tbody>
</table>
Culturing Mycoplasma

- Can be grown in liquid or solid media
- Facultative anaerobe – require 5-10% CO₂
- Optimal temperature for growth 35-37°C
- Generally slow grower (generation interval up to 9hrs)
- For culturing ureaplasmas, urea is added to the medium and thallium acetate is removed, which is toxic for these organisms
Mycoplasmal colonies

- *Mycoplasma* colonies are small and can only be observed under a light microscope at low magnification.

- **Dienes stain** is used for staining mycoplasmal microcolonies.

- **Nipple Shaped colony** or Umbonate micro-colonies – *Fried egg appearance*

- Because of tiny colonies of *Ureaplasma*—they were also called **T-mycoplasma**

<table>
<thead>
<tr>
<th>Genus</th>
<th>Colony size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplama</td>
<td>0.1-0.6 mm</td>
</tr>
<tr>
<td>Ureaplasma</td>
<td>0.02-0.06 mm</td>
</tr>
<tr>
<td>Acholeplasma</td>
<td>Upto 1.5 mm</td>
</tr>
</tbody>
</table>
Requirement for Sterol and Urea hydrolysis

- Mycoplasmas require Sterol except members of genus Acholeplasma.

- The requirement of sterol is ascertained performing “Digitonin” sensitivity.

- Digitonin impregnated discs placed on solid media inhibits growth of isolates that require sterol.

- Members of genus Ureaplasma produce Urease and thus capable of hydolysis “urea”.

![Image of a petri dish showing growth inhibition]
<table>
<thead>
<tr>
<th>Isolate</th>
<th>Effect of digiton</th>
<th>Requirement for cholesterol</th>
<th>Urease production</th>
<th>Colony size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma species</td>
<td>Growth inhibition</td>
<td>+</td>
<td>–</td>
<td>0.1 – 0.6 mm</td>
</tr>
<tr>
<td>Ureaplasma species</td>
<td>Growth inhibition</td>
<td>+</td>
<td>+</td>
<td>0.02 – 0.06 mm</td>
</tr>
<tr>
<td>Acholeplasma species</td>
<td>No growth inhibition</td>
<td>–</td>
<td>–</td>
<td>up to 1.5 mm</td>
</tr>
</tbody>
</table>
### Mycoplasma and L form of bacteria

*Mycoplasma* and L type bacteria are similar:
- they both lack a cell wall and the cell is pleomorphic
- they can both pass through an antimicrobial filter

The main differences between the two are:

<table>
<thead>
<tr>
<th>Mycoplasma</th>
<th>L –form of Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycoplasma</em> are independent microbes</td>
<td>L-type bacteria are variants of normal bacterial cells that have a cell wall</td>
</tr>
<tr>
<td></td>
<td>(most L-type cells will revert to their original form)</td>
</tr>
<tr>
<td><em>Mycoplasma</em> growth requires cholesterol</td>
<td>L-type bacteria does not sterol</td>
</tr>
<tr>
<td>(10–20% serum in the medium)</td>
<td></td>
</tr>
<tr>
<td><em>Mycoplasma</em> do not fade easily after Dienes staining</td>
<td>L-type bacteria fade easily after Diane staining</td>
</tr>
</tbody>
</table>
Habitat

• Mycoplasmas are found on mucosal surfaces of the respiratory, intestinal and genital tracts

• Members of Anaeroplasma are strict anaerobes – remain in rumen of cattle and sheep

• Members of Spiroplasma causes disease in plants.

• Factors such as extremes of age, stress and intercurrent infection may predispose disease condition.

• In addition, mycoplasmas may exacerbate disease initiated by other pathogens, particularly in the respiratory tract.

• Mycoplasmal infections cause respiratory diseases of major economic importance in farm animals especially in ruminants, pigs and poultry
<table>
<thead>
<tr>
<th>Mycoplasma species</th>
<th>Hosts</th>
<th>Disease conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. mycoides</em> subsp. <em>mycoides</em> (small colony type)</td>
<td>Cattle</td>
<td>Contagious bovine pleuropneumonia</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
<td>Cattle</td>
<td>Mastitis, pneumonia, arthritis</td>
</tr>
<tr>
<td><em>M. agalactiae</em></td>
<td>Sheep, goats</td>
<td>Contagious agalactia</td>
</tr>
<tr>
<td><em>M. capricolum</em> subsp. <em>capripneumoniae</em> (F38)</td>
<td>Goats</td>
<td>Contagious caprine pleuropneumonia</td>
</tr>
<tr>
<td><em>M. capricolum</em> subsp. <em>capricolum</em></td>
<td>Sheep, goats</td>
<td>Septicaemia, mastitis, polyarthritis, pneumonia</td>
</tr>
<tr>
<td><em>M. mycoides</em> subsp. <em>mycoides</em> (large colony type)</td>
<td>Goats, sheep</td>
<td>Pleuropneumonia, mastitis, septicaemia, polyarthritis</td>
</tr>
<tr>
<td><em>M. mycoides</em> subsp. <em>capri</em></td>
<td>Goats</td>
<td>Septicaemia, pleuropneumonia, arthritis, mastitis</td>
</tr>
<tr>
<td><strong>M. hyopneumoniae</strong></td>
<td>Pigs</td>
<td>Enzootic pneumonia</td>
</tr>
<tr>
<td>----------------------</td>
<td>------</td>
<td>-------------------</td>
</tr>
<tr>
<td><strong>M. hyorhinis</strong></td>
<td>Pigs (3-10 weeks of age)</td>
<td>Polyserositis</td>
</tr>
<tr>
<td><strong>M. hyosynoviae</strong></td>
<td>Pigs (10-30 weeks of age)</td>
<td>Polyarthritis</td>
</tr>
<tr>
<td><strong>M. gallisepticum</strong></td>
<td>Chickens, Turkeys</td>
<td>Chronic respiratory disease, Infectious sinusitis</td>
</tr>
<tr>
<td><strong>M. synoviae</strong></td>
<td>Poultry</td>
<td>Infectious synovitis</td>
</tr>
<tr>
<td><strong>M. meleagris</strong></td>
<td>Turkeys</td>
<td>Airsacculitis, bone deformities, reduced hatchability and growth rate</td>
</tr>
</tbody>
</table>
Contagious Bovine Pleuro-pneumonia (CBPP)

- A severe contagious disease of cattle caused by *M. mycoides subspecies mycoides (small colony type)*

- Transmission is by aerosols - requires close contact

- In severe outbreaks the mortality rate may be high

- Death can occur 1 to 3 weeks after the onset of clinical signs.
CBPP- Symptoms

- Acute form - sudden **onset of high fever, anorexia, depression, drop in milk yield, accelerated respiration and coughing.**

- Animals adopt a **characteristic stance** with the head and neck extended and elbows abducted.

- **Expiratory grunting and mucopurulent nasal discharge** may be present.

- Arthritis, synovitis and endocarditis may be present in affected calves.
CBPP- PM findings

- *Pneumonic lungs have a marbled appearance.*
- Grey and red consolidated lobules
- *Interlobular septa* are distended and oedematous.
- *Sero fibrinous exudate in the pleural cavity.*
- In chronic cases, *fibrous encapsulation of necrotic foci* is commonly found.
- These necrotic foci contain viable mycoplasmas
CBPP- Diagnosis and Control

Diagnosis
• Clinical signs and characteristic PM findings helps in presumptive diagnosis.
• PCR, FAT can be used on pleural fluid to confirm the presence of the pathogen.
• Broncho-alveolar lavage, pleural fluid, lung tissue or the broncho-pulmonary lymph nodes

Serological tests:
• Rapid field serum agglutination test
• Passive haemagglutination screening test
• Complement fixation test
• Dot-blot technique for confirmation

Treatment and control
• Treatment is generally unsatisfactory
<table>
<thead>
<tr>
<th>Pneumonia, Mastitis, Polyarthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>- <em>Mycoplasma bovis</em> causes severe pneumonia in calves</td>
</tr>
<tr>
<td>- It can exacerbate respiratory disease caused by <em>Pasteurella</em> and <em>Mannheimia</em> species</td>
</tr>
<tr>
<td>- Most important mycoplasmal species causing <em>mastitis</em>.</td>
</tr>
<tr>
<td>- Mycoplasmal mastitis is common in large daily herds.</td>
</tr>
<tr>
<td>- Milking machines, milkers' hands and cloths which are then important sources of infection Causes severe mastitis, systemic involvement is uncommon.</td>
</tr>
<tr>
<td>- subclinical carriers are important sources of infection</td>
</tr>
<tr>
<td>- Dramatic loss of milk production and the serous or purulent mastitic exudates</td>
</tr>
<tr>
<td>- Infection often results in agalactia</td>
</tr>
<tr>
<td>Mycoplasmal infections in Goats</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
</tbody>
</table>
| **Contagious caprine pleuropneumonia** | - Caused by *M. capricolum* subspecies *capripneumoniae* (*Mycoplasma* strain F38)
- Similar in many respects to CBPP
- *Highly contagious disease*; transmission through *aerosols*.
- The disease is characterized by *pneumonia*, *fibrinous pleurisy*, *profuse pleural exudate*
- *Marbled appearance* on the cut surface of affected lungs is seen.
- Inactivated vaccines give satisfactory protection.
- Pleuropneumonia in goats can occasionally be caused by
  - *M. mycoides* subspecies *capri*
  - *M. mycoides* subspecies *mycoides* (large colony type)
  - *M. capricolum* subspecies *capricolum*
| **Contagious agalactia of sheep and goats** | - Caused by *M. agalactiae*
- Characterized by *mastitis, arthritis* and *conjunctivitis*.
- Observed soon after parturition
- *Pregnant animals may abort*
- Can be fatal in young animals due to pneumonic complications.
- The organism is shed in milk
- Organism may remain localized in the supra-mammary lymph nodes
| **Mastitis, Pneumonia, Arthritis** | - *M. mycoides* subspecies *capri*
- *M. mycoides* subspecies *mycoides* (large colony type)
- *M. capricolum* subspecies *capricolum* |
### Mycoplasmal infections in Pigs

| Enzootic pneumonia | • Caused by *M. hyopneumoniae*,  
|                     | • Affects intensively reared pigs.  
|                     | • Extremes of temperature, sudden change in temperature, poor ventilation, overcrowding  
|                     | • Pigs of all ages are susceptible  
|                     | • Characterized by coughing, poor growth rates and, in some cases, respiratory distress.  
|                     | • At post mortem, *pulmonary consolidation is confined to the apical and cardiac lobes* with clear demarcation from normal lung tissue. |
| Polyserositis       | • *Caused by Mycoplasma hyorhinis*  
|                     | • Chronic progressive polyserositis in pigs up to 10 weeks of age.  
|                     | • It is characterized by *fever, laboured breathing, lameness and swollen joints*. |
| Polyarthritis       | • Caused by *M. hyosynoviae*  
|                     | • Affects pigs from 10 to 30 weeks of age.  
|                     | • This self-limiting arthritis and synovitis produces transient lameness. |
Poultry infections

- *Mycoplasma gallisepticum* causes *chronic respiratory disease* in chickens and *infectious sinusitis* in turkeys.

**Transmission:**
- Through infection of the embryo in the egg
- By aerosols

- **In chickens:** affects upper respiratory tract.
- **In turkeys:** Causes swelling of the paranasal sinuses

**Diagnosis:**
- Isolation and identification of the pathogen
- Flock testing using the serum plate agglutination test
- Haemagglutination inhibition and ELISA tests
- Specific-pathogen-free (SPF) flocks
- Eggs used for hatching should be dipped in a tylosin
## Poultry infections

| **Mycoplasma gallisepticum** | *Chronic respiratory disease* in chickens  
*Infectious sinusitis* in turkeys |
|-----------------------------|----------------------------------------------------------------------------------|
| **Mycoplasma meleagridis**  | Causes airsacculitis in young poults and joint and bone deformities in growers  
*Transmission is mainly* through eggs |
| **Mycoplasma synoviae**     | *Infectious synovitis*  
- In chickens and turkeys  
- Transmitted mainly by aerosols.  
- Arthritis and respiratory signs are the main clinical features |
Diagnosis of Mycoplasmal infections

- Suitable samples include mucosal scrapings, tracheal exudates, aspirates, pneumonic tissue, mastitic milk and fluids from joints or body cavities.

- The presence of Mycoplasma in samples can be demonstrated by:
  - Fluorescent antibody techniques
  - Peroxidase-antiperoxidase procedures on paraffin embedded tissues
  - Polymerase chain reaction techniques
Diagnosis of Mycoplasmal infections

- Isolation can be attempted in mycoplasma medium under 10% CO$_2$ at 37° C for up to 14 days.

- Identification criteria for isolates:
  - 'Fried-egg' microcolonies
  - Microcolony size
  - Cholesterol requirement for growth (digitonin sensitivity test)
  - Biochemical profile including urease production
  - Fluorescent antibody technique on microcolonies
  - Growth inhibition test with specific antisera
Serological tests

- Complement fixation tests

- Tests based on ELISA

- Rapid plate agglutination tests

- Haemagglutination-inhibition tests
THANKS