PDA Mycoplasma Filtration Training: Mycoplasma

Barbara J. Potts, Ph.D.
Potts & Nelson Consulting.
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Mycoplasma

- **Smallest free-living microorganisms**
- **Pleomorphic**
  - **Vary in size and shape from 0.2µm upwards**
  - **Pliability allows mycoplasma to pass through small pore filtration devices.**
- **Derived from ancestral anaerobic bacteria (clostridia) by gene deletion.**
- **Contains no cell wall hence, they do not take up Gram stain.**
- **Assume various shapes from round to filamentous.**
- **Can be seen by dark-field and phase-contrast light microscopy.**
- **One mycoplasma can initiate an infection resulting in 10^9 CFU/mL within 48-72 hours.**

Bacteria

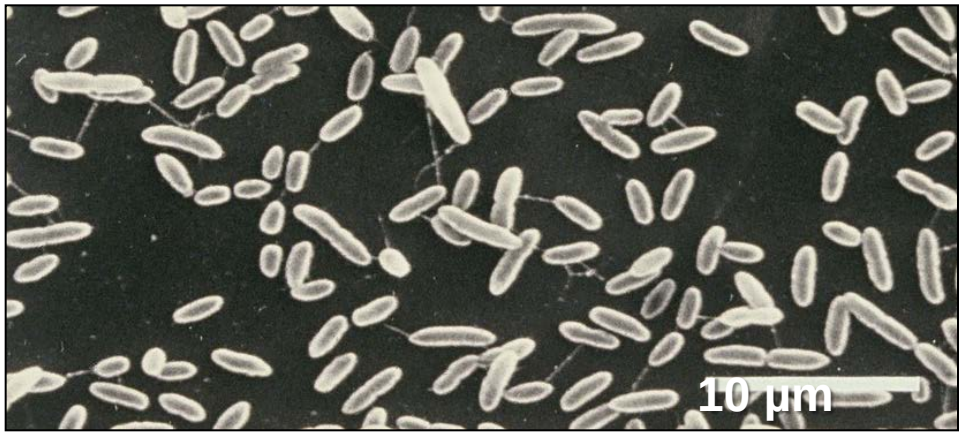
- **Prokaryotic**
  - **Absence of a membrane surrounding the nuclear region.**
  - **Absence of sub cellular organelles.**
- **Much smaller dimensions than most eukaryotic cells, (plants, animals) which have a membrane bounded nucleus. (1µm compared to 10µm).**
- **Thrive in a range of environments that are extreme.**
  - **Can use an infinite array of energy sources.**
- **Can be seen by using light microscopy.**
- **Have a cell wall surrounding the cytoplasmic membrane.**
  - **Gram Stain of cell wall will identify**
    - **Gram positive bacteria (e.g. *Bacillus*)**
    - **Gram negative bacteria (e.g. *E.coli*)**
- **One bacterial cell can initiate an infection.**

Morphological Examination of Viruses, Mycoplasma, Bacteria and Fungi/Mold

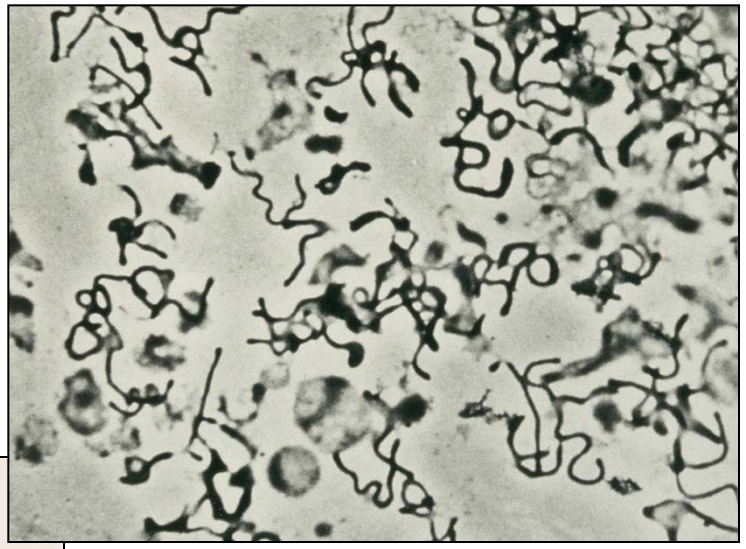
Specimen	Size	Microscopic Range
Fungi/Mold	100µm	Phase contrast
CHO cells	10µm	Phase contrast
Bacteria	1µm	Phase contrast
Mycoplasma	0.2µm & above	Phase contrast
Viruses	200nm-17nm	Electron microscopy

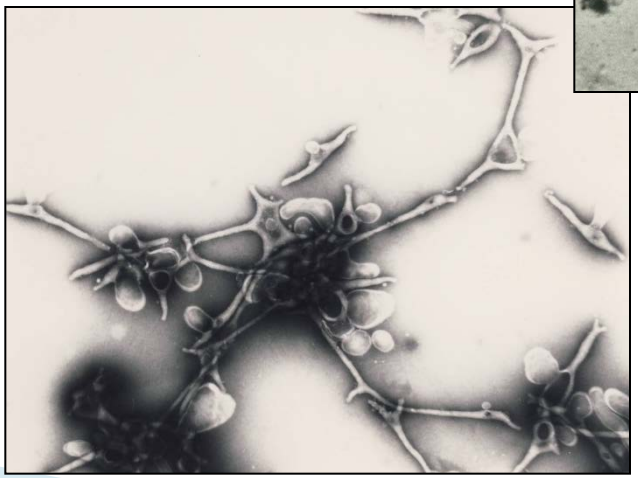
Reference: Microbiology and Microbial Infections Vol. 2 Systemic Bacteriology Ed.A. Balows, B.I. Duerden. 1998, Oxford University Press Inc. N.Y.

Bacterial Cell Morphology



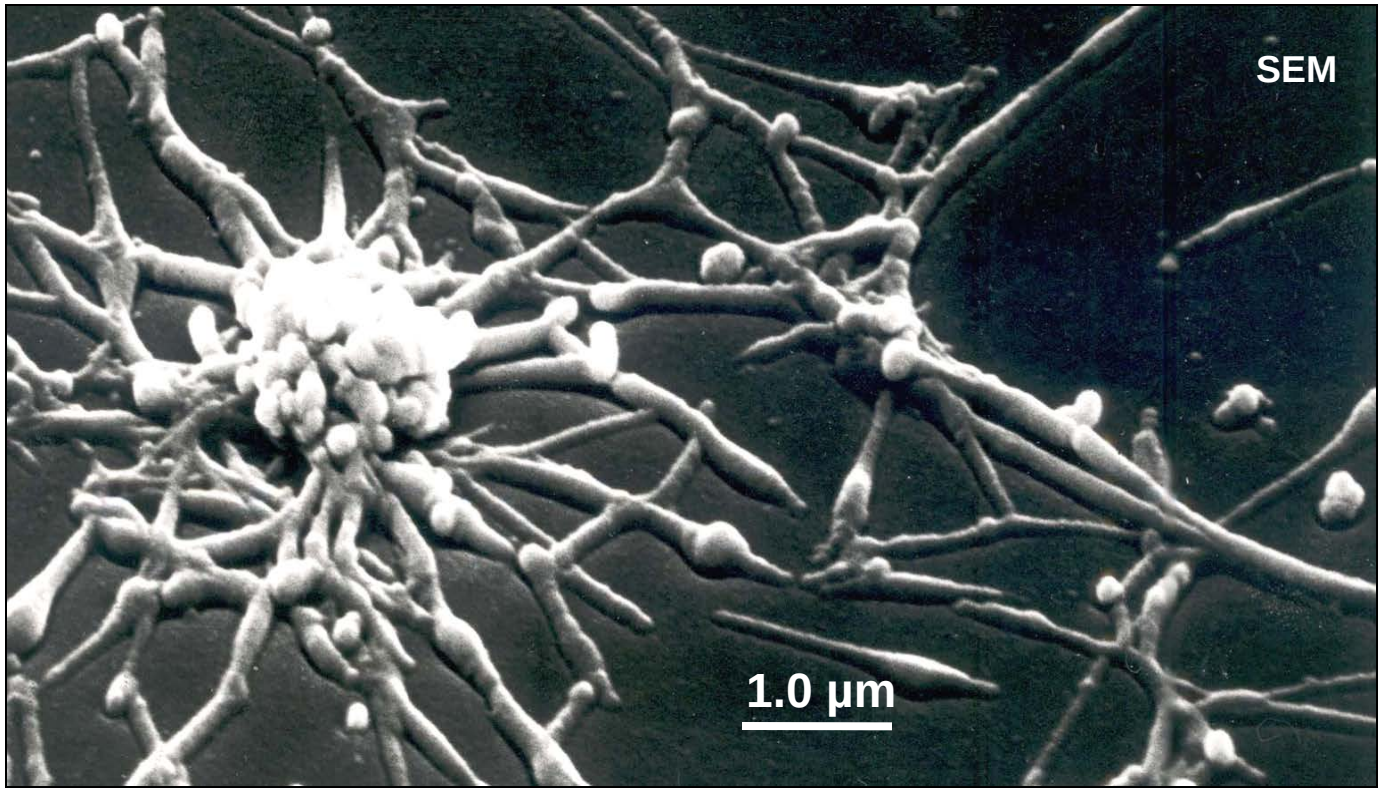
Mycoplasma Cell Morphology
Typical Pleomorphism





Photomicrographs courtesy of Prof. Renate Rosengarten

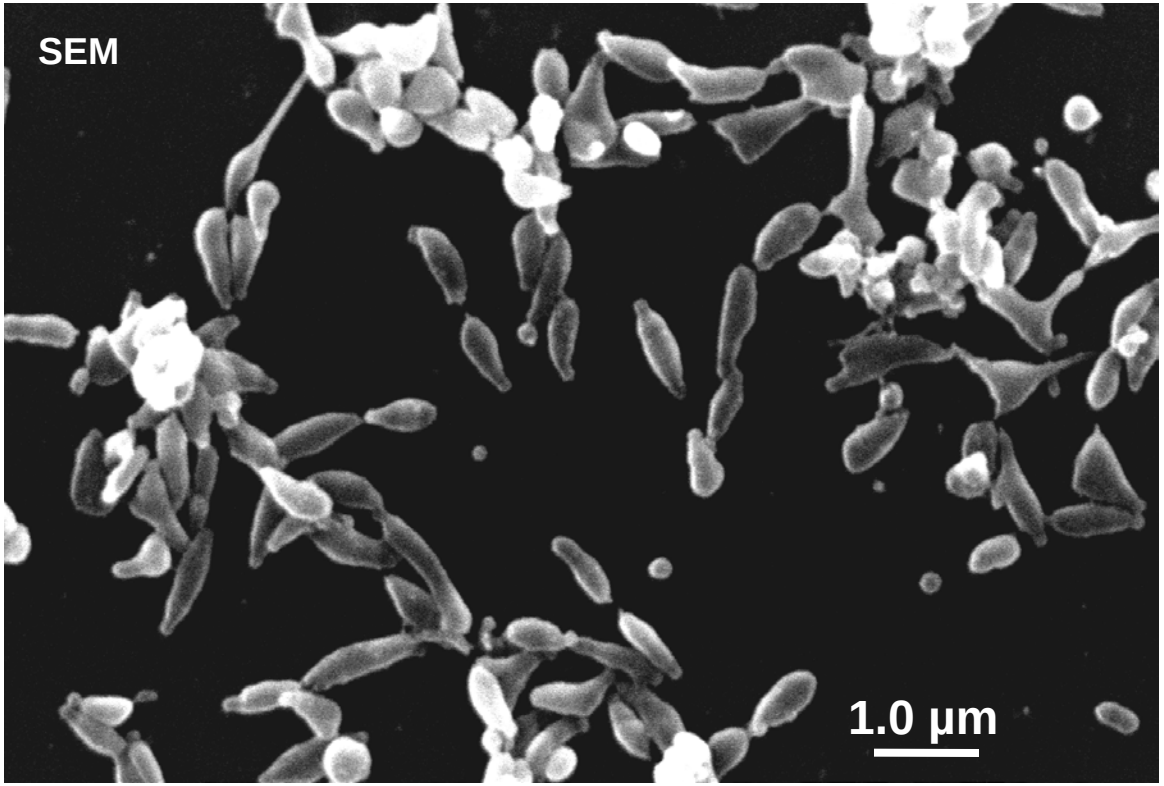
Mycoplasma Cell Morphology
Microcolonies and Biofilms



Mycoplasma pneumoniae

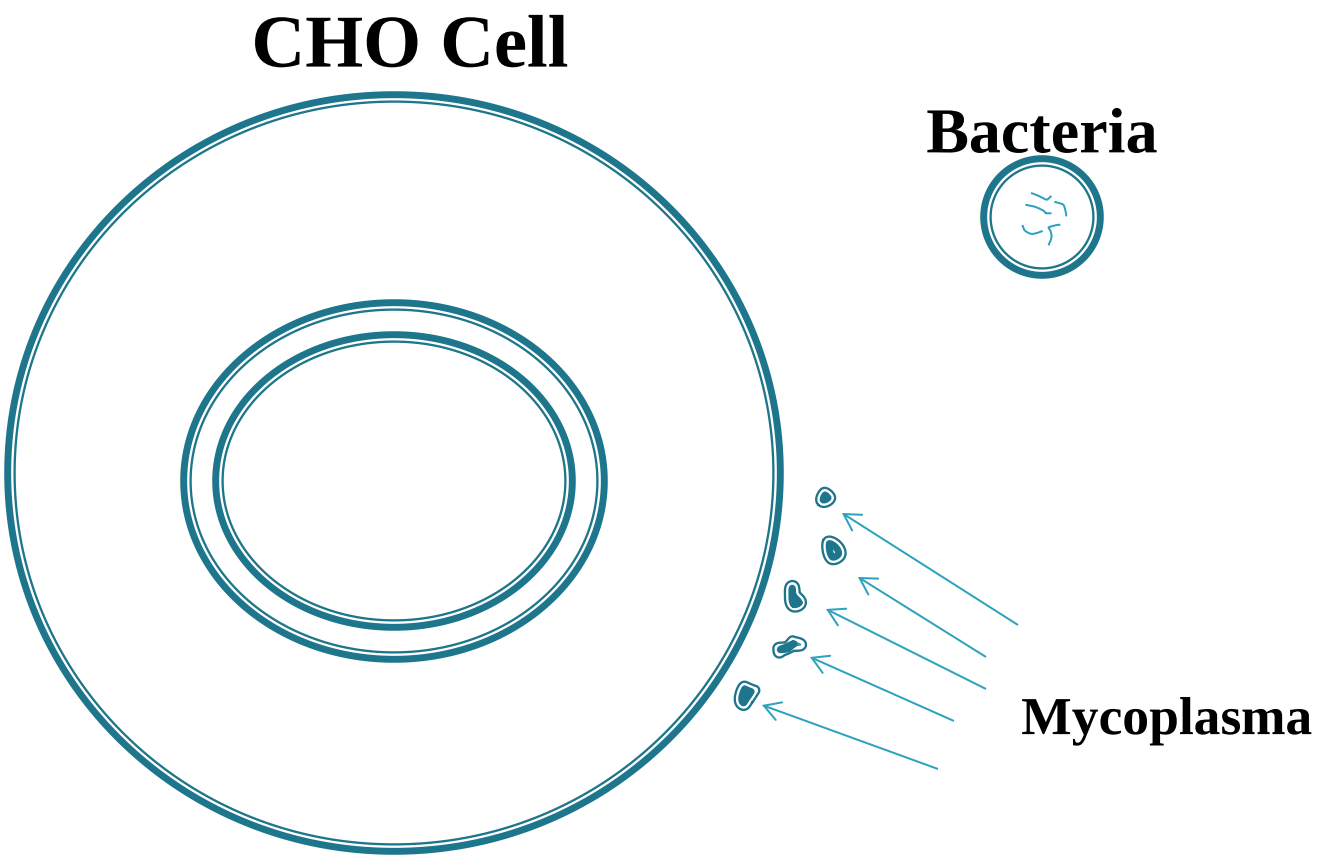
Photomicrograph courtesy of Prof. Renate Rosengarten

Mycoplasma Cell Morphology
Microcolonies and Biofilms



Mycoplasma gallisepticum

Photomicrograph courtesy of Prof. Renate Rosengarten


CHO Cell
Bacteria
Mycoplasma

Mycoplasma Contamination Risks

1. **Pre Banks and Cell Banks**
2. **Complex media**
3. **Peptones – plant and animal sourced**
4. **Water ?**
5. **Tryptic soy broth used for media fills**
6. **Analysts/environment**
7. **Bovine serum**

Steps in Production	Filtration	Filter Decision Makers
1. Pre Bank	X	R&D
2. Master Cell Bank	X	Process Development: Early stage cell culture staff
3. Working Cell Bank	X	
4. Unprocessed Bulk (cells plus media)	X	
5. Recovery Line		Process Development: Late stage purification staff
Protein A Column	X	
Low pH	X	
Anion exchange column	X	
Cation exchange column	X	
Virus filters	X	
Non-virus filters	X	

Steps in Production	Filtration	Filter Decision Makers
6. Processed Bulk	X	Process Development Final Production formulation staff
7. Final Vial	X Validated sterile filtered	Process Development and QA sterile fill staff
8. Sterility Assurance *media fill*	Gamma irradiated or sterile autoclave or filtration	Process Development and QA sterile fill staff

Filter Decision Makers	Technical Expertise
1. Process Development: Early stage cell culture staff. (mycoplasma contamination risk)	• Cell culture growth • Transfections/selections media • Expression systems • Plasmid design, glycosylation, etc…
2. Process Development: Late stage purification staff	• Analytical chemistry • Column performance • Product characterization e.g. aggregation • Ion exchange process • Capillary electrophoresis • Virus removal/inactivation • Virus filters

Filter Decision Makers	Technical Expertise
3. Process Development: Final product formulation	• Formulation chemistry • Product characterization
4. Process Development: QA and QC sterile fill and Sterility Assurance staff (Mycoplasma contamination risk)	• Performance of sterile filters • Lypholization/liquid products • Capping, Labeling, Microbiology

Summary

- **Filter trains may need to eliminate mycoplasmas as well as other micro-organisms**
- **The possible presence of these organisms in the bioburden changes the requirements for filter specification**
- **The personnel responsible for choosing, ordering and validating filters need to be aware of:**

  1. Mycoplasma risks for the product/component to be filtered

  2. The properties of these organisms which affect their retention by the filter media

I would like to thank Dr Barbara Potts for providing the slides for this section and Renate Rosengarten for the photographs