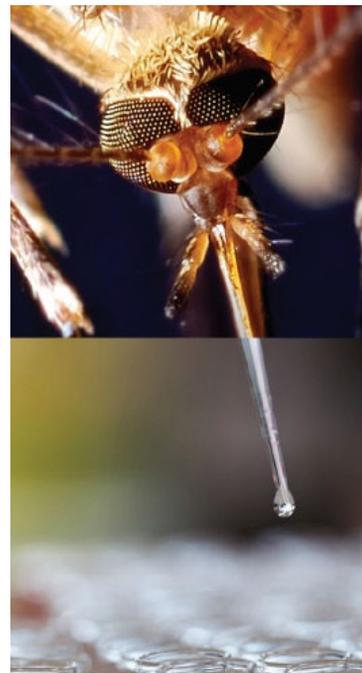
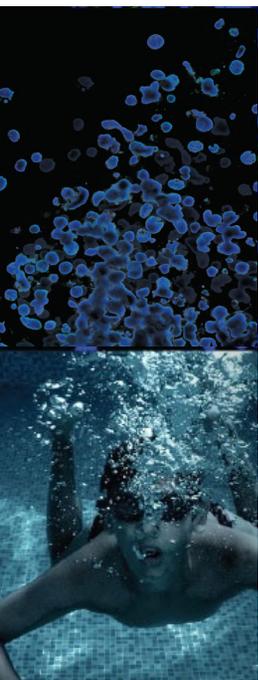




# Tips and Techniques for Successfully Growing Bacteria in Culture

Nancy Krueger, MS  
*Lead Biologist, ATCC*

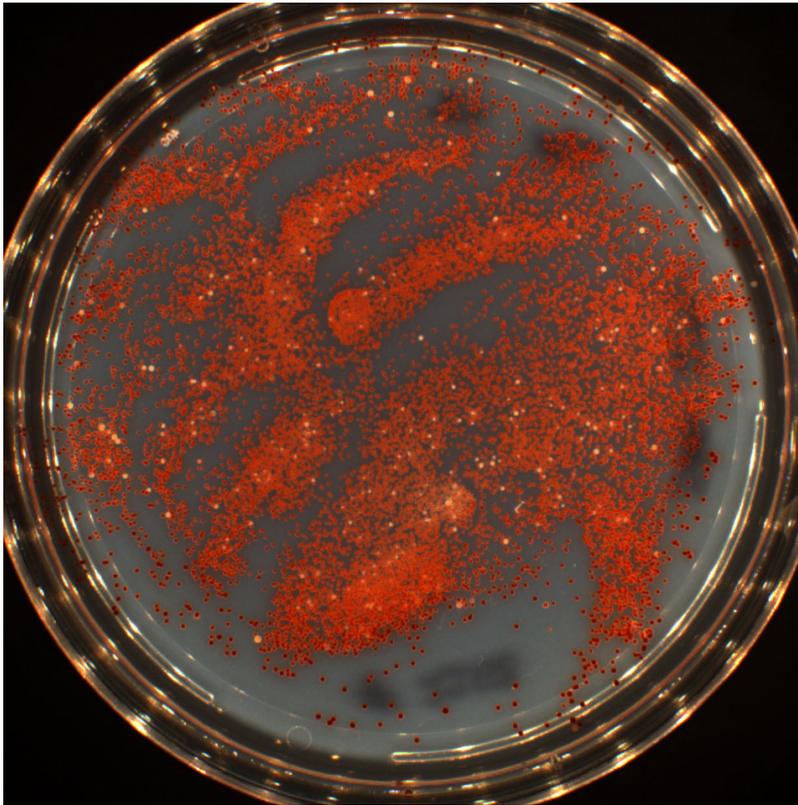
Credible Leads to Incredible™



# About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for bacterial culture – the “*gold standard*”
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

# Agenda



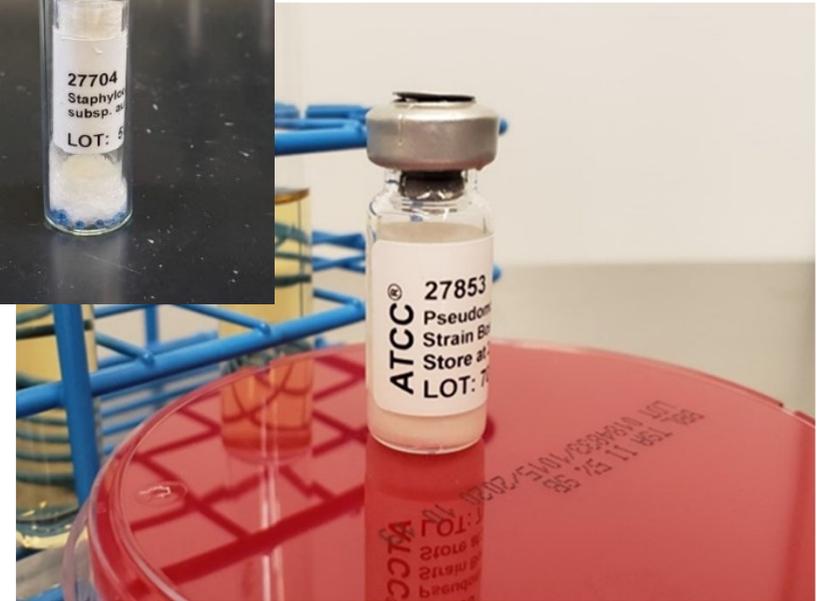
1. Propagation Methods
2. Nutritional and Atmospheric Considerations
3. New bacterial isolates
4. Troubleshooting
5. Quality Control Testing

*Actinocrinis puniceicyclus* (ATCC® BAA-2771™)

# Propagation Methods

## *Starting a culture from a preserved state*

- Freeze-dried
  - Batch vials
  - Serum vials
- Frozen
  - Cryovials
  - Mini's



# Propagation Methods

## Basic Method

- Use a single tube of broth: 5 to 6 mL
- Rehydrate the entire pellet
- Aseptically transfer this aliquot back into the broth tube and mix well
- Inoculate a secondary broth
- Inoculate an agar slant and/or plate
- Incubate all tubes and plate



# Propagation Methods

## Open a batch vial

- Product Information Sheet
  - Includes specific detail for the item
  - Follows the procedure tested at ATCC
  - Contact Customer Care if there isn't one available

**ATCC**  
Product Sheet  
**Escherichia coli (ATCC® 25922™)**

Please read this **FIRST**

Storage Temp.  
**Frozen: -80°C or colder**  
**Freeze-Dried: 2°C to 8°C**  
**Live Culture: See Propagation Section**

Biosafety Level  
**1**

**Intended Use**  
This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

**Citation of Strain**  
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Escherichia coli* (ATCC® 25922™)

**Description**  
**Designation:** FDA strain Seattle 1946 [DSM 1103, NCIB 12210]  
**Deposited Name:** *Escherichia coli* (Migula) Castellani and Chalmers  
**Antigenic Properties:** Serotype O6, Biotype 1  
**Product Description:** Does not produce verotoxin. This organism is a CLSI control strain for antimicrobial susceptibility testing. It is used for media testing, as a negative control for LT toxin production, and as a quality control strain for Abbott, API, Autobac, BBL, bioMérieux Vitek, Biosynth, Difco, IDS, Micro-Media, MicroScan™, Roche Diagnostics, and Sensititre products. Used in susceptibility disc testing of neomycin, colistin [colimycin], kanamycin, cephalixin, gentamicins, cefamandole, cephalothin, tetracycline, cephaloglycin, cephaloridine [cephalomylin], nalidixic acid, and chloramphenicol.

**Propagation**  
**Medium**  
ATCC® Medium 18: Trypticase Soy Agar/Broth  
**Growth Conditions**  
**Temperature:** 37°C  
**Atmosphere:** Aerobic  
**Propagation Procedure**  
1. Open vial according to enclosed instructions.  
2. Using a single tube of #18 broth (5 to 6 mL), withdraw approximately 0.5 to 1.0 mL with a Pasteur or 1.0 mL pipette. Rehydrate the entire pellet.  
3. Aseptically transfer this aliquot back into the broth tube. Mix well.  
4. Use several drops of the suspension to inoculate a #18 agar slant and/or plate.  
5. Incubate the tubes and plate at 37°C for 24 hours.

**Notes**  
ATCC® 25922™ is a recommended reference strain for antibiotic susceptibility testing. It has been found that passage in broth often results in a change in MIC levels. Therefore, it is best to keep it on agar and to make stocks for storage immediately. Repeated passage is discouraged. Purified genomic DNA of this strain is available as ATCC® 25922D-5™. Additional information on this culture is available on the ATCC® web site at [www.atcc.org](http://www.atcc.org).

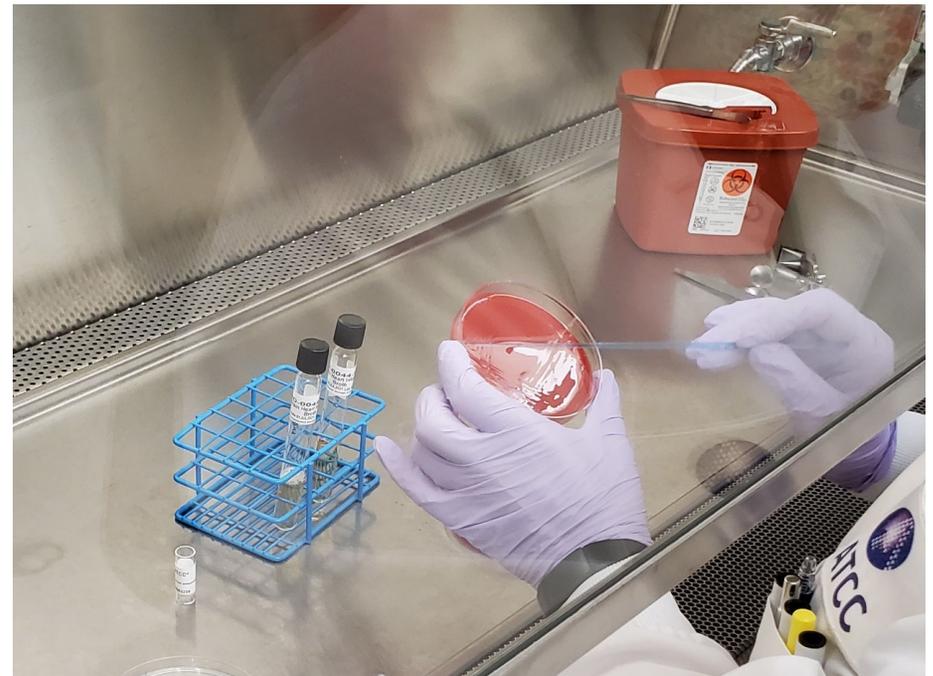
**References**  
References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

**Biosafety Level: 1**

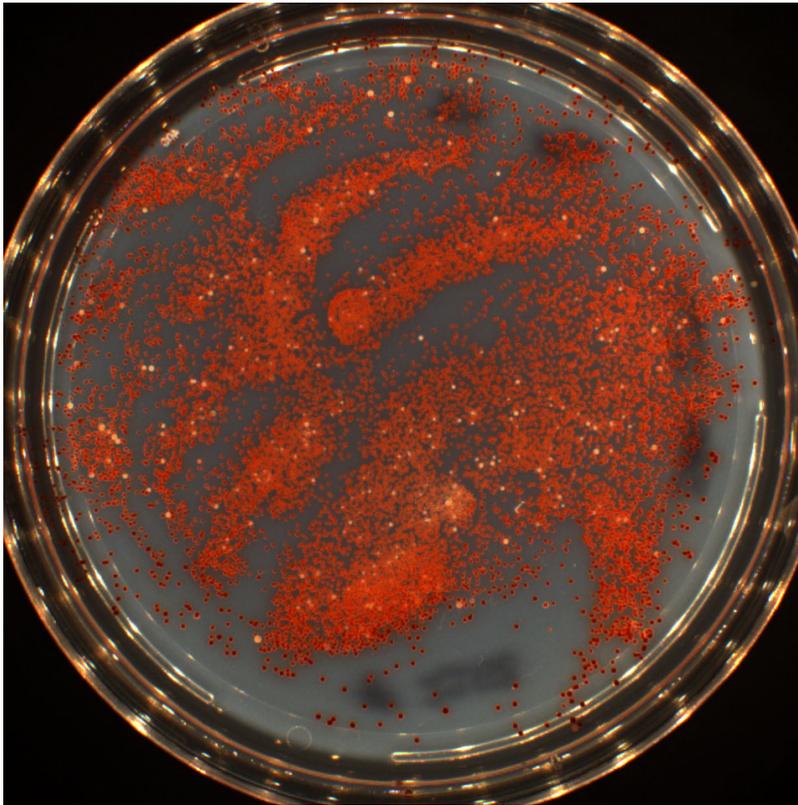
# Propagation Methods

## *Open a batch vial*

- Considerations for recovery
  - Typical cell count
  - Thaw – refreeze
  - Over dilution



# Agenda

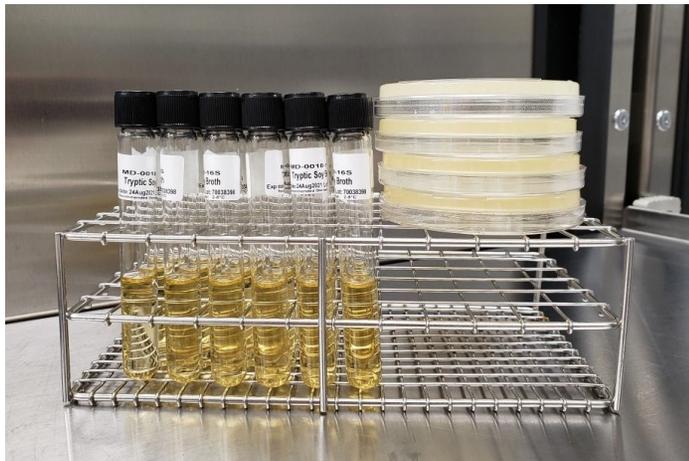


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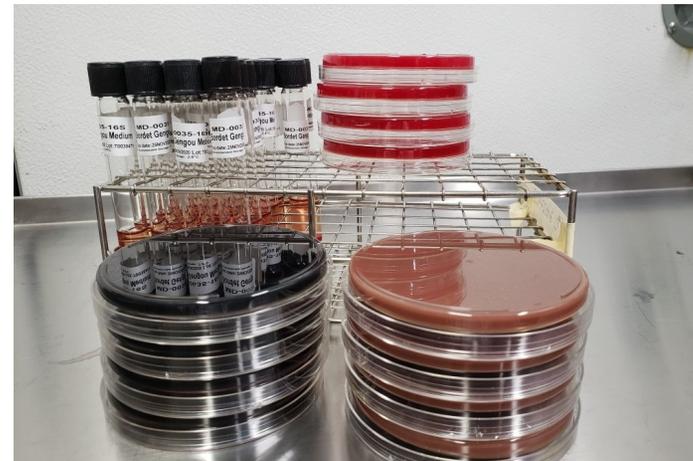
*Actinocrinis puniceicyclus* (ATCC® BAA-2771™)

# Nutritional Requirements

- Common growth media
  - Nutrient
  - Tryptic Soy
  - LB



- Nutrient rich media
  - Chocolate (GC)
  - Bordet-Gengou
  - Buffered Charcoal Yeast Extract (CYE)
  - Brucella



# Facultative

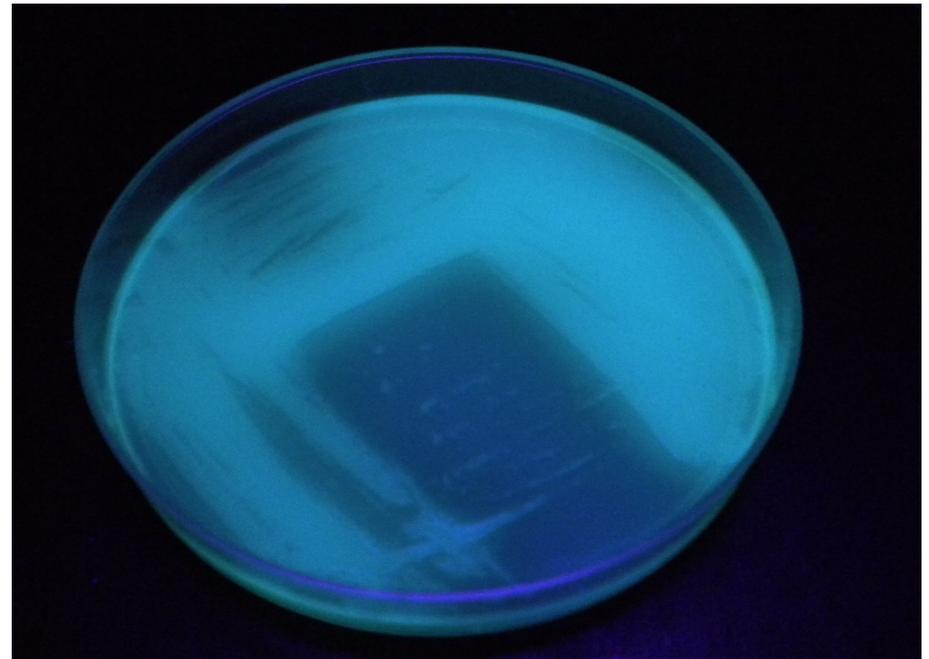


*Klebsiella pneumoniae* (ATCC® BAA-1898™)  
MacConkey agar, positive for lactose fermentation

- This group includes:
  - Escherichia*
  - Serratia*
  - Klebsiella*
  - Enterobacter*
- Gram-negative
- Generate ATP by aerobic respiration in the presence of oxygen – switch to fermentation in its absence
- Ferment carbohydrates
- Many are pathogenic

# Non-Enteric

- This group includes:
  - Pseudomonas*
  - Acinetobacter*
  - Burkholderia*
- Gram-negative
- Large diverse group
- Found in a wide variety of habitats
- Can be opportunistic pathogens
- Do not ferment carbohydrates

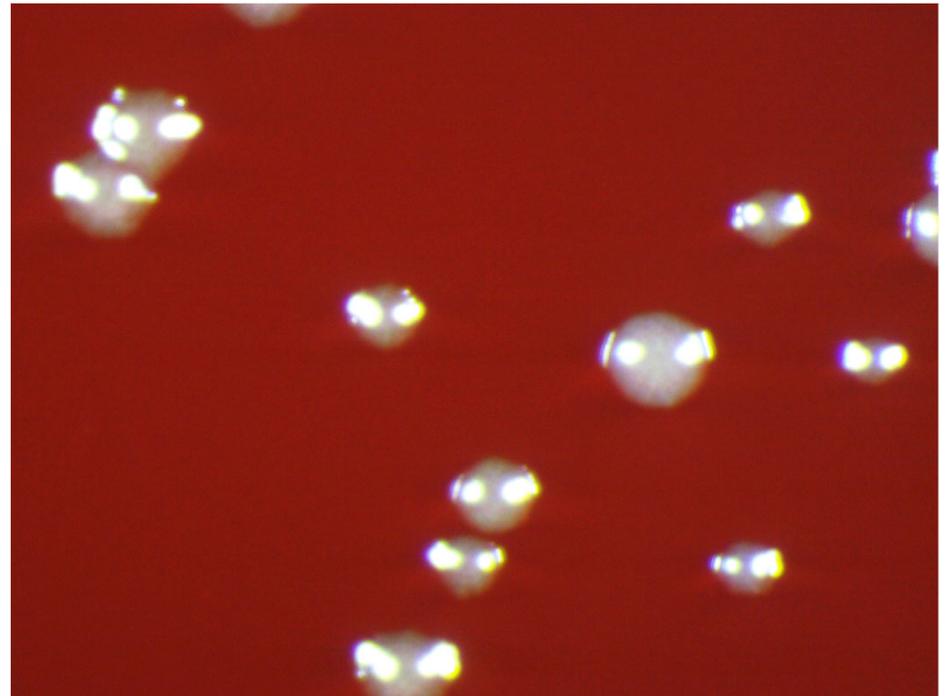


*Pseudomonas chlororaphis* subsp. *chlororaphis*  
(ATCC® 9446™)

Pseudomonas F agar, positive for fluorescence

# Fastidious

- This group includes
  - Bordetella*
  - Moraxella*
  - Francisella*
- Gram-negative
- Slow-growing
- Have complex or specific nutritional requirements
- May require additional CO<sub>2</sub>

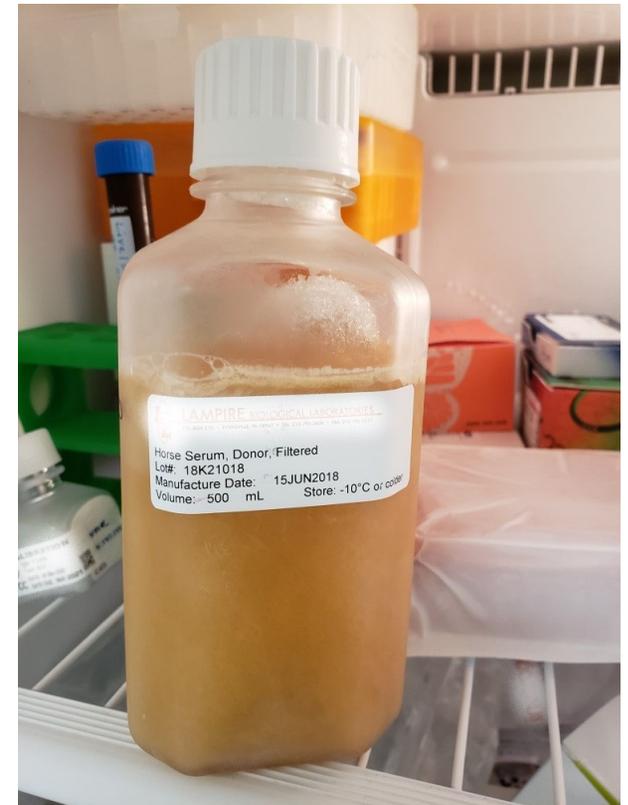


*Bordetella pertussis* (ATCC® 9306™)  
Bordet-Gengou agar

# Nutritional Requirements

## *Supplements*

- Bases for blood agar media:
  - Brucella
  - Columbia based blood agar
  - Tryptic soy based blood agar
  - Brain heart infusion w/ 0.5% yeast extract
- Supplements to enhance growth:
  - 5% sheep, horse, or rabbit blood
  - Vitamin K1 (1  $\mu\text{g}/\text{mL}$ )
  - Hemin (5  $\mu\text{g}/\text{mL}$ )
  - Fetal Bovine Serum (ATCC<sup>®</sup> 30-2020<sup>™</sup>)
  - Horse serum



# Atmospheric Conditions

- Aerobic/Ambient
- Microaerophilic or Anaerobic
  - Automatic jar system
  - Jars and gas generating sachets
  - 5% CO<sub>2</sub> Incubators
- Anaerobic
  - Anaerobic Chamber
  - Needle and gas exchange



# Extremophiles

- What are extremophiles?
  - Thermophile – 45°C and up
  - Psychrophile – 15°C and below
  - Halophile – high salt concentration
  - Acidophile – pH 3.0 or below
  - Alkaliphile – pH 9.0 or above
- These are just a few examples



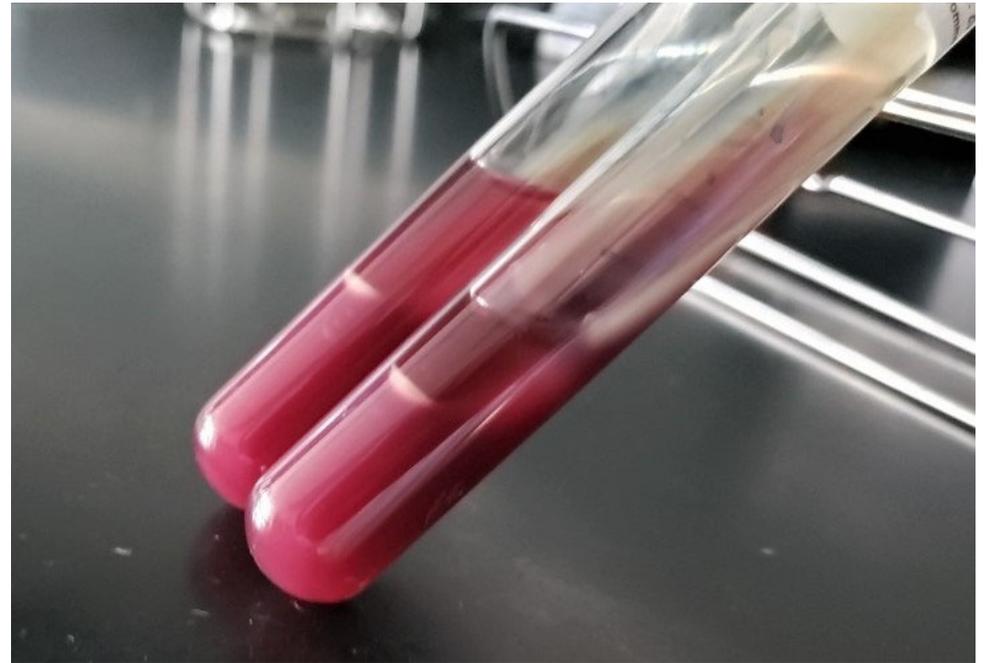
# Extremophiles

- *Methanosarcina barkeri* (ATCC® BAA-2329™)
  - Grows in a gas mixture of 80% H<sub>2</sub> - 20% CO<sub>2</sub> (5 PSI)
  - Requires the use of Hungate or Balch tubes
  - Growth inhibited by both nitrogen and oxygen



# Microaerophilic

- This group includes:
  - Helicobacter*
  - Neisseria*
  - Campylobacter*
- Require a reduced oxygen concentration to grow.
- Require nutrient rich media.
- May grow best in a biphasic environment



*Campylobacter hyointestinalis* (ATCC® 35217™)  
Biphasic growth

# Microaerophilic



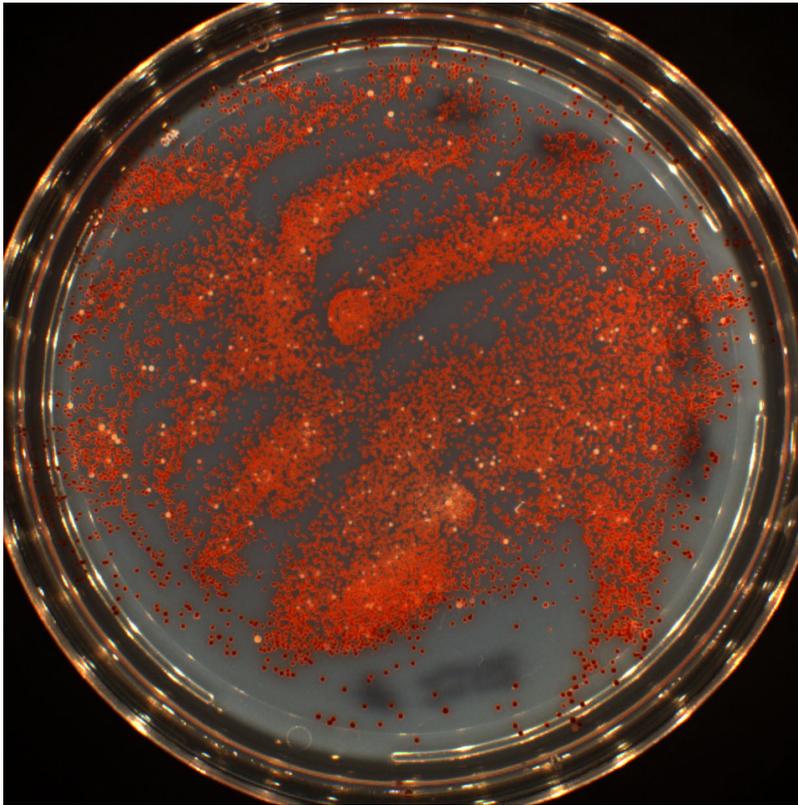
*Magnetospirillum magnetotacticum*

Image courtesy of

<https://en.wikipedia.org/wiki/Magnetospirillum>

- *Magnetospirillum magnetotacticum*
  - Unusual microaerophile
  - Produces magnetite
  - Highly motile

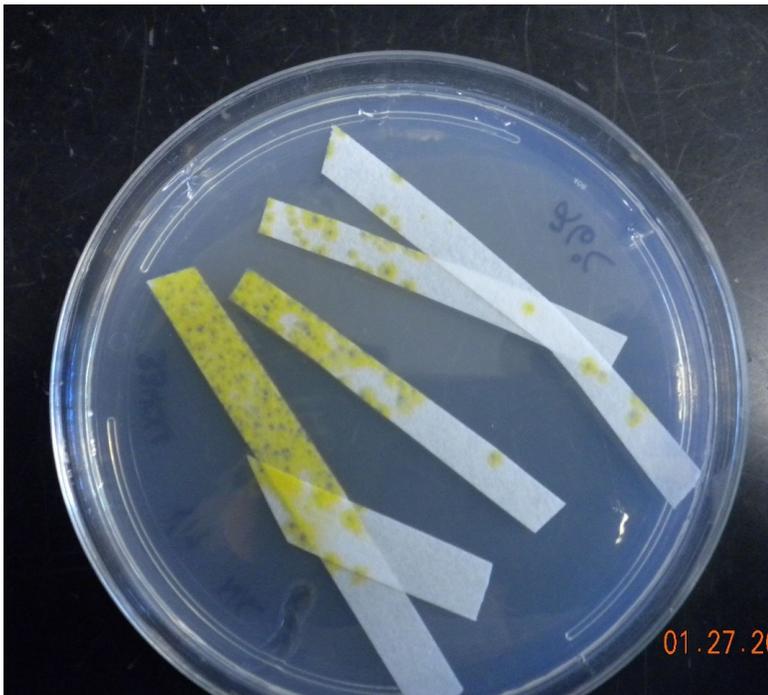
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2. Nutritional and Atmospheric Considerations
3. New bacterial isolates
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5. Quality Control Testing

*Actinocrinis puniceicyclus* (ATCC® BAA-2771™)

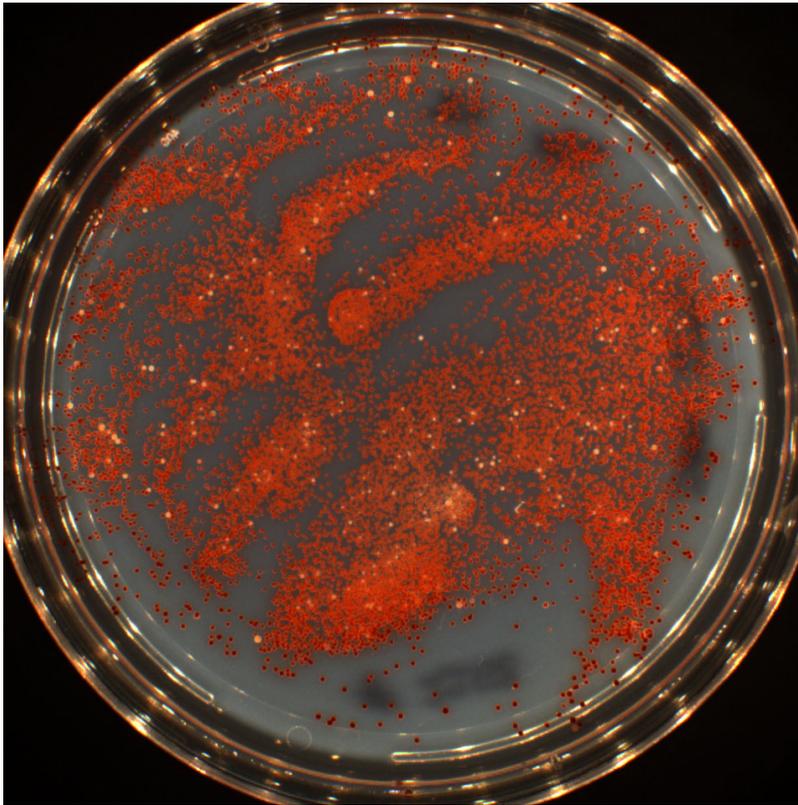
# New Bacterial Isolates



*Cytophaga hutchinsonii* Winogradsky  
(ATCC® 33406™)

- Unique characteristics
- May require specific medium
- May have other requirements
  - Light/dark
  - Shaking
  - Atmosphere
  - Additives
- When depositing at ATCC
  - Provide details
  - Specific formulations

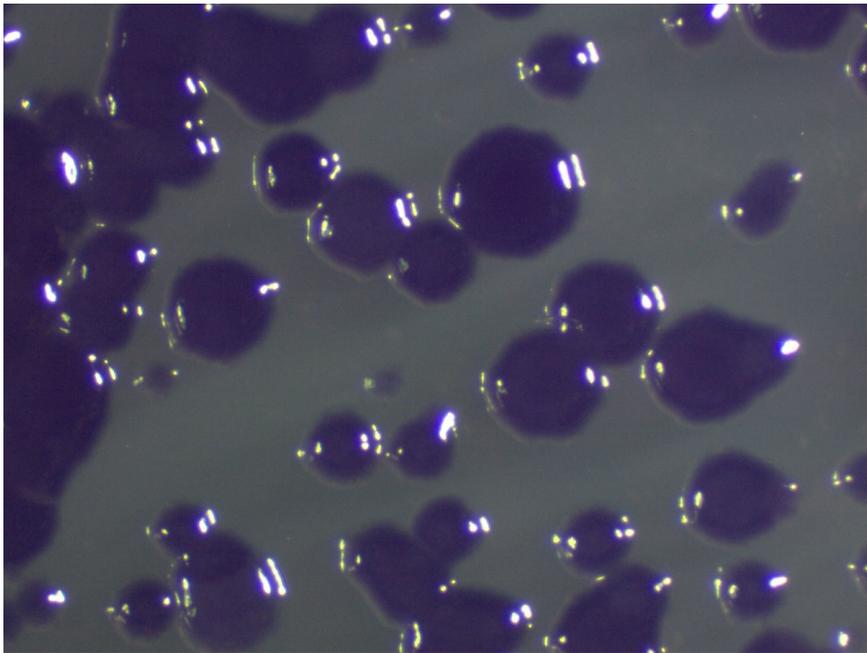
# Agenda



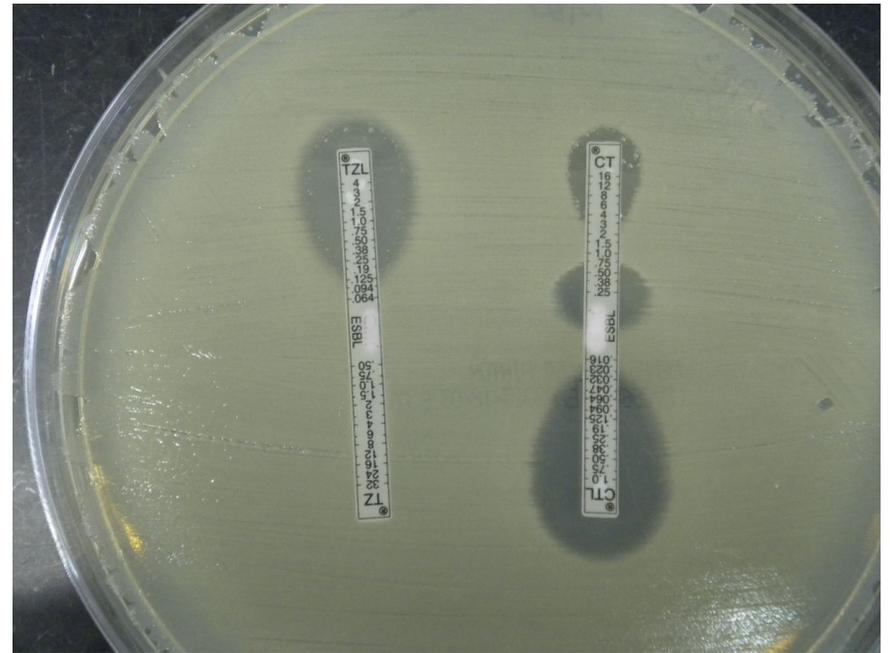
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*Actinocrinis puniceicyclus* (ATCC® BAA-2771™)

# Troubleshooting



*Chromobacterium violaceum* Bergonzini  
(ATCC® 31532™)



Antibiotic susceptibility test strips

# More Troubleshooting

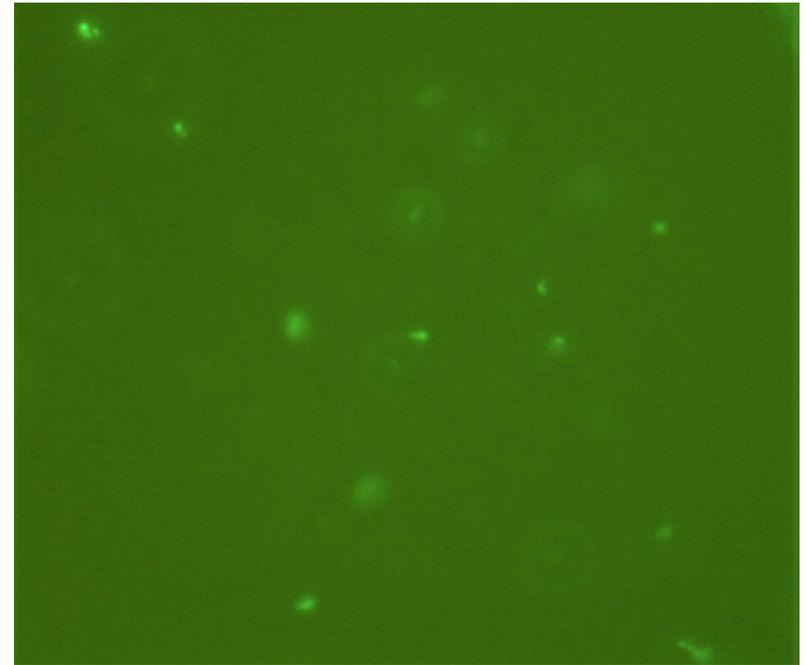
- Medium
  - Components
  - Commercially-made medium



# Mollicutes

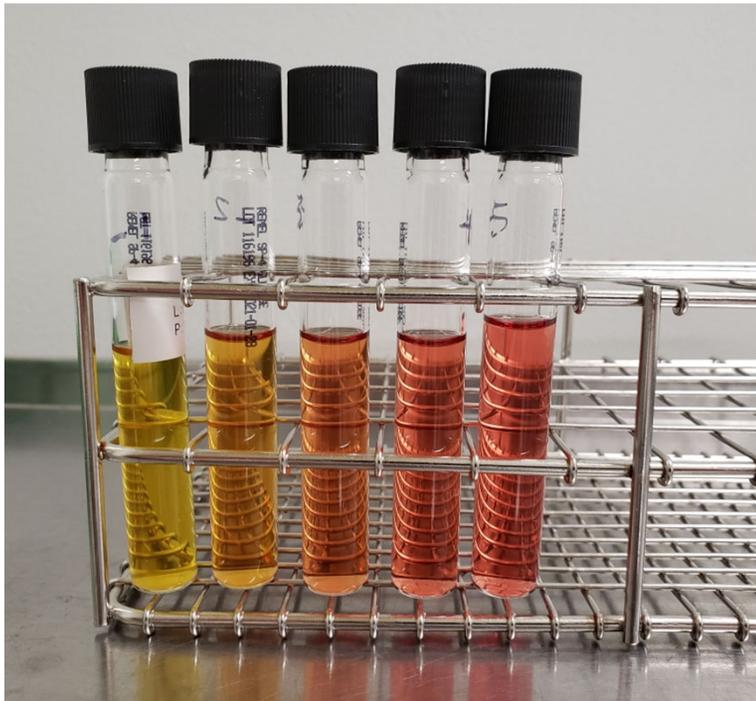
## *Why they are so challenging*

- Mycoplasma and Ureaplasma
  - Smallest bacteria
  - Depend on their hosts for nutrition
  - Lack a cell wall
  - Do not grow well on common media
  - Sensitive to overgrowth



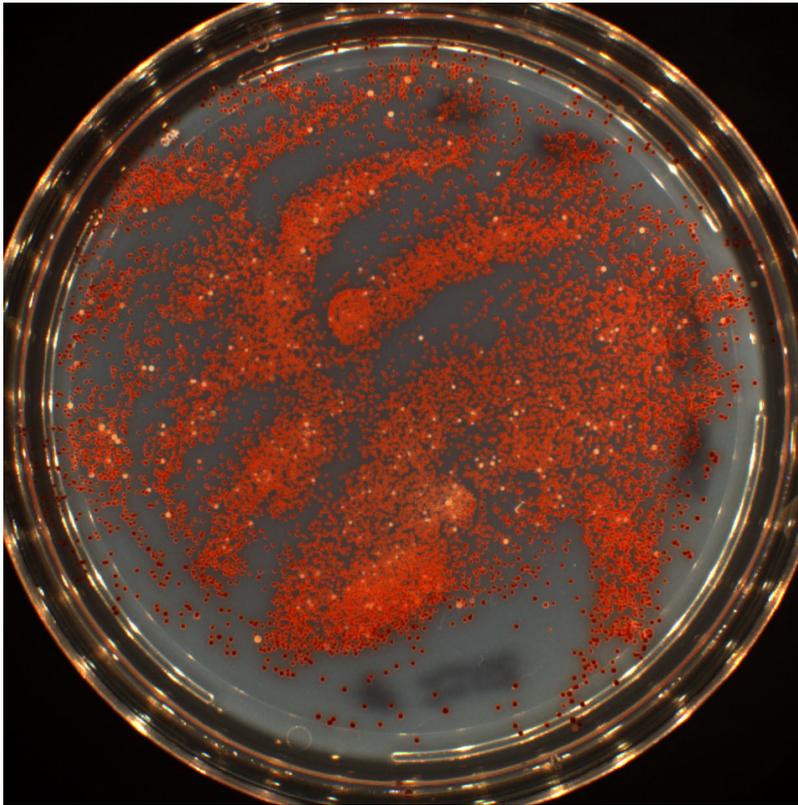
*Mycoplasma hyopneumoniae* (ATCC® 25095™)

# Mollicutes



- Grow initial cultures in a serial dilution
- Transfer every 24 hours
- Most do not grow well on agar

# Agenda

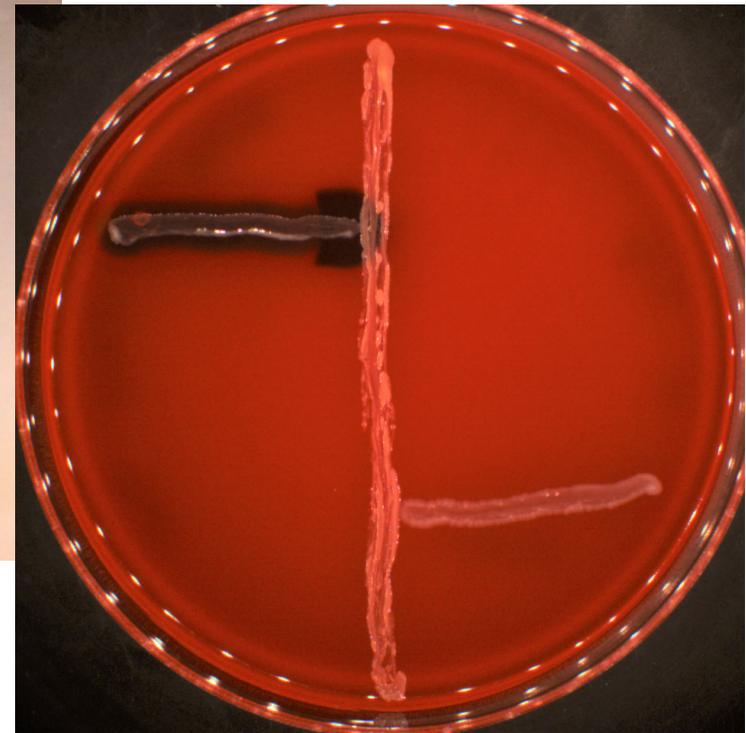
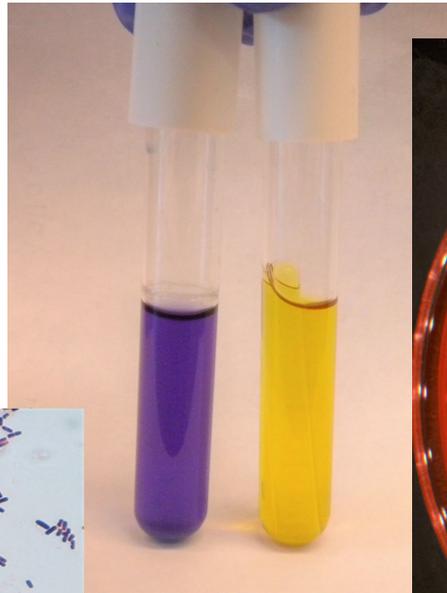
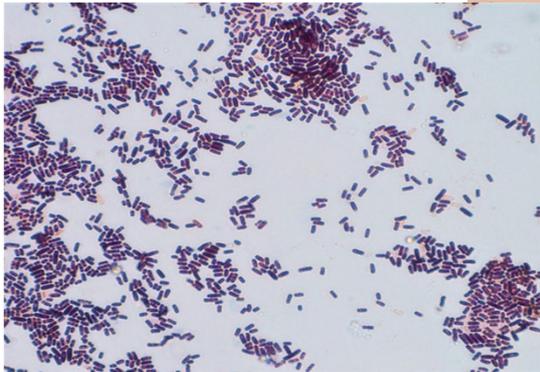


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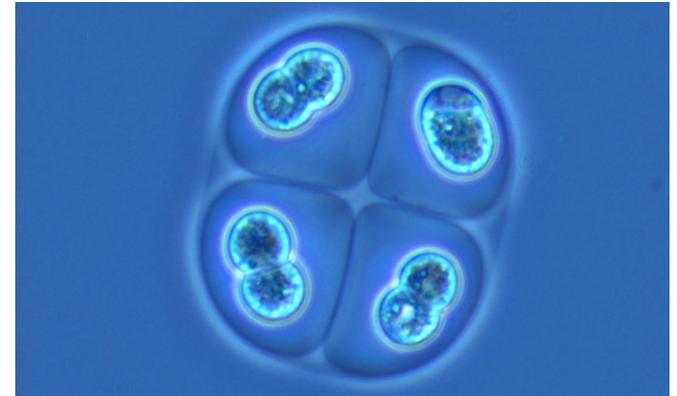
# Quality Control

- Viability
- Purity
- Sequencing
- Phenotypic testing
- Additional tests

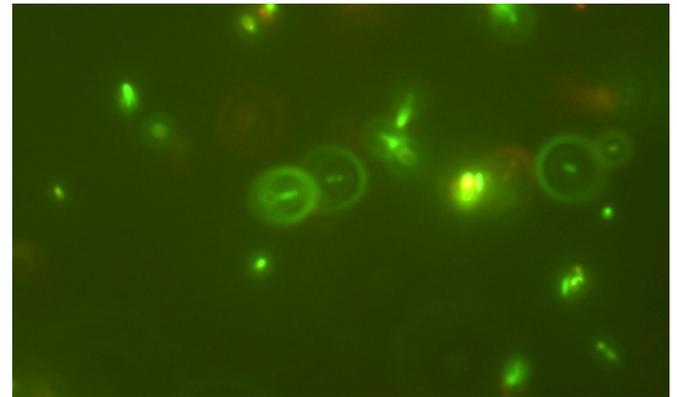


# Conclusion

- Follow a standard propagation method
- Use appropriate:
  - Medium
  - Growth conditions
  - Length of incubation
- Consider specific techniques necessary for specialized strains



*Gloeotheca* sp. (ATCC® 27152™)



*Porphyromonas catoniae*  
(ATCC® 51270™)

# Coming soon

## Upcoming event

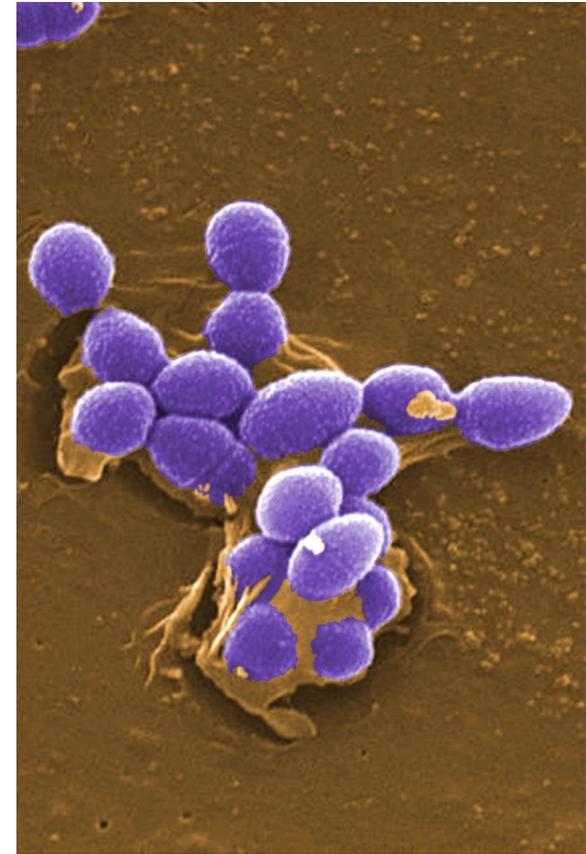
Modeling toxicity with Neural Progenitor Cell-derived Neurospheres

Brian Shapiro, February 24, 12:00 EST

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[www.atcc.org/microbes](http://www.atcc.org/microbes)



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