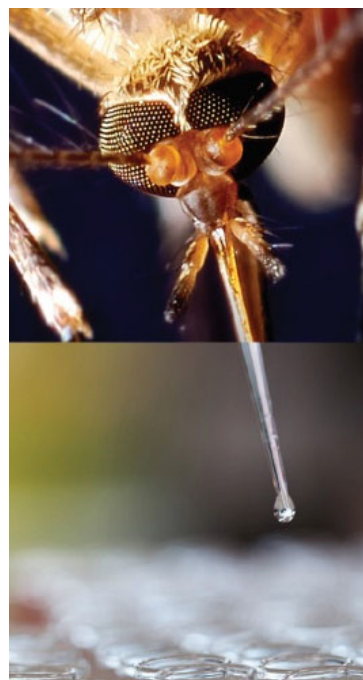
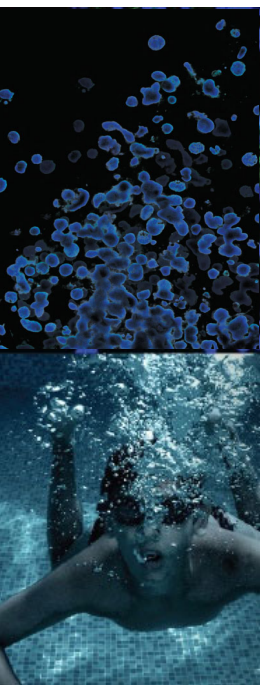




Bacterial Culture Fundamentals: How to Address Common Propagation Challenges

Nancy Krueger, MS
Senior Biologist, ATCC

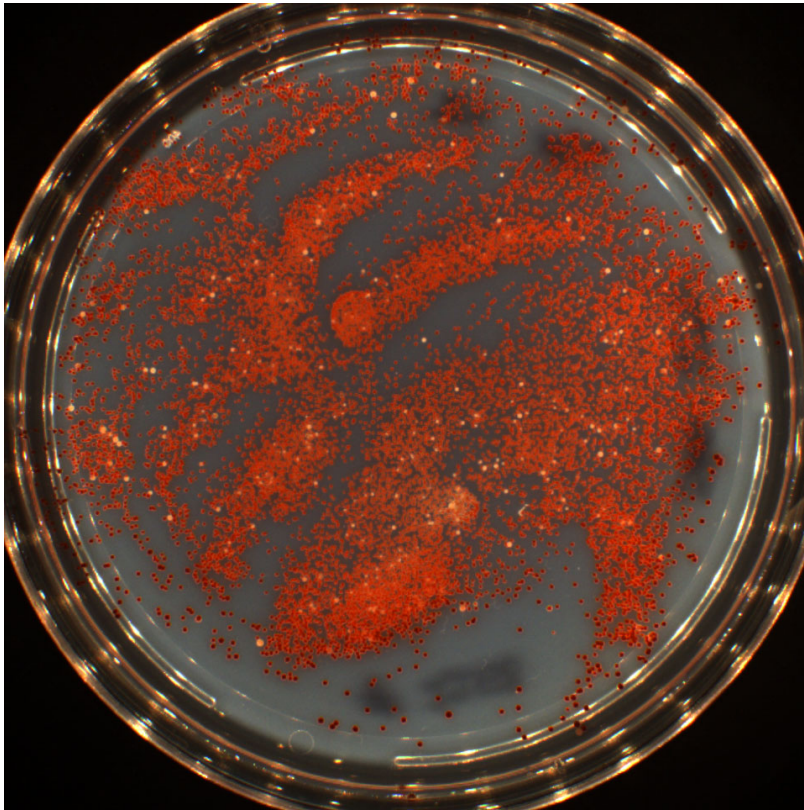
Credible Leads to Incredible™



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



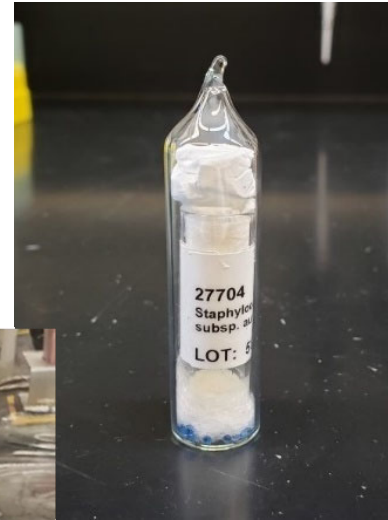
1. Propagation Methods
2. Nutritional and Atmospheric Considerations
3. New bacterial isolates
4. Troubleshooting
5. Quality Control Testing

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

The image shows a product sheet for ATCC® 25922™, which is *Escherichia coli*. The sheet is organized into several sections: Description, Propagation, Intended Use, Citation of Strain, Notes, and References. A prominent black box highlights storage and biosafety information. The ATCC logo is at the top left, and the ATCC logo is also at the bottom right of the page.

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

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.

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™)

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.

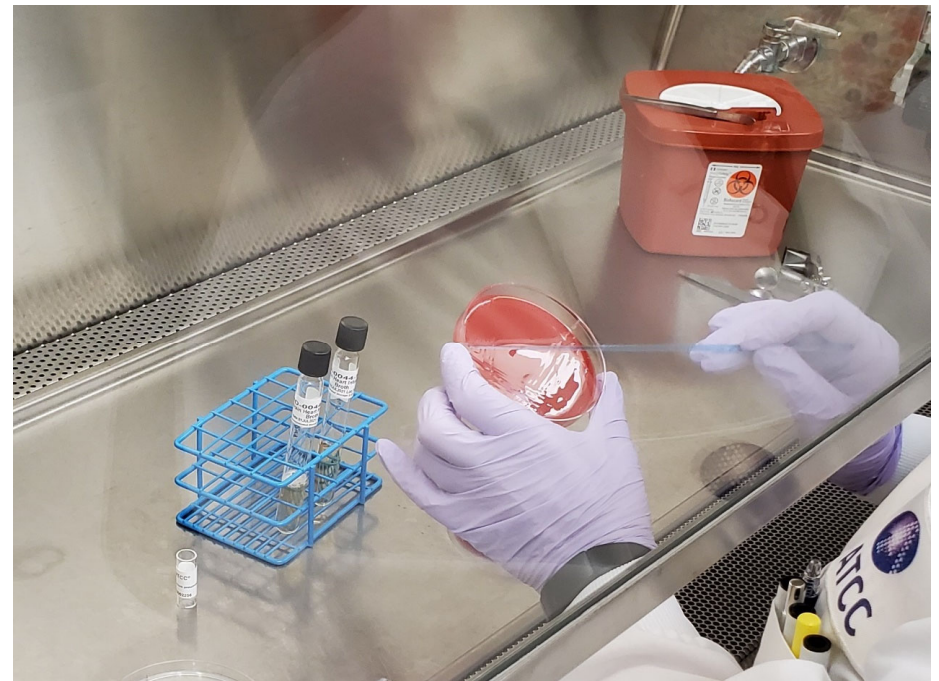
References
References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1

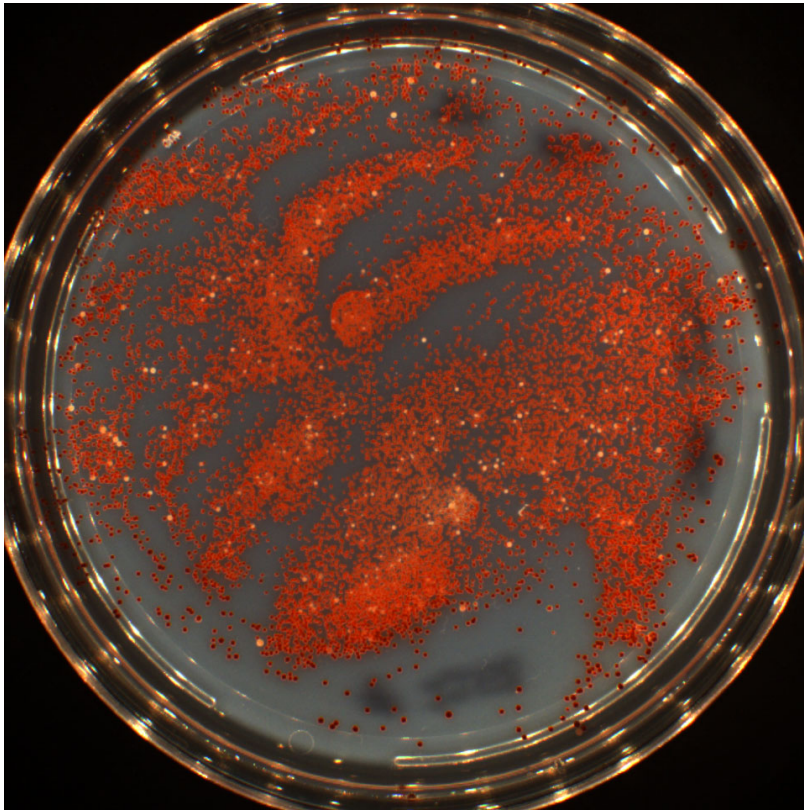
Propagation Methods

Open a batch vial

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



Agenda

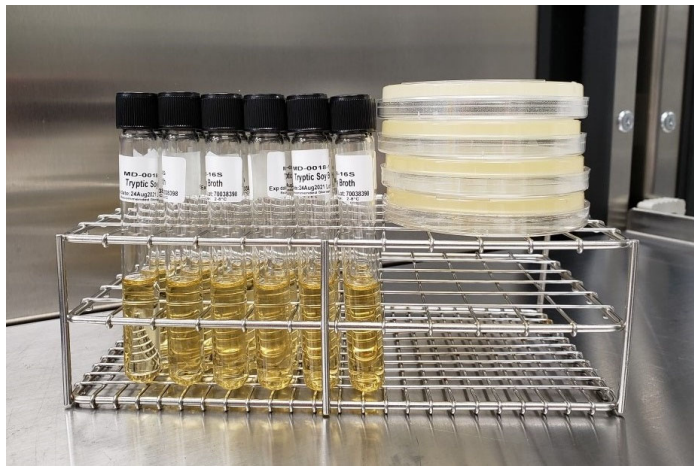


1. Propagation Methods
2. Nutritional and Atmospheric Considerations
3. New bacterial isolates
4. Troubleshooting
5. Quality Control Testing

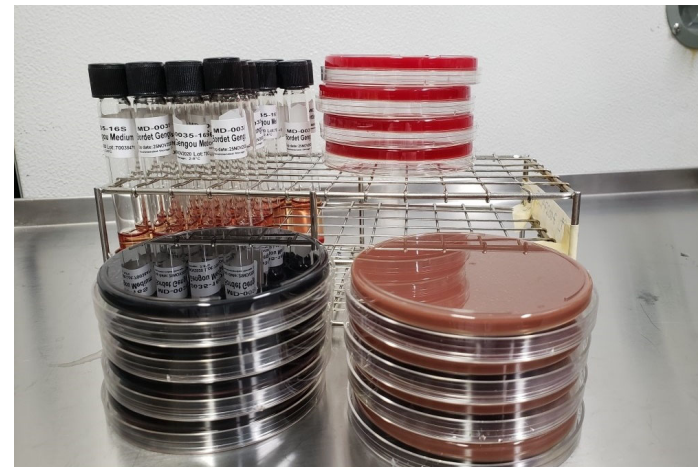
Actinocrinis puniceicyclus (ATCC® BAA-2771™)

Nutritional Requirements

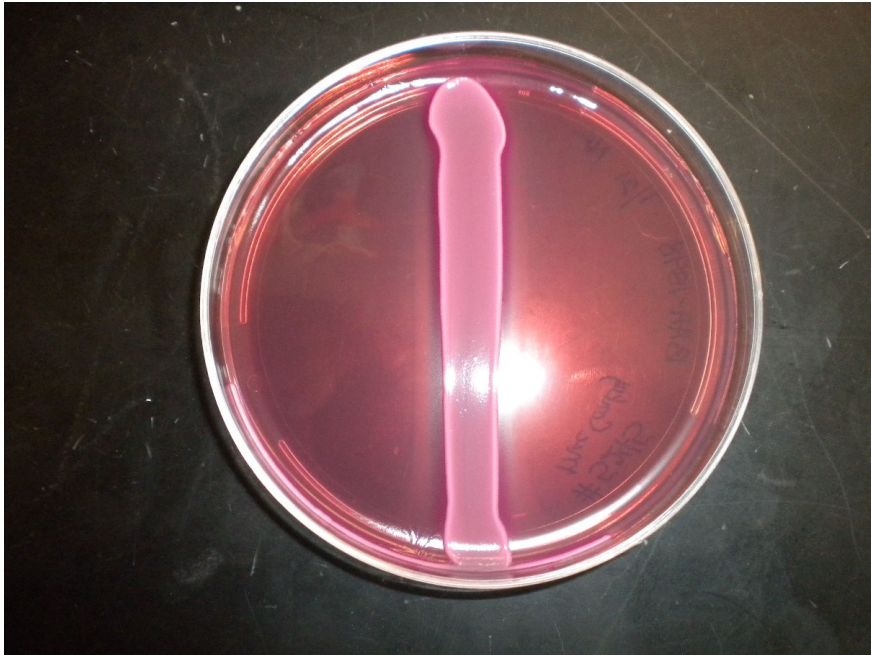
- Common growth media
 - Nutrient
 - Tryptic Soy
 - LB



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



Facultative

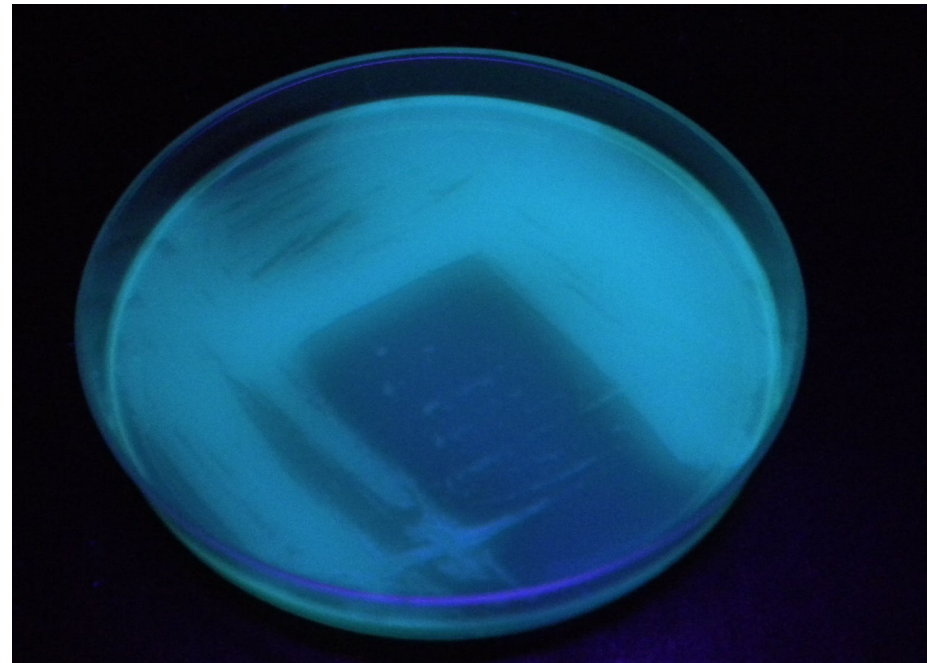


Klebsiella pneumoniae (ATCC® BAA-1898™)
MacConkey agar, positive for lactose fermentation

- 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

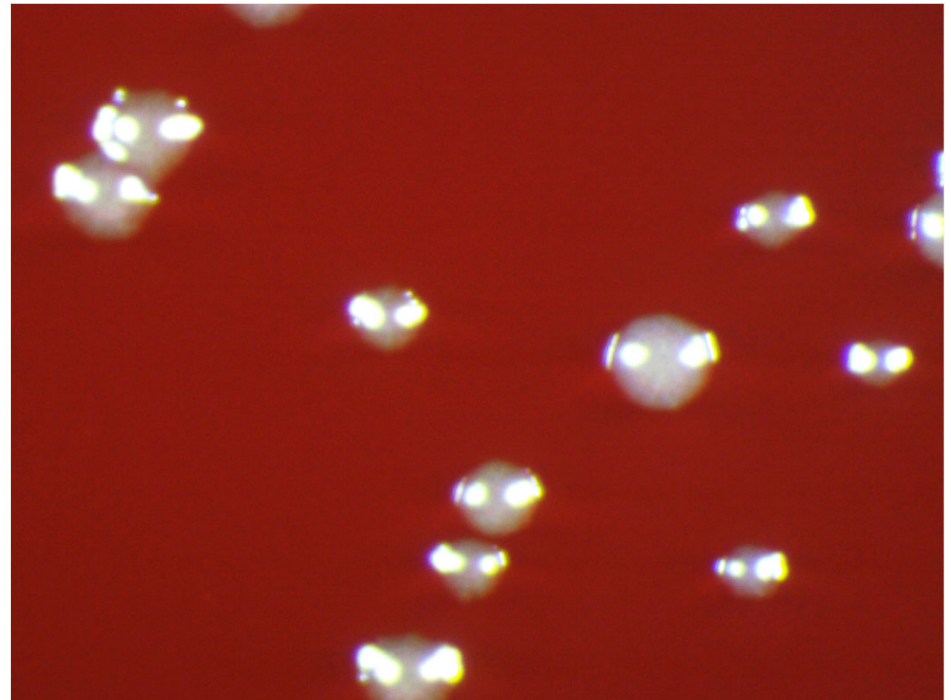


Pseudomonas chlororaphis subsp. *chlororaphis*
(ATCC® 9446™)

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 CO₂

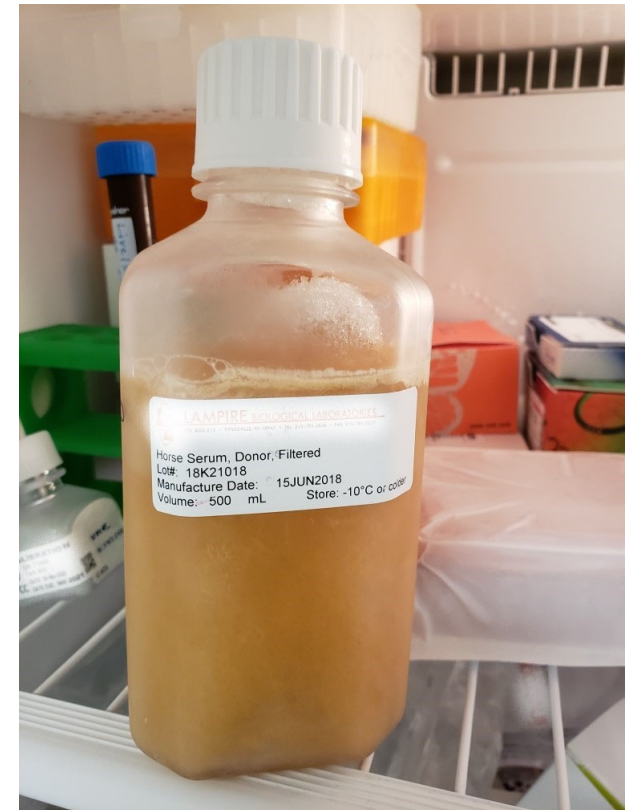


Bordetella pertussis (ATCC® 9306™)
Bordet-Gengou agar

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[®] 30-2020[™])
 - Horse serum



Atmospheric Conditions

- Aerobic/Ambient
- Microaerophilic or Anaerobic
 - Automatic jar system
 - Jars and gas generating sachets
 - 5% CO₂ 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



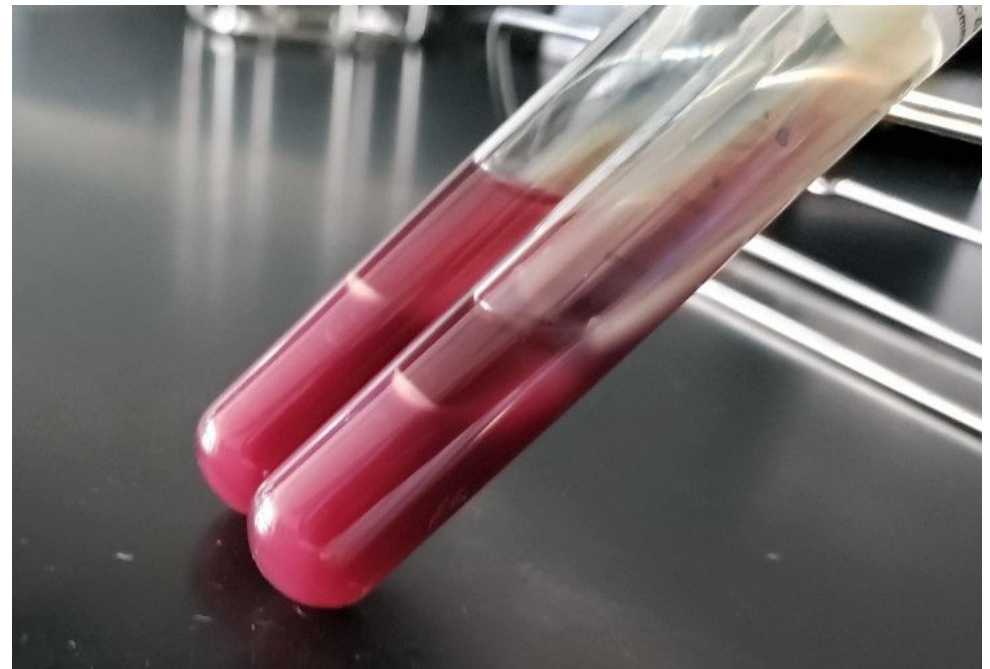
Extremophiles

- *Methanosarcina barkeri* (ATCC® BAA-2329™)
 - Grows in a gas mixture of 80% H₂ - 20% CO₂ (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



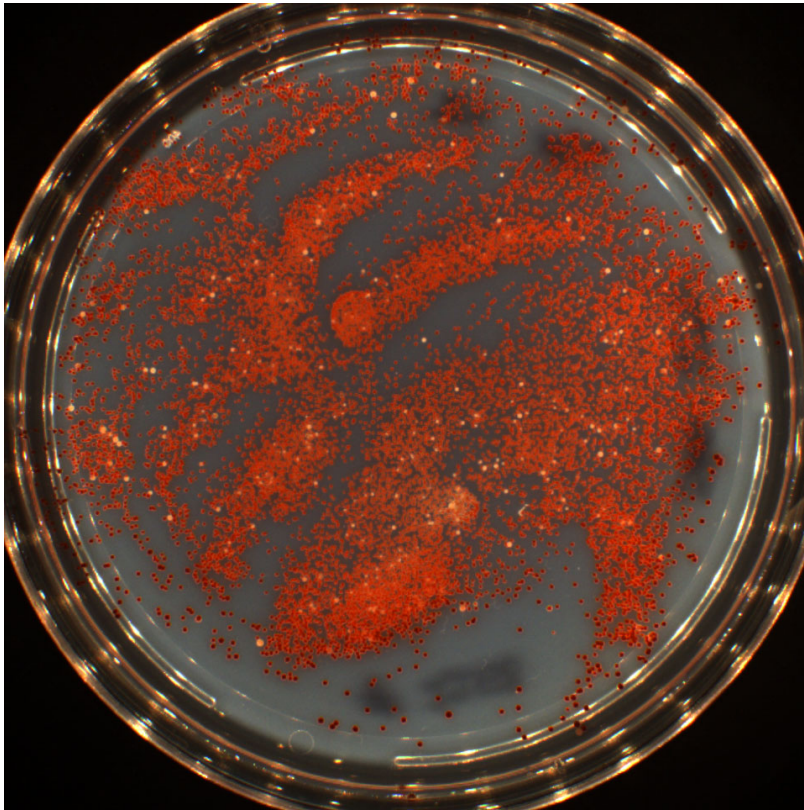
Magnetospirillum magnetotacticum

Image courtesy of

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

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

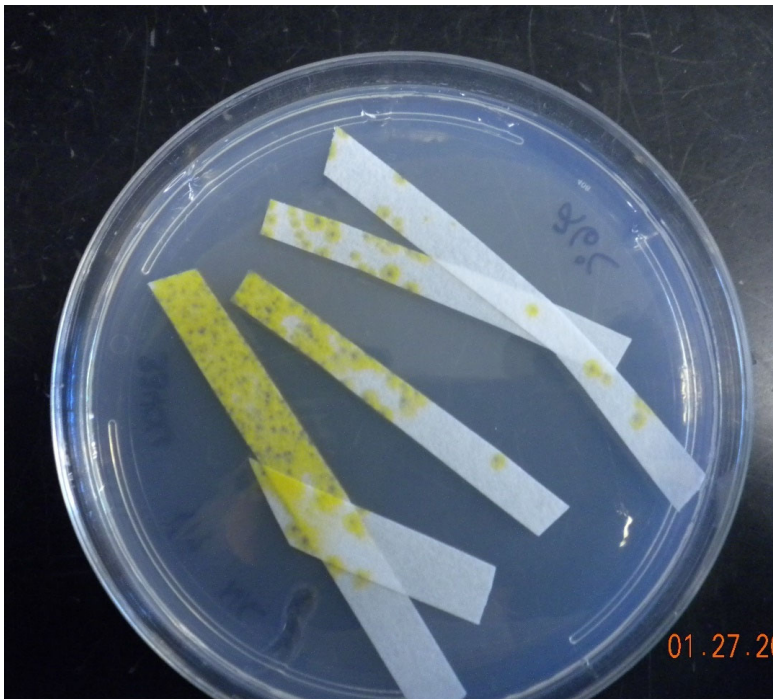
Agenda



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

Actinocrinis puniceicyclus (ATCC® BAA-2771™)

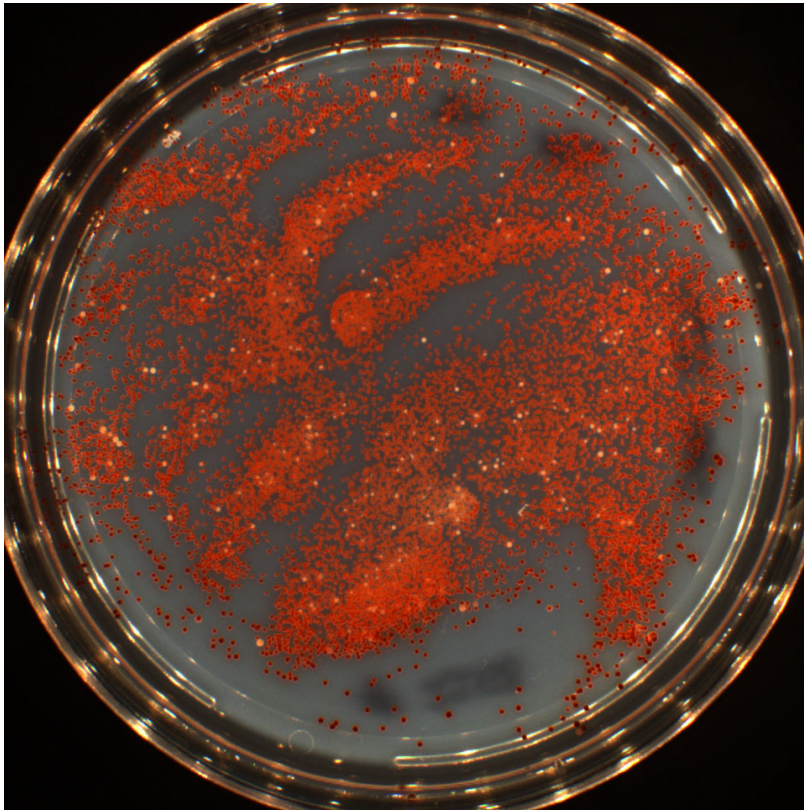
New Bacterial Isolates



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

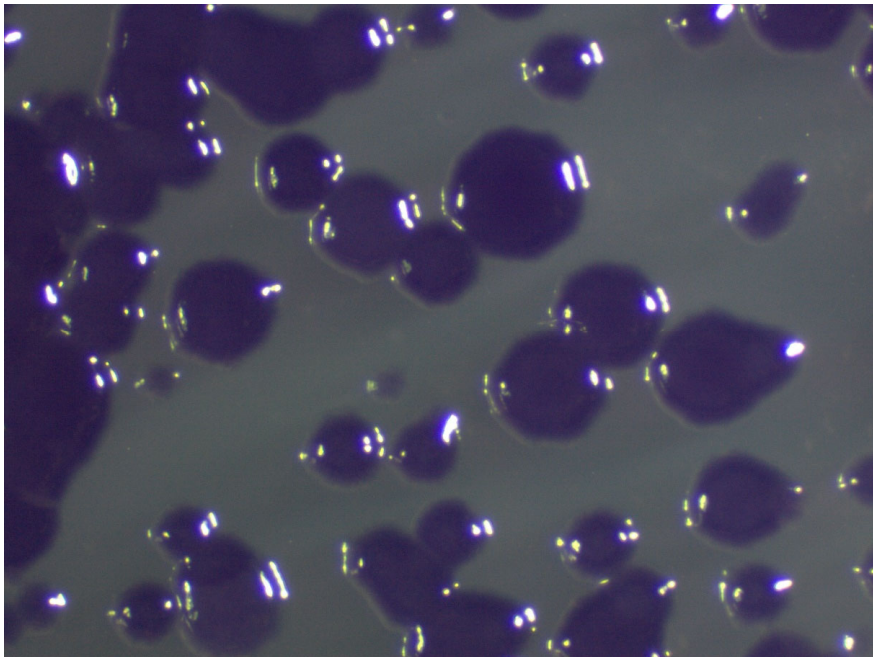
Agenda



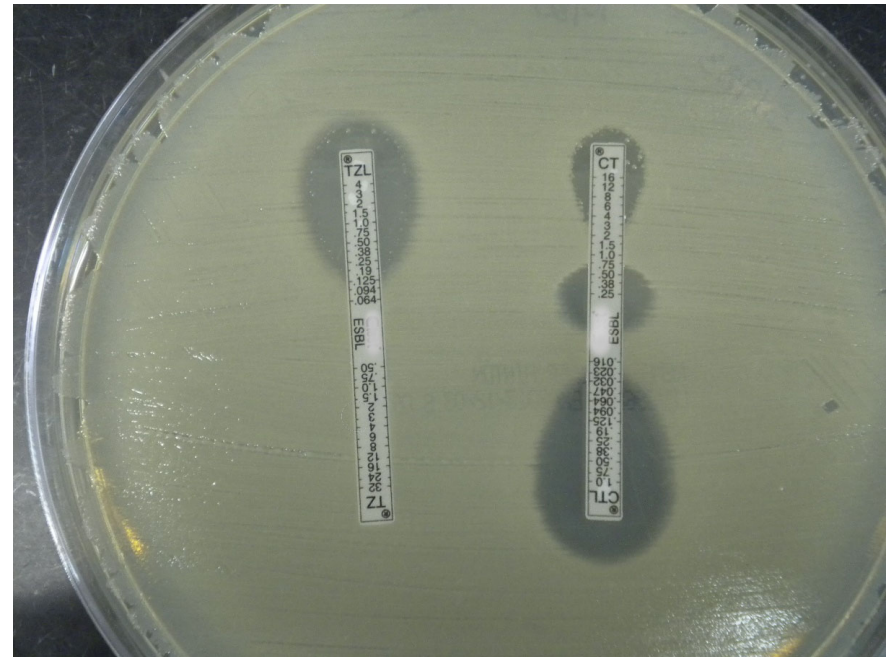
1. Propagation Methods
2. Nutritional and Atmospheric Considerations
3. New bacterial isolates
4. Troubleshooting
5. Quality Control Testing

Actinocrinis puniceicyclus (ATCC® BAA-2771™)

Troubleshooting



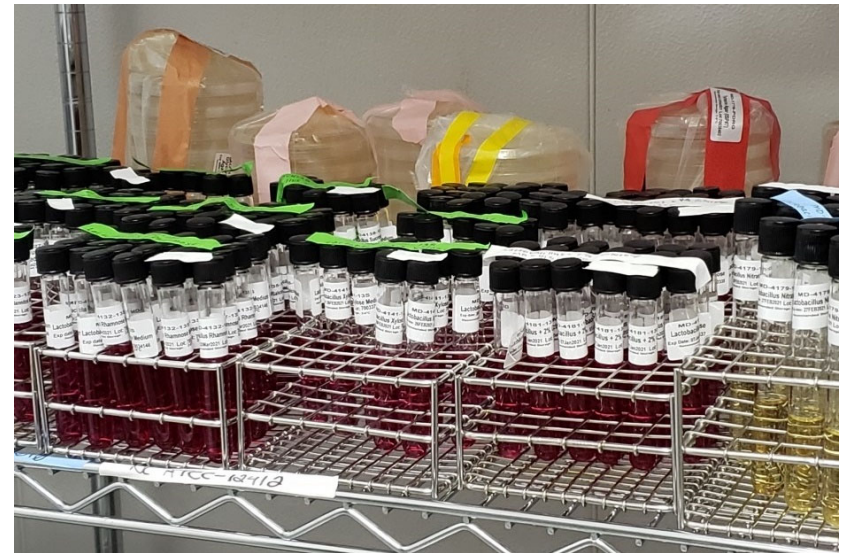
Chromobacterium violaceum Bergonzini
(ATCC® 31532™)



Antibiotic susceptibility test strips

More Troubleshooting

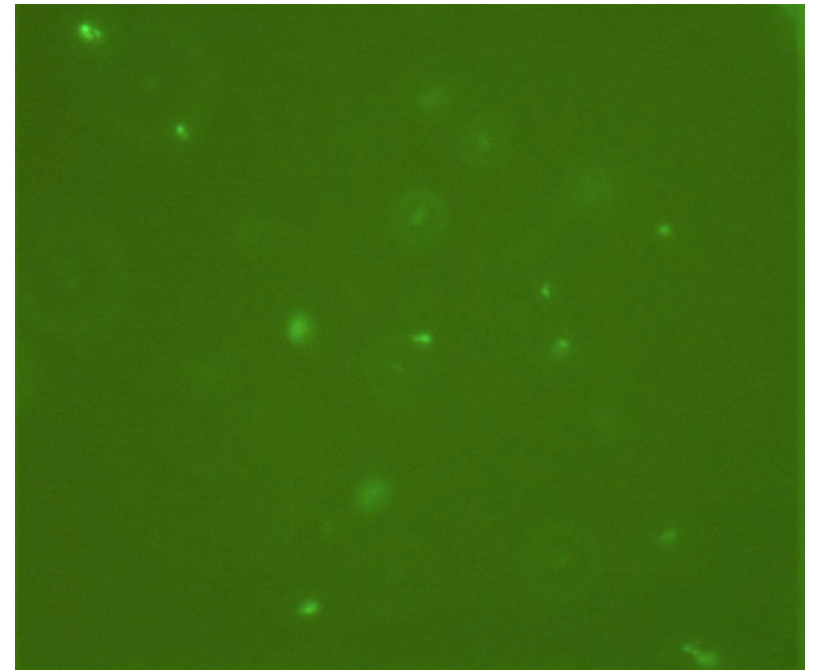
- Medium
 - Components
 - Commercially-made medium



Mollicutes

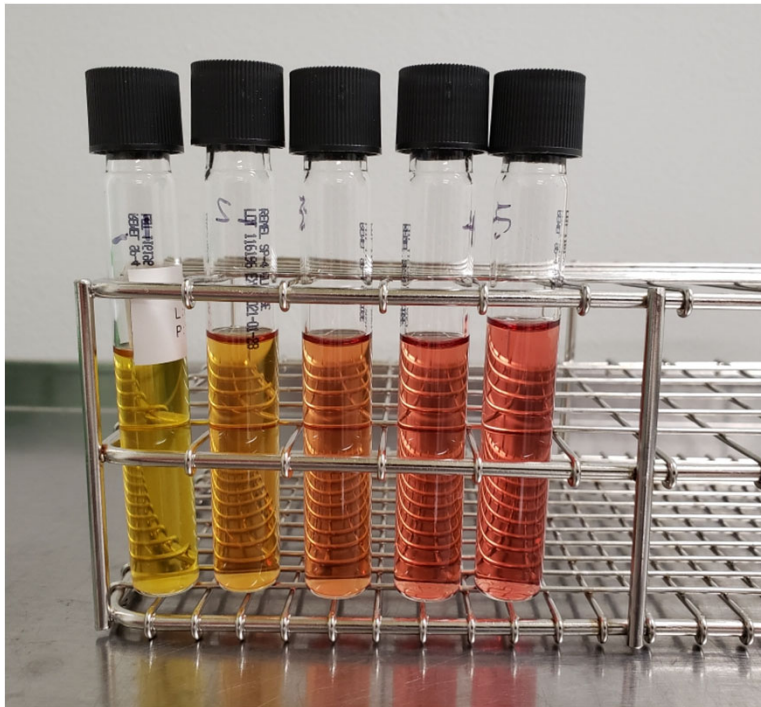
Why they are so challenging

- 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



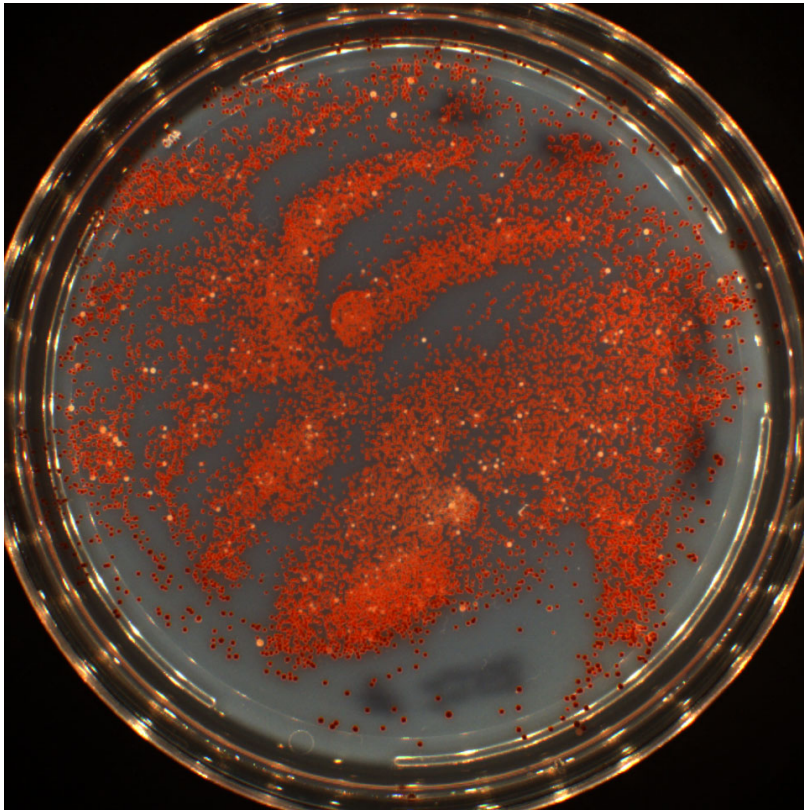
Mycoplasma hyopneumoniae (ATCC® 25095™)

Mollicutes



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

Agenda

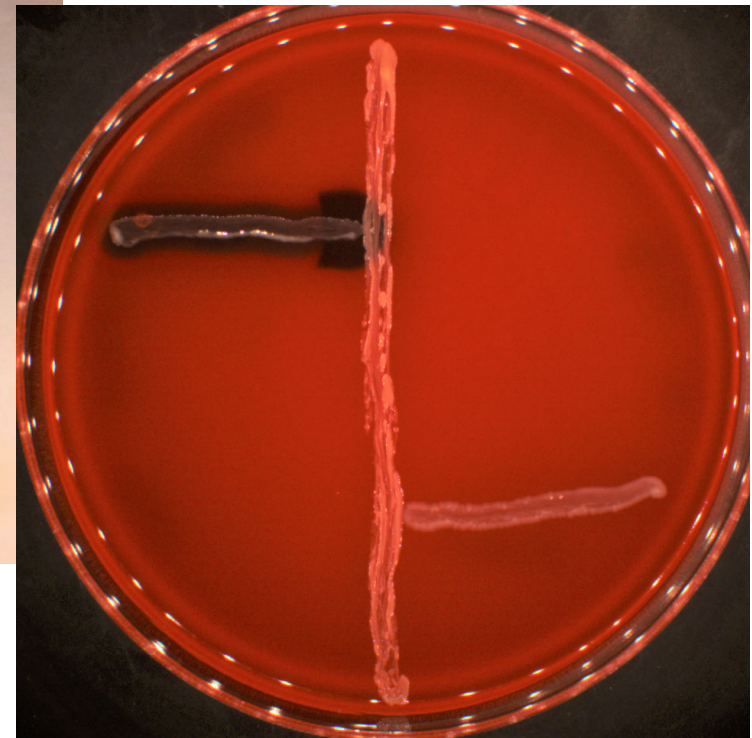
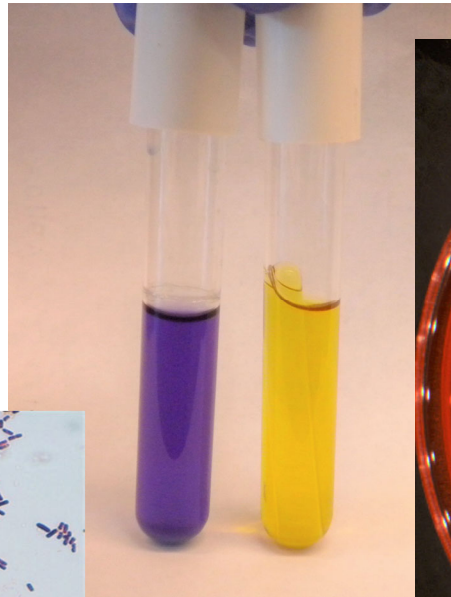
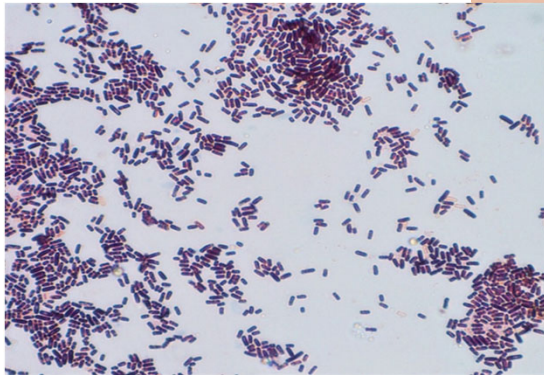


1. Propagation Methods
2. Nutritional and Atmospheric Considerations
3. New bacterial isolates
4. Troubleshooting
5. Quality Control Testing

Actinocrinis puniceicyclus (ATCC® BAA-2771™)

Quality Control

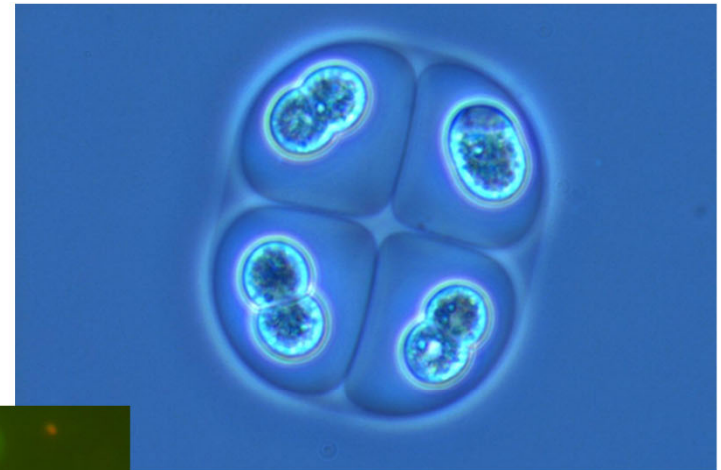
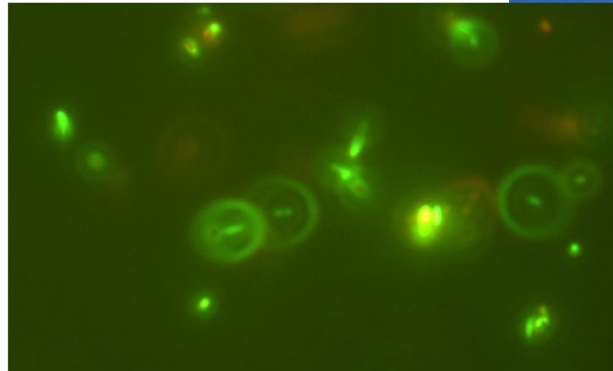
- Viability
- Purity
- Sequencing
- Phenotypic testing
- Additional tests



Conclusion

- Follow a standard propagation method
- Use appropriate medium, growth conditions and length of incubation
- Consider specific techniques necessary for specialized strains.

Porphyromonas catoniae
(ATCC® 51270™)



Gloeotheca sp. (ATCC® 27152™)

From Our Lab To Yours

Safety Tips for Returning to the Lab

To help you get back to work, we have put together a free kit containing best practices to help keep your lab infection-free:

- Poster
- Infographic
- Decals

Order the kit here:

www.atcc.org/backtothelab



© 2020 American Type Culture Collection. The ATCC trademark and trade name, and any other trademarks listed in this publication are trademarks owned by the American Type Culture Collection unless indicated otherwise.