Tips and Techniques for Successfully Growing Bacteria in Culture

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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
Propagation Methods

Open a batch vial

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

- 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

*Klebsiella pneumoniae* (ATCC® BAA-1898™) MacConkey agar, positive for lactose fermentation
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$_2$

*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 µg/mL)
  - Hemin (5 µg/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*
  - Unusual microaerophile
  - Produces magnetite
  - Highly motile

*Magnetospirillum magnetotacticum*
Image courtesy of https://en.wikipedia.org/wiki/Magnetospirillum
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*Actinocrinis puniceicyclus* (ATCC® BAA-2771™)
New Bacterial Isolates

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

**Cytophaga hutchinsonii Winogradsky**
(ATCC® 33406™)
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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
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*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
  - Length of incubation
- Consider specific techniques necessary for specialized strains

Gloeothece 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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