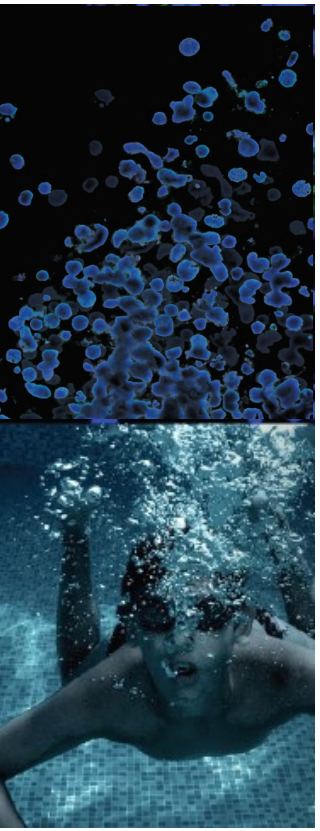
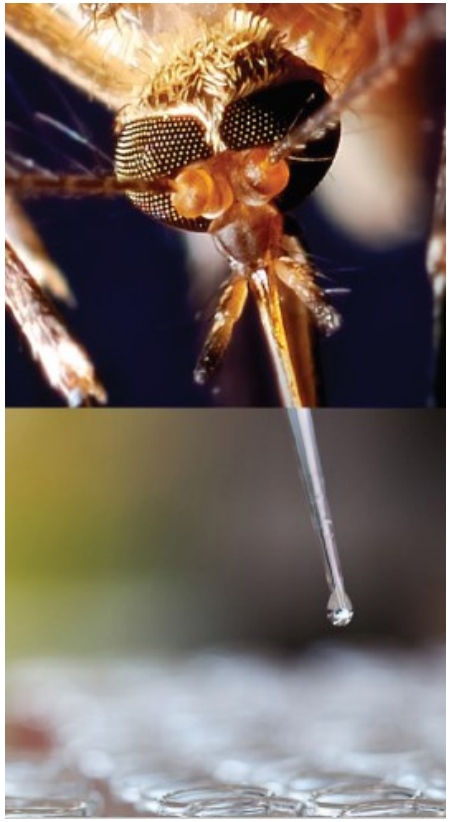
Bacteriology Fundamentals

ATCC's best practices for optimal growth and propagation of bacteria and bacteriophages



Jeanette Rimbey, MSc
Lead Biologist, Bacteriology
ATCC

Credible Leads to Incredible®



Speaker information

Jeanette Rimbey, MSc

Lead Biologist, Bacteriology

ATCC

Jeanette Rimbey is a team lead within ATCC with 15 years' experience as a microbiology scientist in the fields of bacteriology, genetics, veterinary, and virology. Ms. Rimbey achieved a Master of Science from Texas Tech University and began her scientific journey at the University of Missouri, where she explored bacteriophage applications to develop innovative methods for combating pathogens that threaten global food security. On a related note, her mentor was awarded the 2018 Nobel Prize in Chemistry for his work in developing phage display technology. She has contributed significantly to microbiology research, including work on temperature-specific biofilm adaptations in antibiotic-resistant microorganisms. During the COVID-19 pandemic, she served on the COVID-19 diagnostic task force as a molecular biologist, supporting critical front-line efforts in Colorado. Her passion for science drives her commitment to advancing microbiology and giving back to the scientific community.

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 premier biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microorganisms
  - Genomic & synthetic nucleic acids
  - Media/reagents

- ATCC® collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 20 international distributors
- Talented team of 600+ employees, over one-third with advanced degrees

bsi. ISO 9001 Quality Management

bsi. ISO 13485 Medical Devices Quality Management

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

ATCC®

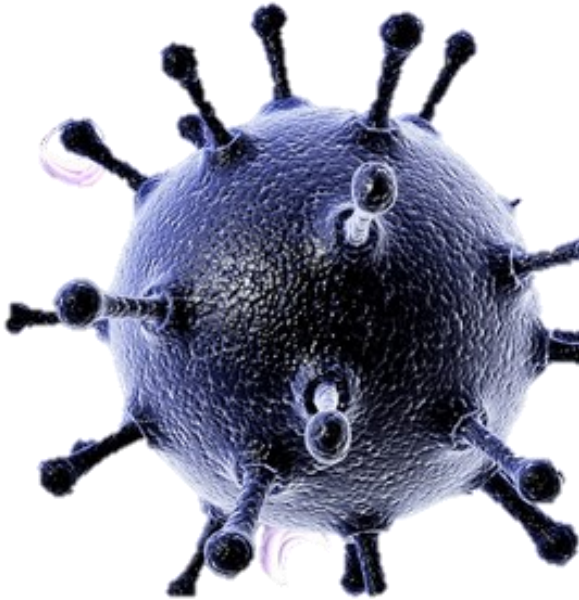
Agenda

1. ATCC® Bioproduction
2. Microbial cultivation and propagation
3. Troubleshooting
4. Quality assurance
5. Depositing at ATCC®
6. Resources

ATCC® Bioproduction

Comprehensive microbial collection

- Comprehensive microbial collection with enhanced authentication
  - 70,000+ bacteria, fungi, viruses, and protozoa
  - 8,000+ microbial type strains
- Brand recognition
  - Organizations and regulatory agencies specify ATCC® cultures in their standards and guidelines
  - USP, ISO, FDA, CLSI, USDA, ASTM, AOAC, WHO
  - 475+ strains recommended for use in quality control
- ATCC® has live microbes and derivatives, including inactivated materials and nucleic acids
- Variety of advanced techniques used to characterize and authenticate biomaterials—no single method of identification is sufficient



Types of vials to store and ship microbes

Batch vials: lyophilized format with flame-sealed external glass shell (glass ampoule)

“Mini” vials: 0.2 mL of frozen material sold in packs of 6

Serum/preceptrol vials: lyophilized material sealed with rubber plug and metal crimp

1.0 mL Frozen/cryovials: 0.5 mL of frozen material

How to open a glass ampoule

1. Disinfect the outside of the ampoule with freshly prepared 70% ethanol.
2. Wipe off the ampoule with a sterile towel or gauze to dry residual ethanol.
3. Score the neck of the ampoule with a vial scorer until a fracture line is made. Apply gentle pressure when rotating.
4. Hold the vial upright and lightly tap open the vial using a metal file along the fracture line.
5. Immediately, relocate vial to a Biosafety cabinet (BSC) prior to rehydrating the material.

Vial scorer

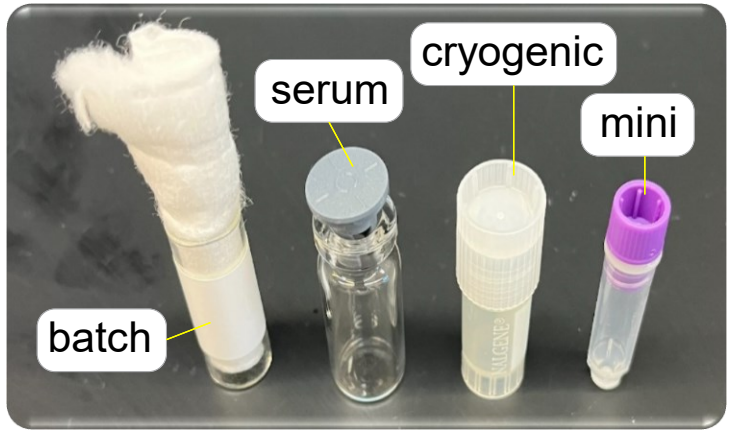
Initiating frozen and lyophilized cultures

Rehydrating pellets

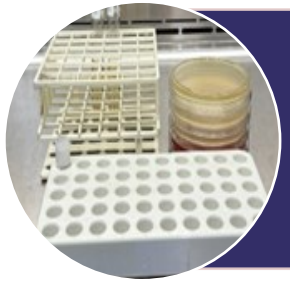
1. Lyophilized vials should be stored at 4°C.
2. Open the vial according the enclosed instructions.
3. Working in a Biosafety Cabinet (BSC), use a single tube containing 5-6 mL of broth and rehydrate the entire pellet.
4. Aseptically transfer the aliquot back into the broth tube and mix well.
5. Use several drops of the suspension to inoculate the recommended agar plate.
6. Incubate at the suggested temperature and atmospheric conditions.

Thawing Frozen Vials

1. Frozen vials should be stored at -80°C.
2. Open the vial according the enclosed instructions.
3. Thaw vial in a 37°C water bath for ~3 mins.
4. Working in a BSC, unscrew the vial and transfer the contents to a sterile test tube containing the appropriate growth medium. Proceed as in previous steps 5 & 6.

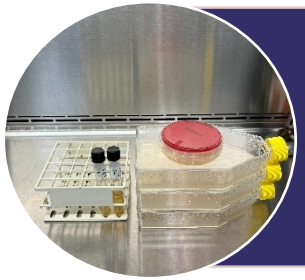


Bioproduction process of BSL-1 and BSL-2 microbes

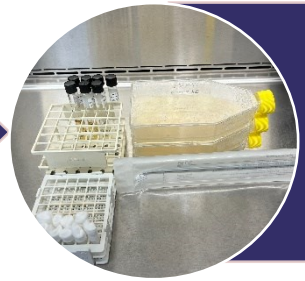


1. Open

2. Observations



3. Transfer



4. Harvest

5. Preservation

6. QC

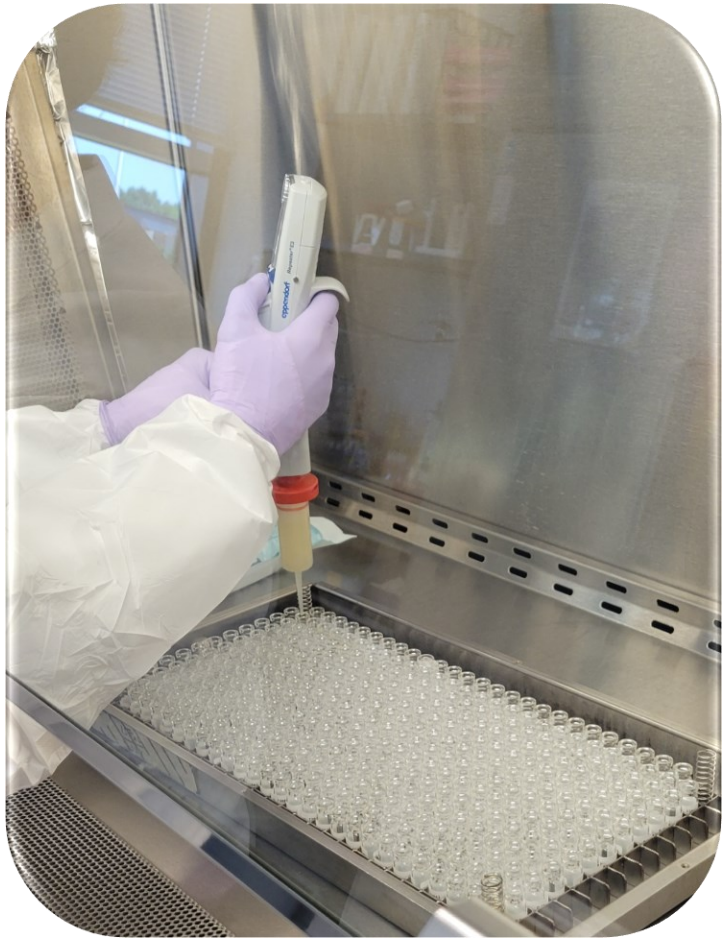
7. QA

8. Distribution

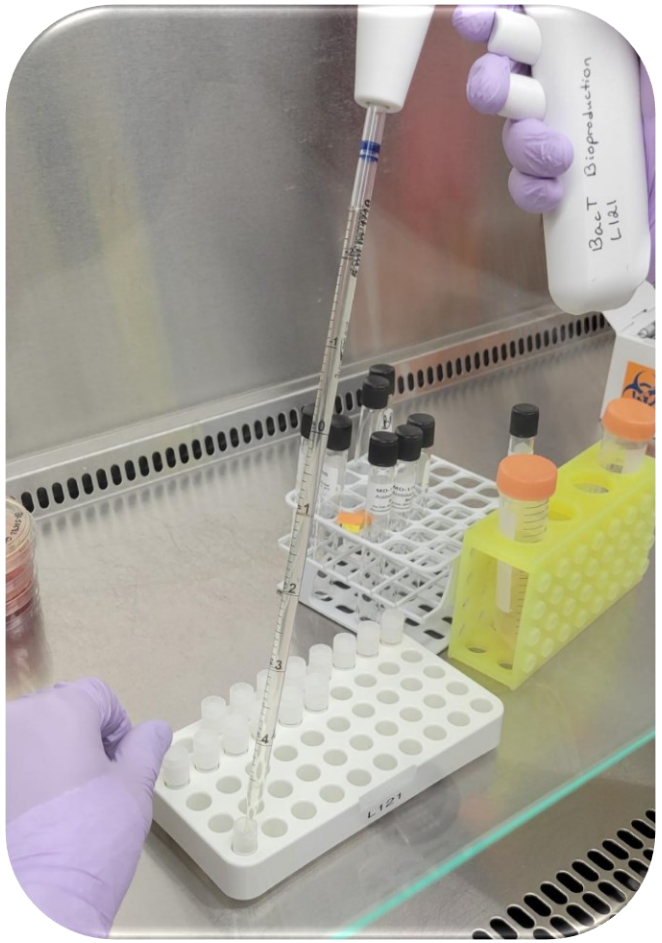
Vialing on harvest day

Batch vial harvest

Serum vial harvest



Cryovial harvest



Microbial cultivation and propagation

Microbe-specific atmospheric conditions

Aerobic

- Pathogenic or commensal bacterial strains grow well at body temperature of 37°C
- Environmental strains thrive at lower temperatures, often 25°C to 30°C

Microaerophilic

- May require 5% CO_2 incubation
- Most rely on 3-5% O_2 and 10% CO_2

Anaerobic

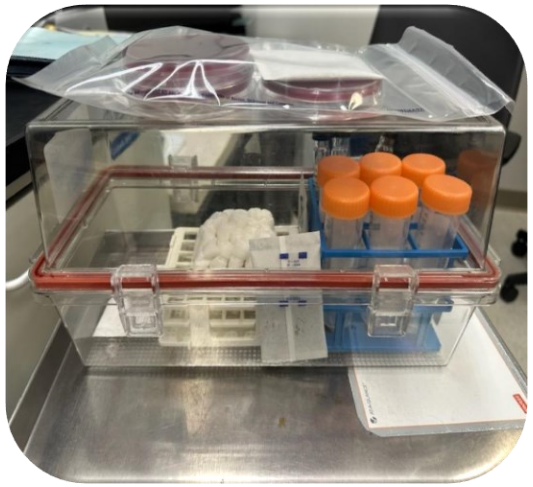
- Establishing a great anaerobic cultivation practice is key to maintain good growth.

Anaerobic chamber

Needle & gas exchange

Anaerobic jars

Anaerobic box with gas generating sachets



Aerobic bacteria and its many applications

Escherichia coli, Salmonella enterica, and Listeria monocytogenes

- Reference strains needed for the testing of food production.
- Wide assortment of food testing solutions support QC testing, process validation, and development of new methods for testing food products.

Staphylococcus aureus

- Antimicrobial resistant strains for research and drug development for clinical medicine.

Pseudomonas aeruginosa

- Specific cystic fibrosis strains from phase III clinical trial studies that provide a unique model system for better understanding of the molecular pathology associated with cystic fibrosis.

ATCC®

Common aerobic media

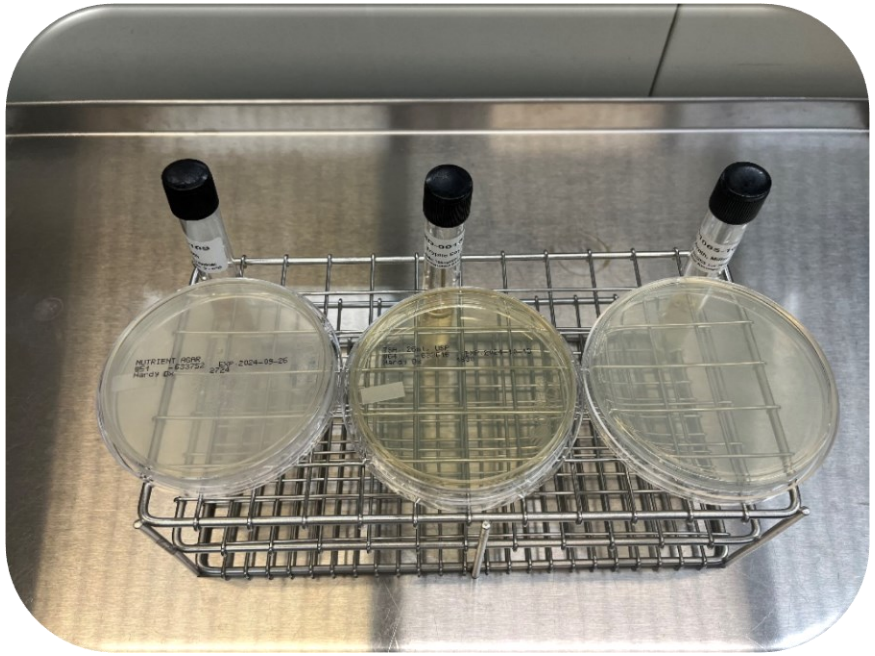
General purpose, non-selective, and nutrient-rich

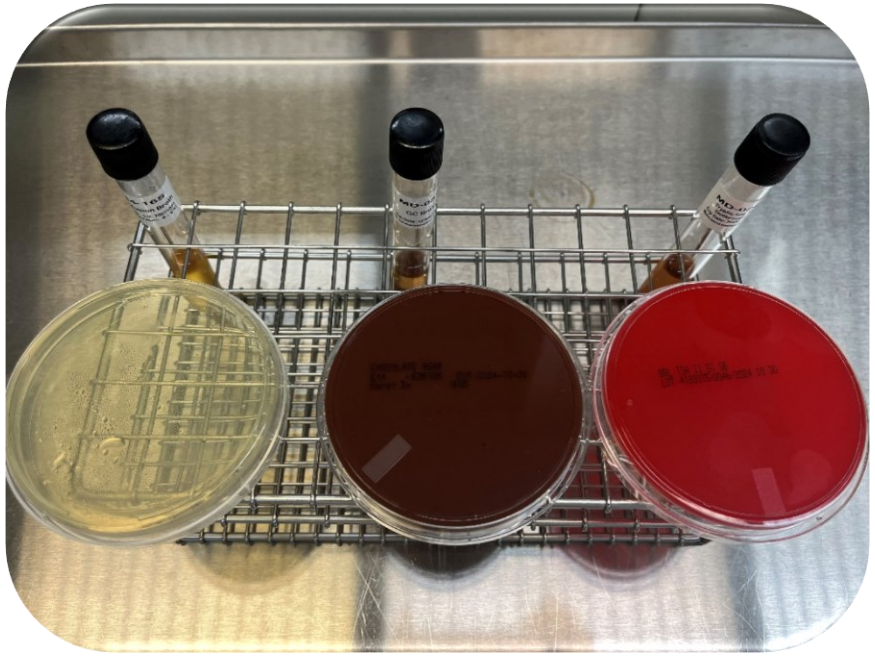
General purpose

- Nutrient Agar/Broth
- Tryptic Soy Agar/Broth (TSA/TSB)
- Luria-Bertani broth and agar (LB)

Nutrient-Rich Media

- Brain-Heart Infusion (BHI)
- Chocolate Agar Base (GC medium)
- TSA w/ 5% Sheep's Blood (Blood agar)





Specialized group-specific media

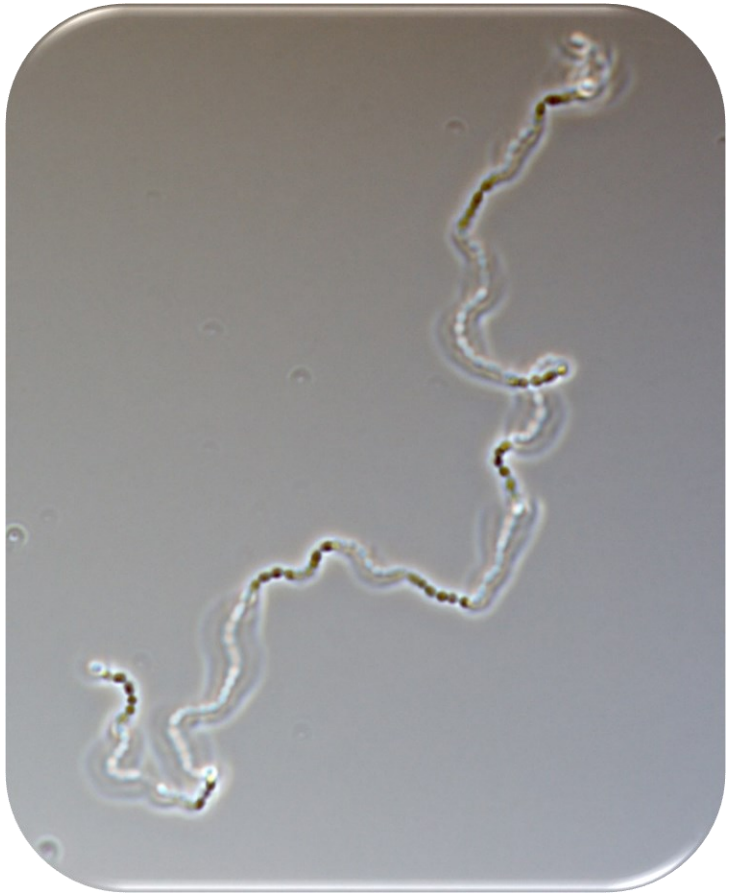
- Charcoal Yeast Extract
  - *Legionella* and related genera
- Marine Media
  - *Vibrio* and related marine genera
- Group-specific supplemented media
  - *Haemophilus* Test Media
  - *Azotobacter* Supplement Media
  - Lactobacilli MRS Media
- Bordet-Gengou - used to isolate *Bordetella pertussis*
- Additional supplements
  - Fetal Bovine Serum (FBS)
    - *Mycoplasma* growth
  - Horse Serum
    - *Ureaplasma* growth

Lactobacilli MRS Media

Anaerobes

- Facultative anaerobes
  - Grows with or without the presence of oxygen.
- Aerotolerant anaerobes
  - Uninhibited by oxygen but can generate energy without using oxygen via fermentation.
- Strict anaerobes (obligate)
  - Only grows in the absence of oxygen. Oxygen is toxic to this organisms. Picture to the right is an example of a strict anaerobe.
- Methanogens
  - Class of strict anaerobes that require nitrogen or ammonia sources in their media.
  - Methane produced as a byproduct of their metabolism.

Coprococcus catus (ATCC® 27761™)

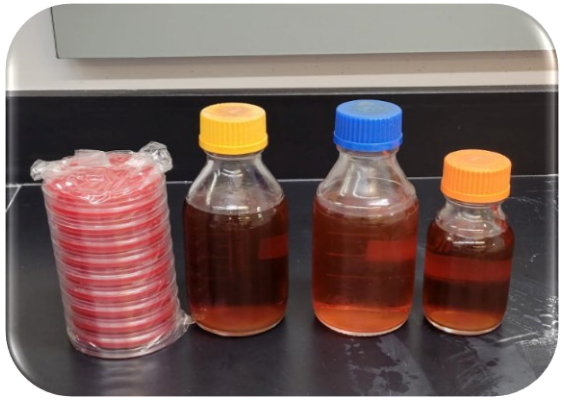


Media for anaerobes

Nutritional requirements is key to establishing healthy growth

- The superior choice is using Pre-Reduced Anaerobically Sterilized (PRAS) media.
- At ATCC®, we recommend using pre-reduced media that was either freshly prepared or previously prepared and stored under anaerobic conditions.
- Common broth medias are chopped meat, reinforced clostridial, and peptone yeast extract broth with glucose (PYG).
- Media should be prepared with reducing agents and stored in anaerobic environments.
- Resazurin, an indicator compound, is added to media that has been reduced of oxygen.

Bottled broth + TSA blood plates



Broth media: chopped meat

Reducing anaerobic media

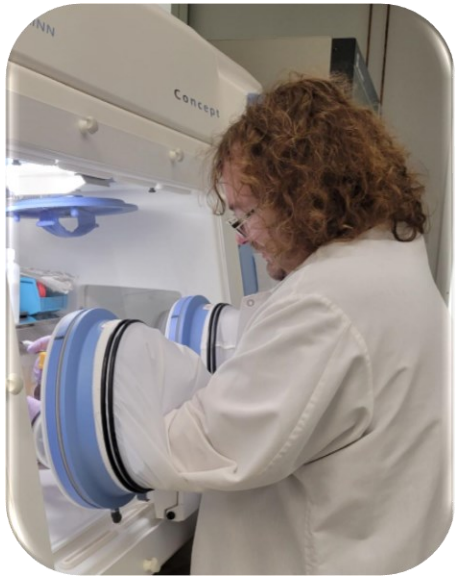
- Standard anaerobic gas mixture is 80% N_2, 10% CO_2, and 10% H_2 for most anaerobes.
- Media should be prepped with reducing agents and stored in an anaerobic environment.
- Common reducing agents are 3% cysteine HCl, 5% coenzyme M, or 15% sodium sulfide.

Gas exchange through a needle and syringe system

Pre-reduce media bottles

Gas generating system

Anaerobic chamber



Learn more about anaerobes

Scan to get the blog

Overcoming the Challenges of Growing Anaerobic Bacteria

June 12, 2024 | Jeanette Rimbey, MS

Explore the best approaches for achieving successful growth of anaerobic bacteria.

Read our blog post to learn more about our anaerobic laboratory practices

- Get more details on anaerobes
- Learn how to select the right media
- Find tips on achieving the best growth conditions

ATCC

Extremophiles

Temperature and pH

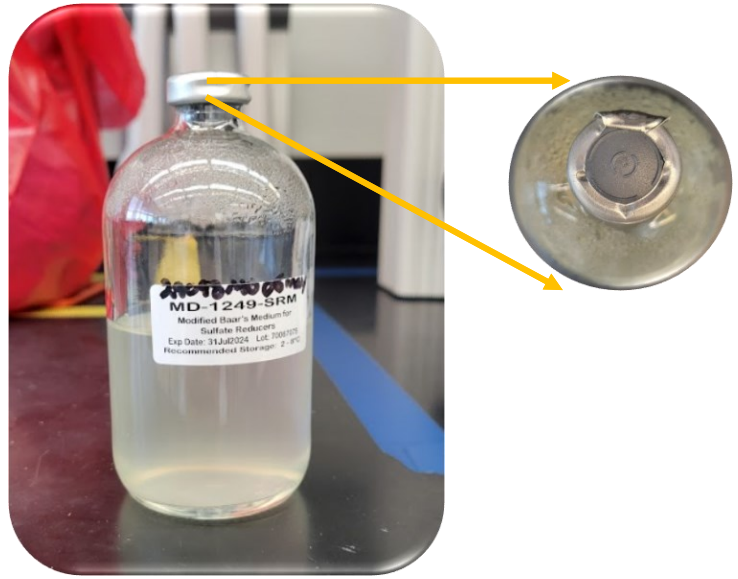
- Thermophile – 45°C and up
- Psychrophile – 15°C and below
- Halophile – prefer high salt concentration
- Acidophile – pH 3.0 or below
- Alkaliphile – pH 9.0 or above

Haloferax volcanii
(ATCC® 29605™)

Yellowstone National Park

Unique growth requirements

- Environmental anaerobes that grow in niche environments
  - Acidic, excessively hot, or irregular in air pressure
- Growth may require Balch tubes/SRM: crimped aluminum seal for culturing
  - Maintains additional pressure
- May also require gas mixtures free of carbon dioxide (CO_2)



Balch/SRM bottle

Microaerophiles

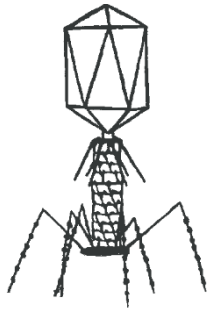
Bacteria requiring lower levels of oxygen vs those in normal air

- *Examples:*
  - *Helicobacter*
  - *Neisseria*
  - *Campylobacter*
- Requires nutrient rich media - blood based media or serum
- Beneficial to use a reliable gas generating system
- Atmospheric conditions is between 5-10% O_2 and 8-10% CO_2
- May grow best in a biphasic environment
- Growth tip
  - Make sure media is pre warmed before inoculation to avoid growth issues
- Fun history fact
  - Microaerophiles were historically cultivated in candle jars!

Biphasic growth

Bacteriophages - "Bacteria eater"

- ATCC uses Broth propagation methods routinely, but Adams Agar Overlay Method is acceptable as well.



- Propagation Steps:

Day 1

Propagate host following basic cultivation practices

Day 2

Amplification of host + bacteriophage using the respective bacterial host strain. Host should be in early log phase prior to adding lysate.

Day 3

Filter the culture to rid of bacteria cells and perform a spot titer to determine the plaque forming units (pfu).

Day 4

After 24-hour incubation, lysis should be visible. At the higher dilutions, individual plaques should be countable.

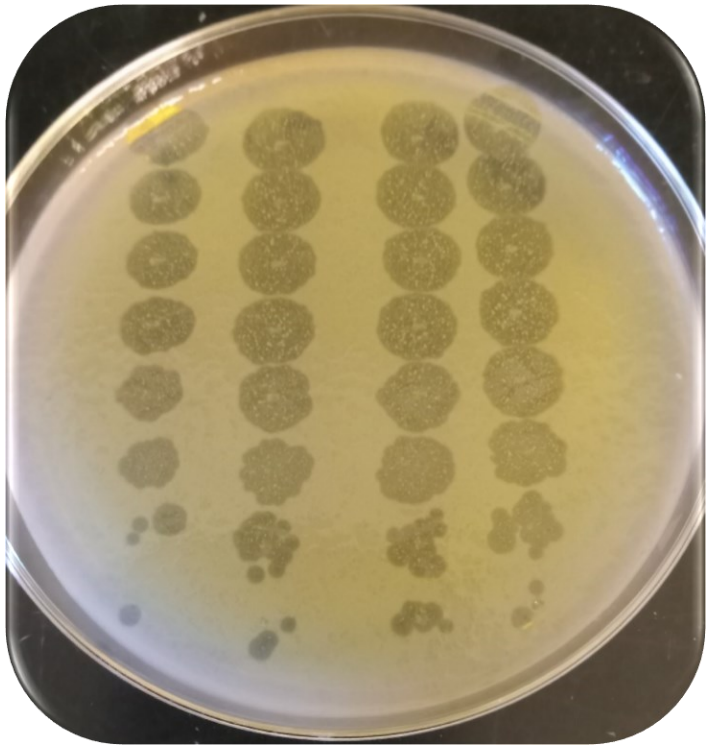
- Method suggestions to consider
  - Make sure host is at an optimal Optical Density (0.1-0.3) prior to introducing the lysate.
  - Consider the appropriate ratio of virus-to-cells called multiplicity of infection (MOI) to enhance lysate growth.
  - Try adding MgCl + CaCl (2^+ salt) to media which increases the potential for phage aggregation through cation binding.

Plaques

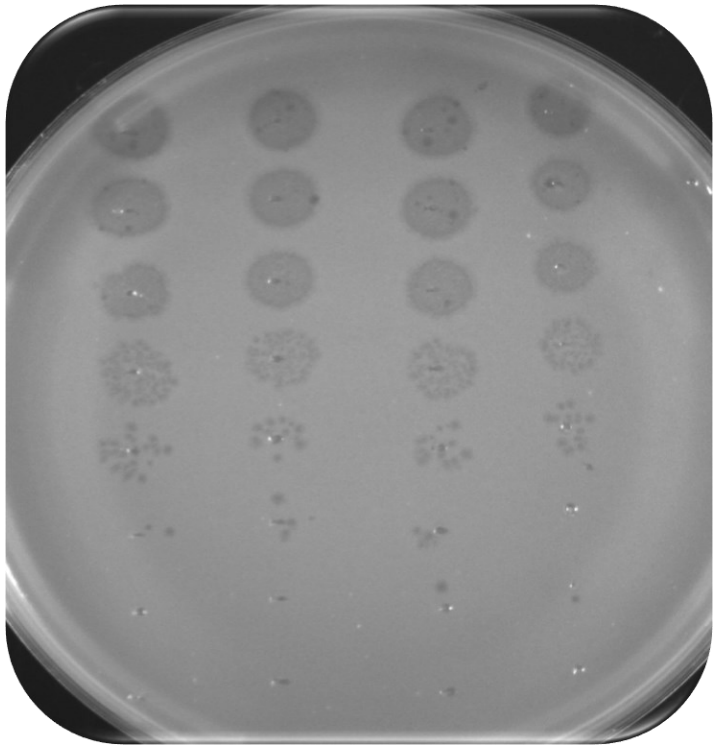
Zones of clearing are observed within the lawn of bacterial growth on a plate.

Escherichia coli
bacteriophage T5
(ATCC® 11303-B5™)

Pseudoalteromonas espejiana
bacteriophage PM2
(ATCC® 27025-B1™)



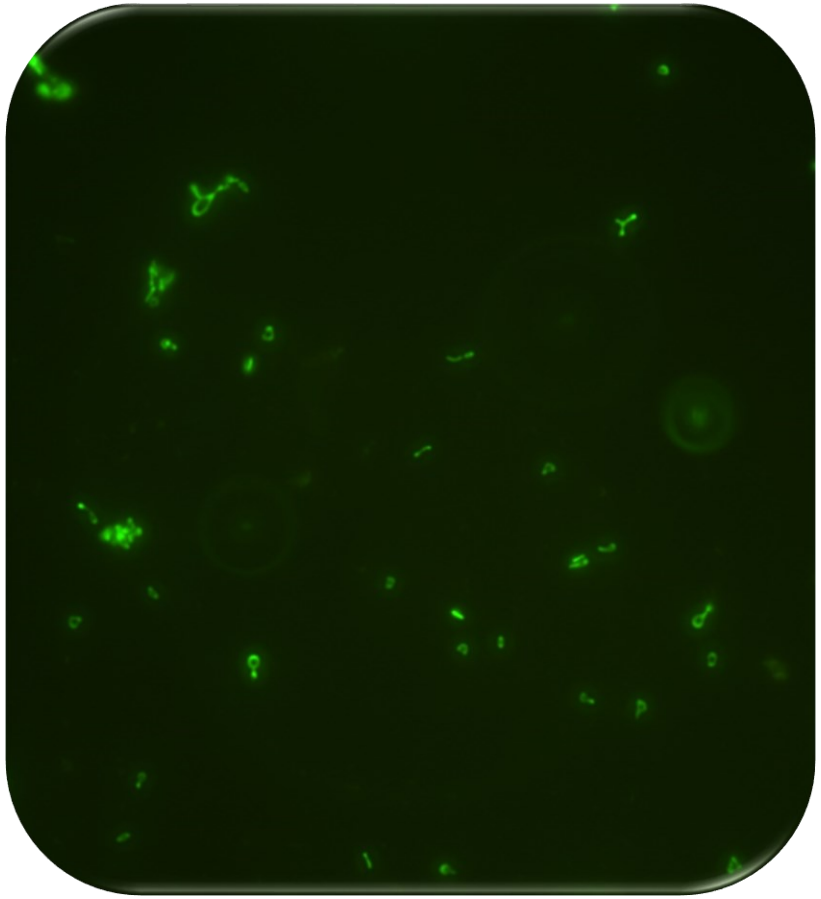
Escherichia coli
bacteriophage Mu-1
(ATCC® 23724-B9™)



Mollicutes

- Very small, pleomorphic cells without a cell wall
- Genera include:
  - *Mycoplasma*, *Ureaplasma*, *Spiroplasma*, and *Acholeplasma*
- Most are aerobic or facultative anaerobes
- Shape depends on osmotic pressure, nutritional quality of the culture medium, and growth phase
- Most media includes pH indicator to track growth cycle
  - Unfavorable pH will inhibit growth
- If the organism is struggling to grow, try adding 10% fetal bovine serum as a supplement
- It's recommended to use at least a 10% inoculum when scaling up growth with mollicutes

Mycoplasma ovipneumoniae
(ATCC® 29419™)



Troubleshooting

Bacteriology troubleshooting

Growth optimization

- Growth optimization assays are performed to test different media with kinetic experiments.
- Comparing preservation methodologies to determine best vial type for numerous organisms.

Specialty troubleshooting

- Antibiotic resistance testing
- Construct PCR protocols to test for the presence/absence of various genes of interest
- Whole-genome sequencing is conducted if suspicious of hidden contaminants

New accessions

Our team determines the ideal growth conditions for new deposits based on depositor notes and research.

Quality Assurance

Advancing authentication through credible standards

Quality control

MALDI-TOF MS

- **Phenotypic analysis –** Cell morphology, colony description, viability, and purity.
- **Genotypic analysis –** Sequencing conserved regions of the genome and whole-genome sequencing (ATCC® Genome Portal).
- **Proteomic analysis –** MALDI-TOF MS
- **Functional testing –** Antimicrobial susceptibility testing, serotyping, virulence detection

ATCC uses a variety of advanced techniques to characterize and authenticate biomaterials—no single method of identification is sufficient. Researchers look to ATCC for a wide range of authentication resources to safeguard reproducibility and meet requirements for funding, publication, and quality control.

ATCC® Genome Portal

A cloud-based platform that enables users to easily browse authenticated and traceable reference genomes and metadata.

Download whole-genome sequences and annotations from your browser or via our secure API.

Search for nucleotide sequences or genes within genomes.

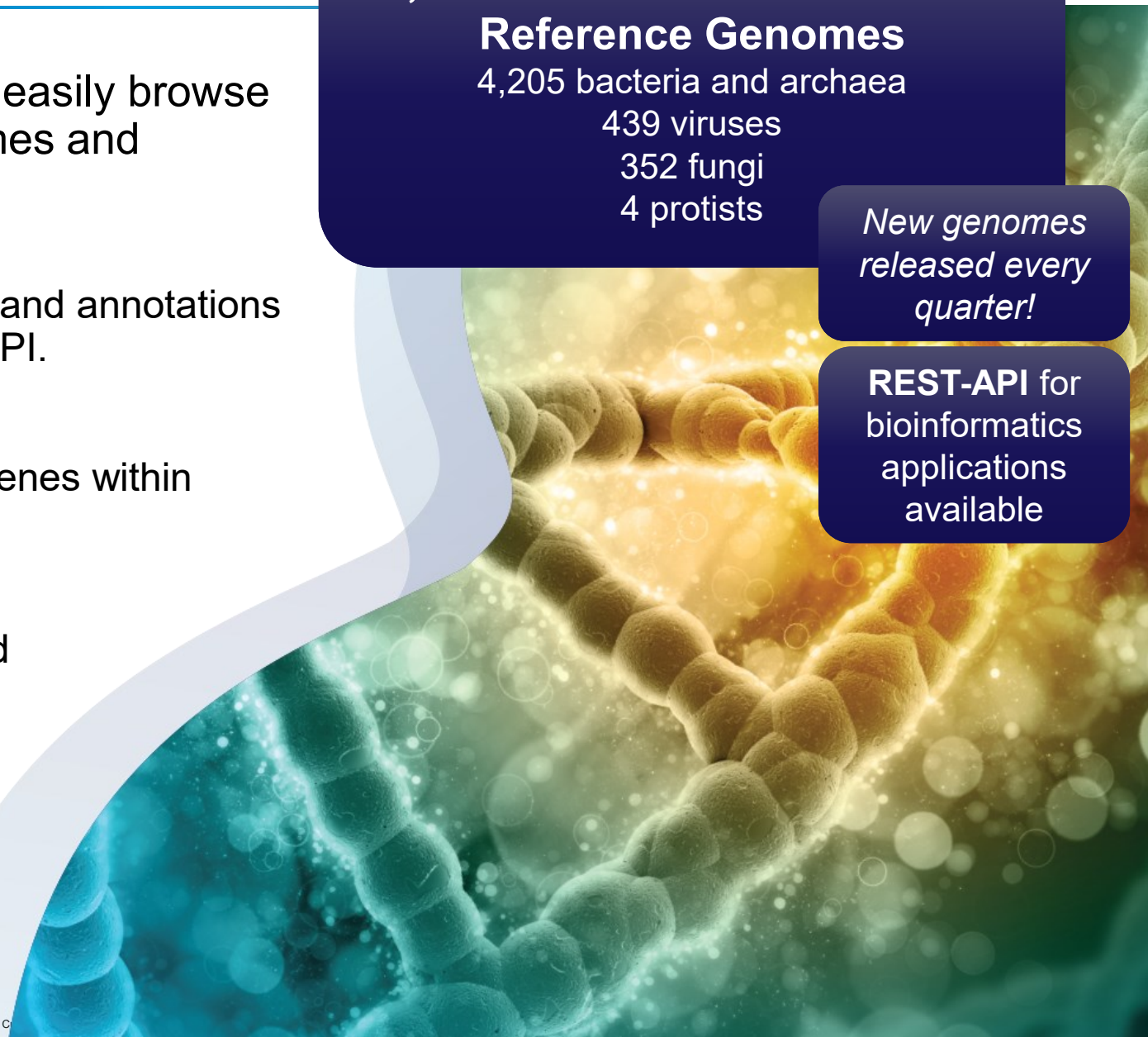
View genome assembly metadata and quality metrics.

5,000 Authenticated Microbial Reference Genomes

4,205 bacteria and archaea
439 viruses
352 fungi
4 protists

New genomes released every quarter!

REST-API for bioinformatics applications available



genomes.atcc.org

Depositing at ATCC®

How to deposit at ATCC®

1. Visit www.atcc.org
2. Select “Services”
3. Select “Depositing with ATCC”
4. Choose the type of deposit
5. Fill out the electronic deposit request form provided
6. Submission is sent to our Content & Accessioning team for review.
7. A project manager will reach out with further details once a deposit is approved.

Deposit service options

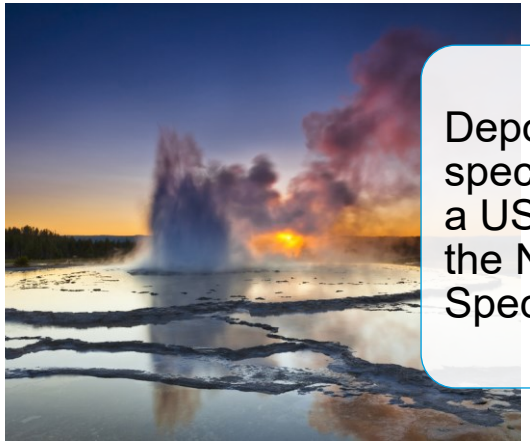
General Collection Deposit

Preserve your research biomaterials and make them available to researchers worldwide by depositing them into the ATCC General Collection.

Patent Deposit

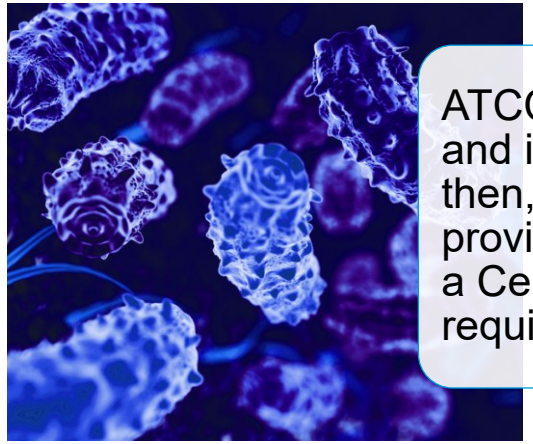
ATCC is an International Depositary Authority under the Budapest Treaty and also accepts deposits under the rules of the US Patent and Trademark Office.

National Park Service Deposit



Deposit biological specimens collected from a US national park into the National Park Service Special Collection.

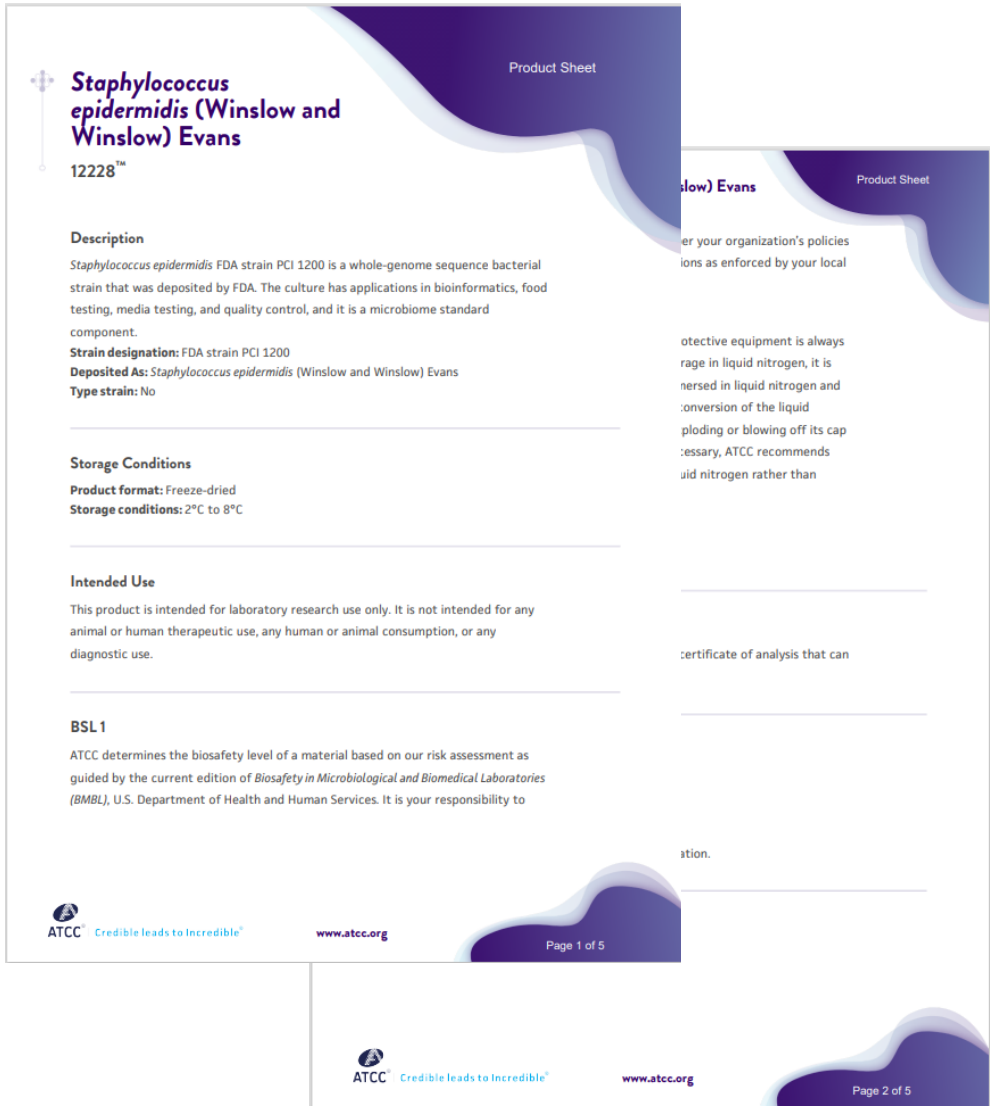
Type strain deposit



ATCC conducts viability and identity testing, and then, if applicable, provides depositors with a Certificate of Deposit required for publication.

Resources

Access detailed product sheets



- Propagation instructions
- Maintenance media
- Atmospheric needs
- Growth temperature
- Incubation time
- Helpful handling notes
- Growth optimization assay results

Get expert tips in our culture guides

View all culture guides at
www.atcc.org/guides

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The science and innovation blog from inside ATCC, the world's premier biomaterial organization

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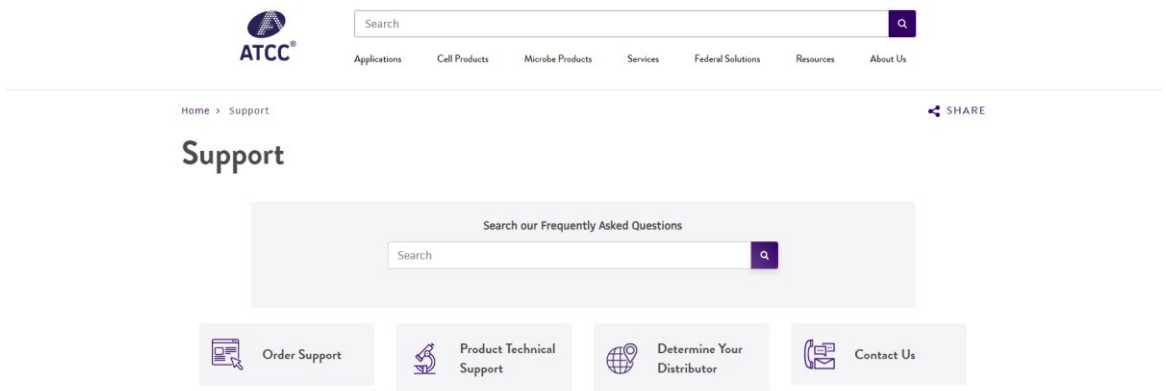

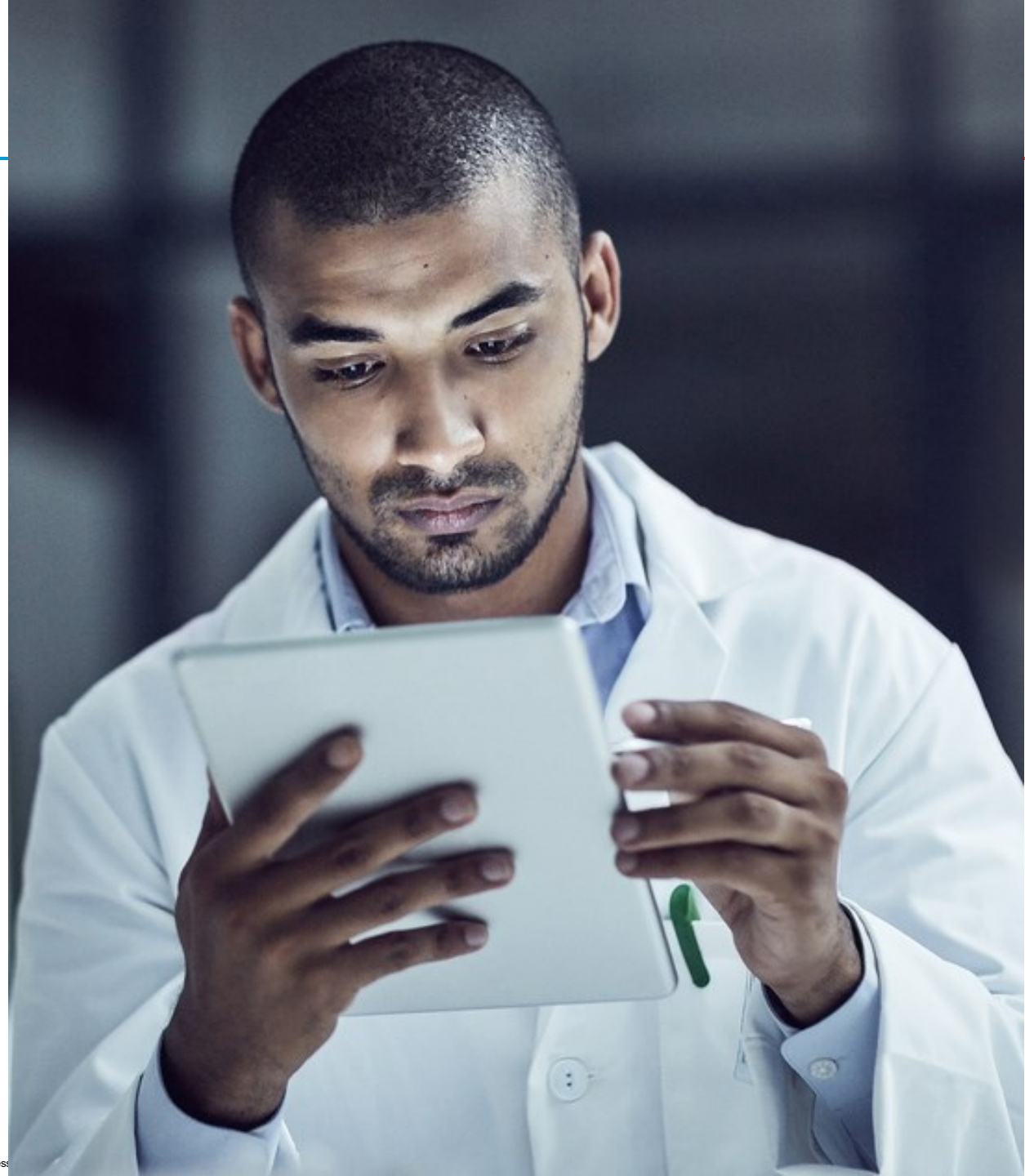

Conclusions

Things to consider…

- Follow a standard propagation method. ATCC® product sheets provide helpful handling information.
- Use the appropriate medium, growth conditions, and length of incubation.
- Reminder that certain organisms prefer shaking incubation over traditional static incubation.
- Make sure your media is room temperature before inoculation.
- Commercial vs. self-made media
  - Commercially made media will often help with poor growth.
  - Don't forget to reduce anaerobic media prior to inoculation.
- Consider specific techniques necessary for specialized strains.
- Bacteriophages – If you don't see plaques, confirm that the host is viable and check the NaCl concentration of the media.

Acknowledgments

Thank You!

- Graham Hogg
- Nancy Krueger
- Joe Thiriot
- ATCC® Bacteriology Team
- Microbiology Bioproduction Teams: Bacteriology | Mycology | Protistology | Molecular Genomics | MSAT-BacT

Learn more about Bacteriology from ATCC

Submit your questions for the ATCC team

- Jeanette Rimbey, Lead Biologist Bacteriology (presenter)
- Graham Hogg, Biologist
- Joe Thiriot, Lab Operations
- Genome Portal Team

View all culture guides at
www.atcc.org/guides