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NEWSLETTER OF THE AMERICAN TYPE CULTURE COLLECTION

In This Issue



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# Culturing Extremophiles: Creating A Home Away From Home

Extremophiles represent the wide range of diversity of bacteria and archaea. They thrive in inhospitable environments that would be deadly to most other organisms. Interest in these organisms has spread from taxonomy to biotechnology as scientists study the enzymes that function under such extreme conditions.

At ATCC we broadly define extremophiles to include microbes that grow at extremes of temperature, pH, salt concentration, and radiation, as well as organisms that grow on unusual energy sources or detoxify hazardous chemicals. ATCC has hundreds of extremophiles in its collection. These fascinating organisms have unusual metabolic requirements that are sometimes difficult to reproduce in the laboratory, and the appropriate media are often complex.

For complete formulations of the media suggested here (or any medium recommended for an ATCC strain), visit our Web site at www.atcc.org. Click on Search Catalogs in the upper right corner of the home page, and choose Media Formulations from the list at left. Enter ATCC's medium number in the query window, and when the record is retrieved, click on the link to reveal the complete formulation.

In this article we describe four different types of extremophiles and explain the cultivation procedures for a specific ATCC strain as an

*(continued on page 3)*

# Verify Cell Line Identity with DNA Profiling

How can you be sure your cell lines truly are what you think they are? If you don't get them from a reliable source (such as ATCC), there is a real chance they might be different than what you expect. In one report, up to 35% of the animal cell lines surveyed were cross-contaminated with other cell lines (1). In the late 1960s, many human cell lines thought to be of various origins were actually HeLa cells (2, 3).

To overcome this problem, we at ATCC have taken several steps to ensure the authenticity of our cell lines. These include isoenzyme analysis, cytogenetics, and DNA profiling.

Since the mid-1990s, ATCC has made a major investment in STR (short tandem repeat) profiling of our human cell lines (4). We analyze each new cell line and we are assembling an STR database of the nearly 1600 human cell lines already in our collection. We anticipate this information will become a

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## Cell Line Profiling *(continued from page 1)*

standard in the scientific community for identifying human cell lines.

Beginning in June 2001, ATCC will make this STR database available to cell biologists everywhere via our Web site. Once you profile your cells, you may compare that data to our reference database. We are providing this information to the scientific community as a service to ensure the quality and integrity of research generated from human cell lines.

STR loci are among the most informative polymorphic markers in the genome. The profiling process used at ATCC involves simultaneously amplifying eight STR loci and the amelogenin gene in a multiplex PCR reaction (Promega GenePrint® PowerPlex® v1.2 system). The amplicons are separated by electrophoresis and analyzed using Genotyper® 2.0 software from Applied Biosystems. Each peak in the resulting electropherogram represents an allele that is alphanumerically scored and entered into our database.

Initially we plan to post the alphanumeric fingerprints of over 200 cell lines on our Web site. The database will be updated monthly. As in the past, when we find an inconsistency in the fingerprint of a cell line (i.e., the profile is similar or identical to that of an unrelated cell line) we will post a note on our Web site under "Misidentified Cell Lines" (www.atcc.org/SearchCatalogs/probline.cfm).

Maintaining cell line integrity is an important standard to bear, but ATCC is up to the task. And we're willing to show you the data to prove it.

**References**


1. Hukku B et al. Cell characterization by use of multiple genetic markers. In: Acton RT and Lynn JD (eds). Eukaryotic Cell Cultures. New York: Plenum Publishing; 1984: pp. 13-31.
2. Nelson-Rees W et al. Science 212: 446-452, 1981.
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# Culturing Extremophiles *(continued from page 1)*

example of each type.

## Halophiles

Halophilic archaea are extremophiles that require environments with salt concentrations exceeding 150 g/liter. They inhabit natural salt lakes, marine salterns, solar salt evaporation ponds, and many other hypersaline bodies of water. To protect themselves from the intense light that often occurs in these harsh environments, they contain carotenoids and other pigments. When grown in dense cultures these organisms may display a beautiful array of colors: yellow, pink, orange, red and even purple. Halophiles have also made a number of unique biochemical adaptations to growing at high salt concentrations. Perhaps the most notable of these is the production of bacteriorhodopsin, a light-driven proton pump that helps supply some of these organisms with energy.

### *Halobacterium salinarum* ATCC 33170

*Halobacterium salinarium* ATCC 33170, formerly the type strain of *Halobacterium cutirubrum*, is unlike the typical *Halobacterium* in terms of its cell morphology and purple membrane. This organism is a Gram-negative, aerobic, pleomorphic rod which also occurs as oval or coccoid shape depending on the growth conditions. The colonies on solid medium are translucent, red-orange, and flat. In a broth formula of Van Niel's yeast agar with 25% NaCl (ATCC Medium 217), growth takes about 4 days in cultures shaken at 150 rpm at 35°C. Growth is evident when the medium becomes turbid and red-orange.

## Methanogens

The methanogens are an abundant and ecologically diverse group of archaea. They are strict anaerobes and generally do not tolerate oxygen even for short periods of time. To carry out methanogenesis they produce unique cofactors, such as coenzyme F420. This chemical makes the cells autofluoresce under excitation by shortwave UV light, which is a diagnostic feature.

Methanogens are found in a variety of environments including flooded soils, anoxic sediments, anaerobic digestors, and the gastrointestinal tracts of animals. They are generally absent from environments with high $O_2$ tension. However, in some oxygenated environments methanogenic bacteria can be found in microenvironments where they may be protected by the activity of other microorganisms.

*Methanosarcina* is one of the more metabolically diverse genera of the methanogenic bacteria. They are able to utilize acetate and a variety of C-1 methyl compounds but not formate, under an atmosphere of 80%$H_2$-20%$CO_2$. The cells are large, Gram-variable cocci and are generally nonmotile.

### *Methanosarcina mazei* ATCC BAA-159

Like most methanogens, *Methanosarcina mazei* ATCC BAA-159 is not tolerant of air and requires handling with extreme care to obtain growth. It is grown in ATCC Medium 1439 in Balch test tubes (see note A below) to maintain strict anaerobic conditions. A redox indicator, such as resazurin (see note B), should be included in the medium. If the medium does not remain reduced the resazurin will cause the medium to turn pink.

To culture *M. mazei:*

1) Prepare two tubes of ATCC Medium 1439 for inoculation by exchanging the atmosphere above the medium for a gas mixture of 80% $H_2$-20% $CO_2$. Add enough gas mixture to overpressurize the tube If the medium is pink, reduce it by adding a small volume of reducing agent (see note C) and allowing the medium to sit at room temperature until it becomes colorless.
2) Once the medium is reduced thaw the frozen vial of ATCC BAA-159 under a gentle stream of oxygen-free gas. Using an anaerobic 1-ml syringe (see note D) with a 22-gauge needle, draw up the thawed culture (or transfer from a growing culture) and then use the syringe to inoculate the tubes of prepared medium.
3) Incubate the tubes at 30°C. The gas in the tube should be exchanged every 24 to 48 hours for fresh 80% $H_2$-20% $CO_2$. Growth will be detected within 48 hours and optimum cell density should be obtained within a week. The cells appear as large cocci, single and in pairs, that are nonmotile. The cells autofluoresce when viewed under an epifluorescent microscope using a UV filter cube.

**Notes for culturing strict anaerobes**

A. Balch tubes (Bellco Glass, Inc.) are special test tubes that are designed to be pressurized and are suitable for anaerobic work.
B. Resazurin is a commonly used redox indicator. It is

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## Culturing Extremophiles *(continued from page 3)*

pink when the redox potential is above –50 mv and colorless when the redox potential is below –110 mv (i.e., highly reduced). Most strict anaerobes require highly reduced media for optimum growth.

C. To obtain a fully reduced medium it is often necessary to decrease the redox potential by adding a reducing agent. Common reducing agents are sodium sulfide, cysteine, dithiothreitol and titanium citrate.

D. Syringes can be made anaerobic by displacing the dead space in the syringe with sterile oxygen-free gas or a reducing agent.

### Anoxygenic phototrophs

*Rhodospirillum* are nonsulfur purple bacteria that can be found in stagnant water bodies, lakes, waste-water ponds, sewage treatment plants, coastal lagoons, sediment, moist soil, and paddy fields, growing best where there is a significant amount of soluble organic matter. *Rhodospirillum* can also be found in almost any anoxic environment that is exposed to sufficient light to allow photosynthesis.

Because they are metabolically diverse, *Rodospirillum* are able to grow anaerobically in the light and many species are also able to grow aerobically in the dark on plates. When growing heterotrophically most *Rhodospirillum* species are able to utilize a variety of carbon sources.

#### *Rhodospirillum indiensis* ATCC BAA-36

*R. indiensis* ATCC BAA-36 can grow both anaerobically in the light and aerobically in the dark on agar. Unlike many other species of this genus, however, ATCC BAA-36 is also able to grow aerobically under light on agar. Another unique feature of *R. indiensis* is the remarkable changes in morphology the cells can undergo. Depending upon the stage of growth, the cell morphology changes from spirilla to thick rods and finally to sarcina. These morphological changes are only observed when strain BAA-36 is grown aerobically on plates (Figure 1).

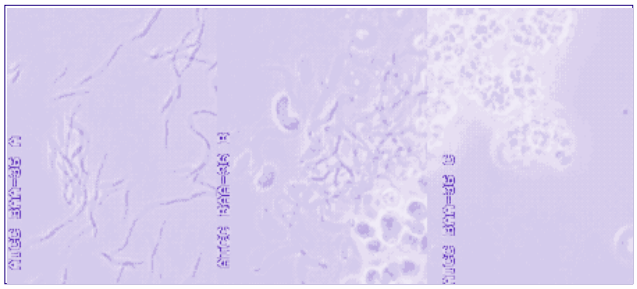

*Figure 1.* Rhodospirillum *undergoes distinctive morphological changes when grown aerobically, from slender rods to thick rods to sarcina. DNA fingerprint analysis verifies that all three forms are the same organism.*

**Phototrophic growth.** Put 6 to 8 ml of ATCC Medium 550 (R8AH Medium) into a 13 × 100-mm sterile screw-capped test tube. Add 3.0% sterile cysteine solution (0.1 ml for each 5 to 10 ml of medium) and then fill the test tube to capacity with additional medium. Seal the test tube with a screw cap and let stand for 20 to 30 minutes at room temperature, after which the medium will be reduced.

Use this medium to rehydrate the freeze-dried pellet from ATCC or if subculturing use a large amount of inoculum from a slant or plate. Be sure that the test tube is filled to capacity and seal the tube. Place the culture at 26 to 30°C within 6 inches of a tungsten lamp.

Initial growth from a freeze-dried culture may take 7 to 10 days to appear, and once growth has been established the culture should be transferred every 3 to 5 days. As the cell density increases the culture will develop a deep red color. If a large inoculum is used when subculturing, the medium does not need to be reduced, but it may take the culture much longer to establish growth as the cells switch from aerobic to anaerobic respiration.

**Aerobic growth.** Inoculate slants and plates of trypticase soy agar (BBL 11043) or test tubes of trypticase soy broth (BBL 11768). Incubate at 30°C. Growth on agar should be detected within two days with the appearance of faint clear pinpoint colonies. At this stage if the cells are picked from the plate and examined by phase contrast microscopy the cells will appear as typical *Rhodospirillum*. After 3 to 4 days the colonies will increase in size. If cells are picked from an area of heavy growth and examined microscopically they will appear as a mixture of large fat rods and spirilla. Cells taken from an area of heavy growth after 6 to 7 days will appear like typical sarcina. Normally seeing cell variation like this would be of concern, but DNA fingerprints of the different morphologies show they are the same organism.

## Sulfur oxidizers

The members of the genus *Thiothrix* are filamentous bacteria capable of oxidizing reduced sulfur compounds. They are found primarily in flowing waters containing sulfide, such as activated-sludge wastewater treatment plants. The physiological study of *Thiothrix* has gained interest over the past 15 years because it is the causative agent in filamentous sludge bulking (poor solid separation) that occurs at wastewater treatment plants.

Finding the optimal conditions for growth of *Thiothrix* can be a challenge. These organisms are difficult to grow in the laboratory, especially on solid media; many have not yet been isolated in pure culture. Because of their unusual, multistage life cycle, the cell morphology varies according to culture conditions. Under poor growth conditions, individual cells become round and form gonidia, which may allow *Thiothrix* to disperse in nature (Figure 2).

### *Thiothrix eikelboomii* ATCC 49788

*Thiothrix eikelboomii* is a Gram-negative rod that forms highly variable multicellular filaments. ATCC 49788, the type strain, was isolated from a wastewater activated sludge site.

This strain does not require inorganic sulfur. We recommend ATCC Medium 1820 (LTH Medium) and incubation at 20°C. The initial growth after thawing does not give the silky mat-like mass that is characteristic of *Thiothrix*; instead the broth is flocculent. However, after several transfers the mat-like mass reappears, possibly because the cryoprotectant has been diluted out.

**Learn more about the cultivation of ATCC's extremophiles in our online catalog of bacteria at www.atcc.org. Search for a genus, species, or strain for recommended medium and growth conditions. For specific questions contact a technical service representative at tech@atcc.org or 800-638-6597.**

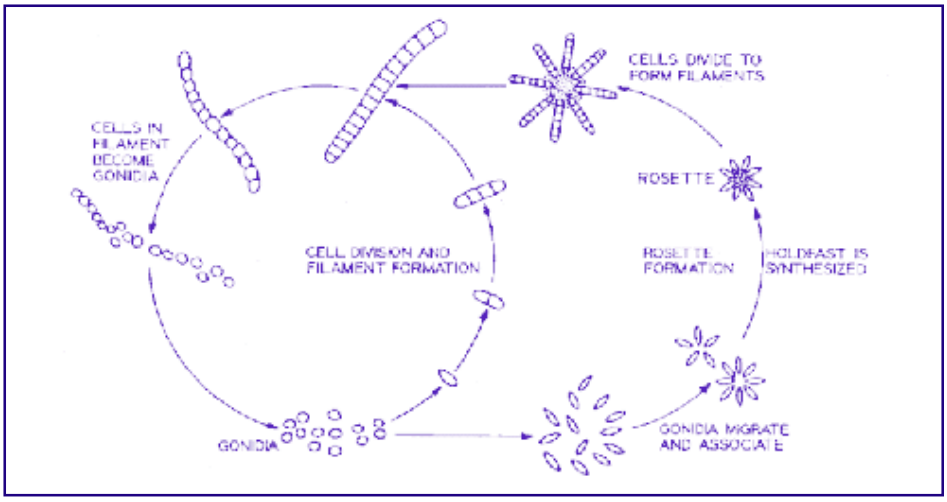


*Figure 2. The morphology of* Thiothrix *changes during its life cycle, appearing as long filaments and individual gonidia. (Reprinted with permission from The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community. Springer-Verlag New York, Inc.)*

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You can also find us at the following meetings in 2001. Stop by our booth and say hello. We'd love a chance to talk to you about your laboratory's needs.

| | | |
|---|---|---|
| Harvard Medical School | September 19-20 | Boston, Massachusetts |
| NIH Research Festival | October 11-12 | Bethesda, Maryland |
| Society for Neuroscience | November 10-15 | San Diego, California |
| American Society for Cell Biology | December 8-12 | Washington, D.C. |

# ATCC Now Offers cGMP-Compliant Safe Deposit

ATCC has been offering safe deposit for back-up safekeeping of valuable cells and organisms for nearly 25 years. Now we are pleased to announce a new cGMP-compliant safe deposit service that can provide a secure back-up storage site at ATCC for cell banks in compliance with current good manufacturing practices.

The new service offers the following enhanced features:

- Dedicated and validated all-vapor LN freezer which is fully alarmed and has controlled and restricted access.
- Tracking label for your shipment to ensure handling immediately upon receipt at ATCC.
- Separate storage boxes for each bank or multiple banks in one box at depositor's option. Only one customer per storage box.
- Direct QA oversight of all freezer entry and retrieval activities.
- Dedicated and trained staff.
- Annual third-party quality audit report for all depositors included in the deposit fee.

Our standard safe deposit service is not cGMP compliant and will be continued to be offered. If you have material stored at ATCC now that you determine should be handled under cGMP conditions, we can arrange transfer of your deposits to the new service. Currently we are only accepting nonmicrobial banks, but we are planning to expand the program soon to include microbial banks.

Your proprietary materials deserve secure, dependable care. Trust the experts in cell storage—now offering cGMP-compliant safe deposit to meet the needs of your company. For more information on this new service, contact ATCC's Laboratory Services department at 703-365-2700 ext. 519 or by e-mail at applied-sci@atcc.org.

## Order Online! Order Online!

If you know your ATCC account number, why not give our online ordering system a try? Our servers are secure for online transactions and you can use a credit card or purchase order number. The electronic system prompts you for all necessary information, so your order will not be delayed because it's incomplete. Just go to our Web site at **www.atcc.org** and click on View Cart in the upper right corner to get started.

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Description and online ordering of ATCC's reagents and kits:
www.atcc.org/Products/Biomaterials_Culture.cfm

Check the status of an order online:
www.atcc.org/Order/OnlineStatus.cfm

Description of lab services offered by ATCC:
www.atcc.org/ProgramsAndServices/BioSciLabSvcs.cfm

## In Brief

### Distributor Update

ATCC has added another authorized distributor to facilitate overseas shipment of materials. Customers in Taiwan Republic of China can now order from Union Biotech, Inc. The e-mail address is ubi@ms9.hinet.net and the telephone number is (02)2368-3600. Union Biotech joins LGC in Europe, Summit Pharmaceuticals in Japan, and Koram Biotech in Korea as exclusive distributors of ATCC products.

### Viral Diseases Examined

Dr. Raymond Cypess, president and CEO of ATCC, was an invited presenter at The National Academies in Washington, D.C., on February 1-2. The meeting, titled "The Consequences of Viral Disease Eradication," was a workshop sponsored by the Forum on Emerging Infections. Frank Simione, vice president of safety and regulatory affairs, joined Dr. Cypess in presenting "Laboratory Security and Regulations Governing Viral Pathogenesis in a Post-Immunization Era." Their paper will be published in the proceedings of the workshop.

### Malaria Workshops Held

ATCC recently hosted two workshops for malaria bioinformatics under the Malaria Research and Reference Reagent Resource Center (MR4). These courses, held last November and this spring, were sponsored by ATCC, MR4, NIH, NIAID, and WHO/TDR. Scientists from malaria-stricken areas participated in the workshops, which consisted of lectures and practical exercises. Instructors included staff from ATCC, government agencies, private research institutions, and several universities.

### Call for Manuscripts

ATCC is looking for technical articles (800 to 1500 words) to publish in this newsletter. An honorarium will be provided after publication. For more details contact the editor at news@atcc.org.

# Cooperative Model Serves Science and Scientists

When ATCC was founded in 1925, a joint committee of scientists from various agencies decided there was a need for a national culture repository to ensure availability of microorganisms to researchers. This simple directive relied on striking a balance between what scientists will share and what they need.

Many microbiologists in ATCC's early years made their entire collections available for distribution. The bacteriology collection, for example, has grown a hundred-fold on the basis of donating and distributing cultures. Scientists depend on ATCC to provide excellent care of their strains, relieving them from responding to requests for cultures and worrying about catastrophic culture loss, and scientists purchasing strains can be assured of the identity and integrity of materials.

Biological resource centers like ATCC are cornerstones for microbial biodiversity, as evidenced by the excellent representation of type strains. Over 3,700 of the strains in the bacteriology collection are type strains, and acquiring newly described species continues to be a priority. Most recently ATCC has been acquiring organisms that display unusual metabolic characteristics, antimicrobic resistance, and extreme growth requirements. In each case, however, it is critical to remember that in order for any organism to be made available to scientists through ATCC someone must first decide to share the strain.

Microbial strains that are the subjects of genome sequencing projects are especially significant. Over 120 different organisms are currently being sequenced, and more than 40 bacteria and archaea have complete genomic sequence information available. These organisms are valuable resources for scientists participating in various areas of genome-related research. However, ATCC has acquired only a fraction of these sequenced strains from various sources, even after directly requesting them. Because most of these sequencing projects are funded by government agencies, it should be mandated that the strains used in these projects be deposited in culture collections and made available to the scientific community.

ATCC's distribution policy ensures that qualified microbiologists everywhere will have equal access to these strains within the limits of regulatory guidelines. Continued cooperation between depositors and recipients of cultures will ensure that biological resource centers will serve researchers with timely, useful materials.

# ATCC Is Awarded Ellison Medical Foundation Grant

ATCC has been awarded a grant by the Ellison Medical Foundation to provide ATCC biomaterials at no cost to scientists in regions of the world most heavily burdened by AIDS/HIV and other infectious diseases. Laboratories in these areas sometimes lack the financial resources to purchase high-quality materials and pay the costs of shipping from the United States, thus this grant will provide an opportunity for those researchers to have equitable access.

Scientists located in the countries listed below are encouraged to submit orders for biomaterials to ATCC and to reference the **Ellison Medical Foundation/ATCC Fund** on the order. ATCC will fulfill orders for reasonable quantities of ATCC biomaterials within the limitations of available funds. All restrictions, requirements, and limitations with respect to use, safety, and compliance with United States law that apply to regular orders will also apply to orders accepted under this program.

Providing the tools of research to scientists in disease-stricken areas is a significant step in the global battle against infectious diseases. We applaud the vision of the Ellison Medical Foundation and thank them for this most generous grant.

Scientists in the following countries may be eligible for these funds:

Benin
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Burundi
Cambodia
Cameroon
Central African Republic
Chad
Comoros
Congo
Djibouti
Equatorial Guinea
Eritrea
Ethiopia
Gabon
The Gambia
Ghana
Guinea
Guinea-Bissau
Haiti
Kenya
Laos
Lesotho
Madagascar
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