



The American Type Culture Collection: Germ Plasm Resources for Plant Pathologists

The American Type Culture Collection (ATCC) is a private, nonprofit organization dedicated to the acquisition, preservation, authentication, and distribution—the “APAD” activities—of diverse biological materials. During its 67-year history (1), ATCC has accessioned and conserved more than 50,000 cultures, consisting of algae and protozoans, bacteria (including rickettsiae and chlamydiae), bacteriophages, fungi and yeasts, animal and plant viruses and antisera, mycoviruses, molecularly cloned viruses, plant and animal cell lines (including human tumor cells), cloned genes (including oncogenes), plasmids, DNA probes, gene libraries, and hybridomas. In 1990 alone, more than 127,000 cultures were distributed to scientists in more than 50 countries. Approximately 16% of the total ATCC collection consists of microorganisms that are of importance to plant pathologists: 1,051 bacteria, 11,522 filamentous fungi, 373 viruses and viroids, and 11 yeasts.

The American Phytopathological Society was one of the organizations instrumental in the founding of ATCC in 1925. Today, ATCC is affiliated with 22 professional scientific organizations, the primary users of its cultures and services. Representatives from each of these organizations serve on the ATCC board of directors (see Box 1).

Dr. McDaniel's address is: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

ATCC, one of the many valuable resources for general and specialized microbial germ plasm in the world (2), provides a broad range of well-characterized bacterial, fungal, and viral plant pathogens and associated data for research, education, agriculture, and industry. In 1989, in recognition of this important role, the American Phytopathological Society officially designated ATCC as a national repository of plant pathogens and related biological materials. This article describes ATCC germ plasm resources of interest to plant pathologists and the APAD activities of the Bacteriology, Mycology and Botany, and Virology departments.

General Facilities

ATCC employs a staff of 220 individuals. The facility is located in Rockville, Maryland, on approximately 4 acres of land and consists of three buildings. The Carlson building (Fig. 1) (53,000 ft²) houses laboratories for the scientific departments, a library, conference/seminar areas, a workshop laboratory, and a greenhouse. The second building (16,000 ft²) contains the offices for the Accounting, Bioinformatics, Shipping, and Sales and Marketing departments, and the third building (30,000 ft²) houses the administrative offices and the Maintenance and Engineering, Materials Management, and Publications departments.

The ATCC animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care and registered by the U.S. Department of Agriculture (USDA). The greenhouse and all laboratories in which plant

pathogens are handled are inspected by state and federal (USDA Animal and Plant Health Inspection Service/Plant Protection and Quarantine) officials for compliance with quarantine regulations. Buildings have restricted access and are monitored 24 hours a day. In emergency situations, an auxiliary generator supplies power to freezers, refrigerators, and other critical instruments. For added security, a duplicate supply of all freeze-dried cultures is stored in Blacksburg, Virginia; backup liquid nitrogen storage for frozen cultures is located in Frederick, Maryland.

The scientific departments are supported by the Bioinformatics, Manufacturing, Sales and Marketing, and Publications departments. The Bioinformatics Department assists in developing computerized data management systems. The Manufacturing Department preserves cultures by freezing and freeze-drying. Storage units consist of walk-in



Fig. 1. The largest of the three buildings that make up the ATCC facility in Rockville, Maryland, the Carlson building houses laboratories for the scientific departments, a library, conference/seminar areas, a workshop laboratory, and a greenhouse.

cold rooms, mechanical freezers (-70 to -135°C), and vacuum-insulated refrigerators cooled with liquid nitrogen. The Sales and Marketing Department uses a computerized inventory, order processing, and invoicing system to provide customers with current information on usage, availability, and replacement of cultures. The Publications Department is responsible for production of catalogues, annual catalogue updates, newsletters, technical manuals, and other informational brochures.

Collection Facilities

The Bacteriology Department has a staff of 23, including nine individuals associated with the media and glassware units. The department is provided with laminar flow hoods and such equipment as gas-liquid chromatographs, spectrophotometers, electrophoresis units, and various types of microscopes. A comprehensive battery of characterization tests

ensures the authenticity of the department's holdings.

The Mycology and Botany Department includes two scientists, a collection manager, and eight other staff members working in four distinct areas: the APAD service laboratory, the molecular technology research laboratory, the information management laboratory, and the plant tissue laboratory, which occupies seven rooms. Facilities and equipment enable the staff to characterize fungi on the basis of anatomical structures, cell division, colony morphology, mating studies, morphogenesis, ultrastructure, anamorph-teleomorph connections, antigenic relationships, electrophoretic patterns, enzyme profiles, lipid composition, nutritional requirements, temperature requirements, and other physiological data.

A collection manager and two technicians make up the plant virology staff within the Virology Department. Laboratories are in a segregated area that is under negative pressure in relation to adjoining areas; entrance is through an air lock. The plant virology section consists of a greenhouse and two laboratories, where staff authenticate cultures by traditional or molecular virology

techniques; a nearby university provides access to a transmission electron microscope facility. The plastic-enveloped greenhouse, used only by the plant virology section, is a restricted area in which positive pressure relative to the corridor of the attached building is maintained. The greenhouse cubicles are temperature-controlled and have individual HEPA filters through which air is recirculated. All fluids passing into the drains of the greenhouse and the adjacent laboratory are sterilized before passing from the area. The area contains two growth chambers for controlled-environment studies.

Holdings

Scientists throughout the world donate cultures to ATCC. Acceptance is based on many criteria, including historical significance, amenability to preservation, level of characterization, and value to the scientific community (see Box 2). Scientists wishing to deposit cultures should contact the appropriate ATCC department head or collection manager. Before being accessioned and catalogued, a strain is subjected to a series of tests to check viability, purity, identity, preferred

Box 1

Organizations affiliated with ATCC

American Association of Immunologists
American Institute of Biological Sciences
American Phytopathological Society
American Society for Biochemistry and Molecular Biology
American Society for Cell Biology
American Society of Human Genetics
American Society for Microbiology
American Society of Parasitologists
American Society of Tropical Medicine and Hygiene
American Society for Virology
American Society of Zoologists
Canadian Federation of Biological Societies
Canadian Society of Microbiologists
Genetics Society of America
Infectious Diseases Society of America
Mycological Society of America
National Research Council of National Academy of Sciences
Pharmaceutical Manufacturers Association
Reticuloendothelial Society
Society for Industrial Microbiology
Society of Protozoologists
Tissue Culture Association

Box 2

ATCC criteria for evaluating cultures for accession and deaccession

Value to the scientific and industrial/technological communities

Reference strains (e.g., type cultures)
Human, animal, or plant pathogens
Educational strains
Production of primary and secondary metabolites
Fermentation products
Genetic stocks
Carriers of plasmids
Testing strains
Historical value

Distribution potential

What is demand for culture at present?
What may be demand in future?

Taxonomic diversity

How many other strains of culture are in the collection?
What are distinguishing characteristics?
Do other strains duplicate these traits?

Maintenance

Can culture be maintained in the laboratory?
Can culture be preserved in lyophilized state and/or liquid nitrogen?
If a genetic strain, can it be maintained/preserved without reversion?
Can culture survive shipping?
Can characteristics be verified?

Documentation of strain

Origin
Identification
Literature citations
Literature citations giving ATCC number

temperature and medium for growth and/or sporulation, and methods of preservation. No fee is charged for deposits accepted into the collection, and no cultures are purchased from investigators. A depositor has a lifetime right to secure a culture of that deposit without charge.

The Bacteriology Department maintains approximately 700 plant pathogens, including 21 strains of *Clavibacter*, 10 of *Curtobacterium*, 149 of *Erwinia*, 160 of *Pseudomonas*, 58 of *Spiroplasma*, 6 of *Streptomyces*, and 166 of *Xanthomonas*.

The Mycology and Botany Department maintains almost 17,000 cultures of interest to plant pathologists, representing approximately 76% of the entire fungi/yeast collection (Table 1).

The Virology Department's collection contains approximately 400 plant viruses and viroids and 70 molecularly cloned viruses and viroids currently in distribution. In addition, approximately 200 polyclonal and 25 monoclonal antisera are available for use in research and diagnostic assays. Most of the viruses are mechanically transmissible because ATCC does not have a USDA permit for using insect vectors in virus propagation. During the past 3 years, however, nonmechanically transmissible

viruses have been acquired and preserved for use as serological controls and for hybridization and cloning studies. Each culture is shipped with a product sheet providing basic information; more detailed information may be obtained by contacting the collection staff.

At present, there is no central repository for collecting, maintaining, authenticating, and distributing phytopathogenic mycoplasma-like organisms (MLOs). Because MLOs cannot be sustained in axenic culture and must be maintained *in planta* or in insect vectors by individual scientists, microbial germ plasm may be lost as scientists retire or change projects. In 1990, members of the ATCC plant virus collection staff and Advisory Committee developed plans to acquire selected MLOs and thereby prevent loss of valuable germ plasm. In 1991, four advisors with expertise with MLOs and spiroplasmas joined the Advisory Committee to provide advice and assistance in this project. ATCC space limitations require that MLOs be acquired, stored, and distributed in insect vectors until other suitable means become available or until a collection is established to which MLO cultures can be transferred. Monoclonal antisera to MLOs and molecularly cloned MLO DNA will also become available. Recently, courtesy of Agriculture Canada and L. N. Chiykowski, ATCC obtained four of Dr. Chiykowski's valuable MLOs before his retirement.

Databases and Catalogues

Information on cultures is gathered and updated through direct contact with depositors and computer-based litera-

ture searches. Data from accession forms and reprints are stored by means of database software, such as MicroIS, Paradox, and Q&A. Reprints are transferred to microfiche records. Computer database information, backed up and stored off-site as a safety precaution, is retrieved for reports, product sheets, and the catalogues and updates. Strain features have been computer-coded for the Bacteriology Department to assist in storage and analysis of the voluminous data, and such coding is being devised for the Mycology and Botany Department (5,6).

The Bioinformatics Department also provides information through on-line systems and is actively involved in establishing international networks of microbial and cell line information resources. Data relating to phytopathogenic bacteria may be obtained on-line by means of the Committee on Data for Science and Technology/Microbial Strain Data Network (CODATA/MSDN) on BT Tymnet. ATCC catalogue information on all ATCC cultures will soon be accessible from BIOSIS, with customers also maintaining access through the CODATA/MSDN network. In addition, a connection to the Internet system is being developed.

Each collection issues a catalogue of its holdings every 4 years and an update of newly released cultures every year. Catalogues (Fig. 2) are concise compilations of the data and literature references of greatest interest to the customers. No charge is made for catalogues shipped within the United States; postage is charged for shipments elsewhere. Scientists requiring additional information about a strain may contact the ATCC technical services section of the appropriate department during customer call-in hours (see Box 3).

APAD Activities

Although APAD activities differ slightly according to the type of organism collected, the criteria used to evaluate cultures for accession are the same. Cultures for potential accession are selected by the collection manager and department head. Each collection has an advisory committee, composed of external scientists with recognized expertise in many disciplines, that meets regularly with ATCC staff to provide advice and assistance in acquisition and authentication of cultures. Several taxonomic committees of the International Union of Microbiological Societies also assist the staff.

Acquisition. The Bacteriology Department attempts to acquire the type strain, plus two additional authentic strains, if available, of every named species of bacteria, with emphasis on those currently regarded as valid and those newly named and described. In addition, strains that have special applications, serve as

Table 1. ATCC fungi and yeast cultures

Primary use ^a	Number of strains ^b
Pathogens of plants ^c	6,194
Type and authentic cultures*	3,058
Tools for genetic studies*	2,926
Producers of vitamins, enzymes, and other biochemicals	2,637
Pathogens of man and animals	1,714
Inhabitants of soil and water*	1,602
Edible mushrooms and food yeasts	1,356
Inhabitants of wood*	1,198
Producers of antibiotics and mycotoxins*	1,099
Producers of fermentation and flavor	647
Mycorrhizae*	325
Marine fungi	291
Cultures used in bioassays	288
Entomogenous fungi*	253
Hosts of mycoviruses*	161
Nematode-destroying fungi*	135
Mycoparasites*	78
Pathogens of fish	77
Rust fungi of plants*	68
Total	24,107

^a Determined at time of deposition.

^b Maintained as of 31 December 1991.

^c * = Important in plant pathology.



Fig. 2. ATCC catalogues are concise compilations of pertinent data and literature citations. Each collection issues a catalogue of its holdings every 4 years and an update of newly released cultures every year.

bioassay or quality control cultures, or are currently important in research are also sought.

The Mycology and Botany Department employs a very selective policy and limits accessions to approximately 500 new strains per year because of the space, staff, and time needed to maintain the expanding collection of fungal strains.

The Virology Department attempts to obtain the type strain of viruses. If a type strain has not been designated by the International Committee on Taxonomy of Viruses of the International Union of Microbiological Societies, Virology Division, at least two well-characterized strains or isolates of the virus are selected for accession. Viral strains of economic importance to the agricultural community are considered for accession, as are molecularly cloned viruses and antisera. The latest reports published by the International Committee on Taxonomy of Viruses are consulted to determine the taxonomic status of and appropriate references to published research on plant viruses.

Preservation. All departments use the seed stock system to maintain their distribution stock. As each culture is accepted for deposit, some ampules are set apart as seed stock and others are designated as order stock for distribution. When the order stock becomes depleted, an ampule of seed stock is opened and new culture specimens are prepared from it and freeze-dried or frozen as new order stock. The seed stock is always the closest material available to the original deposit.

The majority of bacteria and bacteriophages are freeze-dried at either 5 C or -60 C. The type and neotype strains, certain special application strains, and strains not amenable to freeze-drying (lyophilization) are preserved in liquid nitrogen at -196 C (Fig. 3) or its vapor phase at -150 to -180 C. Fungi and yeasts are also preserved by freeze-drying and/or storage in liquid nitrogen. After preservation, purity and viability are rechecked. Freeze-dried cultures are kept at 5 C. Cryopreserved cultures are stored in liquid nitrogen or in liquid nitrogen vapor, the preferred method for long-term conservation of living fungi.

Most plant virus and viroid cultures are preserved by freeze-drying and cryogenic storage. Others are more stable when infected tissues are slowly dried over a chemical desiccant and stored at 4 C, and a few are stored in highly purified form in liquid nitrogen vapor. All virus and viroid samples are stored as seed stock in liquid nitrogen. Polyclonal antisera are usually freeze-dried, although some are mixed with glycerol (1:1, v/v), and all are stored at -70 C. Monoclonal antisera are stored at -70 C as ascites fluid. Molecularly cloned viruses that are transformed into an appropriate host (e.g., an *Escherichia*

coli K-12 derivative) are freeze-dried and distributed by the Molecular and Plasmid Biology Department.

Authentication. The Bacteriology Department uses a comprehensive battery of tests to characterize the various taxa and to authenticate strains. Included are tests for cell wall and whole cell fatty acids, fermentation products, specific enzymes, metabolic end products, antibiotic sensitivity, and carbon and nitrogen substrate utilization.

The methods used to authenticate fungi vary widely and range from microscopic examination to lengthy, sophisticated procedures. Most groups of filamentous fungi are identified on the basis of external morphology, whereas the yeasts and, recently, some fungi are subjected to biochemical tests. In addition, the molecular technology research laboratory uses isoenzyme, restriction fragment length polymorphism, and random amplified polymorphic DNA marker analyses as well as ribotyping (restriction analysis of PCR-amplified ribosomal DNA [7]) to resolve some of the taxonomic problems associated with selected strains (4).

The techniques used to authenticate cultures in the plant virus collection include transmission electron microscopy, host range and infectivity tests, and serological studies, including enzyme-linked immunosorbent assay, Ouchterlony gel double-diffusion tests, and electroblot immunoassay. Also useful are profiles of dsRNA and determinations of virion protein capsid subunit and nucleic acid species and relative molecular weights. The Molecular and Plasmid Biology Department authenticates molecularly cloned viruses by analyzing restriction fragment patterns. A sample

of each processed culture or reagent is sent to the donor for evaluation and approval before the material is released for general distribution.

Distribution. The ATCC distributes cultures (for a fee) to scientists and educators worldwide who have the appropriate quarantine permits and documentation. Prices reflect the ATCC cost of preparing, testing, preserving, maintaining, and shipping cultures or reagents. Shipment of plant pathogens within the United States is regulated by one or more of the following: the USDA and the individual state departments of agriculture, the Public Health Service of the Department of Health and Human Services, and the U.S. Department of Transportation. International shipments are regulated by the Customs Service of the U.S. Department of Treasury and by the U.S. Department of Commerce.

Federal and state laws prohibit—and set criminal penalties (39 CFR 111, *Federal Register* 4 December 1989) for—knowingly mailing conventional plant pathogens between states without the appropriate permits (Form PPQ 526) from the USDA Animal and Plant Health Inspection Service/Plant Protection and Quarantine unit (Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782). Requirements for shipment of genetically modified microorganisms are listed in the 20 April 1988 *Federal Register* (7 CFR 340). Questions about shipping genetically modified microorganisms should be directed to the USDA Biotechnology, Biologics and Environmental Protection office (Federal Building, 6506 Belcrest Road, Hyattsville, MD 20782).

Cultures must be prepared and packaged in accordance with specific stan-

Box 3

How to contact ATCC

Toll-free number in continental United States: 800-638-6597, 8:30 a.m. to 5:30 p.m. EST

In Maryland: 301-881-2600 (collect) between 8:30 a.m. and 4:30 p.m. EST for main switchboard and between 1:30 and 4:30 p.m. EST for technical services

Electronic mail address: Dialcom 142: CDT0109

Telefax: 301-231-5826

Telex: 908768 ATCCROVE for ATCC sales and 898055 ATCCNORTH for director's office



Fig. 3. One method of preserving cultures at ATCC is storage in liquid nitrogen. This technician is removing a canister containing culture vials from a vat of liquid nitrogen.

dards set forth by the Public Health Service, the International Air Transport Association, and the U.S. Department of Transportation. The proper techniques are described in *Packaging & Shipping of Biological Materials at ATCC* (2nd ed.), available from ATCC. Bacterial and fungal cultures are shipped as freeze-dried material, as agar slants, or, rarely, as broth cultures (maximum volume, 5 ml). A few customers request that cultures be shipped in frozen form, which requires use of dry ice and special handling. Plant viruses are usually distributed in freeze-dried plant tissues. Occasionally, a plant virus is supplied as a highly purified preparation and must be shipped on dry ice. Polyclonal antisera are shipped freeze-dried or mixed with glycerol (1:1, v/v), and monoclonal antisera are shipped on dry ice in ascites fluid; both types usually contain an antimicrobial agent, such as sodium azide.

Deaccession of cultures. Department heads and collection managers routinely examine collection holdings to determine their relevance to the scientific community. Taxonomically significant strains, i.e., type, neotype, reference, and genotype strains, must be retained. All others are periodically reviewed for possible "deaccessioning", i.e., discarding. Strains with little scientific or historical significance as judged by documentation data, distribution rate, and other pertinent criteria are candidates for deaccessioning. The same criteria used for accessioning a culture are used for deaccessioning one (see Box 2).

Department heads and collection managers, in consultation with the appropriate advisors, compile a list of deaccession candidates. This list is published in the appropriate catalogue, in the ATCC *Quarterly Newsletter*, and in official newsletters and journals of pertinent scientific societies. After a reasonable waiting period, response from the scientific community is reviewed and a final decision made.

Services

Established in 1949 as a depository for strains that were cited in U.S. patents, ATCC was designated in 1981 as the first International Depository Authority under the International Budapest Treaty (3). In addition to its patent deposit service, ATCC offers a "safety deposit" service to other laboratories for storage of cultures as insurance against loss.

The ATCC catalogues and the *Quarterly Newsletter* (free subscription upon request) list and provide information on available cultures. The ATCC also publishes technical manuals on quality control measures, freezing and freeze-drying, packaging and shipping of biological materials, and specific uses of ATCC strains. The catalogues and manuals serve as general reference documents.

Conferences and training courses are arranged by ATCC in direct response to needs identified by the collection staff or an outside source. Subjects include quality control measures, managing strain data, obtaining patents in biotechnology, and identifying, preserving, and maintaining cultures. The ATCC workshop program provides hands-on laboratory experience in such areas such as cytogenic technology, diagnostic virology, fermentation microbiology, recombinant DNA technology, hybridomas and monoclonal antibody technology, hybridoma data management, DNA sequencing, and polymerase chain reaction (PCR) technology.

Many of ATCC staff with expertise in specialized areas are available for consulting work. They can recommend strains for specific uses; preserving, packaging, and shipping techniques; laboratory practices and quality control procedures; and recording, managing, and administering nonclinical experiments. ATCC scientists can often provide specialized bibliographies to outside investigators. Sponsored visiting scientists are welcome to conduct research of mutual interest.

Funding

At present, the collections in the Bacteriology and the Mycology and Botany departments are partially supported by grants from the National Science Foundation and by a grant from the National Institutes of Health that contributes toward each APAD activity. Neither department receives financial support directly related to their plant pathogen collections. The sale of cultures to scientists in the health, medical, and biotechnology fields helps pay for the APAD activities. In the past decade, the USDA has given partial, but intermittent, support to the collection in the Virology Department. A 2-year grant recently awarded by the National Science Foundation is intended to provide interim support for the plant virus collection until stable, longer-term funding is identified.

Meeting the Challenges

Rapid advances in biotechnology are providing means for detailed molecular characterization of microorganisms and will allow further elucidation of microbial taxonomic and ecological relationships. New strains of microorganisms and viruses are also being produced through genetic engineering. Such modified organisms show specialized characteristics that may necessitate the implementation of new authentication protocols. ATCC has adopted various tools of molecular biology for authenticating cultures and will continue to develop and update its extensive database systems to provide plant pathologists with the most

recent technical information on ATCC cultures.

Culture collections are more than repositories for biological material. Collections are valuable resources for maintaining and distributing standard cultures for comparison and identification purposes, for assembling information on culture use and appropriate model systems for research, and for sharing knowledge with visiting scientists and with colleagues during sponsored workshops and seminars. Because it is difficult to predict which plant pathogens will become important to education, research, agriculture, and industry, culture

collections should be maintained, supported, and developed to meet the challenges of the future.

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Larry L. McDaniel

Dr. McDaniel is an associate collection manager in the Virology Department at ATCC. His primary research interests are in host-pathogen interactions at the cellular level and serological characterization of plant viruses. He received an M.S. degree in plant pathology in 1977 from West Virginia University, where his research involved studies of the nutritional requirements of a mycoparasite. He then entered The Ohio State University and began investigation of a new strain of maize dwarf mosaic virus. He received a Ph.D. degree in 1982 from OSU and remained in a postdoctoral position to study a maize mosaic virus isolate. In 1985 he moved to the University of Florida at Fort Lauderdale to study phytopathogenic mycoplasma-like organisms, and in 1988 he joined ATCC to manage the plant virus collection.



Shung-Chang Jong

Dr. Jong is a senior staff scientist and head of the Mycology and Botany Department at ATCC, where he is in charge of the collections of fungi, yeasts, plant tissues cultures, and seeds. He received a B.S. degree from National Taiwan University and an M.S. degree from Western Illinois University. He joined the staff of ATCC after obtaining his Ph.D. degree in plant pathology at Washington State University in 1969. In 1988, he received the Agricultural Award for International Science and Technology Cooperation from the Ministry of Agriculture in China. He served as a technical advisor to the Department of Agriculture in Bangkok, Thailand, under the USAID Agricultural Technology Transfer Program in 1989 and to the Ministry of Light Industry in Beijing, China, under the UNDP Food Technology Development Program in 1990. Dr. Jong is a fellow of the American Academy of Microbiology and the Washington Academy of Sciences. Currently, he serves on the executive board of the World Federation for Culture Collections and chairs its Committee on Endangered Culture Collections.



Robert L. Gherna

Dr. Gherna is head of the Department of Bacteriology at ATCC. He received an A.B. degree in 1960 and a Ph.D. degree in 1964 from the University of Southern California. His research interests are in systematic bacteriology, sulfur metabolism, and cell wall structure composition of bacteria. He served on the executive board of the United States Federation for Culture Collections during 1975–1979 and as vice-president during 1978–1979. He is a member of the Subcommittee on the Phototrophic Bacteria of the International Committee on Systematic Bacteriology of the International Union of Microbiological Societies.