



AUTHENTICATION OF PROKARYOTES AT ATCC

When a new strain arrives at ATCC, the goal is to ensure it matches the depositor's description, that it is pure and that its classification is consistent with the description. Once the strain becomes a catalog item, our goal is to ensure that the item does not change; subsequent passage of the strain is minimized so that its description is always consistent with the original description. The work done to meet these goals is known as authentication (Figure 1), and here we provide an overview of the authentication process at ATCC.

Every new prokaryote is characterized using a polyphasic approach¹ that elucidates both phenotypic and genotypic traits. The diversity of organisms received by ATCC creates a constant challenge in that the approaches to characterization are driven by the properties of the organisms to be characterized. Thus, the need for specificity must be balanced with the need for efficiency. By using several diverse identification strategies at the phenotypic and genotypic levels that are common to physiologically similar groups of prokaryotes, we have been able to streamline the authentication process while still ensuring thorough characterization of every strain. Even with these refinements there are 15 different polyphasic characterization schema encompassing nearly 250 individual tests that are performed on a routine basis on newly deposited organisms.

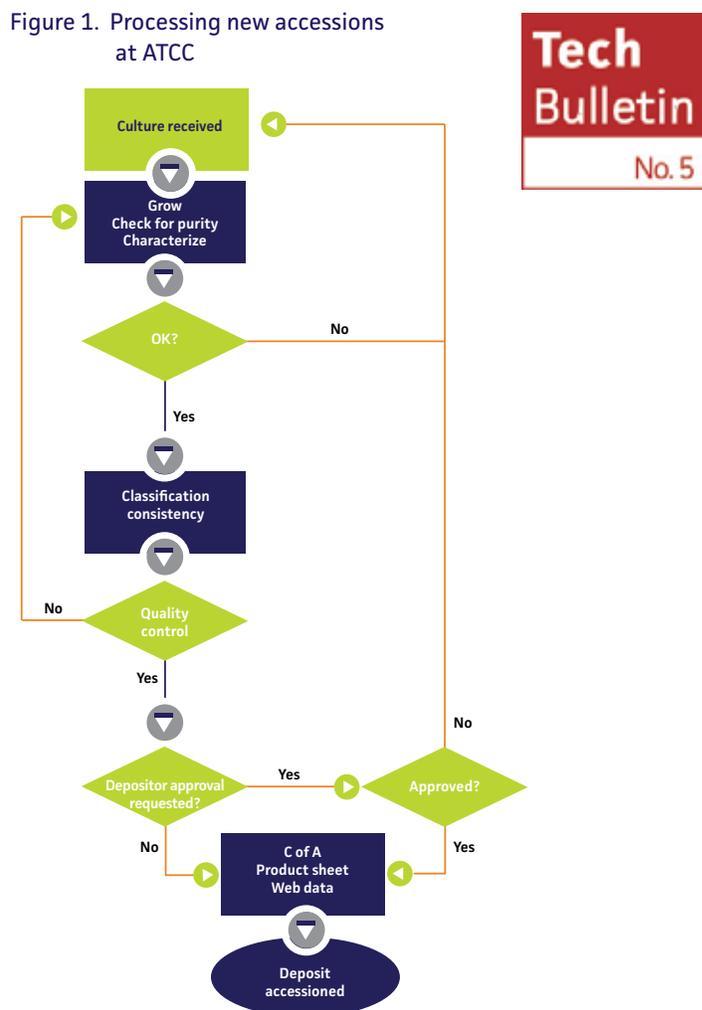
ATCC is an ISO 9001:2008 certified as well as ISO/IEC 17025:2005 and ISO Guide 34:2009 accredited organization and quality control begins when a new strain arrives at ATCC. Testing protocols and results become part of the laboratory record of the strain at each step of the authentication process (Figure 1).

The first step in authentication is to check the growth, purity, and cell and colony morphologies of each culture that arrives at ATCC for deposit. If these match the description given by the depositor, cultures then undergo further characterization to verify the classification of the new deposit.

PHENOTYPIC TESTING

Historically, suites of biochemical and physiological tests were used to gather sets of phenotypic traits and dozens of schema were developed. Numerical taxonomy combined the traits to yield an identity for the organism. The biochemical tests were and still are instrumental for authenticating many important phenotypic properties of ATCC microbes². Standard sets of growth and

Figure 1. Processing new accessions at ATCC



Tech
Bulletin
No. 5

biochemical assays based on the capabilities of the organisms have been developed for different bacterial groups. Commercial rapid test methods commonly found in clinical laboratories such as API® strips and VITEK® 2 cards (bioMérieux VITEK® Inc., Hazelwood, MO) or the Remel RapID™ panels (Thermo Fisher Scientific, Lenexa, KS) are also used. API strips have replaced a number of biochemical tests that had been required in the past and the VITEK 2 Microbial Identification system further automates the quality control process, minimizing manual steps. The Biolog Gen III Microbial ID

System is utilized for analysis of environmental isolates. As new instrumentation is developed, we evaluate these methods, looking for a balance among selectivity, throughput and cost, in an effort to ensure the best quality for our authentication procedures.

We have adopted the Biolog phenotype array system for use in difficult cases where phenotypic data outside the standard schema is required. The Biolog system (Biolog, Inc., Hayward, CA) tests the metabolic phenotype of an organism using a 96-well plate format. Each well contains a different carbon source or chemical sensitivity assay. The test organism is inoculated into the plate and incubated, usually for 24 hours. The pattern of wells that are positive or negative for substrate utilization is then analyzed on a plate reader and matched to a database which provides a presumptive identification for the organism.

We also test specific phenotypes when they are important for the item being deposited. For example, the serology of a clinical isolate may be determined as well as its antibiotic susceptibility profile.



GENOTYPIC CHARACTERIZATION

The repertoire of biochemical tests is very limited for some groups, such as extremophiles or fastidious organisms like *Mycobacteria* or *Actinomycetes* and the development of genotypic methods has been a boon to authentication of these groups of organisms. Genotypic methods can be highly specific and sensitive and are largely independent of the physiological or growth state of the organism. They have become a routine method for identifying and classifying prokaryotes in labs around the world. ATCC sequences the SSU (16S) rDNA gene from all newly deposited strains and subsequent replenishments and checks the sequence against public databases such as the NCBI's GenBank® (www.ncbi.nlm.nih.gov/genbank/) or GreenGenes (greengenes.lbl.gov/) as well as commercial databases such as the ABI MicroSEQ® (Life Technologies Corporation, Carlsbad, CA).

Ribotyping has been a part of our QC process for bacteria for more than a decade. This method—like SSU rDNA sequencing—relies on the ribosomal DNA sequences of an organism and often provides strain resolution. The RiboPrinter® microbial characterization system (DuPont Qualicon, Wilmington, DE) is used to automate the process. The system is comprised of a molecular workstation and a database of fingerprints. DNA is automatically extracted from a single colony, a restriction digest of the chromosomal DNA

is carried out, the restriction fragments are separated by gel electrophoresis, and then blotted to a membrane that is used for Southern blot analysis³. Restriction digest fragments are hybridized to a bacterial probe that is based on the conserved regions of the genes for the ribosomal DNA operon. The result is a DNA fingerprint which is strain specific. *EcoRI* is normally used to digest the DNA, but some genera yield better patterns with *PvuII*, *PstI* or some other restriction enzyme. Each fingerprint is stored in a database so it can be accessed for future comparisons and identifications^{4,5}. The system requires only a single colony as inoculum and there are no restrictions on media and growth conditions. We have tested many genera on the RiboPrinter and found that over 80% yield reproducible riboprints. Riboprinting presents challenges with the *Archaea* and other fastidious environmental strains and prokaryotes.



It is often necessary to go beyond the SSU rDNA gene to identify organisms, or to confirm the presence of a gene or genes of special interest. ATCC has sequenced housekeeping genes such a *gyrA* and *rpoB* for species-strain identification and *hsp60* is used to identify some strains of *Mycobacteria*, for example. Many strains of medical interest carry resistance genes of immediate interest to epidemiologists and other public health scientists. For example, we have sequenced the *mecA* gene from *Staphylococcus aureus* to confirm its presence and the presence of the chromosomal cassette associated with its dissemination. We have also confirmed the presence of carbapenemase genes in *Enterobacteriaceae* including the KPC and the NDM-1 metallo-beta-lactamase.

THE FINISHING TOUCHES

After the initial authentication, the strain is sent to the depositor, if requested, for final approval. This provides further assurance that the strain we received is the strain they deposited. When the item is cleared for distribution, a Certificate of Analysis is produced that outlines the quality control tests performed and results, which assure the identity of the strain. A product sheet is also made for the item. It provides strain information and details the growth conditions and media for optimum recovery of the strain. Finally, the web site information for the organism is assembled which includes links to publications and sequence data plus information on strain designations and the provenance of the item. As the organism is distributed, it is necessary to make new distribution stocks from seed material. Our process is designed so that organisms undergo the minimum number of passages possible, to reduce genetic divergence of the strain.

Authentication of microorganisms is a task we take seriously at ATCC. We continue to use a polyphasic approach that balances traditional phenotypic methods with genotypic technologies to ensure the delivery of high quality microbial strains.

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TB-0711-0-02

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