ATCC

WHITE PAPER

AUTHENTICATION OF PROKARYOTES AT ATCC

Authenticated reference materials are essential for assay reproducibility and data integrity. To ensure the quality of these cultures, it is imperative that they are well-characterized and carefully managed through preservation and storage protocols that maintain the genotype and phenotype. At ATCC, we authenticate and characterize our prokaryotic strains through a polyphasic approach that elucidates both phenotypic and genotypic traits.[1] In an effort to ensure the best quality for our authentication procedures, we are constantly evaluating and adopting novel methods and instrumentation that demonstrate improved levels of sensitivity and specificity. Here, we provide a brief overview on our prokaryotic authentication process.

PHENOTYPIC TESTING

MORPHOLOGY AND PURITY

When a new strain arrives at ATCC, it is our goal to ensure that it matches the depositor's description, that it is pure, and that its classification is consistent with the description. The first step in authentication is to check the growth, purity, and morphology 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.

When evaluating cultures for morphology, we observe attributes at both the cellular and colony level. Bacterial species come in a number of cellular morphologies and aggregations. At ATCC, we use different staining techniques to identify cell wall characteristics, general shape, cellular arrangement, and the presence and localization of flagella. At the colony level, we assess common characteristics such as shape, margin, elevation, size, appearance, optical property, texture, and pigmentation.

BIOCHEMICAL ANALYSES

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. These biochemical tests are still instrumental for authenticating many important phenotypic properties of ATCC microbes; however, with advancements in technology and methodology, we have moved toward using automated and rapid testing methods.

Commercial test methods such as API® strips and VITEK® 2 cards (bioMérieux), the Remel RapID™ panels (Thermo Fisher Scientific), and the Biolog Gen III Microbial ID System (Biolog) are frequently used at ATCC for prokaryotic authentication. API strips and Remel RapID panels have replaced a number of biochemical tests that had been required in the past, and they provide rapid and reliable identification of hundreds of species. The VITEK 2 Microbial Identification system further automates the quality control process while minimizing manual steps, and the Biolog Gen III Microbial ID System is used for the analysis of environmental isolates.

PROTEIN ANALYSES

Another method used for prokaryotic identification at ATCC is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), which is a proteotypic method that identifies proteins using peptide mass matching.[2] Here, the mass-spectral pattern of intact prokaryotic cells is obtained through the analysis of ionized components, creating a unique spectrum that represents the protein profile of each sample. These spectral patterns are then compared to a database of stored profiles from known organisms, allowing for the quick and accurate identification of a species.

ANTIMICROBIAL RESISTANCE TESTING

We also test specific phenotypes—such as the antibiotic susceptibility profile of a clinical isolate—when they are important for the item being deposited. For evaluating antibiotic susceptibility/resistance, we employ a variety of methods such as Kirby-Bauer disk diffusion, the Modified Hodge Test, and VITEK® 2 AST Cards (bioMérieux). Which test(s) is used is dependent on the strain. For example, if the item is considered to be a quality control strain by the Clinical Laboratory Standards Institute (CLIS), we evaluate the strain against antibiotics that are considered to be clinically relevant by CLSI. For these analyses, we use the testing method specified by CLSI. For other items that are characterized as multidrug-resistant, we use VITEK® 2 AST Cards to rapidly evaluate the strain against an array of antibiotics simultaneously. In some cases, if a strain is found to be resistant to a particular antibiotic, we perform an ETEST® (bioMérieux) to determine the minimum inhibitory concentration.

GENOTYPIC CHARACTERIZATION

16S rRNA SEQUENCING

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 the authentication of these groups of organisms. Genotypic methods can be highly specific and sensitive and are largely independent of the physiological state of the organism. They have become a routine method for identifying and classifying prokaryotes in labs around the world. ATCC sequences the 16S rRNA gene from all newly deposited strains and subsequent replenishments and checks the sequence against public databases such as GenBank® , GreenGenes, and the Ribosomal Database Project , as well as commercial databases such as the ABI™ MicroSEQ®.

GENES OF INTEREST

It is often necessary to go beyond the 16S rRNA gene to identify organisms or to confirm the presence of a gene of special interest. For example, ATCC has sequenced housekeeping genes such as gyrA and rpoB for species-strain identification. Many strains of medical interest carry resistance genes or toxin 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 Shiga toxin genes in *Escherichia coli* strains and the presence of toxin A and B genes in *Clostridium difficile* via PCR.

WHOLE-GENOME SEQUENCING

ATCC has started performing whole-genome sequencing on many of our bacterial strains using a standardized genome sequencing, assembly, and annotation pipeline. This method provides an accurate means of microbial species identification and enables the identification of key mutations and clinically relevant genetic markers. Our reference-quality whole-genome sequences and corresponding metadata are publicly available on the ATCC Genome Portal.

DIGITAL DNA-DNA HYBRIDIZATION

ATCC has recently begun to employ digital DNA-DNA hybridization (dDDH) in our taxonomic studies. With older bacterial species that were defined decades ago, the methodologies and technologies used for taxonomic identification were limited. With advancements in genetic testing and sequencing, we can now use technology such as dDDH to re-evaluate the taxonomy of different microbial groups. In a recent study, ATCC scientists used dDDH to evaluate the species of the *Mycobacterium tuberculosis* Complex and discovered that all species within the complex were actually a single species—*M. tuberculosis*.[3]

THE FINISHING TOUCHES

When the item is cleared for distribution, we produce a Certificate of Analysis that outlines the quality control tests performed and their results. A product sheet is also made for the item, which provides strain information and details the growth conditions and media required for optimum recovery of the strain. Finally, the website information for the organism is assembled, which includes links to relevant publications and sequence data in addition to information on strain designation 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, thus reducing genotypic and phenotypic divergence of the strain.

Prokaryotic authentication and characterization is a task we take seriously at ATCC. Our polyphasic approach balances traditional phenotypic methods with genotypic technologies to ensure the delivery of high quality, fully authenticated microbial strains.

REFERENCES

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WP-102021-v03