

## Advanced Techniques in Microbial Identification for Non-Sterile Environments

### *Executive Summary*

Assuring compliance to the regulatory guidelines for microbial monitoring during the production of dietary supplements and probiotics requires a thorough understanding of the regulatory environment and understanding the different methodologies available for microbial identification and differential strain typing. Many nutraceutical products have health related claims and are produced with natural ingredients of botanical or animal origin which are known to have microorganisms present. As a result, regulatory agencies are scrutinizing the application of cGMP quality control measures by the manufacturers to ensure product safety for the consumer. Nutritional supplements, herbal medications and natural lotions and body treatments should be tested to determine the microbiological quality of the raw materials and purity of the final formulations. Manufacturers must confirm that the production processes are sufficiently controlled to prevent contamination and adulteration of the product and production environment. Establishing an environmental monitoring program is one of the most important manufacturing production and process control systems. The program should provide reliable and comprehensive information on the status of environmental quality and the state of control of the facilities. Environmental monitoring programs should specifically detect, identify and quantify microorganisms. The identification of microorganisms can be done by different processes, and these methods have different levels of accuracy and reproducibility with DNA sequencing being recognized as the gold standard for microbial identification. Consistent identification methods will yield data that allow for comprehensive and reliable tracking and trending during routine monitoring of, and investigations into excursions in, the state of control of a production environment.

### *Introduction to the Regulatory Environment*

According to the FDA Code of Federal Regulations 21 Part 111, all companies that manufacture, package, label or warehouse dietary supplements, including those companies involved with importing a dietary supplement for distribution within the United States, must comply with the Dietary Supplement Current Good Manufacturing Practices (cGMP) for quality control<sup>1</sup>. As described in the subsequent subparts of 21 CFR Part 111, these companies are required to establish a production and process control system which covers all stages of the manufacturing, packaging, labeling, and holding or warehousing of the dietary supplement to ensure the quality of the product as specified in the master manufacturing record. Additionally, the manufacturer is to establish a quality system for the manufacturing process control points<sup>1</sup>. Both the Federal Regulations<sup>1</sup> and the Dietary Supplement Health and Education Act of 1994 (DSHEA)<sup>2</sup> state that the manufacturer is responsible for establishing

specifications for identity, purity, strength and composition relating to incoming components, as well as limits on those types of contaminants that may adulterate or lead to adulteration of the finished product. Furthermore, 21 CFR Part 111 states that a manufacturer must verify that the established specifications are met on a subset of finished batches of the dietary supplement<sup>1</sup>. As part of the production and process control system, the methods used for the laboratory operations during testing or examination of the specifications must be appropriate and scientifically valid<sup>1</sup>.

### *Keywords*

21 CFR 111, DSHEA, EM, environmental monitoring, microbial identification, strain typing, nutraceutical, probiotic, genotypic, proteotypic, phenotypic, MLST, trending and tracking

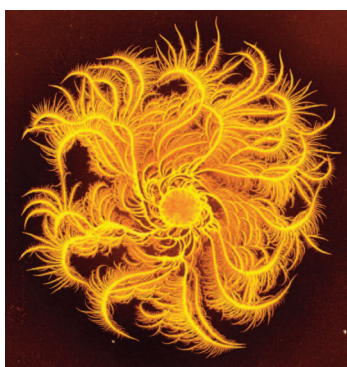
In 2002, the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) published joint Guidelines for the Evaluation of Probiotics in Food<sup>3</sup>. These guidelines should be followed in order to claim that a food has a probiotic effect. They recommend detailed documentation of the probiotic bacterial strain used and statement of the evidence linking a particular strain to a particular health benefit. Documentation should include the identification of the bacteria to the genus, species and subspecies or strain level using the most current, valid methodologies such as genetic testing with 16S rDNA sequencing and using the most current scientifically recognized species nomenclature according to the International Journal of Systematic and Evolutionary Microbiology (IJSEM). In addition, the documentation for a probiotic in food should include the potency or number of viable bacteria per dose and the extent of the research that has been published on health effects for that specific strain. Finally, documentation of the purity of the product is recommended by indicating the presence of contaminating or ineffective bacteria<sup>3</sup>.

Not only do manufacturers of dietary supplements have the responsibility for the documentation of identity, potency and contamination testing on all ingredients and finished products, but they also have substantial requirements for establishing production and process control systems in the manufacturing operations<sup>1</sup>. Manufacturers must take precautions during operations to prevent contamination of components or the final dietary supplement, including performing chemical, microbiological or other testing as necessary. For example, to prevent the use of contaminated components, they must qualify the surface cleaning and sanitizing of product-contact equipment and qualify that the water in the facility does not contaminate the product if it becomes a component of the finished batch of the dietary supplement<sup>1</sup>. The FDA recommends that manufacturers consider identifying areas in processing and production where the risk of microbial contamination is most likely to occur and identify areas where sanitation measures have not been adequate whereby products may become adulterated. Moreover, 21 CFR Part 111 encourages the establishment of a testing program that monitors levels of microorganisms at key places in the facility where the products are processed and produced, such as the surface areas of walls, floors and equipment<sup>1</sup>.

The role of a testing program is to verify compliance to specifications set by the manufacturer and confirm that the manufacturing processes are rigorously controlled to prevent adulteration of the product. Environmental monitoring (EM) is one of the most critical considerations for the establishment of a controlled environment. The EM program should identify the sites at risk of contaminating the product and should facilitate the prioritization of

mitigating the risk. It is a surveillance system that provides a baseline profile of a manufacturing environment and documents consistent quality and control, promptly identifies potential routes of contamination and allows for rapid interventions before product contamination occurs. The objectives of an EM program should be to monitor the effectiveness of cleaning and sanitation through surface monitoring, of gowning and personnel behaviors and to monitor air quality and critical utilities such as water and compressed gasses, focusing on critical control points in the production process. EM programs should specifically detect, quantify and reliably identify organisms including routine flora, organisms of concern and indicator or objectionable organisms. The data gathered in a well-designed and executed EM program provides critical information for tracking and trending, allowing excursions

*Paenibacillus gluconolyticus*



from normal operating conditions to be identified quickly and reliably. Tracking and trending will show 1) if the routine flora has shifted in incidence or number, potentially signifying an adverse trend or a shift in the state of control, 2) if an indicator organism or organism of concern is identified and whose presence and number point to an increased risk of harm or 3) if an objectionable organism that can cause disease, inactivate or lead to deterioration of the product has appeared in the manufacturing environment.

Manufacturing processes are a series of steps which are interlinked while still being independent. All processes have variability. The goal of a production and process control quality system is to understand and reduce the variation and therefore risk. Inaccurate and inconsistent microbial identifications arising from the EM and tracking and trending program can lead to a false sense of control and potentially inappropriate remediation actions. A better understanding of the manufacturing process from the raw materials to the finished product with respect to microbiological quality is gained through an EM program comprising accurate microbial identification and proactive microbial tracking and trending.

### **Methods Available for Microbial Identification**

During the manufacturing of dietary supplements, nutraceuticals and personal care and cosmetic products, the presence of bacteria, filamentous fungi and yeasts are usually a cause for concern. A well-designed EM program should detect the presence of these microorganisms before product contamination occurs. When bacterial or fungal isolates are recovered from a production facility, it is extremely important to be able to accurately identify the organism to the species, and possibly strain level, in order to track the potential origin of the contamination and avoid delays in product release or to complete investigations.

Accuracy of identification is key and dependent on the method used to generate and interpret the data as well as the library database used as a reference. Accurate microbial identification requires significant and continuous process refinements, familiarity and expertise in interpreting data, consistent qualified methods of analysis and timely updates to organism libraries. Regardless of the method used in generating a microbial identification, this information is only as reliable, or accurate, as the reference libraries used for comparison. In order to correctly identify a large percentage of the unknown isolates in EM programs, the libraries must contain all species relevant to the manufacturing environment<sup>4</sup>. Additionally, it is equally critical to continuously update libraries and incorporate new library entries to stay current with an ever evolving microbial world, where taxonomic changes and novel species are described daily. If the library lacks depth of coverage, the interpretation of the data may be inaccurate and unreliable.

### Genotypic Identification

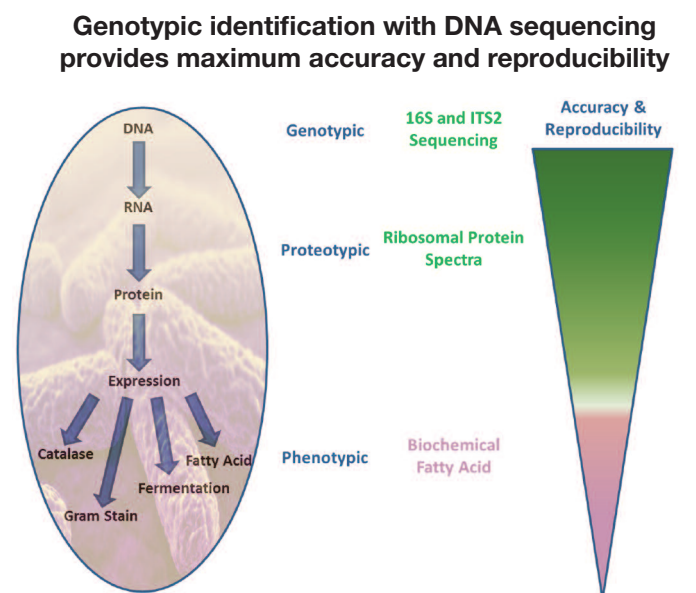
According to the FDA<sup>1</sup> and the FAO<sup>3</sup> guidelines, microbial monitoring of critical components, areas and personnel should include routine identification of isolates to the species level. Three broad categories of identification methods are currently used in commercial settings: genotypic, proteotypic and phenotypic (Figure 1). The FDA states that “genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic techniques. These methods are especially valuable for investigations into failures (e.g., sterility test; media fill contamination)”<sup>5</sup>. Genotypic identification methods involve sequencing different regions of the microorganisms’ ribosomal RNA genes (16S, ITS or D2), resulting in an identification to the species or occasionally the subspecies level. An emerging proteotypic technology in commercial bacterial identification for EM programs is that of matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry which uses a ribosomal protein spectral fingerprint to identify bacteria to the species level. Finally, phenotypic technologies utilize either biochemical reactions or cell-surface component compositions to identify a microorganism. Each technology has advantages and disadvantages. The key is to balance the cost effectiveness of the technologies and to maintain a high level of accurate and reproducible identifications while adhering to manufacturing guidelines and maximizing the state of control of a production environment.

Accurate identification of bacterial and fungal isolates is critical to environmental monitoring programs. For microbial identification of unknown organisms, it is known that DNA sequencing rapidly provides data that are substantially more accurate, robust and reproducible than relying solely on visual phenotypic characteristics, since the sequence-based result is not dependent on age and health of an organism, growth conditions or ancillary testing. In fact, samples can be viable or nonviable cultures or simply genomic DNA material collected from the microbe. Genotypic methods use comparative sequencing

of the ribosomal RNA (rRNA) region. The use of ribosomal DNA sequences for the purposes of microbial taxonomic classification has been in practice for many decades because the technology is inherently stable and thus allows for reproducible data for classification as well as for identification. In all living things, the ribosome, the organelle that is the site of protein synthesis in the cell, contains different sized rRNAs which are transcribed from ribosomal operons in the organism’s genome. The rRNAs fold into elaborate three dimensional structures and are incorporated into an intricate protein-RNA complex that is critical for the ribosomal function and cell survival.

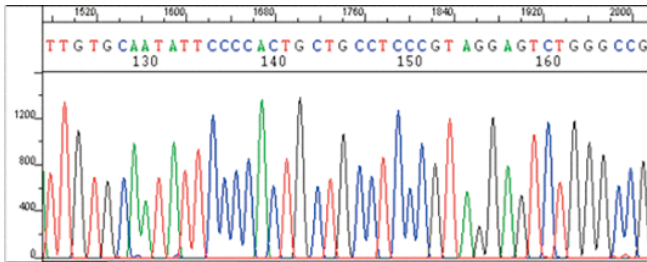
Bacterial isolates are identified using the 16S rDNA region as it is universally distributed among bacteria and contains species-specific variable regions. The identification of fungi, especially filamentous fungi, has historically been a very difficult task. Due to the amount of experience and time required to accurately identify filamentous fungi to the species level, it has been common practice to settle with either identifying these organisms to the genus level, or in some cases, simply identify them as “molds.” Genetic approaches to fungal identification provide a more acceptable, reliable and rapid method for identification to the species level. The internal transcribed spacer (ITS2) region is the ribosomal DNA region that is sequenced by fungal taxonomists, because the ITS2 region has a higher degree of variation between closely related species than other rDNA regions in fungi, such as the D2 region of the large subunit of the ribosome<sup>6</sup>.

To perform bacterial or fungal genotypic identifications, the target regions of the rRNA genes are amplified by PCR and sequenced. The sequence data are then analyzed and aligned in order of increasing genetic distance to relevant sequences against a library database to achieve an identity match. Since interpretation of the data and the database against which the sequence is compared are both essential parts of the identification process, it is important to not neglect the method of data analysis and library coverage

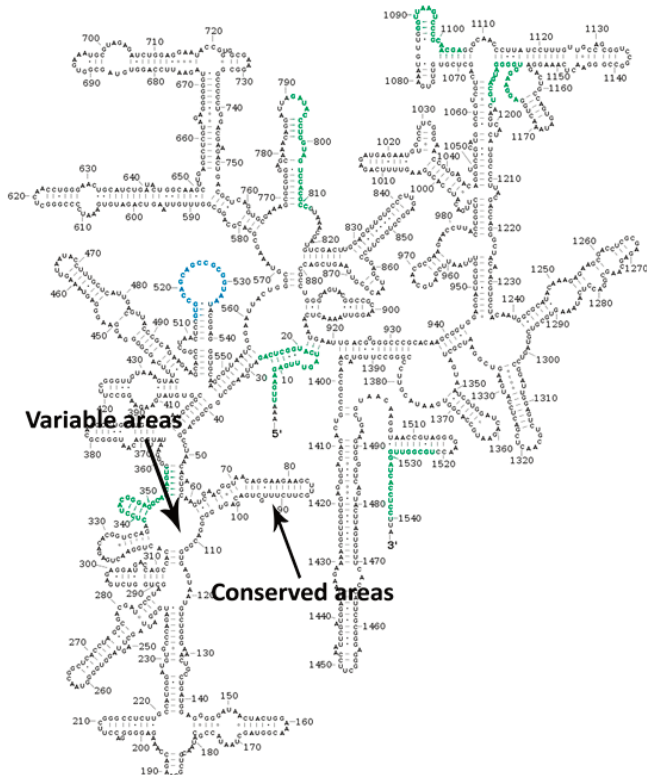


when choosing an identification system or provider. Differences between systems include fully automated or manual interpretation of the data. Manual data analysis and alignment are extremely repeatable and result in the ability to correct for standard sequencing anomalies and sequence variations that can cause the data to appear to be mixed or of poor quality. Poor quality data are usually truncated in fully automated analysis programs which can lead to faulty interpretation of the data and incorrect identification of EM isolates or of bioburden samples taken from final product.

**DNA sequencing chromatogram**



**16S ribosomal RNA structure for *Escherichia coli***



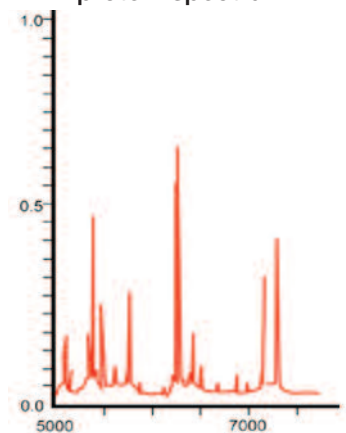
**Proteotypic Identification**

In proteomics, a field devoted to studying the full set of proteins encoded by the genome, “proteotypic” describes a peptide sequence that serves to identify a particular protein. In microbial identification, “proteotypic” is used to indicate a protein spectrum that serves to identify a particular microorganism. For industries that are required to routinely identify microorganisms, the ideal technology is one that is accurate, reproducible, fast and inexpensive. MALDI-TOF mass spectrometry exhibits all these traits. This proteotypic method has demonstrated increased accuracy and reproducibility over phenotypic systems<sup>7</sup>. The analysis of a bacterial sample results in a unique protein spectral fingerprint which is then compared to a validated database for identification. The spectrum is composed of the ribosomal proteins of the bacteria which are translated from DNA but are not subject to the expression variability seen in phenotypic methods. These ribosomal protein molecules are constitutively expressed and structural, so they are consistently present in the cell in very high levels.

MALDI-TOF analyzes a small amount of bacterial sample from a fresh culture which is suspended in a solution containing a matrix and then dried to a target plate. The plate is placed in the vacuum source chamber and irradiated with a pulsed laser beam. The ionization process utilizes the matrix to absorb laser energy while protecting the proteins and transferring ions to the intact molecules. The proteins are released from the matrix and enter the gaseous state as charged molecules. The ions, accelerated by electric fields, are then separated based on the time it takes them to travel a specified distance – time of flight (TOF).

The lower the mass of the ion, the faster it will reach the detector. The protein spectrum or fingerprint that emerges from this process is then compared to a library of spectra from known bacteria. For effective identification, the library needs to contain relevant organisms found in manufacturing environments and must be continually updated to include novel organisms and taxonomic changes. For instances when full sequence data are not required, such as for routine monitoring, but when a repeatable, accurate, cost effective identification is still in demand, the MALDI-TOF technology is a good alternative when supported by a robust, relevant library for EM programs. However, because the technology is in its infancy

**MALDI-TOF ribosomal protein spectrum**



for identifications of microorganisms found in a manufacturing environment with respect to library database development, a polyphasic approach using the proven method of rDNA sequencing is recommended when an identification cannot be made by MALDI-TOF.

## ***Phenotypic Identification***

There are multiple systems which utilize phenotypic characterization to obtain a microbial identification. These systems have been in use for decades and differentiate between organisms based on the results of biochemical tests such as sugar fermentation or physiological properties such as salt or pH tolerance. One phenotypic method provides microbial identification based on cellular fatty acids that are extracted and methylated. The resultant methyl esters are separated by gas chromatography and their patterns are compared against a database. All of these phenotypic systems require a healthy organism, and depending on the technology utilized, may or may not need to be cultured on specific media and require ancillary testing such as Gram stain to achieve an identification. The systems tend to be easy to use and have high throughput and have been, and continue to be, primarily used in clinical settings. However, microorganisms isolated from manufacturing environments on compendial media will likely be physiologically stressed and may not fully express their phenotypic or biochemical characteristics resulting in erroneous identification. For many organisms, traditional phenotypic identification is problematic. Identification can be dependent on media and temperature used to grow the organism and can lead to subjective interpretation of the test results and a higher rate of inaccurate identifications. Not all strains within a given species consistently exhibit a particular characteristic, thereby limiting phenotypic identification methods. Additionally, library databases utilized in support of phenotypic identification are often limited and geared towards clinical isolates.

Despite these major shortcomings that phenotypic processes have in making accurate identifications, these methods still have a role in a microbial quality program. The results from the phenotypic reactions can help determine the biochemical activity of an organism on the product and the resulting stability of the product in the presence of that organism. Understanding the nutrient requirements of an organism can provide insight into controlling or eliminating the organism in the environment or preserving the product.

“Some bacteria are difficult to identify with phenotypic identification schemes commonly used by outside reference laboratories. 16S ribosomal DNA (rDNA)-based identification of bacteria potentially offers a useful alternative when phenotypic characterization methods fail.”<sup>8</sup>

## ***Summary of Identification Methods***

Current available methods of identification range from genotypic and proteotypic to phenotypic, with 16S and ITS2 sequencing being the gold standards for bacterial and fungal identification, respectively, especially when combined with reference quality interpretation methods and curated libraries focused on organisms relevant to the dietary supplements, personal care products and other manufacturing industries. The introduction of the proteotypic MALDI-TOF-based method of identification provides an additional highly accurate, fast and inexpensive option for routine monitoring programs. When identifications are based on phenotypic characteristics, such as with biochemical analysis, the methods are more error prone, variable and subjective.

An EM program should reproducibly detect microorganisms in order to effectively monitor the state of control in the environment. Consistent methods will yield an identification history that allows for comprehensive data comparison and interpretations. DNA sequencing provides the most consistent and unambiguous data set that is reproducible from lab to lab and over time. Genotypic and proteotypic identification are unlike phenotypic which can be affected by differential gene expression resulting in variable characteristics. Additionally, the DNA sequence is stable and unchanging and is a tool for identification as well as a unique descriptor for the microorganism that can be used for tracking and trending.

## ***Methods Available for Microbial Characterization or Strain Typing***

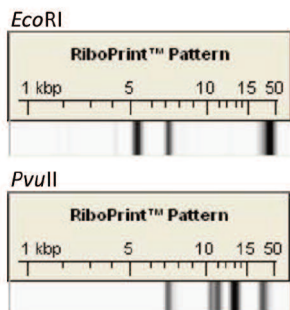
Strain typing or characterization of microorganisms in industrial settings, such as the dietary supplement manufacturing industry, is an important part of a comprehensive environmental monitoring program. Of equal importance to the probiotics and nutraceutical industries is the confirmation of specific bacterial strains used for production. While standard genotypic and proteotypic identification methods increase the ability to accurately and consistently identify and track and trend microorganisms at the species-level, some common microbes cannot be resolved with these approaches alone. Furthermore, strain typing is the best resource in case of a major excursion or sterility failure where characterization to the strain level can be critical.

Subspecies level identification or strain typing of microorganisms, as well as discrimination between closely-related species, is a challenging goal but necessary since this analysis is very important in investigating a root cause. Some of the common approaches used to differentiate closely-related strains compare organisms by considering their genotypic, phenotypic, serological, spatial or temporal characteristics. While the combination of these traits can result in subspecies level identifications, the analysis of multiple characteristics increases the time, labor and expense needed to differentiate isolates, as well as increases the errors that can arise from qualitative and

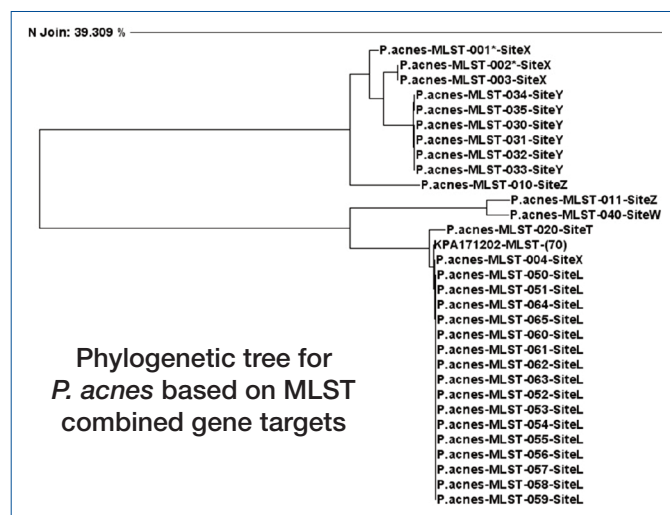
subjective analyses. There are two accepted methods used to accurately and reproducibly differentiate closely-related microorganisms, ribotyping and single- and multi-locus sequence typing (S/MLST).

Ribotyping is an automated process for characterization of bacterial isolates and assesses the genetic relationship between strains of the same species. This fragment-based technology utilizes restriction enzymes (typically *EcoRI* and *PvuII*) to target and cut regions of the ribosomal RNA genes. The DNA fragments are resolved by gel electrophoresis and chemiluminescent probes are used to visualize the banding pattern, resulting in a unique fingerprint of the bacterium. The pattern or fingerprint is compared to others in a database and assigned to a RiboGroup for characterization. This fingerprint can be used for tracking at the subspecies or strain level. These patterns can also be generated from different samples at different times. A historical record can be generated of the operational environment and be used to determine the level of similarity or differences between the historical isolates and the current isolates as part of an investigation to track the source of contamination.

**Banding patterns generated from automated ribotyping after digestion with *EcoRI* and *PvuII***



A more powerful method is emerging in the commercial setting that has increased discrimination at the strain level. This increased resolution is achieved by looking at highly variable single or multiple loci (regions) in the genome. By combining standard genotypic identification methods, such as 16S sequence-based analysis, with multi-locus sequence typing (MLST) or single-locus sequence typing (SLST), it is possible to resolve some of the most difficult organisms to trend and track in the dietary supplement, nutraceutical, and personal care and cosmetics industries.



SLST and MLST are well-established, highly accurate sequence-based methods that can be used to distinguish closely-related microorganisms providing data that resolves differences and/or similarities at the subspecies or strain level. Since the foundation of S/MLST is built upon DNA sequencing results, which can be easily cataloged and referenced, these techniques are highly reproducible, unambiguous and scalable. Given the reproducibility of S/MLST between experiments, and over time, these methods can be used to determine if isolates recovered from one area are the same or different as another isolate – a trait that allows for high-resolution trending and tracking projects.

S/MLST methods involve sequencing one to ten target genes outside of the standard regions sequenced for microbial identification (16S, ITS2 or D2). These target genes are known to harbor moderately to highly variable DNA sequences and are protein-coding or housekeeping genes which encode for the proteins necessary for the normal cellular functions of the bacteria. By using the gene sequence, as opposed to the gene product as in enzyme electrophoresis, more variation can be detected resulting in more potential differences per locus. The addition of multiple loci provides added variation to further differentiate between closely related strains. After sequencing, all the sequences from the multiple gene targets are concatenated (placed end-to-end), aligned and compared to sequences from other organisms. This comparison enables the level of divergence/conservation between microorganisms to be calculated and displayed with an evolutionary phylogenetic tree. The goal is to determine gene combinations that can give a high level of variability to differentiate to the strain or subspecies level. The subspecies or strain level identification makes it possible to definitively track the source of contamination or to verify production strains with certainty.

### **Impact of Identification and Strain Tracking Technologies on Trending and Tracking**

New regulatory guidelines for the manufacturing of dietary supplements and other nutraceutical, cosmetics and personal care products require testing to determine the microbiological quality of the raw materials, purity of the final product and establishment of a quality program, assuring that the manufacturing process is sufficiently controlled to prevent contamination or adulteration of the final product and production environment<sup>1-4</sup>.

Thorough, accurate and reliable identification of the microbial population in the raw materials, final product and manufacturing environment allows for rapid and definitive resolution of sterility failures, alerts and other excursions. Data from EM sample sites should reflect operational considerations and should be proactively used to create tracking and trending reports on a frequent and routine basis, providing detailed analysis regarding the state of environmental control within the manufacturing area. Any significant change in microbial flora should be considered in a review of the ongoing monitoring data and used in investigations of excursions to affect the mitigation process and root cause determination.

Utilizing the most accurate microbial identification methods while conducting gridding studies of the manufacturing plant and performing routine screenings allows reoccurring excursions of the same organism to be recognized and identified. Likewise, an increase in the number of microorganisms recovered in certain areas of the facility may indicate breaches in the HVAC system or other sources of microbial contamination. A recognized shift in the types of organisms recovered from the area may be helpful in locating the source of contamination. EM data can be compared against product bioburden data, and the results of the comparison can impact product development, process validation, production and an uninterrupted supply chain. Assurance of product quality through timely management and review of EM trending data can help to maintain a state of control of the manufacturing process and facilities.

Reliable microbial identification systems are required to give consistent and accurate results for tracking and trending. Identification methods which provide inconsistent identifications or no identification for the same isolate are not useful for tracking isolates to their source or for generating trending reports and can lead to misdirected remediation efforts. Additionally, if a health claim is made for a probiotic, the Latin name (i.e., genus, species and strain designation) of the probiotic microorganism that is the subject of the claim should be declared<sup>3</sup>. Thus, consistent strain typing is critical for the probiotics industry to assure that the exact strain of the microorganism in production is established.

Genotypic methods for identification are based on the rDNA of an organism, which may exhibit but one nucleotide mutation every three million years. The DNA sequence is an unchanging descriptor of the organism and provides a superior tool for tracking and trending. By employing genotypic methods for a monitoring program, sequences will be obtained for the microbial ecology, and if a name changes due to developments in taxonomy that reclassify an organism, the organism can still be tracked since the sequence will not have changed.

If a major excursion occurs, a sterility failure with a product on hold or production that ceases, a thorough characterization of the microbial population in the manufacturing environment through strain typing is the preferred method for sourcing the contaminant. Investigative programs that only utilize the genus and species name of an organism may not provide enough information to make definitive conclusions in an investigation. The species name alone may not provide the appropriate evidence to develop a response to a contamination event if there is the potential for multiple strains as sources for that contamination. Many times it is necessary to differentiate microbial flora to the sub-species or strain level to definitively determine the source of contamination. Once a thorough genetic description is generated, it can contribute to determining a root cause, thus allowing remediation of the situation and determination of a corrective action plan in a timelier manner.

## Conclusions

Assuring compliance to the regulatory guidelines for microbial monitoring during the production of dietary supplements and probiotics requires accurate identification of the organisms in the microbial environment of the production facility and in the components of the product. Accuracy of identification is key and dependent on the method used to generate and interpret the data as well as the database used as reference. Genotypic methods are recognized as the gold standard for identification and strain typing, but the novel mass spectrometry-based technology provides a sound alternative for routine monitoring with accurate bacterial identification to the species level. When these consistent, qualified methods are combined with relevant, up-to-date, validated libraries, identification of organisms from the manufacturing environment to the species or strain level is unsurpassed. By using dependable methods to obtain identifications, you can be certain that tracking the organisms in your environment, or the organisms that are your product, will be accurate and consistent and aid in documenting control of the production environment and leading to overall brand protection and consumer confidence.

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## Glossary of Terms

**Environmental monitoring** - an established program that demonstrates control of a manufacturing environment in compliance with current Good Manufacturing Practices (cGMPs), providing a baseline microbial profile of the facility and allowing for tracking and trending to source contaminations or excursions.

**Fatty Acid** - a long unbranched chain of carbon and hydrogen molecules that has a terminal carboxylic acid group.

**Genome** - an organism's complete set of hereditary information, including both the genes and the non-coding sequences of the DNA/RNA.

**Genotypic** - an identification method using DNA sequence data or fragment analysis.

**Library/Database** - a reference database with known microbial entries used to compare against unknown microbial data for identifications.

**Locus** - (plural loci) the position of a gene or other significant sequence on a chromosome.

**MALDI-TOF** (matrix-assisted laser desorption/ionization-time of flight) - a mass spectrometry method for analysis of complex protein samples, results in a spectrum (plural spectra) of protein peaks.

**MLST/SLST** (multi or single-locus sequence typing) - a technique to characterize the evolutionary relatedness between isolates of microbial species using highly variable DNA sequences.

**Operon** - a functioning unit of genomic DNA containing a cluster of genes the control of a single regulatory signal.

**Phenotypic** - an identification method using biochemical or physiological data.

**Phylogenetic** - the evolutionary relatedness between groups of organisms.

**Phylogenetic tree** (evolutionary tree) - a branching diagram showing the inferred evolutionary relationships between study organisms based upon similarities and differences in their genetic characteristics.

**Polymerase Chain Reaction (PCR)** - a method that results in millions of copies of a specific piece of DNA through a series of repetitive enzymatic reactions.

**Polyphasic** - a method consisting of two or more phases.

**Proteotypic** - a bacterial identification method that uses a ribosomal protein spectral fingerprint.

**rDNA** - the DNA that encode for the ribosomal RNA subunits in an organisms.

**rRNA** - the RNA sequence transcribed from the rDNA and assembles into the ribosomal complex.

**Ribosome** - the organelle responsible for protein synthesis, consists of multiple RNAs and Proteins assembled into a complex.

**Ribotyping** - a method of making a DNA fingerprint of the bacterial ribosomal DNA region for strain characterization.



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