Scientific Contributions

Gene Sequencing: Gold Standard in Culture Identification

by John Williams Jr.

It is estimated that one-quarter of the world's food supply is lost to food spoilage every year. Spoilage bacteria, yeast and mold are most commonly associated with food decomposition. To offset economic losses and brand damage triggered by microbial spoilage, companies are continuously pursuing practical, scientific remedies to assure product quality.

Recent and revolutionary advances in culture identification are providing food processors and manufacturers with insightful and valuable information to safeguard their products and seize new opportunities in our global marketplace.

Type Casting
Culture identification is almost as old as microbiology itself and provides companies with crucial information to prevent food spoilage, improve product formulations, determine microbial agents, validate processes, and pinpoint environmental control problem areas in the plant environment. Today, two types of tests are used to identify microorganisms: phenotypic and genotypic.

Phenotypic Identification
Phenotypic tests focus on the observable physical or biochemical characteristics of an organism. These include biochemical tests, such as carbon utilization tests, and morphological tests (colony and cell size and shape). These are referred to as the conventional or traditional methods of identification; however, in a time-crunched industry, these methods have some limitations.

"Morphological characterizations require a great deal of expertise," explains Dr. Erdogan Ceylan, Operations Manager at the Silliker, Inc. Research Center in South Holland, IL.

"Mold colonies can be quite difficult to identify, especially if you are a novice. In addition, hundreds of bacteria and even yeast can look similar under the microscope."

When there are few unique morphological characteristics exhibited by a microorganism, biochemical tests are used in identification. "The limits, however, are that they are often not highly reproducible. Not all organisms of the same type exhibit the same biochemical patterns either," says Dr. Ceylan.

"Biochemical tests require a reference to compare the unknown against. If you are identifying a large number of microorganisms, you need to perform a larger amount of tests that can be difficult to standardize, require a larger number of reagents, and increase the amount of time it takes to identify the organism."

Many food companies use miniaturized test kits offered by commercial companies for identification. These kits are often easy to use, quick and standardized. However, they are geared for a limited number of organisms and it is difficult to create and expand a database of identified organisms using these kits, according to Dr. Ceylan.

Incubation time poses one of the main limitations for the conventional identification methods. Yeast, for

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example, can require up to four weeks for colonies to develop. The same is true for molds.

Genotypic Identification
Given the limitations of phenotypic identification, genotypic methods are garnering increased attention in the food industry.

"With the genotypic methods, you are looking at the DNA, so the information will not change," says Prof. Daniel Y.C. Fung, Ph.D., Department of Animal Sciences and Industry, Kansas State University in Manhattan, KS. He adds that from a scientific standpoint genotypic methods are more stable.

Genotypic systems are flourishing and several manufacturers, including Amersham Biosciences, LI-COR, Beckman Coulter, MJ Research Inc., and Applied Biosystems, are leading technological advances in this area.

Gold Standard
Silliker has instituted Applied Biosystems' MicroSeq System as its primary methodology for spoilage bacteria, yeast and mold identification. The automated Microseq system is a phylogenetic approach based on ribosomal RNA gene sequences.

In general, gene sequencing involves extracting DNA from an organism. In this case, the DNA codes for the ribosomal RNA of the microorganism to be identified. The DNA is denatured until the two strands separate. A primer (small piece of single-stranded DNA) adheres to a specified location on the single strand and is elongated to a specified point. Various strands of varying lengths of the DNA are made. Gel electrophoresis separates the pieces by size and measures them. From this, the DNA sequence can be read.

"We have turned to gene sequencing because it is basically the same test procedure for all types of organisms," says Dr. Ceylan. "You don't need a variety of biochemical tests to identify an organism. Whether it is a yeast, mold or spoilage bacteria, the same procedure for each one takes only a few days to perform."

The MicroSeq System amplifies and sequences the 16s ribosomal RNA genes of microorganisms, which are universal for spoilage bacteria, yeast and mold. It requires only one polymerase chain reaction and two sequencing reactions to generate sufficient information to identify most organisms accurately to the genus / species level. The system can sequence the ribosomal DNA whether the original cells are dead or alive.

Organisms that are difficult to grow, biochemically inert, highly unusual, or even new and previously uncharacterized, can be identified or classified.

After sequencing, the MicroSeq system identifies the sample to its closest genetic relative using a dedicated software program that contains an expansive database. Gene sequencing is now considered the "gold standard" for microbial identifications and more than 90% of new species are defined by this method.

So, what are the main advantages of gene sequencing? According to industry experts, it offers better reproducibility and more accurately identifies organisms in shorter time frames than traditional phenotypic methods.

"The biggest advantage," states Dr. Ceylan, "is the faster generation of data, particularly for yeast and molds. Turnaround times for these organisms have been reduced drastically from weeks to a few days."

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