Fast action is needed in the event of microbial contamination. Colin Booth, vice-president of science and technology at Oxoid, explains how new ribotyping technology can achieve faster identification and corrective action.

Pinpointing microbial contamination

In many cases of food or pharmaceutical production, contamination problems in the final product may be the first indication that there has been a breakdown in process control. Such an event has enormous financial implications for the company as production ceases, contaminated product is destroyed, and time and resources are ploughed into identifying and correcting the problem. The longer this takes, the longer it is until production can resume, with knock-on effects on revenue and demand.

Presented with a final product contaminant, the first questions to arise are “what is it?” and “where did it come from?”. While the QC laboratory sets about answering these questions, full-scale cleaning and sanitising of the entire processing area may be initiated. This untargeted approach may be warranted with some traditional identification methods, but with the availability of automated “ribotyping” technology it is now possible to pinpoint the source of contamination accurately, to focus corrective action precisely to where it’s needed, and to monitor proactively for problem strains in key areas.

Ribotyping is a method for identifying an isolate by obtaining a genetic fingerprint of the bacterial genome that codes for ribosomal ribonucleic acid (rRNA). Restriction enzymes are used to cut the highly conserved rRNA genes, in addition to less conserved flanking genes and intergenic material. The resulting fragments are separated according to their molecular weight and the resulting pattern, or fingerprint, is used to identify the organism by comparing it to established patterns.

It is in the less conserved, flanking genes and intergenic sections of the genome that small variations between strains occur. The inclusion of this material, therefore, allows characterisation of the organism to strain level.

Ribotyping offers advantages over conventional phenotypic methods, since identification is not affected by culture conditions, growth stages or the stress status of the organism. However, traditionally, ribotyping was a manual method, limited primarily to well-equipped specialised laboratories. It was a time-consuming and labour-intensive method, requiring considerable experience and expertise.

Automated analysis

It wasn’t until an automated ribotyping method became available that the full potential of this genetic fingerprinting technique could be realised.

The DuPont Qualicon Riboprinter Microbial Characterisation System (available in Europe and Australia from Oxoid) is a fully automated ribotyping method. By eliminating the need for manual intervention during the ribotyping process, this system removes the potential for operator error and subjectivity, providing an easy-to-use, standardised method that allows ribotyping to be performed in the routine QC laboratory. Unlike manual ribotyping and many phenotypic identification methods, the Riboprinter System offers same day results. This is an enormous advantage in the pharmaceutical and food production industries where delays are costly.

Pure culture samples are added to the system following heat treatment and the addition of a lysing agent. Cell lysis releases the bacterial DNA (see figure 1). The system then processes the samples automatically, performing the following steps:

1. Extraction of ribosomal RNA
2. Amplification of ribosomal RNA
3. Hybridisation of amplified RNA
4. Detection of hybridised RNA
5. Imaging of hybridised RNA

Figure 1: Sample preparation and analysis
**IDENTIFICATION**

- **DNA fragmentation** using restriction enzymes. The primary enzyme used by the RiboPrinter system is EcoR I. However, it is also supplied for Micrococcus, Kocuria, Pseudomonas and Salmonella. Laboratories also have the flexibility to use other restriction enzyme protocols, if necessary.
- **Separation of fragments** according to their size by gel electrophoresis
- **Transfer of fragments** to a moving membrane
- **Hybridisation of the fragment bands**, causing key fragments to chemiluminesce
- **Capture** of the resulting pattern, or RiboPrint image, using a low-light camera, followed by storage of the digitised image
- **Data analysis** compares the RiboPrint pattern against those in the integral DuPont Identification Database (or the user’s own database) to produce a characterisation or identification report.

This automated ribotyping method can process eight isolates within just eight hours. In addition, new batches can be started every two hours, enabling as many as 32 isolates to be loaded in a normal working day.

**Species identification**

As soon as a RiboPrint pattern is obtained, the system compares it to the patterns stored in the integral database. This powerful database contains more than 6400 patterns, from some 200 genera and more than 1400 species that are of special interest to the pharmaceutical and food industries. Proprietary identification algorithms use fragment size, the number of bands and signal intensity to assign a definitive species-level identification.

If the isolate cannot be identified using the integral database, it may still be possible to obtain an identification by comparing the pattern to the user’s Custom Identification Database (populated with data from local strains and externally generated identifications).

Independently of the identification process, the RiboPrinter System automatically characterises each isolate at the strain level. Each RiboPrint pattern is placed in a RiboGroup, which is a set of statistically identical patterns. If the software recognises a new pattern as indistinguishable from an existing pattern in the database, it assigns that new isolate to the same RiboGroup. However, if the pattern does not match an existing RiboGroup, it is stored as a new RiboGroup.

Each time a new sample is added to an existing RiboGroup, the system software incorporates the sample’s pattern data into a new composite pattern representative of the entire group. Subsequent patterns are then compared against the new RiboGroup. The RiboGroup library is dynamic, changing as more isolates are analysed.

This sub-species characterisation allows companies to pinpoint a source of contamination with greater speed, ease and precision than was possible before.

**Food sector case study**

Staphylococcus contamination was found in a ready-to-eat food product. Conventional methods identified the culprit as *Staphylococcus epidermidis*. However, this species was found to be present in many areas around the production site. Potentially, this could have required the entire site to be cleaned and sanitised. Each isolate was analysed using the automated RiboPrinter system. By comparing the RiboGroup patterns, only one isolate was found to be the same strain as the end product contaminant. The source was identified to be the hands of one employee. Simple and effective measures were implemented to correct the problem, avoiding costly closure of the site and further reduction in output.

**Pharma sector case study**

A bacterial contaminant was found in an asthma inhaler formulation during the final stages of clinical trials. Phenotypic methods failed to identify the organism or to find the source of contamination. The trial was halted and each day that went by increased the delay in bringing the product to market.

Turning to the RiboPrinter System for help, within eight hours the culprit was identified as *Enterobacter cloacae* – an organism that had dangerous implications for the target users of the product. Several different strains of *E. cloacae* were found in several of the raw materials, but only one of these strains matched the contaminant in the final product. The source of the problem was found to be the inert carrier for the active ingredient. Once the company switched to a higher grade product, the problem was resolved and trials could be resumed.

FDA-regulated industries must ensure that any system that stores data is compliant with the Code of Federal Regulations for electronic record security (21 CFR Part 11). The Windows-based RiboPrinter software meets this requirement with four levels of security that determine user access to certain features, along with detailed audit trails to track and record any changes to data.

In addition to answering the important questions, “What is it?” and “Where did it come from?”, with speed and precision, the automated ribotyping method also answers the question, “Have we seen it before?”

Each pattern, or fingerprint, is linked to historical data, so that, should a problem organism recur, operators can find out quickly where and when it appeared before. Historical mapping and trend analyses then allow manufacturers to gain a greater understanding of the microbial environment within their facilities. Such knowledge enables them to take a proactive approach in the control of the aseptic environment, allowing them to anticipate problems before they lead to interruptions in production.

The microbial characterisation that is made possible by automated ribotyping, means that, even in the unlikely event that an identification cannot be obtained, the source of contamination can still be found and eliminated. This precise pinpointing of the source of contamination allows more targeted action and ensures that production is resumed as quickly as possible.

**References:**


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