Expanding the scope of foodborne pathogen detection: a genomic approach

Detecting pathogens in foods before they cause harm – whether to human health or the manufacturer's reputation – is an ongoing issue that industry is seeking to address. Valentin Pflüger and Olaf Degen reveal how a new database from Mabritec, based on Bruker technology, introduces a game-changing solution.

FOOD PRODUCT recall highlights the potential risk that commonly consumed foods and drinks can have on human health – and often makes headlines that can be both financially and reputationally damaging for the businesses concerned. Ferrero's urgent recall of *Salmonella*-contaminated Kinder Surprise eggs just before Easter 2022 is a memorable example, with thousands of products being pulled from the shelves in a blaze of unwelcome publicity.¹

With food product recalls costing companies upwards of \$10,000,000 per case,² detecting pathogens in foods before they cause harm is an ongoing issue affecting the industry. While routine screening for known pathogens like *Salmonella* and *Listeria* is a standard quality control (QC) procedure, unknown and potentially dangerous pathogens are more difficult to identify.

The timely confirmation of the presence of foodborne pathogens and other quality measures is critical to enable manufacturers to make informed decisions surrounding food QC and, if necessary, instigate batch withdrawal. Food quality and safety analysis expert company, Mabritec AG, has developed a whole genome sequencing (WGS)-based method – representing an improvement on state-of-the-art matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) technology, originally developed for advanced clinical research – that uses a biomarker-based approach to microorganism identification for the food industry.

Accurate pathogen identification

Accurate microorganism identification is a well-established component of the food manufacturing process to detect foodborne pathogens, where rapid identification and strain typing tests enable food and beverage manufacturers to make quality and safety decisions more quickly.

Species-level microorganism identification has traditionally been

carried out through time-consuming biochemical assays. However, Bruker's molecular mass spectrometer, the MALDI Biotyper®, enables faster and more efficient analysis than conventional assays or lengthy sequencing methods. Based on Bruker's world-leading MALDI-TOF MS technology, the MALDI Biotyper offers a new approach to microorganism identification in the food safety and quality control industries. Its high species coverage and species-level discrimination, fast speed of analysis with results in minutes, and high-throughput capacity continues to outperform traditional biochemical test methods.

Now, the combination of commercial MALDI-TOF MS systems such as the Bruker MALDI Biotyper with Mabritec's WGS technology introduces a paradigm shift in food quality control: moving from a 'fingerprint' or pattern recognition system, to a biomarker approach, based on a predictive model to expand the reference database used in microorganism identification.



Next-Generation-Sequencing meets MALDI-TOF MS



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The hidden bacterial threat

Because bacterial species identification by MALDI-TOF MS is carried out through reference database matching, it is essential that the species of interest are represented.

The exact number of bacterial species on earth is unknown but estimates suggest that there may be several million. To date, more than 16,000 bacterial species have been described, and of those only 1,500 are classified as obligate human pathogens and 450 as facultative human pathogens.³

The number of known bacterial species is constantly increasing as the description of new species and new potential human pathogens continues. The number of novel described species or reclassified species has grown rapidly in the last decade, driven mainly by the introduction of next-generation sequencing (NGS) technology.

As this number grows, food manufacturers are seeking new ways to identify previously unknown species – which is where a large reference database offers a significant advantage.

MALDI-TOF MS meets NGS: from pattern recognition towards a biomarker-based approach

Commercially available MALDI-TOF MS databases, such as the Bruker MALDI Biotyper reference library, typically cover up to 4,200 bacterial species, but identifying rare or novel human pathogens still presents a challenge. There remains a need for a comprehensive and standardised database extension.

Classical 'fingerprint' MALDI-TOF MS databases rely on the acquisition of spectra from reference isolates, usually multiple strains per species and spectra from different growth conditions.

Mabritec has pioneered a new approach: the MABRITECCENTRAL Database. Changing from a fingerprint to a biomarker approach based on the *in silico* prediction of ribosomal proteins from public WGS data means food manufacturers benefit from this vastly »



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The MALDI-TOF MS workflow for identification of bacteria and fungi species

Identification of bacteria, yeasts and moulds plays a crucial role in industrial, environmental and clinical microbiology. Other methods for identification are often timeconsuming or limited to the available assays or cartridges from different suppliers. However, the benefit of MALDI-TOF MS has revolutionised microbiology by enabling rapid and accurate identification of species starting with colony material.

In industry, species identification is vital for quality control and ensuring the safety of various products, such as food, pharmaceuticals and cosmetics. The MALDI Biotyper allows the identification of contaminants in a few minutes, starting from culture, enabling prompt corrective actions and preventing product recalls. Strain collections of starter cultures for fermentation or R&D projects on microbiomes can be managed with MALDI-TOF MS. In addition, the MALDI Biotyper is validated for confirmation of *Salmonella* spp., *Cronobacter* spp., *Listeria* spp., *Listeria monocytogenes* and *Campylobacter* spp. – independent from the food matrix.

In environmental microbiology, the identification of bacteria and fungi is essential for assessing microbial communities, tracking the spread of signature organisms, and in future more bacteria with antimicrobial resistance. We can find complex microbial samples in many samples such as soil, water or air (eg, in industry on simple sedimentation plates in production rooms).

Bruker works with teams around the world to develop innovative solutions and increase its library scope, and we partner with customers using new ideas to combine genomic and proteomic data in one workflow as presented above. When utilising MALDI-TOF MS, customers have the option to create their own databases for species identification. Building an in-house database empowers customers with flexibility. You can always export data to start analysis with third-party databases. Starting with colony material offers distinct advantages: as this technology continues to advance, it promises to enhance data integration and contribute to a deeper understanding of microbiota in various applications.

extended database of 247,000 WGS entries covering more than 16,000 valid bacterial species and more than 8,000 genomospecies. MABRITECCENTRAL increases the microorganism identification rate from 80 percent to 99 percent in the routine laboratory.

The biomarker approach explained

Ribosomal proteins (the dominant signals in a MALDI-TOF MS spectrum) are highly abundant in vegetative cells, making them ideal biomarkers for species delineation.⁴ There are around 55 phylogenetically conserved ribosomal protein families in bacteria with molecular protein weights in the range of 4-20kDa. Mabritec deduced ribosomal proteins by basic local alignment search tool (BLAST) with ribosomal in-house master templates against an in-house genome database and from the National Center for Biotechnology Information (NCBI) identical protein groups. The resulting protein sequences were quality checked and molecular weights were calculated. Predicted masses were validated by MALDI-TOF MS and post-translational modifications (PTMs) were taken into account if the corresponding information was available.

The MABRITECCENTRAL algorithm allows spectral classification over a broad range of spectra qualities in a dynamic mass range of 4-30kDa. However, routine spectra quality can still be a limitation for the discrimination of such groups as is the case for the *Bacillus cereus* group, for example. Spectra acquired with sinapinic acid matrix with an extended mass range of 4-30kDa, as suggested for some closely related species groups, can also be submitted. MABRITECCENTRAL also supports the discrimination of closely related species groups like *Enterobacter cloacae* complex, *Burkholderia cepacia* complex, *Streptococcus mitis* group or the *Bacillus* cereus group.

In general, the minimum spectral quality required for a comprehensive database is slightly higher than for current commercial systems. This is explained by the higher species coverage, which can sometimes lead to multi-species identifications.

Key requirements for successful microorganism identification

There are two main factors affecting a successful identification. Firstly, the technical quality of the spectra has an impact on the quality of the results. Parameters to be monitored include acquisition speed, signal intensity, number of peaks, upper mass range and peak resolution. Secondly, the biological background of the microorganism to be analysed leads to different spectra. Factors like growth time, growth media, and the structural and metabolic differences between Gram+/- aerobes or anaerobes affect spectra quality. For example, the overall number of mass peaks and especially mass peaks above 10kDa varies largely between taxonomic groups.⁵ Some closely related species groups like Salmonella spp., Bacillus cereus group or Cronobacter spp. can currently only be identified on the genus level with conventional MALDI-TOF MS systems.⁶ Improved spectra quality

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 – especially the detection of mass peaks above 10kDa – can be achieved with the combination of protein extraction, sinapinic acid matrix and an adapted acquisition method.⁷⁸

Ongoing increased identification

Complementary to conventional MALDI-TOF MS systems, the MABRITECCENTRAL database improves food pathogen identification across a wide range of microorganism species.

The MABRITECCENTRAL database can be accessed here⁹ for instant online spectral classification.

Valentin Pflüger



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Valentin is co-founder CEO and former CSO of Mabritec AG, a biotechnology company based in Richen, Switzerland. As a microbiologist

with more than 15 years experience in MALDI-TOF MS-based bacterial identification, he is the driving force behind the development projects at Mabritec AG – especially the MABRITECCENTRAL database. In addition, he has conducted research in the field of microbiology and molecular epidemiology, working at the Swiss Tropical and Public Health Institute and at Mabritec AG. Pflüger has co-authored more than 40 publications in peer-reviewed journals.



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For more information about how the Bruker MALDI Biotyper can resolve microorganism identification challenges in the food industry, visit:

www.bruker.com/en/applications/ microbiology-and-diagnostics/ food-beverage-microbiology



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