

Highlights of Analytical Sciences in Switzerland

Division of Analytical Sciences

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When Time-to-Result Matters: Identification of Microbes Based on MALDI-TOF Protein and Peptide Profiling

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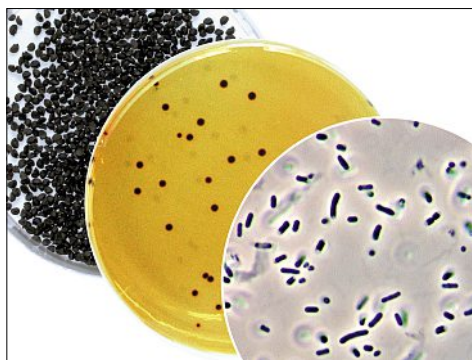
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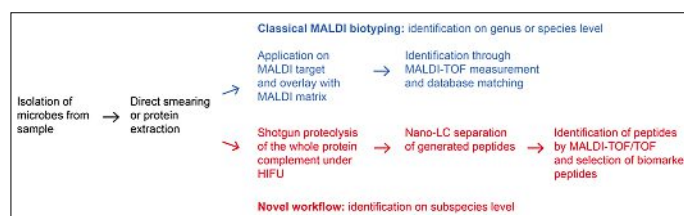
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Rapid and reliable microbial identification is of utmost importance – whether in clinical and veterinary diagnostics or in food safety control and outbreak tracking. Conventional methods assessing phenotypic traits of isolated microbes based on biochemical reactions, Gram staining, colony morphology, or growth pattern are often time-consuming. Molecular biological techniques for assessment of genotypic traits are often expensive. Identification of microbes by MALDI-TOF mass spectrometry (MALDI biotyping), based on profiling of mainly ribosomal proteins and comparison to a reference mass spectra database, has developed from an emerging to a robust leading-edge diagnostic technology and has revolutionized work in microbiological laboratories in recent years. This is due to its short time-to-result, streamlined protocol allowing a cost-effective identification within less than 20 minutes. With MALDI biotyping, an accurate and reliable identification of bacteria and fungi is possible down to genus and species level. For the accurate identification of individual subspecies or serovars, modified methods need to be applied.

In order to further advance the analytical method, the Functional Genomics Center Zurich (FGCZ) and Agroscope have entered a collaboration and developed jointly a novel and ultra-fast workflow that combines the classical MALDI biotyping approach with shotgun trypsin digestion of proteins under High-Intensity-Focused-Ultrasound (HIFU) and subsequent nano-LC separation of resulting peptides. The latter are subsequently identified by MALDI-TOF/TOF mass spectrometry. The use of peptides as biomarkers extends the usable mass range and type of



Onion seeds, agar plate with colonies of *Salmonella* sp. isolated thereof and single *Salmonella* cells under the microscope (from left to right).



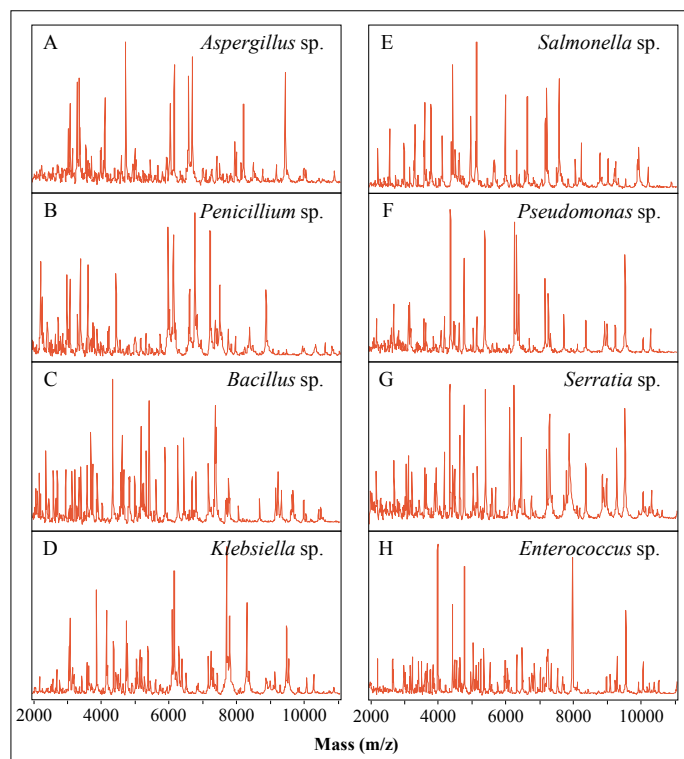
Classical MALDI biotyping and novel workflow.

proteins potentially identified as compared to classical MALDI biotyping, resulting in an increased discrimination power to the subspecies level. The established workflow is currently used for the identification of several pathogenic microbes and will be applied in the near future to selected reaction monitoring (SRM) experiments for an even more rapid identification of microbial strains. **The new combined analytical method will contribute to a more accurate and more sensitive pathogen identification on subspecies level in a multitude of disciplines, such as clinical diagnostics and food safety.**

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References

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MALDI-TOF MS spectra of foodborne spoilage and pathogenic microbes isolated from beans (A), apple juice (B), and onion seeds (C – H).

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