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MALDI-TOF MS identification and tracking of food spoilers and food-borne pathogens

Chris G de Koster¹ and Stanley Brul²

Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) is an established tool for identification of clinical relevant pathogens. MALDI-TOF MS is increasingly accepted as a molecular characterization tool and method for identification of genera, species and isolates of food spoilers and food-borne pathogens in the complex food chain from origin via producers and traders to the consumers. By virtue of its robustness, ease to use, cost per sample, sample throughput MALDI-TOF MS enables tracking and tracing of large sample amounts of food related microbial isolates. Although, food spoiler and foodborne pathogen spectra are underrepresented in commercial available databases the number of entries is rapidly expanding. Instrumental developments such as tandem mass spectrometry with MALDI-TOF/TOF MS instruments enable proteomics approaches for identification of strain specific biomarkers. Multivariate statistics is used to differentiate between spectra of isolates and to correlate clusters to phenotypes. It is envisaged that molecular identification through MALDI-TOF MS will develop into an indispensable tool for tracking and tracing of food spoiling and food-borne pathogenic strains and isolates.

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Introduction

Nowadays consumers demand more and more fresh, exotic and always readily available food products[1]. Such ‘instant availability’ implies that food ingredients as well as finished products often originate from distant and multiple geographic origin and are shipped over the world

[2]. As a result a complex interwoven chain of producers and retailers is between the origin of ingredients at multiple production sites and the consumer [3]. Food spoilers [4] and food-borne pathogens [5,6] can enter the network at multiple nodes. The global food supply network challenges straight forward identification of supply chain entry points of pathogens causing food-borne illnesses. They can be present in ingredients or enter during food processing and packaging. The detection of sources of contamination demands extensive analysis with selective and sensitive identification techniques. Alongside the already mentioned consumer drivers of the food chain current day society asks for food of superior sensory and nutritional quality driven by the wish to prevent disease and to age healthily. Processing conditions that contribute to long lasting microbial stability but detrimentally affect sensory and nutritional food properties are brought back to a minimal extend. Food is hence processed at lower temperatures to avoid loss of organoleptic quality, color deviations and texture. Preservation additives such as sodium, potassium and calcium salts of organic acids and nitrite are usually avoided when a food is to be labeled and sold as a fresh product. All these measures, however, favor microbial growth potentially leading to increased food spoilage and food safety risks. Food spoilage microorganisms produce off-flavor, affect taste, lead to discoloration of food products on the shelf and finally rejection of the product by the consumer. Spoilage leads to losses of food, producers reputations and thus economic value [7]. In addition, foodborne outbreaks of infection illness are a threat for human health. Many foodborne pathogens can impose substantial illness, hospitalizations and even deaths. Human foodborne infections often lead to shut down of the food supply network with serious negative financial consequences for producers. Early, fast and accurate identification of food-spoilers and food-borne pathogens is evidently essential to control and minimize microbiological hazards. In this context subtyping of microbial species is an important tool to attribute food spoilage and outbreaks of food-borne disease to their sources. Such identification of species is the prelude of trouble shooting in a food production environment. Identification and differentiation of strains and isolates is desirable for conclusive actions to tackle outbreak of pathogens [8]. Similar genotypes of bacterial isolates point to a similar source. Identification of isolates can provide information to track and trace them through the food chain from the source of production via processing and suppliers all the way to the consumer [9,10]. For molecular analysis with high specificity and reliability,

Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) is available [11]. This technique is capable of rapid identification of microorganisms at various taxonomic ranks [12,13]. Although the tool is established in clinical microbiology [13,14*,15,16], its acceptance for identification of food spoilers and food-borne pathogens is still challenged [17–23]. This review will address the current status, the art and the potential of MALDI-TOF MS for identification of food spoilers and food-borne pathogens [18].

Phenotypic and molecular characterization of microorganisms

Various phenotypic and molecular approaches have been developed for identification of microorganisms. Phenotypic characterization of microbes is based on staining, colony morphology, microscopic evaluation and differential growth on selective culture media. Generally, phenotypic characterization demands elaborate and time consuming culturing steps. Molecular diagnostics rely mainly on separation of DNA restriction fragments and grouping of patterns thereof or sequencing of 16S ribosomal RNA, DNA sequencing or real-time PCR detection of target genes and MALDI-TOF MS detection of abundant cytosolic proteins [13,24]. Pulsed-field gel electrophoresis (PFGE) separates restriction fragments of genomic DNA. Restriction fragment patterns of isolates are compared and isolates are grouped in pulsotypes. PFGE is a golden standard for the identification of various food-borne pathogens. The PulseNet database is a surveillance network for food-borne disease outbreaks in the USA [25,26]. PCR is one of the most sensitive methods. Identification of microorganisms through PCR requires the selection of genetic markers than can be used to differentiate a large and diverse ensemble of microorganisms. Taxonomic ranks for identification must span a wide range. The success rate for PCR identification depends on the selection of the set of primers. Species for which primers are not selected or available will not be classified. The choice of primers is crucial since primers that are denoted as universal do not always amplify with a similar efficiency. The abundance of amplicons does not necessarily reflect the abundance of microbial species in the food matrix. PCR-based assays often suffer from cross-reactions for phylogenetically closely related species [27]. In bacteria the ribosomal RNA operon comprises variable and conserved sequence domains. This operon consists of a 16S rRNA gene, an internal transcribed spacer region (ITS) and the 23rRNA gene. These sequences are used as molecular markers for the identification of taxa, genera, species and strains. The ITS regions exhibit polymorphic sequences. Bacterial genomes harbor often multiple copies of rRNA operons. Consequently, multiple ITS alleles with highly variable non-coding sequences are present. A strong asset from molecular typing methods such as 16S rRNA sequencing is an amplification step by PCR. This avoids the need for a time consuming step to culture the

microorganisms. This is particularly relevant for situations of outbreaks of food-borne pathogens and where analysis time is critical to avoid costly recall actions of food products. Sequencing of multiple polymorphic loci enables as a fingerprinting method for discrimination between closely related organisms [28]. This multi-locus sequence typing (MLST) is still considered the gold standard for genotyping of many foodborne bacterial pathogens. Finally, next generation sequencing approaches now boast low-costs and hence effectively allows for rapid sequencing of entire genomes. In fact, such whole genome sequencing (WGS) is increasingly used to investigate food-borne outbreaks [10]. A distributed network of laboratories organized in the FDA GenomeTrakr network utilizes whole genome sequencing for pathogen identification. The data of the network are housed in public databases at the National Center for Biotechnology Information (NCBI).

MALDI-TOF MS characterization of microorganisms

While molecular nucleic acid-based identification is based on detection of rRNA sequences such as 16S rRNA and ITS sequencing, MALDI-TOF MS detects predominantly the ribosomal proteins at the protein level. Ribosomal proteins are strongly evolutionary conserved within a genus. At the species level differentiation depends on ribosomal protein sequence divergence [29]. MALDI-TOF MS identification is based on the acquisition of a protein fingerprint of the microorganism [16]. This fingerprint is searched against a library of reference spectra. A workflow consists of sample pretreatment including protein extraction, deposition of the sample with matrix molecules on a target plate, introduction of the plate into a MALDI-TOF mass spectrometer, desorption and ionization of proteins with a laser, mass analysis of the polypeptide ions and database searching of the mass spectra. MALDI-TOF MS detects proteins and consequently requires streaking and colony culturing of the microorganism to obtain a sufficient amount of cells for analysis. The technique is moving toward direct analysis of samples without a culturing step. MALDI is capable of direct identification of human pathogens in a blood culture broth [30]. Furthermore, relatively high bacterial loads of *Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus* can be directly identified in milk omitting a culturing approach or pursuing short culturing times without streaking and colony culturing [31]. The first step is preparation of a sample amendable for MALDI analysis. Sample preparation is known to be a crucial factor that influences abundances of peaks in the spectra. Particularly, protein extraction of microorganisms is a critical step to obtain reproducible results. Various strategies have been exploited for protein extraction [32]. The strategies differ for microorganisms with a thick cell wall and without a thick cell wall and for extraction in the test tube or directly on MALDI target. Proteins are extracted

from cells without a wall with, for example, 50% acetonitrile and 1% aqueous TFA and the debris is spun down [33] or proteins are extracted with acetonitrile, formic acid and water [34]. Yeast proteins are more vigorously extracted from yeast cell pellets with equal volumes of 70% (v/v) formic acid and 100% acetonitrile [35]. The supernatant is deposited on a MALDI target. Protein extractions can be carried out directly on the target plate. Colonies or cell smears from a culture plate are directly transferred to a selected position on an MALDI target plate [19]. Formic acid can be added to cell smears. The addition of formic acid enhances the release of bacterial proteins of particularly Gram-positive species. Enzymes are applied to improve cell wall destruction or cells are disrupted by physical methods such as bead beating, heat and sonification. Deposition of sample or sample pretreatment on target is followed by overlaying a solution of MALDI matrix in acetonitrile and formic acid. Typical MALDI matrices are 2,5-dihydroxybenzoic acid or α -cyano-4-hydroxycinnamic acid. For detection of intact biomarker proteins in a targeted proteomics approach sinapic acid is recommended because of its ionization yield of high mass proteins [36]. The solvent is evaporated at room temperature while the target plate is on the lab table.

The next step is mass analysis of the peptide ions. Because MALDI is a pulsed ionization technique that also can produce high molecular mass ions it is usually coupled with a time of flight mass analyser (TOF). Particularly, ribosomal proteins dominate the MALDI spectrum [37]. Approximately, 50 ribosomal proteins can be detected in the MALDI-TOF MS mass range of m/z 3000–20 000. Besides ribosomal proteins structural proteins such as ribosome modulation factors, carbon storage regulators, cold-shock proteins, DNA binding proteins and RNA chaperones can produce peaks in the MALDI mass spectra [37]. The ribosome constitutes approximately 25% of the bacterial cell mass depending on growth conditions. In fingerprinting MALDI spectra of unknown microorganisms are compared with a library of reference spectra. In a bioinformatics approach proteomics techniques are used to compare biomarker or target protein m/z values with proteomics or genomics databases. A prerequisite is that the genome sequence of the organism is available. Biomarkers are also found by identification of genus, species or strain specific m/z values. Web-based software such as SPECLUST has been used to create a final mass list from a set of spectra and to determine common peak masses in sets of spectra of a taxonomic level [38].

MALDI identification of microorganisms by library searching of spectra requires the following premises: firstly, spectral fingerprints vary between microorganisms. Secondly, the spectra must contain signals that are unique for genera, peaks that specify species and even peaks that

differentiate between strains and isolates. Thirdly, mass spectra of a reference strain or isolate are reproducible and independent of inter and within lab conditions. In a library search approach spectra of identified species and strains are compiled in databases. Specific software is used for comparison of measured fingerprint spectra with reference spectra. A scoring algorithm calculates a measure for the similarity of the unknown profile to available database entries. Multivariate methods such as Principal Component Analysis and hierarchical cluster analysis are used to group spectra of closely related species and to differentiate between strains [39–41]. Library spectra are compiled in commercial databases or released in the format of open-source databases. Reference spectra are compiled in databases by manufacturers of MALDI instrumentation. Two important commercial packages are Biotyper™ and Saramis™. Commercial databases are increasingly expanding with spectra of newly identified organisms and updates are made available by suppliers. The software enable users to build their own databases. Addition of homemade entries, *id est* spectra from reference species, isolates and strains, substantially increase positive identification of microbial species [42]. Reference spectra are also compiled and made available in open-source databases such as SpectraBank, mMASS and MALDIquant [13]. In MALDI fingerprinting of microorganisms spectra should be independent of culture media differences. MALDI ionizes predominantly ribosomal and housekeeping proteins. For ribosomal proteins the effect of growth conditions on the mass spectra and thus on selectivity of identification is expected to be minimal. Furthermore, many genes expressing housekeeping genes are constitutively expressed. Thus *a priori* it is expected that a specific set of constitutively expressed proteins form a MALDI fingerprint regardless of culturing conditions. However, exceptions have been documented. In general, MALDI is with a few exceptions capable of identification of 90–95% of microorganisms at the genus and species taxonomic ranks [12,43]. MALDI as library search method is restricted to analysis of a colony of an organisms. Depending on the species mixed populations up to two or three species can be analyzed. This hampers the profiling of mixtures wherein one species is dominantly present.

Differentiation and identification of strains and isolates

MALDI identification and differentiation of isolates can enable source tracking of food spoilers and food-borne pathogens in a production environment. Culture media can effect the sensitivity for the differentiation at the taxonomic rank of strains and should be evaluated in the course of method development. Various multivariate statistics algorithms are used to cluster spectra and to differentiate between isolates and to correlate isolates with source of isolation. Alternatively multi variate statistics and bioinformatics is used to identify strain or serotype

specific biomarkers as will be exemplified below. The technique has been evaluated as a single identification and source-tracking tool for *Listeria monocytogenes* obtained from different dairy and non-dairy isolates [19]. Because culturing conditions can affect spectral appearance the *L. monocytogenes* isolates were grown on five different media. After 24 hours of incubation more than 90% of the isolates were identified at the genus rank. Species identification varied with the culture media. Successful identification was highest for Agar Listeria Ottaviani Agosti (ALOA) medium, *id est* 91% of 23 *L. monocytogenes* isolates and lowest for Palcam agar (PA) medium, *id est* 50% of 23 species. Chemometric statistical analysis of the mass spectra enabled source tracking of isolates from four different dairy sources [19]. MALDI-TOF MS performed comparably to genotypic methods for *Lactobacilli* identification. The overall success rate for MALDI was higher because several isolates from dairy and meat products belong to other genera than *Lactobacillus* for which no PCR primers were included in the study. The PCR-based assays also suffered from species specific cross reactions [34]. MALDI analysis of *S. aureus* isolates from dairy products and strains and statistical analysis of spectra identified biomarkers specific for *S. aureus*. The isolates covered a wide range of isolation sources. Cluster analysis grouped the strains and isolates into eight groups and can be used as a typing method. The grouping, however, could not be related to the origin of the strains [38].

MALDI-TOF MS fingerprinting appeared to be a rapid and effective analysis tool for the identification of 36 species and strains within the genus *Enterococcus* [33]. All the food isolates could specifically be identified at the species level by matching their mass spectra with the MALDI spectra of reference strains. Interestingly, the spectra have a genus-specific peak at m/z 4426 in common. This peak can be used as a genus specific biomarker. The mass spectrometer and its instrumental characteristics such as mass accuracy and mass resolving power influence the selectivity of identification. Coupling of MALDI with high end and high resolution Fourier Transform Ion Cyclotron Mass Spectrometry (FT-ICR-MS) provides accurate mass measurements with high mass resolving power and dynamic range. Cluster analysis based on MALDI-FT-ICR-MS revealed clustering of *Pseudomonas aeruginosa* isolates into the same three groups as obtained by Amplification Fragment Length Polymorphism (AFLP) data. Interestingly, differentiation between cluster 1 and 2 can be correlated to susceptibility toward ciprofloxacin. Clustering based on MALDI-TOF MS revealed no clear separation compared to AFLP [44**]. Biomarkers for differentiation of strains or serotypes have also been identified. The majority of abundant peaks in a MALDI spectrum arise from ionization of ribosomal proteins. Many of the ribosomal subunit proteins are coded by the highly conserved S10, spc and alpha gene clusters. In a biomarker approach the m/z values of target

gene products are predicted by bioinformatics methods using DNA and protein sequences in genomic databases. The predicted m/z values are compared with the experimental ones to identify ribosomal marker proteins. This biomarker approach has been successfully used by MALDI detection of ribosomal proteins S15 and L25 and acid stress protein H-NS to differentiate between O157, O26 and O111 serogroups of enterohemorrhagic *E. coli* food isolates. A fingerprinting approach to differentiate between these serotypes was less accurate [36].

MALDI identification in the beer brewery

The performance of MALDI is illustrated for identification of microorganisms in a beer manufacturing environment. Various microbial strains cause sediments and haze acidification in a wide assortment of beers such as wheat beer, lager beer and pilsner beers and are notorious beer spoilers. Rapid and reliable differentiation of strains is important for troubleshooting in beer production. Sample preparation must be robust and easy to handle, data acquisition must be straightforward and data-analysis should lead to conclusive actions. Beverage isolates of *Lactobacillus brevis*, *Pediococcus clausenii* and *Leuconostoc mesenteroides* were used to optimize sample preparation with regard to quality of mass spectrometry data. Cell smears are a fast and convenient method for sample preparation of *P. clausenii* and *L. mesenteroides*, but appeared to be less applicable for MALDI identification of *L. brevis*. The influence of culture conditions such as time, availability of oxygen and nutrient media compositions and harvesting at different growth stages on the abundance of signals in the mass spectra do not impair the identification of these species. Although the spectra showed slight differences, all of them were successfully assigned to the correct species through matching with the spectrum of the same strain growing under standard conditions [32]. MALDI-TOF MS analysis has been explored to characterize different *L. brevis* strains. Through cluster analysis mass spectra fingerprints of seventeen *L. brevis* strains have been coupled to their beer spoilage potential [40]. MALDI-TOF analysis successfully assigned 90% of a total of 204 *L. brevis* mass spectra to the correct entry in the database. Among the misclassified mass spectra, the strain TWM 1.1205 was consequently assigned to the strain TMW 1.100 reference spectrum. Interestingly, both MALDI-TOF MS fingerprints and Random Amplification of Polymorphic DNA-PCR patterns showed remarkable similarity. Both isolates exhibited identical growth behavior in beer and similar MIC values for *iso*-alpha hop bitter acids. Comparable results were obtained for two other mismatches. The remaining mismatch is caused by spoor spectrum quality. The spectrum showed a lower total number of picked peaks compared to the spectra that successfully matched with the spectrum of the reference strain. MALDI identification is not restricted to beer-spoiling prokaryotes. Top-fermenting and bottom-fermenting brewer yeast

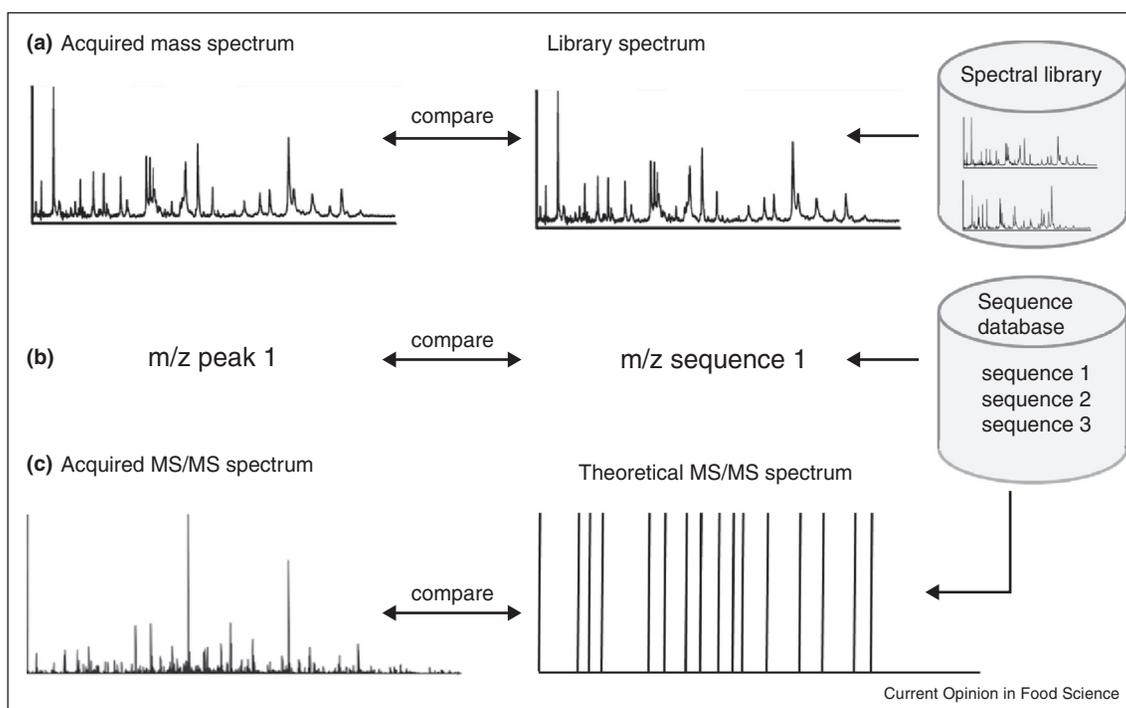
strains and wild yeast *Dekkera/Brettanomyces bruxellensis* were accurately identified after inoculation in beer (Figure 1). In addition, wild yeast contaminations were rapidly detected and differentiated from brewing strains [35]. MALDI-TOF MS spectra of nineteen *Pectinatus* isolates from different breweries were acquired. Principle Component Analysis clusters spectra of *Pectinatus* species. By means of in-house software isolates were differentiated at the subspecies taxonomic rank and in a test 60% could be assigned to the correct reference spectra. Interestingly, isolates from the same brewery group together into the same sub cluster [41]. MALDI-TOF MS is a fast technique to verify the purity of brewer's pitching yeast cultures. Brewing and non-brewing *Saccharomyces cerevisiae* isolates are discriminated by comparing their mass spectra with that of an in-house produced brewer's yeast strain [45]. MALDI enables rapid identification of contaminants from biofilm-covered surfaces on and near machines in a beer filling hall. Isolates were incubated for 24 hours and extracted with formic acid and organic solvent. Almost half of the isolates were identified to the species rank. Interestingly, extension of a commercial database with homemade entries increased the number

of identifications to 89% *Pectinatus* sp. are associated with biofilm formation in breweries. Direct analysis of biofilm masses smeared on MALDI target plate did not result in detectable microbial proteins. The spectrum is dominated by ionization of beer proteins [42] (Figure 2).

MALDI-TOF MS/MS analysis

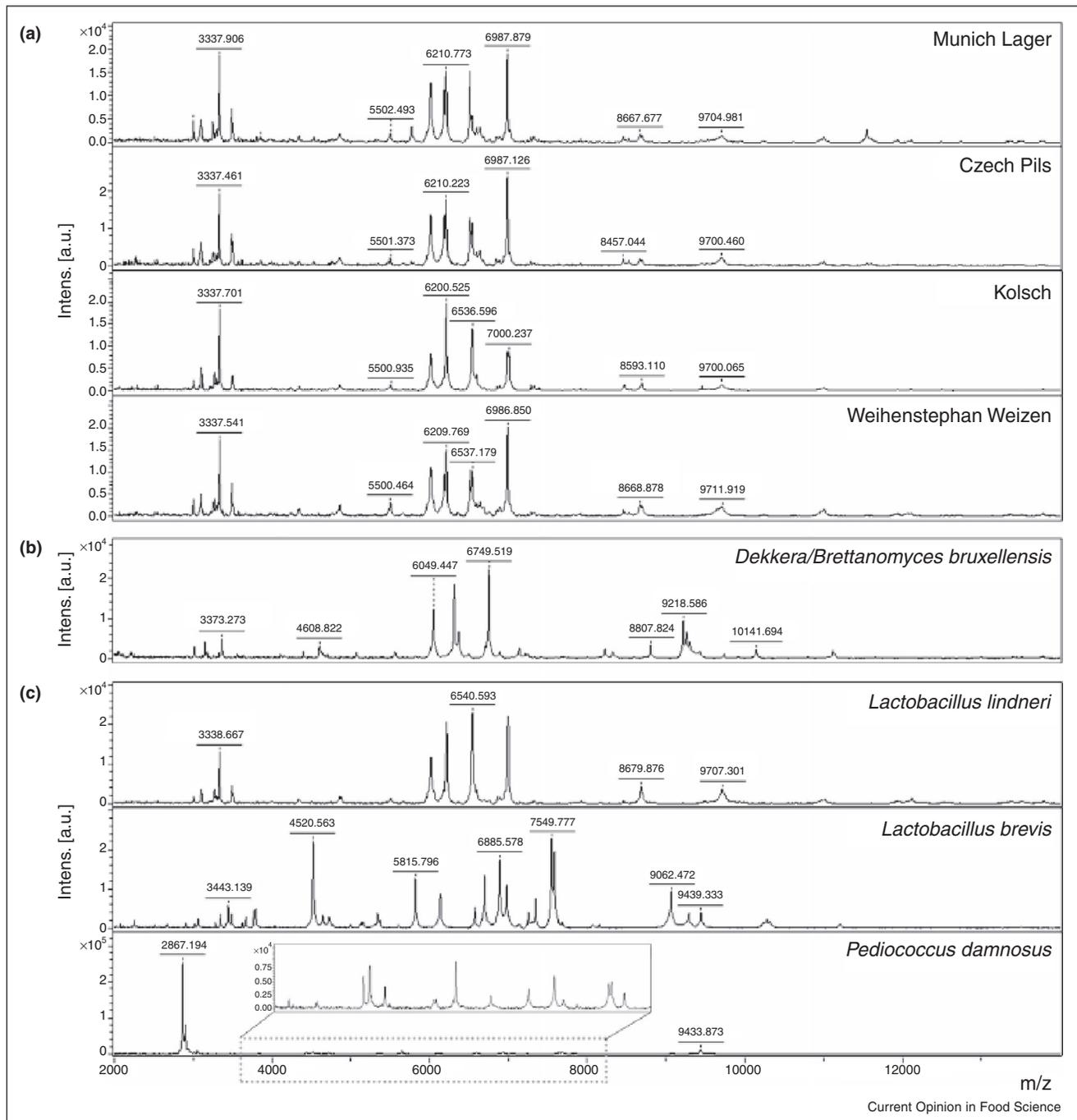
As a fingerprint method MALDI is limited for the identification of individual components in mixed samples. Approximately two or three bacterial species can generally be identified [37]. MALDI is in the majority of applications carried out with a single stage TOF mass analyser. Whilst tandem mass spectrometry (MS/MS) is a corner stone of proteomics, gas-phase sequencing of collisional activated peptide ions is scarcely explored for MALDI identification of microorganisms. The tandem mass spectrometry setup of MALDI-TOF/TOF MS/MS enables high energy collisions of mass selected (poly)peptide ions with neutral atoms. The collisional activated peptide ions dissociate into fragment ions and neutral species. The fragment ions are mass analyzed and peptide sequence information is extracted from the MS/MS spectra. This sequence tag can be searched against

Figure 1



Workflow of MALDI data analysis. **(a)** In a library search workflow MALDI spectra of unknown microorganisms are compared with a library of reference spectra. Various probability scoring algorithms are used to express the degree of similarity between acquired and library spectra. **(b)** In a bioinformatics approach organisms unique m/z values are identified by biostatistics. These biomarkers are used to differentiate between genera, species, or strains. The biomarker m/z values are the end-point of the data analysis or are further downstream also searched against protein sequence databases. The m/z values of protonated proteins in the sequence database are calculated *in silico* and compared with the experimental m/z values. A prerequisite is that the protein sequences of the organism are available. **(c)** In MALDI-TOF MS/MS spectra are theoretically predicted from protein sequences in the database. The abundances of the fragment ion peaks cannot be predicted and are scaled to an arbitrary intensity. The m/z values of the acquired MS/MS spectrum are compared with the m/z values of the theoretical MS/MS spectrum. A prerequisite is that the protein sequences of the organism are available.

Figure 2

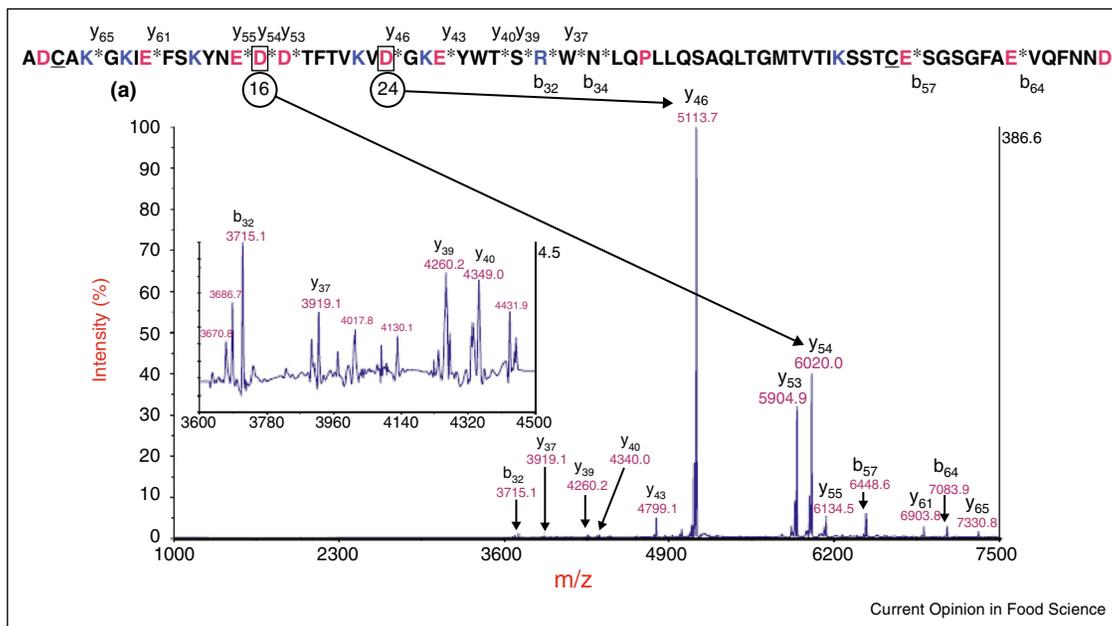


MALDI-TOF MS spectra of beer yeast and beer spoiling bacterial strains. **(a)** commercially available brewing yeasts, **(b)** wild yeast and **(c)** beer spoiling bacteria. The inset in *Pediococcus damnosus* spectrum represents an extension. (reprinted with permission from Springer).

genomic and proteomics databases. A premise is that a sequence is available. In bottom-up proteomics proteins are digested with a protease and the tryptic peptides are subjected to MS/MS. In a top-down proteomics strategy intact proteins are dissociated and sequenced through MS/MS analysis. Top-down proteomic identification of multiple Stx subtypes expressed in a single bacterial

strain was performed through MALDI-TOF/TOF MS/MS. Shiga toxin-producing *E. coli* (STEC) are linked to outbreaks of food-borne illness. The shiga toxins are grouped in Stx 1 and Stx 2 types that are further divided in subtypes. STEC Stx 1 and 2 toxin types and subtypes are coded by multiple *stx* genes. Approximately, 90 STEC strains with distinct Stx2 primary sequences are known.

Figure 3



MALDI-TOF MS/MS of the peak at m/z 7819 upon ionization of a *E. coli* O157:H-Strain E32511 cell lysate. An asterisk * indicated the position of fragmentation. Fragment ions are indicated using the nomenclature of Roepstorff&Fohlmann. The MS/MS spectrum contains sequence specific information. The amino acid sequence Stx2a is shown. Reprinted with permission from Springer.

MALDI-TOF MS of an unfractionated *E. coli* O157:H-strain E32511 cell lysate clearly shows signals from Stx2a B-subunit at m/z 7818.6. The peak at m/z 7773.6 is caused by ionization of the Stx2c B-subunit. The amino acid sequence of Stx2a B-subunit ions is elucidated by MS/MS analysis (Figure 3). Top-down analysis software confirmed the Stx2A B subunit identification [46**]. The acquisition of the shiga toxin profiles requires a beat-beating step. An improvement of the method is bacteriophage induced cell lysis triggered by antibiotics. Bacteriophage enhanced lysis enhanced the detection sensitivity enabling detection of Shiga 1 and 2 toxins from three clinical strains [47]. A bottom up proteomics approach increased the number of detected *Salmonella* subspecies proteins. MS/MS of tryptic peptides enabled identification of subspecies specific biomarkers [48]. Proteins were isolated with a classical MALDI-TOF MS identification formic acid/organic solvent extraction protocol. The subsequently upon tryptic digestion of the pellet fraction obtained peptides are fractionated by LC, detected and sequenced by MALDI-TOF/TOF MS. This bottom-up proteomics method clearly extended the mass range of detected proteins and gave rise to more taxonomic resolving power to differentiate *Salmonella* subspecies.

Conclusions

MALDI databases have been compiled over many years for identifications of pathogenic microorganisms in the clinical lab. MALDI-TOF MS has matured to the status

of a proven technology for rapid identification of pathogens and is as such accepted as a robust molecular identification method in clinical microbiology. Particularly, with respect to analysis cost, sample throughput and ease of use, the technique outcompetes other geno-typing and pheno-typing methods. A prerequisite is a large sample volume. The technique is gaining interest for identification of food spoilers and food-borne pathogens in the food production chain. Tracking demands large scale sampling. Microorganisms that are important in the food chain are currently underrepresented in the MALDI databases. This still somewhat hampers analysis and leads to lower performance compared to other techniques such as molecular identification through PCR amplification. However, it may be expected that this will be rapidly solved the upcoming period for important food spoilers and food-borne pathogens. Hence the technique will exhibit a comparable evolution as was seen in its application to clinical microbiology. MALDI-TOF MS can be expected to go beyond the identification of genera and species. Particularly, the differentiation of isolates is of importance for tracking the source of contaminations.

MALDI-TOF MS identification of microorganisms is generally capable of differentiation at the genus and species level. Robust differentiation of subspecies or subtypes demands more selectivity than provided by a fingerprint of m/z values of intact proteins. Although substantial contributions have been published, the

application of MS/MS is still underappreciated in MALDI identification studies. A development to pursue is further development of gas phase sequencing of MALDI desorbed peptide ions through collisional activation tandem mass spectrometry. Through MS/MS a sequence tag is obtained that can be used for protein identification by genomic and proteomics database searching. This will also expand the toolbox to identify species in mixed populations of microorganisms. It will introduce more selectivity and contribute to lower false positive rate of identification. Protein sequence tags enable interrogation of bioinformatics databases and can be used to identify species that are not present in a protein fingerprint library. Although its potential for identification of microorganisms, the costs of current MALDI-TOF MS/MS instrumentation is prohibitive for its introduction in a routine quality control lab. Another promising development is tryptic digestions in combination with established protein extraction protocols. Although an additional time consuming peptide separation step is introduced the data information content increases significantly. The LC separation and spotting of target plates can be automated and thus should not hamper progress. For identification of biomarkers out of multiple sets of proteins analogy can be sought by statistical methods in clinical proteomics.

In summary, MALDI-TOF MS has been successfully challenged as a tool for molecular identification of food spoilers and food-borne pathogens. Tracking and tracing of strains and isolates will benefit from wide spread application of MS/MS and accurate mass measurements. It is envisaged that molecular identification through MALDI-TOF MS will rapidly establish a robust position for surveillance of microbial pathogens in the complex interwoven network of origin, producers, traders and consumers.

Acknowledgements

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