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Identification and differentiation of food-related bacteria: A comparison of FTIR spectroscopy and MALDI-TOF mass spectrometry

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ABSTRACT

The food industry requires easy, accurate, and cost-effective techniques for microbial identification to ensure safe products and identify microbial contaminations. In this work, FTIR spectroscopy and MALDI-TOF mass spectrometry were assessed for their suitability and applicability for routine microbial diagnostics of food-related microorganisms by analyzing their robustness according to changes in incubation time and medium, identification accuracy and their ability to differentiate isolates down to the strain level. Changes in the protocol lead to a significantly impaired performance of FTIR spectroscopy, whereas they had only little effects on MALDI-TOF MS. Identification accuracy was tested using 174 food-related bacteria (93 species) from an in-house strain collection and 40 fresh isolates from routine food analyses. For MALDI-TOF MS, weaknesses in the identification of bacilli and pseudomonads were observed; FTIR spectroscopy had most difficulties in identifying pseudomonads and enterobacteria. In general, MALDI-TOF MS obtained better results (52–85% correct at species level), since the analysis of mainly ribosomal proteins is more robust and seems to be more reliable. FTIR spectroscopy suffers from the fact that it generates a whole-cell fingerprint and intraspecies diversity may lead to overlapping species borders which complicates identification. In the present study values between 56% and 67% correct species identification were obtained. On the opposite, this high sensitivity offers the opportunity of typing below the species level which was not possible using MALDI-TOF MS. Using fresh isolates from routine diagnostics, both techniques performed well with 88% (MALDI-TOF) and 75% (FTIR) correct identifications at species level, respectively.

1. Introduction

Bacteria play an important role in the food industry. They are used as starter cultures for cheese, yogurt or other fermented foods but they are also responsible for food-spoilage and food-poisoning. The identification and differentiation of food-related bacteria is indispensable for quality assurance and it is the basis for ensuring that safe and wholesome foods are available for the consumers. Besides the detection of pathogens, for which a number of specific detection systems are available, the identification of non-pathogenic bacteria frequently occurring as contaminants also is of considerable importance.

The prerequisite of any identification method is its accuracy. In addition it should be easy to perform, fast and inexpensive. The most accurate methods are based on genetic markers, but are often quite cost-intensive (Montville and Matthews, 2005; Sutherland and Rafii, 2006) and difficult to implement in routine food analysis. However, there are bioanalytical alternatives such as Fourier Transform Infrared (FTIR) spectroscopy or Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Both methods follow a standardized laboratory protocol and record spectra of whole bacterial cells which resemble a phenotypic fingerprint. FTIR spectroscopy generates over-all biochemical fingerprints whereas MALDI-TOF MS records more specific protein mass spectra. In both cases the unknown spectra are compared to a reference database to obtain identification (Helm et al., 1991a; Krishnamurthy et al., 1996; Naumann et al., 1991).

Originally introduced by Naumann and co-workers (Helm et al., 1991a); Naumann et al., 1991), FTIR spectroscopy has gained growing interest in microbial identification (for review see Wenning and Scherer, 2013). It is a very cost-efficient technique and allows a rapid and simple identification of microorganisms within 24 h (Wenning et al., 2008). Absorption of infrared light by cellular compounds results in a fingerprint-like spectrum that can be identified by comparison to spectral databases. Given the availability of well-composed databases, FTIR has been shown to be a reliable technique at both genus and species levels as well as down to the strain level (Büchl et al., 2010; Kümmerle et al., 1998;

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Oberreuter et al., 2002; Rebuffo-Scheer et al., 2007a,b; Wenning et al., 2010) and it has already been employed for identification of many different pathogenic and non-pathogenic species in various fields such as in clinical applications (Becker et al., 2006; Helm et al., 1991b; Horbach et al., 1988; Miguel Gomez et al., 2003; Preisner et al., 2010), and food industry (Amiel et al., 2000; Guibet et al., 2003; Lefier et al., 2000; Lucia et al., 2001) and identification of food pathogens or spoilage microorganisms in consumer products (Beattie et al., 1998; Kümmerle et al., 1998; Lefier et al., 1997; Lin et al., 1998).

Mass spectrometry has been used for bacterial characterization since 1975 (Anhalt and Fenselau, 1975), but a significant progress was marked by the introduction of MALDI-TOF MS (Hillenkamp et al., 1991; Karas and Hillenkamp, 1988). Today, MALDI-TOF MS represents the most frequently used MS technique for a rapid and specific identification of bacteria. The MALDI-TOF technique is a soft ionization method allowing desorption of peptides and proteins from whole cells of cultured microorganisms. Ions are separated and detected according to their molecular mass and charge. Due to the fact that bacterial cells have a high content of proteins (up to 60–70% dry weight in *Escherichia coli*) and these proteins directly represent genetic information of the organism, the profiles of proteins are useful for identification of bacteria. The potential of MALDI-TOF MS for bacterial identification is well documented (Carbonnelle et al., 2012; Sogawa et al., 2011; Steensels et al., 2011) and the technique was used to identify food poisoning, food-borne bacteria and starter cultures (Böhme et al., 2010; Mazzeo et al., 2006; Pavlovic et al., 2013; Stephan et al., 2010; Zeller-Péronnet et al., 2013), environmental bacteria (Dubois et al., 2010; Ruelle et al., 2004), clinical bacteria (Carbonnelle et al., 2011) as well as *Enterobacteriaceae* (Lynn et al., 1999; Pavlovic et al., 2012). In addition to the identification of bacteria, spores have been identified (Hathout et al., 1999) and *Staphylococcus aureus* strains were differentiated on the basis of biomarkers according to resistance or sensitivity towards antibiotics (Edwards-Jones et al., 2000).

Most diagnostic tools are used for detection and identification of pathogens and application in clinical microbiology. The aim of the present study was to assess FTIR spectroscopy and MALDI-TOF mass spectrometry for their suitability and applicability in routine microbial identification of food-related microorganisms by analyzing their robustness according to changes in incubation time and medium, identification accuracy and their ability to differentiate isolates down to the strain level.

2. Material and methods

2.1. Bacterial strains

Altogether 174 bacterial strains from 93 species belonging to four taxonomic groups (bacilli (n = 25), staphylococci (n = 50), strictly oxidative Gram-negative bacteria (n = 48) and enterobacteria (n = 51)) were used to test the two methods (Supplementary Table S1). All isolates were part of the in-house Weihenstephan (WS) strain collection of the Department of Microbiology at the Central Institute for Food and Nutrition Research (ZIEL) and were selected to cover many frequently as well as a number of less frequently occurring species in food industry. With only few exceptions strains had been isolated from food samples and had been identified by *rpoB* (staphylococci), *atpD* (enterobacteria), *rpoD* (*Pseudomonas* spp.) and 16S rRNA (all other bacteria) gene sequencing. Furthermore, 40 fresh isolates from routine food analyses carried out by ZIEL and the Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL) were used as further test organisms (Supplementary Table S1). They were identified by *rpoB* (staphylococci) and 16S rRNA (all other bacteria) gene sequencing (see below).

For strain typing ten strains of each *Bacillus cereus*, *E. coli*, *Pseudomonas aeruginosa*, *S. aureus* and *Staphylococcus epidermidis* were used. Each strain originated from a separate sample to ensure that unrelated strains were analyzed.

2.2. RpoB, rpoD, atpD and 16S rRNA gene sequence analysis

For DNA extraction of Gram-positive bacteria the High Pure PCR Template Kit (Roche, Mannheim, Germany) was used, following the protocol of the supplier. Gram-negative bacteria were lysed by boiling some bacterial colonies in 400 μL of PCR-grade water at 99 °C for 15 min with a subsequent centrifugation at 14,000 ×*g* for 2 min. 5 μL of the extract was used in each PCR.

PCR for *rpoB* gene sequencing was performed according to Mellmann et al. (2006), the one for *rpoD* sequencing according to Mulet et al. (2009, 2010) and that for *atpD* sequencing according to Paradis et al. (2005).

The reaction mixture for 16S rRNA gene sequencing contained 1 U *Taq* polymerase (Applied Biosystems, USA), 1× *Taq* buffer, 2.5 mM $MgCl_2$, 0.05 mM of each dNTP (Applied Biosystems, USA) and 0.4 μM of the primers fD1-forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and 800r reverse (5′-GAGTACCAGGGTATCTAATCC-3′). Thermal cycling was performed using a MasterCycler Gradient (Eppendorf, Hamburg, Germany) thermocycler. PCR mixtures were subjected to 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 55 °C for 30 s and DNA extension at 72 °C for 90 s. Every amplification program began with a denaturation step of 98 °C for 2 min and ended with a final elongation step of 72 °C for 7 min.

Amplicons were purified for sequencing using the QIAquick PCR purification kit (Qiagen, Hilden, Germany). The sequencing was performed at Eurofins MWG Operon (Ebersberg, Germany) and sequences were identified with the BlastN database at NCBI (http://ncbi.nlm.nih.gov/blast/Blast.cgi) and the EzTaxon-e server (Kim et al., 2012).

2.3. FTIR sample preparation, measurement and data analysis

Sample preparation for FTIR spectroscopy measurements was performed according to the methods of Oberreuter et al. (2002). The strains were streaked on tryptic soy agar (TSA) (Oxoid, Wesel, Germany) using a Drigalski spatula and incubated for 24 ± 0.5 h at 30 ± 2 °C for cocci, enterobacteria and strictly oxidative Gram-negatives or at 25 ± 2 °C for spore forming bacteria. One loop full of biomass was suspended in 100 μL of water. 25 μL of the bacterial suspension was placed on a 96-well zinc selenide sample carrier and dried for 45 min at 42 °C. The spectra of the dried biofilms were recorded in transmission between wavenumbers of 4000 and 700 cm^{-1} using the Tensor™ 27 spectrometer coupled to the HTS-XT high-throughput device (both Bruker Optics, Ettlingen, Germany). The following measurement parameters were used: 6 cm^{-1} resolution, 10 kHz scan speed, Blackman-Harris 3-Term apodization, zerofilling of 4. Each strain was analyzed three times independently. Data processing was performed using the OPUS™ V. 5.5 software (Bruker). First (staphylococci) or second (bacilli and all Gram-negative bacteria) derivatives of the spectra were calculated, smoothed and vector normalized. The staphylococci were identified using an extended version of the database described by Oberreuter et al. (2002); all other species were identified by unpublished in-house databases using the parameters described by Kümmerle et al. (1998). At the time of the study, the FTIR databases contained spectra of 905 bacilli, 610 enterobacteria, 1425 strictly oxidative Gram-negative bacteria and 562 staphylococci. Identification results are displayed by the software as a list of most similar spectra each given with a distance measure (hit quality; HQ) to the unknown spectrum indicating the degree of dissimilarity. Only the first hit in the list was counted as identification, the HQ needed to be <1.5. Hits with HQ > 1.5 were counted as not identified.