Food Microbiology 38 (2014) 36–43

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Identification of meat-associated pathogens via Raman microspectroscopy

Susann Meisel [a], Stephan Stöckel [a], Petra Rösch [a,*], Jürgen Popp [a,b,*]

[a] Institute of Physical Chemistry and Abbe Center of Photonics, University of Jena, Helmholtzweg 4, D-07743 Jena, Germany
[b] Institute of Photonic Technology, Albert-Einstein-Straße 9, D-07745 Jena, Germany

ARTICLE INFO

Article history:
Received 30 April 2013
Received in revised form
17 July 2013
Accepted 14 August 2013
Available online 22 August 2013

Keywords:
Food-borne pathogens
Raman microspectroscopy
Support vector machine
Three level classification model
Minced beef
Chicken breast

ABSTRACT

The development of fast and reliable sensing techniques to detect food-borne microorganisms is a permanent concern in food industry and health care. For this reason, Raman microspectroscopy was applied to rapidly detect pathogens in meat, which could be a promising supplement to currently established methods.

In this context, a spectral database of 19 species of the most important harmful and non-pathogenic bacteria associated with meat and poultry was established. To create a meat-like environment the microbial species were prepared on three different agar types.

The whole amount of Raman data was taken as a basis to build up a three level classification model by means of support vector machines. Subsequent to a first classifier that differentiates between Raman spectra of Gram-positive and Gram-negative bacteria, two decision knots regarding bacterial genus and species follow. The different steps of the classification model achieved accuracies in the range of 90.6%–99.5%. This database was then challenged with independently prepared test samples. By doing so, beef and poultry samples were spiked with different pathogens associated with food-borne diseases and then identified. The test samples were correctly assigned to their genus and for the most part down to the species-level i.e. a differentiation from closely-related non-pathogenic members was achieved.

1. Introduction

The presence of bacteria in food is natural and unavoidable (Mandal et al., 2011). While some of these bacteria are involved in spoilage processes, like *Pseudomonas* spp. (Doulgeraki et al., 2012; Gram et al., 2002; Liu et al., 2006), other bacteria are harmful to humans. Meat and poultry provide a good nutrient source for the growth of microorganisms in particular for pathogens like *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Yersinia enterocolitica*, due to high water activity and the presence of proteins and carbohydrates (Naidoo and Lindsay, 2010; Nicolaou et al., 2012). These pathogens can cause gastrointestinal human diseases after consumption of undercooked or raw meat products (Mandal et al., 2011).

Since food-borne illnesses are a permanent problem for public health, rapid and reliable detection systems for spoilage and pathogenic microorganisms in meat products are required (Velusamy et al., 2010). Food-borne outbreaks, like e.g. the *E. coli* outbreak in Germany 2011 (Abu Sin et al., 2013; Krause et al., 2013), exemplify the need for fast and accurate identification systems for contaminated food.

Conventional identification methods based on a pre-enrichment by bacterial cultivation with specific cultivation media are laborious and time-consuming before a reliable identification result is reached (Roda et al., 2012). To reduce the analysis time various sophisticated methods, like bioluminescence or staining procedures (Junillon et al., 2012; Karoui and Blecker, 2011), immunological methods (Amoako et al., 2012; Zhao et al., 2009), nucleic acid based techniques (Garrido et al., 2013; Kawasaki et al., 2012; Kim et al., 2007), biosensors (Byrne et al., 2009; Pedrero et al., 2009; Velusamy et al., 2010), bioimaging (Kemper et al., 2013; Khlebtsov et al., 2013; Lin et al., 2012) or mass spectrometry (Nicolaou et al., 2012) were evaluated. While some of these methods mainly determine the cell quantity, others require pre-cultivation steps, complicated separation techniques or cost-intensive materials.

Vibrational spectroscopic approaches (IR absorption and Raman spectroscopy) have shown their great potential to rapidly identify

* Corresponding authors. Institute of Physical Chemistry and Abbe Center of Photonics, University of Jena, Helmholtzweg 4, D-07743 Jena, Germany.
E-mail addresses: petra.roesch@uni-jena.de (P. Rösch), juergen.popp@uni-jena.de (J. Popp).

0740-0020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2013.08.007

Table 1
List of used bacteria, abbreviations, number of spectra in the database, standard deviation of means (SDM) per species and numbering of the independent identification samples from blood agar (BA), minced beef (beef) and chicken breast (chicken).

Genus	Species	Strain	Source[a]	Abbr.	No. of spectra	SDM	Independent identification samples		
							BA	Beef	Chicken
Escherichia (3)	*E. coli*	DSMZ 501	DSMZ	Ecol1	128	0.13			
	E. coli	DSMZ 10806	DSMZ	Ecol2	281		#1	#2	
	E. coli	ATCC 25922	ATCC	Ecol3	120				
Listeria (5)	*L. innocua*	DSMZ 20649	DSMZ	Linn	202	0.16			
	L. grayi	DSMZ 20601	DSMZ	Lgra	175	0.14			
	L. monocytogenes	DSMZ 20600	DSMZ	Lmon1	191	0.15			
	L. monocytogenes	ATCC BAA-751	DSMZ	Lmon2	267		#3		#4
	L. welshimeri	DSMZ 20650	DSMZ	Lwel	172	0.15			
Pseudomonas (3)	*P. aeruginosa*	DSMZ 50071	DSMZ	Paer	193	0.18	#5	#6	
	P. putida	DSMZ 291	DSMZ	Pput	226	0.12			
	P. stutzeri	DSMZ 5190	DSMZ	Pstu	223	0.15			
Salmonella (2)	*S. enterica*	DSMZ 17058	DSMZ	Sent	163	0.12			
	S. typhimurium	HS/STS-TSF	IMM	Styp	282		#7		#8
Staphylococcus (3)	*S. aureus*	ATCC 29213	DSMZ	Saur	286	0.14	#9	#10	
	S. cohnii	DSMZ 20261	DSMZ	Scoh	202	0.14			
	S. epidermidis	DSMZ 3270	DSMZ	Sepi	153	0.14			
Yersinia (8)	*Y. aldovae*	DSMZ 18303	DSMZ	Yald	171	0.13			
	Y. bercovieri	DSMZ 18528	DSMZ	Yber	170	0.14			
	Y. enterocolitica	HS/RKIW/03	IMM	Yent03	213	0.14			
	Y. enterocolitica	DSMZ 4780	DSMZ	Yent08	132				
	Y. enterocolitica	HS/RKIW/09	IMM	Yent09	185		#11		#12
	Y. mollaretii	DSMZ 18520	DSMZ	Ymol	181	0.13			
	Y. rohdei	DSMZ 18270	DSMZ	Yroh	188	0.12			
	Y. ruckeri	DSMZ 18506	DSMZ	Yruc	118	0.13			

[a] DSMZ (German Collection of microorganisms and cells); ATCC (American Type Culture Collection); IMM (Institute of Medical Microbiology).

microorganisms even if they are embedded in complex matrices (Alexandrakis et al., 2008; Amamcharla et al., 2010; Ammor et al., 2009; Argyri et al., 2010; Davis et al., 2010; Ellis et al., 2004; Gaus et al., 2006; Maquelin et al., 2002; Tarcea et al., 2007; Walter et al., 2011). Especially Raman microspectroscopy with Raman excitation wavelengths in the visible wavelength region is a very promising method to detect microorganisms on a single-cell level with minimal sample preparation. Thus, by applying single-cell Raman microspectroscopy the time-consuming pre-cultivation step can be avoided and therefore the detection process of microorganisms is significantly accelerated (Harz et al., 2009). In doing so, the bacterial Raman spectra are classified or identified by applying innovative chemometric approaches like e.g. discriminant analysis or support vector machines (Bocklitz et al., 2009; Meisel et al., 2012; Rösch et al., 2005; Schmid et al., 2009; Stöckel et al., 2012b).

Here we utilized Raman microspectroscopy in combination with chemometrics to detect food-borne pathogens from spiked meat and poultry products. Therefore we constructed in a first step a Raman spectra database consisting of 19 different species (24 strains), including the most important food-borne pathogens *E. coli*, *L. monocytogenes*, *P. aeruginosa*, *Salmonella* spp., *S. aureus* or *Y. enterocolitica* as well as closely-related non-pathogenic representatives of the same genera. Recently we have shown that besides the right choice of bacterial representatives also the preparation of the microorganisms is crucial to establish a reliable Raman database. In other words, Raman databases, which contain Raman data of bacteria cultivated under different conditions, allow for a more sufficient and reliable identification of real-world samples (Meisel et al., 2012). Therefore the microorganisms were prepared on three different meat-like media: Columbia blood agar, which is a standard agar for microbial clinical purposes, brain heart infusion agar (main ingredients: beef heart and calf brain infusions) and Müller–Hinton agar (main ingredient: beef infusion). Afterwards a three level classification model – including the spectral variations of the different species and nutrient sources – was developed to subsequently validate the database with bacterial test samples directly from meat and poultry. In this context, pieces of minced beef and chicken breast were spiked with pathogenic microorganisms. In order to separate the target bacteria from the complex structured meat surface for the Raman spectroscopic measurements an additional isolation and concentration procedure was applied.

2. Material and methods

2.1. Species and strains used

An overview of the species and strains used throughout this study is provided in Table 1. Most of the non-pathogenic strains were obtained from the German Collection of Microorganisms and Cell Culture GmbH, Braunschweig, Germany (DSMZ) and American Type Culture Collection (ATCC). All pathogenic and a few non-pathogenic strains were provided by the Institute of Medical Microbiology, Jena, Germany (IMM).

The data collection encompasses Gram-positive (five *Listeria* spp. and three *Staphylococcus* spp.) as well as Gram-negative representatives (two *Salmonella enterica* type strains, eight *Yersinia* spp., three *Pseudomonas* spp. and three *E. coli* type strains).

2.2. Sample preparation

To design a Raman database of meat pathogens, all species were cultivated on three different media mainly consisting of meat ingredients. To account for the biological variability, at least four independently cultivated batches of each species were prepared.

The bacterial species were prepared on Columbia blood agar (BA), brain heart infusion agar (BHI) and Müller–Hinton agar (MHA). The compositions of the cultivation media are as follows: (1) BA consists of a Columbia blood agar base and 5% sheep blood. (2) BHI includes beef heart (5 g/l), calf brains (12.5 g/l) and some