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Analytical Methods

Rapid identification of *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Shigella sonnei* in foods by solid phase microextraction coupled with gas chromatography–mass spectrometry

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ABSTRACT

A novel approach for rapid identification of three foodborne pathogens including *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Shigella sonnei* in foods by solid phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS) was established. After cultivation 24, 18 and 20 h for *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Shigella sonnei*, respectively, the microbial volatile organic compounds (MVOCs) were extracted with a SPME device equipped with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coated fibers. The DB-1701P column was applied for separation of MVOCs. A total of 17, 13 and 14 volatile organic compounds were identified as characteristic MVOCs of *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Shigella sonnei*, respectively. Similarity of the MVOC chromatographic fingerprints for the bacteria were calculated and compared, and the results showed that the established method is stable, reproducible, accurate and has the potential to identify the three bacteria in food samples.

1. Introduction

Foodborne diseases encompass a wide spectrum of illness and are an emerging public health concern worldwide. A WHO report, '*WHO estimates of the global burden of foodborne diseases*', presents that diarrhoeal diseases are responsible for more than half of the global burden of foodborne diseases. Diarrhoea is often caused by eating raw or undercooked meat, eggs, fresh products and dairy products contaminated by microorganisms, which can cause not only short-term symptoms but also chronic conditions.

Among the pathogenic microorganisms of greatest concern in foods, *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Shigella* spp. are typical. According to USFDA, an estimated 185,000 foodborne cases of staphylococcal food poisoning, 89,600 foodborne cases of shigellosis and 3600 foodborne cases of vibriosis form *Vibrio parahaemolyticus* occur annually in the USA. According to the data in *China's Health and Family Planning Statistical Yearbook* (2012–2016), *Staphylococcus aureus* caused 27–56 foodborne cases between 2011 and 2015 in China, leading to 814 patients annually. Meanwhile, *Vibrio parahaemolyticus* caused 72–147 foodborne cases and led to 1689 patients annually. The contamination of *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Shigella sonnei* in foods resulted in considerable food poisoning incidents. The approach for quick detection of these three foodborne pathogens plays an essential role in food safety monitoring. However, the conventional methods composed of cultivation and biochemical tests for detection of bacteria are still the most common approaches today. A major disadvantage of the conventional approaches is time-consuming, so they cannot meet the requirements of food safety monitoring during the public health emergency.

Since 1990s, the microbial volatile organic compounds (MVOCs) have drawn researchers' attention as indicators of microbial growth in human body, environment and foodstuff. The MVOCs are volatile

Abbreviations: MVOCs, microbial volatile organic compounds; *S. a*, *Staphylococcus aureus*; *S. s*, *Shigella sonnei*; *V. p*, *Vibrio parahaemolyticus*; SPME, solid phase microextraction; GC–MS, gas chromatography–mass spectrometry; PDMS, polydimethylsiloxane; DVB, divinylbenzene; CAR, carboxen; PA, polyacrylate; AMDIS, Automated Mass Spectral Deconvolution Identification System; NIST, National Institute of Standards and Technology; TCM, Traditional Chinese Medicine

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metabolites produced by bacteria, fungi and molds during metabolism. Lemfack (Lemfack et al., 2018) established an open database available online for MVOCs (http://bioinformatics.charite.de/mvoc). The information from the database shows that approximately 2000 volatile organic compounds from microorganisms have been identified as MVOCs including alcohols, aldehydes, hydrocarbons, acids, ethers, esters, ketones, terpenoids, as well as sulfur containing and amino compounds. The production of MVOCs is greatly affected by microbial species and strains, growth phase and cultural conditions including substrate, nutrients, pH, humidity and temperature (Korpi, Jarnberg, & Pasanen, 2009).

In the past two decades, there has been an increasing interest in applications of MVOCs in diagnosis of respiratory infections (Koo et al., 2014), urinary tract infections (Aathithan, Plant, Chaudry, & French, 2001) and gastrointestinal diseases as well (Garner et al., 2007). Besides, MVOCs were also regarded as indicators of microbial growth in indoor air and applied in environmental monitoring (Ryan & Beaucham, 2013).

Meanwhile, detection of MVOCs is now becoming a novel and effective approach to investigate the microbial growth in foods (Wang, Li, Yang, Ruan, & Sun, 2016). Some researchers have already investigated the MVOCs in meat products (Parlapani, Mallouchos, Haroutounian, & Boziaris, 2014), fruit (Moalemiyan, Vikram, & Kushalappa, 2007), dairy products (Hettinga, van Valenberg, Lam, & van Hooijdonk, 2009), grain (Salvador et al., 2013) and beverage (Iamanaka et al., 2014).

Many factors such as cultivation condition of microorganism, sampling technique, analytical method and approaches for data analysis can affect the profile of MVOCs. By far, the sampling techniques for MVOCs mainly include solid phase microextraction (SPME) (Salvador et al., 2013), purge and trap (Holm et al., 2013) and needle trap (Zscheppank, Wiegand, Lenzen, Wingender, & Telgheder, 2014). Gas chromatography coupled with mass spectrometry (GC–MS) is the most frequently used technique for determination of MVOCs (Morales-Valle, Silva, Oliveira, Venancio, & Lima, 2010) and multidimensional GC coupled with high-resolution mass spectrometry could greatly improve the separation and identification of complex MVOCs (Johanningsmeier & McFeeters, 2011). Statistical analysis approaches such as principle component analysis (PCA) (Salvador et al., 2013), discriminant analysis (DA) (Moalemiyan et al., 2007), probabilistic neural networks (PNN) (Hettinga, van Valenberg, Lam, & van Hooijdonk, 2008) have already been applied to reveal the relationship of MVOCs and microbial genera. Since different microorganism could share the same MVOCs and the methodology used for MVOCs analysis varies between the studies, it is difficult to make a reliable list of MVOCs for the relevant microbial species. Among the MVOCs identified in foods so far, none has been verified as a product of solely microbial strain, thus profiles of MVOCs are the basis of identification of microbial species. Although the studies have successfully achieved to describe the differences and characteristics of the MVOCs profiles among different microorganisms via statistical analysis, how to interpret the profiles into a definite indicator of relevant microbial species still remains to be investigated. The results of statistical analysis could not directly be used for identification of microorganisms by far, novel approaches are still required.

The similarity match of chromatographic fingerprints is an effective approach for comparison of chromatograms with complicated components, which is capable of analyzing both the specificities and entirety of the chromatographic fingerprints. Nowadays, this approach has been widely applied in the quality control of Chinese traditional medicine (Wang et al., 2017) and food (Gorska-Horczyczak, Horczyczak, Guzek, Wojtasik-Kalinowska, & Wierzbicka, 2017). In our study, we proposed a novel approach for identification of bacterial species based on the similarity match of MVOCs chromatographic fingerprints. The calculation of similarity match of chromatographic fingerprints was performed by a software “Similarity Evaluation System for Chromatographic Fingerprints of TCM” (Version 2004A, Chinese Pharmacopoeia Commission). The results of similarity match can offer a definite number that represents the similarity of the chromatogram of pure strain culture and the chromatogram of samples. In other words, the results of similarity match could be considered as the probability that the sample is contaminated by specific microorganism.

In our study, we established a fast and effective method to identify three foodborne pathogens including *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Shigella sonnei* based on their profiles of MVOCs by SPME coupled with GC–MS. A novel approach based on similarity match was applied to reveal the relationship between the profile of MVOCs and the relevant bacterium. The established method was successfully applied to identification of the three foodborne bacteria in simulated samples.

2. Material and methods

2.1. Reagents and instrumentation

The strain of *Staphylococcus aureus* (CICC 10384, Beijing, China), *Vibrio parahaemolyticus* (CICC 21617, Beijing, China) and *Shigella sonnei* (CICC 21535, Beijing, China) were all purchased from China Center of Industrial Culture Collection. All the strains used in this study were positive control strains for National food safety standard of China.

7.5% Sodium chloride broth for cultivation of *Staphylococcus aureus* was purchased from Beijing Land Bridge Technology Co., Ltd., the ingredients of this broth were peptone (10.0 g/L), beef extract powder (5.0 g/L), sodium chloride (75.0 g/L). The pH value of the broth was 7.4 ± 0.1 (25 °C). 3% Sodium chloride alkaline peptone water for cultivation of *Vibrio parahaemolyticus* and *Shigella* broth for cultivation of *Shigella sonnei* were both purchased from Qingdao Hopebio-Technology Co., Ltd.. 3% Sodium chloride alkaline peptone water was composed of peptone (10.0 g/L), sodium chloride (30 g/L) and its pH value was 8.5 ± 0.2 (25 °C). The *Shigella* broth was composed of tryptone (20 g/L), dipotassium hydrogen phosphate (2.0 g/L), potassium dihydrogen phosphate (2.0 g/L), sodium chloride (5.0 g/L), glucose (1.0 g/L) and Tween-80 (1.5 mL/L) and its pH value was 7.0 ± 0.2 (25 °C). The novobiocin for cultivation of *Shigella sonnei* was purchased from Beijing Solarbio Life Sciences. 5 mL novobiocin (25.0 mg/L) was added into 225 mL *Shigella* broth and homogenized before cultivation of *Shigella sonnei*.

The bacteria were cultured on a MAXQ 4000 lab shaker (Thermo Scientific). All the sterilization was performed in a G154DW autoclave (Zealway Instrument Inc.).

The SPME device for extraction was equipped with DVB/CAR/PDMS (50/30 μm)-coated fibers and a manual holder, which was purchased from Supelco. The fibers were conditioned according to the manufacture’s instruction before use.

The separation of MVOCs was performed on a medium polar capillary chromatographic column (DB-1701P, 30 m × 250 μm, 0.25 μm, J&W, Agilent, USA) installed in a 7890A gas chromatograph (Agilent, USA). The detection of MVOCs was carried out by a 5975C mass spectrometer (Agilent, USA).

2.2. Growth of bacteria and sample preparation

The apparatus, culture medium and reagents used in the experiment were all sterilized in the autoclave at 121 °C for 15 min. All the bacterial strains were revived for 18 h at 37 °C. The concentration of bacterial culture was adjusted to 0.132 (equivalent to 0.5 McFarland units) by measuring the absorbance of the incubated bacterial culture at the wavelength of 600 nm after diluted with sterilized normal saline, an aliquot of 100 μL this bacterial culture was used as standard bacterial culture for subsequent inoculation. After revived, the bacterial strains were inoculated into 20 mL glass vials. These glass vials are specific for SPME sealed up with PTFE caps. *Staphylococcus aureus* were inoculated in 10 mL 7.5% sodium chloride broth for 24 h at 37 °C. *Vibrio parahaemolyticus* were inoculated in 3% sodium chloride alkaline peptone