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Detection of *Salmonella* spp. in spices and herbs

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ABSTRACT

The detection of microbial contaminations in spices and herbs is a challenging task due to their strong antimicrobial effects, which potentially increase the risk for false-negative results. Therefore, the present study mainly focuses on the detection of *Salmonella* spiked to cinnamon and oregano. Both condiments completely inhibited the proliferation of *Salmonella* at a 1:10 (w/w) dilution. Consequently, the supplementation of the buffered peptone water with K_2SO_3 as well as the application of higher initial dilutions was investigated. While no detrimental effect of K_2SO_3 was observed during the growth of 14 different *Salmonella* isolates, it even improved their detection in condiments. An effect, which was also determined with increased dilution ratios. For detection, a quantitative approach via enumeration of the colony-forming units (CFUs), and qualitative approaches via the culture-based detection according to ISO 6579 and via the nucleic acid-based detection with the 3M Molecular Detection System (MDS) were performed. Subsequently, the limit of detection (LOD) was determined, which was <5 CFU 25 g^{-1} for both qualitative approaches. Furthermore, the persistence of *Salmonella* DNA spiked to parsley was determined with the MDS. Despite of the modifications, the LOD for *Salmonella* spiked to oregano was significantly lower prior than after enrichment, pointing to the requirement for further improvements. Last but not least, a ring trial was performed, which emphasized the importance for a reliable detection.

1. Introduction

Salmonella spp. are important foodborne pathogens with estimated 1.0 million illnesses per year in the United States (Scallan et al., 2011). In Europe almost 90,000 confirmed cases and 1049 outbreaks of salmonellosis were reported for 2014 (EFSA & ECDC, 2015). Moreover, the matrix-pathogen combination of *Salmonella* in spices and herbs is among the most relevant according to the European Food Safety Authority (EFSA, 2013) and Da Silva Felício et al. (2015). Spices and herbs are ready-to-eat foods without further need for cooking or processing to fulfill their criteria from a microbiological point of view, and thus are considered as critical foods (EC, 2005). The food category herbs and spices is among those with the highest number of notifications according to the European Rapid Alert System for Food and Feed (RASFF, 2016). More than 500 notifications are directed to >80 different *Salmonella* serovars in >60 herbs and spices from 2003 to date. Additionally, *Salmonella* are generally heterogeneously distributed among several subsamples within these foods, which is also emphasized by RASFF notifications from 2003 to date where in only 6% of the cases all subsamples (3, 4 or 5) were positive, while in 45% only one subsample (out of 3, 4, 5 or 10) was positively confirmed (RASFF, 2016). Besides that, also the minimal infectious dose for susceptible persons is remarkably low with ≤10 colony-forming units (CFUs) g^{-1} product (Hammack, 2012), while for healthy people it is considered to be several logs higher (Bell & Kyriakides, 2002; WHO & FAO, 2002). After ingestion and passage of the stomach, nontyphoidal *Salmonella* adhere to epithelial cells of the intestine, followed by an invasion into the cells and expression of virulence factors. Diarrhea and vomiting, and also often fever, can be detected 6–72 h after ingestion (Hammack, 2012). Consequently, a sensitive and fast detection of *Salmonella* spp. is required to respond to outbreaks or food recalls, however, some rapid detection approaches might lack specificity and selectivity (Lee, Runyon, Herrman, Phillips, & Hsieh, 2015). Thus, besides the conventional culture-based methods, in the last decade especially rapid detection methods were investigated and continuously improved. Among them are different types of immunology-based, nucleic acid-based and biochemical assays as well as biosensors (Almeida, Cerqueira, Azevedo, & Vieira, 2013; Cheung & Kam, 2012; Lee et al., 2015; Zheng, Mikš-Krajnik, Yang, Xu, & Yuk, 2014).

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In the present study the detection of *Salmonella* Oranienburg in artificially spiked condiments was investigated. For this purpose, the common ISO 6579 and a modification thereof was used. The latter implied the application of K_2SO_3 to the buffered peptone water for the non-selective enrichment step as well as increased initial dilution ratios. Furthermore, an alternative detection with the 3M Molecular Detection System (MDS) was compared to the ISO. Last but not least, a ring trial for detection of *S.* Oranienburg, artificially spiked to cinnamon, was run among seven partners of the EU project SPICED.

2. Material and methods

2.1. Salmonella strains

All *Salmonella* strains within this study belong to the species *enterica* and subspecies *enterica*. Therefore, subsequently it is only referred to the serotype name. *S.* Oranienburg and *S.* Mbdanka were isolated from ground cumin and rucola salad, respectively, during routine analyses in the laboratory of the Austrian Agency for Food and Health Safety Ltd. (AGES). After biochemical and serological confirmation with API 20 E (bioMérieux), oxidase test (Merck) and omnivalent agglutination (Sifin), the subsequent serotyping was performed by the AGES national reference laboratory (NRL) for *Salmonella*. The other twelve *Salmonella* isolates were either derived from the veterinary laboratory of AGES after serotyping by the NRL or directly by the NRL, which were *S.* Abony, *S.* Abortusequi, *S.* Adelaide, *S.* Agona, *S.* Choleraesuis, *S.* Cotham, *S.* Enteritidis (two isolates), *S.* Gallinarum, *S.* Heidelberg, *S.* Poona and *S.* Typhimurium.

2.2. Matrices: spices and herbs

The condiments cinnamon, oregano and parsley were obtained from FUCHS Gewürze (Germany). All samples were dried, and then grounded to particle sizes of <1 mm. Cinnamon and parsley were steam treated to improve the microbiological status, while oregano was untreated. The sample batches were specifically prepared for the EU project SPICED (Grant Agreement: 312631), and the microbiological status of the condiments is shown elsewhere (Lins, 2017).

2.3. Culture-based detection of Salmonella spp.

The culture-based detection of *Salmonella* spp. was performed according to ISO 6579:2002-Cor.1:2004-Amd.1:2007 with a dilution of 1:10 (w/w) with buffered peptone water (BPW) (Merck) for a non-selective enrichment, shortly mentioned as 'ISO 6579' (Fig. 1). Alternatively, a 1:20 (w/w) dilution and supplementation of the BPW with 0.5% (w/v) K_2SO_3 (Sigma-Aldrich) to reduce potential antimicrobial effects of the matrices, as for example endogenous propyl disulfides (D'Aoust, 2009), was done according to ISO 6887-4:2003-Cor.1:2004-Amd.1:2011 and referred to as 'mISO' for the modification of the ISO 6579. Presumptive *Salmonella* colonies were streaked on xylose lysine deoxycholate (XLD) agar according to ISO 6579 as well as chromID *Salmonella* (SM2) agar (bioMérieux) as a second selective agar. If a CFU value was ≥20, a weighed arithmetic mean with the CFU of the prior dilution was calculated. Additionally, qualitative analyses according to the above mentioned ISO norms were done with Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) broth and a Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar (bioMérieux).

Furthermore, a 'Washing & Shaking' (W&S) method was applied during the ring trial (see below) to reduce potential growth- and polymerase-chain-reaction (PCR)-inhibiting substances from cinnamon, which was principally performed according to Vitullo et al. (2011).

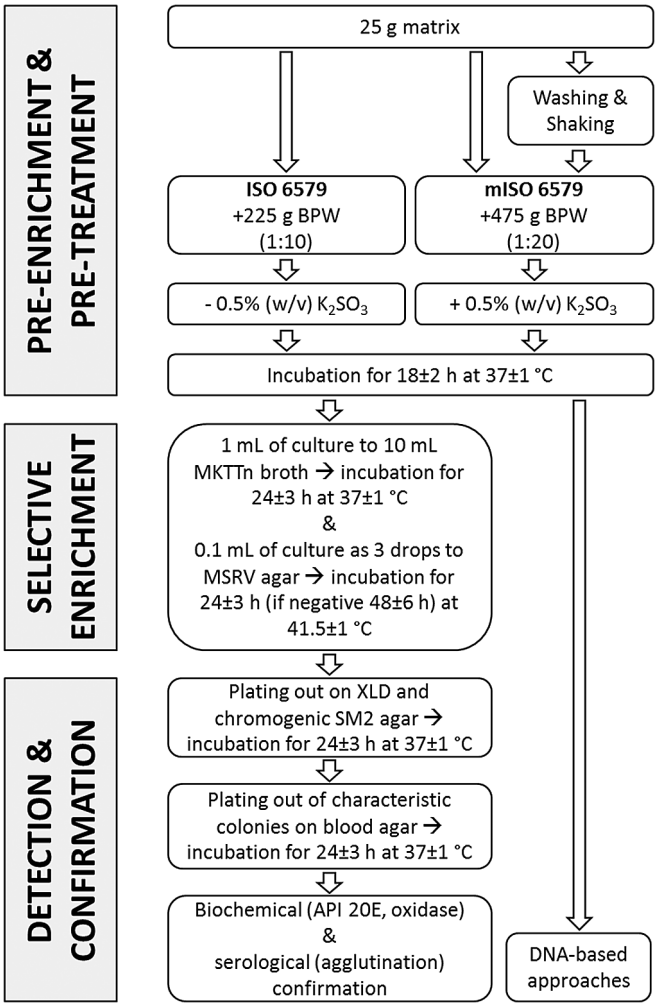

Fig. 1. Methodological scheme. **BPW**, buffered peptone water; **ISO 6579**, strictly sticking to the ISO; **mISO 6579**, modified ISO with 1:20 (w/w) dilution with BPW supplemented with 0.5% (w/v) K_2SO_3; **MKTTn**, Muller-Kauffmann Tetrathionate-Novobiocin broth; **MSRV**, Modified Semi-solid Rappaport-Vassiliadis agar; **XLD**, xylose lysine deoxycholate agar.

2.4. Nucleic acid-based detection of Salmonella spp.

In addition, a nucleic acid-based detection was performed with the MDS from the company 3M. Its principle relies on a loop-mediated isothermal amplification (LAMP), which does not undergo classic denaturation, annealing and elongation steps along with a set cycle number (3M, 2014; Bird et al., 2014). During the LAMP, DNA is constantly amplified, resulting in a cauliflower-like structure. It is assumed that LAMP is more specific, rapid, simple and less expensive than a conventional PCR (Cheung & Kam, 2012; Loff, Mare, de Kwaadsteniet, & Khan, 2014). Bioluminescence detection is used instead of fluorescence, given in Relative Light Units (RLU), which makes the detection more robust. This system uses multiple primers to recognize distinct regions. Pyrophosphate ions are evolved as a by-product of the DNA amplification of the *Bst* DNA polymerase, which is converted with adenosine phosphosulfate (APS) into ATP. Subsequently, ATP reacts with luciferase, producing light, which is continuously detected. Furthermore, positive and negative controls are available as well as matrix control kits to investigate if the matrix itself might interfere and inhibit