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Capture and identification of bacteria from fish muscle based on immunomagnetic beads and MALDI-TOF MS

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ABSTRACT

In the present study, *E. coli* was taken as a model bacterium, anti-*E. coli* functionalized magnetic beads were constructed and used to capture *E. coli* from aqueous extracts of fish sarcoplasmic protein (FSP) and fish muscle protein of sablefish. The excellency of the reproducibility of the present protocol was demonstrated by capturing *E. coli* from sablefish FSP extracts. The presence of 10 CFU/mL *E. coli* is still detectable. A microbial safety test on the surface of fish muscle was successfully performed. The bacterial identification accuracy from samples with different matrices was found to be excellent with RSD = 3%. High specific detection of target bacteria in complex biological samples was testified by spiking *Staphylococcus aureus* and *Klebsiella pneumoniae* in samples as interference. Ten biomarker ions were discovered for *E. coli*'s recognition. It is promising to apply the present protocol in bacterial analysis in muscle food samples to ensure their safety.

Introduction

The rapid, accurate detection of bacteria with high sensitivity is the focus of various fields like clinical diagnosis, food industry and environmental quality control. Different methods have been developed for bacterial detection. Magnetic bead (MB) based procedures have been employed to assist the detection of bacteria. The surface of MB containing aldehyde group was modified with an oriented layer of human immunoglobulin G (IgG) linked by covalent linkage, and then used for capturing staphylococcus protein A (SPA) from pulverator-treated bacterial cell lysate for MS analysis (Chen et al. 2007). MB-based electrochemical impedance system, using interdigitated gold electrodes as sensing elements, was built for the detection of *Escherichia coli* O157:H7 (Wang et al. 2020). Janus magnetic mesoporous silica nanoparticles (Janus M−MSNs) were used for the foodborne bacterial enrichment and direct identification from milk samples (Chang et al. 2018). *Escherichia coli* (*E. coli*) was detected in a sandwich reaction with *E. coli*-specific either aptamer or antibody(Ab)-modified magnetic beads and Ab/aptamer reporter molecules linked to cellulase (Pankratov et al. 2020). Gold nanoparticle-coated starch magnetic beads (AuNP@SMBs) were synthesized for separating and concentrating the target pathogenic bacteria, *E. coli* O157:H7, from an aqueous sample as well as providing a hotspot for surface-enhanced Raman scattering (SERS)-based detection (You et al. 2020).

Detecting food-borne pathogens is for securing food safety. Rapid and reliable methods are always needed for detecting food-borne pathogens to ensure food safety. Bacterial detection in animal muscle food samples have gained an increasing attention. High-throughput

Abbreviations: Ab, antibody; ACN, acetonitrile; anti-*E. coli*@MBs, anti-*Escherichia coli* (*E. coli*) functionalized magnetic beads (MBs) *anti*-E. coli *attached MBs E*. coli *antibody modified magnetic beads*; anti-*E. coli*@MBs@bacteria, anti-*E. coli*@MBs attached with bacteria conjugates *bacteria captured by anti*-E. coli@*MBs*; AuNP@SMBs, gold nanoparticle-coated starch magnetic beads; BSA, bovine serum albumin; CFU, colony forming units; CHCA, α-cyano-4-hydroxycinnamic acid; DI water, deionized water; *E. coli*, *Escherichia coli*; EDC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydro-chloride; EDTA, ethylene diamine tetraacetic acid; FcMBL@Fe_3O_4, fragment crystallizable mannose binding lectin-modified Fe_3O_4; FSP, fish sarcoplasmic protein; HCl, hydrochloric acid; IgG, immunoglobulin G; IgG@Fe_3O_4, immunoglobulin G-modified Fe_3O_4; Janus M-MSNs, Janus magnetic mesoporous silica nanoparticles; LB, Luria-Bertani; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MBs, magnetic beads; MES, 4-morpholineethanesulfonic acid; MEST, MES with Tween 20; *m/z*, mass-to-charge ratio; OD, optical density; PBS, phosphate buffered saline; PBST, phosphate-buffered saline with Tween 20; PCA, principal component analysis; PCR, polymerase chain reaction; PLS-DA, partial least square-discriminant analysis; RPA, recombinase polymerase amplification; SA, sinapinic acid; SERS, surface-enhanced Raman scattering; SPA, staphylococcus protein; STEC, Shiga toxin-producing *Escherichia coli*; sulfo-NHS, sulfo-*N*-hydroxysuccinimide; TFA, trifluoroacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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sequencing analysis of the bacterial communities on meat product (chilled pork) was used to assess the utilized different bacterial extraction methods (Zhou et al. 2020). Avian pathogenic *E. coli* isolates from duck were identified by immunoproteomics (Bao et al. 2013).

Seafood is a source of high quality proteins valued for their functional properties and nutritional value (Tahergorabi, Hosseini and Jaczynski 2011). Bacteria can survive well in aquatic environments independent of their hosts, thus bacterial diseases have become major impediments to aquaculture development (Ben Hamed et al. 2018). Fish infections caused by pathogenic bacteria are quite common in seafood. Most current health risks associated with seafood safety originate in the environment and should be dealt with by control of harvest or at the point of capture. The safety of seafood needs to be ensured to prevent illness resulting from seafood consumption which is associated primarily with environmental contamination. Moreover, microorganisms are the major cause of spoilage of most seafood products (Gram and Dalgaard 2002). Microbiome patterns as well reveal the transmission of pathogenic bacteria in fish marketed for human consumption (Foysal et al. 2019).

Complex food backgrounds can interfere with the identification of bacteria (Chen, Yu and Ho 2019). The suppression of microbial signals may occur due to the matrix interference from food backgrounds and leads to false results. Spiked microorganisms from apple juice/lettuce were enriched by magnetic zirconia nanoparticles, and identified by nanoLC-MS (Chen et al. 2019). Though, the interaction between bacteria and the magnetic zirconia nanoparticles was electrostatic and non-specific, which may complex the interpretation of data when multiple bacteria species are involved. Sun *et al* utilized rabbit immunoglobulin G-modified Fe_3O_4 (IgG@Fe_3O_4) and fragment crystallizable mannose binding lectin-modified Fe_3O_4 (FcMBL@Fe_3O_4) to capture bacteria from aqueous solution and bovine blood for their matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification (Sun sunet al. 2021). However, there is a lack of studies on the detection of bacteria in seafood matrices based on MBs to the best knowledge of the authors.

Bacterial detection in seafood samples is particularly important, and is gaining an increased attention. Specific detection of *Vibrio parahaemolyticus* in seafood was performed by recombinase polymerase amplification (RPA) assay (Geng et al. 2019). Three foodborne pathogens in seafood were simultaneously detected by multiplex RPA assay (Ma et al. 2020). Multiplex polymerase chain reaction (PCR) was used to detect Salmonella contamination in seafoods (Sahu et al. 2019). Shiga-toxin-producing *Escherichia coli* (STEC) in shellfish was detected by real-time PCR (Balière et al. 2015). MALDI-TOF MS has become a method of choice for routine identification of pathogens in clinical settings and has largely replaced conventional biochemical assays (Jadhav, Shah and Palombo 2021). Proteomics-based MALDI-TOF MS approach has been developed to directly detect *Listeria monocytogenes* from foods such as broths (Jadhav et al. 2014). However, MALDI-TOF MS for pathogens in seafoods requires further development.

On the other hand, various bacteria including food spoilage bacteria and pathogens can form biofilms on different food processing surfaces, leading to potential food contamination or spoilage. Surface hygiene is commonly measured as a part of the quality system of food processing. Møretrø et al summarized the residential bacteria on surfaces in the food industry and their implications for food safety and quality (Møretrø and Langsrud 2017).

The bacterial detection on the surface of samples plays an essential role in food safety control. Methods have been developed to extract and detect bacteria from food contact surfaces at retail outlets for food safety control (de Oliveira, Tikekar and Nitin 2018, Kalchayanand et al. 2018, Manafi, Aliakbarlu and Dastmalchi Saei 2020). Bae et al studied the pathogenic bacteria on the surface of stainless steel (Bae, Baek and Lee 2012). Leff *et al* studied the surface bacterial communities of fruits and vegetables (Leff and Fierer 2013).

Principal component analysis (PCA), as one of the most popular multivariate statistical techniques, can help to identify correlations between data points. Partial least squares discriminant analysis (PLS-DA) can be thought of as a supervised version of PCA in the sense that it achieves dimensionality reduction but also with full awareness of the class labels. PLS-DA can also be used for feature selection as well as for classification. Variables importance in projection (VIP) scores indicate the importance of each variable in the projection used in the PLS-DA model and is often used for variable selection. VIP analysis has emerged as a potential method for screening independent variables. Mao *et al* identified the key aromatic compounds in Congou black tea by VIP analysis (Mao et al. 2018). Zhang *et al* screened 38 peaks with VIP values greater than 1.0 out as potential biomarkers to differentiate *E. coli* (Zhang et al. 2018).

The aim of the present study was to develop a protocol for detecting bacteria from complex food matrices. The detection of *E. coli* was taken as a model system. Experimentally, anti-*E. coli* functionalized magnetic beads (anti-*E. coli*@MBs) were constructed for capturing *E. coli* from fish protein extracts where sablefish was taken as a model. During the experiment, the use of magnetic beads (MBs) in the detection of *E. coli* from different media, including water and protein extracts of fish muscle samples, were compared. The reproducibility, sensitivity and specificity of the present protocol for capturing bacteria from complex sample matrices were investigated. The spiked bacteria on muscle surface of sablefish were detected by the developed protocol to extend its application for bacterial safety test of fresh produce. The mass spectra of bacteria captured from different samples by MBs was compared by multivariate statistical methods. The discovery of biomarker ions that associate with the MALDI-TOF MS detection of bacteria was explored by assistance of variables importance in projection (VIP) analysis. It is promising to apply the present protocol for the measurement of bacteria in seafood matrices.

Materials and methods

Chemicals and fish samples

Acetonitrile (ACN, 99.90%), ethylene diamine tetraacetic acid (EDTA, ≥99.0%), tris(hydroxymethyl)aminomethane-(Tris) (≥99.5%) and 2-propanol (99.80%) were purchased from Shanghai Titan Scientific Co., Ltd (Shanghai, China). Bovine serum albumin (BSA) was from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Carboxyl magnetic beads (50 mg/mL, 300 nm in diameter) were purchased from Biomag Biotechnology Co., Ltd. (Wuxi, China). α-Cyano-4-hydroxycynnamic acid (CHCA), sinapinic acid (SA, 99.0%), cytochrome *C* from equine heart (≥95%), and myoglobin from equine skeletal (≥95%) were obtained from Sigma-Aldrich Inc. (St. Louis, USA). Hydrochloric acid (HCl, 36–38%) was obtained from Collins Reagent Co., Ltd (Shanghai, China). 4-Morpholineethanesulfonic acid (MES, ≥99%) was from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Sulfo-*N*-hydroxysuccinimide (sulfo-NHS, 99%), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydro-chloride (EDC, 99.0%), soy peptone and phosphate buffered saline (PBS) were from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Trifluoroacetic acid (TFA, ≥99.9%) was purchased from Fisher Scientific Inc. (Loughborough, UK). Tween 20 was from Beijing Coolaber Technologay Co., Ltd. (Beijing, China).

Activated *Escherichia coli*. (*E. coli*. strain ATCC25922), *Staphylococcus aureus* (*S. aureus*. strain ATCC25923) and *Klebsiella pneumoniae* (*K. pneumoniae*. strain CICC20239) used in this study were kindly provided by Dr. L. Qiao at Fudan University (Shanghai, China). Anti-*E. coli* (5 mg/mL) in PBS (10 mM, pH 7.2, with 1% (m/m) BSA) was obtained from Abcam (Cambridge Science park, Cambridge, UK). Agar was from Shanghai Biyuntian Bio-Technology Co., Ltd. (Shanghai, China). Yeast extract was obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). All reagents were used as received without further

purification unless otherwise specified. Deionized (DI) water (18.2 MΩ cm) was purified by a Smart-Q deionized water system (Hitech pure water technology, Shanghai, China), and used to prepare all the aqueous solutions.

Sablefish slices were bought in October 2020 from Ftabest free trade import food center (Pudong New Area, Shanghai, China), stored at –20 °C in sealed bag and used in two months to guarantee the quality of the sample.

Functionalization of MBs

The carboxyl magnetic beads (MBs) were modified with anti-*E. coli* to form anti-*E. coli* functionalized MBs (anti-*E. coli*@MBs) via addition of sulfo-NHS to EDC reaction (Khaled et al., 2005). In detail, 2 μL of obtained MBs (50 mg/mL) were added to 200 μL of MEST (100 mM MES with 0.05% (v/v) Tween 20, pH 5.0) and washed two times by MEST buffer, and the supernatant was discarded. The pellets were collected and mixed with 500 μL of N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydro-chloride (EDC, 4 mg/mL in MEST buffer (100 mM MES with 0.05% (v/v) Tween 20, pH 5.0) on a incubator (Eppendorf ThermoMixer C, Eppendorf, Hamburg, Germany) for 15 min at 600 rpm and room temperature. Then 500 μL 10 mg/mL sulfo-*N*-hydroxysuccinimide (sulfo-NHS) solutions in MEST buffer (100 mM MES with 0.05% (v/v) Tween 20, pH 5.0) was added to the resultant suspension and shook on an incubator for 15 min to activate carboxyl groups on the surface of magnetic beads. The activated MBs were separated from the reaction solution using a magnetic separation rack and the supernatant was discarded. The pellets were collected and washed with 500 μL of phosphate buffered saline with Tween 20 (PBST, 10 mM PBS with 0.05% (v/v) Tween 20, pH 7.4) for three times and the supernatant was discarded. Subsequently, the pellets were collected, and added with 500 μL PBST (10 mM PBS with 0.05% (v/v) Tween 20, pH 7.4) and 5 μL of 5 mg/mL anti-*E. coli* in PBS (10 mM, 1% (m/m) BSA, pH 7.2) for the attachment of anti-*E. coli* onto the surface of activated magnetic beads. 500 μL of BSA solution (1% (m/m) in PBST buffer) was then added to block the unspecific adsorption sites on the surface of MBs. The prepared anti-*E. coli* functionalized magnetic beads (anti-*E. coli*@MBs) were stored in PBST (10 mM with 0.05% Tween 20, pH 7.2) at 4 °C for at most 12 h prior to further use.

Culture of bacteria

The activated bacteria were cultured with Luria-Bertani (LB) liquid medium (10 mg/mL soy peptone, 10 mg/mL NaCl, and 5 mg/mL yeast extract) at 37 °C for 12 h. The concentrations of *E. coli* bacterial suspension were determined by measuring the optical density of bacteria at an absorbance of 600 nm (OD_{600}) on a UV–Vis spectrometer (UV-1900 UV–VIS-NIR spectrophotometer, Shimadzu®, Kyoto, Japan). The OD_{600} value of 0.101 ± 0.003 corresponds to the concentrations of 10^8 CFU/mL for *E. coli*. The concentration of *E. coli* in a bacterial suspension was calculated by using an equation of $y = 0.0657 \times 10^{-8}x + 0.046$ ($r^2 = 9917$), where y is the measured OD_{600} of the unknown bacterial suspension, x is the concentration of bacteria with unit CFU/mL.

Extraction of proteins from fish muscle samples

Fish sarcoplasmic proteins (FSP) of sablefish were extracted by referring to a method adopted by a previous report (Stephansen, Chronakis and Jessen 2014). In brief, 6 g of sablefish muscle sample was obtained from four slices of sablefish muscle samples, and mixed with 40 mL of buffer solution (20 mM Tris-HCl containing 5 mM EDTA, pH 7.5), and homogenized for 10 min with a homogenizer (F6/10, Shanghai Jingxin Technology Co., Ltd., Shanghai, China). Then, the resultant sample was centrifuged at 6,797 g and 4 °C for 30 min on a centrifuge system (5810 R®, Eppendorf, Hamburg, Germany).

Fish muscle proteins were extracted by using a method adopted by our previous report (Wang and Bi 2021). In brief, 2 g of fish muscle tissue sample and 10 mL of 0.1 % (v/v) TFA aqueous solution were put in a beaker, and mixed thoroughly with a glass rod, and homogenized for 2 min. The mixture was then placed on a hot plate (RH digital, IKA, Staufen, Germany), and heated at 80 °C for 2 min. The resultant sample was cooled to room temperature (ca. 10 min) and centrifuged at 20,000 g for 5 min on a centrifuge system, and filtered by using 0.22 μm filter membrane prior to further analysis.

Capture of bacteria by anti-E. coli@MBs

Bacteria aqueous suspension with different cell density such as 10^8, 10^6, and 10^4 CFU/mL were prepared in water or spiked in fish muscle protein extracts for further analysis. 20 μL of the above prepared anti-*E. coli*@MBs were added to 1 mL bacteria suspension to capture the bacteria. After incubation at 37 °C for 30 min with consecutive shaking on an incubator, the anti-*E. coli*@MBs attached with bacteria conjugates (anti-*E. coli*@MBs@bacteria) were collected by standing for 1 min on a magnetic beads separation rack (BMB-01, 16 holes for 1.5 to 2 mL tubes, BioMag Scientific Inc., Wuxi, China). The pellets, anti-*E. coli*@MBs@bacteria conjugates, were collected and washed once with 200 μL of PBST (10 mM PBS with 0.05% (v/v) Tween 20, pH 7.2) and then once with 200 μL of DI water, afterwards, they were resuspended in 2 μL of DI water prior to MALDI-TOF MS analysis.

MALDI-TOF MS analysis

0.5 μL of anti-*E. coli*@MBs@bacteria sample suspension in water was deposited on a sample spot of a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI) target plate with at least four repetitions droplet-by-droplet (Zhu et al. 2016). Matrix of α-cyano-4-hydroxycinnamic acid (CHCA, 1 μL, 10 mg/mL in 50% ACN, 49.9% H_2O, 0.1% TFA, v/v) and sinapinic acid (SA, 1 μL, 15 mg/mL in 50% ACN, 49.9% H_2O, 0.1% TFA, v/v) was respectively deposited to overlay the dried sample spots also with the droplet-by-droplet protocol for MALDI-TOF MS analysis.

MALDI-TOF MS analysis was performed on a Bruker MicroFlex LRF mass spectrometer (Bremen, Germany) under linear positive mode. The instrumental parameters were set as: 45% laser intensity, laser attenuator with 35% offset and 40% range, accumulation from 400 laser shots, 10.3 × detector gain, and 150 ns delayed extraction time. Aqueous solution containing cytochrome *C* and myoglobin was used for external mass calibration. All experiments were at least triplicated on at least -three independent occasions.

Culture of bacteria on the surface of fish muscle

Frozen fish sample was thawed at 4 °C for 3 h. The fish muscle tissues were cut into manageable portions (usually around 3 cm in width and 1.5 cm in thickness) with a scalpel, then their outer surface sterilized by immersion in boiling water for 20 s, then they were transferred to sterile Petri dishes (high-impact polystyrene, size 100 mm × 20.5 mm, Titan, Shanghai, China).

E. coli was inoculated onto the surface of fish muscle piece on a sterile operating table and cultured at 37 °C for 12 h. The fish muscle pieces were placed in DI water and placed on a sterile operating table for 30 min to make the bacteria diffuse into the water. The absorbance at 600 nm (OD_{600}) of the resultant suspension was measured to determine the bacterial concentration, and then diluted to 10^8 CFU/mL. 20 μL anti-*E. coli*@MBs were added to 1 mL bacteria suspension for the capture of the bacteria prior to MALDI-TOF MS analysis.

Specificity detection of bacteria captured by MBs

10^8 CFU/mL of *S. aureus*, *E. coli* and *K. pneumoniae* were spiked in FSP extract of sablefish, respectively. The obtained suspensions were

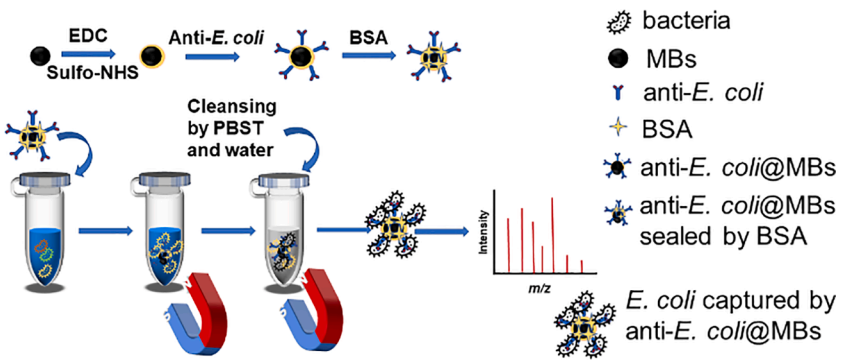


Fig. 1. Schematic diagram of processes of measuring bacteria from fish muscle extracts by using magnetic beads (MBs) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Modification of MBs with anti-bacterial antibodies via the reaction of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydro-chloride (EDC) and sulfo-*N*-hydroxysuccinimide (sulfo-NHS), and used for bacterial capture from different media. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to characterize the captured bacteria.

mixed together with ratio of 1:1:1 to get a sample containing multiple species of bacteria. FSP extract of sablefish spiked with *S. aureus* and *K. pneumoniae*, respectively, were studied as control. 20 μL anti-*E. coli*@MBs were added to 1 mL bacteria suspension for the capture of the bacteria.

Calculation of similarity scores between mass spectra

Raw mass spectral data were extracted by using a Flex Analysis 3.4 user manual revision 1 software (Bruker Daltonik GmbH, Bremen, Germany). And processed by R studio 1.1.419 software (RStudio Inc.). The averaged mass spectrum was obtained by processing several mass spectral data with mMass open source MS tool (*http://www.mmass.org/*). A public on-line platform (BacteriaMS, *http://bacteriams.com/*) was used to calculate the similarity scores between two mass spectra by using cosine correlation method.

Results and discussion

Measurement of bacteria in fish muscle protein extracts by MBs and MALDI-TOF MS

Seafood products are widely consumed all around the world and play a significant role on the economic market. Foodborne pathogens can contaminate seafood and thus pose a risk to human health (Bonnin-Jusserand et al. 2019). The present study was aimed at detecting bacteria from seafood matrices. Fig. 1 schematically illustrates the work flow of detecting bacteria from seafood protein extracts by magnetic beads (MBs). MBs were functionalized with anti-bacterial antibodies to capture the bacterial in seafood matrices.

On the other hand, immunoglobulin G (IgG) has affinity to protein A, protein G, protein L and glycans on the surface of bacterial cells, and therefore it is applicable to the preconcentration of bacterial species. The present study utilizes anti-*E. coli*, belonging to IgG group, attached MBs (anti-*E. coli*@MBs) to enrich or capture *E. coli*, which is taken as a bacteria model, from seafood matrices.

During the experiment, N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydro-chloride (EDC) and sulfo-*N*-hydroxysuccinimide (sulfo-NHS) solutions were added in a buffer with carboxyl-modified magnetic beads to activate carboxyl groups on the surface of magnetic beads. Then antibodies, herein anti-*E. coli*, were attached onto the surface of activated magnetic beads *via* addition of sulfo-NHS to EDC reaction. BSA was then added to block the unspecific adsorption sites on the surface of MBs. The functionalized magnetic beads were added in seafood tissue protein extracts for capturing the spiked bacteria. The anti-*E. coli*@MBs captured bacteria (anti-*E. coli*@MBs@bacteria) were rinsed with buffer and water, respectively, and deposited on matrix-assisted laser desorption/ionization (MALDI) target plate for the identification of captured bacteria via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Capture of bacteria from fish sarcoplasmic protein (FSP) extracts

The study of marine fish products with high values deserves increased attention since marine fish products with high values are increasingly appreciated in China in the last three decades (Xiong et al. 2016). Bacteria of seafood and aquaculture can form economic and biological threat to aquatic animals and humans. Control of bacteria especially indigenous pathogenic bacteria in seafood is an important concern for aquaculture and in the seafood sector.

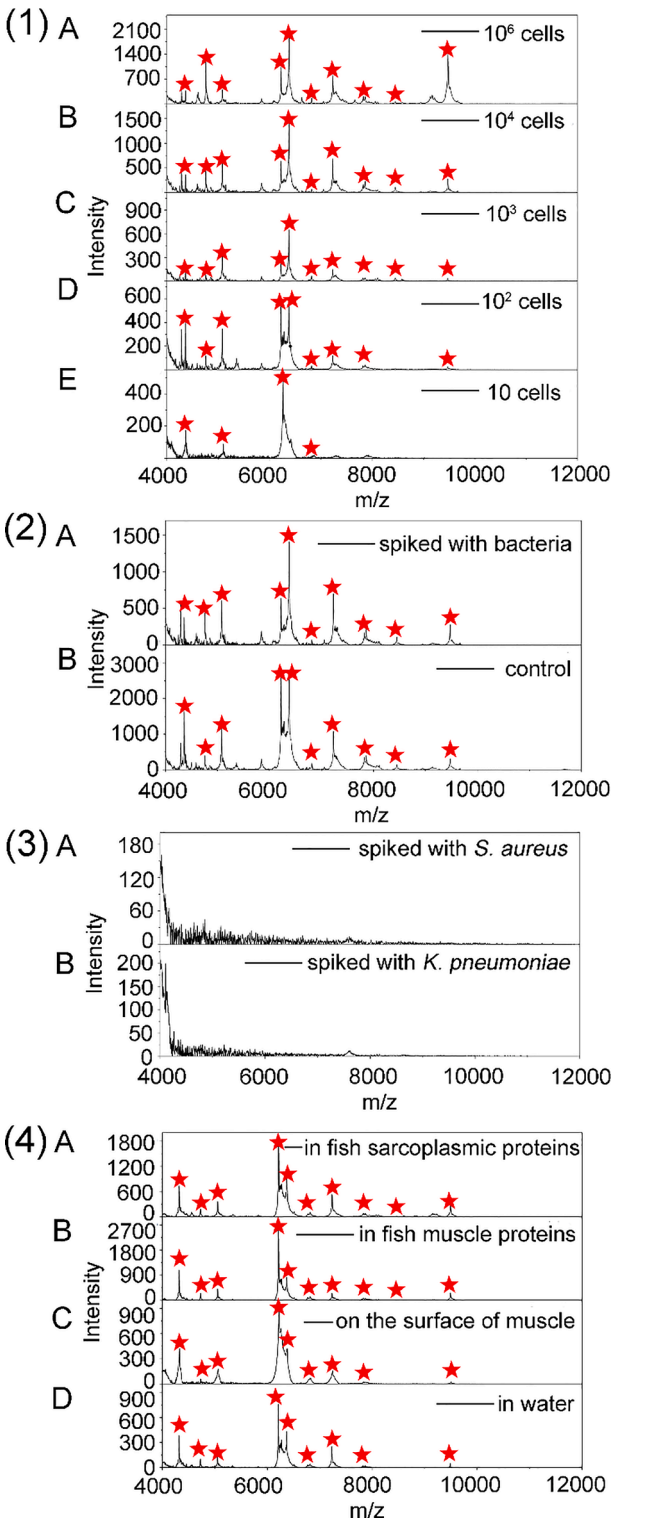
Escherichia coli (*E. coli*) is frequently used as a model organism and applied in biotechnology (Idalia and Bernardo 2017). *E. coli* are generally harmless, but some *E. coli* are pathogenic and can contaminate food, water and the environment. The Food and Agriculture Organization (FAO) reported that hundreds of thousands of people are made ill by *E. coli* each year, with hundreds of them dying.

Herein, *E. coli* was selected as a model of bacteria and spiked in protein extracts of sablefish muscle to verify the capture of bacteria by anti-*E. coli* functionalized MBs (anti-*E. coli*@MBs). During the experiment, fish sarcoplasmic proteins (FSP) of sablefish were extracted to demonstrate the feasibility of applying the method in sensing bacteria from sample with complex matrices. FSP solution was spiked with standard *E. coli* strains with known concentrations to mimic samples of proteins with bacteria. Anti-*E. coli*@MBs were added to capture the bacteria from the protein extracts spiked with *E. coli*. As shown in Fig. 2 (1), bacteria can be captured by the anti-*E. coli*@MBs sorbent and identified by MALDI-TOF MS analysis. Ten ions with high or relatively high signal-to-noise ratio (S/N) specific for *E. coli* are listed in Table 1. The results illustrate that the magnetic beads functionalized with anti-*E. coli* can successfully capture bacteria in the FSP sample.

Reproducibility of capturing bacteria from fish sarcoplasmic proteins (FSP) by MBs

Functional molecules such as proteins are important factors that can impact the accuracy of identification of molecules based on MALDI-TOF MS, and may result in errors during comparison with the standard bacterial spectra in the database (Sun et al. 2021). Reproducibility of anti-*E. coli* functionalized MBs (anti-*E. coli*@MBs) for the capture of bacteria from seafood matrices was investigated. Experimentally, the *E. coli* concentration was controlled to reach 10^7 CFU/mL in the fish sarcoplasmic proteins (FSP) extracts of sablefish muscle which was taken as a model of matrices with complex compounds, then *E. coli* were captured by anti-*E. coli*@MBs and detected by MALDI-TOF MS. In addition, newly extracted FSP of sablefish was used in each experimental run.

Fig. 3 compares the obtained mass spectra of the bacteria in FSP extracts of sablefish muscle captured by anti-*E. coli*@MBs in the three independent experimental runs. The averaged similarity score among the obtained mass spectra in the three runs can be calculated with the value of 0.95 ± 0.03 (RSD = 3.2%), illustrating the present protocol is



(caption on next column)

Fig. 2. (1) Representative matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra of *E. coli* in 1 mL fish sarcoplasmic proteins (FSP) of sablefish at different cell density. Bacteria suspension with (A)10^6, (B) 10^4, (C)10^3, (D)10^2 and (E)10 cells were captured by anti-*E coli* functionalized MBs (anti-*E. coli*@MBs). Experimentally, the obtained magnetic beads are activated and functionalized with anti-*E. coli*, and then added in solutions of FSP spiked with *E. coli*. α-Cyano-4-hydroxycinnamic acid (CHCA) matrix was preferentially used in performing MALDI-TOF MS (Appendix A). The cell density in the bacteria suspension is indicated in the top right corner of each inlet. (2) MALDI-TOF mass spectra for bacteria captured by magnetic beads from samples spiked with *E. coli*, *S. aureus*, and *K. pneumoniae*. *E. coli*, *S. aureus*, and *K. pneumoniae* were added in the solution of fish sarcoplasmic protein (FSP) extracts of sablefish at 10^8 CFU/mL as a sample spiked with bacteria (A). *E. coli* were spiked in the solution of FSP extracts of sablefish at 10^8 CFU/mL as control (B). The magnetic beads were functionalized with *E. coli* antibody (anti-*E. coli*@MBs) and used to capture bacteria in FSP sample. Anti-*E. coli*@MBs captured bacteria (anti-*E. coli*@MBs@bacteria) were rinsed and deposited on MALDI target plate for MALDI-TOF MS analysis. α-Cyano-4-hydroxycinnamic acid (CHCA) was used as matrix. (3) MALDI-TOF mass spectra of *S. aureus* (A), and *K. pneumoniae* (B) captured by 20 μL anti-*E. coli*@MBs from 1 mL fish sarcoplasmic protein (FSP) extracts of sablefish spiked with 10^8 CFU/mL of bacteria, respectively. α-Cyano-4-hydroxycinnamic acid (CHCA) was used as matrix. (4) MALDI-TOF mass spectra for the samples of bacteria captured by magnetic beads from different complex samples. *E. coli* was added to the solution in different media to arrive at a bacterial concentration of 10^7 CFU/mL. The obtained magnetic beads were activated and conjugated with *anti-E. coli* antibody, and then added in samples of (A) fish sarcoplasmic protein (FSP), (B) fish protein extracted by using TFA solution, (C) fish muscle surface and (D) water. Anti-*E. coli*@MBs captured bacteria (anti-*E. coli*@MBs@bacteria) were rinsed and deposited on MALDI target plate and covered by the α-cyano-4-hydroxycinnamic acid (CHCA) matrix for MALDI-TOF MS analysis. Peaks corresponding to bacterial cellular components are denoted by red stars.

highly reproducible for capturing bacteria from complex matrices.

Sensitivity of MBs for capturing bacteria from fish sarcoplasmic proteins

Different magnetic particles based bacterial capture and/or enrichment has been used for sample pre-treatment. Lin *et al* made detection limit down to 7.4 × 10^4 CFU/mL by using magnetic nanoparticles modified with vancomycin to capture bacteria in human urinate (Lin et al. 2005). Chen *et al* presented a method for bacterial identification in food matrices using nanoLC-MS, and the minimum concentration of *E. coli* detected was 5 × 10^3 CFU/mL by the assistance of magnetic zirconia nanoparticles (Chen et al. 2019). Amoxicillin functionalized magnetic nanoparticles were used to detect *E. coli* in human blood, and a LOD of 10^3 CFU/mL was achieved (Hasan, Guo and Wu 2016). Zhu *et al* used magnetic beads modified with specific antibodies to capture target bacteria at concentrations as low as 500 CFU/mL in serum for MALDI-TOF MS identification (Zhu et al. 2016).

Table 2 compares the present approach and the reported methods for the analysis of pathogenic bacteria based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), including the detected bacteria and limit of detection (LOD) accomplished under each method. In the present study, the bacterial concentration in the fish sarcoplasmic proteins (FSP) of sablefish was adjusted to verify the sensitivity of magnetic beads for capturing bacteria. As shown in Fig. 2(1) E, the bacterial suspension with a minimum bacterial concentration of 10 CFU/mL in the FSP solution can still be detectable, illustrating the relatively high sensitivity of the present protocol for bacterial detection.

Table 1
The star labeled ions in Fig 2. The positions of ions were calculated from six mass spectra. The underlined ions are the ones with close mass-to-charge ratio (m/z) that were previously reported.

ions with m/z =	signal-to-noise ratio (S/N)
6255.14±1.81	39
6411.14±1.24	22
7274.28±2.49	11
9554.14±1.95	9
4365.85±1.72	8
4759.37±1.39	6
7873.23±1.16	5
6847.93±2.25	3
5381.71±1.85	3
8604.85±2.23	3

Specificity of MBs for capturing bacteria from fish sarcoplasmic protein (FSP) of sablefish

To investigate the specificity of the present protocol for *E. coli* detection, *E. coli* antibody modified magnetic beads (anti-*E. coli*@MBs) were used to capture bacteria from bacterial suspensions with and without *E. coli*. During the experiment, bacteria, *S. aureus*, *E. coli*, and *K. pneumoniae* were spiked in FSP extracts of sablefish to investigate the specificity of the present protocol for *E. coli* measurement. As shown in Fig. 2(2) only signals specific to *E. coli* can be detected in samples containing *S. aureus*, *E. coli*, and *K. pneumoniae*. In addition, in bio-samples with *S. aureus* and *K. pneumoniae*, respectively, as shown in Fig. 2(3), there are no signals specific to bacteria *S. aureus* and *K. pneumoniae*, showing no bacteria were captured by the utilized anti-*E. coli*@MBs. The results illustrate the high specificity of the present protocol for *E. coli* detection. It can be found that the specificity of the anti-bacterial antibodies coupled with the immunomagnetic properties of the beads, allows a target bacterium to be captured. By changing the anti-bacterial antibodies on the magnetic beads, different bacteria may be detected. The present protocol holds potential to be applied in pathogenic and spoilage bacterial detection in seafood industry for specific recognition.

Microbial safety test on the surface of fish muscle

Herein, the developed protocol was used to test the bacteria on the surface of the fish muscle. As shown in Fig. 2(4)C, the present protocol can be used to sense the bacteria on the surface of fish muscle product for its bacterial control. It is promising to perform the microbial safety test of fresh produce based on the present protocol.

Comparison of bacterial capture from different complex samples by MBs

To verify the feasibility of this method for capturing *E. coli* from different complex samples, fish muscle proteins were extracted and spiked with bacteria, and anti-bacterial antibody modified magnetic beads were utilized for the bacterial capture. Fig. 2(4) illustrates the mass spectra of captured bacteria from different media, including the fish protein extracts of sablefish, surface of sablefish muscle and water. As shown in Fig. 2(4), *E. coli* exhibits peaks near m/z 4365.85 ± 1.72, 4759.57 ± 1.39, 5381.71 ± 1.85, 6255.14 ± 1.81, 6411.14 ± 1.24, 6847.93 ± 2.25 7274.28 ± 2.49, 7873.23 ± 1.16, 8604.85 ± 2.23 and 9554.14 ± 1.95 in four analyzed media. The results shown in Fig. 2(4) also illustrate that the present method can detect *E. coli* from samples with different complexity of matrices, illustrating the broad suitability of the present protocol for bacterial detection.

As shown in Appendix B, the mass spectra of *E. coli* captured from

Table 2
Comparison of the current approaches for the analysis of pathogenic bacteria using functionalized magnetic particles coupled matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

No.	MBs	detected bacteria	LOD (CFU/mL)	Reference
1	immunomagnetic beads	*Salmonella choleraesuis*, *Shigella boydii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus circulans*	1×10^7	Madonna et al. 2001
2	self-regulating magnetic nanoparticles	*Staphylococcus saprophyticus*, *Staphylococcus aureus*	7.4×10^4; 7.8×10^4	Lin et al. 2005
3	200 nm carboxyl MBs	*Staphylococcus aureus*, *Kocuria rosea*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, Enterobacter cloacae, *Pseudomonas aeruginosa*	10^3	Hasan et al. 2016
4	specific antibody-modified MBs	*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*	8×10^3; 5×10^2; 4.5×10^2	Zhu et al. 2016
5	300 nm carboxyl MBs	*Escherichia coli*	10	this work

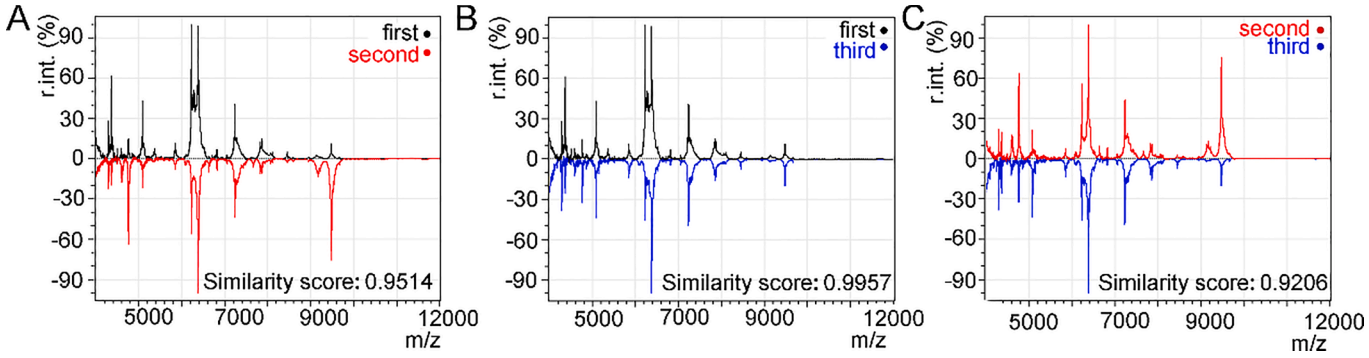


Fig. 3. Comparison of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra of bacteria captured by antibodies functionalized magnetic beads from extracts of sablefish muscle in three independent runs. Fish sarcoplasmic proteins (FSP) of sablefish were extracted. The magnetic beads are activated and modified with anti-*E. coli*, and then added in solutions of FSP spiked with *E. coli*. The spiked bacterial concentration was 10^7 CFU/mL. Bacteria captured by anti-*E. coli*@MBs (anti-*E. coli*@MBs@bacteria) were cleaned and deposited on MALDI target plate for α-cyano-4-hydroxycinnamic acid (CHCA) assisted MALDI-TOF mass spectrometry analysis.

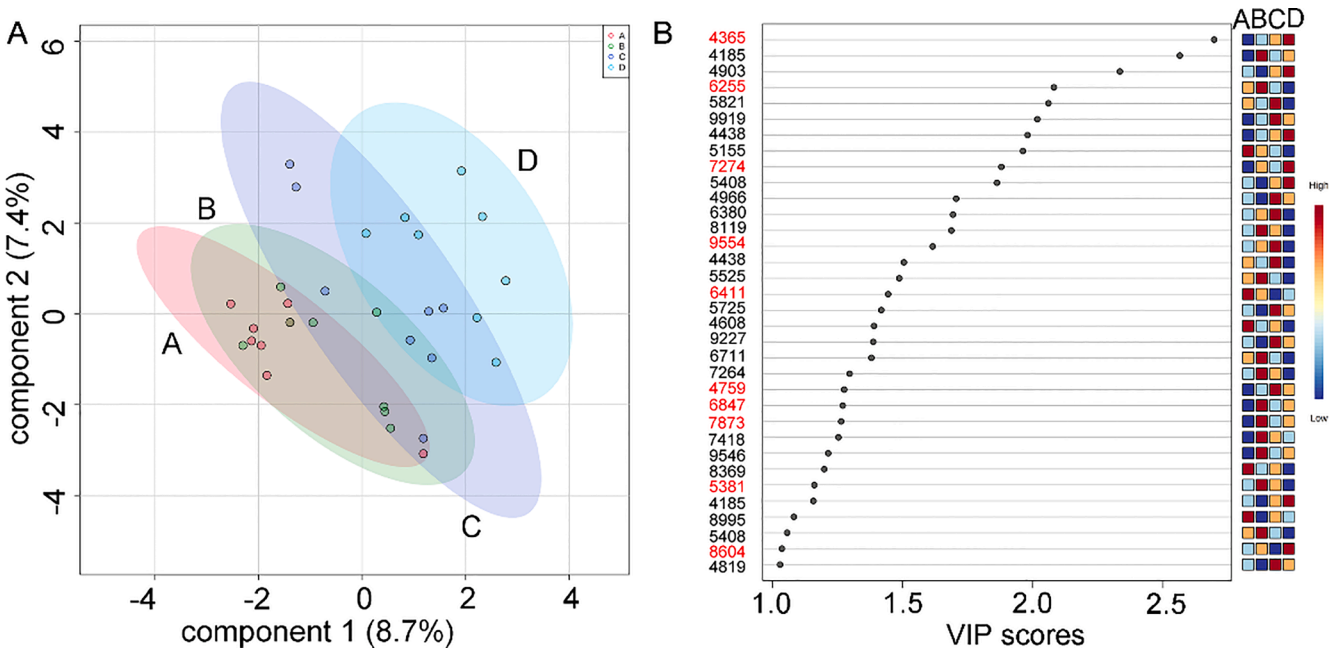


Fig. 4. (A) Plot of partial least-squares discriminant analysis (PLS-DA) of mass spectra for the *E. coli* captured by magnetic beads from samples with different complexities of matrices including water, fish muscle surface, extracts of fish sarcoplasmic protein (FSP) and fish muscle protein. (B) Plot of variable of importance in projection (VIP) scores of mass spectral signals with VIP values more than 1.0 among different complex samples. Ten ions that contribute significantly to differentiate *E. coli* were screened out with relative abundances indicated in red (high) or blue (low) color. *m/z* values for each ion are listed on the y-axis. Inlet or capital letter indicates the capture of *E. coli* from water (A), the surface of fish muscle (B), fish sarcoplasmic proteins (FSP) (C), and in fish muscle proteins (D). Star labeled peaks in Fig. 2 are denoted in red color in Fig. 4B.

different media were compared. The averaged similarity score among the obtained mass spectra was calculated with the value of 0.90 ± 0.03 and relative standard deviation (RSD) of 3.0%, illustrating the high similarity among the captured bacteria by functionalized magnetic beads.

Discovery of biomarker ions for E. coli identification by MBs

Herein, partial least squares discriminant analysis (PLS-DA) was used to classify the *E. coli* captured from different complex samples by magnetic beads. As shown in Fig. 4 A, it can be found that the points representing the information of mass spectra of bacteria cluster in very close areas, showing the high similarity of these datasets.

As shown in Appendix C, Table C.1 lists the peaks of *E. coli.* directly analyzed by MALDI-TOF MS. Ions with peaks at *m/z* = 4365, 5381, 6255, 6411, 6849, 7274, 7871, 8604 and 9554 were reported in previous studies (Yan et al. 2020, Feng et al. 2020, Božik et al. 2018). In the present study, based on the big datasets of mass spectra and 34 biomarker ions discovered by assistance of variables importance in projection (VIP) analysis, the ions with *m/z* = 4365, 4759, 5381, 6255, 6411, 6847, 7274, 7873, 8604 and 9554 with relatively large signal-to-noise ratio (S/N) are preferentially recommended as the biomarker ions for the identification of *E. coli* from different food matrices *via* MALDI-TOF MS. It is promising to apply this method for discovering biomarker ions based on the VIP analysis and use of anti-bacterial antibodies for different bacterial identification *via* MALDI-TOF MS.

Conclusions

Seafood is one of the leading products implicated in foodborne outbreaks worldwide. In the present study, the enrichment and/or capture of *E. coli* and the identification of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) by carboxyl magnetic beads modified with anti-*E. coli* (anti-*E. coli*@MBs) provide a rapid method for the identification of bacteria in aquatic products. The detection limit (LOD) of this method was found to be 10 CFU/mL. The averaged similarity score among the obtained mass spectra in the three independent experiments of capturing *E. coli* from sablefish FSP extracts was calculated with the value of 0.95 ± 0.03, showing the excellent reproducibility of the present protocol for bacterial capture. The developed method was successfully applied for microbial safety test on the surface of fish muscle. The bacterial identification accuracy from samples with different matrices was found to be good with 3% RSD. Magnetic beads functionalized with anti-*E. coli* (anti-*E. coli*@MBs) only provide high specific detection of *E. coli* in complex biological samples when spiking *S. aureus* and *K. pneumoniae* in samples as interference. Based on variable importance in projection (VIP) scores, the biomarker ions discovered for *E. coli*s recognition include *m/z* = 4365, 4759, 5381, 6255, 6411, 6847, 7274, 7873, 8604 and 9554. The present protocol is expected to be further proved by the detection of other foodborne pathogens.

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CRediT authorship contribution statement

Zhaoliang Chai: Validation, Software, Visualization, Writing – original draft. **Hongyan Bi:** Supervision, Funding acquisition, Investigation, Methodology, Project administration, Resources, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100225.

References

Bae, Y. M., Baek, S. Y., & Lee, S. Y. (2012). Resistance of pathogenic bacteria on the surface of stainless steel depending on attachment form and efficacy of chemical sanitizers. *International Journal of Food Microbiology, 153*, 465–473. https://doi.org/10.1016/j.ijfoodmicro.2011.12.017

Balière, C., Rincé, A., Thevenot, D., & Gourmelon, M. (2015). Successful detection of pathogenic Shiga-toxin-producing *Escherichia coli* in shellfish, environmental waters and sediment using the ISO/TS-13136 method. *Letters in Applied Microbiology, 60*, 315–320. https://doi.org/10.1111/lam.12386

Bao, Y., Zhai, Z., Wang, S., Ma, J., Zhang, W., & Lu, C. (2013). Chaperonin GroEL: A novel phylogenetically conserved protein with strong immunoreactivity of Avian Pathogenic *Escherichia coli* isolates from duck identified by immunoproteomics. *Vaccine, 31*, 2947–2953. https://doi.org/10.1016/j.vaccine.2013.04.042

Ben Hamed, S., Tavares Ranzani-Paiva, M. J., Tachibana, L., de Carla Dias, D., Ishikawa, C. M., & Esteban, M. A. (2018). Fish pathogen bacteria: Adhesion, parameters influencing virulence and interaction with host cells. *Fish Shellfish Immunol, 80*, 550–562. https://doi.org/10.1016/j.fsi.2018.06.053

Bonnin-Jusserand, M., Copin, S., Le Bris, C., Brauge, T., Gay, M., Brisabois, A., … Midelet-Bourdin, G. (2019). Vibrio species involved in seafood-borne outbreaks (*Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus*): Review of microbiological versus recent molecular detection methods in seafood products. *Critical Reviews in Food Science and Nutrition, 59*, 597–610. https://doi.org/10.1080/10408398.2017.1384715

Božik, M., Cejnar, P., Maršík, P., & Klouček, P. (2018). Data on low-molecular weight proteins of *Escherichia coli* treated by essential oils components, tetracycline, chlorine and peroxide by MALDI-TOF MS. *Data in Brief, 21*, 962–965. https://doi.org/10.1016/j.dib.2018.10.050

Chang, Z. M., Wang, Z., Shao, D., Yue, J., Lu, M. M., Li, L., … Dong, W. F. (2018). Fluorescent-magnetic Janus nanorods for selective capture and rapid identification of foodborne bacteria. *Sensors and Actuators B-Chemical, 260*, 1004–1011. https://doi.org/10.1016/j.snb.2018.01.123

Chen, C.-T., Yu, J.-W., & Ho, Y.-P. (2019). Identification of bacteria in juice/lettuce using magnetic nanoparticles and selected reaction monitoring mass spectrometry. *Journal of Food and Drug Analysis, 27*, 575–584. https://doi.org/10.1016/j.jfda.2018.09.006

Chen, L., Deng, L., Liu, L., & Peng, Z. (2007). Immunomagnetic separation and MS/SPR end-detection combined procedure for rapid detection of Staphylococcus aureus and protein A. *Biosensors and Bioelectronics, 22*, 1487–1492. https://doi.org/10.1016/j.bios.2006.06.038

de Oliveira, E. F., Tikekar, R., & Nitin, N. (2018). Combination of aerosolized curcumin and UV-A light for the inactivation of bacteria on fresh produce surfaces. *Food Research International, 114*, 133–139. https://doi.org/10.1016/j.foodres.2018.07.054

Feng, B., Shi, H., Xu, F., Hu, F., He, J., Yang, H., … Yu, S. (2020). FTIR-assisted MALDI-TOF MS for the identification and typing of bacteria. *Analytica Chimica Acta, 1111*, 75–82. https://doi.org/10.1016/j.aca.2020.03.037

Foysal, M. J., Momtaz, F., Robiul Kawser, A. Q. M., Chaklader, M. R., Siddik, M. A. B., Lamichhane, B., … Fotedar, R. (2019). Microbiome patterns reveal the transmission of pathogenic bacteria in hilsa fish (*Tenualosa ilisha*) marketed for human consumption in Bangladesh. *Journal of Applied Microbiology, 126*, 1879–1890. https://doi.org/10.1111/jam.14257

Geng, Y., Tan, K., Liu, L., Sun, X. X., Zhao, B., & Wang, J. (2019). Development and evaluation of a rapid and sensitive RPA assay for specific detection of *Vibrio parahaemolyticus* in seafood. *BMC Microbiology, 19*, 186. https://doi.org/10.1186/s12866-019-1562-z

Gram, L., & Dalgaard, P. (2002). Fish spoilage bacteria-problems and solutions. *Current Opinion in Biotechnology, 13*, 262–266. https://doi.org/10.1016/S0958-1669(02)00309-9

Hasan, N., Guo, Z., & Wu, H. F. (2016). Large protein analysis of *Staphylococcus aureus* and *Escherichia coli* by MALDI TOF mass spectrometry using amoxicillin functionalized magnetic nanoparticles. *Analytical and Bioanalytical Chemistry, 408*, 6269–6281.

Idalia, V.-M.-N., & Bernardo, F. (2017). Escherichia coli as a Model Organism and Its Application in Biotechnology. In S. Amidou (Ed.), *Escherichia coli* (pp. 251–274). E-Publishing Inc.

Jadhav, S., Sevior, D., Bhave, M., & Palombo, E. A. (2014). Detection of *Listeria monocytogenes* from selective enrichment broth using MALDI–TOF Mass Spectrometry. *Journal of Proteomics, 97*, 100–106. https://doi.org/10.1016/j.jprot.2013.09.014

Jadhav, S. R., Shah, R. M., & Palombo, E. A. (2021). MALDI-ToF MS: A rapid methodology for identifying and subtyping listeria monocytogenes. *Methods in Molecular Biology, 2220*, 17–29. https://doi.org/10.1007/978-1-0716-0982-8_2

Kalchayanand, N., Arthur, T. M., Bosilevac, J. M., Schmidt, J. W., Shackelford, S. D., Brown, T., & Wheeler, T. L. (2018). Surface pH of Fresh Beef as a Parameter To Validate Effectiveness of Lactic Acid Treatment against *Escherichia coli* O157:H7 and Salmonella. *Journal of Food Protection, 81*, 1126–1133. https://doi.org/10.4315/0362-028X.JFP-17-469

Khaled, A., Mahmoud, Y.-T., Long, G., Schatte, H.-B., & Kraatz. (2005). Rearrangement of the active ester intermediate during HOBt/EDC amide coupling. *European Journal of Inorganic Chemistry*. https://doi.org/10.1002/ejic.200400504

Leff, J. W., & Fierer, N. (2013). Bacterial communities associated with the surfaces of fresh fruits and vegetables. *PLoS One, 8*, Article e59310. https://doi.org/10.1371/journal.pone.0059310

Lin, Y. S., Tsai, P. J., Weng, M. F., & Chen, Y. C. (2005). Affinity capture using vancomycin-bound magnetic nanoparticles for the MALDI-MS analysis of bacteria. *Analytical Chemistry, 77*, 1753–1760. https://doi.org/10.1021/ac048990k

Ma, B., Li, J., Chen, K., Yu, X., Sun, C., & Zhang, M. (2020). Multiplex recombinase polymerase amplification assay for the simultaneous detection of three foodborne pathogens in seafood. *Foods, 9*(3), 278. https://doi.org/10.3390/foods9030278

Madonna, A. J., Basile, F., Furlong, E., & Voorhees, K. J. (2001). Detection of bacteria from biological mixtures using immunomagnetic separation combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry, 15*, 1068–1074. https://doi.org/10.1002/rcm.344

Manafi, L., Aliakbarlu, J., & Dastmalchi Saei, H. (2020). Antibiotic resistance and biofilm formation ability of Salmonella serotypes isolated from beef, mutton, and meat contact surfaces at retail. *Journal of Food Sciences, 85*, 2516–2522. https://doi.org/10.1111/1750-3841.15335

Mao, S., Lu, C., Li, M., Ye, Y., Wei, X., & Tong, H. (2018). Identification of key aromatic compounds in Congou black tea by partial least-square regression with variable importance of projection scores and gas chromatography–mass spectrometry/gas chromatography–olfactometry. *Journal of the Science of Food and Agriculture, 98*. https://doi.org/10.1002/jsfa.9066

Møretrø, T., & Langsrud, S. (2017). Residential bacteria on surfaces in the food industry and their implications for food safety and quality. *Comprehensive Reviews in Food Science and Food Safety, 16*, 1022–1041. https://doi.org/10.1111/1541-4337.12283

Pankratov, D., Bendixen, M., Shipovskov, S., Gosewinkel, U., & Ferapontova, E. E. (2020). Cellulase-linked immunomagnetic microbial assay on electrodes: Specific and sensitive detection of a single bacterial cell. *Analytical Chemistry, 92*, 12451–12459. https://doi.org/10.1021/acs.analchem.0c02262

Sahu, B., Singh, S. D., Behera, B. K., Panda, S. K., Das, A., & Parida, P. K. (2019). Rapid detection of Salmonella contamination in seafoods using multiplex PCR. *Brazilian Journal of Microbiology, 50*, 807–816. https://doi.org/10.1007/s42770-019-00072-8

Stephansen, K., Chronakis, I. S., & Jessen, F. (2014). Bioactive electrospun fish sarcoplasmic proteins as a drug delivery system. *Colloids Surf B Biointerfaces, 122*, 158–165. https://doi.org/10.1016/j.colsurfb.2014.06.053

Sun, J., Shi, H., Xue, Y., Cheng, W., Yu, M., Ding, C., … Yu, S. (2021). Releasing bacteria from functional magnetic beads is beneficial to MALDI-TOF MS based identification. *Talanta, 225*, Article 121968. https://doi.org/10.1016/j.talanta.2020.121968

Tahergorabi, R., Hosseini, S. V., & Jaczynski, J. (2011). 6 – Seafood proteins. In P. A. Williams, & G. O. Phillips (Eds.), *Handbook of food proteins seafood proteins* (pp. 116–149). E-Publishing Inc.

Wang, C., & Bi, H. (2021). Super-fast seafood authenticity analysis by one-step pretreatment and comparison of mass spectral patterns. *Food Control, 123*, Article 107751. https://doi.org/10.1016/j.foodcont.2020.107751

Wang, S. J., Sun, C. Y., Hu, Q. S., Li, S., Wang, C. B., Wang, P., & Zhou, L. (2020). A homogeneous magnetic bead-based impedance immunosensor for highly sensitive detection of *Escherichia coli* O157:H7. *Biochemical Engineering Journal, 156*, 6. https://doi.org/10.1016/j.bej.2020.107513

Xiong, X., D'Amico, P., Guardone, L., Castigliego, L., Guidi, A., Gianfaldoni, D., & Armani, A. (2016). The uncertainty of seafood labeling in China: A case study on Cod, Salmon and Tuna. *Marine Policy, 68*, 123–135. https://doi.org/10.1016/j.marpol.2016.02.024

Yan, W., Qian, J., Ge, Y., Ye, K., Zhou, C., & Zhang, H. (2020). Principal component analysis of MALDI-TOF MS of whole-cell foodborne pathogenic bacteria. *Analytical Biochemistry, 592*, Article 113582. https://doi.org/10.1016/j.ab.2020.113582

You, S. M., Luo, K., Jung, J. Y., Jeong, K. B., Lee, E. S., Oh, M. H., & Kim, Y. R. (2020). Gold nanoparticle-coated starch magnetic beads for the separation, concentration, and SERS-based detection of E. coli O157:H7. *ACS Applied Materials & Interfaces, 12*, 18292–18300. https://doi.org/10.1021/acsami.0c00418

Zhang, R., Qin, Q., Liu, B., & Qiao, L. (2018). TiO_2-assisted laser desorption/ionization mass spectrometry for rapid profiling of candidate metabolite biomarkers from antimicrobial-resistant bacteria. *Analytical Chemistry, 90*, 3863–3870. https://doi.org/10.1021/acs.analchem.7b04565

Zhou, C., Wang, J., Li, R., & Ye, K. (2020). High-throughput sequencing analysis of the bacterial community for assessing the differences in extraction methods of bacteria separation from chilled pork. *LWT – Food Science and Technology, 134*, Article 110213. https://doi.org/10.1016/j.lwt.2020.110213

Zhu, Y., Qiao, L., Prudent, M., Bondarenko, A., Gasilova, N., Möller, S. B., … Girault, H. H. (2016). Sensitive and fast identification of bacteria in blood samples by immunoaffinity mass spectrometry for quick BSI diagnosis. *Chemical Science, 7*, 2987–2995. https://doi.org/10.1039/c5sc04919a