ESTABLISHMENT AND THOROUGH EXTERNAL VALIDATION OF AN FTIR SPECTROSCOPY CLASSIFIER FOR *SALMONELLA* SEROGROUP DIFFERENTIATION [1]

P1-38

Helene Oberreuter[1], Miriam Cordovana[2], Martin Dyk[1], Olaf Degen[2], Jörg Rau[1]
[1] CVUA Stuttgart, Fellbach, Germany, helene.oberreuter@cvuas.bwl.de
[2] Bruker Daltonics, Bremen, Germany, olaf.degen@bruker.com

Introduction

As one of the most relevant food-borne pathogens, the reliable detection, confirmation and subtyping of *Salmonella* strains is very important. *Salmonella* serotype determination by rabbit antisera posts the worldwide-accepted standard but is labor intensive, costly and needs extensive experience. As an alternative, Fourier-transform infrared (FTIR) spectroscopy has previously been used successfully to distinguish between strains of different serogroups in various bacteria. An FTIR classifier is an AI-based tool used in FTIR spectroscopy to analyze and classify different materials based on their infrared spectra.

Purpose

In the current study an FTIR Classifier operating on an IR Biotyper® spectrometer (Bruker, Germany) was designed to distinguish between 36 different *Salmonella* serogroups. Subsequently, the differentiation performance of this classifier was determined by a thorough external validation.

Methods

The external single-lab validation was carried out in accordance with the *Guidelines for Validating Species Identifications Using MALDI-TOF-MS* issued by the German Federal Office of Consumer Protection [2]: The most common *Salmonella* serogroups in Europe, O:4 (B), O:7 (C1), O:8 (C2-C3) and O:9 (D1) were chosen as target parameters and validated using a total of *n*=1039 infrared absorbance spectra from a total of *n*=167 strains pertaining to *n*=39 serogroups.

Results

In summary, serogroups O:4, O:6,7 and O:9 perfectly met the adapted *Guideline* requirements and each resulted in a >99% inclusivity. Serogroup O:8 arrived at a 96.1% true-positive rate due to a single deviating strain.

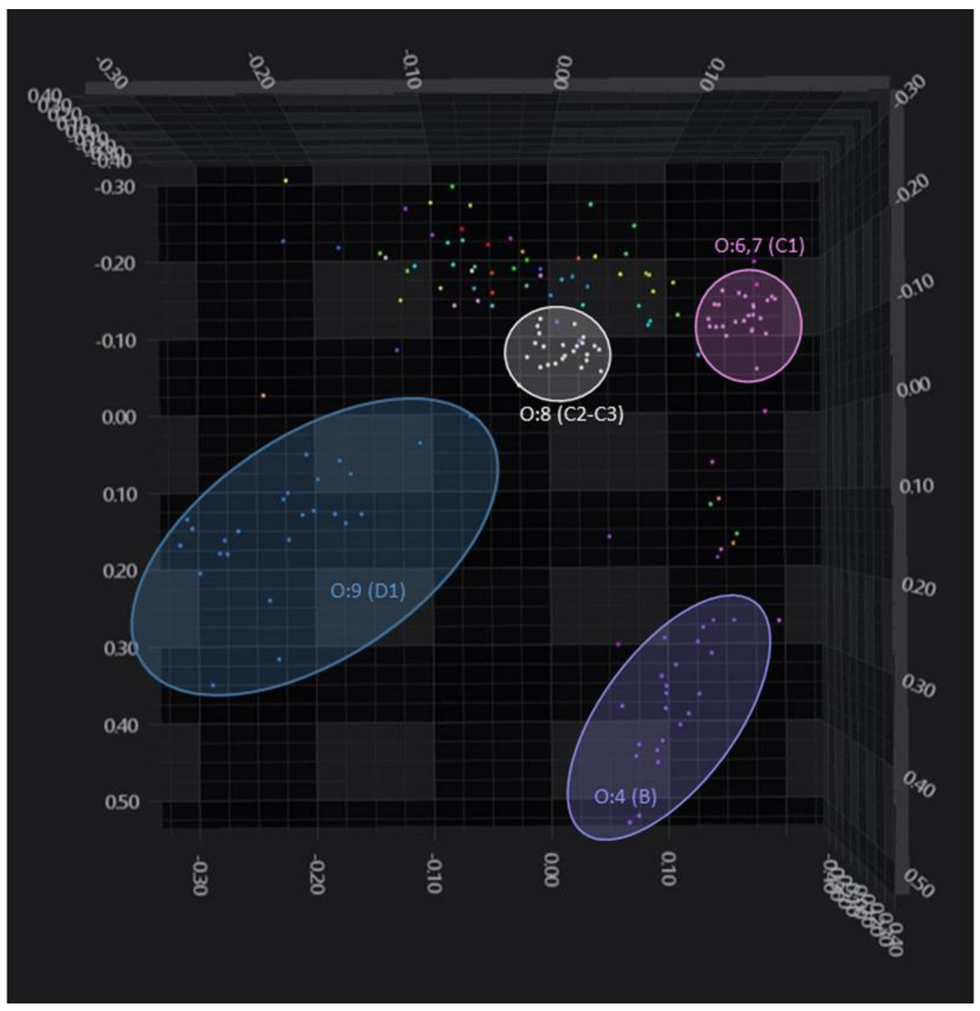

Fig. 2: 3D-scatter plot of the validation strains' average spectra (Tab. 1), 2nd derivative, spectral range 1300-800 cm^{-1}, dimensionality reduction algorithm PCA, displaying Principal Components PC 1, PC 2 and PC 3. Colorful non-marked dots denote strains not pertaining to serogroups O:4, O:7, O:8 or O:9.

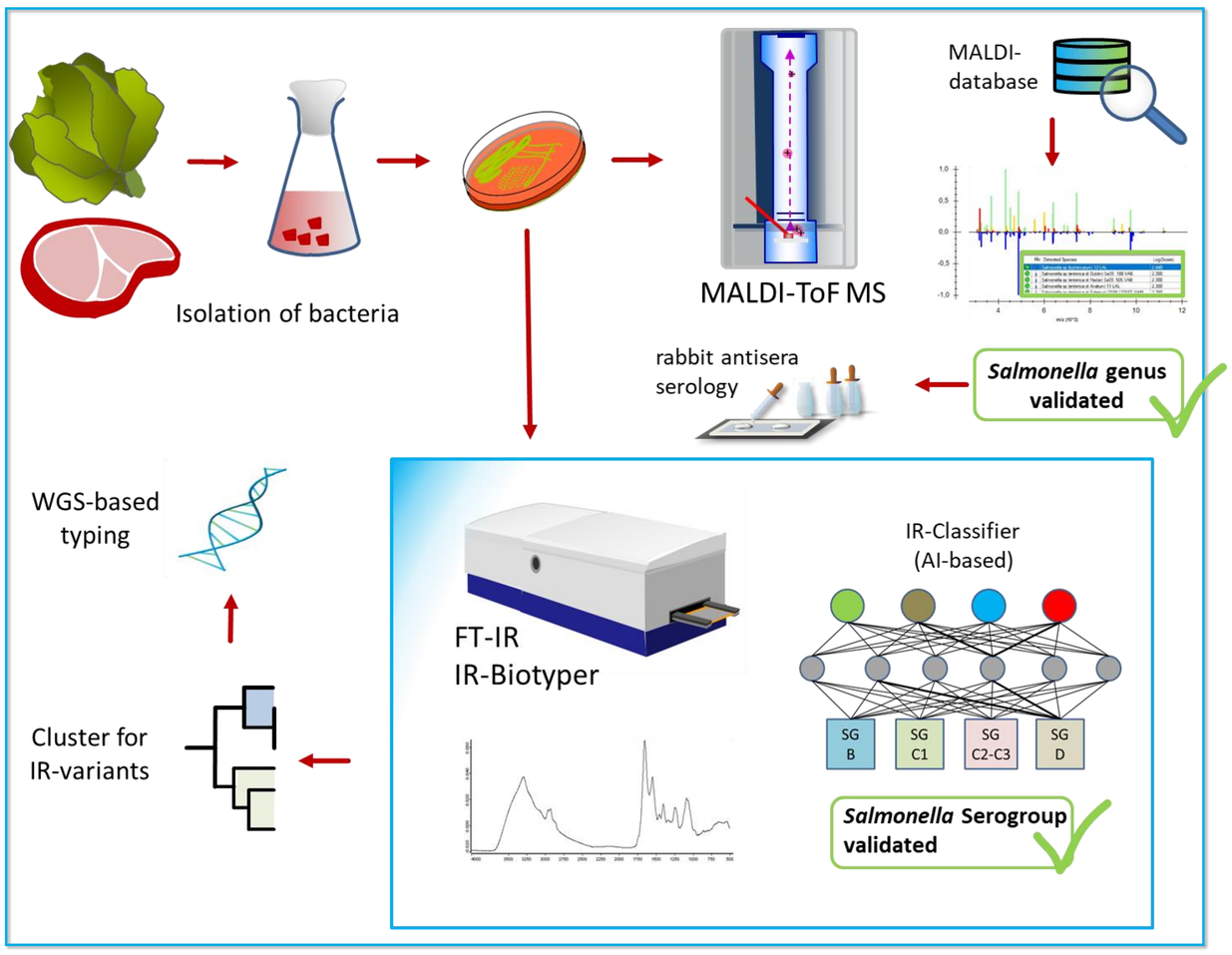

Fig. 1: Workflow for *Salmonella* differentiation from of food products and animal sources

Target parameter	SG O:4 (B)	SG O:7 (C1)	SG O:8 (C2-C3)	SG O:9 (D)
No. of isolates of the target parameter	25	25	25	25
No. of spectra of the target parameter	162	150	159	159
No. of identified spectra	162	148	154	159
Identification rate (%)	100	98.7	96.9	100
True positives	162	147	148	159
False negatives	0	1	6	0
True positive rate (%) of identified spectra	100	99.3	96.1	100
False negative rate (%) of identified spectra	0	0.7	3.9	0
No. of isolates of the non-target parameter group	142	142	142	142
No. of spectra of the non-target parameter group	877	889	880	880
No. of identified spectra	840	854	848	843
Identification rate (%)	95.8	96.1	96.4	95.8
True negatives	839	854	848	837
False positives	1	0	0	6
True negative rate (%) of identified spectra	99.9	100	100	99.3
False positive rate (%) of identified spectra	0.1	0	0	0.7

Tab. 1: Results of *Salmonella* serogroup (SG) differentiation using the *Salmonella serogroup classifier v3* on the IR Biotyper (Bruker Daltonics). *True*: The individual spectrum was correctly allocated to its serogroup; *false*: The individual spectrum was falsely assigned to another serogroup. *Positive* refers to the target parameter (= parameter of interest); *negative* refers to the non-target parameter (= control group).

Significance

This validated classification method can thus be used in routine analysis for quick and easy differentiation of the most common *Salmonella* serogroups in food surveillance. In addition, using the cluster analysis tools of the IR Biotyper, a preselection of isolates before subjecting them to thorough serotyping or whole genome sequence analysis decreases the workload in current routine analyses.

References

[1] A corresponding preprint to this study is available at https://doi.org/10.1101/2025.03.18.643663
[2] J. Rau *et al*.: *Guidelines for validating species identifications using […] MALDI-TOF-MS in a single laboratory or in laboratory networks*, coordinated by the Federal Office of Consumer Protection and Food Safety MALDI-TOF-MS working group pursuant to § 64 of the German Food and Feed Code (LFGB), Berlin (BVL), Editor. 28 Oct 2022