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Establishment of three libraries of reference MALDI-ToF spectra for *Salmonella*, *Yersinia* and *Campylobacter* strains included in the CARE collection

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Context and Objectives

The provision of reference strains (RM) is an essential component for

- the research,
- the advancement of life sciences,
- the support of the biotechnology sector,
- the further development of the bio-economy.

In particular, regarding the zoonotic and foodborne pathogens, there is a need to make easily visible and accessible the offer of Reference strains for validation methods and Proficiency testing. Phenotypic and genomic data must be coupled for checking authenticity of RM and promoting their use as control strains.

The One Health European joint integrative project "CARE" (OHEJP CARE) aims to create a visible and accessible collection of highly characterized foodborne pathogenic bacterial strains (MALDI-TOF identification, serotypes, WGS, AMR profiles) that can be used as RM.

MALDI-TOF Mass spectrometry is now commonly used for bacterial identification. In order to increase the robustness and reliability of identifications by this method, OHEJP CARE partners decided to increase the number of reference spectra already existing and provided by the Bruker commercial database. The work is focusing on three pathogens of public health importance included in the CARE collection: *Salmonella, Yersinia* and *Campylobacter*.

Material and Methods

STRAINS

Growth conditions

Salmonella strains: Drigalski or TSYE at 37°C during 18 H in 3 separate series

Yersinia strains: TSA at 30°C for 24 H

Campylobacter strains : on going, not presented here

Highly characterized strains (phenotypically and genotypically) 58 Salmonella French strains

(Salmonella Network, Food safety laboratory, Maisons-Alfort, ANSES France)

- ✓ Serotyping by glass slide agglutination (White-Kauffmann-Le Minor scheme¹)
 - + checking by SISTR2 prediction from the genome
- → 25 serovars including regulated Enteritidis, S. 4,5,12:i:-, Hadar, Infantis, Kentucky, Virchow and high pathogenic Salmonella serovar Newport
- ✓ MLST profiles determined by MLST seeman tool3
- → 35 MLST profiles identified (Table 1)

22 Yersinia strains (Y. pseudotuberculosis and Y. enterocolitica)

- human clinical strains isolated between 1966 and 2019
- from France, Belgium and Russia
- selected according to their antibiotic resistance profiles
- various serotypes and biotypes

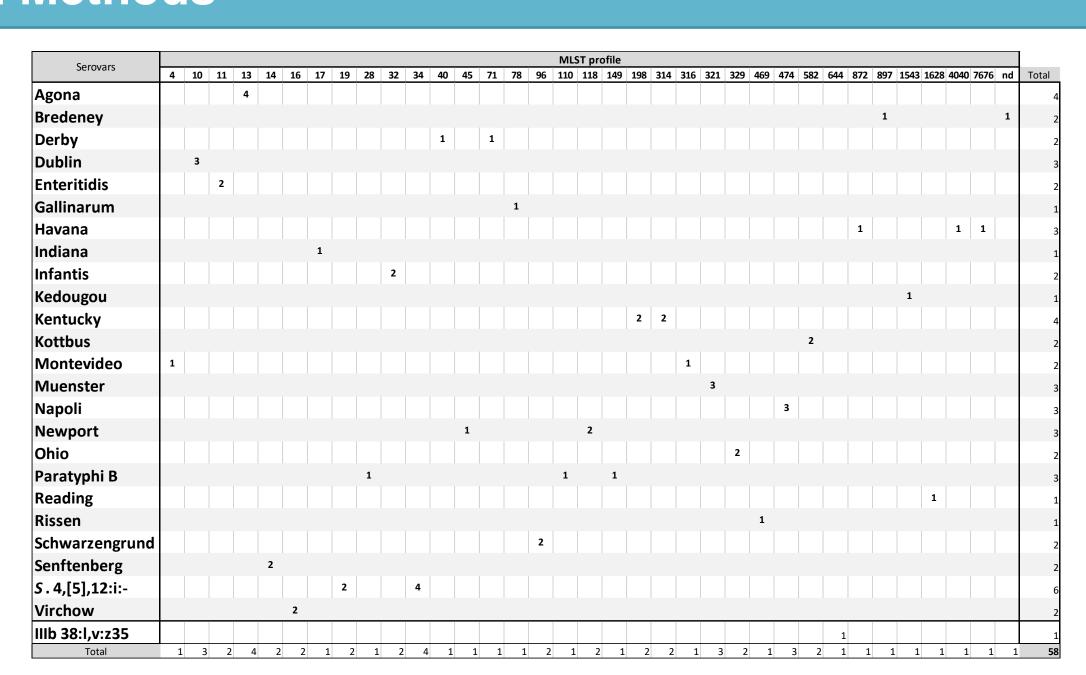


Table 1: List of the 25 serovars analysed in this study with the number of strains included and the corresponding MLST profiles

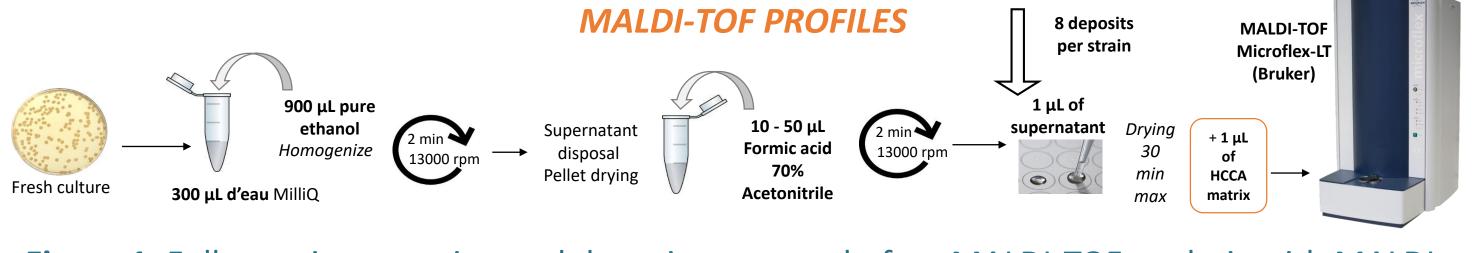


Figure 1. Full protein extraction and deposit on target before MALDI-TOF analysis with MALDI-TOF Microflex-LT (Bruker) and Bruker MBT explorer software (4 independent experiments)

- **32** spectra for each strain before removal of suboptimal spectra either by in-house Anses tool (QualiBioMaldi-Tof) for *Salmonella*, or with the MALDI Biotyper Flexanalysis software (Bruker) for Yersinia.
- At least 20 spectra per strain selected with an intensity between 8000 and 10000 AU
- Comparison with spectra from the commercialized Bruker Daltonic Data Base covering 3239 species/entries (9607 MSP)

Results

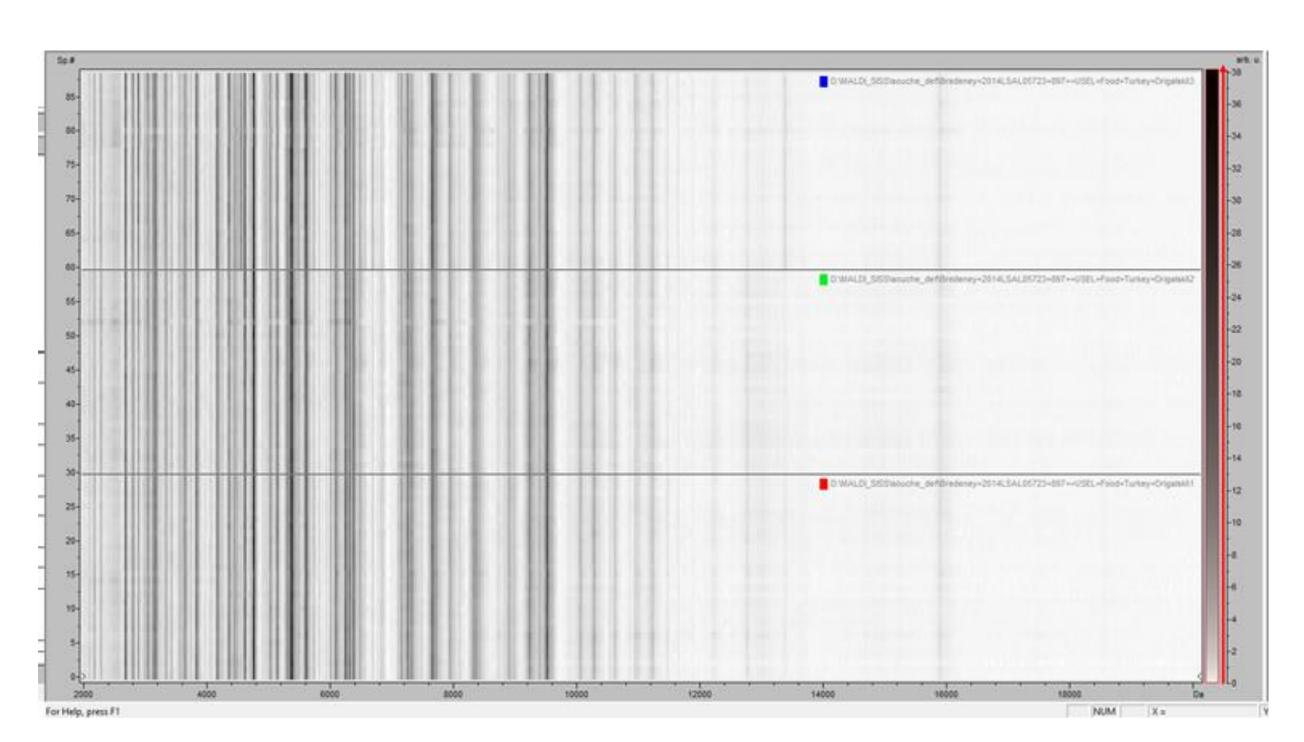


Figure 2. Example of results obtained for the *Salmonella Bredeney* strain 2014LSAL05723 grown on Drigalski medium at 37°C during 18 H in 3 separate series. The results are visulaized by Pseudo-gel displays (Gel View, Bruker Daltonics). The fact of making several series makes it possible to test the robustness of the produced reference spectra.

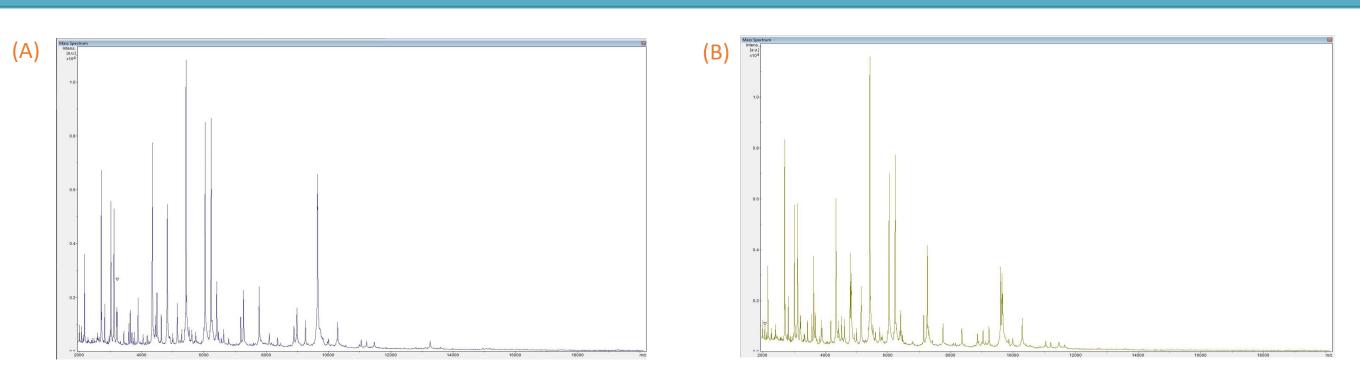


Figure. 3 Mass spectra for Yersinia pseudotuberculosis (A) and Y. enterocolitica (B)

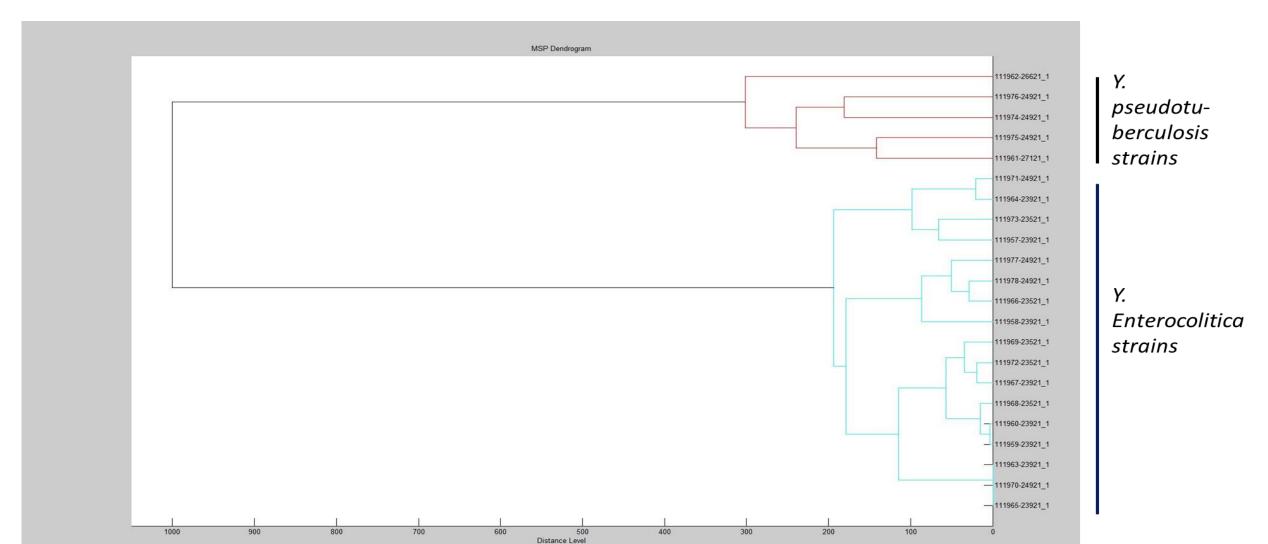


Figure. 4 Dendrogram analysis of the custom MSP of Y. pseudotuberculosis and Y. enterocolitica

Conclusion and Perspectives

This work will contribute to the characterization of the reference material of the CARE collection implemented within the framework of the One Health European Joint Program (OHEJP). The accumulation of standardized spectra for each bacterium of interest is essential to generate data for subsequent studies such as the highlighting of biomarkers allowing a more acute identification like *Salmonella*

serovars identification. Work is in progress to obtain reference spectra for five species of *Campylobacter*. The objective of the CARE project is to provide free access to the produced reference MALDI-TOF spectra via its catalog site currently under construction.







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^{1.} Grimont, P., and Weill, F.-X. (2007). Antigenic Formulae of the Salmonella serovars. [Online]. Paris: WHO Collaborating Center for Reference and Research on Salmonella, Institut Pasteur. 9th Edn. Available at: https://www.pasteur.fr/sites/default/files/veng_0.pdf [accessed Marc 04, 2022]. 2. Yoshida, C. E.; Kruczkiewicz, P.; Laing, C. R.; Lingohr, E. J.; Gannon, V. P.; Nash, J. H.; Taboada, E. N. (2016). The Salmonella In Silico Typing Resource (SISTR): An Open Web-Accessible Tool for Rapidly Typing and Subtyping Draft Salmonella Genome Assemblies. PLoS One, 11 (1), e0147101. 3. https://github.com/tseemann/mlst