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Rapid screening of epidemiologically important *Salmonella enterica* subsp. *enterica* serovars by Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry.

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Salmonella spp. is one of the most widely distributed foodborne pathogens in the European Union (EU) resulting in significant morbidity, mortality and social and economic cost. Accurate and rapid typing of this pathogen is essential for effective surveillance nevertheless conventional serotyping, which enables to type the 2600 serovars known, is a laborious procedure, time consuming and expensive. Private and public laboratories implied in surveillance need a rapid and not expensive prescreening method for at least the 25 serovars, the most frequently isolated [1].

MALDI-TOF mass spectrometry could represent an alternative. In the last decade this technique was considered as revolutionary allowing making the diagnosis of pathogens more easily and quickly compared to conventional methods however even if its ability to discriminate pathogens at genus and specie level was well established, at serovars level it remains scarce and needs to be explored.

The **aim of this study** was to evaluate the potential of MALDI-TOF mass spectrometry as prescreening method for typing of the 5 *Salmonella* serovars considered to be of public health significance under EU regulations (the regulated serovars): Enteritidis, Hadar, Virchow, Infantis, Typhimurium and its variant S.4,[5],12:i:-

Method employed:

The strains were grown 24 h at 36 °C over Drigalski medium. The mass spectrometry MALDI-TOF identification was realized with a Microflex LT® (Bruker Daltonics) after proteomic extraction with ethanol, formic acid and acetonitrile.

Analysis via alignment of identified peaks lists

1. Analysis



2. Manual Reprocessing



3. Automatic Reprocessing

Serovar

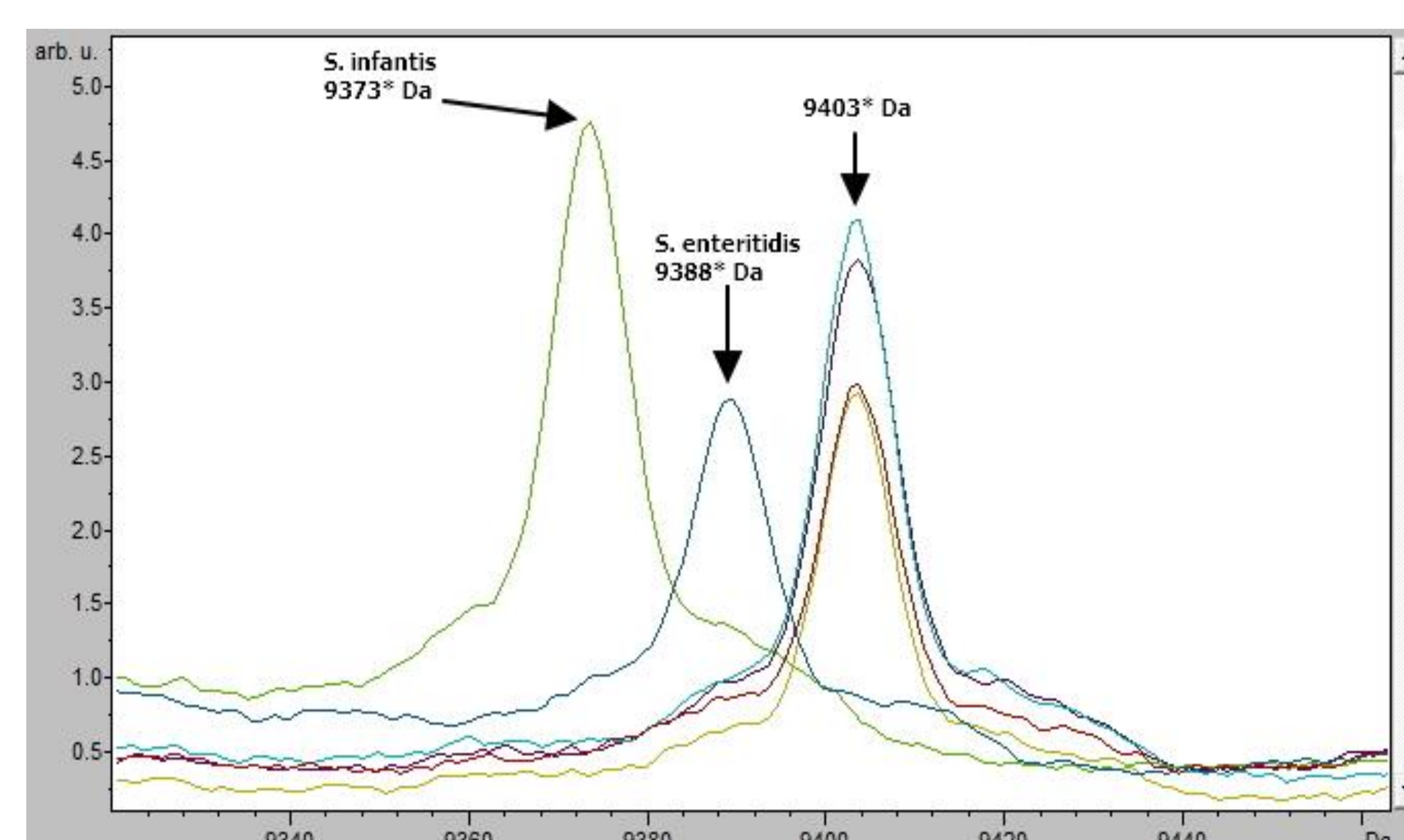
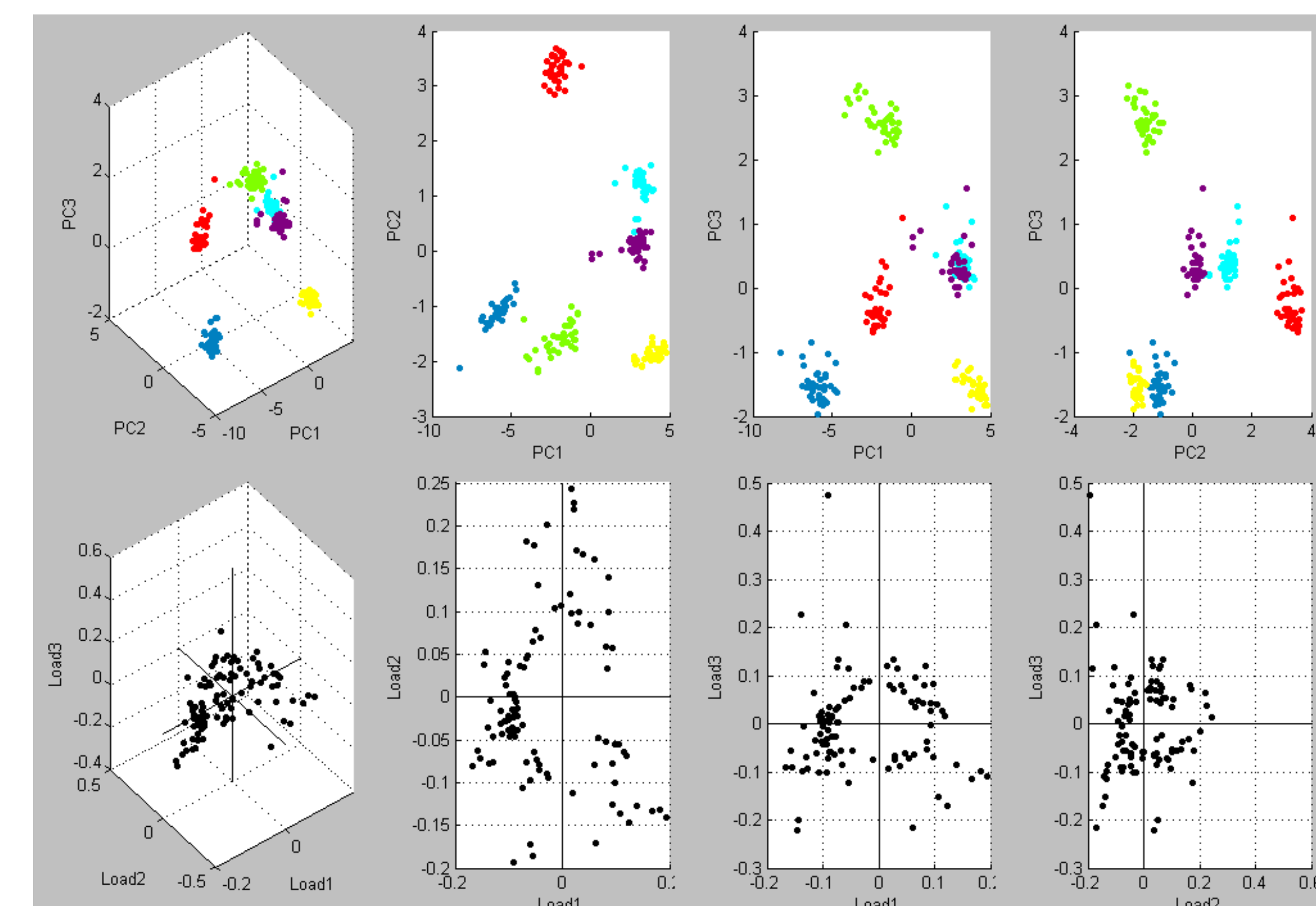
Identification of characteristic mass via the analysis of chromatograms

Creation of reference spectra

Results:

Statistical analysis via ClinProTools:

- Global ACP from 36 raw spectra : **the 5 serovars are distinct with the exception of the variant S.4,[5],12:i:-**



- Characteristics biomarkers via alignment of spectra were found for the 5 serovars

- A mapping of the specific mass for each regulated serovars was realized

Biomarqueurs	Serovars discriminés	S.4,[5],12:i:-	Enteritidis	Hadar	Infantis	Typhimurium	Virchow
1	Enteritidis (label)						
2	Enteritidis (label)						
3	Infantis (label)						
4	Infantis (label)						
5	S.4,[5],12:i:- Typhimurium/Virchow						
6	Enteritidis/Hadar/Infantis						
7	Hadar						
8	S.4,[5],12:i:- Enteritidis						
9	S.4,[5],12:i:- Typhimurium						
10	S.4,[5],12:i:- Typhimurium/Virchow						
11	Infantis						
12	Enteritidis						
13	Enteritidis/Hadar/Infantis						
14	Virchow (label)						
15	Virchow (label)						
16	Hadar						
17	Enteritidis (label)						
18	Enteritidis (label)						
19	Enteritidis						
20	Infantis						
21	S.4,[5],12:i:- Typhimurium/Virchow						
22	Enteritidis/Hadar/Infantis						
23	Enteritidis						
24	Infantis						
25	S.4,[5],12:i:- Typhimurium/Virchow						
26	S.4,[5],12:i:- Typhimurium						
27	Infantis						
28	S.4,[5],12:i:- Typhimurium/Virchow						
29	Enteritidis						
30	Infantis						
31	Enteritidis/Hadar/Infantis						
32	Enteritidis (label)						
33	Virchow						
34	Virchow (label)						
35	S.4,[5],12:i:- Typhimurium						
36	Hadar						
37	Hadar (label)						
38	Enteritidis/Hadar						

Conclusion and outlook:

MALDI-TOF/MS seems to be a possible methodology which could be used for the prescreening of the 5 regulated serovars. Nevertheless the specificity of each mass, selected for the characterization, must be evaluated compared with other serovars and additional robustness tests are essential before using these methodology routinely. Both these tests are actually in process in our laboratory onto 20 others serovars, the most frequently isolated [1].

References:
[1] Frédérique Moury, Sylvine Frémy, Claude Oudart, Christine Piquet, Catherine Pires Gomes, Corinne Danan, Sophie Granier, Renaud Lailier, Anne Brisabois. Inventaire des *Salmonella* d'origine non humaine - Réseau *Salmonella* 2011. Edité en décembre 2013.