



Application of MALDI-TOF MS for the subtyping of *Arcobacter butzleri* strains and comparison with their MLST and PFGE types

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ARTICLE INFO

Keywords:

MALDI-TOF MS

Subtyping

Arcobacter butzleri

PFGE

MLST

ABSTRACT

For the first time, this study evaluated the use of MALDI-TOF as a typing tool for *Arcobacter butzleri*. A total of 104 *A. butzleri* strains isolated from different sources in an artisanal dairy plant in Italy were identified and typed using MALDI-TOF and compared with their multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE) profiles found in previous studies. MALDI-TOF correctly identified all the isolates to species level. No clearly delineated clusters appeared on dendrograms based on either the complete spectra or the significant peaks, but nine clusters were defined using the cophenetic correlation. Interestingly, MALDI-TOF proved able to discriminate *A. butzleri* strains below species level, confirming its potential use for epidemiological surveys. As expected, the comparative analysis with PFGE and MLST showed that the discriminatory index was lower for MALDI-TOF but roughly comparable to sequence types and pulsotypes. MALDI-TOF appears to be a relatively low cost answer to the urgent need for more rapid, less expensive typing tools suitable for source attribution studies, readily allowing multiple typing methods to be combined. This study provides insights into MALDI-TOF as potential epidemiological tool. Its application in healthcare surveillance systems awaits further exploration to encourage interaction and convergence studies between primary care in humans and animal and food veterinary authorities as part of the One Health concept.

1. Introduction

The genus *Arcobacter* is an unusual taxon within the epsilon sub-division of Proteobacteria containing both pathogenic and free-living species found in a wide range of environments (Miller et al., 2007). It has long been considered an emerging human enteric pathogen linked to gastrointestinal illnesses (Collado and Figueras, 2011; Hsu and Lee, 2015). Although several aspects of *Arcobacter* epidemiology and virulence are starting to be clarified, key reservoirs and mechanisms of transmission have yet to be fully determined (Collado and Figueras, 2011). *Arcobacter* species are ubiquitous in animals, in a variety of foods of animal and non-animal origin, and in both aquatic and food-processing environments (Collado and Figueras, 2011; Merga et al., 2013), usually showing a high genotype diversity in all these sources. *Arcobacter butzleri* is the best characterized of all *Arcobacter* species. It is probably an environmental organism (Miller et al., 2007) with some

level of niche adaptation (Merga et al., 2013) and with the ability to survive in the adverse conditions imposed by food processing and storage (Collado and Figueras, 2011; Ferreira et al., 2015; Giacometti et al., 2013; Giacometti et al., 2015; Hausdorf et al., 2013; Rasmussen et al., 2013; Scarano et al., 2014; Serraino and Giacometti, 2014; Shah et al., 2013) that may cause disease through ingestion of contaminated water or food (Collado and Figueras, 2011; Miller et al., 2007).

Source-attribution studies for the burden of human illness require bacterial typing to identify sources and routes of product contamination. Bacterial typing is also a prerequisite for targeted control measures (Dieckmann et al., 2016) and for source-tracking studies to determine the origin of a specific strain by grouping the sources (Santos et al., 2016). The term subtyping refers to characterisation beyond the species or subspecies level, allowing the determination of clonal relationships and the phylogenetic relatedness of bacterial strains (Dieckmann et al., 2016). Nowadays, the genotyping methods most commonly used are

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<https://doi.org/10.1016/j.ijfoodmicro.2018.04.026>

Received 10 October 2017; Received in revised form 5 April 2018; Accepted 13 April 2018

Available online 16 April 2018

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based on DNA banding patterns, such as pulsed field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP), PCR-restriction fragment-length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), enterobacterial repetitive intergenic consensus (ERIC-PCR), multiple locus variable number of tandem repeats analysis (MLVA), multilocus sequence typing (MLST) and 16S rRNA gene sequencing. All these techniques possess different discriminatory powers, and their use depends on the main objective to be achieved. In spite of their recognized resolution, many of these approaches often lack reproducibility within and among laboratories, whereas others are discriminatory and reproducible but expensive, laborious and time-consuming - all undesirable factors for the identification of contamination sources (Santos et al., 2016).

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has wrought the most radical change in the diagnostic microbiology workflow in the last decade (Fournier et al., 2013) and has become a routine tool for microorganism identification in clinical microbiology laboratories worldwide. However, beyond microbe identification, whose importance for human health care is unquestionable, MALDI-TOF MS has proved to have great potential for epidemiological strain typing and antimicrobial susceptibility/resistance detection (Sanguinetti and Posteraro, 2016). This phenotyping technique is based on the detection of a large number of spectral features originating from proteins, namely highly abundant ribosomal and nucleic acid-binding proteins. Though several attempts have been made to apply MALDI-TOF MS to higher resolution microbial discrimination, they have not yielded uniform success, and the limits of the taxonomic resolution of MALDI-TOF MS profiling might be determined in large part by the nature of the particular bacterium profiled (Ghyssels et al., 2011; Sandrin et al., 2013). Hence, both the taxonomic resolution of MALDI-TOF MS and whether MALDI-TOF MS analysis will overlap other subtyping techniques need to be evaluated individually for a particular genus or species of interest. No such studies have hitherto been performed on *A. butzleri* isolates.

The aim of the present study was to evaluate the ability of MALDI-TOF technology to characterize *A. butzleri* isolates according to their different pattern of TOF peaks, and to perform a comparative analysis of their previously obtained MLST and PFGE profiles (De Cesare et al., 2015; Giacometti et al., 2013).

2. Material and methods

2.1. Strains tested

A set of 104 *A. butzleri* strains, of which 102 were collected from different sources in an artisanal dairy plant in four samplings in the Emilia Romagna Region between October and December 2012, and the references strains *A. butzleri* DSM 8739^T and *A. cryaerophilus* DSM 7289^T previously characterized by pulsed-field gel electrophoresis (PFGE) (Giacometti et al., 2013) and multilocus sequence typing (MLST) (De Cesare et al., 2015) were selected and analysed. Overall, the strains were obtained from food samples (i.e. raw cow and buffalo milk and ricotta cheese) (N = 28); food contact surfaces (i.e. bulk tank valve, cheese vat, drainage table, milk pump and mozzarella molding roller) (N = 45) and non-food contact surfaces (i.e. floors of cooler room and floor drain) (N = 29). The strain details were previously described (Giacometti et al., 2013). Extrapolating the results from the cited studies, the strains collated and used for this study showed a high diversity of 34 PFGE profiles and 21 sequence types (STs) respectively. Table 1 briefly reports the characteristics of the 102 *A. butzleri* strains included in this study and their MLST and PFGE results.

2.2. Sample preparation

Strains were cultured on nutrient agar (Oxoid, Basingstoke, United Kingdom) supplemented with 5% laked horse blood (Oxoid,

Table 1

Typology, source, sampling time, multilocus sequence typing and pulsed-field gel electrophoresis results of the 102 *A. butzleri* strains included in this study.

Strain	Source	Sampling	ST	Pulsotype
Food isolates				
56	Raw cow milk	II	437	1
45	Raw cow milk	III	423	2
162	Raw cow milk	III	423	1
210	Raw cow milk	IV	437	7
224	Raw cow milk	IV	438	6
127	Raw WB milk	I	420	33
132	Raw WB milk	I	420	32
149	Raw WB milk	I	432	12
68	Raw WB milk	II	425	26
77	Raw WB milk	II	425	26
91	Raw WB milk	II	425	26
95	Raw WB milk	II	428	26
35	Raw WB milk	III	435	25
37	Raw WB milk	III	422	5
40	Raw WB milk	III	423	2
42	Raw WB milk	III	424	4
108	Raw WB milk	III	429	28
39	Raw WB milk	IV	422	5
185	Raw WB milk	IV	427	10
207	Raw WB milk	IV	436	34
219	Raw WB milk	IV	436	14
220	Raw WB milk	IV	436	14
71	Ricotta cheese	II	66	11
101	Ricotta cheese	II	66	11
117	Ricotta cheese	II	66	11
163	Ricotta cheese	II	66	9
198	Ricotta cheese	II	66	11
205	Ricotta cheese	II	66	29
Isolates from food contact surfaces				
93	Bulk tank valve	I	420	33
124	Bulk tank valve	II	430	16
125	Bulk tank valve	II	429	28
109	Bulk tank valve	III	430	16
172	Bulk tank valve	III	434	17
183	Bulk tank valve	IV	430	16
190	Bulk tank valve	IV	430	20
92	Cheese vat	I	427	9
114	Cheese vat	I	427	8
123	Cheese vat	I	419	4
99	Cheese vat	II	421	22
120	Cheese vat	II	431	22
121	Cheese vat	II	435	25
20	Cheese vat	III	434	19
21	Cheese vat	III	434	19
197	Cheese vat	IV	430	16
199	Cheese vat	IV	430	16
239	Cheese vat	IV	430	18
7	Drainage table	III	435	25
10	Drainage table	III	434	15
11	Drainage table	III	435	22
201	Drainage table	IV	435	25
216	Drainage table	IV	435	25
217	Drainage table	IV	435	25
113	Milk pump	I	421	22
159	Milk pump	I	433	20
3	Milk pump	II	419	25
58	Milk pump	II	419	22
4	Milk pump	III	419	21
49	Milk pump	III	435	25
60	Milk pump	III	419	23
64	Milk pump	III	420	32
86	Milk pump	III	438	24
158	Milk pump	IV	427	8
187	Milk pump	IV	427	9
195	Milk pump	IV	427	8
196	Milk pump	IV	427	9
106	Mozzarella cheese molding roller	I	421	24
46	Mozzarella cheese molding roller	II	435	27
47	Mozzarella cheese molding roller	II	435	25
62	Mozzarella cheese molding roller	II	420	32
12	Mozzarella Cheese molding roller	III	420	30
48	Mozzarella cheese molding roller	III	435	25

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