Food Microbiology

Phenotype and genomic background of Arcobater butzleri strains and taxogenomic assessment of the species

--Manuscript Draft--

Manuscript Number:	FM_2019_668R1
Article Type:	Research Paper
Keywords:	lithotrophic bacteria; antibiotic and heavy metal resistance.; Shellfish; Aliarcobacter butzleri; Vegetables; Arcobacter butzleri
Corresponding Author:	Vincenzina Fusco Institute of Sciences of Food Production - National Research Council of Italy (CNR-ISPA) Bari, Italy
First Author:	Francesca Fanelli
Order of Authors:	Francesca Fanelli
	Daniele Chieffi
	Angela Di Pinto
	Anna Mottola
	Federico Baruzzi
	Vincenzina Fusco
Abstract:	In this study the phenotypic and genomic characterization of two Arcobacter butzleri (Ab) strains (Ab 34_O and Ab 39_O) isolated from pre-cut ready-to-eat vegetables was performed. Results provided useful data about their taxonomy and their overall virulence potential with particular reference to the antibiotic and heavy metal susceptibility. These features were moreover compared with those of two Ab strains isolated from shellfish and a genotaxonomic assessment of the Ab species was performed. The two Ab isolated from vegetables were confirmed to belong to the Aliarcobacter butzleri species by 16S rRNA gene sequence analysis, MLST and genomic analyses. The genome-based taxonomic assessment of the Ab species brought to the light the possibility to define different subspecies reflecting the source of isolation, even though further genomes from different sources should be available to support this hypothesis. The strains isolated from vegetables in the same geographic area shared the same distribution of COGs with a prevalence of the cluster “inorganic ion transport and metabolism”, consistent with the lithotrophic nature of Arcobacter spp.. None of the Ab strains (from shellfish and from vegetables) metabolized carbohydrates but utilized organic acids and amino acids as carbon sources. The metabolic fingerprinting of Ab resulted less discriminatory than the genome-based approach. The Ab strains isolated from vegetables and those isolated from shellfish endowed multiple resistance to several antibiotics and heavy metals.
Response to Reviewers:	Dear Editor, We do wish to thank very much you and reviewers for the attention you had in reviewing our manuscript and for the comments to it. However, within a general consideration on the comments you sent us, we have to say that a difficult point to resolve was to make a decision about your request to reduce the article to a short research note. Based on all reviewers’ comments, and given our response to them (see the enclosed detailed response to reviewers), we do believe that our manuscript should be published as full research article, as it contains valuable and interesting information about the genomic and phenotypic features of Arcobacter butzleri strains as well as the antibiotic and heavy metal resistance profiles of these strains, providing novel insight into the taxogenomic and pathogenicity of this species. Looking at the comments of reviewers, the reviewer#1 affirms that “the matter is interesting but does not warrant a full publication”, but provides no explanation about this decision.

The reviewer #2 is favorable to the publication of this manuscript in form of a full research article. Indeed, he/she found our manuscript valuable and required only few minor revisions that we included in the revised manuscript (highlighted in yellow).

As for the reviewer #3, he/she states that “Arcobacter butzleri is uncommonly reported from vegetables and the report is of interest from that respect”.

Then, he/she consider our manuscript “potentially publishable in a much reduced and focused form” on the basis of incorrect objections.

Indeed, this reviewer states that “Genomes are incomplete and this has been found to result in missing key information such as plasmid content (On et al 2019).” But a plethora of - almost all - bacterial genomes to date available, whose analyses have been published in a copious number of full articles, are incomplete and anyway we found in our “incomplete genomes” many virulence and antibiotic and heavy metal resistance determinants, providing novel insights.
Moreover, this reviewer incorrectly criticizes that “The minimal standards paper cited by the authors recommends Formula 3 for Arcobacter and not Formula 2 as used by the authors (124-127) since this performed better for these and related taxa”, which is just the opposite suggested by the cited authors and done by us! We correctly used the Formula 2 as the authors we cited (124-127) i.e. Auch et al., 2010 and Meier-Kolthoff et al., 2013 suggest the use of Formula 2 for incomplete genome sequences (“The e-mail sent to the user includes the results for all three distance formulas. Considering error ratios at 70% DDH, we recommend formula (2). This formula must be used if incomplete genome sequences are submitted to the server [Auch et al., 2010]” and “When dealing with incomplete genomes it is highly recommended to use formula d4 “(corresponding to formula 2)”, as it is independent of sequence length, and thus not directly affected by the removal of HSPs due to the removal of parts of the genome“. “i.e. formula (2) in either its original or logarithmized variant, is robust against the use of incomplete genomes” [Meier-Kolthoff et al., 201]).
In addition, reviewer #3 incorrectly affirms that “Using Biolog under anaerobic conditions risks false positives since the tetrazolium marker dye reduces and thus changes colour under conditions of anaerobiosis, a risk unnecessary for Arcobacter since they can grow aerobically.” Indeed, based on his/her objection no anaerobic bacterium could be identified by the Biolog and all the numerous full research articles published in which the Biolog has been used for anaerobic bacteria should have not been published as full research articles! On the contrary, the Biolog is just made for both aerobic and anaerobic microorganism identifications (https://www.biolog.com/products-portfolio-overview/microbial-identification. Moreover, we used the anaerobiosis for our Biolog experiments because DSMZ (https://www.dsmz.de/collection/catalogue/details/culture/DSM-8739)as well as ATCC (https://www.lgcstandards-atcc.org/Products/All/49616.aspx#culturemethod) require “Microaerophilic, 3-5% O2-10% CO2” cultivation conditions for the Arcobacter butzleri type strain. Therefore, for all, the type strain and the A. butzleri strains we tested in our study we used the Biolog under anaerobic conditions.

Last but not least, reviewer #3 incorrectly states that “The authors' own DDH data do not support subdivision of the species into source-specific groups since the most genetically closely related strains (L214-217) are from other sources! Subspecies or genomospecies must be supported by genomic separation (Wayne et al. 1987).”
Apart that for the definition of a subspecies we mentioned Chun et al. (2018) (L223), who set the range of genomic indexes to be used and is surely more update than Wayne et al., 1987!, the latest sentence is true as it is truly true that our strains isolated from shellfish (Ab 55 and Ab 6V) cluster on the basis of a genomic separation (see Figures 1 and 2)! So, it is possible to hypothesize the definition of subspecies.
In this regard, however, we only propose this subdivision (L25-27) and in lines 228-229 we only say “Ab strains can be grouped into two large clades and five different subgroups which, in part, resemble characteristic based on host/geographical distribution”). The other genomes clusterization is clearly associated with the source of isolation (Fig. 1-2), although to confirm this hypothesis other genomes should be available to include them in the analysis. In this regard, we added this statement in the text: “even though further genomes from different sources should be available to support this hypothesis” (L229-230). The fact that the new genomes of A. butzleri from

	vegetable (Ab34_O and Ab_39_O) (L214-217) have as closest relatives Ab-ED1 and Ab7h1h for Ab34_O, JV22 and AbNCTC 12481for Ab39_O is not in contrast with what above-mentioned, since it should not be excluded that, even if isolated from pre-cut-ready- to-eat vegetable, these strains could share the same origin (i.e. water contamination, faecal contamination ecc.).

Dear Editor,
We do wish to thank very much you and reviewers for the attention you had in reviewing our manuscript and for the comments to it.
However, within a general consideration on the comments you sent us, we have to say that a difficult point to resolve was to make a decision about your request to reduce the article to a short research note. Based on all reviewers' comments, and given our response to them (see the enclosed detailed response to reviewers), we do believe that our manuscript should be published as full research article, as it contains valuable and interesting information about the genomic and phenotypic features of *Arcobacter butzleri* strains as well as the antibiotic and heavy metal resistance profiles of these strains, providing novel insight into the taxogenomic and pathogenicity of this species.

Looking at the comments of reviewers, the reviewer#1 affirms that "the matter is interesting but does not warrant a full publication", but provides no explanation about this decision.

The reviewer #2 is favorable to the publication of this manuscript in form of a full research article. Indeed, he/she found our manuscript valuable and required only few minor revisions that we included in the revised manuscript (highlighted in yellow).

As for the reviewer #3, he/she states that "Arcobacter butzleri is uncommonly reported from vegetables and the report **is of interest** from that respect".

Then, he/she consider our manuscript "potentially publishable in a much reduced and focused form" **on the basis of incorrect objections**.

Indeed, this reviewer states that "Genomes are incomplete and this has been found to result in missing key information such as plasmid content (On et al 2019)." But a plethora of - almost all - bacterial genomes to date available, whose analyses have been published in a copious number of full articles, are incomplete and anyway we found in our "incomplete genomes" many virulence and antibiotic and heavy metal resistance determinants, providing novel insights.

Moreover, this reviewer incorrectly criticizes that "The minimal standards paper cited by the authors recommends Formula 3 for Arcobacter and not Formula 2 as used by the authors (124-127) since this performed better for these and related taxa", **which is just the opposite suggested by the cited authors and done by us**! We correctly used the Formula 2 as the authors we cited (124-127) i.e. Auch et al., 2010 and Meier-Kolthoff et al., 2013 **suggest the use of Formula 2 for incomplete genome sequences (**"The e-mail sent to the user includes the results for all three distance formulas. Considering error ratios at 70% DDH, we recommend formula (2). This formula must be used if incomplete genome sequences are submitted to the server [Auch et al., 2010]" and "When dealing **with incomplete genomes** it is highly recommended to use formula d_4 "*(corresponding to formula 2)"*, as it is independent of sequence length, and thus not directly affected by the removal of HSPs due to the removal of parts of the genome". "i.e. formula (2) in either its original or logarithmized variant, is robust against the use of incomplete genomes" [Meier-Kolthoff et al., 201]).

In addition, reviewer #3 **incorrectly** affirms that "Using Biolog under anaerobic conditions risks false positives since the tetrazolium marker dye reduces and thus changes colour under conditions of anaerobiosis, a risk unnecessary for Arcobacter since they can grow aerobically." Indeed, based on his/her objection no anaerobic bacterium could be identified by the Biolog and all the numerous full research articles published in which the Biolog has been used for anaerobic bacteria should have not been

published as full research articles! On the contrary, the Biolog is just made for both aerobic and anaerobic microorganism identifications (https://www.biolog.com/products-portfolio-overview/microbial-identification. Moreover, we used the anaerobiosis for our Biolog experiments because DSMZ (https://www.dsmz.de/collection/catalogue/details/culture/DSM-8739)as well as ATCC (https://www.lgcstandards-atcc.org/Products/All/49616.aspx#culturemethod) require "Microaerophilic, 3-5% O2-10% CO2" cultivation conditions for *the Arcobacter butzleri* type strain. Therefore, for all, the type strain and the *A. butzleri* strains we tested in our study we used the Biolog under anaerobic conditions.

Last but not least, reviewer #3 **incorrectly** states that "The authors' own DDH data do not support subdivision of the species into source-specific groups since the most genetically closely related strains (L214-217) are from other sources! Subspecies or genomospecies must be supported by genomic separation (Wayne et al. 1987)."
Apart that for the definition of a subspecies we mentioned Chun et al. (2018) (L223), who set the range of genomic indexes to be used and is surely **more update** than Wayne et al., 1987!, the latest sentence **is true as it is truly true** that our strains isolated from shellfish (Ab 55 and Ab 6V) cluster on the basis of a genomic separation (see Figures 1 and 2)! So, it is possible to hypothesize the definition of subspecies.
In this regard, however, we only propose this subdivision (L25-27) and in lines 228-229 we only say "Ab strains can be grouped into two large clades and five different subgroups which, **in part**, resemble characteristic based on host/geographical distribution"). The other genomes clusterization is clearly associated with the source of isolation (Fig. 1-2), although to confirm this hypothesis other genomes should be available to include them in the analysis. In this regard, we added this statement in the text: "even though further genomes from different sources should be available to support this hypothesis" (L229-230). The fact that the new genomes of *A. butzleri* from vegetable (Ab34_O and Ab_39_O) (L214-217) have as closest relatives Ab-ED1 and Ab7h1h for Ab34_O, JV22 and AbNCTC 12481for Ab39_O is not in contrast with what above-mentioned, since it should not be excluded that, even if isolated from pre-cut-ready- to-eat vegetable, these strains could share the same origin (i.e. water contamination, faecal contamination ecc.).

Detailed response to reviewers' comments

Reviewer #1 The matter is interesting but doe snot warrant a full publication
Thanks for considering our manuscript interesting but we do not understand why it not warrant a full publication since you did not provide any explanation about it.

Reviewer #2: * Summary of the article
In this article the scientist analysed the Phenotype and genomic background of 2 Arcobater butzleri strains and did antaxogenomic assessment of the species with special emphasis on antibiotic resistance, virulence factor and a new aspect heavy metal resistance

* Main impression
- Analysis of only 2 main isolates, the Aliiacrobacter are new but good discussed
- Comprehensive analysis of all genomic features
- Adding new aspects by heavy metal analysis
* Article conforms to the journal-specific instructions
yes
* Give specific comments and suggestions about e.g. title, abstract: Does the title accurately reflect the content? Is the abstract complete and stand-alone?
Yes and yes
Many thanks for your positive comments about our manuscript.

* Carefully review the methodology, statistical errors, results, conclusion/discussion, and references.
Done as notices in the text
We have revised the text (highlighted in yellow in the revised manuscript) following your suggestions.
As for the availability of the two genomes of our A. butzleri strains isolated from vegetables, we have deposited in GenBank both genomes which will be released upon the publication of this manuscript.

Reviewer #3: First of all, the spelling of the genus "Arcobacter" needs checking and correcting throughout. It is misspelled as "Arcobater" several times including the title and abstract.
We do apologize for this inconvenient! We corrected the text accordingly (highlighted in yellow in the revised manuscript).

The authors have gone to significant lengths to characterise two strains isolated from vegetables. They make a close comparison with another two strains they recovered previously from shellfish and then undertake a phylogenetic analysis with other strain sequences to conclude the species could be divided into several subgroups or possibly subspecies.
Arcobacter butzleri is uncommonly reported from vegetables and the report is of interest from that respect. However, any hypotheses about the taxonomic subdivision of the species based on so few strains are preliminary at best. The authors' own DDH data do not support subdivision of the species into source-specific groups since the most genetically closely related strains (L214-217) are from other sources! Subspecies or genomospecies must be supported by genomic separation (Wayne et al. 1987).
I have minor remarks on the methods used.
Apart that for the definition of a subspecies we mentioned Chun et al. (2018) (L223), who set the range of genomic indexes to be used and is surely more update than Wayne et al., 1987!, the latest sentence is true as it is truly true that our strains isolated from shellfish (Ab 55 and Ab 6V) cluster on the basis of a

genomic separation (see Figures 1 and 2)! So, it is possible to hypothesize the definition of subspecies. In this regard, however, we only propose this subdivision (L25-27) and in lines 228-229 we only said "*Ab* strains can be grouped into two large clades and five different subgroups which, **in part**, resemble characteristic based on host/geographical distribution"). The other genomes clusterization is clearly associated with the source of isolation (Fig. 1-2), although to confirm this hypothesis other genomes should be available to include them in the analysis. In this regard, we added this statement in the text: "even though further genomes from different sources should be available to support this hypothesis" (L229-230). The fact that the new genomes of *A. butzleri* from vegetable (Ab34_O and Ab_39_O) (L214-217) have as closest relatives Ab-ED1 and Ab7h1h for Ab34_O, JV22 and AbNCTC 12481for Ab39_O is not in contrast with what above-mentioned, since it should not be excluded that, even if isolated from pre-cut-ready- to-eat vegetable, these strains could share the same origin (i.e. water contamination, faecal contamination ecc.).

Genomes are incomplete and this has been found to result in missing key information such as plasmid content (On et al 2019).

A plethora of - almost all - bacterial genomes to date available, whose analyses have been published in a copious number of full articles, are incomplete and anyway we found in our "incomplete genomes" many virulence and antibiotic and heavy metal resistance determinants providing novel insights.

The minimal standards paper cited by the authors recommends Formula 3 for Arcobacter and not Formula 2 as used by the authors (124-127) since this performed better for these and related taxa.

This is just the opposite suggested by the cited authors and done by us! We correctly used the Formula 2 as the authors we cited (124-127) i.e. Auch et al., 2010 and Meier-Kolthoff et al., 2013 suggest **the use of Formula 2 for incomplete genome sequences (**"The e-mail sent to the user includes the results for all three distance formulas. Considering error ratios at 70% DDH, we recommend formula (2). **This formula must be used if incomplete genome sequences are submitted to the server** [**Auch et al., 2010**]" and "When dealing **with incomplete genomes** it is highly recommended to use formula d_4 "*(corresponding to formula 2)"*, as it is independent of sequence length, and thus not directly affected by the removal of HSPs due to the removal of parts of the genome". "i.e. formula (2) in either its original or logarithmized variant, is robust against the use of incomplete genomes" [Meier-Kolthoff et al., 2013]).

Using Biolog under anaerobic conditions risks false positives since the tetrazolium marker dye reduces and thus changes colour under conditions of anaerobiosis, a risk unnecessary for Arcobacter since they can grow aerobically.

Based on your objection, no anaerobic bacterium could be identified by the Biolog and all the full research articles published in which the Biolog has been used for anaerobic bacteria should have not been published as full research articles! On the contrary, the Biolog is just made for both aerobic and anaerobic microorganism identifications (https://www.biolog.com/products-portfolio-overview/microbial-identification. Moreover, we used the anaerobiosis for our Biolog experiments because DSMZ (https://www.dsmz.de/collection/catalogue/details/culture/DSM-8739) as well as ATCC (https://www.lgcstandards-atcc.org/Products/All/49616.aspx#culturemethod) require "Microaerophilic, 3-5% O2-10% CO2" cultivation conditions for the *Arcobacter butzleri* type strain. Therefore, for all, the type strain and the *A. butzleri*, strains we tested in our study we used the Biolog under anaerobic conditions.

These aside, the level of detail in the report I simply find unnecessary for just two strains in which the suggestion of a proposed potential revision of A. butzleri to encompass source-specific genomospecies is not credible. The paper is potentially publishable in a much reduced and focused form.

On the basis of our response to all comments and given yours and reviewer#2's positive comments, we do believe that this manuscript should be published as a full research article since it contains valuable and interesting information about the genomic and phenotypic features of *Arcobacter butzleri* strains as well as the antibiotic and heavy metal resistance profiles of these strains, providing novel insight into the taxogenomic and pathogenicity of this species.

Highlights

- Two *Arcobacter butzleri* strains from vegetables were genome sequenced.
- Genotaxonomics suggested different subspecies reflecting the source of isolation.
- Phenotypic features of these strains and those isolated from shellfish were assessed.
- No one metabolized carbohydrates but used organic acids and aminoacids.
- All strains were multiple resistant to several antibiotics and heavy metals.

Phenotype and genomic background of *Arcobacter butzleri* strains and taxogenomic assessment of the species

Francesca Fanelli[a†], Daniele Chieffi[a†], Angela Di Pinto[b], Anna Mottola[b], Federico Baruzzi[a], Vincenzina Fusco[a*]

[a]*Institute of Sciences of Food Production of the National Research Council of Italy (CNR-ISPA), Bari, 70126, Italy;* [b]*Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano, Bari, 70010, Italy.*

[†]These authors equally contributed to this work.

Correspondent Footnote

*Corresponding author. Dr. Fusco Vincenzina, Ph.D. Mailing address: National Research Council of Italy, Institute of Sciences of Food Production, Via G. Amendola 122/O, 70126, Bari, Italy. Phone: +39-080-5929322. Fax: +39-080-5929374. E-mail: vincenzina.fusco@ispa.cnr.it

Abstract

In this study the phenotypic and genomic characterization of two *Arcobacter butzleri* (*Ab*) strains (*Ab* 34_O and *Ab* 39_O) isolated from pre-cut ready-to-eat vegetables was performed. Results provided useful data about their taxonomy and their overall virulence potential with particular reference to the antibiotic and heavy metal susceptibility. These features were moreover compared with those of two *Ab* strains isolated from shellfish and a genotaxonomic assessment of the *Ab* species was performed. The two *Ab* isolated from vegetables were confirmed to belong to the *Aliarcobacter butzleri* species by 16S rRNA gene sequence analysis, MLST and genomic analyses. The genome-based taxonomic assessment of the *Ab* species brought to the light the possibility to define different subspecies reflecting the source of isolation, even though further genomes from different sources should be available to support this hypothesis. The strains isolated from vegetables in the same geographic area shared the same distribution of COGs with a prevalence of the cluster "inorganic ion transport and metabolism", consistent with the lithotrophic nature of *Arcobacter* spp.. None of the *Ab* strains (from shellfish and from vegetables) metabolized carbohydrates but utilized organic acids and amino acids as carbon sources. The metabolic fingerprinting of *Ab* resulted less discriminatory than the genome-based approach. The *Ab* strains isolated from vegetables and those isolated from shellfish endowed multiple resistance to several antibiotics and heavy metals.

Key words: *Arcobacter butzleri; Aliarcobacter butzleri;* lithotrophic bacteria; vegetables; shellfish; antibiotic and heavy metal resistance.

1. Introduction

Arcobacter (*A.*) *butzleri*, recently proposed as *Aliarcobacter butzleri* (Pérez-Cataluña et al. 2018; 2019a,b), is one of the most widespread species of the genus *Arcobacter* and can be frequently found at various stage of the food chain, with fecal (directly from the animal or via the use of manure as fertilizer) and cross contaminations as the most likely routs of contaminations (Ferreira et al., 2019). Fresh vegetables are among the main foods where *A. butzleri* may occur, mainly carried through irrigation water, soil or manure, whereas cross-contamination is most likely the main rout of *A. butzleri* contamination in processed and ready-to-eat vegetables (González and Ferrús, 2011; Hausdorf et al., 2013; Kim et al., 2019; Mottola et al., 2016a; Winters and Slavik, 2000;)

Together with *A. cryaerophilus*, *A. thereius* and *A. skirrowii*, *A. butzleri* is among the *Arcobacter* species recognized as human pathogens (Ferreira et al., 2016). In particular, *A. butzleri* (*Ab*) is an emerging water and food-borne pathogen able to cause abortion and stillbirth, mastitis and enteritis in sheep, pigs and cows whereas in human may cause bacteremia, enteritis, septicemia and severe diarrhea (Fanelli et al., 2019; Flynn et al., 2018; Franz et al., 2018, Fusco et al., 2018). Although self-limiting, symptoms' protraction and severity might recall after antibiotic treatment, which could be complicated by the resistance of the *Ab* strains to antibiotic(s). Thus, knowledge about the occurrence and genetic determinants of (multiple) antibiotic resistant *Ab* strains are needed to choose the adequate antibiotic treatment. Moreover, knowing the phenotypic traits and the genomic background of antibiotic and heavy metal resistant strains of this species as well as their virulence potential and their ability to inhabit different ecological niches may provide further insight into the fitness and evolution of this species.

Apart from the *Ab* RM4018, isolated from human feces, and *Ab* ED-1, isolated from microbial fuel cells (Miller et al., 2007; Pérez-Cataluña et al., 2018), only two further strains of this species, which were isolated from shellfish (Mottola et al., 2016b), have been genomically characterized (Fanelli et al., 2019). Moreover, as highlighted by Fanelli et al. (2019), only an exiguous number of strains has

been characterized for the main phenotypic traits that characterize this species (Pérez-Cataluña et al., 2018; Vandamme et al., 1992) and none of the strains used was isolated from vegetables.

Herein, we report the phenotypic and genomic characterization of two *Ab* strains isolated from pre-cut ready-to-eat vegetables (Mottola et al., 2016a), providing useful info about their taxonomy and their genotypic and phenotypic traits with particular reference to their overall virulence potential as well as their antibiotic and heavy metal susceptibility. Moreover, we compared these features with those of the *Ab* strains isolated from shellfish (Fanelli et al., 2019) and carried out a genome-based taxonomic assessment of the *Ab* species.

2. Materials and Methods

2.1 Bacterial Strains

Ab strains 34_O and 39_O were originally isolated from pre-cut ready-to-eat vegetables obtained from supermarkets in the Apulia region (Italy) in 2016 (Mottola et al., 2016a). These strains were previously identified and typed by MLST (Mottola et al., 2016a; Mottola, 2017). Allelic profiles and sequences are available on the *Arcobacter* MLST database (https://pubmlst.org/Arcobacter/MLST) under the ID numbers 837 (*Ab* 34_O) and 838 (*Ab* 39_O).

Pure cultures, provided by the Food Safety Section of the Department of Veterinary Medicine of Bari, were maintained in the Microbial Culture Collection of the Institute of Sciences of Food Production, CNR, Bari (www.ispa.cnr.it/Collection). Bacterial strains were maintained at -80°C as pure stock cultures in Brain Heart Infusion broth (BHI; Oxoid S.p.A., Rodano, Milan, Italy) supplemented with glycerol (30% vol/vol).

2.2 Genome sequencing and assembly

DNA isolation was performed by using the Wizard® Genomic DNA Purification Kit (Promega), as modified by Ercolini et al. (2005). The integrity, purity and quantity of DNA were assessed as previously described by Fusco et al. (2011), by agarose gel electrophoresis, by NanoDrop-2000

(Thermo Fisher Scientific, Wilmington, DE, USA) and by Qubit 3.0 fluorometer (Life Technologies). DNA was then subjected to whole genome shotgun sequencing using the Ion S5™ library preparation workflow (Thermo Fisher Scientific, Waltman, MA, USA). 400 bp mate-paired reads were generated on the Ion S5™ System (Thermo Fisher Scientific). Duplicate reads were removed by FilterDuplicates (v5.0.0.0) Ionplugin. *De novo* assembly was performed by AssemblerSpades (v.5.0) Ionplugin™ (Gurevich et al., 2013).

2.3 Bioinformatic methods

Genes were predicted and annotated using PROKKA pipeline implemented in the Galaxy platform (Galaxy Tool Version 1.0.0; Afgan et al., 2016). The predicted proteins were submitted to the PFAM annotator tool within the Galaxy platform in order to predict the pfam domains. Protein ID used in the manuscript indicated those obtained by NCBI (National Center for Biotechnology Information) Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016).

Predicted proteins were assigned to Clusters of Orthologous Groups (COG) functional categories by Web CD-Search Tool (Marchler-Bauer et al., 2017) using an Expected value threshold of 0.01. COG ID were then manually mapped into functional categories (https://www.ncbi.nlm.nih.gov/COG/).

All the proteins sequences used in this study were retrieved from GenBank (NCBI). The homology-based relationship of *Ab* 34_O and *Ab* 39_O predicted proteins towards selected proteins was determined by BLASTP algorithm on the NCBI site (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Gene models were manually determined, and clustering and orientation were subsequently deduced for the closely linked genes.

Antibiotic resistance genes were predicted by BLASTP search against the Antibiotic Resistance genes Database ARDB (Liu et al., 2009), beta lactamase database (Naas et al., 2017) and The Comprehensive Antibiotic Resistance Database (CARD; Jia e t al. 2017). Genes associated with antibiotic resistance were also retrieved by keywords terms search within UniProtID entry list obtained by functional annotation.

Functional annotation, subsystem prediction and metabolic reconstruction comparison were also performed using the RAST server (Aziz et al., 2008) and by using The Proteome Comparison Service integrated in Patric (www.patric.org; Wattam et al., 2017). Genes involved in the mechanism of resistance to heavy metals were retrieved by homology by BLASTP search against *Ab* 34_O and *Ab* 39_O proteomes.

Genetic divergence was calculated by the ANI/AAI calculator (Goris et al. 2007; Rodriguez and Konstantinidis, 2016) which estimates the average nucleotide/aminoacid identity (ANI/AAI) using both best hits (one-way ANI) and reciprocal best hits (two-way ANI) between genomic datasets. The Genome-to-Genome Distance Calculator (GGDC) (Meier-Kolthoff et al, 2013; 2014) web service was used to report digital DDH for the accurate delineation of prokaryotic subspecies and to calculate differences in G+C genomic content (available at ggdc.dsmz.de). Formula 2 alone was used for analysis, providing an estimation of DDH independent of genome lengths, as recommended by the authors of GGDC for use with any incomplete genomes (Auch et al., 2010; Meier-Kolthoff et al, 2013).

Phylogenetic tree was built by using The Phylogenetic Tree Building Service implemented in Patric platform (www.patric.org) using as out-group the *Campylobacter jejuni* subsp. *jejuni* NCTC 11168 and the Maximum Likelihood method (Stamatakis et al., 2014).

Phylogenetic analysis was also performed by Multilocus Sequence Analysis (MLSA) using a concatenated dataset of 13 housekeeping genes (*atp*A, *atp*D, *dna*A, *dna*J, *dna*K, *fts*Z, *gyr*A, *hsp*60, *rad*A, *rec*A, *rpo*B, *rpo*D, and *tsf*) obtained from the genomes using BLASTn search (Pérez-Cataluña et al., 2018a). Phylogenies based on the concatenated sequences was constructed with MEGA version 7.0 (Kumar et al., 2016) using the Maximum-Likelihood (ML) algorithms.

2.4 Metabolic fingerprinting

Ab strains LMG 10828^{T} (ATCC 49616, RM4018), 55 and 6V (Fanelli et al., 2019), 34_O and 39_O were grown on Brain Heart Infusion (BHI) (Oxoid, Basingstoke, United Kingdom) agar amended with 0.6 % yeast extract (YE) (Biolife, Milan, Italy) incubated at 37 °C for 48 h under microaerophilic atmosphere (CampyGen^{TM} Compact, Oxoid, Basingstoke, United Kingdom). One single colony for each strain was inoculated in 20 ml of BHI broth with 0.6% YE and incubated at 37 °C for 48 h in anaerobiosis (AnaeroGen^{TM}; Oxoid, Basingstoke, United Kingdom), then subcultured at 1% and incubated under the same conditions. Bacterial cells were recovered after centrifugation (10,000 rpm x 10 min. at 4 °C), washed twice with sterile potassium phosphate buffer (50 mM, pH 7.0) and resuspended in sterile 0.9 % NaCl solution adjusting optical density (600 nm) to 0.4. Each bacterial suspension was inoculated into Biolog AN MicroPlate^{TM} (Biolog, Hayward, CA, USA) (100 µl per well) and incubated at 37 °C for 24 h under anaerobic conditions as recommended by the supplier also for microaerophilic bacteria. Microplates were spectrophotometrically read using Biolog Microstation and MicroLog 3 software (Biolog, Hayward, CA, USA). Absorbance values related to the metabolic activities of *Ab* strains were clustered using the graphical analysis program PermutMatrix v. 1.9.3 (Caraux and Pinloche, 2005) applying Euclidean distance as dissimilarity measure.

2.5 Heavy metal and antibiotic susceptibility testing

For heavy metal susceptibility test, *Ab* strains LMG 10828^{T} (ATCC 49616, RM4018), 55 and 6V (Fanelli et al., 2019), 34_O and 39_O were grown on Brain Heart Infusion (BHI) (Oxoid, Basingstoke, United Kingdom) agar amended with 0.6 % yeast extract (YE) (Biolife, Milan, Italy) incubated at 37 °C for 48 h under microaerophilic atmosphere (CampyGen^{TM} Compact, Oxoid, Basingstoke, United Kingdom). One single colony for each strain was inoculated in 20 ml of BHI broth with 0.6% YE and incubated at 37 °C for 48 h, then subcultured at 1% and incubated under the same conditions. Microbial cells were recovered by centrifugation (16,000 rcf x 6 min), washed in sterile 0.9% NaCl solution and resuspended in the same solution reaching the optical density (600

nm) of 0.5. Two microlitres of this suspension were spotted on cation adjusted Mueller Hinton agar (Liofilchem, Teramo, Italy) containing twofold serial dilution of the following heavy metal salts: sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$), cadmium acetate dihydrate [$Cd(CH_3COO)_2 \cdot 2H_2O$] and potassium chromate (K_2CrO_4) (final concentrations ranging from 32 mM to 0.0625 mM); zinc chloride ($ZnCl_2$) and copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) (final concentrations ranging from 16 mM to 0.0625 mM); cobalt dichloride hexahydrate ($CoCl_2 \cdot 6H_2O$) (final concentrations ranging from 64 mM to 0.0625 mM). Inoculated plates were incubated at 37 °C for 48 h under microaerophilic atmosphere. The test was performed in two biological replicates. Minimal inhibitory concentrations (MICs) were recorded as the lowest concentration of heavy metal salts that completely inhibits bacterial growth. Strains were considered resistant for MIC values > 1 mM as suggested by Otth et al. (2005).

The antibiotic susceptibility tests for *A. butzleri* strains LMG 10828^T (ATCC 49616, RM4018), 34_O and 39_O were performed by disk diffusion and broth microdilution methods as previously described in Fanelli et al. (2019). Antibiotic disks with the following antibiotic concentrations were used: ampicillin (10 µg/disk), cefotaxime (30 µg/disk), chloramphenicol (30 µg/disk), ciprofloxacin (5 µg/disk), erythromycin (15 µg/disk), gentamicin (10 µg/disk), kanamycin (30 µg/disk), nalidixic acid (30 µg/disk), streptomycin (10 µg/disk), tetracycline (30 µg/disk), vancomycin (30 µg/disk), and penicillin G (10 units/disk) (Biolab Zrt., Hungary). Broth microdilution method was performed to assess MIC and minimal bactericidal concentration (MBC) for those antibiotics which the tested *Ab* strains did not provide inhibition zone at all. As reported in our previous study (Fanelli et al., 2019) since breakpoint values have not been established for *Arcobacter* spp., classification of strains as susceptible, resistant, or intermediate was defined according to zone diameter and MIC interpretive standards for *Staphylococcus* spp. (erythromycin, penicillin, and vancomycin) and *Enterobacteriaceae* (ampicillin, gentamicin, cefotaxime, ciprofloxacin, tetracycline, chloramphenicol, nalidixic acid, kanamycin, and streptomycin) reported in CLSI performance standards for antimicrobial susceptibility testing (CLSI, 2015).

3. Results and discussion

3.1 General features of A. butzleri 34_O and 39_O genomes

Ab 34_O and *Ab* 39_O genomes were sequenced using a whole genome shotgun approach on an Ion S5™ platform (Thermo Fisher Scientific) generating around 472,441 and 460,707 reads with a median length of 318 and 317 bp, respectively (Table 1). Genomes were assembled using the Spades v5.0 software for a total of 48 and 30 large contigs (>500 bp) and a GC% of 26.91 and 26.79, respectively. The overall contiguity of the assembly is good, with a N50 of 102 Kbp and 192 Kbp for *Ab* 34_O and *Ab* 39_O, respectively; the longest assembled fragment is 301 Kbp in length for *Ab* 34_O and 459 Kbp for *Ab* 39_O (performed by QUAST (Bankevich et al., 2012), available at http://quast.sourceforge.net/quast) while the total length of the assembly was around of 2.2 Mb for both genomes. These Whole Genome Shotgun projects have been deposited at DDBJ/ENA/GenBank under the accessions QYZU00000000 (*Ab* 34_O) and QYZV00000000 (*Ab* 39_O). The versions described in this paper are QYZU01000000 (*Ab* 34_O) and QYZV01000000 (*Ab* 39_O).

Table 1. Summary of *Ab* 34_O and 39_O genome sequencing and assembly results

	Ab 34_O	*Ab* 39_O
Total sequenced bases	150,108,618	146,031,107
Mean read length	318	317
Total length	2,144,826	2,254,940
Number of scaffolds	48	30
Largest contig	301,400	459,205
Number reads	472,441	460,707
N50	102,534	192,281
Genome size	2,154,716	2,267,903
GC content	26.91%	26.79%
Predicted genes	2,239	2,313

CDS	2,091	2,123
tRNA	44	46
ncRNAs	2	2
rRNA	1, 1, 1 (5S, 16S, 23S)	1, 1, 1 (5S, 16S, 23S)

3.2 Genome-based analysis

The *in silico* MLST of the housekeeping genes retrieved from genomic sequences, confirmed *in vitro* results achieved by Mottola (2017): *Ab* 34_O and *Ab* 39_O define two novel sequence types, namely ST651 and ST653, respectively, as they both harbor a new *gly*A allele (Table 2).

Table 2. Allelic profile of *A. butzleri* isolates

				MLST							
id	isolate	species	source	*aspA*	*atpA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	ST
837	34_O	*Arcobacter butzleri*	vegetable	268	186	153	123	**635**	306	210	**651**
838	39_O	*Arcobacter butzleri*	vegetable	3	42	2	15	**684**	21	4	**653**

Novel alleles and novel Sequence Type (ST) are indicated in bold

Both *Ab* 34_O and *Ab* 39_O 16S rRNA gene sequences show 100% identity with the type strain *Ab* RM4018 (Miller et al., 2007). ANI, AAI and DDH analyses were performed with 24 strains within the *Arcobacter* group (Table S1). *Campylobacter jejuni subsp. jejuni* NCTC 11168 and *Helicobacter pylori* 26695 were included as out-groups.

Ab 34_O and *Ab* 39_O share 97.22% nucleotide identity (Table S2) and are comprised in the cluster including all the *Ab* species. According to the ANI, the closest relatives are *Ab* ED-1 and *Ab* 7h1h for *Ab* 34_O (97.39% and 97.29% ANI respectively) and *Ab* JV22 and *Ab* NCTC 12481 for *Ab* 39_O (98.43% and 97.95% ANI respectively).

The same clustering is obtained by using AAI (Table S3) with 96.88% between *Ab* 34_O and *Ab* 39_O, 97.71% between *Ab* 34_O and *Ab* RM4018, and 98.06% between *Ab* 39_O and *Ab* JV22.

DDH analysis confirmed the clustering obtained by ANI and AAI analysis, with values of 76.80 between *Ab* 34_O and *Ab* 39_O, 77.4% between *Ab* 34_O *Ab* ED-1, and 86.8% between *Ab* 39_O and *Ab* JV22 (Table S4).

These ANI and DDH values are within the range suggested by Chun et al. (2018) and, more specifically for *Arcobacter* spp., by On et al. (2017), to include *Ab* 34_O and *Ab* 39_O into the *Aliarcobacter* gen. nov. as *Aliarcobacter butzleri* comb. nov. (Pérez-Cataluña et al., 2018; 2019a, b). Phylogenetic analysis based on the concatenated dataset of 13 housekeeping genes (Fig. 1) shows that *Ab* strains can be grouped into two large clades and five different subgroups which, in part, resemble characteristic based on host/geographical distribution, even though further genomes from different sources should be available to support this hypothesis. The strains in the first subgroup are all derived from animal fecal sample, with the exception of *Ab* S2 005 003 R2 45 and *Ab* L352, which was collected by a diarrheic human stool sample. Within the second subgroup we can locate *Ab* JV22 and *Ab* 39_O, which have also the highest ANI, AAI and DDH reciprocal values. The third subgroup comprised the type strain *Ab* RM4018 and *Ab* NCTC12481, which were both isolated from human clinical samples in USA (Miller et al., 2007). The fourth subgroup includes *Ab* 55 and *Ab* 6V, which were isolated from shellfish (Mottola et al., 2016b) and recently characterized (Fanelli et al., 2019). The fifth subgroup comprises *Ab* ED-1, *Ab* L349, *Ab* 34_O, *Ab* S2 012 000 R2 80 isolated from a metagenomic sample from hospital surfaces, and *Ab* L351 and *Ab* L350, whose 13 gene sequences are identical and descend from the same node. *Ab* L349 was isolated from a diarrheic human stool sample, *Ab* L350 and *Ab* L351 from a healthy human stool, all from Canada.

This internal relationship was also confirmed by RAxML genome-based analysis, which is shown in Fig. 2. Even with this approach we obtained the same grouping retrieved by housekeeping evaluation, e.g. the subgroup comprising only *Ab* 55 and *Ab* 6V, the one including only *Ab* RM4018 and *Ab* NCTC12481 or the close relationship of *Ab* L351 and *Ab* L350, confirming that these sequences, utilized for MLSA, are informative and well support the phylogenetic results.

3.3 Protein functional classification

1309 and 1371 UniProtKB AC/ID identifiers retrieved by PFAM annotator tools (Galaxy Tool Version 1.0.0) were successfully mapped to 1181 and 1211 UniProtKB IDs (The UniProt Consortium, 2017) for *Ab* 34_O and *Ab* 39_O, respectively.

In both strains, the retrieved list included 37 genes associated with antibiotic resistance, including beta-lactamase, multidrug efflux pump and resistance proteins, and 3 with antibiotic biosynthesis related to bacteriocin, 25 for *Ab* 34_O and 36 for *Ab* 39_O putatively involved in pathogenesis (including regulators, lipoproteins, VOC family protein), 27 with metal resistance, 6 associated with drug transmembrane transporter activity, 23 with virulence, 4 with hemolysis, and 2 and 3 with quorum sensing (*lux*S, coding for the S-ribosylhomocysteine lyase, and *tqs*A, coding for the transport of quorum-sensing signal protein, in both strains and *maz*F coding for the endoribonuclease toxin MazF only in *Ab* 34_O).

Predicted genes were assigned to the clusters of orthologous groups (COG) classification (Fig. S1). Despite the group of general function prediction, which was the largest, the highest count for both strain was related to inorganic ion transport and metabolism (890 counts for *Ab* 34_O corresponding to 11.24%, and 967 corresponding to 11.75% for *Ab* 39_O) followed by amino acid transport (10.6%) and metabolism and signal transduction mechanisms (9.62% for *Ab* 34_O and 10.64% for *Ab* 39_O). These strains isolated from ready-to-eat vegetables in the same geographic area share the same distribution of COGs, as emerged from Fig. S1, indicating a limited functional variability, which is different from that found in the two *Ab* strains isolated from shellfish (Fanelli et al., 2019), thus supporting the possibility that the latter strains belong to different subspecies.

Moreover, the prevalence of the cluster “inorganic ion transport and metabolism” is consistent with the lithotrophic nature of *Arcobacter* spp. [i.e. ability to use inorganic substrates as a source of electron donors to drive energy acquisition, using either organic carbon or carbon dioxide as a source of carbon for constructing cellular materials (Ehrlich and Newman 2008)], which is more accentuated in plant-associated strains of the *Ab* species (Kalenitchenko et al., 2016).

3.4 Metabolic fingerprinting

Biolog AN MicroPlates™, also used by other authors to investigate the metabolic properties of microaerophilic, anaerobic or facultative anaerobic Gram positive and negative bacterial strains (Kiely et al., 2010; Siragusa et al., 2013; Wang et al., 2017), allowed us to metabolically characterize the *Ab* strains 34_O, 39_O and LMG10828^T (ATCC 49616, RM4018) along with *Ab* 55 and 6V (Fanelli et al., 2019) by the utilization of 95 carbon sources (Table 3). As expected and reported in the *Arcobacter* genus description by Vandamme et. al. (1992), none of the *Ab* strains metabolized carbohydrates but utilized organic acids and amino acids as carbon sources. Particularly, the tested *Ab* strains did used 16 (*Ab* 39_O), 15 (*Ab* 55), 14 (*Ab* LMG10828^T), 13 (*Ab* 6V) and 12 (*Ab* 34_O) substrates (Table 3), with *A. butzleri* 39_O and 34_O as the most and the least metabolically versatile strains, respectively. All the strains used α-hydroxybutyric acid, D,L-lactic acid, D-lactic acid methyl ester, L- malic acid, pyruvic acid, succinic acid and succinic acid mono-methyl ester, while succinamic acid and L-glutamine were metabolized only by *Ab* 55, and *Ab* LMG 10828^T was the only strain that utilized L-alanyl-L-Histidine. Although several strains from different isolation source are needed to build up a robust metabolic relationship, PermutMatrix analysis (Fig. 3) shows that among the five tested *Ab* strains, 39_O and 55 are the most metabolically related strains followed by 34_O and 6V, while LMG10828^T, that is not a food-borne strain, being isolated from human diarrheal feces (Kiehlbauch et al., 1991), turned out to be the most metabolically different. According to these findings it seems that the metabolic properties of *Ab* are not correlated with the two different source of isolation, i.e. shellfish (*A. butzleri* 55 and 6V) (Fanelli et al., 2019) and pre-cut ready-to-eat vegetables (*Ab* 34_O and 39_O). Merga et al. (2013) on the basis of genotypic and phenotypic differences between one *Ab* strain isolated from a cattle stool sample (*Ab* 7h1h) and *Ab* RM4018, hypothesized a probable niche adaptation in *Ab* but the comparison between only two strains allowed only a tentative evidence as also referred by the same authors (Merga et al., 2013), suggesting the need of comparative studies with strains from other sources as herein reported.

Table 3. Anaerobic metabolism of 95 carbon sources by *A. butzleri* strains LMG10828^T, 34_O, 39_O, 55 and 6V

Carbon source	LMG 10828^T	34_O	39_O	55	6V
Acetic Acid	+[a]	+	+	-[b]	-
Formic Acid	+	+	+	-	-
Fumaric Acid	+	+	+	+	-
α-Hydroxybutyric Acid	+	+	+	+	+
Itaconic Acid	+	-	-	-	+
α-Ketobutyric Acid	-	-	+	+	+
D,L-Lactic Acid	+	+	+	+	+
L-Lactic Acid	-	+	+	+	+
D-Lactic Acid Methyl Ester	+	+	+	+	+
L-Malic Acid	+	+	+	+	+
Propionic Acid	+	+	+	-	-
Pyruvic Acid	+	+	+	+	+
Pyruvic Acid Methyl Ester	-	-	+	+	+
Succinamic Acid	-	-	-	+	-
Succinic Acid	+	+	+	+	+
Succinic Acid Mono-Methyl Ester	+	+	+	+	+
L-Alanyl-L-Histidine	+	-	-	-	-
L-Glutamic Acid	+	-	+	+	+
L-Glutamine	-	-	-	+	-
L-Threonine	-	-	+	+	+

[a]+: utilization/oxidation of the carbon source
[b]-: not utilization/oxidation of the carbon source
The strains LMG 10828^T, 34_O, 39_O, 55 and 6V resulted all negative for: 3-Methyl-D-Glucose, Adonitol, Amygdalin, Arbutin, D,L-α-Glycerol Phosphate, D-Arabitol, D-Cellobiose, Dextrin, D-Fructose, D-Galactose, D-Galacturonic acid, D-Gluconic Acid, D-Glucosaminic Acid, D-Malic Acid, D-Mannitol, D-Mannose, D-Melezitose, D-Melibiose, D-Raffinose, D-Saccharic Acid, D-Sorbitol, D-Trehalose, Dulcitol, Gentibiose, Glucose- 1-Phosphate, Glucose- 6-Phosphate, Glycerol, Glyoxylic Acid, i-Erythritol, Lactulose, L-Alaninamide, L-Alanine, L-Alanyl-L-Glutamine, L-Fucose, L-Rhamnose, Maltose, Maltotriose, m-Inositol, m-Tartaric Acid, N-Acetyl-D-Galactosamine, N-Acetyl-D-Glucosamine, N-Acetyl-β-D-Mannosamine, Palatinose, Salicin, Stachyose, Sucrose, Turanose, Urocanic Acid, α-Cyclodextrin, α-D-Glucose, α-D-Lactose, α-Ketovaleric Acid, α-Methyl-D-Galactoside, α-Methyl-D-Glucoside, β-Cyclodextrin, β-Hydroxybutyric Acid, β-Methyl-D-Galactoside, β-Methyl-D-Glucoside.

3.5 Virulence determinants

The availability of an increasing number of *Arcobacter* spp. genome sequences has widened the knowledge about the pathogenic potential of this genus and greatly helped in exploring and identifying virulence associated genes, homologues to those described for both plant and animal pathogens, which confers this genus an important endowment for host invasion and colonization (Ferreira et al. 2016).

Table 4 shows the virulence determinants retrieved in the two *Ab* strains isolated from vegetables along with those found in the shellfish isolates by Fanelli et al. (2019) and those retrieved in the type

strain *Ab* RM4018. In this case, we investigated the presence of virulence determinants additional to those reported by Miller et al. (2007) in the genomic sequence of *Ab* RM4018.

Bacterial pathogenicity, the ability to grow, survive, colonize and persist in different tissues and ecosystem, aside from motility and chemotaxis, mainly rely on the ability to adhere to various surfaces and co-aggregate forming biofilms (Cepas et al., 2019; Díaz-Guerrero et al., 2018; Matilla and Krell, 2018; Tiwari et al., 2017).

Although still limited, several virulence determinants have been identified in *Ab*. Among these, lipopolisaccharides (LPS) (in the smooth form, i.e. possessing the polysaccharide region) or lipooligosaccharides (LOS) (in the rough form, i.e lacking the polysaccharide), which are major components of the outer leaflet of the outer membrane of most Gram-negative bacteria, play a pivotal role in pathogenesis, participating in host-pathogen interactions with the innate immune system, conferring antibiotic, serum and bile resistance, resistance to phagocytic killing, adhesion, invasion and survival in host cells and endotoxicity to the bacterial pathogens (Maldonado et al., 2016). In the genomes of *Ab* 39_O and *Ab* 34_O, we identified the '*waa*' gene cluster, harboring w*aa*C and *waa*F genes, responsible for assembly and phosphorylation of the inner-core region. *waa*C and *waa*F genes encode a heptosyltransferase I and a heptosyltransferase I that catalyze the transfer of the first and the second heptose to the inner-core region of the LOS/LPS, respectively. Orthologs of these genes have been found in numerous pathogens such as *Salmonella typhimurium* (Sirisena et al., 1992), *Neisseria gonorrhoeae* (Petricoin et al., 1991), *N. meningitidis* (Stojiljkovic et al., 1997), *Bordetella pertussis* (Allen et al., 1998), *Aeromonas hydriphila* (Jimenez et al., 2008), *Campylobacter* spp. (Klena et al., 1998; Kanipes et al., 2006; Richards et al., 2013), *Klebsiella pneumoniae* (Noah et al., 2001), *Serratia marcescens* (Coderch et al., 2004), *Pseudomonas aeruginosa* (de Kievit and Lam, 1997) and *Arcobacter thereius* (Rovetto et al., 2007). Attempts to construct knockout mutants of *waa*C and *waa*F in *P. aeruginosa* failed, leading to consider these genes essential for cellular viability (de Kievit and Lam, 1997), whereas mutation of *waa*C in *Campylobacter jejuni* 81-176 affects the structure of LOS and capsular carbohydrate (Kanipes at al., 2006) while Wang et al. (2016) demonstrated that the

deletion of the *waa*C, *waa*f or *waa*F genes in *Escherichia coli* W3110 disables the flagella biosynthesis. These finding lead to hypothesize that these genes in *Ab* could be involved in the organization of the outer part of the microbial cell.

As shown in Fig. 4, *Ab* 39_O shares the same content and organization of the *Ab* RM4018 cluster, while for *Ab* 34_O we retrieved some differences: the *alP* gene encoding an alkaline phosphatase family protein (WP_012147774.1 in *Ab* RM4018), due to shifts in the open reading frame, has been predicted as two different genes, putatively coding for a LTA synthase family protein and an hypothetical protein. Between the gene encoding the glycosyltransferase (D5K91_07595), orthologue of the glycosyltransferase (WP_012147769.1) in *Ab* RM4018, and the orthologue (DSK91_07585) of the hypothetical protein (WP_012147768.1) in *Ab* RM4018, it is annotated a gene putatively coding for a protein which has no orthologue in *Ab* species but has the 88% identity with glycosyltransferase family 2 protein (WP_024775442.1) of *Arcobacter cibarius* LMG 21996. Furthermore *Ab* 34_O seems to lack both the MBOAT family protein (WP_012147763.1) and the hypothetical protein WP_012147762.1 of *Ab* RM4018 comprised between the glycosyltransferase and the phosphoethanolamine transferase.

The components of the flagellum of *Ab* strains are reported in supplementary Table S5. As reported by Chaban et al. (2018), the architecture of flagellar motors in *A. butzleri* has a diverse (Rossmann and Beeby, 2018) motor structure, which is shared also by *Ab* 34_O and *Ab* 39_O. A core structure (inner membrane stator complexes MotA4B2 and the C-ring), a dedicated type III secretion system (T3SS) export apparatus and the inner membrane MS-ring and the P- and L-rings constitute the bacterial flagellar motor, while homologues of the accessory proteins FlgP, FlgQ, FlgT are not found in the *Arcobacter*-type motor accessory proteins (Chaban et al. 2018). Nevertheless, as for the two *Ab* strains isolated from shellfish (Fanelli et al., 2019), we found the homologue FlgO, an outer membrane protein required for flagellar motility in *Vibrio cholerae*, highly conserved in *Vibrio* spp. (Zhu et al., 2017). Moreover, in *Ab* 34_O, the *flhB* gene coding for the flagellar biosynthetic protein FlhB (D5K91_06835) of T3SS is a pseudogene frameshifted.

Among recognized virulence determinants, the genome of *Ab* 34_O harbors *cj1349* and *cadF* (encoding the fibronectin-binding proteins CadF and Cj1349), *cia*B (encoding the *Campylobacter jejuni* invasion antigen B), *mviN* (encoding a protein essential for the peptidoglycan biosynthesis), *pldA* (encoding phospholipase A), the hemolysin gene *tlyA*, *gyrA* (although we did not found any mutation in this gene) and the *hecB* gene (encoding a hemolysin activation protein) (Table 4). It lacks *irg*A (iron-regulating outer membrane protein) and the gene coding for the hydrolase IroE, while the sequence of *hecA* gene, putatively coding for a member of the filamentous hemagglutinin (FHA) family, is interrupted by a stop codon. Additionally, we identified a gene coding for the DNA binding protein of the conserved virulence factor B (CvfB) superfamily (protein ID D5K91_00265), which contributes to the expression of virulence factors and to pathogenicity in *Staphylococcus aureus* (Junecko et al., 2012; Matsumoto et al., 2006), a VOC family virulence protein (D5K91_03755), *virF* (D5K91_04440) of the AraC family of transcriptional regulators, a virulence transcriptional regulatory protein PhoP (D5K91_10615) and ShlB/FhaC/HecB family hemolysin secretion/activation protein (D5K91_06285).

In the genome of *Ab* 39_O we identified *cadF, cj1349*, *ciaB*, *mviN*, *pldA, tlyA, gyrA* (also in this case with no mutation), *hec*B genes. *hec*A gene coding for a filamentous hemagglutinin N-terminal domain-containing protein (D5R49_02690) resulted incomplete, partial in the middle of a contig. It lacks *iroE* gene but we identified additional virulence determinants such as *virF* (D5R49_06790), the gene coding for the virulence sensor protein BvgS precursor (D5R49_03150), one VOC family protein with a Glyoxalase/Bleomycin resistance protein/Dihydroxybiphenyl dioxygenase domain (D5R49_07635), and the DNA binding protein (D5R49_00290) of the CvfB superfamily, whereas the gene encoding the virulence sensor protein PhoQ is frameshifted.

Both strains lack the *iro*E gene, which encodes a siderophore esterase found also in uropathogenic *E. coli* (Larsen et al., 2006) and catalyses the degradation of salmochelins and enterobactin required for iron acquisition thus contributing to the competition with the host (Fischbach et al., 2006), although its role in pathogenesis in not clearly demonstrated (Caza et al., 2015). The lack of *iro*E gene, together

with the probable lack of functionality of *hec*A in *Ab* 34_O, are the only differences towards the two *Ab* strains isolate from shellfish, leading to suppose that they might share a comparable virulence potential.

Table 4. Virulence determinants identified in *A. butzleri* isolates

Virulence determinant	*A. butlzeri* strain				
	RM4018	34_O	39_O	55	6V
*waa*C-*waa*F cluster	ABV68062.1-ABV68040.1	D5K91_07645-D5K91_07535	DSR49_09855-DSR49_09965	D3M61_00195-D3M61_00085	D3M75_05185-D3M75_05185
*cj*1349	ABV66352.1	D5K91_02870	D5R49_05805	D3M61_05835	D3M75_01765
*cad*F	ABV66756.1	D5K91_00640	D5R49_00740	D3M61_01000	D3M75_00225
*cia*B	ABV67798.1	D5K91_04550	D5R49_06440	D3M61_06575	D3M75_04485
mvi*N*	ABV67138.1	D5K91_06545	D5R49_02405	D3M61_02290	D3M75_04090
*irg*A	ABV66991.1	na	D5R49_08395	D3M61_09625	D3M75_06715
iroE	ABV66992.1	na	na	D3M61_09620	D3M75_06710
pldA	ABV67121.1	D5K91_06630	D5R49_02320	D3M61_02040	D3M75_04175
*tly*A	ABV68075.1	D5K91_07700	D5R49_10875	D3M61_10630	D3M75_09375
*gyr*A	ABV68029.1	D5K91_07480	D5R49_10020	D3M61_00030	D3M75_11235
*hec*A	ABV67200.1	D5K91_06280[a] internal stop	D5R49_02690[b] middle contig	D3M61_02570	na
*hec*B	ABV67199.1	D5K91_06285	D5R49_02685	D3M61_02565	na
CvfB	ABV66823.1	D5K91_00265	D5R49_00290	D3M61_00610	D3M75_05605
*vir*F (*Ara*C transcriptional regulator)	ABV67819.1	D5K91_04440	D5R49_06790	D3M61_06685	D3M75_04600
*Bvg*S precursor	ABV67286.1	na	D5R49_03105	na	D3M75_03370
VOC family protein	ABV68289.1	D5K91_03755	D5R49_07635	D3M61_07955	D3M75_08585
phoQ	ABV68106.1	D5K91_10615	D5R49_10105	D3M61_10465	D3M75_03320
Virulence associated protein-*Vir*E superfamily	na	na	na	D3M61_07785	na

[a] pseudogene, internal stop;
[b] pseudogene, partial in the middle of a contig

3.6 Antibiotic susceptibility and genetic determinants

Table 5 shows a list of genes involved in antibiotic resistance identified in of *Ab* 34_O and *Ab* 39_O genomes and compared to those found in the type strain *Ab* RM4018 and *Ab* 55 and *Ab* 6V isolated from shellfish (Fanelli et al., 2019). This list comprises transporters, multidrug efflux pumps (operon

*emr*AB), multidrug resistance protein (*mtdE*, *mexA* and *mexB*) and methyl- and acetyltransferase (*rlmN*, *bpD*, *lpxD*) and other enzymes with some differences between the strains.

In none of the two *Ab* strains isolated from vegetables we retrieved the gene coding for the bifunctional polymyxin resistance protein *arnA* we previously identified in *Ab* 55 (Fanelli et al., 2019), while both genomes harbour the *arnB* gene coding for the UDP-4-amino-4-deoxy-L-arabinose-oxoglutarate aminotransferase which belongs to the DegT/DnrJ/EryC1/StrS aminotransferase family protein and is required for resistance to polymyxin and cationic antimicrobial peptides (Lee and Sousa, 2014).

Only in *Ab* 39_O we retrieved the *hlp*A gene. The predicted Serine/threonine-protein kinase HipA (D5R49_10660) is the toxic component of a type II toxin-antitoxin (TA) system, and it is involved in multidrug tolerance (Schumacher et al., 2009). A second toxic component of a type II toxin-antitoxin (TA) system, RelE (mRNA interferase toxin), which plays a role in bacterial resistance to antibiotics, was again retrieved only in *Ab* 39_O, *Ab* 55 and *Ab* 6V; overexpression of this protein induces persistent resistance to ciprofloxacin and ampicillin (Maisonneuve et al., 2011).

Table 5. Antibiotic resistance genes in *Ab* 34_O, *Ab* 39_O and *Ab* RM4018 found in this study and retrieved by Fanelli et al (2019) in *Ab* 55 and *Ab* 6V.

Gene name	**Product**	***Ab* RM4018**	***Ab* 34_O**	***Ab* 39_O**	***Ab 55***	**Ab 6V**
-	putative metallo-hydrolase (metallo Beta-lactamase)	P[a]	P	P	P	P
*acr*B	Multidrug efflux pump acriflavin resistance protein AcrB	P	P	P	P	P
*arn*A	Bifunctional polymyxin resistance protein ArnA	Na[b]	Na	Na	P	Na
*arn*B	UDP-4-amino-4-deoxy-L-arabinose—oxoglutarate (polymyxin resistance)	P	P	P	P	P
*arp*C	Antibiotic efflux pump outer membrane protein ArpC	P	P	P	P	P
bcr	Bicyclomycin resistance protein	P	P	P	P	P
*bep*D	Efflux pump periplasmic linker BepD precursor	P	P	P	P	P

*bep*E	Efflux pump membrane transporter BepE	P	P	P	P	P
bla	Beta-lactamase OXA-15 precursor	P	P	P	P	P
bla2	Beta-lactamase 2 precursor	P	Na	Na	P	P
cat3	Chloramphenicol acetyltransferase 3	P	P	P	P	P
*ept*A	Phosphoethanolamine transferase EptA (polymyxin resistance)	P	P	P	P	P
fsr	Fosmidomycin resistance protein	P	Na	P	P	P
*hcp*C	Putative beta-lactamase HcpC precursor	Na	P	P	P	P
*hlp*A	Serine/threonine-protein kinase HipA (methicillin resistance)	Na	Na	P	Na	P
*ile*S	Isoleucine-tRNA ligase (mupirocine resistance)	P	P	P	P	P
*lpx*D	UDP-3-O-(3-hydroxymyristoyl)glucosamine N-acyltransferase	P	P	P	P	P
*mac*A	Macrolide export protein MacA	P	P	P	P	P
*mac*B	Macrolide export ATP-binding/permease protein MacB	P	P	P	P	p
*mdt*B	Multidrug resistance protein MdtB	P	Na	P	P	P
*mdt*E	Multidrug resistance protein MdtE precursor	P	Na	P	P	Na
*mex*A	Multidrug resistance protein MexA	P	P	P	P	P
*mex*B	Multidrug resistance protein MexB	P	P	P	P	P
*opr*M	Outer membrane protein OprM	P	P	P	P	P
pbp	Beta-lactam-inducible penicillin-binding protein	P	P	P	P	P
*rel*E	mRNA interferase toxin RelE (ciprofloxacin and ampicillin)	Na	Na	P	P	P
*rlm*N	putative dual-specificity RNA methyltransferase RlmN (ribosome target antibiotics)	P	P	P	P	P
*stt*H	Streptothricin hydrolase	P	P	P	P	P
*tet*A	Tetracycline resistance protein, class C	P	Na	P	P	P
*tol*C	TolC family protein	P	P	P	P	P
*upp*P	Undecaprenyl-diphosphatase (bacitracin resistance)	P	P	P	P	P
*wbp*D	Group B chloramphenicol acetyltransferase	P	P	P	P	P

[a]P: Present.

[b]Na: Not annotated.

All genomes harbor the *upp*P gene encoding an undecaprenyl-diphosphatase, whose over-expression in *Escherichia coli* is associated with bacitracin resistance (Cain et al., 1993), and the *bcr* gene encoding a putative translocase involved in sulfonamide and byclomicin resistance (Nichols and Guay, 1989). The gene encoding the phosphoetanolamine transferase EptA was detected in both *Ab* 34_O (D5K91_03280) and *Ab* 39_O (D5R49_06240) as in the other *Ab* strains analyzed. It catalyses the addition of a phosphoethanolamine moiety to the lipid A, which is required for resistance to polymyxin. The transporter conferring resistance against fosmidomycin, encoded by the *fsr* gene (Fujisaki et al., 1996), was only found in *Ab* 39_O (D5R49_03895) and not in *Ab* 34_O.

The multidrug resistance protein MdtE precursor is present in *Ab* RM4018, *Ab* 39_O and *Ab* 55, while *mdt*B was absent in *Ab* 34_O.

In all genomes we identified the streptothricin conferring resistance hydrolase gene *sttH* (D5K91_08335 for *Ab* 34_O, D5R49_01845 for *Ab* 39_O) (Hamano et al., 2006).

Moreover, in all strains we retrieved gene sequences of *arp*C, encoding the antibiotic efflux pump outer membrane protein which has been demonstrated conferring resistance to numerous structurally unrelated antibiotics such as carbenicillin, chloramphenicol, erythromycin, novobiocin, streptomycin and tetracycline in *P. putida* (Kieboom et al., 2001), and the 23S rRNA methyltransferase *rlm*N, which specifically methylates position 2 of adenine 2503 in 23S rRNA and position 2 of adenine 37 in tRNAs, which confers resistance to some classes of antibiotics in *E. coli* (Toh et al., 2008).

Results of the disk diffusion tests are shown in Table 6. In Table 7 MICs and MBCs values, obtained by broth microdilution method, for the antibiotics to which the tested *Ab* strains did not provide inhibition zone at all are reported. Both tables also includes the results obtained by Fanelli et al. (2019) for *Ab* 55 and 6V isolated from shellfish.

According to MIC interpretive standards (CLSI, 2015), the tested strains were resistant towards cefotaxime, ampicillin, penicillin G and vancomicyn. These results, along with those obtained by disk diffusion susceptibility test, were similar to those previously reported by Fanelli et al. (2019) for *Ab*

type strain LMG 10828, herein used also as reference strain, and *Ab* 55 and 6V isolated from shellfish. Particularly, *Ab* 34_O and 39_O resulted susceptible as *Ab* 55, *Ab* 6V and LMG 10828^T towards the three tested aminoglycosides antibiotics, i.e. gentamicin, kanamycin and streptomycin and to the hydrophilic fluoroquinolone ciprofloxacin. The latter susceptibility may be due to the absence of any mutation in *gyr*A gene in the quinolone resistance determining region (Abdelbaqui et al., 2007).

Like *Ab* 55, *Ab* 6V and LMG 10828^T, *Ab* 34_O and *Ab* 39_O resulted resistant towards the three tested β-lactams antibiotics, namely ampicillin, penicillin G and cefotaxime, and towards vancomycin. As discussed by Fanelli et al. (2019), vancomycin resistance rather than molecular based (reviewed by Ahmed and Baptiste, 2018), as we did not identify any element of the vancomycin resistance operon in the genomic sequences of the analyzed *Ab* strains, can be due to the intrinsic characteristic of Gram-negative bacteria porins, which do not allow high weight molecules, such as glycopeptides, to pass through them (Quintiliani and Courvalin, 1995).

The ß-lactam resistance can be ascribed to the combined presence of ß-lactamase genes, to the reduced affinity to the penicillin-binding proteins and to the action of proteins regulating the outer membrane permeability (Georgopapadakou et al., 1993). In the genomes of *Ab* 34_O and *Ab* 39_O we identified only two of the three putative ß-lactamases orthologues to that of *A. butlzeri* RM4018 (Miller et al., 2007) (MBL fold metallo-hydrolase D5K91_08125 for *Ab* 34_O and D5R49_04415 for *Ab* 39_O; class D beta-lactamase D5K91_09720 *Ab* 34_O and D5R49_10630 for *Ab* 39_O) as well as penicillin binding proteins (D5K91_09860, D5K91_02225 and the frameshifted D5K91_09815 for *Ab* 34_O and D5R49_10250, D5R49_10295 and D5R49_02950 for *Ab* 39_O). Multialignement of the OXA beta lactamases from *Ab* species is shown in Fig. 5. Aminoacidic sequences shows few differences between *Ab* 34_O and *Ab* 39_O: in *Ab* 39_O there is an asparagine at position 128 (while there is a lysine in *Ab* 34_O as in *Ab* 55 and *Ab* 6V; in *Ab* 34_O there are a lysine and an serine in position 155 and 174 respectively, while in Ab 39_O there are an arginine and an alanine. Finally, in *Ab* 39_O there is a T at position 236 while in the other *Ab* strains there is an alanine. We did not identify MBL of type 2.

In addition, both genomes harbor few putative beta-lactamase-HcpC precursors (D5K91_01815, D5K91_04700; D5R49_03430, D5R49_06300) with a sel1 repeat domain, and one beta-lactamase HcpB-like with a sel1 repeat domain (D5R49_07790; the D5K91_00255 in *Ab* 34_O is frameshifted). In both strains we identified the *lrgAB* operon (D5K91_08490- D5K91_08495 for *Ab*34_O; D5R49_02215-D5R49_02210 for *Ab*39_O), which was associated to the enhanced ß-lactam resistance in *Ab* RM4018 (Miller et al., 2007) and which modulates penicillin resistance in *Staphylococcus* spp. (Bayles, 2000).

Only slight differences were observed for susceptibility to nalidixic acid, chloramphenicol, tetracycline and erytromycin to which none of the tested *Ab* strains was sensitive but classification as resistant or intermediate resistant was differently displayed for *Ab* 34_O, *Ab* 39_O, *Ab* 55, *Ab* 6V and LMG 10828^T as reported in Table 6. Regarding the hydrophobic quinolone nalidixic acid *Ab* 34_O, 39_O, LMG 10828^T and 6V were intermediate resistant while *Ab* 55 was resistant (Table 6). The absence of mutations in the *gyr*A gene as above discussed, suggests that mechanisms of hydrophobic quinolone uptake, may intervene in nalidixic acid putative resistance (Miller et al., 2007).

As concerns chloramphenicol, *Ab* 34_O, *Ab* 55 and *Ab* 6V resulted intermediate resistant while LMG 10828^T and *Ab* 39_O were resistant (Table 6). All strains harbor one chloramphenicol acetyltransferase gene (*cat*3): the coded enzyme (D5K91_05815; D5R49_08130) catalyses the transfer of an acetyl remnant of acetyl CoA to chloramphenicol, which is the most common mechanism for inactivating this antibiotic and conferring resistance in bacteria (Schwarz et al., 2004). *Ab* 34_O, *Ab* 39_O and LMG 10828^T were intermediate resistant towards tetracycline while *Ab* 55 and *Ab* 6V were resistant (Table 6). Tetracycline resistance is generally determined by the action of efflux pumps, modification of the 16S rRNA at the binding site or protection from the ribosome binding. The main proteins involved in these mechanisms are Tet(O) and Tet(M), paralogues of the translational GTPase EF-G, which catalyses by hydrolysis the tetracycline removal from its binding site (Chopra and Roberts, 2001). Both the genomic sequences of our strains harbor the Elongation factor (D5K91_07060 for *Ab* 34_O; D5R49_09200 for *Ab* 39_O) with the same C-terminus domain

of the ribosomal protection proteins, while only in the genome of *Ab* 39_O we detected the tetracycline resistance protein D5R49_01485, a predicted MFS transporter of the efflux pump. Furthermore, the above mentioned multidrug efflux systems are homologs of the CmeABC transporter of *C. jejuni*, which is known to be involved in macrolide and tetracycline resistance (Gibreel et al., 2007).

Lastly, *Ab* 39_O, *Ab* 55 and *Ab* 6V were resistant towards erythromycin, while *Ab* 34_O and LMG 10828^{T} were intermediate resistant. CARD analysis identified in both strains, by using perfect and strict selection criteria, *ade*F gene, encoding a strict resistance-nodulation-cell division (RND) antibiotic efflux pump, which confer resistance to fluoroquinolone antibiotics and erythromycin. AdeF is the membrane fusion protein of the multidrug efflux complex AdeFGH in *Acinetobacter baumanii* (Coyne, et al. 2010) and has the same structure and function of the RND family AcrAB-TolC export system described in *Escherichia coli* and Gram-negative bacteria, which is involved in resistance to macrolide and several unrelated toxic compounds, such as dyes, detergents and antibiotics (Chollet et al., 2004; Du et al., 2014), acting for metal and multidrug transporter.

The components of this tripartite efflux pump are generally coded by an operon and comprise a component of the outer membrane channel (in this case TolC), an efflux pump periplasmic linker (AcrA), an efflux pump membrane transporter (AdeF=AcrB), and an HTH-type transcriptional regulator (MprA).

Ab 34_O has a single copy of contiguous genes coding for this system (D5K91_09085 - D5K91_09100) while these genes are in two different loci in *Ab* 39_O (D5R49_00830 - D5R49_00845; D5R49_09690 - D5R49_09705). The presence of this system may also explain the resistance and intermediate resistance of *Ab* 39_O and *Ab* 34_O, respectively, towards erythromycin, also considering that we did not identify any specific resistance mechanism towards erythromycin, such as the presence of the *erm* (erythromycin resistance methylase) class gene, which protect the antibiotic binding to the ribosomes by post transcriptional methylation of 23S rRNA.

The antibiotic susceptibility results herein reported, are mostly in agreement with antibiotic resistance or susceptibility prevalence reported for other *Ab* isolated from seafood and water sources (Collado et al., 2014; Rathlavath et al., 2017; Šilha et al., 2017) as well as from other various sources such as meat (beef, pork and chicken), slaughterhouses, milk, cheeses and dairy plant, animal stools (cattles, sheeps, pigs and pultry), and humans (Aski et al., 2016; Elmali and Can, 2017; Ferreira et al., 2013; 2017; Kabeya et al., 2004; Kayman et al., 2012; Rahimi, 2014; Scanlon et al., 2013; Šilha et al., 2017; Soma et al., 2017;Van den Abeele et al., 2016; Vicente-Martins et al., 2018; Yesilmen et al., 2014; Zacharow et al., 2015), as widely discussed in our previous study (Fanelli et al., 2019). Conversely a very limited comparison can be accomplished with *Ab* isolated from vegetables. As far as we know, only one study by González et al. (2017) reported antibiotic susceptibility of *Ab* isolates from vegetables but only two quinolone antibiotics were tested, among them ciprofloxacin. Anyway, results here reported are in agreement with those of González et al. (2017): indeed, fifteen (88.24%) out of seventeen *Ab* isolates from fresh vegetables analyzed by González et al. (2017) were susceptible to ciprofloxacin.

According to the proposed definition for multidrug-resistant (MDR) bacteria reported by Magiorakos et al. (2012), on the basis of our disk diffusion susceptibility test, all the five *Ab* strains can be classified as MDR considering criteria either for *Staphylococcus aureus* and *Enterobacteriaceae*, since, as far as we know, specific criteria for MDR *Ab* have not been proposed yet. Indeed, the tested *Ab* strains resulted not susceptible to one antimicrobial agent belonging to three of the required classes of antibiotics used to classify *S. aureus* as MDR, i.e. macrolides, phenicols and tetracyclines, and to four classes of antibiotics proposed for MDR classification of *Enterobacteriaceae*, i.e. penicillins, phenicols, tetracyclines and extended spectrum, 3rd and 4th generation cephalosporins.

Table 6. Disk diffusion susceptibility test for *A. butzleri*

		A. butzleri strain				
Class	Antibiotics	LMG 10828^{T} (RM4018)	34_O	39_O	[a]55	[a]6V

β-lactams	Ampicillin 10 µg /disk	R*	R*	R*	R*	R*
	Penicillin G 10 units/disk	R*	R*	R*	R*	R*
	Cefotaxime 30 µg/disk	R*	R*	R*	R*	R*
Glycopeptides	Vancomycin 30 µg/disk	R*	R*	R*	R*	R*
Quinolones	Ciprofloxacin 5 µg/disk	S	S	S	S	S
	Nalidixic acid 30 µg/disk	I	I	I	R	I
Aminoglycosides	Gentamicin 10 µg/disk	S	S	S	S	S
	Kanamycin 30 µg/disk	S	S	S	S	S
	Streptomycin 10 µg/disk	S	S	S	S	S
Phenicol	Chloramphenicol 30 µg/disk	R	I	R	I	I
Tetracycline	Tetracycline 30 µg/disk	I	I	I	R	R
Macrolide	Erytromycin 15 µg/disk	I	I	R	R	R

Classification as S (susceptible), I (intermediate) and R (resistant) was carried out according to zone diameter interpretive standards for *Staphylococcus* spp. (erytromycin and penicillin G) and *Enterobacteriaceae* (ampicillin, gentamicin, cefotaxime, ciprofloxacin, tetracycline, chloramphenicol, nalidixic acid, kanamycin and streptomycin) (CLSI, 2015). To our knowledge, no vancomycin reference interpretive criteria are reported for *A. butzleri.*

*no inhibition zone detected.

[a] disk diffusion susceptibility results reported by Fanelli et al. (2019)

Table 7. Cefotaxime, ampicillin, penicillin G and vancomycin MIC and MBC values (in µg/ml) for *A. butzleri*

A. butzleri strain	Antibiotics							
	β-lactams						Glycopeptide	
	Cefotaxime		Ampicillin		Penicillin G		Vancomycin	
	MIC[a]	MBC[b]	MIC[a]	MBC[b]	MIC[a]	MBC[b]	MIC[a]	MBC[b]
LMG 10828^T (RM4018)	16	16	32	32	128	256	2,048	> 2,048
34_O	128	128	128	128	128	256	2,048	> 2,048
39_O	32	32	256	256	512	512	> 2,048	> 2,048

[a]minimal inhibitory concentration

[b]minimal bactericidal concentration

3.7 Heavy metal susceptibility

Heavy metals are naturally present in the environment and geological or anthropological activities may considerably accelerate their release and accumulation in different environments also including

agricultural and urban soils (Brandt et al., 2010; Zhang et al., 2018). The exposition to these compounds, which being non-biodegradable remain and accumulate in the environment for extended periods of time, have generated resistance mechanism in several bacteria species (Xavier et al., 2019). Mechanisms of heavy metal resistance in bacteria include extracellular barrier, active transport of metal ions (efflux), extracellular sequestration, intracellular sequestration, reduction of metal ions (Bruins et al., 2000).

Results of heavy metal susceptibility testing are reported in Table 8.

Table 8. Heavy metal minimum inhibitory concentrations (MICs) for *Arcobacter butzleri*

A. butzleri strain	Minimum Inhibitory Concentration (MIC) (mM)					
	heavy metal salt					
	sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$)	zinc chloride ($ZnCl_2$)	cobalt dichloride hexahydrate ($CoCl_2 \cdot 6H_2O$)	copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	cadmium acetate dihydrate [$Cd(CH_3COO)_2 \cdot 2H_2O$]	potassium chromate (K_2CrO_4)
LMG 10828^T (RM4018)	> 32*	4*	4*	2*	0.25	0.125
34_O	16*	4*	4*	2*	0.25	0.125
39_O	> 32*	4*	4*	2*	0.25	0.5
55	> 32*	4*	4*	2*	0.25	0.25
6V	> 32*	4*	4*	2*	0.25	0.25

*resistant strain (MIC > 1 mM) (Otth et al., 2005)

To our knowledge, no standardized protocols for testing bacterial susceptibility to heavy metals are available, and no reference breakpoints to classify bacteria as resistant, intermediate resistant or sensible to heavy metals have been established as previously reported also by other authors (Chenia and Jacobs, 2017; Ug and Ceylan, 2003). However, the agar dilution method employed within this study for heavy metal susceptibility testing has been previously used by several researchers (Aarestrup and Hasman, 2004; Chenia and Jacobs, 2017; Fard et al., 2011; Hasman et al., 2006; Matyar at al., 2008; Otth et al., 2005; Ug and Ceylan, 2003).

Knowledge about *Ab* susceptibility to heavy metals is still very limited, in fact, as far as we know, the *in vitro* susceptibility of *Ab* to heavy metals was assessed only in two studies, particularly by Otth et al (2005) and Schroeder-Tucker et al. (1996), but the latter tested the susceptibility only towards

one heavy metal salt, namely cadmium chloride, using disk diffusion test. According to Otth et al. (2005) we considered MIC > 1 mM as interpretive criterion to classify *Ab* isolates as resistant to heavy metals. This same interpretive criterion was as well used to assess resistance of *Staphylococcus* spp. towards some heavy metal salts, such as zinc, cobalt, copper and chrome salts (Ug and Ceylan, 2003).

Ab 55, 6V, 34_O, 39_O and LMG 10828^T were resistant towards cobalt and molybdate salts and susceptible towards chromate salt (Table 8), in accordance with results obtained by Otth et al. (2005) for fifty *Ab* strains tested in their study. In our study, MIC values for chromate and molybdate salts were similar to those of Otth et al. (2005), ranging from 0.125 to 0.5 mM and 0.04 to 0.16 mM, respectively. Moreover, high MIC values for molybdate, > 32 mM and ≥ 80 mM, respectively, were obtained in this study and in that of Otth et al. (2005), with the exception of *Ab* 34_O which showed more sensitivity to this heavy metal (16 mM MIC).

Our *Ab* strains showed lower MIC values for cobalt (4 mM) than those found by Otth et al. (2005) (≥ 80 mM) for the fifty *Ab* strains tested in their study.

These differences in MIC values could be due to certain differences in the experimental conditions, such as the chosen salt or the incubation condition. Growth conditions recommended for *Ab* type strain LMG 10828^T by BCCM/LMG Bacteria Collection (http://bccm.belspo.be) were used in the present study [37°C in microaerophilic atmosphere instead of 26 °C in aerobiosis used by Otth et al. (2005)] as well as in other previous studies that tested the antimicrobial susceptibility of *Ab* (Aski et al., 2016; Fanelli et al., 2019; Ferreira et al., 2013). As far as we know, we reported for the first time MIC values of zinc, copper and cadmium for *Ab*. The strains tested in this study were all resistant to zinc (4 mM MIC) and copper (2 mM MIC) whereas they were all susceptible to cadmium (0.25 mM MIC). Similar findings were reported also for *Campylobacter* spp., which are taxonomically related to *Arcobacter* spp. (Perez-Cataluña et al., 2018a). Particularly Baserisalehi et al. (2007) reported that MICs for cadmium ranged between 0.01 to 1 mM for the six *Campylobacter* spp. isolates they tested and Kaakoush et al. (2008) reported that cadmium was lethal at 1 mM for *C. jejuni* NCTC 11168, the

only strain they tested, concluding that this heavy metal is highly toxic for *C. jejuni*. Baserisalehi et al. (2007) also reported high MIC values of zinc and copper for the six *Campylobacter* spp. they tested, that ranged between 10 to 100 mM for both heavy metals.

Table 9. RND efflux pumps and metal resistance genes in *Ab* 34_O and *Ab* 39_O

Gene/pump	Product	substrate	*Ab* 34_O	*Ab* 39_O
*ars*C	Arsenate reductase	arsenic	D5K91_04925	D5R49_04920
*ars*3	Arsenic resistance protein		D5K91_00250	D5R49_00275
*ars*B	Arsenical pump membrane protein		D5K91_06775	D5R49_09480
*mop*A	LysR family transcriptional regulator	molybdenum	D5K91_03135	D5R49_06090
*mod*A	molybdate ABC transporter substrate-binding protein		D5K91_03140	D5R49_06095
*mod*B	Molybdate ABC transporter, permease protein		D5K91_03150	D5R49_06100
*mod*C	Molybdate/tungstate binding, C-terminal		D5K91_03145	D5R49_06105
*mer*T	Mercuric transport protein	mercuric	D5K91_10875	D5R49_11280
Cation efflux system	metal efflux RND transporter	cobalt, zinc and cadmium, silver, copper, nickel, various toxins	D5K91_00270	D5R49_00295
	metal ABC transporter ATP-binding protein		D5K91_00275	D5R49_00300
	heavy metal translocating P-type ATPase	copper, cadmium	D5K91_00315 D5K91_00645	D5R49_00340 D5R49_00745
	Metal sensing transcriptional repressor		D5K91_00650	D5R49_00750
	cation diffusion facilitator family transporter	cadmium, zinc, cobalt	D5K91_01295	D5R49_01425
*cor*C	HlyC/CorC family transporter	magnesium, cobalt	D5K91_01595	D5R49_03695
	ABC/ECF transporter, transmembrane component	cobalt, nickel	D5K91_06295	D5R49_02675
	cation ABC transporter substrate-binding protein	zinc	D5K91_07400	D5R49_10485
*rcn*A	nickel/cobalt efflux protein RcnA	cobalt, nickel	D5K91_07405	D5R49_10490*
*znt*B	zinc transporter ZntB	zinc	D5K91_10365	D5R49_10780
*cad*A	P-type ATPase subfamily IB cation trasport	copper, cadmium	D5K91_10645	D5R49_10065
RND efflux pump	efflux RND transporter periplasmic adaptor subunit	cobalt, nickel	D5K91_10805	no
	efflux RND transporter permease subunit		D5K91_10810	no
*cop*Z	copper chaperone	copper, mercuric	D5K91_10870	D5R49_11275
*cso*R	Metal-sensitive transcriptional repressor	copper, nickel, cobalt	D5K91_00650	D5R49_00750
*czc*B	Cobalt-zinc-cadmium resistance protein CzcB	cobalt, zinc, cadmium	No	D5R49_00840

609

The presence of genetic determinants of metal resistance is believed to be ancient as the appearance and toxicity of metals in the environment (Jenkins and Stekel, 2010). Genetic determinants of heavy metal resistance can be localized both on bacterial chromosomes and on extrachromosomal genetic elements. Horizontal gene transfer plays an important role in the spread of heavy metal resistance in nature (Heuer and Smalla, 2012).

Metal resistance and antibiotic resistance genes often co-occur together on mobile elements, such as genomic islands, plasmid or transposable elements, and their genetic linkage often determine their co-resistance and co-selection and the risk of horizontal transfer between bacteria, as increasingly reported (Bengtsson-Palme and Larsson, 2015; Zhao et al., 2019). It is also of great concern since this mechanism can promote antibiotic resistance also in the absence of antibiotic exposure in a metal selective environment. Another mechanism of co-selection is cross-resistance due to the occurrence of single genes encoding resistance to both antibiotics and metals (Zhao et al., 2019).

Strains *Ab* 34_O and *Ab* 39_O were isolated from pre-cut ready-to-eat vegetables (Mottola et al., 2016b) thus it is hardly improbable that they were under a selective antibiotic pressure. The finding of several antimicrobial resistant genes in these strains could be a consequence of exposure to different heavy metal salts in soil or water environments, based on the role of Cu in the spread of antibiotic resistances in bacteria and their antibiotic resistance genes (Berg et al. ,2005; Xu et al., 2017).

Our study found resistance to Cu, Co, Zn, and Mo salts that could correlate with resistance to β-lactams, chloramphenicol and tetracycline. In fact, it is well known that reduction of membrane permeability and rapid efflux mechanisms (whose genes were also found by genomic analysis) are antibiotic- and metal-resistance shared systems as reviewed by Baker-Austin et al. (2006).

In *Escherichia coli* genetic determinants of metal resistance are well characterized and usually organized in operons, while knowledge in *Arcobacter* spp. is limited. As for toxic compounds and antibiotics, in Gram-negative bacteria metal uptake and control is exerted by RND (Resistance-Nodulation-Division) multidrug efflux pumps which are generally composed by an inner membrane

transporter, an outer membrane channel and a periplasmic adaptor protein, as previously stated (Daury et al., 2016). Bacterial multidrug efflux pumps are generally chromosomally encoded with a conserved organization both at the genetic and at the protein levels (Blanco et al., 2016).

The RND pumps act in synergy with other pumps, which are responsible of drug extrusion into the periplasm (Lee et al., 2000).

In both *Ab* 34_O and *Ab* 39_O we identified several RND efflux pumps (Table 9), which are generally located close to genes involved in antibiotic resistance and metal sensing transcriptional regulators. Furthermore, we identified specific metal resistance proteins such as the arsenate reductase, the arsenical membrane protein and the arsenical resistance protein (D5K91_04925, D5K91_03135 and D5K91_06775 in *Ab* 34_O, D5R49_04920, D5R49_00275 and D5R49_09480 in *Ab* 39_O, respectively).

We also retrieved in both strains *mer*T gene, encoding a mercuric transport protein (D5K91_10875 in Ab 34_O; D5R49_11280 in Ab 39_O), as well as transporters for copper, cadmium, zinc, nickel and cobalt (Table 9).

In both strains we identify genes encoding the *mod*ABC system, involved in the molybdenum acquisition and transport, located in an operon as in other bacterial species (Kashyap, et al., 2006; Xia et al., 2018). The operon comprises a gene coding for ModE, a transcriptional regulator, ModA, a molybdenum binding protein, ModB, the transmembrane component of the permease, and ModC P-type ATPase.

Conclusion

To the best of our knowledge, this the first study reporting the genomes of *Ab* strains isolated from vegetables. Genomic analyses allowed us to confirm the amendment of *Arcobacter butzleri* as *Aliarcobacter butzleri*, comb. nov. (Perez- Pérez-Cataluña et al. 2018). Moreover, the genotaxonomic assessment of the *Ab* species supports the division of the *Ab* species in different subspecies. Several antibiotic, virulence and metal resistance determinants were retrieved in both strains and were

compared to those found in the type strain and in the strains isolated from shellfish (Fanelli et al., 2019). Less virulence and antibiotic resistance genes were found in *Ab* 34_O than in *Ab* 39_O while all the five strains endowed multiple resistance to several antibiotics and heavy metals. The metabolic fingerprinting of all the assayed strains resulted less discriminatory than the taxonomic approach although providing useful information about the phenotypic traits of the *Ab* species. Overall, this study provided further knowledge that may contribute to obtain an updated description of the species and to clarify the role of genetic endowment, as well as of the ecological niches the strain come from, in the pathogenesis of *Ab* illness. In addition these findings are relevant in terms of food safety, as they enable us to assess the possible public health implications of food-poisoning illnesses caused by *Arcobacter*-contaminated foods, and highlight the need for additional data in order to better assess the human health risks arising from the consumption of such foods.

Figure captions

Fig. 1. Phylogenetic tree based on the concatenated dataset of 13 housekeeping genes

Fig. 2. Phylogenetic tree based on RAxML analysis

Fig. 3. PermutMatrix analysis of *A. butzleri* metabolic patterns determined by Biolog system. The colours scale from green to red indicates the utilization or oxidation of each carbon source ranging from the lowest to the highest value. Euclidean distance was used to calculate the percentage of dissimilarity.

Fig. 4. Genomic organization of waaC/waaF gene cluster in *A. thereius* LMG 24486, *A. butzleri* ED-1, *A. butlzeri* 34_O, *A. butzleri* 39_O, and *A. butzleri* RM4018. Gene clustering is represented by the arrows superposed on the black horizontal line. Intergenic spaces are not drawn in scale. For *A. thereius* LMG 24486, *Ab* ED-1, and *Ab* RM4018, the locus tag of each gene is indicated below the respective gene arrow; for *Ab* 34_O and *Ab* 39_O, protein ID is indicated below the respective gene arrow. Red arrows in *Ab* ED-1 and indicate genes with no orthologue in *Ab* 34_O, 39_O and RM4018. DSR49_09915* indicates pseudogene (frameshifted). lAaT: lipid A biosynthesis acylTransferase; dK:

diacylglycerol kinase; yejM: inner membrane protein yejM; O-aL: O-antigen ligase; hp: hypotetical protein; gT: glycosyltransferase; pgaB: poly-beta-1,6-*N*-acetyl-D-glucosamine *N*-deacetylase; eptA: phosphoethanolamine transferase eptA; rfaF: lipopolysaccharide heptosyltransferase II; aT: acetyltransferase; sunS: glycosyltransferase sunS; aLP: alkaline phosphatase family protein; gPtT: glucose-1-phosphate thymidylyltransferase rfbA; yrbL-phoP: regulatory network protein; degT: DegT/DnrJ/EryC1/StrS family aminotransferase; rfbA: glucose-1-phosphate thymidyl transferase; rfbB: dTDP-glucose 4,6-dehydratase 1; rfbC: dTDP-4-dehydrorhamnose 3,5-epimerase; rfbD: dTDP-4-dehydrorhamnose reductase; mbOat: membrane bound *O*-acyl transferase; yrbL-phoP: YrbL-PhoP reg domain containing protein.

Fig. 5. Multialignment of OXA beta-lactamase from *A. butzleri* obtained by using T-Coffee web server (Di Tommaso et al., 2011). *Ab* 34_O protein ID: D5K91_097; *Ab* 39_O protein ID: D5R49_106; *Ab* 55 protein ID: D3M61_10735; *Ab* 6V: D3M75_10375; OXA-491, Accession: ANW35665.1; OXA-464: ANW35663.1; OXA-490: ANW35664.1. *A. butzleri* RM4018: WP_012013127.1; *A. butzleri* S2_012_000_R2_80: PZP12670.1; *A. butzleri* L348: WP_046997374.1; *A. butzleri* L353: WP_050071304.1; *A. butzleri* ED-1: WP_014468976.1; *A. buzleri* L355: WP_046997672.1; *A. butzleri* JV22: EFU68937.1; *A. butzleri* L349: WP_046993700.1; *A. butzleri* L.: WP_014474670.1.

Supplementary material

Fig. S1. COGs functional classification of genes present in *Ab* 34_O and *Ab* 39_O genomes.

Table S1: Strains used in this study, source of isolation and accession numbers of available genomes or reference sequences.

Table S2: ANI values for *A. butzleri* strains and outgroups.

Table S3: AAI values for *A. butzleri* strains and outgroups.

Table S4: DDH values for *A. butzleri* strains and outgroups.

Table S5: Flagellum proteins in *A. butzleri* genomes.

Acknowledgments

The use of the Biolog Microstation (Biolog, Hayward, CA, USA) and the Varioskan Flash (Thermo Fischer Scientific) spectrofluorometer was possible thank to the laboratory network project "Biodiversity for food production and safety enhancement of typical Apulian foods - BioNet-PTP" (Cod. 73) - POR Puglia FESR 2000-2006.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Aarestrup, F.M., Hasman, H. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. *Vet. Microbiol.* **2004**, *100*, 83−89. doi: 10.1016/j.vetmic.2004.01.013.

Abdelbaqi, K., Ménard, A., Prouzet-Mauleon, V., Bringaud, F., Lehours, P., and Mégraud F. Nucleotide sequence of the *gyr*A gene of *Arcobacter* species and characterization of human ciprofloxacin-resistant clinical isolates. *FEMS Immunol. Med. Microbiol.* **2007** *49*, 337-45. doi: 10.1111/j.1574-695X.2006.00208.x.

Ahmed, M.O., Baptiste, K.E. Vancomycin-Resistant *Enterococci*: a review of antimicrobial resistance mechanisms and perspectives of human and animal health. *Microbiol. Drug Resist.* **2018** *24*, 590-606. doi: 10.1089/mdr.2017.0147.

Allen, A.G., Isobe, T., Maskell, D.J. Identification and cloning of *waa*F (*rfa*F) from *Bordetella pertussis* and use to generate mutants of *Bordetella* spp. with deep rough lipopolysaccharide. *J. Bacteriol.* **1998**, *180*, 35-40. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC106845

Aski, H. S., Tabatabaei, M., Khoshbakht, R., and Raeisi, M. Occurrence and antimicrobial resistance of emergent *Arcobacter* spp. isolated from cattle and sheep in Iran. *Comp. Immunol. Microbiol. Infect. Dis*. **2016,** *44*, 37-40.

Auch, A.F., von Jan, M., Klenk, H.P., and Göker, M. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand. Genomic Sci.* **2010** *2*, 117-34. doi: 10.4056/sigs.531120.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek, R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., and Zagnitko, O. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* **2008** *8*, 9:75. doi: 10.1186/1471-2164-9-75.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*. **2012**, 19(5):455-77. doi: 10.1089/cmb.2012.0021.

Baker-Austin, C., Wright, M. S., Stepanauskas, R., McArthur, J. V. Co-selection of antibiotic and metal resistance. *Trends Microbiol.* **2006** *14*, 176-182. https://doi.org/10.1016/j.tim.2006.02.006.

Baserisalehi, M., Bahador, N., Kapadnis, B.P. Effect of heavy metals and detergents on thermophilic *Campylobacter* spp. isolated from environmental samples. *Nat. Environ. Pollution Technol.* **2007**, *6*, 327-332. http://dx.doi.org/10.1016/j.cimid.2015.12.002

Bayles, K.W. The bactericidal action of penicillin: new clues to an unsolved mystery. *Trends Microbiol* **2000** *8*, 274-278. https://doi.org/10.1016/S0966-842X(00)01762-5.

Bengtsson-Palme, J., Larsson, D.G.J. Antibiotic resistance genes in the environment prioritizing risks. *Nat Rev Microbiol*. **2015**, *13*, 396. doi: 10.1038/nrmicro3399-c1.

Berg, J., Tom-Petersen, A., Nybroe, O. Copper amendment of agricultural soil selects for bacterial antibiotics resistance in the field. *Lett Appl Microbiol* **2005** *40*, 146–151. https://doi.org/10.1111/j.1472-765X.2004.01650.x

Blanco, P., Hernando-Amado, S., Reales-Calderon, J.A., Corona, F., Lira, F., Alcalde-Rico, M., Bernardini, A., Sanchez, M.B., Martinez, J.L. Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. Microorganisms **2016**, *4*, 14. doi: 10.3390/microorganisms4010014.

Brandt, K.K., Frandsen, R.J.N., Holm, P.E., Nybroe, O. Development of pollution-induced community tolerance is linked to structural and functional resilience of a soil bacterial community following a five-year field exposure to copper. *Soil Biol. Biochem*. **2010**, *42*, 748-757. https://doi.org/10.1016/j.soilbio.2010.01.008

Bruins, M.R., Kapil, S., Oehme, F.W. Microbial resistance to metals in the environment. *Ecotoxicol. Environ. Safety* **2000,** *45*, 198-207. https://doi.org/10.1006/eesa.1999.1860

Cain, B.D., Norton, P.J., Eubanks, W., Nick., H.S., Allen, C.M. Amplification of the *bac*A gene confers bacitracin resistance to *Escherichia coli. J. Bacteriol.* **1993**, *175*, 3784-9. doi: 10.1128/jb.175.12.3784-3789.1993.

Caraux, G., Pinloche, S. Permutmatrix: A graphical environment to arrange gene expression profiles in optimal linear order. *Bioinformatics* **2005**, *21*, 1280-1281. https://doi.org/10.1093/bioinformatics/bti141

Caza, M., Garénaux, A., Lépine, F., Dozois, C.M. Catecholate siderophore esterases Fes, IroD and IroE are required for salmochelins secretion following utilization, but only IroD contributes to virulence of extra-intestinal pathogenic *Escherichia coli*. *Mol Microbiol.* **2015**, *97*, 717-32. doi: 10.1111/mmi.13059. doi: 10.1111/mmi.13059. Epub 2015 Jun 6.

Cepas, V., López, Y., Muñoz, E., Rolo, D., Ardanuy, C., Martí, S., Xercavins, M., Horcajada, J.P., Bosch, J., Soto, S.M. Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. *J. Microbial Drug Res.* **2019**, *25*, 72-79. doi: 10.1089/mdr.2018.0027.

Chaban, B., Coleman, I., and Beeby, M. Evolution of higher torque in *Campylobacter*-type bacterial flagellar motors. *Sci. Rep.* **2018** *8*, 97. doi:10.1038/s41598-017-18115-1.

Chenia, H.Y., Jacobs, A. Antimicrobial resistance, heavy metal resistance and integron content in bacteria isolated from a South African tilapia aquaculture system. *Dis. Aquat. Organ.* **2017**, *126*, 199-209. doi: 10.3354/dao03173. doi: 10.3354/dao03173.

Chollet, R., Chevalier, J., Bryskier, A., Pagès, J.M. The AcrAB-TolC pump is involved in macrolide resistance but not in telithromycin efflux in *Enterobacter aerogenes* and *Escherichia coli*. *Antimicrob Agents Chemother.* **2004**, *48*, 3621-4. doi: 10.1128/AAC.48.9.3621-3624.2004.

Chopra, I., Roberts, M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.* **2001,** *65*, 232-60. doi: 10.1128/MMBR.65.2.232-260.2001.

Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., da Costa, M.S., Rooney, A.P., Yi, H., Xu, X.W., De Meyer, S., Trujillo, M.E. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int. J. Syst. Evol. Microbiol.* **2018,** *68*, 461-6. doi: 10.1099/ijsem.0.002516.

CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. *CLSI document M100-S25*. **2015.** Wayne, PA: Clinical and Laboratory Standards Institute.

Coderch, N., Piqué, N., Lindner, B., Abitiu, N., Merino, S., Izquierdo, L., Jimenez, N., Tomás, J.M., Holst, O., Regué, M. Genetic and structural characterization of the core region of the lipopolysaccharide from *Serratia marcescens* N28b (serovar O4). *J Bacteriol.* **2004**, *186*, 978-988. doi: 10.1128/JB.186.4.978-988.2004.

Collado, L., Jara, R., Vásquez, N., and Telsaint, C. Antimicrobial resistance and virulence genes of *Arcobacter* isolates recovered from edible bivalve molluscs. *Food Control*. **2014**, *46*, 508-512. https://doi.org/10.1016/j.foodcont.2014.06.013.

Coyne, S., Rosenfeld, N., Lambert, T., Courvalin, P., Périchon, B. Overexpression of resistance-nodulation-cell division pump AdeFGH confers multidrug resistance in *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. **2010**, *54*(10), 4389-93. doi: 10.1128/AAC.00155-10.

Daury, L., Orange, F., Taveau, J. C., Verchère, A., Monlezun, L., Gounou, C., Lambert, O. Tripartite assembly of RND multidrug efflux pumps. *Nature comm.* **2016**, *7*, 10731. doi: 10.1038/ncomms10731.

de Kievit, T.R., Lam, J.S. Isolation and characterization of two genes, *waa*C (*rfa*C) and *waa*F (*rfa*F), involved in *Pseudomonas aeruginosa* serotype O5 inner-core biosynthesis. *J Bacteriol*. **1997**, *179*, 3451-57. doi: 10.1128/jb.179.11.3451-3457.1997.

Díaz-Guerrero, M.Á., Gaytán, M.O., González-Pedrajo, B. Structure: function of transmembrane appendages in Gram-Negative Bacteria. In: Geiger O. (eds) Biogenesis of Fatty Acids, Lipids and Membranes. Handbook of Hydrocarbon and Lipid Microbiology. **2018**, Springer, Cham

Du, D., Wang, Z., James, N.R., Voss, J.E., Klimont, E., Ohene-Agyei, T., Venter, H., Chiu, W., Luisi. B.F. Structure of the AcrAB-TolC multidrug efflux pump. *Nature* **2014**, 509, 512-5. doi: 10.1038/nature13205.

Ehrlich, H.L., Newman, D.K. Geomicrobiology. **2008.** CRC Press, Boca Raton.

Elmali, M., Can, H.Y. Occurrence and antimicrobial resistance of *Arcobacter* species in food and slaughterhouse samples. *Food Sci. Technol*. **2017**, *37*, 280-5. Doi: http://dx.doi.org/10.1590/1678-457X.19516.

Ercolini, D., Fusco, V., Blaiotta, G., Sarghini, F., and Coppola, S. Response of *Escherichia coli* O157:H7, *Salmonella Thyphimurium*, *Listeria monocytogenes* and *Staphylococcus aureus* to the

stresses occurring in model manufactures of Grana Padano cheese. *J. Dairy Sci.* **2005** *88*, 3818-3825. doi: 10.3168/jds.S0022-0302(05)73067-8.

Fanelli, F., Di Pinto, A., Mottola, A., Mule' G., Chieffi D., Baruzzi, F., Tantillo G., Fusco, V. Genomic characterization of *Arcobacter butzleri* isolated from shellfish: novel insight into antibiotic resistance and virulence determinants. *Front. Microbiol.* **2019**, *10*:670. https://doi.org/10.3389/fmicb.2019.00670

Fard, R.M.N., Heuzenroeder, M.W., Barton, M.D. Antimicrobial and heavy metal resistance in commensal enterococci isolated from pigs. *Vet. Microbiol.* **2011**, *148*, 276-82. doi: 10.1016/j.vetmic.2010.09.002.

Ferreira, S., Fraqueza, M. J., Queiroz, J. A., Domingues, F.C., Oleastro, M. Genetic diversity, antibiotic resistance and biofilm-forming ability of *Arcobacter butzleri* isolated from poultry and environment from a Portuguese slaughterhouse. *Int. J. Food Microbiol.* **2013**, *162*, 82-88. doi: 10.1016/j.ijfoodmicro.2013.01.003.

Ferreira, S., Oleastro, M., Domingues, F.C. Current insights on *Arcobacter butzleri* in food chain. *Curr. Opinion Food Sci.* **2019**, *26*, 9-17. https://doi.org/10.1016/j.cofs.2019.02.013

Ferreira, S., Oleastro, M., Domingues, F.C. Occurrence, genetic diversity and antibiotic resistance of *Arcobacter* sp. in a dairy plant. *J. Appl. Microbiol*. **2017**, *123*, 1019-26. doi: 10.1111/jam.13538.

Ferreira, S., Queiroz, J.A., Oleastro, M., Domingues, F.C. Insights in the pathogenesis and resistance of *Arcobacter*: a review. *Crit. Rev. Microbiol.* **2016**, *42*, 364-83. doi: 10.3109/1040841X.2014.954523.

Fischbach, M.A., Lin, H., Liu, D.R., Walsh, C.T. How pathogenic bacteria evade mammalian sabotage in the battle for iron. *Nat. Chem. Biol.* **2006**, *2*, 132-8. doi:10.1038/nchembio771.

Flynn, K., Villarreal, B.P., Barranco, A., Belc, N., Bjornsdottir, B., Fusco, V., Rainieri, S., Smaradottir, S.E., Smeu, I., Teixeira, P., Jörundsdóttir, H.Ó. An introduction to current food safety needs. *Trends Food Sci. Technol.* **2018**, *84*, 1-3. doi: https://doi.org/10.1016/j.tifs.2018.09.012.

Franz, C.M.A.P., den Besten, H.M.W., Böhnlein, C., Gareis, M., Zwietering, M.H., Fusco, V. Microbial food safety in the 21st century: emerging challenges and foodborne pathogenic bacteria. *Trends Food Sci. Technol.* **2018**, *81*, 155-8. Doi: 10.1016/j.tifs.2018.09.019.

Fujisaki, S., Ohnuma, S., Horiuchi, T., Takahashi, I., Tsukui, S., Nishimura, Y., Nishino, T., Kitabatake, M., Inokuchi, H. Cloning of a gene from *Escherichia coli* that confers resistance to fosmidomycin as a consequence of amplification. *Gene* **1996**, *175*, 83-7. Doi: 10.1016/0378-1119(96)00128-x

Fusco V., Quero G.M., Morea M., Blaiotta G., Visconti A. Rapid and reliable identification of *Staphylococcus aureus* harbouring the *enterotoxin gene cluster* (*egc*) and quantitative detection in raw milk by real time PCR. *Int. J. Food Microbiol.* **2011** *144*, 528-537. doi: 10.1016/j.ijfoodmicro.2010.11.016.

Fusco, V., Abriouel, H., Benomar, N., Kabisch, J., Chieffi, D., Cho, G.-S., Franz, C.M.A.P., Opportunistic foodborne pathogens. **2018.** Chapter 10. In: Food safety and preservation: modern biological approaches to improving consumer health. 1st Edition. Editors: Alexandru Grumezescu Alina Maria Holban. Academic Press. ISBN: 9780128149560. pp 269-306. https://www.elsevier.com/books/food-safety-and-preservation/grumezescu/978-0-12-814956-0

Georgopapadakou, N.H. Penicillin-binding proteins and bacterial resistance to beta-lactams. *Antimicrob. Agents Chemother.* **1993** *37*, 2045-53. PMID: 8257121.

Gibreel, A., Wetsch, N.M., Taylor, D.E. Contribution of the CmeABC Efflux pump to macrolide and tetracycline resistance in *Campylobacter jejuni*. *Antimicrob Agents Chemother.* **2007**, *51*, 3212-16. doi: 10.1128/AAC.01592-06.

González, A., Ferrús, M.A. Study of *Arcobacter* spp. contamination in fresh lettuces detected by different cultural and molecular methods. *Int J Food Microbiol.* **2011**, *145*, 311-314. doi: 10.1016/j.ijfoodmicro.2010.11.018.

González, A., Morejón, I.F.B., Ferrús, M.A. Isolation, molecular identification and quinolone-susceptibility testing of *Arcobacter* spp. isolated from fresh vegetables in Spain. *Food Microbiol.* **2017**, *65*, 279e283. doi: 10.1016/j.fm.2017.02.011.

Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., and Tiedje, J.M. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int. J. Syst. Evol. Microbiol.* **2007** *57*, 81-91. doi: 10.1099/ijs.0.64483-0.

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G. QUAST: quality assessment tool for genome assemblies, *Bioinformatics*, **2013**, *29*, 1072-5. https://doi.org/10.1093/bioinformatics/btt086.

Hamano, Y., Matsuura, N., Kitamura, M., Takagi, H. A novel enzyme conferring streptothricin resistance alters the toxicity of streptothricin D from broad-spectrum to bacteria-specific. *J Biol Chem.* **2006**, *281*, 16842-8. doi: 10.1074/jbc.M602294200.

Hasman, H., Kempf, I., Chidaine, B., Cariolet, R., Ersbøll, A.K., Houe, H., Bruun, Hansen, H.C., Aarestrup, F.M. Copper resistance in *Enterococcus faecium*, mediated by the tcrB gene, is selected by supplementation of pig feed with copper sulfate. *Appl. Environ. Microbiol.* **2006**, *72*, 5784–9. doi: 10.1128/AEM.02979-05.

Hausdorf, L., Neumann, M., Bergmann, I., Sobiella, K., Mundt, K., Fröhling, A., Schlüter, O., Klocke, M. Occurrence and genetic diversity of *Arcobacter* spp. in a spinach processing plant and evaluation of two *Arcobacter*-specific quantitative PCR assays. Syst. *Appl. Microbiol.* **2013**, *36*, 235-43. doi: 10.1016/j.syapm.2013.02.003.

Heuer H, Smalla K. Plasmids foster diversification and adaptation of bacterial populations in soil. *FEMS Microbiol Rev.* **2012**, *36*, 1083-104 doi: 10.1111/j.1574-6976.2012.00337.x.

Jenkins, D.J., Stekel, D.J. *De novo* evolution of complex, global and hierarchical gene regulatory mechanisms. *J Mol Evol*. **2010**, *71*(2), 128–140. doi: 10.1007/s00239-010-9369-4.

Jia, B., Raphenya, A.R., Alcock, B., Waglechner, N., Guo, P., Tsang, K.K., Lago, B.A., Dave, B.M., Pereira, S., Sharma, A.N., Doshi, S., Courtot, M., Lo, R., Williams, L.E., Frye, J.G., Elsayegh, T., Sardar, D., Westman, E.L., Pawlowski, A.C., Johnson, T.A., Brinkman, F.S., Wright, G.D., McArthur, A.G. CARD 2017: expansion and model-centric curation of the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res.* **2017**, *45*, D566-573. doi: 10.1093/nar/gkw1004.

Jimenez, N., Canals, R., Lacasta, A., Kondakova, A.N., Lindner, B., Knirel, Y.A., Merino, S., Regué, M., Tomás, J.M. Molecular analysis of three *Aeromonas hydrophila* AH-3 (serotype O34) lipopolysaccharide core biosynthesis gene clusters. *J Bacteriol*. **2008**, *190*, 3176-84. doi: 10.1128/JB.01874-07.

Junecko, J.M., Zielinska, A.K., Mrak, L.N., Ryan, D.C., Graham, J.W., Smeltzer, M.S., and Lee, C.Y. Transcribing virulence in *Staphylococcus aureus*. *World J. Clin. Infect. Dis.* **2012** *2*, 63-76. doi: 10.5495/wjcid.v2.i4.63.

Kaakoush, N. O.; Raftery, M.; Mendz, G. L. Molecular responses of *Campylobacter jejuni* to cadmium stress. *FEBS J*. **2008**, *275*, 5021-33. doi: 10.1111/j.1742-4658.2008.06636.x.

Kabeya, H., Maruyama, S., Morita, Y., Ohsuga, T., Ozawa, S., Kobayashi, Y., et al. Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. *Int. J. Food Microbiol*. **2004**, *90*, 303-8. https://doi.org/10.1016/S0168-1605(03)00322-2

Kalenitchenko, D., Dupraz, M., Le Bris, N., Petetin, C., Rose, C., West, N.J., Galand, P.E. Ecological succession leads to chemosynthesis in mats colonizing wood in sea water. *ISME J.* **2016**, *10*, 2246-58. doi: 10.1038/ismej.2016.12.

Kanipes, M.I., Papp-Szabo, E., Guerry, P., Monteiro, M.A. Mutation of *waa*C, encoding heptosyltransferase I in *Campylobacter jejuni* 81-176, affects the structure of both lipooligosaccharide and capsular carbohydrate. *J Bacteriol*. **2006**, *188*, 3273-9. doi: 10.1128/JB.188.9.3273-3279.2006.

Kashyap, D.R., Botero, L.M., Lehr, C., Hassett, D.J., McDermott, T.R. A Na+:H+ antiporter and a molybdate transporter are essential for arsenite oxidation in *Agrobacterium tumefaciens. J Bacteriol.* **2006**, *188,* 1577-84. Doi: 10.1128/JB.188.4.1577-1584.2006.

Kayman, T., Abay, S., Hizlisoy, H., Atabay, H. I., Diker, K. S., Aydin, F. Emerging pathogen *Arcobacter* spp. in acute gastroenteritis: molecular identification, antibiotic susceptibilities and genotyping of the isolated *Arcobacters*. *J. Med. Microbiol*. **2012**, *61*, 1439-44. doi: 10.1099/jmm.0.044594-0.

Kiehlbauch, J.A., Brenner, D.J., Nicholson, M.A., Baker, C.N., Patton, C.M., Steigerwalt, A.G., Wachsmuth, I.K. *Campylobacter butzleri* sp. nov. isolated from humans and animals with diarrheal illness. *J. Clin. Microbiol*. **1991**, *29*, 376-85. PMID: 2007646

Kieboom, J., de Bont, J. Identification and molecular characterization of an efflux system involved in *Pseudomonas putida* S12 multidrug resistance. *Microbiol*. **2001**, *147*(Pt 1), 43-51. doi: 10.1099/00221287-147-1-43.

Kiely, P., Call, D., Yates, M., Regan, J., Logan, B. Anodic biofilms in microbial fuel cells harbor low numbers of higher-power-producing bacteria than abundant genera. *Appl Microbiol Biotech.* **2010**, *88*, 371-80. doi: 10.1007/s00253-010-2757-2.

Kim, N.H., Park, S.M., Kim, H.W., Cho, T.J., Kim, S.H., Choi, C., Rhee, M.S. Prevalence of pathogenic *Arcobacter* species in South Korea: comparison of two protocols for isolating the bacteria from foods and examination of nine putative virulence genes. *Food Microbiol.* **2019**, *78*, 18-24. https://doi.org/10.1016/j.fm.2018.09.008

Klena, J.D., Gray, S.A., Konkel, M.E. Cloning, sequencing, and characterization of the lipopolysaccharide biosynthetic enzyme heptosyltransferase I gene (*waa*C) from *Campylobacter jejuni* and *Campylobacter coli*. *Gene* **1998**, *222*, 177-185. doi: 10.1016/s0378-1119(98)00501-0.

Kumar, S., Stecher, G., Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol*. **2016**, *33*,1870-4. doi: 10.1093/molbev/msw054.

Larsen, N.A., Lin, H., Wei, R., Fischbach, M.A., Walsh, C.T. Structural characterization of enterobactin hydrolase IroE. *Biochemistry* **2006**, *45*, 10184-90. https://doi.org/10.1021/bi060950i

Lee, A., Mao, W., Warren, M.S., Mistry, A., Hoshino, K., Okumura, R., Ishida, H., Lomovskaya, O. Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. *J Bacteriol.* **2000**, *182*, 3142-50. Doi: 10.1128/jb.182.11.3142-3150.2000.

Lee, M., and Sousa, M.C. Structural basis for substrate specificity in ArnB. A key enzyme in the polymyxin resistance pathway of Gram-negative bacteria. *Biochemistry* **2014** *53*, 796-805. doi: 10.1021/bi4015677.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. **2012**, *18*, 268-81. doi: 10.1111/j.1469-0691.2011.03570.x.

Maisonneuve E., Shakespeare L.J., Joergensen M.G., Gerdes K. Bacterial persistence by RNA endonucleases. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 13206-11. doi: 10.1073/pnas.1100186108.

Maldonado, R.F., Sá-Correia, I., Valvano, M.A. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. *FEMS Microbiol Rev.* **2016**, *40*, 480-93. doi: 10.1093/femsre/fuw007.

Marchler-Bauer, A., Bo, Y., Han, L., He, J., Lanczycki, C.J., Lu, S., Chitsaz, F., Derbyshire, M.K., Geer, R.C., Gonzales, N.R., Gwadz, M., Hurwitz, D.I., Lu, F., Marchler, G.H., Song, J.S., Thanki, N., Wang, Z, Yamashita, R.A., Zhang, D., Zheng, C., Geer, L.Y., and Bryant, SH. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Res.* **2017** *45*, 200-3. doi: 10.1093/nar/gkw1129.

Matilla, M.A., Krell, T. The effect of bacterial chemotaxis on host infection and pathogenicity. *FEMS Microbiol. Rev.* **2018** *1*, 42(1). doi: 10.1093/femsre/fux052.

Matsumoto, Y., Xu, Q., Miyazaki, S., Kaito, C., Farr, C.L., Axelrod, H.L., Chiu, H.J., Klock, H.E., Knuth, M.W., Miller, M.D., Elsliger, M.A., Deacon, A.M., Godzik, A., Lesley, S.A., Sekimizu, K., and Wilson, I.A. Structure of a virulence regulatory factor CvfB reveals a novel winged helix RNA binding module. *Structure* **2010** *18*, 537-47. doi: 10.1016/j.str.2010.02.007.

Matyar, F., Kaya, A., Dinçer, S. *Sci Total Environ* **2008**, *407*, 279-85. doi: 10.1016/j.scitotenv.2008.08.014.

Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.-P., and Göker, M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* **2013** *14*, 60. doi: 10.1186/1471-2105-14-60.

Meier-Kolthoff, J.P., Göker, M., and Klenk, H.-P. Taxonomic use of DNA G+C content and DNA-DNA hybridization in the genomic age. *Int. J. Syst. Evol. Microbiol.* **2014** *64*, 352-6. doi: 10.1099/ijs.0.056994-0.

Merga, J.Y., Williams, N.J., Miller, W.G., Leatherbarrow, A.J.H., Bennett, M., Hall, N., Ashelford, K.E., Winstanley, C. Exploring the diversity of *Arcobacter butzleri* from cattle in the UK using MLST and whole genome sequencing. *PLoS ONE* **2013**, *8*:e55240. doi: 10.1371/journal.pone.0055240.

Miller, W.G., Parker, C.T., Rubenfield, M., Mendz, G.L., Wösten, M.M., Ussery, D.W., Stolz, J.F., Binnewies, T.T., Hallin, P.F., Wang, G., Malek, J.A., Rogosin, A., Stanker, L.H., and Mandrell, R.E. The complete genome sequence and analysis of the epsilonproteobacterium *Arcobacter butzleri*. *PloS One* **2007**, *2*, e1358. doi: 10.1371/journal.pone.0001358.

Mottola A. Emerging pathogen *Arcobacter* spp. in food: occurrence and genetic diversity. PhD Thesis. **2017**. University of Bari "Aldo Moro".

Mottola, A., Bonerba, E., Bozzo, G., Marchetti, P., Celano, G.V., Colao, V., Terio, V., Tantillo, G., Figueras, M.J., Di Pinto, A. Occurrence of emerging food-borne pathogenic *Arcobacter* spp.

isolated from pre-cut (ready-to-eat) vegetables. *Int J Food Microbiol*. **2016a**, *236*, 33-7. doi: 10.1016/j.ijfoodmicro.2016.07.012.

Mottola, A., Bonerba, E., Figueras, M.J., Pérez-Cataluña, A., Marchetti, P., Serraino, A., Bozzo, G., Terio, V., Tantillo, G., Di Pinto, A. Occurrence of potentially pathogenic arcobacters in shellfish. *Food Microbiol*. **2016b** *57*, 23–27.

Naas, T., Oueslati, S., Bonnin, R.A., Dabos, M.L. Zavala, A., Dortet, L., Retailleau, P., and Iorga, B.I. Beta-Lactamase DataBase (BLDB) – Structure and Function. *J. Enzyme Inhib. Med. Chem.* **2017** *32*, 917-919. doi: 10.1080/14756366.2017.1344235.

Nichols, B.P., Guay, G.G. Gene amplification contributes to sulfonamide resistance in *Escherichia coli*. *Antimicrob Agents Chemother*. **1989**, *33*, 2042-8. PMID: 2694948

Noah, C., Brabetz, W., Gronow, S., Brade, H. Cloning, sequencing, and functional analysis of three glycosyltransferases involved in the biosynthesis of the inner core region of *Klebsiella pneumoniae* lipopolysaccharide. *J Endotoxin Res.* **2001**, *7*, 25-33. https://doi.org/10.1177/09680519010070010401

On, S.L.W., Miller, W.G., Houf, K., Fox, J.G., and Vandamme, P. Minimal standards for describing new species belonging to the families *Campylobacteraceae* and *Helicobacteraceae*: *Campylobacter*, *Arcobacter*, *Helicobacter* and *Wolinella* spp. *Int. J. Syst. Evol. Microbiol.* **2017** *67*, 5296-5311. doi: 10.1099/ijsem.0.002255.

Otth, L., Solís, G., Wilson, M., Fernández, H. Susceptibility of *Arcobacter butzleri* to heavy metals. *Braz. J. Microbiol.* **2005**, *36*, 286-8. http://dx.doi.org/10.1590/S1517-83822005000300015

Perez-Cataluña, A., Salas-Masso, N., Dieguez, A. L., Balboa, S., Lema, A., Romalde, J. L., Figueras, M. J. Revisiting the taxonomy of the genus *Arcobacter*: getting order from the chaos. *Front. Microbiol*. **2018**, *9*, 2077. doi: 10.3389/fmicb.2018.02077.

Pérez-Cataluña, A., Salas-Massó, N., Diéguez, A.L., Balboa, S., Lema, A., Romalde, J.L., Figueras, M.J. Erratum: Corrigendum: Revisiting the taxonomy of the genus *Arcobacter*: getting order from the caos. Front Microbiol. **2019a**, *10*, 121. doi: 10.3389/fmicb.2019.00121.

Pérez-Cataluña, A., Salas-Massó, N., Diéguez, A.L., Balboa, S., Lema, A., Romalde, J.L., Figueras, M.J. Corrigendum (2): Revisiting the taxonomy of the genus *Arcobacter*: getting order from the chaos. Front Microbiol. **2019b**, *10*, 2253.

Petricoin, E.F. 3rd, Danaher, R.J., Stein, D.C. Analysis of the *lsi* region involved in lipooligosaccharide biosynthesis in *Neisseria gonorrhoeae*. *J Bacteriol*. **1991**, *173*, 7896-902. PMID: 1744044

Quintiliani, Jr.R., Courvalin, P. Mechanisms of resistance to antimicrobial agents. P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Yolken (Eds.), Manual of clinical microbiology (6th ed.), ASM Press, Washington, DC **1995**, pp. 1319.

Rahimi, E. Prevalence and antimicrobial resistance of *Arcobacter* species isolated from poultry meat in Iran. *Br. Poult. Sci*. **2014**, *55*, 174-80. doi: 10.1080/00071668.2013.878783.

Rathlavath, S., Kohli, V., Singh, A. S., Lekshmi, M., Tripathi, G., Kumar, S., et al. Virulence genotypes and antimicrobial susceptibility patterns of *Arcobacter butzleri* isolated from seafood and its environment. *Int. J. Food Microbiol*. **2017**, *263*, 32–37. doi: 10.1016/j.ijfoodmicro.2017.10.005.

Richards, V.P., Lefébure, T., Pavinski Bitar, P.D., and Stanhope, M.J. Comparative characterization of the virulence gene clusters (lipooligosaccharide [LOS] and capsular polysaccharide [CPS]) for *Campylobacter coli*, *Campylobacter jejuni* subsp. *jejuni* and related *Campylobacter* species. *Infect. Genet. Evol.* **2013** *14*, 200-213. doi: 10.1016/j.meegid.2012.12.010.

Rodriguez-R, L.M., and Konstantinidis, K.T. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ. Preprints* **2016** *4*:e1900v1. https://doi.org/10.7287/peerj.preprints.1900v1.

Rossmann, F.M., and Beeby, M. Insights into the evolution of bacterial flagellar motors from high-throughput in situ electron cryotomography and subtomogram averaging. *Acta Crystallogr. D. Struct. Biol*. **2018** *74*, 585-594. doi: 10.1107/S2059798318007945.

Rovetto, F., Carlier, A., Van den Abeele, A.M., Illeghems, K., Van Nieuwerburgh, F., Cocolin, L., and Houf, K. Characterization of the emerging zoonotic pathogen *Arcobacter thereius* by whole genome sequencing and comparative genomics. *PLoS One* **2017** *12*(7), e0180493. doi: 10.1371/journal.pone.0180493.

Scanlon, K. A., Cagney, C., Walsh, D., McNulty, D., Carroll, A., McNamara, E. B., et al. Occurrence and characteristics of fastidious *Campylobacteraceae* species in porcine samples. *Int. J. Food Microbiol*. **2013**, *163*, 6-13. doi: 10.1016/j.ijfoodmicro.2013.02.004.

Schroeder-Tucker, L., Wesley, I.V., Kiehlbauch, J.A., Larson, D.J.,Thomas, L.A., Erickson, G.A., Phenotypic and ribosomal RNA characterization of *Arcobacter* species isolated from porcine aborted fetuses. *J. Vet. Diagn. Investigation* **1996**, *8*, 186-95. doi: 10.1177/104063879600800208

Schumacher M.A., Piro K.M., Xu W., Hansen S., Lewis K., Brennan R.G. Molecular mechanisms of HipA-mediated multidrug tolerance and its neutralization by HipB. *Sci.* **2009**, *323*, 396-401. doi: 10.1126/science.1163806.

Schwarz S., Kehrenberg C., Doublet B., Cloeckaert A. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol. Rev.* **2004**, *28*, 519-42. Doi: 10.1016/j.femsre.2004.04.001.

Šilha, D., Pejchalová, M., and Šilhová, L. Susceptibility to 18 drugs and multidrug resistance of *Arcobacter* isolates from different sources within the Czech Republic. *J. Glob. Antimicrob. Resist*. **2017**, *9*, 74-77. doi: 10.1016/j.jgar.2017.01.006.

Siragusa, S.; De Angelis, M.; Calasso, M.; Campanella, D.; Minervini, F.; Di Cagno, R.; Gobbetti, M. Fermentation and proteome profiles of *Lactobacillus plantarum* strains during growth under food-like conditions. *J. Proteomics*. **2013**, *96*, 366-80. doi: 10.1016/j.jprot.2013.11.003.

Sirisena, D.M., Brozek, K.A., MacLachlan, P.R., Sanderson, K.E., Raetz, CR. The *rfa*C gene of *Salmonella typhimurium*. Cloning, sequencing, and enzymatic function in heptose transfer to lipopolysaccharide. *J Biol Chem*. **1992**, *267*, 18874-84. http://www.jbc.org/content/267/26/18874.long

Soma, S. M., Srinivasa, R. T., Bindu, K. C. H., Subramanyam, K. V., and Mohammad, S. N. Antibiogram of *Arcobacter* species isolated from animals, foods of animal origin and humans in Andhra Pradesh, India. *Int. J. Sci. Environ. Tech*. **2017**, *6*, 1260-69. doi: 10.14202/vetworld.2017.342-347.

Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014** *30*, 1312-13. doi: 10.1093/bioinformatics/btu033.

Stojiljkovic, I., Hwa, V., Larson, J., Lin, L., So, M., Nassif, X. Cloning and characterization of the Neisseria meningitidis *rfa*C gene encoding alpha-1,5 heptosyltransferase I. *FEMS Microbiol Lett.* **1997**, *151*, 41-9. Doi: 10.1111/j.1574-6968.1997.tb10392.x.

Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E.P., Zaslavsky, L., Lomsadze, A., Pruitt, K.D., Borodovsky, M., and Ostell, J. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* **2016** *44*, 6614-24. doi: 10.1093/nar/gkw569.

Tiwari, S., Jamal, S.B., Hassan, S.S., Carvalho, P.V.S.D., Almeida, S., Barh, D., Ghosh, P., Silva, A., Castro, T.L.P., and Azevedo, V. Two-component signal transduction systems of pathogenic bacteria as targets for antimicrobial therapy: an overview. *Front. Microbiol.* **2017** *8*, 1878. doi: 10.3389/fmicb.2017.01878.

Toh, S.M., Xiong, L., Bae, T., Mankin, A.S. The methyltransferase YfgB/RlmN is responsible for modification of adenosine 2503 in 23S rRNA. *RNA*. **2008**, *14*, 98-106. doi: 10.1261/rna.814408.

Ug, A., Ceylan, O. Occurrence of resistance to antibiotics, metals, and plasmids in clinical strains of *Staphylococcus* spp. *Arch Med Res*. **2003**, *34*, 130-6. doi: 10.1016/S0188-4409(03)00006-7.

Van den Abeele, A.M., Vogelaers, D., Vanleare, E., Houf, K. Antimicrobial susceptibility testing of *Arcobacter butzleri* and *Arcobacter cryaerophilus* strains isolated from Belgian patients. *J. Antimicrob. Chemother*. **2016**, *71*, 1241-44 doi: 10.1093/jac/dkv483.

Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., Dewettinck, D., Vlaes, L., Van Den Borre, C., Higgins, R., Hommez, J., Kersters, K., Butzler, J. P., Goossens, H. Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int. J. Syst. Bacteriol*. **1992**, *42*, 344-5. doi: 10.1099/00207713-42-3-344.

Vicente-Martins, S., Oleastro, M., Domingues, F. C., Ferreira, S. *Arcobacter* spp. at retail food from Portugal: prevalence, genotyping and antibiotics resistance. *Food Control*. **2018**, *85*, 107-12. https://doi.org/10.1016/j.foodcont.2017.09.024

Wang, Y., Deng, H., Li, Z., Tan, Y., Han, Y., Wang, X., Du, Z., Liu, Y., Yang, R., Bai, Y., Bi, Y., Zhi, F. Safety evaluation of a novel strain of *Bacteroides fragilis*. *Front. Microbiol*. **2017**, *8*,435. doi: 10.3389/fmicb.2017.00435.

Wang, Z., Wang, J., Ren, G., Li, Y., Wang, X. Deletion of the genes *waa*C, *waa*F, or *waa*G in *Escherichia coli* W3110 disables the flagella biosynthesis. *J Basic Microbiol.* **2016**, *56*, 1021-35. doi: 10.1002/jobm.201600065.

Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, et al. Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. *Nucleic Acids Res.* **2017** *45*, D535-D42. doi: 10.1093/nar/gkw1017.

Winters, D.K., Slavik, M.F. Multiplex PCR detection of *Campylobacter jejuni* and *Arcobacter butzleri* in food products. *Mol Cell Probes.* **2000**, *14*, 95-99. doi: 10.1006/mcpr.2000.0290.

Xavier J. C., Costa P. E.S., Coutinho H. D.M., Verde L. C.L., Hissa D. C., Melo V. M.M., Falcão R. M., Balbino V. Q., Mendonça L. A.R., Lima M. G.S. Evaluation of the microbial diversity and heavy metal resistance genes of a microbial community on contaminated environment. *Appl. Geochem.* **2019**, *105*, 1-6. https://doi.org/10.1016/j.apgeochem.2019.04.012

Xia Z, Lei L, Zhang HY, Wei HL. Characterization of the ModABC molybdate transport system of *Pseudomonas putida* in nicotine degradation. *Front Microbiol.* **2018**, *9*:3030. doi: 10.3389/fmicb.2018.03030. (published correction appears in Front Microbiol. 2018 9:3213).

Xu, Y., Xu, J., Mao, D.Q., Luo, Y. Effect of the selective pressure of sub-lethal level of heavy metals on the fate and distribution of ARGs in the catchment scale. *Environ Pollut* **2017** *220*, 900-8. https://doi.org/10.1016/j.envpol.2016.10.074.

Yesilmen, S., Vural, A., Erkan, M. E., and Yildirim, I. H. Prevalence and antimicrobial susceptibility of *Arcobacter* species in cow milk, water buffalo milk and fresh village cheese. *Int. J. Food Microbiol*. **2014**, *188*, 11-4. doi: 10.1016/j.ijfoodmicro.2014.07.006.

Zacharow, I., Bystroń, J., Wałecka-Zacharska, E., Podkowik, M., and Bania, J. Prevalence and antimicrobial resistance of *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolates from retail meat in lower Silesia region, Poland. *Pol. J. Vet. Sci*. **2015**, *18*, 63-9. doi: https://doi.org/10.1515/pjvs-2015-0008.

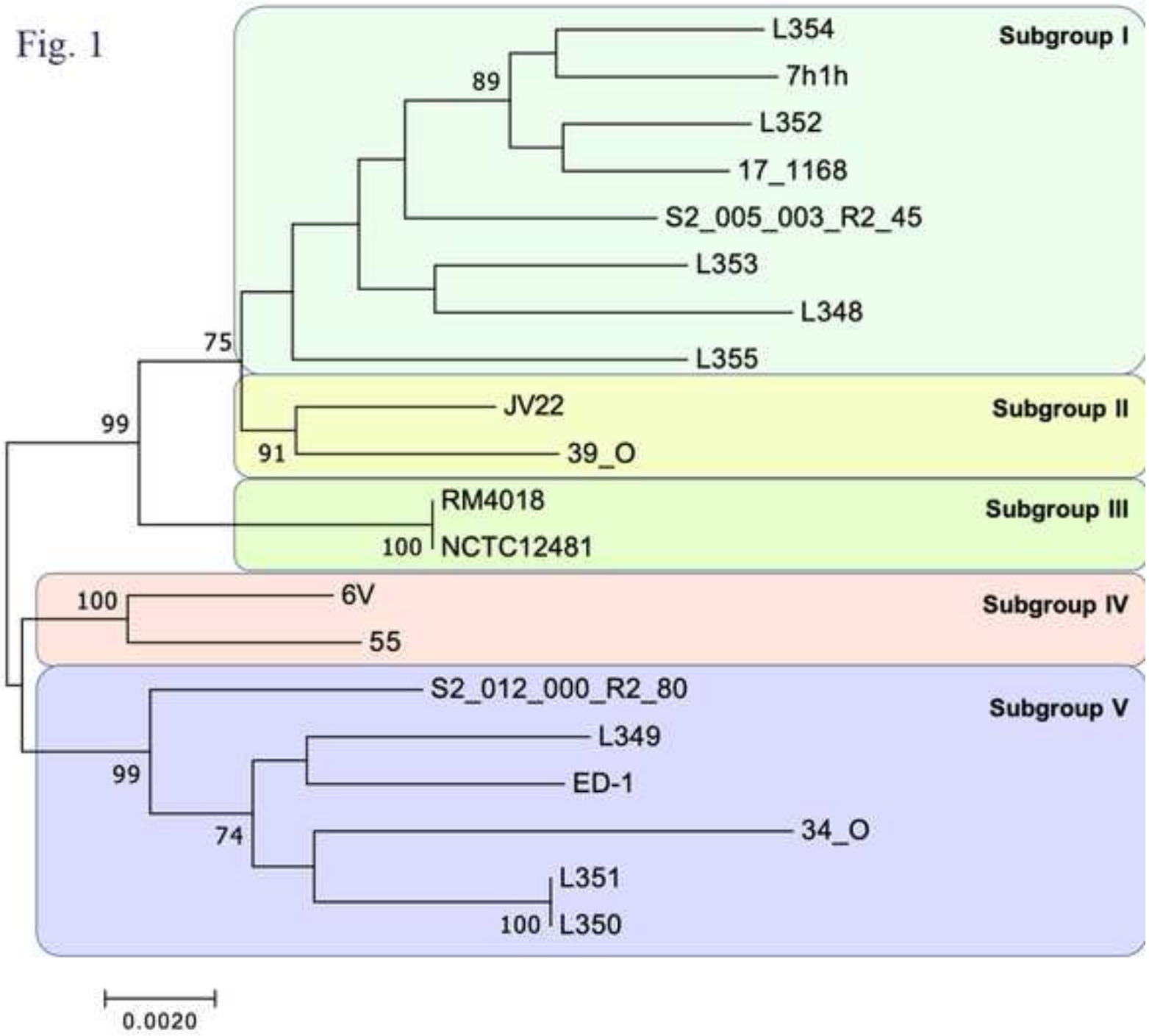
Zhang, Y., Gu, A.Z., Cen, T., Li, X., He, M., Li, D., Chen, J. Sub-inhibitory concentrations of heavy metals facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes in water environment. *Environ Pollut.* **2018**, *237*, doi: 10.1016/j.envpol.2018.01.032.

Zhao, Yi, Cocerva, T., Cox, S., Tardif, S., Su, J.-Q., Zhu, Y.-G., Brandt, K.K. Evidence for co-selection of antibiotic resistance genes and mobile genetic elements in metal polluted urban soils. *Sci. Total Environ.* **2019**, *656*, 512-520. https://doi.org/10.1016/j.scitotenv.2018.11.372

Zhu, S., Nishikino, T., Hu, B., Kojima, S., Homma, M., and Liu, J. Molecular architecture of the sheathed polar flagellum in *Vibrio alginolyticus*. *Proc. Natl. Acad. Sci. U S A.* **2017** *114*, 10966-71. doi: 10.1073/pnas.1712489114.

Figure

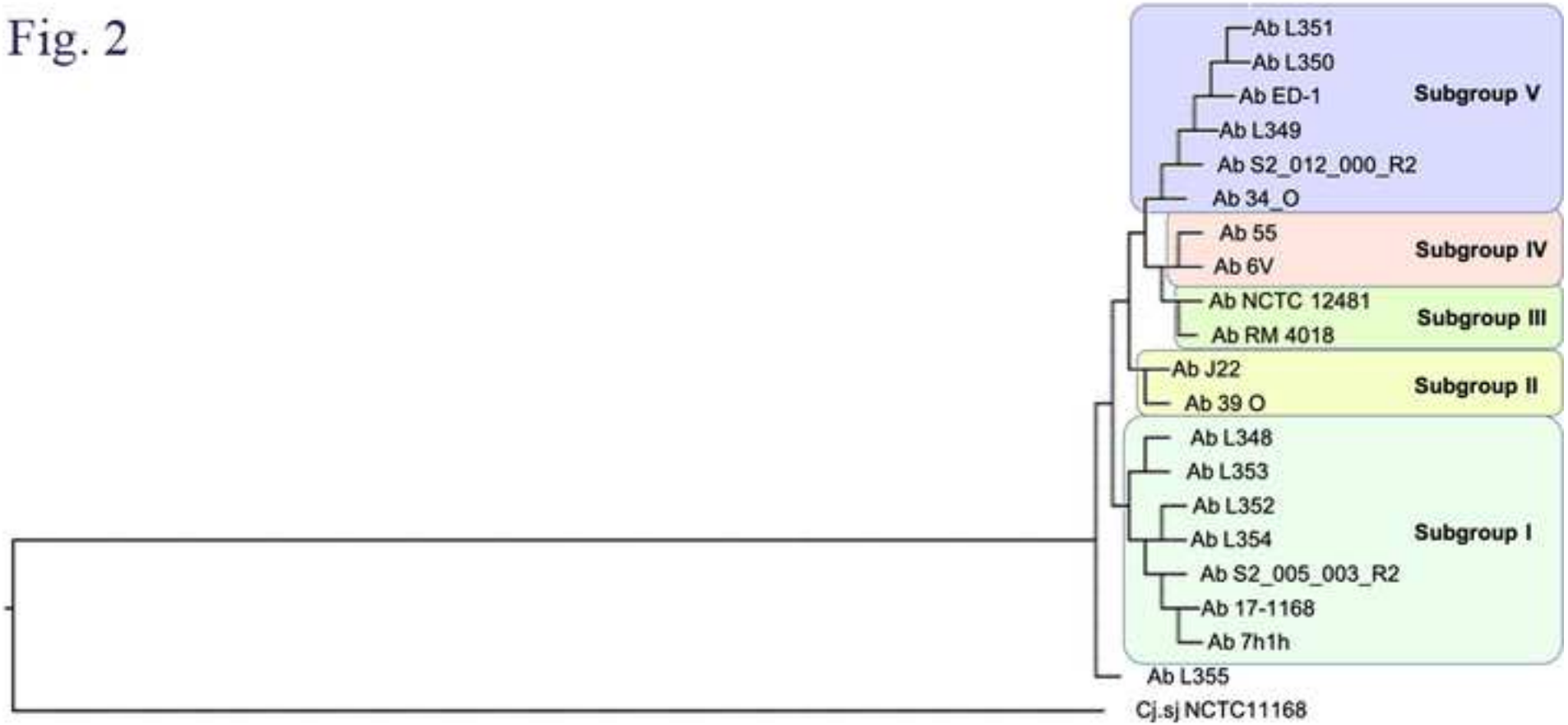
Click here to download Figure Fig 1.tif



Figure

Click here to download Figure Fig 2.tif

Fig. 2



Figure

Click here to download Figure Fig 3.tif

Rows : - Objective function : R=0.600
- Sum of all pairwise distances of neighboring rows (path length): S=23.355
- Linkage rule: McQuitty's criteria
- Tree Seriation rule: Multiple-fragment heuristic (MF)

Columns : - Objective function : R=0.901
- Sum of all pairwise distances of neighboring columns (path length): S=13.481
- Linkage rule: McQuitty's criteria
- Tree Seriation rule: Multiple-fragment heuristic (MF)

Dissimilarity : - Euclidean distance

The colors scale:

Min = -2.63 0.00 Max = 2.63

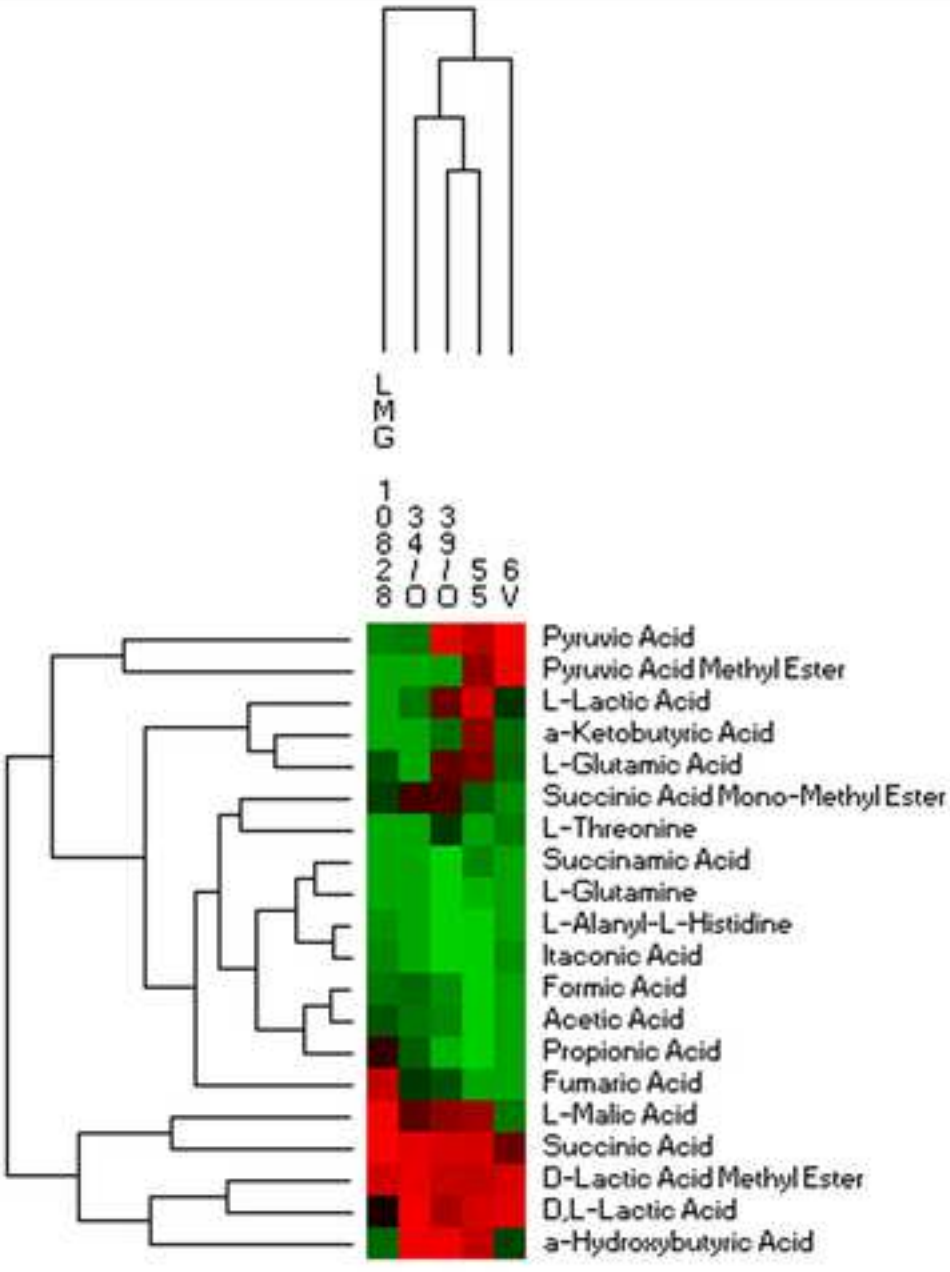


Fig. 3

Figure

Click here to download Figure Fig 4.tif

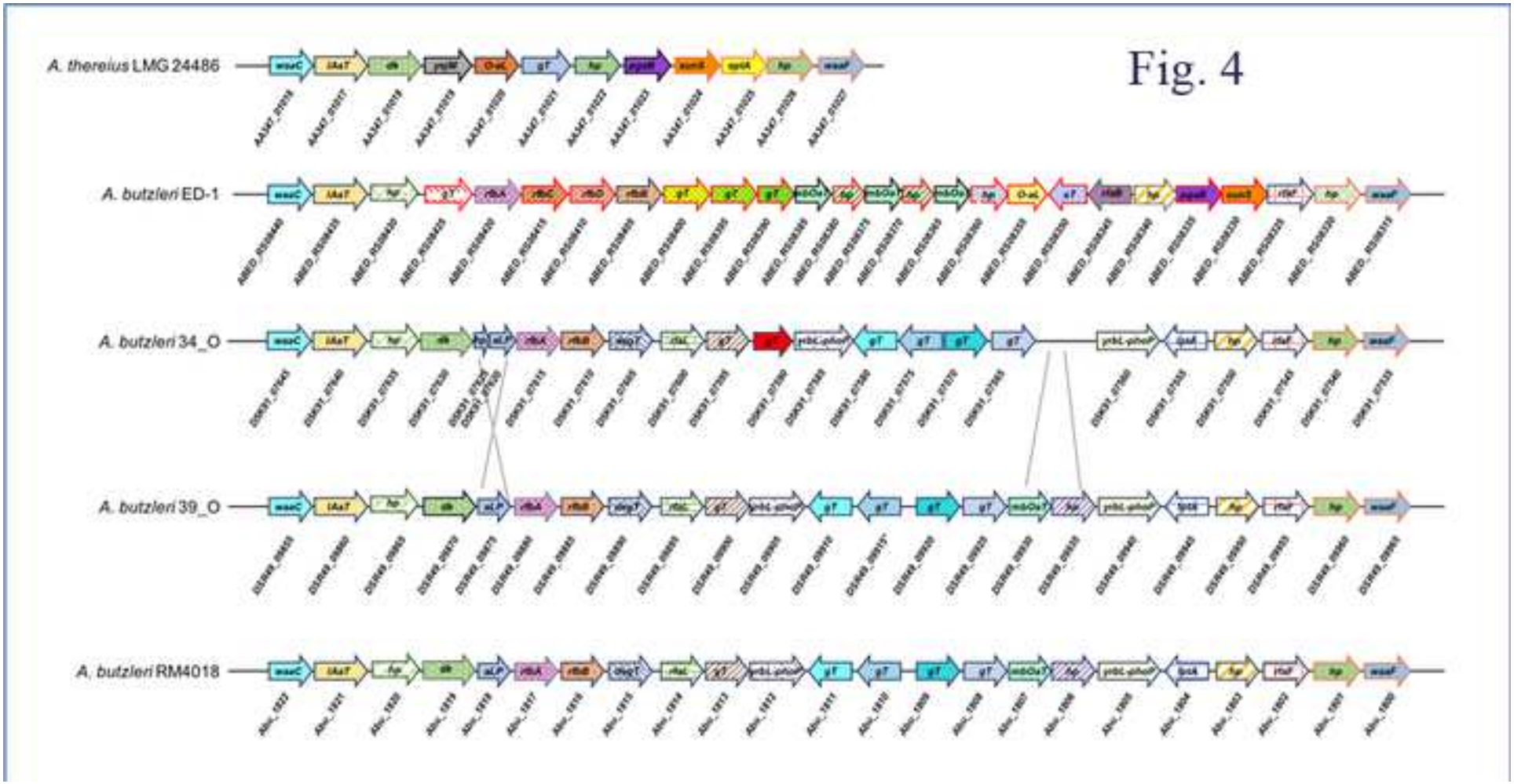
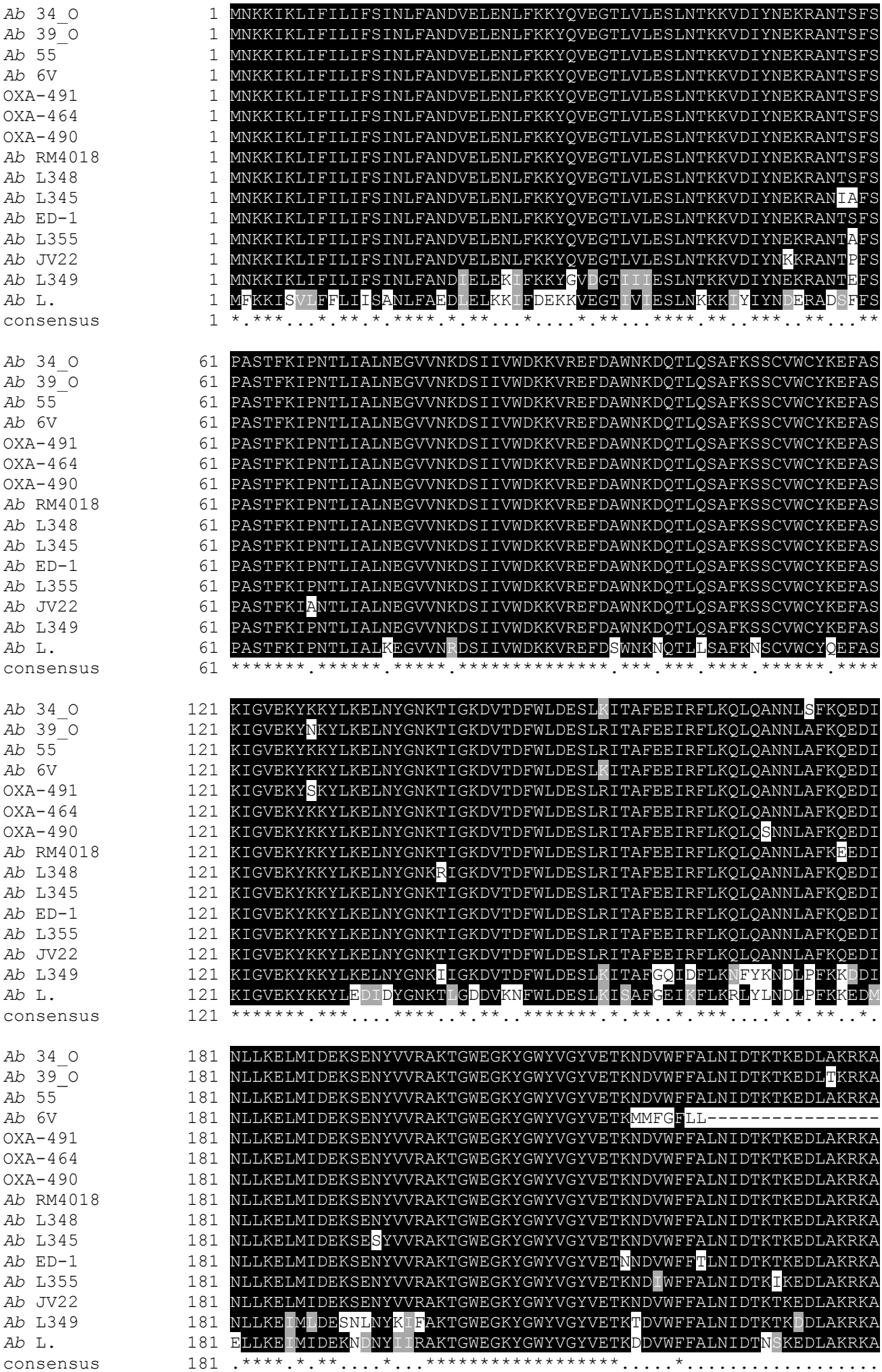


Fig. 4

Figure


Ab 34_O 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
Ab 39_O 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
Ab 55 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
Ab 6V 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
OXA-491 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
OXA-464 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
OXA-490 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
Ab RM4018 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
Ab L348 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
Ab L345 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANIAFS
Ab ED-1 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTSFS
Ab L355 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNEKRANTAFS
Ab JV22 1 MNKKIKLIFILIFSINLFANDVELENLFKKYQVEGTLVLESLNTKKVDIYNKKRANTPFS
Ab L349 1 MNKKIKLIFILIFSINLFANDIELEKIFKKYGVDGTIIIESLNTKKVDIYNEKRANTEFS
Ab L. 1 MFKKISVLFFLIISANLFAEDLELKKIFDEKKVEGTIVIESLNKKKIYIYNDERADSFFS
consensus 1 *.***...*.**.*.****.*.**...*....*.**...****.**..***..**...**
Ab 34_O 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab 39_O 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab 55 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab 6V 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
OXA-491 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
OXA-464 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
OXA-490 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab RM4018 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab L348 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab L345 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab ED-1 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab L355 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab JV22 61 PASTFKIANTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab L349 61 PASTFKIPNTLIALNEGVVNKDSIIVWDKKVREFDAWNKDQTLQSAFKSSCVWCYKEFAS
Ab L. 61 PASTFKIPNTLIALKEGVVNRDSIIVWDKKVREFDSWNKNQTLLSAFKNSCVWCYQEFAS
consensus 61 *******.******.*****.**************.***.***.****.******.****
Ab 34_O 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLKITAFEEIRFLKQLQANNLSFKQEDI
Ab 39_O 121 KIGVEKYNKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKQEDI
Ab 55 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKQEDI
Ab 6V 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLKITAFEEIRFLKQLQANNLAFKQEDI
OXA-491 121 KIGVEKYSKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKQEDI
OXA-464 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKQEDI
OXA-490 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQSNNLAFKQEDI
Ab RM4018 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKEEDI
Ab L348 121 KIGVEKYKKYLKELNYGNKRIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKQEDI
Ab L345 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKQEDI
Ab ED-1 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKQEDI
Ab L355 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKQEDI
Ab JV22 121 KIGVEKYKKYLKELNYGNKTIGKDVTDFWLDESLRITAFEEIRFLKQLQANNLAFKQEDI
Ab L349 121 KIGVEKYKKYLKELNYGNKIIGKDVTDFWLDESLKITAFGQIDFLKNFYKNDLPFKKDDI
Ab L. 121 KIGVEKYKKYLEDIDYGNKTLGDDVKNFWLDESLKISAFGEIKFLKRLYLNDLPFKKEDM
consensus 121 *******.***....****..*.**..*******.*.**..*.***....*.*.**..*.
Ab 34_O 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLAKRKA
Ab 39_O 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLTKRKA
Ab 55 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLAKRKA
Ab 6V 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKMMFGFLL----------------
OXA-491 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLAKRKA
OXA-464 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLAKRKA
OXA-490 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLAKRKA
Ab RM4018 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLAKRKA
Ab L348 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLAKRKA
Ab L345 181 NLLKELMIDEKSESYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLAKRKA
Ab ED-1 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETNNDVWFFTLNIDTKTKEDLAKRKA
Ab L355 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDIWFFALNIDTKIKEDLAKRKA
Ab JV22 181 NLLKELMIDEKSENYVVRAKTGWEGKYGWYVGYVETKNDVWFFALNIDTKTKEDLAKRKA
Ab L349 181 NLLKEIMLDESNLNYKIFAKTGWEGKYGWYVGYVETKTDVWFFALNIDTKTKDDLAKRKA
Ab L. 181 ELLKEIMIDEKNDNYIIRAKTGWEGKYGWYVGYVETKDDVWFFALNIDTNSKEDLAKRKA
consensus 181 .****.*.**....*...******************.....*.................

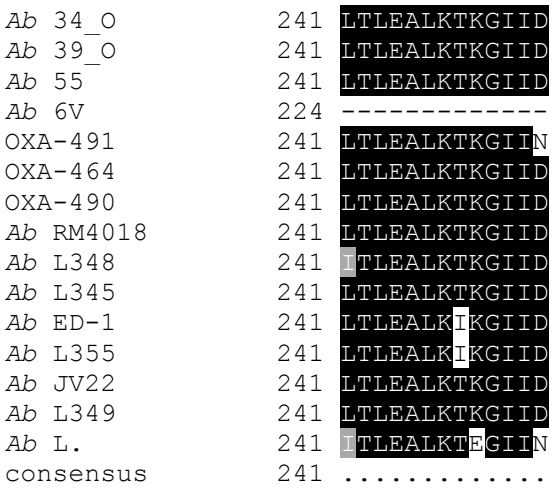
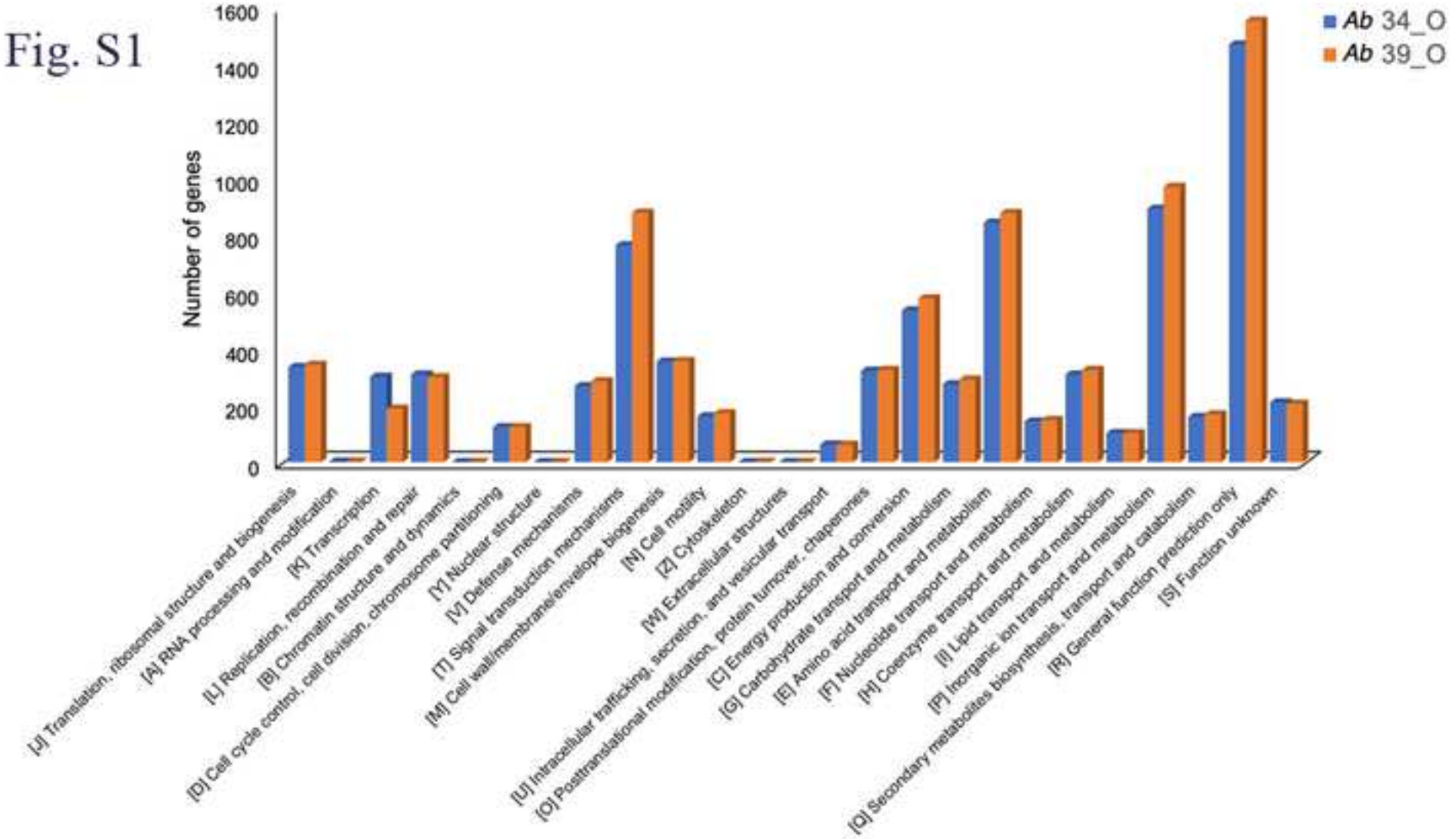


Fig. 4. Multialignment of OXA beta-lactamase from *Arcobacter butzleri* obtained by using T-Coffee web server (Di Tommaso et al., 2011). *Ab* 34_O protein ID: D5K91_097; *Ab* 39_O protein ID: D5R49_106; *Ab* 55 protein ID: D3M61_10735; *Ab* 6V: D3M75_10375; OXA-491, Accession: ANW35665.1; OXA-464: ANW35663.1; OXA-490: ANW35664.1. *A. butzleri* RM4018: WP_012013127.1; *A. butzleri* S2_012_000_R2_80: PZP12670.1; *A. butzleri* L348: WP_046997374.1; *A. butzleri* L353: WP_050071304.1; *A. butzleri* ED-1: WP_014468976.1; *A. buzleri* L355: WP_046997672.1; *A. butzleri* JV22: EFU68937.1; *A. butzleri* L349: WP_046993700.1; *A. butzleri* L.: WP_014474670.1.

Figure

Click here to download Figure Fig S1.tif



Table

Table

Table

Click here to access/download

Table

Table S3 AAI.xlsx

Table

Table

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.