Aus dem Institut für Lebensmittelsicherheit und -hygiene
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Prevalence and cold shock response of *Arcobacter* spp.

Inaugural-Dissertation
zur Erlangung des Grades eines
PhD of Biomedical Science
an der
Freien Universität Berlin

vorgelegt von
Xiaochen Zhang
M.Sc. aus Shanghai, China

Berlin 2021
Journal-Nr.: 4260

Aus dem Institut für Lebensmittelsicherheit und -hygiene

des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

Prevalence and cold shock response of *Arcobacter* spp.

Inaugural-Dissertation

zur Erlangung des Grades eines

PhD of Biomedical Science

an der

Freien Universität Berlin

vorgelegt von

Xiaochen Zhang

M.Sc.

aus Shanghai, China

Berlin 2021

Journal-Nr.: 4260

Gedruckt mit Genehmigung

des Fachbereichs für Veterinärmedizin

der Freien Universität Berlin

Dekan: Univ.-Prof. Dr. Jürgen Zentek

Erster Gutachter: Univ.-Prof. Dr. Thomas Alter

Zweiter Gutachter: PD Dr. Roland Bücker

Dritter Gutachter: PD Dr. Felix Reich

Deskriptoren (nach CAB-Thesaurus)

arcobacter, *Arcobacter butzleri*, seafood, cold shock, cold tolerance, growth curve, gene expression, polymerase chain reaction, prevalence, epidemiology, germany

Tag der Promotion: 08.02.2021

Table of Contents

List of Tables

List of Figures

List of Abbreviations

A.	*Arcobacter*
°C	degree Celsius
AC	aerotolerant *Campylobacter*
aceE	Pyruvate dehydrogenase E1 component (gene)
AceE	Pyruvate dehydrogenase E1 component
aceF	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (gene)
AceF	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex
AFLP	amplified fragment gel polymorphism
AraC	HTH araC/xylS-type domain-containing protein
aspA	aspartate ammonia-lyase (gene)
ATCC	American Type Culture Collection
atpA	ATP synthase subunit alpha (gene)
BHI	Brain Heart Infusion
bp	base pair
C.	*Campylobacter*
C/RFLP	Clonal-Restriction Fragment Length Polymorphism
Caco-2	Human colorectal adenocarcinoma cell line
cadF	outer membrane fibronectin-binding protein (gene)
CAT	cefoperazone, amphotericin B and teicoplannin
CCUG	Culture Collection University of Gothenburg
CFU	colony forming unit
ciaB	*Campylobacter* invasion antigen B (gene)
cj1349	Putative fibronectin (gene)
CMJ	chicken meat juice
cspA	Cold shock protein CspA (gene)

CspA	Cold shock protein CspA
d	day
deaD	ATP-dependent RNA helicase DeaD (gene)
DeaD	ATP-dependent RNA helicase DeaD
DGGE	Denaturing Gradient Gel Electrophoresis
dnaA	Chromosomal replication initiator protein DnaA (gene)
DnaA	Chromosomal replication initiator protein DnaA
E. coli	*Escherichia coli*
e.g.	exempli gratia
EMJH	Ellinghausen McCullough Johnson Harris
ERIC-PCR	enterobacterial intergenic consensus sequence polymerase chain reaction
et al.	et alii
FISH	fluorescence in situ hybridization
g	gram
glnA	glutamine synthetase (gene)
gltA	citrate synthase (gene)
glyA	serine hydroxymethyltransferase (gene)
groEL	60 kDa chaperonin (gene)
gyrA	DNA gyrase subunit A (gene)
GyrA	DNA gyrase subunit A
h	hour
hecA	filamentous hemagglutinin (gene)
hecB	hemolysin activation protein (gene)
hsp60	Heat shock protein 60 (gene)
HT-29	Human colorectal adenocarcinoma cell line
ICMSF	International Commission on Microbiological Specifications for Foods
IF2	Translation initiation factor IF-2
infB	Translation initiation factor IF-2 (gene)

irgA	Iron-regulated outer membrane virulence protein (gene)
iroE	iron-related hydrolase (gene)
JM	Johnson-Murano
l	liter
LAMP	loop-mediated isothermal amplification
LPSN	list of prokaryotic names with standing in nomenclature
MALDI-TOF	Matrix-associated laser desorption/ionization–time of flight
max.	maximum
mg	milligram
MHB	Mueller-Hinton Blood
min.	minimum
ml	milliliter
MLST	multilocus sequence type
mPCR	multiplex polymerase chain reaction
MS	mass spectrometry
mviN	virulence factor MviN (gene)
NCBI	National Center for Biotechnology Information
NCTC	National Collection of Type Cultures
NGS	Next-Generation Sequencing
nusA	Transcription termination/antitermination protein NusA (gene)
NusA	Transcription termination/antitermination protein NusA
PFGE	pulsed-field gel electrophoresis
pgm	phosphoglycerate mutase (gene)
phoQ	histidine kinase PhoQ (gene)
pldA	outer membrane phospholipase A (gene)
pnp	Polyribonucleotide nucleotidyltransferase (gene)
PNPase	Polyribonucleotide nucleotidyltransferase
PRACS	partial 16S rDNA amplification, cloning followed by Sanger sequencing
qPCR	quantitive polymerase chain reaction

RAPD-PCR	randomly amplified polymorphic DNA-PCR
rbfA	30S ribosome-binding factor (gene)
RbfA	30S ribosome-binding factor
recA	Recombinase RecA (gene)
RecA	Recombinase RecA
RFLP	restriction fragment length polymorphism
RNase R	Ribonuclease R
rnr	Ribonuclease R (gene)
rpoB	DNA-dependent RNA Polymerase Beta Subunit (gene)
rpoC	DNA-dependent RNA Polymerase Beta Subunit (gene)
tig	Trigger factor (gene)
tkt	transketolase (gene)
tlyA	hemolysin (gene)
virF	Virulence regulon transcriptional activator VirF (gene)
VOC	VOC family virulence protein
vol	volume
vs.	versus
WGS	whole genome sequencing
wt	weight
µm	micrometer

Introduction

Arcobacter (*A.*) is a genus of Gram-negative, non-spore forming, curved bacteria belonging to the family *Campylobacteraceae* (Vandamme, Falsen et al. 1991). Up to date, 28 *Arcobacter* spp. were identified from a wide range of habitats and hosts, with half of them recovered from aquatic environment and water-borne animals (Table 1.1). Among all *Arcobacter* spp., *A. butzleri* was the most important and predominant species associated with human gastrointestinal disorders. However, the prevalence of *Arcobacter* in food products such as seafood is still scarce, and little is known about the pathogenic mechanisms of this organism. Since the complete genome sequence of *A. butzleri* RM4018 (a clinical strain) was published, the genetic information revealed the presence of several putative virulence factors presenting homology with *Campylobacter* (*C.*) *jejuni* virulence determinants, which contribute to further understanding of its pathogenicity (Miller, Parker et al. 2007).

The genetic information also revealed a set of genes associated with the growth and survival of the bacteria under diverse environmental conditions. *A. butzleri* was reported to grow as low as 10°C in Ellinghausen McCullough Johnson Harris (EMJH) medium (D'Sa and Harrison 2005), chicken meat juice medium (CMJ) and Brain Heart Infusion (BHI) (Kjeldgaard, Jorgensen et al. 2009). Thus, the presence of cold-related genes in *A. butzleri* was hypothesized. The response of mesophilic bacteria to a sudden temperature downshift was intensively studied in *Escherichia* (*E.*) *coli*. After a cold shock from 37°C to 10°C of *E. coli*, bulk transcription and translation slows or comes to an almost complete stop, while a set of cold-shock proteins is preferentially and transiently expressed (Jones, VanBogelen et al. 1987, Yamanaka 1999, Phadtare 2004, Barria, Malecki et al. 2013). These proteins are involved in fundamental functions such as DNA packaging, transcription, RNA degradation, translation, ribosome assembly, etc. (Gualerzi, Giuliodori et al. 2003).

In this thesis, the prevalence of *Arcobacter* spp. in seafood, and the occurrence of its putative virulence genes were initially investigated (chapter 2). Moreover, the growth capabilities of *A.*

butzleri derived from different origins (human, seafood and chicken) as well as the expression profiles of cold-related genes were investigated (chapter 3).

Chapter 1 Literature overview

1.1 Taxonomy

Arcobacter was first isolated from aborted bovine and porcine fetuses, separated from *C. fetus* by growing at 30°C and the aerotolerance (Ellis, Neill et al. 1977, Neill, Ellis et al. 1978). Later in 1991, Vandamme et al. proposed the genus *Arcobacter* after hybridization and immunotyping analysis among DNAs from more than 70 strains of *Campylobacter* spp. and related taxa (Vandamme, Falsen et al. 1991). It was described for the first time as *Arcobacter* (Ar'co. bac.ter. L. n. *arcus*, bow; Gr. n. *bacter*, rod; M. L. masc. n. *Arcobacter*, bow-shaped rod), which is gram-negative non-spore forming rods (width, 0.2 to 0.9 μm; length, 1 to 3 μm) that are usually curved, S-shaped, or helical. They are motile (with a darting, corkscrew like motion) by means of a single polar, unsheathed flagellum. This genus initially included two species, *Arcobacter nitrofigilis* and *Arcobacter cryaerophilus* (Vandamme, Falsen et al. 1991). In 1992, Vandamme et al. reclassified *C. butzleri* as *Arcobacter butzleri* and proposed a novel species, *Arcobacter skirrowi* (Vandamme, Pugina et al. 1992). Thirteen years later, five new species of *Arcobacter* have been isolated and defined, denominated as *A. halophilus* (from hypersaline lagoon), *A. cibarius* (from broiler carcasses), *A. mytili* (from mussel), *A. thereius* (from porcine abortions) and *A. marinus* (from seawater), respectively. Later on, more newly reported *Arcobacter* spp. were isolated from different matrices, especially from water-related sources. So far, 28 species of *Arcobacter* were classified according to the list of prokaryotic names with standing in nomenclature (LPSN), as shown in Table 1.

Table 1.1 List of published *Arcobacter* spp. according to LPSN (last check in July 2020)

No.	Year	Name	First isolated from	Reference
1	1983	*A. nitrofigilis*	roots of *Spartina alterniflora*	(Mcclung, Patriquin et al. 1983)
2	1985	*A. cryaerophilus*	animal abortion	(Neill, Campbell et al.

				1985)
3	1991	*A. butzleri*	human stool	(Kiehlbauch, Brenner et al. 1991)
4	1992	*A. skirrowii*	preputial fluid of bull	(Vandamme, Pugina et al. 1992)
5	2005	*A. halophilus*	hypersaline lagoon	(Donachie, Bowman et al. 2005)
6	2005	*A. cibarius*	broiler carcass	(Houf, On et al. 2005)
7	2009	*A. mytili*	mussel	(Collado, Cleenwerck et al. 2009)
8	2009	*A. thereius*	porcine abortion	(Houf, On et al. 2009)
9	2010	*A. marinus*	seawater	(Kim, Hwang et al. 2010)
10	2011	*A. defluvii*	sewage	(Collado, Levican et al. 2011)
11	2011	*A. trophiarium*	pig feces	(De Smet, De Zutter et al. 2011, De Smet, Vandamme et al. 2011)
12	2011	*A. molluscorum*	shellfish (mussel and oyster)	(Figueras, Collado et al. 2011)
13	2011	*A. ellisii*	mussel	(Figueras, Levican et al. 2011)
14	2012	*A. bivalviorum*	shellfish	(Levican, Collado et al. 2012)
15	2012	*A. venerupis*	shellfish	(Levican, Collado et al.

				2012)
16	2013	*A. cloacae*	sewage	(Levican, Collado et al. 2013)
17	2013	*A. suis*	pork meat	(Levican, Collado et al. 2013)
18	2013	*A. anaerophilus*	estuarine sediment	(Sasi Jyothsna, Rahul et al. 2013)
19	2015	*A. aquimarinus*	seawater	(Levican, Rubio-Arcos et al. 2015)
20	2015	*A. ebronensis*	mussel	(Levican, Rubio-Arcos et al. 2015)
21	2015	*A. lanthieri*	pig and daily cattle manure	(Whiteduck-Leveillee, Whiteduck-Leveillee et al. 2015)
22	2016	*A. pacificus*	seawater	(Zhang, Yu et al. 2016)
23	2016	*A. faecis*	human waste septic tank	(Whiteduck-Leveillee, Whiteduck-Leveillee et al. 2016)
24	2016	*A. acticola*	seawater	(Park, Jung et al. 2016)
25	2017	*A. lekithochrous* *A. haliotis*	molluscan hatchery abalone	(Dieguez, Balboa et al. 2017) (Tanaka, Cleenwerck et al. 2017)
26	2018	*A. canalis*	water canal contaminated with urban	(Perez-Cataluna,

			sewage water	Salas-Masso et al. 2018)
27	2019	*A. lacus*	reclaimed water	(Perez-Cataluna, Salas-Masso et al. 2019)
28	2019	*A. caeni*	reclaimed water	(Perez-Cataluna, Salas-Masso et al. 2019)

1.2 Occurrence of *Arcobacter* spp.

1.2.1 Human related *arcobacters*

Although human infection by *Arcobacter* spp. was only rarely reported to date, *Arcobacter* spp. has been associated with abdominal pain, diarrhea and occasionally bacteremia in humans. *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* were classified as serious hazards by the International Commission on Microbiological Specifications for Foods (ICMSF) (Foods 2003). Since the emerging of new described species in this genus, *A. thereius*, *A. mytili* and *A. lanthieri* were also isolated from patients with symptoms of enteritis and bacteremia, respectively (Van den Abeele, Vogelaers et al. 2014, Vasiljevic, Fenwick et al. 2019, Bruckner, Fiebiger et al. 2020).

Among them, *A. butzleri* was predominantly isolated, followed by *A. cryaerophilus*. *A. butzleri* was mostly isolated from patients with persistent watery diarrhea and seldom with bacteremia (Lastovica and le Roux 2000, Lau, Woo et al. 2002, Jiang, Dupont et al. 2010). Early in the autumn of 1983, an outbreak of recurrent abdominal cramps associated with *A. butzleri* in an Italian nursery and primary school was described. None of the affected children had diarrhea. After whole-cell protein analysis, conventional phenotypic tests and serotyping, all outbreak-related strains were identical, suggesting a person-to-person transmission (Vandamme, Pugina et al. 1992).

A. cryaerophilus was reported in diarrhea stool samples from patients in South Africa, Belgium, Denmark and England and seldom reported in blood culture of patient with

bacteremia (Hsueh, Teng et al. 1997, Tompkins, Hudson et al. 1999, Engberg, On et al. 2000, Woo, Chong et al. 2001, Vandenberg, Dediste et al. 2004, Samie, Obi et al. 2007). It is noteworthy that Figueras et al. reported a persistent case of a 26-year-old male with bloody diarrhea, which was attributed to *Campylobacter,* but in fact was caused by *A. cryaerophilus* according to the *rpoB* sequence (Figueras, Levican et al. 2014). This case report demonstrates that *Arcobacter* species might have been misidentified as *Campylobacter* spp. which might have contributed to underestimation of their prevalence in humans (Figueras, Levican et al. 2014).

A. skirrowii was reported sporadically compared with *A. butzleri* and *A. cryaerophilus*. The first report of *A. skirrowii* from an elderly patient with chronic diarrhea was published in 2004 (Wybo et al. J. Clin. Microbiol. 2004). A 73-year-old man was admitted to the hospital after two months of persisting diarrhea. Stool specimens were cultured for conventional enteric pathogens and *Campylobacter* species but showed negative results. After multiplex polymerase chain reaction (mPCR) assay, *A. skirrowii* was confirmed. In South Africa, *A. skirrowii* was found in 1.9% of stool samples from 322 patients with gastrointestinal complaints/diarrhea and school children. Co-infection of *A. skirrowii* with other organisms such as *A. butzleri*, *C. jejuni*, *C. coli* and *Helicobacter pylori* was also detected in six individuals while three of them suffered from diarrheal or bloody diarrheal (Samie, Obi et al. 2007).

Van den Abeele et al. examined fecal samples from 6,774 patients with enteritis in Belgium from 2008 to 2013 and firstly isolated two *A. thereius* from patients with acute gastroenteritis and chronic colitis. Meanwhile, in this study, *Arcobacter* species were the fourth most common pathogen group isolated from fecal samples from persons with acute enteric disease (Van den Abeele, Vogelaers et al. 2014). Furthermore, Rovetto et al. reported the presence of virulence associated genes and antibiotic resistance genes in *A. thereius* by whole genome sequencing (WGS), supporting the classification of *A. thereius* as a pathogen (Rovetto, Carlier et al. 2017).

Very recently, Vasiljevic et al. reported the first case of a 65-year-old man with bacteremia due to *A. mytili*. Direct transmission of this bacterium, by handling live Maryland crab with wounded hands, was hypothesized as no gastrointestinal symptoms were observed. Thus, *Arcobacter* spp. should be considered as cause of gastroenteritis or bacteremia in patients with a history of exposure to seafood and/or the aquatic environment (Vasiljevic, Fenwick et al. 2019). Another recent study by Brueckner et al. investigated 4,336 human stool samples and firstly isolated two *A. lanthieri* from human specimens (Bruckner, Fiebiger et al. 2020).

1.2.2 *Arcobacter* in animal and food products

Arcobacter was isolated from a wide range of animals and related food matrices; with *A. butzleri* and *A. cryaerophilus* as the most prevalent species.

Poultry

Poultry and its relative products were thought to be the major source of food-borne *Arcobacter* infection. *Arcobacter* was reported with prevalences of 15% to 100% in poultry products, such as chicken, turkeys, ducks and geese in Thailand, Japan, Denmark, the U.S., The Netherlands, Turkey, Spain, Costa Rica, Germany and South Korea (Wesley and Baetz 1999, Morita, Maruyama et al. 2004, Atabay, Waino et al. 2006, Son, Englen et al. 2007, Atabay, Unver et al. 2008, Ho, Lipman et al. 2008, Gonzalez, Suski et al. 2010, Bogantes, Fallas-Padilla et al. 2015, Lehmann, Alter et al. 2015, Kim, Park et al. 2019). However, the origin of contamination is yet undefined. Ho et al. detected *Arcobacter* spp. in 20% to 85% of the intestinal systems of laying hens, and in 3.3% to 51% in broilers. Some *Arcobacter* genotypes showed similar ERIC-PCR patterns between the intestinal and carcass samples of the same flock. Therefore, these authors postulated the poultry intestines as a reservoir of *Arcobacter* and that feacal content could further contaminate the slaughter houses during processing (Ho, Lipman et al. 2008). Atabay et al. examined four parts of the intestine of birds at the abattoir (60 samples) for *Arcobacter*. They isolated only one *Arcobacter* strain in one sample, which supported the idea that the poultry intestine may not be the natural source of *Arcobacter* (Atabay and Corry 1997). In 2006, Atabay et al. tested the occurrence of *Arcobacter* in broiler chickens, ducks and turkeys raised in Denmark by cultural and mPCR

methods, detecting again high carriage rates of *Arcobacter* spp. on chicken carcasses (100%) and a low prevalence in chicken intestine (4.3%). Besides, the authors also found very high prevalence of *Arcobacter* spp. in duck flocks (75%), but quite low rates in turkey flocks (11%) (Atabay, Waino et al. 2006). Another study carried out in Chile reported a significantly lower prevalence (30%) in chicken samples compared with the recovery of that from chicken meat products (92%), indicating that poultry may be contaminated in processing plants by external sources as well (Fernandez, Villanueva et al. 2015).

Khoshbakht et al. (Khoshbakht, Tabatabaei et al. 2014) made a more comprehensive investigation on the prevalence of *Arcobacter* spp. from chicken carcasses at different steps in the broiler processing line (before and after scalding, after evisceration and 24h post-chilling) as well as in the environment of an Iranian slaughterhouse (plucker, scalder, vent cutter, hock cutter, shackle washer, stunner, lung sucker, chiller tank water and processing water). Results showed that broiler carcasses showed increasing occurrence of *Arcobacter* from the scalding step (30 to 48%) and a further increase after evisceration (73%). Only by 24h chilling, the prevalence of contaminated carcasses significantly decreased to 18%. It seems that the supplies and equipment in the slaughterhouse plants can increase and accumulate the *Arcobacter* contamination (Khoshbakht, Tabatabaei et al. 2014). However, the main source of its contamination is still unclear.

Livestock

Livestock is considered as another common reservoir of *Arcobacter*. *Arcobacter* were sporadically isolated from pig or bovine fetuses, ovine aborted fetuses and diarrheic feces (Vandamme, Pugina et al. 1992). Among all *Arcobacter* spp., *A. cryaerophilus*, *A. butzleri* and *A. skirrowii* were most frequently associated with intestinal disorders and reproductive disorders such as mastitis, infertility or late-term abortion in cattle, sheep and pigs (On, Jensen et al. 2002, Collado and Figueras 2011, Bath, Leask et al. 2013, Ferreira, Queiroz et al. 2016). However, they are also found in clinically healthy animals. In Japan, *Arcobacter* species were isolated from 3.6% (12/332) to 10.0% (25/250) of clinically healthy cattle and pig fecal samples, respectively. The same group also determined *Arcobacter* spp. in 2.2% and

7.0% in 90 beef and 100 pork samples respectively, collected from retail shops, which is corresponding with their animal occurrence (Kabeya, Maruyama et al. 2003, Morita, Maruyama et al. 2004). In Belgium, *Arcobacter* spp. were isolated from feces of healthy cattle on three unrelated farms with prevalence ranging from 7.5 to 15%, while the occurrence in 16 to 85% of healthy pigs was determined by mPCR (Van Driessche, Houf et al. 2004, Van Driessche, Houf et al. 2005). In Iran, *Arcobacter* spp. was detected in fecal samples of healthy cattle (12%) and sheep (18.5%) in an industrial abattoir by mPCR, with only *A. butzleri* and *A. cryaerophilus* detected. In addition, co-colonization of these two species was found in 25% of the positive samples (Aski, Tabatabaei et al. 2016).

Dairy products such as milk and cheese were increasingly considered as an important reservoir of *Arcobacter* spp. with high risk of human transmission because raw milk contaminated with *Arcobacter* may imported into the diary plants and spread to the environment during cheese processing (Giacometti, Lucchi et al. 2015, Chieffi, Fanelli et al. 2020). Most of investigations were carried out in Italy as producing and consuming raw milk and cheese is a widespread tradition (Giacometti, Lucchi et al. 2013). In Italy, *Arcobacter* spp. was isolated from raw milk (4.1% to 100%), cheese (0 to 33.3%) and dairy plant environment (66.6% to 75%) with *A. butzleri* as the predominant species (Giacometti, Lucchi et al. 2013, Scarano, Giacometti et al. 2014, Caruso, Latorre et al. 2018, Traversa, Gallina et al. 2019, Marta, Giovanni et al. 2020).

Vegetables

Fresh vegetables as ready-to-eat food products present a greater risk of *Arcobacter* spp. than those products subjected to high temperature cooking (Winters and Slavik 2000). In 2011, the first investigation of *Arcobacter* in fresh vegetables was reported by González and Ferrús. 20% of 50 lettuce samples were *Arcobacter* positive by real-time PCR after enrichment, with *A. butzleri* as the only species isolated (Gonzalez and Ferrus 2011). *Arcobacter* was also found in a carrot-processing plant and in a spinach-processing plant (Hausdorf, Frohling et al. 2011, Hausdorf, Neumann et al. 2013). By far, the prevalence of *Arcobacter* in fresh vegetables range from 4.4% to 27.5% in South Korea, Spain and Italy with only *A. butzleri* and *A.*

cryaerophilus being reported (Mottola, Bonerba et al. 2016a, Gonzalez, Bayas Morejon et al. 2017, Kim, Park et al. 2019).

Seafood

Seafood is another potential source of *Arcobacter* transmission to human due to the insufficient cooking or inappropriate consumption of them (Girbau, Guerra et al. 2015). Table 1.2 lists the occurrence of *Arcobacter* in seafood from different countries in the last decade.

Table 1.2 Prevalence of *Arcobacter* spp. in seafood

Country	Source	Species	Prevalence	Reference
Spain	Mussels	*A. butzleri*	17.8%	(Collado, Guarro et al. 2009)
	n = 56	*A. cryaerophilus*	14.3%	
		A. mytili	5.4%	
		A. nitrofigilis	3.5%	
		Arcobacter sp.	1.8%	
	Clams	*A. butzleri*,	40%	
	n = 5	*A. cryaerophilus*	80%	
		A. skirrowii	20%	
India	Fish	*A. butzleri*	43.8%	(Patyal, Rathore et al. 2011)
	n = 75	*A. cryaerophilus*	31.3%	
		A. skirrowii	62.5%	

Thailand	Fish n = 10	*A. butzleri*	90%	(Bodhidatta, Srijan et al. 2013)
Chile	Mussels n = 22	*A. butzleri*	22.7%	(Fernandez, Villanueva et al. 2015)
Germany	Fish n = 50	*A. butzleri* *A. cryaerophilus*	32% 2%	(Lehmann, Alter et al. 2015)
Italy	Mussels n = 42 Clams n = 28	*A. butzleri* *A. cryaerophilus* *A. butzleri* *A. cryaerophilus*	16.7% 7.1% 17.9% 3.6%	(Mottola, Bonerba et al. 2016b)
Portugal	Fish n = 25	*A. butzleri* *A. cryaerophilus* *A. skirrowii* *A. defluvii*	16% 24% 8% 4%	(Vicente-Martins, Oleastro et al. 2018)
Japan	Japanese giant abalone n = 9	*Arcobacter* spp.	22.2%	(Mizutani, Iehata et al. 2019)

1.2.3 *Arcobacter* from the water environment

Contaminated water plays an important role in transmission of pathogens like *Arcobacter* to animals and humans (Jacob, Woodward et al. 1998, Rice, Rodgers et al. 1999, Celik and Unver 2015). A wide range of waterbodies has been reported for *Arcobacter* contamination, including drinking water, ground water, waste water/sewage and natural fresh water like rivers or seas (Jacob, Woodward et al. 1998, Diergaardt, Venter et al. 2004, Morita, Maruyama et al. 2004, Collado, Inza et al. 2008, Ertas, Dogruer et al. 2010, Collado, Kasimir et al. 2010, Merga, Royden et al. 2014, Celik and Unver 2015, Levican, Collado et al. 2016, Talay, Molva et al. 2016, Cui, Huang et al. 2019). It should be noted that there were already three drinking water outbreaks associated with *Arcobacter* spp. in the U.S. and Slovenia, either due to inefficient disinfecting water treatment or contamination from sewage. The presence of fecal pollution seemed to be related with all these outbreaks (Rice, Rodgers et al. 1999, Fong, Mansfield et al. 2007, Kopilovic, Ucakar et al. 2008). The previous studies showed that *Arcobacter* spp. could survive in sewage systems at low temperatures (D'Sa and Harrison 2005, Collado and Figueras 2011), but this remains to be further demonstrated.

1.3 Isolation, detection and genotyping of *Arcobacter*

1.3.1 Cultural isolation of *Arcobacter*

The first *Arcobacter* reported was isolated using semi-solid EMJH supplemented with 5-fluorouracil (100 mg/l) and rabbit serum (Ellis, Neill et al. 1977). Since then, various media and procedures were developed and applied for isolating and culturing *Arcobacter* spp. from different origins such as food, animal feces, clinical samples and water (Atabay and Corry 1997, Johnson and Murano 1999, Houf, Devriese et al. 2001a, Atabay, Aydin et al. 2003, van Driessche, Houf et al. 2003, Scullion, Harrington et al. 2004, Houf and Stephan 2007, Merga, Leatherbarrow et al. 2011, Fallas-Padilla, Rodriguez-Rodriguez et al. 2014, Silha, Silhova-Hruskova et al. 2015, Levican, Collado et al. 2016, Salas-Masso, Andree et al. 2016). These isolation methods are usually composed of a 48h-enrichment step in a broth supplemented with several antibiotics followed by cultivation on agar media (with or without antibiotics) for additional 48-72h incubation. One common protocol is using an enrichment

broth containing the selective supplements cefoperazone, amphotericin B and teicoplannin (CAT), followed by membrane filtration on blood agar according to Atabay and Corry (Atabay and Corry 1997). Johnson and Murano (Johnson and Murano 1999) developed Johnson-Murano (JM) broth and plates for isolation of arcobacters from poultry with aerobic incubations. Houf et al. proposed an *Arcobacter* selective isolation protocol by using *Arcobacter* broth with a five-antibiotic cocktail: amphotericin B (10 mg/l), cefoperazone (16 mg/l), 5-fluorouracil (100 mg/l), novobiocin (32 mg/l) and trimethoprim (64 mg/l), based on a previous *Arcobacter* antimicrobial susceptibility study (Houf, Devriese et al. 2001b). Later, van Driessche et al. refined this method for better isolation from fecal samples by increasing the concentration of novobiocin to 64 mg/l and addition of 100 mg/l cycloheximide (van Driessche, Houf et al. 2003). Diegaardt et al. isolated arcobacters from various water samples by enrichment in Bolton broth prior to plating on blood agar with no antibiotics but filtration through a 0.6 µm membrane (Diergaardt, Venter et al. 2004).

It should be noted that using different atmosphere might cause bias in the detection. Levican et al. investigated the impact of atmosphere on the *Arcobacter* detection rate. They found that using only aerobic condition increased the detection rate compared to only microaerobic condition (41.1% vs. 23.2%). However, the species diversity was higher after microaerobic incubation. Thus, the author suggested that a combination of two incubation atmospheres in the detection process would increase both the detection rate and the detected diversity (Levican, Collado et al. 2014). Bias also happens when using different formula of media. There is one study comparing this isolation protocol with a new selection method by supplementing the *Arcobacter*-CAT enrichment broth with 2.5% NaCl followed by culturing on Marine Agar (Salas-Masso, Andree et al. 2016). This protocol yielded ca. 40% more positive samples and a higher diversity of known (11 vs. 7) and unknown (7 vs. 2) *Arcobacter* species, where the most prevalent species were *A. molluscorum*, *A. bivalviorum* and *A. mytili*, instead of *A. butzleri* as usually reported (Salas-Masso, Andree et al. 2016). Therefore, a standard isolation protocol becomes an urgent need in *Arcobacter* investigation. However, no standard method has been defined for isolating *Arcobacter* spp. from different sources so far.

1.3.2 Molecular identification of *Arcobacter* spp.

For a better evaluation of the *Arcobacter* spp. from different origins, several molecular detection methods have been developed. Table 1.3 shows an overview of molecular diagnostic PCR-based assays for detection of *Arcobacter* including PCR, mPCR, and real-time PCR. Among them, the most globally applied method is the mPCR designed by Houf et al. (Houf, Tutenel et al. 2000) in 2000, which was later proved to be reliable on genus level but sometimes failed in species discrimination (Levican and Figueras 2013).Thus, a combination of Houf's mPCR assay and 16S rRNA and/or *rpoB* gene sequencing is usually recommended (Collado, Guarro et al. 2009, Levican and Figueras 2013). Another DNA banding pattern-based method: restriction fragment length polymorphism (RFLP) was also used for *Arcobacter* identification. The latest 16S rDNA-RFLP method was updated by Figueras et al. which was able to identify 10 of 17 described *Arcobacter* species but failed to identify *A. thereius* using a single endonuclease *Mse*I (Figueras, Levican et al. 2012).

Table 1.3 Summary of PCR assays based diagnostic methods for *Arcobacter* detection including their advantages and limitations.

Diagnostic methods	**Target gene**	**Advantage**	**Limitation**	**Reference of comment**
PCR	23S rRNA (Kabeya, Kobayashi et al. 2003)	Genus-specific that able to discriminate *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*	Does not allow the concurrent identification of *A. cryaerophilus* and *A. skirrowii*	(Levican and Figueras 2013)
	gyrA gene (Pentimalli, Pegels et	Species-specific for *A. butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. cibarius*	Multiplex detection of the four species is not possible	(Levican and Figueras

	al. 2009)			2013)
mPCR	16S rRNA and 23S rRNA (Houf, Tutenel et al. 2000)	Rapid, simultaneous detection of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* specific detection of *A. butzleri*	Unable to identify other *Arcobacter* spp. except *A. butzleri*	(Levican and Figueras 2013)
	23S rRNA and *gyrA* gene (Douidah, De Zutter et al. 2010, De Smet, Vandamme et al. 2011)	Rapid, simultaneous detection of *A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, *A. cibarius* and *A. thereius*	Unable to identify *A. trophiarum* Misidentified four of non-target species (*A. defluvii*, *A. ellisii*, *A. venerupis*, and *A. suis*) as *A. butzleri*	(Levican and Figueras 2013)
real-time PCR	*rpoB*/*C* and 23S RNA (Brightwell, Mowat et al. 2007)	Sensitive differentiate *A. butzleri* and *A. cryaerophilus.*	Unable to detect other *Arcobacter* spp.	(Ramees, Dhama et al. 2017)
	23S rRNA (Gonzalez,	Rapid detection of *Arcobacter* spp. with low	Non-species specific	(Ramees, Dhama et

	Suski et al. 2010)	detection limit (56 CFU/ml for *A. butzleri*)		al. 2017)
	16S rRNA (Shrestha, Tanaka et al. 2018)	High sensitivity of *Arcobacter* spp. from water samples with lower quantification limits as 10 copies per reaction	Non-species specific	(Shrestha, Tanaka et al. 2018)
real-time mPCR	*hsp60* gene (de Boer, Ott et al. 2013)	Rapid detection of *Campylobacter* species and *A. butzleri* with high sensitivity from stool samples	Does not include detection of other *Arcobacter* spp.	(Ramees, Dhama et al. 2017)

In recent years, some fast and robust methods were developed for rapid species identification in *Arcobacter*. One of them is Matrix-associated laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS), which has been shown to be a rapid and sensitive method for identification of microorganisms. This method analyzes the protein profiles of bacterial macromolecule from intact bacteria without prior purification steps (reviewed by Carbonnelle et al. (Carbonnelle, Mesquita et al. 2011)). An *Arcobacter* MALDI-TOF reference database was established firstly by Alispahic et al. (Alispahic, Hummel et al. 2010). Van den Abeele et al. developed an in-house novel reference spectrum specifically for clinical usage and compared it to the only commercial one by Bruker Daltonics (Germany), reporting that some spectra of *A. skirrowii* in the commercial database are of suboptimal quality. They

demonstrated the identification quality is not only improved by adding extra strains of certain species but also by using newly obtained spectra of the already included reference strain. Their novel database showed significantly improved sensitivity with conservation of excellent specificity but only for the clinically relevant species *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* (Van den Abeele, Vogelaers et al. 2018).

Another powerful technique developed in recent years is Next-Generation Sequencing (NGS). This method allows higher-throughput analysis and is getting also cost effective especially for tasks like characterizing complex microbial consortia (Samarajeewa, Hammad et al. 2015, Mitchell and Simner 2019). Samarajeewa et al. assessed the commercial microbial consortia products with NGS based on Ion Torrent technology with a previous PCR amplification of 16S rDNA and compared it with other three genomic methods: Denaturing Gradient Gel Electrophoresis (DGGE), Clonal-Restriction Fragment Length Polymorphism (C/RFLP) and partial 16S rDNA amplification, cloning followed by Sanger sequencing (PRACS). Ion Torrent technology successfully showed better performance in initial screening by detecting more bacteria but with different proportions of the microbial composition compared with the other three methods. The author suggested combining two or more methods for reliable identification (Samarajeewa, Hammad et al. 2015). Later, two studies in 2019 used 16S rRNA NGS based on the Illumina Miseq platform for wastewater sample evaluation. Cui et al. evaluated the diversity of enteric and environmental pathogens with a self-built reference database and reported that *Arcobacter* and *Bacteroides* were the predominant genera in the urban rivers. They further verified the NGS results using quantitative PCR (qPCR), demonstrating the consistence and thus suggested a combination of these two methods could exploit both advantages to the full (Cui, Huang et al. 2019). Meanwhile, Greay et al. screened the wastewater sample with the same method targeting the V4 region of 16S but failed in differentiating *Enterobacteriaceae*. Only three pathogens could be identified to species level including *Arcobacter venerupis* by NCBI nr/nt database (Greay, Gofton et al. 2019).

Other diagnostic techniques applied especially for field application are loop-mediated isothermal amplification (LAMP) and fluorescence in situ hybridization (FISH). The former

method showed higher sensitivity and less detection time than mPCR, but with the shortage that the continuous amplification target gene product by LAMP may be confused with nonspecific PCR products when using gel electrophoresis (Wang, Seo et al. 2014). The latter method for detecting *Arcobacter* was reported firstly by Moreno et al. in wastewater samples (Moreno, Botella et al. 2003). Fera et al. detected *Arcobacter* spp. in all estuarine water samples without/with enrichment. Although FISH did not improve the detection of *Arcobacter* spp. compared with PCR, it provided more information about cell morphology and viability of single cells while it needed no DNA extraction (Fera, Gugliandolo et al. 2010).

1.3.3 Genotyping of *Arcobacter*

Genotyping is important for recognizing outbreaks of infection, determining the sources of outbreaks or studying the transmission routes of *Arcobacter*, while phenotypic testing is difficult due to the insufficient typing ability and discriminatory power (On 1996, Olive and Bean 1999). Thus, several techniques have been developed and applied to investigate the genetic diversity of arcobacters.

The most commonly used method for genotyping *Arcobacter* is the ERIC-PCR (Vandamme, Giesendorf et al. 1993, Ramees, Dhama et al. 2017). It is widely applied in characterizing *Arcobacter* spp. -especially *A. butzleri*- isolated from food samples like poultry, cattle, beef, sheep, pork, etc. and water samples (Houf, De Zutter et al. 2002, Van Driessche, Houf et al. 2005, Aydin, Gumussoy et al. 2007, Van Driessche and Houf 2007, De Smet, De Zutter et al. 2010, Kayman, Abay et al. 2012, Douidah, De Zutter et al. 2014, Levican, Collado et al. 2014, Ramees, Rathore et al. 2014, Lehmann, Alter et al. 2015, Levican, Collado et al. 2016, Ferreira, Oleastro et al. 2017, Rathlavath, Kumar et al. 2017). Some of these studies evaluated ERIC-PCR with other genotyping methods in parallel. Houf et al. tested the typeability and discriminatory power of both ERIC-PCR and randomly amplified polymorphic DNA-PCR (RAPD-PCR), and found both methods were useful for characterization of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* isolates. They suggested ERIC-PCR to be the first choice due to its better reproducibility and higher differentiation resolution compared with RAPD-PCR (Houf, De Zutter et al. 2002). Douidah et al. compared ERIC-PCR with

pulsed-field gel electrophoresis (PFGE) and amplified fragment gel polymorphism (AFLP) in characterizing six human and animal-associated *Arcobacter* species, and suggested PFGE with *Nru*I enzyme was the best choice when the isolates were not a large number and unrelated. However, ERIC-PCR has the advantage that it is rapid and easy to perform (Douidah, De Zutter et al. 2014). In spite of the disadvantage of this method with reproducibility, ERIC-PCR is still the most common method used for first large-scale screening of isolates.

PFGE has also been used for epidemiological studies and investigations of transmission routes of *Arcobacter* in foods, slaughter houses, breeding farms or dairy plants by using endonucleases, e.g.*Ava*I, *Eag*I, *Sac*II, *Sma*I and *Kpn*I (Hume, Harvey et al. 2001, Rivas, Fegan et al. 2004, Ho, Lipman et al. 2006, Gonzalez, Ferrus et al. 2007, Shah, Saleha et al. 2012, Ferreira, Fraqueza et al. 2013, De Cesare, Parisi et al. 2016, Giacometti, Piva et al. 2018). Recently, two studies compared the discriminatory power of PFGE and multi locus sequence type (MLST), demonstrated that both methods presented good concordance between each other while the former one presented a little higher discriminatory power (De Cesare, Parisi et al. 2016, Giacometti, Piva et al. 2018).

Another commonly used method is AFLP. The first use of AFLP was carried out by On et al. on 72 isolates of *A. butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. nitrofigilis* using the restriction enzymes *Bgl*II and *Csp*6I, followed by PCR amplification and detection of fragments in the whole bacterial DNA. 62 distinct types were defined from 72 isolates with a reproducibility proved to be 93% by the duplicates of 20 isolates (On, Harrington et al. 2003). Debruyne et al. optimized the AFLP using *Hind*III and *Hha*I combined with hsp60 gene sequence analyses, revealing intraspecies relationship particularly in *A. cryaerophilus* (Debruyne, Houf et al. 2010). Later AFLP have been applied in genotyping inter- and intraspecies of *Arcobacter* isolates from various samples for epidemiological purposes (Gilbert, Kik et al. 2014, Oliveira, Pressinotti et al. 2017, Gobbi, Spindola et al. 2018).

The most recent method developed for *Arcobacter* genotyping is MLST. The first *Arcobacter* MLST scheme was created by Miller et al. using the seven loci: *aspA*, *atpA*, *glnA*, *gltA*, *pgm*,

tkt, and *glyA* (http://pubmlst.org/arcobacter/). However, no correlation between the MLST sequence type and the host or geographical source was observed (Miller, Wesley et al. 2009). Other groups analyzed the diversity of the isolates from animal and human feces or food products by MLST and a considerable amount of diversity was found between the isolates of *A. butzleri* (Merga, Leatherbarrow et al. 2011, Merga, Williams et al. 2013, Rasmussen, Kjeldgaard et al. 2013, Alonso, Girbau et al. 2014, Perez-Cataluna, Tapiol et al. 2017, Marta, Giovanni et al. 2020). Comparison between MLST with other molecular typing method such as PFGE for typing *A. butzleri* isolates showed good correlation between each other but lower discriminatory index than that of MLST, while higher than MALDI-TOF (De Cesare, Parisi et al. 2016, Giacometti, Piva et al. 2018).

1.4 Virulence factors of *Arcobacter* spp.

The pathogenicity and virulence mechanisms of *Arcobacter* species are still unclear. However, in recent years, more investigations on virulence genes distribution have been reported since ten putative virulence determinants homologs to those of *C. jejuni* and other bacteria were detected in the genome of *A. butzleri* reference strain RM4018. These ten genes encode for the *Campylobacter* invasive antigen B (*ciaB*); fibronectin binding proteins (cj1349 and *cadF*); the virulence factor MviN (*mviN*); the outer membrane phospholipase A (*pldA*); a hemolysin (*tlyA*); the filamentous hemagglutinin (*hecA*) and the hemolysin activation protein (*hecB*); the iron-regulated outer membrane protein IrgA (*irgA*), and the periplasmic enzyme IroE (*iroE*) (Miller, Parker et al. 2007). Douidah et al. developed a PCR assay for nine putative virulence genes except *iroE* based on the genome of *A. butzleri* ATCC 49616 and validated it in 30 reference strains including *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*. Six genes (*cadF*, *ciaB*, cj1349, *mviN*, *pldA*, and *tlyA*) were detected in all *A. butzleri* strains and *ciaB* was the most prevalent gene in both *A. cryaerophilus* and *A. skirrowii* (Douidah, de Zutter et al. 2012). Whiteduck-Léveillée et al. further optimized three mPCR for eight virulence genes (except *cadF*) with a more rapid detection on the basis of Douidah's study and evaluated this method in *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* isolated from fecal sources. The occurrence of *ciaB* were significantly higher than others in both *A. butzleri* and *A. cryaerophilus*, while

among three *A. skirrowii* isolates only *ciaB* and *mviN* were detected (Whiteduck-Leveillee, Cloutier et al. 2016). Similar occurrence of these putative virulence genes in isolates from different sources was also reported in many other studies, with *cadF*, *ciaB*, cj1349, *mviN*, *pldA*, and *tlyA* as the most frequently detected among all ten genes and with the highest occurrence usually reported for *ciaB* and *mviN* (Karadas, Sharbati et al. 2013, Levican, Alkeskas et al. 2013, Tabatabaei, Shirzad Aski et al. 2014, Girbau, Guerra et al. 2015, Zacharow, Bystron et al. 2015, Piva, Gariano et al. 2017). However, the correlation between the presence of *A. butzleri* virulence genes and the adherence/invasion phenotype in human cell lines is not yet clarified (Karadas, Sharbati et al. 2013, Levican, Alkeskas et al. 2013).

1.5 The cold adaptation of *A. butzleri*

Even though *Arcobacter* is regarded as emerging pathogen which adapted to environment with a wide temperature range, the knowledge about its adaptation to cold is still scarce. Among all species, *A. butzleri* is the most frequently isolated from food chain and associated with human illness (Ferreira, Oleastro et al. 2019). Previous surveillance investigation of *A. butzleri* in food products, farm and processing plants have demonstrated that the commonly used freezing processes or freezing temperatures are insufficient to inactivate these bacteria (reviewed in 1.2.2). The persistence and adaptation of *A. butzleri* to cold remains to be further evaluated.

1.5.1 The growth and survival of *A. butzleri* at cold

The persistence of *A. butzleri* in food products at cold temperatures, applied during meat processing, transportation and fridge storage has been seldom studied despite the vast reports on prevalence carried out all over the world. *In vitro* experiments were evaluated in two types of media: broth media or food matrix. Hilton et al. determined by impedance microbiology that the temperature range for growth of the reference strain *A. butzleri* NCTC 12481 was 15 to 39°C. They found that this strain could survive better under chilling conditions (4°C) compared to freezing (-20°C) with a gradual decrease in viability (Hilton, Mackey et al. 2001). D'Sa and Harrison investigated the growth and survival of six human isolates of *A. butzleri* and *A. cryaerophilus* under different conditions with selected pH and

NaCl contents. They noticed some *A. butzleri* strains could grow at 10°C for minimum incubation of seven days in EMJH PLM-5 medium (D'Sa and Harrison 2005). Van Driessche and Houf assessed the growth of three human associated *Arcobacter* spp. at 4°C, 7°C and 20°C in water with and without organic material. All *A. butzleri* strains incubated at 4°C and 7°C showed a 5% (without organic material) to 15% (with organic material) increase in cell numbers at day 7. The *A. butzleri* isolates remained culturable until day 205 with organic materials with around 40% of the initial inoculum (Vandrissche and Houf 2008). Kjeldgaard et al. examined the growth behavior of the reference strain *A. butzleri* ATCC 49616 in CMJ and BHI, confirming that *A. butzleri* is able to multiply at 10 °C in both media (Kjeldgaard, Jorgensen et al. 2009). In food matrices, *A. butzleri* isolates were reported to survive at temperatures below 10°C on chicken legs, milk and ricotta cheese within the experimental period from 6 days to 22 days, sometimes with an initial cell number increase. However, none of them have reported a consistent growth of *A. butzleri* under 10°C (Badilla-Ramirez, Fallas-Padilla et al. 2016, Giacometti, Serraino et al. 2014, Giacometti, Losio et al. 2015).

1.5.2 Cold-related genes in *E.coli*

Little is known about the cellular processes involved in adaptation to cold temperature for *A. butzleri*. Previous studies focused on the phenotypic response of *A. butzleri* encountering cold temperatures in different broth media or food matrix demonstrating that *A. butzleri* could adapt to cold temperatures. Thus, the presence of cold-related genes in *A. butzleri* is presumed. The function of the cold related proteins was well studied in *E. coli*, which was first reported by Jones et al. (Jones, VanBogelen et al. 1987). After a temperature downshift from 37°C to 10°C, transcription and translation processes for a majority of genes slow down or come to an almost complete stop, while a set of cold-shock proteins is preferentially and transiently expressed. Table 1.5 listed part of these cold-shock related genes as reviewed by Gualerzi et al and Barria et al. (Gualerzi, Giuliodori et al. 2003, Barria, Malecki et al. 2013).

Table 1.4 Cold-related genes in *E. coli*

Gene	Product	Description/function
aceE	AceE	Pyruvate dehydrogenase
aceF	AceF	Pyruvate dehydrogenase
cspA	CspA	Cold-inducible RNA chaperone and anti-terminator; transcriptional enhancer
deaD	DeaD	ATP-dependent RNA helicase; facilitates translation of mRNAs with 5' secondary structures
dnaA	DnaA	DNA binding and replication (initiation); global transcriptional regulator
gyrA	GyrA	DNA gyrase subunit A; DNA binding/cleaving/rejoining subunit of gyrase
infB	IF2	Translation initiation; fMet-tRNA binding; protein chaperone
nusA	NusA	Transcription termination/antitermination/elongation L factor
pnp	PNPase	3'-5' exonuclease; degradosome component; required for growth at low temperatures
rnr	RNase R	3'-5' exonucleases;
rbfA	RbfA	Ribosome assembly/maturation; cold shock adaptation protein
recA	RecA	General recombination and DNA repair; induction of the SOS response
tig	Trigger factor	Protein-folding chaperone, multiple stress protein, ribosome-binding

1.6 Objectives of the study

Since only limited data on the prevalence of *Arcobacter* spp. in seafood are available for Germany, and data on molecular mechanisms involved in the survival and adaptation of *A. butzleri* to cold is still scarce, this study was conducted with the following objectives:

- to evaluate the prevalence of *Arcobacter* spp. in retail seafood in Berlin, Germany,
- to characterize the *Arcobacter* spp. from retail seafood by virulence gene detection and ERIC-PCR genotyping,
- to evaluate the growth of *A. butzleri* at cold, as well as to investigate the presence and transcriptional expression of cold-related genes in *A. butzleri.*

Chapter 2 Characterization of *Arcobacter* spp. isolated from retail seafood in Germany

This chapter has been published in: Food Microbiology (2019)

Manuscript received at Food Microbiology: 13 March 2018

Accepted: 21 February 2019

Available online: 22 February 2019

Authors: Xiaochen Zhang, Thomas Alter and Greta Gölz

Food Microbiology: Volume 82, September 2019, Pages 254-258

doi: https://doi.org/10.1016/j.fm.2019.02.010

Chapter 3 The transcriptional response of *Arcobacter butzleri* to cold shock

This chapter has been accepted in: FEBS Open Bio (2020)

Manuscript received at FEBS Open Bio: 13 May 2020

Accepted: 17 August 2020

First published: 18 August 2020

Authors: Xiaochen Zhang, Yulan Su, Thomas Alter and Greta Gölz

doi: https://doi.org/10.1002/2211-5463.12959

Running heading: Cold response of *Arcobacter butzleri*

3.1 Abstract

Arcobacter (*A.*) *butzleri* is an emerging zoonotic pathogen associated with gastrointestinal diseases, such as abdominal cramps and diarrhea, and is widely detected in animals, showing a high prevalence in poultry and seafood. The survival and adaptation of *A. butzleri* to cold temperatures is poorly studied but it might be of interest for food safety considerations. To address this, growth patterns of eight *A. butzleri* isolates were determined at 8°C for 28 days. *A. butzleri* isolates showed strain-dependent behavior: six isolates were unculturable after day 18, one exhibited declining but detectable cell counts until day 28 and one grew to stationary phase level. Out of 13 *A. butzleri* cold shock-related genes homologous to *E. coli*, 10 were up-regulated in response to a temperature downshift to 8°C, as demonstrated by RT-qPCR. Additionally, we compared these data with the cold shock response in *E. coli*.

Overall, we provide a deeper insight into the environmental adaptation capacities of *A. butzleri*, which we find shares similarities with the *E. coli* cold shock response.

Keywords: *A. butzleri*; cold adaptation; temporal transcriptional expression; cold shock-related genes

3.2 Introduction

Arcobacter spp. is an emerging zoonotic pathogen associated with gastrointestinal diseases such as abdominal cramps and diarrhea [1-3]. Among the 29 species within the genus of *Arcobacter* described so far [4], *Arcobacter* (*A.*) *butzleri* is the most widely detected species in animals, environment and foods, with high prevalences in poultry meat and seafood samples [5-10]. Although the good environmental adaptability of *A. butzleri* is well known, the survival and adaptation of *A. butzleri* to cold is poorly studied so far but might be of interest for food safety considerations. *A. butzleri* has an optimal growth temperature of 30°C in both aerobic and microaerobic conditions but is likely to be exposed to a wide temperature range during its transmission cycle. The minimum growth temperature of *A. butzleri* was initially reported to be 15°C [11]. Later, some studies reported lower limits as 10°C e. g. in Ellinghausen McCullough Johnson Harris (EMJH) medium [12], in chicken meat juice as well as in Brain Heart Infusion (BHI) broth [13]. Van Driessche and Houf [14] investigated the survival capability of *A. butzleri* in water with/without organic material at minimum 4°C and reported that all tested *A. butzleri* strains survived for at least 203 days when organic material is present in the water, indicating the best cold adaptation among all human related *Arcobacter* species.

With the complete genome of the *A. butzleri* RM4018 sequenced in 2007 [15], understanding of adaptation to environmental stress could be improved. Hitherto, no data describing the cold shock response of *Arcobacter* spp. have been reported so far. Since the previous phenotypic studies suggest that *A. butzleri* could adapt to cold temperatures, the presence of cold-related genes is presumed.

The function of the cold related proteins is best studied in *Escherichia coli*. It was first reported by Jones et al. [16] that after a cold shock from 37°C to 10°C, transcription and translation processes for a majority of genes slow down or come to an almost complete stop, while a set of cold-shock proteins is preferentially and transiently expressed [17-20]. These proteins were classified in two groups according to their expression pattern [21]. The class I proteins include: CspA (the major cold-shock protein of *E. coli,* encoded by *cspA*), DeaD (reassigned as CsdA, a DEAD-box protein, encoded by *deaD*), NusA (the transcription termination/antitermination protein, encoded by *nusA*), RbfA (the Ribosome Binding Factor A, encoded by *rbfA*) and RNase R (encoded by *rnr*). The induction of the expression level of these proteins vary, but

are more pronounced compared with Class II proteins which include PNPase (the Polynucleotide phosphorylase, encoded by *pnp*), IF-2 (the translation initiation factors IF2, encoded by *infB*), GyrA (the DNA gyrase subunit A, encoded by *gyrA*), RecA (a DNA-dependent ATPase, encoded by *recA*), DnaA (the replication-initiator protein, encoded by *dnaA*), Trigger factor (encoded by *tig*) and pyruvate dehydrogenase (encoded by *aceE* and *aceF*). All above mentioned genes were reviewed by Gualerzi [18] and Barria [20].
As little is known about the adaption of *A. butzleri* to cold, the growth capabilities of eight *A. butzleri* isolates derived from human, mussels and chicken were investigated at 8°C and expression on transcriptional level of the previously described 13 cold related genes were studied in three *A. butzleri* strains showing different growth and survival capability after the temperature down shift from 28°C to 8°C.

3.3 Materials and Methods

3.3.1 Bacterial strains and growth conditions

The *A. butzleri* strains used in this study were previously described [10, 22]. All isolates from human feaces (H1, H2, H3), mussels (M3, M4) and chicken meat (C1, C2, C3) were routinely grown in Brucella Broth (BB; BD, Heidelberg, Germany) or on Mueller-Hinton Blood agar (MHB; Oxoid, Wesel, Germany). The working cultures were maintained under aerobic atmosphere at optimal (28°C) or cold (8°C) temperatures.

3.3.2 Growth curve

Briefly, all *A. butzleri* strains were recovered on MHB agars under microaerobic conditions (6 % O_2, 10 % CO_2) for 72 h at 28°C. For each strain 5 ml BB were inoculated and incubated overnight (18 h) under microaerobic conditions at 28°C to reach approx. 8 $\log_{10}$ colony forming units (CFU) mL^{-1}. These pre-cultures were subsequently diluted 1:10,000 in 5 ml pre-cooled (8°C) or pre-warmed (28°C) BB, respectively. The cultures were incubated at the respective temperatures under aerobic conditions and the bacterial load determined over 28 d. At each indicated time point, serial dilutions of the cultures were plated on MHB plates and incubated for 48 h at 28°C under microaerobic conditions before enumeration.

3.3.3 RNA Extraction

To screen the transcriptional expression-pattern of the presumed cold-related genes in *A. butzleri* at a very early stage after the sudden temperature downshift, the RNA of *A. butzleri* isolates from different growth modes at cold temperatures (H2 and C2) and the reference

strain (H1/*A. butzleri* RM4018) were analyzed. Briefly, pre-cultures were prepared as described above but in a volume of 300 ml fresh BB. After overnight incubation, the cultures reached the post-logarithmic phase and were harvested by centrifugation (5 min; 7,500 × g) at room temperature and resuspended in 200 ml fresh pre-cooled (8°C) BB. The master culture was split equally into 8 working cultures. All working cultures were incubated at 8°C except the reference sample (0 min), which was further processed immediately. Samples were collected at 5, 30, 60, 120, 180, 240 and 360 min after the temperature downshift. Bacteria from each sample were harvested by centrifugation (5 min, 7500 × g) at 8°C, the pellet resuspended in 1 ml RNA Protect (Qiagen, Hilden, Germany) and stored at -80°C until further processing. RNAs were isolated by peqGOLD Bacteria RNA Kit (VWR, Radnor, USA) following the manufacturer's instructions (Thermo Scientific, Waltham, USA). Afterwards, the RNA was quantified by a Nanodrop 2000 (Thermo Scientific) and subsequently subjected to DNase treatment. Briefly, a total volume of 40 µl containing 750 ng RNA, 4 U DNase I, 1 × Reaction Buffer with $MgCl_2$, 40 U RiboLock (all from Thermo Scientific) and nuclease-free water was incubated at 37°C for 15 min. The DNase digestion reaction was terminated by addition of 4.5 mM EDTA (Thermo Scientific) and incubation at 65°C for 10 min. All samples were placed on ice for further steps afterwards.

3.3.4 cDNA transcription

The complementary DNA (cDNA) of the RNA samples were synthesized using Maxima H Minus First Strand cDNA Synthesis Kit (Thermo Scientific), following the manufacturer's instructions. Briefly, the cDNA synthesis mix contained 220 ng RNA, 0.5 mM dNTP Mix and 5 µM Random Hexamer Primer in a total volume of 15 µl. The mix was pre-heated to 65 °C for 5 min, before addition of 4 µl 5 × RT Buffer and 1 µl Maxima H Minus Enzyme Mix, and incubated at 25°C (10 min) followed by 15 min incubation at 50°C. The reaction was terminated by 5 min incubation at 85°C. To verify the efficiency of the DNase digestion, a control without addition of the reverse transcription enzyme (NRT) of each sample were carried out in parallel. The cDNA was diluted in water at a ratio of 1:10 for RT-qPCR.

3.3.5 Reverse transcription-quantitative PCR (RT-qPCR)

The gene expression profile was analyzed by RT-qPCR. Primers used in this assay were designed using the software Primer3 (http://frodo.wi.mit.edu/) and are listed in Tab. 1. The RT-qPCR was carried out using SsoFastTM EvaGreen Supermix® (Bio-Rad, Munic, Germany) and the CFX connect real time system (Bio-Rad), according to the manufacturer's instructions. Briefly, 7.5 µl SsoFast EvaGreen Supermix, 36 nmol of each forward and reverse primer, and 1 µl of cDNA was mixed in a total volume of 15 µl. The RT-qPCR was started by

an initial preheating step for 30 s at 95°C, followed by 40 cycles of 5s denaturation at 95°C, 5s annealing (corresponding annealing temperatures for each gene is shown in Tab. 1). In this study, *rpoA* was used as housekeeping gene for normalization of the expression. Fold changes were calculated according to the $\Delta\Delta C_T$ method [23] and genes with an expression ratio ≥ 2.0 were regarded as up-regulated and those ≤ 0.5 as down-regulated [24]. The expression of each gene was analyzed using three independent cDNA samples with two technical replicates in each run.

3.3.6 Statistical analysis

Temporal expression patterns of the selected *A. butzleri* genes were analyzed and compared by K-means clustering by Genesis 1.8.1 (TU Graz) with 3 clusters, maximum 10,000 iterations and 5 runs.

The differences in temporal expression pattern among three *A. butzleri* isolates were analyzed by two-way ANOVA (GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com).

3.4 Results and discussion

3.4.1 Growth curves of A. butzleri isolates

As *A. butzleri* is highly prevalent in chicken meat and seafood products, *A. butzleri* strains isolated from these matrices as well as from human stool specimen were included in our cold adaptation study. The growth of eight *A. butzleri* isolates from human (H), mussels (M) and chicken (C) were determined at 28°C and 8°C for 28 d or until undetectable on MHB plates. No difference in the growth capabilities of all eight strains could be determined during incubation at 28°C under aerobic conditions (data not shown). For 6 of the 8 investigated isolates (75%), stable CFU counts of approx. 4 $\log_{10}$ CFU mL^{-1} were determined until 8 d after incubation at 8°C, followed by declining CFU counts until 22 d, while afterwards, no colony counts could be determined (Fig. 2). This behavior is represented by strain C2 in Fig. 1. For the reference strain H1 (*A. butzleri* RM4018), also a declining tendency of cell counts by 2 $\log_{10}$ level was determined although colonies were still detectable on MHB plates at day 28 (Fig. 1). Overall, we determined only survival for strain H1 at 8°C, however, in two of eight replicates we observed slightly growing capabilities of this strain. In contrast, the strain H2 was able to grow at 8°C, reaching stationary phase with 9 $\log_{10}$ CFU mL^{-1} around 13 d in all replicates (Fig. 1). No correlation between the strain origin and the growth behavior at 8°C was observed, which is comparable with results of a previous study of Driessche and Houf [14].

To the best of our knowledge, 8°C was the lowest temperature ever been reported for the observation of continued growing up to 5 $\log_{10}$ level of *A. butzleri* under aerobic conditions in media. In consistence with a previous report, a low temperature resulted in a delayed growth in any medium [13]. In the study of Kjeldgaard et al. [13] *A. butzleri* strain ATCC 49616 (H1 in our study) was able to grow at 10 °C in BHI broth and chicken meat juice and was able to survive in both media when stored at 5°C. A recent study by Silha et al. [25] observed non-significant multiplication with gradually decreasing viability until 14 d of *A. butzleri* CCUG 30484 (H1 in our study) at 5°C in BHI culture.

In previous reports, *A. butzleri* isolates were inoculated in different food matrices to investigate their growth and survival capability at different refrigeration temperatures. In ultrahigh-temperature treated, pasteurized and raw milk, *A. butzleri* could remain viable at both 4°C and 10°C until 6 d [26]. *A. butzleri* RM4018 count remained stable during 5 d storage at 6°C in artisanal ricotta cheese, but decreased by 2.5 $\log_{10}$ level during 22 d storage in industrial ricotta cheese. However, *A. butzleri* count increased up to 8 $\log_{10}$ CFU g^{-1} in industrial cheese sample at 7 d and remained stable until the end of the shelf life (22 d) when stored at 12°C [27]. These results confirmed the hypothesis that the critical growing temperature of the strain *A. butzleri* RM4018 was between 6 to 12°C at least in cheese media. Other studies also demonstrate the growing ability of *A. butzleri* at even lower temperatures, but only over a short time period. Driessche and Houf [14] reported slightly increasing cell counts of some *A. butzleri* strains within 7 d at 4°C and 7°C in pure water before the counts declined. Likewise, Badilla et al. [28] tested the survival rate of *A. butzleri* on chicken legs. Their results showed that *A. butzleri* had a short growing capacity until 3 d at 4°C and 10°C followed by declining tendency afterwards.

Taken all together, we suggest that even though cell counts of the majority of *A. butzleri* isolates start to decline after 8 days storage at 8°C some strains were able to keep stable cell count or could even grow at this temperature.

3.4.2 The expression profile of cold shock-related genes in *A. butzleri* at 8°C

Even though several studies have described the prolonged survival or growth at lower temperatures for *A. butzleri*, the knowledge of the cellular processes involved in adaptation to cold temperature for this species is still scarce. In general, it is known that one of the most important cold shock response mechanisms of bacterial species is to sustain the translational processes, which are strongly impeded at low temperatures [29]. For several proteins involved in the cold stress response of *E. coli*, homologues genes could be determined in the genome of *A. butzleri*, including the genes encoding for RNA modifying proteins (*cspA*, *rnr*, *deaD* and *pnp*), translational regulators (*infB*, *rbfA*), chromosome modulators (*gyrA, recA*),

transcriptional regulators (*nusA, dnaA*), a protein chaperon (*tig*) and metabolic enzymes (*aceE*, *aceF*). The expression profiles of these putative cold-related genes were investigated by RT-qPCR after a temperature down shift to 8°C at aerobic condition for the *A. butzleri* isolates H2, C2 (representing different growth tendencies at cold temperatures) and the reference strain H1. Temporal expression patterns of the genes were analyzed and compared by K-means clustering. As shown in Fig. 3, the expression level of 10 out of the 13 investigated genes (77%) were up-regulated in response to the cold shock, and they were grouped into the high up-regulated cluster I (> 4.5 $\log_2$ fold change) or the moderate up-regulated cluster II (1.5 - 3 $\log_2$ fold change). For all three strains the expression pattern of *cspA*, *rnr* and *dnaA* were grouped in the high up-regulated cluster I (Fig. 3A) while *deaD*, *gyrA*, *infB*, *pnp* and *rbfA* were grouped in the moderate up-regulated cluster II (Fig. 3B). Cluster III comprises the genes *aceE*, *aceF* and *tig,* whose expression levels were non-regulated within the first 6 h after the cold shock in all three isolates (Fig. 3C). In contrast to the above mentioned similarities in the gene expression pattern, differences could be observed for the expression pattern of *recA* and *nusA* in the three investigated *A. butzleri* strains (Fig. 3D). For the strains H1 and H2, the expression pattern of both genes were grouped into the moderately up-regulated cluster II, while *recA* was included in the high up-regulated cluster I and *nusA* in the non-regulated cluster III for the strain C2 (Fig. 3D).

To the best of our knowledge no data describing the cold shock response of *Arcobacter* spp. have been published so far, therefore we compared our data with *E. coli,* for which the cold shock response has been intensively studied [19]. Recently, a comprehensive study by Zhang et al. [30] demonstrated a two-member mRNA surveillance system, composed of the exonuclease RNase R and the RNA chaperon CspA, enabling recovery of translation during cold-acclimation in *E. coli*. Enhanced mRNA level for *cspA* and *rnr* were also determined for all three *A. butzleri* isolates, suggesting utilization of a similar control mechanism. However, similar to *E. coli* the mRNA level reached their maximum at 4 h after the cold shock and then decreased in the strains H1 and H2, while it did not decline in the strain C2 until the end of the assay.

Furthermore, we determined a similar tendency of gene expression of the other 11 corresponding genes investigated in our study compared to *E. coli* [30]. Enhanced expression level for *deaD* and *pnp*, both encoding for components of the RNA degradosome [31], have also been determined for all three *A. butzleri* isolates. However, for *E. coli* higher expression level for *deaD* could be determined compared to the mRNA level of *deaD* in *A. butzleri*.

Similar to our results for *A. butzleri* also no changes in mRNA level of the genes *aceE*, *aceF* (both involved in the pyruvate metabolism) and *tig* (a protein chaperon, active at the later stage of adaptation to cold temperatures [32]) were reported for *E. coli* within the investigated 6 h after the cold shock [30].

The expression level of *dnaA*, the chromosomal replication initiator protein was increased in all three *A. butzleri* strains as well as in *E. coli*. However, for *A. butzleri* the expression pattern was grouped into the highly regulated cluster, while its expression pattern in *E. coli* is only moderately enhanced. However, the precise role of enhanced *dnaA* mRNA/ protein level has not been clarified in detail yet.

In *E. coli* the genes *nusA-infB-rbfA* are located in an operon and mRNA levels for all three genes increased after cold shock [33]. In *A. butzleri*, only *infB* and *rbfA* (Abu_2043 and Abu_2044) are located in direct neighborhood while *nusA* (Abu_0246) is located at a different region on the chromosome. Moderate enhanced expression levels of *rbfA* (encoding a protein, rendering the ribosomes to translate also non-cold-shock mRNAs under cold conditions [31]) and *infB* (encoding the translation initiation factor IF2) were determined for all three *A. butzleri* isolates, which is also in concordance to data reported for *E. coli* [30]. In contrast, differences in the *nusA* expression pattern between the three *A. butzleri* strains were determined. The protein NusA is a transcription factor, functionally involved in the regulation of pausing of the RNA-Polymerase during RNA chain elongation as well as in termination of transcription [34, 35]. Comparable to *E. coli*, the expression level of *nusA* was moderately increased in the strains H1 and H2 while it was not increased in the strain C2.

Further, the expression of *recA* is only moderately induced in the two *A. butzleri* strains H1 and H2 as well as in *E. coli* [30], but highly induced in the *A. butzleri* strain C2. RecA is the major DNA recombinase involved in homologues recombination and DNA repair [36]. Another protein involved in the chromosomal structure is the DNA gyrase alpha subunit (GyrA), which regulates the chromosomal DNA supercoiling upon cold shock and provides a level of DNA supercoiling and favors its efficient transcription [32]. The expression of *gyrA* is regulated by CspA [37] and consistently we determined moderately enhanced expression level for *gyrA* in all three *A. butzleri* isolates.

Taken together, we determined several similarities in the cold shock response of *A. butzleri* with *E. coli*. However, whether and how the differences in the expression levels of *nusA* and *recA* between the three *A. butzleri* might be responsible for the different growth behavior determined at low temperatures under aerobic conditions has to be elucidated in further studies.

3.5 Conclusion

Overall, our data indicate that most of the analysed *A. butzleri* isolates from human, mussel and chicken were not able to grow at 8°C under aerobic conditions. However, one *A. butzleri* isolate was able to grow to stationary phase until the end of the assay.

Furthermore, the presence of 13 homologues genes encoding cold-shock related proteins in *E. coli* were determined in the genome of *A. butzleri*, including *cspA*, *rnr*, *deaD*, *pnp*, *infB*, *rbfA*, *gyrA*, *recA*, *nusA*, *dnaA*, *tig*, *aceE* and *aceF*. The temporal transcriptional expression profiles of these genes in the early cold stress response of *A. butzleri* was reported for the first time and show high similarities compared to *E. coli*. These data provide a deeper insight into the good environmental adaptation capacities of *A. butzleri*.

3.6 Reference

1. Vandamme, P, Pugina, P, Benzi, G, Van Etterijck, R, Vlaes, L, Kersters, K, Butzler, J P, Lior, H & Lauwers, S (1992) Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. *J Clin Microbiol.* **30**: 2335-2337
2. Lerner, J, Brumberger, V & Preac-Mursic, V (1994) Severe diarrhea associated with *Arcobacter butzleri. Eur J Clin Microbiol Infect Dis.* **13**: 660-662
3. Jiang, ZD, Dupont, HL, Brown, EL, Nandy, RK, Ramamurthy, T, Sinha, A, Ghosh, S, Guin, S, Gurleen, K, Rodrigues, S, Chen, JJ, McKenzie, R & Steffen, R (2010) Microbial etiology of travelers' diarrhea in Mexico, Guatemala, and India: importance of enterotoxigenic *Bacteroides fragilis* and *Arcobacter* species. *J Clin Microbiol.* **48**: 1417-1419
4. Ferreira, S, Oleastro, M & Domingues, F (2019) Current insights on *Arcobacter butzleri* in food chain. *Curr Opin Food Sci.* **26**: 9-17
5. Kabeya, H, Maruyama, S, Morita, Y, Kubo, M, Yamamoto, K, Arai, S, Izumi, T, Kobayashi, Y, Katsube, Y & Mikami, T (2003) Distribution of *Arcobacter* species among livestock in Japan. *Vet Microbiol.* **93**: 153-158
6. Houf, K, De Zutter, L, Van Hoof, J & Vandamme, P (2002) Occurrence and distribution of *Arcobacter* species in poultry processing. *J Food Prot.* **65**: 1233-1239
7. Nieva-Echevarria, B, Martinez-Malaxetxebarria, I, Girbau, C, Alonso, R & Fernandez-Astorga, A (2013) Prevalence and genetic diversity of arcobacter in food products in the north of Spain. *J Food Prot.* **76**: 1447-1450
8. Gonzalez, A, Bayas Morejon, IF & Ferrus, MA (2017) Isolation, molecular identification and quinolone-susceptibility testing of *Arcobacter* spp. isolated from fresh vegetables in Spain. *Food Microbiol.* **65**: 279-283
9. Fera, MT, Maugeri, TL, Gugliandolo, C, Beninati, C, Giannone, M, La Camera, E & Carbone, M (2004) Detection of *Arcobacter* spp. in the coastal environment of the Mediterranean Sea. *Appl Environ Microbiol.* **70**: 1271-1276
10. Zhang, X, Alter, T & Golz, G (2019) Characterization of *Arcobacter* spp. isolated from retail seafood in Germany. *Food Microbiol.* **82**: 254-258

11. Kiehlbauch, JA, Brenner, DJ, Nicholson, MA, Baker, CN, Patton, CM, Steigerwalt, AG & Wachsmuth, IK (1991) *Campylobacter butzleri* sp. nov. isolated from humans and animals with diarrheal illness. *J Clin Microbiol.* **29**: 376-385

12. D'Sa, EM & Harrison, MA (2005) Effect of pH, NaCl content, and temperature on growth and survival of *Arcobacter* spp. *J Food Prot.* **68**: 18-25

13. Kjeldgaard, J, Jorgensen, K & Ingmer, H (2009) Growth and survival at chiller temperatures of *Arcobacter butzleri*. *Int J Food Microbiol.* **131**: 256-259

14. Van Driessche, E & Houf, K (2008) Survival capacity in water of *Arcobacter* species under different temperature conditions. *J Appl Microbiol.* **105**: 443-451

15. Miller, WG, Parker, CT, Rubenfield, M, Mendz, GL, Wosten, MM, Ussery, DW, Stolz, JF, Binnewies, TT, Hallin, PF, Wang, G, Malek, JA, Rogosin, A, Stanker, LH & Mandrell, RE (2007) The complete genome sequence and analysis of the epsilonproteobacterium *Arcobacter butzleri*. *PLoS One.* **2**: e1358, doi: 10.1371/journal.pone.0001358

16. Jones, P G, VanBogelen, RA & Neidhardt, FC (1987) Induction of proteins in response to low temperature in *Escherichia coli*. *J Bacteriol.* **169**: 2092-2095

17. Yamanaka, K (1999) Cold shock response in *Escherichia coli*. *J Mol Microbiol Biotechnol.* **1**: 193-202

18. Gualerzi, CO, Giuliodori, AM & Pon, CL (2003) Transcriptional and post-transcriptional control of cold-shock genes. *J Mol Biol.* **331**: 527-539

19. Phadtare, S (2004) Recent developments in bacterial cold-shock response. *Curr Issues Mol Biol.* **6**: 125-136

20. Barria, C, Malecki, M & Arraiano, CM (2013) Bacterial adaptation to cold. *Microbiology.* **159**: 2437-2443

21. Thieringer, HA, Jones, PG & Inouye, M (1998) Cold shock and adaptation. *Bioessays.* **20**: 49-57

22. Karadas, G, Sharbati, S, Hanel, I, Messelhausser, U, Glocker, E, Alter, T & Golz, G (2013) Presence of virulence genes, adhesion and invasion of *Arcobacter butzleri*. *J Appl Microbiol.* **115**: 583-590

23. Livak, KJ & Schmittgen, TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods.* **25**: 402-408

24. Han, YH, Liu, WZ, Shi, YZ, Lu, LQ, Xiao, SD & Zhang, QH (2009) Gene expression profile of *Helicobacter pylori* in response to growth temperature variation. *J Microbiol.* **47**: 455-465

25. Šilha, D, Morávková, K, Škodová, G & Vytřasová, J (2019) Viability and biofilm formation of *Arcobacter* spp. at various processing temperatures. *J Food Nutr Res.* **58**: 208-213

26. Giacometti, F, Serraino, A, Pasquali, F, De Cesare, A, Bonerba, E & Rosmini, R (2014) Behavior of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in ultrahigh-temperature,

pasteurized, and raw cow's milk under different temperature conditions. *Foodborne Pathog Dis.* **11**: 15-20

27. Giacometti, F, Losio, MN, Daminelli, P, Cosciani-Cunico, E, Dalzini, E & Serraino, A (2015) Short communication: *Arcobacter butzleri* and *Arcobacter cryaerophilus* survival and growth in artisanal and industrial ricotta cheese. *J Dairy Sci.* **98**: 6776-6781

28. Badilla-Ramirez, Y, Fallas-Padilla, KL, Fernandez-Jaramillo, H & Arias-Echandi, ML (2016) Survival capacity of *Arcobacter butzleri* inoculated in poultry meat at two different refrigeration temperatures. *Rev Inst Med Trop Sp.* **58**: 22, doi:10.1590/S1678-9946201658022

29. Lim, B & Gross, CA (2011) Cellular Response to Heat Shock and Cold Shock. *Bacterial Stress Responses, 2nd Edition*, 93-114

30. Zhang, Y, Burkhardt, DH, Rouskin, S, Li, GW, Weissman, JS & Gross, CA (2018) A Stress Response that Monitors and Regulates mRNA Structure Is Central to Cold Shock Adaptation. *Mol Cell.* **70**: 274-286

31. Phadtare, S & Severinov, K (2010) RNA remodeling and gene regulation by cold shock proteins. *RNA Biol.* **7**: 788-795

32. Golovlev, EL (2003) Bacterial cold shock response at the level of DNA transcription, translation, and chromosome dynamics. *Microbiology.* **72**: 1-7

33. Brandi, A, Giangrossi, M, Paoloni, S, Spurio, R, Giuliodori, AM, Pon, CL & Gualerzi, CO (2019) Transcriptional and post-transcriptional events trigger de novo *infB* expression in cold stressed *Escherichia coli*. *Nucleic Acids Res.* **47**: 4638-4651

34. Greenblatt, J & Li, J (1981) Interaction of the sigma factor and the *nusA* gene protein of *E. coli* with RNA polymerase in the initiation-termination cycle of transcription. *Cell.* **24**: 421-428

35. Strauss, M, Vitiello, C, Schweimer, K, Gottesman, M, Rosch, P & Knauer, SH (2016) Transcription is regulated by NusA:NusG interaction. *Nucleic Acids Res.* **44**: 5971-5982

36. Cox, MM (2007) Motoring along with the bacterial RecA protein. *Nat Rev Mol Cell Biol.* **8**: 127-138

37. Jones, PG, Krah, R, Tafuri, SR & Wolffe, AP (1992) DNA gyrase, CS7.4, and the cold shock response in *Escherichia coli*. *J Bacteriol.* **174**: 5798-5802

3.7 List of Tables and Figures

Table 3.1 List of primers and annealing temperatures used in the RT-qPCR assays

Primer ID	Sequence (5′ - 3′)	Target gene	Amplicon	Annealing	Strain
Abu_1472 f	AGC CTG AAT CAC TTG GAG CT	*aceE*	164	60°C	C2, H1, H2
Abu_1472 r	AAC TTC CCA TCC AGC TCC TC				
Abu_1473 f	GGA GTT GCT GTT GAT ACA CCA	*aceF*	200	60°C	C2, H1, H2
Abu_1473 r	GGC GTG AAG TAT GTT CCA CC				
Abu_0042 f	TGG ACA AGC GCA TAC AGG TA	*deaD*	195	60°C	C2, H1
Abu_0042 r	GCT TGT CCA CCG TAA ACA GT				
deaD 1-F	ACAGCAGCTTTTGGACTTCC	*deaD*	167	55°C	H2
deaD 1-R	GCTTGACCACCGTAAACAGT				
Abu_0001 f	CGT TGT GGG ACC ATC AAA CC	*dnaA*	108	60°C	C2, H1, H2
Abu_0001 r	CCA AGT CCC GTT CCA CCA TA				
Abu_2043 f	TGA TAC TCC AGG TCA CGC AG	*infB*	91	60°C	C2, H1
Abu_2043 r	GTC ATC AGC AGC AAC AAC GA				
infB 1-F	AGGATTTAATGTACGACCAA	*infB*	129	52°C	H2
infB 1-R	CTCATCATCCCTGAAAGTG				
Abu_1183 f	TGC TGG ACC TAA AGA TGG ACA	*pnp*	108	60°C	C2, H1, H2
Abu_1183 r	GCT TCA CCA ACG CTA AAT CCT				
Abu_2241 f	TAA ATG CAC AAA CGC CAC CA	*recA*	131	60°C	C2, H1, H2
Abu_2241 r	AGC TTT AGG TGT TGG AGG ACT				
Abu_1702 f	ACC GTC AAA TGC AAC ACC AT	*tig*	112	60°C	C2, H1, H2
Abu_1702 r	TGC TCA ATC ATC TGC ACC AT				
Abu_0246 f	ACC TTT TAT TTC GCC AAT TTC GA	*nusA*	176	55°C	C2, H2
Abu_0246 r	TGG GAA GAA ATG CTG CAA CA				
nusA 1-F	TGG AGA TGT TGT AAA AGC TGT TG	*nusA*	240	55°C	H1
nus-A 1-R	ACT CCA ACA ACT GCA CCA AT				
rbfA 1-R	CGC AAT AGT GTA TTT TGA TGG T	*rbfA*	127	55°C	C2, H1, H2

rbfA 1-F	ACA TTT ATA CCA ACT TGT ACT TGC				
cspA 1-R	TGG CAA ATC AAA ATA TCG GAA CA	*cspA*	197	55°C	C2, H1, H2
cspA 1-F	TGC TTG AGG ACC TTT ATC ATT				
rnr 1-F	AGG GCA TTT TGG TTT AGG ATT CT	*rnr*	205	55°C	C2, H1, H2
rnr 1-R	AGC CCA TCT TGC GTA TTT TCT				
gyrA 1-F	TGG ACG TGC ATT ACC TGA TG	*gyrA*	192	55°C	C2, H1
gyrA 1-R	TGT GCC ATT CTT ACA AGT GCA				
gyrA 2-F	TTGGACGTGCGTTACCTGAT	*gyrA*	193	55°C	H2
gyrA 2-R	TGTGCCATTCTTACAAGTGCA				
AB_rpoA_exF	TAGCCCACCCTTTGAGAAGA	*rpoA*	50	60°C, 55°C	C2, H1, H2
AB_rpoA_exR	CGCACAACCAACTGATGAAC			or 52°C *	

* depending on the corresponding protocol used

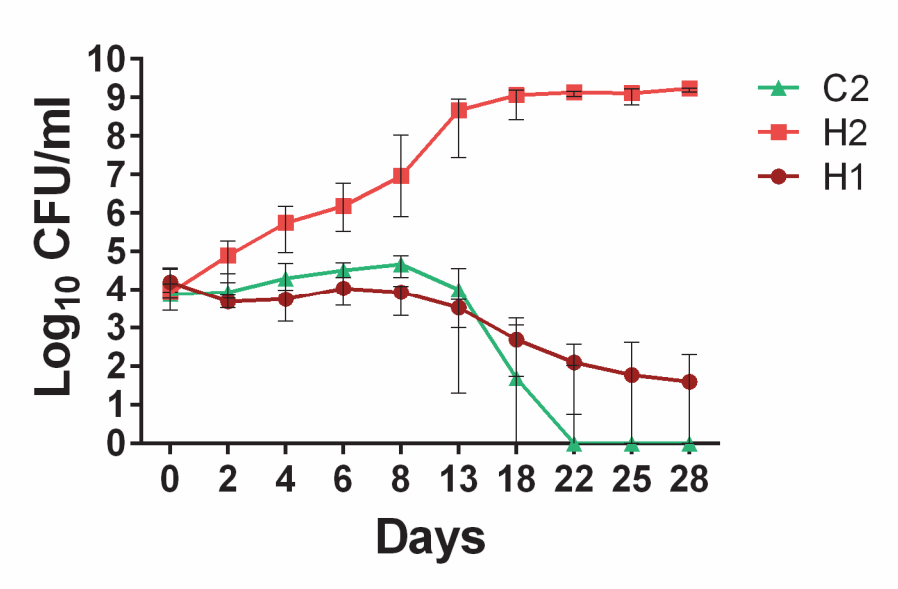

Figure 3.1: Growth curve of three *Arcobacter butzleri* isolates at 8 °C, shown as median ± interquartile range (n=4).

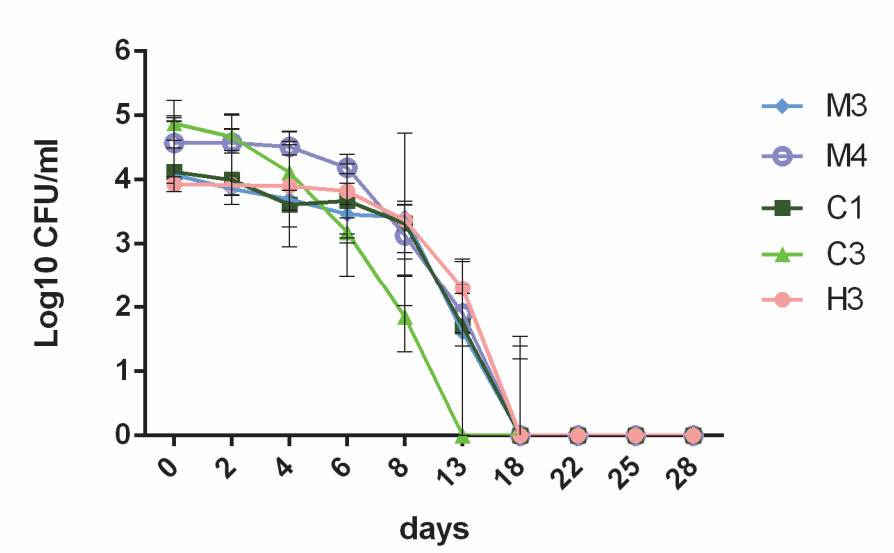

Figure 3.2: Growth curve of five *Arcobacter butzleri* isolates without growing capability at 8 °C, shown as median ± interquartile range (n=3).

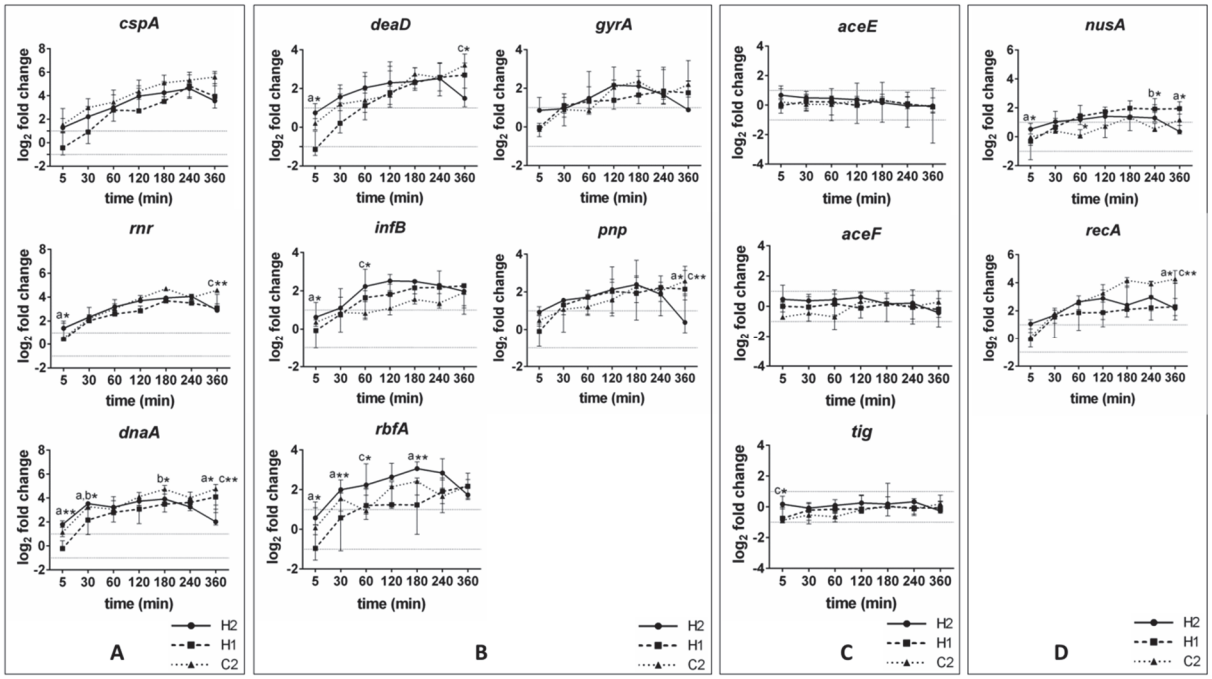

Figure 3.3: Temporal expression pattern of 13 cold-related genes in three *A. butzleri* isolates after temperature downshift to 8°C.

The expression level was analyzed by RT-qPCR and shown as median ± interquartile range of $\log_2$ fold changes (n=3). The dotted lines show the threshold (-1 to 1) for the relevant up and down regulation. Similar expression pattern were analyzed by K-means clustering. Cluster I include the genes with high up-regulated expression (A), cluster II with moderate up-regulated expression (B) and cluster III with non-regulated expression (C) in all three *A. butzleri* strains. (D) Genes belonging to different clusters in strain H1, H2 and C2.

Chapter 4 General Discussion

The genus *Arcobacter* is a common inhabitant of diverse habitats especially in water environment and seafood (Collado and Figueras 2011, Ferreira, Queiroz et al. 2016). Previous data showed that *Arcobacter* were vastly distributed in seafood like shellfish. Consuming these foods raw or undercooked might cause high risks. After sequencing the genome of *A. butzleri* RM4018, the presence of different putative virulence factors was described (Miller, Parker et al. 2007). Therefore, to evaluate the potential public health risk of *Arcobacter,* prevalence study in food is needed. In this doctoral thesis, the occurrence of *Arcobacter* in retail seafood in Berlin, Germany was reported in Chapter II. Moreover, the intra- and interspecies genetic heterogeneity was evaluated by ERIC-PCR. In addition, all isolates were tested for the presence of 10 putative virulence genes by PCR. In conclusion, this study figured out that: first, the high occurrence of virulence factors revealed in the species *A. butzleri*. Second, the highest detection from all seafood types indicates *A. butzleri* seemed to be the most prevalent species.

As the genome sequence of *A. butzleri* demonstrated the presence of several cold shock and stress-response genes, a higher ability of *A. butzleri* to survive at cold temperatures compared with other related organisms such as *Campylobacter* was presumed (Ferreira, Queiroz et al. 2016). However, studies concerning on this aspect are still scarce. For this purpose, the growth of eight *A. butzleri* strains from different food origins was tested at 8°C, reporting three different growth phenotypes at this temperature (Chapter III). Further, in the reference genome of *A. butzleri*, 13 homologs of cold shock-related genes of *E. coli* were determined. In addition, the transcriptional expression of these genes was investigated for 6h after cold shock in isolates with different growth phenotypes.

4.1 Prevalence of *Arcobacter* in seafood

The overall prevalence of *Arcobacter* spp. in seafood in Germany was 17.6% (56/318): 17.0% for bivalves, 27.4% for cephalopods and 8.5% for shrimps. The prevalence of *Arcobacter* spp. in bivalves, which included mussels and clams, is in accordance with previous studies in India and Sicilia region, Italy with 14.7% and 16.7% prevalence, respectively (Laishram, Rathlavath et al. 2016, Noto, Sciortino et al. 2018). However lower than in other studies with a prevalence ranging from 22.8% to 68% in Spain, Chile, Italy and India (Mottola, Bonerba et al. 2016b, Leoni, Chierichetti et al. 2017, Morejon, Gonzalez et al. 2017, Rathlavath, Kumar et al. 2017). The difference in the occurrence of *Arcobacter* spp. in these studies compared to the prevalence in our study could be explained as followed. First, bivalve samples were collected

either by harvesting directly or by local fish markets. Second, geographic location differences and third, more importantly, that in our study no samples were collected in the summer season due to the unavailability of bivalves in retail markets in Germany. Levican et al. determined a significant higher prevalence of *Arcobacter* spp. during summer than in other seasons ($P < 0.05$), associated with an increase in water temperature (23°C to 27°C), while no significant correlation between the salinity with the occurrence of *Arcobacter* was found (Levican, Collado et al. 2014).

The prevalence of *Arcobacter* in shrimp in our study was 8.5% (9/106). Few studies investigate the prevalence of *Arcobacter* in this food matrix despite shrimp being a popular food in many countries. Collado et al. investigated different types of food including 17 frozen shrimp samples and no arcobacters were found (Collado, Guarro et al. 2009). Similar to their samples, the shrimp samples in our study were purchased in retail markets and mostly were purchased frozen. However, *Arcobacter* could be isolated from frozen shrimp samples in our study, indicating that they are able to survive in frozen seafood samples. The possible reason for this difference in prevalence (0 vs. 8.5%) might be the low sample number (n=17) or different geographical sample origin. Our finding suggested that frozen shrimp might also be a reservoir of arcobacters.

Our study also detected arcobacters in cephalopods (squids and octopuses) with a prevalence of 27.4% (29/106) for the first time. Unfortunately, data on origin and packing/transportation condition of cephalopods in retail markets were not available, thus the temperature applied from fishing vessels to transport were not clear.

4.2 Genotyping by ERIC-PCR

The ERIC-PCR was applied in this study for genotyping all identified *Arcobacter* isolates and high inter- and intraspecies heterogeneity were observed. It is not surprising to see that isolates belong to the same species of *Arcobacter* were clustered with less similarity (min. 45.9%), as these seafood products were imported from different locations and origins all over the world. This high heterogeneity was in accordance with ERIC-PCR results of isolates from variant origins including pigs, pork, poultry, bovine products, seafood, water and stool samples of patients (Van Driessche, Houf et al. 2004, Aydin, Gumussoy et al. 2007, Van Driessche and Houf 2007, Collado, Kasimir et al. 2010, De Smet, De Zutter et al. 2010, Kayman, Abay et al. 2012, Rathlavath, Kumar et al. 2017). ERIC-PCR could be used as a preliminary screening for prevalence study. In addition, new generation of molecular genotyping methods based on DNA sequences have been developed as introduced in

Chapter 1 (1.3). The most promising candidate for *Arcobacter* genotyping known as MLST (Chieffi, Fanelli et al. 2020), which could avoid the bias by subjective interpretation of DNA bands. Further efforts should be drawn in continuously enlarging the *Arcobacter* MLST Databases as now the number of sequences included in the database is 2,661 with 831 MLST profiles (PubMLST accessed on July 20, 2020, https://pubmlst.org/ arcobacter/).

4.3 Presence of putative virulence genes

Although *Arcobacter* were isolated from clinical samples (with *A. butzleri* as dominant species), the pathogenicity of *Arcobacter* is still not understood (Ferreira, Queiroz et al. 2016, Ramees, Dhama et al. 2017). Several homologues to the virulence genes in *Campylobacter* and other bacteria have been detected in *A. butzleri*. The occurrence of the ten putative virulence genes was investigated by PCR according to Whiteduck-Leveillee and Karadas (Karadas, Sharbati et al. 2013, Whiteduck-Leveillee, Cloutier et al. 2016). In all of our 62 *Arcobacter* isolates, *ciaB* which encodes the host cell invasion protein in *Campylobacter* spp. was detected, followed by five other most prevalent genes: *mviN* (76%), *cj1349* (69%), *pldA* (55%), *tlyA* (55%) and *cadF* (55%). The highest prevalence of these six genes was in accordance with other studies (Douidah, de Zutter et al. 2012, Tabatabaei, Shirzad Aski et al. 2014, Lehmann, Alter et al. 2015, Zacharow, Bystron et al. 2015, Whiteduck-Leveillee, Cloutier et al. 2016).The gene *hecB* (18%) and *irgA* (2%) was only detected in *A. butzleri,* while *iroE* (18%) was detected in both, *A. butzleri* and *A. cryaerophilus*. The *hecA* (10%) was detected in five *A. butzleri* and the one *A. thereius* isolate. A very recent study by Isidro et al. (Isidro, Ferreira et al. 2020) showed that *hecA* was extremely polymorphic in sequence and size and PCR yielded false negative results by commonly used primers. Some authors tried to deduce any connection between the virulence genes and the adherence/invasion phenotypes of *Arcobacter* spp., especially for the three human-related species *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*. Levican et al. investigated 60 *Arcobacter* strains from various sources including 16 species to adhere and invade the human intestinal cell line Caco-2. They found that all invasive strains were positive for *ciaB* and strains absent of any virulence genes showed little or no invasion of Caco-2 cells (Levican, Alkeskas et al. 2013). Meanwhile Karadas et al. found no functional correlation between the sequence of *ciaB*, *cadF* or cj1349 and the adhesive/invasive phenotype in 6 *A. butzleri* isolates. (Karadas, Sharbati et al. 2013) Recently, Fanelli et al. (Fanelli, Di Pinto et al. 2019, Fanelli, Chieffi et al. 2020) identified additional virulence associated genes in five isolates from shellfish and vegetables through genomic analysis, such as *virF* (one of the AraC family of transcriptional regulator), *pho*Q (encoding a virulence transcriptional regulatory protein) and a VOC family virulence protein

which was shared by all isolates. With higher numbers of *A. butzleri* and other *Arcobacter* spp. genome sequences characterized, the list of putative virulence genes were enlarging and the function of these genes need to be further clarified.

To sum up, our results from bivalves, shrimps and cephalopods demonstrated an overall *Arcobacter* prevalence of 17.6% in the retail seafood in Berlin, Germany. High prevalence (27.4%) was observed in cephalopods (squids and octopuses) for the first time. It is also worth noting that frozen shrimps could also be a reservoir of *Arcobacter* spp., though only 8.5% of the samples were detected positive. In all seafood sampled in this study, only the mussels were sold in enclosed package. Other seafood in retail markets in Germany are sometimes sold without commercial packaging, especially for squids and octopuses which are only sold by weight. Thus, transmission of *Arcobacter* by consuming undercooked seafood or by cross-contamination during storage and handling could happen consequently.

4.4 Growth and survival of *A. butzleri* at cold

A. butzleri could be isolated from livestock animals and also from environments with lower temperatures. Nevertheless, the cold adaptation and survival mechanisms of *A. butzleri* at chilling temperature needs further investigation. For this purpose, we investigate the survival and growth capacity of eight *A. butzleri* strains isolates from human, mussel and chicken at 8°C under aerobic condition to mimic natural environments for food storage at chilling temperatures. Our results showed that most of the isolates (6/8) could only survive at 8°C until day 8 and gradually lose their viability until non-recoverable on MHB agar. The reference strain *A. butzleri* CCUG 30485 (H1) showed a more robust resistance to cold as it declined from 4 $\log_{10}$ CFU ml^{-1} to 2 $\log_{10}$ CFU ml^{-1} at day 28 (the end of the growth assay).The isolate H2 from human sample showed growing capability at 8°C under aerobic conditions and this is the lowest growing limit described for *A. butzleri* as far as we know. No correlation between the growth and survival behavior and the origin of *A. butzleri* isolates was observed, and the growth of *A. butzleri* at 8°C under aerobic circumstance seemed to be strain-dependent. Several studies already reported the survival of *A. butzleri* in different broth or water at cold temperatures. Hilton et al. (Hilton, Mackey et al. 2001) reported a temperature range for growth of *A. butzleri* NCTC 12481 (H1 in our study) of between 15 to 39°C. This isolate showed only gradual decrease in viability at 4°C, but significant decrease from by 2 log level at -20°C after 24h. However, D'Sa (D'Sa and Harrison 2005) noted the growth of some *A. butzleri* isolates in Ellinghausen McCullough Johnson Harris medium at 10°C.

Van Driessche and Houf (Van Driessche and Houf 2008) assessed the survival capacity of arcobacters in water with and without organic material and found *A. butzleri* showed significantly higher surviving capacity than *A. cryaerophilus* and *A. skirrowii*. With 1% organic material, *A. butzleri* could survive up to 203d at both 4°C and 7°C. Kjeldgaard et al. (Kjeldgaard, Jorgensen et al. 2009) examined the growth behavior of the reference *A. butzleri* strain in both CMJ and BHI broth at 5°C, 10°C and 15°C. The lowest growing limit was observed at 10°C in both CMJ and BHI liquid media without lag in CMJ but with ≥ 1 day in BHI broth. They also found better survival of this strain in BHI at 5°C compared with the same strain reported by Hilton et al. (Hilton, Mackey et al. 2001) at 4°C. Their result indicated that even the same isolate could behave differently depending on the broth applied or the labs where the tests were performed. This is also in accordance with a recent published report by Silha et al. (Silha, Morávková et al. 2019) that the same reference strain could survive for 14d at 5°C. Actually, the strain H1 showed only viability in majority of the replicates in our study. However, in two of eight replicates we similarly observed growing capabilities of this strain at 8°C. Thus, for the reference strain *A. butzleri* NCTC 12481 we hypothesize the lowest growing limit might be between 8°C to 10°C denpending on the liquid broth applied.

4.5 Transcriptional regulation of cold-related genes in *A. butzleri*

Although the growth and viability of *A. butzleri* at cold temperatures has been already reported, the knowledge of the cellular processes involved in cold shock adaptation or response and the underlying mechanisms on for example, transcriptional level is still scarce. From the growing/surviving phenotype of some *A. butzleri* strains at chilling temperatures, the presence of cold-related genes was assessed. Since the cold shock proteins was first described and intensively studied in *E. coli* (Jones, VanBogelen et al. 1987, Jones and Inouye 1994), we blasted these cold-shock related genes of *E. coli* against the genome of *A. butzleri* RM4018 and determined 13 homologue genes. These genes encoding proteins involved in many cellular functions, such as RNA modification (*cspA*, *rnr*, *deaD* and *pnp*), transcriptional and translational regulation (*nusA*, *dnaA*, *infB* and *rbfA*), chromosome modulation (*gyrA* and *recA*), a protein chaperon (*tig*) and metabolic enzymes (*aceE* and *aceF*) (Gualerzi, Giuliodori et al. 2003, Phadtare and Severinov 2004, Barria, Malecki et al. 2013). A 6h-temporal transcriptional expression pattern of these genes in three *A. butzleri* strains with different growth mode was investigated after a temperature downshift to 8°C by RT-qPCR. The transcriptional profiles of 13 genes in each strain were afterwards analyzed and compared by K-means clustering and grouped into high up-regulated cluster I (> 4.5 $\log_2$ fold change), moderate up-regulated cluster II (1.5 – 3 $\log_2$ fold change) and non-regulated cluster III. For

all three strains, *cspA*, *rnr* and *dnaA* were grouped in the high up-regulated cluster I, *deaD*, *gyrA*, *infB*, *pnp* and *rbfA* were in the moderate up-regulated cluster II and the genes *aceE*, *aceF* and *tig* were grouped in the non-regulated cluster III. This is the first report of the transcription expression profile of 13 cold-related genes in *A. butzleri* so far. The transcriptional profiles of the genes *recA* and *nusA* differed between the strain C2 (less cold-resistant) and the other two isolates. For strain C2, they were grouped in cluster I and cluster III, respectively, while grouped in cluster II for the other two strains. However, none of the other genes showed significant differences among three strains both by clustering and expression level. The gene product of *recA* is involved in general recombination and DNA repairing (Merrin, Kumar et al. 2011) while NusA is a transcription factor, involved in the anti-terminator function during cold (Bae, Xia et al. 2000). Since the actual function of these gene homologues has not yet been clarified in *A. butzleri*, we could not build a certain correlation between the growing phenotype with the difference in clustering. A recent study about cold shock response of *E. coli* by Zhang et al. demonstrated the two-member mRNA surveillance system composed by the exonuclease RNase R and the RNA chaperon CspA, facilitating translation recovery after cold shock in *E. col* (Zhang, Burkhardt et al. 2018). In this study, a similar temporal transcription expression profile of these genes was provided. The non-regulated group in *E. coli* includes the gene *aceE*, *aceF* and *tig*, which is the same as in *A. butzleri*. Highest induction of expression was observed for *rnr*, with around 45 fold up-regulation followed by *cspA* (30 fold), *deaD* (24 fold), *dnaA* (13 fold), *recA* (8 fold), *nusA* (7 fold), *rbfA* (6 fold), *infB* (5.5 fold), *pnp* (4.5 fold) and *gyrA* (3 fold). By comparing our data with that in *E. coli*, there are some differences in the expression level between the two genera. But it is interesting to see similar tendencies of the transcriptional expression such as the enhanced mRNA level of *cspA* and *rnr*. This similarity in transcriptional expression of these two most important cold-related genes might suggest a similar function as demonstrated in *E. coli* (Zhang, Burkhardt et al. 2018). But apparently further investigation in *A. butzleri* for elucidating this hypothesis is needed.

To conclude, our work on the growth and survival of *A. butzleri* isolated from human, mussel and chicken at cold temperatures contributes to the knowledge of cold adaptation of this species. The growth temperature limit of *A. butzleri* was determined as 8°C in aerobic condition for one isolate while most other isolates could not grow at such temperatures. The growth behavior of *A. butzleri* seemed to be strain-dependent. Combined with the presence and transcriptional expression profile of 13 gene homologues to the cold-related genes in *E. coli*, our data provide more information about the good environmental adaptation of *A. butzleri*.

Chapter 5 Summary

Arcobacter (A.) spp. was regarded as an emerging pathogen associated with enteritis, diarrhea and occasionally bacteremia in human. It has been widely isolated from animals, foods and water environment. In the recent ten years, more than half of the newly described species of this genus were recovered from aquatic environments and water-borne animals. Therefore, the occurrence of *Arcobacter* in seafood has raised increasing interest for food safety concern. Consuming undercooked or preparing raw seafood was regarded as a potential risk of human transmission. Despite the frequent reports of this genus from food, the knowledge of its pathogenicity and the cold stress response is still scarce.

This work aimed to evaluate the prevalence of *Arcobacter* spp. in retail seafood in Berlin, and to characterize all isolates by the genetic diversity and the distribution of 10 putative virulence genes. In addition, the growth of *A. butzleri* isolates from different origins was tested at chilling temperature. The presence and transcriptional expression profile of 13 cold-related genes homologues in *E. coli* were preliminarily reported in *A. butzleri*.

Chapter 1 introduces a comprehensive literature review on the taxonomy, occurrence, current isolation and typing methods of *Arcobacter* spp. The information about virulence determinants, cold adaptation of *A. butzleri* as well as cold-related genes in *E. coli* is also provided. In the first study (**Chapter 2**), the overall prevalence of *Arcobacter* in retail seafood was 17.6%. For each food matrix, cephalopod was found to have the highest prevalence (27.4%) and species diversity (6 species) followed by bivalves (17% and 5 species) and shrimp (8.5% and 4 species). High genetic heterogeneity was observed both at inter- and intra-species level. *A. butzleri* was found to carry most putative virulence genes and the gene *ciaB* was 100% detected in all *Arcobacter* isolates. No correlation between the ERIC-PCR pattern/virulence gene distribution and the source of the isolates can be deduced. The cold adaptation of *A. butzleri* was further investigated in **Chapter 3**. The growth of eight *A. butzleri* isolates from human, mussel and chicken was tested at 8°C under aerobic condition to mimic natural environment for food storage at chilling temperatures. One *A. butzleri* strain (H2) showed growing capability at this temperature while most of others (6/8) were not culturable after 28 days under these culture conditions. No correlation between the growth and / or survival behavior and the source of *A. butzleri* isolates was observed, and the growth of *A. butzleri* at 8°C under aerobic circumstance in our study seemed to be strain-dependent. The transcriptional expression pattern of 13 cold-related genes in *E. coli* was also investigated in three *A. butzleri* strains with different growth mode after a temperature downshift to 8°C by RT-qPCR. Several similarities in the cold-related genes expression pattern of *A. butzleri* with

E. coli were determined. For elucidating whether similar functions of these genes in *A. butzleri* at cold were utilized, further investigation was needed.

To summarize, our research provide more information to estimate whether consumption/handling of seafood have a potential risk for human health and help to better understanding the survival strategy of *A. butzleri* encountering to cold stress.

Chapter 6 Zusammenfassung

Prävalenz und Kälteschock-Antwort von *Arcobacter* spp.

Arcobacter (A.) spp. gilt als ein neu auftretender Erreger, der mit Enteritis, Durchfall und gelegentlich Bakteriämie beim Menschen in Verbindung gebracht wird. *Arcobacter* wurde häufig aus Tieren, Lebensmitteln und Gewässern isoliert. In den letzten zehn Jahren wurde mehr als die Hälfte der neu beschriebenen Arten dieser Gattung aus der aquatischen Umwelt und von im Wasser lebenden Tieren isoliert. Daher hat das Vorkommen von *Arcobacter* in Meeresfrüchten ein wachsendes Interesse in Fragen der Lebensmittelsicherheit geweckt. Der Verzehr von zu wenig gekochten oder roh zubereiteten Meeresfrüchten wurde als potentielles Risiko einer Übertragung auf den Menschen angesehen. Trotz der häufigen Berichte über den Nachweis von *Arcobacter* in Lebensmitteln, sind die Kenntnisse über ihre pathogenes Potential und die Kältestress-Reaktion noch nicht ausreichend geklärt.

Ziel dieser Arbeit war es, die Prävalenz von *Arcobacter* spp. in Meeresfrüchten aus dem Berliner Einzelhandel zu ermitteln und alle Isolate anhand des ERIC-PCR-Musters und des Vorkommens von 10 mutmaßlichen Virulenzgenen zu charakterisieren. Darüber hinaus wurde das Wachstum von *A. butzleri*-Isolaten unterschiedlicher Herkunft bei Kühltemperatur untersucht. Das Vorhandensein von 13 kälteassoziierten Genen, die Homologien zu *E. coli* Genen aufweisen und deren transkriptionelles Expressionsprofil nach einem Kälteschock, wurden erstmal für *A. butzleri* beschrieben.

Kapitel 1 führt in eine umfassende Literaturübersicht zur Taxonomie, zum Vorkommen sowie zu aktuellen Nachweis- und Typisierungsmethoden von *Arcobacter* spp. ein. Weiterhin werden Informationen zu putativen Virulenzfaktoren von *A. butzleri*, der Kälteadaptation von *A. butzleri* sowie zu Kältestress assoziierten Genen in *E. coli* gegeben. In der ersten Studie (**Kapitel 2**) konnte gezeigt werden, dass die Gesamtprävalenz von *Arcobacter* spp. in Meeresfrüchten aus dem Einzelhandel bei 17,6% lag. Die höchste *Arcobacter*-Prävalenz (27,4%) und Speziesdiversiät (6 Spezies) wurde in Cephalopoden, gefolgt von Muscheln (17% und 5 Arten) und Garnelen (8,5% und 4 Arten) nachgewiesen. Es wurde eine hohe genetische Heterogenität sowohl zwischen als auch innerhalb der Spezies beobachtet. , Die zehn putativen Virulenzgene konnten deutlich häufiger in *A. butzleri* isolaten detektiert werden als in den anderen *Arcobacter* Spezies. Nur das Gen *ciaB* wurde in allen *Arcobacter*-Isolaten, unabhängig von der Spezies, zu 100% nachgewiesen. Es lässt sich keine Korrelation

zwischen dem ERIC-PCR-Muster/Virulenzgenverteilung und der Quelle der Isolate ableiten. Die Kälteadaptation von *A. butzleri* wird in **Kapitel 3** dargestellt. Das Wachstum von acht *A. butzleri*-Isolaten aus Mensch, Muschel und Huhn wurde bei 8°C unter aeroben Bedingungen getestet, um die natürliche Umgebung während der Lagerung von Lebensmitteln bei Kühltemperaturen zu imitieren. Einer dieser *A. butzleri* Stämme (H2) zeigte bei dieser Temperatur noch Wachstumsfähigkeiten, während die meisten anderen (6/8) nach 28 Tagen nicht mehr kulturell nachweisbar waren. Es wurde keine Korrelation zwischen dem Wachstums- bzw. Überlebensverhalten und der Quelle von *A. butzleri*-Isolaten beobachtet, und das Wachstum von *A. butzleri* bei 8°C unter aeroben Bedingungen schien in unserer Studie stammabhängig zu sein. Das Vorhandensein und das transkriptionelle Expressionsmuster von 13 kälteverwandten Genen in *E. coli* wurde auch bei drei *A. butzleri* mit unterschiedlichem Wachstumsmodus (C2, H2 und H1) nach einer Temperaturabsenkung auf 8°C mittels RT-qPCR untersucht. Es wurden mehrere Übereinstimmungen in der Expression der kälteassoziierten Gene zwischen *A. butzleri* und *E. coli* festgestellt. Zur Klärung der Frage, obdieser Gene in *A. butzleri* ähnliche Funktionen in der Kältestressantwort haben, wären weitere Untersuchungen erforderlich.

Zusammenfassend lässt sich sagen, dass diese Studien weitere Informationen liefern, um das potentielle Risiko durch den Verzehr bzw. die Handhabung von Meeresfrüchte für die menschliche Gesundheit abzuschätzen und zu einem besseren Verständnis der Überlebensstrategie von *A. butzleri* bei Kältestress beitragen.

Reference

Alispahic, M., K. Hummel, D. Jandreski-Cvetkovic, K. Nobauer, E. Razzazi-Fazeli, M. Hess and C. Hess (2010). "Species-specific identification and differentiation of *Arcobacter*, *Helicobacter* and *Campylobacter* by full-spectral matrix-associated laser desorption/ionization time of flight mass spectrometry analysis." J Med Microbiol **59**(Pt 3): 295-301.

Alonso, R., C. Girbau, I. Martinez-Malaxetxebarria and A. Fernandez-Astorga (2014). "Multilocus sequence typing reveals genetic diversity of foodborne *Arcobacter butzleri* isolates in the North of Spain." Int J Food Microbiol **191**: 125-128.

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

Atabay, H. I., F. Aydin, K. Houf, M. Sahin and P. Vandamme (2003). "The prevalence of *Arcobacter* spp. on chicken carcasses sold in retail markets in Turkey, and identification of the isolates using SDS-PAGE." Int J Food Microbiol **81**(1): 21-28.

Atabay, H. I. and J. E. Corry (1997). "The prevalence of campylobacters and arcobacters in broiler chickens." J Appl Microbiol **83**(5): 619-626.

Atabay, H. I., A. Unver, M. Sahin, S. Otlu, M. Elmali and H. Yaman (2008). "Isolation of various *Arcobacter* species from domestic geese (Anser anser)." Vet Microbiol **128**(3-4): 400-405.

Atabay, H. I., M. Waino and M. Madsen (2006). "Detection and diversity of various *Arcobacter* species in Danish poultry." Int J Food Microbiol **109**(1-2): 139-145.

Aydin, F., K. S. Gumussoy, H. I. Atabay, T. Ica and S. Abay (2007). "Prevalence and distribution of *Arcobacter* species in various sources in Turkey and molecular analysis of isolated strains by ERIC-PCR." J Appl Microbiol **103**(1): 27-35.

Badilla-Ramirez, Y., K. L. Fallas-Padilla, H. Fernandez-Jaramillo and M. L. Arias-Echandi (2016). "SURVIVAL CAPACITY OF *Arcobacter butzleri* INOCULATED IN POULTRY MEAT AT TWO DIFFERENT REFRIGERATION TEMPERATURES." Rev Inst Med Trop Sao Paulo **58**:22

Bae, W., B. Xia, M. Inouye and K. Severinov (2000). "*Escherichia coli* CspA-family RNA chaperones are transcription antiterminators." Proc Natl Acad Sci USA **97**(14): 7784-7789.

Barria, C., M. Malecki and C. M. Arraiano (2013). "Bacterial adaptation to cold." Microbiol **159**(Pt 12): 2437-2443.

Bath, G. F., R. Leask, K. P. Pettey and D. J. Coetzee (2013). "Abortions in sheep associated with *Arcobacter skirrowii* infection." J S Afr Vet Assoc **84**(1).

Bodhidatta, L., A. Srijan, O. Serichantalergs, A. Bangtrakulnonth, B. Wongstitwilairung, P. McDaniel and C. J. Mason (2013). "Bacterial pathogens isolated from raw meat and poultry

compared with pathogens isolated from children in the same area of rural Thailand." SE Asian J Trop Med **44**(2): 259-272.

Bogantes, E. V., K. L. Fallas-Padilla, C. E. Rodriguez-Rodriguez, H. F. Jaramillo and M. L. Echandi (2015). "Zoonotic species of the genus *Arcobacter* in poultry from different regions of Costa Rica." J Food Prot **78**(4): 808-811.

Brightwell, G., E. Mowat, R. Clemens, J. Boerema, D. J. Pulford and S. L. On (2007). "Development of a multiplex and real time PCR assay for the specific detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus*." J Microbiol Methods **68**(2): 318-325.

Bruckner, V., U. Fiebiger, R. Ignatius, J. Friesen, M. Eisenblatter, M. Hock, T. Alter, S. Bereswill, G. Golz and M. M. Heimesaat (2020). "Prevalence and antimicrobial susceptibility of *Arcobacter* species in human stool samples derived from out- and inpatients: the prospective German *Arcobacter* prevalence study Arcopath." Gut Pathog **12**. 10.1186/s13099-020-00360-x.

Carbonnelle, E., C. Mesquita, E. Bille, N. Day, B. Dauphin, J. L. Beretti, A. Ferroni, L. Gutmann and X. Nassif (2011). "MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory." Clin Biochem **44**(1): 104-109.

Caruso, M., L. Latorre, G. Santagada, R. Fraccalvieri, L. M. Difato, A. Miccolupo, L. Capozzi, E. Bonerba, A. Mottola and A. Parisi (2018). "*Arcobacter* spp. in bovine milk: An emerging pathogen with potential zoonotic risk." Ital J Food Saf **7**(4): 7685.

Celik, E. and A. Unver (2015). "Isolation and Identification of *Arcobacter* spp. by Multiplex PCR from Water Sources in Kars Region." Curr Microbiol **71**(5): 546-550.

Chieffi, D., F. Fanelli and V. Fusco (2020). "*Arcobacter butzleri*: Up-to-date taxonomy, ecology, and pathogenicity of an emerging pathogen." Compr Rev Food Sci Food Saf **19**(4): 2071-2109.

Collado, L., I. Cleenwerck, S. Van Trappen, P. De Vos and M. J. Figueras (2009). "*Arcobacter mytili* sp nov., an indoxyl acetate-hydrolysis-negative bacterium isolated from mussels." Int J Syst Evol Microbiol **59**: 1391-1396.

Collado, L. and M. J. Figueras (2011). "Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*." Clin Microbiol Rev **24**(1): 174-192.

Collado, L., J. Guarro and M. J. Figueras (2009). "Prevalence of *Arcobacter* in meat and shellfish." J Food Prot **72**(5): 1102-1106.

Collado, L., I. Inza, J. Guarro and M. J. Figueras (2008). "Presence of *Arcobacter* spp. in environmental waters correlates with high levels of fecal pollution." Environ Microbiol **10**(6): 1635-1640.

Collado, L., G. Kasimir, U. Perez, A. Bosch, R. Pinto, G. Saucedo, J. M. Huguet and M. J. Figueras (2010). "Occurrence and diversity of *Arcobacter* spp. along the Llobregat River

catchment, at sewage effluents and in a drinking water treatment plant." Water Res **44**(12): 3696-3702.

Collado, L., A. Levican, J. Perez and M. J. Figueras (2011). "*Arcobacter defluvii* sp nov., isolated from sewage samples." Int J Syst Evol Microbiol **61**: 2155-2161.

Cui, Q., Y. Huang, H. Wang and T. Fang (2019). "Diversity and abundance of bacterial pathogens in urban rivers impacted by domestic sewage." Environ Pollut **249**: 24-35.

D'Sa, E. M. and M. A. Harrison (2005). "Effect of pH, NaCl content, and temperature on growth and survival of *Arcobacter* spp." J Food Prot **68**(1): 18-25.

de Boer, R. F., A. Ott, P. Guren, E. van Zanten, A. van Belkum and A. M. Kooistra-Smid (2013). "Detection of *Campylobacter* species and *Arcobacter butzleri* in stool samples by use of real-time multiplex PCR." J Clin Microbiol **51**(1): 253-259.

De Cesare, A., A. Parisi, F. Giacometti, A. Serraino, S. Piva, M. Caruso, E. P. De Santis and G. Manfreda (2016). "Multilocus sequence typing of *Arcobacter butzleri* isolates collected from dairy plants and their products, and comparison with their PFGE types." J Appl Microbiol **120**(1): 165-174.

De Smet, S., L. De Zutter, J. Van Hende and K. Houf (2010). "*Arcobacter* contamination on pre- and post-chilled bovine carcasses and in minced beef at retail." J Appl Microbiol **108**(1): 299-305.

De Smet, S., P. Vandamme, L. De Zutter, S. L. W. On, L. Douidah and K. Houf (2011). "*Arcobacter trophiarum* sp. nov., isolated from fattening pigs." Int J Syst Evol Microbiol **61**: 356-361.

Debruyne, L., K. Houf, L. Douidah, S. De Smet and P. Vandamme (2010). "Reassessment of the taxonomy of *Arcobacter cryaerophilus*." Syst Appl Microbiol **33**(1): 7-14.

Dieguez, A. L., S. Balboa, T. Magnesen and J. L. Romalde (2017). "*Arcobacter lekithochrous* sp. nov., isolated from a molluscan hatchery." Int J Syst Evol Microbiol **67**(5): 1327-1332.

Diergaardt, S. M., S. N. Venter, A. Spreeth, J. Theron and V. S. Brozel (2004). "The occurrence of campylobacters in water sources in South Africa." Water Res **38**(10): 2589-2595.

Donachie, S. P., J. P. Bowman, S. L. W. On and M. Alam (2005). "*Arcobacter halophilus* sp. nov., the first obligate halophile in the genus *Arcobacter*." Int J Syst Evol Microbiol **55**(Pt 3): 1271-1277.

Douidah, L., L. de Zutter, J. Bare, P. De Vos, P. Vandamme, O. Vandenberg, A. M. Van den Abeele and K. Houf (2012). "Occurrence of putative virulence genes in arcobacter species isolated from humans and animals." J Clin Microbiol **50**(3): 735-741.

Douidah, L., L. De Zutter, J. Bare and K. Houf (2014). "Towards a typing strategy for *Arcobacter* species isolated from humans and animals and assessment of the in vitro genomic stability." Foodborne Pathog Dis **11**(4): 272-280.

Douidah, L., L. De Zutter, P. Vandamme and K. Houf (2010). "Identification of five human and mammal associated *Arcobacter* species by a novel multiplex-PCR assay." J Microbiol Methods **80**(3): 281-286.

Ellis, W. A., S. D. Neill, J. J. O'Brien, H. W. Ferguson and J. Hanna (1977). "Isolation of Spirillum/Vibrio-like organisms from bovine fetuses." Vet Rec **100**(21): 451-452.

Engberg, J., S. L. On, C. S. Harrington and P. Gerner-Smidt (2000). "Prevalence of *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Sutterella* spp. in human fecal samples as estimated by a reevaluation of isolation methods for *Campylobacters*." J Clin Microbiol **38**(1): 286-291.

Ertas, N., Y. Dogruer, Z. Gonulalan, A. Guner and I. Ulger (2010). "Prevalence of arcobacter species in drinking water, spring water, and raw milk as determined by multiplex PCR." J Food Prot **73**(11): 2099-2102.

Fallas-Padilla, K. L., C. E. Rodriguez-Rodriguez, H. Fernandez Jaramillo and M. L. Arias Echandi (2014). "*Arcobacter*: comparison of isolation methods, diversity, and potential pathogenic factors in commercially retailed chicken breast meat from Costa Rica." J Food Prot **77**(6): 880-884.

Fanelli, F., D. Chieffi, A. Di Pinto, A. Mottola, F. Baruzzi and V. Fusco (2020). "Phenotype and genomic background of *Arcobacter butzleri* strains and taxogenomic assessment of the species." Food Microbiol **89**: 103416.

Fanelli, F., A. Di Pinto, A. Mottola, G. Mule, D. Chieffi, F. Baruzzi, G. Tantillo and V. Fusco (2019). "Genomic Characterization of *Arcobacter butzleri* Isolated From Shellfish: Novel Insight Into Antibiotic Resistance and Virulence Determinants." Front Microbiol **10**: 670. https://doi.org/10.3389/fmicb.2019.00670

Fera, M. T., C. Gugliandolo, V. Lentini, A. Favaloro, D. Bonanno, E. La Camera and T. L. Maugeri (2010). "Specific detection of *Arcobacter* spp. in estuarine waters of Southern Italy by PCR and fluorescent in situ hybridization." Lett Appl Microbiol **50**(1): 65-70.

Fernandez, H., M. P. Villanueva, I. Mansilla, M. Gonzalez and F. Latif (2015). "*Arcobacter butzleri* and *A. cryaerophilus* in human, animals and food sources, in southern Chile." Braz J Microbiol **46**(1): 145-147.

Ferreira, S., M. J. Fraqueza, J. A. Queiroz, F. C. Domingues and M. Oleastro (2013). "Genetic diversity, antibiotic resistance and biofilm-forming ability of *Arcobacter butzleri* isolated from poultry and environment from a Portuguese slaughterhouse." Int J Food Microbiol **162**(1): 82-88.

Ferreira, S., M. Oleastro and F. C. Domingues (2017). "Occurrence, genetic diversity and antibiotic resistance of *Arcobacter* sp. in a dairy plant." J Appl Microbiol **123**(4): 1019-1026.

Ferreira, S., M. Oleastro and F. Domingues (2019). "Current insights on *Arcobacter butzleri* in food chain." Curr Opin Food Sci **26**: 9-17.

Ferreira, S., J. A. Queiroz, M. Oleastro and F. C. Domingues (2016). "Insights in the pathogenesis and resistance of *Arcobacter*: A review." Crit Rev Microbiol **42**(3): 364-383.

Figueras, M. J., L. Collado, A. Levican, J. Perez, M. J. Solsona and C. Yustes (2011). "*Arcobacter molluscorum* sp. nov., a new species isolated from shellfish." Syst Appl Microbiol **34**(2): 105-109.

Figueras, M. J., A. Levican and L. Collado (2012). "Updated 16S rRNA-RFLP method for the identification of all currently characterised *Arcobacter* spp." BMC Microbiol **12**: 292.

Figueras, M. J., A. Levican, L. Collado, M. I. Inza and C. Yustes (2011). "*Arcobacter ellisii* sp. nov., isolated from mussels." Syst Appl Microbiol **34**(6): 414-418.

Figueras, M. J., A. Levican, I. Pujol, F. Ballester, M. J. Rabada Quilez and F. Gomez-Bertomeu (2014). "A severe case of persistent diarrhoea associated with *Arcobacter cryaerophilus* but attributed to *Campylobacter* sp. and a review of the clinical incidence of *Arcobacter* spp." New Microbes New Infect **2**(2): 31-37.

Fong, T. T., L. S. Mansfield, D. L. Wilson, D. J. Schwab, S. L. Molloy and J. B. Rose (2007). "Massive microbiological groundwater contamination associated with a waterborne outbreak in Lake Erie, South Bass Island, Ohio." Environ Health Perspect **115**(6): 856-864.

Foods, I. C. F. S. O. J. I. J. o. F. M. (2003). "Microorganisms in foods 7: microbiological testing in food safety management." **89**(2): 291-292.

Giacometti, F., M. N. Losio, P. Daminelli, E. Cosciani-Cunico, E. Dalzini and A. Serraino (2015). "Short communication: *Arcobacter butzleri* and *Arcobacter cryaerophilus* survival and growth in artisanal and industrial ricotta cheese." J Dairy Sci **98**(10): 6776-6781.

Giacometti, F., A. Lucchi, A. Di Francesco, M. Delogu, E. Grilli, I. Guarniero, L. Stancampiano, G. Manfreda, G. Merialdi and A. Serraino (2015). "*Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* Circulation in a Dairy Farm and Sources of Milk Contamination." Appl Environ Microbiol **81**(15): 5055-5063.

Giacometti, F., A. Lucchi, G. Manfreda, D. Florio, R. G. Zanoni and A. Serraino (2013). "Occurrence and genetic diversity of *Arcobacter butzleri* in an artisanal dairy plant in Italy." Appl Environ Microbiol **79**(21): 6665-6669.

Giacometti, F., S. Piva, K. Vranckx, K. De Bruyne, I. Drigo, A. Lucchi, G. Manfreda and A. Serraino (2018). "Application of MALDI-TOF MS for the subtyping of *Arcobacter butzleri* strains and comparison with their MLST and PFGE types." Int J Food Microbiol **277**: 50-57.

Giacometti, F., A. Serraino, F. Pasquali, A. De Cesare, E. Bonerba and R. Rosmini (2014). "Behavior of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in ultrahigh-temperature, pasteurized, and raw cow's milk under different temperature conditions." Foodborne Pathog Dis **11**(1): 15-20.

Gilbert, M. J., M. Kik, A. J. Timmerman, T. T. Severs, J. G. Kusters, B. Duim and J. A. Wagenaar (2014). "Occurrence, diversity, and host association of intestinal *Campylobacter*, *Arcobacter*, and *Helicobacter* in reptiles." PLoS One **9**(7): e101599.

Girbau, C., C. Guerra, I. Martinez-Malaxetxebarria, R. Alonso and A. Fernandez-Astorga (2015). "Prevalence of ten putative virulence genes in the emerging foodborne pathogen *Arcobacter* isolated from food products." Food Microbiol **52**: 146-149.

Gobbi, D. D. S., M. G. Spindola, L. Z. Moreno, C. E. C. Matajira, M. G. X. Oliveira, R. Paixao, T. S. P. Ferreira and A. M. Moreno (2018). "Isolation and molecular characterization of *Arcobacte butzleri* and *Arcobacter cryaerophilus* from the pork production chain in Brazil." Pesqui Vet Bras **38**(3): 393-399.

Gonzalez, A., I. F. Bayas Morejon and M. A. Ferrus (2017). "Isolation, molecular identification and quinolone-susceptibility testing of *Arcobacter* spp. isolated from fresh vegetables in Spain." Food Microbiol **65**: 279-283.

Gonzalez, A. and M. A. Ferrus (2011). "Study of *Arcobacter* spp. contamination in fresh lettuces detected by different cultural and molecular methods." Int J Food Microbiol **145**(1): 311-314.

Gonzalez, A., M. A. Ferrus, R. Gonzalez and J. Hernandez (2007). "Molecular fingerprinting of *Campylobacter* and *Arcobacter* isolated from chicken and water." Int Microbiol **10**(2): 85-90.

Gonzalez, A., J. Suski and M. A. Ferrus (2010). "Rapid and Accurate Detection of *Arcobacter* Contamination in Commercial Chicken Products and Wastewater Samples by Real-Time Polymerase Chain Reaction." Foodborne Pathog Dis **7**(3): 327-338.

Greay, T. L., A. W. Gofton, A. Zahedi, A. Paparini, K. L. Linge, C. A. Joll and U. M. Ryan (2019). "Evaluation of 16S next-generation sequencing of hypervariable region 4 in wastewater samples: An unsuitable approach for bacterial enteric pathogen identification." Sci Total Environ **670**: 1111-1124.

Gualerzi, C. O., A. M. Giuliodori and C. L. Pon (2003). "Transcriptional and post-transcriptional control of cold-shock genes." J Mol Biol **331**(3): 527-539.

Hausdorf, L., A. Frohling, O. Schluter and M. Klocke (2011). "Analysis of the bacterial community within carrot wash water." Can J Microbiol **57**(5): 447-452.

Hausdorf, L., M. Neumann, I. Bergmann, K. Sobiella, K. Mundt, A. Frohling, O. Schluter and M. Klocke (2013). "Occurrence and genetic diversity of *Arcobacter* spp. in a spinach-processing plant and evaluation of two *Arcobacter*-specific quantitative PCR assays." Syst Appl Microbiol **36**(4): 235-243.

Hilton, C. L., B. M. Mackey, A. J. Hargreaves and S. J. Forsythe (2001). "The recovery of *Arcobacter butzleri* NCTC 12481 from various temperature treatments." J Appl Microbiol **91**(5): 929-932.

Ho, H. T., L. J. Lipman and W. Gaastra (2008). "The introduction of *Arcobacter* spp. in poultry slaughterhouses." Int J Food Microbiol **125**(3): 223-229.

Ho, T. K., L. J. Lipman, L. van der Graaf-van Bloois, M. van Bergen and W. Gaastra (2006). "Potential routes of acquisition of *Arcobacter* species by piglets." Vet Microbiol **114**(1-2): 123-133.

Houf, K., L. De Zutter, J. Van Hoof and P. Vandamme (2002). "Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods." Appl Environ Microbiol **68**(5): 2172-2178.

Houf, K., L. A. Devriese, L. De Zutter, J. Van Hoof and P. Vandamme (2001a). "Development of a new protocol for the isolation and quantification of *Arcobacter* species from poultry products." Int J Food Microbiol **71**(2-3): 189-196.

Houf, K., L. A. Devriese, L. De Zutter, J. Van Hoof and P. Vandamme (2001b). "Susceptibility of *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* to antimicrobial agents used in selective media." J Clin Microbiol **39**(4): 1654-1656.

Houf, K., S. L. W. On, T. Coenye, L. Debruyne, S. De Smet and P. Vandamme (2009). "*Arcobacter thereius* sp nov., isolated from pigs and ducks." Int J Syst Evol Microbiol **59**: 2599-2604.

Houf, K., S. L. W. On, T. Coenye, J. Mast, J. Van Hoof and P. Vandamme (2005). "*Arcobacter cibarius* sp nov., isolated from broiler carcasses." Int J Syst Evol Microbiol **55**: 713-717.

Houf, K. and R. Stephan (2007). "Isolation and characterization of the emerging foodborn pathogen *Arcobacter* from human stool." J Microbiol Methods **68**(2): 408-413.

Houf, K., A. Tutenel, L. De Zutter, J. Van Hoof and P. Vandamme (2000). "Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*." FEMS Microbiol Lett **193**(1): 89-94.

Hsueh, P. R., L. J. Teng, P. C. Yang, S. K. Wang, S. C. Chang, S. W. Ho, W. C. Hsieh and K. T. Luh (1997). "Bacteremia caused by *Arcobacter cryaerophilus* 1B." J Clin Microbiol **35**(2): 489-491.

Hume, M. E., R. B. Harvey, L. H. Stanker, R. E. Droleskey, T. L. Poole and H. B. Zhang (2001). "Genotypic variation among arcobacter isolates from a farrow-to-finish swine facility." J Food Prot **64**(5): 645-651.

Isidro, J., S. Ferreira, M. Pinto, F. Domingues, M. Oleastro, J. P. Gomes and V. Borges (2020). "Virulence and antibiotic resistance plasticity of *Arcobacter butzleri*: Insights on the genomic diversity of an emerging human pathogen." Infect Genet Evol **80**: 104213.

Jacob, J., D. Woodward, I. Feuerpfeil and W. M. Johnson (1998). "Isolation of *Arcobacter butzleri* in raw water and drinking water treatment plants in Germany." Zentralbl Hyg Umweltmed **201**(2): 189-198.

Jiang, Z. D., H. L. Dupont, E. L. Brown, R. K. Nandy, T. Ramamurthy, A. Sinha, S. Ghosh, S. Guin, K. Gurleen, S. Rodrigues, J. J. Chen, R. McKenzie and R. Steffen (2010). "Microbial etiology of travelers' diarrhea in Mexico, Guatemala, and India: importance of enterotoxigenic *Bacteroides fragilis* and *Arcobacter* species." J Clin Microbiol **48**(4): 1417-1419.

Johnson, L. G. and E. A. Murano (1999). "Development of a new medium for the isolation of *Arcobacter* spp." J Food Prot **62**(5): 456-462.

Jones, P. G. and M. Inouye (1994). "The cold-shock response-a hot topic." Mol Microbiol **11**(5): 811-818.

Jones, P. G., R. A. VanBogelen and F. C. Neidhardt (1987). "Induction of proteins in response to low temperature in *Escherichia coli*." J Bacteriol **169**(5): 2092-2095.

Kabeya, H., Y. Kobayashi, S. Maruyama and T. Mikami (2003). "One-step polymerase chain reaction-based typing of *Arcobacter* species." Int J Food Microbiol **81**(2): 163-168.

Kabeya, H., S. Maruyama, Y. Morita, M. Kubo, K. Yamamoto, S. Arai, T. Izumi, Y. Kobayashi, Y. Katsube and T. Mikami (2003). "Distribution of *Arcobacter* species among livestock in Japan." Vet Microbiol **93**(2): 153-158.

Karadas, G., S. Sharbati, I. Hanel, U. Messelhausser, E. Glocker, T. Alter and G. Golz (2013). "Presence of virulence genes, adhesion and invasion of *Arcobacter butzleri*." J Appl Microbiol **115**(2): 583-590.

Kayman, T., S. Abay, H. Hizlisoy, H. I. Atabay, K. S. Diker and F. Aydin (2012). "Emerging pathogen *Arcobacter* spp. in acute gastroenteritis: molecular identification, antibiotic susceptibilities and genotyping of the isolated arcobacters." J Med Microbiol **61**(Pt 10): 1439-1444.

Khoshbakht, R., M. Tabatabaei, H. S. Aski and S. Seifi (2014). "Occurrence of *Arcobacter* in Iranian poultry and slaughterhouse samples implicates contamination by processing equipment and procedures." Br Poult Sci **55**(6): 732-736.

Kiehlbauch, J. A., D. J. Brenner, M. A. Nicholson, C. N. Baker, C. M. Patton, A. G. Steigerwalt and I. K. Wachsmuth (1991). "*Campylobacter butzleri* sp. nov. isolated from humans and animals with diarrheal illness." J Clin Microbiol **29**(2): 376-385.

Kim, H. M., C. Y. Hwang and B. C. Cho (2010). "*Arcobacter marinus* sp nov." Int J Syst Evol Microbiol **60**: 531-536.

Kim, N. H., S. M. Park, H. W. Kim, T. J. Cho, S. H. Kim, C. Choi and M. S. Rhee (2019). "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 **78**: 18-24.

Kjeldgaard, J., K. Jorgensen and H. Ingmer (2009). "Growth and survival at chiller temperatures of *Arcobacter butzleri*." Int J Food Microbiol **131**(2-3): 256-259.

Kopilovic, B., V. Ucakar, N. Koren, M. Krek and A. Kraigher (2008). "Waterborne outbreak of acute gastroenteritis in a costal area in Slovenia in June and July 2008." Euro Surveill **13**(34).
Laishram, M., S. Rathlavath, M. Lekshmi, S. Kumar and B. B. Nayak (2016). "Isolation and characterization of *Arcobacter* spp. from fresh seafood and the aquatic environment." Int J Food Microbiol **232**: 87-89.
Lastovica, A. J. and E. le Roux (2000). "Efficient isolation of campylobacteria from stools." J Clin Microbiol **38**(7): 2798-2798.
Lau, S. K., P. C. Woo, J. L. Teng, K. W. Leung and K. Y. Yuen (2002). "Identification by 16S ribosomal RNA gene sequencing of *Arcobacter butzleri* bacteraemia in a patient with acute gangrenous appendicitis." Mol Pathol **55**(3): 182-185.
Lehmann, D., T. Alter, L. Lehmann, S. Uherkova, T. Seidler and G. Golz (2015). "Prevalence, virulence gene distribution and genetic diversity of *Arcobacter* in food samples in Germany." Berl Munch Tierarztl Wochenschr **128**(3-4): 163-168.
Leoni, F., S. Chierichetti, S. Santarelli, G. Talevi, L. Masini, C. Bartolini, E. Rocchegiani, M. Naceur Haouet and D. Ottaviani (2017). "Occurrence of *Arcobacter* spp. and correlation with the bacterial indicator of faecal contamination *Escherichia coli* in bivalve molluscs from the Central Adriatic, Italy." Int J Food Microbiol **245**: 6-12.
Levican, A., A. Alkeskas, C. Gunter, S. J. Forsythe and M. J. Figueras (2013). "Adherence to and invasion of human intestinal cells by *Arcobacter* species and their virulence genotypes." Appl Environ Microbiol **79**(16): 4951-4957.
Levican, A., L. Collado, C. Aguilar, C. Yustes, A. L. Dieguez, J. L. Romalde and M. J. Figueras (2012). "*Arcobacter bivalviorum* sp. nov. and Arcobacter venerupis sp. nov., new species isolated from shellfish." Syst Appl Microbiol **35**(3): 133-138.
Levican, A., L. Collado and M. J. Figueras (2013). "*Arcobacter cloacae* sp. nov. and Arcobacter suis sp. nov., two new species isolated from food and sewage." Syst Appl Microbiol **36**(1): 22-27.
Levican, A., L. Collado and M. J. Figueras (2016). "The Use of Two Culturing Methods in Parallel Reveals a High Prevalence and Diversity of *Arcobacter* spp. in a Wastewater Treatment Plant." Biomed Res Int **2016**: 8132058.
Levican, A., L. Collado, C. Yustes, C. Aguilar and M. J. Figueras (2014). "Higher water temperature and incubation under aerobic and microaerobic conditions increase the recovery and diversity of *Arcobacter* spp. from shellfish." Appl Environ Microbiol **80**(1): 385-391.
Levican, A. and M. J. Figueras (2013). "Performance of five molecular methods for monitoring *Arcobacter* spp." BMC Microbiol**13**: 220.
Levican, A., S. Rubio-Arcos, A. Martinez-Murcia, L. Collado and M. J. Figueras (2015). "*Arcobacter ebronensis* sp. nov. and *Arcobacter aquimarinus* sp. nov., two new species isolated from marine environment." Syst Appl Microbiol **38**(1): 30-35.

Marta, C., N. Giovanni, M. Angela, C. Loredana, B. Elisabetta, D. Laura, M. Anna, D. P. Angela, S. Gianfranco and P. Antonio (2020). "Large genetic diversity of *Arcobacter butzleri* isolated from raw milk in Southern Italy." Food Microbiol **89**: 103403.

Mcclung, C. R., D. G. Patriquin and R. E. Davis (1983). "*Campylobacter-Nitrofigilis* Sp-Nov, a Nitrogen-Fixing Bacterium Associated with Roots of Spartina-Alterniflora Loisel." Int J Syst Bacteriol **33**(3): 605-612.

Merga, J. Y., A. J. Leatherbarrow, C. Winstanley, M. Bennett, C. A. Hart, W. G. Miller and N. J. Williams (2011). "Comparison of *Arcobacter* isolation methods, and diversity of *Arcobacter* spp. in Cheshire, United Kingdom." Appl Environ Microbiol **77**(5): 1646-1650.

Merga, J. Y., A. Royden, A. K. Pandey and N. J. Williams (2014). "*Arcobacter* spp. isolated from untreated domestic effluent." Lett Appl Microbiol **59**(1): 122-126.

Merga, J. Y., N. J. Williams, W. G. Miller, A. J. Leatherbarrow, M. Bennett, N. Hall, K. E. Ashelford and C. Winstanley (2013). "Exploring the diversity of *Arcobacter butzleri* from cattle in the UK using MLST and whole genome sequencing." PLoS One **8**(2): e55240.

Merrin, J., P. Kumar and A. Libchaber (2011). "Effects of pressure and temperature on the binding of RecA protein to single-stranded DNA." Proc Natl Acad Sci USA **108**(50): 19913-19918.

Miller, W. G., C. T. Parker, M. Rubenfield, G. L. Mendz, M. M. Wosten, D. W. Ussery, J. F. Stolz, T. T. Binnewies, P. F. Hallin, G. Wang, J. A. Malek, A. Rogosin, L. H. Stanker and R. E. Mandrell (2007). "The complete genome sequence and analysis of the epsilonproteobacterium *Arcobacter butzleri*." PLoS One **2**(12): e1358.

Miller, W. G., I. V. Wesley, S. L. On, K. Houf, F. Megraud, G. Wang, E. Yee, A. Srijan and C. J. Mason (2009). "First multi-locus sequence typing scheme for *Arcobacter* spp." BMC Microbiol **9**: 196.

Mitchell, S. L. and P. J. Simner (2019). "Next-Generation Sequencing in Clinical Microbiology: Are We There Yet?" Clin Lab Med **39**(3): 405-418.

Mizutani, Y., S. Iehata, T. Mori, R. Oh, S. Fukuzaki and R. Tanaka (2019). "Diversity, enumeration, and isolation of *Arcobacter* spp. in the giant abalone, Haliotis gigantea." Microbiologyopen **8**(10): e890.

Morejon, I. F., A. Gonzalez and M. A. Ferrus (2017). "Detection, Identification, and Antimicrobial Susceptibility of *Arcobacter* spp. Isolated from Shellfish in Spain." Foodborne Pathog Dis **14**(4): 238-243.

Moreno, Y., S. Botella, J. L. Alonso, M. A. Ferrus, M. Hernandez and J. Hernandez (2003). "Specific detection of *Arcobacter* and *Campylobacter* strains in water and sewage by PCR and fluorescent in situ hybridization." Appl Environ Microbiol **69**(2): 1181-1186.

Morita, Y., S. Maruyama, H. Kabeya, S. Boonmar, B. Nimsuphan, A. Nagai, K. Kozawa, T. Nakajima, T. Mikami and H. Kimura (2004). "Isolation and phylogenetic analysis of arcobacter

spp. in ground chicken meat and environmental water in Japan and Thailand." Microbiol Immunol **48**(7): 527-533.

Mottola, A., E. Bonerba, G. Bozzo, P. Marchetti, G. V. Celano, V. Colao, V. Terio, G. Tantillo, M. J. Figueras and A. Di Pinto (2016a). "Occurrence of emerging food-borne pathogenic *Arcobacter* spp. isolated from pre-cut (ready-to-eat) vegetables." Int J Food Microbiol **236**: 33-37.

Mottola, A., E. Bonerba, M. J. Figueras, A. Perez-Cataluna, P. Marchetti, A. Serraino, G. Bozzo, V. Terio, G. Tantillo and A. Di Pinto (2016b). "Occurrence of potentially pathogenic arcobacters in shellfish." Food Microbiol **57**: 23-27.

Neill, S. D., J. N. Campbell, J. J. Obrien, S. T. C. Weatherup and W. A. Ellis (1985). "Taxonomic Position of *Campylobacter-Cryaerophila* Sp-Nov." Int J Syst Bacteriol **35**(3): 342-356.

Neill, S. D., W. A. Ellis and J. J. O'Brien (1978). "The biochemical characteristics of *Campylobacter*-like organisms from cattle and pigs." Res Vet Sci **25**(3): 368-372.

Noto, A. M. D., S. Sciortino, C. Cardamone, C. Ciravolo, C. Napoli, V. Alio, P. Arculeo, G. Olive, D. M. and P. Bean (1999). "Principles and applications of methods for DNA-based typing of microbial organisms." J Clin Microbiol **37**(6): 1661-1669.

Oliveri and A. Costa (2018). "Detection of *Arcobacter* spp. in food products collected from Sicilia region: A preliminary study." Ital J Food Saf **7**(2): 7171.

Oliveira, M. G., L. N. Pressinotti, G. S. Carvalho, M. C. Oliveira, L. Z. Moreno, C. E. Matajira, A. S. Bergamo, V. M. Aleixo, A. C. Veiga, E. de Souza Corsino, A. P. Christ, M. I. Sato, A. M. Moreno and T. Knobl (2017). "*Arcobacter* spp. in fecal samples from Brazilian farmed caimans (Caiman yacare, Daudin 1802)." Trop Anim Health Prod **49**(4): 777-782.

On, S. L. (1996). "Identification methods for campylobacters, helicobacters, and related organisms." Clin Microbiol Rev **9**(3): 405-422.

On, S. L., C. S. Harrington and H. I. Atabay (2003). "Differentiation of *Arcobacter* species by numerical analysis of AFLP profiles and description of a novel *Arcobacter* from pig abortions and turkey faeces." J Appl Microbiol **95**(5): 1096-1105.

On, S. L. W., T. K. Jensen, V. Bille-Hansen, S. E. Jorsal and P. Vandamme (2002). "Prevalence and diversity of *Arcobacter* spp. isolated from the internal organs of spontaneous porcine abortions in Denmark." Vet Microbiol **85**(2): 159-167.

Park, S., Y. T. Jung, S. Kim and J. H. Yoon (2016). "*Arcobacter acticola* sp. nov., isolated from seawater on the East Sea in South Korea." J Microbiol **54**(10): 655-659.

Patyal, A., R. S. Rathore, H. V. Mohan, K. Dhama and A. Kumar (2011). "Prevalence of *Arcobacter* spp. in humans, animals and foods of animal origin including sea food from India." Transbound Emerg Dis **58**(5): 402-410.

Pentimalli, D., N. Pegels, T. Garcia, R. Martin and I. Gonzalez (2009). "Specific PCR Detection of *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter skirrowii*, and *Arcobacter cibarius* in Chicken Meat." J Food Prot **72**(7): 1491-1495.

Perez-Cataluna, A., N. Salas-Masso and M. J. Figueras (2018). "*Arcobacter canalis* sp. nov., isolated from a water canal contaminated with urban sewage." Int J Syst Evol Microbiol **68**(4): 1258-1264.

Perez-Cataluna, A., N. Salas-Masso and M. J. Figueras (2019). "*Arcobacter lacus* sp. nov. and *Arcobacter caeni* sp. nov., two novel species isolated from reclaimed water." Int J Syst Evol Microbiol **69**(11): 3326-3331.

Perez-Cataluna, A., J. Tapiol, C. Benavent, C. Sarvise, F. Gomez, B. Martinez, M. Terron-Puig, G. Recio, A. Vilanova, I. Pujol, F. Ballester, A. Rezusta and M. J. Figueras (2017). "Antimicrobial susceptibility, virulence potential and sequence types associated with *Arcobacter* strains recovered from human faeces." J Med Microbiol **66**(12): 1736-1743.

Phadtare, S. (2004). "Recent developments in bacterial cold-shock response." Curr Issues Mol Biol **6**(2): 125-136.

Piva, S., G. R. Gariano, P. Bonilauri, F. Giacometti, L. Decastelli, D. Florio, E. Massella and A. Serraino (2017). "Occurrence of putative virulence genes on *Arcobacter butzleri* isolated from three different environmental sites throughout the dairy chain." J Appl Microbiol **122**(4): 1071-1077.

Ramees, T. P., K. Dhama, K. Karthik, R. S. Rathore, A. Kumar, M. Saminathan, R. Tiwari, Y. S. Malik and R. K. Singh (2017). "*Arcobacter*: an emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control - a comprehensive review." Vet Q **37**(1): 136-161.

Ramees, T. P., R. S. Rathore, P. S. Bagalkot, B. Sailo, H. V. Mohan, A. Kumar, K. Dhama and R. K. Singh (2014). "Genotyping and genetic diversity of *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolated from different sources by using ERIC-PCR from India." Vet Q **34**(4): 211-217.

Rasmussen, L. H., J. Kjeldgaard, J. P. Christensen and H. Ingmer (2013). "Multilocus sequence typing and biocide tolerance of *Arcobacter butzleri* from Danish broiler carcasses." BMC Res Notes **6**: 322.

Rathlavath, S., S. Kumar and B. B. Nayak (2017). "Comparative isolation and genetic diversity of *Arcobacter* sp. from fish and the coastal environment." Lett Appl Microbiol **65**(1): 42-49.

Rice, E. W., M. R. Rodgers, I. V. Wesley, C. H. Johnson and S. A. Tanner (1999). "Isolation of *Arcobacter butzleri* from ground water." Lett Appl Microbiol **28**(1): 31-35.

Rivas, L., N. Fegan and P. Vanderlinde (2004). "Isolation and characterisation of *Arcobacter butzleri* from meat." Int J Food Microbiol **91**(1): 31-41.

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

Salas-Masso, N., K. B. Andree, M. D. Furones and M. J. Figueras (2016). "Enhanced recovery of *Arcobacter* spp. using NaCl in culture media and re-assessment of the traits of *Arcobacter marinus* and *Arcobacter halophilus* isolated from marine water and shellfish." Sci Total Environ **566-567**: 1355-1361.

Samarajeewa, A. D., A. Hammad, L. Masson, I. U. Khan, R. Scroggins and L. A. Beaudette (2015). "Comparative assessment of next-generation sequencing, denaturing gradient gel electrophoresis, clonal restriction fragment length polymorphism and cloning-sequencing as methods for characterizing commercial microbial consortia." J Microbiol Methods **108**: 103-111.

Samie, A., C. L. Obi, L. J. Barrett, S. M. Powell and R. L. Guerrant (2007). "Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: Studies using molecular diagnostic methods." J Infect **54**(6): 558-566.

Sasi Jyothsna, T. S., K. Rahul, E. V. Ramaprasad, C. Sasikala and V. Ramana Ch (2013). "*Arcobacter anaerophilus* sp. nov., isolated from an estuarine sediment and emended description of the genus Arcobacter." Int J Syst Evol Microbiol **63**(Pt 12): 4619-4625.

Scarano, C., F. Giacometti, G. Manfreda, A. Lucchi, E. Pes, C. Spanu, E. P. De Santis and A. Serraino (2014). "*Arcobacter butzleri* in sheep ricotta cheese at retail and related sources of contamination in an industrial dairy plant." Appl Environ Microbiol **80**(22): 7036-7041.

Scullion, R., C. S. Harrington and R. H. Madden (2004). "A comparison of three methods for the isolation of *Arcobacter* spp. from retail raw poultry in Northern Ireland." J Food Prot **67**(4): 799-804.

Shah, A. H., A. A. Saleha, Z. Zunita, Y. K. Cheah, M. Murugaiyah and N. A. Korejo (2012). "Genetic characterization of *Arcobacter* isolates from various sources." Vet Microbiol**160**(3-4): 355-361.

Shrestha, R. G., Y. Tanaka, B. Malla, S. Tandukar, D. Bhandari, D. Inoue, K. Sei, J. B. Sherchand and E. Haramoto (2018). "Development of a Quantitative PCR Assay for *Arcobacter* spp. and its Application to Environmental Water Samples." Microbes Environ **33**(3): 309-316.

Silha, D., K. Morávková, G. Škodová and J. Vytřasová (2019). "Viability and biofilm formation of *Arcobacter* spp. at various processing temperatures." J Food and Nutr. Res **58**(3): 208-213.

Silha, D., L. Silhova-Hruskova and J. Vytrasova (2015). "Modified isolation method of *Arcobacter* spp. from different environmental and food samples." Folia Microbiol (Praha) **60**(6): 515-521.

Son, I., M. D. Englen, M. E. Berrang, P. J. Fedorka-Cray and M. A. Harrison (2007). "Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing." Int J Food Microbiol **113**(1): 16-22.

Tabatabaei, M., H. Shirzad Aski, H. Shayegh and R. Khoshbakht (2014). "Occurrence of six virulence-associated genes in *Arcobacter* species isolated from various sources in Shiraz, Southern Iran." Microb Pathog **66**: 1-4.

Talay, F., C. Molva and H. I. Atabay (2016). "Isolation and identification of *Arcobacter* species from environmental and drinking water samples." Folia Microbiol (Praha) **61**(6): 479-484.

Tanaka, R., I. Cleenwerck, Y. Mizutani, S. Iehata, P. Bossier and P. Vandamme (2017). "*Arcobacter haliotis* sp. nov., isolated from abalone species Haliotis gigantea." Int J Syst Evol Microbiol **67**(8): 3050-3056.

Tompkins, D. S., M. J. Hudson, H. R. Smith, R. P. Eglin, J. G. Wheeler, M. M. Brett, R. J. Owen, J. S. Brazier, P. Cumberland, V. King and P. E. Cook (1999). "A study of infectious intestinal disease in England: microbiological findings in cases and controls." Commun Dis Public Health **2**(2): 108-113.

Traversa, A., S. Gallina, F. Martucci, C. Boteva, E. Baioni, C. Maurella, L. Chiavacci, E. Benvenuto, I. Ferrero, E. Ferrero, F. Giacometti, S. Piva, F. Chiesa, D. M. Bianchi, A. Serraino and L. Decastelli (2019). "*Arcobacter* spp. in raw milk from vending machines in Piedmont and occurrence of virulence genes in isolates." Ital J Food Saf **8**(4): 7859.

Van den Abeele, A. M., D. Vogelaers, J. Van Hende and K. Houf (2014). "Prevalence of *Arcobacter* Species among Humans, Belgium, 2008-2013." Emerg Infect Dis **20**(10): 1731-1734.

Van den Abeele, A. M., D. Vogelaers, P. Vandamme, E. Vanlaere and K. Houf (2018). "Filling the gaps in clinical proteomics: a do-it-yourself guide for the identification of the emerging pathogen *Arcobacter* by matrix-assisted laser desorption ionization-time of flight mass spectrometry." J Microbiol Methods **152**: 92-97.

Van Driessche, E. and K. Houf (2007). "Characterization of the *Arcobacter* contamination on Belgian pork carcasses and raw retail pork." Int J Food Microbiol **118**(1): 20-26.

Van Driessche, E. and K. Houf (2008). "Survival capacity in water of *Arcobacter* species under different temperature conditions." J Appl Microbiol **105**(2): 443-451.

van Driessche, E., K. Houf, J. van Hoof, L. De Zutter and P. Vandamme (2003). "Isolation of *Arcobacter* species from animal feces." FEMS Microbiol Lett **229**(2): 243-248.

Van Driessche, E., K. Houf, F. Vangroenweghe, L. De Zutter and J. Van Hoof (2005). "Prevalence, enumeration and strain variation of *Arcobacter* species in the faeces of healthy cattle in Belgium." Vet Microbiol **105**(2): 149-154.

Van Driessche, E., K. Houf, F. Vangroenweghe, N. Nollet, L. De Zutter, P. Vandamme and J. Van Hoof (2004). "Occurrence and strain diversity of *Arcobacter* species isolated from healthy Belgian pigs." Res Microbiol **155**(8): 662-666.

Vandamme, P., E. Falsen, R. Rossau, B. Hoste, P. Segers, R. Tytgat and J. De Ley (1991). "Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov." Int J Syst Bacteriol **41**(1): 88-103.

Vandamme, P., B. A. Giesendorf, A. van Belkum, D. Pierard, S. Lauwers, K. Kersters, J. P. Butzler, H. Goossens and W. G. Quint (1993). "Discrimination of epidemic and sporadic isolates of *Arcobacter butzleri* by polymerase chain reaction-mediated DNA fingerprinting." J Clin Microbiol **31**(12): 3317-3319.

Vandamme, P., P. Pugina, G. Benzi, R. Van Etterijck, L. Vlaes, K. Kersters, J. P. Butzler, H. Lior and S. Lauwers (1992). "Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school." J Clin Microbiol **30**(9): 2335-2337.

Vandenberg, O., A. Dediste, K. Houf, S. Ibekwem, H. Souayah, S. Cadranel, N. Douat, G. Zissis, J. P. Butzler and P. Vandamme (2004). "*Arcobacter* species in humans." Emerg Infect Dis **10**(10): 1863-1867.

Vasiljevic, M., A. J. Fenwick, S. Nematollahi, V. P. Gundareddy, M. Romagnoli, J. Zenilman and K. C. Carroll (2019). "First Case Report of Human Bacteremia With *Malacobacter* (*Arcobacter*) *mytili*." Open Forum Infect Dis **6**(7).

Vicente-Martins, S., M. Oleastro, F. C. Domingues and S. Ferreira (2018). "*Arcobacter* spp. at retail food from Portugal: Prevalence, genotyping and antibiotics resistance." Food Control **85**: 107-112.

Wang, X., D. J. Seo, M. H. Lee and C. Choi (2014). "Comparison of conventional PCR, multiplex PCR, and loop-mediated isothermal amplification assays for rapid detection of *Arcobacter* species." J Clin Microbiol **52**(2): 557-563.

Wesley, I. V. and A. L. Baetz (1999). "Natural and experimental infections of *Arcobacter* in poultry." Poult Sci **78**(4): 536-545.

Whiteduck-Leveillee, J., M. Cloutier, E. Topp, D. R. Lapen, G. Talbot, R. Villemur and I. U. Khan (2016). "Development and evaluation of multiplex PCR assays for rapid detection of virulence-associated genes in *Arcobacter* species." J Microbiol Methods **121**: 59-65.

Whiteduck-Leveillee, K., J. Whiteduck-Leveillee, M. Cloutier, J. T. Tambong, R. Xu, E. Topp, M. T. Arts, J. Chao, Z. Adam, C. Andre Levesque, D. R. Lapen, R. Villemur, G. Talbot and I. U. H. Khan (2015). "*Arcobacter lanthieri* sp. nov., isolated from pig and dairy cattle manure." Int J Syst Evol Microbiol **65**(8): 2709-2716.

Whiteduck-Leveillee, K., J. Whiteduck-Leveillee, M. Cloutier, J. T. Tambong, R. Xu, E. Topp, M. T. Arts, J. Chao, Z. Adam, C. A. Levesque, D. R. Lapen, R. Villemur and I. U. Khan (2016).

"Identification, characterization and description of *Arcobacter faecis* sp. nov., isolated from a human waste septic tank." Syst Appl Microbiol **39**(2): 93-99.

Winters, D. K. and M. F. Slavik (2000). "Multiplex PCR detection of *Campylobacter jejuni* and *Arcobacter butzleri* in food products." Mol Cell Probes **14**(2): 95-99.

Woo, P. C. Y., K. T. K. Chong, K. W. Leung, T. L. Que and K. Y. Yuen (2001). "Identification of *Arcobacter cryaerophilus* isolated from a traffic accident victim with bacteremia by 16S ribosomal RNA gene sequencing." Diagn Microbiol Infect Dis **40**(3): 125-127.

Wybo, I., J. Breynaert, S. Lauwers, F. Lindenburg and K. Houf (2004). "Isolation of *Arcobacter skirrowii* from a patient with chronic diarrhea." J Clin Microbiol **42**(4): 1851-1852.

Yamanaka, K. (1999). "Cold shock response in *Escherichia coli*." J Mol Microbiol Biotechnol **1**(2): 193-202.

Zacharow, I., J. Bystron, E. Walecka-Zacharska, M. Podkowik and J. Bania (2015). "Genetic Diversity and Incidence of Virulence-Associated Genes of *Arcobacter butzleri* and *Arcobacter cryaerophilus* Isolates from Pork, Beef, and Chicken Meat in Poland." Biomed Res Int **2015**: 956507.

Zhang, Y., D. H. Burkhardt, S. Rouskin, G. W. Li, J. S. Weissman and C. A. Gross (2018). "A Stress Response that Monitors and Regulates mRNA Structure Is Central to Cold Shock Adaptation." Mol Cell **70**(2): 274-286.

Zhang, Z., C. Yu, X. Wang, S. Yu and X. H. Zhang (2016). "*Arcobacter pacificus* sp. nov., isolated from seawater of the South Pacific Gyre." Int J Syst Evol Microbiol **66**(2): 542-547.

List of Publication

Publications

Zhang, X., Alter, T., Gölz, G. (2019). Characterization of Arcobacter spp. isolated from retail seafood in Germany. Food Microbiol. **82**: 254-258.

Zhang, X., Su, Y., Alter, T., Gölz, G. (2020). The transcriptional response of Arcobacter butzleri to cold shock. FEBS Open Bio. **10**: 2089-2096.

Posters

Zhang, X., Alter, T., Gölz, G. (2016). Prevalence of Arcobacter spp. in seafood in retails of Germany. (Poster/Abstract). Nationales Symposium für Zoonosenforschung, Berlin, Deutschland, 10/2016.

Zhang, X., Alter, T., Gölz, G. (2017). Prevalence and characterization of Arcobacter spp. isolated from retail seafood in Germany. (Poster/Abstract). CHRO, 19th International Workshop on Campylobacter, Helicobacter and Related Organisms, Nantes, France, 09/2017.

Zhang, X., Alter, T., Gölz, G. (2017). Prevalence and characterization of Arcobacter spp. isolated from retail seafood in Germany. (Poster/Abstract). 10th DRS Doktorandensymposium, Berlin, Germany, 09/2017.

Gölz, G.; Zhang, X.; Markgraf, A.; Brückner, V.; Karadas, G.; Heimesaat, M. M.; Bücker, R.; Bereswill, S.; Alter, T. (2018). Prevalence and pathogenic potential of Arcobacter butzleri. (Poster/Abstract). Food Micro Conference 2018, Fu Berlin, Germany, 09/2018.

Zhang, X., Su, Y., Alter, T., Gölz, G. (2019). Adaption capabilities of Arcobacter butzleri to cold shock. (Poster/Abstract). 20th International Workshop on Campylobacter, Helicobacter and Related Organisms, Belfast, UK, 09/2019.

Acknowledgement

First of all, I would like to acknowledge the China Scholarship Council (CSC) for providing the scholarship which enabled me to join in the doctoral program in Freie Universität Berlin, Germany.

I would like to sincerely gratitude to my supervisors, Dr. Greta Goelz, who generously providing me valuable academic guidance, and help me in all aspects in the time in Berlin. And Prof. Dr. Thomas Alter, Institute of Food Safety and Food Hygiene, Freie Universität Berlin for his generous support, valuable guidance during my entire study.
I would like to thank my heartfelt thanks to Chenyang Li, Thi Thu Tra Vu, Antje Schönknecht and De Xi, who accompany with me in this institute for the most of the time during my PhD period and assist me a lot for my research work. I especially acknowledge Yulan Su for his support in the second part of my project. I am also thankful to Dr. Josef Kleer, Ms. Christine Zilk, Ms. Bettina Sahrada, Christoph Püning and Vanessa Brückner for their technical assistance for my experiments. Their love and tolerance make me feel always relax at work.
My appreciations are to all my colleagues at Institute of Food Safety and Food Hygiene, Freie Universität Berlin for the cooperation and friendship.

Last, but not least, I would like to express my deepest gratitude to my beloved family, my parents who gave me great support when I feel upset and homesick abroad. And I want to thank my husband, Ruijie Ye, for his academic support, uplifting encouragement, intimate care all these years in Germany.

Funding Sources

This work is financially supported by China Scholarship Council (CSC).

Conflict of Interest

The author declared no conflict of interest.

Selbständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen Anspruch genommen habe.

Berlin 08.02.2021

Xiaochen Zhang

Druck: Mensch und Buch Verlag Berlin