Occurrence of potentially pathogenic *Arcobacters* in shellfish.

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Abstract

Considering that several recent cases of human gastroenteritis have been associated with species from the *Arcobacter* genus, and that few data are currently available about the occurrence of this genus in Italian shellfish, the aim of the present study was to evaluate the occurrence of *Arcobacter* spp. and the presence of virulence-associated genes. The approach consisted of cultural and biomolecular (multiplex-PCR and 16S-RFLP) methods identifying isolates, followed by PCR assays aimed at the *cadF*, *ciaB*, *cjl349*, *irgA*, *hecA* putative virulence genes. *Arcobacter* spp. was detected in 16/70 (22.8%) shellfish samples. Specifically, *Arcobacter* spp. was highlighted in 10/42 (23.8%) mussel and in 6/28 (21.4%) clam samples. Subsequently, biomolecular assays revealed *A. butzleri* in 12/16 (75%) and *A. cryaerophilus* 1B in 4/16 (25%) isolates. PCRs aimed at the five putative virulence genes demonstrated widespread distribution of these genes among *Arcobacter* isolates and some differences from the results published by other authors. Our research provides more information regarding the health risks associated with the consumption of raw bivalve molluscs and underlines the need to implement an adequate control plan by performing intensive and continuous monitoring in order to guarantee human health.

Keywords: *Arcobacter*, food-borne pathogen, bivalve molluscs, putative virulence factors

1. Introduction

Each year, food-borne and water-borne zoonotic diseases affect tens of millions of people (Painter et al., 2013). Most reports of food and water-borne illnesses are associated with the consumption of food of animal origin (eggs, mixed foods, fish, molluscs, chicken, dairy) or of contaminated water (EFSA and ECDC, 2015). The prevalent etiological agents involved in the outbreaks are bacteria (*Camplylobacter jejuni, Salmonella enteritidis* and *S. typhimurium*, verocytotoxigenic *E. coli,*

45 *Listeria monocytogenes*), viruses (caliciviruses) and parasites (*Cryptosporidium parvum,*

46 *Cryptosporidium hominis)* (EFSA and ECDC, 2015). Despite advances in food safety, in some

47 cases, the causative agent associated with disease may be underestimated or unknown due to the

48 lack of a specific protocol for their detection and identification in clinical laboratories (EFSA and

49 ECDC, 2015; Figueras et al., 2014; Levican et al., 2013; Ramees et al., 2014).

50 Recently, several cases of human gastroenteritis have been associated with some species belonging

51 to the *Arcobacter* genus, a *Campylobacter*-like organism, able to grow and survive both in aerobic

52 and microaerophilic conditions, at temperatures below 30 °C, which differentiates them from the

53 *Campylobacter* species (Figueras et al., 2014; Van den Abeele et al., 2014).

54 *Arcobacter* is a bacterial genus of Gram-negative, slightly curved rods, positive for oxidase and

55 usually motile, found both in animal and environmental sources (González and Ferrús, 2011;

56 Tabatabaei et al., 2014). This genus currently encompasses 22 species (Levican et al.; 2015; Nieva-

57 Echevarría et al., 2013; Whiteduck-Léveillée et al., 2015; Zhang et al., 2015), some of which have

58 been associated with human and animal illnesses: *A. butzleri* and *A. cryaerophilus* have been

59 associated with diarrhea and enterocholitis and occasionally with bacteriemia, endocarditis, and

60 peritonitis (Collado and Figueras, 2011; Figueras et al., 2014; Hsueh et al. 1997; Kayman et al.,

61 2012; Lau et al., 2002; Lerner at al., 1994; McGregor et al., 2015; On et al., 1995;Woo et al.,

62 2001;Yan et al., 2000). Moreover, *A. butzleri* is the species recognised as the etiological agent of

63 traveller's diarrhea (Jiang et al., 2010). Despite the high persistence and prevalence of these two

64 species in clinical cases, food, and the environment, *A. skirrowii* and *A. thereius* have also been

65 recovered in stool samples of patients with diarrhea or enterocolitis (Van den Abeele et al., 2014;

66 Wybo et al., 2004). The probable human pathogenic potential of *Arcobacter* may be associated with

67 the ability of some species to survive under common food storage conditions and with their

68 environmental survival strategies. For example, *A. butzleri* has the ability to persist in seawater in a

69 VBNC (viable but non-cultivable) state that allows it to survive in the absence of nutrients (Fera et
70 al., 2008). Moreover, *A. butzleri* can persist on surfaces given its ability to form biofilm (Assanta et
71 al., 2002; Kjeldgaard et al., 2009). These survival strategies, together with the virulence potential of
72 some *Arcobacter* spp. that may have a whole suite of putative virulence genes (*cadF, cj1349, ciaB,*
73 *mviN, pldA, tlyA, hecA, hecB* and *irgA*), underlining their importance in its pathogenicity (Douidah
74 et al*.,* 2012, Ferreira et al*.,* 2015; Levican et al., 2013;).

75 Considering the high prevalence of *Arcobacter* species in seawater (Collado et al., 2008; Fera et al.,
76 2008) and the ability of bivalve shellfish, as a result of their filter-feeding activity, to bio-
77 concentrate pathogens, the aim of the present study was to assess the presence of *Arcobacter*
78 species carrying virulence-associated genes in shellfish from Southern Italy in order to evaluate the
79 potential routes of *Arcobacter* spp.

80

81 ## 2. Materials and methods

82 ### *2.1 Sampling*

83 A total of 70 shellfish samples were obtained from a local fish market in the Apulia region (SE
84 Italy) between January 2014 and February 2015. The samples were made up of 42 mussel (*Mytilus*
85 *galloprovincialis*) and 28 clam (*Tapes philippinarum*) samples, which were taken to the laboratory
86 in cooled containers (4 °C) and processed within 24 h of purchase.

87

88 ### *2.2 Cultural Analysis*

89 Shellfish were aseptically prepared for analysis according to the UNI EN ISO 6887-3:2003 standard
90 procedure (UNI EN ISO 6887-3:2003, 2003). For *Arcobacter* isolation, 10 g of flesh and intra-
91 valvular liquid were added to 90 mL (1:10 wt/vol) of *Arcobacter* broth (Oxoid, Basingstoke, UK)

92 supplemented with Cefoperazone, Amphotericin B and Teicoplanin (CAT selective supplement
93 SR0174E; Oxoid, Basingstoke, UK), in sterile bags and homogenized using a stomacher (PBI
94 International, Milan, Italy) at 11 000 rev min $^{-1}$ for 1 min, as previously reported by other authors
95 (Bonerba et al., 2015; Collado et al., 2009). Then the bags were closed and incubated at 30 °C under
96 aerobic conditions for 48 h. After enrichment, 200 µL of the broth was inoculated by passive
97 filtration onto the surface of a 0.45µm membrane filter (Sartorius Stedim Biotech GmbH,
98 Germany), placed onto selective agar plates prepared by suspending 24 g of *Arcobacter* broth
99 (Oxoid, Basingstoke, United Kingdom) and 12 g of agar technical no. 3 (Oxoid, Basingstoke,
100 United Kingdom) and supplemented with a selective antibiotic mix supplement (cefoperazone [16
101 mg/liter], amphotericin B [10 mg/liter], 5-fluorouracil [100 mg/liter], novobiocin [32 mg/liter], and
102 trimethoprim [64 mg/liter]) as described by Houf et al. (2001). Plates were incubated at room
103 temperature for 30 min. After filtration, the filters were removed and the dishes were incubated at
104 30 °C under both aerobic and microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2), produced by
105 the Campy*Gen* gas generating system (Oxoid, Basingstoke, UK). After incubation, typical
106 *Arcobacter* colonies (i.e. small colorless, translucent, convex with an intact edge) were picked,
107 subcultured onto Blood Agar and incubated for 48h at 30 °C. The colonies were confirmed
108 morphologically by Gram staining and by determination of oxidase (Oxidase strips, Oxoid
109 Microbact, Basingstoke, UK) and catalase activity.
110

111 *2.3 Biomolecular analysis*

112 *2.3.1 DNA extraction and purification*

113 The colonies identified as *Arcobacter* spp. were transferred onto *Arcobacter* broth (BA) (Oxoid,
114 Basingstoke, UK) and incubated at 30 °C for 48h. One milliliter BA pure culture of presumptive
115 *Arcobacter* spp. was centrifuged at 7500 rpm for 10 min at room temperature. DNA extraction and

116 purification was performed with the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) and
117 eluted with 80 µL AE Elution Buffer (QIAGEN, Hilden, Germany). The DNA concentration and
118 purity were established by evaluating the ratio A_{260nm}/A_{280nm} using a NanoDrop 2000/2000c
119 Spectrophotometer (Thermo Scientific, MA, USA).
120

121 *2.3.2 m-PCR*

122 In order to determine the identity of the *Arcobacter* isolates, a multiplex-PCR assay was performed
123 as described by Houf et al. (2000). Briefly, 2µL (50 ng) of DNA template were added to 48 µL of
124 the reaction mixture containing 5µL 10X PCR Rxn Buffer (Invitrogen, Carlsbad, CA, USA), 1.5 U
125 of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.2 mmol of each deoxyribonucleotide
126 triphosphate (appl. Biosystems), 1.3 µL of 50 mmol $MgCl_2$ (Invitrogen, Carlsbad, CA, USA). Then
127 50 pmol of the primers ARCO, BUTZ, CRY1, CRY2 and 25 pmol of primer SKIR were added
128 (**Table 1**). The amplification followed conditions described by Houf et al. (2000) and involved an
129 initial denaturation step at 94 °C for 5 min, followed by 32 cycles of denaturation at 94 °C for 45
130 sec, primer annealing at 61 °C for 45 sec, and chain extension at 72 °C for 30 sec. The final
131 extension was carried out at 72 °C for 1 min. The amplifications were carried out in a Mastercycler
132 personal (Eppendorf, Milan, Italy). The positive (*A. butzleri* ATCC 49616^T, *A. cryaerophilus*
133 ATCC 43158^T, and *A. skirrowii* ATCC 51132^T) and negative controls (no added template) were
134 included to verify the purity of the extraction reagents. All reactions were performed in duplicate.
135 The generated m-PCR products were separated by gel electrophoresis on 1.5% (w/v) agarose NA
136 (Pharmacia, Uppsala, Sweden) gel in 1X Tris-borate-EDTA (TBE) buffer containing 0.089 M Tris,
137 0.089 M boric acid, 0.002 M EDTA, pH 8.0 (USB, Cleveland, OH, USA), and stained with
138 RedSafe™ gel (INtRON Biotechnology). A Gene Ruler™ 50 bp DNA Ladder Plus (Invitrogen,
139 Life Thecnologies, Italy) was used as the molecular weight marker. Image acquisition was
140 performed using UVITEC (Eppendorf).

141

142 *2.3.3 16S rDNA-RFLP*

143 The 16S rDNA-RFLP assay described by Figueras et al. (2008) was performed to confirm the m-
144 PCR results (Levican and Figueras, 2013). Briefly, the amplification of 1026 bp of the 16S rRNA
145 gene was carried out with a 50µL PCR mixure containing 5 µL (100 ng) of DNA template, 0.5 µM
146 each of primers CAH16S1am and CAH16S1b (**Table 1**), 5µL 10X PCR Buffer (Invitrogen,
147 Carlsbad, CA, USA), 1.5 µL of 50 mmol $MgCl_2$ (Invitrogen, Carlsbad, CA, USA), 200 µM of each
148 deoxyribonucleotide triphosphate (Applied Biosystems) and 2.5 U of Taq DNA polymerase
149 (Invitrogen, Carlsbad, CA, USA). The amplification profile involved an initial denaturation step at
150 95 °C for 2 min, followed by 31 cycles of denaturation at 94 °C, 30s, primer annealing at 55 °C,
151 30s, and chain extension at 72 °C for 1.30 min. The final extension was carried out at 72 °C for 10
152 min. The PCR reactions were processed in a 2720 Thermal Cycler (Appl. Biosystem). PCR-
153 amplified products were analyzed by electrophoresis on 2 % (w/v) agarose NA (Pharmacia,
154 Uppsala, Sweden) gel in 1X Tris-borate-EDTA (TBE) buffer containing 0.089 M Tris, 0.089 M
155 boric acid, 0.002 M EDTA, pH 8.0 (USB, Cleveland, OH, USA), and stained with RedSafe™ gel
156 (INtRON Biotechnology). A Gene Ruler™ 100 bp DNA Ladder Plus (MBI Fermentas, Vilnius,
157 Lithuania) was used as the molecular weight marker. Image acquisition was performed using
158 UVITEC (Eppendorf). The amplicon 1026 bp was then digested using the *MseI* endonuclease
159 (Fermentas, Schwerte, Germany). Restriction fragments were separated in 3.5% (w/v) agarose gel
160 electrophoresis in in 1X Tris-borate-EDTA (TBE) buffer containing 0.089 M Tris, 0.089 M boric
161 acid, 0.002 M EDTA, pH 8.0 (USB, Cleveland, OH, USA), and stained with RedSafe™ gel
162 (INtRON Biotechnology). A Gene Ruler™ 100 bp DNA Ladder Plus (MBI Fermentas, Vilnius,
163 Lithuania) was used as the molecular weight marker. Image acquisition was performed using
164 UVITEC (Eppendorf).

165

166 *2.3.4 Detection of virulence genes*

167 Five putative *Arcobacter* virulence genes (*ciaB, cadF, cj1349*, *hecA* and *irgA*) were detected using
168 the primers and conditions designed by Douidah et al., 2012 (**Table 1**). *A. butzleri* LMG10828^T and
169 *A. thereius* LMG24486^T were used as positive and negative control, respectively. Briefly, the PCR
170 reactions were performed in a final volume of 50 µL, containing 2µL of DNA extract (50 ng/µL),
171 1.5U of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA Invitrogen, Carlsbad, CA, USA),
172 0.2 µmol of each deoxyribonucleotide triphosphate (Appl. Biosystems), 0.2 mmol $MgCl_2$ for the
173 primer sets for *ciaB, cj1349, hecA* and *irgA*. For the primer sets for *cadF*, 5 µL 10X PCR buffer and
174 1.5 mmol $MgCl_2$ were used. 2 µL of DNA template were added in the PCR reaction. PCR consisted
175 in an initial denaturation step at 94 °C for 30 sec, followed by 32 cycles of denaturation at 94 °C for
176 45 sec, primer annealing at 56 °C for 45 sec for primers designed for *ciaB, cj1349, hecA* and *irgA*
177 and at 55 °C for the primer sets for *cadF*, while an extension step was performed at 72 °C for 45
178 sec. Final extension was carried out at 72 °C for 3 min. PCR-amplified products were detected by
179 electrophoresis on 1.5 % (w/v) agarose gel gel in 1X Tris-borate-EDTA (TBE) buffer containing
180 0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8.0 (USB, Cleveland, OH, USA), and stained
181 with RedSafe^{TM} gel (INtRON Biotechnology). A Gene RulerTM100 bp DNA Ladder Plus (MBI
182 Fermentas, Vilnius, Lithuania) was used as the molecular weight marker. Image acquisition was
183 performed using UVITEC (Eppendorf).

184

185 **3. Results**

186 *3.1 Cultural Analysis*

187 The cultural analysis carried out on shellfish showed typical small, smooth, translucent, and watery
188 colonies in 16 (22.8%) of the 70 shellfish samples. All organisms resulted Gram-negative, slightly
189 curved rods, oxidase- and catalase-positive and were presumptively identified as *Arcobacter* spp.

190 Specifically, *Arcobacter* spp. were highlighted in 10/42 (23.8%) mussel and in 6/28 (21.4%) clam
191 samples.
192

193 *3.2 Molecular analysis*

194 *3.2.1 m-PCR*

195 The m-PCR carried out on the 16 isolates showed the characteristic amplicon of *A. butzleri* in 12/16
196 (75%) and the *A. cryaerophilus* amplicon in 4/16 (25%).
197

198 *3.2.2 16S rDNA-RFLP*

199 Digestion of the 16S rRNA gene with the endonuclease *MseI* produced the expected species-
200 specific *A. butzleri* and *A. cryaerophilus* 1B RFLP patterns, thus confirming the m-PCR results
201 (**Table2**).
202

203 3.2.3 *Detection of virulence genes*

204 *Arcobacter* isolates tested by PCR for the presence of putative virulence genes showed amplicons
205 with expected sizes for the different virulence genes (**Table 3**). Indeed, all (12/12) isolates of *A.*
206 *butzleri* harbored *cadF*, *hecA* and *irgA* genes but the *ciaB* gene was present only in 5/12 and *cj1349*
207 only in 2/12 of the isolates. All five investigated virulence genes (*cadF*, *ciaB*, *cj1349*, *irgA* and
208 *hecA*) were simultaneously detected in only 2/12 *A. butzleri* strains.
209 Among the *A. cryaerophilus* isolates, 4/4 strains were positive for the *cadF* gene and 3/4 and 1/4 for
210 the *hecA* and *ciaB* genes, respectively. None of the strains possessed the *cj1349* or *irgA* genes.
211

212 **4. Discussion**

213 This is the first study of the occurrence of putative virulence genes in potentially pathogenic
214 *Arcobacter* species in shellfish from Italy by cultural methods and biomolecular analysis. In

215 agreement with previous studies carried out in different geographical areas (Collado et al., 2014;
216 Collado et al., 2009; Levican et al., 2014), this study confirms that potentially pathogenic
217 *Arcobacters* are frequently found in edible lamellibranch mollusc samples. In this sense, *A. butzleri*
218 was the most prevalent species (75%) found in this and other studies (Collado et al., 2014; Nieva-
219 Echevarria et al., 2013;). Although both *A. butzleri* and *A. cryaerophilus* were associated with
220 several cases of human disease (Collado and Figueras, 2011; Figueras et al., 2014; Hsueh et al.
221 1997; Kayman et al., 2012; Lau et al., 2002; Lerner et al., 1994; McGregor et al., 2015; On et al.,
222 1995; Woo et al., 2001; Yan et al., 2000), little is known about the route of infection and
223 transmission of this species. Moreover, the mechanism of pathogenicity related to this genus still
224 needs to be fully understood. The present research evaluated the presence of five putative virulence
225 factors in isolates from shellfish. In agreement with previous studies carried out by Douidah et al.
226 (2012), Karadas et al. (2013) and Tabatabaei et al. (2014), all analyzed strains harbored a high
227 occurrence of the *cadF* gene. However, in contrast with the same authors who reported a high
228 occurrence of the *ciaB* and *cj1349* genes and a low presence of the *irgA* gene in all *A. butzleri*
229 isolates tested, the present study showed a high occurrence of various different genes. All the
230 analyzed *A. butzleri* strains harbored the *irgA* and *hec* genes. In this study, all the strains of *A.*
231 *cryaerophilus* investigated harbored at least one gene i.e. *cadF (4/4), hecA (3/4)* and *ciaB* (1/4),
232 while none of the strains studied had the *cj1349* or *irgA* genes. The *cadF* gene was widely
233 distributed among *Arcobacter* isolates. Our results were similar to those reported by Levican et al.
234 (2014), who found that the *irgA* gene was absent in all *A. cryaerophilus* isolates studied. However,
235 the detection of *ciaB* was lower in our study. When comparing our results for *A. cryaerophilus*
236 recovered from mussels and clams with those obtained by Collado et al. (2014) for the same strain
237 and shellfish species, *cadF* and *hecA* were present in most of our samples but absent in Collado et
238 al.'s (2014).

239 Similarly, in *A. cryaerophilus* recovered from a gastroenteritis case, Figueras et al. (2014) detected
240 only the *ciaB* gene, again in contrast with our results.
241 The different results observed in the present study in relation to those found by other authors could
242 be linked to several factors such as the different identification and cultural methodology used or the
243 climatic and geographical areas where samples were collected. Moreover, further investigations into
244 the presence and distribution of virulence factors are required. Especially, further comparison of the
245 data between *Arcobacters* isolated from food and strains detected in clinical cases will be required
246 if we are to understand whether there is a correlation between contaminated food consumption and
247 disease. However, International Standard procedures are required to facilitate the comparison of
248 data observed by other researchers.
249 The results of the present study provided much information regarding the health risks associated
250 with the consumption of raw bivalve molluscs. As previously observed for *Vibrio,* this study
251 confirms that standard shellfish purification technologies are inefficient at removing all pathogenic
252 and potentially pathogenic bacteria (Carraro et al., 2015; Di Pinto et al., 2005; Di Pinto et al., 2006;
253 Tantillo et al., 2004). Moreover, even though the legislation regulating food safety requires
254 assessment of *E. coli* and *Salmonella* contamination (EC Regulation no. 1441/2007), the increase in
255 food-borne disease related to the consumption of raw shellfish suggests that further epidemiological
256 data are required to establish more specific microbiological criteria in seafood and to apply new
257 depuration technologies in order to guarantee food safety. Therefore, effective national and
258 European food control systems are essential to protect consumer health by implementing routine
259 research into the emerging pathogens in this food chain.
260

261 **5. Conclusions**

262 In summary, the results in the present paper demonstrate that bivalve molluscs are potential
263 pathogenic *Arcobacter* hosts. Furthermore, additional studies are needed to provide data that will

264 help extend knowledge and confirm the role played by contaminated shellfish consumption in
265 human disease.
266
267

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Table 1
Oligonucleotide primers

Method	Primer Sequence 5' to 3'	Primer sequence (5'- 3')	Gene	Amplicon Size (bp)	References
m-PCR	ARCO (R)	CGTATTCACCGTAGCATAGC	16S rRNA		Houf *et al.* (2000)
	BUTZ (F)	CCTGGACTTGACATAGTAAGAATGA	16S rRNA	401	Houf *et al.* (2000)
	SKIRR (F)	GGCGATTTACTGGAACACA	16S rRNA	641	Houf *et al.* (2000)
	CRY 1 (F)	TGCTGGAGCGGATAGAAGTA	23S rRNA	257	Houf *et al.* (2000)
	CRY 2 (R)	AACAACCTACGTCCTTCGAC	23S rRNA		
16S rRNA	CAH16S1am (F)	AACACATGCAAGTCGAACGA	16S rRNA	1026	Figueras *et al.* (2008)
	CAH16S1b (R)	TTAACCCAACATCTCACGAC	16S rRNA		Marshall *et al.* (1999)
Virulence genes:					
cadF	cadF (F)	TTACTCCTACACCGTAGT	*cadF*	283	Duidhal *et al.* (2012)
	cadF (R)	AAACTATGCTAACGCTGGTT			
ciaB	ciaB (F)	TGGGCAGATGTGGATAGAGCTTGGA	*ciaB*	284	Duidhal *et al.* (2012)
	ciaB (R)	TAGTGCTGGTCGTCCCACATAAAG			
cj1349	cj1349 (F)	CCAGAAATCACTGGCTTTTGAG	*cj1349*	659	Duidhal *et al.* (2012)
	cj1349 (R)	GGGCATAAGTTAGATGAGGTTCC			
irgA	irgA (F)	TGCAGAGGATACTTGGAGCGTAACT	*irgA*	437	Duidhal *et al.* (2012)
	irgA (R)	GTATAACCCCATTGATGAGGAGCA			
hecA	hecA (F)	GTGGAAGTACAACGATAGCAGGCTC	*hecA*	537	Duidhal *et al.* (2012)
	hecA (R)	GTCTGTTTTAGTTGCTCTGCACTC			

Table 2
Molecular identification results

Sample n.	Isolate	Country	Strain source	Collection date	m-PCR Houf 2000	16S rDNA-RLFP
1	4	Torre a mare (BA)	*Mussels- Mytilus galloprovincialis*	January-2014	*A. butzleri*	*A. butzleri*
2	7	Bari	*Mussels- Mytilus galloprovincialis*	February-2014	*A. cryaerophilus*	*A. cryaerophilus 1B*

3	21	Noci (BA)	*Mussels- Mytilus galloprovincialis*	May-2014	*A. cryaerophilus*	*A. cryaerophilus 1B*
4	24	Noci (BA)	*Mussels- Mytilus galloprovincialis*	May-2014	*A. cryaerophilus*	*A. cryaerophilus 1B*
5	25	Noci (BA)	*Mussels- Mytilus galloprovincialis*	September-2014	*A. butzleri*	*A. butzleri*
6	28	Noci (BA)	*Mussels- Mytilus galloprovincialis*	September-2014	*A. butzleri*	*A. butzleri*
7	34	Noci (BA)	*Mussels- Mytilus galloprovincialis*	October-2014	*A. butzleri*	*A. butzleri*
8	37	Noci (BA)	*Mussels- Mytilus galloprovincialis*	October-2014	*A. butzleri*	*A. butzleri*
9	38	Noicattaro (BA)	*Mussels- Mytilus galloprovincialis*	January-2015	*A. butzleri*	*A. butzleri*
10	39	Noicattaro (BA)	*Mussels- Mytilus galloprovincialis*	January-2015	*A. butzleri*	*A. butzleri*
11	1V	Noci (BA)	*Clams-Tapes philippinarum*	September-2014	*A. butzleri*	*A. butzleri*
12	5V	Noci (BA)	*Clams-Tapes philippinarum*	October-2014	*A. butzleri*	*A. butzleri*
13	6V	Noicattaro (BA)	*Clams-Tapes philippinarum*	October-2014	*A. butzleri*	*A. butzleri*
14	11V	Noicattaro (BA)	*Clams-Tapes philippinarum*	December-2014	*A. butzleri*	*A. butzleri*
15	15V	Valenzano (BA)	*Clams-Tapes philippinarum*	January-2015	*A. butzleri*	*A. butzleri*
16	16V	Altamura (BA)	*Clams-Tapes philippinarum*	February-2015	*A. cryaerophilus*	*A. cryaerophilus 1B*

416

417 **Table 3**
418 Occurrence of virulence-associated genes in *Arcobacter* isolates
419

Species	Isolate	Strain source	*cadF*	*ciaB*	*cj1349*	*irgA*	*hecA*
A. butzleri	4	*Mussels- Mytilus galloprovincialis*	+	-	-	+	+
	25	*Mussels- Mytilus galloprovincialis*	+	-	-	+	+
	28	*Mussels- Mytilus galloprovincialis*	+	-	-	+	+
	34	*Mussels- Mytilus galloprovincialis*	+	-	-	+	+
	37	*Mussels- Mytilus galloprovincialis*	+	+	+	+	+
	38	*Mussels- Mytilus galloprovincialis*	+	+	+	+	+
	39	*Mussels- Mytilus galloprovincialis*	+	+	-	+	+
	1V	*Clams-Tapes philippinarum*	+	-	-	+	+
	5V	*Clams-Tapes philippinarum*	+	-	-	+	+
	6V	*Clams-Tapes philippinarum*	+	+	-	+	+
	11V	*Clams-Tapes philippinarum*	+	-	-	+	+
	15V	*Clams-Tapes philippinarum*	+	+	-	+	+
A. cryaerophilus 1B	21	*Mussels- Mytilus galloprovincialis*	+	-	-	-	+
	24	*Mussels- Mytilus galloprovincialis*	+	+	-	-	+
	7	*Mussels- Mytilus galloprovincialis*	+	-	-	-	+
	16V	*Clams-Tapes philippinarum*	+	-	-	-	-

420