Influence of cell history on the subsequent inactivation of campylobacter jejuni during cold storage under modified atmosphere

Benjamin Duque, Nabila Haddad, Albert Rossero, Jeanne Marie Membre, Sandrine Guillou

Influence of cell history on the subsequent inactivation of *Campylobacter jejuni* during cold storage under modified atmosphere

Benjamin Duqué, Nabila Haddad, Albert Rossero, Jeanne-Marie Membré and Sandrine Guillou*

SECALIM, INRA, Oniris, Université Bretagne Loire, 44307, Nantes, France

*Corresponding author. SECALIM, INRA, Oniris, Université Bretagne Loire, 44307, Nantes, France. Tel.: +33 240 687 763

E-mail address: sandrine.guillou@oniris-nantes.fr (S. Guillou)

Abstract

Worldwide, *Campylobacter* infections are the main cause of human bacterial enteritis and broiler meat is considered as the most important source of human campylobacteriosis. Some mitigation strategies have been focused on reduction of *Campylobacte*r at the slaughtering steps. This study aimed to determine the influence of consecutive stresses inspired by slaughtering steps on the subsequent inactivation of *Campylobacter jejuni* during cold storage under different modified atmospheres. Using a full experimental design, three strains of *C. jejuni* of poultry origin were submitted to consecutive heat (46°, 50° or 54°C for 4 min) and cold (-4° or 3°C for 2 h) stresses by plunging cultures into baths at appropriate temperatures. Cultures were then stored at 6°C during seven days under modified atmospheres (70% O_2 / 30% CO_2 or 50% CO_2 / 50% N_2). Inactivation of *C. jejuni* induced by cold storage was shown to depend significantly ($P<0.0001$) upon the heat stress previously applied. It was shown to be the highest under the atmosphere enriched in oxygen, after application of 54°C. Strain inactivation variability was also quantified. These results show that consecutive stresses influence further inactivation of *C. jejuni* during storage and consequently the contamination level at consumer's plate.

Keywords: foodborne pathogen; slaughter process; stresses; food safety; strain variability

1. Introduction

Since 2005, *Campylobacter* has been the main cause of bacterial enteritis worldwide in humans (EFSA and EDC 2018). Despite being largely under-estimated, the number of reported confirmed cases of *Campylobacteriosis* was 246,158 in 2017 with an EU notification rate of 64.8 per 100,000 population (EFSA and EDC 2018). Infection with *C. jejuni* can lead to chronic sequelae, such as Guillain-Barré syndrome (GBS), characterized by the damage of the peripheral nervous system leading to a reversible neuromuscular paralysis (Nyati and Nyati 2013).

The main reservoirs for *C. jejuni* are avian species and farmed poultry (Young et al. 2007). In food, the occurrence of *Campylobacter* remains high in broiler meat (EFSA and EDC 2018) and it is considered to be the most important single source of human *Campylobacteriosis*. In 2017, 37.4% of the 13,445 samples of fresh broiler meat (single or batch, aggregated data from all sampling stages) were found to be *Campylobacter* positive (EFSA and EDC 2018).

Several microbiological risk assessment studies have been performed to assess the potential effects of control measures on prevention/reduction of *Campylobacter* concentration in broiler meat production. Some studies focused more on pre-slaughter stages associated with broiler rearing (Hermans et al. 2011, Lin 2009) including the use of probiotics (Saint-Cyr et al. 2017) or vaccine (Meunier et al. 2017). Another effective strategy to reduce the number of campylobacteriosis cases may reside in limiting the entry of chicken carcasses highly contaminated by *Campylobacter* into the market. In this context, the European Commission has just set a microbiological process hygiene criterion for *Campylobacter* in broiler carcasses (European Commision 2017). A limit of 1000 CFU.g^{-1} on carcasses after chilling has been defined. The EFSA (European Food Safety Authority) determined that more than 50% human risk reduction could be achieved if broiler carcasses complied with this new microbiological criteria of 1000 CFU.g^{-1} (European Commision 2017). For food producers, the compliance with this hygienic criterion requires improvements in slaughter hygiene to limit cross-contamination but also investigation of new control measures which may favor

58 *Campylobacter* inactivation following slaughter. Focusing on the susceptibility of the bacteria
59 to the processing steps may represent one way to tackle *Campylobacter* risk.
60 *C. jejuni* is able to survive to slaughterhouse environments and during poultry
61 processing. In the poultry farm-to-fork chain, several steps may be stressful for bacteria
62 regarding its growth requirements (microaerophily and thermotolerance), such as scalding,
63 chilling and storage. Scalding consists in immersing chicken carcasses into a hot water bath,
64 which may induce heat stress to *C. jejuni*. At the end of the slaughter process, the chilling
65 step enables quick refrigeration of chicken carcasses, which is necessary to lower the growth
66 rate of pathogenic and spoilage microorganisms (James et al. 2006), is supposed to
67 generate cold stress to *C. jejuni*. At last, chicken cuts are generally conditioned under
68 modified atmosphere and stored under chilled temperature until consumption. Depending on
69 the producer, the gas mix of the package may contain high concentrations of oxygen or not.
70 This latter step induces both cold and possibly oxidative stresses. However, if the influence
71 of single stresses on *Campylobacter* behavior is well-described, the effect of stresses
72 occurring sequentially still needs to be more deeply understood in order to point out
73 conditions of slaughter steps that favor *Campylobacter* inactivation during poultry products
74 storage.
75 Indeed, the exposure to a stress may condition the bacterial behavior following the exposure
76 to another stress. Increased resistance has been for example already reported in *Bacillus*
77 (den Besten et al. 2010, Desriac et al. 2013). For instance, after being subjected to mild acid
78 or oxidative stress, *C. jejuni* appeared to be more resistant to a subsequent lethal stress
79 (Murphy et al. 2003a, Murphy et al. 2003b, Oh et al. 2015). Such cross-protection response
80 from one stress to another has also been reported, like genes involved in heat shock
81 response in *C. jejuni* NCTC 11168 which were upregulated in response to acid stress (Reid
82 et al. 2008b). Additionally, starved *C. jejuni* cells were able to better withstand heat stress
83 (Klančnik et al. 2009).
84 Besides, it is well established that strains have different behavior when subjected to
85 stress (Chan et al. 2001, Cools et al. 2003, Newell et al. 2001) and this is highly relevant for

86 poultry product in the sense that poultry meat is naturally contaminated by different strains of
87 *C. jejuni* and often more than one species (Colles et al. 2003, Johnsen et al. 2006, Rivoal et
88 al. 2005)
89 In the current study, several strains of *C. jejuni* were submitted to stresses inspired from
90 those encountered during slaughter, and subsequently incubated under modified
91 atmosphere. The effect of cell history *i.e.* the effect of hot and cold temperature applied
92 following successive heat and cold stresses, on the subsequent inactivation of *C. jejuni*
93 strains during chilled storage under various modified atmospheres, was then quantitatively
94 analyzed.
95

2. Materials and methods

2.1 Bacterial strains and culture conditions

Experiments were performed on three *C. jejuni* strains isolated from poultry products. *C. jejuni* C09MJLT205 was isolated from chicken legs distributed at the market level (Guyard-Nicodeme et al. 2013). *C. jejuni* RM1221 was isolated by Fouts et al. (2005). The strain *C. jejuni* C97Anses640 was isolated from a poultry product (Guyard-Nicodeme et al. 2016). All strains were stored at -80°C in brain heart infusion (BHI, Biomérieux, Marcy l'Etoile, France) supplemented with 20% (v/v) glycerol. *C. jejuni* strains were routinely cultured on Karmali (Oxoid, Dardilly, France) agar plates at 42°C for 48-72 h under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2). Then, one or two colonies were transferred into 20 mL of Mueller-Hinton broth (MH, Oxoid, Dardilly, France) and incubated for 20 h under the same conditions under agitation (90 rpm). A $1/100^{th}$ dilution was transferred into a fresh MH broth and incubated under the same conditions for 18 h, so that the culture reached stationary phase. Finally, a $1/10^{th}$ dilution was carried out to obtain what we called the 'starter culture' further used for challenge-test experiments.

To enumerate viable *C. jejuni* cells, suspensions were serially tenfold diluted and surface plated on Columbia sheep blood agar plates (Biomérieux, Marcy l'Etoile, France) and incubated for 48 h at 42°C under microaerophilic conditions using a SPIRAL plater (EasySpiral Interscience, Saint Nom, France).

2.2 Experimental procedure

C. jejuni strains were submitted to stresses inspired from conditions encountered during slaughter chicken process. Slaughter is associated with several steps considered as stressful for *C. jejuni*. These steps are scalding, chilling, and are followed by cold storage under modified atmosphere. Scalding consists in immersing chicken carcasses into hot water bath. Then, chicken carcasses are submitted to a quick refrigeration during the chilling step.

122 At last, after cutting carcasses in pieces, chicken cuts are conditioned under modified
123 atmosphere and stored under chilled temperature.
124 The three previously mentioned stresses were reproduced as closely as possible in
125 the laboratory, and named as heat stress, cold stress and storage stress, respectively. The
126 first two stresses, *i.e.* heat and cold stresses were assumed to influence the future behavior
127 of *C. jejuni* during chilled storage under modified atmosphere. As such, they were considered
128 as participating to *C. jejuni* adaptive history (Fig. 1).
129 Ten mL of starter culture with an initial concentration of 8 $\log_{10}$ CFU.mL^{-1} from each strain
130 were transferred into a glass tube and submitted to consecutive heat, cold temperature and
131 storage at 6°C under modified atmospheres according to a full experimental design.
132 Heat stress consisted in immersing *C. jejuni* cultures in hot water baths at 46°, 50° or
133 54°C for 4 min. 54°C was chosen as the maximal observed temperature of scalding baths in
134 French chicken slaughterhouses, 46 °C, the maximum growth temperature (Hazeleger et al.
135 1998) of *C. jejuni* and 50°C, an intermediate temperature.
136 After exposure to heat stress, tubes were immediately cooled for 5 min in a water bath
137 at 22°C, corresponding to the conditions of ambient temperature occurring during the five
138 minutes between scalding and chilling steps in slaughterhouse.
139 Cold stress was applied immediately after cooling by plunging the previous cultures into cold
140 water with ethylene glycol at -4° or 3°C for 2 h. The temperature of 3°C was chosen because
141 it represents the targeted core temperature of poultry carcasses during the chilling step and -
142 4°C is the minimal surface temperature measured during different visits performed in several
143 slaughterhouses. Strains were exposed to cold stress for 2 h similarly as chicken carcasses
144 during the chilling step.
145 At last, cultures were stored at 6°C for seven days under the modified atmospheres
146 (70% O_2 / 30% CO_2 or 50% N_2 / 50% CO_2) commonly used for the packaging of poultry cuts.
147 These two atmospheres were generated by filling jars with the *ad hoc* gas mixture (Air
148 Liquide, Carquefou, France). Cultures were placed in bottles with porous silicone caps
149 (Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Allemagne) enabling gas exchange.

150 The temperature of 6°C was chosen according to the average temperature of consumer
151 refrigerators (Laguerre et al. 2001).
152 During experiments, the temperature applied during heat and cold stress was
153 recorded using a temperature probe Kistock KTT220 (KIMO, Montpon, France) placed in a
154 control tube. The gas composition (% O_2 and % CO_2) of the jar atmosphere was measured
155 using the Oxybaby M+ device (WITT-GASETECHNIK GmbH & Co KG, Witten, Germany). It
156 was performed just after filling with gas mixture and regularly over time to check the stability
157 of the gas composition.
158 After each step, viable counts of *C. jejuni* were enumerated to determine the viability loss
159 resulting from the application of each step, *i.e.* $\Delta\log_{10}$. A total of 152 (3 strains x 4 repetitions
160 x 12 experimental conditions + 8 controls) experiments were performed.
161

162 *2.3 Statistical analysis*

163 Four independent replicates were carried out and statistical analysis was performed on
164 $\Delta\log_{10}$ transformed values. An ANOVA (α=5%) was performed using the software XLSTAT
165 (version 19.03.45028), an Addin of the Microsoft Excel software.
166

3. Results

3.1 Cumulative effect of subsequent heat, cold and storage stresses on inactivation of C. jejuni (Δlog_{total})

From the starter culture with an initial concentration of 8 log_{10} CFU.mL^{-1}, the mean cumulative *C. jejuni* inactivation, due to the consecutive heat, cold and storage stresses, varied from 0.02 to 4.9 log_{10} CFU.mL^{-1} as function of strains and atmosphere conditions. Detailed results are available in supplementary material Table S1.

During experiments, controls were performed and submitted only to the cold storage under modified atmosphere. The inactivation induced by the only storage step varied from 0.9 to 1.69 log_{10} CFU.mL^{-1} with the 70%O_2 / 30% CO_2 atmosphere and from 0.1 to 0.3 log_{10} CFU.mL^{-1} with the 50% N_2 / 50% CO_2 atmosphere.

For the bacterial samples undergoing all steps, the storage step with the 70%O_2 / 30% CO_2 atmosphere, had the main impact on *C. jejuni* inactivation, the bacterial population decrease varied from 1.0 to 3.9 log_{10} CFU.mL^{-1} (Fig. 2a). In addition, the highest inactivation during storage was reached after the application of the highest temperature during heat stress. Heat only produced an inactivation following application of 54°C with a mean inactivation between 0.8 and 0.9 log_{10} CFU.mL^{-1}, whereas the inactivation was negligible when 50° and 46°C were applied. In our conditions, cold temperature had also a negligible impact on the *C. jejuni* inactivation, indeed a maximum of 0.3 log_{10} CFU.mL^{-1} reduction was achieved. The highest cumulative inactivation was obtained for the C97anses640 strain. This strain appeared to be less resistant than C09MJLT205 and RM1221 strains, especially during storage. For instance, the inactivation of strain C97anses640 at 50°C / -4°C was 3.3 log_{10} CFU.mL^{-1} (mean value) compared to 1.2 log_{10} CFU.mL^{-1} for both C09MJLT205 and RM1221 strains (Fig. 2b).

Using the 50% N_2 / 50% CO_2 atmosphere, the cumulative inactivation of *C. jejuni* was mainly attributable to the storage step and varied from 0.1 to 0.9 log_{10} CFU.mL^{-1} (Fig. 2b). However, especially at 54°C, the heat stress contributed equally with the cold storage to the

194 cumulative inactivation, except for the strain RM1221. Indeed, for the latter, the inactivation
195 in the hot water bath was higher than during the storage step, *i.e.* mean values of 1.5 and 0.9
196 log_{10} CFU.mL^{-1}, respectively.
197
198 *3.2 Effect of heat/cold temperature, modified atmosphere, strains and interactions*
199 *on subsequent inactivation during storage affected by cell history ($\Delta log_{storage}$)*
200 When subjected to mild stress, bacteria may become more resistant to another stress
201 applied subsequently. In this study, we tested if the application of successive heat and cold
202 temperature enhanced the resistance of *C. jejuni* during storage. In other words, we studied
203 the effect of cell adaptive story on the subsequent behavior of *C. jejuni*.
204 The influence of the different factors and their interaction on inactivation of *C. jejuni*
205 during cold storage was analysed by ANOVA. The ANOVA highlighted that the temperature
206 of the cold bath did not influence significantly the inactivation during storage. Therefore, a
207 new ANOVA was performed without considering cold stress. The model was significant ($p <$
208 0.0001) as indicated in Table 1.The ANOVA showed that there were significant effects of
209 strain, temperature applied during heat stress, and packaging atmosphere. During the cold
210 storage, strains C09MJLT205 and RM1221 exhibited similar inactivation around 1.1 log10
211 CFU.mL^{-1}. In contrast, the strain C97anses640 appeared to be less resistant to storage than
212 the two other strains with a mean inactivation of 1.8 log10 CFU.mL^{-1} (Fig. 3a).

213 Regarding heat stress, the highest temperature of 54°C induced the highest inactivation
214 associated with storage. Indeed, the mortality reached a mean of 1.9 log_{10} CFU.mL^{-1}
215 compared to that obtained at 46° and 50°C (1 log_{10} CFU.mL^{-1}) (Fig. 3b).
216 Inactivation of *C. jejuni* during storage was more than five times higher under the
217 modified atmosphere 70% O_2 / 30% CO_2 than under the atmosphere 50% N_2 / 50% CO_2 ($p <$
218 0.0001), with a mean inactivation of 2.2 log_{10} CFU.mL^{-1} and 0.4 log_{10} CFU.mL^{-1}, respectively
219 (Fig. 3c).

220 The ANOVA showed significant interactions between strains and atmosphere,
221 temperature applied during heat stress and atmosphere.
222 While the mean inactivation resulting from storage under the atmosphere 50% N_2 / 50% CO_2
223 was low and similar no matter the strain used, (between 0.3 and 0.5 log_{10} CFU.mL^{-1}), the
224 inactivation was largely higher under 70% O_2 / 30% CO_2 and different between strains ($p <$
225 0.0001). Indeed, strain C97anses640 appeared to be less resistant than the strains
226 C09MJLT205 and RM1221. The mean inactivation of strain C97anses640 was 3.2 log_{10}
227 CFU.mL^{-1} compared to that of C09MJLT205 and RM1221 strains, *i.e.* 1.9 and 1.7 log_{10}
228 CFU.mL^{-1}, respectively (Fig. 3d). Besides, this study pointed out that similar inactivation
229 during heat treatment does not necessarily lead to similar inactivation during storage. Indeed,
230 under 70% O_2 / 30% CO_2 atmosphere, the inactivation during heat stress was similar for
231 each strain but higher for the strain C97anses640 during the storage. Thus, this result
232 highlights the importance of the cell history, and the difficulty to predict future behavior while
233 considering only a previous phenotypic response to a specific stress.
234 A significant interaction between atmosphere and heat stress was characterized by a
235 similar mean inactivation at 46° and 50°C under the atmosphere 70% O_2 / 30% CO_2, (1.7
236 and 2.1 log_{10} CFU.mL^{-1}, respectively), compared to that obtained at 54°C, *i.e.* 3.0 log_{10}
237 CFU.mL^{-1} (Fig. 3e). Under the atmosphere 50% N_2 / 50% CO_2, the inactivation following
238 application of 54°C was also the highest and was 0.8 log_{10} CFU.mL^{-1}. In contrast, the
239 inactivation resulting from application of 46°C was slightly higher than that obtained following
240 application of 50°C, *i.e.* 0.4 and 0.1 log_{10} CFU.mL^{-1} respectively.
241

242 **4. Discussion**

243 The objective of this study was to assess the effect of the cell history on the subsequent
244 survival of three *C. jejuni* strains. Stressful steps from slaughterhouses were identified and
245 then reproduced in laboratory.
246 The first step was to immerse the bacteria in hot water bath at temperature ranging from
247 46° to 54°C. The inactivation during this step varied between 0 and 1.5 log_{10}CFU.mL^{-1}

248 according to strain and temperature. From heat inactivation, it was possible to calculate the
249 decimal reduction time or D-value. These D-values corresponds to the time necessary to kill
250 90% of an initial population or decrease the initial population by one log. The D-value is a
251 common metric and enables to compare more easily results from different studies. In our
252 experimentations, heat stress was applied during 4 min. We measured that one minute was
253 the time necessary to reach the temperature desired. Thus, once reached, the set
254 temperature was maintained during 3 min. For a selected temperature, D-values were
255 calculated by dividing the 3-min constant temperature application by Δlog lost following
256 application of the heat stress.

257 All collected D-values from different studies in the literature were gathered in Table 2. We
258 can see an important variability between results of decimal reduction times for *C. jejuni*,
259 mainly at 50°C. It appears that variability was higher at 50°C than at 55°C, which was also
260 mentioned by McCarthy et al. (2018). In our study, *C. jejuni* was submitted to heat stress in
261 suspension rather than on chicken muscle like in the real scalding step. However, the high
262 variability of collected D-values could not enable to visualize a difference of heat resistance
263 between meat matrices and laboratory media, as it could have been expected. Indeed, it has
264 been shown that the heat resistance was higher for Salmonella when attached to muscle as
265 opposed to free cells (Humphrey et al. 2007). *C. jejuni* cells could also be better recovered
266 from 5-min boiling if they were previously attached to chicken muscle as opposed to carrots
267 (de Jong et al. 2012). $D_{50°C}$-values varied from 3.5 to 39 min which corresponds to an
268 inactivation from 0.08 to 0.9 $\log_{10}$ in 3 min. $D_{55°C}$-values varied between 1.5 and 2.8 min and
269 were found in the range of what was observed in previous studies (mean $D_{55°C}$ of 1.5 ± 1.3
270 min). In light of the variation illustrated in Table 2, heat strain sensitivity, experimental
271 method, and variables such as medium may greatly influence the inactivation of *C. jejuni*. In
272 our study, for 46°C and 50°C, no inactivation has been observed. Indeed, we considered that
273 inactivation was significant if Δlog was greater than 0.5 $\log_{10}$CFU.mL^{-1} *i.e.* the experimental
274 error due to microbial enumeration (Pujol et al. 2012). These results are in accordance with
275 the very high D-values found by Nguyen et al. (2006).

276 To our knowledge the effect of cold temperature applied for a short period (*i.e*. two or
277 several hours) on *C. jejuni* has not yet been reported. In this study, application of a cold
278 temperature during two hours had no significant effect on the survival of *C. jejuni*. However,
279 these results have to be interpreted with caution, especially regarding possible extrapolation
280 with the chilling step of the slaughter process. Unlike our experimental conditions, the cold
281 temperature applied during the chilling step is generally associated with desiccation due to
282 ventilation. In laboratory, such conditions could not be mimicked. Nevertheless, the potential
283 influence of desiccation might induce microbial inactivation. For instance, Zoz et al. (2016)
284 have highlighted the impact of relative humidity on survival of *Listeria monocytogenes*.
285 Likewise, a study led by (Rivoal et al. 2016), investigated the relation between temperature,
286 chilling time and air velocity on the inactivation of eight strains of *C. jejuni* by the use of a
287 miniaturized chilling room prototype. This work highlighted the significant effect of the
288 duration of the chilling step on the inactivation of *C. jejuni*. A significant interaction between
289 the temperature and the air flow during the process was also pointed out by the authors.
290 However, it is important to reinforce here that carcasses contaminated with more than 3 log
291 $CFU.g^{-1}$ of *Campylobacter* could not be significantly decontaminated during the chilling step
292 in slaughtering house.
293 After the application of hot and cold temperature at the slaughterhouse, *C. jejuni* was
294 submitted to storage for several days under the same conditions as those found at retailers'
295 and then at consumers' home. During storage, *C. jejuni* undergoes a combination of cold
296 temperature and modified atmosphere. It was found in the current study that the inactivation
297 varied from -1.0 to -3.9 $\log_{10}$ $CFU.mL^{-1}$ in the atmosphere 70% O_2 / 30% CO_2 and from 0 to -
298 0.9 $\log_{10}$ $CFU.mL^{-1}$ in the atmosphere 50% N_2 / 50% CO_2. The inactivation of *C. jejuni* was
299 significantly influenced by the atmosphere, with a higher effect of the atmosphere 70% O_2 /
300 30% CO_2. Indeed, *Campylobacter* are microaerophilic organisms. The presence of O_2 in high
301 concentrations is toxic for the pathogen. For many years, studies have investigated the effect
302 of storage during several days under aerobiosis condition or modified atmosphere. On
303 various meat matrices, the reduction of *Campylobacter* count in aerobiosis at 4°C varied from

304 0.3 to 2 $\log_{10}$ CFU.g^{-1} (Bhaduri and Cottrell 2004, Blankenship and Craven 1982, Eideh and
305 Al-Qadiri 2011, Koidis and Doyle 1983, Phebus et al. 1991, Vashin and Stoyanchev 2011). In
306 broth, similar results have also been found (Chaisowwong et al. 2012, Chan et al. 2001,
307 Garenaux et al. 2008). However, the inactivation of *C. jejuni* was higher in our work
308 compared to what is commonly observed in the literature. Indeed, before the storage step,
309 bacteria were subjected to heat and cold stress which may induce a hurdle effect. Besides,
310 one of the atmospheres used during our experiments had a concentration in O_2 much higher
311 than in air. In other studies, it has been found that the inactivation increased with
312 atmospheres enriched in O_2 compared to atmospheres containing only CO_2 or N_2. Boysen et
313 al. (2007) showed that in broth, the inactivation under atmosphere 70% O_2 / 30% CO_2 was
314 higher than in atmosphere 70% N_2 / 30% CO_2 and 100% N_2, with a reduction of 0.5 to 2 $\log_{10}$
315 CFU.mL^{-1} and 0.3 to 0.8 $\log_{10}$ CFU.mL^{-1} respectively.
316 Such results have also been found when *C. jejuni* was submitted to paraquat, a chemical
317 oxidizing agent (Garenaux et al. 2008). After 7 days of exposition, the population was
318 reduced by 3 $\log_{10}$ CFU.mL^{-1}.
319 In this work, thermal steps were applied successively in order to assess the impact of
320 cell history on the subsequent inactivation of *C. jejuni* during storage. Some studies have
321 already investigated the potential effect of cell history or adaptation of one stress to another
322 stress. Indeed, microorganisms enduring stressful environments can protect or crossprotect
323 themselves to survive subsequent homogeneous or heterogeneous stresses, which is called
324 adaptive tolerance response (ATR). It has been shown that *C. jejuni* can induce an ATR after
325 aerobic or acid stress (Gaynor et al. 2005, Jones et al. 1993, Ma et al. 2008, Martinez-
326 Rodriguez and Mackey 2005, Murphy et al. 2003a, Murphy et al. 2003b, Reid et al. 2008a).
327 On the other hand, in our study it has been shown that after successive application of heat
328 and cold temperature, if an ATR was induced, it was insufficient to produce an adaptation
329 effect since the inactivation was higher for stressed bacteria compared to unstressed ones.
330 This study was conducted on three strains isolated from poultry and more particularly
331 on product from retailed market. Tackling strain variability is not easy because the features of

the strains which are associated with highest or lowest sensitivity to processing steps are not known. The choice was done in this study to select strains (i) representative of strains commonly found in poultry, and (ii) exhibiting *a priori* different behaviors regarding selected stresses (data not shown). Firstly, the representativeness of strains in poultry was sought by determining the strain genotype by MultiLocus Sequence Typing (MLST). The strain C97anses640 belonged to the Sequence Type-45 complex (ST-45), the strain C09MJLT205, to the ST-21 complex and the strain RM121, to the ST-354 complex. The ST-21 and ST-45 are the two main clonal complexes encountered in chicken (and poultry meat) worldwide. Interestingly, they are also commonly found among human isolates (Colles et al. 2003, de Haan et al. 2010, Dingle et al. 2002, Guyard-Nicodeme et al. 2015, Habib et al. 2009, Levesque et al. 2008, Ragimbeau et al. 2008, Sheppard et al. 2009). Secondly, the behavior variability between strains was determined regarding their inactivation during cold storage under modified atmosphere. High response variability between *C. jejuni* strains has also been reported in various studies (Habib et al. 2010, Ligowska et al. 2011, Vashin and Stoyanchev 2011). These findings highlighted the difficulty and the importance to take into account strain variability in microbial inactivation studies, and, more generally in exposure assessment.

The experimental laboratory conditions applied during the present study differ from what *C. jejuni* cells really experience during the slaughtering steps because they are not attached on muscle but free in liquid suspension, and because the chilling step does not induce desiccation. Here reside some limitations of laboratory experiments. They offer the possibility of replicating standardized procedures and measures, which is necessary to obtain the proof of concept. Passed this step, a validation step in laboratory on chicken muscle will be necessary and is currently being processed.

In conclusion, to better predict *Campylobacter* contamination in poultry processing, it is essential to assess its behavior after applying not only one stress but the whole sequence of stresses encountered during the process. Indeed, cell history plays an important role and may induce some physiological responses such as ATR which influences *C. jejuni*

360 further behavior regarding subsequent stresses. In some cases, the bacteria is able to better
361 resist to another stress whereas sometimes, the application of successive stresses induces a
362 hurdle effect, and in contrast, enhances bacterial inactivation. Finally, this study pointed out
363 that there is no clear correlation between the amount of log inactivation observed in
364 preliminary processing steps and the amount of log inactivation occurring at the latest
365 processing steps such as storage, rendering it very difficult to predict the microbial behavior
366 during the storage at retail and consumer's house from phenotypic information resulting from
367 application of previous slaughtering steps.
368 A new generation of predictive models, including cell history information, will be necessary to
369 progress along this route, and omics methods might help. These new tools consider not only
370 the bacterial behavioral response, but also the adaptation response depending on the
371 bacterial gene expression and regulation. The investigation of predictive models including
372 gene expression would enable to fine-tune existing exposure assessment models and
373 consider strain-dependent physiological response.
374

Acknowledgements

We are grateful to the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) of Ploufragan who realized the MLST analysis.

We also especially thank the Regions Pays de La Loire and Bretagne through the “Pôle Agronomique Ouest” for their financial support in the framework of the Biomics project.

381 **References**

382 Berrang, M.E. and Dickens, J.A., 2000. Presence and level of *Campylobacter spp*. on broiler
383 carcasses throughout the processing plant. Applied Poultry Science 9, 43-47.

384 Bhaduri, S. and Cottrell, B., 2004. Survival of cold-stressed *Campylobacter jejuni* on ground
385 chicken and chicken skin during frozen storage. Applied and Environmental
386 Microbiology 70, 7103-7109.

387 Blankenship, L.C. and Craven, S.E., 1982. *Campylobacter jejuni* survival in chicken meat as
388 a function of temperature. Applied and Environmental Microbiology 44, 88-92.

389 Boysen, L., Knochel, S. and Rosenquist, H., 2007. Survival of *Campylobacter jejuni* in
390 different gas mixtures. FEMS Microbiology Letters 266, 152-157.

391 Chaisowwong, W., Kusumoto, A., Hashimoto, M., Harada, T., Maklon, K. and Kawamoto, K.,
392 2012. Physiological Characterization of *Campylobacter jejuni* under Cold Stresses
393 Conditions: Its Potential for Public Threat. Journal of Veterinary Medical Science 74,
394 43-50.

395 Chan, K.F., Le Tran, H., Kanenaka, R.Y. and Kathariou, S., 2001. Survival of Clinical and
396 Poultry-Derived Isolates of *Campylobacter jejuni* at a Low Temperature (4 C). Applied
397 and Environmental Microbiology 67, 4186-4191.

398 Colles, F.M., Jones, K., Harding, R.M. and Maiden, M.C.J., 2003. Genetic Diversity of
399 *Campylobacter jejuni* isolates from Farm Animals and the Farm Environment. Applied
400 and Environmental Microbiology 69, 7409-7413.

401 Cools, I., Uyttendaele, M., Caro, C., D'Haese, E., Nelis, H.J. and Debevere, J., 2003.
402 Survival of *Campylobacter jejuni* strains of different origin in drinking water. Journal of
403 Applied Microbiology 94, 886-892.

404 de Haan, C.P., Kivisto, R., Hakkinen, M., Rautelin, H. and Hanninen, M.L., 2010. Decreasing
405 trend of overlapping multilocus sequence types between human and chicken
406 *Campylobacter jejuni* isolates over a decade in Finland. Applied and Environmental
407 Microbiology 76, 5228-5236.

de Jong, A.E., van Asselt, E.D., Zwietering, M.H., Nauta, M.J. and de Jonge, R., 2012. Extreme Heat Resistance of Food Borne Pathogens *Campylobacter jejuni*, *Escherichia coli*, and *Salmonella typhimurium* on Chicken Breast Fillet during Cooking. Int J Microbiol 2012, 196841.

den Besten, H.M., Arvind, A., Gaballo, H.M., Moezelaar, R., Zwietering, M.H. and Abee, T., 2010. Short- and long-term biomarkers for bacterial robustness: a framework for quantifying correlations between cellular indicators and adaptive behavior. PLoS One 5, e13746.

Desriac, N., Broussolle, V., Postollec, F., Mathot, A.G., Sohier, D., Coroller, L. and Leguerinel, I., 2013. *Bacillus cereus* cell response upon exposure to acid environment: toward the identification of potential biomarkers. Frontiers in Microbiology 4, 284.

Dingle, K.E., Colles, F.M., Ure, R., Wagenaar, J.A., Duim, B., Bolton, F.J., Fox, A., Wareing, D.R.A. and Maiden, M.C.J., 2002. Molecular Characterization of *Campylobacter jejuni* Clones A Basis for Epidemiologic Investigation. Emerging Infectious Diseases 8, 949-955.

Doyle, M.P. and Roman, D.J., 1981. Growth and survival of *Campylobacter fetus* subsp. *jejuni* as a Function of Temperature and pH. Journal of Food Protection 44, 596-601.

Duffy, L.L., Blackall, P.J., Cobbold, R.N. and Fegan, N., 2014. Quantitative effects of in-line operations on *Campylobacter* and *Escherichia coli* through two Australian broiler processing plants. International Journal of Food Microbiology 188, 128-134.

EFSA and EDC, 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal 16, 262.

Eideh, A.M.F. and Al-Qadiri, H.M., 2011. Effect of Refrigerated and Frozen Storage on the Survival of *Campylobacter jejuni* in Cooked Chicken Meat Breast. Journal of Food Science 76, 17-21.

434 European Commision, 2017. Commission regulation (EU) 2017/1495 of 23 August 2017
435 amending regulation (EC) no 2073/2005 as regards *Campylobacter* in broiler
436 carcases. Official Journal of the European Union L 218, 1-6.
437 Fouts, D.E., Mongodin, E.F., Mandrell, R.E., Miller, W.G., Rasko, D.A., Ravel, J., Brinkac,
438 L.M., DeBoy, R.T., Parker, C.T., Daugherty, S.C., Dodson, R.J., Durkin, A.S.,
439 Madupu, R., Sullivan, S.A., Shetty, J.U., Ayodeji, M.A., Shvartsbeyn, A., Schatz,
440 M.C., Badger, J.H., Fraser, C.M. and Nelson, K.E., 2005. Major structural differences
441 and novel potential virulence mechanisms from the genomes of multiple
442 *Campylobacter* species. PLoS Biology 3, e15.
443 Garenaux, A., Jugiau, F., Rama, F., de Jonge, R., Denis, M., Federighi, M. and Ritz, M.,
444 2008. Survival of Campylobacter jejuni strains from different origins under oxidative
445 stress conditions: effect of temperature. Curr Microbiol 56, 293-297.
446 Gaynor, E.C., Wells, D.H., MacKichan, J.K. and Falkow, S., 2005. The *Campylobacter jejuni*
447 stringent response controls specific stress survival and virulence-associated
448 phenotypes. Molecular Microbiology 56, 8-27.
449 Guyard-Nicodeme, M., Keita, A., Quesne, S., Amelot, M., Poezevara, T., Le Berre, B.,
450 Sanchez, J., Vesseur, P., Martin, A., Medel, P. and Chemaly, M., 2016. Efficacy of
451 feed additives against *Campylobacter* in live broilers during the entire rearing period.
452 Poultry Science 95, 298-305.
453 Guyard-Nicodeme, M., Rivoal, K., Houard, E., Rose, V., Quesne, S., Mourand, G., Rouxel,
454 S., Kempf, I., Guillier, L., Gauchard, F. and Chemaly, M., 2015. Prevalence and
455 characterization of *Campylobacter jejuni* from chicken meat sold in French retail
456 outlets. International Journal of Food Microbiology 203, 8-14.
457 Guyard-Nicodeme, M., Tresse, O., Houard, E., Jugiau, F., Courtillon, C., El Manaa, K.,
458 Laisney, M.J. and Chemaly, M., 2013. Characterization of *Campylobacter* spp.
459 transferred from naturally contaminated chicken legs to cooked chicken slices via a
460 cutting board. International Journal of Food Microbiology 164, 7-14.

461 Habib, I., Miller, W.G., Uyttendaele, M., Houf, K. and De Zutter, L., 2009. Clonal population
462 structure and antimicrobial resistance of *Campylobacter jejuni* in chicken meat from
463 Belgium. Applied and Environmental Microbiology 75, 4264-4272.
464 Habib, I., Uyttendaele, M. and De Zutter, L., 2010. Survival of poultry-derived *Campylobacter*
465 *jejuni* of multilocus sequence type clonal complexes 21 and 45 under freeze, chill,
466 oxidative, acid and heat stresses. Food Microbiology 27, 829-834.
467 Hazeleger, W.C., Wouters, J.A., Rombouts, F.M. and Abee, T., 1998. Physiological Activity
468 of *Campylobacter jejuni* Far below the Minimal Growth Temperature. Applied and
469 Environmental Microbiology 64, 3917-3922.
470 Hermans, D., Van Deun, K., Messens, W., Martel, A., Van Immerseel, F., Haesebrouck, F.,
471 Rasschaert, G., Heyndrickx, M. and Pasmans, F., 2011. *Campylobacter* control in
472 poultry by current intervention measures ineffective: urgent need for intensified
473 fundamental research. Veterinary Microbiology 152, 219-228.
474 Humphrey, T., O'Brien, S. and Madsen, M., 2007. Campylobacters as zoonotic pathogens: A
475 food production perspective. International Journal of Food Microbiology 117, 237-257.
476 James, C., Vincent, C., de Andrade Lima, T. and James, S., 2006. The primary chilling of
477 poultry carcasses - a review. International Journal of Refrigeration 29, 847-862.
478 Johnsen, G., Kruse, H. and Hofshagen, M., 2006. Genotyping of *Campylobacter jejuni* from
479 Broiler Carcasses and slaughterhouse environment by amplified fragment length
480 polymorphism. Poultry Science 85, 2278-2284.
481 Jones, D.M., Sutcliffe, E.M., Rios, R., Fox, A.J. and Curry, A., 1993. *Campylobacter jejuni*
482 adapts to aerobic metabolism in the environment. Journal of Medical Microbiology 38,
483 145-150.
484 Klančnik, A., Guzej, B., Jamnik, P., Vuckovic, D., Abram, M. and Mozina, S.S., 2009. Stress
485 response and pathogenic potential of *Campylobacter jejuni* cells exposed to
486 starvation. Research in Microbiology 160, 345-352.
487 Koidis, P. and Doyle, M.P., 1983. Survival of *Campylobacter jejuni* in fresh and heated red
488 meat. Journal Food of Protection 46, 771-774.

489 Laguerre, O., Derens, E. and Palagos, B., 2001. Study of domestic refrigerator temperature
490 and analysis of factors affecting temperature: a French survey. International Journal
491 of Refrigeration 25, 653-659.
492 Levesque, S., Frost, E., Arbeit, R.D. and Michaud, S., 2008. Multilocus sequence typing of
493 *Campylobacter jejuni* isolates from humans, chickens, raw milk, and environmental
494 water in Quebec, Canada. Journal of Clinical Microbiology 46, 3404-3411.
495 Ligowska, M., Cohn, M.T., Stabler, R.A., Wren, B.W. and Brøndsted, L., 2011. Effect of
496 chicken meat environment on gene expression of *Campylobacter jejuni* and its
497 relevance to survival in food. International Journal of Food Microbiology 145, 111-
498 115.
499 Lin, J., 2009. Novel approaches for *Campylobacter* control in poultry. Foodborne pathogens
500 and disease 6, 755-765.
501 Ma, Y., Hanning, I. and Slavik, M., 2008. Stress-induced adaptive tolerance response and
502 virulence gene expression in *Campylobacter jejuni*. Journal of Food Safety 29, 126-
503 143.
504 Martinez-Rodriguez, A. and Mackey, B.M., 2005. Physiological changes in *Campylobacter*
505 *jejuni* on entry into stationary phase. International Journal of Food Microbiology 101,
506 1-8.
507 McCarthy, Z., Smith, B., Fazil, A., Wu, J., Ryan, S.D. and Munther, D., 2018. pH dependent
508 *C. jejuni* thermal inactivation models and application to poultry scalding. Journal of
509 Food Engineering 223, 1-9.
510 Meunier, M., Guyard-Nicodeme, M., Vigouroux, E., Poezevara, T., Beven, V., Quesne, S.,
511 Bigault, L., Amelot, M., Dory, D. and Chemaly, M., 2017. Promising new vaccine
512 candidates against *Campylobacter* in broilers. PLoS One 12, e0188472.
513 Murphy, C., Carroll, C. and Jordan, K.N., 2003a. Identification of a novel stress resistance
514 mechanism in *Campylobacter jejuni*. Journal of Applied Microbiology 95, 704-708.

515 Murphy, C., Carroll, C. and Jordan, K.N., 2003b. Induction of an adaptive tolerance response
516 in the foodborne pathogen, *Campylobacter jejuni*. FEMS Microbiology Letters 223,
517 89-93.
518 Newell, D.G., Shreeve, J.E., Toszeghy, M., Domingue, G., Bull, S., Humphrey, T. and Mead,
519 G., 2001. Changes in the carriage of *Campylobacter* strains by poultry carcasses
520 during processing in abattoirs. Applied and Environmental Microbiology 67, 2636-
521 2640.
522 Nguyen, H.T., Corry, J.E. and Miles, C.A., 2006. Heat resistance and mechanism of heat
523 inactivation in thermophilic *Campylobacters*. Applied and Environmental Microbiology
524 72, 908-913.
525 Nyati, K.K. and Nyati, R., 2013. Role of *Campylobacter jejuni* infection in the pathogenesis of
526 Guillain-Barre syndrome: an update. Biomed Research International 2013, 852195.
527 Oh, E., McMullen, L. and Jeon, B., 2015. Impact of oxidative stress defense on bacterial
528 survival and morphological change in *Campylobacter jejuni* under aerobic conditions.
529 Frontiers in Microbiology 6, 295.
530 Osiriphun, S., Tuitemwong, P., Koetsinchai, W., Tuitemwong, K. and Erickson, L.E., 2012.
531 Model of inactivation of *Campylobacter jejuni* in poultry scalding. Journal of Food
532 Engineering 110, 38-43.
533 Phebus, R.K., Draughon, F.A. and Mount, J.R., 1991. Survival of *Campylobacter jejuni* in
534 modified atmosphere packaged turkey roll. Journal of Food Protection 54, 194-199.
535 Pujol, L., Kan-King-Yu, D., Le Marc, Y., Johnston, M.D., Rama-Heuzard, F., Guillou, S.,
536 McClure, P. and Membre, J.M., 2012. Establishing equivalence for microbial-growth-
537 inhibitory effects ("iso-hurdle rules") by analyzing disparate *Listeria monocytogenes*
538 data with a gamma-type predictive model. Applied and Environmental Microbiology
539 78, 1069-1080.
540 Ragimbeau, C., Schneider, F., Losch, S., Even, J. and Mossong, J., 2008. Multilocus
541 sequence typing, pulsed-field gel electrophoresis, and fla short variable region typing

542 of clonal complexes of *Campylobacter jejuni* strains of human, bovine, and poultry
543 origins in Luxembourg. Applied and Environmental Microbiology 74, 7715-7722.
544 Reid, A.N., Pandey, R., Palyada, K., Naikare, H. and Stintzi, A., 2008a. Identification of
545 *Campylobacter jejuni* genes involved in the response to acidic pH and stomach
546 transit. Applied and Environmental Microbiology 74, 1583-1597.
547 Reid, A.N., Pandey, R., Palyada, K., Whitworth, L., Doukhanine, E. and Stintzi, A., 2008b.
548 Identification of *Campylobacter jejuni* genes contributing to acid adaptation by
549 transcriptional profiling and genome-wide mutagenesis. Applied and Environmental
550 Microbiology 74, 1598-1612.
551 Rivoal, K., Poezevara, T., Quesne, S. and Chemaly, M., 2016. Optimisation des conditions
552 de refroidissement des volailles à l'abattoir pour contrôler la contamination en
553 *Campylobacter*. In: Agence nationale de sécurité sanitaire de l'alimentation, d. l. e. e.
554 d. t. (ed.), Journée d'information et d'échanges sur *Campylobacter*, Ploufragan.
555 Rivoal, K., Ragimbeau, C., Salvat, G., Colin, P. and Ermel, G., 2005. Genomic diversity of
556 *Campylobacter coli* and *Campylobacter jejuni* isolates recovered from free-range
557 broiler farms and comparison with isolates of various origins. Applied and
558 Environmental Microbiology 71, 6216-6227.
559 Saint-Cyr, M.J., Haddad, N., Taminiau, B., Poezevara, T., Quesne, S., Amelot, M., Daube,
560 G., Chemaly, M., Dousset, X. and Guyard-Nicodeme, M., 2017. Use of the potential
561 probiotic strain *Lactobacillus salivarius* SMXD51 to control *Campylobacter jejuni* in
562 broilers. International Journal of Food Microbiology 247, 9-17.
563 Sheppard, S.K., Dallas, J.F., MacRae, M., McCarthy, N.D., Sproston, E.L., Gormley, F.J.,
564 Strachan, N.J., Ogden, I.D., Maiden, M.C. and Forbes, K.J., 2009. *Campylobacter*
565 genotypes from food animals, environmental sources and clinical disease in Scotland
566 2005/6. International Journal of Food Microbiology 134, 96-103.
567 Sörqvist, S., 1989. Heat resistance of *Campylobacter* and *Yersinia* strains by three methods.
568 Journal of Applied Bacteriology 67, 543-549.

569 Vashin, I.T. and Stoyanchev, T.T., 2011. Influence of temperature on *Campylobacter jejuni*
570 survival rate in pork meat. Bulgarian Journal of Veterinary Medicine 14, 25-30.
571 Waterman, S.C., 1982. The heat-sensitivity of *Campylobacter jejuni* in milk. Journal of
572 Hygiene 88, 529-533.
573 Young, K.T., Davis, L.M. and Dirita, V.J., 2007. *Campylobacter jejuni*: molecular biology and
574 pathogenesis. Nature Reviews Microbiology 5, 665-679.
575 Zoz, F., Iaconelli, C., Lang, E., Iddir, H., Guyot, S., Grandvalet, C., Gervais, P. and Beney, L.,
576 2016. Control of Relative Air Humidity as a Potential Means to Improve Hygiene on
577 Surfaces: A Preliminary Approach with *Listeria monocytogenes*. PLoS One 11, e0148418.

578

579

580

581 **Table 1: Results from ANOVA of the influence of the different factors and their interaction on**

582 **inactivation of *C. jejuni* during cold storage**

Source	DF	Sum Sq	Mean Sq	F value	P
Model	9	160.941	17.882	35.661	**< 0.0001**
Error	124	62.181	0.501		
Total	133	223.122			
Heat	2	19.254	9.627	19.198	**< 0.0001**
Atmosphere	1	110.907	110.907	221.169	**< 0.0001**
Strain	2	16.725	8.363	16.677	**< 0.0001**
Heat*Atmosphere	2	6.419	3.210	6.401	**0.002**
Atmosphere*Strain	2	12.806	6.403	12.768	**< 0.0001**

583 **Table 2: D-values (min) of *C. jejuni* collected from different studies**

	pH	Medium	Strain	$D_{50°C}$ (min)	$D_{55°C}$ (min)	Reference
Lab medium	7.2	Mueller-hinton broth	C97anses640	No inactivation	2.5*	This study
			C09MJLT205	No inactivation	2.8*	
			RM1221	No inactivation	1.5*	
	7.4	Heart infusion broth	AR6	36	5.3	(Nguyen et al. 2006)
			L51	39	4.6	
	ND	1% peptone solution	H-840	ND	0.6	(Blankenship and Craven 1982)
			composite of 5 strains	ND	1.1	
	ND	Physiological saline	1503	ND	1.14	(Sörqvist 1989)
Food matrix	6.8	Skim milk	FRI-CF3	5.4	0.7	(Doyle and Roman 1981)
			FRI-CF6	4.7	1	
			FRI-CF8	3.5	0.9	
			FRI-CF12	4.4	1	
			FRI-CF16	5.1	0.9	
	7.2	Milk	24791	5.7	ND	(Waterman 1982)
			16000	ND	ND	
			21033	7.2	ND	
			172589	ND	ND	
			16509	7.3	1.1	
			5388	36	ND	
	ND	Chicken carcasses	H-840	ND	2.1	(Blankenship and Craven 1982)
			composite of 5 strains	ND	2.3	
	8	Chicken carcasses	Endogeneous flora	ND	0.8*	(Osiriphun et al. 2012)
	6	Lamb meat	FRI-CF8	5.9	1	(Koidis and Doyle 1983)
			FRI-CF31P	11.2	1.2	

			FRI-CF401S	13.2	ND	
			FRI-CF402S	11.1	ND	
			FRI-CF403S	8.96	ND	
			FRI-CF404S	13.3	1.23	
	ND	Chicken carcasses	Endogeneous flora	ND	0.9	(Berrang and Dickens 2000)
	ND	Chicken carcasses	Endogeneous flora	ND	1.4	(Duffy et al. 2014)

584

585 ND: Not Determined

586 *$D_{55°C}$-values were calculated using the formula $\log D_{55°C} = \log D_{54°C} - (T_{55°C} - T_{54°C}) / Z$

587 Z = 4.99°C was estimated by calculating the mean Z reported from different studies (Blankenship and Craven 1982, Doyle and Roman 1981, Sörqvist 1989, Waterman 1982).

588 **Figures legends:**

589 **Figure 1.** Global scheme of experimental procedure. The different factors are represented with their associated levels. After each step, the

590 inactivation (viable bacterial population N lost) was calculated (Δlog).

591 **Figure 2.** Contribution of factors (temperature applied during hot and cold bath, strain and gas composition of the atmosphere) on the

592 inactivation of *C. jejuni* induced by each step of the experimental procedure (hot bath in white, cold bath in black, storage at 6°C in grey) under

593 atmosphere 70% O_2 / 30% CO_2 (a) and atmosphere 50% N_2 / 50% CO_2 (b).

594 **Figure 3.** Effect of factors and interactions on inactivation during storage at 6°C under modified atmosphere: strain effect (a), mean effect of

595 temperature applied during heat stress all strains combined (b), mean effect of the gas atmosphere composition all strains combined (c),

596 interaction between strains and atmosphere (d) and mean interaction between temperature of heat stress and atmosphere all strains combined

597 (e).

598 A,B,C Different capital letters indicate significant difference according the Least Significant test ($P < 0.05$) in inactivation.

599

600

Figure 1

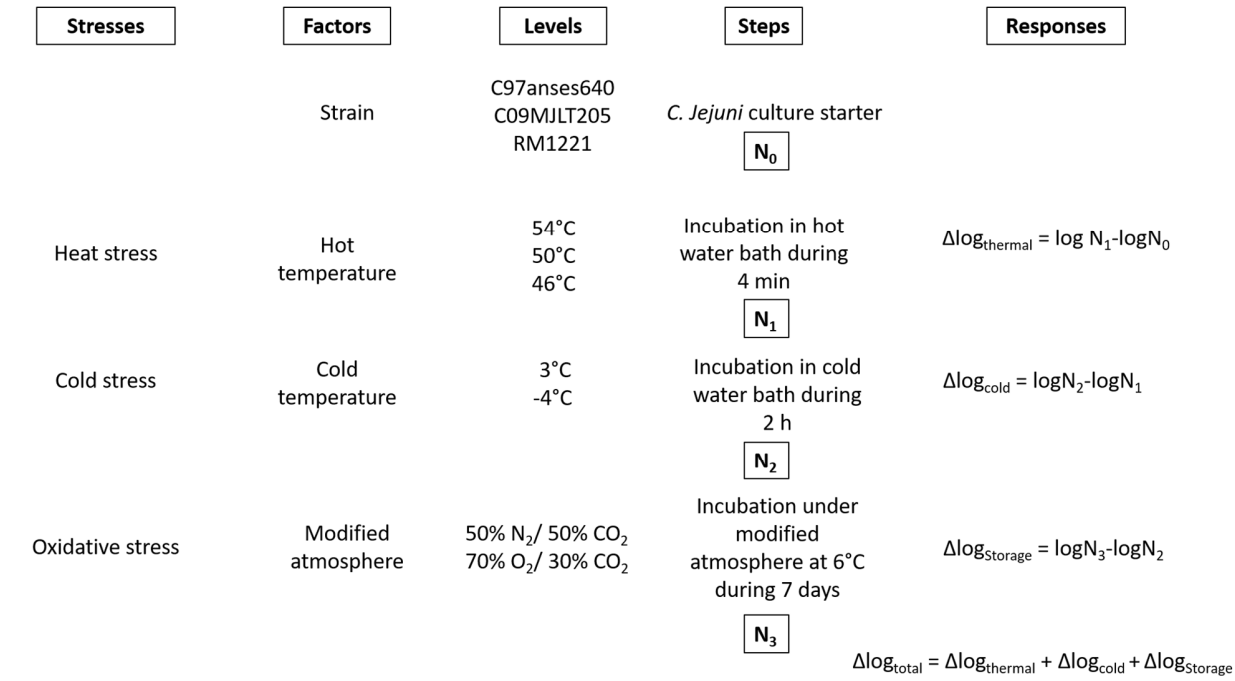

Stresses
Factors
Levels
Steps
Responses
Strain
C97anses640
C09MJLT205
RM1221
C. Jejuni culture starter
N0
Heat stress
Hot temperature
54°C
50°C
46°C
Incubation in hot water bath during 4 min
N1
Δlogthermal = log N1-logN0
Cold stress
Cold temperature
3°C
-4°C
Incubation in cold water bath during 2 h
N2
Δlogcold = logN2-logN1
Oxidative stress
Modified atmosphere
50% N2/ 50% CO2
70% O2/ 30% CO2
Incubation under modified atmosphere at 6°C during 7 days
N3
Δlogstorage = logN3-logN2
Δlogtotal = Δlogthermal + Δlogcold + Δlogstorage

Figure 2

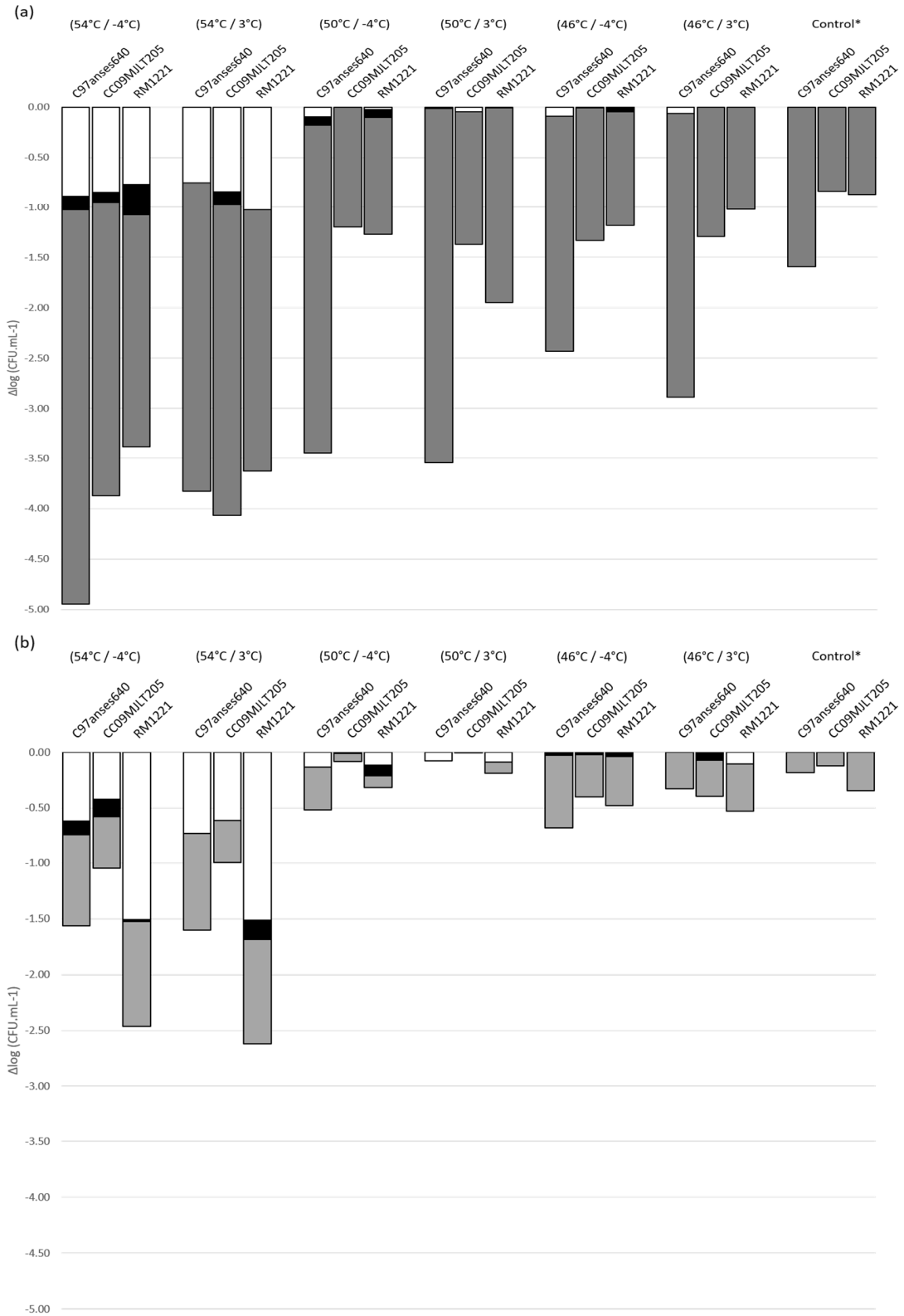
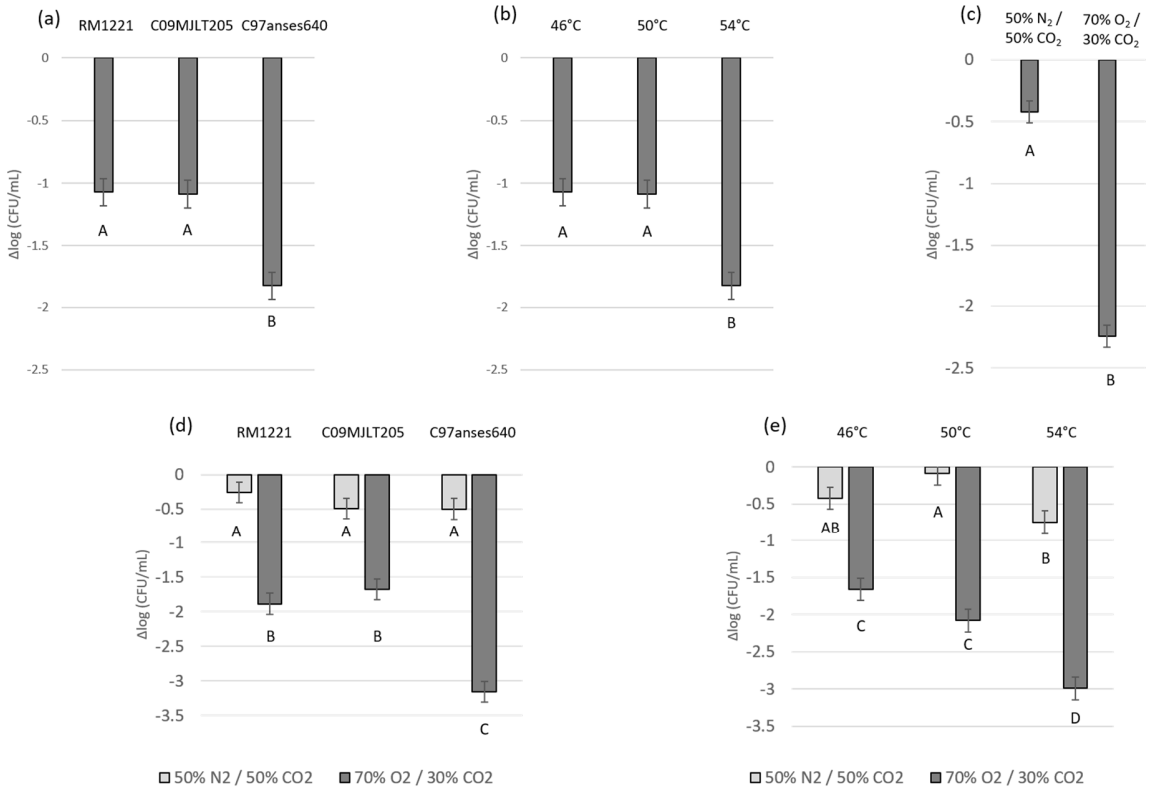

(a)
(54°C / -4°C)
(54°C / 3°C)
(50°C / -4°C)
(50°C / 3°C)
(46°C / -4°C)
(46°C / 3°C)
Control*
C97anses640
CC09MJLT205
RM1221
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
-3.50
-4.00
-4.50
-5.00
Δlog (CFU.mL-1)
(b)
(54°C / -4°C)
(54°C / 3°C)
(50°C / -4°C)
(50°C / 3°C)
(46°C / -4°C)
(46°C / 3°C)
Control*
C97anses640
CC09MJLT205
RM1221
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
-3.50
-4.00
-4.50
-5.00
Δlog (CFU.mL-1)

Figure 3


(a)
RM1221
C09MJLT205
C97anses640
Δlog (CFU/mL)
0
-0.5
-1
-1.5
-2
-2.5
A
A
B
(b)
46°C
50°C
54°C
A
A
B
(c)
50% N2 / 50% CO2
70% O2 / 30% CO2
A
B
(d)
RM1221
C09MJLT205
C97anses640
-3
-3.5
A
A
A
B
B
C
50% N2 / 50% CO2
70% O2 / 30% CO2
(e)
46°C
50°C
54°C
AB
A
B
C
C
D
50% N2 / 50% CO2
70% O2 / 30% CO2