Description of Campylobacter jejuni Bf, an atypical aero-tolerant strain

Ramila Christiane Rodrigues, Anne-Lise Pocheron, Michel Hernould, Nabila Haddad, Odile Tresse, Jean-Michel Cappelier

▶ **To cite this version:**

HAL Id: hal-02631314
https://hal.inrae.fr/hal-02631314v1

Submitted on 27 May 2020

Gut Pathogens

CrossMark

Description of *Campylobacter jejuni* Bf, an atypical aero-tolerant strain

Ramila Cristiane Rodrigues[1,2], Anne-Lise Pocheron[1,2], Mathieu Hernould[1,2], Nabila Haddad[1,2], Odile Tresse[1,2] and Jean-Michel Cappelier[1,2*]

Abstract

Background: *Campylobacter jejuni* is a leading cause of bacterial enteritis worldwide. This microaerophilic bacterium can survive in aerobic environments, suggesting it has protective mechanisms against oxidative stress. The clinical *C. jejuni* Bf strain is characterized by an increased resistance to oxygen. This study aimed to characterize the behavior of the clinical *C. jejuni* Bf strain under an aerobic atmosphere and in response to ROS-promoter agents.

Methods: Growth was studied in both aerobic and microaerobic conditions using classic cultivable methods. Electronic microscopy and *mreB* gene expression were used to evaluate the morphology of this strain under aerobic conditions. The survival under oxidative stress was tested in the presence of different concentrations of hydrogen peroxide (H_2O_2) and paraquat (PQ).

Results: The results showed that *C. jejuni* Bf strain can grow aerobically, unlike other strains of *C. jejuni* tested. Cells of *C. jejuni* Bf exposed to oxidative stress presented changes in morphology and the gene *mreB*, responsible for maintaining the bacillary cell morphology, was down-expressed. In aerobically acclimated conditions, *C. jejuni* Bf exhibited a higher survival rate of 52 % in the presence of H_2O_2 (1 mM) compared to the reference strain NCTC 11168. Concentrations above 1 mM PQ were lethal for the reference strain but not for *C. jejuni* Bf.

Conclusions: Taken together, these data highlight the resistance to oxidative stress conditions of *C. jejuni* Bf, indicating that this microorganism seems more adapted to survival in hostile environmental conditions.

Keywords: *Campylobacter jejuni*, Oxygen tolerance, Hydrogen peroxide, Paraquat, Cell shape

Background

Campylobacter jejuni was the most commonly reported bacteria associated with human intestinal infections in the European Union (EU) in 2013 [1]. In 2013, 64.8 confirmed campylobacteriosis cases per 100,000 inhabitants were reported in the EU, exceeding the number of salmonellosis cases with a notification rate of 20.4 per 100,000 population [1]. *C. jejuni* was isolated in 80.6 % of confirmed cases of campylobacteriosis [1]. In the United States, this pathogen was the second most commonly reported bacterial pathogen (behind *Salmonella*) with 6621 infections in 2013 and an incidence of 13.82 cases per 100,000 population [2]. *C. jejuni* is described as a non-spore-forming Gram-negative bacterium, highly motile, with a spiral shape [3, 4].

In most cases, people infected by *C. jejuni* often have watery diarrhea, cramp, abdominal pain and fever, which may be accompanied by nausea and vomiting. Campylobacteriosis may also result in post-infection complications such as Guillain–Barré Syndrome (GBS) and its variant form, Miller Fisher Syndrome (MFS), a chronic and potentially fatal form of paralysis [5, 6].

Unlike most pathogens, *Campylobacter* species show fastidious growth requirements and an unusual sensitivity to environmental stress. *Campylobacter* is considered as an obligate microaerobic bacterium. According to Kaakoush et al. [7], *Campylobacter* requires low oxygen concentration, between 2–10 %, for optimal growth. Moreover, *Campylobacter* seems to be sensitive to high

*Correspondence: jean-michel.cappelier@oniris-nantes.fr
[1] ONIRIS National College of Veterinary Medicine, Food Science and Engineering, Route de Gachet - La Chantrerie, BP 40706, 44307 Nantes Cedex 03, France
Full list of author information is available at the end of the article

Rodrigues *et al. Gut Pathog (2015) 7:30*

Page 2 of 12

oxygen tensions therefore, theoretically, it cannot grow under an ambient atmosphere, during food processing for instance.

As a microaerophilic bacterium, the main stress encountered by *C. jejuni* is the oxygen concentration in the ambient atmosphere. Under conditions of oxidative stress, oxygen can be reduced to a variety of toxic intermediate products called reactive oxygen species (ROS) including the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (^-OH) [8]. Oxidative stress is produced when ROS accumulates in the bacteria to a concentration level that exceeds the defense capacity of the cell. However, despite this fastidious requirement, the increasing incidence of human enteric campylobacteriosis indicates that *Campylobacter* has developed mechanisms to survive oxidative stress throughout the whole food chain.

In our laboratory, the clinical *C. jejuni* Bf strain was isolated from a French patient in 1994. No link was established between the illness and the food ingested, but the patient was working in a wastewater treatment plant, suggesting that this strain may be present in the environment. Some studies have evaluated the ability of this strain to enter a viable but non-culturable (VBNC) state [9–12], its increased resistance to oxygen and its ability to grow under an ambient atmosphere (unpublished data). However, no study has been performed to evaluate the resistance to oxygen of *C. jejuni* Bf.

In order to provide data about the oxidative stress resistance of *C. jejuni*, we proposed to characterize the behavior of the clinical *C. jejuni* Bf strain under an aerobic atmosphere and in response to ROS-promoter agents. We evaluated the atypical ability of *C. jejuni* Bf to multiply in an ambient atmosphere, in contrast to several other strains of *C. jejuni* tested. We also studied the morphological changes of this strain under aerobic conditions.

Results

Multilocus sequence typing of *C. jejuni* Bf

Using 16S rRNA PCR (Table 1), this isolate was shown to belong to the *Campylobacter* genus (data not shown). Moreover, the MLST analysis revealed that this isolate is a *C. jejuni* strain, which is a member of the clonal complex ST-403.

Growth/survival of *C. jejuni* strains in microaerobic (MAC) and aerobic conditions (AC)

Cultivation of *C. jejuni* Bf in aerobic conditions in BHI broth using shaking flasks or closed tubes showed large variations in cell numbers in AC (see Additional files 1, 2). These results demonstrate the low reproducibility of *C. jejuni* Bf cultivation under aerobic conditions for suspended cells. A better reproducibility in AC was obtained with cells growing

Table 1 Primers used in this study

Primer	Sequence 5′ → 3′	Product size (bp)
mreB F	TTCGTACGGCTGGAGATAAG	234
mreB R	GTGAAAGAACAGGCGAAGAG	
rrs F	AAGGGCCATGATGACTTGACG	207
rrs R	AGCGCAACCCACGTATTTAG	
341 F	CCTACGGGAGGCACGAG	439
758 R	CTACCAGGGTATCTAATCC	
MD16S F	ATCTAATGGCTTAACCATTAAAC	856
MD16S R	GGACGGTAACTAGTTTAGTATT	
aspA F	CCAACTGCAAGATGCTGTACC	625
aspA R	GATGGTATTACCGCAAATGAA	
glnA F	GCTCAATTCATGGATGGC	722
glnA R	GTTCATATGAGCTTATGGAA	
gltA F	GTGGCTATCCTATAGAGTGGC	575
gltA R	CAGGTATTGGTGCGCTTTGG	
glyA F	AGCCTAATTCAGGTTCTCAA	667
glyA R	GCGATGGAACGGATAATCACCT	
pgm F	TTGGAACTGATGGAGTTCG	1298
pgm R	GAAAAAGAAATGAAAAATGTTGTG	
tkt F	TGCACCTTTGGGCTTAGC	702
tkt R	GCACCTTTGGGTGAAGAAGT	
uncA F	AAAGTACAGTGGCACAAGTGG	611
uncA R	GCTAGTGATTTAGATGAGGCA	

on solid surfaces. Consequently, the growth rate in MAC and AC of the 10 strains was compared using solid surfaces.

To compare the growth and/or survival of *C. jejuni* Bf on solid surfaces according to the gas composition, ten strains of *C. jejuni* were cultured on Karmali agar in AC or MAC for 48 h at 42 °C (Table 2).

In MAC, the number of cells increased gradually from approximately 10^6 CFU mL^{-1} to more than 10^{10} CFU mL^{-1} after 24 h of incubation (Fig. 1a). The same trend was observed for *C. jejuni* Bf strain, with a maximum number of cells of approximately 10^9 CFU mL^{-1} after 24 h of incubation.

The decrease in the number of cells in MAC was heterogeneous among strains. After 48 h of incubation, the strains showed a decrease in cells to reach approximately 10^8 CFU mL^{-1}, except for *C. jejuni* subsp. *doylei* 269.97, which showed a cell number of 10^7 CFU mL^{-1} (Fig. 1a).

All strains tested in this study presented a statistically higher specific growth rate under MAC than that of *C. jejuni* Bf strain (Table 3).

The incubation of the strains under AC led to a decrease in the number of cells of the strains tested, with complete loss of cultivability after approximately 24 h of incubation (Fig. 1b). Only *C. jejuni* NCTC 11168 strain showed a total loss of cultivability after approximately 34 h of incubation under AC. In contrast to the other strains, *C.*

Rodrigues *et al. Gut Pathog (2015) 7:30*

Page 3 of 12

Table 2 Strains used in this study

Strain of *C. jejuni*	Origin	Clonal complex	RefSeq	References
Bf	Human	ST-403	ND	This study
subsp. *jejuni* NCTC 11168 = ATCC 700819	Human	ST-21	NC_002163.1	Gundogdu et al. [53] Parkhill [54]
RM1221	Chicken	ST-354	NC_003912.7	Fouts et al. [55]
subsp. *jejuni* 81-176	Human	ST-42	NC_008787.1 NC_008790.1 NC_008770.1	Hofreuter et al. [56]
subsp. *doylei* 269.97, RM 4099	Chicken	ND	NC_009707.1	Fouts et al. [57]
subsp. *jejuni* 81116; NCTC 11828	Human	ST-283	NC_009839.1	Pearson et al. [58]
subsp. *jejuni* 00-2538	Human	ST-21	NC_022351.2	Clark et al. [59]
subsp. *jejuni* 00-2544	Human	ST-21	NC_022353.2 NC_022354.1	Clark et al. [59]
subsp. *jejuni* 00-2426	Human	ST-21	NC_022352.2	Clark et al. [59]
subsp. *jejuni* 00-2425	Human	ST-21	NC_022362.2	Clark et al. [59]

ND not determined

jejuni Bf strain in an ambient atmosphere showed a lag phase of around 10 h, which could be identified as an adaptation phase to the stress condition submitted, followed by a 2.5 log cell proliferation (Fig. 1b). Under AC, this strain showed a decrease in cell number after 24 h of incubation and subsequently remained in cell multiplication until the time of analysis (48 h) (Fig. 1b).

Moreover, no significant difference was observed in the number of cells of *C. jejuni* Bf strain cultured in AC and in aerobically acclimated conditions, AAC (Fig. 2). In the two conditions tested, *C. jejuni* Bf showed, on average, an increase of 2.8 log after 48 h of culture in AC at 42 °C (Fig. 2).

The great majority of strains showed death times (D-values) below 1.5 h, with a maximum value of 1.54 h for *C. jejuni* 00-2426 strain and a minimum value of 0.48 h for *C. jejuni* 81-176 strain. However, strains isolated from chicken, *C. jejuni* subsp. *doylei* and RM 1221, showed higher values of death times (2.27 and 1.93, respectively) (Table 3). These results suggest that the strain origin probably influenced the survival capacity under AC, even though only two strains of animal origin were tested.

According to the results obtained in Fig. 1b, and the similar overall response to AC of the different *C. jejuni* strains tested, we selected *C. jejuni* NCTC 11168 for further comparative analyses with *C. jejun*i Bf.

Cell morphology of *C. jejuni* NCTC 11168 and *C. jejuni* Bf in aerobic conditions

To characterize the effect of oxidative stress on the modification of the cell format of *C. jejuni*, its cell morphology was assessed.

After 12 h of incubation in MAC, the cellular morphology of *C. jejuni* NCTC 11168 and *C. jejuni* Bf exhibited an elongated spiral shape as observed using SEM (Fig. 3) with a size of approximately 2.5 and 1.5 μm, respectively (Fig. 3a, b), while after 12 h of incubation under AC, *C. jejuni* Bf showed a reduced cell size (approximately 0.5 μm), with a predominance of coccoid cells (Fig. 3d). Despite this oxidative stress condition, cells of *C. jejuni* NCTC 11168 kept their spiral shape, with a reduction in cell size to 2.0 μm (Fig. 3c).

An incubation of 24 h under MAC led to a coccoid shape (approximately 0.5 μm) of the *C. jejuni* NCTC 11168 and Bf strains (Fig. 3e, f). *C. jejuni* Bf maintained this shape when it was exposed to an ambient atmosphere, unlike *C. jejuni* NCTC 11168, which exhibited elongated filaments, approximately 20.0 μm in size (Fig. 3g, h). Moreover, under both atmosphere conditions with an incubation of 24 h, cells of *C. jejuni* Bf seemed to interact by close contact and fiber-like structures, like a cobweb (Fig. 3f, h).

Effect of oxidative stress on the expression of the *mreB* gene in *C. jejuni* NCTC 11168 and *C. jejuni* Bf

The role of oxidative stress in the regulation of the *mreB* gene in *C. jejuni* was assessed at the transcript level, using qRT-PCR.

After 24 h of incubation under MAC, the *mreB* gene expression in the strain *C. jejuni* NCTC 11168 fell by 94.1 % compared with 12 h of incubation while *mreB* expression in *C. jejuni* Bf was reduced by 90.0 %. The expression of *mreB* under AC after 24 h of incubation in *C. jejuni* NCTC 11168 and *C. jejuni* Bf showed an increase in the relative expression of 13.9 and 0.8 times, respectively, when compared with 12 h of incubation.

After 12 h of incubation under AC, *mreB* gene expression significantly decreased in both strains tested in comparison with MAC. In fact, *C. jejuni* NCTC 11168 and *C.*
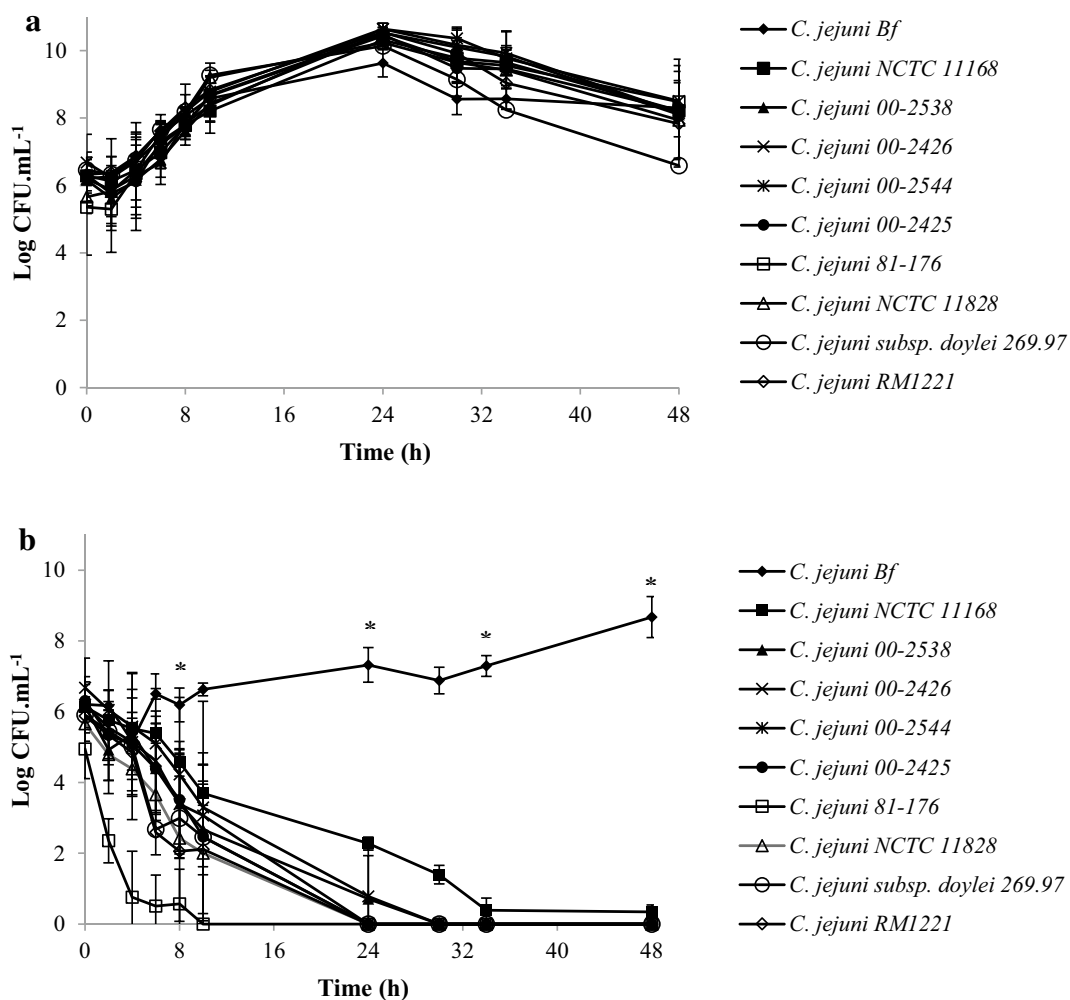
Rodrigues *et al. Gut Pathog* (2015) 7:30

Page 4 of 12

Fig. 1 Growth and survival of *C. jejuni* strains under MAC (**a**) and AC (**b**) at 42 °C. The number of colony-forming units was determined by plating serial dilutions on Karmali agar using the microdroplet technique followed by incubation at 42 °C for 24 h in MAC. Plotted points are the mean measurements from three independent experiments. *Error bars* represent standard deviations, although they are too small to be visible in most cases. *Statistically significant difference between the strains in each condition tested (*P* < 0.05)

jejuni Bf cultured aerobically had a reduced *mreB* gene expression of 86.3 and 91.7 %, respectively (Fig. 4a).

However, after 24 h of incubation, the *C. jejuni* NCTC 11168 strain grown aerobically showed a 1.9-fold increase in the relative *mreB* gene expression when compared to MAC for 12 h. On the other hand, *C. jejuni* NCTC 11168 and *C. jejuni* Bf grown in MAC and *C. jejuni* Bf grown in AC presented a reduced expression of the gene of 94.1, 90.3 and 89.7 %, respectively (Fig. 4b).

Effects of oxidative stress reagents on the growth of *C. jejuni*

To evaluate the resistance to oxidative stress and the aerotolerance of *C. jejuni*, NCTC 11168 and Bf strains were exposed to oxidative stress reagents (hydrogen peroxide—H_2O_2 and paraquat—PQ) in different concentrations.

Campylobacter jejuni Bf strain cultivated in MAC or AAC showed a higher survival rate in the presence of H_2O_2 and PQ than *C. jejuni* NCTC 11168 strain (Fig. 5).

Indeed, *C. jejuni* Bf strain cultivated in MAC and AAC exhibited a higher survival rate of 38.6 and 52 %, respectively, in the presence of H_2O_2 (1 mM) compared to the reference strain *C. jejuni* NCTC 11168 (Fig. 5A). The two *C. jejuni* strains evaluated showed no significant difference in survival at concentrations below 0.5 mM H_2O_2.

In the presence of 0.5 mM paraquat, *C. jejuni* NCTC 11168 strain showed a decrease of 4.7 log, while the *C. jejuni* Bf strain cultivated in MAC or AAC showed a limited reduction of 1.5 and 1.4 log, respectively.

Rodrigues *et al. Gut Pathog* (2015) 7:30

Page 5 of 12

Table 3 Growth rate after 24 h of incubation (μ_{max}) and death times (D-values) in *C. jejuni* strains cultured in MAC and AC

Strain of *C. jejuni*	Rate of growth (μ_{max}) in microaerobic conditions	Death times (D-values) in aerobic conditions
Bf	0.5763	**
subsp. *jejuni* NCTC 11168	0.7714*	0.9737
subsp. *jejuni* 00-2538	0.9198*	1.1039
subsp. *jejuni* 00-2426	0.8964*	1.5442
subsp. *jejuni* 00-2544	0.8303*	1.4554
subsp. *jejuni* 00-2425	0.9085*	1.3785
subsp. *jejuni* 81-176	0.9477*	0.4765
subsp. *jejuni* NCTC 11828	0.7899*	1.3417
subsp. *doylei* 269.97	0.7765*	2.2691
subsp. *jejuni* RM 1221	0.6793*	1.9276

* Statistically significant difference between the strains ($P < 0.05$)

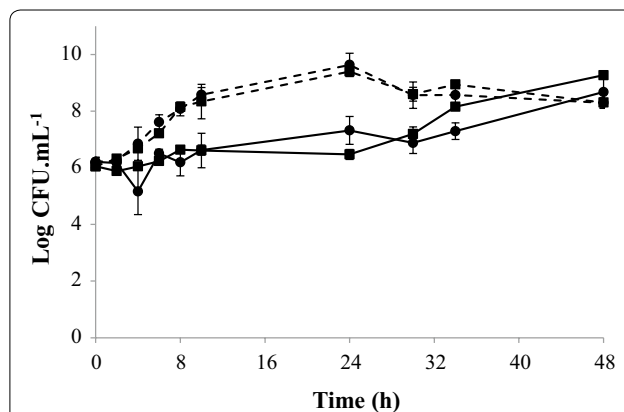** Not determined because Bf is able to grow under AC

Fig. 2 Effect of acclimation to aerobiosis on *C. jejuni* Bf growth under MAC (*dotted line*) and AC (*continuous line*) after aerobiosis acclimation (*square*) or without acclimation (*circle*) at 42 °C. The number of colony-forming units was determined by plating serial dilutions on Karmali agar using the microdroplet technique followed by incubation at 42 °C for 24 h in MAC. Plotted points are the mean measurements from three independent experiments. *Error bars* represent standard deviations, although they are too small to be visible in most cases

Concentrations above 1 mM PQ were lethal for the reference strain but not for aerobically-acclimated *C. jejuni* Bf (Fig. 5B).

Discussion

In this study, some phenotypic and genotypic characteristics of the atypical *C. jejuni* Bf strain were investigated, in order to provide data about the aerotolerance of this strain. It was shown that this strain can grow under aerobic conditions (AC), unlike other strains of *C. jejuni*. Oxidative stress changes the morphology of this strain and the transcription of the *mreB* gene, responsible for maintaining the bacillary cell morphology, is down-expressed. Moreover, *C. jejuni* Bf strain is less susceptible to oxidative stress mediated by ROS-promoter agents, in comparison with the other *C. jejuni* strains tested. Taken together, these data highlight the resistance to oxidative stress conditions of *C. jejuni* Bf.

The clonal complex ST-403, to which the *C. jejuni* Bf strain belongs, has been isolated from humans [13–15], but the origins of this complex are unclear, although it has been found in porcine isolates and occasionally in cattle [16–18]. This clonal complex is associated with Guillain–Barré Syndrome or enteritis as indicated by ST-403 *C. jejuni* HS:23 isolated from patients with these diseases [19].

As a microaerophilic microorganism, *C. jejuni* requires 2–10 % O_2 for optimal growth [7]. However, we report here that the *C. jejuni* Bf strain can survive and multiply under certain AC, unlike several other strains of *C. jejuni*. Although some studies have already reported the ability of *C. jejuni* strains to survive oxygen [7, 19, 20], this is the first report demonstrating the capacity of a *C. jejuni* strain to multiply under atmospheric conditions, with a significant and continuous growth. Further studies are required to define whether this aero-tolerant feature observed in the current study is specific to *C. jejuni* Bf or could be generalized. Furthermore, this property could explain the persistence and spread of this organism in the food chain.

Many studies have investigated the survival of *C. jejuni* under oxidative stress. Chynoweth et al. [20] observed that strains of *C. jejuni* isolated from different sources showed the ability to adapt in atmospheric oxygen. Growth was limited to a few colonies on nutrient agar in atmospheric oxygen. Kaakoush et al. [7] evaluated the growth of *C. jejuni* strain NCTC 11168 at several cell densities in BHI broth cultures. As no growth under AC using initial cell densities lower than 10^4 CFU mL^{-1} and growth in a 19 % O_2 atmosphere with *Campylobacter* densities higher than 10^5 CFU mL^{-1} were observed, they concluded that oxygen plays a growth-limiting role in high-density bacterial cultures under MAC and is toxic in low-density cultures under AC in C. *jejuni* NCTC 11168 [7]. However, the experiments conducted by Kaakoush et al. [7] were performed in liquid medium. Notably, oxygen transfer in liquid medium is low ($k_H = 9.28 \times 10^{-4}$ mol L^{-1} atm^{-1} in water at 42 °C) and the oxygen demand by the microorganism with cell densities around 10^5–10^6 CFU mL^{-1} is higher than for lower densities (lower than 10^4 CFU mL^{-1}). These

Rodrigues *et al. Gut Pathog* (2015) 7:30
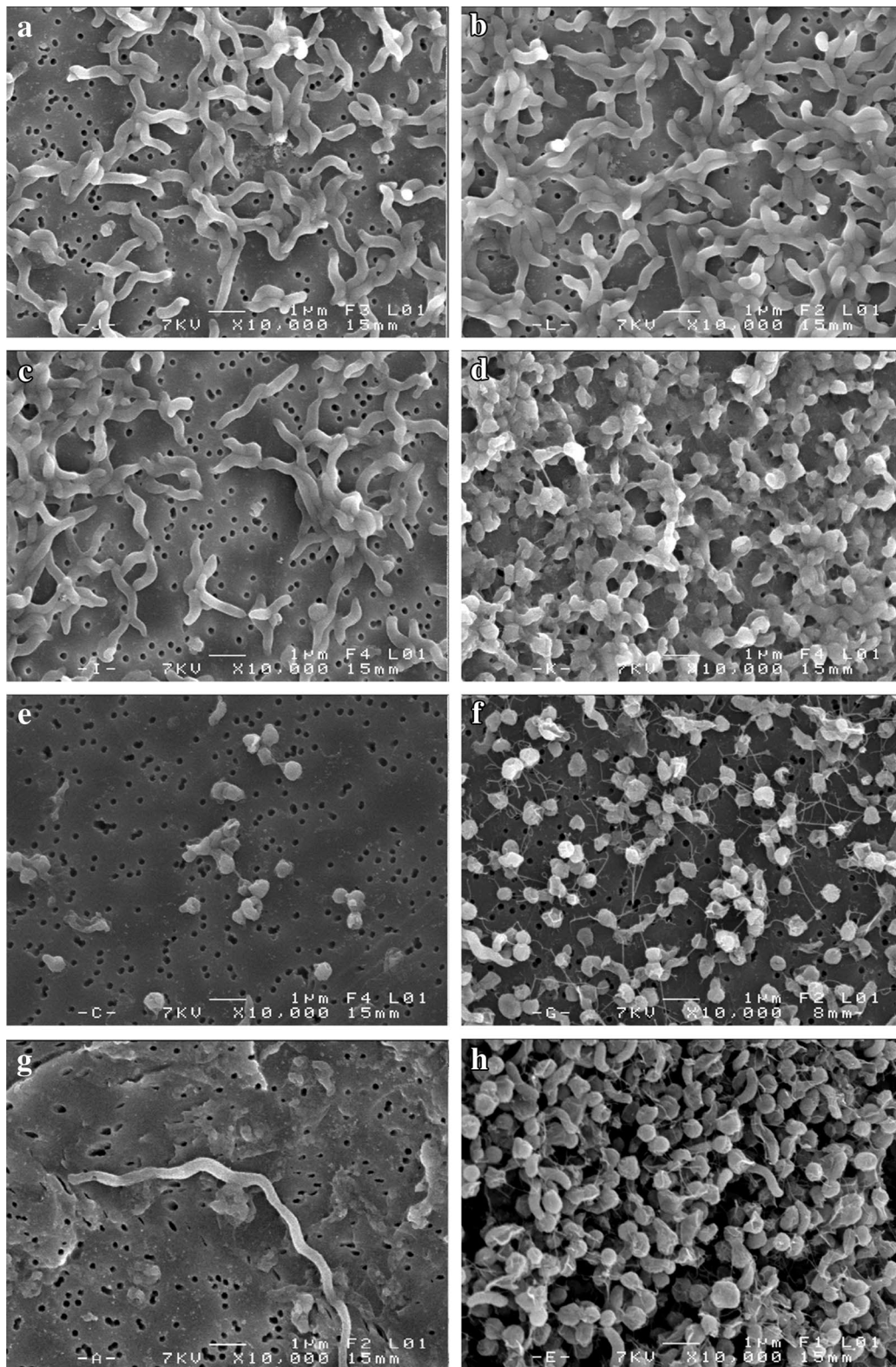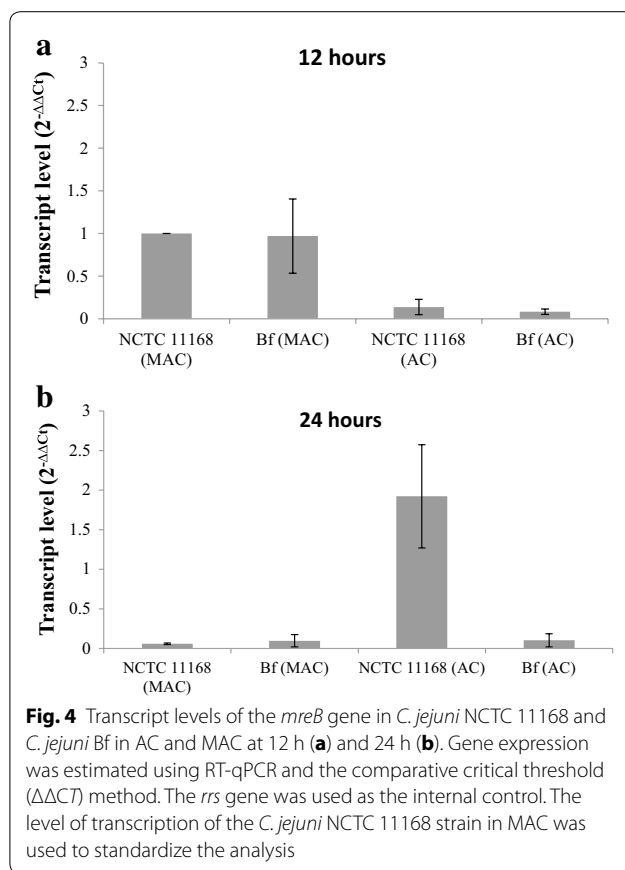
Page 6 of 12

Fig. 3 Micrographs of *C. jejuni* NCTC 11168 and *C. jejuni* Bf in MAC (**a**, **b**, respectively), and AC (**c**, **d**, respectively) after 12 h of incubation, and *C. jejuni* NCTC 11168 and *C. jejuni* Bf in MAC (**e**, **f**, respectively), and AC (**g**, **h**, respectively) after 24 h of incubation using SEM on polycarbonate membranes. Magnification: ×10,000

Rodrigues *et al. Gut Pathog* (2015) 7:30

Page 7 of 12

Fig. 4 Transcript levels of the *mreB* gene in *C. jejuni* NCTC 11168 and *C. jejuni* Bf in AC and MAC at 12 h (**a**) and 24 h (**b**). Gene expression was estimated using RT-qPCR and the comparative critical threshold (ΔΔC*T*) method. The *rrs* gene was used as the internal control. The level of transcription of the *C. jejuni* NCTC 11168 strain in MAC was used to standardize the analysis
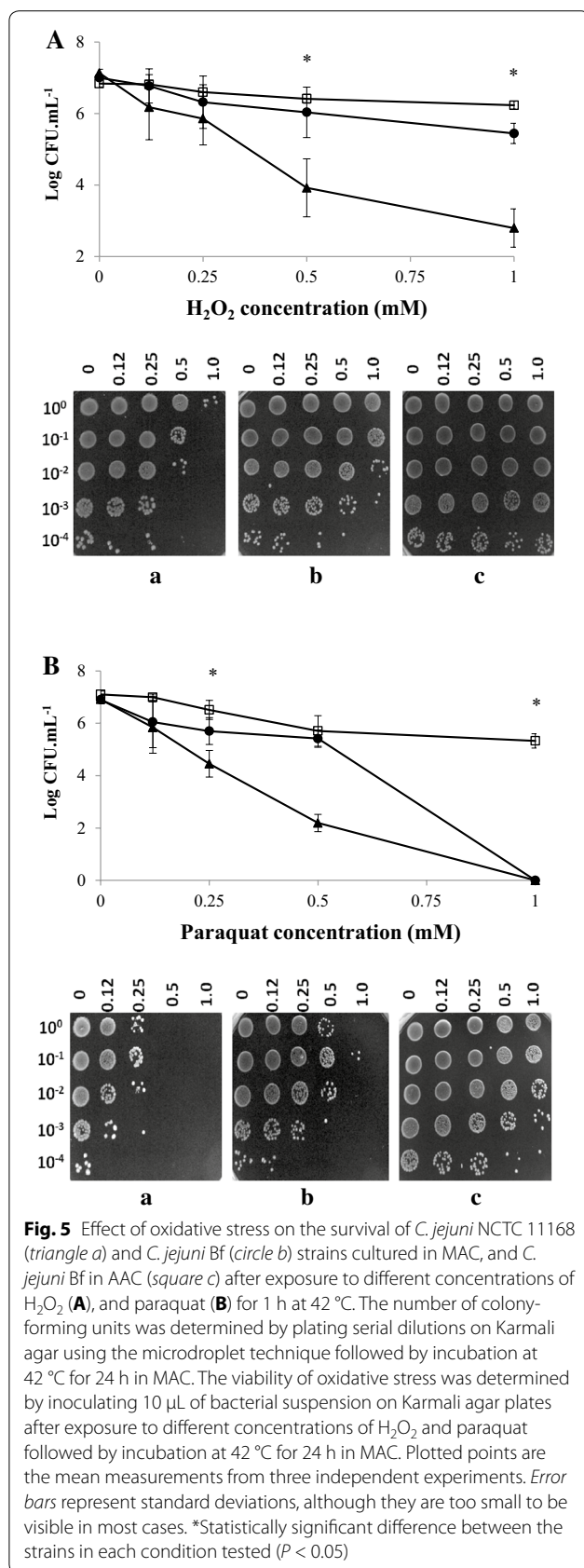
two factors lead to a decrease in the dissolved oxygen concentration in liquid medium, which can induce the growth of *Campylobacter* in AC. In addition, we observed a low reproducibility of *C. jejuni* Bf cultivation under AC in liquid medium (BHI). Due to the absence of a robust biological reproducibility of these results, the growth curve in AC and MAC was performed on a solid surface using Karmali agar. Growth on the agar plate was reproducible for all strains used in the conditions tested, demonstrating the validity of the method. Although not a routine technique, the microbial surface growth of different strains has previously been carried out by many researchers [21–25]. Furthermore, Fujikawa and Morozumi [26] observed that the growth curves of *Escherichia coli* on a nutrient agar plate at constant temperatures were very similar to those in a liquid.

Jones et al. [19] reported that the *C. jejuni* 13997 strain grown under MAC on blood agar plates and subsequently stored under AC survived better than on plates stored under MAC. These results suggest the ability of this *C. jejuni* strain to adapt to an ambient gaseous atmosphere. However, these authors observed only a better survival of the *C. jejuni* strain tested, but no cellular growth under AC [19].

The viability of the reference strains of *C. jejuni* (NCTC 11168, NCTC 11828 and 81-176) under an atmospheric level of oxygen has also been evaluated in the literature [27–29]. After 9 h of exposure under atmospheric conditions, the viability of *C. jejuni* NCTC 11828 and *C. jejuni* 81-176 decreased to 10 and 50 %, respectively [28, 29] while *C. jejuni* NCTC 11168 exposed to ambient oxygen at 37 °C showed a reduction of approximately 2 log after 48 h of culture [27]. In the present study, *C. jejuni* NCTC 11828 and 81-176 strains presented a reduction of 54.4 and 88.6 % viability after 8 h of exposure to atmospheric oxygen, respectively, and *C. jejuni* NCTC 11168 showed a greater reduction (5.8 log) after 48 h of culture in AC at 42 °C.

In order to verify if the ability to grow in certain AC is due to an increase in oxidative stress resistance, we tested the behavior of *C. jejuni* Bf in the presence of H_2O_2 and paraquat. *C. jejuni* Bf was less susceptible to stress conditions induced by H_2O_2 and paraquat than the reference strain *C. jejuni* NCTC 11168, for which the lethal effect of paraquat was concentration-dependent. Unlike the results observed with *C. jejuni* Bf, previous studies have shown the sensitivity of *C. jejuni* referent strains to oxidative stress induced by H_2O_2 and paraquat. Garénaux et al. [30] observed that 1 h of exposure to 500 µM paraquat led to a 0.5 $\log_{10}$ reduction in the *C. jejuni* NCTC 11168 population. This same strain exposed to the final concentration of 1 mM H_2O_2 and paraquat for 1 h showed reduced viability (0.5 and 0.1 %, respectively) at 42 °C under MAC [31]. This resistance to oxidative stress induced by ROS-promoter agents has also been observed in other strains of *C. jejuni* [13, 29].

In the presence of oxygen, the microorganism undergoes genetic and physiological changes in response to oxidative stress. Some studies have reported a transition from the spiral to the coccoid form in cells of *C. jejuni* submitted to oxidative stress conditions [32, 33]. In addition, *C. jejuni* cells may be seen as filaments, doughnuts and straight rods [34–36]. According to Griffiths [35], the rod form of *C. jejuni* is considered to be the usual viable form in the exponential growth phase, whilst filaments and coccoid forms are associated with the stationary growth phase. We have confirmed this phenotype in *C. jejuni* Bf and *C. jejuni* NCTC 11168 submitted to oxidative stress conditions. According to our results, the stationary growth phase (after 24 h of incubation) and particularly oxidative stress conditions are the stimuli for cell transition from the spiral to the coccoid form. The presence of fiber-like structures in *C. jejuni* Bf cells under AC and MAC after 24 h of incubation probably plays a role in protecting them against oxidative stress [37]. Other studies have obtained

Rodrigues *et al. Gut Pathog* (2015) 7:30

Page 8 of 12

Fig. 5 Effect of oxidative stress on the survival of *C. jejuni* NCTC 11168 (*triangle a*) and *C. jejuni* Bf (*circle b*) strains cultured in MAC, and *C. jejuni* Bf in AAC (*square c*) after exposure to different concentrations of H$_2$O$_2$ (**A**), and paraquat (**B**) for 1 h at 42 °C. The number of colony-forming units was determined by plating serial dilutions on Karmali agar using the microdroplet technique followed by incubation at 42 °C for 24 h in MAC. The viability of oxidative stress was determined by inoculating 10 µL of bacterial suspension on Karmali agar plates after exposure to different concentrations of H$_2$O$_2$ and paraquat followed by incubation at 42 °C for 24 h in MAC. Plotted points are the mean measurements from three independent experiments. *Error bars* represent standard deviations, although they are too small to be visible in most cases. *Statistically significant difference between the strains in each condition tested (*P* < 0.05)

similar results of cell morphology modification from spiral to coccoid forms after exposure to atmospheric oxygen [38, 39].

Cell size reduction and transition to coccoid forms might be associated with a modification of *mreB* gene expression, responsible for maintaining the bacillary cell morphology by forming helical filaments underneath the cell membrane [40–44]. It was suggested that the *mreB*-like gene present in the genome of *C. jejuni* NCTC 11168 plays a similar role in the maintenance of the bacterial cell shape [33]. The reduction in *mreB* expression in *C. jejuni* Bf and NCTC 11168 shown in this work during oxidative stress and stationary growth phase most likely leads to the morphological change in the cells under stress conditions. In *E. coli*, *Bacillus subtilis* and *Caulobacter crescentus*, the inactivation of *mreB* genes resulted in coccoid form formation [42, 45, 46]. Transcriptional down-regulation of *Helicobacter hepaticus mreB* and *mreC* genes also induced the formation of spherical cells [47].

This study clearly demonstrates that exposure to oxidative stress plays an important role in the morphological changes of *C. jejuni* under AC. These changes could contribute to the adaptation and survival of these strains in stressful conditions. Although the Bf strain exposed to AC presents a large amount of coccoid cells, this strain is able to multiply. This could be due to the presence of shorter spiral forms, which have previously been observed after exposure to atmosphere oxygen concentration [38].

Conclusions

In conclusion, *C. jejuni* Bf can grow and multiply under AC, unlike other strains of *C. jejuni* and showed a great resistance to compounds that induce oxidative stress, such as hydrogen peroxide and paraquat. This strain is therefore more adapted to survive oxidative stress conditions. During the transmission of *C. jejuni* to food preparation surfaces, the resistance of this strain to oxidative stress may be a key factor in explaining its spread and survival in the environment. The more this pathogen can survive in its environment, the greater is the probability of being exposed to this hazard.

However, it is important to note that we cannot say whether this property of oxidative stress resistance is intrinsic to or recently gained by a sub-type of *C. jejuni*, considering that only a total of ten strains of *C. jejuni* were tested. In addition, further studies are required to determine the cellular mechanisms that contribute to the resistance of this strain to oxidative stress. Moreover, the impact on different biological processes of *C. jejuni* Bf growth capacity under AC, such as its persistence in the environment, its virulence, or biofilm formation, could be investigated.

Rodrigues *et al. Gut Pathog* (2015) 7:30

Page 9 of 12

Methods

Bacterial strains and growth conditions

Strains used in this study are presented in Table 2. The genomes of the selected strains are entirely sequenced and available on NCBI. The cultures were stored at −80 °C in microtubes containing Brain–Heart Infusion medium (BHI) containing 20 % sterile glycerol. Before each experiment, the strains were grown at 42 °C on Karmali agar plates (Oxoid, Dardilly, France) for 48 h under microaerobic conditions, namely MAC (10 % CO_2, 5 % O_2 and 85 % N_2) obtained using gas replacement jars (GRJ) 4× flushed/filled with a MACS MICS system (Whitley jar gassing system) at −50 kPa vacuum.

To prepare cells cultured in aerobic conditions (ambient atmosphere), namely AC, the strains were grown first in MAC at 42 °C on Karmali agar for 48 h. They were then harvested and resuspended in peptone saline solution and standardized to an optical density at 600 nm (OD_{600nm}) of 0.03 ($\approx 10^6$ CFU mL^{-1}). Each bacterial suspension, containing approximately 10^6 CFU mL^{-1}, was then inoculated onto Karmali agar plates and incubated at 42 °C under AC for 24 h.

To prepare cells in aerobically acclimated conditions, namely AAC, *C. jejuni* Bf was first grown in MAC on Karmali agar for 48 h. Then, colonies were recovered and subcultured three times successively on Karmali agar plates followed by incubation at 42 °C for 24 h in AC.

DNA extraction, 16S rDNA and MLST typing of *C. jejuni* Bf

DNA of *C. jejuni* Bf was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Charbonnières, France) according to the manufacturer's instructions. DNA integrity and quantity were determined using an Experion™ Automated Electrophoresis System (BioRad, Marnes la Coquette, France) and NanoDrop 2000c (Fisher Scientific, Illkirch, France), respectively. A DNA fragment of 16S rDNA was amplified using primers specific to *C. jejuni* MD16S1 and MD16S2 [48] and sequenced using the Sanger protocol by Biofidal (Vaux-en-Velin, France). MLST was achieved according to Dingle et al. [49]. Briefly, the seven housekeeping genes for MLST (*aspA, glnA, gltA, glyA, pgm, tkt* and *uncA*) were amplified by PCR using the primers mentioned in Table 1. The PCR products were sequenced by Biofidal (Vaux-en-Velin, France). All allelic sequences were queried against the *C. jejuni* MLST database (http://pubmlst.org/campylobacter) to retrieve sequence types (STs) and determine the clonal complex of *C. jejuni* Bf. The nucleotide sequence data were submitted to the *Campylobacter* PubMLST database for allele assignments.

Growth curve of *C. jejuni* strains

Growth curves of *C. jejuni* NCTC 11168 (reference strain) and Bf were obtained from liquid cultures (using BHI) and the growth curve of *C. jejuni* strains shown in Table 2 were obtained from solid cultures. Bacteria were cultivated in MAC at 42 °C for 48 h. Then, colonies were transferred into a flask containing 10 mL of BHI broth grown in MAC at 42 °C with shaking (80 rpm) for 24 h. After incubation, aliquots corresponding to 2 % of the cultures ($\approx 10^6$ CFU mL^{-1}) of *C. jejuni* NCTC 11168 and *C. jejuni* Bf were transferred to a flask containing 100 mL of BHI broth and incubated at 37 or 42 °C under shaking for 9 h in MAC and AC.

Alternatively, *C. jejuni* NCTC 11168 and Bf were cultivated in MAC at 42 °C for 48 h. Then, colonies were transferred to 50 mL of BHI in a hermetic closed tube in order to prevent a large exchange of oxygen between the culture medium and the environment. The tube was incubated under AC at 42 °C with shaking (80 rpm) for 24 h. After incubation, aliquots containing 10^6 cells mL^{-1} were inoculated in a flask containing 25 mL of BHI broth and incubated at 42 °C with shaking (80 rpm) for 18 days under AC.

Campylobacter jejuni NCTC 11168 and Bf were enumerated on Karmali agar plates using the microdroplet technique [50]. Briefly, 20 μL of serial dilutions were deposited on Karmali agar plates and incubated at 42 °C for 48 h. *C. jejuni* plate counts were expressed in CFU mL^{-1}.

To determine the growth curve of *C. jejuni* strains in solid cultures, bacteria were cultivated in MAC at 42 °C for 48 h. However, *C. jejuni* Bf was cultured in AC and AAC as well. The cells obtained in these different conditions were resuspended in peptone saline solution and standardized to an OD_{600nm} of 0.03. Then, cells were plated on Karmali agar plates and incubated at 42 °C in AC and MAC for 48 h. After incubation, cells were recovered in peptone saline solution. *C. jejuni* was enumerated on Karmali agar plates using the microdroplet technique followed by incubation at 42 °C for 48 h in MAC. The experiment was performed in triplicate using three biological replicates. The D-value was calculated according to the formula:

$$\log \left(\frac{N}{N_0} \right) = -\frac{t}{D};$$

where N_0 is the initial number of cells, N is the number of cells after time t of exposure to oxygen at 42 °C and D is the rate constant for inactivation of cells. Statistically significant differences were calculated using the Student *t* test. Values <0.05 were considered statistically significant.

Rodrigues *et al. Gut Pathog* (2015) 7:30

Page 10 of 12

Scanning electron microscopy

Campylobacter jejuni NCTC 11168 and *C. jejuni* Bf cells were cultivated in MAC at 42 °C for 48 h. Then, cells were resuspended in peptone saline solution, and standardized to an OD_{600nm} of 0.01 ($\approx 10^4$ CFU mL^{-1}). The cells were plated onto Karmali agar and incubated at 42 °C in AC and MAC for 12 and 24 h. After incubation, cells were recovered in peptone saline solution and aliquots of the cell suspensions were filtered on a white polycarbonate membrane (Millipore) with a 0.22 μm pore size. The membrane containing the specimens was fixed with 2.5 % (v/v) glutaraldehyde solution for 48 h at 4 °C. Then, the samples were washed in 0.2 mol L^{-1} sodium cacodylate (pH 7.2), and dehydrated in serial concentrations of ethanol. The membranes were transferred to a critical point dryer, and the specimens were subsequently sputter-coated and observed under a scanning electron microscope (Jeol JSM 6301F).

Bacterial RNA isolation and purification

Expression of the *mreB* gene, responsible for maintaining the bacillary cell morphology of the bacteria [40, 41, 51], was compared between MAC and AC. Bacterial RNA was obtained from *C. jejuni* NCTC 11168 and *C. jejuni* Bf strains cultured for 12 and 24 h in AC and MAC, at 42 °C.

After the incubation time, RNA Protect Reagent (Qiagen, Hilden) was added to the cells. Cells were centrifuged at 3300×*g* for 6 min at 4 °C, and then resuspended in 1 mL of Extract-All (Eurobio, France) and 0.2 mL of chloroform. After centrifugation at 12,000×*g* for 15 min at 4 °C, the aqueous phase was removed. Total RNA was precipitated in isopropanol, rinsed in cold ethanol 75 %, then solubilized in 50 μL of Rnase-free water (Qiagen, Hilden). DNA was removed by digestion using RQ1 RNase-free DNase (Promega, USA), and then total DNA removal was verified by PCR using 341F/758R primers. The quality and quantity of RNA were checked using a NanoDrop spectrophotometer (NanoDrop® 2000, Thermo Scientific). The integrity of RNA samples was verified on a 1 % agarose gel; electrophoresis was carried out in Tris−acetate−EDTA buffer for 50 min at 90 V. RNA concentrations were then standardized to 100 ng for each sample prior to reverse transcription.

Quantitative real-time reverse transcription PCR

The cDNA synthesis was performed using the RevertAid H Minus First-Strand cDNA synthesis kit (Euromedex) with the random hexamer primer according to the manufacturer's instructions. The quantitative real-time PCR assay was performed with SYBR Green I (Applied Biosystems, USA) using an MJ Research PTC-200 Thermal Cycler and *mreB* and *rrs* primers (Table 1). The *rrs* gene was used as the internal control [52]. The composition of the PCR mix was as follows: 5.0 μL of sample, reverse primer (1 μM), forward primer (1 μM), and 12.5 μL of SYBR Green I Master Mix. The amplification program included an initial denaturing step at 95 °C (10 min), followed by 40 cycles at 95 °C (15 s) and 60 °C (1 min). A negative control (without cDNA) was included in each run. Relative quantification of *mreB* expression was calculated according to the $2^{-\Delta\Delta Ct}$ method. The experiments were performed in triplicate from three independent cultures.

Survival of *C. jejuni* NCTC 11168 and *C. jejuni* Bf in oxidative stress conditions

Campylobacter jejuni NCTC 11168 and *C. jejuni* Bf grown in MAC and *C. jejuni* Bf in AAC were resuspended in peptone saline solution, and standardized to an OD_{600nm} of 0.03. Subsequently, hydrogen peroxide (H_2O_2) (Sigma-Aldrich, Saint-Quentin Fallavier, France) or paraquat (PQ) (MP Biomedicals, Illkirch, France) solutions were used to induce a superoxidative and a peroxide stress, respectively. Concentrations of 0.12, 0.25, 0.50 and 1 mM of H_2O_2 or PQ were added to the bacterial suspension, which were incubated at 42 °C for 1 h under MAC. The ability of the strains to resist oxidative stress was assessed by plating serial dilutions on Karmali agar plates using the microdroplet technique followed by incubation at 42 °C for 48 h under MAC. The experiment was performed in triplicate from three independent cultures. Statistically significant differences were calculated using the Student *t* test. *P* values <0.05 were considered statistically significant.

Additional files

Additional file 1. Growth and survival of *C. jejuni* NCTC 11168 (●) and Bf (■) under MAC (continuous lines) and AC (dashed lines) at 37°C (A) and 42°C (B) in BHI broth. The number of colony-forming units was determined by plating serial dilutions on Karmali agar using the microdroplet technique followed by incubation at 42°C for 24 h in MAC. Plotted points are the mean measurements from three independent experiments. Error bars represent standard deviations, although they are too small to be visible in some cases.

Additional file 2. Growth and survival of *C. jejuni* NCTC and Bf in AC at 42°C in BHI broth.

Rodrigues *et al. Gut Pathog* (2015) 7:30

Page 11 of 12

Author details
[1] ONIRIS National College of Veterinary Medicine, Food Science and Engineering, Route de Gachet - La Chantrerie, BP 40706, 44307 Nantes Cedex 03, France. [2] Institut National de la Recherche Agronomique (INRA), Rue de la Geraudière, 44000 Nantes, France.

Competing interests
The authors declare that they have no competing interests.

References
1. European Food Safety Authority—EFSA. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J. 2015;13:3991–4153.
2. Center for Disease Control and Prevention—CDC. In: Incidence and trends of infection with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 U.S. 2014;63:328–332. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6315a3.htm?s_cid=mm6315a3w. Accessed 15 Jan 2015.
3. Blaser MJ, Taylor DN, Feldman RA. Epidemiology of *Campylobacter jejuni* infections. Epidemiol Rev. 1983;5:157–76.
4. Tauxe RV. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin I, Blaser MJ, Tompkins LS, editors. *Campylobacter jejuni*: current status and future trends. Washington, DC: American Society for Microbiology; 1992. p. 9–19.
5. Altekruse SF, Stern NJ, Fields PI, Swerdlow DL. *Campylobacter jejuni* an emerging foodborne pathogen. Emerg Infect Dis. 1999;5:28–35.
6. Kuwabara S. Does *Campylobacter jejuni* infection elicit axonal or demyelinating Guillaine Barré syndrome, or both? J Neurol Neurosurg Psychiatry. 2011;82:238.
7. Kaakoush NO, Miller WG, De Reuse H, Mendz GL. Oxygen requirement and tolerance of *Campylobacter jejuni*. Res Microbiol. 2007;158:644–50.
8. Cabiscol E, Piulats E, Echave P, Herrero E, Ros J. Oxidative stress promotes specific protein damage in *Saccharomyces cerevisiae*. J Biol Chem. 2000;275:27393–8.
9. Federighi M, Tholozan JL, Cappelier JM, Tissier JP, Jouve JL. Evidence of non-coccoid viable but non-culturable *Campylobacter jejuni* cells in microcosm water by direct viable count, CTC-DAPI double staining, and scanning electron microscopy. Food Microbiol. 1998;15:539–50.
10. Cappelier JM, Minet J, Magras C, Colwell RR, Federighi M. Recovery in embryonated eggs of viable but nonculturable *Campylobacter jejuni* cells and maintenance of ability to adhere to HeLa cells after resuscitation. Appl Environ Microbiol. 1999;65:5154–7.
11. Tholozan JL, Cappelier JM, Tissier JP, Delattre G, Federighi M. Physiological characterization of viable-but-nonculturable *Campylobacter jejuni* cells. Appl Environ Microbiol. 1999;65:1110–6.
12. Cappelier JM, Rossero A, Federighi M. Demonstration of a protein synthesis in starved *Campylobacter jejuni* cells. Int J Food Microbiol. 2000;55:63–7.
13. Dingle KE, Colles FM, Ure R, Wagenaar JA, Duim B, Bolton FJ, et al. Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. Emerg Infect Dis. 2002;8:949–55.
14. Duim B, Godschalk PC, van den Braak N, Dingle KE, Dijkstra JR, Leyde E, et al. Molecular evidence for dissemination of unique *Campylobacter jejuni* clones in Curacao, Netherlands Antilles. J Clin Microbiol. 2003;41:5593–7.
15. Dingle KE, McCarthy ND, Cody AJ, Peto TE, Maiden MC. Extended sequence typing of *Campylobacter* spp., United Kingdom. Emerg Infect Dis. 2008;14:1620–2.
16. French N, Barrigas M, Brown P, Ribiero P, Williams N, Leatherbarrow H, et al. Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. Environ Microbiol. 2005;7:1116–26.
17. Kwan PS, Birtles A, Bolton FJ, French NP, Robinson SE, Newbold LS, et al. Longitudinal study of the molecular epidemiology of *Campylobacter jejuni* in cattle on dairy farms. Appl Environ Microbiol. 2008;74:3626–33.
18. Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, et al. Tracing the source of campylobacteriosis. PLoS Genet. 2008;4:e1000203.
19. Jones DM, Sutcliffe EM, Rios R, Fox AJ, Curry A. *Campylobacter jejuni* adapts to aerobic metabolism in the environment. J Med Microbiol. 1993;38:145–50.
20. Chynoweth RW, Hudson JA, Thom K. Aerobic growth and survival of *Campylobacter jejuni* in food and stream water. Lett Appl Microbiol. 1998;27:341–4.
21. McKay AL, Peters AC. The effect of sodium chloride concentration and pH on the growth of *Salmonella typhimurium* colonies on solid medium. J Appl Microbiol. 1995;79:353–9.
22. Amanatidou A, Smid EJ, Gorris LGM. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated micro-organisms. J Appl Microbiol. 1999;86:429–38.
23. Ingham SC, Losinski JA, Dropp BK, Vivio LL, Buege DR. Evaluation of *Staphylococcus aureus* growth potential in ham during a slow-cooking process: use of predictions derived from the U.S. Department of Agriculture Pathogen Program Modeling Program 6.1 Predictive Model and an inoculation study. J Food Protect. 2004;67:1512–6.
24. Koutsoumanis KP, Kendall PA, Sofos JN. A comparative study on growth limits of *Listeria monocytogenes* as affected by temperature, pH and a_w when grown in suspension or on a solid surface. Food Microbiol. 2004;21:415–22.
25. Meldrum RJ, Brocklehurst TF, Wilson DR, Wilson PDG. The effects of cell immobilization, pH and sucrose on the growth of *Listeria monocytogenes* Scott A at 10 °C. Food Microbiol. 2003;20:97–103.
26. Fujikawa H, Morozumi S. Modeling surface growth of *Escherichia coli* on agar plates. Appl Environ Microbiol. 2005;71:7920–6.
27. Kassem II, Khatri M, Sanad YM, Wolboldt M, Saif YM, Olson JW, et al. The impairment of methylmenaquinol:fumarate reductase affects hydrogen peroxide susceptibility and accumulation in *Campylobacter jejuni*. Microbiologyopen. 2014;3:168–81.
28. Baillon ML, van Vliet AH, Ketley JM, Constantinidou C, Penn CW. An iron-regulated alkyl hydroperoxide reductase (AhpC) confers aerotolerance and oxidative stress resistance to the microaerophilic pathogen *Campylobacter jejuni*. J Bacteriol. 1999;181:4798–804.
29. Fields JA, Thompson SA. *Campylobacter jejuni* CsrA mediates oxidative stress responses, biofilm formation, and host cell invasion. J Bacteriol. 2008;190:3411–6.
30. Garénaux A, Guillou S, Ermel G, Wren B, Federighi M, Ritz M. Role of the Cj1371 periplasmic protein and the Cj0355c two-component regulator in the *Campylobacter jejuni* NCTC 11168 response to oxidative stress caused by paraquat. Res Microbiol. 2008;159:718–26.
31. Hwang S, Kim M, Ryu S, Jeon B. Regulation of oxidative stress response by CosR, an essential response regulator in *Campylobacter jejuni*. PLoS One. 2011;6:e22300.
32. Moran AP, Upton ME. Factors affecting production of coccoid forms by *Campylobacter jejuni* on solid media during incubation. J Appl Bacteriol. 1987;62:527–37.
33. Ikeda N, Karlyshev AV. Putative mechanisms and biological role of coccoid form formation in *Campylobacter jejuni*. Eur J Microbiol Immunol. 2012;2:41–9.
34. Ng LK, Sherburne R, Taylor DE, Stiles ME. Morphological forms and viability of *Campylobacter* species studied by electron microscopy. J Bacteriol. 1985;164:338–43.
35. Griffiths PL. Morphological changes of *Campylobacter jejuni* growing in liquid culture. Lett Appl Microbiol. 1993;17:152–5.
36. Cameron A, Frirdich E, Huynh S, Parker CT, Gaynor EC. Hyperosmotic stress response of *Campylobacter jejuni*. J Bacteriol. 2012;194:6116–30.

Rodrigues *et al. Gut Pathog* (2015) 7:30

Page 12 of 12

37. Hilbert F, Scherwitzel M, Paulsen P, Szostak MP. Survival of *Campylobacter jejuni* under conditions of atmospheric oxygen tension with the support of *Pseudomonas* spp. Appl Environ Microbiol. 2010;76:5911–7.

38. Klancnik A, Zorman T, Mozina SS. Effects of low temperature, starvation and oxidative stress on the physiology of *Campylobacter jejuni* cells. Croat Chem Acta. 2008;81:41–6.

39. Lee YD, Choi JP, Mok CK, Ji GE, Kim HY, Noh BS, et al. Expression of flagellin proteins of *Campylobacter jejuni* within microaerobic and aerobic exposures. Microbiol Biotechnol. 2004;14:1227–31.

40. Daniel RA, Errington J. Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. Cell. 2003;113:767–76.

41. Graumann PL. Cytoskeletal elements in bacteria. Annu Rev Microbiol. 2007;61:589–618.

42. Figge RM, Divakaruni AV, Gober JW. MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in *Caulobacter crescentus*. Mol Microbiol. 2004;51:1321–32.

43. Divakaruni AV, Baida C, White CL, Gober JW. The cell shape proteins MreB and MreC control cell morphogenesis by positioning cell wall synthetic complexes. Mol Microbiol. 2007;66:174–88.

44. Wang S, Arellano-Santoyo H, Combs PA, Shaevitz JW. An actin-like cytoskeleton contributes to cell mechanics in bacteria. Proc Natl Acad Sci. 2010;107:9182–5.

45. Bendezú FO, Hale CA, Bernhardt TG, de Boer PA. RodZ (YfgA) is required for proper assembly of the MreB actin cytoskeleton and cell shape in *E. coli*. EMBO J. 2008;28:193–204.

46. Kawai Y, Daniel RA, Errington J. Regulation of cell wall morphogenesis in *Bacillus subtilis* by recruitment of PBP1 to the MreB helix. Mol Microbiol. 2009;71:1131–44.

47. Okoli AS, Wilkins MR, Raftery MJ, Mendz GL. Response of *Helicobacter hepaticus* to bovine bile. J Proteome Res. 2010;9:1374–84.

48. Denis M, Soumet C, Rivoal K, Ermel G, Blivet D, Salvat G, et al. Development of a m-PCR for simultaneous identification of *Campylobacter jejuni* and *Campylobacter coli*. Lett Appl Microbiol. 1999;29:406–10.

49. Dingle KE, Colles FM, Wareing DRA, Ure R, Fox AJ, Bolton FE, et al. Multilocus sequence typing system for *Campylobacter jejuni*. J Clin Microbiol. 2001;39:14–23.

50. Morton RD. Aerobic plate count. In: Downes FP, Ito K, editors. Compendium of methods for the microbiological examination of foods. Washington: American Public Health Association; 2001. p. 63–7.

51. van den Ent F, Johnson CM, Persons L, de Boer P, Löwe J. Bacterial actin MreB assembles in complex with cell shape protein RodZ. EMBO J. 2010;29:1081–90.

52. Hyytiäinen H, Juntunen P, Akrenius N, Hanninen ML. Importance of RNA stabilization: evaluation of *ansB*, *ggt*, and *rpoA* transcripts in microaerophilic *Campylobacter jejuni* 81-176. Arch Microbiol. 2012;194:803–8.

53. Gundogdu O, Bentley SD, Holden MT, Parkhill J, Dorrell N, Wren BW. Re-annotation and re-analysis of the *Campylobacter jejuni* NCTC 11168 genome sequence. BMC Genomics. 2007;8:162.

54. Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, et al. The genome se ll BG quence of the foodborne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature. 2000;403:665–8.

55. Fouts DE, Mongodin EF, Mandrell RE, Miller WG, Rasko DA, Ravel J, et al. Major structural differences and novel potential virulence mechanisms from the genomes of multiple campylobacter species. PLoS Biol. 2005;3:e15.

56. Hofreuter D, Tsai J, Watson RO, Novik V, Altman B, Benitez M, et al. Unique features of a highly pathogenic *Campylobacter jejuni* strain. Infect Immun. 2006;74:4694–707.

57. Fouts DE, Mongodin EF, Puiu D, Sebastian Y, Miller WG, Mandrell RE, et al. Complete genome sequence of *Campylobacter jejuni* subsp *doylei* 269.97 isolated from human blood. Submitted (JUL-2007) to the EMBL/GenBank/DDBJ databases. 2007.

58. Pearson BM, Gaskin DJ, Segers RP, Wells JM, Nuijten PJ, van Vliet AH. The complete genome sequence of *Campylobacter jejuni* strain 81116 (NCTC11828). J Bacteriol. 2007;189:8402–3.

59. Clark CG, Chong PM, McCorrister SJ, Simon P, Walker M, Lee DM, et al. The CJIE1 prophage of *Campylobacter jejuni* affects protein expression in growth media with and without bile salts. BMC Microbiol. 2014;14:70–92.