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Survival of *Campylobacter jejuni* under Conditions of Atmospheric Oxygen Tension with the Support of *Pseudomonas* spp.[▽]

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***Campylobacter jejuni* is a major food-borne pathogen. Despite causing enteritis in humans, it is a well-adapted intestinal microorganism in animals, hardly ever generating disease symptoms. Nevertheless, as a true microaerophilic microorganism it is still puzzling how *Campylobacter* cells can survive on chicken meat, the main source of human infection. In this study, we demonstrate that *C. jejuni* is able to withstand conditions of atmospheric oxygen tension when cocultured with *Pseudomonas* species, major food-spoiling bacteria that are frequently found on chicken meat in rather high numbers. Using an *in vitro* survival assay, interactions of 145 *C. jejuni* wild-type strains and field isolates from chicken meat, broiler feces, and human clinical samples with type strains and food isolates of *Pseudomonas* spp., *Proteus mirabilis*, *Citrobacter freundii*, *Micrococcus luteus*, and *Enterococcus faecalis* were studied. When inoculated alone or in coculture with *Proteus mirabilis*, *Citrobacter freundii*, *Micrococcus luteus*, or *Enterococcus faecalis* type strains, *Campylobacter* cells were able to survive ambient oxygen levels for no more than 18 h. In contrast, *Campylobacter* bacteria inoculated with type strains or wild-type isolates of *Pseudomonas* showed a prolonged aerobic survival of up to >48 h. This microbial commensalism was diverse in *C. jejuni* isolates from different sources; isolates from chicken meat and humans in coculture with *Pseudomonas putida* were able to use this survival support better than fecal isolates from broilers. Scanning electron microscopy revealed the development of fiberlike structures braiding *P. putida* and *C. jejuni* cells. Hence, it seems that microaerophilic *C. jejuni* is able to survive ambient atmospheric oxygen tension by metabolic commensalism with *Pseudomonas* spp. This bacterium-bacterium interaction might set the basis for survival of *C. jejuni* on chicken meat and thus be the prerequisite step in the pathway toward human infection.**

Campylobacter food-borne infections are the most prevalent bacterial enteric infections in humans in industrialized and developing countries (1). It has been shown that most human infections are related to poultry meat and food produced from cattle or sheep (34, 41). *Campylobacter jejuni*, the species most frequently causing human disease, can be isolated from the animal intestinal tract at levels of up to 10^9 CFU per gram of feces and can thus be called a well-adapted intestinal microorganism (30, 37). Nevertheless, because it causes human disease as a food-borne pathogen, it has to survive outside the gut. By cross-contamination at the level of the abattoir, *Campylobacter* bacteria hit the meat surface and have to adapt to different environmental challenges. *C. jejuni* is a true microaerophilic bacterium; thus, on the one hand it requires oxygen, but on the other hand it cannot grow under normal atmospheric oxygen tension conditions (15). Despite its sensitivity to high oxygen tension *in vitro*, viable and culturable *Campylobacter* bacteria can be isolated from nonskinned chicken meat at frequencies of 10^4 CFU/g (9, 19). Assumptions on the mechanisms by which *Campylobacter* cells survive on meat surfaces are diverse, for example, by growing in biofilms, entering a "viable but nonculturable state," or interacting with other microorganisms.

For instance, *C. jejuni* is able to resist protozoa digestion and can parasitize inside protozoa, e.g., *Tetrahymena pyriformis* (35). This mechanism provides survival in harsh environments and resistance to antimicrobial substances and thus enhances the potential for transmission. But bacterium-bacterium interaction has also been demonstrated to be of a high level of importance for intestinal survival and uptake (20). Accordingly, members of *Campylobacter* have been identified to initiate cellular uptake of commensal bacteria into enterocytes (14). However, a bacterial community can also mean competition, e.g., bacteriocin production by *Lactobacillus salivarius* that is effective against *Campylobacter* colonization (36).

Meat surfaces harbor numerous bacterial species (24). Some of these bacteria have adapted to this specific environmental niche and are well-known spoilage bacteria. Most relevant species belong to the family *Pseudomonadaceae*. But also different members of the *Enterobacteriaceae* can be found on meat. To date, information regarding the interaction between spoilage bacteria and pathogens is of increasing importance for public health safety measures.

Hence, experimental data on the survival of *C. jejuni* isolates in the presence of selected meat-spoiling bacteria were analyzed and clearly demonstrated a specific interaction with type strains and isolates of *Pseudomonas putida*, *Pseudomonas fragi*, and *Pseudomonas fluorescens* from chicken meat surfaces.

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TABLE 1. Bacterial strains used in this study

Species	Strain name[a]	Origin[c]
Campylobacter jejuni	DSM 4688^T	DSMZ
	NCTC 11168	NCTC
	NCTC 12662	NCTC
Campylobacter coli	DSM 4689	DSMZ
	FC56	This study
Citrobacter freundii	ATCC 8090^T	ATCC
Enterococcus faecalis	ATCC 19433^T	ATCC
Micrococcus luteus	ATCC 4698^T	ATCC
Proteus mirabilis	ATCC 29906^T	ATCC
Pseudomonas chlororaphis	FP64d	This study
Pseudomonas fluorescens	151/3	This study
	223/2A[b]	This study
	224/PS3	This study
	409/1	This study
	409/2	This study
	427/PS2	This study
	FP22a	This study
	FP56aII	This study
Pseudomonas fragi	DSM 3456^T	DSMZ
Pseudomonas putida	DSM 50198	DSMZ
	FP56bII	This study
	FP64b	This study
	FP64c	This study

[a] T, type strain.
[b] API 20 NE identification only 63%.
[c] ATCC, American Type Culture Collection, Rockville, MD; DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany; NCTC, National Collection of Type Cultures, Colindale, London, United Kingdom.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The type strains and isolates of *Campylobacter*, *Pseudomonas*, and meat-spoiling bacteria used in this study are listed in Table 1. All strains were kept as glycerol stock culture broths at −80°C. Strains of *Pseudomonas* spp. were plated on glutamate-starch-phenol red (GSP) agar (Merck, Darmstadt, Germany) according to Kielwein (16) and incubated aerobically at 25°C for 48 h for further use. Strains of *C. jejuni* and *Campylobacter coli* were grown on Campylosel agar (bioMerieux, Marcy l'Etoile, France) or on modified charcoal-cefoperazone-deoxycholate (mCCD; CM739) agar with SR155E supplement (both from Oxoid, Basingstoke, England) and grown at 42°C for 48 h under microaerobic conditions (10% CO_2, 5% O_2, and 85% N_2) by using a microbiological culture jar equipped with a Campy gas generating kit (BR0060A; Oxoid) unless stated otherwise. All other strains used were grown on Luria-Bertani (LB) agar (Merck, Darmstadt, Germany) and incubated at the appropriate temperature and time for further use.

Field isolates of *C. jejuni* (n = 142) have been isolated in different previous studies in Austria from 2001 to 2008.

Identification of *Campylobacter jejuni*. Identification of *Campylobacter jejuni* isolated from chicken meat and broiler feces was done by hippurate hydrolysis according to the methods of Hwang and Ederer (12). Additionally, all isolates were further characterized by PCR analysis of the hippuricase gene *hip* (18) and the flagellin gene *fla* (40). The *Campylobacter* strains *C. jejuni* DSM 4688 and *C. coli* DSM 4689 from the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen [DSMZ], Braunschweig, Germany) were used as positive and negative controls, respectively. For DNA extraction and detection, standard molecular biological techniques (33) were used.

***flaA* SVR sequence typing.** The method described by Meinersmann et al. (22) and primers and conditions reported by Dingle et al. (7) were used to amplify a 621-bp fragment of the gene *flaA* by PCR analysis, and the 321-bp short variable region (SVR) sequence as well as the peptide sequence encoded by the SVR nucleotide sequence were used to type six *C. jejuni* isolates (see Fig. 2). The sequence was determined by using the BigDye Terminator v3.1 cycle sequencing kit and an Applied Biosystems 310 ABI Prism genetic analyzer.

For phylogenetic analysis, sequences were aligned using ClustalW, and a dendrogram was generated using neighbor-joining analysis of MEGA v4.0 (38).

Isolation and identification of *Pseudomonas* spp. *Pseudomonas* spp. were isolated from chicken meat surfaces by serial dilution of the meat rinsates (25 g chicken meat in 250 ml peptone water) and by plating $10^{-2}/10^{-4}$ dilutions onto GSP agar plates. After an aerobic incubation at 25°C for 48 h, single colonies were picked and identified by the API 20 NE system (bioMerieux, Marcy l'Etoile, France).

Survival assays. For the survival assay, suspensions of *C. jejuni* and the respective supporter strains were made: from a *C. jejuni* culture freshly grown on Campylosel agar plate, bacterial cells were harvested and suspended in 500 μl Mueller-Hinton (MH) broth (Merck, Darmstadt, Germany) to give a final optical density at 600 nm (OD_{600}) of 0.3 as determined by the use of a Biomek plate reader (Beckman Coulter, Krefeld, Germany). A culture of the supporter strain adjusted to a final OD_{600} of 0.4 was achieved the same way. A starting OD_{600} of 0.3 and 0.4 for the *Campylobacter* and supporter strains, respectively, was chosen as the best from a series of different combinations of various bacterial concentrations.

The bacterial suspensions were further diluted 1:10 (vol/vol), and 10 μl was used for each assay. The survival assays were performed in 96-well microtiter plates, and each assay was performed in duplicate. For the assay, 10-μl samples each of *C. jejuni* and of the supporter strains to be tested, alone or in combination, were transferred to the wells and filled up to 100 μl with MH broth. The microtiter plates were incubated aerobically at 35°C for 48 h. Viable cell counts at selected time points were determined by removing 10-μl aliquots, and serial dilutions were plated on GSP agar and mCCD agar. Controls of *Campylobacter* isolates alone and *Pseudomonas* isolates alone were included in duplicate for each assay and for every isolate tested. Mean CFU values were calculated from four independent assays.

For mass survival assays of large series of *Campylobacter* and supporter strains, the same microtiter plate format was chosen, but instead of following the CFU over time only two time points were chosen. Ten-microliter samples collected after 32 and 48 h of aerobic incubation were plated on GSP agar and on mCCD agar and incubated (either aerobically at 25°C [GSP] or microaerobically at 42°C [mCCD], respectively). In each experiment, control samples of each *Pseudomonas* and *Campylobacter* strain were plated on GSP and mCCD agar. Thus, possible growth of *Pseudomonas* cells on mCCD or *Campylobacter* cells on GSP agar would have been detected as well as survival of culturable *Campylobacter* spp. at the different time points. All *Campylobacter* spp. were also grown without the support of *Pseudomonas*, and they were tested for viability at the 18-h time point.

Swarming ability. A statistically significant set of *C. jejuni* isolates was tested for swarming ability performed on soft agar plates composed of Bolton broth (Oxoid, Basingstoke, England) containing 0.22% agar (LP0011; Oxoid). Colonies were harvested, suspensions were made (1:1,000 [wt/vol]) in sterile saline, and the OD_{600} was determined. For each suspension, aliquots of 5 μl were spotted onto the center of the soft agar plates. Swarming ability was measured in millimeter diameter growth from the center after 24 and 48 h of microaerobic incubation at 42°C and 35°C.

Scanning electron microscopy (SEM). *Campylobacter jejuni* alone or in conjunction with *Pseudomonas putida* (DSM 50198) from microtiter plates at time point 32 h were spotted onto poly-L-lysine-coated cover slides. The cover slides were then washed three times with phosphate-buffered saline (PBS) followed by 2 washes with cacodylate buffer (0.1 M sodium cacodylate, pH 7.4) for 10 min. Samples were fixed in 2.5% glutaraldehyde in cacodylate buffer for 2 h at 4°C and washed three times in cacodylate buffer. The samples were then dehydrated with graded series of ethanol concentrations. Next, the specimens were critical point dried in a Bal-Tec CPD030 (Leica Microsystems GmbH, Wetzlar, Germany), and after being mounted, specimens were sputter coated with gold-palladium in a Polaron SC7640 (Quorum Technologies Ltd., Newhaven, United Kingdom). The samples were finally viewed on a Jeol JSM 5410LV scanning electron microscope (Jeol Ltd., Tokyo, Japan), operated at 10 to 15 kV. Samples of four independent experiments were analyzed.

Statistical analysis. The abilities of two different *Pseudomonas* species isolates to improve aerobic survival of *Campylobacter* were compared by the chi-square test (32). Also the influence of the source of the *Campylobacter* isolates on their

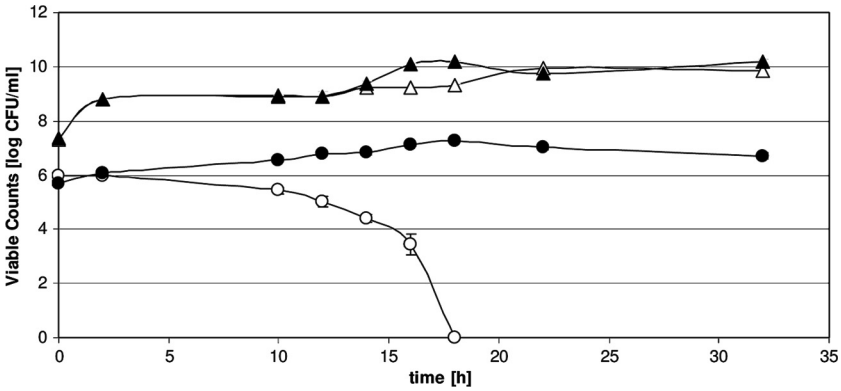

FIG. 1. Viable cell counts of *C. jejuni* and *P. putida* under aerobic growth conditions. *C. jejuni* DSM 4688 (circles) and *P. putida* DSM 50198 (triangles) were grown at 35°C under aerobic conditions alone (open symbols) or in combination (filled symbols). Coincubation with *P. putida* prolonged the survival of *C. jejuni* for at least 14 h. Viability of *P. putida* seemed unaffected by coincubation with *C. jejuni*. Data correspond to mean values of four independent experiments.

improved aerobic tolerance was statistically analyzed by the chi-square test. The significance of differences in swarming ability of *Campylobacter* isolates showing different aerotolerance when coincubated with *Pseudomonas* was assessed by comparing box plots and the 95% confidence intervals of the medians (StatGraphics; Statistical Graphics Corp., Princeton, NJ).

Nucleotide sequence accession numbers. Nucleotide sequences have been deposited in the GenBank database under accession numbers HM581947 to HM581952.

RESULTS

Interaction between spoilage bacteria and *Campylobacter jejuni*. To study the relevance of bacterial interactions for the survival of *C. jejuni* under aerobic growth conditions, field isolates and type strains of *C. jejuni* and spoilage bacteria commonly found on meat (Table 1) were coincubated and the viability was determined at different time points.

Incubation of *C. jejuni* type strain DSM 4688 with type strains of *Proteus mirabilis*, *Citrobacter freundii*, *Micrococcus luteus*, and *Enterococcus faecalis* did not result in a prolonged survival of the aerobic growth conditions for *C. jejuni* DSM 4688. However, when *Pseudomonas putida* DSM 50198 was present in the mixed growth culture, high numbers of viable *C. jejuni* DSM 4688 cells were detected even after 32 h, whereas 18 h of aerobic growth conditions sufficed to eliminate all *Campylobacter* cells (all type strains and all isolates used) grown alone (Fig. 1). Viability of *P. putida* DSM 50198 seemed unaffected by coincubation with *C. jejuni*. We also included two *Campylobacter coli* strains, DSM 4689 and one field isolate, FC56, in the study. Both strains were able to survive under the tested conditions with *P. putida* DSM 50198 for more than 42 h but did not survive without *P. putida* DSM 50198 more than 18 h.

Survival ability is dependent on *Campylobacter jejuni* and *Pseudomonas* strains. To analyze if the enhanced aerotolerance is a general phenomenon in *C. jejuni*, selected strains isolated from chicken meat were examined. When cocultivated with *P. putida* DSM 50198, all tested *C. jejuni* strains exhibited an increased aerobic tolerance but differed in the overall extent (Fig. 2A). Field isolates GC8 and EuC245 survived the aerobic incubation for only a short period of time, having lost viability after 32 h, while GC162 and EuC196 survived for at least 40 h. Field isolates GC3 and EuC300 as well as type strain DSM 4688 revealed a growth phase for the first 18 h and only a modest decrease in viability for less than 2 $\log_{10}$ units at time point 48 h (Fig. 2A). However, despite the fact that aerobic survival in the presence of *P. putida* was not of the same quality in all *C. jejuni* strains, all strains benefitted from the coincubation, as no *C. jejuni* strain was able to stay alive for more than 18 h when grown alone (Fig. 2B).

flaA SVR sequencing of these field isolates revealed that both GC8 and EuC245, the two isolates having lost viability already after 32 h, belong to the same *flaA* SVR group although they were isolated from very different sources, turkey meat and chicken feces. The other field isolates all belonged to different *flaA* SVR groups (GenBank accession no. HM581947 to HM581952).

When all the different *Pseudomonas* strains (Table 1) were analyzed for their capacities to enhance aerobic tolerance of *C. jejuni* type strain DSM 4688, all *Pseudomonas* strains supported the aerobic survival of *C. jejuni* DSM 4688 for at least 48 h.

Interestingly, the interactions between *C. jejuni* and the supporting *Pseudomonas* strains seem to be very specific. When testing either *P. putida* DSM 50198 or the meat isolate FP56bII for enhancement of the aerobic survival of 142 *C. jejuni* field isolates, two isolates exhibited aerobic survival for less than 32 h with DSM 50198. When coincubated with *P. putida* FP56bII, the same two isolates survived for more than 48 h (Table 2). Eight additional isolates which survived for less than 32 h with DSM 50198 showed an increased aerobic survival in the presence of FP56bII, although the viable counts were highly reduced at 32 h (reduction to 10 to 100 CFU/ml).

Survival ability of *Campylobacter jejuni* isolates from different sources. To see whether *C. jejuni* isolates from different sources differed in their abilities to use *Pseudomonas* species as viability supporters, isolates from chicken meat, broiler feces, and human clinical samples were cocultured with *P. putida* DSM 50198 or with the meat isolate FP56bII. *C. jejuni* isolates from broiler fecal samples differed significantly ($P < 0.01$) in aerobic survival from chicken meat isolates and from human isolates (Table 3). Whereas all 42 *C. jejuni* strains isolated from

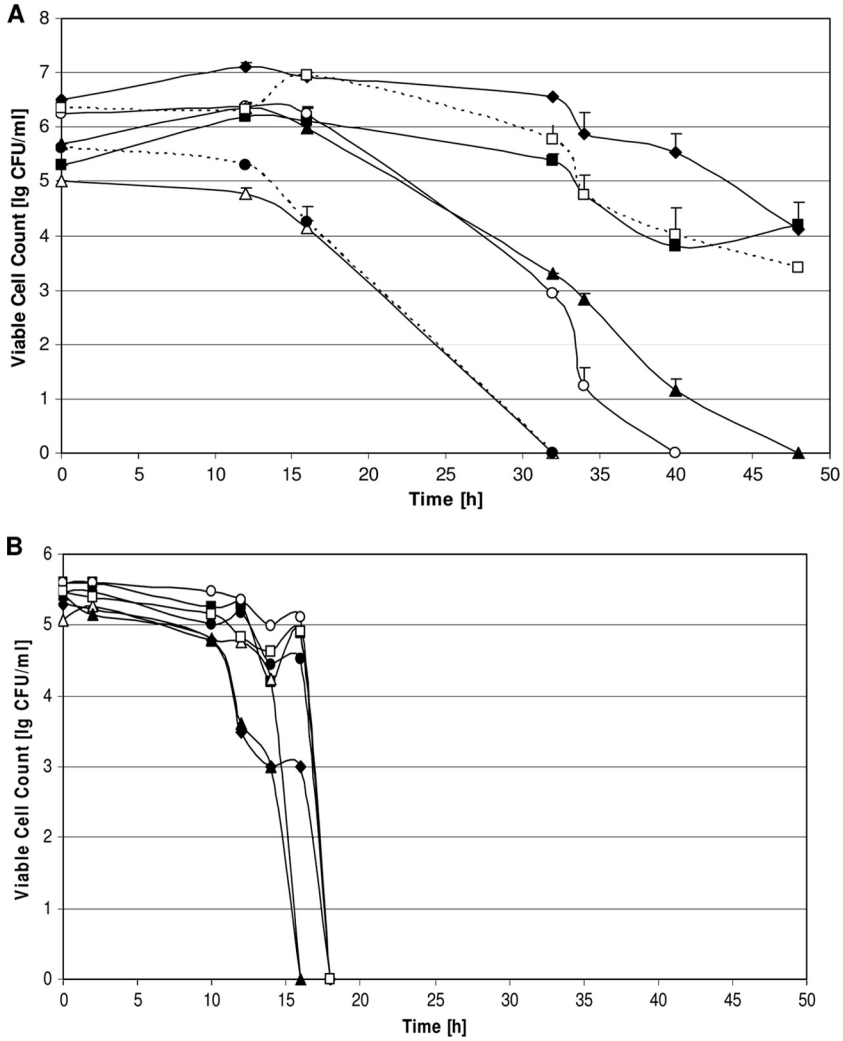

FIG. 2. Viable cell counts of *C. jejuni* strains coincubated with *P. putida* DSM 50198. (A) *C. jejuni* strains DSM 4688 (filled diamonds), EuC300 (open squares), GC3 (filled squares), EuC196 (filled triangles), GC162 (open circles), EuC245 (filled circles), and GC8 (open triangles) were coincubated with *P. putida* DSM 50198, and some survived the aerobic growth conditions for more than 48 h. (B) When incubated without the support of *P. putida*, none of the *C. jejuni* strains could survive 18 h of aerobic incubation.

TABLE 2. Survival of *Campylobacter jejuni* strains with *P. putida* strain DSM 50198 or FP56bII

Survival of *C. jejuni* when coincubated with DSM 50198	No. of *C. jejuni* strains with indicated result when coincubated with FP56bII[a]			Total *C. jejuni* strains
	Survival of >48 h	Highly reduced CFU at 32 h[b]	Survival of <32 h	
Survival of >48 h	89	3	0	92
Highly reduced CFU at 32 h	22	6	0	28
Survival of <32 h	2	8	12	22
Total	113	17	12	142

[a] Chi-square = 101.45; $P = 0.000$.
[b] "Highly reduced" indicates reduction to 10 to 100 CFU/ml.

human clinical samples and 45 out of 47 chicken meat isolates showed prolonged aerobic survival in the presence of *P. putida* DSM 50198, 20 out of 53 *C. jejuni* strains isolated from broiler fecal samples could not even survive 32 h. Without coincubation with a *Pseudomonas* supporter, no strain survived 18 h of aerobic growth conditions. When coincubated with FP56bII, the results were similar.

Scanning electron microscopy of *Campylobacter jejuni* coincubated with *Pseudomonas putida*. Grown under conditions of a microaerobic atmosphere, *C. jejuni* displayed its characteristic spiral morphology (Fig. 3A). Whereas it is known that *C. jejuni* cells undergo a morphological change from spiral to coccoid under normal oxygen tension (5), *C. jejuni* cells remained in the spiral morphology despite atmospheric oxygen tension when cocultured with *P. putida* (Fig. 3B to D). Moreover, *C. jejuni* and *P. putida* seem to interact by close contact and fiberlike structures, like a cobweb. The

TABLE 3. Survival of *Campylobacter jejuni* isolates from different sources cocultured with either *P. putida* strain DSM 50198 or FP56bII

Source of *C. jejuni*	No. of *C. jejuni* strains with indicated result when coincubated with:			
	DSM 50198[a]		FP56bII[b]	
	Survival of ≥32 h[c]	Survival of <32 h	Survival of ≥32 h[c]	Survival of <32 h
Broiler feces	33	20	42	11
Chicken meat	45	2	46	1
Humans	42	0	42	0

[a] Chi-square = 32.26; $P < 0.01$. Within "Source," broiler feces differ from chicken meat and human stool samples.
[b] Chi-square = 16.68; $P < 0.01$. Within "Source," broiler feces differ from chicken meat and human stool samples.
[c] Combined from "Survival of >48 h" and "Highly reduced CFU at 32 h" (see Table 2).

fiberlike structures appear to aggregate *C. jejuni* with *P. putida* (Fig. 3B to D).

Swarming capability of *C. jejuni* isolates. The swarming abilities of *C. jejuni* isolates grouped according to their previously demonstrated aerotolerance—survival for more than 48 h, survival but highly reduced CFU at 32 h, and survival for less than 32 h—were determined 24 and 48 h after plating. Whereas all isolates were able to swarm, no statistically significant difference between the groups was detected (i.e., box plots comprising the central 50% of samples as well as the 95% confidence interval [CI] for the median clearly overlapped).

DISCUSSION

C. jejuni is a major food-borne pathogen whose principle reservoir is in the intestinal tracts of warm-blooded animals.

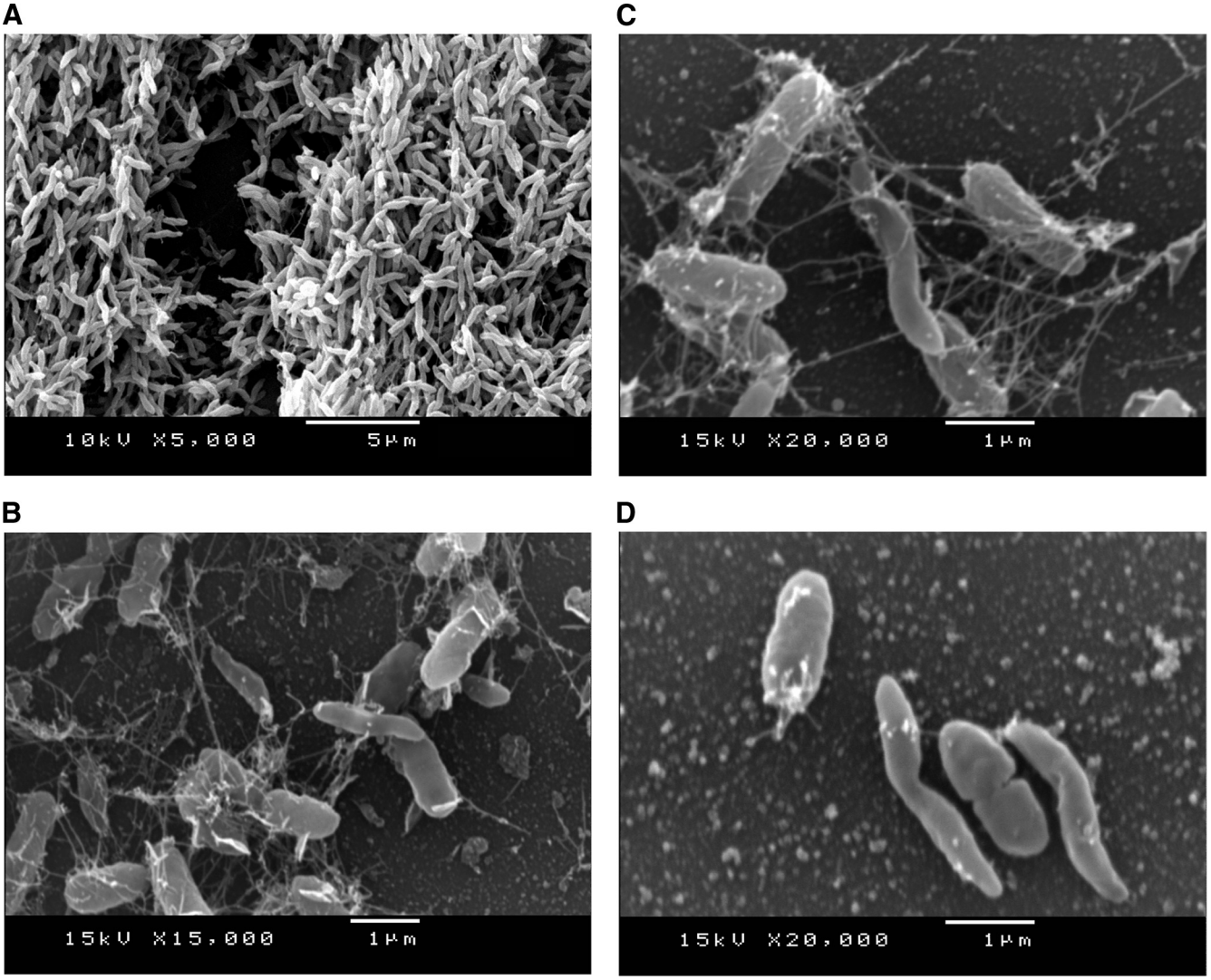

FIG. 3. Representative scanning electron microscopy. (A) SEM of *C. jejuni* DSM 4688 cultured under microaerobic conditions. (B and C) SEM of *P. putida* DSM 50198 and *C. jejuni* DSM 4688 cocultured under aerobic conditions for 32 h. *C. jejuni* cells are spiral shaped when cocultured with *P. putida*. (B to D) Cells of *Pseudomonas* and *Campylobacter* can be distinguished by the shape and thickness of the cells; fiberlike structures form cobwebs around the bacteria. (D) Both *P. putida* and *C. jejuni* seem to interact by close contact.

Colonization in food-producing animals has been studied and reaches up to 10^7 to 10^9 CFU per gram of intestinal content. Especially high rates of culturable *Campylobacter* spp. are found in the cecal and fecal contents of broilers reaching slaughter age (30), and thus chicken meat has been detected as the main source of human disease (34, 41). At slaughter, chicken meat can readily be contaminated by fecal contamination at the slaughter processing steps: scalding, defeathering, evisceration, and washing (25). Contamination of chicken meat with *Campylobacter* spp., determined by using cultural methods, ranges from 30 to 90% (4, 23, 29, 30). Thus, *Campylobacter* spp. can stay not only viable but also culturable on chicken meat surfaces despite their sensitivity to atmospheric oxygen tension. In general, *Campylobacter* species require 3 to 15% oxygen and 3 to 5% carbon dioxide for growth as they have a respiratory metabolism, resulting in the production of toxic reactive oxygen species, such as hydroxyl radical and hydrogen peroxide, that might lead to nucleic acid and protein injury (15, 28). Recent reports indicate that *C. jejuni* contains a range of enzymes involved in oxidative stress resistance. Another possible mechanism of dealing with high oxygen tension seems to be metabolic commensalism with aerobic microorganisms found on foods. This study confirms that theory by showing a beneficial effect for the aerobic survival of *C. jejuni* when incubated with well-adapted food-spoiling bacteria: *Pseudomonas* species. Several tested *Pseudomonas* species regularly found on chicken meat, like *P. putida*, *P. fragi*, *P. fluorescens*, and *Pseudomonas chlororaphis* in levels of up to 10^3 to 10^4 CFU/g (10, 21), were demonstrated to support growth and/or survival of *C. jejuni in vitro* under conditions of atmospheric oxygen tension.

Whether *C. jejuni* or *P. putida* has a higher impact on the enhanced aerotolerance of *C. jejuni* is still unclear. Different *C. jejuni* strains coincubated with *P. putida* DSM 50198 exhibited prolonged aerobic survival to different extents (Fig. 2A); however, it also made a difference whether *C. jejuni* field isolates were coincubated with either *P. putida* FP56bII or DSM 50198 (Tables 2 and 3).

A very good supporter was found in *P. putida* FP56bII, isolated from chicken meat which was already naturally contaminated with *Campylobacter* bacteria. The contaminating *Campylobacter* strain was identified as *C. coli* FC56 (Table 1), and also this *C. coli* strain had the ability to survive for a prolonged period of time, more than 48 h in combination with FP56bII and other *P. putida* strains (data not shown). Without the support of *Pseudomonas* also, this *C. coli* strain failed to survive aerobic growth for more than 18 h when tested under the conditions described above.

Metabolic commensalism regarding oxygen depletion is known from bacterial communities building up dental plaques. Primary plaque-colonizing species tend to be facultative anaerobes, like streptococci, paving the way for fastidious microorganisms and obligate anaerobes by reducing oxygen tension (for a review, see reference 17). The strictly anaerobic bacterium *Porphyromonas gingivalis* can survive oxygen levels of 20% when grown together with *Fusobacterium nucleatum* (6). In this case, *Porphyromonas gingivalis* benefits from the NADH oxidase/peroxidase activity of *F. nucleatum*, which creates an optimum microenvironment by locally reducing oxygen levels.

Although some studies have identified mechanisms that enable *C. jejuni* to cope with reactive oxygen species, like hydrogen peroxide (2, 3, 31, 39), it might be a better way for *C. jejuni* to sense the environment for low oxygen tension, actively move to that site (11), and make close contact with strictly aerobic bacteria like members of the *Pseudomonadaceae*, thus coming to a microenvironment of lowered oxygen levels.

To make close contact to other bacterial cells as seen in the SEM pictures of *C. jejuni* laying side by side with *P. putida* (Fig. 3D), motility might be advantageous. It is known that the flagellated species of *Campylobacter* not only move by flagella but also use the flagella apparatus to colonize surfaces and epithelial cells (27). However, motility is also important for bacterium-bacterium metabolic interaction. The formation of microcolonies consisting of *Burkholderia* and *Pseudomonas* species, which are able to metabolically interact with each other, seems to benefit from motility of *Pseudomonas* cells (26). Very recently, it has been documented that oxygen sensing drives motility in microbial communities (8).

Nevertheless, the swarming ability of *C. jejuni* isolates tested *in vitro* was independent of the rate of aerobic survival in the presence of *P. putida*. Strains of *C. jejuni* surviving for less than 32 h had no different swarming activity than *C. jejuni* strains surviving for more than 42 h under conditions of atmospheric oxygen tension in coculture with *P. putida*. However, as motility of *Campylobacter* might be affected by quorum sensing (13), the *in vitro* conditions used might not allow for the essential signals that trigger this special movement.

Even though all *C. jejuni* strains ($n = 145$) tested had the ability to survive under conditions of atmospheric oxygen tension for a longer period in coculture with *Pseudomonas* spp. than without, some strains were even able to multiply in the first 18 h and to survive for more than 48 h, while others could survive only up to 30 h. By testing *C. jejuni* isolated from three different sources, chicken feces, chicken meat, and human isolates, we could identify a higher percentage of *C. jejuni* isolates from chicken meat that were able to benefit from cocultivation with *Pseudomonas* spp. more effectively than isolates from chicken feces. The biological relevance of this unexpected finding might point to a possible survival mechanism for *C. jejuni* on chicken meat. New studies have to be designed to determine whether *Pseudomonas* exhibits the same supporting activity for aerobic survival of *C. jejuni* on chicken meat.

All isolates from human clinical samples used in this study showed a high level of ability to survive in coculture with *Pseudomonas* species for a prolonged period of time. Therefore, it is notable that *Campylobacter* species maintain these characteristics during the passage through the human host, suggesting it to be a highly important feature for the pathway of human infection.

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