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Isolation, Identification, and Characterization of Aero-Adaptive *Campylobacter jejuni*

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Abstract *Campylobacter* is one of the emerging foodborne pathogens, and its worldwide incidence rate is extremely high. This study was undertaken to isolate and identify *Campylobacter* strains from chicken carcasses in the local markets, and analyze their characteristics regarding oxygen tolerance. They were isolated after aerobic enrichment and identified by biochemical, physiological, and morphological characteristics, PCR, and 16S rDNA sequencing. Their oxygen tolerances were analyzed in terms of the cell surface hydrophobicity, cell fatty acid composition, and oxidoreductase. Five strains of *C. jejuni* were isolated and identified from 61 isolates from 50 chickens. Among them, *C. jejuni* IC21 grew well in Brucella broth and commercial milk under aerobic condition. However, in the aerobic exposure, the cell surface hydrophobicity of *C. jejuni* IC21 was almost the same as the other isolates, even though its morphology changed from the spiral-bacilli form into the coccoid form. Fatty acid analyses showed that all *Campylobacter* strains had a high composition of $C_{19:1}$, cyclopropane fatty acid, and that the amount of the other fatty acids were very similar between them. Interestingly, however, only oxidoreductase activities of *C. jejuni* IC21 increased highly under aerobic exposure even though its activities were almost the same as the other *C. jejuni* strains just after microaerobic culture. It had 11.8 times higher catalase activity, 4.4 times higher for SOD, and 2.0 times higher for NADH oxidase activities. Therefore, in the case of the aero-adaptive *C. jejuni* IC21, expression of oxidoreductase significantly increased under oxidative stressed condition, which might allow it to survive for a longer time and grow on food under aerobic exposure. Such new strain might be one of the explanations for the increase of campylobacteriosis.

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Recently, the incidence rate of food poisoning due to *Campylobacter* has increased more than any other foodborne pathogens such as *Salmonella*. It has been estimated that *Campylobacter* would annually cause more than two million cases of diarrhea in the United States and the number of cases reported has been around 50,000 per annum in England and Wales [6, 9, 30]. *Campylobacter* causes short-term symptoms of diarrhea, fever, and headache as well as long-term symptoms such as Miller-Fisher Syndrome (MFS) and Gullain-Barre Syndrome (GBS) which are severe nervous diseases [2, 11].

Campylobacter is Gram-negative and spiral-bacilli form, and contains a single polar flagellum on one or both ends that produce characteristic cork-screw like darting motility. *Campylobacter* grows characteristically in the microaerobic condition and temperature ranging from 37°C to 42°C. It has a circular chromosome of 1.6–1.7 Mbps, which has previously been reported to encode 1,654 proteins [24, 26]. Microorganisms in food are exposed to various environmental stresses during harvesting, processing, and distribution, and adaptive responses of the foodborne pathogens on these stresses are considered the new potential hazards to food hygiene [37]. It might be due to a genetically programmed physiological response of some bacteria, which enhances survival during environmental stress such as nutritional starvation, osmotic pressure, heat, cold, and oxidative stress. These kinds of characteristics of many foodborne pathogens, including *Salmonella*, *E. coli*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, and *Enterococcus faecalis*, have been reported [7, 27, 29]. Generally, microaerobic *Campylobacter* are sensitive to oxidative stress, but aerotolerant *Campylobacter*

can subsist on oxidative stress, as well as external stresses such as osmotic pressure, nutritional starvation, and heat shock [2, 14, 28]. The morphology of *Campylobacter* changes from spiral-bacilli form to coccoid form, which is usually viable but a nonculturable (VBNC) cell by the external stress [25, 34]. VBNC cells have the metabolic activity, but mostly cannot grow on the culture media. Regarding the pathogenesis of VBNC cell, there are still many different outcomes depending on induction treatment and virulence assay test; therefore, its virulence is not yet certain [5, 15, 22]. However, the hazard of *Campylobacter* has rapidly increased worldwide, so that physiological properties by external stresses and virulence as well as removal from food are being investigated. Therefore, the purpose of this study was to investigate whether an adapted *Campylobacter* would survive on oxidative stress under aerobic cultivation. Thus, *Campylobacter* strains were isolated after aerobic enrichment from chicken carcasses from local markets and identified, and the characteristic of their aero-adaptive growth was analyzed. In particular, morphological changes and physiological characteristics of aero-adaptively growing *Campylobacter* and microaerobic *Campylobacter* were analyzed. Through these analyses, the hazard due to possible survival and growth of *Campylobacter jejuni* under aerobic exposure were confirmed.

MATERIALS AND METHODS

Strains and Media

Campylobacter spp. were isolated from 50 chicken carcasses purchased from local markets. The strains used were *Campylobacter jejuni* ATCC 43429 and *C. coli* ATCC 43472. Chemical reagents and media were purchased from Difco (Detroit, MI, U.S.A.), BBL (MD, U.S.A.), Oxoid (Hampshire, U.K.), and Sigma (St. Louis, MO, U.S.A.).

Isolation of *Campylobacter*

Isolation of *Campylobacter* followed the methods of Tran [35] and Blaser *et al.* [4]. After the chicken carcasses were homogenized in a stomacher, the homogenates were filtered through a cheesecloth and centrifuged (10,000 ×*g*, 20 min) at 4°C and the supernatant was removed. Then, the pellet was resuspended in a minimal volume and incubated in aerobic condition for 48 h at 37°C in an enrichment broth [Bolton broth with sodium cefoperazone 32 μg/ml, trimethoprim lactate 11.4 μg/ml, vancomycin 10 μg/ml, cycloheximide 100 μg/ml, and 5% sheep blood(Komed, Seongnam, Korea)]. Then, it was streaked in the selective media (Brucella agar with vancomycin 10 μg/ml, trimethoprim lactate 5 μg/ml, polymixin B 2,500 IU, amphotericin B 2 μg/ml, cephalothin 15 μg/ml, and 10% sheep blood) and incubated at 37°C in a microaerobic chamber (Bug box, Ruskin Tech. Co., U.K.), which was flushed with a gas mixture of 5% O_2, 10% CO_2, and 85% N_2. The presumptive colonies were picked up to Brucella blood agar and further identified by biochemical, morphological, molecular biological, or genetical characterizations [10].

Scanning Electron Microscopy and Confocal Laser Scanning Microscopy Studies

The morphology of the isolated *Campylobacter* was observed by a scanning electron microscope. *Campylobacter* strains were microaerobically inoculated in Brucella blood agar for 48 h at 37°C. Then, the pellets were harvested and washed twice with phosphate buffered saline (PBS), and fixed with 2.5% glutaraldehyde. The cells were dehydrated once in 50, 70, 80, and 90% ethanol and twice in 100% ethanol, and finally three times with 100% ethanol for 15 min. The specimens were dried in a critical point drier (Polaron, Watford, England) with CO_2 as a transition fluid. They were then mounted on metal studs and coated with gold for 5 min and viewed with the scanning electron microscope (JSM 5410LV, JEOL, Tokyo, Japan). *Campylobacter* from Brucella blood agar were microaerobically inoculated into FBP-Brucella (supplemented with Brucella broth: 0.9 mM ferrous sulfate, 1.3 mM sodium metabisulfite, 2.3 mM sodium pyruvate) with 3% bovine calf serum for 48 h at 37°C. In the case of the morphology analysis, aerobic incubation was further conducted for 48 h. One ml of the culture was harvested, washed twice with PBS (pH 7.0), and resuspended again in PBS. It was stained with 0.025% acridine orange and the morphology was observed on the slide glass by confocal laser scanning microscopy (OLYMPUS, New York, U.S.A.) with an Ar laser, Kr/Ar laser light source.

PCR and DNA Sequencing

PCR was carried out for further identification of the *Campylobacter* strain. The primers [12, 21] were synthesized commercially (Genotech and Bioneer, Daejon, Korea). Whole cells of *Campylobacter* were used without DNA extraction as the DNA template for the PCR [32]. It was performed in a Gene cycler (BioRad, Hercules, CA, U.S.A.), and the PCR products were analyzed using 1% agarose gel in a TAE buffer containing 0.5 μg/ml of ethidium bromide. The gel was visualized and photographed under a UV transilluminator (Seolinbiotech, Suwon, Korea) after electrophoresis at 5 V/cm. 16s rRNA sequencing reactions were performed in a MJ Research Gradient Cycler using a dye terminator cycle sequencing ready reaction kit with AmpliTaqr DNA polymerase (Biosystems, Foster City, CA, U.S.A.), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using CAH 16S1a (5'-AATACATGAA-AGTCGAAGGA-3') and CAH 16S1b (5'-TTAACCCAA-CATCTCACGAC-3'). The fluorescent-labeled fragments

were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in a sequencer (Biosystems ABI 3700, Foster City, CA, U.S.A.). All primary readings were edited to remove vector sequences and unreliable data using the program Factura (Perkin-Elmer, Norwalk, CT, U.S.A.). Sequences longer than 100 nucleotides were further analyzed. Local homology searches were performed in a PC computer running Linux, using the BLAST suite of programs. BLAST searches against the National Center for Biotechnology Information (NCBI) nonredundant protein database were performed locally with database. The BLAST programs and the Netblast client are distributed by the NCBI (ftp://ncbi.nlm.nih.gov).

Incubation of Isolated *C. jejuni* in Aerobic Condition

Campylobacter from Brucella blood agar was microaerobically inoculated in FBP-Brucella broth and further in aerobic condition for 48 h at 37°C. Its growth was measured by absorbance at 550 nm. For the growth in a commercial milk broth, 100 ml of milk was put into a sterilized flask, sterilized for 30 min at 63°C, and cooled down to room temperature. *Campylobacter* at 10^4–10^5 CFU/ml was inoculated into the milk and incubated aerobically for 48 h at 37°C. The numbers of colony forming units on Brucella blood agar were counted after the agar plates were incubated in a microaerobic chamber at 37°C for 48 h.

Cell Surface Hydrophobicity

Campylobacter was inoculated into FBP-Brucella broth and incubated microaerobically for 48 h at 37°C, and then further aerobically for 48 h. After centrifugation of the culture for 5 min at 10,000 ×*g*, the cells were washed twice with 50 mM potassium phosphate buffer (pH 7.0). The suspension was adjusted to 1.0 (A_i) at 600 nm, and 3 ml of n-hexadecane was added to 3 ml of the suspension and then vortexed for 120 sec. In order to separate the layers, the suspension was left for 20 min and CSH was calculated from the hydrophilic layers at the bottom by measuring absorbance (A_f) at 600 nm [CSH (%)=$(A_i-A_f)\times100/A_i$] [18].

Analysis of Cell Fatty Acid Composition

In order to analyze the fatty acid composition of *Campylobacter*, gas chromatography (HP 6890, Hewlett Packard, Delaware, U.S.A.) was used. After the microaerobic culture, *Campylobacter* was harvested by centrifugation at 5,000 ×*g*. Fatty acids from these suspensions were methylated and extracted. Then, the samples were analyzed using GC and via Sherlock, which is a *Campylobacter* identification program from MIDI. The GC column used Ultra 2 (25 m, 0.2 mm, Hewlett Packard, Delaware, U.S.A.) and the gas composition was air (300 ml/min), hydrogen (25 ml/min), and nitrogen (25 ml/min).

Determination of Superoxide Dismutase, Catalase, and NADH Oxidase

To determine the enzyme activities, *Campylobacter* cells were inoculated and incubated microaerobically on the broth for 48 h at 37°C, and aerobically for further 48 h. After centrifugation (10,000 ×*g*, 5 min, 4°C) of the culture, it was washed twice with sterile saline and resuspended in 1/30 volume of the original culture. After sonication for 5 min, it was then centrifuged (25,000 ×*g*, 20 min, 4°C), and the supernatant was used as the cell-free extract. Protein concentration of the extract was quantified by the Bradford method using a Bio-Rad protein kit (Bio-Rad Laboratory, California, U.S.A.). The activity of superoxide dismutase (SOD) was measured by cytochrome c reduced at 550 nm by the McCord and Fridovich methods [20] by UV spectrophotometery (Shimadzu, Tokyo, Japan). The reaction solution contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM cytochrome c, 0.1 mM xanthine, and xanthine oxidase (0.35 unit/ml), and 10–120 µl of the cell-free extract were added to the above afterwards. The reduced cytochrome c was measured at 550 nm, and one unit of SOD was defined as the amount of

Table 1. Biochemical and physiological characteristics of *Campylobacter jejuni* isolated from chicken carcasses.

Tests	Isolates						
	C. jejuni	*C. coli*	*C.* IC21	*C.* IC22	*C.* IC24	*C.* IC25	*C.* IC28
Gram stain	–[1)]	–	–	–	–	–	–
Catalase	+[2)]	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+
H_2S(TSI)	–	–	–	–	–	–	–
Motility	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+
Nalidixic acid*	S[3)]	S	S	R	R	R	R
Cephalothine	R[4)]	R	R	R	R	R	R
Growth at 37°C and 42°C	+	+	+	+	+	+	+

[1)]Negative; [2)]Positive; [3)]Sensitive; [4)]Resistant.

Table 2. Levels of 16S rDNA sequence similarity.

Species or strain	% Similarity in :																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 *C. coli* ATCC 33559																				
2 *C. concisus* ATCC 33237	93.5																			
3 *C. curvus* ATCC 35224	93.0	97.0																		
4 *C. helveticus* NCTC1 2470	95.5	91.9	91.8																	
5 *C. fetus* subsp. *venerealis* ATCC 1943	95.0	95.1	94.4	92.7																
6 *C. fetus* ATCC 27374	94.9	95.0	94.3	92.6	99.9															
7 *C. hominis* NCTC 13146	89.9	92.9	92.7	88.8	91.6	91.5														
8 *C. hyointestinalis* NCTC 11608	94.9	94.0	93.3	92.9	98.2	98.2	90.8													
9 *C. jejuni* subsp. *jejuni* ATCC 33560	98.5	93.2	92.7	96.7	94.2	94.1	90.3	94.1												
10 *C. jejuni* subsp. *doylei* CCUG 24567	98.5	93.3	92.8	96.5	94.2	94.1	90.4	94.1	99.9											
11 *C. lanienae* NCTC 13004	96.3	94.2	94.1	93.9	96.7	96.7	90.5	97.0	95.4	95.3										
12 *C. lari* CCUG 23947	97.4	93.6	92.3	95.9	94.1	94.0	90.1	94.1	98.5	98.4	95.2									
13 *C. mucosalis* CCUG 6822	93.6	95.7	94.7	91.9	96.9	96.8	91.2	95.3	93.5	93.4	95.4	93.4								
14 *C. rectus* ATCC 33238	92.6	95.3	96.4	91.1	94.4	94.3	93.3	93.3	92.3	92.2	92.1	92.1	94.2							
15 *C. showae* CCUG 3054	91.4	94.4	96.0	90.7	93.3	93.2	92.8	92.1	91.1	91.2	91.1	91.1	93.5	98.0						
16 *C. upsaliensis* CCUG 14913	94.4	92.0	92.0	98.1	92.9	92.8	89.0	92.4	95.7	95.8	93.0	95.1	92.7	91.3	90.4					
17 *Campylobacter* IC21	97.5	92.1	87.1	96.0	93.1	92.8	89.0	92.8	99.1	99.0	93.4	97.1	91.8	91.7	89.8	95.7				
18 *Campylobacter* IC22	96.6	90.9	90.8	94.3	91.9	91.6	87.3	91.2	97.7	97.4	92.1	95.5	91.0	90.7	88.9	94.2	98.4			
19 *Campylobacter* IC24	95.6	90.3	89.9	93.2	90.9	90.7	86.9	90.6	96.4	96.2	91.1	94.4	89.7	90.2	88.5	93.0	97.7	98.9		
20 *Campylobacter* IC25	96.6	91.2	91.0	94.9	92.2	91.8	87.9	91.7	97.6	97.8	92.5	96.0	91.1	91.3	89.5	94.4	99.0	99.3	98.6	
21 *Campylobacter* IC28	95.8	90.6	90.5	93.8	91.4	91.1	87.3	90.6	97.0	96.8	92.0	94.9	90.0	90.1	88.4	93.3	98.6	98.9	97.9	98.5

enzyme required to inhibit cytochrome c reduction rate by 50% per min at 25°C. Catalase activity was assayed by the Beers and Sizer method [3] by measuring absorbance at 240 nm and 25°C. The reaction mixture contained 1 ml of 0.05 M H_2O_2, 1.9 ml of distilled water, and 100 µl of the cell-free extract. One unit of catalase was to decompose one micromole of hydrogen peroxide per minute at 25°C. Oxidation speed of NADH was measured via the Anders and Hogg method [19]. The reduction of absorbance was measured at 340 nm for 3 min at 25°C, and the reaction mixture contained 0.3 mM EDTA, 500 µM FAD, 3 mM β-NADH, 50 mM potassium phosphate buffer (pH 7.0), and 100 µl of the cell-free extract. One unit of NADH oxidase was defined as the oxidation ratio of NADH to NAD per min at 25°C.

RESULTS AND DISCUSSION

Isolation and Identification

After aerobic enrichment, 61 presumptive colonies on the selective media for *Campylobacter* were isolated from 50 chicken carcasses. These colonies were incubated in Brucella blood agar, and biochemical and physiological characteristics were analyzed. As a result, 5 isolates were found to match with the characteristics of *Campylobacter* such as Gram (-), catalase (+), oxidase (+), motility (+), nitrate reduction (+), and H_2S (-) test except for the resistance to nalidixic acid (Table 1). Morphologies of all the isolates showed the typical spiral-bacilli forms under the scanning electron microscope (data not shown). PCR products using the primer set of pA and pB for *Campylobacter* were confirmed: The isolates showed the product of 426 bp, which was the same as that of *C. jejuni* ATCC 43429 (data not shown). These 5 *Campylobacter* isolates were further identified by sequencing PCR products from the primers of CAH 16S1a and CAH 16S1b. The analyses are shown in Table 2, and Fig. 1 shows the systematical homology by drawing the dendrogram and 16S rDNA similarity of the 5 *Campylobacter* isolates. Through the comparisons of the systematical homology, they were identified as *C. jejuni*.

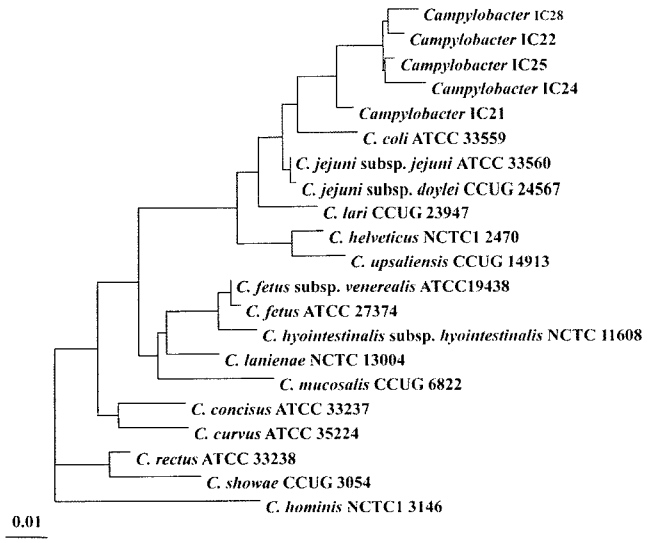

Fig. 1. Dendrogram showing relationships among five isolates of *Campylobacter jejuni* based on 16S rDNA sequence.

Aerobic Growth of Isolated *C. jejuni* in Brucella Broth and in Commercial Milk

When *C. jejuni* IC21 and *C. jejuni* ATCC 43429 were aerobically cultured in FBP-Brucella broth with 3% bovine calf serum, the growth of *C. jejuni* IC21 was remarkably high [Fig. 2(A)], showing 1.0 O.D.550 difference than *C. jejuni* ATCC 43429. The viability counting of these strains after aerobic incubation in the commercial milk was the same as that on FBP-Brucella media [Fig. 2(B)]. The number of *C. jejuni* ATCC 43429 cells decreased from 10^4 CFU/ml at the beginning to 10^3 CFU/ml after 48 h incubation and, interestingly, that of *C. jejuni* IC21 increased up to 10^8 CFU/ml, indicating that *C. jejuni* IC21 was able to grow under the high oxygen content of air. *Campylobacter* is known to survive microbial competition and express oxidoreductase and other physiological properties in aerobic condition. However, Chynoweth *et al.* [8] and Jones *et al.* [13] reported that *Campylobacter* cells

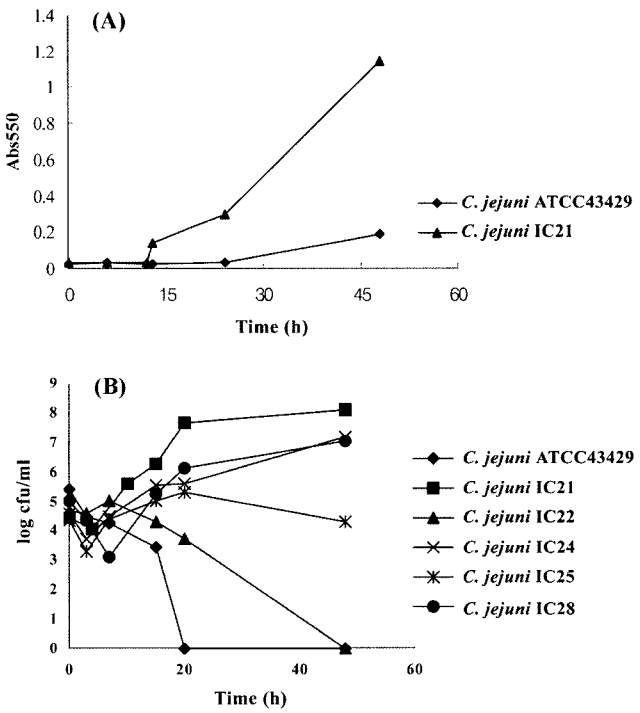

Fig. 2. Growth of *Campylobacter* on brucella broth (A) and commercial milk (B) under aerobic exposure.

were viable in aerobic condition when subcultured in aerobic condition and exposed and then adapted afterward. Microaerophilic *C. jejuni* maintained the initial bacteria number at a certain level, but the growth did not seem to occur at the aerobic exposure. In this study, the initial bacteria number was maintained until 18 h of culture when *C. jejuni* ATCC 43429 was incubated in the milk media under aerobic condition; therefore, it might be able to survive because it was adapted to oxygen, in support of the Chynoweth *et al.* and Jones *et al.* reports. The robust growth of *C. jejuni* IC21 was observed, so that this strain could tentatively be called the aero-adaptively growing *C. jejuni* IC21. Therefore, the aerobic growth phenomenon of the aero-adaptive *C. jejuni* IC21 was analyzed in terms of cell surface hydrophobicity, fatty acid composition, and oxidoreductase activities such as SOD, catalase, and NADH oxidase.

Morphological Change of *C. jejuni* Under Aerobic Condition and Cell Surface Hydrophobicity

Campylobacter jejuni ATCC43429 and *C. jejuni* IC21 were microaerobically incubated for 48 h and then aerobically exposed for further 48 h, and their morphological changes were examined by a confocal laser scanning microscope. After microaerobic incubation, the typical spiral-bacilli forms of *Campylobacter* were shown; however, the typical forms were changed to the coccoid forms and only some cells of both strains remained in the spiral-bacilli forms under aerobic exposure (Fig. 3). The morphology of *C. jejuni* IC21 could be confirmed as the typical morphological

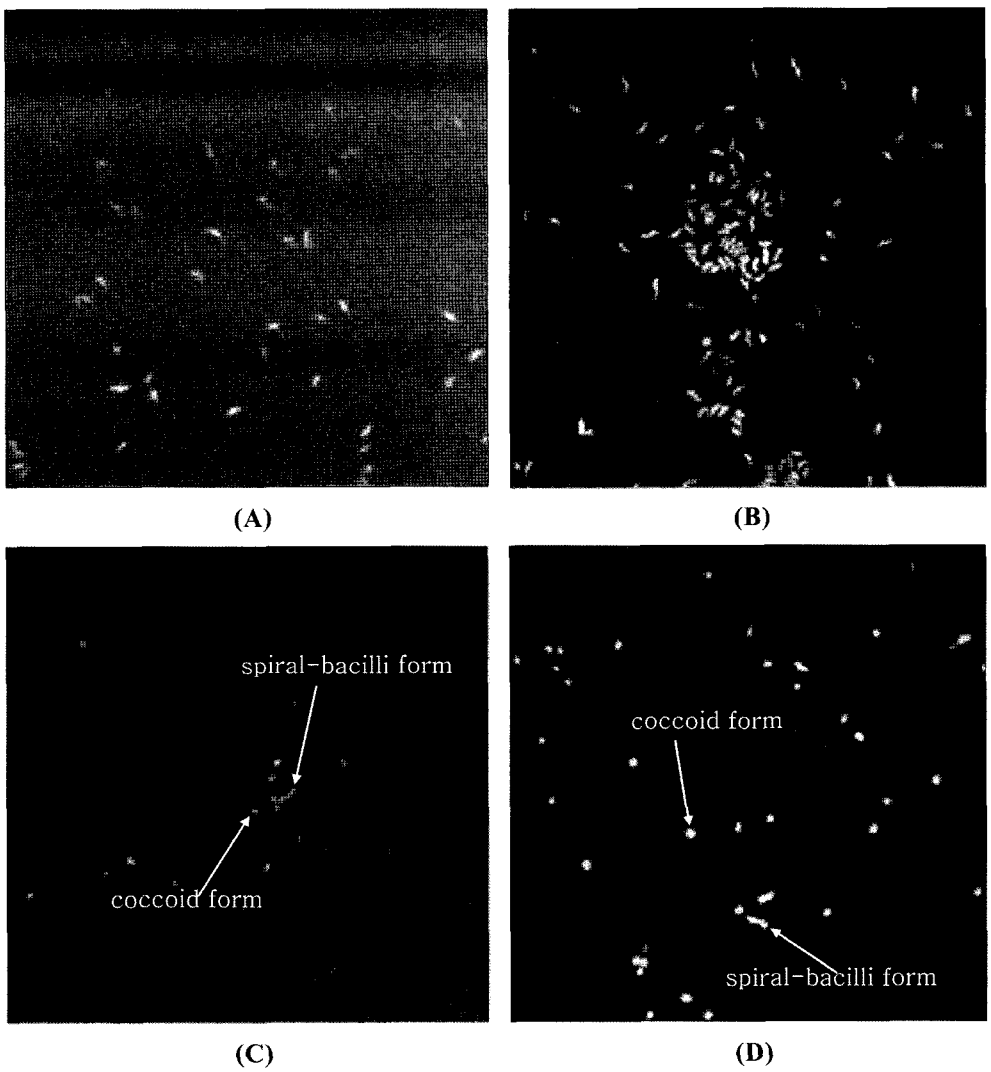

Fig. 3. Morphological change of *Campylobacter jejuni*, as seen by confocal scanning microscopy under microaerobic and aerobic exposures (×1,500).
(A) and (C), *C. jejuni* ATCC43429; (B) and (D), *C. jejuni* IC21. The bacteria were exposed microaerobically (A, B) for further 48 h and aerobically (C, D) for 48h after microaerobic culture.

Table 3. The cellular fatty acid composition of *Campylobacter* spp.

(Percentage of total chromatic area)

Isolates	Component			
	$C_{14:0}$ FAME*	$C_{16:0}$ FAME	$C_{18:0}$ FAME	$C_{19:1}$ cyclo 11,12 FAME
C. jejuni ATCC43429	4.87	40.28	3.22	4.61
C. jejuni IC21	8.72	37.53	0	7.20
C. jejuni IC22	7.17	35.57	0	5.87
C. jejuni IC24	6.50	40.00	0	5.27
C. jejuni IC25	5.48	12.27	0	6.43
C. jejuni IC28	7.20	34.50	0	3.96

*FAME (Fatty acid methyl ester): a methylated form of typical ester linkage of fatty acid.

change of other *C. jejuni*, even though it had high growth activity under the aerobic culture condition. To discern any morphological changes, cell surface hydrophobicity (CSH) was analyzed after *Campylobacter jejuni*, *C. coli*, and *C. jejuni* IC21 were incubated microaerobically and aerobically. Generally, it has been reported that high hydrophobicity of the cell wall and membrane, including the lipopolysccharide, would greatly contribute to stress and cell adherence in Gram-negative bacteria [16, 36]. However, in this experiment, CSH showed no notable differences under aerobic condition, compared with microaerobic condition; therefore, CSH might not contribute to the aero-adaptive growth of *C. jejuni* IC21 (data not shown).

Analysis of Total Fatty Acid Composition of *C. jejuni*

As shown in Table 3, the fatty acid composition of *Campylobacter jejuni* ATCC 43429 and isolated *C. jejuni* were analyzed by gas chromatography. The fatty acid composition of isolated *Campylobacter* whole cell showed a higher composition of $C_{14:0}$ and $C_{19:1}$, and no detected $C_{18:0}$ in *Campylobacter jejuni* ATCC 43429. In particular, the amount of $C_{19:1}$ in *C. jejuni* IC21 was 1.6 times higher than that it *Campylobacter jejuni* ATCC 43429. Generally, when oxidatively stressed, the amount of unsaturated fatty acid would go down and that of saturated fatty acid would go up, and this would be one mechanism to protect the organism from the oxidative radicals [1, 31]. Therefore, the high compositions of the cyclic form, such as cyclopropane $C_{19:1}$, in *Campylobacter* might contribute to the aerotolerance during the aerobic exposure. In the present study, there was no notable difference in fatty acid composition between *Campylobacter jejuni* ATCC 43429 and *C. jejuni* IC21.

Activities of Superoxide Dismutase, Catalase, and NADH Oxidase

To determine the activities of superoxide dismutase (SOD), catalase, and NADH oxidase as oxidoreductases, *C. jejuni* ATCC43429, *C. coli*, and *C. jejuni* IC21 were inoculated, and microaerobically incubated on the Brucella broth for 48 h at 37°C and aerobically for a further 48 h. Under aerobic exposure, the activities of oxidative enzymes in *C. jejuni* ATCC43429 were 7.8 units of SOD, 0.8 unit of catalase, and 9.5 units of NADH oxidase, which were 1.1, 2.6, and 4.8 time higher than those activities under microaerobic condition, respectively (Table 4). In *C. coli*, there were 9.6 units of SOD, 17.4 units of catalase, and 10.2 units of NADH oxidase, which were 1.0, 29.9, and 3.9 times higher, respectively. The catalase activity in *C. coli* increased very high, but its growth seemed to be lower than that of *C. jejuni* IC21. In *C. jejuni* IC21, these were 34.4 units of SOD, 9.4 units of catalase, and 19.1 units of NADH oxidase, which showed 4.4, 34.4, and 3.9 times increase in comparison with the activities under microaerobic growth, respectively. The oxidoreductase activities of both *C. jejuni* ATCC43429 and *C. jejuni* IC21 under microaerobic growth were almost the same, but they increased several

Table 4. Activities of superoxide dismutase (SOD), catalase, and NADH oxidase in *Campylobacter* exposed to each condition.

Strains	Condition*	Specific activity (unit/mg protein)		
		SOD	Catalase	NADH oxidase
C. jejuni ATCC43429	Microaerobic	6.8	0.3	2.0
	Aerobic	7.8	0.8	9.5
C. coli ATCC43472	Microaerobic	9.2	0.6	2.6
	Aerobic	9.6	17.4	10.2
C. jejuni IC21	Microaerobic	7.8	0.2	4.9
	Aerobic	34.4	9.4	19.1

**C. jejuni* ATCC43429, *C. coli*, and *C. jejuni* IC21 were inoculated and incubated microaerobically (Microaerobic) on the Brucella broth for 48 h at 37°C, and then aerobically (Aerobic) for a further 48 h.

times under aerobic exposure and might contribute to the protection from oxidative radicals of the aerobic O_2 content. Interestingly, the activities of SOD and catalase were higher in *C. jejuni* IC21 than in *C. jejuni* ATCC43429, which might seem important for the aero-adaptive growth. Generally, when *Campylobacter* cells are oxidatively stressed under aerobic condition, operons such as KatA, KatE, or SodB are in operation to express proteins such as catalase and SOD [25]. In the case of NADH oxidase, a known terminal oxygen oxidoreductase in bacteria, this would importantly work on the oxygen resistance mechanism in anaerobic and aerobic bacteria such as lactic acid bacteria [1]. It was reported that NAD(P)H oxidase would protect microaerobic *Campylobacter* [17, 33], and similar results were also obtained in the present experiment. In this study, however, SOD would be the most important oxidoreductase between them, since it is known to work first when oxidative radicals appear in the cell. Therefore, the high expression levels of SOD and catalase during aerobic exposure would be crucial for the aero-adaptive growth of *C. jejuni* IC21.

The possibility that microaerobic *Campylobacter jejuni* could survive and grow against oxidative stress under aerobic cultivation was analyzed. After aerobic enrichment, *Campylobacter* strains were isolated and identified, and characteristics of its aero-adaptive growth were analyzed. One of the isolates, which was identified as *Campylobacter jejuni* IC21, grew well in aerobic exposure of the milk; however, its cell fatty acid composition and cell surface hydrophobicity were not different from the other *Campylobacter* isolates. Furthermore, its oxidoreductase activities such as SOD, catalase, and NADH oxidase were almost the same after microaerobic cultivation, but its activities increased highly under aerobic exposure. The increased SOD activity in the present study seems to be very important; nevertheless, no study on the increase in expression of *Campylobacter* oxidoreductase under aerobic stress has ever been presented. Additional studies on the high enzyme expression phenomenon, which has been observed only in *C. jejuni* IC21, are needed, and study on such kind of phenomenon should be done either by adaptation or by mutation. Since PCR-RFLP analysis using the pg50 and pg3 set of PCR primer showed that only *C. jejuni* IC21 had a pattern different from the other *C. jejuni*, some kind of mutation is expected. When the morphology of *C. jejuni* IC21 under aerobic exposure was examined, they were almost the coccoid forms like the other *Campylobacter* isolates. Therefore, they seemed to survive and proliferate because of high expression of oxidoreductase even though morphological changes occurred [8, 13, 33]. Consequently, when *Campylobacter jejuni* is present in food, it might have various mechanisms for survival and adaptation. At exposure to aerobic conditions, such aero-adaptive *Campylobacter* strains would exist longer and even grow, indicating the hazard increasing. Through these analyses, the hazard arising from the possibilities of longer survival and growth of *Campylobacter jejuni* under aerobic exposure was confirmed, and a high level of food hygiene is required in the case of *Campylobacter*-contaminated foods.

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