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Temperature-Dependent Membrane Fatty Acid and Cell Physiology Changes in Coccoid Forms of *Campylobacter jejuni*

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The effect of temperature and the availability of nutrients on the transition of spiral *Campylobacter jejuni* cells to coccoid forms was investigated. Ageing of spiral *C. jejuni* cells in either nutrient-poor or nutrient-rich environments resulted in the formation of nonculturable coccoid cells at 4, 12, and 25°C after different periods, with the cells incubated at 4°C in nutrient-deficient media remaining culturable the longest. To study the phenomenon, ATP levels, protein profiles, and fatty acid compositions were monitored under conditions where the transition from spiral to coccoid cells occurred. During storage, the levels of intracellular ATP were highest in cells incubated at low temperatures (4 and 12°C) and remained constant after a small initial decrease. During the transformation from spiral to coccoid forms, no alteration in protein profiles could be detected; indeed, inhibition of protein synthesis by chloramphenicol did not influence the transition. Furthermore, DNA damage by gamma irradiation had no effect on the process. Membrane fatty acid composition of cocci formed at low temperatures was found to be almost identical to that of spiral cells, whereas that of cocci formed at 25°C was clearly different. Combining these results, it is concluded that the formation of cocci is not an active process. However, distinctions between cocci formed at different temperatures were observed. Cocci formed at 4°C show characteristics comparable to those of spirals, and these cocci may well play a role in the contamination cycle of *C. jejuni*. However, spiral campylobacters can also play an important role in this cycle, since these cells remain culturable for a long period, especially under nutrient-poor and low-temperature conditions.

Campylobacter jejuni is an important cause of food-borne infections and is associated mostly with chicken products (22). Although much research has been carried out, the environmental contamination cycle of this pathogen has not been completely elucidated (9, 30). When cells of this microorganism are excreted in the environment, they usually do not grow, since the minimum growth temperature for *C. jejuni* is 32°C (5); therefore, survival rather than growth is important under these conditions. It is generally known that *Campylobacter* cells are transformed from the normal spiral form into a coccoid form under unfavorable conditions. Although this coccoid form, which is evident at both growth and environmental temperatures (26), is not culturable by available techniques, it may nevertheless play a role in *Campylobacter* survival during the contamination cycle.

The transformation to a viable nonculturable form under unfavorable environmental conditions has been described for a variety of pathogenic microorganisms such as *Legionella*, *Vibrio*, *Salmonella*, and *Shigella* spp., and it appears to be reversible when the conditions improve (15). Resuscitation is influenced by various specific conditions for different bacteria. For *Campylobacter* spp., Rollins and Colwell (26) have provided preliminary evidence which suggests that reversion from coccoid to spiral forms occurred following animal passage. These results agree with observations by Colwell et al. (4) that a nonculturable *Vibrio cholerae* strain was found to be pathogenic in animal passage experiments. Infectivity of *Campylobacter* cocci has also been reported by Jones et al. (10), Saha et al. (27), and Stern et al. (29), who observed the appearance of spiral forms after administration of a cell suspension which apparently consisted only of nonculturable coccoid forms to mice, rats, and chickens, respectively. Further evidence for a role of nonculturable forms in the contamination cycle of *Campylobacter* spp. was given by Pearson et al. (23), who demonstrated that nonculturable forms of the bacterium, observed in water supplies by direct immunofluorescence microscopy, gave rise to *Campylobacter* colonization of chickens following consumption of this water. It is notable, however, that Medema et al. (16) could not isolate *Campylobacter* cells from the ceca of chickens, 7 days after ingestion of nonculturable forms. Also, in previous experiments in our laboratory, when cocci were given to mice, rabbits, and humans, no *Campylobacter*-specific antibodies could be detected and, furthermore, the bacterium could not be isolated from stool samples in a 30-day period after administration (1).

Another approach to investigate the viability of coccoid forms is to determine the physiological state of the cells. Indeed, viability of coccoid forms could be deduced from the work of Rollins and Colwell (26), who used a slight modification of the method of Kogure et al. (12) to determine the direct viable count in order to observe cell elongation. Moran and Upton (19–21), however, have concluded that the coccoid form is a degenerative state on the basis of the observation of decreased levels of peptides, nucleic acids, and superoxide dismutase in cocci. These authors also described a lack of cell integrity visible by electron microscopy.

Such contradictory results may well be caused by the different conditions under which cocci were formed and also by the different conditions under which reversion experiments or viability assays were performed. In this study, we therefore analyze the process of ageing of spiral *C. jejuni* cells under different environmental conditions, such as decreased nutrient

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availability and low temperature. Samples were taken at regular time intervals, and culturability and cell morphology were examined. To monitor metabolic changes, intracellular ATP concentrations were determined and membrane fatty acid analyses were performed. Furthermore, the effect of chloramphenicol-induced inhibition of protein synthesis and DNA-damaging gamma irradiation on the process of coccus formation was investigated. We demonstrate that formation of coccoid forms of *C. jejuni* is not an active process. Cocci identified following incubation of the bacterium at 4°C have physiological characteristics comparable to those of spirals; thus, these cocci in particular may well play a role in environmental contamination cycles of this human pathogen.

MATERIALS AND METHODS

***Campylobacter* strains and culture conditions.** The following *C. jejuni* strains were used: 104 (from sewage), 85Y500 (from human feces), LU101090 (from chicken liver), B258 (from chicken feces), and ATCC 33560 (from bovine feces). Strains 104 and LU101090 were isolated in our laboratory; strains 85Y500 and B258 were obtained from the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. All strains were maintained in 15 to 20% (vol/vol) glycerol in brain heart infusion broth (BHI; Difco no. 0037-01-6) with glass beads at −80°C. For culturing, one glass bead was removed and put in a tube with 10 ml of BHI. After 30 to 48 h, the strains were subcultured in 50 to 500 ml of BHI for 20 to 28 h. Plate counts were performed by spread plating decimal dilutions of cell suspensions on Columbia agar base (Oxoid no. CM331) with 5% (vol/vol) lysed, defibrinated horse blood (CAB). All cultures were incubated microaerobically at 37°C (unless otherwise stated) in jars or incubators flushed with a gas mixture of 5% O_2, 10% CO_2, and 85% N_2.

Survival curves of *C. jejuni*. Cells grown at 37°C in BHI were harvested by centrifugation at 3,000 × *g* and resuspended in either BHI (pH 7.4), 50 mM potassium phosphate buffer (PPB [pH 7]), or 50 mM potassium phosphate buffer containing 0.85% (wt/vol) NaCl (PPBS [pH 7]). Cell suspensions were incubated stationary in the dark at 4, 12, and 25°C. Samples were taken regularly to determine plate counts on CAB and for examination of morphology by phase-contrast microscopy (Zeiss standard 20; total magnification, ×1,600).

ATP measurements. For determination of ATP concentration, 1 ml of cell suspension was centrifuged at 20,000 × *g*. The supernatant was frozen (for determination of extracellular ATP concentration), and the pellet was resuspended in 1 ml of fresh BHI or buffer (for determination of intracellular ATP concentration) and subsequently frozen. For ATP analysis, frozen samples of cells and supernatants of cells were thawed at 4°C and the ATP concentration of 100 μl of sample was determined in a Biocounter M2010 (Lumac, Landgraaf, The Netherlands), as specified by the manufacturer, with the NRB/LUMIT-PM kit (LUMAC no. 9268-7). Standard curves were constructed with ATP (Sigma no. A 5394) diluted in BHI, PPBS, and PPB. Cell volumes were estimated to be approximately the same for spiral and coccoid cells, although minor differences might arise in the cell length of the spirals (6) and in the diameter of the cocci (data not shown). From the observed dimensions, the internal volume of one (spiral or coccoid) cell was estimated to be 6×10^{-16} liter.

Two-dimensional gel electrophoresis. Cell suspensions of spirals and cocci formed in BHI following incubation at 4 and 25°C (strains 104, B258, and ATCC 33560) were harvested and frozen as a pellet. For protein analysis, pellets were thawed at 4°C and incubated for 30 min at 37°C in lysis buffer (9 M urea, 2% [vol/vol] Triton X-100, 2% [vol/vol] 2-mercaptoethanol, 2% [vol/vol] Pharmalyte 3-10, 8 mM phenylmethylsulfonyl fluoride). Two-dimensional gel electrophoresis was performed with Immobiline DryStrip (pH 3.0 to 10.5) (no. 18-1016-61; Pharmacia Biotech AB, Uppsala, Sweden) and ExcelGel SDS gradient 8-18 (no. 80-1255-53, Pharmacia Biotech) as specified by the manufacturer. After two-dimensional electrophoresis, the gels were silver stained and recorded with a Sony XC 77CE camera, and image analysis was performed with the Gemini program (Applied Imaging, Tyne and Wear, England).

Chloramphenicol treatment. Addition of chloramphenicol at 10 μg/ml resulted in growth inhibition of *C. jejuni*, indicating that protein synthesis was indeed inhibited under these conditions. An overnight culture was used to inoculate (0.1%, vol/vol) six 150-ml volumes of BHI which were incubated for 20 h microaerobically at 37°C. Chloramphenicol (10 μg/ml) was added to three cultures, and all were incubated for another 5 h at 37°C to allow the antibiotic to enter the cells. Cell suspensions with and without chloramphenicol were subsequently incubated stationary in the dark at 4, 25, and 37°C. Samples were removed regularly (every 2 days during the first week, twice during the second week, and once a week subsequently) and assessed microscopically to determine cell morphology and for the ability to grow on agar surfaces.

Irradiation of cells. Overnight *C. jejuni* cultures were exposed to ionizing radiation (gamma irradiation, 2 kGy at the pilot irradiation plant PROVO, Wageningen, The Netherlands). Cell suspensions (100 ml) were subsequently incubated stationary at 4 or 37°C in the dark. Samples were taken at regular intervals to determine plate counts and morphology (see above).

Fatty acid analysis. To determine the fatty acid composition, 500 ml of various strains (spirals or cocci formed at different temperatures) was harvested by centrifugation at 3,000 × *g* as described above. Fatty acids from these cell suspensions were methylated and extracted as described by Miller and Berger (17). For separation, detection, and identification, The Midi microbial identification system was used. This system consists of a Hewlett-Packard HP5890A gas-liquid chromatograph with a 25-m by 0.2-mm 5% methylphenyl silicone fused-silica capillary column, H_2 as the carrier gas, a flame ionization detector, an automatic sampler, an integrator, and a program which identifies the fatty acids by using data in a fatty acid library and a calibration mix of known fatty acids (Microbial ID, Inc.). Principal-component analysis was performed on the data, and fatty acid compositions of the same cell types were averaged from spiral *C. jejuni* suspensions ($n = 11$) and from coccus suspensions formed at 4, 12, or 25°C ($n = 10$, $n = 10$, and $n = 7$, respectively). Statistical analysis of the data was performed with the Student *t* distribution (two-tailed) with a significance level of 0.01.

RESULTS

Effect of temperature on survival of *C. jejuni* in different media. Survival curves of *C. jejuni* 104 cells resuspended in PPBS and in BHI show the same characteristics: a decrease in culturability below the detection limit ($\log N/\text{ml} = 1.7$) in 40 to 70, 15 to 20, and 6 to 8 days at 4, 12, and 25°C, respectively (Fig. 1). Cells incubated in PPBS remained culturable for a longer period than did cells incubated in BHI. In contrast, following resuspension in PPB, culturability dropped below the detection limit in 30, 45, and 5 days at 4, 12, and 25°C, respectively. Cells maintained in this buffer at 12°C remained culturable significantly longer than did cells at 4°C. Similar results were obtained with the other *C. jejuni* strains (data not shown).

ATP measurements. During the first week of incubation, a decrease in intracellular ATP level (ATP_{in}), an indication of the cells' energetic state, and an increase in extracellular ATP level (ATP_{ex}), an indication of ATP leakage, were observed in all experiments. After this first week, the levels remained quite constant for several weeks. To facilitate comparison, the ratios of ATP_{in} to ATP_{ex} were calculated, and these data are shown in Fig. 2. The ratio is lower for cells stored at 25°C in all media when compared with the ratios measured for cells stored at 4 and 12°C.

Cell protein composition analysis. To explore the possible synthesis of new (stress) proteins as means of adaptation, two-dimensional protein profiles of spirals and cocci formed at 4 and 25°C were compared. No major differences in protein patterns of spirals and the various cocci of strain ATCC 33560 could be detected (Fig. 3). Image analysis indicated similar amounts of spots for spirals (315 spots) and cocci formed at 4°C (306 spots) and 25°C (310 spots). Minor differences observed might be due to limited proteolysis and protein turnover. This might also explain the bulk increase of low-molecular-weight compounds in cocci formed at 25°C. Similar results were obtained with the other strains.

Effect of chloramphenicol on formation of coccoid cells. Addition of chloramphenicol (10 μg/ml) to a growing culture inhibited growth immediately, indicating that protein synthesis was blocked (data not shown). The ability of chloramphenicol-treated cells to form colonies on agar plates results from dilution of the antibiotic-containing medium to subinhibitory levels during plating. No irreversible damage due to chloramphenicol treatment was apparent. Cocci appeared in both treated and untreated cell suspensions after 14 and 4 days of incubation at 4 and 25°C, respectively, whereas the culturability dropped below the detection limit in 43 and 10 days, respectively (Table 1). At 37°C, cell counts from suspensions without chloramphenicol remained high for several

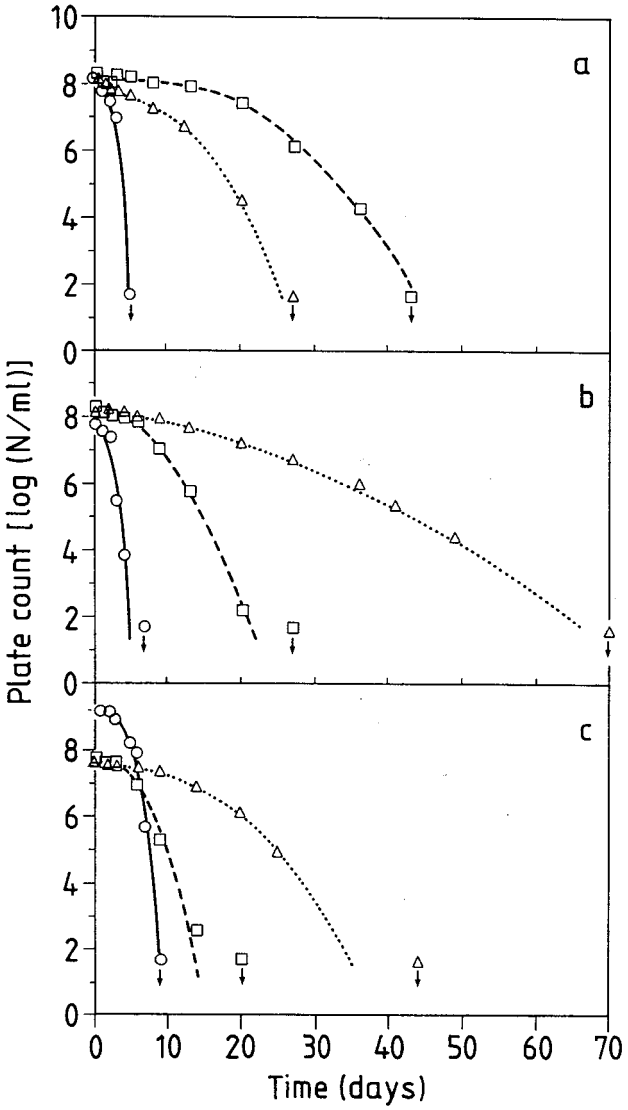

FIG. 1. Survival curves of *C. jejuni* 104 in PPB (a), PPBS (b), and BHI (c). Cells were incubated at 4°C (△), 12°C (□), and 25°C (○). Points with downward-pointing arrows indicate values below the detection limit (log N = 1.7).

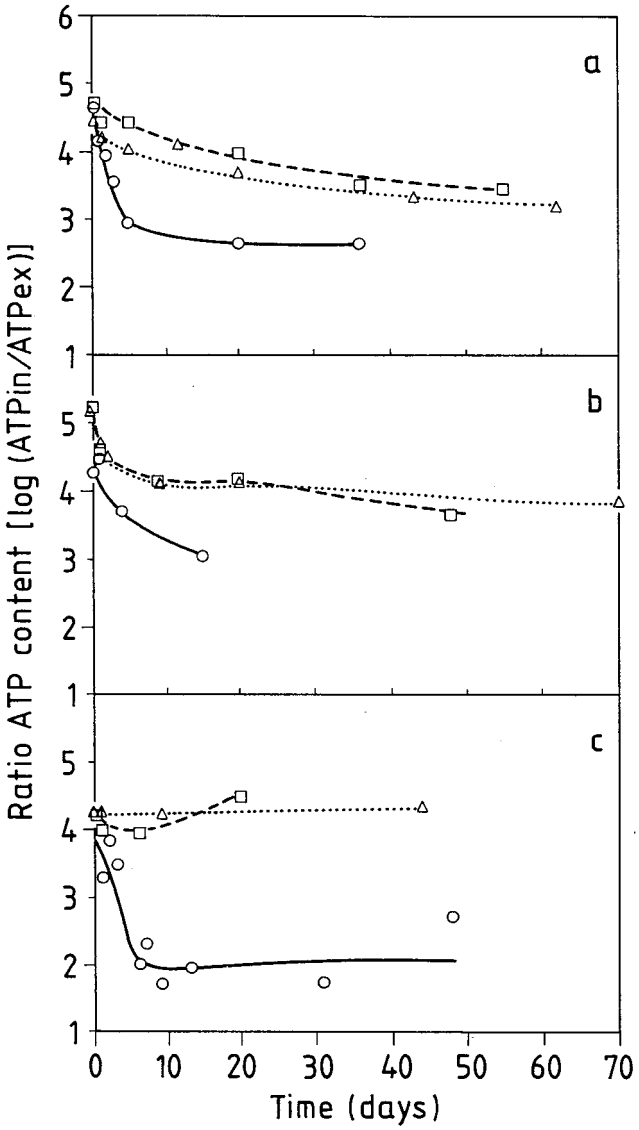

FIG. 2. ATP content of *C. jejuni* 104 during ageing of cells in PPB (a), PPBS (b), and BHI (c). Cells were incubated at 4°C (△), 12°C (□), and 25°C (○). The ATP_{in}/ATP_{ex} ratio was determined as described in Materials and Methods.

weeks. Addition of chloramphenicol caused nonculturability in 6 days at 37°C. Microscopic analysis indicated that coccoid forms arose as frequently following chloramphenicol treatment as without the antibiotic at all temperatures tested, indicating that protein synthesis is not essential for the formation of cocci. Similar results were obtained with the other four strains tested.

Effect of irradiation. The effect of irradiation resulting in DNA damage was examined to investigate whether intact DNA is needed for the formation of cocci. Before irradiation, plate counts on CAB of all five strains were approximately log N/ml = 8.6 to 8.9. Immediately after irradiation (2 kGy), all five, which were observed to be still in the spiral form, yielded no colonies, probably as a result of DNA damage. Microscopic analysis, however, showed that the cells were still as motile as nonirradiated cells, indicating that cellular energetics had not been affected. Strikingly, in both the irradiated and the nonirradiated cell suspensions, cocci were observed after 3 to 5 days at 37°C and after 2 to 3 weeks at 4°C. Furthermore, no differences were observed in one-dimensional protein analysis of cocci resulting from irradiated and untreated cultures (data not shown).

Fatty acid analysis. Although *C. jejuni* is unable to grow at temperatures below 32°C, it was of interest to discover whether adaptation to lower temperatures could be observed to result in changes in the cytoplasmic membrane. Principal-component analysis was used to compare the fatty acid compositions of the various samples, and a two-dimensional reproduction of a multidimensional analysis was obtained. Analysis of fatty acid profiles of the spiral and various coccoid cell suspensions showed that those of cocci formed at 25°C grouped in a distinct cluster (Fig. 4). Fatty acids profiles of cocci formed at 4 and 12°C could not easily be discriminated from those of spirals. Only minor differences in fatty acid patterns between strains occurred, and fatty acid compositions of the same cell types were therefore averaged to facilitate comparison. Significant differences (significance level, 0.01; two-tailed) in amounts of indi-

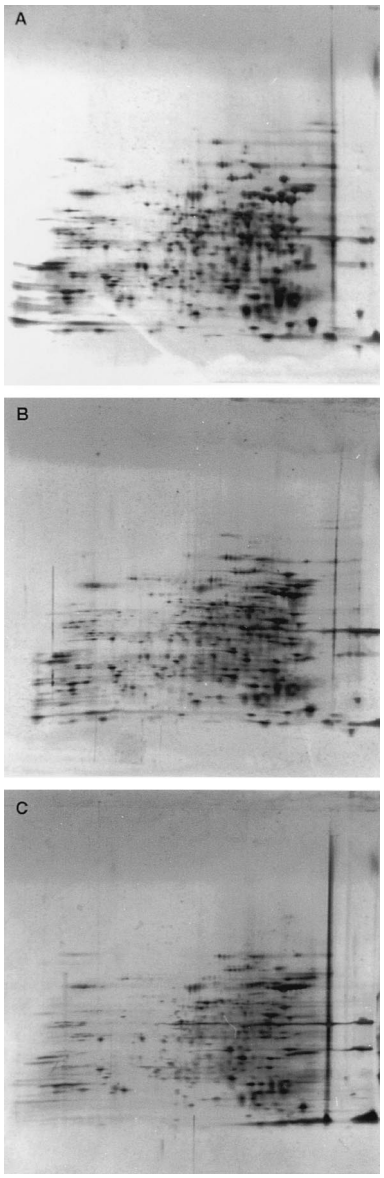

FIG. 3. Two-dimensional protein profiles of *C. jejuni* ATCC 33560. Profiles are shown for spirals (A) and for cocci formed at 4°C (B) and 25°C (C). First dimension, isoelectric focusing pH 10.5 (left) to 3 (right); second dimension, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8 to 18%) high molecular weight (top) to low molecular weight (bottom). Proteins were visualized with silver stain.

vidual fatty acids (contributing more than 1% of the total amount of phospholipids) could be observed (Fig. 5). Cocci formed at 25°C have a different fatty acid composition from that of spiral cells: a significant increase in the amount of 16:0 and 18:0 fatty acids and a decreased concentration of 14:0, 16:1, and 19:0 fatty acids. Cocci formed at 12°C show significant increase in 16:0, 18:1, and 18:0 and a decrease in 14:0 and 16:1 fatty acids. The fatty acid composition of cocci formed at 4°C is quite similar to that of spirals; only the amount of 14:0 fatty acids is decreased and the amount of 18:1 fatty acids is increased. These results indicate that the fatty acid composition of cocci is strongly influenced by the temperatures at which they are formed.

TABLE 1. Culturability and transformation to coccoid forms of *C. jejuni* 104 during ageing of cultures in the absence and presence of chloramphenicol

Storage temp (°C)	Presence of chloramphenicol (10 μg/ml)	Time (days) required for:	
		Observation of cocci	Onset of nonculturability[a]
4	−	14	43
	+	14	43
25	−	4	10
	+	4	10
37	−	1	>40
	+	1	6

[a] Time after which plate counts were below the detection limit (more than 99% of cells were in the coccoid form).

DISCUSSION

To study the process of ageing of *Campylobacter jejuni* cells, the effect of temperature and resuspension and storage media on culturability was analyzed. BHI was used as an environment rich in nutrients; PPBS was used as a poor environment but with addition of saline to prevent osmotic shock. PPB was used as an environment comparable to water, containing a low concentration of phosphate buffer to keep the pH at 7. In all media, the culturability decreased faster at higher storage temperatures (Fig. 1), which can be explained by the higher metabolic activity of the cells at these temperatures. Cells remain culturable for a longer period in PPB and PPBS than in BHI. This is probably due to low metabolic activity resulting from decreased nutrient availability, leading to slower transforma-

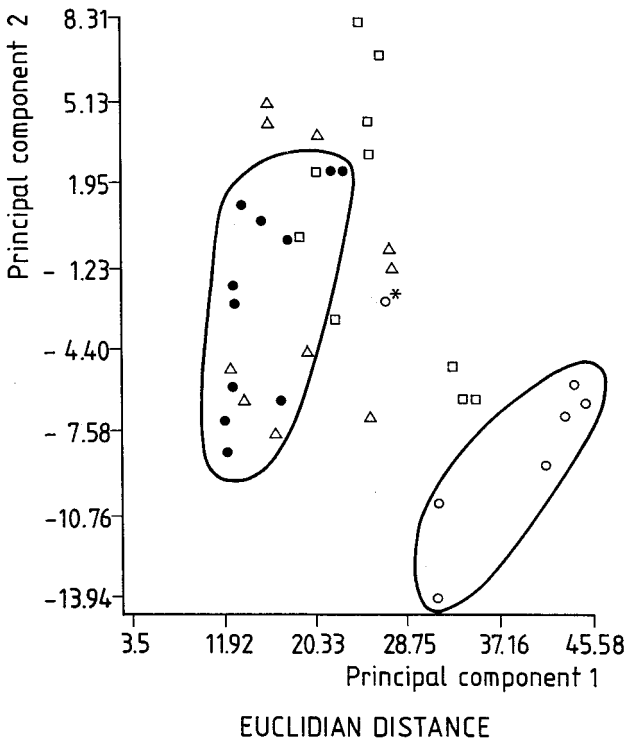

FIG. 4. Two-dimensional principal-component analysis of fatty acids of spirals and coccoid cells of *C. jejuni*. Fatty acids of spirals (●) and of cocci formed at 4°C (△), 12°C (□), and 25°C (○) are shown. The spot marked by an asterisk is closer to the 25°C cluster than to the spiral cluster when compared in three-dimensional space.

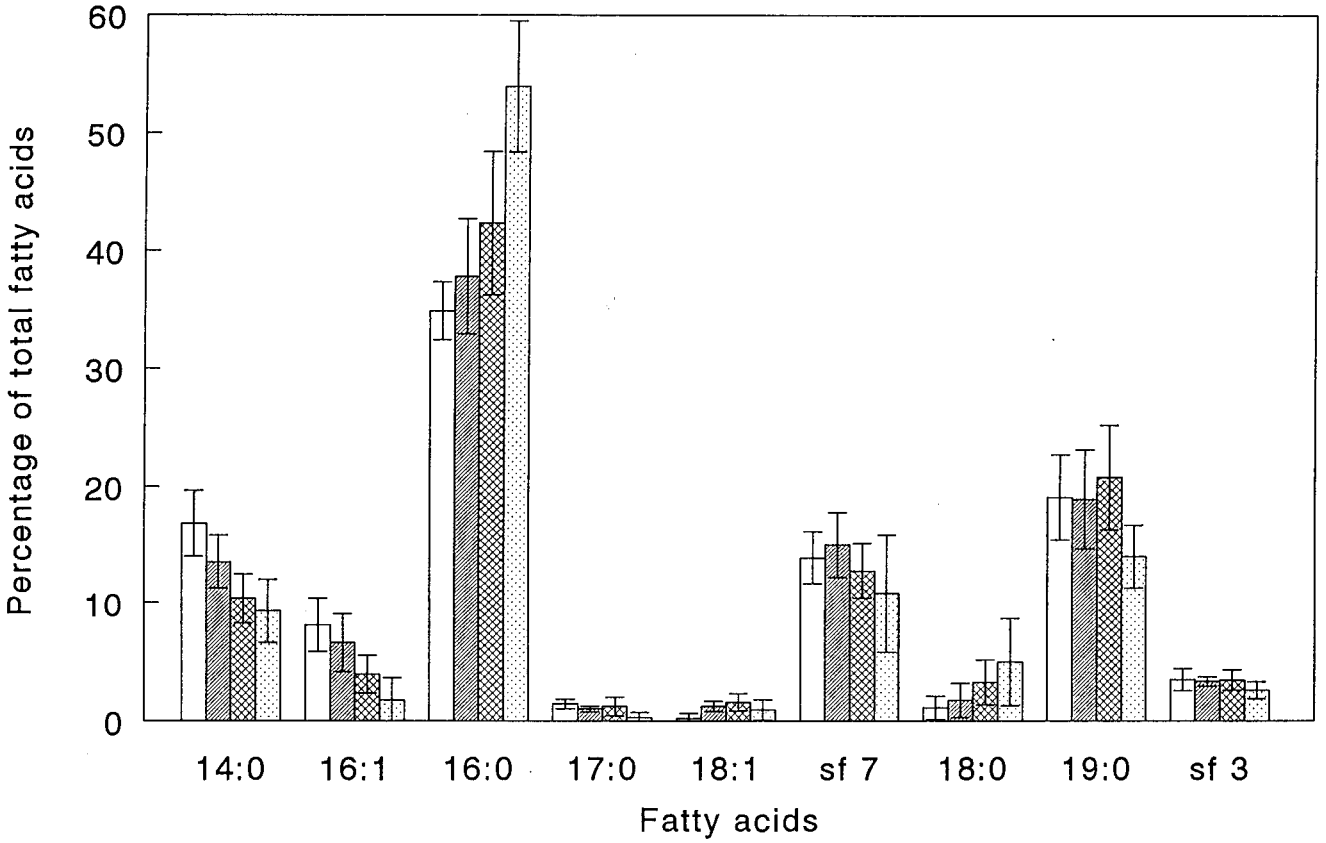

FIG. 5. Fatty acid composition of *C. jejuni* spirals and various coccoid forms. Because of the closely eluting fatty acids, no discrimination could be made between 12:0 aldeh., 16:1 iso, or 14:0 3OH (sf3) and between 18:1 ω7c/ω9t/ω12t, 18:1 ω9c/ω12t/ω7c, or 18:1 ω12t/ω9t/ω7c (sf7); spirals (□), cocci formed at 4°C (▨), 12°C (▩) and 25°C (⁞⁞); 16:1 = 16:1 ω7c; 18:1 = 18:1 ω9c; 17:0 = 17:0 cyclo; 19:0 = 19:0 cyclo ω8c.

tion to a nonculturable state. The remarkable fact that cells in PPB become nonculturable more slowly at 12°C than at 4°C is difficult to explain, but this phenomenon occurred with all the strains tested. Perhaps the combination of osmotic shock (transfer from BHI to PPB) and the greater temperature shock (from the growth temperature of 37°C to the storage temperature of 4°C) is responsible for the observed results. Apparently, temperature is a major factor determining the rate at which cells become nonculturable whereas the presence of nutrients seems to stimulate rather than to retard the process of coccus formation. This is in agreement with results of experiments performed by Rollins and Colwell (26), who observed, with *C. jejuni* cells suspended in a stream water microcosm at low temperature, that the culturability was extended by comparison with that observed at higher temperatures.

To determine the energetic state of the cells during ageing, ATP concentrations were determined at regular time intervals. The intracellular ATP concentration decreased while the extracellular ATP concentration increased during the first week of incubation. Subsequently, levels remained generally constant, indicating that the cells are able to maintain a certain level of ATP while their culturability is below the detection limit. Apparently, there is no strict correlation between the ratio ATP_{in}/ATP_{ex} and culturability. Cells incubated at 4 and 12°C show comparable ATP_{in}/ATP_{ex} ratios in all media tested. At both temperatures, the ratios decreased during the first week and then remained quite constant at a log ratio of 3.5 to 4. In contrast, cells kept at 25°C showed a lower log ratio of 2 and relatively more ATP present outside the cells as a result of increased ATP leakage and/or cell lysis. These observations suggest that cocci formed at 25°C are more seriously damaged than cocci formed at low temperatures. While ATP levels and, in some instances, adenylate energy charge have been related to cell viability during starvation of *Escherichia*, *Zymomonas*, and *Peptococcus* spp. (11), apparently in *C. jejuni* no such relationship exists between ATP levels and viability.

Whole-cell protein profiles of spirals and cocci formed at different temperatures were compared to explore the possibility of new proteins being formed during the process of coccus development. The induction of stress proteins in starvation situations has been described for several microorganisms, such as *Escherichia* (25) and *Vibrio* (8) spp. However, in *C. jejuni*, the appearance of newly synthesized proteins in coccoid forms could not be detected. This was confirmed in experiments in which protein synthesis was blocked by chloramphenicol (reference 28 and data not shown), when formation of coccoid cells proceeded at a rate comparable to that with untreated cells (Table 1). Exposure of cells to gamma irradiation induces single- and/or double-strand breaks in the DNA, thereby preventing DNA transcription (32). *C. jejuni* was not able to form colonies after gamma irradiation (2 kGy), which is consistent with a D_{10} value of 0.2 (2). However, irradiation of the cells did not influence the process of coccus formation (data not shown). Furthermore, the protein composition of these coccoid cells was essentially the same as that of coccoid cells derived from untreated cultures (see above). These results indicate that coccus formation can occur in the presence of protein synthesis inhibitors and gamma irradiation; therefore,

de novo synthesis of proteins may not be required for the transition of spirals to coccoid forms, suggesting that this is a passive process in *C. jejuni.*

Fatty acid analysis is currently employed for identification of a wide variety of bacteria, since it involves a relatively simple sample preparation and has a high reproducibility (33). This method has also been used successfully for the identification of *Campylobacter* species (13, 31) and for differentiation to subspecies level (3). The fatty acid compositions of spirals described in this paper were comparable to those previously published for *Campylobacter* species (3, 18), the predominant species in spirals being 14:0, 16:1, 16:0, 18:1, and 19:0. Linder and Oliver (14) described an increase in the amount of short-chain fatty acids in nonculturable cells of *Vibrio vulnificus* formed at 5°C, which was explained by the need for the microorganism to maintain fluidity of the membrane at this low temperature. A similar phenomenon has been described for *Listeria monocytogenes*, in that cells grown at low temperatures exhibit increased amounts of short-chain length fatty acids (24). Significantly, however, *L. monocytogenes* and *V. vulnificus* are able to grow at these low temperatures whereas *C. jejuni* grows only at temperatures above 32°C. Therefore, we investigated whether an adaptation of the fatty acid composition could be observed in coccoid cells formed at temperatures below 32°C, i.e., those to which *C. jejuni* will be exposed in the environment. Strikingly, the fatty acid composition of cocci formed at 4°C and to a lesser extent at 12°C showed a remarkable similarity to the fatty acid composition of spirals. Apparently, *C. jejuni* does not adapt the composition of fatty acids in the membrane during transition to the coccoid form at low temperatures. The fatty acid composition of cocci formed at 25°C does differ significantly, however, from that of spirals (Fig. 4 and 5). The observed increase in the amounts of 16:0 and 18:0 fatty acids and the concomitant decrease in the amounts of 14:0, 16:1, and 19:0 fatty acids at 25°C are not in line with an active adaptation of the fatty acid composition to low temperatures (see above) and might be caused by nonspecific reactions. The induced changes in the fatty acid composition in these cocci apparently result in leaky membranes, such that ATP is lost from the cells (Fig. 2). We have previously shown by a PCR technique that cocci formed at high temperatures (25 and 37°C) had a significantly reduced DNA content compared with cocci formed at low temperatures, at which DNA levels were comparable to those of spiral cells (7). The transformation from spirals to coccoid cells has previously been studied extensively at higher temperatures, ranging from 30 to 42°C, and in these studies a significant degeneration of these coccoid cells was observed (19–21). Notably, the conditions applied in these studies do not mimic the environmental conditions to which *C. jejuni* cells are exposed upon excretion by the human or animal host into the environment, i.e., low nutrient concentrations and low temperature.

In conclusion, there are many different coccoid forms of *C. jejuni*, and their distinct characteristics are determined by the conditions under which they are formed. Furthermore, a lack of de novo protein synthesis is not required for the transition of spirals to coccoid forms, indicating that this is a passive process. Cocci formed at high temperatures show significant degeneration, whereas cocci formed at 4°C show characteristics comparable to those of spirals. The latter cocci may well play a role in the contamination cycle of *C. jejuni*. Furthermore, an important role of spiral campylobacters in this cycle cannot be excluded, since these forms remain culturable for more than 2 months under environmental conditions.

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