THE DEVELOPMENT AND VIABILITY OF COCCAL *CAMPYLOBACTER JEJUNI* CELLS IN CHEMICALLY DEFINED MEDIA

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ABSTRACT

Campylobacter jejuni for near a decade is the most leading cause of gastrointestinal infections in humans worldwide (Colles et al 2008).In spite of increasing incidence of illnesses caused by C. jejuni as a food-borne pathogen there are quite many areas of the physiology of C. jejuni that is still poorly defined. The study of some aspects of physiology of C. jejuni is vital in understanding mechanisms of pathogenicity of C. jejuni and how the bacterium survives in the environment. The objective of the experiment was to investigate how long it took for spiral C. jejuni cells in chemically defined medium under microaerophilic condition to transform to coccal cells and to find out if coccal C. jejuni cells were viable. The experiment was carried out at the University of Reading, United Kingdom. C. jejuni NCTC 11168 culture was inoculated in both chemically defined medium and complex medium (Bolton broth Oxoid CM983 + 5 % v/v laked horse blood Oxoid SR48) and incubated under microaerophilic conditions at 37^{o}C for 3 – 12 days. Viable cells of C. jejuni were estimated by making spread plates on Bolton broth agar and Nutrient agar (Oxoid CM3). The turbidity of C. jejuni in media was measured with a spectrophotometer. Microscopic enumeration in a counting chamber and Most Probable Number were used to estimate total cells counts whereas PC_Image Analysis and scanning electron microscopy were used to record changes in morphology. It took 9 days for spiral C. jejuni cells in chemically defined medium and Bolton broth + laked horse blood under microaerophilic condition to transform to coccal cells. Nine days old C. jejuni coccal cells formed colonies on selective agar media. Viable counts by Most Probable Number method were identical to total counts obtained by microscopical counting indicating that all the cells were viable. The coccal C. jejuni cells were viable.

Key words: Campylobacter jejuni; Viability of coccal cells; Defined Media

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INTRODUCTION

The morphology of *Campylobacter* spp. is curved, S-shaped, or spiral rods (Nachamkin 2001, Karmali *et al.* 1981). Their dimensions are 0.2 to 0.9 µm in width and 0.5 to 5 µm in length. They can form spherical or coccoid bodies in old cultures, under certain environmental stresses and especially when the culture is exposed to air for a long period (Rollins and Colwell 1986). There is controversy surrounding the concept of viable but non-culturable, arising from the definition of viability. Harvey and Leach (1998) suggest that coccoid cells represent a degenerative form and are, therefore, dead. Nevertheless, a better understanding of the viability of coccoid cells is vital to know if they play any role in virulence.

Temperature can bring about transition of spiral cells to coccal cells (Karmali *et al*., 1981). Thomas *et al*., (2002) observed that the rate of loss of culturability was much higher at temperatures above 20 ^{o}C than at 10 ^{o}C. In some *Campylobacter* spp. Cultures 5 – 25 % of cells were still culturable after 60 days. Viability of coccoid *Campylobacter* spp. cells also depends on the methods used to detect if the cells are viable. Talibart *et al.*, (2000) observed that some *Campylobacter* spp. did not grow on agar medium after 14-21 days and some after 30 days. Some strains remained culturable after 35-60 days on agar medium. Bovil and Mackey (1997) compared viability of *C. jejuni* using plate counts and most probable number methods and observed that plate counts under estimated viability of dormant *C. jejuni* cells up to 5-23 fold compared to most probable method.

Obiri-Danso *et al.*,(2000) observed that loss of culturability of *C. jejuni* in sea water and river water was most rapid at 37^{o}C and slowest at 4^{o}C. *C. jejuni* grew in chemically defined medium (CDM) and old cultures formed coccal bodies. The objectives of the study was to find out how long it took for spiral *C. jejuni* culture in chemically defined medium under microaerophilic condition to transform to coccal cells, if coccal *C. jejuni* cells form colonies on selective agar media and also to investigate if coccal *C. jejuni* cells were viable.

METHODOLOGY

Cultures

Campylobacter jejuni NCTC 11168 was obtained from the National Collection of Type Cultures and Pathogenic Fungi Centre, UK.

Medium components

Purified water

The source of purified water was reverse osmosis ion exchange unit (Purite RO50).

Stock salts solutions

Separate stock solutions were made as described by Owens and Keddie (1969. The solutions were stored under refrigeration at 4 ^{o}C.

Trace metals mixture

The trace metals mixture was similar to that of Owens and Keddie (1969) but with the addition of H_3BO_3. The solution was sterilised by filtration through a 25 mm diameter membrane with 0.45 μm pores (Metricel GN6). The solutions were stored in a refrigerator at 4 ^{o}C.

Sodium pyruvate, 1 mol l^{-1} stock solution

Sodium pyruvate (11.0 g) was suspended in 100 ml purified water. The solution was sterilised by filtration through a 25 mm diameter membrane with 0.45 μm (Metricel GN6) pores.

Double strength Mineral base E solution

Double Strength Mineral base E was prepared by separately adding the following stock solutions to *ca* 88 ml purified water; 1.15 mol l^{-1} KH_2PO_4, 2 ml; 1.15 mol l^{-1} K_2HPO_4, 2.4 ml; 0.023 mol l^{-1} $CaCl_2.6H_2O$, 2 ml; 0.081 mol l^{-1} $MgSO_4.7H_2O$, 2 ml; 0.171 mol l^{-1} NaCl, 1 ml; 0.378 mol l^{-1} $(NH_4)_2SO_4$, 2 ml; and Trace metals mixture, 0.6 ml. The volume of the mixture was made to 100 ml with purified water and sterilised by autoclaving at 121 ^{o}C for 15 min (Owens and Keddie, 1969).

Double strength Medium base (2MB).

Double strength Medium base (2MB) was prepared by aseptically combining the following sterile solutions: Double strength mineral base E, 50 ml; MEM (minimum essential medium), Sigma M7145 amino acids, 1.5 ml; MEM, Sigma M7020 amino acids, 1.5 ml; Grace's vitamins mixture, Sigma G9897, 2.0 ml and 1 mol l^{-1} KOH, 0.2 ml. The pH of medium was adjusted with 1 mol l^{-1} KOH to 7.0. The final volume of medium was made to 100 ml with purified water.

Chemically Defined Medium (CDM)

The medium was prepared by aseptically combining the following sterile solutions: 2MB, 25 ml; MEM, Sigma M7145 amino acids, 0.75 ml; MEM, Sigma M7020 amino acids, 0.75 ml; 1 mol l^{-1} Sodium pyruvate, 2.5 ml; Grace's vitamins mixture, Sigma G9897, 1.0 ml and 1 mol l^{-1} KOH, 0.2 ml. The pH of medium was adjusted with 1 mol l^{-1} KOH to 7.0. The final volume of medium was made to 50 ml with purified water. The solution was dispensed in 5 ml quantities into sterile screw-capped plastic bottles. The bottles were stored in a refrigerator at 4 ^{o}C.

Complex Media

Bolton broth agar (Medium BLBA)

Bolton broth (1.38 g; Oxoid CM983) and 0.75 g purified agar (Oxoid L28) were suspended in 50 ml purified water. The mixture was heated to dissolve the agar and sterilised by autoclaving at 121 ^{o}C for 15 min. The medium was cooled to
50 ^{o}C, 2.5 ml laked horse blood (Oxoid SR48) was added aseptically and the medium was dispensed in 15-20 ml quantities into sterile Petri dishes.

Bolton broth (Medium BLBB)

Bolton broth (Oxoid CM983) + (5 % v/v) laked horse blood (Oxoid SR48) was prepared as described in the Oxoid Manual, (1999).

Bolton broth (BB)

The procedure for preparation of BB was the same as for Medium BLBB but BB did not contain laked horse blood (Oxoid SR48).

Nutrient agar (NA)

The procedure for preparation of NA (Oxoid CM3) was as described in the Oxoid Manual, (1999).

Creation of microaerophilic conditions

Campy*Gen*^{TM} sachets (Oxoid CN35A) were used to create microaerophilic conditions for growth of *C. jejuni* NCTC 11168. The rest of the procedure was as described in Oxoid Manual, (1999).

Determination of development of coccal *C. jejuni* NCTC 11168 cells in media CDM and Bolton broth.

C. jejuni NCTC 11168 growth from a region of multiple isolated colonies on medium BLBA was suspended in five millilitres mineral Base E to make a just visibly turbid suspension. Forty two bottles, each containing four millilitres sterile Medium CDM, and Forty two bottles, each containing four millilitres sterile Bolton broth were inoculated with 0.05 ml of suspension. Forty two bottles, each containing four millilitres sterile Medium CDM, and Forty two bottles, each containing four millilitres sterile Bolton broth were inoculated with 0.05 ml of 24 h old *C. jejuni* NCTC 11168 in medium BLBB. The cultures were incubated under microaerophilic conditions at 37 ^{o}C for 72 h. At 0 h, 6 h, 12 h, 18 h, 24 h 48 h and 72 h, separate cultures sampled and cell morphology, cell count and turbidity were determined.

Determination of the time it takes for spiral *C. jejuni* NCTC 11168 cells in medium CDM under microaerophilic conditions to transform to coccal cells.

The procedure was the same as under determination of development of coccal (above) but the length of sampling was up to 9 days. The cell morphology was determined by scanning electron microscopy. The cell count was determined by Thoma cell counting chamber. When all the cells had transformed from spiral to coccal cells, dilutions were made in Bolton broth and spread on plates of medium BLBA. The cultures were incubated under microaerophilic conditions at 37 ^{o}C for 48 h and colonies counted.

Growth of *C. jejuni* spp. in media CMP13 and Bolton broth.

The turbidity of cultures in media CDM and Bolton broth was measured with a spectrophotometer (550 nm, 1 cm light path Pye Unicam PU 8620).

Determination of *C. jejuni* NCTC 11168 viable population.

The culture was diluted in 4.5 ml medium BLBB and viable cells were estimated by making spread plates on Media BLBA and NA, and incubating under microaerophilic conditions at 37 ^{o}C for 48 h. The procedure was repeated at 0 h, 6 h, 12 h, 18 h, 24 h 48 h and 72 h.

Microscopic enumeration in a counting chamber

Total count of cultures was estimated using a Thoma cell counting chamber. The conversion factor per small square of Thoma counting chamber (cells ml^{-1}) was equal to $2n \times 10^{7}$, where *n* was the mean number of organisms per small square.

Most Probable Number Count

Sterile medium BLBB (4.5 ml) was used to determine the viable counts of *C. jejuni*. Inoculum dilutions from 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} were used to determine the viable counts of *C. jejuni*. Three tubes were inoculated from each dilution with 0.5 ml of culture. The growth of *C. jejuni* in the media was recognised by observing colour change (from dark red to light red) due to break down of substrate and checking for the presence of cells by phase contrast microscopy.

PC_Image Analysis and measurement parameters

The cells had transformed to coccal by day 9. Using PC_Image Analysis and measurement programme the following cell parameters were measured; length, breadth, circularity, area and perimeter. The length and circularity were the two variables that were the most sensitive parameters reflecting changes in cell morphology. To differentiate between spiral and coccal cells, the predicting values derived by dividing length by circularity were used. The highest value when all the cells were coccal at 9 days was adopted as cut point. The value derived by dividing length by circularity was adopted as factor to use, since the two variables were the most sensitive parameters reflecting changes in cell morphology. It was much easier dealing with whole numbers than fractions. All values derived by dividing length by circularity between 0- 5.4 were considered to represent coccal cells. The figure of 5.4 was the highest value obtained by dividing length by circularity from PC_Image

Analysis and measurement at 9 days. All values derived by dividing length by circularity > 5.5 represented spiral cells.

Determination of *C. jejuni* cell morphology by scanning electron microscopy.

Two, Three, Nine and Twelve days old cultures (0.5 ml of each) of *C. jejuni* NCTC 11168 in Media CDM and BLBB were filtered through 25 mm, 0.2 μm pore size Track-Etch membrane filters (Nuclepore Whatman, Kent UK), and washed with 0.1 mol l^{-1} KH_2PO_4 (pH 7.2) buffer. The cells were fixed for 15 min in 3 % (v/v) glutaraldehyde in 0.1 mol l^{-1} KH_2PO_4 (pH 7.2) at room temperature. The filters were washed twice in buffer for 15 min and postfixed in 1 % (v/v) aqueous osmium tetroxide for 15 min. Unbound osmium tetroxide was removed by washing the filters in water (3 x 15 min). The cells were dehydrated through graded alcohol beginning with 30 % (v/v) ethanol, followed by ethanol at 50 %, 70 %, 95 % and 100 % concentration. The filters were finally washed through absolutely dry ethanol (with copper sulphate) and dried in a critical point drier (EMITECK K850 Ashford, Kent, UK). The specimens were mounted on copper tape and sputter (EMSCOPE SC500, UK) coated with gold (~ 40 nm) for examination using a scanning electron microscopy (LEO-1450 Variable Pressure-SEM, Leo Electron Microscopy LTD, Cambridge CB1 3QH, UK) operated at 20 kv. The experiment was performed at School of Plant Sciences (The University of Reading, Reading, Berkshire, United Kingdom) assisted by Dr Lynda Bonner.

RESULTS AND DISCUSSION

Development of coccal *C. jejuni* NCTC 11168 cells in media CDM and Bolton broth.

Development of C. jejuni cocci cells from 24 h old C. jejuni cells in medium BLBB. C. jejuni (24 h old) cells from medium BLBB used as an inoculum were almost all spiral in shape (Fig. 1). The initial population of inoculum from medium BLBB was 4 x 10^7 cfu ml^{-1} (by Thoma counting chamber method), 4.8 x 10^6 cfu ml^{-1} (by Most Probable Number method) and 1 x 10^6 cfu ml^{-1} (by spread plate method on medium BLBA). This was the *C. jejuni* NCTC 11168 culture that was used to inoculate four millilitres of medium CDM. The amount of inoculum used to inoculate each four millilitres of medium was 0.05 ml.

The proportion of spiral *C. jejuni* cells in medium CDM over the incubation period of 72 h at 37 ^{o}C under microaerophilic conditions was as shown in Table 1. More than 70 % - 100 % of *C. jejuni* cells were spiral (Table 1).

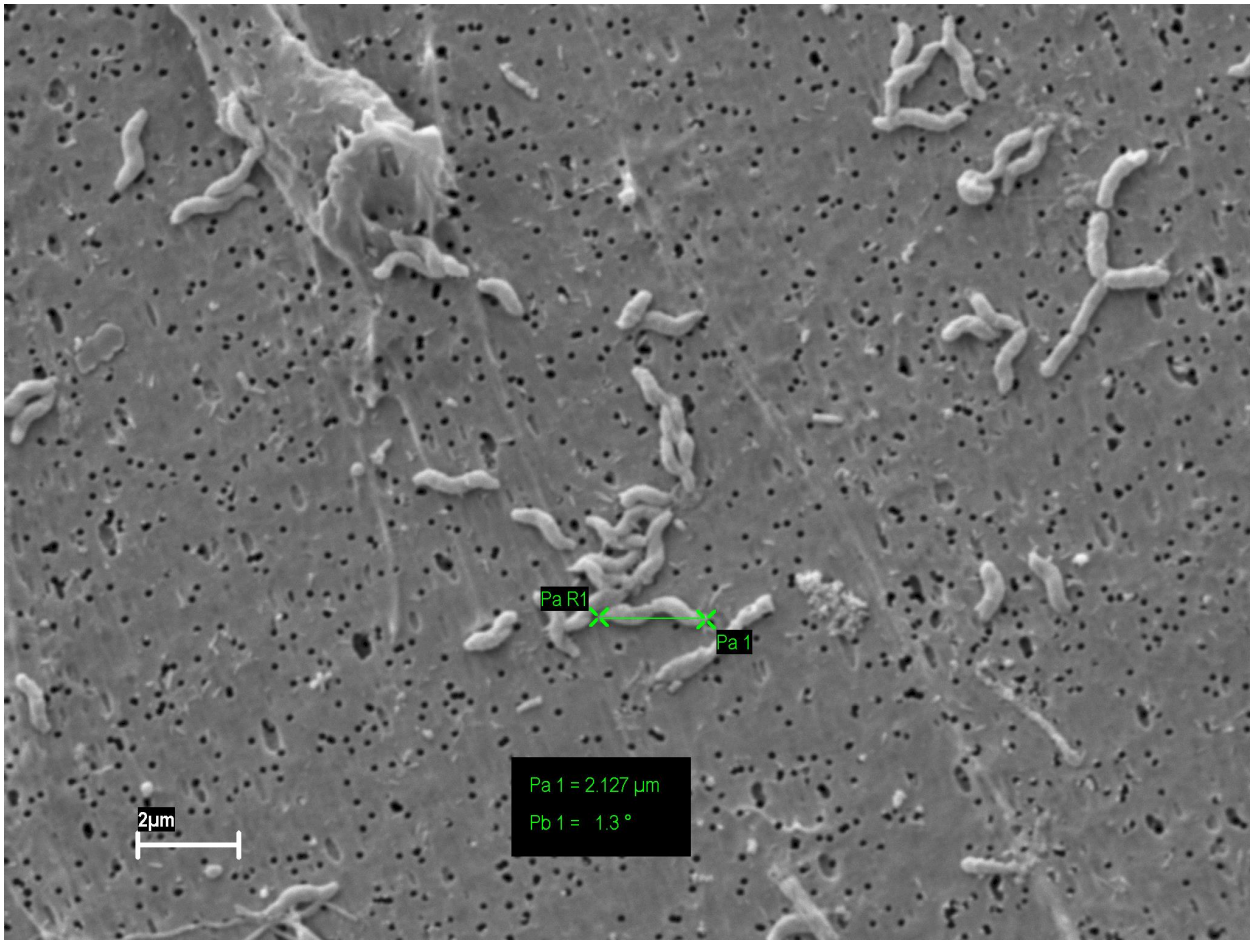


Fig. 1 Scanning electron microscope images of 24-48 h old *C. jejuni* NCTC 11168 cells in Medium BLBB at 37 ^{o}C under microaerophilic conditions

Development of C. jejuni coccal cells from 48 h old C. jejuni colonies suspension.

C. jejuni (48 h old) cells in mineral base E used were used as inoculums. Most of the cells in the inoculums were cocci in shape. The initial population of the inoculum from medium BLBB was 3.8 x 10^{9} cfu ml^{-1} (by Thoma counting chamber method), 9.2 x 10^{6} cfu ml^{-1} (by Most Probable Number method) and 8.1 x 10^{5} cfu ml^{-1} (by spread plate method on medium BLBA). This was the cells suspension of *C. jejuni* NCTC 11168 culture that was used to inoculate four millilitres of medium CDM. The amount of inoculum transferred into each four millilitres of medium was 0.05 ml.

The proportion of spiral *C. jejuni* cells in medium CDM over the incubation period of 72 h at 37 ^{o}C under microaerophilic conditions was as shown in Table 2. More than 70 % - 100 % of *C. jejuni* cells were spiral (Table 2).

Table 1. Development of *C. jejuni* NCTC 11168 coccal cells in media CDM and Bolton broth under microaerophilic conditions at 37 ^{o}C for 72 h.

		Growth in media expressed as optical density (OD), pH, Total count (microscopic count) and Viable count (most probable number and colony count)*					
					Viable count by		
Medium[†‡]	Incubation duration	OD_{550}	pH	Total count (cfu ml^{-1})	Most probable number (cfu ml^{-1})	Spread plate (cfu ml^{-1})	Cell morphology
CDM	0 h	0.03	6.3	$4.0x10^7$	$9.2x10^6$	$3.6x10^5$	All spiral
	6 h	0.07	6.3	$1.0x10^8$	$9.2x10^6$	$2.9x10^5$	73% spiral
	12 h	0.06	6.3	$3.2x10^8$	$9.2x10^7$	$1.3x10^5$	70% spiral
	18 h	0.06	6.2	$8.0x10^7$	$2.2x10^7$	$1.6x10^5$	78% spiral
	24 h	0.06	6.2	$8.0x10^7$	$2.2x10^7$	$6.5x10^5$	74% spiral
	48 h	0.14	6.1	$2.8x10^8$	$2.2x10^8$	$1.3x10^6$	82% spiral
	72 h	0.15	6.2	$3.8x10^8$	$4.2x10^8$	$1.0x10^6$	86% spiral
Bolton broth	0 h	0.03	7.2	$4.0x10^7$	$4.8x10^7$	$4.2x10^5$	All spiral
	6 h	0.03	6.7	$8.0x10^7$	$9.2x10^7$	$5.3x10^5$	79% spiral
	12 h	0.14	6.7	$2.8x10^8$	$9.2x10^7$	$9.1x10^5$	80% spiral
	18 h	0.18	7.0	$4.0x10^8$	$4.8x10^8$	$5.2x10^6$	All spiral
	24 h	0.38	6.6	$8.0x10^8$	$9.2x10^8$	$1.9x10^6$	All spiral
	48 h	0.64	6.9	$6.0x10^8$	$2.2x10^9$	$2.9x10^7$	73% spiral
	72 h	0.40	6.9	$3.2x10^9$	$4.8x10^9$	$1.3x10^7$	83% spiral

*, Source of inoculum, 24 h old *C. jejuni* in medium BLBB.
†, Initial pH of medium CDM was 7.0
‡, Added ingredients in medium: CDM, pyruvate, 50 mmol l^{-1}.

The proportion of spiral *C. jejuni* cells in medium CDM (Table 2) at time 0 – 18 h was extremely low and this also indicated a period when there was negligible growth of *C. jejuni* in the medium. This was a general observation that when *C. jejuni* NCTC 11168 from 37 ^{o}C was left to stand for some time in the laboratory at ~ 19 ^{o}C, (room

temperature) the culture lost mobility and eventually the proportion of spiral cells gradually reduced.

Table 2. Development of *C. jejuni* NCTC 11168 coccal cells in media CDM and Bolton broth under microaerophilic conditions at 37 ^{o}C for 72 h.

		Growth in media expressed as optical density (OD), pH, Total count (microscopic count) and Viable count (most probable number and colony count)*					
					Viable count by		
Medium†‡	Incubation duration	OD_{550}	pH	Total count (cfu ml^{-1})	Most probable number (cfu ml^{-1})	Spread plate (cfu ml^{-1})	Cell morphology
CDM	0 h	0.02	6.3	$6.0x10^7$	$4.8x10^6$	$7.0x10^5$	5.0 % spiral
	6 h	0.02	6.3	$8.0x10^7$	$4.8x10^6$	$2.0x10^5$	6.6 % spiral
	12 h	0.03	6.3	$4.0x10^7$	$4.2x10^6$	$1.0x10^5$	6.6 % spiral
	18 h	0.05	6.2	$4.0x10^7$	$9.2x10^6$	$1.2x10^6$	7.7 % spiral
	24 h	0.14	6.2	$1.0x10^8$	$9.2x10^6$	$2.5x10^6$	23% spiral
	48 h	0.30	6.1	$1.0x10^8$	$3.0x10^7$	$9.0x10^6$	25% spiral
	72 h	0.45	6.1	$2.8x10^8$	$4.8x10^7$	$1.2x10^6$	31% spiral
Bolton Broth	0 h	0.02	7.0	$6.0x10^7$	$4.2x10^5$	$1.0x10^6$	5.0 % spiral
	6 h	0.03	6.9	$1.0x10^8$	$9.2x10^5$	$2.4x10^5$	10 % spiral
	12 h	0.05	6.9	$6.0x10^7$	$4.2x10^6$	$1.2x10^6$	9.5 % spiral
	18 h	0.09	6.8	$6.0x10^7$	$3.0x10^7$	$1.6x10^6$	23% spiral
	24 h	0.74	6.7	$1.2x10^9$	$9.2x10^8$	$5.6x10^6$	76% spiral
	48 h	0.76	6.8	$6.0x10^8$	$2.2x10^8$	$4.9x10^7$	73% spiral
	72 h	0.70	6.6	$1.6x10^8$	$4.8x10^7$	$5.3x10^6$	80% spiral

*, Source of inoculum, 48 h old isolated *C. jejuni* colonies on medium BLBA suspended in five millilitres single strength mineral base E.
†, Initial pH of medium CDM was 7.0
‡, Added ingredients in medium: CDM, pyruvate, 50 mmol l^{-1}.

By 72 h at 37 ^{o}C under microaerophilic incubation conditions most *C. jejuni* cells in Medium CDM and BLBB were spiral (Table 1 and Fig. 1). *C. jejuni* spp. can form coccal cells with age and by prolonged exposed to air (Rollins and Colwell 1986; Karmali *et al.* 1981 and Talibart *et al.*, 2000). Rollins and Colwell (1986) observed

that *C. jejuni* cells in logarithmic growth were spiral. This experiment also showed that the morphology of 2 days to 4 days old *C. jejuni* cells (stationary phase) used as inocula throughout the study were mostly spiral. *C. jejuni* NCTC 11168 in medium CDM at 37 ^{o}C for 24-48 h under continuous bubbling of gas (5 % O_2, 10 % CO_2 & 85 % N_2) reach stationary growth phase by 24 h (Simatende 2004). This observation is supported by Rollins and Colwell (1986), because the 24th hour mark is a transition growth point from logarithmic growth to stationary phase.

Determination of the time it takes for spiral *C. jejuni* NCTC 11168 cells in medium CDM under microaerophilic conditions to transform to coccal cells.

C. jejuni NCTC 11168 cells in media CDM and BLBB under microaerophilic conditions took 9 days to transform to coccal cells (Table 3, Table 4 and Fig. 2). These results were consistent with the finding by Karmali *et al.* (1981) who observed that *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* retained normal morphology for 4 days, and some did so even for 6 days.

Determination the ability of *C. jejuni* NCTC 11168 cells to form colonies on Medium BLBA.

Ability of spiral cells to form colonies. The 72 h old *C. jejuni* cells in medium CDM was mostly spiral (86 %) (Table 1). Less than 0.3 % of cells formed colonies on Medium BLBA ($1x10^6/3.8x10^8$ x 100), whereas, the Most Probable Number counts were almost identical to the microscopical total count and. The value of $3.8x10^8$ was the total count of 72 h old *C. jejuni* in medium CDM at 37 ^{o}C (Table 1).

Ability of spiral cells to form colonies from 48 h old colonies suspension inoculum. The 72 h old *C. jejuni* cells in medium CDM was mostly spiral (83 %) (Table 2). Only 0.4 % of cells formed colonies on Medium BLBA ($1.2x10^6/2.8x10^8$ x 100), whereas, the Most Probable Number counts was one logarithmic lower than the microscopical total count. The value of $2.8x10^8$ was the total count of 72 h old *C. jejuni* in medium CDM at 37 ^{o}C (Table 2).

The viable counts by Most Probable Number method at 0 h, 6 h, 12 h, 18 h, 24 h, 48 h, and 72 h were identical to total counts obtained from microscopical counting

(Table 1 and 2). All *C. jejuni* cultures contained more than 80 % spiral cells (Table 1 and 2). All spiral cells were, therefore, able to form colonies on Medium BLBA.

In terms of viable counts by spread plate method only between 0.3 % and 0.4 % of 72 h old *C. jejuni* cells formed colonies on Medium BLBA. Talibart *et al.*, (2000) observed that with reference to viability of *Campylobacter* spp. a lot depends on the methods employed to examine if the cells are viable.

The 9 days old *C. jejuni* cells in media CDM and BLBB formed colonies on medium BLBA. The average colony count from the culture in CDM was 3.9×10^7 cfu ml^{-1} and from BLBB, 9.1×10^7 cfu ml^{-1}. The microscopical total count for culture in medium CDM was 2.3×10^9 and 1.4×10^{10} in medium BLBB.

The 9 days old *C. jejuni* culture in medium CDM had almost 100 % coccal cells. The total cell count estimated by Thoma chamber was 2.3×10^9 cfu ml^{-1} and the viable count on BLBA spread plate was 3.9×10^7 cfu ml^{-1}. This meant that less than 1.7 % of the cells in the culture formed colonies. The trend was the same for 9 d old *C. jejuni* in medium BLBB that had almost 100 % coccal cells at 9 days, but only 0.6 % of the cells in the culture formed colonies on agar medium BLBA. It was striking to note that in the 9 days culture (coccal cells) a higher percentage (1.7 %) of the cells formed colonies on agar media compared to 72 h old culture (with mostly spiral cells), where 0.3-0.4 % of cells formed colonies on agar media.

Although, the percentage of spiral cells estimated by PC_Image Analysis was 0 % at 9 days, there could still have been some very few spiral cells in the medium (Fig. 2). (The viable count values by Most Probable Number for *C. jejuni*. at 9 days were not estimated).

Plate counts underestimate viable counts of *C. jejuni* by up to 23 fold (Bovil and Mackey 1997). Therefore, plate counts values cannot be entirely used to assess the viability of *C. jejuni* culture.

Table 3. Times for spiral *Campylobacter jejuni* NCTC 11168 cells in medium CDM to transform to coccal cells under microaerophilic conditions at 37 ^{o}C.

			Cell parameters as measured by PC-Image Analysis		Length/Circularity			
			Length (μm)	Circularity[†‡]	0-5.4	>5.5		
Incubation Duration	OD_{550}	Microscope count (cfu ml^{-1})	Range	Range	No. of cells		Total number of cells counted	Proportion of spiral cells[*]
0 d	0.01	2.7×10^8	0.69-3.07	0.07-0.17	2	19	21	90 % spiral
0.2 d	0.04	8.2×10^8	0.62-1.98	0.05-0.20	6	10	16	63 % spiral
0.5 d	0.07	8.5×10^8	0.79-2.31	0.04-0.14	1	16	17	94 % spiral
0.8 d	0.09	8.9×10^8	0.50-3.31	0.02-0.27	7	23	30	77 % spiral
1 d	0.10	9.3×10^8	0.68-1.91	0.05-0.18	1	12	13	92 % spiral
2 d	0.44	1.2×10^9	0.60-1.70	0.06-0.19	3	25	28	89 % spiral
3 d	0.53	6.5×10^9	0.69-1.99	0.05-0.15	0	33	33	100% spiral
4 d	0.58	8.3×10^9	0.73-2.01	0.04-0.16	1	24	25	96 % spiral
8 d	0.51	1.7×10^9	0.33-1.00	0.11-0.44	22	4	26	15 % spiral
9 d	0.57	2.3×10^9	0.48-0.73	0.15-0.25	13	0	13	0 % spiral

[*], Cells > 5.5/Total number of cells x 100.
[†], Circularity, 4πArea/Perimeter^2.
[‡], Poor circularity reading due to poor images.

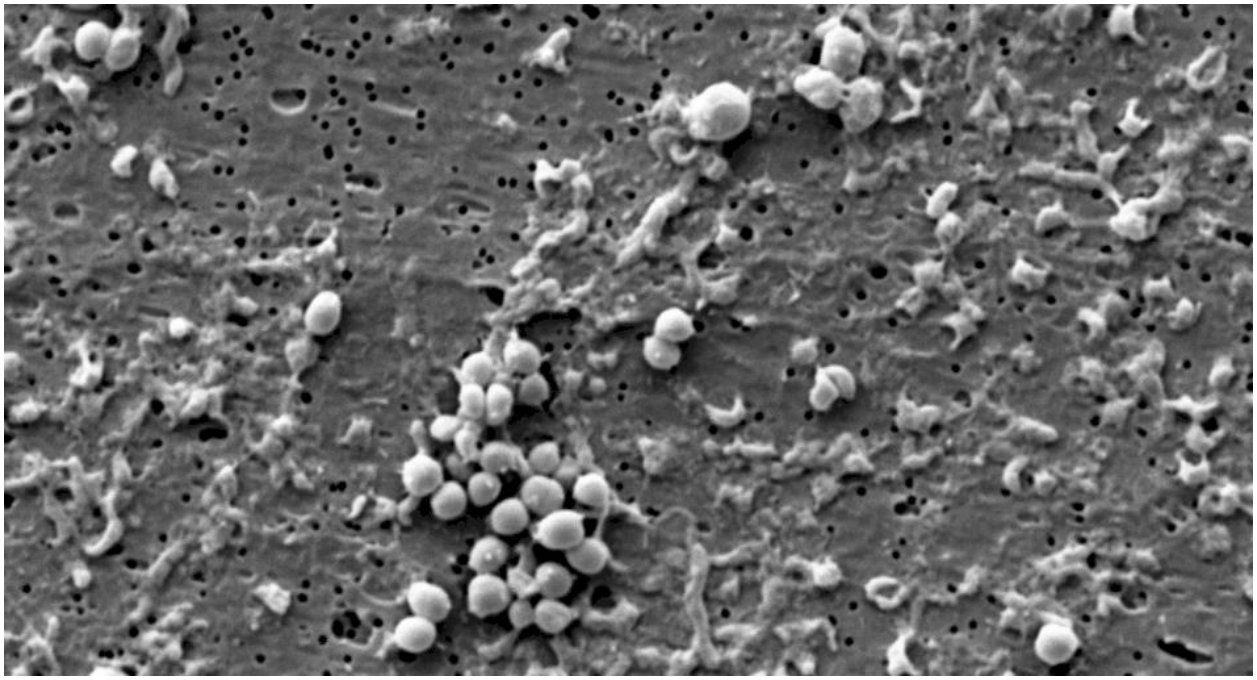
Table 4. Times for spiral *Campylobacter jejuni* NCTC 11168 cells in medium BLBB to transform to coccal cells under microaerophilic conditions at 37 ^{o}C.

		Cell parameters as measured by PC-Image Analysis		Length/Circularity			
		Length (μm)	Circularity[†‡]	0-5.4	>5.5		
Incubation Duration	Microscope count (cfu ml^{-1})	Range	Range	No. of cells		Total number of cells counted	Proportion of spiral cell[*]
0 d	8.8 x 10^{8}	0.69-3.07	0.07-0.17	2	19	21	90 % spiral
0.2 d	1.1 x 10^{9}	0.59-1.87	0.05-0.22	2	10	12	83 % spiral
0.5 d	2.3 x 10^{9}	0.48-1.25	0.08-0.27	17	14	31	45 % spiral
0.8 d	5.8 x 10^{9}	0.83-2.07	0.05-0.14	0	17	17	100% spiral
1 d	5.3 x 10^{9}	0.79-1.57	0.07-0.13	0	13	13	100% spiral
2 d	7.3 x 10^{9}	0.67-1.90	0.06-0.17	1	13	14	93 % spiral
3 d	5.2 x 10^{9}	0.42-1.62	0.06-0.31	7	18	25	72 % spiral
4 d	6.3 x 10^{9}	0.59-1.88	0.06-0.18	1	9	10	90 % spiral
8 d	1.3 x 10^{10}	0.38-1.08	0.10-0.33	25	6	31	19 % spiral
9 d	1.4 x 10^{10}	0.43-0.79	0.15-0.20	13	0	13	0 % spiral

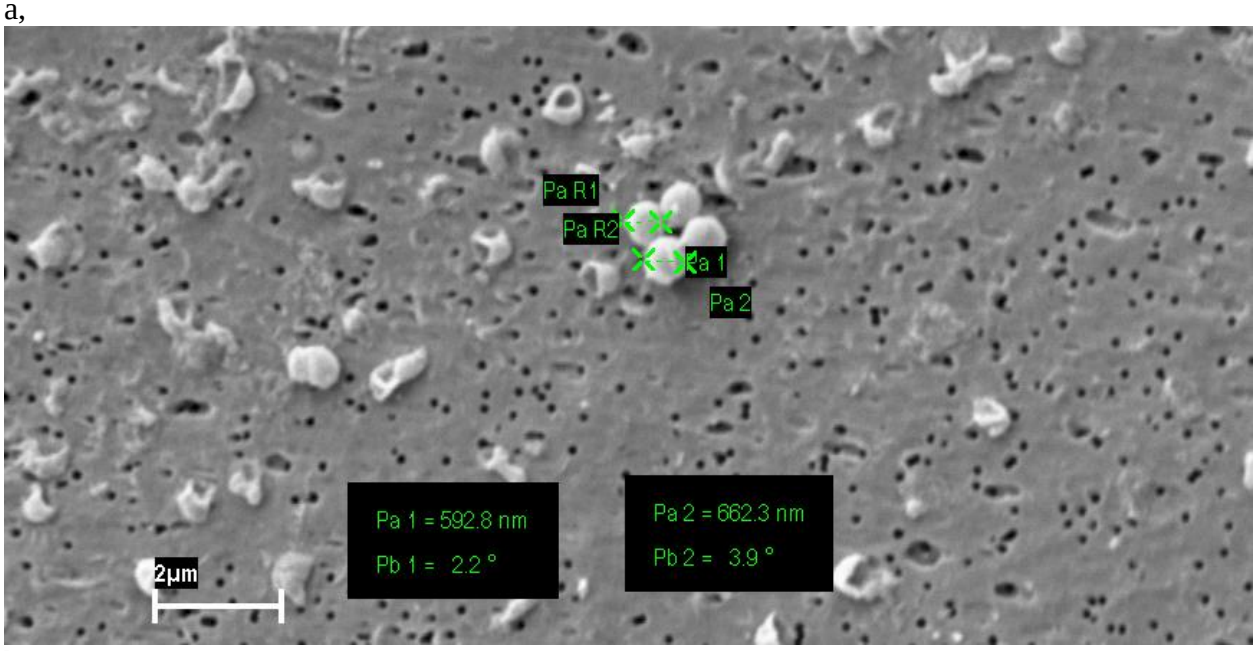
*, Cells > 5.5/Total number of cells x 100.

†, Circularity, 4πArea/Perimeter^{2}.

‡, Poor circularity reading due to poor images.



a,



b,

Fig. 2 Scanning electron microscope images of 9-12 d old *C. jejuni* NCTC 11168 cells in Medium BLBB at 37 ^{o}C under microaerophilic conditions. a, 9 d and b, 12 d.

The time taken by *C. jejuni* NCTC 11168 in chemically defined medium CDM and medium BLBB to transform from spiral cells to coccal cells was the same (P > 0.05). There was no significant difference in the proportion of spiral and coccal cells between the two media (P > 0.05; Table 5). In other words, differences in media composition did not affect the rate at which cells transformed from spiral to coccal and had no influence on the proportion of cells of each type (P > 0.05). Although not many cells were presented in Table 3 and 4 several slides were made for PC_Image

analysis but fewer cells were measured because some images on slides were poor. This also accounted for some poor circularity measurement (Table 3 & 4). Unlike length where a cord was drawn across the cell, circularity was computed automatically.

Viability of coccal *C. jejuni* NCTC 11168 cells

The fact that Most Probable Number estimates of 72 h old *C. jejuni* with 86 % spiral cells was almost identical to total counts obtained from microscopical counting meant that all the cells were viable.

Table 5. Effect of medium on transformation of spiral *C. jejuni* NCTC 11168 cells to coccal cells in liquid media under microaerophilic condition at 37 ^{o}C.

	NCTC 11168		
	% spiral cells in medium		
Incubation Duration (d)	CDM (A)	BLBB (B)	A – B*
0	90	90	0
0.2	63	83	-20
0.5	94	45	49
0.8	77	100	-23
1	92	100	-8
2	89	93	-4
3	100	72	28
4	96	90	6
8	15	19	-4
9	0	0	0
			2.4†

* Paired T-test, no significance difference in proportion of spiral cells and coccal cells between mean A-B of both media ($P > 0.05$).
† Mean A-B.

CONCLUSIONS AND RECOMMENDATION

It can be concluded that by 9 days almost all spiral *C. jejuni* had transformed to coccal cells in chemically defined media and medium BLBB. The spiral *C. jejuni*

cells formed colonies on Medium BLBA. The coccal *C. jejuni* cells were viable. As a recommendation there is need to carry out more research on viability of coccal (viable but non-culturable) *C. jejuni* cells using much more sensitive methods like florescent dyes.

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