

Effect of Ferrous Sulfate, Sodium Metabisulfite, and Sodium Pyruvate on Survival of *Campylobacter jejuni*

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A combination of ferrous sulfate, sodium metabisulfite and sodium pyruvate, incorporated in solid medium, maintained the characteristic morphology, motility, and viability of six isolates of *Campylobacter jejuni* stored at room temperature and 4°C for up to 20 and 30 days, respectively, under normal atmospheric conditions.

Campylobacter jejuni is recognized as a significant enteric pathogen in humans (1, 2, 6, 8). This organism is microaerophilic and requires special media for optimal growth and isolation from clinical and environmental sources. Cells from fresh cultures are curved or spiral, motile, gram-negative, and nonsporulating rods. In aged cultures, cells transform to coccoid forms which are nonviable (9). This transformation is more rapid on solid media than in broth or semisolid media and is enhanced by exposure to room air and temperature (7, 9). The addition of various agents, namely a combination of ferrous sulfate, sodium metabisulfite, and sodium pyruvate (FBP) effective in destroying the toxic hydrogen peroxide and superoxide anions to culture media (9) is shown to increase aerotolerance and growth of this organism (3, 4). Its effect on the morphological transformations and the survival of *C. jejuni* on solid media, however, has not yet been fully investigated. The purpose of this study was, therefore, to observe the effect of FBP supplement on morphology, motility, and viability of *C. jejuni* after exposure to atmospheric air at room temperature or at 4°C during prolonged storage on plate media.

Six *C. jejuni* isolates, E 1833, E 1523, 591, 592, 6845, and 6309, from stool specimens with less than three passages were stored at -70°C in semisolid thioglycollate medium containing 10% defibrinated horse blood. Frozen isolates, thawed at room temperature, were subcultured on campylobacter blood agar plates (8), and incubated at 42°C for 48 h in an atmosphere consisting of 5% O₂, 10% CO₂, and 85% N₂. A loopful of the fresh culture was suspended in 5 ml of brucella broth (GIBCO Laboratories) supplemented with 0.025% (wt/vol) each of ferrous sulfate, sodium metabisulfite, and sodium pyruvate (Br. broth-FBP). A drop of this suspension was streaked in duplicate on each of the following four agar plates having a pH of 7.4: (i)

brucella broth supplemented with 1.5% Oxoid agar no. 1 (Br.A); (ii) Br.A supplemented with 0.025% FBP (Br.A-FBP); (iii) Columbia agar (GIBCO) supplemented with 7% lysed horse blood (BA); and (iv) BA supplemented with 10 mg of vancomycin, 2,500 U of polymyxin B, and 5 mg of trimethoprim per liter (Campy-BA). Plates were incubated at 42°C for 24 h in evacuation replacement jars containing an atmosphere of 5% O₂, 10% CO₂, and 85% N₂ and checked for the presence of growth. Culture plates were then sealed with parafilm to retain moisture. One of the two plates of each medium was stored at 4°C and the other on the laboratory bench top (room temperature). A loopful of each culture from each plate was withdrawn at 1, 2, 3, 5, 7, 10, 12, 15, 20, 25 and 30 days and was suspended in 5.0 ml of Br. broth-FBP. Cell suspensions were observed for typical corkscrew type motility by using a phase contrast microscope (5) and cell morphology by Gram stain. Viability of the culture was tested by subculturing onto Campy-BA plate (8) and into a biphasic system (10). The biphasic medium was prepared in tissue culture flasks with 10 ml of Br.A-FBP in the solid phase and 10 ml of Br. broth-FBP in the liquid phase. Both were incubated at 42°C for 48 h under microaerophilic conditions. Colonies on the Campy-BA plate and turbidity in the biphasic system suggested that the suspension contained viable organisms. Viability of the culture in the biphasic system was confirmed by subculturing 0.05 ml of the liquid onto Campy-BA plate and observing typical colonies after 48 h at 42°C.

Observations on cell morphology, motility, and viability of *C. jejuni* isolate 591 are summarized in Table 1. Observations on the remaining five isolates studied were similar to those for 591. It is to be noted that at any time interval in which the presence of motile and spiral form of cells was observed under direct microscopy,

TABLE 1. Morphology and motility characteristics of *C. jejuni* 591 at room temperature and 4°C^a

Observation interval (days)	BA, Campy-BA, Br.A				Br.A-FBP			
	22°C		4°C		22°C		4°C	
	M	Mt	M	Mt	M	Mt	M	Mt
1	S	3+	S	4+	S	4+	S	4+
2	S/C	2+	S	4+	S	4+	S	4+
3	C	—	S/C	3+	S/C	3+	S	4+
5			S/C	3+	S/C	3+	S	4+
7			S/C	2+	S/C	2+	S	4+
10			C/S	+	S/C	2+	S	4+
12			C	—	C/S	+	S/C	3+
15					C/S	+	S/C	2+
20					C/S	+	S/C	2+
25							S/C	2+
30							S/C	2+

^a M, Morphology; S, spiral; C, coccoid; S/C, predominantly spiral; C/S, predominantly coccoid; Mt, motility; 4+, 100% motile; 3+, ≥ 75% motile; 2+, ≥ 50% motile; +, ≥ 10% motile; —, nonmotile.

subculture always showed growth by direct plating or through passage in the biphasic system. Cultures on BA, Campy-BA and Br.A transformed completely to the coccoid form on day 3 of storage at room temperature, and viable forms were not recovered either by direct subculture onto a Campy-BA plate or by passaging through the biphasic system. On Br.A-FBP, a few spiral cells were still observed for up to 20 days of storage at room temperature, and viable forms could be recovered for up to 10 days of storage by direct subculture onto a Campy-BA plate. However, prior passage into the biphasic system helped in the recovery of viable organisms from Br.A-FBP plates even up to 20 days of storage.

In comparison to cultures stored at room temperature, those stored at 4°C consisted of predominantly spiral motile cells for up to 7 days, and viable cells were recovered for up to 10 days from all the media without FBP. Observations were discontinued after 12 days when complete transformation to the coccoid form occurred and no viable cells were recovered even after passage into the biphasic system. Cultures on Br.A-FBP plates were, however, still viable on day 30 of storage as confirmed by direct subculture on the Campy-BA agar plates.

The results of the present study support earli-

er observations that *C. jejuni* transform rapidly to coccoid forms when exposed to ambient temperature and atmosphere on conventional media (5, 7). In addition to the ability of FBP supplement to increase the aerotolerance of *C. jejuni* and thereby enabling the strains to grow at elevated oxygen tension (3), our findings show that FBP supplement helps in maintaining the viability and characteristic cell morphology during prolonged storage. The suggestion of Karmali (5) to use rapid coccal transformation as one of the criteria to differentiate *C. jejuni* from *Campylobacter fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* may not be valid when plating media are supplemented with FBP. The recovery of viable cultures from Br.A-FBP plates for up to 30 days of storage at 4°C suggests the possibility of maintaining primary cultures on plates for longer periods of time. This procedure could minimize alteration in genetic or metabolic characteristics which might otherwise occur during frequent subculturing.

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