

Communication

[Comunicação]

Survival rates of thermotolerant *Campylobacter* species in a transport and enrichment medium under different environmental conditions

[Taxas de sobrevivência de espécies termotolerantes de *Campylobacter* mantidas em um meio de transporte sob diferentes condições ambientais]

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Thermotolerant campylobacters are important zoonotic agents of human diarrhea, being isolated as enteric pathogens practically in all Central and South America (Fernández, 1992a).

The ability of campylobacters to survive in clinical samples seems to be a difficult event but, if a transport and enrichment medium is used, it should be possible to maintain viable cells for a longer period. Several transport media have been developed for fecal samples (Luechtefeld et al., 1981; Wang et al., 1983; Aho et al., 1988; Fernández, 1992b) and the importance of the transporting power at the same time with the enrichment capacity of some media have been shown (Hutchinson and Bolton, 1983; Sjögren et al., 1985). The use of a transport media might be advantageous (i) for stool samples when campylobacters are present in small numbers, especially when the patient has been treated with antibiotics or when the stool samples can not be processed within 24 h, and (ii) for food and environmental samples. A transport medium is also necessary when rectal swabs are being used, not only because of the small number of bacteria but also because of their increased exposure to oxygen (Palumbo, 1986).

Fernández (1992b) evaluated an enrichment medium (TEC) for the isolation of thermotolerant *Campylobacter* species. This

medium was also used as a transport medium for both human and animal fecal samples as well as for pure cultures of campylobacters, with good results. However, there is no information about the best atmospheric conditions for maintaining viable cells during transport. For this reason, the optimal atmospheric conditions (temperature and oxygen concentration) for survival of campylobacters in this transport and enrichment medium were determined.

Ten *Campylobacter* strains (6 *C. jejuni* subsp. *jejuni* and 4 *C. coli* isolated from chicken and pig feces, respectively) were studied. They were spread on blood agar plates and incubated at 42°C for 48h under microaerobic conditions. Aliquots (2ml) of suspensions of each strain prepared in sterile distilled water (10⁹ CFU/ml) were seeded onto 2ml of TEC medium (Fernández, 1992a) consisting of: *Brucella* broth 2.8g%, agar 0.15g%, ferrous sulphate 0.05g%, sodium metabisulfite 0.05g%, sodium pyruvate 0.05g%, trimethoprim 1mg%, rifampicin 1.5mg%, colistin 1000IU%, amphotericine 1mg% and defibrinated horse blood 3ml%. The inoculated tubes were incubated at different atmospheric conditions: a) aerobically at room temperature ($\pm 28^{\circ}\text{C}$), b) microaerobically at room temperature, c) aerobically under refrigeration (4°C) and d) microaerobically under refrigeration.

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Survival rates...

Viable counts, carried out using a modified Miles and Misra method (Tresierra-Ayala et al., 1999) were done on 0, 1, 3, 5, 7 and 9 days after inoculation or until no bacterial survival was detected. At selected days, a 0.1ml sample of TEC medium from each atmospheric conditions was obtained, log₁₀ serially diluted in 0.1% peptone water and 20µl from each dilution were seeded in quintuplicate on blood agar plates. After 36h incubation at 42°C under microaerobic conditions, viable counts were determined and the results were corrected using the linear regression method.

The use of transport and enrichment media improves campylobacters isolation procedures contributing to develop more accurate epidemiological studies of these bacteria.

As shown in Table 1, most of the strains were viable over five days in TEC medium, but maintenance under microaerobic conditions at room temperature allowed longer survival periods (between 7 and 15 days). Blaser et al. (1980) reported that campylobacters could be

recovered after 3 weeks at 4°C from feces containing 6x10⁶ to 9x10⁹ organisms but, when the same samples were stored at 25°C, no viable organisms could be recovered after 1 week. Contrarily, Ladrón de Guevara et al. (1989) reported that they lost about 16% of their positive samples after 24h storage at 4°C. Monhort et al. (1989) reported that campylobacters survive in animal feces at least for 3h at 4°C but for less than 2h at 25°C stating that the organisms may be killed by toxic metabolites.

C. coli showed longer periods of viability than *C. jejuni* subsp. *jejuni* (Table 2). This difference is statistically significant [*Snedecor variance F test* ($P<0.05$); *Student t test* ($P<0.10$)] for the microaerobic atmosphere plus room temperature storage conditions. Some authors suggested that *C. coli* strains could be more resistant than *C. jejuni* to environmental conditions (Tresierra-Ayala et al., 1999; Fernández et al., 2003) being necessary more studies to elucidate this phenomenon.

Table 1. Survival of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli* strains in TEC medium, under different incubation conditions

Organism	Strain	Survival time (days)			
		A - RT	M - RT	A - RF	M - RF
<i>C. jejuni</i> subsp. <i>jejuni</i>	1	5	7	3	3
	2	5	9	9	5
	3	7	9	5	5
	4	7	9	7	7
	5	7	9	5	5
	6	9	10	7	7
<i>C. coli</i>	1	10	15	9	9
	2	7	10	5	5
	3	7	10	5	7
	4	10	15	7	7

A-RT: aerobically and room temperature; M-RT: microaerobically and room temperature; A-RF: aerobically and refrigeration temperature; M-RF: microaerobically and refrigeration temperature

Table 2. Average of the survival times of *C. jejuni* subsp. *jejuni* and *C. coli* strains in TEC medium, under different incubation conditions

Organism	N	Survival time (days)			
		A-RT	M-RT*	A-RF	M-RF
<i>C. jejuni</i> subsp. <i>jejuni</i>	6	6.7	8.8	6.0	5.3
<i>C. coli</i>	4	8.5	12.5	6.5	7.0

A=aerobically; RT=room temperature; M=microaerobically; RF= refrigeration temperature

* For values in this column only, there is a significant difference (*F test*, $P<0.05$; *Student t test*, $P<0.10$) among the two species.

In all the atmospheric conditions decreasing of the number of viable cells was observed. This could be because both species are unable to multiply below 30°C. However, when inoculated TEC medium was incubated at 42°C, microbial population increased.

Although the highest survival times were obtained microaerobically at room temperature,

the use of TEC medium associated to aerobic conditions at room temperature could be an efficient method for transporting samples, especially applicable in field studies, since it avoids the use of special atmosphere and temperature conditions.

Keywords: *Campylobacter jejuni* subsp. *jejuni*, *C. coli*, transport medium

RESUMO

Determinou-se a sobrevivência de *Campylobacter jejuni* subsp. *jejuni* e *C. coli* no meio de transporte e enriquecimento TEC, mantido sob diferentes condições de temperatura e concentração de oxigênio. A sobrevivência da maioria das amostras foi superior a cinco dias, obtendo-se os períodos de sobrevivência mais prolongados (sete a 15 dias), quando o meio foi incubado em microaerofilia à temperatura ambiente, condições nas quais o tempo de sobrevivência de *C. coli* foi superior ao de *C. jejuni* subsp. *jejuni*.

Palavras chave: *Campylobacter jejuni* subsp. *jejuni*, *C. coli*, meio de transporte

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