Letter to the Editor

Modification and Evaluation of Brucella Broth Based *Campylobacter jejuni* Transport Medium*

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Reliable transport of *Campylobacter jejuni* isolates is critical to microbial epidemiology research, especially in developing countries without a good temperature control mailing system. Various factors, including oxygen, temperature, transport medium composition, could affect the survival of *C. jejuni.* In this study, the protective effects of different ingredients in *C. jejuni* transport media at 4 °C and 25 °C and under aerobic condition were quantitatively evaluated respectively. The results showed that enriched medium, supplementation with 5% blood and being kept at 4 °C could improve the viability of different *C. jejuni* strains during transport. In addition, supplementation with 25 mmol/L L-fucose in Wang's transport medium could significantly improve the survival of *C. jejuni* at both 4 °C and 25 °C. To the best of our knowledge, this is the first report to evaluate the protective effect of L-fucose in enriched *C. jejuni* transport medium which is feasible in developing countries without an effective cold chain mailing system. These data will be good reference for *C. jejuni* transport medium improvement in future.

Campylobacter jejuni is one of the major pathogens causing foodborne bacterial infections worldwide[1]. It has been identified from a variety of sources, including foods, water, animals, and human being samples[2]. Different surveillance networks have been established to study the epidemiology and health impact of this pathogen[3-4]. Since *C. jejuni* requires microaerophilic condition to grow and is sensitive to oxygen at high concentration[5], therefore, the exchange of the bacterial cultures among laboratories needs dependable and fast transportation means. Studies have shown that various factors, including oxygen, temperature, medium composition, could affect the survival of *C. jejuni* during transport[5]. Different transport media have been developed and qualitatively evaluated for *C. jejuni* shipment[6], but quantitative evaluation data of transport medium were limited and the reliable shipment of *C. jejuni* isolates is still a technical barrier, especially in developing countries without good temperature control mailing system. In this study, the protective effects of different ingredients in *C. jejuni* transport media at 4 °C and 25 °C and under aerobic condition were quantitatively evaluated. The purpose of this study was to improve the protective effect of transport medium under aerobic conditions for the short-term shipment of *C. jejuni* strains.

Bacterial Strains: Six *C. jejuni* strains were used in this study for transport medium evaluation, including strain ATCC33291, strain ATCC33560, strain C10-1 and strain C12-1 (two strains from WHO external quality control system), strain FC13771 and strain FC13775 (two human strains from China CDC). All the strains were kept in Brucella broth (BD, Beijing, China) with 5% laked sheep blood and 50% glycerol at -80 °C freezer and cultured on Mueller-Hinton (MH, BD, Beijing) agar with 5% laked sheep blood under microaerophilic condition (5% O_2, 10% CO_2, 85% N_2) at 42 °C.

Transport Medium Preparation and Evaluation: Six kinds of transport media were evaluated in this study, including Cary-Blair medium, Brucella broth with 0.4% agar, Brucella broth with 0.4% agar supplemented with (a) 5% fresh sheep blood, (b) 5% laked sheep blood, (c) 5% laked sheep blood and FBP (a combination of 0.05% ferrous sulphate, 0.02% sodium bisulphite and 0.05% sodium pyruvate), or (d) 5% laked sheep blood and 25 mmol/L L-fucose, respectively. Approximately 10^8 CFU/mL of each *C. jejuni* strain fresh culture were inoculated in each kind of transport medium and dispensed into 2 mL sterile tubes with 1 mL each. The inoculated transport media were kept at either 4 °C or 25 °C.

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The viability of different *C. jejuni* strains in the inoculated transport media was measured by plate counting method.

Survival of Strain ATCC33291 in Transport Media at 4 °C: In Cary-Blair medium kept at 4 °C, the survival rate of strain ATCC33291 showed a substantial decline on the fifth day (8.8%) and no alive bacteria were found on the seventh day. But in Brucella media supplemented with 5% laked or non-laked sheep blood, the substaintal decline of survival rate was found on the 14th day (4.2%-8.6%). When 25 mmol/L L-fucose was added in Brucella media with 5% laked sheep blood, the survival rate of strain ATCC33291 was still 42.6% on the 14th day (Figure 1 A). Supplementation with FBP in transport media did not improve the survival of strain ATCC33291 at 4 °C.

Survival of Strain ATCC33291 in Transport Media at 25 °C: In Cary-Blair medium, 0.5% of bacteria survived after 24 h and no alive bacteria were found after 48 h. In Brucella media, 5.4% of bacteria survived after 24 h and no alive bacteria were detected on the third day. In Brucella media supplemented with 5% laked sheep blood, the survival rate was 25% after 48 h and 7.1% on the third day. When 5% laked sheep blood and 25 mmol/L L-fucose were added in Brucella media, the survival was further improved and 14.8% of cells survived on the third day and alive bacteria were even found on the fifth day (0.1%, 4.0×10^5 CFU/mL) (Figure 1B).

Survival of Other Five C. jejuni strains in Brucella Media at 25 °C: The survival of the other five *C. jejuni* strains except ATCC33291 were further determined in Brucella media with 5% laked sheep blood and 25 mmol/L L-fucose and in Brucella media with only 5% laked sheep blood. Supplementation with 25 mmol/L L-fucose in Brucella media could improve the survival of all tested strains. After being kept at 25 °C for 72 h, the survival rates of the five strains in media with 25 mmol/L L-fucose were 1.3 to >10 times higher than those in media without L-fucose. Four out of the five strains in Brucella media with 5% laked sheep blood and 25 mmol/L L-fucose had alive bacteria to be found on the fifth day compared with one strain survived in Brucella media with only 5% laked sheep blood. Strain FC13775 could survive in Brucella media for more than 10 d at 25 °C and showed the best viability in the transport media compared with the other strains in this study (Table 1).

In this study, the protective effects of different ingredients in *C. jejuni* transport media were evaluated quantitatively. Enriched medium, supplementation with blood and L-fucose and refrigeration could improve the viability of *C. jejuni* strain during transport. Especially, the supplementation with 25 mmol/L L-fucose in enriched transport medium could improve the survival of *C. jejuni* at both 4 °C and 25 °C. To the best of our knowledge, this is the first report of using

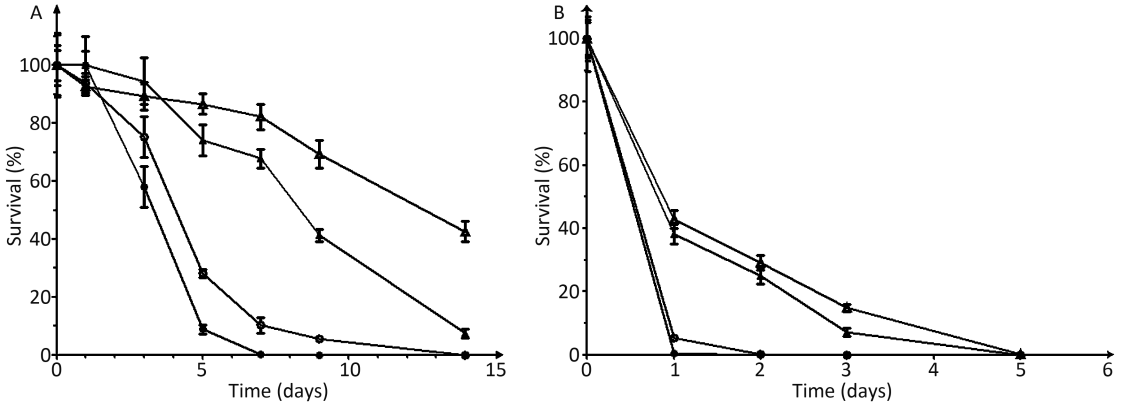

Figure 1. Survival rate of *C. jejuni* ATCC33291 in transport media at 4 °C (A) and 25 °C (B). Cary-Blair medium; Brucella broth with 0.4% agar; Brucella broth with 0.4% agar and 5% laked sheep blood; Brucella broth with 0.4% agar, 5% laked sheep blood and 25 mmol/L L-fucose.

Table 1. Survival Rate of *C. jejuni* Strains on Different Day at 25 °C

Medium Category	Strains Code	Counting (CFU/mL) and survival rate (%) of *C. jejuni* stains on different media						
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 7	Day 11
Brucella broth with 0.4% agar and 5% laked sheep blood	ATCC33560	$6.0\times10^{8}\pm4.3\times10^{7}$	$2.0\times10^{8}\pm1.3\times10^{7}$ (33.2±0.3)	$8.4\times10^{6}\pm5.1\times10^{5}$ (1.4±0.1)	$4.3\times10^{6}\pm5.6\times10^{5}$ (0.7±0.1)	ND	-**	-
	FC13771	$2.6\times10^{8}\pm2.1\times10^{7}$	$6.2\times10^{7}\pm1.8\times10^{6}$ (24.2±0.1)	$2.4\times10^{7}\pm3.2\times10^{6}$ (9.4±0.2)	$3.9\times10^{6}\pm4.8\times10^{5}$ (1.5±0.1)	ND	-	-
	FC13775	$5.2\times10^{8}\pm3.2\times10^{7}$	$1.2\times10^{8}\pm1.7\times10^{7}$ (22.4±0.5)	$1.0\times10^{8}\pm2.6\times10^{7}$ (19.7±0.1)	$5.8\times10^{7}\pm3.3\times10^{6}$ (11.3±0.1)	$3.4\times10^{7}\pm2.7\times10^{6}$ (6.6±0.1)	$2.5\times10^{7}\pm1.2\times10^{6}$ (4.8±0.4)	$5.0\times10^{2}\pm0.5\times10^{1}$ (0.0±0.0)
	C10-1	$2.6\times10^{8}\pm2.8\times10^{7}$	$8.3\times10^{7}\pm2.2\times10^{6}$ (32.2±0.1)	$8.1\times10^{6}\pm4.9\times10^{5}$ (3.1±0.3)	$6.0\times10^{3}\pm1.4\times10^{2}$ (0.0±0.0)	ND	-	-
	C12-1	$5.8\times10^{8}\pm3.7\times10^{7}$	$1.0\times10^{8}\pm0.7\times10^{7}$ (17.1±0.2)	$1.5\times10^{3}\pm0.5\times10^{2}$ (0.0±0.0)	ND*	ND	-	-
Brucella broth with 0.4% agar, 5% laked sheep blood and 25mmol/L L-fucose	ATCC33560	$6.0\times10^{8}\pm4.3\times10^{7}$	$2.0\times10^{8}\pm2.1\times10^{7}$ (34.1±0.5)	$9.4\times10^{7}\pm3.7\times10^{6}$ (15.8±0.1)	$5.7\times10^{6}\pm2.7\times10^{5}$ (1.0±0.1)	$1.0\times10^{3}\pm0.6\times10^{2}$ (0.0±0.0)	-	-
	FC13771	$5.6\times10^{8}\pm4.7\times10^{7}$	$1.9\times10^{8}\pm1.4\times10^{7}$ (34.2±0.3)	$1.2\times10^{8}\pm0.5\times10^{7}$ (21.8±0.1)	$8.0\times10^{7}\pm3.9\times10^{6}$ (14.2±0.2)	$4.1\times10^{4}\pm1.2\times10^{3}$ (0.0±0.0)	-	-
	FC13775	$7.7\times10^{8}\pm3.6\times10^{7}$	$3.4\times10^{8}\pm1.6\times10^{7}$ (43.7±0.4)	$2.8\times10^{8}\pm1.3\times10^{7}$ (35.9±0.3)	$2.1\times10^{8}\pm1.6\times10^{7}$ (27.2±0.4)	$2.1\times10^{8}\pm7.1\times10^{6}$ (26.9±0.4)	$8.4\times10^{7}\pm4.3\times10^{6}$ (10.9±0.1)	$1.6\times10^{7}\pm0.8\times10^{6}$ (2.1±0.2)
	C10-1	$6.7\times10^{8}\pm2.3\times10^{7}$	$1.7\times10^{8}\pm0.9\times10^{7}$ (25.2±0.4)	$1.4\times10^{8}\pm0.4\times10^{7}$ (20.9±0.2)	$2.4\times10^{7}\pm1.2\times10^{6}$ (3.6±0.1)	ND	-	-
	C12-1	$5.8\times10^{8}\pm1.9\times10^{7}$	$1.1\times10^{8}\pm0.5\times10^{7}$ (19.1±0.3)	$2.3\times10^{3}\pm1.7\times10^{2}$ (0.0±0.0)	$1.8\times10^{3}\pm0.5\times10^{2}$ (0.0±0.0)	ND	-	-

Note. * 'ND', no growth was detected. ** '-', not tested.

L-fucose in enriched *C. jejuni* transport medium which is feasible in pathogen delivery in developing countries without effective cold chain mailing system.

Previous studies have qualitatively evaluated the protective effects of different *C. jejuni* transport media by swab inoculation and enrichment detection method[7-9], limited quantitative data were available for the protective effect evaluation of *C. jejuni* transport medium. In this study, the protective effects of different *C. jejuni* transport media were quantitatively compared. Although *C. jejuni* could survive in Cary-Blair transport medium for more than 10 d at 4 °C, but the alive bacteria were dramatically decreased on the fifth day (Figure 1A). At 25 °C, no alive *C. jejuni* ATCC33291 were found in Cary Blair media after 48 h, which was similar to a previous study[10]. Our data showed enriched medium, blood and 25 mmol/L L-fucose were important for the survival of *C. jejuni* at both 4 °C and 25 °C during transport. Brucella broth is an enriched non-selective medium intended for the cultivation of most anaerobic bacteria and microaerophilic bacteria from clinical specimens. And blood is an ingredient widely used in *C. jejuni* culture medium. The USDA/FSIS laboratory guide book recommends using Brucella medium supplemented with 5% lysed horse blood (Wang's media) to transport *Campylobacter* in ice package. It also recommends to use a sterile cotton swab and dispenses the entire lawn of bacteria from three or four plates into a single cryovial containing the Wang's transport medium. Our data showed that if *C. jejuni* culture was homogenously mixed in the transport media, inoculation with 10^8 CFU/mL of *C. jejuni* was enough for culture delivery, instead of the high concentration of culture from three to four plates as recommended in the USDA/FSIS laboratory guide book. Our data also showed both fresh and laked sheep blood had a good protective effect for *C. jejuni* in transport medium which had also been used in other studies[10]. Since sheep blood is easier to obtain than horse blood in China, these data would be a good reference for transport medium improvement.

A recent study reported a new metabolic pathway in *C. jejuni* strains that could utilize L-fucose as a substrate for growth[11]. This pathway was linked to an 11-open reading frame plasticity region of the bacterial chromosome which was widely distributed in the strains from various host species[12]. Our data showed supplementation with 25 mmol/L L-fucose could improve the survival of *C. jejuni* strain in transport medium at both 4 °C and 25 °C, which might be related with this new recognized metabolic pathway. FBP has been proved to be an efficient *Campylobacter* growth supplement, but it did not show any protective effect for *C. jejuni* in transport medium. Since the L-fucose pathway was widely distributed in *C. jejuni* strains and related with its pathogenicity, L-fucose might be a good supplement ingredient in *C. jejuni* transport medium in future.

In conclusion, enriched medium, supplementation with 5% sheep blood and 25 mmol/L L-fucose and low temperature could improve the survival of *C. jejuni* strain at both 4 °C and 25 °C, these data will be good reference for *C. jejuni* transport medium improvement in future.

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REFERENCES

1. Silva J, Leite D, Fernandes M, et al. *Campylobacter* spp. as a foodborne pathogen: a review. Front Microbiol, 2011; 2, 200.
2. Taylor EV, Herman KM, Ailes EC, et al. Common source outbreaks of *Campylobacter* infection in the USA, 1997-2008. Epidemiol Infect, 2012; 141, 987-96.
3. Hurd S, Patrick M, Hatch J, et al. Clinical laboratory practices for the isolation and identification of *Campylobacter* in Foodborne Diseases Active Surveillance Network (Food Net) sites: baseline information for understanding changes in surveillance data. Clin Infect Dis, 2012; 54 Suppl 5, 440-5.
4. Gautret P, Cramer JP, Field V, et al. Infectious diseases among travellers and migrants in Europe, Eurotravnet 2010. Euro Surveill, 2012; 17, 16-26.
5. Garenaux A, Jugiau F, Rama F, et al. Survival of *C. jejuni* strains from different origins under oxidative stress conditions: effect of temperature. Curr Microbiol, 2008; 56, 293-7.
6. Wang WL, Reller LB, Smallwood B, et al. Evaluation of transport media for *C. jejuni* in human fecal specimens. J Clin Microbiol, 1983; 18, 803-7.

7. Omurtag I, Aydin F, Paulsen P, et al. Simple media and conditions for inter-laboratory transport of *C. jejuni* isolates. Vet Q, 2011; 31, 73-5.
8. Sjogren E, Lindblom GB, and Kaijser B. Comparison of different procedures, transport media, and enrichment media for isolation of *Campylobacter* species from healthy laying hens and humans with diarrhea. J Clin Microbiol, 1987; 25, 1966-8.
9. Vidal AB, Rodgers J, Arnold M, et al. Comparison of Different Sampling Strategies and Laboratory Methods for the Detection of *C. jejuni* and *C.coli* from Broiler Flocks at Primary Production. Zoonoses Public Health, 2013; 60, 412-25.
10. Wasfy M, Oyofo B, Elgindy A, et al. Comparison of preservation media for storage of stool samples. J Clin Microbiol, 1995; 33, 2176-8.
11. Stahl M, Friis LM, Nothaft H, et al. L-fucose utilization provides *C. jejuni* with a competitive advantage. Proc Natl Acad Sci USA, 2011; 108, 7194-9.
12. Muraoka WT and Zhang Q. Phenotypic and genotypic evidence for L-fucose utilization by *C. jejuni*. J Bacteriol, 2011; 193, 1065-75.