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Improved Preservation Medium for *Campylobacter jejuni*

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An egg-based medium was found to be superior to the conventional Wang transport medium and the recently developed biphasic medium for the preservation of *Campylobacter jejuni* in the laboratory. Strains of *C. jejuni* preserved in egg-based medium maintained at 4°C were viable for over 3 months. The survival of *C. jejuni* in egg-based medium held at room temperature (27 ± 2°C) was also relatively longer than in Wang transport medium and biphasic medium.

Worldwide recognition of *Campylobacter jejuni* as an important enteric pathogen has generated considerable interest in recent years among scientists in both developed and developing countries. As a result, techniques for the isolation and identification of this organism from clinical specimens have been simplified considerably and are now well within the reach of most enteric laboratories. However, maintenance of stock cultures of *C. jejuni* in the laboratory remains an unresolved problem. *C. jejuni* is known to rapidly lose viability, usually 72 h after primary isolation, and subsequently degenerates into nonmotile coccoid forms which fail to grow on subculture (2).

In the conventional Wang transport medium (WTM) (4) and the biphasic preservation medium (BPM) developed in this laboratory (3), *C. jejuni* isolates can be preserved for a short period (ca. 1 month). However, survival of *C. jejuni* in these media often exhibits a marked degree of strain to strain variation. The unpredictability of growth of *C. jejuni* on subculture from preservation media is, in fact, one of the serious difficulties encountered during preservation and transportation of this organism.

In the course of our search for a suitable preservation medium for *C. jejuni*, we observed that an egg-based medium (EM) performed consistently well in maintaining the viability of this organism over a considerable period of time. The efficacy of this medium for preservation of *C. jejuni* was evaluated with the WTM and the BPM with a number of *C. jejuni* strains isolated from different sources.

EM was a modification of the Boeck and Drbohlav (1) medium originally devised for cultivation of *Entamoeba histolytica*. Fresh hen eggs, washed thoroughly with detergent and water, were wiped with 70% ethanol. The contents of six eggs were poured into a presterilized, wide-mouth, stoppered bottle containing sterile glass beads and 100 ml of sterile 1/40 M phosphate-buffered saline (pH 7). The contents of the bottle were thoroughly emulsified by shaking and subsequently filtered through a sterile muslin cloth. The emulsified egg (5 ml) was aseptically dispensed into screw-cap tubes (16 by 150 mm). Egg slopes were prepared by keeping the tubes at 80°C for 1 h for three successive days in an inspissator. Sterility of the medium was checked by incubating the slants at 37°C for 48 h. The egg slant was overlaid with 5 ml of thioglycolate broth (BBL Microbiology Systems) supplemented with 0.025% each of ferrous sulfate, sodium metabisulfite, and sodium pyruvate (Oxoid freeze-dried *Campylobacter* growth supplement, SR 84) a posteriori to inoculation of the culture on the surface of the slope. WTM and BPM were prepared as described previously (3, 4).

Ten strains of *C. jejuni* (one reference culture, three strains from human diarrhea, two strains from chicken intestine, and four strains from asymptomatic healthy carriers) were used in this study. None of the test strains were passaged for more than six generations on artificial media. All strains were checked for purity on blood agar plates. A heavy inoculum of a 48-h-old culture was inoculated into the test media and incubated for 48 h at 42°C in candle extinction jars with caps loosely screwed. Postincubation, one batch of the test strains was kept refrigerated (4°C), whereas the other was kept at room temperature (27 ± 2°C). Viability checks were made every 10 days from both the batches by streaking a loopful of the test material withdrawn from the solid-liquid interface (in the case of EM and BPM) or from the lower portions of the test tube (in the case of WTM) on blood agar plates.

The ability of the three preservation media to support viability of *C. jejuni* is shown in Table 1, which represents results of three independent series of experiments conducted separately. When kept refrigerated, all the strains inoculated in EM were viable for over 3 months. Although few isolates lost viability thereafter, 70% of the strains could be recovered from EM for another month and a half. In contrast, survival of *C. jejuni* in WTM and BPM was markedly shorter, with some cultures losing viability as early as 10 days. The amount of growth, in terms of the number of colonies appearing on the blood agar plates during viability checks, was also consistently higher for EM as compared with WTM and BPM.

The ability of EM to support viability of *C. jejuni* when held at room temperature was also relatively better than on WTM and BPM. A sharp decline in viability of *C. jejuni* in

TABLE 1. Ability of the three preservation media to support viability of *C. jejuni*

Medium	Holding temp	% Recovery of *C. jejuni* on the following days postincubation:					
		10	20	40	60	90	120
WTM	Room temp	40	10	0	—[a]	—	—
	4°C	80	80	60	0	—	—
BPM	Room temp	10	0	—	—	—	—
	4°C	100	80	80	30	0	—
EM	Room temp	100	100	20	0	—	—
	4°C	100	100	100	100	100	80

[a] —, Not done.

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EM was observed after 20 to 30 days. Another feature which obliterated the viability of a few cultures of *C. jejuni* preserved in EM held at room temperature was the copious production of H_2S. The reaction was aerogenic with the insoluble black pigment appearing along the slant and ultimately resulted in the crumbling of the egg slant. The appearance of the black precipitate in EM was delayed and occurred at various periods ranging from 5 to 15 days. The survival of *C. jejuni* in WTM and BPM held at room temperature was very much lower (Table 1).

In consideration of the longer duration of survival of *C. jejuni* in EM at room temperature, this medium might also substitute for WTM as a transport medium for sending pure cultures between laboratories. The transit time for parcels dispatched by airmail postage generally never exceeds 3 weeks, even between distantly located countries. This is of special importance since serological typing of *C. jejuni* is still in the developmental stages and is performed by few laboratories, thereby necessitating the dispatch of cultures to such laboratories for serotyping.

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