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EVALUATION OF DIFFERENT SELECTIVE MEDIA FOR RECOVERY OF CAMPYLOBACTER JEJUNI

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INTRODUCTION

Campylobacter jejuni is apathogen affecting many animals species. It is transmitted to human through ingestion of contaminated foods, particularly raw milk or water (Doyle, 1981, Lovett et al. 1983, Carler et al. 1987).

Campylobacter jejuni is one of the important causes of human gastroenteritis. It was isolated from foods and other sources with special requirment (Blaser et al., 1979 and Rosef, 1981) as selective media (Tanner and Bullin, 1977, Blaser et al., 1979; Rosef, 1981 and Chan and Mackenzie 1982).

Special techniques (Doyle and Roman, 1982 and Fricker 1987), microaerophilic condition (Tanner and Bullin 1977; Stern, 1982 and Lovett et al. 1983). Addition of antimicrobials (Blaser et al. 1979 and Oesterom et al., 1981), incubation temperature (Doyle and Roman, 1982; Lovett et al., 1983; Hunt et al., 1985 and Heisick 1985). For isolation and identification of *C. jejuni* selective plating solid media are necessary. Several solid media have been used to achieve such goal (George et al. 1978 , Lauwers et al. , 1978 Skirrow, 1977 and Stern, 1982).

This work was done to compare different selective enrichment broths (Preston, brucella and thioglycollate) with and without antibiotic supplement to evaluate their recoveries of *C. jejuni* on three solid media Campylobacter agar base, brucella agar base and Campylobacter blood free.

MATERIAL AND METHODS

For strains of *C. jejuni* were isolated from market raw milk were identified morphologically and confirmed by biochemcial reaction according to Bergy's Manual of Systematic Bacteriology (1984) in paralled with a strain of *C. jejuni* (WA2) obtained from Food Research Inistitute. University of Wisconsion, Modison. U.S.A. Raw milk was enriched in Preston broth (Bolton and Robertson, 1982) supplemented with polymyxin B 2500 IU, (inhibitory to Enterobacteriace and Pseudomonas species), Rifampicin 5 mg, trimethoprim lactate 5 mg (acts

against Proteus species), and actidione 50 mg (Boef et al., 1984). Enrichment both was incubated at 42°C for 18 hours under microaerxophilic conditions (5% O_2 : 10% CO_2 : 85% N_2) by oxoid gas generating Kits in Gas pakjar. After enrichment a loopful (3 mm) of enrichment broth was streaked onto plates of campylobacter blood free selective agar base (Oxoid, CM 739) supplemented with cefoperazon *(actt against Streptococcus faecalis, Enterobacter species,* Serratia species, *Pseudemonas aeruginosa, Y. enterocolitica and Bacteroids Fragilis)* Stern, 1982.

Plates were incubated at 42°C for 48 hours under microaerophilic conditions. Typical colonies were microscopically examined and biochemically tested according to Bergy's Manual of Systematic Bacteriology (1984).

To study the efficiency of the selective media which gave optimum recovery of *C. jejuni*.

Ten experiments (frequencies) were done on three enrichment broths. Preston broth (Bolton and Robertson, 1981), brucella broth (Finegold and Martin 1982) and thioglycollate broth (Blaser et al., 1979), were used. Enrichment broths have been inoculated, each, with a loopful (3mm), of the strain of *C. jejuni* , Enrichment broths were incubated at 37°C and 42°C under microaerophilic conditions (Hunt, 1985) . Growth rates have been evaluated after 24 hrs and 48 hrs visually (turbidity test) and detection of optical densities (OD) through spectrophotometer (C = CIL instruments, equipped with CE 595 double beam digital U.V.).

Plates of campylobacter agar base + 5% sheep blood, brucella agar base + 5% sheep blood and campylobcter blood free agar were subcultured, surface plating, from the enrichment broths. The plates were incubated according to the original incubation temperature of the enrichment broth at 37°C and 42°C under microaerophilic conditions. Recovery rates were determined by colony forming unit/ml (CFUI) after 48 hours.

RESULTS AND DISCUSSION

The highest growth rate of C. jejuni was observed in thiogly collate broth at 37°C indicated by turbidity (++++) and optical densities 0.973 and 1.246 for the the broth with and without antibotic supplement, respectively. While the highest growth rate at 42°C was in brucella broth with supplement (OD 1.414) and without supplement (OD 1.556) (Table 1). This results agree with Blaser et al. 1979; Chan and Mackenzie, 1982 and Doyle and Roman (1982). No significant effect of the antibiotic

Table (1): Growth of C jejuni in different enrichment broth with or without antibiotics supplement at 37 and 42 °C for 24 hours.

Enrichment broth	37 °C				42 °C			
	With supplement		Without supplement		With supplement		Without supplement	
	Turbidity	O.D.	Turbidity	O.D.	Turbidity	O.D.	Turbidity	O.D.
Preston	+++	0.803	++++	0.929	++	0.546	++	0.590
Brucella	++	0.636	+++	0.701	++++	1.414	++++	1.556
Thioglycollate	++++	0.973	++++	1.246	++	0.430	+++	0.737

Table (2) : Growth of C. jejuni previously enriched in three enrichment broths with or without antibiotics supplement and subsequently subcultured on three solid media at 37°c for 48h.

solid media	Mean recovery rates of *C. jejuni* (CFU/ml) previously enriched					
	Preston broth		Brucella broth		Thioglycollate broth	
	with supplement	without supplement	with supplement	without supplement	with supplement	without supplement
Brucella agar base + 5% sheep blood	24×10^8	38×10^8	25×10^{10}	42×10^{10}	37×10^7	75×10^7
Compy agar base +5% sheep blood	40×10^8	57×10^8	$37\ 10^{10}$	57×10^{10}	25×10^8	41×10^8
Compy blood free	70×10^6	89×10^7	30×10^8	60×10^8	16×10^6	80×10^6

* Preston supplement (Oxoid, SR 117).

supplements on the the growth rates of *C. jejuni* within the enrichment broth whether supplemented or not, as indicated by the turbidity and/or OD.

Typical surface colonies of C. jejuni obtained were round, small, translucent, grey, buffy or tan mucoid and non hemolytic on the selective agar plates.

Results of the growth of Campylobacter jejuni on different solid media at different temperatures showed that brucella broth medium, with and without antibotic supplement, gave higher counts of *C. jejuni* on the three solid media incubated at 37°C for 48 than gave the other enrichment broths (Table 2, Fig.1). On the other hand brucella agar base and campylobacter agar base, both with 5% sheep blood, seeded with *C. jejuni* from brucella broth, showed higher recovery rates ($\approx$ x 10^{10} CFU/ml) than campylobacter blood free ($\approx$ x 10^8 CFU/ml. These results seemed to agree with the observation done by Blaser et al., 1979 and Lauwers et al., 1978).

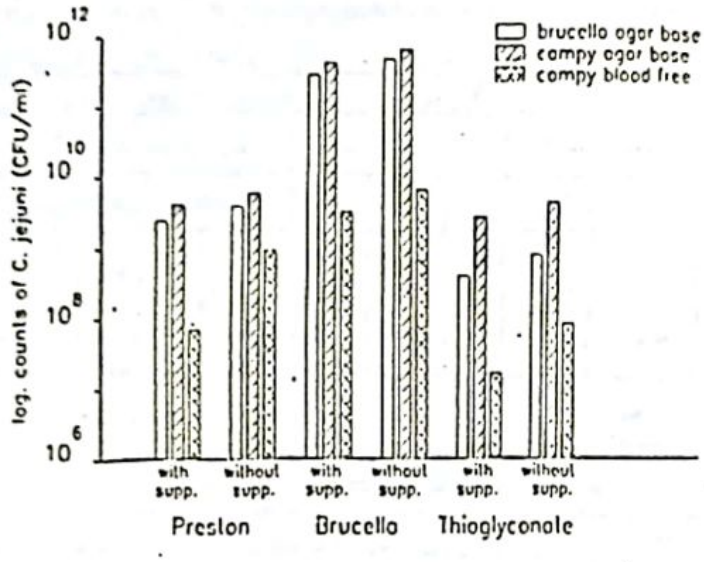

Fig 1. Growth of C. jejuni at 37 C in different broths and solid media.

Table (3) : Growth of C. jejuni previously enriched in three enrichment broths with or without antibiotics supplement and subsequently subcultured on three solid media at 42°c for 48h.

solid media	Mean recovery rates of C. jejuni (CFU/ml) previously enriched					
	Preston broth		Brucella broth		Thioglycollate broth	
	with supplement	without supplement	with supplement	without supplement	with supplement	without supplement
Brucella agar base + 5% sheep blood	75×10^{9}	30×10^{11}	39×10^{11}	75×10^{11}	10×10^{10}	17×10^{11}
Campy agar base +5% sheep blood	50×10^{9}	18×10^{10}	27×10^{11}	46×10^{11}	40×10^{11}	55×10^{11}
Campy blood free	10×10^{8}	27×10^{8}	20×10^{8}	50×10^{10}	90×10^{8}	17×10^{9}

* Preston supplement (Oxoid, SR 117).

Campylobacter recovery rates obtained from preston and thioglycollate enrichment broths on the solid media (≈ $\times 10^{6}$ - ≈ $\times 10^{8}$ CFU/ml) showed similar trends. Also the obtained results on recovering camplyobacter onto solid media emphasized that slight inhibition effect due to adding antibotic supplement to the enrichment broths (Fig. 1) since counts of *C. jejuni* (CFU) enriched in, broths with suplement and subcultured onto the solid media were in the same long Cycles.

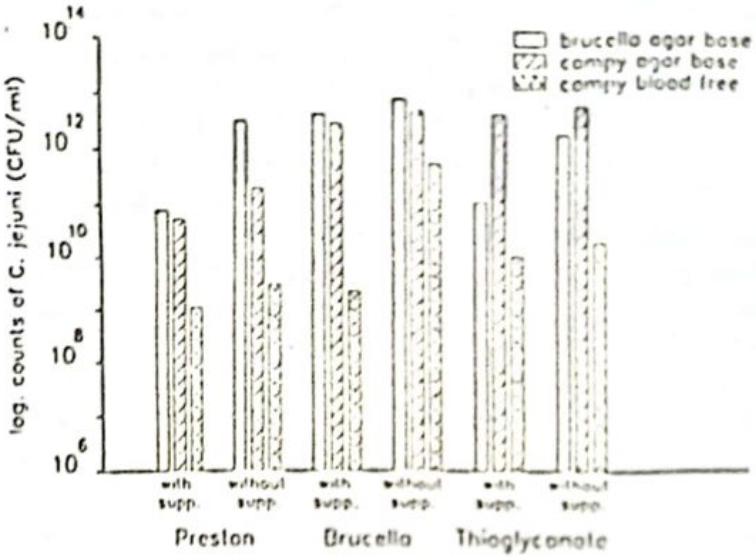

Fig 2. Growth of C. jejuni at 42 C in different broths and solid media.

At higher temperature 42°C, results as shown in (Table 3 and Fig. 2) indicate that higher recovery rates were obtained (≈ x 10^8 to ≈ x 10^{11} CFU/ml) than that at 37°C (≈ x 10^6 to ≈ 10^{10} CFU/ml). These findings agreed with Norman, 1982 who found that the optimum temperature for growth of *C. jejuni* about 42°C. *C. jejuni* was highly recovered from brucella broth and thioglycollate broth subcultured onto brucella agar base and campylobacter agar base (≈ x10^{11} CFU/ml) at 42°C while , *C. jejuni* enriched in preston broth and subcultured onto campylobacter blood free medium at 42°C showed the least recovery rate at this temperature.

SUMMARY

Evaluation of different selective broths and media for recovery of *Campylobacter jejuni* were studied. The enrichment of *Campylobacter jejuni* on preston, brucella and thioglycollate broths revealed that the highest rate of reisolation were obtained on brucella and/or thioglycollate broths with or without antibiotic supplement, while the selective media which gave the highest recovery rate were on brucella agar base and campylobacter agar base, enriched with 5% sheep red cells.

The optimum temperature for reisolation of *C. jejuni* either from enrichment or selective solid media was 42°C.

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