EFFECT OF SUPPLEMENTS ON *CAMPYLOBACTER* GROWTH IN ENRICHMENT MEDIA[1]

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ABSTRACT

Supplementation of Niroomand and Fung broth (NFB) and Hunt broth (HB) with Oxyrase®, sheep blood, hemin or yeast extract for recovery of Campylobacter *was evaluated. Five strains of* C. jejuni *and one strain of* C. coli *were cultured in HB with or without Oxyrase®, NFB with or without Oxyrase®, NFB without hemin, and NFB plus yeast extract without hemin. The HB without Oxyrase® was evacuated and flushed three times with mixed gas (5% O_2, 10% CO_2, and 85% N_2) prior to incubation. The effect of blood in HB supplemented with Oxyrase® on the growth of* Campylobacter *was also examined. Hunt broth showed significantly better performance than NFB ($p < 0.05$). Supplementation of 0.2% hemin and 0.6% yeast extract stimulated growth by up to 2 log CFU/mL depending on strains. Addition of 7% lysed blood enhanced growth of* C. jejuni, *and differences were significant at 6 h and 24 h of incubation. At 20 h of incubation, broth containing 0.6 units/mL of Oxyrase® yielded* Campylobacter *counts of 8.6 to 10.9 log CFU/mL, and the broth flushed with mixed gas provided counts of 9.4 to 11.0 log CFU/mL. No statistical difference ($p > 0.05$) was found between the Oxyrase® method and gas replacement method at incubation times of 12 h, 20 h, 28 h, and 36 h for all strains tested. Dissolved oxygen levels of all enrichment media were less than 1.5 mg/L. Using Oxyrase®-supplemented HB without the cumbersome gassing step provides a simple and time-saving procedure for culturing* Campylobacter jejuni *and* C. coli.

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INTRODUCTION

Campylobacter jejuni is the leading cause of diarrheal illness worldwide. About 2.5 million cases of human campylobacteriosis were estimated to occur in the United States annually (Nachamkin *et al.* 1998). Campylobacters have been isolated from a variety of foods and water, especially from poultry origins, because the bacteria are commensal in the intestinal tracts of poultry, cattle, swine, and other wild and domestic animals. The consumption of contaminated foods that are undercooked and the ingestion of contaminated water commonly are associated with the infection in humans. As few as 500 cells of *C. jejuni* are able to produce symptoms in humans (Medema *et al.* 1996). The predominant clinical symptoms of *Campylobacter* enteritis are diarrhea, abdominal pain, fever, and malaise. The disease is generally self-limited and typically subsides within 1 week. However, severe complications may occur after the onset of infection, including reactive arthritis and Guillain-Barré syndrome (Blaser 1997). Estimated total annual costs from *Campylobacter* infection in the Unites States are \$1.5 to \$8.0 billion, and 55% to 70% of costs probably are attributable to foodborne sources (Buzby *et al.* 1997).

Campylobacter spp. are fastidious, microaerophilic, gram-negative, curved to spiral bacteria. Older and stressed microorganisms undergo morphological transformation into a coccoid form, which is difficult to culture and termed "nonculturable". Enrichment procedures usually are used for resuscitating and increasing target pathogens from food samples which normally contain low numbers of the pathogen. Enrichment media used for recovery of *C. jejuni* have included lysed horse blood, hematin solution, charcoal, and/or FBP (ferrous sulfate, sodium metabisulphite, and sodium pyruvate) as oxygen scavengers to protect the organisms from the toxicity of oxygen derivatives (Corry 1995). One common method for generating microaerobic conditions within enrichment broth involves evacuation and gas replacement with a mixture of gas containing 5% O_2, 10% CO_2, and 85% N_2, which is laborious, expensive, and time-consuming. An alternative way to achieve a microaerobic environment for *Campylobacter* growth by supplementation with Oxyrase® has been proposed (Niroomand and Fung 1994). Oxyrase® is a mixture of oxygen membrane fragments isolated from *Escherichia coli* and functions as an oxygen-quenching agent to remove oxygen from media. It has been demonstrated to stimulate growth of pathogenic facultative anaerobes, such as *E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Yersinia enterocolita* (Yu and Fung 1991; Tuitemwong *et al.* 1995; Niroomand and Fung 1992).

Niroomand and Fung (1994) first proposed an Oxyrase®-brucella enrichment medium (Niroomand and Fung broth, NFB) for culturing *Campylobacter* species. Media incorporating with Oxyrase® have been used for isolation of campylobacters from food samples, such as shellfish and chicken carcasses

(Abeyta *et al.* 1995; Raben and Slavik 1994; Tran 1995). In an attempt to improve enrichment for the detection of *Campylobacter*, supplementation with blood, hemin, or yeast extract in NFB and a conventional Hunt broth was evaluated using a pure culture system. In addition, the gas replacement method and the Oxyrase® method were compared.

MATERIALS AND METHODS

Bacterial Cultures

Five strains of *C. jejuni* and one strain of *C. coli* were studied. *C. jejuni* ATCC 33291 and ATCC 29428 were obtained form MicroBiologics Inc., St. Cloud, MN. *C. jejuni* #105 and #103 were obtained from culture collections of the Food Microbiology Laboratory, Kansas State University, and *C. jejuni* #101 and *C. coli* #102 were provided by Dr. J. Stanley Bailey at the United States Department of Agriculture (USDA), GA. The bacteria were maintained on brucella agar (Difco Laboratories, Sparks, MD) containing 7% defibrinated sheep blood (Remel, Lenexa, KS) in anaerobic jars with CampyPak (BBL, Cockeysville, MD) at 4C under microaerophilic atmosphere and subcultured every 2 weeks.

Preparation of Inoculum

Campylobacter strains from stock cultures were subcultured on brucella blood agar under microaerophilic atmosphere at 37C for 48 h. The 48 h cultures were transferred into 0.1% peptone water (Difco) and were serially diluted to achieve an initial bacterial count of about 2-3 log CFU/mL in 250 mL-Erlenmeyer flasks containing 75 mL of tested media.

Preparation of Enrichment Media

The various formulations of enrichment broth in 250 mL flasks are described below. Abbreviations for supplement additions are as follows: with addition of Oxyrase® = O, with addition of hemin = H, and with addition of yeast extract = Y.

(1) Hunt broth (HB) was prepared as described in the Bacteriological Analytical Manual (Food and Drug Administration 1995) with some modifications. Nutrient broth No. 2 (Oxoid) with 0.6% yeast extract (Difco) added was prepared, and 75 mL aliquots of the broth were distributed in 250 mL screw-capped Erlenmeyer flasks. After autoclaving at 121C, 15 lb pressure for 15 min, all flasks were kept in the dark at room temperature before use.

The FBP solution consisting of 6.25% each of ferrous sulfate (Sigma Scientific, St. Louis, MO); sodium metabisulfite (Fisher Scientific, Fair Lawn, NJ); and sodium pyruvate (Sigma) was prepared in a volumetric flask and sterilized by filtration through a 0.45 μm-filter (Nalgene, Rochester, NY). The solution was distributed into 6 mL portions in tightly capped sterile containers at -20C. Defibrinated sheep blood cells (Remel) were frozen and thawed and then kept at 4C before use. Sterile oxygen-reducing membrane fragments (Oxyrase®) derived from *Escherichia coli* b/r strain and designed for anaerobic cultivation of bacteria were obtained from Oxyrase Inc., Mansfield, OH in a frozen state. The enzyme contains activity of 30 units/mL in a quantity of 50 mL per bottle, and it was thawed at 4C overnight prior to experiments.

Before an experiment, 93 mL of HB was supplemented with 0.3 mL (final concentration of 0.025%) of FBP solution, 5.2 mL (final concentration of 7%) of lysed sheep blood, and 1.5 mL (final concentration of 0.6 units/mL) of Oxyrase® (HBO). The HB without Oxyrase® (HB&Gas) was evaluated and flushed three times with a mixture of gases including 5% O_2, 10% CO_2, and 85% N_2 (Compressed Gas, Linweld, Lincoln, NE) prior to incubation. HBO without the supplement of lysed blood also was evaluated.

(2) Niroomand and Fung (NF) broth was prepared according to their formulation (Niroomand and Fung 1994). Brucella broth (Difco) was prepared according to the manufacturer's procedure. Seventy-five mL aliquots of brucella broth were distributed in 250 mL screw-capped Erlenmeyer flasks and sterilized by autoclaving for 15 min. All flasks were protected from light and kept at room temperature. Hemin solution was prepared fresh before use by dissolving 0.32 g of bovine hemin (Sigma) in 10 mL of 0.15 N sodium hydroxide and then filtering through 0.45 μm-filter (Nalgene).

Prior to inoculation, the NF broth was supplemented with 0.3 mL of FBP solution, 6.2 mL (final concentration of 0.2%) of hemin solution, and 1.5 mL of Oxyrase® (NFOH) or not supplemented with Oxyrase® (NFH&No Oxy). The NFOH broth without the addition of 0.2% hemin (NFO&No hemin) and the NFO&No hemin broth supplemented with 0.6% yeast extract (NFOY) also were evaluated.

Campylobacter Growth Study

The prepared suspensions of *C. jejuni* and *C. coli* were inoculated into flasks containing 75 mL of various enrichment broths. The flasks were incubated in a shaking water bath (Model G 76 New Brunswick Scientific Co., Inc., Edison, NJ) at 100 rpm at 42C. Bacterial counts were performed before (0 h) and at 6, 12, 20, 28, and 36 h of incubation. When the HB&Gas flasks were opened for sampling, the gas replacement step with mixed gas was repeated for the subsequent incubation time. One mL from each flask was withdrawn

aseptically and 100 μL of appropriate dilutions in peptone water was spread on brucella blood agar plates in duplicate. All plates were incubated under microaerophilic atmosphere at 37C for 48 h before counting. Growth curves presented show averages of cell numbers of three replications of each strain that was studied in different times.

Dissolved Oxygen Measurement

The dissolved oxygen (DO) of each culture before (0 h) and at incubation times of 6, 12, 20, 28, and 36 h was monitored by a Dissolved Oxygen Meter YSI 500 with a YSI 5010 probe (Yellow Springs Instrument Co., Yellow Springs, OH) which is a Clark-type polarographic sensor of DO with a temperature sensor. The DO values of each 2 mL of sample in %-air saturation and mg/L and temperature were recorded.

Statistical Analysis

Results of *Campylobacter* counts for each enrichment medium were the means from three separate experiments. Bacterial numbers were transformed to $\log_{10}$ CFU/mL prior to analysis. Statistically significant differences among enrichment media at each incubation time were determined by using the least-squares means and analysis of variance techniques from the SAS Mixed procedure (version 6.12; SAS Institute, Cary, NC) at p-value ≤ 0.05.

RESULTS AND DISCUSSION

The efficiency of various formulations of enrichment media for recovery of all *Campylobacter* strains tested is presented in Table 1. Growth curves of each strain are showed in Fig. 1 to Fig. 6. The performance of HB was significantly better than that of NFB for all tested strains. The viable counts cultured in HB&Gas and HBO increased by 3 to 5 log CFU/mL at 12 h of incubation and by 5 to 7 log CFU/mL at 20 h depending on strains and initial concentrations of inocula. HBO (added Oxyrase®) provided *Campylobacter* growth as effective as HB&Gas (flushed with mixed gas) with no statistically significant differences ($p > 0.05$) for all strains, indicating the stimulatory effect of Oxyrase® for *Campylobacter* growth in pure culture systems under atmospheric conditions. In addition, NFOH gave higher bacterial counts than NFH&No Oxy at every incubation periods and for all strains, with some statistically significant differences, also supporting the positive effect of Oxyrase®. In addition to contributing a microaerophilic environment, Oxyrase® also enhanced growth of *C. jejuni* in pure cultures (Wang *et al.* 1998).

TABLE 1.
GROWTH OF FIVE STRAINS OF *CAMPYLOBACTER* AT VARIOUS INCUBATION PERIODS IN SIX DIFFERENT ENRICHMENT MEDIA

Strain	Time (h)	*Campylobacter* count (log CFU/ml)					
		HB&Gas	HBO	NFOH	NFO&No hemin	NFOY	NFH&No Oxy
C. jejuni	0	3.52	3.52	3.52	3.52	3.52	3.52
ATCC 33291	6	6.21^{a}	6.21^{a}	5.44^{ab}	4.40^{b}	4.76^{b}	4.60^{b}
	12	8.66^{a}	7.96^{a}	6.31^{b}	5.32^{b}	5.91^{b}	5.75^{b}
	20	9.96^{a}	9.59^{a}	7.63^{b}	6.58^{b}	7.18^{b}	6.87^{b}
	28	9.97^{a}	10.2^{a}	7.37^{b}	6.96^{b}	7.47^{b}	6.61^{b}
	36	9.58^{a}	9.96^{a}	7.90^{ab}	7.73^{ab}	8.19^{ab}	6.90^{b}
C. jejuni	0	3.76	3.76	3.76	3.76	3.76	3.76
ATCC 29428	6	5.00^{ab}	5.56^{a}	4.04^{c}	3.33^{d}	4.88^{b}	4.11^{c}
	12	6.56^{ab}	6.91^{a}	5.05^{bcd}	2.93^{e}	5.84^{abc}	4.60^{cd}
	20	11.02^{a}	10.39^{a}	4.76^{bc}	2.24^{d}	6.93^{b}	3.92^{cd}
	28	11.44^{a}	10.69^{ab}	5.91^{c}	2.00^{d}	8.46^{bc}	2.00^{d}
	36	10.94^{a}	11.50^{a}	7.35^{ab}	3.00^{b}	7.37^{ab}	3.00^{b}
C. jejuni	0	3.73	3.73	3.73	3.73	3.73	3.73
# 105	6	5.93^{a}	5.33^{b}	4.48^{c}	3.58^{d}	4.45^{c}	4.02^{cd}
	12	8.47^{a}	8.15^{a}	6.51^{ab}	6.52^{ab}	6.32^{ab}	5.56^{b}
	20	9.42^{a}	9.13^{ab}	8.68^{ab}	6.90^{c}	7.48^{bc}	7.55^{bc}
	28	10.59^{ab}	10.40^{ab}	10.65^{a}	8.56^{b}	9.41^{ab}	8.92^{b}
	36	8.52^{a}	9.20^{a}	8.70^{a}	9.63^{a}	9.62^{a}	8.34^{a}
C. jejuni	0	1.79	1.79	1.79	1.79	1.79	1.79
# 103	6	3.73^{a}	3.58^{a}	2.89^{ab}	2.24^{b}	2.69^{ab}	2.00^{b}
	12	6.22^{a}	5.44^{a}	4.23^{ab}	3.00^{b}	3.51^{b}	3.00^{b}
	20	9.43^{a}	8.02^{a}	5.09^{b}	3.98^{b}	4.74^{b}	4.48^{b}
	28	10.32^{a}	8.83^{ab}	7.93^{abc}	4.96^{c}	6.47^{bc}	5.66^{c}
	36	10.39^{a}	10.6^{a}	8.79^{ab}	5.76^{b}	6.59^{ab}	5.83^{b}
C. jejuni	0	3.77	3.77	3.77	3.77	3.77	3.77
# 101	6	6.33^{a}	5.85^{a}	4.77^{b}	4.49^{c}	4.51^{c}	4.71^{bc}
	12	8.25^{a}	8.07^{a}	7.32^{b}	6.09^{d}	6.35^{d}	6.86^{c}
	20	11.04^{a}	10.94^{a}	9.52^{b}	8.63^{b}	8.63^{b}	9.12^{b}
	28	10.95^{ab}	11.42^{a}	11.31^{ab}	9.98^{cd}	9.30^{d}	10.20^{bc}
	36	11.09^{ab}	11.20^{a}	10.70^{abc}	9.73^{c}	9.21^{c}	9.93^{bc}
C. coli	0	2.89	2.89	2.89	2.89	2.89	2.89
# 102	6	4.20^{a}	3.44^{b}	2.61^{bc}	1.72^{d}	1.94^{d}	2.33^{cd}
	12	8.30^{a}	6.40^{a}	3.53^{b}	1.63^{c}	2.32^{bc}	3.01^{b}
	20	9.97^{a}	8.56^{a}	5.66^{b}	2.59^{d}	3.80^{c}	4.39^{bc}
	28	9.92^{ab}	10.52^{a}	8.07^{abc}	3.38^{d}	6.26^{c}	6.46^{bc}
	36	10.71^{a}	10.78^{a}	8.54^{a}	5.36^{b}	7.73^{a}	6.86^{ab}

[a-e]: values followed by the same letter in a row are not significantly different ($p>0.05$).

HB&Gas: Hunt enrichment broth flushing with mixed gas.

HBO: Hunt enrichment broth with 0.6 units/ml of Oxyrase.

NFOH: Niroomand and Fung broth with 0.6 units/ml of Oxyrase and hemin.

NFO&No hemin: NFOH broth without hemin.

NFOY: NFO broth with yeast extract but without hemin.

NFH&No Oxy: Niroomand and Fung broth without Oxyrase.

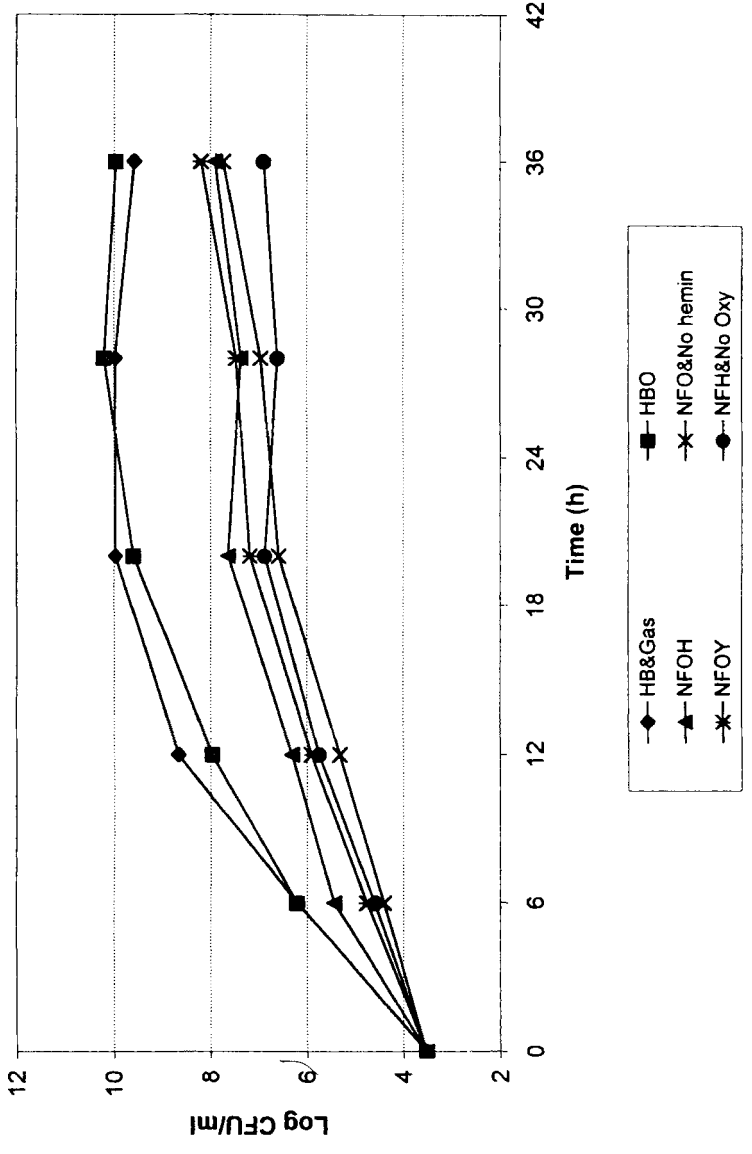

FIG. 1. GROWTH CURVE OF *C. JEJUNI* ATCC 33291

Suspensions of *Campylobacter* cells were inoculated into six enrichment media including HB with gassing but no Oxyrase® (HB&Gas), HB with Oxyrase® (HBO), NF with Oxyrase® and hemin (NFOH), NF with Oxyrase® but no hemin (NFO&No hemin), NF with Oxyrase® and yeast extract (NFOY), and NF with hemin and without Oxyrase® (NFH&No Oxy). After inoculation, HB&Gas was evacuated and flushed three times with a mixture of gas (5% O_2, 10% CO_2, and 85% N_2). *Campylobacter* counts were performed before (0 h) and at incubation times of 6, 12, 20, 28, and 36 h.

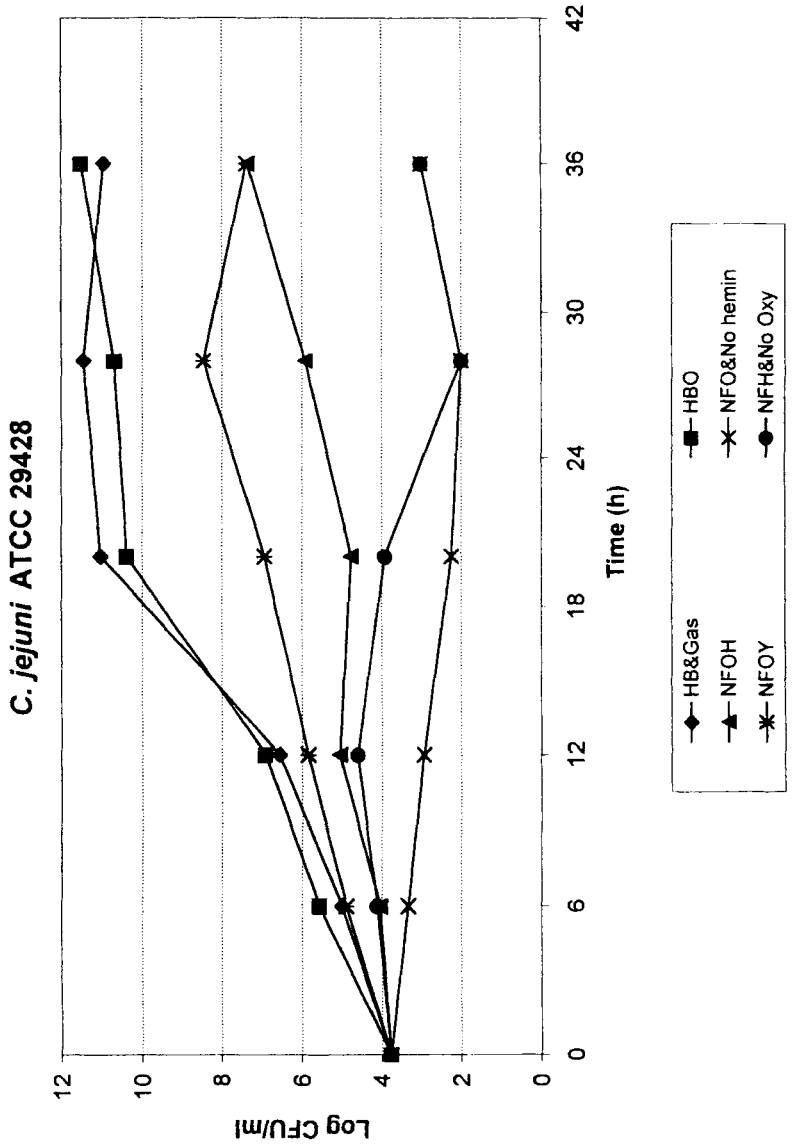

FIG. 2. GROWTH CURVE OF *C. JEJUNI* ATCC 29428

Suspensions of *Campylobacter* cells were inoculated into six enrichment media including HB with gassing but no Oxyrase® (HB&Gas), HB with Oxyrase® (HBO), NF with Oxyrase® and hemin (NFOH), NF with Oxyrase® but no hemin (NFO&No hemin), NF with Oxyrase® and yeast extract (NFOY), and NF with hemin and without Oxyrase® (NFH&No Oxy). After inoculation, HB&Gas was evacuated and flushed three times with a mixture of gas (5% O_2, 10% CO_2, and 85% N_2). *Campylobacter* counts were performed before (0 h) and at incubation times of 6, 12, 20, 28, and 36 h.

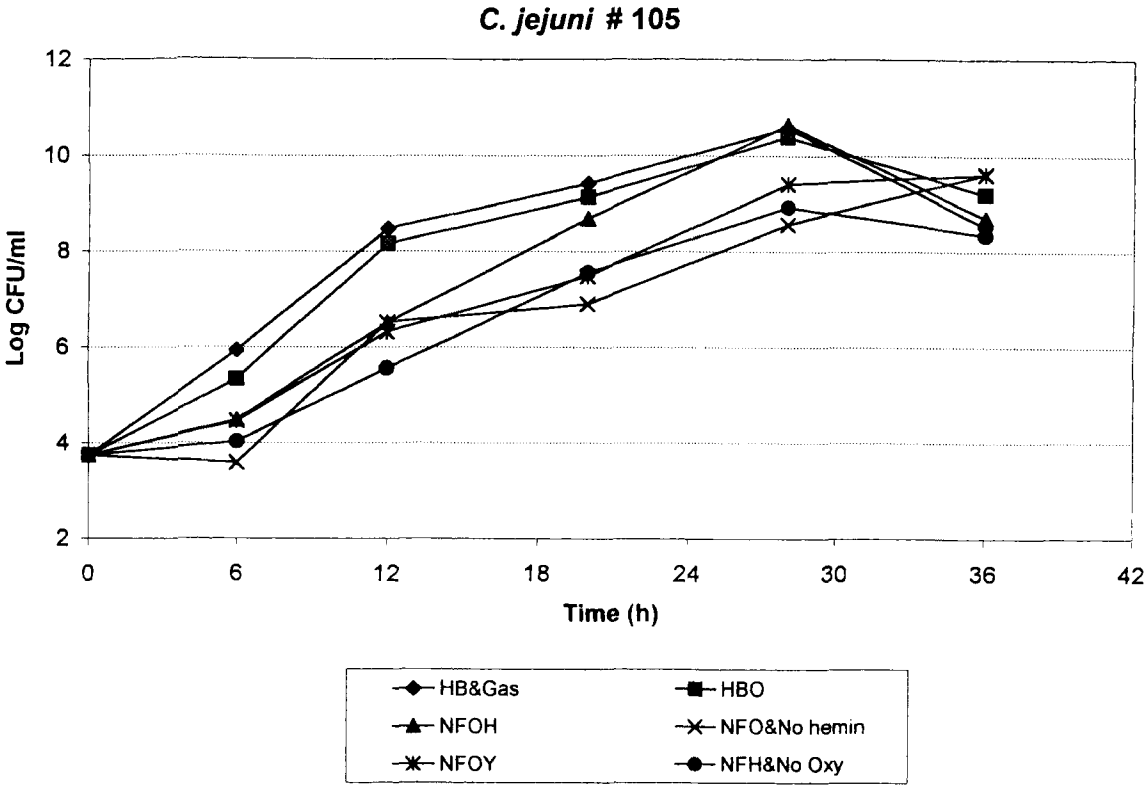

FIG. 3. GROWTH CURVE OF *C. JEJUNI* #105

Suspensions of *Campylobacter* cells were inoculated into six enrichment media including HB with gassing but no Oxyrase® (HB&Gas), HB with Oxyrase® (HBO), NF with Oxyrase® and hemin (NFOH), NF with Oxyrase® but no hemin (NFO&No hemin), NF with Oxyrase® and yeast extract (NFOY), and NF with hemin and without Oxyrase® (NFH&No Oxy). After inoculation, HB&Gas was evacuated and flushed three times with a mixture of gas (5% O_2, 10% CO_2, and 85% N_2). *Campylobacter* counts were performed before (0 h) and at incubation times of 6, 12, 20, 28, and 36 h.

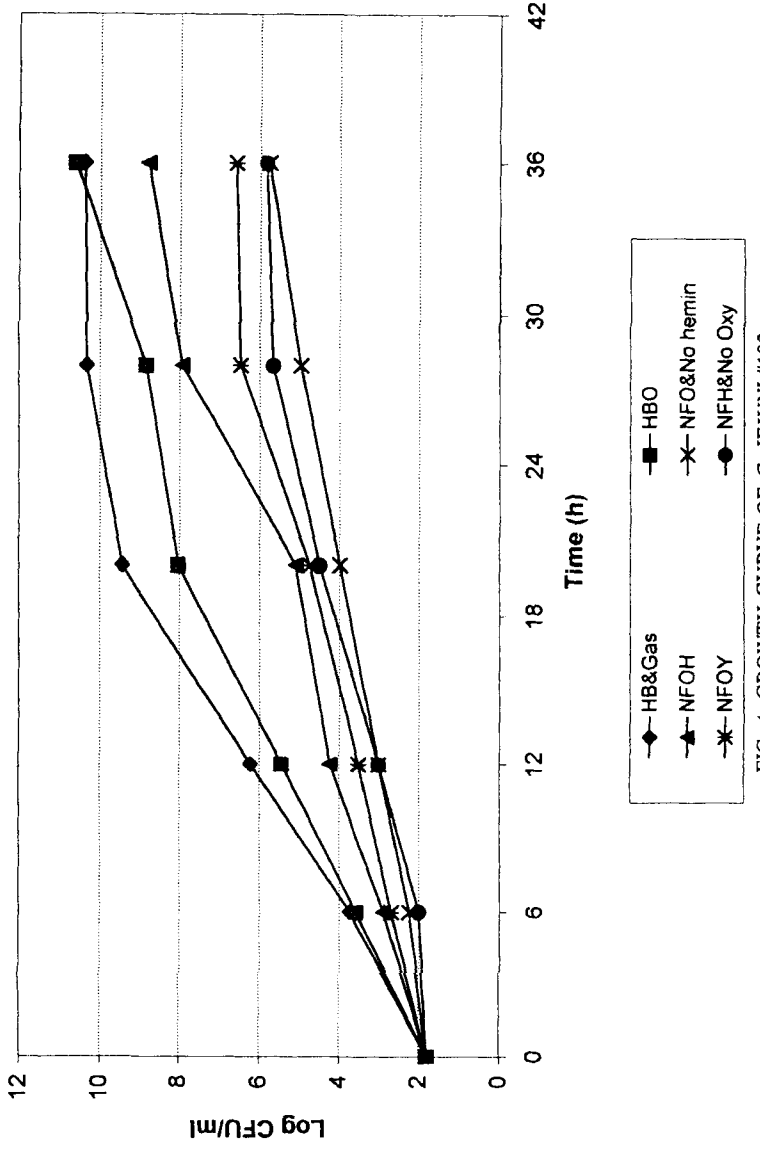

FIG. 4. GROWTH CURVE OF *C. JEJUNI* #103

Suspensions of Campylobacter cells were inoculated into six enrichment media including HB with gassing but no Oxyrase® (HB&Gas), HB with Oxyrase® (HBO), NF with Oxyrase® and hemin (NFOH), NF with Oxyrase® but no hemin (NFO&No hemin), NF with Oxyrase® and yeast extract (NFOY), and NF with hemin and without Oxyrase® (NFH&No Oxy). After inoculation, HB&Gas was evacuated and flushed three times with a mixture of gas (5% O_2, 10% CO_2, and 85% N_2). Campylobacter counts were performed before (0 h) and at incubation times of 6, 12, 20, 28, and 36 h.

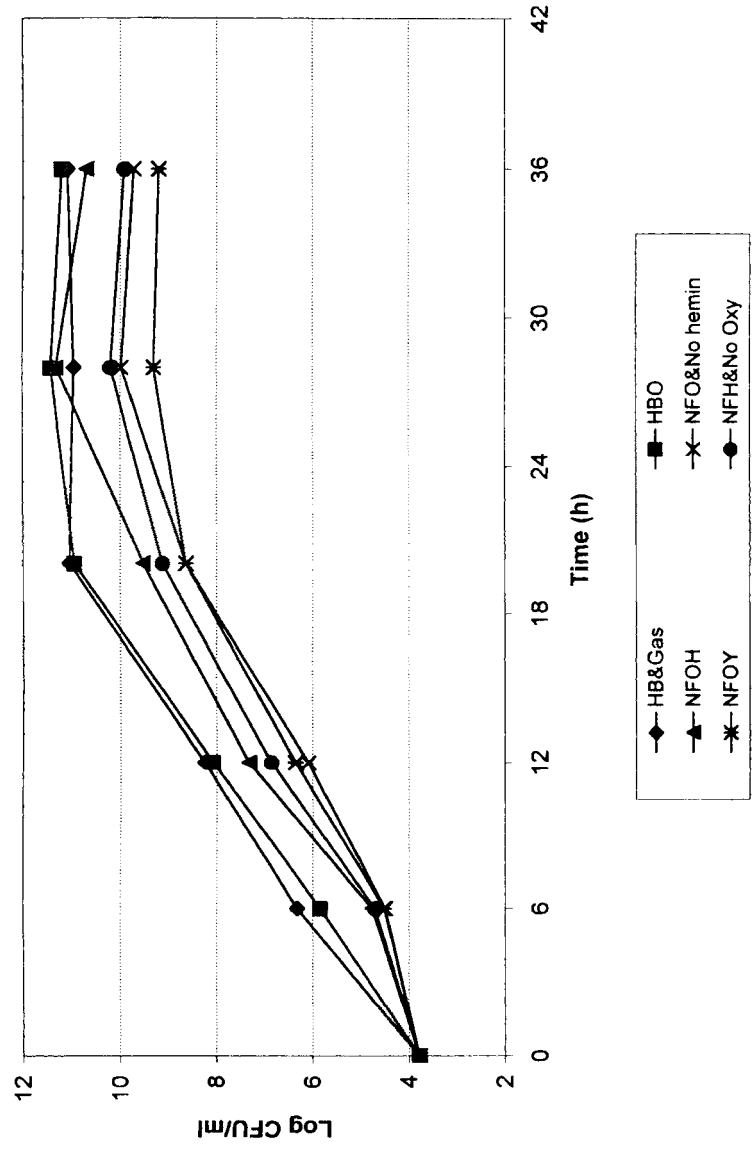

FIG. 5. GROWTH CURVE OF *C. JEJUNI* #101

Suspensions of *Campylobacter* cells were inoculated into six enrichment media including HB with gassing but no Oxyrase® (HB&Gas), HB with Oxyrase® (HBO), NF with Oxyrase® and hemin (NFOH), NF with Oxyrase® but no hemin (NFO&No hemin), NF with Oxyrase® and yeast extract (NFOY), and NF with hemin and without Oxyrase® (NFH&No Oxy). After inoculation, HB&Gas was evacuated and flushed three times with a mixture of gas (5% O_2, 10% CO_2, and 85% N_2). *Campylobacter* counts were performed before (0 h) and at incubation times of 6, 12, 20, 28, and 36 h.

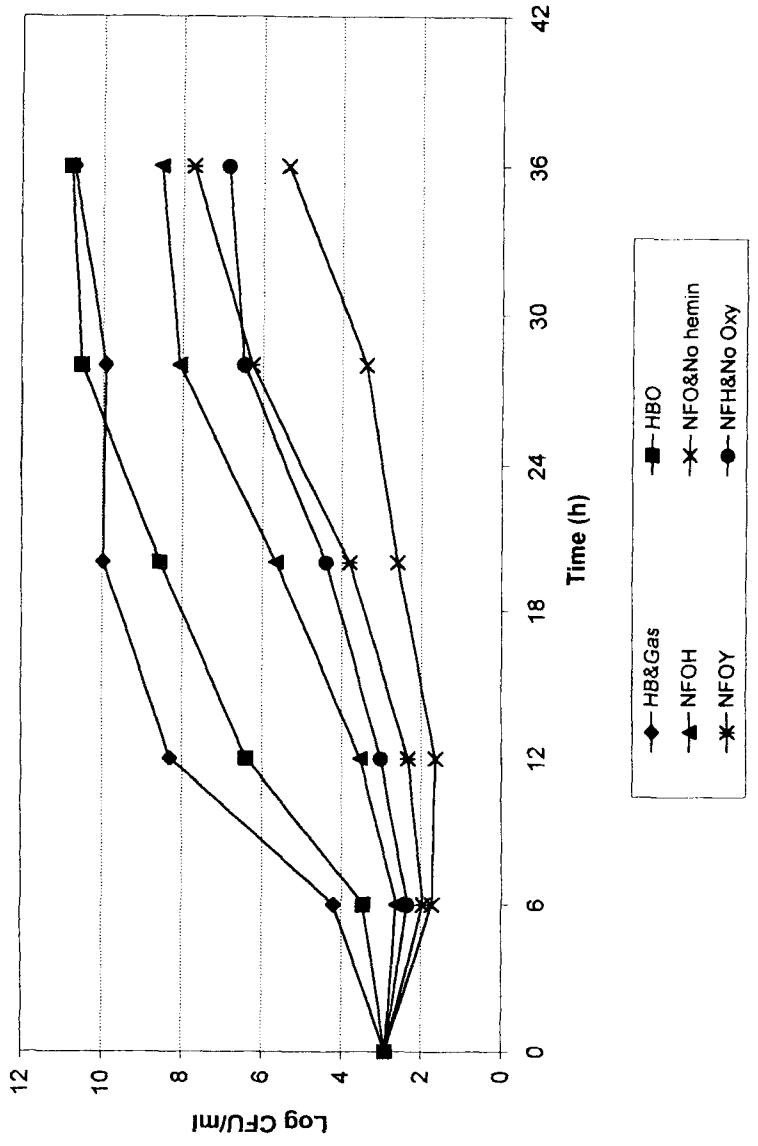

FIG. 6. GROWTH CURVE OF *C. COLI* #102

Suspensions of *Campylobacter* cells were inoculated into six enrichment media including HB with gassing but no Oxyrase® (HB&Gas), HB with Oxyrase® (HBO), NF with Oxyrase® and hemin (NFOH), NF with Oxyrase® but no hemin (NFO&No hemin), NF with Oxyrase® and yeast extract (NFOY), and NF with hemin and without Oxyrase® (NFH&No Oxy). After inoculation, HB&Gas was evacuated and flushed three times with a mixture of gas (5% O_2, 10% CO_2, and 85% N_2). *Campylobacter* counts were performed before (0 h) and at incubation times of 6, 12, 20, 28, and 36 h.

Several reports have indicated the stimulating effect of oxygen-reducing membrane fragments on facultative anaerobic bacteria. *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Streptococcus faecalis*, and *Listeria monocytogenes* grew more rapidly in brain heart infusion broth containing 0.1 units/mL of Oxyrase® than in the broth without Oxyrase® (Yu and Fung 1991). *Salmonella paratyphimurium*, *S. arizonae*, and *L. monocytogenes* had shorter lag phases and faster growth rates in the "Universal Preenrichment Medium" containing 0.3 units/mL of enzyme than in the medium without Oxyrase® (Niroomand and Fung 1992). Oxyrase® was noted to provide the most growth stimulation for *E. coli* O157:H7 during the first incubation period of 6 to 8 h (Phebus *et al.* 1993).

In the first study of the effect of Oxyrase® on *Campylobacter* growth using NF enrichment broth, Niroomand and Fung (1994) reported that the optimal concentration of Oxyrase® to provide the fastest growth and shortest generation time was at least 0.6 units/mL at 42C under both shaking and nonshaking conditions. The generation times of five strains of *C. jejuni* and one strain of *C. coli* ranged from 46.2 to 79.9 min in the presence of 0.6 units/mL of enzyme, whereas they ranged from 62.9 to 133 min in the absence of enzyme. Abeyta *et al.* (1995) showed that HB with a high concentration of Oxyrase® (0.6 units/mL) could recover low levels of campylobacters ($\leq$ 10 cells) and performed better than NFB.

The comparison among NFOH, NFOY, and NFO&No hemin showed that supplementation with 0.2% alkaline hematin solution or 0.6% yeast extract stimulated the growth of all strain tested. Growth was increased by about 1 to 2 log CFU/mL, with variation among strains and incubation times and some statistically significant differences ($p<0.05$). The addition of 7% lysed sheep blood into HBO enhanced the growth of *C. jejuni* and *C. coli* with significant differences ($p<0.05$) at 6 h and 24 h of incubation (Fig. 7.) The results are means of *Campylobacter* counts from five strains examined, excluding *C. jejuni* ATCC 29428. No significant difference was found between cultures on HBO with and without blood at 48 h of incubation.

Dissolved oxygen levels of each enrichment medium over incubation periods are presented in Fig. 8. The results are averaged from three strains: *C. jejuni* ATCC 29428, #105, and #101. The DO values of all cultures were lower than 1.44 mg/L or 22.6% air saturation at all incubation times. The DO levels of HB&Gas ranged from 0.2% to 11.1% air saturation or 0.01 to 0.71 mg/L and varied with incubation times. HBO contained DO levels ranging from 0.0% to 15.3% or 0.0 to 0.90 mg/L. The amount of DO in NFOH, NFO&No hemin, NFOY and NFH&No Oxy were higher than that of HB&G and HBO. Therefore, the low levels of DO in both HB&Gas and HBO might be attributed to the higher *Campylobacter* counts in these media. The DO levels of all media decreased as the numbers of *Campylobacter* cells increased. However, all the

DO values increased after first 6 h of incubation. An explanation is that at the beginning period, an oxygen gradient occurs between the head space gas and the liquid layer. Oxygen is transported from the gas phase through the liquid phase resulting in increasing of DO levels. At the same time, oxygen is being consumed by the catalytic reaction of Oxyrase® and/or campylobacters. Randers-Eichhorn *et al.* (1996) indicated that diffusivity of oxygen in water depends on temperature and viscosity, and diffusivity of oxygen in air is proportional to temperature and pressure. At 37C, diffusivity of oxygen in water is approximately 0.103 cm^2/h, and the diffusivity in air is about 720 cm^2/h. The concentration of oxygen in air is 8.23×10^{-3} mmol O_2/mL at 37C at 1 atm. The slightly inconsistent low levels of DO observed in these experiments might have been due to sensitivity of the electrode and liquid preparation before testing. The Clark electrode used in this study was likely to be inaccurate at low oxygen tensions, because its measurement depends on oxygen consumption by the electrode.

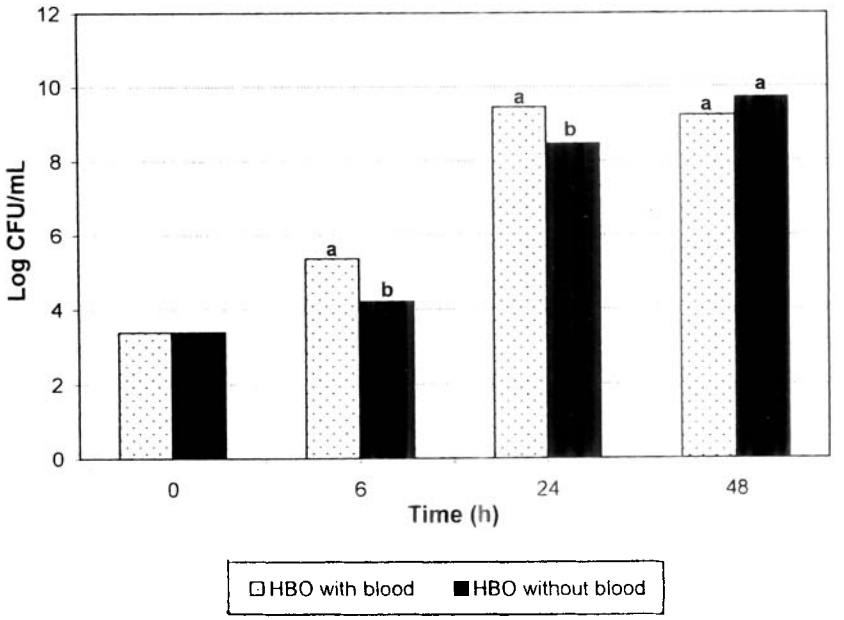

FIG. 7. EFFECT OF BLOOD ON GROWTH OF *CAMPYLOBACTER*

Campylobacter cells were inoculated into 75 mL of HB containing 0.6 units/mL of Oxyrase® and supplemented with 7% (v/v) lysed sheep blood or not supplemented. The 250 mL-Erlenmeyer flasks were incubated aerobically at 42C in a shaking water bath at 100 rpm. Samples were spread on duplicate brucella blood agar plates, which were incubated at 37C under microaerobic conditions. *Campylobacter* counts were determined before (0 h) and at 6, 24, and 48 h of incubation.

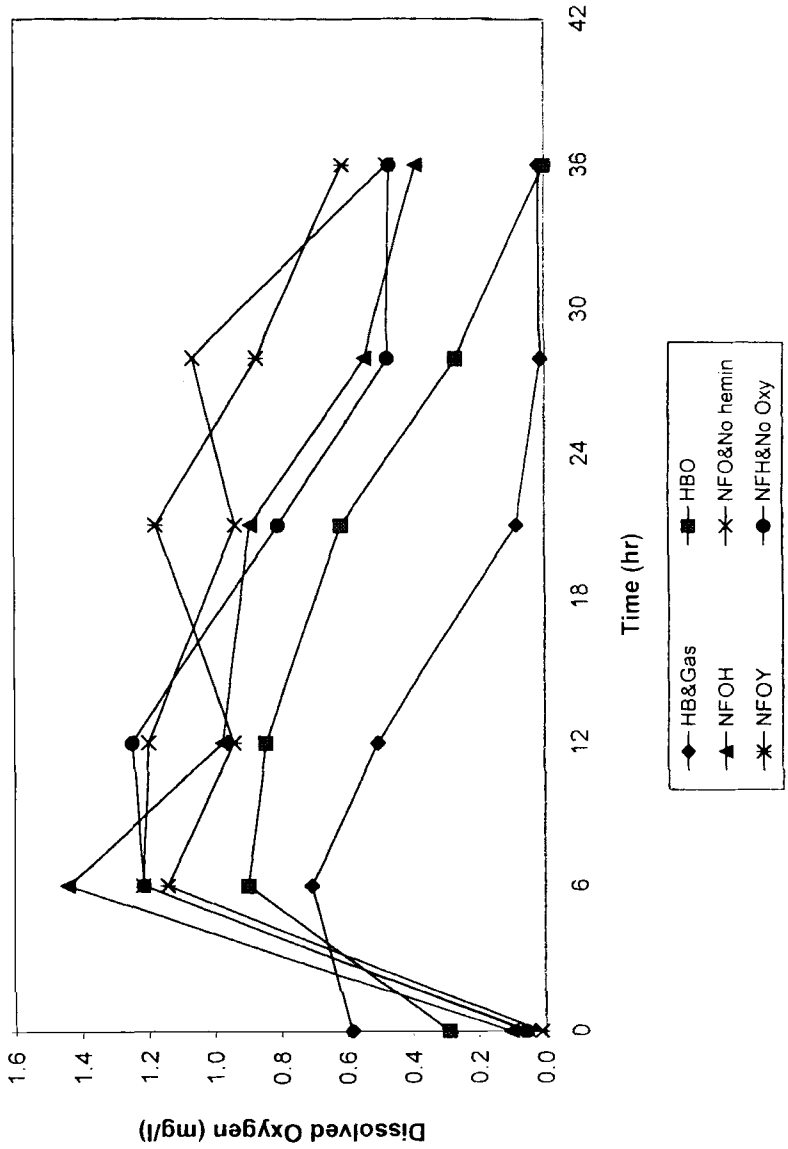

FIG. 8. DISSOLVED OXYGEN LEVELS

Two mL of culture fluid was withdraw from various enrichment media before (0 h) and at 6, 12, 20, 28, and 36 h of incubation. The probe of a Dissolved Oxygen Meter YSI 500 was placed immediately into the vial, and the dissolved oxygen values were recorded in %-air saturation and mg/L. Temperature compensation of the dissolved oxygen reading is determined automatically by the instrument.

Border *et al.* (1974) found that addition of hematin to brucella agar allowed growth of *C. fetus* and was a satisfactory substitute for defibrinated blood. The authors suggested that hematin might be useful as a precursor of heme compounds and possibly for the destruction of peroxide before an adequate amount of catalase is produced by campylobacters. Wesley *et al.* (1983) also showed the same growth stimulation for *C. jejuni* with incorporation of 0.2% hematin into liquid and solid media compared with the addition of blood. Bolton and Coats (1983) indicated that hematin and blood improved the aerotolerance of basal media and might act as quenching agents of photochemically generated toxic oxygen derivatives rather than as enrichment factors.

In conclusion, addition of 0.6 units/mL of Oxyrase® to Hunt enrichment medium was as efficient as use of the evacuation and gas replacement method for recovery of *C. jejuni* and *C. coli* in pure culture experiments. The performance of Hunt broth was significantly better than that of Niroomand and Fung broth. Supplementation of 7% lysed sheep blood, 0.2% hemin, or 0.6% yeast extract into both enrichment media stimulated *Campylobacter* growth. Using Oxyrase®-supplemented enrichment broth without the cumbersome gassing step provided a simple procedure for recovery of *Campylobacter* growth.

REFERENCES

ABEYTA JR., C., KAYSNER, C.A., HUNT, J.M. and WEKELL, M.M. 1995. A method for isolation of *Campylobacter* spp. (*jejuni, coli* and *lari*) from shellfish and the marine environment. J. Rapid Methods Automation Microbiology *4*, 51–64.

BLASER, M.J. 1997. Epidemiologic and clinical features of *Campylobacter jejuni* infections. J. Infect. Dis. *176*(Suppl 2), S103–105.

BOLTON, F.J., COATES, D., HINCHLFFE, P.M. and ROBERTSON, L. 1983. Comparison of selective media for isolation of *Campylobacter jejuni/coli*. J. Clin. Pathol. *36*, 78–83.

BORDER, M.M., FIREHAMMER, B.D. and MYERS, L.L. 1974. Tube culture method for viable counts of *Campylobacter fetus* (*Vibrio fetus*). Appl. Microbiol. *28*, 730–732.

BUZBY, J.C., ALLOS, B.M. and ROBERTS, T. 1997. The economic burden of *Campylobacter* associated Guillain-Barré syndrome. J. Infect. Dis. *176*(Suppl 2), S192–197.

CORRY, J.E.L., POST, D.E., COLIN, P. and LAISNEY, M.J. 1995. Culture media for the isolation of campylobacters. Intern. J. Food Microbiol. *26*, 43–76.

Food and Drug Administration. 1995. *Bacteriological Analytical Manual.* 8th Ed., pp. 7.01–7.27, Association of Official Analytical Chemists International, Gaithersburg, MD.

MEDEMA, G.J., TEUNIS, P.F.M., HAVELAAR, A.H. and HAAS, C.N. 1996. Assessment of the dose-response relationship of *Campylobacter jejuni*. Intern. J. Food Microbiol. *30*, 101–111.

NACHAMKIN, I., ALLOS, B.M. and HO, T. 1998. *Campylobacter* species and Guillain-Barré syndrome. Clin. Microbiol. Rev. *11*, 555–567.

NIROOMAND, F. and FUNG, D.Y.C. 1992. Effect of Oxyrase™ on growth of *Salmonella* spp. and *Listeria monocytogenes* in the universal preenrichment medium. J. Rapid Methods Automation Microbiology *1*, 241–247.

NIROOMAND, F. and FUNG, D.Y.C. 1994. Effect of oxygen reducing membrane fragments on growth of *Campylobacter* spp. J. Rapid Methods Automation Microbiology *2*, 247–277.

PHEBUS, R.K., THIPPAREDDI, H., KONE, K., FUNG, D.Y.C. and KASTNER, C.L. 1993. Use of Oxyrase™ enzyme in enrichments to enhance the recovery of *Escherichia coli* O157:H7 from culture media and ground beef. J. Rapid Methods Automation Microbiology *1*, 249–260.

RABEN, D. and SLAVIK, M. 1994. Evaluation of Oxyrase™ method for *Campylobacter* isolation from chicken carcasses. J. Rapid Methods Automation Microbiology *2*, 279–286.

RANDERS-EICHHORN, L., BARTLERR, R.A., FREY, D.D. and RAO, G. 1996. Noninvasive oxygen measurements and mass transfer considerations in tissue culture flasks. Biotechnol. Bioengineering *51*, 466–478.

TRAN, T.T. 1995. Evaluation of Oxyrase® enrichment method for isolation of *Campylobacter jejuni* from inoculated foods. Lett. Appl. Microbiol. *21*, 345–347.

TUITEMWONG, K., FUNG, D.Y.C. and TUITEMWONG, P. 1995. Rapid detection of *Listeria monocytogenes* using a reflectance colorimetric method with membrane fractions from oxidative bacteria. J. Rapid Methods Automation Microbiology *3*, 185–202.

WANG, H., FARBER, J.M., MALIK, N. and SANDERS, G. 1999. Improved PCR detection of *Campylobacter jejuni* from chicken rinses by a simple sample preparation procedure. Intern. J. Food Microbiol. *52*, 39–45.

WESLEY, I.V., SWAMINATHAN, B. and STADELMAN, W.J. 1983. Isolation and enumeration of *Campylobacter jejuni* from poultry products by a selective enrichment method. Appl. Environ. Microbiol. *46*, 1097–1102.

YU, L.S.L. and FUNG, D.Y.C. 1991. Effect of Oxyrase™ enzyme on *Listeria monocytogenes* and other facultative anaerobes. J. Food Safety *11*, 163–175.