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Growth of *Campylobacter* incubated aerobically in fumarate-pyruvate media or media supplemented with dairy, meat, or soy extracts and peptones☆

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ABSTRACT

The ability of *Campylobacter* to grow aerobically in media supplemented with fumarate-pyruvate or with dairy, meat, or soy extracts or peptones was examined. Optical densities (OD) of *Campylobacter* cultured in basal media, media supplemented with fumarate-pyruvate or with 1.0, 2.5, 5.0, or 7.5% beef extract was measured. Growth was also compared in media supplemented with other extracts or peptones. Finally, cfu/mL of *Campylobacter* recovered from basal media or media supplemented with fumarate-pyruvate, casamino acids, beef extract, soytone, or beef extract and soytone was determined. Results indicated that OD of cultures grown in media supplemented with fumarate-pyruvate or with 5.0 or 7.5% beef extract were higher than OD of isolates grown in basal media or media supplemented with lower concentrations of beef extract. Highest OD were produced by isolates grown in media supplemented with beef extract, peptone from meat, polypeptone, proteose peptone, or soytone. Also, more cfu/mL were recovered from media with fumarate-pyruvate, beef extract, soytone, or beef extract-soytone than from basal media or media with casamino acids. Findings indicate that media supplemented with organic acids, vitamins, and minerals and media supplemented with extracts or peptones containing these metabolites can support aerobic growth of *Campylobacter*.

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1. Introduction

Most methods for culturing *Campylobacter* require the production of artificial atmospheres containing increased concentrations of carbon dioxide (CO_2), decreased concentrations of oxygen (O_2), and the utilization of media supplemented with blood (Bolton and Coates, 1983a; Krieg and Hoffman, 1986; Macé et al., 2015). Since *Campylobacter* continues to be a major cause of foodborne, bacterial illnesses in humans (Park, 2002; Lin, 2009), there is still a significant need for research on this pathogen; however, costs of equipment required to establish microaerophilic atmospheres and dependence on inconsistent blood supplies may make studies on this pathogen difficult for some laboratories.

Research on media formulated to simplify methods for growing *Campylobacter* previously have been reported. Blood-free media supplemented with ferrous sulfate, sodium metabisulphite, sodium pyruvate, sodium carbonate, alpha-ketoglutaric acid, and activated charcoal (Bolton and Coates, 1983b; Tran, 1998; Verhoeff-Bakkenes et al., 2008)) have been described. Also, media supplemented with mixtures of organic acids associated with the Krebs Cycle, a mineral-vitamin mixture, and bicarbonate have been shown to support growth of *Campylobacter* without the requirement for generating microaerobic atmospheres (Hinton, 2013).

There are several bacteriological media supplements that contain amino acids, organic acids, vitamins, and minerals (Gray et al., 2008) that have been demonstrated to support the growth of *Campylobacter* without requiring the production of artificial atmospheres. The purpose of this study was to examine the growth of *Campylobacter* incubated under aerobic conditions in media supplemented with dairy, meat, and soy extracts or peptones.

☆ Mention of trade names or commercial products does not imply recommendation or endorsement by the U. S. Department of Agriculture.

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2. Materials and methods

2.1. Media preparation

Basal (BA) broth medium was prepared by dissolving 10.0 g of tryptose (Becton, Dickinson, and Co., Sparks, MD), 5.0 g of yeast extract (Becton, Dickinson, and Co.), and 1.5 g of Bacto agar (Becton, Dickinson, and Co.) in 700 ml of distilled water. Seven ml aliquots of the basal medium was dispensed in test tubes and sterilized by autoclaving at 121 C for 15 min. On the day of the experiments, basal medium was supplemented with 1 ml of a mineral-vitamin solution (Hinton, 2013); 1 ml of filter sterilized, 0.5% (w/v) sodium bicarbonate (NaHCO3) (Spectrum Chemical Manufacturing, Corp, Gardena, CA, PubChem CID: 516892) solution; and 1 ml of sterile distilled water to produce a final volume of 10 ml per tube. Fumarate-pyruvate (FP) medium was prepared by supplementing 7 ml of sterile basal medium with 1 ml of mineral-vitamin solution, 1 ml of 0.5% NaHCO3, and 1 ml of a solution containing 300 mM sodium fumarate (Sigma–Aldrich, St. Louis, MO, PubChem CID: 10197734) and 1000 mM sodium pyruvate (Sigma–Aldrich, PubChem CID: 23662274) to produce a final volume of 10 ml of FP media per test tube.

Beef extract (BE) media were prepared by supplementing basal media with various concentrations of beef extract. The components of basal medium and 10, 25, 50, or 75 g of beef extract (Remel® Products, Lenexa, KS) were dissolved in 900 ml of water. The media was dispensed in 9 ml aliquots in test tubes, then sterilized by autoclaving. On the day of the experiment, 1 ml of 0.5% NaHCO3 solution was added to each test tube of media to produce 10 ml volumes of BE media.

Basal media supplemented with 5.0% (w/v) dairy, meat, or soy extracts or peptones were prepared by dissolving components of the basal medium and 50 g of Bacto™ Casamino Acids (CA, Becton, Dickinson, and Co.); Remel® Beef Extract (BE); Lactalbumin Hysrolysate (LA, Sigma–Aldrich); Peptone from Meat, Peptic Digest (PD, Sigma–Aldrich); Peptone from Animal Tissue (PA, Sigma–Aldrich); Bacto™ Poly-peptone (PY, Becton, Dickinson, and Co.); Bacto™ Proteose Peptone (PP, Becton, Dickinson, and Co.); Bacto™ Proteose Peptone #2 (P2, Becton, Dickinson, and Co.); Bacto™ Proteose Peptone #3 (P3, Becton, Dickinson, and Co.); Bacto™ Soytone (SY, Becton, Dickinson, and Co.); N-Z Soytone (NZ, Sigma–Aldrich); or Bacto™ Tryptone (TR, Becton, Dickinson, and Co.) in 900 ml of water. Media were dispensed in test tubes in 9 ml aliquots and sterilized by autoclaving. On the day of the experiment 1 ml of filter sterilized 0.5% NaHCO3 solution was added to each test tube of media.

2.2. Campylobacter isolates and measurement of OD of Campylobacter cultures

Campylobacter coli ATCC® 33559, *Campylobacter fetus* ATCC® 27349, *Campylobacter jejuni* ATCC® 33560, *Campylobacter jejuni* ATCC® 49349, and *Campylobacter lari* ATCC® 35221 were obtained from the American Type Culture Collection, Manassas, VA. C. *jejuni* 3b was isolated from a commercially processed broiler chicken carcass and identified using the MIDI Sherlock Microbial ID System (MIDI Inc., Newark, DE), and *Campylobacter coli* gent^R was isolated from a processed poultry carcass then selected for gentamicin resistance (Cox et al., 2003). All other cultures were provided by Dr. Richard Meinersmann of the Bacterial Epidemiology and Antimicrobial Resistance Unit of the U. S. National Poultry Research Center, Agricultural Research Service, Athens, GA.

Fresh cultures of *Campylobacter* isolates were grown microaerophilically in Petri dishes on selective Oxoid Blood Agar Base 2 (Oxoid Limited, Wade Road, Basingstoke, Hampshire, UK) supplemented with 7% lysed horse blood (Lampshire Biological, Pipersville, PA) and *Campylobacter* Selective Supplement (Blaser-Wang) (Oxoid). Inoculated media was incubated in BBL GasPak Jars (Becton, Dickinson, and Co.) with activated CampyPak Plus Hydrogen + CO_2 Disposable Gas Generator Envelopes with Integral Palladium Catalyst (Becton, Dickinson, and Co.) for 48 h at 37 °C. Cultures were harvested from incubated plates by adding approximately 10 ml of 0.1% Bacto™ Peptone (Becton, Dickinson, and Co.) solution to the surface of the agar then using sterile spreaders to remove bacterial growth from the agar surface. The harvested bacterial suspensions contained approximately 10^8 cfu/ml.

Studies comparing of growth of *Campylobacter* cultures in during incubation in FP media and media supplemented with various concentrations of beef extract were conducted using the Bioscreen C Microbiology Reader (Growth Curves USA, Piscataway, NJ) operated by Growth Curves Software, v. 2.28 (Transgalactic Ltd., Helsinki, Finland). Freshly harvested *Campylobacter* cultures were serially diluted in 0.1% Bacto™ Peptone solutions to produce suspensions containing approximately 10^6 cfu/mL, 0.1 ml of the suspensions were added to 10 ml of the broth media, and 0.2 ml of the inoculated media was pipetted into wells of a Honeycomb 2 cuvette plate (Labsystems, Inc., Franklin, MA). The filled plates were placed in the incubator tray of the Bioscreen microbiology reader which had been programmed to incubate the media at 37 °C for 48 h and to measure culture absorbance (optical density, OD) 600 nm following shaking at medium speed for 10 s.

The Bioscreen was also used to compare the growth of *Campylobacter* isolates in media supplemented with dairy, meat, or soy extracts or peptones. Ten ml of CA, BE, LA, PD, PA, PY, PP, P2, P3, SY, NZ, or TR media were inoculated with 10^4 *Campylobacter*/ml, and 0.2 ml of the inoculated media was added to the wells of Honeycomb plates. The plates were placed into the Bioscreen and cultures OD were measured as described above.

2.3. Enumeration of Campylobacter after incubation in media in cell culture flasks

Cfu/mL of *Campylobacter* recovered from media incubated at 37 °C for 24 and 48 h was also determined. Ten ml of BA, FP, 5.0% CA, 5.0% BE, 5.0% SY, or 2.5% BE-2.5% SY media were transferred to sterile, 12.5 cm^2 tissue culture flasks with plugged caps (Becton, Dickinson, and Co.). Media in the flasks were inoculated with 0.1 ml suspensions of *Campylobacter* to produce a final concentration of approximately 10^4 cfu/mL. Inoculated media were incubated at 37 °C, and cfu/mL of *Campylobacter* in the media was determined after 24 and 48 h by plating serial dilutions of the media on Oxoid selective *Campylobacter* agar medium described above. Inoculated plates were incubated microaerophilically in a GasPak Jar with catalyst at 37 °C for 48 h. Cfu on the inoculated plates were counted, and numbers were converted to log cfu/mL. Random colonies were selected from the plates, and identity of the isolates was confirmed as *Campylobacter* by testing for agglutination using the Latex-CAMPY(jcl)™ *Campylobacter* Culture Confirmation Test, Latex-CAMPY (jcl) ™ (Integrated Diagnostics, Inc. Baltimore, MD).

2.4. Statistical analysis

GraphPad InStat, Version 3.0 for Windows (GraphPad Software, San Diego, CA) was used to perform statistical analyses of data on culture OD and cfu/mL. One-Way Analysis of Variance (ANOVA) of group means of absorbances of each culture and log cfu/mL was performed to determine significant differences. When ANOVA detected significant variations in group means, the Tukey–Kramer Multiple Comparison test was used to determine which means differed significantly. All significant differences were determined at