Growth and Laboratory Maintenance of *Campylobacter jejuni*

Lindsay Davis[1] and Victor DiRita[1, 2]

[1]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan
[2]Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, Michigan

ABSTRACT

Campylobacter jejuni is a fastidious organism, growing in microaerophilic conditions with a temperature range between 37° and 42°C. Multiple types of media can be used to cultivate it; however, Mueller Hinton broth and agar support the best *C. jejuni* growth. Optimum atmosphere for *C. jejuni* is 85% N_2, 10% CO_2, and 5% O_2. *Curr. Protoc. Microbiol.* 10:8A.1.1-8A.1.7. © 2008 by John Wiley & Sons, Inc.

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INTRODUCTION

Campylobacter jejuni is one of the major causes of bacterial gastroenteritis worldwide and is primarily acquired through the ingestion of contaminated poultry products. Research on *C. jejuni* has been greatly impaired due to poor culturing techniques and genetic tools. *C. jejuni* was successfully isolated in the early 1970s, utilizing specialty agar and microaerophilic atmospheric conditions. Since its isolation, it has been connected with bacterial gastroenteritis as well as the neurological disorders Guillen-Barré Syndrome and Fisher Syndrome in humans. It is now one of the leading causes of gastroenteritis in both the developed and developing worlds. (Young et al., 2007).

C. jejuni is a fastidious organism, requiring modified atmospheric conditions, a longer growing time, a narrow temperature range, and specialized media when compared to such bacteria as *E. coli*. All these factors must be taken into consideration when working with *C. jejuni.*

STRATEGIC PLANNING

Atmosphere

Campylobacter jejuni requires microaerophilic conditions for growth. Optimum growth is maintained in a tri-gas incubator (e.g., Thermo Forma Series II Water-Jacketed CO_2 Incubator), with 85% N_2, 10% CO_2, and 5% O_2. If a tri-gas incubator is unavailable, specific gas packs (i.e., BBL Campy Pak Plus) or formulated compressed air can be applied with sealable gas chambers or plastic bags, respectively. Gas packs are expensive, and growth in plastic bags is suboptimal compared to that obtained by using a tri-gas incubator. Notes concerning the differences are in the specific protocols outlined. *C. jejuni* is also able to grow in anaerobic conditions.

Temperature

C. jejuni has a narrow temperature range of growth, with optimum growth occurring between 37° and 42°C. The bacteria are able to survive at 7°C and perform vital cellular processes such as protein synthesis; however, growth and recovery at low temperatures

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is difficult (Hazeleger et al., 1998). Significant loss of viable bacteria occurs when the bacteria are left at room temperature and atmosphere for only 10 min. Therefore, when working with *C. jejuni*, the amount of time the bacteria are out of incubators and microaerophilic atmosphere must be limited.

Media

Many types of media have been used to culture *C. jejuni*. Mueller-Hinton medium, blood agar, Columbia blood agar, and BBL medium are commonly used. However, Mueller Hinton (MH) has the highest recovery rate and is recommended in this unit (Ng et al., 1985). *Campylobacter* defined medium was developed by Leach and colleagues (Leach, 1997). Although a minimal medium has not been developed that supports *C. jejuni* growth, the defined medium can be used to adjust components based on experimental needs.

Growth Conditions

C. jejuni can grow in both static and shaking broth cultures. The stringent growth conditions do not lend themselves well to a shaking apparatus. Some tri-gas incubators are equipped to have an internal shaker; however, most are not, and the gas packs in bags or jars do not always allow shaking conditions. *C. jejuni* grown in shaking cultures grow faster than those in static culture. Aggregates form when *C. jejuni* is grown in static broth (Joshua et al., 2006). This aggregation phenotype has been studied extensively, and several genes involved in this have been identified. Based on availability, the protocols suggested in this unit use static growth as the standard.

Strain Selection

Several isolates of *C. jejuni* have been used in previous research (Table 8A.1.1). Strain selection is important, and depends upon the proposed research. Strains have been isolated from chicken, human, and environmental sources. Phenotypic variation among strains from varying sources has been observed for many traits including invasion, cytolethal distending toxin (cdt) production, chick colonization, lipooligosaccharide (LOS)

Table 8A.1.1 Most Commonly Used *C. jejuni* Strains[a]

	Sources	Sequences	Notes
260.94	Human	N	GBS[b]-associated; O:41 serotype
480	Human	N	Electrocompetent; highly invasive
811681116	Human	N	Lab strain
11168	Human	Y	Gaynor (2004)
81-176	Human	Y	Contains pVir, highly virulent (Bacon, 2000)
BTI	Chicken	N	—
CG8245	Human	N	—
F38011	Human	N	—
M1	Environment	N	—
RM1221	Human	Y	—

[a]The most commonly used isolates of *C. jejuni* are listed here, including their source of isolation and whether their genome has been sequenced to date. Strains can be acquired from individual labs working on the particular isolate.
[b]GBS, Guillain-Barré Syndrome.

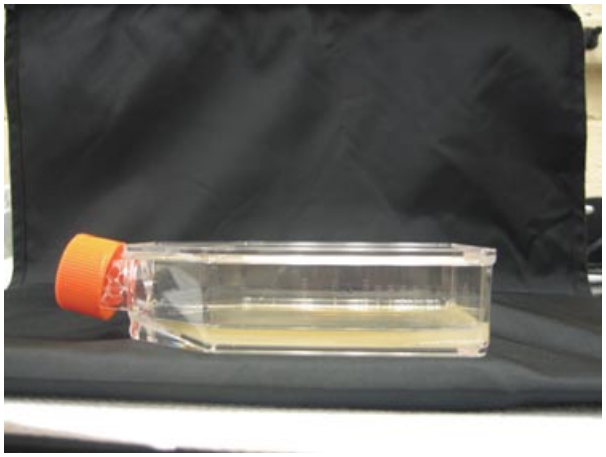

Figure 8A.1.1 Preparation of biphasic medium. In a 75-cm^2 tissue culture flask, 20 ml of MH agar were poured and solidified, followed by 20 ml of MH broth.

production, and natural transformation. The ability of *C. jejuni* to undergo natural transformation has led to a high degree of horizontal gene transfer between *C. jejuni* strains, leading to high genetic diversity among *C. jejuni* strains. *C. jejuni* strains 81-176 and 11168 are well characterized strains, and the most commonly used in pathogenesis studies.

Growth Curves

Mueller Hinton agar is the recommended medium for standard growth curves of *C. jejuni*. Biphasic MH medium, prepared in tissue culture flasks (Fig. 8A.1.1), is the classic way to perform growth curves. The biphasic medium is made using 50% MH agar and 50% MH (poured after the agar solidifies). Note that MH agar should never be microwaved for *C. jejuni* agar plates and biphasic media.

Biphasic medium is recommended for growing conditions outside of a tri-gas incubator, such as in bags or sealed containers. If a tri-gas incubator is used, growth curves can be performed in MH broth alone. However, no volume below 1 ml should be used, due to poor recovery and inconsistent growth.

Each time point should be an individual sample. Static growth of *C. jejuni* results in aggregative bacterial raft formation, and disruption of this growth has led to variable and inconsistent growth. Therefore, each time-point should be separate and undisturbed. Furthermore, each time-point should be done in triplicate to obtain a statistically significant average. Growth curves are normally performed for 36 or 48 hr.

The inoculum for a growth curve is 1×10^6 cfu/ml. From 16- to 18-hr plates, *C. jejuni* should be resuspended to an OD_{600} of 0.4. Dilute this suspension 1:10. Inoculate 80 µl of this dilution into 20 ml of medium. A standard growth curve, measured in cfu/ml over 48 hr, is shown in Figure 8A.1.2.

CAUTION: Campylobacter jejuni is a Biosafety Level 2 (BSL-2) pathogen. Follow appropriate guidelines and regulations for the handling of pathogenic microorganisms. Proper hand washing is essential, as *C. jejuni* has been shown to cause gastroenteritis at a small dose (10 to 100 organisms). See *UNIT 1A.1* and other pertinent resources for more information.

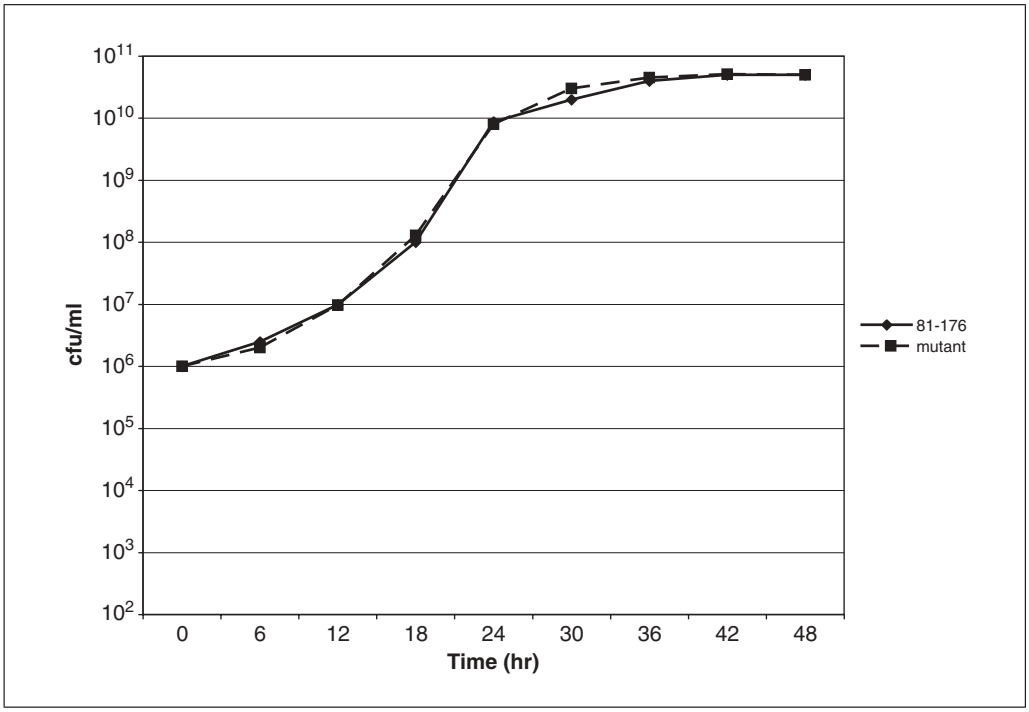

Figure 8A.1.2 Growth curve in biphasic medium of two different isolates of *C. jejuni* 81-176.

BASIC PROTOCOL 1

GROWTH OF *C. JEJUNI* FROM A FROZEN STOCK

C. jejuni is ill equipped for adjusting to changing environments in the lab, especially after being taken out of the freezer. The protocol below supports the best usable *C. jejuni* growth from frozen stocks. Time for growth depends on atmospheric conditions. The protocol outlined below uses Mueller Hinton (MH) broth or MH agar containing 10 μg/ml trimethoprim (TMP) for growing strain 81-176 in a tri-gas incubator. Selective antibiotics can be added to the medium; concentrations commonly used for culturing of and cloning in *C. jejuni* are listed in Table 8A.1.2.

Materials

C. jejuni frozen stock (Basic Protocol 2)
100 × 15–mm Mueller Hinton (MH) agar plates (BD Biosciences, cat. no. 22520; plates are poured in lab) containing 10 μg/ml trimethoprim (antibiotics added in lab)
Mueller Hinton (MH) broth (BD Biosciences, cat. no. 275730) containing 10 μg/ml trimethoprim
Equipment for maintaining *Campylobacter*-specific microaerophilic atmosphere (see Strategic Planning)

Additional reagents and equipment for streaking bacteria (*APPENDIX 4A*)

1. From frozen stock (in MH broth plus 20% glycerol; see Basic Protocol 2), heavily streak out *C. jejuni* strain on 100 × 15–mm Mueller Hinton agar plates containing 10 μg/ml trimethoprim. Grow for 16 to 20 hr in microaerophilic conditions (see Strategic Planning).

   Growth in a tri-gas incubator allows more rapid growth, while bag plating may require up to 48 hr of growth. See Strategic Planning for additional discussion of atmospheric conditions required for growing C. jejuni.

   Streaking of bacteria on agar plates is described in APPENDIX 4A.

Table 8A.1.2 Concentrations of Antibiotics Used in *C. jejuni* Research

Antibiotic	Concentration
Kanamycin	50 μg/ml
Chloramphenicol	15-20 μg/ml
Streptomycin	100 μg/ml to 2 mg/ml
Trimethoprim	10 μg/ml
Cefoperazone	20 μg/ml
Nalidixic acid	30 μg/ml
Tetracycline	12.5 μg/ml

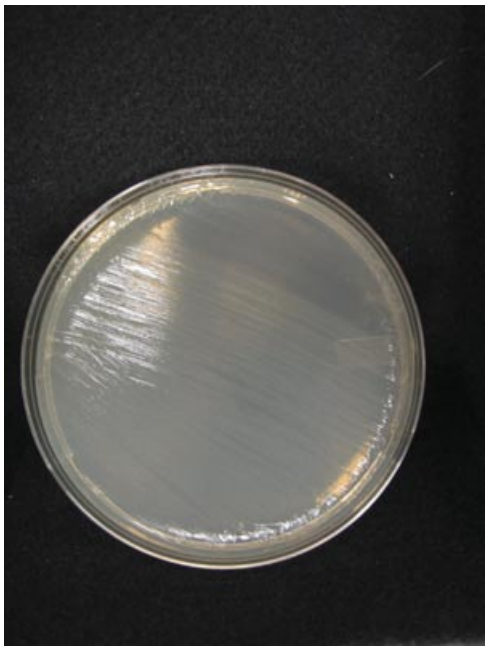

Figure 8A.1.3 Growth of *C. jejuni* 81-176 on an Mueller Hinton agar plate containing 10 μg/ml trimethoprim after 24 hr in a tri-gas incubator.

2. Restreak *C. jejuni* onto a new Mueller Hinton plate containing 10 μg/ml trimethoprim. Grow for 16 to 20 hr in microaerophilic conditions.

   Single colonies will not be observed until culture has been incubated >24 hr. Scrape up a large amount of cells from the lawn (see Figure 8A.1.3).

   After this incubation, C. jejuni is ready to be used.

   For single colonies, streak for isolated colonies on plates and grow for 48 hr or until individual colonies are observed.

3. *Optional:* If large amounts are needed, restreak a heavy inoculum onto several Mueller Hinton plates containing 10 μg/ml trimethoprim or inoculate into Mueller Hinton broth containing 10 μg/ml trimethoprim.

4. *Optional:* Passage *C. jejuni* an additional two to three times.

   Further passages from frozen stock are not recommended.

PRESERVATION OF *C. JEJUNI*

Stocks of *C. jejuni* should be kept in 20% glycerol stocks, stored at −80°C. Incubations can be performed in a tri-gas incubator or in the other recommended conditions at 37°C.

Materials

C. jejuni organisms (cannot be purchased from ATCC or similar sources; must be obtained from individual labs)
100 × 15–mm Mueller Hinton (MH) agar plates (BD Biosciences, cat. no. 22520; plates are poured in lab) containing 10 μg/ml trimethoprim (antibiotics added in lab)
Mueller Hinton (MH) broth (BD Biosciences, cat. no. 275730) containing 20% (v/v) glycerol

Sterile cotton swabs
2-ml cryotubes

1. Heavily streak out *C. jejuni* strain on an MH agar plate containing 10 μg/ml trimethoprim. Grow for 18 to 20 hr.

   After making a mutant (see UNIT 8A.2), streak for single colonies, which takes 36 to 48 hr. Pick a single colony and streak out. Finally, restreak it heavily onto a MH agar plate and carry out the following steps to preserve.

2. Swab bacteria using a sterile cotton swab from plate.

   C. jejuni will appear a pink-peach color (Fig. 8A.1.3).

3. Transfer a sufficient quantity of bacteria to 1.5 ml of Mueller Hinton broth containing 20% (v/v) glycerol in a 2-ml cryotube. Immediately freeze at –80°C.

   The frozen stock should contain a large amount of bacteria; roughly an OD_{600} of 1.5 or more

4. Never thaw frozen stocks. Instead, scrape off an ice chip from the frozen stock using a sterile implement and plate directly onto MH agar plates containing 10 μg/ml trimethoprim.

COMMENTARY

Background Information

Campylobacter jejuni is the causative agent of campylobacteriosis, a self-limiting gastroenteritis. It is one of the major causes of bacterial-associated gastroenteritis in the United States. *C. jejuni* has also been linked to Guillain-Barré Syndrome (GBS), an acute autoimmune neuropathy resulting in flaccid paralysis. Research on *C. jejuni* has been greatly impaired due to poor culturing techniques and genetic tools. *C. jejuni* was successfully isolated in the early 1970s, utilizing specialty agar and microaerophilic atmospheric conditions. Originally thought to be primarily a pathogen of animals, *C. jejuni* is now considered one of the main causes of foodborne bacterial gastroenteritis in humans. The original culturing techniques have expanded to include a number of types of media that *C. jejuni* can use. Although *C. jejuni* is a fastidious organism, its manipulation has become more standardized in recent years. A narrow temperature range, specific media, and specific atmospheric conditions must all be taken into account when growing *C. jejuni.*

Critical Parameters and Troubleshooting

If no growth is seen from the initial streaking of the frozen stock, or from subsequent re-streaks, the inoculum size or selective antibiotics may be the problem. Try to inoculate the plates with a larger amount of bacteria. A usual amount of frozen stock to be struck out is a chunk of frozen sample the size of a grain of rice. Due to the quantity of passages and relatively short bench life of *C. jejuni*, it is recommended that two sets of frozen stocks be kept with highly used strains: a backup stock and a frequently used stock. This will help diminish the possibility of contamination.

Anticipated Results

A large inoculum of frozen stock of *C. jejuni* 81-176 should grow after 18 to 24 hr. The growth will appear as a hazy lawn. The bacteria will have a pinkish-cream color when scraping the cells from the plate. After re-streaking from the overnight plate, growth of *C. jejuni* 81-176 should be observed after 18 to 20 hr. The growth will be hazy. To isolate single colonies, streak for isolation and allow growth for 48 hr.

Time Considerations

Following the protocol, large amounts of viable *C. jejuni* will be available after 36 to 48 hr in a tri-gas incubator. If gas jars are used, growth will be slower, with usable growth appearing after 40 to 48 hr, depending on the amount being streaked.

Freezing a newly constructed *C. jejuni* strain may take 4 to 5 days. Streak from a single colony. Growth will be visible after 48 hr. Heavily restreak the growth onto a new MH agar plate and incubate for another 24 to 36 hr.

Literature Cited

Bacon, D. 2000. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. *Infect. Immun.* 68:4384-4390.

Gaynor, E. 2004. The genome-sequenced variant of *Campylobacter jejuni* NCTC11168 and the original clonal clinical isolate differ markedly in colonization, gene expression, and virulence-associated phenotypes. *J. Bacteriol.* 186: 503-517.

Hazeleger, W.C. 1998. Physiological activity of *Campylobacter jejuni* far below the minimal growth temperature. *Appl. Environ. Microbiol.* 64:3917-3922.

Joshua, G.W., Guthrie-Irons, C., Karlyshev, A.V., and Wren, B.W. 2006. Biofilm formation in *Campylobacter jejuni*. *Microbiology* 152:387-396.

Leach, S. 1997. Changes with growth rate in the membrane lipid composition of and amino acid utilization by continuous cultures of *Campylobacter jejuni*. *J. Appl. Microbiol.* 82: 631-640.

Ng, L.-K. 1985. Comparison of basal media for culturing *Campylobacter jejuni* and *Campylobacter coli*. *J. Clin. Microbiol.* 21:226-230.

Young, K., Davis, L.M., and DiRita, V.J. 2007. *Campylobacter jejuni*: Molecular biology and pathogenesis. *Nat. Rev. Microbiol.* 5:665-679.