INSTITUT PASTEUR

Research in Microbiology 162 (2011) 173–179

www.elsevier.com/locate/resmic

MCLMAN, a new minimal medium for *Campylobacter jejuni* NCTC 11168

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Received 29 March 2010; accepted 19 September 2010
Available online 7 December 2010

Abstract

The design of a defined synthetic minimal medium for *Campylobacter jejuni* strain NCTC 11168 that includes only inorganic salts and necessary amino acids and vitamins is useful in physiological assays and responses to exogeneous agents. In silico genomic analysis of biosynthesis pathways was preliminarily performed prior to experimental assays to determine (i) amino acids and vitamins necessary for improving the growth of *C. jejuni* strains, and (ii) the most appropriate sources of carbon, nitrogen and sulfur. The different sources of carbon, nitrogen and sulfur were analyzed by comparing growth parameters. A new minimal medium that contains inorganic salts, the amino acids L-cysteine, L-leucine, L-methionine and L-aspartic acid (nitrogen source), the vitamin niacinamide and lactate as a carbon source, named MCLMAN (medium cysteine leucine methionine aspartic acid niacinamide), was checked on some *C. jejuni* strains and showed similar growth ratios and final biomass when compared to the most frequently used medium, MEM (modified Eagle's medium), primarily designed for eukaryote cell culture and more complex than MCLMAN. Our results show that *C. jejuni* presents auxotrophy for cysteine and methionine and can be inhibited by ammonium sulfate. A simple minimal medium containing few amino acids and vitamins will facilitate physiological studies of different functions in *C. jejuni* strains submitted to different stresses.

Keywords: *Campylobacter*; Minimal medium; Cysteine biosynthesis

1. Introduction

Campylobacter jejuni is the most common cause of food-borne bacterial enteritis in humans in developed countries (Oberhelman and Taylor, 2000). The symptoms of campylobacteriosis are severe abdominal pain and acute diarrhea. Post-infectious complications such as Guillain-Barré syndrome (GBS) occur in rare cases (Moore et al., 2005). Members of the genus *Campylobacter* are readily isolated from animal reservoirs, including farm animals and wild birds (Frost, 2001). The microaerophilic nature of this chemo-organotrophic bacterium that uses organic and amino acids as carbon/energy source could explain the relative fragility of this species and the need for complex media for recovering isolates from clinical and environmental sources (Corry et al., 1995).

Although the genomic sequences of several strains are available (Fouts et al., 2005; Gundogdu et al., 2007; Parker et al., 2006; Parkhill et al., 2000; Pearson et al., 2007), the mechanisms involved in *C. jejuni* virulence and environmental stress resistance are not fully understood or identified (Garenaux et al., 2008), possibly because of the reliance on complex substrates present in current media for culturing *C. jejuni*. The development of a simple defined minimal medium with well known concentrations of substrates (iron, magnesium, sulfate, phosphate, amino acids and vitamins) that supports the growth of *C. jejuni* is primordial to the design of reproducible assays related to biochemical, physiological, genetic and expression analyses for a better knowledge of adaptation, stress resistance and virulence mechanisms of this bacterium.

The metabolism and assimilation of different sources of energy, carbon and nitrogen depends on the availability of

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doi:10.1016/j.resmic.2010.09.024

these different sources. Westfall et al. (1986) observed significantly different kinetics of substrate utilization between standard nutritive media, i.e. brain heart infusion broth with 1% yeast extract, and minimal essential medium (MEM). L-serine is often added to the culture medium for *C. jejuni* so as to improve its growth because of its preferential use in either complex or defined media (Leach et al., 1997). MEM supplemented with 20 mM sodium pyruvate was used to cultivate *C. jejuni* strains in a study on iron acquisition (Naikare et al., 2006). In another study on iron-scavenging mechanisms, Miller et al. (2008) also used MEM to cultivate *C. jejuni* strains, along with a basal defined medium named SAPI containing only glucose and ammonium nitrate for assays. Velayudhan and Kelly (2002) also used MEM supplemented with different carbon sources to study mutants in gluconeogenic and anaplerotic enzymes. They showed that supplementation of MEM with sodium pyruvate enhanced growth of reference strain NCTC 11168.

In order to determine the required amino acids and vitamins for *C. jejuni* strain NCTC 11168, an in silico analysis of its genome was done in order to understand the true requirements of this strain. Then, various defined media based on the composition of MEM were assayed for studying the different sources of nitrogen, sulfur and carbon necessary to improve the growth of this microorganism and to discard the unnecessary elements.

2. Materials and methods

2.1. Bacterial strains

C. jejuni strains NCTC 11168 (Parkhill et al., 2000), BKR314, AKR6, Cj480, AKR672, E6-B1, BKR266, E12-K3, MJL640, BKR589, BKR290, BKR522, BKR646, BKR293, BKR146, BKR543, BKR396, AKR546, BKR645, A728, A922, A928 (Rivoal et al., 2005), 81-176 (Korlath et al., 1985), F38011 (Ziprin et al., 1999) and Bf (Cappelier et al., 2000) were stored at −80 °C in *Brucella* broth (Difco), supplemented with 20% glycerol (v/v).

2.2. Media and culture conditions

After streaking on Karmali agar plates (Oxoid), bacterial cells were transferred to *Brucella* broth and incubated under microaerobic conditions (10% O_2 [v/v], 5% CO_2 [v/v], and 85% N_2 [v/v]) using Gaspak (Campy*Gen*™ Oxoid) for 8 h with shaking (150 rpm). A second culture at an initial OD_{600nm} of 0.00125 was inoculated and incubated overnight at 37 °C under the same conditions. Then, cells were collected, washed and resuspended in 1 ml physiological water (0.9% NaCl). Cultures of *C. jejuni* NCTC 11168 were inoculated at an initial OD_{600nm} of 0.05 either in the minimal medium (MEMα; Sigma–Aldrich, Table 1), supplemented by 10 mM sodium pyruvate, or in the tested minimal media defined in different parts of the study. Incubation of the cultures was performed microaerobically at 37 °C for 24 h with shaking (150 rpm). For assays on sulfur requirements, O_2-limited atmosphere (1% O_2 [v/v]) obtained with Anaero*Gen*™ (Oxoid) was used.

2.3. Chemicals

All chemicals (inorganic salts, amino acids and vitamins) used in this study were purchased from Sigma. L-amino acids are symbolized by the 3-letter codes.

2.4. Determination of growth rate and relative fitness index

The OD (600 nm) of the cultures was monitored with a GENESYS 20 spectrophotometer (Thermo Spectronic). The

Table 1
Composition of MEM and MCLMAN minimal media.

	MEM[a]	MCLMAN		MEM[a]	MCLMAN
Inorganic salts			*Amino acids*		
$CaCl_2.2H_2O$	1.8 mM	1.8 mM	L-Arginine·HCl	0.4 mM	–
$Fe(NO_3)_3.9H_2O$	0.25 μM	0.25 μM	L-Cystine·2HCl	0.3 mM	–
$MgSO_4$	1.75 mM	1.75 mM	L-Cysteine HCl	–	0.2 mM
KCl	5.4 mM	5.4 mM	L-Lysine·HCl	0.8 mM	–
$NaHCO_3$	44 mM	44 mM	L-Methionine	0.2 mM	0.2 mM
NaCl	0.1 M	0.1 M	L-Serine	0.4 mM	–
NaH_2PO_4	0.9 mM	0.9 mM	L-Glycine	0.4 mM	–
Vitamins			L-Histidine·HCl	0.2 mM	–
Choline-Cl	29 μM	–	L-Isoleucine	0.8 mM	–
Folic acid	9 μM	–	L-Leucine	0.8 mM	0.8 mM
Myo-inositol	40 μM	–	L-phenylalanine	0.7 mM	–
Niacinamide	33 μM	33 μM	L-Threonine	0.8 mM	–
Pyridoxine·HCl	20 μM	–	L-Tryptophan	0.08 mM	–
Riboflavin	1 μM	–	L-Tyrosine	0.6 mM	–
Thiamine·HCl	13 μM	–	L-Valine	0.8 mM	–
D-pantothenic ac	18 μM	–	L-Glutamine	4 mM	–
Other component			L-Aspartic acid	–	10 mM
Na-Lactate	–	10 mM			

[a] The molarities of the different elements were calculated from information provided by Sigma–Aldrich (http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/Formulation/d5796for.Par.0001.File.tmp/d5796for.pdf).