Original Article

In Vitro Protein Expression Profile of *Campylobacter jejuni* Strain NCTC11168 by Two-dimensional Gel Electrophoresis and Mass Spectrometry*

ZHANG Mao Jun#, GU Yi Xin, DI Xiao, ZHAO Fei, YOU Yuan Hai, MENG Fan Liang, and ZHANG Jian Zhong

Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China

Abstract

Objective To investigate the protein expression profiles of the major food-borne pathogen *Campylobacter jejuni* NCTC11168.

Methods Membrane and soluble cellular proteins were extracted from the genome-sequenced *C. jejuni* strain NCTC11168. Protein expression profiles were determined using two-dimensional gel electrophoresis (2-DE). All the detected spots on the 2-DE map were subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF) analysis.

Results A total of 537 and 333 spots were detected from the whole cell and membrane-associated proteins of *C. jejuni* NCTC11168 cultured on Columbia agar medium at 42 ℃ by 2-DE and Coomassie Brilliant Blue staining, respectively. Analyses of whole cell and membrane-associated proteins included 399 and 133 spots, respectively, which included 182 and 53 functional proteins identified by MALDI-TOF/TOF analysis.

Conclusion The comprehensive expression protein profiles of *C. jeuni* NCTC11168 obtained in this study will be useful for elucidating the roles of these proteins in further pathogenesis investigation.

Key words: *Campylobacter jejuni*; Two-dimensional gel electrophoresis; MALDI-TOF; Soluble cellular protein; Membrane protein

Biomed Environ Sci, 2013; 26(1):48-53 *doi: 10.3967/0895-3988.2013.01.006* *ISSN:0895-3988*

www.besjournal.com(full text) *CN: 11-2816/Q*

INTRODUCTION

Campylobacter jejuni causes human gastroenteritis worldwide and is a common cause of traveler's diarrhea. In addition, *C. jejuni* infection has been associated with the development of Guillain-Barre syndrome, the leading cause of acute flaccid paralysis worldwide[1-3]. Using the genome sequence of *C. jejuni*, an extensive proteome expression database of the pathogen has been useful for the investigation of microbial physiology and pathogenesis. Currently, proteomics analysis is a key analytical method for investigating bacterial proteomes[4-5]. Techniques such as two-dimensional gel electrophoresis (2-DE) and two-dimensional difference gel electrophoresis (2-DE DIGE) are widely used to determine bacterial gene regulation and the stress response under different conditions. However, most studies on *C. jejuni* have a focus on the regulation of specific proteins[6-7]. In the present study, 2-DE and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF) protein analyses were used to determine a comprehensive protein expression profile and protein identification for the genome-sequenced *C. jejuni* strain NCTC11168. The data obtained in this study will be helpful for the further investigation of *C. jejuni*.

*This work was supported by The General Program of National Natural Science Foundation of China (81071314).

#Correspondence should be addressed to ZHANG Mao Jun. Tel: 86-10-58900755; E-mail: zhangmaojun@icdc.cn

Biographical note of the first author: ZHANG Mao Jun, female, born in 1969, Ph.D., majoring in molecular microbiology.

Received: November 21, 2011; Accepted: October 23, 2012

MATERIALS AND METHODS

Bacterial Culture and Sample Preparation

C. jejuni NCTC11168 was grown on *Campylobacter* Columbia agar (Oxoid, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (10% CO_2, 5% O_2 and 85% N_2) at 42 °C for 24 h. Whole cell proteins were extracted using a standard protocol modified in our laboratory[8-9]. Briefly, bacterial cells were harvested from the agar plates into normal saline [0.9% (w/v) NaCl] and washed three times in saline by centrifugation at 1500 g at 4 °C for 10 min. The bacterial pellet was suspended in lysate buffer [6 mol/L urea, 2 mol/L thiourea, 4.0% (w/v) CHAPS, 40 mmol/L DL-dithiothreitol (DTT) and 0.5% (v/v) immobilized pH gradient (IPG) pH 3-10 buffer] and sonicated on ice three times for 30 s at 30 s intervals until the suspension became clear. Cell debris was collected by centrifugation at 134 000 ×g at 4 °C for 15 min.

Outer membrane proteins were extracted as previously described with some modifications[10-11]. Briefly, bacterial pellets were re-suspended in 50 mmol/L Tris-buffer (pH 7.5) and sonicated on ice several times for 30 s at 30 s intervals. Lysates were centrifuged at 5000 ×g for 10 min to remove particulate matter, then the supernatants were collected and mixed with 5 mL of 0.1 mol/L Na_2CO_3 (pH 11) at 4 °C for 1 h with shaking. The mixed supernatant was further centrifuged at 100 000 ×g for 1 h and the pellets washed with 50 mmol/L Tris-buffer. Supernatants were then centrifuged at 100 000 ×g for 1 h and re-suspended in lysate buffer [6 mol/L urea, 2 mol/L thiourea, 4.0% (w/v) 3-3-Cholamidopropyl dimethylammonio propanes ulfonic acid (CHAPS), 40 mmol/L dithiothreitol (DTT) and 0.5% (v/v) immobilized pH gradient (IPG) buffer, pH 3-10]. Both the membrane and cellular protein supernatants were cleaned using the 2-D clean-up kit (Amersham Biosciences, Piscataway, NJ, USA) and proteins were quantified using the 2-D quant kit (GE Healthcare Bio-Science Corp. Piscataway NJ08855, USA) according to the manufacturer's instructions. Proteins were then subjected to 2-DE. Sample preparations and 2-DE were repeated at least three times using the same standard protocols.

Two-Dimensional Polyacrylamide Gel Electrophoresis

One-dimensional isoelectric focusing (IEF) and two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were performed according to the manufacturer's instructions (Amersham Biosciences). Equal amounts of protein (450 μg in 450 μL rehydration buffer for membrane protein and 800 μg in 450 μL rehydration buffer for cellular proteins) were loaded onto dry 24 cm IPG strips (pH 3-10 NL; Amersham Biosciences). IEF was carried out for 90 000 Vh over a 24 h period. Thereafter, the strips were equilibrated twice in equilibration buffer [50 mmol/L Tris-HCl, pH 6.8, 6 mol/L urea, 30% glycerol, 2% SDS, with 0.25% DTT for the first time, and 4.8% indole-3-acetic acid (IAA) for the second time]. Strips were then subjected to SDS-PAGE using 12.5% gels at 2.5 watts per gel for 30 min followed by 18 watts per gel until the bromophenol blue tracking dye reached the bottom of the gel.

Detection of Protein Spots on 2-DE Gels

Gels subjected to 2-DE were stained with Coomassie Brilliant Blue (CBB) G-250 (BioRad, Hercules, CA, USA). Individual gels were imaged using an ImageScanner (Amersham Biosciences) with an 8-bit grayscale 400 dots-per-inch (dpi) transparency adapter. Spot detection, quantification (% volume) and pattern matching were completed using the ImageMaster 2D Platinum software (Amersham Biosciences).

Identification of Proteins by MALDI-TOF-TOF Analysis

The entire visualized spots on 2-DE gels of *C. jejuni* cellular and membrane protein fractions were excised from the gel. In-gel digestion was performed as previously described[10]. Protein identification was carried out using tandem matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF/TOF) mass spectrometry (4700 MALDI-TOF/TOF mass spectrometer; Applied Biosystems, Foster City, CA, USA). Briefly, the protein samples were mixed (1:1) with a saturated matrix solution (α-cyano-4-hydroxycinnamic acid prepared in 50% acetonitrile/0.1% trifluoroacetic acid) on a sample plate. The mass spectra were obtained by using the 4000 Series Explorer software (version 3.0) in the positive-ion reflector mode with a mass accuracy of approximately 20 ppm. The MALDI tandem mass spectrometer was equipped with a 200-Hz frequency-tripled Nd:YAG laser operating at a wavelength of 355 nm. The MS spectra were acquired in the mass range between 800-4000 Da using 1500 laser shots. MS/MS spectra were acquired using 2000 laser shots with air as the