BIOMEDICAL AND ENVIRONMENTAL SCIENCES **18**, 341-344 (2005)

Comparative Proteomic Analysis of *B. henselae* Houston and *B. henselae* Marseille by Two-dimensional Gel Electrophoresis

SU-QING ZHAO[*,#], YAN-FEI CAI[◊], AND ZHEN-YU ZHU[□,1]

[*]*Unite des Rickettsies, Faculte de Medecine, Universite de la Mediterranee, 13385 Marseille Cedex 05, France;* [#]*Faculty of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou 510090, Guangdong, China;* [◊]*College of Resource and Environment, South China Agriculture University, Guangzhou 510642, Guangdong, China;* [□]*Department of Biochemistry, Zhongshan Medical College, Sun Yat-Sen University, Guangzhou 510080, Guangdong, China*

Objective To compare the protein difference between *B. henselae* Houston and *B. henselae* Marseille by two-dimensional gel electrophoresis. **Method** Protein samples were prepared by vorterx, ultrasonic treatment, and centrifugation. Protein concentrations were determined by Bradford method. Protein difference was compared by the first IEF and the second SDS-polyacrylamide gel electrophoresis. **Results** Protein concentrations in samples of *Bartonella henselae* Houston and *Bartonella henselae* Marseille were 2.117 μg/μL and 2.200 μg/μL respectively. Sample protein of 40 μg for IPG strips loading was perfect. The results of 2-DE in pH 4 to 7 IPG strips showed that the total protein spots of *Bartonella henselae* Houston and *Bartonella henselae* Marseille were 375 and 379 respectively, 95% of the spots were the same between the two strains of *Bartonella henselae*. **Conclusion** The procedure of 2-DE may prove successful for the proteomic analysis of *Bartonella henselae*. *Bartonella henselae* Houston and *Bartonella henselae* Marseille are different genotypes.

Key words: Protein; *B. henselae*; Two-dimensional gel electrophoresis

INTRODUCTION

The species name of *B. henselae* was first proposed in 1992[1]. It is a gram-negative, oxidase-negative, fastidious, aerobic, rod-shaped and slow-growing bacterium. Improvements in the techniques used to isolate *B. henselae* and new methods for the identification and detection of the organism have enabled us to further determine clinical manifestations of infections including cat scratch disease (CSD)[2-5], BA[6], peliosis hepatitis[6], septicemia[7], endocarditis[8-9], and neurological disorders[10].

In 1996, Drancourt *et al*. [11] reported a new serotype of *B. henselae* named Marseille, which is also a new genotype. The authors found that two isolates of *B. henselae*, from a patient with endocarditis and a patient with CSD, are genetically different from all previously isolated strains by sequence analysis of the 16S rRNA-encoding gene. Investigators in the Netherlands[12] also demonstrated two restriction fragment length polymorphism (RFLP) patterns of *B. henselae* DNA in samples from CSD patients, which are shown by analysis of the 16S-23S rRNA gene spacer PCR fragments and 16S rRNA gene PCR products digested with *Alu*I. The presence of two genotypes is later confirmed in France and Germany[13-16] based on sequencing of the 16S rRNA-encoding gene. Based on 16S rRNA gene differences, genotypes I and II are proposed. *Bartonella henselae* Houston is genotype Ⅰ, and *Bartonella henselae* Marseille is genotype Ⅱ. The reports of previous studies are concentrated on culturing, gene sequence analysis by PCR method, serological diagnosis and prevalence of *Bartonella henselae*. Few studies about protein difference between the two genotypes are reported. So we studied protein difference between *B. henselae* Houston and *B. henselae* Marseille with the methods of 2-dimensional gel electrophoresis. The results of these studies are described herein.

[1]Correspondence should be addressed to Zhen-Yu ZHU. Email: zhuqun@gzsums.edu.cn

Biographical note of the first author: Su-Qing ZHAO, male, born in 1969, Ph. D., associate professor, majoring in biochemistry. E-mail: suqingz@hotmail.com

0895-3988/2005
CN 11-2816/Q

MATERIALS AND METHODS

Chemicals

Chemicals were purchased from Merck-Eurolabo (Leuven, Belgium), except for CHAPS, sodium dodecyl sulfate, potassium ferricyanide, urea, and trifluoroacetic acid (TFA), which were from Sigma-Aldrich (Steinheim, Germany). Dithiothreitol (DTT) was from Serva (Heidelberg, Germany), Tris were from ICN (Aurora, OH, USA), and sodium thiosulfate and ammonium hydrogen carbonate were from UCB (Brussels, Belgium).

Bacterial Cultures

B. henselae Houston and *B. henselae* Marseille were obtained from Unite des Rickettsies of Universite of Mediterrance, Faculte de Medecine, Marseille, France. The two *Bartonella* strains were grown on Columbia sheep blood agar (BioMerieux, Marcy l'Etoile, France) at 37℃ in a 5% carbon dioxide incubator, harvested after 8 days of culture, suspended in deionized water and stored at -70℃.

Sample Protein Preparation

One mL of the above lyophilized bacteria of *Bartonella henselae* Houston and *Bartonella henselae* Marseille was dissolved in 3 mL solubilization mixture containing 7 mol/L urea, 2mol/L thiourea, 4% CHAPS (W/V), vortexed on ice with ice-cold reagents for 30 min, ultrosonic treatment lasted for 1 min by output power 600 W, then 100 000 ×g centrifugation for 30 min at 4℃. The pellets were discarded and the supernatant was collected for protein determination.

Total Protein Determination

Protein was quantified by the Bradford method using the BioRad protein assay (BioRad, Richmond, CA, USA) with bovine serum albumin as a standard. All samples and bovine serum albumin standards contained 10 μL of a 10:1 (vol/vol) mixture of sample buffers 1 and 2.

Isoelectrofocusing

Eighteen-centimeter immobilized pH gradient (IPG) strips (pH 3 to 10, 4 to 7) were rehydrated overnight at room temperature with 360 μL of sample containing protein. Isoelectric focusing (IEF) was conducted at 20℃ for 7 h (1 V, 1 min; 500 V, 30 min; 3500 V, 6.5 h).

SDS-polyacrylamide Gel Electrophoresis

After IEF, each IPG strip was washed for 15 min in 10 mL of equilibration buffer 1 (6 mol/L urea, 133 mmol/L DTT, 30% glycerol, 50 mmol/L Tris-acetate, pH 7.0) and then for 15 min in 10 mL of equilibration buffer 2 (6 mol/L urea, 2.5% iodoacetamide, 30% glycerol, 50 mmol/L Tris-acetate, pH 7.0). The IPG strips were loaded onto 10% precast Duracryl gels (22 cm by 23 cm by 1 mm; Tris/Tricine/SDS chemistry). Electrophoresis was carried out for 5 to 6 h (500 V; 14 000 to 20 000 mW/gel) at 15℃. All subsequent operations were carried out in an automated staining apparatus by silver staining method. At last, the gels were scanned with ImageScanner Ⅱ and saved in the computer. Total protein spots were analyzed by the software of ImageMaster Labscan v 3.00.

RESULTS

Total Protein Concentration Determination

The protein concentration of samples was assayed using Bradford method, and the standard BSA assay curve is shown in Fig.1. The results showed that the protein concentration of samples of *Bartonella henselae* Houston and *Bartonella henselae* Marseille was 2.117 μg/μL and 2.200 μg/μL respectively.

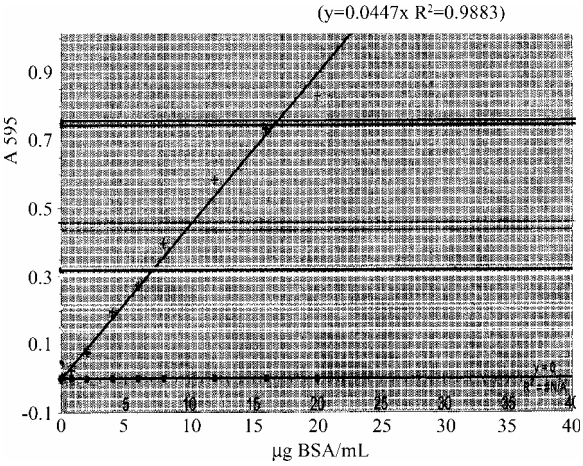

FIG.1. Standard BSA assay curve.

Comparison of 2-DE in Different Protein Load Condition

Eighteen-centimeter immobilized pH gradient (IPG) strips (pH 3 to 10) were rehydrated overnight at room temperature with 350 μL of sample containing total protein of 40 μg, 80 μg, 120 μg respectively, and then isoelectric focusing (IEF), sodium dodecyl sulfate poly acrylamined gel

electrophoresis (SDS-PAGE), and silver staining were carried out. The results showed that the 120 μg sample protein for IPG loading (Figs. 2A, 2B) was too much, and the spots on gels were not separated well and overlapped, and that the background was dark. Sample protein of 80 μg for IPG loading (Figs. 2C, 2D) was better than that of 120 μg for IPG loading, but the background was still dark and the spots were overlapped. The spots on gels were clear and separated well if the IPG strips were loaded with sample protein of 40 μg (Figs. 2E, 2F). Consequently, the sample protein of 40 μg for IPG strip loading was perfect in the following experiments.

Comparison of 2-DE in Protein Spot

The results of 2-DE in pH 4 to 7 IPG strips are shown in Figs. 3A, 3B. The total protein spots of *Bartonella henselae* Houston and *Bartonella henselae* Marseille were 375 and 379 respectively. Five protein spots of *Bartonella henselae* Marseille could not be found on the gel of *Bartonella henselae* Houston. Seven protein spots of *Bartonella henselae* Houston could neither be found on the gel of *Bartonella henselae* Marseille. Furthermore, the spot difference was found to be about at 60 000 Da. No other spot difference could be found on the gel. 95% spots were the same between the two strains of *Bartonella henselae*.

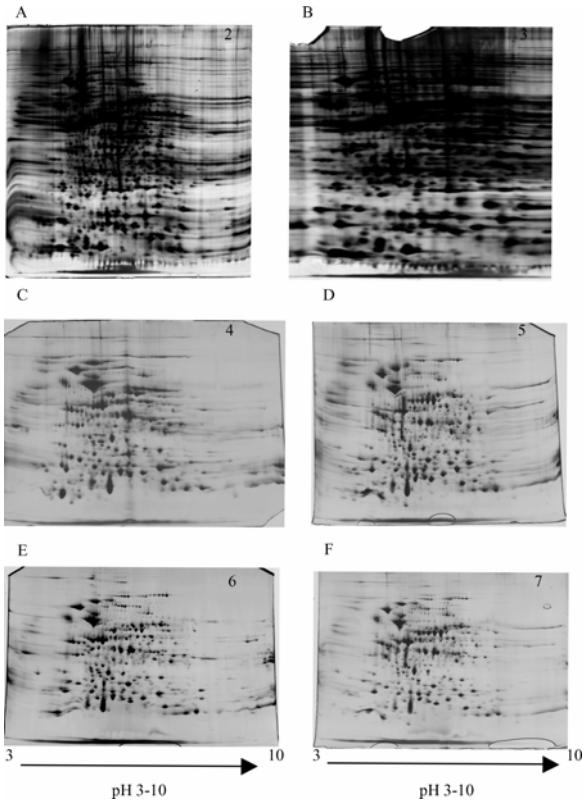

FIG. 2. Different protein loading tests. (A, B: 120 μg protein loading; C, D: 80 μg protein loading; E, F: 40 μg protein loading).

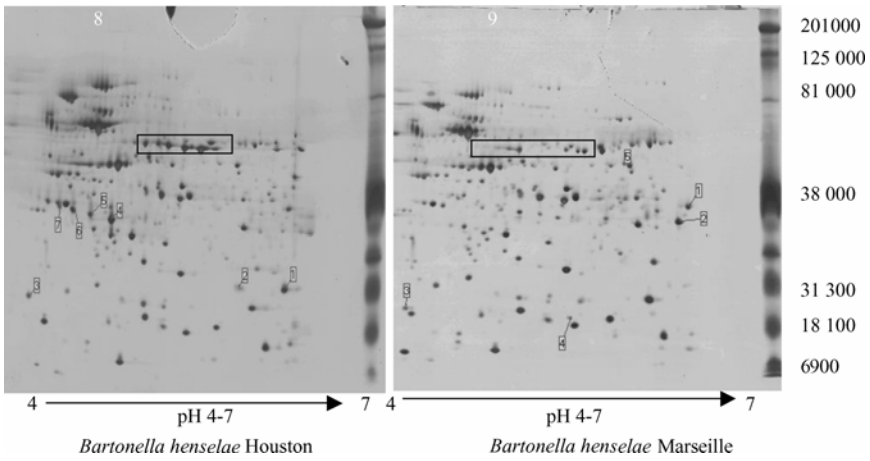

FIG. 3. Protein spots of *Bartonella henselae* Houston (A) and *Bartonella henselae* Marseille (B).

DISCUSSION

With the current interest in proteomics, two dimensional gel electrophoresis (2-DE) has emerged as a powerful technique for the separation and characterization of proteins and elucidation of protein-gene linkages[17]. In order to gain clear and separated good spots in gels of 2-DE, preparation of protein samples is a crucial step[18]. By studying different protein load conditions, the best sample protein for IPG strip loading has been found. Clear and separated good protein spots on gels were gained when the IPG strips were loaded with sample protein of 40 μg (Figs. 2C, 2D). So in this research, the protein samples of *Bartonella henselae* Houston and

Bartonella henselae Marseille were prepared successfully. The solubilization mixture containing 7 mol/L urea, 2 mol/L thiourea, 4% CHAPS (W/V) and ultrosonic treatment were suitable conditions for protein sample preparation of *Bartonella henselae*. Protein spots of *Bartonella henselae* Houston and *Bartonella henselae* Marseille were compared with 2-DE method, 5% protein spot difference was found and proved that *Bartonella henselae* Houston and *Bartonella henselae* Marseille were different genotypes. The result is consistent with sequencing analysis of the 16S rRNA-encoding gene. In addition, the procedure of 2-DE may prove useful for the proteomic analysis of *Bartonella henselae*. So the research is significant for vaccine development against cat scratch disease by peptide sequencing of the antigenic proteins in the future.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge professor Didier RAOULT and associate professor Florence FENOLLAR and associate professor Pierre-Edouard FOURNIER in Faculty of Medecine in University of Mediterrane of France for their sincere help in this study.

REFERENCES

1. Regnery, R. L., B. E., Anderson, B. E., J. E., Clarridge, M. C., Rodriguez-Barradas, D. C., Jones, and J. H., Carr (1992). Characterization of a novel *Rochalimaea* species, *R. henselae* sp. nov., isolated from blood of a febrile, human immunodeficiency virus-positive patient. *J. Clin. Microbiol.* **30**, 265-274.
2. Drancourt, M., R. J., Birtles, G., Chaumentin, F., Vandenesh, J., Etienne, and D. Raoult (1996). New serotype of *Bartonella henselae* in endocarditis and cat scratch disease. *Lancet* **347**, 441-443. (Erratum, *Lancet* **347**, 842.)
3. Gradon, J. D. and D. S. Stein (1993). Association between *Rochalimaea* infection and cat-scratch disease. *Clin. Infect. Dis.* **17**, 287-288.
4. Koehler, J. E., C. A., Glaser, and W., Tappero (1994). *Rochalimaea henselae* infection: a new zoonosis with the domestic cat as a reservoir. *JAMA* **271**, 531-535.
5. Sander, A., M., Ruess, K., Deichmann, N., Böhm, and W., Bredt (1998). Two different genotypes of *Bartonella henselae* in children with cat-scratch disease and their pet cats. *Scand. J. Infect. Dis.* **30**, 387-391.
6. Slater, L. N., D. F., Welch, and K. W., Min (1992). Rochalimaea henselae causes bacillary angiomatosis and peliosis hepatis. *Arch. Intern. Med.* **152**, 602-606.
7. Slater, L. N., D. F., Welch, D., Hensel, and D. W., Coody (1990). A newly recognized fastidious gram-negative pathogen as a cause of fever and bacteremia. *N. Engl. J. Med.* **323**, 1587-1593.
8. Raoult, D., P. E., Fournier, M., Drancourt, T. J., Marrie, J., Etienne, J., Cosserat, P., Cacoub, Y., Poinsignon, P., Leclercq, and A. M., Sefton (1996). Diagnosis of 22 new cases of *Bartonella* endocarditis. *Ann. Intern. Med.* **125**, 646-652.
9. Fournier, P. E., H., Lelievre, S. J., Eykyn, J. L., Mainardi, T. J., Marrie, F., Bruneel, C., Roure, J., Nash, D., Clave, E., James, C., Benoit-Lemercier, L., Deforges, H., Tissot-Dupont, and D., Raoult (2001). Epidemiologic and clinical characteristics of *Bartonella uintana* and *Bartonella henselae* endocarditis. A study of 48 patients. *Medicine* **80**, 245-251.
10. Maurin, M., R. J., Birtles, and D., Raoult (1997). Current knowledge of *Bartonella* species. *Eur. J. Clin. Microbiol. Infect. Dis.* **16**, 487-506.
11. Drancourt, M., R. J., Birtles, G., Chaumentin, F., Vandenesh, J., Etienne, and D., Raoult (1996). New serotype of *Bartonella henselae* in endocarditis and cat scratch disease. *Lancet* **347**, 441-443. (Erratum, *Lancet* **347**, 842.)
12. Bergmans, A. M. C., J. F. P., Schellekens, J. D. A., van Embden, and L. M., Schouls (1996). Predominance of two *Bartonella henselae* variants among cat-scratch disease patients in The Netherlands. *J. Clin. Microbiol.* **34**, 254-260.
13. Gurfield, A. N., H. J., Boulouis, B. B., Chomel, R., Heller, R. W., Kasten, K., Yamamoto, and Y., Piemont (1997). Coinfection with *Bartonella clarridgeiae* and *Bartonella henselae* and with different *Bartonella henselae* strains in domestic cats. *J. Clin. Microbiol.* **35**, 2120-2123.
14. Heller, R., M., Artois, V., Xemar, D., De Briel, H., Gehin, B., Jaulhac, H., Monteil, and Y., Piemont (1997). Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in stray cats. *J. Clin. Microbiol.* **35**, 1327-1331.
15. Sander, A., C., Bühler, K., Pelz, E., Von Cramm, and W., Bredt (1997). Detection and identification of two *Bartonella henselae* variants in domestic cats in Germany. *J. Clin. Microbiol.* **35**, 584-587.
16. Sander, A., M., Ruess, K., Deichmann, N., Böhm, and W., Bredt (1998). Two different genotypes of *Bartonella henselae* in children with cat-scratch disease and their pet cats. *Scand. J. Infect. Dis.* **30**, 387-391.
17. Santoni, W., Rouquie, D., Doumas, P., Mansion, M., Boutry, M., Degand, H., Dupree, P., Packman, L., Sherrier, J., Prime, T., Bauw, G., Posada, E., Rouze, P., Dehais, P., Sahnoun, I., Barlier, I., and Rossignol, M. (1998). Use of a proteome strategy for tagging proteins present at the plasma membrane. *Plant J.* **16**, 633-641.
18. Daniel, Lafitte, Bertrand, Dussol, and Soren, Andersen (2002). Optimized preparation of urine samples for two-dimensional electrophoresis and initial application to patient samples. *Clinical Biochemistry* **8**, 581-589.

(*Received October* 21, 2004 *Accepted June* 21, 2005)