

## An improved procedure for the pulsed-field gel electrophoresis analysis of *Vibrio vulnificus*

Follow this and additional works at: <https://www.jfda-online.com/journal>

### Recommended Citation

Wong, H.-C. and Kuo, C.-J. (2006) "An improved procedure for the pulsed-field gel electrophoresis analysis of *Vibrio vulnificus*," *Journal of Food and Drug Analysis*: Vol. 14 : Iss. 2 , Article 1.  
Available at: <https://doi.org/10.38212/2224-6614.2480>

This Original Article is brought to you for free and open access by Journal of Food and Drug Analysis. It has been accepted for inclusion in Journal of Food and Drug Analysis by an authorized editor of Journal of Food and Drug Analysis.

# An Improved Procedure for the Pulsed-field Gel Electrophoresis Analysis of *Vibrio vulnificus*

HIN-CHUNG WONG\* AND CHENG-JU KUO

Department of Microbiology, Soochow University, Taipei 111, Taiwan, R.O.C.

(Received: March 9, 2006; Accepted: April 12, 2006)

## ABSTRACT

*Vibrio vulnificus* is a marine bacterium that causes septicemia with high mortality via wound infection or seafood ingestion. Subspecies typing of *V. vulnificus* can be performed by pulsed-field gel electrophoresis (PFGE). However, this method is hampered by the degradation of chromosomal DNA in about 12% of strains. This study presents a modified PFGE procedure. The outer membrane of *V. vulnificus* cells was lysed utilizing a sucrose-EDTA method and components in the periplasmic space were removed prior to making plugs. Cytoplasmic digestive enzymes were inactivated by an extended proteinase K reaction. Experimental results indicated that five out of eight strains exhibiting smeared DNA in a previous study (Appl. Environ. Microbiol. 70: 5153-5158, 2004) achieved clear banding patterns. This modified PFGE procedure can be applied to improve PFGE typing of untypeable strains of *V. vulnificus*.

Key words: *Vibrio vulnificus*, pulsed-field gel electrophoresis, DNase

## INTRODUCTION

*Vibrio vulnificus* is an autochthonous marine and estuarine bacterium commonly associated with shellfish<sup>(1)</sup>. This bacterium frequently causes septicemia with high mortality in susceptible persons via wound infection or ingesting contaminated seafood<sup>(2)</sup>. *V. vulnificus* strains are subgrouped into biotypes 1 and 2. Biotype 1 strains are ubiquitous in marine environments and occasional on human, whereas biotype 2 strains are generally restricted to diseased eels<sup>(3)</sup>.

In addition to biotyping, several molecular techniques have been developed to examine the intraspecific diversity of *V. vulnificus*, including amplified fragment length polymorphism<sup>(4)</sup>, randomly amplified polymorphic DNA<sup>(5)</sup>, pulsed-field gel electrophoresis (PFGE), and ribotyping<sup>(6)</sup>. PFGE is considered a reliable approach for differentiating strains in many other pathogenic bacteria, including several *Vibrio* species such as *V. cholerae*<sup>(7)</sup>, *V. anguillarum*<sup>(8)</sup>, and *V. parahaemolyticus*<sup>(9)</sup>. We successfully employed PFGE following *Sfi*I or *Not*I digestion to characterize *V. vulnificus* strains obtained in the United States and Taiwan<sup>(10)</sup>.

The conventional PFGE procedure is limited in that a markedly high percentage (about 12%) of the *V. vulnificus* strains formed smeared DNA and, consequently, were untypeable<sup>(10)</sup>. Degradation of the chromosomal DNA may result from the presence of highly active digestive enzymes. *V. vulnificus* has several extracellular enzymes, namely, protease<sup>(11)</sup>, phospholipase<sup>(12)</sup>, cytolysin<sup>(13)</sup>, and DNase<sup>(14)</sup>, etc. DNase exhibits multiple functions in bacteria, such as lysing intestinal mucosal linings for

the adherence of bacteria, providing carbon and nitrogen sources for bacterial growth, and preventing intrusion of foreign DNA<sup>(15)</sup>. Degradation of DNA in PFGE is likely due to the presence of DNase<sup>(16)</sup>. The nuclease of *V. vulnificus* is a heat-stable alkaline-tolerant periplasmic protein (25 kDa) that can digest RNA and DNA<sup>(17)</sup>. The aim of this study is to improve PFGE analysis of *V. vulnificus* by removing the periplasmic components.

## MATERIALS AND METHODS

### I. Bacterial Strains and Cultivation

Eight strains of *V. vulnificus* that exhibited DNA degradation in a previous study were examined<sup>(10)</sup>. Strain VJ11 was a clinical isolate from Taiwan and strains LSU549 and ORL8074CDC were clinical isolates from the USA. Environmental strains CG41, CG54, CG63 and CG65 were from Taiwan, and SS109A-4B2 was an environmental strain from the USA. The clinical strain YJ03 from Taiwan had a clear PFGE pattern and was used as a positive control in this study<sup>(10)</sup>.

Stock cultures were maintained in Tryptic Soy Broth (Difco, Becton-Dickinson Diagnostic Systems, Sparks, NJ, USA) with 10% glycerol at -85°C. *V. vulnificus* was cultured at 37°C in Luria-Bertani Broth (LB, Difco) or Agar (LA, Difco) with a supplement of 1% NaCl.

### II. Removal of Periplasmic Components

Bacteria on LA-1% NaCl were inoculated to 5 mL LB-1% NaCl, and incubated at 37°C and shaken at 160 rpm for 24 hr. Bacterial cells at the stationary

\* Author for correspondence. Tel: +886-2-8819471 ext. 6852; Fax: +886-2-8831193; E-mail: wonghc@scu.edu.tw

phase were harvested by centrifugation, resuspended in 2 mL of sucrose-EDTA buffer (100 mM Tris-HCl, 250 mM sucrose, 10 mM EDTA) containing 50 µg/mL lysozyme<sup>(16)</sup>, and incubated at 25°C for 30 min to disrupt the outer membrane. Cells were then harvested and resuspended in buffer containing 10 mM Tris-Base, 10 mM EDTA and 0.83 M NaCl at pH 8.0.

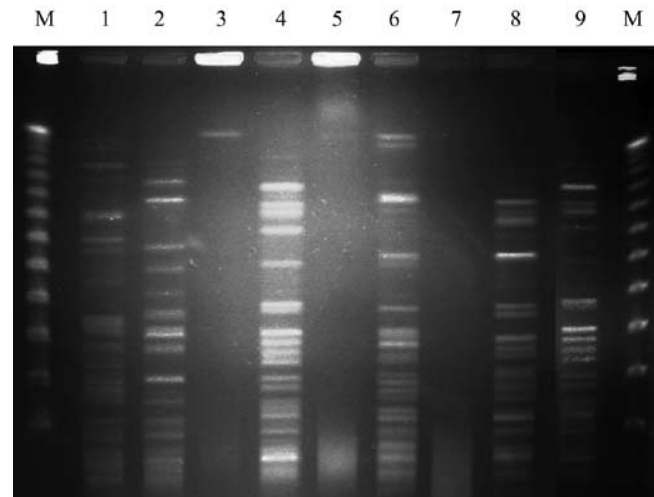
### III. Pulsed-field Gel Electrophoresis

DNA extraction, DNA digestion and PFGE were performed according to the method previously described<sup>(9)</sup> with modifications. Agarose plugs were prepared by mixing an equal volume of bacterial suspensions with 1.5% low-melting agarose (FMC Corp., Rockland, ME, USA). Cells in the agarose plugs were lysed with a lysis solution containing 2.5 mg/mL lysozyme, 1% N-sodium lauroyl sarcosine and 0.1 M EDTA, and incubated at 37°C for 24 hr. The plugs were washed in TE buffer (10 mM Tris-HCl, 1 mM EDTA) three times for 20 min each. The cells were then treated with proteinase K (0.5 mg/mL in 0.5 M EDTA and 1% N-sodium lauroyl sarcosine) at 45°C for 72 hr, and washed three times for 30 min each with TE buffer. One plug section (4 × 9 × 1.2 mm) was equilibrated with an enzyme buffer and then placed in 100 µL fresh buffer containing 10 units of SfiI (New England Biolabs, Beverly, MA, USA) and incubated at 37°C for 4 hr.

High molecular-weight restriction fragments were resolved in 1% agarose gel in 0.5% Tris-borate-EDTA buffer using a CHEF apparatus (CHEF-DR II; Bio-Rad Laboratories, Richmond, CA, USA). Running conditions were 200 V for 24 hr at 14°C, with a 1-40 sec pulse time. A lambda ladder PFGE marker (New England Biolabs, Beverly, MA, USA) was used as molecular size markers. Following electrophoresis, gels were stained in ethidium bromide (Sigma Co., St. Louis, MO, USA), destained in distilled water, and photographed over a UV transilluminator V028490 (Vilber Lourmat, Torey, France).

## RESULTS AND DISCUSSION

Degradation of DNA, a principal drawback of PFGE, occurs in *V. vulnificus* and many other species including *Campylobacter concisus*<sup>(18)</sup>, *Salmonella enterica*<sup>(19)</sup> and *V. parahaemolyticus*<sup>(20)</sup>. Thus, application of this reliable and highly discriminative molecular method is impaired. Several modifications have been applied to minimize DNA degradation. Koort *et al.*<sup>(21)</sup> altered the running buffer from 0.5× Tris-borate-EDTA to HEPES to decrease DNA degradation during electrophoresis of restricted *Escherichia coli* DNA. As chromosomal DNA of *Clostridium difficile* is highly sensitive to degradation, thiourea was added to the running buffer to minimize the DNA degradation of *C. difficile* during electrophoresis<sup>(22)</sup>. DNase activity in *C. botulinum* cells was inactivated by a formaldehyde solution<sup>(23)</sup>.



**Figure 1.** Banding patterns of *Vibrio vulnificus* strains analyzed via a modified PFGE procedure. M, Molecular-sized ladder markers starting at 48.5 kb with increments of 48.5 kb. Lane 1, strain YJ03; 2, strain YJ11; 3, strain SS109A-4B2; 4, strain CG41; 5, strain LSU549; 6, strain CG54; 7, strain CG63; 8, strain CG65; 9, ORL8074CDC. Lane 9 is from another running gel. Except strain YJ03, all other strains exhibited DNA degradation in previous PFGE analysis<sup>(10)</sup>.

In this study, two novel modifications were adopted to improve PFGE for *V. vulnificus*. The sucrose-EDTA method was applied to eliminate periplasmic components, including periplasmic DNase<sup>(16)</sup>. The incubation time for the proteinase K reaction was increased from 48 to 72 hr to ensure complete inactivation of the degradative enzymes. These modifications likely removed or deactivated nuclease and other digestive enzymes in periplasmic and cytoplasmic fractions. Results in at least triplicate experiments indicated that five of the eight strains that had smeared DNAs, namely, YJ11, CG41, CG54, CG65 and ORL8074CDC, achieved clear banding patterns, on the other hand, SS109A-4B2, LSU549 and CG63 did not show clear patterns (Figure 1). Other problems may hinder the PFGE process. In addition to smeared DNA, most chromosomal DNA of SS109A-4B2 and LSU549 were stuck in the loading wells and did not migrate into the running gel. These two strains formed a thick capsule likely interfered with the lysis of outer membrane, proteinase digestion, restricted enzyme activity or the migration of the restricted DNA fragments. One may also try to use thiourea or formaldehyde to inhibit DNA degradation of strains SS109A-4B2, LSU549 and CG63<sup>(23)</sup>.

In conclusion, this modified PFGE procedure enhances PFGE typing of previously untypeable strains of *V. vulnificus* and other pathogens.

## ACKNOWLEDGEMENTS

The authors would like to thank the Department

of Health, ROC, for financially supporting this research under Contract Nos. DOH92-TD-1081 and DOH91-DC-1006. J. D. Oliver and L.-I. Hor are appreciated for providing the bacterial strains.

## REFERENCES

- Linkous, D. A. and Oliver, J. D. 1999. Pathogenesis of *Vibrio vulnificus*. FEMS Microbiol. Lett. 174: 207-214.
- Jackson, J. K., Murphree, R. L. and Tamplin, M. L. 1997. Evidence that mortality from *Vibrio vulnificus* infection results from single strains among heterogeneous populations in shellfish. J. Clin. Microbiol. 35: 2098-2101.
- Hoi, L., Dalsgaard, I., DePaola, A., Siebeling, R. J. and Dalsgaard, A. 1998. Heterogeneity among isolates of *Vibrio vulnificus* recovered from Eels (*Anguilla anguilla*) in Denmark. Appl. Environ. Microbiol. 64: 4676-4682.
- Arias, C. R., Verdonck, L., Swings, J., Garay, E. and Aznar, R. 1997. Intraspecific differentiation of *Vibrio vulnificus* biotypes by amplified fragment length polymorphism and ribotyping. Appl. Environ. Microbiol. 63: 2600-2606.
- Aznar, R., Ludwig, W. and Schleifer, K. H. 1993. Ribotyping and randomly amplified polymorphic DNA analysis of *Vibrio vulnificus* biotypes. Syst. Appl. Microbiol. 16: 303-309.
- Hoi, L., Dalsgaard, A., Larsen, J. L., Warner, J. M. and Oliver, J. D. 1997. Comparison of ribotyping and randomly amplified polymorphic DNA PCR for characterization of *Vibrio vulnificus*. Appl. Environ. Microbiol. 63: 1674-1678.
- Mahalingam, S., Cheong, Y. M., Kan, S., Yassin, R. M., Vadivelu, J. and Pang, T. 1994. Molecular epidemiologic analysis of *Vibrio cholerae* O1 isolates by pulsed-field gel electrophoresis. J. Clin. Microbiol. 32: 2975-2979.
- Skov, M. N., Pedersen, K. and Larsen, J. L. 1995. Comparison of pulsed-field gel electrophoresis, ribotyping, and plasmid profiling for typing of *Vibrio anguillarum* serovar O1. Appl. Environ. Microbiol. 61: 1540-1545.
- Wong, H., Lu, K. T., Pan, T. M., Lee, C. L. and Shih, D. Y. C. 1996. Subspecies typing of *Vibrio parahaemolyticus* by pulsed-field gel electrophoresis. J. Clin. Microbiol. 34: 1535-1539.
- Wong, H. C., Chen, S. Y., Chen, M. Y., Oliver, J. D., Hor, L. I. and Tsai, W. C. 2004. Pulsed-field gel electrophoresis analysis of *Vibrio vulnificus* strains isolated from Taiwan and United States. Appl. Environ. Microbiol. 70: 5153-5158.
- Watanabe, H., Miyoshi, S., Kawase, T., Tomochika, K. and Shinoda, S. 2004. High growing ability of *Vibrio vulnificus* biotype 1 is essential for production of a toxic metalloprotease causing systemic diseases in humans. Microb. Pathog. 36: 117-123.
- Testa, J., Daniel, L. W. and Kreger, A. S. 1984. Extracellular phospholipase A2 and lysophospholipase produced by *Vibrio vulnificus*. Infect. Immun. 45: 458-463.
- Wright, A. C. and Morris, J. G., Jr. 1991. The extracellular cytotoxin of *Vibrio vulnificus*: Inactivation and relationship to virulence in mice. Infect. Immun. 59: 192-197.
- Biosca, E. G., Oliver, J. D. and Amaro, C. 1996. Phenotypic characterization of *Vibrio vulnificus* biotype 2, a lipopolysaccharide-based homogeneous O serogroup within *Vibrio vulnificus*. Appl. Environ. Microbiol. 62: 918-927.
- Focareta, T. and Manning, P. A. 1991. Distinguishing between the extracellular DNases of *Vibrio cholerae* and development of a transformation system. Mol. Microbiol. 5: 2547-2555.
- Moulard, M., Condemine, G. and Robert-Baudouy, J. 1993. Characterization of the nucM gene coding for a nuclease of the phytopathogenic bacteria *Erwinia chrysanthemi*. Mol. Microbiol. 8: 685-695.
- Wu, S. I., Lo, S. K., Shao, C. P., Tsai, H. W. and Hor, L. I. 2001. Cloning and characterization of a periplasmic nuclease of *Vibrio vulnificus* and its role in preventing uptake of foreign DNA. Appl. Environ. Microbiol. 67: 82-88.
- Matsheka, M. I., Elisha, B. G., Lastovica, A. L. and On, S. L. 2002. Genetic heterogeneity of *Campylobacter concisus* determined by pulsed field gel electrophoresis-based macrorestriction profiling. FEMS Microbiol. Lett. 211: 17-22.
- Murase, T., Nagato, M., Shiota, K., Katoh, H. and Otsuki, K. 2004. Pulsed-field gel electrophoresis-based subtyping of DNA degradation-sensitive *Salmonella enterica* subsp. *enterica* serovar Livingstone and serovar Cerro isolates obtained from a chicken layer farm. Vet. Microbiol. 99: 139-143.
- Marshall, S., Clark, C. G., Wang, G., Mulvey, M., Kelly, M. T. and Johnson, W. M. 1999. Comparison of molecular methods for typing *Vibrio parahaemolyticus*. J. Clin. Microbiol. 37: 2473-2478.
- Koort, J. M., Lukinmaa, S., Rantala, M., Unkila, E. and Siitonen, A. 2002. Technical improvement to prevent DNA degradation of enteric pathogens in pulsed-field gel electrophoresis. J. Clin. Microbiol. 40: 3497-3498.
- Corkill, J. E., Graham, R., Hart, C. A. and Stubbs, S. 2000. Pulsed-field gel electrophoresis of degradation-sensitive DNAs from *Clostridium difficile* PCR ribotype 1 strains. J. Clin. Microbiol. 38: 2791-2792.
- Hielm, S., Bjorkroth, J., Hyytia, E. and Korkeala, H. 1998. Genomic analysis of *Clostridium botulinum* group II by pulsed-field gel electrophoresis. Appl. Environ. Microbiol. 64: 703-708.