Comparison of Repetitive Sequence-based Polymerase Chain Reaction (rep-PCR) and Pulsed-Field Gel Electrophoresis (PFGE) for Genetic Characterization of *Arcobacter* spp.

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Abstract

*Arcobacter* has been associated with foodborne illness in humans. Recently, this organism has been receiving more attention as a pathogen of public health concern. The contamination of *Arcobacter* is frequently observed in foods of animal origin especially poultry products; however, the source of contamination as well as the molecular epidemiology of *Arcobacter* is not clearly understood. In the present study, we compared the use of repetitive sequence-based polymerase chain reaction (rep-PCR) and pulsed-field gel electrophoresis (PFGE) for genetic characterization of *Arcobacter*. Thirty *Arcobacter butzleri* isolates from retail chicken carcasses and 3 *Arcobacter* reference strains were typed with rep-PCR and PFGE. Rep-PCR yielded 27 fingerprint patterns, while PFGE yielded 29 PFGE patterns. Two pairs of *Arcobacter* isolates that exhibited the same rep-PCR pattern yielded different PFGE patterns. Discriminatory power determined by Simpson’s index of diversity of rep-PCR was as high as 0.989, comparable to 0.992 as obtained by PFGE. Concordance of the two methods as determined by Adjusted Rand coefficient was 0.798. Prediction of PFGE results by rep-PCR results was quantified by Wallace coefficient, which showed the value of 0.667. Together, our study shows that rep-PCR can be used as an effective screening tool for studying genetic profiles of *Arcobacter*.

Keywords: *Arcobacter*, genetic profiles, PFGE, rep-PCR

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Introduction

Arcobacter is a genus of gram-negative bacterium belonging to the family Campylobacteraceae. This organism has been implicated in human foodborne diarrheal illness and occasionally in bacteremic infections (Ho et al., 2006). Consumption of contaminated foods of animal origin especially chicken products has been considered as the most important risk factor for transmission of this organism to humans (Collado and Figueras, 2011). Although contamination of Arcobacter in chicken meat is commonly observed (Kabeya et al., 2004; Rivas et al., 2004), the source of contamination is not clearly elucidated (Collado and Figueras, 2011). Several molecular genotyping techniques such as repetitive sequence-based polymerase chain reaction (rep-PCR), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST) have been used for typing Arcobacter strains in epidemiological studies to trace the sources of contamination or to determine genetic relatedness of Arcobacter isolated strains. (Atabay et al., 2002; Houf et al., 2002; Rivas et al., 2004; Gonzalez et al., 2007; Miller et al., 2009). Although many molecular typing techniques have been applied, a standard typing technique for Arcobacter has not yet been established. PFGE is considered as the gold standard method for typing of several organisms including those in the family Campylobacteraceae since the technique has good discriminatory power and reproducibility (Majella et al., 2006). Nevertheless, PFGE is technically-demanding, time-consuming, and labor-intensive which may not be suitable for screening a large number of samples. Rep-PCR, on the other hand, is a simple and rapid typing technique which has high throughput ability. This technique has also been used for strain characterization of several organisms (Versalovic, 1994). Since the ability of rep-PCR to characterize Arcobacter strains has never been compared with that of PFGE, the objective of the present study was to determine the discriminatory ability and the concordance of rep-PCR and PFGE in differentiating between Arcobacter isolates in order to assess the use of rep-PCR as an alternative genotypic tool for studying genetic profiles of Arcobacter.
Materials and Methods

Bacterial strains and growth conditions: Thirty A. butzleri isolates used in this study were obtained from a strain collection of the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University. These Arcobacter isolates were recovered from retail chicken carcasses in Bangkok during 2010-2011. In addition, three Arcobacter strains including A. butzleri NCTC 12481, A. cryaerophilus NCTC 11885, and A. skirrowii CV1103 were also used in this study as quality control organisms. Prior to strain characterization, each Arcobacter isolate was subcultured on blood agar (CM0271, Oxoid, Hampshire, UK) supplemented with 5% defibrinated sheep blood and incubated at 30°C for 48 hours under aerobic conditions.

Rep-PCR: Whole cell lysate of the test strains was used as template for rep-PCR amplification. The cells were lysed using alkaline PEG solution as described by Chomczynski and Rymaszewski (2006). In brief, a quarter loopful of each strain was collected and suspended in 500 µl of the alkaline PEG solution and heated at 90°C for 10 min. Then, the mixture was centrifuged at 12,000 rpm for 5 min to pellet the cell debris. Two microliters of the supernatant were used as DNA template for rep-PCR.

Rep-PCR amplifications were performed in 25 µl reaction volumes. Each reaction contained 0.625 U of Ex Taq (Takara Bio Inc., Shiga, Japan), 2.5 µl of 10x Ex Taq buffer (Takara), 1 µl of 20 mM (GTG)$_{5}$ primer (5’-GTG GTG GTG GTG GTG-3’), and 2 µl of DNA template. The PCR consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 45 sec, 40°C for 1 min, and 65°C for 10 min, and a final extension at 65°C for 20 min. To evaluate the reproducibility of the technique, A. butzleri NCTC 12481 was included in every batch of rep-PCR amplification. The PCR products were separated in 1% agarose gels (UltraPureTM Agarose, Invitrogen, California, USA) in 0.5x Tris-borate-EDTA buffer at 135 V for 2.2 hours. The gels were stained with 5 µg/ml ethidium bromide for 5 min and then destained in tap water for 20 min. The gel images were visualized and captured by gel scanner (Typhoon 9410, Amersham Pharmacia Biotech Inc., New Jersey, USA).

PFGE: PFGE was performed according to CDC’s standardized PulseNet protocol for Campylobacter jejuni (Ribot et al., 2001), except for the electrophoresis conditions that the protocol for separation of restriction fragments of Arcobacter was used as previously described (Son et al., 2006). The Arcobacter strains tested in the present study were restricted with KpnI (New England Biolabs, Canada), Salmonella Braenderup H9812 restricted with XbaI was used as a molecular marker as recommended by PulseNet.

Analysis of rep-PCR and PFGE patterns: All rep-PCR and PFGE profiles were analyzed using the program GelCompar II® version 5.10 (Applied Maths BVBA, Kortrijk, Belgium). The gel images were normalized by aligning the bands of the size marker in each gel. The optimization and band position tolerance setting was 1%. Similarity of the band patterns was calculated using Pearson’s correlation coefficient and then clustered using dendrogram generated by unweighted pair group of arithmetic mean (UPGMA) method according to the rep-PCR results. Isolates that had a similarity value higher than 94% were considered the same rep-PCR type. Isolates that had PFGE patterns showing the same number of bands with the same size of the corresponding bands or showing less than two band differences were considered indistinguishable isolates (Tenover et al., 1995).

Determination of discriminatory power and concordance between rep-PCR and PFGE: Simpson’s index of diversity (SID), Adjusted Rand coefficient, and Wallace coefficient were calculated using the online tool for quantitative assessment of classification agreement available at http://darwin.phyloviz.net/ComparingPartitions/.

The Simpson’s index of diversity demonstrates the discriminatory ability of typing techniques (Hunter and Gaston, 1988). To determine the concordance between rep-PCR and PFGE, Adjusted Rand coefficient and Wallace coefficient were calculated. The Adjusted Rand coefficient provides overall concordance of two typing techniques (Hubert and Arabie, 1985), while the Wallace coefficient shows directional information on clustering concordance between different typing methods which can be used for predicting results of one technique by results of another technique (Carrico et al., 2006).

Results and Discussion

Of the 33 Arcobacter isolates analyzed, 27 rep-PCR patterns and 29 PFGE patterns were obtained. The DNA fingerprints generated by rep-PCR consisted of 8-15 fragments with the size ranging from 300-9,000 bp, whereas PFGE profiles of KpnI-digested Arcobacter genomic DNA were composed of 10-20 fragments with the size ranging from 10-500 kbp (Figure 1). The rep-PCR profiles of A. butzleri NCTC 12481 generated from different PCR amplifications were indistinguishable and clustered together at >94% similarity value (data not shown), suggesting that the technique had good reproducibility. Figure 1 shows the dendrogram constructed based on the rep-PCR results. A. butzleri isolates examined in the present study as well as A. butzleri reference strain NCTC 12481 were grouped together. This A. butzleri cluster was only 16.2% similar to A. cryaerophilus NCTC 11885 and A. skirrowii CV1103 reference strains. All Arcobacter isolates exhibiting different rep-PCR patterns also revealed different PFGE patterns. Four pairs of Arcobacter isolates that had identical rep-PCR pattern (R5, R16, R17, and R21) also had indistinguishable PFGE pattern. However, two pairs of Arcobacter isolates that had the same rep-PCR pattern (R9 and R19) were distinguished by PFGE (P9, P10, P20, and P21) (Fig 1). The Simpson’s index of diversity of rep-PCR and PFGE was 0.989 and 0.992, respectively, indicating the high discriminatory
The quantitative assessment of concordance between rep-PCR and PFGE was carried out by determining the Adjusted Rand and Wallace coefficients (Table 1). The Adjusted Rand coefficient was 0.798, which demonstrated the good congruence between rep-PCR and PFGE. The Wallace coefficient of PFGE to rep-PCR was 1.000, which indicated that if the isolates were identified to be of the same PFGE type, those isolates had 100% chances to be identified as the same rep-PCR type. On the other hand, the Wallace coefficient of rep-PCR to PFGE was 0.667, indicating that if the isolates were identified as the same rep-PCR type, those isolates had 66.7% chances to be identified as the same PFGE type.

With the high discriminatory power and good correlation with the PFGE, the gold standard typing technique for bacteria in the family Campylobacteraceae, the rep-PCR technique as proposed in the present study can be used as a rapid and effective screening tool for studying genetic profiles of Arcobacter especially when large numbers of isolates are needed to be investigated. After the rep-PCR screening, an additional technique such as PFGE can be performed if a more thorough investigation of specific isolates is still required.

Figure 1 UPGMA dendrogram of Arcobacter isolates constructed based on the results of rep-PCR. The corresponding PFGE patterns were shown for each isolate. The circle (●) in front of the strain ID indicates strains that shared the same rep-PCR pattern (R9 and R19), but had different PFGE patterns (P9, P10, P20, and P21). Arcobacter reference strains (A. cryaerophilus NCTC 11885, A. skirrowii CV1103, and A. butzleri NCTC 12481) are underlined.

Table 1 Adjusted Rand and Wallace coefficients

<table>
<thead>
<tr>
<th>Typing method</th>
<th>Adjusted Rand coefficient (95% confidence interval)</th>
<th>Wallace coefficient (95% confidence interval)</th>
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<tr>
<td></td>
<td>PFGE</td>
<td>Rep-PCR</td>
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<tr>
<td>PFGE</td>
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<td>1.000 (1.000-1.000)</td>
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<tr>
<td>Rep-PCR</td>
<td>0.798 (0.391-1.000)</td>
<td>1.000 (1.000-1.000)</td>
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a Wallace coefficient of PFGE to rep-PCR

b Wallace coefficient of rep-PCR to PFGE
Acknowledgments

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References


