

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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บทคัดย่อ

การเปรียบเทียบเทคนิค repetitive sequence-based PCR (rep-PCR) และ pulsed-field gel electrophoresis (PFGE) ในการศึกษาลักษณะทางพันธุกรรมของเชื้ออาร์โคแบคเตอร์

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เชื้ออาร์โคแบคเตอร์เป็นเชื้อแบคทีเรียที่ทำให้เกิดโรคอาหารเป็นพิษในมนุษย์ ในช่วงระยะเวลา 2-3 ปีที่ผ่านมา เชื้อนี้ได้รับความสนใจเพิ่มมากขึ้นในฐานะเชื้อแบคทีเรียก่อโรคที่มีความสำคัญทางสาธารณสุข การปนเปื้อนของเชื้ออาร์โคแบคเตอร์มักพบได้ในอาหารที่มาจากสัตว์ โดยเฉพาะเนื้อสัตว์ปีก อย่างไรก็ตามแหล่งที่มาของการปนเปื้อนรวมถึงระดับโมเลกุลของเชื้ออาร์โคแบคเตอร์ยังไม่เป็นที่ทราบแน่ชัด การศึกษาในครั้งนี้เป็นการเปรียบเทียบการใช้เทคนิค repetitive sequence-based polymerase chain reaction (rep-PCR) และเทคนิค pulsed-field gel electrophoresis (PFGE) ในการศึกษาลักษณะทางพันธุกรรมของเชื้ออาร์โคแบคเตอร์ โดยทำการศึกษาเชื้ออาร์โคแบคเตอร์จำนวน 30 ตัวอย่างที่แยกได้จากเนื้อไก่ และเชื้ออาร์โคแบคเตอร์สายพันธุ์มาตรฐานอีกจำนวน 3 สายพันธุ์ ผลการจำแนกสายพันธุ์ด้วยเทคนิค rep-PCR พบว่าเชื้ออาร์โคแบคเตอร์ที่ทดสอบให้รูปแบบลายพิมพ์สารพันธุกรรมจำนวน 27 แบบ ส่วนเทคนิค PFGE นั้นให้รูปแบบลายพิมพ์สารพันธุกรรมจำนวน 29 แบบ โดยเชื้ออาร์โคแบคเตอร์ 2 คู่ที่ไม่สามารถจำแนกลักษณะทางพันธุกรรมออกจากกันได้ด้วยเทคนิค rep-PCR สามารถจำแนกลักษณะทางพันธุกรรมพันธุ์ออกจากกันได้ด้วยเทคนิค PFGE ค่าแสดงความสามารถในการจำแนกเชื้อ (Simpson's index of diversity) ของเทคนิค rep-PCR และ PFGE มีค่า 0.989 และ 0.992 ตามลำดับ ค่าสัมประสิทธิ์ความสอดคล้องของการจำแนกสายพันธุ์ (Adjusted Rand coefficient) ของเชื้ออาร์โคแบคเตอร์ด้วยวิธี rep-PCR และ PFGE มีค่าเท่ากับ 0.798 และค่าสัมประสิทธิ์ในการทำนายผลการจำแนกสายพันธุ์ (Wallace coefficient) ของเทคนิค PFGE ด้วยเทคนิค rep-PCR มีค่าเท่ากับ 0.667 จากผลการศึกษาที่กล่าวมาข้างต้นแสดงให้เห็นว่าเทคนิค rep-PCR สามารถใช้เป็นเทคนิคในการศึกษาลักษณะทางพันธุกรรมเบื้องต้นของเชื้ออาร์โคแบคเตอร์ที่มีประสิทธิภาพ

คำสำคัญ: เชื้ออาร์โคแบคเตอร์ ลักษณะทางพันธุกรรม 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 multi-locus 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 typing of *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 *Arcobacter* colonies on blood agar were washed and resuspended 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), a deoxynucleotide triphosphates mixture containing each deoxynucleoside triphosphates at a final concentration of 0.2 mM (Takara), 1 µl of 20 µM (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

power of these two techniques. 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.

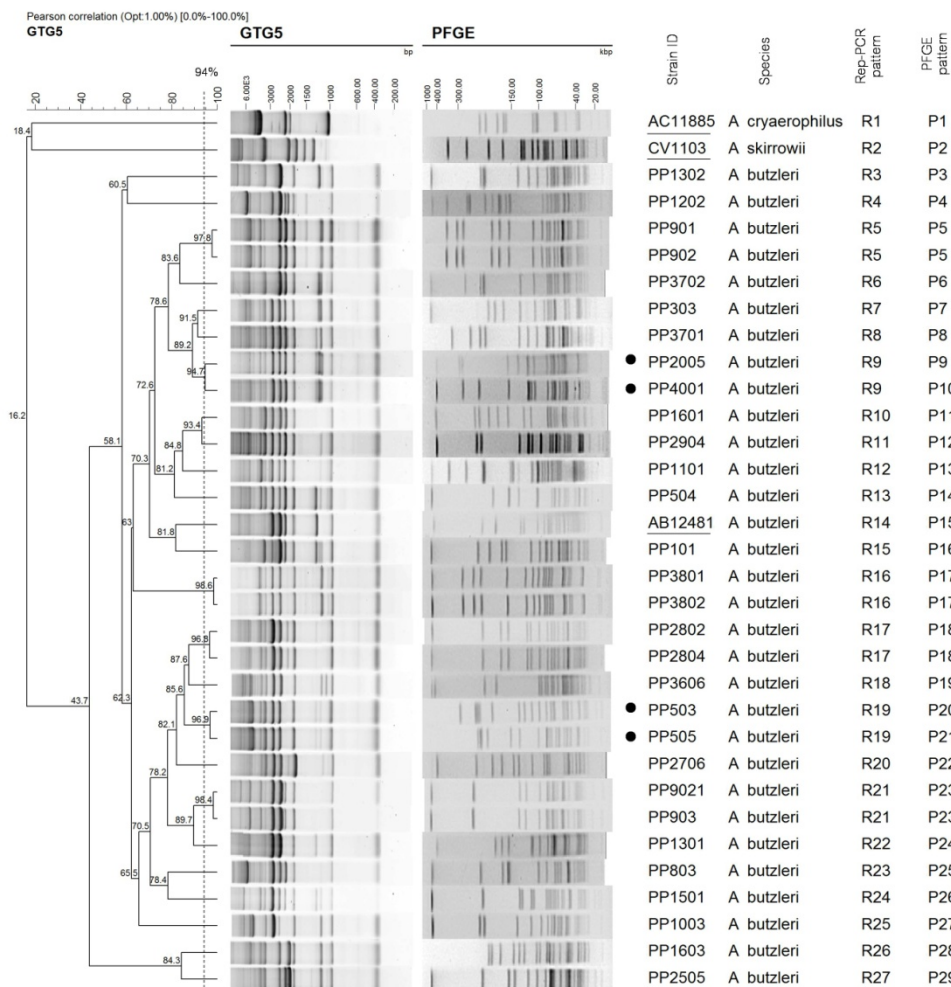


Figure 1 UPGMA dendrogram of *Arcobacter* isolates constructed based on the results of rep-PCR. The corresponding PFGE patterns were shown for each isolate. The circle (●) in front of the strain ID indicates strains that shared the same rep-PCR pattern (R9 and R19), but had different PFGE patterns (P9, 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

| Typing method | Adjusted Rand coefficient (95% confidence interval) | | Wallace coefficient (95% confidence interval) | |
|---------------|--|------------------------|--|-------------------------------------|
| | PFGE | Rep-PCR | PFGE | Rep-PCR |
| PFGE | 1.000 (1.000-1.000) | | 1.000 (1.000-1.000) | 1.000 ^a (1.000-1.000) |
| Rep-PCR | 0.798 (0.391-1.000) | 1.000 (1.000-1.000) | 0.667 ^b (0.431-0.902) | 1.000 (1.000-1.000) |

^a Wallace coefficient of PFGE to rep-PCR

^b Wallace coefficient of rep-PCR to PFGE

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References

- Atabay HI, Bang DD, Aydin F, Erdogan HM and Madsen M 2002. Discrimination of *Arcobacter butzleri* isolates by polymerase chain reaction-mediated DNA fingerprinting. *Lett Appl Microbiol.* 35(2): 141-145.
- Carrico JA, Silva-Costa C, Melo-Cristino J, Pinto FR, de Lencastre H, Almeida JS and Ramirez M 2006. Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant *Streptococcus pyogenes*. *J Clin Microbiol.* 44(7): 2524-2532.
- Chomczynski P and Rymaszewski M 2006. Alkaline polyethylene glycol-based method for direct PCR from bacteria, eukaryotic tissue samples, and whole blood. *Bio Techniques.* 40(4): 454-458.
- Collado L and Figueras MJ 2011. Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clin Microbiol Rev.* 24(1): 174-192.
- Gonzalez A, Ferrus MA, Gonzalez R and Hernandez J 2007. Molecular fingerprinting of *Campylobacter* and *Arcobacter* isolated from chicken and water. *Int Microbiol.* 10(2): 85-90.
- Ho HT, Lipman LJ and Gaastra W 2006. *Arcobacter*, what is known and unknown about a potential foodborne zoonotic agent! *Vet Microbiol.* 115(1-3): 1-13.
- Houf K, De Zutter L, Van Hoof J and Vandamme P 2002. Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods. *Appl Environ Microbiol.* 68(5): 2172-2178.
- Hubert L and Arabie P 1985. Comparing partitions. *J Classif.* 2(1): 193-218.
- Hunter PR and Gaston MA 1988. Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. *J Clin Microbiol.* 26(11): 2465-2466.
- Kabeya H, Maruyama S, Morita Y, Ohsuga T, Ozawa S, Kobayashi Y, Abe M, Katsube Y and Mikami T 2004. Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. *Int J Food Microbiol.* 90(3): 303-308.
- Majella M, Lahiff S, Morris D 2006. Molecular diagnostics and epidemiological typing methods for common foodborne. In: *Molecular Diagnostics Current Technology and Application*. JR Rao, CC Fleming, JE Moore (eds). Norfolk: Horizon BioScience: 93-122.
- Miller WG, Wesley IV, On SL, Houf K, Megraud F, Wang G, Yee E, Srijan A and Mason CJ 2009. First multi-locus sequence typing scheme for *Arcobacter* spp. *BMC Microbiol.* 14(9): 196.
- Ribot EM, Fitzgerald C, Kubota K, Swaminathan B and Barrett TJ 2001. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. *J Clin Microbiol.* 39(5): 1889-1894.
- Rivas L, Fegan N and Vanderlinde P 2004. Isolation and characterisation of *Arcobacter butzleri* from meat. *Int J Food Microbiol.* 91(1): 31-41.
- Son I, Englen MD, Berrang ME, Fedorka-Cray PJ and Harrison MA 2006. Genetic diversity of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *J Food Prot.* 69(5): 1028-1033.
- Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH and Swaminathan B 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. *J Clin Microbiol.* 33(9): 2233-2239.
- Versalovic J 1994. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods Mol Cell Biol.* 5: 25-40.

