Pakistan Journal of Biological Sciences 3 (1): 35-37, 2000

Enterobacterial Repetitive Intragenic Consensus (ERIC) Genotyping of *Escherichia coli* O157:H7

Ooi Wai Ling[1], Son Radu[1], Gulam Rusul[2], Mohamed Ismail Abdul Karim[1], Endang Purwati[3] and Samuel Lihan[1]
[1]Department of Biotechnology, [2]Department of Food Science,
Faculty of Food Science and Biotechnology, University Putra Malaysia, Selangor, Malaysia
[3]Fakultas Peternakan, Universitas Andalas, Kampus Limau Manis, Padang, Indonesia

Abstract: A total of 30 strains of *Escherichia coli* O157:H7 isolated from beef and chicken burger were characterized by Enterobacterial Repetitive Intragenic Consensus (ERIC) genotyping. The ERIC polymorphism patterns obtained as illustrated in a dendrogram showed a significant discriminatory fingerprint among the 30 *E. coli* O157:H7 strains. Nearly every isolates had a unique fingerprint and that there were no bands that were highly conserved among the isolates. This study suggests that there is considerable genetic heterogeneity among the *E. coli* O157:H7 strains by ERIC PCR, and that this has application in screening strains from clinical or food samples to detect a virulent strain with a known fingerprint, and to trace its dissemination.

Key words: Escherichia coli O157:H7, ERIC-PCR

Introduction

Various dispersed repetitive DNA sequences had been described presently in eubacteria. Consensus oligonucleotides are used in accordance to PCR amplification to access for the distribution and evolutionary conservative region of distinct prokaryotic repetitive elements. A new family of repetitive elements, the enterobacterial repetitive intergenic consensus (ERIC) sequences was recently being defined using the genomic sequence information obtained primarily from both the organism of *E. coli* and S. typhimurium (Sharples and Lolyd, 1990). The ERIC gene sequence is also being known otherwise as intergenic repeat units (IRUs). Like their repetitive extragenic palindromic elements (REP) counterparts, these larger 126 base pairs ERIC elements contain a highly conserved central inverted repeat and are located in extragenic regions. But these consensus sequence are not at all related or having any relationship to the REP consensus sequence (Sharples and Lolyd, 1990). The bacteria *Escherichia coli* O157:H7 is an important pathogen worldwide. Apart from causing the common type of food related illness, *E. coli* O157:H7 is able to cause a more serious illness like hemorrhagic colitis, hemorrhagic uremic syndrome (HUS) and thrombotic thrombocytopaenia purpura (TTP) (Padye and Doyle, 1992). Thus, subtyping is required to differentiate outbreak from background strains belonging to the same serotype. In the present study, 30 strains of *E. coli* O157:H7 were fingerprinted by ERIC PCR methods, to evaluate the ability of this fingerprinting to discriminate among the strains studied.

Materials and Methods

Bacterial strains: The *E. coli* O157:H7 strains were isolated from chicken burger and tenderloin beef purchased from supermarkets in Selangor and Kuala Lumpur as described previously (So *et al.*, 1998a), except that an immunomagnetic separation (IMS) procedure (Dynal UK Ltd) was used after the enrichment steps prior to plating on the selective agar. In addition, the strains were confirmed as *E. coli* O157:H7 by PCR assay for the flagellar H7 genes amplification as described elsewhere (Gannon *et al.*, 1992).

ERIC-PCR amplification: Prior to amplification, genomic DNA of the *E. coli* O157:H7 strains was extracted as described previously (Son *et al.*, 1998b). ERIC-PCR was conducted in a reaction mixtures consisting of 2.5 μl 10x reaction buffer, 1 mM (final conc.) of each dNTP, 2 μM primer, 2 mM MgCl2, 20-30 ng genomic DNA and one unit Taq polymerase (Promega), made up to 25 μl with sterile distilled water. The ERIC primers used are ERICIR (5' ATGTAAGCTCCTGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGTGAGCG-3') (Versalovic *et al.*, 1990). Amplification were performed for 30 cycles at 90 C for 30s, 55 C for 1 min and 65 C for 8 min. A final elongation step at 65 C for 10 min was included. The PCR amplification products were visualized by ruing 15 μl of the reaction on a 1.2 per cent agarose gel and detected by staining with ethidium bromide. DNA ladder (Promega) was used as DNA size markers.

Results and Discussion

Three strains of *E. coli* O157:H7 were isolated from chicken burger (No. 9,10 and 30) and another 27 strains were isolated from beef. An ATCC *E. coli* O157:H7 strain EDL933 was included as the positive control in PCR assay for the identification of the strains. All were positive for the H7 flagellar genes (data not shown). The fingerprint data obtained from ERIC PCR did not correlate with the sample

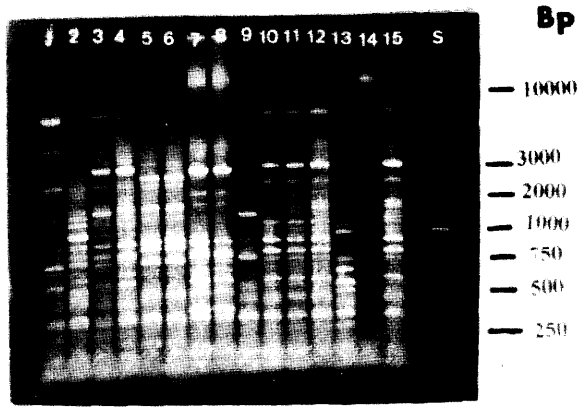

Fig. 1a: ERIC-PCR profiles of the *Escherichia coli* O157:H7 strains. Lanes 1-15, strain No. 1 to 15, respectively, according to Fig. 2; lane S, molecular weight size (base pairs, Bp) are indicated by nuymbers on the left.

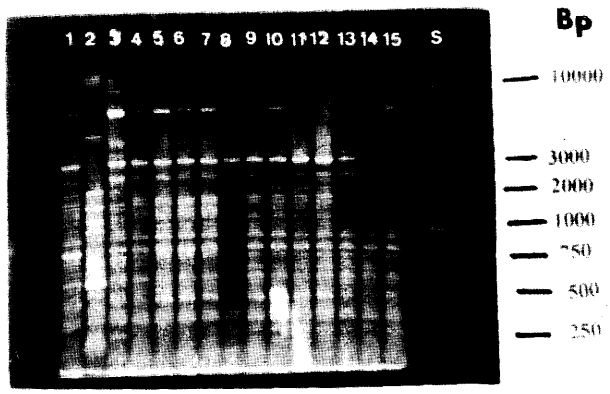

Fig. 1b: ERIC-PCR profiles of *Echerichia coli* O157H:7 strains. Lanes 1-15, strain No. 16 to 30, respectively, according to Fig. 2; lane S, molecular weight size (base pairs, Bp) are indicated by numbers on the left.

source. ERIC PCR produced complex patterns (Fig. 1a and 1b). Many bands smaller than 750 bp were difficult to resolve. Bands between about 750 bp and 7000 bp were less numerous, better resolved, and are useful for matching fingerprints. No bands was common to all strains. The fingerprints were scored and matched by computer using

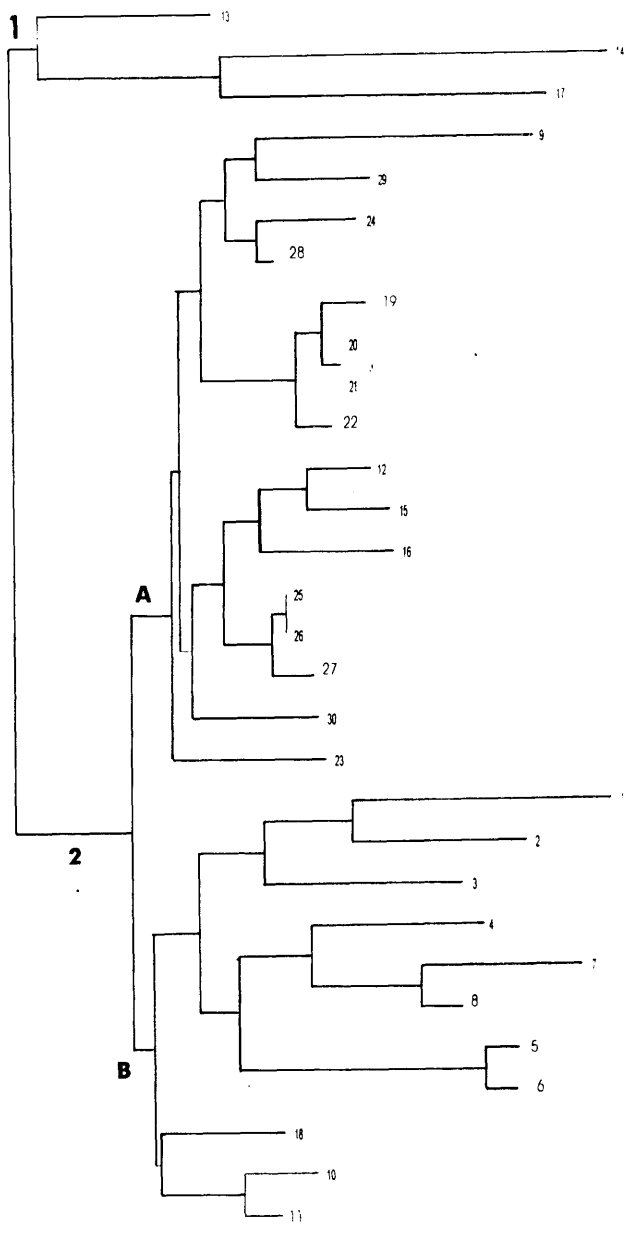

Fig. 2: The dendrogram generated from ERIC genotyping profile.

the RAP Distance software package based on the data retrieved from the presence or absence of banding pattern, and the similarities were displayed as dendrogram. Though the dendrogram in Fig. 2 illustrated that nearly every strain had a unique fingerprint, the *E. coli* O157:H7 strains were grouped together on three major branches (1,2A and 2B) indicating their clonal lineage or possible sources of origin. Whether the ERIC PCR produces useful and valid results depends on the quality of the fingerprints and the degree to which the fingerprints reflect the true genetic relatedness of a group of bacterial strains. Versalovic *et al.* (1991) demonstrated that ERIC 1R and ERIC2 primers revealed species-specific band patterns of different bacterial genomes. In addition, it has been reported that gram negative enteric bacteria species and its close relative in the same phyla tested for ERIC gene sequence were found to yield amplification patterns of greatest complexity in comparison to the gram positive bacteria. This could probably explain for the unique and complex fingerprint observed for almost every strain. PCR fingerprints patterns have been used as estimators of genetic relatedness, but nucleotide divergence is the true measure of relatedness. Our results are in general agreement with several other researchers who have reported obtaining reproducible and reliable PCR fingerprints (Akopyanz *et al.*, 1992; Woods *et al.*, 1994; Fadl *et al.*, 1995). The advantages of ERIC PCR over other general PCR methods is the ability of this technique to generate distinctive banding profile between varied bacterial species and strains that contain the repetitive elements.

An outcome of this study is the better characterization of *E. coli* O157:H7, which is known to be potentially pathogenic in colonizing humans and many increase the risk of disease. If genotypes of *E. coli* O157:H7 that are pathogenic for humans are present, detection of the presence in a population of specific genes sequences, such as by ERIC PCR genotyping is important to determine the risk posed by various source of transmission.

Acknowledgement

Funding for this research was made possible by grants from the International Foundation for Science (IFS), Sweden and the Malaysian Government through IRPA.

References

Akopyanz, N., N. O. Bukanov, T. U. Westblom, S. Kresovich and D. E. Berg, 1992. DNA diversity among clinical isolates of Helicobacter pylori detected by PCR-based PARD fingerprinting. Nucleic Acid Res., 20: 5137-5142.

Fadl, A. A., A. V. Nguyen and M. I. Khan, 1995. Analysis of Salmonella enteritidis isolates by arbitrarily primed PCR. J. Clin. Microbiol., 33: 987-989.

Gannon, V. P. J., R. K. King, J. Y. Kim and E.J.G. Thomas, 1992. Rapid and sensitive method for detection of Shiga-like toxin producing *Escherichia coli* in ground beef using polymerase chain reaction. Appl. Environ. Microbiol., 58: 3809-3915.

Padye, N. V. and M. P. Doyle, 1992. *Escherichia coli* O157:H7 epidemiology, pathogenesis and methods for detection in foods. J. Food Protection, 55: 555-556.

Sharples, S. J. and R. G. Lloyd, 1990. A novel repeated DNA sequence located in the intergenic regions of bacteria chromosomes. Nucleic Acid Res., 18: 6503-6508.

Son, R., E. H. Nasreldin, H. Zaiton, L. Rusul and G. F. Nimita, 1998b. Characterization of Vibrio vulnificus isolated from cockles (Anadara granosa): antimicrobial resistance, plasmid profiles and random amplification of polymorphic DNA analysis. FEMS Microbiol. Lett., 165: 139-143.

Son, R., A. M. Sahilah, G. Rusul, A. Zainori, T. Morigaki, N. Asai, Y. B. Kim, J. B. Okuda and M. Nishibuchi, 1998a. Detection of Escherichia coli O157:H7 in the beef marketed in Malaysia. Appl. Environ. Microbiol., 64: 1153-1156.

Versalovic, J., J. Koeuth and J. R. Lupski, 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acid Res., 19: 6823-6831.

Woods, J. P., D. Kersulyte, R. W. Tolan, C. M. Berg and D. E. Berg, 1994. Use of arbitrarily primed polymerase chain reaction analysis to type disease and carrier strains of Neisseria meningitidis isolated during a university outbreak. J. Infect. Dis., 169: 1384-1389.