

## Differentiation of *Salmonella typhimurium* from Gram-negative Intestinal Microbes by Randomly Amplified Polymorphic DNA (RAPD) Fingerprinting

Un-Ho Jin, Tae-Wook Chung, June-Ki Kim, Kyung-Soo Nam<sup>1</sup>,  
Sang-Do Ha<sup>2</sup>, and Cheorl-Ho Kim\*

Department of Biochemistry, Molecular Biology and Pathology, College of Oriental Medicine,  
<sup>1</sup>Department of Pharmacology, College of Medicine, Dongguk University, Kyungju, Kyungpook 780-714;  
<sup>2</sup>Department of Industry and Technology, Korea Health Industry Development Institute,  
Dongjac-Ku, Seoul 151-714, Korea

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In order to rapidly identify and differentiate *Salmonella typhimurium* from the intestinal gram-negative bacteria, randomly amplified polymorphic DNA (RAPD) fingerprinting of *Salmonella typhimurium* was carried out using random primers designated OPA-13 (5'-CAGCACCCAC-3'), OPB-10 (5'-CGT-CTGGGAC-3'), OPB-18 (5'-CCACAGCAGT-3'), and OPJ-10 (5'-AAGCCCGAGG-3'), and its patterns compared with 6 representative intestinal, gram-negative bacterial strains, *Vibrio parahaemolyticus*, *V. vulnificus*, *Enterobacter cloacae*, *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, and *Proteus* sp., which are often found in foods. *S. typhimurium* had unique and distinct fingerprinting patterns. RAPD fingerprinting is thus concluded to be a rapid and sensitive method for the identification of *S. typhimurium* compared to conventional culturing procedures or immunoassays.

**Key words:** Randomly amplified polymorphic DNA (RAPD), fingerprinting, *Salmonella typhimurium*

Salmonellosis (typhoid fever) is an important infectious disease caused by the consumption of contaminated meat, poultry, meat products, and raw milk (2, 6). Although *S. typhimurium* is usually destroyed by cooking, typhoid fever caused by the bacterium may occur frequently by the ingestion of raw vegetables. Thus far, *S. typhimurium* has been detected using culturing and immunoassay techniques (10) and tests based on DNA restriction enzyme analysis, Southern hybridization, and PCR have been used to detect *S. typhimurium* in food and blood samples (3, 4, 9). For each method, inoculation of nutrient broth with food samples is a common step. In addition, classic techniques require multiple subcultures with various selective and indicator media. These methods may take as long as a week to complete and are often limited by poor sensitivity (1, 5).

In various foods, it is still difficult to detect *S. typhimurium*. Although many approaches for its rapid detection have been reported (7, 8, 14), contamination by other microbes tend to influence the sensitivity of detection. Recently, randomly amplified polymorphic DNA (RAPD) fingerprinting has been used for identification of microbes (11-13), but not specifically for *S. typhimurium* detection.

Therefore, we developed a RAPD fingerprinting method to differentiate *S. typhimurium* from several other intestinal microbes commonly found in food.

### Materials and Methods

#### Bacterial strains and media

The reference strains, as shown in Table 1, of *S. typhimurium*

**Table 1.** The reference strains tested by PCR

Species	Sources
<i>S. typhimurium</i> (ATCC 14028)	KMHW <sup>a</sup>
<i>S. typhimurium</i> A (wild type from poultry)	KMHW
<i>S. typhimurium</i> B (wild type from poultry)	KMHW
<i>S. typhimurium</i> C (wild type from poultry)	KMHW
<i>S. typhimurium</i> D (wild type from poultry)	KMHW
<i>S. montevideo</i> (wild type from poultry)	KMHW
<i>S. muenchen</i> (wild type from poultry)	KMHW
<i>V. parahaemolyticus</i> (ATCC 27519)	KMHW
<i>V. vulnificus</i> (ATCC 29307)	KMHW
<i>Enterobacter cloacae</i> (ATCC 13047)	KMHW
<i>E. coli</i> O157:H7	KMHW
<i>P. aeruginosa</i> (ATCC 27582)	KMHW
<i>Proteus</i> spp. (N 13838)	KMHW

<sup>a</sup> KMHW, Korean Ministry of Health and Welfare

\* To whom correspondence should be addressed.  
(Tel) 82-561-770-2663; (Fax) 82-561-770-2281  
(E-mail) chkimbio@mail.dongguk.ac.kr

*imurium* (ATCC 14028), *V. parahaemolyticus* (ATCC 27519), *V. vulnificus* (ATCC 29307), *Enterobacter cloacae* (ATCC 13047), *E. coli* O157:H7, *P. aeruginosa* (ATCC 27582), and *Proteus* sp. (N 13838) used in this study were obtained from the Korean Ministry of Health and Welfare. *S. typhimurium* was grown in tryptic soy broth (Difco Laboratories) or chicken meats containing 20 µg/ml of nalidixic acid and novobiocin (Sigma). Other strains were grown in LB broth.

#### Chromosomal DNA extraction

DNA samples were purified from bacterial strains using the standard protocol of Takara Biotechnology, Inc. (Okayama, Japan). This involved treating cells with lysozyme and STET solution (0.5% SDS, 50 mM Tris-HCl, pH 8.0, 0.4 M EDTA, 1 mg/ml proteinase K), and repeatedly extracting with phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v), followed by final extraction using chloroform-isoamyl alcohol (24:1, v/v). The DNA was concentrated by precipitation with absolute ethanol and treated with RNase A.

#### RAPD fingerprinting

PCR was carried out in 50 µl reaction mixtures containing 0.3 µg of each chromosomal DNA, 10 mM Tris-HCl (pH 8.3), 5 mM KCl, 1.5 mM MgCl<sub>2</sub>, 100 mM of each dNTP, 25 pmol primer, and 0.5 U of *Taq* DNA polymerase (Takara Co., Okayama, Japan). The mixture was overlaid with 50 µl of mineral oil (Sigma), and PCR was performed in a Takara PCR thermal cycler 480 as follows: initial denaturation at 95°C for 5 min, 45 cycles of denaturation at 95°C for 1 min, annealing of primer at 36°C for 1 min, and extension at 72°C for 2 min without a final extension step during the last cycle. The PCR products were separated by gel electrophoresis in 1.5% agarose (Sigma) containing ethidium bromide with 1 × TAE buffer, and visualized under a UV transilluminator. The primers used were randomly constructed 10mer primers, OPA-13 (5'-CAGCACCCAC-3'), OPB-10 (5'-CGTCTGGGAC-3'), OPB-18 (5'-CCACAGCAGT-3') and OPJ-10 (5'-AAGCCCCGAGG-3'). They were synthesized by Takara Co., LTD (Okayama, Japan).

## Results and Discussion

In this study, a RAPD fingerprinting method for the detection of *S. typhimurium* based on short DNA primers was developed. Primers used in the study were OPA-13, OPB-10, OPB-18, and OPJ-10. RAPD fingerprinting using these primers produced characteristic band patterns for different bacterial strains, particularly those of *S. typhimurium*. RAPD fingerprinting patterns 1-3 kb in length by each of the 4 primers was detected in *S. typhimurium*, yielding different bands from *V. parahaemolyticus*, *V. vulnificus*, *Enterobacter cloacae*, *E. coli* O157:H7, *P. aeruginosa*, and

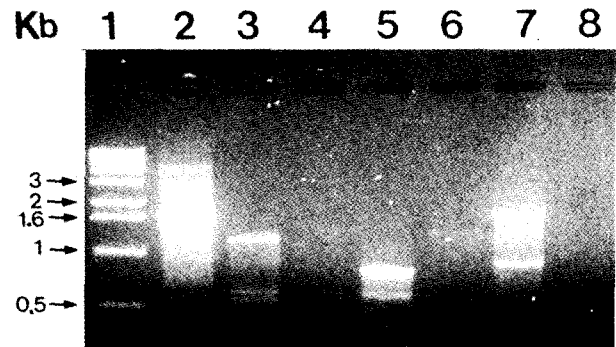


Fig. 1. Agarose gel electrophoresis of RAPD fingerprinting product using the OPA-13 primer. Lanes 1, DNA size marker (Boehringer manheim marker); 2, *S. typhimurium*; 3, *V. parahaemolyticus*; 4, *V. vulnificus*; 5, *Enterobacter cloacae*; 6, *E. coli* O157:H7; 7, *P. aeruginosa*; 8, *Proteus* sp. (N 13838).

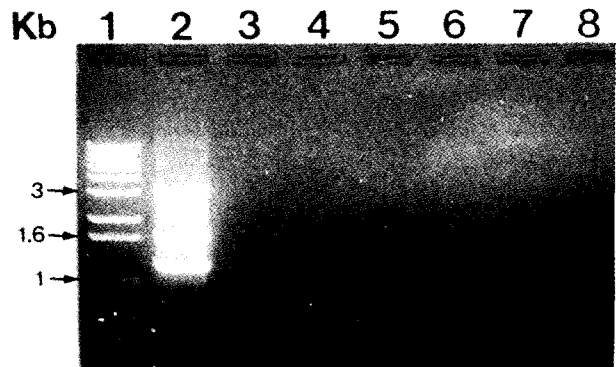
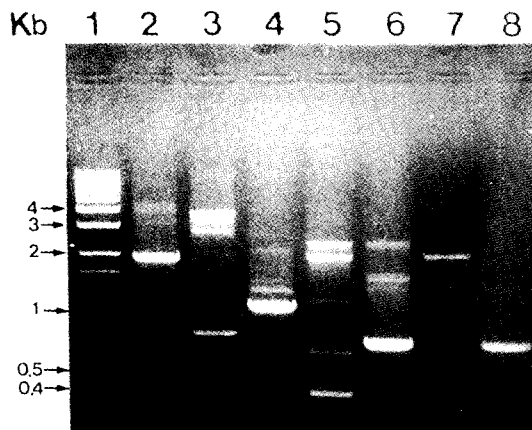


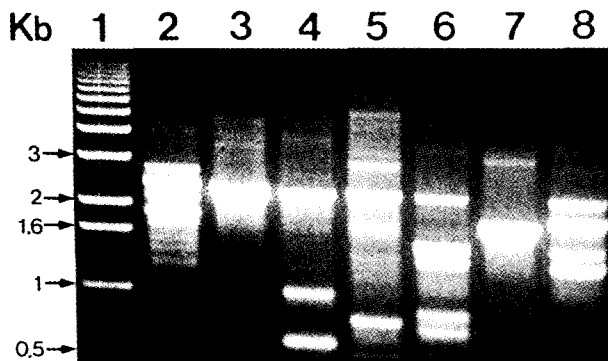
Fig. 2. Agarose gel electrophoresis of RAPD fingerprinting product using the OPB-10 primer. Lanes 1, DNA size marker (Boehringer manheim marker); 2, *S. typhimurium*; 3, *V. parahaemolyticus*; 4, *V. vulnificus*; 5, *Enterobacter cloacae*; 6, *E. coli* O157:H7; 7, *P. aeruginosa*; 8, *Proteus* sp. (N 13838).

*Proteus* sp. N13838. Using primer OPA-13, distinct bands were produced from *S. typhimurium*, *V. parahaemolyticus*, *Enterobacter cloacae*, and *P. aeruginosa* (Fig. 1). In contrast, the OPB-10 primer amplified DNA bands of 3 kb, 1.6 kb, 1.2 kb only from *S. typhimurium*, not from the other microbes (Fig. 2). Thus, it is suggested that the OPB-10 primer is a useful primer for the specific detection of the *Salmonella* sp. by PCR.

On the other hand, when the OPB-18 primer was used for RAPD fingerprinting, all strains showed banding patterns, as shown in Fig. 3. For example, the OPB-18 primer produced a single 1.9 kb DNA band from *S. typhimurium*, and also produced a 2.0 kb DNA fragment as a single band from *P. aeruginosa*, showing a difference between *S. typhimurium* and *P. aeruginosa*. However, various DNA bands were observed from other strains such as *V. parahaemolyticus*, *V. vulnificus*, *Enterobacter cloacae*, *E. coli* O157:H7, and *Proteus* sp., indicating that each strain produced a distinct RAPD fingerprinting pattern. As shown in Fig. 4, OPJ-10 primer produced distinct fingerprinting patterns among 7 intestinal microbes. Four major bands (2.7, 2.4, 2.1, and 1.8



**Fig. 3.** Agarose gel electrophoresis of RAPD fingerprinting product using the OPB-18 primer. Lanes 1, DNA size marker (Boehringer manheim marker); 2, *S. typhimurium*; 3, *V. parahaemolyticus*; 4, *V. vulnificus*; 5, *Enterobacter cloacae*; 6, *E. coli* O157:H7; 7, *P. aeruginosa*; 8, *Proteus* sp. (N 13838).



**Fig. 4.** Agarose gel electrophoresis of RAPD fingerprinting product using the OPJ-10 primer. Lanes 1, DNA size marker (Boehringer manheim marker); 2, *S. typhimurium*; 3, *V. parahaemolyticus*; 4, *V. vulnificus*; 5, *Enterobacter cloacae*; 6, *E. coli* O157:H7; 7, *P. aeruginosa*; 8, *Proteus* sp. (N 13838).

kb) from *S. typhimurium*, one band (2.1 kb) from *V. parahaemolyticus*, 3 major bands (2.1, 1.0, and 0.6 kb) from *V. vulnificus*, 3 major bands (3.0, 2.1, and 0.8 kb) from *Enterobacter cloacae*, 5 major bands (2.1, 1.5, 1.4, 0.9, and 0.7 kb) from *E. coli* O157:H7, one band (1.6 kb) from *P. aeruginosa*, and 4 major bands (2.1, 1.7, 1.5, and 1.4 kb) from *Proteus* sp. were detected respectively. This pattern showed that the 2.1 kb DNA band was a common fragment among tested microbes except in the case of *P. aeruginosa*.

In summary, *S. typhimurium* DNA had a distinct and characteristic RAPD fingerprinting pattern, and the control bacterial strains did not produce any amplification with the OPB-10 primer used in this study, confirming the specificity of the primer. Although there are several methods for the specific detection of *S. typhimurium* (1, 3, 4, 5, 9, 10), the development of rapid, sensitive, reproducible, and reliable methods are widely needed in *Salmonella* diagnosis. Thus, the RAPD fingerprinting method

developed in this study can serve as a detection method in rapidly characterizing *S. typhimurium* and differentiating *Salmonella* sp. from other intestinal bacterial strains.

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