

## Molecular typing of enterohemorrhagic *Escherichia coli* O157 : H7 isolates derived from an outbreak in Okayama, Japan in 1997 by pulsed-field gel electrophoresis

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### Abstract

Thirteen enterohemorrhagic *Escherichia coli* O157 : H7 (EHEC) isolates derived from the patients of an outbreak in the R-hospital in Okayama Japan and one isolated from the ingredients of Japanese noodles in June 1997 were analyzed by molecular typing using pulsed-field gel electrophoresis (PFGE). The PFGE patterns of the patients were almost the same as the patterns of the Japanese noodle ingredients. Therefore, the EHEC O157 : H7 derived from the food was considered to have caused the outbreak in the R-hospital. The molecular typing of isolates from the patients and the Japanese noodle ingredients was almost the same as that of isolates from outbreaks in Hiroshima and Fukuoka prefectures classified as type Ia in 1996 by PFGE analysis. These results indicate that EHEC O157 : H7 strains with a similar PFGE type Ia pattern have already spread throughout western Japan since last year.

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**Key words :** EHEC O157 : H7, PFGE, outbreak, molecular typing

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### Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) O157 : H7 infections occurs in both animals and humans. In humans, the clinical manifestations of EHEC disease are abdominal cramps, diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome (HUS).<sup>1)</sup> Recently, EHEC O157 : H7 infection has been frequently reported and has been epidemiologically linked to undercooked ground beef, other foods and water<sup>2-5)</sup>. In Japan, an EHEC O157 : H7 outbreaks occurred at a kindergarten in Urawa city, Saitama prefecture in 1990 and in primary schools in Okayama, Gifu, Hiroshima, Aichi, Fukuoka, Osaka and Gunma prefectures in 1996.<sup>6)</sup>

An outbreak of EHEC O157 : H7 infection involving 21 patients occurred in the R-hospital in Okayama city on June 25, 1997. The EHEC O157 : H7 was isolated from the ingredients of Japanese noodles in the evening meal on June 19, 1997. To determine whether the EHEC O157 : H7 derived from the Japanese noodle ingredients caused the outbreak, we performed pulsed-field gel electrophoresis (PFGE). In addition, molecular typing of isolates derived from the outbreaks in 1996 were compared to the outbreak isolates in the R-hospital.

### Materials and Methods

Thirteen of 21 EHEC O157 : H7 isolates der-

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ived from the patients, one from the Japanese noodle ingredients in the R-hospital and 4 isolates from three of the outbreaks in 1996 were analyzed.

PFGE was performed as described elsewhere with minor modifications.<sup>4)</sup>

In brief, bacterial cells on an agar medium were washed once in 75mM NaCl-25mM EDTA solution and were suspended in the 100 $\mu$ l water. This cell suspension was mixed with an equal volume of melted 2% chromosomal grade agarose (Bio-Rad Laboratories, Hercules, Calif.), and 100 $\mu$ l of the mixture was dispensed into 1.5mm-thick molds (Bio-Rad). After solidification, the plugs were transferred to tubes containing lysis buffer (50mM Tris [pH8.0], 50mM EDTA, 1% N-laurylsarcosine, and 1mg of proteinase K per ml), and the tubes were incubated overnight at 50°C. After lysis, the plugs were washed twice for 30min at 50°C in 10mM Tris-1mM EDTA (pH8.0) (TE) containing 4mM 4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride (pefabloc SC) and twice in TE without pefabloc SC. After appropriate preparations for restriction endonuclease digestion were made, the DNAs in each plug were digested with 30U of Xba I (Boehringer Mannheim, Germany) at 37°C for 4h. PFGE was performed with a 1% agarose gel by using a CHEF DR III apparatus (Bio-Rad Laboratories) in a 0.5 $\times$ TBE (Tris-borate-EDTA) buffer at 13°C at 200V. To separate a whole genome, a linearly ramped switching time from 4 to 8s was applied for 12h and then a linearly ramped switching time from 8 to 50s was applied for 10h. For separation of fragments of less than 100Kb, a constant 4s switching time was applied for 20h. After PFGE, the gels were stained with etidium bromide and were photographed under UV transillumination.

## Results and Discussion

Thirteen of the 21 EHEC O157 : H7 isolates derived from the patients and one from the Japanese noodle ingredients were analyzed. The PFGE patterns of the patients with the exception of one case (Fig. 1a, b, line 9) were similar to the pattern of the Japanese noodle ingredients (Fig. 1a, b, line 14). Therefore, the EHEC O157 : H7 derived from those ingredients was considered to have caused the outbreak in the R-hospital in Okayama city, on June 25, 1997. The one variant case (line 9) showed deletion of about a 70Kb band. A mutation or deletion may be seen in the Xba I restriction sites of the 70Kb fragment. However, the other band fragments of the band pattern appear to be the same as those of the other patients. The characteristic banding patterns observed in the less than 100-Kb DNA bands of EHEC O157 : H7 isolates were useful for classifying the isolates.

Izumiya et al. applied molecular typing to 825 EHEC O157 : H7 isolates, most of which were from 19 outbreaks and 608 sporadic cases in Japan, mainly between May and August 1996.<sup>6)</sup> These were classified into six types (type I to V and ND [nondescript]). Fifty isolates from seven outbreaks and 60 isolates from patients with sporadic cases of infection showed almost identical PFGE patterns which differed in only 1 of 22 DNA fragments. They were classified into types Ia, Ib and Ic. The sizes of the different bands in the patterns Ia, Ib, and Ic, were 75 and 50Kb. Ninety-nine isolates from 10 other outbreaks and 156 isolates from patients in Kinki area with sporadic cases of infection showed identical PFGE patterns. They were classified into type II. Type IV EHEC isolates, which had only the stx 2 gene, caused another outbreak in a primary school. EHEC isolates of two other types, types

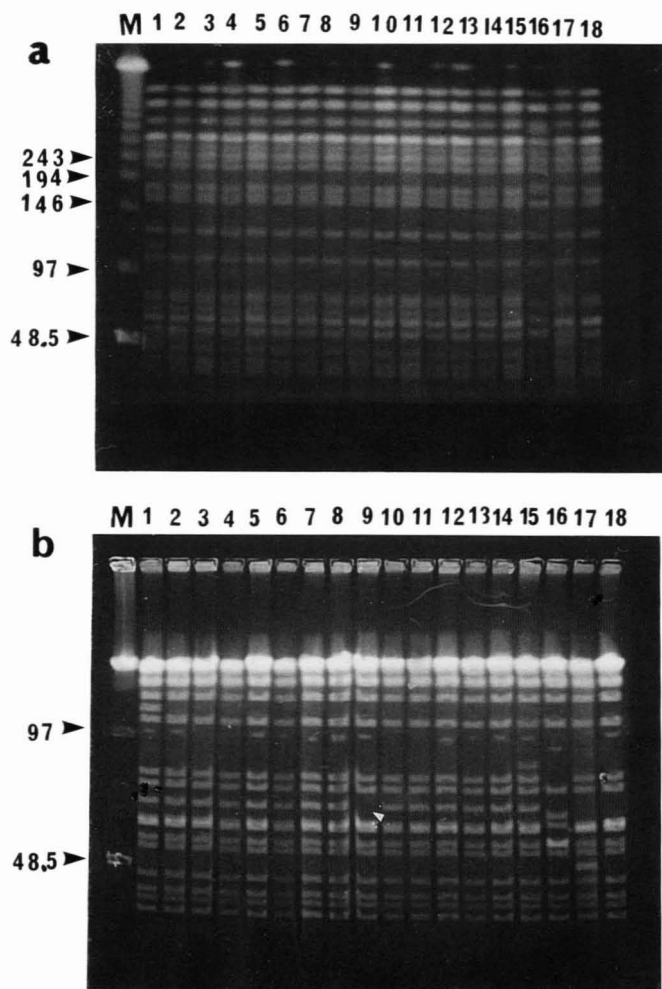


Fig. 1 (a) PFGE patterns of 18 EHEC O157 : H7 isolates showing separation of the whole genome. Lanes 1 to 13 show 13 strains isolated from the patients in the R-hospital in Okayama prefecture in 1997. Lane 14 shows the strain isolated from the Japanese noodle ingredients and lane 15 shows a strain isolated from the outbreak in Hiroshima prefecture (type Ia). Lanes 16, 17 and 18 show strains isolated from outbreaks in Osaka (Sakai city-type IIa), Okayama (Niimi-type Ib) and Okayama (Oku-type Ic) respectively, in 1996. (b) The PFGE patterns of 18 EHEC O157 : H7 were isolated by separation of fragments of less than 100 Kb. Lanes 1 to 18 show the same cases in (a). The white arrowhead in lane 9 indicates deletion of about a 70 Kb fragment. M indicates the bacteriophage lambda DNA ladder standard for PFGE applications (Bio-Rad). The numbers on the left indicate molecular sizes (in kilobases).

III and V, were not related to the outbreak but were isolated in several parts of Japan. ND EHEC isolates included a variety of patterns which could not be classified into either of the types mentioned above. In this study, we

compared the molecular typing of EHEC O157 : H7 isolates from 13 patients in the R-hospital in Okayama city on June 25, 1997 with that of isolates derived from the outbreaks in Japan in 1996 (lines 15 to 18). The PFGE band patterns

of patients and the Japanese noodle ingredients in the R-hospital were almost the same as type Ia (lane 15). The case in lane 15 was isolated from the outbreak in Hiroshima prefecture in 1996. The PFGE band pattern was type Ia. Also, EHEC O157: H7 of type Ia was isolated from the patients of the outbreak in Fukuoka prefecture in 1996.<sup>6)</sup> These results indicate that EHEC O157: H7 strains having almost the same PFGE type Ia pattern have already spread to western Japan since last year.

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(原 著)

## 1997年岡山県下に発生した集団食中毒患者から 分離された腸管出血性大腸菌 EHEC O157 : H7 の パルスフィールドゲル電気泳動法による遺伝子解析

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### 要 約

1997年6月に岡山県下のR-病院で腸管出血性大腸菌 EHEC O157 : H7 による集団食中毒が発生した。その患者および食材である日本そばから分離された EHEC O157 : H7 の菌株をパルスフィールドゲル電気泳動法 (PFGE) を使用して遺伝子解析を行った。13名の患者から分離された菌株の遺伝子型は日本そばから分離された菌株と一致した。従って日本そばから分離された EHEC O157 : H7 が集団食中毒の原因であることが解った。さらにこれらの菌株の遺伝子型は1996年に広島県および福岡県の西日本に集団食中毒が発生した菌株のタイプ Ia 型と類似していた。このことはこの遺伝子タイプの類似した菌株の感染が西日本で去年から広がっていることを示している。

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**キーワード** : 腸管出血性大腸菌 EHEC O157 : H7, パルスフィールドゲル電気泳動法,  
集団感染, 遺伝子解析

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