Short Communication

Characteristics of *Listeria monocytogenes* Isolated from Imported Meat in Japan

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**SUMMARY:** The genomic structure of the *iap* region in *Listeria monocytogenes* (serovar 4b), isolated from chicken imported into Japan, was compared with those from Japanese strains. The isolate was similar to the Japanese strains in a comparatively new, rare group. Such strains might be imported from foreign countries.

Listeriosis, caused by *Listeria monocytogenes*, is known to be transferred via contaminated foods. The authors have been investigating isolates from the Japanese environment and raw non-processed foods since 1988, and have classified the isolates using the *iap* region, as reported by Köhler et al. (1) and Rasmussen et al. (2). Japanese *L. monocytogenes* strains have three evolutionary streams of A, B and C groups (3), similar to those in other countries (2, 4). However, the numbers and rates for each lineage are not equal among the groups: A (32%; 28 isolates), B (66%; 58 isolates) and C (2%; only 2 isolates). The figures suggest that the first two lineages, the A and B groups, are dominant in Japan. We recently found one isolate that was very similar to the rare isolates belonging to the C group. Thus, in the present study, molecular biological analysis of this isolate was performed and its detailed genomic character was compared with those of Japanese isolates.

We attempted to isolate *L. monocytogenes* from a randomly selected total of 51 samples of raw and non-processed pork, beef and chicken, imported from 11 countries (USA, Canada, Denmark, Australia, Mexico, France, Thailand, Ireland, China, New Zealand and Brazil) from 2001-2003. While in bond, an appropriate volume of meat was removed under the routine quarantine condition of asepsis, put into sterilized plastic bags, and transferred to the laboratory through the Yokohama Quarantine Station, Yokohama, Japan. Details for the isolation methods have been described previously (5). One strain, serovar 4b, was obtained from chicken imported on 1 March 2001 from Correa (Rio Grande do Sul, Brazil). Chromosomal DNA was extracted from the strain and the target 687 bp (position 836-1522), within the *iap* gene of *L. monocytogenes*, was amplified by polymerase chain reaction (PCR) using two primers, SI3A and SI3B (5). Determination of the DNA sequence was performed for the 407 bp (position 1116-1522) in the amplified product. The sequence was edited and aligned, then compared with *L. monocytogenes* EGD-e (GenBank accession no. AL591824), and compared with 88 Japanese strains (3) using the computer software DNAsis pro (Ver. 2.00.000.002; Hitachi Software Japan, Tokyo, Japan). Phylogenetic analysis and the genetic distance estimation were also performed using DNAsis pro.

Figure 1 shows the typical cleavage pattern for the PCR amplified *iap* region 678 bp of serovar 4b isolates after digestion with *Fnu*4HI and *Alw*NI. In the figure, all strains, except No. 10, are Japanese strains of meat or human origin. Three similarly sized fragments were obtained from seven Japanese strains, except for strain Nos. 5, 8 and 10. Nos. 5 and 8 were isolated in 1998 from Japanese chicken and beef, respectively, while No. 10 (YC16C10) was an isolate from foreign chicken. After *Fnu*4HI digestion, three different isolates were cleaved into five smaller fragments, designated a through e. Although the size was different in each isolate, the size of No. 10 seemed to be similar to that of No. 8. After *Alw*NI digestion, only two fragments were obtained from Nos. 5 and 10, while in No. 8, three fragments of a size similar to α and γ, but smaller than β, were obtained. These results suggested that the rare isolates, Nos. 5 and 8, are closely related to the No. 10 isolate from imported chicken, though they were not the same type.

The nucleotide sequences of the three isolates were also

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characteristic, having more than 50 point-mutations and characteristic insertions and deletions in the iap region (Fig. 2), though Japanese isolates usually have mutations of less than 30 in this region (5). An unrooted tree for the diversity within the 407 bp iap region was originally established using the 88 Japanese and EGD-e strains (3); isolate No. 10 was inserted into this tree. Isolate No. 10 (YC16C10), together with Nos. 5 (125C1) and 8 (133B1), belongs to group C (Fig. 2, solid circle). In Figure 2, the solid circles A and B consist of 28 and 58 isolates containing the EGD-e, respectively. Lineage C was very far from lineages A and B, at a distance of 0.115, and No. 10 was closer to No. 8 than No. 5. Many foods have been imported into Japan from foreign countries. In the present study, we found a rare isolate from imported meat, though Japan has had no major outbreak of listeriosis, and we could find no references stating that such an isolate is common in other countries, including Brazil. The present results suggest that new types of \textit{L. monocytogenes}, such as Nos. 5 and 8, might have been recently imported from other countries. The isolates having serovar 4b generally have a high virulence to humans, and have caused food poisoning in foreign counties. Thus, for the sake of food hygiene, the continuous inspection of Japanese commercial and imported meats is necessary.

The characteristics of \textit{L. monocytogenes} isolates from imported chicken were investigated, and the strain was found to belong to a minor lineage in Japan.

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**REFERENCES**


