

## Short Communication

# Analysis of the Molecular Evolution of *Listeria monocytogenes* Isolated from Japanese Meats and Environment

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**SUMMARY:** Food contaminated by *Listeria monocytogenes* is a problem on a worldwide level because it is a serious food-borne pathogen. Although 3 evolutionary divisions have been reported for *L. monocytogenes*, the evolution of Japanese isolates has not yet been clarified. Thus, in order to determine the lineage of these Japanese isolates, we classified and conducted phylogenetic analysis of 407 bp (position 1116-1522) of the *iap* gene derived from 88 isolates from Japanese listeriosis patients, foods and environment. The isolates were classified into 18 types commonly accompanied by serotypes, and the types were divided into 3 lineages. Our results suggest that these Japanese isolates belong to the 3 lineages of *L. monocytogenes* isolated in other countries.

*Listeria monocytogenes* causes a serious food-borne illness and there have been many reports of such outbreaks in the United States and Europe (1-3). In Japan, sporadic cases have occasionally been reported but no large outbreak has yet occurred. Contaminated food is believed to be the primary source of human exposure to *L. monocytogenes* and has been repeatedly linked to both sporadic cases and large outbreaks of listeriosis (4). Some Japanese food such as meat, milk and cheese, has also been contaminated by *L. monocytogenes* (5). We have previously reported the genetic diversity of the *iap* gene encoding an extracellular protein p60 among the isolates from various foods and listeriosis patients (6-8). In the present study, the *iap* gene cluster sequence from 88 isolates derived from meat, milk and the environment was used to establish a phylogeny for *L. monocytogenes* isolated in Japan.

The *L. monocytogenes* strains isolated from food, the environment and listeriosis patients during 1988-2003 are listed in Table 1. The isolates included 81 and 7 strains from food/environment and patients, respectively. The method of isolation has been described in previous reports (6,7). Serovars were determined using the Sahumy method developed in our laboratory (9), which gave the following numbers of isolates: serovar 1/2a, 32 strains; 1/2b, 26 strains; 1/2c, 4 strains; and 4b, 26 strains. The methods of determining the DNA sequence in the *iap* region (407 bp; position 1116-1522) from each isolate have been described in previous reports (6,7). The sequences were edited and aligned before comparison with the *L. monocytogenes* strain EGD-e (GenBank accession no. AL591824) using the DNAsis pro computer software (Ver.2.00.000.002; Hitachi Software Japan, Tokyo, Japan). Phylogenetic analysis and genetic distance estimation were also performed using DNAsis pro.

Eighty-nine isolates were classified into 19 types (Fig. 1) that differed in their EGD-e strain (0 type). Types 1 and 5-10

consisted of 15 isolates of serovar 1/2a only, type 3 included one isolate of serovar 1/2c, and then types 11 and 15-18 included 8 isolates of serovar 4b only. The remaining 5 types consisted of multiple serovars: 2 serovars each in type 2 (1/2a, 1/2c), type 4 (1/2a, 4b) and type 14 (1/2a, 1/2b), and then 3 serovars of 1/2a, 1/2b and 4b in types 12 and 13. The human isolates were classified into types 1, 5, 10 and 15-16 corresponding to the serovar. Thirteen of 18 types contained a sole serovar suggesting that the isolates from the Japanese foods and environment are commonly associated with the lineage, as suggested by a previous report (4).

Although three evolutionary divisions have been reported for *L. monocytogenes* (4,10), the details of this division have not yet been clarified for Japanese isolates. Nineteen types containing the EGD-e strain (type 0) were resolved into 3 large *L. monocytogenes* lineages in the present study: the A group (types 0-11), the B group (types 12-16) at a distance of 0.038, and the C group (types 17 and 18), containing serovar 4b only at a distance of 0.115. Groups A, B and C seem to correspond to Groups 2, 1 and 3, respectively, in the results of Ward et al. (4) and Rasmussen et al. (10), though different regions were analyzed in these two studies. These results suggest that the Japanese isolates also have 3 evolutionary streams.

The present results demonstrate that *L. monocytogenes* isolates from Japanese foods and the environment can be classified into 18 types commonly associated with serotypes, and that the identified types may be divided into 3 lineages similar to those of *L. monocytogenes* isolated in other countries.

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Table 1. Sources and serovars of the *L. monocytogenes* isolates

origin	serovar	No.	year	p <sup>2)</sup>	strain		
human <sup>1)</sup>	1/2a	1	1989	S1	H1		
		2	1988	S2	H3		
		3	1996	T1	HM1		
		4	1996	T1	HM2		
		5	2002	F1	12H		
	4b	6	1989	S3	H2		
		7	2000	F2	11H		
beef	1/2a	8	2000	T2	312B1		
		9	1999	C1	173B3		
	4b	10	1998	T3	82B1		
		11	1998	S4	133B1		
		12	2001	T4	392B1		
chicken	1/2a	13	2003	T4	468B1		
		14	1998	T4	80C1		
		15	1989	S5	SC23		
		16	1998	T4	89C5		
		17	2000	T5	265C1		
		18	2000	T6	268C1		
		19	1999	S4	213C1		
		20	1999	C1	221C1		
		21	2000	T7	307C1		
		22	1999	T5	186C1		
	1/2b	23	1999	T8	188C3		
		24	1999	T6	223C3		
		25	1998	T4	72C1		
		26	1996	T4	9C1		
		27	1998	T4	79C1		
chicken	1/2b	28	1996	S6	6C1		
		29	1996	G	17C1		
		30	1997	T4	66C3		
		1/2c	31	1997	T4	69C3	
			32	1998	T4	74C1	
			33	1998	T4	77C1	
		chicken	1/2b	34	1998	T4	87C1
				35	1998	T4	89C4
				36	1998	T4	91C3
				37	1998	T4	93C1
	38			1998	T4	95C1	
	39			1998	T4	97C1	
	40			1998	T4	99C4	
41	1998			T4	103C1		
42	1998			T4	105C2		
43	1998			T4	106C3		
chicken	1/2c	44	1998	T4	109C1		
		45	1998	T4	116C1		
		46	1996	C2	23C1		
		47	1997	S4	28C1		
		48	1989	S5	SC32		
		49	1997	T9	42C1		
		50	1998	T9	125C1		
		51	1997	T4	229C1		
		52	2000	T3	295C1		
		53	2001	T4	355C4		
pork	1/2a	54	2003	T4	490C1		
		55	1996	T4	11P1		
		56	1998	T4	76P2		
		57	1998	T4	78P5		
		58	1999	S4	183P1		
		59	1997	T3	63P1		
		60	1998	T4	81P1		
		pork	1/2b	61	1998	T4	100P3
				62	1998	T4	102P1
				63	1998	T4	104P6
64	1998			T4	112P4		
65	1998			T4	114P3		
66	1998			T4	75P1		
67	1998			T4	76P1		
68	1998			T4	78P1		
69	1998			T4	112P3		
70	2003			T4	489P1		
sheep	1/2a	71		T <sup>3)</sup>	IID566		
	4b	72		T <sup>3)</sup>	IID571		
pro- cessed meat	1/2a	73	1989	S7	Hu53		
	4b	74	1989	S7	Hu120		
		75	1989	S7	Hu80		
milk	1/2a	76	1989	S8	LM51		
		77	1989	S8	LM41		
		78	1989	S8	LM61		
		79	1989	S8	LM71		
		80	1989	S8	LM87		
		81	1989	S8	LM103		
cheese	4b	82	1989	S9	CH191		
		83	1989	S9	CH115		
		84	1989	S9	CH188		
		85	1989	S9	CH190		
environ- ment	4b	86	1989	S9	CHF201		
		87	1989	S9	CHF208		
		88	1989	S9	CHF231		

<sup>1)</sup>: Isolates from listeriosis patients who caused meningitis and/or septicemia.

<sup>2)</sup>: Different number shows the different place or shop. p, place; S, Saitama; T, Tokyo; F, Fukuoka; C, Chiba; G, Gunma.

<sup>3)</sup>: The isolates were supplied from the University of Tokyo.

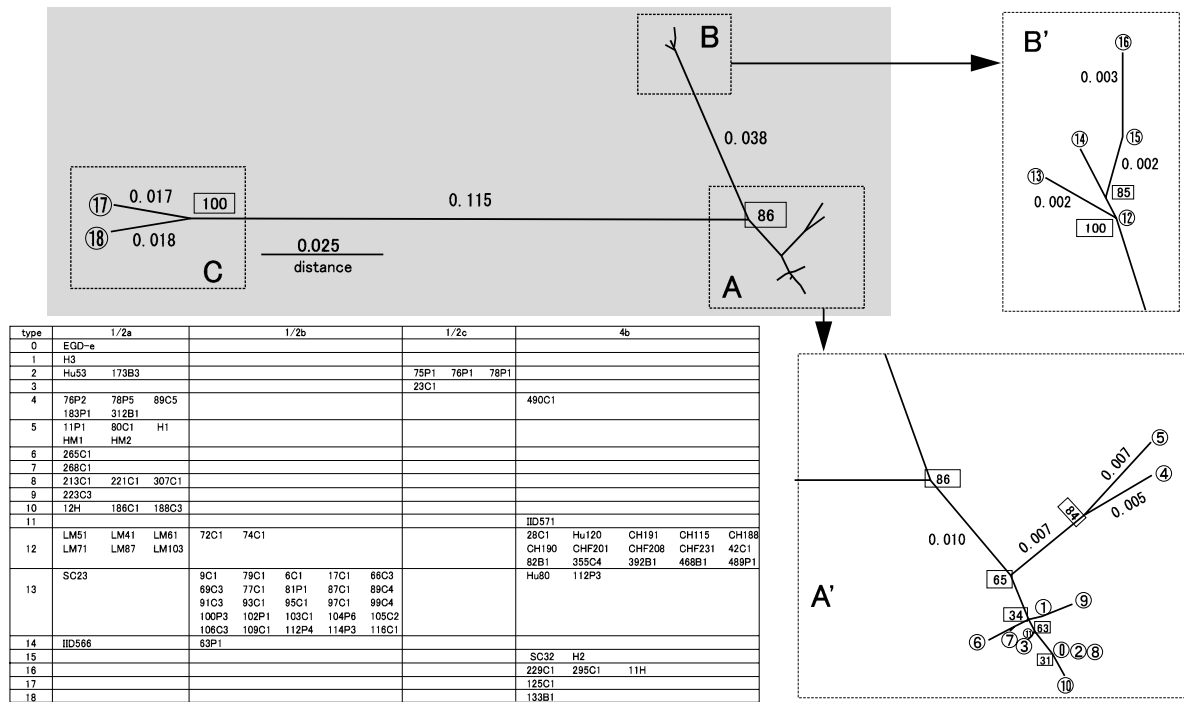


Fig. 1. Unrooted tree for *Listeria monocytogenes* isolated from Japanese foods and the environment. A' and B' show the magnified branches of A and B, respectively. Number, distance. Number in circle, type. Number in square, bootstrap rate (%). The percent was obtained by 1,000 neighbor-joining bootstrap replications.

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