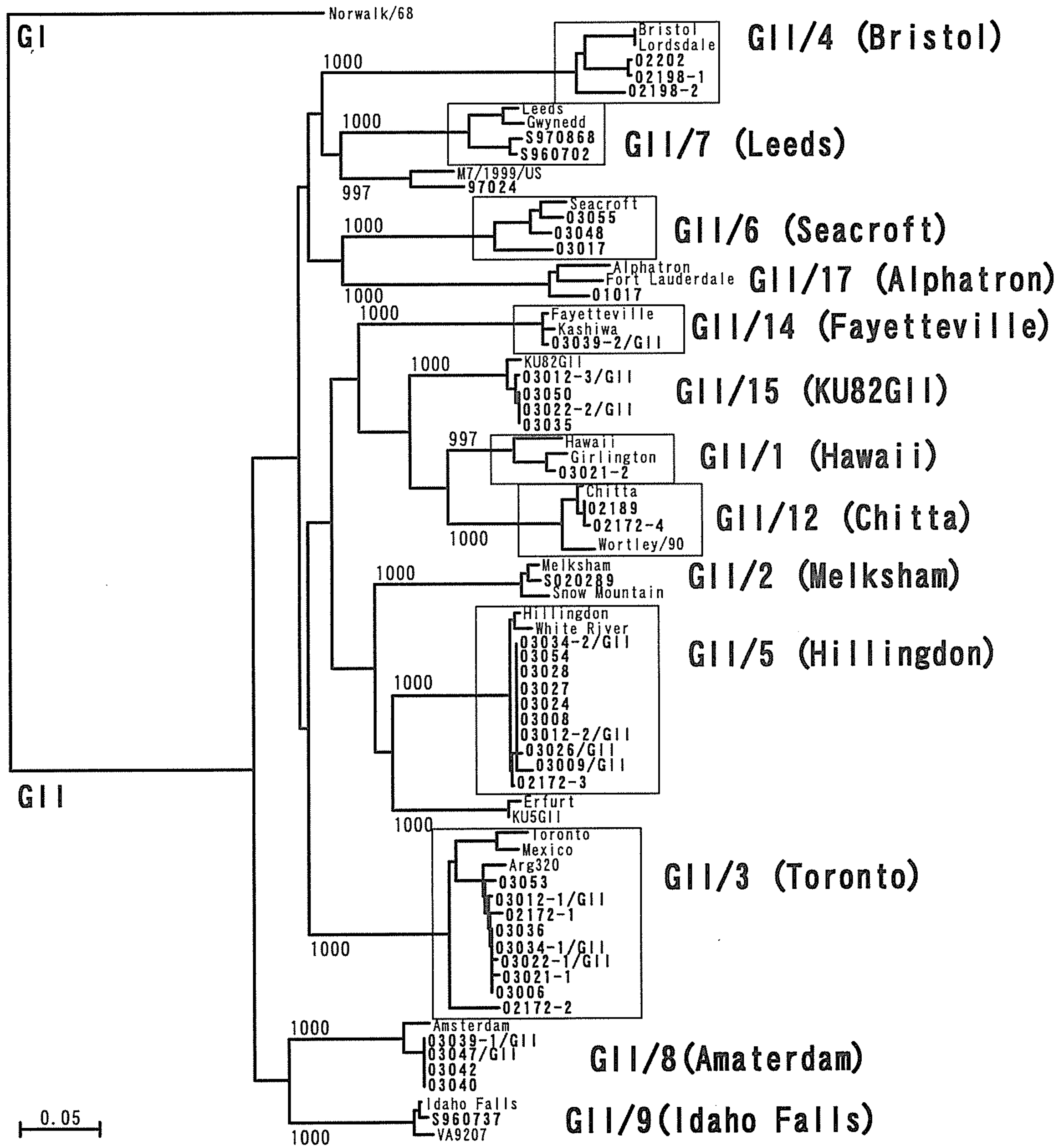
B) genogroup II NVs



図ノロウイルスのキャプシッド NS 領域の分子系樹

□ NV-AD で検出されたノロウイルスの遺伝子型

表　糞便材料中のノロウイルス遺伝子コピー数とNV-AD測定値

検体番号	遺伝子型	コピー数/100μl	OD
04165	GI/1	$2.1x10^6$	0.525
04221	GI/1	$6.8x10^6$	0.641
04545	GI/2	$0.4x10^5$	0.019
05112	GI/3	$2.8x10^7$	0.104
04787	GI/nt	$2.5x10^5$	0.362
03390	GII/3	$1.0x10^5$	0.378
04701	GII/3	$2.1x10^7$	1.143
04703	GII/3	$1.1x10^7$	0.545
04063	GII/4	$6.9x10^5$	0.363
Kod1	GII/4	$1.5x10^7$	0.829
04169	GII/5	$7.0x10^6$	0.151
04506	GII/6	$8.0x10^6$	0.801
05097	GII/nt	$1.0x10^5$	0.106
04804	GI/1	$1.4x10^2$	0.029
	GII/6	$2.2x10^5$	
Nam1	GII/nt	NT	0.209
Hiji1	GII/nt	NT	0.211
Shib1	ND	NT	0.103
Kod3	ND	NT	0.136

nt：遺伝子未型別

NT：未試験

OD：430nm/650nm 測定値、0.140 以下陰性

平成18年度厚生労働科学研究費補助金(食品の安全・安心確保推進研究事業)
分担研究報告書

食品における微生物迅速検査法の開発及びその精度評価システムに関する研究

ノロウィルス迅速検査法の検討と評価

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研究要旨
東京都内で発生した胃腸炎集団発生31事例の患者226名のふん便材料を用いて、市販のノロウイルス検出用ＥＬＩＳＡキットとリアルタイムＰＣＲ法のウイルス検出率の比較を行った。リアルタイムPCR法の陽性率は144/226件(63.7%)に対し、ELISA法の陽性率は62/226件(27.4%)は顕著に低かった。その原因として、特定の遺伝子型（ＧⅠ-3型、ＧⅡ-2型、ＧⅡ-3型、ＧⅡ-6型等）の検出率が低いことが影響していた。このため、調理従事者のウイルス保有調査にＥＬＩＳＡキットを用いることは不適切と考えられた。ＥＬＩＳＡ検出成績の評価を進めるために、ノロウイルス検出例に占めるこれらの遺伝子型の割合を把握していく必要がある。

A. 研究目的

ノロウイルス食中毒は1997年に食中毒病因物質として食品衛生法に加えられて以来、発生事件数は増加傾向にある。東京都内における食中毒発生件数は、毎年約100事件前後であるが、病因物質別の食中毒発生数では、2001年からノロウイルスが最も多い状況が続いている（図1）。

近年、ノロウイルス食中毒の発生要因に変化が認められる。2000年にはノロウイルスによる食中毒事件は21件あり、このうち二枚貝関連事件は9事件(42.9%)であったが、2005年には7事件(21.2%)まで減少していた(表1)。2006年度においては、二枚貝関連事件はわずか2事例しか報告されていない。このように、ウイルス汚染された二枚貝を推定原因食品とする事例は減少し、調理従事者によって汚染された食品を原因とする事例が増加していると考えられる。このため、食中毒発生予防のため、調理従事者のウイルス保有を簡便に検査し、食品のウイルス汚染機会を減らしていくことが求められている。

現在までに酵素抗体法のほか、核酸増幅法ではRT-LAMP法、TRC法等、様々な検査

キットが市販されているが、その評価は定まっていない。今年度は96穴マイクロプレートを用いて多数検体同時処理が可能で、核酸抽出を必要としない簡便な検査法である酵素抗体法のノロウイルス検出キットについて、その実用性評価を行った。

B. 研究方法

平成18年度に東京都内で発生した胃腸炎集団発生31事例の患者ふん便226件について、ELISA法およびリアルタイムPCR法によるノロウイルス検出率を比較した。表2に示すように、用いた検査材料はノロウイルスＧⅠが4事例53件（遺伝子型の内訳はＧⅠ-3型2事例26件、ＧⅠ-4型1事例14件、ＧⅠ-8型1事例13件）、ＧⅡが27事例173件（ＧⅡ-1型2事例12件、ＧⅡ-2型4事例12件、ＧⅡ-3型3事例23件、ＧⅡ-4型5事例39件、ＧⅡ-5型4事例27件、ＧⅡ-6型6事例41件、ＧⅡ-7型2事例12件、ＧⅡ-14型1事例8件）、陰性対照として7事例29件（A群・C群ロタウイルス2事例9件、カンピロバクター等の食中毒菌5事例20件）である。

ノロウイルス抗原検出キットは、14種類の遺伝子型に対する抗体を含み、ノロウイルスＧⅠ・ＧⅡを同時に検出するキットである。検査の術式はELISAキットの添付書に従って行った。

リアルタイムPCR法は、ノロウイルス遺伝子のORF1とORF2のオーバーラップ部位を含んだ領域を標的として設定され、ノロウイルスＧⅠ・ノロウイルスＧⅡを分けて検出するものである。

検査の術式は厚生労働省通達「ノロウイルス検査法」に従って実施した。

C. 研究結果

ノロウイルスが検出された集団事例数を遺伝子型別に比較し、表2に示した。リアルタイムPCR法は31事例全て陽性となったが、ELISA法による陽性事例数は22事例(71.0%)であった。遺伝子型別で見ると、ＧⅡ－2型は4事例全てELISA陰性であった。また、陰性対象としたA群・C群ロタウイルス、カンピロバクター等の食中毒菌による7事例は両法共に陰性であった。

ノロウイルス陽性検体数を遺伝子型別に比較し、表3に示した。リアルタイムPCR法は226件中144件(63.7%)がノロウイルス陽性であったが、ELISA法による陽性数は62件(27.4%)と顕著に少なかった。遺伝子型別にノロウイルス陽性数を見ると、ＧⅠ-3型(15:2、リアルタイムPCR陽性数:ELISA陽性数)、ＧⅡ-2型(9:0)、ＧⅡ-3型(16:4)、ＧⅡ-6型(28:2)など特定の遺伝子型においてELISA法陽性数が少なかった。

ELISA法陽性率が良好であったＧⅠ-4型、ＧⅠ-8型、ＧⅡ-4型、ＧⅡ-5型、ＧⅡ-7型、ＧⅡ-14型合計113件について両検査法の一致率を表4に示した。陽性一致率は65.7%、陰性一致率は86.0%、総合の一致率は73.5%であった。

D. 考察

ノロウイルス陽性率を検体数で比較すると、リアルタイムPCR法144件(63.7%)に対してELISA法62件(27.4%)は半分以下であった。ＧⅠ-3型、ＧⅡ-2型、ＧⅡ-3型、ＧⅡ-6型など特定の遺伝子型に対するELISA法陽性率が低かったことが影響していると考えられた。このため、調理従事者

のノロウイルス保有状況調査を ELISA 法で行うことは、不適当と考えられた。ノロウイルスは遺伝子変異が激しく、近年の流行ウイルスに検出用抗体が適合していないと推測された。検出ウイルスの遺伝子型解析を継続し、抗体の適合性の向上を続けていくことが必要であろう。

一方、近年ヒトーヒト感染事例が増加し、高齢者福祉施設・病院等でノロウイルスGⅡ-4型による胃腸炎集団発生が多数報告され、いくつかの集団発生においては死亡者も報告されている。GⅡ-4 型に関する ELISA 法の検出率は良好であったことから、これらの事例に対しては、ELISA 法の特徴である検体前処理（核酸抽出）が不要なこと、96 穴マイクロプレートにより多数検体同時処理が可能なことが活用できると考えられる。集団発生の原因究明と感染防止に有効であると考えられた。

E. 結論

評価の対象とした ELISA キットは、調理従事者のウイルス保有を調査するためには不適であった。近年の病院内・高齢者施設内集団事例の主流行遺伝子型であるGⅡ-4型の検出率は良好であったことから、これらの集団事例の原因解明には有効と考えられた。

F. 健康危機情報

平成 17 年に全国で発生した食中毒事件 1,545 件のうち、ノロウイルスによるものは 274 件でカンピロバクター(645 件)に次いで第 2 位、患者数は 27,012 人のうち 8,727 人で第 1 位であった。原因食品のうち、二枚貝が関与する事例は減少傾向にあり、調理従事者のウイルス保有状況を把握するために簡易･迅速な検査法を確立し、食中毒予防に活用していく必要がある。

G. 学会発表

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　吉田　靖子
　平成 18 年 6 月　東京

第 27 回食品微生物学会
　ノロウイルス検出用 ELISA キットの評価
　林　志直、森　功次、野口やよい、吉田靖子、
　山田　澄夫
　平成 18 年 9 月　大阪

H. 知的財産権の出願・登録状況

1．特許取得
　なし
2．実用新案登録
　なし
3．その他
　なし

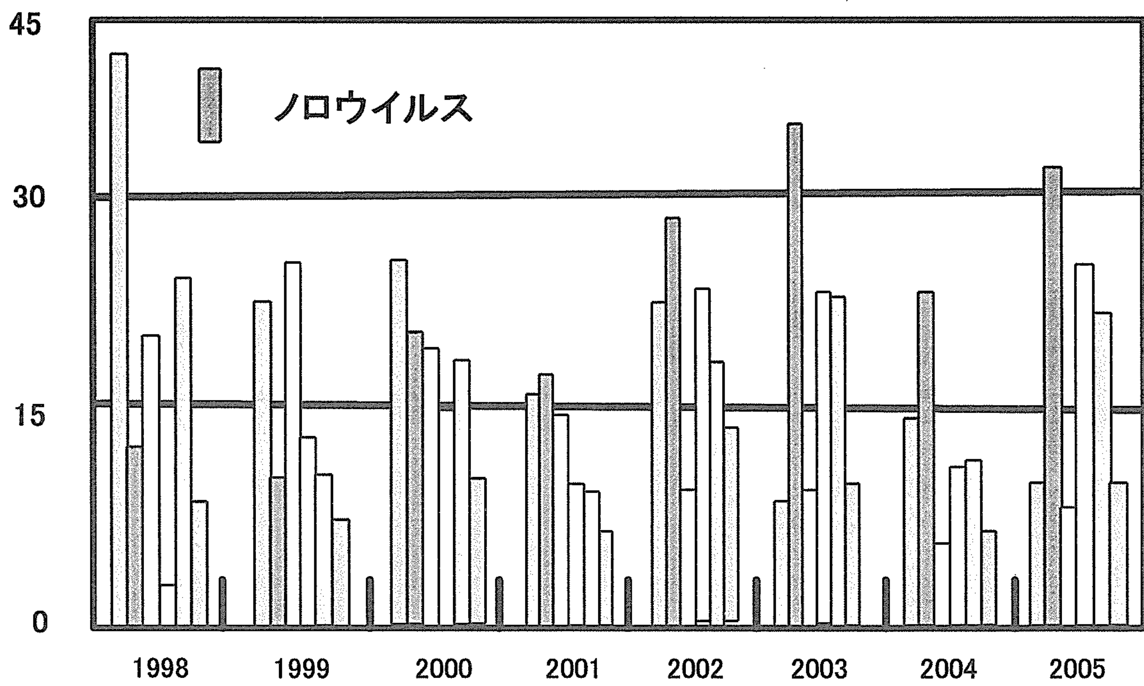

図1.　東京都内の食中毒事件数

表2. 二枚貝関連事件数の推移

年次	2000	2001	2002	2003	2004	2005
食中毒事件数	110	77	118	103	79	99
NV関連事件数	21	17	30	34	26	33
二枚貝関連(%)	9 (42.9)	9 (52.9)	15 (50.0)	9 (26.5)	6 (23.1)	7 (21.2)

表3.検査材料の遺伝子型と試料数

遺伝子型	事例数	試料数
GⅠ-3	2	26
GⅠ-4	1	14
GⅠ-8	1	13
GⅡ-1	2	12
GⅡ-2	4	12
GⅡ-3	3	23
GⅡ-4	5	39
GⅡ-5	4	27
GⅡ-6	6	41
GⅡ-7	2	12
GⅡ-14	1	8
合計	31	226
陰性対照		
A群ロタ	1	5
C群ロタ	1	4
ｶﾝﾋﾟﾛﾊﾞｸﾀｰ	1	5
腸炎ﾋﾞﾌﾞﾘｵ	1	5
ｻﾙﾓﾈﾗ	1	3
黄色ﾌﾞﾄﾞｳ球菌	1	3
ウェルシュ菌	1	4

表4.ノロウイルス陽性率の比較

遺伝子型	事例数	ﾘｱﾙﾀｲﾑPCR(%)	ELISA(%)
GⅠ-3	2	2 (100)	1(50.0)
GⅠ-4	1	1 (100)	1(100)
GⅠ-8	1	1 (100)	1(100)
GⅡ-1	2	2 (100)	1(50.0)
GⅡ-2	4	4 (100)	0
GⅡ-3	3	3 (100)	3(100)
GⅡ-4	5	5 (100)	5(100)
GⅡ-5	4	4 (100)	4(100)
GⅡ-6	6	6 (100)	2(33.3)
GⅡ-7	2	2 (100)	2(100)
GⅡ-14	1	1 (100)	1(100)
合計	31	31 (100)	22(71.0)
陰性対照	7	0	0

表5.遺伝子型別のノロウイルス陽性率

遺伝子型	検体数	リアルタイムPCR(%)	ELISA(%)
GⅠ-3	25	15 (60.0)	2 (10.4)
GⅠ-4	14	8 (57.1)	8 (57.1)
GⅠ-8	13	8 (61.5)	7 (53.8)
GⅡ-1	12	6 (50.0)	2 (16.7)
GⅡ-2	12	9 (75.0)	0
GⅡ-3	23	16 (69.6)	4 (17.4)
GⅡ-4	39	24 (61.5)	15 (38.5)
GⅡ-5	27	19 (70.4)	12 (44.4)
GⅡ-6	41	28 (68.3)	2 (4.9)
GⅡ-7	12	7 (58.3)	6 (50.0)
GⅡ-14	8	4 (50.0)	3 (37.5)
合計	226	144 (63.7)	62 (27.4)

表6. ELISA法とリアルタイムPCR法の比較

ELISA法 ＼ リアルタイムPCR法	陽性	陰性	合計
陽性	46	6	52
陰性	24	37	61
合計	70	43	113

陽性一致率　46／ 70　65. 7%
陰性一致率　37／ 43　86. 0%
一致率　83／113　73. 5%

Ⅲ　研究成果の刊行物に関する一覧表

Ⅲ　研究成果の刊行物に関する一覧表

発表者氏名	論文タイトル名	発表雑誌	巻号	ページ	出版年
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丸山弓美,木村凡,藤井建夫,徳永宜則,松林　潤,相川保史	食卓用ドライアイス装置内の魚介類における腸炎ビブリオの増殖抑制	食品衛生学雑誌	46	213－217	2006
藤川　浩・矢野一好・諸角　聖・木村凡・藤井建夫	各種温度条件下における微生物増殖予測プログラの開発	食品衛生学雑誌	47	288-292	2006
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K. Fujioka, I. Kozone, M. Saito, H. Matsuoka	Rapid Evaluation of the Efficacy of Microbial Cell Removal from Fabrics	J. Ind. Microbiol. Biotechnol.	33	995-1002	2006

Ⅳ　研究成果の刊行物・別刷

Microbes Environ. Vol. 21, No. 2, 69–77, 2006
http://wwwsoc.nii.ac.jp/jsme2/

Microbes and Environments

Minireview

Recent Advances in the Study of the Genotypic Diversity and Ecology of *Listeria monocytogenes*

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Listeria monocytogenes, an intracellular pathogen, is the causative agent of listeriosis, a serious epidemic and sporadic food-borne disease. The clinical manifestations of listeriosis include meningitis, meningoencephalitis, septicemia, spontaneous abortion, perinatal infections, and gastroenteritis. Although rare in comparison to other food-borne diseases, listeriosis has a high rate of lethality (about 30%), making *L. monocytogenes* an important pathogen. *L. monocytogenes* can survive in a broad range of ecological niches, including farm environments and food-processing plants and in a wide range of hosts, including humans and many species of mammals. Furthermore, the capacity to adapt and survive under extreme conditions allows this bacterium to exist ubiquitously in the environment and to survive and proliferate under conditions within the food supply. Although the study of *L. monocytogenes* has already been extensively reviewed, knowledge about this pathogen has been expanding rapidly. Against the background of the growing body of information on this bacterium, the present review mostly discusses advances made in the study of this pathogen over the last 5 years.

Key words: *Listeria monocytogenes*, food pathogens

Introduction

Listeria monocytogenes, a Gram-positive foodborne pathogen, is responsible for listeriosis, which has an overall mortality rate in humans of 30%, manifesting as clinically asymptomatic fecal carriage, febrile gastroenteritis, severe mother-to-child infections, and central nervous system infections[59]. Although the incidence of listeriosis is low[13], *Listeria monocytogenes* is second only to *Salmonella* spp. in the estimated number of food-related deaths it causes in the United States[38], and food-borne transmission is the main route of listeriosis infection[13].

Six species of the genus *Listeria* are currently recognized: *Listeria monocytogenes*, *Listeria innocua*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria welshimeri* and *Listeria grayi*. Of these, only two species are considered to be pathogenic, *L. monocytogenes* in humans and *L. ivanovii* in other mammals. Most human infections by *L. monocytogenes* are attributed to the consumption of contaminated food. This pathogen usually affects susceptible individuals such as the elderly, pregnant women, newborn babies or fetuses. Symptoms are flu-like for healthy persons, but severe complications, such as meningitis, septicaemia, spontaneous abortion or listeriosis of the newborn[64], may occur. Pregnant women are most susceptible to this pathogen, accounting for approximately 35% of all cases worldwide[56]. Active surveillance in the United States showed that *L. monocytogenes* is the second leading cause of bacterial meningitis in patients younger than 1 month or older than 60 years[55]. The number of cases of listeriosis averaged 100 per year from 1993 to 1997 in the USA[4]. A recent study in Japan[26,45]

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estimated that the number of listeriosis cases has averaged 83 per year since 1996.

Genetic Properties of L. monocytogenes

While many different strains of *L. monocytogenes* have been isolated from food and food processing plant environments, only a few virulent strains are known to cause listeriosis[58]. Therefore, accurate tracing of *L. monocyotoges* strains is very important in terms of clinical epidemiology and food safety. Molecular typing can be used to trace *L. monocytogenes* contamination in food-processing plants. Over the last decade, a vast number of reports of inexpensive and rapid methods to type *Listeria* spp. have been published[8,20,22,62,66]. The overall goal has been to develop methods that are more discriminatory than existing serotyping and phage-typing methods. Ribotyping[8] and pulsed-field gel electrophoresis of macrorestriction enzyme-digested chromosomal DNA[22] has demonstrated good discrimination of *Listeria* spp. However, since the results are difficult to standardize among laboratories, cooperative studies using these methods are difficult.

Recently, multi-locus sequence typing (MLST) was developed for analyzing the population genetics of bacteria with the advantages of (i) providing unambiguous DNA sequence data that can be easily exchanged and compared via worldwide web databases; (ii) combining PCR and automated DNA sequencing to reduce labor and analysis time; and (iii) providing a discriminatory power comparable to or greater than that provided by fragment-based methods[34]. As the target of MLST is slowly diversifying housekeeping genes with limited sequence variation, MLST sometimes lacks the discriminatory power required for evaluating the local epidemiology of *L. monocytogenes* strains[3]. Consequently, to overcome the problem of low-resolution, a multi-virulence-locus sequence typing (MVLST) scheme was developed for subtyping *L. monocytogenes*[39]. Using molecular typing methods of MLST or MLVST, as well as restriction fragment length polymorphism analysis and ribotyping, three major phylogenetic divisions within the species have been identified[39,41,61,63] (Fig. 1): Lineage I consists of serotypes 1/2b, 3b, 4b, 4d and 4e, and Lineage II consists of serotypes 1/2a, 1/2c, 3a and 3c[41,63]. Epidemic strains are mostly found in Lineage I and sporadic strains are found in Lineages I and II, while Lineage III strains are extremely rare and are mostly animal pathogens[28].

The complete genomic sequences of *L. monocytogenes* strain EGDe and *L. innocua* strain CLIP 11262 were also recently determined[21]. Analysis of these sequences revealed 10.5 and 14%, respectively, to be species-specific sequence for each strain[21]. Among the most striking findings of recent studies is the degree of divergence within *L. monocytogenes*; one study[9] found that the genetic divergence between Lineages I and II of *L. monocytogenes* was nearly as great (about 8%) as interspecies differences between *L. monocytogenes* EGDe serovar 1/2a strain and *L. innocua* (10%). These results are consistent with a previous report[25] identifying a difference of 39 specific gene fragments between the epidemic *L. monocytogenes* strain F.4565 and *L. monocytogenes* strain EGDe determined by subtractive hy-

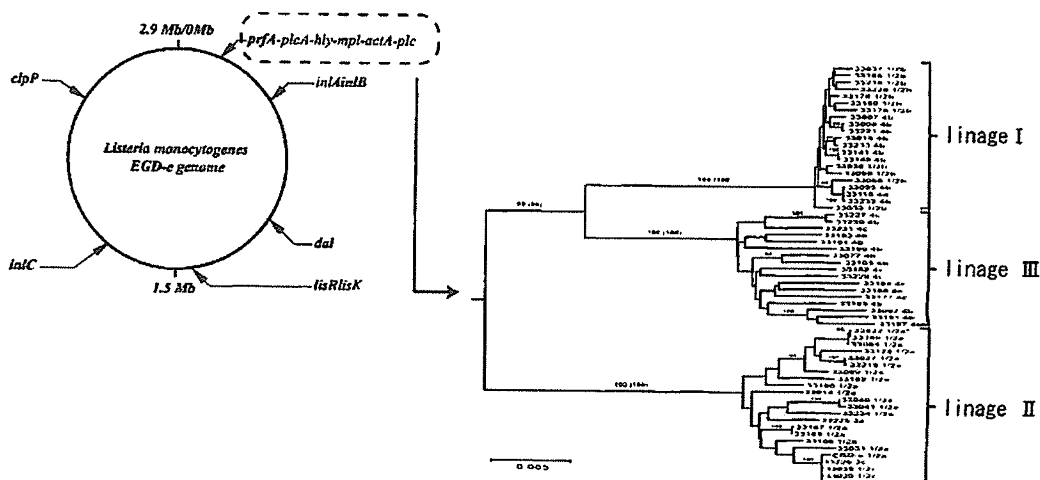

Fig. 1. Neighbor-joining phylogram inferred from an analysis of the combined pVGC sequence data demonstrating three lineages in *L. monocytogenes* populations (redrawn from ref. 61).

bridization. Based on obvious patterns of gene presence or absence among *L. monocytogenes* serovars and *Listeria* spp., it is suggested that early divergence of the ancestral *L. monocytogenes* serovar 1/2c strains from the serovar 1/2b strains led to the establishment of two major phylogenetic lineages. It is also suggested that one group comprising the serogroup 4 strains branched off the serovar 1/2b ancestral lineage, producing (mostly by gene loss) the species *L. innocua*. This is of particular importance since strains of serovar 4b mainly represent epidemic *L. monocytogenes* strains and are isolated from severe invasive human cases more frequently than are strains of other serovars, such as serovar 1/2a. Another recent study[1] using DNA microarrays with 20 strains of *L. monocytogenes* representing six serovars revealed that a majority of epidemic strains of Lineage I grouped together, forming a cluster clearly distinct from the two other Lineage I clusters, which included primarily sporadic and environmental strains. The precise characterization of *L. monocytogenes* is essential for epidemic study of this species. Thus, selective markers for different subpopulations are an essential for the construction of rapid, accurate identification and subtyping methods, which should be powerful tools in public health and food industry investigations.

L. monocytogenes *in hosts*

All pathogenic bacteria must take on and overcome host defense systems and must circumvent many different stresses in order to arrive at the site of infection. *L. monocytogenes* is no exception. These mechanisms include compromising the acid barrier of the stomach, the physical barrier of the epithelial cells lining the gastrointestinal tract, and various immune defenses including the initial onslaught of macrophages. Survival in the presence of bile salt and acidic pH, along with the adhesion of protein and virulence factors required for colonization are important aspects of virulence and have been reviewed elsewhere[11,19,47].

The mechanisms by which *L. monocytogenes* invades mammalian cells have been elucidated by a series of detailed and intricate experiments. *L. monocytogenes* has the amazing ability to cross three significant barriers in humans, namely the intestinal barrier, the blood-brain barrier and the fetoplacental barrier (Fig. 2). Several steps involving a number of specialized molecules have been identified in the infection cells by this pathogen[10]: (1) internalin (also called InlA) and InlB (another member of the internalin multigene family, characterized by the presence of leucine-rich repeats), which are responsible for the internalization of *L. monocytogenes* in cultured non-phagocytic cells; (2) listeriolysin, which acts in concert with two phospholipases (PlcA and PlcB) to allow escape from the phagocytic vacuole; and (3) ActA, which mediates actin-based intracytoplasmic movement and cell-cell spreading. Firstly, live bacteria delay phagosomal maturation and targeting by the degradative pathway through rapid lysation of the membrane of the acidified phagosome by listeriolysin O (LLO) acting in concert with the phospholipases PlcA and PlcB[36]. Then, the bacteria reside freely in the cytoplasm, where they replicate and acquire F-actin-based intracellular motility based on expression of the ActA protein[30]. Subsequently, they invade adjacent cells by cell-to-cell spread[51].

The entry of *L. monocytogenes* into cultured human epithelial cells is mediated by the interaction of an *L. monocytogenes* surface protein, internalin and its human receptor, E-cadherin[32]. In a transgenic mouse model that expresses human E-cadherin in enterocytes, it was demonstrated that *L. monocytogenes* could cross the intestinal barrier assisted by internalin. Epidemiological evidence also suggests that internalin allows this pathogen not only to cross the intestinal barrier, but also to cross the placental and blood-brain barriers.

Particularly important among recent findings is the observation that some *L. monocytogenes* isolates express a truncated nonfunctional form of internalin[53]. *L. monocytogenes* strains carrying a truncated *inlA* also were significantly less capable of invading Caco-2 cells than isolates with homologous 3' *inlA* sequences without a truncation[53]. A recent study used an immunoblot assay to investigate the expression of internalin in 300 clinical strains obtained in France in a single year and a representative set of 150 strains obtained from food products during the same period[27]. This study demonstrates the critical role of internalin in the pathogenesis of human listeriosis. In another recent study, the truncation of this gene in a number of food and environmental isolates was confirmed with *L. monocytogenes* isolated from the U.S.A[42]. Truncations were also found in *prfA*[52], which regulates the expression of a set of virulence factors, including listeriolysin O (LLO), actin polymerization protein ActA, phospholipases (PlcA and PlcB), and internalins. These recent findings support the usefulness of studying the expression of internalin and other virulence genes as markers of virulence in humans. Although the present prevalent opinion is that all strains of *L. monocytogenes* should be considered to be pathogenic[37], it now seems extremely important to determine whether assessment of the truncation of internalin or other virulence or virulence-associated genes provides a new tool for assessing

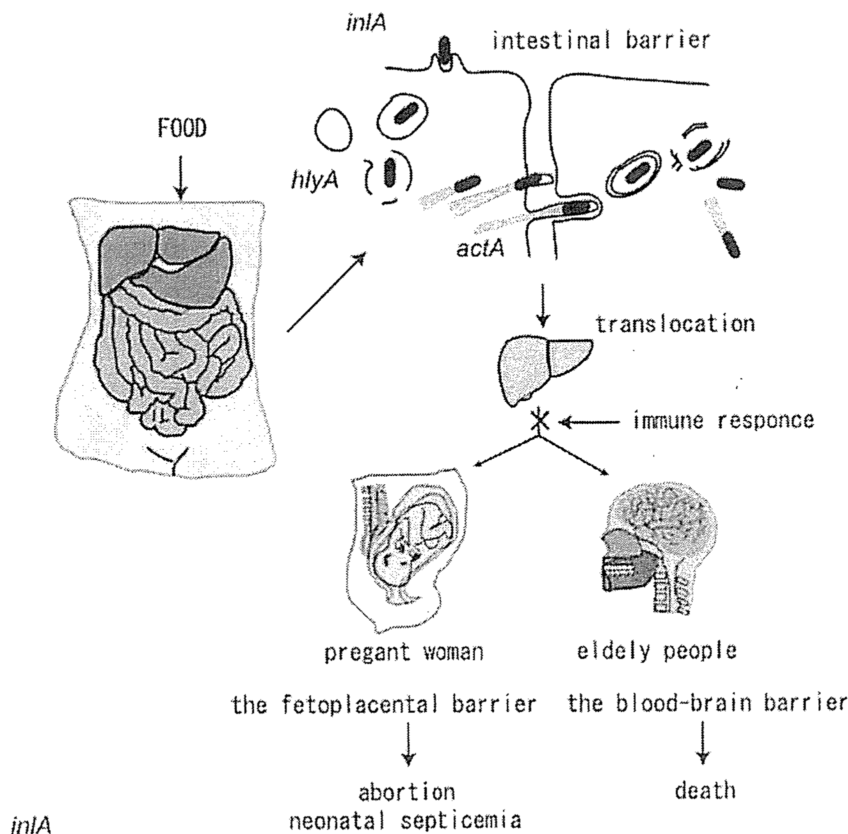

Fig. 2. A schematic representation of how *L. monocytogenes* invades its host. Following ingestion of contaminated food, *L. monocytogenes* cross the intestinal barrier and gain access to the liver and spleen via the bloodstream. In immunocompromised individuals and pregnant women, bacteria can cross the tight blood-brain barrier and the maternofetal barrier, respectively and reach the central nervous system and the placenta.

the risk associated with consumption of food products contaminated with *L. monocytogenes*. An evaluation of any genetic differences between *L. monocytogenes* populations found in humans and foods will be an important area of study to evaluate the risk of this pathogen in the future.

L. monocyotogenes *in food products and ecosystems*

While *L. monocytogenes* is best known as a food-borne pathogen, in nature it has been found in association with plants and decaying plant tissue. It has been hypothesized, that, as an ecological system, livestock farms may function as natural reservoirs for *L. monocytogenes*, and ultimately, as a primary source of *L. monocytogenes* contaminating food-processing plants (Fig. 3). Our understanding of the transmission of *L. monocytogenes* in the farm ecosystem is limited. Many authors agree that a likely scenario for *L. monocytogenes* transmission on farms includes the initial contamination of crops and soil by wildlife, birds or manure used to fertilise fields. Recently, a case-control study of listeriosis in ruminants (cattle, sheep and goats) was conducted on 24 cases and 28 control farms[43]. The study results indicated that the epidemiology and transmission of *L. monocytogenes* differed between small-ruminant and cattle farms and that cattle contribute to the amplification and dispersal of *L. monocytogenes* into the farm environment.

L. monocytogenes is noted for its ability to grow under a wide range of environmental conditions. In particular, it can grow at low or high osmolarity[5,57], it can effectively adapt to acidic conditions[7], and it can grow at temperatures as low as −0.1°C[60]. Miller[40], reported growth of *L. monocytogenes* Scott A in brain heart infusion broth, pH 7.4, at 28°C as having a water activity (a_w) value of 0.92 with NaCl as a humectant and 0.90 with glycerol as a humectant. The mini-

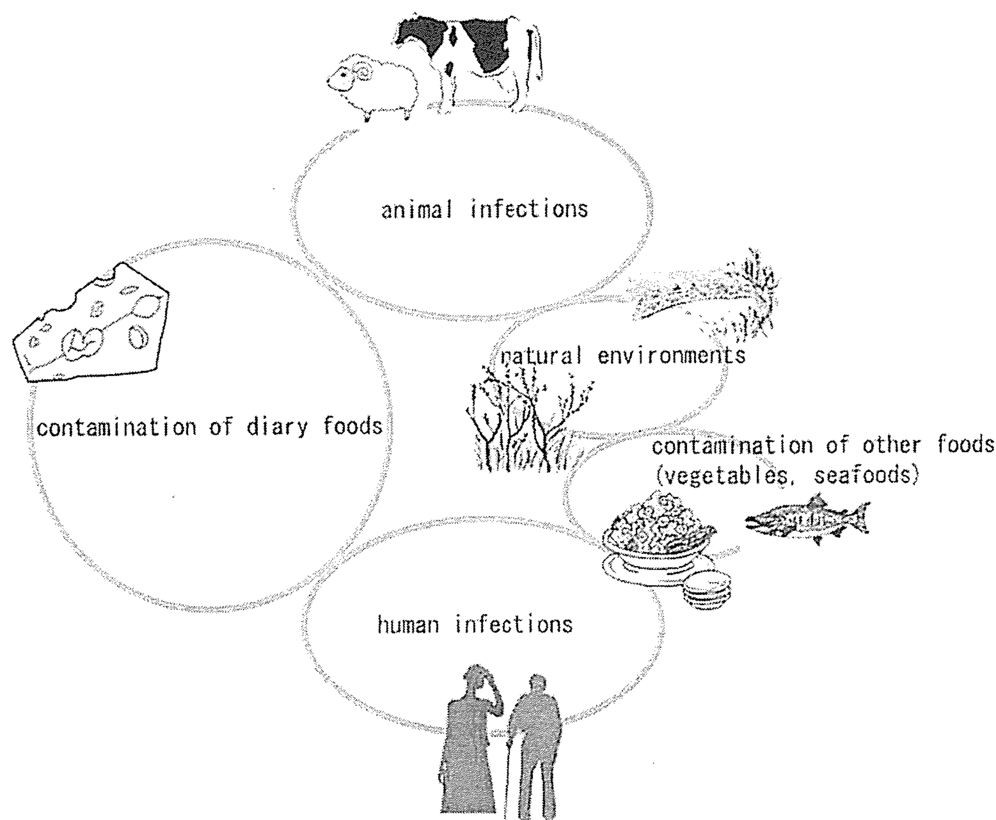

Fig. 3. Different (saprophytic and infective) lifestyles of *L. monocytogenes*. The bacterium is widely distributed in nature, is able to withstand suboptimal conditions encountered during a saprophytic lifestyle, and eventually adapts to the environmental stresses encountered during the infection of a host.

mum pH for the growth of *L. monocytogenes* was 4.3 using HCl as the acidulant[14]. Of the acid-resistance mechanisms characterized thus far, that of *L. monocytogenes* is most dependent on the glutamate decarboxylase (GAD) system[6]. The GAD system operates by converting a molecule of glutamate to γ-aminobutyrate (GABA), thus consuming an intracellular proton and alleviating acidification of the cytoplasm[20]. The intracellular GABA is then exchanged for an extracellular glutamate via an antiporter, and the system is thus primed to consume another intracellular proton. A recent study demonstrated that *L. monocytogenes* possesses a total of three glutamate decarboxylase homologs and two transporters[45]. *L. monocytogenes* has been reported to grow at temperatures of less than 0°C in laboratory media broth[60]. Cold-stress (cold-shock and cold acclimation) proteins whose synthesis is increased after temperature downshifts have been isolated, but for the most part, their identity and functions remain undetermined. A recent study of gene expression in *L. monocytogenes* in response to growth at 10°C showed that the pathogen's acclimation involves amino acid starvation, oxidative stress, aberrant protein synthesis, cell surface remodeling, alterations in degradative metabolism, and induction of global regulatory responses[55].

In general, to survive in extreme environments, the ability to respond rapidly to changes in the environment is necessary. In bacteria, these responses are frequently enacted at the transcriptional level. Global changes in transcription are often coordinated by specific sigma factors whose levels and activities fluctuate in response to environmental cues. In *L. monocytogenes*, the gene encoding σ^B (*sigB*) was identified based on its homology to the *sigB* gene from *Bacillus subtilis*. Phenotypic characterization of *L. monocytogenes* strains lacking *sigB* has shown that σ^B plays a role in resistance to various forms of environmental stress, including osmotic, oxidative, and acid stress[16, 18]. Also, a broad role for σ^B-dependent genes in virulence has recently been sug-

gested for Gram-positive bacteria. A recent study has indicated that, σ^B also contributes to the regulation of virulence gene expression in *L. monocytogenes*[29].

L. monocytogenes is able to grow in a wide range of environmental conditions and is almost ubiquitous in the environment, which makes the control of this pathogen problematic in the food industry. Vegetables, cheese and meat products have been sources of several outbreaks and sporadic cases[54]. Seafood products have not been linked to large outbreaks of listeriosis, but five cases of febrile gastroenteritis in Finland were associated with cold smoked salmon contaminated with *L. monocytogenes*[12]. Other contaminated seafood products, such as smoked mussels, have been assumed to be sources for sporadic cases of listeriosis[2]. Our recent study revealed that many seafood products eaten raw in Japan, such as raw fish, are widely contaminated with this pathogen[23].

Little is known about the physiology of *L. monocyotogenes* in food products, plants, animals, and other natural environments. Among them, the capacity of this organism to grow in a fresh-cut produce environment, including cabbage, has been well documented[15,49]. During adaptation to growth in a plant environment, bacteria must be capable of growth with a limited supply of nutrients, biosynthesis, and/or the transport of building blocks necessary for growth, such as amino acids and nucleotides. Potential forms of stress, such as fluctuations in pH and osmolarity, must be addressed. In a recent study[46], the differential display of reverse transcription-PCR fragments amplified with a set of 81 arbitrary primers allowed the isolation and identification of 32 *L. monocytogenes* gene fragments that showed higher levels of expression under cabbage-associated conditions. This study is only an initial step toward a more detailed understanding of the physiological strategy of *L. monocytogenes* in natural environments.

Of special interest: why has the incidence of listeriosis in Japan been so low?

In Japan, the incidence of listeriosis has remained very low and there have not been any outbreaks of this disease. In February 2001, *L. monocytogenes* serotype 1/2b was isolated from a washed-type cheese during routine monitoring of domestic cheeses[35]. Studies with people who had consumed cheese from the plant revealed that 86 had been infected with *L. monocytogenes*. Based on the epidemiological and genetic evidence, it appears that the outbreak was caused by cheese. That study was the first to document an incidence of food-borne listeriosis in Japan. However, no other food-borne outbreaks have been recognized in Japan in the past 40 years. One hypothesis for this low incidence of listeriosis is that foods distributed in Japan have lower levels of contamination. Recently, Okutani *et al.*[44] reviewed data on Japanese foods contaminated with *L. monocytogenes*, mainly from Japanese reports, and found that the proportion of *L. monocytogenes*, *Listeria* spp. isolated from foods (meats, natural cheeses, seafood and other ready-to-eat foods, for example) in Japan is similar to reports from other countries. Moreover, in Japan, there is significant consumption of ready-to-eat fish products, including raw fish products, and recent studies have revealed that these products are widely contaminated by *L. monocytogenes*[23,24,31,65]. However, these products have not been implicated in clinical listeriosis in humans to date. Thus, factors other than the contamination rate might be responsible for the low occurrence of listeriosis. At present, almost no information on this issue can be found in published reports. Okutani *et al.*[44] carefully discussed this matter in discriminating each type of contaminated food in Japan. However, a direct relationship between the proportion of food contaminated with *L. monocytogenes*, which is almost the same as that in other countries, and the low incidence of listeriosis in Japan could not be identified. They concluded that other factors need to be analyzed in order to resolve this contradiction. One solution to this problem may be a genetic approach. A genetic difference between *L. monocytogenes* populations in humans and in fish products consumed in Japan may account for this difference. Further study will be necessary to evaluate this risk based on the functionality of virulence genes such as *InlA* or *PrfA* or other virulence genes (full-length vs. truncated), as discussed in the above section, in order to differentiate seafood isolates from clinical isolates. At present, however, this is an area of considerable uncertainty, despite great advances in research on the genetic basis of the virulence of this bacterium.

Conclusion

In the last 15 years, listeriosis has changed from being an infectious disease of limited importance to one of the most topical food-borne infections. These pathogens are now a major concern for public health authorities and the food industry. Over the same period, the work of various groups in Europe and the United States has made these pathogens one of the best-characterized groups of intracellular parasites at both the molecular and cellular levels. Despite the major progress made towards understanding the mechanisms of virulence of *Listeria* spp., current insight into the mecha-