Prevalence of the *lmo0036-0043* gene cluster encoding arginine deiminase and agmatine deiminase systems in *Listeria monocytogenes*

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SUMMARY

Arginine deiminase and agmatine deiminase systems are involved in acid tolerance, and their encoding genes form the cluster *lmo0036-0043* in *Listeria monocytogenes*. While *lmo0042* and *lmo0043* were conserved in all *L. monocytogenes* strains, the *lmo0036-0041* region of this cluster was identified in all lineages I and II, and the majority of lineage IV (83.3%) strains, but absent in all lineage III and a small fraction of lineage IV (16.7%) strains, suggesting that the presence of the complete *lmo0036-0043* cluster is dependent on lineages. *lmo0036-0043*-complete and -deficient lineage IV strains exhibit specific *ascB-dapE* profiles, which might represent two subpopulations with distinct genetic characteristics.

KEY WORDS: Listeria monocytogenes, *lmo0036-0043* gene cluster, Arginine deiminase and agmatine deiminase systems, Lineage, *ascB-dapE* profile.

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Listeria monocytogenes is a significant pathogen for both humans and animals that comprises four phylogenetic lineages with different but overlapping ecological niches (Liu, 2006; Orsi *et al.*, 2010). While lineages I and II account for at least 95% of strains from foods and patients, lineages III and IV (previously designated sublineage II-IB) are predominantly isolated from ruminants and other non-primate mammals (Orsi *et al.*, 2010; Swaminathan and Gerner-Smidt, 2007). Lineage III is further distinguished into three subpopulations, IIIA-1, IIIA-2 and IIIC, with IIIA-2 strains constituting possible evolutionary intermediates between *L. monocytogenes* and non-pathogenic species *L. innocua* (Chen *et al.*, 2009a; Liu *et al.*, 2006; Zhao *et al.*, 2010).

The virulence potential of *L. monocytogenes* is linked to its ability to survive acidic conditions encountered both in natural environments and subsequently within the host (Gandhi and Chikindas, 2007; Ryan *et al.*, 2008; Sleator *et al.*, 2009). It is important to understand the mechanisms which *L. monocytogenes* utilizes to overcome this hurdle, as they may in turn point to strategies for controlling this pathogen. The arginine deiminase (ADI) and agmatine deiminase (AgDI) systems have been characterized in several Gram positive and Gram negative bacteria, e.g., *Streptococcus pyogenes*, *Streptococcus mutans*, *Streptococcus suis*, *Enterococcus faecalis*, and *Lactobacillus brevis*, and implicated in bacterial resistance to acidic environments (Degnan *et al.*, 2000; Griswold *et al.*, 2004; Gruening *et al.*, 2006; Llacer *et al.*, 2007; Lucas *et al.*, 2007). Homologues of ADI and AgDI systems (Chen *et al.*, 2009b; Ryan *et al.*, 2009) were also found in

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the genomes of *L. monocytogenes*. Additionally, the glutamate decarboxylase (GAD) system mediates another pathway involved in acid tolerance in L. monocytogenes (Cotter *et al.*, 2001; Cotter *et al.*, 2005). In contrast to three GAD homologs scattered in distinct loci (Cotter *et al.*, 2005), the genes encoding ADI and AgDI systems form the cluster *lmo0036-0043* (Figure 1A) (Chen *et al.*, 2009b; Chen *et al.*, 2011a). Ryan *et al.* identified the functional ADI (encoded by *lmo0043*) in initiating the ADI pathway (2009). Chen *et al.* determined the role of AgDI (encoded by *lmo0038*) in acid stress response, which triggers the AgDI pathway (2009b). Chen *et al.* further revealed that Lmo0036 represents the first example of carbamoyltransferase which is able to catalyze reversible ornithine and putrescine carbamoyltransfer reactions, promoting ADI and AgDI pathways (2011a). Additionally, this cluster contains *lmo0040*, *lmo0037*, *lmo0039* and *lmo0041*, encoding the other paralog of AgDI, a membrane-bound transporter, carbamate kinase and a putative transcriptional regulator respectively (Chen *et al.*, 2011a; Ryan *et al.*, 2009). Using the murine model of infection, we have established roles for the ADI and AgDI systems in the virulence of *L. monocytogenes* (Chen *et al.*, 2009b; Chen *et al.*, 2011a; Ryan *et al.*, 2009). To our knowledge, *L. monocytogenes* represents the first example harboring ADI and AgDI systems in the same genetic locus.

Given their importance in mediating bacterial acid tolerance, it is critical to clarify the prevalence of these systems. Chen *et al.* revealed that

TABLE 1 - *Distribution of arginine deiminase (ADI) and agmatine deiminase (AgDI) systems in L. monocytogenes strains.*

Lineage/ Subgroup	*Serovar*	*ascB-dapE profile*	*Subtotal*	*No. (%) of Listeria strains*		
				gadD1 positive	*lmo0036-0041 positive*	*lmo0042-0043 positive*
I	1/2b	*inlC2DE*	28	0/28 (0%)	28/28 (100%)	28/28 (100%)
		inlGC2DE	1	1/1 (100%)	1/1 (100%)	1/1 (100%)
	4b	*inlC2DE*	29	0/29 (0%)	29/29 (100%)	29/29 (100%)
Subtotal			**58**	**1/58 (1.7%)**	**58/58 (100%)**	**58/58 (100%)**
II	1/2a	*inlC2DE*	17	0/17 (0%)	17/17 (100%)	17/17 (100%)
		inlGC2DE	35	35/35 (100%)	35/35 (100%)	35/35 (100%)
		inlGHE	2	2/2 (100%)	2/2 (100%)	2/2 (100%)
	1/2c	*inlGC2DE*	3	3/3 (100%)	3/3 (100%)	3/3 (100%)
		inlGHE	15	15/15 (100%)	15/15 (100%)	15/15 (100%)
Subtotal			**72**	**55/72 (76.4%)**	**72/72 (100%)**	**72/72 (100%)**
IIIA	4a	*inlGC2DE*	3	3/3 (100%)	0/3 (0%)	3/3 (100%)
		None	17	0/17 (0%)	0/17 (0%)	17/17 (100%)
	4b	*inlGC2DE*	2	2/2 (100%)	0/2 (0%)	2/2 (100%)
	4c	*inlGC2DE*	1	1/1 (100%)	0/1 (0%)	1/1 (100%)
		None	5	0/5 (0%)	0/5 (0%)	5/5 (100%)
Subtotal			**28**	**6/28 (21.4%)**	**0/28 (0%)**	**28/28 (100%)**
IIIC	4a	*inlC2*	4	0/4 (0%)	0/4 (0%)	4/4 (100%)
	4b	*inlC2*	1	0/1 (0%)	0/1 (0%)	1/1 (100%)
	4c	*inlC2*	2	0/2 (0%)	0/2 (0%)	2/2 (100%)
Subtotal			**7**	**0/7 (0%)**	**0/7 (0%)**	**7/7 (100%)**
IV	4a	None	6	0/6 (0%)	4/6 (66.7%)	6/6 (100%)
	4b	None	4	0/4 (0%)	4/4 (100%)	4/4 (100%)
	4c	None	2	0/2 (0%)	2/2 (100%)	2/2 (100%)
Subtotal			**12**	**0/12 (0%)**	**10/12 (83.3%)**	**12/12 (100%)**
Total			**177**	**62/177 (35.0%)**	**140/177 (79.1%)**	**177/177 (100%)**

the presence of *gadD1* is not dependent on lineages or serovars but correlates with *ascB-dapE* internalin profiles (2012a). Preliminary studies showed that the complete *lmo0036-0043* cluster was only identified in lineages I and II (Chen *et al.*, 2009b). To examine whether ADI and AgDI systems are specific to lineage, serovar or certain subpopulation, we performed a systematic investigation on the prevalence of *lmo0036-0043* cluster in *L. monocytogenes* strains.

A total of 177 *L. monocytogenes* strains were examined. These included 58 lineage I (29 serovar 1/2b, 29 serovar 4b), 72 lineage II (54 serovar 1/2a, 18 serovar 1/2c), 35 lineage III (24 serovar 4a, three serovar 4b, eight serovar 4c) and 12 lineage IV (six serovar 4a, four serovar 4b, two serovar 4c) strains (Table 1), typed by phylogenetic analysis of *actA* (Wiedmann *et al.*, 1997) and classical serotyping (Schonberg *et al.*, 1996). Thirty-five lineage III strains represented sublineages IIIA-1 (n=6), IIIA-2 (n=22), and IIIC (n=7) characterized by polyphasic approaches as previously described (Zhao *et al.*, 2011). *ascB-dapE* internalin profiles were characterized as previously shown (Chen *et al.*, 2009c; Chen *et al.*, 2012a; Chen *et al.*, 2012b). Also analyzed were ten *L. innocua* strains belonging to four evolutionary subgroups (Chen *et al.*, 2010), as well as three *L. welshimeri*, two *L. ivanovii*, two *L. seeligeri* and two *L. grayi* strains.

Listeria strains were retrieved from glycerol stocks maintained at -80°C, and cultured in brain heart infusion broth (BHI; Oxoid, England) at 37°C. Primers were designed using Primer 3, and synthesized by Invitrogen Biotechnology (Shanghai, China). The specificities of primers targeting *lmo0036-0043* genes were verified using *L. monocytogenes* strains F2365 (lineage I) (Nelson *et al.*, 2004), EGD-e (lineage II) (Glaser *et al.*, 2001) and M7 (lineage III) (Chen *et al.*, 2011b), whose complete genome sequences were known, and strains belonging to another 14 bacterial genera, as positive and negative controls respectively. The genetic organization of the *lmo0036-0041* region was further determined by long-distance PCR targeting *lmo0035* and *lmo0042*. The PCR reaction was conducted using the PT-200 thermal cycler (MJ, USA). For DNA sequencing analysis, PCR fragments were purified using the AxyPrep DNA Gel Extraction Kit (Axygen, USA) and ligated into pMD18-T (TaKaRa). The recombinant plasmids were sequenced by dideoxy method on ABI-PRISM 377 DNA sequencer.

Systematic investigations were conducted on the eight genes within the *lmo0036-0043* cluster. While *lmo0042* and *lmo0043* were conserved in all *L. monocytogenes* strains, the *lmo0036-0041* region of this cluster was identified in 140 *L. monocytogenes* strains (79.1%), including all lineages I (58/58, 100%) and II (72/72, 100%) strains, and the majority of lineage IV (10/12, 83.3%) strains (Table 1).

All sublineages IIIA-1 (6/6, 100%), IIIA-2 (22/22, 100%) and IIIC (7/7, 100%), and a small fraction of lineage IV (2/12, 16.7%) strains lacked the *lmo0036-0041* region (Table 1). Long-distance PCR targeting *lmo0035* and *lmo0042* further confirmed the above results. Two *lmo0036-0041*-negative lineage IV strains belonged to serovar 4a, however, the other four lineage IV serovar 4a strains and all 24 lineage III serovar 4a strains contained this region (Table 1), suggesting the presence of a complete *lmo0036-0043* cluster was dependent on lineages but not on serovars. In addition, *L. ivanovii*, which mainly infects ungulated animals, also carried the complete *lmo0036-0043* cluster, while another four nonpathogenic *Listeria* species, representing saprophytes that have adapted to free-living in soil and decaying vegetation, lacked the *lmo0036-0041* region (data not shown).

Our previous studies demonstrated that the *ascB-dapE* locus exhibits great diversity of internalin profiles, which serve as a potential marker for understanding the population structure and evolutionary history of *L. monocytogenes* (Chen *et al.*, 2009c; Chen *et al.*, 2012a; Chen *et al.*, 2012c). Here, we examined the genetic relationship between the presence of ADI and AgDI systems and *ascB-dapE* profiles using a larger strain collection. The presence of a complete *lmo0036-0043* cluster in *L. monocytogenes* was not fully related to *ascB-dapE* profiles (Table 1).

Interestingly, *lmo0036-0043*-complete and -deficient lineage IV strains exhibited a specific genetic organization of the *ascB-dapE* locus (Figure 1B). Ten *lmo0036-0043*-complete strains, regardless of serovars, harbored two genes encoding putative ABC transporters and one gene encoding a hypothetical protein (Figure 1B). Small stretches reminiscent of *inlG* (two segments with lengths

of 119 bp and 40 bp) and *inlE* (two segments with lengths of 155 bp and 203 bp) were found between these genes and *dapE* (Figure 1B). On the other hand, two *lmo0036-0043*-deficient lineage IV strains bore nothing except for shorter relics of *inlE* (22 bp) (Figure 1B).

ADI and AgDI systems are involved in listerial acid tolerance, and contribute to the enhanced adaption to acidic conditions in the host gastrointestinal tract, which is the key stage to initiate listerial infection (Chen *et al.*, 2009b; Chen *et al.*, 2011a; Ryan *et al.*, 2009).

Molecular evolution of ADI and AgDI systems might be a consequence of adaption to various environments that present various stress conditions for bacterial growth and survival. The *lmo0036-0043* cluster encoding ADI and AgDI systems is conserved in *L. monocytogenes* lineages I and II and the majority of lineage IV strains, but lacks the *lmo0036-0041* region in all lineage III and a small portion of lineage IV strains, suggesting that the presence of a complete *lmo0036-0043* cluster is dependent on lineages. *lmo0036-0043*-complete and -deficient lineage IV strains exhibit specific *ascB-dapE* profiles and might represent two subpopulations with distinct genetic characteristics adapting to different environmental niches. Further studies should be carried out to determine the expression and regulation of these genes under different conditions, and to examine the host specificities and pathogenicity of *lmo0036-0043*-complete and -deficient strains, with the purpose of establishing potential roles of ADI and AgDI systems in listerial survival under environmental stresses and pathogenesis within the host.

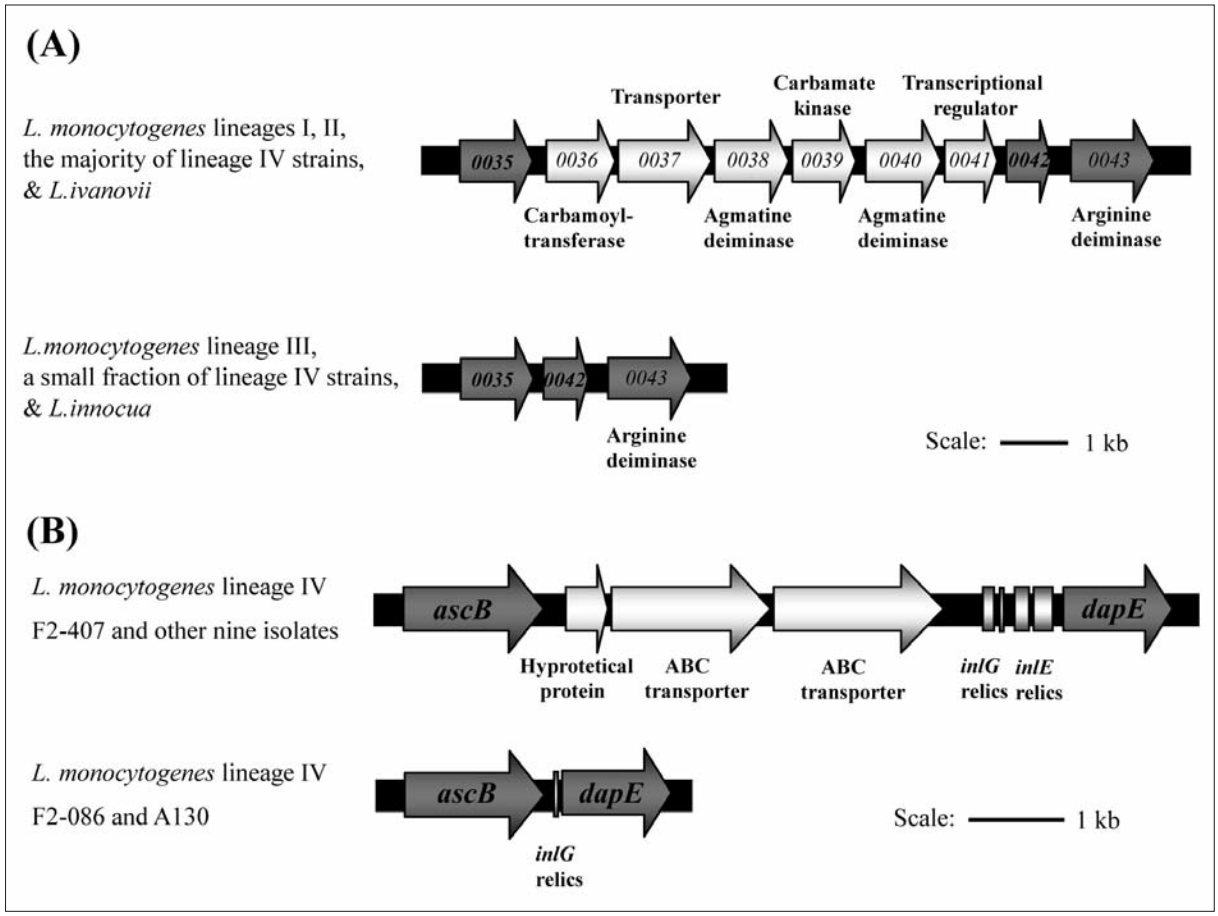

FIGURE 1 - *Genomic organization of (A) the lmo0036-0043 cluster encoding arginine deiminase and agmatine deiminase systems in L. monocytogenes and (B) the ascB-dapE locus among L. monocytogenes lineage IV strains. The diversity of gene contents (in white) is delimited by conseved genes (in dark grey). Arrows indicate gene orientation.*

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REFERENCES

CHEN J., JIANG L., CHEN X., LUO X., CHEN Y., YU Y., TIAN G., LIU D., FANG W. (2009a). *Listeria monocytogenes* serovar 4a is a possible evolutionary intermediate between *L. monocytogenes* serovars 1/2a and 4b and *L. innocua*. *J. Microbiol. Biotechnol.* **19**, 238-249.

CHEN J., JIANG L., CHEN Q., ZHAO H., LUO X., CHEN X., FANG W. (2009b). *lmo0038* is involved in acid and heat stress responses and specific for *L. monocytogenes* lineages I and II, and *L. ivanovii*. *Foodborne Pathog. Dis.* **6**, 365-376.

CHEN J., LUO X., JIANG L., JIN P., WEI W., LIU D., FANG W. (2009c). Molecular characteristics and virulence potential of *Listeria monocytogenes* isolates from Chinese food systems. *Food Microbiol.* **26**, 103-111.

CHEN J., CHEN Q., JIANG L., CHENG C., BAI F., WANG J., MO F., FANG W. (2010). Internalin profiling and multilocus sequence typing suggest four *Listeria innocua* subgroups with different evolutionary distances from *Listeria monocytogenes*. *BMC Microbiol.* **10**, 97.

CHEN J., CHENG C., XIA Y., ZHAO H., FANG C., SHAN Y., WU B., FANG W. (2011a). Lmo0036, an ornithine and putrescine carbamoyltransferase in *Listeria monocytogenes*, participates in arginine deiminase and agmatine deiminase pathways and mediates acid tolerance. *Microbiology.* **157**, 3150-3161.

CHEN J., XIA Y., CHENG C., FANG C., SHAN Y., JIN G., FANG W. (2011b). Genome sequence of a non-pathogenic *Listeria monocytogenes* serovar 4a strain M7. *J. Bacteriol.* **193**, 5019-5020.

CHEN J., FANG C., ZHENG T., ZHU N., BEI Y., FANG W. (2012a). Genomic presence of GadD1 glutamate decarboxylase correlates with the organization of *ascB-dapE* internalin cluster in *Listeria monocytogenes*. *Foodborne Pathog. Dis.* **9**, 175-178.

CHEN J., CHENG C., LV Y., FANG W. (2012b). Genetic diversity of internalin genes in the *ascB-dapE* locus among *Listeria monocytogenes* lineages III and IV strains. *J. Basic Microb.* In press.

CHEN J., FANG C., ZHU N., LV Y., CHENG C., BEI Y., ZHENG T., FANG W. (2012c). Genetic organization of *ascB-dapE* internalin cluster serves as a potential marker for *Listeria monocytogenes* sublineages IIA, IIB and IIC. *J. Microbiol. Biotechnol.* **22**, 575-584.

COTTER P.D., GAHAN C.G., HILL C. (2001). A glutamate decarboxylase system protects *Listeria monocytogenes* in gastric fluid. *Mol. Microbiol.* **40**, 465-475.

COTTER P. D., RYAN S., GAHAN C.G., HILL C. (2005). Presence of GadD1 glutamate decarboxylase in selected *Listeria monocytogenes* strains is associated with an ability to grow at low pH. *Appl. Environ. Microbiol.* **71**, 2832-2839.

DEGNAN B.A., FONTAINE M.C., DOEBEREINER A.H., LEE J.J., MASTROENI P., DOUGAN G., GOODACRE J.A., KEHOE M.A. (2000). Characterization of an isogenic mutant of *Streptococcus pyogenes* Manfredo lacking the ability to make streptococcal acid glycoprotein. *Infect. Immun.* **68**, 2441-2448.

GANDHI M., CHIKINDAS M.L. (2007). *Listeria*: A foodborne pathogen that knows how to survive. *Int. J. Food Microbiol.* **113**, 1-15.

GLASER P., FRANGEUL L., BUCHRIESER C., RUSNIOK C., AMEND A., BAQUERO F., BERCHE P., BLOECKER H., BRANDT P., CHAKRATORY T., CHARBIT A., CHETOUANI F., COUVE E., DE DARUVAR A., DEHOUX P., DOMANN E., DOMINGUEZ-BERNAL G., DUCHAUD E., DURANT L., DUSSURGET O., ENTIAN K.D., FSIHI H., PORTILLO F.G., GARRIDO P., GAUTIER L., GOEBEL W., GOMEZ-LOPEZ N., HAIN T., HAUF J., JACKSON D., JONES L.M., KAERST U., KREFT J., KUHN M., KUNST F., KURAPKAT G., MADUENO E., MAITOURNAM A., VICENTE J.M., NG E., NEDJARI H., NORDSIEK G., NOVELLA S., DE PABLOS B., PEREZ-DIAZ J.C., PURCELL R., REMMEL B., ROSE M., SCHLUETER T., SIMOES N., TIERREZ A., VAZQUEZ-BOLAND J.A., VOSS H., WEHLAND J., COSSART P. (2001). Comparative genomics of *Listeria* species. *Science* **294**, 849-852.

GRISWOLD A.R., CHEN Y.Y.M., BURNE, B.A. (2004). Analysis of an agmatine deiminase gene cluster in *Streptococcus mutans* UA159. *J. Bacteriol.* **186**, 1902-1904.

GRUENING P., FULDE M., VALENTIN-WEIGAND P., GOETHE R. (2006). Structure, regulation, and putative function of the arginine deiminase system of *Streptococcus suis*. *J. Bacteriol.* **188**, 361-369.

LIU D. (2006). Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J. Med. Microbiol.* **55**, 645-659.

LIU D., LAWRENCE M.L., WIEDMANN M., GORSKI L., MANDRELL R.E., AINSWORTH A.J., AUSTIN F.K. (2006). *Listeria monocytogenes* subgroups IIIA, IIIB, and IIIC delineate genetically distinct populations with varied pathogenic potential. *J. Clin. Microbiol.* **44**, 4229-4233.

Llacer J.L., Polo L.M., Tavarez S., Alarcon B., Hilario R., Rubio V. (2007). The gene cluster for agmatine catabolism of *Enterococcus faecalis*: study of recombinant putrescine transcarbamylase and agmatine deiminase and a snapshot of agmatine deiminase catalyzing its reaction. *J. Bacteriol.* **189**, 1254-1265.

Lucas P.M., Blancato V.S., Claisse O., Magni C., Lolkema, J.S., Lonvaud-Funel A. (2007). Agmatine deiminase pathway genes in *Lactobacillus brevis* are linked to the tyrosine decarboxylation operon in a putative acid resistance locus. *Microbiology.* **153**, 2221-2230.

Nelson K.E., Fouts D.E., Mongodin E.F., Ravel J., DeBoy R.T., Kolonay J.F., Rasko D.A., Angiuoli S.V., Gill S.R., Paulsen I.T., Peterson J., White O., Nelson W.C., Nierman W., Beanan M.J., Brinkac L.M., Daugherty S.C., Dodson R.J., Durkin A.S., Madupu R., Haft D.H., Selengut J., Aken S.V., Khouri H., Fedorova N., Forberger H., Tran B., Kathariou S., Wonderling L.D., Uhlich G.A., Bayles D.O., Luchansky J.B., Fraser C.M. (2004). Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. *Nucleic Acids Res.* **32**, 2386-2395.

Orsi R.H., den Bakker H.C., Wiedmann M. (2010). *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. *Int. J. Med. Microbiol.* **301**, 79-96.

Ryan S., Hill C., Gahan C.G. (2008). Acid stress responses in *Listeria monocytogenes*. *Adv. Appl. Microbiol.* **65**, 67-91.

Ryan S., Begley M., Gahan C.G., Hill C. (2009). Molecular characterization of the arginine deiminase system in *Listeria monocytogenes*: regulation and role in acid tolerance. *Environ. Microbiol.* **11**, 432-445.

Schonberg A., Bannerman E., Courtieu A.L., Kiss R., McLauchlin J., Shah S., Whihelms D. (1996). Serotyping of 80 strains from the WHO multicentre international typing study of *Listeria monocytogenes*. *Int. J. Food Microbiol.* **32**, 279-287.

Sleator R.D., Watson D., Hill C., Gahan C.G. (2009). The interaction between *Listeria monocytogenes* and the host gastrointestinal tract. *Microbiology* **155**, 2463-2475.

Swaminathan B., Gerner-Smidt P. (2007). The epidemiology of human listeriosis. *Microbes Infect.* **9**, 236-1243.

Wiedmann M., Bruce J.L., Keatine C., Johnson A.E., McDonough P.L., Batt C.A. (1997). Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. *Infect. Immun.* **65**, 2707-2716.

Zhao H., Chen J., Fang C., Xia Y., Cheng C., Jiang L., Fang W. (2011). Deciphering the biodiversity in *Listeria monocytogenes* lineage III strains by polyphasic approaches. *J. Microbiol.* **49**, 759-767.