Biochemical and Biophysical Research Communications 454 (2014) 399–403

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Comparative analysis of CRISPR loci in different *Listeria monocytogenes* lineages

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ARTICLE INFO

Article history:
Received 9 September 2014
Available online 13 October 2014

Keywords:
Listeria monocytogenes
Lineage
CRISPR
Self-targeting spacer

ABSTRACT

Listeria monocytogenes, an important food-borne pathogen, causes high mortality rate of listeriosis. Pan-genomic comparisons revealed the species genome of *L. monocytogenes* is highly stable but not completely clonal. The population structure of this species displays at least four evolutionary lineages (I–IV). Isolates of different lineages displayed distinct genetic, phenotypic and ecologic characteristics, which appear to affect their ability to be transmitted through foods and to cause human disease, as well as their ability to thrive in markedly phage-rich environments. CRISPR (clustered regularly interspaced short palindrome repeats), a recently described adaptive immunity system, not only confers defense against invading elements derived from bacteriophages or plasmids in many bacteria and archaeal, but also displays strains-level variations in almost any given endowed species. This work was aimed to investigate CRISPR diversity in *L. monocytogenes* strains of different lineages and estimated the potential practicability of the CRISPR-based approach to resolve this species' biodiversity. Only a third of strains contained all three CRISPR loci (here defined as LMa, LMb and LMc) at same time. Combined the strain-level variations in presence/absence of each CRISPR locus and its relative size and spacer arrangements, a total of 29 CRISPR genotypes and 11 groups were defined within a collection of 128 strains covering all serotypes. The CRISPR-based approach showed powerful ability to subtype the more commonly food-borne isolates of serotype 1/2a (lineage II) and serotypes 1/2b (lineage I), but limited by the absence of typical CRISPR structure in many lineage I isolates. Strikingly, we found a long associated cas1 gene as well as two self-targeting LMb spacers accidently homologous with endogenous genes in a fraction of serotype 1/2a isolations, demonstrated that CRISPR I B system might involve in bacterial physiology besides antiviral immunity.

1. Introduction

The Gram-positive bacterium *Listeria monocytogenes* is one of the leading causes of death from food-borne pathogens. Compared with other microorganisms, the ability to survive under the salinity, alkalinity and cold stress environment makes *L. monocytogenes* a significant challenge in food production. A large number of genotyping and phylogenetic studies [1–4] have shown that isolates of *L. monocytogenes* form at least four evolutionary lineages (lineages I–IV). While lineages I isolates are associated with most human clinical cases and outbreaks, lineage II isolates seem to be widespread, especially in foods, and mainly common in sporadic human clinical cases. Whilst isolates of lineage III and IV were rare and predominantly isolated from animal sources. ~98% of human listeriosis caused by the more common serotype 1/2a and 1/2c (lineage II) and serotype1/2b and 4b (lineage I) [5]. Lineage II isolates seem to own more mobile elements and higher recombination rate than lineage, but lineage I seemed to be higher pathogenic [3,5]. Overall, the four *L. monocytogenes* lineages identified so far represent different genetic, phenotypic and ecologic characteristics, which appear to affect their ability to be transmitted through foods and to cause human disease as well as their ability to thrive in environment surround abundant and ubiquitous phages [6–8].

Pan-genomic comparisons revealed the species genome of *L. monocytogenes* is highly stable but not completely clonal [9]. One important examples is the adaptive immunity CRISPR/Cas system, composed of the clustered regularly interspaced short palindromic repeats and CRISPR-associated genes (cas), could obtain via

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http://dx.doi.org/10.1016/j.bbrc.2014.10.018
0006-291X/

horizontal gene transfer (HGT) [6,10]. The CRISPR/*cas* system was confirmed to confer resistance to exogenous genetic elements such as plasmids and phages in many bacteria and archaeal [11–13]. Strain-level variations in presence/absence of a given CRISPR locus and the number of repeat-spacer units in that locus were exploited for genotyping in several instances [14–17]. Prior studies indicated the presence of three CRISPR loci in *L. monocytogenes* genome [7,9,18]. Locus 1, described as CRISPR RliB, probably involved in strain 1/2a EGD-e virulence, which completely devoid of *cas* genes [19]; locus 2 located approximately ~10 kb downstream of locus 1, associated with *cas* subset *"Tneap"* (*cas2*, *cas1*, (*cas4*), *cas3*, *cas5t*, *cst1*, *cst2*, *cas6*), *belongs to* CRISPR/Cas systems type-I (subtype I-B) according the current classification method [9,20,21]; while locus 3 located in strain 1/2a EGD-e at about 2.7 Mb, associated with *cas* subset *"Nmeni"* (*cas2*, *csn 2*, *can1*, *cas1*), belongs to type-II (subtype II-A). Noticeably, the defective cas-less CRISPR RliB array was the substrate of polynucleotide phosphorylase (PNPase) and probably functionally linked with locus 2 array, as they share many similar features, however the machinery behind has not been thoroughly [18]. The most understanding locus 3 thus hypothesize to be able to silence foreign elements in a host which is able to supply an RNase III enzyme as well as a trans-encoded sRNA (tracrRNA) [22].

Here, we investigated the CRISPR/CAS systems in 128 *L. monocytogenes* strains, including 38 strains with completed genomes in NCBI database and a collection of 90 isolates from North China food (NCFLM) (Table s2) [23]. Combined collections are thought to achieve full coverage of all serotypes, thus providing a reliable frame work for understanding CRISPR polymorphism among distinct serotypes or lineages. We also inquired the CRISPR structure in protecting the pathogen against foreign stressors by relating the spacers with the sequences of known bacteriophages or with plasmid in NCBI database. The CRISPR/Cas system maybe is the key point of the protective system in *L. monocytogenes*, which would be helpful for the future the food processing industry.

2. Materials and methods

2.1. Strains and growth conditions

All of these strains were routinely growing overnight (16–18 h) at 37 °C in Luria–Bertani (LB) broth prior to DNA extraction. The samples were randomly collected from the markets from May to October of each year. Strain isolation and identification were performed as described previously [23].

2.2. Genomic DNA extraction

Chromosomal DNA from each of the *L. monocytogenes* isolates was extracted by using Qiagen Dneasy Blood and Tissue Kit.

2.3. PCR amplification

Oligonucleotide primers (Table 1) were designed following the alignment of conserved sequences that flank each CRISPR locus of available genomes. PCRs for CRISPR loci amplification of NCFLM strains were performed with *ExTaq* polymerase (Takara) and corresponding primer pairs in a Mastercycler Gradient (Eppendorf) thermal cycler under the following conditions: 5 min at 95 °C, 30 s at 95 °C, 30 cycles of 30 s at 95 °C for denaturation, followed by 30 s at 60 °C for annealing; and 1 min (CRISPR LMa)/8 min (CRISPR LMb)/6 min (CRISPR LMb) at 72 °C for extension. Then PCR products were purified using the MinElute PCR cleanup kit (Qiagen). The PCR products were cloned in plasmid pMD20-T using the pMD20-T cloning kit (Takara Bio Inc., Shiga, Japan), then sequenced by Invitrogen Biotechnology Co., Ltd. (Guangzhou, China).

2.4. Bioinformatic analysis

CRISPR spacers and repeats were identified using CRISPRFinder (Grissa et al., 2007 [20]). The similarity of each spacer to sequences deposited in GenBank was analyzed by BLASTN, using an *E-value* cutoff of 0.1. Only sequences pointing to elements outside the CRISPR loci were considered as legitimate hits. Considering that phage sequences evolve rapidly and that sampling of the viral and plasmid world is still incomplete, we searched for proto-spacers with a less stringent identity criterion (i.e. .94% ⩽ 2 base mismatch).

3. Results and discussion

3.1. Organizations diversity of CRISPR arrays and cas genes

Genome screen of all 38 completed *L. monocytogenes* genomes, only three CRISPR loci (here defined as LMa, LMb, LMc, respectively) are found in the same corresponding genomic locus, but not each strain harbored all of them (Fig. 1, Table s2). Conceivably, PCR amplifications of the three CRISPR region were not obtained for all NCFLM stains. Besides the ubiquitous LMa region, PCR amplifications for LMb and LMc regions gave products for only 35 and 15 strains, respectively. Together all 128 *L. monocytogenes* analyzed, 53 strains (41.4%) were found to encompass *cas* genes, in other wards had the CRISPR-based immunity function. Among them, only a fraction of strains (32.08%) contained two set *cas* genes in one genome. Table s2 summarizes the most relevant features of the CRISPR loci found in all analyzed strains.

Intriguingly, the typical repeat of both CRISPR LMa (GTTTTAGTTACTTATTGTGAAATGTAAAT) and that of LMb (GTTTTAACTACTTATTATGAAATGTAAAT) were 29 bp long and different in only three un-adjacent nucleotides, belonging to Kunin's cluster1 [25]. While the typical repeat of LMc (GTTTTGTTAGCATTCAAAATAACATAGCTCTAAAAC) was 36 bp long belonging to Kunin's cluster 10.

Table 1
Primers used for amplifications of CRISPR loci.

CRISPR loci	Name	Sequence (5′–3′)	Used for
Locus 1	P1	TTGAGGTAAGATGGGAGTAAG	Amplification and sequencing of CRISPR LMa locus
	P2	ACAGATTGCTCGTTTGACTA	
Locus 2	P3	TGCTACCACATCGCCAGACACCAC AACC	Amplification and sequencing of CRISPR LMb locus
	P4	TGCTCGCAGACCTGAACTCCACCAACG	
Locus 1 + locus 2	*Imp-c*	AAACTCGCAGCCAAGACAT	Amplification of the sequence from CRISPR LMa to LMb
	Pm-c	AGAACCGCATCAGACCAAC	
	Rppk-c	AACCGTAAACCAAGACCACAT	
Locus 3	P5	GGGTCTATTTGGGCTGGTG	Amplification and sequencing of CRISPR LMc locus
	P6	GCGACGATTAGCGAGTTG	
	LMcc	CGTTCACGGTGGTGGTTAC	Amplification and sequencing of CRISPR LMc locus and *cas* genes

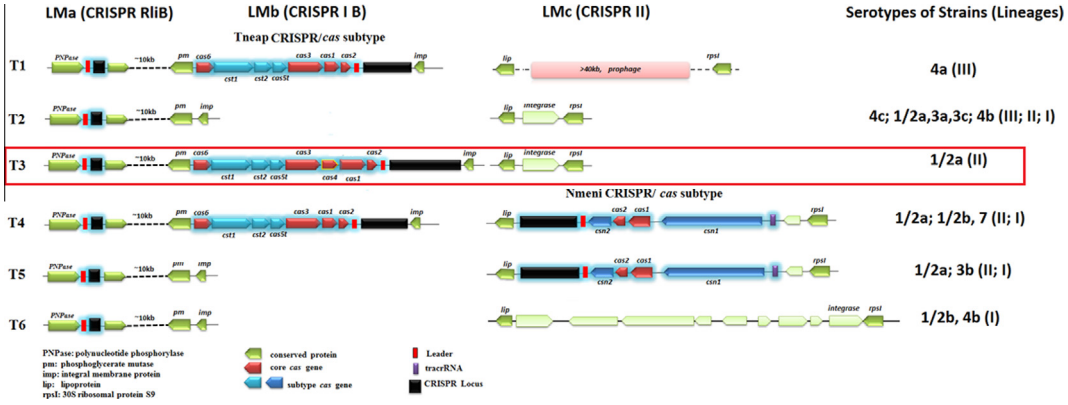

Fig. 1. CRISPR regions in *Listeria monocytogenes* genomes. Genetic elements are arranged according to their relative position in the chromosome. Light blue shadows represent CRISPR/cas locus. A thin black line connects core genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Unlike other repeat clusters, neither cluster 1 nor cluster 10 can form a secondary hairpin [25]. The machinery why *L. monocytogenes* prefer non-repeat RNA hairpin was unknown at present, but other elements (spacers for CRISPR RliB; trancRNA for CRISPRII) were confirmed to be needed when transcribed pro-RNA processed into mature crRNA [18,22].

3.2. Arrangement diversity in CRISPR arrays

The numbers of spacers in each CRISPR array displayed large variations among the different *L. monocytogenes* stains. Two extremes were found: (i) little or no: in two strains (*La111* and *N53-1*) [26], they retained only five and four repeats respectively in CRISPR LMa, and the spacers degenerated to 1–4 base pairs residues (Fig. s1); (ii) large more than fifty, some strains (C1-387, Finland1998 and 20 NCFLM trains) contained over 50 spacers (even achieved 58) in CRISPR LMb. This supported the formerly guess by accident that *cas*4 which only contained by the up 22 larger LMb arrays, might involve in spacer acquisition.

In detail, 15 distinct genotypes were resolved for CRISPR LMa, as well as twelve were resolved for both LMb and LMc (Fig. 2). The new defined CRISPR RliB (LMa) occupied all analyzed *L. monocytogenes* genomes, contained between zero and ten spacers. A charming set of nine alternative arrangements of 16 spacers (a1–a16 color background in Fig. 2) might divergent from a same ancestor clone by homologous recombinant. Combined the variation from strain *La111* to strain *N53-1* (Fig. s1), two nearly unchanged strains at 99% sequence identity isolated at two different fish processing plants sampled 6 years apart [26], it conceivably speculate that the CRISPR LMa should be degenerating gradually through random

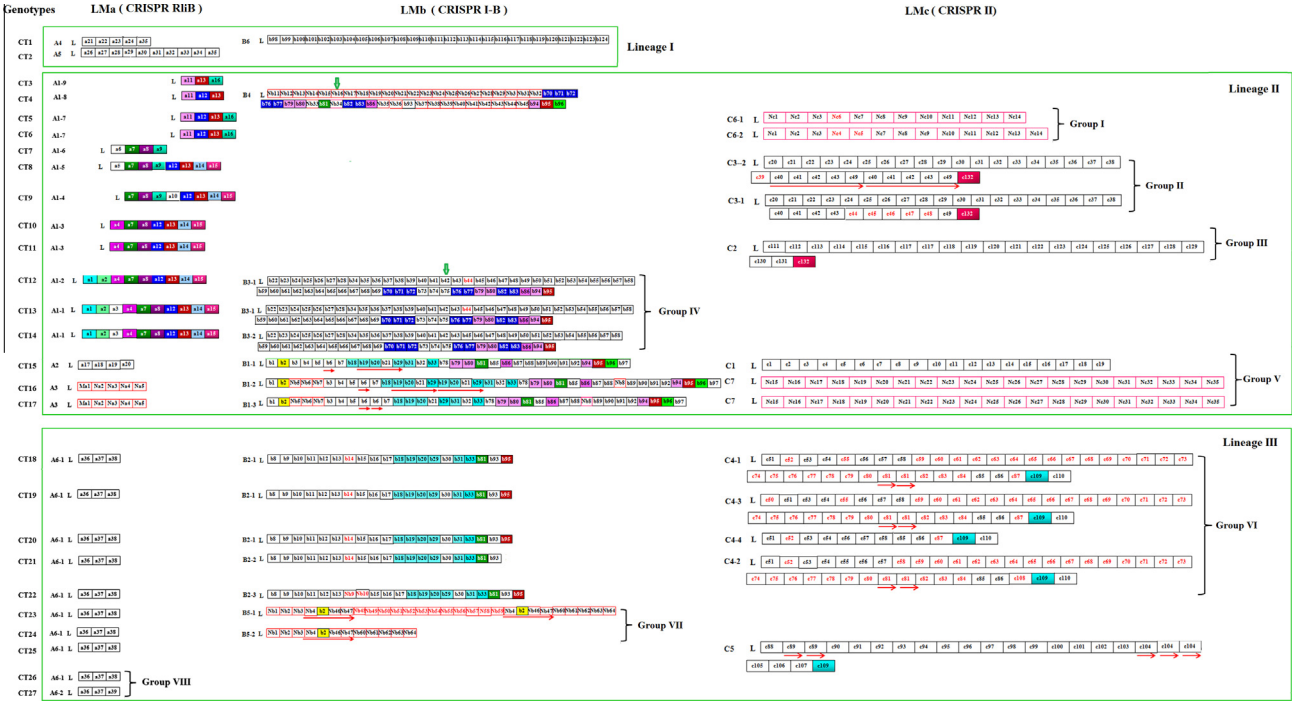

Fig. 2. Graphic representation of spacers across three CRISPR loci in all *Listeria monocytogenes* genomes analyzed. Repeats are not included. Published spacers show in black border; the newly discovered spacers in this study show in red border; unique spacers appear in white background; identical spacers in multiple genotypes are marked using identical number and a same color background scheme, respectively. Red font represent the spacer varied in subtypes. Duplication events within the same CRISPR are marked by red arrows. The self-targeting spacers are marked by green arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

spacers deletion. In contrast, the relatively least probable LMc array (18.75%) (CRISPR II), contained between 12 and 40 spacers in only a fraction of lineage I and II strains rather than lineage III ones (Fig. 2). At this array, almost each host showed its own unique genotype, while no spacers were shared among strains with distinct serotypes. Between them was the LMb array (CRISPR I B), which contained between 12 and 58 spacers (Fig. 2). Unlike LMc locus, LMb had more common spacers shared among strains of different serotypes even different evolution lineages (color background in Fig. 2). This suggested that the LMb array might inserted into the *L. monocytogenes* before LMc, and the latter has not had time to add more same spacers.

When all three CRISPR loci were combined, the 128 strains could be assigned to six CRISPR loci patterns (T1–6) (Fig. 1) and 28 distinct genotypes (CT0, CT1–27) (Table s2, Fig. 2). In several cases, variations between strains were obtained due to mere deletion or addition, as well as duplication of one or more spacers, while in others entire locus were added or at some point divergent evolution occurred at the leader end of the array, leading to the formation of eight major genotypic groups following cluster analysis (Fig. 2).

Irrespective of serotypes, a low degree of genetic homogeneity among *L. monocytogenes* strains from same country but distinct incidents is reflected, herein. On the other hand, the CRISPR/Cas content does not seem to be equally frequent among isolates of different serotypes, even belonging to same lineage, as the homologous recombination (HR) rate was not equally among isolates of different lineages [1,27]. Strains of the representative 1/2a serotype of lineage II, which usually displayed high recombination rate [27], showed the most variations. Altogether, 55 strains contained 106 CRISPR arrays displayed fourteen different genotypes (CT4–17) (Fig. 2, Table s2). Whilst all isolates of another lineage II serovar (1/2c) shared only one CRISPR array (CT7, Fig. 2). The probably explanation is that all 25 NCFLM isolates was a same clone, which also confirmed by the approach of pulsed-field gel electrophoresis (PFGE) [23]. Although very few evidences of HR have been observed among lineage I isolates by now [1,8], there were also many strains contained two or three CRISPR loci. For examples, fourteen analyzed 1/2b strains contained total 26 CRISPR loci displayed eight genotypes (CT18–24, 27) (Table s2), as well as only one strain of serovar 3b or 7 (lineage I) showed one or two big functional CRISPR loci, respectively. But we did not found any *cas* genes in all serotype 4b (lineage I) isolates as before. Conclusion the results mentioned above, we found that strains of a certain lineage can be divided into distinct CRISPR genotype groups, otherwise we did not found any group comprised strains belonging to different lineages. Furthermore, lineage I and II strains may diverge from a same ancestor, as they shared nine spacers with each other at the opposite end of leader in LMb array (Fig. 2). However, No spacer was found to share between lineage III and the lineage I or II in all three CRISPR loci, implied a big phylogenic niche between them. Intriguingly, the perfectly identity CRISPR arrangements (LMa and LMb) shared by all three serotype 4a (lineage III) isolates, which isolated time spanned half a century and geographically across three continents (Fig. 2, Table s2). This demonstrated the poor environmental adaptation ability of isolates of lineage III in another side, and also echoed the past studies that the source and the numbers of lineage III isolations were less [28]. Since the spacers of CRISPR are considered as historical imprint remained in host DNA from intruders [29], the above results suggested that strains of a certain lineage probably diverge to different genotypes if their encounter with different invaders, while it seems to be inevitable to divergent evolution between different lineages strains as they differed in phage specificity and HR rate caused by the different cell wall constitution and different living environments.

Compared the lineage III, strains of lineage II and I seemed to more variable, especially the ones of serotype 1/2a (lineage II) and 1/2b (lineage I). The CRISPR-based genotyping scheme at this point seemed to be more powerful than PFGE (Table s2). For example, PFGE I type and O type strains can be divided into two distinct genotypes [23]. However, its applications was limited by the absence of typical CRISPR structure in many lineage I isolates, especially all strains of serovar 4b, the most common serotype associated with human listeriosis [28]. To some extent, CRISPR-based genotyping is only suitable for typing *L. monocytogenes* isolated from a small background.

3.3. Analysis of CRISPR spacers

A total of 399 different CRISPR spacers were identified, and were numbered as progressively (in 5′ to 3′ orientation with the locus name ahead). The spacers' average length of both CRISPR LMa and LMb were 36 bp, ranging from 33 to 42, only about one third (80/231) in total were of that long; However, over 90% of the LMc spaces were 30 bp long, with only twelve variants. It suggested that LMc array (CRISPR II) seemed to be more active [16]. Two notable findings must be mentioned (Supplemental Table s1): (i) only two bases (GC) were different between LMa spacer a9 and LMb spacer Nb20 at the 3′ end. This might echoed the previous hypothesis that CRISPR LMa and LMb shared the *Tneap* set of *cas* genes adjacent to LMb array (Sesto et al., 2014 [18]). But it is also possible that CRISPR LMa own the similar set of *cas* genes a long time ago. In addition, the fact that spacer a9 and Nb20 were not contained by the same genome, suggested they acquired independently. (ii) Two LMc spacer alleles (c60 and c132) may target the same proto-spacer, only one SNP was present in the middle (G/A). Spacer c60 shared 100% identity with the fragment of bacteria phage A500. Compared with c60, c132 located on the farthest end of leader, thus added earlier than the former before the adaptive mutation of the phage A500 took place. Furthermore, spacer duplications are easy encountered in both CRISPR LMb and LMc, even repeated five units (c40-c41-c42-c43-c49) in LMc array in strains *SLCC5850* CRISPR. However, we did not find two duplication items in one CRISPR array or in two arrays of a same genome. To be worth, duplicated spacers might greatly reinforce the host defense ability to the respective phages.

3.4. Targets of CRISPR/CAS system

We searched NCBI blastn database (http://blast.st-va.ncbi.nlm.nih.gov/) for homologous proto-spacers and the responding adjacent motifs (PAMs) [30] for all the 399 unique spacers. Only about one third spacers (166) showed matches above the selected cutoff, with 19% of them showed 100% identity (Supplemental Table s1). Almost all matches were similar to bacteriophage or prophage sequences, with only three noteworthy exceptions (spacer b42, Nb16 and c101), which pointed to chromosomal DNA excluding prophages. The first two located in LMb array showed 100% identity with fragment ubiquitous in all complete *L. monocytogenes* genomes, and will illustrate below. The last one (c101) was LMc spacer of strain *SLCC2540*, the only analyzed strain of serotype 3b (Fig. 2, Table s2), and showed 97% identity with sequences of two Gram-negative plant pathogen genomes (*Erwinia carotovora* subsp. *atroseptica SCRI1043* and *Pectobacterium atrosepticum* strain *JG10–08*), apparently provided one more evidence for the previous presumption that CRISPR can transmit among prokaryotes across species. We yet did not find any spacers showed homologous to the plasmid sequences [7,18].

In accordance with Sesto's study [18], CRISPR LMa and LMb shared an identical PAM "CCA" at the 5′ end, while CRISPR LMc spacers showed homologous to proto-spacers which possessing

"NGG" at the 3′ end (data not show). Unfortunately, no preference for a proto-spacers strand (sense or antisense) of a gene or a phenomenon were found in our research supported the currently accepted perception of DNA, not RNA as the CRISPR immune target in eubacterium, obtained randomly, non-directional [29].

Furthermore, several self-targeting spacers [31] were found. Besides ones mentioned in Sesto's report [18] presumably inactive or weak negative fitness effects, we found other two interesting items. Both are LMb (CRISPR I-B) spacers and perfect matched to their endogenous genes conserved in all complete *L. monocytogenes genomes* with consensus PAM (CCA) at the 5′ end. One (b42) from strain *Finland1998* and *C1–387* as well as eight NCFLM strains (*LM11*, *LM18*, *LM86*, *LM55–59*) (CT12–14, Fig. 2), targeted *aadB* gene (ATP-dependent nuclease). Another one showed homologous with the diguanylate cyclase (GGDEF domain) gene, just found in four NCFLM strains (CT4, Fig. 2)), whose PCR productions showed 99% identity with that of *C1–387*. Both targeting genes conserved in all 1/2a strains (>95%), presumably involve in the many bacterial physiology, such as cell differentiation, biofilm formation and virulence generation. Furthermore, adjacent the arrays contained each self-targeting spacer, we found a longer *cas1* gene (Fig. s2), which seem to exhibit nuclease activity as *YgbT* gene in *Escherichia coli* [24]. These indicated that in addition to antiviral immunity, the LMb loci (CRISPR I B) might also have a function in bacterial physiology regulations [32,33] in a fraction of serotype 1/2a isolations. But the functional mechanism needs further research.

In conclusion, CRISPR of *L. monocytogenes* isolates showed some lineages related features, since isolates of different lineages encountered distinct environments and displayed distinct HR rates. What's more, some of the lineage I strains may have mechanisms that limit the acquisition of foreign DNA by horizontal gene transfer [3,8], which leads to the lineage I strains to obtain CRISPR structure is very difficult, resulting CRISPR-based genotyping application has been limited in *L. monocytogenes* species.

Acknowledgments

This work was supported by the Major State Basic Research Development Program of China (973 Program) (No. 2010CB35704; 2012CB723705), the National Key Technology R&D Program (No. 2012BAD28B09; 2014BAD13B00) and the National Natural Science Foundation of China (No. 31201363), and grants from the University Research Committee and Research Coordinating Council of South China University of Technology.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.018.

References

[1] H.C. den Bakker, X. Didelot, E.D. Fortes, Lineage specific recombination rates and microevolution in *Listeria monocytogenes*, BMC Evol. Biol. 8 (2008) 277.
[2] D. Liu, M.L. Lawrence, M. Wiedmann, *Listeria monocytogenes* subgroups IIIA, IIIB, and IIIC delineate genetically distinct populations with varied pathogenic potential, J. Clin. Microbiol. 44 (2006) 4229–4233.
[3] R.H. Orsi, H.C. den Bakker, M. Wiedmann, *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics, Int. J. Med. Microbiol. 301 (2011) 79–96.
[4] T.J. Ward, T.F. Ducey, T. Usgaard, Multilocus genotyping assays for single nucleotide polymorphism -based subtyping of *Listeria monocytogenes* isolates, Appl. Environ. Microbiol. 74 (2008) 7629–7642.
[5] R.W. Pinner, A. Schuchat, B. Swaminathan, Role of foods in sporadic listeriosis. II. Microbiologic and epidemiologic investigation, JAMA 267 (1992) 2046–2050.
[6] H. Deveau, J.E. Garneau, S. Moineau, CRISPR/Cas system and its role in phage-bacteria interactions, Annu. Rev. Microbiol. 64 (2010) 475–493.
[7] C. Kuenne, A. Billion, M.A. Mraheil, Reassessment of the *Listeria monocytogenes* pan-genome reveals dynamic integration hotspots and mobile genetic elements as major components of the accessory genome, BMC Genomics 14 (2013) 47.
[8] M. Ragon, T. Wirth, F. Hollandt, A new perspective on *Listeria monocytogenes* evolution, PLoS Pathog. 4 (2008) e1000146.
[9] P. Horvath, R. Barrangou, CRISPR/Cas, the immune system of bacteria and archaea, Science 327 (2010) 167–170.
[10] L.A. Marraffini, E.J. Sontheimer, CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea, Nat. Rev. Genet. 11 (2010) 181–190.
[11] D.H. Haft, J. Selengut, E.F. Mongodin, A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes, PLoS Comput. Biol. 1 (2005) e60.
[12] R. Barrangou, C. Fremaux, H. Deveau, CRISPR provides acquired resistance against viruses in prokaryotes, Science 315 (2007) 1709–1712.
[13] L.A. Marraffini, E.J. Sontheimer, CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA, Science 322 (2009) 1843–1845.
[14] Y. Cui, Y.J. Li, O. Gorgé, Insight into microevolution of *Yersinia pestis* by clustered regularly interspaced short palindromic repeats, PLoS ONE 3 (2008) e2652.
[15] R. Fabio, H.M. Theo, D. Brion, Diversity, evolution, and functionality of clustered regularly interspaced short palindromic repeat (CRISPR) regions in the fire blight pathogen *Erwinia amylovora*, Appl. Environ. Microbiol. 77 (2011) 3819–3829.
[16] P. Horvath, A.R. Dennis, C.M. Anne-Claire, Diversity, activity, and evolution of CRISPR loci in *Streptococcus thermophilus*, J. Bacteriol. 190 (2008) 1401–1412.
[17] C. Pourcel, G. Salvignol, G. Vergnaud, CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA and provide additional tools for evolutionary studies, Microbiology 151 (2005) 653–663.
[18] N. Sesto, M. Touchon, J.M. Andrade, A PNPase dependent CRISPR system in listeria, PLoS Genet 10 (1) (2014) e1004065–4065.
[19] A. Toledo-Arana, O. Dussurget, G. Nikitas, The Listeria transcriptional landscape from saprophytism to virulence, Nature 459 (2009) 950–956.
[20] I. Grissa, G. Vergnaud, C. Pourcel, CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats, Nucleic Acids Res. 35 (2007) 52–57.
[21] K.S. Makarova, D.H. Haft, R. Barrangou, Evolution and classification of the CRISPR-Cas systems, Nat. Rev. Microbiol. 9 (2011) 467–477.
[22] D. Elitza, C. Krzysztof, M.S. Cynthia, CRISPR RNA maturation by *trans*-encoded small RNA and host factor RNase III, Nature 471 (2011) 602–607.
[23] He. Yan, B.N. Sucharit, Z.Y. Mo, Prevalence and characterization of antimicrobial resistance of foodborne *Listeria monocytogenes* isolates in Hebei province of Northern China, 2005–2007, Int. J. Food Microbiol. 144 (2010) 310–316.
[24] M. Babu, N. Beloglazova, F. Robert, A dual function of the CRISPR-Cas system in bacterial antivirus immunity and DNA repair, Mol. Microbiol. 79 (2011) 484–502.
[25] V. Kunin, R. Sorek, P. Hugenholtz, Evolutionary conservation of sequence and secondary structures in CRISPR repeats, Genome Biol. 8 (2007). R61.
[26] A. Holch, K. Webb, O. Lukjancenko, Genome sequencing identifies two nearly unchanged strains of persistent *Listeria monocytogenes* isolated at two different fish processing plants sampled 6 years apart, Appl. Environ. Microbiol. 79 (2013) 2944–2951.
[27] Y.H. Tsai, R.H. Orsi, K.K. Nightingale, *Listeria monocytogenes* internalins are highly diverse and evolved by recombination and positive selection, Infect. Genet. Evol. 6 (2006) 378–389.
[28] G.T. Jeffers, J.L. Bruce, P.L. McDonough, Comparative genetic characterization of *Listeria monocytogenes* isolates from human and animal listeriosis cases, Microbiology 147 (2001) 1095–1104.
[29] A. Bolotin, B. Quinquis, A. Sorokin, S.D. Ehrlich, Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extra chromosomal origin, Microbiology 151 (2005) 2551–2561.
[30] F.J.M. Mojica, C. Díez-Villaseñor, J. García-Martínez, Short motif sequences determine the targets of the prokaryotic CRISPR defence system, Microbiology 155 (2009) 733–740
[31] A. Stern, L. Keren, O. Wurtzel, Self-targeting by CRISPR: gene regulation or autoimmunity?, Trends Genet 26 (2010) 335–340.
[32] T.R. Sampson, D.S. Weiss, Alternative Roles for CRISPR/Cas Systems in Bacterial Pathogenesis, PLoS Pathog. 9 (2013) e1003621.
[33] R.S. Timothy, S.W. Davi, CRISPR-Cas systems: new players in gene regulation and bacterial physiology, Cell. Infect. Microbiol. 4 (2014) 37.