Biochemical and Biophysical Research Communications 464 (2015) 894–900

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Analysis of the features of 45 identified CRISPR loci in 32 *Staphylococcus aureus*

Siyu Yang, Jing Liu, Fuye Shao, Pengfei Wang, Guangcai Duan, Haiyan Yang*

Department of Epidemiology, College of Public Health, Zhengzhou University, Zhengzhou, Henan, China

ARTICLE INFO

Article history:
Received 18 June 2015
Accepted 13 July 2015
Available online 17 July 2015

Keywords:
CRISPR/Cas
Staphylococcus aureus
Genomes
Spacer
MLST

ABSTRACT

Staphylococcus aureus (*S. aureus*) is a common pathogen that can cause serious infections, even death. Because of the horizontal gene transfer (HGT) of antibiotic resistance genes, the drug resistant condition is becoming increasingly prevalent. Recently, an adaptive immunity system, named clustered regularly interspaced short palindromic repeats (CRISPR), was discovered and demonstrated to confer a defense against foreign invading elements that may carry the antibiotic resistance genes. In this study, we reveal the features of 45 identified CRISPR loci and the CRISPR associated gene (Cas) in 32 *S. aureus* strains from CRISPR database. Five spacers of *S. aureus* 08BA02176 and MSHR1132 were homologous with foreign genetic sequences from phages or plasmids, even containing a spacer sequence identical to part of some phages' genomes containing lukPV gene that encodes the PVL toxin. Many *S. aureus* strains with the same CRISPR type shared the same MLST type. CRISPR loci that had 3 or more similar protein loci mostly belonged to the same CRISPR type. We came to the conclusion that the CRISPR/Cas of strains 08BA02176 and MSHR1132 were inherited from a common ancestor or recombined from *Staphylococcus lugdunensis*. CRISPR loci can be mobilized and can transfer among different but closely related species, and the same types of MLST strains exhibit a higher affinity to the same types of CRISPR loci. Bacteriophages may be the predominant challenge facing *S. aureus*. The CRISPR/Cas structure may limit the transmission of bacterial virulence among *S. aureus*.

1. Introduction

Staphylococcus aureus is an important pathogen that can lead to a series of diseases, including endocarditis, pneumonia, toxic shock syndrome and food poisoning, thereby resulting in serious health problems [1–3]. In recent years, the drug resistance rates and the drug resistance degree of the *S. aureus* have been increasing sharply [3], which is directly linked to the HGT of antibiotic resistance genes by mobile genetic elements (MGE), including bacteriophages, plasmids, *S. aureus* pathogenicity islands, transposons and *staphylococcal* cassette chromosomes (SCC) [2]. During the evolution course of microorganisms, many mechanisms, such as abortive infection, adsorption inhibition, and the blocking of foreign DNA injection, were formed to make microorganisms' genomes relatively stable [4]. CRISPR is a newly identified prokaryotic adaptive defense system that provides resistance against alien replicons, such as viruses and plasmids [5,6]. CRISPR loci can not only counteract multiple routes of HGT but also limit the spread of antibiotic resistance in pathogenic bacteria [7].

CRISPR, which is made up of direct repeats of almost identical sequences separated by unique spacer sequences sharing similar lengths, is found in approximately 40% of bacteria analyzed to date [5]. In a certain CRISPR locus, the repeats always have palindromic structures and may develop RNA secondary structures composed of “loops” and “stems” [5]. Although spacers in one locus share a similar sequence length, they are quite diverse in the nucleotide composition [6,8]. Researchers have found that parts of the spacers derive from foreign gene elements outside their own genome [7,9–11] and confer immunity against viruses and plasmids containing regions complementary to the spacers. Cas genes were always found near a repeat locus [12]. Through CRISPR, bacteria and archaea acquire resistance to invading viruses and plasmids by 3

Abbreviations: S. aureus, *Staphylococcus aureus*; HGT, horizontal gene transfer; CRISPR, clustered regularly interspaced short palindromic repeats; MLST, multilocus sequence type; MGE, mobile genetic elements; Cas genes, CRISPR-associated genes; MFE, minimum free energy; CDRs, consensus direct repeat sequences; CDSs, Cas coding domain sequences.

* Corresponding author. Department of Epidemiology, College of Public Health, Zhengzhou University, No. 100 of Science Road, Zhengzhou 450001, China.
E-mail address: yhy@zzu.edu.cn (H. Yang).

http://dx.doi.org/10.1016/j.bbrc.2015.07.062
0006-291X/

steps to degrade the invading nucleic acid by dedicated nucleases [4,13–15].

Analyzing the structure systematically may help us explore more potential functions in bacteria. However, there is seldom a systematic bioinformatics analysis of the structure of the *S. aureus* stains with genome sequences that are published. In this study, we used 45 identified CRISPR loci from the CRISPR database to explore the structural features of CRISPR and its possible functions in *S. aureus* using bioinformatics. We analyzed the homology between spacers and the foreign invading elements (bacteriophages and plasmids), as well as the factors that may influence the stability of the repeat sequence's RNA secondary structure. We further compared the CRISPR type with the MLST type of *S. aureus*. The CRISPR/Cas system may play a key role in limiting the transformation of toxin genes in *S. aureus*.

2. Materials and methods

2.1. Data source

All information about the *S. aureus* CRISPR loci was available through the CRISPR database (http://crispr.u-psud.fr/) and the most recent update time was 2014/08/05. The information about the MLST of the total 32 *S. aureus* was searched through NCBI's PubMed (http://www.ncbi.nlm.nih.gov/pubmed/). The proteins adjacent to the CRISPR loci were searched through the NCBI Nucleotide database.

2.2. Analysis methods

Multi-sequence alignment was performed using Clustal X. The following grouping standard was that the sequence should be identical. TreeView 1.6.6 was used to draw the unrooted tree plots. The RNA secondary structures of the repeats as well as the minimum free energy (MFE) were predicted by RNA fold Web Server (http://rna.tbi.univie.ac.at/). As for the Fold algorithms, we chose the MFE and the output options were set as default. The homologous sequences of spacers were searched by NCBI BLAST (Default Parameters, nr database, mismatches $\leq$ 3). The Cas1 and Cas2 proteins in the remaining 30 *S. aureus* genomes and the multi-sequences alignment of the proteins were searched by BLASTP (identity $\geq$ 90%, cover rate $\geq$ 90%). The SPSS21.0 software was used to analyze the differences among the MFE of different RNA secondary structures.

3. Results

3.1. CRISPR loci of S. aureus in the CRISPR database

Thirty-nine *S. aureus* strains have been found to hold 234 CRISPR loci in the CRISPR database. Forty-five CRISPR loci were identified and the remaining 189 loci were questionable structures. Our research focused on the information of the 45 CRISPR loci in 32 strains. Each of 19 strains held only one CRISPR locus, accounting for 59.4%. The remaining 40.6% of strains all contained 2 CRISRR loci in their genome. The numbers of spacers in different loci range from 1 to 15. The loci with 2 spacers were the majority.

3.2. Repeat sequences

3.2.1. Grouping of repeat sequences

Repeat sequences are always similar and even identical in one CRISPR loci, so we chose the consensus direct repeat sequences (CDRs) of each locus as the representatives to alignment. There were 15 groups and the CDRs in the same group were all the same (Supplemental table 1).

3.2.2. Clustering of repeat sequences

Fifteen CDRs were chosen from 15 groups as the deputations. According to the unrooted tree (Supplemental Figure 1), the 45 CDRs sequences were divided into 3 clusters. The first cluster included groups 1–5, 7, 8 and 15 and 37 CDRs that all had a short sequence "TGTTGGGGCCCCGC." The second cluster embodied 5 CDRs from groups 6, 9, 10 and 11 with a common sequence: "TGCAAGTTGGCGGGGCC(C/A)CAA(C/T)A(T/C)AG". Groups 12, 13 and 14 involving 3 CDRs were assigned to the last cluster with a common sequence "TCGATAACTACCCCGAA (G/T) AA" (Fig. 1).

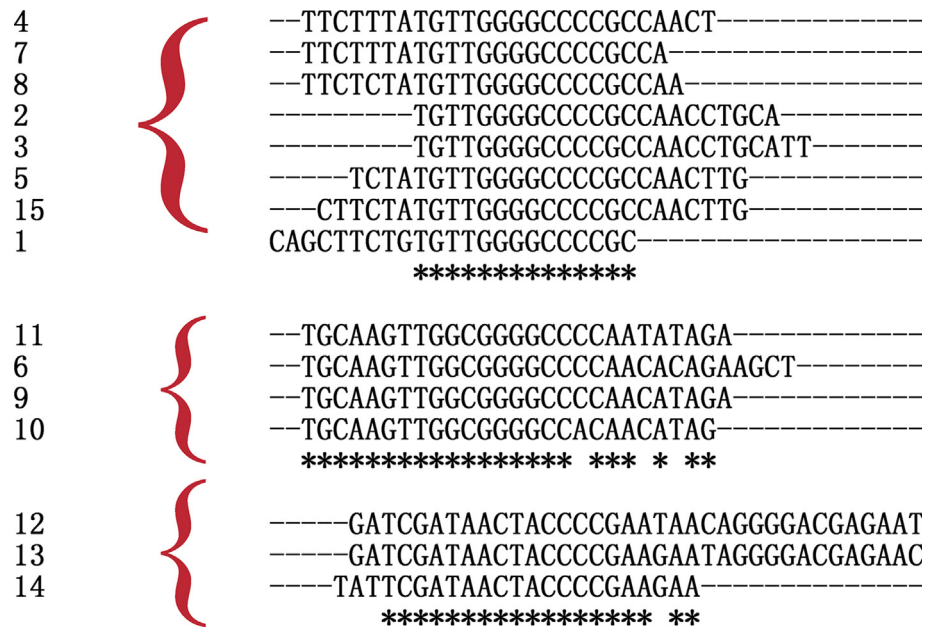

Fig. 1. The clustering result of the repeat sequences. "*" represented the similar base pairs. The numbers from 1 to 15 represented the 15 groups of repeat sequences.

3.2.3. RNA secondary structure of repeat sequences

The secondary structures as well as the MFE of 15 representative CDRs were predicted by RNA fold (Fig. 2).

All of the 45 CDRS could form conservative dumbbell-shaped RNA secondary structures with two "rings" at both poles and a "stem" in the middle. The "rings" were predominant in the structures. The RNA secondary structures could be classified into 3 categories according to their stem length (Table 1).

3.2.4. Stability of RNA secondary structure of repeat sequences

In the first cluster, the difference between the MFE of categories A and C was significant ($U = 0.000$, $P = 0.002$, $\alpha = 0.05$, Supplemental Figure 2-1). The MFE of category A was lower than category C, meaning that the RNA secondary structures with the "stems" of 5 base pairs might be more stable than the structures of 3 base pairs "stems". The secondary cluster contained 5 sequences and the lengths of the "stems" were 3, 4, 5, 5 and 5 base pairs, respectively, with the corresponding MFEs −2.30 kcal/mol, −3.50 kcal/mol, −4.30 kcal/mol, −4.30 kcal/mol and −4.30 kcal/mol. The structures with a longer "stem" tended to have a lower MFE and a more stable structure in the second cluster (Supplemental Figure 2-2). In the third cluster, there were 3 sequences from group 12, 13 and 14, and they all had "stems" 4 base pairs long. Their MFEs were all −8.20 kcal/mol, except for group 14, in which a high "GC" ratio sequence "TAGGGGACGAGAAC" was lost in comparison with the other two sequences.

3.3. Spacers

3.3.1. Multi-sequence alignment of spacers

Seventy-five spacers of the 45 CRISPR loci in our research were divided into 45 groups (Fig. 3) according to the results of the multi-sequence alignment.

3.3.2. The homologous analysis of spacers

The blast results were shown in Table 2.

Four spacers were homologous with some phages genomes, and only 1 (NC_018608_1N) showed a high sequence similarity with sequences of the plasmid SAP020A. All 5 spacers belonged to the genomes of *S. aureus* 08BA02176 and *Staphylococcus aureus subsp. aureus* MSHR1132. Spacer NC_016941_1F was identical to a phage that carries the lukPV gene encoding the PVL toxin.

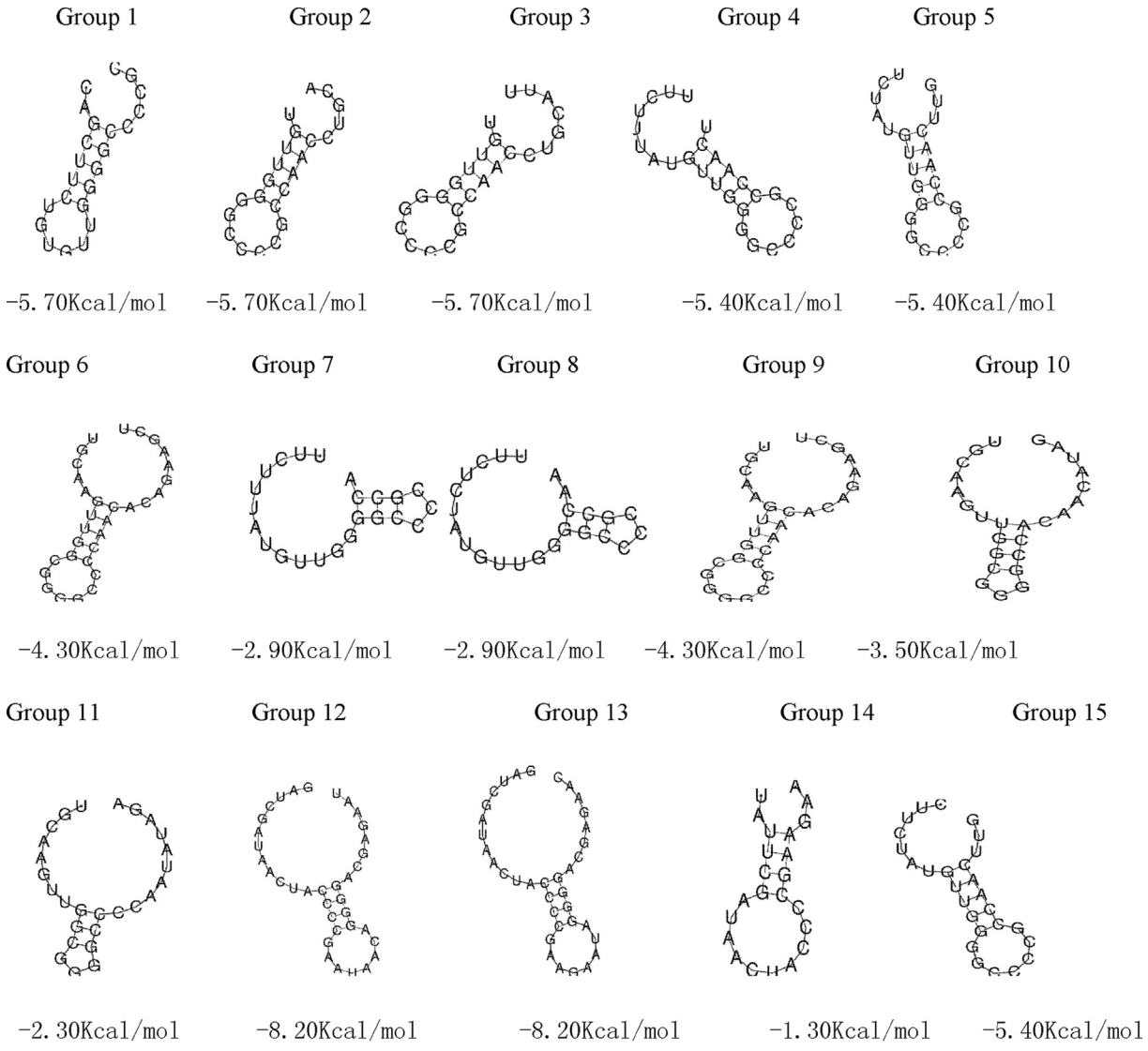

Fig. 2. The RNA secondary structures and the MFE of the 15 group of CDRs.

Table 1
Three categories of RNA secondary structures.

Category	Stem length (base pairs)	Groups	Number of CDRs	Percentage of the total CDRs number
A	5	1–6, 9, 15	37	82.2%
B	4	10, 12, 13, 14	4	8.9%
C	3	7, 8, 11	4	8.9%

3.4. CRISPR and MLST

3.4.1. Typing and clustering of CRISPR loci

The CRISPR loci were typed according to the standard that the same type of CRISPR loci should be equally long and share the same CDRs and spacers. Moreover, the order and amounts of spacers should also be the same.

There were in total 17 types (Supplementary Table 2). The 17 types were chosen and divided into 3 clusters on the basis of the unrooted tree (Supplemental Figure 3). Group 1–5, group 7–10 and group 17 belonged to cluster 1. The second cluster contained group 6 and groups 11–13. The remaining 3 groups (Group 14–16) were included in the third cluster.

3.4.2. The CRISPR types and the MLST types

The results of the CRISPR types and the MLST types of strains were shown in Table 3a, Table 3b and Table 3c, and the references to the strains' MLST types were shown in Supplemental Table 3.

In the first cluster, 6 out of 8 strains containing CRISPR type A + E shared the same MLST type 5 with an agreement rate of 75.0%. Five of 6 CRISPR type B strains were ST8 with an agreement rate of 83.3%. Three strains of CRISPR type C were all ST59, the agreement rate reaching 100%. Three out of 4 strains with CRISPRs of type D were ST239, and the agreement rate was 75.0%. In the second cluster, 2 strains of CRISPR type A had MLST type 398.

3.4.3. The similarities between proteins flanking the CRISPR loci

We searched the Cas1 and Cas2 proteins in the CRISPR database and found that the two core Cas proteins existed only in two strains (*Staphylococcus aureus* 08BA02176 and *Staphylococcus aureus subsp. aureus* MSHR1132) but not the remaining 30 *Staphylococcus aureus*.

We searched 6 protein loci adjacent to each CRISPR loci (3 upstream and 3 downstream). The CRISPR loci with the more than 3 protein loci having similar proteins were shown (Fig. 4). We found that the CRISPR loci with 3 or more flanking protein loci that can find the similar proteins mostly belonged to the same CRISPR type.

4. Discussion

CRISPR is a widespread system providing acquired resistance against phages and viruses, possibly by an RNA interference-like mechanism [15–17]. There were more questionable structures located in the *S. aureus* genomes. In each of identified loci, only a small number of spacers were located, except for the loci in the

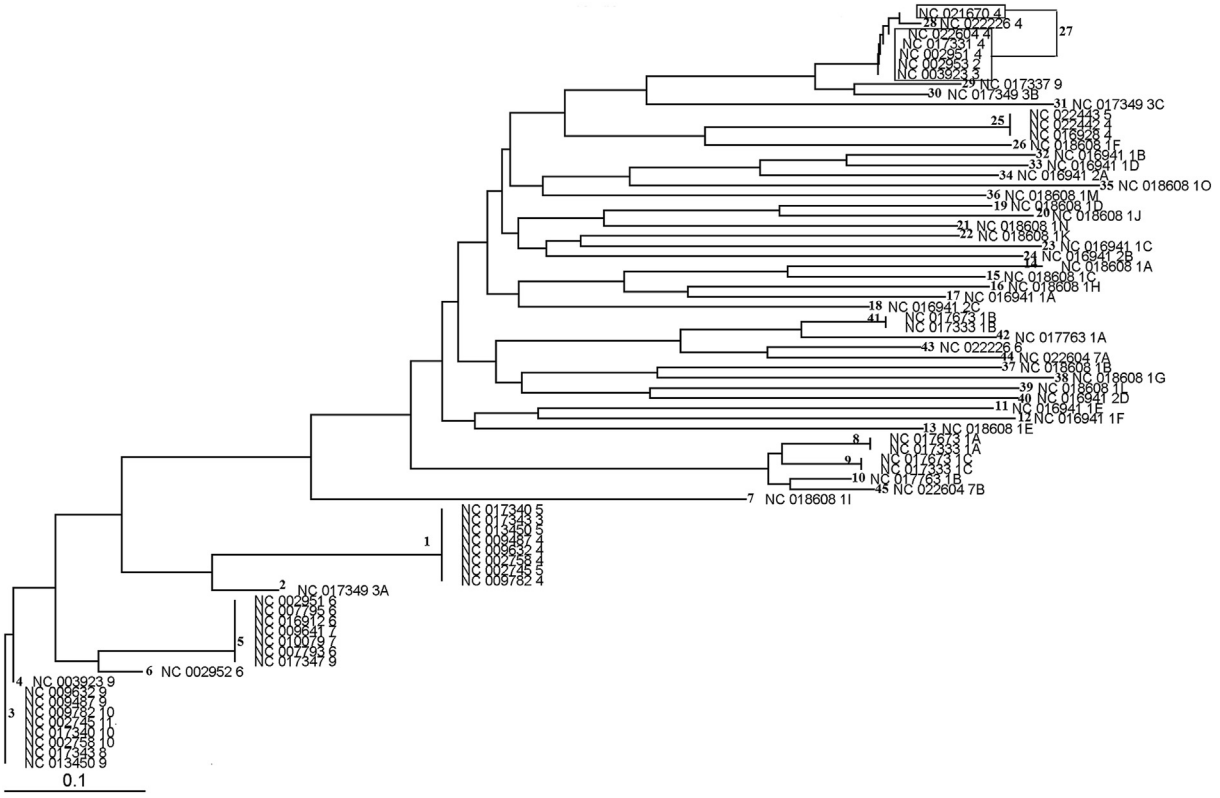

Fig. 3. Grouping results of 75 spacers. The numbers from 1 to 45 represented the 45 groups. The evolutionary distance scale is 0.1. The spacer ID represented the corresponding spacers.

Table 2
Genetic elements exhibiting similarity to spacer sequences.

Strains	Spacer id	Sequences	Similar phage GI	Product	Similar plasmid
subsp. aureus MSHR1132	NC_016941_1F	CACGCTGTAGTGAAGTATAGAAACGGCATGAGTACAAT	589626950, 402761649, 402761585, 514343602, 398256436, 475990627, 456174244, 349732033, 349731990, 302749846, 215260398, 116235513, 154818103, 119225778, 62086035, 62086034, 62086018, 46917482, 809088637,475908549, 121309263, 8918747, 62086038, 62086030, 8918415	putative phi PVL-like protein	–
08BA02176	NC_018608_1N	TCATCTTTCATGTCACTGATTAATTCATTTGTA	–	–	plasmid SAP020A
08BA02176	NC_018608_1K	TTTTCTTTAACTGTTTTTACTGCCCATTTAATAGT	735998439, 525336474	–	–
subsp. aureus MSHR1132	NC_016941_1D	GTTTTTCATAGTTAATCAATCCCTTTTCTTTTTT	398255565, 670139430	–	–
08BA02176	NC_018608_1M	AAGTTAACGGCATTACCTAATAAAAATATTTTAGG	365189246, 365189224,	–	–

Similarity was identified by BLAST/nr with default settings. Only hits similar over the whole length of the query are shown. In general, these contain no more than 3 mismatches from the query.

strain 08BA02176 and strain MSHR1132, whose repeat sequences showed strikingly variation from that of the other *S. aureus*. Moreover, the Cas genes were only found in the genomes of the two strains.

Considering this phenomenon, we analyzed the identified CRISPRs in 5 strains (*Staphylococcus epidermidis* RP62A, *Staphylococcus lugdunensis* HKU09-01, *S. lugdunensis* N920143, *Staphylococcus pasteuri* SP1, *Staphylococcus pseudintermedius* ED99), which were closely related with *S. aureus* species. The multi-sequence alignment of the CRISPR loci showed the CRISPR loci in the 2 *S. aureus* strains had high similarity with the *S. lugdunensis* HKU09-01 and *S. lugdunensis* N920143. The Cas coding domain sequences (CDSs) in MSHR1132 were homologous and syntenic with those in the genomes of *S. epidermidis* RP62A and *S.*

Table 3a
The MLST type and CRISPR type in CRISPR cluster 1.

Strain	CRISPR ID	CRISPR type	CRISPR of strain	MLST type	Agreement rate
Sa 04-02981	NC_017340_5	A	A + E	ST225	75.0%
	NC_017340_10	E			
Sasa ED98	NC_013450_5	A	A + E	ST 5	
	NC_013450_9	E			
Sasa JH1	NC_009632_4	A	A + E	ST 5	
	NC_009632_9	E			
Sasa JH9	NC_009487_4	A	A + E	ST5	
	NC_009487_9	E			
Sasa Mu3	NC_009782_4	A	A + E	ST 5	
	NC_009782_10	E			
Sasa Mu50	NC_002758_4	A	A + E	ST 5	
	NC_002758_10	E			
Sasa N315	NC_002745_5	A	A + E	ST 5	
	NC_002745_11	E			
Sasa ECT-R2	NC_017343_3	A	A + E	–	
	NC_017343_8	E			
Sasa VC40	NC_016912_6	B	B	ST8	83.3%
Sasa NCTC 8325	NC_007795_6	B	B	ST8	
Sasa str.Newman	NC_009641_7	B	B	ST8	
Sasa USA300	NC_007793_6	B	B	ST8	
Sasa USA300_TCH1516	NC_010079_7	B	B	ST8	
Sasa T0131	NC_017347_9	B	B	ST239	
Sasa M013	NC_016928_4	C	C	ST59	100%
Sasa SA40	NC_022443_5	C	C	ST59	
Sasa SA957	NC_022442_4	C	C	ST59	
Sasa MSSA476	NC_002953_2	D	D	ST1	75.0%
Sasa TW20	NC_017331_4	D	D	ST239	
Sasa Z172	NC_022604_4	D	D	ST239	
Sa Bmb9393	NC_021670_4	D	D	ST239	
Sasa COL	NC_002951_4	D	D + B	ST250	
	NC_002951_6	B			
Sasa MW2	NC_003923_3	D	D + J	–	
	NC_003923_9	J			
Sasa LGA251	NC_017349_3	H	H	ST425	
Sasa MRSA252	NC_002952_6	F	F	ST 36	
Sasa ED133	NC_017337_9	I	I	ST133	
Sasa CN1	NC_022226_4	G	G	ST72	

"*Sa*" represents *Staphylococcus aureus*.
"*Sasa*" represents *Staphylococcus aureus* subsp. *aureus*.
"A", "B", "C", "D", "E", "F", "G", "H", "I" and "J" represent group 1, 2, 3, 4, 5, 7, 8, 9, 10 and 17, respectively.

Table 3b
The MLST type and CRISPR type in CRISPR cluster 2.

Strain	CRISPR ID	CRISPR type	CRISPR of strain	MLST type	Agreement rate
Sasa 71193	NC_017673_1	a	a	ST398	100%
Sasa S0385	NC_017333_1	a	a	ST398	
Sasa HO5096 0412	NC_017763_1	b	b	ST22	
Sasa CN1	NC_022226_6	c	c	ST72	
Sasa Z172	NC_022604_7	d	d	ST239	

"*Sasa*" represents *Staphylococcus aureus* subsp. *aureus*.
"a", "b", "c" and "d" represent group 6, 11, 12 and 13.

Table 3c
The MLST type and CRISPR type in CRISPR cluster 3.

Strain	CRISPR ID	CRISPR type	CRISPR of strain	MLST type
Sasa MSHR1132	NC_016941_1	II	II	ST1850
	NC_016941_2	III	III	
Sasa 08BA02176	NC_018608_1	I	I	ST398

"*Sasa*" represents *Staphylococcus aureus* subsp. *aureus*.
"I," "II" and "III," represent groups 14, 15 and 16, respectively.

lugdunensis HKU09-01. We infer that the CRISPR loci in *S. aureus* 08BA02176 and MSHR1132 may partly belong to the CRISPR of *S. lugdunensis* species, and not only CRISPR loci but also the Cas proteins adjacent to them can be mobilized, meaning the CRISPR/Cas structure can transfer among different but closely related species. We also found the CRISPR locus in the strain 08BA02176 was located in the J1 region of a SCC*mec* V element and the CRISPR loci in the strain MSHR1132 adjacent to a SCC*mec* IVa structure were inserted into the orfX region [18–21]. This makes it possible that the loci in the two *S. aureus* strains were obtained through recombination from different but closely related species. Strong evidence for HGT of CRISPR/Cas in other genera may also support our viewpoints [12,22].

A previous study showed that the longer CRISPR arrays appeared to be more active than short arrays in spacer acquisition [23]. Moreover, there were no Cas 1 or Cas 2 proteins, and the probable proto-spacer from the plasmids and phages were not found in the strains genomes except for stain 08BA02176 and MSHR1132. We can infer the CRISPR loci in the two strains were more active and the CRISPR loci of the other strains in the database were inactive or have no function at present.

Mojica et al. first reported the palindromic structure of repeats [24], and an experiment demonstrated the transcription of CRISPRs into non-messenger RNAs [25]. Our study showed the CDRs in the 32 *S. aureus* could all form stable RNA secondary structures of stem-loops. One study showed that stem-loop structures of some repeats may serve to facilitate recognition-mediated contact between the spacer-targeted foreign RNA or DNA and Cas-encoded proteins [5]. We infer that the stability of RNA secondary structures may influence the function of CRISPR. Moreover, we found the influencing factors of the RNA secondary structures may be the following: in the same cluster, the first may be the length of the "stems". The longer the "stem," the higher the stability of the secondary structure becomes, resulting in a higher possibility of a functional structure. Other factors, such as the length of the repeat sequence and its "GC" content, may influence the stability, as well. The longer the repeat sequence is the more stable the structure may be. In the same cluster, repeats of equal length having a higher "GC" content are the more stable.

Researchers speculated that spacers have an extra-chromosomal origin and may show us the history of past challenges from mobile genetic elements [9]. Our results are consistent with this speculation. There were more phages than plasmids that are homologous with the spacers, indicating the high occurrence frequency of phages' attack in the two strains' evolutionary history and the importance of phages in the process of gaining new spacers. We also found that Spacer NC_016941_1F was identical to part of phage sequences that contained the lukPV genes encoding the PVL toxin. A study screened 126 CC75 isolates of their collection, in which the strain MSHR1132 also belonged to, for the luk*PV* genes, and all were negative [19]. Thus, this is evidence for the potential of CRISPRs in *S. aureus* to impede the acquisition of toxin

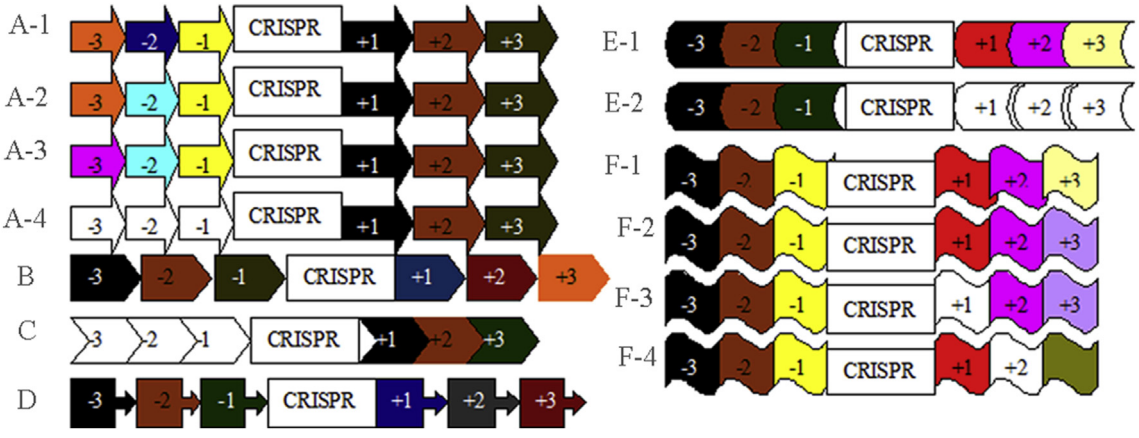

Fig. 4. The similarities between proteins flanking the CRISPR loci. The "CRISPR" markers represented for different CRISPR Loci. "−3" to "+3" were the protein loci adjacent to the CRISPR and different shapes represented different proteins. "A", "B", "C", "D", "E", "F" were 5 groups and in the same group, the same color represented the same proteins. The CRISPRS IDs of each group were: A-1: NC_017340_5, NC_009782_4, NC_002758_4; A-2: NC_013450_5; A-3: NC_017343_3, NC_009632_4, NC_009487_4; A-4: NC_002745_5; B: NC_017340_10, NC_017343_8, NC_009632_9, NC_009487_9, NC_009782_10, NC_002758_10, NC_013450_9, NC_003923_9, NC_002745_11, NC_002952_6; C: NC_018608_1, NC_016941_2; D: NC_021670_4, NC_022226_4, NC_002951_4, NC_002953_2, NC_003923_3, NC_017331_4, NC_022604_4; E-1: NC_017673_1, NC_017333_1; E-2: NC_017763_1; F-1: NC_002951_6, NC_007795_6; F-2: NC_022443_5, NC_022442_4,NC_017347_9, NC_007793_6, NC_016928_4; F-3: NC_009641_7; F-4: NC_010079_7, NC_016912_6.

genes and reveals the possibility of using CRISPR to limit the transmission of bacterial virulence among *S. aureus*.

During our work, we found the same type of CRISPR loci could appear in different *S. aureus* strains that most had the same MLST type, and that the same types of CRISPR loci mostly shared some similar, even identical, proteins adjacent to the CRISPR loci.

The same MLST type strains may be closely related with each other and be the descendants of a same ancestor. The strains can inherit the same CRISPR loci, as well as certain important conserved proteins adjacent to the CRISPR loci from the common ancestor, similar to JH9, which is a progeny of the isolate JH1 [26]. Another possibility may be that because the CRISPR loci are able to be mobilized, the same type of MLST strains may show higher affinity to the same type of CRISPR loci, as well as certain conserved proteins that may bear some important functions.

In conclusion, our research primarily analyzed the structure of repeat sequences and spacers, as well as the flanking conserved proteins, and this study speculated on the functions of each part, which helps to elucidate the associations between CRISPR structures and their possible functions. CRISPR's possible functions in preventing the transition of some toxin genes demonstrated its importance in limiting the infection of *S. aureus*.

Acknowledgments

Our study was funded by grants from the National Natural Science Foundation of China (81401700) and was supported by the Important National Science and Technology Specific Projects (2013ZX10004607).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.07.062.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.07.062.

References

[1] B. Shopsin, B.N. Kreiswirth, Molecular epidemiology of methicillin-resistant Staphylococcus aureus, Emerg. Infect. Dis. 7 (2001) 323–326.
[2] J.A. Lindsay, Staphylococcus aureus genomics and the impact of horizontal gene transfer, Int. J. Med. Microbiol. 304 (2014) 103–109.
[3] E.A. Larkin, R.J. Carman, T. Krakauer, et al., Staphylococcus aureus: the toxic presence of a pathogen extraordinaire, Curr. Med. Chem. 16 (2009) 4003–4019.
[4] J.M. Sturino, T.R. Klaenhammer, Engineered bacteriophage-defence systems in bioprocessing, Nat. Rev. Microbiol. 4 (2006) 395–404.
[5] V. Kunin, R. Sorek, P. Hugenholtz, Evolutionary conservation of sequence and secondary structures in CRISPR repeats, Genome Biol. 8 (2007) R61.
[6] G. Gasiunas, T. Sinkunas, V. Siksnys, Molecular mechanisms of CRISPR-mediated microbial immunity, Cell Mol. Life Sci. 71 (2014) 449–465.
[7] L.A. Marraffini, E.J. Sontheimer, CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA, Science 322 (2008) 1843–1845.
[8] R. Barrangou, C. Fremaux, H. Deveau, et al., CRISPR provides acquired resistance against viruses in prokaryotes, Science 315 (2007) 1709–1712.
[9] A. Bolotin, B. Quinquis, A. Sorokin, et al., Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin, Microbiology 151 (2005) 2551–2561.
[10] F.J. Mojica, C. Diez-Villasenor, J. Garcia-Martinez, et al., Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements, J. Mol. Evol. 60 (2005) 174–182.
[11] S.J. Brouns, M.M. Jore, M. Lundgren, et al., Small CRISPR RNAs guide antiviral defense in prokaryotes, Science 321 (2008) 960–964.
[12] D.H. Haft, J. Selengut, E.F. Mongodin, et al., A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes, PLoS Comput. Biol. 1 (2005) e60.
[13] B. Wiedenheft, In defense of phage: viral suppressors of CRISPR-mediated adaptive immunity in bacteria, RNA Biol. 10 (2013) 886–890.
[14] L.A. Marraffini, E.J. Sontheimer, CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea, Nat. Rev. Genet. 11 (2010) 181–190.
[15] E. Deltcheva, K. Chylinski, C.M. Sharma, et al., CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III, Nature 471 (2011) 602–607.
[16] R. Sorek, V. Kunin, P. Hugenholtz, CRISPR—a widespread system that provides acquired resistance against phages in bacteria and archaea, Nat. Rev. Microbiol. 6 (2008) 181–186.
[17] P. Mali, L. Yang, K.M. Esvelt, et al., RNA-guided human genome engineering via Cas9, Science 339 (2013) 823–826.
[18] G.R. Golding, L. Bryden, P.N. Levett, et al., whole-genome sequence of livestock-associated st398 methicillin-resistant staphylococcus aureus Isolated from Humans in Canada, J. Bacteriol. 194 (2012) 6627–6628.
[19] D.C. Holt, M.T. Holden, S.Y. Tong, et al., A very early-branching Staphylococcus aureus lineage lacking the carotenoid pigment staphyloxanthin, Genome Biol. E vol 3 (2011) 881–895.
[20] G.R. Golding, L. Bryden, P.N. Levett, et al., Livestock-associated methicillin-resistant Staphylococcus aureus sequence type 398 in humans, Canada, Emerg. Infect. Dis. 16 (2010) 587–594.
[21] A.M. Hanssen, J.U. Ericson Sollid, SCCmec in staphylococci: genes on the move, FEMS Immunol. Med. Microbiol. 46 (2006) 8–20.
[22] M. Touchon, E.P. Rocha, The small, slow and specialized CRISPR and anti-CRISPR of Escherichia and Salmonella, PLoS One 5 (2010) e11126.
[23] P. Horvath, D.A. Romero, A.C. Coute-Monvoisin, et al., Diversity, activity, and evolution of CRISPR loci in Streptococcus thermophilus, J. Bacteriol. 190 (2008) 1401–1412.
[24] F.J. Mojica, C. Diez-Villasenor, E. Soria, et al., Biological significance of a family of regularly spaced repeats in the genomes of Archaea, bacteria and mitochondria, Mol. Microbiol. 36 (2000) 244–246.
[25] T.H. Tang, J.P. Bachellerie, T. Rozhdestvensky, et al., Identification of 86 candidates for small non-messenger RNAs from the archaeon Archaeoglobus fulgidus, Proc. Natl. Acad. Sci. U. S. A 99 (2002) 7536–7541.
[26] K. Sieradzki, T. Leski, J. Dick, et al., Evolution of a vancomycin-intermediate Staphylococcus aureus strain in vivo: multiple changes in the antibiotic resistance phenotypes of a single lineage of methicillin-resistant S. aureus under the impact of antibiotics administered for chemotherapy, J. Clin. Microbiol. 41 (2003) 1687–1693.