Characterization of a functional insertion sequence ISSau2 from Staphylococcus aureus

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Abstract

Background: ISSau2 has been suggested as a member of the IS150 f subgroup in the IS3 family. It encodes a fusion transposase OrfAB produced by programmed −1 translational frameshifting with two overlapping reading frames orfA and orfB. To better characterize ISSau2, the binding and cleaving activities of the ISSau2 transposase and its transposition frequency were studied.

Results: The purified ISSau2 transposase OrfAB was a functional protein in vitro since it bound specifically to ISSau2 terminal inverted repeat sequences (IRs) and cleaved the transposon ends at the artificial mini-transposon pUC19-IRR. In addition, the transposition frequency of ISSau2 in vivo was approximately 1.76 ± 0.13 × 10−3, based on a GFP hop-on assay. Furthermore, OrfB cleaved IRs with the similar catalytic activity of OrfAB, while OrfA had no catalytic activity. Finally, either OrfA or OrfB significantly reduced the transposition of ISSau2 induced by OrfAB.

Conclusion: We have confirmed that ISSau2 is a member of IS150/IS3 family. The ISSau2 transposase OrfAB could bind to and cleave the specific fragments containing the terminal inverted repeat sequences and induce the transposition, suggesting that ISSau2 is at least partially functional. Meanwhile, both OrfA and OrfB inhibited the transposition by ISSau2. Our results will help understand biological roles of ISSau2 in its host S. aureus.

Keywords: ISSau2, Functional insertion sequence, IS150, Transposition frequency

Background

Insertion sequences are ubiquitous in prokaryote and eukaryotes. They exert a major effect on genome evolution. Previously, we have suggested that an insertion sequence ISSau2 in Staphylococcus aureus was probably a member of the IS150 subgroup in the IS3 family based on the sequence structure and searching results from the ISfinder database [1]. Members of the IS3 family have a general structure, consisting of a single transposase gene flanked by terminal inverted repeats (IRs) and the transposase gene contains two open reading frames, orfA and orfB. The transposase contains a DNA-binding helix-turn-helix (HTH) motif which specifically recognizes the transposon inverted repeats [2] and a DDE domain which catalyzes transposition reactions [3]. The IS3 family can be further divided into six subgroups (IS150, IS407, IS51, IS3, IS2, and IS911) based on the structure of insertion sequences and alignment of their OrfB sequences [4]. Whether ISSau2 functions as a member of the IS150 subgroup remains to be determined.

While searching the ISfinder database, 11 subgroups/groups of insertion sequences contain two open reading frames, orfA and orfB. Among them, 8 (IS1, IS150, IS407, IS51, IS3, IS2, IS427, and IS630) definitely use OrfB as the transposase. At the same time, which protein (OrfA, OrfB or OrfAB) functions as a transposase in the other 3 subgroups/groups (IS21, IS605, IS607) remains elusive [4, 5]. For the vast majority of insertion sequences in the IS3 family, the OrfAB transposase is produced by programmed −1 translational frameshifting with the motif AaG as the frameshifting region [5, 6]. Translational frameshifting is essential for expression of OrfAB in the IS3 family [7]. A bioinformatics analysis
revealed that ISSau2 contains both orfA and orfB [8]. A frameshift could occur at the A,p,G sequence site to produce a single functional OrfAB. This needs to be experimentally confirmed.

The transposition of IS3-IS150 subgroup elements, catalyzed by the OrfAB transposase, occurs by a cut and paste mechanism. In the first step of transposition, the transposase OrfAB specifically binds to one IR and cleaves it to generate a “figure of eight” loop [9–11]. In the second step of transposition, the figure of eight is processed into a transposon circle and completes the transposition process to another position of the genome. However, only a few studies have proved the transposition function of IS150 elements either in vitro or in vivo. To better understand the biological activity of ISSau2, the binding and cleaving activities of the ISSau2 transposases in vitro and the transposition of ISSau2 in vivo deserve exploration.

Besides OrfAB, OrfA and OrfB are also produced during the transposition of the IS3 family suggesting potential roles of OrfA and OrfB for transposition in nature. OrfA contains a HTH motif and is considered to compete with OrfAB for the DNA binding site, while OrfB might compete for the catalytic site to reduce the transposase activity [4]. It has been reported that both OrfA and OrfB of the IS3 subgroup inhibited transposition, based on generation of circles or linear molecules using an artificial plasmid product in vitro [10]. In addition, OrfB of IS629, a member of the IS3 family, was also able to reduce the transposition frequency [12]. However, there was no study on the function of OrfA or OrfB of the IS150 subgroup. It would be interesting to detect whether the OrfA or OrfB of ISSau2 has the same inhibition activity.

In our previous work, the distribution and sequence diversity of ISSau2 were determined [1]. The goals of this study were to determine binding and cleaving activities of the transposase OrfAB of ISSau2 and its transposition frequency and to investigate the inhibitory function of OrfA and OrfB in the transposition of ISSau2.

**Results**

**ISSau2 belongs to the IS150 subgroup of the IS3 family**

ISSau2 from bovine *S. aureus* isolates was flanked by imperfect inverted 42 bp nucleotide repeats (IRL and IRR) (Fig. 1). Examination of ISSau2 OrfAB revealed a HTH motif within the N-terminal region and an Aspartate-Aspartate-Glutamate (DDE) catalytic domain in the C-terminal of OrfAB. There were 60 amino acid residues between the two D residues and 35 amino acid residues between the second D and E residues. A lysine (K) residue was six amino acids downstream of the E residue. For members of the IS150 subgroup, lysine (K) residue should be six amino acids downstream of the E residue. To sub-categorize ISSau2, a phylogenetic tree was constructed based on the amino acid sequence alignment of OrfB (Fig. 2). The result from the phylogenetic analysis confirmed that ISSau2 belongs to the IS150 subgroup of the IS3 family.

**Translational frameshift of OrfAB occurred in ISSau2**

To verify the frameshifting phenomenon during expression of ISSau2, the whole transposase sequence was inserted into pET28b to create ISSau2-pET28b, which was transformed into *E. coli* BL21 (DE3) for expression. As shown in Fig. 3a, three proteins were expressed with similar concentrations and expected to be OrfAB, OrfB and OrfA from top to bottom. The results indicated that OrfAB of ISSau2 was produced by −1 translational frameshifting.

**OrfAB bound to ISSau2 IRSs in vitro**

In order to ascertain the binding between transposase OrfAB and the target DNA, an ISSau2 mutant, containing
a single guanine insertion in the A₆G sequence to generate A₆G₂, was inserted into pET32a to create pET32-OrfAB and expressed in E. coli BL21 (DE3). The transposase OrfAB was expressed according to the Western blot analysis (Fig. 3b) and purified.

To test whether the transposase can bind to DNA, an electrophoretic mobility shift assay (EMSA) was carried out with the purified transposase OrfAB and the biotin-labeled 42 bp oligonucleotides (IRL and IRR) which included the putative transposase binding sites. Transposase OrfAB bound to IRL42 in the presence of an excess of the poly (dI-dC) competitor and Mg²⁺ (Fig. 3c) and also bound to IS₅ Sau2 IRR42 with a similar affinity (Fig. 3d). Crude extracts from E. coli with pET32a failed to bind to both left and right IS₅ Sau2 inverted repeat sequences as shown in lane 2 of Fig. 3c-d. Therefore, the binding was specifically carried out by the purified IS₅ Sau2 transposase OrfAB.

**ISSau2 transposase OrfAB cleaved transposon ends**

To test whether the ISSau2 transposase had a catalytic activity, a plasmid (pUC19-IRL-gfp-IRR), an artificial mini-transposon with IRL and IRR flanking a gfp gene, was incubated in a buffer with purified full-length transposase OrfAB. The reaction produced linearized plasmid and two fragments of the correct size to be excised mini-transposon (ETF) and the vector backbone (Fig. 4, lane 3). Restriction digestion confirmed that these products were produced by double-strand cleavage at IRR and IRL. As shown in lane 3 and 5 of Fig. 4, both the linearized plasmid (LN) and nicked plasmid (OC) were also produced by transposase OrfAB in the cleavage reaction. These results indicated that transposase OrfAB cleaved both single and double strands in vitro.

To test which domain in the transposase OrfAB is required for cleavage, purified OrfA and OrfB were used in the cleavage assay. OrfA did not have the catalytic activity to cleave transposon end (Fig. 4, lane 4), while OrfB and OrfAB had the similar activity for the cleavage (Fig. 4, lane 3 and lane 5). These results proved that OrfB was involved in the cleavage reaction and in ISSau2 transposition reactions.

**Measurement of transposition frequency of ISSau2 in E. coli**

To measure the transposition frequency of ISSau2 in vivo, a modified GFP hop-on assay was used. A GFP hop-on assay plasmid pET28a-ISgfp was constructed. It contained a gfp gene which lacks transcriptional and translational signals and was located between IRL and IRR of ISSau2. The gfp gene in the GFP-hopper was not expressed at its original location in the plasmid. In the
presence of pET32a-OrfAB, it could be expected that expression of the IS\textsubscript{Sau2} transposase gene led to transposition of IS\textsubscript{gfp}, into an expressed gene in the bacterial genome in the correct orientation and reading frame, resulting in the fusion of the gene and \textit{gfp} gene and expression of a green fluorescent fusion protein.

To detect transpositional events of IS\textsubscript{gfp} by FACS, we introduced both pET28a-IS\textsubscript{gfp} and pET32a-OrfAB into \textit{E. coli} DH5\textalpha. The strain harboring pET28a-IS\textsubscript{gfp} acted as a negative control. Per $10^6$ events through the FACS, 622.32 ± 18.84 events were detected in the negative control group (Fig. 5a). As shown in Fig. 5b, 2381.82 ± 105.68 events for $10^6$ total events were detected with the transposase induction and the number was significantly higher than that in the negative control group, demonstrating that the appearance of the extra fluorescent signals depended on the OrfAB transposase. The transposition frequency of the IS\textsubscript{gfp}, representing the transposition frequency of ISS\textsubscript{au2} in vivo, was approximately $1.76 \times 10^{-3}$.

**Effect of OrfA and OrfB on the transposition of ISS\textsubscript{au2}**

To investigate whether OrfA or OrfB affected the transposition activity, pET32a-OrfA and pET32a-OrfB were introduced into the strains harboring the pET28a-IS\textsubscript{gfp} and pET32a-OrfAB. As shown in Fig. 5c and D, OrfA (1209.85 ± 3.66 events for $10^6$ total events) and OrfB (1341.95 ± 4.32 events for $10^6$ total events) significantly decreased the transposition frequency of IS\textsubscript{gfp}. These data suggested that OrfA and OrfB were the inhibitors for the transposition of ISS\textsubscript{au2}.

**Discussion**

Until now, only 138 insertion sequences have been identified as members of IS150 subgroup in the IS3 family (data from ISfinder). There were only a few studies on IS150 insertion sequences. Early studies identified an unknown insertion sequence based on the homology analyses of nucleotide sequences or protein sequences [13, 14]. Later on, presumed members have also been
required to possess the common features of IS150: 1200–1400 bp in length, containing two orf, a HTH motif, a D (57–60) D (35) E motif, and an A
G frameshifting window (OrfAB as a transposase) [4, 9]. In accordance with these, our results confirmed that ISSau2 belongs to the IS150 subgroup of IS3 family not only supported by the sequences analysis (Fig. 1) but also by results from the molecular experiments (Fig. 3a). First, ISSau2 contains two open reading frames encoded two proteins. Secondly, OrfA contains a HTH motif, while OrfB contains a D (60) D (35) E motif and the OrfAB transposase produced by programmed −1 translational frameshifting with the motif A6G (Fig. 1). Additionally, three proteins were expressed with similar concentrations (Fig. 3a) and this is consistent with the idea that the frequency of frameshifting in IS150 is approximately 50% [15]. Last, the classification was further confirmed by the phylogenetic analysis using OrfB protein sequence (Fig. 2).

ISSau2 was first found in HA-MRSA252 strain by the complete genome sequence analysis [16]. Our previous study found that ISSau2 was only present in S. aureus [1]. Except our work, only another paper analyzed the insertion sites of ISSau2 in CC30 S. aureus by bioinformatics analysis [8] without any other functional studies. Our results in this study confirmed that ISSau2 was at least partially functional with binding and cleavage activities by the transposase OrfAB and the transposase was able to catalyze the transposition in vivo.

The ISSau2 transposase OrfAB bound to the inverted repeat sequences, where it carries out cleavage and strand transfer reactions. The conserved HTH motif in the N terminal of OrfAB is supposed to bind the IRs specifically. The low binding of purified OrfAB to the IRL or IRR (Fig. 3c and d) was presumably due to the fact that the full-length transposase binds poorly to the

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**Fig. 4** In vitro excision of ISSau2. The artificial mini-transposon pUC19-IRL-gfp-IRR was incubated for 16 h in the absence or presence of OrfAB, OrfA or OrfB. Products were separated by agarose gel electrophoresis. SC, LN and OC represent supercoiled, linear and open circle pUC19-IRL-gfp-IRR, respectively; Vector stands for the vector backbone; ETF stands for the excised mini-transposon fragment.

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**Fig. 5** Flow cytometric analysis of ISgfp transposition and measurement of the transposition frequency of ISSau2. 

**a** E. coli strain harboring pET28a-ISgfp as a negative control.

**b** E. coli strain harboring pET28a-ISgfp and pET32a-OrfAB.

**c** E. coli strain harboring pET28a-ISgfp, pET32a-OrfA and pET32a-OrfB.

**d** E. coli strain harboring pET28a-ISgfp, pET32a-OrfAB and pET32a-OrfB. Experiments were performed in triplicates and representative results from one experiment are shown. The range of gated events is indicated as marker 1 (M1).
ends in vitro [17]. Additionally, we showed that OrfAB was able to cleave the transposon ends, otherwise, OrfB alone could also cleave the transposon in vitro (Fig. 4) with an explicable reason that it contained the DDE domain which catalyzes transposition reactions [3]. Our results at least partly support the idea that the transposition occurred by a cut and paste mechanism like other members in the IS3 family [18].

The transposition frequency of ISSau2 was estimated using a modified “GFP hop-on assay” method, which has been used to determine the transposition of transposons in both *Bacillus subtilis* and *E. coli* [19, 20]. This method, making it possible to detect transposition at the single cell level, was more efficient to estimate the transposition frequency than other methods [19, 20], such as papillation assays [21, 22], mutation accumulation experiment [23] and mating-out assay [24]. Thus, the GFP hop-on assay was adapted to measure the transposition frequency of ISSau2 in the presence of OrfA, OrfB or OrfAB. As shown in Fig. 5, OrfAB of ISSau2 induced transposition at the frequency of 5.76 ± 0.13 × 10^{-3}. Nevertheless, the observed transposition frequency (1.76 ± 0.13 × 10^{-3}) might be over-estimated than the real transposition in bacteria, due to the laboratory conditions which had few limiting factors and thus favored the transposition. In nature, the transposition rates of insertion sequences generally must be maintained at a low level, acting as a possible strategy to limit any negative effects on the host genome [25]. These data demonstrated that the transposition of ISSau2 depended on the transposase OrfAB and indicated that ISSau2 was at least partially functional.

It is interesting that both OrfA and OrfB inhibited significantly the transposition of ISSau2 induced by OrfAB. In the IS3 family, OrfA inhibits reactions promoted by transposases by binding to transposon ends [26]. OrfB might form a complex with OrfA to inhibit the transposition of ISSau2 by blocking formation of an active transpososome consisting of transposase, two terminal IRs and target DNA or by preventing the transposase from catalyzing the strand transfer reaction [26].

In summary, we characterized a functional insertion sequence ISSau2 in *S. aureus*. To the best of our knowledge, this is the first study to investigate the transposition of ISSau2 and to characterize the effects of OrfA and OrfB of ISSau2 on transposition. Our results provide a solid basis for future studies of the molecular mechanisms involved in ISSau2 transposition and its biological function in its host *S. aureus*. Meanwhile, we now have an experimental system that allow us to characterize any novel insertion sequence in bacterial cells and even in other eukaryotic cells especially for the insertion sequences which contain two *orf* genes and use the fusion OrfAB as the transposase.

**Conclusion**

We have confirmed that ISSau2 is a member of IS150/IS3 family. The ISSau2 transposase OrfAB could bind to and cleave the specific fragments containing the terminal inverted repeat sequences and induce the transposition, suggesting that ISSau2 was functional. Meanwhile, both OrfA and OrfB were the inhibitors for transposition of ISSau2. Our results will help understand biological roles of ISSau2 in its host *S. aureus*.

**Methods**

**Construction of prokaryotic expression vectors**

A known ISSau2 transposase sequence, located in *sdrC* of *S. aureus* E48 [27], was isolated by PCR with the specific primers pairs ISSau2-F/ISSau2-R (Table 1) using the *Pfu* polymerase, and inserted between *Bam*H I and *Xho* I sites of pET28b to create pET28-ISSau2. Within pET28-ISSau2, an N-terminal 6-His tag and a C-terminal 6-His tag were linked to either side of the ISSau2 sequence. Meanwhile, a G residue was inserted downstream of A6G2 in the ISSau2 sequence, forming an A6G2 region [2] by overlap PCR using the two primer pairs ISSau2-F/OrfAB-overlap-R and OrfAB-overlap-F/ISSau2-R (Table 1). In theory, addition of the G would not change the OrfAB amino acid sequence [28]. The mutated sequence was inserted between *Bam*H I and *Xho* I sites of pET32a, which contains an N-terminal 6-His tag, to create pET32-OrfAB. The *orfA* region (753 bp) and *orfB* region (891 bp) of ISSau2 were amplified by PCR using the primers ISSau2-F/OrfA-R and OrfB-F/ISSau2-R respectively (Table 1) and inserted into pET32a to construct pET32a-OrfA and pET32a-OrfB.

**Expression and purification of ISSau2 transposase**

The constructed expression vectors were transformed into BL21 (DE3) competent cells, separately. The overnight culture of BL21 (DE3) was diluted 1:50 in fresh LB broth containing different antibiotics (pET28-ISSau2: 50 μg/mL kanamycin; pET32-OrfAB: 100 μg/mL ampicillin) with vigorous shaking at 37 °C to an OD₆₀₀ of 0.4–0.6. Expression of the transposase was induced by the addition of Isopropyl-β-D-thiogalactopyranoside (IPTG) to 0.2 mM. Bacterial cells were grown for 6 h at 28 °C and harvested by centrifugation. All the following steps were carried out on ice. Cultured bacterial cells (BL21 with pET32-OrfAB) were suspended in a PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, and 2 mM KH₂PO₄) and disrupted by an Ultrasonic Processor. The supernatant containing soluble transposase OrfAB was collected by centrifugation at 13,000 g for 45 min. After filtration by 0.22 μm filter, the supernatant was applied to a nickel-nitrioltriacetic
Table 1 Plasmids and primers used in this study

| Plasmid          | Genotype/Description                              |
|------------------|--------------------------------------------------|
| pET28a-SSau2     | pET28b derive with SSau2 transposase sequence under control of T7 promoter, Kan^R |
| pET32-OrfAB      | pET32a derive with SSau2 transposase sequence with an insertion of G residue in the A_G region to generate the A_G region, Amp^R |
| pET32a-OrfA      | pET32a derive with OrfA sequence (the first orf of SSau2, 753 bp in length), Amp^R |
| pET32a-OrfB      | pET32a derive with OrfB sequence (the second orf of SSau2, 891 bp in length), Amp^R |
| pUC19-I RL-gfp-IRR | pUC19 derive with the gfp sequence from PGLO vector flanked by IRL (42 bp) and IRR (42 bp) of IRL42, Kan^R |
| pET28a-I sgfp    | pET28a derive with the gfp sequence from PGLO vector flanked by IRL (42 bp) and IRR (42 bp) of IRR42, Kan^R |

Primer

| Primer         | Sequence 5'-3'                                 |
|----------------|-----------------------------------------------|
| SSau2-F        | CGCGGATCAGTAAAACCTTTGAAGGAGC                  |
| SSau2-R        | CCGCTGACGATTAAAAATGGCTGATCTGTA               |
| OrfAB-overlap-R| ATGGTCCAAACCTTTTTAAACGT                      |
| OrfAB-overlap-F| ACGTTTTAAAAAGGTTCACGCT                      |
| OrfA-R         | CCGCTGAGAGCGCTCAATCTGGTTGTAT                |
| OrfB-F         | CGCGGATCCGTTTTAAAAAAGTTTCAAGGCA             |
| IRL-gfp-R      | CGCGGAATCTCAAGAACGCGCTTCCCTCCCTCCAGTAGA     |
| IRR-gfp-R      | CGCGGAATCTCAAGAACGCGCTTCCCTCCCTCCAGTAGA     |
| D-orfA-F       | ATGAAAAACTTTGAAGGAGC                        |
| D-orfA-R       | ACGCTTCAATGGTTGTAT                          |
| D-orfB-F       | CGTTTTAAAAAGGTTCACGAC                      |
| D-orfB-R       | CATTAAAAATGGCTGATCTGTA                      |
| D-I sgfp-F     | AATGAAAAACTTTGAAGGAGC                       |
| D-I sgfp-R     | TGCAGTTCATGTAGTACGCT                      |

*All the plasmids were constructed and all primers were designed in this study*

Electrophoretic mobility shift assays (EMSA)

Two 42 bp biotin-labeled DNA fragments containing IRL and IRR were generated by oligonucleotide hybridization (heated together at 95 °C for 10 min and cooled to room temperature) respectively to generate dsDNA IRL42 and IRR42. For EMSA, 2 μL 10 x binding buffer, 1 μg/μL Poly (dI-dC), 50% Glycerol, 1% NP-40, 1 M KCl, 100 mM MgCl2, 200 mM EDTA (EMSA kit, Thermo) and 0.42 μg OrfAB were incubated at room temperature for 20 min. Binding was initiated by addition of biotin end-labeled target DNA. Binding reactions were allowed at room temperature for 20 min and the products were then run on 5% polyacrylamide gels in 0.5 x TBE buffer (100 V) at 0 °C. The biotin end-labeled DNA was detected using the Streptavidin-Horseradish Peroxidase Conjugate and the Chemiluminescent Substrate.

In vitro transposition assays

An in vitro transposition assay was performed as described [29]. The artificial mini transposon plasmid (pUC19-I RL-gfp-IRR) contained IRL42 and IRR42 flanking the gfp gene which lacks transcription and translation start signals, the fusion sequence was amplified by PCR using the specific primer pairs IRL-gfp-F/I RL-gfp-R (Table 1) with the pGLO plasmid DNA as the template. The gfp gene was amplified so as to remove the transcriptional and translational signals. Standard in vitro transposition assays contained 0.12 pmol of pUC19-I RL-gfp-IRR in 20 mL of transposase assay buffer (10 mM MgCl2, 1 mM DTT, 0.1 mg/mL BSA, 50 mM Tris-HCl pH 7.5 and 50 mM NaCl). Reactions were started by the addition of 1–2 pmol of purified transposed OrfAB and incubated at 30 °C for 16 h. Reactions were stopped by heating at 75 °C for 10 min, and products were analyzed by agarose gel electrophoresis.

Detection of GFP-hopper transposition

The plasmid pET28a-I sgfp was generated from pUC19-I RL-gfp-IRR. The GFP hop-on assay was performed as described [20] with minor modifications. The plasmid pET28a-I sgfp as well as pET32a-OrfAB or pET32a-OrfA or pET32a-OrfB was introduced into E. coli DH5α. The transformed colonies were cultured in 5 mL LB medium under the selective pressure (both 50 μg/mL kanamycin and 100 μg/mL ampicillin) at 37 °C. Harvested cells were then resuspended in a sheath fluid (BD Biosciences, USA) at OD600 = 0.3. Flow cytometry was performed on a FACSCalibur cytometer with the CELL-QUEST software (BD Biosciences). GFP fluorescence was detected with a FL1 detector and its intensity was expressed as an arbitrary logarithmic value (FL1-H) within the range of 10⁰ - 10⁴. The transposition-frequency was estimated as the ratio between the number of events within region M1 (> 10² FL1-H) in the presence of OrfAB minus the background number and the number of total events.

To investigate whether OrfA or OrfB has the activity to inhibit the transposition of I sgfp induced by OrfAB, the pET32a-OrfA or pET32a-OrfB was first introduced
into \textit{E. coli} DH5\textalpha{} confirmed and verified by PCR with the primer pairs OrfA-F/OrfA-R or OrfB-F/OrfB-R (Table 1) and then the pET28a-ISgfp and pET32a-OrfAB were introduced into \textit{E. coli} DH5\textalpha{} by electroporation and confirmed by PCR using the primers Isgf-F/Isgf-R and OrfA-F/OrfB-R (Table 1). The GFP fluorescence of the cells containing pET28a-ISgfp, pET32a-OrfAB and pET32a-OrfA (or OrfB) were detected by FACScalibur cytomter.

### Abbreviations

DDE: Aspartate-Aspartate-Glutamate; \textit{E. coli}: \textit{Escherichia coli};
EMSA: Electrophoretic Mobility Shift Assay; FACS: Fluorescence Activated Cell Sorting; GFP: Green Fluorescent Protein; HA-MRSA: Healthcare-Associated Methicillin Resistant \textit{Staphylococcus aureus}; HTH: Helix-Turn-Helix; IPTG: Isopropyl-\textbeta{}-D-thiogalactopyranoside; IRs: Inverted Repeat sequences; ORF: Open Reading Frame; PBS: Phosphate Buffer solution; \textit{S. aureus}: \textit{Staphylococcus aureus}

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### Authors' contributions

XZ LW LX conceived and designed the experiments. LW WS performed the program: analysis of data.

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Competing interests

The authors declare that they have no competing interests.

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