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Full Length Research Paper

First observation of excision and integration in Class 1 integron in *staphylococci*

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Class 1 integron had been well-known as a mobile genetic element involved in the antibiotic resistance of various bacteria. Up to date, most of the relevant publications concentrated on gram-negative bacteria, with limited exceptions. However, in recent studies, class 1 integron had been detected in methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase negative staphylococci (MRCNS). So in this study, we tested in *S. aureus,* the class 1 integron mediated excision and integration. We first asked 8 plasmids from previous studies, then established some transformants and perform the excision and integration reaction. As the results revealed, we observed positive excision assay, which had been confirmed by further integration assays. This observation may raise the public attention of integron as a novel antibiotic resistance determinant in gram-positive bacteria, especially in staphylococci.

Key words: *Staphylococcus aureus*, class 1 integron, mobile genetic element, excision and integration, antibiotic resistance.

INTRODUCTION

In recent years, the role of integrons and gene cassettes in the dissemination of resistance genes has been well established, which is responsible for the facile spread of resistance genes and the rapid evolution of resistance to a wide range of unrelated antibiotics among diverse bacteria (Tauch et al., 2002; Yang et al., 2004; Yu et al., 2003). An integron comprises three elements including the integrase gene (*intI*) encoding an integrase, a recombination site, *attI* and a promoter gene. Several classes of integrons have been reported on the basis of the *intI* gene and classes 1 to 3 are so-called multi-resistant integron. Of these, the class 1 integron platform is the most ubiquitous among resistant clinical isolates of gram-negative bacteria and is found to be associated with the Tn21 transposon family, while the class 2 integrons are associated with the Tn*7* transposon family (Arakawa et al., 1995; Barlow et al., 2004; Correia et al., 2003; Hall and Stokes, 1993; Nandi et al., 2004; Nesvera et al., 1998; Nield et al., 2001; Sun et al., 2002). Class 1 integron can capture the gene cassettes, which also contain *attC* site for recombination, via a site-specific recombination event between *attI* and *attC*. Its 3' conserved segment (3'CS) possesses qacE≥1 and *sul1*

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genes, encoding resistance to quaternary ammonium salts and sulfonamide, respectively (Recchia and Hall, 1997). Although, the role of class 1 integron in the spread of antibiotic resistance genes in gram-negative bacteria is clear, little is known about the prevalence of class 1 integron in gram-positive bacteria, especially in *Staphylococcus aureus* (Clark et al., 1999; Nandi et al., 2004). Increasng antibiotic resistance in gram-positive bacteria has become a great concern.

In the past decades, methicillin-resistant *S. aureus* (MRSA) has become one of the most prevalent pathogens that cause nosocomial infections throughout the world. Since it can spread easily by direct or indirect contact between patients and environment or through patients or medical personnel, MRSA is an important risk factor for nosocomial infection and MRSA mediated nosocomial outbreaks are common in hospitals. As consequence, MRSA mediated nosocomial infections continue to be a challenge for clinicians, hospital epidemiologists and administrators since such infections may lead to a serious problem for therapy of patients and infection control. MRSA shows resistance to practically all β-lactam antibiotics, which is caused by *mecA* gene. The *mecA* gene is carried by a mobile genetic element, designated by staphylococcal cassette chromosome *mec* (SCC*mec*), which contains the *mec* gene complex (the *mecA* gene and its regulators) and the *ccr* gene complex encoding site-specific recombinases responsible for the mobility of SCC*mec* (Ito et al., 2001; Kuroda et al., 2001; Xu et al., 2007, 2008a,b, 2010c, 2011a and b).

In recent years, class 1 integron had been found commonly existed in MRSA and methicillin-resistant coagulase negative staphylococci (MRCNS) strains (Xu et al., 2007, 2008a and b, 2009, 2010a and b). In this study, we investigated class 1 integron-mediated recombination in staphylococci, including class 1 integron mediated excision of different gene cassettes with 2 *att* sites and 3 arrays of cassettes and further integration of them by site-specific recombination.

MATERIALS AND METHODS

Bacteria and plasmids

Plasmid-free *S. aureus* strain RN4220 was used as recipient bacterial, with storage, culturing and processing performed as described previously (Hauschild et al., 2003). *S. aureus* strain RN4220 was routinely cultured in Luria-Bertani (LB) medium or agar supplemented with or without appropriate antibiotics as ampicillin (100 μg/ml), chloramphenicol (25 μg/ml), sulfamethoxazole (25 μg/ml) or trimethoprim (25 μg/ml). Plasmids R388 (a 33 kb IncW plasmid containing In3 with cassettes *dfrB2-orfA*, antibiotic phenotype including Tpr (trimethoprim resistan) Sur (sulfamethoxazole resistant)) and pSU2056 (1,176 bp *Rsa*I-*Bam*HI fragment of In2 in pUC9, antibiotic phenotype including Apr(ampicillin resistant)) had been acquired from Hall et al. (1991). PLQ425 (with bla_{imp} as gene cassette and GTTAGAG as associated *attC*), PLQ431 (with *pse-1* as gene cassette and GTTAGCC as associated *attC*), PLQ438 (with bla_{imp} as gene cassette and GTTAGAA as associated *attC*), PLQ440 (with *aacA1a-orfG* + *orfH* as gene cassette and GTTAGGG as associated *attC*), PLQ443 (with *aadA1* as gene cassette and GTTAAAC as associated *attC*) and PLQ444 (with *aacA1a-orfG* + *orfH* as gene cassette and GTTAGGGG as associated *attC*) were obtained from Leon and Roy (2003).

Electroporation of cassettes carried plasmids into RN4220

To establish different transformants for the excision and integration assay, the aforementioned plasmids had been transformed into RN4220 by electroporation, which had been performed as described previously (Hauschild et al., 2003). In brief, 100 μl of the competent cells and 1 or 2 μg of each plasmid were subjected to electroporation, 200 μl aliquots were then streaked on plated supplemented with proper antibiotics according to relevant phenotypes.

Excision and integration reaction

In vivo excision and integration tests were performed by double transformants (pSU2056 with one of the cassette containing PLQ clones) and triple transformants (R388, pSU2056 with one of the cassette containing PLQ clones), respectively. Growth condition and induction of *IntI1* in transformants were performed as described previously (Leon and Roy, 2003; Martinez and de la Cruz, 1990). In brief, transformants were grown overnight at 37°C in blood medium supplemented with selective antibiotics (ampicillin 50 g/ml, chloramphenicol 50 g/ml) and 2% of the overnight culture was then inoculated in 15 ml of new medium (without antibiotic). Isopropyl-β-D-thiogalactopyranoside (IPTG) was added (with final concentration of 0.4 mM for overnight induction) when the optical density at 600 nm reached 0.6. Plasmid isolation was performed as described previously (Schwarz et al., 1989). Excision events were determined by polymerase chain reaction (PCR) amplification and further confirmed by excision-integration tests, with different primer pairs as indicated (Table 1). All experimental procedure had been performed as described previously with slight changes (Leon and Roy, 2003). In brief, for excision assays, sixty-four colonies had been randomly selected and further subjected to PCR amplification for each transformant. For integration assays, ten colonies recovered had been selected to sequencing. In detail, PCR products were purified from agarose gels and the DNA fragments were ligated with the pGEM-T easy vector (Promega, Madison, WI, USA). The ligation mixture was transformed into *E. coli* DH5α strain and the recombinants were selected on Luria agar containing ampicillin (100 μg/ml). Recombinant plasmid DNA was purified by standard method and subjected to sequencing and further analyses. The nucleotide sequences of the insert DNA was determined using the BigDye terminator cycle sequencing FS ready reaction kit on an ABI PRISM 310 genetic analyzer (Perkin-Elmer Japan Applied Biosystems, Tokyo, Japan). The nucleotide sequences were analyzed and compared using GenBank database by using the BLAST algorithm, which is available through the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov). Definition of excision and integration efficiency was according to previous report (Leon and Roy, 2003).

RESULTS

Transformants in this study

Twelve different types of transformants had been

Table 1. Primes used for the different mobile gene cassettes constructs.

Primer	Nucleotide sequence (5' - 3')	Temperature (℃)	Function	Reference
pACYC184-5'	TGTAGCACCTGAAGTCAGCC	55	Excision test	Leon and Roy (2003)
pACYC184-3'	AGTGATCGAAGTTAGGCTG			
ATTINEU	AGGCGTGATAGATTCTC	48	Integration test	Leon and Roy (2003)
AADA1	TGCATGACGCCAACTAC	48		
BLA_{IMP}	TGCGGTAGCAATGCTGC	48		
AACA1a	TAATTGCTGCATTCCTCCGC	48		
PSE-1	CGGATGGTATTAAAAGC	44		

Table 2. List of transformants and its characteristics.

Transformant	Plasmid	Phenotype
RN4220-PLQ425	pSU2056 and PLQ425	Ap^r, Cm^r
RN4220-PLQ431	pSU2056 and PLQ431	Ap^r, Cm^r
RN4220-PLQ438	pSU2056 and PLQ438	Ap^r, Cm^r
RN4220-PLQ440	pSU2056 and PLQ440	Ap^r, Cm^r
RN4220-PLQ443	pSU2056 and PLQ443	Ap^r, Cm^r
RN4220-PLQ444	pSU2056 and PLQ444	Ap^r, Cm^r
RN4220-R388-PLQ425	pSU2056 and PLQ425	Ap^r, Cm^r, Su^r, Tr^r
RN4220-R388-PLQ431	pSU2056 and PLQ431	Ap^r, Cm^r, Su^r, Tr^r
RN4220-R388-PLQ438	pSU2056 and PLQ438	Ap^r, Cm^r, Su^r, Tr^r
RN4220-R388-PLQ440	pSU2056 and PLQ440	Ap^r, Cm^r, Su^r, Tr^r
RN4220-R388-PLQ443	pSU2056 and PLQ443	Ap^r, Cm^r, Su^r, Tr^r
RN4220-R388-PLQ444	pSU2056 and PLQ444	Ap^r, Cm^r, Su^r, Tr^r

constructed in this study (Table 2), including RN4220-PLQ425 (Ap^r, Cm^r, with pSU2056 and PLQ425), RN4220-PLQ431 (Ap^r, Cm^r, with pSU2056 and PLQ431), RN4220-PLQ438 (Ap^r, Cm^r, with pSU2056 and PLQ438), RN4220-PLQ440 (Ap^r, Cm^r, with pSU2056 and PLQ440), RN4220-PLQ443 (Ap^r, Cm^r, with pSU2056 and PLQ443), RN4220-PLQ444 (Ap^r, Cm^r, with pSU2056 and PLQ444), RN4220-R388-PLQ425 (Ap^r, Cm^r, Su^r, Tr^r, with R388, pSU2056 and PLQ425), RN4220-R388-PLQ431 (Ap^r, Cm^r, Su^r, Tr^r, with R388, pSU2056 and PLQ431), RN4220-R388-PLQ438 (Ap^r, Cm^r, Su^r, Tr^r, with R388, pSU2056 and PLQ438), RN4220-R388-PLQ440 (Ap^r, Cm^r, Su^r, Tr^r, with R388, pSU2056 and PLQ440), RN4220-R388-PLQ443 (Ap^r, Cm^r, Su^r, Tr^r, with R388, pSU2056 and PLQ443), RN4220-R388-PLQ444 (Ap^r, Cm^r, Su^r, Tr^r, with R388, pSU2056 and PLQ444).

Excision and integration reaction

According to the results, excision efficacy ranged from 7.8 to 62.5%, which was thoroughly lower than previous reports when the assays induced in bacteria other than *S. aureus* (Leon and Roy, 2003). Two major factors affecting the excision included *att* site and gene cassettes. As the results showed, greater efficacy had been observed when *attC* site on the left-hand neighbour comparing with *attI*, based on 7.8% (5/64) for RN4220-PLQ425 with 31.2% (20/64) for RN4220-PLQ438, 25% (16/64) for RN4220-PLQ440 with 53.1% (34/64) for RN4220-PLQ444. In addition, transformants containing *aadA1* and *aacA1a-orfG+orfH* obtained higher efficacy than *pse-1* and bla_{imp}, as excision efficacy was 15.6% (10/64) for RN4220-PLQ431 and 31.25% for RN4220-PLQ438, comparing with 53.1% for RN4220-PLQ444 and 62.5% (40/64) for RN4220-PLQ443. These results were similar to those published previously (Hansson et al., 1997; Leon and Roy, 2003) and had also been further confirmed by subsequent integrations of excised cassettes into relevant *attI* site. Amplicons obtained were around expected 200 bp and sequencing amplicons confirm that, the excised cassettes were integrated specifically into the G/TTRRRY consensus site of the *attI1* or *attC* site.

DISCUSSION

Since the first description of class 1 integron in 1989 (Stokes and Hall, 1989) integrons have been identified as a primary source of resistance genes and were suspected to serve as reservoirs of antimicrobial resistance genes within microbial populations (Xu et al., 2007,

2009). However, most of these studies were done in gram-negative bacteria with only a few exceptions. While in recent years, integrons had been identified as a novel and unexpected antibiotic determinant in gram-positive organism, especially in MRSA and MRCNS (Xu et al., 2007, 2008a and b, 2009, 2010). The Integron system had been well-known as a mobile genetic element, playing key role in antibiotic resistance in various bacteria (Rowe-Magnus and Mazel, 2001). In this study, we tested and investigated the class 1 integron-mediated excision and integration in *S. aureus* RN4220 and positive result had been observed for both assays. Up to date, no report is available on the dissemination of class 1 integron in any species of staphylococci strain and we are convinced that, the observation of the class 1 integron-mediated excision and integration in *S. aureus* may definitely raise the attention of integron as the novel antibiotic mechanism in gram-positive bacteria, especially in staphylococci, which had been neglected throughout the time.

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