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A novel integrative conjugative element mediates transfer of multi-drug resistance between *Streptococcus suis* strains of different serotypes

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ABSTRACT

Streptococcus suis represents a key antibiotic resistance gene reservoir and an important pathogen for humans and animals. Resistance can be spread through horizontal gene transfer of chromosome-borne mobile genetic elements; however, the exact mechanism by which this occurs remains poorly understood. In the present study, we identified and characterized a novel 82-kb integrative conjugative element (ICE) named ICE*Ssu*CZ130302 from the virulent *S. suis* strain CZ130302. It carries genes that provide resistance to multiple antibiotics, such as tetracycline, doxycycline, erythromycin, lincomycin, neomycin, and kanamycin. It also contains a nisin biosynthesis gene cluster, a toxin-antitoxin system, a type IV secretion system, and an integrase and excisase system. The mobile element can be excised from the chromosome, circulized, and transferred via conjugation from serotype Chz strain CZ130302 to serotype 2 strain P1/7, where it confers resistance to the aforementioned antimicrobial agents. The full length ICE, where multiple antimicrobial resistance genes accumulated, was further identified to be naturally transferred between different serotypes strains of *S. suis.* This finding illustrates how such elements represent a potential means by which antimicrobial resistance is introduced to a wide range of bacteria of veterinary and medical significance.

1. Introduction

Streptococcus suis is an important pathogen in the swine industry, responsible for a variety of serious diseases, including meningitis, septicemia, and endocarditis, which can result in sudden death (Lee et al., 2008). *S. suis* can cause human infection, as evidenced by two extensive outbreaks caused by serotype 2 in China (Yang et al., 2006). Given the bacterium's widespread presence in the environment, it may represent a critical source of antimicrobial resistance genes, which can be delivered to other streptococcal pathogens in the food chain. Previous studies have described *S. suis* as a key antimicrobial resistance reservoir and gene delivery system driving the evolution of antimicrobial resistance (Palmieri et al., 2011; Huang et al., 2016b). Plasmids, genomic islands (GIs), integrative conjugative elements (ICEs), transposons, phages, and chimeric elements carry single or multiple antimicrobial resistance genes that can be transferred to a wide range of species. In recent years, drug-resistant *S. suis* isolates have come to encompass a gradually wider spectrum, indicating the continued diversification of the corresponding resistance mechanisms (Martel et al., 2001; Gurung et al., 2015; Huang et al., 2016b; Hernandez-Garcia et al., 2017). Horizontal gene transfer remains a crucial driving force in bacterial evolution, especially in antimicrobial resistance. ICEs harboring multiple resistance and virulence genes are regarded as increasingly common vehicles of lateral gene transfer among bacterial pathogens. Persistent findings of ICEs have been reported in *S. suis*, (ICE*Ssu*HB1011, ICE*Ssu*ZJ20091101, ICE*Ssu*T15, ICE*Ssu*D12, ICE*Ssu*JH1308, ICE*Ssu*SC260, ICE*Ssu*JH130) (Huang et al., 2016b, c); however, there are only limited reports of excised forms of ICEs from the chromosome and of transferable multidrug resistance in *S. suis* through ICE transfer in *vivo*.

In the present study, an 82-kb integrative conjugative element (ICE) has been identified in the *S. suis* serotype Chz strain CZ130302, but not in the virulent strain P1/7 of serotype 2. Further analysis led to the characterization of ICE*Ssu*CZ130302, which carries multiple antimicrobial resistance genes and potential virulence genes. Notably, we provide the first report of the excision and integration forms of ICE*Ssu*CZ130302 and characterize the transferability of *S. suis* serotype Chz CZ130302 to serotype 2 virulent strain P1/7.

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2. Materials and methods

2.1. Bacterial strains, plasmids, and culture conditions

The novel Chz serotype of *S. suis* strain CZ130302 was isolated from the brain of a piglet with acute meningitis in Changzhou, China (Pan et al., 2015; Pan et al., 2017). *S. suis* serotype 2 strain P1/7 (Clifton-Hadley, 1983, 1984) was isolated from an ante-mortem blood culture in Europe. The strain P1/7 used in this study has been reconstructed with the spectinomycin resistance (*spc*) gene. The transconjugant P1/7 strain contained ICE*Ssu*CZ130302. The strain *S. pneumoniae* ATCC 49619 gifted by Prof. Jingren ZHANG (Tsinghua University) was used for quality control. Plasmid pSET-4 s carrying the *spc* gene was a gift of Daisuke Takamatsu (Takamatsu et al., 2001). Bacteria were cultured in Todd Hewitt Broth (THB, Becton-Dickinson) or agar medium containing 6% (v/v) sheep blood at 37 °C in 5% CO_2 atmosphere. *Escherichia coli* strains were cultured in Luria-Bertani (Becton-Dickinson) medium at 37 °C. Finally, 100 μg/mL spectinomycin (Sigma) was added to *S. suis* medium; whereas 50 μg/mL spectinomycin or 100 μg/mL ampicillin (Sigma) were added to *E. coli* medium.

2.2. Discovery and structure of the 82 K ICE

Mauve (Darling et al., 2004) software was used to compare and analyze the genomes of *S. suis* CZ130302 and *S. suis* P1/7. The reference genomes of ICE*Sa*2603 and ICE*Ssu*05ZYH33-1 were obtained from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/). The ICEberg tool (Bi et al., 2012) (http://db-mml.sjtu.edu.cn/ICEberg/) was used to search for previously reported ICEs and the Antibiotic Resistance Genes Database (ARDB, http://ardb.cbcb.umd.edu/) was used to predict resistance genes.

2.3. Construction of gene deletion mutants

To investigate the contribution of GIs, the 82 K deletion mutant was constructed by peptide pheromone-induced DNA transformation (Zaccaria et al., 2014). The strategy used to construct the 82 K deletion mutant is shown in Fig. 1A and the primers used to this end are listed in Table S1. Fragments AB (upstream, 1040 bp) and CD (downstream, 1009 bp) were amplified from *S. suis* CZ130302 using primers Δ82 K-A (*Bam*HI site at its 5′ end) and Δ82 K-B, and Δ82 K-C and Δ82 K-D (*Sal*I site at its 3′ end) respectively. The *spc* sequence (1133 bp) with the promoter was amplified from plasmid pSET-4 s using primers Spc-F1 and Spc-R1. These three fragments were fused by PCR and ligated into pUC19 to form the recombinant plasmid pUC19-AB-Spc-CD. The mutant CZ13-Δ82 K was obtained following homologous recombination and resistance screening. Mutant strains of individual resistance genes were constructed as previously described (Zaccaria et al., 2014).

2.4. Determination of minimal inhibitory concentrations (MICs)

MICs of different classes of antimicrobial agents, including beta-lactams, aminoglycosides, tetracyclines, amide alcohols, macrolides, lincosamides, polypeptides, and fluoroquinolones against wild-type and mutant strains or transconjugants were determined according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI, 2015). The strain *S. pneumoniae* ATCC 49619 was used for quality control purposes.

2.5. PCR assays

Briefly, 30 primers pairs were synthesized to verify the deletion of the whole 82 K ICE from the CZ130302 genome (Table S1). Primers T1/T2 or T9/T10 served mainly to highlight the existence of homologous arms at the ends, whereas the products identified by primer sets T3/T4 to T7/T8 represented the genes located from the homologous arm to the GI. To investigate the possible forms and patterns of ICE*Ssu*CZ130302 in horizontal transmission of *S. suis* resistance traits, combination primers P1–P4 were designed and used in PCR analysis. Primer pairs P1/P2 and P3/P4 identified the integrated form, whereas P2/P3 and P4/P1 could be used to identify the extrachromosomal circular form and excised form, respectively. The pairs S2-F/S2-R and Chz-F/Chz-R were used as typing primers to distinguish between serotypes Chz and 2.

2.6. Conjugation experiments

Conjugation experiments were performed as previously described (Li et al., 2011) with some modifications. Reconstructed *S. suis* P1/7 (tetracycline- and erythromycin-susceptible but spectinomycin-resistant; Fig. 1B) was used as recipient and *S. suis* CZ130302 (tetracycline- and erythromycin-resistant but spectinomycin-susceptible) was utilized as donor. Briefly, donor and recipient bacteria were grown with appropriate antibiotics separately in THB medium at 37 °C with 5% CO_2. They were centrifuged to remove the antibiotics, harvested at a suitable phase, and mixed *S. suis* strain P1/7 strain and *S. suis* strain CZ130302 at a 1:5 ratio. The mixtures were placed on sterile nitrocellulose filters (0.45 mm, Millipore) on THB plates and incubated at 37 °C with 5% CO_2 overnight. Bacteria were removed by washing the

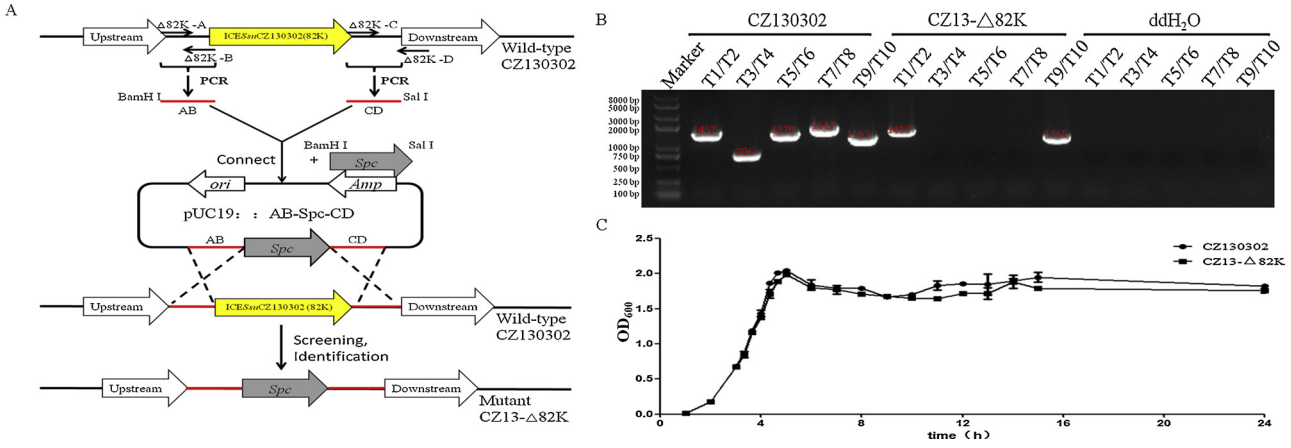

Fig. 1. Construction and identification of mutant strains. (A) Strategy for constructing mutant strains. Fragments AB, Spc, and CD (both ends with *Bam*HI and *Sal*I restriction sites) were fused and ligated with pUC19 to form an intermediate vector. Mutant strains were obtained by transformation, screening, and identification. (B) Strains CZ130302 and CZ13-Δ82 K; and ddH_2O (negative control) were used as templates for primer pairs from T1/T2 to T9/T10; T1/T2 and T9/T10 amplified the homologous arms adjacent to the 82 K ICE. (C) Growth curves of wild-type CZ130302 and mutant strain CZ13-Δ82 K under the same conditions *in vitro*.

filters in 1 mL THB medium. Transconjugants were selected on THB plates containing appropriate antibiotics and further confirmed by PCR analysis. The procedure was repeated three times and the conjugation rate was calculated based on colony-forming units (CFUs) with the formula (CFUs of transconjugants/CFUs of recipients)*100%.

3. Results

3.1. Characterization of the 82-kb ICE

A unique element (CVO91_RS06510 - CVO91_RS06980) was found in the genome of CZ130302, but not in P1/7 (Figure S1). The size of this element was about 82 kb, including 81 putative open reading frames (ORFs), of which 76 were located on the negative strand. Its G + C content was 37.8%, lower than the average G + C percentage of *S. suis* CZ130302 (41.2%). The element was located between *hyd* (CVO91_RS06505) and *rpl*L (CVO91_RS06985), which encoded a predicted hydrolase and 50S ribosomal protein L7/L12, respectively. These genes presented high homology with *hyd* (SSU_RS04320) and *rpl*L (SSU_RS04325) from strain P1/7. Two direct repeats (*att*L and *att*R) were located at both ends of the element. The length of the *att* sequence (5′-TTATTTAAGAGTAAC-3′) was 15 bp, and corresponded to the recognition site of the *att* integrase from the ICE*Sa*2603 family ($Int_{ICESa2603}$) (Huang et al., 2016a). Sequence alignment revealed 97% sequence identity to the integrase of $Int_{ICESa2603}$. The result was analyzed by NCBI and ACT software and is shown in Fig. 2. Three predicted proteins (relaxase, MobC, and Tn*5252* ORF10) may participate in the DNA mobile and processing module (blue boxes). The integration and excision modules were identified next to the *att*L site (purple boxes), and similar functional integrase genes are labeled with lilac. A large antibiotic-biosynthesis cluster is noted in brown. Toxin-antitoxin family proteins were predicted in the clusters of antimicrobial resistance genes (red boxes). Moreover, 5′-GTTATTGTG-3′, an inverted repeat sequence, represented a binding site for relaxase and 5′-CTCG/CAAA was predicted as the precise nickase cleavage site. Subsequently, there was a gap at the origin of transfer (*ori*T) (Fig. 2, red arrow) in the circular DNA, which, compared with *ori*T sequences of ICE*Sa*2603 and ICE*Ssu*05ZYH33-1, was characterized by four point mutations (Figure S2). Initially, an approximately 200-bp, highly conserved region, with 92–100% identity to ICE*Sa*2603 was thought to be the putative *ori*T region in the 82 kb ICE. The mating pair formation module was associated mainly with the type IV secretion system (T4SS) which was mapped in the ICE (orange boxes) and included the conjugative transfer protein G (TraG). The single DNA is synthesized by rolling loop replication and is transferred to host bacteria through a junction channel with the help of TraG (Nguyen and Vedantam, 2011). A series of complex proteins, such as VirB4, Tn5252, and VirB6 were included in the T4SS. The T4SS can form a transmembrane secretory pathway together with some extracellular components, such as fimbriae (Christie et al., 2005), to mediate the exchange of proteins and DNA between cells and the environment, thus spreading resistance and pathogenicity traits (Li et al., 2011; Zhao et al., 2011; Jiang et al., 2016). As the 82 K element harbored core modules of an ICE, it was consequently named ICE*Ssu*CZ130302.

3.2. Multiple drug resistance modules are harbored in the 82 K ICE

Based on the ARDB database, multiple antimicrobial resistance genes or resistance gene clusters were predicted in the 82 K ICE (Fig. 2, red frame). The *tet*(O) gene, encoding a ribosomal protective protein, confers resistance to tetracycline and doxycycline. The *erm*(B) gene encoding an rRNA methyltransferase, conferring resistance to macrolides and lincosamides, such as erythromycin and lincomycin, and two *aphA* genes, each encoding an aminoglycoside 3′- phosphotransferase, mediating resistance to neomycin or kanamycin resistance (yellow boxes) are indicated in Fig. 2. Interestingly, two almost exactly analogous structure units were found in front of the *aph*A genes. The modules consist of the SNF2 family of proteins (pink), toxin -antitoxin pairs (red), and the *parA* gene (green). SNF2 proteins are ATP-dependent chromatin-remodeling factors associated with transcription,

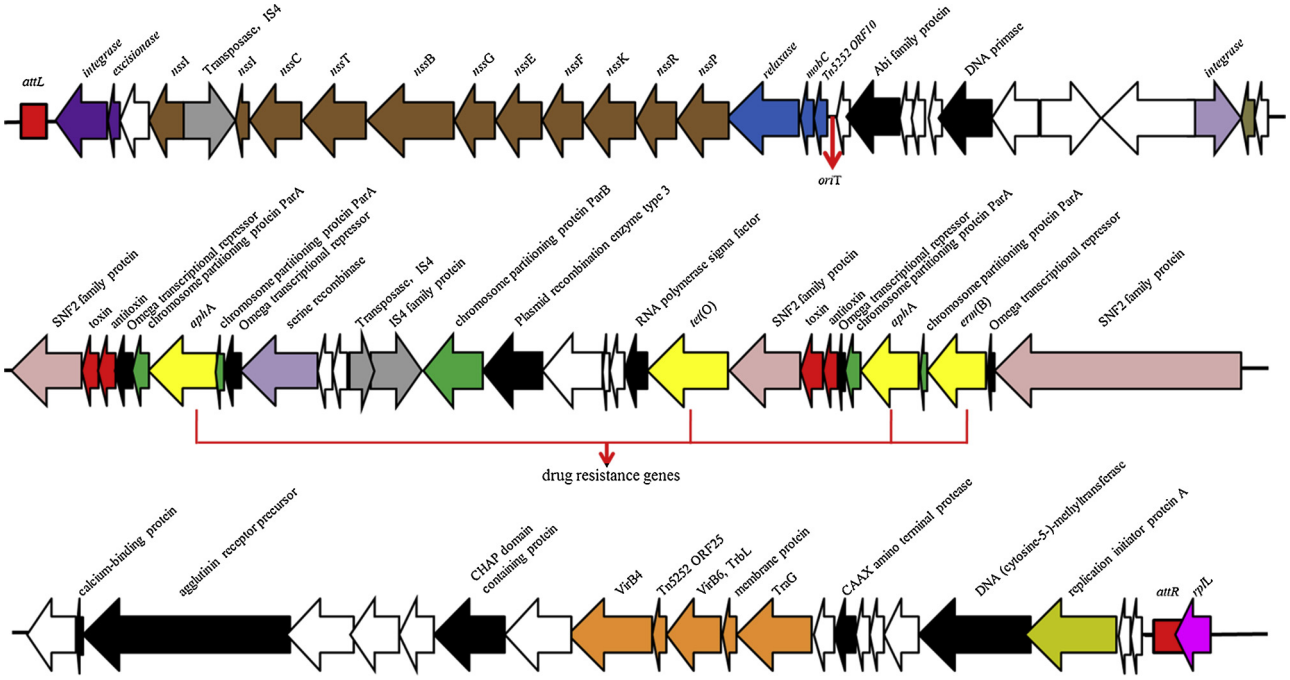

Fig. 2. Gene analysis of ICE*Ssu*CZ130302. Genetic map of the 82 K ICE in *S. suis* CZ130302. The direction of the arrow indicates the direction of transcription. Direct repeats *att*L and *att*R (red color) are located at the terminals of 82 K ICE. Genes are shown in different colors: DNA mobile and processing module (blue), integration and excision module (purple), drug resistance modules (yellow), and other functional genes (other colors). Nisin family proteins are labeled in brown, T4SS is marked in red, integrase is in lilac, and putative proteins are in white. The red frame indicates drug-resistant gene modules predicted by the ARDB database. The red arrow denotes the origin of transfer (*ori*T) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Comparison of minimum inhibitory concentrations (MICs) of several antibiotics against different strains.

Antibiotics	MICs (μg/mL)				
	CZ130302	CZ13-Δ82 K	P1/7 [a]	Transconjugant	*S. pneumoniae* ATCC 49619
tetracycline	64	4	0.5	128	0.125
doxycycline	16	0.125	0.125	64	2
erythromycin	1,024	0.015625	0.015625	2048	0.125
lincomycin	512	8	1	512	0.5
kanamycin	> 8,192	2,048	16	> 8,192	256
neomycin	4,096	128	128	32,768	16

[a] *S. suis* P1/7 has been reconstructed with the *spc* gene.

replication, homologous recombination, and DNA repair (Hu et al., 2013). A large antimicrobial-biosynthesis cluster was identified in the ICE (Fig. 2, brown boxes); this provides an important means for bacteria to produce the bacteriocin nisin (Wirawan et al., 2006; Wu et al., 2014) and inhibit growth of surrounding bacteria. In conclusion, multiple resistance genes were predicted in a dense region of the ICE, flanked by helper genes, which possibly enhanced their recombination.

3.3. 82-kb ICE is important for multidrug resistance in S. suis CZ130302 strain

The 82-kb ICE deletion mutant was constructed successfully and labeled with the spectinomycin resistance expression cassette to assess drug resistance (Fig. 1B). All PCR products were of the expected sizes. Hereditary stability of the mutant strain was examined for 10 generations *in vitro*. Growth curves showed that absence of the 82-kb ICE did not affect bacterial proliferation (Figure S2). The susceptibility of CZ130302 and CZ13-Δ82 K to a series of antimicrobial agents was compared, and the results are listed in Table 1. The mutant CZ13-Δ82 K strain presented increased susceptibility to tetracycline, macrolides, lincosamides, and some aminoglycosides. Genes conferring erythromycin and lincomycin resistance encode ribosome protective protein (CVO91_RS06800), an rRNA methyltransferase (CVO91_RS06855), and two aminoglycoside 3′-phosphotransferases (CVO91_RS06710 and CVO91_RS06835). These findings were consistent with the prediction of genetic annotation and the ARDB database. At the same time, these four drug-resistance genes were individually mutated and susceptibility to antibiotics was similar to that of CZ13-Δ82 K (data not shown). Overall, the 82-kb ICE harbors a multidrug resistance island and each resistance gene exerts an influence on *S. suis* strain CZ130302.

3.4. Mobilization of ICESsuCZ130302

Due to the integrase gene recognizing the *att* site at the 3′ end of *rpl*L, ICE*Ssu*CZ130302 could be integrated into bacterial chromosomes. Excision enzymes induced ICE to be excised from the bacterial chromosome, after which the mobile element formed a circular intermediate, which was transferred by conjugation and was finally integrated into the recipient bacteria (Fig. 3A). To verify the formation of circular DNA from the genome of CZ130302, specific primers (P1–P4) were designed. If ICE*Ssu*CZ130302 is present in the *S. suis* CZ130302 genome, primer pairs P1/P2 and P3/ P4 would yield positive PCR products, whereas the primer pair P4/P1 would not.

After excision from the donor chromosome and before integration into the recipient chromosome, the entire ICE would circularize to generate a short-living form, which could be visualized by PCR using P2/P3. Once the ICE has integrated into the chromosomal DNA, it would be present as a linear element. After the circular intermediate was excised from the chromosome, PCR products could be obtained using primers P4/P1. *S. suis* strains CZ130302 and P1/7, as well as ddH_2O were used as templates and the corresponding PCR products are shown in Fig. 3B. Three forms of ICE*Ssu*CZ130302 could be detected simultaneously when strain CZ130302 was cultured to logarithmic phase. Results indicated that ICE*Ssu*CZ130302 integrated on the chromosome maintained the ability to form circular DNA and be excised from chromosomes. However, a product was obtained by the primer pair P4/P1 in *S. suis* P1/7, indicating the absence of an ICE. Therefore, *S. suis* serotype Chz strain CZ130302 and *S. suis* serotype 2 strain P1/7 (spectinomycin-resistant) were selected, respectively, as donor and receptor for conjugation experiments. A total of 66 transconjugants were obtained successfully through double antibiotic plates and verified by PCR (Fig. 4). *S. suis* CZ130302, *S. suis* P1/7, and the P1/7 transconjugant containing ICE*Ssu*CZ130302 were used as templates. Primer pairs S2-F/S2-R and Chz-F/Chz-R were used to distinguish between serotypes 2 and serotype Chz. Other primer pairs were applied to detect the integrity and accurate insertion site of ICE*Ssu*CZ130302. In addition, the conjugation rate was calculated to be about 6.6×10^{-6}. Finally, compared with *S. suis* P1/7, the transconjugant exhibited significantly increased resistance to tetracycline, erythromycin, lincomycin, kanamycin, and neomycin (Table 1).

4. Discussion

The present study shows that chromosomal ICE*Ssu*CZ130302 could be transferred from strain CZ130302 to strain P1/7 in *vivo*, thus carrying antibiotic resistance genes to the recipient cell. With this single passage, P1/7, which was previously susceptible to antimicrobial agents, became resistant to tetracycline, doxycycline, erythromycin, lincomycin, kanamycin, and neomycin. Over the years, the drug resistance spectrum of *S. suis* has shown that drug resistance strategies in this strain have evolved rapidly and that this is particularly true for the spread of resistance to other strains. Aarestrup et al. (1998) demonstrated that the time-associated frequency of macrolides and tetracycline resistance in *S. suis* serotype 2 and 7 isolates had significantly increased during 15 years of continuous recordings. Palmieri et al. (2011) reported that almost all ribosomal protection genes, such as *tet* (M/O/32/Q/T/W), and efflux genes, such as *tet*(K/L/B/40), could be detected in *S. suis* strains. More than 90% of *S. suis* isolates are resistant to tetracycline and more than 70% are resistant to macrolides. Hernandez-Garcia et al. (2017) collected 405 isolates of *S. suis* and found that from 2009 to 2011 to 2013–2014, the level of resistance to numerous antimicrobial classes, including aminoglycosides, cephalosporins, fluoroquinolones, pleuromutilins, potentiated sulphonamides, and tetracyclines, had increased. Strain P1/7, which was isolated in 1981 (Clifton-Hadley, 1984), remains susceptible to tetracycline, but strain CZ130302 exhibits strong resistance to tetracycline, doxycycline, erythromycin, lincomycin, kanamycin, and neomycin (Pan et al., 2015).

Antimicrobial resistance arises through various mechanisms, including the selection of naturally occurring resistant mutants and horizontal gene transfer. Horizontal gene transfer is a crucial way to exchange resistance genes among different bacterial species or genera. Many drug resistance gens are located on ICEs, which can be passed through horizontal gene transfer to a new host cell. ICEs represent a type of mobile genetic element (MGE) and aid bacterial proliferation in

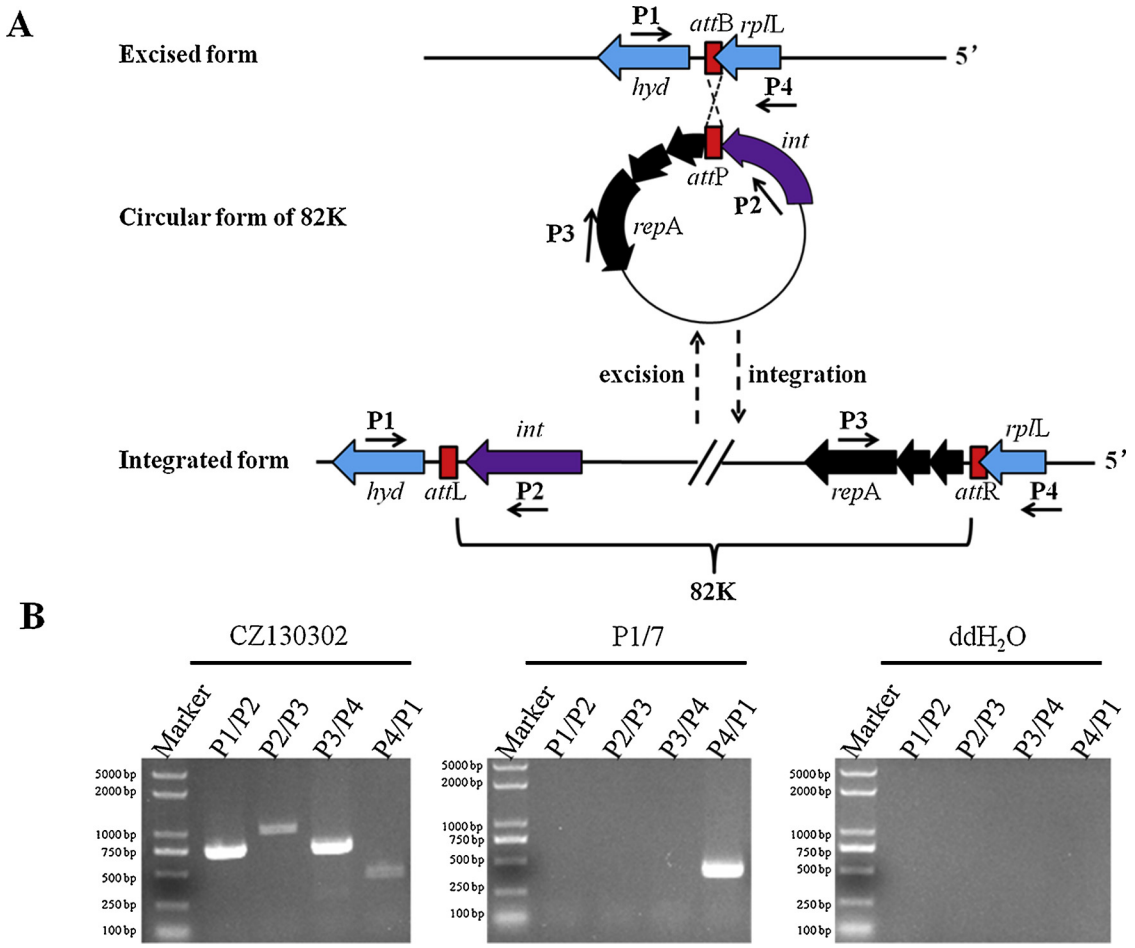

Fig. 3. Detection of the circular form of ICE*Ssu*CZ130302. (A) Schematic diagram of the integration and excision forms of ICE*Ssu*CZ130302 in the chromosome of *S. suis* CZ130302. The left and right red rectangles represent junctions (*att*L and *att*R), which can be formed by recombination. The direction and location of primers used to check the integration and excision forms of 82 K are indicated by thin arrows. (B) PCR analysis to detect the circular form of ICE*Ssu*CZ130302 in *S. suis* CZ130302; primer pairs are indicated above the lanes (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

a multifunctional manner, as they provide resistance to antimicrobial agents, immunity, heavy metals, and the capacity to degrade aromatic compounds (Ravatn et al., 1998; Huang et al., 2016c; Colombi et al., 2017). In addition, ICEs enhance the ability of the organism to colonize a eukaryotic host, fix nitrogen, promote biofilm formation, and insert virulence genes (Drenkard and Ausubel, 2002; He et al., 2004; Wozniak and Waldor, 2010). Normally, ICEs have been difficult to manufacture experimentally because they are integrated into the chromosome of an organism. Previous studies revealed different ICEs in most *S. suis* clinical isolates (Huang et al., 2016b). *S. suis* serotype 2 P1/7 is regarded as a particularly virulent strain (Holden et al., 2009). Interestingly, blasting the genome of P1/7 in the ICEberg database has failed to yield any type of ICE or ICE-like mobile elements (Bi et al., 2012). Moreover, this was confirmed in the present study by comparing the identity and similarity of flanking sequences of ICE*Ssu*CZ130302 between P1/7 and CZ130302.

By PCR mapping, we identified that ICE*Ssu*CZ130302 could be excised from the chromosome of the host cell, after which the linear ICE could be circularized and then transferred into the recipient cell for genetic integration.

Subsequently, the excised circles integrate into the chromosome of the recipient cell by transposable elements. The exact mechanism of chromosome-borne MGEs horizontal gene transfer and the key steps of ICEs excision, maintenance, circling, conjugative transfer, and integration remain poorly understood. The conjugation process in two organisms is difficult to simulate because ICEs are always linked to the bacterial chromosome under controlled laboratory conditions. In the present study, strain CZ130302 acquired broad spectrum antimicrobial resistance compared with the other isolates of *S. suis*.

Among *Streptococcus* species, novel ICE families are regularly identified in clinical isolates. Some conserved functional genes, such as those coding for integrases, relaxases, type IV coupling proteins, mating pair formation proteins, and ATPase (VirB4/TraU) proteins are always regarded as key genes. Integrases are associated with the recombination of excised circular DNA in the chromosome of the recipient cell. Relaxase is important for providing stability to the excised DNA-protein complex. Some mobile elements lacking nearby relaxases are not classified as part of the ICE family. ATPase participated in the co-transporting process, whereby the excised element is carried with the carrier across the cell membrane. Besides the drug resistance locus, the toxin-antitoxin system was also identified in ICE*Ssu*CZ130302. T4SS, which guides excision of the circular form of ICE from the donor cell. Type IV coupling proteins guide the DNA-protein complex across the channel and attachment to the recipient cell. Mating pair formation proteins construct a series of channels. At present, ICE family classification has not been carried out across the different serotype *S. suis* strains. Huang et al. (2016b) analyzed the core genes and proteins of 15 ICEs in the ICE*Sa*2603 family or ICE*Sa*2603-like family. They identified the integrase of the ICE*Sa*2603-like family as belonging to the SR group and the ICE*Sa*2603 family integrase as belonging to the tyrosine group. In

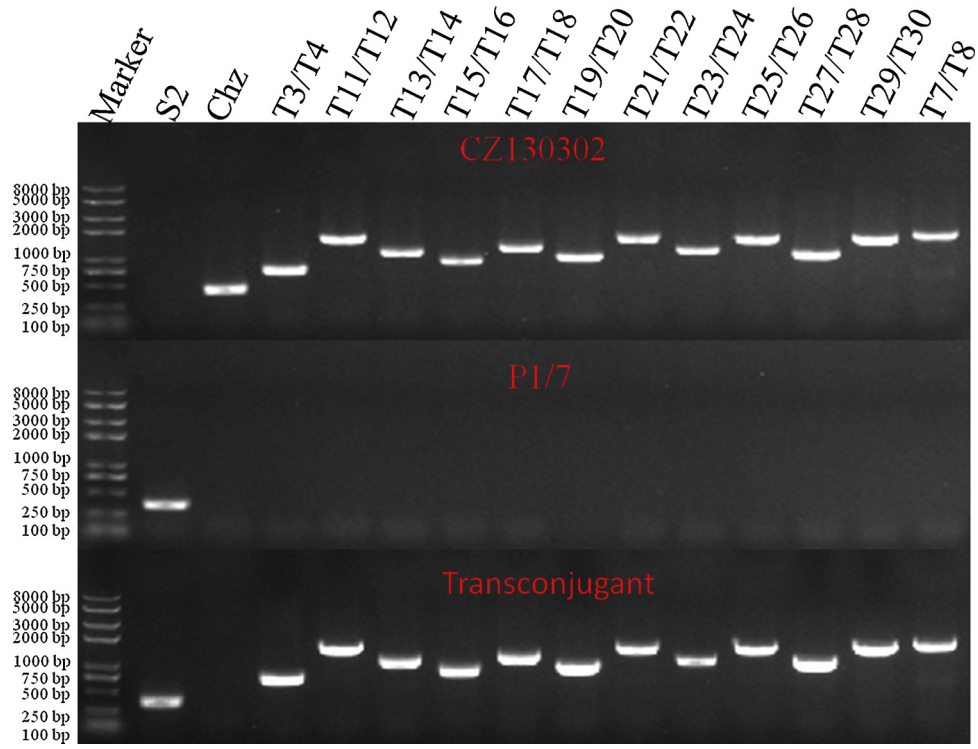

Fig. 4. ICE*Ssu*CZ130302 is transferable. PCR analysis to confirm the successful attainment of a transconjugant. To distinguish between the ICE*Ssu*CZ130302 transconjugant and *S. suis* serotypes Chz (strain CZ130302) and 2 (strain P1/7), 14 different pairs of primers were used.

our study, the integrase of ICE*Ssu*CZ130302 exhibited significant homology (97%) with the tyrosine family site-specific integrase of ICE*Sa*2603.

In 2005, outbreaks of fatal human streptococcal toxic shock syndrome in China presented a serious public health challenge, causing the death of 43 patients. Isolates from the blood of these patients harbored a candidate MGE named 89 K, which differed from classical virulent strains (Shi et al., 2016). The MGE included about 80 predicted genes thought to be required for full bacterial virulence. Here, the 82-kb ICE was identified and comparison with 89 K suggested that the core genes, including integrase and T4SS, which were highly homologous at the nucleotide and amino acid levels with over 96% identity; yet, the average similarity of other genes was only 37% (data not shown). For example, functional genes such as the tetracycline resistance gene *tet*(O) in the 82-kb ICE was replaced by the tetracycline resistance gene *tet*(M) in 89 K MGE. Significantly, more and more ICEs or ICE-like mobile elements are identified and analyzed by scanning bacterial genomes. This will likely reveal the detailed mechanisms with which organisms such as *S. suis* capture large MGEs via horizontal transfer.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2018.11.028.

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