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CRISPR-based genome editing of clinically important *Escherichia coli* SE15 isolated from indwelling urinary catheters of patients

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Abstract

Urinary tract infections (UTIs) are clinically important problems that lead to serious morbidity and mortality, and indwelling urinary catheters are a major factor of UTIs. In this study, we applied clustered regularly interspaced short palindromic repeats (CRISPR) genome editing to generate Δ*luxS* mutant strains from clinical isolates of *Escherichia coli* SE15, which is one of major pathogens and can cause colonization and biofilm formation in the catheter. A major regulatory pathway of such biofilm formation on medical devices is the quorum sensing mechanism via small molecule autoinducer-2 synthesized by LuxS enzyme. Here, we used the CRISPR-Cas9 system for precise deletion of *luxS* gene in clinical isolate *E. coli* SE15. To this end, we constructed a donor DNA for homologous recombination to delete 93 bases in the chromosomal target (*luxS*) and observed the success rate of *luxS* deletion to be 22.7 %. We conducted biofilm assay to observe decreased biofilm formation in the *E. coil* SE15 Δ*luxS* mutants compared to wild-type *E. coil* SE15. Quantitative real-time PCR analysis of *E. coil* SE15 Δ*luxS* mutants showed that the expression of *luxS* was below detection level. We also observed that the relative mRNA levels of biofilm-formation-related genes, such as *mqsR*, *pgaBC* and *csgEF*, were significantly decreased in *E. coil* SE15 Δ*luxS* mutants compared to wild-type. We conclude that genome editing by CRISPR-Cas9 system is an effective tool to dissect the molecular mechanism of biofilm formation in medically important strains, and the study may serve as a basis for developing novel medical intervention against UTIs caused by biofilm.

INTRODUCTION

Urinary tract infections (UTIs) are the most common nosocomial infection, accounting for over 40 % of all hospital-associated infections, and can cause serious morbidity and mortality. An estimated 80 % of all nosocomial UTIs are catheter-associated urinary tract infections (CA-UTIs) related to use of an indwelling urinary catheter [1]. The pathogenesis of CA-UTIs involves bacterial biofilm formation on the inner surfaces of an indwelling urinary catheter [2, 3].

In the previous studies, *Escherichia coli* was reported to be the causative pathogen in 70–95 % of UTIs and was isolated from the urine of 20–50 % of patients with indwelling urinary catheters [4, 5]. Such *E. coli* strains, named uropathogenic *E. coli* strains, are able to colonize and form biofilm in urinary tracts and artificial conditions such as catheter. The biofilm formation by *E. coli* on the surface of the catheter can trigger CA-UTIs, one of the most common nosocomial infections [6, 7].

Such biofilm formation is mainly regulated by a cell-to-cell communication called quorum sensing (QS). Upon activation of QS mechanism, bacteria secrete autoinducers (AIs) to the extracellular environment, and once a high cell density is reached, they upregulate biofilm formation and development [8]. The QS system in *E. coli* has been an issue of major interest, and several intra–extracellular signalling systems, such as LuxR homology SdiA, LuxS/AI-2 system, AI-3 and indole, have been reported [2, 9–11]. Notably, among the QS systems, the LuxS/AI-2 system is uniquely linked to central metabolism and AI-2 directly stimulates biofilm in *E. coli* [12, 13].

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Keywords: CRISPR-Cas9 system; *E. coli* SE15; *luxS* gene; biofilm; UTIs.

Abbreviations: AI, autoinducer; CA-UTIs, catheter-associated urinary tract infections; CRISPR, clustered regularly interspaced short palindromic repeat; crRNA, CRISPR RNA; HDR, homology directed repair; PAM, protospacer adjacent motif; qRT-PCR, quantitative real time-polymerase chain reaction; QS, quorum sensing; RT-PCR, reverse transcriptase-polymerase chain reaction; sgRNA, single guide RNA; tracrRNA, trans-activating crRNA; UTI, urinary tract infection.

Several technologies for targeted gene editing such as homologous recombination, allelic replacement, integrases and directed nucleases have been available in eukaryotes and prokaryotes [14]. In bacterial cells, mutagenesis of a gene generally required introduction of a selection marker in the edited site or needed a two-step process that included a counter-selection system for homologous recombination [15, 16]. In addition, phage recombination proteins have been applied for genome editing to improve homologous recombination by linear DNA or oligonucleotides [17]. However, since this method lacks a mutant selection step, the efficiency can be relatively low for mutant construction. Therefore, many colonies had to be analysed to find a genetically engineered colony. Thus, new tools with easy operation and high efficiency were still required for genome editing in prokaryotic organisms.

The clustered regularly interspaced short palindromic repeats (CRISPR)-associated systems, which are bacterial adaptive immunity systems against invading DNA from virus, were recently developed as an efficient genome engineering method with wide applications [18]. The CRISPR-based genome editing technology has been widely applied to many organisms, including *E. coli* [19, 20], *Saccharomyces cerevisiae* [21], *Streptomyces* spp. [22], human cells [23], mouse [24] and zebrafish [25].

Currently, the most common CRISPR genome editing technology is based on the type II CRISPR-Cas system from *Streptococcus pyogenes*. This system uses the endonuclease Cas protein 9 (SpCas9) and two RNAs [maturation CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA)] to specify the target of any 20 nt DNA sequence [26]. The target DNA sequence, called protospacer, is however limited by the requirement of a protospacer-adjacent motif NGG, where N represents any nucleotide at the 3′ end of the target sequence [27]. For genome editing, SpCas9 and the two RNAs (crRNA and tracrRNA) are sufficient to induce dsDNA cleavage at the target site [28], and it was also found that a single guide RNA (sgRNA) can efficiently replace crRNA and tracrRNA [29]. Thus, in CRISPR-based genome editing, CRISPR-Cas9 induces double-strand DNA break at the target DNA in a programmable manner, and the DNA break is repaired via indel-forming non-homologous end joining or homology-directed repair [19, 30].

To date, there has been no study reporting on the application of CRISPR genome editing to medically important bacterial strains isolated from patients. Therefore, we performed CRISPR-Cas9-based genome editing in an *E. coli* SE15 strain isolated from a patient's indwelling urinary catheter. The delivery of CRISPR-Cas9 and donor DNA template, with homology to *luxS* gene, facilitated highly efficient selection of bacterial colonies that underwent homologous recombination. As a result, we obtained *luxS* mutant *E. coli* SE15 clones with a precise 93 base deletion at the target genomic locus. The *luxS* mutant *E. coli* SE15 clones showed decreased biofilm formation, and the overall gene expression in the biofilm pathway was repressed. In conclusion, we report the first application of CRISPR-Cas9 to a medically important bacterial strain.

METHODS

Bacteria and culture conditions

In one of our previous studies, *E. coli* SE15 was isolated from the indwelling catheter of UTI patients in a hospital in South Korea [31]. All strains and plasmids used in this study are listed in Table 1. *E. coli* strain DH5α was used for cloning vectors. The strains were routinely cultured at 37 °C with shaking at 150 r.p.m. in Luria–Bertani (LB) medium with antimicrobial agents added when necessary. Because pCas vector has temperature-sensitive replication (RepA101ts), pCas electro-transformed *E. coli* SE15 were cultured at 30 °C. The antimicrobial agents used were spectinomycin and kanamycin.

Construction of *luxS* mutant using CRISPR-Cas9 system

Primers used for preparation of the sgRNA and N_{20} sequences are listed in Table 2. Plasmids and genomic DNA were extracted using the NucleoSpin kit (Macherey–Nagel) according to the manufacturer's instructions. In this study, we used two plasmid systems (separate expression of SpCas9 and the sgRNA in pCas and pTargetF, respectively) for genome editing using CRISPR-Cas9 system in *E. coli* SE15, as described previously [32]. In short, *E. coli* SE15 *luxS* mutant strains were constructed by utilizing the three elements: Cas9 constitutive expression plasmid with λ-Red recombineering system, pTarterF with target (*luxS*) sgRNA plasmid and donor template DNA. The donor DNA

Table 1. List of the bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype	Reference
Strains		
E. coli SE15	Clinical isolates*	In this study
E. coli DH5α	F$^-$ *supE44 recA1* Δ*lacU169* (ϕ80 *lacZ*ΔM15) *gyrA96 thi-1 hsdR17 endA1*	[43]
Plasmids		
pCas	*repA101*(Ts) *kan* P_{cas}-*cas9* P_{araB}-*Red lacI*^{q} P_{trc}-sgRNA-*pMB1*	[32]
pTargetF	*pMB1 aadA* sgRNA-*pMB1*	[32]

**E. coli* SE15 was isolates from indwelling urinary catheter of patients and deposited in Korea Environmental Microorganism Bank (KEMB 9000-004).

Table 2. Primer list for quantitative real-time PCR

The sgRNA sequence is underlined. Bold represents N_{20} sequence of target gene (*luxS*).

Gene locus		Oligonucleotide sequence (5′ → 3′)
sgRNA-*luxS*	Forward	GTCCTAGGTATAATACTAGT**GCTTCACAGTCGATCATACC**GTTTTAGAGCTAGAAATAGC
	Reverse	ACTAGTATTATACCTAGGACTGAG
Fragment A	Forward	ATGATGATAACAAATGCGCGTCTTTCATATACTCAGACTCGCCTGGGAACAAAGAGTTCA
	Reverse	CGGCATTTAGCCACCTCCGGTAATTTTTTTTTAAAATTTTCTGAACTCTTTGTTCCCAGGC
Fragment B	Forward	ACCGTGTTCGATCTGCGCTTCTGCGTGCCGAACAAAGAAGTGATGCCAGAAAGAGGGATC
	Reverse	GGTTACGCATAAAACCAGCAAACAGGTGCTCCAGGGTATGGATCCCTCTTTCTGGCATCA
luxS	Forward	TCG GAT GAC TGA CAG GTA ACA
	Reverse	TTA CCG GAG GTG GCT AAA TG
recA	Forward	ACA GAT TTC CAG TGC CTG CT
	Reverse	GTT CCA TGG ATG TGG AAA CC
rpoS	Forward	GAA TCC ACC AGG TTG CGT AT
	Reverse	GCG ACT CAG CTT TAC CTT GG
mqsR	Forward	CGC AGG CGA CCT ACA CTT AT
	Reverse	GCC GTA GTT GAC CAC TGT CC
pgaB	Forward	TTA AAT TGC TCC GGG TTT TG
	Reverse	TGA CAG GGT CAG AGC ACA AG
pgaC	Forward	AAA GCG GCA TGT ATG GTT TC
	Reverse	TTA TTG GCG GCG TCT ATT TC
csgE	Forward	ATC TGG CGA CGA TTT AGT GC
	Reverse	ATT AAT GAA ACG CCC AGT GC
csgF	Forward	TGA TGG CGT TTC AAT ACC AA
	Reverse	TGC GTG TCA AAC ATG CAG TA

contained *luxS* homologous sequences with 93 bp deletion. The DNA sequence of *luxS* gene in *E. coli* SE15 was determined by PCR using *luxS* primer.

The pTargetF which encodes the sgRNA targeting the *luxS* gene (target sequence selected by DNA2.0 software) was constructed by site-directed mutagenesis. Briefly, the guide RNA target sequence in pTargetF was mutagenized by PCR to target *luxS*, and the reaction was treated with DpnI (NEB) to eliminate the wild-type pTargetF. We performed cloning for a pTargetF-*luxS* spectinomycin-resistant plasmid harbouring a spacer (for guiding Cas9 cleavage of the wild-type SE15) in *E. coli* DH5*α* and selected on LB plate with 50 μg ml^{-1} spectinomycin. The transformants were confirmed by sequencing.

The pCas kanamycin-resistant plasmid was first introduced into *E. coli* SE15 by electro-transformation. Then, the *E. coli* SE15 cells harbouring pCas were prepared for co-transformation of the pTargetF-*luxS* and a donor DNA (Fig. 1). The co-electro-transformation was performed as previously described [32]. Briefly, cells were mixed with pTargetF, and donor DNA was incubated on ice for 5 min. Co-electro-transformation was conducted in a 2 mm Micro Pulser cuvette (Bio-Rad), and the cells were immediately resuspended with 1 ml pre-warmed LB medium. Cells were then recovered at 30 °C for 1 h before being spread onto LB plates supplemented with spectinomycin and kanamycin and incubated overnight at 30 °C. The transformants were confirmed by colony PCR and DNA sequencing (Macrogen).

Confirmation of *luxS* gene deletion in *E. coli* SE15

To confirm the deletion in *luxS* sequences and loss of RNA expression in the mutant strain SE15, we performed both reverse transcriptase PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR). Total RNA was isolated from both wild-type *E. coli* SE15 and Δ*luxS* strain SE15 using the NucleoSpin kit (Macherey–Nagel) according to the manufacturer's instructions. Synthesis of cDNA was performed by reverse transcription using Superscript II reverse transcriptase (Invitrogen) with random hexamer primer. Then, 2 μl of the cDNA was used for PCR amplification with *luxS* primer sets, using 16S rRNA primer sets as internal control gene. Then, the PCR products were separated by DNA electrophoresis [1 % (w/v) agarose gel], and the gel was stained with RedSafe staining kit (iNtRON Biotechnology) and the bands were visualized by UV lamp.

Biofilm formation assay using crystal violet

Biofilm formation was measured using the microtitre dish assay system, performed as described previously [33]. Wild-type *E. coli* SE15 and Δ*luxS* SE15 were grown in 96-well plates at 37 °C without shaking in TSB (tryptic soy broth) medium supplemented with 0.2 % (w/v) glucose, 1 mM $MgSO_4$ and 1 mM $FeSO_4$. To quantify biofilm formation of *E. coli* SE15 and Δ*luxS* SE15, 150 μl of 0.1% crystal violet solution was added to the wells, and the samples were then washed in distilled water and solubilized in 30 % (v/v) glacial acetic acid. Finally, the absorbance was quantified using a plate reader at 595 nm using 30 % (v/v) glacial acetic acid

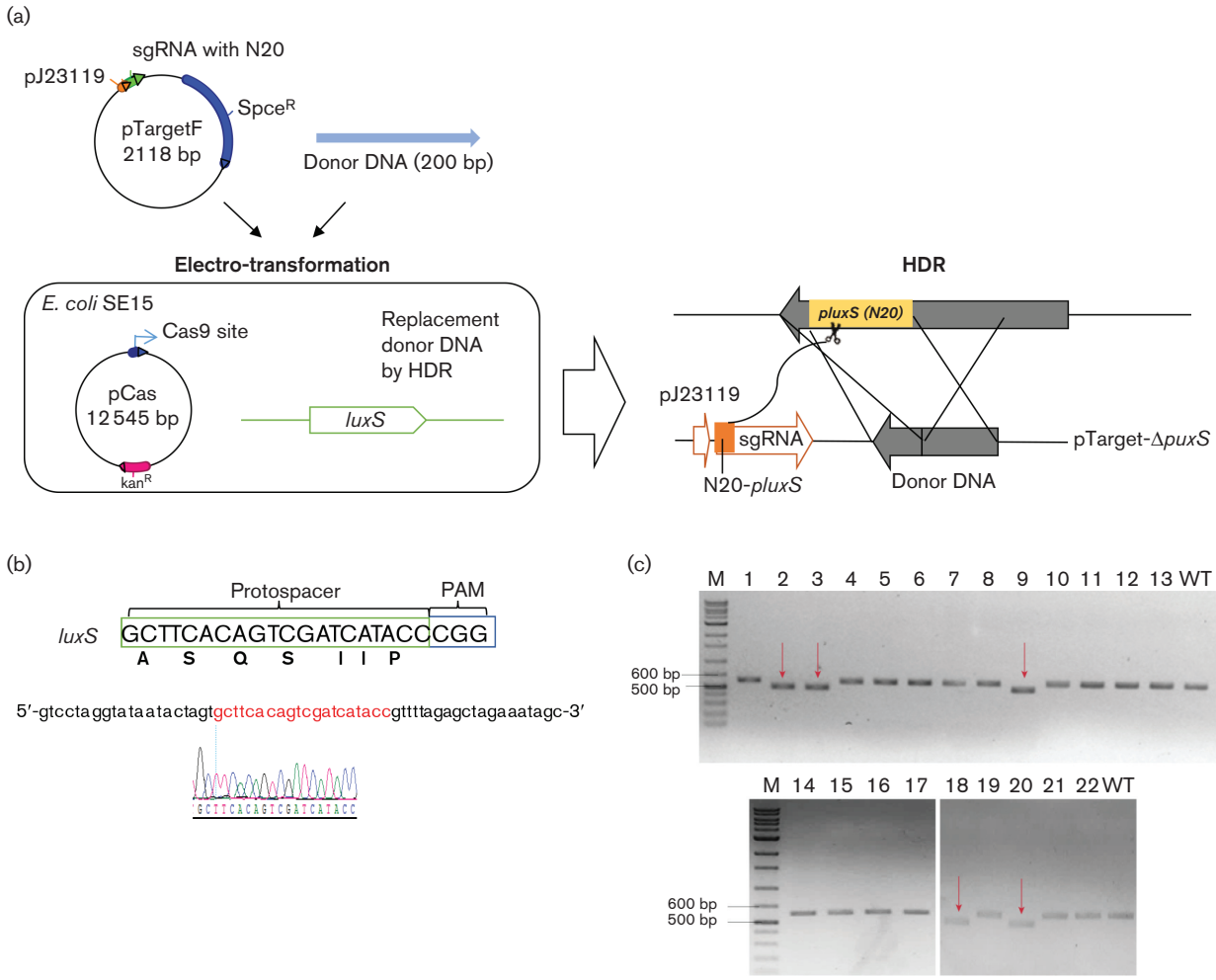

Fig. 1. CRISPR-Cas9 system for genome editing in *E. coli* SE15. (a) Experimental scheme of the CRISPR-Cas9 system to induce *luxS* mutation. (b) Sequence of sgRNA. (c) DNA electrophoresis result of picked colonies with spectinomycin and kanamycin resistance. Five samples (red arrows) were selected for DNA sequencing. HDR, Homology directed repair; PAM, protospacer adjacent motif.

as the blank. Each data point was the average from five replicate wells, and the standard deviations were calculated.

Verification of *luxS* related novel genes by qRT-PCR

Primers for genes used in this study are listed in Table 2. Wild-type *E. coli* SE15 and Δ*luxS* SE15 cultures were centrifuged and stored frozen until RNA extraction. Reverse transcription using Superscript II reverse transcriptase (Invitrogen) was performed with random hexamer primer. The cDNA was used as a template for qRT-PCR, which was conducted in a total of 20 µl reaction mixture containing the SYBR Green 2× mix (Bio-Rad), 0.2 µM target primers and cDNA (1000 ng). The SYBR-Green-labelled PCR products were detected with a CFX96 Touch detection system (Bio-Rad).

Data were gathered during the annealing step, and melting curves were obtained following completion of the reaction. Raw data were analysed by using the BioCFX Manage (Bio-Rad), and then relative quantitation with the threshold cycle (C_t) method was performed on Microsoft Excel. The comparative threshold method ($\Delta\Delta C_t$ analysis) was applied to evaluate the profile in gene expression. Computer programs CFX manager (Bio-Rad) and Excel (Microsoft) were used to calculate: $\Delta\Delta C_t = \Delta C_t$, sample $-\Delta\Delta C_t$, reference.

RESULTS AND DISCUSSION

Genome editing using CRISPR-Cas9 system for construction of *luxS* mutant in *E. coli* SE15

We first sought to delete a large part of *luxS* gene in *E. coli* SE15 using CRISPR. The traditional dsDNA-mediated recombineering tool gives relatively low efficiency $>10^{-4}$. Genome editing by small single-strand DNA yields higher mutation rate, but the efficiency reduces significantly with larger replacements and insertions (<2 % for >20 bp) [34]. Since the CRISPR-Cas9 genome editing technique is a

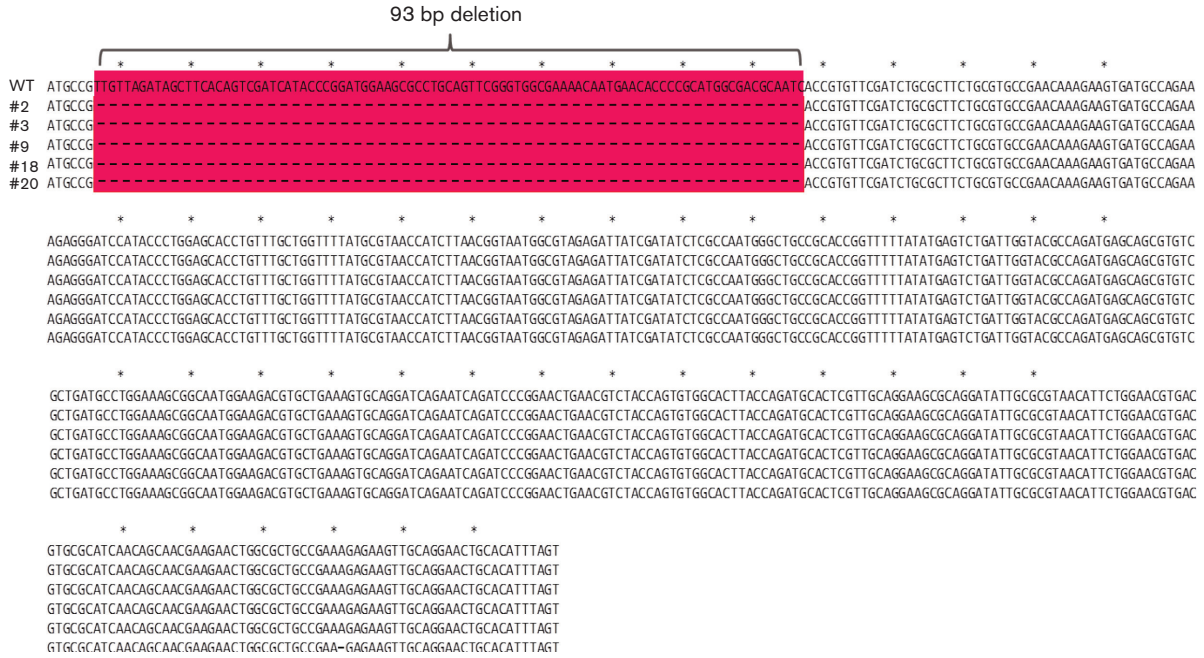

Fig. 2. Sequence alignment of wild-type SE15 and *luxS*-deleted mutant candidate strains. Pink box represents the deleted region (93 bp).

powerful tool to construct knockout mutant, we performed CRISPR-assisted genome editing using the two-plasmid-based CRISPR-Cas9 system [32] to mutagenize the *luxS* gene in *E. coli* SE15. The two-plasmid system consisted of a pCas kanamycin-resistant plasmid expressing the Cas9 and λ-Red recombineering and a pTargetF spectinomycin-resistant plasmid carrying the sgRNA with target *luxS* gene spacer (N_{20}) [32, 35]. The pCas kanamycin-resistant plasmid was firstly inserted into *E. coli* SE15 by electroporation for constitutive expression of Cas9 protein. Also, we prepared a pTargetF::*luxS* spectinomycin-resistant plasmid harbouring a spacer (guiding Cas9 cleavage of the wild-type SE15). Then, the SE15 harbouring pCas was co-transformed with pTargetF::*luxS* plasmid and donor DNA, a DNA fragment with sequences homologous to the upstream (100 bp) and downstream (100 bp) regions of the *luxS* locus and 93 bp deletion (Fig. 1). We only selected 22 colonies that were spectinomycin and kanamycin

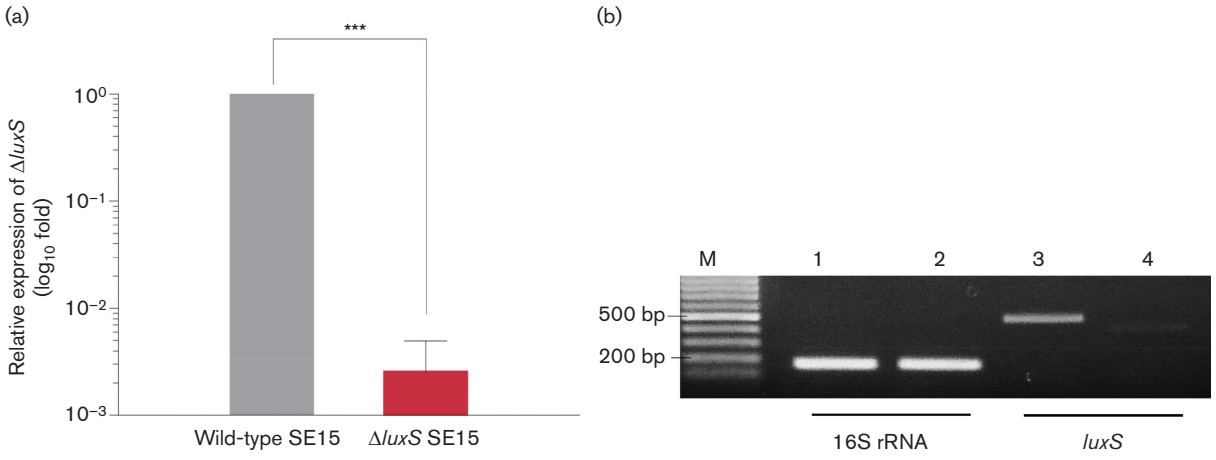

Fig. 3. qRT-PCR (a) and RT-PCR (b) of *luxS* mRNA from wild-type SE15 and Δ*luxS* SE15. A smaller-size PCR product corresponding to truncated *luxS* mRNA was detected by RT-PCR of the Δ*luxS* SE15 compared to wild-type. 16S rRNA (200 bp) was used as an internal reference. Error bars represent the standard deviation of the mean for three independent experiments (***P<0.001). M, Marker; Lane 1 and 3, wild type *E. coli* SE15; Lane 2 and 4, Δ*luxS* mutant SE15.

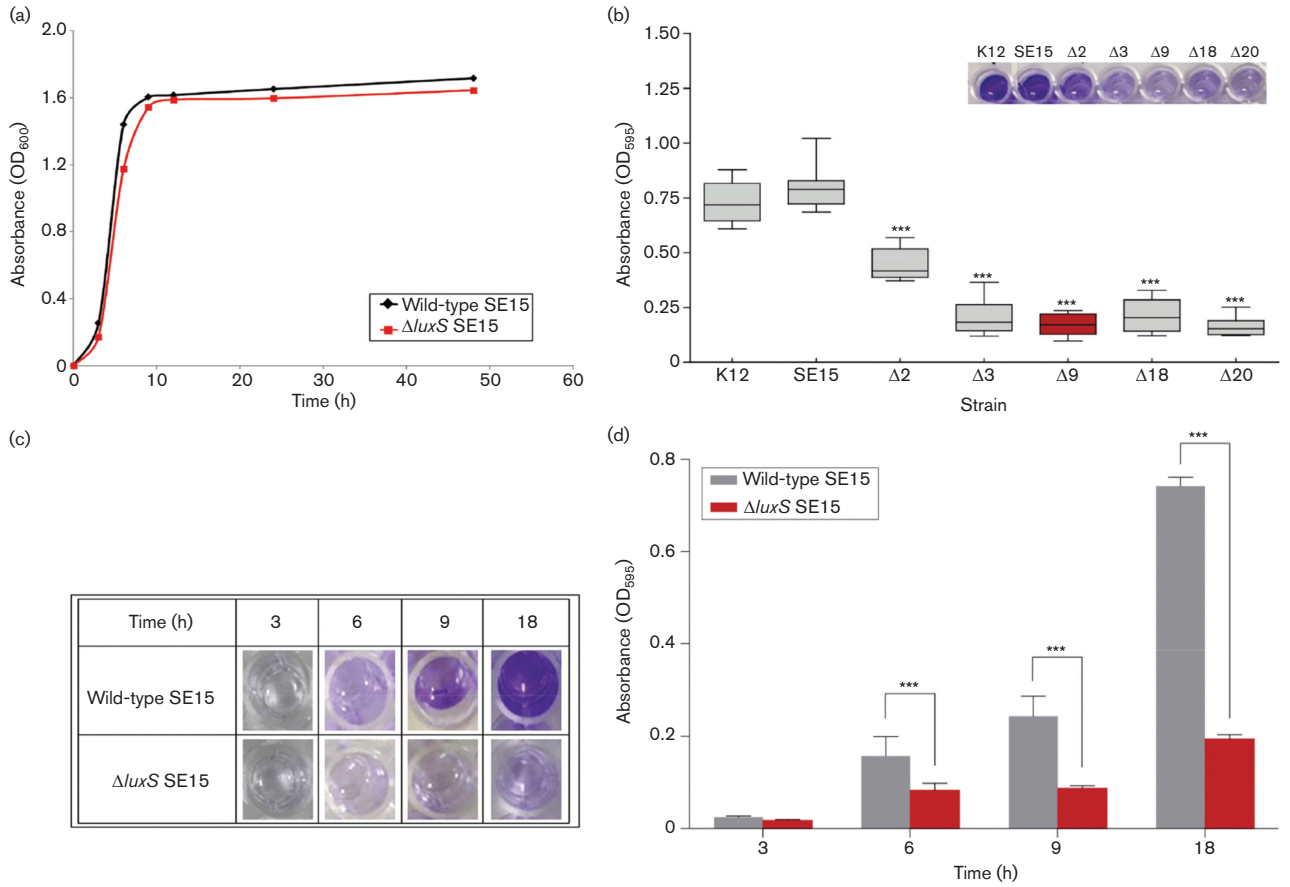

Fig. 4. The biofilm formation was regulated by *luxS*-dependent QS system. (a) Growth curve of wild-type SE15 and Δ*luxS* SE15 in TSB supplemented with 1 mM $MgSO_4$ and 2 % (w/v) glucose. (b) Biofilm formation in wild-type SE15 and Δ*luxS* SE15. Shown is the quantification of crystal-violet-dyed attached cells forming biofilms in wild-type SE15 and Δ*luxS* mutant candidates (five colonies). (c) Time course of crystal violet dye staining of wild-type SE15 and Δ*luxS* SE15 (#9 colony). (d) Quantification of the crystal violet stain time course. Error bars represent the standard deviation of the mean for five independent experiments (***P<0.001).

resistant and, to check for mutation, conducted PCR with primers targeting *luxS* (Table 2). We resolved the PCR amplicons with electrophoresis and found that the PCR fragments of five mutant candidate colonies (#2, #3, #9, #18 and #20) were smaller (470 bp) than wild-type (563 bp), consistent with 93 bp deletion in *luxS* (Fig. 1c). The mutant candidate clones were validated by sequencing, and the results showed that 100 % of five colonies contained the expected 93 bp deletion in *luxS* gene locus (Fig. 2), confirming that we obtained *luxS* deletion efficiency of 22.7 % (5 mutants/22 colonies).

Next, we compared the *luxS* mRNA expression levels between wild-type SE15 and Δ*luxS* SE15 by performing RT-PCR and qRT-PCR (Fig. 3). qRT-PCR showed that the *luxS* gene mRNA expression was 2500-fold lower in Δ*luxS* SE15 compared to wild-type SE15 (P<0.001). Agarose gel electrophoresis of RT-PCR showed no detectable signal of *luxS* for Δ*luxS* SE15 mutants.

These results suggested that the CRISPR-Cas9 system of genome editing is a useful platform not only for a single gene but also for accurate large-scale bacterial genome manipulations.

Effect on the biofilm formation by *luxS* deletion

QS-mechanism-associated genes are essential for biofilm formation, and LuxS/AI-2 is one of the QS mechanisms that is closely associated with biofilm formation in *E. coli* strains [36, 37]. In the report of Niu, AI-2 was able to control biofilm formation of *E. coli* via regulating motility [38]. In this study, we investigated whether biofilm formation is influenced by the mutation of *luxS* in *E. coli* SE15 strain isolated from the indwelling catheters. No difference in the growth of Δ*luxS* mutant and *E. coli* SE15 was observed, but we found significant changes of biofilm formation between Δ*luxS* mutant and *E. coli* SE15 (Fig. 4a). To assess the biofilm formation, we applied crystal violet assay to wild-type SE15 and mutant candidates (#Δ2, #Δ3, #Δ9, #Δ18 and #Δ20) grown in 96-well plates (Fig. 4b). All mutant candidates showed dramatically decreased biofilm formation compared to wild-type SE15 by crystal violet quantification (absorbance at 595 nm): wild-type SE15 (0.798±0.099), Δ2

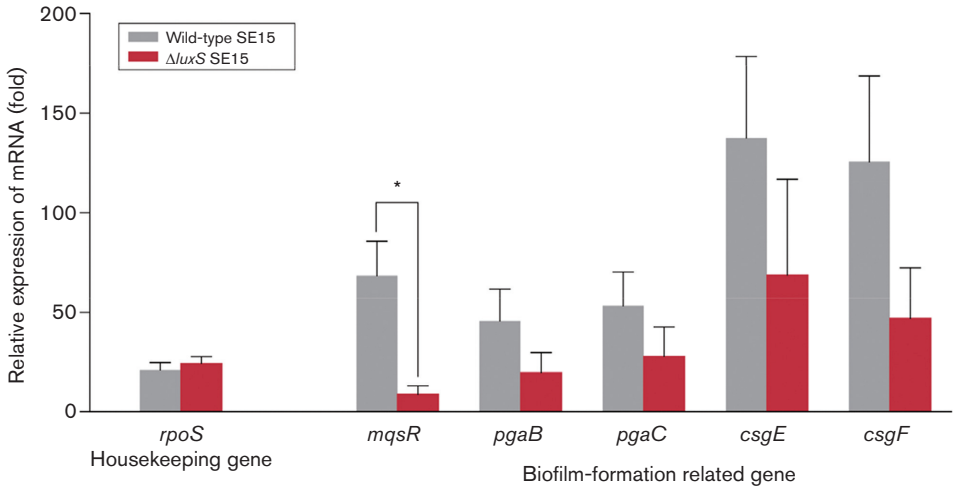

Fig. 5. Downregulation of biofilm-related genes by *luxS* gene mutation. Shown is the decrease in induction of *luxS*-dependent QS genes in Δ*luxS* mutant. Relative expression levels were internally normalized to *recA* gene and compared between early phase and stationary phase. Induction of *rpoS*, a housekeeping gene, was not changed. Error bars represent SD for triplicate experiments. (*P<0.05).

(0.445±0.072), Δ3 (0.203±0.075), Δ9 (0.174±0.050), Δ18 (0.216±0.078) and Δ20 (0.164±0.041). We further assessed the impact of *luxS* mutation on biofilm formation in a time-dependent manner, by comparing a mutant *E. coli* SE15 (Δ9) and wild-type. For Δ*luxS* SE15, we observed a significantly reduced biofilm formation throughout the time course (0.168±0.021 at 18 h) compared to wild-type (0.742 ±0.042 at 18 h). (Fig. 4c, d). These results support the hypothesis that AI-2/LuxS-dependent QS is significantly correlated to biofilm formation.

Biofilm-related genes that depend on QS signal are regulated by *luxS* deletion

It has been reported that extracellular AI-2 concentration reaches its peak in mid-late exponential phase and quickly reduces on initiation of stationary phase [8, 39]. The report of Ren *et al.* [40] demonstrated that several genes, including biofilm-related genes, were repressed in stationary phase of *E. coli* to adjust to high cell density [40]. Besides, synthesized AI-2 molecules directly stimulate biofilm formation in *E. coli* by increasing bacterial motility, which is regulated by several factors including MqsR protein [41]. Therefore, we sought to analyse the induction of genes, such as *mqsR*, *pgaBC* and *csgEF*, which are associated with biofilm formation. To this end, we conducted qRT-PCR to quantify the changes in the gene expression levels from low density (3 h) to stationary phase (12 h) and compared the fold changes between wild-type SE15 and Δ*luxS* SE15 (Fig. 5). We found that, in Δ*luxS* SE15, biofilm-formation-associated genes (*mqsR*, *pgaBC* and *csgEF*) were significantly less induced compared to wild-type SE15, while the induction of *rpoS*, a housekeeping gene, was not affected. The *mqsR* mRNA expression in Δ*luxS* mutant SE15 was induced only 8.8-fold on the initiation of stationary phase compared with 68.3-fold induction in wild-type *E. coli* SE15. Induction of *pgaB* and *pgaC* were 19.7- and 27.9-fold in Δ*luxS* mutant compared with 68.3- and 45.9-fold in wild-type. The induction of *csgE* and *csgF* gene expression also showed a similar trend: 68.5- and 47.0-fold in Δ*luxS* mutant SE15 and 137.6- and 125.5-fold in wild-type. The results showed that CRISPR-mediated knockout of *luxS* efficiently decreased the biofilm formation of the medically relevant *E. coli* strain via repression of gene expression in the pathway.

Biofilm formation on the surface of urinary catheters is a key factor in the pathogenesis of CA-UTIs [37], and the control of biofilm formation by QS mechanism suggested encouraging approaches in treating urogenital infection from *E. coli* [42]. Here, we sought to develop a novel method to prevent CA-UTI via controlling the biofilm formation on the surface of urinary catheters. In this study, we demonstrated the first application of CRISPR-Cas9 system to construct *luxS* mutants from the clinical isolate *E. coli* SE15 found on indwelling urinary catheters of patients. We observed that the bacterial *luxS* deletion efficiency was 22.7 % and also found that *luxS* deletion led to reduced biofilm formation and effective downregulation of biofilm-related genes in wild-type *E. coli* SE15. Overall, we propose a strategy to use the CRISPR-Cas9 system of genome editing to make a platform for accurate large-scale bacterial genome manipulations. We conclude that the CRISPR-Cas9 system will be useful for investigating the molecular mechanism of how AI-2-dependent QS regulates biofilm formation in clinically important bacteria including and not limited to *E. coli* SE15.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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