Development and Application of Two Inducible Expression Systems for *Streptococcus suis*

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ABSTRACT

*Streptococcus suis* is an important zoonotic bacterial pathogen posing a threat to the pig industry as well as public health, for which the mechanisms of growth and cell division remain largely unknown. Developing convenient genetic tools that can achieve strictly controlled gene expression is of great value for investigating these fundamental physiological processes of *S. suis*. In this study, we first identified three strong constitutive promoters, *P_g*, *P_t*, and *P_e*, in *S. suis*. Promoter *P_g* was used to drive the expression of repressor genes *tetR* and *lacI*, and the operator sequences were added within promoters *P_t* and *P_e*. By optimizing the insertion sites of the operator sequence, we successfully constructed an anhydrotetracycline (ATc)-inducible expression system and an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible expression system in *S. suis*. We showed that these two systems provided inducer-concentration- and induction-time-dependent expression of the reporter gene. By using these tools, we investigated the subcellular localization of a key cell division protein, FtsZ, which showed that it could be correctly localized to the midcell region. In addition, we constructed a conditional knockout strain for the *glmS* gene, which is an essential gene, and showed that our ATc-inducible promoter could provide strictly controlled expression of *glmS* in trans, suggesting that our inducible expression systems can be used for deletion of essential genes in *S. suis*. Therefore, for the first time we developed two inducible expression systems in *S. suis* and showed their applications in the study of an important cell division protein and an essential gene. These genetic tools will further facilitate the functional study of other important genes of *S. suis*.

IMPORTANCE

*Streptococcus suis* is an important zoonotic bacterial pathogen. Studying the mechanisms of cell growth and division is important for the identification of novel antimicrobial drug targets. Inducible expression systems can provide strictly controlled expression of the protein of interest and are useful tools to study the functions of physiologically important proteins. However, there is a lack of convenient genetic tools that can achieve inducible protein expression in *S. suis*. In this study, we developed two (ATc-inducible and IPTG-inducible) inducible expression systems and showed their applications in a subcellular localization study of a cell division protein and the construction of conditional knockout of essential genes in *S. suis*. These systems will be useful for functional studies of important proteins of *S. suis*.

KEYWORDS

*Streptococcus suis*, genetic tool, inducible expression system, subcellular localization, conditional gene knockout

*Streptococcus suis* is an important zoonotic pathogen causing meningitis, arthritis, endocarditis, pneumonia, and septicemia in pigs; it can also lead to streptococcal toxic shock-like syndrome (STSLS) in humans, with very high mortality rates (1–3). Performing studies regarding the etiology as well as the mechanisms of pathogenesis of *S. suis* is critical for developing novel strategies to prevent and control the disease.
To date, extensive studies have been carried out and advances have been made regarding the pathobiology of this important pathogen (4–7). However, it needs to be pointed out that convenient genetic tools for manipulating the genome of *S. suis* are a prerequisite for these studies. The most commonly used genetic tools for *S. suis* have been developed by D. Takamatsu and colleagues, who established a set of genetic tool systems including the temperature-sensitive (Ts) suicide vectors pSET4s, pSET5s, and pSET6s for gene knockout and the replicating vectors pSET1, pSET2, and pSET3 for gene expression (8, 9). However, there is still a lack of genetic tools that can provide inducible expression of proteins in *S. suis*.

Bacteria replicate through binary fission, which involves DNA replication and segregation, cell growth, and cell division (10). These are the most fundamental and important physiological processes, which are attracting antimicrobial drug targets (11–13). As an oval coccus, *S. suis* undertakes a usual pattern of elongation and division, the molecular mechanisms of which are not yet fully understood. There are several dozen proteins that participate in cell growth and division, and many of them are reported to be essential and need precise regulation (10, 14). FtsZ is one of the most important proteins during bacterial cell division and serves as a scaffold to recruit other cell division proteins (15). Proper subcellular localization of FtsZ ensures the subsequent assembly of other cell division proteins (16). GlmS is a glutamine-fructose-6-phosphate aminotransferase involved in the conversion of fructose-6-phosphate (Fru-6P) to glucosamine-6-phosphate (GlcN-6P), which is an initial substrate for peptidoglycan synthesis (17, 18). In *Streptococcus mutans*, *glmS* is essential when cells are cultured in tryptic soy broth (TSB), while a *glmS* deletion mutant could survive in the presence of *N*-acetylglucosamine (GlcNAc) (19). Considering the importance and essentiality of the genes related to cell growth and division, achieving regulated or induced expression and conditional gene knockout is critical to elucidating the functions of these genes.

Inducible expression systems usually act at the level of transcription initiation by repressor proteins responding to small molecules. TetR and LacI are the most commonly used repressor proteins, for which the repression effect can be relieved by the presence of anhydrotetracycline (ATc) and isopropyl-β-D-thiogalactopyranoside (IPTG), respectively (20, 21). TetR can bind to the operator sequence tetO, which inhibits the expression of the tetracycline resistance gene in the absence of tetracycline. Once tetracycline is present, the binding affinity is significantly reduced, leading to the expression of the resistance gene (22, 23). The IPTG-inducible expression system comes from the lactose operon of *Escherichia coli*. When the inducer is present, the lac repressor binds to the inducer and reduces the binding to the operator sequence (20).

Here, we identified three constitutive promoters, Pg, Pe, and Pt, from the *S. suis* genome that can be expressed steadily under different culture conditions. Based on them, we successfully developed two inducible expression systems in which the expression can be induced by ATc or IPTG. We demonstrate that the systems are useful tools that can be used to study the subcellular localization of cell division proteins and to construct conditional knockout mutants of essential genes. For the first time, we provide these genetic tools, which will greatly facilitate studies concerning essential genes or genes whose expression needs fine regulation in such an important zoonotic bacterial pathogen.

**RESULTS**

**Construction of a stable expression system.** Constitutive promoters can be used to engineer efficient inducible promoters (24). In *S. suis*, we selected three promoters, Pg, Pe, and Pt, from the genome that drive the expression of the housekeeping gene *gapdH*, which has been used as an internal reference for quantitative reverse transcription-PCR (RT-qPCR) (25), the protein enolase, which has been used as an internal reference for Western blotting, and the elongation factor Tu, which has been reported to be constitutively expressed in other bacteria (26). The *gfp*
A gene was cloned downstream of the promoters to test their activity, resulting in three plasmids, i.e., pSET2-Pg-gfp, pSET2-Pt-gfp, and pSET2-Pe-gfp. The plasmids were introduced individually into S. suis, and the cells were cultured for different periods of time (optical density at 600 nm [OD600] of 0.3 to 0.9), at a pH of 6 or 8, or at different temperatures (28°C or 42°C). Fluorescence intensity results indicated that the expression of promoters Pg, Pt, and Pe was relatively stable under most conditions except for growth at 42°C or at pH 8 (Fig. 1). Since previous studies showed that high temperatures and alkaline medium can interfere with the fluorescence intensity of green fluorescent protein (GFP) (26, 27), we further verified the expression level by Western blotting, which showed that GFP was expressed at similar levels under different growth conditions (Fig. 1D). These data suggest that promoters Pg, Pt, and Pe are constitutive promoters in S. suis.

**Construction of ATc- and IPTG-inducible expression systems.** An inducible expression system harbors the element for expression of a repressor protein and a promoter inserted with an operator sequence. To construct an ATc-inducible expression system, the repressor gene tetR was driven by promoter Pg, and the operator sequence was inserted into promoters Pt and Pe. The putative −10 and −35 regions and the transcription start site of the promoters were analyzed by using Softberry BPROM software (28) (Fig. 2A). First, the operator sequence tetO was inserted at the +27 position of promoter Pt and at the +2 position of promoter Pe, which were subsequently cloned.
FIG 2  Construction and evaluation of the ATc- and IPTG-inducible expression systems. (A) Characterization of ATc-inducible promoters. The putative −10 and −35 regions and the transcription start site of the promoters were analyzed by using Softberry BPIROM software. The expression of tetR was driven by P₉, and that of gfp was driven by Pₑ. The operator sequence was inserted into different positions of Pₑ. (B) Detection of the expression of the ATc-inducible expression systems. S. suis cells harboring the indicated plasmid were grown to the mid-log phase and induced with or without 200 ng/mL ATc for 60 min at 37°C. The cells were harvested and washed with PBS, and the fluorescence intensity was measured using a spectrometer. − ATc, average expression level in the absence of inducer; + ATc, average expression level in the presence of inducer; fold, fold change with ATc, compared with the value without inducer. (Continued on next page)
Inducible Expression Systems for S. suis

System to study the subcellular localization of an essential cell division protein, FtsZ, in the presence of inducer ATc. Therefore, we used the above-established inducible expression system to exert their functions, and it is important to be able to characterize the subcellular localization of these proteins. Therefore, we used the above-established inducible expression system.

FIG 2 Legend (Continued)
ATc. Statistical analysis was performed using the unpaired Student’s t test to compare the activity of promoters P\textsubscript{at2}, P\textsubscript{at3}, and P\textsubscript{at4} with or without 200 ng/mL ATc. **, P < 0.01; ***, P < 0.001; ns, not significant. The data are presented as the mean ± standard deviation. (C) Characterization of IPTG-inducible promoters. The expression of lacZ was driven by P\textsubscript{p} and that of gfp was driven by P\textsubscript{at}. The operator sequence was inserted into different positions of P\textsubscript{p}, (D) Detection of the expression of the IPTG-inducible expression systems. S. suis cells harboring the indicated plasmid were grown to the mid-log phase and induced with or without 0.2 mM IPTG for 60 min at 37°C. – IPTG, average expression level in the absence of inducer; + IPTG, average expression level in the presence of inducer; fold, fold change with IPTG, compared with the value without IPTG. Statistical analysis was performed using the unpaired Student’s t test to compare the activity of promoters P\textsubscript{at1} and P\textsubscript{at2} with or without 0.2 mM IPTG.
S. suis. A ftsZ-gfp fusion was cloned into pSSTete2-gfp and pSSlace1-gfp to replace gfp, resulting in plasmids pSSTete2-ftsZ_gfp and pSSlace1-ftsZ_gfp, respectively. The plasmids were transformed into S. suis, and the cells were cultured in the presence of inducer, followed by examination by fluorescence microscopy. It was shown in Fig. 4 that, by using either pSSTete2-ftsZ_gfp or pSSlace1-ftsZ_gfp in the presence of inducer, a clear FtsZ-GFP signal that was localized at the middle of the cells was observed (Fig. 4), which was consistent with the localization pattern of FtsZ reported previously for S. suis (29, 30). In addition, by using plasmid pSSlace1-ftsZ_gfp, a weak FtsZ-GFP signal was observed even without induction, indicating basal expression, which was consistent with the observations above. These results suggest that the inducible expression systems can be used for subcellular localization studies for cell division proteins.

Use of the inducible expression system for conditional gene knockout in *S. suis*. It is difficult to study the functions of essential genes because knockout mutants cannot be obtained. By using an inducible expression system, however, essential gene mutants can be constructed (31). In *S. suis*, there were so far no available tools to construct knockout mutants for essential genes. The glmS gene is an essential gene that encodes glutamine-fructose-6-phosphate aminotransferase, which is involved in the conversion of Fru-6P to GlcN-6P (17). A previous study reported that the glmS deletion mutant was viable in the presence of GlcNAc (19) but lethal under normal growth conditions. Therefore, we constructed an *S. suis* glmS gene deletion mutant (ΔglmS) in the presence of GlcNAc. It was shown that, consistent with the previous finding, the *S. suis* ΔglmS mutant could not...
grow in the absence of GlcNAc (Fig. 5). We next constructed an inducible expression plasmid encoding \textit{glmS}, pSSTete2-\textit{glmS}, which was transformed into the \textit{S. suis} \textit{D}\textit{glmS} strain. As shown in Fig. 5, the \textit{S. suis} \textit{D}\textit{glmS} strain and the \textit{D}\textit{glmS} strain harboring pSSTete2-\textit{glmS} but without ATc induction could not grow on tryptic soy agar (TSA) plates in the absence of GlcNAc but could grow when GlcNAc was supplemented (Fig. 5). However, it was seen that the \textit{D}\textit{glmS} strain harboring pSSTete2-\textit{glmS} could grow in the presence of 150 ng/mL ATc on TSA plates without GlcNAc (Fig. 5). These results suggest that pSSTete2-\textit{glmS} can provide strict control of expression that is inducer dependent. Therefore, this inducible plasmid system enables conditional deletion of essential genes.

**DISCUSSION**

Bacterial resistance to antibiotics has become a serious threat to public health, and it is urgent to develop new drugs to deal with antimicrobial resistance. Common antibiotic drug targets are the protein machineries of fundamental biological processes, such as DNA and RNA synthesis, cell division, bacterial metabolism, and protein synthesis (11–13). Most of the coding genes are essential and cannot be knocked out using the traditional gene deletion approaches. This makes it difficult to study the functions of these essential genes. Unlike model microorganisms, \textit{S. suis} is an oval coccus in which the mechanism of cell growth and division is distinct from that of typical bacilli or cocci (32). Here, we successfully developed two (ATc-inducible and IPTG-inducible) inducible expression systems that can be used for regulated
expression and conditional knockout of essential genes in the important zoonotic pathogen \textit{S. suis}.

Bacterial gene expression is influenced by a variety of environmental stimuli, including nutrient availability, ions, and temperature, on the basis of which several inducible expression systems have been developed, for example, the IPTG-inducible system (21), the ATc-inducible system (20), the arabinose-inducible system (33), and the Zn-inducible system (31). An inducible expression system usually includes a repressor protein that is normally expressed and binds the operator sequence within the promoter region, leading to repression of the target gene. Once the inducer is present, the binding between the repressor protein and the operator sequence is relieved and the target gene starts to be expressed. Therefore, to construct inducible expression systems in \textit{S. suis}, we first identified three strong constitutive promoters, \( P_g, P_t, \) and \( P_e \). To ensure efficient gene repression in the absence of inducer, the relatively stronger promoter \( P_g \) was used to drive the expression of the repressor gene. However, when the operator sequence was inserted into \( P_t \), its activity was disrupted. Therefore, we then optimized the insertion site of the operator sequence with promoter \( P_e \) and two inducible expression plasmids, pSSTete2-\textit{gfp} (ATc inducible) and pSSlace1-\textit{gfp} (IPTG inducible), were successfully constructed in \textit{S. suis}.

We next evaluated the regulatory capacity of these two inducible expression systems. Both of the plasmids showed inducer-concentration-dependent and induction-time-dependent expression of the reporter gene, indicating successful construction of the expression systems. However, it was noted that, compared with pSSTete2-\textit{lacZ}, pSSlace1-\textit{lacZ} showed higher basal expression in the absence of inducer. This is consistent with previous findings that the \textit{lac} promoter shows leaky expression even in the presence of the repressor Lacl (34). This can be resolved by increasing the expression of the repressor or by inserting more operator sequences into the promoter.

\textit{S. suis} is an important zoonotic pathogen posing a severe threat to public health. To date, there are still many unknowns regarding the fundamental biology and pathogenesis of this pathogen that deserve deep investigation. Unfortunately, very limited genetic tools have been developed and no inducible expression systems are available for \textit{S. suis}. The easiest way to develop inducible expression systems is to directly clone an inducible expression system that has already been developed from other bacteria. For example, the tetracycline-inducible promoter \( P_{\text{tet}} \) from \textit{Staphylococcus aureus} has been applied to \textit{Streptococcus pneumoniae} and \textit{Streptococcus agalactiae} (35, 36). However, further optimizations are still needed; otherwise, they may not be able to provide ideal expression levels (35, 37). Another way to develop novel inducible expression systems is to screen the natural inducible expression systems in the bacterial genome by adding different inducers. By using this strategy, the ComS peptide-regulated system (38), the zinc-inducible promoter \( P_{\text{zcu}} \) (31), the maltose-inducible promoter \( P_M \) (39), and the fucose-inducible promoter \( P_{\text{fuc}} \) in \textit{S. pneumoniae} (40) were developed.

Bacterial cell division is one of the most fundamental physiological processes. Proteins involved in this process need precise temporal and spatial regulation.
null mutants of essential genes. These systems provide useful tools that will further facili-
ty division protein FtsZ, and we demonstrated their potential use in the construction of
We showed their practical applications in the subcellular localization study of the critical
MATERIALS AND METHODS
be exploited to construct gene deletion mutants of essential genes in
Inducible Expression Systems for
Bacterial strain or plasmid Description Reference or source

| Bacterial strains |
|-------------------|
| E. coli MC1061 | Cloning host for recombinant vector pSET2 |
| S. suis SC19 | S. suis serotype 2, wild type |
| ΔglmS | S. suis SC19 glmS deletion mutant |
| ΔglmS:pSSTete2-glmS | ΔglmS strain carrying recombinant plasmid pSSTete2-glmS |

| Plasmids |
|-------------------|
| pSET4s | S. suis Ts suicide vector; Spc' |
| pSET2 | S. suis expression vector; Spc' |
| pET28a | Expression vector; Kan' |
| pRAB11 | E. coli/S. aureus shuttle vector, containing a tetracycline-inducible promoter; Chl' |
| pSET2-P_gfp | pSET2 with P_gfp fusion cloned at BamHI site; Spc' |
| pSET2-P_gfp | pSET2 with P_gfp fusion cloned at BamHI site; Spc' |
| pSET2-P_gfp | pSET2 with P_gfp fusion cloned at BamHI site; Spc' |
| pSSTete1-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSTete2-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSTete3-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSTete4-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSLace1-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSLace2-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSLace3-lacZ | pSET2 with P_tetR fusion and P_lacZ fusion; Spc' |
| pSSLace4-lacZ | pSET2 with P_tetR fusion and P_lacZ fusion; Spc' |
| pSSTete2-ftsZ_gfp | pSET2 with P_tetR fusion and P_ftsZ_gfp fusion; Spc' |
| pSSLace1-ftsZ_gfp | pSET2 with P_tetR fusion and P_ftsZ_gfp fusion; Spc' |
| pSET4s-glmS | Derived from pSET4s for deleting glmS in SC-19; Spc' |
| pSSTete2-glmS | pSET2 with P_tetR fusion and P_glmS fusion; Spc' |

*Spc*, spectinomycin resistance; Kan', kanamycin resistance; Chl', chloramphenicol resistance.

Examining the subcellular localizations of important cell division proteins is critical
to studying the details of the cell division process. FtsZ plays a central role during
cell division; it forms a ring structure and orchestrates cell division. Therefore, it is
important to investigate its subcellular localization. However, it has been reported
that overexpression of FtsZ in bacteria results in abnormal cell division (41, 42).
Therefore, care is needed when performing localization studies with FtsZ. Utilizing
the inducible expression systems we established in this study, it is feasible to
achieve fine control of FtsZ expression, and our results showed that FtsZ-GFP
expressed from both of the plasmids was localized correctly to the midcell region,
suggesting that our inducible expression systems provide powerful tools for func-
tional and localization studies of cell division proteins in S. suis.

Many important proteins involved in cell growth and division are essential, and null
mutants cannot be obtained using normal genetic tools, which hinders the functional
study of these proteins. Recently, however, a conditional knockout strategy that can be
used to construct deletion mutants of essential genes has been developed (43). In this
study, by using the inducible expression system, we showed that, in the absence of in-
ducer, pSSTete2-glmS barely expressed glmS, indicating that the growth of S. suis
ΔglmS needs GlcNAc. Once induced, expression from pSSTete2-glmS complemented
the need of S. suis ΔglmS for GlcNAc. Therefore, our inducible expression systems can
be exploited to construct gene deletion mutants of essential genes in S. suis.

In summary, we successfully constructed two inducible expression systems in S. suis.
We showed their practical applications in the subcellular localization study of the critical
cell division protein FtsZ, and we demonstrated their potential use in the construction of
null mutants of essential genes. These systems provide useful tools that will further facilit-
tate the functional study of important proteins of S. suis.

MATERIALS AND METHODS
Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. S. suis strains in this study are derivatives of S. suis SC19, a highly virulent serotype 2

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**TABLE 1** Bacterial strains and plasmids used in the present study

| Bacterial strain or plasmid | Description | Reference or source |
|-----------------------------|-------------|---------------------|
| E. coli MC1061              | Cloning host for recombinant vector pSET2 | 9 |
| S. suis SC19               | S. suis serotype 2, wild type | 44 |
| ΔglmS                      | S. suis SC19 glmS deletion mutant | This study |
| ΔglmS:pSSTete2-glmS        | ΔglmS strain carrying recombinant plasmid pSSTete2-glmS | This study |

| Plasmids |
|-------------------|
| pSET4s | S. suis Ts suicide vector; Spc' |
| pSET2 | S. suis expression vector; Spc' |
| pET28a | Expression vector; Kan' |
| pRAB11 | E. coli/S. aureus shuttle vector, containing a tetracycline-inducible promoter; Chl' |
| pSET2-P_gfp | pSET2 with P_gfp fusion cloned at BamHI site; Spc' |
| pSET2-P_gfp | pSET2 with P_gfp fusion cloned at BamHI site; Spc' |
| pSET2-P_gfp | pSET2 with P_gfp fusion cloned at BamHI site; Spc' |
| pSSTete1-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSTete2-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSTete3-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSTete4-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSLace1-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSLace2-gfp | pSET2 with P_tetR fusion and P_gfp fusion; Spc' |
| pSSLace3-lacZ | pSET2 with P_tetR fusion and P_lacZ fusion; Spc' |
| pSSLace4-lacZ | pSET2 with P_tetR fusion and P_lacZ fusion; Spc' |
| pSSTete2-ftsz_gfp | pSET2 with P_tetR fusion and P_ftsz_gfp fusion; Spc' |
| pSSLace1-ftsz_gfp | pSET2 with P_tetR fusion and P_ftsz_gfp fusion; Spc' |
| pSET4s-glmS | Derived from pSET4s for deleting glmS in SC-19; Spc' |
| pSSTete2-glmS | pSET2 with P_tetR fusion and P_glmS fusion; Spc' |

*Spc*, spectinomycin resistance; Kan', kanamycin resistance; Chl', chloramphenicol resistance.
strain that was isolated from a diseased pig during the *S. suis* outbreak in 2005 in the Sichuan Province of 37°C for 15 min. The cells were cultured in fresh TSB with a ratio of 50:1 and grown for 0.5 h. To induce protein expression, ATc was supplemented at 100 ng/mL, followed by incubation at 37°C for 15 min. The cells were collected by centrifugation, washed with PBS three times, and resuspended in 500 μL of PBS. Fluorescent dye AF647 (catalog number A20006; Thermo Fisher Scientific) was added to the cell lysate, followed by incubation at 37°C for 15 min, and the reaction was terminated by the addition of 200 μL of sodium carbonate (Na2CO3). The absorbance was measured at the wavelength of 420 nm and 550 nm, respectively. The β-galactosidase activity was calculated as $1,000 \times (OD_{550} - [1.75 \times OD_{420}] / (T \times V \times OD_{550})$, in which $T$ represents the reaction time and $V$ represents the volume of the reaction (47).

**Construction of a glmS deletion/complementation strain.** In order to construct the *glmS* deletion strain of *S. suis*, the regions upstream and downstream of the *glmS* gene were amplified by PCR using primers GU-F/GU-R and GD-F/GD-R, respectively (Table 2). The PCR products were cloned into the Ts plasmid pSET4s via the BamHI site, resulting in plasmids pSET2-Pg-gfp, pSET2-Pt-gfp, and pSET2-Pe-gfp. The recombinant plasmids were transformed into *S. suis* by electroporation (2.5 kV, 25 μF, and 200 Ω). To construct ATc- and IPTG-inducible expression plasmids, the *reT* gene and *locI* gene were amplified from plasmids pRA811 and pET-28a, respectively (37). The promoter fragments with the operator sequence inserted were synthesized by Sangon Biotech Company (Shanghai, China).

**Inducible expression of GFP.** *S. suis* cells cultured overnight were transferred to 4 mL of TSB and grown at 37°C to the mid-log phase (OD550 of ~0.5). 200 ng/mL ATc or 0.2 mM IPTG was then added, followed by another 1 h of incubation to induce expression. The cells were then harvested, washed three times with phosphate-buffered saline (PBS), resuspended in PBS, and diluted to give an OD600 of about 0.5. The suspension (200 μL) in four replicates was subjected to measurement of GFP fluorescence intensity in 96-well plates using a microplate reader (TECAN SPARK10M, Switzerland) (excitation at 485 nm and emission at 535 nm). *S. suis* strain SC19 was used as the negative control. The expression of GFP was further verified by Western blotting as follows. *S. suis* cells cultured overnight were transferred to 40 mL of TSB and grown under different culture conditions. The samples were collected by centrifugation, washed three times with PBS, and lysed with lysozyme treatment, followed by homogenization. The concentration of total protein was measured using a micro-bicinchoninic acid (BCA) protein assay kit (CwbioTech, Beijing, China) and normalized to 15 mg for each sample. The samples were subjected to 12% SDS-PAGE analysis, followed by transfer to a polyvinylidene fluoride (PVDF) membrane by electrophoretic transfer. The GFP protein was probed with anti-GFP antibody (catalog number 50430-2-AP; Proteintech) (1:10,000 dilution) as the primary antibody and horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (catalog number SA00001-2; Proteintech) (1:10,000 dilution) as the secondary antibody. The HylA protein as a control was detected with mouse anti-HylA serum (developed in the authors' laboratory) (1:10,000 dilution) and the secondary antibody. Chemiluminescent signals of the protein bands were detected using a Western enhanced chemiluminescence (ECL) substrate kit (catalog number 1705060; Bio-Rad) and the ChemiDoc Touch imaging system (Bio-Rad).

**Determination of β-galactosidase activity.** In order to determine the optimal induction conditions, *locZ* was used as a reporter gene; it was amplified by PCR amplification from the chromosomal DNA of *E. coli* BTH101 (45) and cloned into the inducible plasmids. *S. suis* cells cultured overnight were transferred to 80 mL of TSB and grown at 37°C to the mid-log phase (OD550 of ~0.5). The samples were then induced with the addition of ATc and IPTG, respectively, with different inducer concentrations and induction times. The samples were collected by centrifugation, washed three times with PBS, and resuspended with 2-buffer (46). The OD550 was recorded. The cell suspension was lysed with lysozyme treatment, followed by homogenization, o-Nitrophenyl-β-D-galactopyranoside (ONPG) was added to the cell lysate, followed by incubation at 37°C for 15 min, and the reaction was terminated by the addition of Na2CO3. The absorbance was measured at the wavelength of 420 nm and 550 nm, respectively. The β-galactosidase activity was calculated as $1,000 \times (OD_{550} - [1.75 \times OD_{420}] / (T \times V \times OD_{550})$, in which $T$ represents the reaction time and $V$ represents the volume of the reaction (47).
**TABLE 2** Primers used in the present study

| Primer name | Primer sequence |
|-------------|-----------------|
| P1-F        | 5′-TCAAGCAGCCCTGAAGAACATGAGATGAAAGCCGTAAGAC-3′ |
| P1-R        | 5′-TTTTCACATGATGAGATGAAAGCCGTAAGAC-3′ |
| gGFP-F      | 5′-GGGACGCTTGACGCTAGCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| GFP-R       | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| FtsZ-F      | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| lacZ-F      | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| lacZ-R      | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| lacI-F      | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| tetRzD-R    | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| tetRzR      | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| PrF         | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| PrR         | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| ppGFP-F     | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| ppGFP-R     | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| GG-R        | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |
| GG-F        | 5′-TACAGTCTTGGTACGATGAAAGCCGTAAGAC-3′ |

488 nm, and emission at 500 to 545 nm were used. For AF647 imaging, a 20-ms exposure time, excitation at 647 nm, and emission at 663 to 738 nm were used.

**Data availability.** The sequences of plasmids pSSTete2-gfp and pSSlace1-gfp are available in the NCBI GenBank database with the accession numbers ON391044 and ON391045, respectively.

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**REFERENCES**

1. Staats JJ, Feder I, Okwumabua O, Chengappa MM. 1997. *Streptococcus suis*: past and present. Vet Res Commun 21:381–407. https://doi.org/10.1023/a:1005870317757.

2. Tang J, Wang C, Feng Y, Yang W, Song H, Chen Z, Yu H, Pan X, Zhou X, Wang H, Wu B, Wang H, Zhao H, Lin Y, Yue J, Wu Z, He X, Gao F, Khan AH, Wang J, Zhao GP, Wang Y, Wang X, Chen Z, Gao GF. 2006. *Streptococcus suis* toxic shock syndrome caused by *Streptococcus suis* serotype 2. PLoS Med 3:e151. https://doi.org/10.1371/journal.pmed.0030151.

3. Goyette-Desjardins G, Auger JP, Xu J, Segura M, Gottschalk M. 2014. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent: an update on the worldwide distribution based on serotyping and sequence typing. Emerg Microbes Infect 3:e81. https://doi.org/10.1002/epi.2014.45.

4. Fittipaldi N, Segura M, Grenier D, Gottschalk M. 2012. Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. Future Microbiol 7:259–279. https://doi.org/10.2217/fmb.11.149.

5. Hatrongjit R, Fittipaldi N, Gottschalk M, Kerdsin A. 2020. Tools for molecular epidemiology of *Streptococcus suis*. Pathogens 9:81. https://doi.org/10.3390/pathogens9020081.

6. Segura M, Aragon V, Brockmeier S, Gebhart C, Greeff A, Kerdsin A, O’Dea M, Okura M, Saléry M, Schultsz C, Valentin-Weigand P, Weinert L, Wells J, Gottschalk M. 2020. Update on *Streptococcus suis* research and prevention in the era of antimicrobial restriction: 4th International Workshop on *S. suis*. Pathogens 9:374. https://doi.org/10.3390/pathogens9050374.

7. Segura M, Fittipaldi N, Calzas C, Gottschalk M. 2017. Critical *Streptococcus suis* virulence factors: are they all really critical? Trends Microbiol 25:585–599. https://doi.org/10.1016/j.tim.2017.02.005.

8. Takamatsu D, Osaki M, Sekizaki T. 2001. Thermosensitive suicide vectors *Streptococcus suis*–*Escherichia coli* shuttle cloning vectors. Plasmid 45:140–148. https://doi.org/10.1006/plas.2000.11532.

9. Takamatsu D, Osaki M, Sekizaki T. 2001. Construction and characterization of *Streptococcus suis*–*Escherichia coli* shuttle cloning vectors. Plasmid 45:101–113. https://doi.org/10.1006/plas.2000.1510.
10. Engan AJ, Vollmer W. 2013. The physiology of bacterial cell division. Ann N Y Acad Sci 1277:8–28. https://dx.doi.org/10.1111/j.1749-6632.2012.06618.x.

11. den Blaauwen T, Andreu JM, Monasterio O. 2014. Bacterial cell division proteins as antibiotic targets. Biochem J 555:72–80. https://dx.doi.org/10.1042/BJ20140307.

12. Foster TJ. 2017. Antibiotic resistance in Staphylococcus aureus: current status and future prospects. FEMS Microbiol Rev 41:430–449. https://dx.doi.org/10.1093/femsre/fux007.

13. Misra HS, Maurya GK, Chaudhary R, Misra CS. 2018. Interdependence of bacterial cell division and genome segregation and its potential in drug development. Microbiol Res 208:12–24. https://dx.doi.org/10.1016/j.micres.2017.12.013.

14. Luktenhaus J, Pichoff S, Du S. 2012. Bacterial cytokinesis: from Z ring to divisome. Cytoskeleton (Hoboken) 69:77–80. https://dx.doi.org/10.1002/cm.21054.

15. Huang KH, Durand-Heredia J, Janakiraman A. 2013. FtsZ ring stability: of bundle and future prospects. FEMS Microbiol Rev 41:430–486. https://dx.doi.org/10.1111/j.1551-7052.2009.00687.x.

16. Winkler ME, Bouloc P. 2014. A tetracycline-inducible expression vector for Streptococcus agalactiae allowing controllable gene expression. J Microbiol Methods 96:168–173. https://dx.doi.org/10.1016/j.mimet.2013.10.020.

17. Ward JE, Lutkenhaus J. 1985. Overproduction of FtsZ induces minicell formation. J Bacteriol 163:591–596. https://dx.doi.org/10.1128/JB.185.6.6314-6316.1992.

18. Li H, Liao JC. 2015. A synthetic anhydrotetracycline-controllable gene expression system in E. coli and future prospects. Mol Microbiol Rev 73:157–171. https://dx.doi.org/10.1128/MMBR.69.2.326-356.2005.

19. Winkler ME. 2016. Suppression of a deletion mutation in the gene encoding PBP2b reveals a new lytic transglycosylase involved in peptidoglycan dynamics. J Bacteriol 198:2051–2058. https://dx.doi.org/10.1128/JB.185.6.2051-2058.2013.

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