The recombination regulator RecX negatively regulates heat-stable antifungal factor (HSAF) biosynthesis in *Lysobacter enzymogenes*

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Abstract

Bacteria often use multiple transcription factors to regulate specific biological processes. Biosynthesis of heat-stable antifungal factor (HSAF) is regulated by multiple factors in *Lysobacter enzymogenes*. However, the mechanism of HSAF biosynthesis regulation remains largely unknown. In this study, we screened a potential HSAF biosynthesis regulator, RecX, by a DNA pull-down assay. Deletion of *recX* resulted in a significant increase in the production of HSAF, and overexpression of *recX* significantly suppressed HSAF production. Importantly, our results showe that RecX directly binds to the promoter region of the *lafB* gene to inhibit its transcription and thus decreases HSAF production in *L. enzymogenes*. These findings reveal the novel mechanism of RecX regulation of antifungal antibiotic production in *L. enzymogenes*.

Keywords Heat-stable antifungal factor (HSAF), Biosynthesis regulator, RecX, *lafB* gene, Transcription, *Lysobacter enzymogenes*

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Background

Lysobacter is a group of gram-negative bacteria belonging to the family *Xanthomonadaceae* (Christensen and Cook 1978). A representative and well-studied species of this genus is *L. enzymogenes*, which is a biocontrol bacterium that has been used to control crop fungal diseases (Qian et al. 2009; Odhiambo et al. 2017; Zhao et al. 2017, 2019a). This bacterium is able to achieve such broad-spectrum antifungal activity by secreting an antifungal antibiotic called HSAF (heat-stable antifungal factor), which is a polycyclic tetramate macrolactam and is regarded as an attractive agent for the biological control in fungal diseases of agriculturally important plants (Li et al. 2006, 2018; Yu et al. 2007; Lou et al. 2011). HSAF inhibits the growth of a wide range of fungal species, and its chemical structure and mode of action are distinct

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from those existing antifungal drugs and fungicides (Li et al. 2006; Zhao et al. 2019a).

Previous studies have shown that the production of HSAF by the *L. enzymogenes* wild-type strain is low. Understanding the biosynthetic and regulatory mechanisms of HSAF is important to improve its yield in *L. enzymogenes* strains. The HSAF biosynthesis operon constitutes a single transcription unit (ORF1–ORF7) (Wang et al. 2017a). The single-module hybrid PKS/NRPS protein lafB (ORF6) is the key enzyme for HSAF biosynthesis in *L. enzymogenes* (Lou et al. 2011; Xu et al. 2018, 2021a, 2021b; Li et al. 2021). Our previous work revealed that the biosynthesis of HSAF is regulated by multiple factors. Our collaborators discovered several small-molecule signal factors, such as diffusible signal factor (DSF) and diffusible factor (DF), that positively regulate the biosynthesis of HSAF in *L. enzymogenes* (Qian et al. 2013; Han et al. 2015). In particular, 4-hydroxybenzoic acid (4-HBA) serves as a DF that connects the metabolic shikimate pathway to the HSAF biosynthetic pathway in *L. enzymogenes* (Zhou et al. 2013a, 2013b; Su et al. 2017). Cyclic dimeric GMP (c-di-GMP) is a ubiquitous dinucleotide secondary messenger in a diverse range of bacteria (Romling et al. 2005). This compound hinders HSAF production by decreasing the expression of the HSAF biosynthesis gene (Chen et al. 2017; Xu et al. 2018). Spermidine also has also been shown to play a significant role in the regulation of HSAF biosynthesis (Chen et al. 2018; Zhao et al. 2019b). In addition to chemical signals, other factors also affect the biosynthesis of HSAF in *L. enzymogenes*. The global transcriptional regulator Clp is a direct regulator of HSAF biosynthesis and positively regulates HSAF production in a c-di-GMP-dependent manner (Xu et al. 2018). The TetR family protein LetR is involved in the transcriptional repression of HSAF production in *L. enzymogenes* (Wang et al. 2017a). LysR-family transcription factor (LysR$_{Le}$) can bind to the promoter of the HSAF biosynthesis gene (p*lafB*) and positively regulate HSAF production (Qian et al. 2014; Su et al. 2017). Previous results showed that LarR, a member of the MarR family, is involved in interplay with LysR$_{Le}$ during 4-HBA-regulated HSAF production (Su et al. 2018). In addition, evidence has shown that other response regulators mediate the regulation of HSAF biosynthesis, such as PilG, PilR, Lsp, and TonB-dependent receptors (Zhou et al. 2015; Wang et al. 2016, 2017b; Chen et al. 2017). RpfB1 and RpfB2 increase the metabolism of free fatty acids by inducing the activity of fatty acyl-CoA ligase which blocks the biosynthesis of HSAF in *L. enzymogenes* (Li et al. 2020a). Recent evidence indicates that the DSF type-based quorum-sensing (QS) system component RpfG interacts with three hybrid two-component system proteins (HtsH1, HtsH2, and HtsH3) to regulate the

biosynthesis of HSAF in *L. enzymogenes* (Li et al. 2021). However, the mechanism underlying the regulation of HSAF biosynthesis in *L. enzymogenes* remains largely unknown.

In this study, we describe the role of the recombination regulator RecX in HASF biosynthesis. Deletion of *recX* increased production of the antifungal antibiotic HSAF in *L. enzymogenes*. We also demonstrate that RecX directly bound to the promoter of the HSAF biosynthesis gene (p*lafB*) to negatively regulate HSAF biosynthesis. Overall, the results of the present study facilitate the current understanding of RecX-mediated regulation of HASF biosynthesis in *L. enzymogenes*.

Results

DNA pull-down screening for potential regulators of HSAF biosynthesis in *L. enzymogenes*

To investigate the novel genes that regulate HSAF biosynthesis in *L. enzymogenes*, we performed DNA pull-down analyses using biotin-conjugated p*lafB* as a probe. A total of 340 proteins capable of directly binding p*lafB* were identified (Fig. 1a). These proteins include the canonical HSAF synthesis regulator protein Clp (Xu et al. 2018), other regulator proteins, such as LysR$_{Le}$ (Su et al. 2018) and some two-component system regulators (Chen et al. 2017). Furthermore, we identified 8 uncharacterized
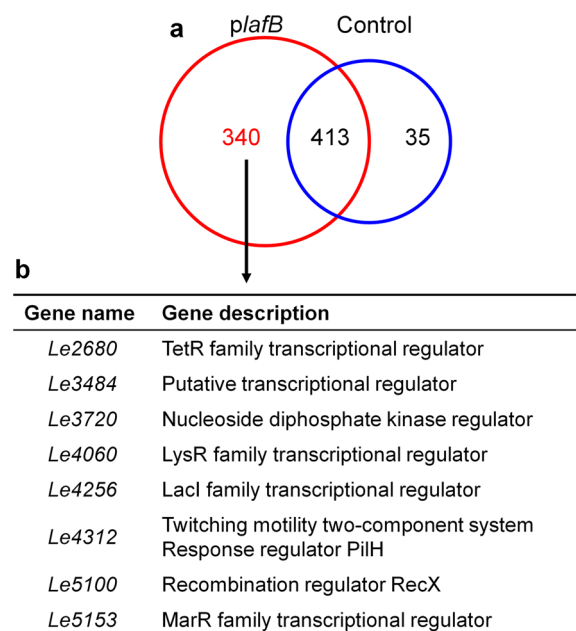

Gene name	Gene description
Le2680	TetR family transcriptional regulator
Le3484	Putative transcriptional regulator
Le3720	Nucleoside diphosphate kinase regulator
Le4060	LysR family transcriptional regulator
Le4256	LacI family transcriptional regulator
Le4312	Twitching motility two-component system Response regulator PilH
Le5100	Recombination regulator RecX
Le5153	MarR family transcriptional regulator

Fig. 1 DNA pull-down screen to identify potential transcription factors the regulate HSAF biosynthesis. **a** The Venn diagrams show the potential *lafB* promoter-binding proteins in the total protein extracts that were pulled down using the 5-biotinylated probe. **b** Eight potential transcription factors were identified that might regulate HSAF biosynthesis

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regulator proteins that may regulate HSAF synthesis by directly binding p*lafB* (Fig. 1b). To identify the physiological functions of these 8 proteins in HSAF production, the genes *Le2680*, *Le3484*, *Le3720*, *Le4060*, *Le4256*, *Le4312*, *Le5100*, and *Le5153* were deleted using a two-step homologous recombination approach to construct the corresponding single knockout strain, respectively. We compared the growth rates of the generated knockout mutants in the medium used to measure HSAF production (0.1% TSB) and found that none of the mutants showed significant growth defects when compared to the wild-type strain (Additional file 1: Figure S1a). As a control, all test mutants and the wild-type strain were grown in nutrient-rich conditions (LB), and we found no significant differences in their colony morphologies (Additional file 1: Figure S1b). When cultured on media used to indicate protease and cellulase production, all knockout mutants produced wild-type levels of these two extracellular enzymes (Additional file 1: Figure S1c, d), suggesting that these 8 genes are not involved in regulating the production of extracellular enzymes in *L. enzymogenes*.

To evaluated the physiological functions of the *L. enzymogenes* genes *Le2680*, *Le3484*, *Le3720*, *Le4060*, *Le4256*, *Le4312*, *Le5100*, and *Le5153* in HSAF production, we quantified HSAF production in the corresponding mutant strains by high-performance liquid chromatography (HPLC). The Δ*3484* strain had lower HSAF levels and deletion of *Le5100* resulted in 1.75-fold increase in HSAF production compared to the wild-type (Fig. 2).

The *recX* gene in the *L. enzymogenes* genome is conserved among different bacteria species.

In this study, we focused on one of the newly identified regulators, *Le5100*, that was found to be involved in the

regulation of HSAF biosynthesis; *Le3484* will be investigated in a separate study. The *Le5100* gene is located downstream of the *rexA* gene (Fig. 3a). The *Le5100* gene encodes a typical RecX family protein with pfam-RecX domains (Fig. 3b). To investigate the function of the *Le5100* gene in *L. enzymogenes*, protein sequence alignments of *L. enzymogenes* OH11 *Le5100* with RecX proteins from *Xanthomonas campestris* pv. *campestris*, *X. oryzae* pv. *oryzae*, *Stenotrophomonas maltophilia*, and *L. enzymogenes* C3 were performed (Fig. 3c). The results showed that the *L. enzymogenes* OH11 *Le5100* protein shares 30.5, 31.1, 33.5, and 89.4% identity residues with *X. campestris* pv. *campestris* RecX, *X. oryzae* pv. *oryzae* RecX, *S. maltophilia* RecX, and *L. enzymogenes* C3 RecX, respectively (Fig. 3c). Based on these criteria, it seems reasonable that *Le5100*, namely, RecX, could be a functional RecX family member that plays a crucial role in HSAF production in *L. enzymogenes*.

RecX is involved in HSAF biosynthesis
To ascertain the role of RecX in the regulation of HSAF biosynthesis, we constructed a *recX*-overexpressing strain in a wild-type OH11 background and complemented the *recX* mutant with plasmid-borne *recX*. HSAF production was significantly decreased in the *recX*-overexpressing strain (OH11/*recX*) when compared with the wild-type (Fig. 4). The complemented strain Δ*recX*/*recX*, containing a plasmid-borne *recX*, had a similar level of HSAF production as the wild-type (Fig. 4). Importantly, the *recX* mutation did not impair bacterial growth (Additional file 1: Figure S1a, b), implying that *L. enzymogenes* RecX plays a specific role in regulating HSAF production.

RecX directly binds to the promoter of the HSAF biosynthesis gene (p*lafB*)
As mentioned earlier, deletion of *recX* resulted in increased HSAF production (Fig. 2) and DNA pull-down assays confirmed that RecX directly binds to the promoter of the HSAF biosynthesis gene. Thus, we wondered whether RecX might directly target HSAF biosynthesis gene promoters. To test this hypothesis, we performed an *Escherichia coli*-based one-hybrid assay and found that RecX could directly bind to the promoter of the *L. enzymogenes* HSAF biosynthesis gene *lafB* (Fig. 5a). To further verify this binding, an electrophoretic mobility shift assay (EMSA) was performed. The RecX protein had a monomeric molecular weight of 35.8 kDa and N-terminal-His 6-tagged version of the protein was purified by nickel chelate chromatography and showed a single band when run on an SDS-gel (Fig. 5b). A PCR-amplified 590 bp DNA fragment from p*lafB* was used as a probe. The addition of purified RecX protein, ranging in concentration from 0 to 4 μM, to the reaction mixtures
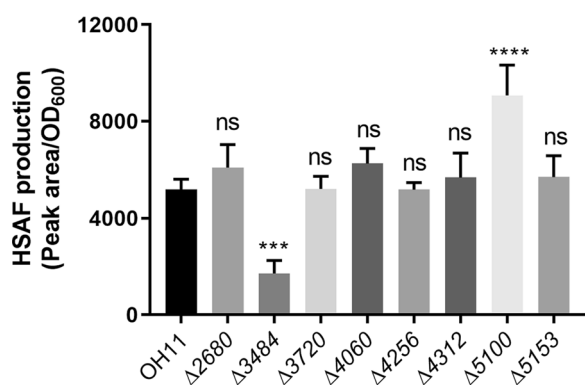

Fig. 2 Quantification of HSAF produced by the *Lysobacter enzymogenes* knockout mutants of the 8 potential transcription factors. Error bars, means ± standard deviation (n = 3). ***$P < 0.001$, ****$P < 0.0001$, assessed by one-way ANOVA. All experiments were repeated three times with similar results
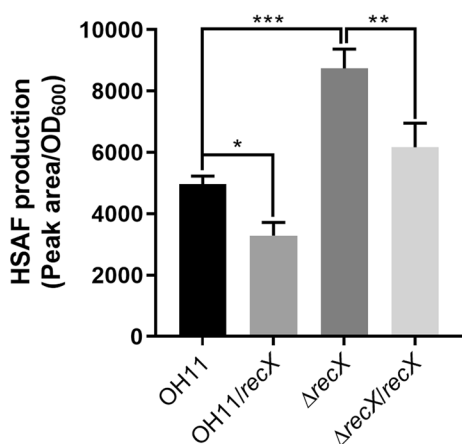
Fig. 3 Identification and sequence characterization of RecX in *Lysobacter enzymogenes*. **a** Genomic localization of *recX*. *csrA*: carbon storage regulator; *alaS*: alanyl-tRNA synthetase; *recX*: regulatory protein; *recA*: recombination protein; *lexA*: repressor LexA; *HP*: hypothetical protein; *marR*: MarR family N-acetyltransferase transcriptional regulator; *cinA*: competence damage-inducible protein A. **b** Bioinformatics analyses of the domain organization of RecX. **c** Alignment of *L. enzymogenes*, *Xanthomonas campestris* pv. *campestris*, *X. oryzae* pv. *Oryzae*, and *Stenotrophomonas maltophilia* RecX sequences. The alignment was performed with Clustal W based on identical residues

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Fig. 4 Quantification of HSAF produced by the *Lysobacter enzymogenes* Δ*recX* mutant. Error bars, mean ± standard deviation (n = 3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, assessed by one-way ANOVA. All experiments were repeated three times with similar results
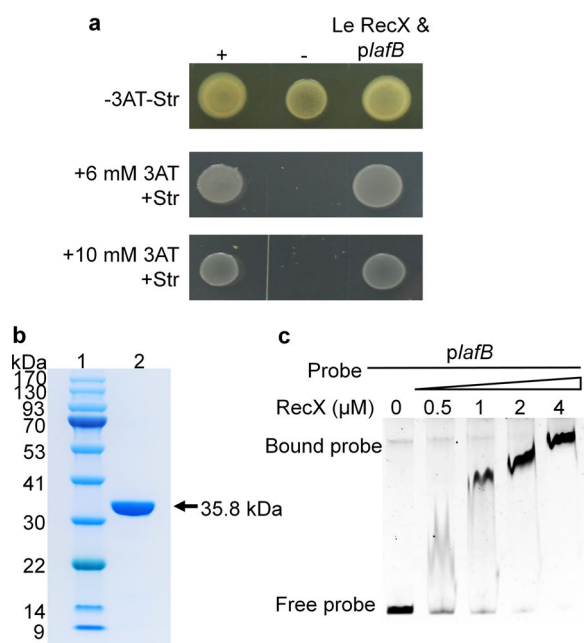

Fig. 5 RecX directly bound the promoter of the HSAF biosynthesis gene (p*lafB*). **a** Direct physical interaction between RecX and the *lafB* promoter region was detected in *E. coli*. Experiments were performed according to the procedures described in the 'Experimental procedures' section. +, cotransformant containing pBX-R2031 and pTRG-R3133, used as a positive control; −, cotransformant containing pBXcmT-p*lafB* and the empty pTRG, used as a negative control; *L. enzymogenes* RecX and p*lafB*, cotransformant harboring pTRG-RecX and pBXcmT-p*lafB*. -3AT-Str, plate with no selective medium (3AT 3-amino-1,2,4-triazole and Str streptomycin) and + 3AT + Str, plate with M9-based selective medium. **b** The purified protein was analyzed by 12% SDS-PAGE. Lane 1, molecular mass markers; Lane 2, RecX protein. **c** Gel shift assay showing that RecX directly bound p*lafB*. RecX (0, 0.5, 1, 2, or 4 µM) was added to reaction mixtures containing 50 ng of probe DNA, and the reaction mixtures were separated on polyacrylamide gels

(20 µL at 28°C for 25 min) caused a mobility shift of the p*lafB* DNA fragment. EMSA indicated strong RecX binding with the p*lafB* probe in a dose-dependent manner (Fig. 5c). These results suggest that RecX directly binds to the promoter region of the HSAF biosynthesis gene *lafB* in *L. enzymogenes*.

RecX negatively regulates the expression of the HSAF biosynthesis gene

Our results confirmed that deletion of *recX* resulted in an increase in the production of HSAF in *L. enzymogenes* (Fig. 4). Therefore, we hypothesized that *L. enzymogenes* RecX negatively regulates the expression of the HSAF biosynthesis gene to decrease HSAF production. To test this hypothesis, we performed reverse transcription-quantitative PCR (RT-qPCR) and found that the expression of the HSAF biosynthesis gene *lafB* in the Δ*recX* mutant strain was increased compared with that in the wild-type *L. enzymogenes* strain, indicating that RecX negatively regulates the expression of *lafB* (Fig. 6a). To further study the regulation of *lafB* by RecX, we constructed a p*lafB*-lacZ reporter systems in the *recX* mutant strain. Consistent with the RT-qPCR results, deletion of *recX* resulted in increased expression levels of the HSAF biosynthesis gene *lafB* (Fig. 6b). Taken together, these results showed that RecX can directly target the promoter of the HSAF biosynthesis gene to decrease the expression and production of HSAF in *L. enzymogenes*.

Discussion

Previous studies have indicated that the biosynthesis of HSAF is regulated by multiple factors, including small-molecule compounds, transcription factors and other proteins. However, the mechanism underlying the regulation of HSAF biosynthesis remains largely unknown in *L. enzymogenes*. In this study, we screened a novel transcription factor, RecX, that can regulate HSAF biosynthesis in *L. enzymogenes*. This study provides biochemical, genetic, and physiological evidence to demonstrate that RecX directly binds the promoter of the HSAF biosynthesis gene (p*lafB*) to negatively regulate HSAF biosynthesis in *L. enzymogenes* (Fig. 7).

Several studies have demonstrated an alternative role for *recX* in the DNA repair of recombination performed by RecA (Pages et al. 2003; Ragone et al. 2008; Prasad et al. 2019). The absence of RecX severely impaired the natural transformations in both plasmid and chromosomal DNA. A previous study indicated that RecX suppresses the negative effect exerted by RecA during plasmid transformation, preventing RecA missense recombination in single-stranded DNA tracts, and modulating DNA strand exchange (Cardenas et al. 2012). RecX abrogates
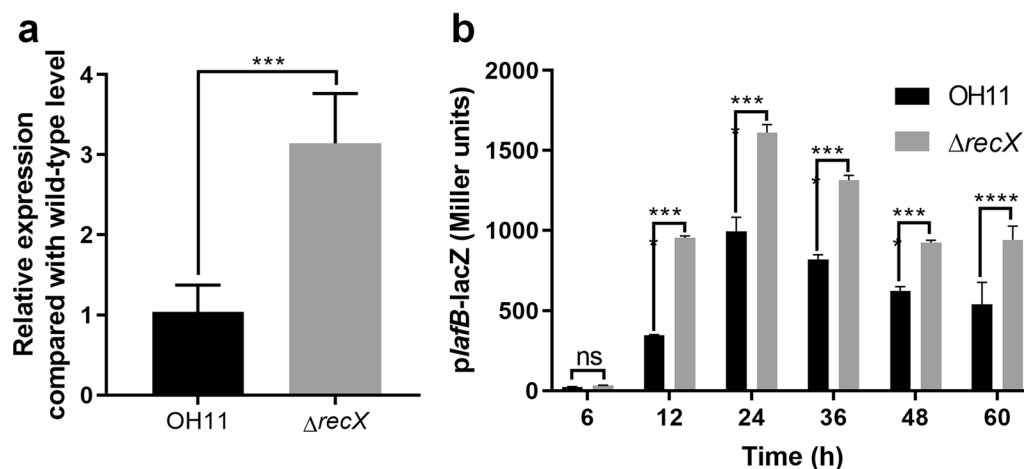
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Fig. 6 *Lysobacter enzymogenes* RecX negatively regulated *lafB* expression. **a** RT-qPCR analyses of *lafB* mRNA in the wild-type and *recX* mutants grown in 10% TSB. **b** The effect of RecX on *lafB* gene expression was measured by assessing the *β*-galactosidase activity of the *lafB*-lacZ transcriptional fusion in the *L. enzymogenes* wild-type and Δ*recX* strains. Error bars, means ± standard deviation (n = 3). ***$P < 0.001$, ****$P < 0.0001$, assessed by one-way ANOVA. All experiments were repeated three times with similar results

RecA·ATP-hydrolysis, and strand exchange promoted by RecA and RecX might act as an anti-recombinase to inhibit inappropriate recombination repair during normal DNA metabolism (Venkatesh et al. 2002; Yakimov et al. 2017). Previous work revealed that RecX is also involved in regulating the *Mycobacterium smegmatis* stress response (Prasad et al. 2019). However, the regulatory mechanisms of RecX have been less studied in the biocontrol bacterium *L. enzymogenes*.

Our data show that the in-frame deletion of the *recX* coding sequence significantly increases HSAF production (Fig. 4), and the overexpression of *recX* in the wild-type OH11 strain and the Δ*recX* mutant strain significantly suppressed HSAF production (Fig. 4). Moreover, a DNA pull-down assay confirmed that RecX directly binds p*lafB* (Fig. 1). To confirm this result, we performed an *E. coli*-based one-hybrid assay and EMSA. The results demonstrate that RecX could directly target the promoter of the HSAF biosynthesis gene (Fig. 5). We further analyzed the transcription level of the HSAF biosynthesis-related gene *lafB* in the *recX* mutant. Knockout of *recX* significantly increased the transcription level of *lafB* (Fig. 6). These results suggest that RecX can directly regulate the expression of the HSAF biosynthesis gene and decrease the production of the antifungal factor HSAF in *L. enzymogenes*. However, this study investigated the role of RecX only in relation to HSAF biosynthesis in *L. enzymogenes*. To determine whether RecX regulates recombination DNA repair in *L. enzymogenes*, we will need additional research.

Conclusions

In this study, we screened a potential HSAF biosynthesis regulator, RecX, by a DNA pull-down assay. One of the notable results of this study is that the recombination regulator RecX negatively regulated the expression of the HSAF biosynthesis gene by directly binding to its promoters to block the biosynthesis of HSAF in *L. enzymogenes*.
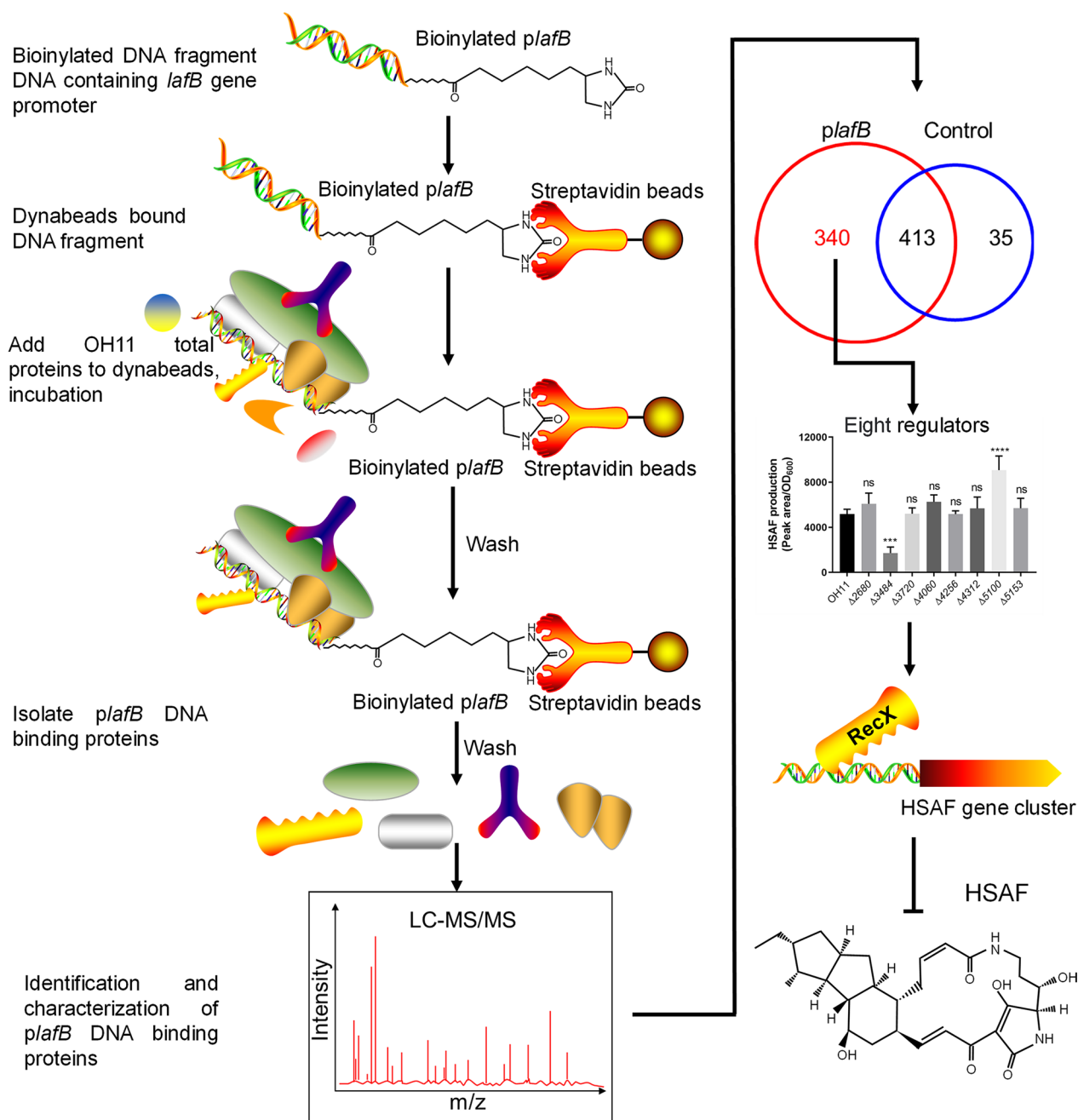
Methods

Bacterial strains, plasmids, and growth conditions

The strains and plasmids used in this study are shown in Additional file 2: Table S1. *E. coli* strains were grown in Luria–Bertani medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, pH 7.0) at 37℃. *L. enzymogenes* strains were grown in Luria–Bertani medium and 10% TSB at 28℃. For the preparation of the culture media, tryptone, peptone, beef extract, and yeast extract were purchased from Sangon Biotech (Shanghai, China). When needed, antibiotics were added (30 μg/mL kanamycin sulfate, 50 μg/mL gentamycin) to the *E. coli* or *L. enzymogenes* cultures.

DNA pull-down assays

The promoter of the *lafB* gene was amplified by PCR using the 5-biotin-labeled primers listed in Additional file 2: Table S2. The 5-biotinylated DNA of the promoter of the *lafB* gene was immobilized to streptavidin beads using Dynabeads™ M270 Streptavidin based on the manufacturer's protocol (Thermo Fisher Scientific Inc., San Jose, CA, USA). Proteins in the nuclear fraction

Fig. 7 Model of speculation about RecX regulation mechanism. This study revealed the role of RecX in negatively regulating the production of HSAF in *Lysobacter enzymogenes* using a DNA pull-down screening approach

were incubated with 5-biotinylated DNA beads on a rotating shaker at 4°C overnight in 500 µL protein binding buffer containing 5% glycerol, 0.05% Triton X-100, 25 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM dithiothreitol, and 1 mM EDTA. Following this incubation, the supernatant was removed. The beads were washed three times with cold protein binding buffer. After the last wash, the pull-down mixture was resuspended in

distilled water at 90°C for 2 min to break the streptavidin and biotin bond. The eluted samples containing the bound proteins were subjected to SDS-PAGE followed by Coomassie blue staining and LC-MS/MS analyses. The proteins that were eluted from the beads without the biotinylated DNA probe were used as controls.

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Gene deletion and complementation

The in-frame deletions in *L. enzymogenes* OH11 were generated via double-crossover homologous recombination (Xu et al. 2018; Li et al. 2020a) using the primers listed in Additional file 2: Table S2. In brief, the flanking regions of each gene were PCR-amplified and cloned into the suicide vector pEX18Gm (Additional file 2: Table S1). The deletion constructs were transformed into the wild-type strain by electroporation, and gentamycin was used to select for integration of the nonreplicating plasmid into the recipient chromosome. A single-crossover integrant colony was spread on LB medium without gentamycin and incubated at 28℃ for 3 days, and after appropriate dilution, the culture was spread on LB plates containing 15% sucrose. Colonies sensitive to gentamycin were screened by PCR using the primers listed in Additional file 2: Table S2, and the gene deletion strains were obtained.

For gene complementation constructs, DNA fragments containing the full-length genes along with their promoters were PCR-amplified and cloned into the versatile plasmid pBBR1MCS5 (Kovach et al. 1995). The resulting plasmids were transferred into the *L. enzymogenes* strain by electroporation, and the transformants were selected on LB plates containing gentamycin.

HSAF extraction and quantification

HSAF extraction and quantification were performed as follows (Tang et al. 2018; Li et al. 2021). HSAF was extracted from 4 mL *L. enzymogenes* cultures grown in 10% TSB for 48 h at 28℃ with shaking (at 180 rpm) and adjusted to pH 2.5 with HCl. Same volume of ethyl acetate was added to the acidified broth and the mixture was shaken in a vortexer at 2000 rpm for 1 min. The ethyl acetate fractions were collected, and ventilated to dryness in a fume hood. The residue was dissolved in 100 μL of methanol. The crude extract was subjected to 0.22-μm Mini-star filtration, and the filtrate was concentrated to 100 μL. The extract (20 μL) was used for HPLC analysis using a C18 reversed-phase HPLC column (4.6 × 250 mm, Agilent Technologies Inc.) with detection at 318 nm. Pure water and acetonitrile containing 0.04% (v/v) Trifluoroacetic acid (TFA) were used as the A and B mobile phases, respectively. The gradient program used a flow rate of 1 mL/min. The experiments were performed three times, and three replicates were used in each sample.

Reverse transcription-quantitative PCR (RT-qRCR)

The bacterial cells were collected when the optical density (OD_{600}) reached 1.0 in 10% TSB. Total RNA was extracted using a TRIzol-based method (Life Technologies, CA, USA). RNA quality control was performed via the following several steps: (1) the degree of RNA degradation and potential contamination were monitored on 1% agarose gels; (2) the RNA purity (OD_{260}/OD_{280}, OD_{260}/OD_{230}) was checked using a NanoPhotometer® spectrophotometer (IMPLEN, CA, USA); and (3) the RNA integrity was measured using a Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA). The primers used in this assay are listed in Additional file 2: Table S2. cDNA was synthesized from each RNA sample (400 ng) using the TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) Kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. RT-qPCR was performed using TransStart Top Green qPCR SuperMix (TransGen Biotech) on a QuantStudio TM 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with the following thermal cycling parameters: denaturation at 94℃ for 30 s, followed by 40 cycles of 94℃ for 5 s and 60℃ for 34 s. Gene expression analyses were performed using the $2^{-\Delta\Delta CT}$ method with 16S rRNA as the endogenous control, and the expression level in the wild-type was set to a value of 1. The experiments were performed three times, and three replicates were used in each run.

Bacterial one-hybrid assays

Bacterial one-hybrid assays were performed as previously reported (Xu et al. 2016; Wang et al. 2018; Li et al. 2021). In brief, the bacterial one-hybrid reporter system contained the following three components: the plasmids pBXcmT and pTRG, which are used to clone the target DNA and to express the target protein, respectively, and the *E. coli* XL1-Blue MRF′ kan strain, which is the host strain used for the propagation of the pBXcmT and pTRG recombinants (Guo et al. 2009). In this study, the promoter of the HSAF biosynthesis gene (p*lafB*) was cloned into the pBXcmT plasmid to generate the recombinant vector pBXcmT-p*lafB*. Similarly, the coding regions of *recX* were cloned into the pTRG plasmid to create the final construct pTRG-*recX*. The two recombinant vectors were transformed into the XL1-Blue MRF′ kan strain. If direct physical binding occurred between RecX and p*lafB*, the positive-transformant *E. coli* strain containing both pBXcmT-p*lafB* and pTRG-RecX would grow well on the selective medium, that is, minimal medium containing 6 mM or 10 mM 3-amino-1,2,4-triazole, 8 μg/mL streptomycin, 12.5 μg/mL tetracycline, 34 μg/mL chloramphenicol, and 30 μg/mL kanamycin. Furthermore, transformants containing both pBX-R2031/pTRG-R3133 served as a positive control (Guo et al. 2009), and co-transformants containing either empty pTRG and pBXcmT-p*lafB* were used as negative controls. All

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co-transformants were spotted onto selective medium, grown at 28°C for 3–4 days, and then photographed.

Protein expression and purification

Protein expression and purification were performed as previously reported (Li et al. 2017, 2021, 2022). To clone the *recX* gene, genomic DNA extracted from *L. enzymogenes* was used for PCR amplification using *Pfu* DNA polymerase, and the primers are listed in Additional file 2: Table S2. The PCR products were inserted into pET-28b (+) to produce the plasmid pET-*recX*. The *recX* gene was verified by nucleotide sequencing by GenScript (Nanjing, Jiangsu, China). *L. enzymogenes recX* with a vector-encoded His_6-tagged N-terminus was expressed in *E. coli* BL21 (DE3) and purified with Ni-nitrilotriacetic acid (NTA) agarose (Qiagen, Chatsworth, CA, USA) using a nickel-ion affinity column (Qiagen, Chatsworth, CA, USA). The protein purity was monitored by SDS-PAGE.

Electrophoretic mobility gel shift assays (EMSAs)

Electrophoretic mobility gel shift assays were performed as previously described (Hirakawa et al. 2015; Shao et al. 2018; Li et al. 2021). For RecX gel shift assays, we used DNA fragments that included p*lafB* as a probe. The probe DNA (50 ng) was mixed with protein in a 20 μL reaction mixture containing 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM dithiothreitol, and 0.4% glycerol. After incubation for 30 min at 28°C, samples were electrophoresed on a 5% nondenaturing acrylamide gel in $0.5 \times$ TBE buffer at 4°C. The gel was soaked in 10,000-fold-diluted SYBR Green I nucleic acid dye (Sangon Biotech, Shanghai, China), and the DNA was visualized at 300 nm.

Construction of reporter strains and *β*-galactosidase measurement assays

The p*lafB*-lacZ reporter was introduced into the *L. enzymogenes* wild type and *recX* mutant strains by electroporation. The transconjugants were selected on LB agar plates supplemented with gentamycin, and X-Gal. To measure *β*-galactosidase activities, the overnight cultured bacteria were diluted to the same cell density ($OD_{600} \approx 0.01$) in 10% TSB medium. The inoculated cultures were then incubated at 28°C and 180 rpm. The cells were harvested to assess *β*-galactosidase activities according to previous studies (Wang et al. 2019; Li et al. 2020b, 2022; Wang et al. 2021).

Statistics and reproducibility

The experimental datasets were subjected to analyses of variance using GraphPad Prism 7.0. The significance of the treatment effects was determined by the *F* value ($P = 0.05$). If a significant *F* value was obtained, separation of means was accomplished by Fisher's protected least significant difference at $P \leq 0.05$.

Abbreviations

4-HBA	4-Hydroxybenzoic acid
C-di-GMP	Cyclic dimeric GMP
Clp	Global transcriptional regulator
DF	Diffusible factor
DSF	Diffusible signal factor
EMSAs	Electrophoretic mobility gel shift assays
HPLC	High-performance liquid chromatography
HSAF	Heat-stable antifungal factor
LarR	A member of the MarR protein family
LetR	TetR family protein
$LysR_{Le}$	LysR-family transcription factor
NTA	Ni-nitrilotriacetic acid
p*lafB*	Promoter of the HSAF biosynthesis gene
QS	Quorum-sensing
RecX	Recombination regulator
RpfB	Fatty acyl-CoA ligase
TFA	Trifluoroacetic acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-023-00160-6.

Additional file 1. Figure S1. Effects of 8 potential transcription factors on the growth and production of extracellular cellulase and protease in *Lysobacter enzymogenes*.

Additional file 2. Table S1. Bacterial strains and plasmids used in this study. **Table S2.** Sequences of the PCR primers used in this work.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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