Development of a synthetic oxytetracycline-inducible expression system for streptomycetes using *de novo* characterized genetic parts

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Abstract

Precise control of gene expression using exogenous factors is of great significance. To develop ideal inducible expression systems for streptomycetes, new genetic parts, oxytetracycline responsive repressor OtrR, operator *otrO* and promoter *otrB*p from *Streptomyces rimosus*, were selected *de novo* and characterized *in vivo* and *in vitro*. OtrR showed strong affinity to *otrO* (K_D = 1.7×10^{-10} M) and oxytetracycline induced dissociation of the OtrR/DNA complex in a concentration-dependent manner. Based on these genetic parts, a synthetic inducible expression system Potr* was optimized. Induction of Potr* with 0.01-4 μM of oxytetracycline triggered a wide-range expression level of *gfp* reporter gene in different *Streptomyces* species. Benchmarking Potr* against the widely used constitutive promoters *ermE** and *kasO*p* revealed greatly enhanced levels of expression when Potr* was fully induced. Finally, Potr* was used as a tool to activate and optimize the expression of the silent jadomycin biosynthetic gene cluster in *Streptomyces venezuelae.* Altogether, the synthetic Potr* presents a new versatile tool for fine-tuning gene expression in streptomycetes.

Keywords

Inducible expression system, streptomycetes, genetic parts, wide-range strength, biosynthetic gene cluster, natural product

Introduction

The Gram-positive streptomycetes are well-known producers of an immense diversity of biologically active compounds, which have been applied in human medicine,

animal health, and plant crop protection [1]. In the past decades, a suite of genetic tools such as plasmids and constitutive promoters have been specifically developed for streptomycetes [2-8]. These tools have greatly facilitated genetic manipulations of this genus. However, only a limited number of inducible expression systems are available for the precise control of gene expression in streptomycetes [5].

Inducible expression systems are versatile genetic tools for controlling gene expression upon induction with chemical or physical inducers, and they are typically used for the functional characterization of genes and for the production of medically or industrially important proteins [9, 10]. In many cases, inducible expression systems have also been employed to optimize the levels of pathway expression in order to achieve economically viable titers, yields and productivities of products of interest [8, 11, 12]. However, the existing inducible expression systems have their limitations and could not meet the need of synthetic biology to precise control gene expression in streptomycetes. The thiostrepton-inducible *ptipA* was the first inducible expression system developed from *Streptomyces lividans* [13]. It depends on the presence of the activator TipAL but also relies on the resistance gene *tsr*, and it often show high level of leaky expression [14, 15]. Other inducible expression systems developed for streptomycetes include the ε-caprolactam-inducible expression system based on the nitrilase promoter/operator P*nitA*/NitR from *Rhodococcus rhodochrous* [16], the synthetic tetracycline (Tc)-inducible expression system based on the repressor TetR/operator *tetO* from *Escherichia coli* [17] and the cumate-inducible system from *Pseudomonas putida* [18]. While the P*nitA*/NitR inducible expression system is

excellent for protein overproduction from high copy number plasmids, it is unclear whether this system will also perform well to control expression in routine genetic analysis and metabolic pathway engineering [17]. Importantly, the inducers ε-caprolactam and cumate are degradable in some *Streptomyces* species [16, 19], which will limit their applications. In addition, the above-mentioned genetic parts for inducible expression systems are not perfectly compatible in streptomycetes, which are characterized by large and high-GC content genomes and intricate regulatory networks [20]. The regulatory networks of streptomycetes result in background noise when using these genetic parts [17, 18]. For example, each streptomycetes genome contains over 100 TetR family regulators, and thus TetR homologs SCO0253 perturbs the work of the synthetic Tc-inducible expression system developed by TetR repressor and *tetO* operator in *Streptomyces coelicolor* [17]. Therefore, although several inducible expression systems have been reported for streptomycetes [15-18], there is still a definitive lack of tightly inducible expression system suitable for elaborate studies in streptomycetes.

Ideally, an inducible expression system should be completely turned off when repressed, and tunable to different strengths when induced. In addition, the inducer should be easily available and have little pleiotropic effects on growth [9]. *Streptomyces* species exhibit marked regulatory capacity and flexibility [20]. They typically have more than 8000 protein-coding genes, and >10% of the coding genes are predicted to be transcription factors [20]. The native regulators and operators in the genomes of streptomycetes are therefore rich repositories of genetic parts that fulfill the

requirements for the development of inducible expression systems.

In the present study, we aimed to find unique genetic parts that respond to small molecules in streptomycetes and to develop ideal inducible expression systems. Interestingly, regulatory genes situated in biosynthetic gene clusters (BGCs) that direct secondary metabolite biosynthesis do often interact with the products of that pathway and regulate the transcription of biosynthetic genes and/or resistance genes [8,21]. For example, ActR [22], KijA8 [23], LanK [24], SimR [25], and VarR [26] have all been reported to bind the end products or intermediates of the corresponding BGCs and induce the expression of divergently adjacent exporter genes. The regulatory mechanism is similar to the Tc-inducible repressor (TetR) and exporter (TetA) from the *E. coli* transposon Tn10 [23]. The well-characterized repressor TetR and operator *tetO* have been widely used as genetic parts to construct inducible expression systems in prokaryotes [27], mammalian cells [28] and other organisms (such as *Drosophila* [29]). Here we therefore focused on the pairs of antibiotic exporter and regulatory genes from BGCs of streptomycetes to identify the candidate genetic parts. By combining the rareness of the regulators and the availability of the inducers, the oxytetracycline (OTC)-inducible regulator OtrR, operator *otrO* and promoter *otrB*p from *Streptomyces rimosus* were selected and characterized *de novo*. Using these well-characterized genetic parts, a synthetic and tightly controlled inducible expression system Potr* with wide-range OTC-inducible strength was developed. Potr* showed negligible leaky expression levels in the absence of the inducer OTC and high levels of induced expression in *Streptomyces venezuelae, Streptomyces*

coelicolor and *Streptomyces albus*. Furthermore, Potr* was used to activate the silent jadomycin BGC and to optimize the production of this antibiotic in *S. venezuelae* ISP5230, exemplifying the usefulness of this system in eliciting the production of silent and possibly cryptic antibiotic-like natural products in streptomycetes.

Results and Discussion

Search for parts in genomes of streptomycetes

To develop a strict inducible expression system with minimum interference from endogenous regulators of streptomycetes, pairs of antibiotic exporter and regulator genes in the genomes of streptomycetes were selected to investigate candidate genetic parts. For ideal genetic parts, the inducers should be highly stable and easily available. Aromatic polyketides, synthesized by type II polyketide synthases in streptomycetes, are recalcitrant to be degraded in these producers [30]. Therefore, we searched all type II polyketide BGCs with identified products from the genomes of streptomycetes, and extracted sequences of exporters as well as the divergently arranged putative regulators (Table 1). The regulators were identified as TetR, ArsR, MarR or DeoR family (Table 1). Previous studies have reported that intrinsic large numbers of TetR homologs may perturb the inducible expression system developed through other TetR family regulators [17], therefore this family regulators were excluded from this study. Considering the availability and cost of the inducers, we selected genetic parts from the commercialized type II polyketide BGCs. Only OTC and chlortetracycline (CTC) meet the criteria of being readily available as well as low cost. The putative regulators

of OTC and CTC exporters (OtrR and CtcS) belong to MarR family, which have many less members (approximate 20-30 members) compared with the TetR family in streptomycetes. To avoid the interference of native regulators in streptomycetes, the sequences of OtrR and CtcS were used as queries to conduct a BLASTp search against the local protein database containing the sequences of 569,791 proteins from streptomycetes deposited in UniProt. Highly homologous orthologs of OtrR and CtcS (> 30% identities) were not observed in most genomes of streptomycetes, except that of their native hosts, *Streptomyces monomycini* and *Streptomyces griseoflavus.* The results suggest that OtrR and CtcS are suitable candidates with minimum interference from other endogenous regulators in streptomycetes. Since OTC is known to be less toxic to microbial cells than CTC, we decided to investigate the regulatory mechanism of the OTC exporter to identify inducible genetic parts (Fig. 1).

Characterization of the regulatory mechanism of OtrR

The MarR family transcriptional regulators exist as dimers and bind palindromic sequences within target promoters, resulting in either transcriptional repression or activation [31]. To confirm the regulatory role of OtrR on the putative target promoter *otrB*p, a Lux reporter system was constructed in heterologous host *E. coli* (Fig. 2a). The *lux* genes were directly controlled by *otrB*p, endowing the strains with the ability of bioluminescence (Fig. 2b). When an OtrR expression plasmid was transferred into *E. coli* harboring the *lux* reporter plasmid, the bioluminescence was severely repressed (Fig. 2b), indicating that OtrR could repress the expression of *otrB*p. Furthermore, when OTC was added, the bioluminescence was restored in a

dose-dependent manner (Fig. 2b). These results suggest that OtrR can sense and respond to OTC to de-repress the transcription of *otrB*p. More importantly, the concentration of OTC needed to completely unlock *otrB*p is approximately 2.5 μM, which is a concentration much lower than the minimal inhibitory concentration (MIC, approximate 10-30 μM).

To further confirm the direct interaction of OtrR with *otrB*p, OtrR was expressed and purified from *E. coli* (Supplementary Fig. S1) and an electrophoretic mobility shift assay (EMSA) was performed with a probe of *otrB*p (P-*otrB*). As shown in Fig. 2c, OtrR could bind P-*otrB* in a concentration-dependent manner. The binding was shown to be specific, with no retardation was observed for the negative control probe of *hrdB* promoter (P-*hrdB*) (Supplementary Fig. S2). These results demonstrate that OtrR represses *otrB*p by direct interaction. The responses of OtrR/P-*otrB* complexes to OTC were also analyzed by EMSA *in vitro*. We observed that OtrR and P-*otrB* complexes would dissociate in the presence of OTC, and this dissociation effect also occurred in an OTC concentration-dependent manner (Fig. 2d). This data along with the results obtained from the *in vivo* Lux reporter system undoubtedly indicate that OtrR represses the transcription of *otrB*p by direct binding, and OTC releases this repression effect by attenuating the binding of OtrR, thus inducing the transcription of *otrB*p.

Determination of OtrR operator *otrO* and the core promoter of *otrB*p

To develop a new OTC-inducible expression system, it is necessary to identify the

operator of OtrR. DNase I footprinting was performed to locate the binding sites of OtrR in the *otrB*p region using a capillary sequencer. As shown in Fig. 3a, a fully protected region was observed in the presence of OtrR, and this region encompasses two 6-nt inverted repeats (GACAAG) with 2-bp spacing (Fig. 3b). To further confirm whether the identified region containing the palindromic sequence is the operator of OtrR (designated as *otrO*), we amplified the probes with (PR) or without (PL) the palindromic sequence (Supplementary Fig. S3a) and tested their binding with OtrR by EMSA. The results showed that the retardation was only observed with the PR probe (Supplementary Fig. S3b and c). Furthermore, the binding ability of the 20-bp probe (P20) containing the palindromic sequence with OtrR was examined using EMSA, and the retardation was readily detected (Supplementary Fig S3d). These results suggest that this palindromic sequence is the core region of *otrO*. To determine the equilibrium dissociation constant (K_D) between the interaction of OtrR and *otrO*, surface plasmon resonance (SPR) was performed. The sequence containing *otrO* was labeled with biotin and immobilized onto a streptavidin sensor chip, and subsequently OtrR was injected. The sensorgrams showed increased binding with the increasing OtrR concentrations (Fig. 3c). The best fit for OtrR yielded K_D value of 0.17 nM. The K_D of interaction between OtrR and *otrO* is similar to that of the interaction between Tc-inducible repressor TetR and operator *tetO*, which have been successfully used as genetic parts for the development of inducible expression systems and the construction of genetic circuits in many organisms [27-29]. Therefore, the new genetic parts OtrR and *otrO* show good potential for widespread application. Notably, OtrR,

containing only 165 amino acids, is smaller than TetR, which will enable a much easier and economical application in other organism. In addition, the operator of OtrR is very unique. A search using *otrO* sequence did not give closely matched sequences in the genomes of streptomycetes, suggesting that OtrR and *otrO* are ideal parts that do not interplay with host DNA or regulators in streptomycetes.

To determine the loci of the *otrO* relative to the the transcriptional start site (TSS) of *otrB*p, rapid amplification of 5'-cDNA ends (5'-RACE) was performed (Fig. 3d). Then the putative -10 and -35 regions were identified based on the determined TSS (Fig. 3d), which shares high nucleotide sequence identity with the core promoter recognized by the essential sigma factor HrdB [32]. After defining the core region of *otrB*p, *otrO* site was found to overlap with -35 region of *otrB*p (Fig. 3d).

Development of the OTC-inducible expression system for streptomycetes

OtrR and *otrB*p were identified from *Streptomyces* species, and thus the native *otrR-otrB*p inducible expression system (designated as Potr) should be compatible in streptomycetes. The performance of Potr was firstly evaluated in *S. venezuelae*, which grows in a diffuse and homogenous manner in a variety of liquid media [33]. Such growth characteristics facilitate accurate data normalization. Strain *S. venezuelae* WVR2006::pIJ-Potr was incubated in MYM medium supplemented with OTC at concentrations ranging from 10 nM to 6 μM. We observed that GFP fluorescence increased with increasing concentrations of OTC and Potr was fully induced at 4 μM OTC (Fig. 4a). Surprisingly, the fully induced fluorescence was stronger than that

driven by the widely used constitutive promoters *kasO*p* [2] and *ermE*p* [34] (Fig. 4b), suggesting that the activity of *otrB*p was very strong. Recently, Bai et al. [35] has shown that *kasO*p* exhibited the strongest activity among all the tested constitutive promoters in streptomycetes using a flow cytometry-based quantitative method. At the fully induced state, we observed that the activity of native *otrB*p was stronger than that of *kasO*p* (Fig. 4b). Hence, the activity of *otrB*p does not require further optimization. However, we observed that the leaky expression without OTC induction was higher compared with the control (Fig. 4b). Thus, Potr should be engineered to reduce leaky expression.

Zhang et al. [36] reported that the integration of the same operators at two locations, flanking the -35 and the -10 regions of the target promoter, can achieve tighter control of the promoter activity. To reduce the leaky expression of Potr, we engineered the inducible expression system (designated as Potr*) by inserting another operator downstream of the TSS of *otrB*p (Fig. 4c). After examining the GFP fluorescence of *S. venezuelae* WVR2006::pIJ-Potr* (map of pIJ-Potr*, see Supplementary Fig. S4), we observed that Potr* showed nearly the same GFP-response curve as Potr to OTC (Fig. 4d), whereas the leaky expression was significantly reduced to a level similar to the control (Fig. 4e).

To examine the activity of Potr* induced by different doses of OTC at the transcription level, real-time quantitative PCR (RT-qPCR) was performed to assess the transcript levels of the *gfp* gene in *S. venezuelae* WVR2006::pIJ-Potr*. The results showed that the transcript abundance of *gfp* increased in an OTC

concentration-dependent manner (Fig. 5a), suggesting that the inducible activity of Potr* was sensitively controlled at the transcriptional level in *S. venezuelae*.

To evaluate the growth inhibitory effects by OTC, the growth of *S. venezuelae* was assayed in the presence OTC. The results indicate OTC does not have significant effects on growth at lower than 6 μM (Supplementary Fig. S5). As a concentration less than 4 μM OTC is sufficient to induce Potr or Potr* to a fully expressed level, the inducer can be used at a concentration where the growth is hardly affected.

Evaluation of the performance of Potr* in different *Streptomyces* species

To evaluate the inducible behavior of Potr* in other *Streptomyces* species, the production of GFP fluorescence under the control of Potr* was further evaluated in *S. coelicolor* M1146::pIJ-Potr* and *S. albus* J1074::pIJ-Potr*. It was shown that the expression of *gfp* was sensitively induced in a OTC concentration-dependent manner in the two hosts; the leaky expression of *gfp* was negligible without OTC and the fully induced output of *gfp* was much higher than that of the constitutive promoters *kasO*p* [2] and *ermE*p* [34] (Fig. 5b and c). When grown in liquid culture, *S. coelicolor* and *S. albus* form large pellets or clumps, meaning that the GFP fluorescence assay might have margins of error in the data normalization of these strains. Therefore, we further performed RT-qPCR to more accurately evaluate the transcription levels of *gfp* reporter gene in *S. coelicolor* M1146::pIJ-Potr* and *S. albus* J1074::pIJ-Potr* after induction using different OTC concentrations. The results showed that the transcript levels of the *gfp* gene also increased in an OTC concentration-dependent manner in

the two *Streptomyces* strains (Fig. 5d and e), consistent with the results obtained in *S. venezuelae*. The highly consistent results generated in different *Streptomyces* species indicate that Potr* is a tightly controlled inducible expression system with wide-range strength in streptomycetes.

Application of Potr* in activation and optimization of the silent jadomycin BGC

The potential for the production of high-value secondary metabolites is even larger than previously realized in streptomycetes [21]. However, accessing this rich resource to identify new compounds through the activation of cryptic pathways is still a challenge. We hypothesized that a cryptic gene cluster under the control of an inducible expression system will facilitate the identification of corresponding product, as the production will increase with the inducer in a concentration-dependent manner. To demonstrate this application, we designed a plug-and-play plasmid pIW01 (Supplementary Fig. S6) using the newly developed inducible expression system Potr*. This plasmid was integrated into the genome of *S. venezuelae* ISP5230 to drive the expression of silent jadomycin BGC [37] (Fig. 6a). As expected, jadomycin B production gradually increased with the OTC inducer (Fig. 6b), while the control without OTC induction showed almost no production, similar to the results of wild type *S. venezuelae* ISP5230. These results indicate that the inducible expression system can be applied in the activation and identification of silent and possibly cryptic natural products in streptomycetes.

To improve the production of jadomycin, the expression levels of jadomycin BGC

were optimized by adding different doses of OTC at different growth time points. Accordingly, jadomycin B production under different induction conditions was examined. It was found that an induction dose of 0.75 μM OTC and time of 4 h was optimal (Fig. 6c). The use of an inducible expression system to optimize the production levels of secondary metabolites in streptomycetes is a promising approach for the improvement of antibiotics production. Previously, overexpression of BGCs or activators through constitutive strong promoters is a prefered approach [38]. A potential disadvantage of these approaches is that the constitutive expression of BGCs leads to the synthesis of the products at early growth stage, which could reduce the growth of host cell and concomitantly decrease the overall production level [39]. The strategy of fine-tuning the expression of BGCs could coordinate the biomass growth and desired product accumulation. Our results suggest that the inducible expression system Potr* can be applied as an optimizing tool in rational engineering.

Overall, new genetic parts (repressor OtrR, operator *otrO* and promoter *otrB*p) were identified and characterized *de novo*. They have been shown to fulfill the requirements of inducible expression system. Then the synthetic inducible expression system Potr* was developed using these genetic parts, and it showed tightly tunable strength though the inducer OTC in streptomycetes. Potr* was also designed as a tool to activate silent gene clusters and to optimize the production of secondary metabolites. The present work suggests that the synthetic inducible expression system Potr* will play important roles on fine-tuning gene expression in streptomycetes and the newly characterized genetic parts will have potential to be used in the control of

gene expression and construction of genetic circuits in synthetic biology.

Methods

Bacterial strains and culture conditions

The strains and plasmids used in the present study are listed in Supplementary Table S1. *E. coli* JM109 and DH5α were used for cloning and bioluminescence assays, respectively; BL21(DE3) was used for the recombinant expression of OtrR protein. *E. coli* strains were grown in Luria-Bertani (LB) medium containing ampicillin (100 μg/ml), hygromycin (50 μg/ml), kanamycin (25 μg/ml) or chloramphenicol (25 μg/ml) when necessary. For spore preparations, *Streptomyces coelicolor* M1146 [40], *Streptomyces albus* J1074 [41], *Streptomyces rimosus* M4018 [39] and their derivatives were maintained on mannitol-soya flour (MS) agar plates [7], while *Streptomyces venezuelae* ISP5230, *S. venezuelae* WVR2006 [42] and its derivatives were grown on maltose-yeast extract-malt extract (MYM) agar plates [7]. For DNA and total RNA preparation, *S. rimosus* M4018 was grown in TSB medium [39]. For GFP reporter assays, the derivatives of *S. coelicolor* M1146 and *S. albus* J1074 were grown in supplemented minimum medium (SMM) [7], while the derivatives of *S. venezuelae* WVR2006 were grown in MYM liquid medium.

Construction of plasmids

All primers used in the present study are listed in Supplementary Table S2. For the construction of pET-OtrR, *otrR* was amplified from the genomic DNA of *S. rimosus* 4018 with primer pair OtrRF1/OtrRR1. The product was digested with NdeI/XhoI and

inserted into the corresponding sites of pET-23b. For the construction of pLux-otrBp, *otrB*p was amplified from genomic DNA of *S. rimosus* 4018 with primer pair PBF1/PBR1. The product was digested with BamHI/XhoI and inserted into the corresponding sites of pCS26-Pac [43]. For the construction of pOtrR, *otrR* was amplified using the primer pair OtrRF2/OtrRR2, and a fragment of plasmid backbone was amplified from pACYC184 using the primer pair 184F/184R. The two fragments were assembled using the In-Fusion cloning kit (Clontech) to generate pOtrR. For the construction of pIJ-Potr, a 637-bp fragment containing *otrR* and *otrB*p was amplified from *S. rimosus* 4018 genomic DNA using the primer pair PBF2/PBR2, and enzymatically assembled with pIJ8660::BsaI-sfgfp [35] digested by BsaI to generate pIJ-Potr [44]. To construct pIJ-Potr*, a fragment of plasmid backbone was amplified from pIJ-Potr using the primer pair IJF2/IJR2 and subsequently self-ligated through enzymatic assembly to generate pIJ-Potr*. For the construction of plug-and-play plasmids, a fragment of the plasmid backbone was amplified from pIJ-Potr* using the primer pair IJF3/IJR3 and subsequently self-ligated to remove the sequences of *attP* and integrase gene to generate pIW. A fragment of plasmid backbone was amplified from pIW using the primer pair IWF1/IWR1 to remove the *gfp* gene, followed by self-ligation through enzymatic assembly to generate pIW01. For the construction of pIW01-jad, 5'-partial *jadJ* was amplified from *S. venezuelae* ISP5230 genomic DNA using the primer pair JF/JR, and a fragment of plasmid backbone was amplified from pIW01 using the primer pair IWF/IWR. Subsequently, the two fragments were enzymatically assembled to generate pIW01-jad.

Expression and purification of OtrR

The plasmid pET-OtrR was introduced into *E. coli* BL21 (DE3) for recombinant expression of OtrR. These proteins were purified as previously described [45].

EMSA

A probe for *otrB*p was amplified using the primers PBF4/PBR4 (Table S2). The probe was incubated with varying concentrations of purified OtrR at 25℃ for 30 min in 20 μl of buffer containing 20 mM Tris-base (pH 7.5), 2 mM dithiothreitol, 5 mM $MgCl_2$, 0.5 mg/ml calf BSA and 5% glycerol. After incubation and electrophoresis, the non-denaturing 4% polyacrylamide gels were stained with SYBR Gold Nucleic Acid Gel Stain (Invitrogen) for 30 min in TBE (89 mM Tris-base, 89 mM boric acid, and 1 mM EDTA, pH 8.0), and photographed under an ultraviolet trans-illuminator using Bio-Rad GelDoc XR.

Luciferase assays *in vivo* using Lux reporter systems

The plasmid pLux-otrBp and control pCS26-Pac [43] were introduced into *E. coli* DH5α. Subsequently, pOtrR was introduced into DH5α harboring pLux-otrBp to examine the effects of OtrR on *otrB*p. For luciferase assays, approximately 10^6 cells were inoculated into 5 ml of LB for 12 h. Then the bioluminescence of *E. coli* cultures was measured using an EnSpire Multimode Reader (PerkinElmer).

DNase I footprinting assay

DNase I footprinting assays were performed through fluorescent labeling and

capillary analyses [46]. Briefly, DNA fragments were prepared through PCR using fluorescence-labeled primers PBF5/PBR4. After purification from agarose gel, the labeled DNA fragments (120 ng) and respective concentrations of proteins were added to a final reaction volume of 50 μl, and incubated at 25°C for 20 min. Then the fragments were digested with DNase I (Promega) for 1 min at 25°C and terminated using stop buffer (Promega). After phenol-chloroform extraction and ethanol precipitation, the samples were loaded onto an Applied Biosystems 3730 DNA genetic analyzer (Applied Biosystems) together with the internal-lane size standard ROX-500 (Applied Biosystems). A dye primer-based sequencing kit (Thermo) was used to precisely determine the sequences after aligning the results of the capillary electrophoresis reactions. The electrophoregrams were subsequently analyzed with the GeneMarker v1.8 software.

Determination of TSS

5'-RACE experiments were conducted to map the transcriptional start points of *otrB*p according to the manufacturer's instructions (RLM-RACE kit, Ambion). The total RNA was extracted from *S. rimosus* M4018. GSPB1 was used as special outer *otrB* primer, and GSPB2 was used as special inner *otrB* primer, respectively.

SPR assays

The SPR assays were conducted using a Biacore 3000 System (GE Healthcare). The experiments were performed in PBS buffer (9 mM PBS, pH 7.4, 1% DMSO and 0.05% Tween 20) with a flow rate of 30 μl/min at 25°C. The probe was obtained after

annealing the biotin-labeled forward primer SPRF and the unlabeled reverse primer SPRR, and then immobilized on a streptavidin sensor chip. OtrR was diluted to different concentrations in PBS buffer and subsequently injected. At the end of each cycle, 0.4% SDS was used to regenerate the surface. The data fitting for the binding model was conducted using BIA evaluation 4.1 software (GE Healthcare).

Quantitative measurement of GFP fluorescence

For *S. venezuelae*, 1 ml of the 24-h seed cultures of the recombinant strains was inoculated into 50 ml of MYM medium for 6 h, then induced by different doses of OTC for 18 h. 200-μl aliquots of the cultures were washed twice with PBS (pH 7.2) and re-suspended with 1ml PBS, then GFP fluorescence was detected (excitation at 485 nm; emission at 512 nm, Synergy H4 Multi-Mode Reader). All fluorescence values were normalized to cell growth (OD_{600}). For *S. coelicolor* and *S. albus*, approximately 2×10^8 spores were inoculated into 50 ml of SMM medium for 48 and 42 h, respectively. Subsequently, the cells were washed twice with PBS (pH 7.2). The pellets were re-suspended in 1 ml R buffer (20 mM Tris-HCl, 0.5 M NaCl, and 10% (v/v) glycerol, pH 7.4) and subjected to ultra-sonication to generate a cell extract [3]. The fluorescence intensity was quantified after normalizing the fluorescence intensity to the protein concentration in the 200-μl sample [3]. Each value and error bar represents the average and standard deviation of three experimental replicates, respectively.

Transcriptional analysis using RT-qPCR

Total RNA was isolated from *Streptomyces* samples using a standard procedure [7] at the same time as GFP fluorescence assay. The RNA samples were then treated with RNase-free DNase (Progema) and checked by PCR to eliminate the possibility of chromosomal DNA contamination. First-strand cDNA of *gfp* gene was performed using a Superscript III first-strand Synthesis System (Invitrogen) with 500 ng of total RNA according to the manufacturer's instructions. All cDNA synthesis reactions included a replicate reaction without reverse transcriptase to ensure the complete removal of contaminating DNA from the RNA samples. The primers of *gfp* and *hrdB* are listed in Supplementary Table S2. RT-qPCR was performed using the ABI 7500 Detection System and SYBR Green PCR Master Mix (Applied Biosystems). The results were analyzed using ABI 7500 software v2.0.1 and the relative expression levels of the target genes were normalized to endogenous *hrdB* levels. All samples were analyzed in triplicate.

Activation of jadomycin BGC and optimization of jadomycin production

The plasmid pIW01-jad was introduced into *E. coli* ET12567 (pUZ8002) and conjugated into *S. venezuelae* ISP5230. Recombinant strains were incubated in 96 deep-well plates after the introduction of OTC at different time points and dose at 30^{o}C with shaking (900 rpm), then the production of jadomycin B was examined. The HPLC condition for the detection of jadomycin B was the same as previously described [42].

Supporting Information Available

Supplementary Figures, Tables and Sequences. This material is available free of

charge via the Internet at http://pubs.acs.org.

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Author Contributions

K.Y., W.W. and C.C. conceived the project. W.W., T.Y. and Y.L. performed the experiment. S.L., S.Y., C.C. and K.S. assisted with the primary data analysis, W.W., K.Y. and C.C. wrote the manuscript.

Notes: The authors declare no competing interest.

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References

1. Newman, D. J., and Cragg, G. M. (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010, *J. Nat. Prod. 75*, 311-335.

2. Wang, W., Li, X., Wang, J., Xiang, S., Feng, X., and Yang, K. (2013) An

engineered strong promoter for streptomycetes, *Appl. Environ. Microbiol. 79*, 4484-4492.

3. Li, S., Wang, J., Li, X., Yin, S., Wang, W., and Yang, K. (2015) Genome-wide identification and evaluation of constitutive promoters in streptomycetes, *Microb. Cell Fact. 14*, 172.

4. Luo, Y., Zhang, L., Barton, K. W., and Zhao, H. (2015) Systematic identification of a panel of strong constitutive promoters from *Streptomyces albus*, *ACS Synth. Biol. 4*, 1001-1010.

5. Medema, M. H., Breitling, R., and Takano, E. (2011) Synthetic biology in *Streptomyces* bacteria, *Methods Enzymol. 497*, 485-502.

6. Siegl, T., Tokovenko, B., Myronovskyi, M., and Luzhetskyy, A. (2013) Design, construction and characterisation of a synthetic promoter library for fine-tuned gene expression in actinomycetes, *Metab. Eng. 19*, 98-106.

7. Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F., and Hopwood, D. A. (2000) *Practical Streptomyces Genetics*, The John Innes Foundation, Norwich, UK.

8. Smanski, M. J., Zhou, H., Claesen, J., Shen, B., Fischbach, M. A., and Voigt, C. A. (2016) Synthetic biology to access and expand nature's chemical diversity, *Nat. Rev. Micro. 14*, 135-149.

9. Terpe, K. (2006) Overview of bacterial expression systems for heterologous protein production: from molecular and biochemical fundamentals to commercial systems, *Appl. Microbiol. Biotechnol. 72*, 211-222.

10. Heiss, S., Hormann, A., Tauer, C., Sonnleitner, M., Egger, E., Grabherr, R.,

and Heinl, S. (2016) Evaluation of novel inducible promoter/repressor systems for recombinant protein expression in *Lactobacillus plantarum*, *Microb. Cell Fact. 15*, 50.

11. Lan, E. I., and Liao, J. C. (2011) Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide, *Metab. Eng. 13*, 353-363.

12. Martin, V. J., Pitera, D. J., Withers, S. T., Newman, J. D., and Keasling, J. D. (2003) Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids, *Nat. Biotechnol. 21*, 796-802.

13. Takano, E., White, J., Thompson, C. J., and Bibb, M. J. (1995) Construction of thiostrepton-inducible, high-copy-number expression vectors for use in *Streptomyces* spp, *Gene 166*, 133-137.

14. Ali, N., Herron, P. R., Evans, M. C., and Dyson, P. J. (2002) Osmotic regulation of the *Streptomyces lividans* thiostrepton-inducible promoter, *ptipA*, *Microbiology 148*, 381-390.

15. Murakami, T., Holt, T. G., and Thompson, C. J. (1989) Thiostrepton-induced gene expression in *Streptomyces lividans*, *J. Bacteriol. 171*, 1459-1466.

16. Herai, S., Hashimoto, Y., Higashibata, H., Maseda, H., Ikeda, H., Omura, S., and Kobayashi, M. (2004) Hyper-inducible expression system for streptomycetes, *Proc. Natl. Acad. Sci. U.S.A. 101*, 14031-14035.

17. Rodriguez-Garcia, A., Combes, P., Perez-Redondo, R., Smith, M. C., and Smith, M. C. (2005) Natural and synthetic tetracycline-inducible promoters for use in the antibiotic-producing bacteria *Streptomyces*, *Nucleic Acids Res. 33*,

e87.

18. Horbal, L., Fedorenko, V., and Luzhetskyy, A. (2014) Novel and tightly regulated resorcinol and cumate-inducible expression systems for *Streptomyces* and other actinobacteria, *Appl. Microbiol. Biotechnol. 98*, 8641-8655.

19. Davis, J. R., and Sello, J. K. (2010) Regulation of genes in *Streptomyces* bacteria required for catabolism of lignin-derived aromatic compounds, *Appl. Microbiol. Biotechnol. 86*, 921-929.

20. Romero-Rodriguez, A., Robledo-Casados, I., and Sanchez, S. (2015) An overview on transcriptional regulators in *Streptomyces*, *Biochim. Biophys. Acta. 1849*, 1017-1039.

21. Liu, G., Chater, K. F., Chandra, G., Niu, G., and Tan, H. (2013) Molecular regulation of antibiotic biosynthesis in *Streptomyces*, *Microbiol. Mol. Biol. Rev. 77*, 112-143.

22. Tahlan, K., Ahn, S. K., Sing, A., Bodnaruk, T. D., Willems, A. R., Davidson, A. R., and Nodwell, J. R. (2007) Initiation of actinorhodin export in *Streptomyces coelicolor*, *Mol. Microbiol. 63*, 951-961.

23. Cuthbertson, L., and Nodwell, J. R. (2013) The TetR family of regulators, *Microbiol. Mol. Biol. Rev. 77*, 440-475.

24. Ostash, I., Ostash, B., Luzhetskyy, A., Bechthold, A., Walker, S., and Fedorenko, V. (2008) Coordination of export and glycosylation of landomycins in *Streptomyces cyanogenus* S136, *FEMS Microbiol. Lett. 285*,

195-202.

25. Le, T. B., Schumacher, M. A., Lawson, D. M., Brennan, R. G., and Buttner, M. J. (2011) The crystal structure of the TetR family transcriptional repressor SimR bound to DNA and the role of a flexible N-terminal extension in minor groove binding, *Nucleic Acids Res. 39*, 9433-9447.

26. Namwat, W., Lee, C. K., Kinoshita, H., Yamada, Y., and Nihira, T. (2001) Identification of the *varR* gene as a transcriptional regulator of virginiamycin S resistance in *Streptomyces virginiae*, *J. Bacteriol. 183*, 2025-2031.

27. Bertram, R., and Hillen, W. (2008) The application of Tet repressor in prokaryotic gene regulation and expression, *Microb. Biotechnol. 1*, 2-16.

28. Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W., and Bujard, H. (1995) Transcriptional activation by tetracyclines in mammalian cells, *Science 268*, 1766-1769.

29. Stebbins, M. J., Urlinger, S., Byrne, G., Bello, B., Hillen, W., and Yin, J. C. (2001) Tetracycline-inducible systems for *Drosophila*, *Proc. Natl. Acad. Sci. U.S.A. 98*, 10775-10780.

30. Hertweck, C., Luzhetskyy, A., Rebets, Y., and Bechthold, A. (2007) Type II polyketide synthases: gaining a deeper insight into enzymatic teamwork, *Nat. Prod. Rep. 24*, 162-190.

31. Perera, I. C., and Grove, A. (2010) Molecular mechanisms of ligand-mediated attenuation of DNA binding by MarR family transcriptional regulators, *J. Mol. Cell. Biol. 2*, 243-254.

32. Strohl, W. R. (1992) Compilation and analysis of DNA sequences associated with apparent streptomycete promoters, *Nucleic Acids Res. 20*, 961-974.

33. Pullan, S. T., Chandra, G., Bibb, M. J., and Merrick, M. (2011) Genome-wide analysis of the role of GlnR in *Streptomyces venezuelae* provides new insights into global nitrogen regulation in actinomycetes, *BMC Genomics 12*, 175.

34. Bibb, M. J., Janssen, G. R., and Ward, J. M. (1985) Cloning and analysis of the promoter region of the erythromycin resistance gene (*ermE*) of *Streptomyces erythraeus*, *Gene 38*, 215-226.

35. Bai, C., Zhang, Y., Zhao, X., Hu, Y., Xiang, S., Miao, J., Lou, C., and Zhang, L. (2015) Exploiting a precise design of universal synthetic modular regulatory elements to unlock the microbial natural products in *Streptomyces*, *Proc. Natl. Acad. Sci. U.S.A. 112*, 12181-12186.

36. Zhang, F., Carothers, J. M., and Keasling, J. D. (2012) Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids, *Nat. Biotechnol. 30*, 354-359.

37. Doull, J., Singh, A., Hoare, M., and Ayer, S. (1994) Conditions for the production of jadomycin B by *Streptomyces venezuelae* ISP5230: Effects of heat shock, ethanol treatment and phage infection, *J. Ind. Microb. 13*, 120-125.

38. Hwang, K. S., Kim, H. U., Charusanti, P., Palsson, B. O., and Lee, S. Y. (2014) Systems biology and biotechnology of *Streptomyces* species for the production of secondary metabolites, *Biotechnol. Adv. 32*, 255-268.

39. Yin, S., Wang, W., Wang, X., Zhu, Y., Jia, X., Li, S., Yuan, F., Zhang, Y., and Yang, K. (2015) Identification of a cluster-situated activator of oxytetracycline biosynthesis and manipulation of its expression for improved oxytetracycline production in *Streptomyces rimosus*, *Microb. Cell Fact. 14*, 46.

40. Gomez-Escribano, J. P., and Bibb, M. J. (2011) Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters, *Microb. Biotechnol. 4*, 207-215.

41. Chater, K. F., and Wilde, L. C. (1980) *Streptomyces albus* G mutants defective in the SalGI restriction-modification system, *J. Gen. Microbiol. 116*, 323-334.

42. Fan, K., Pan, G., Peng, X., Zheng, J., Gao, W., Wang, J., Wang, W., Li, Y., and Yang, K. (2012) Identification of JadG as the B ring opening oxygenase catalyzing the oxidative C-C bond cleavage reaction in jadomycin biosynthesis, *Chem. Biol. 19*, 1381-1390.

43. Bjarnason, J., Southward, C. M., and Surette, M. G. (2003) Genomic profiling of iron-responsive genes in *Salmonella enterica* serovar typhimurium by high-throughput screening of a random promoter library, *J. Bacteriol. 185*, 4973-4982.

44. Gibson, D. G., Young, L., Chuang, R. Y., Venter, J. C., Hutchison, C. A., 3rd, and Smith, H. O. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases, *Nat. Methods 6*, 343-345.

45. Wang, W., Li, X., Li, Y., Li, S., Fan, K., and Yang, K. (2015) A genetic

biosensor for identification of transcriptional repressors of target promoters, *Sci. Rep. 5*, 15887.

46. Zianni, M., Tessanne, K., Merighi, M., Laguna, R., and Tabita, F. R. (2006) Identification of the DNA bases of a DNase I footprint by the use of dye primer sequencing on an automated capillary DNA analysis instrument, *J. Biomol. Tech. 17*, 103-113.

Figure legends and tables

Figure 1 Position and arrangement of *otrR* and *otrB* genes in the OTC BGC.

Figure 2 Identification of the repression effect of OtrR and the de-repression effect of OTC. **(a)** Schematic representation of the Lux reporter system. **(b)** OtrR represses the bioluminescence controlled by *otrB*p and OTC induces the bioluminescence *in vivo*. All values are presented in relative light units (RLU) and represented as the means ± SD from at least three independent experiments. **(c)** EMSA of the interaction between the P-*otrB* and OtrR. Each lane contains 6 ng of P-*otrB* and different amounts of OtrR. **(d)** Dissociation effect of the DNA-binding activity of OtrR in the presence of OTC. Each lane contains 6 ng of P-*otrB*, 0.15 μM OtrR and different amounts of OTC.

Figure 3 Characterization of the interaction between the operator *otrO* and OtrR. **(a)** Capillary electrophoregram of the DNase I digestion reaction of *otrB*p protected by 0.15 μM OtrR. **(b)** Capillary electrophoregram of sequencing reactions of *otrB*p aligned by GeneMarker v1.8 software. The sequence around the protected region is

indicated below the electrophoregrams, and the palindromic sequence of the protected region is indicated with arrows. **(c)** SPR analysis of the equilibrium dissociation constant (K_D) of the interaction between OtrR and *otrO*. **(d)** Nucleotide sequences of the core region of *otrB*p. The transcriptional start site (TSS) is indicated with an asterisk. The operator *otrO* characterized with inverted repeat sequences is indicated with arrows. The presumptive -10 and -35 regions of *otrB*p are indicated with boxes.

Figure 4 Development of the inducible expression system Potr*. **(a)** Expression levels of Potr in response to different doses of OTC measured by GFP fluorescence. The inducer concentrations were 0, 0.01, 0.02, 0.04, 0.08, 0.2, 0.4, 0.8, 1.6, 2, 3, 4, 5, and 6 μM OTC. The values are presented as the means ± SD of three independent experiments. **(b)** Activity of the on and off state of Potr. 4 μM OTC was used to fully induce the activity of Potr. **(c)** Core promoter sequences of the native Potr and the developed Potr*. The TSS is indicated with an asterisk. The presumptive -10 and -35 regions are indicated with boxes, and the operator *otrO* is underlined. **(d)** Expression levels of Potr* in response to different doses of OTC measured by GFP reporter. The same OTC concentrations as in A were used. **(e)** Activity of the on and off state of Potr*. 4 μM OTC was used to fully induce the activity.

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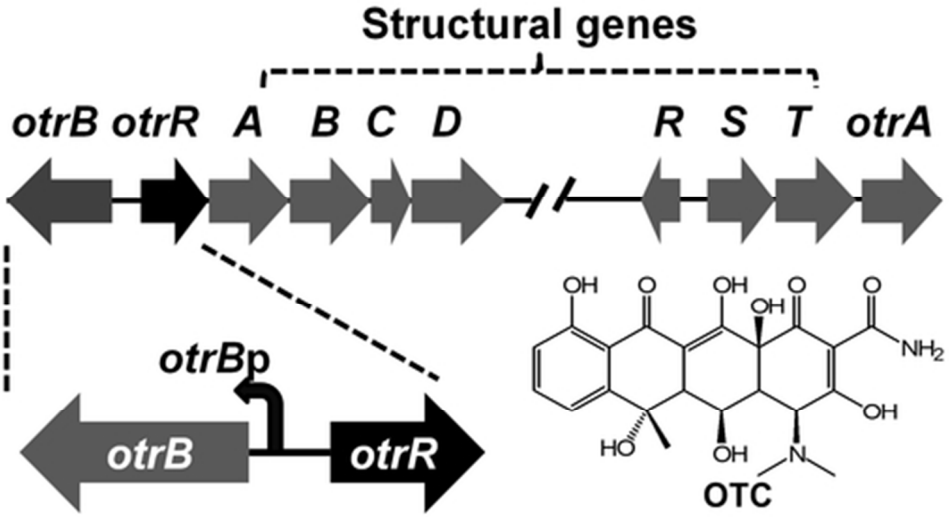
Table 1 Pairs of antibiotic exporter and regulatory genes of in type II polyketide synthesis clusters.

Streptomyces species	Type II polyketide	Transporter	Regulator/family	Effector[a]	GenBank accession number
S. coelicolor	Actinorhodin	ActII-ORF2	ActII-ORF1/TetR family	(S)-DNPA; kalafungin	M64683
S. sp. CM020	Alnumycin	AlnT1	AlnR8/MarR family	NA	EU852062
S. maritimus	Enterocin	EncT	EncS/TetR family	NA	AF254925
S. griseoflavus	Gilvocarcin	GilJ	GilI/DeoR family	NA	AY233211
S. cyanogenus	Landomycin	LanJ	LanK/TetR family	landomycin A and B	AF080235
S. glaucescens	Tetracenomycin	TcmA	TcmR/TetR family	Tetracenomycin	M80674
S. antibioticus	Oviedomycin	OvmE	Orf3/ArsR family	NA	AJ632203
S. rimosus	Oxytetracycline	OtrB	OtrR/MarR family	NA	DQ143963
S. aureofaciens	Chlortetracycline	CtcR	CtcS/MarR family	NA	HM627755
S. steffisburgensis	Steffimycin	SfrB	StfRIII/MarR family	NA	AM156932
S. fradiae Tü2717	Urdamycin	UrdJ	UrdK/TetR family	NA	AF164961
S. antibioticus	Simocyclinone	SimX	SimR/TetR family	Simocyclinone D8 and C4	AF324838

[a] NA indicate the unknown effector.



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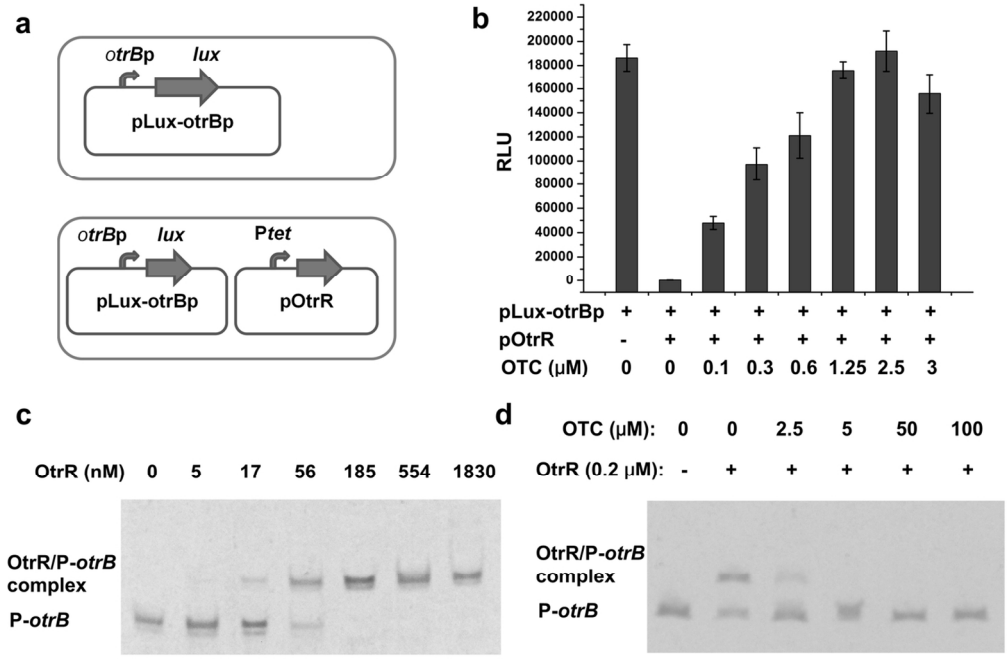

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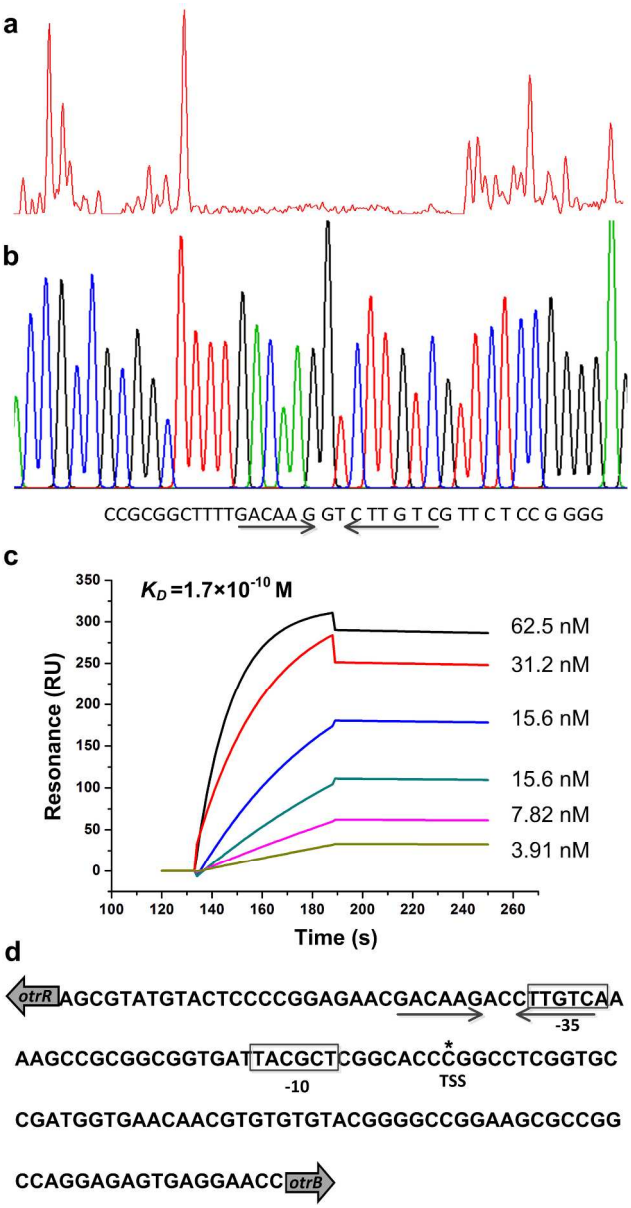

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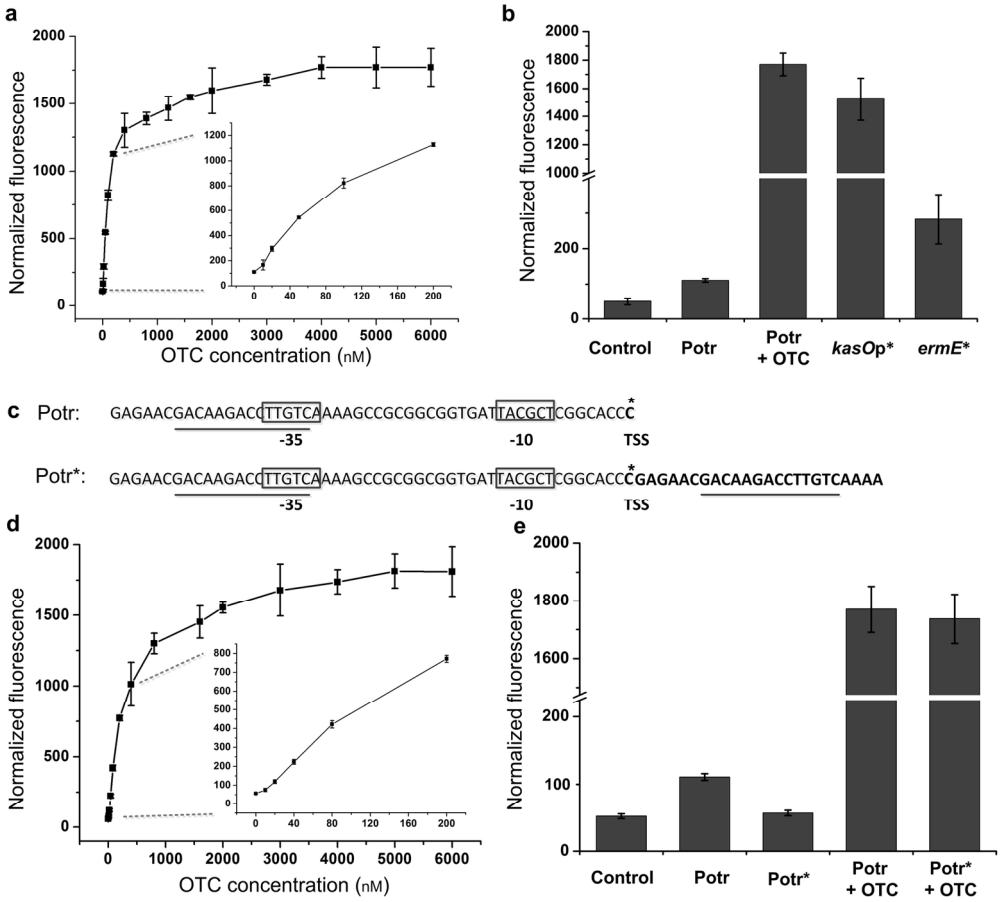

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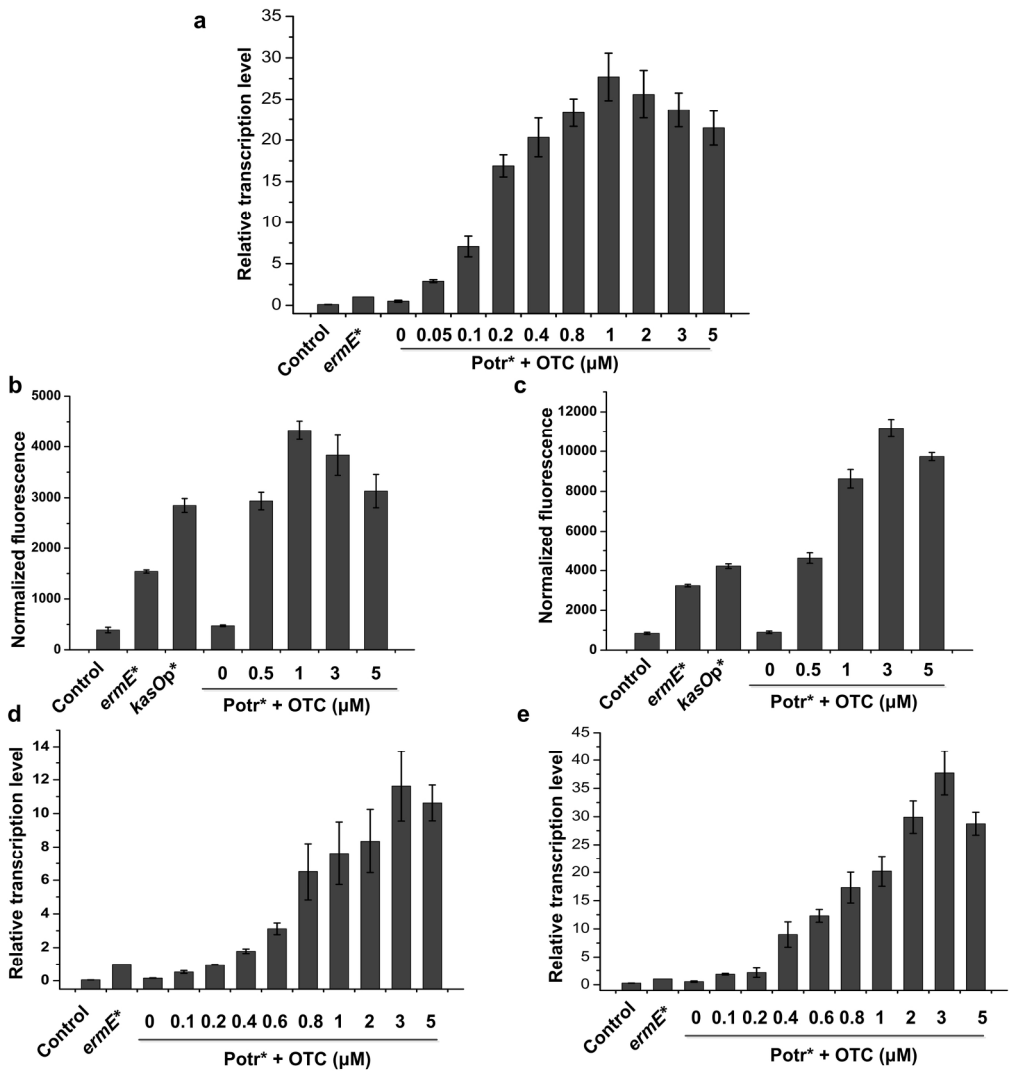

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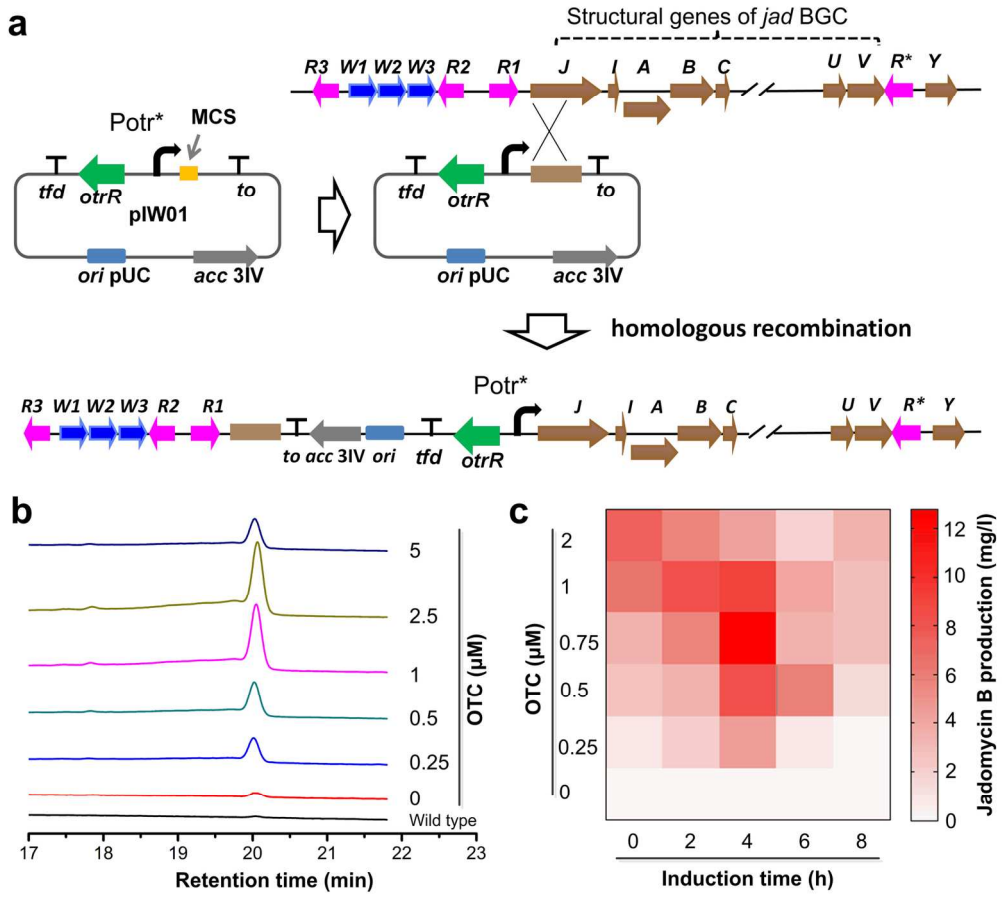

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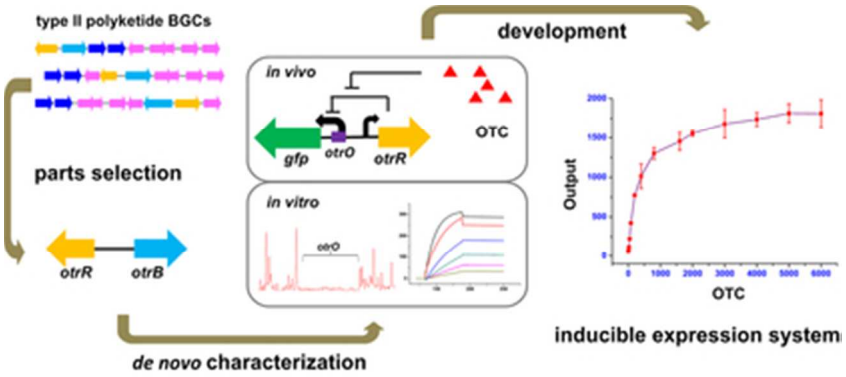

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Author(s): Wang, Weishan; Yang, Tongjian; Li, Yihong; Li, Shanshan; Yin, Shouliang; Styles, Kathryn ; Corre, Christophe; Yang, Keqian
35x15mm (300 x 300 DPI)