Cloning and identification of a new repressor of 3,17β-Hydroxysteroid dehydrogenase of Comamonas testosteroni

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Abstract

Background 3,17β-hydroxysteroid dehydrogenase (3,17β-HSD) is a key enzyme in the metabolic pathway for steroid compounds catabolism in Comamonas testosteroni. Tetracycline repressor (TetR) family, repressors existing in most microorganisms, may play key roles in regulating the expression of 3,17β-HSD. Previous reports showed that three tetR genes are located in the contig58 of C. testosteroni ATCC 11996 (GenBank:AHIL01000049.1), among which the first tetR gene encoded a potential repressor of 3,17β-HSD by sensing environmental signals. However, whether the other proposed tetR genes act as repressors of 3,17β-HSD are still unknown.

Methods and Results In the present study, we cloned the second tetR gene and analyzed the regulatory mechanism of the protein on 3,17β-HSD using electrophoretic mobility shift assay (EMSA), gold nanoparticles (AuNPs)-based assay, and loss-of-function analysis. The results showed that the second tetR gene was 660-bp, encoding a 26 kD protein, which could regulate the expression of 3,17β-HSD gene via binding to the conserved consensus sequences located 1100-bp upstream of the 3,17β-HSD gene. Furthermore, the mutant strain of C. testosteroni with the second tetR gene knocked-out mutant expresses good biological genetic stability, and the expression of 3,17β-HSD in the mutant strain is slightly higher than that in the wild type under testosterone induction.

Conclusions The second tetR gene acts as a negative regulator in 3,17β-HSD expression, and the mutant has potential application in bioremediation of steroids contaminated environment.

Keywords Comamonas testosteroni · Tetracycline repressor (TetR) · Steroid hormones · Electrophoretic mobility shift assay · Gold nanoparticles (AuNPs)-based assay

Introduction

Comamonas testosteroni is a gram-negative bacterium with various steroid compounds or polycyclic aromatic hydrocarbons as its carbon source [1–4]. Studies have shown that dozens of enzymes in C. testosteroni are involved in steroid catabolic or metabolic pathway [5–9]. Among the enzymes, 3,17β- hydroxysteroid dehydrogenase (3,17β-HSD) and other hydroxyl dehydrogenases are the key enzymes to decompose steroids [5, 6]. 3,17β-HSD is a member of short-chain dehydrogenase/reductase (SDR) superfamily, which can catalyze the oxidation of hydroxyl groups from some steroids into oxygen (or keto) groups [5, 6]. The expression of 3,17β-HSD is mainly regulated by activators and suppressors [5, 6]. Activator can promote protein expression, while suppressor, on the contrary, can bind to activators or operators to block protein expression [5, 6].

The tetracycline repressor (TetR) family contains a kind of transcriptional repressors [10, 11]. TetR repressors show a characteristic helix-turn-helix (HTH) structural motif for binding to DNA operators inhibiting specific gene expression [10, 12, 13]. The regulatory network involved by the TetR family members can be simple or complex [10]. For example, TetR inhibits membrane-associated protein (TetA) transcription by binding to the target
operator upstream of the tetA gene, while the expression of the TetR family member is regulated by another regulator through a series of regulatory cascades [10]. Moreover, the TetR family member also can trigger a cell response to react to environmental signals [10].

Sequence analysis shows there are three tetR genes located in the contig58 of C. testosteroni ATCC 11,996 (GenBank: AHIL01000049.1), 79,551–80,072, 135,580–136,239 (complement), and 181,113–181,673 (complement) [14]. Previously report showed that the tetR genes located on 79,551–80,072 (locus tag: CTA TCC11996_22802) of C. testosteroni ATCC 11,996 contig58 encoded a potential repressor, which can repress the expression of 3,17β-HSD protein by sensing environmental signals [1, 2, 14]. However, whether the other proposed tetR genes acts as repressors of 3,17β-HSD is still unknown. In the present study, we cloned the second tetR gene located on 135,580–136,239 (complement) of C. testosteroni ATCC 11,996 contig58, followed by analyzing the regulation mechanism of the protein on 3,17β-HSD using electrophoretic mobility shift assay (EMSA), gold nanoparticles (AuNPs)-based assay, and loss-of-function analysis. Our results suggest that the protein encoded by the second tetR gene acts as another repressor of 3,17β-HSD.

Materials and methods

Reagents

Gold nanoparticles (AuNPs) was kindly provided by Prof Zhenxin Wang (National Analytical Research Center of Electrochemistry and Spectroscopy, Changchun, China). Hind III, EcoR I, Nde I, BamH I, testosterone, and IPTG were purchased from Sangon Biotech (Shanghai, China). DNA marker and protein marker were purchased from Thermo (USA).

Probes

Three fragments (double stranded) were selected, synthesized, and used as probes (Probe 1: 5′-AAGGAG GCGCAAGGCCTCCTTGGCTT-3′; Probe 2: 5′-AGC GGGCCCTGGGCCGCT-3′; Probe 3: 5′-TGCGATGC CCCTGATAAG-3′). Two probes were conserved core consensus sequences of C. testosteroni located 1100-bp upstream of the 3,17β-HSD gene, while the third probe was a non-conserved sequence of C. testosteroni located 1100-bp upstream of the gene, which was used as a negative control.

Construction of C. testosteroni with tetR gene knocked-out

To generate a recombinant C. testosteroni with the second tetR gene located on 135580–136239 (complement) of C. testosteroni ATCC 11996 contig58 knocked-out, a 301-bp fragment from 5′ terminal of the tetR gene with a G insertion after start codon ATG were amplified (Forward primer: 5′-CGCAAGGCTTATGGCTGATCTCATGCAA ATAAAT-3′, reverse primer: 5′-TTATGAATTCGCTGCGGCAGGCTGCGATGCA T-3′) and subcloned into pCR2.1-TOPO plasmid (kindly provided by Prof. Xiong) with Hind III and EcoR I (Restriction enzymes were underlined. Bold letters indicate mutated sites) and transformed into Escherichia coli DH5α [2], resulting a recombinant plasmid pCR2.1-TetR. The plasmid was identified by PCR with primer described above and sequencing [2]. Then, the plasmid pCR2.1-TetR was electrically transformed into C. testosteroni, followed by selection using kanamycin (100 µg/mL) and ampicillin (100 µg/mL). The positive clone was identified by PCR with primers F1 (5′-TTGATA AA AATA AAAAAGCGGAT-3′, come from upstream of tetR gene ) and R1 (5′-CAGTGAATGTAA TACGATC TACTAAT-3′, come from kanamycin gene of pCR2.1-TOPO ), and further confirmed by sequencing (data not shown).

To evaluate the growth characteristics of the recombinant C. testosteroni, the recombinants and wild type strain were cultured for 12 h in LB media containing kanamycin (100 µg/mL) and ampicillin (100 µg/mL), and the growth curves were generated based on the absorbance of cultures on OD595.

ELISA of 3,17β-HSD

The expression level of 3,17β-HSD in the strains was examined to evaluate genetic stability according to the ELISA protocol described by Xiong et al. [5]. The standard curve of 3,17β-HSD was generated by examining the absorbance of the purified 3,17β-HSD protein (kindly provided by Prof. Xiong) was diluted to 1.95, 3.9, 7.813, 15.625, 31.5, 62.5, and 250 ng/mL. The mutant and wild type strains were cultured in LB containing kanamycin (100 µg/mL) and ampicillin (100 µg/mL), and the growth curves were generated based on the absorbance of cultures on OD595.

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Cloning and expression of tetR gene

Primers were designed and synthesized according to the second tetR gene located on 135,580–136,239 (complement) of C. testosteroni ATCC 11,996 contig58 (GenBank: AHIL01000049.1). Forward primer was 5’-CAACATATG CTGCACTCTCATGCAA-3’ (BamH I was underlined), and the reverse primer was 5’-ATCGGATCCTCAACGCTC TCAATGAATAAG-3’ (Nde I was underlined). The tetR gene (660 bp) was amplified with these primers and cloned into pET-15b with Nde I and Bam HI, followed by identification via double digestion with NdeI and BamHI, and sequencing. The resulting plasmid was designated as pET-15b-TetR.

To obtain the TetR protein, the recombinant plasmid pET-15b-TetR was transformed into E. coli BL21(DE3). The positive colony was cultured in LB to OD600 of 0.6, followed by induction with 0.5 mmol/L IPTG at 37 °C for 4 h, respectively. Thereafter, cells were centrifuged at 4 °C and 8000×g for 15 min. Centrifuged cells were resuspended in 10 times lysis buffer, disrupted by sonication (20 kHz, 30 × 5 s), and centrifuged at 4 °C and 8000×g for 15 min. Then, the supernatants were collected and examined with SDS-PAGE or affinity chromatography (Fig. 1b). The results suggested that the TetR protein was cloned and expressed successfully in E. coli BL21(DE3).

Electrophoretic mobility shift assay (EMSA)

Electrophoretic mobility shift assay (EMSA) was performed to examine the binding of the TetR to the 3,17β-HSD DNA according to the protocol described by Hellman and Fried [16]. Briefly, three probes (45 μmol/L) were incubated with TetR in different proportions (molar concentration, 1:0, 1:1, 1:2, and 1:3) for 20 min, followed by electrophoretic analysis on 12% native polyacrylamide gel. The bands were stained with Ethidium bromide (EB) and examined under Ultraviolet (UV)-light.

Gold nanoparticles (AuNPs)-based assay

Gold nanoparticles (AuNPs)-based assay was performed to further confirm interaction between TetR and probes according to the protocol described previously [17]. Briefly, DNA probes 2 (45 μM) and TetR protein (0, 200, 400, 600, and 800 nM) was respectively mixed with AuNPs for 20 min in buffer solution (pH7.4, 10 mM Tris-HCl, 80 mM KCl). Second, DNA probes 2 (0, 50, 300, 500, 700, 800, 1000 nM) was mixed with TetR protein (500 nM) for 20 min in buffer solution (pH7.4, 10 mM Tris-HCl, 80 mM KCl). Third, TetR protein (500 nM) 1:1 mol concentration proportions mixed with three DNA probes (1:2:3) for 20 min in buffer solution (pH7.4, 10 mM Tris-HCl, 80 mM KCl). Then, 25 μL mixture was incubated with 75 μL AuNPs (13 nm in diameter), followed by examined the UV-visible absorption spectra of the AuNPs samples from 400 to 900 nm. Zeta potential of the surface charge of AuNPs under different sample (water, TetR protein, TetR-DNA mixture) treatments was measured using a ZETA PLUS zeta potential analyzer (Brookhaven Instruments, USA).

Moreover, 125nM TetR was 1:8 mol concentration ratio mixed with testosterone (1 mM) for 40 min, followed by incubation with 125nM probe 2 for 20 min. centrifuged at 14,000 rpm for 10 min. Then, the mixture was added in 0.5 nM AuNPs binding buffer (10 mM Tris-HCl, 80 mM KCl) and analyzed for UV-visible spectra.

Statistical analysis

Statistical analysis was conducted using GraphPad software 5.0 (SanDiego, USA) with a one-way (ANOVA). P value < 0.05 is considered as significant difference. For each group of independent analysis, at least 3 independent experiments were evaluated. The results are expressed as mean ± standard deviation (SD) of three replicates.

Results

Cloning and expression of C. testosteroni tetR gene

The tetR gene located on 135,580–136,239 (complement) of C. testosteroni ATCC 11,996 contig58 was amplified and subcloned into expression plasmid pET-15b, resulting a recombinant plasmid pET-15b-TetR. The recombinant plasmid pET-15b-TetR can be digested into two fragments, including a 660-bp tetR gene and a 5708-bp vector (Fig. 1a). The recombinant plasmid pET-15b-TetR was further confirmed by sequencing.

To obtain TetR protein, the recombinant plasmid pET-15b-TetR was transformed in to E. coli BL21(DE3), followed by induction with IPTG. The optimized expression condition is 0.5 mmol/L IPTG, at 37 °C for 4 h (Supplemental Fig. 1). Thereafter, the protein was purified using affinity chromatography (Fig. 1b). The results suggested that the TetR protein was cloned and expressed successfully in E. coli BL21(DE3).

Construction of recombinant C. testosteroni with tetR gene mutated

The C. testosteroni with the second tetR gene mutated was generated via homologous recombination by electrically transforming the recombinant plasmid pCR2.1-TetR into C. testosteroni. As shown in Fig. 2a, after homologous integration, an additional ‘G’ after start codon ATG was inserted
in the \textit{tetR} gene of the knock-out mutant, which caused the frameshift of the gene. The positive clone was identified by PCR (Fig. 2b) and further confirmed by sequencing (data not shown). Moreover, the strains in lanes 2, 4 and 5 can not continue to grow after multiple subcultures, which should be false positive. Only strain in lane 1, 3 can be subcultured for multiple times and still be alive as a gene knockout strain. Therefore, the strain in lane 1 was used in the following studies.

Subsequently, the growth characteristics of the recombinant \textit{C. testosteroni} were evaluated (Fig. 2c). As shown in Fig. 2c, no significant difference was observed in the recombinant \textit{C. testosteroni} compared with that of the wild-type \textit{C. testosteroni}, suggesting the deletion of \textit{tetR} gene has little effect on the normal growth of the bacteria.

\textbf{TetR protein decreases the levels of 3,17β-HSD in \textit{C. testosteroni}}

To examine the effect of TetR protein on the expression of 3,17β-HSD protein, the levels of 3,17β-HSD expressed in the mutant \textit{C. testosteroni} and the wild type strains were evaluated. As shown in Fig. 3a, the expression of 3,17β-HSD in wild-type and mutant strains without 1 mM testosterone induction was less different, but the expression of 3,17β-HSD protein in mutant and wild-type strains under the testosterone induction is higher than that of the groups without the induction, and the expression of 3,17β-HSD protein in mutant strains is slightly higher than that of the wild strains. Therefore, it is speculated that TetR can reduce the expression of 3,17β-HSD in \textit{C. testosteroni}, but cannot completely inhibit the expression, suggesting the TetR is an repressor of 3,17β-HSD. Meanwhile, testosterone can induce 3,17β-HSD of both wild-type and mutant strains. Moreover, the mutant \textit{C. testosteroni} were cultured continuously for five generations, and the expression of 3,17β-HSD in each two generations was detected (Fig. 3b). The expression of 3,17β-HSD in the mutant was stable during the continuous culture of five generations, which was slightly higher than that in wild-type cells. Taken together, the mutant strains present good genetic stability, and have potential in theoretical research and practical application.

\textbf{TetR protein interacts with DNA upstream of 3,17β-HSD gene}

As reported, proteins of the TetR family have been found in 115 genera of proteobacteria, cyanobacteria, and archaea, composing a complex regulatory network for gene expression [10]. Some TetRs can bind to target operators to inhibit transcription, while others may involve a series of regulatory cascades, in which the expression of TetR family members is regulated by another regulator, or TetR family members trigger cell response to environmental damage [10].

To further evaluate the effect of TetR on 3,17β-HSD expression, electrophoretic mobility shift assay (EMSA) was conducted to examine the binding of the TetR to the 3,17β-HSD DNA. Three fragments were selected, synthesized, and used as double stranded probes, respectively. Two probes were conserved core consensus sequences located 1100-bp upstream of the 3,17β-HSD gene, while the third probe was a non-conserved sequence located 1100-bp upstream of the gene, which was used as a negative control. Thereafter, probes (45 µmol/L) were incubated with the purified TetR
Fig. 2 Construction of recombinant C. testosteroni. a Schematic diagram of construction of tetR gene knocked-out C. testosteroni. Left arm, left homologous sequence; right arm, right homologous sequence. b Identification of recombinant C. testosteroni by PCR. Lane 1 and 3, positive clones; 2, 4, and 5, negative clones; M, DL2000 marker. c Growth curve of the mutant and wild type C. testosteroni

Fig. 3 TetR protein decreases the levels of 3,17β-HSD in C. testosteroni. a Effect of testosterone on the expression of 3,17β-HSD. CT-, the wild type strain without testosterone stimulation; MT-, the mutant strain without testosterone stimulation; CT+, the wild type strain induced by testosterone; MT+, the mutant strain induced by testosterone. b Expression of 3,17β-HSD in different generations of wild-type and mutated C. testosteroni. The results are expressed as mean ± standard deviation (SD) of three replicates. *p < 0.05; **p < 0.01
in different proportions, followed by electrophoretic analysis. As shown in Fig. 4, with the increase of protein concentration, gel block appeared in the electrophoresis band, while the mobility shift of the negative control protein phaC (polyhydroxyalkanoate synthetase) was similar in three gels, suggesting the phaC did not interact with the three probes. The mobility shift of the DNA bands of probe 1 and probe 2 decreased gradually with the increase of TetR protein concentration, indicating that TetR protein could bind to probe 1 (Fig. 4a) and probe 2 (Fig. 4b). Moreover, smeary bands were detected Fig. 4a, b, indicating that the protein may form polymers and bind to the probes with the increase of protein concentration. The negative DNA probe 3 does not bind to either phaC protein or TetR protein (Fig. 4c). Therefore, it can be concluded that the TetR protein can bind to two palindromic sequences upstream of the 3,17β-HSD gene.

**Interaction between the TetR protein and DNA upstream of 3,17β-HSD gene was further confirmed by AuNPs-based assay**

It is known that AuNPs is stable in water and has a clear surface plasmon peak at 520 nm. When AuNPs was exposed to buffer solution containing 80 mM KCl, a large number of nanoparticles gathered, and the absorbance at 520 nm decreased, while the absorption peak appeared at 750 nm [17]. Therefore, gold nanoparticles (AuNPs)-based assay was performed to further confirm the above results. Firstly, we determined a suitable TetR protein concentration for DNA binding (Fig. 5a). As shown in Fig. 5a, the higher the TetR protein concentration, the more stable the particles were. Thus, 500 nM TetR was selected to detect the formation of protein-DNA complex. In addition, when the concentration of TetR protein is 500 nM, the ability of TetR-DNA complex to protect AuNPs is also enhanced with the increase of the concentration of DNA probe 2 (Fig. 5b). To examine the TetR-DNA complex formation, the TetR protein (500 nM) were incubated with probe 1, 2, and probe 3 at 1:1 mol ratio, respectively, followed by incubating with AuNPs. As shown in Fig. 5c, The AuNPs are more stable in the TetR-probe 1 (a) and TetR-probe 2 (b) groups compared with that of the TetR-negative probe (c) and buffer (d) groups, suggesting that protein-DNA complexes were formed between TetR and probes. The degree of stabilization is probe 2 > probe 1 > negative control DNA probe. Moreover, the insets in the upper right corner of Fig. 5c are the color photos of the respective AuNPs solutions. It can be observed that both a and b groups are pink with little difference, indicating that protein-DNA complex has a good protective effect on AuNPs, while c is blue, suggesting an incomplete protection. Group d is dark blue due to denaturation of AuNPs in buffer solution. Therefore, combining the results of EMSA (Fig. 4) and AuNPs-based assay, it can be concluded that probe 2 has higher apparent affinity than probe 1. Probe 2 was used in the following studies.
indicating that testosterone inhibits interaction between TetR and target DNA.

In order to prove that the potential electrostatic effect produced by protein-DNA complex is the reason for the stability of AuNPs, the Zeta potential of the surface charge of AuNPs under different sample treatments was measured (Fig. 5e). TetR has a weak positive charge, and AuNPs coated with TetR showed a much lower negative charge density than AuNPs coated with citrate ions. When the TetR-DNA complex is formed and coated on the AuNPs, the particles get more negative charges from the double-stranded DNA (dsDNA) in the TetR-DNA complex, which leads to the enhanced stability of the AuNPs nanoparticles.

Taken together, these results demonstrate that the TetR protein of *C. testosteroni* interacts with DNA upstream of the bacterial 3,17β-HSD gene, thus negatively regulating the expression of the 3,17β-HSD gene.

**Discussion**

Transcriptional regulation is an important way of biological regulation, most of which is carried out by the interaction between cis-acting elements and trans-acting factors. Notably, the regulation of gene expression in bacteria is mainly worked at transcription level. Previous reports showed that three *tetR* genes were located in the contig58 of *C. testosteroni* ATCC 11,996 (GenBank: AHIL01000049.1), 79,551–80,072, 181,113–181,673 (complement), and 135,580–136,239 (complement) [14]. In this study, we cloned the second *tetR* gene located on 135,580–136,239 (complement) of *C. testosteroni* ATCC 11,996 contig58. The results demonstrate that the second *tetR* gene was 660-bp, encoding a 26 kD protein, which could be expressed in *E. coli* induced by 0.5 mmol/L IPTG at 27 °C for 4 h from pET-15b-TetR (Fig. 1).

To evaluate the effect of second *tetR* gene, the gene was knocked-out via homologous recombination. Expectedly, the mutated strain has little difference on the growth compared to that of the wild-type *C. testosteroni* (Fig. 2c), and the expression of 3,17β-HSD in wild-type and mutant strains

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**Fig. 5** Results of gold nanoparticle colorimetry. 

a Absorbance of AuNPs (1.5 nM) in buffer solution (10 mM Tris–HCl, 80 mM KCl) containing different concentrations of TetR (0, 200, 400, 600, and 800 nM) and probe 2 (45 µM). 

b Optimization of probe 2 with AuNPs (0.5 nM). 500 nM TetR was mixed with different concentrations of probe 2 (0, 50, 300, 500, 700, 800, and 1000 nM) in buffer solution (10 mM Tris–HCl, 80 mM KCl).

c Absorbance of AuNPs (1.5 nM) in binding buffer (10 mM Tris–HCl, 80 mM KCl) containing TetR-probe 1 (a), TetR-probe 2 (b), TetR-negative probe (c), and buffer (d). 500 nM TetR was mixed with 500 nM probes. AuNPs in water are used as reference. The insets are the color photos of the respective AuNPs solutions.

d Testosterone inhibits interaction between TetR (125 nM) and target DNA (125 nM). TetR was mixed with 8 times of testosterone (a) or without testosterone (b) for 40 min, followed by incubation with probe 2 for 20 min. Then, the mixture was added in AuNPs (0.5 nM) binding buffer (10 mM Tris–HCl, 80 mM KCl), and analyzed for UV-visible spectra assay.

e Zeta potential of different AuNPs samples in water and buffer solution (10 mM Tris–HCl, 80 mM KCl). The experiment were repeated three times.
without testosterone induction is similar (Fig. 3a), suggesting the deletion of second tetR gene has little effect on the normal growth of the bacteria. However, a slight increase of 3,17β-HSD protein was observed in the mutant group compared with that of the wild-type group under the testosterone induction (Fig. 3a, b), suggesting that the second TetR is a repressor of 3,17β-HSD, and the repressor activity is further regulated by testosterone.

To further confirm this hypothesis, the effect of the second TetR on 3,17β-HSD expression was evaluated using EMSA and AuNPs-based assay. As results showed (Figs. 4, 5), the TetR can specifically interact with two probes (probe 1 and probe 2) derived from the conserved consensus sequence located 1100-bp upstream of the 3,17β-HSD gene, but not with the non-palindromic sequence (probe 3). However, this interaction can be disrupted by testosterone (Fig. 5d). Moreover, due to the large size and concentrated negative charge of protein-DNA complex, protein-DNA complex can be coated on negatively charged AuNPs to provide protection against salt (such as KCl)-induced aggregation through electrostriction protection [17]. When the TetR-DNA complex is formed and coated on the AuNPs, the AuNPs particles get more negative charges from the double-stranded DNA (dsDNA) in the TetR-DNA complex than that of the TetR coated AuNPs, resulting in the enhanced stability of the AuNPs nanoparticles which was similar to that of the AuNPs coated with citric acid (Fig. 5e). These results indicate that the TetR protein encoded by the second tetR gene of C. testosteroni acts as another negative regulator, which interacts with consensus DNA upstream of the bacterial 3,17β-HSD gene, thus negatively regulating the expression of the 3,17β-HSD gene. After induction of testosterone, the inhibitory effect weakened and the level of 3,17β-HSD increased.

Notably, TetR protein family contains a kind of transcriptional repressors. We and other group [1, 2, 14] proved that two of three tetR genes in the contig58 of C. testosteroni ATCC 11,996 (GenBank: AHIL01000049.1) encode repressors regulating the expression of the 3,17β-HSD gene. Moreover, it was reported that LuxR protein also acted as a repressor in the expression of the 3,17β-HSD gene [18, 19]. However, these repressors can only partially inhibit the expression of the 3,17β-HSD gene, indicating that the expression of the 3,17β-HSD gene may be synergistically regulated by several regulatory factors. The detailed regulatory mechanism of the 3,17β-HSD gene remains to be elucidated, and further confirmatory experiments are still in progress.

Conclusions

In conclusion, we confirmed that the second tetR gene in the contig58 of C. testosteroni regulates the expression of 3,17β-HSD gene via binding to the conserved core consensus sequences located 1100-bp upstream of the 3,17β-HSD gene. Furthermore, the mutant strain of C. testosteroni with the second tetR gene knocked-out has good biological genetic stability, and the expression of 3,17β-HSD in the mutant strain is slightly higher than that in the wild type under testosterone induction, suggesting the mutant generated in this study might be used to treat environmental pollution caused by steroid hormones. The further study can help us to explore the application of the tetR gene mutant.

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Author contribution HX and XG conceived and designed research. XW and XQ conducted experiments. CL contributed new reagents or analytical tools. YY and GY analyzed data. HX and XW wrote the manuscript. All authors read and approved the manuscript.

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Data availability All datasets for this study are included in the manuscript files.

Declarations

Conflict of interest The authors declare no competing interests.

Consent to publish The Author state herein that this manuscript has not been published elsewhere and that it has not been submitted simultaneously for publication elsewhere.

References

1. Pan T, Huang P, Xiong G, Maser E (2015) Isolation and identification of a repressor TetR for 3,17beta-HSD expressional regulation in Comamonas testosteroni. Chem Biol Interact 234:205–212
2. Wu Y, Huang P, Xiong G, Maser E (2015) Identification and isolation of a regulator protein for 3,17beta-HSD expressional regulation in Comamonas testosteroni. Chem Biol Interact 234:197–204
3. Cuthbertson L, Hodwell JR (2013) The TetR family of regulators. Microbiol Mol Biol Rev 77(3):440–475
4. Ahn SK, Cuthbertson L, Hodwell JR (2012) Genome context as a predictive tool for identifying regulatory targets of the TetR family transcriptional regulators. PLoS ONE 7(11):e50562
5. Xiong G, Maser E (2015) Construction of a biosensor mutant of Comamonas testosteroni for testosterone determination by cloning the EGFP gene downstream to the regulatory region of the 3,17beta-HSD gene. Chem Biol Interact 234:188–196
6. Fujii K, Kikuchi S, Satomi M, Ushio-Sata N, Morita N (2002) Degradation of 17beta-estradiol by a gram-negative bacterium isolated from activated sludge in a sewage treatment plant in Tokyo, Japan. Appl Environ Microbiol 68(4):2057–2060
7. Horinouchi M, Koshino H, Malon M, Hirota H, Hayashi T (2018) Steroid degradation in Comamonas testosteroni em openTA441: identification of metabolites and the genes involved in the reactions necessary before D-ring cleavage. Appl Environ Microbiol 84(22):e01324
8. Liu C, Liu K, Zhao C, Gong P, Yu Y (2020) The characterization of a short chain dehydrogenase/reductase (SDRxD) in Comamonas testosteroni. Toxicol Rep 7:460–467
9. Horinouchi M, Koshino H, Malon M, Hirota H, Hayashi T (2019) Identification of 9-oxo-1,2,3,4,5,6,10,19-octanor-13,17-secoandro st-8(14)-ene-7,17-dioic acid as a metabolite of steroid degradation in Comamonas testosteroni TA441 and the genes involved in the conversion. J Steroid Biochem Mol Biol 185:268–276
10. Ramos JL, Martinez-Bueno M, Molina-Henares AJ, Teran W, Watanabe K, Zhang X, Gallegos MT, Brenner R, Toles R (2005) The TetR family of transcriptional repressors. Microbiol Mol Biol Rev 69(2):326–356
11. Berens C, Hillen W (2003) Gene regulation by tetracyclines. Constraints of resistance regulation in bacteria shape TetR for application in eukaryotes. Eur J Biochem 270(15):3109–3121
12. Kisker C, Hinrichs W, Tovar K, Hillen W, Saenger W (1995) The complex formed between Tet repressor and tetracycline-Mg\(^{2+}\) reveals mechanism of antibiotic resistance. J Mol Biol 247(2):260–280
13. Hinrichs W, Kisker C, Duvel M, Muller A, Tovar K, Hillen W, Saenger W (1994) Structure of the Tet repressor-tetracycline complex and regulation of antibiotic resistance. Science 264(5157):418–420
14. Gong W, Kisiela M, Schilhabel MB, Xiong G, Maser E (2012) Genome sequence of Comamonas testosteroni ATCC 11996, a representative strain involved in steroid degradation. J Bacteriol 194(6):1633–1634
15. Peng Z, Ma T, Pang D, Su D, Chen F, Chen X, Guo N, Ouyang T, Ouyang H, Ren L (2016) Expression, purification and antibody preparation of PCV2 Rep and ORF3 proteins. Int J Biol Macromol 86:277–281
16. Hellman LM, Fried MG (2007) Electrophoretic mobility shift assay (EMSA) for detecting protein-nucleic acid interactions. Nat Protoc 2(8):1849–1861
17. Aung KM, New SY, Hong S, Sutarlie L, Lim MG, Tan SK, Cheung E, Su X (2014) Studying forkhead box protein A1-DNA interaction and ligand inhibition using gold nanoparticles, electrophoretic mobility shift assay, and fluorescence anisotropy. Anal Biochem 448:95–104
18. Ji Y, Yang J, Gao L, Xiong G, Yu Y, Zhang Y (2020) Characterization of a LuxR repressor for 3,17beta-HSD in Comamonas testosteroni. Chem Biol Interact 336:109271
19. Ji Y, Pan T, Zhang Y, Xiong G, Yu Y (2017) Functional analysis of a novel repressor LuxR in Comamonas testosteroni. Chem Biol Interact 276:113–120

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