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

Weiqi Xie
Changchun University Science and Technology
Qin Xia
Changchun University of Science and Technology
Ling Chen
Changchun University of Science and Technology
Guangming Xiong
Medical School Schleswig-Holstein
Yuwei Gao
Academy of Military Medical Sciences
Yuanhua Yu
Changchun University of Science and Technology
Xiuxia He (hexx@cust.edu.cn)
Changchun University of Science and Technology

Research Article

Keywords: Comamonas testosteroni, tetracycline repressor (TetR), steroid hormones, electrophoretic mobility shift assay, gold nanoparticles (AuNPs)-based assay

Posted Date: April 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-277512/v2

Abstract

3,17β-hydroxysteroid dehydrogenase (3,17β-HSD) is a key enzyme that can degrade steroid compounds 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 were 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 acts as repressors of 3,17β-HSD is still unknown. In the present study, we cloned the second *TetR* gene and analyzed 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. 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 knockout 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 can efficiently degrade steroids as carbon sources. The mutant generated in this study can be used to treat environmental pollution caused by steroid hormones.

Introduction

Comamonas testosteroni is a gram-negative bacterium with various steroid compounds or polycyclic aromatic hydrocarbons as its carbon source (12, 15). Studies have shown that dozens of enzymes in *C. testosteroni* are involved in steroid metabolism pathway (16, 3, 6, 11, 7). Among the enzymes, 3,17β-hydroxysteroid dehydrogenase (3,17β-HSD) and other hydroxyl dehydrogenases are the key enzymes to decompose steroids (16, 3). 3,17β-HSD is a member of short-chain dehydrogenase/reductase (SDR) superfamily, which can catalyze the conversion of hydroxyl groups of steroids into oxygen groups (16, 3). The expression of 3,17β-HSD is mainly regulated by activators and suppressors (16, 3). Activator can promote protein expression, while suppressor, on the contrary, can bind to activators or operators to block protein expression (16, 3).

The tetracycline repressor (TetR) family contains a kind of transcriptional repressors (14, 2). TetR works as a homodimer and binds to two α-helix-β-turn-α-helix motifs (HTH) of two conserved DNA operators, thus blocking the expression of the target genes (10, 5, 14). The regulatory network involved by the TetR family members can be simple or complex (14). 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 (14). Moreover, the TetR family member also can trigger a cell response to react to environmental signals (14).

Sequence analysis shows that there are three *TetR* genes located in the contig58 of *C. testosteroni* ATCC 11996 (GenBank: AHIL01000049.1), 79551–80072, 135580–136239 (complement), and 181113–

181673 (complement) (4). Previously report showed that the *TetR* genes located on 79551–80072 of *C. testosteroni* ATCC 11996 contig58 encoded a potential repressor, which can repress the expression of 3,17β-HSD protein by sensing environmental signals (12, 15, 4). 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 135580–136239 (complement) of *C. testosteroni* ATCC 11996 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).

*Hin*d III, *Eco*R I, *Nde* I, *Bam*H 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 (Table 1). 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.

Table 1 Probes used in this study

Probe	Sequence
Probe 1	5'-AAGGAGGCGCAAGCGCCTCCTTGCTT-3'
Probe 2	5'-AGCGGGCCATTGGCCCGCT-3'
Probe 3	5'-TCGCGATGCCCCTGATAGAC-3'

Construction of TetR gene-deleted C. testosteroni

To generate a recombinant *C. testosteroni* with the second *TetR* gene located on 135580-136239 (complement) of *C. testosteroni* ATCC 11996 contig58 deleted, a 301-bp fragment from 5’ terminal of the *TetR* gene with a G insertion after start codon ATG were amplified (Forward primer: 5’-CGCAAGCTT**ATGG**CTGCATCTCATGCAAATAAGT-3’, reverse primer: 5’-TATGAATTCTGGCTGGCGGCAGCCTGTGCCAT-3’) and subcloned into pK18 plasmid (kindly provided by Prof. Xiong) with *Hin*d III and *Eco*R I (Restriction enzymes were underlined. Bold letters indicate mutated

sites.) (15), resulting a recombinant plasmid pK18-TetR. The plasmid was identified by PCR with primer described above and sequencing (15). Then, the plasmid pK18-TetR was electrically transformed into *C. testosteroni*, followed by selection using kanamycin and ampicillin (100 μg/mL). The positive clone was identified by PCR with primers F1 and R1, 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, and the growth curves were generated based on the absorbance of cultures on OD595.

The level of 3,17β-HSD (kindly provided by Prof. Xiong) was examined according to the protocol described by Xiong *et al.* (16). Briefly, the standard curve of 3,17β-HSD was generated by examining the absorbance of 3,17β-HSD at 1.95, 3.9, 7.813, 15.625, 31.5, 62.5, and 250 ng/mL. Then, the mutant and wild type strains were cultured in LB containing Amp (100 μg/mL), Kan (100 μg/mL), and 1 mmol/L testosterone for 15 h. The level of 3,17β-HSD was examined using ELISA based on the standard curve mentioned above.

Cloning and expression of TetR gene

Primers were designed and synthesized according to the second *TetR* gene located on 135580-136239 (complement) of *C. testosteroni* ATCC 11996 contig58 (GenBank: AHIL01000049.1). Forward primer was 5'-CAACATATGCTGCATCTCATGCAA-3' (*Bam*H I was underlined), and the reverse primer was 5'-ATCGGATCCTCAACGCTCTCCAATGAATAAG-3' (*Nde* I was underlined). The *TetR* gene (660 bp) was amplified with primers described above and cloned into pET-15b with *Nde* I and *Bam* HI, followed by identification via double digestion with *Nde* I and *Bam* HI, 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*. The positive colony was cultured in LB to OD_{600} of 0.6, followed by induction with 0.25 mmol/L, 0.5 mmol/L or 1 mmol/L IPTG at 16 °C, 27 °C or, 37 °C for 4 h, respectively. To optimize the inductin time, the recombinant strain was induced with 0.5 mmol/L IPTG, at 27 ℃ for 2, 4, or 6 h. 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, and centrifuged at 4 °C and 8000×g for 15 min. Then, the supernatants were collected and examined wiht PAGE or purified using HisPur™ Ni-NTA Resin (Thermoscientific, USA) according to protocol described previously (13).

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. Briefly, three probes (45 μmol/L) were incubated with TetR in different proportions (1:0, 1:1, 1:2, and 1:3) for 20 min, followed by electrophoretic analysis on 12 % denatured 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 (1). Briefly, TetR protein (0, 200, 400, 600, and 800 nM) was 1:1 mixed with DNA probes (45 μM) 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 dimeter), followed by examined the UV-visible absorption spectra of the AuNPs samples from 400 to 900 nm. Furthermore, TetR-DNA mixture or TetR protein was incubated with AuNPs in buffer solution (pH7.4, 10 mM Tris-HCl, 80 mM KCl). Zeta potential of the surface charge of AuNPs under different sample treatments was measured using a ZETA PLUS zeta potential analyzer (Brookhaven Instruments, USA).

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

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).

Results

Cloning and expression of C. testosteroni TetR gene

The *TetR* gene located on 135580-136239 (complement) of *C. testosteroni* ATCC 11996 contig58 was amplified and subcloned into expression plasmid pET-15b, resulting a recombinant plasmid pET-15b-TetR. As shown in Fig. 1A, the recombinant plasmid pET-15b-TetR can be digested into two fragments, including a 660-bp *TetR* gene and a 5708-bp vector. 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*, followed by induction with IPTG. The Fig. 1B and C showed that the TetR protein can be expressed in the recombinant plasmid-transformed *E. coli*. The optimized expression condition is 0.5 mmol/L IPTG, at 27 °C for 4 h. Thereafter, the protein was purified using affinity chromatography (Fig. 1D). These results suggested that the TetR protein was cloned and expressed sucessfully in *E. coli*.

Construction of recombinant C. testosteroni with TetR gene mutated

As shown in Fig. 2A, the recombinant *C. testosteroni* with the second *TetR* gene mutated was generated via homologous recombination. Briefly, a 301-bp fragment from 5’ terminal of *TetR* gene with a G insertion after start codon ATG were amplified and subcloned into pK18 plasmid (kindly provided by Prof.

Xiong) (15), resulting a recombinant plasmid pK18-TetR. The plasmid was identified by PCR (Fig. 2B) and sequencing (data not shown). Then, the plasmid pK18-TetR was electrically transformed into *C. testosteroni*, followed by selection using kanamycin and ampicillin (100 μg/mL). The positive clone was identified by PCR (Fig. 2C) and further confirmed by sequencing (data not shown).

Subsequently, the growth characteristics of the recombinant *C. testosteroni* was evaluated (Fig. 2D). As shown in Fig. 2D, no significant difference was observed in the recombinant *C. testosteroni* compared with that of the wild-type *C. testosteroni*, suggesting the deletion of *TetR* gene has little effect on the normal growth of the bacteria.

TetR protein decreases the levels of 3,17β-HSD in 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 *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 testosterone induction is not much 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 *C. testosteroni*, but cannot completely inhibit it, suggesting the TetR is an inhibitor of 3,17β-HSD. Meanwhile, testosterone can induce 3,17β-HSD of both wild-type and mutant strains.

Moreover, the mutant *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 have good genetic stability, and have potential application in theoretical research and practical application.

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 (14). 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 (14).

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 in different proportions, followed by

electrophoretic analysis. As shown in Fig. 4, the mobility shift of the negative control protein phaC was similar in three gels, suggesting the phaC did not interact with the three probes. However, 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 and 4B, 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 (1). 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. To examine the TetR-DNA complex formation, the TetR protein (500 nM) were incubated with probe 1, 2, and probe 3 at 1:1 ratio, respectively, followed by incubating with AuNPs. As shown in Fig. 5B, 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. 5 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. 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. 5C). 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.

To further confirm these results, TetR protein was mixed with 8 times of testosterone, followed incubation with probe 2. Then, the mixture was added in AuNPs binding buffer, and analyzed for UV-visible spectra assay. As shown in Fig. 5D, TetR-DNA complex can efficiently bind to AuNPs in the group without testosterone (b, blue line), while the protective effect of TetR-DNA complex on AuNPs was released after testosterone was added (a, pink line), indicating that testosterone inhibits interaction between TetR and target DNA.

In order to prove that the potential electrosteric 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 11996 (GenBank: AHIL01000049.1), 79551–80072, 181113–181673 (complement), and 135580–136239 (complement) (4). In this study, we cloned the second *TetR* gene located on 135580–136239 (complement) of *C. testosteroni* ATCC 11996 contig58. The results demonstrate that the second *TetR* gene was 660-bp, encoding a 26 kD protein, which could be expressed in *E. coli* inducted by 0.5 mmol/L IPTG at 27 ℃ for 4 h (Fig. 1).

To evaluate the effect of second *TetR* gene, the gene were deleted via homologous recombination. Expectedly, the mutated strain has little difference on the growth compared to that of the wild-type *C. testosteroni* (Fig. 2D), and the expression of 3,17β-HSD in wild-type and mutant strains 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, significant 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 and 3B), suggesting that the second TetR is an inhibitor 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 and 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 (1). 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 testosterone was added, 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 (12, 15, 4) proved that two of three *TetR* genes in the contig58 of *C. testosteroni* ATCC 11996 (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 (9, 8). 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.

Conclusion

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 knockout 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 can degrade steroids as carbon sources. The mutant generated in this study can be used to treat environmental pollution caused by steroid hormones.

Declarations

Ethics Approval: Not applicable.

Consent to Participate: Not applicable.

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.

Authors Contributions: 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.

Funding: None.

Competing Interests: The authors declare no competing interests.

Availability of data and materials: All datasets for this study are included in the manuscript files.

References

1. 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
2. Berens C, Hillen W (2003) Gene regulation by tetracyclines. Constraints of resistance regulation in bacteria shape TetR for application in eukaryotes. Eur J Biochem 270:3109–3121
3. 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 68:2057–2060
4. 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 194:1633–1634
5. 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 264:418–420
6. Horinouchi M, Koshino H, Malon M, Hirota H, Hayashi T (2018) Steroid Degradation in Comamonas testosteroni TA441: Identification of Metabolites and the Genes Involved in the Reactions Necessary before D-Ring Cleavage. Appl Environ Microbiol **84**.(22):e01324–e01318
7. 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-secoandrost-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
8. 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
9. 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 ATCC11996. *Chemico-Biological Interactions*, 109271
10. 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 247:260–280
11. Liu C, Liu K, Zhao C, Gong P, Yu Y (2020) The characterization of a short chain dehydrogenase/reductase (SDRx) in Comamonas testosteroni. Toxicology Reports 7:460–467
12. 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
13. 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. I*nternational Journal of Biological Macromolecules* 86:277–281
14. Ramos JL, Martinez-Bueno M, Molina-Henares AJ, Teran W, Watanabe K, Zhang X, Gallegos MT, Brennan R, Tobes R (2005) The TetR family of transcriptional repressors. Microbiol Mol Biol Rev 69:326–356

15. 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
16. 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

Figures

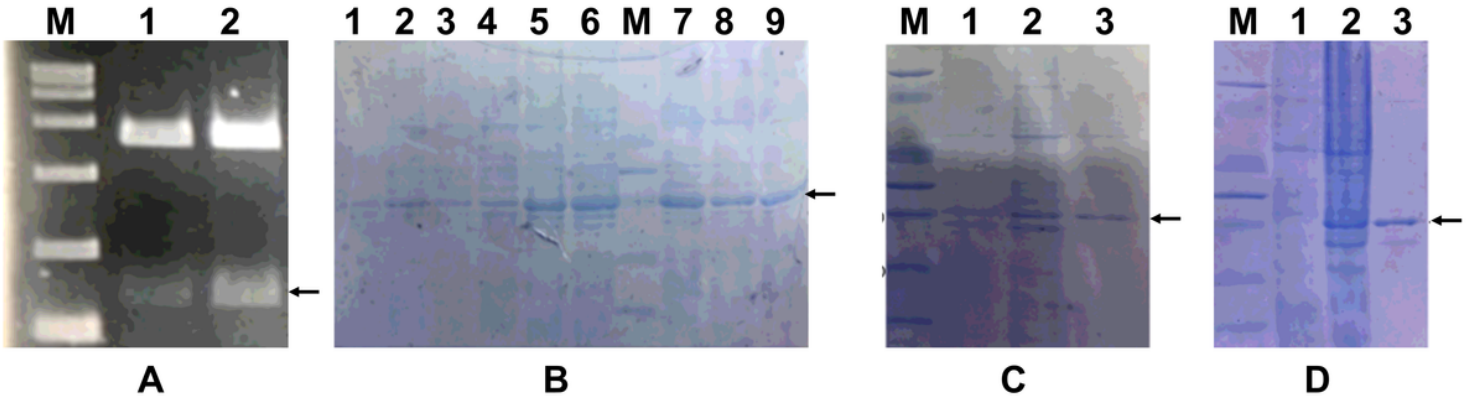

Figure 1

Cloning and expression of TetR gene. (A) Identification of recombinant plasmid pET-15b-TetR. M, DL15000 Marker (from top to bottom: 7500, 5000, 2500, 1000, and 250 bp)；1-2：pET-15b-TetR double digested with Nde I and BamH I. Arrow indicates 600 bp. (B) Optimization of expression conditions. M, protein Maker；1-3: 16 °C；4-6: 27 °C；7-9: 37 °C；1, 4, 7: 0.2 mmol/L IPTG；2, 5, 8: 0.5 mmol/L IPTG；3, 6, 9: 1 mmol/L IPTG. (C) Induction time. M: Maker; 1: 2 h; 2: 4 h; 3: 6 h. (D) Purification of TetR protein. M: protein Maker; 1: wild-type bacteria; 2: induced without purified TetR; 3: purified TetR. Arrow indicates target protein TetR.

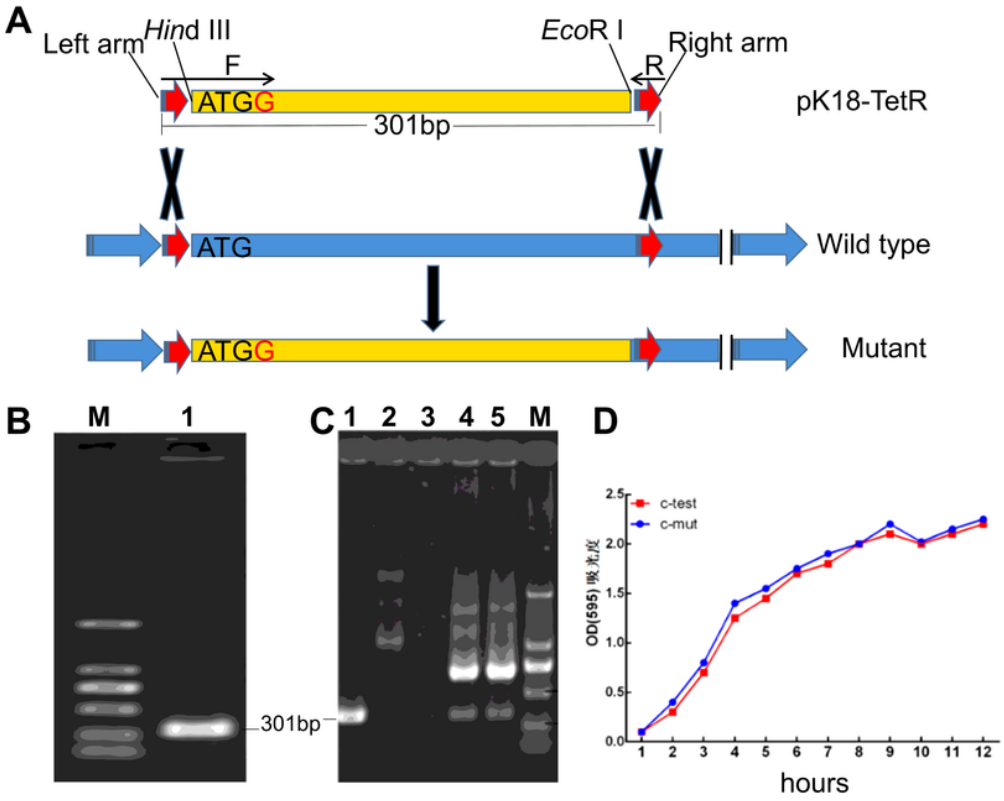

Figure 2

Construction of recombinant C. testosteroni. (A) Schematic diagram of construction of TetR gene-deleted C. testosteroni. (B) Identification of pK18-TetR by PCR. M, DL2000 Marker (From top to bottom: 2000, 1000, 750, 500, 250, 100 bp); 1, TetR gene. (C) Identification of recombinant C. testosteroni by PCR. Lane 1 and 3, positive clones; 2, 4, and 5, negative clones; M, DL2000 marker. (D) Growth curve of the mutant and wild type C. testosteroni.

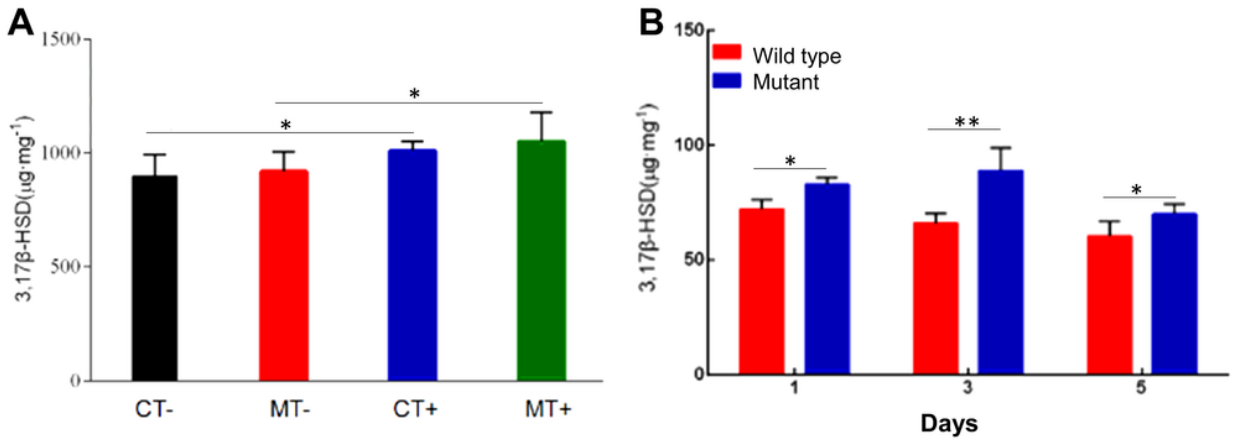

Figure 3

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; MT-the mutant strain; 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.

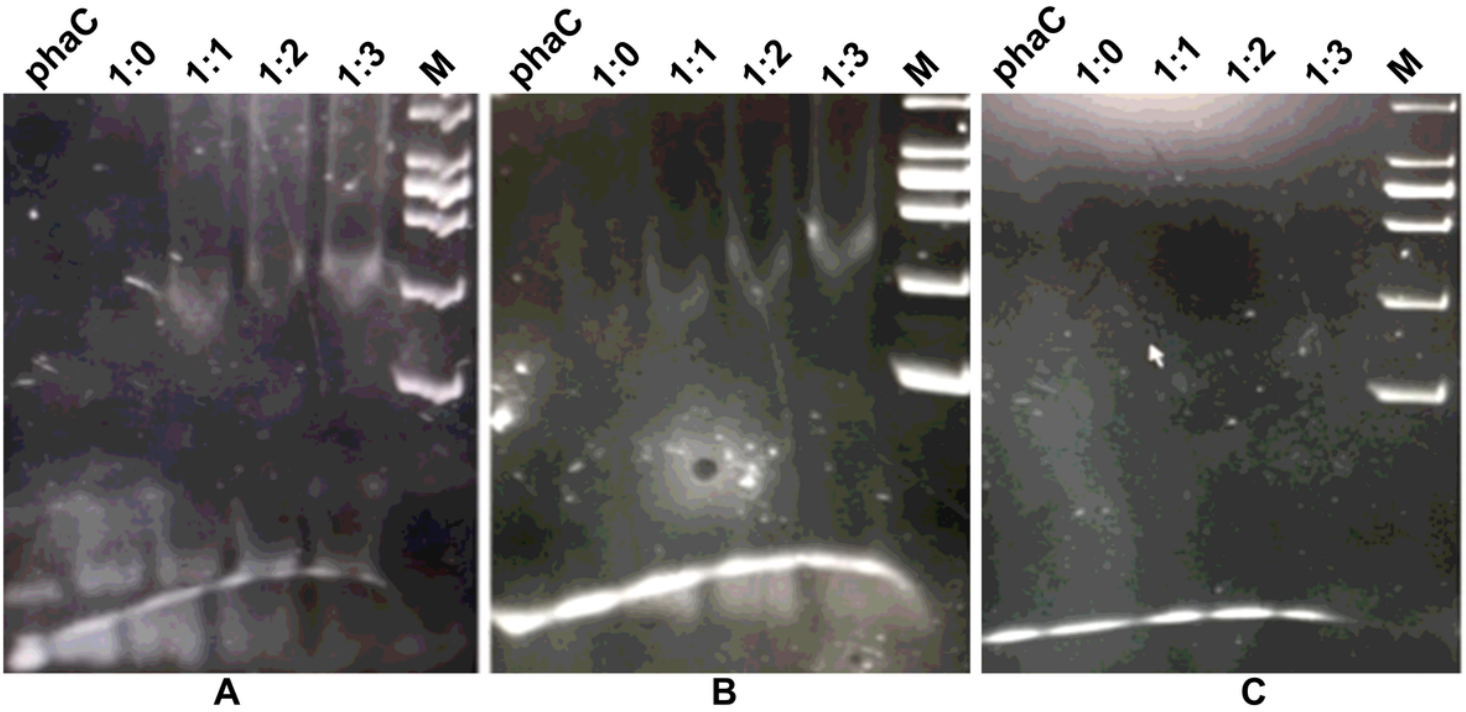

Figure 4

EMSA assay. TetR was incubated with probe 1, 2, and 3 in different proportions, respectively, followed by electrophoretic analysis. Protein phaC was used as a negative control. M, DNA Maker. (A) Probe 1. (B) Probe 2. (C) Probe 3.

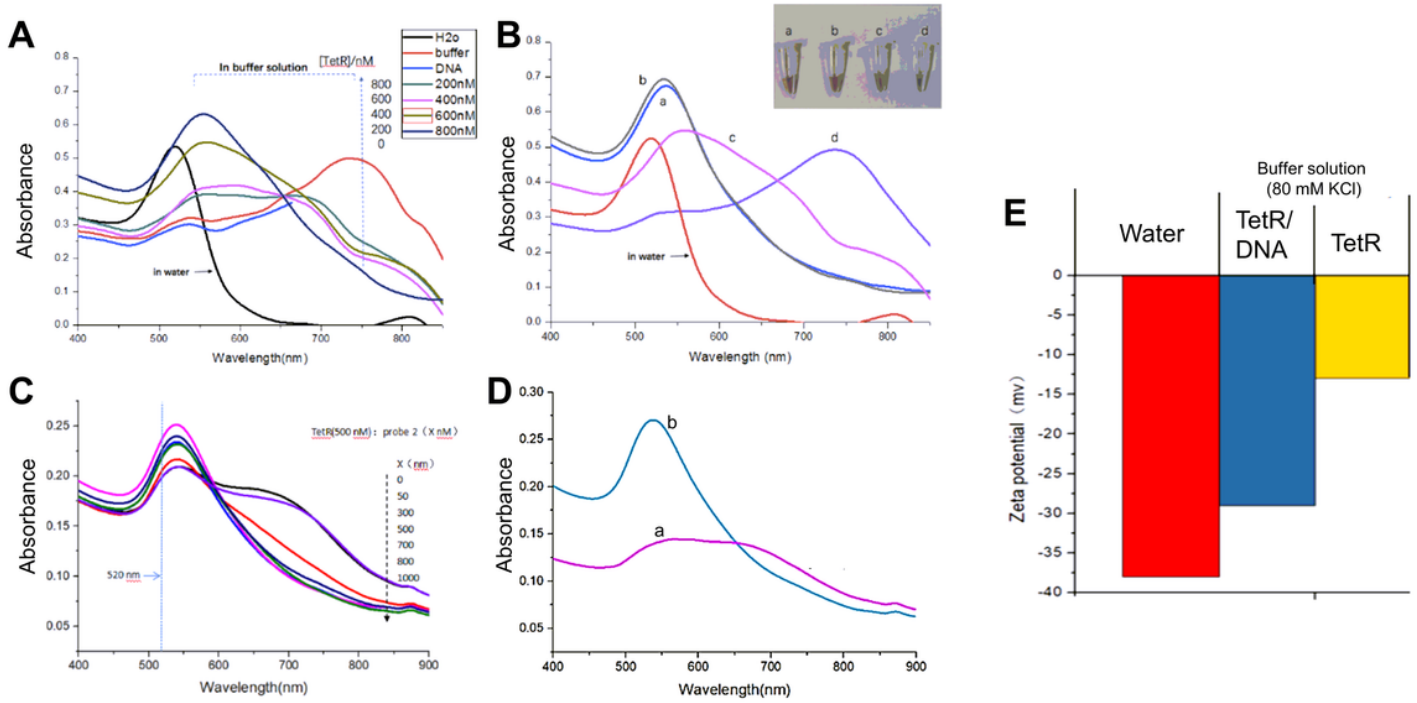

Figure 5

Results of Gold Nanoparticle Colorimetry. (A) Absorbance of AuNPs in buffer solution (10 mM Tris–HCl, 80 mM KCl) containing different concentrations of TetR (0, 200, 400, 600, and 800 nM). (B) Absorbance of AuNPs 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. (C) Optimization of probe 2 with AuNPs. 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). (D) Testosterone inhibits interaction between TetR and target DNA. 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 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).