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The characterization of a short chain dehydrogenase/reductase (SDRx) in Comamonas testosteroni

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ABSTRACT

C. testosteroni is a research topic that can degrade steroid hormones into water and carbon dioxide through a series of enzymes in the body. Short-chain dehydrogenase (SDR) are a class of NAD (P) H-dependent oxidoreductases in C. *testosteroni*. Its main function is catalyzing the redox of the hydroxyl/ketone group of the hormone. In this paper, a SDR gene(*SDRx*) is cloned from C. *testosteroni* ATCC11996 and expressed. The polyclonal antibody was prepared and the *SDRx* gene knocked out by homologous recombination. Wild type and mutant C. *testosteroni* induced by testosterone, estradiol, estrone and estriol. The growth curves of the bacteria were measured by spectrophotometer. ELISA established the expression of SDRx protein, and high-performance liquid chromatography(HPLC) detected the contents of various hormones. The results show that the growth of wild type was faster than mutant type induced by testosterone. The concentration of SDRx is 0.318 mg/ml under testosterone induction. It has a great change in steroid hormones residue in culture medium measured by HPLC: Testosterone residue in the mutant type group was 42.4 % more than the wild type in culture medium. The same thing happens with induced by estrone. In summary, this *SDRx* gene involved in the degradation of testosterone and estradiol, and effects the growth of *C. testosteroni*.

1. Introduction

That reduction of carbonyl groups in aldehydes, ketones, and quinones to their corresponding hydroxyl derivatives plays an important role in the metabolism of many endogenous(biogenic aldehydes, steroidal compounds, prostaglandins, lipid peroxidation product reactions) and exogenous(pharmacological drug metabolism, carcinogenic substances, toxic substances) compounds [1]. Its biochemical reaction mainly relies on carbonyl reductase. As early as the 1970s, it has purified a good deal of carbonyl reductase, including its characteristics, substrate specificity, and kinetic constant. In recent years, through sequence comparison, chemical degeneration and site-directed mutagenesis studies, combined with crystallographic analysis and bio-informatics data, scientists have recognized the important recognition structures and characteristics of these enzymes [2]. We divide these carbonyl reductases into two large protein super-families–aldehyde-keto reductase(AKR) and short-chain dehydrogenase/reductase (SDR)–which are catalyzed by the two superfamilies different positions of the core.

The AKR super-family is a growing family of super-enzymes. They can degrade different endogenous and exogenous substrates [3]. These include aldose reductase (EC 1.1.1.21), acetaldehyde reductase (EC 1.1.1.2) and some HSD (EC 1.1.1. x), which are formed from monomers (α/β) barrels of the protein of approximately 320 amino acids, which could combine with an auxiliary substrate without Rossmann-fold [4–6]. The active site member of AKR contains a conserved four-unit of amino acids Tyr, His, Asp, and Lys [7]. In almost organism, AKRs play an important role in metabolizing steroids, sugars, prostaglandins, polycyclic aromatic hydrocarbons, and a wide variety of non-steroidal aldehydes and ketones [8,9]. The amino acid sequences of different members of AKR are homologous. The family consists of 14 members: AKR1-AKR14. Studies have shown that AKR1 and AKR7 derived from mammals. AKR2-AKR5 and AKR8-AKR14 are mainly present in plants, yeasts, and bacteria. It composes the AKR6 of a beta subunit of K^+ ion channels [10].

The second family of carbonyl reductases is the short-chain dehydrogenase/reductase (SDR). As an enzyme widely present in prokaryotic and eukaryotic cells, they are involved in the metabolism of aromatic hydrocarbons including steroids, sugars, hormones, nitrogen fixation, and antibiotic synthesis [11–14]. It composes the SDR family of the average length of 250–350 amino acid, and most of its members are medium or long-chain dehydrogenase families [15]. The SDR family consists of large members of about 3000 known forms, including variants of the species [16]. Their homology is usually 15–30%, and their

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common sequences reveal their early origins and evolutionary trends [17]. It considers the folded structure of the protein as a conserved sequence, largely overlapping peptide backbone. It divides SDR into two parts, with about 250 amino acids conserved and 350 amino acids non-conserved [18,19]. The conserved regions contain sequences that determine protein structure, such as folding, active sites, substrates and substrate binding regions, and reaction mechanism determining regions [20,21]. The positively charged lysine side chain is located close to the hydroxyl oxidation of tyrosine and promotes proton transfer [22]. It also involves serine in the catalytic reaction of intermediates through a stable or partial proton delivery network. When protons transfer from tyrosine to the oxygen atom of the 20-ketosteroid, they replenish the protons in the solvent into the catalytic chamber, and the serine regulating substrate forms reaction state [23,24]. Conservative lysine residues play a dual role in catalytic reactions. It locates the nicotinamide ribose group of the cofactor to form a hydrogen bond and reduces the pKa value of the catalytic tyrosine through electrostatic interaction, which promotes the reaction [25–28].

C. *testosteroni* is an aerobic Gram-negative bacterium. It bases its growth on steroids as the sole carbon source. The metabolic process is a complex process. There are hundreds of enzymes involved in the degradation of steroids, and SDR is one of the most important ones [29,30]. Therefore, this paper cloned a short-chain dehydrogenase/reductase(named SDRx) of C. *testosteroni* bacteria and expressed in *E. coli*, prepared polyclonal antibodies, and research the expression of SDRx under the induction of different steroid hormones. Knock out the *SDRx* gene by homologous recombination, then study the growth of mutant and wild type C. *testosteroni* under different induction steroid hormones.

2. Materials and method

2.1. Materials

C. *testosteroni* ATCC11996 (Deutsche Sammlung für Mikroorganismen). Plasmid pET-15b containing the ampicillin resistance gene (Shanghai qincheng biotechnology co. China). *Escherichia coli* (BL21, Promega, Madison, USA) and Plasmid pCR2.1-TOPO (Thermo Fisher Scientific, California, USA).

Ampicillin and kanamycin (Sangon, Shanghai, China). Restriction enzymes, DNA ligase, and DNA polymerase were obtained from Boehringer Mannheim, Biolabs, MBI, and Amersham, and used according to the manufacturers instructions. Recombinant DNA work was carried out following standard techniques, according to Sambrook and Russel.

2.2. Isolation of chromosomal DNA

The chromosomal DNA of C. *testosteroni* was isolated through chloroform extraction. The culture conditions of C. *testosteroni* was grown in a shaker (180 rpm), in LB medium 27℃. Harvested 1 ml of overnight bacteria cell culture after centrifugation at 13,000 rpm for 1 min, then re-suspended in 1 ml distilled water, containing 100 μg lysozyme. To lyse C. *testosteroni* freeze (-20℃, 30 min) and thaw (25℃, 30 min) three times. Recovered DNA from the lysate by chloroform extraction, followed by ethanol precipitation. It suspended the DNA in a TE buffer (10 mmol Tris－HCl, 1 mmol EDTA, pH 8.0) and stored at 4℃. The purified chromosomal DNA was used for *SDRx* gene PCR (Fig. 1).

2.3. The SDRx gene clone

Search *SDRx* gene in Gen Bank (LN879547.1)and design the primer by Primer 5. Such the forward primer is 5′-CATATGAATCCATGCAT CAG TGGTTTG-3′ (Italics is NdeI), reverse primer is 5′-GGATCCTTAA ATATTAATTACTTTGGCTGC-3′ (Italics is BamHI) and synthesis by Sangon Shanghai. C. *testosteroni* chromosomal DNA is template, and PCR was taken at the following conditions: 95℃ 60 s, 50℃ 45 s, 72℃ 60 s; 30 cycles. Agarose gel electrophoresis identified the products. If the results were correct, recycled and purified the target DNA, following the SanPrep Column Plasmid Mini-Preps Kit by Sangon (Sangon, Shanghai), -20℃ conserved (Fig. 2).

2.4. Transformation of BL21

The *SDRx* gene was cloned into plasmid pET-15b to yield plasmid. The plasmid pET-15b-SDRx was used for SDRx functional characterization studying (Fig. 3).

The pET-15b-SDRx transferred into *E.coli* BL21 cells via the calcium chloride method. The cells were cultured in LB agar plates in the presence of Ampicillin at 37℃ overnight. They got 2 ml bacteria culture medium centrifuge 8000 rpm, 2 min, plasmid extracted by kit Sangon (Sangon, Shanghai). Double enzyme digested by BamHI and NdeI, The products were identified by 1% agarose gel electrophoresis (Fig. 4).

2.5. Separation and purification of SDRx

SDRx protein was over-expression in *E.coli* strain BL21(DE3) with plasmid pET-SDRx. 1 ml BL21 was added to 100 ml medium containing 100 μg/ml Amp(37℃, 180 rpm, 14 h). Then transferred 50 ml BL21 to 1 L LB medium with a final concentration of 100 μg/ml Amp(37℃, 180 rpm, 2 h). IPTG with a final concentration of 0.5 mmoL/L was added, and the culture temperature was 27℃, 160 rpm. Oscillated the culture for 12 h. Used the freezing and thawing technique; broke cells and separated supernatant. Add 5 ml 8 M urea into the sediment. for dissolution. The recombinant protein was purified through Ni-chelating affinity chromatography. The products were concentrated by the hyperosmotic glucose solution and explained by SDS-PAGE gel scan analysis. The result is shown in (Fig. 5). Then, the total protein content was measured using Coomassie brilliant blue staining.

2.6. Generation of SDRx gene knock-out mutant of C. testosteroni

To understand the biological function of SDRx protein, a mutant of C. *testosteroni* with the *SDRx* gene knock out by homologous integration. PCR generated the promoter with forward primer 5′-*GAGCTC* GGGGC TGATG CTGTTGACCC GCACGCCGTA A-3′ (Italics is BamHI), and reverse primer 5′-*GGATCC* TGGGCAGATT GATGGACTGG TGAC -3′(XholI). The resulting 318 bp PCR fragment clones into plasmid pCR2.1-TOPO to yield pTOPO-SDRx with restriction enzymes BamHI and XholI. Because of its sensitivity to kanamycin, only the mutant of *C. testosteroni,* in which pTOPO-SDRx was integrated into the chromosomal DNA, could grow up in kanamycin medium (Fig. 6A). *C. testosteroni* cells were transformed with 10 μg of pTOPO-SDRx by electroporation (1.8 kV, 1 mm cuvette, Bio-Rad), which interrupted the *SDRx* gene upon integration of the plasmid into the chromosomal DNA. The *C. testosteroni cultures and spreads on* LB agar plates under 30 μg kanamycin pressure, and cultured in a 27℃ incubator for 12 h. The plasmid integration proves by PCR. The forward primer 5′-*CATATG* AATCCAT GCA TCAGTGGTTT G-3′ is in *SDRx* gene and reverse primer 5′-TCAG AAGAAC TCGTCAAGAA GGCG-3′ is in kanamycin. The PCR products were confirmed by Sangon (Shanghai). PCR identified the successful knock-out insertion of plasmid. (Fig. 6B)

2.7. The expression of SDRx induction

Because SDRx is a new protein of short chain dehydrogenase/reductase, The ELISA method was used to detect the protein SDRx content of C. *testosteroni* in different steroid hormone inductions. Rabbit antibodies against SDRx was prepared according to the standard method.

The rabbits Yisi Changchun, Inspection number: SCXK JI 2016-0003 were immunised with the methods of Freund's adjuvant and given carrots after immunization as a prize. Blood was collected from the rabbit's ears in aseptic conditions. ELISA detected separation of rabbit

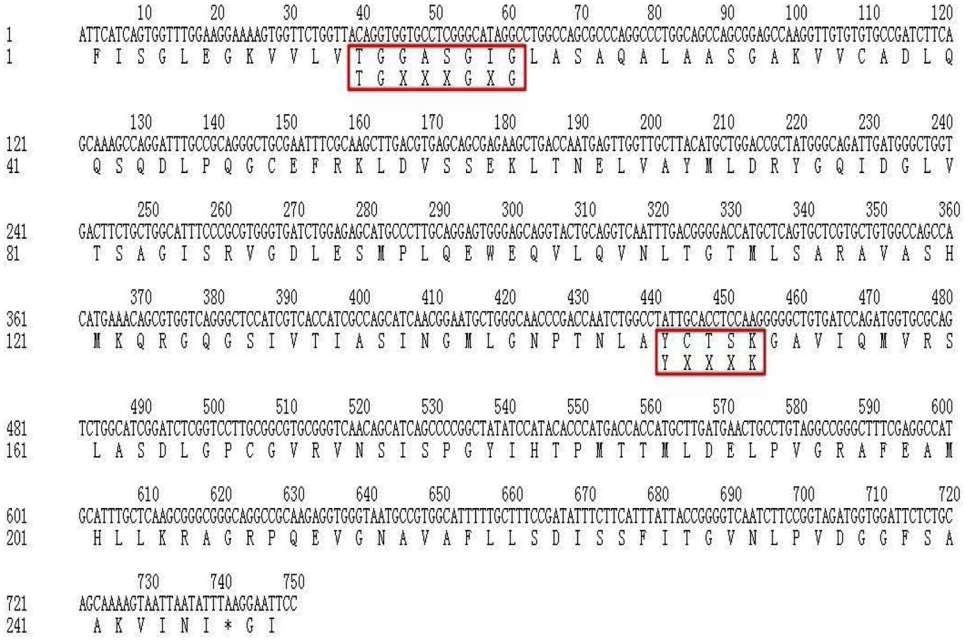

Fig. 1. Nucleotide sequence and amino acids sequence of *SDRx*.
NAD(P)H binding domain and active center are boxed. Asterisk represents the stop codon.

serum titer. 96-well plate coated with SDRx protein antigen of SDRx concentrated at 20 μg /ml. Blank groups, negative groups, and positive groups were used in the experiment. The positive serum was diluted with PBS-T at the ratio of 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32,000 and 1:64000, and added in the 96-well plate. Each well was 100 μl, 4℃, overnight, then washed with PBS-T three times. The secondary antibody Goat anti-rabbit, HRP conjugate, Beijing Zhongshan Biotechnology Company. Diluted ratio:1:4 000 was added about 100 μl, 37°C, reaction for 30 min, then washed with PBS-T three times, and ddH_2O for twice. Finally, 100 μl TMB was added, light was avoided for 10 min, and 50 μl, 2 mol H_2SO_4 was added into each well. The data was detected under the wavelengths of 450 nm (Table 2). The antibody was standby in -20 ℃.

The quantity standard curve of SDRx was established by ELISA. The SDRx protein concentration was coated in 96-well plate with 0.8 μg/ml, 0.4 μg/ml, 0.2 μg/ml, 0.1 μg/ml, 0.05 μg/ml, 0.025 μg/ml, 0.0125 μg/ml, and 0.00625 μg /ml. Immobilization, closing, washing, and rabbit serum antibody diluted at ratio of 1:16000, 4°C, overnight, then IgG-HRP 100 μl was added at 37°C, and reacted for 30 min. When the reaction ended the data was recorded in OD_{450} (Fig. 7).

To determine SDRx protein whether disruption or high expression of *SDRx* gene, it was incubated with 0.5 mM alcohol, estrone, testosterone, estradiol and estriol respectively, in 100 μl of inorganic medium for 3 h at 27 ℃. Then, 1 ml bacteria liquid at 13 000 rpm for 30 s. After centrifugation, 200 μl, 8 M urea was added to in the supernatant bacteria. The mixture was stirred until uniform. Supernatant was collected by freeze-thaw crushing method at 13 000 rpm for 2 min. Diluted samples treated with different steroid hormones to 20 μg/ml with PBS-T. The expression of SDRx was measured by ELISA, and calculated with SDRx standard curve (Fig. 8).

2.8. Growth of C. testosteroni and mutant C. testosteroni in different steroid hormones

The bacteria incubated with 0.5 mM alcohol, estrone, testosterone, estradiol and estriol respectively, in 100 μl of 5 mL inorganic medium. The growth of wild-type and *SDRx* gene knock-out mutant C. *testosteroni* detected by the measurement of the optic density after 14 h, 180 rpm, at 27°C, each steroid hormone has three parallel groups. Culture medium diluted 10 times with inorganic medium. Record the optic density in λ = 595 nm (Fig. 9). The sample of high performance liquid chromatography was collected after 4°C, 12 000 rpm for 10 min about 1 ml induced culture liquid.

The remaining alcohol, estrone, testosterone, estradiol, and estriol in the medium were extracted with ethyl acetate. The hormone diluted with 100 μl HPLC running buffer(Methanol: H_2O in 80:20), then 50 μl sample was injected into HPLC(SHIMADZU LC-20A chromatography), speed 1 ml/min. The conditions were as the Table 1. The results were shown in Fig. 10.

3. Results

3.1. SDRx gene analysis of C. testosteroni

In this paper, a short chain dehydrogenase short gene (*SDRx*) of SDR families was identified by bioinformatics studies in the *C. testosteroni* genome sequence. The sequencing result was consistent with conservative regions. The gene was submitted to Gen bank with accession ID: LN879547.1. The gene is 750 bp, make up of 250 amino acids and with a molecular mass of 27.5 kDa using OMIGA software. SDRx is located in C. *testosteroni* LN879547 in NCBI. Because it belongs to short the chain dehydrogenase family, the gene contains two conserved motifs that all SDR family members have: one is the TGxGxxxG motif related to NADP(H) binding (39 bp to 63 bp), the other is the YxxxK motif related to its catalytic activity (441–456). The nucleotide sequence and the deduced amino acids of *SDRx* shown in Fig. 1. In the study of evolutionary relationships, it found that SDRx has certain homology with 3α-HSD of Stenotrophomonas. They are involved in redox reactions in the body and degradation of steroid hormones (Fig. 2).

3.2. Cloning SDRx gene of C. testosteroni

The *SDRx* gene from ATCC11996 was amplified by PCR, and the results are shown in Fig. 3. The gene fragment was 750 bp and the molecular mass was correct, this suggesting that the *SDRx* gene had successfully cloned out.

The PCR products of *SDRx* gene were cloned and linked to the pET-

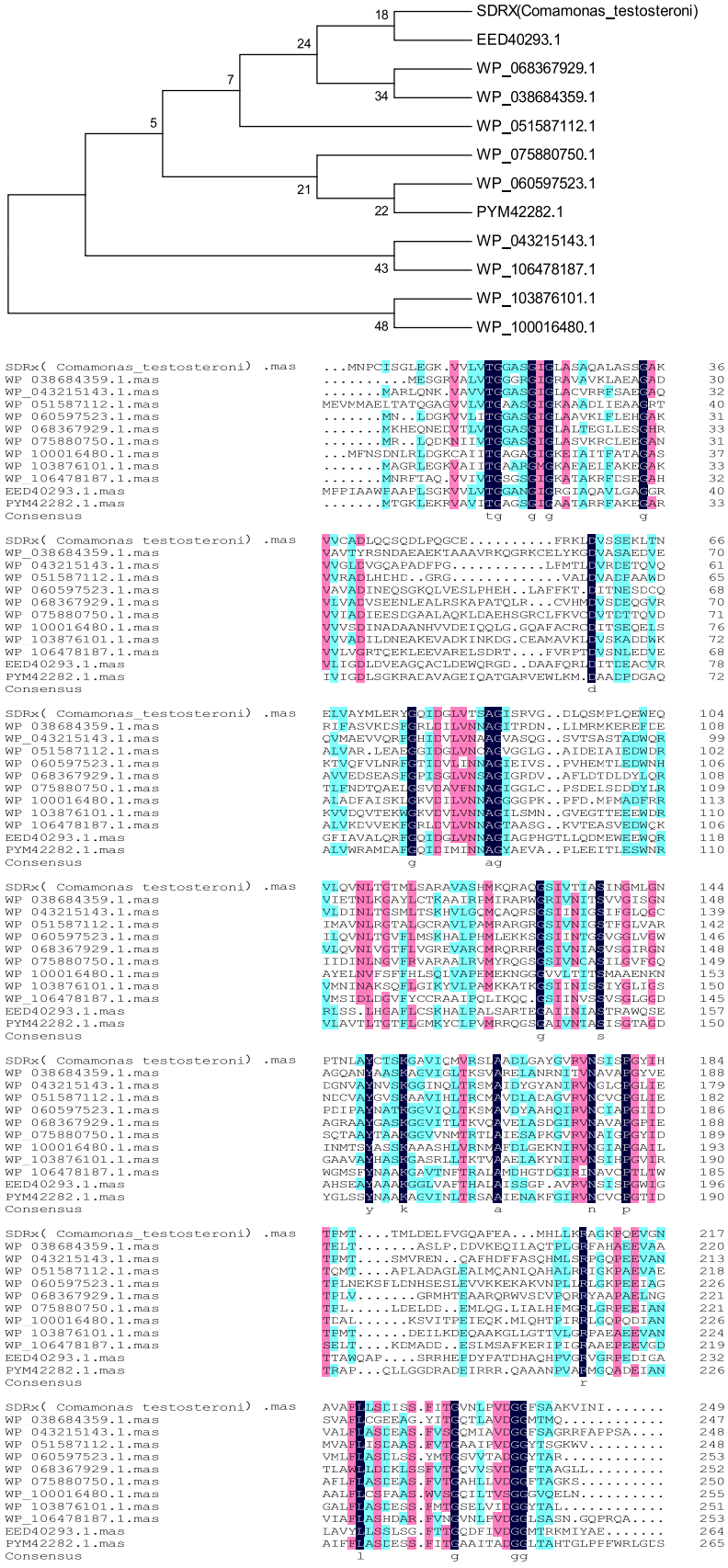

Fig. 2. Phylogenetic tree analysis and amino-acid sequence alignments of the *SDRx* gene.

The Phylogenetic tree of *SDRx* gene made by MEGA 5.1, Accession numbers of the full-length proteins are SDR family oxidoreductase [C. testosteroni]; 3-oxoacyl-reductase [Rubrobacter], WP_038684359.1; SDR family oxidoreductase [Pseudomonas], WP_043215143.1; SDR family oxidoreductase [Novosphingobium], WP_051587112.1; SDR family oxidoreductase [Bacillus], WP_060597523.1; SDR family oxidoreductase [Kerstersia gyiorum], WP_068367929.1; SDR family oxidoreductase [Halomonas], WP_075880750.1; 7-alpha-hydroxysteroid dehydrogenase [E.coli], WP_100016480.1; SDR family oxidoreductase [Petrotoga], WP_103876101.1; SDR family oxidoreductase [Halomonadaceae], WP_106478187.1; 3α-HSD [Stenotrophomonas], EED40293.1; oxoacyl-ACP reductase [Candidatus Rokubacteria], PYM42282.1; Note: The tree in A shows that SDRx and Stenotrophomonas have a close provenance relationship. In B, in the alignment table amino acids of SDRx and other SDR in different species, the consensus amino acids marked in the last line. The common site TGxxxGxG and YxxxK existed in the amino acids.

15b vector (named pET15b-SDRx). The plasmid was shown in Fig. 4A. In order to identify the connection between the *SDRx* gene and the plasmid, NdeI and BamHI were used in the pET15b-SDRx combined with the restriction enzyme digestion. The results showed that there were lines at 750 bp and 5700 bp lineal plasmid (Fig. 4B). The sequencing was correct by Shanghai Sangon.

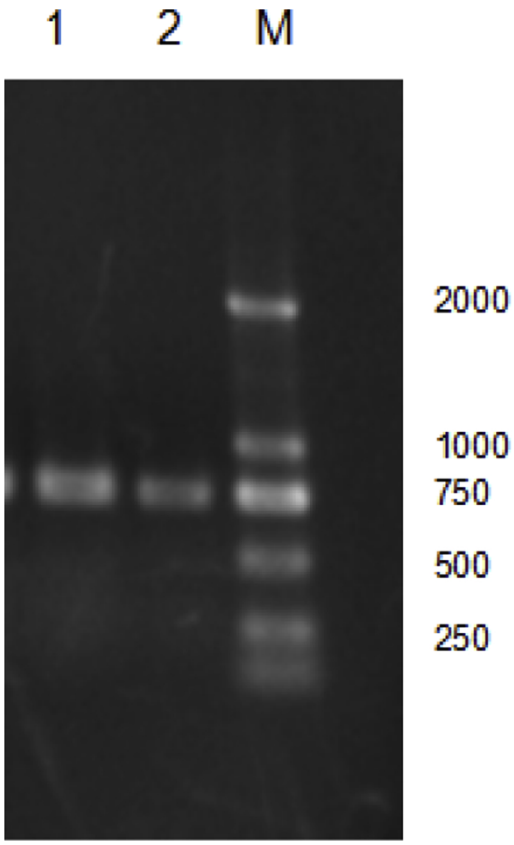

Fig. 3. The *SDRx* gene identified in gel electrophoresis.
M: DNA Marker DL2000; 1,2 were *SDRx* gene.

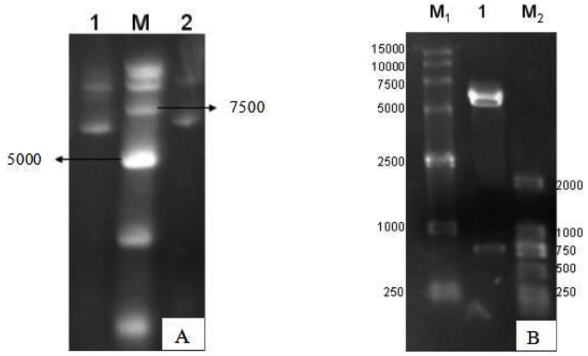

Fig. 4. The Identification of recombinant vector and the double enzyme digestion.
A: M:DL15000 Marker, 1: pET15b-SDRx recombinant plasmid, 2: pET15b vector as a negative control; B: M1: DL15000 Marker, M2: DL2000 Marker, 1: pET15b-SDRx vector with the restriction enzyme digestion.

3.3. Expressing SDRx protein of C. testosteroni

The plasmid pET15b-SDRx was transferred into *E.coli* (BL21), the SDRx protein induced expression and purified by nickel column. In Fig. 5B, it is obvious that the means of removing other proteins in the total protein were primarily great. In the 8th band at 27.2 kDa. The amino acid sequence was input into the Swiss model to predict the 3D structure of the protein (Fig. 5A), and the content of the SDRx protein was measured by Coomath Bright Blue method: the concentration is about 2.64 mg/ml.

3.4. Growth of SDRx gene knock-out mutants

The target *SDRx* gene was linked to the pCR2.1-TOPO plasmid, which was used as the gene target vector, and the results were identified by agarose electrophoresis (Fig. 6). To understand the biological function of the SDRx protein, the mutant C. *testosteroni* was built by homologous recombination. The method of the mutant strain is shown in Fig. 6A. Using the whole genome, cloning a part gene of SDRx 318 bp was obtained by PCR, then insert into the pCR2.1-TOPO plasmid (named pTOPO-SDRx), as a homologous recombination with SDRx. The pCR2.1-TOPO plasmid was inserted into the *SDRx* gene. Then SDRx function was lost(named MC.T.). The mutant C. *testosteroni* strain filtrated out under antibiotic pressure. *SDRx* gene was successfully knocked out and identified by PCR (Fig. 6B).

3.5. The expression of SDRx protein in different condition

Indirect ELISA test tested serum titer. The absorbance value was recorded at 450 nm wavelengths. The serum of the immunization rabbits was negative groups (N groups). The serum of immunized rabbits was positive groups (P groups). The OD_{450} value got in the well of negative groups and positive groups. The P/N value was taken as the titer criterion. When the effective value is P/N $>$ 2.1, the dilution ratio of the serum was regarded as the titer of rabbit polyclonal antibody. According to the data in Table 2, when the antigen (SDRx) concentrated at 20 μg /ml, the high titer of the serum can reach a maximum of 1:16,000. Carry 3 groups, two parallel samples of each group, the above OD_{450} data are average for each group.

The quantity standard curve of SDRx was established by ELISA. The standard curve was made by the Origin 8 scatter plot method. Through logarithmic curves analysis of models, this model proved to have a good curve fitting characteristics of relatively high precision models. The standard curve equation was y = 0.2359Ln (x)-0.3764, R^2 = 0.9592.

According to the standard curve and its formula in Fig. 7, indirect ELISA measured the absorption value. The quantity of SDRx in the total protein of 20 μg was the Y axis. The steroid hormone was the X axis. Origin software processed the data (Fig. 8). It is obvious that the SDRx protein could be induced by some steroid hormones, especially testosterone and estrone. The protein content of SDRx was 5.36 ± 0.4 μg/ml and 4.89 ± 0.5 μg/ml. But in the ethanol control group, there was about 2.92 ± 0.3 μg/ml of SDRx. Estradiol group was 3.28 ± 0.3 μg/ml, and 3.76 ± 0.2 μg/ml in estriol group.

3.6. Growth of mutants and wild-type C. testosteroni in different conditions

From the chart(Fig. 9), wild type and mutant type C. *testosteroni* could promote growth by adding steroid hormones. But when the *SDRx* gene was knocked out, the density of bacteria of wild type was much higher than of mutant type. No matter in the hormone-induced group or the control group, the growth of mutant type was generally inhibited. In the comparison of the OD values of the bacteria induced by the same hormone in the experiment, it was found that the growth density of the wild type in testosterone group decreased from 5.38 ± 0.6–4.06 ± 0.4 compared with mutant type. In the estrone group, the growth density of the wild type decreased from 4.99 ± 0.3–3.71 ± 0.2 compared with the growth after mutation, and the growth rate was only 74.3 % of the wild type. This shows that the *SDRx* gene plays an important role in degrading steroid hormones and affects the growth of C. *testosteroni*.

After steroid induction, there was a significant difference between the mutant strain of the *SDRx* gene knockout and the wild type C.*testosteroni* in steroid hormone degradation (Fig. 10). The results showed that the wild type and mutant type had no effect on the degradation of ethanol. C.*testosteroni* and mutant strains could use estradiol and estriol with little difference. This showed that SDRx had little effect on the degradation of estradiol and estriol, hormone residues

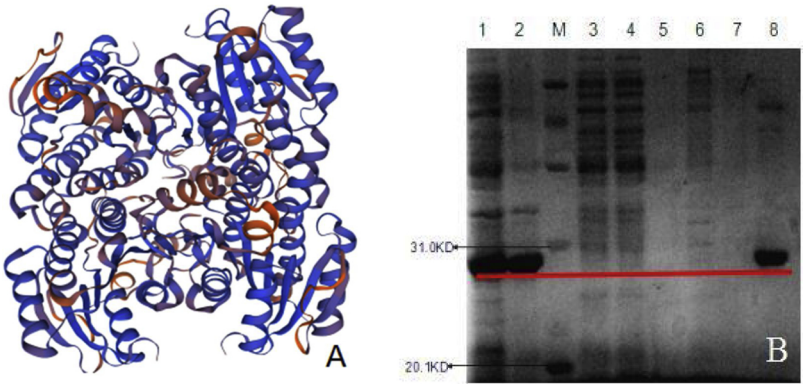

Fig. 5. Isolation and purification of SDRx protein by SDS-PAGE. A: The 3D structure of SDRx protein got through computational simulation by Swiss-model software. B: 1,2:Soluble total protein; M: Protein Marker; 3,4: Sample of protein after nickel column; 5: Elution NPI 1 washed out protein; 6: Elution NPI 20 washed out protein; 7: Elution NPI 30 washed out protein; 8: The purified and concentrated SDRx protein.

71.3 % and 82.6 % in the culture medium of mutant type. This showed that SDRx had little effect on the degradation of estradiol and estriol. However, there were significant differences between the mutant and wild type in the utilization of testosterone and estrone, especially as the residual mutant of testosterone in the culture medium was 42.4 % more than the wild type, and in the estrone group wild type is 21.6 % less than the mutant type. Therefore, SDRx played an important role in the utilization of testosterone and estrone.

The steroid hormone groups were X axis and the percentage of residual hormone in the medium was in the vertical coordinate. In each group, the sample extracted from the mixture of hormone and inorganic medium is a control. HPLC determined the residual hormone content.

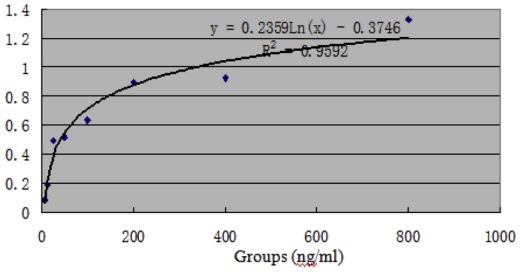

Fig. 7. Established the quantity standard curve of SDRx by ELISA.

4. Discussion

Hormones are increasingly used in our daily lives. Although they can reduce the suffering caused by some diseases or bring huge economic benefits, they can also be a huge threat. Hormones spread into the soil and water. This kind of pollution seriously affects human health and destroys the balance of ecology. Hormonal pollution has become one of the biggest problems to the environment [31].

There are many effective ways to do this in nature. People are surprised by the adaptability of microorganisms to their environment. They can survive on the catabolism of hormones. These microorganisms produce enzymes which can use hormones [32]. Studying the survival of these microorganisms is of great significance to our understanding of nature.

Short-chain dehydrogenase (SDR) is one of the most important enzymes involved in hormone metabolism. We found SDRs in microorganisms, including bacteria and archaea, using bioinformatics methods. In recent years, SDR enzymes were also found in the human body. This suggests that we use hormones in the same way as other microbes. The mechanisms of regulation induction and expression of these enzymes is still unclear, which is the motivation for our efforts. The physiological functions of SDR enzymes make some SDR members attractive metabolic targets. It is composed of 250–300 amino acids, and usually contains two structures: binding coenzyme domains and substrates [33]· In order to take part in the metabolism of steroid hormones, many microbes under environmental selection could express SDR enzymes like C. *testosteroni*.

In this paper, we have identified a potential short chain dehydrogenase in C. *testosteroni* using the bioinformatics methods and studied its effects on hormone metabolism. At first, the *SDRx* gene was amplified from the C. *testosteroni* chromosome DNA by PCR, and connected to the plasmid, pET15b. Then the recombinant plasmid was transformed into *E.coli*. The SDRx protein was expressed under these conditions,as well as separated and purified. The concentration of proteins, measured using Coomassie brilliant blue staining, was about 2.64 mg/ml. Next, the ELISA method used to explore the change of SDRx protein expression in the process of hormone induction(ethanol,

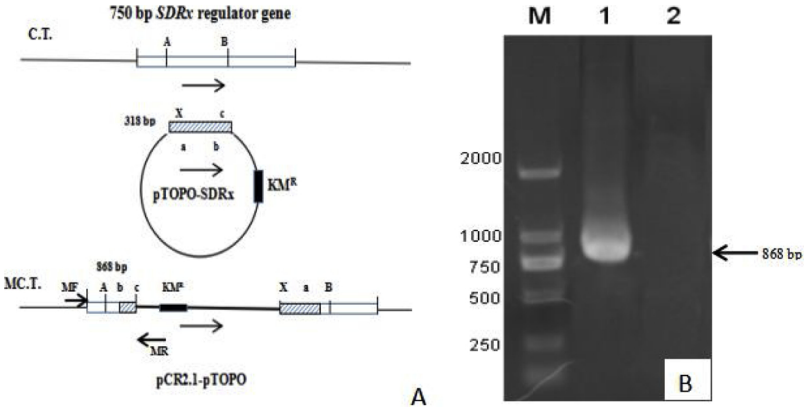

Fig. 6. Preparation of *SDRx* knock-out mutant.
A: Schematic diagram of the method for preparing mutant strains, B: PCR detects *SDR*x knock-out mutants, M: DL 2000 DNA Marker; 1: *SDR*x gene knock-out mutants, 2: PCR base on wide type C. *testosteroni*.

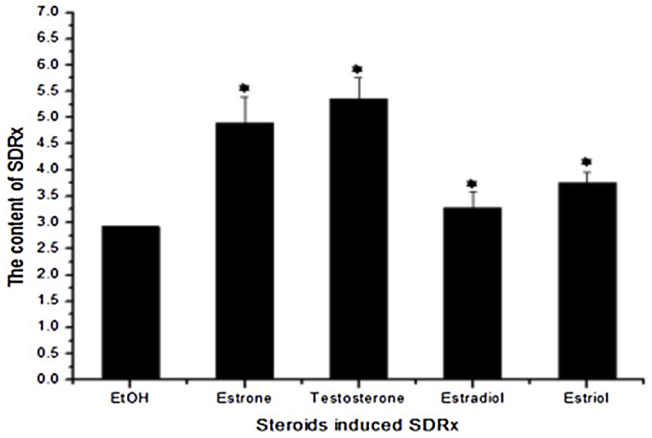

Fig. 8. The expression of SDRx with different steroid hormone treatments.
The experiment was performed three times, and the average value was calculated and plotted in the figure. The asterisk represents a significant difference between the experimental group and the control group, *P < 0.05.

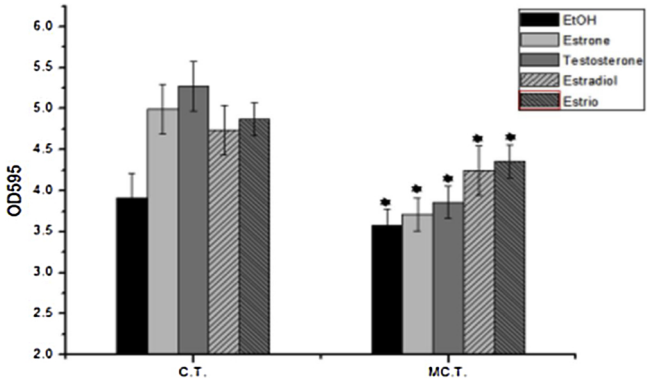

Fig. 9. The growth of wild type and mutant type C. *testosteroni* in different hormonal induction.
The experiment was performed three times. The asterisk represents a significant difference between the experimental group and the control group, *P < 0.05.

Table 1
The conditions of steroid hormone in HPLC.

Steroid hormone	Wavelength (nm)	Temperature (℃)
Estrone	241	40
Testosterone	250	40
Estradiol	280	40
Estriol	230	40

estradiol, estriol, testosterone and estrone). SDRx was the antigen for rabbit immunity, used to prepare the polyclonal antibody. ELISA results showed SDRx expression, induced by testosterone and estrone, is higher than the estradiol and estriol groups. Lastly, the bacterial growth situations were detected between wide and mutant types C. *testosteroni* in hormone induction by spectrophotometer. The growth density of bacteria showed that testosterone and estrone have positive facilitation on the growth of bacteria. However, the result for the mutant type is much fewer then in the wide group in the same situation. At the same time,

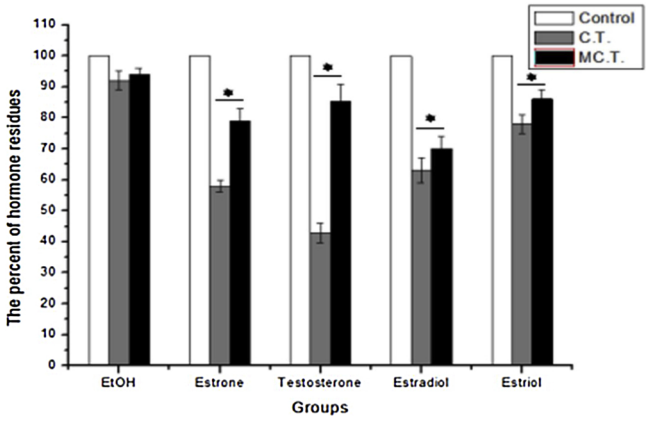

Fig. 10. The residual hormones in the culture medium.
The experiment was performed three times. The asterisk represents a significant difference between the experimental group and the control group, *P < 0.05.

hormone residue from the culture medium was analyzed by HPLC. The result found that least testosterone remained than other groups. After *SDRx* gene knockout, testosterone use declined to 42.4 %. The results also explain why the growth in the testosterone group was worse than others, when SDRx was knock out, testosterone was used decreased, lead to inhibition of bacterial growth. So the gene(Accession ID: LN879547.1) belongs to the short chain dehydrogenase family, and takes part in the degradation of hormones in C. *testosteroni*, especially testosterone hormone.

Curiously, when the SDRx protein was eliminated, the mutant type still continue to grow, but much less then wide type. This may tell us that the SDRx protein might not be the key enzyme to growth. In other word, there some other enzymes could use testosterone hormones. As we know, microbes can respond quickly to changes in the environment. Nutritional supply can change at any time. They must have the ability to switch between different metabolic substrates to survive [34]. Microorganisms have a near-ghost-like instinct for food use, and they manage nutrition like an economist. In a suitable environment, they can reproduce and metabolize some secondary products. However, in the absence of food, it is not necessary to synthesize a large number of related enzymes, so they have created a regulatory mechanism that blocks the enzymes synthesis pathway in the hostile environment, but at the same time they are prepared to synthesize when survival space improved [35]. Therefore, the metabolism of microorganisms must never been only one way. The food will be equipped with corresponding enzymes, and the biochemical reactions involves many biological enzymes [36]. There are many ways to use hormones from the growth of C. *testosteroni*. In conclusion, This SDRx can be considered one of the most complex enzyme families involved in hormone degradation.

Although there are so many enzymes join in biochemical reactions, each enzyme plays an extremely important role. In our bodies, they take park in hormone metabolism, maintain the hormone balance of living organisms, and provide an important role for life and health [37,38]. Research the function these enzymes have a great significance for understanding the relationship between life and hormones, but these are still a long way for us.

Table 2
The titer detection of rabbit polyclonal antibody by ELISA.

Sample	Blank	Negative	1:1 000	1:2 000	1:4 000	1:8 000	1:16 000	1:32 000	1:64 000
OD_{450}	0.063	0.21 ± 0.34	3.59 ± 0.62	2.78 ± 0.57	1.64 ± 0.44	0.92 ± 0.42	0.51 ± 0.07	0.25 ± 0.02	0.13 ± 0.01
P/N	——	——	17.09	13.24	7.81	4.38	2.43	1.19	0.62

CRediT authorship contribution statement

Chuanzhi Liu: Software, Methodology, Writing - review & editing. **Kai Liu:** Data curation, Writing - original draft. **Chunru Zhao:** Visualization, Investigation. **Ping Gong:** Validation. **Yuanhua Yu:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.02.015.

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