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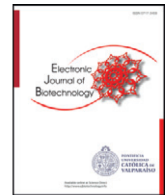

Research article

Enhanced alkaline catalase production by *Serratia marcescens* FZSF01: Enzyme purification, characterization, and recombinant expression

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ABSTRACT

Background: Catalase (CAT) is an important enzyme that degrades H_2O_2 into H_2O and O_2. To obtain an efficient catalase, in this study, a new strain of high catalase-producing *Serratia marcescens,* named FZSF01, was screened and its catalase was purified and characterized.
Results: After optimization of fermentation conditions, the yield of catalase produced by this strain was as high as 51,468 U/ml. This catalase was further purified using two steps: DEAE-fast flow and Sephedex-G150. The purified catalase showed a specific activity of 197,575 U/mg with a molecular mass of 58 kDa. This catalase exhibited high activity at 20–70°C and pH 5.0–11.0. *Km* of the catalase was approximately 68 mM, and *Vmax* was 1886.8 mol/min mg. This catalase was further identified by LC–MS/MS, and the encoding gene was cloned and expressed in *Escherichia coli* BL21 (DE3) with a production of 17,267 ± 2037 U/ml.
Conclusions: To our knowledge, these results represent one of the highest fermentation levels reported among current catalase-producing strains. This FZSF01 catalase may be suitable for several industrial applications that comprise exposure to alkaline conditions and under a wide range of temperatures.

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1. Introduction

Hydrogen peroxide is increasingly used in many commercial processes such as bleaching (textile and paper making industry) [1], sterilization of medical devices [2], and wastewater treatment [3]. During the production process, residual H_2O_2 can, however, have adverse effects on human health and the environment [4]. H_2O_2 could be scavenged by hydrosulfite, but it has the disadvantage of adding salt ions to the production process and thus the environment [4]. Although H_2O_2 can also self-degrade, it takes time. Catalase (EC 1.11.1.6) may be the best alternative as it effectively catalyzes H_2O_2 into H_2O and O_2 with no salt ion production. Catalases are widely found in most plants, animals, and microbes and are important protective enzymes in cells [5,6]. Catalases are also used in biosensor development, bioremediation, food processing, biomedical, and other areas [4]. Microbes are the preferred source of catalase for research and applications because of their high activity levels, rapid cultivation, and easy handling [4].

Commonly used commercial catalases produce enzymatic activities greater than 50,000 U/ml (e.g., Novozymes [7]). Catalases that show high activity levels and tolerance to either low or high temperatures or extreme pH are highly valued in the industry. Properties, such as levels of activity and reaction to temperatures and pH, are therefore important to examine as they vary among bacterial strains. Although many bacteria produce catalase, including *Thermoascus aurantiacus*, *Acinetobacter* sp., and *Rhizobium radiobacter*, the level of enzyme activity remains low, from 100 to 1000 U/ml [4].

Catalases can be divided into four classes: class 1 monofunctional heme catalases, class 2 catalase–peroxidases, class 3 non-heme catalases, and class 4 minor catalases [4]. Catalase of class 1 is part of the heme-containing monofunctional enzymes and widely used in textile, dairy, and paper industries [9]. This class of catalase includes many highly effective bacterial catalases, such as acid-stable catalase from *Bacillus altitudinis* SYBC hb4 [8], *Bacillus subtilis* [9], *Pigmentiphaga* sp. strain DL-8 [9], and alkali-tolerant catalase from *Acinetobacter gyllenbergii* 2P01AA [11].

To develop a new strain of catalase efficient enough for industrial application in terms of both properties and production, we screened and identified a strain of *Serratia marcescens*, named FZSF01, which produced high-activity heme-containing monofunctional catalase. The enzyme properties were studied, and the fermentation conditions of enzyme production were optimized. As we could reach more than

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50,000 U/ml in liquid fermentation, we further purified, identified, and characterized the heme-containing monofunctional catalase from FZSF01. We also sequenced, cloned, and studied the recombinant expression of this catalase and compared it with other related catalase sequences. This comprehensive study represents a major step toward defining a new catalase that can be effectively used in the industry.

2. Materials and methods

2.1. Screening of high catalase-producing strains

Samples from rice field soil of an industrial farm in Fuzhou, China, were collected and immediately diluted with sterilized water and spread on nutrient agar plates. Agar plates were incubated at 30°C until colonies formed. A total of 36 strains were initially used to screen for their catalase-producing ability. A loopful of pure culture of each strain was transferred into 50 ml of nutrient broth (1% beef extract, 0.3% soy peptone, 0.2% NaCl, and pH 7.2). Catalase activity of each strain was determined after incubation at 35°C, 180 rpm for 12 h according to the reported method [12].

2.2. Bacterial strain identification through 16S rRNA sequencing

The strain's genome was extracted using Bacteria Genomic DNA Extraction Kit Ver.3.0 (TaKaRa Bio Inc., Dalian, China). A pair of universal primers [13] was used to amplify the 16S rRNA gene. The obtained 16S rRNA sequence was compared with available sequences in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) and Ezbiocloud (www.ezbiocloud.net), and then those sequences with high similarity to the amplified 16S rRNA sequence were chosen in Ezbiocloud to construct a phylogenetic tree. After editing with ClustalX 2.0.11, a phylogenetic tree was constructed with MEGA 4.0.2 [14].

2.3. Method for catalase assay

Ultrasonication was applied to prepare crude enzyme from the fermentation broth, with the following parameters: 400 W, with every 1 s of ultrasonication followed by 3-s pulse until the fermentation became clear. Crude catalase was diluted with 50 mM Na_2HPO_4/NaH_2PO_4 buffer (pH 7.0) for subsequent assays. Purified catalase was diluted with proper buffers for its enzymatic characterization including temperature, pH, and kinetic parameters.

Catalase activity was assayed by spectrophotometry at an absorbance of 240 nm [15]. For all the assays performed in this study, 0.1 ml of enzyme was injected in a quartz cuvette (1 cm × 1 cm) first, followed by the rapid addition of 2.9 ml of 50 mM Na_2HPO_4/NaH_2PO_4 buffer (pH 7.0, containing 30mM H_2O_2 [16]). The decrease in absorbance at 240 nm was monitored every 10 s for 1 min, as described in Hildebrandt and Roots [17]. Molar absorptivity of H_2O_2 at 240 nm was 43.6 mol/cm and one unit of catalase activity was defined as the amount of catalase needed to degrade 1 μM H_2O_2 per minute. The reaction temperature was kept at 30°C.

2.4. Optimization of catalase fermentation conditions with strain FZSF01

Optimization of catalase fermentation was performed with a batch culture method in 250 ml shake flasks containing 50 ml of medium.

Seed broth was prepared by transferring a loopful of pure culture into 50 ml of seed medium (1% beef extract, 0.3% soy peptone, 0.2% NaCl, and pH 7.2) and cultured at 37°C, 180 rpm for 24 h. For the nitrogen source optimization, fermentation media were prepared with one of the following: 1% (m/v) of soy peptone, yeast extract, beef extract, malt extract, or tryptone; then 1 ml of seed broth was transferred into 50 ml of the different media above. After culturing at 30°C for 24 h, catalase production with different kinds of nitrogen source was measured. For carbon source optimization, 3% (m/v) of cane sugar, maltose, glycerin, glucose, lactose, or citric acid was added to the basic medium (1% of optimal nitrogen source). Once the optimum nitrogen and carbon sources were identified, additional media were prepared using one of the following mineral salts at a dilution of 0.2% m/v: sodium citrate, NaH_2PO_4, Na_2HPO_4, $ZnSO_4$, $(NH_3)_2SO_4$, NH_3NO_3, $MgSO_4$, NaCl, CaCl, $MnCl_2$, KCl, $FeCl_2$, $FeCl_3$, or $CuSO_4$. All fermentation media were autoclaved at 115°C for 20 min. Fermentation conditions were 2% (v/v) inoculum size, 50 ml of fermentation volume, 30°C, and 180 rpm for 24 h. Growth of the strain was indirectly monitored by measuring the *OD*600 value of the fermentation broth. Catalase activity under different fermentation conditions was assayed as described above.

To determine the optimum fermentation temperature to produce catalase, FZSF01 was cultured at different temperatures from 20°C to 40°C (in 5°C increment). Catalase production curve was obtained by measuring the catalase activity of the fermentation broth every 5 h under determined optimum fermentation medium and temperature conditions. Catalase activity assay was performed as described above.

2.5. Catalase purification

After fermenting for 55 h, the broth was centrifuged at 12,000 rpm for 5 min and the sediment suspended in 50 mM PBS (pH 7.0). Because the catalase enzyme was found to be intracellular, suspended cells were disrupted with an ultrasonic instrument at 0°C until the solution became clear and then centrifuged at 13,000 rpm for 30min. The supernatant was considered the crude catalase, which was used for purification. Protein quantification of crude catalase and purified portions was done following the Bradford method [18].

The catalase was purified by slowly loading 8 ml of crude catalase into a DEAE Sepharose fast flow column (2 cm × 30 cm). Both crude catalase and the DEAE column were prepared using 50 mM PBS (pH 7.0). Catalase was extracted by adding NaCl solutions prepared with 50 mM PBS (pH 7.0) at increasing concentrations (0, 100, 200, and 400 mM) at a flow rate of 1 ml/min. Approximately 40 ml extracted samples showing catalase activity were merged. This resulting catalase solution was desalted and concentrated to 5 ml using a Millipore concentrator (UFC903096). Finally, 2 ml of concentrated fractions was loaded on a Sephadex G150 column (2 cm × 100 cm) calibrated using 50 mM PBS (pH 7.0). The enzyme was eluted with 50 mM PBS (pH 7.0) at a flow rate of 1 ml/min. Each fraction with catalase activity (up to 8 ml) was collected for purity and protein concentration assessment.

2.6. Gel electrophoresis of DNA and protein

DNA samples were electrophoresed with 1% of agarose gel and stained with ethidium bromide. Protein samples were examined through SDS-polyacrylamide gel electrophoresis (SDS-PAGE), according to Laemmli [19], and the gel was stained with Coomassie brilliant blue-R250.

2.7. Characterization of purified catalase

The effects of pH and temperature on the purified catalase were assessed as Zhang et al. [8] described.

The optimal reaction temperatures for catalase enzymatic activity were assayed at different temperatures, ranging from 0°C to 90°C (in 10°C increment). Temperature stability was assayed by incubating the catalase at different temperatures (40°C, 50°C, 60°C, 70°C, and 80°C) for different periods of time (15, 30, 60, and 120 min).

Optimal pH of the catalase was determined following the same procedure using different pH buffers: 50 mM citrate–phosphate buffer (pH 3.0–6.0), 50 mM NaH_2PO_4-Na_2HPO_4 buffer (pH 7.0–8.0), and 50 mM glycine buffer (pH 9.0–11.0).

To calculate the *Km* and *Vmax* values of the purified enzyme, reaction speeds (V) in various concentrations of H_2O_2 (from 5 mM to 20 mM)

were assayed at 30°C. The kinetic constants *Km* and *Vmax* were obtained using the Michaelis–Menten equation, and a double-reciprocal Lineweaver–Burk plot [20] was graphed on SigmaPlot 12.0.

2.8. Catalase identification with LC–MS/MS

The purified protein was digested with trypsin and analyzed using a RPLC–MS/MS on a Thermo Fisher Orbitrap Fusion Mass Spectrometer (Thermo Fisher, San Jose, CA). The data were treated with ProteinPilot 5.0 software (AB SCIEX), and database UniProt (http://www.uniprot.org) was chosen to identify the catalase.

2.9. Gene cloning analysis of the catalase

The genome of the strain FZSF01 was extracted using the Bacteria Genomic DNA Extraction Kit Ver.3.0 (TaKaRa Bio Inc., Dalian, China). All primers are shown in Supplementary Table 1. Partial sequence of the catalase was obtained by PCR using the degenerate primers F1 and R1 designed from the catalase gene sequences of *Serratia* spp. strains in GenBank. Complete sequence of the catalase gene was obtained by hiTALE-PCR [21]. The primers KatF and KatR were used to amplify the whole sequence of the catalase gene.

The obtained catalase gene was compared to those of other *Serratia* spp. strains by first querying NCBI database using the BLASTN program with an E value $<10^{-5}$. Nucleotide sequences that showed high similarity with this catalase gene in GenBank were downloaded and selected to construct a phylogenetic tree with MEGA 4.0.2 [14]. Protein modeling was performed on SWISS-MODEL [22,23] with 1e 93.1.A as the template.

2.10. Gene expression in Escherichia coli BL21 (DE3)

Expression vector was constructed with the pEASY-E2 expression vector (pEASY™-E2 Expression Kit, TransGen Biotech, China) [24]. Primers (KatF and KatR) used to amplify the catalase gene are listed in Table S1. Termination codon was on the 5′ region of primer KatR to avoid expression of the His-tag. Recombinant catalase expression was induced in Luria–Bertani (LB) broth containing 100 μg/ml of ampicillin and 1 mM of IPTG. Incubation conditions of the recombinant *E. coli* were 37°C and 220 rpm for 16 h.

3. Results and discussion

3.1. Bacterial screening and identification

Nine strains among 36 tested showed significant catalase activity (Table S2), and strain No. 4 with the highest activity was named FZSF01. After testing three times, the same strain, FZSF01, remained the most stable, with crude catalase production of approximately 1800 U/ml. The initial catalase activity of *S. marcescens* SYBC01 is 1888.9 U/ml [25] and that of *S. marcescens* SYBC08 is 1401 U/ml [26]. These values indicated that FZSF01 had high H_2O_2 catalytic capability.

The 16S rRNA gene sequenced in this study was 1410 bp (accession number in GenBank: KT749869). On the basis of the 16S rRNA sequence phylogenetic tree (Fig. 1), FZSF01 showed over 99.65% similarity with strain *S. marcescens* ATCC13880 (T), 99.5% with strain *S. marcescens* KRED (T), 99.5% with *Serratia nematodiphila* DSM 21420 (T), and 98.65% with *Serratia ureilytica* NiVa 51 (T). According to the 16S rRNA gene sequence and its morphological features (Fig. S1), this strain was named *S. marcescens* FZSF01.

Because of potential similarities between FZSF01 and *S. marcescens* SYBC01 and *S. marcescens* SYBC08, an additional phylogenetic comparison was conducted separately. The results showed that the 16SrRNA gene sequence of FZSF01 was similar to those of strains *S. marcescens* SYBC01 at 99.1% [25] and *S. marcescens* SYBC08 at 99.5% [26]. These two strains also produce catalases [25,26].

3.2. High production of catalase with optimum conditions

FZSF01 catalase production was optimized at pH 7.0 in 2.5% (w/v) soya peptone (Fig. S3), 9% (w/v) cane sugar (Fig. S4), and 0.2% (w/v) KCl (Table S3). No biomass and catalase production were detected with citric acid. These results differed from those of *S. marcescens* SYBC08, which can grow on citric acid medium and produce catalase [26]. An optimum fermentation temperature of FZSF01 was 30°C (Fig. S2), which was similar with SYBC08 [26]. In this study, the highest catalytic activity of the crude catalase extracted from strain FZSF01 reached 51,468 U/ml after 55 h under optimized fermentation medium and conditions (Fig. 2), which was 28.6-fold the initial level (1800 U/ml). This was lower than some recently reported strains [31,32] but higher than many other reported high catalase-producing strains (Table 1), such as recombinant strain *B. subtilis* WSHDZ-01 (39,117 U/ml) [33], *B. subtilis* WSHDZ-01 (28,990 U/ml) [34], *Exiguobacterium oxidotolerans* T-2-2T (22,000 U/ml) [35], *S. marcescens* SYBC08 (20,353 U/ml) [26], and *R. radiobacter* strain 2-1 (17,035 U/ml) [36]. High production of catalase is a precondition for potential industrial-scale application [33]. Although many catalase-producing microbes have been isolated from various species, successfully purified, and characterized, few have the ability to produce more than 10,000 U/ml in fermentation broth [4]. This is the case for *Penicillium* spp. (400û1400 U/ml) [27], *Penicillium variabile* (P16) (700 U/ml) [28], *Alternaria alternata* (461 U/ml) [29], and *Saccharomyces cerevisiae* (561 U/ml) [6]. Future research on optimizing the production conditions may further enhance the performance of FZSF01.

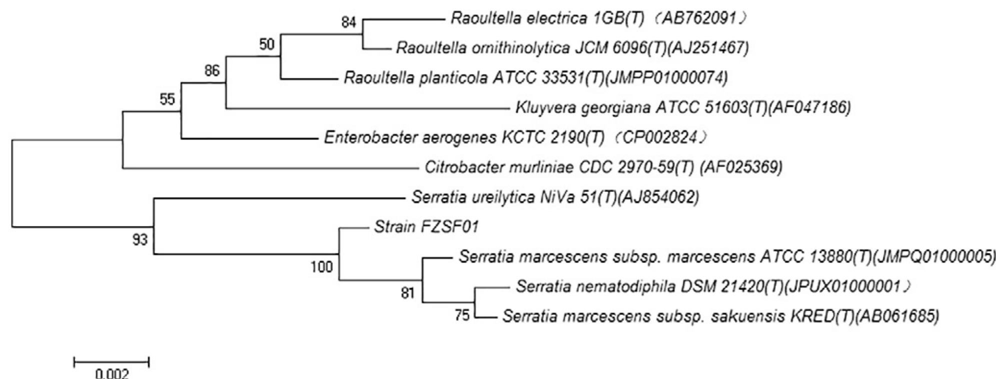


Fig. 1. Phylogenetic tree of *Serratia marcescens* FZSF01 showing the relationship with other strains using 16S rRNA gene sequencing. Most related 10 type strains with more than 97.8% similarity were chosen to construct the phylogenetic tree. Phylogenetic tree was constructed with MEGA 4.0.2 with the Neighborhood-Joining method and the branch points represent values from 1000 replicate test.

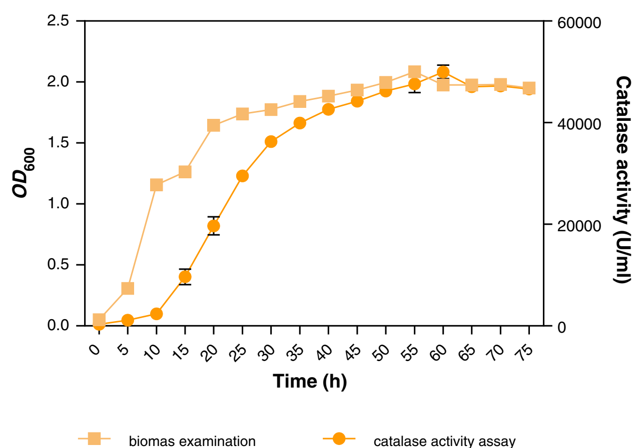


Fig. 2. Growth and catalase production curve of strain FZSF01. The strain was fermented in a 250-ml flask with 50 ml liquid medium at 30 °C. Samples were loaded and used for biomass examination and catalase activity assay.

3.3. Purification of catalase

Purification of the catalase of FZSF01 from fermentation broth was accomplished in three steps (Table 2). The DEAE-Sepharose recovered 42.5% of catalase from the extract, reaching 5.5-fold purity. The use of Millipore for concentrating the enzyme was effective, with a loss of only 7.1%. Highly purified catalase was obtained with 10-fold purity following the use of the Sephadex-G150 column. Specific activity of the purified catalase was 197,575 U/mg, which was similar to that of *S. marcescens* SYBC08 (199,585 U/mg) [37]. FZSF01 catalase had a higher value than many other catalases, such as *Bifidobacterium asteroids* JCM8230 (6400 U/mg) [38], *Comamonas terrigena* N_3H (55,900 U/mg) [39], and *Deinococcus radiodurans* (68,800 U/mg) [40]. *Vibrio rumoiensis* (395,000 U/mg) [41] and *Neurospora crassa* (1,404,891 U/mg) [42] are two catalases with specific activities higher than that of FZSF01 catalase. The purified catalase showed a molecular mass of 58 kDa on SDS-PAGE gel (Fig. 3), which was the same as that of catalase from *S. marcescens* SYBC08 [37].

3.4. Effects of temperature and pH on catalase activity

The effects of temperature on the activity and stability of the purified catalase showed that the optimum reaction temperature of the catalase was 50°C, and a high level of activity (>80%) was maintained between 20°C and 80°C (Fig. 4A). Many catalases show high activity levels (>80% activity) over a wide range of temperatures: for example, *S. marcescens* SYBC08 at temperatures ranging from 0°C to 70°C [37], *Psychrobacter piscatorii* T-3 from 10°C to 60°C [43], and *B. altitudinis* SYBC hb4 from 20°C to 40°C [8]. However, the optimum reaction temperature of FZSF01 catalase was higher than that of *S. marcescens* SYBC08 (20°C) [37], *P. piscatorii* T-3 (45°C) [43], and *B. altitudinis* SYBC hb4 (30°C) [8]. Therefore, FZSF01 catalase could be used under temperature conditions that could help save energy, particularly in industries where high temperature conditions are unavoidable [25].

The FZSF01 catalase retained over 90% of its residual activity after 120 min under 50°C and over 70% at 60°C (Fig. 4B). The thermostability of FZSF01 catalase was very similar to that of *S. marcescens* SYBC08 [37]. The FZSF01's stability was better than other catalases, such as those of *V. rumoiensis* S-1T (65°C, 10 min, 0%) [41], *Vibrio salmonicida* (60°C, 20 min, 0%) [44], and *Halomonas* spp. SK1 (55°C, 30 min, 0%) [45]. This suggested that FZSF01 could be advantageous in high-temperature processes because it can be inactivated (0% of residual activity) by treating at a high temperature of 80°C, for a long period of time, 120 min.

The effect of pH on FZSF01 catalase showed that the enzyme was active in a wide range of pH values, from 5.0 to 11.0 (Fig. 5). Under alkaline conditions (pH 7.0–11.0), the relative activities of the catalase were higher than 85%, with highest activities at pH 10.0 and 11.0. These values differ from those of *S. marcescens* SYBC08, which exhibits maximal activity between pH 7.0 and 9.0 [37]. FZSF01 catalase appeared to be more active under alkaline conditions than that of *Rhodospirillum rubrum* S1 (optimal at pH 7.0 and 8.0) [46] and *B. altitudinis* SYBC hb4 (optimal at pH 5.0) [8]. Catalase from *Acinetobacter* sp. YS0810 [47] also has its maximal activity at pH 11.0, but its relative activity is much lower at pH 5.0 and 6.0 than FZSF01 catalase. Alkaline catalases are usually suitable for textile and paper processing industries as these processes are performed under alkaline conditions [1].

3.5. Kinetics of the catalase

Km of this catalase was 68 mM, and *Vmax* was 1886.8 mol/min mg (Fig. 6). The *Km* value was similar with that of *S. marcescens* SYBC 08 (78 mM) [37] and *V. rumoiensis* (70 mM) [41]. With a value of 589 mM, the catalase produced by *B. asteroids* JCM8230 [38] has a higher *Km*. Considering that *Km* reflects the catalase affinity to H_2O_2, FZSF01 catalase may be considered average in terms of performance.

Table 2
Summary of purification of catalase from *Serratia marcescens* FZSF01.

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (Fold)	Yield (%)
Crude extract	372	7,244,179	19,473	1	100
DEAE-Sepharose	28.6	3,077,231	107,532	5.5	42.5
Millipore	25.4	2,858,027	112,520	5.8	39.5
Sephadex-G150	9.6	1,896,720	197,575	10.1	26.2

Table 1
Comparison of fermentation levels of different high catalase-producing strains.

Strains	Medium	Fermentation modes	Time (h)	Activity (U/ml)	Reference
Geobacillus sp.	Yeast extract, sucrose	1.0 l bioreactor	12 h	105,000 U/mg	[30]
Recombinant *E coli* BL21	Luria–Bertani (LB)	Shake flask 10-l fermenter	50 h	78,762	[31]
Ureibacillus thermosphaericus	Yeast powder, maltodextrin	Shake flask	32 h	57,630	[32]
Serratia marcescens FZSF01	Soya peptone, cane sugar	Shake flask	55 h	51,468	This study
Recombinant *Bacillus* sp. WSHDZ-01	Glucose, $NaNO_3$	3-l STR	48 h	39,117	[33]
Recombinant *E coli* BL21	LB medium	Shake flask	20 h	35,831	[24]
Bacillus sp. WSHDZ-01	Glucose, $NaNO_3$, H_2O_2, ethanol	Shake flask	50 h	28,990	[34]
Exiguobacterium oxidotolerans T-2-2T	Polypeptone, yeast extract	Shake flask	24 h	22,000	[35]
Serratia marcescens SYBC08	Corn steep liquor, citric acid	7-l fermenter	36 h	20,353	[26]
Recombinant *E coli* BL21	LB medium	Shake flask	16 h	17,267	This study
Rhizobium radiobacter strain 2-1	Polypeptone, yeast extract	Shake flask	22 h	17,035	[36]
M. luteus	Polypeptone, yeast extract	Shake flask	22 h	6920	[36]

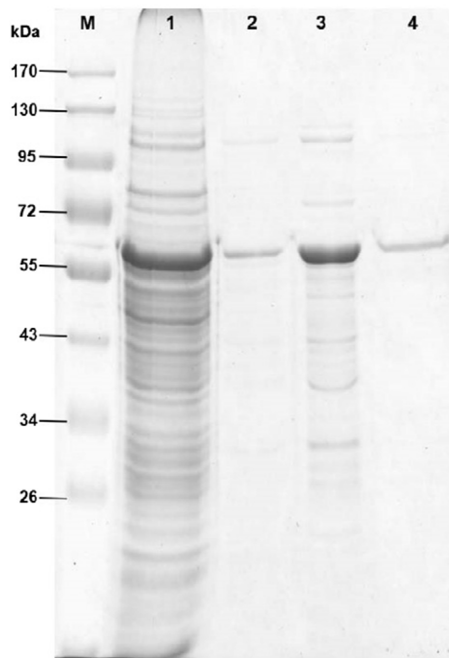


Fig. 3. Electrophoretic analysis of catalase from *Serratia marcescens* FZSF01 by SDS-PAGE. Lane M, marker; Lane 1, crude catalase prepared by centrifugation and ultrasonication of the fermentation broth; Lane 2, portion with catalase activity after DEAE-Sepharose step; Lane 3, concentration of the DEAE-Sepharose portion with Millipore filtration; Lane 4, portion with catalase activity after sephadex-G150 step.

3.6. Identification with LC–MS/MS

LC–MS/MS results showed that FZSF01 catalase highly matched heme-containing monofunctional catalases, such as *S. marcescens* RSC-14 (UniProt number A0A0M4JH38), *S. marcescens* SmUNAM836 (UniProt number A0A0M4RXQ9), *S. marcescens* SYBC08 (UniProt number D7RJ66), and *S. marcescens* SM39 (UniProt numberW0SWB1) (Table S4). Because of their similarities, these gene sequences were then selected for the next phase of cloning and analysis of the FZSF01 catalase gene.

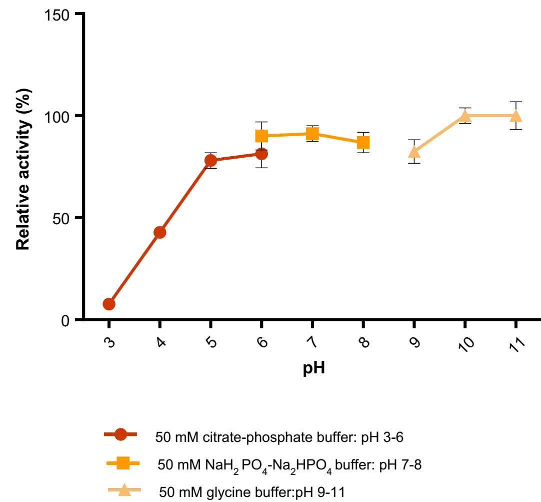
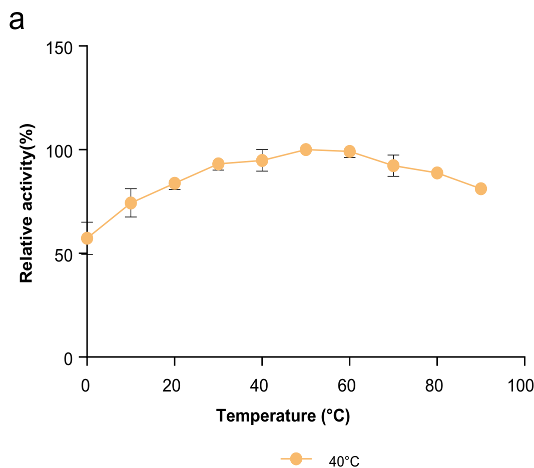


Fig. 5. Effect of pH on the catalase activity. Activities were measured with 20 mM of H_2O_2 prepared with different pH buffers from 3.0 to 11.0. The relative activity at optimum pH was defined as 100%, and relative activity at other pH values was calculated; 50 mM citrate–phosphate buffer: pH 3.0–6.0; 50 mM NaH_2PO_4–Na_2HPO_4 buffer: pH 7.0–8.0; and 50 mM glycine buffer: pH 9.0–11.0.

3.7. Cloning and analysis of the catalase gene

Partial sequence of the catalase gene (1352 bp) was obtained with the degenerate primers F1 and R1. HiTAILE-PCR primers were designed from this partial sequence and the whole sequence was obtained with HiTAILE-PCR. The entire FZSF01 catalase gene contained 1473 bp. Nucleotide and amino sequences were both compared with those of other species using BLAST in NCBI and showed similarities more than 90% (96.5%–98.5%) with 11 sequences, all belonging to the genus *Serratia*. FZSF01 gene was submitted to GenBank (Accession number KU350659) and a phylogenetic tree of this gene was constructed with those 11 similar catalase gene sequences (Fig. 7). These nucleotide sequences separated into two groups on the phylogenetic tree, with FZSF01 and SYBC08 on different branches (Fig. 7). The results suggest that these two strains may have evolved from others over a long period of time. Among these 11 sequences, only the sequence of *S. marcescens* SYBC08 (96.5% similarity) was ever



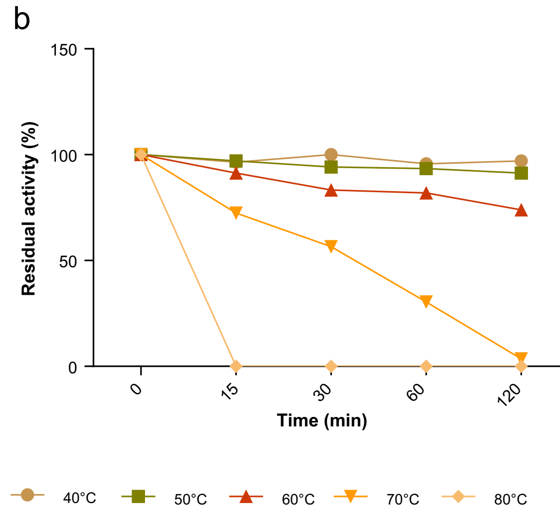


Fig. 4. Effect of temperature on the catalase from *Serratia marcescens* FZSF01. (a) Optimum reaction temperature of the catalase; 20 mM of H_2O_2 solutions was incubated at temperatures from 0°C to 90°C, and catalase activity at different temperatures was measured. Activity at optimum reaction temperature (50°C) was defined as 100% and relative activity at different temperatures was calculated. (b) Thermostability of the catalase; catalase was incubated at temperatures from 40°C to 80°C at different time periods, and the residual activities were measured. Catalase activity with no temperature incubation was defined as 100% activity.

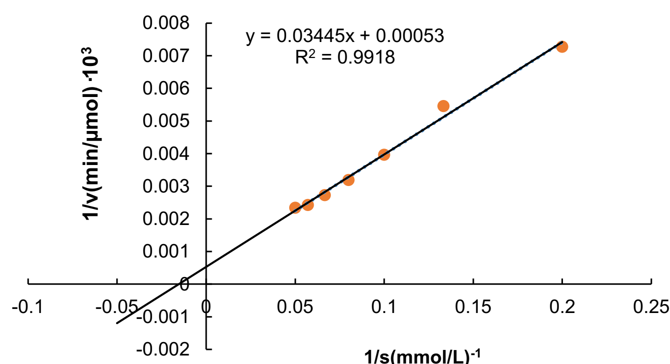


Fig. 6. Lineweaver–Burk plot of the catalase from *Serratia marcescens* FZSF01.

cloned and enzymatically characterized [37]. Therefore, FZSF01 catalase represented a second catalase of genus *Serratia* to be examined further in depth.

FZSF01 nucleotide sequence-encoded protein had 478 amino acids with three catalase-typical domains and conserved residuals: heme-binding domain (H_{54}, S_{93}, N_{127}, F_{132}, F_{140}, R_{333}, and Y_{337}), NADPH-binding domain (P_{130}, H_{173}, I_{177}, S_{180}, R_{182}, H_{192}, $H2_{14}$, and $VWPH_{281–284}$), and tetramer ($GN_{120–121}$, $DPLKF_{136–140}$, H_{145}, $PHTNLR_{151–156}$, and D_{163}) domain. These domains are highly conserved in *Serratia* spp. and others, such as *Danio rerio*, *Rugosa rugosa*, *Methanosarcina barkeri* strain, and *Drosophila melanogaster* (analyzed in NCBI, data not shown). FZSF01 catalase contained 29 amino acid sequences with more than 97% similarity with other *Serratia* spp. Among these 29 catalase sequences, eight amino acids were weakly conserved (Table S5) and could result in differential enzymatic characteristic when compared to others such as SYBC08 catalase.

Further analysis between FZSF01 and SYBC08 strains showed that there were nine amino acids in FZSF01 catalase that differed from those in strain SYBC08 (Fig. 8). Using the structure of *V. salmonicida* catalase [48], the aspect of structure location of three of these nine amino acid residual sites in FZSF01 and SYBC08 was further analyzed (Fig. S5). For catalase, one amino acid mutation may result in significant change in enzymatic properties. For example, mutation from Lys114 to Tyr increases the catalytic efficiency of *B. subtilis* catalase by 5.3-fold [9]. Mutating Arg260 into alanine in *E. coli* increases the activity of the HPII catalase by 3-fold [49]. In this study, how the substitution of amino acid residuals has had affected the enzyme property remains unknown, and further investigation is necessary to better understand the role of these three amino acids.

3.8. Expression of the catalase gene in E. coli

The recombinant plasmid of the catalase gene was successfully transferred into *E. coli* BL21 (DE3), and the recombinant protein was consistent with the purified catalase in terms of molecular size (Fig. 9). The catalase was mainly expressed as an inclusion body. The fermentation broth showed limited results with lower solubility and level of activity at 17,267 ± 2037 U/ml. This level was lower than that in the original broth, suggesting that this type of recombinant might not be optimal, and further research would be required to determine a better recombination system. Some bacteria (e.g., *Pigmentiphaga* sp. DL-8 [10], *Proteus mirabilis* [50], *Mycobacterium tuberculosis* [51], and *Lactobacillus plantarum* [52]) have been previously used for recombinant catalase production in *E. coli*, with production ranging from 431 U/mg [51] to 95,000 U/mg [10]. A study reports that the recombinant alkaline catalase (KatA) in *E. coli* BL21 is a highly soluble form that can reach 78,762 U/ml of fermentation broth [31]. Recombinant catalase may present advantages if it is possible to enhance its activity and solubility. Shi et al. [53] have transferred *Kat*A gene from *B. subtilis* into *B. subtilis* WB600, and its recombinant can achieve catalase activity levels of 3500 U/ml, compared to 0 U/ml initially. Xu et al. [33] improved the catalase production of *B. subtilis* WSHDZ-01 by self-cloning the catalase gene, achieving a level of 39,117 U/ml, which is more than four times greater than the original strain.

4. Conclusion

In this study, we identified and genetically and enzymatically characterized a new catalase-producing strain, *S. marcescens* FZSF01. This catalase reached activity levels of 51,468 U/ml, which might represent one of the most productive enzymes recorded from a bacterium to date. It also exhibited broad levels of activity, from 0°C to 60°C and pH 5.0 to 11.0. These features suggest that this enzyme can efficiently remove residual H_2O_2 after cotton fabric bleaching, which will save much power and water than the traditional procedure, which involves several times of washing with lots of water. Furthermore, we could encode its gene in *E. coli* BL21, but the solubility and level of activity remained low. Further research would be needed to enhance the effectiveness of the recombinant to effectively produce high level of

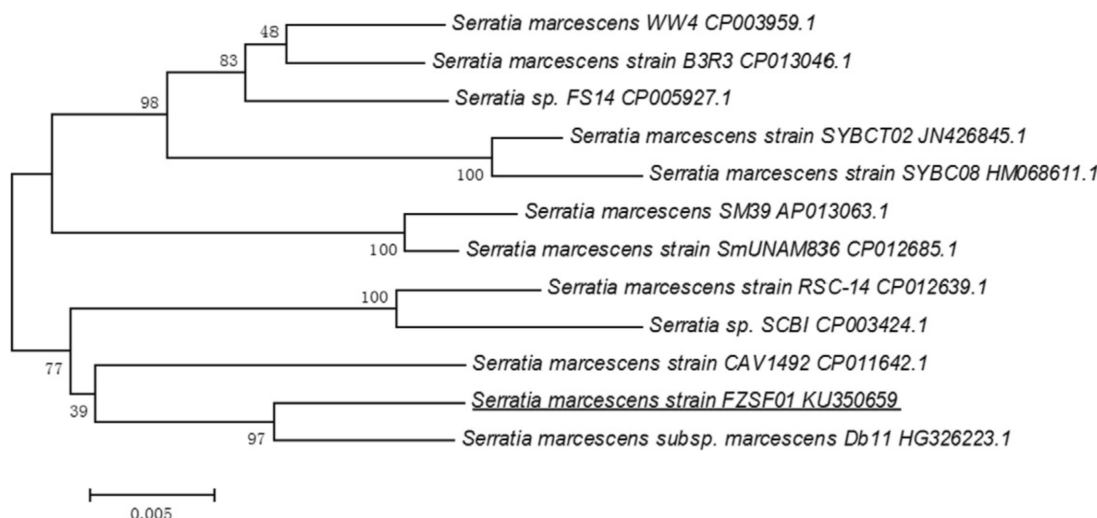


Fig. 7. Phylogenetic tree of *Serratia marcescens* FZSF01 catalase nucleotide sequence showing the relationship with other strains on catalase nucleotide sequences. Catalase nucleotide sequences with similarity greater than 90% were chosen to construct the phylogenetic tree. Branch points represent the values when test was repeated for 1000 times.

```
SYBC08    MSKKGLGTAVGAPVVDNNNVITAGKRGPMLLQDVWFLEKLAHFDREVIPERRMHAKGSGA    60
FZSF01    MSRKGLTTAAGAPVVDNNNVITAGKRGPMLLQDVWFLEKLAHFDREVIPERRMHAKGSGA    60
Consensusms kgl ta gapvvdnnnvitagkrgpmllqdvwfleklahfdreviperrmhakgsga

SYBC08    YGTFTVTHDITRYTRAKIFSEIGKQTDMFIRFSTVAGERGAADAERDIRGFAMKFYTEEG    120
FZSF01    YGTFTVTHDITRYTRAKIFSEIGKQTDMFIRFSTVAGERGAADAERDIRGFAMKFYTEEG    120
Consensusygtftvthditrytrakifseigkqtdmfirfstvagergaadaerdirgfamkfyteeg

SYBC08    NWDLVGNDTPVFYLRDPLKFPDLNHVVKRDPHTNLRNPVYKWDFFSHLPESLHQLTIDFS    180
FZSF01    NWDLVGNDTPVFYLRDPLKFPDLNHVVKRDPHTNLRNPVYKWDFFSHLPESLHQLTIDFS    180
Consensusnwdlvgndtpvfylrdplkfpdlnhvvkrdphtnlrnpvykwdffshlpeslhqltidfs

SYBC08    DRGIPKSYRHMHGFGSHTFSFINAANERFWVKFHFRCEQGIENLMDEEAEAIIAKDRESS    240
FZSF01    DRGIPKSYRHMHGFGSHTFSFINAANERFWVKFHFRCEQGIENLMDEEAEAIIAKDRESS    240
Consensusdrgipksyrhmhgfgshtfsfinaanerfwvkfhfrceqgienlmdeeaeaiiakdress

SYBC08    QRDLFEAIKRGDFPRWKLQIQIMPEHEASQTPYNPFDLTKVWPHGDYPLIDVGYFELNRN    300
FZSF01    QRDLFDAIKRGDFPRWKLQIQIMPEHEASQTPYNPFDLTKVWPHGDYPLIDVGFFELNRN    300
Consensusqrdlf aikrgdfprwklqiqimpeheasqtpynpfdltkvwphgdyplidvg felnrn

SYBC08    PDNYFSEVEQVAMNPANVVPGISFSPDKMLQGRLFSYGDAHRYRLGVNHHQIPVNGAKCP    360
FZSF01    PDNYFSEVEQVAMNPANVVPGISFSPDKMLQGRLFSYGDAHRYRLGVNHHQIPVNGAKCP    360
Consensuspdnyfseveqvamnpanvvpgisfspdkmlqgrlfsygdahryrlgvnhhqipvngakcp

SYBC08    FHNYHRDGAMRVDGNSGNGATYEPNSFGLFQEQPDFSEPPLSIEGAADHWNHREDDDYYS    420
FZSF01    FHNYHRDGAMRVDGNSGNGATYEPNSFGLFQEQPDFSEPPLSIEGAADHWNHREDDDYYS    420
Consensusfhnyhrdgamrvdgnsgngatyepnsfglfqeqpdfsepplsiegaadhwnhredddyys

SYBC08    QPRALFNLLSAEEHQRMFTRIAGELSQVPEQIQRRQVELFTKVHPDYGAGSAQALGL       477
FZSF01    QPRALFNLLSAEEHQRMFTRIAGELSQVPEHIQRRQVELFTKVHPDYGAGVTKALGL       477
Consensusqpralfnllsaeehqrmftriagelsqvpe iqrrqvelftkvhpdygag   algl
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Fig. 8. Multiple sequence alignment of FZSF01 and SYBC08 catalase amino acid sequences. The homologous amino acid residuals are given in box with gray color and the different residuals are blank.

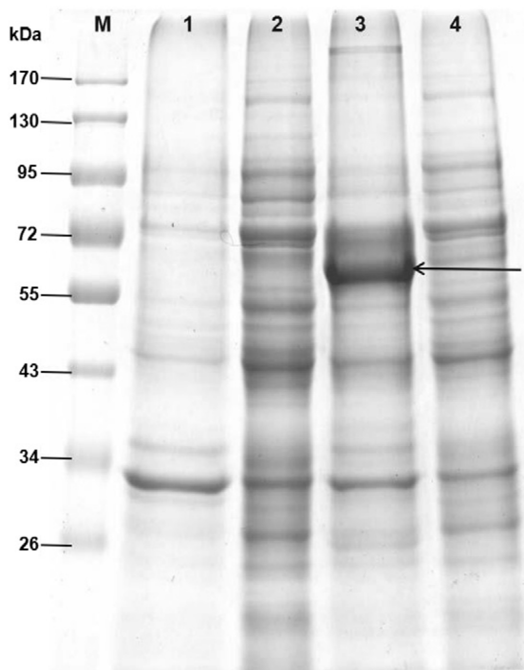

Fig. 9. SDS-PAGE analysis of recombinant catalase of *Serratia marcescens* FZSF01 expressed in *Escherichia coli*. Lane M, marker; Lane 1, sediment of the recombinant *E. coli* without IPTG induction; Lane 2, supernatant portion of the recombinant *E. coli* without IPTG induction; Lane 3, sediment portion of the recombinant *E. coli* with 1 mM IPTG induction; Lane 4, supernatant portion of the recombinant *E. coli* with 1 mM IPTG induction. The arrow showed position of the recombinant catalase.

catalase activity. In summary, *S. marcescens* FZSF01 and its recombinant *E. coli* may represent a competitive alternative in industrial catalase production.

Conflict of interest

None declared.

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Supplementary data

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