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Heterologous expression and characterization of a recombinant thermostable alkylsulfatase (*sdsAP*)

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Abstract A novel alkylsulfatase gene, *sdsAP*, was cloned from a newly isolated bacterium *Pseudomonas* sp. S9. It encoded a protein of 675 amino acids with a calculated molecular mass of 74.9 kDa. The protein contained a typical N-terminal signal peptide of 41 amino acid residues, followed by a metallo-β-lactamase like domain at the N-terminus and a SCP-2-like domain at the C-terminus. This domain organization mode suggested that it belonged to the type III sulfatase. The mature alkylsulfatase was overexpressed in *Escherichia coli*. The optimal temperature and pH of the recombinant SdsAP were 70°C and 9.0, respectively. Notably, at optimal conditions, the purified recombinant SdsAP had a high specific activity of 23.25 μmol min^{-1} mg^{-1}, a $K_{m\ (app)}$ of 264.3 μmol, and a $V_{max\ (app)}$ of 33.8 μmol min^{-1} mg^{-1} for SDS. Additionally, it still retained more than 90% activity after incubation at 65°C for 1 h, which was much different from other alkylsulfatases reported. The recombinant enzyme hydrolyzed the primary alkyl sulfate such as sodium octyl sulfate and sodium dodecyl sulfate (SDS). It was a Zn^{2+}-containing and Ca^{2+} activated alkylsulfatase. This is the first report to explore the various characteristics of the heterologous recombinant alkylsulfatase in details. These favorable properties could make SdsAP attractive to be useful in the degradation of SDS-containing waste.

Keywords Alkylsulfatase · *Pseudomonas* sp. · Characterization · Mangrove · Thermostability · SDS

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Introduction

Sulfatase (EC 3.1.6.X) is a family of hydrolytic enzyme that cleaves the sulfate ester bond to liberate inorganic sulfate and the corresponding alcohol (Pogorevc and Faber 2003). At present, there are at least three mechanistically distinct groups of sulfatases. The first type (type I), was named as arylsulfatases. The arylsulfatases from eukaryotes were the best studied. They undergo a unique post-translational modification to produce formylglycine residue in their active site (Berteau et al. 2006; Marquordt et al. 2003). The Fe^{2+} α-ketoglutarate-dependent dioxygenase superfamily constitutes the second type (type II) of sulfatases. These enzymes require α-ketoglutarate as a cosubstrate, and oxygen and Fe^{2+} are also needed for their activity (Kahnert and Kertesz 2000). In 2006, alkylsulfatases (type III) were defined as members of the metallo-β-lactamase superfamily (Hagelueken et al. 2006). These alkylsulfatases harbor Zn^{2+}-binding motif and could degrade sodium dodecyl sulfate (SDS).

Alkylsulfatases have mostly been found in Gram-negative bacteria; the only exception to date is *Bacillius cereus* (Singh et al. 1998). In 1980s, most of the work on

alkylsulfatases was carried out with a Gram-negative bacteria, *Pseudomonas* sp. strain C12B (=NCIMB 11753 = ATCC 43648). It was shown that under appropriate culture conditions *Pseudomonas* sp. strain C12B secreted up to five different alkylsulfatases (three secondary alkylsulfatases designated S1, S2, S3, and two primary alkylsulfatases designated P1 and P2, which are specific for the hydrolysis of *sec*- and *prim*-alkyl sulfates, respectively) (Dodgson and White 1983). These five enzymes have been purified to homogeneity, respectively. S1, S2, and S3 in strain C12B exhibited positional and stereo-specificity, S1 being active towards S-alkyl 2-sulfates, S2 towards R-alkyl 2-sulfates (White 1991), and S3 towards symmetrical and near-symmetrical secondary alkyl sulfates. P1 and P2 were both able to hydrolyse primary alkyl sulfate esters of chain lengths C_6–C_{12} (Bateman et al. 1986). The P1 enzyme was tetrameric and appeared during the early stationary phase of strain C12B growth; in contrast, the P2 enzyme was dimeric and appeared in mid-exponential growth phase (Cloves et al. 1980a, b). The three secondary alkylsulfatases and P2 enzyme splitted C–O bond of the C–O–S linkage, but only P1 enzyme splitted O–S bond (Bartholomew et al. 1978; Cloves et al. 1980a; Dodgson et al. 1974). However, the first gene of alkylsulfatase was not cloned and sequenced until 1992 (Davison et al. 1992).

SDS, an anionic surfactant, has wide industrial application as detergent ingredients. Excessive use of detergents has detrimentally affected environment. SDS has been considered to be biodegradable by aerobic processes (Cserháti et al. 2002). Till now, many SDS-degrading strains have been found in *Pseudomonas* (Lillis et al. 1983; Davison et al. 1992) as well as in *Klebsiella* (Shukor et al. 2009), *Vibrio* (Bryant et al. 1987), *Acinetobacter*, *Pantoea* (Abboud et al. 2007), and other genus. However, few alkylsulfatases to date have been used extensively for applications of SDS biodegradation.

In this report, the isolation and identification of an alkylsulfatase-producing bacterium *Pseudomonas* sp. S9 from mangrove soil of Xiamen, China, was described, and an alkylsulfatase gene was cloned and expressed in *Escherichia coli*. The characteristics of the purified heterologous recombinant alkylsulfatase were first explored in detail. The noticeable thermal stability and higher specific activities make it an ideal candidate for the application in disposing SDS-containing waste.

Materials and methods

Bacterial strains, plasmids, and culture conditions

E. coli DH5α and *E. coli* BL21 (DE3) were used as the hosts for cloning and expression, which were routinely grown at 37°C in Luria–Bertani (LB) broth supplemented with ampicillin (100 μg ml^{-1}) when required. *E. coli* DH5α electro competent cells for genomic library construction were purchased from TaKaRa, and the plasmid pUC19/*Bam*HI was purchased from Fermentas. The alkylsulfatase-producing bacterial strains were cultured at 30°C using LB broth supplemented with 0.1% (w/v) filter-sterilized SDS.

Isolation and identification of SDS-degrading strain S9

Strain S9 was isolated from a mangrove soil sample taken from the coastal area of Xiamen, China. Soil samples around mangrove roots were collected, diluted with sterile water, and plated onto solid LB medium containing 0.1% SDS. After incubation at 30°C for 3–5 days, colonies with opaque halos were picked out and purified under the same conditions. The strain S9 responsible for the biggest opaque halos was isolated.

16S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal primers 27F and 1492R (Weisburg et al. 1991). The PCR was programmed as follows: denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 54°C for 1 min, 72°C for 1.5 min, and with a final extension at 72°C for 10 min. The purification of PCR products was performed using the Gel Extraction Kit (Omega Bio-Tek) according to the manufacturer's instruction and cloned into pMD18-T (TaKaRa) for sequencing. The 16S rRNA gene sequence was analyzed by using the EzTaxon database (Chun et al. 2007) to obtain the closely matched species.

Cloning of the *sdsAP* gene

The chromosomal DNA of *Pseudomonas* sp. S9 was extracted using the method described before (Syn and Swarup 2000). The genomic DNA was partially digested with *Sau*3AI and separated on a 0.7% agarose gel. Then the fragments with their size ranging from 3 to 10 kb were collected, purified, and ligated into *Bam*HI-linearized pUC19 vector. The ligation mixture was transformed into *E. coli* DH5α to construct a genomic DNA library. Positive clones expressing alkylsulfatase were screened out when they generated opaque halos after incubation on LB plates supplemented with SDS (0.1%; w/v) and ampicillin (100 μg ml^{-1}) at 37°C for 16–24 h. Positive clones were sequenced to obtain the *sdsAP* gene.

Sequence analysis and classification of *sdsAP* gene

Gene sequence assembly and analysis were performed with the DNAMAN Software (Lynnon BioSoft). The signal

peptide in the deduced amino acid sequence was predicted using SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). The alignments of DNA and protein sequences were conducted with blastn and blastp programs, respectively (http://www.ncbi.nlm.nih.gov/BLAST/).

Expression and purification of recombinant SdsAP

The *sdsAP* gene without the signal sequence was amplified by PCR using primers sds-F: 5′-ATAGGATCCGCAG AAACAGCTAAGCCTG-3′ and sds-R: 5′-AACGAATTC TTATGGCGTCACAATGTTG-3′ containing *Bam*HI and *Eco*RI site (underline), respectively. The chromosomal DNA of *Pseudomonas* sp. S9 was used as the PCR template. The PCR product was purified and digested with *Bam*HI and *Eco*RI. Then it was cloned into the pET-His expression vector, which was digested with the same restriction enzymes. The plasmid pET-His-SdsAP was analyzed by digestion with restriction enzymes and DNA sequencing, and then transferred into *E. coli* BL21 (DE3). The *E. coli* BL21 (DE3) cells containing the alkylsulfatase gene were grown at 37°C in LB medium supplemented with ampicillin (100 μg ml^{-1}) until the cell density reached $OD_{600} = 0.4–0.6$. Then, a final concentration of 50 μM isopropyl-β-D-galactopyranoside (IPTG) was added. The cultivation was continued at 16°C and 200 rpm for 10–12 h. *E. coli* BL21 (DE3) containing pET-His vector was used as the negative control.

The induced *E. coli* BL21 (DE3) cells bearing the alkylsulfatase gene (pET-His-SdsAP) were harvested by centrifugation and the His-tagged alkylsulfatase was purified with nickel-nitrilotriacetic acid agarose under native conditions according to the recommendations of the manufacturer (QIAGEN, Valencia).

SDS–PAGE and molecular weight analysis

SDS–PAGE was performed on a 10% gel (Laemmli 1970) and the protein concentration was assayed by the Bradford method (Bradford 1976).The purified recombinant SdsAP was subjected to gel filtration using a Sepharose G-150 coloumn (1 cm × 100 cm). The elution volume of standard proteins of Ovalbumin (48 kDa), Albumin (63.5 kDa), Adolase (177 kDa), and Catalase (206 kDa) (1 mg each) were first detected with the flow rate of 250 μl min^{-1}, followed by gel filtration of recombinant SdsAP under the same condition. The molecular weight curve of standard proteins was thus constructed based on the data above and the molecular weight of SdsAP was calculated.

Enzyme activity assay

The activity of alkylsulfatase was assayed using stains-all solution method (Rusconi et al. 2001). 10 μl diluted enzyme solution (150 μg ml^{-1}) was mixed with 490 μl of 50 mM Tris–HCl buffer (pH 9.0) containing SDS with a final concentration of 0.01% (w/v). After incubation at 70°C for 5 min, the reaction was terminated by adding 10 μl sample solution to 200 μl stains-all solution. The absorbance of SDS quantitation was measured at 438 nm and compared with the standard curve of SDS. Enzyme activity (*U*) was defined as the amount of enzyme that degraded 1 μmol SDS per minute.

Effects of pH and temperature on SdsAP activity and stability

The optimal temperature of SdsAP was determined by carrying out the enzyme activity assay at different temperatures (30–90°C) in 50 mM Tris–HCl buffer (pH 9.0) for 5 min. The optimal pH of SdsAP was assayed at 70°C in a pH range of 4.0–11.0. The relative activity was defined as the percentage of activity determined with respect to the maximum SdsAP activity. Furthermore, the thermostability of recombinant SdsAP was determined by measuring the residual activity of the enzyme, exposed to three different temperatures: 60, 65, and 70°C in Tris–HCl buffer, 50 mM, pH 9.0 for 1 h. Samples were removed at certain time intervals and the remaining activity was assayed under standard conditions. The pH stability of the SdsAP was determined by pre-incubating the enzyme in each solution pH (4.0–11.0) at room temperature for 1 h and then measuring the residual enzyme activity under the standard condition.

Effects of various reagents on SdsAP activity

To analyze the effects of metal ions, chelators, and reducing reagents on SdsAP activity, different concentrations (10, 100 mM) of various ions and reagents were added to the reaction mixture, and the alkylsulfatase activity was assayed at pH 9.0 and 70°C. The relative activity was defined as the percentage of activity determined with respect to that measured under the standard condition described previously.

Strain and nucleotide sequence accession numbers

Strain S9 was deposited in China General Microbiological Culture Collection Center under the accession number 4026. The 16S rRNA gene and the *sdsAP* gene sequences from strain S9 were submitted to GenBank database under accession numbers HQ189532 and HQ189533, respectively.

Results

Isolation and identification of strain S9

Eight alkylsulfatase-producing bacterial strains were screened from the mangrove soil. Strain S9 responsible for the biggest opaque halos was selected for further study. Analysis of 16S rRNA gene sequences of strain S9 showed 99% sequence similarity to the sequence of *Pseudomonas segetis* strain FR1439^T. Of the 100 BLAST sequence matches, all of which showed between 95 and 99% sequence similarity, 97 bacteria belong to the genus *Pseudomonas*, one was *Flavimonas oryzihabitans* IAM 1568^T, one was *Azomonas macrocytogenes* IAM 15003^T, and one was *Azomonas insignis* NCIB 9963. These results indicated that strain S9 should represent a *Pseudomonas* species, named *Pseudomonas* sp. S9.

Cloning, sequencing and analysis of the alkylsulfatase-encoding gene

The genomic library of *Pseudomonas* sp. S9 was spread on LB plates supplemented with SDS (0.1%; w/v) and ampicillin (100 μg ml^{-1}). Among these transformants, two clones expressing alkylsulfatase activity generated opaque halos. A positive clone, named pUB-PS9, was inserted with a smaller fragment, approximately 3 kb. The insert contained an open reading frame (ORF) of 2,025 bp, designated as *sdsAP*, encoding a polypeptide of 675 amino acid residues with an estimated molecular mass of 74.9 kDa and a pI of 5.83. Further analysis with the SignalP 3.0 server showed that it contained an N-terminal signal peptide of 41 amino acid residues and the most likely cleavage site was between the amino acids Ala^{41} and Ala^{42} that possessed a typical AXA motif for signal peptidase I (Tuteja 2005).

The predicted translational ATG start codon is preceded by a putative AAGGAG Shine–Dalagarno sequence (SD), between nucleotide 7 and 13 upstream (Fig. 1). An inverted repeat sequence, rich in GC, was found downstream from the stop codon TAA that was identified as a transcription termination signal.

Two functional domains were found in the coding region. A metallo-beta-lactamase superfamily domain was located between 141 and 367 amino acid residues. And a SCP-2 sterol transfer family domain, binding sterols, was located between 557 and 663 amino acid residues. The N-terminal domain of SdsAP also has a Zn^{2+}-binding motif (THxHxDHxGG-102-E-17-AE-44-H) (Fig. 1).

Comparison of the deduced amino acid sequence of SdsAP with other proteins in the NCBI database showed SdsAP shared 68, 67, 66, 60, and 42% identity with *Cupriavidus metallidurans* alkyl sulfatase (ABF12261), *Salmonella enterica* alkyl/aryl sulfatase (EDZ09460), and *Enterobacter cloacae* alkyl sulfatase (CBK85007), *Pseudovibrio* sp. alkyl/aryl-sulfatase (EEA95975), and *Pseudomonas aeruginosa* PAO1 SdsA1 (AAG04129), respectively (Fig. 2).

Expression and purification of SdsAP protein in *E. coli*

The *sdsAP* gene, excluding the putative signal peptide sequence, was cloned into the pET-His expression system and conditionally expressed in the *E. coli* BL21 (DE3) as an N-terminally His-tagged recombinant protein. In comparison with non-induced cells, the heterologous protein was expressed at high levels by induced cells with 50 μM IPTG. To maintain its bioactivity, the recombinant protein was purified from the supernatant under native conditions. The monomeric molecular weight of the purified enzyme (including the six-His tag) was found to be approximately 70 kDa on SDS–PAGE (Fig. 3). We also analyzed the molecular weight of native recombinant SdsAP by gel filtration. The molecular weight of native recombinant SdsAP was detected to be 147 kDa (Supplementary Fig. S1). Hence, the SdsAP in physiological conditions is in the form of dimer.

Properties of the recombinant SdsAP

Both temperature and pH effects on the activity of the recombinant SdsAP were measured. The enzyme exhibited the maximum SdsAP activity at pH 9.0. It was very stable within the range of the tested pH, retaining more than 90% activity after incubation at a wide pH range of 6.0–11.0 for 1 h (Fig. 4). The optimal temperature for SdsAP activity was 70°C, and the activity dropped sharply at temperature 90°C, possibly due to heat inactivation of the enzyme. The effect of temperature on recombinant SdsAP stability was investigated at 60, 65, and 70°C. At 60 and 65°C, the alkylsulfatase maintained more than 90% of its original activity after 1 h of incubation (Fig. 5). SDS was stable under the all detected conditions (Supplementary Fig. S2).

Under the optimal condition, the SdsAP had a high specific activity of 23.25 μmol min^{-1} mg^{-1}, a $K_{m\ (app)}$ of 264.3 μmol, and a $V_{max\ (app)}$ of 33.8 μmol min^{-1} mg^{-1} for SDS.

Effect of various additives on SdsAP activity

The effects of various additives on SdsAP activity like metal ions and other chemical reagents are shown in Table 1. The results suggested that Ca^{2+} slightly activated SdsAP, and the non-significant activation or inhibition of SdsAP was observed by metal ions (Na^+, K^+, Mg^{2+}), the reducing reagents (β-Mercaptoethanol, β-Me and DL-Dithiothreitol, DTT) and urea. Cu^{2+}, Zn^{2+}, Fe^{2+}, Co^{2+}

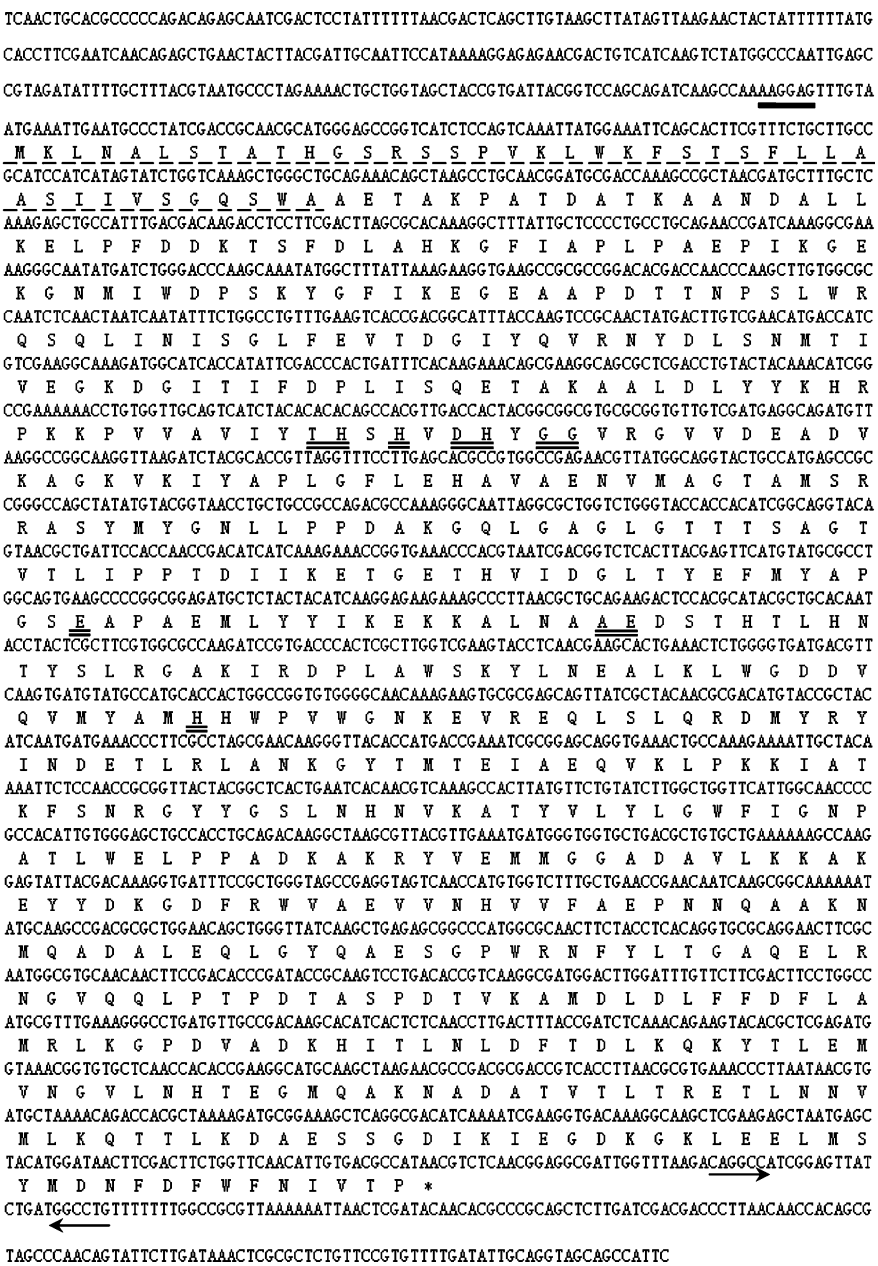

Fig. 1 Nucleotide sequence of *sdsAP* gene and deduced amino acids sequence. The putative ribosome binding site is *underlined*. The amino acids *dashed underline* at the N terminus represent the deduced signal peptide. Zn^{2+}-binding motif is *double underlined*. The translational stop codon is indicated by an *asterisk* (*). A potential transcriptional termination signal is convergent *arrowed*

and Mn^{2+}, as well as chelator (ethylenediaminetetraacetic acid, EDTA) exhibited an apparent negative effect on the enzyme activity.

Discussion

Enzymes of microorganisms existing in extreme environments have drawn considerable attention for their potential values in industry and biotechnology. The unique swampy, saline, and partially anaerobic environment of mangrove ecosystem determines the diversity and uniqueness of the native microorganisms (Jiang et al. 2006; Gao et al. 2010). In fact, unique fungi and bacteria have already been isolated from mangrove ecosystems (Takeuchi and Hatano 1998; Lin et al. 2002; Arunasri et al. 2005). Here, an alkylsulfatase-producing bacterium S9 was isolated from the mangrove soil. *Pseudomonas* sp. S9 has demonstrated optimum growth in M9 minimal medium containing SDS concentration of 0.1% (w/v) as a sole carbon source and it could be tolerance to 0.4% SDS (data not shown). Previous study showed that *Citrobacter braakii* could grow optimally at surfactant concentration of 1.0 g l^{-1} (0.1%) (Dhouib et al. 2003). Bacterial growth on higher SDS concentration at 4 g l^{-1} (0.4%) has been reported (Abboud et al. 2007). To date, the most tolerant SDS-degrading bacterium is *Pseudomonas* sp. ATCC19151 that could grow on 4% SDS (Jovcic et al. 2010). These bacterium

Fig. 2 Alignment of amino acid sequences of SdsAP and alkylsulfatases. Amino acids conserved in at least 75% are *highlighted*

successfully degraded SDS make them a good candidate for bioremediation of wastewaters.

By screening a genomic library, the *sdsAP* gene of *Pseudomonas* sp. S9 was isolated. Conserved domains analysis implied that the *sdsAP* had its metallo-β-lactamase like domain in the N-terminal and SCP-2-like domain in the C-terminal, which was similar to the molecular organization of type III sulfatase (Hagelueken et al. 2006). In the N-terminal metallo-β-lactamase domain of SdsAP, there was a Zn^{2+}-binding motif that was almost the same as the motif (THxHxDHxGG-102-E-18-AE-44-H) of SdsA from *Pseudomonas aeruginosa* (Hagelueken et al. 2006). According to the atomic absorption spectrophotometry analysis, SdsAP contained two Zn atoms in a dimer enzyme (data not shown), compared with four Zn atoms in SdsA1 from *Pseudomonas aeruginosa* (Hagelueken et al. 2006). Two Zn ions were fully occupied in the structure, and the other two were half-occupied in SdsA1. So we suspected that the two half-occupied Zn may be lost in the SdsAP maturing process. According to the substrate

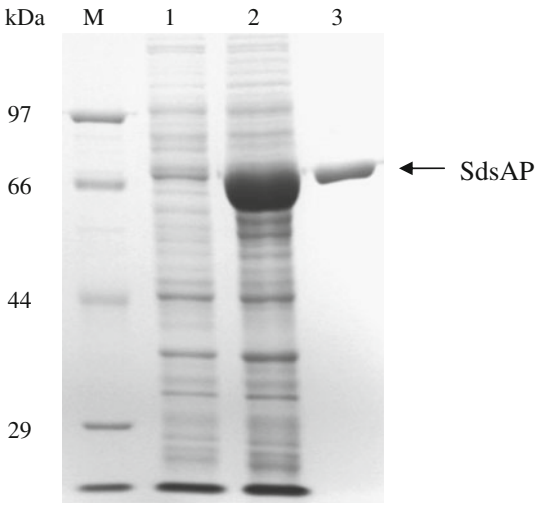

Fig. 3 SDS-PAGE analysis of recombinant SdsAP protein. *M* molecular mass marker, *lane 1* total cell protein extracts without IPTG induction, *lane 2* total cell protein extracts induced by IPTG, *lane 3* purified SdsAP

specificity analysis, SdsAP had high activity against SDS and sodium octyl sulfate, but no obvious activity was detected against *p*-nitrophenyl sulfate (*p*NPS) and glucose-3-sulfate, which were substrates of arylsulfatses (data not shown). Similar result has been reported in some bacterial alkylsulfatases (Hagelueken et al. 2006; Cloves et al. 1980a). Although there were some similar characters between the SdsAP and SdsA1, their amino acid sequences shared only 42% identity (Fig. 2). Therefore, these data make it clear that the SdsAP was a novel modular alkylsulfatase (type III).

Alkylsulfatases often function in the form of dimer or tetramer. The central dimerization domain ensures resistance to high concentration of SDS, and the C-terminal domain provided a hydrophobic groove, presumably to recruit long aliphatic substrates (Hagelueken et al. 2006). The molecular mass of the native SdsAP enzyme (ca. 147 kDa as determined by the elution profile on a size exclusion column and 70 kDa as determined by SDS–PAGE) suggested that the SdsAP formed a dimer. Furthermore, the maximum activity of most alkylsulfatases always occurred around 30–40°C and these enzymes were not stable at high temperature (Cloves et al. 1980b; Pogorevc and Faber 2003). The SdsAP protein showed optimal activity at pH 9.0 and 70°C, and stability analysis showed that more than 90% activity of SdsAP still retained after pretreatment at 65°C for 1 h. These remarkable properties were much different from other alkylsulfatases. Similar to those were previously reported (Cloves et al. 1980b; Kahnert and Kertesz 2000), SdsAP was also inhibited by the high concentration of SDS. The $K_{m\ (app)}$ and $V_{max\ (app)}$ values calculated from Michaelis–Menten

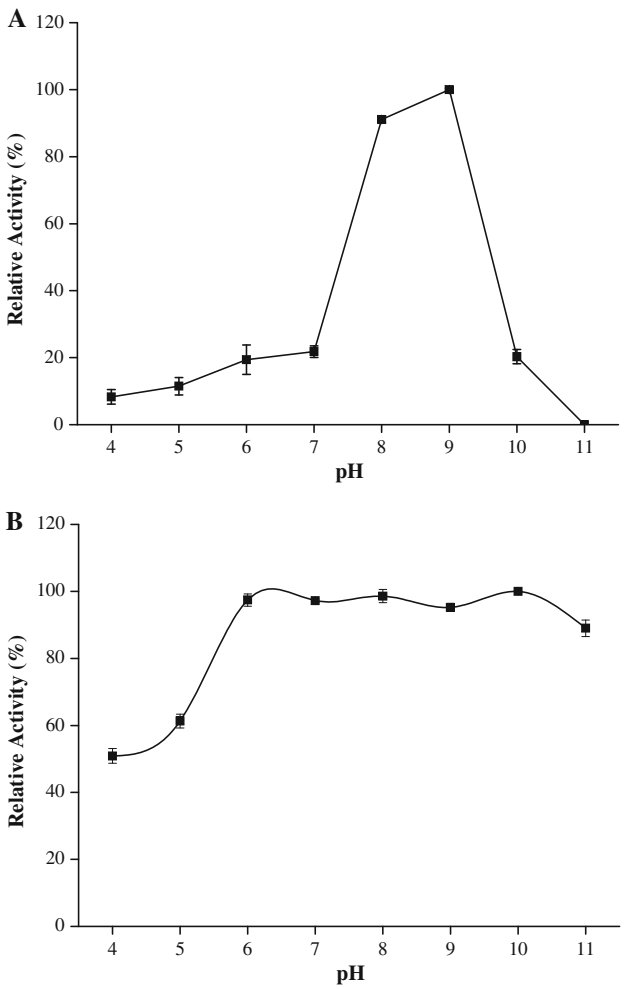

Fig. 4 Effect of pH on activity and stability of recombinant SdsAP. **a** pH effect on the activity of SdsAP. **b** pH effect on the stability of SdsAP. pH profiles were measured in 50 mM of different buffers: acetic acid buffer (pH 4.0–5.0), sodium phosphate buffer (pH 6.0–7.0), Tris–HCl buffer (pH 8.0 and 9.0) and glycine–NaOH buffer (pH 10.0–11.0)

plot gave values of 264.3 μmol and 33.8 μmol min^{-1} mg^{-1}, respectively. Lillis et al. (1983) reported that the K_m and V_{max} of alkylsulfatase from *Pseudomonas putida* FLA were 1.0 mM and 10.9 μmol SDS per minute, respectively. The K_m and V_{max} of the alkylsulfatase from the *Klebsiella oxytoca* strain DRY14 gave values of 0.1 mM SDS and 1.07 μmol min^{-1} mg^{-1} protein, respectively (Shukor et al. 2009). A lower K_m value of 0.034 mM was demonstrated by *Pseudomonas putida* S-313 (Kahnert and Kertesz 2000). These evidences suggest that SdsAP is a distinctive alkylsulfatase, an attractive candidate for SDS biodegradation.

In our study, EDTA strongly inhibited the enzyme activity; therefore, we assumed that SdsAP possessed metal ions as cofactors for catalytic activity. We also discovered

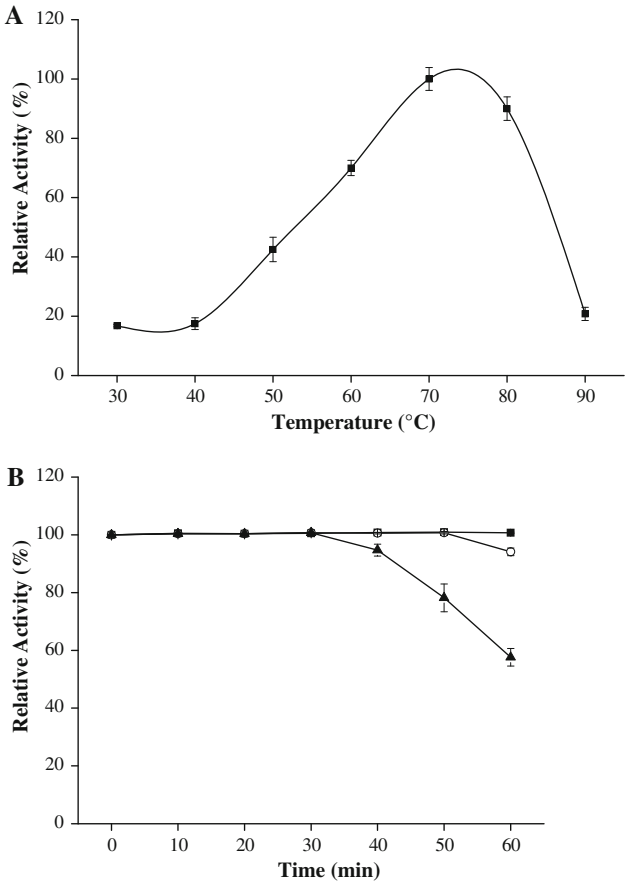

Fig. 5 Effect of temperature on activity and stability of recombinant SdsAP. **a** Temperature effect on the activity of SdsAP. Temperature profiles were measured at different temperatures (30–90°C) in 50 mM Tris–HCl buffer (pH 9.0). **b** Temperature effect on the stability of SdsAP. SdsAP stability was measured after incubating at temperatures of 60, 65, and 70°C at different times (*filled square* 60°C, *open circle* 65°C, *filled triangle* 70°C)

Table 1 Effect of different metal ions and chemical reagents on the purified SdsAP activity

Agent	Conc. (mM)	Relative activity (%)	SD	Agent	Conc. (mM)	Relative activity (%)	SD
Na	100	99.2	0.1	Co	10	68.7	1.2
K	100	99.1	0.4	Mn	10	66.1	1.8
Mg	100	99.8	0.2	Urea	10	100.2	0.2
Ca	100	100.3	0.1	DTT	10	99.8	0.1
Cu	10	82.9	4.9	β-Me	10	99.9	0.4
Zn	10	77.8	1.6	EDTA	10	25.8	3.2
Fe	10	55.4	4.6				

The values are averages of results from experiments performed in triplicate. The activity measured under the standard condition was set as 100%

that Ca^{2+}, Mn^{2+} would completely reactivate the activity of SdsAP after EDTA treatment, and Mg^{2+}, Cu^{2+}, Zn^{2+} would partly restore enzyme activity. Additionally, Ca^{2+} slightly activated the SdsAP activity, whereas Zn^{2+} inhibited the activity. Similar results have been reported in other enzymes. For example, aminopeptidase A (EC 3.4.11.7), a zinc-metallopeptidase family M1, is also activated by Ca^{2+} and can be reactivated by Ca^{2+} and Mn^{2+} (Tsujimoto et al. 2008; Danielsen et al. 1980). Ca^{2+}, which binds to the enzyme through aspartic acids, forms a bridge between an aspartic acid of the enzyme and an acidic N-terminal amino acid of the substrate (Goto et al. 2007; Claperon et al. 2008). In vitro, Ca^{2+} can likely be replaced with Mn^{2+} (Mikoulinskaia et al. 2009). Moreover, this Zn^{2+}-containing Ca^{2+}-activated aminopeptidase A is inhibited by Zn^{2+}, too (Larsen and Auld 1989), and such a feature can also be found among other Zn^{2+}-containing metalloenzymes (Wang and Cooper 1993). Further research on the mechanism of how SdsAP is activated by Ca^{2+} and inhibited by Zn^{2+} is needed.

In conclusion, the characterization of a novel type III sulfatase gene (*sdsAP*) from *Pseudomonas* genus was studied. The recombinant alkylsulfatase (SdsAP) was overexpressed in *E. coli* and purified under native conditions. Characterization studies showed that the enzyme possessed thermostability at high temperature and high K_m, V_{max} values for SDS. Therefore, the SdsAP protein may be suitable for applications in the biodegradation of SDS-containing waste water under elevated temperature conditions.

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