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Characterization of a recombinant arylsulfatase enzyme from glucosinolate-metabolizing human gut bacterium *Escherichia coli* VL8

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Abstract

Bacterial arylsulfatases can hydrolyze organosulfur compounds. Thus, the objective of this research project was to characterize a recombinant arylsulfatase (ARS) from *Escherichia coli* VL8, a human gut bacterium able to produce nitrile from desulfo-glucosinolates. The *Ars* gene (Accession no.: LC685335.1) with a length of 1,494 bp corresponding to ARS enzyme with a length of 497 amino acids was cloned from *E. coli* VL8 and expressed in *E. coli* BL21 (DE3) for 16 h at 25°C in lysogeny broth (LB) by induction of isopropyl β-D-1-thiogalactopyranoside (0.5 mM). The recombinant ARS enzyme (57 kDa) was partially purified using Ni^{2+} affinity column chromatography. This recombinant ARS enzyme was able to desulfate synthetic *p*-nitrocatechol sulfate (*p*NCS) substrate to produce *p*-nitrocatechol as an indicator of ARS activity, with optimal conditions at 30°C and pH 6.0, respectively. The ARS enzyme displayed a Michaelis-Menten kinetic constant (K_m) of 1.09 mM and Maximum reaction velocity (V_{max}) of 25.1 U/mg for *p*NCS. ARS enzyme activity toward *p*NCS was not enhanced by any metal ions, while activity was inhibited by Ca^{2+}, Fe^{2+}, $NaHSO_4$ and Na_2SO_4.

Keywords: Desulfo-glucosinolate, *Escherichia coli* VL8, *p*-Nitrocatechol sulfate, Recombinant enzyme, Sulfatase

1. Introduction

Natural sulfated biological molecules have complex chemical compositions and biological functions. These molecules vary from small to complex polymers as sulfamates ($RN(H)SO_3^-$) and sulfate esters ($ROSO_3^-$) [1]. Sulfatase enzymes play a vital role in recycling these compounds. One of the most widely used arylsulfatases (ARSs) in research is derived from the intestinal juice of *Helix pomatia* (Roman snail). This snail sulfatase has 503 amino acids and shares 27% of its amino acid sequences with human lysosomal arylsulfatase A and 52% with human arylsulfatase B [2-4]. The sequence of snail sulfatase [4] also contains residues that make up active sites of ARSs in eukaryotes. Snail sulfatase acts on aryl, steroid and glucosinolate (GSL) substrates [5,6]. Snail sulfatase with GSL-sulfatase activity has been widely used to produce desulfo-glucosinolates (DS-GSLs) from intact GSLs for quantitative GSL analysis of plant extracts by High-performance liquid chromatography (HPLC) [6]. DS-GSL is a pre-cursor of nitrile (NIT) production in certain organisms. *Aspergillus flavus* can convert intact GSLs to NITs via enzyme-catalyzed reactions involving β-*O*-glucosidase and ARS [7], while recombinant β-*O*-glucosidase from *Caldocellum saccharolyticum* can hydrolyze DS-GSLs to produce pure NITs [8]. The recombinant β-*O*-glucosidase from the bacterium Tp8 was cloned into *Escherichia coli* and hydrolyzed a β-thioglucosidic bond of a DS-GSL [9], while a recombinant β-*O*-glucosidase from the gut bacterium, *Enterococcus casseliflavus* CP1, has recently been shown to produce NITs from DS-GSLs [10].

Bacterial arylsulfatases have proved suitable for a variety of applications in industry, agriculture, and the environment [11,12]. They are commonly utilized in the desulfation of agar [13] and have also been used for the degradation of endosulfan, a commonly used insecticide [14]. Bacterial growth has a significant influence on

sulfatase activity and can be used to assess soil quality [15]. GSL degradation by sulfatase from human gut bacteria occurs either alone or in combination with bacterial myrosinase, although bacterial sulfatases do not generally accept GSLs as substrates [16]. In this study, a sulfatase gene (*Ars*) for the ARS enzyme from a GSL-degrading bacterium, *E. coli* VL8 [17], was cloned in *E. coli* DH5 and expressed in *E. coli* BL21 (DE3). The ARS enzyme was characterized for the first time and evaluated for its kinetic activity toward *p*-nitrocatechol sulfate (*p*NCS) as a substrate. Properties of the bacterial ARS enzyme may be useful for future applications.

2. Materials and methods

2.1 Bacteria

E. coli VL8 with GSL-metabolizing ability isolated from human feces [17] and cultured in NB broth pH 7.0 was deposited at the Quadram Institute Bioscience, Norwich, UK. Gene cloning was performed by *E. coli* DH5 (Promega, UK), with gene expression using BL21 (DE3) (Novagen, UK). The two *E. coli* strains were cultured in lysogeny broth (LB) pH 7.0.

2.2 Gene sequences

The *Ars* gene (GenBank: LC685335.1) with a length of 1,494 bp corresponded to the ARS enzyme (GenBank: BDG00987.1) with a length of 497 amino acids. The *Ars* gene sequence was cloned using primers designed from the sulfatase gene of *E. coli* O83:H1 str. NRG 857C (GenBank: NC_017634.1) encoding a sulfatase-like hydrolase of *E. coli* (GenBank: WP_000828472.1). *E. coli* O83:H1 str. NRG 857C showed 98% similarity to the *16S rRNA* gene sequence of *E. coli* VL8 (GenBank: LC682613.1).

Construction of the phylogenetic tree and multiple sequence alignments of sulfatases from several species were carried out using Clustal Omega [18], while the sequence logo of sulfatase motif was created using WebLogo (https://weblogo.berkeley.edu/).

2.3 Reverse transcriptase polymerase chain reaction (RT-PCR)

E. coli VL8 was cultured in NB broth (2 mL) with or without 1 mM gluconasturtiin at time intervals of 24 h. At each time interval, bacterial cultures were collected, and cell pellets were obtained. Complete RNA was harvested from the bacterial pellets using Trizol (Invitrogen, UK). RNA was purified using the Pure Link RNA Minikit (Invitrogen, UK). Total RNA (1 mg) was used for reverse transcription of the *Ars* gene using OneStep RT-PCR (Qiagen, UK). The *Ars* gene primer pairs were used in the PCR (forward primer: 5′ GGTCATATGAAACGCCCCAATTTTCT 3′ and reverse primer: 5′ GTTGAGCTCTCAGAACTTCTG TTTTTTCT 3′). The PCR was conducted in an Eppendorf Thermal Cycler MasterCycler Personal 5332 (50°C for 30 min, 95°C for 15 min, followed by 28 three-step cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min). The products were examined on 0.8% agarose gel for cDNA content.

2.4 Inducibility of a native ARS enzyme of E. coli VL8

Cell-free extracts (200 µL) from *E. coli* VL8 cultures (both induced and non-induced with 1 mM gluconasturtiin overnight in 200 mL NB broth) were assayed for sulfatase activity using 1 mM *p*NCS as a substrate. Cell-free extracts from *E. coli* BL21(DE3) grown in LB broth were also tested as a negative control. Protein concentrations were measured using the Bradford assay, as directed by the manufacturer (Bio-Rad, UK).

2.5 Recombinant plasmid construction

Genomic DNA from *E. coli* VL8 was obtained using a Wizard Genomic DNA Purification Kit (Promega, UK). The *Ars* gene with a length of 1,494 bp corresponding to sulfatase (ARS) from *E. coli* VL8 was cloned by Pfu DNA polymerase (Promega, UK) using a gene-specific pair of forward primer: 5′GGTGGTCATATGAAACGCCCCAATTTTCT 3′ (Ndel site) and reverse primer: 5′GTTGAGCTCTCAGAA CTTCTGTTTTTTCT 3′(SacI site) (95°C for 1 min, followed by 28 three-step cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 2 min/kb and final extension at 72°C for 5 min). The PCR product was confirmed on 0.8% agarose gel and obtained according to the QIAquick PCR Purification Kit (Qiagen, UK) manual. The purified gene fragment was cut with both *NdeI* and *SacI* (Fermentas, UK), and inserted into a pET28b+ vector of approximately 5.4 kb (Novagen, UK) previously digested with identical restriction enzymes. Ligation of a recombinant DNA and a pET28b+ vector was performed using T4 DNA Ligase (Promega, UK) according to the producers' specifications. A 3:1 ratio of desired PCR insert to pET28b (+) vector (50 ng) was well-mixed by pipetting in a microcentrifuge tube and left at 4°C overnight. The combination was then introduced to competent

E. coli DH5 cells for gene cloning. QIAprep Spin Miniprep Kit (Qiagen, UK) was used to extract the recombinant plasmid, which was subsequently transformed into *E. coli* BL21 (DE3) cells.

2.6 Expression and purification of a recombinant ARS enzyme

Positive colonies harboring the *Ars* gene were screened and selected using gene-specific primers under the thermocycle program detailed above. The pET28b (+) - *Ars* gene recombinant plasmids were extracted from *E. coli* BL21 (DE3) and sent for DNA sequencing to confirm the DNA sequence of the *Ars* gene of *E. coli* VL8.

The positive clone was cultured (1 L of LB medium mixed with 50 µg/mL of kanamycin) at 37°C and 200 rpm to reach an OD_{600nm} of ~ 0.6. The inducer, 0.5 mM of isopropyl β-D-1-thiogalactopyranoside (IPTG), was used to induce the T7/lac promoter for 16 h at 25°C and 200 rpm. Induced cells were pelleted at 10,000g for 20 min, resuspended in 0.1 M citrate phosphate buffer pH 7.0 (10 mL), placed on ice, and disrupted by two shots of a 30k psi disruption cycle in a tissue disrupter (Constant Cell Disruption Systems, UK). After spinning down at 16,100 g for 30 min at 4°C, the soluble protein was restored. The supernatant was obtained after filtration through a 0.2 µm syringe filter and then added to a Ni^{2+}-attached 4 mL Profinity IMAC column (Bio-Rad, UK) pre-equilibrated with 50 mM sodium phosphate pH 8.0 containing 300 mM NaCl and washed with 50 mM sodium phosphate pH 8.0 containing 300 mM NaCl and 5 mM imidazole. Next, 50 mM sodium phosphate pH 8.0 containing 300 mM NaCl and 500 mM imidazole was used to elute the ARS enzyme. Active fractions were pooled and desalted against 100 mM citrate phosphate pH 7.0 using Amicon Ultra-15 Centrifugal Filter Units with 10K MWCO (Millipore, UK). Protein concentrations were measured using the Bradford reagent (Sigma, UK), with bovine serum albumin acting as a standard. The proteins were analyzed using a denatured, discontinuous SDS-PAGE. Molecular weight of the ARS enzyme was measured by comparison with molecular weight markers.

2.7 Characterization of a recombinant ARS enzyme

The *p*NCS-based enzymatic assay of sulfatase was conducted using spectrophotometric/colorimetric detection. In the reaction combination, 1 mL of ARS activity was evaluated in 50 mM sodium acetate buffer pH 5.0 (890 µL), 1 mM *p*NCS (100 µL) and ARS enzyme solution (20 µg/10 µL). The mixture was left at 37°C for 5 min before addition of 5 mL of 0.1 M NaOH to stop the reaction. A LKB Novaspec II Spectrophotometer (Pharmacia, UK) was used to record the absorbance at 510 nm. A calibration curve of known *p*NC amounts as a function of absorbance at 510 nm was used to calculate the amount of *p*-nitrocatechol (*p*NC) product generated by the reaction. One unit of ARS activity was defined as the enzyme quantity that liberated 1 µmol of *p*NC per min. Total and specific activities of the recombinant ARS enzyme were determined. Optimal pH for the partially purified ARS enzyme in 0.1 M citrate phosphate buffer (pH 3.0-8.0) at 37°C was examined using the *p*NCS substrate. The ideal temperature was determined by incubating 0.1 M citrate phosphate buffer (pH 7.0) containing *p*NCS for 5 min at various temperatures (4-80°C) before adding the enzyme. Activities were obtained under the optimal conditions. The activities of Na_2SO_4, $NaHSO_4$, $CoCl_2$, $CaCl_2$, $MgCl_2$, $FeSO_4$, $NiCl_2$, $MnCl_2$, $FeCl_3$, each at 1 mM, were tested to determine their effect on recombinant ARS enzyme activity. Percentage activities of the recombinant ARS enzyme with metal ion co-factors were compared against the control with no addition of metal ions.

The apparent enzyme activities, a Michaelis-Menten kinetic constant (K_m) and Maximum reaction velocity (V_{max}), of the *in vitro* incubations of crude extracts of the recombinant ARS enzyme with varied concentrations of *p*NCS in sodium acetate buffer (50 mM, pH 6.0) were determined for 15 min at 30°C. The K_m and V_{max} values and graphs were created using GraphPad Prism 6 (demo version) and estimated at 95% confidence.

2.8 Statistical analyses

All measurements were recorded in triplicate and reported as means ± standard deviation (SD). The Statistical Package for Social Science (SPSS) software (demo version) was used to identify significant differences using one-way analysis of variance (ANOVA), with multiple comparison at $p < 0.0001$ and Duncan's multiple range test at $p < 0.05$.

3. Results and discussion

3.1 ARS contained the signature CXPXR sulfatase motif

The phylogenetic tree of amino acid sequences of sulfatase enzymes from *E. coli* VL8, *E. coli* O83:H1 str. NRG 857C (NCBI accession no. WP_000828472.1), *Bacteroides thetaiotaomicron* VPI-5482 (NCBI accession no. AAO78455.1) [19], *Pedobacter yulinensis* (NCBI accession no. WP_107215127.1) [20] and the snail *H.*

pomatia sulfatase (Uniprot: Q9NJU8) showed that *E. coli* VL8 and *E. coli* O83:H1 were evolutionarily similar. Likewise, sulfatases from *P. yulinensis* and the snail were similar. However, glycosaminoglycan sulfatase from *B. thetaiotaomicron* was evolutionarily distant from the others (Figure 1A). Multiple sequence alignments of these sulfatases showed the consensus "C/S-X-P-X-R" sulfatase signature (Figure 1B) as an active site near the N' terminus, except for *B. thetaiotaomicron* VPI-5482. *Vibrio parahemeolyticus*, *Vibrio vulnificus* and *B. thetaiotaomicron* are less frequent examples of genomes containing putative serine sulfatases with a "S-X-A-X-R" pattern [19].

These sulfatases include conserved active site regions that are essential for orchestrating the calcium ion required for sulfate ester hydrolysis (Figure 1B). ARS enzymes (EC 3.1.6.1) are present in all kingdoms of life and stimulate the hydrolysis of a wide variety of sulfate esters. The signature sequence, "C/S-X-P-X-R", is conserved throughout the enzyme family and plays a crucial role in guiding post-translational modification [21]. The sequence logo for sulfatase signature "C/S-X-P-X-R" also indicated that the bacterial sulfatase ARS enzyme from *E. coli* VL8 was a "Cys-type" sulfatase (Figure 1C).

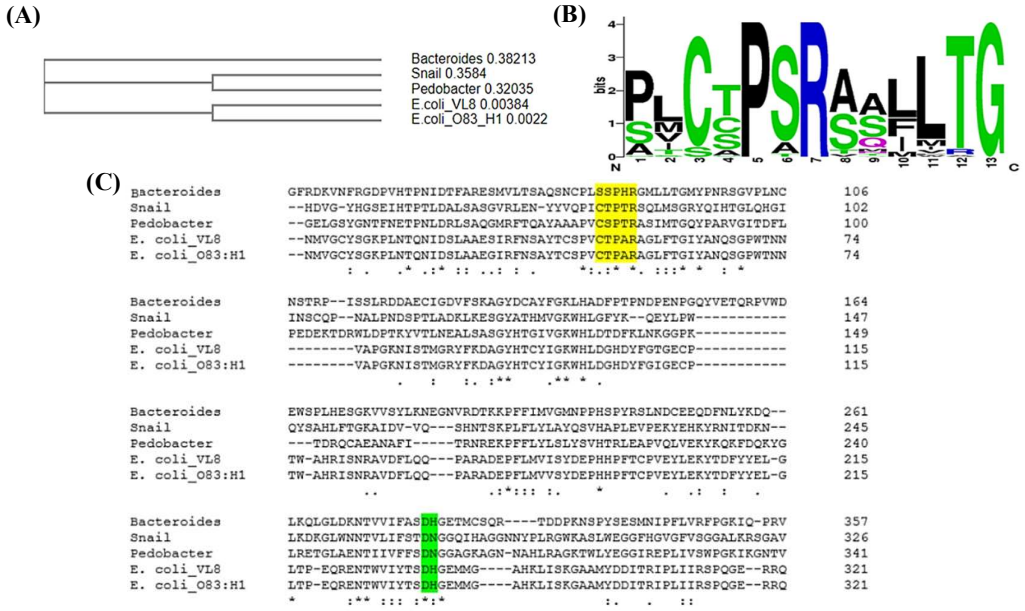

Figure 1 Sulfatase phylogenetic tree, alignments, and sequence logo. (A) Phylogenetic tree of amino acid sequences of sulfatases. (B) The sequence logo for sulfatase signature "CXPXR" indicated that the sulfatase ARS enzyme of *E. coli* VL8 was a "Cys-type" sulfatase. (C) Sequence alignment of sulfatases from bacteria and a snail. The conserved "CXPXR" sulfatase motif (Cys to FGly post-translational modification) is shown in yellow, while residues involved in calcium binding are indicated in green. The symbol "*" indicated that the residues in that column were identical in all alignment sequences, ":" indicated that conserved substitutions were found and "." indicated that semi-conserved substitutions were found.

3.2 Inducibility of a native ARS enzyme of E. coli VL8

The recombinant ARS enzyme is a member of the sulfatase family, which is distinguished by a sulfatase domain at the amino terminus. This protein is soluble in the cell crude extracts, suggesting its location in the periplasmic space or in the cytosol, consistent with previous observations of bacterial sulfatases. Glycosaminoglycan sulfatases from the marine bacteria *Photobacterium* sp. FC615 belonging to a diverse and well-preserved sulfatase family are reported to catalyze the hydrolysis of sulfate esters from glycosaminoglycan and are soluble proteins [19]. *Pseudomonas aeruginosa* ARS was also found to be soluble [22]. Until now, virtually every active, recombinantly expressed sulfatase published in the literature has had a Cys in the active site sequence. As a consequence, the *E. coli* host may contain a Cys-specific altering machinery. Whilst having at least one sulfatase-related gene as the *aslA* gene [23], *E. coli* BL21(DE3) has not yet produced successful endogenous sulfatases.

A sulfatase from *H. pomatia* was constitutively expressed, while a bacterial sulfatase from *Mycobacterium bovis* BCG was also constitutively expressed [24]. However, little is known about sulfatase enzyme expression systems in *E. coli* VL8. Therefore, here, a native ARS enzyme of *E. coli* VL8 was tested for the expression system (constitutive or inducible). We hypothesized that the GSL substrate was able to induce sulfatase in bacterial metabolism of the GSL pathway. Therefore, 1 mM gluconasturtiin was added to *E. coli* VL8 cells, with

no addition to the other cells. Protein extracts from *E. coli* VL8 cells (with/without 1 mM gluconasturtiin overnight) were assayed for sulfatase activity using 1 mM *p*NCS substrate in *p*NCS-based enzymatic assay of sulfatase. As a negative control, protein extract from *E. coli* BL21(DE3) host was also assayed. Results showed that cell-free extracts from both groups of VL8 cells, with and without 1 mM gluconasturtiin overnight, showed weak sulfatase activity of 0.07-0.09 µmol/min/mg using *p*NCS substrate in sodium acetate buffer (50 mM, pH 5.0), as observed by color change from yellow (*p*NCS substrate) to red orange (*p*NC product) (Figure 2A). By contrast, the BL21(DE3) host did not exhibit active endogenous sulfatase activity (as indicated by no change in yellow) and was suitable for heterologous expression of the recombinant ARS enzyme from *E. coli* VL8. From this finding, a native ARS enzyme from *E. coli* VL8 was constitutively expressed. This was also reconfirmed by RT-PCR analysis showing no significant difference in the *Ars* transcript levels from 0 h to 24 h in *E. coli* VL8 cells with and without 1 mM gluconasturtiin addition (Figure 2B). The experimental variations of *Ars* transcript levels within each sample were normalized with *16S rRNA* transcripts as a reference gene (data not shown).

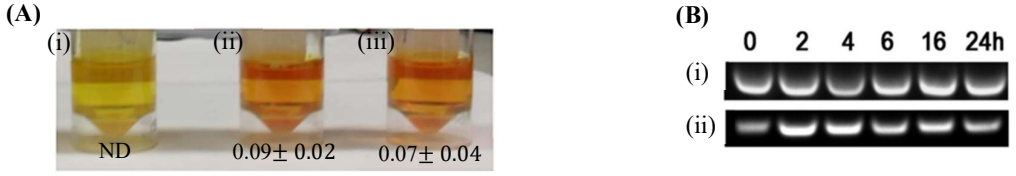

Figure 2 Inducibility test of a native ARS enzyme of *E. coli* VL8. (A) Bacterial protein extracts were assayed for endogenous sulfatase activity using 1 mM *p*NCS substrate. (i) In *E. coli* BL21(DE3) host no sulfatase activity was detected. (ii) Overnight addition of GSL and 1 mM gluconasturtiin to *E. coli* VL8 showed weak sulfatase activity. (iii) No addition of 1 mM gluconasturtiin to *E. coli* VL8 also showed weak sulfatase activity. All values are the mean±SD of three replicates. (B) RT-PCR analysis of *Ars* transcripts from *E. coli* VL8 cells over 24 h. (i) *Ars* transcripts from *E. coli* VL8 cells without gluconasturtiin addition. (ii) *Ars* transcripts from *E. coli* VL8 with gluconasturtiin addition. Experimental variations of *Ars* transcript levels within each sample were normalized with *16S rRNA* transcripts as a reference gene (data not shown).

3.3 Purification of the recombinant ARS enzyme

The recombinant ARS enzyme was purified using Ni^{2+}-affinity column chromatography. The eluted fractions were investigated using SDS-PAGE (Figure 3A). Soluble recombinant ARS enzymes with molecular weight of approximately 57 kDa (as predicted by UniProt) were found in the three eluted fractions (E1, E2 and E3). Protein homogeneity or purity was evaluated by the fold purification. The use of Ni^{2+}-affinity column chromatography purification resulted in partially purified recombinant ARS enzyme with 61% purity assessment by SDS-PAGE (Figure 4A, fraction E2). Relative ARS activity of the E2 fraction was highest (Figure 3B) and this fraction was used for its kinetic analysis.

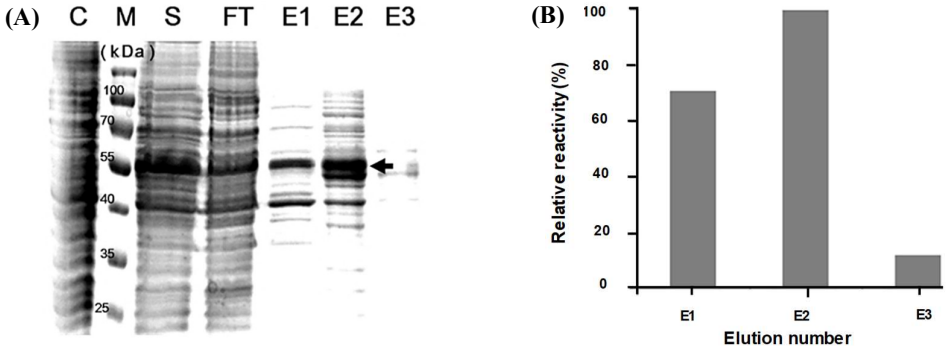

Figure 3 Purification of the recombinant ARS enzyme expressed in BL21(DE3). (A) SDS-PAGE analysis of protein fractions obtained by Ni^{2+}-affinity column chromatography; C = total proteins from an empty pET28b+ vector expressed in BL21 (DE3) as a negative control; M = PageRuler pre-stained protein ladders (Thermo Scientific, UK); S = supernatant of crude extracts from recombinant bacteria expressing ARS enzyme; FT = flow-through fraction during purification; E1 = elution fraction no. 1; E2 = elution fraction no. 2; E3 = elution fraction no. 3. The arrow indicates the sulfatase protein band of size 57 kDa. (B) Relative activity of each eluted protein fraction with the same volume of 10 µL.

Table 1 Purification scheme of the recombinant ARS enzyme using Ni^{2+}-affinity column chromatography.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purity (Fold)	Yield (%)
Cell-free extract	268	206	1.3	1	100
Ni^{2+}-affinity column chromatography	125	12	10.4	8	47

One unit (U) of sulfatase was defined as the amount of enzyme liberating 1 μmol/min of pNC product.

The purification scheme of the recombinant ARS enzyme by Ni^{2+}-affinity column chromatography is shown in Table 1. Enzyme purity increased by eight folds, while specific activity increased by ten folds.

The molecular weight of the recombinant ARS enzyme was similar to values reported in previous studies. A sulfatase protein of 511 amino acids from *Flammeovirga pacifica*, isolated from deep-sea sediments of the West Pacific Ocean and encoded by sulfatase gene *ary423* (1,536 bp an open reading frame (ORF)) showed a molecular mass of 56 kDa [25]. This ARS featured the sulfatase consensus sequence as a conserved C-X-A-X-R pattern. The recombinant enzyme was able to hydrolyze sulfate ester linkages in *p*-nitrophenyl sulfate (*p*NPS) at specific activity of 64.8 U/mg [25].

Other studies found bacterial recombinant sulfatase with lower molecular weight. *Marinomonas* sp. FW-1, from the shore of Qingdao, China, was capable of hydrolyzing pNPS and agar sulfate ester linkages. Using ion exchange and gel filtration chromatographies, an ARS with recovery of 13% yield and a fold of 12 was purified to homogeneity. The enzyme was made up of a single polypeptide chain with molecular mass of 33 kDa determined by SDS-PAGE [26].

3.4 Optimal temperature and pH of the recombinant ARS enzyme

The recombinant ARS enzyme showed optimal activity at 30°C and pH 6.0 (Figure 4) using 1 mM *p*NCS as the substrate. Interestingly, 40% of the maximal sulfatase activity remained at 4°C. However, enzyme activity of the recombinant ARS enzyme decreased sharply at 50°C and 80°C, possibly related to thermal denaturation (Figure 4A). Alkalinity increased above pH 6.0 and enzyme activity dropped considerably, indicating that ARS did not belong to the category of alkaline ARS (Figure 4B). Its optimal pH at mildly acidic pH 5.5-6.0 may be useful for testing acidic soil contamination.

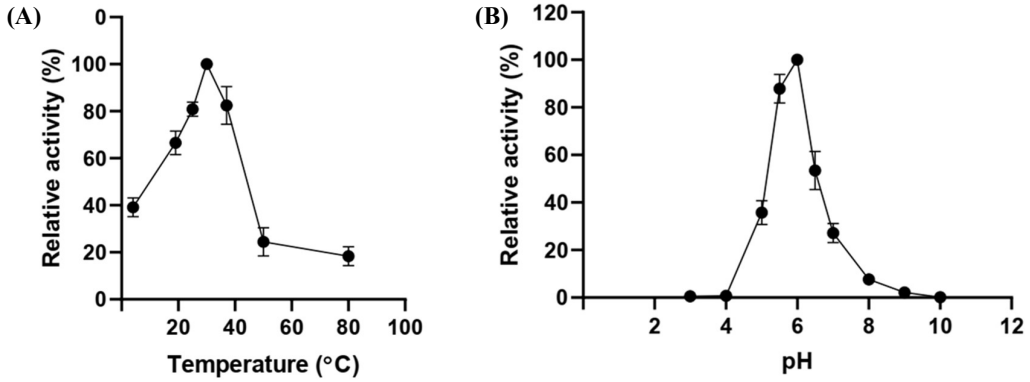

Figure 4 Optimal temperature and pH of the recombinant ARS enzyme. (A) Optimal temperature. (B) Optimal pH. Values are averages of three replicates.

Bacterial ARSs can be split into two categories as those with optimal pH activity at 6.5-7.1 and those with ideal pH activity at 8.3-9.0 [26]. The first group includes ARSs from *Sphingomonas* sp. AS6330 [27] and *P. carrageenovora* [28], which have optimal pH values at 6.7 and 7.5, respectively while the second category includes ARSs from *Pseudomonas testosteroni* [29]. However, our recombinant ARS enzyme showed maximal activity at pH 6.0 and was not in either category. Previous findings showed that *Schistocerca gregaria* (desert locust) sulfatase had optimal pH value at 6.5 for ARS activity [30], while *H. pomatia* (snail) sulfatase had optimal pH of 7.2 at 30°C. The partially purified recombinant ARS enzyme exhibited optimal ARS activity at similar pH and temperature values of pH 6.0 and 30°C to sulfatases from the desert locust and the snail, respectively. Discrepancies in optimal pH value may be related to enzyme origin.

3.5 Apparent activities of the recombinant ARS enzyme

The kinetic parameters of the recombinant ARS enzyme were determined using *p*NCS as a substrate, while the partially purified recombinant ARS enzyme was used for the *p*NCS substrate. The Michaelis-Menten kinetic curve and the Lineweaver-Burk plot showed that ARS activity of the recombinant ARS enzyme had a Michaelis-Menten constant (K_m) of 1.09 mM, with V_{max} of 25.1 U/mg for *p*NCS (Figure 5, Table 2). Similar to a previous study, the snail ARS had a K_m value of 2.5 mM for *p*NPS [31].

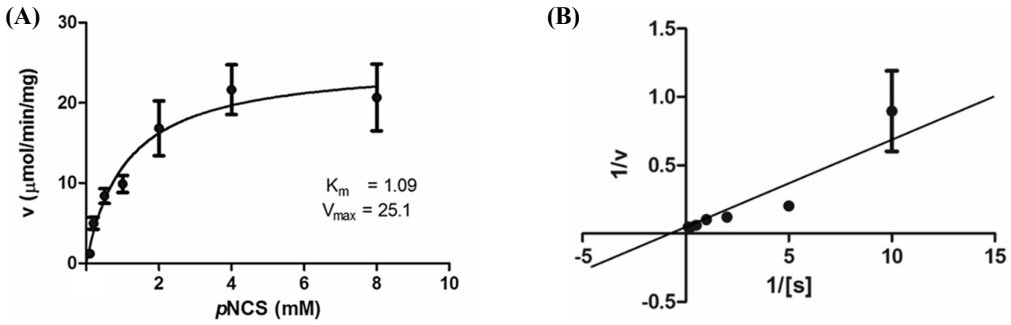

Figure 5 Apparent enzyme activities of a partially purified recombinant ARS enzyme for *p*NCS substrate. (A) Recombinant ARS enzyme Michaelis-Menten curve for *p*NCS. (B) Lineweaver-Burk plot of graph (A). ARS activity was determined by the production of *p*NC from *p*NCS by the recombinant ARS enzyme (100 μg). Values are means±SD of three replicates.

Table 2 Apparent enzyme activities of the recombinant ARS enzyme.

Apparent specific activity (U/mg) *	Relative activity (%) **	K_m (mM)	V_{max} (U/mg)	k_{cat} (s^{-1})	K_{cat}/K_m (M^{-1} s^{-1})
10.4 ± 0.25	51.0 ± 1.4	1.09	25.1	232	2.13×10^5

*Values determined are apparent specific activities since the ARS enzymes were not 100% pure.

**Activity produced by crude extracts of the recombinant ARS enzyme (1000 μg) in (%) relative to activity produced by purified *H. pomatia* sulfatase (100 μg). Values are means ± SD of triplicate measurements. One unit (U) of sulfatase was defined as the amount of enzyme liberating 1 μmol min^{-1} of *p*NC product. Values of K_m and V_{max} were estimated at 95% confidence using GraphPad Prism 6.

Higher K_m and lower V_{max} values of our recombinant ARS enzyme presented its inferior property to other bacterial ARSs previously reported. The lower K_m and higher V_{max} values of *Marinomonas* ARS toward *p*NPS were calculated as 13.73 and 270.27 μM/min, respectively. The ARSs from *Sphingomonas* sp. AS6330 and *P. carrageenovora* displayed the K_m values toward *p*NPS hydrolysis of 1150 and 54.9 μM, respectively [27,28]. Results of optimal conditions for enzyme activity and kinetic parameters showed that our recombinant ARS enzyme had characteristics more similar to the snail ARS than to those of certain other bacteria.

3.6 Effects of metal ions on activity of the recombinant ARS enzyme

Various compounds including Na_2SO_4, $NaHSO_4$, $CoCl_2$, $CaCl_2$, $MgCl_2$, $FeSO_4$, $NiCl_2$, $MnCl_2$ and $FeCl_3$ at 1.0 mM were tested for their effects on ARS activity of our recombinant ARS enzyme using *p*NCS as a substrate. Results showed no significant effects for most of the compounds and metal ions tested, except for Fe^{2+}, $NaHSO_4$ and Na_2SO_4 that reduced ARS activity by 20, 75 and 50%, respectively (Figure 6).

Activity of the recombinant ARS enzyme was not influenced by metal ions, indicating that metal ions played minimal roles during the catalytic process of this enzyme toward *p*NCS substrate. This differed from *P. carrageenovora* with its ARS activity promoted by Mg^{2+} [28]. Activity of the recombinant Ary423 ARS from *F. pacifica* was also enhanced by Mg^{2+} [25]. The same trend occurred in *Pseudomonas jessenii*, with alkylsulfatase activity enhanced by the addition of K^+ and Mg^{2+} [32].

However, Na_2HSO_4 and Na_2SO_4 inhibited the action of the recombinant ARS enzyme. This result concurred with a previous report showing that snail sulfatase was inhibited by K_2SO_4 when using *p*NPS as a substrate [31]. Likewise, Na_2SO_3 significantly inhibited activity of the desert locust sulfatase, while Na_2HPO_4 and NaF showed slight inhibition [30].

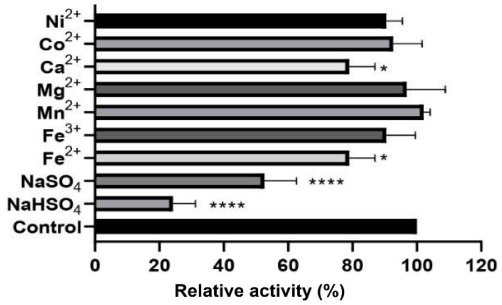

Figure 6 Influence of compounds on recombinant ARS enzyme activity showing relative activity (%). The control contained only the recombinant ARS enzyme with no other compounds. Values are means ± SD of triplicate measurements. Asterisks indicate significant differences ($p< 0.0001$) compared to the control using one way ANOVA multiple comparison.

4. Conclusion

This report presents the first characterization of recombinant ARS enzyme activity from *E. coli* VL8. Human gut microbiota are valuable sources for the discovery of new enzymes. Our findings improve the knowledge of bacterial sulfatase activity with applications in industry, agriculture, and the environment.

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6. References

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