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Expression and purification of an ArsM-elastin-like polypeptide fusion and its enzymatic properties

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Abstract

Enzymes could act as a useful tool for environmental bioremediation. Arsenic (As) biomethylation, which can convert highly toxic arsenite [As(III)] into low-toxic volatile trimethylarsine, is considered to be an effective strategy for As removal from contaminated environments. As(III) S-adenosylmethyltransferase (ArsM) is a key enzyme for As methylation; its properties and preparation are crucial for its wide application. Currently, ArsM is usually purified as a His-tag fusion protein restricting widespread use due to high costs. In this study, to greatly reduce the cost and simplify the ArsM preparation process, an Elastin-like polypeptide (ELP) tag was introduced to construct an engineered *Escherichia coli* (ArsM-ELP). Consequently, a cost-effective and simple non-chromatographic purification approach could be used for ArsM purification. The enzymatic properties of ArsM-ELP were systematically investigated. The results showed that the As methylation rate of purified ArsM-ELP (> 35.49%) was higher than that of *E. coli* (ArsM-ELP) (> 10.39%) when exposed to 25 μmol/L and 100 μmol/L As(III), respectively. The purified ArsM-ELP was obtained after three round inverse transition cycling treatment in 2.0 mol/L NaCl at 32 °C for 10 min with the yield reaching more than 9.6% of the total protein. The optimal reaction temperature, pH, and time of ArsM-ELP were 30 °C, 7.5 and 30 min, respectively. The enzyme activity was maintained at over 50% at 45 °C for 12 h. The enzyme specific activity was 438.8 ± 2.1 U/μmol. ArsM-ELP had high selectivity for As(III). 2-Mercaptoethanol could promote enzyme activity, whereas SDS, EDTA, Fe^{2+}, and Cu^{2+} inhibited enzyme activity, and Mg^{2+}, Zn^{2+}, Ca^{2+}, and K^{+} had no significant effects on it.

Keywords Arsenic · As(III) S-adenosylmethionine (SAM) methyltransferase · Elastin-like polypeptide tag · *Rhodopseudomonas palustris*

Introduction

Arsenic (As) contamination has become a major public health problem in the world (Zhou et al. 2018; Zhu et al. 2014). Due to As being ubiquitous in nature, microorganisms have acquired strategies for As detoxification, such as arsenate [As(V)] reduction and arsenite [As(III)] oxidation and methylation as well as organic As demethylation (Zhu et al. 2017). These mechanisms not only play a key role in driving the As biogeological cycle (Liu et al. 2014; Rensing and Rosen 2009), but also inspired people to employ these mechanisms to remove As, especially As volatilization by As methylation. As methylation can convert highly toxic As(III) to low-toxic volatile trimethylarsine [TMAs(III)] (Qin et al. 2006, 2009; Zhao et al. 2015) and was found to be widespread in natural environment; therefore, this detoxification mechanism is considered to be an effective strategy for As removal. As(III) S-adenosylmethionine (SAM) methyltransferase (termed as

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ArsM in microbes, As_3MT in higher organisms) is the key enzyme for As methylation. Since Qin et al. (2006) identified and characterized ArsM from *Rhodopseudomonas palustris* CGA009 by heterologous expression in As sensitive *Escherichia coli* AW3110, many novel *arsM* genes or As-methylating microbes have been reported, such as *Cyanidioschyzon* sp. 5508 (Qin et al. 2009), *Methanosarcina acetivorans* C2A (Wang et al. 2014b), *Pseudomonas alcaligenes* NBRC14159 (Zhang et al. 2015), *Streptomyces* sp. GSRB54 (Kuramata et al. 2015), *Asibacter rosenii* SM-1 (Huang et al. 2016), *Synechocystis* sp. PCC 6803 (Xue et al. 2017), and *Bacillus* sp. CX-1 (Huang et al. 2018). These works provided a better understanding of bacterial As methylation pathways. However, the majority of naturally existing microorganisms exhibited a relatively limited ability to volatilize As (Wang et al. 2014a) except for *A. rosenii* SM-1 (Huang et al. 2016); therefore, many genetically engineered As-methylating organisms harboring *arsM* were constructed, such as *E. coli* AW3110 (Yuan et al. 2008), *Sphingomonas desiccabilis* (Liu et al. 2011), *Bacillus idriensis* (Liu et al. 2011), *Pseudomonas putida* KT2440 (Chen et al. 2013, 2014), *Bacillus subtilis* 168 (Huang et al. 2015), *Saccharomyces cerevisiae* (Verma et al. 2016), and *Rhizobium leguminosarum* R3 (Zhang et al. 2017). These engineered organisms displayed different capacities to volatilize As. For example, engineered strain *P. putida* KT2440 containing *arsM* converted 25 μM As(III) to volatile TMAs(III) ($10 \pm 1.2\%$) and dimethylarsine [Me_2AsH] ($21 \pm 1.0\%$) (Chen et al. 2014). Therefore, the As-methylating or As-volatizing organisms would have potential application value for the bioremediation of As-contaminated environments.

In addition to microbes, enzymes play a main role in bioremediation (Rao et al. 2014). Regarding ArsM, the reported studies focused on *arsM* function validation (Guo et al. 2016; Huang et al. 2016, 2018; Qin et al. 2006, 2009; Verma et al. 2016; Wang et al. 2014b; Ye et al. 2014; Yin et al. 2011) and the investigation on determining ArsM reaction temperature and pH (Guo et al. 2016; Qin et al. 2009; Wang et al. 2014b; Xue et al. 2017; Ye et al. 2014; Zhang et al. 2015). So far, the detailed ArsM enzymatic properties are still poorly understood. In addition, ArsM was obtained as a His-tag fusion protein with higher cost (Guo et al. 2016; Huang et al. 2016, 2018; Qin et al. 2006, 2009; Wang et al. 2014b; Ye et al. 2014; Yin et al. 2011). Therefore, it is of great significance to develop a simple, cost-effective, large-scale ArsM preparation method and explore ArsM enzymatic properties.

Elastin-like polypeptide (ELP), as a non-chromatographic protein purification tag, has been widely used in various protein purification due to its simple, low-cost, easily large-scale preparation (Trabbic-Carlson et al. 2004). ELP are polymers composed of repeats of the pentapeptide (Val-Pro-Gly-Xaa-Gly)n, where Xaa is the amino acid except Pro (Meyer and Chilkoti 1999). The ELP fusion proteins can be purified by using non-chromatographic technique termed as inverse thermal cycling (ITC), that is, the ELP fusion proteins are soluble at temperatures lower than their transition temperature (T_t) and become insoluble aggregates above the T_t (Li and Zhang 2014; Roberts et al. 2015). In this study, we cloned *arsM* from *R. palustris* CGA009, a good model organism for studying As detoxification (Qin et al. 2006; Zhao et al. 2015), and then *arsM* was fused with the ELP-tag to construct an engineered *E. coli* (ArsM-ELP) with a high As resistance. ArsM-ELP was purified by the ITC system, and its enzymatic properties including temperature, pH, temperature stability, selectivity, and interference factors were investigated. This work provided a novel, simply, non-chromatographic ArsM purification method and obtained an engineered *E. coli* (ArsM-ELP) with a higher As methylation rate, which laid a good foundation for a biotechnological application of ArsM.

Material and methods

Strains, plasmids, and reagents

Rhodopseudomonas palustris CGA009 (ATCC BAA-98) was obtained from the American Type Culture Collection (ATCC, USA). *Escherichia coli* DH5α was obtained from Tiangen Biochemical Technology Co., Ltd. (Beijing, China). Strain *E. coli* AW3110 (DE3) (Δ*arsRBC*) (Carlin et al. 1995) was a present by Professor Yongguan Zhu of the Institute of Urban Environment, Chinese Academy of Science; pET-22b-ELP (KV_8F-40) (Huang et al. 2011), by Professor Guangya Zhang of Huaqiao University. As(V) (Na_3AsO_4) and As(III) ($NaAsO_2$) were obtained from Merck (Darmstadt, Germany). Unless specified otherwise, all chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and all reagents used in this study were of analytical or better grade.

Construction and identification of engineered *E. coli* (ArsM-ELP)

The *arsM* (GenBank accession no. BX571963.1) was amplified by PCR from *R. palustris* CGA009 using the following primers: *arsM*-F: 5′-CATGCATATGCCACTGACATGCAAGACGTGAAG-3′ and *arsM*-R: 5′-CCGGGATCCCCCGCAGCAGCGCGCC-3′. The primers were designed by Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA) and synthesized by Suzhou Genewiz Biotechnology Co., Ltd. (Suzhou, China). The *arsM* fragment and pET-22b-ELP plasmid were double-

digested with *Nde*I and *Bam*HI and were recovered by agarose gel electrophoresis. The gene fragment was ligated with pET-22b-ELP plasmid at 16 °C for 20 h and transformed into *E. coli* DH5α competent cells. DH5α cells were plated on Luria-Bertani (LB) solid medium containing 100 μg/mL ampicillin and incubated overnight at 37 °C. Positive clones were screened to identify the recombinant plasmids (pET-22b-*arsM*-ELP) by double restriction enzyme digestion with *Nde*I and *Bam*HI to obtain the cloned *arsM*. The identified plasmid (pET-22b-*arsM*-ELP) was transformed into competent *E. coli* AW3110 (DE3) cells. The engineered *E. coli* AW3110 (pET-22b-*arsM*-ELP) was screened for ampicillin resistance, verified by double enzyme digestion and sequencing by Suzhou Genewiz Biotechnology Co., Ltd. (Suzhou, China). The *E. coli* AW3110 (DE3) strain harboring the pET-22b-*arsM*-ELP and pET-22b-ELP were named *E. coli* (ArsM-ELP) and *E. coli* (pET-22b-ELP), respectively. The ELP gene was ligated to the C-terminus of the *arsM*.

Purification and identification of ArsM

A single colony of engineered *E. coli* (ArsM-ELP) was picked and inoculated in 100 mL LB liquid medium containing ampicillin (100 μg/mL), and incubated at 37 °C until optical density at 600 nm (OD_{600}) of 0.4~0.6. The cells were induced by 0.2 mmol/L of isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16 h at 30 °C.

The total protein extract was prepared as follows: 5 mL of cells was harvested by centrifugation and washed with 50.0 mmol/L phosphate-buffered saline (PBS) buffer (137.0 mmol/L NaCl, 2.7 mmol/L KCl, 10.0 mmol/L $Na_2HPO_4 \cdot 12H_2O$, 2.0 mmol/L KH_2PO_4, pH 7.4), then resuspended in PBS buffer. The cells were disrupted by a sonicator (Scientz-IID, Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, China) (300 W output, pulse 2 s, stop 2 s, cycle 90 times), and the supernatant was collected by centrifugation at 13,000×*g* for 25 min at 4 °C and submitted for measuring total protein content as described by Bradford (1976) using bovine serum albumin (Sigma, Shanghai, China) as a standard. Expressed ArsM-ELP proteins were identified by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

As resistance in engineered *E. coli* (ArsM-ELP)

Single colonies of *E. coli* (pET-22b-ELP) and *E. coli* (ArsM-ELP) were picked and inoculated in 20 mL LB medium supplemented with the appropriate antibiotics, respectively, and incubated at 37 °C overnight. Two percent (*V*/*V*) inocula were transferred to 100 mL liquid LB medium containing 0.2 mmol/L of IPTG and As(III) (50 and 70 μmol/L) and incubated at 37 °C for 22 h. As(III) at 50 and 70 μmol/L were chosen as described previously (Qin et al. 2006). Control experiments without As(III) were carried out under the same conditions. Each experiment was repeated in triplicate. The bacterial growth was monitored by measuring OD_{600}. The growth inhibition rate was used to assess the calculated growth based on the growth of the corresponding As-free medium.

The optimization of expression of ArsM-ELP by *E. coli* (ArsM-ELP)

To enhance the protein expression level of *E. coli* (ArsM-ELP), the temperature, IPTG concentration, and expression time of ArsM-ELP were optimized by a single-factor experiment. The recombinant *E. coli* (ArsM-ELP) was inoculated in a 250 mL flask containing 100 mL of LB medium and cultured in a shaker at 200 r/min at 37 °C until the culture reached an OD_{600} of approximately 0.5~0.6. 0.8 mmol/L of IPTG was added to induce for 12 h at 200 r/min at 16, 20, 24, 30, and 37 °C, respectively. The optimized temperature was used to optimize IPTG concentration and the expression time of ArsM-ELP. The total protein content was determined as described by Bradford (1976), and the ArsM-ELP protein was checked by SDS-PAGE and analyzed by a Gel-Pro Analyzer 4 (Media Cybernetics, Rockville, MD, USA).

The optimization of purification conditions for ArsM-ELP

The purification procedures of the ELP-tag fusion protein were performed as described previously (Huang et al. 2011). In this study, the effects of NaCl concentration, T_t, phase transition time, and total protein content on enzyme purification effectiveness were investigated, respectively. Briefly, the cell lysate was centrifuged at 12000 r/min for 15 min at 4 °C to obtain the supernatant. The inverse phase transition of ELP fusion proteins was triggered by the addition of NaCl (1.0~2.5 mol/L); the aggregated ELP protein precipitation was collected by centrifugation and then was resolubilized in cold PBS buffer (pH 7.4) and ice bath for 15 min, and centrifuged again to remove particulate contaminants. This aggregation and resolubilization process was repeated for three times, and the purified protein content was determined as described by Bradford (1976). The optimized NaCl concentrations were designed to optimize T_t, the phase transition time, and the total protein content. The purified protein purity was ascertained by SDS-PAGE.

The relationship between NaCl concentration and T_t

NaCl was dissolved in 50.0 mmol/L PBS buffer (pH 7.4) to prepare different concentration NaCl solution (0, 0.5, 1, 1.5, 2, and 2.5 mol/L). The purified ArsM-ELP was set at a final concentration of 1.0 mg/mL. The reaction system was

measured by an ultraviolet-visible spectrophotometer (Specord 200, Analytical Jena, Jena, Germany). The programed heating temperature was set to 1.0 °C/min, and the absorbance was monitored by measuring OD_{350}. The temperature in corresponding to half of the maximum value of OD_{350} is defined as the T_t (Meyer and Chilkoti 1999).

Determination of the recovery rate of purified ArsM-ELP

The purified ArsM-ELP was formulated into gradient enzyme solutions containing 2.0 mol/L NaCl in 50.0 mmol/L PBS buffer (pH 7.4) and was submitted for one round of ITC purification treatment. The protein concentration was determined and the protein recovery rate was calculated.

The enzymatic properties of ArsM-ELP

The optimal reaction time of ArsM-ELP was determined in the presence of 5.0 μmol/L ArsM-ELP at 37 °C, pH 7.4. Subsequently, based on the optimal reaction time, the effects of temperature and pH on enzyme activity were investigated. Under optimal temperature and pH conditions, the thermal stability, substrate selectivity, and interference factors on enzyme were investigated, respectively. KCl [K^+], $CaCl_2$ [Ca^{2+}], $FeSO_4$ [Fe^{2+}], $FeCl_3$ [Fe^{3+}], $Cr_2(SO4)_3$ [Cr^{3+}], and Na_3AsO_4 [As(V)] were selected to replace As(III) as the substrates. 0.3 mmol/L of $MgCl_2$ [Mg^{2+}], $ZnSO_4$ [Zn^{2+}], $CuSO_4$ [Cu^{2+}], $CaCl_2$ [Ca^{2+}], $FeSO_4$ [Fe^{2+}], KCl [K^+], 2-mercaptoethanol (2-Me), sodium dodecyl sulfate (SDS), and ethylene diamine tetraacetic acid (EDTA) was used to assess the interference effect on ArsM-ELP enzyme activity. The enzyme activity of ArsM-ELP measured under the optimized conditions was defined as 100%.

ArsM-ELP enzyme activity assay

EPIgeneous™ Methyltransferase Assay kit (Cisbio Bioassays, Bedford, MA, USA) was used to assay the enzyme activity of ArsM-ELP. The conversion of SAM to S-adenosylhomocysteine (SAH) was measured according to the assay kit instruction. The reaction time was set at 30 min (Dong et al. 2015). The reaction system had a volume of 10.0 μL of 50 mmol/L PBS buffer (pH 7.4) containing 5.0 μmol/L ArsM-ELP, 10 μmol/L As(III), 20 μmol/L SAM, and 4 mmol/L glutathione (GSH). ArsM-ELP enzyme at 5.0 μmol/L and As(III) at 10 μmol/L were chosen as described previously (Dong et al. 2015). SAM at 20 μmol/L was used to assay the enzyme activity of ArsM-ELP according to the Instruction of EPIgeneous™ Methyltransferase Assay kit (Cisbio Bioassays, Bedford, MA, USA). The reactions were performed at 37 °C for 30 min. At the end of the reaction, the ruthenium-labeled SAH antibody and SAH-d2 tracer were added and incubated at 37 °C for 1 h. The fluorescence intensity was measured at both 665 and 620 nm with excitation at 337 nm in a multi-function microplate reader (SpectraMax M5, Molecular Devices, Silicon Valley, CA, USA). The blank control (without As, without enzyme) and the As-free control were set up. The homogeneous time-resolved fluorescence (HTRF) signal was calculated from the ratio of emission at 665 and 620 nm. The yield of SAH was calculated from the SAH standard curve. The enzyme activity was evaluated as the difference in SAH content between the experimental group and the As-free control group.

As volatilization of engineered *E. coli* (ArsM-ELP) and ArsM-ELP

The difference in total As content before and after bacterial growth was used to assess the As volatilization of *E. coli* (ArsM-ELP). 20 mL of cells of *E. coli* (ArsM-ELP) and *E. coli* AW3110 (harboring empty plasmid) (OD_{600} about 0.5) was cultured in LB liquid medium containing 0.20 mmol/L IPTG and 25 μmol/L As(III). Control experiments without As(III) were performed under the same conditions. Each experiment was repeated in triplicate. These experiments were performed by shaking at 200 r/min, at 37 °C for 8 h and 24 h and submitted for total As measurement. 0.50 mL of the sample was digested with 6 mL of HNO_3 with a microwave digestion apparatus (Multiwave Pro, Anton Paar GmbH, Graz Austria) for microwave-assisted digestion. The digestion parameters were set as described previously (Zhu et al. 2008). The digested sample was diluted to 50.0 mL and filtered through a 0.22 μM microporous membrane. The total As content was analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent 7800, Agilent Technologies, Tokyo, JHS, Japan). The As mixture standards were used as external standard samples and measured as described previously (Oliveira et al. 2017).

The difference in total As content was used to assess the As volatilization rate of ArsM-ELP. The ArsM-ELP reaction system was set at 5.0 mL containing 2.0 mmol/L SAM, 20.0 mmol/L GSH, 100.0 μmol/L As(III), and 50.0 μmol/L ArsM-ELP. After incubating for 3 h and 12 h, 0.5 mL of sample was taken and 3% HNO_3 was added to make a volume of 50.0 mL. The total As content was determined by ICP-MS.

Results

Construction and identification of engineered *E. coli* (ArsM-ELP)

The engineered *E. coli* (ArsM-ELP) cell was obtained by recombinant DNA techniques. The PCR-amplified *arsM* was ligated into the pET-22b-ELP vector and heterologously

expressed in the As(III)-hypersensitive *E. coli* AW3110 (DE3), which lacks the *arsRBC* operon and has no *arsM* (Carlin et al. 1995). The sequencing results showed that the *arsM* sequence cloned into the pET-22b-ELP vector and expressed in *E. coli* (ArsM-ELP) was identical to those deposited in GenBank. A protein band of 49.48 kDa was visible in *E. coli* (ArsM-ELP) after IPTG induction, whereas the protein bands in *E. coli* (pET-22b-ELP) and in *E. coli* (ArsM-ELP) were not obvious without ITPG induction (control) (Fig. 1). Due to the presence of ELP at the C-terminus of ArsM, the molecular weight of the expressed ArsM was in accordance with the expected molecular weight of the expressed protein (49.48 kDa), indicating that *arsM* was functionally expressed.

As resistance and volatilization by engineered *E. coli* (ArsM-ELP)

ArsM could methylate As(III) to form methylated As species with lower toxicity and volatile As species under aerobic conditions, thereby conferring As resistance to As(III)-sensitive *E. coli* AW3110 (Qin et al. 2006). To examine the functional activity of the ArsM-ELP fusion protein, we determined the As resistance and As volatilization rate of the engineered *E. coli* (ArsM-ELP) (Fig. 2). In the absence of As(III), no

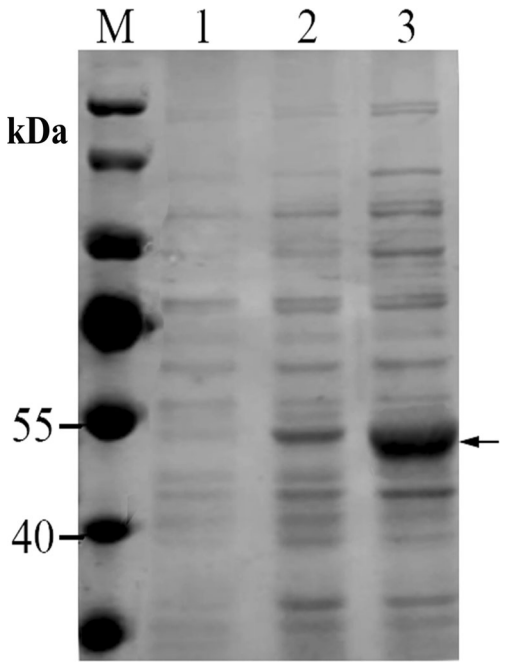

Fig. 1 SDS-PAGE analysis of ArsM-ELP in engineered *E. coli* (ArsM-ELP). Lane M: protein marker. Lane 1: cell lysate of *E. coli* (pET-22b-ELP). Lane 2: cell lysate of *E. coli* (ArsM-ELP) without IPTG induction. Lane 3: cell lysate of *E. coli* (ArsM-ELP) induced by IPTG. Samples were harvested after induction with 0.2 mmol/L IPTG at 30 °C for 16 h, and the cellular proteins were analyzed by SDS-PAGE

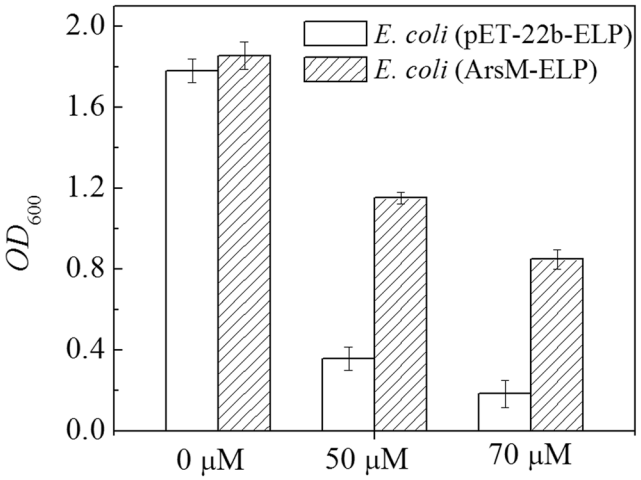

Fig. 2 As(III) resistance of the engineered *E. coli* (ArsM-ELP) and the control strain *E. coli* (pET-22b-ELP). The inocula of 2.0% (*V*/V) were incubated for 22 h in 100 mL LB medium in the presence of 0.2 mmol/L IPTG and different As(III) concentrations. Data shown were the mean values (± standard deviations) obtained from three independent experiments

significant difference ($P > 0.05$) in cell growth between the engineered strain and the control could be observed. After exposure to 50 μM and 70 μM As(III), the inhibition rate of the cell growth displayed a difference between the control strain (79.9% and 89.8%, respectively) and engineered strain (37.0% and 54.3%, respectively), indicating that the engineered *E. coli* (ArsM-ELP) possessed higher As resistance than the control strain in the presence of As(III). Furthermore, the expressed ArsM-ELP fusion protein was functionally active in vivo.

To examine the As volatilization rate of engineered *E. coli* (ArsM-ELP), the difference in total As content before and after bacterial growth was used to assess the As volatilization rate (Fig. 3). After incubation for 8 h and 24 h, the total As content in the medium decreased by 10.39% and 13.42% when incubated with the engineered strain, respectively, whereas the total As content decreased by only 0.61% and 0.53% with the control strain, respectively. These results demonstrated that the engineered *E. coli* (ArsM-ELP) had a significant As volatilization capacity and the ArsM-ELP fusion protein was responsible for As(III) methylation. Therefore, we further purified the ArsM-ELP fusion protein and studied its enzymatic properties.

The expression of ArsM-ELP by *E. coli* (ArsM-ELP)

As shown in Fig. 4, the highest expression level of ArsM-ELP (0.14 g/L) was observed at 30 °C, which accounted for 23.4% of the total protein content (Fig. 4a2). Under optimized conditions at 30 °C, 0.2~1.0 mmol/L of IPTG induced a high expression level of ArsM-ELP of about 0.14 g/L (Fig. 4b2). Under the optimized 30 °C and 0.2 mmol/L of IPTG

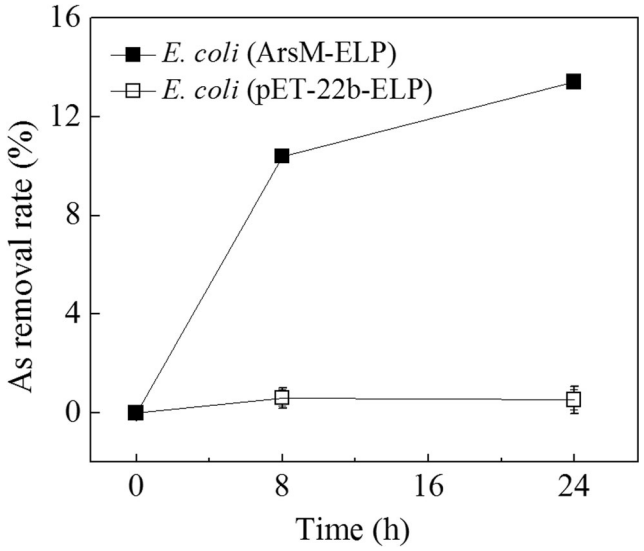

Fig. 3 As volatilization rate comparison between the engineered *E. coli* (ArsM-ELP) and the control *E. coli* (pET-22b-ELP). 100 mL of culture (OD_{600} = 0.5) was incubated for 8 h and 24 h at 37 °C in the presence of 0.2 mmol/L IPTG and 25.0 μmol/L As(III). The As volatilization rate was assessed by the difference in total As content before and after bacterial growth. The data shown were the mean (± standard deviation) obtained from three independent experiments

condition, the high expression level of ArsM-ELP (0.25 g/L) was observed at 16 h of culture time, accounting for 32.2% of the total protein content (Fig. 4c2). Collectively, the optimal conditions for ArsM-ELP expression in recombinant *E. coli* (ArsM-ELP) were that 0.2 mmol/L of IPTG induced *E. coli* (ArsM-ELP) for 16 h at 30 °C; the total protein yield reached 0.79 g/L.

The purification of ArsM-ELP

The highest yield of ArsM-ELP (9.46%) was obtained in the presence of 2.5 mol/L NaCl after three rounds of ITC purification (Fig. 5a). However, considering the negative influence of high concentration of NaCl on enzymatic activity, we selected 2.0 mol/L NaCl for subsequent experiments. In the presence of 2.0 mol/L NaCl, the optimal T_t was optimized at 32 °C (Fig. 5b). Under the above-optimized condition, the phase transition time for ArsM-ELP purification was optimized at 5 min (Fig. 5c). In order to ensure the complete precipitation of ArsM-ELP, the phase transition time was prolonged to 10 min.

The effect of total protein content on ArsM-ELP purification was investigated based on the optimal NaCl concentration, T_t, and phase transition time (Fig. 5d, e). The 6.0~16.0 mg/mL of total protein extract was selected for ArsM-ELP purification. After three rounds of ITC, a single protein band was observed by SDS-PAGE; the yield of ArsM-ELP reached 9.6~10.8% of the total protein. Two rounds of ITC were enough to satisfy the purity

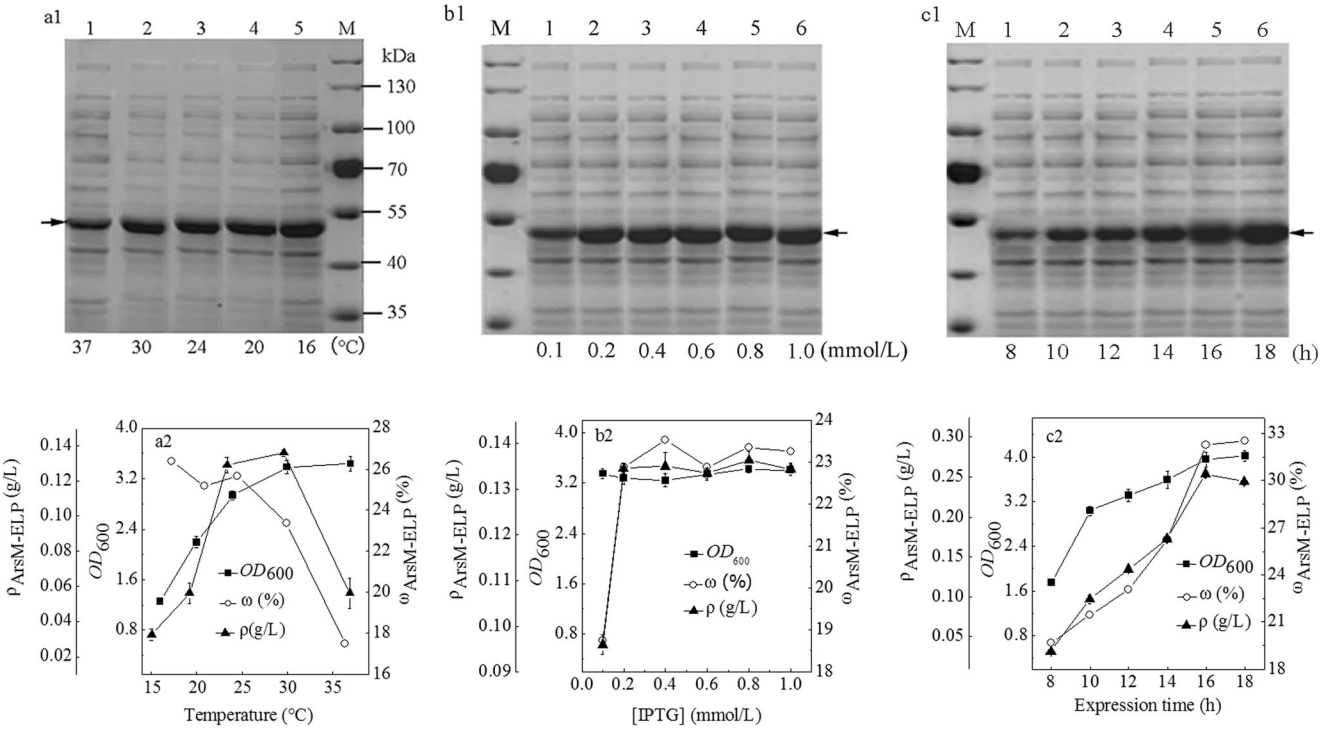

Fig. 4 The effect of culture temperature (**a**), IPTG concentration (**b**), and the expression time (**c**) on the ArsM-ELP amount in *E. coli* (ArsM-ELP). **a** *E. coli* (ArsM-ELP) was induced for 12 h at 0.8 mmol/L IPTG; **b** *E. coli* (ArsM-ELP) was induced for 12 h at 30 °C; **c** *E. coli* (ArsM-ELP) was cultured at 30 °C and 0.2 mol/L IPTG. $\rho_{ArsM\text{-}ELP}$ represents the ArsM-ELP amount in recombinant *E. coli* (ArsM-ELP). $\omega_{ArsM\text{-}ELP}$ represents the ratio of ArsM-ELP to total protein content in recombinant *E. coli* (ArsM-ELP)

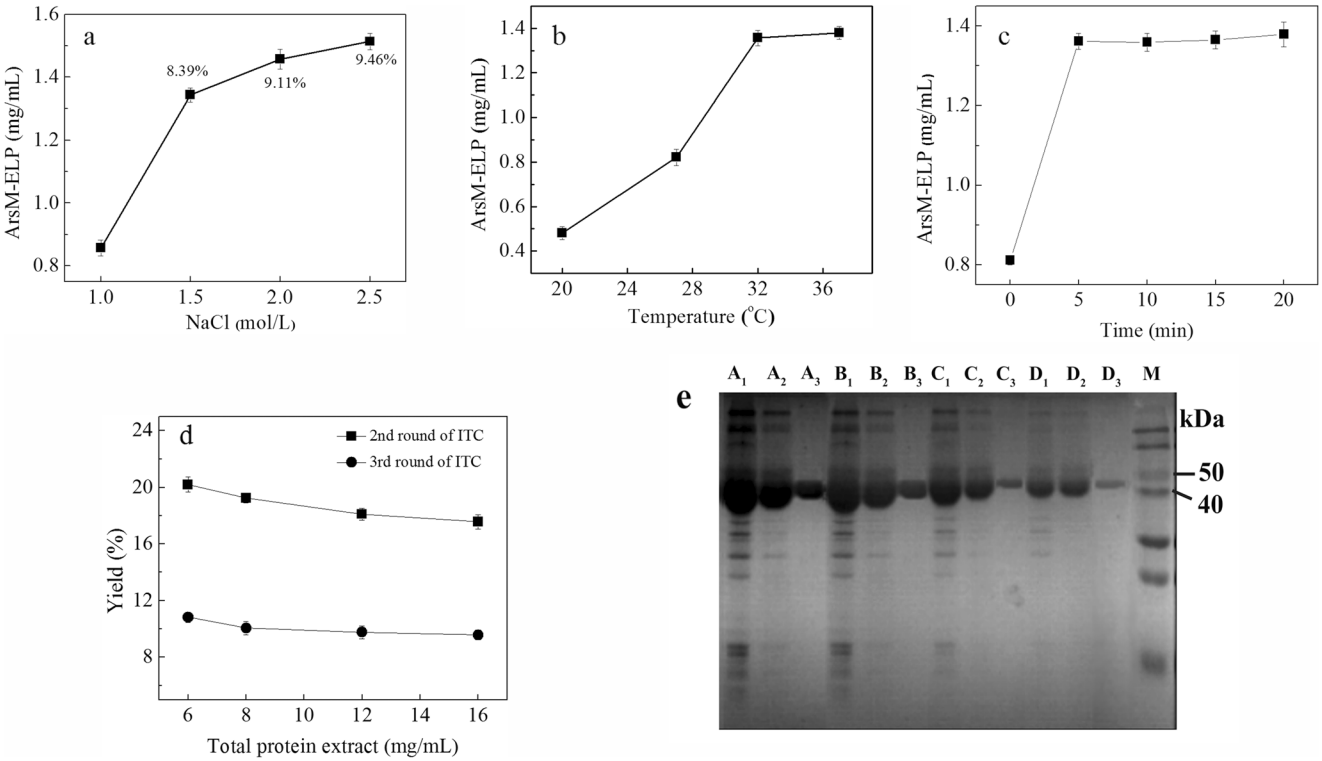

Fig. 5 Optimization of ITC conditions for ArsM-ELP purification. **a** NaCl concentration; **b** phase transition temperature; **c** phase transition time; **d** ITC treatment times; **e** total protein extract concentration: Lane M: protein marker; Lane A–D: 16, 12, 8, and 6 mg/mL, respectively; subscript 1, 2, and 3 represented ITC round times, respectively

requirement for ArsM-ELP since a protein yield of 17.6~20.2% had been achieved, and the purity requirement is not that stringent in practical applications. Therefore, the ArsM-ELP content available in 1 L of *E. coli* culture ranged from 0.14 to 0.16 g.

The relationship between NaCl concentration and T_t

A linear negative correlation between T_t and NaCl concentration was observed in the presence of 1.0 mg/mL of purified ArsM-ELP (Fig. 6); this phenomenon was consistent with the reported literature (Huang et al. 2011; Lim et al. 2007; Meyer and Chilkoti 1999; Reguera et al. 2007). The T_t was 31.7 °C in the presence of 2.0 mol/L NaCl, which was consistent with the optimized T_t (32 °C). The result shown here (T_t = 31.7 °C, 2.0 mol/L NaCl) was much better compared with the reported ELP transition data (T_t = 35 °C, 2.5 mol/L NaCl) because the lower salinity could prevent the denaturation of the spatial structure of ArsM caused by higher salt concentration.

The recovery rate of purified ArsM-ELP

To determine whether ArsM-ELP could be recovered and reused, the recovery rate of different concentrations of ArsM-ELP was investigated (Fig. 7). Under the optimized NaCl concentration, T_t, and phase transition time conditions, a recovery rate of 85.2~96.0% for ArsM-ELP was achieved in the range of 1.0~1.5 mg/mL ArsM-ELP after one round of ITC treatment. A positive linear relationship was observed between the recovery rate and increasing

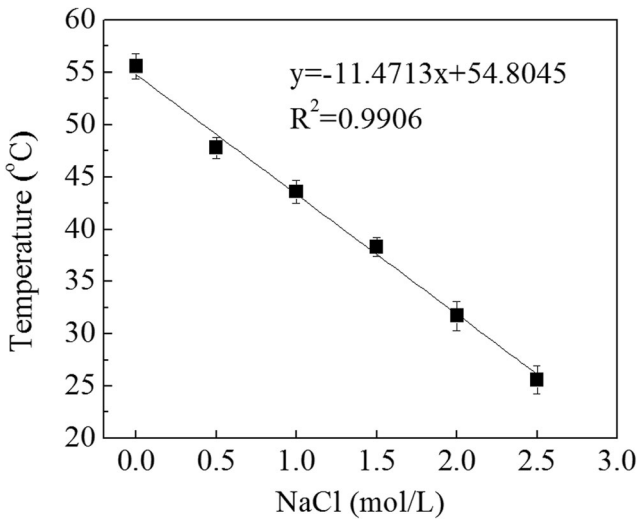

Fig. 6 The relationship between NaCl concentration and T_t in the presence of 1.0 mg/mL of purified ArsM-ELP

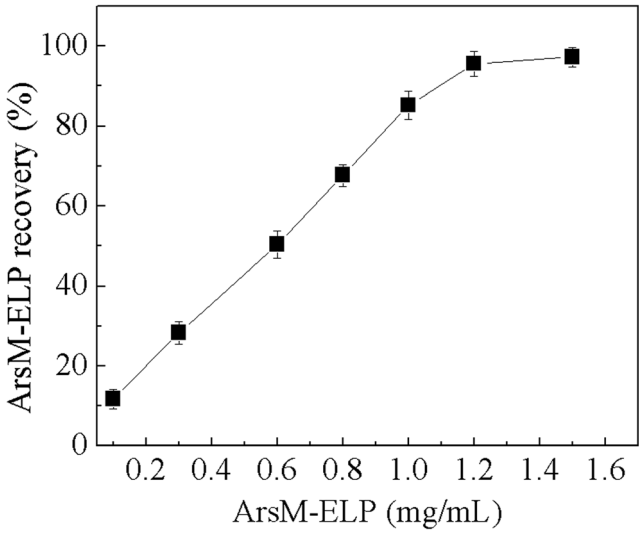

Fig. 7 The relationship between the recovery rate and increasing concentrations of purified ArsM-ELP

enzyme concentrations, which would be of great significance for bioremediation of As-contaminated environments by using ArsM-ELP.

The enzymatic properties of ArsM-ELP

The ArsM-ELP is able to catalyze the demethylation of SAM to SAH and in turn methylate As; therefore, the change in SAH yield can reflect the enzyme activity of ArsM-ELP in vitro. When exposed to 10 μmol/L As(III), catalytic activity of ArsM-ELP increased linearly within 30 min ($R^2 = 0.9981$) (Fig. 8a). More than 95% of enzymatic activity was observed in the range of 25~35 °C; the SAH yield reached 648.7 nmoles under the optimal 30 °C condition (Fig. 8b). More than 75% and 50% of enzyme activity were retained at 35 °C and 45 °C for 12 h, respectively. The enzyme activity was retained at more than 60% at 55 °C for 2 h (Fig. 8c). The highest enzyme activity was observed at pH 7.5, and the SAH yield reached 658.23 nmoles, the enzyme activity was still retained above 70% in the range of pH 5.0~9.0, indicating that the ArsM-ELP had an even broader pH tolerance (Fig. 8d).

The substrate specificity for ArsM-ELP was also examined under the above-optimized conditions (Fig. 8e). It was shown that the catalytic activity of ArsM-ELP was only observed when exposed to As (III), but not for other ions including K^+, Ca^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, and even As(V), indicating that ArsM-ELP had a narrow selectivity for substrates.

The interference factors on ArsM-ELP enzyme activity were further investigated (Fig. 8f). It could be shown that ArsM-ELP enzyme activity increased by14.71% after the addition of 2-Me, whereas SDS, EDTA, Fe^{2+}, and Cu^{2+} significantly inhibited enzyme activity (decreased by 55.74%, 39.49%, 11.67%, and 14.71%, respectively). The other ions including Mg^{2+}, Zn^{2+}, Ca^{2+}, and K^+ had no significant effect on the enzyme activity ($P > 0.05$). Under optimized conditions, the specific activity of the enzyme was 438.8 ± 2.1 U/μmol.

As volatilization rate by purified ArsM-ELP

The As volatilization by ArsM-ELP is shown in Fig. 9. The initial value of As(III) was 99.45 ± 0.21 μmol/L. The As content in the medium decreased by 35.49% and 38.35% after incubation for 3 h and 12 h, respectively, indicating that ArsM-ELP catalyzed the conversion of As(III) to volatile As.

Discussion

Arsenic (As) contamination affects an increasing number of people and has been given more attention as approaches are needed to mitigate the negative effects of this health crisis (Zhou et al. 2018; Zhu et al. 2014). Genetically engineered bacteria display more efficient enrichment and immobilization of heavy metals and are gradually emerging as an effective tool for remediation of heavy metal pollutants (Singh et al. 2011). For example, arsenic regulatory protein gene (*arsR*), phytochelatin gene, and metallothionein gene were used to construct engineered *E. coli* which showed a high metal-binding capacity (Ke et al. 2018; Sauge-Merle et al. 2003; Singh et al. 2008). Recently, many As-methylating microbes and ArsM enzyme were shown to convert highly toxic As(III) into less toxic pentavalent or volatile methylated As species; the volatile As species was removed from environment via volatilization. Therefore, As methylation was considered to be an effective method for the remediation of As contamination (Ye et al. 2012). However, except for a wild strain displaying high As(III) volatilization (such as the *A. rosenii* SM-1, Huang et al. 2016), most naturally occurring microbes showed a limited As volatilization capacity (Wang et al. 2014a). In an attempt to obtain high As volatilizing microbes, *arsM* was used to construct engineered strains (Chen et al. 2013, 2014; Huang. et al. 2015; Liu et al. 2011; Zhu et al. 2008), and the As volatilization rate of these engineered strains was generally in the range of 0.01~13% except for *P. putida* KT2440 (31%) (Chen et al. 2014). The reason for a lower As volatilization rate might be because ArsM was poorly expressed in vivo. In addition, As(III) could not be transported by a specific transporter into the cells leading to lower As(III) concentrations. However, lower concentration of As(III) in vivo did not efficiently induce transcription of *arsM* to produce sufficient ArsM for a high rate of As(III) methylation. On the other hand, perhaps the ArsM activity in vivo was not enough to catalyze the conversion of As(III) to TMA(III) gas, leading only to the formation of MMA(V) or DMA(V) intermediates. In contrast, the purified ArsM was

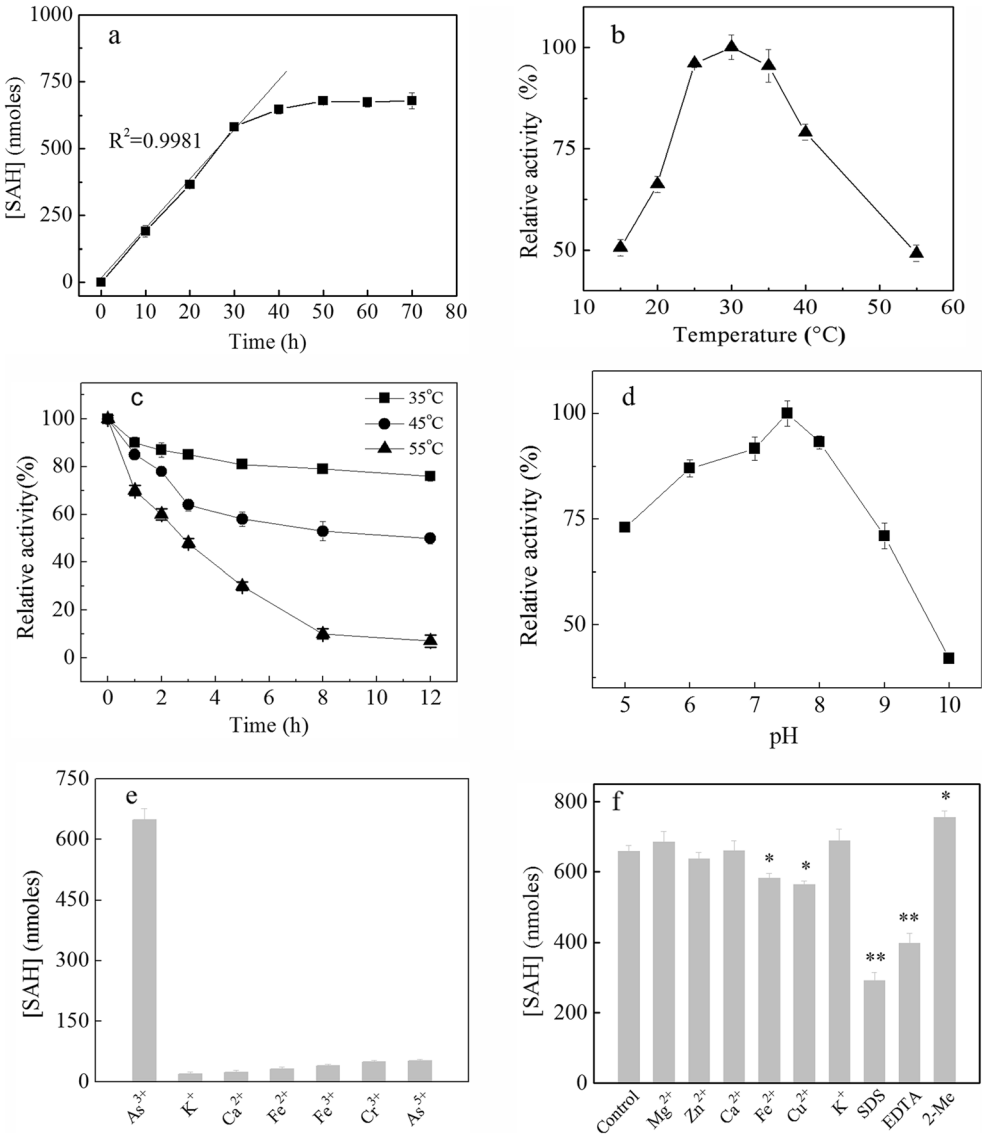

Fig. 8 ArsM-ELP enzymatic properties. **a** reaction time curve; **b** the effects of temperature on the enzyme activity; **c** the thermal stability; **d** the effects of pH on enzyme activity; **e** substrate selectivity; **f** the effects of interference factor on enzyme activity. $*P < 0.05$; $**P < 0.01$. The data shown were the mean (± standard deviation) obtained from three independent experiments

able to contact directly with As(III), therefore leading to a high As methylation rate. In previous studies, the determination of As methylation was mainly used to identify the function of putative *arsM* genes, and ArsM was purified as a fusion protein using His tags (Guo et al. 2016; Huang et al. 2016, 2018; Qin et al. 2006, 2009; Wang et al. 2014b; Ye et al. 2014; Yin et al. 2011). Since the His-tag affinity chromatography purification technique is cost-expensive (Kosobokova et al. 2016), the application of ArsM purified using His-tag in remediation of As contamination is limited. Thus, it was necessary to explore a simple and cost-effective preparation of ArsM purification.

ArsM encoded on the genome of *R. palustris* CGA009 was considered a suitable candidate for an applied As methylation process. Since Qin et al. (2006) firstly validated the ArsM function, many engineered bacteria (Chen et al. 2014; Liu et al. 2011; Yuan et al. 2008) or plants (Meng et al. 2011) harboring *arsM* derived from CGA009 were developed. In this study, we fused *arsM* from *R. palustris* CGA009 with ELP-tag and heterologously expressed it in *E. coli*. The engineered *E. coli* (ArsM-ELP) could volatilize 10.39% and 13.42% of ~25 μmol/L As after 8 h and 24 h, respectively, showing a high level of As volatilization traits compared to most of the reported strains.

Compared to the His-tag affinity chromatography, the ELP-tag purification approach is non-chromatographic (Meyer and Chilkoti 1999; Roberts et al. 2015), convenient, low cost, and easy to realize at a large scale (Trabbic-Carlson et al. 2004). So far, many recombinant proteins such as chloramphenicol acetyltransferase, blue fluorescent protein, thioredoxin, calmodulin, and xylanase have been successfully purified by using ITC (Li and Zhang 2014; Lim et al. 2007; Trabbic-Carlson et al. 2004). In this study, the purified ArsM-

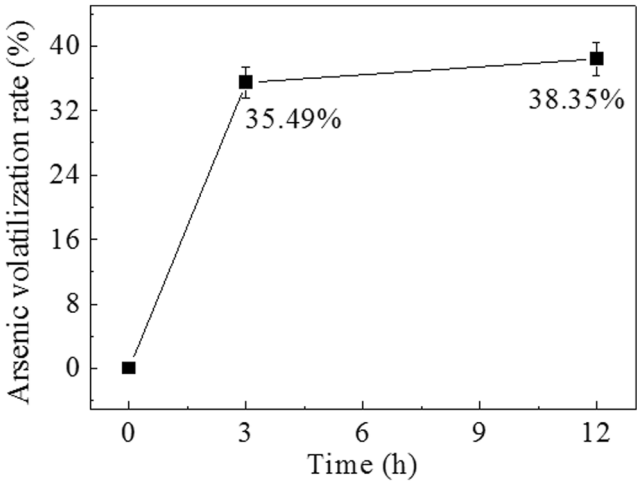

Fig. 9 As(III) volatilization by ArsM-ELP. The reaction system (5.00 mL) contained 2.0 mmol/L of SAM, 20.0 mmol/L of GSH, 100 μmol/L of As (III), and 50 μmol/L of ArsM-ELP. The As volatilization rate was measured after incubation for 3 h and 12 h. Percentage indicates As volatilization. The data shown were the mean (± standard deviation) obtained from three independent experiments

ELP was obtained after ITC purification in the presence of 2.0 mol/L NaCl at 32 °C for 10 min. Both the NaCl concentration and T_t were decreased (2.0 mol/L NaCl at ~32 °C) compared to the reported ITC purification system (2.5 mol/L NaCl at 35 °C, Huang et al. 2011) and the phase transition time was shorter (~10 min). A good enzyme activity of purified ArsM-ELP was observed under optimized ITC conditions.

Studies have shown that enzymes play a major role in bioremediation processes and were shown to have a significant advantage over microbes and plants in remediation of heavy metal contaminated soils (Rao et al. 2014). For example, the conversion rate of As(III) to TMA(III) by NsArsM derived from *Nostoc* sp. PCC7120 (< 2.0% of total As, after 12 h) was higher than that of *E. coli* AW3110 harboring *NsarsM* (< 1% of total As) (Yin et al. 2011). When WaArsM derived from *Westerdykella aurantiaca* used As(III) (100 ppb) and As(V) (200 ppb) as substrates respectively, 29.3% and 13.95% of As volatilization rate were achieved after 3 h, respectively. *Saccharomyces cerevisiae* harboring *WaarsM* volatilized 29.0% and 11.0% of total As when incubated with As(III) (2 ppm) and As(V) (20 ppm) for 24 h, respectively (Verma et al. 2016). Compared to the engineered organisms, the As(III) methylation reaction catalyzed by ArsM needed less time and resulted in higher As volatilization, highlighting the potential advantage of ArsM as an enzyme preparation in the remediation of environmental As contamination. In this study, the purified ArsM-ELP could reduce the initial As content (~100 μmol/L) by 35.49% after 3 h; the engineered *E. coli* (ArsM-ELP) reduced 13.42% of the initial As content (~25 μmol/L) after 24 h, indicating that the ArsM-ELP had a higher volatilization rate for As(III) than the engineered *E. coli* (ArsM-ELP), which was consistent with the reported literature (Verma et al. 2016; Yin et al. 2011). Probably As(III) is able to react with ArsM directly in vitro, whereas the expression of ArsM in vivo is regulated by As(III), which needed across the cell membrane to induce and react with ArsM. In addition, As(III)-pump protein (ArsB) would pump out As(III), the substrate of ArsM. Therefore, the enzyme has an advantage in the remediation of environmental As contamination compared to the engineered strain. However, current studies mainly focused on the characterization of As-methylating microbes, less was known about using an ArsM strategy to address As pollution. Therefore, this study is of great potential application value for future As pollution bioremediation.

Collectively, this study provided a novel, simple, cost-effective strategy for ArsM enzyme preparation at a large scale. The purified, stable, specific, higher activity of the ArsM-ELP could be obtained by using a modified ELP non-chromatography protein purification technique. The ELP-tag is not only used for protein purification in large scale, but also for the protein recovery and recycles. Both purified ArsM-ELP and engineered *E. coli* (ArsM-ELP) could perform As(III) methylation, and the As(III) methylation rate by ArsM-ELP was higher than that of *E. coli* (ArsM-ELP). The ArsM-ELP would not only be used for bioremediation of As contamination environment by As methylation, but also for the standards' preparation of various As methylated intermediate products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any study with human participants or animals performed by any of the authors.

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