Bioadsorption and microbe-mediated reduction of Sb(V) by a marine bacterium in the presence of sulfite/thiosulfate and the mechanism study

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HIGHLIGHTS
• A novel shewanella subspecies was reported for Sb(V) reduction for the first time.
• The presence of SO₃²⁻/S₂O₃²⁻ can lead to the formation of Sb₂S₃, while SO₄²⁻ can’t.
• Gene S.CNZ.1GM004058/001069 should responsible for SO₃²⁻/S₂O₃²⁻ bioreduction.

GRAPHICAL ABSTRACT

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ABSTRACT

Microbe-Sb interactions remain poorly understood and the molecular mechanism of microbe-mediated Sb(V) reduction remains unclear. In the present study, we investigated the process and mechanism of Sb(V) bioreduction by Shewanella sp. CNZ-1, which was isolated from the sediment of the Bohai Strait, in the absence or presence of sulfate/sulfite/thiosulfate. Results demonstrate that Sb(V) could be reduced to Sb(III) (including Sb₂O₃ and Sb(III)(aq) etc.) by CNZ-1 cells. Kinetic studies are carried out using the Langmuir-Freundlich dual model and Monod model and the results reveal that Sb removal by strain CNZ-1 is a fast bioadsorption (KLF, 1/n and a are 8.03, 0.0032 and 0.95, respectively; R² = 0.98) and slow bioreduction (R² = 0.95) process. Moreover, the supplementation of SO₃²⁻ and S₂O₃²⁻ into the reaction system can lead to the formation of Sb₂S₃ along with the reduction of SO₃²⁻ and S₂O₃²⁻, while SO₄²⁻ can’t. The precipitates of Sb₂S₃ and Sb₂O₃ are further characterized and confirmed by SEM-EDX, XPS and XRD. In addition, global transcriptome assays reveal that genes encoding dehydrogenase, cytochrome, reductase, stress resistance protein, membrane proteins and transporters play key roles during the transformation of SO₃²⁻/S₂O₃²⁻ by CNZ-1 cells and subsequent RT-qPCR assays show that the genes S.CNZ.1GM004058 and S.CNZ.1GM001069 should account for the reduction of SO₃²⁻ and S₂O₃²⁻ by the CNZ-1 strain, respectively.

1. Introduction

Recently, antimony (Sb) has received much attention because of its increasing environmental concerns [1–4]. Sb is the ninth-most mined metal worldwide, and it is used in a diverse array of industries, including the manufacturing of semiconductors, lead acid automobile batteries, printing presses, ammunition, glassware, ceramics and paint pigments [1,5]. The wide use of Sb and Sb-contained compounds has
increased its transfer into the surrounding environment. Considering their toxicity and suspected carcinogenicity to humans, Sb and Sb-containing compounds have been classified as pollutants of priority interest by both the EPA (U.S.A) [6] and European Union [7] since the 1970s. Sb can exist in a variety of oxidation states in nature but is most commonly found in either antimonite (Sb(III)) or antimonate (Sb(V)) [1,8]. Although trivalent Sb compounds are generally considered to be more toxic than pentavalent Sb compounds, the mobility of Sb(III) in the environment is significantly inferior compared to that of Sb(V) [9–10]. Therefore, it is of great significance to study the migration and transformation of trivalent and pentavalent Sb compounds in different natural water conditions for predicting their potential environmental risks.

As previous studies indicated, Sb(V) and Sb(III) are thermodynamically stable in aerobic and anaerobic conditions, respectively [4]. However, Sb(V) has been found in anoxic condition [11,12] and Sb (III) has also been detected in various oxygen-contained systems, including fresh water [13], marine [14] and ground water [15]. These observations were explained by various abiotic and biological mechanisms [5,16]. Compared to those abiotic hypotheses, the involvement of microorganisms in the biogeochemical cycle of Sb seems more reasonable. There are a great deal of studies, which often focused on the bioavailability of Sb and the interactions of Sb and microorganisms from different habitats, that were carried out to illuminate the microbe-mediated transformation process and mechanism between Sb(V) and Sb(III) [4,5,17–20]. Early in 1974, Lyalikova found that Sb(III) could be oxidized to Sb(V) by a chemoautotrophic bacterium [18]. Henceforth, more than 60 Sb(III) oxidizing strains were isolated from contaminated sediments and mining soil [16]. Moreover, in terms of molecular mechanisms, genes (ars operon) and enzymes (AioB4 and AnoA) related to Sb(III) resistance and oxidation were discovered in succession [19–21]. Compared to the detailed data on the microbial oxidation of Sb(III), the knowledge of the microbial reduction of Sb(V) is rather insufficient. In 2014, Kulp et al. reported the anaerobic bioreduction of Sb(V) by a natural microbiological population obtained from the stibnite mine site [4]. Subsequently, Abin et al. [5] and Nguyen et al. [22] reported that Sb(V) could be reduced to Sb(III) by the MLFW-2 and JUK-1 bacteria by dissimilatory reduction using Sb(V) as a terminal electron acceptor. In addition, Lai et al. found that the autotrophic bio-reduction of Sb(V) could be driven by either H2 or CH4 [23,24]. However, up until now, the molecular mechanism of microbe-mediated Sb(V) reduction is still not clear enough, and further studies need to be performed to make this process better understood.

In the present study, we reported that a marine bacterium Shewanella sp. CNZ-1 is capable of reducing Sb(V) to Sb(III) under anoxic conditions. Since Sb(III) can easily precipitate with sulfide in a reducing environment, the effects of sulfate, sulfite and thiosulfate on Sb(V) reduction by the CNZ-1 strain were investigated. Moreover, the genome sequencing, whole transcriptome and real-time quantitative polymerase chain reaction (RT-qPCR) experiments were carried out for seeking related genes that are involved in strain CNZ-1 mediated Sb(V) reduction in the presence of sulfate and thiosulfate. These studies are beneficial for understanding the fate of Sb in the marine environment in the presence of sulfate, sulfite and thiosulfate.

2. Materials and methods

2.1. Chemicals

K5Sb(OH)6 and Sb2O3 were purchased from the Shanghai Macklin Biochemical Co., Ltd (China). Sodium lactate, sodium sulfate, sodium sulfite, sodium thiosulfate, thiocarbamide and ascorbic acid were purchased from the China National Pharmaceutical Group Co., Ltd (China). All other reagents used in this study were of the highest analytical grade. Sterile stock solutions of K5Sb(OH)6 (10 mM) and Sb2O3 (treated with 2 M HCl, 10 mM) were prepared using deionized water.

2.2. Bacterial strain and culture

Shewanella sp. CNZ-1 was isolated from marine sediment (N 38° 30.29′, E 121° 14.10′, Bohai Strait, China) by Zhang et al. and stored in our lab at −80°C [25]. Strain CNZ-1 (GenBank accession number KX384589) was proven to be a member of the electrochemical active bacteria. Luria-Bertani (LB) broth contains (g/L) yeast extract 5, peptone 10 and NaCl 10. Mineral salt medium (MSM) contains (g/L) sodium lactate 2 (approximately 17.85 mM), NH4Cl 1.0, Na2HPO4 0.8, KH2PO4 0.2, MgCl2 0.2, CaCl2·2H2O 0.1 and NaCl 20. The pH value was maintained at 7.2 ± 0.2 using phosphate buffer included in the MSM.

2.3. Sb(V) reduction assays

Unless otherwise noted, all Sb(V) reduction assays in the present study were carried out at 20 ± 2°C. The Sb(V) reduction system contains 100 mL of deoxygenated sterile MSM, CNZ-1 cells (OD600 = ~0.2) and Sb(V) (0.2, 0.4, 0.8, 1.2 and 1.6 mM, respectively) in a 135 mL serum bottle. Strain CNZ-1 was first cultured for 2 h (logarithmic phase) in 100 mL of LB (v/v = 1%) in a rotary incubator shaker (150 r.p.m., 20°C); secondly, the cell pellet was harvested by centrifugation (10,000 r.p.m., 5 min), washed by MSM twice and then added into the Sb(V) reduction system at a final dry cell weight of 0.1 g cell/L (OD600 = ~0.2). After 10 min of N2 exposure, all experimental systems were transferred into an anaerobic chamber. Samples were taken periodically with a sterile needle and a syringe for the analysis of Sb species. Assays were run in triplicate.

2.4. Effects of sulfate, sulfite, thiosulfate on the bioreduction of Sb(V)

To investigate the effects of sulfate, sulfite and thiosulfate on the reduction of Sb(V) through strain CNZ-1, MSM supplemented with 0.8 mM antimonite, 0.1 g/L CNZ-1 cells and varied concentrations (1.41–9.52 mM) of sulfate, sulfite and thiosulfate (0 g/L, 0.2 g/L, 0.4 g/L, 0.8 g/L and 1.2 g/L, respectively) were divided into fifteen sets and tested in parallel. All fifteen sets of triplicate bottles were incubated according to Section 2.3. Samples were taken periodically with a sterile needle and a syringe for the analysis of the Sb species, SO42− concentration and HS− concentration.

2.5. Characterization of the reduced products

Scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDX, Hitachi S-4800, Japan) was employed to identify the surface morphology of the reduced product. To identify the structures and valence states of Sb species, the reduced products were further analyzed by using X-ray diffraction patterns (XRD, BRUKER D8 ADVANCE, Germany), X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, England) and Fourier transform infrared spectroscopy (FTIR, Jasco FT/IR-4100, Japan). For SEM-EDX analysis, the CNZ-1 cells associated with the reduced products, which were exposed to 0.8 mM Sb(V) for 338 h, were first fixed with 3% (v/v) glutaraldehyde. The samples were then dried with ethanol in ambient conditions and mounted on an aluminum stub. For XRD, XPS and FTIR analyses, the cells were first exposed to 0.8 mM Sb(V) for 338 h, then separated by centrifugation (10,000 r.p.m., 4 °C, 5 min) and finally dried for 24 h in a freeze-dryer.

2.6. Genome sequencing assays

In order to further study the molecular mechanism of Sb(V) reduction by strain CNZ-1 in the presence of sulfate, sulfite and thiosulfate, the draft genome of strain CNZ-1 was sequenced using Illumina HiSeq 2500 with a PE150 strategy at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). Gene prediction and functional annotation were performed using the NCBI Prokaryotic Genome Annotation Pipeline.
2.7. Whole transcriptome assays

The whole transcriptome assays were performed according to the following steps: first, the total RNA from cells obtained from systems with or without sulfate/thiosulfate supplementation was isolated by anEasy Pure RNA Kit (TransGen, Biotech, China); second, cDNA was then transcribed from the total RNA, followed by cDNA library sequencing with an Illumina HiSeq 2500 system (Novogene Bioinformatics Technology Co., Ltd., Beijing, China); third, reads generated by the sequencing machines were cleaned by discarding the adaptor and low-quality sequences; and finally, the clean reads were assembled and then mapped to the genome sequence of Shewanella sp. CNZ-1. Comparative analysis of the transcriptomes was performed as described in a previous study [26].

2.8. RT-qPCR assays

Furthermore, RT-qPCR assays were performed to (i) identify the key genes responsible for Sb(V) reduction and SO$_4^{2−}$/S$_2$O$_3^{2−}$-assisted Sb(V) reduction by the CNZ-1 strain and (ii) verify the reliability of global transcriptome assays. RT-qPCR (ABI Prism 7500 Fast, Applied Biosystems, CA, USA) assays were carried out to study the related genes responsible for the reduction of Sb(V) in the presence of sulfate, sulfite and thiosulfate. CNZ-1 cells grown in MSM medium with or without Sb(V) supplementation were extracted at 48 h. The primer pairs for the fifteen genes were designed as described by Wan et al. [27] and synthesized by Qingdao Ruibiotech. Co. Ltd. The results were analyzed using the 2$^{-\Delta \Delta Ct}$ method and normalized using the 16s rRNA gene as an endogenous control. RT-qPCR data were analyzed by the ABI Prism 7500 Fast software (Applied Biosystems, CA, USA).

2.9. Analytical methods

The liquid samples were taken from sealed serum bottles using sterile syringes. All samples for Sb analysis were diluted 1000 times to guarantee final concentrations in the range of 0–150 μg/L. The Sb species were analyzed using an atomic fluorescence spectrometer (AFS, AFS-930, China) according to the method described by Kong et al. [28]. The concentrations of cells were determined by a UV–Vis spectrophotometer at 600 nm. Concentrations of sulfate in liquid samples were measured by suppressed conductivity ion chromatography (IC, Dionex ICS3000, USA). The concentrations of sulfite and thiosulfate were calculated as corresponding sulfate concentrations after their oxidation using 35% H$_2$O$_2$. The dissolved sulfide concentration was determined using an ultraviolet visible spectrometer (UV–Vis, PerkinElmer Lambda 365, USA) based on methylene blue formation [29]. Dissolved oxygen, oxidation–reduction potential (ORP), temperature and pH values were measured using the related probes.

The equilibrium study was performed by the Langmuir-Freundlich dual model

\[ Q_e = K_{L,F} C^{1/n} / (1 + a C^{1/n}) \]  

where $Q_e$ is the equilibrium Sb(V) concentration in the biomass (mg/g), $C_e$ is the equilibrium Sb(V) concentration in solution, and $K_{L,F}$, $1/n$ and $a$ are the Langmuir-Freundlich adsorption constants.

The approximate Sb(V) reduction rates were calculated using equation (2). The half saturation constant $K_s$ (mg Sb/L) and the maximum Sb(V) utilization rate $V_{max}$ (mg Sb(V)/(L.h)) were obtained via fitting the concentration and reduction rate data to a nonlinear Monod equation

\[ V_{reduction} = C_t / t \]  

\[ V_{reduction} = V_{max} C_t / (K_s + C_t) \]  

where $V_{reduction}$ is the approximate Sb(V) reduction rate (calculated as the generation rate of Sb(III)); $C_t$ and $C_i$ (mg/L) are the initial TSB and Sb(III) at time $t$; and $t$ (h) is the reaction time.

3. Results and discussion

3.1. Sb(V) reduction process by the CNZ-1 strain

In the present study, we first studied whether or not strain CNZ-1 is capable of reducing Sb(V) to Sb(III). The evolution of total Sb (TSb) and Sb(III) over the course of incubation in the inoculum amended with both Sb(V), CNZ-1 cells and sodium lactate, the inoculum amended with Sb(V) and CNZ-1 cells and the inoculum amended with only Sb(V) is shown in Fig. 1a and b. In the absence of CNZ-1 cells, no obvious Sb(V) loss and Sb(III) accumulation occurred during the entire process. As can be seen in Fig. 1a, TSB in the inoculum amended with both Sb(V) and sodium lactate (2 g/L) first decreased in the range of 0–74 h and then increased to a steady level in the range of 74–338 h; the Sb(III) in this inoculum increased gradually (Fig. 1b). We inferred that the decrease in TSB was due to both biodesorption of Sb(V) and bioreduction of Sb(V) to some insoluble Sb(III) (such as Sb$_2$O$_3$) by strain CNZ-1, which was confirmed by subsequent analyses. Additionally, the subsequent increase of TSB should be attributed to the accumulation of soluble Sb(III) in the system. In addition, in the absence of sodium lactate, TSB in the inoculum amended with only Sb(V) was dramatically decreased first during 0–98 h and then decreased slightly until 338 h (Fig. 1a); Sb(III) in this inoculum increased at first and then decreased to a steady level (Fig. 1b). Under these conditions, the decrease of TSB is
due to the biosorption of Sb(V) by CNZ-1 cells and also attributed to that a small amount of Sb(V) was reduced to insoluble Sb(III) through the endogenous metabolism of CNZ-1 cells. Similarly, the increase of Sb (III) and its subsequent decrease in the system amended with CNZ-1 cells and Sb(V) can be explained as follows: a small amount of soluble Sb(III) was first accumulated, and this Sb(III) was subsequently immobilized or transformed by strain CNZ-1.

To study the effect of the initial Sb(V) concentration on Sb(V) reduction by strain CNZ-1, 0.2, 0.4, 0.8, 1.2 and 1.6 mM Sb(V) were respectively supplemented in the reaction system in the presence or absence of an electron donor. The results showed that a similar reaction trend to that described in Fig. 1 was observed at all tested Sb(V) concentrations (Fig. S1). In addition, the result of positive control assay showed that the heat-killed CNZ-1 cells can’t reduce Sb(V) to Sb(III) (Fig. S1d). On the basis of these data, kinetic analyses were further carried out by using Langmuir-Freundlich and Monod equations (Fig. S2). For the data obtained in the system without supplementation with an electron donor, we found that they could be well described by the Langmuir-Freundlich equation (R² = 0.98, Fig. S2a). The values of Kp, 1/n and α are 8.03, 0.0032 and 0.95, respectively. For the data obtained in the system supplemented with an electron donor, the Sb(V) reduction rate was calculated based on the initial Sb(III) accumulation data and the values are 0.018, 0.046, 0.095, 0.12 and 0.15 mgSb(V)/(L·h) in the presence of 0.2, 0.4, 0.8, 1.2 and 1.6 mM Sb(V), respectively. The data were further analyzed by using the Monod equation, and the results showed that the Sb(V) reduction rate has a reasonable correlation with the initial concentration of Sb(V) (R² = 0.95, Fig. S2b). Above all, the removal of Sb(V) by strain CNZ-1 is a fast biosorption and slow bioreduction process. Additionally, the Sb(V) reduction and Sb(III) accumulation processes were dependent on the presence of an electron donor (sodium lactate in the present study).

Since microorganisms were found to play key roles in influencing the fate of antimony in the natural environment, increasing attention has been focused on studying the molecular mechanisms during the biotransformation of Sb species [19-21]. Compared to the detailed data on the microbial oxidation of Sb(III), the knowledge of the microbial reduction of Sb(V) is more limited [16]. To the best of our knowledge, there are only two previous reported strains (Firmicutes bacterium MLFW-2 and Sinorhizobium sp. JUK-1) that are capable of reducing Sb(V) to Sb(III), and the two strains were isolated from the shore of Mono lake and the vicinity of an antimony oxide-producing factory, respectively [5,22]. In view that the natural conditions of marine sediment are much different from the other two mentioned habitats (lakeside and land), we thus conducted our present experiment anaerobically at 20 °C in the presence of 2% NaCl in order to unravel the possible mechanism of Sb-transportation in marine sediment.

The Shewanella genus was reported to be capable of reducing various metal ions (such as Fe(III) [30], Cr(VI) [31], Au(III) [32], Pd(II) [33] and U(VI) [34]). Thus far, however, no Shewanella resources were explored for Sb(V) reduction. In the present study, a marine bacterium (Shewanella sp. CNZ-1), isolated from the sediment of the Bohai Straits, was first proven to be capable of reducing Sb(V) to Sb(III) in the presence of lactate. Moreover, the homology between strain CNZ-1 (GenBank accession number KX384589) and a Shewanella algae MARS 14 (GenBank accession number LN795823.1) is 99%, but the average nucleotide identity (ANI) value between the genome sequences of CNZ-1 and its MARS 14 is ~93.4% (<95%), indicating that CNZ-1 is likely a novel subspecies of the genus Shewanella. Accordingly, the current study is a prominent contribution to the list of Shewanella strains with the ability for Sb(V) reduction.

3.2. Effects of SO₄²⁻, SO₃²⁻ and S₂O₃²⁻

Antimony is a sulfur-affinitive element, and previous studies showed that the addition of SO₄²⁻ could facilitate the precipitation of Sb(III) in the form of Sb₂S₃ through sulfate-reducing bacteria (SRB) [17,35]. Accordingly, the effects of SO₄²⁻, SO₃²⁻ and S₂O₃²⁻ on Sb(V) reduction by strain CNZ-1 were further investigated. First, we determined the reducibility of systems supplemented with 0.8 g/L SO₄²⁻, SO₃²⁻ and S₂O₃²⁻ after Sb(V) reduction. Results showed that the presence of Sb(V) led to a dramatic decrease of the redox potential (ORP) value in the reaction system, and the presence of SO₄²⁻ and S₂O₃²⁻ can further reduce it (Table S1). Meanwhile, the cell concentration in the system supplemented with SO₄²⁻ was lower than that of SO₄²⁻/S₂O₃²⁻-supplemented systems. It seems that the supplementation of SO₄²⁻ has a negative effect on CNZ-1 cells, which could further affect the bioreduction of Sb(V) (Table S1).

To study the effects of sulfate, sulfite and thiosulfate on the bioreduction of Sb(V) in depth, the biological transformation of Sb(V) (0.8mM) was evaluated in the presence of different initial concentrations of SO₄²⁻, SO₃²⁻ and S₂O₃²⁻ (0, 0.2, 0.4, 0.8 and 1.2 g/L). Interestingly, the results of sulfate, sulfite and thiosulfate on the bioreduction of Sb(V) by strain CNZ-1 are much different. The presence of SO₄²⁻ (0.2–1.2 g/L) has almost no influence on Sb(V) reduction by strain CNZ-1 (data not shown). However, the addition of SO₃²⁻ (0.2–1.2 g/L) and S₂O₃²⁻ (0.2–1.2 g/L) can dramatically improve the removal efficiency of TSB (Fig. 2a). The maximum removal efficiency of TSB in the control system (Sb(V)+MSM+CNZ-1 cells) is approximately 58%. In the systems supplemented with SO₃²⁻ and S₂O₃²⁻ (0.2–1.2 g/L), the maximum removal efficiencies of TSB increased to 85% and 88%, respectively. Further study found that the CNZ-1 concentration in the system amended with sulfite/or thiosulfate is negatively correlated with the concentration of sulfite/or thiosulfate (Fig. 2b and c). Additionally, although small accumulations of aqueous Sb(III) were observed during the course of Sb(V) bioreduction in the presence of SO₄²⁻/or S₂O₃²⁻ (0.2–1.2 g/L), tiny concentrations of Sb(III) (0.63–2.59 mg/L and 0.58–2.29 mg/L) were observed at last in both systems supplemented with SO₃²⁻ and S₂O₃²⁻ (Fig. 2b and c). Fig. 2d shows the evolution of SO₄²⁻, SO₃²⁻ and S₂O₃²⁻ over the course of incubation. As can be seen, no reduction of sulfate was recorded in the system amended with 1.2 g/L SO₄²⁻ while obvious decrease of sulfite/thiosulfate concentrations were observed in the system amended with 1.2 g/L SO₃²⁻ or S₂O₃²⁻.

Surprisingly, the supplementation of sulfate (SO₄²⁻) into the reaction system could not lead to the formation of Sb₂S₃, which is much different from previous studies [17,35]. This phenomenon may be explained by that the ability for sulfate reduction of different bacteria is much different, since the SRB can reduce SO₄²⁻ to S²⁻ while Shewanella sp. CNZ-1 cannot. To verify this viewpoint, the concentrations of HS⁻ were further analyzed. Results showed that 2.64 ± 0.37 mg/L and 1.87 ± 0.15 mg/L HS⁻ were detected in systems amended with 1.2 g/L SO₄²⁻ (approximately 9.52 mM) and S₂O₃²⁻ (approximately 7.59 mM) at 98h, respectively (Fig. S3). Meanwhile, no HS⁻ was observed in the system amended with 1.2 g/L SO₄²⁻ (approximately 8.45 mM, Fig. S3). The concentrations of SO₄²⁻ were or S₂O₃²⁻ was reduced to S²⁻ by strain CNZ-1 but the SO₄²⁻ was not during the Sb(V) reduction process by CNZ-1 cells. As a consequence, the presence of SO₄²⁻/S₂O₃²⁻ could facilitate Sb(III) removal from the present experimental system though a certain manner. We believe that genus Shewanella and SRB are of equal importance for microbe-mediated reduction of Sb(V), while SRB play a leading role due to the widespread sulfate in actual marine environment.

3.3. Characterization of the reduced products

3.3.1. SEM-EDX analyses

The SEM-EDX results of the SO₄²⁻/SO₃²⁻/S₂O₃²⁻-treated CNZ-1 cells are shown in Fig. 4. As can be seen, the surface morphology of CNZ-1 cells that were obtained in the culture supplemented with SO₄²⁻ (Fig. 3a) is much different from that of the CNZ-1 cells that were obtained in the inoculum amended with SO₃²⁻ or S₂O₃²⁻ (Fig. 3d/g). In addition, some rhabdoid or irregular agglomerates could be found
around cells that were obtained in the inoculum amended with SO$_3^{2−}$ or S$_2$O$_3^{2−}$. The elements Sb, O and S are confirmed according to the EDX analyses on the surface of CNZ-1 cells (Fig. 3b, e, h) and agglomerates (Fig. 3c, f, i), indicating the possible formation of Sb-O and Sb-S compounds (SbO$_3$ and Sb$_2$S$_3$ are confirmed by subsequent XPS and XRD results) in SO$_4^{2−}$- and SO$_3^{2−}$/S$_2$O$_3^{2−}$-supplemented systems, respectively.

3.3.2. XPS analyses

XPS analyses were performed for determining the valence state of Sb species in different cultures. As previous studies reported [36,37], the Sb 3d$_{3/2}$ and Sb 3d$_{5/2}$ peaks are representative for Sb species, and the distance between the Sb 3d$_{5/2}$ and Sb 3d$_{3/2}$ peaks is approximately 9.3 eV. As shown in Fig. 4a and b, no Sb and S species were found on the surface of CNZ-1 cells that were obtained in the control system (CNZ-1 cells). Although some Sb(III) was dissolved into the solution after reduction, insoluble Sb$_2$O$_3$ was detected on the surface of precipitations that were obtained in system 0 (CNZ-1 cells+Sb(V)) (data not shown, similar to Fig. 4c) and system 1 (CNZ-1 cells+Sb(V)+SO$_4^{2−}$) (Fig. 4c). However, no sulfide was observed in system 1 (CNZ-1 cells + Sb(V) + SO$_4^{2−}$) (Fig. 4d), indicating that SO$_4^{2−}$ was not utilized by CNZ-1 cells. In addition, Sb(III) could be clearly observed on the surface of precipitations that were obtained in SO$_3^{2−}$- or S$_2$O$_3^{2−}$-supplemented systems (Fig. 4e/g). Meanwhile, the results of S 2d spectra also confirmed the presence of sulfide in the inoculum supplemented with SO$_3^{2−}$ or S$_2$O$_3^{2−}$ (Fig. 4f, h). On the basis of these results, we conclude that the solid state of Sb$_2$O$_3$ was formed in the SO$_4^{2−}$-supplemented system, while the solid state of Sb$_2$S$_3$ was formed in systems supplemented with SO$_3^{2−}$ or S$_2$O$_3^{2−}$. Particularly, sulfur, as a likely intermediate product, is observed in system supplemented with S$_2$O$_3^{2−}$ (Fig. 4h).

3.3.3. XRD analyses

The solid agglomerates collected from different cultures were characterized by XRD. The XRD patterns in Fig. 5 indicated that Sb$_2$O$_3$ (JCPDS 05-0534) was formed in all Sb(V)-supplemented systems and Sb$_2$S$_3$ (JCPDS 42-1393) was formed in both SO$_3^{2−}$- and S$_2$O$_3^{2−}$-supplemented systems. Moreover, sulfur (JCPDS 78-1889) was only present in the system supplemented with S$_2$O$_3^{2−}$, which is consistent with the XPS and SEM results. Additionally, no sulfur was identified in the SO$_3^{2−}$-supplemented system. A possible explanation is that the quantity of sulfur formed in the inoculum supplemented with SO$_3^{2−}$ is too little, and simultaneously, the produced sulfur may react with other species or is used as an electron donor by CNZ-1 cells [17].

In brief, the addition of SO$_4^{2−}$, SO$_3^{2−}$ and S$_2$O$_3^{2−}$ affected Sb(V) reduction through strain CNZ-1. On the basis of the SEM-EDX, XPS and XRD results as well as previous studies [17,35], possible reactions involved in the CNZ-1 mediated Sb(V) reduction in the presence of SO$_4^{2−}$, SO$_3^{2−}$ and S$_2$O$_3^{2−}$ are listed below (reactions a-i):

Fx1

3.3.4. FTIR analyses

FTIR spectra can reveal possible physical and chemical interactions between Sb species and chemical groups on the cell surface of CNZ-1, and we further investigated this process with FTIR analyses, with the results shown in Fig. S4. As can be seen, some peaks increased or decreased after treatment with Sb(V) for 338 h in the presence of SO$_4^{2−}$, SO$_3^{2−}$ and S$_2$O$_3^{2−}$. These changes are center at 2750–3250 cm$^{-1}$ and 1250–1500 cm$^{-1}$, which are attributed to the N–H in plane bending and C–N stretching of amides and carboxyl groups, respectively.
Previous studies [38,39] also reported that amine groups on the surface of bacteria cells were involved in the reduction of metal ions, which is consistent with our present study. Additionally, the changes between 250 and 750 cm\(^{-1}\) are related to the addition of SO\(_4^{2-}\), SO\(_3^{2-}\) and S\(_2\)O\(_3^{2-}\). These findings imply that some superficial structural properties of CNZ-1 cells account for the interactions between Sb(V) and strain CNZ-1, including the adsorption of Sb(V).

### 3.4. Study of the molecular mechanisms

The molecular mechanisms of microbial Sb(V) reduction in the presence of SO\(_4^{2-}\), SO\(_3^{2-}\) and S\(_2\)O\(_3^{2-}\) remain unknown. To clarify the molecular mechanisms responsible for Sb(V) reduction by CNZ-1 cells in the absence/presence of SO\(_4^{2-}\), SO\(_3^{2-}\) and S\(_2\)O\(_3^{2-}\) at the genomic level, genome sequencing, global transcriptome and RT-qPCR analyses were thus carried out to reveal the response of *Shewanella* sp. CNZ-1 exposed to Sb(V) in the absence or presence of SO\(_4^{2-}\), SO\(_3^{2-}\) and S\(_2\)O\(_3^{2-}\) as well as to identify the key genes responsible for Sb(V) reduction and SO\(_3^{2-}\)/S\(_2\)O\(_3^{2-}\)-assisted Sb(V) reduction by strain CNZ-1.

#### 3.4.1. Genome sequencing assays

The overview of the draft genome for strain CNZ-1 is listed in Fig. S5. The draft genome of strain CNZ-1 is deposited at GeneBank under accession number QGDA00000000. The final draft genome of strain CNZ-1 yielded 19 contigs (>500 bp; max length, 1,701,328; min length, 447; \(N_{50}\), 1,409,432 bp) and a total assembled length of 4.79 Mb. The genomic sequence of strain CNZ-1 is 4,562,856 bp in length, with a G + C content of 52.23%. Previous studies reported that the *Shewanella* genus could reduce various metal ions (such as Fe(III), Cr(VI), Au(III), Pd(II), U(VI) and so on) and xenobiotics (such as azo dyes and nitroaromatics) in the presence of electron donors along with the transfer of energy [25,30–34]. The analysis of functional annotation reveals that the genes belonging to “Energy production and conversion (249, GOG function classification)” and “Energy metabolism (54, KEGG pathway annotation)” are present in the genome of strain CNZ-1 (Figs. S6 and S7). In addition, Zhang et al. revealed that genes encoding some cytochromes and reductase/dehydrogenase (nrfABCD, nirBD, frdBCD, dmsABC etc.) generally played key roles during the bioreduction of azo dyes and quinone compounds [40,41]. In the present study, 46 genes
Intensity (a.u.)

B.E. (eV)

(a) CNZ-1 cells only

(b) CNZ-1 cells only

(c) CNZ-1 cells + Sb(V) + SO$_4^{2-}$

(d) CNZ-1 cells + Sb(III) + SO$_4^{2-}$

(e) CNZ-1 cells + Sb(V) + SO$_3^{2-}$

(f) CNZ-1 cells + Sb(III) + SO$_3^{2-}$

(g) CNZ-1 cells + Sb(V) + S$_2$O$_3^{2-}$

(h) CNZ-1 cells + Sb(III) + S$_2$O$_3^{2-}$

Intensity (a.u.)

B.E. (eV)

(a) O 1s

(b) S 2p

(c) Sb$_2$O$_3$

(d) Sb$_2$S$_3$

(e) S$_2$O$_3^{2-}$

(f) S$_2$O$_4^{2-}$

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Fig. 4. XPS patterns of precipitates obtained from different systems. (a, b) O 1s and S 2p spectra of precipitates obtained from control system; Sb 3d and S 2p spectra of precipitates obtained form (c, d) SO\textsubscript{2}\textsuperscript{2−}, (e, f) SO\textsubscript{3}\textsuperscript{2−} and (g, h) S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−}-supplemented systems, respectively.

Fig. 5. X-ray diffraction patterns of precipitates obtained from different systems.

Fig. 6. RT-qPCR results of genes S.CNZ.1GM001569 and S.CNZ.1GM004058 in SO\textsubscript{3}\textsuperscript{2−}, SO\textsubscript{3}\textsuperscript{2−} and S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−}-supplemented systems compared to control system.

encoding cytochromes and 95 genes encoding reductase, which should attract more attention in the following studies, are observed in the draft genome of strain CNZ-1.

3.4.2. Global transcriptome assays

Global transcriptome assays were carried out to unravel the detailed response in gene levels of CNZ-1 cells when facing SO\textsubscript{3}\textsuperscript{2−} and S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−} in the presence of Sb(V). To identify co-regulated patterns of gene expression, we classify all differentially expressed genes in response to SO\textsubscript{3}\textsuperscript{2−} and S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−} into diverse hierarchical clusters based on their expression log ratio. As shown in Fig. S8a, an obvious distinction of the expression levels could be observed between SO\textsubscript{3}\textsuperscript{2−}-induced and S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−}-induced genes, indicating the different molecular response of CNZ-1 cells when facing SO\textsubscript{3}\textsuperscript{2−} and S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−} in the presence of Sb(V). Subsequently, statistical analysis of differentially expressed genes of three contrasts is performed, and the outcome is visualized in volcano plots (Fig. S9). Although the number of up-regulated genes in contrasts system 3/system 1 (S\textsubscript{3}/S\textsubscript{1}, 580 up-regulated genes) and system 4/system 1 (S\textsubscript{4}/S\textsubscript{1}, 570 up-regulated genes) are similar, the number of up-regulated genes in contrast group S\textsubscript{3}/S\textsubscript{1} (570 down-regulated genes) and S\textsubscript{4}/S\textsubscript{1} (156 up-regulated genes) are much different (Fig. S9a-b). The comparison of the gene expression profiles between system 4 and system 3 reveals that 432 genes are up-regulated and 262 genes are down-regulated (p value < 0.05, Fig. S9c).

In addition, the shared sets of differentially expressed genes in the same direction (up-regulated or down-regulated (p value < 0.05)) are screened at contrasts S\textsubscript{3}/S\textsubscript{1}, S\textsubscript{4}/S\textsubscript{1} and S\textsubscript{4}/S\textsubscript{3}, respectively. The Venn plot indicates that contrasts S\textsubscript{3}/S\textsubscript{1} and S\textsubscript{4}/S\textsubscript{1} shared 369 differentially expressed genes (251 up-regulated and 118 down-regulated, Fig. 7b and Table S2). Among them, 85 up-regulated genes encoding dehydrogenase (highlighted with green), cytochromes (highlighted with black), reductase (highlighted with yellow), stress resistance protein (highlighted with red), membrane proteins and transporters (highlighted with blue) should play key roles during the transformation of SO\textsubscript{3}\textsuperscript{2−}/S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−} inside CNZ-1 cells (Table S2). Additionally, the three groups share 78 differentially expressed genes (35 up-regulated and 43 down-regulated, Fig. S8 b and Table S3). Compared to SO\textsubscript{3}\textsuperscript{2−}, 12 up-regulated genes encoding lactate/formate utilization protein (highlighted with green), thiosulfate/polysulfide reductase (highlighted with yellow), phage/heat shock protein (highlighted with red) and inner membrane proteins (highlighted with blue) are more responsive to S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−} (Table S3).

On the basis of the above results, we speculate that the addition of SO\textsubscript{3}\textsuperscript{2−}/S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−} will lead to increasing environmental stress on CNZ-1 cells. It is well known that maintaining various balances in the cell is an important challenge for microorganisms facing complex environments. Thus, CNZ-1 cells exposed to SO\textsubscript{3}\textsuperscript{2−}/S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−} also need to regulate their gene expression levels for freedom from stressful situations. The up-regulation of genes S.CNZ.1GM000441/000566/000789/002733-35/003,515 etc. (annotated as uspE/cspA/ibpA/pspABC etc., Table S2) can support the above standpoint, since previous studies reported that proteins encoded by these genes are involved in the resistance to diverse environmental stress [40–43]. Besides, the addition of SO\textsubscript{3}\textsuperscript{2−}/S\textsubscript{2}O\textsubscript{3}\textsuperscript{2−} will affect the activities of enzymes (including dehydrogenase, cytochromes and reductase) that are involved in the electron transfer pathway for Sb(V) reduction by CNZ-1 cells, and these effects may have a further impact on the environmental fate of Sb(V) in the presence of strain CNZ-1.

3.4.3. RT-qPCR assays

Two previous studies reported that reduction processes involving electron transfer are generally performed by diverse nonspecific reductases [38,39]. On the basis of previous studies and the results of global transcriptome assays, we thus tested some functional genes (such as the genes encoding cytochromes and their synergic reductase) that have been reported to play important roles in other redox reactions by using RT-qPCR. As shown below, fifteen genes encoding cytochromes (S.CNZ.1GM 000200/001,406/001833/001928/002308/002362) and their synergic reductases (S.CNZ.1GM 000200/001,406/001833/001931/003323/003324/003602/003971/004058) were selected to further study their possible roles during Sb(V) reduction by strain CNZ-1 cells, and these effects may have a further impact on the environmental fate of Sb(V) in the presence of strain CNZ-1.
genes tested, the general patterns of differential expression obtained from global transcriptome assays and RT-qPCR assays are similar.

As shown in Table S5, the expression of genes S.CNZ.1GM 000200/002021, S.CNZ.1GM 001833, S.CNZ.1GM 002308, S.CNZ.1GM 003323/003324, and S.CNZ.1GM 003/971 (annotated as napA/B, cytB, cytB, dmA/B, frdA) were 2.22–4.68-fold up-regulated by induction with the supplementation of Sb(V) compared to the control system with only CNZ-1 cells added, indicating the involvement of these genes during Sb (V) reduction by strain CNZ-1. These results verify our previous conjecture and further emphasize the importance of those nonspecific reductases during electron transfer. Additionally, the expression of genes S.CNZ.1GM001569 and S.CNZ.1GM004058 (annotated as phsA and sirA) were 2.63/12.02 and 3.31/2.83 up-regulated when co-incubated with SO$_3^{2-}$/S$_2$O$_3^{2-}$, respectively, compared to system 1 (cells + Sb(V)) (Fig. 6). PsrA was identified as the thiosulfate and polysulfide reductase in Shewanella MR-1 [44] and SirA was proven to be capable of catalyzing sulfite reduction in Shewanella spp. [45]. Thus, we conclude that genes S.CNZ.1GM001569 and S.CNZ.1GM004058 perform the same functions as phsA and sirA in CNZ-1. Surprisingly, all tested genes were down-regulated by co-incubation with SO$_4^{2-}$ compared to system 1 (Table S5). A possible explanation is that the addition of SO$_4^{2-}$ inhibits the Sb(V) reduction process by CNZ-1 cells, since Meng et al. reported that a decreased decolorization efficiency of Acid Red 27 could be observed after adding SO$_4^{2-}$ in the presence of Shewanella aquimarina cells [46]. On the basis of the above results and previous study [45], a possible network of Sb(V) response genes in CNZ-1 cells along with the electron transfer pathway responsible for Sb(V) reduction by CNZ-1 cells in the presence of SO$_3^{2-}$/or S$_2$O$_3^{2-}$ is proposed (Fig. 7).

4. Conclusions

In conclusion, we illuminate the process and mechanism of Sb(V) bioreduction by a novel marine Shewanella subspecies in the presence of sulfite/thiosulfate. Sb removal by strain CNZ-1 is a combined process including both bioadsorption and bioreduction. Moreover, the supplementation of SO$_3^{2-}$ and S$_2$O$_3^{2-}$ into the reaction system can lead to the formation of Sb$_2$S$_3$ along with the reduction of SO$_3^{2-}$ and S$_2$O$_3^{2-}$, while SO$_4^{2-}$ can’t. In addition, molecular studies show that the genes encoding dehydrogenase, cytochromes, reductase, stress resistance protein, membrane proteins and transporters play key roles during the transformation of SO$_3^{2-}$/S$_2$O$_3^{2-}$ by CNZ-1 cells, and genes S.CNZ.1GM004058 and S.CNZ.1GM001069 should responsible for the reduction of SO$_4^{2-}$ and S$_2$O$_4^{2-}$ by strain CNZ-1, respectively.

Conflicts of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data (includes Tables S1–S5 and Figs. S1–S10) to this article can be found online at https://doi.org/10.1016/j.cej.2018.11.168.

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Fig. 7. A possible network of Sb(V) response genes in CNZ-1 cell along with electron transfer pathway responsible for Sb(V) reduction by CNZ-1 cell in the presence of SO$_3^{2-}$/or S$_2$O$_3^{2-}$.
