**Table S1 Protein sequencing of the peptides digested by trypsin using LC-MS/MS**

| Uniprot Accession No. | Coverage (%) | PTM | Avg-Mass | Description |
|---|---|---|---|---|
| tr\|A0A1J9W043\|A0A1J9W043_9BACI | 60 | Y | 42997 | Elongation factor Tu OS=Bacillus paramycoides OX=2026194 GN=tuf PE=3 SV=1 |
| tr\|A0A1J9VZ58\|A0A1J9VZ58_9BACI | 49 | Y | 40165 | Alanine dehydrogenase OS=Bacillus paramycoides OX=2026194 GN=BAU28_09470 PE=3 SV=1 |
| tr\|A0A1J9W3N8\|A0A1J9W3N8_9BACI | 56 | Y | 40189 | Alanine dehydrogenase OS=Bacillus paramycoides OX=2026194 GN=BAU28_21080 PE=3 SV=1 |

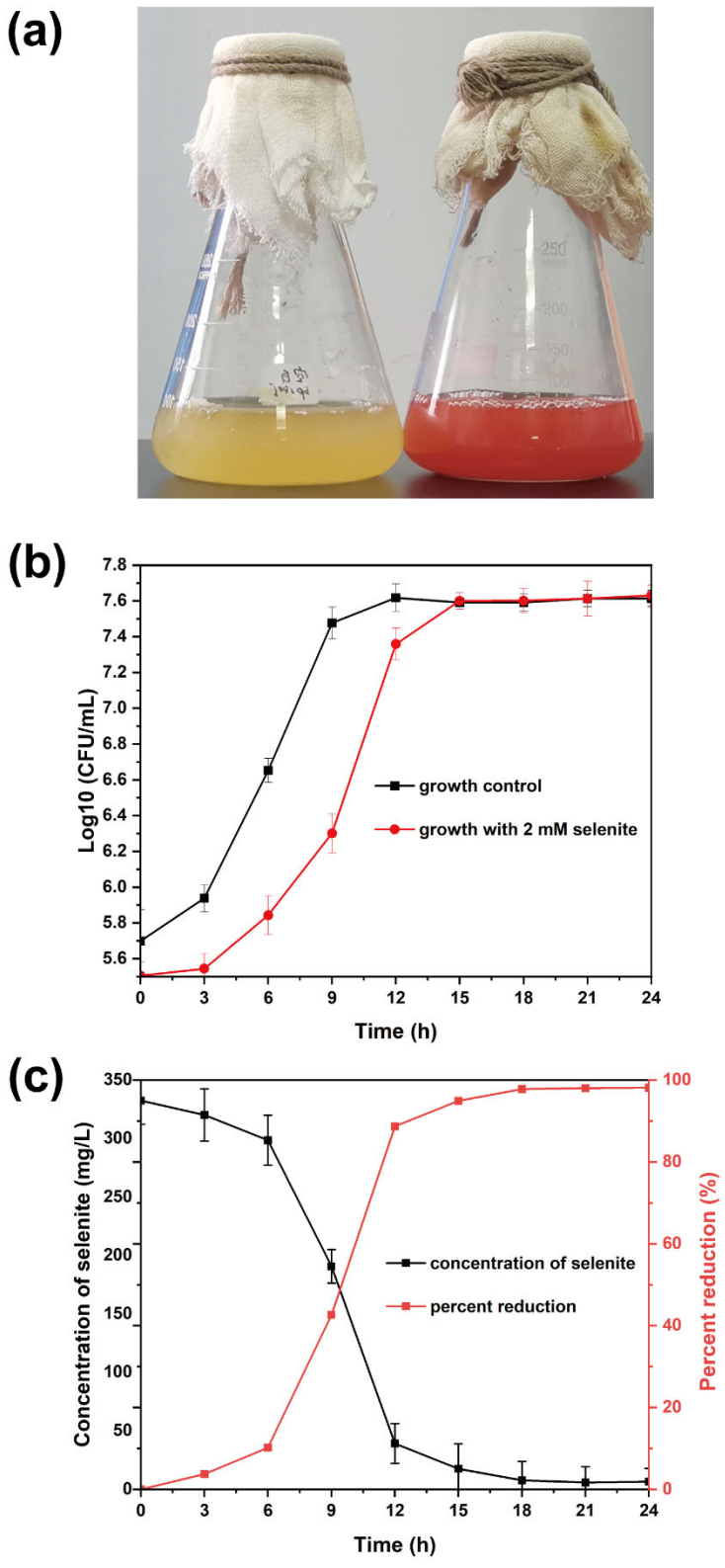


**Fig. S1 (a) Images of cultures with 2.0 mM selenite (on the right) and without selenite (on the left) aerobically grown for 12 h. (b) Growth curve. Each test was performed in triplicate, and the data were presented as the mean ± standard deviation. (c) Time courses of selenite reduction and percent reduction by strain *B. paramycoides* 24522.**

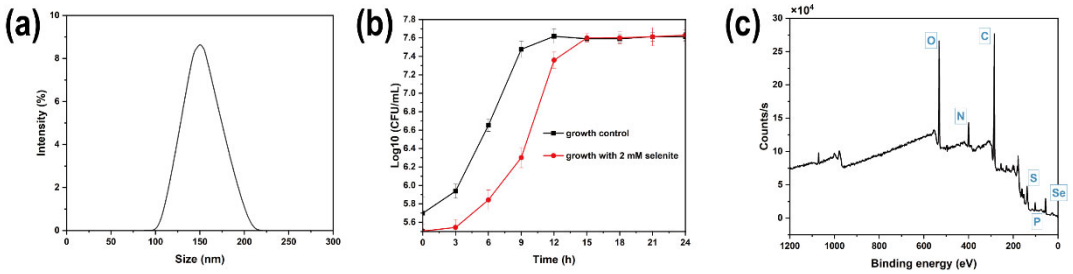


**Fig. S2 (a) DLS profile of SeNPs. (b) Zeta-potential analysis of SeNPs purified from *B. paramycoides* 24522 grown in LB containing 2.0 mM selenite after 24 h of incubation. (c) XRD pattern of SeNPs.**

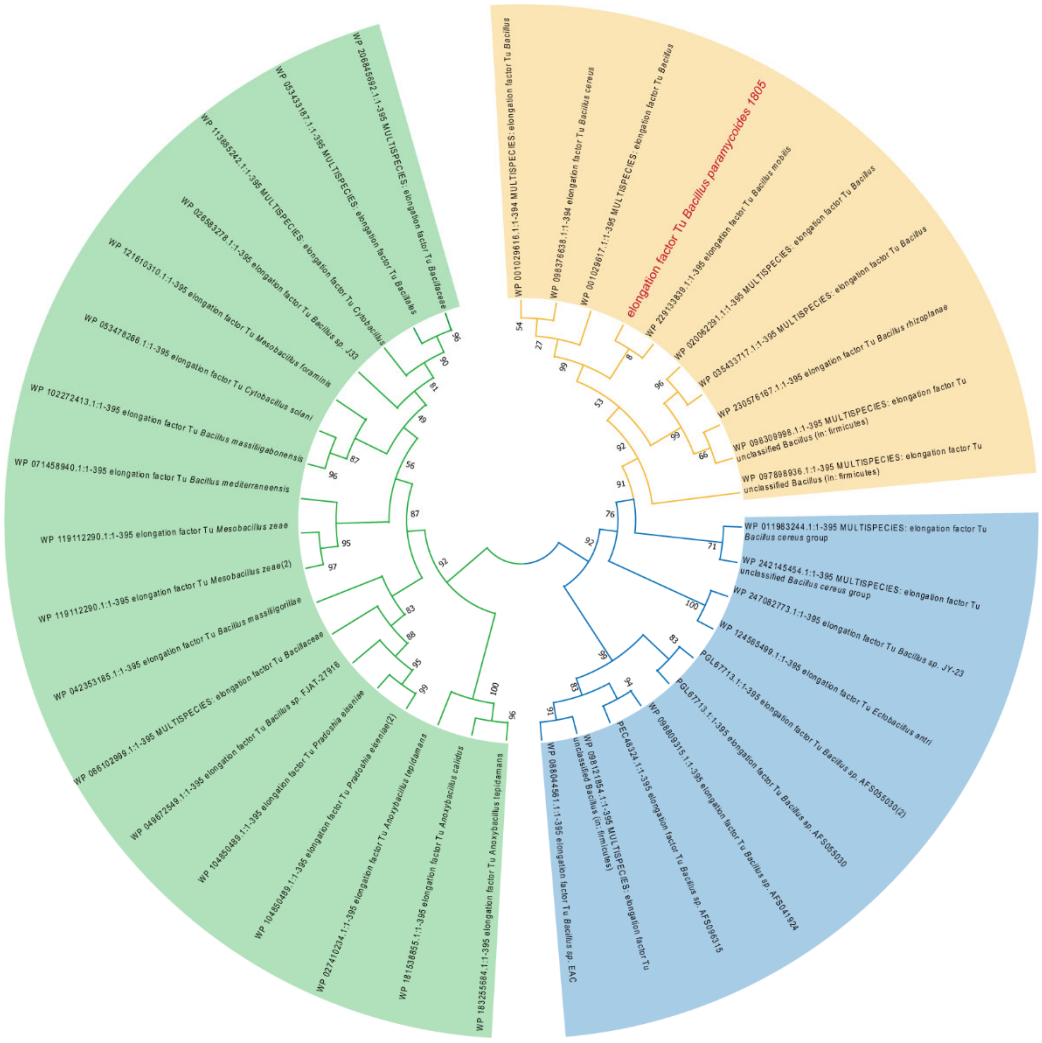


**Fig. S3 Phylogeny of amino acid sequences of EF-Tu from 37 *Bacillus* species. Numbers on the nodes were bootstrap percentages deduced from 1000 replications using the maximum likelihood method performed in MEGA 11.**

## Materials and methods

### Materials

Sodium selenite ($Na_2SeO_3$), sodium dodecyl sulfate (SDS), 1×phosphate buffer saline (1×PBS, 10 g/L NaCl, 1.44 g/L $Na_2HPO_4$, 1 g/L KCl, 1.2 g/L $KH_2PO_4$, pH=7.4), glycerol, n-hexane, lysozyme, and deoxyribonuclease I (DNase I) were purchased from Beijing Solarbio Science & Technology Co., Ltd.

### SeNPs synthesis

*B. paramycoides* 24522 specimens preserved in our laboratory of Huaiyin Institute of Technology were precultured for 24 h at 30 °C under aerobic conditions on an orbital shaker (200 rpm) with 100 mL of Luria-Bertani medium (5 g/L tryptone, 10 g/L yeast extract, and 10 g/L NaCl, pH=6). Bacterial growth was carried out in 250 mL Erlenmeyer flasks containing 100 mL of LB supplemented with 2.0 mM selenite ($SeO_3^{2-}$) at 37 °C on an orbital shaker (140 rpm).

### Selenite reduction

After a period of incubation in LB medium with 2.0 mM selenite, the ability of strain *B. paramycoides* 24522 to biotransform selenite was evaluated. After that, 1 mL of culture mixture was taken every 3 h and centrifuged for 20 min at 12,000 g. After being transferred to a new tube, the supernatant was digested overnight in 5% (v/v) $HNO_3$, after which it was diluted progressively with 20% HCl and 5% HCl to the proper selenium content. Atomic fluorescence spectrometer (AFS; Beijing Beifen-Ruili Analytical Instrument (Group) Co. Ltd) was applied to determine the remaining selenite.

To further understand the *B. paramycoides* 24522 growth dynamics when placed under 2.0 mM selenite, a sample of 100 μL of the *B. paramycoides* 24522 culture was taken every three hours. Following successive dilutions with physiological saline, samples were spotted onto LB agar plates. After a 24-hour incubation period at 30 °C, colony-forming units per milliliter (CFU/mL) were counted. As a negative control, *B. paramycoides* 24522 was cultivated in LB medium without selenite.

### Localization of biocatalytic selenite reduction

different cellular compartments [cytoplasm, periplasm, membrane, culture supernatant, and extracellular polysaccharide (EPS) fractions] were examined for selenite reduction. The overall protein concentration of the four parts of *B. paramycoides* 24522 was determined utilizing the

Bradford technique with bovine serum albumin as the reference (adjusted to 2 mg/mL). In each well, a mixture (190 μL of the four separated fractions or EPS, along with 10 μL of 2.0 mM sodium selenite solution) was added, then it was incubated for no less than 72 h at 37 °C. Two negative controls were included: one well without selenite and another well without the four separated fractions or EPS.

**Preparation of SeNPs**

*B. paramycoides* 24522 was cultured in modified LB medium (with 2.0 mM selenite supplement) for 24 h at 37 °C. Centrifugation was used to separate the bacteria-containing SeNPs for 20 min at 5,000 g, followed by three washes with 1×PBS. The bacterial cells were then disrupted by an ultrasonic cell crusher for 50 min at 350 W. Centrifugation was performed at 11,000 g for 50 min to remove impermissible cell fragments and possible inclusion bodies, and the supernatant was resuspended in dd$H_2O$. Finally, the purified SeNPs were obtained through amendment with a 1/1 vol of n-hexane for 24 h.

**Characterization of SeNPs**

Dynamic light scattering (DLS; Nano ZS90, Malvern Instruments Ltd.) was used to determine the zeta potential and particle size of the purified SeNPs. For TEM observations, 24-hour cultures of bacteria containing SeNPs were fixed overnight at 4 °C in a refrigerated fixative solution. X-ray photoelectron spectroscopic (XPS) measurements were carried out using a scanning XPS Microprobe (PHI Versaprobe III, US).

**Isolation and identification of the SeNPs surface protein**

Purified SeNPs (1 mL) were pelleted by centrifugation (11,000 g, 3 min), underwent treatment with 100 μL 4% (w/v) SDS (containing 200 mM Tris-HCl, pH = 7.0), and then heated to 100 °C for 5 min prior to centrifugation at 25,000 g for 20 min. SDS-PAGE was applied to separate the separated proteins from the supernatant. Figuring out the protein band using LC-MS/MS equipment (Thermo EASY nLC and Thermo Scientific QE, Thermo Scientific, USA) after it has been cut and trypsinized. Then, a phylogenetic tree was generated to compare the protein sequences with different species by maximum likelihood techniques.

**qPCR**

The mRNA expression of target proteins that might be accountable for selenite reduction was identified by qPCR. After *B. paramycoides* 24522 incubation in LB medium supplemented with 2.0

mM selenite for 24 h at 30 °C, bacterial cells were harvested through centrifugation (5,000 g × 5 min), and the total bacterial RNA was extracted using RNAiso Plus (TAKARA). Then, cDNA was synthesized using the PrimeScriptTM RT Reagent Kit (TAKARA) and subjected to qPCR analysis using the HieffTM qPCR system (YEASEN). The gene-specific primers were supplied by Tsingke Biotechnology Co., Ltd (Table S2). RNA transcript levels were calculated as a difference from control cells that had not been treated with 2.0 mM selenite using the $2^{-\Delta\Delta Ct}$ method.

**Table S2 Primers used for qPCR analysis of EF-Tu gene**

| Primers | Sequences |
|---|---|
| Bacillus paramycoides-gene-F1 | GGTGGACGTCACACTCCATT |
| Bacillus paramycoides-gene-R1 | TTCGATGTTGTCACCAGGCA |

Forward Primer: Bacillus paramycoides-gene-F1, Reverse Primer: Bacillus paramycoides-gene-R1.