

A Novel Selenite-Reducing Bacterium *Bacillus pseudomycooides* SA14 Isolated from Se-Enriched Soil and Its Potential Se Biofortification on *Brassica chinensis* L.

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ABSTRACT: Microbial participation in biofortification can improve the availability of selenium (Se) in soil and contribute to the enrichment of Se in crops. In this study, a selenite (Se(IV)) reducing strain was isolated from Se-rich soil, and its Se transformation and bio-enhancement ability were studied. The strain was identified as *Bacillus pseudomycooides* and could reduce more than 93.48% of 1.0 mM Se(IV) in 54 h. The results of scanning electron microscope (SEM) and energy dispersive X-ray spectrometry (EDS) showed that Se(IV) was reduced to Se(0), and Se nanoparticles (SeNPs) were eventually formed. In pot experiments, *B. pseudomycooides* SA14 could promote the bioavailable Se in soils and the concentration of Se in *Brassica chinensis* L.. The concentrations of water-soluble Se, ion exchange Se and carbonate-binding Se in soil were increased by 23.13%, 22.05% and 30.89%, respectively. The Se concentration of *Brassica chinensis* L. in pot experiments was increased by 145.05%. The relative abundance of *Bacillus* in soil increased from 0.97% to 2.08% in the pot experiments. As far as we know, this is the first report of Se reduction by *B. pseudomycooides*. This study might provide a prospective strategy for microbial fortification of Se in crops.

KEY WORDS: Se(IV) reduction, *Bacillus pseudomycooides*, bioavailability, biofortification, dominant community, natural environment.

0 INTRODUCTION

Selenium (Se) is an essential trace element for life (Zhao et al., 2020; Chauhan et al., 2019; El-Ramady et al., 2015). Insufficient Se intake by humans will lead to some endemic epidemics, such as Keshan disease and Kashin-Beck disease, whereas excessive Se intake can easily lead to selenosis (Wang Q et al., 2022; Wang X et al., 2019; Zhang X et al., 2019). Generally, Se is absorbed by humans through eating Se-enriched plants and crops, which absorb and transfer Se from soils (D'Amato et al., 2020). Previous studies have shown that Se-rich soils are generally distributed in spots rather than flakes, and Se deficiency in soils is a global problem (Araújo do Nascimento et al., 2021; Zhong et al., 2021).

In China, 72% of areas are Se-deficient, whereas the levels of Se in soils in Jinhua City of Zhejiang Province, Guizhou Province, Enshi City of Hubei Province, and parts of southwestern Shanxi Province are higher than the global average (0.40 mg/kg) (Li et al., 2012). Se enrichment in soils is affected by many factors, such as soil parent materials, soil physical and chemical properties, farming pattern, and microbial activity (Wang Y H et al., 2022; Sharma et al., 2015). The utilization efficiency of Se by plants is determined mainly by the bioavailable Se forms rather than the total Se concentrations in soils, which means that the high concentration of bioavailable Se in soils is more conducive to the enrichment of Se in plants (Wang Q et al., 2022; Zhou et al., 2021; Dinh et al., 2019; Li et al., 2017).

Se biofortification is a promising strategy for Se enrichment in agricultural products. Traditional methods of agronomic Se bio-enhancement mainly include direct application of Se fertilizer into soils, the spray of Se fertilizer on foliage, and soaking of plant seeds in Se solutions (Li et al., 2021a, b). However, the direct inorganic Se fertiliz-

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er might not be an efficient and safe strategy due to its low utilization rate and uncontrolled amount of Se absorbed by plants (Muleya et al., 2021; Zhang et al., 2018; Wang et al., 2017). Application of inorganic Se fertilizers may also result in soil compaction and pH imbalance (Chen et al., 2021; Huang Y P et al., 2021; Zhang et al., 2020). Microorganisms play an essential role in improving the bioavailability of Se in the soil. The concentrations of organic matter, alkali hydrolyzed nitrogen, available phosphorus, and available potassium could be increased by adding microbial agents (Chen et al., 2021; Deng et al., 2021; Huang C L et al., 2021). However, at present, only a limited study focused on microbial agents for Se enrichment in crops. Microorganisms, including Se-oxidizing bacteria and Se-reducing bacteria, can play an essential role in the Se cycle in soils (Zhang J et al., 2019; Turanov et al., 2011). In anaerobic conditions, $\text{SeO}_4^{2-}/\text{SeO}_3^{2-}$ can be used as terminal electron acceptors by microorganisms and reduced to elemental Se via dissimilatory reduction (Bao et al., 2014; Stolz et al., 2006; Hapuarachchi et al., 2004; Kashiwa et al., 2001; Huber et al., 2000; Ike et al., 2000). Some microorganisms can also reduce $\text{SeO}_4^{2-}/\text{SeO}_3^{2-}$ to elemental Se under aerobic or microaerophilic conditions (Wadgaonkar et al., 2019; Song et al., 2017; Kuroda et al., 2011; Tejo Prakash et al., 2009; Hunter and Kuykendall, 2007). There are many pathways for the reduction of Se(IV), such as Painter-type reaction, thioredoxin system, dissimilatory reduction, siderophore-mediated reduction, and sulfate reduction (Wang D et al., 2022; Pettine et al., 2012; Hockin and Gadd, 2003; Lloyd, 2003; Stolz et al., 2002; Painter, 1941). Besides, microbial communities, including nitrogen-fixing bacteria and dissimilatory iron-reducing bacteria, can also enhance the availability of Se in soil (Wang Z et al., 2022). So far, many Se-reducing strains have been isolated from soil and water, such as *Enterobacter cloacae* SLD1a-1 (Losi and Frankenberger, 1997), *Streptomyces* sp. ES2-5 (Tan et al., 2016), *Comamonas testosteroni* S44 (Zheng et al., 2014), *Citrobacter* sp. NDSe-5 (Won et al., 2021) and *Pseudomonas* sp. NDSe-2 (Won et al., 2021). These strains could reduce 40%-98% of 1.0 mM Se(IV). However, whether these functional strains can improve soil Se availability and their fortification of Se in crops are still not well understood.

The current study described a novel Se(IV)-reducing strain isolated from Se-enriched soil, which could produce Se nanoparticles (SeNPs) and further promote bioavailable forms of Se in soil and the Se concentrations in *Brassica chinensis* L. plants. Results of this study can help us better understand the microbially mediated Se transformation and might provide a prospective strategy for microbial fortification of Se in agricultural products.

1 MATERIALS METHODS

1.1 Soil Collection and Strain Isolation

Soil samples were collected from a Se-rich area located in Jinhua City of Zhejiang Province, China. Soils for strain isolation were collected from 10–20 cm of plant

roots in dry land, which were mainly grown with vegetables. The total Se concentration in this soil was 0.46 mg/kg, while the bioavailable Se (the sum of soluble Se, exchangeable Se and carbonate-bound Se) was 0.11 mg/kg. To avoid contamination, soil samples for strain isolation were quickly placed into 50 mL sterile polypropylene tubes and stored in ice boxes (4 °C). Bulk soil was collected at the same site for pot experiments. All samples were transported to the lab within two days for further experiments.

Se(IV)-reducing bacterium was isolated on 1/10 TSA tryptic soy agar (TSA, pH 7.3, Difco) medium containing 1.0 mM Na_2SeO_3 (Tan et al., 2016). Weighed 10 g of collected soil and mixed with 90 mL sterile pure water, shook at 150 rpm for 5 min, and settled for 10 min. The supernatant was taken for gradient dilution and spread evenly onto a solid 1/10 TSA medium. After 48 h of incubation at 30 °C, visualized colonies with reddish color were selected and separated for several times until a single colony appeared on the solid medium. The single colony was cultured into log-phase in liquid 1/10 TSA medium at 30 °C for subsequent experiments.

1.2 Strain Identification

Physiological and biochemical identification of the strain was carried out according to previous studies (Huang C L et al., 2021; Jiang et al., 2015). Colony morphology was determined visually, and microscopic characteristics were observed under the optical microscope and compared with the “Bergey’s Manual of Systematic Bacteriology 9th Edition”. The detailed strain morphology was further observed with 100 kV TEM (Hitachi HT-7700, Japan). Microbiochemical identification tubes (Hangzhou Microbial Reagent Co., LTD, China) were used to identify the chemical and carbon metabolic characteristics of the strain compared with the characteristics of other isolates identified as the same genera.

The genomic DNA of the strain was extracted using miniBEST Bacteria Genomic DNA Extraction Kit (Takara, Japan) according to the manufacturer’s instructions. The DNA concentration and quality were determined by NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Universal primer set 27F (5'-AGAGTTTGATCCTGGCT-CAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used to amplify bacterial 16S rRNA genes (Weisburg et al., 1991). Detailed information was provided in the Supporting Information. The 16S rRNA gene sequence has been deposited in the GenBank database under accession number MW846086.

1.3 Se(IV) Reduction Assay

Se(IV) reduction of the strain was performed with chemical defined medium (CDM) modified with 1.0 mM Se(IV) under aerobic conditions. Cells in 5 mL cultures were centrifuged, washed with physiological saline, and inoculated into 50 mL CDM containing 1.0 mM Se(IV). A sterile medium was used as a control. All experiments were conducted in triplicate under 150 rpm at 30 °C. It

was taking 1 mL of the mixed solution every 6 h to determine Se(IV) concentration and biomass variations. The sampled liquid suspensions were used to determine Se (IV) concentration after being filtered with 0.22 μm filters to exclude cells. The samples were centrifuged to obtain cell deposits and washed three times with physiological saline. The intracellular protein of bacterial cells was extracted by ultrasonic fragmentation for 3 min (300 W power, 3 s/7 s), and its biomass concentration was quantified with Pierce® BCA Protein Assay Kit (Thermo Scientific). The Se concentrations were determined by hydride generation-atomic fluorescence spectrometry (HG-AFS, Hai Guang AFS-9780, Beijing, China).

Log-phase (24 h) cells of *B. pseudomycoides* SA14 were obtained by growth at 30 °C, shaking at 150 rpm in 100 mL CDM broth. The modified method was based on a previous study (Zheng et al., 2014). The cells in 100 mL CDM cultures were harvested by centrifugation for 10 min at 8 000 $\times$ g, the supernatant was removed. After being harvested, the cells were suspended in fresh sterile CDM broth. The suspension was placed on ice and treated with ultrasound for 20 min (20 amplitude micron, 5 s/5 s). This experiment set up four different systems: no cell control, live cells, heat-deactivated cells, and broken-cells suspension. Each design was added with 0.2 mM Se(IV) and incubated at room temperature for 12 h.

1.4 Identification of Reduction Products of the Strain

Strain *B. pseudomycoides* SA14 was incubated in CDM broth with 1.0 mM Se(IV) for 6 h. The bacterial suspension was centrifuged (8 000 $\times$ g, 4 °C, 10 min) and washed three times with phosphate buffer (PBS, pH = 7). The strain bullets were prepared for TEM observation (Hitachi HT-7700, Japan) and EDS analysis (Oxford X-Max, UK).

SeNPs produced by the strain were collected according to the method described by Dobias et al. (2011). Briefly, the cultured suspension was centrifuged at 4 °C, 8 000 $\times$ g for 10 min to remove biomass. The supernatant was then centrifuged at 4 °C, 20 000 $\times$ g for 15 min to collect SeNPs. The obtained SeNPs were observed with the scanning electron microscope (SEM) (Hitachi SU8010, Japan) and analyzed by spot scanning and area scanning with energy dispersive spectroscopy (EDS) (Hitachi SU8010 with accessory XPS, Japan).

1.5 Pot Experiments

Brassica chinensis L. was selected for pot experiments. Details about the cultivation and management of the plants were provided in the Supporting Information. Four experimental groups were designed to investigate the effects of the strain on Se enrichment in soils and plants, which included the P + B group (*Brassica chinensis* L. planted with SeRB added), P group (only *Brassica chinensis* L. planted without SeRB added), B group (only SeRB added without *Brassica chinensis* L. planted) and Control group (no *Brassica chinensis* L. planted and no

SeRB added). Duplicates were set for each group. The cultivation conditions of these pots were: 25 °C for 14 h in light (240 $\mu\text{mol}/\text{m}^2/\text{s}$) and 20 °C for 10 h in darkness, with 60%–70% of field capacity.

About 10 g of soils samples adjacent to the plant roots were taken on the 1st, 5th, 10th, 15th, and 30th day, respectively, after the SeRB was added. Samples were quickly stored in the refrigerator at -20 °C for subsequent Se form determination and microbial communities analysis. After 30 days of cultivation, the total Se concentrations of the above-ground parts of *Brassica chinensis* L. in the P group and P + B group were measured using AFS. Details about the extraction and determination of Se concentrations in soils and plants were provided in the Supporting Information.

To explore whether the SeRB added to the soil could survive and its influence on the original soil microbial communities, the soil microbial communities of the P group and P + B group on the 1st day and 30th day were analyzed. Soil genomic DNA was extracted according to the method mentioned above. The forward primer 341F (5'-CCTACGGGNGGCWGCAG-3') and reverse primer 805R (5'-GACTACHVGGGTATCTAATCC-3') were used to amplify the V3-V4 region of the microbial 16S rRNA genes. After purification, 25 ng of purified amplicon were sequenced on the illumine Miseq platform. The raw sequencing data have been uploaded to NCBI's Short Read Archive database with the accession number PRJ-NA730710.

2 RESULTS AND DISCUSSION

2.1 Bacterial Identification

A novel isolated Se(IV)-reducing strain was obtained and referred to as SA14. The 16S rRNA gene sequence of SA14 showed 99% sequence similarity with the other *B. pseudomycoides* strains from the GenBank database, such as *B. pseudomycoides* strain 74 (MH910178) and *B. pseudomycoides* strain JBRI-MO-0005 (MK302220). The neighbor-joining phylogenetic tree was constructed based on the 16S rRNA gene sequences of these closely related strains from the GenBank database (Figure 1). Phenotypic analysis showed that the strain was a gram-positive bacterium (Figure S1a). The strain was rod-shaped and 2.8 μm $\times$ 1.2 μm (Figure S1b). Its optimal growth conditions were temperature of 30 °C and pH of 7.0 (Figure S2). The primary biochemical and physiological characteristics of SA14 were not the same of isolates from the same genus (Table 1). Compared to *B. oceanii* SW109T and *B. oceanii* W006 (Song et al., 2016), strain SA14 was positive for hydrolysis of arginine, ornithine and lysine, utilization of urea and sucrose, and negative for activity of oxidase. Compared to *B. halodurans* LMG 7121T (Nielsen et al., 1995), strain SA14 was detected positive for hydrolysis of arginine, utilization of citrate and urea, and negative for utilization of maltose, activity of β -galactosidase and oxidase. Similarly, compared to *B. okuhidensis* DSM 13666T (Li et al., 2002), strain SA14 was positive for hydrolysis of arginine, utilization of urea,

and negative for utilization of maltose, activity of β -galactosidase and oxidase. Consequently, strain SA14 was characterized as *B. pseudomycooides* based on the phylogenetic, morphologic and physiologic characteristics.

2.2 Se(IV) Reduction and Biogenesis of Elemental Se Nanoparticles

B. pseudomycooides SA14 could reduce more than 93.48% of 1.0 mM Se(IV) within 54 h (Figure 2a). The maximal reduction rate was 1.65 $\mu\text{M}/(\mu\text{g protein}\cdot\text{h})$ (Fig-

ure 2b). The strain presented a distinct stronger Se(IV) reducing ability than *B. cereus* AJK3, *Comamonas testosteroni* S44, *Comamonas testosteroni* SE26, and *Chitinophaga* sp. SE06 were reported in previous studies (Huang C L et al., 2021; Zheng et al., 2014). As far as we know, this was the first report of Se(IV) reduction by *B. pseudomycooides*. Accompanied by Se reduction, the cultured suspension changed into red color, which indicated the possible production of SeNPs. We could observe SeNPs produced inside and outside of cells by TEM and EDS (Figure 3). The live cells and broken-cells suspension showed Se(IV)-reducing ability while no cell control and heat-deactivated cells did not (Figure S5). There are many vacuole-like structures in the cells, which may be related to the detoxification of Se(IV) (Figures S1a, 3a and 3b) (Yin et al., 2019). The red products produced by the *B. pseudomycooides* SA14 were further analyzed by SEM-EDS. The results showed a strong absorption peak in 1.37 keV, and weak absorption peaks in 11.22 and 12.49 keV, which belonged to the Se element (Figure S3 and S4). In the spot scanning, the Se element accounted for 37.26% (spot 1) and 42.24% (spot 2) of the total element concentrations, respectively (Figure S4). Flat scanning was further used to analyze the distribution of Se in cell products. Strong Se signals (in red, Figure S4c) were shown in the elemental maps. Carbon (C) (Figure S4d), oxygen (O) (Figure S4f) signals were also exhibited in the elemental maps because nano-Se produced by microbial reduction of Se(IV) was usually coated with a protein layer which functioned as the capping agent during the formation of particles (Zhang et al., 2012). These results showed that the reduction of Se(IV) by *B. pseudomycooides* SA14 was an enzymatic reaction, and SeNPs were synthesized by microorganisms in the cell and then released out of the cell.

Table 1 Biochemical characteristics of the strain SA14

Characteristics	SE14	Species ^a			
		1	2	3	4
Oxygen requirement	A	A	A	A	A
Arginine	+	-	-	-	-
Ornithine	+	-	-	ND	ND
Lysine	+	-	-	ND	ND
Citrate	+	+	W	-	+
Nitrate reduction	+	+	+	+	+
Urea	+	-	-	-	-
Peptone	-	-	-	-	-
β -Galactosidase	-	-	-	+	+
Sucrose	+	-	-	+	+
Maltose	-	-	-	+	+
Hydrogen sulfide	-	-	-	-	-
Oxidase	-	+	+	+	+

ND, not determined; W, weakly positive; A, aerobic; +, positive; -, negative. ^a. Species were as follows: 1. *B. oceanii* SW109T (Song et al., 2016); 2. *B. oceanii* W006 (Song et al., 2016); 3. *B. halodurans* LMG 7121T (Nielsen et al., 1995); 4. *B. okuhidensis* DSM 13666T (Li et al., 2002).

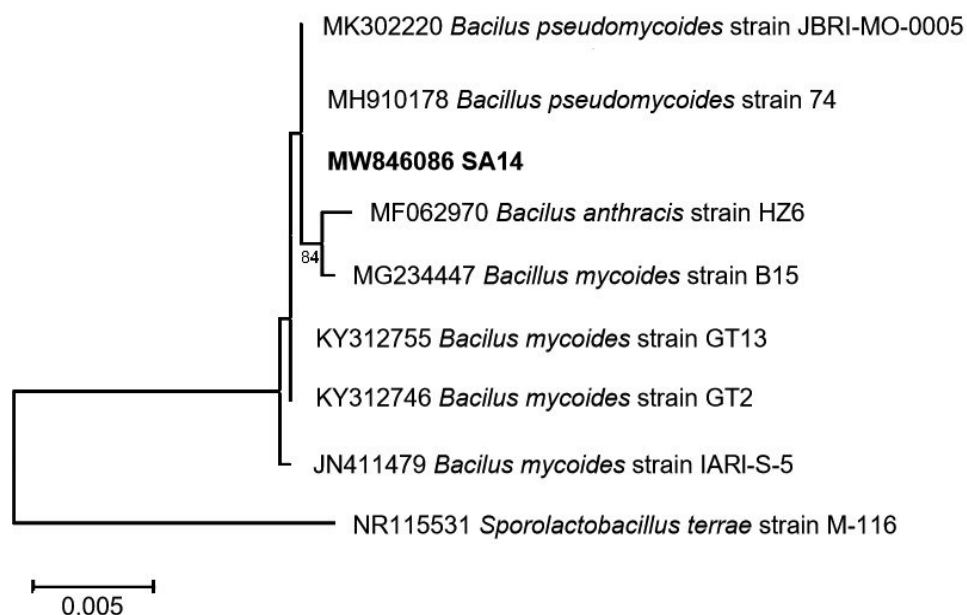


Figure 1. Neighbor-joining tree of 16S rRNA gene sequences showed the phylogeny of strain SA14 and closely related strains from the GenBank database. The GenBank accession numbers were shown before the strain name.

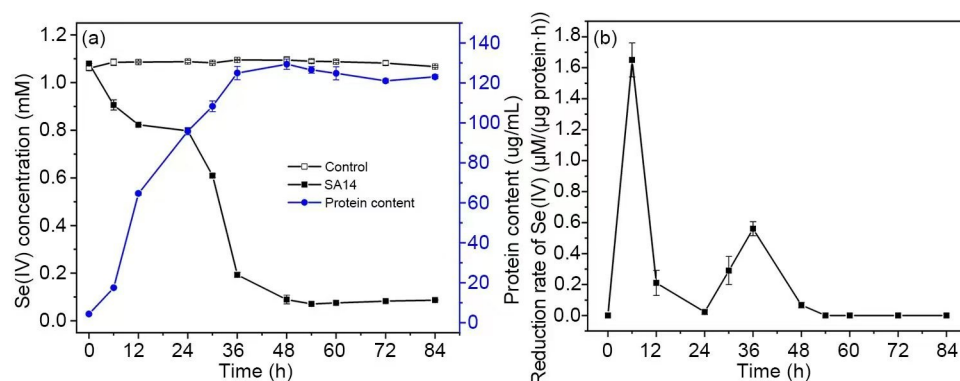


Figure 2. The growth curve, Se(IV) reduction ability (a) and reduction rate (b) of the *B. pseudomycoides* SA14 in CDM broth at 1.0 mM concentration of Se(IV). The bacterial biomass was characterized with total protein concentrations.

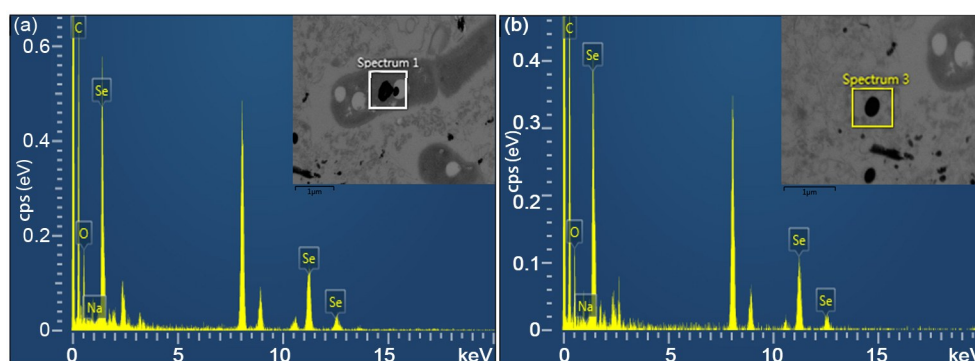


Figure 3. TEM micrographs and EDS analysis of *B. pseudomycoides* SA14 cells grown in presence of 1.0 mM Se(IV). Intracellular particles were marked by a white square (a) and extracellular particles were marked by a yellow square (b). The emission spectrums for Se element were shown at 1.37, 11.22, and 12.49 keV.

2.3 Effects of the Strain Addition on Se Concentrations in Soils and *Brassica chinensis* L.

Pot experiments were carried out to investigate the effects of the SeRB on the concentrations and forms of Se in soils. The concentrations of seven different forms of Se were detected in the *Brassica chinensis* L. rhizosphere soils when the *Brassica chinensis* L. plants were incubated with/without the SeRB. Soluble Se indicated 26.72% and 23.13% increase in group B and P + B, respectively, compared to 3.76% decrease in group P (Figure 4a). Exchangeable Se decreased by 3.13% in group P, whereas it increased by 30.63% in group B and 22.05% in group P + B (Figure 4b). Carbonate-bound Se increased 10.18% in group B and 30.89% in group P + B, respectively (Figure 4c). The concentration of bioavailable Se (the sum of soluble Se, exchangeable Se and carbonate-bound Se) in group B and group P + B increased obviously (Figure 4d). In contrast, humic acid-bound Se, which accounted for the largest proportion of Se at the beginning, decreased by 4.82% and 14.39% in group B and P + B, respectively (Figure S6b). The concentrations of organic matter (OM)-bound and residual Se showed no apparent variations among the four groups, while Fe-Mn-bound Se showed a slight decrease (Figures S6a, S6c and S6d). No apparent variation of Se forms was found in controls. In acidic soils with strong re-

ducibility, Se mainly exists in the form of Se (IV), which is easily absorbed by clay minerals, ferromanganese, hydroxides, humic acid, and organic matter (Kamei-Ishikawa et al., 2007). Therefore, the addition of Se(IV)-reducing strain might, on the one hand, break down the humic acid-bound Se in the soil and release more bioavailable Se (Kulikova and Perminova., 2021; Ryu et al., 2021; Wang et al., 2016), on the other hand, produce SeNPs which showed a higher bioavailability than that of Se(IV) in soils (Golubkina et al., 2012).

The effects of SeRB on Se uptake by *Brassica chinensis* L. plants and the condition of plants growth were also investigated (Figure 5). Inoculated plants showed significantly higher Se concentrations than uninoculated controls. The total Se concentrations of plants in group P + B with SeRB were 59.48 μg/kg, which was 2.46 times higher than the Se concentrations of plants in group P (Figure 5a). The fresh and dry weights of plants in group P + B were higher than that in group P, indicating better growth conditions in group P + B (Figure 5b). The amendment of SeRB showed a better promotion of plant Se concentration compared with a previous study. For example, using soil Se fertilizer and foliar Se fertilizer increased 15.7% and 28.1% of the total Se concentration in oat, adding Se fertilizer to the soil increased the total Se concentration of maize by 20 μg/kg (Li et al., 2021a;

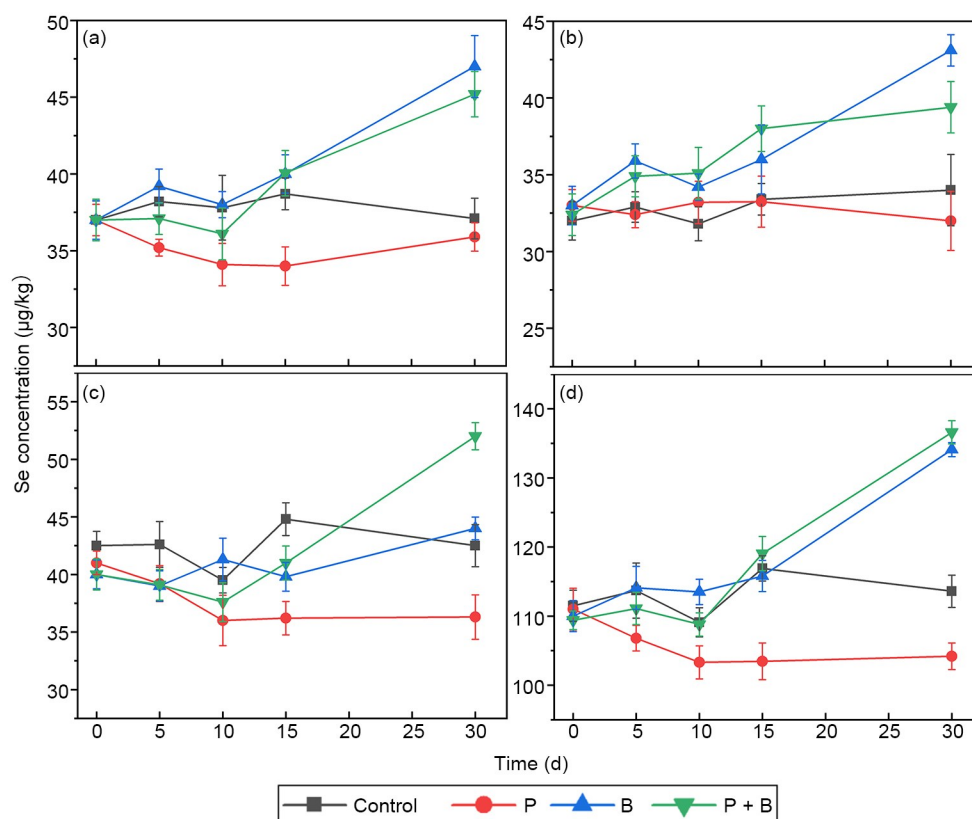


Figure 4. The concentrations of (a) soluble Se, (b) exchangeable Se, (c) carbonate-bound Se, and (d) available Se (the sum of soluble Se, exchangeable Se and carbonate-bound Se concentrations) in *Brassica chinensis* L. rhizosphere soils inoculated with *B. pseudomycoloides* SA14 in pot experiments.

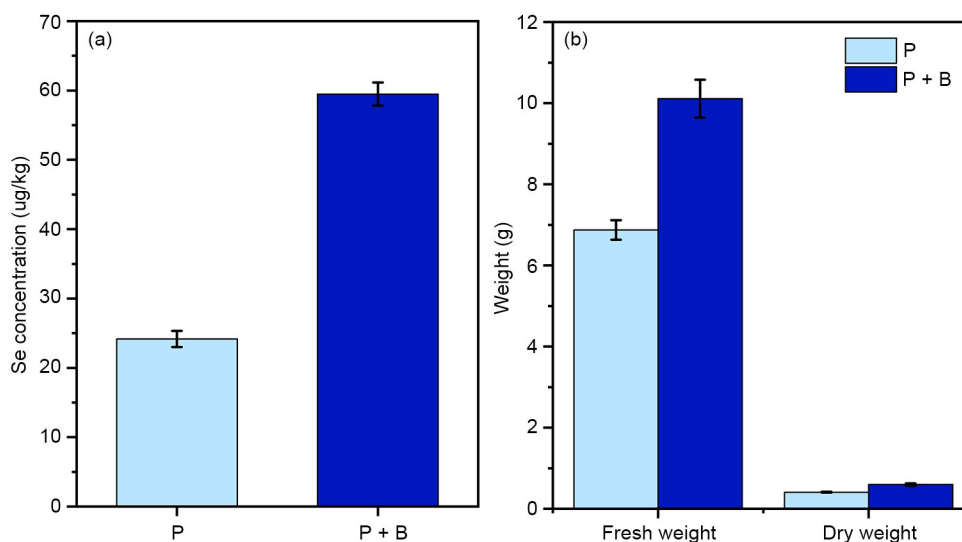


Figure 5. The total Se concentrations and growth state of *Brassica chinensis* L. plants amended with *B. pseudomycoloides* SA14 in pot experiments. P: group P (only *Brassica chinensis* L. planted without SeRB added), P + B: group P + B (*Brassica chinensis* L. planted with SeRB added). The total Se concentrations in *Brassica chinensis* L. plants (a). The fresh weight and dry weight of *Brassica chinensis* L. plants (b).

Chilimba et al., 2012). These results indicated that adding SeRB could effectively promote Se enrichment in plants, which might have great application potential.

2.4 Changes of Soil Microbial Communities in Soils

This study explored the changes of microbial com-

munities by analyzing the composition of microbial communities in soils at the 1st and 30th day in group P and group P + B. Microbial taxonomic analysis showed that four soil samples contained 516 OTUs, which could be divided into 9 phyla (Figure 6a). In the four soil samples, the main dominant phyla included *Proteobacteria*, *Actino-*

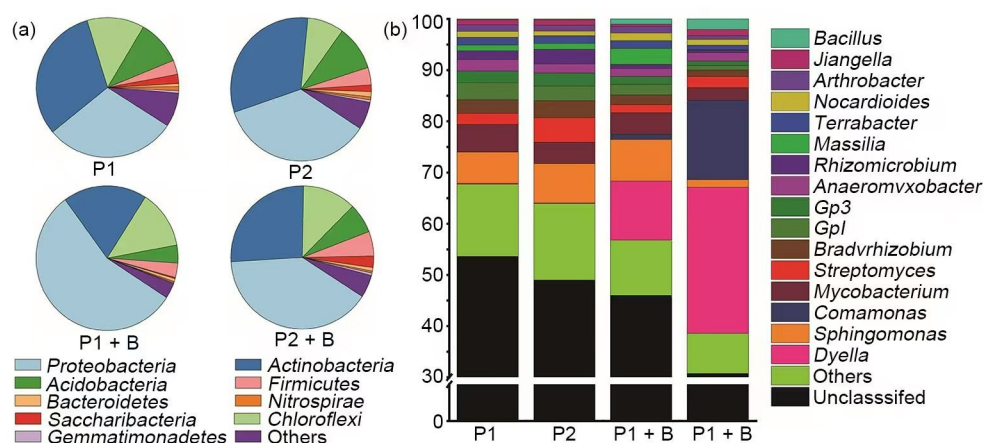


Figure 6. The microbial communities composition of potting soils at (a) phylum and (b) genus levels. Only the top 9 phyla and top 16 genera in abundance were presented. P1 and P2 refer to the soil of group P at 1st and 30th days, respectively; P1 + B and P2 + B refer to the soil of group P + B at 1st and 30th days, respectively.

bacteria, *Chloroflexi*, *Acidobacteria*, and *Firmicutes*. Compared with 1st day, the relative abundance of dominant phyla, including *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, varied greatly. The relative abundance of *Firmicutes* to which the added SeRB belonged increased from 3.23% to 5.55% in group P + B. In contrast, the relative abundance of each phylum in group P without SeRB varied a little, which might be because group P was thought to be less disturbed, so the microbial communities in the soil remained relatively stable.

The top 16 genera with relatively high abundances were selected to analyze the variation of soil microbial communities at the genus level (Figure 6b). There were differences in the microbial communities between group P1 and group P1 + B on 1st day. This may be because the samples collected on 1st day were taken 24 h after SeRB was added to group P + B, and the initial addition of SeRB had some effect on the soil microbial communities. Compared with group P1, the relative abundance of *Bacillus*, *Comamonas*, and *Dyella* in group P1 + B was significantly increased. After 30 days cultivation, the relative abundance of *Bacillus*, *Comamonas* and *Dyella* was further increased. The relative abundance of *Bacillus* increased from 0.97% to 2.08%. The relative abundance of *Comamonas* increased from 1.03% to 15.50%. The relative abundance of *Dyella* increased from 11.51% to 28.48%. The relative abundance of *Bacillus*, *Comamonas*, and *Dyella* in group P after cultivation showed no apparent variation from that before cultivation. The microbial communities structure of group P remained stable. SeRB belonged to *Bacillus* (Figure 1). Its addition increased the relative abundance of *Bacillus* in group P1 + B. *Bacillus*, *Comamonas*, and *Dyella* are common genera in Se-rich soil, and all possess the function of Se metabolism (Wang Y H et al., 2022; Ullah et al., 2020; Zheng et al., 2014). *Comamonas* and *Dyella* were mainly distributed in soils enriched with high concentrations of exchangeable Se and carbonate-bound Se (Wang Y H et al., 2022). The addition of *B. pseudomycoides* SA14 pro-

moted the increase of exchangeable Se and carbonate-bound Se (Figures 4b and 4c), thus increasing the relative abundances of *Comamonas* and *Dyella*. Previous studies indicated that *Bacillus*, *Comamonas*, and *Dyella* could promote plant growth by secreting indole-3-acetic acid (IAA), gibberellin and dissolving phosphorus in the soil, which is consistent with the better growth of *Brassica chinensis* L. planted with SeRB in the present study (Figure 5b) (Domínguez-Castillo et al., 2021; Rolewicz et al., 2018; Erturk et al., 2010).

3 CONCLUSION

A new isolate of *B. pseudomycoides* from Se-rich soils was found with strong Se(IV)-reducing capacity. The strain could reduce more than 93.48% of 1.0 mM Se(IV) within 54 h and generate SeNPs. Amendment of the strain to soils could distinctly increase the bioavailable Se in soils and Se concentrations in *Brassica chinensis* L.. Microbial communities analysis showed that *Bacillus*, which the strain belonged to become the dominant communities in soils after the strain addition. This study expands the current understanding of microbially-mediated Se transformation and may provide a reliable strategy for microbial fortification of Se in agricultural products.

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Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES CITED

- Araújo do Nascimento, C. W., Viera da Silva, F. B., de Brito Fabricio Neta, A., et al., 2021. Geopedology-Climate Interactions Govern the Spatial Distribution of Selenium in Soils: A Case Study in Northeastern Brazil. *Geoderma*, 399: 115119. <https://doi.org/10.1016/j.geoderma.2021.115119>
- Bao, P., Su, J. Q., Hu, Z. Y., et al., 2014. Genome Sequence of the Anaerobic Bacterium Bacillus Sp. Strain ZYK, a Selenite and Nitrate Reducer from Paddy Soil. *Standards in Genomic Sciences*, 9(3): 646–654. <https://doi.org/10.4056/sigs.3817480>
- Chauhan, R., Awasthi, S., Srivastava, S., et al., 2019. Understanding Selenium Metabolism in Plants and Its Role as a Beneficial Element. *Critical Reviews in Environmental Science and Technology*, 49(21): 1937 – 1958. <https://doi.org/10.1080/10643389.2019.1598240>
- Chen, Y. H., Li, S. S., Liu, N., et al., 2021. Effects of Different Types of Microbial Inoculants on Available Nitrogen and Phosphorus, Soil Microbial Community, and Wheat Growth in High-P Soil. *Environmental Science and Pollution Research International*, 28 (18): 23036–23047. <https://doi.org/10.1007/s11356-020-12203-y>
- Chilimba, A. D. C., Young, S. D., Black, C. R., et al., 2012. Agronomic Biofortification of Maize with Selenium (Se) in Malawi. *Field Crops Research*, 125: 118 – 128. <https://doi.org/10.1016/j.fcr.2011.08.014>
- D'Amato, R., Regni, L., Falcinelli, B., et al., 2020. Current Knowledge on Selenium Biofortification to Improve the Nutraceutical Profile of Food: A Comprehensive Review. *Journal of Agricultural and Food Chemistry*, 68(14): 4075–4097. <https://doi.org/10.1021/acs.jafc.0c00172>
- Deng, L., Wang, T., Luo, W., et al., 2021. Effects of a Compound Microbial Agent and Plants on Soil Properties, Enzyme Activities, and Bacterial Composition of Pisha Sandstone. *Environmental Science and Pollution Research International*, 28 (38): 53353–53364. <https://doi.org/10.1007/s11356-021-14533-x>
- Dinh, Q. T., Wang, M. K., Tran, T. A. T., et al., 2019. Bioavailability of Selenium in Soil-Plant System and a Regulatory Approach. *Critical Reviews in Environmental Science and Technology*, 49 (6): 443–517. <https://doi.org/10.1080/10643389.2018.1550987>
- Dobias, J., Suvorova, E. I., Bernier-Latmani, R., 2011. Role of Proteins in Controlling Selenium Nanoparticle Size. *Nanotechnology*, 22(19): 195605. <https://doi.org/10.1088/0957-4484/22/19/195605>
- Domínguez-Castillo, C., Alatorre-Cruz, J. M., Castañeda-Antonio, D., et al., 2021. Potential Seed Germination-Enhancing Plant Growth-Promoting Rhizobacteria for Restoration of Pinus Chiapensis Ecosystems. *Journal of Forestry Research*, 32(5): 2143–2153. <https://doi.org/10.1007/s11676-020-01250-3>
- El-Ramady, H., Abdalla, N., Alshaal, T., et al., 2015. Selenium in Soils under Climate Change, Implication for Human Health. *Environmental Chemistry Letters*, 13(1): 1 – 19. <https://doi.org/10.1007/s10311-014-0480-4>
- Erturk, Y., Ercisli, S., Haznedar, A., et al., 2010. Effects of Plant Growth Promoting Rhizobacteria (PGPR) on Rooting and Root Growth of Kiwifruit (*Actinidia Deliciosa*) Stem Cuttings. *Biological Research*, 43(1): 91–98
- Golubkina, N. A., Folmanis, G. E., Tananaev, I. G., 2012. Comparative Evaluation of Selenium Accumulation by Allium Species after Foliar Application of Selenium Nanoparticles, Sodium Selenite and Sodium Selenate. *Doklady Biological Sciences*, 444: 176–179. <https://doi.org/10.1134/S0012496612030076>
- Hapuarachchi, S., Swearingen, J., Chasteen, T. G., 2004. Determination of Elemental and Precipitated Selenium Production by a Facultative Anaerobe Grown under Sequential Anaerobic/Aerobic Conditions. *Process Biochemistry*, 39(11): 1607–1613. [https://doi.org/10.1016/S0032-9592\(03\)00298-X](https://doi.org/10.1016/S0032-9592(03)00298-X)
- Hockin, S. L., Gadd, G. M., 2003. Linked Redox Precipitation of Sulfur and Selenium under Anaerobic Conditions by Sulfate-Reducing Bacterial Biofilms. *Applied and Environmental Microbiology*, 69(12): 7063 – 7072. <https://doi.org/10.1128/AEM.69.12.7063-7072.2003>
- Huang, C. L., Wang, H. L., Shi, X. Y., et al., 2021. Two New Selenite Reducing Bacterial Isolates from Paddy Soil and the Potential Se Biofortification of Paddy Rice. *Ecotoxicology*, 30(7): 1465 – 1475. <https://doi.org/10.1007/s10646-020-02273-6>
- Huang, Y. P., Wang, Q. Q., Zhang, W. J., et al., 2021. Stoichiometric Imbalance of Soil Carbon and Nutrients Drives Microbial Community Structure under Long-Term Fertilization. *Applied Soil Ecology*, 168: 104119. <https://doi.org/10.1016/j.apsoil.2021.104119>
- Huber, R., Sacher, M., Vollmann, A., et al., 2000. Respiration of Arsenate and Selenate by Hyperthermophilic Archaea. *Systematic and Applied Microbiology*, 23(3): 305 – 314. [https://doi.org/10.1016/S0723-2020\(00\)80058-2](https://doi.org/10.1016/S0723-2020(00)80058-2)
- Hunter, W. J., Kuykendall, L. D., 2007. Reduction of Selenite to Elemental Red Selenium by Rhizobium Sp. Strain B1. *Current Microbiology*, 55(4): 344 – 349. <https://doi.org/10.1007/s00284-007-0202-2>
- Ike, M., Takahashi, K., Fujita, T., et al., 2000. Selenate Reduction by Bacteria Isolated from Aquatic Environment Free from Selenium Contamination. *Water Research*, 34(11): 3019–3025. [https://doi.org/10.1016/S0043-1354\(00\)00041-5](https://doi.org/10.1016/S0043-1354(00)00041-5)
- Jiang, D. W., Li, P., Jiang, Z., et al., 2015. Chemolithoautotrophic Arsenite Oxidation by a Thermophilic Anoxybacillus Flavithermus Strain TCC9-4 from a Hot Spring in Tengchong of Yunnan, China. *Frontiers in Microbiology*, 6: 360. <https://doi.org/10.3389/fmicb.2015.00360>
- Kamei-Ishikawa, N., Tagami, K., Uchida, S., 2007. Sorption Kinetics of Selenium on Humic Acid. *Journal of Radioanalytical and Nuclear Chemistry*, 274(3): 555 – 561. <https://doi.org/10.1007/s10967-006-6951-8>
- Kashiwa, M., Ike, M., Mihara, H., et al., 2001. Removal of Soluble Selenium by a Selenate-Reducing Bacterium Bacillus Sp. SF-1. *BioFactors*, 14(1/2/3/4): 261 – 265. <https://doi.org/10.1002/biof.5520140132>
- Kulikova, N. A., Perminova, I. V., 2021. Interactions between Humic Substances and Microorganisms and Their Implications for Nature-Like Bioremediation Technologies. *Molecules*, 26(9): 2706. <https://doi.org/10.3390/molecules26092706>
- Kuroda, M., Notaguchi, E., Sato, A., et al., 2011. Characterization of Pseudomonas Stutzeri NT-I Capable of Removing Soluble Selenium from the Aqueous Phase under Aerobic Conditions. *Journal of Bioscience and Bioengineering*, 112(3): 259 – 264. <https://doi.org/10.1016/j.jbiosc.2011.05.012>
- Li, J. H., Yang, W. P., Guo, A. N., et al., 2021a. Combined Foliar and Soil Selenium Fertilizer Increased the Grain Yield, Quality, Total

- Se, and Organic Se Content in Naked Oats. *Journal of Cereal Science*, 100: 103265. <https://doi.org/10.1016/j.jcs.2021.103265>
- Li, J. H., Yang, W. P., Guo, A. N., et al., 2021b. Combined Foliar and Soil Selenium Fertilizer Improves Selenium Transport and the Diversity of Rhizosphere Bacterial Community in Oats. *Environmental Science and Pollution Research International*, 28 (45): 64407–64418. <https://doi.org/10.1007/s11356-021-15439-4>
- Li, S. H., Xiao, T. F., Zheng, B. S., 2012. Medical Geology of Arsenic, Selenium and Thallium in China. *Science of the Total Environment*, 421: 31–40. <https://doi.org/10.1016/j.scitotenv.2011.02.040>
- Li, Z., Kawamura, Y., Shida, O., et al., 2002. *Bacillus Okuhidensis* Sp. Nov., Isolated from the Okuhida Spa Area of Japan. *International Journal of Systematic and Evolutionary Microbiology*, 52(pt 4): 1205–1209. <https://doi.org/10.1099/00207713-52-4-1205>
- Li, Z., Liang, D. L., Peng, Q., et al., 2017. Interaction between Selenium and Soil Organic Matter and Its Impact on Soil Selenium Bioavailability: A Review. *Geoderma*, 295: 69 – 79. <https://doi.org/10.1016/j.geoderma.2017.02.019>
- Lloyd, J. R., 2003. Microbial Reduction of Metals and Radionuclides. *FEMS Microbiology Reviews*, 27(2/3): 411–425. [https://doi.org/10.1016/S0168-6445\(03\)00044-5](https://doi.org/10.1016/S0168-6445(03)00044-5)
- Losi, M. E., Frankenberger, W. T., 1997. Reduction of Selenium Oxyanions by *Enterobacter Cloacae* SLD1a-1: Isolation and Growth of the Bacterium and Its Expulsion of Selenium Particles. *Applied and Environmental Microbiology*, 63(8): 3079–3084. <https://doi.org/10.1128/aem.63.8.3079-3084.1997>
- Muleya, M., Young, S. D., Reina, S. V., et al., 2021. Selenium Speciation and Bioaccessibility in Se-Fertilised Crops of Dietary Importance in Malawi. *Journal of Food Composition and Analysis*, 98: 103841. <https://doi.org/10.1016/j.jfca.2021.103841>
- Nielsen, P., Fritze, D., Priest, F. G., 1995. Phenetic Diversity of Alkaliphilic *Bacillus* Strains: Proposal for Nine New Species. *Microbiology*, 141(7): 1745 – 1761. <https://doi.org/10.1099/13500872-141-7-1745>
- Painter, E. P., 1941. The Chemistry and Toxicity of Selenium Compounds, with Special Reference to the Selenium Problem. *Chemical Reviews*, 28(2): 179 – 213. <https://doi.org/10.1021/cr60090a001>
- Pettine, M., Gennari, F., Campanella, L., et al., 2012. The Reduction of Selenium(IV) by Hydrogen Sulfide in Aqueous Solutions. *Geochimica et Cosmochimica Acta*, 83: 37–47. <https://doi.org/10.1016/j.gca.2011.12.024>
- Rolewicz, M., Rusek, P., Borowik, K., 2018. Obtaining of Granular Fertilizers Based on Ashes from Combustion of Waste Residues and Ground Bones Using Phosphorous Solubilization by Bacteria *Bacillus Megaterium*. *Journal of Environmental Management*, 216: 128–132. <https://doi.org/10.1016/j.jenvman.2017.05.004>
- Ryu, J. H., Jung, J. H., Park, K. Y., et al., 2021. Humic Acid Removal and Microbial Community Function in Membrane Bioreactor. *Journal of Hazardous Materials*, 417: 126088. <https://doi.org/10.1016/j.jhazmat.2021.126088>
- Sharma, V. K., McDonald, T. J., Sohn, M., et al., 2015. Biogeochemistry of Selenium. a Review. *Environmental Chemistry Letters*, 13(1): 49–58. <https://doi.org/10.1007/s10311-014-0487-x>
- Song, D. G., Li, X. X., Cheng, Y. Z., et al., 2017. Aerobic Biogenesis of Selenium Nanoparticles by *Enterobacter Cloacae* Z0206 as a Consequence of Fumarate Reductase Mediated Selenite Reduction. *Scientific Reports*, 7(1): 3239. <https://doi.org/10.1038/s41598-017-03558-3>
- Song, L., Liu, H. C., Wang, J., et al., 2016. *Bacillus Oceani* Sp. Nov., Isolated from Seawater. *International Journal of Systematic and Evolutionary Microbiology*, 66(2): 796 – 800. <https://doi.org/10.1099/ijsem.0.000793>
- Stolz, J. F., Basu, P., Oremland, R. S., 2002. Microbial Transformation of Elements: The Case of Arsenic and Selenium. *International Microbiology*, 5(4): 201 – 207. <https://doi.org/10.1007/s10123-002-0091-y>
- Stolz, J. F., Basu, P., Santini, J. M., et al., 2006. Arsenic and Selenium in Microbial Metabolism. *Annual Review of Microbiology*, 60: 107 – 130. <https://doi.org/10.1146/annurev.micro.60.080805.142053>
- Tan, Y. Q., Yao, R., Wang, R., et al., 2016. Reduction of Selenite to Se(0) Nanoparticles by Filamentous Bacterium *Streptomyces* Sp. ES2-5 Isolated from a Selenium Mining Soil. *Microbial Cell Factories*, 15(1): 157. <https://doi.org/10.1186/s12934-016-0554-z>
- Tejo Prakash, N., Sharma, N., Prakash, R., et al., 2009. Aerobic Microbial Manufacture of Nanoscale Selenium: Exploiting Nature's Bio-Nanomineralization Potential. *Biotechnology Letters*, 31(12): 1857–1862. <https://doi.org/10.1007/s10529-009-0096-0>
- Turanov, A. A., Xu, X. M., Carlson, B. A., et al., 2011. Biosynthesis of Selenocysteine, the 21st Amino Acid in the Genetic Code, and a Novel Pathway for Cysteine Biosynthesis. *Advances in Nutrition*, 2(2): 122–128. <https://doi.org/10.3945/an.110.000265>
- Ullah, A., Sun, B., Wang, F. H., et al., 2020. Isolation of Selenium-Resistant Bacteria and Advancement under Enrichment Conditions for Selected Probiotic *Bacillus Subtilis* (BSN313). *Journal of Food Biochemistry*, 44(6): e13227. <https://doi.org/10.1111/jfbc.13227>
- Wadgaonkar, S. L., Nanchaiah, Y. V., Jacob, C., et al., 2019. Microbial Transformation of Se Oxyanions in Cultures of *Delftia Lacustris* Grown under Aerobic Conditions. *Journal of Microbiology*, 57(5): 362 – 371. <https://doi.org/10.1007/s12275-019-8427-x>
- Wang, D., Rensing, C., Zheng, S. X., 2022. Microbial Reduction and Resistance to Selenium: Mechanisms, Applications and Prospects. *Journal of Hazardous Materials*, 421: 126684. <https://doi.org/10.1016/j.jhazmat.2021.126684>
- Wang, Q., Yu, Y., Li, J. X., et al., 2017. Effects of Different Forms of Selenium Fertilizers on Se Accumulation, Distribution, and Residual Effect in Winter Wheat-Summer Maize Rotation System. *Journal of Agricultural and Food Chemistry*, 65(6): 1116 – 1123. <https://doi.org/10.1021/acs.jafc.6b05149>
- Wang, Q., Zhan, S., Han, F., et al., 2022. The Possible Mechanism of Physiological Adaptation to the Low-Se Diet and Its Health Risk in the Traditional Endemic Areas of Keshan Diseases. *Biological Trace Element Research*, 200(5): 2069–2083. <https://doi.org/10.1007/s12011-021-02851-7>
- Wang, S. M., Cui, J. T., Li, X. Y., Cheng, W., 2016. Effect of Microorganism on the Degradation and Formation of Humic Acid in Landfill Leachate. *Science & Technology Vision*, 7: 86–87 (in Chinese with English Abstract)
- Wang, X., Ning, Y. J., Zhang, P., et al., 2019. Hair Multi-Bioelement

- Profile of Kashin-Beck Disease in the Endemic Regions of China. *Journal of Trace Elements in Medicine and Biology*, 54: 79–97. <https://doi.org/10.1016/j.jtemb.2019.04.002>
- Wang, Y. H., Shi, X. Y., Huang, X. X., et al., 2022. Linking Microbial Community Composition to Farming Pattern in Selenium-Enriched Region: Potential Role of Microorganisms on Se Geochemistry. *Journal of Environmental Sciences*, 112: 269–279. <https://doi.org/10.1016/j.jes.2021.05.015>
- Wang, Z., Huang, W., Pang, F., 2022. Selenium in Soil-Plant-Microbe: A Review. *Bulletin of Environmental Contamination and Toxicology*, 108(2): 167–181. <https://doi.org/10.1007/s00128-021-03386-2>
- Weisburg, W. G., Barns, S. M., Pelletier, D. A., et al., 1991. 16S Ribosomal DNA Amplification for Phylogenetic Study. *Journal of Bacteriology*, 173(2): 697–703. <https://doi.org/10.1128/jb.173.2.697-703.1991>
- Won, S., Ha, M. G., Nguyen, D. D., et al., 2021. Biological Selenite Removal and Recovery of Selenium Nanoparticles by Haloalkaliphilic Bacteria Isolated from the Nakdong River. *Environmental Pollution*, 280: 117001. <https://doi.org/10.1016/j.envpol.2021.117001>
- Yin, K., Wang, Q. N., Lv, M., et al., 2019. Microorganism Remediation Strategies towards Heavy Metals. *Chemical Engineering Journal*, 360: 1553–1563. <https://doi.org/10.1016/j.cej.2018.10.226>
- Zhang, G. L., Zhou, L. L., Cai, D. Q., et al., 2018. Anion-Responsive Carbon Nanosystem for Controlling Selenium Fertilizer Release and Improving Selenium Utilization Efficiency in Vegetables. *Carbon*, 129: 711–719. <https://doi.org/10.1016/j.carbon.2017.12.062>
- Zhang, J., Wang, Y., Shao, Z. Y., et al., 2019. Two Selenium Tolerant *Lysinibacillus* Sp. Strains Are Capable of Reducing Selenite to Elemental Se Efficiently under Aerobic Conditions. *Journal of Environmental Sciences*, 77: 238–249. <https://doi.org/10.1016/j.jes.2018.08.002>
- Zhang, L., Li, D. P., Gao, P., 2012. Expulsion of Selenium/Protein Nanoparticles through Vesicle-Like Structures by *Saccharomyces Cerevisiae* under Microaerophilic Environment. *World Journal of Microbiology & Biotechnology*, 28(12): 3381–3386. <https://doi.org/10.1007/s11274-012-1150-y>
- Zhang, X. M., Guo, J. H., Vogt, R. D., et al., 2020. Soil Acidification as an Additional Driver to Organic Carbon Accumulation in Major Chinese Croplands. *Geoderma*, 366: 114234. <https://doi.org/10.1016/j.geoderma.2020.114234>
- Zhang, X., Wang, T., Li, S. E., et al., 2019. A Spatial Ecology Study of Keshan Disease and Hair Selenium. *Biological Trace Element Research*, 189(2): 370–378. <https://doi.org/10.1007/s12011-018-1495-7>
- Zhao, B., Xing, C., Zhou, S. B., et al., 2020. Sources, Fraction Distribution and Health Risk Assessment of Selenium (Se) in Dashan Village, a Se-Rich Area in Anhui Province, China. *Bulletin of Environmental Contamination and Toxicology*, 104(4): 545–550. <https://doi.org/10.1007/s00128-020-02827-8>
- Zheng, S. X., Su, J., Wang, L., et al., 2014. Selenite Reduction by the Obligate Aerobic Bacterium *Comamonas testosteroni* S44 Isolated from a Metal-Contaminated Soil. *BMC Microbiology*, 14: 204. <https://doi.org/10.1186/s12866-014-0204-8>
- Zhong, X. L., Gan, Y. Q., Deng, Y. M., 2021. Distribution, Origin and Speciation of Soil Selenium in the Black Soil Region of Northeast China. *Environmental Geochemistry and Health*, 43 (3): 1257–1271. <https://doi.org/10.1007/s10653-020-00691-3>
- Zhou, F., Li, Y. N., Ma, Y. Z., et al., 2021. Selenium Bioaccessibility in Native Seleniferous Soil and Associated Plants: Comparison between in Vitro Assays and Chemical Extraction Methods. *Science of the Total Environment*, 762: 143119. <https://doi.org/10.1016/j.scitotenv.2020.143119>