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Cadmium detoxification by *Stenotrophomonas* sp. via cell wall exfoliation and regeneration mediated by *mtgA*



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ABSTRACT

Understanding bacterial strategies for coping with heavy metal stress is essential for elucidating their resilience in contaminated environments. However, whether cell wall exfoliation contributes to bacterial tolerance under heavy metal stress, such as cadmium (Cd) exposure, remains unclear and requires further investigation. In this study, we reveal a novel self-protective mechanism in *Stenotrophomonas* sp. H225 isolated from a Cd-contaminated farmland soil, which underwent controlled cell wall exfoliation and regeneration in response to Cd stress up to 200 mg L⁻¹. Transmission electron microscopy and energy-dispersive X-ray spectroscopy analyses revealed that the exfoliated cell wall fragments served as extracellular Cd sinks, thereby reducing intracellular Cd accumulation. Fourier-transform infrared spectroscopy and enzyme-linked immunosorbent assay indicated progressive peptidoglycan (PG) degradation, with exfoliated PG concentration in solution increasing from 148 ng mL⁻¹ at 0 mg L⁻¹ Cd to 240 ng mL⁻¹ at 200 mg L⁻¹ Cd. This degradation was counteracted by the compensatory upregulation of PG biosynthesis genes, with the enrichment ratio reaching up to 0.83, facilitating cell wall reconstruction. Transcriptomic analysis and gene knockout experiments identified *mtgA* (encoding a monofunctional transglycosylase) as a key determinant in cell wall repair and Cd resistance. To our knowledge, this is the first mechanistic evidence that bacteria can mitigate heavy metal toxicity through dynamic cell wall remodeling involving exfoliation and regeneration. This finding enhances our understanding of microbial survival strategies under environmental stress and highlights potential targets for engineering metal-tolerant strains for bioremediation applications.

Key Words: bioremediation, Cd resistance, Cd stress, cell wall remodeling, detoxification strategy, gene knockout, heavy metal, *phpC* gene, peptidoglycan

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INTRODUCTION

Cadmium (Cd) pollution has emerged as a critical global issue, primarily caused by industrial emissions, mining activities, and agricultural runoff (Phaenark *et al.*, 2024). Due to its high environmental persistence and bio-accumulative properties, Cd poses serious risks to ecosystems and human health worldwide (Essien *et al.*, 2024). Ranked seventh on the 2016 Hazardous Substances Priority List, Cd has no known biological function and is toxic even at trace concentrations (Cheremisnoff, 2016). It enters the food chain through contaminated crops such as rice and wheat, with daily human intake reaching up to 30 µg, raising serious food safety concerns (Nriagu and Pacyna, 1988; Joseph, 2009). Chronic exposure to Cd is linked to kidney disease, osteoporosis, and cancer, thereby raising a significant public health concern (Satarug and Moore, 2004; Rasin *et al.*, 2025). Regions with

extensive industrialization and mining, including Thailand's industrial hubs and mining-intensive areas in Latin America, often report elevated Cd levels surpassing environmental safety thresholds (Guarín *et al.*, 2024; Niampradit *et al.*, 2024). Despite continued remediation efforts, effectively controlling Cd contamination remains a major challenge, underscoring the urgent need for innovative and sustainable bioremediation strategies.

Bacterial cell walls act as the first barrier against Cd toxicity. The peptidoglycan (PG) layer, along with surface functional groups such as carboxyl, amine, phosphate, and hydroxyl, can bind Cd cations and help reduce their entry into the cell (Vijayaraghavan and Yun, 2008). A wide range of bacteria has demonstrated the ability to bind Cd on their cell surfaces, including *Bacillus*, *Stenotrophomonas*, *Azotobacter*, *Escherichia coli*, *Mycobacterium*, and *Serratia*

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(Kenney and Fein, 2011; Chakravarty and Banerjee, 2012; Kumar *et al.*, 2021; Cao *et al.*, 2022). Recent studies highlight an overlooked bacterial adaptation: cell wall exfoliation. Under stress, some bacteria shed the damaged parts of their cell wall as a survival strategy. Similar responses have been observed under antibiotic and osmotic stresses, where shedding damaged cell wall material helps maintain cellular integrity (Ramijan *et al.*, 2018). For example, *Lysinibacillus* sp. has been reported to exfoliate iron-bound surface layers, and certain *Actinobacteria* can shift into wall-deficient forms under high osmotic pressure (Chandramohan *et al.*, 2019). Likewise, *Bacillus subtilis* and *Staphylococcus aureus* have been shown to partially shed and regenerate their cell walls after prolonged antibiotic exposure (Kawai *et al.*, 2018; Ramijan *et al.*, 2018). However, whether cell wall exfoliation contributes to bacterial tolerance under heavy metal stress, such as Cd exposure, remains unclear and requires further investigation.

Understanding how bacterial cell wall remodeling is regulated at the genetic and biochemical levels under Cd stress is essential for improving microbial bioremediation strategies. This study identified the genes of *pbpC* (encoding penicillin-binding protein 3) and *mtgA* (encoding a monofunctional transglycosylase) as key regulators of cell wall synthesis in *Stenotrophomonas* sp. H225. These genes appeared to limit bacterial survival under high Cd stress, likely due to their critical roles in maintaining cell wall structure and function (Di Berardino *et al.*, 1996; Vollmer *et al.*, 1999). The gene *pbpC* is essential for PG assembly in *Clavibacter michiganensis*, and its deletion results in reduced cell wall thickness and mechanical resistance under copper stress (Chen *et al.*, 2022). Similarly, deletion of the penicillin-binding protein 5 in *Escherichia coli* increases susceptibility to β -lactam antibiotics, indicating its role in preserving normal cell shape (Sarkar *et al.*, 2010). Although previous studies have demonstrated that *pbpC* and *mtgA* participate in PG biosynthesis, their expressions were found to be suppressed under high Cd concentrations. However, it remains unclear whether targeted modification or deletion of these genes could enhance the stress resistance of beneficial bacterial strains, which warrants further investigation.

In this study, the detoxification mechanism of strain H225 against Cd stress was investigated through cell wall exfoliation and regeneration. Transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDS), Fourier-transform infrared (FTIR) spectroscopy, and enzyme-linked immunosorbent assay (ELISA) were applied to characterize structural changes and PG release under 0–200 mg L⁻¹ Cd exposure. Transcriptomic analysis and gene knockout experiments targeting *pbpC* and *mtgA* were conducted to assess their roles in cell wall remodeling. Growth assays and morphological observations were used to evaluate the Cd resistance of knockout strains.

MATERIALS AND METHODS

Bacterial strain and Cd-stress incubation

Bacterial strain H225, with accession number 25640 in the China General Microbiological Culture Collection Center and Genbank number OP522361 in the National Center for Biotechnology Information (NCBI), was collected from a Cd-contaminated farmland soil in Xiaguang Town, Kaihua County, Quzhou, China. The soil, developed from black shale, had a Cd concentration of 4.447 mg kg⁻¹, with a pH of 5. One ring of colonies was collected and inoculated into 100 mL Luria-Bertani (LB) liquid medium, and then cultured in a shaker at 180 r min⁻¹ and 28 °C for 48 h to serve as a seed culture medium.

A 1 mL H225 seed solution was inoculated into a series of 100 mL LB liquid media. Then, the Cd concentrations of the media were adjusted to 0, 25, 50, 75, 100, 150, and 200 mg L⁻¹ by adding an appropriate amount of a 10 000 mg L⁻¹ CdCl₂ stock solution. Each treatment (Cd concentration) had three replicates. All treatments were cultured in a shaker at 180 r min⁻¹ and 28 °C for 48 h. Bacterial growth was assessed by measuring the optical density at 600 nm (OD₆₀₀) after a 10-fold dilution of 5 mL H225 culture. In addition, 20 mL H225 culture was centrifuged at 4 500 r min⁻¹ for 10 min and dried to determine the conversion factor between OD₆₀₀ and dry cell weight for establishing Cd exposure conditions and quantify the growth response of H225 at varying Cd concentrations.

Determination of residual, adsorbed, and intracellular Cd

To elucidate the mechanisms of Cd uptake and immobilization by H225, a 30 mL aliquot of the culture solution was centrifuged at 10 000 r min⁻¹ for 10 min. The Cd in the supernatant was residual Cd. The pellet was extracted with 10 mL 20 mmol L⁻¹ Na₂EDTA for 30 min and centrifuged at 10 000 r min⁻¹ for 10 min, and the Cd in the supernatant was adsorbed Cd. Finally, the pellet was extracted with 10 mL 50% HNO₃ for 30 min and centrifuged at 10 000 r min⁻¹ for 10 min, and the extracted Cd was intracellular Cd. Before Cd quantification with an inductively coupled plasma-atomic emission spectrometer, the supernatant and extract were passed through 0.22- μ m membrane filters.

TEM observation, FTIR analysis, and exfoliated PG quantification

To examine the morphological changes and exfoliated cell wall fragments of H225 under Cd stress, bacterial pellets obtained from centrifugation (4 500 r min⁻¹, 10 min) were fixed with 2.5% (volume/volume) glutaraldehyde at 4 °C for 4 h, washed three times with pH 7.2 phosphate-buffered saline, and post-fixed with 2.5% (volume/volume) osmium

tetroxide at 4 °C. After a graded ethanol dehydration series (30%, 50%, 70%, 90%, and 100%), samples were infiltrated with acetone-resin mixtures and embedded in epoxy resin using embedding molds. Ultrathin sections were cut using an ultramicrotome and mounted on copper grids for TEM observation.

To identify the functional group changes of H225 during Cd exposure, bacterial pellet obtained from centrifugation (4 500 r min⁻¹, 10 min) was washed three times with deionized water. After vacuum drying, 1 mg pellet was thoroughly ground with 100 mg KBr for FTIR spectral analysis (Kamnev *et al.*, 1999).

Culture solution of H225 was centrifuged at 4 500 r min⁻¹ for 10 min, and the exfoliated PG in the supernatant was quantified using ELISA. A 50 µL aliquot of each supernatant was transferred to a 96-well microplate pre-coated with PG antibody, and then 100 µL horseradish peroxidase-conjugated detection antibody was added. The plate was sealed and incubated in a 37 °C water bath for 60 min. After incubation, the wells were washed five times with washing buffer. Color development was initiated by adding 3,3',5,5'-tetramethylbenzidine (TMB). Upon horseradish peroxidase catalysis, TMB was oxidized to produce a blue color, which turned yellow upon acid termination. Absorbance was measured at 450 nm using a microplate reader, and PG concentration was calculated based on the standard curve.

Transcriptomic analysis

To comprehensively investigate the gene response of H225 to Cd stress, transcriptomic analysis was conducted. Particular attention was paid to genes involved in PG biosynthesis and stress adaptation. A 30 mL aliquot of the culture solution was centrifuged at 4 000 r min⁻¹ and 4 °C for 10 min. The bacterial pellet was washed three times with 5 mL phosphate-buffered saline (pH 7.2) and centrifuged at 1 500 r min⁻¹ and 4 °C for 10 min to collect the bacterial cells, which were then immediately frozen in liquid nitrogen. Cells were cryopreserved at -80 °C before being transported on dry ice to Beijing Genomics 176 Institute for RNA sequencing.

The TRIzol protocol was used to isolate total RNA from each sample (Chomczynski, 1993). Purification of the poly-A-containing mRNA molecules was conducted using poly-T oligo-attached magnetic beads. After mRNA fragmentation, first-strand cDNA was synthesized using reverse transcriptase and random primers, followed by second-strand cDNA synthesis using DNA polymerase I and RNase H. Then, the cDNA fragments were purified and enriched *via* polymerase chain reaction (PCR) amplification. Finally, the library was loaded into the BGISEQ-500 platform. Raw reads from RNA sequencing were quality-checked using FastQC

(version 0.11.9) and subsequently cleaned with SOAPnuke (Chen *et al.*, 2018). Clean reads were aligned to the reference genome sequence using HISAT (Kim *et al.*, 2015). Gene and transcript expression levels were calculated using RSEM (Bo and Colin, 2011). For protein function prediction, uni-genes were aligned against the NCBI Nonredundant Protein Database, Swiss-Prot, and Kyoto Encyclopedia of Genes and Genomes (KEGG) using the DIAMOND software (version 2.0.4, “more-sensitive” mode) with a cutoff *E*-value of 1×10^{-5} .

Gene knock and functional validation

To generate gene deletion mutants of 2998 (*pbpC*) and 3035 (*mtgA*) and evaluate the roles of these genes in Cd resistance, the plasmid backbone of pK18 was amplified using pK18*mobsacB* (Harighi, 2009) as a template. The upstream and downstream fragments of the target gene (TG) were inserted into the backbone, resulting in constructs pK-TG-LF/LR and pK-TG-RF/RR (Table I). A strong promoter, the *sac* promoter, was also incorporated into the vector. The *sacB* gene, located on the vector, was highly expressed under the control of the *sac* promoter. The constructed vector was introduced into the recipient strain H225 *via* triparental conjugation. The donor strain, *Escherichia coli* DH5α (Woodcock *et al.*, 1989), harbored the recombinant plasmid pK18*mobsacB*-TG, while the helper plasmid pRK600, carrying a chloramphenicol resistance gene, was used to facilitate conjugation. Selection for successful transformants was conducted using tetracycline (Tc) resistance (70 µg mL⁻¹) encoded by the constructed vector and gentamicin resistance (30 µg mL⁻¹) inherent to H225. Under antibiotic pressure, the upstream and downstream fragments on the vector were integrated into the genome of the recipient strain through homologous recombination.

During the first homologous recombination event, Tc resistance was used for positive selection. Three pairs of primers (pK-VF/VR, 2998-OVF/OVR, and 3035-OVF/OVR) were used to verify gene knockout and recombination. The recombinant strains were then subjected to counterselection on a sucrose-containing medium. Cells expressing *sacB* were eliminated due to their toxicity, while mutants with successful gene deletion survived. The knockouts were confirmed by PCR using the same primer sets.

To assess the morphological and growth responses of knockout strains under Cd stress (100 mg L⁻¹) and verify gene function, the successfully constructed knockout strains Δ3035 and Δ2998 were examined using TEM. Subsequently, the knockout strains were inoculated into LB medium containing 0–200 mg L⁻¹ Cd and cultured in a shaker at 180 r min⁻¹ and 28 °C for 48 h. A 200 µL aliquot of each culture was then transferred to a 96-well plate, with six replicates per culture, and incubated at 28 °C. During the growth phase, OD₆₀₀ was measured hourly using a microplate reader. Growth curves of the knockout strains were plotted using OriginPro 2025 (OriginLab, USA).

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 (IBM, USA), with differences among treatments assessed by one-way analysis of variance followed by Ryan-Einot-Gabriel-Welsch multiple range test, least significant difference, or Duncan's *post hoc* test. A *P* value of < 0.05 was considered statistically significant. Gene expression was analyzed using the R software. The batch effect was removed from the visualization of batch-controlled gene expression using the ComBat function in the Bioconductor *sva* package. Heatmaps were plotted using R package *gplots*.

RESULTS

H225 biomass

For H225 biomass determination, both OD₆₀₀ and dry weight were measured. The dry weight measurements closely

mirrored the OD₆₀₀ values (Fig. 1), with a strong positive correlation ($R^2 = 0.9934$) between the two independent measurements. At 25 mg L⁻¹ Cd, a slight increase in OD₆₀₀ (0.662) was observed compared to 0 mg L⁻¹ Cd (0.612), suggesting a mild stimulatory effect. However, as Cd concentration increased to 75 mg L⁻¹ and above, both OD₆₀₀ and dry weight declined progressively, indicating a dose-dependent inhibitory effect of Cd. At 200 mg L⁻¹ Cd, only a few cells survived, with an OD₆₀₀ of 0.11. These results suggest that the toxicity of Cd to H225 biomass was characterized by non-inhibitory at low concentrations and lethal at high concentrations.

Cd partitioning

With Cd concentration increasing from 25 to 200 mg L⁻¹, the residual Cd in solution increased exponentially from 0.47 to 165 mg L⁻¹ (Fig. 2). Under low Cd stress

TABLE I

Strains, plasmids, and primers used in this study

Type	Name	Sequence	Usage
Strain	H225		Host for routine cloning
	<i>Escherichia coli</i> DH5α		Donor strain for conjugation
Plasmid	pK18 <i>mobsacB</i>		Allelic exchange vector
	pK-2998-LF	gcgagtcagtgagcaggaaggtcaacgaccgtctgtagaa	Construction of the recombinant vector
	pK-2998-LR	AAACAAATAGGGGTTCGCGGatgcgcgaccttggtcgccgttc	pK18 <i>mobsacB</i> -2998
	pK-3035-LF	gcgagtcagtgagcaggaaggggtgggttcagggccgagctgc	Construction of the recombinant vector
	pK-3035-LR	AAATAAACAAATAGGGGTTCGCGGaacgtacaatccggacatgaac	pK18 <i>mobsacB</i> -3035
Primer	pK-2998-RF	AGCCGGGCCACCTCGACCTGAatgcgaattgcatcaggacgg	Construction of the recombinant vector
	pK-2998-RR	gtttgcgtattggcgctcttcgagcgcagcaaggcggtcggtg	pK18 <i>mobsacB</i> -2998
	pK-3035-RF	AGCCGGGCCACCTCGACCTGAaattactggtgcatccttctctatc	Construction of the recombinant vector
	pK-3035-RR	gcgtattggcgctcttcgacaagccgtttactgaagccgttc	pK18 <i>mobsacB</i> -3035
	TC-F	CGCGGAACCCCTATTTGTTTAT	Construction of the recombinant vectors
	TC-R	TCAGGTTCGAGGTGGCCCGGCTCCAT	pK18 <i>mobsacB</i> -2998/3035
	pK-F	cggagagcgcccaataacgc	
	pK-R	cttctcgctcactgactcgt	
	pK-VF	cttgagcgtcgtttttgtgat	Recombinant vector verification
	pK-VR	taccgagctcgaattcgtaa	
	2998-OVF	aatgtcttgacccccctgtc	H225 Δ2998 verification
	2998-OVR	ctgtccggtggcaatcagtt	
	3035-OVF	acgatcacgcagcagatct	H225 Δ3035 verification
	3035-OVR	accttgcgcgtgccctgttt	

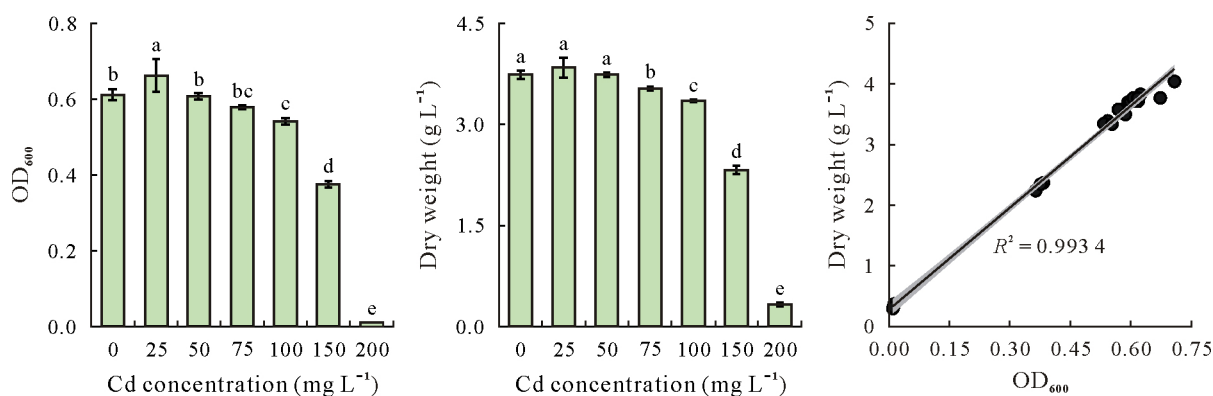


Fig. 1 Optical density at 600 nm (OD₆₀₀) after 10-fold dilution and dry weight of *Stenotrophomonas* sp. H225 after 48 h of incubation (180 r min⁻¹, 28 °C) under 0–200 mg L⁻¹ Cd and correlation between the OD₆₀₀ and dry weight. Error bars are standard deviations of the means (*n* = 3). Different letters above the bars indicate significant differences between different Cd concentrations at *P* < 0.05. The shaded area represents the 95% confidence interval of the fit.

(25 mg L⁻¹), only 2% of the total Cd remained in solution. In contrast, more than 60% of the Cd remained unremoved at concentrations ≥ 50 mg L⁻¹. The adsorbed Cd dropped from 2.2 to 0.9 mg g⁻¹ with Cd increasing from 25 to 50 mg L⁻¹. Then, it increased continuously to 53.9 mg g⁻¹ at 200 mg L⁻¹ Cd. The intracellular Cd concentration was high (4.2 mg g⁻¹) at 25 mg L⁻¹ Cd, decreased to 1.2 mg g⁻¹ at 50 mg L⁻¹ Cd, rose continuously up to 4.5 mg g⁻¹ until 150 mg L⁻¹ Cd, and then declined slightly to 2.3 mg g⁻¹ at 200 mg L⁻¹ Cd.

The removal efficiency of Cd, defined as the total proportion of adsorbed and intracellular Cd, achieved its peak value of 98.0% at 25 mg L⁻¹ Cd (Fig. 2). It declined sharply to 16.1% at 50 mg L⁻¹ Cd, was 31.8%–37.7% at 75–150 mg L⁻¹ Cd, and then dropped to 10.2% at 200 mg L⁻¹ Cd. These results indicate that H225 can efficiently remove Cd

at low concentrations mainly *via* intracellular accumulation, but its removal capacity is significantly reduced under high Cd stress, leading to high residual Cd in the environment.

Cell wall exfoliation of chelated Cd

In the Cd-containing systems, H225 underwent sloughing and exfoliation at all Cd concentrations (Fig. S1, see Supplementary Material for Fig. S1). The TEM images showed that exposure to 100–200 mg L⁻¹ Cd caused extensive exfoliation and fragmentation of H225 cell walls, breaking the originally intact walls into detached fragments (Fig. 3). While some cells retained structural integrity, those with wall exfoliation exposed their inner and outer membranes, which may serve as additional Cd-binding sites. To determine whether the exfoliated cell wall structures contained Cd, EDS was performed. The results showed that Cd

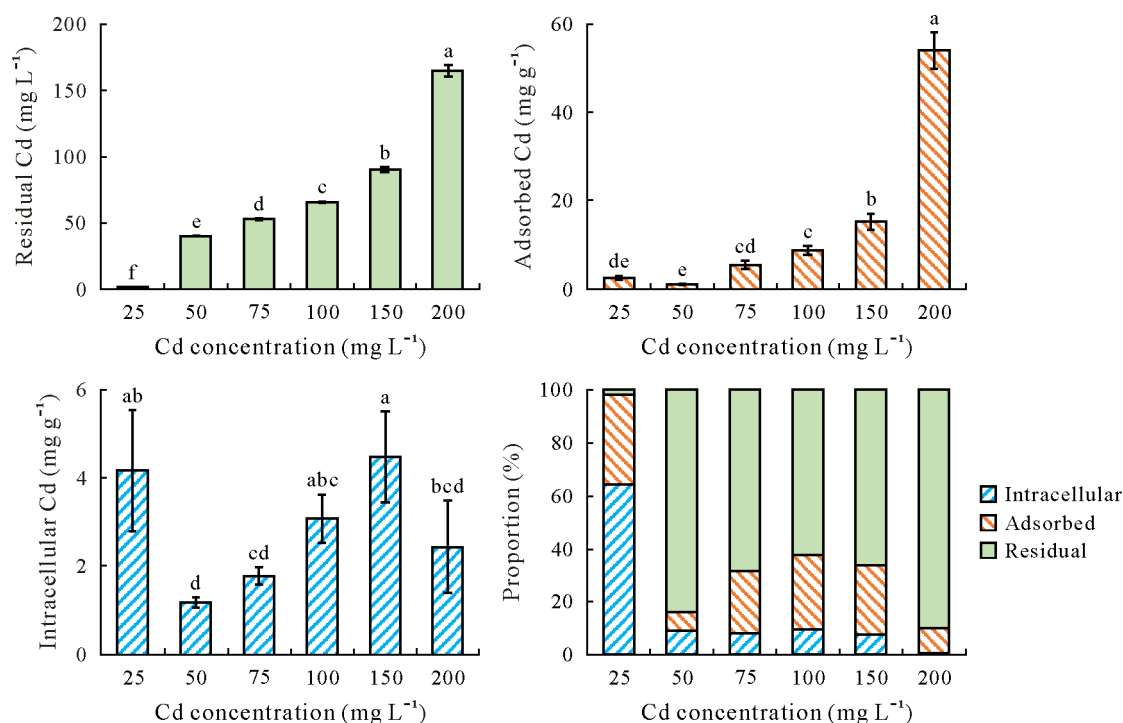


Fig. 2 Concentrations and proportions of residual, adsorbed, and intracellular Cd after 48 h of incubation (180 r min⁻¹, 28 °C) of *Stenotrophomonas* sp. H225 under 0–200 mg L⁻¹ Cd. Error bars are standard deviations of the means ($n = 3$). Different letters above the bars indicate significant differences between different Cd concentrations at $P < 0.05$.

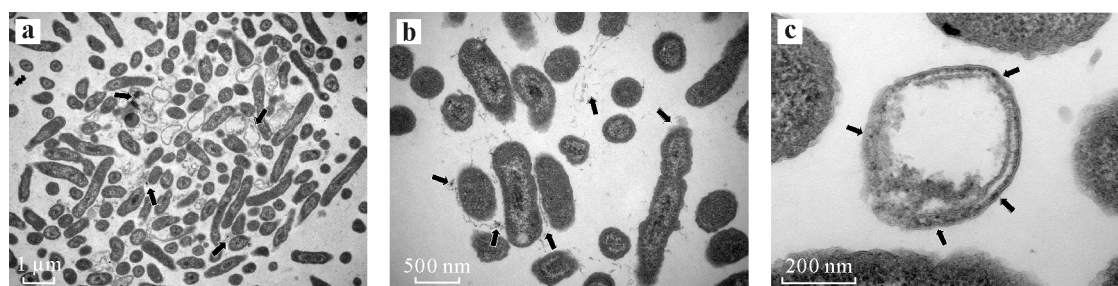


Fig. 3 Transmission electron microscopy images of *Stenotrophomonas* sp. H225 cells after 48 h of incubation (180 r min⁻¹, 28 °C) under 100 (a), 150 (b), and 200 (c) mg L⁻¹ Cd. The arrows in the images point to exfoliated cell wall fragments.

signals (cyan dots) were spatially co-localized with the morphological outlines of fragmented cell wall structures seen in the corresponding TEM images (Fig. 4). At 0 mg L⁻¹ Cd, only sparse or background-level Cd signals were detected. In contrast, under 200 mg L⁻¹ Cd stress, the exfoliated structures observed in TEM images exhibited a high density of Cd signals, indicating that these detached fragments function as Cd-binding sites. The spatial consistency between the fragment morphology and EDS-detected Cd signals strongly supports the conclusion that the exfoliated cell wall segments acted as extracellular Cd sinks. The FTIR spectra (Fig. 5) of H225 cells revealed a progressive decrease in the intensity of amide I peak (1 654 cm⁻¹) as Cd concentration increased, indicating a reduction in PG content of H225 cell wall. Quantification of PG in solution confirmed a dose-dependent increase in the exfoliated PG, rising from 148 ng mL⁻¹ at 0 mg L⁻¹ Cd to 156–240 ng mL⁻¹ at 25–200 mg L⁻¹ Cd. Collectively, these results support cell wall exfoliation as a potential detoxification mechanism employed by H225 under Cd stress.

Mapping of upregulated genes

Differentially expressed genes (DEGs) with a log₂(fold change) > 1 under Cd stress compared to 0 mg L⁻¹ Cd were subjected to KEGG pathway enrichment analysis, with a significance threshold of $P < 0.05$. The number of significantly enriched metabolism-related pathways progressively increased with Cd concentration from 25 to 150 mg L⁻¹, followed by a slight decline at 200 mg L⁻¹ Cd (Fig. 6). At low Cd concentrations (25 and 50 mg L⁻¹), pathways involved in PG biosynthesis, lysine biosynthesis, and mineral absorption were significantly enriched. At moderate Cd levels (75 and 100 mg L⁻¹), additional pathways became enriched, including nonribosomal peptide biosynthesis, adenosine triphosphate-binding cassette transporters, fatty acid degradation, and pyrimidine metabolism. At high Cd concentrations (150 and 200 mg L⁻¹), enrichment was observed in pathways related to ribosome function, the tricarboxylic acid cycle, carbon and pyruvate metabolism, and microbial adaptation to environmental stress. Notably, the enrichment ratio for PG biosynthesis increased markedly

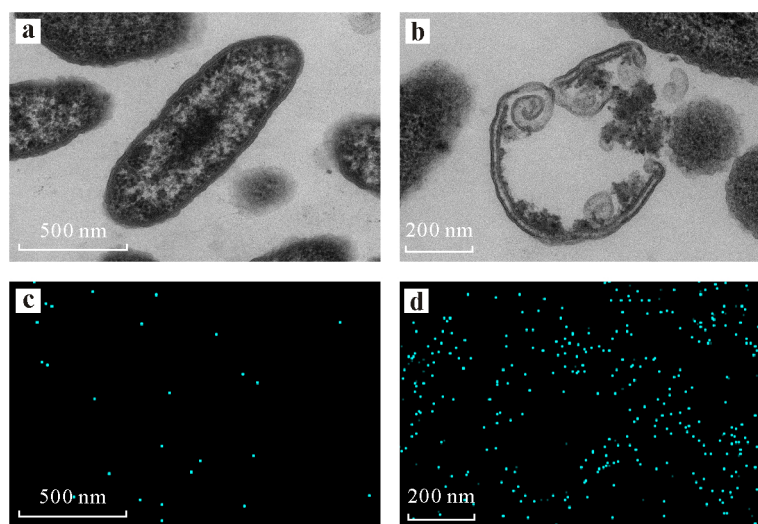


Fig. 4 Transmission electron microscopy images of *Stenotrophomonas* sp. H225 cells (a and b) and the corresponding energy-dispersive X-ray spectroscopy Cd maps (c and d) after 48 h of incubation (180 r min⁻¹, 28 °C) under 0 (a and c) and 200 (b and d) mg L⁻¹ Cd.

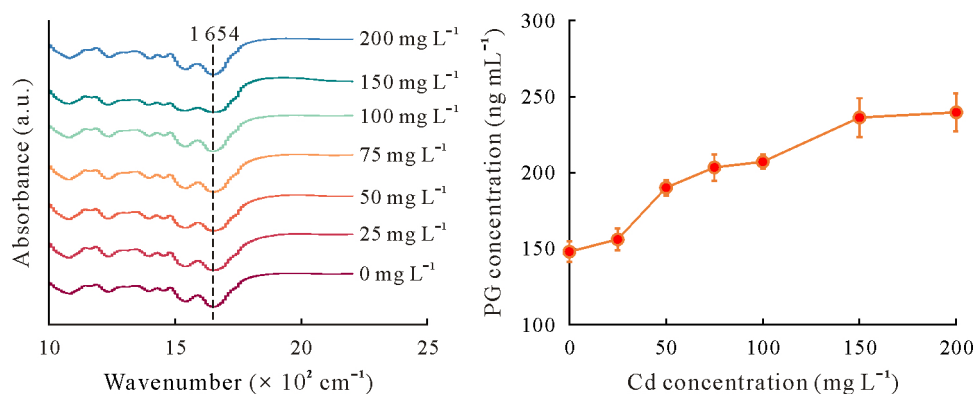


Fig. 5 Fourier-transform infrared spectra of *Stenotrophomonas* sp. H225 cells and exfoliated peptidoglycan (PG) concentrations in solution after 48 h of incubation (180 r min⁻¹, 28 °C) under 0–200 mg L⁻¹ Cd. Error bars are standard deviations of the means ($n = 3$). a.u. = arbitrary unit.

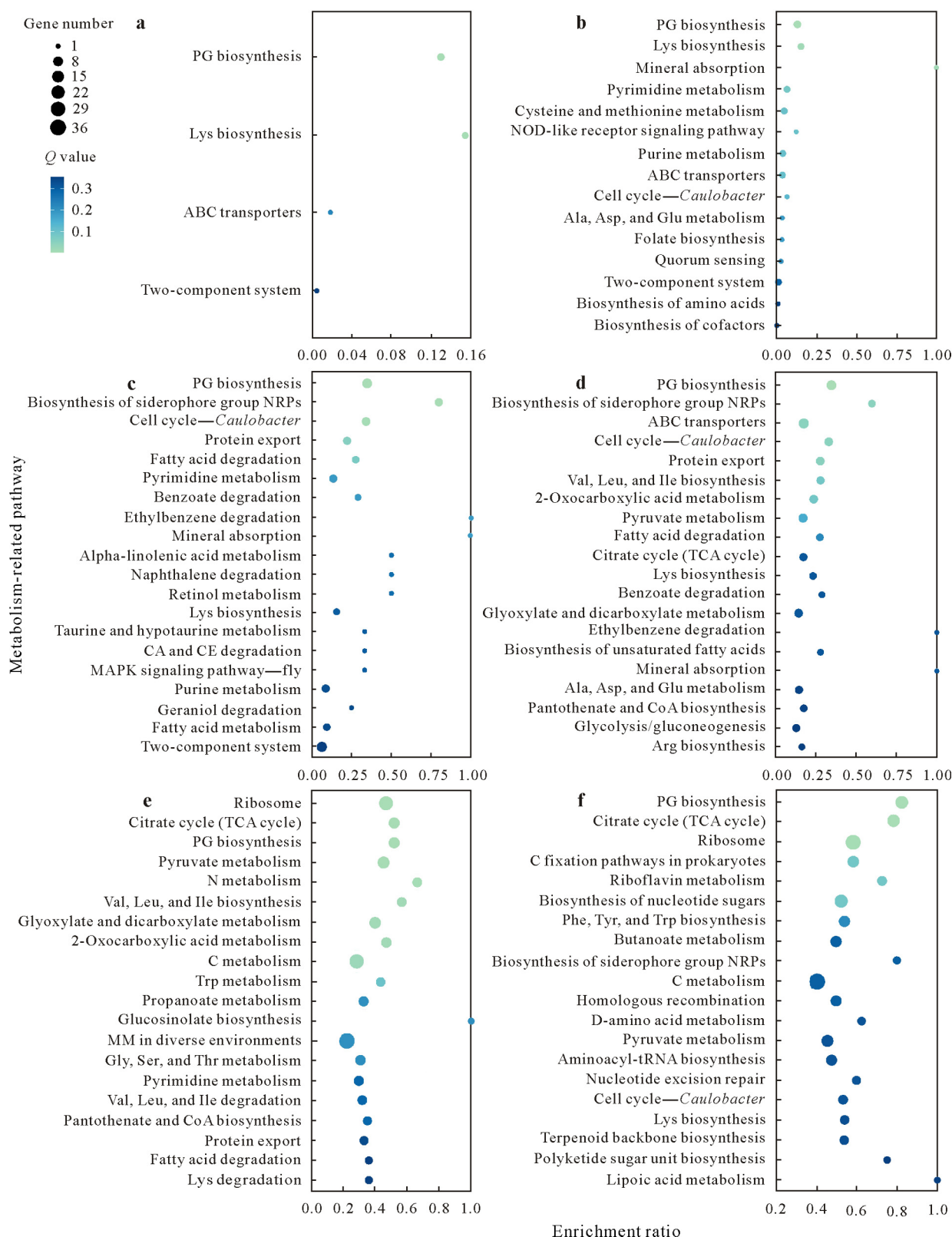


Fig. 6 Kyoto Encyclopedia of Genes and Genomes enrichment analysis of differentially expressed genes involved in metabolism-related pathways in *Stenotrophomonas* sp. H225 after 48 h of incubation (180 r min⁻¹, 28 °C) under 25 (a), 50 (b), 75 (c), 100 (d), 150 (e), and 200 (f) mg L⁻¹ Cd compared to 0 mg L⁻¹ Cd. PG = peptidoglycan; Lys = lysine; ABC = adenosine triphosphate-binding cassette; NOD = nucleotide-binding oligomerization domain; Ala = alanine; Asp = aspartate; Glu = glutamate; NRPs = nonribosomal peptides; CA = chloroalkane; CE = chloroalkene; MAPK = mitogen-activated protein kinase; Val = valine; Leu = leucine; Ile = isoleucine; TCA = tricarboxylic acid cycle; CoA = coenzyme A; Arg = arginine; Trp = tryptophan; MM = microbial metabolism; Gly = glycine; Ser = serine; Thr = threonine; Phe = phenylalanine; Tyr = tyrosine.

from 0.13 (25 mg L⁻¹ Cd) to a peak of 0.83 (150 mg L⁻¹ Cd), indicating a potential role of PG biosynthesis in the adaptive response of H225 to Cd stress.

H225 cell wall regeneration

The TEM images revealed the exfoliation of cell wall under Cd stress, which was accompanied by a distinct upregulation of PG biosynthesis genes (Fig. 7). In the cytoplasm, genes involved in Park's nucleotide synthesis showed significant upregulation with increasing Cd concentration. Notably, *murB*, which encodes uridine diphosphate-N-acetylmuramic acid dehydrogenase, exhibited a marked induction when Cd concentration exceeded 150 mg L⁻¹. At the cell membrane level, the expression of *uppS* (encoding undecaprenyl diphosphate synthase) and *bacA* (encoding undecaprenyl-diphosphatase), which regulate the formation of lipid carriers, was positively correlated with Cd concentration. In the outer membrane, the genes encoding glycosyltransferases (Gtase), specifically *mrcA* (encoding penicillin-binding protein 1a) and *mrcB* (encoding penicillin-binding protein 1b), were significantly upregulated. The expression of *mrcA* was notably higher than that of *mrcB* across all Cd concentrations. Additionally, the genes regulating transpeptidase activity, *e.g.*, *dacCAD*, *ftsI*, and *mrda*, exhibited a pronounced induction at 200 mg L⁻¹ Cd, though changes were minimal at low Cd concentrations. Overall, the PG biosynthesis genes displayed a consistent trend of upregulation in response to increasing Cd concentration, suggesting a stress-induced remodeling of the bacterial cell wall structure.

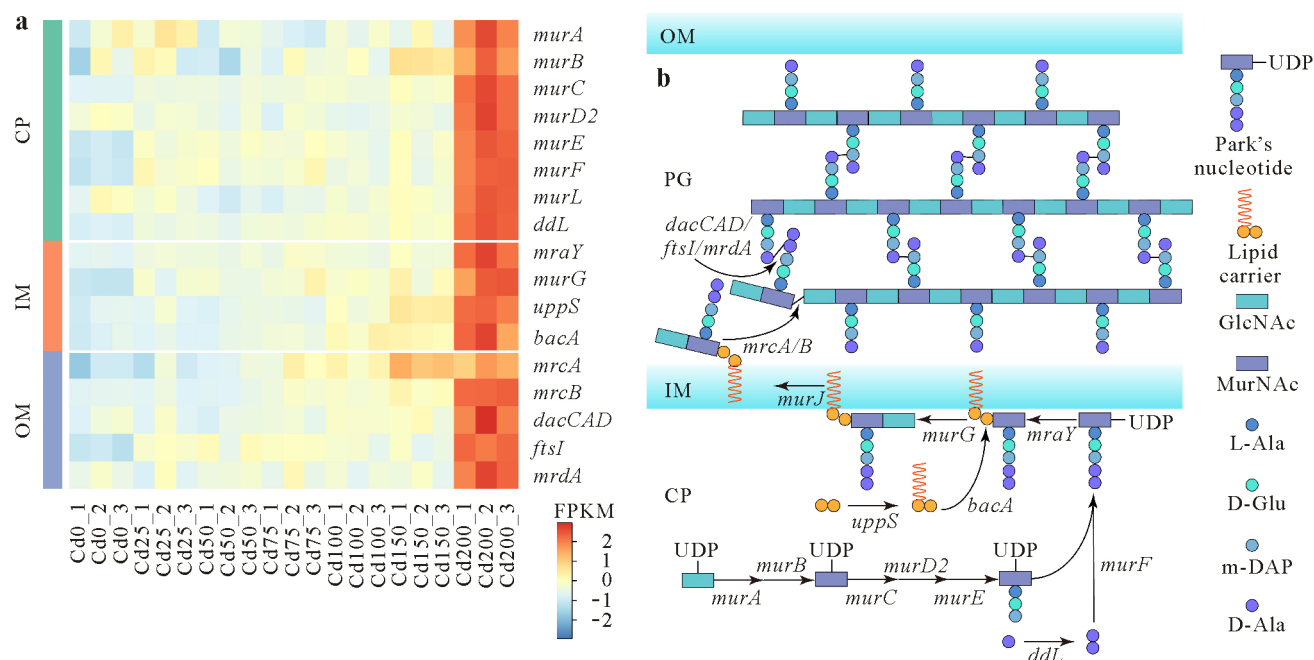


Fig. 7 Differentially expressed genes in the cytoplasm (CP), inner membrane (IM), and outer membrane (OM) of *Stenotrophomonas* sp. H225 cells after 48 h of incubation (180 r min⁻¹, 28 °C) under 0–200 mg L⁻¹ Cd (a) and schematic representation of the peptidoglycan (PG) biosynthesis pathway in H225 cells (b). FPKM = fragments per kilobase of transcript per million mapped reads; Cd x . y = the y th replicate of the x mg L⁻¹ Cd treatment (x = 0, 25, 50, 75, 100, 150, and 200; y = 1, 2, and 3); UDP = uridine diphosphate; GlcNAc = N-acetylglucosamine; MurNAc = N-acetylmuramic acid; L-Ala = L-alanine; D-Glu = D-glutamic acid; m-DAP = meso-diaminopimelic acid; D-Ala = D-alanine.

Validation of the knockout of key genes involved in cell wall exfoliation

The KEGG pathway analysis revealed significant changes in the expressions of PG biosynthesis genes in H225 under Cd stress (Fig. S2, see Supplementary Material for Fig. S2). Among the DEGs, *pbpC* and *mtgA*, both involved in PG cross-linking, were consistently downregulated across the Cd concentrations. Given their essential role in bacterial cell wall integrity, targeted gene knockouts were performed to investigate their functional relevance under Cd exposure.

The construction of recombinant plasmid pK18*mob-sacB*-TG and the homologous recombination strategy used for gene knockouts were detailed in Fig. S3 (see Supplementary Material for Fig. S3). The upstream and downstream homologous fragments of *pbpC* and *mtgA*, along with the Tc resistance gene, were amplified and fused *via* overlapping extension PCR (Fig. 8a–c). Transformants harboring the recombinant plasmids were verified using pK18*mob-sacB*-specific primers, confirming the successful construction of pK18*mob-sacB*- Δ 2998 and pK18*mob-sacB*- Δ 3035 (Fig. 8d).

To ensure transformant viability, H225 recipient strains carrying the knockout plasmids were plated on selective media containing Tc (70 μ g mL⁻¹) and gentamicin (30 μ g mL⁻¹). No growth was observed in control strains lacking knockout plasmids, validating the pK18*mob-sacB*-based selection system (Fig. 8e). The PCR verification confirmed successful homologous recombination, identifying both sin-

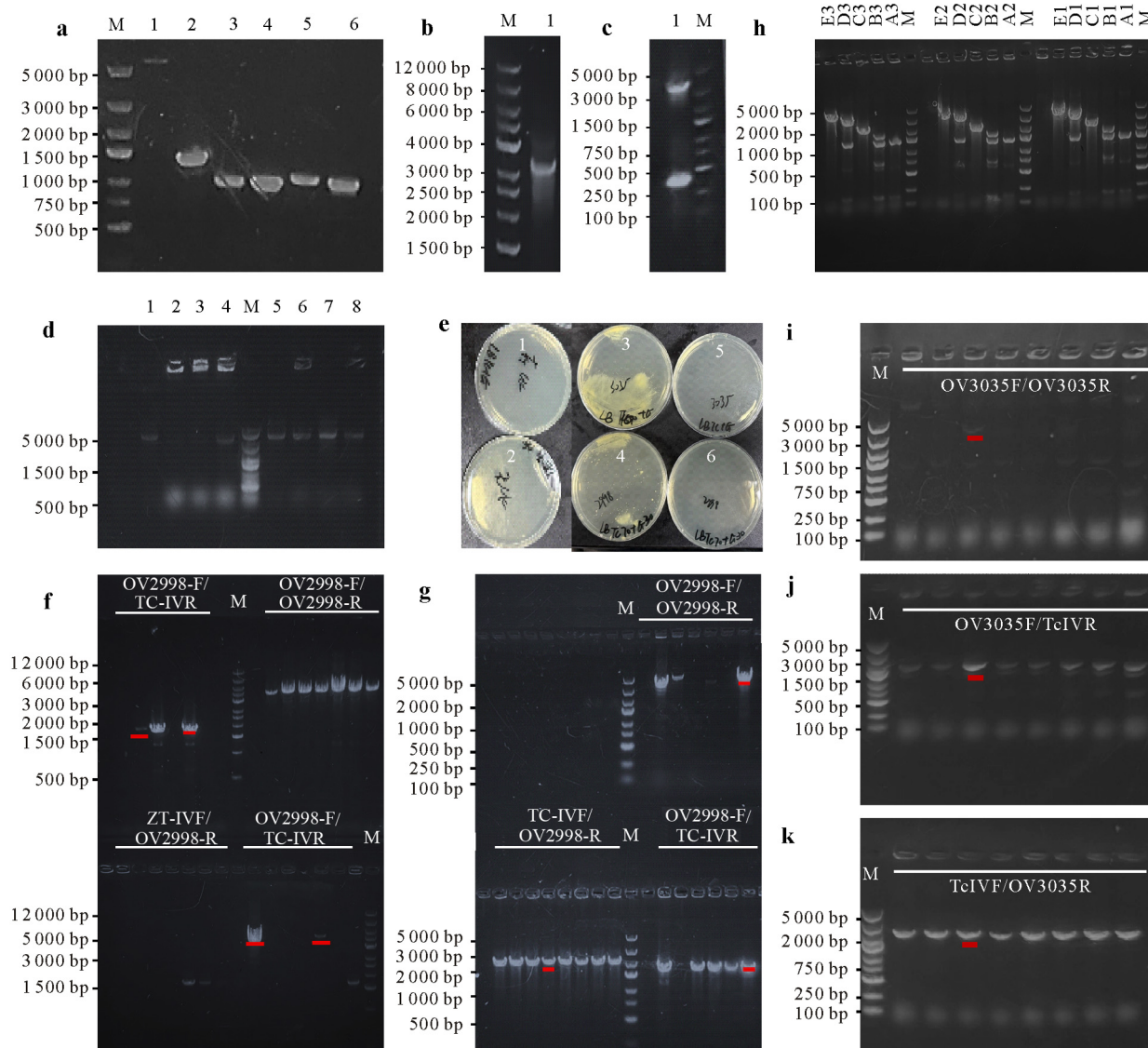


Fig. 8 Gene cloning and phenotypic analysis of *Stenotrophomonas* sp. H225 with 2998 (*pbpC*) and 3035 (*mtgA*) gene knockouts showing polymerase chain reaction (PCR) amplification of vector pK18mobsacB, target genes (2998 and 3035), and tetracycline (Tc) resistance gene (a), overlapping extension PCR for the fusion of homologous arms (upstream and downstream fragments) with the Tc gene for genes 2998 (b) and 3035 (c), verification of recombinant transformants using the pK18mobsacB-specific primers PK-VF/VR with expected fragment sizes (ca. 3 800 bp for Δ3035, ca. 3 980 bp for Δ2998) (d), phenotypic validation of control and experimental strains after homologous recombination with no growth in control strains (e), positive and negative selections of Δ2998 transformants using Tc (f) and sucrose (g), respectively, and PCR confirmation of Δ3035 single-crossover transformants (h) and double-crossover transformants using OV3035F/OV3035R primers (i), OV3035F/TcIVR primers (j), and TcIVF/OV3035R primers (k). In all panels, lane M = DNA marker, and for clarity, only the major marker bands are indicated. In panel a, lane 1 = pK18mobsacB; lanes 2 and 3 = upstream and downstream fragments of gene 3035, respectively; lane 4 = Tc gene; lanes 5 and 6 = upstream and downstream fragments of gene 2998, respectively. In panel b, lane 1 = recombination of 2998-LF, Tc, and 2998-RF. In panel c, lane 1 = recombination of 3035-LF, Tc, and 3035-RF. In panel d, lanes 1–4 = transformants carrying pK18mobsacB-3035 (expected fragment size of ca. 3 800 bp); lanes 5–8 = transformants carrying pK18mobsacB-2998 (expected fragment size of ca. 3 980 bp). In panel e, dishes 1 and 2 = control group; dishes 3 and 5 = pK18mobsacB-Δ3035 homologous recombinant strains at 10- and 1 000-fold dilutions, respectively; dishes 4 and 6 = pK18mobsacB-Δ2998 homologous recombinant strains at 10- and 1 000-fold dilutions, respectively. In panel h, A1/2/3 = OV3035F/OV3035R single-crossover transformants; B1/2/3 = OV3035F/TcIVR single-crossover transformants; C1/2/3 = TcIVF/OV3035R single-crossover transformants; D1/2/3 = OV3035F/ZT-IVR single-crossover transformants; E1/2/3 = ZT-IVF/OV3035R single-crossover transformants.

gle- and double-crossover events detected in Δ2998 strain (Fig. 8f, g). Sequencing further confirmed that both *pbpC* and *mtgA* were successfully replaced by the Tc resistance gene (Fig. 8h–k). Thus, both *pbpC* and *mtgA* were successfully knocked out in H225, providing the basis for subsequent functional analyses under Cd stress.

Morphological and growth curve changes of H225 cells after gene knockout

The Δ2998 strain exhibited a phenotypic profile comparable to that of the wild-type strain, whereas the Δ3035 strain showed a marked reduction in Cd-induced cell wall

degradation (Fig. 9). Notably, $\Delta 2998$ cells displayed cell wall degradation even in the absence of Cd stress. However, most cells retained a structurally intact, elliptical morphology. Under 100 mg L^{-1} Cd stress, the $\Delta 2998$ strain continued to exhibit cell wall exfoliation, suggesting that *pbpC* knockout does not prevent structural damage induced by Cd stress. In contrast, the knockout of *mtgA* resulted in a substantial increase in cell wall regeneration under both Cd-free and Cd-stressed conditions. Growth curve analysis further revealed that *pbpC* knockout had no statistically significant effect on H225 cell proliferation (Fig. 10). However, $\Delta 3035$ cells exhibited enhanced growth across multiple Cd concentrations, with OD_{600} values increased by 0.021–0.056 units compared

to both wild-type and $\Delta 2998$ strains. These findings suggest that loss of *mtgA* may confer a protective advantage under Cd stress by promoting cell wall regeneration and enhancing overall growth.

DISCUSSION

Exfoliated cell walls block Cd entry to intracellular space

The bacterial cell wall serves as a critical structural barrier, enabling Gram-negative bacteria to maintain mechanical integrity and adapt to external stressors (Rojas *et al.*, 2018). Herein, we demonstrated that under Cd stress, cell wall fragments with adsorbed Cd were exfoliated from

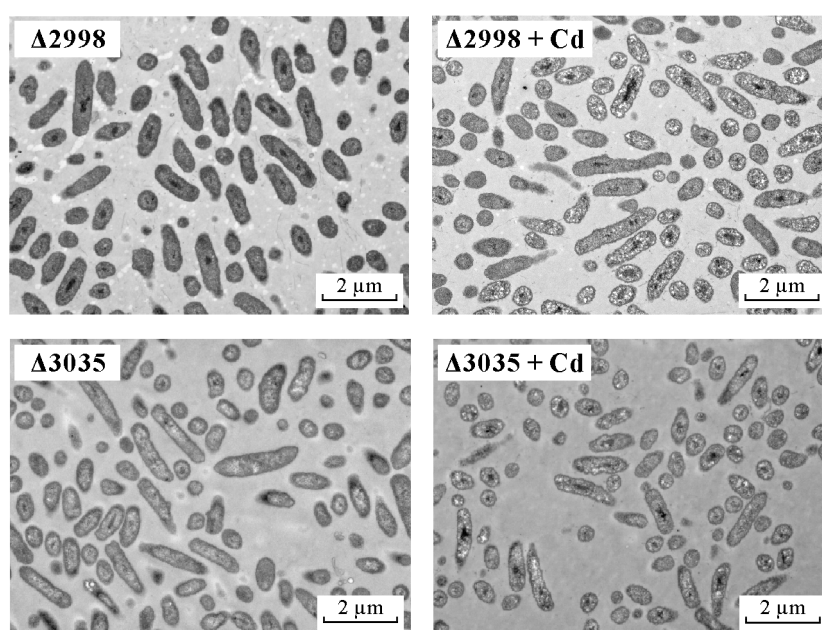


Fig. 9 Transmission electron microscopy images of *Stenotrophomonas* sp. H225 $\Delta 2998$ and $\Delta 3035$ mutants after 48 h of incubation (180 r min^{-1} , 28°C) under Cd-free and Cd-stressed (100 mg L^{-1} Cd, + Cd) conditions. Gene 2998 = *pbpC* (encoding penicillin-binding protein 3); gene 3035 = *mtgA* (encoding a monofunctional transglycosylase).

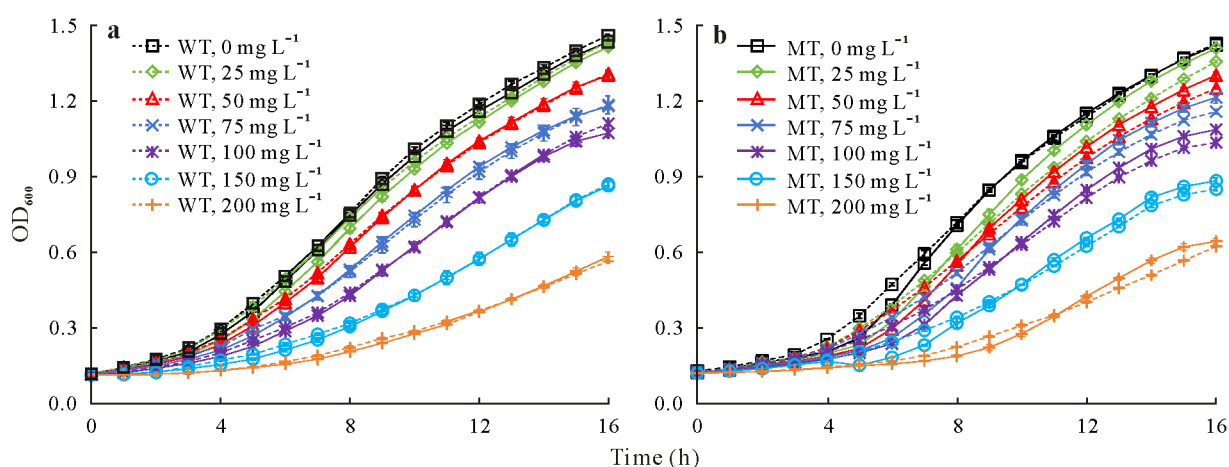


Fig. 10 Growth curves of *Stenotrophomonas* sp. H225 $\Delta 2998$ (a) and $\Delta 3025$ (b) mutants (MT) compared to that of wild-type (WT) strain cultured in Luria-Bertani medium containing $0\text{--}200 \text{ mg L}^{-1}$ Cd for 16 h. OD_{600} = optical density at 600 nm. Error bars are standard deviations of the means ($n = 6$). Gene 2998 = *pbpC* (encoding penicillin-binding protein 3); gene 3035 = *mtgA* (encoding a monofunctional transglycosylase).

bacterial cells, similar to the exfoliation observed in response to antibiotics and iron stress (Ramijan *et al.*, 2018; Chandramohan *et al.*, 2019). Comparable morphological changes were observed in *Stenotrophomonas maltophilia* SeITE02 exposed to selenium (Lampis *et al.*, 2017), further supporting the notion that cell wall remodeling is a general bacterial defense strategy against environmental stressors (Kawai *et al.*, 2018).

The EDS results further confirmed that both the inner and outer sides of the exfoliated cell wall were capable of binding and immobilizing Cd, implicating these structures in extracellular Cd detoxification. The presence of phosphate, hydroxyl, carboxyl, amide I, amide II, and amine groups on the cell wall likely facilitates Cd adsorption (Pabst *et al.*, 2010; Wang *et al.*, 2020). Additionally, FTIR analysis revealed a decreased intensity of the 1 654 cm⁻¹ band (C=O stretching of amide I), indicating that exfoliation intensified with increasing Cd stress (Safari *et al.*, 2013).

The role of bacterial surfaces in Cd sequestration is further supported by findings in *Pseudomonas putida* and *Brevundimonas diminuta*, which were shown to reduce Cd translocation from soil to plants (Xing *et al.*, 2023). Likewise, co-cultures of *Enterobacter* and *Comamonas* were shown to immobilize Cd in the rhizosphere, thereby lowering its bioavailability and subsequent plant uptake (Wang *et al.*, 2020). These findings highlight the importance of surface-mediated metal immobilization as an environmentally relevant detoxification strategy. Together, our TEM, EDS, and FTIR results suggest that bacterial cell wall not only serves as a primary binding site for Cd, but also facilitates its detoxification *via* stress-induced exfoliation, ultimately mitigating intracellular accumulation.

Upregulated PG synthesis genes facilitate cell wall regeneration

Transcriptome analysis revealed significant upregulation of PG biosynthesis genes under Cd stress, suggesting active remodeling of bacterial cell wall in response to metal exposure. As the primary structural component of the bacterial cell wall, PG is synthesized and integrated into pre-existing glycan strands by membrane-bound protein complexes (Meroueh *et al.*, 2006; Typas *et al.*, 2011). The KEGG enrichment analysis revealed a progressive increase in the enrichment of the PG biosynthesis pathway with rising Cd concentration. Further expression profiling confirmed that the key genes involved in PG synthesis in strain H225 exhibited a dose-dependent upregulation, highlighting cell wall remodeling as a critical adaptive mechanism for mitigating Cd toxicity.

The cell wall remodeling response observed in this study is consistent with the report in *Lysinibacillus* sp., where bacteria exfoliated and regenerated mineral S-layers as a survival strategy under iron stress (Chandramohan *et al.*,

2019). Similar cell wall-shedding behaviors under other environmental stresses have also been reported, including antibiotic exposure, where cells produce defective but viable wall structures (Leaver *et al.*, 2009; Claessen and Errington, 2019). These findings suggest that cell wall exfoliation and regeneration may serve as a widespread bacterial response not only to antibiotics but also to heavy metals. Notably, while similar cell wall remodeling responses have been documented for copper exposure (Vincent *et al.*, 2018), Cd stress appears to induce a particularly strong exfoliation effect, possibly due to its high affinity for bacterial surface functional groups and its potential to interfere with PG biosynthesis at multiple stages.

Collectively, these observations support the idea that H225 has evolved a complete mechanism for cell wall exfoliation and regeneration under Cd stress (Fig. S4, see Supplementary Material for Fig. S4). The exfoliated cell wall fragments effectively chelate Cd cations, providing H225 cells with essential time to repair and regenerate their cell walls. This regeneration process continuously generates new binding sites for Cd cations, demonstrating strong sustainability when H225 is employed in heavy metal bioremediation.

Deletion of *mtgA* enhances cell wall regeneration under Cd stress

The *mtgA* gene encodes a monofunctional transglycosylase, which is crucial for PG biosynthesis (Kadoya *et al.*, 2015; Song *et al.*, 2023). Our knockout experiments revealed that deletion of *mtgA* reduced cell wall degradation and significantly enhanced cell wall regeneration under Cd stress. As exfoliation and regeneration are interrelated processes in bacterial adaptation to Cd exposure, exfoliation helps remove Cd-bound fragments, but excessive exfoliation can compromise structural integrity and hinder growth. This suggests that *mtgA* deletion contributes to the maintenance of cell wall integrity by reducing the need for continuous exfoliation and subsequent regeneration.

Interestingly, the Δ *mtgA* strain demonstrated enhanced growth both in the absence and presence of Cd stress. Under Cd exposure, the Δ *mtgA* strain exhibited a significantly higher growth rate than both the wild-type and Δ *pbpC* strains. The assembly of PG is known to depend on the biosynthetic machinery of penicillin-binding proteins (Daniel and Errington, 2003; Chen *et al.*, 2021). Based on prior studies, inhibition of PG synthesis in the Δ *pbpC* mutant was expected to be alleviated by compensatory cross-linking mechanisms, thereby enhancing stress resistance (Song *et al.*, 2023). However, in our study, the Δ *pbpC* strain exhibited no significant difference in growth compared to the wild-type strain. This may be due to the functional redundancy of genes involved in PG synthesis, where other genes with similar functions compensate for the loss of *pbpC* during

the biosynthetic process. While our knockout experiments demonstrate the importance of *mtgA* in Cd resistance, further validation through functional assays or complementation experiments would clarify its mechanistic role.

The absence of *mtgA* may reduce excessive cell wall degradation, helping maintain mechanical stability and enhancing bacterial resistance to external stress. Additionally, an intact cell wall may limit Cd entry into the cytoplasm, consequently reducing Cd toxicity. This finding aligns with previous studies demonstrating that bacteria modulate cell wall synthesis and degradation to maintain structural integrity under environmental stress (Typas *et al.*, 2011). Moreover, cell wall regeneration and repair are crucial for bacterial adaptation to antibiotic stress (Claessen and Errington, 2019). Our study extends these mechanisms by revealing that cell wall remodeling is equally essential for bacterial resistance to heavy metal stress.

Overall, the discovery that *mtgA* deletion enhanced cell wall regeneration and Cd resistance in H225 provides a promising genetic target for engineering robust bacterial strains with improved bioremediation capacity. By minimizing cell wall exfoliation while maintaining structural integrity under Cd exposure, Δ *mtgA* mutants could sustain high survival and metal immobilization efficiency in contaminated environments. This targeted genetic enhancement offers practical potential for improving the long-term effectiveness and resilience of microbial consortia used in heavy metal bioremediation, especially in environments with persistently high Cd levels.

CONCLUSIONS

This study uncovers a novel bacterial defense mechanism against Cd stress through cell wall exfoliation and regeneration. Under high Cd conditions, exfoliated PG binds extracellular Cd, thereby limiting intracellular accumulation. Transcriptome and knockout analyses further reveal that deletion of *mtgA* enhances Cd resistance by reducing excessive exfoliation and promoting structural recovery. These findings advance our understanding of bacterial stress adaptation and highlight *mtgA* as a promising genetic target for engineering metal-tolerant strains with enhanced bioremediation potential.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

REFERENCES

- Bo L, Colin N D. 2011. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics*. **12**: 323.
- Cao X F, Xu L, Chen Y P, Decho A W, Cui Z J, Lead J R. 2022. Contribution, composition, and structure of EPS by *in vivo* exposure to elucidate the mechanisms of nanoparticle-enhanced bioremediation to metals. *Environ Sci Technol*. **56**: 896–906.
- Chakravarty R, Banerjee P C. 2012. Mechanism of cadmium binding on the cell wall of an acidophilic bacterium. *Bioresour Technol*. **108**: 176–183.
- Chandramohan A, Duprat E, Remusat L, Zirah S, Lombard C, Kish A. 2019. Novel mechanism for surface layer shedding and regenerating in bacteria exposed to metal-contaminated conditions. *Front Microbiol*. **9**: 3210.
- Chen X, Bai K H, Lyu Q Y, Jiang N, Li J Q, Luo L X. 2021. Role of penicillin-binding proteins in the viability, morphology, stress tolerance, and pathogenicity of *Clavibacter michiganensis*. *Phytopathology*[®]. **111**: 1301–1312.
- Chen X, Li Y, Bai K H, Gu M, Xu X L, Jiang N, Chen Y, Li J Q, Luo L X. 2022. Class A penicillin-binding protein C is responsible for stress response by regulation of peptidoglycan assembly in *Clavibacter michiganensis*. *Microbiol Spectr*. **10**: e0181622.
- Chen Y X, Chen Y S, Shi C M, Huang Z B, Zhang Y, Li S K, Li Y, Ye J, Yu C, Li Z, Zhang X Q, Wang J, Yang H M, Fang L, Chen Q. 2018. SOAPnuke: A MapReduce acceleration-supported software for integrated quality control and preprocessing of high-throughput sequencing data. *Gigascience*. **7**: gix120.
- Cheremisinoff N P. 2016. Agency for toxic substances and disease registry (ATSDR). In Cheremisinoff N P (ed.) *Pollution Control Handbook for Oil and Gas Engineering*. John Wiley & Sons, Inc., Hoboken. pp. 83–93.
- Chomczynski P. 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *Biotechniques*. **15**: 532–534, 536–537.
- Claessen D, Errington J. 2019. Cell wall deficiency as a coping strategy for stress. *Trends Microbiol*. **27**: 1025–1033.
- Daniel R A, Errington J. 2003. Control of cell morphogenesis in bacteria: Two distinct ways to make a rod-shaped cell. *Cell*. **113**: 767–776.
- Di Berardino M, Dijkstra A, Stüber D, Keck W, Gubler M. 1996. The monofunctional glycosyltransferase of *Escherichia coli* is a member of a new class of peptidoglycan-synthesising enzymes. *FEBS Lett*. **392**: 184–188.
- Essien J P, Ikpe D I, Inam E J, Okon A O, Obong G A, Benson N U. 2024. Correction: Occurrence and spatial distribution of heavy metals in landfill leachates and impacted freshwater ecosystem: An environmental and human health threat. *PLOS ONE*. **19**: e0304882.
- Guarín D, Martín-López J M, Libohova Z, Benavides-Bolaños J, Maximova S N, Guiltinan M J, Spargo J, da Silva M, Fernandez A, Drohan P. 2024. Accumulation of cadmium in soils, litter and leaves in cacao farms in the North Sierra Nevada de Santa Marta, Colombia. *Geoderma Reg*. **36**: e00762.

- Harighi B. 2009. Genetic evidence for CheB- and CheR-dependent chemotaxis system in *A. tumefaciens* toward acetosyringone. *Microbiol Res.* **164**: 634–641.
- Joseph P. 2009. Mechanisms of cadmium carcinogenesis. *Toxicol Appl Pharmacol.* **238**: 272–279.
- Kadoya R, Matsumoto K, Ooi T, Taguchi S. 2015. *mtgA* deletion-triggered cell enlargement of *Escherichia coli* for enhanced intracellular polyester accumulation. *PLOS ONE.* **10**: e0125163.
- Kamnev A A, Antonyuk L P, Matora L Y, Serebrennikova O B, Sumaroka M V, Colina M, Renou-Gonnord M F, Ignatov V V. 1999. Spectroscopic characterization of cell membranes and their constituents of the plant-associated soil bacterium *Azospirillum brasilense*. *J Mol Struct.* **481–481**: 387–393.
- Kawai Y, Mickiewicz K, Errington J. 2018. Lysozyme counteracts β -lactam antibiotics by promoting the emergence of L-form bacteria. *Cell.* **172**: 1038–1049.
- Kenney J P L, Fein J B. 2011. Cell wall reactivity of acidophilic and alkaliphilic bacteria determined by potentiometric titrations and Cd adsorption experiments. *Environ Sci Technol.* **45**: 4446–4452.
- Kim D, Langmead B, Salzberg S L. 2015. HISAT: A fast spliced aligner with low memory requirements. *Nat Methods.* **12**: 357–360.
- Kumar A, Subrahmanyam G, Mondal R, Cabral-Pinto M M S, Shabnam A A, Jigyasu D K, Malyan S K, Fagodiya R K, Khan S A, Kumar A, Yu Z G. 2021. Bio-remediation approaches for alleviation of cadmium contamination in natural resources. *Chemosphere.* **268**: 128855.
- Lampis S, Zonaro E, Bertolini C, Cecconi D, Monti F, Micaroni M, Turner R J, Butler C S, Vallini G. 2017. Selenite biotransformation and detoxification by *Stenotrophomonas maltophilia* SeITE02: Novel clues on the route to bacterial biogenesis of selenium nanoparticles. *J Hazard Mater.* **324**: 3–14.
- Leaver M, Domínguez-Cuevas P, Coxhead J M, Daniel R A, Errington J. 2009. Erratum: Life without a wall or division machine in *Bacillus subtilis*. *Nature.* **460**: 538–538.
- Meroueh S O, Bencze K Z, Hesse D, Lee M, Fisher J F, Stemmler T L, Mobashery S. 2006. Three-dimensional structure of the bacterial cell wall peptidoglycan. *Proc Natl Acad Sci USA.* **103**: 4404–4409.
- Niampradit S, Kiangkoo N, Mingkhwan R, Kliengchuay W, Worakunpiset S, Limpananont Y, Hongsibsong S, Inthorn D, Tantrakarnapa K. 2024. Occurrence, distribution, and ecological risk assessment of heavy metals in Chao Phraya River, Thailand. *Sci Rep.* **14**: 8366.
- Nriagu J O, Pacyna J M. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature.* **333**: 134–139.
- Pabst M W, Miller C D, Dimkpa C O, Anderson A J, Mclean J E. 2010. Defining the surface adsorption and internalization of copper and cadmium in a soil bacterium, *Pseudomonas putida*. *Chemosphere.* **81**: 904–910.
- Phaenark C, Phankamolsil Y, Sawangproh W. 2024. Ecological and health implications of heavy metal bioaccumulation in Thai Fauna: A systematic review. *Ecotox Environ Saf.* **285**: 117086.
- Ramijan K, Ultee E, Willemse J, Zhang Z R, Wondergem J A J, van der Meij A, Heinrich D, Briegel A, van Wezel G P, Claessen D. 2018. Stress-induced formation of cell wall-deficient cells in filamentous actinomycetes. *Nat Commun.* **9**: 5164.
- Rasin P, Ashwathi A V, Basheer S M, Haribabu J, Santibanez J F, Garrote C A, Arulraj A, Mangalaraja R V. 2025. Exposure to cadmium and its impacts on human health: A short review. *J Hazard Mater Adv.* **17**: 100608.
- Rojas E R, Billings G, Odermatt P D, Auer G K, Zhu L, Miguel A, Chang F, Weibel D B, Theriot J A, Huang K C. 2018. The outer membrane is an essential load-bearing element in Gram-negative bacteria. *Nature.* **559**: 617–621.
- Safari M, Sorooshzadeh A, Asgharzadeh A, Saadat S. 2013. The application of adsorption modeling and Fourier transform infrared spectroscopy to the comparison of two species of plant growth-promoting rhizobacteria as biosorbents of cadmium in different pH solutions. *Biorem J.* **17**: 201–211.
- Sarkar S K, Chowdhury C, Ghosh A S. 2010. Deletion of penicillin-binding protein 5 (PBP5) sensitises *Escherichia coli* cells to β -lactam agents. *Int J Antimicrob Ag.* **35**: 244–249.
- Satarug S, Moore M R. 2004. Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. *Environ Health Perspect.* **112**: 1099–1103.
- Song Y L, Sun M Y, Mu G Q, Tuo Y F. 2023. Exopolysaccharide produced by *Lactiplantibacillus plantarum* Y12 exhibits inhibitory effect on the *Shigella flexneri* genes expression related to biofilm formation. *Int J Biol Macromol.* **253**: 127048.
- Typas A, Banzhaf M, Gross C A, Vollmer W. 2011. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat Rev Microbiol.* **10**: 123–136.
- Vijayaraghavan K, Yun Y S. 2008. Bacterial biosorbents and biosorption. *Biotechnol Adv.* **26**: 266–291.
- Vincent M, Duval R E, Hartemann P, Engels-Deutsch M. 2018. Contact killing and antimicrobial properties of copper. *J Appl Microbiol.* **124**: 1032–1046.
- Vollmer W, von Reichenberg M, Hölte J V. 1999. Demonstration of molecular interactions between the murein polymerase PBP1B, the lytic transglycosylase MltA, and the scaffolding protein MipA of *Escherichia coli*. *J Biol Chem.* **274**: 6726–6734.
- Wang X, Hu K, Xu Q, Lu L F, Liao S J, Wang G J. 2020. Immobilization of Cd using mixed *Enterobacter* and *Comamonas* bacterial reagents in pot experiments with *Brassica rapa* L. *Environ Sci Technol.* **54**: 15731–15741.
- Woodcock D M, Crowther P J, Doherty J, Jefferson S, DeCruz E, Noyer-Weidner M, Smith S S, Michael M Z, Graham M W. 1989. Quantitative evaluation of *Escherichia coli* host strains for tolerance to cytosine methylation in plasmid and phage recombinants. *Nucleic Acids Res.* **17**: 3469–3478.
- Xing Y H, Liu S, Tan S X, Jiang Y, Luo X S, Hao X L, Huang Q Y, Chen W L. 2023. Core species derived from multispecies interactions facilitate the immobilization of cadmium. *Environ Sci Technol.* **57**: 4905–4914.