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Cadmium Resistance, Microbial Biosorptive Performance and Mechanisms of a Novel Biocontrol Bacterium Paenibacillus sp. LYX-1

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Title Page

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Cadmium resistance, microbial biosorptive performance and mechanisms of a novel biocontrol bacterium *Paenibacillus* sp. LYX-1

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Abstract: In order to explore whether the newly discovered biocontrol strain *Paenibacillus* sp., LYX-1 having antagonistic effect on peach brown rot was resistant to Cd^{2+}, a series of growth of strain LYX-1 under different Cd concentration and biosorption experiments were conducted to living and dead strain LYX-1. Meanwhile, the Cd^{2+} resistance and biosorption mechanisms were further identified by Cd-resistant genes, TEM, SEM-EDS, FTIR and XPS analysis. The results showed that strain LYX-1 could resist 50 mg/L Cd^{2+} and the adsorption process of both living and dead strain LYX-1 all satisfied the pseudo-second kinetic equation. Under pH 8.0 and at a dose of 1.0 g/L strain, the removal capacities of living and dead cells were as high as 90.39% and 75.67% at 20 mg/L Cd^{2+}, respectively. For the adsorption isotherm test, results revealed that both Langmuir (R^2=0.9704) and Freundlich (R^2=0.9915) model could describe the Cd^{2+} biosorption well for living strain LYX-1. The maximum equilibrium biosorption capacities of living and dead biomass were 30.6790 and 24.3752 mg/g, respectively. The adsorption mechanism was controlled by chemisorption with -OH, -NH, -C=O, O=C-O, C-N, S^{2-} and phosphate functional groups on the cell surface of strain LYX-1, which were further identified by XPS. The insignificant biosorption difference of living and dead biomass was caused by CzcD gene in strain LYX-1 detoxifying

cadmium through the heavy metal efflux system. The above results indicated that strain LYX-1 had higher tolerance and fixed capacity to Cd^{2+}.

Keywords: *Paenibacillus* sp.; Cd resistance; Biosorption; Mechanism

1. Introduction

Heavy metals (HMs) are the main pollutants in the development of mineral resources, textile, fertilizer manufacturing, metal smelting and processing(Jin et al. 2019). Cadmium (Cd) designated as one of the priority pollutants controlled by many countries can participate in food chain cycle, accumulate in the organism and eventually cause irreversible tubular damage in kidney after it enters the environment(Jin et al. 2020). Therefore, effective treatment of heavy metal Cd pollution in soil and water has become an important issue that needs to be solved urgently in the field of environmental protection today.

Compared with conventional chemical precipitation, electrolysis, reverse osmosis, ion exchange, etc., microbial adsorption had significant advantages, mainly including high efficiency, abundant raw materials, coupled with high chemical activity(Li et al. 2018, Shi et al. 2021, Sun et al. 2020). Additionally, in domestic and international literatures, more and more strains employed as environmentally friendly biomasses have been applied in the removal of metal ions, such as *Comamonas* sp. XL8(Shi et al. 2021), *Enterbacter* sp. DNB-S2(Sun et al. 2020), *Serratia liquefaciens* CL-1 and *Bacillus thuringiensis* X3(Han et al. 2018). Besides, numerous evidences have proved that biological cell components (e.g., cell wall polysaccharides, proteins, carboxyl in lipid molecules, hydroxyl, carbonyl, phosphoryl, sulfhydryl) realized the accumulation and purification of heavy metals through chelation, ion exchange, biosorption, inorganic micro-precipitation, redox and diffusion(Huang et al. 2014, Özdemir et al. 2009, Tan et al. 2020, Zhou et al. 2018).

At present, the environment polluted by HMs is widespread, and there are few reports on the adaptability of the currently used biocontrol bacterium as microbial materials for heavy metal pollution control under the stress of HMs. The search and discovery of biocontrol bacterium that have high tolerance and immobilization to HMs

is great significance to the restoration and utilization of heavy metal contaminated soils.

Paenibacillus sp. is a kind of significant disease biological control microorganism, which due to their ability to produce-variety of enzymes of degrading the cell wall, e.g. chitinases, cellulases, proteases and β-1,3-glucanases(Hao et al. 2017). In the previous study, *Paenibacillus* sp. showed diverse antagonistic activities against five phytopathogenic fungi (*Fusarium graminearum*, *Magnaporthe oryzae*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Botrytis cinerea*)(Ali et al. 2020). Researches over the past decades showed that *Paenibacillus* sp. could produce a variety of antibiotics, polymyxin, hydrolases and other antagonistic substances(Ali et al. 2020, Araujo et al. 2020), which was one of the important mechanisms for the biocontrol of *Paenibacillus* sp.(Hao et al. 2017). *Paenibacillus* sp. can not only offer protection against bacteria, fungi, nematodes and viruses, but also has advantages of non-pathogenicity, non-toxicity, good environmental compatibility, less residue, safety for humans and animals. Nevertheless, the survival of biocontrol bacterium employed for prevention and control of plant diseases are prone to be threatened by toxic Cd, pests and diseases in composite pollutant sites(Olaniran et al. 2013). Therefore, finding a *Paenibacillus* sp. with high tolerance and fixation capacity for Cd is of great significance for the simultaneous control of soil Cd pollution and plant rhizosphere disease. However, relevant studies are rarely reported.

In present work, a novel biocontrol strain *Paenibacillus* sp. LYX-1 having antagonistic to peach brown rot and higher Cd resistance was isolated from soil in peach orchard. The Cd resistance, microbial biosorptive performance and mechanisms of *Paenibacillus* sp. LYX-1 were studied, including (1) investigate the Cd resistance of biocontrol *Paenibacillus* sp. LYX-1, (2) compare the biosorption process of living and dead biosorbents, closer to the real-world scenarios where nutrients are deficient, (3) describe the biosorption performance of living and dead cells via kinetic and isotherm models and (4) explore the Cd^{2+} resistance and removal mechanisms through HMs gene coding, TEM, SEM-EDS, FTIR and XPS analysis. The objective of such work is to provide a theoretical basis focusing a *Paenibacillus* sp. LYX-1 with higher Cd resistance to realize the simultaneous control of soil Cd pollution and plant

rhizosphere disease in Cd stress soil in future.

2. Materials and methods

2.1 Isolation and identification of biocontrol strain

Biocontrol strain was isolated as following steps. Ten g of rhizosphere soil in peach orchard in Fenghua city, Zhejiang province, China was initially suspended in 90 mL of sterile water and incubated at 180rpm, 30℃ for 1h. The gradient dilution method was used to dilute the soil into soil suspensions with sterile water. 0.1mL 10^{-4}, 10^{-5} and 10^{-6} gradient dilutions were applied to Luria-Bertani's (LB) agar plate consisted of 10 g/L tryptone, 5.0 g/L yeast extract, 10.0 g/L NaCl and 20.0 g/L agar. There replicates for each gradient were incubated at 30℃. (1) Primer screening: 6mm diameter peach brown rot fungus taken by a hole puncher was placed in the center of the PDA plate. And then, the strains to be screened were placed on 4 corners 3cm away from the center of the plate and incubated at 28℃ for 48h. 24 strains that shrunk the hyphae of the pathogenic bacteria were selected and saved as the re-screening objects. (2) Fine screening: Using the plate confrontation method, the pathogenic bacteria was inoculated in the center of PDA plate, and the re-screening strains were spotted in 3 positions 2.5cm away the pathogenic bacteria cake with a sterile inoculation loop to inoculate only the peach brown rot fungus. Three replicates plates was incubated at 28℃ for 7days to observe the antibacterial effect. And representative single colony that had highest antagonistic was selected and kept at 4℃ to be tested.

The 16S rDNA extracted from the strain with highest antagonistic effect on peach brown rot, as a PCR template, was amplified and sequenced using the universal primers 27F (5′-AGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) according to previous study(Galkiewicz and Kellogg 2008). Nucleotide sequence was compared in GenBank database (Nucleotide Blast) using the BLAST search tool to accurately identify the bacterium(Tan et al. 2020). Meanwhile the Neighbor-joining method was selected to construct sequencing results of biocontrol strain into a Phylogenetic tree through MEGA 7.0 software.

2.2 Cell growth curves under Cd stress

The pure cultured strain LYX-1 at logarithmic growth phase (OD_{600}=1.5±0.2) were seeded in LB medium containing different Cd^{2+} concentrations(0, 5, 10, 20, 50, 100 mg/L) at 2% inoculation amount, meanwhile, conical flasks were incubated at 30℃ with 150 rpm on a shaker. The OD_{600} values were monitored at different time intervals using ultraviolet spectrophotometer (Shimadzu UV-2450, Japan) to construct growth curves. The Cd ion stock solution used in the above experiment was prepared in 1000 mL volumetric flask by dissolving 2.74g $Cd(NO_3)_2 \cdot 4H_2O$ (analytical grade) purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, PRC) in deionized water (Millipore, 18.2Ω).

2.3 Sorbents preparation

The pure cultured strain LYX-1 was seeded in 600 mL sterile LB medium in 1000 mL flasks at 1% inoculation amount and cultured for 48 h at 30℃, 150 rpm on a constant temperature shaker. Living cell adsorbent was prepared by centrifuging the bacterium suspension at 4℃, 8000 rpm for 10 min, while dead cell adsorbent was harvested by centrifuging the bacterial suspension that has been sterilized at 121℃ for 30 min. Both living and dead pellet were washed 3 times and re-suspended with sterile water, then biomass was stored in a 4℃ refrigerator until use. The concentration (grams per liter) of above cell suspension was tested by drying an aliquot to constant weight at 80℃(Huang et al. 2013, Xu et al. 2020).

2.4 Biosorption Cd^{2+} experiments by strain LYX-1

2.4.1 *pH effects*

The initial solution pH values for sorption experiment were adjusted using 1 M NaOH and 1 M HNO_3. Sorption experiments were conducted in 50 mL centrifuge tubes containing 20 mL Cd^{2+} aqueous solution, 1.0 g/L living and dead sorbents, which were agitated at a speed of 180 rpm for 6 h. The metal solutions used for the sorption experiments were diluted from 1000ppm (metal concentration) $Cd(NO_3)_2$ stock solution. All experiments were performed at ambient conditions and in triplicate. The sorbents was removed by centrifugation at 10000rpm for 10min and the supernatant filtrated through 0.22 μm filter (PVDF, Sterile) was used to measured remaining Cd^{2+} via ICP-MS (Perkin Elmer 600X, USA). The pH was measured by pH meter (PHS-25,

INESA, China)(Xu et al. 2020).

2.4.2 ***Biosorbent dosage effects***

To examine the effects of the biosorbent dosage, the biosorption experiments were carried out at different dosage(0.2-3.0g/L). The experiments were performed at optimal pH 8, 30℃, 180rpm and 50mg/L Cd^{2+} for 6h. All experiments were performed at ambient conditions and in triplicate.

2.4.3 ***Contact time effects and sorption kinetic characteristics***

The optimal pH(8.0) and biomass(1.0g/L) was mixed with different contact time (0-300min) at 50 mg/L Cd^{2+}. All experiments were performed at 30℃, 180rpm for 6h and in triplicate. To determine the rate-limiting steps by of Cd^{2+} biosorption by the strain LYX-1, Pseudo-first and pseudo-second order rate equation were used to fit the kinetic experimental data to study the biosorption mechanism.

Pseudo-first order kinetic model has long been widely applied(Ho 2006), The form is:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (1)$$

Where q_e and q_t are the amounts of the Cd^{2+} sorbed at equilibrium and at any time t (mg/g), k_1 is the pseudo-first order rate constant of sorption (1/min).

The pseudo-second order kinetic equation(Ho and McKay 1999) is shown below:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \quad (2)$$

Where k_2 is the pseudo-second order rate constant, g/ (mg min), respectively, q_e and q_t (mg/g) were defined elsewhere.

2.4.4 ***Initial Cd^{2+} concentrations and sorption isotherm experiments***

The optimal pH(8.0) and biomass(1.0g/L) was mixed with different concentrations of Cd^{2+} (0-50mg/L). All experiments were performed at 30℃ and 180rpm in triplicate. Langmuir and Freundlich isotherm equations were frequently used to analyze equilibrium data in related research (Masoudzadeh et al. 2011, Ozturk 2007, Zhou et al. 2018). The Cd biosorptive performance of the strain LYX-1 was simulated by using Langmuir and Freundlich isotherm equations, as follows(Freundlich 1906, Langmuir 1918)

Langmuir equation: $q_e = \frac{q_{max} k_L C_e}{1+K_L C_e}$ (3)

Freundlich equation: $q_e = K_f C_e^{\frac{1}{n}}$ (4)

Where q_e is the amount of Cd sorbed at equilibrium (mg/g), C_e is the Cd equilibrium concentration (mg/L), q_{max} is saturated sorption capacity of aqueous solution for Cd^{2+} and K_L is a Langmuir constant related to the sorption strength. Here K_F is Freundlich coefficient indicating sorption capacity, n, a constant related to Cd ion concentration, characterizes adsorption intensity, respectively, q_e and C_e were described earlier.

Another dimensionless constant separation factor R_L of Langmuir isotherm model can be expressed as following：

$R_L = \frac{1}{1+K_L C_0}$ (5)

Where C_0 is the highest Cd^{2+} concentration (mg/L), K_L is a Langmuir constant, R_L reflects the nature of biosorption process (R_L>1: unfavorable; R_L=1: linear; 0<R_L<1: favorable; R_L=0: irreversible).

The Dubinin-Radushkevich (D-R) was selected to study the nature of the sorption phenomena either physical or chemical sorption, govern the characteristic of not assuming a homogeneous surface or constant adsorption potential(Dubey and Gupta 2005, Zhou et al. 2018). It can be expressed as:

$\ln q_e = \ln q_{max} - \beta\varepsilon^2$ (6)

Where q_e (mg/g) and q_{max} (mg/g) are described above, β (mol^2/kJ^2) is the parameter with respect to the mean free energy of sorption per molecule of the adsorbate, ε (kJ/mol) is the adsorption potential, and the mean free energy E (kJ/mol) can be computed using following formula(Kaur et al. 2015):

$E = \frac{1}{\sqrt{2\beta}}$ (7)

For the adsorption from aqueous solution, the adsorption potential can be defined as (Hu and Zhang 2019):

$\varepsilon = RT \ln (1+1/C_e)$ (8)

2.5 PCR amplification of Cd-resistant genes

According to the current research on the mechanism of strain resistance to Cd, its resistance was mainly controlled by universal cadA and Czc systems(Ayangbenro et al. 2019, NIES et al. 1898, Wei et al. 2009). We could screen for the resistance genes to further explore the mechanism of reducing or eliminating heavy metal toxicity by biocontrol *Paenibacillus* sp., LYX-1 through PCR amplification of Cd^{2+}-related genomes (cadA, CzcA, CzcB and CzcD). The genomic DNA of strain LYX-1. used as a template, amplified as follows: pre-denaturation at 98℃ for 3 min; 35 cycles of denaturation at 98℃ for 10s, annealing at X℃ for 10s, extension at 72℃ for 15 s; followed by a final extension step at 72℃ for 3 min. The primer sequences selected with reference to the previous studies of the above-mentioned enzyme genes were listed in **Table S1.** All primers used for PCR were synthesized by Tsingke biotechnology Co., Ltd. (Beijing, China).

2.6 Characterization to biosorption mechanisms of Cd^{2+} by strain LYX-1

Samples of living and dead cells before and after 50 mg/LCd^{2+} biosorption were collected and freeze-dried for the following tests. Cells were immobilized in glutaraldehyde at 4℃ overnight and then washed using phosphate buffer (pH 7.2) for 3 times. The washed biomass was finally dehydrated in alcohol, and followed by immobilization and being cut into 70-90 nm shin sheets for transmission electron microscopy (H-7650, Japan), scanning electron microscope coupled with energy dispersive spectroscopy (Nova Nano 450, USA) analysis. Freeze-dried biomass were prepared for Fourier Transform Infrared Spectrometry (Thermo Scientific Nicolet iN10, USA) and X-ray photo-electron spectroscopy (Thermo Scientific K-Alpha, USA) to study the fundamental properties and underlying encapsulation mechanisms of adsorbents before and after biosorption according to previous methods(Huang et al. 2020).

2.7 Statistical analysis

All experiments were performed in triplicates. Statistical analysis was carried out using SPSS 20.0 software. All drawing and model fittings were generated using Origin 2018 software. The phylogenetic tree was constructed by MEGA 7.0.

3. Results and discussion

3.1 The isolation and identification of biocontrol strain Paenibacillus sp. *LYX-1*

Biocontrol strain *Paenibacillus* sp. LYX-1 with antagonistic to peach brown rot was isolated from rhizosphere soil in peach orchard in Fenghua city, Zhejiang province, China. The significant antagonism to peach brown rot could be seen in plate antagonism test from **Fig. S1**. The 16S rDNA sequences of LYX-1 aligned with the published sequences in NCBI database using BLAST (https://www.ncbi.nlm.nih.gov) had the highest similarity (99.62%) with *Paenibacillus* sp.. The phylogenetic tree constructed also indicated that the strain had 99% threshold with *Paenibacillus* sp. (**Fig. 1a**). Thus, it was named *Paenibacillus* sp.. The strain LYX-1 and its GenBank accession number was MZ234160. The strain LYX-1 has also been deposited in China General Microbiological Culture Collection Center (CGMCC accession No. 22158) as a patent strain. The strain on LB medium was round, light yellow with smooth edges(**Fig. 1b**). **Fig. 1c** proved that strain LYX-1 was Gram-positive. Biocontrol strain *Paenibacillus* sp. LYX-1 was also identified with higher Cd^{2+} resistance(**Fig. 1d**).

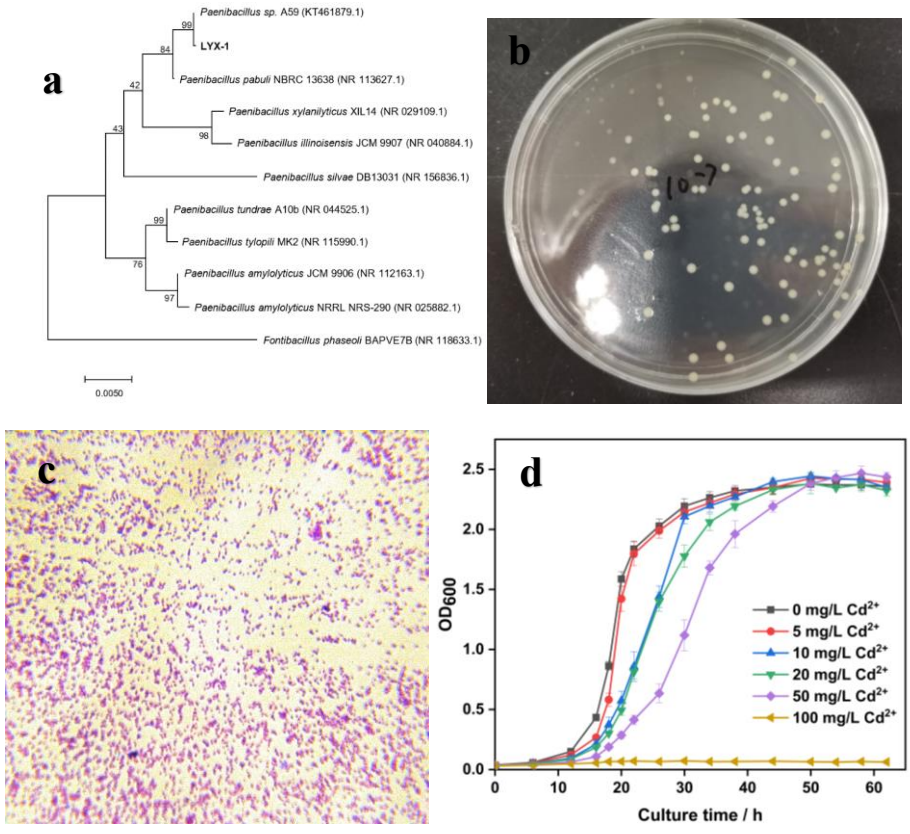


Fig. 1. (a) the neighbor-joining tree based on 16S rDNA gene sequences, showing the phylogenetic relationships

between strain LYX-1 with other strains, (b) the colony morphology, (c) gram staining characteristics and (d) growth curves in presence of different initial Cd^{2+} concentrations of the *Paenibacillus* sp. strain LYX-1.

3.2 Effects of Cd^{2+} concentrations on growth curves of Paenibacillus sp. *LYX-1*

Microbial growth was influenced directly by the microbial activity under different concentrations of heavy metal(Huang et al. 2014, Sun et al. 2020, Tan et al. 2020). As shown in **Fig. 1d**, the growth of *Paenibacillus* sp. LYX-1 was barely affected and the maximum OD_{600} value even exceeded 2.4 under 20 mg/L Cd^{2+}. As the concentration increased, the logarithmic growth stage had obvious delays, which might due to stressed metabolic pathway and internal biochemical reactions(Sun et al. 2020). Increasing the Cd^{2+} concentrations did not significantly decreased the strain LYX-1 activity (**Fig. 2**), suggesting that the stain LYX-1 had a good environmental adaptability that could continue to grow in the presence of 50 mg/L Cd^{2+}. And, the stain could not grow when Cd^{2+} reached 100 mg/L. In sum, the strain LYX-1 had high Cd resistance (50mg/L).

3.3 Impact factors optimization to Cd^{2+} adsorption by Paenibacillus sp. *LYX-1*

The Cd^{2+} biosorption performance of living and dead biomass was complex, and the removal efficiency of Cd^{2+} was closely related to pH, initial Cd^{2+} concentrations, doses and contact time(Yu and Fein 2017, Zhou et al. 2014). To investigate the sorption conditions of living and dead sorbents, effects of these four independent variables were illustrated.

3.3.1 *Effects of different pH*

Earlier studies have shown that pH was an important factor affecting biosorption process, because it could directly influence the solubility of metal ions, ionization state of functional groups on the microorganism surface(Altowayti et al. 2019, Şahin and Öztürk 2005). As the initial pH increased, Cd^{2+} removal showed a rising tendency (**Fig. 2a**). Due to the competitive adsorption between hydrogen ions and adsorbed Cd^{2+} ions(Zeng et al. 2021), the equilibrium adsorption capacity of heavy metal was just 7.95 mg/g and 6.48 mg/g for living and dead cells at the pH 3.0. When the pH reached 4.0, more charged functional groups on the cell surface, such as carboxyl, amino, and hydroxyl began to expose, which strengthened the binding of Cd^{2+} to the adsorption site, thereby increasing the amount of adsorption((Li et al. 2018, Özdemir et al. 2009).

Meanwhile, as the solution pH increased, more Cd^{2+} would be fixed on to the negatively-charged cell surface due to the electrostatic adsorption. Notably, the presence of a highest point between pH 3 and 8, the optimum pH value for sorption was determined at 8.0 for both living cells and dead cells, which suggested that the LYX-1 had an excellent pH buffering performance. It was calculated that when pH=8.0, heavy metal ions not reached the precipitation equilibrium constant, so hydroxide precipitation not generated in the solution.

3.3.2 *Effects of initial Cd^{2+} concentrations*

Fig. 2b showed the adsorption capacity of *Paenibacillus* sp. at different Cd^{2+} concentrations ranging from 0 to 50 mg/L. In general, with the increase of the initial concentrations of Cd ions, the biosorption capacity gradually increased for both living and dead cells, which implied that an increase in the initial Cd^{2+} concentration generated a diving force and reduced the mass transfer resistance between Cd ions and sorbents(Masoudzadeh et al. 2011). Owing to the limited biosorption sites of sorbents(Xu et al. 2020), the sorption capacity reached saturation in higher concentration treatment (more than 30 mg/L). In addition to the adsorption of living cells, there was also intracellular accumulation(Li et al. 2010). Herein, the adsorption capacity of living cells(18.019 mg/g) was slightly higher than dead cells (14.847 mg/g) when Cd^{2+} concentration was 20 mg/L. Note that, the removal capacities of living and dead cells were as high as 90.39% and 75.67% under the 20 mg/L Cd^{2+}, respectively. Thus, the *Paenibacillus* sp., a promising and highly efficient biocontrol biosorbent, could be applied for heavy metal sewage treatment.

3.3.3 *Effects of adsorbent dose*

The variation of equilibrium uptake for Cd ions at different biosorbent dose for each living biomass and dead biomass were shown in **Fig. 2c**, which indicated that Cd^{2+} was adsorbed by the living biomass more strongly than dead biomass and two adsorbents had similar patterns for Cd removal. The adsorption capacity increased gradually as the biomass dose increased from 0.2 to 1.0 g/L, which could be explained that more binding sites were available and thus the biosorption capacity went up with the increase of dose(Sun et al. 2011). However, the biomass of strain LYX-1 presented

a decline trend with an increased biosorbent dose from 1.0g/L to 3.0 g/L. It has been reported that biosorbent generated aggregation under high biosorbent concentrations, causing the decrease in effective contact area between cells and metal ions(Zhu et al. 2016). Therefore, the optimum living and dead cells dosage of all following experiments was selected as 1.0 g/L. Therefore, it was obvious that redundant adsorbents were not necessary for an efficient biosorption performance under specific initial concentration.

3.3.4 *Effects of contact time*

As reported by Sun(Sun et al. 2011), contact time had a significant effect on the equilibrium biosorption. Cd^{2+} biosorption by *Paenibacillus* sp. LYX-1 under different contact time (0-300 min) at 50 mg/L initial Cd^{2+}, pH 8.0, 30℃ and 180 rpm were studied. The effect of contact time on the amount biosorbed was presented in **Fig. 2d**. For Cd^{2+}, saturation levels of living and dead biomass were obtained after 120 min and 60 min, respectively, which indicated that LYX-1 had a higher adsorption efficiency. The biosorption capacity of the two adsorbents for Cd increased with the extension of time. It showed a fast adsorption process within 30 minutes and a slow adsorption process within 30-60 minutes for dead biosorbent. This was because the adsorption sites on the bacterial surface gradually reached saturation. After reaching 60 and 120 minutes, the adsorption curve showed a relatively gentle trend, indicating that the adsorbent and adsorbate reached adsorption equilibrium. It was also noticeable that the experimental results were consistent with previous studies on the biosorption of Cd ions (Li et al. 2010, Xu et al. 2020). The shaking time was determined to be 3 h for the rest of the batch experiments to make sure the equilibrium was reached.

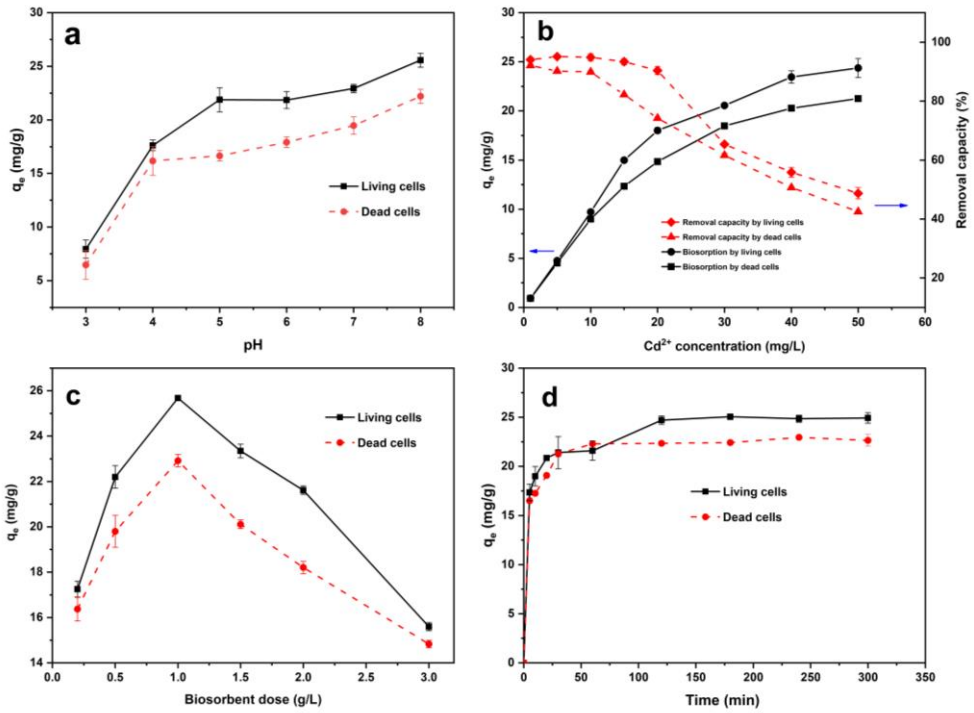


Fig. 2. Effects of environmental factor on Cd^{2+} biosorption by living and dead biocontrol strain LYX-1 (a) pH (b) initial Cd^{2+} concentrations (c) biosorbent dose (d) contact time.

3.4 Adsorption kinetic characteristics

According to formulas (1) and (2), the experimental data was fitted to obtain the kinetic fitting diagrams. As depicted in **Fig. 3a** and **3b**, clearly, the effect of the pseudo-second-order rate equation was better than pseudo-first-order rate equation, which suggested that the influence of the mass diffusion step on the adsorption rate could be negligible and rate-limiting step was a chemical adsorption process(Ho 2006, Ho and McKay 1999). This result was consistent with numerous reports on the (Abdolali et al. 2016, Feng et al. 2011, Xu et al. 2020). The equilibrium adsorption capacity of ions calculated by the pseudo-second-order rate equation was basically consistent with the measured value (**Table 1**). Thus, the pseudo-second-order kinetic parameters could be used to calculate the equilibrium adsorption capacity and removal rate.

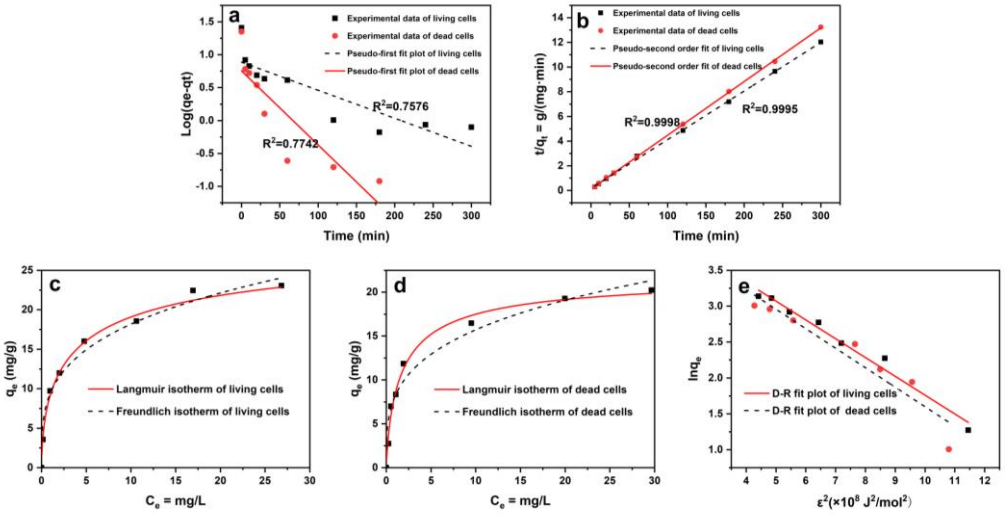


Fig. 3. The linear form of (a) Pseudo-first and (b) Pseudo-second order model for Cd^{2+} adsorption onto living and dead cells; biosorption isotherm images of Langmuir (q_{max}: mg/g, b: L/mg), Freundlich (K_f, n_f ($0.1<1/n_f<1$)) for (c) Cd^{2+} biosorption onto living cells, (d) Cd^{2+} biosorption onto dead cells and Dubinin-Radushkevich (D-R) for (e) Cd^{2+} biosorption onto both living and dead cells.

Table 1 Kinetic parameters obtained for the adsorption of Cd^{2+} using the linear method and isothermal constants, coefficient of determination (R^2) for the models fitted to equilibrium Cd^{2+} biosorption onto biomass *Paenibacillus* sp. LYX-1 strain (data calculated by equation of regression line obtained by software Origin 2018).

Models	Parameters	biosorbents	
		Living biomass	dead biomass
Pseudo-first order kinetic	$q_{e,exp}$ (mg/g)	23.2854	23.2854
	$q_{e,cal}$ (mg/g)	7.7250	5.7030
	k_1 (min^{-1})	9.8338×10^{-3}	2.5930×10^{-2}
	R^2	0.7273	0.7365
Pseudo-second order kinetic	$q_{e,exp}$ (mg/g)	23.7158	20.5453
	$q_{e,cal}$ (mg/g)	23.3614	19.9568
	k_2(mg/g min)	8.5471×10^{-3}	1.5590×10^{-2}
	R^2	0.9894	0.9798
Langmuir	q_{max} (mg/g)	30.6790	24.3752
	K_L (L/g)	0.4130	0.6244
	R_L	0.0461	0.0310
	R^2	0.9704	0.9871
Freundlich	K_F (mg/g)	9.4936	8.2306
	n	3.48831	4.5157
	R^2	0.9915	0.9378
Dubinin-Redushckevich (D-R)	β (mol^2/kJ)	2.6201×10^{-9}	2.7162×10^{-9}
	E (kJ/mol)	13.8313	13.5676
	R^2	0.9757	0.9065

3.5 Isothermal adsorption characteristics

The adsorption isotherm to some extent reflected the strength of interaction,

surface properties and affinity of adsorbent for different heavy metal ions(Mohapatra et al. 2019). In this study, Langmuir, Freundlich and D-R isotherm models were selected to simulate favorable equilibrium uptake curve and reveal the adsorption behavior of living and dead cells *Paenibacillus* sp. LYX-1(**Fig. 3**). In case of Langmuir isotherm equation, the biosorption feature was determined by both q_{max} and K_L value(Ranjan et al. 2009). As shown in **Fig. 3c** and **table 1**, the regression coefficient R^2 of Langmuir and Freundlich equations were 0.9704 and 0.9915, respectively, indicating that these two models could describe the adsorption process of living biomass well. This result suggested that Cd^{2+} biosorption by living adsorbent was more likely to be monolayer and heterogenous surface adsorption, besides, other intracellular accumulation mechanisms might be involved in removal process of Cd^{2+} by living cells. In addition, the Langmuir model (R^2=0.9871) was more applicable to simulate the dead cell adsorption process than Freundlich (R^2=0.9478), indicating that the nature of biosorption by dead cells was a monolayer and non-heterogenous adsorption. As seen in **table 1**, the maximum adsorption capacity of living and dead cells for Cd sorption, estimated from the Langmuir q_{max} parameter, was found to be 30.6790 mg/g and 24.3752 mg/g. The comparison of R_L for living biomass (R_L=0.0461) and dead biomass (R_L=0.0310) showed that living cells had a higher adsorption capacity. The high adsorption properties of *Paenibacillus* sp. meant that it had a strong fixation ability to Cd and might reduce the bioavailability of Cd^{2+} in the contaminated soil. Therefore, the biocontrol LYX-1 might control the migration of heavy metal pollutants into plants while exerting its biocontrol effect on pathogenic bacterium.

For D-R model, the plots of $\ln q_e$ versus ε^2 were shown in **Fig. 3e**, and parameters were presented in **Table 1**. Additionally, the magnitude of E might give useful information about the type of adsorption process (physical or chemical)(Ishola et al. 2014, Tran et al. 2016). Physical adsorption arises from relatively weak interactions such as van der waals force, while chemical adsorption involves stronger chemical interactions (chemical bonding) with attendant transfer of electrons between the adsorbent and adsorbate(Tran et al. 2017). In essence, the value of E for living and dead biomass were found to be with the range from 8 kJ/mol to 16 kJ/mol, indicating that

409 chemisorption (ion exchange complexation and precipitation) controlled the adsorption
410 process.

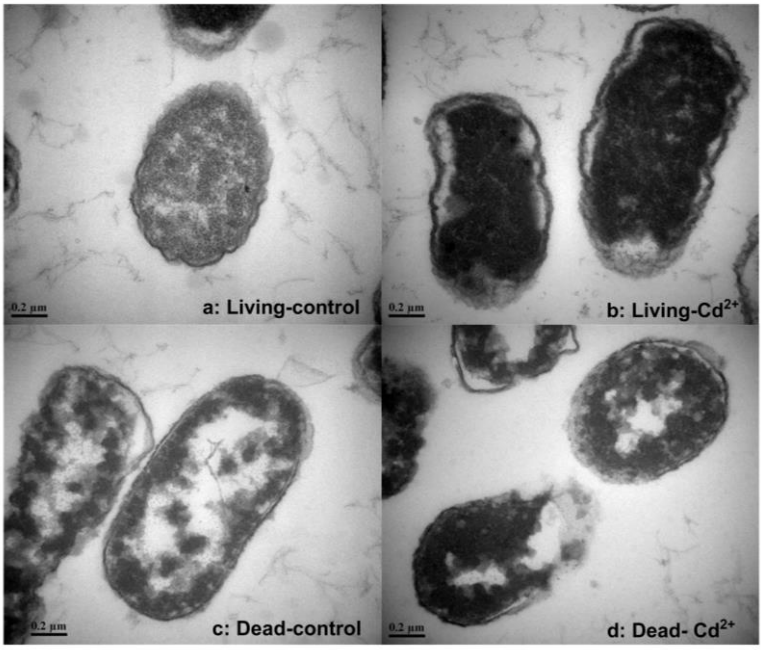
411 ***3.6 Mechanisms of Cd^{2+} resistance and biosorption by strain LYX-1***

412 **3.6.1** ***Amplification of Cd-resistant genes***

413 *Paenibacillus* sp. LYX-1 resistant to Cd^{2+} was chosen for sequence analysis of the
414 cadA, CzcA, CzcB, CzcD, which had the potential to reduce or eliminate heavy metal
415 Cd^{2+} toxicity(Wei et al. 2009). **Fig. S2** showed that *Paenibacillus* sp. LYX-1 processed
416 Cd^{2+}-resistant genes (CzcD) that could modify the protein, determine the specificity to
417 the metal substrate, and realized the resistance to Cd through efflux system(Legatzki et
418 al. 2003). The detection of CzcD demonstrated that *Paenibacillus* sp. had ability to
419 resist 50 mg/L Cd^{2+} (**Fig. 2**). Besides, the function of efflux CzcD gene further
420 explained the insignificant difference in the maximum adsorption capacity of living
421 (30.6790mg/g) and dead biosorbent (24.3752mg/g) in **Table 1**.

422 **3.6.2** ***TEM analysis***

423 In order to study the intracellular accumulation and extracellular adsorption of
424 Cd^{2+} by biocontrol strain LYX-1, TEM was used to observe the changes of bacterial
425 surface morphology in the presence of Cd^{2+}. It could be seen from the **Fig. 4a** that the
426 living control cells were full of intracellular and had uniform cytoplasm, complete cell
427 wall structure, clear boundaries. On the one hand, when under the stress of 50 mg/L
428 Cd^{2+}, living biomass had a clear gap between the cell wall and cytoplasm of living
429 biomass with high-density black particular matter appearing inside and outside the cell
430 (**Fig. 4b**). This might be attributed to the contraction of the cytoplasm to increase the
431 intracellular accumulation of Cd^{2+} and form a resistance mechanism(Sun et al. 2020).
432 Additionally, this also verified the results of the previous isotherm and kinetic
433 experiments that not only fast surface adsorption occurred in living cells, but also
434 relatively slow intracellular accumulation (**Fig. 3**). After the high temperature heat
435 treatment, the cytoplasm of dead cells severely shrank and appeared different size
436 vacuoles (**Fig. 4c**), besides, **Fig. 4d** appeared high-density black particulate matter,
437 indicating that the adsorption of Cd by dead cells was mainly concentrated on the outer
438 edge and periplasmic space, which was consistent with Huang et al. (Huang et al. 2013).



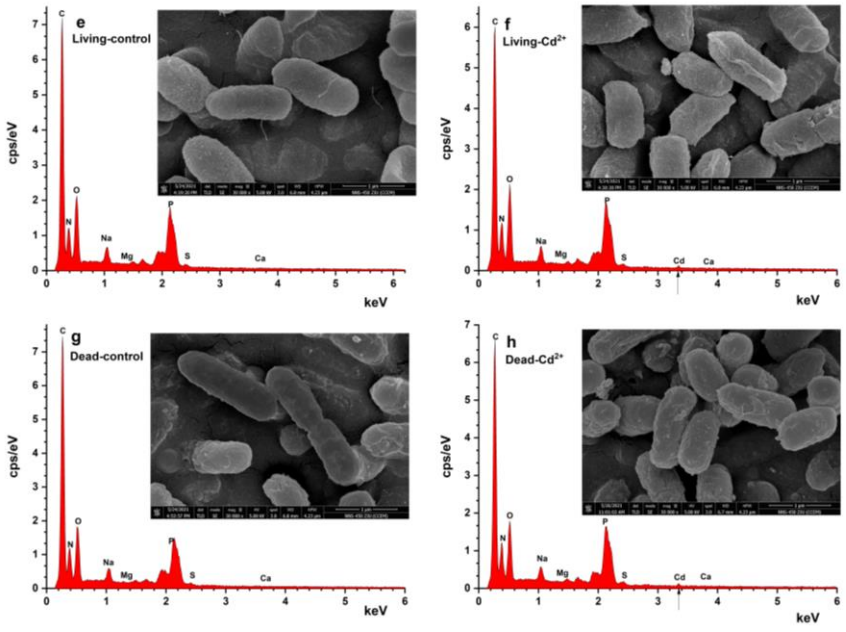


Fig. 4. Submicroscopic structures of *Paenibacillus* sp., LYX-1 (a) nature living cells, (b) 50 mg/L Cd^{2+}-loaded living cells, (c) dead cells, (d) 50 mg/L Cd^{2+}-loaded dead cells; scanning electron micrograph (SEM) and energy dispersive spectrometer (EDS) of the (e) living cells without Cd^{2+} treatment; (f) living cells treated with 50 mg/L Cd^{2+}, (g) dead cells without Cd^{2+} treatment, (h) dead cells treated with 50 mg/L Cd^{2}.

3.6.3 *SEM-EDS analysis*

Based on the micro-characterization SEM-EDS study of biomass, Cd removal mechanism by the strain *Paenibacillus* sp. was further analyzed. **Fig. 4(e)-(h)** showed surface characteristic changes of living and dead cells morphology before and after Cd^{2+} removal. We found that the surface of living cells appeared smooth and short-rod

shapes, while living cells after absorbing Cd^{2+} became rough and ruptured, accompanied by a large amount of flocculent sedimentation (**Fig. 4e and f**). Compared with living cells, dead cells seemed to be rougher, which might be caused by temperature heat treatment. After the biosorption of Cd, precipitated materials appeared on the surface of dead cells, indicating that numerous Cd^{2+} ions were adsorbed on the surface of dead biomass. The EDS analysis further verified the adsorption of Cd^{2+} by the biosorbents. No Cd peaks were detected in pristine cells. However, the peaks of Cd^{2+} with all the other elements was detected in the EDS analysis of two biomass samples after Cd biosorption. Elemental variation of strain LYX-1 before and after biosorption was in **Table S2**. Presence of Cd on cell surface after adsorption as seen from EDS images (**Fig. 4g and 4h**) proved the biosorption of Cd^{2+} by living and dead biosorbents of *Paenibacillus* sp..

3.6.4 *FTIR analysis*

In order to confirm the changes of functional groups involved in the Cd binding of biomass, the FTIR analysis was carried out. The FTIR spectrum of raw and Cd^{2+}-loaded living and dead biosorbent were presented in **Fig. 5**, where characteristic transmittance peaks listed in were identified according to previous reports (Chen et al. 2012, Huang et al. 2016, Sun et al. 2020).

For untreated living and dead cells, in **Table S3,** the peaks observed at 3429.88, 2923.73, 1647.93, 1544.78, 1236,90, 1069.97, 672.87 (living) and 3413.90, 2921.66, 1643.37, 1540.02, 1245.74, 1054.21 and 633.56 (dead) were assigned to associated O-H, N-H., $-CH_2$, amide I (-C=O/C-N), amide II (C-N/N-H), S^{2-}, C-N and phosphate, or sulfate functional groups, respectively(Peng et al. 2020). The free Cd^{2+} could be fixed by the abundant oxygen-containing and amino functional groups via the formation of surface complexation, which could be described:

$$Cd^{2+} + 2OH^{-}/\text{LYX-1} = Cd(OH)_2(s)/\text{LYX-1} \quad (9)$$

$$Cd^{2+} + PO_4^{3-}/\text{LYX-1} = Cd_3(PO_4)_2(s)/\text{LYX-1} \quad (10)$$

$$Cd^{2+} + \text{-CO/LYX-1} \rightarrow Cd(CO)^{2+}/\text{LYX-1} \quad (11)$$

$$Cd^{2+} + \text{-COOH/LYX-1} \rightarrow Cd(COO)_2/\text{LYX-1} + 2H^{+} \quad (12)$$

$$Cd^{2+} + \text{-}NH_2/\text{LYX-1} \rightarrow Cd(NH_2)^{2+}/\text{LYX-1} \quad (13)$$

After Cd^{2+} biosorption, there was only slight shift of groups between control and Cd^{2+} treated groups. However, attributed to interaction between adsorbents and heavy metal, -OH, -NH, $-CH_2$, -C=O, O=C-O, C-N, S^{2-} and phosphate functional groups were red or blue shifted when compared to nature living and dead cells, suggesting that those shifted bonds and correspond functional groups might be involved in Cd biosorption(Xu et al. 2020).

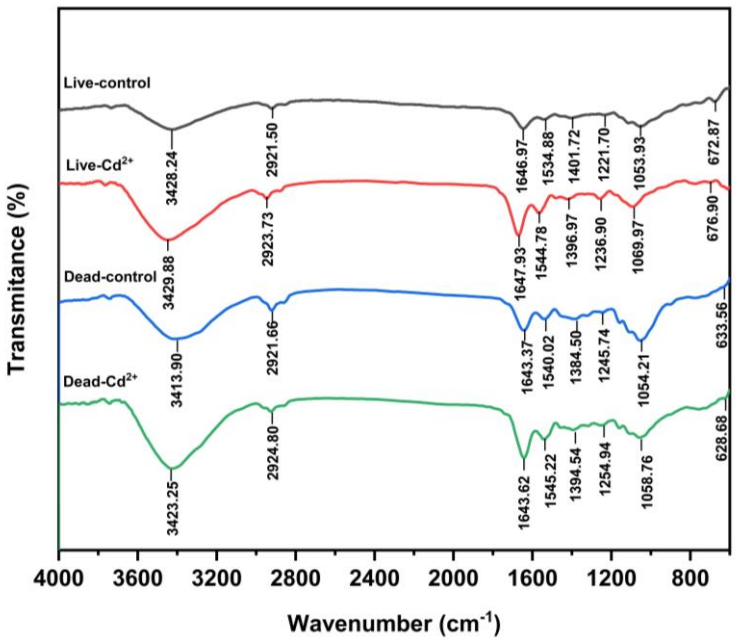


Fig. 5. FTIR spectra of the living and dead cell before and after adsorption of Cd^{2+}.

3.6.5 *XPS analysis*

Full-range XPS spectra of *Paenibacillus* sp. before and after biosorption were shown on **Fig. 6a**, which proved Cd^{2+} were adsorbed onto the strain LYX-1. The peaks of C-O at 286.31eV and 286.39eV (BE) of living and dead cells, respectively, were slightly reduced (**Fig. 6b**), which indicated that surface reducing groups such as C-OH could form precipitation with Cd^{2+} or provide protons to promote the formation of CO-Me (CO-Cd^{2+})**(Ho et al. 2017)**. By comparing the O 1s spectra of the control and experimental groups, the peaks of O 1s at 532.60eV and 532.62eV of living and dead cells were also considered as C-O-Cd^{2+}(**Fig. 6c**). Peaks of Cd detected after the adsorption could be divided into peaks at 405.39ev and 411.98ev, corresponding to Cd $3d_{5/2}$ and Cd $3d_{3/2}$ (**Fig. 6d**), respectively. There peaks (404.85eV, 405.30 eV, 405.78 eV for living cells, 404.80 eV, 405.30 eV and 405.78 eV for dead cells) could be possibly

ascribed to $Cd(OH)_2$, $CdCO_3$, and CdS. **Fig. 6e** and **Fig. 6f** also demonstrated the generation of CdS precipitation. XPS results also identified that Chemisorption controlled the biosorption process of Cd^{2+} by LYX-1 (**Fig.3e**).

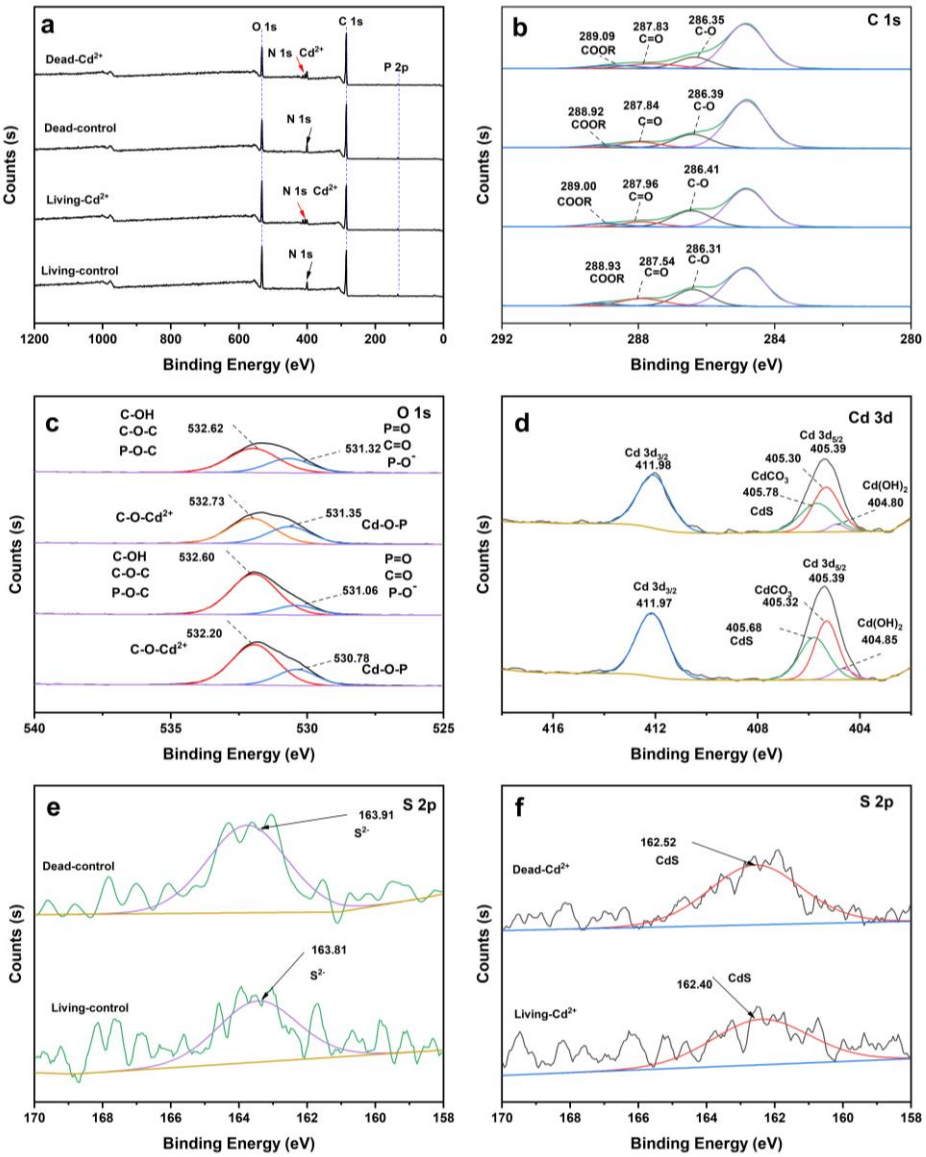


Fig. 6. XPS spectra of Paenibacillus sp. LYX-1 living and dead cells before and after adsorption of Cd^{2+}. (a) Wide scan, (b) C 1s spectra, (c) O 1s spectra, (d) Cd 3d spectra, (e) S 2p spectra before biosorption, (f) S 2p spectra after biosorption.

4. Conclusions

This study successfully isolated and identified a novel biocontrol bacterium with high-Cd resistance from soil contaminated by HMs, named *Paenibacillus* sp. LYX-1. PCR amplification showed that LYX-1 had the CzcD gene responsible for Cd detoxification by removing Cd^{2+} from the cytoplasm or periplasmic to extracellular

medium. pH, initial Cd^{2+} concentration, adsorbent dose and contact time had significant effects on the Cd capacity of living and dead biomass in batch adsorption test. Pseudo-second order kinetic model was more suitable to two biosorbents. The fitting of Langmuir and Freundlich isotherm showed that there was a monolayer and heterogenous surface process for living cells, while monolayer and non-heterogenous process for dead cells. The E value of two biomasses ranged from 8 kJ/mol to 16 kJ/mol, revealing that chemisorption controlled the adsorption of Cd^{2+} by *Paenibacillus* sp. The maximum capacity obtained were 30.6790 mg/g (living biomass) and 24.3752 mg/g (dead biomass), respectively. TEM, SEM-EDS, FTIR and XPS characterization indicated that extracellular precipitation, ion exchange, and intracellular accumulation might be the main mechanisms for the adsorption of Cd^{2+} by living cells, while there was no intracellular accumulation for dead cells. -OH, -NH, -C=O, O=C-O, C-N, S^{2-} and phosphate functional groups participated in the adsorption process of Cd. Therefore, this novel biocontrol *Paenibacillus* sp. can be applied as a promising adsorbent to be used in the simultaneous control of plant rhizosphere diseases and Cd pollution in soil.

Compliance with ethics guidelines

This article dose not contain any studies with human or animal subjects performed by either of authors.

Consent for publication

All authors have read the submitted version of the manuscript and agree to submit the work to Environmental Science and Pollution Research, and we all agree that the transfer of copyright from the author to Environmental Science and Pollution Research.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Contributors

Yixin Luo, Min Liao and Xiaomei Xie conceived the idea. Min Liao and Xiaomei Xie designed the research. Yixin Luo, Yuhao Zhang, Na Xu, Xiaomei Xie and Qiyan Fan performed the experiment. Yixin Luo, Min Liao and Xiaomei Xie analysed the data and wrote the manuscript. All authors contributed to the discussion of the manuscript.

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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