Characterization of three Pb-resistant fungi and their potential Pb$^{2+}$ ions adsorption capacities

Xin Sun, Fei Han, Hui Wang, Fupeng Song, Xiumin Cui, Yanhong Lou and Yuping Zhuge

ABSTRACT

Bioremediation is preferred in heavy metal remediation, and the high-performance microbe is of prime importance. In the present research, three Pb-resistant microbes were isolated and growth characteristics and adsorption capacities were evaluated. The results showed that R. oryzae SD-1, T. asperellum SD-5, and M. irregularis SD-8 can grow well under 100 mg L$^{-1}$ Pb$^{2+}$ ions stress. There is a higher minimum inhibitory concentration (MIC) of Pb but lower MICs of Cd and Zn in T. asperellum SD-5. However, there were similar MICs of Cu among the three microbes. R. oryzae SD-1 exhibited a higher adsorption capacity and removal rate relative to the other two microbes under various Pb$^{2+}$ ion levels. The Langmuir equation was fitted for the adsorption capacity of T. asperellum SD-5 and M. irregularis SD-8, and their maximum adsorption capacities were approximately 456.62 mg g$^{-1}$ and 93.62 mg g$^{-1}$. Moreover, the Elovich equation and the double constant equation can describe the adsorption process of Pb$^{2+}$ ions in Pb-resistant microbes well. The strongest adsorption capacity under lower Pb$^{2+}$ ion level was observed in M. irregularis SD-8, while the strongest adsorption capacities under higher Pb$^{2+}$ ion levels were seen in R. oryzae SD-1 and T. asperellum SD-5. Therefore, three novel Pb-resistant microbes may be used as efficient, easily cultivated materials for Pb-contaminated soil remediation.

Key words | adsorption kinetics, biosorption, Pb-resistant fungus

INTRODUCTION

Lead (Pb), as a hazardous environmental pollutant, has been widely used in dyes and paints since ancient times, and lead batteries, automobile exhaust, and industrial activities that accompanied the development of modern society are recognized as important sources of Pb pollution. Soils contaminated with Pb$^{2+}$ ions are now distributed on a global scale, and high levels of Pb$^{2+}$ ions are commonly encountered in urban, agricultural, and mining soils (Park et al. 2011). The Pb$^{2+}$ ion, a two-valent cation with a small hydrated ion radius, has a strong exchange capacity and is easily adsorbed by soil colloids. It is well-documented that Pb$^{2+}$ ions could accumulate in the human body through the food chain, and excessive Pb$^{2+}$ ions intake is harmful to skeletal, blood circulatory, and enzymatic activities in the human body, especially in children (Rai et al. 2006). Therefore, Pb is recognized as the most common heavy metal contaminant by the environmental protection agency (EPA), and it has generated worldwide concern (Bing et al. 2001).

With the increasing awareness of environmental protection, the use of traditional physical and chemical remediation methods such as electrochemical treatment, ion exchange, and physical precipitation to remove heavy metals from contaminated soil does not meet environmental requirements, and hence biological approaches have been considered as environmentally friendly ecological remediation methods for heavy metal contamination (Solgi et al. 2012). However, heavy metals are toxic to microorganisms by inhibiting enzymatic activities, disrupting membrane functions, and damaging nucleic acids, but some microorganisms can resist heavy metal toxicity through changes in physiological metabolism and ecological structure. Some micro-organisms, such as Pseudomonas marginalis (Roane 1999), Pseudomonas aeruginosa (Sinha & Mukherjee 2008), and Aspergillus aculeatus (Xie et al. 2014), were reported to have the ability to resist and detoxify metals and were utilized to remediate metal-contaminated soils. Researchers reported that the toxicity of heavy metals in...
the soil could be minimized by microorganisms via adsorption to cell surfaces, complexation by exopolysaccharides, intracellular accumulation or precipitation (Pan et al. 2009). Heavy metal removal by microorganisms is recognized as a complex process that depends on the chemistry of metal ions, cell wall composition, cell physiology, and physicochemical factors such as pH, temperature, metal concentration, and ionic strength (Abdalla et al. 2012).

Both living and non-living microbial biomass have been employed to remove toxic metal ions from different matrices. Velmurugan et al. (2010) isolated a new biosorbent microbe, Penicillium sp. MRF-1, that could remove lead from aqueous solutions. Deng et al. (2014) revealed that an endophytic fungal strain (MXSF51) isolated from Portulaca oleracea growing in polluted soils had the potential to remove metal ions from soils containing multiple heavy metals. Therefore, understanding the characteristics of heavy metal-resistant microbes and clarifying suitable environmental factors are necessary for the effective utilization of bioremediation.

The present study was conducted to isolate and characterize Pb-resistant microbial strains from Pb-contaminated soil that originated from a lead-acid battery plant and to assess their capacity to adsorb Pb$^{2+}$ ions. Adsorption kinetics and isothermal adsorption equations were also evaluated, and the results could provide material and theoretical support for bioremediation of heavy metal pollution.

**MATERIALS AND METHODS**

**Isolation of Pb-resistant microbial strains**

Mixed soil samples were collected from the 0–20 cm soil layers from a lead-acid battery factory in Tai’an, Shandong Province, China. Sterile bags and an ice incubator were used to keep the samples fresh and transfer them to the laboratory. All samples were sieved through 2 mm mesh, and 10.0 g soil samples were weighed and transferred to a 250 ml Erlenmeyer flask that contained 90 ml of sterile water under sterile conditions. The bottle was then kept on an oscillation incubator (with 150 r min$^{-1}$ at 30°C) for 30 min to completely separate microorganisms from the soil samples, and the flask was then held stationary for 20 min. One millilitre of microbial suspension was obtained and transferred to Luria–Bertani (LB) medium that contained 500 mg L$^{-1}$ Pb$^{2+}$ ions for incubation at 30°C. After 7 days of incubation, the fungal community was separated using the plate streak method and then inoculated on the solid medium with 800, 1,000, 1,200, or 1,500 mg L$^{-1}$ Pb$^{2+}$ ions to select Pb-resistant microbial strains. The same method was used for the isolation (the plate was streaked more than three times) and purification of Pb-resistant microbial strains.

**Identification of Pb-resistant microbial strains**

The three Pb-resistant microbial strains that survived under 1,500 mg L$^{-1}$ Pb$^{2+}$ ions were fungi, based on identification provided by Personal Biotechnology Co., Ltd (Shanghai, China). An 18S rDNA fungi identification kit (TIANGEN, DP502, Beijing) was employed for Pb-resistant fungi identification. After the extraction of total DNA, the internal transcribed spacer 1 (ITS1)-5.8S-ITS2-28S region of nuclear rDNA was amplified with ITS1 and ITS4 primers. The amplification reaction was carried out according to the method described by Baldoni (2022). Sequencing of the samples was carried out using an ABI 3730-XL Genetic Analyzer (Applied Biosystems). The consensus sequence was deposited in GenBank, and a comparative search of GenBank sequences was carried out using the BLASTn tool. The additional sequences retrieved from GenBank included those of Brazilian species described for these genera (Gomes et al. 2013). To identify the fungi, all of the sequences were aligned using the program BioEdit version 7.2.5 with the ClustalW algorithm (Hall 1999).

**Growth characteristics of Pb-resistant microbial strains**

Liquid-culture experiments were conducted to assess the growth characteristics of Pb-resistant fungi under 100 mg L$^{-1}$ Pb concentrations. An inoculating loop was used to inoculate the strains into liquid medium (pH = 7), and the strains were then cultured in an oscillation incubator at a constant temperature (150 rpm min$^{-1}$ at 30°C). Meanwhile, the control group was treated with no Pb$^{2+}$ ions. OD$_{600}$ (optical density at 600 nm) was determined after 2, 4, 8, 12, 24, 36, 48, 60, and 72 h to monitor the growth of the microbial strains.

**Minimal inhibitory concentration**

Minimal inhibitory concentration (MIC) was defined as the lowest concentration of heavy metals that inhibited microbial strain growth (Froidevaux et al. 2001). The agar dilution method described by Abdel-Rahman et al. (2016a, 2017) was used to determine the MICs of Cu, Cd, and Zn for the Pb-resistant microbial strains. For MIC
determination, the Pb-resistant fungi were incubated in a growth incubator until they entered the logarithmic phase and then diluted 10 times. Ten microlitres of diluted microbial strain suspension was sampled and inoculated onto nutrient agar plates that contained various concentrations (50, 100, 150, 200, 250, 300, 350, 400, 450, 500 mg L\(^{-1}\)) of Cu, Cd, and Zn. Analytical pure metal salts from Sinopharm Chemical Reagent Co., Ltd were used to configure heavy metal solutions. Cu was provided by CuSO\(_4\)·5H\(_2\)O (AR, >99%), Cd was provided by CdSO\(_4\)·8H\(_2\)O (AR, >99%), and Zn was provided by ZnSO\(_4\)·7H\(_2\)O (AR, >99.5%). After incubation at 30°C for 3 days, strain growth was determined by visual inspection.

**Dissolution ability of insoluble lead**

0.2 g of dry mycelium was weighed and added to a flask which contained 150 mL PbCO\(_3\) solution (1,000 mg L\(^{-1}\)), and setting uninoculated control group. All flasks were then cultured in an incubator at a constant temperature (150 rpm min\(^{-1}\) at 30°C). The pH was determined after 0, 12, 24, 36, 48 and 60 h. At the same time, 10 mL filtrate solution was sampled at different time points for determination of Pb\(^{2+}\) ion concentration. The Pb\(^{2+}\) ion concentrations were determined by atomic absorption spectrometry (AAS, AA-7000, Japan).

**Biosorption experiment**

The kinetic biosorption experiment was carried out as follows: the Pb-resistant microbial strains were pre-cultured for 7 days in the solid culture medium and then isolated and washed using sterile water to remove the media from the mycelium. In all, 0.2 g of dry mycelium was weighed and added to a series of flasks, with each flask containing 100 mL of sterilized, double-distilled water with a certain Pb\(^{2+}\) ion level. Nine Pb\(^{2+}\) ion treatments were employed: 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 mg L\(^{-1}\). All flasks were cultured in an oscillation incubator at a constant temperature (150 rpm min\(^{-1}\) at 30°C). After 24 h of incubation, 10 mL supernatants were collected for determination of Pb\(^{2+}\) ion concentration. The Pb\(^{2+}\) ion concentrations were determined by atomic absorption spectrometry (AAS, AA-7000, Japan).

**Isolation and identification of Pb-resistant microbial strains**

Pb-resistant microbial strains were isolated from Pb-contaminated soil by using a spread-plate procedure with pH-neutral SLP medium. Three Pb-resistant fungal strains, designated SD-1, SD-5, and SD-8, were the best at solubilizing Pb in the solution culture with 1,500 mg kg\(^{-1}\) Pb\(^{2+}\) ions. Based on the analysis of ITS rRNA gene sequences, the SD-1, SD-5, and SD-8 microbial strains were identified as *Rhizopus oryzae* (99% identity), *Trichoderma asperellum* (100%), and *Mucor irregularis* (100%), respectively. The ITS1-5.8S rDNA-ITS2 nucleotide sequence data were deposited in GenBank with the accession numbers KY807765 (R. oryzae SD-1), KY807766 (T. asperellum SD-5), and KY807767 (M. irregularis SD-8). Fortunately, *Trichoderma asperellum* was reported by Tan & Ting (2012) to perform well in the adsorption of copper ions at a concentration of 134.22 mg L\(^{-1}\). However, little is known about the heavy metal resistance of *R. oryzae* and *M. irregularis*.

**Minimum inhibitory concentration of heavy metals**

To our knowledge, although the growth of microbial strains was inhibited due to heavy metal toxicity, the microbial strains also adjusted their structural and physiological conditions in order to adapt to this adverse environment (Waranusantigul et al. 2011). Diverse MICs of metals (Pb, Cd, Cu, and Zn) were observed among three Pb-resistant fungal strains (*Rhizopus oryzae*, *Trichoderma asperellum* and *Mucor irregularis*) (Table 1). The MICs of Pb(II) for the three Pb-resistant fungal strains (*Rhizopus oryzae, Trichoderma asperellum* and *Mucor irregularis*) were 1,750, 2,000, and 1,550 mg L\(^{-1}\), respectively. For Cd(II), the highest MIC was observed in *Rhizopus oryzae* (250 mg L\(^{-1}\)),
and the lowest MIC was observed in *T. asperellum* SD-5 (50 mg L⁻¹). The three Pb-resistant fungal strains (*Rhizopus oryzae*, *Trichoderma asperellum*, and *Mucor irregularis*) had similar MIC of Cu(II). However, the MIC for *T. asperellum* SD-5 (300 mg L⁻¹) was lower than that for *R. oryzae* SD-1 and *M. irregularis* SD-8 (400 mg L⁻¹).

Growth characteristics of Pb-resistant microbial strains under Pb stress

The growth rates of the three strains in the Pb²⁺ ion solution (100 mg L⁻¹) decreased significantly, and the contents of bacteria were also remarkably inhibited (Figure 1). Compared with the control, the growth rates of *R. oryzae* SD-1, *T. asperellum* SD-5, and *M. irregularis* SD-8 were inhibited by 7.61%, 11.85%, and 10.87%, respectively (Figure 1). Notably, the growth of *R. oryzae* SD-1 was slower than that of *T. asperellum* SD-5 and *M. irregularis* SD-8. After 72 h of cultivation, the absorbance value of *R. oryzae* SD-1 was lower than that of the strains *T. asperellum* SD-5 and *M. irregularis* SD-8, and the slopes were reduced by 13.72% and 10.73%, respectively.

Adsorption characteristics of Pb-resistant fungal strains

Ability to adsorb Pb²⁺ ions

The adsorption capacities of *R. oryzae* SD-1 and *T. asperellum* SD-5 showed increasing trends with an increase in Pb²⁺ ion concentration, and the highest adsorption capacity of these strains was observed at the 500 mg L⁻¹ Pb²⁺ ion concentration, with capacities of 270.56 mg g⁻¹ and 165.47 mg g⁻¹, respectively (Figure 2). For *M. irregularis* SD-8, when the initial concentration of Pb²⁺ ions was 50–300 mg L⁻¹, the adsorption capacity increased with increasing Pb²⁺ ion concentration, but when the concentration of Pb²⁺ ions was more than 300 mg L⁻¹, the adsorption capacity (approximately 70 mg g⁻¹) did not change significantly. This phenomenon was attributed to the enhancement of the driving force required to overcome the resistance of mass transfer between the aqueous and solid-metal ions; the collision between metal ions and the adsorbent may have intensified when exposed to a higher Pb²⁺ ion regime (Chen et al. 2005). The two strains *R. oryzae* SD-1 and *T. asperellum* SD-5 excavated in this study had higher adsorption capacity for lead than FBILS.

### Table 1 | Minimal inhibitory concentrations of Pb, Cd, Cu and Zn for *R. oryzae* SD-1, *T. asperellum* SD-5, and *M. irregularis* SD-8

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pb (mg L⁻¹)</th>
<th>Cd (mg L⁻¹)</th>
<th>Cu (mg L⁻¹)</th>
<th>Zn (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. oryzae</em> SD-1</td>
<td>1,750</td>
<td>8.45</td>
<td>250</td>
<td>2.23</td>
</tr>
<tr>
<td><em>T. asperellum</em> SD-5</td>
<td>2,000</td>
<td>9.66</td>
<td>50</td>
<td>0.45</td>
</tr>
<tr>
<td><em>M. irregularis</em> SD-8</td>
<td>1,550</td>
<td>7.49</td>
<td>100</td>
<td>0.89</td>
</tr>
</tbody>
</table>

**Figure 1** | Growth of *R. oryzae* SD-1, *T. asperellum* SD-5, and *M. irregularis* SD-8 in nutrient broth containing 0 or 100 mg L⁻¹ of Pb at 30 °C. The vertical line on each bar showed the standard deviation (n = 3).

**Figure 2** | Effects of the initial concentration of Pb²⁺ ions on the adsorption amounts. The vertical line on each bar showed the standard deviation (n = 3).
Phanerochaete Chrysosporium which can adsorb lead up to 135.3 mg g\(^{-1}\), as reported by Iqbal. However, the adsorption capacity of M. irregularis SD-8 of lead was less than FBILS. No significant differences in adsorption capacity were found among R. oryzae SD-1, T. asperellum SD-5, and M. irregularis when the Pb\(^{2+}\) ion concentration was 50–200 mg L\(^{-1}\) (Figure 2). However, the adsorption capacity of R. oryzae SD-1 was highest when the Pb\(^{2+}\) ion concentration exceeded 200 mg L\(^{-1}\), and the lowest adsorption capacity was observed in M. irregularis SD-8. The removal rates of Pb\(^{2+}\) ions by R. oryzae SD-1 and T. asperellum SD-5 were increased gradually with an increase in the initial concentration of lead ions, and the highest removal rate of lead by R. oryzae SD-1 was 33.76% in the 450 mg L\(^{-1}\) lead concentration and by T. asperellum SD-5 it was 18.71% in the 250 mg L\(^{-1}\) lead concentration (Figure 3). The removal rate by M. irregularis SD-8 reached the highest level of 17.37% in the 100 mg L\(^{-1}\) lead concentration, after which the removal rate decreased gradually with an increase in lead concentration (Figure 3). The average removal rate by R. oryzae SD-1 under different initial concentrations of Pb was 52.16% higher than that of T. asperellum SD-5 and 92.04% higher than that of M. irregularis SD-8 (Figure 3). Generally, the capacity to adsorb lead is as follows: R. oryzae SD-1 > T. asperellum SD-5 > M. irregularis SD-8. Therefore, in the complex heavy pollution environment, R. oryzae SD-1 and T. asperellum SD-5 were preferred for utilization due to their stable adsorption capacities. Microbial remediation was also recognized as greener and environmentally friendly technology compared with general physical and chemical adsorbents (Bing et al. 2001; Yari et al. 2005). Notably, the present results of the three Pb-resistant strains on lead removal ability were conducted under solution conditions, and we also applied the sawdust as a carrier to apply the strain to the lead-contaminated soil where the strain was located, and found that the lead contamination has a good repair effect (unpublished).

Previous studies have clearly revealed that pH has great influence on the biosorption of metal ions from aqueous solutions (Yan & Viraraghavan 2005). In fact, the effect of pH on the biosorption process is varied, especially on the different morphology of lead ions (Fan et al. 2008). Therefore, in order to compare the adsorption of lead ions by three different fungi, we adjusted the initial pH of the solution to 7.0. The dissolution test of lead carbonate showed the law of pH change during the adsorption process. The three strains had a dissolution effect on insoluble lead, and the solution pH decreased gradually (Figure 4). These results indicated that all three strains could secrete a lot of organic acids, but T. asperellum SD-5 had the worst dissolution effect on lead carbonate. At 0–48 hours, the lead concentration of R. oryzae SD-1 and M. irregularis SD-8 increased rapidly, while pH decreased to less than 5 (Figure 5). We also believe that the actual solution concentration may be higher than our measurements. In other words, there is a simultaneous adsorption process during the dissolution of the strain, which is why the concentration of lead ions in the lead carbonate solution added with strain T. asperellum SD-5 decreases at 24 hours.

However, the adsorption function of microorganisms is affected by various factors, such as soil texture, temperature and metal ion (Goyal et al. 2003; Piotrowska-Seget et al. 2005; Sahin & Ozturk 2005), especially the nutrients...
limitation. It has been reported that a *Trichoderma* strain can effectively remediate heavy metal pollution in tailings soil, especially the removal ability for Pb is stronger than that of Cd, As, Zn and Cu (Babu et al. 2014). In addition, a *Trichoderma asperellum* strain has also been used to adsorb As in soil (Wang et al. 2015). Zhang et al. (2018) has found that Mucor bacteria could repair lead-contaminated soil containing 1,000 mg kg\(^{-1}\) lead. It has also been found that *Rhizopus* has an adsorption effect on Cr, Fe and Cu (Sag & Kutsal 1998). However, few studies have reported the remediation of Pb in soil by *Rhizopus oryzae*, Furthermore, the impact on other more extensive soil environments needs further exploration.

### Adsorption kinetics

In the first five minutes of Pb\(^{2+}\) ion exposure, the three Pb-resistant fungal strains exhibited higher adsorption rates, and no significant changes in adsorption rates were detected when the strains were exposed to a Pb\(^{2+}\) ion regime for more than 4 h (Figure 6). Shin et al. (2012) also reported that the concentration of Pb\(^{2+}\) ions in solution becomes stable after removal by *Bacillus* sp. MN3-4 for 24 h. Concurrently, there may be a certain resistance mechanism in fungal strains such as the active transport system that can remove Pb\(^{2+}\) ions from the cytoplasm under Pb\(^{2+}\) ion stress. From 4 hours to 24 hours, the concentration of Pb\(^{2+}\) ions is in equilibrium and a stable state. This equilibrium state may be maintained for a long time without the influence of other factors except for nutrient deficiency.

Notably, the adsorption rate of *M. irregularis* SD-8 was significantly higher than that of *R. oryzae* SD-1 and *T. asperellum* SD-5 in the first 30 minutes. The Elovich equation (\(Y = a + k \ln t\)) and double constant equation (\(\ln Y = a + k \ln t\)) were employed to evaluate the efficiency of the three Pb-resistant fungi strains as biosorbents.

The Elovich equation describes a series of adsorption processes, such as bulk or interfacial diffusion and surface activation and deactivation, and the double constant
equation, also called the power function equation, was derived from the Freundlich equation in 1974. Both the Elovich equation and the double-constant equation can describe the adsorption kinetics of Pb\(^{2+}\) ions on mycelia (Table 2). In the equation, the intercept (absolute value \(a\)) can reflect the adsorption capacity, and the larger the intercept is, the stronger the adsorption capacity. The slope (absolute value \(k\)) can reflect the rate of adsorption, and the larger the \(K\) value is, the faster the adsorption reaction. The adsorption rate and adsorption capacity of \(M.\) irregularis SD-8 were higher than those of \(R.\) oryzae SD-1 and \(T.\) asperellum SD-5 in a Pb\(^{2+}\) ion concentration of 100 mg L\(^{-1}\) (Table 2).

### Adsorption Isotherm

Two isotherm models (Langmuir and Freundlich) were used for modelling the adsorption data of the three Pb-resistant fungal strains used as biosorbents (Iqbal & Edyvean 2004; Abdel-Rahman et al. 2016b). The Langmuir isothermal equation could be used as a token for Pb\(^{2+}\) ion isothermal adsorption properties (Abdel-Rahman et al. 2016c). According to the isothermal equation used in traditional studies to assess the performance of different adsorbents, the relationship between equilibrium adsorption and equilibrium concentration is well demonstrated (Figure 7). The Langmuir isotherm describes strong monolayer sorption onto specific surface binding sites in the biomass and can be described by the following equations:

\[
1/Q_e = 1/Q_{\text{max}} + 1/(K_L \times Q_{\text{max}} \times C_e) \\
\ln Q_e = \ln K_F + a \ln C_e.
\]

In the formulae, \(Q_e\) (mg g\(^{-1}\)) is the equilibrium adsorption amount, \(Q_{\text{max}}\) (mg g\(^{-1}\)) is the maximum adsorption capacity, \(K_L\) (L mg\(^{-1}\)) is the Langmuir constant, \(K_F\) (L mg\(^{-1}\)) is the Freundlich constant, and \(a\) and \(k\) are the intercept and slope, respectively.

### Table 2: Fitting and parameters of two adsorption kinetic models

<table>
<thead>
<tr>
<th>Strain</th>
<th>Equation model</th>
<th>Expression</th>
<th>Adsorption constants</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R.) oryzae SD-1</td>
<td>Elovich equation</td>
<td>(Y = a + k\ln t)</td>
<td>(a = 28.7469,) (k = 0.4390)</td>
<td>0.9332</td>
</tr>
<tr>
<td></td>
<td>Double constant</td>
<td>(\ln Y = a + k\ln t)</td>
<td>(a = 3.5544,) (k = 0.0163)</td>
<td>0.9999</td>
</tr>
<tr>
<td>(T.) asperellum SD-5</td>
<td>Elovich equation</td>
<td>(Y = a + k\ln t)</td>
<td>(a = 23.6619,) (k = 0.2296)</td>
<td>0.8342</td>
</tr>
<tr>
<td></td>
<td>Double constant</td>
<td>(\ln Y = a + k\ln t)</td>
<td>(a = 3.1534,) (k = 0.0118)</td>
<td>0.9997</td>
</tr>
<tr>
<td>(M.) irregularis SD-8</td>
<td>Elovich equation</td>
<td>(Y = a + k\ln t)</td>
<td>(a = 33.9707,) (k = -3.8571)</td>
<td>0.9118</td>
</tr>
<tr>
<td></td>
<td>Double constant</td>
<td>(\ln Y = a + k\ln t)</td>
<td>(a = 3.4949,) (k = -0.1276)</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

Figure 7 | Adsorption isotherms of the (a) Langmuir and (b) Freundlich equations.
capacity, \( C_e \) (mg L\(^{-1}\)) is the equilibrium concentration, and \( K_L \) and \( K_F \) are the adsorption constants.

Based on the results of the Langmuir equation-fitting of \( R.\) oryzae SD-1 adsorption, the maximum adsorption capacity and equilibrium adsorption coefficient were negative, indicating that the Langmuir equation is not suitable for describing the adsorption characteristics of \( R.\) oryzae SD-1 (Tab 3). For \( T.\) asperellum SD-5 and \( M.\) irregularis SD-8, the Langmuir equation better describes the adsorption of \( Pb^{2+} \) ions than does the Freundlich isotherm; the maximum adsorption capacity for each of the strains was 456.62 mg g\(^{-1}\) and 93.62 mg g\(^{-1}\), respectively (Table 3). The \( K_L \) value is the ratio of positive and negative reaction rate constants and is a measure of the stability of the adsorbate and adsorbent; the larger the \( K_L \) value is, the greater the stability of the combination. The \( K_L \) values of the \( T.\) asperellum SD-5 and \( M.\) irregularis SD-8 strains were 0.0011 and 0.013, respectively, and \( M.\) irregularis SD-8 has the higher stability of binding with \( Pb^{2+} \) ions.

The adsorption behaviour of the three strains can be characterized by the Freundlich equation. The \( a \) value in the Freundlich equation can be used to measure the influence of adsorbate concentration on adsorption capacity. The larger the \( a \) value is, the greater the influence of the adsorbate concentration on the adsorption capacity. The \( K_F \) value indicates the affinity of the adsorption system for the metal ion. The higher the \( K_F \) value is, the stronger the affinities of the adsorbent and adsorbate. The results indicate that the effect of \( Pb^{2+} \) ion concentration on the adsorption capacity of \( M.\) irregularis SD-8 is the highest in the same way that \( M.\) irregularis SD-8 had the highest affinity for \( Pb^{2+} \) ions (Table 3).

### CONCLUSION

Generally, this study found that three novel \( Pb \)-resistant microbes (\( Rhizopus oryzae \), \( Trichoderma asperellum \), and \( Mucor irregularis \)) survived in 1,500 mg kg\(^{-1}\) \( Pb^{2+} \) ion regimes, and higher MIC of \( Pb \), \( Cu \), and \( Zn \). Meanwhile, \( Pb^{2+} \) ions were removed successfully due to the higher adsorbing ability. However, significant differences in adsorption capacity were observed among the three \( Pb \)-resistant microbes, and the strongest \( Pb^{2+} \) ion adsorption capacity under lower \( Pb^{2+} \) ion levels was observed in \( M.\) irregularis SD-8 while the strongest \( Pb^{2+} \) ion adsorption capacity under higher \( Pb^{2+} \) ion levels was observed in \( R.\) oryzae SD-1 and \( T.\) asperellum SD-5. Furthermore, the remediation effects and mechanism in \( Pb \)-contaminated soils needs to be explored further to extend the utilization of these three \( Pb \)-resistant microbes.

### ACKNOWLEDGEMENTS

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**Table 3** | Langmuir and Freundlich isothermal adsorption parameters of \( Pb^{2+} \)

<table>
<thead>
<tr>
<th>Strains</th>
<th>( Q_{max} )</th>
<th>( K_L )</th>
<th>( R^2 )</th>
<th>( K_F )</th>
<th>( a )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R.) oryzae SD-1</td>
<td>–216.45</td>
<td>–0.0017</td>
<td>0.9806</td>
<td>0.3098</td>
<td>0.710</td>
<td>0.9558</td>
</tr>
<tr>
<td>( T.) asperellum SD-5</td>
<td>456.62</td>
<td>0.0011</td>
<td>0.9468</td>
<td>0.7687</td>
<td>1.054</td>
<td>0.9405</td>
</tr>
<tr>
<td>( M.) irregularis SD-8</td>
<td>93.62</td>
<td>0.0130</td>
<td>0.9610</td>
<td>2.605</td>
<td>2.700</td>
<td>0.8386</td>
</tr>
</tbody>
</table>

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