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RESEARCH ARTICLE

Characteristics of metal-tolerant plant growth-promoting yeast (*Cryptococcus* sp. NSE1) and its influence on Cd hyperaccumulator *Sedum plumbizincicola*

Wuxing Liu[1] · Beibei Wang[1,2] · Qingling Wang[1] · Jinyu Hou[1,2] · Longhua Wu[1] · Jennifer L. Wood[3] · Yongming Luo[1,4] · Ashley E. Franks[3]

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Abstract Plant growth-promoting yeasts are often over looked as a mechanism to improve phytoremediation of heavy metals. In this study, *Cryptococcus* sp. NSE1, a Cd-tolerant yeast with plant growth capabilities, was isolated from the rhizosphere of the heavy metal hyperaccumulator *Sedum plumbizincicola*. The yeast exhibited strong tolerance to a range of heavy metals including Cd, Cu, and Zn on plate assays. The adsorption rate Cd, Cu, Zn by NSE1 was 26.1, 13.2, and 25.2 %, respectively. Irregular spines were formed on the surface of NSE1 when grown in MSM medium supplemented with 200 mg L^{-1} Cd. NSE1 was capable of utilizing 1-aminocyclopropane-1-carboxylate (ACC) as a sole nitrogen source and was capable of solubilization of inorganic phosphate at rates of 195.2 mg L^{-1}. Field experiments demonstrated that NSE1 increased phytoremediation by increasing the biomass of Cd hyperaccumulator *S. plumbizincicola* (46 %, $p < 0.05$) during phytoremediation. Overall, Cd accumulation by *S. plumbizincicola* was increased from 19.6 to 31.1 mg m^{-2} though no difference in the concentration of Cd in the shoot biomass was observed between NSE1 and control. A Cd accumulation ratio of 38.0 % for NSE1 and 17.2 % for control was observed. The HCl-extractable Cd and $CaCl_2$-extractable Cd concentration in the soil of the NSE1 treatment were reduced by 39.2 and 29.5 %, respectively. Community-level physiology profiling, assessed using Biolog Eco plates, indicated functional changes to the rhizosphere community inoculated with NSE1 by average well color development (AWCD) and measurement of richness (diversity). Values of Shannon-Weiner index, Simpson index, and McIntosh index showed a slight but no significant increases. These results indicate that inoculation of NSE1 could increase the shoot biomass of *S. plumbizincicola*, enhance the Cd accumulation in *S. plumbizincicola*, and decrease the available heavy metal content in soils significantly without overall significant changes to the microbial community.

Keywords Cd-contaminated soil · Phytoremediation · *Cryptococcus* sp. · Plant growth promoting · Microbial community

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✉ Wuxing Liu
liuwuxin@issas.ac.cn

[1] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] Department of Physiology, Anatomy and Microbiology, La Trobe University, Melbourne, VIC 3086, Australia

[4] Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

Introduction

Soil contamination by heavy metals has increased considerably during the last few decades. Among heavy metals, cadmium (Cd) is one of the most toxic contaminants due to its high mobility and toxicity in soils (Guo et al. 2010). Cd is readily taken up by plants and results in growth inhibition, alterations in nutrient uptake, and activated oxygen metabolism, chlorosis, and even plant death (Sandalio et al. 2001). Cd may affect a wide range of living organisms, including humans, through accumulation within the food chain. Cd is

a carcinogen and acute exposure may lead to flu-like symptoms, renal tube dysfunction, softening of bones, kidney failures, and pain (Żukowska and Biziuk 2008). This pain associated with the mass cadmium poisoning in the Toyama Prefecture of Japan in 1912 led to the name itai-itai disease (it hurts-it hurts disease) (Sato et al. 2010). Cd contamination therefore must be removed due to concerns for both environment quality and human health.

Techniques for the remediation of heavy metal-contaminated soils include immobilization and phytoremediation. Immobilization can reduce the mobility and bioavailability of heavy metals through addition of remediation agents to contaminated soils. Researchers have reported that amendment with dithiocarbamate chitosan (DTC-CTS) is effective in immobilizing Cd and mitigating accumulation in plants (Yin et al. 2015), and thus potentially alleviate the toxicity of Cd to plants. Phytoremediation is advantageous as it physically removes the heavy metal contaminant from the soil through accumulation in plant biomass. Phytoextraction suffers from several main limitations such as the low biomass of the hyperaccumulators and low bioavailability of heavy metals (Chen et al. 2010). The use of plant growth-promoting rhizobacteria (PGPR) to improve phytoremediation was first proposed by Kloepper et al. (1980) and has since been reported to abate heavy metal stress, promote biomass production, and improve phytoremediation in contaminated soils (Kumar et al. 2015; Marques et al. 2013).

Previous research into the use of PGPR has focused on bacteria (Vessey 2003) or mycorrhizal fungi (Johansson et al. 2004), while relatively fewer attempts were made to use yeasts as plant growth promoter. Yeasts are unicellular fungi which can proliferate rapidly on simple carbohydrates (Mukherjee and Sen 2015). Yeasts have shown adaption to environmental niches including an ability to colonize the rhizosphere in association with plant roots (Amprayn et al. 2012; Botha 2011; Cloete et al. 2009). A diverse range of yeasts have been reported to exhibit plant growth-promoting characteristics, including phosphate solubilization (Alonso et al. 2008; Falih and Wainwright 1995), siderophore production (Sansone et al. 2005), phytohormone production (Nassar et al. 2005), pathogen inhibition (El-Tarabily and Sivasithamparam 2006; Sansone et al. 2005), N and S oxidation (Botha 2011) and stimulation of mycorrhizal-root colonization (Alonso et al. 2008). In addition, several yeasts have received considerable attention and have been applied for the management of heavy metal pollution as their strong resistance and specific metal sorption capacities toward a broad range of heavy metals (Bankar et al. 2012). Therefore, yeasts may be able to play a potential role in the enhancement of plant growth under heavy metal stress.

Sedum plumbizincicola is a Cd hyperaccumulator plant noted for its remarkable Cd extraction capacity from polluted soils (Wu et al. 2008). However, the potential effect of exogenous yeast with plant growth-promoting (PGP) abilities on the growth of *S. plumbizincicola*, Cd uptake, and changes in the microbial diversity of the rhizosphere has not been previously studied. Therefore, to improve our understanding of the potential for exogenous yeast and to improve Cd phytoremediation by *S. plumbizincicola* on heavy metal-contaminated farmland, we (1) isolated a metal-tolerant plant growth-promoting yeast, (2) evaluated PGP characteristics of the isolated strain, (3) observe the effect of the yeast inoculant on Cd hyperaccumulator *S. plumbizincicola* growth and absorption of Cd, and (4) examined the influence of yeast inoculation on the functional diversity indices and activity of the soil microbial community after phytoremediation.

Materials and methods

Isolation of Cd-tolerant strain

Fresh root samples (1 g) were taken from *S. arboretum* that had been grown for 120 days in 20 mg kg^{-1} Cd-contaminated soils in a glasshouse. Roots were washed in sterile water before homogenization in 1 mL of sterile water using sterile mortar and pestle. Serial dilutions of this suspension were prepared (10^{-1}–10^{-3}) and 100 μL was spread onto the SMN medium agar (Belimov et al. 2001) supplemented with 20 mg L^{-1} of $CdCl_2$. The SMN medium's pH was adjusted to 7.0 before autoclaving and ACC (0.5 g L^{-1}) was added as the sole nitrogen source after filter sterilization. After incubation for 7 days at 30 °C, individual colonies of distinct morphology were isolated through streak plating on the YPD medium (1 % yeast extract, 2 % peptone, 2 % dextrose) supplemented with 20 mg L^{-1} of $CdCl_2$. The yeast was selected according to the morphology and microscopic examination.

Identification of isolated yeast strain

DNA extraction

DNA was extracted from yeast cells using a detergent and mechanical-based disruption technique to overcome the more robust cell wall of eukaryotes. The method was adapted from Cheng and Jiang (2006). Briefly, cells were grown overnight in 10 mL of LB then transferred in 1-mL allocates to an Eppendorf tubes and washed in 0.5 mL dH_2O (3000 rpm, 3 min). Cells were then re-pelleted and combined with 300 μL of acid-washed glass beads (Sigma), 200 μL of detergent lysis buffer (2 % Triton X-100, 1 % SDS, 100 mM NaCl, 10 mM Tris–HCl pH 8, 1 mM EDTA), and 200 μL 1:1 phenol/chloroform (bottom layer) for DNA extraction.

Eppendorf tubes were sealed with parafilm and vortexed for 3–4 min on a multi-head vortex at maximum speed. TE (200 μL) (10 mM Tris pH 8, 1 mM EDTA) was added and the samples centrifuged (13,000 rpm, 5 min). The aqueous layer was transferred to a fresh Eppendorf tube and 1 mL of ice cold 100 % ethyl alcohol (EtOH) was added. Samples were mixed by inversion and the DNA pelleted (13,000 rpm, 2 min, 4 °C). The supernatant was discarded and the pellet was resuspended in 400 μL TE (pH 8) with 3 μL of RNase (10 mg mL^{-1} Promega) and incubated for 5 min at 37 °C. DNA was precipitated via the addition of 10 μL of 4 M ammonium acetate and 1 mL of ice cold 100 % EtOH. Samples were mixed by inversion and then centrifuged (13,000 rpm, 2 min, 4 °C). The supernatant was decanted and the pellet air-dried before being resuspended in 50 μL TE (pH 8) and stored at −20 °C for future use.

PCR of 26S rRNA genes

Polymerase chain reaction (PCR) procedures were adapted from Sambrook and Russell (2001). Primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG) and NL4 (5′-GGTCCGTGTTTCAAGACGG) (Kurtzman and Robnett 1997) were used to amplify the D1/D2 variable domain at the 5′ end of the 28S rRNA gene from the gDNA extract.

Amplifications were carried out in 20-μL reaction mixes on a CFX Connect Real-Time PCR Detection System (BioRad). The reaction mixture utilized reagents and buffers supplied by Qiagen and contained 2 μL of 10× PCR buffer, 4-μL of Q solution, 0.4 μL $MgCl_2$ (25 mM), 0.5 μL of Taq DNA polymerase, 9.1 μL dH_2O, 1 μL of each primer (10 mM), 1 μL dNTP mix (10 mM), and 1 μL of gDNA (50 ng) template. Cycle settings used for amplification were as follows: initial denaturation (95 °C for 180 s) followed by 30 cycles of denaturation (95 for 60 s), annealing (52 °C for 45 s), and extensions of 72 °C for 120 s before a final extension (72 °C for 300 s).

Amplified replicate reaction mixes were pooled for purification and analyzed by DNA electrophoresis (1 % Agarose gel with 0.1 μL mL^{-1} Syber Safe, run at 100 V for 45 min). The amplified product was excised from the gel, cleaned up using the WIZARD® SV Gel & PCR Cleanup system (Promega), and sequenced by the Australian Genome Research Facility (AGRF) via Sanger sequencing using the AB 3730*xl* platform. Four replicate 20-μL reactions were used for each sequencing run. Primers for these reactions were the external primers (NL1 and NL2) as well as primers internal to the sequencing region: NL2A (5′-CTT GTT CGC TAT CGG TCT C) and NL3A (5′-GAG ACC GAT AGC GAA CAA G) (Kurtzman and Robnett 1997).

Characteristics of the isolate strain

Heavy metal tolerance and biosorption experiment

Minimal inhibitory concentrations (MICs) for each metal were determined by the plate dilution method (Malik and Jaiswal 2000). In order to avoid precipitation of heavy metals, tolerance to heavy metals was tested in sucrose-minimal salts low phosphate (SLP) medium (Jiang et al. 2008) supplemented with the following metal cations: Cd^{2+} ($CdCl_2$), Cu^{2+} ($CuCl_2$), and Zn^{2+} ($ZnCl_2$) at a range of concentrations (0–3000 mg L^{-1}). The SLP agar plates without metals were used as controls. The experiments were carried out in triplicate. Cultures were incubated at 28 °C for 7 days. The absorptivity to heavy metals (Cu, Zn, Cd) of NSE1 was determined in the YPD medium supplemented with 100 mg L^{-1} of heavy metals.

Effects on the morphology of cadmium

The Cd-tolerant PGP yeast NSE1 was inoculated in mineral salt medium (MSM) supplemented with 200 mg L^{-1} Cd and the control without Cd. After 3 days, the microbial morphology was observed by scanning electron microscope (SEM) according to Staniszewska et al. (2013).

Determination of yeast phosphorus solubilizing

A modified PVK medium was used for all experiments unless otherwise stated, containing (g L^{-1} in distilled water) glucose 10.0, $(NH_4)_2SO_4$ 0.5, NaCl 0.2, $MgSO_4$ 0.1, KCl 0.2, yeast extract 0.5, $MnSO_4$ 0.002, and $FeSO_4$ 0.002. The pH was adjusted to 7.0 for all experiments before autoclaving. Phosphate (PO_4^{3-}) was added at 5.0 g L^{-1} as Ca_3 $(PO_4)_2$. All experiments were conducted with triplicate sample flasks for each time sample, with 25 mL of liquid growth media in 100-mL flasks. Flasks were incubated aerobically with shaking (160 rpm) for 6 days at 28 °C. Every 12 h, 300 μL of culture suspension was taken for $OD_{600\ nm}$ determination. The remainder of the sample was centrifuged at 8000 rpm for 10 min, the pH was determined potentiometrically, and soluble P in the supernatant was quantified by the ammonium molybdate spectrophotometric method as described by Payne (1994).

Field experiments

Microbial inoculum preparation

Field inoculum was prepared by mixing NSE1 with sterilized wheat bran as an inoculation matrix. In brief, NSE1 was grown in a fermenter (East Biotech Equipment and Technology Co., Ltd.) containing YBD media (4 L), 28 °C,

180 r min^{-1}, and 4 L min^{-1} of air flow, to a density of 10^8 colony-forming units (CFUs) mL^{-1} then mixed with wheat bran in ratio of 2:3 before storage at 4 °C and application into the field. Sterilized NSE1 mixed with wheat bran was utilized as a negative control.

Site selection and experimental design

Field experiments were conducted at Xiangtan city (27° 52′ N and 112° 53′ E) in Hunan province, China, to evaluate the effects of NSE1 inoculation on Cd hyperaccumulator *S. plumbizincicola* biomass and Cd uptake. Field experiments were carried out from April 13 to July 16, 2014. Field sites are in a temperate zone with a moist sub-tropical monsoon climate with 1640–1700 h of annual average sunshine. The average annual temperature and precipitation are 16.7–17.4 °C and 1200~1500 mm, respectively. The basic soil properties at the start of the experiment were as follows: pH (1:2.5 soil/water) 4.53, organic matter 40.2 g kg^{-1}, total K 10.4 g kg^{-1}, total P 0.51 g kg^{-1}, available P 15.7 mg kg^{-1}, available K 162 mg kg^{-1}, total Cd 0.70 mg kg^{-1} (determined using routine methods (Lu 1999)).

Stem cuttings (10 cm) of the Cd hyperaccumulator *S. plumbizincicola* were prepared from the middle section of 1-year-old plant shoots. The experiment was conducted in a split-plot design with three replications. The unit sub-plot size was 1.6 × 3.1 m about 5 m^2 and every adjacent plot was separated by a gap in 30 cm. Plant spacing and row spacing in each sub-plot were both 20 cm with 105 *S. plumbizincicola* cuttings planted in every area. Approximately 3 g of microbial inoculum was added to each plant in the soil around the plant stem.

Sampling and analyses of heavy metal

At harvest, the plants were cut at the soil surface and washed with de-ionized water before being oven-dried (105 °C for 0.5 h and subsequently at 85 °C for 5–6 h). Dry plant samples (~0.5 g) were digested using a mixture of 6 mL HNO_3 and 4 mL $HClO_4$, and concentrations of Cd was determined using flame atomic absorption spectrometry (AAS, Varian SpectrAA 220 FS). The soil samples from the plant root in each pot were collected; stored at 4 °C; and partly were air dried, ground, and passed through a 60- and 100-mesh sieve. Soil sub-samples were digested with 5 ml HNO_3 and 5 mL HCl. The concentration of total Cd was measured with a graphite furnace atomic absorption spectrophotometry (GF-AAS) after dilution. The measurements of HCl-extractable and $CaCl_2$-extractable Cd in soil (pH < 7.0) were obtained by equilibrating the soil in 0.1 M HCl and 0.01 M $CaCl_2$ solution, respectively, and were finally analyzed by AAS.

Microbial CLPP analysis

The microbial community function was analyzed using Biolog Eco™ plates adapted from Bundy et al. (2004). An aliquot of 10 g fresh soil was shaken with 100 mL of autoclave-sterilized saline solution (0.85 % NaCl, *w/v*) for 60 min and then aliquots of 10^{-3} dilutions were used to inoculate ECO plates (150 μL per well) and incubated at 25 °C. The plates were read every 12 h ($OD_{590\ nm}$) over 192 h using a BIOLOG automated plate reader. All wells were blanked to the control wells. For the BIOLOG data, average well color development (AWCD) was calculated as described by Garland and Mills (1991). Microbial diversity indices, such as Shannon-Weiner index (Spellerberg and Fedor 2003), McIntosh index, Simpson index, richness and substrate evenness, were calculated according to Gomez et al. (2006).

Statistical analysis

Statistical analysis was conducted with SPSS 17.0 software. Duncan's multiple range tests were used to compare the means of treatments; variability in the data was expressed as the standard errors. All analyses were performed at the $p < 0.05$ level.

Results

Identification and characteristics of the yeast rhizosphere isolate NSE1

A Cd-tolerant isolate NSE1, which could use ACC as the sole nitrogen source, was obtained from the root of the Cd hyperaccumulator *Carpobrotus rossii* grown in a Cd-contaminated soil. The isolate NSE1 was preliminarily identified as *Cryptococcus* sp. by 26S rRNA gene sequence homology (99 % similarity) (GenBank accession number 1859883) (Fig S1). NSE1 showed strong tolerance to the heavy metals Cd, Zn, and Cu with MICs of 1000 mg L^{-1} Cd, 3000 mg L^{-1} Cu, and 3000 mg L^{-1} Zn on plate assays (Table 1). The adsorption rate of NSE1 to Cd, Cu, and Zn and were 26.1, 13.2, and 25.2 %, respectively (Table 1).

Distinct morphological changes of NSE1 in MSM supplemented with 200 mg L^{-1} Cd was observed (Fig. 1). Some irregular spines were formed on the surface compared with the control treatment, which may be polysaccharide substances, and play a key role in the chelation and absorption Cd in yeast.

Determination of yeast phosphorus solubilizing

The growth of NSE1 in the PVK medium was associated with an accumulation of soluble P (Fig. 2 and Fig. 3). P in the

Table 1 Minimal inhibitory concentrations (MICs) and adsorptivity to heavy metals of NSE1

	Cd	Cu	Zn
MICs (mg L^{-1})	1000	3000	3000
Adsorption rate (%)	26.1	13.2	25.2

medium increased over time, while the medium's pH (Fig. 2) quickly decreased from 7.0 to 5.1 within the first 36 h, and finally dropped to 4.5 at the end of the experiment. However, there is a slight fluctuation at 84 h (pH 4.9). Soluble P in the solution did not change according to a sigmoid curve but steadily increased over time. In the first 72 h, soluble P reached 105.4 mg L^{-1} at 72 h but was followed with a sudden decrease at 84 h (40.8 mg L^{-1}). According to the experiment, the solubilizing ability of NSE1 can reach 195.2 mg L^{-1}, which is accompanied by a significant drop in pH (to 5.1) from an initial pH of 7.0 after 72 h.

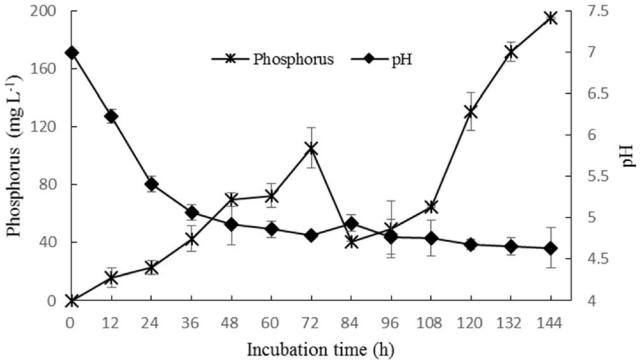

Fig. 2 Phosphorus concentration in culture solution with $Ca_3(PO_4)_2$ and pH varied with incubation time

Effects of microbial inoculation on *S. plumbizincicola* biomass and Cd extraction

Compared with the control group, the dry biomass yield of the inoculated treatment (NSE1) was increased significantly ($p < 0.05$) by 46 % and up to 308 g m^{-2} (Table 2). While the Cd concentration in shoots of NSE1 treatment was not significantly different from that of the control, the total Cadmium extracted (the shoot weight × Cd concentration) rose significantly from 19.6 to 31.1 mg m^{-2} (Table 2), an increase of 58.8 %. This demonstrated that an increase in plant biomass is beneficial to total heavy metal extraction.

Total Cd in the soil treatment inoculated with NSE1 (0.315 mg kg^{-1}) was lower than the control group (0.438 mg kg^{-1}). *S. plumbizincicola* in the inoculated treatment (NSE1) and the control removed about 38.0 and 17.2 % Cd, respectively, in 0.2-m tillage layer of soil bulk with a bulk density of 1.3 g cm^{-3}. Furthermore, the soil HCl-extractable Cd and $CaCl_2$-extractable concentration in NSE1 treatments were reduced by 39.2 and 29.5 %, respectively, compared to the control. It can be concluded that the inoculation of yeast NSE1 could significantly enhance the accumulation of heavy metals cadmium in hyperaccumulator and simultaneously decrease the available heavy metal content in soils ($p < 0.05$).

Analysis of microbial community function

The activity of soil microbial communities evaluated by AWCD was always higher in the inoculated soil than that in the control plot (Fig. 4). No differences in the AWCD was observed within the first 24 h but increased afterwards, and the AWCD of NSE1 was higher than that of the control with the increase of incubation time. A single time point absorbance at 144 h, when the optical density did not increase for the highest number of wells over all plates, was used to calculate diversity indices of the microbial community. The values of Shannon-Weiner index, Simpson index, McIntosh index, and richness were shown in Table 3; the value of richness (calculated using an OD of 0.25 as threshold for positive response (Garland 1997)) for NSE1 was 24.5, which was significantly ($p < 0.05$) greater than 20.5 for the control. The Shannon-Weiner index (provided information on the distribution of carbon source utilization and potential metabolic diversity (Gomez et al. 2006; Garland 1997)), McIntosh index

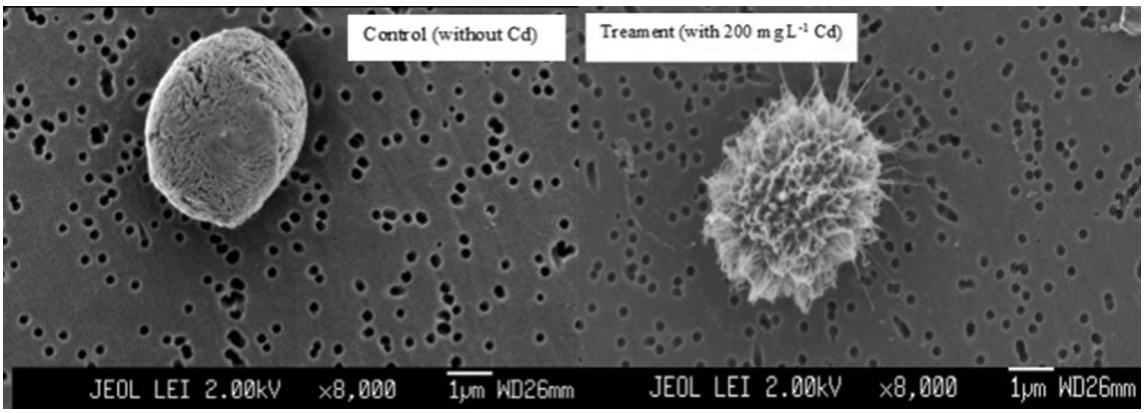

Fig. 1 Effects of cadmium on the morphology of NSE1

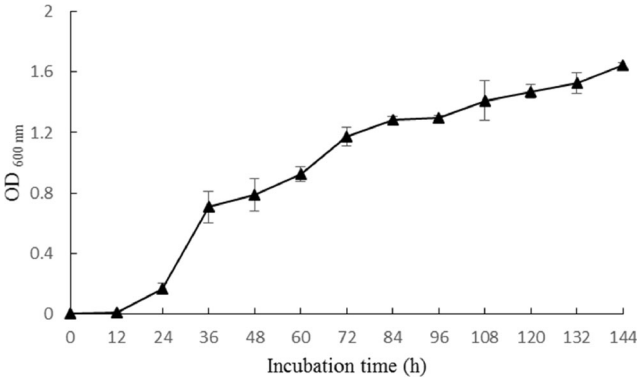

Fig. 3 Growth dynamic of *Cryptococcus* sp. NSE1 varied with incubation time

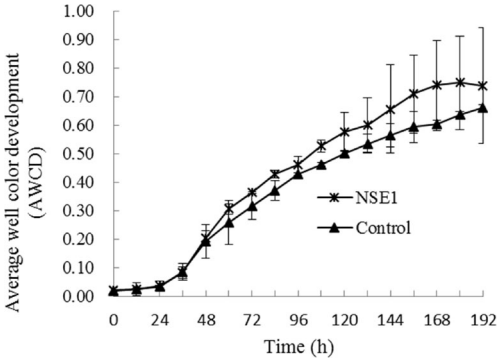

Fig. 4 Variation in average well color development (AWCD) over time in Biolog ECO plates

(provides information on species richness (Zak et al. 1994)), and Simpson index (which quantifies the number of species as well as the relative abundance of each species (Garland 1996)) showed no significant difference between the inoculated NSE1 and the control by analysis of variance (ANOVA) for microbial community diversity indexes ($p < 0.05$) (Table 3). On account of the uncontrollable factors in field trials, the error is large. However, inoculation with NSE1 into the heavy metal-contaminated soil could result in a slight increase in microbial activity and diversity.

Discussion

In this investigation, *Cryptococcus* sp. NSE1, a yeast with high Cd resistances (1000 mg L^{-1}) and the ability to utilize ACC as sole nitrogen source, improved phytoremediation by the Cd hyperaccumulator *S. plumbizincicola*. NSE1 was also found to be resistant and able to absorb zinc, copper, and cadmium (Table 1). NSE1 formed irregular spines on the cell surface when exposed to Cd, which may provide insight into the yeast mechanism for the absorption of heavy metals. The cell wall of yeast mainly consists of glucan, mannosan, protein, amino glucose, phosphoric acid, and lipoid, with a large number of chemical functional groups containing nitrogen, oxygen, sulfur, and phosphorus, which can adsorb Cd^{2+} by Van der Waals electrostatic adsorption or covalent binding to depress the toxicity of heavy metals. Therefore, we proposed that the changes of morphology could have played a role in the process of adsorption of Cd, due to the chelation and complexation of polysaccharide substances. This mechanism needs to be explored further to provide definite details.

While eukaryotic species, such as *Cryptococcus* sp., have been shown to assist phytoremediation and possess antifungal activities (Cloete et al. 2009), plant growth promotion has typically been investigated using rhizosphere-associated PGP bacteria. Yeasts possess analogous beneficial bioactivity to PGP bacteria (Agamy et al. 2013) and have to date been relatively under investigated. NSE1 was isolated from the SMN medium containing ACC as a sole carbon source, implying NSE1 is capable of producing ACC deaminase and lowering plant ethylene levels which is similar to the study of Amprayn et al. (2012). P is one of the major essential macronutrients for biological growth and development (Ehrlich 1990). *Cryptococcus* sp. NSE1 was capable of increasing the availability of dissolved inorganic phosphate (Fig. 2). A large portion of soluble inorganic phosphate, applied to soils as chemical fertilizer, is rapidly immobilized soon after application and is unavailable to plants (Rodriguez and Fraga 1999). PGP strains can exert a positive effect on plant growth through the solubilization of inorganic phosphate and mineralization of organic phosphate, which convert insoluble inorganic P into a form (e.g., $H_2PO_4^-$) usable by plants in acid soils (Lynch 1984). According to the experiment, NSE1 possesses a strong solubilizing ability that can reach 195.2 mg L^{-1} in the PVK medium (Fig. 2). Within

Table 2 Effects of NSE1 on plant biomass, Cd plant concentration, and extractable Cd soil fractions

Treatments	Dry biomass (g m^{-2})	Concentration in plant (mg g^{-1})	Accumulation of Cd (mg m^{-2})	Total Cd in soil (mg kg^{-1})	Extractable in soil	
					HCl (mg kg^{-1})	$CaCl_2$ (mg kg^{-1})
NSE1	308 ± 16a	0.099 ± 0.007a	31.10 ± 0.693a	0.315 ± 0.067a	0.253 ± 0.029a	0.067 ± 0.007a
Control	211 ± 12b	0.093 ± 0.007a	19.58 ± 0.899b	0.438 ± 0.075a	0.416 ± 0.028b	0.095 ± 0.009b

Each treatment contained three replicates. Data were expressed as mean ± standard error. Different letters are statistically different at $p < 0.05$ according to ANOVA (similarly hereinafter)

Table 3 Effect of NSE1 on microbial diversity indexes in soil

Treatments	Shannon-Wiener index	McIntosh index	Richness	Simpson index
NSE1	2.69 ± 0.277a	5.52 ± 0.719a	24.50 ± 0.707a	0.95 ± 0.010a
Control	2.65 ± 0.322a	4.07 ± 0.450a	20.50 ± 0.7077b	0.95 ± 0.007a

Different letters are statistically different at $p < 0.05$ according to ANOVA

these experiments, the pH value of the solution changed inversely to the concentration of soluble P. The solubilization of P is commonly associated with a decrease in pH (Puente et al. 2004) and has been reported in fungi as a result of production of various organic acids, including citric acid, gluconic acid (Reyes et al. 2001; Whitelaw et al. 1999), and oxalic acid (Gharieb 2000). As seen in Fig. 2, soluble P in the solution which rose slowly over time had a peak of 105.4 mg L^{-1} at 72 h and was followed by a sudden decrease at 84 h (40.8 mg L^{-1}). Previously, the phosphate solubilization capacity of the phosphate-solubilizing bacteria has been reported to range from 8 to 233.35 mg L^{-1} (Nautiyal 1999; Liu et al. 2015a; Yu et al. 2011). NSE1 was able to solubilize 195.2 mg L^{-1} when cultured for 6 days, placing it at the higher end of this range.

Interestingly, a sudden repeatable decrease occurred at 84 h during the process of cultivation, accompanied by a slight rise of pH (Fig. 2). In former studies, Illmer and Schinner (1995), and supported by others (de Freitas et al. 1997), found a similar phenomenon of *Penicillium aurantiogriseum* and *Pseudomonas* sp., which they proposed was likely due to the release of protons accompanying respiration or NH_4^+ assimilation as the most probable reason for P solubilization without acid production. Whitelaw et al. (1999) proposed that fungus *Penicillium radicum* may consume more P than it was releasing, resulting in the observed decrease during exponential growth. Therefore, the specific phosphate solubilization mechanism in the soil needs to be further studied.

A number of studies have demonstrated the importance of bacterial inoculation for plant growth and heavy metal accumulation during phytoremediation in heavy metal-polluted environments (Sheng and Xia 2006). NSE1 was able to improve phytoremediation in conjunction with *S. plumbizincicola* when inoculated into contaminated soil in this study. The dry biomass yield of *S. plumbizincicola* (308 g m^{-2}) of treatment (NSE1) was significantly ($p < 0.05$) increased compared with that of the control (211 g m^{-2}). However, the concentration of Cd in the plant biomass showed no significant difference between the control (0.093 mg g^{-1}) and NSE1 (0.099 mg g^{-1}). It seems most likely that inoculation of *S. plumbizincicola* with *Cryptococcus.* sp. NSE1 did not significantly influence the concentrations of Cd in shoot systems (Table 2) but promoted the growth of plants which consequently increased the total Cd uptake, even under field conditions. Various microbial species have been reported capable of protecting plants against the inhibitory effects of cadmium and improving phytoremediation by increasing plant biomass rather than due to an increase in concentration of cadmium in the root and shoot systems. Dell'Amico et al. (2008) also found the similar phenomenon; the strains did not influence the concentration in the shoots or roots but increased the plant biomass and consequently the total cadmium accumulation. Liu et al. (2015a) found inoculation of NSX2 and LCR1 significantly increased the dry weight of plant shoots other than the Cd concentrations in shoots. NSE1 may have exhibited beneficial effects in our experiment due to its resistance and adsorption of Cd, as well as PGP abilities including ACC deaminase production and phosphate solubilization abilities, thus protecting the plants against the inhibitory effects of cadmium while promoting the plant growth and increasing the overall uptake of Cd by increasing the biomass.

Phytoremediation also depends on the activity of the rhizosphere-associated microbial community (Ma et al. 2009). While the function of the microbial community as a whole can enhance the phytoremediation of contaminated soils (Reichman 2007), plants also provide additional surfaces for microbial colonization and organic compounds in root exudates that form a feedback mechanism between the plant and the community (Liu et al. 2015b). The addition of inoculants or an increase in plant growth has the potential to change the community function through changes to this feedback mechanism. Biolog Eco plates were utilized to evaluate qualitatively and quantitatively the community-level physiological profile of the microbial communities in the presence and absence of the NSE1 inoculum. Average well color development (AWCD) was calculated as an indicator of rate of carbon utilization, richness values were calculated as the number of oxidized C substrates, and the Shannon-Weiner index values were calculated using an OD of 0.25 as threshold for positive response. In this present study, the average microbial utilization and microbial diversity was higher in the communities supplemented with NSE1 (Fig. 4 and Table 3). The richness values showed significant difference ($p < 0.05$) between NSE1 and the control. According to Table 3, carbon utilization, metabolic activity, and biological diversity of NSE1 were slightly higher than that of the control treatment. Overall, it appears that the addition of NSE1 may have provided a slight beneficial effect for the microbial community function indirectly through promoting the plant growth or directly by increasing available phosphate while sequestering local Cd.

Conclusions

In this study, a Cd-tolerant plant growth-promoting yeast, NSE1, which can also absorb heavy metals, was isolated and identified as *Cryptococcus* sp. Irregular spines were formed on the surface in the MSM medium containing 200 mg L^{-1} Cd. The irregular spines are speculated to be polysaccharide substances that may form a Cd-polysaccharide complex reducing Cd bioavailability. NSE1 exhibited plant growth-promoting properties, ACC deaminase, and inorganic phosphate solubilization. We speculate that NSE1 inoculation could alleviate Cd toxicity to the plants and enhance the phytoremediation efficiency of *S. plumbizincicola* in Cd-contaminated soil by increasing biomass. Moreover, the microbial function diversity of inoculated NSE1 was slightly higher than that of the control treatment. This system has the potential to improve the removal efficiency of phytoremediation in a Cd-contaminated area through application of eukaryotic and bacterial inoculums. Further study on the mechanisms is needed.

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