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ORIGINAL PAPER

Molecular mechanisms of heavy metals resistance of *Stenotrophomonas rhizophila* JC1 by whole genome sequencing

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Abstract

In this study, a higher metal ions-resistant bacterium, *Stenotrophomonas rhizophila* JC1 was isolated from contaminated soil in Jinchang city, Gansu Province, China. The Pb^{2+} (120 mg/L) and Cu^{2+} (80 mg/L) removal rate of the strain reached at 76.9% and 83.4%, respectively. The genome comprises 4268161 bp in a circular chromosome with 67.52% G + C content and encodes 3719 proteins. The genome function analysis showed *czc* operon, *mer* operon, *cop* operon, arsenic detoxification system in strain JC1 were contributed to the removal of heavy metals. Three efflux systems (i.e., RND, CDF, and P-ATPase) on strain JC1 genome could trigger the removal of divalent cations from cells. cAMP pathway and ABC transporter pathway might be involved in the transport and metabolism of heavy metals. The homology analysis exhibited multi-gene families such as ABC transporters, heavy metal-associated domain, copper resistance protein, carbohydrate-binding domain were distributed across 410 orthologous groups. In addition, heavy metal-responsive transcription regulator, thioredoxin, heavy metal transport/detoxification protein, divalent-cation resistance protein CutA, arsenate reductase also played important roles in the heavy metals adsorption and detoxification process. The complete genome data provides insight into the exploration of the interaction mechanism between microorganisms and heavy metals.

Keywords *Stenotrophomonas rhizophila* JC1 · Heavy metals · Genome · Adsorption · Detoxification

Introduction

Nowadays, lots of researches have focused on application of microbial remediation in heavy metal contaminated soils (Kang and Noh 2016). It will alter the original equilibrium between ions by replacing the essential elements at the cell membrane binding site, and disrupt the normal function of the enzyme, hinder the synthesis of proteins, impair the function of cells and even cause the death of organisms when the concentrations of heavy metals reach at certain levels (Li et al. 2019). To counter such effects, microorganisms have evoluted a series of resistance and response mechanisms via their own physiological and biochemical characteristics to strictly regulate metal homeostasis (Pal et al. 2017; Peng et al. 2018).

Usually, the genomic analysis was carried out to reveal the heavy metal resistance mechanism of microorganisms. Several transport protein-encoding genes in *Pseudaminobacter manganicus* JH-7T that related to heavy metal resistance of the strain were predicted, including Mn^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Cr^{6+}, Hg^{2+} and As^{3+} plasma transport and efflux protein genes (Li 2017). Among them, MntH is a divalent cation transporter that can selectively react to Mn^{2+}, Cd^{2+}, Zn^{2+}, thereby reducing their intracellular accumulation. In addition, Zn^{2+}/Co^{2+}/Cd^{2+}, Ni^{2+}/Co^{2+}, Zn^{2+}, Co^{2+} and Cu^{2+}/Ag^{+} transporters in *Cupriavidus metallidurans* CH34 were predicted that they may remove transition metals outside

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from the periplasm (Nies 2003). Meanwhile, CadA, PbrA, CzcP and ZntA in the genome were predicted to be Zn^{2+}/Cd^{2+}/Pb^{2+}-exporting proteins, which were responsible for Cd2 +, Zn^{2+}, Pb^{2+}detoxification. In *E. coli*, CopA together with the periplasmic copper-containing Cu^{+}-oxidase CueO and the RND-driven efflux system Cus was responsible for copper and silver resistance (Grass et al. 2004; Roberts et al. 2002). Metallothionein, whose induction and specific conglutination with heavy metals or bioaccumulation are one of the common mechanisms of heavy metal resistance and detoxification in microorganisms (Doering et al. 2015). For example, spoVG was significantly upregulated under heavy metal exposure (Nagamine et al. 2005). Besides metallothionein, the antioxidant pathway of microorganisms was also involved in heavy metal resistance and detoxification mechanisms. It was also speculated in other report that the important way to resist Cd biotoxicity is antioxidant stress of *Schizosaccharomyces pombe* due to the secretion of glutaredoxin and catalase increased significantly when *S. pombe* was exposed to Cd (Barak et al. 2006). The research revealed the expression of resistance gene *cup*1 encoding metallothionein in *Saccharomyces cerevisiae* could be induced at a high concentration of copper (Xiong et al. 2019). Due to contributions of CCc2P and PcalP, copper homeostasis in *S. cerevisiae* cells can be maintained well. CCc2P is one of the enzymes that excretes cytoplasmic copper ion to extracellular surroundings through the secretory pathway (Chen et al. 2006). Moreover, the Cup/Cop clusters contain genes encoding for sensor, chaperone, efflux, cytochrome C, and oxidoreductase that regulate the cyto- and periplasmic detoxifications of Cu^{+}/Cu^{2+} (Ha et al. 2011).

Stenotrophomonas rhizophila was known to be able to restore hexavalent chromium (Wu et al. 2019), and further used as a type of iron carrier high-yield microbial inoculum for heavy metal remediation of contaminated soil in farmlands. In this study, we aimed to evaluate the adsorption efficiency of the JC1 strain at different metal concentrations as well as to explore the possible resistance mechanism by sequencing the whole genome of this strain.

Material and methods

Isolation and identification of strain

The strain JC1 was isolated from heavy metals-contaminated soil in Jinchang (102°04′ to 102°43′ east longitude and 37°47′ to 39°00′ north longitude) area of Gansu province. After isolation, it was first purified to obtain a pure culture and then stored in cryovials with 20% (V/V) of glycerol at − 80 °C for further study. The strain was cultured to the logarithmic phase in a beef extract peptone liquid medium. Genomic DNA of the JC1 strain was extracted using Ezup Column Bacteria Genomic DNA Purification Kit (Sangon, Shanghai, China). The 16S rRNA gene of the JC1 strain was amplified by PCR with universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′). PCR amplification was conducted according to the method described by Klemetsen (Klemetsen et al. 2019). The PCR products were pooled and purified using a gel extraction kit (Sangon, Shanghai, China), and then sequenced at Sangon Biological Engineering Co., Ltd. The sequence of 16S rRNA gene of this strain was compared through NCBI GeneBank (https://www.ncbi.nlm.nih.gov/) and EzTaxon (http://www.eztaxon.org/). The MEGA6.0 Software was used to align the sequence, and then the maximum reduction method was applied to construct a phylogenetic tree. The bootstrap value was selected as 1000, and Kimura's two-parameter model was introduced as the calculation method parameter.

Meanwhile, the morphological characteristics of the tested strains were determined via single-colony observation, Gram staining and scanning electron microscopy (SEM) (Sun 2016). The growth characteristics of the strain were determined in the presence of oxygen, pH, temperature and NaCl concentration being as variables, respectively. Physiological and biochemical characteristics were determined according to the method described by Guibaud (Guibuad et al. 2008) and no-intestinal gram-negative bacteria identification system (API 20 NE) was employed to detect the metabolism of strain JC1 (Li 2017).

Experimental treatments

The JC1 strain was cultured in LB liquid medium at 170 rpm and 35 °C. The cell suspension was prepared to an optical density of 0.6–0.8 at 600 nm (OD_{600}). One-percent aliquot of the cell suspensions was added into fresh liquid medium for respective treatment with Pb^{2+}, Cu^{2+}, Cr^{6+}, Ni^{2+} under different concentrations, i.e., 0 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L. The cells were harvested by centrifugation at 10,000 rpm after culturing for 24 h. Each treatment contained three biological replicates.

Determination of metal ions-removal efficiency

Several indexes concerning the growth of this bacterium were determined after 24 h of cultivation. The metal ion concentration in the liquid medium and that on the surface or inside of bacterial cells were determined via graphite furnace atomic absorption spectrometry (Wang et al. 2020). Metal ions-removal efficiency (%) was determined based on the following formula:

$$\text{Removal efficiency } (\%) = (X-Y)/X$$

where *X* denotes the concentration of metal ions in the control and *Y* denotes the concentration of metal ions in the treatment.

Whole-genome sequencing and assembly

The Heliscope/Helicos Genetic Analysis System was used for whole-genome sequencing. Clean data could be obtained for analysis through quality control of the original lower-level data and by removing low-quality sequences. Statistics on clean data were performed to obtain valuable information such as total data, read length, quality value distribution, etc.

The SMRT Link v5.0.1 Software was used to assemble the reads to obtain preliminary assembly results. The reads to the assembled sequence were compared, the distribution of sequencing depth was evaluated, the preliminary assembled sequence was distinguished as the chromosomal or the plasmid sequence according to the sequence length and alignment, and whether the sequence could form a loop was checked.

The analysis of genome function

The amino acid sequence of the target species was analysed with the Gene Ontology (GO) database (http://geneontology.org/) and Pfam-A database (http://pfam.xfam.org/), respectively. The Carbohydrate-Active enZYmes (CAZy) database (http://www.cazy.org/) was employed to identify the genes in the genome that encode glycosylhydrolases. The Transporter Classification Database (TCDB) (http://www.tcdb.org/analyze.php) was used to predict the genes that encode transport proteins. In addition, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www.kegg.jp/kegg/pathway.html) was used to speculate the metabolic pathways and genes encoding key enzymes in the genome.

Results

Isolation and identification of the strain

Morphological characteristics

The single-colony of *S. rhizophila* JC1 appeared milky white and round, whose surface was moist, smooth, slightly convex, and opaque when cultured in LB solid medium for 24 h. Based on the staining results, the cell was observed to be Gram-negative and have no capsule or spore. The results of SEM showed that the cell were short and rod-shaped, with a length of about 1.8 ~ 2.3 μm (Fig. 1).

The growth characteristics

S. rhizophila JC1 is strictly aerobic, and its growth temperature range is 10–40 °C, whose optimum growth temperature is 37 °C. The pH growth range is 2–10 and the optimum pH is 7.0. The tolerance range of NaCl is 0% ~ 7% and the optimal NaCl concentration is 1%, indicating that *S. rhizophila* JC1 is a kind of moderately salt-tolerant bacterium.

Physiological and biochemical characteristics

The results of physiological and biochemical determinations (Table 1) showed that the strain JC1 having nitrate reducibility, but not nitrite reducibility. Sugars (i.e., sucrose, fructose, glucose, maltose, etc.) and alcohols (i.e., inositol, mannitol, sorbitol) could be used as its carbon source. While, acid can be produced only when mannitol was metabolized, and neither acid nor gas could be produced by other. Contact enzyme testing (i.e., Lysine decarboxylase, Ornithine decarboxylase, Arginine dihydrolase) was negative.

Phylogenetic analysis based on 16S rRNA sequence

The complete 16S rRNA gene sequence of strain JC1 with a full length of 1429 bp was obtained and submitted to the

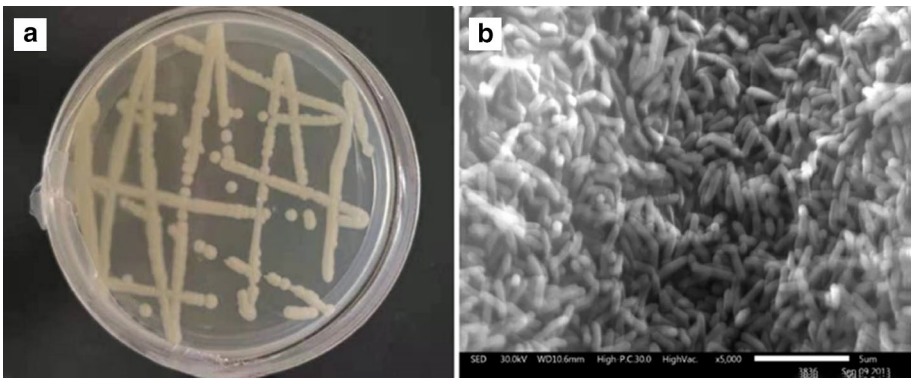

Fig. 1 The morphological features of strain JC1

Table 1 Physiological-biochemical features of strain JC1

Items	Description	Items	Description
Lysine decarboxylase	–	Arabinose	+
Ornithine decarboxylase	–	Sucrose	–
Arginine dihydrolase	–	Fructose	–
Oxidase	–	Glucose	–
Cellulase	+	Lactose	–
Amylase	–	Galactose	–
Protease	+	Xylan	–
Xylanase	–	Phosphorus dissolving	–
Inositol	–	Starch hydrolysis	–
Mannitol	+	Urease determination	–
Sorbitol	–	Nitrite reduction	–
Citrate utilization	–	Nitrate reduction	+
Indole	–	a-ketolactose	–
Methyl red test	+	Pyocyanin	–
a-ketolactose	–	Pyocyanin	–

Note: "–" represents the negative result; "+" represents the positive result

NCBI database (CPO50062). The similarity analysis in GeneBank and EzTaxon revealed that the similarity between strain JC1 and *S. rhizophila* OTU2 reached at 99.98%, followed by that with *S. maltophilia* NM62_B4-13 (99.93%). While the similarity with other strains was lower than 99.9%, most of whom belong to strains of the *Stenotrophomonas spp.* The phylogenetic and evolutionary analysis showed that strain JC1 had stable aggregation with a strain of *S. rhizophila* (Fig. 2).

Combined with morphological structure, physiological and biochemical characteristics and phylogenetic results, the strain JC1 was identified as *Stenotrophomonas rhizophila* JC1.

Metal ions-removal efficiency

Although this tested strain could survive in the presence of several kinds of heavy metal ions after adaptation for various degrees of periods, the adsorptions and resistances to different heavy metal ions exhibited relatively large differences. In detail, when *S. rhizophila* JC1 was cultured respectively in the presence of 40 ~ 120 mg/L Pb^{2+}, 40 ~ 160 mg/L Cu^{2+}, 40 mg/L Cr^{6+} or 40 mg/L Ni^{2+} for 24 h, there was no significant difference in OD_{600} value. However, when the concentrations of Cr^{6+} and Ni^{2+} were increased to 120 mg/L and 80 mg/L, respectively, the bacterium hardly grew. The Pb^{2+} bio-removal efficiency was determined to be 76.9% in the presence of 120 mg/L Pb^{2+}, while it reduced to 25.9% in the

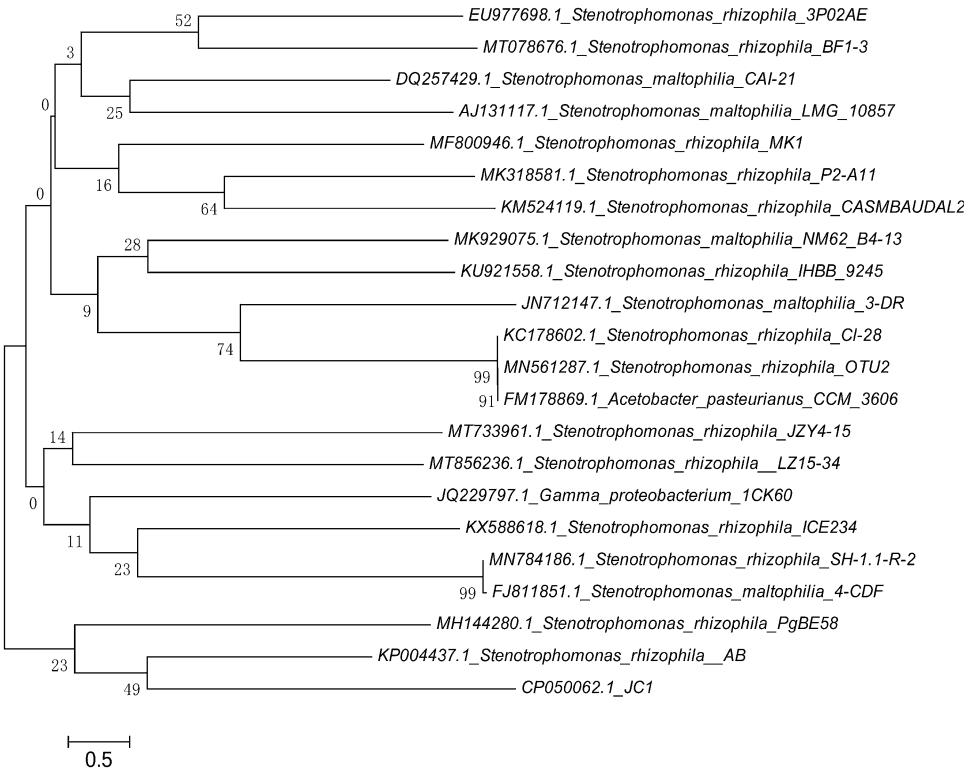

Fig. 2 The phylogenetic analysis of strain JC1based on 16S rRNA sequences

presence of 160 mg/L Pb^{2+} as expected. Interestingly, it was noticed that Cu^{2+} within the concentration of 40~160 mg/L had no significant inhibition effect on the growth of the bacterium, but the strain only showed good metal ion-removal ability with 83.4% in the presence of 80 mg/L Cu^{2+}. As far as Cr^{6+} and Ni^{2+} were concerned, the cell had almost no removal ability (Fig. 3).

Genome assembly

There were 113,175 high-quality reads obtained, and these reads were assembled using the SMRT Link v5.0.1 System. The assembly process revealed that the initially assembled sequence was a circular chromosome sequence. The sum of the contig length was 4,268,161 bp and the G + C content was 67.52%, indicating good-quality assembly for further downstream analysis (Table S1). The GeneMarkS Software (http://topaz.gatech.edu/) used to predict the encoding gene revealed that the genome of *S. rhizophila* JC1 harbored 3719 protein-encoding sequences. The average gene length was 1003 bp, with 288 genes spacing at every 1 Mb of the genome, which indicates that *S. rhizophila* JC1 has a dense gene similar to that of other bacteria. The average distance between the genes was 1.12 kb with a G + C content of 64.1% in the gene internal region. The results of non-encoding RNA annotation revealed that there were 68 tRNAs and 10 rRNAs. Consequently, we predicted that the genome of *S. rhizophila* JC1 may contain several genes related to material transport, which may play an important role in the absorption and exportation of heavy metals.

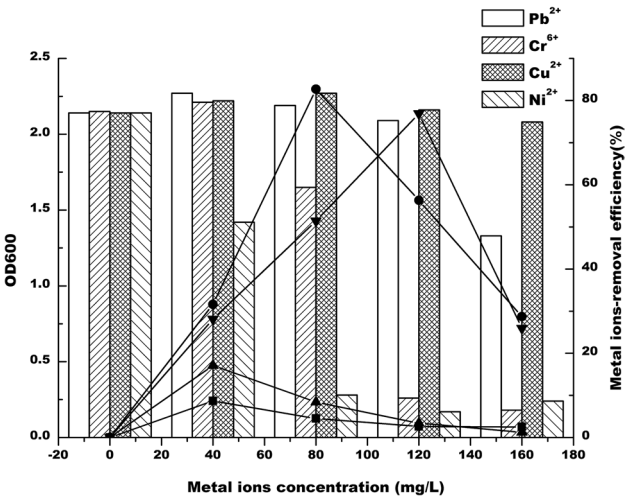

Fig. 3 The metal ions removal efficiency of strain JC1 under different concentrations of Pb^{2+}, Cr^{6+}, Cu^{2+}, Ni^{2+}

Gene ontology (GO) annotation

GO analysis of 9889 genes of *S. rhizophila* JC1 revealed that 51.6%,19.69% and 28.68% of genes were annotated with biological, cellular, and molecular functions, respectively (Fig. 4). Among the biological processes, approximately 17.09% of genes were involved in transport, followed by transcription (11.99%), biosynthetic process (8.37%), redox process (7.3%), DNA-dependent regulation of transcription (5.97%), transmembrane transportation (4.3%), translation (4.13%), carbohydrate metabolic process (2.77%), DNA repair (0.97%) and protein folding (0.64%), in turn. With respect to cellular components, 9.4%,5.2% and 2.5% of genes were found to be associated with the nucleus, cytosol, and cytoplasm, respectively. Similarly, as to molecular functions, around 7.22% of genes were involved in the catalytic activity, followed by DNA binding (6.35%), nucleic

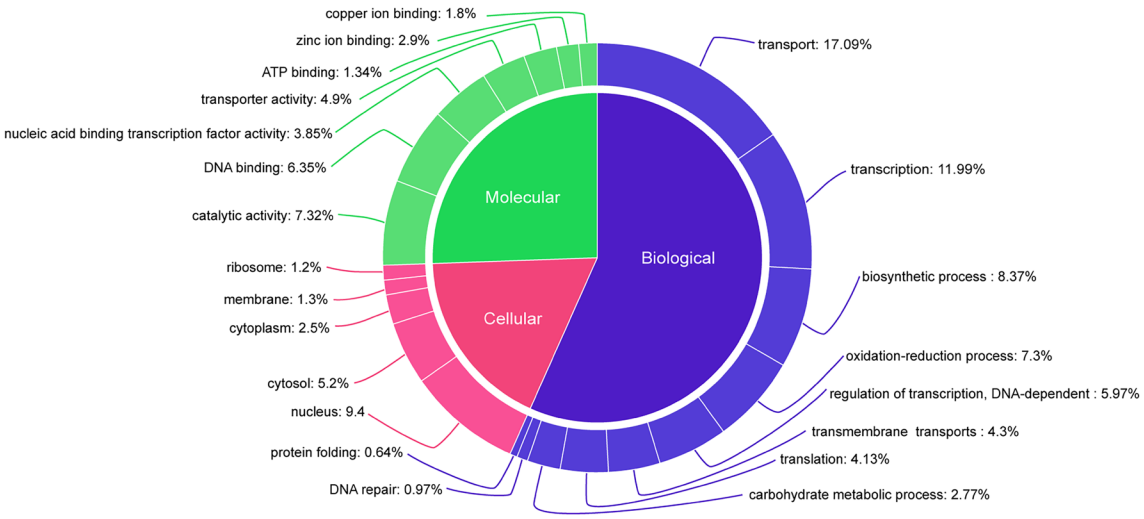

Fig. 4 The gene ontology (GO) annotation of strain JC1

acid-binding transcription factor activity (3.85%), and transportation activity (4.9%).

Protein family (Pfam) domains and putative interaction with heavy metals

The Pfam analysis identified 1608 protein families containing 4549 proteins in *S. rhizophila* JC1. Accordingly, it was found that some protein families were directly related with the adsorption and transportation of heavy metals. The first family is metalloenzyme superfamily. This family includes phosphopentomutase and 2, 3-bisphosphoglycerate-independent phosphoglycerate mutase. The alignment contains the most conserved residues that were probably involved in metal binding and catalysis. The second family is plastocyanin/azurin family. This family is associated with copper resistance and contains sulfocyanin domain, CzcE, Cu-oxidase 2, Cu-oxidase 3, cupredoxin 1 and plysatocyanin-like domain. In addition, copper resistance operons, i.e., CopB, CopC and CopD, were riched in *S. rhizophila* JC1. It had been demonstrated CopC together with CopD are responsible for copper resistance by sequestration of copper in the periplasm (Mishra 2017). The third family is mercuric resistance operon regulatory proteins. The previous report showed that, in the absence of mercury, *merR* represses transcription by binding tightly as a dimer to the *mer* operon region; in the presence of mercury, the dimeric complex binds with a single ion and becomes a potent transcriptional activator while the remaining binds to the mer site (Hou et al. 2001). Moreover, other proteins or enzymes existed in *S. rhizophila* JC1, including heavy-metal-associated (HMA) domain, ion transport protein, zinc-binding dehydrogenase, mechanosensitive ion channel, Mgt C family, ect., probably play a crucial role in resistance maintenance to heavy metals as well.

Transport proteins

Several heavy metal transport-related genes were identified via the analysis of transport proteins in *S. rhizophila* JC1, which mainly included the genes that constituted the three efflux systems. For the cation diffusion facilitator (CDF) system, 2 protein-encoding genes were identified in the genome of *S. rhizophila* JC1. Of which, CzcD/ZitB (JC1_GM001116) behaved as the cobalt-zinc-cadmium efflux system protein that contains cation efflux, cation transporter ATPase C terminus, and dimerization domain of zinc transporter; another hypothetical protein (JC1_GM001883) is an integral membrane protein that was found to increase resistance to divalent metal ions such as cadmium, zinc and cobalt. Six resistance-nodulation-division (RND)system proteins, including two types of *czcCBA* operons, i.e., one possible Cu^{+}/Ag^{+} exporting protein, another possible copper resistance protein, were also identified in *S. rhizophila* JC1. As a member of the RND system, *czcCBA* operons form a complete cobalt-zinc-cadmium resistance system that transports Cd^{2+} and Zn^{2+}, probably Co^{2+}, because of a downstream gene of *czcA* encoding Cd^{2+}/Zn^{2+} exporting ATPase in the genome of *S. rhizophila* JC1. In addition to the CDF and RND systems, the P-ATPase efflux system containing 5 proteins was identified in the genome of this strain. Among them, CopA contains a polypeptide domain of an approximate length of 50 amino acid residues and two cysteines. It is presumed that CopA domain is a Cu^{+} exporting ATPase that transports Cu^{+} or Ag^{+}, but cannot transport divalent ions. ZntA (JC1_GM001891) is a P5-type ATPase cation transporter that contains the plasma membrane calcium transporter ATPase C-terminal domain and Ca^{2+}-ATPase N-terminal autoinhibitory domain.

Besides, there were several specific heavy metal efflux proteins in the genome of *S. rhizophila* JC1, such as putative metal chaperone YciC, TerC, ZiP, and gold resistance ATPase, which indicated *S. rhizophila* JC1 might be an ideal biomaterial for heavy metal pollutant remediation.

Secretory proteins and their functions in heavy metal transportation and detoxification

The secretome analysis of the proteome of *S. rhizophila* JC1 revealed 510 proteins harboring signal peptides (SPs) (Additional file 1). When the heavy metal response transcriptional regulator received stimulation signals triggered by heavy metals, the related genes activated the heavy metal sensor histidine kinase, whose member contains a sensor histidine kinase domain and another domain found in bacterial signal protein associated with heavy metal resistance efflux systems for Cu^{2+}, Ag^{2+}, Cd^{6+}, and/or Zn^{2+} (Mugerfeld et al. 2009). In addition, α-*N*-acetylglucosaminidase was detected in the genome of *S. rhizophila* JC1, which has proven to be extremely important for microbial ATP supply in the process of resisting Pb^{2+} biotoxicity(Raimunda et al. 2012). Further analyses demonstrated that some heavy metal detoxification-related proteins, such as divalent-cation resistance protein CutA, thioredoxin and arsenate reductase, had positive effects on heavy metal detoxification through interaction with other functional proteins.

CAZymes and putative functions in heavy metal detoxification

The analysis of CAZymes of *S. rhizophila* JC1 revealed 2716 proteins encoding CAZymes that were distributed across 142 CAZyme protein families (Table S2). Out of these 2716 proteins, only 232 proteins had SPs (Additional file 2).

Further analysis of CAZymes based on the catalytic activity showed that the metallopeptidase was abundant in the genome of *S. rhizophila* JC1. Among them, M1-M3 and

M13 peptidases were the members of the gluzincin peptidase family (thermolysin-like proteinases, TLPs), which contained the HEXXH motif as a part of their active site. Peptidases in this family bound to a single catalytic zinc ion, which was tetrahedrally coordinated by three amino acid ligands and a water molecule that formed the nucleophile on activation during catalysis. Meanwhile, M14-M15, M20, M23-M24, M40, M48, and M60 peptidases were distributed and every peptidase had an ion binding site, which hydrolyzed single C-terminal amino acids from the polypeptide chains and possessed a recognition site for the free C-terminal carboxyl group.

In addition, there were a series of protease families distributed in *S. rhizophila* JC1 cell. For example, heavy metal translocation P-type ATPase was a superenzyme family that contained a conserved ATP domain, an aspartic acid residue (a phosphatase binding domain), and a phosphatase domain, which contribute to transporting cations into and outside of the cells. A branch of the P-type ATPase included the soft metal cationic transport ATP pump, which could be subdivided into monovalent and bivalent pumps. In particular, in the sixth transmembrane segment, the N-terminal and cys-pro-cys (histidine) were rich in metal-binding sequences. These pumps are often referred to as cpx-type pumps or soft metal P-ATPase (Máthé et al. 2012). Further analysis revealed that the P-ATPase in the genome of *S. rhizophila* JC1 contained a copA/copB copper pump and a K^+ transporting pump, while H^+ transporting pump was an F-type ATPase.

Moreover, arsenate reductase catalyzes arsenical resistance protein ArsH for As (III) and Sb (III) detoxification. RNA binding proteins (RIP) metalloprotease RseP includes a region that hits the PDZ domain, which is found in a number of proteins targeted to the membrane by binding to a peptide ligand. The N-terminal region of this family contains a perfectly conserved motif HEXGH that is found in several metalloproteinases, in which Glu is the active site and His residues coordinate with the metal cation(Molina et al. 2019).

Genes/gene clusters related to heavy metal resistance in *S. rhizophila* JC1 genome

The analysis of the KEGG indicated a series of genes/gene clusters involved in heavy metal adsorption and detoxification that were distributed in the genome of *S. rhizophila* JC1 (Fig. 5).

The *pco/cop* clusters (GM001901–GM001908) contained genes encoding for copper resistance proteins and Cu^{2+} exporting ATPase. In the process of detoxification of copper, Cu^{2+} was bound by copA and copC in the periplasmic space and blocked in the cytoplasm, so that the concentration of free Cu^{2+} in the cytoplasm was

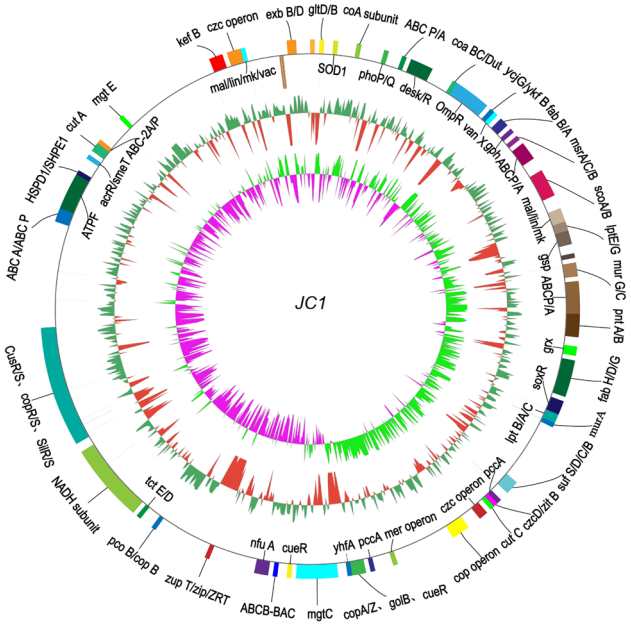

Fig. 5 The genes distribution that related to heavy metals detoxication on strain JC1 chromosome

controlled. CopZ, GolB and PccA were copper chaperones and CueR was a copper efflux regulator. Except that, the copper homeostasis protein encoded by *cutC* contributed to maintaining the copper concentration equilibrium in the cytoplasm and extracellular membrane.

The *czcCBA* operon (GM001887–GM001891) contained genes encoding for chaperone-, efflux-, cytochrome C-, and oxido-reductase proteins that contributed to Co-Zn-Cd adsorption and detoxification. Among them, CzcC was an outer membrane protein, CzcB was a membrane fusion protein, CzcA was responsible for Co-Zn-Cd transportation, and CzcD was a regulatory protein. Its downstream gene encoding protein (ZntA) could hydrolyze ATP to provide energy for the transportation of Cd^{2+}/Zn^{2+}. Similarly to the previous studies (Kang and Noh 2016), the *mer* cluster conferred mercury resistance;, *merP* encoded periplasmic mercuric ion binding protein located in the periplasmic space, *merR* regulated the *mer* operon, and *merT* encoded mercuric transferase that was responsible for mercuric transportation.

Except for genes/gene clusters related to specific metal resistance, we also found some genes/gene clusters related to the complexation and redox of heavy metals. For example, GM000547–GM000550 encoding enzymes were related to glucose dehydration, and GM001273–GM001278 encoding proteins were related to phosphate transportation, all of which could provide heavy metal-binding groups such as –COOH, –OH and –SH.

Phylogenetic relationship, orthology and multigene families of *S. rhizophila*

The orthologous genes result from the formation of species, and orthologous relationship between species contribute to the reconstruction of the evolution of species. Moreover, orthology is the most effective method to identify similarities and differences between, as well as the transfer of functional gene information, from model organisms to uncharacterized newly sequenced genomes (Gabaldón and koonin 2013).

To explore and predict the role of related genes in heavy metal adsorption and detoxification in *S. rhizophila,* homology analysis was performed in 11 strains (*Stenotrophomonas rhizophila, Stenotrophomonas maltophilia, Acetobacter pasteurianus, Achromobacter xylosoxidans, Bacillus megaterium, Citrobacter freundii, Escherichia coli, Pseudomonas stutzeri, Ralstonia. metallidurans, Staphylococcus aureus,* and *Acidithiobacillus. ferrooxidans*) that have been reported to have effects on heavy metals. The clustering of proteomes resulted in 1340 orthologous groups (Fig S1), of which 410 were core orthologous groups among the 11 strains. There were 5636 gene families after clustering, consisting of 34,491 genes and 2112 single-copy orthologous genes, indicating that they are essential genes. There were 192 single-copy orthologous groups, consisting of 309 genes, found distributed (just single-copy gene) in the genome of *S. rhizophila* JC1. The multigene family coded HMA domain (PF00403), copper resistance protein (PF05425), ABC transporter transmembrane region (PF00664), ABC transporter (PF00005), glycosyl hydrolases family (PF04616), oxaloacetate decarboxylase (PF04277), ion transport protein (PF00520), carbohydrate-binding domain (PF02839), and 4′-phosphopantetheinyl transferase superfamily (PF01648), respectively. ABC transporters play important roles in utilizing the energy of ATP hydrolysis to transport substances. Many researchers have revealed that ABC transporters can translocate toxic substances across membranes and impose survivability to the bacteria under harsh environmental conditions. The HMA domain is known to produce some receptor proteins to traffic metal ions within cells and sequester metals in the cell compartment(Wolf et al. 2002).

Further analysis revealed that some homologous gene clusters (Additional file 3) were involved in CAMP metabolism and ABC transportation (Fig. 6). Among these gene clusters, two (JC1_GM000266, JC1_GM000266) OmpR families, two (JC1_GM003241, JC1_GM003242) multidrug efflux pumps, two (JC1_GM002784, JC1_GM003331) lipid A ethanolaminephosphotransferases, and UDP-N-acetylglucosamine acyltransferases (JC1_GM001219) belong to CAMP resistance pathway. Four (JC1_GM000287, JC1_GM000527, JC1_GM000680, JC1_GM000947) putative ABC transport system permeases, four (JC1_GM001274, JC1_GM001275, JC1_GM001276, JC1_GM001277) phosphate transport system ATP-binding proteins, ABC (JC1_GM001201), and lipopolysaccharide transport system

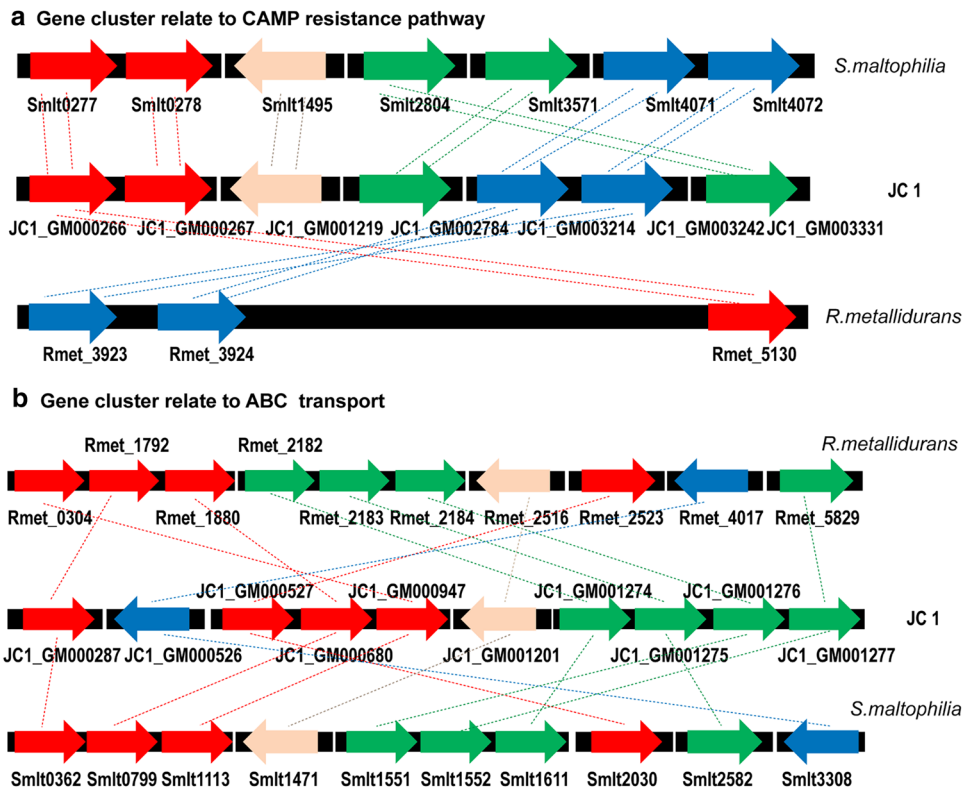

Fig. 6 Organization of putative gene clusters that related to CAMP and ABC pathway

permease (JC1_GM001277) belong to ABC transportation pathway.

Phylogenetic relationship between *S. rhizophila* and other heavy metal resistant bacteria was predicted by protein similarity of 100 randomly chosen single-copy orthologous genes from orthoMCL analysis (Whelan and Goldman 2001). The results showed that *S. rhizophila* was closely related to *S. maltophilia* (has a detoxification effect on mercury), followed by *P. stutzeri*, *E. coli, C. freundii, R. tallidurans, A. xylosoxidans, A. pasteurianus, B. megaterium,* and *S. aureus* (Fig S2). The closer genetic relationship to *S. maltophilia* implies the shared gene arsenal are required for adaptation to a single host.

Discussion

Until now, *Pseudomonas* spp., *Stenotrophomonas* spp., and *Arthrobacter* spp. have been confirmed to have good resistance to heavy metals(Prapagdee and Wankumoha 2017). These bacteria that remove heavy metals mainly rely on extracellular adsorption and intracellular uptake (Peng et al. 2018). Extracellular adsorption is mainly accomplished by some anion groups such as –COOH, -OH and -SH (Pau et al. 2013). For intracellular uptake, a number of genes have been found to be involved in the bacterial resistance to heavy metals, such as antioxidant-related, metal binding, metal transporter genes (Li et al. 2018). In this study, we isolated a strain of Gram-negative bacterium from soil contaminated with heavy metals and identified it as *S. rhizophila* JC1. Heavy metal treatment experiments showed that the growth of *S. rhizophila* JC1 was not significantly affected in the presence of 40–120 mg/L Pb^{2+}, 40–160 mg/L Cu^{2+}, 40 mg/L Cr^{6+} or 40 mg/L Ni^{2+}, indicating the maximum resistance capacity for 120 mg/L Pb^{2+} and 80 mg/L Cu^{2+}, but very low resistance capacity for Cr^{6+} and Ni^{2+}. This phenomenon indicated that there were specific response mechanisms to different heavy metal stresses in the *S. rhizophila* JC1. We speculated that the stress of some metals might be more likely to activate the explants in the genome of the strain, and normal growth of the strain was the result of the balance between endocytosis and explants (Nies 2003).

The expression and regulation of related genes are necessary for microorganisms to survive in a certain concentration of heavy metal environment. The existence of a number of genes/gene clusters provides guarantee for the resistance to heavy metals by *S. rhizophila* JC1. The *czcCBA* operon, combined with its downstream genes *zntA* and *czcD*, of *S. rhizophila* JC1 could mediate the detoxification of Cd^{2+} or Zn^{2+}, which was similar to the findings from the study on *R. metallidurans* CH34, *R. metallidurans,* and *A. eutrophus* (Grass et al.2000). It is possible that the gene *czcD* can also bind with Cu^{2+} and Ni^{2+} because it contains a cation efflux domain and cation transporter ATPase C terminus (Anton et al. 2004). Heavy-metal transporting P-type ATPase was important for the balance and resistance of metals in cells. Five predicted P-type ATPases promote the efflux of related heavy metals out of *S. rhizophila* JC1. Among them, contrary to CopB, CopA is a Cu^{+} exporting ATPase that can transport Cu^{+} or Ag^{+}, but cannot transport the divalent metal ions. However, in the paper regarding *Bradyrhizobium liaoningense* by Liang (Liang et al. 2016), CopA was found to be responsible for resistances to Cu^{+}, Zn^{2+}, and Cd^{2+}, and in the research of *Salmonella enterica* by Pontel (2007), CopA was reported to be the gold (Au^{2+}) resistance ATPase. A review of related heavy metal resistance genes in the genome of *S. rhizophila* JC1 revealed that it seems to resist most heavy metals, including Cu^{2+}, Pb^{2+}, Cd^{2+}, and Ag^{+}. However, judging from the effect of heavy metal treatment, it only showed some resistance and adsorption capacities to Cu^{2+} and Pb^{2+}. Therefore, we speculated that in addition to the characteristics of microorganisms, the effects of heavy on microorganisms may also be related to the atomic structure and charging properties of heavy metals.

Conclusions

In the present study, the lead (120 mg/L) and copper (80 mg/L) removal rate of *S. rhizophila* JC1 reached at 76.9% and 83.4%, respectively. A large number of genes related to heavy metal transport or detoxification were predicted, such as *czc*CBA operons, zntA, *pco/cop* gene clusters, etc. In addition, CAMP metabolism and ABC transportation of *S. rhizophila* JC1were found to be closely related to bacterial metal tolerance and biosorption.

Although how correlative genes and proteins play roles in the bio-adsorption and bio-detoxification of heavy metals remains unclear, the practicability of this genome from our sequencing effort can help researchers perform relevant research in an efficient manner to analyze the relationship, as well as help to articulate better environmental modification.

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Declarations

Conflict of interest All authors declare no conflicts of interest.

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