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Original Research

The Isolation and Characterization of *Glutamicibacter* DC1 to Induce Carbonate Precipitation of Some Heavy Metals at Low-Temperature

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Abstract

The psychrotolerant bacteria able to induce carbonate precipitation of heavy metals at low temperatures play an essential role in the remediation of heavy metal contaminated soil in cold regions. In this study, a psychrotolerant bacterial strain DC1 with carbonate mineralization potential, identified as *Glutamicibacter* sp., was isolated from tailing soil. The optimal temperatures and pH range for the growth of strain DC1 were 10-30ºC and 6-8, respectively. Besides, strain DC1 showed a high degree of resistance to various metals (Pb>Cu>Ni>Cd). Furthermore, strain DC1 produced a large amount of urease (266.91 U/mL), which induced the fermentation suspension's pH (pH 9.5 at 10ºC). Moreover, the fermentation suspension of strain DC1 could generate carbonate precipitates of various metals (Pb, Cd, Ni, and Cu) to remove them from the solutions. Based on the analyses of XRD, SEM, and EDS, the carbonate precipitates of Pb and Cd have been identified as $PbCO_3$ and $CdCO_3$ mineral crystals, respectively. In contrast, no mineral crystals of Ni and Cu existed. Furthermore, the carbonate minerals of Cd and Pb with regular crystal improved resistance to dilute acid. Thus, this study showed that strain DC1 without acid production could induce carbonate precipitates of Pb, Cd, Cu, and Ni at low-temperature.

Keywords: psychrotolerant bacterium, *Glutamicibacter* sp., microbially induced carbonate precipitate, heavy metals

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Introduction

In recent years, with economic and social development, heavy metals have been released into the environment in many ways and caused series of pollutions [1]. Arable soil pollution caused by co-existing heavy metals has attracted the researcher's attention because of its difficulty in remediation for safe farming [2, 3]. Heavy metal-polluted soil is currently remediated through physical, chemical, and biological methods [4-6]. The physicochemical process has many disadvantages, such as high cost, secondary pollution, which affect soil production function [7]. Hence, it was not suitable for the remediation of arable soil. The biological method based on microbial growth and metabolism is attracting more and more attention in the remediation of heavy metal contaminated arable soil due to its advantages, such as low cost and eco-friendliness [8, 9]. Among the microorganisms used for remediation of heavy metal polluted soil, the one with the capacity to immobilize heavy metal ions by hydrolyzing urea to induce carbonate precipitate are becoming the researcher's targets. For example, *Kocuria flava* sp. and *Sporosarcina koreensis* sp. were reported to be involved in high urease production and high removal efficiency of Cu [10, 11]; *Lysinibacillus sphaericus* sp. was found with the feasibility of Cd bioremediation via carbonate precipitation [12], and *Bacillus cereus* sp. was used to remediate Cr in contaminated soil by inducing stable carbonate mineral [13]. However, most studies on heavy metal remediation by microbially induced carbonate precipitation are focused on individual heavy metal remediation. At the same time, there is a lack of microorganisms that can simultaneously immobilize various or co-existing heavy metals. Kang and So [14] have reported that the mix of multiple bacteria could remove Pb, Cd, and Cu mixture contaminated soils via microbially induced carbonate precipitation. Thus, this kind of microorganisms may have significant application significance in the remediation of heavy metals contaminated arable soil.

The remediation of heavy metal polluted arable soil is generally carried out before planting or after harvest. However, in areas with a long period of low temperatures, such as northeast China where the annual temperature during the year is no more than 15ºC, the microorganisms used for soil remediation should adapt to the low-temperature environment. Unfortunately, most of the microorganisms used for remediation of heavy metal polluted soil are mesophilic, and most of the researches has been carried out under temperature conditions of over 20ºC [10, 12, 14]. Temperature is one factor that affects the growth and metabolism of microorganisms [15]. As far as we know, there is a kind of psychrotolerant bacteria that can grow at low-temperature and assure their metabolisms in a cold region [16]. These psychrotolerant bacteria may play a vital role in the remediation of heavy metals contaminated soil in the cold environment. For example, Kumari et al. [13] found that a psychrotolerant *Exiguobacterium undae* YR10 strain could immobilize Cd in soil by biomineralization at 10ºC. However, the knowledge of the psychrotolerant bacteria and their metabolism for heavy metal carbonate precipitation is unclear.

Therefore, we carried out the following research to provide highly efficient bacteria for remediation of arable soil polluted by various heavy metals. Firstly, we isolated and characterized the psychrotolerant bacteria that hydrolyzed urea and resisted different heavy metals at low temperatures. Then, the removal of heavy metals in solution by the psychrotolerant bacterium through inducing carbonate precipitation was studied. Also, the characteristics of carbonate precipitates were analyzed. Finally, the resistance of these carbonate precipitates to a weak acid solution was conducted. The current research would provide the theoretical basis for remediation of arable soil polluted by various heavy metals in a cold region.

Materials and Methods

Isolation of Psychrotolerant Bacteria

The soil sample in this study was collected from the Hongqiling tailings area of Jilin Province, China (N 42°40', E 125°39'). For the isolation of bacteria, 10 g of the soil sample was added in 90 mL of sterile water sterilized and incubated at 10°C for 30 min in a shaker incubator (160 rpm). Then, 1 mL of the suspension was diluted with the concentration gradients of 10^{-5}, 10^{-6}, and 10^{-7} to prepare for separating the bacterial colony in a nutrient agar medium. These nutrient agar plates were incubated at 10ºC for 5 d in an incubator. Each purified colony that grew in the plates was transferred to the urea agar plate (described by Alizadeh et al. [17]) for urease determination. Next, the colonies with positive urease were collected and incubated into nutrient broth (NB) medium (containing 3 g/L of beef extract, 5 g/L of peptone, 5 g/L of sodium chloride with pH 7.0-7.2) at 10ºC, 160 rpm for 48 h. After that, the bacterial fermentation suspensions were centrifuged at 8000 rpm (4ºC) to collect the bacterial cells. These bacterial cells were diluted with sterile water until OD_{600} of 0.3 (2.0×10^8 CFU/mL) as a bacterial suspension for the following experiments.

To observe the growth and urease activity of these selected bacterial strains, the bacterial suspensions (2% of volume) were incubated in 200 mL of nutrient broth-urea (NBU) medium (containing 3 g/L of beef extract, 5 g/L of peptone, 5 g/L of sodium chloride, and 15 g/L urea with pH of 7.0-7.2) at 10°C, 160 rpm for 54 h. Then, at designated intervals (every 6 h for 54 h), the OD_{600}, pH, and urease activity of the selected bacterial strains were determined.

The Resistance of Isolated Psychrotolerant Bacteria to Heavy Metals Assay

One strain with high growth ability and high urease production was selected for the study of heavy metals resistances from the isolated bacterial strains. Based on the preliminary experiments of heavy metal resistance, we conducted the concentration of heavy metals as Cd(II) concentrations (0-30 mg/L), Pb(II) concentrations (0-600 mg/L), Cu(II) concentrations (0-130 mg/L), and Ni(II) concentrations (0-120 mg/L). The bacterial cell suspension with a volume ratio of 2% was inoculated in 200 mL of NBU medium and incubated at 10ºC, 160 rpm for 48 h. The OD_{600} of the bacterial fermentation suspension was measured at a regular interval time.

Identification of Psychrotolerant Bacteria

The bacterial genomic DNA was extracted from the bacterial cells suspended in sterilized water and boiled at 100ºC for 5 min. The 16S rRNA genes were amplified by polymerase chain reaction (PCR) protocol with universal primers, 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') as described in Al-Dhabaan [18]. Then, purified PCR products were sequenced directly and compared to the GenBank database using the Basic Local Alignment Search Tool (BLAST). Nucleotide sequences were aligned and matched with 16S rRNA, deposited in the NCBI GenBank under accession number MN659446.1 (*Glutamicibacter* sp. strain DC1). A phylogenetic tree was generated using the Neighbour-Joining method in MEGA version 7.0 software [19]. The obtained 16S rDNA sequences. Besides, the partial morphological, physiological, and biochemical properties of strain DC1 were analyzed.

The Bacterial Growth and pH Variation Assay

The bacterial cell suspension (2% of volume ratio) was inoculated in 200 mL of NBU medium with the initial pH of 5, 6, 7, 8, and 9, respectively, and incubated at 10ºC, 160 rpm for 48 h. Then, at a regular interval time (every 6h for 48 h), the OD_{600} and pH were determined to observe the bacterial growth and alkali production and/or change of pH.

Characterization of Heavy Metals Carbonate Precipitates Induced by Strain DC1

In this experiment, the bacterial cell suspension (2% of volume ratio) was inoculated and incubated in 200 mL of NBU medium at 10ºC, 160 rpm for 48 h to collect the bacterial fermentation suspension. Then, 5 mL of 0.5 mol/L $CdCl_2$, 10 mL of 0.5 mol/L $PbNO_3$, 10 mL of 0.5 mol/L $NiCl_2$, and 10 mL of 0.5 mol/L $CuCl_2$ were added in 200 mL of bacterial fermentation suspension, respectively, and stored at 10ºC for 24 h. Afterward, the suspension liquids were collected to determine the heavy metal concentrations that remained in the solution. The precipitates obtained from the bottom of the flasks were dried at 50ºC for 24 h. The characteristics of the precipitates were analyzed using X-ray diffraction (XRD), scanning electron microscope (SEM), and energy spectrum (EDS).

Stability of Mineralization Products in Acid Solution Assay

The dried precipitates collected from section 2.4 were used in this experiment. The 0.5 mol/L hydrochloric acid (HCl) was used to adjust the pH of 5.0 as the acid solution in this experiment. First, 0.2 g of four precipitate products were added into 100 mL of the acid solution (pH 5.0), respectively, in a shaker (160 rpm, 10ºC) for 5 h. Then, the supernatants were filtered through a 0.45 μm membrane, and the concentration of heavy metals (Cu, Pb, Cd, and Ni) were measured, respectively.

The dissolution percentage of the heavy metals contained in precipitate products in acid solution was calculated as the followed equation:

$$\text{Dissolution rate (\%)} = (C_0 \times m)/(C_t \times V) \times 100$$

Where C_0 (mg/g) represents the content of heavy metals in the precipitate products, m (g) represents the mass of precipitate products, C_t (mg/L) represents the concentration of heavy metals in the acid solution after 5 h, and V (L) represents the volume of the acid solution.

All the experiments were carried out with three replicates.

Analytical Methods

The urease activity was determined according to the phenol-hypochlorite assay method by Achal and Pan [2]. The optical density of the color complex was measured at 630 nm (Spectrophotometer, Spectrum Shanghai 722E, China) against the blank (1 mL phenol nitroprusside sodium +1 mL sodium hypochlorite + distilled water). Ammonium chloride (50-1,000 μM) was used as the standard. One unit of urease is defined as the amount of enzyme hydrolyzing (1 μmole urea/min).

After filtered, heavy metal concentrations were analyzed using Graphite Furnace Atomic Absorption Spectrometry (GFAAS) (SP-3590AA, Shanghai, China). The heavy metals removal rate was calculated based on the following equation:

$$\text{Removal efficiency (\%)} = (C_0\text{-}C_t)/C_0 \times 100$$

Where C_0 and C_t are the concentration at initial and at time t of heavy metals ions (mg/L).

The dried precipitates (section 2.4) were examined using the SEM-EDS system (X-550, SHIMADZU, Japan), according to Zhao et al. [20]. Also, the XRD was used to identify the characteristic of these precipitates described by Zhao et al. [20]. Furthermore, the heavy metals precipitates were compared with the International Centre for Diffraction Data (ICDD) mineral crystal database to identify their formula.

Statistical Analyses

The data were analyzed by analysis of variance (ANOVA), and the means were compared by Duncan's test ($p<0.05$) using SPSS software (version 25.0). Error bars on graphs show the standard deviations of measurements. All the graphs were plotted by using Excel software 2013. In addition, the XRD patterns were analyzed using Jade5 software.

Results and Discussion

Isolation of Psychrotolerant Bacteria

Six bacterial strains with positive urease activity were isolated from the tailings area. These bacterial strains determined their growth and production abilities under low-temperature conditions (data not shown). The bacterial strain DC1, which showed the best production rate of alkaline, and urease (Fig. 1), was selected for further study. The strain DC1 grew slowly in the first 8 h, then increased rapidly from 8 to 48 h, and reached the maximum growth value of 1.521 at 48 h under the temperature of 10ºC (Fig. 1a). After that, the growth of the strain was relatively stable. This indicated that this bacterial strain might be psychrotolerant due to its characteristics similar to psychrotolerant bacteria [16].

During the bacterial growth, the pH of the fermentation suspension increased quickly in the first 48 h, then gradually increased and reached a maximum value of 9.56 at 56 h (Fig. 1b). Afterward, the pH of the fermentation suspension seemed no change. There is an observation that the production of urease increased during the enhancement of bacterial growth. The maximum value of urease activity was 266.91 U/mL at 48 h, and then it was stable during the stationary phase of bacterial growth (Fig. 1c). These phenomenons were similar to some previous studies, which reported the bacterial mechanism of urea hydrolysis [14, 21, 22]. The increase of pH and urease production indicated that strain DC1 would have the ability to induce carbonate precipitation [23, 24].

The Growth of Strain DC1 in Different Levels of Heavy Metals

The strain DC1 was tested with the resistance to some common heavy metals (Cd, Pb, Cu, and Ni). The results showed that in the stress conditions of different heavy metals, the strain DC1 could grow in a diverse range of heavy metals concentrations (Fig. 2). The highest concentration for CD1 growth was found to Pb, with the concentration up to 550 mg/L. On the other hand, the lower concentration for its development was observed with Cu concentration (up to 130 mg/L) and Ni concentration (120 mg/L), while strain DC1 could grow in the medium with low Cd content (20 mg/L).

This strain showed the resistance to various heavy metals at different levels of concentrations. The minimum inhibitory concentrations of Cd, Pb, Cu, and Ni by strain DC1 at 10ºC were 20, 550, 130, and 120 mg/L, respectively. This difference in resistance of strain DC1 to various heavy metals might be because of the adaptation of strain DC1 to different pollution areas with elevated heavy metal levels. Gadd [25] demonstrated that microbial cells grown at high metal concentrations had been related to the bacterial mechanisms of resistance and environmental factors. Moreover, heavy metal-resistant bacteria have been reported to participate in heavy metal remediation in contaminated environments [14, 26]. Therefore, our results showed that strain DC1 would potentially immobilize heavy metals in a cold environment.

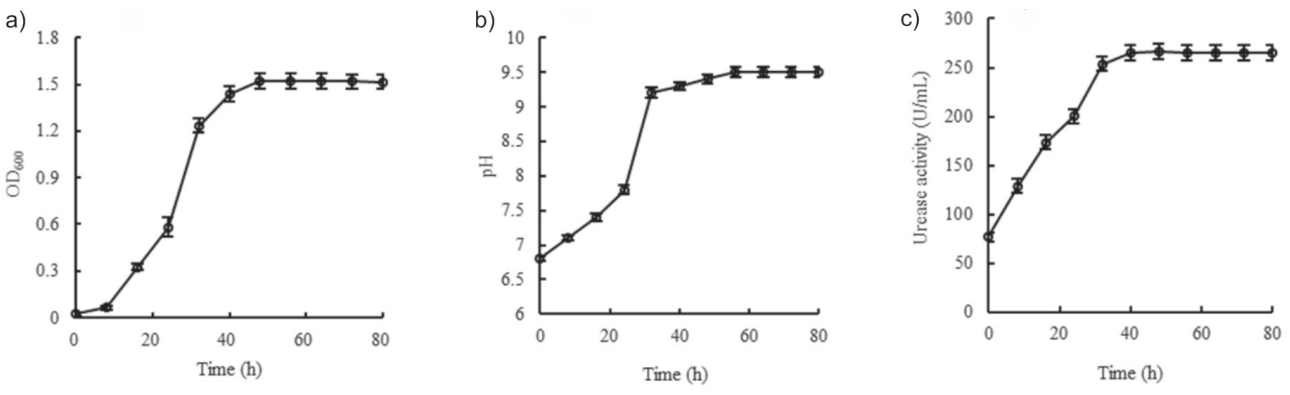

Fig. 1. The bacterial growth a), the change of fermentation suspension pH b), and the urease activity c) of strain DC1 after 80 h of incubation. Each value is the mean of triplicates. Error bars represent standard deviation.

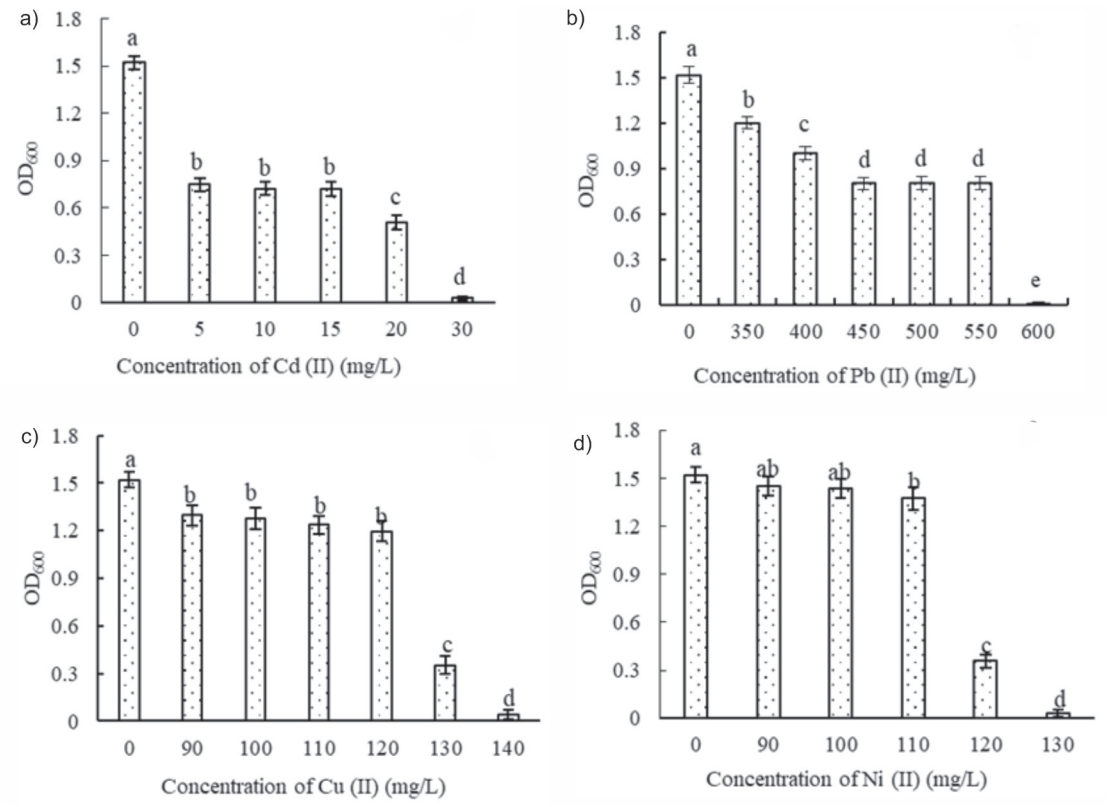

Fig. 2. The growth of strain DC1 in different levels of heavy metals a) Cd (II), b) Pb (II), c) Cu (II), d) Ni (II). Each value is the mean of triplicates. Error bars represent standard deviation. The letters a, b, c, etc. represent the significant difference between treatments based on Duncan's test result ($p<0.05$).

Identification of Strain DC1

Strain DC1 was identified based on colony characteristics, cell morphology, gram staining, and 16S rDNA gene sequence analyses. The results showed that the surface of the strain DC1 was wet, smooth, and yellow opaque on the NB medium. Strain DC1 is a Gram-positive with short and small rod cells. Also, it could utilize glucose to grow but not to produce acid. Besides, the phylogenetic analysis based on 16S rDNA sequences showed that DC1 was homologous to *Paeniglutamicibacter psychrophenolicus* AG31 (Gene bank: NR 027226.1) and *Glutamicibacter soli* SYB2 (Gene bank: NR 044338.1) (Fig. 3). Therefore, strain DC1 was identified as belonging to the *Glutamicibacter* genus.

Strain DC1 could grow in glucose medium but could not assimilate glucose, which has rarely been found with *Glutamicibacter* strains since most of the tested strains utilized this carbon source [27]. This might be due to the adaptation of strain DC1 in the environment containing harmful heavy metals. Also, the lack of acid production can reduce the heavy metal toxicity to limit the damages caused to the strain body. Besides, strain DC1 could hydrolyze urea to produce the urease enzyme. This result is similar to the previous study reported about *Peaniglutamicibacter psychrophenolicus* [28]. However, in this previous study, the authors demonstrated that *P. psychrophenolicus* sp. could grow in various temperatures (1-25°C) and different pH (6-10) as the psychrotolerant bacteria [28]. Hence, strain DC1 would have the ability of a psychrotolerant strain to survive at low-temperature. Moreover, most of the studies on microorganisms that can induce carbonate mineralization by hydrolyzing urea were reported for *Bacillus* sp. [13, 29, 30], *Sporosarcina ginsengisoli* sp. [31], *Lysinibacillus sphaericus* sp. [12], *Kocuria flava* sp. [10]. While, the remediation ability of heavy metal by bacterial-induced carbonate mineralization using *Glutamicibacter* sp. has been rarely reported, especially at low temperatures. Therefore, it is necessary to study the growth metabolism of strain DC1 and its potential for heavy metal remediation under low-temperature conditions.

Effects of the Environmental Factors on the Growth of Strain DC1 and pH Change of the Fermentation Suspension

The growth of strain DC1 and pH variation of the fermentation suspension at different temperatures (Fig. 4) revealed that strain DC1 had the strongest growth with the OD_{600} value of 1.623 and the highest pH value (9.5) at 25ºC. Besides, this strain showed good growth and raising pH under the temperature conditions from 10ºC to 30ºC (Fig. 4a and b) with no significant

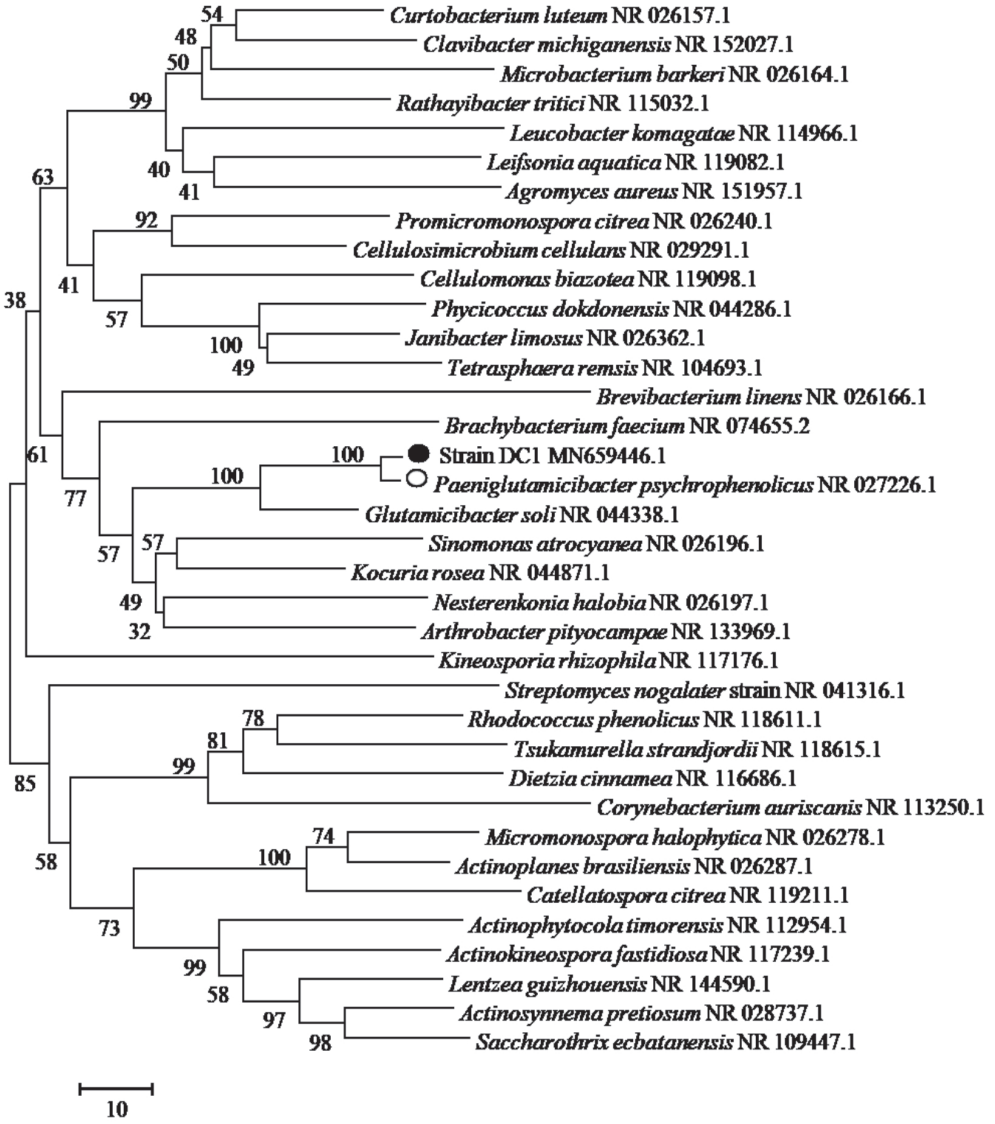

Fig. 3. Phylogenetic relationship based on 16S rRNA sequences of *Glutamicibacter* strain DC1 with available strains at Gen Bank. The numerical values at nodes are percentage bootstrap values based on 1000 replicates.

differences at 10, 25, and 30ºC (Fig. 4b). However, at lower (5ºC) or higher (35-40 ºC) temperatures, the growth of strain DC1 was significantly affected, inducing the decrease in the pH of fermentation suspension (Fig. 4b). This showed that strain DC1 could grow in an extensive range of temperatures (5-40ºC), indicating the ability of a psychrotolerant bacterium [32].

Moreover, the initial pH of the solution also affected the growth of strain DC1 and the change of pH of the fermentation suspension. The strain DC1 was observed with the most substantial growth capacity when the initials pH were 7 and 8 (Fig. 4c). With the lower initial pH of 5 or 6, the bacterial growths were slowly in the first 72 h, then reached the stable stage with similar values when the pH were 7 and 8. The strain DC1 showed the weakest growth when the initial pH was 9, but after 96 h, no significant difference in the bacterial growth was observed with another initial pH. During bacterial growth, the pH of fermentation suspensions was changed in specific regular (Fig. 4d). With all the initials pH, the final pH of fermentation suspension was going to 7.4-7.8.

These results indicated that the strain DC1 could apply for heavy metal remediation under the normal temperature condition and play a crucial role in the remediation of heavy metal polluted soil by inducing carbonate precipitation in the cold regions. Besides, the effect of initial pH on strain DC1 showed that the optimum pH for bacterial growth was 7-8, the optimum pH for most of the psychrotolerant bacteria growth [28, 32]. We found that with a weak acid (pH = 5-6) or weak alkaline (pH = 9), the bacterial growth was slow, but it still could reach the maximum value after 120 h, similar to the growth under 7 or 8. This indicated that strain DC1 is strongly adapted to grow in low-temperature soil with an extensive pH range.

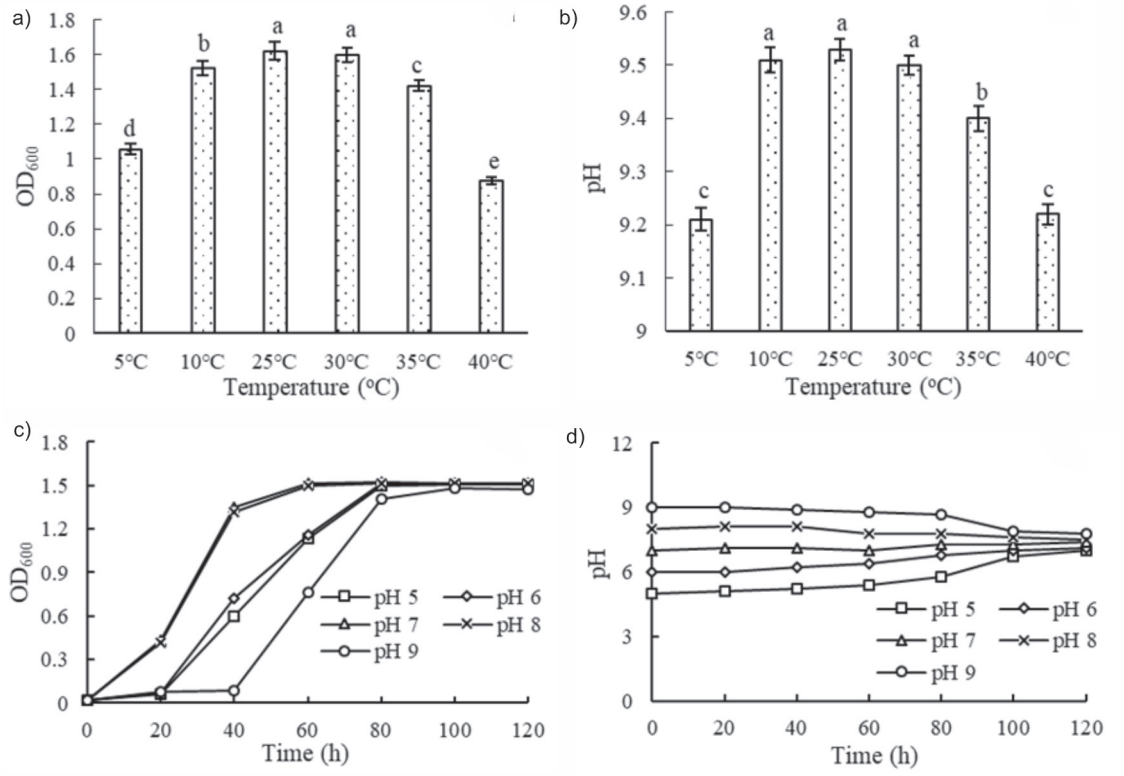

Fig. 4. The growth rate of strain DC1 under different temperatures a) and the influence of various temperatures on the change of pH in the fermentation suspension b). Effects of different initial pH on cell growth c) and pH of fermentation suspension d). Each value is the mean of triplicates. Error bars represent standard deviation. The letters a, b, c, etc. represent the significantly different based on Duncan's test result ($p<0.05$).

The Removal Efficiency of Heavy Metals in Fermentation Suspension at 10ºC

Fig.5 shows the removal efficiencies of four heavy metals (Cd, Pb, Cu, and Ni) in the fermentation suspensions. As can be seen, the highest removal efficiencies have been observed for Cd (II) and Pb (II), with values of 92.67% and 93.67%, respectively. On the other hand, for Cu (II) and Ni (II), the removal efficiencies were less than 50% (specifically, 48.67 % of Cu (II) and 43.33% of Ni (II)).

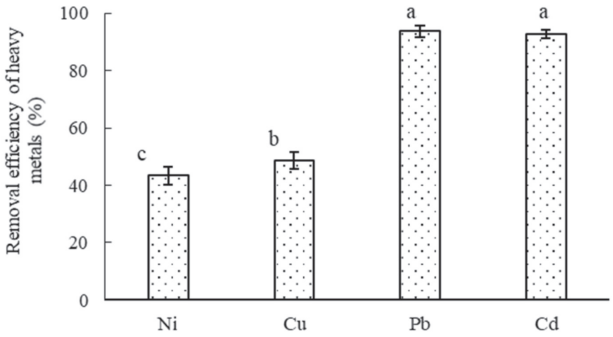

Fig. 5. The removal efficiencies of heavy metals in the fermentation suspension by strain DC1. Each value is the mean of triplicates. Error bars represent standard deviation. The letters a, b, and c represent the significantly different based on Duncan's test result ($p<0.05$).

This could be explained by the different removal mechanisms of strain DC1 to various heavy metals. For example, the previous study of Li et al. [33] showed that the removal rates of Pb and Cd by *T. tumescens* A12 were significantly higher than the ones of Cu and Ni, but all of them could be removed at more than 90%. This contrasts with our results, which could be explained by the type of bacteria that have different metabolism for the removal of heavy metals. Also, the low-temperature condition might affect the removal rate of heavy metals by strain DC1. These results also indicated that strain DC1 could precipitate heavy metals in solution [33].

Characteristics of the Heavy Metals Carbonate Precipitates

The carbonate precipitates of four heavy metals (Cd, Pb, Cu, and Ni) were characterized using XRD, SEM-EDS analyses (Fig. 6). The XRD pattern of Cd and Pb precipitates showed the peaks of Cd and Pb carbonate minerals, respectively (Fig. 6a and b). According to the PDF card database of Jade5 software, we found that they were $CdCO_3$ (PDF 42-1342) and $PbCO_3$ (PDF 47-1734). In comparison, there was no carbonate mineral structure formed in Cu and Ni-containing precipitates (Fig. 6c and d).

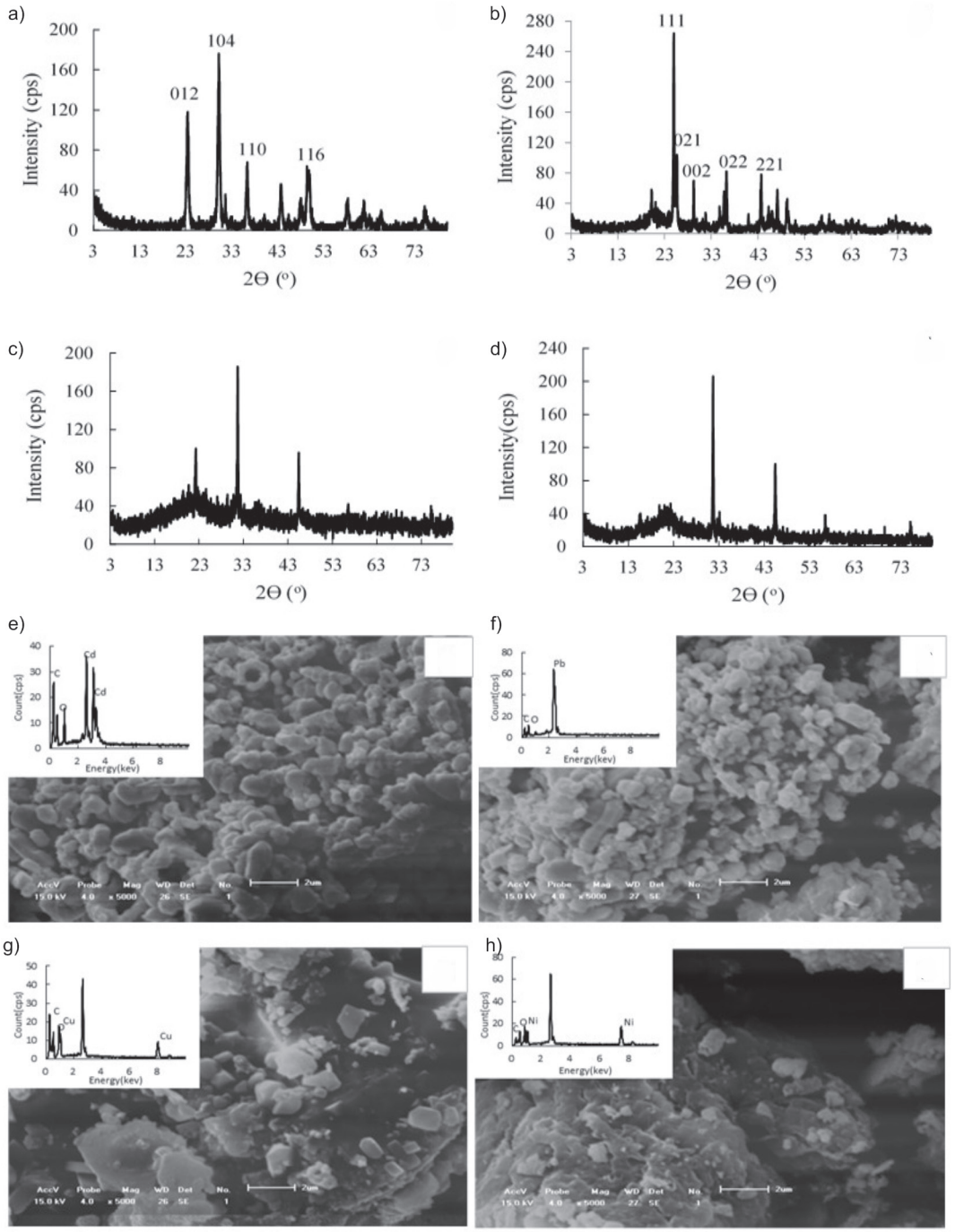

Fig. 6. The characteristics of heavy metals containing carbonate precipitates by strain DC1. The XRD patterns of carbonate precipitates of a) Cd, b) Pb, c) Cu, d) Ni; the SEM-EDS of carbonate precipitates of e) Cd, f) Pb, g) Cu, h) Ni.

The morphological characteristics of four carbonate precipitates using SEM-EDS analyses showed that Cd and Pb carbonate precipitates were regular shapes with rhombohedra, spherical crystal (Fig. 6e), and small spherical, cube crystal (Fig. 6f), respectively. Besides, the EDS analysis showed that the carbonate precipitates of Cd and Pb mainly contain C, O, Cd elements (Fig. 6e) or C, O, and Pb elements (Fig. 6f), respectively, which further proven by the XRD results. However, the carbonate precipitates of Cu and Ni mainly contain C, O, and Cu elements or C, O, and Ni elements, respectively, but no regular crystal structure was found (Fig 6g, h).

These results were related to the removal rates, strain DC1 absorbed more Cd (II) and Pb (II) to form the carbonate minerals, and the few amounts of Cu (II) and Ni (II) absorbed were not enough to create the stable mineral structure. In addition, most of the studies on Cd or Pb carbonate precipitate by ureolytic bacteria were reported with the regular mineral crystal of $CdCO_3$ or $PbCO_3$ [11-13, 29, 34, 35]. In this study, we found similar results that strain DC1 could induce

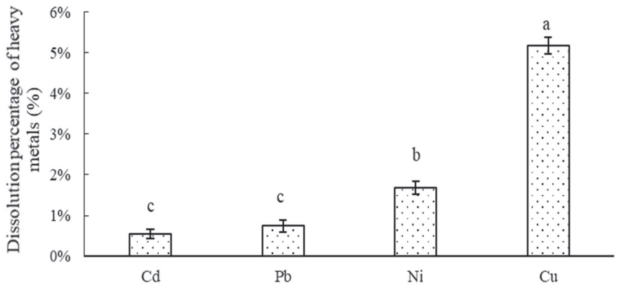

Fig. 7. The dissolution efficiencies of the heavy metals (Cd, Pb, Ni, Cu) containing precipitate products in acid solution. Each value is the mean of triplicates. Error bars represent standard deviation. The letters a, b, and c represent the significantly different based on Duncan's test result ($p<0.05$).

carbonate minerals of Cd and Pb with the rhombohedra and spherical crystal of $CdCO_3$ and tiny spherical and cube crystals of $PbCO_3$. This could be concluded that most of the ureolytic bacteria could form the carbonate minerals of Cd or Pb, but the morphologies of mineral induced by different microorganisms were different [13; 33]. Moreover, the results of Cu and Ni-carbonate precipitates showed the absence of formed mineral crystal, which is similar to Achal et al. [10], who demonstrated that *Kocuria flava* CR1 could remove the high efficiency of Cu from the solution, but no Cu-crystal mineral was recorded. In opposition to this phenomenon, Li et al. [33] reported that *T. tumescens* A12 induced carbonate mineral precipitation of various heavy metals (Ni, Cu, Co, Pb…) to remove these heavy metals from both liquid and porous media. *Furthermore, Bacillus cereus* NS4 was also found to reduce the soluble-exchangeable Ni from the soil and form stable mineral crustal of $NiCO_3$ in remediated soil [30]. However, these above previous studies were conducted under the normal temperature of 27-30ºC, while our study was carried out at low temperature (10ºC), so the temperature might affect the bacterial metabolism and the formation of carbonate mineralization of Cu and Ni.

The Stability of Heavy Metals Precipitates in Acid Solution

The stabilities of the four precipitate products in acid solution were expressed by the dissolution efficiencies of the heavy metals that existed in these products in the acid solution (Fig. 7). The results showed that Cu-containing products had the highest dissolution efficiency (5.17%) in acid solution. The second high dissolution efficiency with a value of 1.68% was found in the Ni-containing precipitate product. The precipitate products of Cd and Pb with the dissolution rate of 0.55% and 0.74% were the least dissolved in acid solution.

The carbonate precipitates of Pb, Cd, Ni, and Cu showed different resistance levels to dilute acid. The carbonate precipitates of Pb and Cd with mineral crystal were more resistant to acid than that of Ni and Cu in the non-mineral crystal structure. This indicates that the regular mineral crystal structure is helpful to improve the stability of carbonate precipitate induced by microorganisms. Therefore, strain DC1 could be considered as an element for remediation of Pb and Cd polluted arable soil, even in areas with severe soil acidification under low-temperature conditions.

Conclusion

In conclusion, a psychrotolerant bacterium, identified as *Glutamicibacter* DC1, was isolated from the tailing area. Strain DC1 showed strong growth ability and high urease production at 10 °C. Meanwhile, it could grow at high concentrations of Pb, Cd, Ni, and Cu respectively. The fermentation suspension could remove most of the heavy metals, in the order of Cd, Pb>Cu>Ni, by inducing the formation of carbonate precipitates. The carbonate precipitates of Cd and Pb were the regular mineral crystals of $PbCO_3$ and $CdCO_3$. In contrast, the ones of Cu and Ni did not show any mineral crystals. Moreover, the carbonate precipitates of Cd and Pb were more resistant to the diluted acid solution than those of Cu and Ni. For the environmental and agricultural practice, strain DC1 can be applied to the soil together with organic fertilizer or alone for the immobilization of Pb, Cd, Cu, and Ni.

In some cases, heavy metals are usually co-existing in soil. Besides, the growth of plants maybe affects the stability of carbonate precipitates of heavy metals. Therefore, our future research work will focus on the carbonate precipitation of co-existing Pb, Cd, Cu, and Ni. Meanwhile, the effect of plant growth on the stability of carbonate minerals will be further studied.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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