Biosynthesis of Pd and Au as nanoparticles by a marine bacterium Bacillus sp. GP and their enhanced catalytic performance using metal oxides for 4-nitrophenol reduction

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1. Introduction

Noble metal nanoparticles (NPs) have been widely used in biome-dicine, chemicals, catalysts, sensors and biosensors due to their unique physicochemical properties [1–6]. As the wide use of noble metal NPs, it’s necessary to develop economical, environmentally safe and efficient recovery methods for their sustainable application. Microbial recovery of noble metal-containing wastewater under mild conditions is emerging as a clean alternative to traditional physical and chemical reclaiming methods. Previous studies showed that biogenic noble metal NPs could be produced intracellular or extracellular by many terrestrial bacteria, including Klebsiella pneumonia, Shewanella oneidensis, Stenotrophomonas, Marinobacter Pelagius, Desulfovibrio desulfuricans, Cupriavidus necator, Pseudomonas putida, Paracoccus denitrificans, Escherichia coli and so on [1,2,6–9]. However, from a practical application perspective, terrestrial bacteria may not survive under extreme conditions (high concentration of salt and metal ions, poor nutrition, low temperature and so on) of those noble metal-containing wastewaters. Therefore, more hardy bacterial resource for producing noble metal NPs need to be further developed. Marine bacteria are the most abundant microorganisms on earth and can thrive at extreme environments and a wide range of acidity, alkalinity, temperatures, and salinity [10]. Moreover, marine ecosystems are thought to be excellent resource of metal-tolerant microorganisms due to continuously release of metals via volcanoes, natural weathering of rocks as well as numerous anthropogenic activities, including mining, combustion of fuels and urban sewage, industrial and agricultural practices. Accordingly, various marine algae, spermato- phytes, yeast, fungi and animals are being explored as the potential biofactories for metallic NPs synthesis in recent years [11]. So far, however, limited marine bacteria with the ability of producing metallic NPs were isolated [12–16]. Thus, it’s a beneficial attempt to further explore functional bacteria from the marine environment for noble metal biosynthesis.

In this study, we isolated 7 marine bacteria from the sediment of Bohai Straits (N 38° 30.29′, E 121° 14.10′, China) and Bacillus sp. GP (GP) was selected for further study due to its potential ability for metal ions reduction (take Pd(II) and Au(III) ions for example). Subsequently, we investigated (i) Pd(II)/Au(III) bioreduction process and mechanism through GP; (ii) the catalytic performance of Pd/Au-NPs on 4-NP reduction; (iii) effects of metal oxides on Pd/Au-NPs mediated 4-NP reduction.

Keywords: Marine bacterium; Pd(II); Au(III); Catalytic reduction; Metal oxides

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ABSTRACT

Recovery of noble metals using marine bacteria is becoming an attractive research area because the marine microbes can better adapt to unfavorable environment than terrestrial microorganisms. In this study, we first reported that a marine Bacillus sp. GP was capable of producing Pd and Au NPs in the presence of sodium lactate. Ultraviolet visible spectrometer (UV–vis), transmission electron microscopy (TEM), X-ray diffraction patterns (XRD), X-ray photoelectron spectroscopy (XPS) and fourier transform infrared spectroscopy (FTIR) analyses were employed to explain the process and mechanism of Pd(II)/Au(III) reduction through GP. Additionally, we also found that bio-Pd/Au NPs could be used as catalysts in chemical reduction of 4-nitrophenol (4-NP). Moreover, the catalytic activity of bio-Pd NPs could be enhanced by Fe3O4, Al2O3 and SiO2, which is beneficial for practical application. The k1 (k2) values of Fe3O4, Al2O3 and SiO2 supplemental systems were approximately 1.28–1.69 (1.15–1.69), 1.42–1.75 (1.53–1.91) and 1.07–1.73 (1.14–1.49) fold, respectively, compared to that of control systems.

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2. Materials and methods

2.1. Chemicals

Na₂PdCl₄ and HAuCl₄ were used to prepare Pd(II) and Au(III) solution and purchased from Shanghai Macklin Biochemical Co., Ltd (China). Acid red 18 (AR 18) and 4-NP used in this study was purchased from Macklin Biochemical Co., Ltd (China) and Tokyo Chemical Industry Co., Ltd (Tokyo, Japan), respectively. All other reagents used in this study were of the highest analytical grade.

2.2. Isolation, identification and growth medium of Bacillus sp. GP

All bacteria were isolated using spread plate technique from the marine sediment, which was taken in the middle of Bohai straits (N 38°30′29′′, E 121°14′10′′, China). The extracellular electron transfer capacity of these bacteria was first tested using a AR 18 reduction system described by Zhang et al. [17]. The experimental systems contained 100 mL deoxygenated sterilized mineral salt medium (MSM), 0.05 mM AR 18, 20 mM sodium lactate and 0.5 g/L strain cells. The tested strain was first cultured overnight in 100 mL LB in a rotary incubator shaker at 150 rpm, 30 °C, harvested by centrifugation (5 min, 10,000 rpm) and washed twice with MSM. Then, the cell pellets were resuspended with MSM and held in an anaerobic chamber. Samples were periodically taken with a sterile needle and a syringe for the analysis of AR 18.

Strain GP was finally selected for further study due to that it could decolour AR 18 fastest in all 7 candidates. Scanning electron microscope (SEM) and 16S rRNA gene sequencing analysis were carried out to characterize strain GP. LB medium (LB) contains (g/L): 10 peptone; 5 yeast extract; 0.1 ferric citrate, 20 NaCl, 5.98 MgCl₂, 3.24 Na₂SO₄, 1.8 CaCl₂, 0.55 KCl, 0.16 yeast extract, 10 NaCl (pH 7.2); The modified 2216E medium (2216E) used in this study contains (g/L): 5 peptone, 1 yeast extract, 0.1 ferric citrate, 20 NaCl, 5.98 MgCl₂, 3.24 Na₂SO₄, 1.8 CaCl₂, 0.55 KCl, 0.16 Na₂CO₃, 0.08 KBr, 0.034 SrCl₂, 0.022 H₃BO₃, 0.004 Na₂SiO₃, 0.0024 NaF, 0.0016 NaNO₃, 0.008 Na₃PO₄, aged-seawater; The mineral salt medium (MSM) used in this study contains (g/L): 1 NH₄Cl, 0.8 Na₂HPO₄, 0.2 KH₂PO₄, 0.2 MgCl₂7H₂O, 0.1 CaCl₂2H₂O, 20 NaCl (pH 7.2).

2.3. Preparation of bio-Pd-NPs and bio-Au-NPs

Effects of pH (3.84–9.56) and NaCl concentration (2–9%, wt%) on growth of GP were first investigated using 2216E under anaerobic conditions. Effect of carbon source, including sodium lactate, maltose, potassium acetate, lactose, glucose and saccharose, on GP growth was also studied using MSM under anaerobic conditions. Under the obtained optimal conditions, bio-Pd-NPs or bio-Au-NPs were prepared by GP cells as following procedures: (i) After culturing 12 h in 100 mL LB medium (LB) contains (g/L): 10 peptone, 5 yeast extract, 0.1 ferric citrate, 20 NaCl, 5.98 MgCl₂, 3.24 Na₂SO₄, 1.8 CaCl₂, 0.55 KCl, 0.16 yeast extract, 10 NaCl (pH 7.2); The modified 2216E medium (2216E) used in this study contains (g/L): 5 peptone, 1 yeast extract, 0.1 ferric citrate, 20 NaCl, 5.98 MgCl₂, 3.24 Na₂SO₄, 1.8 CaCl₂, 0.55 KCl, 0.16 Na₂CO₃, 0.08 KBr, 0.034 SrCl₂, 0.022 H₃BO₃, 0.004 Na₂SiO₃, 0.0024 NaF, 0.0016 NaNO₃, 0.008 Na₃PO₄, aged-seawater; The mineral salt medium (MSM) used in this study contains (g/L): 1 NH₄Cl, 0.8 Na₂HPO₄, 0.2 KH₂PO₄, 0.2 MgCl₂7H₂O, 0.1 CaCl₂2H₂O, 20 NaCl (pH 7.2).

2.4. Characterisation of bio-Pd/Au NPs

To identify the location of the reduced product, GP cell attached with Pd/Au NPs were characterised by TEM (JEM-1400, Japan) and FTIR (Jasco FT/IR-4100, Japan). To identify the structure and valence state of Pd/Au-NPs, the reduced product was further analysed by using UV-vis (PerkinElmer Lambda 365, USA), XRD (BRUKER D8 ADVANCE, Germany) and XPS (ESCALAB 250Xi, England). For TEM analysis, samples were prepared as described by Wu et al. [18]. For XRD, XPS and FTIR analyses, GP cells were exposed to 50 mg/L Pd(II)/Au(III) for 48 h and then separated by centrifugation at 10,000 rpm at 4 °C for 5 min. The pellet was washed with deionised water and dried in an oven at 60 °C. The supernatant was used for subsequent UV-vis analysis.

2.5. The catalytic performance of bio-Pd-NPs/bio-Au-NPs

The catalytic performance of bio-Pd-NPs was further evaluated in 4-NP chemical reduction system. For 4-NP chemical reduction system, the electron donor was NaBH₄ and the electron acceptor was 4-NP. The total volume of the reaction mixture was 3 mL, which contained 8.3 mg/L bio-Pd/Au-NPs, 16.67–66.68 mg/L metal oxides, 1.5 g/L NaBH₄, and 150 mg/L 4-NP in ultrapure water. Control assays without bio-Pd/Au-NPs and with GP powders (drying in the oven at 60 °C for 24 h) only were also performed. The assay mixtures were incubated at room temperature. Each test was conducted three times and the mean was taken to eradicate any discrepancies.

2.6. Analytical methods

The concentration of Pd(II) and Au(III) were determined by inductively coupled plasma mass spectrometer (ICP-MS). The concentrations of cells, 4-NP and AR 18 were determined by UV-vis spectrophotometer at their characteristic absorption peaks (600 nm, 400 nm and 506 nm, respectively). The AR 18 reduction rate and 4-NP reduction efficiency were calculated by using Eqs. (I) and (II), respectively, as follows:

Reduction rate = \frac{C_t - C_i}{C_t} \times 100\% 

Reduction efficiency (%) = \frac{C_i - C_t}{C_i} \times 100\%

Where $C_i$ (mg/L) and $C_t$ (mg/L) are the initial and residual AR 18/4-NP concentrations of cells, 4-NP and AR 18 at time zero and $t$, respectively; $m$ (g cell/L) is the dry weight of the cells; $t$ (h/ min) is the reaction time.

A zero-order model (III) was applied to describe the kinetics of 4-NP reduction. The zero-order rate constant $k_1$ (mol/L min⁻¹) was determined using Eq. (III).

$C_i - C_t = k_1 t$ (III)

A pseudo-first-order model (IV) was used to describe the kinetics of 4-NP reduction. The first-order rate constant $k_2$ (min⁻¹) was determined using Eq. (IV).

$\ln C_i / C_t = k_2 t$ (IV)

3. Results and discussion

3.1. Identification of Bacillus sp. GP and its growth characteristics

Seven facultative anaerobic strains were isolated from the marine sediment and 7# (strain GP) was selected for the following studies. As shown in Fig. S1, the fastest decolouration of AR 18 could be found in the presence of GP cells compared to the other 6 candidates. The reduction rate of AR 18 in GP supplemented system was 11.95 μmol/(h g cell), indicating high reductase activity of GP cells [17]. Previous studies showed that the capacity of bacteria in reducing metal ions was related to their reductase activity [7,8]. Thus, strain GP was chosen for reducing noble metal (Pd(II) and Au(III)) ions in the present study. When strain GP grown on an LB agar plate under aerobic conditions, its colony was white and circular in shape (Fig. 1a). The SEM analysis showed that the morphology of strain GP was long and thin with...
dimensions of $3 \times 0.4 \mu m^2$ (Fig. 1b). On the basis of the sequencing of the 16S rDNA gene, the homology between strain GP (GenBank accession number KY952129) and a Bacillus flexus NBRC 15715 (GenBank accession number NZ_BCVD01000224.1) is 99%. Thus, it could be concluded that strain GP belongs to the genus Bacillus. The phylogenetic tree of strain GP was shown in Fig. 1c. NaCl resistance assay (1–9%) indicated that anaerobic growth of GP in the 2216E had only slight delay with the increase of NaCl concentration from 2 to 5%.
When adding 7% NaCl, strain GP could recover the growth after 20 h incubation. When adding 9% NaCl, growth of strain GP was inhibited severely. Effect of pH on anaerobic growth of GP was also investigated and the result was shown in Fig. 2b. As can be seen, strain GP grew best at pH of 7.31, whereas it failed to survive at pH of 3.84 (Fig. 2b). Effect of carbon source on anaerobic growth of GP was studied for subsequent biosynthesis of Pd-NPs. In the six tested carbon sources, GP grew best with sodium lactate and could not grow with lactose (Table S1). The generational interval was 8.1, 9.1, 39.3, 8.4 and 8.1 h⁻¹ in the presence of sodium lactate, maltose, potassium acetate, glucose and saccharose, respectively. On the basis of above results, the optimum conditions for Pd/Au NPs biosynthesis through strain GP were as follows: 2% NaCl, pH of 7.31 and sodium lactate served as carbon source.

3.2. Pd(II)/Au(III) reduction by GP cells and characterization of bio-Pd/Au-NPs

The capacity of *Bacillus* sp. GP for reducing Pd(II)/Au(III) ions was first examined. After 48 h of reaction, the color of Pd(II) solution changed from pale yellow to black brown and the color of Au(III) solution changed from pale yellow to purple in the presence of GP cells, indicating the formation of bio-Pd NPs (Fig. 3a) and bio-Au NPs (Fig. 3b). TEM images of thin sections of GP cells treated with Pd(II) and Au(III) solutions were obtained to further clarify the formation of Pd/Au NPs (Fig. 3c and d). In addition, particle size analysis showed that the size distribution of bio-Pd NPs and bio-Au NPs was 15–40 nm and 5–30 nm, respectively (Fig. S2). It was observed that Pd NPs were mainly located on the surface of GP cells. Unlike bio-Pd NPs, some bio-Au NPs were found around GP cells and some bio-Au NPs were located on the surface of GP cells. A possible explanation is that the mechanism of Pd(II) reduction through GP and the mechanism of Au(III) reduction through GP are different. The UV–vis sorption results showed that broad absorption bands extending throughout ~300–500 nm and ~550 nm were appeared (Fig. 3a and b), respectively, indicating the formation of bio-Pd NPs and bio-Au NPs, which was consisted with previous studies [19,20].

XRD patterns of different bio-Pd/Au NPs were presented in Fig. 2c and d, the diffraction peaks at ~40°, 46° and 68° could be indexed to the (111), (200) and (220) reflections of the fcc Pd (JCPDS 46-1043) structure (Fig. 4a) and the diffraction peaks at ~38°, 45° and 65° could be indexed to the (111) and (200) reflections of the fcc Au (JCPDS No.04-0783) structure (Fig. 4b) [7,21]. In addition, other diffraction peaks of Pd and Au NPs were maybe attributed to the protein on the surface of GP cells. XPS was used to further characterize the chemical state information and electronic properties of bio-Pd/Au NPs. Fig. 4c...
and d revealed that Pd(II) and Au(III) ions absorbed on the surface of GP cells were reduced to Pd⁰ NPs and Au⁰ NPs, respectively, after 48 h co-culture with stain GP. Before the reduction reaction, the binding energies of Pd(II) 3d⁵/₂ and Pd(II) 3d³/₂ appeared at 343.45 eV and 338.05 eV, respectively. After the reduction reaction, the binding energies of Pd⁰ 3d⁵/₂ and Pd⁰ 3d³/₂ shifted to 334.35 eV and 339.7 eV, respectively, indicating the complete reduction of Pd(II) (Fig. 4c). Before the reduction reaction, the binding energies of Au(III) 4f⁷/₂ appeared at ∼87.2 eV. After the reduction reaction, the binding energies of Au⁰ 4f⁷/₂ shifted to 83.2 eV, indicating the complete reduction of Au(III) (Fig. 4d).

FTIR spectra can reveal possible physical and chemical interactions between Pd(II)/Au(III) ions and chemical groups on the cell surface, FTIR analysis was thus carried out and the results were shown in Fig. 5. As can be seen, some characteristic peaks decreased or disappeared after treating with GP cells for 48 h. These changes are centered at 1050–1650 cm⁻¹, attributed to N–H in plane bending and C–N stretching of amides and carboxyl groups. It’s inferred that some cellular proteins, mainly functional amino groups [22], were involved in Pd(II) and Au(III) reduction through GP. Our current data imply that

Fig. 4. The XRD spectra of bio-Pd-NPs (a) and bio-Au-NPs (b) produced by GP; The XPS spectra of bio-Pd-NPs (c) and bio-Au-NPs (d) produced by GP.

Fig. 5. The FTIR spectra of bio-Pd-NPs and bio-Au-NPs produced by GP.
more general and structural properties of the cells account for the observed formation of Pd⁰/Au⁰ NPs, which may have implications for their potential biotechnological applications.

3.3. Enhanced 4-NP reduction in the presence of bio-Pd/Au-NPs

The catalytic activity of bio-Pd/Au NPs was evaluated in a model reduction reaction. Results showed that no reduction occurred in control assays in 16 min (< 1%) and 4-NP was reduced completely in 13–15 min with the addition of bio-Pd/Au NPs produced by GP cells (−8.3 mg/L, Fig. 6). These results indicated that both bio-Pd NPs and bio-Au NPs could be used as catalysts for heterogeneous catalysis, at least for 4-NP reduction. Considering the trace amount of bio-Pd/Au NPs (~25 µg) used in the catalytic reduction, the catalytic activity of bio-Pd/Au NPs should be considered eligible. However, the catalytic activity of bio-Pd/Au-NPs produced by GP is quiet different because the bio-Pd/Au NPs obtained in different conditions (pH values, concentrations of metal ions and so on) are in various size and shape. Burda et al. pointed out that the phase and morphology variations in Au NPs can alter their physical and chemical properties [23]. It seemed that the catalytic activity of biogenic Au NPs was weaker than those of previous reported Au NPs synthesized by chemical methods. Therefore, we further developed new strategies for enhancing the catalytic performance of biogenic noble metal ions.

3.4. Effects of metal oxides on bio-Pd-NPs mediated 4-NP reduction

Previous studies reported that multiple interfacial reactions can take place between NPs and metal oxides after their combination (such as enhanced electron transport). Special structure of alloy could improve the electron transport and create active sites with high electron density for enhanced electron transport (enhanced reduction). Special structure of alloy could improve the electron transport and create active sites with high electron density for enhanced electron transport (enhanced reduction). Special structure of alloy could improve the electron transport and create active sites with high electron density for enhanced electron transport (enhanced reduction).

3.5. Implications

So far, microbial reduction of noble metal ions had attracted a lot of attention recently [1,2,5]. Compared to terrestrial microbes, marine bacteria can better adapt to bad environment (elevated or low temperature, alkaline or acidic pH, high pressure, or high salt concentration and so on) because they get exposure to such unfavorable conditions naturally. Hence, any marine bacteria having the potential for bioremediation can become the ideal candidates for the biological treatment of polluted extreme habitats [26]. Accordingly, in the present study, we investigated a marine bacterium Bacillus sp. GP for noble metal recovery. Results showed that GP could produce bio-Pd NPs and bio-Au NPs in the presence of sodium lactate, which contributed to the list of marine bacteria with the ability for bio-Pd/Au NPs production. To date, only four species of Bacillus were ever reported that could produce noble metal NPs [10,27–29]. Gopinath et al. reported particularly that the biogenic synthesis of Ag NPs could be achieved by using 100 mL of cell free supernatant of Bacillus sp. GP-23 [30]. Nevertheless, it’s worth noting that Bacillus sp. GP reported in the present study was different from Bacillus sp. GP-23. On the basis of the sequencing of the 16S rDNA gene, the homology between strain GP-23 (GenBank accession number JX156301) and a Bacillus subtilis subsp. (GenBank accession number NC_000964) is 98% and the homology between strain GP (GenBank accession number KY952129) and a Bacillus flexus NBRCC 15715 (GenBank accession number NZ_BCV01000224.1) is 99%. Accordingly, Bacillus flexus was first reported that could reduce Pd(II) and Au(III) in the present study. Moreover, the size of NPs produced by GP and GP-23 was different, resulting to different properties of NPs.

As stated in the present paper, Pd(II) and Au(III) reduction processes by GP were very simple, which is beneficial for further practical application. In addition, the as-prepared bio-Pd/Au NPs could be used as catalysts for 4-NP reduction and the bio-Pd-NPs mediated 4-NP reduction could be enhanced by some metal oxides in a dose-dependent manner. These findings will offer a theoretical foundation for developing novel bio-remediation technologies.

4. Conclusions

In the present study, we demonstrated that a marine bacterium Bacillus sp. GP has potential application in noble metal recovery. Strain GP could reduce Pd(II) and Au(III) to Pd⁰ and Au⁰, respectively, in 48 h with the addition of sodium lactate, the reduction process and mechanism were further explained by UV–vis, XRD, XPS and FTIR analyses. In addition, the catalytic activity of bio-Pd-NPs for 4-NP reduction could be enhanced by some metal oxides (Fe₃O₄, Al₂O₃ and SiO₂), which is beneficial for further practical application. The k₁ (k₂) values of Fe₃O₄, Al₂O₃ and SiO₂ supplemented systems were approximately 1.28–1.69 (1.15–1.69), 1.42–1.75 (1.53–1.91) and 1.07–1.73 (1.14–1.49) fold, respectively, compared to that of control systems. A possible explanation is that metal ions in these metal oxides promoted the reaction as a Lewis acid or the possible formation of alloy perturbs the reactivity of the noble metal sites [24,25]. In addition, bio-Pd-NPs mediated 4-NP reduction could be enhanced in dose-dependent manner of metal oxides (Table 1). These findings will benefit further practical application of bio-Pd NPs and bio-Au NPs that produced by strain GP.

Conflict of interest

None.

Acknowledgements

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Fig. 7. Plots of $C_t - C_0$ vs. time (a, b, c) and $\ln(C_t/C_0)$ vs. time (d, e, d) for the reduction of 4-NP by NaBH₄ in the presence of Fe₃O₄ (a, d), Al₂O₃ (b, e) and SiO₂ (c, f).
Catalysts and metal oxides are 5 g/L and 10 g/L, respectively.

Control system was supplemented with bio-Pd-NPs only, the initial concentration of di-

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.enzmictec.2018.03.002.

References

Table 1

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Control system was supplemented with bio-Pd-NPs only, the initial concentration of catalysts and metal oxides are 5 g/L and 10 g/L, respectively.