Green synthesis of gold nanoparticles using fungus *Mariannaea* sp. HJ and their catalysis in reduction of 4-nitrophenol

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Abstract In the present study, biosynthesis of gold nanoparticles (AuNPs) by the cells (cells-AuNPs) and cell-free extracts (extracts-AuNPs) of a new fungus *Mariannaea* sp. HJ was reported. The as-synthesized particles were characterized by UV-vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). The effects of different parameters on AuNP biosynthesis were investigated, and initial gold ion concentration of 2 mM, pH 7, was demonstrated to be suitable for both cells-AuNP and extracts-AuNP syntheses. The cells-AuNPs were of various shapes, including sphere, hexagon, and irregular shapes, with an average size of 37.4 nm, while the extracts-AuNPs were almost spherical and pseudo-spherical with an average size of 11.7 nm. XRD pattern suggested that the crystal structure of both AuNPs was face-centered cubic. FTIR spectra implied that some biomolecules from the fungal cell walls or cell-free extracts were involved in

the formation of AuNPs. The as-synthesized AuNPs were demonstrated to have excellent catalytic activities for the reduction of 4-nitrophenol with the catalytic rate constants of $5.7 \times 10^{-3}/s$ for cells-AuNPs and $24.7 \times 10^{-3}/s$ for extracts-AuNPs. To the best of our knowledge, this is the first report on AuNP biosynthesis by *Mariannaea* sp.

Keywords Gold nanoparticles · Biosynthesis · *Mariannaea* sp. · 4-Nitrophenol · Catalytic activity

Introduction

Gold nanoparticles (AuNPs) have shown remarkable potentials for numerous applications in photonics, catalysis, imaging technology, drug delivery, and space science due to their good stability, biocompatibility, and oxidation resistance which are different from bulk gold (Barabadi et al. 2017; Daniel and Astruc 2004; Schröfel et al. 2014). Various methods have been developed to produce AuNPs, among which the biological synthesis of AuNPs as an emerging highlight has attracted more and more attention due to its advantages of clean, cost-effective, and environmentally friendly nature (Mandal et al. 2006; Narayanan and Sakthivel 2010; Pereira et al. 2015; Lallawmawma et al. 2015). However, accurate control of size, morphology, and monodispersity of biosynthesized nanoparticles is still one of the big challenges.

A number of microorganisms have been reported to have the capacity of reducing gold ions into AuNPs, including bacteria, fungi, yeasts, and actinomyces (Cai et al. 2011; Shedbalkar et al. 2014; Narayanan and Sakthivel 2011; Pimprikar et al. 2009; Manivasagan et al. 2015). The active molecules present in microbial

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system can be used as reducing and stabilizing agents for the biosynthesis process; thus, no other chemical reagent is required. Among the microbes, fungi are considered as superior microbial resources for the green synthesis of AuNPs due to their high production of biomass, easy handling, and high metal tolerance (Kitching et al. 2015; Das et al. 2012). Mukherjee et al. (2001) firstly reported that the eukaryotic organisms could synthesize AuNPs by the co-incubation of fungus *Verticillium* sp. and HAuCl_4 . Since then, several fungal species have been demonstrated to successfully synthesize AuNPs through intra- or extracellular manners, such as *Botrytis cinerea*, *Candida albicans*, *Penicillium brevicompactum*, *Trichoderma harzianum*, and *Helminthosporium solani* (Castro et al. 2014; Ahmad et al. 2013; Mishra et al. 2011; Tripathi et al. 2014; Kumar et al. 2008). However, the fungal species reported for AuNP biosynthesis are still limited (Kitching et al. 2015).

AuNPs possess a high catalytic activity in the reduction of organic contaminants in water owing to their large specific surface area, plenty of active sites, and low coordination number (Narayanan and Sakthivel 2011). As is well-known, 4-nitrophenol (4-NP) is one of the industrial pollutants with teratogenic, carcinogenic, and mutagenic toxicities, which is commonly used as a basic composition of various explosives, pesticides, and solvents (Goi and Trapido 2002; Zhao et al. 2015). Previous reports have suggested that the biogenetic AuNPs could effectively catalyze 4-NP reduction in a short span of time at room temperature (Narayanan and Sakthivel 2011; Mishra et al. 2014). In addition, the biosynthesized AuNPs usually present much better catalytic activity than AuNPs synthesized by chemical methods (Das et al. 2012). Thus, biological synthesis of AuNPs provides a nontoxic and feasible method for 4-NP degradation.

In this study, a green and cost-effective approach for the synthesis of AuNPs by cells (cells-AuNPs) and cell-free extracts (extracts-AuNPs) of the fungus *Mariannaea* sp. was reported for the first time. The fungus *Mariannaea* was isolated from activated sludge of laboratory reactor, which has been widely found in leaves of conifer trees, decaying wood, and soil (Okuda and Yamamoto 2000; Cai et al. 2010; Zeng and Zhuang 2014; Tang et al. 2012). As far as we know, there is no report on the AuNPs synthesized by *Mariannaea* sp. Herein, the effects of initial gold ion concentration and pH on AuNP synthesis were investigated. The as-synthesized AuNPs were analyzed by UV-vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), dynamic light scattering (DLS), and Fourier transform infrared spectroscopy (FTIR). The catalytic activities of cells-AuNP and extracts-AuNP bioconjugates were also evaluated for the reduction of 4-NP in the presence of NaBH_4 .

Materials and methods

Chemicals and culture media

Activated sludge was collected from the secondary sedimentation tank of Chunliu river wastewater treatment plant (Dalian, China). Gold(III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was purchased from J&K Scientific Ltd. (Shanghai, China). 4-NP was obtained from Sinopharm Chemical Reagent Beijing Co., Ltd. (China). All other reagents were of analytical grade.

The synthetic wastewater used in the bioreactor consisted of 20 mg/L KH_2PO_4 , 90 mg/L NH_4Cl , 10 mg/L NaCl , 12.5 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 12 mg/L CaCl_2 , 10 mg/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 500 mg/L glucose. The culture medium used for the isolation and cultivation of strains was the modified Martin medium, which contained 1.0 g/L NH_4SO_4 , 1.0 g/L K_2HPO_4 , 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 1.0 g/L glucose. Solid medium was prepared by adding 20 g/L agar to the modified Martin medium. The modified Martin media were autoclaved at 115 °C for 20 min prior to use.

Isolation and identification of *Mariannaea* sp. HJ

A sequencing batch reactor (SBR) was constructed and seeded with the activated sludge, which was domesticated with the synthetic wastewater containing 50 mg/L tetracycline for 2 months. Subsequently, 1 mL reaction mixture was plated on the modified Martin agar medium supplemented with 50 mg/L tetracycline and incubated at 30 °C for 3 days. A single colony was picked and cultured in the modified Martin medium containing 50 mg/L tetracycline at 30 °C with continuous shaking for 48 h. Then, the culture was further plated on the modified Martin agar medium with tetracycline (50 mg/L). After about 1-month screening, the fastest-growing fungus was selected and designated as HJ. The colony pattern of strain HJ was observed via potato dextrose agar (PDA) plates, and the morphological characteristics were observed by scanning electron microscopy (SEM, Hitachi SU8020, Japan).

The 26S ribosomal RNA (rRNA) gene of the strain HJ was amplified and sequenced by TaKaRa Biotechnology Co., Ltd. (Dalian, China). The obtained sequence was analyzed using the BLAST program in NCBI and aligned with reference sequences using Clustal X 1.8. The aligned sequences were used to construct a phylogenetic tree using the neighbor-joining method by MEGA 5.1.

Biosynthesis of AuNPs by cells and cell-free extracts of strain HJ

The fungal strain HJ was inoculated into the modified Martin medium and incubated at 30 °C with shaking. After 48 h of

fermentation, the culture medium was filtered with qualitative filter papers and washed thoroughly with sterile ultrapure water for three times. Then, 0.5-g fungal mycelia (wet weight) were resuspended in 5 mL of HAuCl₄ solution with an initial gold ion concentration of 2.0 mM and incubated at 30 °C for 12 h in dark condition. At a certain time interval, the mixture was collected and treated by sonication for 30 min (Ultrasonic Processor CPX 750, USA) to disrupt the fungal mycelia and release the nanoparticles into aqueous solutions. The mixture was then centrifuged at 3000×g for 10 min to remove the cell debris, and the supernatant was monitored using UV-vis spectroscopy. Different HAuCl₄ concentrations of 0.1, 0.5, 1.0, 2.0, and 3.0 mM were used to check the effects of initial gold ion concentration on cells-AuNP synthesis. The fungal mycelia resuspended in 2.0 mM HAuCl₄ solutions were incubated at pH 2, 4, 6, 7, 8, and 10 to investigate the effects of pH, which were adjusted using KOH or HCl.

For extracts-AuNPs, the harvested mycelia were resuspended in phosphate sodium buffer (PBS, 50 mM, pH 7) and then lysed by sonication for 40 min. The cell debris were removed by centrifugation at 10,000×g for 20 min, and the supernatant was filtered through 0.45-μm Millipore filters, which was donated as the cell-free extracts. The concentration of protein in the cell-free extracts was regulated to 100 mg/L based on Bradford assays (Bradford 1976). Biosynthesis of extracts-AuNPs was performed by mixing the cell-free extracts with 2.0 mM HAuCl₄. Then, the reaction mixture was incubated at 30 °C for 6 days in dark condition. Different HAuCl₄ concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, and 5.0 mM were added to PBS to investigate the effects of initial gold ion concentration on extracts-AuNP synthesis. As for the effects of solution pH, reactions were conducted at different pH of 5, 6, 7, 8, and 9, which were adjusted using Na₂HPO₄ and NaH₂PO₄ in PBS. The control experiments were carried out without the addition of HAuCl₄. The formation of AuNPs was monitored by UV-vis spectroscopy, and all experiments were carried out in triplicate.

Characterization of cells-AuNPs and extracts-AuNPs

The color change of the reaction solution was the preliminary indicator of AuNP formation. Further, the formation of AuNPs was monitored by UV-vis spectroscopy (Metash UV-9000, Shanghai, China) at a resolution of 1 nm between 400 and 800 nm. TEM observation was carried out by Tecnai G220 S-Twin (FEI, USA) at an accelerating voltage of 120 kV. XRD analysis was operated using D/max-2400 X-ray diffractometer (Rigaku, Japan) with Cu Kα radiation ($\lambda = 0.1541$ nm). About 20 representative TEM images including more than 400 particles were used to obtain the histograms of AuNPs. DLS measurement was performed by Zetasizer Nano ZS (Marvin Instruments, UK) to analyze the average particle size, polydispersity, and zeta potential of

AuNPs. The diffracted patterns were recorded at 2θ from 20° to 90° with a scanning speed of 5°/min. FTIR spectra of cells-AuNPs, extracts-AuNPs, and the cells and the cell-free extracts of strain HJ were collected with a Shimadzu IRPrestige-21 FTIR spectrophotometer (Japan) with the wavelength ranging from 750 to 4000/cm. The gold concentration in the reaction solutions after the biosynthesis of AuNPs was measured using a Perkin-Elmer Optima 2000DV (USA) inductively coupled plasma-optical emission spectrometer (ICP-OES).

Toxicity of AuNPs against *Arthrobacter* sp. W1 and *Escherichia coli* BL21 (DE3)

The toxicity of both cells-AuNPs and extracts-AuNPs was investigated using two tested bacteria, *Arthrobacter* sp. W1 (gram-positive bacteria) and *Escherichia coli* BL21 (DE3) (gram-negative bacteria), which were derived from our lab. The paper disc diffusion method was performed to evaluate the antibacterial activities of Au³⁺, cells-AuNPs, and extracts-AuNPs. The two bacteria were grown aerobically in Luria-Bertani (LB) broth until they reached the late log phase, and then 200 μL of each bacterium was inoculated on LB agar plates evenly. Subsequently, the paper discs containing the sample solution were placed at the LB agar plates and incubated for 24 h at 37 °C. The inhibition zone was the standard measure of the antibacterial activity (Huang et al. 2016).

Catalytic activities of cells-AuNPs and extracts-AuNPs in the reduction of 4-NP

For catalytic reactions, the cells-AuNPs and extracts-AuNPs were used for the reduction of 4-NP with an excess quantity of NaBH₄. Typically, 50 μL cells-AuNPs (0.394 mg/mL) or 101 μL extracts-AuNPs (0.195 mg/mL) were mixed with 100 μL 0.1 M NaBH₄ and 100 μL 2.0 mM 4-NP, respectively. Sterile ultrapure water was added to give a final volume of 2 mL. The reduction of 4-NP was monitored by measuring the UV-vis spectra of the mixture at a time interval of 1 min for cells-AuNP system and 10 s for extracts-AuNP system. Blank controls without the addition of AuNPs were also conducted. Each reaction was performed in triplicate, and the average value was used for calculation.

Results

Isolation and identification of *Mariannaea* sp. HJ

A fungal strain, designated as HJ, was isolated from the activated sludge. As shown in Fig. 1a, the colony of the strain HJ on PDA agar plate was zonate- and mat-like with a regular edge. SEM images of the strain HJ showed that the

Fig. 1 Morphological and phylogenetic analyses of strain HJ. **a** Colonial morphology of strain HJ on PDA agar plate. **b** SEM images of strain HJ. The inset shows the spores of strain HJ. **c** Phylogenetic tree of strain HJ and related species based on 26S rRNA gene sequence analysis

conidiophores were spheroidic and formed in clusters (Fig. 1b). To taxonomically identify the strain HJ, the 26S rRNA gene was amplified and a 521-bp amplicon was obtained, which was deposited in GenBank under the accession number KP330204. Based on the sequence analysis, the strain HJ exhibited high similarity (99%) to several *Mariannaea* sp. strains. Therefore, the strain HJ was identified as *Mariannaea* sp. and deposited in the China General Microbiological Culture Collection Center under accession number CGMCC 10030. The phylogenetic trees of the isolated HJ and other *Mariannaea* sp. strains are shown in Fig. 1c.

Biosynthesis and characterization of cells-AuNPs

When the mycelium of the strain HJ was exposed to HAuCl_4 aqueous solution, a rapid color change of the reaction solution from pale yellow to purple was observed within 3 h. In the meanwhile, a strong absorption peak at around 543 nm was observed due to the surface plasmon resonance (SPR) of AuNPs (Fig. 2a) (Eustis and El-Sayed 2006). The SPR peak intensity gradually increased versus the incubation time and reached maximum after 9 h. Further increasing the incubation

time, almost no enhancement of SPR intensity was observed. In the blank experiments, no color change or absorption peak was observed.

The effects of the initial gold ion concentration and pH on cells-AuNP synthesis are depicted in Figs. 2b and S1. When the initial gold ion concentration was 0.1 mM, no significant SPR peak was observed. Then, with the initial gold ion concentration increasing from 0.5 to 2.0 mM, the intensity of SPR peaks at around 543 nm increased without an obvious shift in peak position (Fig. S1a). Further increasing the initial gold ion concentration to 3.0 mM, the SPR peak intensity showed a significant decrease, indicating the lower production of cells-AuNPs (Pimprikar et al. 2009). The cells of the strain HJ could synthesize AuNPs at a broad pH range (4–10), and pH 7 was the most suitable (Fig. S1b). When the pH was below or above 7, the SPR peak intensity decreased slightly, and the SPR peaks had no change in position and shape except for pH 2. When pH 2 was used, no SPR peak was observed, indicating no cells-AuNP formation. Thus, 2.0 mM HAuCl_4 and pH 7 were preferable to cells-AuNP synthesis.

TEM measurement was carried out to estimate the morphology and size of cells-AuNPs (Fig. 3a). From the

Fig. 2 Biosynthesis of AuNPs by cells of strain HJ. **a** UV-vis spectra of AuNPs synthesized at various times with a time interval of 3 h. The *inset* shows the maximum intensity of SPR peaks at different incubation times. **b** Simultaneous effects of pH and HAuCl₄ concentration on SPR peak intensities of AuNPs

representative TEM images, it was found that multiple shapes of nanoparticles were produced, including sphere, trigon, pentagon, hexagon, and irregular shapes. Through measuring more than 400 particles, the size of cells-AuNPs was calculated to be in the range of 4–120 nm and the average size was 37.4 nm, with a sigma value of 3.29. The large variation in particle size was attributed to the presence of trigon, hexagon, and irregular shapes. DLS data indicated that the average size of cells-AuNPs was 104.3 nm, which was larger than that observed by TEM. Polydispersity index (PDI) of cells-AuNPs was 0.379, indicating the formation of nano-sized monodispersed particles. The zeta potential measurement showed a mean value of −12.95 mV for cells-AuNPs, indicating the stability of the synthesized nanoparticles.

XRD analysis was performed to investigate the crystal structure of cells-AuNPs (Fig. 3b). Five distinct intense diffraction peaks were detected at $2\theta = 238.02^\circ$, 44.19° , 64.46° , 77.51° , and 81.68° , corresponding to the (111), (200), (220), (311), and (222) planes of the standard face-centered cubic

Fig. 3 TEM (**a**) and XRD (**b**) analyses of AuNPs synthesized by strain HJ cells. The *inset* shows the size distribution histogram determined from TEM images

phase of gold, respectively (JCPDS No. 04-0783). The diffraction peak corresponding to the (111) phase was distinctly more intense than the rest of peaks, indicating that (111) was the primary orientation. No impure peaks were detected, suggesting the high purity of the biogenetic AuNPs.

Biosynthesis and characterization of extracts-AuNPs

Biosynthesis of extracts-AuNPs was revealed from the changes in color and SPR peak of the reaction solution (Fig. 4a). The color of the reaction solution changed from pale yellow to purple-red within 1 day, and a weak SPR peak emerged at about 532 nm, indicating the formation of AuNPs (Ahmad et al. 2013). Incubation for a longer time led to the increase in color intensity and SPR peak intensity, which reached saturation after 5 days.

Fig. 4 Biosynthesis of AuNPs by cell-free extracts of strain HJ. **a** UV-vis spectra of AuNPs synthesized at various times with a time interval of 1 day. The *inset* shows the maximum intensity of SPR peaks at different incubation times. **b** Simultaneous effects of pH and HAuCl₄ concentration on SPR peak intensities of AuNPs

The effects of the initial gold ion concentration and pH on extracts-AuNP synthesis are shown in Figs. 4b and S2. When 0.1 mM HAuCl₄ was used, no SPR peak was observed. At HAuCl₄ concentration of 0.5 mM, an obvious SPR peak at around 532 nm occurred, which slightly redshifted to about 556 nm with the increase of HAuCl₄ concentration to 5.0 mM (Fig. S2a). It was observed that with 2.0 mM HAuCl₄ used, the SPR peak was stronger and narrower than others, indicating the formation of extracts-AuNPs with higher production and smaller size distribution (Pimprikar et al. 2009). As shown in Fig. S2b, no absorption peaks of the reaction solution were observed at pH 5 and pH 6. At pH 7, a distinct SPR peak was recorded at about 535 nm, but then, the intensity of SPR peaks decreased with the increase of pH (8 and 9). Therefore, the maximum intensity of SPR peak was obtained at 2.0 mM HAuCl₄ and pH 7 (Fig. 4b).

TEM images revealed that the extracts-AuNPs were almost spherical and pseudo-spherical with a particle size distribution

of 4 to 25 nm, and the average size was calculated to be 11.7 nm (Fig. 5a). Corresponding histograms indicated that there was a good uniform size distribution with 57% of extracts-AuNPs distributing between 10 and 15 nm with a sigma value of 25.49. According to DLS analysis, the average size of extracts-AuNPs was 45.4 nm, and the polydispersity index (PDI) was 0.476. Meanwhile, the zeta potential value of extracts-AuNPs was −43.7 mV.

XRD pattern of extracts-AuNPs exhibited three peaks at 2θ values of 37.94°, 44.06°, and 63.94°, which were indexed to the (111), (200), and (220) reflections of the face-centered cubic phase of metal gold, respectively (JCPDS No. 04-0783) (Fig. 5b). It was noted that the diffraction peak corresponding to the (111) plane was more intense than the others, indicating that the (111) plane was the primary orientation.

FTIR analysis of cells-AuNPs and extracts-AuNPs

FTIR analysis was carried out to identify the possible functional groups of the biomolecules that were involved in the formation of cells-AuNPs and extracts-AuNPs. As shown in Fig. 6a, it was observed that both strain HJ cells and cells-AuNPs showed obvious absorption bands at ~3440, ~2930, ~2850, ~1645, ~1540, and ~1040/cm. The strong absorption band at ~3440/cm was attributed to the stretching vibration of O–H groups present in carbohydrates or proteins (Shi et al. 2015). The two bands observed at ~2930 and ~2850/cm referred to aliphatic C–H stretching vibration. The bands at ~1645 and ~1540/cm were assigned to amide I and amide II, respectively (Zhang et al. 2016). The band at ~1040/cm corresponded to C–N stretching of aliphatic amines. The other bands observed in the FTIR spectra of strain HJ cells located at ~1750 and ~1400/cm were confirmed to COO[−] symmetric stretching vibration from carboxyl side groups (Reddy et al. 2012), which were not observed in that of cells-AuNPs. Similarly, the FTIR spectra of cell-free extracts and extracts-AuNPs closely resembled the one of strain HJ cells, which showed obvious absorption bands at ~3440, ~2930, ~2850, ~1745, ~1645, ~1540, ~1400, and ~1040/cm (Fig. 6b). The slight shifts of absorption bands should be due to the reduction of Au³⁺ to Au⁰ and the capping of AuNPs by some biomolecules.

Toxicity of AuNPs

The antibacterial effect of the synthesized nanoparticles was evaluated against gram-positive (*Arthrobacter* sp. W1) and gram-negative (*E. coli*) bacterial strains. Au³⁺ was used as control. The inhibition zone results using disc diffusion assay are shown in Fig. S4. It was evident from the zone of inhibition that Au³⁺ possessed potent bactericidal activity, but the cells-AuNPs and extracts-AuNPs showed no apparent antibacterial activity.

Fig. 5 TEM (a) and XRD (b) analyses of AuNPs synthesized by cell-free extracts of strain HJ. The inset shows the size distribution histogram determined from TEM images

Catalytic activities of cells-AuNPs and extracts-AuNPs

The catalytic activities of cells-AuNPs and extracts-AuNPs were evaluated in the reduction of 4-NP using excess NaBH_4 . In aqueous media containing 0.1 mM 4-NP and 100 mM NaBH_4 , UV-vis spectra showed an absorption peak at 400 nm due to the formation of 4-nitrophenolate ion (Das et al. 2012; Narayanan and Sakthivel 2011). After the addition of cells-AuNP (0.394 mg/mL) or extracts-AuNP (0.195 mg/mL) bioconjugates, the intensity of absorption peaks at 400 nm decreased rapidly, and a new peak appeared at around 300 nm (Fig. S3), corresponding to the reduction products, 4-aminophenol (4-AP). For cells-AuNP and extracts-AuNP systems, the color of the reaction mixture gradually changed from yellow to colorless within 480 and 120 s, respectively, while the control experiment without AuNPs had no color change even after several days. Since the concentration of NaBH_4 was significantly higher than that of 4-NP, the apparent rate

Fig. 6 FTIR spectra of cells-AuNPs (a) and extracts-AuNPs (b)

constant (k) could be estimated by the pseudo-first-order approach (Gu et al. 2014). Figure 7 shows the plots of A_t/A_0 and $\ln(A_t/A_0)$ versus time, and good linear correlations were observed. R^2 values of Fig. 7a, b were 0.98 and 0.9933, and the rate constants for 4-NP reduction catalyzed by cells-AuNPs and extracts-AuNPs were calculated to be 5.7×10^{-3} and $24.7 \times 10^{-3}/\text{s}$, respectively.

Discussion

Mariannaea species were previously reported to be widely existing in the soil, rotten wood, and leaves of conifer trees (Okuda and Yamamoto 2000; Cai et al. 2010; Zeng and Zhuang 2014; Tang et al. 2012). Some natural organic compounds with antimicrobial activities could be isolated from the fungus *Mariannaea*, such as prenylated phenylpropanoids (Fukuda et al. 2011). However, very limited features of the

Fig. 7 Catalytic reduction of 4-NP by cells-AuNPs (a) and extracts-AuNPs (b)

fungus *Mariannaea* have been characterized, and no literature concerning the biosynthesis of metal nanoparticles by *Mariannaea* spp. has been reported. Herein, the biosynthesis of AuNPs using cells and cell-free extracts of *Mariannaea* sp. HJ was investigated for the first time.

Previous studies have postulated that fungi could secrete abundant intracellular or extracellular proteins, which could act as reducing, capping, and stabilizing agents for the synthesis of AuNPs (Kitching et al. 2015). But there were still relatively few studies concerning the synthesis of AuNPs by fungi. For example, Das et al. (2012) reported the formation of spherical AuNPs ranging from 5 to 65 nm using *Rhizopus oryzae* protein extracts, while the cell-free extracts of *Trichoderma* sp. could synthesize AuNPs with the majority being spherical (60%) in the range of 20–30 nm (Mishra et al. 2014). The cells of *Magnusiomyces ingens* LH-F1 could produce various shaped nanoparticles with an average particle size of 80.1 nm, and some plate-shaped particles larger than 200 nm were also observed (Zhang et al. 2016). Our results indicated that both cells and cell-free extracts of *Mariannaea* sp. HJ exhibited good abilities for the synthesis of AuNPs, providing a novel microbial resource for the green synthesis of metal nanoparticles.

Different reaction parameters, such as gold ion concentration and pH, had a significant impact on the reduction and stabilization of AuNPs. The formation of both cells-AuNPs and extracts-AuNPs was favored at 2 mM HAuCl₄. The nucleation and growth processes of nanocrystal were generally important for the formation of AuNPs (Jana et al. 2001). Lower HAuCl₄ concentration might decrease the formation of initial metal nuclei, resulting in the low production of biosynthesized AuNPs. But higher HAuCl₄ concentration could result in the formation of AuNPs with multiple shapes and large size, leading to the decrease in intensity and redshift of the SPR peak (Mishra et al. 2016). Mishra et al. (2011) reported that the concentration of gold salt had an effect on the size and shape of biosynthesized AuNPs, and low or high HAuCl₄ concentration would prevent the formation of AuNPs with uniform size and high yield. Meanwhile, a neutral condition was more suitable for the formation of cells-AuNPs and extracts-AuNPs. Acidic and alkaline conditions might change the activity of proteins or affect the aggregation of nanoparticles, posing negative impacts on the biosynthesis of AuNPs (Mishra et al. 2016). Sneha et al. (2010) reported that the aggregation of AuNPs was observed at low pH, resulting in the formation of large nanoparticles and platelets.

XRD analysis revealed the face-centered cubic phase of cells-AuNPs and extracts-AuNPs with the (111) plane as the basal plane, while the shape and size of these nanoparticles were quite different as shown by TEM analysis. The cells-AuNPs contained various shapes with a wide size distribution (4–120 nm) and larger average particle size (37.4 nm), while the extracts-AuNPs were more uniform with a narrower size distribution (4–25 nm) and smaller average particle size (11.7 nm). The sigma value of extracts-AuNPs was 7.7-fold higher than that of cells-AuNPs, further indicating the better uniform size distribution of extracts-AuNPs. However, the particle size observed in the DLS measurement was larger than that in TEM, which was probably due to the enveloping of bioorganic compounds on the AuNPs (Zhang et al. 2016). In addition, the biosynthesis rate of cells-AuNPs was much faster than that of extracts-AuNPs and many other reported AuNPs synthesized by different fungi, such as *M. ingens* LH-F1 (Zhang et al. 2016) and *Verticillium* sp. (Mukherjee et al. 2001), indicating that the cells of the strain HJ were more favorable to the reduction of Au³⁺ to Au⁰. Xie et al. (2007) indicated that the reduction rate greatly affected the size and morphology of AuNPs and a slow reduction process was generally favorable for anisotropic growth and larger nanoparticles, which was in contrast to the present results. Compared with physical and chemical methods, the biosynthesis of AuNPs is considered as an environment-friendly manner without the consumption of high energy and toxic chemical reagents. Since no additional chemical reagents were used in the present study, the reduction and stabilization of AuNPs should be driven by the preexisting biomolecules in the fungal

Table 1 Comparison of the rate constants of different biogenetic AuNPs for 4-NP catalytic reduction

Biological systems	Size (nm)	Morphology	k ($10^{-3}/s$)	References
<i>Cylindrocladium floridanum</i>	5–35	Sphere	0.1–0.5	Narayanan and Sakthivel (2011)
<i>Sterculia acuminata</i> extracts	18.8	Sphere	1.8	Bogireddy et al. (2015)
<i>Escherichia coli</i>	50	Sphere	0.21	Srivastava et al. (2013)
<i>Pichia pastoris</i>	14.8 ± 3.9	Sphere	8.1	Lin et al. (2013)
<i>Breynia rhamnoides</i> extracts	25	Sphere	9.2	Gangula et al. (2011)
<i>Mariannaea</i> sp.	37.4	Multiple shapes	5.7	This study
<i>Mariannaea</i> sp. extracts	11.7	Sphere	24.7	This study

system. It is possible that some biomolecules derived from the fungal cell walls could be used as reducing and capping agents to form the anisotropic nanoparticles (Xie et al. 2007). The capping of polyphenolic constituents in the cell-free extracts might result in the large negative potential value of extracts-AuNPs, which could make the nanoparticles more stable (Edison and Sethuraman 2012). The FTIR spectra demonstrated that hydroxyl, amine, and carboxyl groups might play an important role in the formation of both cells-AuNPs and extracts-AuNPs. However, the absence of carboxyl groups in cells-AuNPs suggested that these groups might be mainly involved in the reduction process rather than the capping process. A previous study by Shi et al. (2015) reported that hydroxyl, amine, and carboxyl present in the intracellular protein extracts of *Pycnoporus sanguineus* were involved in the reduction of Au^{3+} and stabilization of AuNPs. Zhang et al. (2016) demonstrated that some amide and carboxyl groups originated from *M. ingens* LH-F1 were absorbed on the surface of AuNPs, serving as reducing and capping agents for the formation of AuNPs. However, the mechanism of AuNP biosynthesis was not fully explored and needed further investigation.

The antibacterial test indicated the low toxicity and good biocompatibility of the biosynthesized AuNPs, which could be used as an environment-friendly catalyst for nitroaromatic compound degradation. The cells-AuNPs and extracts-AuNPs possessed excellent catalytic activities for the reduction of 4-NP into 4-AP in the presence of excess $NaBH_4$, and the catalytic rate constant of extracts-AuNPs ($24.7 \times 10^{-3}/s$) was 4.3-fold higher than that of cells-AuNPs ($5.7 \times 10^{-3}/s$). The rate constants of both cells-AuNPs and extracts-AuNPs for 4-NP reduction were comparable with or even higher than that of other biogenetic AuNPs (Table 1). For example, the nanogold biocomposite synthesized by the fungus *Cylindrocladium floridanum* could catalyze the reduction of 4-NP with rate constants of $0.1–0.5 \times 10^{-3}/s$ (Narayanan and Sakthivel 2011), while the AuNPs synthesized by *Sterculia acuminata* extracts could be used for 4-NP reduction with a rate constant of $1.8 \times 10^{-3}/s$ (Bogireddy et al. 2015). The high catalytic activity of extracts-AuNPs could be associated with their uniform shapes and small particle sizes. According to previous

reports, the size and shape of nanoparticles could influence the available active surface area for the absorption of reactants (4-NP and $NaBH_4$), which further affected the electron transfer process during 4-NP reduction, leading to differentiated catalytic activities (Kundu et al. 2009a, 2009b). Aromal and Philip (2012) found that the catalytic activity of AuNPs for 4-NP reduction was size-dependent, and the speed of catalytic reduction increased with the decrease in particle size. Reddy et al. (2012) revealed that AuNPs with various shapes, including quasi-spheres, triangles, and pentagonals, showed a lower catalytic activity for the reduction of *p*-nitroaniline to *p*-phenylenediamine than that of the uniform spherical nanoparticles. Thus, it is significant to accurately control the size and shape of biosynthesized AuNPs in order to improve their catalytic activities. Nevertheless, the present study demonstrated the potential application of biogenetic AuNPs in catalysis.

In conclusion, a low-cost and environment-friendly process for AuNP synthesis was developed using the cells and extracts of a novel fungus *Mariannaea* sp. HJ. Various reaction conditions, including initial gold ion concentration and pH, could affect the shape and size of AuNPs, and the as-synthesized AuNPs were characterized by UV-vis spectroscopy, TEM, XRD, and FTIR analyses. The AuNP bioconjugates could be used as an efficient heterogeneous catalyst for the reduction of 4-NP. The present study has demonstrated the ability of AuNPs synthesized by the cells and extracts of *Mariannaea* sp. for the first time, which should provide insights into the green synthesis of metal nanoparticles.

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References

- Ahmad T, Wani IA, Manzoor N, Ahmed J, Asiri AM (2013) Biosynthesis, structural characterization and antimicrobial activity

- of gold and silver nanoparticles. *Colloids Surf B Biointerfaces* 107: 227–234
- Aromal SA, Philip D (2012) Green synthesis of gold nanoparticles using *Trigonella foenum-graecum* and its size-dependent catalytic activity. *Spectrochim Acta A Mol Biomol Spectrosc* 97:1–5
- Barabadi H, Honary S, Mohammadi MA, Ahmadvpour E, Rahimi MT, Alizadeh A, Naghibi F, Saravanan M (2017) Green chemical synthesis of gold nanoparticles by using *Penicillium aculeatum* and their scolicidal activity against hydatid cyst protoscolices of *Echinococcus granulosus*. *Environ Sci Pollut Res* 2017:5800–5810
- Bogireddy NKR, Anand KKH, Mandal BK (2015) Gold nanoparticles—synthesis by *Sterculia acuminata* extract and its catalytic efficiency in alleviating different organic dyes. *J Mol Liq* 211:868–875
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Cai F, Li J, Sun JS, Ji YL (2011) Biosynthesis of gold nanoparticles by biosorption using *Magnetospirillum gryphiswaldense* MSR-1. *Chem Eng J* 175:70–75
- Cai L, Kurniawati E, Hyde KD (2010) Morphological and molecular characterization of *Mariannaea aquatica* sp. nov. collected from freshwater habitats. *Mycol Prog* 9:337–343
- Castro ME, Cottet L, Castillo A (2014) Biosynthesis of gold nanoparticles by extracellular molecules produced by the phytopathogenic fungus *Botrytis cinerea*. *Mater Lett* 115:42–44
- Daniel MC, Astruc D (2004) Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem Rev* 104:293–346
- Das SK, Dickinson C, Lafir F, Brougham DF, Marsili E (2012) Synthesis, characterization and catalytic activity of gold nanoparticles biosynthesized with *Rhizopus oryzae* protein extract. *Green Chem* 14:1322–1334
- Edison TJI, Sethuraman MG (2012) Instant green synthesis of silver nanoparticles using *Terminalia chebula* fruit extract and evaluation of their catalytic activity on reduction of methylene blue. *Process Biochem* 47:1351–1357
- Eustis S, El-Sayed MA (2006) Why gold nanoparticles are more precious than pretty gold: noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. *Chem Soc Rev* 35:209–217
- Fukuda T, Sudoh Y, Tsuchiya Y, Okuda T, Fujimori F, Igarashi Y (2011) Marianins A and B, prenylated phenylpropanoids from *Mariannaea camptospora*. *J Nat Prod* 74:1327–1330
- Gangula A, Podila R, Ramakrishna M, Karanam L, Janardhana C, Rao A (2011) Catalytic reduction of 4-nitrophenol using biogenic gold and silver nanoparticles derived from *Breynia rhamnoides*. *Langmuir* 27:15268–15274
- Goi A, Trapido M (2002) Hydrogen peroxide photolysis, Fenton reagent and photo-Fenton for the degradation of nitrophenols: a comparative study. *Chemosphere* 46:913–922
- Gu S, Wunder S, Lu Y, Ballauf M (2014) Kinetic analysis of the catalytic reduction of 4-nitrophenol by metallic nanoparticles. *J Phys Chem C* 118:18618–18625
- Huang XQ, Chen X, Chen QC, Yu QQ, Sun DD, Liu J (2016) Investigation of functional selenium nanoparticles as potent antimicrobial agents against superbugs. *Acta Biomater* 30:397–407
- Jana NR, Gearheart L, Murphy CJ (2001) Seeding growth for size control of 5–40 nm diameter gold nanoparticles. *Langmuir* 17:6782–6786
- Kitching M, Ramani M, Marsili E (2015) Fungal biosynthesis of gold nanoparticles: mechanism and scale up. *Microb Biotechnol* 8:904–917
- Kumar SA, Peter YA, Nadeau JL (2008) Facile biosynthesis, separation and conjugation of gold nanoparticles to doxorubicin. *Nanotechnology* 19:495101
- Kundu S, Lau S, Liang H (2009a) Shape-controlled catalysis by cetyltrimethylammonium bromide terminated gold nanospheres, nanorods, and nanoprisms. *J Phys Chem C* 113:5150–5156
- Kundu S, Wang K, Liang H (2009b) Size-selective synthesis and catalytic application of polyelectrolyte encapsulated gold nanoparticles using microwave irradiation. *J Phys Chem C* 113:5157–5163
- Lallawmawma H, Sathishkumar G, Sarathbabu S, Ghatak S, Sivaramakrishnan S, Gurusubramanian G, Kumar NS (2015) Synthesis of silver and gold nanoparticles using *Jasminum nervosum* leaf extract and its larvicidal activity against filarial and arboviral vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Environ Sci Pollut Res* 22:17753–17768
- Lin LQ, Wu WW, Huang JL, Sun DH, Waithera NM, Zhou Y, Wang HT, Li QB (2013) Catalytic gold nanoparticles immobilized on yeast: from biosorption to bioreduction. *Chem Eng J* 225:857–864
- Mandal D, Bolander ME, Mukhopadhyay D, Sarkar G, Mukherjee P (2006) The use of microorganisms for the formation of metal nanoparticles and their application. *Appl Microbiol Biotechnol* 69:485–492
- Manivasagan P, Alam MS, Kang KH, Kwak M, Kim SK (2015) Extracellular synthesis of gold bionanoparticles by *Nocardiopsis* sp. and evaluation of its antimicrobial, antioxidant and cytotoxic activities. *Bioprocess Biosyst Eng* 38:1167–1177
- Mishra A, Kumari M, Pandey S, Chaudhry V, Gupta KC, Nautiyal CS (2014) Biocatalytic and antimicrobial activities of gold nanoparticles synthesized by *Trichoderma* sp. *Bioresour Technol* 166:235–242
- Mishra A, Tripathy SK, Wahab R, Jeong SH, Hwang I, Yang YB, Kim YS, Shin HS, Yun SI (2011) Microbial synthesis of gold nanoparticles using the fungus *Penicillium brevicompactum* and their cytotoxic effects against mouse mayo blast cancer C₂C₁₂ cells. *Appl Microbiol Biotechnol* 92:617–630
- Mishra P, Ray S, Sinha S, Das B, Khan MI, Behera SK, Yun SI, Tripathy SK, Mishra A (2016) Facile bio-synthesis of gold nanoparticles by using extract of *Hibiscus sabdariffa* and evaluation of its cytotoxicity against U87 glioblastoma cells under hyperglycemic condition. *Biochem Eng J* 105:264–272
- Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R, Ajayakumar PV, Alam M, Sastry M, Kumar R (2001) Bioreduction of AuCl₄[−] ions by the fungus, *Verticillium* sp. and surface trapping of the gold nanoparticles formed. *Angew Chem Int Ed* 40:3585–3588
- Narayanan KB, Sakthivel N (2010) Biological synthesis of metal nanoparticles by microbes. *Adv Colloid Interf Sci* 156:1–13
- Narayanan KB, Sakthivel N (2011) Synthesis and characterization of nano-gold composite using *Cylindrocylindrium floridanum* and its heterogeneous catalysis in the degradation of 4-nitrophenol. *J Hazard Mater* 189:519–525
- Okuda T, Yamamoto K (2000) Materials for the fungus flora of Japan (56) *Mariannaea camptospora* and *M. elegans* var. *punicea* from Japan. *Mycoscience* 41:411–414
- Pereira L, Mehboob F, Stams AJM, Mota MM, Rijnaarts HHM, Alves MM (2015) Metallic nanoparticles: microbial synthesis and unique properties for biotechnological applications, bioavailability and biotransformation. *Crit Rev Biotechnol* 35:114–128
- Pimprikar PS, Joshi SS, Kumar AR, Zinjarde SS, Kulkarni SK (2009) Influence of biomass and gold salt concentration on nanoparticle synthesis by the tropical marine yeast *Yarrowia lipolytica* NCIM 3589. *Colloids Surf B Biointerfaces* 74:309–316
- Reddy V, Torati RS, Oh S, Kim C (2012) Biosynthesis of gold nanoparticles assisted by *Sapindus mukorossi* gaertn. Fruit pericarp and their catalytic application for the reduction of *p*-nitroaniline. *Ind Eng Chem Res* 52:556–564
- Schröfel A, Kratošová G, Šafařík I, Šafaříková M, Raška I, Šor LM (2014) Applications of biosynthesized metallic nanoparticles—a review. *Acta Biomater* 10:4023–4042

- Shedbalkar U, Singh R, Wadhvani S, Gaidhani S, Chopade BA (2014) Microbial synthesis of gold nanoparticles: current status and future prospects. *Adv Colloid Interf Sci* 209:40–48
- Shi C, Zhu N, Cao Y, Wu P (2015) Biosynthesis of gold nanoparticles assisted by the intracellular protein extract of *Pycnoporus sanguineus* and its catalysis in degradation of 4-nitroaniline. *Nanoscale Res Lett* 10:1–8
- Sneha K, Sathishkumar M, Kim S, Yun YS (2010) Counter ions and temperature incorporated tailoring of biogenic gold nanoparticles. *Process Biochem* 45:1450–1458
- Srivastava SK, Yamada R, Ogino C, Kondo A (2013) Biogenic synthesis and characterization of gold nanoparticles by *Escherichia coli* K12 and its heterogeneous catalysis in degradation of 4-nitrophenol. *Nanoscale Res Lett* 8:1
- Tang L, Hyun MW, Yun YH, Suh DY, Kim SH, Sung GH, Choi HK (2012) *Mariannaea samuelsii* isolated from a bark beetle-infested elm tree in Korea. *Mycobiology* 40:94–99
- Tripathi RM, Gupta RK, Singh P, Bhadwal AS, Shrivastav A, Kumar N, Shrivastav BR (2014) Ultra-sensitive detection of mercury(II) ions in water sample using gold nanoparticles synthesized by *Trichoderma harzianum* and their mechanistic approach. *Sens Actuator B Chem* 204:637–646
- Xie JP, Lee JY, Wang DIC, Ting YP (2007) High-yield synthesis of complex gold nanostructures in a fungal system. *J Phys Chem C* 111:16858–16865
- Zeng ZQ, Zhuang WY (2014) A new holomorphic species of *Mariannaea* and epitypification of *M. samuelsii*. *Mycol Prog* 13:980
- Zhang XW, Qu YY, Shen WL, Wang JW, Li HJ, Zhang ZJ, Li SZ, Zhou JT (2016) Biogenic synthesis of gold nanoparticles by yeast *Magnusiomyces ingens* LH-F1 for catalytic reduction of nitrophenols. *Colloids Surf A Physicochem Eng Asp* 497:280–285
- Zhao PX, Feng XW, Huang DS, Yang GY, Astruc D (2015) Basic concepts and recent advances in nitrophenol reduction by gold-and other transition metal nanoparticles. *Coord Chem Rev* 287:114–136