Effects of Bio-Au Nanoparticles on Electrochemical Activity of *Shewanella oneidensis* Wild Type and Δ*omcA/mtrC* Mutant

Ranran Wu¹, Li Cui¹, Lixiang Chen¹, Chao Wang¹, Changli Cao¹, Guoping Sheng², Hanqing Yu² & Feng Zhao¹

¹Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen, Fujian Province, 361021, China, ²Department of Chemistry, University of Science & Technology of China, Hefei, Anhui Province, 230026, China.

Both *Shewanella oneidensis* MR-1 wild type and its mutant Δ*omcA/mtrC* are capable of transforming AuIII into Au nanoparticles (AuNPs). Cyclic voltammetry reveals a decrease in redox current after the wild type is exposed to AuIII but an increase in oxidation current for the mutant. The peak current of the wild type is much higher than that of the mutant before the exposure of AuIII, but lower than that of the mutant after the formation of AuNPs. This suggests that damage to the electron transfer chain in the mutant could be repaired by AuNPs to a certain extent. Spectroscopy and SDS-PAGE analysis indicate a decrease in cell protein content after the formation of AuNPs, which provides a convenient way to detect intracellular information on cells.

Metal nanoparticles have been widely investigated due to their unique properties in optics, chemistry, electronics, and magnetics. Biosynthesis of metal nanoparticles, as a reliable and eco-friendly method, has led to increasing interest in their synthetic mechanisms and applications. Many bacteria have been identified to be able to reduce metals, such as *Shewanella oneidensis*¹⁻³, *Geobacter sulfurreducens*⁴⁻⁶, *Desulfovibrio desulfuricans*⁷⁻⁹, *Aeromonas hydrophila*¹⁰, and *Pantoea agglomerans*¹¹. Bio-reduced metal nanoparticles have potential uses in biotechnological and environmental processes¹¹. For example, biosynthesized Pd nanoparticles were found to be able to exhibit a high catalytic activity for lactate oxidation and molecular hydrogen production⁶. Bio-reduced Au–Pd nanoparticles showed a comparable catalytic activity to chemosynthetic counterparts in regard to dechlorination of diclofenac and the oxidation of benzyl alcohol¹²⁻¹³.

*Shewanella oneidensis*, a dissimilatory metal-reducing microorganism, has the ability to reduce metal oxides through their respiration¹⁴⁻¹⁶. The process of metal reduction is associated with extracellular electron transfer. One proposed mechanism is Mtr (i.e. metal-reducing) respiratory pathway, which is considered to be a vital anaerobic respiratory pathway¹⁷. In such a process, a series of c-type cytochromes transfer electrons from inner membrane via periplasm to outer membrane¹⁷⁻¹⁹, electrons eventually arriving at metal oxide directly or mediated by electroactive metabolites/secretions¹⁹. Outer membrane cytochromes (OMCs), OmcA and MtrC, located on the surface of the outer membrane are key proteins transferring electrons to external electron acceptor in the Mtr pathway. However, it is not clear whether the two proteins are responsible for metal reduction. Besides, although there have been some studies on the microbial process of transforming AuIII into AuNPs²⁰⁻²¹, little is known about the role of AuNPs in bacterial electron transfer, biochemical properties and physical functions.

When metal nanoparticles are present in cells, it will cause the variation of cell composition and function. Spectroscopy is a sensitive way that can be used to infer structural molecular information or composition from a sample. Surface Enhanced Raman Spectroscopy (SERS), which usually makes use of colloidal Au/Ag particles, has been widely applied to study bacterial compositions²²⁻²⁵. Although the intracellular AuNPs point to a more promising way in understanding cellular processes, there have been only a few studies of acquired SERS spectra from internalized colloids²⁶⁻²⁷. X-ray photoelectron spectroscopy (XPS)²⁸, over a depth of about 5 nm, has been used to study the surface chemical composition of a variety of microbial cells, involved in surface properties and adhesion behaviors²⁹⁻³⁰. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) assay is a visualized and effective method to further study the variation of cell total protein after AuIII reduction.

**OPEN**

**SUBJECT AREAS:**
NANOPARTICLES
ELECTROCATALYSIS
ENVIRONMENTAL BIOTECHNOLOGY
BIOGEOCHEMISTRY

Received 8 April 2013
Accepted 30 October 2013
Published 22 November 2013

Correspondence and requests for materials should be addressed to F.Z. (fzhao@iue.ac.cn)

**SCIENTIFIC REPORTS** | 3 : 3307 | DOI: 10.1038/srep03307

Metal nanoparticles have been widely investigated due to their unique properties in optics, chemistry, electronics, and magnetics. Biosynthesis of metal nanoparticles, as a reliable and eco-friendly method, has led to increasing interest in their synthetic mechanisms and applications. Many bacteria have been identified to be able to reduce metals, such as *Shewanella oneidensis*¹⁻³, *Geobacter sulfurreducens*⁴⁻⁶, *Desulfovibrio desulfuricans*⁷⁻⁹, *Aeromonas hydrophila*¹⁰, and *Pantoea agglomerans*¹¹. Bio-reduced metal nanoparticles have potential uses in biotechnological and environmental processes¹¹. For example, biosynthesized Pd nanoparticles were found to be able to exhibit a high catalytic activity for lactate oxidation and molecular hydrogen production⁶. Bio-reduced Au–Pd nanoparticles showed a comparable catalytic activity to chemosynthetic counterparts in regard to dechlorination of diclofenac and the oxidation of benzyl alcohol¹²⁻¹³.

*Shewanella oneidensis*, a dissimilatory metal-reducing microorganism, has the ability to reduce metal oxides through their respiration¹⁴⁻¹⁶. The process of metal reduction is associated with extracellular electron transfer. One proposed mechanism is Mtr (i.e. metal-reducing) respiratory pathway, which is considered to be a vital anaerobic respiratory pathway¹⁷. In such a process, a series of c-type cytochromes transfer electrons from inner membrane via periplasm to outer membrane¹⁷⁻¹⁹, electrons eventually arriving at metal oxide directly or mediated by electroactive metabolites/secretions¹⁹. Outer membrane cytochromes (OMCs), OmcA and MtrC, located on the surface of the outer membrane are key proteins transferring electrons to external electron acceptor in the Mtr pathway. However, it is not clear whether the two proteins are responsible for metal reduction. Besides, although there have been some studies on the microbial process of transforming AuIII into AuNPs²⁰⁻²¹, little is known about the role of AuNPs in bacterial electron transfer, biochemical properties and physical functions.

When metal nanoparticles are present in cells, it will cause the variation of cell composition and function. Spectroscopy is a sensitive way that can be used to infer structural molecular information or composition from a sample. Surface Enhanced Raman Spectroscopy (SERS), which usually makes use of colloidal Au/Ag particles, has been widely applied to study bacterial compositions²²⁻²⁵. Although the intracellular AuNPs point to a more promising way in understanding cellular processes, there have been only a few studies of acquired SERS spectra from internalized colloids²⁶⁻²⁷. X-ray photoelectron spectroscopy (XPS)²⁸, over a depth of about 5 nm, has been used to study the surface chemical composition of a variety of microbial cells, involved in surface properties and adhesion behaviors²⁹⁻³⁰. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) assay is a visualized and effective method to further study the variation of cell total protein after AuIII reduction.
In this work, *S. oneidensis* MR-1 (wild-type and ΔomcA/mtrC) were used to synthesize AuNPs. We not only proposed the mechanisms of AuIII bio-reduction, but also investigated the roles of AuNPs in biochemical properties and physical functions by imaging, electrochemistry, spectroscopy and gel electrophoresis analysis.

**Results**

**Characterization of biosynthetic AuNPs.** Natural *S. oneidensis* MR-1 cells were rod-shaped with a relatively smooth surface (Fig. 1a). During exposure to an aqueous HAuCl₄ solution (100 mg L⁻¹ AuIII), the color of the cell suspension changed from pale yellow to purple (see Supplementary Fig. S1 online) within 30 min. A control experiment with only lactate and HAuCl₄ aqueous solution under anaerobic condition showed no color change or precipitation. The distinct color change in the bacterial experiment provides a visible signature for the formation of AuNPs. SEM images showed irregular-shaped spots on the bacterial cell surface after the wild type is exposed to AuIII for 1 h (Fig. 1b) and the size distribution is displayed in Fig. 1c. EDX spectroscopy confirmed that these spots were elemental AuNPs (Fig. 1d). Further analysis by TEM reveals that AuNPs were embedded in the membrane (Fig. 1e). The mutant lacking MtrC and OmcA proteins could still synthesize AuNPs in 30 min. Fig. 1f shows the location of biosynthesized AuNPs in a mutant cell.

**Electrochemical properties.** Mediated electron transfer could be excluded since the cell pellets were washed to remove residual compounds. Curve 1 in Fig. 2a shows a direct electron transfer between the bacteria and electrode when the wild type was coated onto a glassy carbon surface. Oxidation peaks appeared at −0.28 and +0.06 V and a reduction peak at −0.25 V. Cyclic voltammetry results also reveal that ΔomcA/mtrC was still capable of reversible electron transfer, although the peak currents and integrated areas were much lower compared to the wild type (Fig. 2b Curve 1).

To explore the roles of the biosynthetic AuNPs in electrochemical characteristics of the strain, *S. oneidensis* MR-1 was exposed to AuIII (10 mg L⁻¹) for 1 h. Large changes could be observed between the Au-free wild type and the Au-wild type cells in Fig. 2a. Redox current was reduced after the wild type synthesized AuNPs. Although the current decreased in magnitude redox peaks were still observed. A comparison between Curve 1 and Curve 2 in Fig. 2a shows a new reduction wave at a potential of −0.5 V, but this did not show in the...
CV for the raw strain. Control experiments were conducted in anaerobic (in the absence of electron acceptor) and aerobic culture conditions. In the two situations, the peak at $-0.5\, \text{V}$ was not observed. Considering that the reduction potential of Au is not at $-0.5\, \text{V}$ and no hydrogen desorption reaction occurs (see Supplementary Fig. S2 online), the influence of reduced Au is also excluded. Hence, the emerging peak may be attributed to the proteins on the bacterial membrane transferring electrons to an exogenous electron acceptor, rather than oxygen, in their metabolism. The addition of lactate leads to an oxidation current response after the wild type synthesis of AuNPs.

In order to investigate whether Mtr pathway was responsible for Au$^{III}$ reduction, ΔomcA/mtrC mutant was used in this study. However, different from the wild type, the mutant showed an increase in oxidation currents after the reduction of Au$^{III}$ (Fig. 2b). The comparison of redox current between the two strains before and after synthesizing AuNPs is shown in Table 1, which is obtained by 10 replicate cyclic voltammetry tests. Although the Au-free ΔomcA/mtrC revealed a lower electrochemical activity compared with the Au-wild type, the redox peaks of the Au-ΔomcA/mtrC were higher than those of the Au-wild type.

Microorganisms obtain electrons from oxidized substrates through their respiratory processes. The addition of lactate leads to an oxidation current response in the Au-wild type and the Au-mutant. A further investigation of potentiotiestic test on Au-cells revealed a clear lactate-oxidation current at 50 s after the addition of lactate (see Supplementary Fig. S3 online). In the benchmark experiment in which Au-free cells were tested, no obvious peak current change could be observed in 50 s or in even at slightly longer time. However, previous work revealed an increasing current in cyclic voltammetry of 30 min after the addition of lactate$^1$. Therefore, the process for lactate oxidation through anaerobic respiration of S. oneidensis was considered to be a slow reaction. Supplementary Fig. S3 shows that after S. oneidensis transformed Au$^{III}$, this process was accelerated (current rapidly increased about 10 nA after 50 s). This indicates that the bio-synthetic AuNPs help S. oneidensis transfer electrons to oxidize lactate. In the control experiment of AuNPs electrodeposited on an identical GC electrode surface, no oxidation current increase was detected in potentiostatic tests after the addition of lactate. The result illustrates that AuNPs themselves could not catalyze lactate oxidation, but electron transfer would be facilitated when they combined with the cell membrane.

**Spectroscopy characteristics.** Raman and SERS analysis are used to detect changes of bacterial compositions after S. oneidensis transformed Au$^{III}$ into AuNPs. No distinct peaks could be observed for the AuNPs-free control samples of the two strains. It means that little chemical information was presented in the absence of AuNPs. After the addition of chemosynthetic AuNPs or the formation of bio-reduced AuNPs, signals were sufficiently enhanced. Representative bands of proteins, nucleic acids and lipids could be observed in Fig. 3. The major bands assignments have been shown in Table 2. The amide III region is represented by bands at 1230–1245 cm$^{-1}$, indicating the enhancement of key proteins characteristics in spectroscopy, and these proteins are more likely to be associated with membrane involved in the reduction of Au$^{III}$. No obvious difference can be detected in spectra between the wild type and the mutant.

When comparing the SERS spectra obtained from the biosynthetic and chemosynthetic AuNPs labelled samples, slight differences could be detected. The different intensities of the bands at 1325–1345 and 1455 cm$^{-1}$ relative to the sharpest and stable band at $\sim736\, \text{cm}^{-1}$ suggest a varying contribution from proteins (amide III) and lipids. The biosynthetic AuNPs labelled sample shows lower intensities at the two bands, that is a decline in protein quantities or protein
structure change has happened in the AuNPs formation process. The variation in amide III content was further confirmed by Fourier transform infrared spectroscopy (FTIR). Detailed information is available in Supplementary Fig. S4 online.

The surface chemical composition of bacteria was further studied by XPS. Table 3 displays mole fraction and atomic mass ratios of main elements with respect to total carbon. Duplicate experiments showed similar data. Notably, N quality which mainly contributed to amine or amide varied a lot. Strains before AuNPs formation are much richer in N content than Au-strains. The variation in N content is also reflected by Fig. 4 and binding energy of N appeared at around 400.0 eV. The main constituents of bacterial cell surface are polysaccharides (Ps), proteins (Pr) and lipids (Lp). According to previous studies, $C_6H_{10}O_5$ represents for polysaccharides and CH$_2$ for saccharides (Ps), proteins (Pr) and lipids (Lp). According to previous studies and XPS results, the chemical compositions were calculated by the following formulas:

$$\frac{[N]}{[C]}_{obs} = 0.279(C_{Pr}/C)$$
$$\frac{[O]}{[C]}_{obs} = 0.325(C_{Pr}/C) + 0.833(C_{Pr}/C)$$
$$\frac{[C]}{[C]}_{obs} = (C_{Ps}/C) + (C_{Pr}/C) + (C_{Lp}/C) = 1$$

 Comparing with the results showed in Table 3, significant differences in protein content were observed. The wild type and mutant are much richer in protein than Au-wild type and Au-mutant, which verifies SERS and FTIR results. Besides, more significant decrease in Au-wild type was observed.

Protein analysis by SDS-PAGE. Total protein contents of both the wild type and the mutant are significantly decreased after the formation of AuNPs (Fig. 5). Notably, the decrease in wild type protein content is much larger. In order to obtain the precise protein variation caused by the biosynthesis of AuNPs, all samples were electrophoresed at the same protein content. The effects of biosynthesis of AuNPs on total cell protein of the wild type and the mutant are shown in Fig. 6. About 35 obvious bands in the gel of the total proteins prepared from the two strains before and after the exposure of Au$^{III}$ and separated by SDS-PAGE could be observed. The protein band patterns of these four samples were similar to each other, but variations in the degree of brightness could still be detected. After the exposure of Au$^{III}$, the bacterial total protein content was dramatically modified at both the wild type and the mutant. Many protein bands from Au$^{III}$ exposed cells were not as distinct as in the unexposed ones, especially in below 35 kDa area. Protein extracts from both Au-wild type and Au-mutant showed 8 proteins (about 80 kDa, 66 kDa, 30 kDa, 26 kDa, 21 kDa, 19 kDa) that were significantly reduced in content compared with the unexposed ones. In addition, the total protein content of Au-wild type was more drastically diminished on the gel than the Au-mutant in the $< 25$ kDa region. That is, small molecular protein concentrations in Au-wild type were significantly lower than that in the Au-mutant.

Discussion

It is found that the mutant could still transform Au$^{III}$ into AuNPs (Fig. 1f) and the color change time in suspension and characteristics (size, shape and location) of bio-AuNPs are basically the same as the wild type. This discovery demonstrates that lack of OmcA and MtrC proteins does not affect the pathway or the location of AuNPs formation, i.e. OmcA and MtrC proteins are not necessary in the Au$^{III}$ reduction process.

Since the bio-reduced AuNPs are embedded in membrane judged by TEM images, there are two possible explanations. One is that Au$^{III}$ ions could get into bacterial outer membrane and be reduced. In Mtr pathway, besides OmcA and MtrC exposed on the outer surface of the outer membrane, there are MtrA facing interior and MtrB functions as a transmembrane sheath which envelops MtrA and

![Figure 4](https://www.nature.com/scientificreports/4)

**Figure 4** | Cell surface chemical composition. Representative N$_1$s spectra obtained for the wild type, Au-wild type, mutant and Au-mutant.

| Table 3 | Cell surface chemical composition and deduced proportions of carbon referred to proteins, polysaccharides, and lipids |
| Strains | % C | % N | % O | N/C | O/C | % C$_{Pr}$ | % C$_{Ps}$ | % C$_{Lp}$ |
|----------|-----|-----|-----|-----|-----|----------|----------|----------|
| Wild type | 65.15 | 7.51 | 26.99 | 0.115 | 0.414 | 41 | 34 | 25 |
| Au-wild type | 73.87 | 3.19 | 22.43 | 0.043 | 0.304 | 15 | 31 | 54 |
| Mutant | 68.94 | 6.73 | 24.03 | 0.098 | 0.348 | 35 | 28 | 37 |
| Au-mutant | 75.63 | 3.26 | 20.71 | 0.043 | 0.274 | 15 | 27 | 58 |

| Table 2 | Tentative band assignments for SERS acquired from S. oneidensis MR-1 wild type with biosynthetic and chemosynthetic AuNPs |
| Au SERS | Biosynthetic | Chemosynthetic | Tentative assignments |
|---------|-----------|-------------|---------------------|
| 736 | 734 | Adenine$^{34}$ |
| 961 | 963 | Lipid(C=C deformation)$^{25,43}$ |
| 1235 | 1245 | Amide III$^{25,44}$ |
| 1318 | 1324 | Amide III$^{25,44}$ |
| 1347 | 1455 | Nucleic acids(A, G)$^{34}$ |
| 1580 | 1584 | Lipid(CH$_2$ deformation)$^{43}$ |

![Figure 3](https://www.nature.com/scientificreports/3/)

**Figure 3** | SERS of S. oneidensis MR-1 wild type. In the presence of (a) chemosynthetic and (b) biosynthetic AuNPs substrate.
MtrC. Both MtrA and MtrC are partially inserted in MtrB, and MtrC are bounded with OmcA. A previous study on Fe\textsuperscript{III} reduction by Mtr\textsuperscript{CAT} proteoliposomes has demonstrated that in the presence of soluble Fe\textsuperscript{III}, limited electron transport via Mtr\textsuperscript{AB} still occurs. However, electrons cannot be transferred to insoluble Fe\textsuperscript{III} in the absence of OmcA and MtrC. This means the two proteins are more important in transferring electrons to insoluble electron acceptors. Since the porin-like outer membrane protein MtrB was near the membrane surface, soluble ions would be capable of penetrating far enough into the MtrB to make electrical contact with Mtr\textsuperscript{A}. Lacking of OmcA and MtrC proteins, Au\textsuperscript{III} ions could easily get into MtrB porin and accept electrons transported by MtrA, thus formed the AuNPs. Another possible explanation is that electrons could be transferred from bacteria to Au\textsuperscript{III} ions through other protein functional groups located on the outer membrane. Apart from Mtr pathway, electrons may also be transported across the bacterial outer membrane by other protein conduits composed of MtrE and DmsF (i.e. dimethyl sulphoxide F), a homologue of MtrB\textsuperscript{C}. However, whether electrons are transferred through this pathway during Au\textsuperscript{III} reduction needs further study. In the both situations, the formation of AuNPs would not be affected when \textit{S. oneidensis} cells lack the OmcA and MtrC proteins. That is, electrons can be transferred to Au\textsuperscript{III} ions by OmcA and MtrC when the two proteins exist and by other proteins/pathways when they are absent.

Considering the increase in redox current of the Au-\textit{OmcA/mtrC}, this demonstrates that a low loading AuNPs may help bacteria transfer electrons when lacking the OmcA and MtrC proteins. Damage to the electron transfer chain in mutant could be repaired to a certain extent after the formation of bio-AuNPs so that the mutant even exhibited a better electrochemical activity than the wild type. Hence, an approach to repairing cell electron transfer damage is proposed; this new discovery may have a promising future in biology and biochemistry fields.

SERS and FTIR studies explain the decrease in current of cyclic voltammogram after the wild type reduced Au\textsuperscript{III}. The variation of amide III intensity is probably because the AuNPs formation and growth, which would probably cause damage to the cell wall structure and so lead to a decline in protein quantities or protein structure change. As a result, accessible electroactive centres would decrease in number. Although a protein quantity decrease could also be observed in SERS and FTIR of biosynthetic Au-\textit{OmcA/mtrC}, oxidation currents of the mutant were increased (Table 1). This discovery illustrates that the proteins affected by AuNPs are involved in electron transfer of the wild type but not in that of the mutant. It means the proteins which lead to a decrease in amide III are very likely part of the Mtr pathway, since only the Mtr pathway had been cut off in the mutant.

### Methods

#### Cultivation and Au\textsuperscript{III} reduction

Strains were grown aerobically at 32°C in 50 mL lactate contained Luria-Bertani medium overnight. The cells were collected by centrifugation at 6000 rpm for 5 min and were washed three times by sterile distilled water. The washed pellets were re-suspended in 50 mL sterile distilled water with lactate (50 mM) as the electron donor in 100 mL serum bottles. HAuCl\textsubscript{4}·4H\textsubscript{2}O was added to a final Au concentration of 10 mg L\textsuperscript{-1}. Anaerobic conditions were maintained by bubbling nitrogen, and then the serum vials were sealed. The cell-resuspended solutions were cultivated at 32°C as before.

#### Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

Cells were fixed in 2.5% (w/v) glutaraldehyde for 4 h. After washing three times with phosphate buffer (50 mM, pH 7.0), the cells were dehydrated in an ethanol series (50%, 70%, 90%, and 100% twice). After drying at 80°C for 12 h, the cells were placed onto a carbon substrate. SEM and EDX images were carried out by a Hitachi S-4800 Scanning electron microscope with an EDX attachment at 5 kV.

Cells were prefixed in 2.5% (w/v) glutaraldehyde for 4 h and fixed in 2% osmium tetroxide for 4 h. Then the cells were washed by phosphate buffer (50 mM, pH 7.0), dehydrated by an ethanol series (50%, 70%, 80%, 90%, and 100% twice), and...
embedded in resin (LR White). The mixtures were polymerized at 60 °C for 24 h. Ultrathin sections of ~60 nm were cut by an ultramicrotome and deposited on carbon-coated copper grids. A Hitachi H-7650 transmission electron microscope was used to acquire TEM images at 80 kV.

Electrode preparation and electrochemical measurements. After exposed to AuIII for 1 h, cells were collected by centrifugation at 6000 rpm for 5 min, and washed three times with phosphate buffer (50 mM, pH 7.0). 5 μl of cells were transferred to the surface of a glassy carbon (GC) electrode by pipette and dried naturally at room temperature.

A three-electrode chamber with the prepared GC working electrode, an Ag/AgCl reference electrode and a Pt wire counter electrode was used to measure electrochemical characteristics in a Faraday cage to exclude interferences. Cyclic voltammetry and potentiostatic measurements were carried out by an Autolab electrochemical workstation. The autolab is equipped with ECD module which can measure electrochemical transfer.

Raman and SERS. Cells were collected by centrifugation at 6000 rpm for 5 min, washed three times with sterile distilled water, and then the pellet was dropped on a clean glass slide. Raman and SERS spectra of washed cells were recorded on a Horiba Jobin Yvon S.A.S. LabRAM Aramis laser confocal Microscope Raman spectrometer, with a 632.8 nm HeNe laser yielded 7.25 mW power at the sample, and the exposure time of each experiment was 1–10 s. Chemosynthetic AuNPs were prepared in accordance with Frens’ method. In short, 100 mL of 0.01% (w/v) HAuCl₄ solution with a 632.8 nm HeNe laser yielded 7.25 mW power at the sample, and the exposure time of each experiment was 1–10 s. Chemosynthetic AuNPs were prepared in accordance with Frens’ method. In short, 100 mL of 0.01% (w/v) HAuCl₄ solution was heated boiling for 1 h. The mixture was then added and mixed. The mixture was kept boiling for 1 h.

X-ray photoelectron spectroscopy (XPS). The cells were dried by freeze drying method. XPS analyses were carried out by a Thermo Escalab 250 electron energy dispersive spectrometer. The angle between the electrostatic lens axis and the sample surface was 0°. C1s spectrum was used as reference to obtain the binding energies of all spectra.

Total cell protein content assay. To ensure that bacteria before and after AuNPs formation have same cell concentrations and growth conditions, each strain was cultured in one bottle. The cells were collected by centrifugation of 200 mL bacterial solution at 6000 rpm for 5 min and washed 3 times by sterile distilled water. The cell pellets were then resuspended in 3 mL NaOH liquid solution (1 M) and heated to boiling water for 30 min. Total cell proteins were obtained in the supernatant after the heated resuspension was centrifuged at 10000 rpm for 20 min. Total protein contents were measured by Bradford Assay.

SDS-PAGE. The cell total proteins were extracted by grinding bacteria in a mortar with liquid nitrogen. Then the disrupted cells were collected by resolving in PBS (50 mM, pH 7.0) and total proteins were obtained from the supernatant by centrifugation at 12000 rpm for 20 min at 4°C. Protein extracts were analyzed using 15% polyacrylamide resolving gels and 5% polyacrylamide stack gels. The protein loading quantity of each sample was 10 μg. All samples were bathed in boiling water for 5 min and electrophoresed with constant voltage until the dye front reached the bottom of the gel. The protein bands were visualized by Coomassie Brilliant Blue G-250 staining, methanol and acetic acid destaining.
Acknowledgments

This study was sponsored by the main Direction Program of Knowledge Innovation (KZCXZ-EW-402) and the Hundred Talents Program of the Chinese Academy of Sciences, and Ministry of Science and Technology (2011AA060907).

Author contributions

R.R.W. and F.Z. designed and performed the experiments. L.C. assisted in Raman experiment, L.X.C. gave a hand with cyclic voltammetry, C.W. and L.X.C. guided the SDS-PAGE assay and C.L.C. helped in cultivating strains. R.R.W., G.P.S., H.Q.Y. and F.Z. analysed the results and wrote the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Wu, R.R. et al. Effects of Bio-Au Nanoparticles on Electrochemical Activity of Shewanella oneidensis Wild Type and DomC/mtrC Mutant. Sci. Rep. 3, 3307; DOI:10.1038/srep03307 (2013).

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported license. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/3.0