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Cellular Localization of Gold and Mechanisms of Gold Resistance in *Rhodobacter sphaeroides*

Hannah Johnson[1,2], Ram C. Kafle[3], Madhusudan Choudhary[1]

[1]Department of Biological Sciences, Sam Houston State University, Huntsville, TX, USA
[2]Advanced Technology Cores, Baylor College of Medicine, Houston, TX, USA
[3]Department of Mathematics and Statistics, Sam Houston State University, Huntsville, TX, USA
Email: hannahj@bcm.edu, rckafle@shsu.edu, mchoudhary@shsu.edu

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Abstract

Heavy metal pollution is a worldwide problem with many associated health risks, including bone loss, kidney damage, and several forms of cancer. There is a great need of bioremediation of these toxic metals from the environment, as well as implementing a monitoring system to control the spreading pollution. This study focuses on the bioremediation potential of *Rhodobacter sphaeroides* in the presence of the toxic gold chloride ($AuCl_3$). Growth characteristics of the bacterial cells exposed to a range of toxic gold concentrations were analyzed through the growth kinetics and the colony forming units under aerobic, photosynthetic, and anaerobic growth conditions. The localization of the gold particles within two cellular fractions, cytoplasm and the plasma membrane, are analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Results of this study demonstrated the photosynthetic growth condition as best suited for the metal tolerance, compared to the aerobic and anaerobic growth conditions. Results also revealed the overall accumulation and localization of gold particles, while not different between the membrane and the cytoplasmic fractions increased at different concentrations of the gold contamination. The results of the localization under photosynthetic growth condition revealed the accumulation reached the highest very quickly, and an overall shift in localization of the gold particles from an equal distribution to an increase within the membrane fraction at the highest concentrations of gold contamination. The localization of the gold particles was validated by Transmission Electron Microscopy (TEM) where the results confirmed the increase in accumulation within the membrane, and photosynthetic membranes, of *R. sphaeroides.*

Keywords

Rhodobacter sphaeroides, Heavy Metal Bioremediation, Inductively

Coupled Plasma (ICP), Transmission Electron Microscopy (TEM)

1. Introduction

The definition of heavy metals has differed over the years, beginning with defining heavy metals as metals with a density of five times greater than water [1] and then as metals with densities above 4 - 5 g/cm^3 [2] [3]. There are about 30 metals and metalloids within the heavy metals group, including zinc (Zn), mercury (Hg), gold (Au), lead (Pb), cadmium (Cd), copper (Cu), silver (Ag), platinum (Pt), arsenic (As) and chromium (Cr), which have densities that are greater than 5 g/cm^3 [4]. Heavy metals may have a nutritional benefit to the organism as cofactors, such as zinc, magnesium, copper, chromium, or nickel, while other metals, such as lead, cadmium, mercury, arsenic, and gold, are not yet identified with any beneficial attributes to the organism [5]. Regardless of the nutritional benefit, all metals lead to toxic effects when accumulated in high concentrations in the cell. There are many factors that contribute to the toxicity of metals, which includes the concentration of the metal, the source of contamination, time of exposure, as well as the chemical structure [6]. There is a world-wide problem of heavy metal contamination that comes from a variety of different sources. These pollutants come from acid rain, the by-products of industrial effluents, gold mines, and metal ions that leach into the soil columns which all have detrimental effects worldwide [7] [8]. Each metal has a different concentration at which it is deemed to be toxic to both the environment and the human body, and there are a multitude of health risks, including bone loss, kidney and liver damage, neurological damage, as well as various types of cancer including skin and lung cancer [9] [10] [11] [12] [13]. Since the metal toxicity is dependent on numerous factors, some metals, such as chromium, cobalt and nickel, actually play important roles in various in metabolic processes as essential micronutrients, stabilizing molecules [14] and catalysts in enzymatic reactions [15]; they also help regulate osmotic balance [16] and are used in redox reactions [17].

Whether essential or non-essential, heavy metals become toxic to organisms at high levels, resulting in bioaccumulation, modifications of conformational structure of nucleic acids and proteins, damage to the DNA and cell membrane, and interference with the oxidative phosphorylation and osmotic balance [16]. Resistance mechanisms to heavy metals have been identified including intracellular and extracellular sequestration, exclusion by permeability barrier, efflux pumps, active transport, reduction of heavy metal ions and cellular targets, and enzymatic detoxification [18]. To better understand the mechanisms of tolerance, analysis of the genomes of the heavy metal resistance genes can provide information on the distribution of the heavy metal genes which confer the ability to tolerate the metal contaminations.

Microorganisms have a wide array of different tolerance mechanisms de-

pending on the organism as well as the heavy metal involved; each mechanism is specific to particular metals or group of metals. The mechanism of tolerance is also attributed to the concentration of the metals. It has been found that when metals are within the nanomolar range, the main mechanism of tolerance includes the activity of the metallothioneins, a class of proteins that is involved in the uptake and transportation of metals, including zinc [19]. As the concentration of the metals reaches the micro-molar to millimolar range, the mechanisms of tolerance become the enzymatic transformation of the metals in attempts to reduce and detoxify them, as well as the export of the metal particles outside of the cell [19]. As the concentration increases to the molar range, the main mechanism of tolerance that has been identified is the precipitation of the toxic metals, such as the Archaea will utilize [19].

Metals are found within the environment in different concentrations. Gold averages about 1 - 5 ppt in natural water [20], but has been found to reach more than 100 ppb in soils [21]. Free gold ions are found in high abundance in aqueous media since the redox potential of Au (I) and Au (III) exceeds water, which could lead to the toxicity of gold on organisms [20]. To combat the toxicity, bacteria actively transports the gold out of the cytoplasm through means of efflux pumps. As previously mentioned, there are different mechanisms of tolerance especially with each specific metal. For example, *Salmonella* contains a *gol*-gene cluster, which codes for a metal exporter, GolT, a transcriptional regulator, GolS, and a metal binding protein GolB. GolS, along with CupR, belongs to the MerR family of regulators that are activated in the presence of heavy metal ions, ranging from the essential ions such as Zn (II) or Cu (I), to the toxic Pb (II) and Au (I) ions. Studies have shown the formation of biofilms, such as *Cupriavidus*, on deposits of Au, which suggests that Au-specific resistance mechanisms are the mechanisms to survive against gold toxicity. This presents a challenge as each metal has a different mechanism found in the different group of bacteria. In order to design and synthetically construct an effective bioremediation tool, the mechanisms of metal transport and the use of efflux pumps needs to be better characterized in a choice of model microorganism.

Different growth conditions may also affect the cell survivability in response to the toxic metal contaminants. *Rhodobacter sphaeroides* is a bacterium that belongs the class Alphaproteobacteria within the phylum of Proteobacteria, which contains many species that are able to survive toxic metal conditions, and has been studied previously in heavy metal resistance studies, including within silver and arsenic [16] [18]. In our previous studies, *Rhodobacter sphaeroides* was analyzed for the survivability and growth characteristics within various concentrations of gold chloride [22]. Gold salts have been shown to be toxic to the bacterial cells at a high concentration, and was used to analyze the survivability within the low contaminations. In this study, the cells were subject to a concentration range of 0.1 - 10.0 μM $AuCl_3$ and the cell density was monitored each day for a total of five days. The experiment included three of the seven growth conditions for the bacterial cells which consisted of aerobic condition

(20% oxygen with light), photosynthetic condition (no oxygen, and constant 3 Watt light), as well as anaerobic condition (no oxygen and no light). Since the optical density readings are unable to discern live from dead cells, a series of colony forming units was performed across the growth conditions and concentrations. It was determined from the growth kinetics, as well as the colony forming units that the photosynthetic growth condition led to a higher survivability and overall within the gold contaminated cells [22]. After it was revealed that the *R. sphaeroides* cells are able to tolerate the gold chloride, it is important to identify the localization of the gold particles within the cells in order to better understand the tolerance mechanisms of *R. sphaeroides*,

To expand this investigation, the current study is focused on the localization of the gold particles within the cellular fraction. If the localization of the particles is determined, it will provide more insight into the mechanism of gold tolerance of *R. sphaeroides*. The hypothesis tested in this study is the localization of the gold particles is highest within the membrane fraction of cells, including the photosynthetic membrane located in the cytoplasm. The concentration of gold particles within the membrane and the cytoplasmic fractions was determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The localization of the gold particles was examined using Transmission Electron Microscopy

The accumulation within the membrane fraction may indicate a tolerance mechanism of sequestration and detoxification in order to maintain cell survivability. The results will shed important insight whether bacterial gold resistance, particularly within *R. sphaeroides,* is due to either cellular adaptation or mutation-selection mechanisms.

2. Materials and Methods

2.1. Bacterial Strain, Gold Solution, and Growth Media

The growth media used in this study consisted of Sistrom (SIS) media [23]. The formula for this media includes nitrilotriacetic acid (10 g/l), $MgSO_4 \cdot 7H_2O$ (29.5 g/l), $CaCl_2$ (3.335 g/l), $FeSO_4 \cdot 7H_2O$ (25 mg/l), $(NH_4)8Mo7 \cdot 4H_2O$ (9.25 mg/l), potassium succinate (10%), L-Glutamic acid (5%), L-Aspartic acid (2%), trace elements, and sodium chloride (5%). This is the minimal media used for *R. sphaeroides* [24]. Cultures began on solid media plates, and then liquid cultures were inoculated. Throughout the course of the study, *R. sphaeroides* cells were contaminated with gold chloride in both the solid media plates as well as liquid cultures. The amount of gold chloride added to each solid media plate and liquid culture was determined based upon the volume of each plate and tube, as well as the concentration of the initial stock. The final concentrations used in all three growth conditions, aerobic, photosynthetic, and anaerobic, of gold chloride were 0.0 μM, 0.1 μM, 0.5 μM, 1.0 μM, and 10.0 μM.

Rhodobacter sphaeroides 2.4.1 cells were obtained from stock cultures stored at −80˚C. The cells were streaked onto SIS minimal media and grown aerobically

at 30˚C. For the photosynthetic growth condition, cells were streaked onto SIS plates and placed inside a photosynthetic box. The photosynthetic box provides constant 10 Watt light conditions, and sealing the plates provides the low oxygen content. Placing the plates in an anaerobic chamber and adding the appropriate anaerobic sachets with an indicator facilitated the anaerobic growth condition. The chamber, which was placed inside an incubator at 30˚C, was covered with aluminum foil to prevent the light penetrating the chamber.

Gold chloride, $AuCl_3$, was purchased in powdered form from Sigma Aldrich. The stock gold solutions were made by suspending the powder into deionized water to a 1.0 mM concentration. The gold chloride concentrations further discussed were diluted from the stock concentration of 1.0 mM gold chloride to final concentrations of 0.1 μM, 0.5 μM, 1.0 μM, and 10.0 μM.

2.2. Sample Preparation

A series of growth kinetics was performed for each concentration under three different growth conditions. For the aerobic growth condition, a sample of *R. sphaeroides* cells was grown in liquid SIS media until the log phase of growth was obtained (0.6 - 0.8 optical density at 600 nm for *R. sphaeroides*). The log phase of growth has been identified as the optimal growth phase to study metal tolerance [25]. Once the log phase of growth was reached, the cells were contaminated with varying concentrations of gold chloride ranging from 0.0 μM to 10.0 μM $AuCl_3$.

The samples were all inoculated with the bacterial cells and the subsequent gold concentrations on the same day, designated as "day zero". The following day, the tubes for the 24-hour time period, "day one" were analyzed, while the rest of the time periods were left untouched. This process repeated until the 120-hour time period, "day five". This methodology allows the samples to remain untouched for the duration of the study. Once all of the tubes were prepared for the aerobic growth condition, the samples were placed in a shaker incubator at 30˚C. The prepared samples for the photosynthetic condition were sealed tightly and placed in a photosynthetic box under a constant 10 Watt light condition. The anaerobic samples were overlaid with sterile mineral oil and then placed in an incubator at 30˚C and covered in aluminum foil to prevent light reaching the tubes. The aerobic growth condition was completed first, then the photosynthetic condition, and then finally anaerobic.

2.3. Subcellular Fractionation

To determine the localization of the gold particles, subcellular fractionation was done following the protocol outlined in [26]. The cells of each sample, from the previous experiments, were pelleted by centrifugation at 5000 g for fifteen minutes at 4˚C. The supernatant was collected and labeled as the media fraction. The pellet was resuspended in SIS media plus 20 μl of cell lysis solution. The sample was sonicated on ice at 50% duty cycle for three, 30 second bursts. The

samples were pelleted again for fifteen minutes at 5000 g, at 4˚C, and the supernatant was collected and labeled as the cytoplasmic fraction. The pellet was re-suspended in SIS media and 20 µl of lysis solution. The samples were sonicated on ice at 50% duty cycle for six, one minute bursts. The samples were centrifuged once more at 10,000 g for 10 minutes at 4˚C; the supernatant was collected and labeled as the membrane fraction. These samples were stored at −20˚C until they could be analyzed.

2.4. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

The previously fractioned samples were loaded into the tubes designed for the equipment within the TRIES analytical laboratory. To each 4 ml sample, 80 µl of 2% Nitric Acid was added and mixed by inversion. A standard calibration curve was made using stock concentrations. The stock solutions were made using: 0.0 ml, 0.00246 ml, 0.0049 ml, 0.0492 ml, 0.492 ml, and 4.92 ml of Au concentrate into 25 ml 2% HNO_3 to make the stock concentrations of 0.0 mM, 0.0005 mM, 0.001 mM, 0.01 mM, 0.1 mM and 1.0 mM respectively. The machine was programmed to take the stock concentration readings and then the standard curve was analyzed for any outliers. If any outliers were present, the standard curve was reset. Once the standard curve was linear, the job file was set up through the Smart Analyzer Vision software. A new "method" was constructed and named (Au_only) rev_2. The plasma was turned on within the machine and left to heat up for 30 minutes. The job file was constructed with a series of steps that included: Pre-flush, Iteration One, Flush, Iteration Two, Flush, Iteration Three, Final Flush. This methodology was repeated for each of the samples. The machine calculates the total amount of gold (mg/L) in the average of the three iterations per sample, and each individual iteration for the samples. The data was stored and exported to an excel sheet for further analysis. Statistical analysis on the localization of the gold particles was completed using the repeated measures analysis [27], as well as the multiple comparison analyses.

2.5. Transmission Electron Microscopy (TEM)

R. sphaeroides cells were grown to the log-phase and exposed to 0.5 µM and 1.0 µM gold contamination under the aerobic and photosynthetic growth conditions. The samples were taken on ice to the Image Analysis Laboratory at the Veterinary Medicine and Biomedical Sciences College at the University of Texas A&M. The cells were pelleted and the supernatant was removed. The cells were fixed in 2% glutaraldehyde and 2.5% paraformaldehyde in a 0.1 M sodium cacodylate buffer for three hours. The cells were washed and enrobed in agar overnight. The enrobed cells were cut into 1 mm cubes and fixed onto a block for imaging. Pre and post stained blocks were imaged.

2.6. Statistical Analysis

Statistical analysis on the localization of the gold particles was completed using

the repeated measures analysis [27]. Restricted Maximum Likelihood approach was applied to study the effect of Aerobic and Photosynthetic growth condition in varying concentration of gold over time. Since we measured the kinetic growth of bacteria under gold conditions within the Cytoplasm and Membrane for aerobic and photosynthetic conditions by taking some portion of sample of cells each day, the variation in growth was assumed to be dependent across the time. The hypothesis tests for the effect of growth period, growth conditions, gold concentrations, fractions of cells (cytoplasm and membrane) and their interactions were performed considering all fixed effects. Pairwise multiple comparisons of growth time, growth conditions, gold concentrations, fractions of cells (cytoplasm and membrane) were also studied using the difference of least squares means [28]. The approximate t tests and their p-values for all pairwise multiple differences of these variables were calculated to test if there is significant difference in means or not.

3. Results and Discussion

3.1. Localization of Gold Particles within Subcellular Fractions

The localization of the gold particles within the subcellular (cytoplasmic and membrane) fractions can be seen in **Figure 1** and **Figure 2** below:

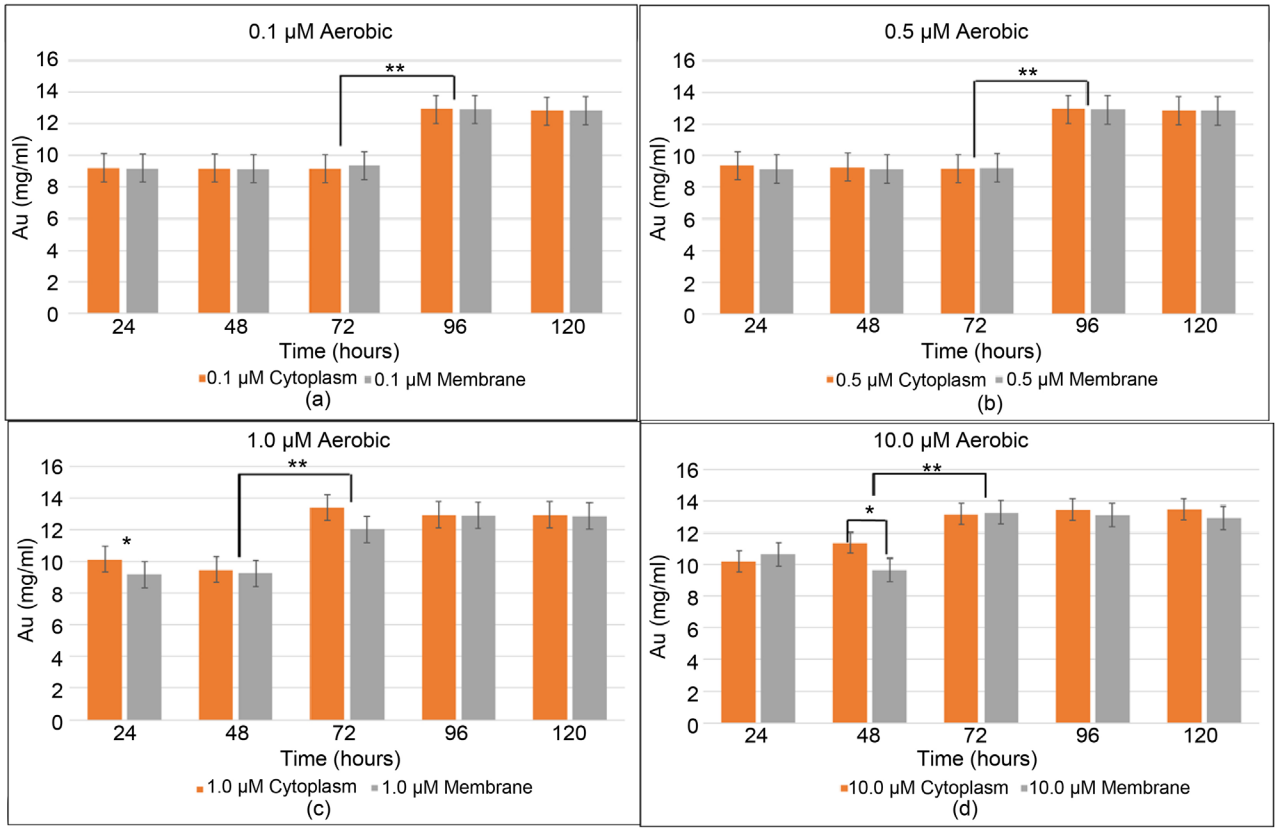

Figure 1. ICP analysis of the aerobic growth condition in varying concentrations of gold contamination. (a) Analysis of the 0.1 μM AuCl3 cellular fractions; (b) Analysis of the 0.5 μM $AuCl_3$ cellular fractions; (c) Analysis of the 1.0 μM $AuCl_3$ cellular fractions; (d) Analysis of the 10.0 μM $AuCl_3$ cellular fractions. *denotes ($p < 0.001$) while **denotes ($p < 0.05$).

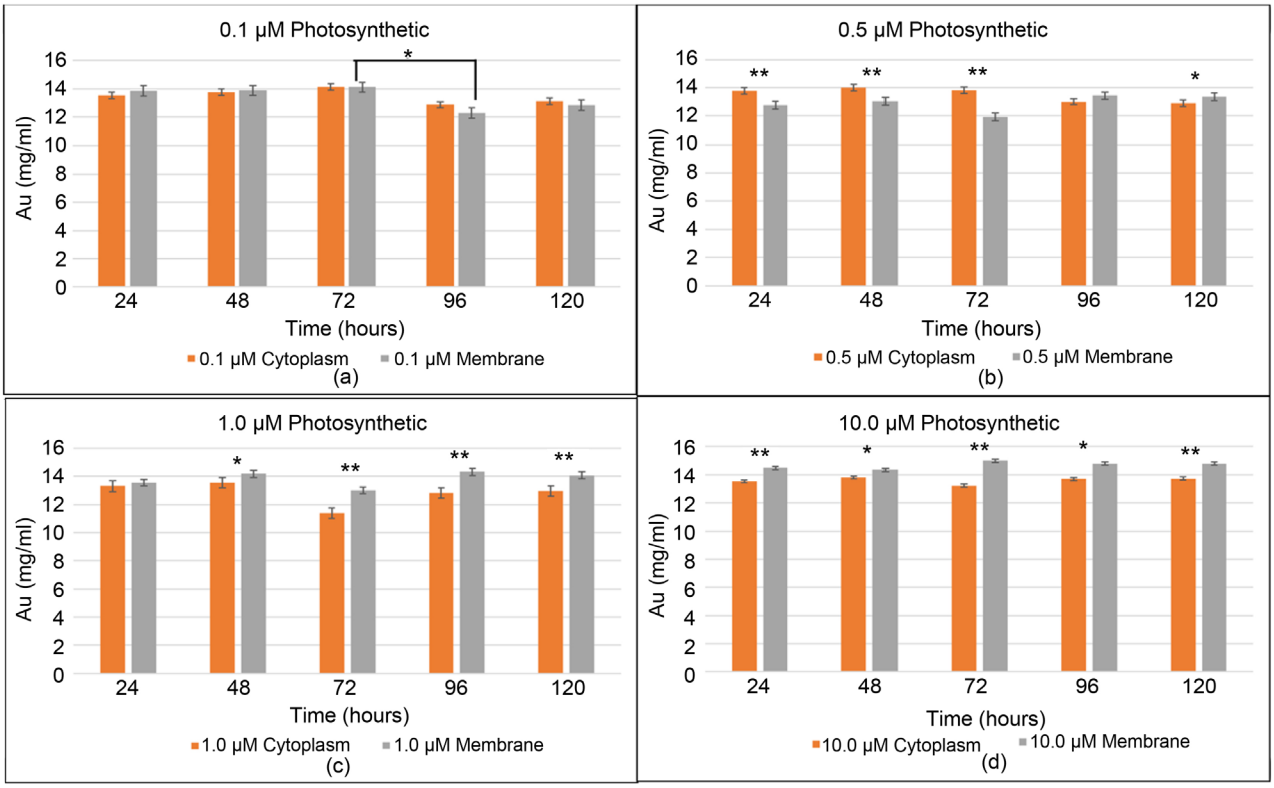

Figure 2. ICP analysis of the photosynthetic growth condition in varying concentrations of gold contamination. (a) Analysis of the 0.1 µM $AuCl_3$ cellular fractions; (b) Analysis of the 0.5 µM $AuCl_3$ cellular fractions; (c) Analysis of the 1.0 µM $AuCl_3$ cellular fractions; (d) Analysis of the 10.0 µM $AuCl_3$ cellular fractions. *denotes ($p < 0.001$) while **denotes ($p < 0.05$).

The results of the localization of the gold particles within the aerobic condition between the cytoplasmic (orange bars) and membrane (grey bars) fractions, reveal no significant difference between the two distinct subcellular fractions. When the statistical analysis was performed between the two fractions, there was no significant differences found, except where the * denotes a significance factor of ($p < 0.05$). Within the aerobic growth condition, the localization of the gold particles does not show a signification shift that favors one fraction over the over, and the overall accumulation does not increase in any specific pattern.

In comparing the two growth conditions, the 0.1 µM gold contamination in the photosynthetic condition reveals an equilibrium between the two cellular fractions, as well as overall accumulation of the gold ions and gold bio-nanoparticles across the entire 120-hour incubation time. At the 0.5 µM gold concentration, a higher accumulation of gold particles is seen within the cytoplasmic fraction for the first 72 hours (p value < 0.0001), and an equilibrium between membrane and cytoplasm is reached at 96 hours, and then gold starts to get sequestered and/or reduced at higher concentration (p value < 0.05) in the membrane fraction. At the 1.0 µM gold concentration, cells revealed an equilibrium state of the gold particles between both cellular fractions in the first 24 hours, and then it is increasingly localized within the membrane fraction (p value < 0.0001) throughout the incubation of 120 hours. At the 10.0 µM gold concentration, gold

is localized at much higher concentration in the membrane fraction throughout the 120 hours post exposure. The results of the localization of the gold particles within cells grown under photosynthetic conditions suggest the photosynthetic condition is better suited for the tolerance of the metal contamination. Cells grown under photosynthetic condition maintain high concentration gold particles within both subcellular fractions compared to the concentration of gold found in aerobically grown cells. As the concentration of the gold increases, the accumulation of the gold particles remains higher within the membrane. Cells which are grown in anaerobic plus light (photosynthetic condition), and produces light harvesting complexes embedded in the photosynthetic vesicles. These vesicles are membranous structure, and contain electron transport carriers and NADP dehydrogenases [29]. The photosynthetic membranes are localized in the cytoplasm, however when cells are sonicated to break the plasma membranes, photosynthetic membranes are pelleted along with the cell membrane. Therefore, it is indicative of the photosynthetic membrane attributing to the enhanced capability of tolerance of *R. sphaeroides* cells to the higher concentrations of gold toxicity, as well as the survivability.

To better understand the tolerance mechanism of *R. sphaeroides* to gold, or any bacterium with metal resistance, it is important to determine both the uptake kinetics and the spatial and temporal distributions of the gold ions and gold nanoparticles within subcellular fractions of the cell. This information will provide further insight into the mechanism that may be operating within the bacterial cell. There are multiple mechanisms of heavy metal resistance in bacteria which include: active transport through efflux pumps, reduction of the metal ions, the production of an extracellular barrier, as well as intra or extracellular sequestration [29]. However, each of these mechanisms may function independently or coordinate with another mechanism to optimize the metal resistance. The uptake and localization of the metal particles and/or ions over time can provide support for the mode and the mechanism of metal tolerance mechanisms [30].

3.2. Transmission Electron Microscopy (TEM)

The localization of the gold nanoparticles is shown in **Figure 3**, depicted by the red arrows as a reference, within the aerobic growth condition inside the cytoplasm and membrane of the cells, which validates the observation of previous ICP-OES data. The photosynthetically grown cells reveal a higher subcellular localization of the gold particles within the membrane, both plasma and photosynthetic membrane, which also independently validates the ICP-OES data under the photosynthetic growth condition. Several cells within the images below can be seen to have a compromised membrane (shown with the yellow arrows) due to the presence of the toxic gold particles. It has been reported in *R. capsulatus* that Au (III) damages the structure of the cell in high concentrations, >80 ppm [31]. The cells shown in the control images can be seen in various stages of

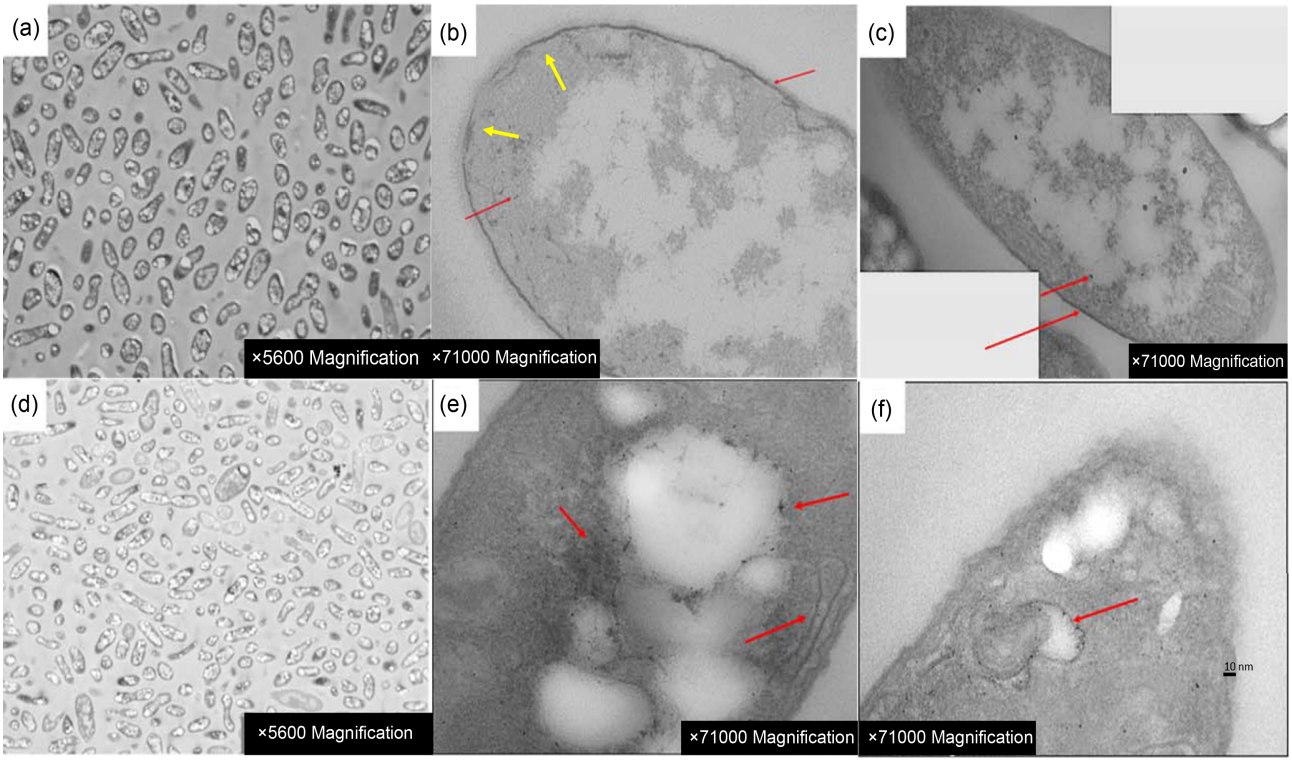

Figure 3. Transmission electron microscopy images of *R. sphaeroides* cells exposed to gold contamination. Top panel ((a), (b), (c)) represents the *R. sphaeroides* cells grown under the aerobic growth condition with the gold contamination of 0.0 μM $AuCl_3$, 0.5 μM $AuCl_3$, and 1.0 μM $AuCl_3$ respectively. Bottom panel ((d), (e), (f)) represents the *R. sphaeroides* cells grown under photosynthetic growth conditions with the gold contamination of 0.0 μM $AuCl_3$, 0.5 μM $AuCl_3$, and 1.0 μM $AuCl_3$ respectively. Red arrows point to detectable gold nanoparticles identified within the cellular membrane and the cytoplasm of the cells. Yellow arrows point to the compromised membranes of the cells. Scale bar corresponds to 10 nm in length.

cellular growth and cell division, representative of the logarithmic phase of growth. This growth phase, as previously mentioned, is the ideal phase to study metal contamination within, and this has been captured in the samples analyzed using the transmission electron microscopy. The aerobically grown cells exposed to the gold contamination can be seen to have the gold particles within the cytoplasm and membrane of the cells. The photosynthetically grown cells were identified to contain the gold particles localizing within the cytoplasm and membrane fractions. The presence of a photosynthetic membrane was identified in several images taken of the photosynthetically grown samples across the gold contamination concentrations. The vesicle structures, photosynthetic membrane, can be seen inside the cytoplasm of the *R. sphaeroides* cells, as seen in Panels E and F. This photosynthetic membrane, as previously mentioned, has been identified as the site of metallic reduction in closely related organisms, such as *R. capsulatus*, and the visualization of the gold particles within the photosynthetic membrane can be seen [31] [32] [33]. The presence of the gold particles within this photosynthetic membrane validates the higher accumulation and localization of the gold particles identified under the photosynthetic growth condition as measured by the ICP methodology.

3.3. Proposed Mechanism of Tolerance within *Rhodobacter sphaeroides*

If the transport system is the significant line of defense against the toxic effects of gold biosorption and bioaccumulation, the efflux pump located in the plasma membrane would play an important role in flushing out the excessive gold ions from the cytoplasm, and after a while it allows to maintain a state of equilibrium between the plasma membrane and the cytoplasm [33]. If, however, the cytoplasmic space is equipped with reducing the gold cations into metallic gold and produces bio-nanoparticles of less toxic effects, a net increase in gold particles can be observed in the cytoplasm. It is possible that photosynthetic vesicles within the cytoplasm are involved in the electron transport chain, and therefore the reduction of gold particles can take place there. Three main mechanisms help the bacterial cells to sequester and/or detoxify the heavy metals within the two cellular fractions (cytoplasmic and membrane) of *R. sphaeroides*. The current study presents the following models of heavy metal resistance in *R. sphaeroides* as illustrated in Figure 4 depicts the results of the localization of gold under different models.

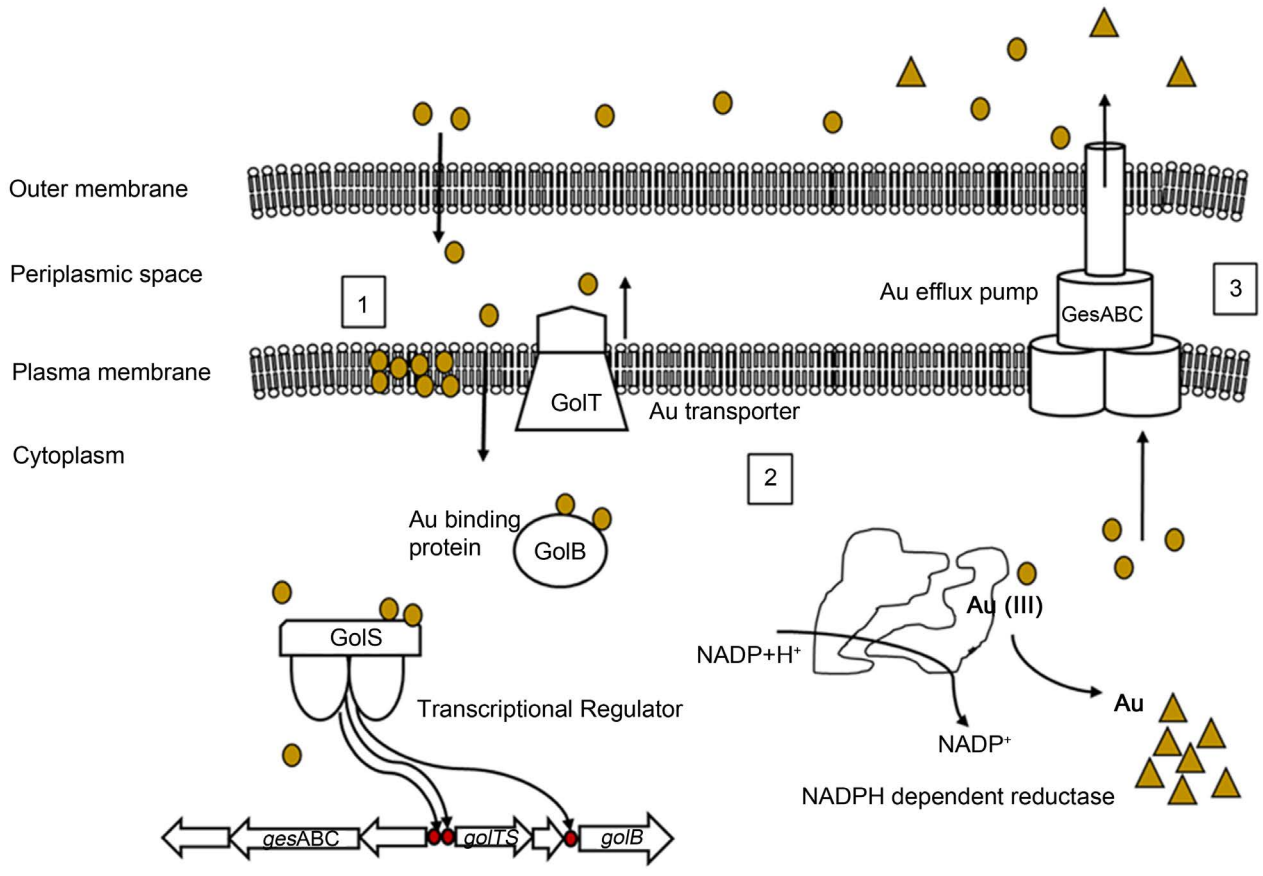

Figure 4. Proposed mechanisms of gold tolerance in *R. sphaeroides* as designed by the authors. Circles represent Au (III) and triangles represent the reduced gold particles, Au. The specific genes and transporters listed here are derived from a bioinformatics search of heavy metal related proteins within *R. sphaeroides* based on the similarity to previously identified heavy metal tolerance genes located in closely related organisms.

The first mechanism is the sequestration of heavy metals within the internal membrane layer, plasma membrane. The second mechanism includes the reduction of the metallic ions into less toxic metallic forms of bio-nanoparticles in the cytoplasm. The third mechanism is to actively transport the metallic ions into the cytoplasm, take out excessive gold ions from the cytoplasm to outside the cell, and over time an equilibrium is maintained between the membrane and the cytoplasm. The results of the Inductively Coupled Plasma suggest that the accumulation of gold into the bacterial cell increases as the incubation time increases as shown in Figure 1 and Figure 2. Based on a pilot experiment, it was noted that 86% - 95% of the gold that is initially introduced into the culture medium is recovered by the ICP analysis (data not shown). The percentage that is lost is due to precipitation within the machine itself, not a result of the bacterial interaction. Based on this knowledge, it can be seen that as time progresses, the cells are interacting more with the gold and increasingly accumulates the higher amounts.

4. Conclusion

In conclusion, the localization of the gold particles within *R. sphaeroides* was found to be in the highest accumulation within the membrane fraction of the cells within the photosynthetic growth condition. The results of the TEM also validated the localization of the gold particles within the membranes of the cells. Across the growth conditions, a shift in the accumulation of particles within the cellular fractions indicates a shift in the tolerance mechanisms within the bacterial cells based on the ICP results. The tolerance mechanism that we propose based on the results of this study includes the sequestration and subsequent detoxification of the gold particles within the membrane (s) of *R. sphaeroides.* Upon analysis of the localization study done through ICP, it can be seen how the accumulation changes between the cytoplasmic fraction and into the membrane fraction over the course of time which would possibly indicate the initial accumulation within the cytoplasmic fraction until the effects of the gold particles become too toxic to the cell, at which time the gold particles are shuttled into the membrane to be detoxified. This mechanism of tolerance will be further studied, with a focus on the photosynthetic growth condition, to further valid and characterize the gold tolerance mechanism within *Rhodobacter sphaeroides.*

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References

[1] Morris, C. (1992) Dictionary of Science and Technology. Academic Press, San Diego.

[2] Jennings, D. (1993) Stress Tolerance of Fungi (Vol. 10). M. Dekker, New York.

[3] Nies, D.H. (1999) Microbial Heavy Metal Resistance. *Applied Microbiology Technology*, **51**, 730-750. https://doi.org/10.1007/s002530051457

[4] Oves, M., Saghir Khan, M., Zaidi, A. and Ahmad, E. (2012) Soil Contamination, Nutritive Value, and Human Health Risk Assessment of Heavy Metals: An Overview. In: Zaidi, A., Wani, P.A. and Khan, M.S., Eds., *Toxicity of Heavy Metals to Legumes and Bioremediation*, Springer, Vienna, 1-27. https://doi.org/10.1007/978-3-7091-0730-0_1

[5] Spiegel, H. (2002) Trace Element Accumulation in Selected Bioindicators Exposed to Emissions along the Industrial Facilities of Danube Lowland. *Turkish Journal of Chemistry*, **26**, 815-823.

[6] Mielke, H. and Reagan, P. (1988) Soil as an Impact Pathway of Human Lead Exposure. *Environmental Health Perspectives*, **106**, 217-229. https://doi.org/10.1289/ehp.98106s1217

[7] Facchinelli, A., Sacchi, E. and Mallen, L. (2001) Multivariate Statistical and GIS-Based Approach to Identify Heavy Metal Sources in Soils. *Environment Pollution*, **114**, 313-324. https://doi.org/10.1016/S0269-7491(00)00243-8

[8] Solgi, E., Esmaili-Sari, A., Riyahi-Bakhtiari, A. and Hadipour, M. (2012) Soil Contamination of Metals in Three Industrial Estates, Arak, Iran. *Bulletin of Environmental Contamination and Toxicology*, **88**, 634-638. https://doi.org/10.1007/s00128-012-0553-7

[9] Kido, S. (2013) Secondary Osteoporosis or Secondary Contributors to Bone Loss in Fracture. *Bone Metabolism and Heavy Metals* (*Cadmium and Iron*) *Clinical Calcium*, **23**, 1299-1306.

[10] Barbier, O., Jacquillet, G., Tauc, M., Cougnon, M. and Poujeol, P. (2005) Effect of Heavy Metals on and Handling by, The Kidney. *Nephron Physiology*, **99**, 105-110. https://doi.org/10.1159/000083981

[11] Clarkson, T. (1987) Metal Toxicity in the Central Nervous System. *Environmental Health Perspectives*, **75**, 59-64. https://doi.org/10.1289/ehp.877559

[12] Jarup, L. (2003) Hazards of Heavy Metal Contamination. *British Medical Bulletin*, **68**, 167-182. https://doi.org/10.1093/bmb/ldg032

[13] Rokadia, H. and Agarwal, S. (2013) Serum Heavy Metals and Obstructive Lung Disease: Results from the National Health and Nutrition Examination Survey. *Chest*, **143**, 388-397. https://doi.org/10.1378/chest.12-0595

[14] Crannell, B., Eighmy, T., Krzanowski, J., Eusden, J., Shaw, E. and Francis, C. (2000) Heavy Metal Stabilization in Municipal Solid Waste Combustion Bottom Ash Using Soluble Phosphate. *Waste Management*, **20**, 135-148. https://doi.org/10.1016/S0956-053X(99)00312-8

[15] Vatamaniuk, O., Mari, S., Lu, Y. and Rea, P. (2000) Mechanism of Heavy Metal Ion

Activation of Phytochelatin (PC) Synthase. *The Journal of Biological Chemistry*, **275**, 31451-31459. https://doi.org/10.1074/jbc.M002997200

[16] Rosen, B. (1996) Bacterial Resistance to Heavy Metals and Metalloids. *Biological Inorganic Chemistry*, **1**, 273-277. https://doi.org/10.1007/s007750050053

[17] Tan, W., Liu, F., Feng, X., Huang, Q. and Li, X. (2005) Adsorption and Redox Reactions of Heavy Metals on Fe-Mn Nodules from Chinese Soils. *Journal of Colloid and Interface Science*, **284**, 600-605. https://doi.org/10.1016/j.jcis.2004.10.049

[18] Jaiswal, S. (2011) Role of Rhizobacteria in Reduction of Arsenic Uptake by Plants: A Review. *Journal of Bioremediation and Biodegradation*, **2**, 126. https://doi.org/10.4172/2155-6199.1000126

[19] Valls, M. and Lorezo, V. (2002) Exploiting the Genetic and Biochemical Capacities of Bacteria for the Remediation of Heavy Metal Pollution. *Microbiology Reviews*, **26**, 327-338. https://doi.org/10.1016/S0168-6445(02)00114-6

[20] Williams-Jones, A., Bowell, R. and Artashes, M. (2009) Gold in Solution. *Elements*, **5**, 281-287. https://doi.org/10.2113/gselements.5.5.281

[21] Reith, F., Etschmann, B., Grosse, C., Moors, H., Benotmane, M., Monsieurs, P. and Brugger, J. (2009) Mechanisms of Gold Biomineralization in the Bacterium Cupriavidus Metallidurans. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 17757-17762. https://doi.org/10.1073/pnas.0904583106

[22] Johnson, H. and Choudhary, M. (2016) Heavy Metal Pollution and Use of Microorganisms for Bioremediation. *ATINER'S Conference Paper Series*, 11*th Annual International Symposium on Environment*, Athens, 23-26 May 2016, ENV2016-2012.

[23] Sistrom, W. (1960) A Requirement for Sodium in the Growth of Rhodopseudomonas Spheroides. *Journal of General Microbiology*, **22**, 778-785. https://doi.org/10.1099/00221287-22-3-778

[24] Imam, S., Yilmaz, S., Sohmen, U., Gorzalski, A., Reed, J., Noguera, D. and Donohue, T. (2011) IRsp1095: A Genome-Scale Reconstruction of the Rhodobacter Sphaeroides Metabolic Network. *Biomed Central Systems Biology*, **5**, 116. https://doi.org/10.1186/1752-0509-5-116

[25] Beggs, W. (1984) Growth Phase in Relation to Ketoconazole and Miconazole Susceptibilities of Candida Albicans. *Antimicrobial Agents and Chemotherapy*, **25**, 316-318. https://doi.org/10.1128/AAC.25.3.316

[26] Anthony, J., Green, H. and Donohue, T. (2003) Purification of Rhodobacter Sphaeroides RNA Polymerase and Its Sigma Factors. *Methods in Enzymology*, **370**, 54-65. https://doi.org/10.1016/S0076-6879(03)70005-6

[27] Diggle, P.J. (1988) An Approach to the Analysis of Repeated Measurements. *Biometrics*, **44**, 959-971. https://doi.org/10.2307/2531727

[28] Maxwell, S.E. (1980) Pairwise Multiple Comparisons in Repeated Measures Designs. *Journal of Educational Statistics*, **5**, 269-287. https://doi.org/10.2307/1164969

[29] Frison, L. and Pocock, S. (1992) Repeated Measures in Clinical Trials: Analysis Using Mean Summary Statistics and Its Implications for Design. *Statistics in Medicine*, **11**, 1685-1704. https://doi.org/10.1002/sim.4780111304

[30] Sener, M., Olsen, J., Hunter, C. and Schulten, K. (2007) Atomic-Level Structural and Functional Model of a Bacterial Photosynthetic Membrane Vesicle. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 15723-15728. https://doi.org/10.1073/pnas.0706861104

[31] Ianeva, O. (2009) Mechanisms of Bacteria Resistance to Heavy Metals. *Microbiol Z*,

71, 54-65.

[32] Munoz, A., Ruiz, E., Abriouel, H., Galvez, A., Ezzouhri, L., Lairini, K. and Espinola, F. (2012) Heavy Metal Tolerance of Microorganisms Isolated from Waste Waters: Identification and Evaluation of Its Potential for Biosorption. *Chemical Engineering Journal*, **210**, 325-332. https://doi.org/10.1016/j.cej.2012.09.007

[33] Feng, Y., Yu, Y., Wang, Y. and Lin, X. (2007) Biosorption and Bioreduction of Trivalent Aurum by Photosynthetic Bacteria *Rhodobacter capsulatas*. *Current Microbiology*, **55**, 402-408. https://doi.org/10.1007/s00284-007-9007-6