EFFECT OF GOLD AND SILVER NANOPARTICLES ON CYANOBACTERIUM *ARTHROSPIRA PLATENSIS* AND MICROALGA *PORPHYRIDIUM CRUENTUM*

Liliana CEPOI, Ludmila RUDI, Tatiana CHIRIAC, Ana VALUTA, Svetlana DJUR, Valery RUDIC

Institute of Microbiology and Biotechnology of Technical University of MOLDOVA, Chisinau, Republic of Moldova, liliana.cepoi@imb.utm.md

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Abstract

The development of nanomaterials for various purposes has led to the diversification of their fields of application but has also raised questions regarding the environmental safety of releasing NPs into the environment. In the biotechnology of microalgae and cyanobacteria, NPs can serve as biotechnological tools to control specific processes. In the field of biosecurity, these microorganisms can be used as models for NP toxicity. Among the metal nanoparticles, gold and silver nanoparticles stand out due to their multiple functionalities, unique properties, and wide practical applications. The effect of 10 nm - sized Au and Ag nanoparticles stabilized in citrate has been studied on cyanobacterium *A. platensis* (spirulina) and microalga *P. cruentum*. Both cultures were grown in media with nanoparticles in different concentrations. It was found that AgNPs reduced the biomass production of *A. platensis* and altered its quality. In the case of *P. cruentum* culture, AgNPs provided a significant increase in the content of phycobiliproteins and lipids, while reducing the carbohydrate content. The effect of AuNPs resulted in increased phycobiliprotein and lipid content in *A. platensis* and *P. cruentum* biomass. In *P. cruentum* culture, AuNPs caused a significant reduction in protein content. In both cultures, nanoparticles induced an increase in the oxidative stress marker – malondialdehyde, and a decrease in the antioxidant activity. These microorganisms can be used as test objects to identify the toxic effects of NPs. At the same time, NPs can serve as tools for directing certain biotechnological processes in the cultivation technologies of spirulina and porphyridium.

Keywords: Gold, silver, nanoparticles**,** spirulina, porphyridium.

1. INTRODUCTION

Research on the use of nanoparticles in microalgae cultivation has mainly focused on assessing their toxicity to aquatic organisms [1]. Analysis of the results raised the question of exploring the benefits of using nanoparticles in the growth of microalgae [2]. The addition of nanoparticles to microalgae cultures is a promising alternative to enhance the growth of microalgae and the accumulation of intracellular compounds [3]. Recent studies have shown that the addition of nanoscale material can improve the efficiency of CO_2 absorption and light conversion in microalgae cultures [2]. However, cellular damage caused by certain nanoparticles due to the release of toxic ions remains a challenge for the use of this technology.

Evaluation and comparison of the action of nanoparticles depending on their type, size, coating, and origin on eukaryotic microalgae and prokaryotic cyanobacteria species contribute to a better understanding of the effects of these nanoparticles and the possibility to manipulate their characteristics for the development of directed cultivation biotechnological processes [4,5].

Cyanobacterium *A. platensis* and red microalga *P. cruentum* are well-known producers of biologically active compounds, including proteins, sulfated polysaccharides, phycobiliproteins, and valuable polyunsaturated fatty acids [6,7].

The present research is focused on analyzing the effect of 10 nm gold and silver nanoparticles the growth and accumulation of valuable biochemical components in *P. cruentum* and *A. platensis* cultures.

2. EXPERIMENTAL DESIGN

Cyanobacterium *A. platensis* and microalga *P. cruentum* were grown on mineral media under laboratory conditions [8,9]. Gold and silver nanoparticles were supplemented to the mineral medium. We used citrate-stabilized Au and Ag nanoparticles with a diameter of 10 nm (SIGMA-ALDRICH CHEMIE GmbH, Germany). Specifications for AuNP: OD 1 and PDI < 0.2. Specification for AgNP: 10 ± 0.2 nm particle size TEM). At the end of the cultivation cycle (6 days for *A. platensis*; 14 days for *P. cruentum*), biomass was separated and standardized to a concentration of 10 mg/mL. The amount of biomass was determined spectrophotometrically at 680 nm for *A. platensis* and 545 nm for *P. cruentum*. The content of proteins (Lowry method), carbohydrates (using the anthrone reagent), and lipids (using the phosphovanillin reagent) was determined in the biomass. The content of phycobiliproteins was determined in aqueous extracts. The values of malondialdehyde content were determined based on the reaction with thiobarbituric acid. The antioxidant activity of the biomass was assessed by the ABTS radical cation (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) in aqueous extracts. [8,9].

3. RESULTS AND DISCUSSION

Figure 1 illustrates the amount of biomass of *A. platensis* and *P. cruentum* and proteins at the end of cultivation cycle in the presence of 10 nm gold and silver nanoparticles.

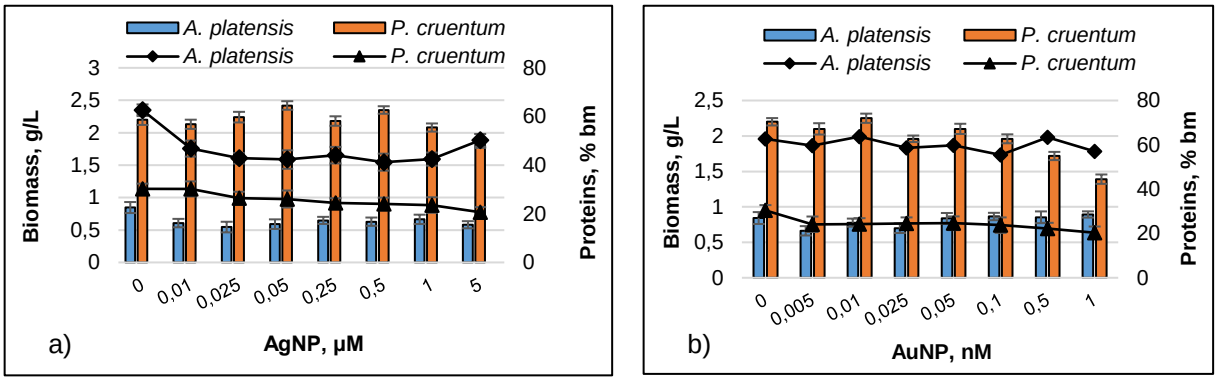

Figure 1 Biomass amount (g/L) and accumulated protein quantity (% bm) during cultivation of cyanobacterium *A. platensis* and red microalga *P. cruentum* in the presence of AgNPs (a) and AuNPs (b)

The culture of *A. platensis* responds to AgNPs in the growth medium by reducing biomass amount at all applied concentrations (**Figure 1a**). Biomass amount was 15.9-30.9% lower compared to control sample. When cyanobacterium was grown on a medium containing AuNPs (0.005-0.025 nM), a decrease in biomass of 7.9-21.4% was observed (**Figure 1b**). Microalga *P. cruentum* responded differently to the presence of nanoparticles. Regarding AgNPs, no significant correlation was detected between biomass amount and nanoparticle concentration within the range of 0.01-0.5 μM in the nutrient medium (**Figure 1a**). Concentrations of 0.05 μM and 0.5 μM stimulated microalgal biomass production by 10% and 6.8%, respectively. A decrease in biomass by 17.3% was found at a concentration of 5 μM AgNPs. In the case of AuNPs, concentrations of 0.5 and 1 nM reduced the amount of biomass by 21.9% and 36.7%, respectively (**Figure 1b**). Such a response of *A. platensis* and *P. cruentum* cultures to the presence of Au and Ag nanoparticles in the growth medium can be considered commonplace. In several studies, toxic effects of Au and Ag nanoparticles on aquatic cultures have been demonstrated, regardless of concentration, size, presence/absence of coatings, or coating type. On the contrary, some studies have reported a stimulatory effect of these nanoparticles on microalgae

growth. Therefore, when using 12 nm Ag nanoparticles stabilized in polyethylene glycol (PEG), in the concentration range of 0.025-0.1 µM, biomass production of *Spirulina platensis* was stimulated by 24.2-31.6%. Concentrations of 0.025-0.5 µM 5 nm AuNPs (PEG) induced an increase in biomass amount by 23.4-35.8% [10]. Gold nanoparticles coated with polyvinylpyrrolidone with a size of 43.67± nm, at a concentration of 0.014 mg/mL, enhanced the productivity of microalgae *Raphidocelis subcapitata* [11]. Silver nanoparticles in concentrations of 10, 40, 75, 150 and 300 µg/L, when applied to the cultivation of microalgae *Chlamydomonas reinhardtii* resulted in identical cell density values [12].

Silver nanoparticles applied to the cultivation medium of *A. platensis* by 19.75-34% and *P. cruentum* (**Figure 1a**) reduced protein content. Gold nanoparticles had a similar effect, but only for *P. cruentum* culture (by 18.5-32.7%) **(Figure 1b**). In a previous study, when AgNPs (PEG) were applied to cultivate *A. platensis* at concentrations ranging between 0.025-0.5 µg/L, no changes in protein content were identified [10]. The decrease in biomass amount of cyanobacterium *A. platensis*, as well as the protein content in biomass, indicates the toxic effect exerted by AgNPs. This implies a potential disruption of the entire biosynthetic process within cyanobacterial cells.

Figure 2 shows the content of phycobiliproteins (% biomass) and carbohydrates (% biomass) accumulated in the biomass of *A. platensis* and *P. cruentum* during their growth in the presence of AgNPs and AuNPs.

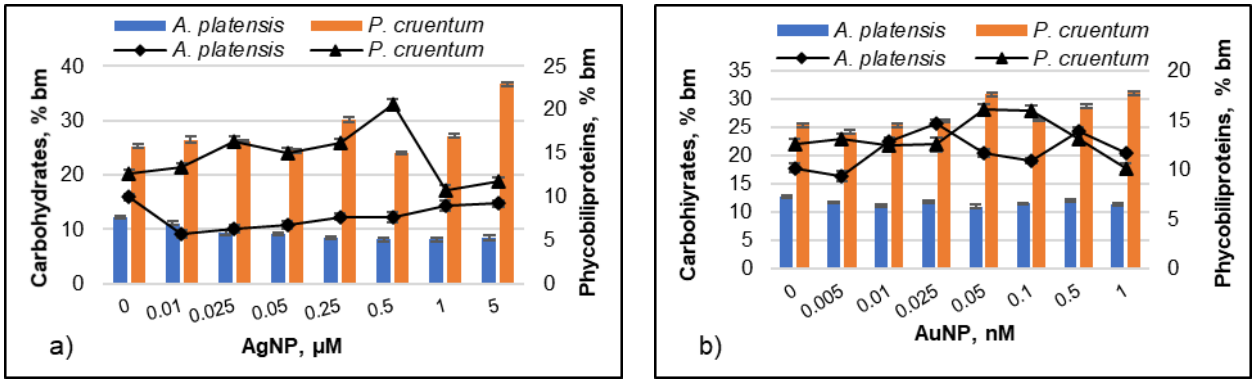

Figure 2 Phycobiliproteins (% bm) and carbohydrate content (% bm) in the biomass of *A. platensis* and *P. cruentum* during cultivation in the presence of AgNPs (a) and AuNPs (b)

In the case of 10 nm AgNPs stabilized in citrate, a toxic effect was detected on *A. platensis* culture even at low concentrations in the range of 0.01-0.5 µM, which led to a decrease in phycobiliprotein content by 22.5-42.3% (**Figure 2a**). Concentrations of 1 and 5 µM had a milder negative impact on phycobiliproteins, with their content in biomass below the control level by 9.5% and 7.3%, respectively. In the biomass of *P. cruentum*, AgNPs in the concentration range of 0.025-0.5 µM induced an increase in phycobiliprotein content by 30-64%. Gold nanoparticles were found to increase phycobiliprotein content in both cultures (**Figure 2b**). The highest values of phycobiliproteins in *A. platensis* biomass – 44.3% and 36.8%, respectively – were recorded when using AuNPs in concentrations of 0.025 nM and 0.5 nM. In the case of *P. cruentum*, an increase of 28.5% and 26.9% was observed for concentrations of 0.05 nM and 0.1 nM, respectively. Application of AuNPs at a concentration of 1 nM reduced the content of phycobiliproteins in microalgae biomass by 19%. The use of AgNP (PEG) nanoparticles with a size of 10 nm during the cultivation of *A. platensis* resulted in a decrease in phycobiliprotein content at low nanoparticle concentrations and an increase of 14% at concentrations of 2.5 µM and 10.0 µM [8]. The content of phycobiliproteins in *Skeletonema costatum* biomass increased with 30% as a result of the application of AgNPs at a concentration of 5 mg/L [13]. The increase in phycobiliprotein content in *P. cruentum* biomass can be interpreted as an adaptation of the culture to the applied NPs' toxicity. Similarly, the increase in carbohydrate production can be assessed as a protective mechanism in response to the altered growth conditions [14].

The alteration of carbohydrate content was analyzed depending on the type of nanoparticles (**Figure 2**). AgNPs reduced the carbohydrate content in biomass of *A.platensis* by 23-33.5% (**Figure 2a**). In the case of microalga *P. cruentum*, AgNPs in concentrations of 0.25 μM and 5 μM stimulated carbohydrate production by 19.5% and 45%, respectively. Gold nanoparticles used for the cultivation of *A. platensis*, showed a trend towards lower carbohydrate content, which decreased by 5.5-13.4% (**Figure 2b**). In the biomass of *P. cruentum*, higher carbohydrate values of 21.5-22.4% were recorded at concentrations of 0.05 nM and 1 nM, respectively. A decrease in carbohydrate quantity was reported when microalgae *Microcystis* and *Oscillatoria* were exposed to nanoparticles due to cell membrane damage [15]. Microalga *P. cruentum* reacted by increasing carbohydrate content in response to the action of 12 nm-sized AgNP (PEG) [9].

Currently, there is an increasing number of publications on the involvement of nanoparticles in lipid synthesis in microalgae [2,16]. The stimulatory effect of nanoparticles on the synthesis of these microalgal and cyanobacterial constituents is considered a result of oxidative stress cell installation and activation of processes to restore the integrity of cell membranes [17]. **Figure 3** shows the content of lipids and MDA in the biomass of cyanobacterium *Arthrospira platensis* and red microalga *P. cruentum* grown in the presence of different concentrations of AgNPs and AuNPs.

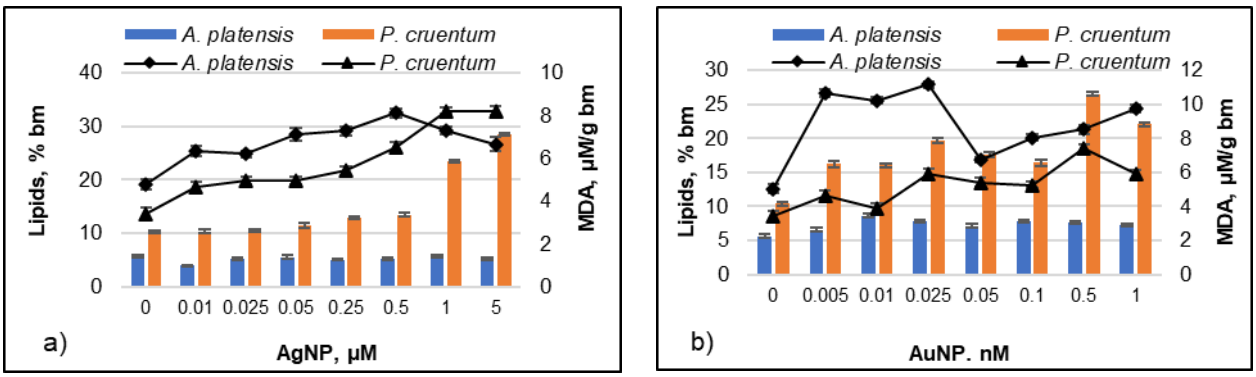

Figure 3 Lipid content (% bm) and MDA (μM/g bm) levels in the biomass of *A. platensis* and *P. cruentum* during cultivation in the presence of AgNPs (a) and AuNPs (b)

The presence of 10 nm AgNPs stabilized in citrate in the cultivation medium of *A. platensis* did not significantly alter the lipid content in the biomass (**Figure 3a**). Nanoparticle concentration of 0.01 μM reduced the lipid content by 31.5%, while other concentrations led to reductions of 7.2-11.3% in lipid content of cyanobacterial biomass. In the case of *P. cruentum*, AgNPs in concentrations of 1 μM and 5 μM induced a 2.3-2.8 fold increase in lipid content. Nanoparticle concentrations of 0.05-0.5 μM stimulated lipid synthesis by 12.3-31.4%. Cultures of *A. platensis* and *P. cruentum* responded to the presence of AuNPs in the nutrient medium by increasing the lipid content, similar to the change observed in the content of phycobiliproteins (**Figure 3b**). In the biomass of *A. platensis*, lipid content increased by 16.3-52.3%. The maximum lipid content in biomass was achieved at a nanoparticle concentration of 0.01 nM. In *P. cruentum* biomass obtained through cultivation with 0.5 μM and 1 nM AuNPs, lipid values were more than 2.4-2.1 times higher compared to control sample. For AuNPs concentrations ranging between 0.005-0.1 nM, lipid accumulation reached 54-89.7%. The inhibitory or stimulatory effect on biosynthetic processes for both types of cultures in response to the presence of Au and Ag nanoparticles in the nutrient media can be considered a result of the oxidative stress induced by these nanoparticles. It has been established that AgNPs at a concentration of 0.2 mg/L reduced lipid content by 5% in *Scenedesmus sp.* biomass, while a concentration of 0.1 mg/L stimulated lipid synthesis in *Thalassiosira sp.* by 33% [17]. An increase of 16% in lipid content was observed in *S. costatum* under the influence of AgNPs at a concentration of 5 mg/L.

The elevated levels of malondialdehyde (MDA) can be used as an indicator of oxidative stress detected in *A. platensis* and *P. cruentum* cultures. It was found that AgNPs caused significant increases (29-69%) in MDA

formation in *A. platensis* biomass. In *P. cruentum* biomass, MDA increased by 26.4-91% when using concentrations of 0.005-0.1 µM and by 2.4 times when it exposed to AgNPs concentrations of 1 and 5 µM (**Figure 3a**). Gold nanoparticles in concentrations of 0.005-0.025 nM induced a 2.0-2.2-fold increase in MDA values in *A. platensis* biomass (**Figure 3b**). Concentrations ranging from 0.05 nM to 1 nM enhanced MDA values by 34-94.4%. Thus, lower concentrations of AuNPs were more actively involved in the oxidative degradation of lipids in cyanobacterial culture. In the case of *P. cruentum*, concentrations of 0.005-0.01 nM led to an increase in MDA values of 13.6-26.4%. For other applied concentrations, MDA content in biomass was higher by 54.5-72.7%.

Figure 4 shows the changes in the antioxidant activity of aqueous extracts derived from *A. platensis* and *P. cruentum* grown in the presence of AgNPs and AuNPs.

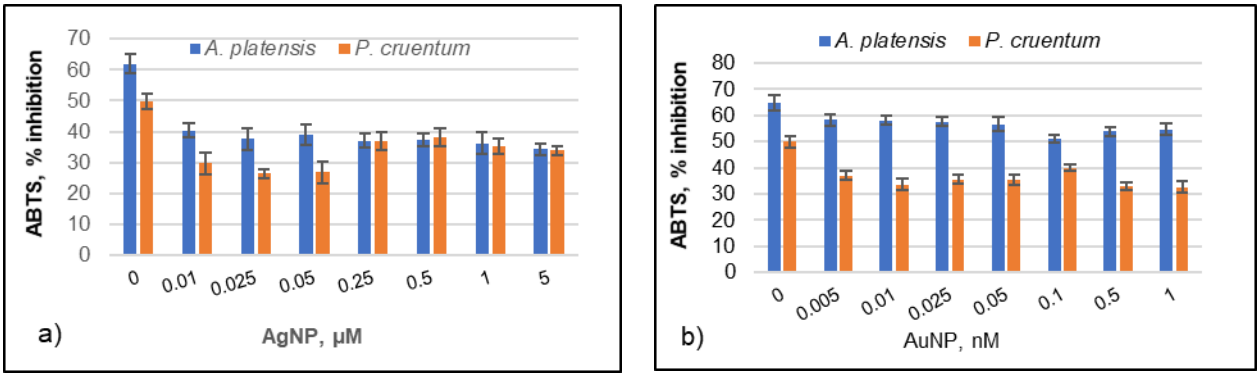

Figure 4 Antioxidant activity (ABTS, % inhibition) of aqueous extracts derived from biomass of *A. platensis* and *P. cruentum* grown in the presence of AgNPs (a) and AuNPs (b)

The antioxidant activity of biomass was another indicator of the oxidative stress caused by the presence of 10 nm-sized Au and Ag nanoparticles stabilized in citrate in the culture medium. The values of ABTS radical scavenging activity decreased for both cultures. AgNPs reduced the antioxidant activity of water extracts from *A. platensis* biomass by 34.8-44.7% and the antioxidant activity of water extracts from *P. cruentum* biomass by 23.5-46.9% (**Figure 4a**). Exposure to AuNPs resulted in a moderate reduction in the antioxidant activity of *A. platensis* biomass by 8.9-20%, while a 20-32.9% reduction in ABTS test values was found for *P. cruentum* (**Figure 4b**)

4. CONCLUSION

One of the most important conclusions drawn from this study is that the outcome of nanoparticle action on microalgae and cyanobacteria depends on the type, size, coating of nanoparticles, and, obviously, the species of microalgae and cyanobacteria, their origin and cultivation conditions.

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