THE DIFFERENCES IN THE EFFECTS OF PVP STABILIZED SILVER NANOPARTICLES ON THE MICROALGA *PORPHYRIDIUM CRUENTUM* AND THE CYANOBACTERIUM *ARTHROSPIRA PLATENSIS*

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Abstract

Nanoparticles are increasingly becoming part of microalgal biotechnologies. Certain types of nanoparticles are suggested as stimulators for lipid synthesis in microalgal biofuel production. The variety of responses from microalgal cultures depends on many factors, such as the type of nanoparticles, their size, concentration, origin, the duration of contact with microorganisms, the age of the culture, and the cultivation conditions. Another determining factor in the relationship between microorganisms and the presence of nanoparticles in their growth environment is the type of microorganisms. Eukaryotic organisms, such as microalgae, respond differently to nanoparticles than prokaryotic organisms, such as cyanobacteria. A study was conducted to determine the differences in the effects of PVP-stabilized silver nanoparticles on the microalga *Porphyridium cruentum* and the cyanobacterium *Arthrospira platensis*. Silver nanoparticles at concentrations ranging from 0.05 mg/L to 5 mg/L were added to the mineral medium from the first day of the cultivation cycle. The microorganism cultures were cultivated under autotrophic conditions. Biochemical tests were performed on the collected biomass. The cyanobacterium *Arthrospira platensis* reacted to AgNP-PVP by reducing biomass content and showing an insignificant change in structural compounds. Reduced antioxidant levels evidenced stress in cyanobacterial culture. For *Porphyridium cruentum*, biomass content increased, while lipid and carbohydrate content decreased. Higher malondialdehyde levels indicated stress in microalgal culture. The variety of responses from microalgae and cyanobacteria cultures to silver nanoparticles supports the idea that diverse mechanisms are involved in maintaining viability, which creates the possibility of remodeling biosynthetic activity depending on the intended purpose

Keywords: *Porphyridium cruentum*, *Arthrospira platensis*, PVP, silver, nanoparticles

1. INTRODUCTION

Silver nanoparticles (AgNPs) are recognized for their antimicrobial properties, which make them widely used in various products such as medical devices, textiles, cosmetics, and food packaging [1,2]. Recently, AgNPs have been exploited in biotechnologies for biofuel production, using microalgae and cyanobacteria as producer substrates [3]. Due to their stimulating effects, which involve reducing toxicity, AgNPs can be employed in phycological technologies to produce biologically active substances [4].

One key aspect of this effect is reducing AgNP aggregation and silver ion (Ag^+) release. Organic coatings and stabilizing substances address this issue [5]. To enhance colloidal stability and control the release of Ag+, nanoparticles are often coated with stabilizers such as polyvinylpyrrolidone (PVP). PVP is a polymer frequently used in nanoparticle synthesis because it prevents aggregation through steric repulsion. It forms a physical

barrier on the surface of the nanoparticles, limiting their direct interactions and thus reducing the risk of forming large aggregates, otherwise decreasing nanoparticle bioavailability [2,5].

One of the most significant effects of PVP is reducing the release of Ag^+ ions, considered the primary cause of acute toxicity to aquatic organisms. Silver nanoparticles coated with PVP have shown a slower release of Ag+ ions, reducing toxicity in microalgae species tests [1]. AgNP-PVP particles have been detected in microalgae's periplasmic space and cytoplasm without significantly affecting their growth or photosynthesis [2]. The reduced aggregation of AgNP-PVP allowed for the maintenance of microalgal viability, lowering the generation of free radicals [6]. These findings suggest that PVP stabilizes AgNPs and moderates their toxicity by reducing oxidative stress.

The stimulating effect of AgNPs depends not only on the type of nanoparticles, their size, and concentration but also on the specific microalgae species with which they interact [7].

This research aimed to evaluate and compare the effects of AgNP-PVP on the cyanobacterium *Arthrospira platensis* (a prokaryotic organism) and the microalga *Porphyridium cruentum* (a eukaryotic organism), focusing on how they induce oxidative stress and influence biomass accumulation and biosynthetic activity.

2. EXPERIMENTAL DESIGN

The cyanobacterium *Arthrospira platensis* CNMN-CB-02 and the microalga *Porphyridium cruentum* CNMN-AR-01, stored in the National Collection of Non-Pathogenic Microorganisms at the Institute of Microbiology and Biotechnology of the Technical University of Moldova, were cultivated in mineral media under photoautotrophic conditions, with continuous illumination and constant temperature. The cultivation cycle lasted 6 days for *Arthrospira platensis* and 12 days for *Porphyridium cruentum*. Silver nanoparticles stabilized with PVP (AgNPs-PVP), with a size of 20 nm ± 2 nm, were added to the cultivation media at various concentrations.

Biomass content was determined spectrophotometrically. In the collected biomass, lipid content was measured using the phospho-vanillin reagent, and carbohydrate content was assessed using the anthrone reagent. The malondialdehyde (MDA) level was quantified using thiobarbituric acid reactive substances (TBARS). Antioxidant activity was evaluated using the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay on 55% ethanol extracts from the collected biomass. All experiments were performed in triplicate, and statistical data analysis was conducted using Microsoft Excel software (Microsoft 365 Excel version 2108).

3. RESULTS AND DISCUSSION

This study investigated the effects of PVP-stabilized AgNPs on the growth and biosynthetic activity of two representative species, the cyanobacterium *Arthrospira platensis* and the microalga *Porphyridium cruentum*. Changes in biomass content primarily indicated the presence or absence of the toxic effect of AgNPs. **Figure 1** shows the effects of AgNPs-PVP on growth and biomass accumulation in *Arthrospira platensis* and *Porphyridium cruentum* cultures.

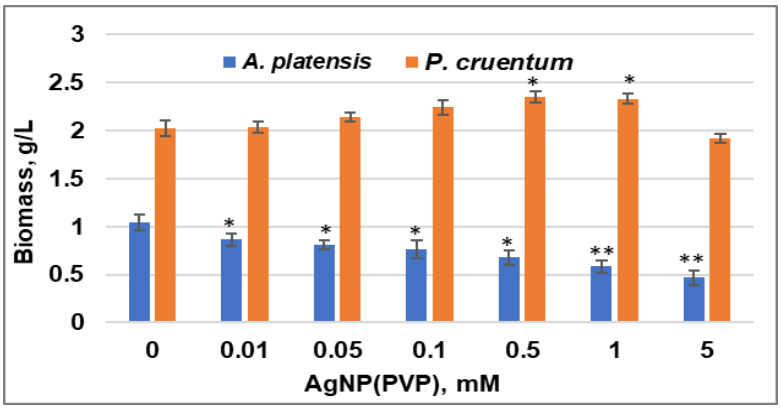

Figure 1 Changes in biomass content (g/L) of *Arthrospira platensis* and *Porphyridium cruentum* cultures under the action of AgNPs-PVP; *p < 0.05; p < 0.01

Applying AgNPs-PVP at the studied concentrations led to a significant reduction in biomass in the *Arthropsira platensis* culture. At a concentration of 0.01 mM AgNPs, biomass decreased by 17.3% ($p < 0.05$), while at 5 mM, the reduction was 55.4% ($p < 0.01$). A strong negative correlation was established between the concentration of nanoparticles in the cultivation medium and the final biomass values of the cyanobacterium, with an $r^2 = -0.8557$. In contrast, applying AgNPs-PVP to the *Porphyridium cruentum* culture resulted in a different response about biomass accumulation. Concentrations ranging from 0.01 mM to 0.1 mM did not affect biomass production or cause a slight, non-significant increase. At concentrations of 0.5 mM and 1.0 mM, the microalgal biomass content increased by 16.4% ($p < 0.05$) and 15.3% ($p < 0.05$), respectively. The correlation between AgNP concentrations and biomass values indicates a strong negative relationship, with an $r^2 = -0.6057$, suggesting a trend of decreasing *Porphyridium cruentum* biomass as AgNP concentration increases.

Figure 2 graphically presents the results regarding the accumulation of lipids, carbohydrates, and oxidative stress severity (MDA) in the biomass of the investigated cultures.

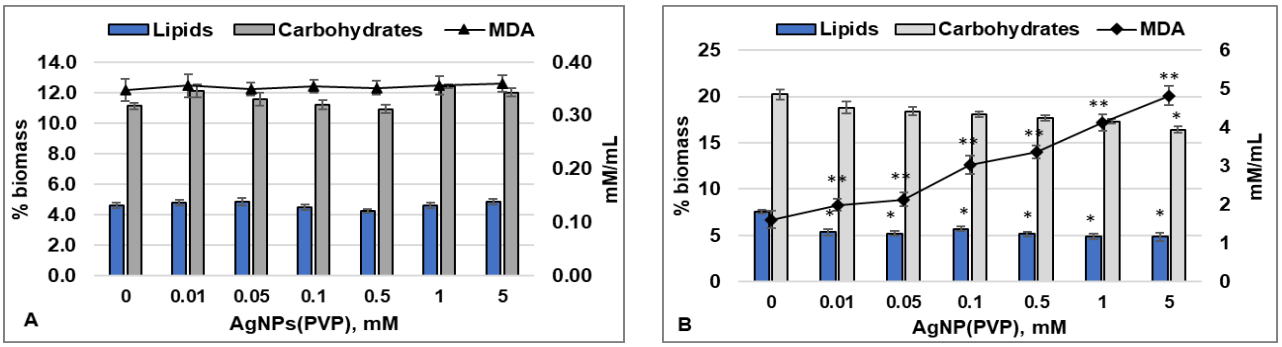

Figure 2 Changes in carbohydrate content (% biomass), lipid content (% biomass), and MDA (mM/mL) in the biomass of (**A**) *Arthrospira platensis* and (**B**) *Porphyridium cruentum* under the action of AgNPs-PVP; *$p < 0.05$; **$p < 0.01$

The carbohydrate and lipid content indices provide valuable information about maintaining cellular membrane integrity, reflecting the biosynthesis of structural and storage components. Malondialdehyde (MDA) is a marker of the severity of oxidative stress in cells. In the case of the cyanobacterium *Arthrospira platensis*, where AgNPs-PVP inhibited biomass production, the biosynthetic activity of the culture was not affected (**Figure 2A**). The lipid and carbohydrate content remained at the same level as the control. Only at the 5 mM concentration of AgNPs-PVP was a significant reduction of 18.8% ($p < 0.05$) observed in carbohydrate content. MDA values did not change, suggesting that, against a backdrop of reduced biosynthetic activity, excessive free radical production did not occur. A completely different results was established in the microalgae *Porphyridium cruentum* culture exposed to the action of AgNPs-PVP (**Figure 2B**). In all experimental variants, the carbohydrate content in the microalgal biomass did not change, remaining at the same level as in the control. In contrast, the lipid content significantly decreased due to AgNPs-PVP exposure, with this effect being observed at all applied concentrations. The reduction in lipid content ranged from 24.3% ($p < 0.05$) to 35.6% ($p < 0.05$) below control values. A strong negative correlation, $r^2 = -0.6204$, was determined between the nanoparticle concentrations and lipid content values. In the final biomass of *Porphyridium cruentum*, MDA test values were significantly higher than the control. AgNPs-PVP concentrations of 0.01 mM and 0.05 mM led to an increase in malondialdehyde content by 23.6% ($p < 0.01$) and 32.3% ($p < 0.01$), respectively. At a concentration of 1.0 mM AgNPs, the MDA value increased by 88.2% ($p < 0.01$). Additionally, concentrations of 0.5 mM and 1.0 mM caused a doubling of malondialdehyde content, which was significant. At 5 mM AgNPs, MDA values tripled compared to the control. Lipid biosynthesis supported microalgal biomass production, but the lipid content decreased due to oxidative degradation of cellular membranes. However, the maintenance of biomass productivity in the presence of AgNPs-PVP suggests that biosynthetic activity was not significantly affected. The reduction in lipid content appears to be a necessary mechanism to mitigate the oxidative stress

induced by the nanoparticles. Notably, the decrease in lipid values is not critical for the *Porphyridium cruentum* culture, indicating some metabolic flexibility of this species in the face of oxidative stress induced by this type of nanoparticles.

Antioxidant tests can verify cell stress, which can either increase or decrease. **Figure 3** analyzes the ABTS antioxidant test values for the hydro-ethanolic extracts from the biomass of the studied cultures.

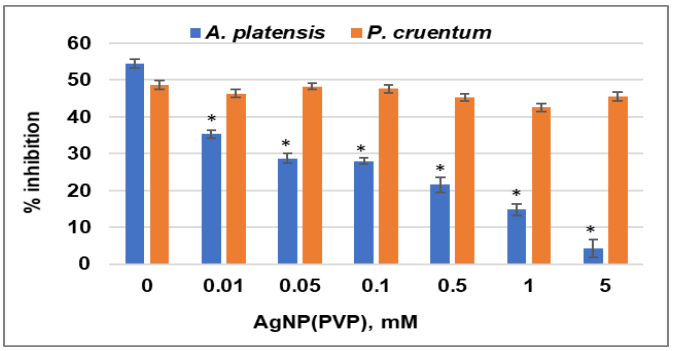

Figure 3 Changes in antioxidant activity (% ABTS inhibition) of *Arthrospira platensis* and *Porphyridium cruentum* biomass obtained from cultivation in the presence of AgNPs-PVP; *p < 0.01

In the samples obtained from *Arthrospira platensis* biomass, antioxidant activity was significantly reduced under the influence of AgNPs-PVP. Thus, the ABTS test value for the sample treated with 0.01 mM AgNPs decreased by 35% ($p < 0.01$). As the nanoparticle concentration increased, antioxidant activity continued to decline, reaching a 92% reduction ($p < 0.01$) at a concentration of 5 mM AgNPs. The relationship between AgNP concentrations and antioxidant test values indicates a strong negative correlation, $r^2 = -0.8829$. For the microalga *Porphyridium cruentum*, the stress experienced by the cells was not reflected in changes in the antioxidant test values, which remained at the control level.

The study highlighted clear differences in the effects of AgNPs-PVP on the cyanobacterium *Arthrospira platensis* and the microalga *Porphyridium cruentum*. In the case of *Arthrospira platensis*, biomass production was reduced, accompanied by stress indicated by a decrease in antioxidant activity without significant changes in structural compounds. On the other hand, *Porphyridium cruentum* showed increased biomass but with a reduction in lipid and carbohydrate content and an increase in oxidative stress, indicated by elevated malondialdehyde levels. These differentiated responses suggest that eukaryotic microalgae and prokaryotic cyanobacteria adopt distinct adaptive strategies in the presence of silver nanoparticles. These results open the possibility of optimizing the use of nanoparticles in microalgal biotechnologies, depending on the type of organism and the specific objectives pursued. The toxicity of AgNPs on aquatic microorganisms has been extensively studied and described in several works. It was reported that *Chlorella vulgaris* significantly loses viable cells at high concentrations of 200 mg/L AgNPs [8]. Another study showed that the freshwater species *Scenedesmus* sp. was more affected by AgNPs compared to the marine diatom *Thalassiosira* sp., suggesting that AgNPs exhibit different toxicity mechanisms for microalgae and are more toxic in freshwater environments than in marine ones [9]. The differences in toxicity between marine and freshwater environments of AgNP-PVP, where marine species seem to be less sensitive to the toxicity of silver nanoparticles compared to freshwater species, are attributed to the greater complexity of the marine environment, which reduces the availability of silver ions released from the nanoparticles. It has been observed that the toxicity of silver nanoparticles is lower than that of directly released silver ions. However, toxicity varies significantly depending on the initial concentration of AgNPs and the exposed microalgae species. It has been established that the toxicity of PVP-coated silver nanoparticles varies considerably depending on testing methodologies, environmental parameters, and microalgae species. Thus, for *Phaeodactylum tricornutum*, the EC50 was 0.06 mg/L, for *Isochrysis galbana* 0.039 mg/L, and for *Tetraselmis suecica* only 0.0052 mg/L [1]. PVP-stabilized

silver nanoparticles are considered less toxic because PVP ensures their stability in the environment, preventing aggregate formation and reducing the release of large amounts of Ag+ ions. However, a positive correlation has been established between AgNP-PVP concentration and growth inhibition of the microalga *Raphidocelis subcapitata* [10], with a response similar to that obtained in this study for *Arthrospira platensis* and AgNPs-PVP. Another study demonstrates the toxicity of Polyvinylpyrrolidone coating substances in combination with Polyethyleneimine, which, when applied to 5 nm silver nanoparticles, significantly inhibited the growth of marine microalgae *Isochrysis galbana*, *Phaeodactylum tricornutum*, and *Tetraselmis suecica*. The coating products are said to promote the release of silver ions [11]. As can be seen, the results are very contradictory. The reactions of aquatic cultures to the action of AgNPs have been investigated depending on their size. Thus, the freshwater microalga *Chlamydomonas reinhardtii* and the marine microalga *Phaeodactylum tricornutum* were sensitive to small-sized AgNPs, resulting in growth inhibition at low concentrations. In contrast, for larger-sized nanoparticles, the cells exhibited a hormetic response [12]. Such an effect can be assumed for the microalga *Porphyridium cruentum*, which tended to reduce biomass production at the high concentrations of AgNPs-PVP applied in this study. Differences in the response of the cyanobacterium *Arthrospira platensis* and the microalga *Porphyridium cruentum* were also observed in the case of the action of citrate-stabilized AgNPs [7]. The lipid peroxidation effect has been described for the microalgae *Chlorella vulgaris* and *Dunaliella tertiolecta*, where exposure to AgNPs led to the formation of cell aggregates due to the amplification of the lipid peroxidation process. At a concentration of 1 mg/L, lipid peroxidation increased by 45% for *Chlorella vulgaris* and by 48% for *Dunaliella tertiolecta*. At 10 mg/L, lipid peroxidation increased fourfold for *Chlorella vulgaris* and fifteenfold for *Dunaliella tertiolecta* [6]. In contrast, AgNPs-PVP did not induce lipid peroxidation in *Chlorella vulgaris* [2]. In the present study, this effect was established for *Arthrospira platensis*, for which MDA values did not change.

4. CONCLUSIONS

The cyanobacterium *Arthrospira platensis* and the microalga *Porphyridium cruentum* exhibit different responses to AgNPs-PVP in their cultivation media. *Arthrospira platensis* experienced a decrease in biomass without significant cellular content changes. At the same time, *Porphyridium cruentum* maintained biomass productivity but showed signs of oxidative stress and a decrease in lipid content. Oxidative stress was confirmed based on different indicators. For *Arthrospira platensis*, oxidative stress manifested through a significant reduction in the antioxidant activity of the biomass, measured by the ABTS test. For *Porphyridium cruentum*, oxidative stress was confirmed by increased malondialdehyde (MDA) levels, a marker of lipid peroxidation. These differences suggest that microalgae and cyanobacteria employ different adaptation mechanisms to nanoparticle-induced stress, offering opportunities for optimizing nanoparticle use depending on the organism studied and the biotechnological goal pursued.

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