Proteomic responses to nanoparticulate and ionic silver in freshwater microbes with different background

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1. Introduction

Intensive production and use of silver nanoparticles (AgNPs) are increasing the probability of their release into freshwaters in significant amounts. Due to the antimicrobial properties of Ag^+, it is relevant to examine whether AgNPs can pose a risk to freshwater microbes in natural ecosystems. In fact, metals are known to induce toxicity through the generation of reactive oxygen species (ROS) [1] and AgNPs were reported to reduce the biomass of aquatic bacteria and fungi on decomposing plant litter [2]. On the other hand, functional proteomics is the most promising next generation risk assessment tool, augmenting measurements of direct and highly sensitive responses at the cellular and sub-cellular levels. In freshwater detrital ecossystems, bacteria and fungi play key roles in plant litter decomposition by transfering nutrients and energy to higher trophic levels [3]. Our goal was to examine the impacts of AgNPs and Ag^+ in freshwater microbes (fungi and bacteria) with different background by assessing their proteomic responses.

2. Materials and methods

Two strains of aquatic fungi, *Articulospora tetracladia*, one isolated from a non-polluted stream (At72) and the other from a metal-polluted stream (At61) and the bacterial strain, *Pseudomonas* sp. M1 (PsM1) isolated from sediments in a metal-polluted stream were used. Microbes were exposed to AgNPs (20 nm; citrate-coated) and to Ag^+ at concentrations inhibiting 20% of biomass production (EC_{20}) at early exponential phase of their growth and used for proteomic analyses. Dynamic light scattering (DLS) was used to monitor the size distribution of AgNPs and the zeta potential was used to determine the surface charge and stability of the nanoparticles in the media. Ag^+ released from AgNPs in the media and the bioaccumulations of total Ag were determined by ICP-MS.

3. Results and discussion

3.1. Characterization of AgNPs and quantification of dissolved Ag^+ in media

Characterization of AgNPs showed increased particle stability and lesser agglomeration with exposure time in At72, while for At61 and PsM1 there was an increase in AgNP agglomeration explaining its lower effects on the growth of these two microbes. The considerably low extracellular concentration of Ag^+ ions released from AgNPs to the medium suggested low involvement of dissolved Ag^+ to the AgNP-induced stress in PsM1. Also, the higher percentage of silver bioaccumulation found in mycelia of At61 (≈40%) compared to At72 (≈20%) is Indicative of its higher tolerance/resistance to AgNPs.

3.2. Proteome analysis in PsM1

A total of 197 proteins were quantified, but only 40 proteins had their content significantly altered by exposure to either or both forms of silver. The content of the majority of these proteins increased (68%) by Ag^+ whereas the opposite trend was observed for AgNPs. The exposure to Ag^+ increased the content of proteins mostly involved in carbohydrate metabolism, outer cell membrane and stress related proteins involved in protein folding and degradation, whereas exposure to AgNPs decreased the content of proteins associated with carbohydrate metabolism. Both forms of silver decreased the content of proteins involved in protein biosynthesis.

3.3. Proteome analysis in fungi

A total of 874 proteins were quantified. Out of these, the content of 361 proteins was altered significantly after exposure to AgNPs and/or Ag^+. Out of 361 proteins, 101 belonged to the strain At72, 189 to the strain At61 and 71 proteins were common to both strains. The fact these 2 strains only share 20% of the proteins, suggests that the biological pathways involved in the interactions with Ag+ and AgNPs are different. Figure 1 shows the dynamic profiles of the proteins significantly altered among the experimental conditions determined by heatmap in both fungal strains, and, by the color patterns we can verify how the levels of the proteins change between the different conditions. Based on the different profiles, several clusters were formed in each strain. Also, the percentages of altered proteins (either increased or decreased) were very similar in both strains, but in At61 these percentages were higher (ca. 20 to 25%) indicating higher responses. The quantitative information from clusters was combined followed by gene ontology analysis to get insights of the altered biological processes. In At72, exposure to Ag^+ increased the content of proteins involved in protein homeostasis while exposure to AgNPs increased the content of proteins related to DNA repair, transport of substances and energy production. In At61, the exposure to AgNPs increased the content of proteins involved in protein synthesis and energy production while both forms of Ag increased the content of proteins related to cell-redox and protein homeostasis, biomass and spores production and also to nucleic acids metabolism. Both Ag forms induced stress-responsive proteins, including catalase and superoxide dismutase, which was consistent with the profile of their enzymatic activities. These results supported the ability of these fungi in initiating an efficient antioxidant response to cope with Ag-induced toxicity.

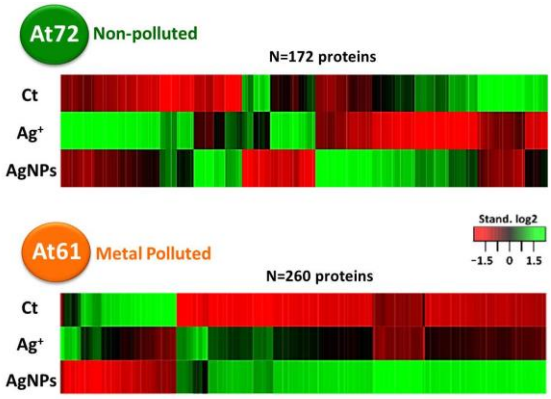

Figure 1: *Dynamic profiles of the proteins with statistical different variation among the experimental conditions determined by heatmap in At72 and At61.*

4. Conclusions

We concluded that Ag^+ and AgNPs induced different metabolic, energetic and stress responses in aquatic microbes with different backgrounds. Overall, the functional proteomic approach can be useful to get a mechanistic insight into the stress induced by AgNPs and Ag^+ in aquatic microbes that play key roles in plant litter decomposition in freshwaters.

5. References

[1] Azevedo M-M, Carvalho A, Pascoal C, Rodrigues F, Cássio F. 2007. Responses of antioxidant defenses to Cu and Zn stress in two aquatic fungi. Sci. Total Environ. 377: 233-243.
[2] Pradhan A, Seena S, Pascoal C, Cássio F. 2011. Can metal nanoparticles be a threat to microbial decomposers of plant litter in streams? Microb. Ecol. 62: 58-68.
[3] Pascoal C, Cássio. 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Appl. Environ. Microbiol. 70: 5266-5273.

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