INFLUENCE OF NANOPARTICLES ON MICROALGAE DETECTED BY IN VIVO IMAGING

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Abstract

Fluorescence imaging is an efficient and powerful technique, which has been often utilized for tracking compounds in animals, however its application on plants or other organisms is not very common. In this work, the fluorescence imaging was used to investigate the effect of selected nanoparticles - NPs (ZnO, CdTe, etc.) on microalgae (*Scenedesmus quadricauda and Chlorella vulgaris*) growth. The growth and metabolic activity of microalgae was influenced by application of nanoparticles into the growing medium (liquid and solid) and therefore the intrinsic fluorescence was affected. Microalgae pigments (chlorophylls, carotenoids) are responsible for the autofluorescence of the species and therefore, the fluorescence can be used as a tool for quick and easy evaluation of expression these compounds. These pigments have main role in light harvesting and minimize photo-oxidative damage of the cells. Such protection mechanism can be disturbed by another stress related compounds like NPs that presumably damage this protection mechanism i) indirectly (reactive oxygen species) and ii) directly (disruption of biomembranes). Owing to broad fluorescence imaging and correlation with total amount of chlorophylls and carotenoids it was possible to evaluate the influence of NPs on individual microalgae just only by *in vivo* imaging.

Keywords: Nanoparticles, Scenedesmus quadricauda, Chlorella vulgaris, fluorescence, abiotic stress, metabolic

1. INTRODUCTION

Algae are a large group of organisms which including unicellular microalgae and more complex multicellular organisms (macroalgae). In recent years, large-scale production of microalgae has been very successful especially for use in many branches, such as food, feed and fuel products [1]. In microalgae, pigments with fluorescent activity, in particular, carotenoids and chlorophylls have an important role in photosynthesis. Greenish, fat-soluble chlorophylls are pigments with porphyrin ring responsible for conversion of solar energy into the chemical energy. Carotenoids (β-carotene, lycopen, astaxanthin, zeaxanthin, vialoxanthin a lutein) perform two important functions: light absorption in range of visible spectrum, where chlorophyll does not (400-530 nm [2]) and, thanks to their antioxidant activity, defence against formation of reactive oxygen species (ROS) [3].

Plant systems are subject abiotic stress, which can be caused by many factors (for example cold, heat, and drought). Therefore, it is important to study of metabolic pathways in plant cells. This can be accomplished by many methods such as quantitative polymerase chain reaction [4], liquid chromatography [5] or mass spectrometry [6]. Use of imaging technologies is more favorable than mentioned methods, mainly for their financial and time-saving benefits. Imaging methods allow imaging in living organisms at the cell (microscopy), organ and/or whole body level. The most commonly used ones include ultrasound, computed tomography and/or magnetic resonance. NMR spectroscopy has contributed to the study of plant primacy or specialized metabolism in very diverse ways [7].

On the other hand, fluorescence imaging is no very common yet even though compared to the above mentioned methods; it exhibits numerous advantages such as variability, multiplexing suitability and/or cost-effectivity. Fluorescence *in vivo* imaging has a great potential for a wide range of molecular diagnostic

application. Fluorescence-based methods are increasingly used for their quantitative sensitivity, natural biological safety and fairly ease of use [8].

In this study, a fluorescence *in vivo* imaging was used and changes in growth microalgae exposed to nanoparticle effects were investigated.

2. MATERIALS AND METHODS

2.1. Preparation of nanoparticle solutions

Solutions of ZnO nanoparticles (NPs) were prepared by mixing a stock solution of the commercial NPs (Houston, USA) with distilled water at four different concentrations (0 mg/L, 10 mg/L, 50 mg/L a 100 mg/L). Solutions were sonicated for 10 minutes before use.

2.2. Preparation of bold´s basal medium (BBM)

Bold´s Basal Medium (BBM) has been prepared according to published method [9]. The final solutions were prepared by mixing 2 mL solutions of NPs (0 mg/L, 10 mg/L, 50 mg/L a 100 mg/L). Volumes were adjusted with BBM medium to 150 mL. One of these solutions (0 mg/L) was used as a control. All solutions were autoclaved and subsequent these media were added to petri dishes.

2.3. Spot test

The stock solution of microalgae (*Scenedesmus quadricauda and Chlorella vulgaris*) was prepared at absorbance of 0.1-0.2 AU. Then, four samples were prepared by subsequent dilution in ratio 1:1 with BBM without NPs. Individual samples (5 µL) were spotted onto petri dishes with NPs containing BBM (three spots per sample). In total, 4 petri dishes containing 12 spots (4 microalgae dilutions, in triplicates) were prepared for each microalgae specie *(Scenedesmus quadricauda and Chlorella vulgaris*)

2.4. *In vivo* fluorescence imaging

Monitoring of influence of nanoparticles on microalgae was performed using an In Vivo Xtreme Imaging System by Bruker (Massachusetts, USA). The parameters used for image acquisition were following: exposition time - 4 s, binning - 4x4 pixels, fStop - 1.1, field of view - 10x10 cm.

Monitoring of influence of nanoparticles on microalgae has been performed using excitation filter of 650 nm and emission filter of 700 nm. Individual samples were measured consecutively for 6 days.

For image post processing and analysis Bruker Molecular Imaging Software (Bruker, Massachusetts, USA) was used. In this software the spectral modeling was performed.

3. RESULTS AND DISCUSSION

In this study, the series of *in vivo* experiments has been done to evaluate the potential of influence of nanoparticles on microalgae. The samples of microalgae were monitored for 6 days and a change in fluorescence intensity was observed in pW/mm^2.

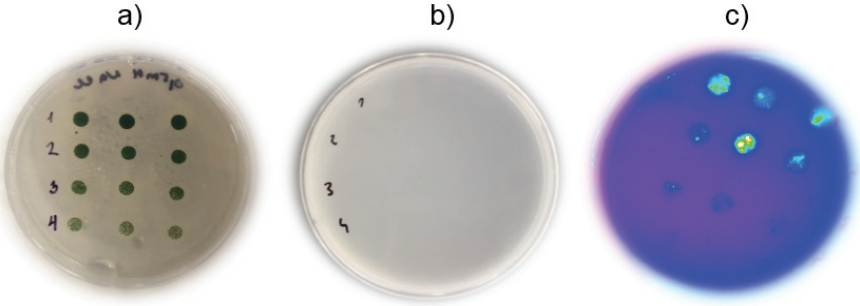

Figure 1 Microalgae *Chlamydomonas reinhardtii* (a) visible to the naked eye, *Chlorella vulgaris* including 100 mg/L NPs (b) invisible to the naked eye and *Chlorella vulgaris* including 100 mg/L NPs by fluorescence imaging (c).

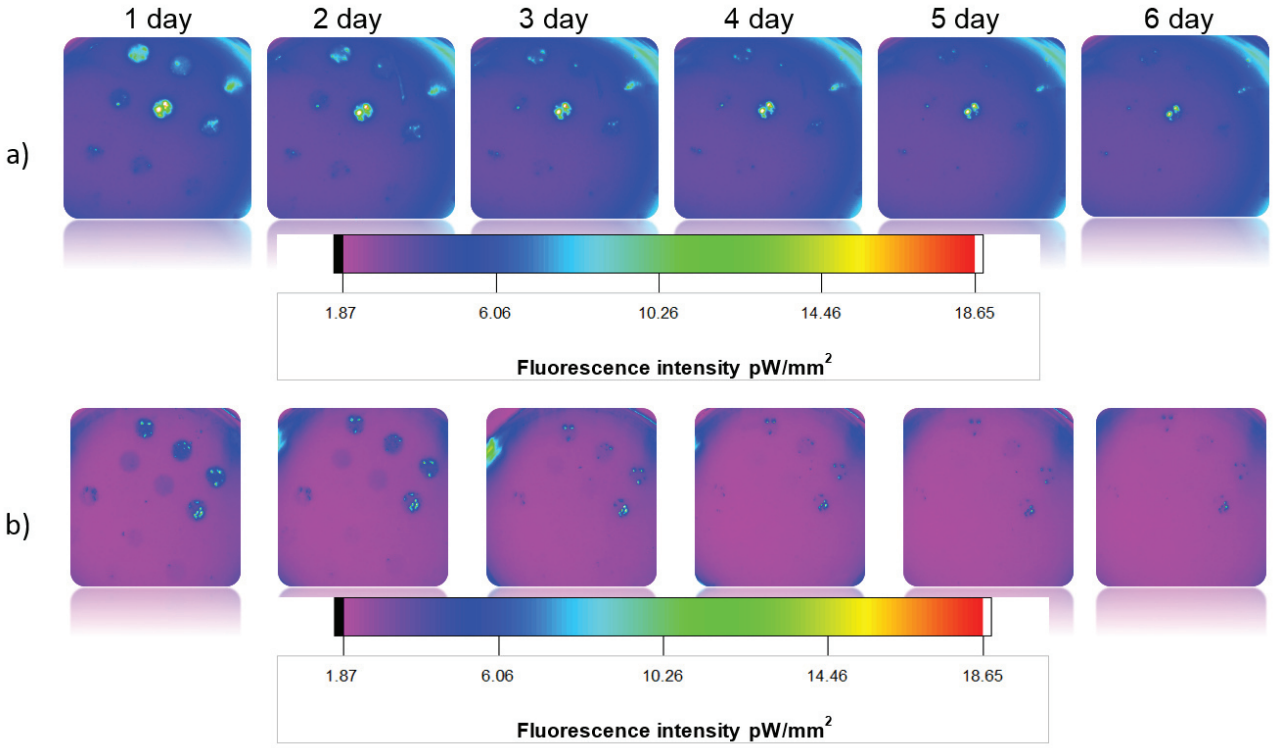

Figure 2 Microalgae *Chlorella vulgaris* including 100 mg/L NPs (a) and microalgae without NPs (b). Fluorescence images captured at time intervals as noted above picture respectively.

Fluorescence intensity was higher in microalgae including NPs (**Figure 2a**) than microalgae without NPs (**Figure 2b**). This could be due to the increased content of photosynthetic pigments, which could be explained by the balancing effect to overcome the toxicity induced by NPs [10]. But images show that fluorescence intensity was gradually fallen. Which could be the fact that metal NP and ions affecting metabolism in the form of excessive increasing levels of reactive oxygen species (ROS) within the microalgae [11].

The images were further analyzed using Bruker Molecular Imaging Software.

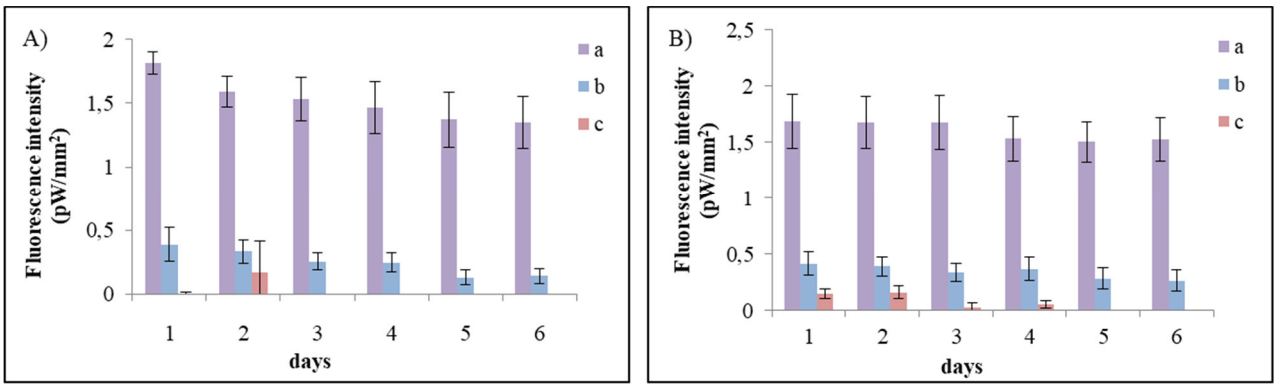

Figure 3 The graph A (*Chlorella vulgaris)* and the graph B (*Scenedesmus quadricauda)* show 3 concentrations of NPs (a-c). The graphs show pure fluorescence intensity with deducted control fluorescence intensity. All 3 NPs concentrations (a-c) gradually killed the cells, therefore fluorescence intensity decreased in time gradually. The highest fluorescence intensity was detected at the highest concentration of NPs (A and B, a).

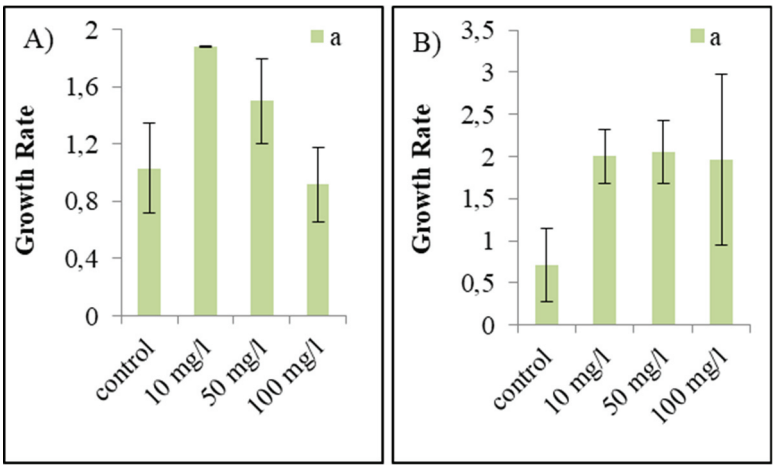

Figure 4 The growth rates of *Chlorella vulgaris* (A) and *Scenedesmus qudricauda* (B)

Based on our results we have come the following hypothesis. The highest concentrations of nanoparticles (100 mg/L) affect probably almost the biosynthesis of plant pigments instantly. For about 4 hours, fluorescence intensity was superior to the other concentrations (**Figure 3a-c**). Which may be caused by stimulated of photosynthesis. It is important to note that the literature is not available for assessment.

If we look at the growth rate (**Figure 4A**), we can see a concentration 10 mg/L the fluorescence intensity increased but concentrations 50 mg/L and 100 mg/L reduced it. We conclude it to reflect the amount of biomass. The smallest concentration of nanoparticles (10 mg/L) increased the amount of biomass, while concentration of NPs reduced it. The growth rate of *Scenedesmus quadricauda* (**Figure 4B**) was level-headed.

4. CONCLUSION

Microalgae are the first trophic level of the food chain, providing biological energy and oxygen for other organisms and playing very important roles in keeping the balance of aquatic ecosystems. Nanoparticles (NPs) are inevitably released into the aquatic environment for being widely used [12]. During this study the effect of nanoparticles on *Scenedesmus quadricauda* and *Chlorella vulgaris* was studied. The NPs can be a stress factor and promote the emergence of ROS. Mentioned changes in growth of microalgae were observed using modern *in vivo* imaging technology. Today, the *in vivo* imaging method is used primarily in animals, whereas in plants it is not so common. Thanks to this technology, we would like to optimize *in vivo* imaging methods in plant materials. The future should be, for example, recognizing the distribution of toxic substances in plants to preserve the safety of agriculture and food.

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