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THE IMPACT OF $CoFeO_4$ NANOPARTICLES ON SOLUBLE PROTEIN CONTENT AT WHITE ROT FUNGUS *Phanerochaete chrysosporium*

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Keywords: *Phanerochaete chrysosporium*, electrophoresis, soluble protein content, $CoFeO_4$ nanoparticles
Abstract: Experimental investigation focused on influence of different CoFeO4 nanoparticles concentrations on soluble protein and electrophoretic pattern of *Phanerochaete chrysosporium* fungus. Number of electrophoretic fraction is higher in older mycelium *P. chrysosporium* 14-day-old compared to that 7-day-old. Moreover, there is an increase in staining intensity of polypeptides of mycelium 14-day-old fact confirmed also by the higher amount of soluble protein. On the other hand, there are no visible differences as regards the different concentrations of nanoparticles effect on the polypeptides pattern.

INTRODUCTION

Nanotechnology, in general and nanomaterials, in particular, have the potential to revolutionize different sectors such as pharmacy and medicine, electronics and agriculture and allied sectors such as food processing, packaging and storage with modern tools (Rai and Ingle, 2012).

Much research has been focused on nanoparticles (dimension < 100nm) over the last decade due to their bulk counterparts. The advancement of research on the synthesis of NPs from natural living sources has attracted considerable attention in the field of nanobiotechnology and its application. It is also known that the shape, morphology and size of nanoparticles play an important role in controlling their special properties (Kamat, 2002; El-Sayed, 2001).

Metal NPs have as possible uses and applications various fields such as electronics, cosmetics, medicine (medical treatment) and biotechnology (Shah and Tokeer, 2010) or solar energy conversion and water treatment (Dubchak et al., 2010). A number of nanoparticles / nanomaterials have an enormous potential for medical applications, such as clinical diagnosis and / or treatment of cancer. The biomedical use of magnetic nanoparticles in diluted suspensions includes nanosized iron oxides as contrast agents in magnetic resonance imaging, in experimental cancer treatment through hyperthermia, in magnetically targeted drug delivery, magnetic separation of biomolecules and cells etc. (Oprica et al., 2015).

On the other hand, nanotechnology may be a better alternative for environmental remediation (Zang Wei-xian, 2003). For transformation and detoxification of pollutants, the nanoremediation methods involve the use of nanomaterials which have properties that allow the chemical reduction and catalyze, lowering the concentration of pollutants (Karn et al., 2009).

Microorganisms have the potential to synthesize NPs intracellulary or extracellularly under ambient conditions without toxic chemically and stringent conditions; even the properties of such nanoparticles to chemically synthesized materials (Baűerlein, 2003). The role of microorganisms in bioremediation, biotransformation, biosorption and biomineralization are well known; however, the microbial-based biogenesis of nanoparticles is a relatively new and largely unexplored area of research. The biogenic potential of different prokaryotes (bacteria and actinomycetes), eukaryotes (fungi and yeast) and viruses have been explored for the synthesis of nanoparticles.

White rot basidiomycetes make an essential contribution to global carbon cycling by efficiently degrading the recalcitrant aromatic biopolymer lignin, which encloses the cellulose and hemicelluloses of vascular plants and is second only to these polysaccharides as a repository of terrestrial biomass (Shary S et al., 2008). The most widely studied white rot organism, *Phanerochaete chrysosporium*, belongs to the homobasidiomycetes. White-rot fungi have the apparently unique ability to degrade lignin to the level of CO_2 (Kirk, Farrell, 1987). Due to the heterogeneity of the substrate, white-rot degradation of lignocellulosic material involves an ensemble of extracellular enzymes.

In order to emphasize the response reaction and particularities detection of P. *chrysosporium* protein spectrum it was analyzed the polypeptide pattern of intracellular protein from fungus grown on media with various concentration of CoFeO4 nanoparticles.

MATERIALS AND METHODS

1. Phanerochaete chrysosporium cultivation.

P. chrysosporium white rot fungus was purchased from the Institute Sciéntifique de Santé Publique, Belgium (HEM no. 5772). This fungus was cultivated in Petri dishes on agarized Sabouraud medium (peptone 10 g/l, glucose 35g/l, agar 2g/l, distilled water up to 1.0 l).

The Erlenmeyer flasks with liquid Sabouraud medium was inoculated with 0,8 cm diameter disks from 7 days' culture of *P. chrysosporium*. In addition, in the medium was added the different concentration of CoFeO4 nanoparticles resulting four variants: V1- 15mg/l, V2- 20mg/l, V3- 30mg/l, V4- 35mg/l and control which not present nanoparticles. All samples were incubated at 28°C in INCUCELL room. The determination of proteins content was assayed in fungus mycelium at 7 days and 14 days after inoculation. All reagents for the culture medium preparation were purchased from Merck.

2. Nanoparticle preparation

Metal salt precursors were Merck chemicals at molar ratio 2:1, each dissolved in deionized water (Kim et al., 2003). In all steps of magnetic nanoparticle suspension synthesis was used deionized water (18.2 MΩ/cm, Barnstead EASYPureII ultrapure water system). Cobalt ferrite coprecipitation was produced by stirring the two stock solutions at 75 °C and by slowly pouring of 2M NaOH (150 mL). To ensure ferrite particles uniform dispersion in deionized water, 12 mL perchloric acid aqueous solution (25%) was added (under continuous stirring at 75 °C - thus modifying the MNPs surface in order to prevent their agglomeration in the presence of ubiquitous gravitational and magnetic fields (Laurent et al., 2008). The final product was a magnetizable nanofluid based on electrostatic stabilization (Gazova et al., 2012) that presented good stability over time at pH close to biological one.

3. Protein assay

The determination of soluble protein content was done according to Bradford method (Bradford, 1976) using 50 mM Tris–HCl buffer, pH 7, with bovine serum albumin as standard. The assay is based on the binding of Coomassie Brilliant Blue G-250 (from Fluka) at aromatic amino acid radicals with the measure of light intensity at 595 nm. The result was expressed in mg protein per g of mycelium.

4. Electrophoresis

Protein electrophoresis was performed in the system of Laemmli (1970) tampons, in the polyacrylamide vertical plates with 0,75 mm thickness, under denaturing conditions and post electrophoresis operations were carried out according to the standard method.

Sample preparation involved protein extraction in Tris-HCl buffer solution, 50 mM pH=7. Protein precipitates were dissolved in Tris-HCl buffer solution (pH=7), contained SDS – 4.25 %, sucrose about 20%, β-mercaptoetanol – 6% and bromophenol blue – 0.004%. Prior to electrophoresis samples were thermally treated at 95°C with a denaturing buffer solution containing sodium dodecyl sulfate (SDS) and β-mercaptoethanol. Aliquots of 10 µl of protein extract were loaded onto the gel and electrophoresed with Tris-glycine buffer (pH=8.3). For the determination of relative molecular mass of separated polypeptide fractions were used protein markers Sigma Wide Range with known molecular weight.

Electrophoresis was performed at 30mA until the bromophenol blue tracking dye entered the resolving gel. The power was decreased to 10mA until the tracking dye reached the bottom of the resolving gel (~ 4 h). After the bromophenol blue reached the bottom of the gel briefly washing with deionized water was carried out and then dying with Coomasie Brilliant Blue R250 was applied. Colored gels were photographed with a high resolution camera (Canon 550D). Images were analyzed with GE Image Quant 8.1 software. Reagents came from Carl Roth and/or Sigma.

5. Statistical analysis

All experiments were carried out with four independent repetitions and the results were expressed as the mean values ± standard deviation (SD). Statistical significance was ensured by four replies of each $CoFeO_4$ nanoparticles concentration and by *t*-test application.

RESULTS AND DISCUSSIONS

Changes of the intracellular soluble protein content of *Phanerochaete chrysosporium* grown on media with different concentrations of $CoFeO_4$ nanoparticles are shown in figure 1. The results obtained evidenced that soluble protein amount increased at 14 days than the 7 days after inoculation.

At 7-day-old culture, the introduction of nanoparticles in the medium determined a slightly increased protein content with increasing the concentration of $CoFeO_4$ nanoparticles. In

contrast, on 14 days it noticed a slight decrease in protein content 14-day-old culture on nanoparticles culture.

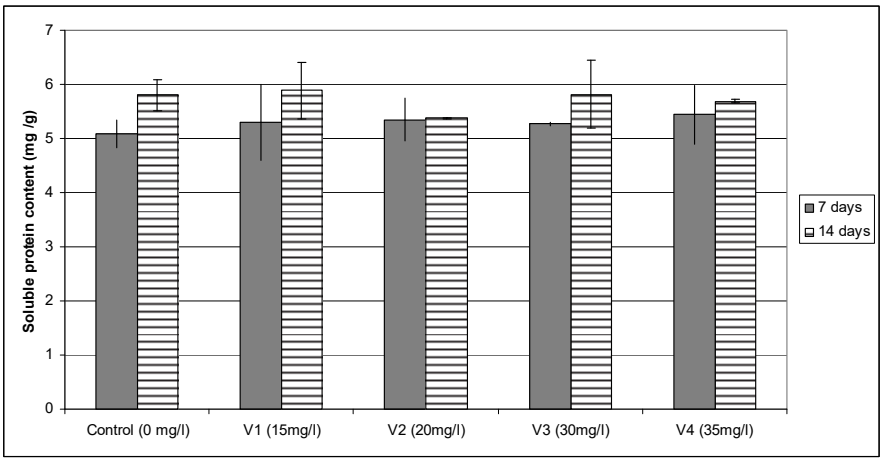

Figure 1. Protein content in mycelium of *Phanerochaete chrysosporium* grown on media with different concentrations of $CoFeO_4$ nanoparticles

The SDS electrophoresis showed distinct protein bands for both ages of *P. chrysosporium* mycelium, namely at 7 and 14 days after inoculation (Figure 2, Figure 3). Using homogeneous gels, proteins in mycelium extract were detected in the entire range detectable by the method.

Comparatively with control, there were no significant differences between mycelium grown on media with different concentrations of $CoFeO_4$ nanoparticles or between the nanoparticles variants. Nevertheless, the electrophoretic pattern showed consistent differences regarding the number of polypeptides and the staining intensity of polypeptides between mycelium 7-day-old and 14-day-old. The overall intensity of the bands of *P. chrysosporium* mycelium at 14 days was stronger both in control and at low nanoparticles concentration (40µl and 60µl) than high nanoparticles concentration (80µl and 100µl).

At 7 days after inoculation, the intensity of the polypeptides from the fungal mycelium was very low and did not show any differences between the various concentrations of nanoparticles or compared to control. At 14 days after inoculation a group of nine polypeptides (with molecular weights between 15-25KDa) it has been emphasized in all variants which have been treated with nanoparticles including the in the control.

In previous research (Oprica et al., 2014) conducted by us regarding influence of electromagnetic fields on protein synthesis of *P. chrysosporium* it was established that there no qualitative modification in protein fraction pattern but the soluble protein amount was diminished with 25%.

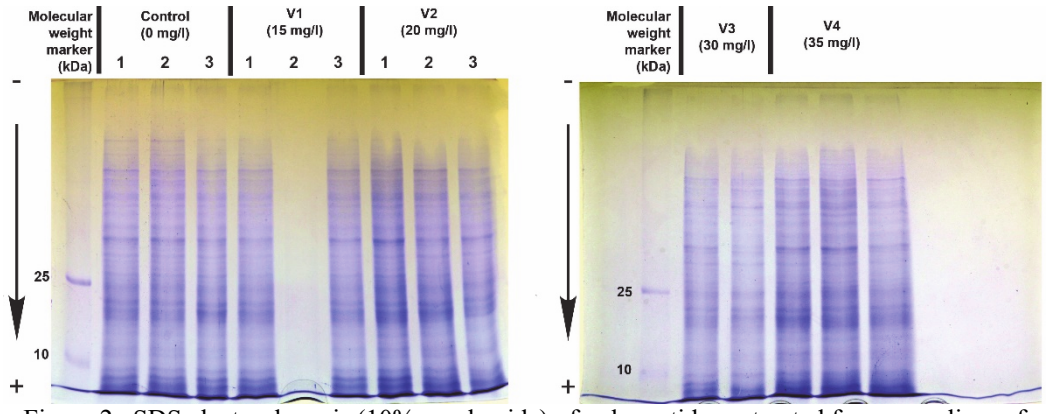

Figure 2. SDS electrophoresis (10% acrylamide) of polypeptides extracted from mycelium of *Phanerochaete chrysosporium* 7-day-old grown on media with different concentration of $CoFeO_4$ nanoparticles (Sigma Wide Range = Molecular weight standards indicated by number on the left of the gel/KDa)

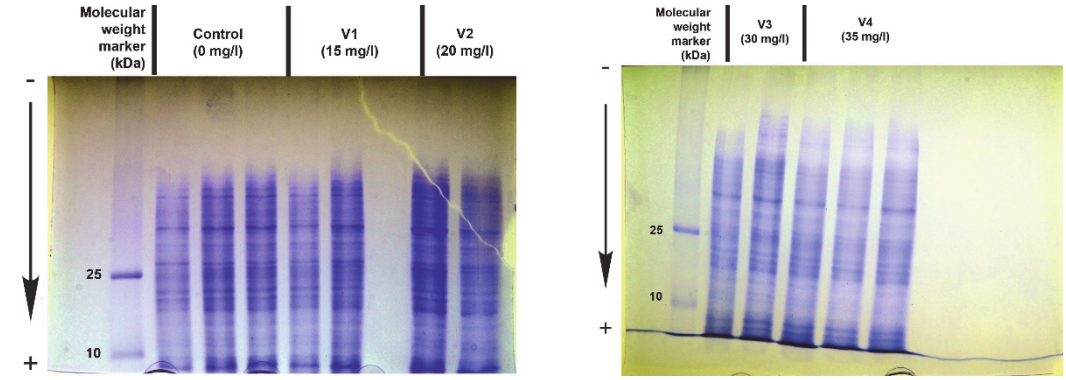

Figure 3. SDS electrophoresis (10% acrylamide) of polypeptides extracted from mycelium of *Phanerochaete chrysosporium* 14-day-old grown on media with different concentration of $CoFeO_4$ nanoparticles (Sigma Wide Range = Molecular weight standards indicated by number on the left of the gel/KDa)

CONCLUSIONS

The comparative analysis of soluble protein pattern from *P. chrysosporium* revealed the presence of common polypeptides both in control and variants with $CoFeO_4$ nanoparticles however, varying by color intensity as well as the bands dimension at 14-day-old mycelium comparatively with 7-day-old mycelium.

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