1st International Conference on Chemo and BioInformatics
ICCBIKG 2021

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BOOK OF PROCEEDINGS

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INFLUENCE OF VANADIUM ON THE GROWTH AND METABOLISM OF COPRINELLUS TRUNCORUM FUNGAL MYCELIUM

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Abstract

Fungi could absorb heavy metals, metalloids, or radionuclides, thus fungal species possess great potential in bioremediation. Since fungi absorb the vanadium, in the present study ability of *Coprinellus truncorum* mycelia for vanadate uptake and its intracellular metabolism were investigated. The submerged cultivated *C. truncorum* was exposed to a rising concentration of vanadate. ^{31}P NMR spectroscopy was used to investigate phosphate metabolism of the mycelium, while the status of vanadium in the cell was followed by ^{51}V NMR spectroscopy. The mycelium could grow, and overcome vanadate presence, up to the concentration of 1.6 mM in the submerged medium. ^{31}P NMR measurements pointed out that vanadate induced changes in the concentration of the crucial metabolite containing phosphorus, particularly sugar phosphates. The major result of vanadate action is evinced through an appearance of a signal positioned at around 2.8 ppm, and an increased signal of hexose-phosphates. Using ^{51}V NMR spectroscopy the presence of vanadate monomer in the mycelia of the fungal cell was confirmed.

Keywords: fungi, phosphate metabolism, vanadium, ^{31}P NMR spectroscopy, ^{51}V NMR spectroscopy

1. Introduction

Fungi primarily play a role in the decomposition of organic matter in nature and can absorb heavy metals and various toxic substances. Fungi participate in the circulation of matter and the flow of energy. As such, their role in biogeotechnological processes is irreplaceable [1]. Since fungi absorb and solubilize heavy metals, metalloids, or radionuclides these compounds are directly or indirectly involved in many aspects of fungal growth and metabolism [2]. The comprehension of their interaction with fungal cell compounds is of great importance for a better understanding of their potential in bioremediation or their possibilities to overcome the certain health problems of today.

Vanadium (V), the transition metal, in higher concentrations is a potent environmental pollutant and toxic for humans and animals. However, it was reported that V at lower concentrations could be used in medicine for treating diabetes [3, 4]. Since fungi absorb V, the effects of its uptake, accumulation, and influence on the phosphate metabolism inside the fungal cells were monitored. The fungi *Coprinellus truncorum* was exposed to rising concentration of vanadate. ^{31}P NMR spectroscopy was used to investigate phosphate metabolism of the mycelium, while the status of vanadium in the cell was followed by ^{51}V NMR spectroscopy.

2. Materials and Methods

2.1. Biological material

Coprinellus. truncorum fruiting body was sampled from Fruška Gora Mountain, Serbia. The fungal mycelia were isolated on Malt agar (Torlak, Serbia) at 26°C and afterward submerged cultivated in fermentation medium at 26°C on an orbital shaker (IKA KS 4000i control, Germany). Biomass of submerged cultivated mycelia was harvested after 3, 5, 7, 14, 21, 28, and 35 days of incubation.

2.2. Influence of sodium metavanadate on cultivated mycelia

The influence of sodium metavanadate ($NaVO_3$) in fermentation medium on mycelial growth was investigated for different concentrations of vanadate (0.3 mM, 1.0 mM, 1.6 mM, and 3.3 mM). The biomass of cultivated mycelia (with and without $NaVO_3$) was collected by filtration (Filters Fioroni, France), lyophilized (ALPHA 2–4 LDplus, Freeze Dryer, Christ GmbH, Switzerland), and measured.

2.3. Nuclear magnetic resonance (NMR) spectroscopy

The submerged cultivated fungal mycelium was harvested, filtered, washed with distilled water, and collected into an experimental medium (110 mM glucose and 13.3 mM asparagine) prior to NMR analysis. Before recording the ^{51}V NMR spectra mycelium was treated with sodium orthovanadate (Na_3VO_4) (Sigma, Taufkirchen, Germany). A stock solution of 200 mM Na_3VO_4 was prepared at pH 10 [5]. All spectra were recorded with an Apollo spectrometer (Tecmag, USA). Detailed experimental conditions were explained in our previous paper [6].

3. Results and Discussion

3.1 Influence of sodium metavanadate on the growth of mycelia

After an incubation period of 3, 5, 7, 14, 21, 28, and 35 days, the biomass was collected and growth curves were constructed (Fig. 1). The highest mycelia biomass yield was observed in the control, whereas the increasing concentration of vanadate (0.3, 1.0, and 1.6 mM) slowed down biomass production and led to complete inhibition of the growth (3.3 mM of vanadate). A similar trend of mycelia growth upon V addition was obtained with *Coprinus comatus* fungal mycelia [7]. After determining an exponential growth phase, the mycelia on the 14th day of submerged cultivation were used for ^{31}P NMR and ^{51}V NMR measurements.

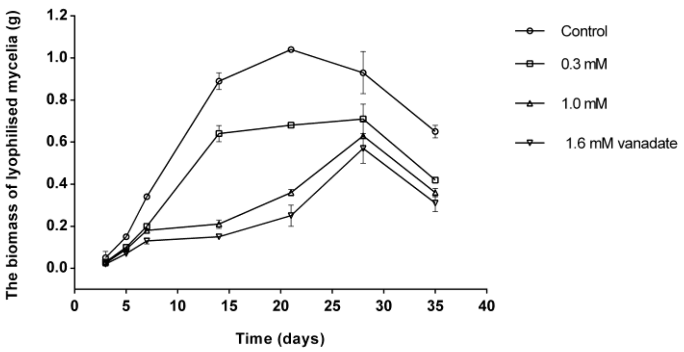

Fig. 1 Growth curves of *C. truncorum* mycelium grown in the fermentation medium and medium enriched with vanadate

3.2 NMR

Determination of the relative content of phosphorus compounds in fungal cells provides key information about cell energy status. According to our knowledge ^{31}P NMR spectrum was assessed for the first time for *C. truncorum* species, and assignments were done according to Žižić et al. [6]. The major peaks in the spectrum with chemical shifts of -22.1 ppm, -12 ppm, -10.2 ppm, and 1.3 ppm was assigned to core-polyphosphates (PPc), uridine diphosphate glucose (UDPG), nicotinamide adenine dinucleotide phosphate (NADP(H)+UDPG) and inorganic phosphate (Pi), respectively (Fig. 2., Control spectra). Upon addition of 10 mM of sodium metavanadate (Na_3VO_4), the appearance of a new signal in the ^{31}P NMR spectrum was noticed. This signal was positioned at around 2.8 ppm, and higher intensity of the signal of hexose-phosphates (HP) was also detected (Fig. 2., +V^{5+} spectra). Thus, the major result of vanadate action is evinced through the ramification of the most downfield shifted signals reflecting an altered activities of enzymes involved in glucose metabolism [4,8].

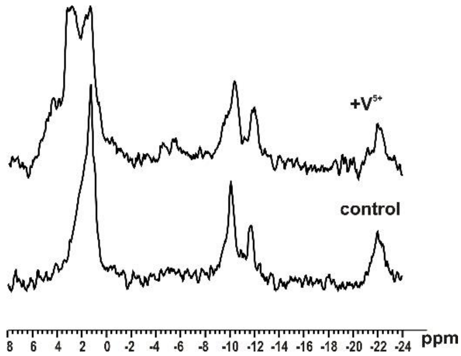

Fig. 2. *C. truncorum* ^{31}P NMR control and vanadate addition spectra

To precisely determine the metabolically active vanadate species in cells we were running ^{51}V NMR measurements (Fig. 3). Monomer at -559 ppm and dimer at -571 ppm indicated the pH around 6.8. After washing the sample, only the signal at -561 ppm remained in the spectrum (Fig. 3. Washed mycelia). This means that vanadate in the form of monomer entered the cell and, that the pH of the intracellular compartment of its accumulation is around 6.8.

Since the accumulation of vanadate has not changed the pH status of the cell, its accumulation in the cytoplasm allows expressing metabolic effects on the compounds located in the cytoplasm, primarily on sugar phosphates, whose concentration is directly dependent on enzymatic activity. Therefore, vanadate influence the action of enzymes of sugar phosphate metabolism (e.g. glucose-6-phosphatase, phosphoglucomutase, and fructose-2,6-biphosphatase) and thereby affecting glycolysis and glycogenesis [8,9].

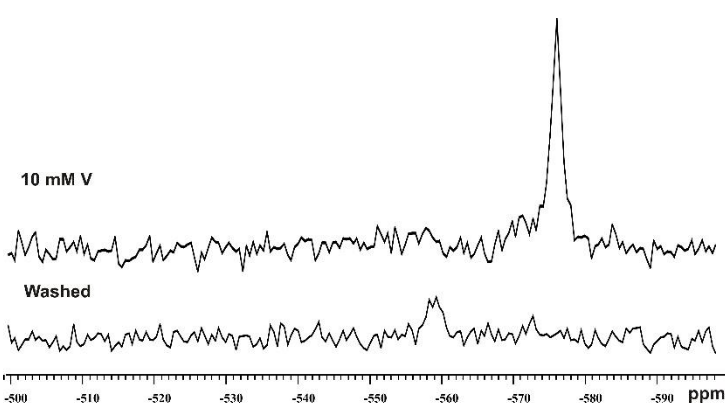

Fig. 3. *C. truncorum* ^{51}V NMR

3. Conclusions

It has been shown that vanadium in the form of sodium orthovanadate influences phosphate metabolism in fungal species inducing changes in the content of sugar phosphates. According to ^{51}V NMR spectroscopy, intracellular vanadate in the form of monomer enters the cell remaining the pH status of the cell constant which allows expressing the metabolic effects on the compounds of phosphate metabolism located in the cytoplasm.

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