Journal of Trace Elements in Medicine and Biology

Uptake of vanadium and its intracellular metabolism by Coprinellus truncorum mycelial biomass

--Manuscript Draft--

Manuscript Number:	JTEMB-D-23-00168R2
Article Type:	Research Paper
Section/Category:	Metabolic processes
Keywords:	Coprinellus truncorum; vanadium; phosphate metabolism; NMR; HPLC; EPR
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Abstract:	BACKGROUND Fungi absorb and solubilize a broad spectrum of heavy metals such as vanadium (V), which makes them a main route of its entry into the biosphere. V as vanadate (V5+) is a potential medical agent due to its many metabolic actions such as interaction with phosphates in the cell, and especially its insulin-mimetic activity. Antidiabetic activity of V-enriched fungi has been studied in recent years, but the biological and chemical bases of vanadium action and status in fungi in general are poorly understood, with almost no information on edible fungi. METHODS This manuscript gives a deeper insight into the interaction of V5+ with Coprinellus truncorum, an edible autochthonous species widely distributed in Europe and North America. Vanadium uptake and accumulation as V5+ was studied by 51V NMR, while the reducing abilities of the mycelium were determined by EPR. 31P NMR was used to determine its effects on the metabolism of phosphate compounds, with particular focus on phosphate sugars identified using HPLC. RESULTS Vanadate enters the mycelium in monomeric form and shows no immediate detrimental effects on intracellular pH or polyphosphate (PPc) levels, even when applied at physiologically high concentrations (20 mM Na3VO4). Once absorbed, it is partially reduced to less toxic vanadyl (V4+) with notable unreduced portion, which leads to a large increase in phosphorylated sugar levels, especially glucose-1-phosphate (G1P) and fructose-6-phosphate (F6P). CONCLUSIONS Preservation of pH and especially PPc reflects maintenance of the energy status of the mycelium, i.e., its tolerance to high V5+ concentrations. Rise in G1P and F6P levels implies that the main targets of V5+ are most likely phosphoglucomutase and phosphoglucokinase(s), enzymes involved in early stages of G6P transformation in glycolysis and glycogen metabolism. This study recommends C. truncorum for further investigation as a potential antidiabetic agent.
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Response to Reviewers:	

Dear Prof Bornhorst,

Please find enclosed the revised version of our Manuscript No. **JTEMB-D-23-00168 R2** entitled: ‘Uptake of vanadium and its intracellular metabolism by Coprinellus truncorum mycelial biomass”.

We hope that you will find the revised version of our manuscript acceptable for publication in the JTEMB journal, as an original contribution.

We have carefully read the text, and consulted native English speakers and revised the manuscript according to their suggestions. The revised manuscript is given in two forms: with track changes and without, for easier reading.

On behalf of all authors,

Milan Žižić

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Uptake of vanadium and its intracellular metabolism by *Coprinellus truncorum* mycelial biomass

Milan Žižić[a,b*], Kristina Atlagić[c], Maja Karaman[d], Miroslav Živić[c], Marina Stanić[a], Vuk Maksimović[a], Joanna Zakrzewska[e]

The work was carried out in laboratories of the Institute of General and Physical Chemistry, Belgrade and Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad

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Short title: Vanadium status in *C. truncorum* biomass

Declarations of interest: none

Highlights

1. Vanadate (V^{5+}) enters the mycelia of C. truncorum in the form of monomer
2. It is partially reduced to vanadyl (V^{4+}) intracellularly
3. ^{31}P NMR and HPLC show that vanadate caused the increase of G6P and F6P content
4. Vanadate targets glycolytic pathway of glucose metabolism

Structured Abstract

BACKGROUND

Fungi absorb and solubilize a broad spectrum of heavy metals such as vanadium (V), which makes them a main route of its entry into the biosphere. V as vanadate (V5+) is a potential medical agent due to its many metabolic actions such as interaction with phosphates in the cell, and especially its insulin-mimetic activity. Antidiabetic activity of V-enriched fungi has been studied in recent years, but the biological and chemical bases of vanadium action and status in fungi in general are poorly understood, with almost no information on edible fungi.

METHODS

This manuscript gives a deeper insight into the interaction of V5+ with Coprinellus truncorum, an edible autochthonous species widely distributed in Europe and North America. Vanadium uptake and accumulation as V5+ was studied by 51V NMR, while the reducing abilities of the mycelium were determined by EPR. 31P NMR was used to determine its effects on the metabolism of phosphate compounds, with particular focus on phosphate sugars identified using HPLC.

RESULTS

Vanadate enters the mycelium in monomeric form and shows no immediate detrimental effects on intracellular pH or polyphosphate (PPc) levels, even when applied at physiologically high concentrations (20 mM Na3VO4). Once absorbed, it is partially reduced to less toxic vanadyl (V4+) with notable unreduced portion, which leads to a large increase in phosphorylated sugar levels, especially glucose-1-phosphate (G1P) and fructose-6-phosphate (F6P).

CONCLUSIONS

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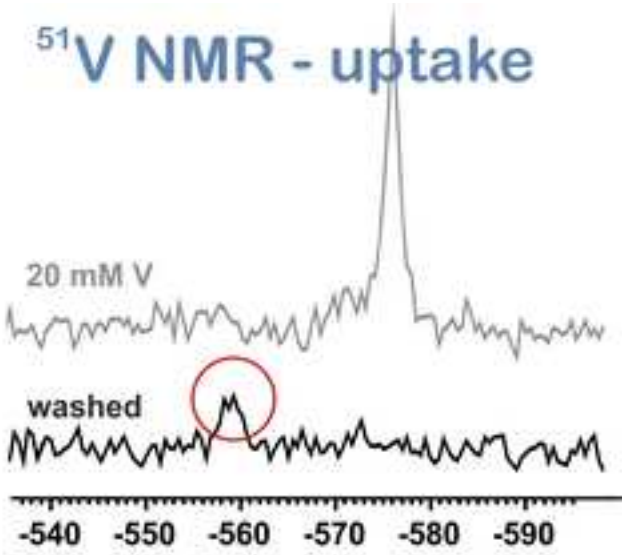
C.truncorum
submerged
mycelium

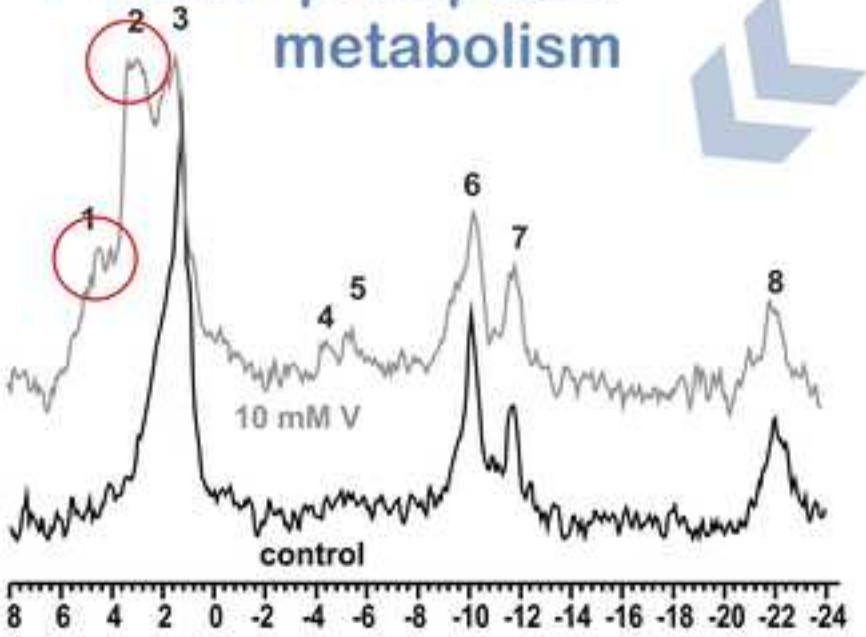
+V^{5+}

^{51}V NMR - uptake



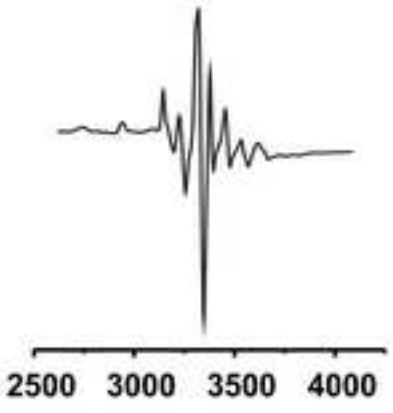
Vanadate enters the cell
in the form of monomer

^{31}P NMR - phosphate
metabolism

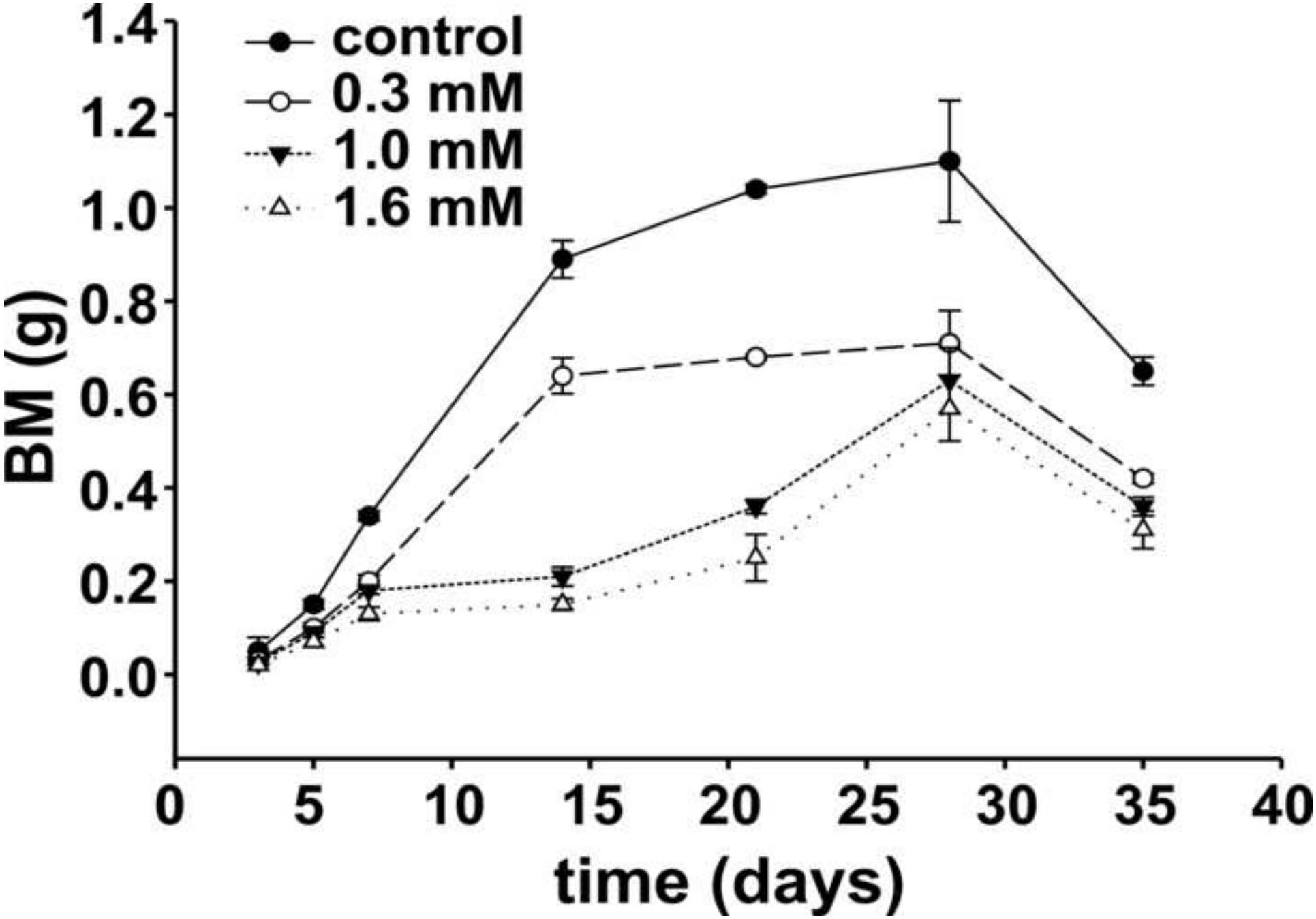


Rise in the content of sugar phosphates
- changes in activities of glycolytic
and glycogen metabolism enzymes

EPR - reduction



Vanadyl signal shows
intracellular reduction
of vanadate



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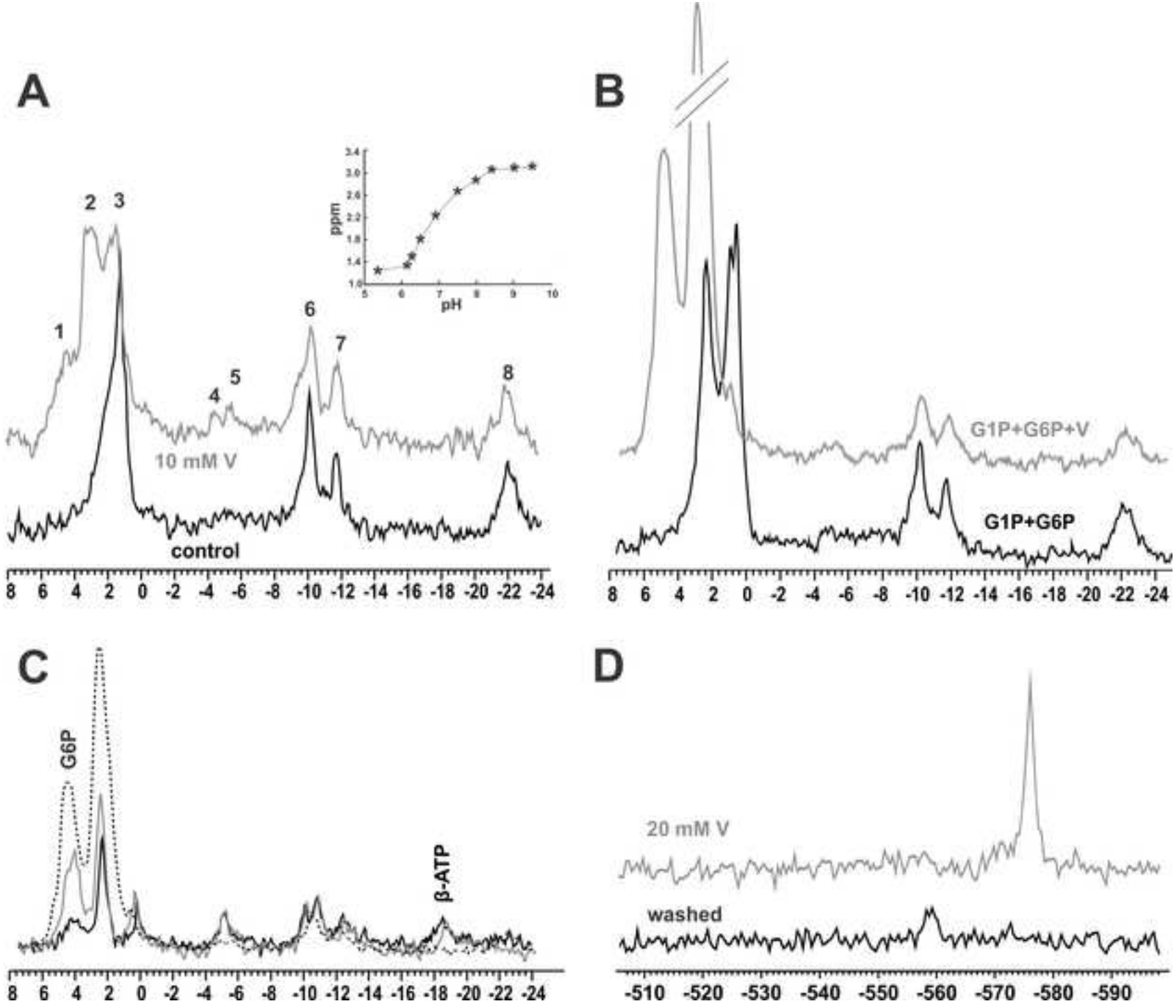

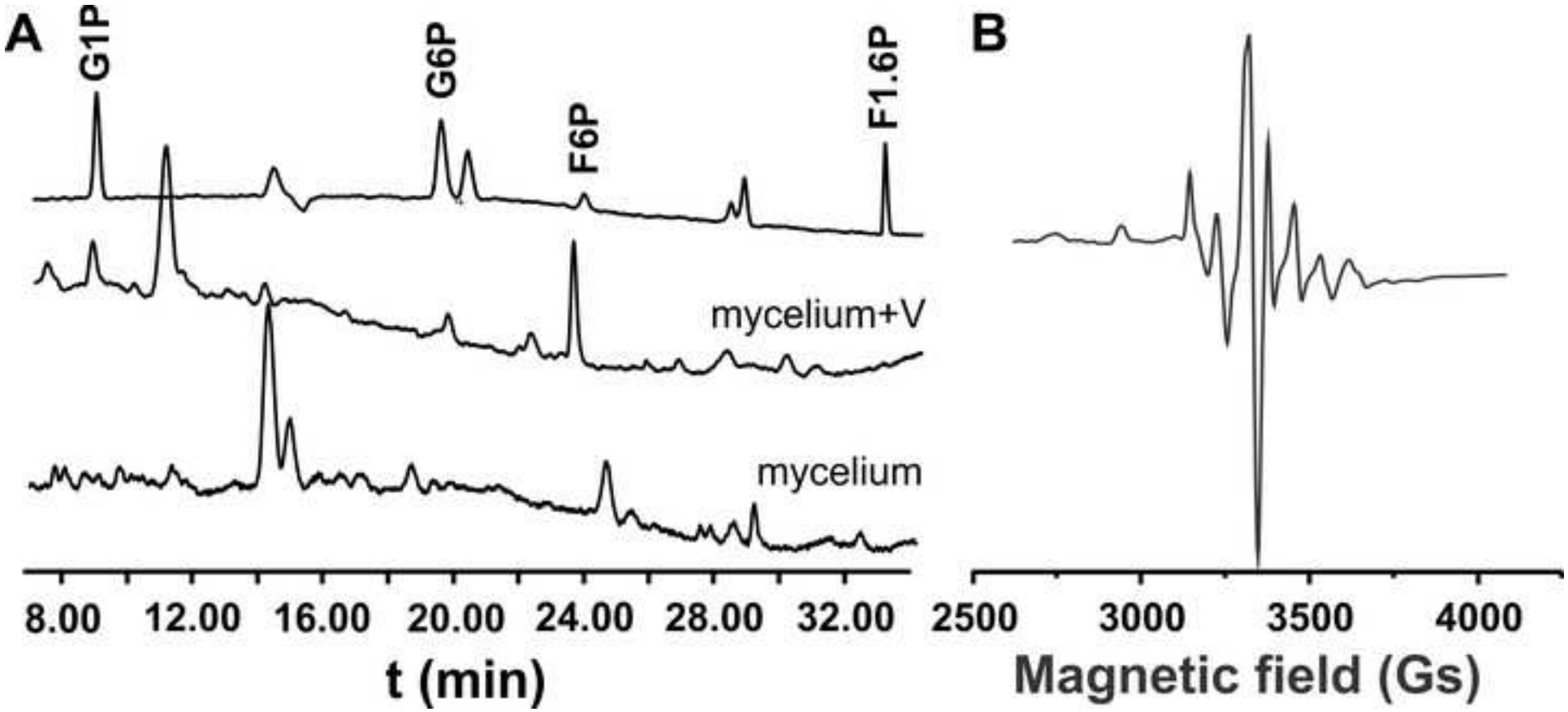

Declaration of Interest Statement

The authors declare no conflict of interest.

Author Statement

No competing financial interests exist.

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Preservation of pH and especially PPc reflects maintenance of the energy status of the mycelium, i.e., its tolerance to high V^{5+} concentrations. Rise in G1P and F6P levels implies that the main targets of V^{5+} are most likely phosphoglucomutase and phosphoglucokinase(s), enzymes involved in early stages of G6P transformation in glycolysis and glycogen metabolism. This study recommends *C. truncorum* for further investigation as a potential antidiabetic agent.

Keywords: *Coprinellus truncorum*, vanadium, phosphate metabolism, NMR, HPLC, EPR

1. Introduction

Vanadium (V) is classified as a dangerous pollutant when distributed in higher concentrations in soil, water and the atmosphere [1–3]. However, in lower concentrations, vanadate (V^{5+}) can serve as a potential medical agent due to its metabolic effects, primarily as insulin-mimetic agent and through interaction with cellular phosphates [4–6]. As a phosphate analogue, V^{5+} may inhibit phosphatases, ribonucleases and ATPases [7, 8]. By inhibiting or activating several enzymes of sugar phosphate metabolism (e.g. glucose-6-phosphatase, phosphoglucomutase and fructose-2,6-biphosphatase), V^{5+} may induce changes in glycolysis and glycogenesis [9, 10].

Fungi absorb and solubilize heavy metals, metalloids, or radionuclides [11–13] including V, and are a main route of V entry into the biosphere. As a result, some V-enriched fungi show strong antidiabetic activity, with *Coprinus comatus* as a representative that has been studied in recent years [14–16].

This study investigates the interaction of *Coprinellus truncorum* (Scop.) Redhead, Vilgalys & Moncalvo 2001, with physiologically high concentrations of vanadate in its environment. *C. truncorum* is an autochthonous, well distributed fungal species in Europe and North America. Its physiology has been far less investigated than its taxonomically related species *C. comatus*, and there are no available data on vanadium uptake or potential antidiabetic activity of *C. truncorum*. The focus of this study was to explore the capacity of its mycelium for V uptake, effects of V on the phosphate metabolism and potential toxicity toward fungal hypha. ^{31}P NMR spectroscopy was used to investigate metabolic changes of the mycelium biomass, while the status of V in the cell was monitored by the combination of ^{51}V NMR with EPR spectroscopy. Finally, HPLC was used to identify sugar phosphate metabolites.

2. Material and methods

2.1. Fungal material

Coprinellus truncorum (Ph. Basidiomycota, Cl. Agaricomycetes, O. Agaricales, Fam. Psathyrellaceae) fruiting bodies were sampled from Fruška Gora mountain (Kamenički park - Northern Serbia). After determination of the species, mycelium was isolated from the fresh material and cultivated for 10 days on Malt agar (Torlak, Serbia) at 26°C and deposited in the FungiCult culture collection of ProFungi laboratory. After drying it was deposited in fungarium collection at the Department of Biology and Ecology, Faculty of Sciences Novi Sad, Serbia (BUNS; No 12 – 00709). Submerged cultivation was carried at 26°C on 100 rpm on an orbital shaker (IKA KS 4000i control, Germany) in fermentation medium as previously described [16].

2.2. Molecular identification of mycelia

Genomic DNA of isolated mycelia was extracted using chloroform-isoamyl alcohol DNA extraction ("CATB") protocol [17]. For PCR amplification of ITS1, ITS2 and 5.8S region, primers ITS1 and ITS4 were used (Biometra T Professional Basis, Germany). Reactions, gel electrophoresis and extraction of PCR products were carried out the same as described in Žižić et al. [16].

2.3. Growth curve and influence of sodium metavanadate on cultivated mycelia

Biomass of submerged cultivated *C. truncorum* was harvested after 3, 5, 7, 14, 21, 28 and 35 days of incubation to define growth phases. Influence of different concentrations (0.01%, 0.03%, 0.05% and 0.1% i.e., 0.3 mM, 1.0 mM, 1.6 mM and 3.3 mM) of sodium metavanadate ($NaVO_3$) in fermentation medium on mycelial growth was also investigated. The biomass of both cultivated mycelia (with and without $NaVO_3$) was collected by filtration at different incubation times (Filters Fioroni, France), lyophilized (ALPHA 2–4 LDplus, Freeze Dryer,

Christ GmbH, Switzerland) and measured to generate growth curves. Results were presented as mean ± standard deviation; three independent measurements were done for each time point.

2.4. Nuclear magnetic resonance (NMR) spectroscopy

For NMR analysis (^{31}P NMR and ^{51}V NMR) mycelium harvested at exponential phase (14th day) was filtered, washed with distilled water and transferred into experimental medium (110 mM glucose and 13.3 mM asparagine). A stock solution of 200 mM Na_3VO_4 (Sigma, Taufkirchen, Germany) was prepared at pH 10 [18]. All spectra were recorded with Apollo spectrometer (Tecmag, USA) at the resonant frequency of 161.978 MHz for ^{31}P, and 105.169 MHz for ^{51}V, other experimental conditions were previously described [10, 19]. For perchloric extracts, control and treated mycelia were suspended in 0.5 M perchloric acid (1:5 w/v), and homogenized in mortar on ice for 15 min. The obtained homogenate was stirred for 15 min on ice and centrifuged at 10000 x g for 12 min. The pellet was discarded, and the supernatant was titrated with 2M KOH until pH was 7. The aliquots were kept at -20°C and thawed just before the experiments. For defining the position of H6P signal, G1P and G6P were added to the extract of control mycelium.

2.5. High-Performance Liquid Chromatography (HPLC)

For HPLC experiments, 14-day old mycelium was treated with 20 mM (80 mmol/gFW) Na_3VO_4 for 10 minutes and then washed with deionized water. The control and V^{5+} treated mycelia were prepared as previously described [10]. HPLC investigation was performed on a Waters Breeze chromatographic system (Waters, Milford, MA) connected to Waters 2465 electrochemical detector with 3 mm gold working electrode and hydrogen referent electrode. All measurement conditions were the same as previously reported in Žižić et al. [10].

2.6. Electron paramagnetic resonance (EPR)

Mycelium was collected by filtration, treated with 10 mM Na_3VO_4, and washed with distilled water. EPR spectra were recorded at 70 K temperature, with Bruker Elexsys-II EPR spectrometer (Bruker, Germany) operating at 9.432 GHz (X-band) equipped with EPR 4112 H V helium cryostat. All measurement conditions and software for analysis were as described in Žižić et al. [16].

3. Results

3.1. Molecular identification of isolated mycelia

The obtained sequence of isolated genomic DNA was compared with the reference sequences in database from the United States National Centre for Biotechnological Information (NCBI). The query sequence showed identity of 99% with *C. truncorum* strain (accession number of sequence JN159562). After confirming the identity, our sequence was registered in NCBI database with accession number MH489093.

3.2. Effect of vanadate on growth of C. truncorum mycelium

Mycelial cultivation in submerged liquid medium showed common growth curve trend with a preparation (lag), acceleration/exponential (log), stationary, and death phase of mycelium (Fig. 1). The control and mycelium treated with 0.3 mM of vanadate reached an exponential phase from 7^{th} to 14^{th} day of growth, while the higher concentration of vanadate in the media (1.0 and 1.6 mM) slowed down the production of biomass. Thus, the highest mycelium biomass yield was observed in control, whereas increasing concentration of vanadate in medium led to decreasing biomass yield, and complete inhibition of the growth at the concentration of 3.3 mM (not shown). Mycelium in exponential phase (14^{th} day of cultivation) was used for NMR, EPR and HPLC assays of acute V effects.

Effects of vanadate on phosphorylated compounds in the mycelium of C. truncorum

Relative content of phosphorylated compounds and energy status of the hyphae were assessed for the first time in *C. truncorum*. Assignment of signals in ^{31}P NMR spectrum of the mycelium from submerged culture was done according to Žižić et al. [10] (Fig. 2A). The major peaks in ^{31}P NMR spectrum of *C. truncorum* mycelium with chemical shifts at -22.1 ppm, -12 ppm, -10.2 ppm, and 1.3 ppm were assigned to core-polyphosphates (PP_c), UDPG, NADP(H)+UDPG and inorganic phosphate (P_i), respectively. Upon addition of vanadate, a new signal emerged at around 2.8 ppm, and barely visible signal(s) of hexose 6 phosphates (H6P) at around 4 ppm became much stronger (Fig. 2A). The new signal could theoretically be attributed to Pi that shifted downfield (left) from the expected position due to pH increase after V^{5+} addition [16]. However, the original Pi signal with similar intensity remained at the same position in the spectrum of V^{5+} treated mycelium, making this assumption unlikely (Fig. 2A). Its non-Lorentzian shape in the spectrum of control mycelium indicated overlapping of at least two signals. Deconvolution of the mentioned region identified three different compounds as most probable contributors. The upfield shoulder of the most prominent signal that resonated at 0.8 ppm was identified as glycerophosphoserine (GPS) [20]. The signals at 1.7 and 1.9 ppm were from Pi located in two cellular compartments - vacuole and cytoplasm, respectively. These signals were used for pH determination according to titration procedure by Hollander et al. [21], which showed that the vacuolar compartment corresponds to pH 6.4 and the cytoplasmic to pH 6.6 (Fig. 2A, inset). The position of the only Lorentzian shaped Pi signal at 2.4 ppm in the spectrum of the neutral (pH 7) perchloric extract of submerged culture (Fig. 2C) corroborates the signal assignment based on the titration curve. The major effect of V^{5+} was related to two signals in the region of phosphorylated sugars (Fig. 2A, gray spectrum), indicating altered activities of enzymes involved in glucose metabolism [4, 10,

22]. According to Navon et al. [20] the position of H6P signals (between 4-4.5 ppm) corresponds approximately to the pH value of Pi-accumulating compartment with pH 6.6. As H6Ps are the intermediates in glycolytic pathway that occurs in the cytoplasm, the more alkaline region of Pi accumulation is further confirmed to be cytoplasm.

Identification of most downfield signals was performed by the addition of G1P and G6P to both control and V^{5+} treated mycelium (Fig. 2B). The signal of G6P appeared at about 3 ppm and 4.2 ppm, while G1P appeared at 1.2 ppm and 2.8 ppm in control and V^{5+} treated sample, respectively. Phosphorylated sugars are large molecules that generally do not pass cellular membrane, so their different positions in ^{31}P NMR spectra of control and V^{5+} treated mycelium can be explained by higher extracellular pH of treated samples, as pH of vanadate stock was 10.

To bypass the influence of V^{5+} on positions of pH-dependent signals in the ^{31}P NMR spectrum, neutral extracts of the control and treated mycelium were prepared (Fig. 2C). All signals in the extract spectra were positioned at ppm values for pH 7 and had almost the same intensities after V^{5+} treatment as in control. Differences compared to *in vivo* experiments were coalescence of two Pi signals due to lack of pH differentiation, and a 96% rise in the intensity of the H6P signal (around 4 ppm) in the spectrum of V^{5+} treated mycelium (Fig. 2C). According to Navon et al., [20] the most prominent spike of this peak corresponds to the chemical shift of fructose-6-phosphate. The intensity of the signal predicted as G1P was also increased but its quantitative determination is rather complex due to partial overlapping with Pi signal. The signal of β-ATP, indiscernible in the *in vivo* spectra, appeared at -19.2 ppm in the spectra of extract.

^{51}V NMR was employed to identify the intracellular V^{5+}species involved in observed changes in ^{31}P NMR spectra, and to corroborate information on intracellular pH [23]. Weak signals of monomer and dimer, accompanied by a strong tetramer signal, were recorded in the spectrum

of V^{5+} treated mycelium (Fig. 2D). As tetramer is not likely to enter the cell [23], monomer and/or dimer were plausible candidates for intracellular accumulation. Their positions at -559 ppm and -571 ppm for monomer and dimer, respectively, also suggest cytoplasmatic pH of 6.6 [23]. After washing the sample with distilled water, only the signal at -559 ppm remained in the spectrum (Fig. 2D), showing that it is the monomer that enters the cell, and that it accumulates in the cytoplasm. The accumulation of V^{5+} in the cytoplasm allows it to have an active role in enzyme regulation and interaction with other compounds located in the cytoplasm, primarily on sugar phosphates (SP), whose concentration is directly dependent on enzymatic activity [10].

3.5. Identification of vanadate induced changes in metabolism of phosphorylated sugars

According to HPLC, V^{5+}caused the increase in G1P, G6P and F6P content, but not that of F1.6BP (Fig. 3A). The largest increase in signal intensity was observed for F6P, which suggests modifications in the glycolytic pathway and confirms the results proposed by NMR. The rise in G6P explains the feature at the top of the H6P signal(s) in both *in vivo* and extracts ^{31}P NMR spectra, where instead of one clearly defined peak, there seem to be 2 or 3. Higher concentration of G1P in the vanadate-treated sample indicates changes in the activity of enzymes involved in the metabolism of glycogen and is in agreement with the emerging of the signal at 2.8 ppm in ^{31}P NMR spectrum, confirming its accumulation induced by V^{5+}.

3.6. Reducing capacity of C. truncorum mycelium towards vanadate

EPR measurements were performed to test whether the reduction of V^{5+} to vanadyl (V^{4+}) occurred and contributed to *C. truncorum* tolerance towards V^{5+}. A characteristic 8-line signal of V^{4+} with the central line position at g=2.0136 emerged in the spectrum after addition of 10 mM V^{5+}, indicating reduction. High concentration of V^{4+} was recorded even after rinsing the

mycelium (Fig. 3B). The observed EPR signal, measured as intensity of central line, was 4 times higher for washed mycelium of *C. truncorum* (Fig. 3B), than for *C. comatus* [16]. This indicates that *C. truncorum* internalizes V^{5+} and reduces it intracellularly to vanadyl with a higher reduction capacity than *C. comatus* [16].

4. Discussion

Vanadium salts and compounds are foremost known as insulin-mimetics, or rather, insulin enhancers, but their impact in the treatment of other metabolic disorders and cancer has also been documented [24]. However, the complex chemistry of V, regarding its oxidation states and coordination forms, its tendency to exchange ligands in different environments and the formation of ROS holds back further progress in applicability of V as therapeutic agent. Absorption of V through digestive system, affected by dietary composition and speciation, is another obstacle as the studies have shown that over 95% of ingested V is excreted by feces [25]. Biofortification of edible fungi by metals and metalloids is a known strategy for overcoming toxicity and speciation problems. For example, V^{5+} enriched *C. comatus* shows augmented hypoglycemic activity [26], and selenium enriched yeasts are used in human nutrition [27]. This manuscript investigates metabolic changes exerted by V^{5+} on edible basidiomycetous fungus *Coprinellus truncorum,* with focus on phosphorylated compounds, especially sugars, and intracellular speciation of V itself.

Phosphates play an important role in several aspects of fungal life and their role as energy storage has been proved as essential for fungal survival. ^{31}P NMR spectroscopy was used as optimal method for *in vivo* monitoring of the changes in phosphates and phosphorylated compounds induced by V^{5+}. The position of Pi signal in the spectrum of control mycelium revealed that, as in the mycelium of *C. comatus* [16], orthophosphates are accumulated in two cellular compartments with different pH (6.4 and 6.6). The pH value of the cytoplasm is

around 6.6, which was confirmed by position of hexose-6-phosphate (H6P) signal(s) in vanadate treated mycelium [20, 28]. Contrary to some other fungal species, the accumulation of Pi in *C. truncorum* is not predominantly related to acidic intracellular compartment, most probably vacuole [29, 30]. The pH difference between two cellular compartments is not significant, which is also opposed to most fungal species that use this difference as a driving force for storing potentially toxic cations within the vacuole [10, 16, 31]. Even though mycelial biomass yield was decreased with the increase of vanadate concentration in submerged substrate, treatment with high concentrations of vanadate did not induce drastic changes in the pH of the mycelium, which excludes the pH-related effect on viability. Similar concentrations of vanadate have been reported to have toxic effects on the growth of most examined fungal species [32] including taxonomically similar *C. comatus*, where there was also a noticeable decrease in PPc signal in NMR spectra with only 2,5 mM vanadate [16]. In *C. truncorum*, this signal remained stable even with 20 mM vanadate exposure, but on the other side of the response spectrum there are *H. polymorpha* and *P. blakesleeanus* whose PPc signal increased with V^{5+} addition, as they use vacuolar polyphosphates for metal(loid) sequestration [30, 33]. The initial step in V^{5+} detoxification for these two strains is the reduction of V^{5+} to a less toxic V^{4+}, which was also observed in *C. truncorum*. Tolerance of fungal species to vanadium in mM concentrations has already been known for many strains but has been exclusively related to vanadium in the 4+ oxidation state [34]. In aerobic conditions, intracellular reducing agents such as glutathione, ascorbate, NADH, phenolic compounds or proteins reduce V^{5+} to V^{4+} [35, 36]. However, *P. blakesleeanus* can bind V^{5+} to an unidentified intracellular biomolecule without reduction, which makes it tolerant to extreme V^{5+} concentrations [19]. Vanadate polymerization has also been reported as an effective mechanism of coping with its toxicity [37], and even though V^{5+} polymers are most stable at acidic pH [23], they show considerable stability in physiological environment [37],

which is highly fortified by their interactions with intracellular macromolecules [38]. Similar processes in *C. truncorum* cannot be excluded, but the intensive reduction of V^{5+} to less toxic V^{4+} form seems to be the predominant way in overcoming toxicity of extracellular V^{5+}. EPR measurements suggest that *C. truncorum* reduces V^{5+} intracellularly, but ^{51}V NMR experiments showed that monomer partially survived the reducing intracellular environment. Pharmacological activity, both toxicity and therapeutic effects of V^{5+} monomer are attributed to its structural and electronic similarities to phosphate [39]. These similarities allow it to affect phosphate utilizing systems [40], on which the insulin-mimetic action of V^{5+} is based [4]. Glycolytic and glycogenolytic paths of phosphate metabolism are likely to be the core of V^{5+} activity due to its impact on many enzymes that participate in these processes [41–44]. The rise in concentrations of F6P and G6P shown by ^{31}P NMR and HPLC demonstrate that phosphoglucomutase [45] and phosphoglucokinase(s) [9, 46] are most likely affected by V^{5+}. Binding of V^{5+} to active site of phosphoglucomutase-type enzyme can be assumed because of prominent rise in concentration of G1P, which also implies changes in the metabolism of glycogen. The role of glycogen is not precisely known for this species, but its accumulation in the first days of mycelial growth has an important function in fruit body development of *Coprinus cinerius* [47], so the interference with this pathway may have consequences on further development stages of the fungus. In addition to monomer, V^{5+} tetramer can impact the process of glucose degradation by inhibiting the main enzyme of phosphate-pentose shunt, 6-phosphogluconate dehydrogenase, in human, mammalian, yeast and bacterial cells [48]. The tetramer signal was indeed registered in ^{51}V NMR spectrum of *C. truncorum*, but it disappeared after rinsing, which means that the tetramer was in extracellular environment where it cannot affect phosphate metabolism. Decamer of V^{5+} is also a known pharmacological agent [38], but this one and other oligomeric species were not detected in ^{51}V NMR spectrum of *C. truncorum*. The presented results indicate that, like for *Coprinus*

cinereus [49], the main path of carbohydrate metabolism in *C. truncorum* takes places via glycolysis.

Previous studies suggested that taxonomically related fungus *Coprinus comatus* rich in vanadium reduced hyperglycemia in alloxan-induced diabetic mice by inhibiting gluconeogenesis, increasing insulin level and recovering the injured β-cells [14, 15, 50]. We have shown here that *C. truncorum* adopts vanadium from the environment in V^{5+} form and is able to partially reduce it to V^{4+}. Both forms are associated with antidiabetic properties [24] and further research of vanadium enriched *C. truncorum* as a health food product or supplement for blood sugar regulation should be considered. Since *C. truncorum* has dietary value as a source of essential nutrients (proteins, phenolic compounds, unsaturated fatty acids such as oleic and linoleic) [51, 52] its nutritional benefits with vanadium enrichment could provide a holistic approach to managing diabetes through diet.

Conclusions

- V^{5+} uptake and its intracellular action and bioavailability in *C. truncorum* point to the tolerance of mycelia to its presence.
- V^{5+} targets glycolytic pathway of glucose metabolism which could be helpful in further investigation of its potential therapeutic/antidiabetic properties.
- Changes in sugar phosphates indicate that vanadate affects enzymes that participate in early stages of G6P transformation in glycolysis and glycogen metabolism.
- *C. truncorum* should be considered for further research as a vehicle for vanadium intake as a means of blood sugar regulation.

Conflict of interests

The authors declare that they have no conflict of interest.

Funding

This work was supported by the Projects of Ministry of Science, Technological Development and Innovation of the Republic of Serbia [grant numbers 451-03-47/2023-01/ 200053; 451-03-47/2023-01/200178; 451-03-47/2023-01/200051; 451-03-47/2023-01/200125].

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FIGURE CAPTIONS:

Fig. 1 Growth curves of *C. truncorum* mycelium with and without vanadate addition

Fig. 2 NMR spectra of *C. truncorum* mycelium from submerged cultures. X-axes are in ppm. A) Solid black line –^{31}P NMR of control mycelium; solid grey line – V^{5+} treated mycelium. Signals can be assigned as: (1) hexose 6 phosphate (H6P), (2) glucose 1 phosphate (G1P), (3) inorganic phosphates, (4) γ-ATP and β-ADP, (5) pyrophosphates and terminal P of polyphosphates (PolyP), (6) α-ATP and NADP+UDPG, (7) second resonance of UDPG, and (8) core PolyP. Inset – pH titration curve. B) Solid black line - ^{31}P NMR spectra of control mycelium after addition of G1P and G6P; solid grey line - ^{31}P NMR spectra of mycelium after addition of G1P, G6P and V^{5+}. C) Solid black line - ^{31}P NMR spectra of perchloric extract of control *C. truncorum* mycelium; solid grey line - ^{31}P NMR spectra of perchloric extract of V^{5+} treated *C. truncorum* mycelium; dashed black line - ^{31}P NMR spectra of perchloric extract of control *C. truncorum* mycelium with added G1P and G6P. D) ^{51}V NMR spectra of the *C. truncorum* mycelium treated with 20 mM V^{5+}, before (upper grey) and after washing (lower black).

Fig. 3 A) HPLC chromatogram of control (lower), V^{5+} treated mycelium (middle), and mixture of G1P, G6P, F6P, F1.6BP standards (upper). B) EPR spectrum of *C truncorum* mycelium treated with 10 mM V^{5+} and washed