Response of soil fungi to chromium(VI)

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Fungi isolated from soil were tested for their resistance to 0.2–2 mM chromium (VI), using $K_2Cr_2O_7$. From all micromycetes tested, most sensitive were *Acremonium* sp. and *Penicillium decumbens* whose growth was inhibited completely at 1 mM chromium in the medium. More tolerant fungi were not affected up to 0.5 mM Cr concentration, and the most resistant *Trichoderma viride* and *Penicillium chrysogenum* were able to develop in the presence of 2 mM chromium in the medium. The metal uptake experiment showed that the both chromium-tolerant fungi, *T. viride* and *P. chrysogenum*, accumulated chromium in their biomass (2.24 and 1.52 mg/ g dry weight, respectively).

Key words: fungi, chromium-resistance, metal-accumulation

INTRODUCTION

Metals are directly or indirectly involved in all aspects of fungal growth, metabolism and differentiation, but they can exhibit a harmful effect above their certain concentration [6, 15]. Heavy metals released into nature affect fungal communities, exhaust the ecosystems, reduce species diversity and shift to more tolerant individuals [1, 2, 6, 10].

The use of chromium in industry (metal refining, electroplating and tanning, etc.) often results in contamination of soils or sediments [4]. Cr (VI) is known to be toxic, mutagenic and carcinogenic and usually exists as an anion of high solubility (CrO_4^{2-}, and $Cr_2O_7^{2-}$) [4, 12].

The aim of the work was to study the sensitivity of soil micromycetes to chromium (VI) and to determine their capability of chromium accumulation by their biomass.

MATERIALS AND METHODS

Micromycetes were isolated from a rhizosphere zone soil under leaf-litter (Verkiai Regional Park). Fungal sensitivity to heavy metals and their mixture was tested on Czapek medium agar consisting of 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.001% FeSO4 · $7H_2O$, 2% glucose and 2% agar, pH 5.5. The medium was amended with 0.2–2 mM Cr^{6+}, using $K_2Cr_2O_7$. All glassware used for metal studies were washed with 4% nitric acid and rinsed 3 times with distilled water.

Cultures were grown on the solid medium at 25+2 °C and inspected during 5 days. Chromium influence on fungal growth was evaluated as changes in radial rate of hyphal extension by measuring diameters of fungal colonies. All experiments were conducted in triplicates.

For metal sorption experiments, fungi were grown in a liquid medium of the same composition with addition of $K_2Cr_2O_7$ at a concentration of 0.2 mM Cr. The cultures were incubated on a rotary shaker for 3 days. The content of biomass was evaluated as dry weight obtained by drying at 105 °C for 8 h. Measurements of metals that remained in the growth medium after cultivation were performed spectrophotometrically [16].

RESULTS AND DISCUSSION

The results show that $K_2Cr_2O_7$ affected all the fungi tested depending on a concentration used. Nevertheless, the lowest, 0.2 mM, chromium concentration had no negative effect on the development of *Alternaria alternata, Trichoderma viride* and *Ulocladium* sp. Low sensitivity at this concentration was showed by *T. koningii, Cladosporium* sp., *Talaromyces luteum* (Table 1). The highest inhibition (51.7–57.7%) was excerted on the genus *Penicillium* fungi: *P. lilacinum, P. decumbens, P. claviforme* and *P. viridicatum*. When the concentration of chromium was raised to 0.5 mM, the fungi that were not affected by the previous $K_2Cr_2O_7$ concentration also exhibited high resistance. The lowest growth inhibition was 21.8–25.7% and the highest reached even 85.7–87.5% (*P. decumbens, P. lilacinum* and *P. paxilli*). The rest micromycetes were also inhibited significantly (50.0–87.5%). When the chromium content reached 1 mM, inhibition evi-

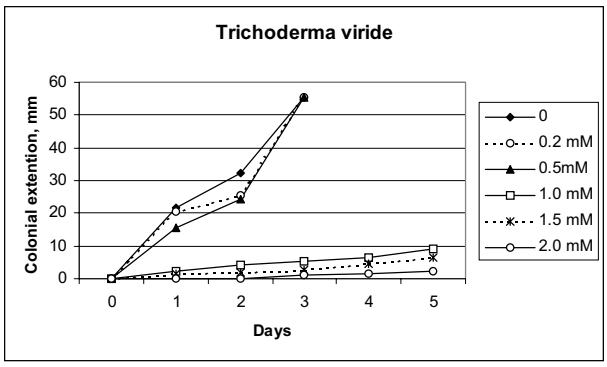
Table 1. **Suppression of fungal growth (colonial extension) on the medium supplemented with $K_2Cr_2O_7$, after a 5-day cultivation, %**

Fungi	Concentration of chromium, mM				
	0.2	0.5	1.0	1.5	2.0
Acremonium sp.	27.5 ± 5.3	77.5 ± 2.5	100	100	100
Alternaria alternata	0	0	92.5 ± 11.3	95.6 ± 10.3	100
Aspergillus niger	8.5 ± 3.0	30.5 ± 4.3	9.1 ± 0.8	98.4 ± 15.3	100
Cladosporium sp.	7.5 ± 1.7	21.8 ± 1.7	48.4 ± 8.2	89.8 ± 8.7	100
Fusarium sp.	15.4 ± 2.7	58.8 ± 5.7	87.7 ± 9,0	100	100
Mucor plumbeus	29.0 ± 2.5	50.8 ± 4.7	81.7 ± 8,7	100	100
Paecilomyces carneus	36.5 ± 4.9	63.6 ± 11.3	87.0 ± 10,3	97.4 ± 8.5	100
Penicillium decumbens	53.5 ± 8.4	85.7 ± 10.0	100	100	100
P. funiculosum	51.3 ± 10.3	87.5 ± 12.8	89.8 ± 11.3	95.8 ± 10.5	100
P. chrysogenum	41.7 ± 5.2	65.7 ± 8.3	81.7 ± 6.5	93.2 ± 7.7	97.5 ± 15.7
P. lilacinum	43.8 ± 6.5	63.6 ± 10.3	87.7 ± 9.7	97.5 ± 8.3	100
P.claviforme	56.8 ± 6.7	78.9 ± 8.0	89.2 ± 11.3	98.5 ± 14.3	100
P. paxilli	29.0 ± 4.2	85.8 ± 7.7	91.3 ± 14.3	100	100
P. viridicatum	57.5 ± 8.5	78.8 ± 4.5	84.9 ± 7.3	97.7 ± 10.3	100
Rhizopus nigricans	9.8 ± 2.1	25.7 ± 1.7	84.3 ± 12.8	97.2 ± 8.7	100
Rhizopus arrhizus	5.5 ± 1.5	45.9 ± 3.7	91.6 ± 11.3	94.8 ± 9.5	100
Talaromyces luteum	7.3 ± 2.1	54.5 ± 6.3	81.3 ± 12.3	95.5 ± 12.5	100
Trichoderma koningii	1.3 ± 0.5	19.5 ± 1.5	85.8 ± 8.0	88.8 ± 9.3	100
T. viride	0	0	83.4 ± 6.5	87.7 ± 7.0	95.9 ± 14.3
Ulocladium sp.	0	0	83.4 ± 8.7	88.0 ± 13.2	100

dently increased – even up to 100% in some fungi (*Acremonium* sp. and *P. decumbens*) and most of the fungi were suppressed by 80–90%. At 1.5 mM chromium growth of *Fusarium* sp., *Mucor plumbeus* and *P. paxilli* was completely inhibited. The highest resistance was manifested by *T. viride, T. koningii* and *Ulocladium* sp. (suppression 87.7–88.8%). Only two of the fungi (*Trichoderma viride* and *P. chrysogenum*) were able to develop on the medium supplemented with 2.0 mM chromium, however, their growth was very slow, with no conidiogenesis and with obviously altered morphology (Figure).

As the results showed the growth of the most sensitive fungi (*Acremonium* sp., *P. decumbens*) was inhibited completely at 1.0 mM chromium in the medium. Some more resistant fungi were not affected up to 0.5 mM Cr concentration (*A. alternata, T. viride, Ulocladium* sp.). The genus *Penicillium* fungi, in comparison with other micromycetes, were affected rather considerably at low concentrations. Nevertheless, at high chromium concentrations the sensitivity of most of these fungi did not differ from other micromycetes; moreover, *P. chrysogenum* was able to develop on the medium in the presence of 2 mM chromium.

The negative effect of $K_2Cr_2O_7$ was considerable in the initial period of fungal growth. If the deve-



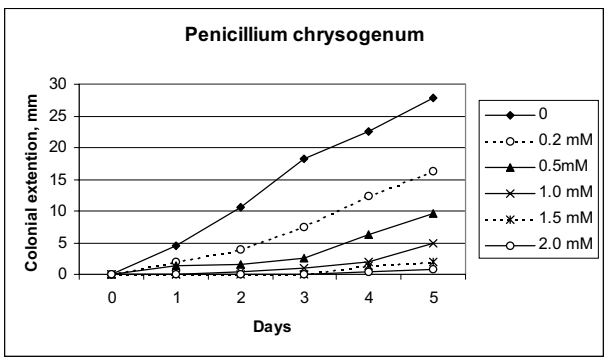


Figure. Colonial growth of *Trichoderma viride* and *Penicillium chrysogenum* on Czapek medium amended with $K_2Cr_2O_7$

Table 2. **Chromium accumulation by fungal biomass on 3-day growth in the medium containing 0.2 mm $K_2Cr_2O_7$**

Fungi	Total biomass, g dry weight	Accumulated chromium, mg/g dry biomass weight	pH after cultivation
Penicillium chrysogenum	0.055 ± 0.012	2.24 ± 0.15	4.7
Trichoderma viride	0.042 ± 0.007	1.52 ± 0.08	6.9

lopment of fungi in a chromium-free medium was observed after a day, $K_2Cr_2O_7$ prolonged the lag-phase depending on the concentrations used. Even the lowest concentration of potassium bichromate slowed down the development of the quite resistant *T. viride* for 1–2 days if compared to the control (Figure). The growth of *P. chrysogenum* at these concentrations was suppressed more considerably also during the whole period of its growth. Development of the both fungi on a medium with 2 mM chromium was observed only after 3–4 days. Not only the growth of the fungi, but also their conidiogenesis was slowed down or even did not occur at 1.5–2 mM Cr in the medium throughout the experiment.

Further studies were done to elucidate the possibilities of most resistant fungi to accumulate chromium in their biomass. *Penicillium chrysogenum* and *Trichoderma viride* were chosen as those capable of tolerating 2 mM, the highest chromium concentration used. The results revealed that the both fungi grown in the medium with addition of 0.2 mM chromium accumulated chromium (Table 2). The uptake of the metal by *P. chrysogenum* was higher and after 3 days reached 2.24 mg/g of dry fungal biomass, *Trichoderma viride* accumulated a slightly smaller amount – 1.52 mg/g. Biomass growth was also more efficient in *P. chrysogenum* (0.055 g) than in *T. viride* (0.042 g). Differences in the uptake, even if not high, could be determined by the different biology (physiological properties, cell wall composition, etc.) of these fungi belonging to different genera.

The experiment showed that the fungi that were rather resistant to $K_2Cr_2O_7$ were able to accumulate chromium by their biomass. It has been reported that Cr(VI) could be sorbed by microorganisms, especially fungi, on the cell wall [5]. The cell wall of the fungi due to its structure can bind considerable amounts of metals. The high binding potency is attributed to amino amides, hydroxyl, carboxyl, sulphydryl and phosphate groups [6, 7, 14]. Some works have also demonstrated the ability of bacteria and yeast to uptake chromium inside the cell by reducing of Cr^{6+} to Cr^{3+} as less toxic and less harmful to the cell [8, 9, 11, 13].

Based on the obtained results, the ability of fungi to develop in the medium supplemented with chromium suggest that fungi can be able to some extent to resist the presence of this metal in natural surroundings. Obviously, the toxicity of Cr(VI) depends on many factors of the surroundings (pH, organic mater, other ions, etc.) [3, 6]. The ability of the fungi to accumulate chromium and grow, as the experiment has demonstrated, help more resistant fungi to survive in a polluted environment.

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DIRVOŽEMIO GRYBŲ REAKCIJA Į CHROMĄ (VI)

S a n t r a u k a

Tirtas iš dirvožemio išskirtų grybų atsparumas 0,2–2 mM chromui (VI), naudojant $K_2Cr_2O_7$. Iš visų tirtų mikromicetų jautriausi buvo *Acremonium* sp. ir *Penicillium decumbens*, kurių vystymasis visiškai nuslopintas, į terpę pridėjus 1 mM chromo. Atsparesni grybai nebuvo veikiami iki 0,5 mM chromo koncentracijos terpėje, o patys atspariausi *Trichoderma viride* ir *Penicillium chrysogenum* vystėsi esant 2 mM metalo terpėje. Metalo kaupimo tyrimai parodė, kad abu chromui atspariausi grybai *T. viride* ir *P. chrysogenum* sukaupė atitinkamai 2,24 ir 1,52 mg chromo /g sausos biomasės svorio.

Raktažodžiai: grybai, atsparumas chromui, metalų akumuliacija