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Zinc influence on the ability of *Penicillium* Link. genus fungi to use natural C sources

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The effect of Zn on the ability of eight fungi from the genus *Penicillium* to assimilate different C sources common in nature or in human environment (starch, skim milk powder, natural leather powder, sunflower oil, chitin and cellulose) was investigated. A 12 mM Zn concentration was found to inhibit assimilation of starch and skim milk by all fungi. Micromycete growth on media containing these C sources and Zn were evaluated to be from 0 to 72% in comparison with their growth on the same but Zn-free substrata. The least growth suppression was noted on the use of chitin and cellulose; fungal colony growth was equal to 71–125%. The fungi most resistant to Zn influence on substrate assimilation were *P. italicum* 170S and *P. aurantiogriseum* 23L.

INTRODUCTION

Fungi in soils are ubiquitous organisms and play a great role in decomposition of residues and humus formation, take part in degradation of various organic and inorganic substances, excrete active metabolites like organic acids, enzymes, antibiotics, etc. (Carlile and Watkinson, 1995). However, various pollutants can significantly influence their physiological and reproductive abilities in the natural environment. In a highly polluted environment, common fungal functioning can be affected and community structures of microorganisms can be disturbed. Zinc is an essential metal for fungi taking part in their biological functions (Gadd, 1993; Лугаускас, 1988), but above a certain concentration zinc, like other heavy metals, becomes toxic (Hughes and Poole, 1989). It was shown that zinc reduced the abundance of *Penicillium, Oidiodendron, Mortierella* and other fungal genera near the zinc smelter (Jordan and Lechevalier, 1975). Zinc was also indicated to affect significantly the appearance of fungi, *e.g.*, Martino et al. showed that in the presence of zinc ions, fungi loss most of their pigmentation (Martino et al., 2000). Nordgren et al. (1983) reported that high Zn concentrations (up to 20 mg/g dry soil) affect the physiological properties. The metal decreased fungal biomass and soil respiration rate.

The aim of this work was to evaluate the effect of zinc on the abilities of fungi to assimilate different natural substrata for their nutrition as C sources, which are common in the natural and anthropogenic environment.

MATERIALS AND METHODS

For the investigation, fungi of the genus *Penicillium* Link. have been chosen as the most widespread fungi in Lithuanian soils (Лугаускас, 1988) and known for their active physiological functions in their habitats (Domsh and Gams, 1980).

Fungi for a control trial were grown on Czapek agar containing glucose (Pitt, 1979). To investigate the assimilation ability of various C sources, glucose was replaced with 1% starch, skim milk powder, natural leather powder, sunflower oil or chitin, and filter paper (cellulose) was added to the agar. The substrata chosen for the experiment were those common in nature or in human environment.

The ability to use these C sources was evaluated as colonial growth (a diameter size) on modified media (containing a substratum tested) in comparison with fungal growth on Czapek (control) medium.

Zinc was added into the media containing the same C sources. Zinc concentration of 12 mM was chosen as close for all the fungi to the concentration reducing fungal growth by 50% (Fig. 1). The zinc inhibition effect (%) was evaluated by comparing fungal colony sizes on media containing zinc with colonies on the same but Zn-free media.

The ratio substratus-Zn *versus* glucose-Zn (control) was calculated as a ratio of growth values desc-

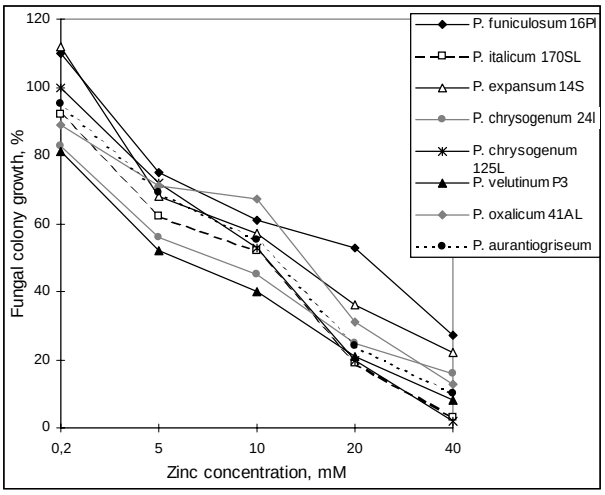


Fig. 1. Effect of Zn on colonial growth of fungi on Czapeck medium in 7 days

ribed above (%) of fungi on various substrata with zinc *versus* fungal growth values on a glucose-containing medium with zinc. All tests were conducted in triplicate. Results were expressed as the mean and standard deviation (Atlas, 1993).

RESULTS AND DISCUSSION

First, fungi were tested for their ability to assimilate various substrata common in the environment as residues in soil: cellulose, chitin, starch, lipids (sunflower oil) and protein-containing natural substrata (leather powder and skim milk). The results showed that starch and skim milk were the most suitable C sources for micromycetes. Fungi *P. funiculosum* 16PL and *P. expansum* 14S grew even better on starch and *P. expansum* 14S and *P. chrysogenum* 125 AL on skim milk than on glucose substratum (Table 1). The rest substrata supported fungal growth less than glucose. Leather was a rather good C source for *P. chrysogenum* 24L, *P. chrysogenum* 125AL and *P. velutinum* P 3. The fungi *P. funiculosum* 16Pl and *P. expansum* 14S developed well on sunflower oil, but the other micromycetes assimilated this substratum moderately or poorly. Cellulose was a hardly available source for all fungi with exception of *P. funicullosum* 16PL whose development was good and did not much differ from the control. Assimilation of chitin was poor by all fungi tested; only *P. itali-*

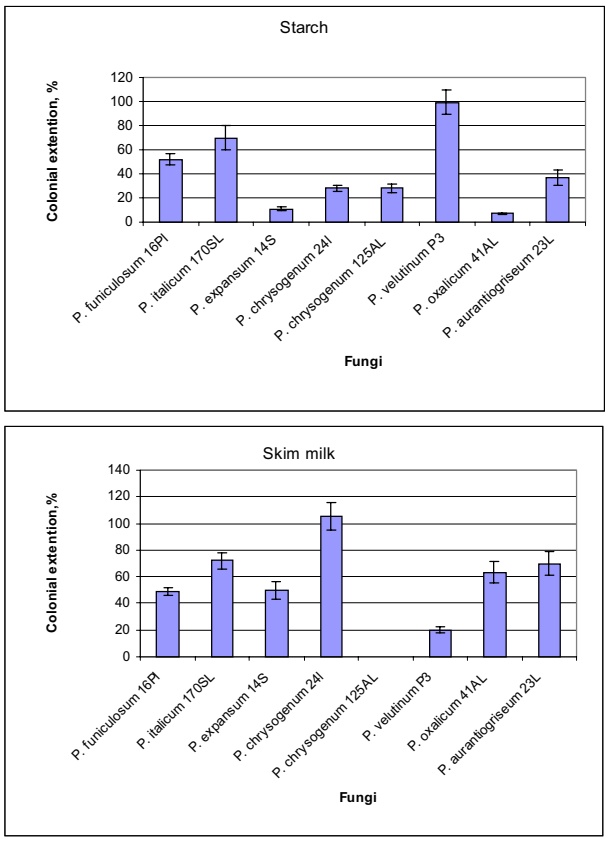


Fig. 2. Growth of *Penicillium* fungi on the media containing starch or skim milk powder as C source in the presence of 12 mM zinc in comparison with their development on the same but Zn-free media

Table 1. **Colony growth values (a diameter) of fungi on different substrata after 7 days, mm**

Fungi	Substrata added into media						
	glucose	starch	skim milk powder	leather powder	sunflower oil	cellulose	chitin
P. funiculosum 60PL	35.7 ± 2.3	39.3 ± 3.3	32.5 ± 2.3	60.5 ± 2.5	29.3 ± 2.5	30.3 ± 2.5	6.4 ± 0.3
P. italicum 170SL	32.0 ± 2.0	32.0 ± 2.7	22.4 ± 1.7	57.6 ± 3.0	57.6 ± 3.3	80.0 ± 3.3	10.2 ± 1.5
P. expansum 14S	38.5 ± 2.2	40.4 ± 3.2	42.4 ± 3.0	19.3 ± 1.4	24.3 ± 1.1	1.2 ± 0.2	0
P. chrysogenum 24L	34.3 ± 3.3	25.71 ± 1.7	40.9 ± 2.5	28.1 ± 2.0	15.4 ± 0.7	1.0 ± 0.0	5.1 ±2.9
P. chrysogenum 125AL	40.3 ± 2.3	32.2 ± 2.7	44.3 ± 1.7	30.2 ± 2.7	4.0 ± 0.7	2.8 ± 0.3	6.0 ± 1.7
P. velutinum P(3)3	42.5 ± 3.5	29.8 ± 1.7	38.3 ± 2.3	28.9 ± 1.5	10.6 ± 1.2	4.3 ±0.3	7.7 ± 0.3
P. oxalicum 41AL	52.2 ± 2.1	37.6 ± 3.0	47.0 ± 3.7	30.3 ± 2.0	13.1 ± 0.7	5.2 ± 1.1	9.4 ± 1.0
P. aurantiogriseum 23L	31.5 ± 2.5	37.6 ± 3.0	22.7 ± 1.2	8.8 ± 0.3	6.3 ± 0.5	2.5 ± 1.5	6.0 ± 1.1

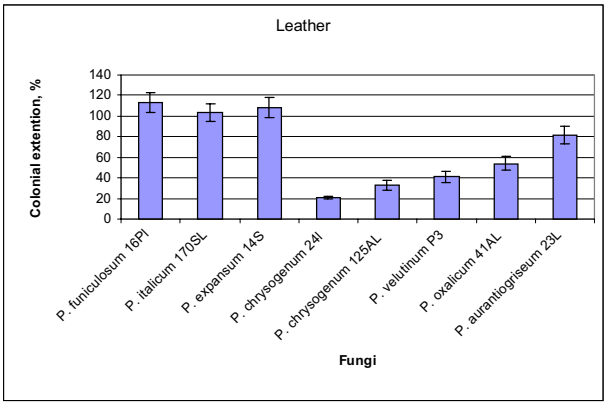
cum 170SL showed a better growth than on leather and sunflower oil substrata.

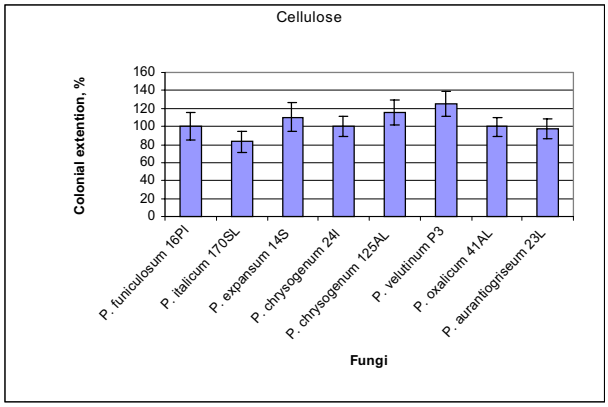
When zinc was added into the media, the metal at a concentration of 12 mM inhibited the growth of fungi in most cases.

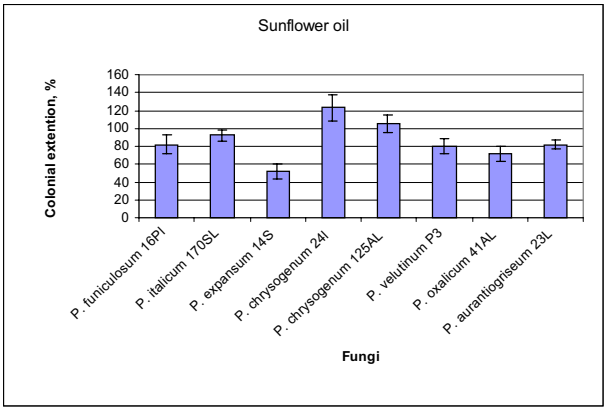
Starch assimilation was supressed in all the fungi: their growth was evaluated to be 7–70% in comparison with a Zn-free starch-containing medium. The micromycetes most resistant to the effect of Zn were *P. velutinum* P3, *P. italicum* 170SL, *P. funiculosu*m 16PL (Fig. 2). The most negative influence of zinc on starch assimilation was seen in cases of *P. oxalicum* 41AL and *P. expansum* 14S; their colony extension on a starch-containing medium with 12 mM Zn reached only 7 and 11% as compared to their growth on the same Zn-free medium.

The growth of the fungi on protein-containing media showed that zinc also strongly inhibited fungal development. In particular, a negative influence of Zn was exerted on *P. chrysogenum* 125AL; its growth on a milk protein-containing medium was totally suppressed (Fig. 3). On the contrary, growth of *P. expansum* 14S was very good and even was slightly stimulated by Zn. The development of the rest micromycetes was slowed down in the presence of zinc, and their growth amounted to 20–72%. The growth of most fungi on the medium with leather substratum was better than on the medium with milk proteins. The highest suppression of growth was observed in *P. chrysogenum* 24L and *P. chrysogenum* 125AL cases (their colony growth equalled 21% and 33%, respectively) (Fig. 4). Nevertheless, the growth of three fungi (*P. funiculosum* 16PL, *P. expansum* 14S and *P. italicum* 170SL) was not suppressed and even stimulated (growth – 103–113%). The influence of Zn was insignificant also on *P. aurantiogriseum* 23L; its development was evaluated to be 82%.

Inhibition of lipid assimilation by 12 mM Zn was not so severe as the inhibition in the previous tests. The most Zn-sensitive fungus on sunflower oil-containing substratum was *P. expansum* 14S: its growth reached 52% in comparison with its development on a Zn-free medium. Fungi *P. chrysogenum* 24L and *P. chrysogenum* 125AL developed even better in the presence of zinc (growth 123–105%). The other micromycetes developed slower under the effect of zinc on this substratum; their development was evaluated to be 72–92%.







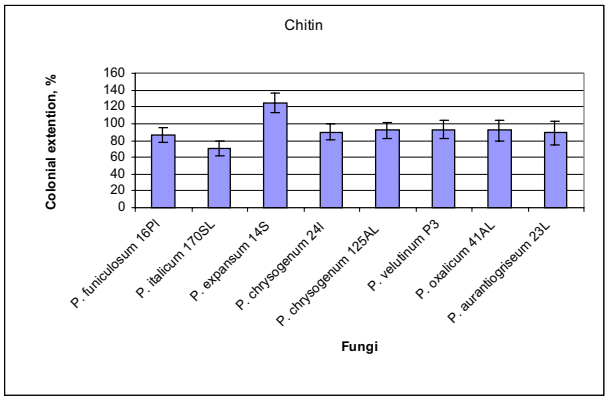


Fig. 3. Growth of fungi on the media containing leather or sunflower oil as C source in the presence of 12 mM zinc in comparison with their development on the same but Zn-free media

Fig. 4. Growth of fungi on the media containing cellulose or chitin as C source in the presence of 12 mM zinc in comparison with their growth on the same but Zn-free media

Zn (12 mM) had a little effect on assimilation of chitin. The strongest inhibition by Zn was seen in the case of *P. italicum* 170SL: its colony growth was 71%. Many fungi grew insignifically slower in the presence of Zn, colony extension being 87% to 93%. The fungus *P. expansum* 14S showed resistance to Zn on this medium and even was stimulated by Zn (growth 125%).

The least inhibition of fungal growth by Zn was observed on a cellulose-containing medium. The growth of only two fungi, *P. italicum* 170SL and *P. aurantiogriseum* 23L, was slightly suppressed (growth 83% and 97%). The other micromycetes did not react to the presence of the metal or were even stimulated up to 125% (*P. velutinum* P. 3).

The individual fungal reaction to Zn when growing on the test substrata was rather variable. Growth variance of *P. italicum* 170S differed not much on all the media with zinc (growth from 70% to 103%). The reaction was similar in the case of *P. aurantiogriseum* 23L, with an exception of starch substratum in the presence of Zn (growth 70–97%). The other micromycetes were affected rather variably on various substrata, *e.g.*, *P. chrysogenum* 24L growth on starch with the presence of Zn was only 28% and on a skim milk medium with Zn 105%, whereas development of *P. velutinum* 3P was otherwise 99% and 20% on the same media, respectively. There were no fungi in all fungi tested, which could survive evidently better in the presence of Zn on all the substrata or a fungus that could grow very poorly on the all media tested.

The effect of Zn on the assimilation of various substrata was compared with its effect on glucose assimilation (medium used for assessment of Zn tolerance) and expressed as the ratio substratum–Zn (a medium containing a particular substratum and supplemented with zinc) *versus* glucose–Zn (control). The comparison showed that the suppression of growth of most fungi by Zn was most evident on a starch-supplemented medium and slightly less on skim milk-containing medium than on a glucose medium, *i.e.* the fungi react more sensitively to Zn on these substrata than their established tolerance in a glucose-containing medium (Table 2). The ratio leather–Zn/glucose–Zn was lower than 1 only in two cases (*P. chrysogenum* 24L and *P. chrysogenum* 125AL), while other fungi showed a higher Zn-tolerance on leather substratum than on glucose medium. Fungi growing on the other substrata with zinc were more resistant than on glucose–Zn medium, particularly *P. chrysogenum* 24L on sunflower oil (2.51 times), *P. velutinum* P3 on cellulose (3.05), and *P. expansum* 14S and *P. velutinum* P3 on chitin (both 2.27). It should be mentioned that on more complicated substrata (cellulose, chitin) fungi were much more tolerant to the same Zn concentration added than on the glucose medium.

The results showed that Zn at a concentration of 12 mM affected significantly fungal possibilities to use the test substrata as C sources. Bewly and Stotzky (1983) also indicated that 5000–10000 ppm Zn had extended the lag phase of microorganisms and reduced the total amount of carbon mineralization. It is reported that Zn ions at 100 μ/ml exerted even a lethal effect on the genus *Penicillium* fungi (Лугаускас, 1988). Growth suppression on different substrata is first of all related to changed or inhibited activity of certain enzymes involved in the processes (Gadd, 1993). It is known that heavy metals can replace essential metal ions or make complexes with protein components, and eventually such changes result in a decreasing enzymatic activity (Горбунова и Терехова, 1995). It was indicated that Zn at 0.2–0.4 μ/l decreased activity of peroxidase, aldolase and acidic phosphatase of *Aspergillus* (Martino et al., 2000). In this experiment the most evident growth inhibition of Zn was observed on media with rather easily assimilated C sources (starch and skim milk), whereas a lower effect of Zn was manifested on the use of such substrata as chitin and cellulose which were less available in a Zn-free medium. Additionally, these complex compounds are able to chelate metals and thus reduce toxicity in the medium (Martin, 1972). Differences in reaction to Zn of individual fungi on various substrata could be related to their biology – production of organic acids or other extracellular components which are known to chelate heavy metals and in this way to

Table 2. **Ratio "substratum-Zn"/"control (glucose)-Zn", showing fungal growth on a medium with the particular C source versus fungal growth on glucose-containing medium**

Fungi	Starch	Skim milk	Leather	Sunflower oil	Cellulose	Chitin
P. funiculosum 60PL	0.90	0.84	1.95	1.41	1.72	1.5
P. italicum 170SL	1.04	1.07	1.54	1.37	1.24	1.06
P. expansum 14S	0.20	1.91	1.96	0.95	2.0	2.27
P. chrysogenum 24L	0.57	1.02	0.43	2.51	2.04	1.84
P. chrysogenum 125AL	0.53	0	0.62	1.98	2.17	1.74
P. velutinum P(3)3	2.41	0.49	1.0	1.95	3.05	2.27
P. oxalicum 41AL	0.12	1.05	0.90	1.20	1.67	1.45
P. aurantiogriseum 23L	0.77	1.46	1.71	1.71	20.2	1.85

reduce their toxicity (Gadd and Griffiths, 1978). The fungi tested could also differ in their binding abilities on cell wall and mechanisms of uptake/efflux and accumulation (Ross, 1975; Winkelmann, 1990). Thus, it could be supposed that Zn in the environment might suppress assimilation of natural substrata and the fungal reaction could differ depending on the origin of substrata and the properties of micromycetes involved in this process.

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CINKO POVEIKIS *Penicillium* Link. GENTIES GRYBŲ GEBĖJIMUI PASISAVINTI NATŪRALIUS C ŠALTINIUS

S a n t r a u k a

Tirtas cinko poveikis 8 *Penicillium* genties grybų gebėjimui asimiliuoti įvairius C šaltinius, dažnai aptinkamus gamtoje ir žmogaus aplinkoje (krakmolą, lieso pieno miltus, susmulkintą natūralią odą, saulėgrąžų aliejų, chitiną ir celiuliozę). Nustatyta, kad 12 mM cinko koncentracija slopino visų mikromicetų krakmolo ir pieno baltymų pasisavinimą. Mikromicetų augimas terpėse su šiais C šaltiniais ir cinku buvo įvertintas nuo 0 iki 72%, palyginus su jų augimu ant tų pačių substratų, bet be cinko. Mažiausias augimo slopinimas buvo nustatytas C šaltiniais naudojant chitiną ir celiuliozę – grybų kolonijų augimas 71–125%. Atspariausi cinko poveikiui grybai buvo *P. italicum* 170S (augimas 70–103%) ir *P. aurantiogriseum* (70–97%).