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THE INFLUENCE OF SELENITE ON FILLAMENTOUS FUNGI HYPHA MORPHOMETRY PARAMETERS

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Abstract

Selenium salts have been known for long time to have a potential for both beneficial and harmful effects on living organisms. It is present in the environment, where it can be readily assimilated by plants and fungi, thus entering the food chain. We investigated the cell growth dynamics in the presence of selenite which is considered to have more toxic potential than selenate. The effects of selenite (1 mM) on the growth of fungi from the activated spores to the end of the exponential growth were measured on several hypha morphological parameters by microscopy *in vivo*. *Phycomyces blaekesleneeanus* was used as model filamentous fungus. The most striking effect of Se^{+4} treatment was inhibition of hypha growth, resulting in more than four times shorter hypha in Se^{+4} –treatment group than in the control (200 ± 50 μm, n = 50 vs 900 ± 100 μm, n = 40 respectively) at the end of exponential growth period under controlled conditions. The Se^{+4} effect was an inhibition and not a simple delay in growth, as hypha length did not change significantly from 27^{th} to 30^{th} hour of culture in Se^{+4}-treatment group. Since the microscopy was performed on live cultured cells, undisturbed cytoplasmic streaming was observed, confirming that hyphae were alive at all time points measured. 30h old spore diameters were also significantly reduced by Se^{+4} treatment ($p = 0.0365$), while hypha diameters were not significantly altered.

Keywords: growth inhibition, *Phycomyces blakesleeanus*, live microscopy

1. Introduction

Selenium salts have been known for long time to have a potential for both beneficial and harmful effects on living organisms. Selenium is essential to many organisms, including some archaea, bacteria, protozoans, green algae and animals. It is present in the environment, where it can be readily assimilated by plants and fungi, thus entering the food chain. The narrow gap between necessary and toxic doses of Se [1], warrants the need for better understanding of the effects of Se salts on living organisms. Fungi are one of the main pathways for Se entrance into ecosystems, due to their intensive exchange with the extracellular milieu and very large surface to volume ratio. Se can be concentrated in their mycelia [2] or fungi can mediate Se concentration from the soil by stimulating its absorption by plant roots as mycorrhizal symbionts [3]. Moreover, fungi are, with rare exceptions, the only kingdom of life entirely devoid of genome encoded selenoproteins which are the basis of selenium beneficial effects in the organisms that do possess the machinery to synthesize them [4]. Therefore, filamentous fungi could be a very useful model system for research on harmful selenium effects.

We investigated the filamentous fungi cell growth dynamics in the presence of selenite. The effects

of selenite (1 mM) on the growth of fungi from the activated spores to the end of the exponential growth phase were measured on several hypha morphological parameters by microscopy *in vivo*. *Phycomyces blakesleeanus* was used as well-defined model for filamentous fungus with rapid growth, since it finishes the exponential growth stage by the 30th hour of culture in control conditions.

2. Material and Methods

The model organism was unicellular wild-type strain of the filamentous fungus *Phycomyces blakesleeanus* (Burgeff) (NRRL 1555(−)), grown in lighted stationary plates from the spore stock as previously described [5]. To observe the effect of treatment with 1 mM sodium selenite, the prepared fungi activated spore culture volume was divided into control culture and a treatment culture (same as control, with addition of sodium selenite in final concentration 1 mM). Live fungi were imaged unstained on the conventional bright field upright microscope, in six randomly chosen fields of view. The morphometric traits were quantified from obtained images using ImageJ software. The data are presented as mean ± SE, and the differences were tested by student t-test at a 95% confidence level. Correlation matrix Principal component analysis (PCA) was done in XLSTAT.

3. Results and Discussion

Morphometric analysis was based on the data extracted from the images like the one in Figure 1. There are numerous small bright and dark structures which were continuously in motion. This feature which was observed in all groups at all growth times imaged. Vigorous movement of cytoplasm and organelles, cytoplasmic streaming, is characteristic for all large cells and is a marker of viability, since in the conditions of ATP depletion the streaming stops.

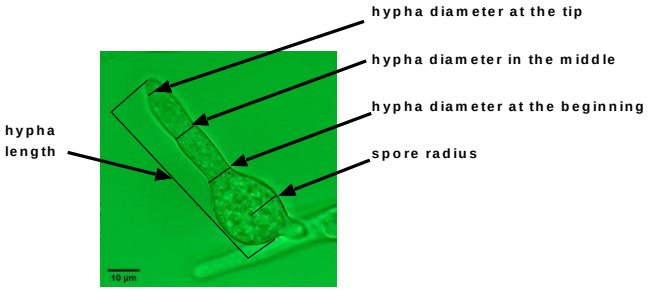

Figure 1. Representative brightfield image used for extraction of morphometric data. Hypha on the picture is from Se^{+4} treated culture (27 h old). The main features measured are marked with hair lines. The inherent variability of the fungi cultures that might obscure some differences between groups was controlled by growing all cultures to be compared from the same initial batch culture, by randomization of the samples and by a large number of hyphae analyzed (n_{total} = 255).

The most striking effect of Se^{+4} treatment was inhibition of hypha growth, with more than four times shorter hyphae in the Se^{+4} –treatment group than in the control (200 ± 50 µm, n = 50 *vs.* 900 ± 100 µm, n = 40 respectively) after 30 h of the beginning of the growth, which corresponded to the end of exponential growth in control conditions (Figure 2A., left panel).

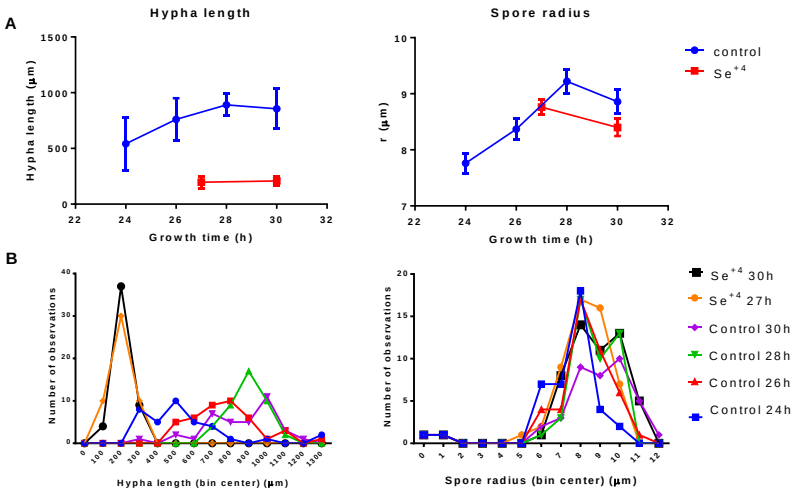

Figure 2. The effect of Se^{+4} on the most prominent hypha morphometric traits, hypha length and the size of the remaining spore "head" of the hypha. A. The hypha length (left panel) and spore radius (right panel) obtained for control and Se^{+4}–treatment group vs. time of growth. The data for Se^{+4}–treated fungi earlier than 27 h is lacking, because at that times the growing hyphae were very scarce. B. Histograms showing the distribution of all values obtained for hypha length (left panel) and spore radius (left panel).

Because hypha length did not increase significantly between 27 and 30 hour of culture in the Se^{+4}–treatment group (Figure 2A left), it seems that the Se^{+4} -mediated effect is an inhibition, and not a simple delay in growth followed by the unchanged speed of growth at later times. Undisturbed cytoplasmic streaming confirmed that hyphae were alive at all time points measured.

From distribution of lengths (Figure 2B left), it can be seen that in control group, "the spread" of the lengths distribution is very wide. In contrast, the Se^{+4} -treated hyphae are less diverse, and seem to be synchronized in growth. Spore size was slightly decreased by the Se^{+4} -treatment but values had similar distributions to controls (Figure 2B right panel). The statistical significance of spore radius decrease was reached with the 30 h old spores ($p = 0.0365$) (Figure 2A, right panel).

The diameters of the specific hypha regions, were not significantly changed by Se^{+4} -treatment (Figure 3A). The spore circumference was measured independently from radius, in case any deviations in shape are found. This parameter was deemed not useful, since it was not sensitive to any perturbation tested (data not shown).

In correlation matrix principal component analysis we used just three non-correlated variables: the hypha diameter at the middle, the hypha diameter at the tip and the spore circumference out of 6 measured. Scree plot showed that first three axes described entire variability and therefore they are retained for presentation. The 3-axis plot shows that the Se^{+4}-treated groups lump together on the opposite side of middle hypha diameter, meaning that it is negatively correlated with the Se^{+4}-treatment. Based on the result of PCA, new variable is defined, the ratio of middle to tip width (Figure 3C). New variable showed that changes in hypha width ratio are significant after Se^{+4} treatment (at 30 h, $p < 0.0001$), and that during growth in control conditions, the hypha width ratio gradually declines in the period of intensive elongation (until 28 h, as can be seen at Figure 2A). At the 30 h point, an abrupt increase in the ratio is evident.

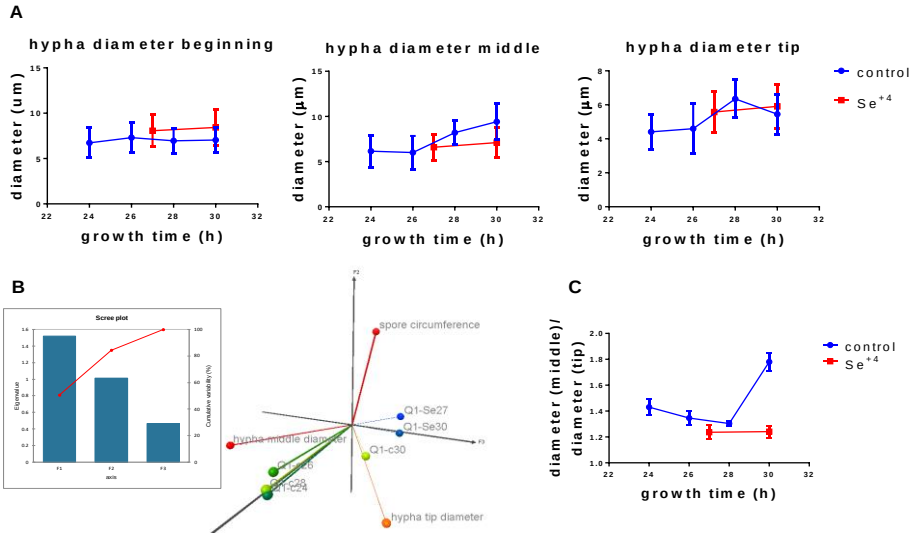

Figure 3. A. Diameters of hyphae measured (beginning, middle, tip) were seemingly unchanged by Se^{+4}. B. Principal Component Analysis (PCA) biplot. Se^{+4}-treated groups: blue symbols; Control groups: green symbols. On the left side, the Scree plot shows variability % explained by F1, F2 and F3. C. Newly generated compound parameter, the ratio of hypha diameter at the middle to hypha diameter at the tip, calculated for each measured hypha individually, plotted against growth time.

3. Conclusions

Hypha morphology can provide a range of useful information if randomly sampled from sister cultures (treatment and matched control), despite the notorious intragroup variability of hypha cultures. Selenite in concentration of 1 mM acted as an inhibitor of hypha elongation. Although diameters of hypha measured (beginning, middle, tip) were seemingly unchanged by Se^{+4}, PCA showed that that is not the case with hypha middle parameter which was negatively correlated with the Se^{+4}-treated groups. Based on the result of PCA, new variable is defined, the ratio of middle to tip width, that seems to be more informative about hypha physiology.

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