Abstract

Over past years, the fact that plants cannot be considered as independent entities has become evident. Similarly, as other multicellular organisms, plants are inhabited by various microorganisms, residing on the plants surface as well as inside plant tissues. These diverse taxonomically microorganisms are involved in many important physiological processes and functions, including plant nutrition, stress responses, resistance against abiotic and biotic stimuli. Thus, plant fitness, according to the holobiont theory, is a resultant of plant-microbial partnership. Fungal endophytes are widely distributed in most, if not all living plants and currently are under intensive studies due to their role in plant nutrition, resistance against biotic stress, e.g. pathogens and herbivores, tolerance to environmental pollution. In addition, endophytic fungi were shown to produce various secondary metabolites and also to affect secondary metabolites profile of the host plant.

On contrary to previous studies conducted in toxic metal (TM) polluted sites in southern Poland, where the role of mycorrhizal fungi in plant tolerance to heavy metals toxicity was comprehensively studied, the aim of this dissertation was to investigate the role of endophytic fungi in plant vegetation under heavy metals stress. Endophytic fungi are a taxonomically diverse group, among which are obligate and facultative symbionts and in contrast to mycorrhizal fungi, able to colonize host roots as well as above-ground parts. In the dissertation, fungal endophytes were isolated from *Arabidopsis arenosa*, collected from the post-mining waste dump. This plant belongs to Brassicaceae family, considered to be non-mycorrhizal, thus the investigations of the role of non-mycorrhizal fungal symbionts in plant tolerance to TM presence was of special interest. Apart from showing the diversity of the mycobiota of *Arabidopsis arenosa*, the results presented here shed new light on the principles of the plant-endophytic fungi symbiosis, the mode of plant tissues colonization by the fungus and the role of plant hormones -strigolactones as factors determining beneficial symbiosis (Fig.1).

In its natural environment *A. arenosa* was inhabited by 11 cultivable fungal endophyte strains. One of them, a fungus, closely related to *Mucor plumbeus* was chosen for detailed studies. The combined use of a GFP-tagged *Mucor* sp. strain and *q*PCR allowed for fungal detection and investigation of plant colonization dynamics. The role of strigolactones (SLs) in the shaping of beneficial plant-endophytic fungus interaction was explored with the use of SLs synthetic analog-GR24 and a close relative of *A. arenosa*, the model plant-*Arabidopsis thaliana*. *A. thaliana* mutants defective in SLs biosynthesis and signaling pathways were utilized to gain an understanding of the importance of SLs in the interaction between plants and the endophytic fungus *Mucor* sp.

The establishment of plant-fungal symbiosis is a complex process, that requires changes in the metabolism of the host plant as well as in fungal partner. Plant hormones seem to be important factors, involved in this cross-talk. The maintenance of a proper balance between growth-related traits and the plant defense mechanisms is crucial in terms of mutualisms or parasitism development. During the *Mucor* sp.-*A. thaliana* interaction SLs proved to be necessary for the mutualistic relationship development. On contrary to wild-type plants, SLs biosynthetic mutants inoculated with the fungus produced significantly less biomass. This negative growth response was accompanied by enhanced accumulation of salicylic acid (SA) and in consequence upregulated expression of defense-related genes, e.g. plant defensins. *Mucor* sp. was shown to be able to regulate SLs levels both by the phytohormones decomposition and by the downregulation of the expression of SLs biosynthesis genes. SLs were also shown to affect growth and metabolism of the fungal endophyte. The radial growth of *Mucor* sp. was shown to be inhibited upon GR24 treatment, while the respiration rate was found to be enhanced. Further, it was found that *Mucor* sp. growing on SLs supplemented medium produced more H_2O_2, also the activity of fungal antioxidant enzymes was affected, in a dose-dependent manner.

In recent years, endophytic fungi were shown to be important for the TM stress alleviation in the host plants, through the regulation of various aspects of plant metabolism including nutrient and water uptake, metal transport or redox balance. Ethylene (ET) was found to play an important role in plant response to TM and it was shown that, ET production and signaling may also be regulated by symbiotic microorganisms. Microarray analysis revealed, that in *A. arenosa* inoculated with the endophyte the ethylene biosynthesis and ET-related signaling pathways/effectors are upregulated. Results of our studies indicate that endophyte induced alterations in plant metal homeostasis, including altered metal distribution within the plant and the expression of metal transporter encoding genes may be a result of the altered ET homeostasis. *Mucor* sp. was also found to improve plant nutrition through stimulation of plant endogenous phosphorus starvation response and improve water uptake by the plant. The endophyte was also shown to affect the elongation of plant root hairs, thus increasing the volume of penetrated soil by the plant and nutrient uptake. A link between these processes and ET biosynthesis or/and signaling is also possible.

The understanding of plant-endophyte interaction and its significance for plant tolerance to TM requires complex knowledge about the metabolic changes, which occur in both symbiotic partners, the tolerance of endophyte to TM and also about the colonization dynamics. *Mucor* sp. was shown to stimulate host plant growth regardless of the toxic metal presence. The fungus was found to be tolerant to Cd, Pb and Zn. Further, it was also shown, that the endophyte improved nitrogen acquisition by the host plant. TM stress alleviation was manifested by a decrease in the activity of catalase in plants inoculated with the endophyte. Studies performed with the use of a GFP-labelled strain and *q*PCR revealed its presence in roots and in above-ground tissues as well. *Mucor* sp. was found inside vascular tissues, what suggest that the fungus reaches above-ground organs through the plant vascular system.

The analysis of the colonization dynamics revealed the transient decrease of fungal abundance on the fifth day after inoculation. This seems to stay in accordance with the transient upregulation of the expression of defense/stress-related genes in plant shoots at the beginning of the interaction when the balance between beneficial and non-beneficial traits is not yet attained.

The results presented here demonstrate the importance of endophytic fungi in plant tolerance to heavy metals toxicity and underline the significance of the understanding of mechanisms determining beneficial plant-endophytic fungi symbiosis establishment.

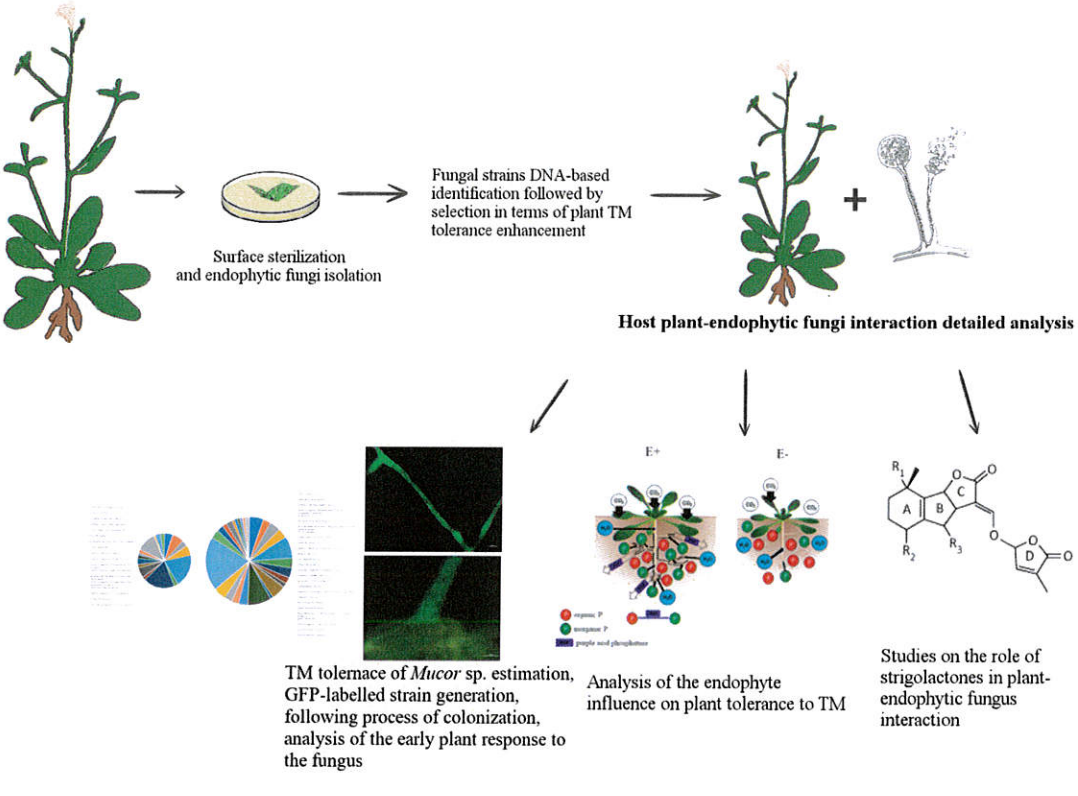

Fig.1. The research scheme of the conducted studies concerning the plant-endophytic fungi interaction in the context of plant survival in TM enriched habitats. *Arabidopsis arenosa* plants collected from post-mining waste were used for endophytic fungi isolation. Plant colonization was traced by the use of GFP-tagged *Mucor* strain and *q*PCR quantification. Detailed analysis of the fungal influence on plant physiology under TM stress was performed. The role of strigolactones in the symbiosis establishment was evaluated. GFP-green fluorescent protein; E+/E-plant inoculated with *Mucor* sp./uninoculated control plants