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Morphological and physiological characterisation and multigene phylogeny of the zygomycetous fungal order Mortierellales

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Mortierellales is one of the largest groups of Zygomycota. These fungi have practical importance as producers of polyunsaturated fatty acids, such as arachidonic acid, and as biotransforming agents of different organic compounds used in the pharmacological and chemical industries. Understanding the evolution of Mortierellales including the origin and evolutionary role of their enzymes may increase the biotechnological relevance of these fungi. Originally, this group was considered as a family of the order Mucorales. In the recent past, phylogeny of Zygomycetes had been analysed in detail revealing the need of the establishment of the new order Mortierellales. However, these works focused mainly on the Mucorales and the phylogenetic relationships within the Mortierellales remained unresolved. Molecular phylogenetic data suggest that the presently accepted, morphology-based taxonomy of the order is highly unnatural.

We inferred phylogenetic relationships of the Mortierellales using a combined matrix of nrITS, 5.8S, nrLSU, nrSSU, EF1-alpha and RPB1 sequences from 106 strains of *Mortierella*- and related taxa. PCR was carried out and the questioned sequences defined. Sequences were aligned by using the softwares MUSCLE and Probalign. The phylogenetic analyses were made by using Maximum Likelihood and Bayesian estimation. Phylogenetic trees were calculated on the strength of the sequences of each ribosomal subunit on its own and on a fourth, combined, large alignment including all of them. Results suggest that the genus *Mortierella* is paraphyletic and includes the genera *Gamsiella, Dissophora* and *Lobosporangium*. The relationships between the larger groups of the genus *Mortierella* also became clearer, as we found that *M. verticillata* and *M. humilis* are closely related to each other. The same conclusion goes to *M. gamsii* and *M. hyalina*. It seems that *G. multidivaricata* and *M. mutabilis* are in close relationship not only on a phylogenetic but also on morphological basis.

Morphological investigations were carried out using both light- and scanning electron microscopic techniques. Our goal was to reveal the fine structure of the observed characters, such as branching of the sporangiophores, ornaments of the sporangia and the mycelial structure. We found that phenotypic traits of these fungi strongly depend on the culturing conditions. We also investigated the carbon source utilization patterns of the fungi by using 67 different carbon sources. This research showed that delimitation of the species is difficult by using only morphological and/or physiological characters but merged with the phylogenetic results they improve the understanding of the relationships within this fungal group.

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Relationship between reactive oxygen (ROS) and reactive nitrogen species (RNS) and auxin in *Arabidopsis* development under copper excess

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Copper (Cu^{2+}) is an essential microelement but its excess influences the shoot and root architecture of plants. This heavy metal induces ROS production leading to oxidative stress condition. Moreover, alterations of nitric oxide (NO) levels can also be detected, which plays a role e.g. in cell death induction. Based on these, the aim of this study was to investigate the morphological and physiological responses and the possible relationship between ROS and RNS during short-term (7-day-long) and longer (17-day-old) copper exposure in the root tips of *Arabidopsis* using microscopic methods.

For the experiments (*Col-0*, WT), NO-overproducer (*nox1*), NO-deficient mutants (*nia1nia2*, *nia1nia2noa1-2*) and the S-nitrosoglutathione reductase (GSNOR)- deficient *gsnor1-3* plants were used. Also *Arabidopsis* plants with low (*vtc2-1* and *vtc2-3*) and high ascorbate content (*miox4*) were treated with 0, 5, 25 and 50 μM $CuSO_4$.

During short-term treatments, Cu^{2+} at a concentration of 50μM resulted in a large reduction in cotyledon area and hypocotyl and primary root lengths, accompanied by an increase in auxin levels. In cotyledons, a low Cu^{2+} concentration promoted NO accumulation, which was arrested by nitric oxide synthase or nitrate reductase inhibitors. The 5μM Cu^{2+}- induced NO synthesis was not detectable in *nia1nia2* or *nia1nia2noa1-2* plants. In roots, Cu^{2+} caused a decrease of the NO level, which was not associated with superoxide and peroxynitrite formation. Inhibition of auxin transport resulted in an increase in NO levels, while exogenous application of an NO donor reduced the auxin-dependent DR5::GUS expression. The elongation processes of *nox1* were not sensitive to Cu^{2+}, but NO-deficient plants showed diverse growth responses.

Copper excess caused the inhibition of stem and root growth of 17-day-old *Arabidopsis*, during which cell elongation, division and expansion were also modified. The symptoms of stress induced morphogenic response (SIMR) were found in the root system of 25 μM

Cu^{2+}- treated plants. In both organs, the decrease of auxin-dependent gene expression was found, which can partly explain the growth inhibitions.

In plant organs, Cu^{2+} treatment results in severe morphological responses during which the endogenous hormonal balance and signal transduction are affected. Auxin and NO negatively regulate each other's level and NO intensifies the metal-induced cotyledon expansion, but mitigates elongation processes under short-term Cu^{2+} exposure. Besides hormonal system, nitric oxide metabolism was also influenced by copper. In the root tips, this heavy metal excess induced NO generation, while NO content in lateral roots was not affected by the treatments. Using *nia1nia2* mutants, nitrate reductase enzyme as a putative source of Cu^{2+}-induced NO was identified in *Arabidopsis* primary roots.

Moreover, ROS levels were also influenced by copper. Under copper treatment, NO might play a protective role by regulating ROS levels possibly through modulation of the antioxidant activity.

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Studies on the cellular functions of newly discovered Prion family protein Shadoo

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The SPRN gene encodes the Shadoo glycoprotein (Sho), a central nervous system-expressed member of the prion protein super family. Sho is highly conserved from fish to mammals. SPRN is conserved in mammals, as is the prion gene PRNP, but in sheep SPRN and PRNP are both marked by polymorphic variation, suggestive of a shared selection pressure within these scrapie disease-prone livestock animals. In rodent models of prion disease there are reduced levels of Sho in infected tissues, defining a form of cross-regulation between full-length Sho holoprotein and PrP^{Sc}. The similarities between Sho and PrP N-terminus are the natively unfolded nature of polypeptide chains, a hydrophobic domain and tandem repeats with positively charged residues. Indeed, scrutiny of Sho's biochemical properties in uninfected cells has revealed overlaps with the properties of PrP^{C}, these including shared protein binding partners.

Prion protein functions as a metal binding protein because divalent cations such as copper, zinc and manganese can bind to the octapeptide repeat sequences in the N-terminus of PrP^{C}. Since the binding of these metals to the octapeptide has been proposed to influence both structural and functional properties of prion protein, alterations in transition metal levels can alter the course of the disease. As a member of the prion protein super family, we thought that Sho protein may behave like PrP as a metal binding protein, although it lacks the octapeptide region.

We carried out experiments on N2a cell lines stably expressing the Sho protein applying various concentrations of transition divalent metals. We could see the membrane internalisation of Sho protein induced by Co^{+2}, Mn^{+2} and Zn^{+2} ions. Also, we observed that the Sho expressing cells showed protection against the cytotoxic effects of Mn^{+2}.

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Identification and characterization of a novel circadian clock mutant in *Arabidopsis thaliana*

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The circadian clock is a biological timing mechanism that provides rhythmicity to gene expression, metabolism, and physiology with ~24h periodicity. The central oscillator of eukaryotic clocks is based on the network of clock genes and proteins, which are interconnected by transcriptional/translational negative feed-back loops.

Current models of the plant circadian clock postulate three interlocked feedback loops. A pair of single Myb-domain transcription factors, *CIRCADIAN CLOCK ASSOCIATED1 (CCA1)* and *LATE ELONGATED HYPOCOTYL (LHY)*, plays central roles in two loops. In one loop, *CCA1* and *LHY* repress the expression of the Pseudo-Response Regulator gene *TIMING OF CAB EXPRESSION 1 (TOC1)*. *TOC1* closes the first loop by inducing *CCA1* and *LHY* transcription for the next cycle. In a second loop, *PRR7* and *PRR9*, are induced by *CCA1* and *LHY*. *CCA1* and *LHY* are subsequently repressed by *PRR7* and *PRR9*. In a third loop, *GIGANTEA (GI)* and, possibly, *PRR5* are positive regulators of *TOC1*. *GI* is negatively regulated by both *CCA1/LHY* and *TOC1*.