PRELIMINARY EVIDENCES FOR THE EXISTANCE OF THE Ca^{++}-NO-cGMP PATHWAY DURING THE SPORULATION OF THE CHYTRIDIOMYCETE *Blastocladiella emersonii*

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The sporulation phase of the aquatic fungus Blastocladiella emersonii can be induced by nutrient starvation at any time during vegetative growth and is dependent on extracellular Ca^{++}. This developmental stage shows a large number of morphological changes, which require the synthesis of many new materials. The present work reports nitric oxide (NO) production as well as its participation in signal transduction pathways during sporulation phase of B. emersonii. The sporulation was delayed by L-NAME suggesting NO production by an enzymatic mechanism from L-arginine via nitric oxide synthase. Confocal microscopy experiments using 10 mM L-NAME and 100 µM DAF-DA showed a remarkable decrease in NO production. The reagent 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) inhibited the sporulation completely when added up to 90 minutes after starvation suggesting that NO acts as a first messenger in cGMP production. In fact, our microarray experiment showed an increase of guanylyl cyclase mRNA levels after the first hour of starvation. Northern blot experiments for the genes encoding a Nitric Oxide Synthase-like protein and a GTP cyclohydrolase during the sporulation showed that both mRNA levels are high already in the beginning of the sporulation and decrease considerably after 120 minutes of starvation. Intracellular concentration of nitrite, nitrate and other nitric oxide derived products were measured using a Nitric Oxide Analyzer. This is the first evidence for the existance of the Ca^{++}-NO-cGMP pathway in a chytridiomycete.
Key words: Calcium, nitric oxide, cGMP and Blastocladiella emersonii
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