Morphological and physiological changes in carbon starving cultures of G protein mutant *Aspergillus nidulans* strains

Thesis of Ph.D. dissertation

Molnár Zsolt

University of Debrecen

Faculty of Sciences

Debrecen, 2007.

1. Introduction

The differentiation and cell death processes of fungi got into the focus of the mycological research in the last few years. Not surprisingly because: The fungi have great economical, industrial, medical and ecological magnitude. The better understanding of the life cycle of fungi would lead us to new strategy, which can revolutionize the protection against fungal infections and biotechnological applications, then again we can obtain valuable information about the cell differentiation of higher eukaryotes through the investigation of fungi.

Many interesting results come to light about the function of the fungal G protein signaling pathways in the last few years. These signaling pathways very often have crucial role in the life cycle of fungi. They play role in maintaining the vegetative growth, regulation of asexual and sexual reproduction, development of stress response (Lee *et al.*, 1994; Hicks *et al.*, 1997; Adams *et al.*, 1998; Rosen *et al.*, 1999; Chang *et al.*, 2004; Han *et al.*, 2004*a*, *b*; Lukov *et al.*, 2005; Yu, 2006), moreover they can play a part in the initialisation of apoptosis too (Leiter *et al.*, 2005).

Instead of *Aspergillus nidulans* is a popular model organism, we know a little about the morphological and physiological changes of carbon starving cultures, and the regulator mechanisms beyond them. The role of the G protein mediated signalling pathways in the regulation of metabolism of the autolysing, carbon-starving cultures is not clear. And although it has a practical importance, the regulation of the autolysis and apoptosis or rather their connection is unmapped.

The aim of my investigations was to get acquainted with the morphological and physiological changes and the autolytic and apoptotic processes of carbon starving cultures of the *Aspergillus nidulans*, an *Ascomycota* filamentous fungus, and the importance of the G protein mediated signaling pathways in the above procedures.

2. Materials and methods

We used the following strains in our experiments:

Code of the strain	**Genotype of the strain**
FGSC 26 **	*biA1, veA1*
FGSC 1035 *	*yA2, fadA^{G203R}*
RJH046 *	*argB2, biA1, pyroA4, veA1, Δflba::argB*
RKH51.117 ***	*pabaA1, yA2*
RKH51.09[b] ***	*pabaA1, yA2, ΔrgsA::argB^{+}*
RMdgB03 ***	*pabaA1, yA2, ΔargB::trpC^{+} ΔganB::argB^{+}, trpC801*
RKH52.02 ***	*pabaA1, yA2, ΔrgsA::argB^{+} ΔganB::argB^{+}*
FGSC 1079 **	*biA1, pabaA1, pyroA1, ΔbrlA, veA+*
FGSC 744 **	*fluG1, pabaA1, yA2*

During our investigation we used minimal medium or agar (Barrat e*t al.,* 1965) supplemented with yeast extract, and if it was necessary we added biotin, pyridoxin or para amino benzoic acid to the media. Every 100 ml of the culture media was inoculated with 50 million spores and was cultivated at 37 ^{o}C, 180 rpm.

After 30 hours we had started the cultivation 0,005 g/l D,L-dihydrosphingosine and 0,3 g/l phytosphingosine were added to the cultures in order to investigate the apoptosis.

For intracellular activity measurements filtered mycelia were washed by distilled water, re-suspended in ice-cold 0.1 M potassium phosphate buffer (pH 7.5) and frozen to –20 °C. Cell disruption was done with an X-press (AB Biox, Göteborg, Sweden), and the cell debris was separated by centrifugation at 10,000 g at 4 °C.

The enzyme activities (glucose-6-phosphate dehydrogenase (G6PD), NADP-specific isocitrate dehydrogenase (NADP-ID), catalase, superoxide dismutase (SOD), glutathion reductase (GR), glutathione peroxidase (GPx) – except γ-glutamil transpeptidase (γGT) activity – were measured by rate assay method (Roggenkamp *et al.,* 1974; Chiu *et al.,* 1976, Pinto *et al*., 1984; Oberley *et al.,* 1984; Bruinenberg *et* al., 1983; Emri *et al.,* 1994).

The reaction compound contained the followings during γGT measurement: 20 mmol/l glycin-glycin, 1 mmol/l γ-glutamil-*p*-nitroanilide and 50% (v/v) sample. The reaction samples

were centrifuged after 1 hour incubation on RT, and the amount of disengaged *p*-nitroanilide was measured by the changes of the absorbance (Emri *et al.,* 1997).

The protein content of the samples was determined with the Folin reaction modified by Peterson (1983).

In order to define the glutathion (GSH) and glutathion-disulfide (GSSG) content of the cells, the cell disruption was carried out by 5% (w/v) 5'-sulfosalicic acid.

To measure the oxidized glutathion content (GSSG) of the cells we used Anderson's „rate assay" method (1985). The reduction of DTNB was tracked photometrically on 412 nm. The GSH content of the sample was depleted by treatment with 2-vinilpiridin (Emri *et al.,* 1997).

The determination of the GSH content was carried out using Anderson's method (1985) too, in such a way that the whole glutathion content (GSH+GSSG) was measured (without 2-vinilpiridin treatment) and in view of the GSSG concentration we were able to calculate the GSH content (Emri *et al.,* 1997).

The cells were disrupted with the method described before at the GSH/GSSG concentration determination during the measurement of the superoxide and peroxide content of the cells.

The superoxide and peroxide production of the cells was investigated by adding dihydroethidium and 2',7'-dichlorofluorescein-diacetate to the cultures and measuring the generated ethidium (Et) or 2',7'-dichlorofluorescein (DCF) concentration with fluorimeter (Carter *et al.,* 1994).

The determination of the glucose content of the media was carried out by Leary's "rate assay" method (1992).

The ammonia concentration of the media was determined by OP-NH3-7113-S type ammonia-sensitive electrode (Pusztahelyi *et al.,* 1997).

The viability of the cultures was characterized with their MTT reduction ability. The mycelia were re-suspended in fresh media containing 5 mg/ml MTT (Metil thiazol tetrazolium) and shaken for 4 hours on 37 °C, then, the amount of the generated MTT formazan was determined photometrically (Lee *et al.,* 1999).

The respiration of the cultures was determined with OXY 320 type polarographic oxygen electrode. The measurement of the alternative respiration was carried out in the presence of KCN, which inhibits the cytochrome dependent pathway, and the remaining activity was regarded the alternative respiration (Bahr and Bonner, 1973).

To determine the dry cell mass (DCM) 5 ml of the culture was filtered through sintered glass filter and it was dried to consistence weight. The filtrate was used in chitinase and proteinase activity determination (Pusztahelyi *et al.,* 1997).

Chitinase activities were measured with carboxymethyl-chitin-remazol brilliant violet (Loewe Biochimica, Sauerlach, Germany) substrate from culture filtrates or soluble intracellular samples (Emri *et al.,* 2004).

Azocasein was the substrate in the extracellular proteinase activity determination. The amount of the disengaged colourful product was measured photometrically (Pusztahelyi *et al.,* 1997).

The morphology of hyphae, pellets and cells was investigated with a OLYMPUS BH-2 microscope equipped with SPlan 20NH phases contrast objective, and with a Carl Zeiss Labovar4 phases contrast microscope (Sipiczki *et al.,* 1998).

To investigate changes in the morphology of nuclei, mycelia were collected, re-suspended in 70 v/v % ethanol for brief fixation and permeabilisation, were stained with DAPI (4',6'-diamidino-2-phenylindole) solution and observed under epifluorescence microscope (Moreno *et al.,* 1991).

The phosphatidylserine exposure on protoplast (Tilburn and munkatársai, 1983; Vágvölgyi and Ferenczy, 1991) surface was determined by Annexin V assay using Vybrant Apoptosis Assay Kit (Molecular Probes, Eugene, OR, USA) (Leiter *et al.,* 2005). The protocol also included propidium iodide staining for filtering necrotic cells, and only Annexin V positive but propidium iodide negative protoplasts were regarded as "apoptotic".

To identify cells undergoing DNA fragmentation, protoplasts were subjected to Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End Labelling (TUNEL) using the APO-BrdU™ TUNEL Assay Kit (Molecular Probes, Eugene, OR, USA) according to the manufacturer's instructions. Nucleus-containing protoplasts were counted using DAPI staining, and ratios of TUNEL positive and DAPI positive protoplasts were calculated (Mousavi *et al.*, 2003; Leiter *et al.,* 2005).

For RT-PCR RNA was extracted from frozen, lyophilized mycelial mats by TRISOL treatment (Invitrogen, Lofer, Austria) as recommended by Chomczynski (1993). The total RNA was DNase treated and only then used for RT-PCR. The RNA concentration was determined spectrophotometrically (λ=280/260). The RNA was run in 1 % agarose gel for quality checking.

RT-PCR reactions employed QuantiTect™SYBR®Green RT-PCR Kit (Qiagen, Hilden, Germany) and were carried out according to the manufacturer recommendations with 400 ng of RNA per reaction, 2.5 mM of Mg^{2+} and 0.5 μM gene specific primers.

The following oligonucleotide PCR primers were employed:

7. *catA* F: 5'-CAA ACG CTC CGC CAT CTA C-3'
   R: 5'-CTT GAG GTG CCC GAA TGT C-3'
8. *catB* F: 5'-CCG AGC CCG ACA ACA CTT AC-3'
   R: 5'-GTT CAG CGA CGA CAA TGA CG-3'
9. *catC* F: 5'-CAG AGC AAG CCG AGA AGT TC-3'
   R: 5'-CAA GGT GGG AGG GAG AGA AG-3'
10. *prtA* F: 5'-TTC TGT CCG TCA AGG TTT TC-3'
    R: 5'-TGA AGG CGT AAG AGT ATC CAC-3'
11. *prtB* F: 5'-GCT TGA ATC TCC TCT GTT TGC-3'
    R: 5'-GTC CAA CCA CCG TAG AAG AAG-3'
12. *chiB* R: 5'-CGGGACGAAGGATCATACG-3'
    F: 5'-TGGTCACCAGGCGAATCTC-3'

To check the homogeneity of the RT-PCR products, the PCR products were subjected to agarose gel-electrophoresis and the melting temperatures were also determined.

3. Results

Pellets were observed in the cultures of wild strains of the *Aspergillus nidulans* after stationary growth phase. Longer or shorter hypha fragments had appeared and later converted into “yeast-like” cells in the cultures of wild strains, similarly experienced in *Penicillium chrysogenum*. These “yeast-like” cells composed a living part of the culture.

Forasmuch the morphological changes observed in *Aspergillus nidulans* cultures are general and can be experienced in other filamentous fungi, like wild strains of *Penicillium chrysogenum* and other *Penicillium* and *Aspergillus* species, the large amount of the “yeast-like” cells, which were produced by the industrial *Penicillium* strain, can be a side effect of the selective breeding.

The autolysis could be characterized, among others, by the induction of the extracellular hydrolytic enzymes. The increase in the chitinase production correlates with the decrease of the biomass and the fragmentation of the pellets and hyphae. The product of the *chiB* gene is responsible for the main part of the chitinase activity in autolysing cultures.

The increase of the ammonia concentration and proteinase activity shows that cells used amino acids and amino-carbohydrates as energy source after depletion of glucose fund. The changes of the proteinase production could be a result of the altered *prtA* expression.

We found no correlation between the proteinase activity and the autolysis of the cultures. It can be interpreted by the involvement of the hydrolytic enzyme in the mobilization of nutrients.

The induction of the hydrolytic enzyme production is an important evidence for the idea that the autolysing cultures generate *de novo* the needful enzymes.

The rate of the cyan-resistant respiration had decreased during the stationary and early autolytic phase then it increased again in the late autolytic phase. It shows that there are great changes in the mitochondria, which is the most important free radical generating cell organ.

The G6PD activity decreased steadily, while another NADPH generating enzyme, the NADP-ID production increased. It seems that the role of pentose-phosphate cycle in NADPH production is markedly reduced, while the role of the NADP-ID grows in the aging cultures.

After the ROS accumulation an increase was observed in the CuZn- and Mn-superoxide dismutase, as well as GPx and catalase activity. The *CatB* could stand beyond the changes of the catalase production in autolysing cultures. Remarkable redox changes take place in carbon starving, aging cultures.

Fast decrease of the GSH and GSSG concentrations are observable in autolysing *Aspergllus nidulans* cultures. The shrinkage of the GSH/GSSG ratio was not caused by the oxidation of GSH or infiltration of GSH into glutathione S-conjugates. The reason of the decrement in the GSH concentration can be: 1. mobilization of GSH by γGT, 2. acceleration of the amino acid transport through the cell and vacualar membranes.

We observed apoptotic-like cell death during the stationary and early autolytic phase, which was escorted by decreased biomass and viability.

FadA^{G203R}, *ΔflbA* together with *ΔganB*, *ΔrgsA* and *ΔganBΔrgsA* mutations did not caused significant changes in the measure of autolysis. Although the effect of FadA and GanB pathways on autolysis was found negligible, the FadA pathways caused numerous changes in the morphology of the fungus and both signaling pathways substantially influenced the physiology.

Mutations of these G-protein pathways generated remarkable changes in the protein production. The fluctuation of expression of *prtA* gene stands at least partially in the transcriptional background of the mentioned changes.

Our findings and other result with *fadA*, *sfaD* (Gβ) and *gpgA* (Gγ) deletion mutants suggest that the FadA pathway directly takes a part in the regulation of extracellular proteinases, while the GanB pathway controls them indirectly through Gβγ subunits.

The γGT activity changes of the mutant strains differed notably from the measured activity at the control strains. The changes of γGT activity were barely traceable or not traceable in the intracellular GSH and GSSG concentration changes. The joint SfaD-GpgA complex, namely the Gβγ subunit, migth activate the γ-glutamil transpeptidase production.

The catalase production decreased slower in the *ΔrgsA* strains cultures than in the control strains, while the *ΔganBΔrgsA* strain showed transient behaviour. The *ΔrgsA* protein expresses its effect on the catalase production, at least partially, through the inhibition of *catB* expression.

The GPx activity was lower in the culture of *ΔrgsA* mutant strain, while in the cultures of the other mutants it was higher than in the control stains. The *ΔflbA* mutation decreased, the *fadA*^{G203R} increased the GPx production. The two RGS proteins induce, while the Gα subunits inhibits the GPx production. The accumulation of free radicals instead of the altered antioxidant enzyme levels, did not change. The antioxidant mechanisms are proven under a G-protein mediated regulation.

The *brlA* and *fluG* mutants showed non-autolytic phenotype. The FluG pathway is indispensable not only for the iniciation of the sporulation but for the start of the autolysis. One of the main importance of the autolysis is to liberate nutrients through degrading one part of the cells. High proteinase production was shown by the *ΔbrlA* mutant, which is related with the connection between the FluG and FadA pathways.

The investigated non-autolytic phenotype is profitable in the industrial point of view and supports the idea, that the hypha fragmentation is an active, enzymatic process and not only a side effect of the shaking and stirring.

Instead of the non-autolytic phenotype the *fluG* and *ΔbrlA* strains have almost the same cell death rate and viability as the control strains. Sphingosine derivatives induced the apoptosis in aging *Aspergillus nidulans* cultures. The sphingosine treatment, used during the stacionary phase, although it doubled the number of the apoptotic cells, had no effect on the autolytic markers. There are two processes in autolytic cultures: 1. cell death, 2. breakdown of dead cell materials.

4. Summary

Most of the mycological investigations dealt with the studying of growing, exponential phase cultures. Only a few studies examine the morphological and physiological changes of the carbon starving cultures and the regulation that lies behind. This is surprising, because the starving of carbon sources is one of the most common stress situations both in the nature and in industrial conditions. Many enzyme and metabolite, which have great practical importance, are produced in carbon starving conditions; moreover the better understanding of the cell death processes occur during carbon starvation can lead us results which can be useful in the protection against fungi. In the center of my investigations was the study of the carbon starving cultures of *Aspergillus nidulans*, as model organism, and the better understanding of the morphology, physiology and cell death processes of the carbon starving cultures. During my work the role of the heterotrimer G protein mediated pathways in carbon starving cultures, had great accent.

The morphology of the *Aspergillus nidulans* cultures was very altered from the industrial *Penicillium chrysogenum* strain, examined former at our department (Pusztahelyi *et al.,* 1997; Pócsi *et al.,* 2007). The pellets disintegrated slowly and the cultures preserved their filamentous morphology after the carbon source had depleted at *Aspergillus nidulans*. Although the "yeast-like" cells, observed at *Penicillium chrysogenum*, appeared but they never became dominant. The changes observed at *Aspergillus nidulans* can be considered general by the literature (Pócsi *et al.*, 2007), while the specific morphology noticed at *Penicillium chrysogenum* is probably a side effect of the industrial breeding.

Instead of the essential morphological differences the physiological changes observed in carbon starving cultures (ROS accumulation, antioxidant enzyme production, induction of apoptotic and autolytic processes, extracellular hydrolase production) were similar to the noticed at *Penicillium chrysogenum* (Sámi *et al.*, 2001). Some elements of the phenomena observed by us were demonstrated at other fungi (Munkers and Minssen, 1976; Dufur *et al.,* 2000; Jakubowski *et al.,* 2000; Gyetvai *et al.,* 2006). All these investigations suggest us that well regulated, complex physiological changes occur in carbon starving fungi cultures. That's why the carbon starving cultures can not considered as mass of declining, necrotic cells, but we can speak about cultures which can actively defend themselves against stress situation and they are able to survive it (Winderickx *et al.,* 2003).

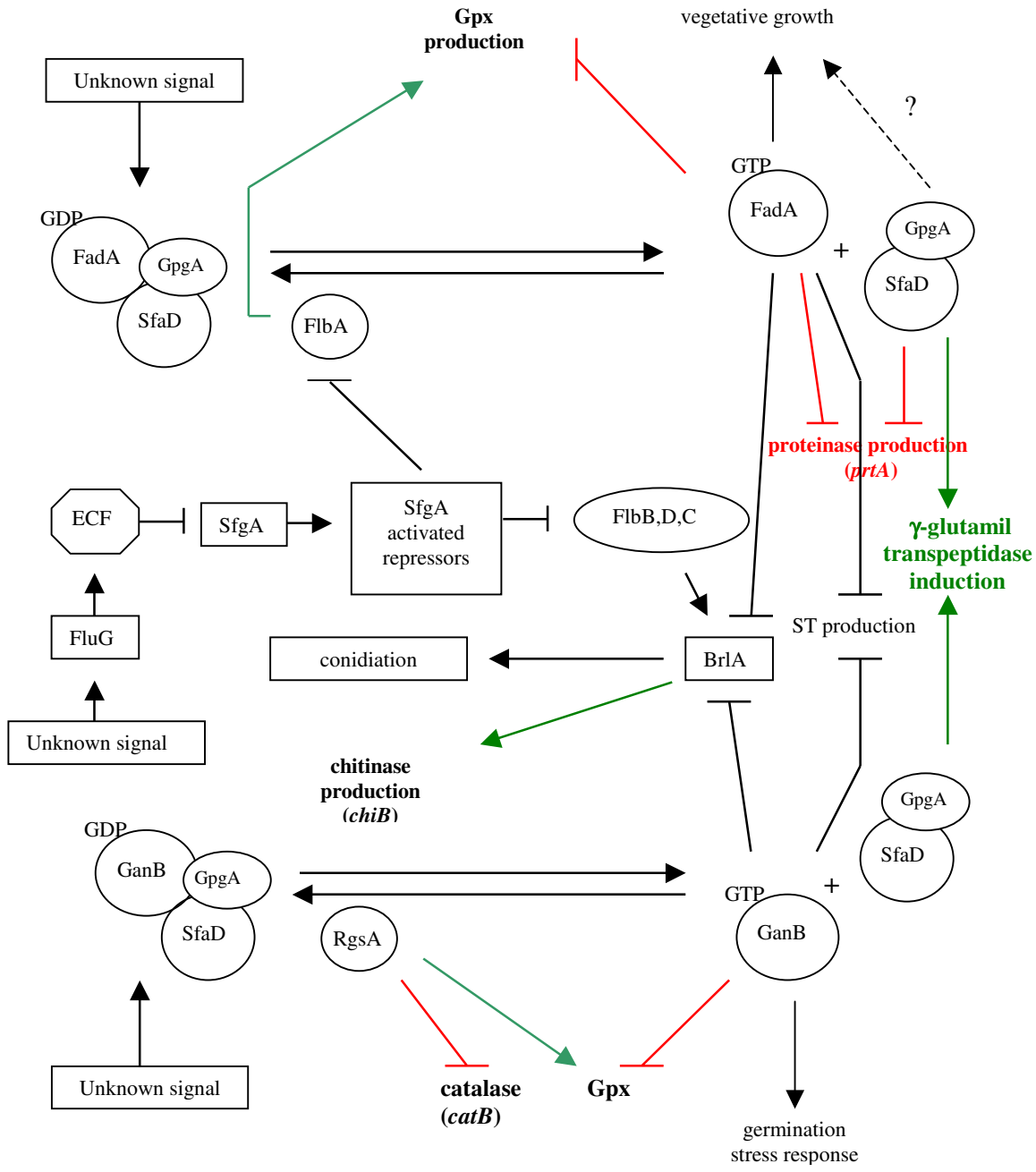
Based on our investigations the FadA/FlbA and GanB/RgsA pathways not only effected an the germinating spores and the physiology of growing hyphae, but the operation of the carbon starving cultures as well (Adams *et al.*, 1998; Chang *et al.*, 2004; Lafon *et al.*, 2005). Their regulative role was significant in the production of extracellular proteinase (*prtA*), in the induction of γGT and GSH degradation, furthermore via the production of some antioxidant enzyme – GPx, catalase (*catB*) – in the creation of ROS. Surprisingly the above heterotrimeric G protein mediated pathways did not influence notably the autolysis of the cultures.

Instead of the FadA/FlbA and GanB/RgsA pathways the FluG-BrlA pathways responsible for the sporulation (Adams *et al.*, 1988; Lee and Adams, 1994*b*, 1996; D'Souza *et al.*, 2001) were essential in the regulation of the autolysis. These pathways influenced the decrease of the DCM, the production of extracellular chitinase (*chiB*) and the fragmentation of hyphae (pellet disintegration, creation of "yeast-like" cells) too. Based on our experiments the autolysis and the sporulation are linked closely together, and regulated by the FluG-BrlA pathway. One of the main physiological importance of the autolysis in carbon starving cultures is probably that it liberates nutrients through the degradation of some cells, which can be used by the autolysing cells to produce conidia after the depletion of the carbon source. As the result of this procedure even the whole vegetative biomass can be utilized for conidia creation. The fungus can reach new productive areas via the conidia, which is a propagator formation. The conidia as persistent formation can ensure survive of the colony after the depletion of the nutrients.

Surprisingly the mutations of the fluG-BlrA pathway did not influence nor the expression of the apoptotic markers neither the cell death, even the induction of the apoptosis was not followed by increased autolysis. According these we could not find close relationship between the expression of the autolytic phenotype, the decrease in the vitality of the cultures and the apoptotic processes. This means in the same time, that the cell death is not caused by the autolyis (expression of autolytic phenotype) in carbon starving cultures, but it mobilizes the materials of the dead cells. Two procedures welter in the autolysing cultures: 1. the destruction of the cells (the decrease of the vitality of the cultures, the expression of the apoptotic markers), 2. disassembly of the materials of the dead cells (expression of autolytic phenotype). The importance of the different regulation of the cell death and cell wall degradation is unknown. A possible explanation is connected to the morphology of the fungus: the remarkable part of the materials of the dead cells does not flow away thanks to the cell wall, but remains easily accessible for the neighbour, living cells. In this way the cell

death is not accompanied necessarily by cell wall disintegration and the fungus can reuse its substance more effectively.

My examinations led me to several results, which are worth to go over in practical aspects: I have demonstrated, that the hydrolyse enzymes are produced *de novo* during the autolysis, namely over production of these enzymes can be generated by the induction the autolysis in the industry. I have proved through the phenotype of the *fluG1* and *ΔbrlA* strains, that the fragmentation of the hyphae is an active process and not a consequence of the mechanical shearing forces caused by the stirring and shaking. Namely the morphology of the cultures can be manipulated by the regulation of the activity of the adequate cell wall degrading enzymes. The phenotype of the *fluG1* mutant is a good example to that how we can create an industrially beneficial strain with the inactivation of only one gene: The cultures, which are stable for long time and keep their intact pellet morphology after depletion of the carbon sources, remain well shakeable and efficiently filterable even when they are old. The lack of the proteinase enzyme production can hinder the proteolytic degradation of the product at the same time.



The role of the G protein mediated and the FluG-BrlA mediated pathways in the life cycle and metabolism of *Aspergillus nidulans* (Seo *et al.*, 2006; Yu, 2006).

Irodalomjegyzék / References

Adams, T. H., Wieser, J. K. and Yu, J. H. 1998. Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol. Biol. Rev., 62, 35-54.

Adams. T. H., Boylan, M. T. and Timberlake, W. E. 1988 *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. Cell., 29;353-62.

Chang, M. H., Chea, K. S., Han, D. M. and Jahng, K. Y. 2004. The GanB Gα-protein negatively regulates asexual sporulation and plays a positive role in conidial germination in *Aspergillus nidulans*. Genetics., 167, 1305-15.

Bahr, J.T. and Bonner, W.D. Jr. 1973. Cyanide-insensitive respiration. I. The steady states of skunk cabbage spadix and bean hypocotyl mitochondria. J. Biol. Chem., 248, 3441-3445.

Barratt, R. W., Johnson, G. B. and Ogata, W. N. 1965. Wild-type and mutant stocks of *Aspergillus nidulans*. Genetics, 52, 233-246.

Bruinenberg, P. M., Van Dijken, J. P. and Scheffers, W. A. 1983. An enzymic analysis of NADPH production and consumption in *Candida utilis*. J. Gen. Microbiol., 129, 965-971.

Carter, W.O, Narayanan, P.K. and Robinson J.P. 1994 Intracellular hydrogen peroxide and superoxide anion detection in endothelial cells. J. Leukoc. Biol., 55, 253-258.

Chiu, D.T.Y., Stults, F. H. and Tappel, A. L. 1976. Purification and properties of rat lung soluble glutathione peroxidase. Biochem. Biophys., Acta, 445, 558-566.

Chomczynski, P. 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques. 15, 532-4, 536-7.

D'Souza, C. A., Lee, B. N. and Adams, T. H. 2001. Characterization of the role of the FluG protein in asexual development of *Aspergillus nidulans*. Genetics. 158, 1027-36.

Dufour, E., Boulay, J., Rincheval, V. and Sainsard-Chanet, A. 2000. A causal link between respiration and senescence in *Podospora anserina*. Proc. Natl. Acad. Sci. USA., 97,4138-43.

Emri, T., Bartók, G. and Szentirmai, A. 1994. Regulation of specific activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in *Penicillium chrysogenum*. FEMS Microbiol. Lett., 117, 67-70.

Emri, T., Pócsi, I. and Szentirmai, A. 1997. Glutathione metabolism and protection against oxidative stress caused by peroxides in *Penicillium chrysogenum*. Free Rad. Biol. Med., 23, 809-814.

Emri, T., Molnár, Z., Pusztahelyi, T. and Pocsi, I. 2004*a*. Physiological and morphological changes in autolyzing *Aspergillus nidulans* cultures. Folia Microbiol., 49, 277-284.

Gyetvai, A., Emri, T., Takacs, K., Dergez, T., Fekete, A., Pesti, M., Pocsi, I. and Lenkey, B. 2006. Lovastatin possesses a fungistatic effect against *Candida albicans*, but does not trigger apoptosis in this opportunistic human pathogen. FEMS Yeast Res., 6(8),1140-8.

Han, K. H., Seo, J. A. and Yu. J. H. 2004a. A putative G-protein-coupled receptor negatively controls sexual developmentin *Aspergillus nidulans*. Mol. Microbiol., 51, 1333-1345.

Han, K. H., Soe, J. A. and Yu J. H. 2004b. Regulators of G-protein signalling in *Aspergillus nidulans*: RgsA downregulates stress response and stimulates asexual sporulation through attenuation of GanB (Gα) signalling. Mol. Microbiol., 53, 529-540.

Hicks, J. K., Yu, J. H., Keller, N.P. and Adams, T. H. 1997. *Aspergillus* sporulation and mycotoxin production both require inactivation of the FadA Gα protein-dependent signaling pathway. EMBO J., 16, 4916-23.

Jakubowski. W., Bilinski, T. and Bartosz, G. 2000. Oxidative stress during aging of stationary cultures of the yeast *Saccharomyces cerevisiae*. Free Radic. Biol. Med., 28, 659-64.

Lafon, A., Seo, J. A., Han, K. J., Yu, J. H. and D'enfert, C. 2005. The heterotrimeric G-protein GanB(α)-SfaD(β)-GpgA(γ) is a carbon source sensor involved in early cAMP-dependent germination in *Aspergillus nidulans*. Genetics, 171, 71-80.

Leary, N. O., Pembroke, A. and Duggan, P. F. 1992. Improving accuracy of glucose oxidase procedure for glucose determinations on discrete analyzers. Clin. Chem., 38**,** 298-302.

Lee, B. N. and Adams, T. H. 1994b. The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal and is related to prokaryotic glutamine synthetase I. Genes Dev., 8, 641-51.

Lee, B. N. and Adams. T. H. 1996. *FluG* and *flbA* function interdependently to initiate conidiophore development in *Aspergillus nidulans* through *brlA* beta activation. EMBO J. 15, 299-309.

Lee, D. G., Shin, S. Y., Maeng, C. Y., Jin, Z. Z., Kim, K. L. and Hahm, K. S. 1999. Isolation and characterisation of a novel antifungal peptide from *Aspergillus niger*. Biochem. Biophys. Res. Comm., 263, 646-651.

Leiter, E., Szappanos, H., Oberparleiter, C., Kaiserer, L., Csernoch, L., Pusztahelyi, T., Emri, T., Pócsi, I., Salvenmoser, W. Marx, F. 2005. The antifungal protein PAF severly affects the integrity of the plasma of *Aspergillus nidulans* and induces an apoptosis-like phenotype. Antimicrob. Agents Chemother., 49, 2445-2453.

Lukov, G. L., Hu, T., Mclaughlin, J. N., Hamm, H. E. and Willardson, B.M. 2005. Phosducin-like protein acts as a molecular chaperone for G-protein βγ dimer assembly. EMBO J., 24, 1965-1975.

Monreno, S., Klar, A. and Nurse, P. 1991. Molecular genetics analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol., 194, 795-823.

Mousavi, S. A. and Robson, G.D. 2003 Entry into the stationary phase is associated with a rapid loss of viability and an apoptotic like phenotype in the opportunistic pathogen *Aspergillus fumigatus*. Fungal Genet. Biol., 39, 221-229.

Munkres, K. D., and M. Minssen. 1976. Aging of *Neurospora crassa*. I. Evidence for the free radical theory of aging from studies of a natural death mutant. Mech. Aging Dev., 5, 79-98.

Oberley, L. W. and Spitz, D. R. 1984. Assay of superoxide dismutase activity in tumour tissue. Method. Enzymol., 105, 457-464.

Peterson, G. L. 1983. Determination of total protein. Method. Enzymol., 91, 86-105.

Pinto, M. C., Mata, A. M. and López-Barea, J. 1984. Reversible inactivation of *Saccharomyces cerevisiae* glutathione reductase under reducing conditions. Archives of Biochem. Biophys., 228, 1-12.

Pócsi, I., Molnár, Zs., Pusztahelyi, T., Varecza, Z. and Emri, T. 2007. Yeast-like cell formation and glutathione metabolism in autolysing cultures of *Penicillium chrysogenum*. ACTA biol. Hung. (in press).

Pusztahelyi, T., Pócsi, I., Kozma, J. and Szentirmai, A. 1997*a*. Aging of *Penicillium chrysogenum* cultures under carbon starvation: I. morphological changes and secondary metabolite production. Biotechnol. Appl. Biochem., 25, 81-86.

Roggenkamp, R., Sahm, H. and Wagner, F. 1974. Microbial assimilation of methanol induction and function of catalase in *Candida boidinii.* FEBS Lett., 41, 283-286.

Rosen, S., Yu, J. H. and Adams, T. H. 1999. The *Aspergillus nidulans sfaD* gene encodes a G-protein beta subunit that is required for normal growth and repression of sporulation. EMBO J., 18, 5592-600.

Sámi, L., Emri, T. and Pócsi I. 2001*a*. Autolysis and ageing of *Penicillium chrysogenum* cultures under carbon starvation: glutathione metabolism and formation of reactive oxygen species. Mycol. Res., 105, 1246-1250.

Seo, J. A. and Yu, J. H. 2006. The phosducin-like protein PhnA is required for Gbetagamma-mediated signaling for vegetative growth, developmental control, and toxin biosynthesis in *Aspergillus nidulans*. Eukaryot Cell., 5, 400-10.

Sipiczki, M., Takeo, K. and Grallert, A. 1998. Growth polarity transitions in a dimorphic fission yeast. Microbiology 144, 3475-85.

Tilburn, J., Scazzocchio, C., Taylor, G. G., Zabicky-Zissman, J. H., Lockington, R. A. and Davies, R. W. 1983. Transformation by integration in *Aspergillus nidulans*. Gene, 26, 205–211.

Vágvölgyi, Cs. and Ferenczy, L. 1991. Isolation of nuclei from *Aspergillus nidulans* protoplasts. FEMS Microbiol. Lett., 66, 247-251.

Winderickx, J., Holsbeeks, I., Lagatie, O., Giots, F., Thevelein, J. and de Winde H. 2003. From feast to famine; adaptation to nutrient availability in yeast. In: Yeast stress responses, Edd.: Hohman, S. and Mager, W.H., Springer-Verlag Berlin Heidelberg, Germany pp.:305-387.

Yu, J. H. 2006. Heterotrimeric G-protein signaling and RGSs in *Aspergillus nidulans*. J. Microbiol., 44, 145-54.

A tézisek alapjául szolgáló tudományos munkák / Publications relevant in the thesis

Közlemények/Publications:

1. Emri. T., Oláh. B., Sámi. L., **Molnár. Zs.**, Nagy. M., Pusztahelyi. T. and Pócsi. I. (2002) Investigation of glutathion metabolism in filamentous fungi. *Acta Microbiol et Immunol. Hung*. 49, 267-276. ***(IF: -)***

2. **Molnár, Z**., Mészáros, E., Szilágyi, Zs., Rosén, S., Emri, T., and Pócsi, I. (2004) Influence of *fadA* and *flbA* mutations on the morphology and physiology of submerged *Aspergillus nidulans* cultures. *Applied Biochemistry and Biotechnology*, 118(1-3):349-60. ***(IF: 0,907)***

3. Emri, T., **Molnar, Z.**, Pusztahelyi, T. and Pócsi, I. (2004) Physiological and morphological changes in ageing *Aspergillus nidulans* cultures. *Folia Microbiologica.* 49(3):277-84. ***(IF: 1,034)***

4. Emri, T., **Molnar, Z.**, Pusztahelyi, T., Varecza, Z. and Pócsi, I. (2005) The *fluG*-BrlA pathway contributes to the initialisation of autolysis in submerged *Aspergillus nidulans* cultures. *Mycol Res*. 109(Pt 7):757-63. ***(IF: 1,572)***

5. Emri, T., **Molnar, Z.** and Pócsi, I. (2005) The appearances of autolytic and apoptotic markers are concomitant but differently regulated in carbon-starving *Aspergillus nidulans* cultures. *FEMS Microbiol Lett*. 251(2):297-303. ***(IF: 2,057)***

6. Molnár, Z., Emri, T., Zavaczki, E., Pusztahelyi, T. and Pócsi I. (2006) Effects of mutations in the GanB/RgsA G protein mediated signalling on the autolysis of *Aspergillus nidulans*. *J. Basic Microbiol.* 46(6):495-503. ***(IF: 1,000)***

7. Pusztahelyi, T., **Molnár, Z.**, Emri, T., Klement, É., Miskei, M., Kerékgyártó, J., Balla, I. and Pócsi, I. (2006) Comparative studies on differential expression of chitinolytic enzymes encoded by *chiA*, *chiB*, *chiC* and *nagA* genes in *Aspergillus nidulans*. *Folia Microbiol.* 51(6):547-54. ***(IF: 1,059)***

8. Pócsi, I., **Molnár, Zs**., Pusztahelyi, T., Varecza, Z. and Emri, T. (2007) Yeast-like cell formation and glutathione metabolism in autolysing cultures of *Penicillium chrysogenum*. *ACTA biol. Hung*. in press

9. Emri, T., **Molnár, Zs.**, Szilágyi, M. and Pócsi, I. (2008) Regulation of autolysis in *Aspergillus nidulans*. *Appl. Biochem. Biotechnol*. közlésre benyújtva

Előadások és poszterek/Presentations and posters:

1. **Molnár, Zs.**, Emri, T. and Pócsi. I. Molnár, Zs., Emri, T. és Pócsi, I. Az *Aspergillus nidulans* autolízisét kísérő morfológiai és redox változások vizsgálata. II. Magyar Mikológiai Konferencia, Szeged 2002. (a poszter elnyerte a "rendezvény legjobb posztere címet")

2. Emri, T., **Molnár, Zs.** és Pócsi, I. Morfológiai és fiziológiai változások vizsgálata öregedő *Aspergillus nidulans* tenyészetekben. A Magyar Mikrobiológiai Társaság 19. Nagygyűlése, Balatonfüred 2002.

3. Emri, T., **Molnár, Zs.** and Pócsi, I. Morphological and physiological changes in ageing *Aspergillus nidulans* cultures. VIII. International Fungal Biology Conference, Guanajuato, Mexico, 2002.

4. Pócsi, I., **Molnár, Zs.**, Mészáros, E. and Emri, T. Influence of *fadA* and *flbA* mutations on the morphology and physiology of submerged *Aspergillus nidulans* cultures. International Conference on Emerging Frontiers at the Interface of Chemistry and Biology, Trivandrum, India 2003. (a poszter elnyerte a "rendezvény legjobb posztere címet")

5. Emri, T., **Molnár, Zs.**, Pusztahelyi, T., Leiter, É. and Pócsi, I. Az autolízis és az apoptózis kapcsolatának vizsgálata *Aspergillus nidulans* fonalas gombákban. A Magyar Mikrobiológiai Társaság 21. Nagygyűlése, Keszthely 2004.

6. Emri, T., Pusztahelyi, T., **Molnár, Zs.** és Pócsi, I. A FluG fehérje nélkülözhetetlen az *Aspergillus nidulans* autolízisének iniciálásában. A Magyar Mikrobiológiai Társaság 21. Nagygyűlése, Keszthely 2004.

7. Pusztahelyi, T., Emri, T., **Molnár, Zs.**, Balla, J. és Pócsi, I. *Aspergillus nidulans* kitináz gének expressziójának változása szénlimitált öregedő tenyészetekben és oxidatív stressz hatására. A Magyar Mikrobiológiai Társaság 21. Nagygyűlése, Keszthely 2004.

8. Pusztahelyi T., Emri, T., **Molnár, Zs.** és Pócsi, I. *Aspergillus nidulans* hidrolitikus enzimek génexpressziójának változása szénlimitált öregedő tenyészetekben és oxidatív stressz hatására. A Magyar Mikrobiológiai Társaság 21. Nagygyűlése, Keszthely 2004.

9. Varecza, Z., Emri, T., **Molnár, Zs.**, Pusztahelyi, T. és Pócsi, I. Élesztőszerű sejtek képződése és glutation metabolizmus *Penicillium chrysogenum* és *Aspergillus nidulans* gombákban. A Magyar Mikrobiológiai Társaság 21. Nagygyűlése, Keszthely 2004.

10. **Molnár Zs.**, Zavaczki E., Dudás G., Emri T. és Pócsi I. A G-protein mediált jelátviteli útvonalak szerepe az *Aspergillus nidulans* autolízisének szabályozásában. A Magyar Mikológiai Társaság 3. Nagygyűlése, Mátraháza 2005.

11. Emri, T., **Molnár, Zs.**, Veres, T., Pusztahelyi, T. and Pócsi, I. (2005) Az autolízis és a sporuláció szénforrás-függő szabályozása az *Aspergillus nidulans* fonalas gombában III. Magyar Mikológiai Konferencia, Mátraháza 2005

12. Pusztahelyi, T., **Molnár, Zs.**, Kelemen, Zs., Emri, T. and Pócsi, I. (2005) A *chiB* gén expresszióját a BrlA transzkripciós faktor szabályozza az *Aspergillus nidulans* fonalas gombában. III. Magyar Mikológiai Konferencia, Mátraháza 2005.

13. Pusztahelyi, T., **Molnár, Zs.**, Kelemen, Zs., Emri, T. and Pócsi, I. (2005) A *chiB* gén expresszióját a BrlA transzkripciós faktor szabályozza az *Aspergillus nidulans* fonalas gombában. III. Magyar Mikológiai Konferencia, Mátraháza 2005

14. **Molnár, Zs.**, Zavaczki, E., Dudás, G., Emri, T. and Pócsi, Involvement of G protein mediated signalling pathways in the regulation of autolysis in *Aspergillus nidulans*. 16th International Congress of the Hungarian Society for Microbiology, Keszhely 2005.

15. Pócsi, I., **Molnár, Zs.** and Emri, T. Regulation of autolysis in *Aspergillus nidulans*. 8th European Conference on Fungal Genetics. Austria, Vienna 2006

A tézisekhez nem kapcsolódó tudományos munkák / Other Publications:

Közlemények/Publications:

1. Emri, T., **Molnar, Z.**, Pusztahelyi, T., Rosén, S. and Pócsi, I. (2004) Effect of vitamin E on the autolysis and sporulation of *Aspergillus nidulans*. *Applied Biochemistry and Biotechnology*, 118(1-3):337-48. ***(IF: 0,907)***

2. Emri, T., **Molnar, Z.**, Veres, T., Pusztahelyi, T., Dudas, G. and Pocsi I. (2006) Glucose repression of autolysis and conidiogenesis in *Emericella nidulans*. *Mycol. Res.* 110(Pt 10):1172-8. ***(IF: 1,572)***

Előadások és poszterek/Presentations and posters:

1. Pócsi, I., **Molnár, Zs.** and Emri, T. Effect of vitamin E on the autolysis and sporulation of *Aspergillus nidulans*. International Conference on Emerging Frontiers at the Interface of Chemistry and Biology, Trivandrum, India 2003.

2. Emri, T., **Molnár, Zs.**, Pócsi, I. Effect of vitamin E and menadion on the autolysis and sporulation of *Aspergilus nidulans* .14th International Congress of the Hungarian Society for Microbiology, Balatonfüred 2003.

3. **Molnár Zs.**, Emri T., Veres T., Pusztahelyi T., Dudás G. és Pócsi I. A 2-dezoxi-D-glükóz és az E-vitamin hatása *Aspergillus nidulans* tenyészetek autolízisére és sporulációjára. A Magyar Mikrobiológiai társaság 15. Nagygyűlése, Keszhely 2004.