REGULATION OF FRUITING BODY FORMATION IN *COPRINOPSIS CINEREA*

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ABSTRACT

Fruiting body formation in the edible dung fungus *Coprinopsis cinerea* is regulated by environmental cues (nutrients, temperature, light, humidity), physiological conditions (mycelial status, age) and genetic control elements (e.g. *A* and *B* mating type genes, *dst1* and *dst2* genes for light regulators). Fruiting body development consists of a series of defined steps occurring in a concerted process over seven days. Following hyphal aggregation, stipe and cap tissues differentiate under the control of light and dark phases. Once light-induced karyogamy takes place in basidia present at the surface of the gills in the cap of mature primordia, stipe elongation and cap expansion start parallel to meiosis in the basidia and subsequent basidiospore formation. All these processes are expected to appoint numerous intracellular as well as extracellular protein functions, many of which might be specific to steps in fruiting. Since the genome of the fungus is available, identification of proteins can now be addressed by large scale proteomic techniques. In order to gain an insight into participating proteins from a first analysed developmental step, we collected immature fruiting bodies after meiosis II. Stipe and caps were separated from each other, and intra- and extracellular proteins from both types of samples were isolated and compared by 1D- and 2D-gel electrophoresis. Whilst there might be contaminating intracellular proteins in the extracellular fractions, there are many proteins not present in the intracellular fractions. Stipe and cap fractions clearly differed from each other, less so in the intracellular and strongly in the extracellular proteome.

Keywords: Secretome; Intracellular proteome, Meiosis II, Rapid stipe elongation, Cap expansion

INTRODUCTION

Coprinopsis cinerea is a saprotrophic fungus, which in nature grows on dung [1, 2]. It can also easily grow on simple artificial media based on e.g. malt extract [3]. *C. cinerea* can readily fructify under laboratory conditions and thus serves as a model basidiomycete for fruiting body development [4]. Although it is quite easy to obtain *C. cinerea* fruiting bodies, this fungus in general is considered to be of little edible value because of the fast autolysis of the cap at maturity. However, *C. cinerea* is cultivated as a speciality in small farming businesses in Thailand and it is consumed by workers on sisal and sugar cane farms in certain African countries and Sri Lanka, where the species fruits abundantly on plant waste heaps [5].

Fruiting body development occurs on the dikaryon, or on specific self-compatible mutants with defects in the mating type loci (Fig. 1), under the control of environmental signals (depletion of nutrients, temperature of 25-28 °C, a light/dark regime following the typical day/night rhythm, humidity >85%), physiological conditions (dikaryotic mycelial status, young

age) and endogenous regulators (master regulators, i.e. the *A* mating type transcription factors and the *B* mating type pheromone-pheromone receptor system, and also putative light regulators encoded by *dst1* and *dst2*). On fully established fresh vegetative mycelium growing on artificial medium (YMG/T; [6]), fruiting body development starts in the dark in the form of intense localized hyphal branching resulting in loose hyphal aggregates about 0.03 mm in size (primary hyphal knots). On receipt of a light signal, the primary hyphal knots aggregate firmly into small round bodies called secondary hyphal knots [7]. After another light signal and a 12 h dark period, i.e. on day 2 of development, differentiation of cap and stipe tissues becomes evident within the now approximately 0.5-1 mm oblong-shaped structures, the outer appearance of which is still relatively homogenous (primordia stage 1). Primordia maturation occurs during the subsequent three days under the control of alternating light and dark conditions (stages 2 to 4). On day 3, the stage 2 primordia are egg-shaped, 1-2 mm in size and clearly demarcated into cap and stipe. At this time, gills develop. With progressing development, the growing primordia reach 2-3 mm on day 4 (stage 3) and contain primary as well as secondary gills with specific hymenial cells defined (probasidia, cystidia). One day later, primordia are 3-6 mm in size with the gills and hymenia fully developed (mature primordia). These structures correspond to the stage when, according to Lu [8], karyogamy and DNA replication in the pre-meiotic S-phase is induced. At this stage, the shaggy outer veil on the cap starts to loosen from the lowest part of the stipe. The bulging cap is slightly pink in colour. After another dark period, the primordia (now 5-7 mm) extend during the following 12 h of day 6 by further cap expansion and the commencement of slow stipe elongation. Early in the morning of that day, synchronized karyogamy occurs, followed some hours later by meiosis, spore formation and eventually pigmentation which will darken the colour of the cap. The spores mature and stain dark brown during the first 6 h in the dark. In parallel, stipes elongate rapidly and the further expanding caps of the maturing fruiting body open promptly like an umbrella in order to release the ripened spores. In the first hours after fruiting body maturation, spores are discharged from the basidia freely into the air. Auto-digestion of the fruiting body pileus starts simultaneously with this spore release. In the early hours of the next light period, the cap autolyses completely, releasing all the remaining spores in droplets of auto-digested fruiting body fluid [4].

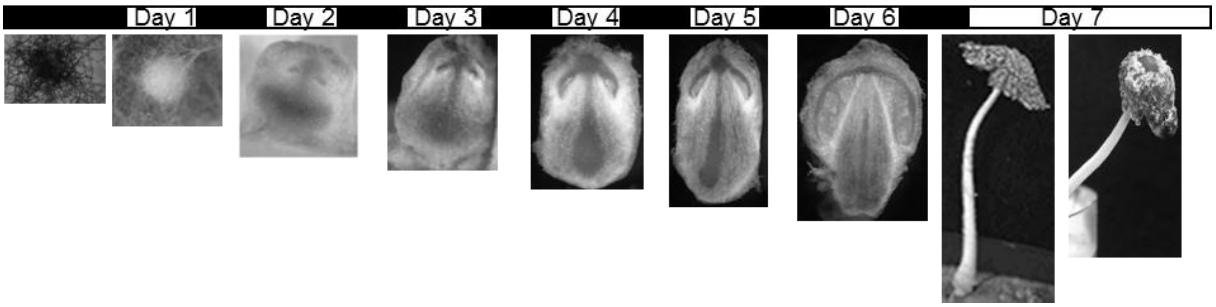

Figure 1: Fruiting body development of the self-compatible *C. cinerea* homokaryon AmutBmut on YMG/T complete medium under standard fruiting conditions
Standard fruiting conditions are defined in [6]. Photos from left to right: primary and secondary hyphal knots, primordium differentiation (stages 1 to 4), mature primordium at karyogamy, mature (~5 cm tall) and autolysing fruiting bodies. Dark blocks in the horizontal bar indicate night phases (12 h darkness), white blocks indicate daylight phases (12 h light); actual days are defined according to the start of the corresponding light period (modified from [5]).

The complexity and rapidity of the fruiting process suggest the occurrence of massive physiological and metabolic changes in the different tissues of the developing structures. To give some examples, in the first stages of the process, new combinations of cellular activities appear.

Correlated with karyogamy, there is massive synchronized DNA replication, while structural reorganisation of the chromosomes, DNA recombination and chromosome migration processes are connected to the two nuclear divisions in meiosis. Also, during cap expansion and stipe elongation, substantial cell wall stretching and restructuring takes place [4]. Multiple sets of proteins are expected to contribute to the different events.

Proteomics providing detailed descriptions of the structure, function and control of complex mixtures of proteins in a determined biological system [9] is a very useful tool for understanding complex molecular processes. The objective of this work is to characterize for the first time the proteome linked to *C. cinerea* fruiting body development. In this study, we chose to analyse the developmental phase directly after meiosis II at the beginning of spore formation, rapid stipe elongation and opening of the cap as a stage at which we expect manifold changes in the overall protein compositions. Thereby, we consider cap and stipe and their intra- and extracellular proteomes separately.

MATERIALS AND METHODS

Strain and culture conditions. *Coprinopsis cinerea* strain AmutBmut (*A43mut, B43mut, pab-1*) is a self-compatible homokaryon that, due to mutations in the *A* and *B* mating type genes, produces fruiting bodies without mating to another compatible monokaryon [10,11]. Stocks were cultivated on YMG/T agar plates (4 g yeast extract, 10 g malt extract, 4 g glucose, 10 mg tryptophan, and 10 g agar per l) for 5 days at 37 ^{O}C in black boxes with wet tissues to create a humid atmosphere [6,12]). About 10 small agar pieces with fresh mycelium were used to inoculate sterile fresh horse dung (about 80 g) in 1 l jars. Cultures were incubated at 37 ^{O}C for 4-5 days until the mycelium fully covered the substrate. For fruiting, cultures were transferred into a climate chamber at a chamber temperature of 25 ^{O}C (i.e. 28 ^{O}C directly on the cultures due to temperature effects caused by the light sources [6]) and 80-90% humidity in a 12 h light/12 h dark cycle. Cultures and development of fruiting structures were observed daily.

Primordia dissection. Primordia were dissected with the help of a razor blade and a needle under a stereo microscope (Stemi 200-C, Zeiss, Göttingen, Germany). For protein analysis, rhizomorphs (basal tissues for anchoring the stipes in the substrate), cap and stipe tissues were separated and directly frozen in liquid nitrogen and stored at 20 ^{O}C until further use. For microscopy, gills were taken from the caps with fine forceps and squashed on glass slides and nuclei in the basidia were stained with DAPI (4′,6-diamidine-2-phenylindole dihydrochloride; Boehringer Mannheim, Germany) following the procedure of Polak et al. [13]. Microscopy of tissues was performed with a Zeiss Axiophot microscope. A computer-linked Soft Imaging ColorView II Mega Pixel digital camera was used for photography and images were analyzed with the AnalySIS® software program (Soft Imaging System, Germany).

Protein isolation. Frozen tissue samples were freeze-dried and mildly ground to a powder using a mortar and pestle. Sample powders in portions of 0.5 g in 10 ml of Tris-HCl buffer, pH 7.5, containing 2% Tween 80 and 1 mM PMSF (phenylmethanesulfonyl fluoride) were incubated at room temperature for 30-40 min with gentle shaking and then centrifuged at 4,000 g at 4 ^{O}C for 20 min. Supernatants with extracellular proteins (and possible intracellular contaminations) from two successive extractions were collected and stored at -20 ^{O}C for later analysis. The remaining mycelial debris were frozen overnight at -20 ^{O}C and freeze-dried. To break all the cells, the dried material was ground three times for 3 min in a horizontally oscillating mill under liquid nitrogen cooling (Mixer Mill 2000, Retsch, Haan, Germany) at an amplitude setting of 80. The broken cells were washed twice with cell breaking buffer (CBB; 20 mM Tris-base [pH 7.5], 200 mM

NaCl, 2 mM EDTA, 0.01 % Tween 80, 1 mM PMSF), centrifuged for 20 min at 650 g at 4 ^{O}C, stored overnight at -20 ^{O}C and then suspended in 10 ml CBB buffer. To separate the cell wall fraction from the intracellular material, the solution was bedded onto 15 ml of 30% sorbitol and centrifuged at 650 g for 20 min. The supernatant with the intracellular proteins was stored at -20 ^{O}C until further use. The remaining pellet was washed with CBB and subsequently with distilled water, frozen at -20 ^{O}C and freeze-dried. Cell wall proteins were extracted by boiling 4x for 30 min 10 mg samples with 1 ml SDS-extraction buffer (60 mM Tris-base, pH 7.5, 50 mM EDTA, 2% SDS, 0.5% 2-mercaptoethanol, 1 mM PMSF) and centrifugation for 10 min at 3,200 g and 4 ^{O}C. The four extracts were pooled together and stored for subsequent gel electrophoresis.

Proteins in solution were precipitated by adding 10% TCA (v/v) from a TCA stock solution (100 g TCA in 45.4 ml water), incubation overnight at 4 ^{O}C on ice and centrifugation at 1,700 g for 20 min at 4 ^{O}C. Pellets were washed 5x with each 10 ml 80% acetone/ 20% 50 mM Tris-buffer, pH 7.5 and centrifuged 20 min at 4,000 g at 4 ^{O}C. Finally, the samples were washed with 10 ml acetone for 30 min at -20 ^{O}C, air dried, and stored at -20 ^{O}C. For gel electrophoresis, samples were re-suspended in 200-300 µl of rehydration buffer [8 M urea, 2% CHAPS, 18 mM DTT, 0.5% buffer for IPG (immobilized pH gradient) (pH 3-10) (Amersham Biosciences, Freiburg, Germany) and centrifuged for 15 min at 4 ^{O}C and 4,000 g. Protein extraction was repeated 3x and protein concentrations of the combined supernatants were determined using a Coomassie Plus-Bradford assay kit (Pierce, Rockford, USA) with bovine serum albumin (BSA) as the standard. All assays were performed in triplicate.

Protein gel electrophoresis. SDS-PAGE was performed with 12% resolving gel and 4% stacking gel as described previously [14]. A protein aliquot of 10-20 µg per sample well was mixed with 30 µl of the loading buffer (0.5 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 18 mM DTT and traces of bromophenol blue) and heated at 95 ^{O}C for 5 min. Upon loading, gel separation was performed at a constant current of 15 mA until the samples reached the resolving gel and continued at 30 mA for further migration of proteins. After the run, gels were fixed in 40% ethanol/10% acetic acid/50% H_2O overnight and subsequently stained with RuBP [ruthenium (II) Tris (bathophenanthroline disulfate)] as described previously [15]. Stained gels were scanned using an FLA-5100 fluorescent reader (Fujifilm, Düsseldorf, Germany). The gels were further stained in colloidal Coomassie solution (10% phosphoric acid, 10% $(NH_4)_2SO_4$ and 0.12% Coomassie Brilliant Blue G250; Serva Electrophoresis GmbH, Heidelberg, Germany) in 20% methanol. The gels were washed several times with water until an optimal contrast between bands and background was reached and then scanned (TMA 1600 scanner, Microtek, Willich, Germany).

For 2D-gel electrophoresis, protein samples were dissolved in loading buffer [8 M urea, 2% CHAPS, 18 mM DTT, 0.5% IPG buffer (pH 3-10), and traces of bromophenol blue]. Aliquots (350 µl) of sample solution containing approximately 100 µg protein were loaded onto a 18 cm IPG-strip, pH range 3-10 (Amersham Biosciences) in ceramic holders, rehydrated and focused using an Ettan IPGphor (Amersham Biosciences) with the following settings: 20 V for 10 h; 150 V for 1 h; 200 V for 1 h, 500 V for 2 h, 1000 V for 1 Vh; gradient 4000 V for 2 h; gradient 8000 V for 1 h; 8000 V for 60,000 Vh). Afterwards, the strips were incubated twice for 10 min in 4 ml of equilibration buffer (6 M urea, 2% SDS, 30% glycerol, 50 mM Tris-base, pH 8.8 and traces of bromophenol blue) containing 20 mM DTT in the first step and 200 mM iodoacetamide in the second. The proteins were then separated in the second dimension on a 12% SDS-PAGE using an Ettan DALTsix electrophoresis chamber (Amersham Biosciences) together with a protein marker 14-116 kDa (Fermentas, St. Leon-Rot, Germany). After separation of the proteins, first for 30 min at 120-130 V and then for 5 h at 300 V, the gels were fixed in 40% ethanol/10% acetic acid/50% dH_2O and stained with RuBP and Coomassie

Brilliant Blue G250 as described above. Prominent protein bands of Coomassie-stained gels were cut with a glass Pasteur pipette and gel pieces were stored at -20 ^{O}C for future analysis.

RESULTS AND DISCUSSION

Material requirements. For 1D- and 2D-gel electrophoresis about 10-20 µg and 100 µg protein, respectively is required for loading. From vegetative mycelium of *C. cinerea* cultures, our laboratory found that the fungus may secrete up to 0.2 µg protein/mg dry biomass and produces up to 0.7 µg intracellular protein/mg dry biomass [16]. In order to estimate how many immature fruiting bodies were needed to be collected, we first separately collected caps and stipes of 60 immature fruiting bodies from YMG/T plates [from day 6 of development (Fig. 1) after 10 h of illumination]. On average, caps and stipes had wet weights of 16 mg and 32 mg, respectively. The corresponding dry weight was about 7-11%. Based on our experience with vegetative mycelium, this corresponded to between 1.5-2.4 µg intracellular protein. We observed that structures of the same age on horse dung are 2-3x times larger and 9-18x heavier than on YMG/T plates, which helped to collect enough material (~10x less mushrooms) for the isolation of protein in amounts sufficient for electrophoretic analysis.

Fruiting body development on horse dung. Fruiting body development on horse dung was observed over time. The process on the natural substrate followed the same time schedule as established previously on YMG/T medium (shown in Fig. 1) but the developing structures were generally larger. Stage 4 primordia (day 5 of development) were 4-6 mm in size, and 9-11 mm at karyogamy on the morning of the next day (Fig. 2). The course of further events over day 6 for fruiting body maturation were defined for the target to best identify the wanted stage directly after meiosis II. Immature fruiting bodies were harvested on day 6 at different times after the light was switched on. Over this time, structures grew into sizes up to about 4 cm in length by both stipe elongation and cap expansion. The colour of the caps changed from pinkish in the first 8 h of illumination to light grey at later times (9 and 12 h of illumination).

When karyogamy was completed after one hour on day 6, the veil and the gills in the now 9-11 mm sized primordia were still connected throughout to the stipes although all the connections were already somewhat loosened which made it easy to separate caps and stipes from each other. Already at this time, a hollow lumen developed throughout the stipes. At hour 3 of day 6, veil connections of the outer edge of the caps separated from the lowest part of the slowly stretching stipes but the lamella were still connected to the stipes over their whole length. At this time, the length of the caps was still longer than the length of free part of the stipes. Separation of gills from the stipes began after about 4 h of illumination at the outer end of the gills and moved up with time to the tip of the stipes. At 10 h of illumination, about one half to two thirds of the length of the still extending gills was free from the hollow stipes. The lamellae were white throughout without any brown colouration typical of spore maturation and staining (Fig. 2). Nuclear staining of basidia was performed at one hour intervals between hours 7 and 10 of day 6. At hour 9, >60% of basidia had completed meiosis II and 100% at hour 10 (Fig. 3). The first sterigmata were seen on some basidia in the 9 h samples whereas, in 10 h samples, most basidia had sterigmata and, in some cases, budding

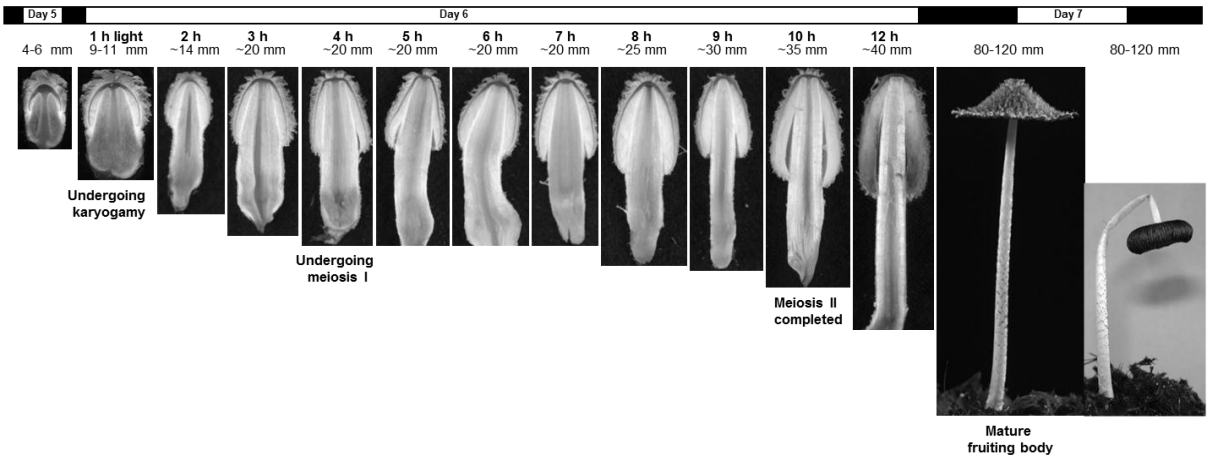

Figure 2: Later stages of primordia and fruiting body development of *C. cinerea* homokaryon AmutBmut on horse dung.

of basidiospores had begun at the tips. Basidiospore maturation started at the outer edges of the gills and moved quickly up to the top of the gills in the center of the fruiting bodies. After 12 h of illumination, gills were dark-stained over half of their length. Stipes rapidly elongated and the expanding caps opened. After 6 h in the dark, the mushrooms were fully developed and the black caps fully opened. Next morning, the mushrooms collapsed due to autolysis.

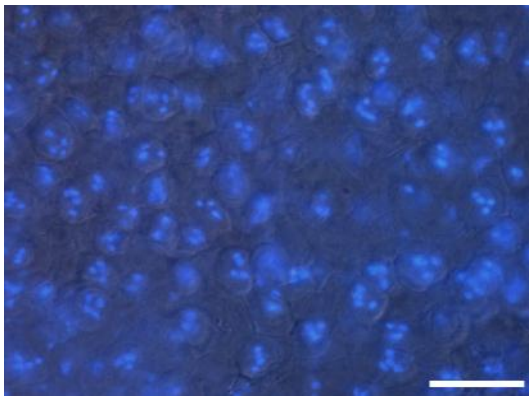

Figure 3: Nuclear staining of basidia in immature fruiting bodies of *C. cinerea* homokaryon AmutBmut at hour 10 of illumination on day 6 of development. Basidia finalised meiosis II as documented by the four nuclei in each basidium. A photo from DAPI stained basidia was overlaid with a differential interference contrast photo from the same basidia set at 30% transparency using the corelDRAW 12 program (Corel Corporation, Unterschleißheim, Germany). Scale bar 20 µm.

Protein characterisation of cap and stipes from immature fruiting bodies after meiosis II. Immature fruiting bodies were collected from horse dung on day 6 of development after 10 h of illumination, representing the stage after meiosis II with sterigmata formation, the onset of basidiospore formation, and the onset of rapid stipe elongation and cap expansion (Figs. 2 and 3). All harvested structures were checked for gill colour. Any immature fruiting bodies showing the first shades of staining at the outermost parts of the lamellae (suggesting onset of basidiospore pigmentation) were not considered. Caps and stipes were separated. Of 60 structures, the average weights of caps and stipes were 293 mg and 290 mg, respectively. Accordingly, caps and stipes from structures grown on horse dung had an 18x higher weight than structures of the same age grown on YMG/T medium.

For protein isolation, caps and stipes of structures were separated, frozen directly upon harvest, and intra-, extracellular and cell wall proteins were isolated as described above. First, the different samples of protein were separated by SDS-PAGE (Fig. 4). The 1D-resolution of the intracellular proteins and the fractions of the cell-wall-bound proteins resembled each other, suggesting a high portion of contamination of intracellular proteins in the cell wall samples. However, the patterns of freely secreted extracellular proteins and of the intracellular proteins differed very much from each other. Patterns from intracellular proteins from caps and stipes looked similar to each other as might be expected from intercellular functions, many of which might cover the normal cellular housekeeping functions. In contrast, the patterns of the extracellular proteins of caps and stipes were very different to each other, although there were also some overlapping banding patterns between the samples. A number of larger and smaller prominent bands were present in both extracellular samples but these did not correspond to each other in size.

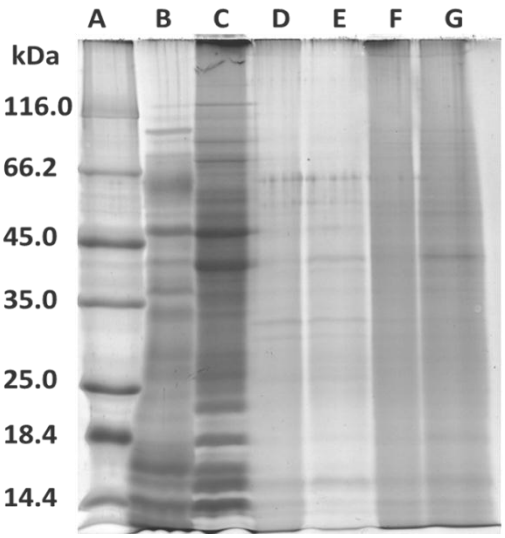

Figure 4: SDS-PAGE of proteins from immature fruiting bodies of *C. cinerea* after meiosis II. **A.** Protein-marker; extracellular proteins from **B.** cap and **C.** stipe; intracellular proteins from **D.** cap and **E.** stipe; proteins from cell walls of **F.** cap and **G.** stipe.

The extracellular proteome, i.e. the free secretome from fruiting body tissues may contain proteins that affect cell wall structure and cell wall formation [17], proteins that help in cell-cell-communication and aggregation, proteins that change hyphal surface properties, proteins required for pigmentation, and proteins that act in defence [18-20]. Obvious differences between the secretomes from cap and stipes suggests that organ-specific, or even tissue-specific, extracellular processes take place during development. This idea was further promoted by the protein patterns obtained after separation of the samples by 2D-gel electrophoresis (Fig. 5).

More than 270 protein spots were visible in the 2D-gel of the free secretome of the caps, and >200 spots were recorded in the 2D-gel of the free secretome of the stipes. Very few strong spots appeared to overlap between the two samples (see white circles in Fig. 5) whilst others were obviously unique to either one or the other of the samples (see black circles in Fig. 5). Due to the preparation technique of grinding frozen tissues prior to washing out the free proteins, intracellular protein contamination was very likely present in the washing buffer. However, the entire protein patterns from the intracellular samples were clearly distinguishable from their extracellular counterparts (compare Figs. 5 and 6), suggesting that the majority of the protein spots seen in the 2D-gels in Fig. 5 were actual secreted proteins.

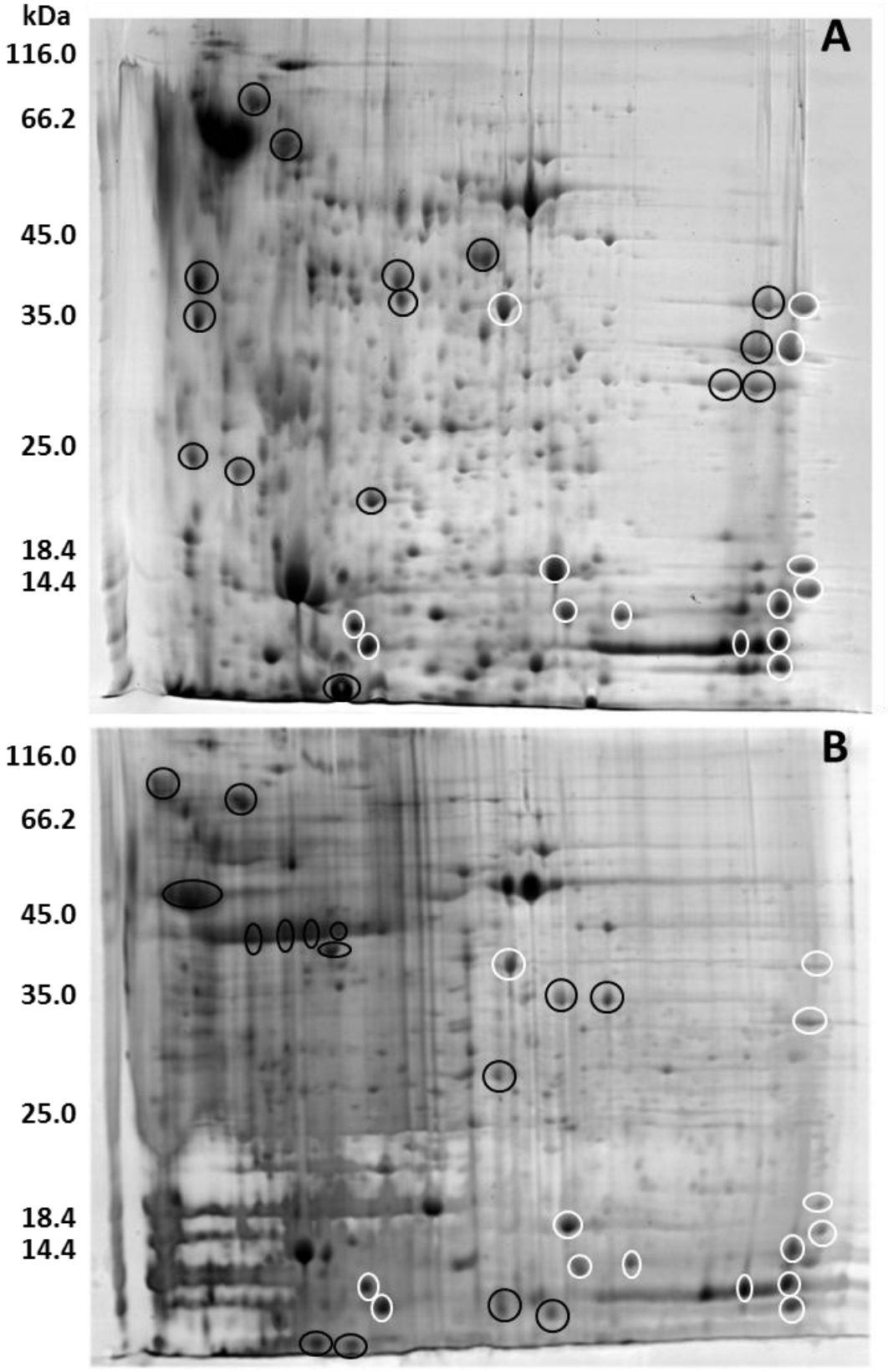

Figure 5: 2D-Gel of the extracellular secreted proteins from immature fruiting bodies of *C. cinerea* after meiosis II. **A.** Caps and **B.** stipes. Gels were stained with RuBP. Examples of protein spots shared between the two samples are encircled in white and unique protein spots in black. Note that the background smear was due to polysaccharides that cannot easily be separated from the proteins [20].

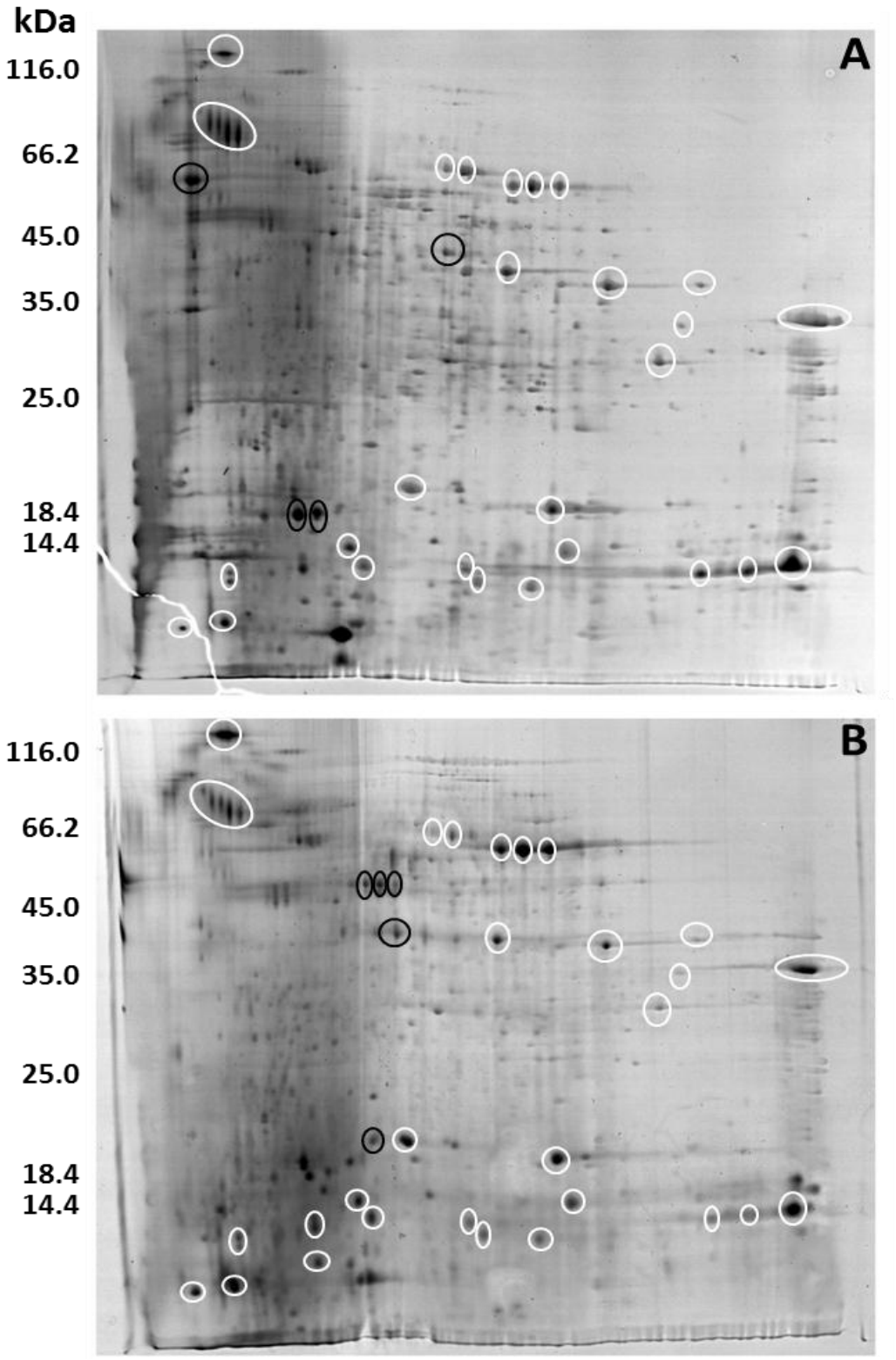

Figure 6: 2D-Gel of the intracellular proteins from immature fruiting bodies of *C. cinerea* after meiosis II. **A.** Caps and **B.** stipes. The gels were stained with RuBP. Examples of protein spots shared between the two samples are encircled in white and unique protein spots in black.

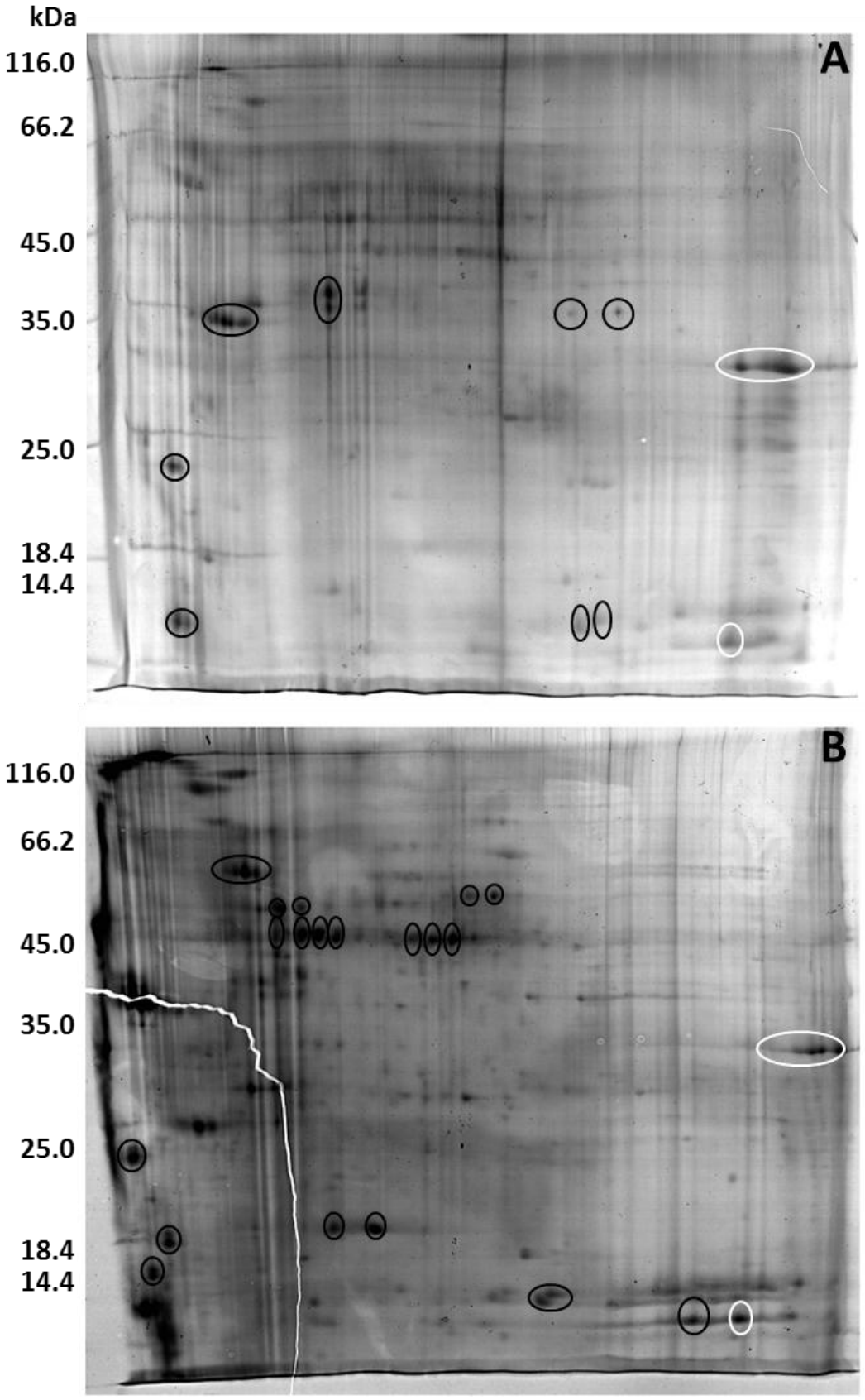

Figure 7: 2D-Gel of the cell wall proteins from immature fruiting bodies of *C. cinerea* after meiosis II. **A.** Caps and **B.** stipes. The gels were stained with RuBP. Examples of protein spots shared between the two samples are white and unique protein spots black encircled. Note that the very strong background and vertical smear were due to high polysaccharides content in protein samples.

In the SDS-soluble protein fraction of the cell walls, despite the likelihood of background spots arising from intracellular contamination (compare Fig. 6), there were lower numbers of strong spots (Fig. 7). Importantly, however, other prominent spots were visible than in the free secretome (compare Fig. 5) indicating a functional separation of the free secretome from the cell-wall-bound proteins. As in the case of the free secretome, the cell-wall-bound secretome of the cap and stipe also differed from each other considerably (Fig. 7).

In contrast to the extracellular samples, the spot patterns of the intracellular proteins of caps and stipes in 2D-gels are much more similar to each other, both in spot positions and in spot densities (see examples of white marked spots in the gel; Fig. 6). Nevertheless, there are also a number of proteins unique to the caps and to the stipes, respectively (see black encircled spots marked in the 2D-gels in Fig. 6). Overall, there were >400 spots visible on the 2D-gels loaded with intracellular proteins.

CONCLUSIONS

Our pioneering protein analysis of extra- and intracellular proteins from cap and stipe tissues of immature fruiting bodies of *C. cinerea* revealed interesting perspectives to be uncovered by proteomic identification of the whole sets of proteins. The annotated genome of *C. cinerea* has been published [22] and has already been used successfully to identify proteins of the secretome of vegetative mycelial cultures of the fungus [16]. The results of this study suggest that the differences in the free and cell-wall-bound secretomes of cap and stipe will be particularly important for the morphology of the fruiting body organs and the formation of their different tissues within the fruiting bodies. Prominent spots from 2D-gels have been picked for future analysis for protein identification.

Our presentation of the whole process of fruiting body development (Fig. 1) indicates that many different developmental stages have to be harvested and fractions of proteins extracted for further proteome definition. Moreover, similar approaches can be taken with characterized or newly developed mutants such as those with mutations in the *dst* (dark stipe) genes. Such mutants are defective in light perception and thus form elongated stipes with underdeveloped caps instead of normal primordia with gills in the cap (Fig. 8A). Also, other mutants that are likewise defective in morphology such as stunted stipe growth (shown in Fig. 8B) or mutants with white caps due to defects in basidiospore formation or pigmentation (Fig. 8B,C).

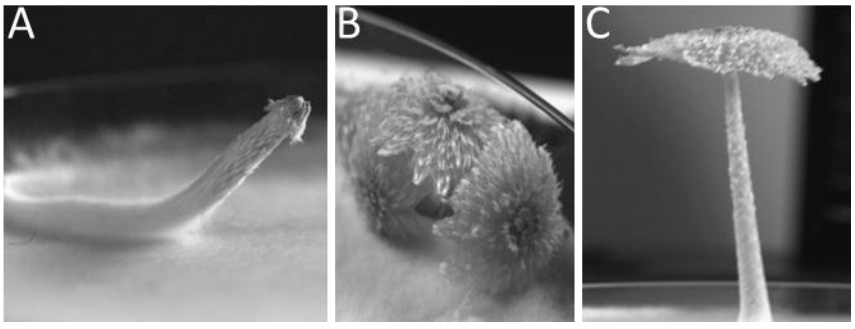

Figure 8: Mutants in fruiting body development of self-compatible homokaryons. **A.** Strain PUK1 with a *dst1* defect forms etiolated stipes in light. **B.** Mutant UFO is a mutant with a defect in stipe elongation (*eln*) forms dumpy mushrooms. The cap is white due to an additional mutation in basidiospore formation (*bad*). **C.** Mutant OU2 with a yet uncharacterized *bad* mutation has normal-sized white mushrooms without spores (after [5]).

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REFERENCES

[1] Buller A.H.R. (1931). *Researches on Fungi.* Vol. IV. *Further Observations on the Coprini together with Some Investigations on Social Organization and Sex in the Hymenomycetes.* Hafner Publishing Co., New York, N.Y.

[2] Uljé C.B. and Noordeloos M.E. (1999). Studies in *Coprinus* V. *Coprinus* section *Coprinus*. Revision of subsection Lanatuli Sing.. *Persoonia* 17: 165-199.

[3] Rao P.S. and Niederpruem D.J. (1969). Carbohydrate metabolism during morphogenesis of *Coprinus lagopus* (*sensu* Buller). *J. Bacteriol.* 100: 1222-1228.

[4] Kües U. (2000). Life history and developmental processes in the basidiomycete *Coprinus cinereus*. *Microbiol. Mol. Biol. Rev.* 64: 316-353.

[5] Kües U. et al. (2007). Mushroom biology and genetics. In: *Wood Production, Wood Technology and Biotechnological Impacts*. Kües U. Ed., Universitätsverlag-Verlag, Göttingen, Germany. ISBN 978-3-940344-11-3.

[6] Granado J.D. et al. (1997). Restriction enzyme-mediated DNA integration in *Coprinus cinereus*. *Mol. Gen. Genet.* 256: 28-36.

[7] Kües U. et al. (2002). Influence of activated *A* and *B* mating-type pathways on developmental processes in the basidiomycete *Coprinus cinereus*. *Mol. Genet. Genom.* 268: 262-271.

[8] Lu B.C. (1974). Meiosis in *Coprinus*. V. The role of light on basidiocarp initiation, mitosis and hymenium differentiation in *Coprinus lagopus*. *Can. J. Bot.* 52: 299-305.

[9] Patterson S.D. and Aebersold R.H. (2003). Proteomics: the first decade and beyond. *Nature Genet. Suppl.* 33: 311-323.

[10] Swamy S. et al. (1984). Morphogenetic effects of mutations at the *A* and *B* incompatibility factors in *Coprinus cinereus*. *J. Gen. Microbiol*. 130: 3219-3224.

[11] Boulianne R.P. et al. (2000). Fruiting body development in *Coprinus cinereus*: regulated expression of two galectins secreted by a non-classical pathway. *Microbiol*. 146: 1841-1853.

[12] Kertesz-Chaloupková K. et al. (1998). Blue light overrides repression of asexual sporulation by mating type genes in the basidiomycete *Coprinus cinereus*. *Fungal Genet. Biol.* 23: 95-109.

[13] Polak E. et al. (1997). Asexual sporulation in *Coprinus cinereus*: Structure and development of oidiophores and oidia in an Amut Bmut homokaryon. *Fungal Genet. Biol.* 22: 112-126.

[14] Laemmli U.K. (1970). Cleavage of structural proteins during assembly of head of bacteriophage T4. *Nature* 227: 680-685.

[15] Lamanda A. et al. (2004). Improved ruthenium II Tris (bathophenantroline disulfonate) staining and destaining protocol for a better signal-to-background ratio and improved baseline resolution. *Proteomics* 4: 599-608.

[16] Fragner D. (2011). *Secretome Analysis in Higher Basidiomycetes – Freely Secreted and Cell Proteins from Coprinopsis cinerea.* PhD Thesis. Georg-August-Universität Göttingen, Göttingen, Germany.

[17] Rast D.M. et al. (2003). Cell wall-associated enzymes in fungi. *Phytochem.* 64: 339-366.

[18] Walser P.J. et al. (2003). Extracellular matrix proteins in mushroom development. *Rec. Res. Dev. Microbiol.* 7: 381 415.

[19] Lakkireddy et al. (2011). Proteins expressed during hyphal aggregation for fruiting body formation in basidiomycetes. (These Proceedings).
[20] Kües U. and Navarro-González M. (2009). Communication of fungi on individual, species, kingdom, and above kingdom level. In: The Mycota, Vol. XV. Anke T. & Weber D. Eds., Springer Verlag, Berlin, 79-106.
[21] Fragner D. et al. (2009). Optimized protocol for the 2-DE of extracellular proteins from higher basidiomycetes inhabiting lignocellulose. *Electrophoresis* 30: 2431-2441.
[22] Stajich J.E. et al. (2010). Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). *Proc. Natl. Acad. Sci. USA* 107: 11889-11894.