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Protein mapping and genome expression variations in the Basidiomycete *Agrocybe aegerita*

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Summary. A procedure suitable for the extraction and mapping of total proteins from the basidiomycete, *Agrocybe aegerita,* was developed. *A. aegerita* mycelia were fragmented either with a Dangoumeau grinder, an X-press bomb or a sonicator and the efficiency of these three disruption methods were compared. The extraction buffer composition was optimized to avoid proteolytic activities. 2D-SDS-PAGE analysis of protein extracts showed that the rate of reproducibility depending on extractions and electrophoretic separations was always greater than 96% for all strains. The differences in efficiency observed between the breaking procedures indicate that the *A. aegerita* cell wall is more mechanically resistant than that of other basidiomycetes. The efficient action of protease inhibitors (PMSF and SDS) showed that *A. aegerita* mycelia contains numerous and/or highly active proteases. Reproducibility of protein extraction and separation methods allowed the establishment and the comparison of standard maps. Qualitative and quantitative variations in gene products between a wild dikaryotic strain and 11 homokaryotic strains from its progeny were examined. The genetic diversity, determined by comparing the distribution of proteic variations in 11 homokaryons from the same progeny, was comparable to that observed between co-isogenic homokaryons of another basidiomycete.

Key words: *Agrocybe aegerita* – Edible mushroom – Protein extraction – Protein mapping – Genome expression variation

Introduction

Separation of proteins by 2D-SDS-PAGE has been intensively used in numerous types of studies and organisms: (1) for characterization of the ascomycete, *Tuber moschatum,* and of the basidiomycete, *Agaricus arvensis* (Mouchès et al. 1978), of clonal cell lines of rat (Garrels 1979), of the C_4 grass plant, *Digitaria sanguinalis* (Harrison and Black 1982) and of mouse liver cells (Anderson et al. 1985); (2) for taxonomic studies in *Tuber moschatum* and *T. melanosporum* (Mouchès et al. 1981), and in the basidiomycete, *Ustilago* (Kim et al. 1984); (3) for the detection of induction, repression or modification of proteins from wild strains of the yeast, *Saccharomyces cerevisiae* (Elliott and McLaughlin 1979), of the zygomycete, *Mucor racemosus* (Hiatt et al. 1980), of the basidiomycete, *Schizophyllum commune* (De Vries and Wessels 1984) or of mutant strains of *Podospora anserina* (Bonneu and Labarère 1983) and *Neurospora crassa* (Bowman Nasrallah and Srb 1983), or of transformed cells of mouse and hamster (Bravo and Celis 1980).

However, sample preparation procedures must be adapted to the type of organism (or cellular fraction) to be analyzed by 2D-SDS-PAGE. Though comparison of 2D patterns of cellular fraction is relatively easy because of the low number of proteins, it is necessary to develop a suitable fractionation procedure to obtain a good reproducibility (Stotish and Somberg 1981). Comparisons are more difficult for total proteins studies in which resolution and reproducibility in 2D patterns depend on the suitability of the sample preparation procedure. For this purpose, and more particularly in the case of basidiomycetes, it is necessary to develop an appropriate method for breaking biological material (Shechter et al. 1972) and to avoid proteolytic degradation during disruption and protein solubilization (Colas des Francs et al. 1985). The inhibition of proteolysis is essential in filamentous fungi, since they are particularly rich in endogenous and exogenous proteases (Siepen and Kula 1976; Labarère 1980).

The experiments reported below were undertaken to investigate genome expression by 2D-PAGE of denatured soluble proteins of a wild dikaryotic strain of *Agrocybe aegerita* and of the homokaryotic strains from its progeny. A procedure for extracting total proteins from *Agrocybe aegerita* mycelium which avoids proteolysis was developed. Protein extraction studies gave information on the mechanical resistance and protease content of *A. aegerita* mycelia. The standard map of each strain was established by recording the spots reproducible according to total extracts and electrophoretic runs. Analysis and comparison of the standard maps allowed us to identify spots presenting qualitative and quantitative variations among the progeny of the dikaryotic parental strain, and to evaluate the level of genetic diversity between these strains. Genetic variability between homokaryotic strains and the differences in genome expression between homokaryons and their dikaryotic parental strain are discussed, and are compared with that previously described in a basidiomycete.

Investigations of the protein content were conducted with the aim of studying gene product variations in wild and mutant strains and during differentiation, genomic interrelations in dikaryons, and of the protecting industrial varieties in the cultivated basidiomycete *Agrocybe aegerita*.

Materials and methods

Strains. The tetrapolar basidiomycete, *Agrocybe aegerita,* belongs to the class of Agaricales. As described in other basidiomycetes (Esser and Stahl 1973; Miyake et al. 1980; Leslie and Leonard 1984), homocaryotic mycelia of *Agrocybe aegerita* are able to produce fruit bodies (Meinhardt and Esser 1981). Homokaryotic strains used in this work were obtained from the germination of single basidiospores collected from a mature basidiocarp of the wild-type strain SM51, as described by Labarère et al. (1989). Strains are listed in Table 2 according to their incompatibility group (loci *A* and *B*) and their ability to fruit (su^+ *fifb*, su^+fi^+ *fb*, su^+fifb^+, $su^+fi^+fb^+$: fruiting pathway suppressed; *su* fi^+*fb*: strains forming initials; *su* fi^+fb^+: homokaryons producing fruit bodies) (Meinhardt and Esser 1981). For the development of protein extraction procedure, we used exclusively the homokaryotic strain F24 (*A1B1 su* fi^+fb).

Culture conditions. Mycelium was grown on solid complete Raper (CR) medium (Raper et al. 1972) in petri dishes, for 8 days at 25 °C in the dark, then harvested with a scalpel (about 0.1 g dried weight per petri dish), resuspended in 10 ml of CR liquid medium and disrupted for 30 s at 4 °C with an homogenizer Polytron (Kinematica, shaft type 10 TS). The suspension obtained was inoculated in 100 ml of CR liquid medium supplemented with 50 µg/ml of ampicillin in Roux culture flasks, and incubated for 6 days at 25 °C in the dark. After incubation, grown mycelium was harvested by filtration on sterilized gauze, thoroughly washed with cold distilled water, dried and weighted. Dried mycelium (about 2 g per flask) was immediately broken for protein extraction.

Breaking methods. For each disrupting procedure study, 2 g of dried mycelia was used; extraction buffer was 0.05 *M* TRIS-HCl (pH 6.8), 2 m*M* EDTA. For Dangoumeau grinding, mycelium was agitated for 5 min in a closed steel bowl containing liquid nitrogen and a steel ball, and broken mycelium was resuspended in 1.6 ml of extraction buffer at 4 °C. For breaking in the 5 ml X-press bomb (Biotec, Sweden), mycelium resuspended in 1.6 ml extraction buffer was placed in the upper compartment of the X-press bomb previously chilled at –25 °C, and hyphal material was broken by four cycles of pressing. For sonication (sonicator Vibracell VC500), mycelium was suspended in 1.6 ml of extraction buffer; various sonication times and temperature conditions were assayed. For the method finally retained, four sonication cycles of 3 min were applied to the mycelial suspension contained in a glass tube refrigerated in an ice-ethanol bath; the suspension was homogenized by vortexing between each sonication cycle. In all cases, conditions of breaking were determined to disrupt more than 90% of hyphae as observed by light microscopy.

Extraction buffers. Three extraction buffers were used for mycelium broken by sonication, their composition depending on the type of added reagents: nucleases (buffer A), PMSF (buffer B) and SDS (buffer C). Buffer A: 0.05 *M* TRIS-HCl (pH 6.8), 2 m*M* EDTA, 5 m*M* $MgCl_2$ with 100 µg/ml of DNase I, or 50 µg/ml of RNase A, or 100 µg/ml of DNase I and 50 µg/ml of RNase A. Buffer B: 0.05 *M* TRIS-HCl (pH 6.8), 2 m*M* EDTA, 5 m*M* $MgCl_2$, 50 µg/ml RNase A with 1.2 m*M*, 2.4 m*M*, 4.8 m*M* or 9.6 m*M* of PMSF. Buffer C: 0.05 *M* TRIS-HCl (pH 6.8), 2 m*M* EDTA, 5 m*M* $MgCl_2$, 50 µg/ml RNase A, 4.8 m*M* PMSF with 0.1%, 0.4%, 1% or 2% of SDS.

Protein sample preparation. After mycelium breaking, proteins were solubilized by adding 1 vol of SDS sample buffer (0.1 *M* TRIS-HCl, 4% SDS, 30% Glycerol, 10% β-mercaptoethanol, pH 6.8). Suspensions were homogenized by vortexing, heated (100 °C for 5 min) and allowed to stand on ice for at least 5 min. They were then clarified by centrifugation (10,000 g, 15 min, 4 °C). The supernatants constituted the total protein extracts.

Two-dimensional electrophoresis. Isolectric focusing (IEF) was carried out according to O'Farrell (1975). Loaded protein quantities were always about 150 µg; the pH gradient was determined on 5 mm gel slices soaked in de-aerated water for 20 h. SDS-PAGE in the second dimension was performed as described by Mouchès et al. (1979) with a separating gel (0.75 mm thick, 120 mm in height and 300 mm in width) containing a 17% – 22% exponential acrylamide gradient and an upper 5% acrylamide stacking gel. The second dimension SDS-polyacrylamide gels were standardized with 3.5 µg the molecular weight marker kit Dalton Mark VII-L from Sigma (about 0.5 µg of albumin bovine, ovalbumin, glyceraldehyde-3-phosphate deshydrogenase, carbonic anhydrase, trypsinogen, trypsin inhibitor and α-lactalbumin) and 0.5 µg of β-galactosidase. Gels were stained by the ultrasensitive silver stain (Oakley et al. 1980). For 2D pattern comparison, protein maps were squared as previously described for plasma membrane from *Podospora anserina* (Bonneu and Labarère 1983).

Establishment and comparison of standard maps. For the establishment of standard maps, three independent protein samples were prepared from each strain, and each sample was analyzed in triplicate. The nine resulting 2D patterns were traced on a transparent overlay sheet and the diagrams were compared by superimposing the transparent sheets. Non-reproducible polypeptide spots were eliminated; their proportion never reached 4%. For comparison of the standard maps and the establishment of the "master map", three types of spots were differentiated: spots common to all maps, spots with qualitative varia-

Table 1. Influence of nucleases and of protease inhibitors on proteic extracts of *Agrocybe aegerita* analyzed by 2D-SDS-PAGE. All total protein extracts were prepared from the F24 homokaryotic strain. Data given in the table correspond to average values derived from three independent extracts

2D-electrophoretic patterns	Buffer A^a+			Buffer B^a+			Buffer C^a+			
	DNase I (100 µg/ml)	RNase A (50 µg/ml)	DNase I (100 µg/ml) + RNase A (50 µg/ml)	PMSF 1.2 m*M*	PMSF 2.4 m*M*	PMSF 4.8 m*M*	PMSF 9.6 m*M*	SDS 0.1%	SDS 0.4%	SDS 1%	SDS 2%
No. of spots	332 ± 8	335 ± 6	329 ± 5	378 ± 6	410 ± 7	453 ± 5	451 ± 7	462 ± 5	488 ± 4	501 ± 5	501 ± 6
Proteolysis inhibition:											
– No. of LMW polypeptides[b]	279 ± 4	283 ± 5	281 ± 7	262 ± 4	253 ± 3	228 ± 4	230 ± 2	224 ± 3	215 ± 3	204 ± 6	207 ± 4
– No. of HMW polypeptides[c]	0	0	0	35 ± 2	42 ± 4	55 ± 3	53 ± 3	60 ± 5	68 ± 3	75 ± 2	75 ± 1
Horizontal streaking	yes	no	no	no	no	no	no	no	no	no	no

[a] Detailed compositions of buffers A, B and C are indicated in "Materials and methods"
[b] Low-molecular-weight (LMW) polypeptides: MW between 10,000 and 30,000 daltons
[c] High-molecular-weight (HMW) polypeptides: MW greater than 60,000 daltons

tions (present or absent depending on the strain) and spots with quantitative variations (clear and reproducible variation of intensity). The master map was constructed using graphics software after scanning of the F24 strain standard map.

Results

Efficiency of three disruption methods

Mycelia were disrupted either in a Dangoumeau grinder, an X-press bomb or by sonication. Suitability of each disrupting procedure was determined by analyzing the resulting protein extracts by 2D-SDS-PAGE. Breaking mycelium in a Dangoumeau grinder for 1, 3 and 5 min allowed disruption of 20%, 50% and 90% of hyphae, respectively. With protein extracts obtained by Dangoumeau grinding, no individual spot could be detected on the 2D patterns, only a highly colored uniform zone beginning from the 60,000 dalton molecular weights and reaching the bottom of the gel was visualized. Proteins of these extracts were so highly degraded that Dangoumeau disrupting was abandoned for breaking *Agrocybe aegerita* mycelia. Protein samples prepared with X-press and sonification methods showed, respectively, the presence of about 250 and 300 well-individualized spots; the polypeptides resolved had an apparent molecular weight ranging between 10,000 and 60,000 daltons, the majority (about 85%) falling between 10,000 and 30,000 daltons. The presence of numerous low-molecular-weight polypeptides reflects a proteolysis during the extraction. With these extracts we could also note a horizontal streaking on the gels, probably due to the presence of nucleic acids in the samples. Sonication was retained for the following procedures, since it allowed the solubilization of the highest number of polypeptides (20% of additional spots compared with the X-Press disrupting).

Influence of the extraction buffer on the stability of proteins

To circumvent horizontal streaking and proteolysis, the influence of two nucleases (DNase I and RNase A) of a large spectra protease inhibitor (PMSF) and of a protein solubilizing agent (SDS) were investigated.

The addition of DNase to the extraction buffer did not eliminate streaking, whereas addition of RNase A lead to the almost total disappearance of the horizontal streaking on 2D patterns (Table 1). Simultaneous use of DNase I and RNase A did not give better results so, in all further experiments, 50 µg/ml of RNase A was added to the extraction buffer.

In order to inhibit proteases liberated during the extraction procedure, for concentrations of PMSF (1.2 m*M*, 2.4 m*M*, 4.8 m*M* and 9.6 m*M*) were added to the extraction buffer. Analysis of 2D patterns showed

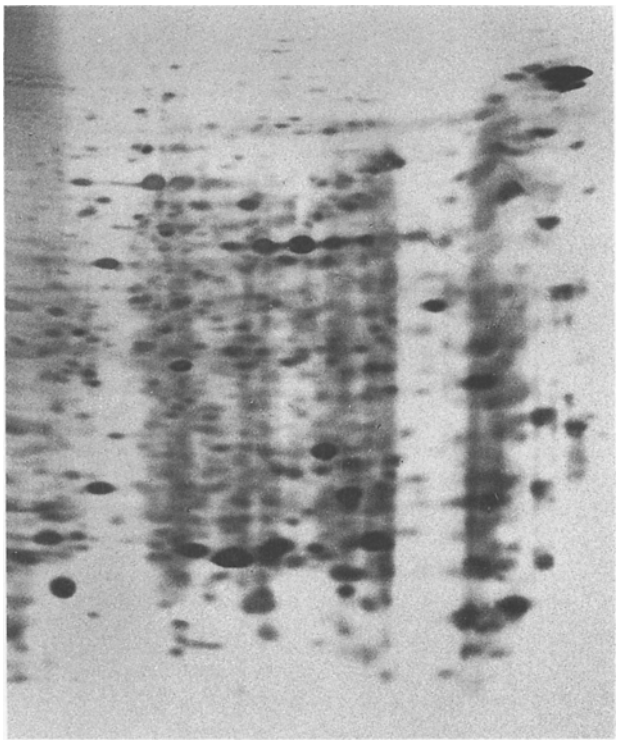

Fig. 1. Two dimensional isoelectric focusing SDS-polyacrylamide gel electrophoresis of total proteins obtained from the F24 homokaryotic strain by the final extraction procedure

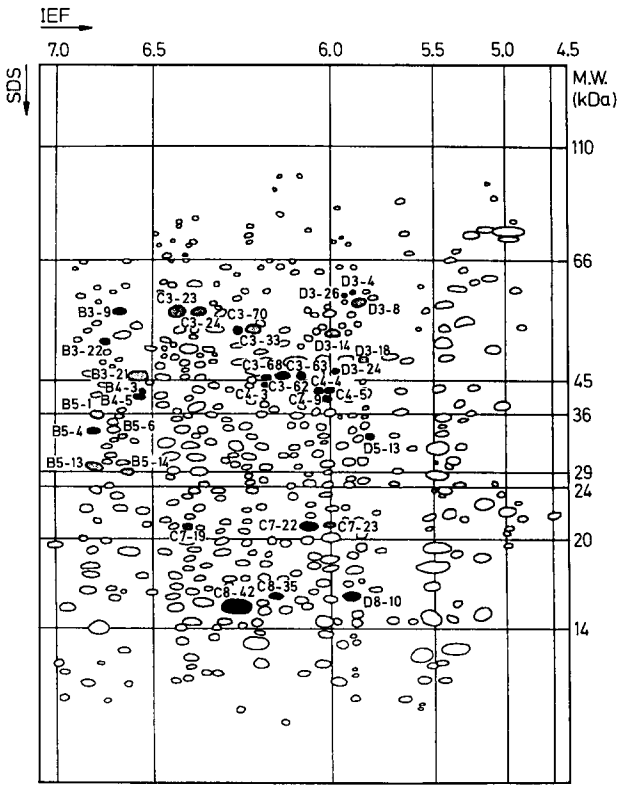

Fig. 2. Master map of polypeptidic spots from *Agrocybe aegerita*, showing spots common to all the strains (o) and spots presenting qualitative (●) and quantitative (⊛) variations. Only spots presenting variations were named (see text for nomenclature)

that in the presence of PMSF, high-molecular-weight (HMW) polypeptides (from 60,000 to 100,000 daltons) could be detected and that low-molecular-weight (LMW) proteins disappeared (Table 1). The number of HMW-appearing and LMW-disappearing spots increased until the PMSF concentration reached 4.8 m*M*. Therefore, 4.8 m*M* of PMSF was considered to be the optimal concentration and retained for following studies.

Beside its solubilizing properties, SDS is also known to inhibit proteolytic degradation (Colas des Francs et al. 1985). In order to further improve the quality of the extraction, the addition of four concentrations of SDS to the extraction buffer were assayed: 0.1%, 0.4%, 1% and 2%. The optimal concentration was determined to be 1% (Table 1). Addition of SDS allowed the solubilization of a new set of proteins (11% of additional spots) and lead to the appearance of about 20 HMW proteins and to the disappearance of some 30 LMW ones. This last point shows that SDS blocks the activity of some proteases which were not inhibited by PMSF.

Accordingly, the extraction buffer finally retained for protein extractions was: 0.05 *M* TRIS-HCl (pH 6.8), 2 m*M* EDTA, 5 m*M* $MgCl_2$, 50 μg/ml RNase A, 1% SDS, 4.8 m*M* PMSF. A 2D pattern of total proteins from the homokaryotic strain F24 using the final extraction method is shown in Fig. 1. The polypeptides resolved have an apparent molecular weight of 10,000–100,000 daltons; they are situated in the pH range 4.5–7.0. Comparison of nine 2D patterns corresponding to three independent extracts from F24 analyzed in triplicate showed that more than 96% of the spots were detected with complete reproducibility. Accordingly, the method described here was employed to establish and compare the standard protein maps of a dikaryotic wild strain and 11 homokaryotic strains of different genotypes, in respect to homokaryotic fruiting and incompatibility of its progeny.

Protein content variations in 11 homokaryotic strains issued from the SM51 dikaryotic strain

Standard maps of 11 homokaryotic strains (listed in Table 2) were compared. Three types of spots could be differentiated on the schematic 2D pattern (Fig. 2): (1) spots of constant intensity common to all the strains, (2) spots with qualitative variations (present or absent ac-

Table 2. Genotypes of the 11 homokaryotic strains of *Agrocybe aegerita* used

Incompatibility alleles	Genes controlling fruiting[a]		
	su^{+}[b]	*su fi*$^{+}$*fb*	*su fi*$^{+}$*fb*$^{+}$
A1 B1	F41	F24	F30
A2 B2	–	F12	F31
A1 B2	F9	F3	F19
A2 B1	F34	F25	F6

[a] For nomenclature see Meinhardt and Esser (1981)
[b] For simplification we named *su*$^{+}$ the following genotypes: *su*$^{+}$ *fi fb*, *su*$^{+}$ *fi*$^{+}$*fb*, *su*$^{+}$*fi fb*$^{+}$, *su*$^{+}$*fi*$^{+}$*fb*$^{+}$

cording to the strains), (3) spots with quantitative variations (whose intensity varied among the different strains).

Gels were squared by drawing vertical lines corresponding to the following pH of the fist dimension: pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5, and horizontal lines corresponding to apparent molecular weights (110,000, 66,000, etc.). Each square was given a letter (A–G from pH 7.5 to 4.0) followed by a number (1–9 from high to low molecular weights). Spots were designated by the name of the square in which they were situated and in each square, spots were enumerated with increasing numbers from left to right and from top to bottom.

Table 3. Qualitative and quantitative variations of polypeptidic spots in total protein standard maps from 11 homokaryotic strains and their parental dikaryotic strain. For each strain, standard maps were established by analysis and comparison of three independent total proteic extracts electrophoresed in triplicate

Spots	Strains											
	A1 B1			*A2 B2*		*A1 B2*			*A2 B1*			*A1 B1/A2 B2*
	F41	F24	F30	F12	F31	F9	F3	F19	F34	F25	F6	SM51
B3-9	+	+	–	–	–	+	+	+	–	–	–	+
B3-22	+	–	–	–	–	–	–	–	–	–	–	–
B4-3	+	+	+	+	+	+	+	+	+	+	+	–
B4-5	+	+	+	+	+	+	+	+	+	+	+	–
B5-4	+	+	–	+	+	+	+	+	+	+	+	+
C3-62	+	+	+	+	+	+	+	+	–	+	+	+
C3-68	+	+	+	+	+	+	+	+	+	+	+	–
C3-70	–	–	–	–	–	+	+	+	–	–	–	–
C4-3	+	+	+	+	+	+	+	+	+	+	+	–
C8-35	–	+	+	–	–	+	+	+	+	–	+	+
C8-42	+	+	+	+	+	+	+	+	–	–	+	+
D3-24	–	–	–	–	–	+	+	–	–	–	–	–
D3-26	–	–	–	–	+	–	–	–	–	+	+	–
C3-63	+	+	+	+	+	+	+	+	–	+	+	+
C4-4	+	+	+	+	+	+	+	+	+	+	+	–
C4-5	+	+	+	+	+	+	+	+	+	+	+	–
C4-9	+	+	+	+	+	+	+	+	+	+	+	–
C7-22	+	+	+	+	+	+	–	+	+	+	+	–
D5-13	–	–	–	–	–	+	–	+	–	–	–	–
D8-10	+	+	+	+	+	+	+	+	+	–	+	+
B3-21	L	L	L	L	L	L	L	L	L	L	H	L
B5-1	L	L	H	L	L	L	L	L	L	L	L	L
B5-6	L	L	L	L	L	L	L	L	L	L	L	H
B5-13	L	L	H	L	L	L	L	L	L	L	L	L
B5-14	L	L	L	L	L	L	L	L	L	L	H	L
C3-23	H	H	H	H	L	H	H	H	L	L	L	H
C3-24	L	L	L	H	H	H	L	H	H	H	H	L
C3-33	H	H	H	H	H	H	H	H	L	L	L	H
C7-19	L	L	L	L	L	L	L	L	L	H	L	L
C7-23	L	L	L	H	H	L	H	L	H	H	H	H
D3-4	L	L	L	L	H	L	L	L	L	H	H	L
D3-8	L	L	L	H	H	L	L	L	H	H	H	L
D3-14	L	L	L	L	L	L	L	L	H	H	H	L
D3-18	L	L	L	L	L	L	L	L	L	H	L	L

+/–: presence/absence of spots
H/L: high/low intensity of spots

Among 467 spots, 433 (93% of spots) were common to the 12 strains studied. Globally, 20 and 14 spots presented qualitative and quantitative variations, respectively. The individual variations of each strain are listed in Table 3. Analysis of qualitative and quantitative variations shows that the number of missing spots and of high-intensity spots range from 2 to 7, depending on the homokaryotic strains.

In the dikaryotic parental strain SM51, a greater diversity in qualitative variations was observed since 12 missing spots are found in this strain. Among these 12 missing spots, 7 (B4-3, B4-5, C3-68, C4-3, C4-4, C4-5 and C4-9) were always present in the 11 homokaryotic strains. Concerning quantitative variations, 4 spots were present in the dikaryotic strain with a high intensity. Spot B5-6 was observed with a high intensity only in the dikaryon.

Discussion

Extraction procedure

Dangoumeau grinding efficiency, measured on the basis of the percentage of broken hyphae, shows that 5 min of breakage (disruption rate: 90%) was necessary to disrupt *Agrocybe aegerita* mycelium, whereas 1 min of breakage, e.g. was sufficient to break ascocarps of *Tuber melanosporum* and *T. moshatum* (Mouchès et al. 1978, 1981). Disrupting *Agrocybe aegerita* mycelium for a long time allows liquid nitrogen to evaporate and biological material to warm up so that endogenous proteases become active. As a result, a good disruption of hyphae with the Dangoumeau grinding is incompatible with the recovery of intact proteins of this fungus. Protein samples obtained after breakage in the X-press and by sonication were suitable, since 250 and 300 individualized spots were revealed without using protease inhibitor. Sonication which allows the solubilization of more proteins was retained. Differences in the efficiency of the three methods assessed indicate that *A. aegerita* cell wall is particularly rigid.

The addition of RNase A to the extraction buffer eliminated streaking, which indicates that extracts contain a great quantity of RNAs. As shown in other organisms, streaking is often due to the presence of nucleic acids (O'Farrell 1975).

Among the 300 resolved spots detected on 2D gels without using protease inhibitors, 85% had an apparent molecular weight of between 10,000 and 30,000 daltons and there was no spot where the molecular weight reached 60,000. Accordingly, liberated proteases were active though the SDS sample buffer was added immediately after breaking. The addition of PMSF and SDS leads to the detection of 50% of additional spots and avoids proteolysis, since we observed the concomitant appearance of HMW polypeptides and the disappearance of LMW ones. PMSF concentrations commonly used to inhibit proteases during breakage of fungi were 0.25 m*M* for *Neurospora crassa* (Stotish and Somberg 1981), 1 m*M* for *S. commune* (De Vries and Wessels 1984), *Ustilago* (Kim et al. 1984) or *Candida albicans* (Elorza et al. 1985). The optimal concentration of PMSF for *A. aegerita* protein extraction which is equal to 4.8 m*M* indicates that *A. aegerita* mycelium probably contains numerous and/or highly active proteinases.

Proteins extracted by the final procedure were resolved in 500 spots, in the pH 4.5–7.0 range and their molecular weights ranged between 10,000 and 100,000 daltons. Ninety-six percent of the spots were recovered with a total reproducibility, the very low number of non-reproducible spots attesting to the good reproducibility of the extraction and of the 2D separation procedures. The non-reproducible spots were discarded for standard map establishment. Knowing that the silver stain we used is comparable in sensitivity to autoradiography of radioactively labeled proteins (Oakley et al. 1980), the 500 polypeptides spots revealed in *A. aegerita* were in accordance with results previously obtained in filamentous fungi: 400–500 spots were detected in the zygomycete, *Mucor racemosus* (Hiatt et al. 1980), 480 in the ascomycete, *Neurospora crassa* (Metzenberg and Nelson 1977), 400 (De Vries and Wessels 1984) and 700 (De Vries et al. 1980) in the basidiomycete, *S. commune*.

Genome expression variations

Analysis and comparison of standard maps of 11 homokaryotic strains and of the dikaryotic parental strain shows that 20 and 14 spots present qualitative and quantitative variations, respectively. All the strains studied differed among each other in their distribution of variations (Table 3).

Qualitative variations among homokaryotic strains concern 13 spots (2.8% of total spots). Studies of two co-isogenic monokaryons of the basidiomycete, *S. commune*, showed that they differed by 2.3% of total spots, whereas the two co-isogenic monokaryons and a non-isogenic one differed by 11% (De Vries et al. 1980). Accordingly, the genetic diversity observed in the 11 *A. aegerita* homokaryons from the same progeny is comparable to that found between two co-isogenic strains of *S. commune*. It should be noted that in both cases, genetic variations are underestimated because of the limits of the protein extraction procedures: De Vries et al. (1980) evaluated that their analysis encompassed about 7.5% of the protein capacity of the poly (A) RNA, knowing the genome size of *S. commune* (Dons et al. 1979) and the rate of single-copy DNA complementary to poly (A) RNA

(Hoge et al. 1982). Diversity among strains is also underestimated since post-translational modifications are not considered. Indeed, their detection requires the use of specific techniques like that previously described for glycoproteins (Clegg 1982) or phosphoproteins (Contor et al. 1987). This fact was confirmed in previous analysis of glycoprotein contents from various *Agrocybe aegerita* homokaryotic strains (data not shown).

Comparison of qualitative variations of the homokaryons and the parental dikaryon shows that a set of seven spots which are always present in the 11 homokaryons seems to be specifically absent in the dikaryotic strain SM51. In *S. commune,* two co-isogenic homokaryons differed by only 2% (of total proteins), whereas between homokaryons and the resulting dikaryon there was a 6.6% and 7.7% difference (De Vries et al. 1980). The greater difference observed between *A. aegerita* homokaryons and their parental dikaryon than between each homokaryon is in accordance with these results. This differential genomic expression could be related to morphological differences between homokaryons and dikaryons during vegetative growth (Casselton 1978).

Only one qualitative variation appeared to be common to strains of the same genotype: the spot C3-70 is present only in the three homokaryons belonging to the *A1B2* incompatibility group. Nevertheless, this finding must be confirmed by further studies. Concerning quantitative variations, 9 of the 11 homokaryons are different from one another; only the homokaryons F24 (*A1B1*) and F41 (*A1B1*) present the same distribution.

The methods for protein extraction and separation reported here, which have a good reproducibility, appear to be suitable for protein analysis in *Agrocybe aegerita.* Our study concerns protein content of strains in the vegetative state (mycelium). Analysis by 2D-PAGE of strains in different states of differentiation will be undertaken, since that seems to be an interesting field of investigation, as previously described in *Saccharomyces cerevisiae* (Elliot and McLaughlin 1979; Trew et al. 1979), *Candida albicans* (Manning and Mitchell 1980) and *S. commune* (De Vries and Wessels 1984). This efficient method to extract and separate proteins from *Agrocybe aegerita* will allow us to characterize *Agrocybe aegerita* mutant strains already selected (Labarère et al. 1989) as well as other types of mutant. Comparison between 2D patterns of homokaryons and dikaryons derived from them seems also to be a way to investigate genetic features of the dikaryotic state and interrelations between the two genomes of the dikaryon. Moreover, these studies could be completed by that of glycoproteins, which present more diversity between *A. aegerita* strains and which are implicated in fundamental cellular processes and, more particularly, in differentiation (Wallace et al. 1984; Lennarz 1985).

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References

Anderson NL, Nance SL, Tollaksen SL, Giere FA, Anderson NG (1985) Quantitative reproducibility of measurements form Coomassie blue stained 2-D gels: analysis of mouse liver protein patterns and a comparison of BALB/C and C57 strains. Electrophoresis 6:592–599

Bonneu M, Labarère J (1983) Electrophoretic analysis of plasma membrane proteins of wild-type and differentiation-deficient mutant strains of *Podospora anserina.* Curr Microbiol 9:133–140

Bowman Nasrallah J, Srb AM (1983) Two-dimensional electrophoresis of plasma membranes, showing differences among wild-type and abnormal ascospore mutant strains of *Neurospora crassa.* J Bacteriol 155:1393–1398

Bravo R, Celis JE (1980) Gene expression in normal and virally transformed mouse 3T3B and hamster BHK21 cells. Exp Cell Res 127:249–260

Casselton LA (1978) Dikaryon formation in higher basidiomycetes. In: Smith JE, Berry DR (eds) The filamentous fungi, vol III. Edward Arnold, London, pp 275–294

Clegg JCS (1982) Glycoprotein detection in nitrocellulose transfers of electrophoretically separated protein mixtures using concanavalin A and peroxidase: application to arenavirus and flavivirus proteins. Anal Biochem 127:389–394

Colas des Francs C, Thiellement H, De Vienne D (1985) Analysis of leaf proteins by two-dimensional gel electrophoresis. Plant Physiol 78:178–182

Contor L, Lamy F, Lecoq RE (1987) Use of electroblotting to detect and analyse phosphotyrosine containing peptides separated by two-dimensional gel electrophoresis. Anal Biochem 160:414–420

De Vries OMH, Wessels JGH (1984) Patterns of polypeptide synthesis in non-fruiting monokaryons and a fruiting dikaryon of *Schizophyllum commune.* J Gen Microbiol 130:145–154

De Vries OMH, Hoge JHC, Wessels JGH (1980) Regulation of the pattern of protein synthesis in *Schizophyllum commune* by the incompatibility genes. Dev Biol 74:22–36

Dons JJM, De Vries OMH, Wessels JGH (1979) Characterization of the genome of the basidiomycete *Schizophyllum commune.* Biochim Biophys Acta 563:100–112

Elliott SG, McLaughlin CS (1979) Synthesis and modification of proteins during the cell cycle of the yeast *Saccharomyces cerevisiae.* J Bacteriol 137:1185–1190

Elorza MV, Murgui A, Sentandreu R (1985) Dimorphism in *Candida albicans:* contribution of mannoproteins to the architecture of yeast and mycelial cell walls. J Gen Microbiol 131:2209–2216

Esser K, Stahl U (1973) Monokaryotic fruiting in the Basidiomycete *Polyporus ciliatus* and its suppression by incompatibility factors. Nature 244:304–305

Garrels JI (1979) Two-dimensional gel electrophoresis and computer analysis of proteins synthesized by clonal cell lines. J Biol Chem 254:7961–7977

Harrison PA, Black CC (1982) Two-dimensional electrophoretic mapping of proteins of bundle sheath and mesophyll cells of the C_4 grass *Digitaria sanguinalis* (L.) Scop. (Cralgrass). Plant Physiol 70:1359–1366

Hiatt WR, Inderlied CB, Sypherd PS (1980) Differential synthesis of polypeptides during morphogenesis of *Mucor.* J. Bacteriol 141:1350–1359

Hoge JHC, Springer J, Zantinge B, Wessels JGH (1982) Absence of differences in polysomal RNAs from vegetative monokaryotic and dikaryotic cells of the fungus *Schizophyllum commune*. Exp Mycol 6:225–232

Kim WK, Rohringer R, Nielsen J (1984) Comparison of polypeptides in *Ustilago* spp. pathogenic on wheat, barley, and oats: a chemotaxonomic study. Can J Bot 62:1434–1437

Labarère J (1980) Proteolytic activities during growth and aging in the fungus *Podospora anserina:* effect of specific mutations. Arch Microbiol 124:269–274

Labarère J, Noël T, Imbernon M (1989) Selection and genetic analysis of antibiotic-resistant mutant strains in *Agrocybe aegerita*, part 1. Mushroom Sci XII, 161–174

Lennarz WJ (1985) Regulation of glycoprotein synthesis in the developing sea-urchin embryo. TIBS 10:248–251

Leslie JF, Leonard TJ (1984) Nuclear control of monokaryotic fruiting in *Schizophyllum commune*. Mycologia 76:760–763

Manning M, Mitchell TG (1980) Morphogenesis of *Candida albicans* and cytoplasmic proteins associated with differences in morphology, strain, or temperature. J Bacteriol 144:258–273

Meinhardt F, Esser K (1981) Genetic studies of the basidiomycete *Agrocybe aegerita*. Theor Appl Genet 60:265–268

Metzenberg RL, Nelson RE (1977) Genetic control of phosphorus metabolism in *Neurospora*. In: Wilcox G, Abelson J, Fox CF (eds) Molecular approaches to eukaryotic genetic systems. Academic Press, New York, pp 253–269

Miyake H, Tanaka K, Ishikawa T (1980) Basidiospore formation in monokaryotic fruiting bodies of a mutant strain of *Coprinus macrorhizus*. Arch Microbiol 126:207–212

Mouchès C, Duthil P, Bové JM, Valjalo J, Delmas J (1978) Caractérisation des champignons supérieurs par électrophorèse de leurs protéines. Mushroom Sci X:491–503

Mouchès C, Vignault JC, Tully JG, Whitcomb RF, Bové JM (1979) Characterization of spiroplasmas by one- and two-dimensional protein analysis on polyacrylamide slab gels. Curr Microbiol 2:69–74

Mouchès C, Duthil P, Poitou N, Delmas J, Bové JM (1981) Caractérisation des espèces truffières par analyse de leurs protéines en gels de polyacrylamide et application de ces techniques à la taxonomie des champignons. Mushroom Sci XI:819–831

Oakley BR, Kirsh DR, Morris R (1980) A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. Anal Biochem 105:361–363

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

Raper CA, Raper JR, Miller RE (1972) Genetic analysis of the life cycle of *Agaricus bisporus*. Mycologia 64:1088–1117

Shechter Y, Landau JW, Dabrowa N (1972) Comparative electrophoresis and numerical taxonomy of some *Candida* species. Mycologia 64:841–854

Siepen J, Kula MR (1976) Specificity of five intracellular proteinases of *Neurospora crassa*. FEBS Lett 66:31–34

Stotish RL, Somberg EW (1981) Electrophoretic analysis of the plasma membrane proteins of a mutant of *Neurospora crassa* which lacks a cell wall. Biochim Biophys Acta 641:289–300

Trew BJ, Friesen JD, Moens PB (1979) Two-dimensional protein patterns during growth and sporulation in *Saccharomyces cerevisiae*. J Bacteriol 138:60–69

Wallace J, Morrissey JH, Newell PC (1984) Monoclonal antibodies specific for stalk differentiation in *Dictyostelium discoïdeum*. Cell Differ 14:205–212