Theor Appl Genet (1988) 76:712–718

DNA polymorphisms in commercial and wild strains of the cultivated mushroom, *Agaricus bisporus*

M. G. Loftus [1], D. Moore [1] and T. J. Elliott [2]

[1] Microbiology Research Group, Department of Cell and Structural Biology, Stopford Building, The University, Manchester M13 9PT, UK
[2] Department of Microbiology and Plant Pathology, AFRC Institute of Horticultural Research, Littlehampton, Sussex BN17 6LP, UK

Received May 10, 1988; Accepted June 28, 1988
Communicated by G. Wenzel

Summary. DNA from the cultivated mushroom, *Agaricus bisporus*, was cloned into the bacteriophage lambda vector EMBL3 creating a partial genomic library. Ten random clones from the library were used to probe for restriction fragment length polymorphisms (RFLPs). Six of the ten probes detected polymorphisms and were used to demonstrate variation in wild and cultivated strains of the mushroom. These results suggest that RFLPs could form a basis for genetic finger-printing and subsequent strain protection in *A. bisporus*. In single spore progeny, RFLPs were used to demonstrate normal meiotic segregation and to differentiate between homokaryons and heterokaryons. RFLPs therefore have great potential in the development of the genetics and breeding of this commercially important species.

Key words: Mushroom – RFLPs – DNA polymorphisms – *Agaricus bisporus*

Introduction

The white button mushroom, *Agaricus bisporus* (Lange) Imbach (syn. *A. brunnescens* Peck; Malloch 1976) is the principal fungal species in cultivation. It is grown worldwide with an annual value in excess of £ 2,000 million, and is a major horticultural crop in Europe, North America and Australasia. Despite its value and importance, progress in directed strain improvement has been limited by its breeding system. The basidia of this species normally bear two basidiospores, in contrast to the classical tetrad of spores of other species (Evans 1959), and these spores may germinate to form self-fertile or self-sterile mycelia; a system of sexuality described as secondary homothallism (Miller 1971; Raper et al. 1972; Elliott 1972). *Agaricus bisporus* also lacks the clamp connections, binucleate hyphal compartments and conjugate nuclear divisions normally associated with basidiomycete dikaryons.

Dramatic improvements in yield and quality have been achieved in many agricultural and horticultural crops through plant breeding involving the systematic assembly of genetic variation, recombination by crossing, and the selection of types with the best combination of features over a number of generations. In the case of the mushroom, however, most yield improvement is due to improved cultivation and the occasional selection of chance variants (Elliott 1985). More systematic programmes have not been launched in *A. bisporus* because homokaryons have proved difficult to produce, and the generation of useful genetic variants, including auxotrophs, has proved to be extremely difficult (Elliott 1985). Similarly, the lack of any morphological distinguishing features between homokaryons and heterokaryons remains a problem as heterokaryosis can only be confirmed by fruiting trials which are both expensive and time-consuming. Nevertheless, one case (Fritsche 1984) indicates what can be achieved. In this instance, putative homokaryons were intercrossed to produce hybrid strains which are commercially successful.

Isoenzyme variants have been used to assign commercial and laboratory strains to different allelic classes (Royse and May 1982a) and to monitor crosses between known homokaryotic lines (May and Royse 1982). However, observation of only five distinct genotypic classes led these authors to conclude that the cultivated mushroom was a 'near monoculture' (Royse and May 1982b). Recently, Castle et al. (1987) reported restriction fragment length polymorphisms (RFLPs) in both *A. brun-*

Offprint requests to D. Moore

nescens and *A. bitorquis*. RFLPs directly reflect DNA structure and permit application of standard methods of genetic analysis to evaluate the extent of genetic variation (Botstein et al. 1980); they might also provide a means of rapidly identifying homokaryons and heterokaryons (Castle et al. 1987). In this paper, we report on the construction of a partial genomic library of *Agaricus bisporus* and the use of ten randomly chosen clones as probes to assess the frequency and segregation of DNA polymorphisms in commercial strains of *A. bisporus*, some single-spore progeny including a unique tetrad, and strains derived from fruit bodies collected in the wild.

Materials and methods

Agaricus bisporus cultures

All mycelial strains came from the cryostat culture collection at the AFRC Institute for Horticultural Research (Littlehampton). Wild strains were collected as fruit bodies in the vicinity of Littlehampton and identified as *A. bisporus* on the basis of overall morphology and 2-spored basidia (Table 1). For DNA preparation, white cultivated strains were obtained as fruit bodies, wild and brown strains were grown as mycelial cultures on malt extract medium (2% malt extract + 1.5% agar, adjusted to pH 6.5 with NaOH) or complete yeast medium (2% glucose, 0.2% peptone, 0.2% yeast extract, 0.1% K_2HPO_4, 0.46% KH_2PO_4, 0.05% $MgSO_4$ and 1.5% agar). The malt extract used was that sold by The Boots Co., Nottingham, England.

Mycelium for DNA extraction was prepared from cultures grown at 25°C for 3–4 weeks on liquid malt extract medium (2% malt extract adjusted to pH 6.5). After harvesting, the mycelium was shock frozen in either liquid nitrogen vapour or in a −70°C freezer, freeze-dried, and then ground to a fine powder using a coffee grinder. Alternatively, whole fresh fruit bodies of *A. bisporus* were freeze-dried and ground with a coffee grinder. Strains used are described in Table 1.

Table 1. Strains of *Agaricus bisporus* used for the isolation of DNA

Code number	Origin
(a) fruit bodies of traditional strains	
A6	Supplied by Arun Valley Mushrooms, Littlehampton, W. Sussex; spawn produced by E. Hauser (England) Ltd., Yaxley, Peterborough PE7 3EJ
D21	Supplied by Stanley-Evans, Shackleford, Surrey; spawn produced by Darmycel (UK), Station Road, Rustington, Littlehampton, W. Sussex BN16 3RF
S22	Supplied by Blueprince Mushrooms, Oxted, Surrey; spawn produced by Darmycel
D649	Supplied by the IHR mushroom unit; spawn produced by Darmycel
(b) fruit bodies of hybrid strains	
U3	Supplied by the IHR mushroom unit; spawn from Darmycel
F44	Supplied by Winterpick Mushrooms, Henfield, W. Sussex; spawn produced by Ukital Spawn Ltd., 224 Redland Road, Bristol BS6 6YR
AX60	Supplied by Arun Valley Mushrooms: spawn from E. Hauser
(c) strains used solely as mycelial cultures	
W5	Isolated from the wild
W8	Isolated from the wild
C13	Brown cultivated variety
C27	Cultivated strain, traditional variety
C43-carb-13	Fungicide-resistant mutant

DNA preparation

Agaricus DNA was prepared following the CTAB (hexadecyltrimethyl-ammonium bromide) method described by Zolan and Pukkila (1986) modified as follows: 10 g of freeze-dried tissue was added to 100 cm^3 of CTAB extraction buffer (1% Sigma CTAB; 50 m*M* *Tris*/HCl, pH 8; 10 m*M* EDTA; 1% beta-mercaptoethanol; 0.7 *M* NaCl). After a 30 min incubation at room temperature, the preparation was successively extracted with phenol-chloroform-isoamyl alcohol (50:25:1) and ether, and the crude DNA was then precipitated with isopropanol. Centrifuged pellets were resuspended in TE buffer (10 m*M* *Tris*/HCl + 1 m*M* EDTA, pH 8.0), 1 g CsCl + 1 mg ethidium bromide per ml of sample was added, and the solution was centrifuged at 120,000 g for 65 h. The DNA band was syringed from the gradient, ethidium bromide removed by partition in isopropanol and the sample dialysed for 48 h with two changes of TE buffer. DNA was precipitated from the dialysed samples with ethanol and resuspended in TE buffer.

Cloning

DNA of *Agaricus bisporus* strain D649 was digested with *Sau*3A (BRL) using conditions chosen by experiment to give the optimum number of fragments of 15–20 kb (Maniatis et al. 1982). The *Agaricus* DNA was treated with calf intestinal alkaline phosphatase (BCL) and then ligated with T4 DNA ligase (BCL) into bacteriophage lambda EMBL3 DNA. The latter was prepared following the methods described by Frischauf et al. (1983).

A ligation of a 3:1 molar ratio of arms to inserts was used for packaging lambda EMBL3. A total of 5,558 recombinant plaques were recovered. According to the equation of Clarke and Carbon (1976), complete representation would require a minimum of 11,200 clones for DNA inserts of 14 kb, so although the *A. bisporus* lambda gene bank used here is only a partial gene library, it is adequate for the detection of RFLPs using randomly selected clones as probes.

Southern blotting

DNA from the seven cultivated mushroom strains was digested, separately, with *Bam*HI, *Eco*RI and *Sal*I according to the supplier's instructions (BCL). DNA from wild and brown strains was only digested with *Eco*RI. Samples of digested DNA were loaded into horizontal 0.7% agarose gels for electrophoresis at 1.0 V cm^{-1} for 16 h. Gels were stained with ethidium bromide and bands visualized with UV for photography.

DNA on the gels was denatured by 30 min exposure to 1.5 *M* NaCl/0.5 *M* NaOH at room temperature, and Southern blots were prepared in the conventional manner using Amersham Hybond N nylon sheets. Following Amersham's recommendation, the DNA was fixed to blots by 4 min exposure on a UV transilluminator.

DNA hybridization

DNA from ten randomly selected lambda clones from the gene library was radioactively labelled by nick translation (Rigby et al. 1977) with an Amersham International Nick Translation Kit (type N.5000) using [^{32}P]-dCTP. Unincorporated nucleotides were removed using the spin column procedure of Maniatis et al. (1982). Filters were placed in heat-sealed polythene bags with 20 ml prehybridization solution per filter (recipe: Wu et al. 1983). Bags were incubated overnight in solution in a 65 °C waterbath. The pre-hybridization solution was discarded and replaced with 5 cm^3 hybridization solution (Wu et al. 1983) per filter. Probe DNA was denatured by boiling for 10 min and was added to the bags, which were re-incubated for 12–16 h at 65 °C.

Unbound probe was washed off using three washes. Filters were washed for 10 min in 500 cm^3 2 × SSC, pre-warmed at 65 °C. A second wash was done with 500 cm^3 2 × SSC containing 0.1% SDS at 65 °C for 30 min. Finally, filters were washed for 20 min in 500 cm^3 0.1 × SSC at 65 °C.

Dried filters were wrapped in Saran Wrap and placed between two Dupont Intensifying Screens (Cronex Xtra Life Hi-Plus). The screens and filters were placed in an X-Ray cassette (Cuthbert Andrews or Kodak) and, loaded with Fuji or Kodak AR Safety X-Ray Film. Autoradiographs were left at −70 °C for up to 3 days for an image to form. X-Ray films were developed using Kodak D-15 developer and universal fixer.

Results

Cultivated strains

The seven cultivated strains showed RFLPs with six of the ten cloned fragments used as hybridization probes. The six polymorphic clones were numbers λAb15, λAb16, λAb19, λAb21, λAb22 and λAb24. Results from probing experiments with five of these are illustrated in Figs. 1 and 2 and Table 2. Only data for blots carrying DNA digested with *Eco*RI are shown; except where indicated in the next section, similar results were obtained with blots carrying DNA digested with *Sal*I and *Bam*HI. Because of technical difficulties, only incomplete results were obtained with clone λAb19, but it produced a pattern of hybridization on *Sal*I blots indicating polymorphism: strains U3 and AX60 (hybrids) had bands of hybridization of 3.9 kb and 2.1 kb and the two traditional cvs 'S22' and 'D649' possessed two bands of DNA hybridization of 3.9 kb and 2.7 kb.

Types of polymorphism

Although RFLPs were found between seven cultivated varieties of *Agaricus bisporus*, no polymorphic differences were found between the differently numbered hybrid cultivars.

The polymorphism among the traditional cultivated strains was of two types. In the first (shown with blots made with all three enzymes when probed with clones λAb15 and λAb21, *Sal*I and *Bam*HI with clone λAb16, *Sal*I with clone λAb19, and *Eco*RI and *Sal*I blots with clone λAb24), traditional strains of mushroom had one

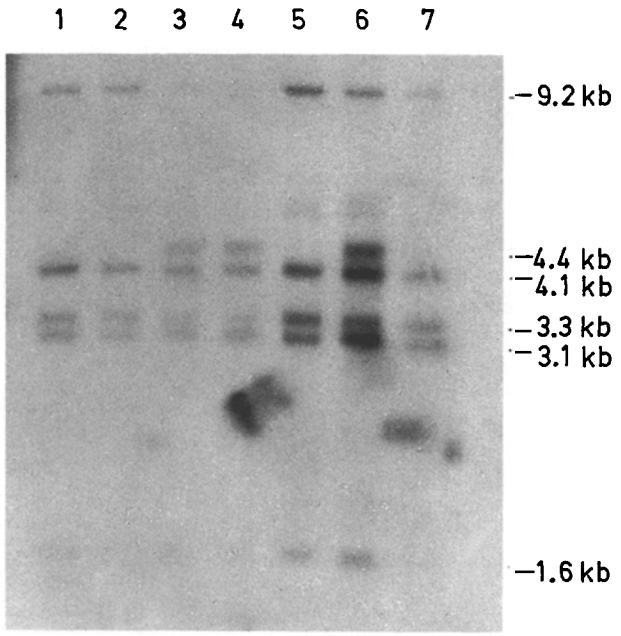

Fig. 1. Autoradiograph of an *Eco*RI Southern blot probed with clone λAb15. DNA samples from seven strains of *Agaricus bisporus* were electrophoresed in the different lanes as follows: *1* = A6, *2* = D21, *3* = U3, *4* = F44, *5* = S22, *6* = AX60, *7* = D649. Note that the strains can be distinguished on the basis of the presence or absence of a 4.4 kb hybridisation signal

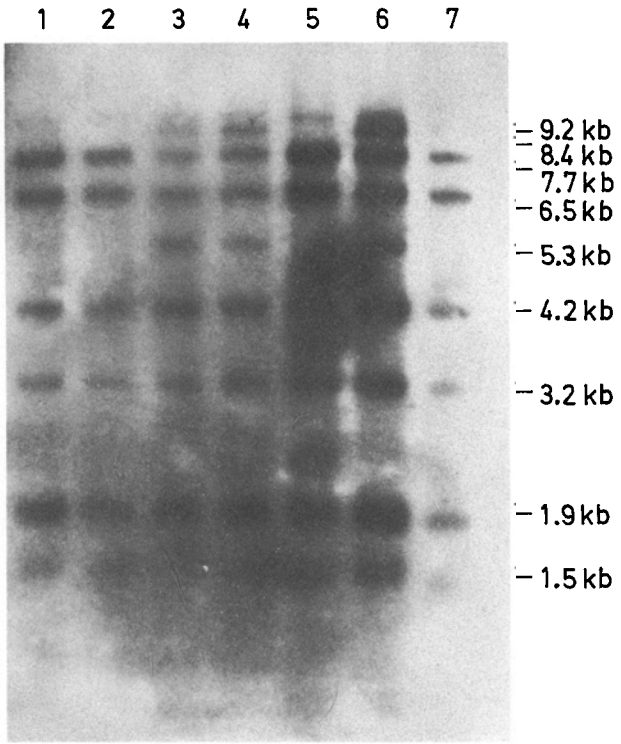

Fig. 2. Autoradiograph of an *Eco*RI Southern blot probed with clone λAb22. DNA samples from seven strains of *Agaricus bisporus* were electrophoresed in the different lanes as follows: *1* = A6, *2* = D21, *3* = U3, *4* = F44, *5* = S22, *6* = AX60, *7* = D649. Note that the strains can be distinguished on the basis of the presence or absence of 9.2, 8.4 and 5.3 kb hybridisation signals

Table 2. Molecular lengths of DNA fragments (kb) detected on autoradiographs of Southern blots of DNA digested with *Eco*RI and probed for hybridisation with radioactively-labelled DNA from cloned genomic fragments of *Agaricus bisporus*

Strain	Bands detected by the probe						Strain	Bands detected by the probe								
(a) probed with clone λAb15							(b) probed with clone λAb16									
A6	9.2		4.1	3.3	3.1	1.6	A6	9.3			6.5	4.9			3.2		2.6
D21	9.2		4.1	3.3	3.1	1.6	D21	9.3		7.0	6.5	4.9		3.4	3.2		2.6
U3	9.2	4.4	4.1	3.3	3.1	1.6	U3	9.3	8.0		6.5	4.9	3.6		3.2	2.8	2.6
F44	9.2	4.4	4.1	3.3	3.1	1.6	F44	not done									
S22	9.2		4.1	3.3	3.1	1.6	S22	not done									
AX60	9.2	4.4	4.1	3.3	3.1	1.6	AX60	9.3	8.0		6.5	4.9	3.6		3.2	2.8	2.6
D649	9.2		4.1	3.3	3.1	1.6	D649	9.3			6.5	4.9			3.2		2.6
(c) probed with clone λAb21							(d) probed with clone λAb22									
A6		13.1		5.5			A6			7.7	6.5		4.2	3.2	1.9		
D21		13.1		5.5			D21			7.7	6.5		4.2	3.2	1.9		
U3	19.9		10.7		5.3		U3	9.2	8.4	7.7	6.5	5.3	4.2	3.2	1.9		
F44	19.9		10.7		5.3		F44	9.2	8.4	7.7	6.5	5.3	4.2	3.2	1.9		
S22		13.1		5.5			S22	9.2		7.7	6.5		4.2	3.2	1.9		
AX60	19.9		10.7		5.3		AX60	9.2	8.4	7.7	6.5	5.3	4.2	3.2	1.9		
D649		13.1		5.5			D649			7.7	6.5		4.2	3.2	1.9		
(e) probed with clone λAb24																	
A6	11.7	8.8	6.0	3.6													
D21	11.7	8.8	6.0	3.6													
U3		8.8	6.0	3.6													
F44		8.8	6.0	3.6													
S22	11.7	8.8	6.0	3.6													
AX60		8.8	6.0	3.6													
D649	11.7	8.8	6.0	3.6													

DNA extracted from strains A6, D21, U3, F44, S22, AX60, and D649 was digested with *Eco*RI, electrophoresed and then Southern blots were probed with ^{32}P-labelled preparations of the cloned fragments. The table records the fragment size (kb) of each band to which the probe hybridised. Autoradiographs corresponding to panels (a) and (d) are shown as Figs. 1 and 2 respectively

pattern of hybridization, and the hybrid cultivars shared a different pattern. The second type of polymorphism encountered revealed genetic differences between the traditional cultivars. There were two examples of this, revealed (1) when *Eco*RI blots were probed with λAb22, and (2) when *Eco*RI blots were probed with λAb16 (Table 2).

In addition to the experiments illustrated in Table 2, one probing experiment was completed which compared cultivated, wild and brown strains, *Eco*RI blots being probed with clone λAb21. Four patterns were observed: (a) U3 possessed three bands of hybridization, at 19.9 kb, 10.7 kb and 5.3 kb; (b) W8, C27 and D649 all had an identical pattern of DNA hybridization, with two bands of 13.1 kb and 5.5 kb; (c) W5 possessed two bands of DNA hybridization of 11.7 kb and 7.7 kb; (d) C13 had two bands of DNA hybridization of 23.0 kb and 12.1 kb.

Identification of homokaryons and heterokaryons.

Probes λAb17, 18, 21 and 22 were used to analyse six single-spore progeny originating from aberrant three-spored basidia, following digestion of their DNA with *Eco*RI, *Sal*I, *Bam*HI and *Pst*I (Table 3). Five were shown to have partial patterns of DNA hybridization, consistent with their being homokaryotic. The sixth had the full pattern of hybridization seen in the parent, which is consistent with its being heterokaryotic. These data conform to the analysis of these strains by the use of fruiting trials conducted at IHR(L).

Segregations of DNA polymorphisms in progeny of Agaricus bisporus.

A complete meiotic tetrad isolated by micromanipulation from a rare four-spored basidium is held in the IHR culture collection. The opportunity was taken to test the meiotic segregation of polymorphisms detected with probe 22 in the members of this tetrad. In addition, a heterologous probe – the rDNA of *Coprinus cinereus* (Cassidy et al. 1984) – was also used. Results are shown in Fig. 3; the polymorphisms segregate in a regular 2:2 pattern in this tetrad of progeny. Isozyme analysis has also shown regular segregation in this tetrad (Royse and May 1982a).

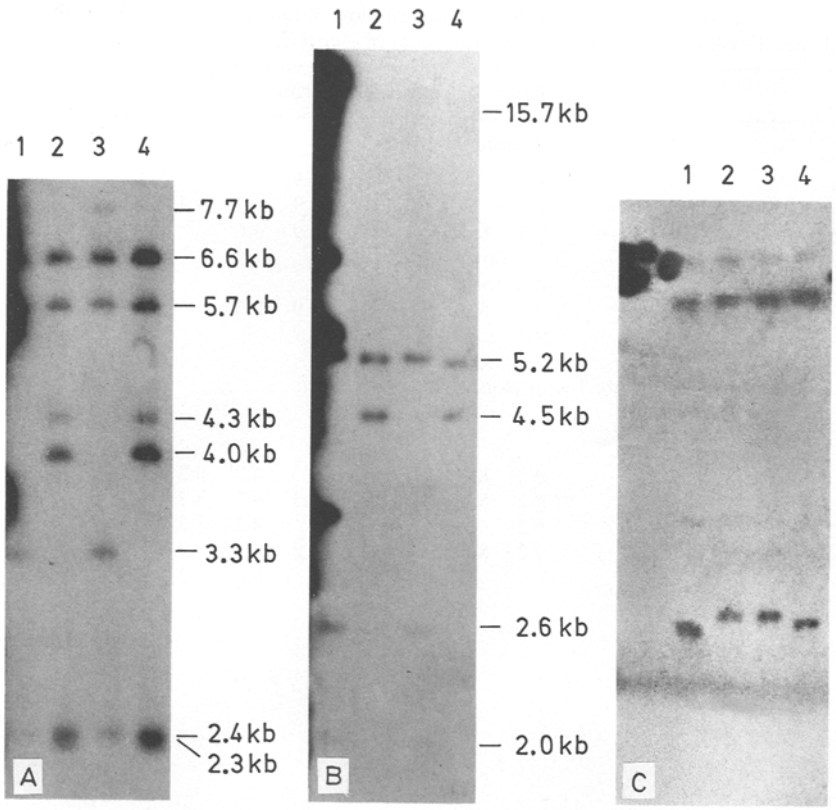

Fig. 3. Autoradiographs showing regular segregation of RFLPs in the four members of a tetrad of progeny of *Agaricus bisporus*. In each case *lanes 1* to *4* were loaded with DNA extracted from one of the tetrad progeny. In *panel A*, the DNA was digested with *Eco*RI and probed with clone λAb22; in *panel B*, the DNA was digested with *Sal*I and probed with clone λAb22, and in *panel C*, the DNA was digested with *Eco*RI and probed with a clone of the rDNA of *Coprinus cinereus*

Table 3. Classification of strains derived from a complete tetrad and some triad spores on the basis of DNA hybridization patterns

Strain	Hybridisation pattern[a]	Status
B237	Partial	Homokaryon
B238	Partial	Homokaryon
B239	Partial	Homokaryon
B240	Partial	Homokaryon
B200	Partial	Homokaryon
B201	Partial	Homokaryon
B202	Partial	Homokaryon
B213	Partial	Homokaryon
B214	Parental	Heterokaryon
B215	Partial	Homokaryon

[a] Parental means that the progeny mycelium showed the complete RFLP pattern of the parental heterokaryon used to produce the fruit bodies; partial means that only some of the bands seen in the parent were seen in the progeny

Discussion

The RFLPs found in the seven cultivars can be classified into two types: in the first type of polymorphism, differences were limited to those between the traditional strains as a group (A6, D21, S22 and D649) and the group of hybrid strains (U3, F44 and AX60). This presumably reflects the different genetic origins of the two groups of cultivars. The second class of RFLPs revealed polymorphisms between individual traditional strains and suggests a degree of genetic variability in the traditional cultivars, though only of a limited extent as RFLPs were found with only 2 out of 27 probe/blot combinations.

No polymorphism was detected between the three hybrid cultivars, an unexpected outcome since all three strains are marketed by different companies as original and independent products. The original hybrid, strain U3, was bred by crossing homokaryons isolated from two strains with contrasting agronomic characteristics (Fritsche 1984). If the same approach was used by the other commercial companies to produce their own strains, then they have selected hybrid heterokaryons which give the same patterns of DNA hybridization with ten probes and three different restriction enzymes. As the growth conditions and flushing (times of fruiting) patterns for each of the three strains are also very similar, the coincidence of their genetic similarity is even more remarkable.

Among the four traditional strains, RFLPs permitted three genotypic classes to be distinguished: S22, D21, and A6/D649. RFLPs thus have potential for the 'genetic fingerprinting' of strains of mushroom in a similar manner to the technique described by Jeffreys et al. (1985) which can distinguish between human individuals.

The results shown here have identified three classes of traditional strains on the basis of randomly chosen RFLPs, and more such probes or a hypervariable fragment could provide a very accurate strain typing method.

Genetic variation in mushroom cultivars.

Klingman (1950) reported that in 1927 a clump of pure white, smooth-capped mushrooms were found in a bed planted with a strain of mushroom with cream-coloured sporophores. Some authors (for example Fritsche 1978) believe that all white mushrooms grown today are derived from this chance cluster. It is difficult to be certain of the origin of the white cultivars because of commercial secrecy and the time that has elapsed since their appearance. If the assertion of Fritsche (1978) is true, then the older, traditional varieties should be isogenic. The genetic differences detected with two probe/blot combinations could have occurred through the multispore selection procedure adopted by spawn makers. Multispore selection is based on germinating a large number of basidiospores from a sporophore with selected desirable characteristics (Elliott 1985). In this way, strains can be improved, and over a number of generations it is possible that slight differences in the sequence of DNA could occur, for example through point mutations and meiotic recombination, and these differences could be detected as RFLPs.

On the basis of the results presented here, the hybrids certainly appear to be isogenic, and the traditional strains appear to be closely related. The isoenzyme analysis of Royse and May (1982a, b) and May and Royse (1982) found variation at 5 structural gene loci in 34 commercial lines. In contrast, we have tested seven cultivated strains and found genetic variation at six out of ten loci. This is a much higher level of variation than that found when isoenzymes were used. However, the differences detected here were largely between the traditional and the hybrid cultivars. If this dichotomy is ignored (hybrid strains were not released until 1981 so were not included in the isozyme analyses), and the traditional strains are considered in isolation, then variation was found for only two out of ten cloned fragments, a level of variation comparable with that found when isoenzymes were examined.

Hintz et al. (1985) examined restriction patterns of mitochondrial DNA of four strains of *A. bisporus* and ten strains of *A. bitorquis*. In *A. bisporus* restriction patterns were identical for each of the four strains, whereas *A. bitorquis* isolates were very polymorphic. The authors concluded that cultivated mushrooms expressed little genetic variability. Thus, both mitochondrial and nuclear genomes of *A. bisporus* show little variation.

Nevertheless, some genetic variation *is* detectable with cloned genomic fragments. For example, clone λAb21 revealed two patterns of DNA hybridization among the seven white cultivars and separated a heterogeneous group consisting of wild strains (W5 and W8), a brown-capped cultivated strain (C13) and some white cultivated varieties (C27, C43-carb-13, U3 and D649) into four classes on the basis of patterns of DNA hybridization. Of the two wild strains, W8 had a similar RFLP pattern to commercial strains C27, C43-carb 13 and D649, and may represent an escape from commercial cultivation. By contrast, W5 had a unique RFLP pattern and is perhaps a truly wild *A. bisporus* and, consequently, a useful potential source of novel variation.

Identification of genetic variation using RFLPs could, therefore, provide the basis for a programme of genetic improvement of the mushroom crop. The demonstration that the clone λAb22 and rDNA polymorphisms segregated 2:2 in the products of a single meiosis is important as it shows that despite the peculiarities of nuclear segregation in this two-spored species, regular segregation of genetic markers can be expected. The approach serves also to demonstrate that clone λAb22 is a genomic (as opposed to mitochondrial) fragment. Thus, as well as showing that there is a useful resource of genetic variation even within the few strains tested here, RFLPs could serve as genetic markers for formal genetic analysis of *A. bisporus* following the approach described by Raeder and Broda (1986) and thereby provide the foundation for breeding programmes.

Acknowledgements. We thank the Science and Engineering Research Council for the award of a CASE research studentship to MGL and Dr. P. J. Pukkila for providing the *Coprinus cinereus* rDNA clone.

References

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Cassidy JR, Moore D, Lu BJ, Pukkila PJ (1984) Unusual organisation and lack of recombination in the ribosomal genes of *Coprinus cinereus*. Curr Genet 8:607–613

Castle AJ, Horgen PA, Anderson JB (1987) Restriction fragment length polymorphisms in the mushrooms *Agaricus brunnescens* and *Agaricus bitorquis*. Appl Environ Microbiol 53:816–822

Clarke L, Carbon J (1976) A colony bank containing synthetic Col EI hybrid plasmids representative of the entire *E. coli* genome. Cell 9:91–99

Elliott TJ (1972) Sex and the single spore. Mushroom Sci 8:11–18

Elliott TJ (1985) The genetics and breeding of species of *Agaricus*. In: Flegg PB, Spencer DM, Wood DA (eds) The Biology and technology of the cultivated mushroom. Wiley, London, pp 111–139

Evans HJ (1959) Nuclear behaviour in the cultivated mushroom. Cromosoma 10:115–135

Frischauf AM, Lehrach H, Poutska A, Murray N (1983) Lambda replacement vectors carrying polylinker sequences. J Mol Biol 170:827–842

Fritsche G (1978) Breeding work. In: Chang ST, Hayes WA (eds) The Biology and cultivation of edible mushrooms. Academic Press, New York, pp 239–250

Fritsche G (1984) Breeding *Agaricus bisporus* at the mushroom experimental station, Horst. Mushroom J 122:49–55

Hintz WE, Mohan M, Anderson JB, Horgen PA (1985) The mitochondrial DNAs of *Agaricus:* heterogeneity in *A. bitorquis* and homogeneity in *A. brunnescens*. Curr Genet 9: 127–132

Jeffreys AJ, Wilson V, Thein SL (1985) Hypervariable minisatellite regions in human DNA. Nature 314:67–73

Kligman AM (1950) Handbook of mushroom culture. Business Press, Lancaster/PA

Malloch D (1976) *Agaricus brunnescens:* the cultivated mushroom. Mycologia 68:910–919

Maniatis T, Fritsche EF, Sambrook J (1982) Molecular cloning – a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor/NY

May B, Royse DJ (1982) Confirmation of crosses between lines of *Agaricus brunnescens* by isozyme analysis. Exp Mycol 6:238–292

Miller RE (1971) Evidence of sexuality in the cultivated mushroom *Agaricus bisporus*. Mycologia 63:630–634

Raeder U, Broda P (1986) Meiotic segregation analysis of restriction site polymorphisms allows rapid genetic mapping. EMBO J 5:1125–1127

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

Rigby PWJ, Dieckmann M, Rhodes C, Berg P (1977) Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113:237–251

Royse DJ, May B (1982a) Use of isozyme variation to identify classes of *Agaricus brunnescens*. Mycologia 74:93–102

Royse DJ, May B (1982b) Genetic relatedness and its application in selective breeding of *Agaricus brunnescens*. Mycologia 74:569–575

Wu MJW, Cassidy JR, Pukkila PJ (1983) Polymorphisms in DNA of *Coprinus cinereus*. Curr Genet 7:385–392

Zolan ME, Pukkila PJ (1986). Inheritance of DNA methylation in *Coprinus cinereus*. Mol Cell Biol 6:195–200