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DNA Amplification Polymorphisms of the Cultivated Mushroom *Agaricus bisporus*

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Single 10-bp primers were used to generate random amplified polymorphic DNA (RAPD) markers from commercial and wild strains of the cultivated mushroom *Agaricus bisporus* via the polymerase chain reaction. Of 20 primers tested, 19 amplified *A. bisporus* DNA, each producing 5 to 15 scorable markers ranging from 0.5 to 3.0 kbp. RAPD markers identified seven distinct genotypes among eight heterokaryotic strains; two of the commercial strains were shown to be related to each other through single-spore descent. Homokaryons recovered from protoplast regenerants of heterokaryotic strains carried a subset of the RAPD markers found in the heterokaryon, and both of the haploid nuclei from two heterokaryons were distinguishable. RAPD markers also served to verify the creation of a hybrid heterokaryon and to analyze meiotic progeny from this new strain: most of the basidiospores displayed RAPD fingerprints identical to that of the parental heterokaryon, although a few selected slow growers were homoallelic at a number of loci that were heteroallelic in the parent, suggesting that they represented rare homokaryotic basidiospores; crossover events between a RAPD marker locus and its respective centromere appeared to be infrequent. These results demonstrate that RAPD markers provide an efficient alternative for strain fingerprinting and a versatile tool for genetic studies and manipulations of *A. bisporus*.

Breeding programs for the commercially valuable but genetically recalcitrant basidiomycete *Agaricus bisporus* (Lange) Imbach (= *A. brunnescens* Peck), commonly known as the button mushroom, have traditionally suffered from an absence of outcrossing between strains and a dearth of genetic markers. An unusual lifestyle, termed secondary homothallism, results in a predominance of two-spored basidia: most basidiospores receive two meiotic nuclei and germinate into heterokaryotic, self-fertile mycelia that are capable of fruiting (4, 15). The lack of a haploid, uninucleate stage renders both hybridizations between strains and the creation of genetically marked lines such as auxotrophs difficult. Consequently, most strain improvement to date has consisted of the evaluation of single-spore progeny from extant cultivars for phenotypic variants, and as a result, commercial strains encompass extremely limited genetic resources (10, 16). A small number of hybridizations between strains have been achieved by isolating uninucleate spores from aberrant three- or four-spored basidia, germinating them to produce homokaryotic mycelia, and attempting to pair different homokaryons via mycelial anastomosis (14). Without genetic markers or reliable morphological indicators of karyogamy, such as clamp connections, growth rate, or mycelial appearance, however, fruiting trials were necessary to confirm each putative homokaryon or heterokaryon, and the 10-year period required to produce the current popular hybrid strains is a reflection of the labor involved in this process (7).

Recently, however, isozyme-encoding loci (12) and restriction fragment length polymorphisms (RFLPs) (1a) have provided simple, stable genetic markers for *A. bisporus*, and studies of their sexual transmission have elucidated interesting aspects of the *A. bisporus* life cycle: over 90% of single-spore isolates (SSIs) that germinate inherit both parental alleles at the isozyme and RFLP loci analyzed, indicating that postmeiotic, nonsister nucleic are preferentially packaged together in basidiospores; the remaining SSIs appear homoallelic at all of the loci scored, suggesting that they represent homokaryotic spores which received a single postmeiotic nucleus (16, 20). These findings of nonrandom segregation of the four meiotic products are consistent with cytological studies which noted specific spindle alignments during meiosis and predicted that levels of heterozygosity at a given locus would be inversely proportional to its distance from its respective centromere (5). In general, however, evidence for recombination, such as single-spore lines that display homoallelism at some fraction of the loci that were heteroallelic in the parental line, is surprisingly rare (8, 16, 20).

Heterokaryotic strains of *A. bisporus*, perhaps as a consequence of this unusual life cycle, appear to be highly heteromorphic at both isozyme (8, 13, 17) and RFLP (1a, 11) loci. These markers, therefore, also provide a reliable means for both identifying homokaryotic single-spore progeny and protoplast regenerants of heterokaryotic strains that function as haploid propagules in a breeding program and for verifying any resulting artificial hybrids (2, 13). In addition, isozymes and RFLPs demonstrate that a high level of genetic diversity, which is lacking in the commercial strains, is present in wild isolates, suggesting that a source of novel germ plasm is available for introgression into the cultivated gene pool of *A. bisporus* (1a, 8, 10, 11). The manipulation of genetic traits via conventional breeding schemes may, however, prove cumbersome owing to the observed lack of recombination in this organism; additional information on linkage relationships and genetic segregation in *A. bisporus* will become available with the construction of a genetic map using both isozyme and DNA markers (9).

To facilitate the breeding and genetic analysis of *A. bisporus* further, we investigated the utility of DNA polymorphisms generated by amplifying genomic DNA with single, arbitrary 10-bp primers via the polymerase chain reaction (21, 22) as genetic markers in this organism. Ran-

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TABLE 1. Strains of *A. bisporus* used in this study

Isolate type and strain no.	Other designation	Origin
Commercial		
AA-1	Horst U1	Cultivated hybrid
AA-2	Amycel 208	Cultivated hybrid
AA-3	Somycel 456	Cultivated brown
Wild[a]		
AA-17	RWK 1533 (ARP-1)	California
AA-18	RWK 1534	California
AA-21	RWK 1537	California
AA-82	FS6 (ARP-7)	California
AA-90	I3	Israel
Homokaryon		
91-5		AA-1 protoplast regenerant
91-51		AA-1 protoplast regenerant
91-95		AA-2 protoplast regenerant
91-86		AA-2 protoplast regenerant

[a] All wild isolates were provided by R. W. Kerrigan (University of Toronto, Mississauga, Ontario, Canada). Isolates with ARP numbers are included in the Agaricus Recovery Program managed by R. W. Kerrigan.

dom amplified polymorphic DNA (RAPD) loci serve as dominant markers which are detected as DNA segments that amplify in one parent but not the other and have been characterized in a number of species, including humans, maize, soybeans, the fungus *Neurospora crassa*, and various prokaryotes. They offer numerous advantages over other DNA markers: they do not require any of the DNA cloning, hybridization, or detection techniques associated with RFLPs, thereby offering great savings in labor and time, and unlike other polymorphism assays based on the polymerase chain reaction, they do not rely on any previous sequence information (21, 22). In addition, RAPDs often identify multiple loci in a single reaction, making them useful for DNA fingerprinting as well as linkage analysis. In this study, we demonstrated that RAPD markers can be used to (i) differentiate between various strains of *A. bisporus*, (ii) identify the two haploid nuclear components of a heterokaryotic strain, (iii) verify artificially synthesized heterokaryons, and (iv) monitor the transmission of genetic loci to progeny isolates.

MATERIALS AND METHODS

Strains. The *A. bisporus* strains used in this study are listed in Table 1. Mycelial cultures of each strain were grown on solid compost extract medium (19) and in liquid MPFYE media (1a). Fruiting trials were performed at the Monterey Mushrooms Inc. pilot production facility.

Protoplast production and regeneration. Protoplasts of strains AA-1 and AA-2 were isolated and regenerated as described by Sonnenberg et al. (19). Novozyme 234 (Novo Biolabs, Bagsvaerd, Denmark), at a concentration of 5 mg/ml, was used as a cell wall-lytic enzyme along with 0.1 mg of chitinase (*Streptomyces griseus*; Sigma) per ml. Regeneration frequencies varied from 1 to 10%, and homokaryotic regenerants were initially selected as slow growers on compost extract medium.

DNA isolation. Total DNA for Southern hybridizations and RAPD reactions was isolated by using a variation of the plant DNA miniprep described by Dellaporta et al. (3). Ten grams of mycelial tissue obtained from a liquid culture or 10 g of fresh cap tissue was freeze-dried overnight and then ground by using a mortar and pestle. The resulting powder was transferred to a 50-ml disposable tube containing 15 ml of cold extraction buffer (100 mM Tris-HCl [pH 8], 50 mM EDTA, 500 mM NaCl, 20 mM Na-bisulfite) and vortexed. Two milliliters of 10% sodium dodecyl sulfate was added, and the solution was mixed well and heated for 15 min at 65°C. Five milliliters of 5 M potassium acetate was then added to the solution, which was again mixed well and placed on ice for 20 min. After the tubes were centrifuged at 13,000 × *g* for 20 min, the supernatant was poured through two layers of cheesecloth into a 50-ml tube containing 10 ml of isopropanol. Precipitated nucleic acids were spooled out with a sterile Pasteur pipet, washed in 70% ethanol, air dried, and suspended in 700 μl of TE (50 mM Tris-HCl [pH 8.0], 10 mM EDTA) in a 1.5-ml microcentrifuge tube. This DNA solution was incubated at 37°C for 1 h with 1 μl of RNase A (10 mg/ml), extracted with phenol-$CHCl_3$, and precipitated with 0.1 volume of 2.5 M sodium acetate and 0.7 volume of isopropanol. The resulting DNA was suspended in 100 μl of TE, and 5 μl was run on a 0.7% agarose gel to assay its concentration and integrity.

Southern hybridizations. From 3 to 5 μg of total DNA from each strain was digested to completion with either restriction enzyme *Eco*RI or *Bam*HI (5 U/μg of DNA) under conditions recommended by the manufacturer (Promega, Madison, Wis.). The digested DNA was size fractionated on 0.7% agarose gels by using 1× TBE (0.089 M Tris base, 0.089 M H_3BO_3, 0.002 M EDTA) buffer, stained with 0.5 mg of ethidium bromide per ml, and transferred to GeneScreen Plus hybridization membranes by using the capillary method (18). The membranes were probed, washed, and exposed to X-ray film as recommended by the manufacturer (Du Pont NEN Inc., Boston, Mass.).

Probe preparation. Two single-copy sequences of *A. bisporus* genomic DNA were used to detect RFLPs. pAg33n10 is a 1.5-kbp *Eco*RI restriction fragment cloned into pUC18 (1a) and kindly provided by J. B. Anderson (University of Toronto, Mississauga, Ontario, Canada); pTRP800 is an 800-bp fragment amplified via the polymerase chain reaction and cloned into pCR1000 (Invitrogen Corp., San Diego, Calif.). The probes were separated from vector sequences by restriction enzyme digests and electrophoresis in low-melting-point agarose (FMC Corp., Rockland, Maine) and labelled with [α-^{32}P]dCTP (3,000 Ci/mmol; Du Pont NEN) by using random primers (6) obtained as a commercial kit (Amersham Inc., Arlington Heights, Ill.).

RAPD reactions. RAPD marker conditions were essentially as described by Williams et al. (22). The amplification reactions were done in 25 μl and consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, each dNTP at 100 μM, and 0.2 μM primer. Approximately 5 ng of DNA and 1 U of native *Taq* DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn.) were used for each reaction. The reactions were performed in a Genetic Thermal Cycler (Precision Scientific Inc., Chicago, Ill.) for 45 cycles by using the following parameters: 92°C, 1 min; 35°C, 1 min; 72°C, 2 min. A 10-μl volume of each reaction was analyzed on a 1.4% agarose gel run at 3 V/cm and stained with ethidium bromide. The 20 primers utilized for this study comprise RAPD primer Kit A from Operon Technologies Inc. (Alameda, Calif.), which consists of random 10-mers of 60 to 70% G+C content that lack self-complementary ends. The sequences of the four primers whose amplification products are shown in Fig. 2, 4, and 5 are as follows: A-02,

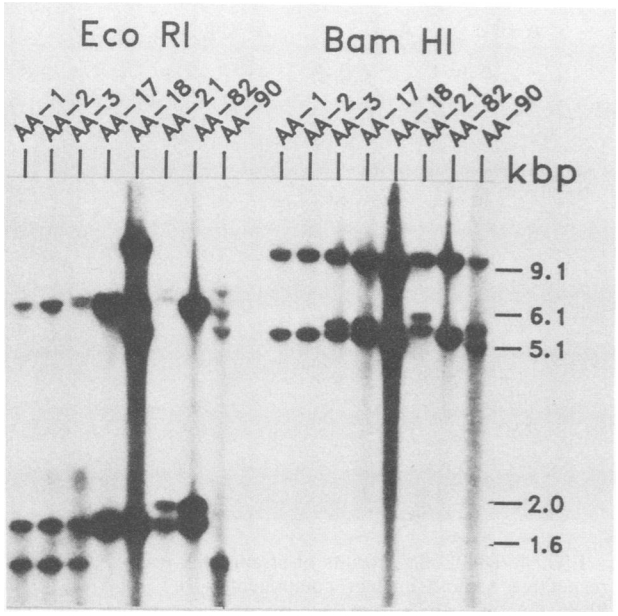

FIG. 1. RFLP fingerprints of eight *A. bisporus* strains. A Southern blot of genomic DNA from each strain, digested with *Eco*RI and *Bam*HI, was hybridized with probe pAg33n10. Strains AA-17, AA-18, and AA-82 each displayed two *Eco*RI bands larger than 6.0 kb that are impossible to distinguish on this autoradiogram.

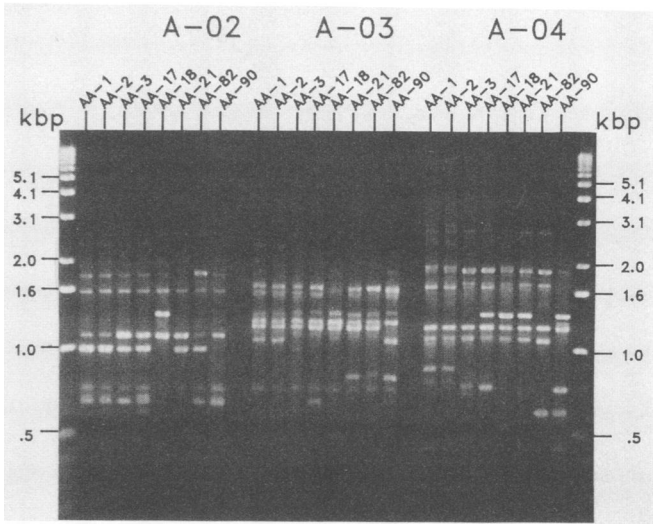

FIG. 2. RAPD fingerprints of eight *A. bisporus* strains. Genomic DNA from each strain was amplified with primers A-02, A-03, and A-04 individually, and the resulting products were separated on a 1.4% agarose gel stained with ethidium bromide. The left- and rightmost lanes contained the 1-kb size marker (BRL, Gaithersburg, Md.).

5′-TGCCGAGCTG-3′; A-03, 5′-AGTCAGCCAC-3′; A-04, 5′-AATCGGGCTG-3′; A-07, 5′-GAAACGGGTG-3′.

RESULTS

Strain fingerprinting. Variation among the eight heterokaryotic strains selected for strain fingerprinting experiments, and described in Table 1, was initially examined by comparing *Eco*RI and *Bam*HI polymorphisms detected by probe pAg33n10 (Fig. 1). *Eco*RI polymorphisms differentiated between all of the strains except AA-1 and AA-2, while *Bam*HI polymorphisms placed the strains into the following five groups: 1, AA-1 and AA-2; 2, AA-3 and AA-17; 3, AA-18 and AA-82; 4, AA-21; 5, AA-90. At least three *Eco*RI restriction fragments in each strain hybridized to pAg33n10, which is only a 1.5-kb *Eco*RI fragment, suggesting that all eight strains are heteroallelic for *Eco*RI polymorphisms at the pAG33n10 locus.

RAPD fingerprints of the eight strains are depicted in Fig. 2. Among the eight strains, primer A-02 amplified an average of 9 scorable loci, A-03 amplified an average of 7 scorable loci, and primer A-04 amplified an average of 11 scorable loci; under different electrophoresis conditions, some of the brighter bands were resolved into two distinct bands (data not shown). Scorable fragments ranged in size from 0.5 to 3.0 kb, and excluding strains AA-1 and AA-2, which contained identical amplification loci for every primer, each strain displayed a unique RAPD fingerprint with each primer. Of the 20 10-mer primers we tested, only one (A-06 [5′-GGTCCCTGAC-3′]) did not amplify *A. bisporus* DNA fragments under the reaction conditions described (data not shown).

These results demonstrate that single 10-mers are capable of amplifying multiple polymorphic loci in *A. bisporus* that readily differentiate between strains. The identical RFLP and RAPD fingerprints displayed by strains AA-1 and AA-2 suggest that these two strains are either identical or closely related, which supports findings by Loftus et al. (11), who found no RFLP variation among three hybrid cultivars marketed in Europe as different strains.

Homokaryon identification. Homokaryotic isolates from strains AA-1 and AA-2 were identified by screening slowly growing protoplast regenerants from the two strains. Figure 3 shows RFLP fingerprints of the two homokaryotic types—isolates 91-5 and 91-51—obtained from strain AA-1 and the two homokaryotic types—isolates 91-95 and 91-86—obtained from strain AA-2. As expected, each homokaryon displayed a subset of the restriction fragments found in the parental heterokaryon. The Southern blot shown in Fig. 3 was hybridized simultaneously with two different probes—pAg33n10 and pTRP800, and although the two parental heterokaryons (AA-1 and AA-2) have identical RFLP patterns, it is evident that alleles of the two probe loci are paired differently in the homokaryons from each strain: the 2.9-kb pTRP800 allele segregated with the 1.8-kb pAg33n10 allele, and the 2.7-kb pTRP800 allele segregated with the 1.5-kb pAg33n10 allele in homokaryons of strain AA-1, while the converse was true for homokaryons from strain AA-2.

RAPD profiles of the two heterokaryons and their corresponding homokaryons were compared (Fig. 4) by using primers A-02, A-03, A-04, and A-07. For each primer, strains AA-1 and AA-2 displayed identical amplification products and the two homokaryotic isolates from each strain displayed a subset of the parental bands; homoallelic amplification polymorphisms in strains AA-1 and AA-2 were found in both of their homokaryotic types, while heteroallelic amplification polymorphisms were found in only one homokaryotic type. This is illustrated for amplification products produced by primer A-02 in Table 2. Among the four primers shown in Fig. 4, 31 RAPD loci in strain AA-1 could be scored with confidence as heteroallelic by noting the segregation of amplification polymorphisms between the two haploid nuclei. All 31 of these loci were also heteroallelic in

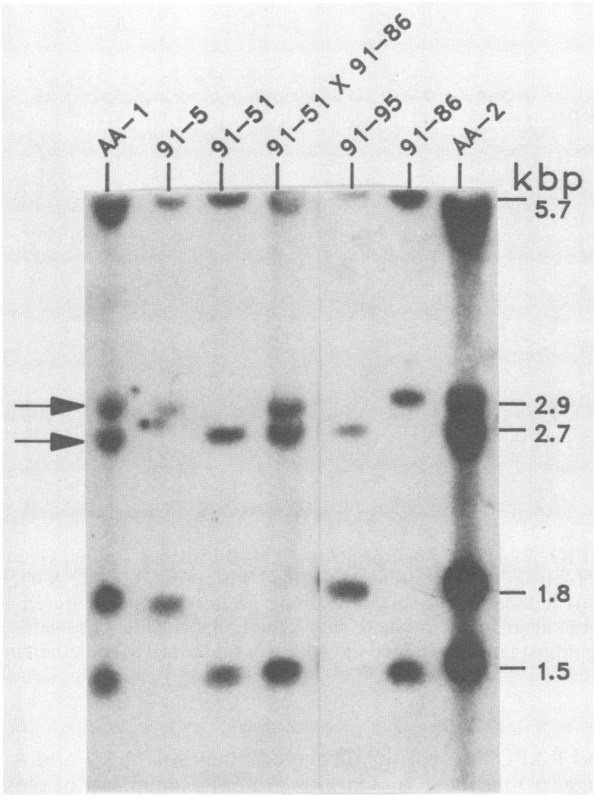

FIG. 3. RFLP fingerprints of strains AA-1 and AA-2; their respective haploid nuclear components (91-5, 91-51, 91-95, and 91-86), identified among protoplast regenerants from each strain; and a hybrid of strains AA-1 and AA-2 produced by crossing 91-51 with 91-86. A Southern blot of *Eco*RI-digested genomic DNA from each isolate was hybridized simultaneously with probes pAg33n10 and pTRP800; the two restriction fragments of 2.9 and 2.7 kb that hybridized to pTRP800 are indicated by the arrows.

strain AA-2, although as was evident for the RFLPs shown in Fig. 3, some polymorphisms segregated among the two haploid nuclei of strain AA-2 in combinations different from those seen with strain AA-1.

Anecdotal evidence suggests that strain AA-2 is related to strain AA-1 via single-spore descent, and the genotypes observed at the DNA loci scored in this study support this assumption: both heterokaryotic strains share identical RFLP and DNA amplification polymorphisms, yet it is obvious that alleles of some loci are present in the two haploid nuclei of each strain in different combinations. These findings are consistent with the meiotic process in *A. bisporus*, wherein chromosomes assort randomly but low levels of recombination and the preferential pairing of postmeiotic nonsister nuclei tend to perpetuate "pseudoclonal" lineages among single-spore isolates (8, 16, 20).

Hybrid identification and genetic segregation. A hybrid heterokaryon was created by plating homokaryons 91-51 and 91-86 on a single petri dish and subculturing mycelium from the zone of anastomosis; the putative hybrid mycelium was fruited, and DNA isolated from the resulting sporophores was analyzed with both RFLPs (Fig. 3) and RAPDs (Fig. 5) to confirm its hybrid status. SSIs from a single 91-51 × 91-86 hybrid mushroom were germinated, and a range of growth rates from very fast to very slow was observed. DNAs

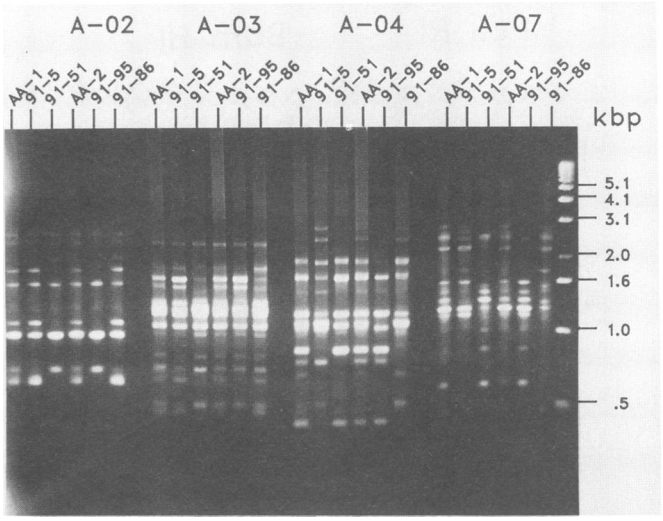

FIG. 4. RAPD fingerprints of strains AA-1 and AA-2 and their respective haploid nuclear components—91-5, 91-51, 91-95, and 91-86. Genomic DNA from each isolate was amplified with primers A-02, A-03, A-04, and A-07 individually. The rightmost lane contained the 1-kb size marker (BRL).

extracted from a total of 19 slowly, moderately, and fast-growing SSIs were subjected to RAPD analysis; amplification products generated by primer A-02 from homokaryon 91-51, homokaryon 91-86, the hybrid heterokaryon, and the 19 SSIs are shown in Fig. 5.

On the basis of the genotypes of the two parental homokaryons, it is evident that primers A-02, A-03, and A-04 together amplify 13 heteroallelic loci in the hybrid heterokaryon. Table 3 summarizes the segregation results for these 13 loci among the 19 SSIs. As described by Summerbell et al. (20), a single crossover between any locus heteroallelic in an *A. bisporus* heterokaryon and its centromere will result in a homoallelic genotype in half of the progeny that receive postmeiotic nonsister nuclei. Since RAPDs are dominant genetic markers, however, only 50% of these homoallelic individuals—those that are homoallelic for the absence of an amplification product—will be recognizable. Five SSIs, 3, 7, 13, 18, and 19, lack amplification products at multiple loci; these isolates are also slow grow-

TABLE 2. Primer A-02 RAPD loci in strains AA-1 and AA-2 and their respective homokaryons

Amplification locus (size [kb])[a]	Presence of amplification product in strain:					
	AA-1	91-5	91-51	AA-2	91-95	91-86
A-02-1 (2.5)	+	+	+	+	+	+
A-02-2 (2.3)	+	+	+	+	+	+
A-02-3 (2.0)	+	−	+	+	−	+
A-02-4 (1.8)	+	+	−	+	−	+
A-02-5 (1.6)	+	+	+	+	+	+
A-02-6 (1.1)	+	+	−	+	−	+
A-02-7 (1.0)	+	+	+	+	+	+
A-02-8 (0.7)	+	−	+	+	+	−
A-02-9 (0.65)	+	+	−	+	−	+
A-02-10 (0.6)	+	+	−	+	−	+
A-02-11 (0.48)	+	−	+	+	+	−

[a] Primer A-02 amplification loci are numbered consecutively, beginning with the largest.

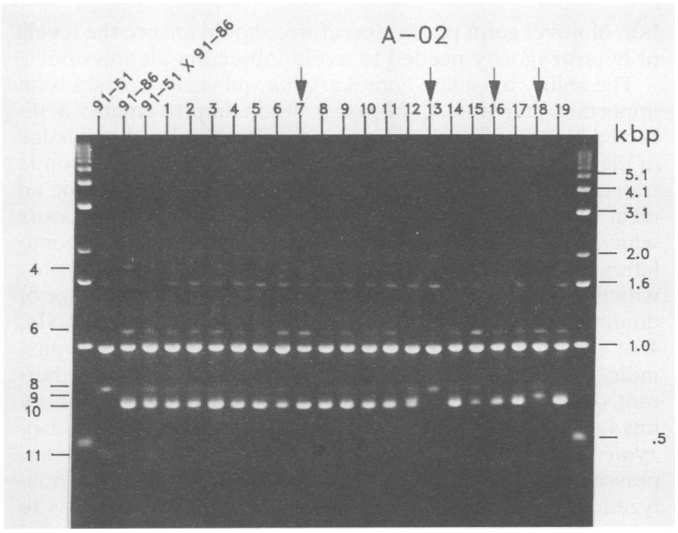

FIG. 5. RAPD fingerprints of homokaryons 91-51 and 91-86, the heterokaryon generated by crossing the two homokaryons, and 19 SSIs collected from the resulting hybrid sporophore. Genomic DNA from each isolate was amplified with primer A-02. Heteroallelic amplification loci in the heterokaryon are numbered on the left; the numbers correspond to A-02 RAPD loci generated in strains AA-1 and AA-2. The SSIs indicated by arrows are homoallelic at one or more of the heteroallelic loci present in the hybrid parent: SSI 13 does not display bands at loci 4, 6, 9, and 10; SSI 16 lacks a band at locus 8; and SSI 18 lacks a band at locus 10. Although loci 9 and 10 are difficult to score on this gel, other gels (not shown) showed that SSI 7 also lacks a band at locus 8 while SSI 15 does contain bands at loci 9 and 10.

ers and therefore probably represent either homokaryons arising from uninucleate basidiospores or heterokaryons composed of postmeiotic sister nuclei. An example of a crossover proximal to a RAPD locus is evident in SSI 16, a fast grower that lacks an amplification product at only one locus (A-02-8). The rest of the SSIs displayed amplification products at all 13 loci and are most likely heterokaryons that received postmeiotic nonsister nuclei. Potential recombination events may be masked in these isolates, although it is reasonable to infer that these events are rare since the number of loci homoallelic for the absence of a RAPD marker is so limited.

DISCUSSION

The limited genetic manipulation of *A. bisporus* to date and the ambiguity surrounding its sexual life cycle underscore the necessity of stable, simply inherited markers for this commercially important organism, and recently, both isozymes and RFLPs have proven invaluable in this respect (1a, 12). In this study, we demonstrated the potential of RAPDs as genetic markers for *A. bisporus*: as previously shown for a range of species from bacteria to humans (21, 22), single, arbitrary 10-bp oligonucleotides are also capable of amplifying multiple fragments of the *A. bisporus* genome via the polymerase chain reaction, and variations in primer-binding sites between strains result in amplification polymorphisms that function as genetic markers. Several features of RAPDs, demonstrated in our results, illustrate their utility for strain characterization and improvement: the high percentage of 10-mers that amplified *A. bisporus* DNA under our conditions, the large number of loci targeted by each primer, and the ease with which these markers are generated. In comparison, isozymes are restricted to a few useful

TABLE 3. Segregation of heteroallelic RAPD loci generated by primers A-02, A-03, and A-04 among 19 SSIs isolated from hybrid mushroom 91-51 × 91-86

Strain or SSI	Growth rate	Presence of amplification product in heteroallelic RAPD loci generated by:												
		A-02					A-03			A-04				
		4	6	8	9	10	5	12	14	1	2	9	11	13
Strains														
91-51	Slow	−	−	+	−	−	−	−	−	−	−	+	−	−
91-86	Slow	+	+	−	+	+	+	+	+	+	+	−	+	+
SSIs														
1	Very fast	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Very slow	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Very slow	+	+	+	+	+	+	−	+	−	−	+	+	+
4	Very fast	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Fast	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Fast	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Very slow	+	+	+	−	+	−	+	+	−	−	+	+	−
8	Very fast	+	+	+	+	+	+	+	+	+	+	+	+	+
9	Very fast	+	+	+	+	+	+	+	+	+	+	+	+	+
10	Very fast	+	+	+	+	+	+	+	+	+	+	+	+	+
11	Fast	+	+	+	+	+	+	+	+	+	+	+	+	+
12	Very fast	+	+	+	+	+	+	+	+	+	+	+	+	+
13	Slow	−	+	+	−	−	−	−	+	+	+	+	+	−
14	Very fast	+	+	+	+	+	+	+	+	+	+	+	+	+
15	Very fast	+	+	+	+	+	+	+	+	+	+	+	+	+
16	Very fast	+	+	−	+	+	+	+	+	+	+	+	+	+
17	Very fast	+	+	+	+	+	+	+	+	+	+	+	+	+
18	Slow	+	+	+	+	−	+	−	+	+	+	+	+	+
19	Very slow	+	+	+	+	+	−	+	+	−	−	+	+	−

loci (16), and RFLP analysis is hindered by a tedious process.

The amount of RAPD variation evident between the heterokaryotic isolates of *A. bisporus* that we tested is consistent with studies of isozyme and RFLP loci which have determined that while commercial cultivars are confined to a narrow genetic base, field-collected isolates are much more heterogeneous (1a, 8, 10, 11, 16). Also comparable to previous marker analysis is the high degree of amplification polymorphism extant in the *A. bisporus* genome: comparison of the two haplotypes that make up strain AA-1, for example, reveals that 31, or about 60%, of the approximately 50 loci amplified in strain AA-1 by primers A-02, A-03, A-04, and A-07 are heteroallelic; RFLP data show that the level of RFLP in *A. bisporus* is equivalent to the amount observed in domestic cultivars of maize, a highly variable species, and much greater than that found in tomatoes, lettuce, or humans (1a).

High levels of heterozygosity are apparently maintained through lineages of *A. bisporus* via its unusual automictic form of reproduction, termed intramixis by Kerrigan (8) and characterized by reduced meiotic recombination accompanied by packaging of complementary postmeiotic nuclei into spores (16, 20). This phenomenon is strikingly illustrated in strain AA-2, a single-spore descendant of strain AA-1 that has remained heteroallelic at all 31 RAPD loci and both RFLP loci scored as heteroallelic in strain AA-1. In addition, we scored 14 heterokaryotic SSIs at 19 loci known to be heteroallelic in a hybrid parent and found evidence for a proximal crossover at only 1 locus in 1 SSI, although when using RAPDs, recombination events that lead to homoallelism for the presence of an amplification product would have been masked. Isozyme and RFLP analyses of SSIs also demonstrate this meiotic process: Summerbell et al. (20) observed recombinant genotypes in only 5 of the 357 SSIs that they collected from 7 different strains and examined at 3 or 4 heteroallelic RFLP loci, while less than 10% of the SSIs obtained from 4 parental strains by Royse and May (16) and scored at 5 heteroallelic isozyme loci showed signs of a recombination event.

One possible consequence of this sexual cycle, which continually restitutes the ancestral genotype, may be accumulation of deleterious mutations that remain masked by complementing alleles (1a, 20), and this is, in fact, suggested by the markedly reduced growth exhibited by homokaryons isolated from vigorous heterokaryotic strains. The presence of these recessive, deleterious alleles among strains that have been maintained and improved via spore selection for a number of years may affect strain improvement programs intended to produce new genotypes through hybridizations between extant cultivars; for example, pairings of homokaryons isolated from strains related to each other through single-spore descent will lead to increased homozygosity in the resulting hybrid and, possibly, inbreeding depression. Isolate 91-51 × 91-86, the product of a cross between homokaryons obtained from strains AA-1 and AA-2, is homoallelic at 1 of the 2 RFLP loci and 15 of the 31 RAPD loci that were shown to be heteroallelic in strains AA-1 and AA-2; in addition, isolate 91-51 × 91-86 grew and fruited very poorly in comparison with the 2 parents (1), which suggests a negative correlation between homozygosity and vigor. Crossing strategies for *A. bisporus*, consequently, may demand careful consideration of the lineages of potential parents: an isozyme survey of 196 strains of, presumably, all cultivars placed them into 23 genotypic classes (10, 16); interclass pairings, as well as the introduction of novel germ plasm, therefore, should ensure the levels of heterozygosity needed to avoid inbreeding depression.

The ability to isolate homokaryons and verify crosses is an important step for *A. bisporus* strain improvement; a directed breeding program, however, also requires knowledge of the genetic determinants that control important agronomic traits, such as color, yield, and shelf life. RAPDs provide an ideal source of markers for genetic mapping of *A. bisporus* which, owing to the intermictic life cycle, requires a population of postmeiotic homokaryons; the use of homokaryons, which are effectively haploid, eliminates the disadvantage of dominant markers for genetic study. In our analysis of SSIs, 4 of the 5 slow growers are most probably homokaryons, indicating that homokaryotic SSIs, produced through aberrant packaging, are selectable via growth rate. Preliminary linkage analysis by Kerrigan et al. (9), utilizing both isozymes and RFLPs, indicates that recombination is suppressed over much of the genome of the population analyzed. Low recombination rates will hamper both efforts to introgress alleles from wild germ plasm into cultivated backgrounds and attempts to identify markers that are physically linked to traits of interest; we hope to exploit the advantages offered by RAPD markers to analyze recombination in a number of different strains, obtain a detailed chromosome map of *A. bisporus*, and design a breeding strategy that considers the genetic peculiarities of this species.

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