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Interspecific Allozyme Variation among *Morchella* spp. and its Inferences for Systematics within the Genus

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Abstract—The genetic variability of 12 enzymes encoded by 12 loci (*Fum, Gapdh, Gpi, Idh, Lap, Mpi, Me, Pgk, Pgd, PepGl, Sod* and *Tpi*) was examined by horizontal starch gel electrophoresis and standard histochemical staining in 19 lines of *Morchella* spp. The lines could be separated into eight genotypic classes based on their multilocus allelic combinations. Several isolates originally recorded as one species based on morphology were classified as another based on electrophoretic banding patterns. This lack of concordance between allozymic and morphological data sets may help to explain the taxonomic difficulties within the genus. In general, two groupings of the six morphologically identified species could be recognized (*M. angusticeps, M. conica* and *M. elata* vs *M. esculenta, M. crassipes* and *M. deliciosa*).

Introduction

Morels (*Morchella* spp.) are some of the most desirable, edible mushrooms known. In the wild, morels may exhibit many shapes, sizes and colors depending upon environmental conditions. These variations have led some mycologists to classify the genus into as many as 50 species while others favor only three to five species [1–5]. Some authors even go so far as to separately list variant forms of the same species [6]. Part of the taxonomic difficulty within the genus can be attributed to the effects of habitat, rainfall and time of seasonal appearance on size and shape of the carpophore [6].

The development of procedures for the electrophoretic analysis of specific enzymes (allozymes) in edible fungi [7–14] has provided an opportunity to assess genetic variability at single Mendelian loci. Allozyme analysis allows the most informative data sets because the phenotypic differences in electrophoretic banding patterns between individuals can be directly correlated to genotypic differences [7, 15, 16]. Recently, we have successfully used allozyme data to examine interspecific genetic variation within the fungal genus *Pleurotus* [17].

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The commercial production of *Morchella* spp. has not yet become a reality although small-scale laboratory production has been accomplished [18, 19]. While experimental studies on the morel have demonstrated heterokaryon formation between monoascosporous strains of *Morchella* [22], the taxonomic difficulties within the genus remain [1, 6, 20]. In this paper we discuss the applications of interspecific allozyme variation in the genus *Morchella.*

Results

Thirty-one enzymes were tested for electrophoretic resolution and variability. Twelve enzymes were sufficiently resolved to allow their use for species comparisons. A list of these enzymes, abbreviations, Enzyme Commission numbers, subunit compositions, loci, alleles with mobility designations and the buffer systems used are presented in Table 1. The inferred genotypes of each isolate at the 12 biochemical loci are listed in Table 2. When isolates with the same multilocus profiles were placed into the same genotypic class, eight genotypic classes were recognized. A UPGMA cluster analysis, based on Roger's coefficients of similarity [21], was performed and the results are presented in Fig. 1.

TABLE 1. ENZYMES, ABBREVIATIONS, ENZYME COMMISSION (EC) NUMBERS, NUMBER OF ENZYME SUBUNITS, LOCI, ALLELES WITH MOBILITY DESIGNATIONS AND BUFFER SYSTEMS USED IN THIS STUDY

Recommended name	Abbrev.	EC no.	Subunits	Locus	Alleles	Buffer
Fumerase	FUM	4.2.1.2	4	*Fum*	1=100, 2=114, 3=171	4
Glyceraldehyde-3-phosphate dehydrogenase	GAPHD	1.2.1.12	4	*Gapdh*	1=100, 2=46, 3=92, 4=133	C
Glucosephosphate isomerase	GPI	5.3.1.9	2	*Gpi*	1=100, 2=117, 3=123	C
Isocitrate dehydrogenase	IDH	1.1.1.42	2	*Idh*	1=100, 2=88	4
Leucine aminopeptidase	LAP	3.4.11.1	1	*Lap*	1=100, 2=114	R
Mannose phosphate isomerase	MPI	5.3.1.8	1	*Mpi*	1=100, 2=110	M
Malic enzyme	ME	1.1.1.40	4	*Me*	1=100, 2=200	C
Phosphoglycerate kinase	PGK	2.7.2.3	1	*Pgk*	1=100, 2=130, 3=170	4
Phosphogluconate dehydrogenase	PGD	1.1.1.44	2	*Pgd*	1=100, 2=107, 3=115	C
Peptidase with glycyl-leucine	PEP-GL	3.4.11-13	2	*PepGl*	1=100, 2=107	R
Superoxide dismutase	SOD	1.15.1.1	2	*Sod*	1=100, 2=179	R
Triosephosphate isomerase	TPI	5.3.1.1	2	*Tpi*	1=100, 2=119, 3=81, 4=148	4

TABLE 2. INFERRED GENOTYPES BASED ON ELECTROPHORETIC BANDING PATTERNS FOR 12 LOCI IN 19 ISOLATES OF *MORCHELLA* SPP.

Genotypic	Loci												PSUMCC
class	*Fum*	*Gapdh*	*Gpi*	*Idh*	*Lap*	*Mpi*	*Me*	*Pgk*	*Pgd*	*PepGl*	*Sod*	*Tpi*	line nos
1	22	11	22	11	11	11	11	11	11	22	22	11	13, 15, 17
2	11	11	22	11	11	11	11	11	11	22	22	22	25, 30
3	11	11	22	11	11	11	11	11	11	22	22	11	5, 327, 374, 402, 690, 692
4	11	22	33	11	22	11	22	33	22	22	22	44	172, 178
5	33	22	11	22	22	11	22	11	33	11	22	33	198
6	33	22	11	22	22	12	22	11	33	11	22	33	27, 331
7	11	33	22	11	11	22	11	22	11	22	11	44	190, 191
8	33	44	11	22	22	11	22	22	33	11	22	44	578

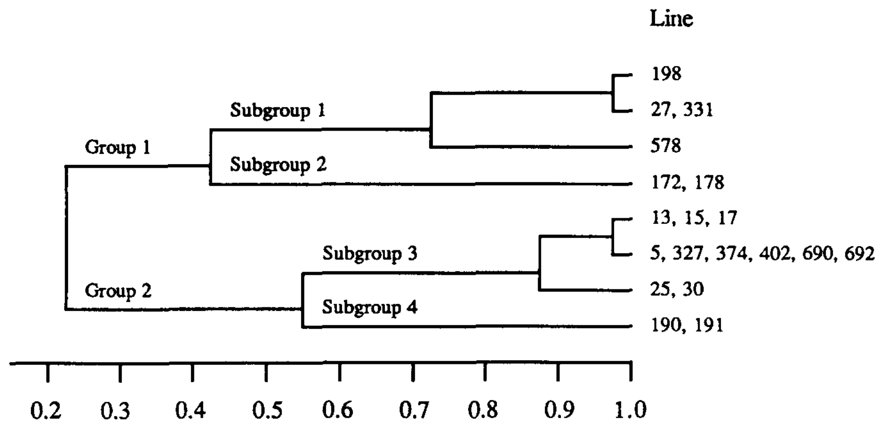

FIG. 1. DENDROGRAM OF GENETIC SIMILARITIES OF 19 LINES OF *MORCHELLA* SPP.

These are relative similarities because only polymorphic loci were used.

The original species identifications of these isolates based on morphological grounds are not in complete congruence with genetic similarities based on allozyme variation (Fig. 1). In general, two groupings of the 19 lines could be recognized. The first group (lines 198, 27, 331, 578, 172 and 178) contained the morphologically identified species of *M. angusticeps, M. conica* and *M. elata.* The remainder of the morphologically identified species (*M. esculenta, M. crassipes* and *M. deliciosa*) was included in the second major group. Within each group, two major subgroups also could be identified, subgroups 1 and 2 in Group 1 and subgroups 3 and 4 in Group 2 (Fig. 1).

Discussion

Interest in the cultivation of morels has increased as a result of recent biochemical and genetic studies [18, 19, 22]. The status of *Morchella* taxonomy makes the application of cultivation and breeding methodologies more difficult. It is important to identify species correctly because optimal cultural techniques will probably differ for each. Effective genetic modifications also require knowledge of the systematic relationships of isolates used in interspecific as well as intraspecific matings. In the results above we have shown that a number of *Morchella* spp. isolates have probably been misidentified. From the six species listed in this study, we propose that only two major species groupings be recognized: *M. angusticeps* (Group 1) containing isolates 27, 172, 178, 198, 331 and 578 and *M. esculenta* (Group 2) containing isolates 5, 13, 15, 17, 25, 30, 190, 191, 327, 374, 402, 690 and 692. Within each of these major species groupings there are two major subgroups (Fig. 1). Upon more rigorous morphological scrutiny these may also be shown to be separate species. It is also possible that current morphological characteristics used to identify field isolates of *Morchella* spp. are not adequate and should be reexamined.

Isolates identified as *M. esculenta* and *M. deliciosa* clustered with genetic similarities as low as 0.55 in our study. This shows the potentially large genetic diversity within a single species. Gessner *et al.* [23] have proposed the possibility of separate gene pools or distinct species for one population identified morphologically as *M. deliciosa* and one population identified morphologically as *M. esculenta* collected only a few hundred meters from each other in Plymouth, Illinois. Nei's Unbiased Identity Values calculated for these two populations in Illinois was 0.89. Our data would support their suggestion that the two populations are merely part of a very variable species-complex, as judged by allozymic data and thus not worthy of specific recognition. Additional breeding and population studies, however, are needed to confirm this contention.

This study shows the potential of allozyme analysis to separate isolates into putative specific classes. These classes could then be subjected to more rigorous morphological, biochemical and breeding analysis to determine a logical systematic classification within the genus. In other organisms, where systematic relationships have been clearer, a high similarity exists between allozyme data and morphological characteristics [24, 25]. Thus, allozyme data obtained for *Morchella* spp. in this study and by Gessner *et al.* [23] may represent a first step in a revised taxonomy of the genus.

Allozyme analysis can provide an unambiguous means of identifying crosses between single ascospore lines. Since *Morchella* spp. do not have hyphal clamp connections, it is difficult to confirm nuclear exchange. One morphological characteristic proposed to recognize crossing is a mycelial meld (aerial ridge or hyphae) between isolates grown in dual culture [22]. Mycelial melding, coupled with allozyme analysis, could be used to routinely produce and confirm crosses. This ability would allow breeders to make more rapid progress in genetic improvement programs for the commercial production of morels.

Experimental

Table 3 shows the sources and species identification based on morphology of the isolates examined. Isolates were assigned numbers and deposited in The Pennysylvania State University Mushroom Culture Collection (PSUMCC). Lines are maintained on potato-dextrose yeast extract agar (PDYA) slants at 4°C [26]. All lines are also maintained in liquid nitrogen.

Mycelial mats growing in potato-dextrose yeast-extract broth (PDYB) were vacuum-dried with a water aspirator on Whatman No. 1 filter paper and then transferred to 1.2 ml liquid

TABLE 3. PENNSYLVANIA STATE UNIVERSITY MUSHROOM CULTURE COLLECTION (PSUMCC) NUMBER, ORIGINAL SPECIES CLASSIFICATION BASED ON MORPHOLOGY AND SOURCE OF *MORCHELLA* SPP. EXAMINED

PSUMCC no.	Species	Source
5	Not determined	L. R. Kneebone, Center County, Pennsylvania
13	*M. esculenta*	L. C. Schisler, Center County, Pennsylvania
15	*M. crassipes*	Pennsylvania
17	*M. crassipes*	Pennsylvania
25	*M. esculenta*	Illinois
27	*M. conica*	Pennsylvania
30	*M. crassipes*	L. C. Schisler, Center County, Pennsylvania (1980)
172	*M. angusticeps*	L. C. Schisler, Werde isolate, British Columbia (1975)
178	*M. angusticeps*	L. C. Schisler, Merritt no. 2, British Columbia (1975)
190	*M. deliciosa*	L. C. Schisler, W. Kettle River, British Columbia (1975)
191	*M. deliciosa*	L. C. Schisler, W. Kettle River, British Columbia (1975)
198	*M. angusticeps*	L. C. Schisler, Juliet Creek, British Columbia (1975)
327	*M. esculenta*	B. Myers, Center County, Pennsylvania (1981)
331	*M. angusticeps*	D. J. Royse, Center County, Pennsylvania (1981)
374	*M. esculenta*	L. C. Schisler, Center County, Pennsylvania (1982)
402	*M. crassipes*	L. C. Schisler, Center County, Pennsylvania (1983)
578	*M. elata*	Centraalbureau voor Schimmelcultures, Baarn, Netherlands (1986)
690	*M. deliciosa*	C. Halbert, Center County, Pennsylvania (1986)
692	*M. esculenta*	Native Plants Inc., Utah (1986)

nitrogen (Nunc) tubes [14]. Three to five drops of extraction buffer (Tris–HCl, 0.05 M, pH 7.1) were added to the samples (about 0.1 g of vacuum-dried mycelium per sample) prior to freezing in a vapor-phase liquid nitrogen freezer until used (up to one month). At the time of enzyme assay, samples were allowed to partially thaw at room temperature and were then transfered immediately to 12×75 mm disposable glass test tubes. The samples were ground with a glass rod, centrifuged at 1000 *g* for 5 min and the supernatants applied to starch gels (14%) with 3×8-mm filter-paper wicks (Schleicher and Schuell No. 470). Horizontal starch gel (Connaught and Sigma starches mixed in a 1:1 ratio by weight) electrophoresis was performed according to May *et al.* [27].

Buffer S4 was adapted from Selander *et al.* [28] as follows: S4 tray buffer (per liter); 27 g Tris, 18.07 g citric acid, 2 g NaOH, pH 6.3; S4 gel buffer (per liter): 970 mg Tris, 630 mg citric acid, 110 mg NaOH. Buffers C, R and M were from Clayton and Tretiak [29], Ridgway *et al.* [30] and Markert and Faulhaber [31], respectively (as adapted in May *et al.* [27]).

The allozyme nomenclature used follows May *et al.* [27] and Royse and May [14]. Enzymes were abbreviated with all capital letters (e.g. FUM); loci were abbreviated using a capital letter followed by lower case letters (eg. Fum). One allele, usually the most common, was designated as 100 and all the other alleles were designated according to migratory ratio of their homomeric protein products to that of allele 100. For gel scoring, the most common allele was designated as 1 and successive alleles were numbered from slowest to fastest. New alleles were given the next highest number, regardless of mobility.

Genetic similarities [21] and cluster analysis by the unweighted pair-group (UPGMA) method [32] were calculated with a computer program ("Genes in Populations") designed by B. May and C. C. Krueger and written by W. Eng of Cornell University.

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