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Physiological and Environmental Studies of Sclerotium Formation and Maturation in Isolates of *Morchella crassipes*

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This study provides a set of nutritional and environmental parameters suitable for the growth of morel (*Morchella crassipes*) sclerotia in the laboratory, using a modification of the jar method of Ower et al. (U.S. patent 4,594,809, June 1986). The optimum nutritional and environmental conditions for morel sclerotium formation and maturation as determined in this study consist of a layer of rye grain supplemented with peptone, yeast extract, trace elements, and Casamino Acids overlaid with perforated aluminum foil and covered with a layer of nutrient-poor soil medium in an 8-oz. (ca. 237-ml) glass jar in the dark. We noted that addition of asparagine or aspartic acid as a nitrogen source to the rye also had a beneficial effect on sclerotium formation, while addition of carbon sources had no significant effect.

In nature, morel fruiting bodies are usually produced during a 2-week period in early spring. The major source of nutrition used at this time to support fruiting-body growth and development is presumed to be sclerotia, the large multicellular storage structures first described by Fron (3). Although he did not realize the significance of the sclerotium in the life cycle, he did record that they could grow to be "quite large and looked and tasted like fruiting bodies." Sclerotia are formed by the *Morchella crassipes* mycelium and serve to protect the fungal cells through the adverse environmental changes brought on by winter. In the spring, a fruiting mycelium is produced as a result of sclerotium germination, which gives rise to the familiar morel fruiting bodies. This important role of the sclerotium in the life cycle of *Morchella* spp. was confirmed by Ower (15) and Ower et al. (R. Ower, G. Mills, and J. Malachowski, U.S. patent 4,594,809, June 1986), who also demonstrated that sclerotia can be cultivated and used to produce morel fruiting bodies under controlled conditions. This important finding underscores the need to determine optimal conditions for sclerotium formation, growth, and maturation so that their production can be controlled.

Although there are no published studies on the nutrition of sclerotia in *Morchella* spp., some clues regarding nutrition may be revealed by the nutritional needs of vegetative hyphae (4). The physiology of *Morchella* vegetative mycelium has been relatively well studied compared with other aspects of the morel life cycle. Fron (3) reported on carbon nutrition and the effects of pH on mycelial growth, although he did not include any quantitative data.

Brock (2) did an extensive study on the nutrition of *Morchella esculenta* in which he compared various carbon and nitrogen sources for their ability to support vegetative growth in liquid culture. Although Brock's study involved only a single stipe culture of *M. esculenta*, which by itself may be too narrow to represent the entire genus, his experiments were repeated by Kaul (7, 8), who extended the study to eight different strains of *Morchella* representing six different species. His results were similar to Brock's, suggesting that the physiology of different morel strains and species is quite similar. For example, in these studies, *M. esculenta* was very similar physiologically to *M. crassipes*. There have also been numerous growth studies of morel mycelium in large batch liquid culture with various waste substrates, including ammonia base waste sulfur liquor (12), citrus wastes (10), cheese whey (9), and peat hydrolysates (13).

Mineral nutrition has also been examined in *Morchella* spp. Robbins and Hervey (17) studied the beneficial effects of wood and tomato extracts on the growth of one isolate each of *M. crassipes* and *M. esculenta*; the minerals of wood ash were found to be the cause of this increased vegetative growth (18). Morels also seem to thrive in the aftermath of forest fires (1, 5, 14, 16), which again points to the effects of ash.

The nutrition of sclerotium formation in *Morchella* spp. has received only scant attention. In these studies, "wheat berries" were the substrate of choice (15; Ower et al., patent, 1986). We have also described another method of sclerotium formation (T. J. Volk and T. J. Leonard, Mycol. Res., in press). We found that morel sclerotia were not true sclerotia (Volk and Leonard, in press), as characterized in *Sclerotinia sclerotiorum*, which differentiates complex tissues such as rind and medulla, but rather were more like the relatively undifferentiated "pseudosclerotia" of *Monilinia fructigena*. Although these structures are more properly termed pseudosclerotia, we have followed the lead of Willetts (23), who referred to any "macroscopic fungal resting structure" as a sclerotium, and we have adopted this terminology for the morel.

Following up on the premise of Hawker (4) that substrates that will support good vegetative growth of fungi will also support good sclerotium formation, we tested *Morchella* spp. on a variety of natural, synthetic, and semisynthetic media with the jar method (Ower et al., patent, 1986). The present experiments test qualitatively and quantitatively the effects of various substrates as well as the effects of growth container size and light regimens on the formation of sclerotia in *Morchella crassipes*.

MATERIALS AND METHODS

Strains. Mycelial subcultures of *M. crassipes* (Vent.) Pers. strains JR1, JR2, JR3, and JR5 (the JR series) were used to study sclerotium formation. These strains were derived from the four nonsister spores of a single ascus, as determined by the incompatibility test described previously (22), collected from a very large (ca. 30 cm in height) fruiting body that has

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TABLE 1. Semisynthetic and synthetic agar media tested for their ability to support sclerotium formation[a]

Media and additions tested	Vegetative growth[b]	Sclerotium formation[c]
Standard agar types		
CYM (for *Schizophyllum commune*)	+++	+
Difco malt extract agar	++	+
Difco mycological agar	++	−
Difco potato dextrose agar	++	−
Difco cornmeal agar	+	+
Difco nutrient agar	±	−
Difco beef extract agar	±	−
Honey-peptone agar	++	−
Nitrogen sources[d]		
Control (no N source)	±	−
Aspartic acid	+	−
Asparagine	+	−
Urea	+	−
Sodium nitrite	+	−
Ammonium tartrate	+	−
Ammonium nitrate	+	±
Ammonium chloride	+	±
Ammonium sulfate	+	−
Sodium nitrate	+	−
Cysteine-hydrochloride	+	−
D-α-Alanine	+	−
L-Glutamic acid	+	−
Glycine	+	−
Leucine	+	−
Histidine	+	−
Carbon sources[e]		
Control (no C source)	±	−
Dextrose	++	+
Fructose	++	−
Sucrose	+	−
Maltose	+	−
Lactose	±	−
Starch	++	−

[a] Combined mycelia from *M. crassipes* JR1, JR2, JR3, and JR5 were used.
[b] Symbols: +++, colonized in 5 to 6 days; ++, colonized in 7 to 8 days; +, colonized in 9 to 10 days; ±, colonized in >10 days; −, no growth.
[c] Symbols: +, one to four 1- to 2-mm sclerotia; ±, one 1-mm sclerotium; −, no sclerotia.
[d] Tested in basal medium with dextrose (30 g) and N source at 250 mg/liter.
[e] Tested in basal medium with asparagine (2 g) and C source at 12 g/liter.

been studied extensively in this laboratory. Although sclerotia can be formed by monoascosporous isolates (Volk and Leonard, in press), this combination of strains was used because they represent all of the meiotic products from the same ascus, and all genes that may be conducive to sclerotium formation and fruiting should be available. The four strains were always used together in order to allow heterokaryon formation (22) should a heterokaryon be essential to fruiting.

Induction of sclerotia on nutrient agar medium. Several standard fungal medium recipes (Difco Laboratories, Detroit, Mich.), as well as CYM (11), were tested (Table 1). Brock's (2) basal medium supplemented with different nitrogen sources (250 mg of nitrogen per liter) with 30 g of dextrose per liter as the carbon source was also tested, as was Brock's basal medium plus different carbon sources (12 g/liter) with 2 g of asparagine per liter as the nitrogen source. In all experiments, the JR series were inoculated together on the center of petri dishes. Vegetative growth was scored as time of colonization of a 9-cm medium-filled petri dish, and sclerotium formation was scored after 25 days with respect to size and number. These two times were chosen after preliminary experiments. Vegetative growth was scored as follows: +++, colonized in 5 to 6 days; ++, colonized in 7 to 8 days; +, colonized in 9 to 10 days; ±, colonized in >10 days; −, no growth. Sclerotium formation was scored as follows: +++, more than five 2-cm sclerotia; ++, one to five 1- to 2-cm sclerotia; +, one to four 2-mm sclerotia; ±, one 1-mm sclerotium; −, no sclerotia.

Sclerotium formation with the jar method. The remainder of the sclerotium experiments were carried out by the procedure of Ower et al. (patent, 1986), with some modifications. The substrate to be tested was used to half-fill a glass jar covered with a layer of perforated aluminum foil, and the remainder of the jar was filled with a nutrient-poor soil consisting of 3 parts potting soil mixture (Riverside Gardens, Watertown, Wis.; a "chemically sterilized virgin soil combined with compost, humus, perlite, sand, and rice hulls"), 1 part peat moss (Premier Brands, Inc., New Rochelle, N.Y.), and 2 parts distilled water. The initial pH of the soil mixture was ca. 5.8, as determined by forming a slurry composed of 10 g of soil mixture and 50 ml of water and reading the pH from a Beckman pH meter. A wide range of well-drained soil types will work well in this regimen. The top of the jar was covered with intact foil, and the jars were autoclaved at 15 lb/in^2 for 30 min. After cooling, the jars were inoculated with 5-day-old CYM-grown JR *Morchella* mycelium on top of the soil layer and mixed slightly with a spatula until covered. Jars were placed in a dark chamber at room temperature (ca. 25°C) at approximately 50% relative humidity. The sclerotia and soil were removed after 25 days, and their combined wet weight from single jars was determined immediately. The wet weight of the sclerotia alone was determined by subtracting the known wet weight of the soil, since the weights of the sclerotia cannot be determined directly; during development, which involves coalescence of several sclerotia, soil particles often become entrapped. Biological efficiency (BE), calculated as the wet weight of sclerotia divided by the dry weight of the substrate, was used as a method of evaluating yield, as is commonly used in the mushroom industry (19). Dry weights of soil plus sclerotia were also determined by drying jar contents at room temperature.

Three sizes of glass jars (8, 16, and 32 oz. [ca. 237, 474, and 948 ml, respectively]) were used to accommodate different amounts of growth medium (100, 200, and 300 g, respectively). A rich growth medium, RPYTC (rye [mushroom rye; Stanford Seed Co., Buffalo, N.Y.], peptone [Difco], yeast extract [Difco], trace elements [6], and Casamino Acids [Difco]), was used as the substrate to determine which container size would provide the greatest sclerotium-to-substrate ratio (i.e., BE).

Four light regimens, ranging from continuous light to continuous darkness, were tested to determine their effect on *Morchella* sclerotium formation in RPYTC medium. The light was provided by a standard fluorescent light (25 W) at a distance of 1 m.

Boiled rye containing 60% water was used as the basal medium in supplementation experiments. In the RPYTC experiment, complex additives were added to the rye in the amount of 1% (wt/wt): peptone, yeast extract, and Casamino Acids, plus 1 ml of trace element stock solution. In experiments to test nitrogen, nitrogen sources were added to the rye in the amount of 250 mg of N per 100 g of substrate. In experiments to test carbon, carbon sources were added to the rye in the amount of 12 g of carbon per kg of substrate. Data in all cases were analyzed with the MGLH and STATS

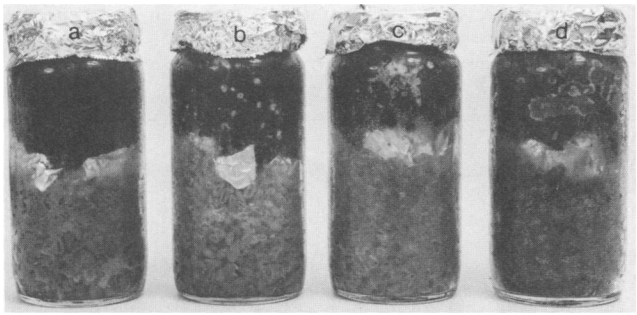

FIG. 1. Production of sclerotia by *M. crassipes* in jars. Upper layer consists of nutrient-poor soil separated from a lower layer of grain medium by perforated aluminum foil. Initial inoculum was 5-day-old combined mycelia of strains JR1, JR2, JR3, and JR5. (a) Mycelium has reached into the grain layer, 5 days after inoculation; sclerotium initials have not yet formed. (b) Small, discrete, light-colored sclerotium initials have developed at the interface of the soil and the glass, 8 days after inoculation. (c) Coalescence of the initials is under way, along with increasing pigmentation, 10 days after inoculation. (d) Sclerotia are nearly mature, 22 days after inoculation, as indicated by the large dark central area. Some light-colored areas of immature tissue are visible on the margin of the sclerotia.

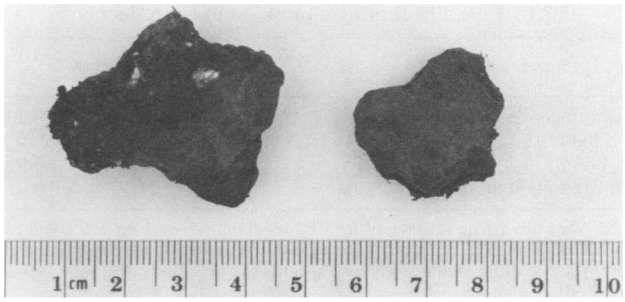

FIG. 2. Sclerotia removed from the jar after 22 days. Note irregular shape and large size.

modules of the Systat statistical package (Systat, Inc., Evanston, Ill.).

RESULTS

Sclerotium formation on nutrient agar medium in petri dishes. Several basal agar media were supplemented singly with simple and complex nutrients to determine the relationship between good vegetative growth and good sclerotium formation (Table 1). Although most of the nutrients fostered about the same level of excellent vegetative growth, with the mycelium colonizing the entire 9-cm petri dish in about 6 days, none of these nutrients provided for good sclerotium formation with these strains. In the small percentage of cases in which sclerotia formed, production was poor (one to four sclerotia per plate) and the sclerotia were uniformly small (1 to 2 mm diameter).

Sclerotium formation on complex medium in jars. Since the JR strains will not form large sclerotia without a "casing layer," the jar method of Ower et al. (patent, 1986) was used to produce sclerotia from these strains. After inoculation of JR mycelia on top of the soil layer, the hyphae grew through the nutrient-poor soil substrate to reach and colonize the nutrient-rich substrate (Fig. 1). As in other sclerotium-forming fungi (20), nutrients were translocated back into the hyphae in the nutrient-poor substrate, as evidenced by the accumulation of oil droplets in the swelling cells (Volk and Leonard, in press), at which time light-colored sclerotial initials formed. These initials grew in size, and where they made contact with each other they often coalesced to form several large sclerotia. Maturation of the sclerotia was considered complete when radial growth of the sclerotia was no longer detectable and the sclerotia became darkly pigmented; in most cases this was 3 to 4 weeks after inoculation at room temperature. Mature sclerotia were often quite large (2 to 5 cm diameter) when they were removed from the container (Fig. 2). Many different substrates were tried in the jar method to determine an optimal basal medium for further studies. Those single ingredients that induced good sclerotium formation (a qualitative judgment based on the formation of at least three sclerotia larger than 2 cm in diameter) included wheat, rye, oats, oatmeal, corn grains, corn extract, corn husks, corn silk, and elm fruits. Those substrates that were not successful in the induction of large sclerotia included rice, millet, cornmeal, corn cobs, jerusalem artichokes, apples, apple juice, turnips, pumpkin, tomato juice, carrots, and fungal fruiting-body or mycelial macerates (*Laetiporus sulfureus*, *Pleurotus ostreatus*, *Agaricus bisporus*, *Trichoderma viride*, and *Armillaria mellea*). Some of these substances were chosen because of various reports on their performance with fruiting in the morel. Others were chosen because of their easy availability. Most of the grains, especially wheat, rye, and corn, were particularly vigorous in their support of sclerotium production.

Effect of container size on sclerotium production. Although larger jars produced quantitatively more and therefore greater weights of sclerotia of approximately the same size (Table 2), the BE in smaller 8-oz. jars was significantly greater than that of either of the two larger jars (16 and 32 oz.). The results were verified with the use of Duncan's multiple-range test.

Effect of light on sclerotium production. The effects of four light regimens on the production of sclerotia by *Morchella* spp. in 8-oz. glass jars is shown in Table 3. Sclerotium yield was maximum in continuous darkness and was completely inhibited by continuous light. Intermediate light conditions caused some inhibition. The biological efficiencies clearly fell into three groups with regard to light duration when analyzed by Duncan's multiple-range test.

Nutrient supplementation of rye medium. Production of sclerotia was tested in rye medium supplemented with such complex nutrient additives as peptone, yeast extract, trace elements, and Casamino Acids (Table 4). The biological efficiencies of this experiment were analyzed by a factorial analysis of variance, which was designed to determine the effects of single additives as well as two-way, three-way, and four-way interactions of the additives. The results of this analysis showed that the three-way and four-way interactions were not statistically significant ($P = 0.896$) and the

TABLE 2. Effect of jar size and substrate amount on sclerotium production in RPYTC medium[a]

Jar size (oz.)	Wet wt (g)			BE (%)
	Soil	Grain	Sclerotia	
8	100	100	36.22	90.1 a
16	200	200	48.56	60.7 b
32	300	300	61.35	51.1 b

[a] Each value is the average for five replicates. Biological efficiency (BE) values followed by the same letter did not differ significantly by Duncan's multiple-range test ($\alpha = 0.05$).

TABLE 3. Effect of light on sclerotium production[a]

Light treatment	Sclerotium wet wt (g)	BE (%)
Continuous darkness	36.22	90.1 a
8 h light, 16 h dark	25.23	63.0 b
16 h light, 8 h dark	24.37	60.9 b
Continuous light	0	0 c

[a] Cells were grown in 100 g of RPYTC medium in 8-oz. jars. Each value is the average for five replicates. Biological efficiency followed by the same letter did not differ significantly in Duncan's multiple-range test ($\alpha = 0.05$).

two-way interactions were marginally significant ($P = 0.139$). Therefore, a reduced model was constructed which took into account only the effects of single additives as well as the two-way interactions. Little accuracy was lost in reducing the model in that the extended model was able to explain 31.6% of the variation and the reduced model was able to explain 29.7% of the variation. The remaining variation is due to error; the replicates within each treatment group varied considerably and the standard deviations were quite high. The analysis of variance ($\alpha = 0.05$) on the reduced model indicated that the Casamino Acids had the most significant effect on sclerotium production ($P = 0.002$), although the addition of yeast extract was also significant ($P = 0.032$). Neither peptone nor the addition of trace elements produced a statistically significant effect ($P = 0.122$ and 0.220, respectively). Among the two-way interactions, only the interaction of the Casamino Acids with the trace elements was significant ($P = 0.043$), although the interaction of Casamino Acids with peptone was nearly significant ($P = 0.052$). Duncan's multiple-range test ($\alpha = 0.05$) revealed that only the addition of all four substances (i.e., RPYTC) yielded a mean that was statistically different from the control mean.

The various nitrogen sources used to supplement rye and their impact on sclerotium formation are shown in Table 5. Two of the nitrogen sources, asparagine and aspartic acid, increased the biological efficiency above that of the control, although sodium nitrate and ammonium chloride had a slight positive effect.

The effect of adding various carbon sources to rye was analyzed in a similar manner (Table 6). An analysis of variance ($\alpha = 0.05$) indicated that none of the carbon sources had a significant effect on sclerotium production.

DISCUSSION

Sclerotium formation in most *Morchella* isolates appears to be under a different type of control than in other sclerotium-forming ascomycetes, e.g., *Sclerotinia gladioli*, *S. cepivorum*, and *S. rolfsii*, as well as those of *Botrytis cinerea*, *B. allii*, and *Rhizoctonia solani* (21), which, in contrast to *Morchella* spp., easily form sclerotia on the same nutrient agar media that support good vegetative growth. Although vegetative growth of our *Morchella* strains on most of the media tested in petri dishes was good, sclerotium production was relatively poor. The few small sclerotia developed only after most of the nutrient reserves were consumed by vegetative growth; therefore, the sclerotia were not able to act as nutrient sinks to further growth, since there did not appear to be any usable nutrients left in the medium. Whether this extends to other morel strains is not clear. Moreover, sclerotia formed in this manner would not be expected to have a large enough nutrient reserve to support fruiting-body formation, since large nutrient reserves are necessary for fruiting of most macrofungi. Therefore, we tried another method reported to produce larger sclerotia, the jar method (Ower et al., patent, 1986).

As expected, the jar method yielded larger and more abundant sclerotia than any of the agar cultures. The primary objective of this portion of the study was to determine favorable substrates which support rapid growth of large sclerotia. As a practical matter, the substrate should be inexpensive and lend itself to large-scale employment. This factor ruled out using as primary substrates the relatively expensive pure nutrients which had supported good vegetative growth in agar culture; nevertheless, their use as sup-

TABLE 4. Effects of various additions to rye on formation of sclerotia[a]

Rank	Medium	BE (%)	Rank	Medium	BE (%)
1	RPYTC	90.6 a	9	RPYT	66.4 b
2	RPTC	76.7 a,b	10	RPY	66.3 b
3	RYT	74.4 a,b	11	RYC	65.5 b
4	RPYC	73.9 a,b	12	RC	64.9 b
5	RYTC	70.9 a,b	13	R	64.7 b
6	RTC	69.4 b	14	RP	63.0 b
7	RY	67.8 b	15	RT	58.7 b
8	RPC	67.2 b	16	RPT	58.7 b

[a] To 40 g (dry weight) of rye (R) in 8-oz. jars were added 1 g of peptone (P), 1 g of yeast extract (Y), 1 ml of a stock solution of trace elements (T), and/or 1 g of Casamino Acids (C). Each value is the average for four or five replicates. Biological efficiency (BE) values followed by the same letter did not differ significantly in Duncan's multiple-range test ($\alpha = 0.05$). See text for additional statistical analysis of data.

TABLE 5. Effects of addition of different nitrogen sources to rye on production of sclerotia[a]

Addition to rye	Sclerotia wet wt (g)	BE (%)
Asparagine	27.25	68.1 a
Aspartic acid	27.13	67.8 a
Sodium nitrate	24.27	60.7 a,b
Ammonium chloride	21.23	53.1 a,b,c
None (control)	19.93	49.8 b,c
Ammonium tartrate	19.22	48.1 c
Sodium nitrite	18.26	45.6 c
Ammonium nitrate	17.74	44.3 c
Urea	17.49	43.7 c

[a] The nitrogen sources listed were added to 40 g (dry weight) of rye at 250 mg per 8-oz. jar. Each value is the average for five replicates. Biological efficiency (BE) values followed by the same letter did not differ significantly in Duncan's multiple-range test ($\alpha = 0.05$).

TABLE 6. Effects of addition of different carbon sources to rye on production of sclerotia[a]

Addition to rye	Sclerotia wet wt (g)	BE (%)
Starch	25.61	64.0
None (control)	25.48	63.7
Lactose	24.66	61.6
Maltose	23.61	59.0
Sucrose	23.17	57.9
Dextrose	21.60	54.0
Fructose	21.46	53.6

[a] The carbon sources listed were added at 12 g/kg (wet weight) of rye in 8-oz. jars containing 40 g (dry weight) of rye. Each value is the average for five replicates. None of the biological efficiency (BE) values were significantly different from one another as determined by Duncan's multiple-range test ($\alpha = 0.05$).

plements to a relatively inexpensive medium formed the basis for later studies. Although most of the grains induced excellent sclerotium formation, rye was chosen as the basal medium to test the addition of growth-promoting substances for use as supplements to induce more vigorous sclerotium formation. Rye is easily available and inexpensive; it is a choice substrate of the mushroom industry, which uses rye instead of wheat, corn, or oats because rye is "cleaner," i.e., contains fewer bacterial contaminants, than other grains and generally causes fewer problems when used on an industrial scale because of its hardness and friability after sterilization.

The increase in biological efficiency exhibited by the smaller jars is probably due to their greater surface-volume ratio, since all other factors were the same except the size of the jar. In addition, sclerotia were initiated and formed most abundantly when in contact with the glass sides of the jar. Replicates of these cultures in plastic bags, by comparison, did not form abundant or large sclerotia (personal observation). The glass-contact induction is also true of the sclerotia of *Sclerotinia* and *Botrytis* spp. (23). Therefore, although it is much more convenient to prepare one large container rather than several smaller ones, the yield in terms of biological efficiency appears to be well worth the trouble.

Light has been shown to affect sclerotium production in *Sclerotinia* spp. (23); continuous light completely inhibits sclerotium production, while periodic light has a less obvious inhibitory effect. This is also true of *Morchella* sclerotia under similar conditions. Ecologically, this would seem to make sense; to enhance viability, the sclerotia of *Morchella* spp. would be expected to form beneath the soil surface to avoid the hard freeze at the soil-air interface, since their viability is impaired at −20°C in the laboratory (personal observation).

Although rye alone as a substrate promoted good sclerotium formation, supplementation with substances known to enhance vegetative growth might be expected to enhance sclerotium formation if these substances are present in negligible or limiting amounts in the rye (21); there may also be catalytic effects. The nutrient agar cultures were examined to determine which nutrients might be useful as supplements. CYM medium, the main ingredients of which are peptone and yeast extract, consistently supported the most rapid and dense growth of *Morchella* vegetative hyphae as well as the most consistent (albeit meager) sclerotium production. Trace elements were added to rye medium because of the reported beneficial effects of minerals on *Morchella* vegetative growth (18). Furthermore, because rye lacks certain amino acids, Casamino Acids (containing all amino acids) would be expected to boost both vegetative growth and sclerotium formation. For these reasons, each of these four additives, peptone, yeast extract, trace elements, and Casamino Acids, was used to supplement the rye separately as well as in all possible combinations to test for effectiveness in promoting sclerotium formation. The Casamino Acids and the yeast extract were the only two additives that had a significant effect separately; Casamino Acids also had a significant effect in combination with the trace elements and a slight positive effect in combination with peptone. Thus, it is somewhat surprising that only the combination of all four additives produced a significantly greater biological efficiency than the control, rye alone. Because of this effect and the high biological efficiencies, over 100% in some of the replicates, RPYTC medium is used extensively for sclerotium production in this laboratory and was used in the environmental experiments in this study.

These results with complex additives to rye medium led to studies to determine whether nutrient supplements could enhance biological efficiency. Addition of two nitrogen sources, asparagine and aspartic acids, produced a significantly greater biological efficiency than in the control. These were also among the best nitrogen sources for vegetative growth in Brock's studies (2). None of the carbon source additions produced a significantly greater biological efficiency; this is interpreted to mean that carbon availability is not a limiting factor in the rye medium.

In order to test the legitimacy of using biological efficiency as a measure of yield, dry weights of the soil layer plus sclerotia were determined. Sclerotium yields measured in dry weight agreed with the data for biological efficiency (data not shown). However, the biological efficiency is reported because it is a much more useful parameter in the mushroom industry, since it is more easily measured.

The results of this study indicate a regimen that produces good sclerotium formation in these isolates of *M. crassipes*. Our data indicate that the jar method of Ower et al. (patent, 1986) with RPYTC medium (rye, peptone, yeast extract, trace elements, and Casamino Acids) as the substrate in 8-oz. glass jars in the dark is the best method. Although this regimen works well with the JR series of *M. crassipes* described in this study as well as several other *Morchella* strains that we tested in preliminary studies, it would be premature to prescribe this regimen for production of sclerotia by all *Morchella* strains. In fact, we only recently isolated an unusual series of strains which do not require a nutrient-poor medium adjacent to the substrate for excellent sclerotium formation. Further experiments are under way to determine the optimal conditions for these soil-free sclerotium-producing strains.

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