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Isolation of Amoebae from Edible Mushrooms

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Isolation of amoebae from the surface and internal tissues of edible mushrooms was investigated. Samples tested over a 3-year period included mushrooms cultivated from six geographic localities. Of 168 mushroom surfaces tested, 161 (96%) yielded amoebae. Of 166 samples of internal stalk and cap tissues tested, only 1 yielded amoebae.

The discovery that amoebae may be isolated from the internal tissues of the cap and stalk, as well as from the surface of the basidiomycete *Laccaria trullisata* collected at the Hempstead Lake State Park on Long Island, New York (4; J. J. Napolitano and V. D. Flanagan, J. Protozool. 27:46A, 1980), has raised new questions about the occurrence of amoebae in and on other kinds of mushrooms.

This report describes results obtained in experiments designed to determine the presence of amoebae in and on edible mushrooms cultivated, packaged, and sold for public consumption. All mushrooms (*Agaricus bisporus*) were purchased either loose or previously packaged from local stores. Some samples were described as washed, and some contained a preservative such as sodium bisulfite. To insure that no single sample would unduly influence the results, trials were restricted to eight or fewer mushrooms from any single batch. Moreover, care was taken to use a variety of suppliers representing different geographic localities. The selection was spread over a 3-year period to allow examination of some samples from the same supplier at different times. All mushrooms were tested as purchased and used directly. Methods for aseptic removal of internal tissues from the cap and stalk were those used in a previous study (4). Isolation of amoebae from the surface was performed by scraping the entire cap with a single-edged razor blade, gathering surface materials onto the blade. This aggregate was transferred by means of a sterile microspatula to the surface of 2% agar (Difco Laboratories) in standard-size plastic petri dishes. An overlay of either *Escherichia coli* (ATCC 25922) or *Proteus mirabilis* (Adelphi University strain) cultivated as in the previous study (4) served as the food source. Cultures prepared in this way were stored in the dark at room temperature. Examination was performed using bright-field optics after a minimum of 3 days of incubation. Dishes yielding amoebae in either trophic or cystic stages were scored as positive. If after 10 days of incubation amoebae in either stage were not visible, cultures were scored as negative. Morphological characteristics such as trophic size, cyst morphology, form of pseudopod, and the length/breadth ratio permitted preliminary assignment of most isolates to either the family or genus level.

Of 168 mushrooms cultivated by 23 different suppliers representing six widely distributed geographical localities sampled over a 3-year period, 161 (96%) contained amoebae on their surfaces as evidenced by the appearance of amoebae in isolation dishes (Table 1). None of 166 samples of internal tissue from the stalk presented amoebae, and only 1 of 166 samples of internal cap tissue yielded amoebae under the conditions utilized in this study.

Information about generic diversity was obtained by direct observation of trophic and cystic stages, as well as by testing for the presence of amoeboflagellates by the introduction of a distilled water overlay directly into the isolation dishes. In this way, it was determined that the most frequently observed species of amoebae belonged to the genus *Acanthamoeba*. Several such species were determined by identification through pseudopodial type, length/breadth ratio, and cyst morphology. Other genera represented among these samples included *Hartmanella* and *Vanella*. No attempt was made in this initial investigation to clone or classify these strains further since their number was large and the determination of species is a long arduous task that requires separate study. In no case were amoeboflagellates noted despite testing of all isolates whose colonization and growth pattern appeared to resemble those typical for *Naegleria* and *Adelphamoeba* species. No testate amoebae were observed in any samples tested.

In addition to amoebae, various ciliates, including holotrichs and hypotrichs, were observed in the isolation dishes. The most frequently encountered ciliate appeared to belong

TABLE 1. Isolation of amoebae from the surface, cap, and stalk of edible mushrooms

Date	Location	Amoebae isolated/samples tested		
		Surface	Cap	Stalk
11/7/79	Evansville, Pa.	6/6	0/6	0/6
11/12/79	Kennett Square, Pa.[a]	5/5	0/5	0/5
11/27/79	Quarryville, Pa.	6/6	1/6	0/6
12/3/79	Evansville, Pa.	6/6	0/6	0/6
12/10/79	Kennett Square, Pa.[a]	6/6	0/6	0/6
1/4/80	East Windsor, Conn.[b]	6/6	0/6	0/6
1/10/80	East Windsor, Conn.[b]	6/6	0/6	0/6
2/22/80	St. Louis, Mo.	7/7	0/7	0/7
3/11/81	Avondale, Pa.	8/8	0/8	0/8
3/18/81	Evansville, Pa.	7/8	0/8	0/8
3/30/81	Oxford, Pa.	7/8	0/8	0/8
5/29/81	St. Louis, Mo.	5/7	0/7	0/7
6/13/81	Monterey, Calif.	8/8	0/8	0/8
6/22/81	Rising Sun, Md.	8/8	0/8	0/8
6/25/81	Oxford, Pa.	8/8	0/8	0/8
7/4/81	St. Louis, Mo.	8/8	0/8	0/8
9/10/81	East Windsor, Conn.	8/8	0/8	0/8
9/28/81	Kennett Square, Pa.[a]	8/8	0/8	0/8
10/3/81	Kirkwood, Pa.	7/8	0/8	0/8
10/17/81	Glen Mills, Pa.	8/8	0/8	0/8
10/18/81	Langdenberg, Pa.	8/8	0/8	0/8
10/26/81	Louisville, Ky.	5/5	0/3	0/3
10/26/81	Lawton, Ky.	6/8	0/8	0/8
10/26/81	Worthington, Pa.	4/4	0/4	0/4

[a] Represents different suppliers.
[b] Represents same supplier.

to the genus *Colpoda*. No identification of other ciliates was attempted. Ciliates appeared in 38 of 166 (23%) surface samples tested. No ciliates were observed from internal stalk and cap tissues.

The discovery that amoebae may be isolated from the surface and internal tissues of the mushroom *L. trullisata* (4; Napolitano and Flanagan, J. Protozool. 27:46A, 1980) led to related questions about the occurrence of similar relationships in other mushrooms. The results obtained in this study begin to answer these questions and indicate that the surface tissues of edible mushrooms also contain amoebae. Since *L. trullisata* resides in a beach sand environment, which is very different from the richer organic environment in which edible mushrooms are usually cultivated, it is suggested that such associations between amoebae and mushrooms may be widespread.

Although amoebae may be isolated from the surface of both kinds of mushrooms, only *L. trullisata* yielded these protozoa from internal tissues. This difference is of interest since it may represent a fundamental distinction between the ways in which amoebae and mushrooms associate in nature. For example, if the association is initially at the mycelial stage, as has been postulated in the case of *L. trullisata* (4), with amoebae encysting before or during transformation to the fruiting stage of the mushroom, the question must be asked as to why this association is so different with edible mushrooms. Are there opportunities provided to amoebae in the vicinity of the growing mycelial stage of *L. trullisata* that are not presented by the same stage in edible mushrooms? It might also be asked whether the mycelial stage of edible mushrooms produces factors or creates conditions that discourage such an association in their environment. The answers to these and other questions posed by this new information about amoebae-mushroom relationships are of great interest to biologists and mushroom growers.

The absence of amoeboflagellates from the surface of mushrooms tested in this study, as well as from the surface of *L. trullisata* and its surrounding carposphere, is noteworthy since it is widely held that these amoebae are universally distributed in soils. The absence of testate amoebae is interesting inasmuch as a large number of such species have been reported from soils in which truffles (*Tuber melanosporum*) grow (1).

Because soil amoebae are recognized as pathogens (3) and because virulent and nonvirulent amoebae have been obtained from lettuce, radishes, and onions (2), the role of vegetables in transmitting amoebae to humans must be evaluated.

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LITERATURE CITED

1. **Bonnet, L.** 1974. Quelques aspects du peuplement Thécamoebien des Sols de Truffières. Protistologica **10**:281–291.
2. **Clurea-Vari Saanen, M.** 1981. L'isolement d'amibes libres dans le sol et les légumes; étude morphologique et pathogenique des souches isolèes. Rev. Med. Suisse Romande. **101**:229–238.
3. **Duma, R. J.** 1972. Primary amoebic meningoencephalitis. Crit. Rev. Clin. Lab. Sci. **3**:163–192.
4. **Napolitano, J. J., and V. D. Flanagan.** 1980. Occurrence of amoebae in and around the mushroom *Laccaria trullisata*. J. Protozool. **28**:494–497.