MOULDS

Their Isolation, Cultivation, and Identification

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UNIVERSITY OF TORONTO PRESS

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Preface

Many of the skills and techniques involved in the isolation, cultivation, and identification of mould fungi are more of an art than a science. Laboratory procedures considered routine by mycologists (scientists who study fungi) seem to be mysterious, well-guarded secrets to the uninitiated. Generally they are passed along from generation to generation of mycologists through a time-honoured system of apprenticeship. Access to these skills is difficult for the beginner; most books on the subject are rather advanced and contain such a quantity of information that it is not clear where to begin.

My experience with these problems comes from two sources: first my work with Canada Agriculture and later as a teacher at the University of Toronto. In working in a government laboratory I was frequently confronted with mould problems that had arisen in agricultural or industrial situations. Most often, the person responsible for the solution of these problems was a bacteriologist who had had only limited experience with fungi. The scattered and highly technical mycological literature was no help whatsoever; what was needed was a simple text demonstrating what moulds are and how they are grown and identified, and indicating which books would be useful for further work.

In university teaching I encountered students just learning about fungi who were, like the bacteriologist, confused by the complex literature in mycology. Identification of moulds was difficult for them, and usually required the use of manuals containing large numbers of fungi in addition to those that are commonly seen in the class-room. What was needed here was a simple means of identifying the common moulds that make up 90 per cent or more of those encountered by the average student. To remedy the situation I designed a simple set of keys that presented the most frequently occurring moulds first so that the most inexperienced student could arrive at a positive identification as quickly as possible. These keys were used in the class-room for several years and were gradually improved.

The text that follows is what I hope will be a point of access to the often bewildering world of moulds. It is intended to be an introduction; in no way does it attempt to replace the many excellent and more detailed works that the beginner will gradually learn to use. In fact, it will have served its purpose most effectively when the reader no longer needs it. In the chapter on identification the necessary literature is cited; for isolation and cultivation, the relevant literature is much more widely scattered, but the reader requiring more detailed information should refer to some of the excellent collections of specialists' techniques that are available, notably those of Booth (1971C), Fuller (1978), and Stevens (1974).

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1 Moulds and their characteristics

Moulds, those dusty little spots that spread over bread, cheese, books, and other things in the home, cause the loss of millions of dollars to our economy every year. To deal successfully with this menace we must understand what moulds are and exactly what they are doing.

Moulds are microscopic, plant-like organisms, composed of long filaments, that grow over the surface and inside nearly all substances of plant or animal origin. Because of their filamentous construction and consistent lack of chlorophyll they are considered by most biologists to be separate from the plant kingdom and members of the kingdom of fungi. They are related to the familiar mushrooms and toadstools, differing only in not having their filaments united into large fruiting structures. For our purposes here, we shall consider as moulds only fungi that are commonly encountered in the home and laboratory and that can be easily grown and studied.

The filaments of the mould fungi are called *hyphae*. When the hyphae are numerous enough to be seen by the naked eye they form what is called a *mycelium*. It is the hyphae and resulting mycelium that invade things in our homes and cause them to decay.

Moulds reproduce by *spores*. Spores are like seeds and germinate to produce a new mould colony when they land in a suitable place. Unlike seeds, they are very simple in structure and never contain an embryo or any sort of preformed offspring. Spores are produced in a variety of ways and occur in a bewildering array of shapes and sizes. In spite of the diversity, the form of spores is quite constant for any given mould, making it one of the most useful features for identification.

The most basic difference between spores lies in their method of initiation, which can be either sexual or asexual. Sexually initiated spores result from a mating between two different organisms or hyphae, whereas asexual spores result from a simple internal division or external modification of an individual hypha. The recognition of a mating and subsequent spore formation is often difficult for an observer, and is usually reserved for patient specialists. However, for practical purposes one can learn to recognize certain indications of the sexual process, namely, the four kinds of sexually determined spores that appear in mould fungi: (1) oospores, (2) zygospores, (3) ascospores, and (4) basidiospores.

Oospores are produced when male gametes (reproductive nuclei) enter a large spherical cell (*oogonium*) and fertilize the eggs within. The result, as seen in routine examination, is numerous oogonia containing one to several spherical and

often brownish eggs. The oogonia are usually penetrated by one or more hyphae (*antheridia*) that give rise to the male nuclei (figure 1C).

Zygospores do not occur inside any kind of enclosing structure, but are produced by the direct fusion of two hyphal protrusions (*suspensors*) from neighbouring filaments. Usually zygospores are recognized as large, nearly spherical, often dark brown or black, rough-walled spores with two connecting hyphae, representing the two mating gametangia (figure 2C; see also the illustration 'zygospores' at the end of the book). Sometimes the zygospore may be surrounded by several finger-like extensions from the two gametangia.

Ascospores are produced within spherical to cylindrical cells called *asci*, most often in groups of four or eight (figure 3A). Usually the asci are produced within some kind of enclosing structure and thus are not found exposed on the hyphae. In a few cases the asci may be borne among hyphae and resemble oogonia with eggs, but they will never be penetrated by any sort of fertilizing hypha. Fertilization occurs early in the life cycle and is not evident at the time ascospores are produced.

Basidiospores are always produced externally on a structure called a *basidium*. Basidia come in a variety of forms, but those commonly encountered on moulds will be club-shaped and bear four or eight spores on sharp projections at the apex. At first it may be difficult to distinguish between a basidiospore and one of the asexually initiated spore types, but one should always suspect the presence of basidia when externally produced spores consistently occur in groups of four or eight (figure 4). As with ascospores, basidiospores are the result of an early fertilization that is not easily observed.

Asexual spores usually occur either in sporangia or as conidia. *Sporangia* are modified hyphae or cells containing numerous spores (*sporangiospores*). They never have more than a single connecting hypha and the spores do not constantly occur in groups of four or eight as do ascospores.

Conidia are the most difficult group to characterize because of their great diversity of form. The only feature that most conidia have in common is that they occur externally on the cells that produce them. These conidium-bearing (*conidiogenous*) cells may occur within rather specialized or characteristic structures that resemble those that frequently bear asci, however, and it is often necessary to break them open to confirm that the spores are truly conidia and not ascospores. Conidia that are borne on the hyphae, without any kind of compound fruiting structure, are the most commonly encountered type. Structures that completely enclose the conidium-bearing cells are called *pycnidia*, and those resulting from a fusion of conidium-bearing cells are called *synnemata* if they are longer than they are broad or *sporodochia* if broader than long.

2 Classification of moulds

Strictly speaking, all fungi should be classified according to their method of sexual reproduction. In many cases this is possible, allowing us to recognize several groups of fungi, of which the following four are of interest to us as moulds. Fungi that are encountered most often or exclusively in the asexual condition create special problems and are discussed separately as *anamorphs*.

Oomycetes (figure 1)

Members of this group all reproduce by oogonia and eggs. The hyphae have few or no cross-walls (*septa*) and thus appear as long, clear tubes. If a hypha is broken, most of the contents run out. Many oomycetes reproduce asexually by *zoospores*, which are motile and can swim quite rapidly. Because of their motile zoospores, oomycetes commonly require water for reproduction and are often encountered in water or wet soil.

Zygomycetes (figure 2)

As their name implies, these fungi all produce zygospores. They resemble the oomycetes in having hyphae that usually lack cross-walls or septa, but differ in lacking motile spores. Asexual reproduction is by sporangia or conidia. The members of this group are usually terrestrial and will be encountered only occasionally in aquatic conditions.

Ascomycetes (figure 3)

All ascomycetes have ascospores borne inside asci. The hyphae always bear numerous septa. Asexual reproduction is by conidia that always lack motility. Although most ascomycetes are terrestrial, some occur in freshwater or marine habitats.

Basidiomycetes (figure 4)

This large group, which includes mushrooms and puffballs, is characterized by the

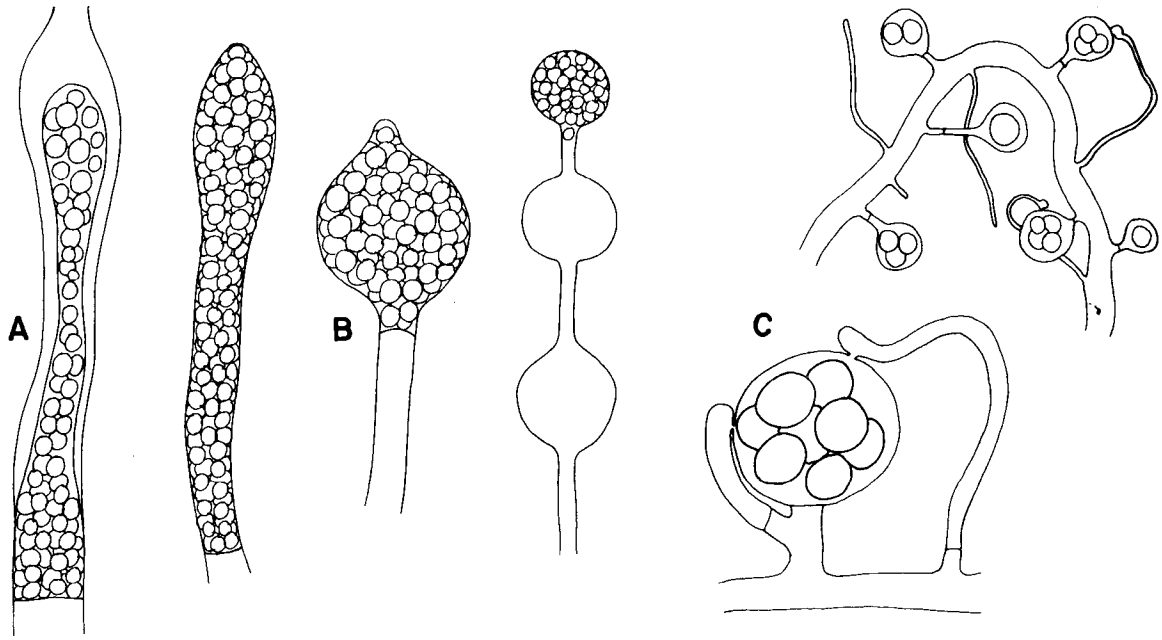

Figure 1. Oomycetes
A: zoosporangia of *Saprolegnia* sp. B: zoosporangia of *Pythium* sp. C: oogonia and antheridia of *Saprolegnia* sp.

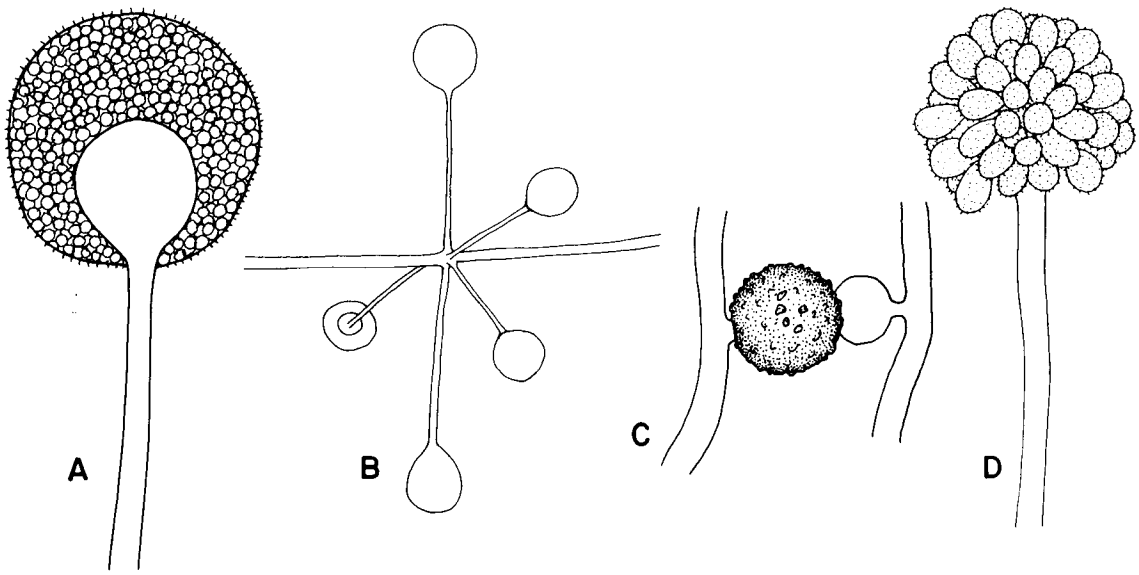

Figure 2. Zygomycetes
A: sporangia of *Mucor* sp. B: whorl of sporangia of *Absidia* sp. C: zygospore of *Zygorhynchus* sp. D: sporangiophore and sporangiola of *Cunninghamella* sp.

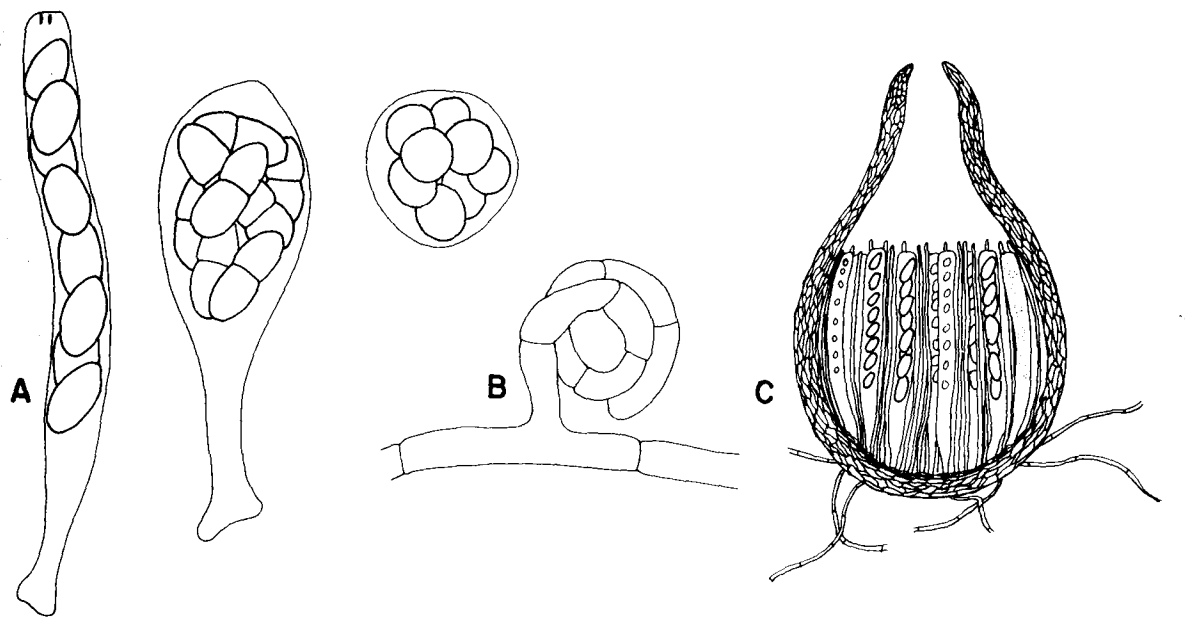

Figure 3. Ascomycetes
A: three kinds of asci: cylindrical, clavate, and spherical. B: initial phase of sexual reproduction. C: cross-section of a flask-shaped perithecium bearing cylindrical asci.

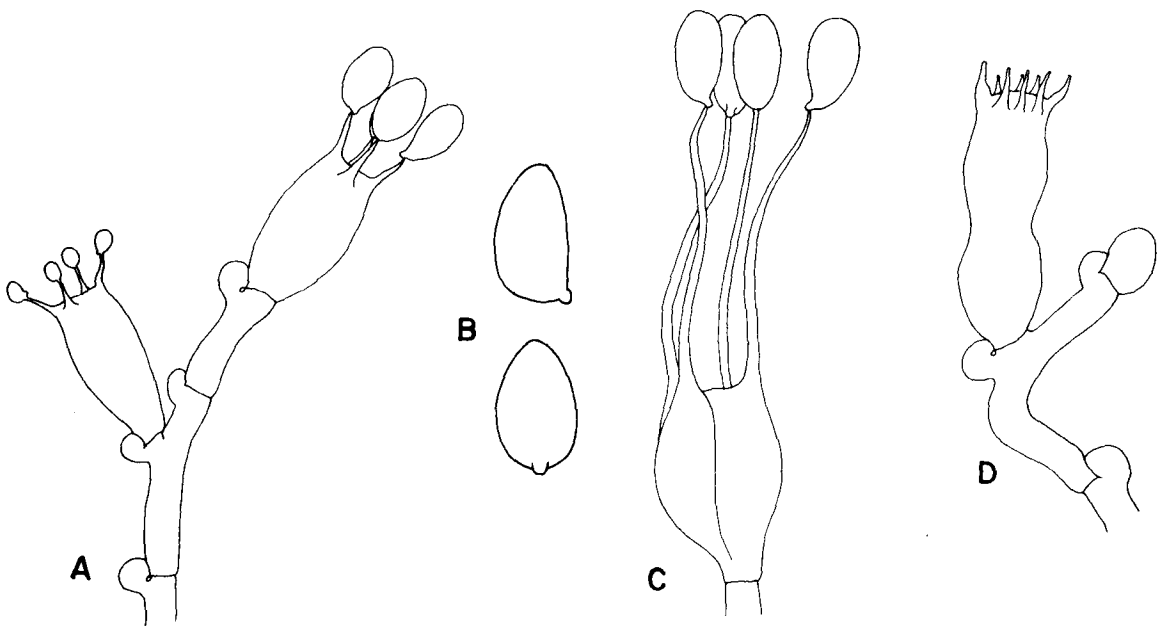

Figure 4. Basidiomycetes
A: four-spored undivided basidium (holobasidium); note the clamp connection at each cross-wall. B: two typical basidiospores, the upper one in side view and the lower in front view. C: a four-celled, cruciate basidium typical of many jelly fungi. D: eight-spored holobasidium typical of species of *Sistotrema*; the spores have been discharged.

presence of basidia and basidiospores. Like ascomycetes, to which they are related, basidiomycetes have hyphae with septa and lack motile spores. The hyphae of many basidiomycetes bear characteristic swellings, called *clamp connections*, that play a specialized role in nuclear migration. Asexual spores, when formed, are produced as conidia. Most basidiomycetes are terrestrial.

Anamorphs

This group comprises the large number of fungi known to reproduce asexually by conidia and is by far the most important in a study of moulds. The asexually reproducing structures of a fungus are called *anamorphs*, which, together with the sexually reproducing structures or *teleomorphs*, make up the *holomorph* or whole fungus. Many fungi will be encountered only as anamorphs and cannot conveniently be classified with the ascomycetes or basidiomycetes to which they undoubtedly belong. Mycologists have long used a system of classification that allows anamorphs to be named separately from the holomorph of which they form a part. As a consequence, many fungi can have two different names. For example, the name *Eurotium repens* pertains to a holomorph with both ascospores and conidia, whereas *Aspergillus repens* pertains only to the anamorph of the same fungus.

Anamorphs show considerable structural diversity and can be classified or described according to three attributes: (1) the method of conidium production (conidiogenesis), (2) the locus of conidiogenesis, and (3) the sequence of conidiogenesis.

METHOD OF CONIDIOGENESIS

Production of a conidium involves the transformation of part of a cell into a separable spore. Sometimes a hypha or cell becomes separated into one or more segments by septa. The separate cells then thicken, swell, and finally separate. This kind of conidiogenesis, where the septa appear before the conidium is initiated, is called *thallic* (figure 5A, C). When the new conidium is initiated, begins to swell or thicken, and *then* is cut off by a septum, the conidiogenesis is called *blastic* (figures 5B, D; 6). When the wall of the conidium is continuous with the cell that produced it, it is called either *holothallic* (when thallic) (figure 5A) or *holoblastic* (when blastic) (figure 5B). When only the inner walls of the conidium-bearing cell are involved in conidiogenesis, the prefix 'entero-' is used, resulting in the terms *enterothallic* (figure 5C) and *enteroblastic* (figure 5D). Although enterothallic types are rare, enteroblastic types are probably the most common of all and are represented by the ubiquitous phialide.

The following genera, illustrated at the end of the book, illustrate features of this section:

Holoblastic: *Botrytis, Geniculifera, Trichocladium*
Enteroblastic: *Acremonium, Bipolaris, Penicillium*
Holothallic: *Geotrichum, Oidiodendron*

LOCUS OF CONIDIOGENESIS

Conidiogenesis usually can be traced to a particular point or locus on the conidiogenous cell. If this point remains stationary and gives rise to more conidia it is said to be *stable* (figure 5D). If subsequent conidia are produced at new points on the cell the conidiogenous locus is said to be *unstable*. Unstable loci may gradually elongate and be called *progressive* (figure 6C), or move toward the base of the conidiogenesis cell and be *retrogressive* (figure 6B). Usually it is necessary to examine several cells to 'reconstruct' the loci of successive spore productions.

The following demonstrate types of loci:

Stable: *Aspergillus, Dactylella*
Unstable-progressive: *Arthrobotrys, Scopulariopsis, Stemphylium*
Unstable-retrogressive: *Arthrinium, Geotrichum*

SEQUENCE OF CONIDIOGENESIS

If only a single conidium is produced on a conidiogenous cell (figure 6A), we can hardly speak of a sequence. If, however, more than one is produced they can occur either *simultaneously* (figure 5A, B, C) or *serially* (i.e. one after another) (figures 5D; 6B, C).

The following illustrate sequence types:

Solitary: *Monacrosporium*
Multiple, simultaneous: *Botrytis, Oedocephalum*
Multiple, serial: *Cladosporium, Phialophora, Ulocladium*

Other mould-like organisms

In routine work we often encounter organisms that are similar to moulds but do not fit our strict definition of the term. Either the organism is not filamentous or it is filamentous but not strictly a fungus. We can put four groups of organisms into this category; bacteria, actinomycetes, yeasts, and slime moulds.

BACTERIA

Bacteria represent a very ancient group of organisms, perhaps as old as four billion years. Colonies of bacteria are composed of minute spore-like cells that together form a slimy mass. Such colonies never contain hyphae and are thus easily distinguished from those of true moulds. Bacterial cells, rarely more than 1 μm in diameter, are difficult to examine, even with a good microscope, and are best seen when stained. Many bacteria are motile and swim vigorously.

ACTINOMYCETES

These organisms are usually classified as bacteria but have filaments like fungi. The filaments are seldom more than 1 μm in diameter, however, and are thus

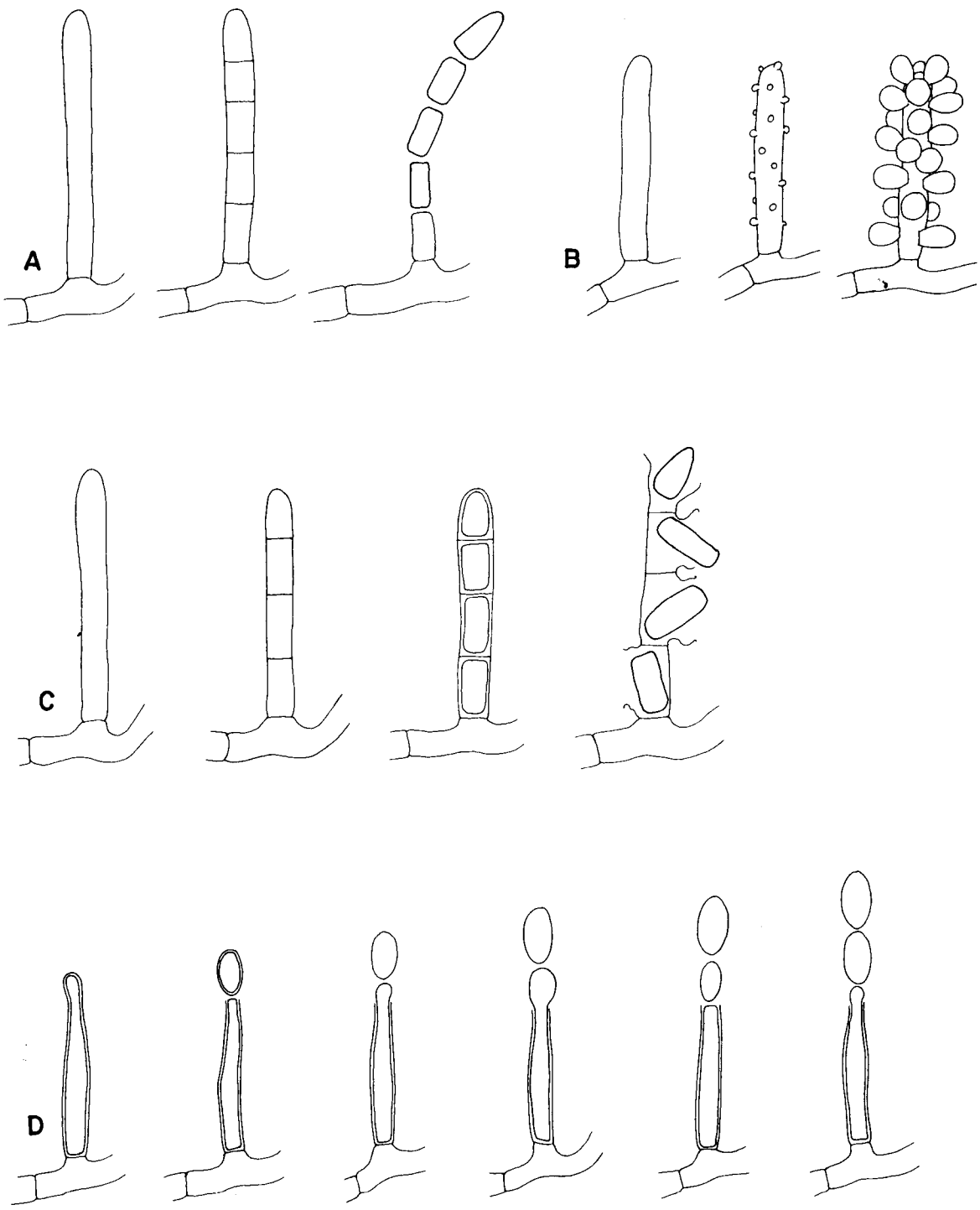

Figure 5. Conidiogenesis I
A: holothallic; the multiple conidia are produced simultaneously. B: holoblastic; the multiple conidia are produced simultaneously. C: enterothallic; the multiple conidia are produced simultaneously. D: enteroblastic; the multiple conidia are produced serially from a stable locus.

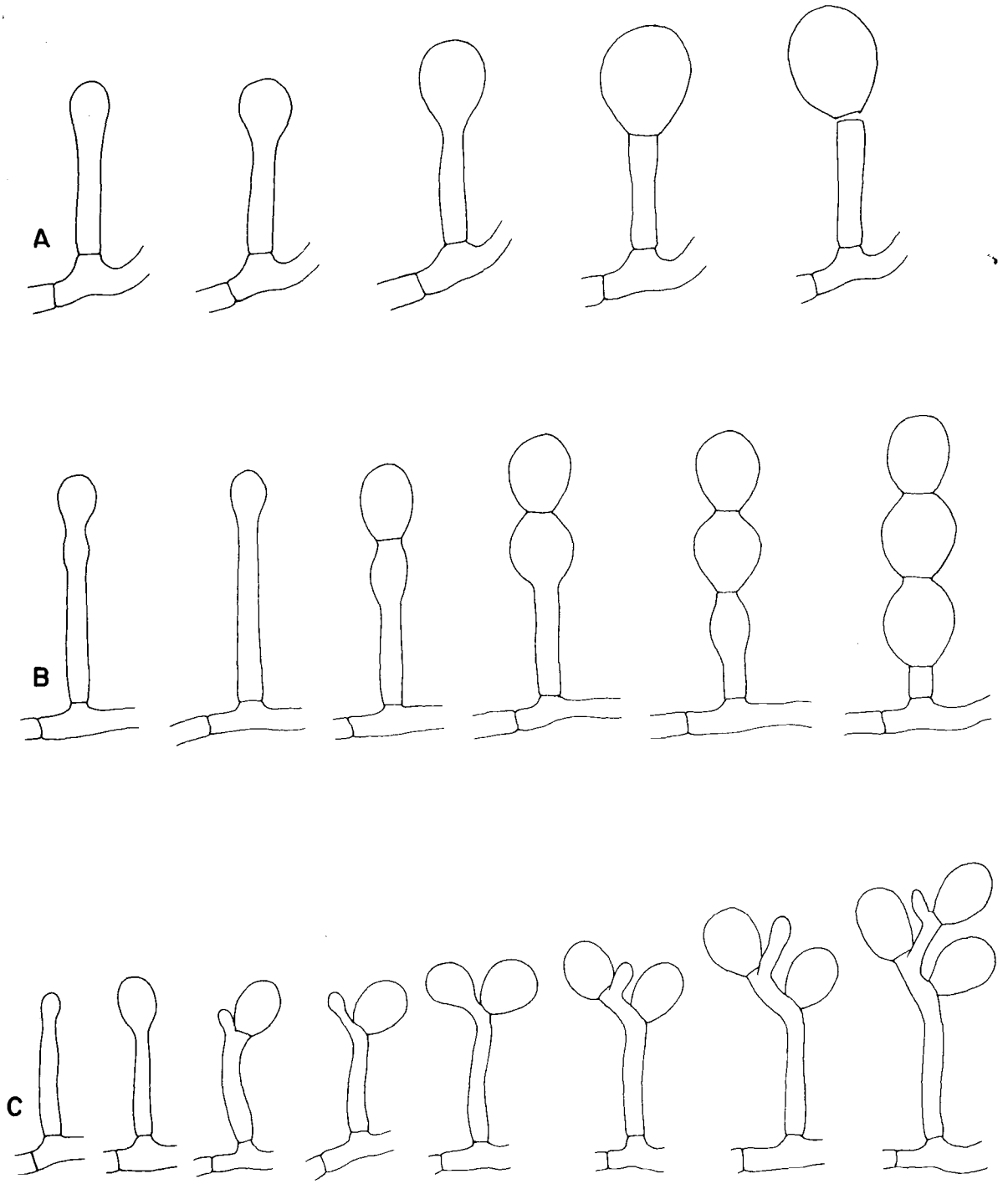

Figure 6. Conidiogenesis II
A: holoblastic; the conidium is solitary. B: holoblastic; the multiple conidia are produced serially from a retrogressive locus. C: holoblastic; the multiple conidia are produced serially from a progressive locus.