Secondary Metabolism and Differentiation in Fungi

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Series Introduction

Mycology is the study of fungi, that vast assemblage of microorganisms which includes such things as molds, yeasts, and mushrooms. All of us in one way or another are influenced by fungi. Think of it for a moment – the good life without penicillin or a fine wine. Consider further the importance of fungi in the decomposition of wastes and the potential hazards of fungi as pathogens to plants and to humans. Yes, fungi are ubiquitous and important.

Mycologists study fungi either in nature or in the laboratory and at different experimental levels ranging from descriptive to molecular and from basic to applied. Since there are so many fungi and so many ways to study them, mycologists often find it difficult to communicate their results even to other mycologists, much less to other scientists or to society in general.

This Series establishes a niche for publication of works dealing with all aspects of mycology. It is not intended to set the fungi apart, but rather to emphasize the study of fungi and of fungal processes as they relate to mankind and to science in general. Such a series of books is long overdue. It is broadly conceived as to scope, and should include textbooks and manuals as well as original and scholarly research works and monographs.

The scope of the Series will be defined by, and hopefully will help define, progress in mycology.

Paul A. Lemke

Foreword

For too many years, microbial secondary metabolism has been considered a laboratory artifact with no significance in nature. It is indeed peculiar that although biologists accepted the concept that plant secondary metabolites had function, the concept of microbial secondary metabolites, and especially of antibiotics, as ecological effectors has been widely disputed. I believe this bias against function for antibiotics is due to two major facts: (1) antibiotics are of major economic significance and patents on them were much more easily obtained if they were not considered products of nature, and (2) experiments to prove their ecological significance were more often unsuccessful than successful. Although we can dismiss point (1) as being of no scientific significance, point (2) deserves some comment. It is clearly much more difficult to do experiments in natural environments than under controlled conditions in the laboratory. Thus, one should expect that it would be quite difficult to prove or disprove a possible function for a compound in an ecological type of experiment. For this reason, I have been much more impressed with the few successful experiments pointing to a real function of an antibiotic in nature than with the larger number of inconclusive experiments. Broadening our scope from antibiotics to secondary metabolites, there is no doubt that functions exist for these compounds. They serve as antagonistic agents, symbiotic agents, sexual hormones, effectors of sporulation and germination, and metal transporters, etc., and in such roles offer the producing organism an opportunity to survive in the competitive arena of nature.

The editors of the present volume approach secondary metabolism in a very mature way, i.e., as a series of chemical processes correlated with a series of morphological processes, both of which are partners in the extremely important act of differentiation. Although this volume deals with fungi, there is no doubt that it contains messages for those scientists and technologists working with unicellular bacteria and actinomycetes producing secondary metabolites. Even more importantly, it could strike a death blow to the concept that secondary metabolites are without function.

Arnold L. Demain

Preface

Cellular differentiation involves molecular and cytological changes during development. In the fungi, chemical changes have traditionally been studied by natural products chemists and categorized as "secondary metabolites," whereas changes in cellular structure have been studied by biologists and termed "morphogenesis." In this volume we view secondary metabolism and morphogenesis as correlated events in the overall process of cellular differentiation.

Many other books have been written about either secondary metabolism or fungal morphogenesis, usually treating these as independent processes. This illustrates one of the failings of contemporary biological science, wherein there is a tendency to reduce problems to constitutive elements which are then studied as isolated events. The result is an ever-narrowing and myopic view of the entire organism. The sum of the parts no longer equals the whole.

This book does not attempt to be an exhaustive review of previous science or thought. Rather, we have chosen topics that highlight current research, with particular emphasis on genetics, molecular biology, and ecology. It is in this latter field, an area of increasing importance, that we have come to see biological systems not as independent and isolated chemical units, but as entities in an interrelated universal whole.

We asked our authors to indulge in "honorable speculation" and told them we welcomed "responsible hypotheses" in the hope that this volume would serve not only as a reference to what has already been done, but as a guideline for future research. Each chapter presents a unique perspective on fungal differentiation, and each is rich with ideas for future experimentation. We can only hope that our readers will find information and inspiration that will be of use either in their own research or in formulating a more profound view of the biological universe.

J. W. Bennett
Alex Ciegler

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1

Differentiation and Secondary Metabolism in Mycelial Fungi

J. W. Bennett / Tulane University, New Orleans, Louisiana

INTRODUCTION

"Differentiation" and "secondary metabolism" are terms which encompass an enormous body of accumulated scientific knowledge and evoke an even larger body of unanswered questions. Neither term is easily defined and many overlapping and sometimes ambiguous definitions have been used by different writers. Table 1 presents a list of pertinent terminology with the definitions that will be employed in this chapter.

Historically, development has been viewed as two separate, although frequently simultaneous, processes: growth and differentiation. A more modern conception might be that there is no such thing as an undifferentiated cell—there are simply changes in states of differentiation. Nevertheless, this arbitrary distinction between undifferentiated and differentiated states is useful for purposes of discussion and will be retained with a recognition of the inherent limitations.

In multicellular plants and animals, after an initial stage of undifferentiated growth, the processes of growth and differentiation occur concomitantly. Indeed, "cellular differentiation is the necessary condition of multicellular life" (Gross, 1968) or, put another way, "in order for organisms to become large, they must divide the labor; the two phenomena are inseparable" (Bonner, 1974, p. 25).

Many aspects of development are common to both plants and animals, but the different nature of the biological systems has profound effects upon the pattern of development. Plant cells generally have rigid cell walls and are not motile. In plants, development is indeterminate, with the growing points remaining permanently embryonic and with specific organ systems exhibiting limited growth; most animals show a distinct embryonic phase which ends when adult structure is achieved. These distinctions are important because most biologists who call themselves "developmentalists" and theorize on the molecular basis of differentiation are trained in the animal sciences. They frequently view

Table 1 Definitions

Major term	Related terms	Definition
Development		Process of growth and differentiation by which the potentialities of a zygote or spore are established; sequence of progressive changes resulting in increased biological complexity.
	Epigenesis	Development involving gradual diversification and differentiation of an initially undifferentiated entity such as a zygote or sphore. Development is an epigenetic phenomenon.
	Morphogenesis	The formation of tissues and organs; the developmental process leading to the characteristic mature form of an organism; those aspects of development related to morphological changes.
Differentiation		Progressive diversification of the structure and function of cells in an organism; acquisition of differences during development.
Growth		An increase in size, especially an increase in the quantity of cellular material; frequently dry weight is used as an equivalent.
Mycelium		The vegetative part (thallus) of a fungus consisting of one or more hyphae; a mass of hyphae.
	Hyphae	Branched filamentous cells; may be septate or aseptate.
Secondary metabolites		Diverse natural products unnecessary for growth, of restricted taxonomic distribution, generally produced during a limited stage of the cell cycle from a few simple precursors derived from primary metabolism; among microorganisms the term "idiolite" may be used as a synonym.

Source: Ainsworth (1971); King (1972); Turner (1971); *Webster's Third* (1972).

differentiation as an ultimately self-limiting process in development whereby differentiation proceeds through a number of significant restrictions in developmental potential (Rutter et al., 1973).

The concept of "pluripotency" is an important one in embryology. Pluripotency is the condition of having a large indeterminate number of possible fates; it is viewed as a property lost during the developmental process in animals. The historical context of "pluripotency" led botanists to adopt the term "totipotency" to express the idea that any somatic, nucleated plant cell could, under the influence of appropriate stimuli, dedifferentiate and regain the ability to act like a zygote and produce an entire new plant (Needham, 1950; Street, 1976; Steward and Mohan Ram, 1961).

Among prokaryotes and unicellular eukaryotes, growth and differentiation are phenomena observed in populations, not simultaneously in single cells. A given cell may divide vegetatively for a number of generations, or it may form a spore. When viewing an entire population, some cells are differentiated, some are not; but when viewing any given cell (organism), it is either vegetative, reproductive, or in transition. Moreover, this transition from a vegetative to a reproductive form is dependent upon the external environment. For this reason, microorganisms have been useful model systems for studying certain forms of differentiation; for example, experimental manipulation of the environment can be used as a "trigger" to initiate the transition between "undifferentiated" and "differentiated" states.

The fungi hold a unique and useful position for experimentalists. Like plants and animals, they are multicellular and eukaryotic; like plants, individual cells are totipotent; and like prokaryotes and unicellular forms, differentiation is generally triggered by changes in the environment. Some of the most elegant experiments on fungal morphogenesis involve the water mold *Blastocladiella emersonii* (Cantino, 1966), the acellular slime molds or Mycomycetes (Alexopoulos, 1962; 1966), and the cellular slime molds or Acrasiales (Ashworth, 1971; Gregg, 1966; Sussman and Brackenbury, 1976). But these are not "mycelial fungi."

What are "mycelial fungi"? Formal fungal taxonomy is by no means settled and a variety of classification schemes have been adopted by different workers. Alexopoulos (1962), Hawker (1966), and others have accepted a general scheme in which the true fungi (Eumycota) are subdivided as follows:

Lower fungi (Phycomycetes). Unicellular or with a mycelium that is generally aseptate.

Higher fungi. The mycelium is regularly septate, with the exception of the yeasts. The higher fungi are usually divided into three classes:

Ascomycetes: Sexual spores (ascospores) borne endogenously.
Basidiomycetes. Sexual spores (basidiospores) born exogenously.
Deuteromycetes. Sexual reproduction lacking. This form class is also called the Fungi Imperfecti.

In the broadest sense, the mycelial fungi encompass all filamentous species of both the lower and the higher fungi. However, perhaps because Vuillemin restricted the term "hyphae" to septate forms (Ainsworth, 1971, p. 281), usage of "mycelial fungi" is often accepted as meaning only the higher fungi. I will follow this limited definition and exclude the Phycomycetes from my discussion. The subject of yeast-mold dimorphism will also be excluded, but it is reviewed by Cutler and Hazen in Chapter 10 of this volume. Breitenback and Lachkovics discuss the role of the yeast genome in differentiation from a contemporary molecular perspective in Chapter 11 of this volume.

MORPHOGENESIS

Spores can be considered as "both a beginning and an end of fungal development" (Ainsworth, 1976, p. 81). Spores possess many attributes in common with the totipotent cells of plants and animals and more attention has been given to the spore than any other fungal structure. Spore morphology and development are basic to fungal systematics and most studies on fungal differentiation involve aspects of spore formation or germination.

"Spore" is a general term for a reproductive structure. In fungi, spores may be produced asexually or sexually, and in many species both forms of reproduction occur. Generally, the expression "vegetative" is associated with the mycelial phase of the fungal life cycle by mycologists. This is in contrast to another common usage of the term which equates "vegetative" to "asexual."

Spores vary in shape, size, ornamentation, origin, modes of liberation, function, and ontogeny. Asexual spores (conidia) have received the most detailed attention by classical mycologists because they provide the basic taxonomic criteria within many groups. The earliest and the majority of studies on spore differentiation are descriptive. Vuillemin was among the first to emphasize spore development as a taxonomic criterion rather than the characteristics of the spores themselves. He distinguished spores which are not separated from the hypae producing them (thallospores) from spores borne upon special hyphae which separate upon maturity (conidiospores) (see Fig. 1).

The extraordinary diversity of spore types in nature has challenged mycologists to find a satisfactory nomenclature. Ainsworth's *Dictionary of the Fungi* (1971) lists 100 spore names. In face of this proliferation, many workers simply

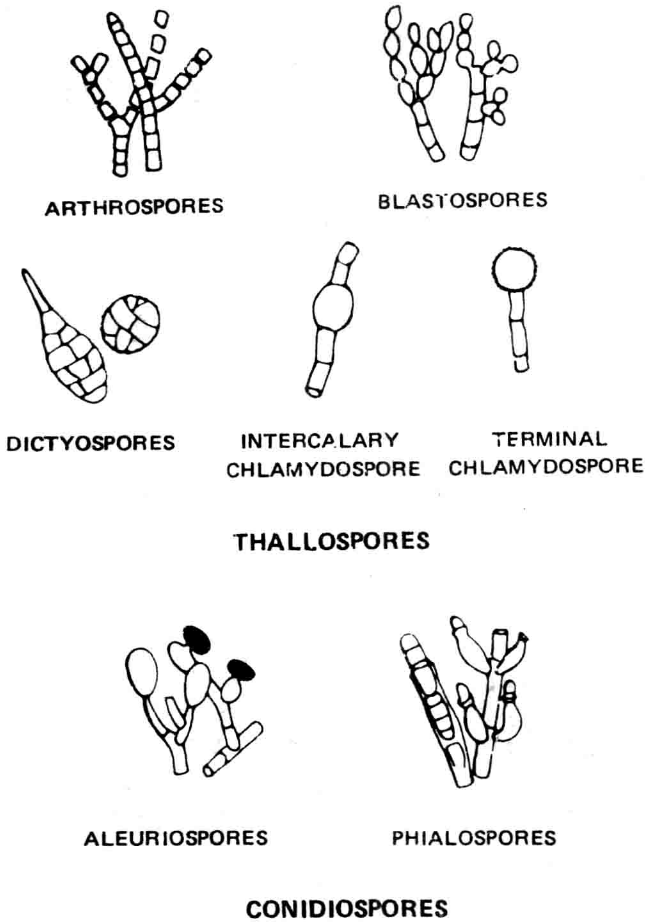

Figure 1 Examples of thallospores and conidiospores; Vuillemin's spore groups (after Tubaki, 1966).

call all asexual fungal spores "conidiospores." This in turn creates a terminological backlash. Vullemin's conidiospores are now designated "phiallospores" or "conidia vera." The major spore types delineated by Tubaki (1966) for the Fungi Imperfecti are illustrated in Fig. 2. An extended treatment of spore types and terminology within this group has been given by Kendrick (1971). Other recent monographs on conidial fungi include Cole and Samson (1979) and Cole and Kendrick (1981).

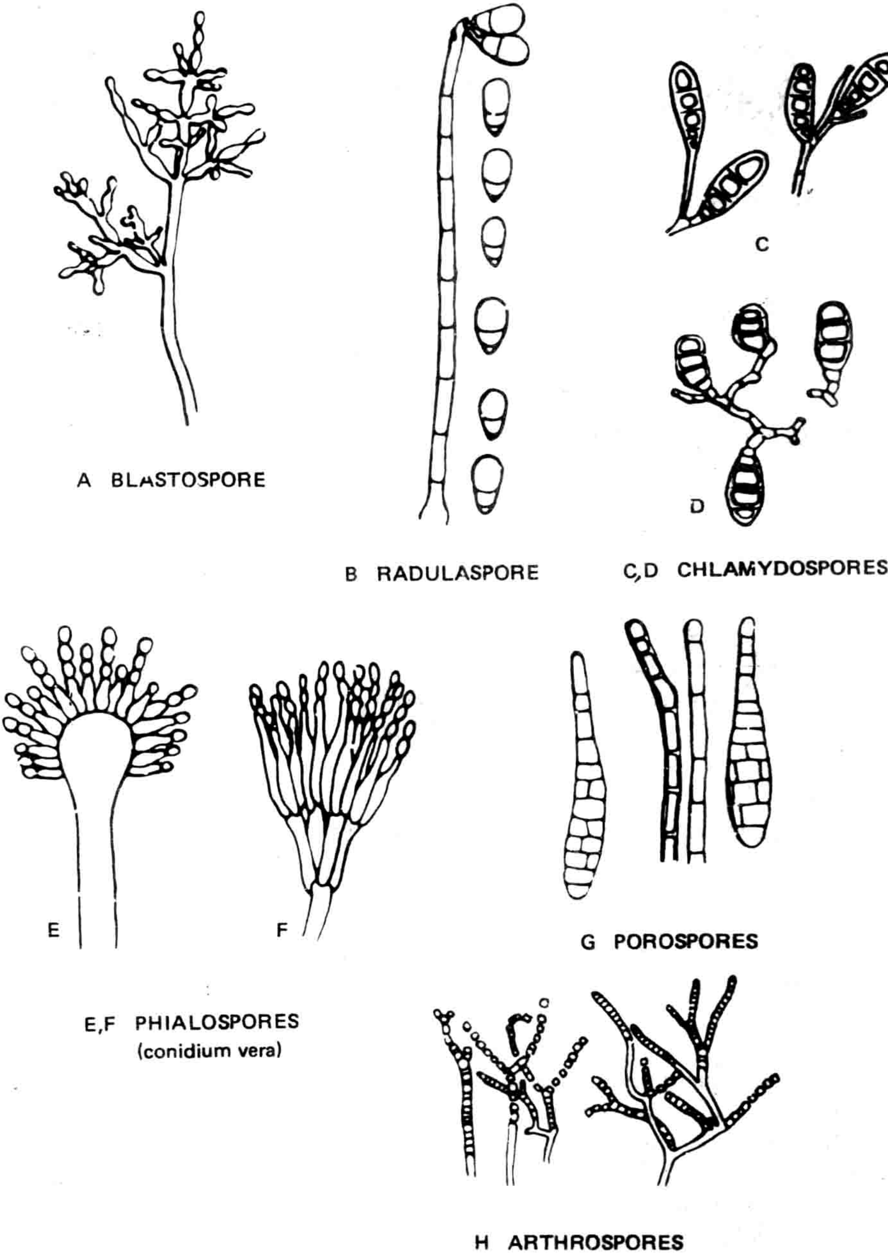
A BLASTOSPORE
B RADULASPORE
C
D
C,D CHLAMYDOSPORES
E
F
E,F PHIALOSPORES
(conidium vera)
G POROSPORES
H ARTHROSPORES

Figure 2