Penicillium and *Acremonium*

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Foreword

Biotechnology is a word that was originally coined to describe the new processes which could be derived from our ability to manipulate, *in vitro*, the genetic material common to all organisms. It has now become a generic term encompassing all "applications" of living systems, including the more traditional fermentation and agricultural industries. Recombinant DNA technology has opened up new opportunities for the exploitation of microorganisms and animal and plant cells as producers or modifiers of chemical and biological products.

This series of handbooks deals exclusively with microorganisms which are at the forefront of the new technologies and brings together in each of its volumes the background information necessary to appreciate the historical development of the organisms making up a particular genus, the degree to which molecular biology has opened up new opportunities, and the place they occupy in today's biotechnology industry. Our aim was to make this primarily a practical approach, with emphasis on methodology, combining for the first time information which has largely been spread across a wide literature base or only touched upon briefly in review articles. Each handbook should provide the reader with a source text, from which the importance of the genus to his or her work can be identified, and a practical guide to the handling and exploitation of the organisms included.

It is perhaps apt that Volume I of the series should deal with a group of organisms involved in producing chemicals which have dominated the fermentation and pharmaceutical industries for the past forty years—the antibiotics. In terms of the degree to which molecular biology has made an impact on this genus to date, it is clear that the major rewards are still to come.

Tony Atkinson
Roger F. Sherwood

Wiltshire

Preface

Fungi have a long-standing importance in biotechnology. Of the filamentous fungi, species of *Penicillium* are probably the best known, having applications in three major areas of industrial activity. In this volume, I have taken the genus *Acremonium* as a "bookfellow," the link being the importance of these fungi as the source of two of the most commercially significant products, the β-lactams penicillin and cephalosporin.

In compiling the book, I have attempted to meet the different needs of its readers. For the industrial microbiologist, there are accounts of the fundamental aspects of the physiology and genetics of these fungi. For the academic microbiologist interested in their industrial use, there are contributions that provide the biochemical background to the various processes involved. There are no descriptions of the different commercial processes, but these are discussed in many other texts. The book opens with a chapter on taxonomy and throughout reflects the clear necessity for careful identification of organisms that are used or may be used in commercial processes.

The authors have working experience with the subject areas of their respective chapters. I am greatly indebted to all of them for taking part in the production of this book despite their busy schedules. They will join me, I am sure, in expressing the wish that all workers in the field of biotechnology find this a useful reference in their day-to-day work.

John F. Peberdy

Nottingham

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Taxonomy of *Penicillium* and *Acremonium*

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A. H. S. ONIONS and B. L. BRADY

1. INTRODUCTION

The fungi form one of the largest groups of organisms, comprising some 65,000 species. They vary from the larger bracket fungi and toadstools to the common soil fungi or recycling organisms. The latter play an important ecological role in breaking down dead plant and animal materials and returning them to the soil. However, from time to time, they attack materials grown or manufactured by man. In the process, they often damage the material either mechanically or enzymatically or by other chemical reactions. Interesting secondary metabolites including the useful antibiotics or undesirable mycotoxins are produced. This potential activity is harnessed in manufacturing processes. Two genera of particular interest in these respects, especially on account of their production of antibiotics and mycotoxins, are *Penicillium* and *Acremonium*.

1.1. Anamorphs and Teleomorphs

Penicillium and *Acremonium* by definition reproduce by vegetatively produced conidia (spores). They belong, therefore, to the class Hyphomycetes of the Deuteromycotina or Fungi Imperfecti. However, several genera of the Ascomycotina (Perfect Fungi) that reproduce sexually produce *Penicillium* or *Acremonium* vegetative asexual structures. The vegetative form is known as the *anamorph*, while the ascosporic form is called the *teleomorph*. According to the Botanical Code (Voss *et al.*, 1983), the teleomorph takes precedence and the teleomorphic name should be used.

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However, some species do not produce a teleomorph, in which case the anamorphic name is applied. Since there are several teleomorphic genera that produce *Penicillium* anamorphs, it is convenient to treat them together, though they may not be truly related. The correct procedure is to use the teleomorph name, but many authors have found it convenient to classify all these organisms together in *Penicillium*. The situation is similar in *Acremonium*.

1.2. Priority of Nomenclature

Fungus names are controlled by the International Code of Botanical Nomenclature (Voss *et al.*, 1983), one of the provisions of which is priority of publication, whereby the earliest name under which a genus or species was described should be used (Hawksworth, 1984).

2. *ACREMONIUM*

2.1. The Names *Cephalosporium* and *Acremonium*

The name *Cephalosporium*, which has long been familiar to biotechnologists and which fathered that of the cephalosporin compounds, has been largely replaced by the name *Acremonium* since the monograph of Gams (1971). *Cephalosporium* was introduced by Corda (1839) for colorless molds with simple unbranched conidiophores and condiogenous cells bearing at the tip a group or "head" of unicellular conidia, from whence the name was derived. At that time, little was known about the interrelationships of these fungi, and the name came to be used for a wide variety of Hyphomycetes. However, there is a suspicion that Corda had applied the name to a member of the Mucorales, but none of his original material is now available for verification. Gams (1968) reexamined specimens of the original (type) material of *Acremonium* Link (1809), which has nomenclatural priority over *Cephalosporium*. This material of *A. alternatum* Link was well preserved and enabled Gams to redescribe the genus from living cultures of later isolates of that species. He applied this name to hyaline fungi that form numerous conidia enteroblastically in basipetal succession from erect conidiogenous cells on sparsely branched conidiophores and included most of the species formerly referred to *Cephalosporium*. However, many authors had come to use the name *Cephalosporium acremonium* for all such molds, and since the history of this name is so confusing, Gams (1971) described a new species *A. strictum* to encompass those fungi, retaining *A. kiliense* Grütz (the earlier name) for very similar forms that additionally produce hyaline chlamydospores, are frequently isolated from the soil, and have been associated with skin infections of man.

At first sight, the morphology of *Acremonium* is simple compared with that of *Penicillium*. It is only when the arrangement, form, and function of the conidiogenous cells (often referred to in earlier work as "phialides") are compared in the individual species that an underlying complexity is revealed.

The conidiogenous cells that generate conidia in both *Penicillium* and *Acremonium* do so by an active wall-building region at the tip of the cell budding off the first conidium, both inner and outer wall layers being involved; a basal cross wall is laid down, and the process is described as "holoblastic." Thereafter, in *Acremonium*, this conidium becomes detached by the separation of the two layers of wall between conidiogenous cell and conidium (schizolysis), and a further conidium is formed at the tip of the conidiogenous cell, the original inner wall layer becoming the outer wall and a new inner wall layer being laid down within it. These and subsequent conidia are said to be formed "enteroblastically," and after they secede, they may remain either clustered in the typical "head" of a "*Cephalosporium*" or loosely connected end-to-end in a "false chain." In *Penicillium*, the second and subsequent conidia produced by a conidiogenous cell are formed from the same two original wall layers, cross walls forming at intervals, but the outer wall layers of the whole chain of conidia remain continuous, and the chains are known as "true chains." The different forms of conidiogenesis are described in detail by Minter *et al.* (1982, 1983a,b).

2.2. Problems of Genus and Species Concepts

In his monograph of the *Cephalosporium*-like fungi, Gams (1971) transferred 18 *Cephalosporium* species to *Acremonium* and described many more *Acremonium* species in the genus, adding others later (Gams and Lacey, 1972; Gams, 1975). He also transferred most of the species of *Paecilomyces* Bainier with solitary conidiogenous cells to the genus *Acremonium* and included many of *Gliomastix* Guéguen, despite their dark-pigmented conidia. Subramanian (1972), recognizing that many of the species first described in *Paecilomyces* and *Gliomastix* formed their conidia in true chains as in *Penicillium*, transferred these to a new genus, *Sagrahamala* Subramanian, while Gams (1978) similarly placed these true chain-formers in another new genus, *Sagenomella* W. Gams. While Subramanian transferred *G. luzulae* to *Sagrahamala*, Gams considered it better left in *Acremonium*, to which he had transferred it from *Gliomastix* in 1971. This dark-spored species is probably best left in *Gliomastix* and is so treated here. Several *Cephalosporium* species, including *C. nordinii*, were transferred by Gams (1971) to *Monocillium* Saksena, a genus in which the wall at the base of the conidiogenous cell is thickened, the distal part expanding in outline before narrowing again at the tip. Some species of *Cephalosporium* were transferred to *Verticillium*, which typically has con-

idiogenous cells that arise together at one level to form whorls or verticils, but in which these cells are sometimes found singly or in "whorls" of only two; *C. aphidicola* Petch was transferred by Gams to *V. lecanii* (Zimm.) Viégas. On account of the distinct collarette at the mouth of the conidiogenous cell in *C. gregatum*, Gams transferred this species to *Phialophora*. Thus, although *Cephalosporium* species are generally speaking now renamed *Acremonium*, there are many exceptions. Furthermore, some combinations into *Acremonium* have never been made, usually because the taxonomic information on the fungus is too scanty. For instance, *C. caerulens* Matsumae, Kamio, and Hata was inadequately described, and the authors made no mention of type material; Gams (1971) suggested that it was a microconidial state of a species of *Fusarium* Link. Gams considered *C. gramineum* to be synonymous with *Hymenula cerealis* Ell. and Ev., a fungus that in nature is sporodochial. *Cephalosporium mycophilum* was never moved to *Acremonium*, since there was no type material available for study; a suggestion by Tubaki (1955) that it is the same as *A. butyri* was discounted by Gams. These controversial species are most appropriately still referred to as "*Cephalosporium*" so long as their position and status are in doubt.

All the fungi dealt with here are conidial states belonging to the class Hyphomycetes. Many Hyphomycetes are anamorphs (conidial states) of members either of the ascomycetes or of the basidiomycetes. *Acremonium* anamorphs are found in many ascomycete orders including the Clavicipitales, Eurotiales, Hypocreales, and Sordariales; "*Cephalosporium salmosynnematum*" is the *Acremonium* anamorph of *Emericellopsis salmosynnemata* Groskl. and Swift, considered by Gams to be synonymous with *E. minima* Stolk in the Eurotiales. *Acremonium* stages are found especially in the younger or juvenile phases of development of other genera. Microconidial stages of *Fusarium* resemble *Acremonium*, and similar simple conidiogenous cells bearing unicellular conidia are found in *Cylindrocarpon*, *Gliocladium*, *Sarocladium*, and *Verticillium* and constitute additional conidial states in *Humicola* and *Thermomyces*.

2.3. Genus Description: *Acremonium* (Figs. 1 and 2)

Colonies on culture media more or less slow-growing, attaining a diameter of less than 25 mm in 10 days on malt extract or oatmeal agars at 20°C. Hyphae thin-walled and hyaline. Typically, the erect conidiogenous cells are formed singly, but conidiophores with simple or verticillate branching occur in some species. Conidiogenous cells usually delimited by a basal septum, occasionally continuous with the hypha from which they are formed, narrowing in shape gradually toward the tip. First conidium formed holoblastically, secession schizolytic, sometimes leaving a short collarette; later conidia produced enteroblastically without further elongation of the tip

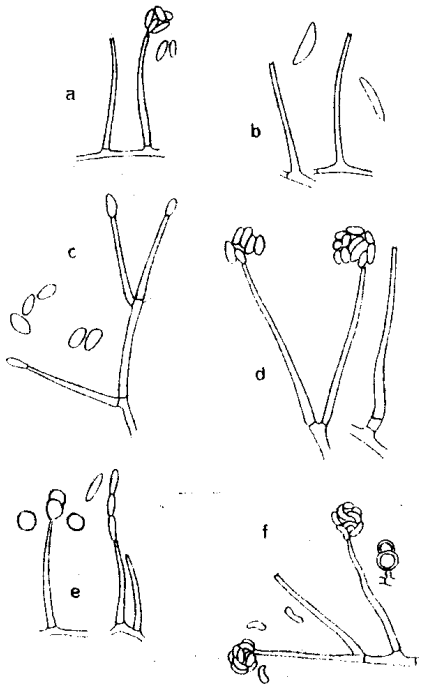

Figure 1. *Acremonium* species. (a) *A. strictum*; (b) *A. coenophialum*; (c) *A. chrysogenum*; (d) *A. crotocinigenum*; (e) *A. fusidioides*; (f) *A. recifei*. Scale bar: 10 μm.

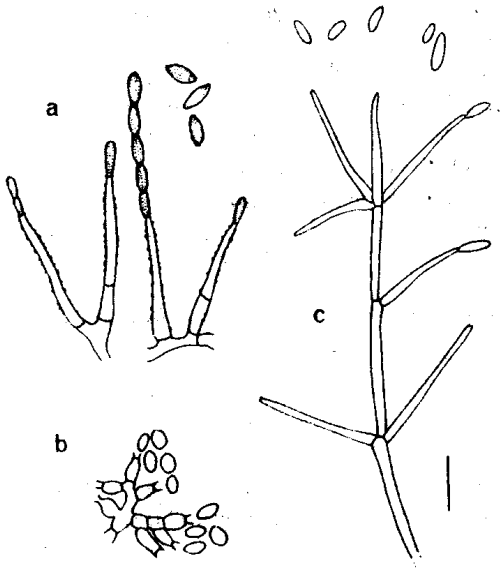

Figure 2. (a) *Gliomastix luzulae*; (b) *Phialophora gregata* (after Gams, 1971); (c) *Verticillium lecanii*. Scale bar: 10 μm.

of the conidiogenous cell. Conidia hyaline, usually consisting of a single cell, rarely two-celled, globose, ovoid, ellipsoidal, cylindrical, or fusiform, collecting after secession at the tip of the conidiogenous cell in a head or a false chain. Chlamydospores sometimes formed. Sclerotia sometimes formed. Some species have a teleomorph; these are in *Cordyceps*, *Emericellopsis*, *Nectria*, *Torrubiella*, *Wallrothiella*, and several other genera.

2.4. Identification of Species (see Section 4.1)

Gams (1971) divides the genus into three sections: *Simplex* (which includes *A. strictum* and *A. fusidioides*), *Gliomastix* (including *A. luzulae*, here kept in the genus *Gliomastix*), and *Nectrioidea* (including *A. chrysogenum*, *A. crotocinigenum*, and *A. recifei*). The characters employed in identification of the species are: appearance of the culture on malt extract or oatmeal agars; presence and form of the conidiophore or its absence; form of the conidiogenous cell; presence or absence of a collarette after the first conidium has seceded; way in which the conidia collect at the tip of the conidiogenous cell, i.e., in "heads" or "false chains"; presence and form of chlamydospores and of sclerotia.

2.4.1. *Acremonium chrysogenum* (Thirum. and Sukap.) W. Gams (Fig. 1c)

Colonies on malt extract agar after 10 days, 8–15 mm in diameter, yeast-like, slimy, chrome yellow or paler yellow, underside an intense chrome yellow, mycelium largely submerged in the medium. Sporulation sparse, conidiophores indistinguishable from the vegetative mycelium. Conidiogenous cells simple, upright, smooth, without an apical collarette, 25–50 μm long, 1.5–2.5 μm at the base, narrowing to 0.6–1.2 μm. Conidia in slimy heads, short or long ellipsoidal with a flattened base, occasionally slightly curved, 4–7.5 × 1.5–3 μm. Chlamydospores absent, although swollen hyphal segments occasionally occur. This species is placed by Gams (1971) in his section *Nectrioidea*. Teleomorph unknown.

2.4.2. *Acremonium cosmophilum* Morgan-Jones and W. Gams (Fig. 1b)

Colonies on malt extract agar very slow-growing, 3 mm in diameter in 10 days, 20 mm in diameter in 2 months, white and cottony on malt extract agar, cream-colored and waxy on cornmeal agar. Conidiophores lacking, conidiogenous cells arising directly from the aerial mycelium, usually not delimited by a basal septum from the subtending hypha, 12–34 μm long, 1.5–2 μm wide at the base, tapering to 0.5–0.8 μm at the tip, without an