IMPROVING THE CLASSIFICATION OF MYCOTOXIN-PRODUCING PENICILLIA ASSOCIATED WITH THE STORAGE OF FOOD

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Abstract

Three hundred and forty eight strains of penicillia were examined by a team of specialists at IMI using morphological, physiological and biochemical criteria. Chief amongst these were the assessment of conidial ornamentation using scanning electron microscopy, growth on specific carbon and nitrogen sources, screening for enzyme production and thin layer chromatography of secondary metabolites. Variation in characteristics both within and between species was considered.

Results were submitted to numerical analysis and clusters produced using the average linkage algorithm. The use of criteria across a range of biological disciplines enabled a number of recently described or previously accepted species to be shown to be either variants or deteriorated examples of established species. In all, thirty seven species, or species complexes were recognised.

This study showed that satisfactory species concepts can be defined for the penicillia using this integrated multi-disciplinary approach. Moreover, reliable identification schemes can be produced.

Introduction

In recent years the taxonomy of the terverticillate penicillia has been considerably revised (Samson *et al.*, 1976; Pitt, 1980). However, areas of uncertainty remain (Onions *et al.*, 1984; Samson and Gams, 1984). A major integrated multidisciplinary study was therefore undertaken to clarify the systematics and species concepts. This involved selecting and applying taxonomic criteria from morphology, including scanning electron microscopy (SEM), physiology and biochemistry. These characters were critically examined for reproducibility and reliability (Bridge *et al.*, 1986*a*), and the data submitted to numerical taxonomy using cluster analysis (Bridge *et al.*, 1989*a*). The full methods and results are published elsewhere (Bridge *et al.*, 1987; 1989*a*, 1989*b*, 1990; Paterson, 1986, Kozakiewicz, 1989) and this paper presents an over-view of the completed study.

Numerical Taxonomy

The 348 strains studied separated into 37 clusters, recognisable as species or species complexes. A simplified version of the resulting dendrogram is given in Figure 1. The majority of clusters represented species concepts in agreement with existing taxonomic schemes (Pitt, 1980;1986; Cruickshank and Pitt, 1987), but there were particular discrepancies. For example, based on morphological characters Pitt (1980) placed *Penicillium hordei* and *Penicillium corymbiferum* in

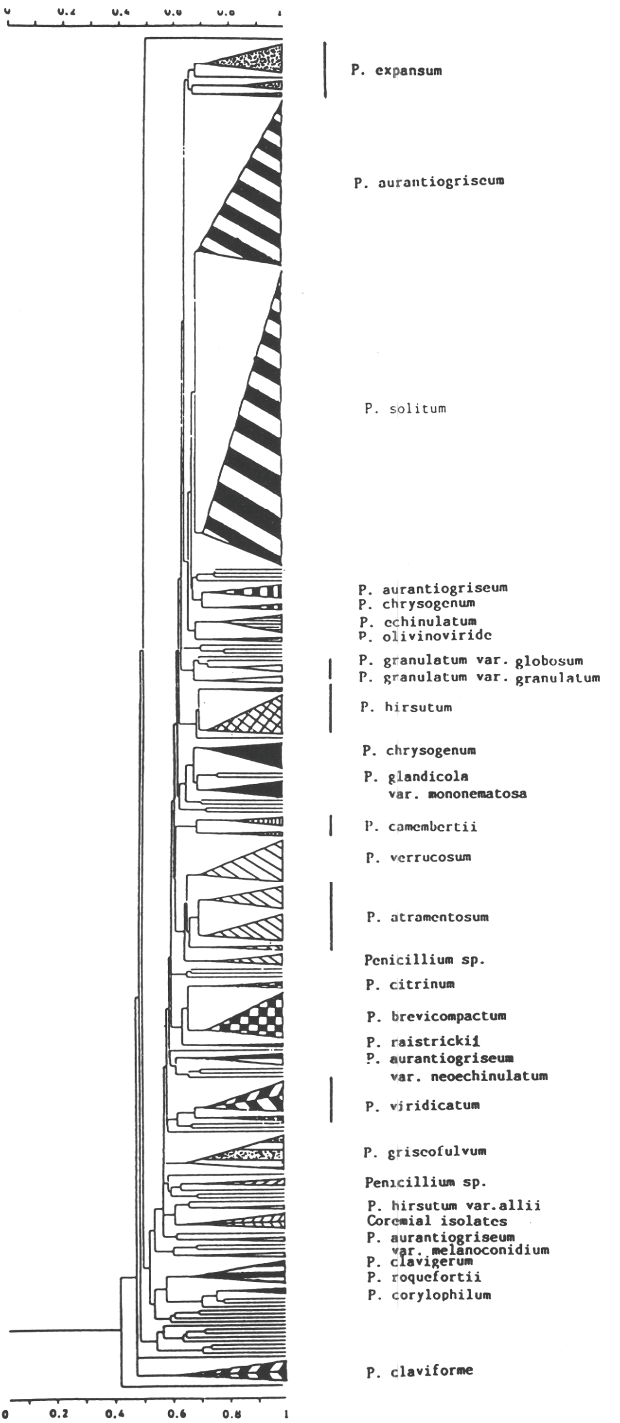

Fig. 1. UPGMA dendrogram based on Gower's coefficient.

synonymy with *P. hirsutum*. Frisvad and Filtenborg (1989), using secondary metabolite profiles, reduced *P. hordei* to a variety of *P. hirsutum*. Cruickshank and Pitt (1987) solely using isozyme patterns suggested that these two taxa may be two distinct species. However, the results of the present multidisciplinary study (Bridge *et al.* 1989*a*, 1989*b*; Kozakiewicz, 1989) showed the three taxa as separating into two clusters on the dendrogram. Moreover, SEM micrographs revealed two conidial ornamentations. One reticulate as in the ex-type culture of *P. hordei* and the other tuberculate (Fig.2). Based on previous work (Kozakiewicz, 1982) this latter information provides sound evidence on which to define species limits. In a subsequent study of the terverticillate penicillia using traditional morphological characters Stolk *et al.* (1990) have reached the same conclusion.
Examination of 13 isolates of *Penicillium camembertii* again revealed two conidial ornamentations (Fig.3); one tuberculate and the other microverrucate, corresponding to two clusters on the dendrogram. The larger, with tuberculate conidia was citrinin negative, the strains having been recently isolated from cheese. The smaller cluster, with micro-verrucate conidia was citrinin-positive and contained two older ex-type isolates. Citrinin production has not been reported in *P. camembertii* before. Interestingly, Abe (1956) in his study of the penicillia also found that his "cheese" isolates separated into two groups. The conidia were either smooth or spinulose.

Sixty-five isolates received as *Penicillium aurantiogriseum* also revealed two conidial ornamentations. One with micro-tuberculate ornamentation and elliptical conidia, as in the ex-type strain of *P. aurantiogriseum* and one with lobate ornamentation and globose conidia. This latter ornamentation is not dissimilar to that of *Penicillium crustosum*, and it is considered that isolates possessing such ornamentation had been originally misidentified. These two species are closely related. The conidial difference, hitherto unknown, is therefore an important diagnostic character. At the physiological level, of the two species *Penicillium crustosum* alone produces penitrem A.

Strains received as *Penicillium brevicompactum, Penicillium stoloniferum* and *Penicillium olsonii* clustered together. These species could not be separated on any characters.

Strains received as *Penicillium chrysogenum* and its synonyms were recovered in two separated clusters. Most of the differences between these clusters were a matter of degree and it was considered that one cluster represented deteriorated lines of *Penicillium chrysogenum* (Fig.1). Included in one of these clusters was the ex-type culture of *P. griseoroseum*, a name which predates *P. chrysogenum*. However, a proposal has been put forward (Frisvad *et al.*, 1990) to conserve *P. chrysogenum* against *P. griseoroseum*.

Finally, strains of *Penicillium expansum* were also recovered in three small clusters (Fig.1). While there were some distinct differences between the groups they were overall very similar and are therefore considered as representing different variants of a single species.

Variation and test reproducibility

Intraspecific variability was studied using single-spore cultures. A number of lines of the same culture were isolated and maintained separately, the differences in characteristics noted. Whilst on the whole only minor differences were apparent, in certain strains significant differences in phenotypic properties were found (Bridge *et al.*, 1986*a*;1987). A possible explanation for this phenomenon is some kind of genetic rearrangement, involving aneuploidy or chromosome duplication and transposition. Support for this supposition comes from the variation in DNA content between conidia of these lines (Bridge *et al.*, 1986*b*) and the descriptions of occurrences in other fungi where differences in chromosome size and number have been demonstrated (Suzuki *et al.*, 1989). However, these phenomena have not been considered in fungal taxonomic studies. Indeed some of the earlier workers in this genus (Biourge, 1923) who recognised many more species, used strains derived from single conidia.

The influence of basal medium and additions to the basal medium were examined as part of an overall assessment of reproducibility. Additions of low levels of pentachlorophenol induced

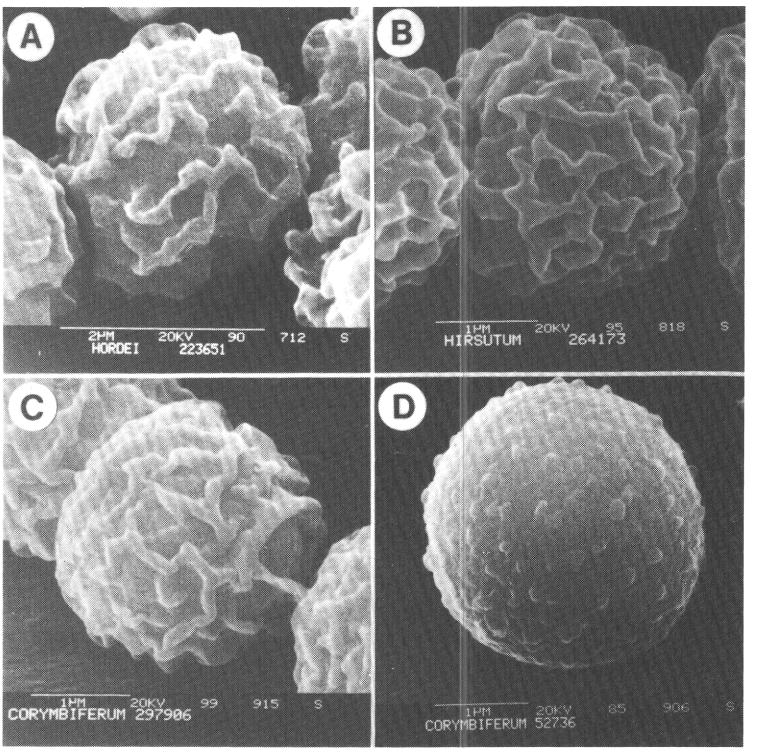

Fig. 2. Taxonomic anomalies in *P. hirsutum* and its synonyms, as revealed by scanning electron microscopy. **A.** *P. hordei* IMI 223651; **B.** *P. hirsutum* IMI 264173; **C.** *P. corymbiferum* IMI 297906; **D.** *P. corymbiferum* IMI 52736.

synnemata in fasciculate penicillia (Bridge *et al.*, 1990). Thus synnematal species such as *Penicillium duclauxii* now grouped in the section *Biverticillium* (Pitt, 1980) may be more closely related to the fasciculate penicillia than previously realised, the separation based merely on a small difference in the control of gene expression.

Conclusions

This study has shown that minor changes to basic media can produce dramatic changes in secondary metabolite profiles, extracellular enzyme production and morphological expression. As a consequence, the variation in results of different workers in different laboratories may not necessarily be due to genuine differences between taxa, but to minor differences in methodology. In support of this conclusion, Filtenborg *et al.* (1990) have shown that the brand of yeast extract employed in YES and CYA agars * can profoundly affect the resulting metabolite profiles.

* YES (2% yeast extract, plus 15% sucrose)
CYA (czapek - yeast extract agar)

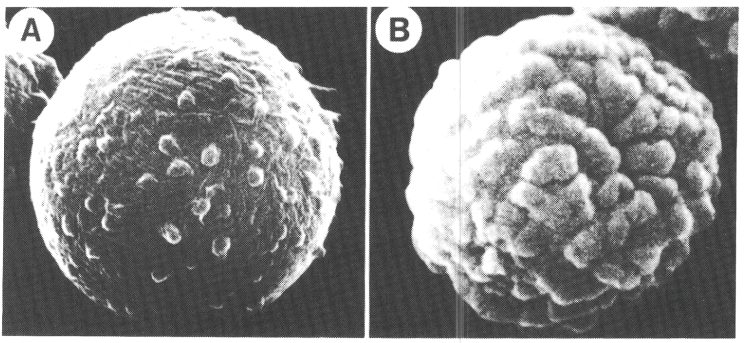

Fig. 3. Taxonomic anomalies in *P. camembertii* and its synonyms, as revealed by scanning electron microscopy. **A.** *P. camembertii* IMI 91924 (A); **B.** *P. caseicola* IMI 28810.

The multidisciplinary approach adopted in this study has undoubtedly improved our understanding of species delimitation in the fasciculate penicillia. This is a way forward which is gaining general acceptance among fungal taxonomists. Indeed, an international working group, the Subcommission of *Penicillium* and *Aspergillus* Systematics (SPAS) has been set up, to undertake studies on nomenclature and taxonomy of these two important genera. It consists of twelve members from seven countries, and comprises taxonomists using morphological parameters and other specialists in the more physiological and biochemical techniques. Hopefully, their efforts will result in an improvement to our understanding of the systematics of *Penicillium*.

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AMELIORATION DE LA CLASSIFICATION DES *PENICILLIA* PRODUCTEURS DE MYCOTOXINES ASSOCIES AUX DENREES STOCKEES

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RESUME

Trois cent quarante-huit souches de *Penicillia* ont été examinées en utilisant des critères morphologiques, physiologiques et biochimiques, par une équipe de spécialistes du CMI. Les principaux examens étaient les mesures des conidies telles que les révélait le microscope à balayage électronique, la croissance sur différentes sources de carbone et d'azote,la recherche d'activités enzymatiques spécifiques et la chromatographie sur couche mince des métabolites secondaires. La variation des propriétés, à la fois dans et entre les espèces, a été prise en considération.

Les résultats ont été soumis à l'analyse numérique et à la recherche de synthèses par utilisation d'algorithmes de liaison. L'utilisation de critères choisis dans un éventail de disciplines biologiques a permis de montrer, parmi des espèces récemment décrites ou connues depuis longtemps, qu'elles étaient, soit des variantes, soit des exemplaires dégénérés d'espèces existantes. En tout, trente-sept espèces, ou groupes d'espèces, ont étés reconnus.

Cette étude a montré que les concepts propres à une espèce peuvent donner satisfaction en étant étendus aux *Penicillia* par l'utilisation d'une approche multidisciplinaire. En outre, il est possible d'établir des modèles d'identification fiables.