A Reappraisal of the Terverticillate *Penicillia* Using Biochemical, Physiological and Morphological Features

I. Numerical Taxonomy

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Three-hundred-and-forty-eight strains representing the major species of terverticillate penicillia, and including representatives of other closely and distantly related species, were included in a numerical taxonomic study. One-hundred characters were derived from morphological features, physiological and biochemical activities and SEM micrographs. Strains were compared by both Gower's coefficient and Pattern difference, and clustered using the average linkage algorithm. Thirty-seven species or species-complex clusters were recovered at approximately 70% similarity; they generally corresponded to existing taxonomic concepts. Several species were shown to contain variants or chemotypes which were often supported by differences in conidial shape and ornamentation. The use of different types of characters enabled a number of new and previously accepted species to be shown to be either variants or deteriorated examples of other species. Variation in properties both between and within species was considered, particularly in relation to strain stability.

INTRODUCTION

The generic name *Penicillium* was introduced by Link (1809), and the species *P. expansum* is now accepted as the lectotype for the genus (Hawksworth, 1986). The genus has been divided into sections or subgenera based on the number and arrangement of branches of the spore-bearing structures, the conidiophores (e.g. Thom, 1930; Pitt, 1980). Despite many studies and monographs (e.g. Dierckx, 1901; Westling, 1911; Biourge, 1923; Thom, 1930; Raper & Thom, 1949; Abe, 1956; Samson *et al.*, 1976; Fassatiouva, 1977; Pitt, 1980), the taxonomy of the section of the genus containing cultures with three or more levels of branches remained unsatisfactory until relatively recently (Onions *et al.*, 1984; Samson & Gams, 1984). The species concepts and names adopted for these fungi have not always been consistent between authors, making identifications of cultures and the interpretation of literature reports difficult (Onions *et al.*, 1984; Samson & Gams, 1984). Another problem is the poor condition of some historically and nomenclaturally important strains in culture, and their generally unremarkable morphological features. Previous taxonomic studies on the terverticillate penicillia have been based almost solely on morphology and gross physiology, and results from more recent biochemical studies have suggested that new species and chemotypes may be necessary to characterize accurately these organisms (Frisvad, 1985; Pitt & Hawksworth, 1986). Since the introduction of a multidisciplinary approach to the taxonomy of *Penicillium* (Abe, 1956; Frisvad, 1981; Bridge *et al.*, 1986) and the start of the present investigation in 1984, several further studies have considered the application of particular new techniques within specific areas of the genus. Additional techniques have been applied in the delimitation of *Penicillium* species in recent

years, such as physiological criteria (Pitt, 1973; Bridge, 1985; Frisvad, 1985), secondary metabolite production (Ciegler *et al.*, 1973; Frisvad, 1986; El-Banna *et al.*, 1987), serology (Polonelli *et al.*, 1986), isozyme electrophoresis (Cruickshank & Pitt, 1987), pyrolysis-GLC (Söderström & Frisvad, 1984), and cross-point separation (Ström, 1986). However, in most cases the new techniques have been used in isolation from each other and some of the deteriorated or markedly atypical strains were not examined.

In general, these latter studies have involved either a large number of strains from a few species, or relatively few strains from a number of species, and have rarely been subsequently checked for reproducibility. The reproducibility and applicability of such approaches was therefore in urgent need of evaluation before such characters became regarded as diagnostic criteria.

The three branched penicillia have been variously grouped under the terms 'Asymmetrica subsection Fasciculata' (Raper & Thom, 1949), 'biverticillate penicillia' (Samson *et al.*, 1976) and 'subgenus *Penicillium*' (Pitt, 1980). Further subdivisions within these taxa have been proposed according to colony texture (e.g. Raper & Thom, 1949) and further details of the branching pattern (Pitt, 1980). Species were originally delineated on the basis of colony morphology, diameter and pigmentation; conidiophore shape and ornamentation; features of the conidiogenous cells; and conidium shape, colour and ornamentation (e.g. Westling, 1911; Raper & Thom, 1949). More recent studies have included some physiological characters such as growth temperatures and growth on nitrite as well as the production of specific secondary metabolites or isozyme electrophoresis (e.g. Abe, 1956; Ciegler *et al.*, 1973; Pitt, 1973; Frisvad, 1981; Frisvad & Filtenborg, 1983; Cruickshank & Pitt, 1987).

Numerical taxonomy based on a mixture of morphological, physiological and biochemical characters, followed by an objective numerical analysis has rarely been attempted in filamentous fungi (Sneath, 1985), and those that have been done have largely been restricted to fewer than 100 cultures, often to help clarify relationships in particular host- or substrate-related situations (e.g. Mueller, 1985; Mordue *et al.*, 1989). Others have not proved entirely satisfactory (e.g. Kendrick & Proctor, 1964).

This paper reports the results of an integrated multidisciplinary study on the terverticillate penicillia, combining characters from morphology, ultrastructure, physiology and biochemistry with critical studies on strain and test variation and numerical taxonomic analysis.

METHODS

Strains studied. The 348 strains studied are given in Table 1. The names given to individual strains were initially those assigned to the cultures by the donors, and apart from checking for purity, initial identification was not carried out on receipt. Strains were maintained during the study on stock slopes of Czapek-Dox agar (Cz; Smith & Onions, 1983) preserved at -20°C . All strains were grown aerobically at 25°C unless stated otherwise.

Inocula. Strains were transferred and inoculated as conidial suspensions. Inoculation of plates for morphological testing and SEM studies was by conidia suspended in a semisolid agar/Tween 80 medium (Pitt, 1980). Inoculation of plates and bottles for physiological testing was by conidia suspended in aqueous Tween 80 (0.02%; Bridge, 1985).

Test selection. One-hundred characters (Table 2) were selected from more than 200 evaluated in previous studies with smaller sets of strains (Bridge *et al.*, 1986, 1987; Paterson, 1986). Tests or procedures that had been shown to be irreproducible, or had appeared of little value in earlier studies were not used.

Colony and cellular morphology. Duplicate plates of Cz agar were three-point-inoculated with a sterile wire as described in Pitt (1980) and incubated for 7 d. Colony characters were recorded directly from these plates with the aid of a binocular dissecting microscope. Slides were prepared in warmed lactophenol cotton blue. The terminology for morphological structures used is that of Minter *et al.* (1986).

Physiological characters. Growth at different temperatures on Cz agar, growth on tannic acid, growth and base production on lactic acid, citric acid, Tween 80 and urea, and hydrolysis of casein, gelatin, RNA, DNA, starch and cellulose were determined as described by Bridge (1985). In addition, growth on acetic acid agar, growth and basic reaction on creatine agar and growth on nitrite-sucrose agar (Frisvad, 1981), and the ability to produce yellow colonies on ammonium oxalate (CZNH₄ agar; Bridge *et al.*, 1986) were assessed.

Secondary metabolites. The production of secondary metabolites on Czapek Yeast agar (CYA; Pitt, 1980) and Yeast Extract Sucrose agar (YES; Davis *et al.*, 1966) was detected by TLC with the agar plug method of Paterson (1986), adapted from the methods of Frisvad (1981).

Table 1. Strains studied arranged in cluster order (S_G coefficient)

IMI no.	Comments	IMI no.	Comments	IMI no.	Comments
Single-member cluster (SM1)					
		91944iii	ex-neotype of <i>P. glabrum</i>		
Cluster 1 (<i>P. expansum</i>)					
28619		297898		174158	
285521		297899		265302	
232297		284419		39803	
		207082			
Cluster 2 (<i>P. expansum</i>)					
285524		246656		279025	
Cluster 3 (<i>P. expansum</i>)					
297959		299046			
Cluster 4 (<i>P. aurantiogriseum</i>)					
89372	ex-type of <i>P. cyclopium</i>	297547		92235	ex-type of <i>P. aurantiogriseum</i> var. <i>poznaniensis</i>
291190	ex-type of <i>P. johanioli</i>	39761	ex-neotype of <i>P. expansum</i>		
40236				243002	
285514		297908		297909	
297971		92199	ex-type of <i>P. brunneoviolaceum</i>	284413	
291194	ex-type of <i>P. polonicum</i>			286084	
159109		89374		291555	
44917		291195	ex-type of <i>P. glaucocoeruleum</i>	286094	
39814	ex-neotype of <i>P. aurantiocandidum</i>	34913	ex-neotype of <i>P. puberulum</i>	280215	
183170				285516	
291201	ex-type of <i>P. ochraceum</i> var. <i>macrosporum</i>	40211		297910	
		297890		285515	
297902		297953		24318	
293185		286106		297951	
204208		195050	ex-neotype of <i>P. aurantiogriseum</i>	297957	
296937					
286763		195050	ex-neotype of <i>P. aurantiogriseum</i>		
Cluster 5.1 (<i>P. solitum</i> var. <i>solitum</i>)					
169087a		291199	ex-type of <i>P. conservandi</i>	92224	ex-type of <i>P. schneeggii</i>
40218	ex-type of <i>P. carneolutescens</i>			224935	
		291198		297969	
39811		297556			
		293201			
Cluster 5.2 (<i>P. solitum</i> var. <i>solitum</i>)					
297980		92225		291543	
285507		68235	ex-type of <i>P. pseudocasei</i>	89386	
68234	ex-type of <i>P. roquefortii</i> var. <i>punctatum</i>			265297	
		293179			
		194737	ex-type of <i>P. flavoglaucum</i>		
Cluster 5.3 (<i>P. solitum</i> var. <i>crustosum</i>)					
206159		209887		262790	
111867		262791		91939	
293722		91939		89385	
295697		297891		39808	
295696		293182		143321	
296059		243053		28040	
286238					

Table 1 (*continued*)

IMI no.	Comments	IMI no.	Comments	IMI no.	Comments
Cluster 5.4 (<i>P. solitum</i> var. <i>crustosum</i>)					
52736		181050		229439	
281775		296938		281551	
89378		293196			
Cluster 5.5 (<i>P. solitum</i> var. <i>solitum</i>)					
206416		154731		321495	
209783		291547		291192	ex-type of <i>P.</i>
287746	ex-type of <i>P. mali</i>	297999			<i>verrucosum</i> var.
293210		351512			<i>melanochlorum</i>
291196		214070		51355	
92276				49096	
Cluster 5.6 (<i>P. solitum</i> var. <i>solitum</i>)					
39810	ex-type of <i>P. solitum</i>	297956		298007	
34911	ex-type of <i>P. gladioli</i>				
Cluster 5.7 (<i>P. solitum</i> var. <i>solitum</i>)					
291197	ex-type of <i>P. casei</i> var. <i>compactum</i>	39822	ex-type of <i>P.</i> <i>lanosogriseum</i>	297997	
92219	ex-neotype of <i>P.</i> <i>griseobrunneum</i>	39820	ex-type of <i>P. biforme</i>	321496	
39766		297892		92264	ex-type of <i>P. olivicolor</i>
		321523	ex-type of <i>P.</i> <i>majusculum</i>	40215	ex-type of <i>P. palitans</i>
SM2					
		285531			
SM3					
		192904	ex-type of <i>P.</i> <i>psittacinum</i>		
SM4					
		39819	ex-type of <i>P.</i> <i>lanosoviride</i>		
SM5					
		89312	ex-type of <i>P. cyclopium</i> var. <i>album</i>		
Cluster 6 (<i>P. aurantiogriseum</i>)					
286235		34913	ex-neotype of <i>P.</i> <i>puberulum</i>	92222	
291191	ex-type of <i>P.</i> <i>janthogenum</i>			297894	
Cluster 7 (weak <i>P. chrysogenum</i>)					
24314	ex-type of <i>P.</i> <i>chrysogenum</i>	91935		39762iii	
Cluster 8 (<i>P. echinulatum</i>)					
285508		68236	ex-type of <i>P. palitans</i> var. <i>echinoconidium</i>	281776	
40028	ex-type of <i>P.</i> <i>echinulatum</i>			297554	
68236	ex-type of <i>P. palitans</i> var. <i>echinoconidium</i>				
Cluster 9 (<i>P. olivinoviride</i>)					
166526(B)		285511		285526	
40223	ex-type of <i>P.</i> <i>olivinoviride</i>	293204		298085	
		59286			

Table 1 (continued)

IMI no.	Comments	IMI no.	Comments	IMI no.	Comments
Cluster 10 (<i>P. granulatum</i> var. <i>globosum</i>)					
298083		297543		299049	
Cluster 11 (<i>P. granulatum</i> var. <i>granulatum</i>)					
40220	ex-neotype of <i>P. granulatum</i>	321513		297543	
Cluster 12 (<i>P. hirsutum</i>)					
285512		196570			
Cluster 13 (<i>P. hordei</i>)					
246204		286971		297900	
296066		284723		297906	
264173		223651		151748	ex-type of <i>P. hordei</i>
291539		40213	ex-neotype of <i>P. corymbiferum</i>		
SM6					
		19759			
Cluster 14 (<i>P. chrysogenum</i>)					
37767		279567		297551	
91020(b)		321521		92220	ex-type of <i>P. griseoroseum</i>
297895		296926			
75832	ex-neotype of <i>P. griseofulvum</i>	321507			
Cluster 15 (<i>P. glandicola</i> var. <i>mononematos</i>)					
296925	ex-type of <i>P. viridicatum</i> var. <i>verrucosoidis</i>	321494		321508	
		321509		39767	ex-type of <i>P. nigricans</i>
		296930		297544	
321492					
321493					
Cluster 16 (<i>P. camembertii</i>)					
91924(A)		129208		287746	ex-type of mutant <i>P. mali</i>
285506					
Cluster 17 (<i>Penicillium</i> sp.)					
28810	ex-type of <i>P. caseicola</i>	27831	ex-type of <i>P. camembertii</i>		
Cluster 18 (<i>P. verrucosum</i>)					
285522		284410		297897	
295238		260915		285523	
186092		297964		295050	
297896		284409		321522	
Cluster 19 (<i>P. atramentosum</i>)					
210922		285530		297984	
210922		295234		192503	
298080					
Cluster 20 (<i>P. atramentosum</i>)					
297983		293209		298081	
321497		321499		192501	
321498		200310	ex-neotype of <i>P. verrucosum</i>		
Cluster 21 (<i>P. atramentosum</i>)					
321515		321516			

Table 1 (*continued*)

IMI no.	Comments	IMI no.	Comments	IMI no.	Comments
Cluster 22 (<i>Penicillium</i> sp.)					
321510		321501		321511	
321500					
		SM7			
		285510			
		SM8			
		296073			
		SM9			
		134650	ex-type of <i>P. majusculum</i>		
Cluster 23 (<i>P. citrinum</i>)					
280297		92196	ex-type of <i>P. citrinum</i>	39812	ex-type of <i>P. commune</i>
Cluster 24 (<i>P. brevicompactum</i>)					
17456		92044		192502	ex-neotype of <i>P. olsonii</i>
17456		285220		200408	
39824	ex-type of <i>P. stoloniferum</i>	40225	ex-neotype of <i>P. brevicompactum</i>	297553	
94149		321514		295048	
				297950	
Cluster 25 (<i>P. raistrickii</i>)					
286753		40221			
SM10					
		68414			
Cluster 26 (<i>P. aurantiogriseum</i> var. <i>neoechinulatum</i>)					
85513	ex-type of <i>P. echinulatum</i> var. <i>discolor</i>	296927		321491	
		321490			
92209ii	ex-type of <i>P. subcinereum</i> , ex-neotype of <i>P. citreonigrum</i>	297546		293178	
Cluster 27 (<i>P. viridicatum</i>)					
39758ii	ex-neotype of <i>P. viridicatum</i>	150406		297907	
173209		284414		293194	
284408		192905		293197	
Cluster 28 (<i>P. viridicatum</i>)					
265301		293187			
293186		297904		291201	ex-type of <i>P. ochraceum</i> var. <i>macrosporum</i>
Cluster 29 (<i>P. griseofulvum</i>)					
285525		39809	ex-type of <i>P. urticae</i>	92273	ex-type of <i>P. flexuosum</i>
205652		297542			
297893		28808	ex-type of <i>P. patulum</i>	296934	
297901		291549		296934	
		285527			
Cluster 30 (<i>Penicillium</i> sp.)					
284411		284415		192216	
174718	ex-type of <i>P. conservandi</i>	92215		297903	

Table 1 (continued)

IMI no.	Comments	IMI no.	Comments	IMI no.	Comments
Cluster 31 (<i>P. hirsutum</i> var. <i>allii</i>)					
297905		321505		321506	
Cluster 32 (coremial isolates)					
297911		321504		321519	
299048		321520			
		297546			
Cluster 33 (<i>P. aurantiogriseum</i> var. <i>melanoconidium</i>)					
321502		321503			
34846	ex-type of <i>P. aurantiovirens</i>	39759	ex-type of <i>P. notatum</i>		
Cluster 34 (<i>P. clavigerum</i>)					
224631		39807	ex-neotype of <i>P. clavigerum</i>		
Cluster 35 (<i>P. roquefortii</i>)					
285518		148775		129207	
285509		24313	ex-type of <i>P. roquefortii</i>	293190	
291202					
Cluster 36 (<i>P. corylophilum</i>)					
39754	ex-neotype of <i>P. corylophilum</i>	39754	ex-neotype of <i>P. corylophilum</i>	296936	
213846		297991		204081	
39739	ex-type of <i>P. shearii</i>	90178		297548	
181051		285455		291193	ex-neotype of <i>P. verrucosum</i> var. <i>verrucosum</i>
39752	ex-type of <i>P. atramentosum</i>	29700			
297966		40238	ex-neotype of <i>P. janthinellum</i>	39733	ex-type of <i>P. javanicum</i>
192332	ex-type of <i>P. oxalicum</i>	197477	ex-neotype of <i>Talaromyces flavus</i>		
Cluster 37 (<i>P. claviforme</i>)					
285528		40237	ex-neotype of <i>P. claviforme</i>	194564	
44744					
39754		40237	ex-neotype of <i>P. claviforme</i>		
SM11					
		193019	ex-neotype of <i>P. funiculosum</i>		

Scanning electron microscopy. SEM of 21-d-old cultures (to ensure mature conidia) from Cz agar was done using a cryo-stage as described in Kozakiewicz (1989). In addition to the conidial shape and ornamentation characters, the length and breadth of conidia were determined from the SEM micrographs with a MADS digitizer.

Coding of results. Missing or inapplicable results (such as base production on carbon source when growth on carbon source was negative) were coded as 'no comparison' (NC). Physiological characters were coded as 0 for no reaction, 1 for a weak (but definite) reaction and 2 for a strong reaction. The one exception was production of yellow colonies on CZNH4 agar, which was coded as 0 for negative and 1 for positive.

Morphological characters were coded as binary with the following exceptions: colony diameter, first-level branch length and conidiogenous cell length were recorded as the mean measurement from 20 samples and a coefficient of variation was determined from these measurements; roughening of the conidiophore on a scale from 0 for smooth to 2 for very rough; colony profile as 1 for flat and spreading, 2 for raised and 3 for high domed; fasciculation on a scale from 0 for not fasciculate up to 3 for very fasciculate and 4 being used only when coremia

were produced. Characters relating to colour were recorded on an 'all present' principle so that a colony with grey-green conidia in the centre and blue-green conidia at the margin was recorded as both blue-green and grey-green. This principle was also used with conidium shape.

Coding of secondary metabolite data was primarily determined by the reliability and reproducibility of the detection. Metabolite results seen in at least 67% of all replicates were coded as 2, and 83% of these were 100% reproducible; metabolite results seen in 50% of replicates were coded as 1, and all other metabolite results were coded as 0.

Reproducibility. Strain reproducibility was tested by subculturing duplicate lines of 10 strains on receipt. These duplicate cultures were maintained and tested separately. Test reproducibility was examined from all tests for each set of data; physiological tests and secondary metabolite screening were repeated for 148 strains; morphology tests were repeated for 52 strains; and SEM tests were repeated for 227 strains. Tests were repeated between 12 and 18 months after the original tests. For comparison, multistate characters were converted to binary (0 = -ve, 1 or 2 = +ve). Standard deviations of measurements suggested that ± 2 mm was a suitable cutoff for comparisons of colony diameters.

Numerical analyses. All computation was done on a DEC VAX cluster through the TAXPAK numerical taxonomy package (M. J. Sackin, unpublished) at the Microbiology Department, Leicester University, UK). Resemblances between strains were calculated with Gower's coefficient (S_G ; cf. Sneath & Sokal, 1973) and the Pattern coefficient (D_{p2} ; Sackin, 1981). Corresponding vigour values and differences in vigour were also calculated (D_{v2} ; Sackin, 1981). In both cases all matching negative results were excluded from calculations in order to avoid excessive weighting where there were multiple characters relating to a single phenomenon, such as colony colour. Clustering was by average linkage (UPGMA; Sneath & Sokal, 1973) and dendrograms were plotted. Overlap between adjacent pairs of taxa was estimated with the TESTDEN program which determined overlap between adjacent groups on the same arm of the dendrogram (Sneath, 1979). In cases where there were four or more degrees of freedom, the apparent overlap was compared for significance against a critical overlap set at both 2.5% and that expected for a rectangular distribution, as described in Sneath (1979). Principal coordinates analysis was done by the method of Gower (1966), with a distance matrix calculated by a transformation of the S_G values [$d = (1 - S_G)^{1/2}$], using a program written by M. J. Sackin (unpublished).

RESULTS AND DISCUSSION

Reproducibility and other analyses

All duplicate strains were recovered in the same clusters as their parental strains with two exceptions (IMI 34913 and IMI 291199), which were found to be either very deteriorated or contaminated at the end of the study. The average similarity for the eight remaining pairs in the Gower's coefficient dendrogram was 0.82, a low figure for numerical taxonomy studies (e.g. Williams *et al.*, 1983; Bridge & May, 1984). The discounting of matching negative results has resulted in generally low similarities throughout the study. Variation in phenotypic properties (often seen as sectoring) within single strains of conidial fungi is well known (e.g. Burnett, 1976; Klittich *et al.*, 1988) and phenotypic variation within some of the strains of *Penicillium* used in this study has been reported (Bridge *et al.*, 1987). Test duplication after more than 12 months maintenance gave 4.4% discrepancy for physiological and SEM characters and 7.6% discrepancy for morphological characters. These figures were considered acceptable in this study in view of the inherent genetic variability and the subjectivity often involved in scoring morphological characters such as shades of colour.

Reproducibility for secondary metabolite production was not entirely satisfactory. Duplicated cultures grown, extracted and tested together very rarely showed discrepancies. However, when individual strains were screened over a period of time, not all metabolites were seen during each screening. This type of discrepancy in secondary metabolite production by fungi has been widely reported and may be due to, amongst other things, variations in relative concentrations of metabolites at different times caused by, for example, instability of enzymes involved in the biosynthetic pathways (e.g. Lam *et al.*, 1988). It was for this reason that the final set of secondary metabolites used was restricted to those that were 50–100% reproducible, and the irreproducibility of the production of a particular metabolite was represented in the scoring of the character, so that their effect on similarity values was minimized when compared to apparent non-producers. Computation of the data without the secondary metabolite results gave a lower value for the cophenetic correlation coefficient and did not improve clustering.

The cophenetic correlation for the dendrogram derived from Gower's coefficient was 0.788.

This would be a relatively low figure for a numerical taxonomy study on bacteria (Jones & Sackin, 1980); however, this statistic has rarely been used with mycological data, and so a fuller interpretation is difficult.

Overlap between pairs of adjacent groups on the same arm of the Gower's coefficient dendrogram was investigated and compared to critical overlaps set for that expected for a rectangular distribution and 2.5%. In this dendrogram clusters appeared either singly or in small groups on distinct arms which were subsequently joined by a number of very close links. The overlap results mirrored the lengths of the stems on the dendrogram; comparisons between clusters on the same arm of the dendrogram showed either 95 or 99% confidence that the groups were distinct. However, comparisons between arms of the dendrogram that were very close together suggested overlap. These results indicate that the species clusters, while representing distinct entities, may be placed either in or just outside a larger indistinct continuum.

Cluster analyses

A simplified dendrogram based on Gower's coefficient and UPGMA clustering is shown in Fig. 1. Two-hundred-and-ninety-seven strains (85%) formed 37 species or species-complex clusters at approximately 70% similarity. The characteristics of these clusters are given in Table 2. In addition, 20 of the strains included as either single representatives of species or as representatives of dissimilar species formed single member or small, loosely defined clusters. In total 317 (91%) of the strains were considered grouped. The twenty most useful differential tests between clusters were calculated from Gyllenberg's Sum of C (see Sneath & Sokal, 1973), and are indicated in Table 2. The dendrogram obtained from the Pattern coefficient was broadly similar, although some clusters merged, and this is considered in the following cluster descriptions. Names were assigned to clusters on the basis of the oldest recognized ex-type strain present; in clusters where no ex-type strains were recovered names are based on species descriptions and strains regarded as typical.

Description and identity of clusters

Single-member cluster 1 (SM1) contained a single strain received as *P. frequentans* (*P. glabrum*), which had been included as an example of a different section of the genus, subgenus *Aspergilloides*. The separation of this strain was therefore as expected.

Cluster 1 contained nine strains and agrees well with the species concept of *P. expansum* (Raper & Thom, 1949; Samson *et al.*, 1976; Fassatova, 1977; Pitt, 1980). Our culture of the ex-neotype strain of *P. expansum* was not recovered in this cluster, although another line of the ex-neotype tested subsequently showed good agreement in all of the typical characters (following paper: Bridge *et al.*, 1989). The ex-neotype strain of *P. expansum* originally used in this study must therefore be considered aberrant.

Cluster 2 contained three strains received as examples of a possible new species, characterized primarily by secondary metabolite profiles (J. C. Frisvad, personal communication). The group showed considerable similarity with *P. expansum*, although strains grew at 37 °C; however, unlike all except one of the other *P. expansum* strains, these three strains were isolated from tropical or equatorial zones and it would seem most likely that they represent a variant of *P. expansum*.

Cluster 3 consisted of two strains supplied as freshly isolated strains of *P. expansum*. Both strains were morphologically very similar to cluster 1, but differed in producing a basic reaction from lactic acid and failing to hydrolyse DNA. These strains represent a variant of *P. expansum*. However, this cluster was moved considerably down the dendrogram in the Pattern representation and so it is unlikely that its separation from cluster 1 is due entirely to the greater vigour of the fresh isolates (see later).

Cluster 4 consisted of 44 strains including 12 ex-type or neotype strains. The cluster contains strains considered representative of *P. aurantiogriseum*, and with the exception of the aberrant *P. expansum* noted above, the remaining ex-type cultures have all been regarded as synonyms of this species (Raper & Thom, 1949; Samson *et al.*, 1976; Fassatova, 1977; Pitt, 1980).

Cluster 5 consisted of 77 strains including 16 ex-type cultures. Many of these strains have been

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

Table 2. *Distribution of positive characters in clusters*

Values are number of strains positive in each cluster. D indicates the 20 most useful diagnostic tests, including fasciculate colonies and coremia production as two characters (test 27).

Cluster	No. of strains	D																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
		Growth at 4 °C		Growth at 30 °C		Growth at 37 °C		Growth on acetic acid		Growth on 1% tannic acid		Growth on 1% lactic acid		Growth on 1% citric acid		Growth on Tween 80		Base from lactic acid		Base from citric acid		Base from Tween 80		Growth on NO2 agar		Growth on 0.2% urea		Growth on creatin		Base from urea		Base from creatin		Gelatin hydrolysis		Casein hydrolysis		DNA hydrolysis		RNA hydrolysis		Cellulose hydrolysis		Starch hydrolysis		Yellow colonies on CZNH4		Yellow mycelium																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			

Table 2 (continued)

Cluster	No. of strains	D																											
		Velvet colonies D	Colony profile*	Fasciculate colonies D	Yellow-green conidia	White conidia	Blue-green conidia	Grey-green conidia D	Green conidia	Dark green conidia	Colony reverse yellow D	Diffusing pink pigment	Roughened conidiophores D	No branches	First-level branches	FLB divergent D	FLB appressed	FLB base cylindrical	Conidigenous cells ampulliform	Second-level branches	FLB number†	FLB length variation‡	CC length variation‡	Colony diameter (mm)	Lobate conidia D				
		25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48				
1.	9	0	1-2	9	0	0	6	9	6	0	3	4	3	0	0	6	9	8	9	9	2	14-25	10-21	25-37	0				
2.	3	0	2	3	0	0	0	3	3	0	3	3	3	0	0	0	3	3	3	3	2-3	9-17	12-14	26-28	0				
3.	2	0	1-2	2	0	0	0	2	2	0	2	0	0	0	0	0	2	2	2	2	2	11-17	12-16	35-37	0				
4.	44	0	1-3	43	1	0	35	36	29	1	24	23	38	0	0	19	44	43	43	44	2-3	10-21	9-14	16-35	3				
5.	77	1	1-2	67	3	0	41	63	40	7	24	1	72	0	1	36	72	77	77	77	1-3	9-29	7-25	16-40	71				
6.	5	3	2	2	1	0	1	5	5	2	0	0	2	1	1	4	4	5	5	5	1-2	12-24	10-17	22-44	0				
7.	3	3	2	0	0	0	2	1	3	0	1	0	0	0	0	3	0	3	3	3	2-3	13-25	10-14	19-36	0				
8.	6	0	2	6	0	0	2	4	4	6	1	0	6	0	0	0	6	6	6	6	2	10-17	8-14	24-28	0				
9.	2	0	2	1	0	0	0	2	1	1	0	0	2	0	0	0	2	2	2	2	2	13-21	10-14	21-23	2				
10.	4	0	1-2	4	0	0	4	4	1	1	4	0	4	0	0	1	4	3	3	4	2	12-27	11-17	12-29	0				
11.	3	0	1-2	3 ^c	0	0	2	3	2	0	3	0	3	0	0	0	3	3	3	3	2-3	12-14	10-13	16-32	0				
12.	2	1	2	2	0	0	0	0	2	0	1	0	2	0	0	0	2	2	2	2	2-3	16-17	15-17	28-30	2				
13.	11	0	3	11	2	0	7	2	11	0	10	1	7	0	0	1	11	11	11	11	2-3	11-22	9-15	25-46	0				
14.	8	5	1-2	0	0	0	7	0	0	0	7	0	0	0	0	8	8	8	8	8	2-3	12-28	9-16	19-33	1				
15.	5	1	1-2	4	0	0	4	5	3	3	3	0	1	0	0	4	5	1	5	5	2-3	12-18	11-22	21-28	0				
16.	4	0	2-3	1	0	4	0	0	0	0	0	0	4	0	0	0	4	4	4	4	2-3	13-20	9-16	20-24	4				
17.	2	0	2	0	0	2	0	0	0	0	0	0	2	0	1	2	2	2	2	2	2	20-28	12-13	9-17	0				
18.	12	0	1-3	10	1	0	4	4	9	0	0	0	11	0	0	2	12	11	12	12	2-3	11-19	9-18	12-19	2				
19.	7	4	2	6	0	0	3	7	4	2	0	1	2	0	1	5	7	7	7	7	2-3	10-23	9-21	14-26	1				
20.	8	5	1-2	8	0	0	5	7	7	1	1	0	3	0	0	1	7	8	8	8	2-3	13-21	9-14	12-21	1				
21.	2	1	1-2	1	0	0	1	0	1	2	0	1	0	0	0	2	2	2	2	2	2-3	14-18	13	20-26	0				
22.	4	0	1-2	4	0	0	0	4	4	0	2	0	1	0	0	0	4	4	4	4	3	10-16	8-14	15-25	0				
23.	3	3	1-2	0	0	0	1	3	2	0	2	0	0	1	2	2	2	2	3	0	2	14-17	9-14	16-19	3				
24.	13	13	1-2	0	0	0	1	12	12	2	0	0	2	0	0	1	13	5	13	13	2-3	11-17	8-15	11-24	12				
25.	2	2	1	0	0	0	1	2	2	1	1	0	2	0	2	0	2	1	2	0	2-3	14-16	14-15	25-27	0				
26.	4	1	1-3	3	0	0	4	3	1	1	4	3	2	0	0	1	4	3	4	4	2-3	12-20	10-14	21-29	0				
27.	9	0	1-2	9	9	0	0	0	0	3	8	5	9	0	0	0	9	9	9	9	2-3	9-18	7-17	27-35	7				
28.	2	0	2	2	1	0	0	0	2	0	0	2	2	0	0	0	2	2	2	2	2	14-17	9-13	25-27	1				
29.	10	0	2	9	0	0	0	10	2	0	0	6	0	0	0	0	9	3	8	9	10	2	15-29	11-17	17-29	0			
30.	3	1	1-2	3	3	0	2	1	1	0	2	1	2	0	0	0	3	0	3	3	2	1-2	12-18	11-13	15-23	0			
31.	3	0	2-3	3	1	0	1	2	3	0	1	3	3	0	0	1	3	3	3	3	3	14-17	10-17	15-22	0				
32.	5	0	2-3	3 ^c	0	0	4	4	0	1	4	0	4	0	0	1	5	5	5	5	3	13-22	11-15	19-22	5				
33.	2	0	2	0	1	0	0	0	1	2	2	0	1	0	0	0	2	1	2	2	3	11-14	8-15	17-23	0				
34.	2	0	2	0 ^c	0	0	2	2	0	0	0	1	0	0	0	0	2	2	2	2	2-3	15-21	11-17	21-23	0				
35.	7	7	1	0	1	0	3	3	7	3	0	0	7	0	0	6	4	6	7	7	1-2	19-26	13-27	23-55	0				
36.	3	1	1	0	0	0	2	3	3	1	0	0	0	0	3	3	0	3	3	0	1-2	13-24	11-13	18-29	0				
37.	6	0	1-2	0 ^c	0	0	5	4	1	0	0	0	0	0	0	5	1	6	2	6	1-2	22-41	9-16	16-24	0				

* Colony profile: 1, flat and spreading; 2, raised; 3, high or domed.

† First-level branch number: 0, none; 1, 1-3 FLB; 2, 3-4 FLB; 3, >4 FLB.

‡ First-level branch and conidigenous cell length; coefficient of variation = SD/mean.

^c Some or all strains produced coremia: *P. granulatum* II, 1/3; coremial strains, 2/5; *P. clavigerum*, 2/2; *P. claviforme*, 6/6.

Cluster	No. of strains	Conidia										Chemical composition																																								
		Reticulate conidia		Microtuberculate conidia D		Echinulate conidia		Microverrucate conidia D		Globose conidia		Elliptical conidia D		Subglobose conidia D		Elongate conidia		Conidia length (µm)		Conidia breadth (µm)		Brevianamide A		Brevianamide B		Brefeldin A		Carlosic acid		Cyclophenol		Citrinin		Compactin		α-Cyclopiazonic acid		Carolic acid		Chaetoglobosin C		Canescin										
		49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	59	60	61	62	63	64	65	66	67	68	69	59	60	61	62	63	64	65	66	67	68	69								
1. <i>P. expansum</i>	9	0	9	0	0	5	7	0	1	2.5–3.0	2.0–3.0	0	0	0	1	8	6	0	1	1	2	0																														
2. <i>P. expansum</i>	3	0	3	0	0	0	2	2	0	3.0	2.0–2.5	0	0	0	0	2	0	1	0	0	0	0																														
3. <i>P. expansum</i>	2	0	0	0	2	0	2	0	0	2.5–3.0	2.0–2.5	1	0	0	0	2	0	0	1	0	0	0																														
4. <i>P. aurantiogriseum</i>	44	0	39	1	1	3	12	34	0	2.0–3.0	2.0–2.5	5	1	3	14	3	1	2	1	10	0	2																														
5. <i>P. solitum</i>	77	0	0	1	21	21	2	54	0	2.0–3.5	1.5–3.5	3	0	3	9	2	1	0	1	7	2	0																														
6. <i>P. aurantiogriseum</i>	5	0	5	0	0	0	4	3	0	2.5–3.5	2.0–3.0	0	0	0	0	2	1	0	0	1	1	0																														
7. <i>P. chrysogenum</i>	3	0	3	0	0	0	0	3	0	2.5–3.0	2.0	0	0	0	0	0	0	0	0	0	0	0																														
8. <i>P. echinulatum</i>	6	0	0	6	0	3	0	3	0	3.0–3.5	3.0	0	0	1	0	0	0	2	0	0	0	3																														
9. <i>P. olivinoviride</i>	2	0	0	0	0	0	0	2	0	3.0	2.5–3.0	0	0	1	0	0	1	0	0	0	0	0																														
10. <i>P. granulatum</i> var. <i>globosum</i>	4	0	4	0	0	4	0	0	0	2.5	2.0–2.1	0	0	0	0	0	1	1	0	2	2	0																														
11. <i>P. granulatum</i> var. <i>granulatum</i>	3	0	3	0	0	0	3	0	0	2.5–3.0	2.0–2.5	0	0	0	0	0	2	0	0	0	0	0																														
12. <i>P. hirsutum</i>	2	0	0	0	0	0	0	2	0	2.5	2.5	0	1	0	1	0	0	1	0	2	2	0																														
13. <i>P. hordei</i>	11	10	1	0	0	2	0	9	0	2.5	2.0–2.5	1	0	0	6	7	8	0	0	6	1	0																														
14. <i>P. chrysogenum</i>	8	0	7	0	0	2	1	6	0	2.5–2.7	2.0–2.5	1	0	0	0	0	0	0	0	0	0	0																														
15. <i>P. glandicola</i> var. <i>mononematos</i>	5	0	5	0	0	0	0	5	0	2.5–3.0	2.0–2.4	0	0	0	0	0	0	0	0	0	0	0																														
16. <i>P. camembertii</i>	4	0	0	0	0	0	2	4	0	2.0–3.7	2.0–3.7	0	0	0	0	0	0	0	0	0	0	0																														
17. <i>Penicillium</i> sp.	2	0	1	0	1	1	0	1	0	2.0	2.0	0	0	0	0	0	2	0	0	0	0	0																														
18. <i>P. verrucosum</i>	12	2	7	0	1	0	0	12	0	2.0–3.0	2.0–2.5	0	1	0	0	1	5	0	1	0	0	0																														
19. <i>P. atramentosum</i>	7	0	1	0	5	7	0	0	0	2.0–3.0	2.0–2.5	1	0	1	0	1	1	0	1	1	0	0																														
20. <i>P. atramentosum</i>	8	0	4	0	3	0	8	0	0	2.5–3.5	2.0–2.5	0	0	0	1	0	0	3	0	0	0	0																														
21. <i>P. atramentosum</i>	2	0	0	0	2	0	2	0	0	2.7–2.7	2.5	0	0	0	0	0	0	0	0	0	0	0																														
22. <i>Penicillium</i> sp.	4	0	0	0	4	0	2	2	0	3.0–3.5	2.2–2.5	0	0	1	1	0	1	0	0	0	0	0																														
23. <i>P. citrinum</i>	3	0	0	0	0	1	0	2	0	2.0	1.5–1.8	0	0	0	0	0	3	0	0	0	0	0																														
24. <i>P. brevicompactum</i>	13	1	0	0	0	1	1	12	0	2.0–3.0	2.0–2.5	3	2	0	0	0	1	1	1	1	0	1																														
25. <i>P. raistrickii</i>	2	0	0	0	2	0	0	2	0	2.0	1.5–2.0	1	0	0	0	0	0	0	0	0	0	0																														
26. <i>P. aurantiogriseum</i> var. <i>neoechinulatum</i>	4	0	0	4	0	2	0	2	0	2.5–3.0	2.5–3.0	0	0	0	3	0	0	0	0	0	0	0																														
27. <i>P. viridicatum</i>	9	0	0	2	0	3	0	7	0	2.0–3.0	2.0–2.5	9	6	0	3	3	2	0	0	0	0	2																														
28. <i>P. viridicatum</i>	2	0	1	0	0	1	0	1	0	2.5	2.0–2.5	0	0	0	0	0	0	0	0	0	0	0																														
29. <i>P. griseofulvum</i>	10	0	10	0	0	0	8	6	0	2.0–3.0	2.0–2.5	0	0	0	2	3	0	0	1	0	1	0																														
30. <i>Penicillium</i> sp.	3	0	3	0	0	0	0	3	0	2.5	2.0–2.5	0	0	0	1	0	0	0	0	0	0	0																														
31. <i>P. hirsutum</i> var. <i>allii</i>	3	0	2	0	1	0	0	3	0	2.5–3.0	2.5	0	0	0	1	0	0	1	0	0	1	0																														
32. Coremial strains	5	0	0	0	0	0	0	5	0	2.5–3.0	2.5–2.7	0	0	1	2	0	1	1	1	1	0	0																														
33. <i>P. aurantiogriseum</i> var. <i>melanoconidium</i>	2	0	2	0	0	0	2	0	0	2.7–3.2	2.5	0	0	0	1	0	0	1	0	0	1	0																														
34. <i>P. clavigerum</i>	2	0	0	0	2	0	2	0	0	2.5–3.0	2.0–2.5	2	0	0	0	1	1	0	0	0	0	0																														
35. <i>P. roquefortii</i>	7	1	6	0	0	6	0	1	0	3.0–4.0	2.5–3.5	0	0	0	0	0	0	1	0	0	0	0																														
36. <i>P. corylophilum</i>	3	0	1	0	2	0	0	3	0	2.0–3.0	2.0–2.5	0	0	0	0	0	0	0	0	0	0	0																														
37. <i>P. claviforme</i>	6	0	5	0	1	0	6	0	0	3.0	2.5	0	0	0	0	0	0	0	0	0	0	0																														

Cluster	No. of strains	2-Carboxy-3,5-dihydroxy-phenylacetyl carbinol										Desacetylpebrolide										Ergosterol										Fulvic acid										Frequentin										Griseofulvin										Gentisyl alcohol										Gentisic acid										5'-Hydroxyaspartin										m-Hydroxybenzoic acid										Myophenolic acid										6-Methylsalicylic acid										Ochratoxin A										Penicillic acid										Patulin										Penitrem A										Palitantin										PR toxin										Rugulovasine A										Roquefortine B										Roquefortine C										Terrestic acid										Terrein										Viomellein										Unknown m										Unknown S										Unknown B										Tenuazonic acid										Mycelianamide										Unknown d										Unknown i																																																																																																																																																																																																																							
		70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89

Fig. 2. Subclusters in cluster 5. ▲, Branch point where $P < 0.90$ that overlap is less than rectangular; ●, branch point where $P > 0.95$ that overlap is less than rectangular; ■, branch point where $P > 0.99$ that overlap is less than rectangular.

in preservation for many years, and certainly a number of the names as received on the cultures may be of doubtful validity. The cluster was recovered as seven smaller clusters in the Gower's coefficient dendrogram (Fig. 2) and most of these could be recognized in the Pattern dendrogram. There were few distinguishing characters between these smaller clusters (Table 3). This area of the genus has been reported by several authors to consist of a number of species, some of which have been considered synonymous with *P. aurantiogriseum*, including *P. commune*, *P. solitum*, *P. palitans* and *P. crustosum* (see Samson *et al.*, 1976; Pitt, 1980; Frisvad, 1985; Cruickshank & Pitt, 1987). In this study the central part of the cluster appeared distinct, with properties gradually varying in subgroups away from this. Overlap statistics suggested that some subgroups were distinct, particularly within the arm containing subgroups 2–5 (see Fig. 2). However, the Euclidean distances, derived from the similarity values, for both inter- and intra-group distances were all very similar (Table 4). A principal coordinates analysis of these strains did separate the central region of cluster 5 but since only 28% of the variation was explained by

Table 3. *Most useful tests available for differentiating between subclusters of cluster 5 (P. solitum)*

+, >85% positive; -, <25% positive; v, 25–85% positive.

	Subclusters						
	1	2	3	4	5	6	7
Production of penitrem A	—	—	—	v	v	—	—
Growth on tannic acid	+	+	+	+	+	—	v
DNA hydrolysis	—	v	v	+	+	+	+
RNA hydrolysis	—	+	+	v	+	v	+
Conidia blue-green	+	v	v	—	v	+	v
FLB divergent	—	+	v	v	—	+	+
Conidia globose	—	—	—	v	v	+	—
Conidia subglobose	+	+	+	v	v	—	+
Colony diameter (mm)	24–32	24–34	27–40	25–38	16–28	21–29	22–34

Table 4. *Inter- and intra-group Euclidean distances for subgroups of cluster 5 (P. solitum)*

Sub-cluster							
1	0						
2	1.67	0					
3	2.18	1.75	0				
4	2.24	1.92	1.63	0			
5	1.71	1.58	2.25	1.76	0		
6	2.45	2.16	2.77	2.25	2.13	0	
7	2.21	1.55	2.25	2.33	2.04	2.28	0
	1	2	3	4	5	6	7
Mean intra-group distances	2.23	1.69	1.65	2.04	1.97	2.44	1.98

the first three dimensions, this separation was at best slight. It therefore seems likely that the cluster represents a large continuum, showing almost random variation in most properties. The central region of the cluster corresponds well with descriptions of *P. crustosum* (e.g. Thom, 1930; Raper & Thom, 1949; Pitt, 1980), and could be distinguished from the rest of the strains by secondary metabolite production, particularly terrestrial acid and roquefortine C, but the overall arrangement of the cluster suggested that this was only at best a variety of the overall taxon.

The number of older ex-type strains within this cluster makes assigning a name difficult. The oldest ex-type strain included is *P. griseobrunneum*. However, this name has previously been considered a synonym of *P. brevicompactum* (Samson *et al.*, 1976; Pitt, 1980) and there may be some doubt about the authenticity of the line included here. Similarly, the next oldest name included in the group, *P. biforme*, has been considered a synonym of *P. verrucosum* var. *cyclopium* by Samson *et al.* (1976), a synonym of *P. camembertii* by Pitt (1980, 1986), and has been included in *P. commune* in later studies (e.g. Polonelli *et al.*, 1987). In the light of this, and the fact that the line used in this study showed both green- and white-spored sectors, it would be unwise to resurrect this old name. The ex-type cultures of *P. solitum*, *P. palitans* and *P. majusculum* were all present in this cluster. *P. palitans* has been considered a synonym of *P. viridicatum* by Pitt (1986), although this author later considered *P. palitans* as a synonym of *P. commune* (Cruickshank & Pitt, 1987). *P. majusculum* was represented by two different lines of the ex-type culture in this study which were recovered in different places, so the authenticity of this strain must be in doubt. The best name, as represented by an ex-type strain, which can be applied to this taxon is *P. solitum*. The central area of the cluster could be distinguished as a variety, and the general description and metabolite production profile match fairly well with *P. crustosum* (Frisvad, 1986; Pitt, 1980, 1986). The following new combination is made ***P. solitum* var. *crustosum* (Thom) comb. nov.** [basonym: *P. crustosum* Thom, *Penicillia*: 399, 1930]. Pitt (1986) designated IMI 91917 as the lectotype, and as herbarium material of this culture is still extant this specimen is acceptable as the nomenclatural type of the name.

Linked above cluster 6 were four single isolates (single-member clusters 2-5; IMI 285531, 192904, 39819 and 89312). IMI 285531 was received as *P. camembertii* III, a name ascribed to certain strains of *P. palitans* (Frisvad, 1986), a taxon recovered in cluster 5; this strain may be an atypical member of this group. The other three strains were all ex-type cultures. The strains IMI 192904 and 39819 were the only isolates of *P. psittacinum* and *P. lanosoviride* included in the study and so little can be concluded from their position. The strain IMI 89312 is of particular interest; it was originally described as a white-spored mutant of *P. cyclopium* (i.e. *P. aurantiogriseum*) although during most of this study the line consistently produced fawn conidia. However, both white and blue-green sectors have been produced by this strain so it appears to be naturally unstable.

Cluster 6 consisted of five strains. There were few distinguishing characters between these and *P. aurantiogriseum* other than colony texture and that these strains have only slightly roughened or smooth conidiophores. The ex-type culture of *P. puberulum* was present in this cluster and strains accepted in *P. puberulum* by Pitt (1980) are typical of the cluster. The ex-type strain was one of the two cases of duplicate cultures not being recovered together, the other line clustering in *P. aurantiogriseum*. Cruickshank & Pitt (1987) have considered *P. puberulum* to be a synonym of *P. aurantiogriseum* and this view is supported here.

Cluster 7 consisted of three strains including the ex-type isolate of *P. chrysogenum*, although this is an old culture that is now in a poor condition and shows few distinctive morphological features. This cluster moved close to other more recent isolates identified as *P. chrysogenum* (cluster 14) with the Pattern coefficient and so is considered to represent older and/or deteriorated representatives of this species.

Cluster 8 consisted of six strains including the ex-type strain of *P. echinulatum*. Echinulate conidia are rare amongst the fasciculate penicillia and so this was a useful diagnostic character for *P. echinulatum* which conformed to existing concepts (see Samson *et al.*, 1976; Pitt, 1980).

Cluster 9 consisted of two unusual strains which were in poor condition, one of which was received as the ex-type strain of *P. olivinoviride*. In the absence of any other material this cluster was tentatively named as *P. olivinoviride*.

Three strains (IMI 285511, 293204 and 59286) were recovered on a separate arm of the dendrogram directly below clusters 8 and 9. These strains also appeared as a weak group in the Pattern dendrogram. Two of them (IMI 285511 and 293204) were received as new chemotype variants of the two species *P. hirsutum* and *P. roquefortii*. The third was received as *P. granulatum*. The strains shared few common properties and so any decision would require the study of further isolates. Below this set of three were two further strains (IMI 285526 and 298085) which were partly attached to cluster 10. These were both phenotypically unusual isolates, which were recovered separately in other parts of the Pattern dendrogram and so their identity remains unclear.

Clusters 10 and 11 consisted of two sets of three strains, with the ex-neotype of *P. granulatum* being recovered in cluster 11. The two clusters were recovered separately in different areas of the Pattern dendrogram. Both groups showed many common properties but differed in conidial shape and the production of base from creatine, other differences being primarily a matter of degree only. The base from creatine reaction has been considered a species-specific character by Frisvad; however, he reported isolates of *P. granulatum* as being both creatine-positive and -negative (Frisvad, 1985, 1986). Pitt (1986) reported the conidia of *P. granulatum* as 'ellipsoidal, less commonly subspheroidal'. These two groups seem to represent varieties of the same species, cluster 11 being *P. granulatum* var. *granulatum* and cluster 10 being a creatine-positive *P. granulatum* variety with globose conidia. This new variety is named here as ***P. granulatum* var. *globosum* var. nov.** [Diagnosis: ab varietate granulato differt quod habet conidia globosa et id quod anglicebase from creatin redditur product. Holotypus: USA, Wisconsin, isolatus ex solo sylvae, 1983. *S. E. Gochenaure* (IMI 299049).]

Seifert & Samson (1986) reexamined early herbarium specimens and considered dried material described in the genus *Coremium* as equivalent to a number of strongly coremial *Penicillium* species. As the coremium species epithets are older, they should have priority over the existing *Penicillium* species, thus renaming *P. granulatum* as *P. glandicola* (Seifert & Samson,

1986). However, the earlier material from the genus *Coremium* is available only as herbarium specimens and so a detailed objective comparison can not be made with the living cultures studied here. In our view it is unwise to resurrect old names in this genus, for which no ex-type cultures are extant, as the full range of parameters used in species separations in the genus cannot be ascertained; thus some doubt must always remain (in the absence of further studies such as DNA homologies) as to whether the specimens are truly conspecific with the available cultures.

Clusters 12 and 13 consisted of 13 strains recovered in both dendrograms as a distinct arm of two strains (cluster 12) and a core group of 11 (cluster 13). The larger group contained the ex-neotype of *P. hirsutum*, which is synonymous with *P. corymbiferum* (Pitt, 1980). The cluster also contained the ex-type strain of *P. hordei*, which has been considered a synonym of *P. hirsutum* (Pitt, 1980), although the species is now regarded as distinct by Frisvad (1985) and Cruickshank & Pitt (1987). Unusually for fasciculate penicillia, these isolates were able to hydrolyse cellulose and produced a yellow pigment from CZNH4 agar. The two strains in cluster 12 differed in conidial ornamentation and in not producing citrinin or any yellow pigmentation. It has been suggested that these two latter properties may be linked (e.g. Smith, 1949). *P. hordei* was differentiated from *P. hirsutum* partly on the basis of the rough-walled conidia of the former (Samson *et al.*, 1976), a property seen in this study, where strains in cluster 13 have distinctly reticulate conidia in comparison to the finely lobate conidia of strains in cluster 12. It therefore seems likely that this small cluster represents *P. hirsutum*, although further work is necessary to establish whether there is in fact a gradation between this and *P. hordei*. At the base of cluster 13 was a single-member cluster (SM6) which contained strain IMI 19759, received as *P. cyclopium*; this remained ungrouped in the Pattern dendrogram.

Cluster 14 consisted of eight strains received as *P. chrysogenum* or related species and the ex-type culture of the older name *P. griseoroseum*. Samson *et al.* (1977) commented on the relationship between *P. griseoroseum* and *P. chrysogenum*, regarding the former as a synonym of the latter, a view supported in other recent work (Cruickshank & Pitt, 1987). This cluster is very similar to cluster 7 which contained the ex-type culture of *P. chrysogenum*, although strains in cluster 14 differed in producing base from lactic acid, having blue-green conidia and some appressed branches. Strains from cluster 14 also failed to produce roquefortine C consistently. In this case, where both clusters contained ex-type strains, they could be considered as two different species. However, the two clusters showed few different characters, most discrepancies probably being due to the vigour of the isolates. This is not a complete explanation as the two clusters were recovered close together but separate in the Pattern dendrogram. The characteristics of both clusters correspond well with the accepted concept of *P. chrysogenum* (e.g. Samson *et al.*, 1977; Pitt, 1980). Our line of the ex-type strain of *P. notatum*, considered a synonym of *P. chrysogenum*, was very deteriorated and was not recovered in this cluster. Given these factors, possible deterioration of some of the older ex-type cultures, and the importance of the name *P. chrysogenum*, it is prudent to consider both clusters as variants of *P. chrysogenum* until further isolates can be studied.

Two strains were recovered loosely linked at the top of cluster 15 (IMI 75832 and 321507). Strain 75832, originally received as the ex-type of *P. griseofulvum*, was found to be contaminated at the end of the study and so must be discounted. Strain 321507 was one of two representatives of *P. chrysogenum* var. *dipodomysis* (Frisvad *et al.*, 1987). The other strain of this species (IMI 321508) was recovered loosely linked at the base of cluster 15. These two isolates moved considerably in the Pattern dendrogram and were recovered in a number of different positions in other representations (unpublished). It is possible that these strains are true 'wanderers' (Sackin, 1985) or that this species is intermediate between several others where minor differences in characters may have significant taxonomic effect. More strains of *P. chrysogenum* var. *dipodomysis* are needed before any firm taxonomic conclusions can be drawn.

Cluster 15 consisted of five strains, four of *P. glandicola* var. *mononematosa*, including the ex-type culture and one strain of *P. flavogenum*. This cluster represents *P. glandicola* var. *mononematosa* but the relationship with *P. flavogenum* is unclear. This isolate appeared at the base of the group as a possible outlier and so may be distinct. However, there are insufficient

strains to make a firm decision. At the base of clusters 14 and 15 there were two pairs of ungrouped strains. The upper pair consisted of *P. chrysogenum* var. *dipodomysis*, discussed above, and a further isolate labelled 'viridicatum VII' (IMI 269930). This strain was considered distinct by J. C. Frisvad (personal communication), but to date no similar strains have been isolated and so its position cannot be properly determined. The second pair of strains were the ex-type culture of *P. nigricans* (IMI 39767), a species considered in synonymy with *P. janczewskii* (Pitt, 1980), and one strain received as *P. cyclopium* (IMI 297544). *P. nigricans* is not terverticillate and was included as an example of a different, distantly related species. However, *P. nigricans* is assigned to the section *Divaricatum* (Pitt, 1980), species of which are characterized by an irregular branching pattern that could be considered terverticillate. Strain 297544, received as *P. cyclopium*, does not belong to that taxon and appears to be a deteriorated isolate of a different species.

Clusters 16 and 17 consisted of six strains split into one group of four and a smaller group of two in both dendrograms. Five of the six strains were received as *P. camembertii* or synonyms, including the ex-type culture. The two clusters were distinguished by the production of citrinin, their conidial ornamentation and several negative physiological characters for cluster 17. Citrinin production has not been reported in *P. camembertii* before. Cluster 16 was citrinin-negative and contained strains recently isolated from cheese, whereas the apparent citrinin-positive cluster 17 consisted of two older ex-type strains. One possible explanation is that cluster 16 represents the true cheese *P. camembertii* strains, whereas the older ex-type strains in cluster 17 represent a different taxon. Included in cluster 16 was one strain (IMI 287746) which was isolated as a white-spored sector from a strain recovered in cluster 5. This white variant remained unstable throughout the work, often reverting to green conidia. *P. camembertii* has been suggested as a domesticated variant of the green-spored *P. commune* (Polonelli *et al.*, 1987; El-Banna *et al.*, 1987), and the placement of this strain in cluster 16 gives some support to that theory.

Cluster 18 consisted of 12 strains but no ex-type cultures. Six of these strains have previously been identified as *P. verrucosum* and the overall morphological description of this cluster matches the species concept of Pitt (1980). The ex-type culture of *P. verrucosum* was recovered in the adjacent cluster 19 in this dendrogram, but was found in this cluster with the Pattern dendrogram. It is likely that the ex-type culture is either deteriorated or in a generally poor condition.

Clusters 19 and 20 consisted of 15 strains split into two clusters at 69% similarity; they differed primarily in conidial shape. These two clusters were recovered as one single cluster in the Pattern dendrogram, along with cluster 21. The only ex-type culture recovered in these clusters was that of *P. verrucosum* (see above). Strain IMI 192503 was described as indistinguishable from the lectotype of *P. atramentosum* by Pitt (1980) and his general description of this species agrees well with the morphological characters recorded here. The ex-lectotype culture of *P. atramentosum* was included in this study (IMI 39752) but proved to be very deteriorated and was loosely linked near the base of the dendrogram. A fresh line of this culture examined subsequently agreed with all the typical properties of the clusters (Bridge *et al.*, 1989). Cluster 19 therefore represents *P. atramentosum*, with cluster 20 being an elliptical-spored variant.

Cluster 21 consisted of only two strains, both received as *P. atramentosum*, which had elliptical conidia and failed to hydrolyse starch. These two characters were the only clear-cut differences between this cluster and clusters 19 and 20; the group can thus be considered a starch-negative variant of *P. atramentosum*.

Cluster 22 consisted of four strains received as two new varieties of *P. glandicola* and *P. coprophilum*. All produced 2-carboxy-3,5-dihydroxyphenylacetyl carbinol, a metabolite not commonly found in this group of penicillia. This cluster may represent two taxa, as the morphology of the strains was quite varied; however, more isolates are needed to make a firm decision.

Three strains (IMI 285510, 296073 and 134650) appeared below cluster 22 as single-member clusters (SM7, 8 and 9), loosely linked to clusters 1–22. These are all apparently unrelated strains

and they included one line of the ex-type culture of *P. majusculum* (IMI 134650). Another line of this culture (IMI 321523), received through a different route had possibly become replaced and was recovered in cluster 5. The three strains appear to be three distinct isolates but some further study is needed to clarify their position.

Cluster 23 consisted of three strains including the ex-type isolate of *P. citrinum* and one isolate obviously replaced by *P. citrinum*. This species was included in the study as an example of a distantly related species. The placement of the species here rather than as a distinct outlier may be due to some of the typical diagnostic properties of this organism, which include the production of citrinin and other yellow pigments, like many terverticillate penicillia, and the general gross colony morphology being similar to those of *P. brevicompactum*.

Cluster 24 consisted of 13 strains, including the ex-neotype cultures of *P. brevicompactum* and *P. olsonii*, and the ex-type culture of *P. stoloniferum*. *P. stoloniferum* was regarded as a synonym of *P. brevicompactum* by Pitt (1980), and the latter is the oldest name for this group. *P. olsonii* is generally regarded as distinct (Pitt, 1980; R. A. Samson, personal communication), although in this study there appeared to be no features that could distinguish it from *P. brevicompactum*.

Cluster 25 consisted of two strains, including the ex-type of *P. raistrickii*. This species was included for comparison and is only distantly related to the terverticillate penicillia.

Loosely linked at the top of cluster 26 was a single-member cluster (SM10) received as *P. corymbiferum* (IMI 68414). This strain was morphologically typical of *P. hirsutum*, with which *P. corymbiferum* is synonymous (Pitt, 1980). However, this strain was atypical both physiologically and biochemically and does not appear to group with any accepted species.

Cluster 26 formed at below 70% similarity and contained three strains received as *P. aurantiogriseum* var. *neoechinulatum* and one strain described as atypical *P. hirsutum*. This group differed from *P. echinulatum* primarily by having yellow mycelium and being creatine-negative.

Below cluster 26 were three loosely linked strains: two of these (IMI 92209ii and 297546) were linked at 70% similarity and were representatives of two species, *P. citreoviride* and *P. raciborski*, which are both unrelated to the terverticillate penicillia and unrelated to each other. The third strain (IMI 293178) was received as *P. camembertii* but had presumably been originally misidentified as it produced green, echinulate conidia.

Cluster 27 contained nine strains including the ex-type strain of *P. viridicatum*. The properties recorded agreed well with the concept of this species given by Pitt (1980).

Linked at the base of cluster 27 was the small cluster 28, consisting of two strains, which were very similar to *P. viridicatum* and joined that cluster at 67% similarity. However, these two strains were found loosely linked at the base of *P. aurantiogriseum* in the Pattern dendrogram. *P. viridicatum* and *P. aurantiogriseum* appear well-separated on the dendrograms but they showed significant overlap (see later). These two strains represent a variant of *P. viridicatum* intermediate between the two taxa.

Below clusters 27 and 28 were three loosely linked strains; one (IMI 291201) was received as the ex-type of *P. ochraceum* var. *macrosporum*. These remained loosely linked in the same position in the Pattern dendrogram and may represent atypical variants of *P. viridicatum*.

Cluster 29 contained ten strains, identified as *P. griseofulvum* or currently accepted synonyms. The ex-type culture of this species was not present in the cluster as discussed above (under cluster 15). However, subsequent testing placed a fresh ex-type line in this cluster.

Below cluster 29 were four loosely linked strains and cluster 30. This cluster consisted of three *P. viridicatum*-like strains and was recovered in a similar position in the Pattern dendrogram. Cluster 30 represents a group of three different strains, all of which were in poor condition.

Cluster 31 consisted of two strains considered a new taxon by J. C. Frisvad (personal communication), *P. hirsutum* var. *allii* and one additional strain. They were loosely linked at 59% similarity but appear distinct.

Cluster 32 consisted of five loosely linked strains that were mainly received as further unpublished varieties of *P. hirsutum*. Cluster 31 and 32 were recovered together in the Pattern dendrogram and strains in both clusters formed coremia. Cluster 32 may be merely a variant of cluster 31.

Cluster 33 consisted of two strains recognized as a new variety, *P. aurantiogriseum* var. *melanoconidium* (J. C. Frisvad, personal communication). Loosely linked to this cluster was a single coremial strain which had been misidentified as *P. claviforme*. Clusters 31–33 may represent a single new taxon or atypical variants of an existing one.

Cluster 34 consisted of two strains (including the ex-type) of *P. clavigerum*. Above this cluster were two loosely linked strains: both were very poor deteriorated cultures.

Cluster 35 consisted of seven strains of *P. roquefortii*, including the ex-type strain. This cluster was distinct in a number of features including growth on acetic acid agar and colony size.

Cluster 36 consisted of three strains, including the ex-type, of *P. corylophilum*. This was a distinct group in both dendrograms and is not closely related to the fasciculate penicillia.

Below cluster 36 were two sets of loosely linked strains. These included the only three authentic lines of *P. italicum* included in the study; the remainder were all either representatives of distantly related species or poorly growing isolates in a deteriorated condition.

Cluster 37 consisted of six strains of the strongly coremial *P. claviforme*. This taxon, included for comparison, was distinct on both dendrograms; it is not closely related to the fasciculate species. As with *P. granulatum*, an earlier species epithet is available from *Coremium vulpinum* (Seifert & Samson, 1986). Again, we consider it unwise to resurrect this name in the absence of extant ex-type cultures.

At the base of the dendrogram was a single-member cluster (SM11), containing a strain of *P. funiculosum*. This was included as an example of a different section of the genus, subgenus *Biverticillium*, and so was expected to be distinct from other strains.

Vigour

The vigour values for the strains used in this study varied from 0.198 to 0.419. However, the mean vigour values for the clusters described ranged only between 0.26 and 0.35. This is a narrow range and indicates that groups formed from deteriorated cultures in this study are not necessarily less vigorous for the characters used. An example of this is cluster 6, considered a deteriorated variant of *P. aurantiogriseum*. The mean vigour for cluster 6 (0.325) is actually higher than that for *P. aurantiogriseum* (0.317). *P. chrysogenum* was recovered in two well-separated clusters in the Gower dendrogram, clusters 7 and 14, and these clusters moved closer together in the Pattern dendrogram. Vigour differences were not apparently responsible for this as the mean vigour of *P. chrysogenum* I, cluster 7, was 0.281 and the mean vigour of *P. chrysogenum* II, cluster 14, was 0.262. In *P. expansum*, clusters 1 and 3, where cluster 3 contained freshly isolated strains, the vigour for this cluster was slightly lower than for the main group (cluster 1). One possible reason for this is that when deteriorated strains start to lose characteristic properties, they may express others: for example, they may stop producing a particular secondary metabolite but instead accumulate a precursor which is seen as a diffusing pigment. This has been suggested as a possibility with some red pigments that may be due to an overproduction of melanin following deregulation of metabolite pathways (see Anné, 1986).

CONCLUSIONS

The fasciculate penicillia have many common properties and overall they appear very similar. Serological studies have shown considerable similarity between these species, most antisera being unable to separate them (Polonelli *et al.*, 1986, 1987). Patterns of extracellular enzymes have shown distinct separations at only the subgenus and section level (Bridge & Hawksworth, 1984) and protoplast fusion studies have resulted in cross-specific heterokaryons (Anné & Peberdy, 1981; Anné, 1986). The mol% G + C ratios for penicillia are all very similar (e.g. Storck & Alexopoulos, 1970), and first-derivative melting curve data from DNA of representative strains of the present study also indicate considerable similarity, particularly between fasciculate strains (Paterson *et al.*, 1990). To date no DNA hybridization data have been published for *Penicillium*. Electrophoretic mobilities of pectinase and amylase enzymes have been proposed as species-specific by Cruickshank & Pitt (1987), who used 181 strains from 38 species or synonyms. In our experience numerical taxonomic studies on pectinase and amylase isozymes from some of the cultures included in this study have shown there may be

considerable heterogeneity in isozyme patterns in many species (accompanying paper: Paterson *et al.*, 1989). This study has shown that properties may vary considerably within some species as was seen in *P. solitum*, although the species remained distinct on other grounds. Interestingly, preliminary results suggest that there is very little difference in mitochondrial DNA restriction fragment length polymorphisms (RFLPs) from strains with diverse names and histories referred to as *P. solitum* here (J. Croft, unpublished results), indicating a common phylogeny (Croft, 1987).

The present results raise a number of interesting points. Firstly, the number and reproducibility of secondary metabolites seen in some of the strains studied here were in general lower than those reported by some workers (e.g. Frisvad *et al.*, 1987), although in some other recent studies low levels of metabolite production have been reported (Land & Hult, 1987). In some cases the production of a metabolite by only 30% of strains in a species has been considered a positive feature (Frisvad & Filtenborg, 1983). The problem of loss of secondary metabolite production has been studied, especially for patulin where it was shown to involve the short half-life of critical enzymes in the pathway (Lam *et al.*, 1988). However, this information is only available for a few metabolites. Second, distinct chemical variants occur in a number of species, and these variants were often also distinct by SEM, such as the globose conidia of the creatine-positive *P. granulatum* or the strains of *P. camembertii* with microtuberculate conidia which showed reduced physiological activity. Thirdly, there is considerable 'background' similarity amongst the fasciculate penicillia, which is supported by inter-specific fusions and serological reactions (Anné, 1986; Polonelli *et al.*, 1986), and some strains must be considered as intermediates between species. While deteriorated strains could usually be identified as such, strain IMI 287746 (grouped in cluster 5) produced a sector which showed the properties of a different species (*P. camembertii*, cluster 16). Studies involving a number of single conidium lines of the same strain of *Penicillium* have shown that there can be considerable phenotypic variation within a single strain, possibly as a result of aneuploidy (e.g. Bridge *et al.*, 1987). It is perhaps relevant that some of the earlier workers in this genus, who recognized many more species, used strains derived from single conidia (e.g. Biourge, 1923). The role of heterokaryosis and parasexual mating has been considered in relation to variation in naturally occurring *Penicillium* species (e.g. Jinks, 1952), and differences seen in isozyme patterns can be due to heterokaryosis or aneuploidy (Anné & Peberdy, 1981; Micales *et al.*, 1986; Paterson *et al.*, 1989a). However, these phenomena have not usually been considered in taxonomic studies.

The taxonomic results obtained in this study are generally in agreement with existing schemes (e.g. Pitt, 1980, 1986; Cruickshank & Pitt, 1987). The main discrepancies concerning *P. solitum* and *P. crustosum* and *P. olsonii* and *P. brevicompactum* have already been mentioned. The one remaining discrepancy concerned the species *P. commune* which was not recovered in this study. Strains previously assigned to *P. commune* were recovered in both the *P. solitum* and *P. aurantiogriseum* clusters. The ex-type culture studied was quite different from these and further studies with another line of this culture showed it to be a typical *P. aurantiogriseum* (Bridge *et al.*, 1989). The exact status of this species is unclear from the literature. Pitt (1980, 1986) considered this species a synonym for *P. puberulum*, which itself is now considered a synonym of *P. aurantiogriseum*. Samson & van Reenen-Hoekstra (1988) give *P. commune* as the correct name for strains previously considered as *P. verrucosum* var. *melanochlorum*, a species previously considered the correct name for *P. palitans* (Samson *et al.*, 1976). However, Frisvad (1985) considered both *P. palitans* and *P. commune* to be distinct species. The comparison between the results presented here for secondary metabolite production and the existing literature is similarly difficult due to the different strain identifications made by different authors. One example of this is the production of ochratoxin A, which has been described from *P. viridicatum* (see Turner, 1971). Subsequently, ochratoxin-producing strains have been re-identified as *P. verrucosum*, although up to 20% of the strains assigned to that species did not produce the secondary metabolite (Pitt, 1987).

This study has shown that an integrated multidisciplinary approach can be applied successfully to elucidate taxonomic problems in *Penicillium*. Species that were initially delineated on simple morphological features were found to be supported by critical SEM studies

and were also determined by combinations of physiological and biochemical characters. The numerical taxonomy approach allows for a considerable number of different features to be considered together in an objective manner. This study also attempted to consider the effect on taxonomic schemes of older and atypical cultures, and the placement of these in relation to inter- and intra-specific variation. This has acted as a test for species concepts based on smaller numbers of characters and will therefore assist in the development of a more acceptable and stable taxonomy for this critical group. As many of the species accepted are clearly very closely related, further work is needed to fully interpret the appropriate taxonomic levels of groups within the genus; genetic and molecular studies particularly may clarify the situation further, especially with regard to strain variation and stability.

Identification matrices and keys to the fasciculate penicillia included in this study are provided in the following paper (Bridge *et al.*, 1989).

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* This book, although dated 1985, was not available until 1986.

† This book, although dated 1979, was not available until 1980.