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Genetic Relationships among *Penicillium* Species by Characterizing RAPD Markers

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Random amplified polymorphic DNA markers were characterized for three taxonomically problematic *Penicillium* species: *P. aurantiogriseum* var. *aurantiogriseum*, *P. verrucosum* and *P. puberulum*, as well as for 25 species of mono-, bi-, and terverticillate Penicillia. The relationship of *P. vulpinum* with bi- and terverticillate *Penicillium* species was investigated and genetic relationships among mono-, bi-, and terverticillate *Penicillium* species were determined from these RAPD markers. Eight species from mono-, eight from bi-, and nine from terverticillate Penicillia were examined. With 14 randomly chosen 10-mer primers, a 310 character by 25 species matrix was generated. Phenetic analysis separated the 25 species into three genetically distinct groups that correspond to the different arrangements of penicilli (mono-, bi-, and terverticillate). The results of this study suggest that *P. aurantiogriseum* var. *aurantiogriseum*, *P. verrucosum*, and *P. puberulum* represent genetically distinct species, and that *P. vulpinum* should be included in terverticillate Penicillia. Phenogram branching patterns indicated that biverticillate species are genetically more similar to monoverticillate species than they are to terverticillate species.

Key words: molecular systematics, *Penicillium*, RAPD markers

Despite many notable works on the identification and taxonomy of *Penicillium* species (1, 2, 6, 7, 13, 15, 17, 20), the state of the taxonomy of these fungi has remained unsatisfactory due to the conflicting species concepts of each taxonomist (3, 12, 18). For example, Raper and Thom (15) described 137 species largely based on the colony texture, while Pitt (13) accepted approximately 150 species according to colony diameter, morphology, and conidia color.

The taxonomic position of *P. vulpinum* (Cooke & Massee) Seifert & Samson has been examined in several studies (9, 10, 13, 19). Pitt (13) considered *P. vulpinum* to be a biverticillate species in subgenus *Biverticillium* due to production of acerose phialides typical of that subgenus. However, his view of this species has not been supported by secondary metabolite profiles (19) or molecular analyses (9, 10). Secondary metabolite profiles have indicated that *P. vulpinum* is more closely related to the terverticillate species than to the biverticillate species (19). Furthermore, sequence analyses of various DNA regions (9, 10) have shown a close phylogenetic relationship between this species and the terverticillate species. However, data from these studies have not been sufficient to draw clear conclusions of taxonomic position of this species.

Penicillium trzebinskii Zaleski, and *P. terlikowskii* Zaleski are now recognized to be synonyms of *P. spinulosum* Thom (14), but there are differing views of the concept of *P. spinulosum* which has been redescribed a number of times since 1901 (14). *Penicillium trzebinskii* was placed in the *P. lividum* Westling series because the former species produced long, rough conidiophores and echinulate conidia (15). However, they suggested that the concept of *P. spinulosum* should be broadened to accomodate *P. trzebinskii* due to the morphological similarities between the two. Raper and Thom (15) also included *P. terlikowskii* in terminal position of *P. adametzi* Zaleski series because *P. terlikowskii* produced larger and more roughened conidia than those produced by species typical of the *P. adametzi* series.

There has also been some confusion concerning *P. aurantiogriseum* Dierckx, *P. cyclopium* Westling, *P. verrucosum* Dierckx, and *P. puberulum* Bainier in the subgenus *Penicillium* (3, 4, 5, 13, 17). Raper and Thom (15) pla-

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ced *P. cyclopium* in close affinity with *P. puberulum* due to their similar fasciculate structures. However, Pitt (13) designated *P. cyclopium* a synonym of *P. aurantiogriseum*. Bridge *et al.* (3), and Cruickshank and Pitt (5) added *P. puberulum* to the synonyms of *P. aurantiogriseum* due to the small differences in biochemical, physiological, and morphological characters. In addition, Samson *et al.* (17) considered *P. aurantiogriseum* to be a variety of *P. verrucosum* because of their morphological similarities. Recently, a comparison of partial nucleotide sequences of the nuclear small subunit (18S) and large subunit (25S) ribosomal RNA genes determined that *P. aurantiogriseum, P. puberulum,* and *P. verrucosum* represent distinct species based on the large number of differing bases among these species (10).

This study attempts to resolve some of the taxonomic controversies concerning mitotic *Penicillium* species through the use of RAPD markers. Thus, we examined the taxonomically problematic species discussed above as well as species agreed upon by all *Penicillium* taxonomists. This study also assesses the degree of genetic variation within each of mono-, bi-, and terverticillate Penicillia, and compares the genetic relationships among them.

Materials and Methods

Strains and cultures

Species of *Penicillium* were obtained from KCTC (Korean Collection for Type Culture), ATCC (American Type Culture Collection), and NRRL (Northern Regional Research Laboratory) as designated in Table 1.

Suspensions of conidia were prepared from 7-10 day-old cultures, grown at 25℃ on Difco potato dextrose agar (PDA) by flooding the plate with 3 ml of sterile water. Flasks containing 100 ml of Difco potato dextrose broth (24 g/L of tap water) were inoculated with 1 ml of the conidial suspension and incubated at 25℃ for 30 hr at 180 rpm. Mycelia were harvested by vacuum filtration, rinsed with distilled water, and ground with dry ice to a fine powder. Genomic DNA was isolated according to the method of Yoon *et al.* (22). DNA solutions containing 100 μg of RNase A/ml final concentration were incubated at 37℃ for 1 hr to digest RNA. The concentration of DNA in each sample was estimated by comparing the intensity of DNA bands in a 0.8% agarose gel with a series of samples of known DNA concentrations under UV light (310 nm) after staining with ethidium bromide.

PCR amplification

Fourteen arbitrarily chosen primers (Operon Technologies) were used to amplify the genomic DNA of the 25 *Penicillium* species listed in Table 1. The primer se-

Table 1. Isolates of *Penicillium* species used and their characteristic penicillus type.

KCTC code	Strain	Source	Type
6012	*Penicillium spinulosum*	Isolate	Monoverticillate
6246	*Penicillium adametzi*	NRRL 737	Monoverticillate
6258	*Penicillium trzebinskii*	NRRL 731	Monoverticillate
6259	*Penicillium vinaceum*	NRRL 739	Monoverticillate
6260	*Penicillium terlikowskii*	NRRL 752	Monoverticillate
6261	*Penicillium lividum*	NRRL 754	Monoverticillate
6262	*Penicillium turbatum*	NRRL 757	Monoverticillate
6270	*Penicillium sclerotiorum*	NRRL 2074	Monoverticillate
6248	*Penicillium allahabadense*	NRRL 3397	Biverticillate
6263	*Penicillium waksmanii*	NRRL 777	Biverticillate
6264	*Penicillium chrzaszozii*	NRRL 903	Biverticillate
6272	*Penicillium godlewskii*	NRRL 2111	Biverticillate
6273	*Penicillium manginii*	NRRL 2134	Biverticillate
6274	*Penicillium kapuscinskii*	NRRL 2147	Biverticillate
6275	*Penicillium megasporum*	NRRL 2232	Biverticillate
6267	*Penicillium vulpinum*	NRRL 2031	Biverticillate
1257	*Penicillium expansum*	Isolate	Terverticillate
6052	*Penicillium chrysogenum*	NRRL 807	Terverticillate
6053	*Penicillium notatum*	NRRL 832	Terverticillate
6114	*Penicillium puberulum*	ATCC 36363	Terverticillate
6256	*Penicillium cyclopium*	NRRL 1888	Terverticillate
6252	*Penicillium aurantiogriseum* var. *aurantiogriseum*	NRRL 2040	Terverticillate
6253	*Penicillium brunneoviolaceum*	NRRL 2137	Terverticillate
6255	*Penicillium carneolutescens*	NRRL 2035	Terverticillate
6265	*Penicillium verrucosum*	NRRL 965	Terverticillate

Table 2. Sequences of primers used in this study.

Primer	Sequence
OPA-01O	C A G G C C C T T C
OPA-10	G T G A T C G C A G
OPB-01	G T T T C G C T C C
OPB-04	G G A C T G G A G T
OPB-05	T G C G C C C T T C
OPB-07	G G T G A C G C A G
OPB-08	G T C C A C A C G G
OPB-10	C T G C T G G G A C
OPB-11	G T A G A C C C G T
OPB-12	C C T T G A C G C A
OPB-14	T C C G C T C T G G
OPB-15	G G A G G G T G T T
OPB-17	A G G G A A C G A G
OPB-18	C C A C A G C A G T

quences and designations are listed in Table 2. The RAPD markers were amplified in 25 μl reactions containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin (Sigma), 0.1% Triton X-100, 100 μM each of dATP, dCTP, dGTP and dTTP (Perkin-Elmer-Cetus), 15 ng of a single primer, 12~25 ng of genomic DNA, and 0.7 unit of *Taq* DNA polymerase (Promega). Control reactions omitting genomic DNA were also prepared in order to distinguish amplified fungal DNA fragments from possible artifacts. The reaction mixtures were overlaid with mineral oil (Sigma) and placed in an MJ Research DNA thermal cycler (Model PTC-100-60), in which the heating/cooling block was preheated to 80℃.

The amplification protocol consisted of an initial denaturation period of 2 min at 94℃ followed by 40 cycles of 1 min at 94℃, 1 min at 36℃, and 2 min at 72℃. An additional extension step of 5 min at 72℃ followed the final cycle and the reaction mixtures were stored at 4℃. Amplified DNA fragments were resolved in a gel consisting of 0.7% NuSieve GTG agarose (FMC Bioproducts) and 0.7% ultra pure agarose (Sigma) at 4.5 V/cm for 3 hr, and were detected by staining with ethidium bromide with UV transillumination as described above. For each gel, a 123 bp DNA ladder (Gibco BRL) was also run to serve as a size marker. Gels were photographed over a UV transilluminator using a Polaroid camera (Model DS-34) and black and white film (Type 667, Polaroid Corp).

Analysis of data

Amplified DNA fragments (bands), reproducible in two to three reactions, were scored for all samples on two possible character states 0 (fragment absent) and 1 (fragment present). A 310 character by 25 species matrix was generated.

Estimation of genetic relationships between all pairs of taxa in the matrix was performed using distance values (D) generated from the following formula (11):

$$D=1-2C_{xy}/(U_x+U_y+2C_{xy})$$

in which C_{xy} equals the number of fragments in common to samples X and Y, and U_x and U_y represent the number of unique bands. D values range from 0 to 1. When the D value for a comparison of two samples is close to 0, a high degree of genetic similarity is indicated. D value close to 1 indicates a low degree of genetic similarity. The matrix of D values is available on request. This method helps avoid biased estimation of genetic relationships since genetic similarity values based on RAPD markers are not appropriate for constructing phenograms (8, 21). This results from two causes. If comigrating DNA bands with small size differences, or representing different sequences, cannot be resolved, their similarity values would be overestimated. Under- or overestimation of similarity values may also result if the samples exhibit different numbers of amplification products, or if length mutations occur in amplified regions. For these reasons, in this study dissimilarity values were used instead of similarity values.

Distance values were subjected to phenetic analyses with the software package NTSYS-pc (version 1.8) developed by Rohlf *et al.* (16). Unweighted pair-group arithmetic average (UPGMA), single linkage, and complete linkage analyses were used to construct phenograms. The goodness of fit of the phenogram was examined on the basis of cophenetic correlation using the program MXCOMP. The average genetic distance value ($\overline{D}$) was used to determine genetic relationships between groups, and to assess the degree of genetic variation within each of these groups. These values were calculated using the following formula:

$$\overline{D}=\frac{\sum D_{xy}}{N}$$

in which Dxy represents the distance value between samples X and Y in a pair of groups to be compared, or between samples X and Y in a group, and N equals the total number of comparisons between or within groups.

Results

DNA polymorphisms and genetic relationships among 25 species

Using 14 primers, a total of 310 different reproducible RAPD markers were generated from the 25 *Penicillium*

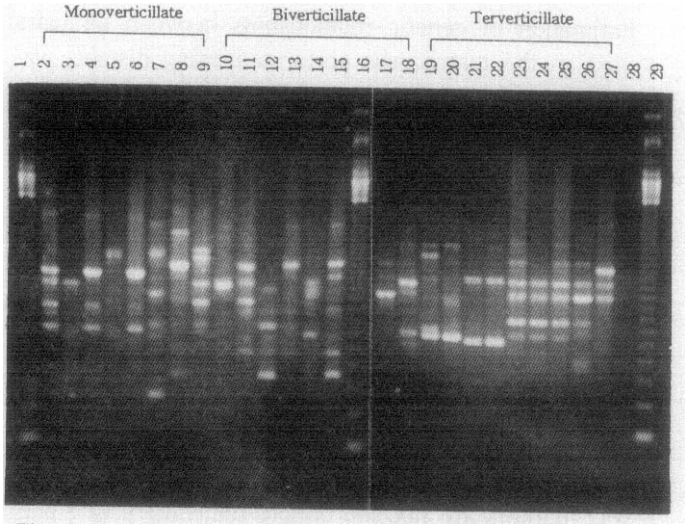

Fig. 1. Amplification products from DNA of 25 *Penicillium* strains and primer OPB-10. Lanes 2~9: PCR products from DNA of eight monoverticillate *Penicillium* species (Lane 2: *P. spinulosum;* Lane 3: *P. adametzi;* Lane 4: *P. trzebinskii;* Lane 5: *P. vinaceum;* Lane 6: *P. terlikowskii;* Lane 7: *P. lividum;* Lane 8: *P. turbatum;* Lane 9: *P. sclerotiorum*); Lanes 10~17: PCR products from DNA of seven biverticillate *Penicillium* species (Lane 10: *P. allahabadense;* Lane 11: *P. waksmanii;* Lane 12: *P. chrzaszozii;* Lane 13: *P. godlewskii;* Lane 14; *P. manginii;* Lane 16: *P. kapuscinskii;* Lane 17: *P. megasporum*); Lane 18: PCR products from DNA of *P. vulpinum;* Lanes 19~27: PCR products from DNA of nine terverticillate *Penicillium* species (Lane 19: *P. expansum;* Lane 20: *P. chrysogenum;* Lane 21: *P. notatum;* Lane 22: *P. puberulum;* Lane 23: *P. cyclopium;* Lane 24: *P. aurantiogriseum* var. *aurantiogriseum;* Lane 25: *P. brunneoviolaceum;* Lane 26: *P. carneolutescens;* Lane 27: *P. verrucosum*); Lane 28: control PCR reaction with no added DNA; Lanes 1, 16 and 29: 123 bp DNA ladders.

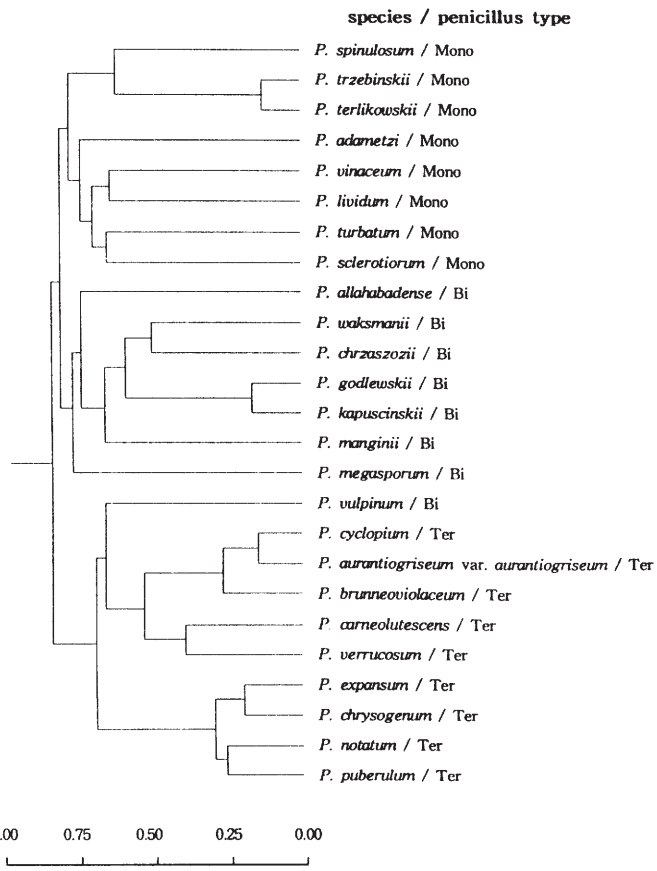

Fig. 2. Phenogram based on RAPD markers amplified from DNA of 25 *Penicillium* strains and 14 arbitrarily chosen 10-mer primers. The phenogram was constructed from distance values using the UPGMA method in the software package NTSYS-pc. Three distinct groups were delimited, corresponding with mono-, bi- and terverticillate species.

species. As shown in Fig. 1, the numbers of amplified RAPD markers varied, depending on the primer and species combination. Phenograms generated from the three analyses showed similar topologies. The cophenetic correlation coefficient for UPGMA was 0.9390, for single linkage was 0.9280, and for complete linkage was 0.9242. These results indicate that the phenogram based on UPGMA analysis exhibits the closest fit to the data (Fig. 2). The 25 species fell into three groups, generally corresponding with the different arrangements of penicilli. Group A consisted of eight monoverticillate species. Group B consisted of seven biverticillate species. Group C consisted of ten terverticillate species including *P. vulpinum*.

In order to assess the degree of genetic variation within each group, average genetic distance values were calculated for each of the three groups. The average genetic distance values within each of groups A, B, and C were 0.7215, 0.6785, and 0.5852, respectively. These results indicate that strains in monoverticillate Penicillia are genetically more variable than those in other Penicillia, whereas strains in terverticillate Penicillia are the least variable.

Genetic relationships among three different groups were determined on the basis of the phenogram (Fig. 2) and the average genetic distance values. The average genetic distance value between mono- and biverticillate groups was 0.7946, between mono- and terverticillate groups was 0.7952, and between bi- and terverticillate groups was 0.8181. These values indicate that each group differed from each other by approximately 80% of RAPD markers generated. Biverticillate strains appear to exhibit a closer genetic relationship with the monoverticillate strains than with terverticillate strains.

Genetic relationships among species within each group

Clustering patterns in the phenogram (Fig. 2) appeared to reflect taxonomic affinity among the strains. *Penicillium spinulosum* clustered with its taxonomic synon-

yms, *P. trzebinskii* and *P. terlikowskii. Penicillium cyclopium,* a synonym of *P. aurantiogriseum* (13) clustered with a variety of the latter, *P. aurantiogriseum* var. *aurantiogriseum.* However, *P. puberulum, P. verrucosum,* and *P. aurantiogriseum* var. *aurantiogriseum* showed much more distant genetic relationships to each other. In addition, *P. vulpinum* which was placed in the subgenus *Biverticillium* by Pitt (13) clustered with terverticillate strains. This indicates that *P. vulpinum* is genetically more closely related to terverticillate species than to biverticillate species. However, *P. chrysogenum* Thom clustered with *P. expansum* Link rather than with *P. notatum* Westling (a synonym of *P. chrysogenum*).

Discussion

Genetic and taxonomic relationships among species within each group

Even though each group included several strains with names considered to be synonyms for one another, the degree of genetic variation among strains within each group was high. As shown in Fig. 2, *P. spinulosum* was genetically more similar to *P. trzebinskii* and *P. terlikowskii* than to other species in this study. This finding agrees with Pitt's (13) concept that *P. trzebinskii* and *P. terlikowskii* are synonyms of *P. spinulosum.* The average genetic distance value between *P. trzebinskii* and *P. terlikowskii* was 0.1250, whereas that between *P. spinulosum* and both *P. trzebinskii* and *P. terlikowskii* was 0.6289. If *P. trzebinskii* and *P. terlikowskii* are actually strains of *P. spinulosum*, the distance value would be expected to be much lower. This discrepancy may result from the use of a different strain of *P. spinulosum* than the one Pitt (13) observed, or from the use of a non-type strain of *P. spinulosum.*

Our data also indicate that *P. aurantiogriseum* var. *aurantiogriseum* is genetically more similar to *P. cyclopium* than it is to *P. verrucosum* or *P. puberulum,* and that *P. aurantiogriseum* var. *aurantiogriseum, P. verrucosum,* and *P. puberulum* are genetically quite distant from each other. These results are consistent with previous studies (10, 13, 15) and provide insight into the species concept of *P. aurantiogriseum.* Pitt (13), and Raper and Thom (15) considered *P. cyclopium* to be a synonym of *P. aurantiogriseum.* Samson *et al.* (17), however, rejected this concept and considered *P. cyclopium* to be a variety of *P. verrucosum.* In addition, Cruickshank and Pitt (4, 5), and Bridge *et al.* (3) placed *P. puberulum* into synonymy with *P. aurantiogriseum* based on biochemical, morphological, and physiological data. A recent study (10), however, delineated *P. aurantiogriseum, P. puberulum,* and *P. verrucosum* into distinct species on the basis of nuclear small (18S) and large (25S) ribosomal RNA sequence data. In that study, two strains were considered to be distinct species if they differed at more than two nucleotide positions. Between thirteen and fifteen nucleotide positions with differing bases in each of the three species were found when they were compared with each of the other two species. The results of our study using RAPD markers are consistent with those of studies (10, 13, 15) indicating that the name *P. cyclopium* should be designated as a synonym of *P. aurantiogriseum,* and that the names *P. aurantiogriseum, P. puberulum,* and *P. verrucosum* should be retained at this point. However, the taxonomic status of *P. puberulum* is not certain, as the strain used in this study is not the type strain. For a clear taxonomic conclusion, the type strain of this species needs to be examined with RAPD's as well as other methods.

Even though the genetic distance between *P. chrysogenum, P. expansum,* and *P. notatum* is low, some discrepancies existed (Fig. 2). The close genetic relationship between *P. chrysogenum* and *P. expansum* shown in the phenogram seems to correspond with Pitt's (1988) observation that they have similar penicillus morphology. However, *P. chrysogenum* clustered with *P. expansum* rather than with *P. notatum* which is considered to be a synonym of *P. chrysogenum.* This discrepancy may also result from the use of a non-type strain of *P. expansum.* Nevertheless, these three strains were more similar to each other than they were to others, indicating a close genetic relationship.

The results indicate clearly that *P. vulpinum* is more similar to terverticillate species than it is to other biverticillate species. These results are consistent with previous studies using secondary metabolite profiles (19) and sequences of nuclear internal transcribed spacer (ITS) (9).

It is noteworthy that Pitt's (14) illustrations included *P. vulpinum* with the biverticillate species because of its typically acerose phialides. Stolk *et al.* (19), on the other hand, stated that this species should not be considered biverticillate because its phialides do not have necks and that it produces patulin and roquefortine C which are not produced by typical biverticillate species. In this study with RAPD markers, *P. vulpinum* seems to be genetically distinct from the biverticillate species, clustering with the terverticillate species. Thus, the results of this study strongly support assertion of Stolk *et al.* (19) that *P. vulpinum* should be included in terverticillate Penicillia.

Our results are consistent with a recent study using mtSrDNA and nuclear ITS sequences which demonstrated a close phylogenetic relationship between *P. vulpi-*

num and the terverticillate species in the subgenus *Penicillium* (9). This study found a close phylogenetic relationship between *P. vulpinum, P. clavigerum* Demelius and species of *Eupenicillium.* However, the taxonomic relationship between *P. vulpinum* and mitotic terverticillate species in the subgenus *Penicillium* was not clearly resolved by that study (9) because typical mitotic terverticillate *Penicillium* species were not included. Meiotic *Eupenicillium* strains were used to infer the phylogenetic relationship between *P. vulpinum* and mitotic terverticillate species. However, the present study, which includes eight biverticillate and nine terverticillate strains, clearly separates *P. vulpinum* from the biverticillate strains and clusters it with terverticillate strains, indicating that *P. vulpinum* is genetically more similar to terverticillate strains than it is to biverticillate strains.

Genetic relationships between groups

A total of 25 strains fell into three groups, corresponding with the different arrangements of penicilli which have been used to delineate subgenera. This overall topology of the phenogram generally agrees with Pitt's (13) classification system. The average genetic distance values between groups were calculated on the basis of the phenogram (Fig. 2). These distance values indicate that biverticillate strains are genetically more similar to monoverticillate strains than they are to terverticillate strains.

Pitt (14) observed intermediate characters in some mono- and biverticillate species. Two metulae were occasionally observed in monoverticillate species such as *P. spinulosum* and *P. thomii* Maire. In addition, biverticillate/monoverticillate penicilli were irregularly produced by biverticillate species, *P. waksmanii.* Thus, these intermediate characters in some mono- and biverticillate species may reflect the close genetic relationship between mono- and biverticillate species shown in the phenogram (Fig. 2).

In conclusion, the results indicate that biverticillate strains, at least in part, are more closely related to mono- than terverticillate strains. Additional data will be required to resolve specific patterns of evolution in these groups. Based on evidence from other studies and the branching patterns of our phenogram, it appears that the name *P. cyclopium* is a synonym of *P. aurantiogriseum,* and that *P. aurantiogriseum, P. verrucosum,* and *P. puberulum* are indeed distinct species. The clustering of *P. vulpinum* with terverticillate strains in the phenogram suggests that this species should be included with the terverticillate Penicillia.

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References

1. **Abe, S.,** 1956. Studies on the classification of the Penicillia. *J. Gen. Appl. Microbiol.* **2**, 1-344.
2. **Biourge, P.,** 1923. Les moisissures du groupe *Penicillium* Link. *Cellule* **33**, 7-331.
3. **Bridge, P.D., D.L. Hawksworth, Z. Kozakiewicz, A.H. S. Onions, R.R.M. Paterson, M.J. Sackin, and P.H.A. Sneath,** 1989. A reappraisal of the terverticillate penicillia using biochemical, physiological and morphological features I. Numerical taxonomy. *J. Gen. Microbiol.* **135**, 2941-2966.
4. **Cruickshank, R.H. and J.I. Pitt,** 1987a. The zymogram technique: Isoenzyme patterns as an aid in *Penicillium* classification. *Microbiol. Sci.* **4**, 14-17.
5. **Cruickshank, R.H. and J.I. Pitt,** 1987b. Identification of species in *Penicillium* subgenus *Penicillium* by enzyme electrophoresis. *Mycologia* **79**, 614-620.
6. **Dierckx, R.P.,** 1901. Un essai de revision du genre *Penicillium* Link. *Ann. Soc. Sci. Bruxelles* **25**, 83-89.
7. **Fassatiova, O.,** 1977. A taxonomic study of *Penicillium* series Expansa Thom emend. Fassatiova. *Acta Unuv. Carol. Biol.* **12**, 283-335.
8. **Hibbett, D.S., and R. Vilgalys,** 1991. Evolutionary relationships of *Lentinus* to the Polyporaceae: Evidence from restriction analysis of enzymatically amplified ribosomal DNA. *Mycologia* **83**, 425-439.
9. **LoBuglio, K.F., J.I. Pitt, and J.W. Taylor,** 1994. Independent origins of the synnematous *Penicillium* species, *P. duclauxii, P. clavigerum,* and *P. vulpinum,* as assessed by two ribosomal DNA regions. *Mycol. Res.* **98**, 250-256.
10. **Logrieco, A., S.W. Peterson, and D.T. Wicklow,** 1990. Ribosomal RNA comparisons among taxa of the terverticillate penicillia, p. 343-355. *In* Samson, A.R. and J.I. Pitt (eds.), Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, New York and London.
11. **Nei, M, and W.H. Li,** 1987. Mathematical model for studying genetic variations in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* **76**, 5269-5273.
12. **Onions, A.H.S., P.D. Bridge, and R.R.M. Paterson,** 1984. Problems and prospects for the taxonomy of *Penicillium. Microbiol. Sci.* **1**, 185-189.
13. **Pitt, J.I.,** 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces.* Academic Press, London.
14. **Pitt, J.I.,** 1988. A laboratory guide to common *Penicillium* species, 2nd ed. CSIRO Division of Food Processing, North Ryde, New South Wales.
15. **Raper, K.B. and C. Thom,** 1949. A manual of the Penicillia. Williams and Wilkins Company, Baltimore.
16. **Rohlf, F., J. Kishpaugh, and D. Kirk,** 1993. NTSYS numerical taxonomy system of multivariate statistical prog-

rams. State University of New York, Stony Brook.

17. **Samson, R.A., A.C. Stolk, and R. Hadlok,** 1976. Revision of the subsection Fasciculata of *Penicillium* and some allied species. *Stud. Mycol. Barrn* **11**, 1-47.
18. **Samson, R.A., and W. Gams,** 1984. The taxonomic situation in the hyphomycete genera *Penicillium, Aspergillus* and *Fusarium. Antonie van Leeuwenhoek* **50**, 815-824.
19. **Stolk, A.C., R.A. Samson, J.C. Frisvad, and O. Filtenborg,** 1990. The systematics of the terverticillate Penicillia, p. 121-137. *In* Samson, A.R. and J.I. Pitt (eds.), Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, New York and London.
20. **Westling, R.,** 1911. Ünber die grünen Spezies der Gattung *Penicillium. Arkiv fur Botanik.* **11**, 1-156.
21. **Yoon, C.-S. and D.A. Glawe,** 1993. Association of random amplified polymorphic DNA markers with stromatal type in *Hypoxylon truncatum sensu* Miller. *Mycologia* **85**, 369-380.
22. **Yoon, C.-S., and D.A. Glawe, and P.D. Shaw,** 1991. A method for rapid small-scale preparation of fungal DNA. *Mycologia* **83**, 835-838.