An Evaluation of Some Physiological and Biochemical Methods as an Aid to the Characterization of Species of *Penicillium* Subsection *Fasciculata*

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Physiological and biochemical test methods have been evaluated for use in the taxonomy of filamentous microfungi, with specific reference to isolates of *Penicillium* subsection *Fasciculata*. Physiological and enzymic tests such as growth in the presence of inhibitory substances, pH limits of growth and the hydrolysis of a variety of substrates proved to be relatively easy to perform and showed promise as differential characters. Carbon and nitrogen source assimilations were technically difficult to perform and few substrates gave differential results. In the few cases where assimilation tests were successful they often involved substrates that may otherwise be inhibitory, such as organic acids and sodium nitrite. Results from assimilation tests appeared to be differential at around species level and positive results were often associated with a significant rise in growth medium pH. Many of the tests described have not previously been applied to filamentous fungi and may have applications in the study of other genera.

INTRODUCTION

The use of biochemical characters in microbial taxonomy is well established, particularly in bacteria (Cowan, 1974; Wilson & Miles, 1975; Buchanan & Gibbons, 1974) and yeasts (Barnett *et al.*, 1983; Kreger-van Rij, 1984). In the taxonomy of the filamentous microfungi, the presence of an abundance of diverse morphological features in most groups has obviated the need to consider biochemical characters (Hawksworth, 1974). In other groups, the lack of suitable methods has prevented their inclusion in taxonomic schemes. However, it has become apparent that fungal classifications based almost exclusively on morphological features may not always result in well defined taxa. This situation is particularly complicated in the fasciculate penicillia (Raper & Thom, 1949; Pitt, 1979; Frisvad, 1981; Onions *et al.*, 1984), where confusion also results from variation in the nomenclatural and species concepts adopted by different authors (see Onions *et al.*, 1984).

Attempts have been made to introduce physiological and biochemical characters into the taxonomy of *Penicillium*, a small number of such characters having been incorporated into schemes primarily based on morphology (Thom, 1910; Abe, 1956; Pitt, 1973). Moreover, hydrocarbon assimilation and specific enzyme activities have been investigated for use as taxonomic characters (Nyns *et al.*, 1968; Bridge & Hawksworth, 1984). The number and types of secondary metabolites produced have also been used to divide the species *Penicillium viridicatum* into subgroups (Ciegler *et al.*, 1973, 1981), and this approach has been extended by the inclusion of a small number of physiological tests to differentiate a number of species within the genus (Frisvad, 1981; Frisvad & Filtenborg, 1983). While these approaches have all achieved some level of separation of species, they have not always achieved either the same separations or shown high levels of agreement with schemes based largely on morphology (Pitt, 1979; Onions *et al.*, 1984).

As part of a major multidisciplinary approach to the taxonomy of critical areas within the genus *Penicillium*, a range of physiological and biochemical tests that may be of value in future studies of this kind have been evaluated and the results of the study are presented here.

Table 1. *Isolates of Penicillium spp. studied*

Study number	IMI number	Name as received*	Equivalents and comments†
PP1	91944	<i>P. frequentans</i>	LSHB P.28; ex Neotype of <i>P. glabrum</i>
PP2	28619	<i>P. expansum</i>	NCTC 5612
PP3	89372	<i>P. cyclopium</i>	CBS 144.45; LSHB P.123; NRRL 1888; ATCC 8731; QM 6839
PP4	280215	<i>P. cyclopium</i>	
PP5	169087a	<i>P. viridicatum</i>	
PP6	39758ii	<i>P. viridicatum</i>	FRR 963; NRRL 963; QM 7683; CBS 390.48; ATCC 10515; IFO 7736; Thom (5740.2); ex Neotype
PP7	173209	<i>P. viridicatum</i>	
PP8	37767	<i>P. chrysogenum</i>	ATCC 10002; UV mutant of IMI 26211
PP9	280297	<i>P. citrinum</i>	
PP10	17456	<i>P. brevicompactum</i>	QM 8406; ATCC 16024
PP11	90178	<i>P. purpurogenum</i>	
PP12	91924	<i>P. camembertii</i>	LSHB Ad.34
PP13	193019	<i>P. funiculosum</i>	FRR 1630; ex Neotype
PP14	68414	<i>P. corymbiferum</i>	CBS 502.75
PP16	285522	<i>P. viridicatum</i> II	W1018
PP19	285525	<i>P. griseofulvum</i>	W1021
PP20	285526	<i>P. concentricum</i> I	W1022
PP22	285507	<i>P. camembertii</i> II	W1002
PP25	285515	<i>P. aurantiogriseum</i> II	W1011
PP27	285508	<i>P. echinulatum</i>	W1003
PP29	285512	<i>P. hirsutum</i> III	W1008
PP30	285528	<i>P. claviforme</i>	W1025
PP33	285510	<i>P. crustosum</i>	W1005
PP34	285518	<i>P. roquefortii</i>	W1014
PP35	285509	<i>P. mali</i>	W1004
PP37	39752	<i>P. atramentosum</i>	Thom (38); Biourge 161; Thom 4733.3; NRRL 795; ATCC 10404; QM 7483; CBS 291.48; IFO 8137; ex Lectotype
PP38	284408	<i>P. viridicatum</i> I	NRRL 3586
PP41	284411	<i>P. viridicatum</i> III	NRRL 3711
PP48	209783	<i>P. crustosum</i>	
PP50	207082	<i>P. patulum</i>	

* Due to the existing confusion in the names of *Penicillium* species, the names given here are those designated by the source collections, and as such they may not be directly comparable.

† LSHB, Biochemistry Department, London School of Hygiene and Tropical Medicine, London, UK; NCTC, National Collection of Type Cultures, London, UK; CBS, Centraalbureau voor Schimmelcultures, Baarn, Netherlands; NRRL, Northern Utilization Research Branch, USDA, Peoria, Illinois, USA; QM, US Army Natick Laboratories, Natick, Massachusetts, USA; FRR, Division of Food Research, Food Research Laboratory, CSIRO, Sydney, Australia; IFO, Institute for Fermentation, Osaka, Japan; IMI, Commonwealth Mycological Institute, Kew, Surrey, UK; W, J. Frisvad, Technical University of Denmark, Lyngby, Denmark.

METHODS

Isolates studied. The full list of *Penicillium* isolates studied is given in Table 1. The isolates were divided into two sets; PP1 to 14 represent most of the fasciculate penicillia and some other dissimilar species of the genus. These isolates were used for all the tests described. Those tests that showed differential results with these 14 isolates were then evaluated with a second set of 16 isolates, PP16 to 50, that represent an expansion of the fasciculate penicillia and also include some isolates that may be close neighbours to this subsection. Isolates were checked for purity on receipt and were maintained on slopes of Czapek Dox (Cz) agar (Smith & Onions, 1983) at -20°C .

Growth conditions. All cultures were incubated aerobically at 25°C on Cz agar slopes unless otherwise stated.

Preparation of inocula. Each strain to be tested was grown for 7 d on Cz agar slopes. Spores were collected by washing from the slope with 0.2% (v/v) aqueous Tween 80. Spores were washed twice in the Tween solution by centrifugation and were resuspended to give a final turbidity equal to tube no. 6 on the McFarland scale (Lennette *et al.*, 1980). This is equivalent to approximately 2×10^6 spores ml^{-1} for the fasciculate penicillia (Bridge & Hawksworth, 1984). Inoculation from the spore suspensions was done using a straight wire to give a single point inoculation unless otherwise stated.

Reproducibility. All tests were repeated after a time interval of at least 3 weeks. Conflicting results were repeated again, where necessary, to check their consistency.

Growth in the presence of inhibitors. Cultures were grown on Cz agar containing filter sterilized solutions (final concentration in parenthesis) of: formalin (0.32 ml l⁻¹); sodium azide (0.1 g l⁻¹); zinc sulphate (3 and 0.2 g l⁻¹); crystal violet (0.05 and 0.01 g l⁻¹); copper sulphate (1 and 0.5 g l⁻¹); stannous chloride (5, 2 and 0.8 g l⁻¹); methylene blue (2 and 0.8 g l⁻¹); sodium selenite (0.32 g l⁻¹); cobalt nitrate (0.5 g l⁻¹) and phenol (0.01 and 0.008 g l⁻¹).

Growth at different temperatures. The ability to grow at 4 °C, 37 °C and 40 °C within 14 d was tested on Cz agar.

Growth at different pH values. The ability to grow at pH 2, pH 10 and pH 12 was tested in liquid medium containing 0.05 g bromocresol purple l⁻¹. For pH 2 the media was supplemented with 10.5 g citric acid l⁻¹ and adjusted with 1 M-HCl. For pH 10 and pH 12 the KH₂PO₄ in the medium was replaced by 3.75 g glycine l⁻¹ and 0.2 g K₂HPO₄ l⁻¹ and adjusted with 1 M-NaOH.

Temperature resistance of spores. This was tested by inoculating one drop from a pasteur pipette into 0.5 ml 0.2% (v/v) Tween solution held at 75 °C in a water bath. After 5 min the suspensions were spread on Cz agar plates and incubated for 7 d.

Assimilation of carbon sources. The ability to assimilate various carbons sources was investigated on three media: Cz agar without sucrose; medium B (LB; Lynch *et al.*, 1981); and nitrate-phosphate medium (NP) which contained NaNO₃ (3 g l⁻¹), K₂HPO₄.3H₂O (1.3 g l⁻¹) and mineral supplement (10 ml l⁻¹; Frisvad, 1981). Solid media contained Oxoid no. 3 agar (12 g l⁻¹); liquid media contained bromocresol purple (0.05 g l⁻¹); all media were adjusted to pH 4.5 with 1 M-HCl or 1 M-NaOH before autoclaving and had a final pH of 4.2–4.8. In solid media, carbon sources (30 g l⁻¹) were lactose, sucrose, soluble starch and ammonium oxalate; in liquid media, carbon sources (10 g l⁻¹) were glucose, lactose, galactose, xylose, mannitol, ammonium tartrate, ammonium oxalate, malic acid, lactic acid, citric acid and tannic acid, all autoclaved at 110 °C for 15 min, and dulcitol, ethanol and ascorbic acid, which were added as filter sterilized solutions. A positive result was the formation of a colony on solid media or a hyphal mat in liquid media within 14 d.

Assimilation of nitrogen sources. The ability to assimilate various nitrogen sources was investigated on three media: Cz agar without NaNO₃; medium A (LA; Lynch *et al.*, 1981); and nitrite sucrose medium (NS; Frisvad, 1981) without NaNO₂. Solid media contained Oxoid no. 3 agar (12 g l⁻¹); liquid media contained bromocresol purple (0.5 g l⁻¹); all media were adjusted to pH 4.5 with 1 M-HCl or NaOH before autoclaving and had a final pH of 4.2–4.8. In solid media, nitrogen sources (3 g l⁻¹) were ammonium oxalate and sodium nitrite; in liquid media nitrogen sources (2 g l⁻¹) were sodium nitrite, ammonium oxalate, creatin and glycine, all autoclaved at 110 °C for 15 min, and urea and thiourea, which were added as filter sterilized solutions. A positive result was the formation of a colony on solid media or a hyphal mat in liquid media within 14 d.

All assimilation tests were compared with positive and negative controls. Negative controls consisted of the basal medium with either no carbon or no nitrogen source. Positive controls contained 30 g sucrose l⁻¹ for the solid test media, 10 g glucose l⁻¹ for the liquid test media and 2.5 g sodium nitrate l⁻¹ for the nitrogen tests. Agar for the solid control media were Oxoid no. 3, Oxoid Ionagar or washed Oxoid no. 3 agar.

Water testing. The concentration of free ammonia in the water used for making culture media was examined using the method of Da Fonseca-Wollheim (1973).

Enzyme activities. Aesculin hydrolysis was tested on Cz agar, with the sucrose content reduced to 5 g l⁻¹ and with the addition of 3 g aesculin l⁻¹ and 0.2 g ferric citrate l⁻¹. Blackening of the colony and the surrounding medium denoted a positive reaction.

Starch hydrolysis was tested on Cz agar with the sucrose replaced by 10 g soluble starch l⁻¹. A positive reaction was indicated by the appearance of a clear zone around the colony after flooding with Gram's iodine solution (Cowan, 1974).

Tween 80 hydrolysis was tested using a method based on that of Sierra (1957). This utilized two components, a 10% (v/v) aqueous Tween 80 solution and a basal medium containing mycological peptone (10 g l⁻¹; Oxoid), NaCl (5 g l⁻¹), CaCl₂.2H₂O (0.1 g l⁻¹), agar (15 g l⁻¹; Oxoid no. 3) and bromocresol purple (0.05 g l⁻¹). The basal medium was adjusted to pH 5.6 with 1 M-NaOH and the two components were autoclaved separately. The basal medium and the Tween solution were cooled and mixed in the proportion 9:1 and poured into petri dishes. A positive result was the appearance of white crystals in the medium and a rise in pH.

Cellulose hydrolysis was tested on LB agar containing 250 ml 4% (w/v) suspension of ball-milled cellulose l⁻¹ (Eggs & Pugh, 1962). A positive result was a clear zone around a streak inoculation within 21 d.

Hydrolysis of polypectate was tested at pH 6 and pH 8 on a modified LB medium containing 6 ml 10% (w/v) CaCl₂.2H₂O l⁻¹ and 0.05 g bromothymol blue l⁻¹. The medium was adjusted to pH 6 or pH 8 with 1 M-NaOH after the addition of 35 g polygalacturonic acid l⁻¹ (Koch Light Labs). A positive result was the liquefaction of the gel.

RNA hydrolysis and phosphatase activity were tested using the methods of Gochenaur (1984) with LB agar as the basal media.

Casein hydrolysis was tested on LA agar containing 10 g glucose l⁻¹ and 25 ml 15% (w/v) skim milk l⁻¹ (Difco) and adjusted to pH 5.6. A positive result was a clear zone around a streak inoculation after flooding with 10% (w/v) HgCl₂.

Table 2. *Physiological reactions of Penicillium isolates*

Isolate number	Temperature resistance	Growth at:				Growth with:*						
		4 °C	37 °C	40 °C	pH 2	Nas	Naa	For	Cvt		Cus	Zns
									0.05	0.01	0.5	3
PP1	+	+	—	—	—	+	—	+	—	—	+	+
PP2	+	+	—	—	+	+	+	—	—	—	—	—
PP3	—	+	—	—	—	+	—	—	—	+	—	—
PP4	+	+	—	—	—	+	—	+	—	+	—	+
PP5	+	+	—	—	+	+	—	+	—	—	+	—
PP6	—	+	—	—	—	+	—	+	—	—	—	—
PP7	+	+	—	—	—	+	—	—	—	—	—	—
PP8	+	—	+	+	—	+	+	+	—	—	+	+
PP9	+	—	+	+	—	+	+	—	+	+	+	+
PP10	+	+	—	—	+	+	+	+	—	+	+	+
PP11	+	—	+	+	+	+	—	+	—	+	+	+
PP12	—	+	+	—	—	—	+	+	—	—	—	—
PP13	—	—	+	+	+	+	+	+	—	+	—	+
PP14	—	+	—	—	+	+	—	+	+	+	—	—
PP16	+	+	—	—	—	+	—	—	+	+	—	+
PP19	+	+	—	—	—	+	—	—	—	+	—	—
PP20	+	+	—	—	—	—	—	—	+	+	—	—
PP22	+	+	—	—	—	+	—	—	—	—	+	+
PP25	+	+	—	—	—	—	—	—	—	+	—	+
PP27	+	+	—	—	—	—	—	—	—	+	+	+
PP29	+	+	—	—	—	+	—	—	+	+	+	—
PP30	+	+	—	—	—	+	+	—	—	+	—	—
PP33	+	+	—	—	+	+	+	—	—	+	+	+
PP34	+	+	—	—	+	+	+	+	—	+	+	+
PP35	+	+	—	—	—	+	+	—	—	—	+	+
PP37	+	+	—	—	—	+	—	—	—	—	+	—
PP38	+	+	—	—	—	—	+	+	—	+	—	+
PP41	—	+	—	—	—	+	—	+	+	+	+	—
PP48	+	+	—	—	—	+	—	—	—	+	—	+
PP50	+	+	+	—	—	+	+	—	—	+	—	+

* Nas, sodium selenite; Naa, sodium azide; For, formalin; Cvt, crystal violet; Cus, copper sulphate; Zns, zinc sulphate. Final concentrations of Cvt, Cus and Zns are given in g l⁻¹.

Gelatin hydrolysis was tested as the ability to liquefy Cz medium solidified with 120 g gelatin l⁻¹. This test was chilled for 1 h at 4 °C after incubation before reading.

Reduction of tetrazolium was tested on Cz agar containing 0.064 g tetrazolium salt l⁻¹ (BDH). A positive result was the appearance of a red colour in the colony.

Reduction of tellurite was tested on Cz agar containing 0.032 g potassium tellurite l⁻¹. A positive result was the blackening of the colony.

RESULTS

Physiological tests. The isolates PP1 to 14 grew at pH 10 and in the presence of lead acetate, cobalt nitrate, phenol (0.008 g l⁻¹), methylene blue (2 and 0.8 g l⁻¹), zinc sulphate (0.2 g l⁻¹) and stannous chloride (2 and 0.8 g l⁻¹). Conversely no isolates grew at pH 12, with phenol (0.01 g l⁻¹), copper sulphate (1 g l⁻¹) or stannous chloride (5 g l⁻¹). The results for the 12 tests that gave differential results are given in Table 2 together with the results for these tests with the second set of isolates.

Assimilation of carbon and nitrogen sources. Attempts to perform assimilation tests on solid media failed to give any differential results with the first set of isolates, all of which showed traces of growth on all control plates. Substitution of the agar with either washed agar or Ionagar and the substitution of the distilled water, which contained 10 mg NH₄⁺ l⁻¹, with distilled-deionised water, which also contained 10 mg NH₄⁺ l⁻¹, did not significantly affect these results.

Table 3. *Assimilation reactions for Penicillium isolates*

Isolate number	Growth on carbon sources				Growth on nitrogen sources		
	Ammonium tartrate	Ammonium oxalate	Citric acid	Tannic acid	Sodium nitrite	Creatin	Ammonium oxalate
PP1	—	+	—	+	—	—	+
PP2	+	+	+	+	—	+	+
PP3	+	—	—	—	—	+	+
PP4	+	—	—	—	—	+	+
PP5	+	+	+	—	—	+	+
PP6	+	—	+	—	+	+	+
PP7	+	+	+	+	—	+	+
PP8	+	—	+	+	—	+	+
PP9	+	+	—	+	+	+	+
PP10	+	+	+	+	+	+	+
PP11	+	—	+	+	+	+	—
PP12	+	+	—	—	+	+	—
PP13	+	—	+	+	—	+	+
PP14	+	—	+	—	+	+	+
PP16	+	—	+	—	—	—	+
PP19	+	—	—	—	—	+	+
PP20	+	+	+	—	+	+	+
PP22	+	—	+	+	—	+	+
PP25	+	+	—	+	—	+	+
PP27	+	—	+	—	—	+	+
PP29	+	—	+	+	—	+	+
PP30	+	+	—	—	—	+	+
PP33	+	—	+	+	+	+	+
PP34	+	—	+	+	+	+	+
PP35	+	—	+	+	+	+	+
PP37	—	—	+	—	+	+	+
PP38	+	+	+	+	—	+	+
PP41	+	—	+	—	—	+	+
PP48	+	+	+	—	+	+	+
PP50	+	+	+	—	+	—	+

The problem was minimized by the use of liquid media. It was subsequently found that significant levels of ammonia ($160 \text{ mg NH}_4^+ \text{ l}^{-1}$) were introduced during autoclaving when using tap water in the autoclave. Liquid media were therefore prepared in a stainless steel autoclave which was cleaned before each use, and distilled water was used in the autoclave boiler. This significantly reduced the amount of trace growth in N-assimilation tests. The isolates PP1 to 14 were tested in liquid media with the 19 carbon and nitrogen sources described. All 14 isolates assimilated glucose, galactose, xylose, mannose, dulcitol, ethanol, ascorbic acid, malic acid, lactic acid, urea and glycine. No isolates were able to assimilate thiourea. The results for the seven differential tests are shown in Table 3, together with the results for the second set of isolates. In total, seven carbon and nitrogen sources resulted in a differential rise in pH sufficient to turn the bromocresol purple indicator bright purple, equivalent to approximately pH 8.5 (Table 4).

Enzyme activities. The results for strains PP1 to 14 with nine of the 12 enzymic tests are given in Table 5, together with the results for the second set of isolates. Three tests (starch hydrolysis, aesculin hydrolysis and phosphatase activity) gave positive results with all of the first set of strains, and were not used for the second set of strains.

DISCUSSION

Physiological tests have been used previously as an aid to *Penicillium* taxonomy (Pitt, 1973, 1979), and the inhibitory effects of a variety of chemicals have been used in industrial applications (de Tilley *et al.*, 1983). In agreement with Pitt (1973, 1979), the results presented

Table 4. *Increase in pH during assimilation tests with Penicillium isolates*

Isolate number	Carbon sources			Nitrogen sources			
	Ammonium tartrate	Citric acid	Lactic acid	Ammonium oxalate	Creatin	Urea	Glycine
PP1	—	—	+	—	—	—	—
PP2	+	+	—	—	—	+	—
PP3	—	—	+	—	—	+	—
PP4	+	—	+	—	—	+	—
PP5	+	—	+	—	—	+	—
PP6	—	+	—	—	—	—	—
PP7	—	+	+	—	—	+	—
PP8	+	+	—	—	—	+	+
PP9	+	—	—	+	+	+	—
PP10	+	—	—	+	—	+	+
PP11	+	—	—	—	—	—	—
PP12	+	—	—	—	—	—	—
PP13	+	+	—	—	—	+	—
PP14	+	+	—	—	—	+	—
PP16	+	—	+	—	—	+	+
PP19	—	—	—	—	—	+	+
PP20	+	—	—	—	—	—	—
PP22	+	—	+	—	—	+	+
PP25	—	—	—	—	—	+	—
PP27	+	+	+	—	—	+	+
PP29	+	+	—	—	—	—	+
PP30	+	—	—	—	—	+	—
PP33	+	—	—	+	—	+	+
PP34	+	—	—	—	+	+	—
PP35	+	—	—	—	—	+	—
PP37	—	+	—	—	—	+	—
PP38	—	+	—	—	—	—	—
PP41	—	—	+	—	—	+	+
PP48	+	—	—	—	—	+	—
PP50	—	—	+	—	—	+	—

+, Increase in pH to approximately pH 8.5 or above.

here show that growth temperatures are of little use in differentiating species within the subsection *Fasciculata*. Although species of *Penicillium* have been reported from extremely acid environments (Sinclair & Herring, 1975), these results indicate that the ability to grow in conditions of high or low pH may be limited to a relatively small number of species. As a result the differential value of this test would be fairly limited.

Resistance to inhibitory compounds, particularly metal ions and dyes, has been used as a taxonomic character in a number of studies on bacteria and yeasts (Goodfellow *et al.*, 1982; Bridge & May, 1984). Resistance to such compounds in the penicillia has been reported in several ecologically orientated studies (Moubasher & Mazen, 1971; Greenaway *et al.*, 1974). The results presented here suggest that resistance to some compounds may be useful differential characters. There may, however, be complications with this type of test. Resistance to some chemicals is plasmid mediated in bacteria (Harwood, 1980), and the discovery of extra-chromosomal elements in filamentous fungi (Hollings, 1982; Hashiba *et al.*, 1984) means that an analogous situation may exist. Should such resistance be unstable or transferable the complications could be considerable.

A major problem experienced with assimilation tests in this study was growth on negative controls, a phenomenon reported in a number of studies with fungi (Dickinson & Boardman, 1970; Dabinett & Wellman, 1978; Lynch *et al.*, 1981). There are at least four separate factors that may account for this: (i) free sugars in agar-solidified acid media due to partial hydrolysis of the agar; (ii) invasion and accumulation of airborne organic materials (Geller, 1983); (iii)

Table 5. Enzyme activities of *Penicillium* isolates

Isolate number	Hydrolysis of :						Reduction of :	
	Tween 80	Cellulose	Pectin pH 6	Pectin pH 8	Gelatin	Casein	RNA	Tetrazolium Tellurite
PP1	—	—	+	+	+	—	—	—
PP2	+	—	+	+	—	+	—	+
PP3	+	—	+	—	—	+	+	+
PP4	+	—	+	+	+	—	+	+
PP5	+	—	+	—	—	+	+	+
PP6	+	—	+	—	+	—	+	—
PP7	+	—	+	+	+	—	+	—
PP8	+	—	—	—	+	—	+	+
PP9	+	—	+	—	+	+	+	+
PP10	+	—	+	—	+	+	+	+
PP11	—	+	—	—	+	—	—	—
PP12	+	—	+	—	—	+	—	+
PP13	—	+	+	—	+	—	—	—
PP14	+	—	+	+	+	+	+	+
PP16	+	—	+	—	—	+	—	+
PP19	+	—	—	—	+	+	+	+
PP20	+	+	+	—	+	+	—	+
PP22	+	—	+	—	—	+	+	+
PP25	+	—	+	+	+	+	—	+
PP27	+	+	+	—	+	+	+	+
PP29	+	—	+	—	+	+	+	+
PP30	+	—	+	—	+	+	—	+
PP33	+	+	—	—	+	+	+	+
PP34	—	—	—	—	+	+	—	+
PP35	+	+	+	—	+	+	+	+
PP37	+	—	+	+	+	—	+	+
PP38	+	—	—	+	+	—	—	—
PP41	+	—	+	+	+	+	+	+
PP48	+	+	+	+	+	—	+	—
PP50	+	+	+	+	+	+	+	+

quality of water and other ingredients; (iv) storage materials within spores allowing some degree of hyphal extension after germination. In this study least growth occurred in liquid media and was always limited to a small hyphal pellet at the bottom of culture bottles after 10 d. As a positive result required the production of a hyphal mat on the surface of the liquid this did not affect the reading of the tests.

During the nitrogen assimilation tests variations in the extent of trace growth were noticed between different batches of media. This was found to be due to an increase in nitrogen content during autoclaving, but this increase only occurred when tap water was used in the autoclave, and the effect was minimized by the use of deionised or distilled water in the boiler. This indicates that there was some carry over of nitrogen from the steam to the media. Further work is necessary to investigate the phenomenon fully and to ascertain its possible implications in other biochemical tests.

The large increases in pH that were observed during growth on some carbon and nitrogen sources are of note and may have one of a number of causes. Where ammonium salts, creatin, glycine and urea were used the pH rise may be due to the accumulation of ammonia in the medium. There are a number of possibilities that could account for the pH rise with the organic acids. The organisms may be particularly prone to autolysis under these conditions and release ammonium compounds on autolysis. The basal medium for these tests utilized ammonium hydrogen phosphate and so a depletion of phosphate during the later growth stages could result in an increase in pH from the remaining free ammonia. Thom (1910) observed a similar occurrence using litmus in carbon and nitrogen assimilation tests and pointed out that the pH rise was dependent on the carbon and nitrogen sources used. Frisvad (1981) used the delayed rise

in pH on a creatin medium as a differential test for some fasciculate penicillia. In neither of these cases was a definite explanation given for the pH rise. The phenomenon may be of use in future taxonomic studies, although further work is necessary to determine the exact mechanisms operating.

The enzymic activity tests gave more encouraging results in respect of differentiating *Penicillium* isolates. All cultures tested showed some level of hydrolytic activity and only three (PP1, PP38 and PP48) showed no reducing ability. The effects of readily available carbon and nitrogen sources on enzymic activities *in vitro* has been reported previously (Gochenaur, 1984) and in this study the media were designed so that the substrate for the enzymes was usually the sole source of nitrogen or carbon in the medium. Some substrates may be degraded by one of a number of different enzymes, e.g. cellulose and polypectate. In tests such as these it is difficult to know which enzyme is responsible for the final result. The specificity of these tests may be increased by control of the pH of medium (Coutts & Smith, 1976; Gochenaur, 1984). With the exception of polypectate, all enzymic test media were used at pH 5-6 (see Gochenaur, 1984). Polypectate was used at both pH 6 and pH 8 to observe the effects of polygalacturonase and pectate lyase respectively.

These results show that physiological and biochemical tests may be used to obtain differential features for isolates of *Penicillium* subsection *Fasciculata*. The results indicate that such tests can contribute to species level separations.

The tests evaluated here are representative of the types of tests that have proved so valuable in other branches of microbiology. These methods, or extensions of them, can therefore be expected to make significant contributions to the taxonomy of filamentous fungi, particularly where existing entirely morphologically-based schemes prove to be unsatisfactory.

The results obtained by physiological and biochemical tests are both reproducible and objective and so may be used in general identification schemes; the tests may also be of value in monitoring or screening for specific attributes.

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