Special Topics

Penicillium solitum Revived, and its Role as a Pathogen of Pomaceous Fruit

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ABSTRACT

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Penicillium solitum, a species neglected in recent taxonomies, is revived. A new description and related taxonomic information are given, based on examination of a number of fresh isolates from pome fruit and wooden fruit bin surfaces in Australia and from processed meats in Germany. Isolates of *P. solitum* were less virulent on apple and pear fruits than those of *P. expansum,* the dominant pathogenic *Penicillium* on pome fruits. *P. solitum* and *P. expansum* showed similar temperature growth curves, but growth of *P. solitum* was slower. All isolates of *P. solitum* from fruit and fruit storage bins in this study were insensitive to benomyl, but isolates from meat and cheese were sensitive to benomyl.

In his revision of the genus *Penicillium,* Pitt (7) placed *P. solitum* Westling in synonymy with *P. aurantiogriseum* Dierckx. Available authentic cultures of *P. solitum* had been maintained in culture for many years and had deteriorated to the point where features distinguishing this species from *P. aurantiogriseum* were not apparent. Recently, a number of fresh isolates were collected that failed to fit the description of *P. aurantiogriseum* but were consistent with assignment to *P. solitum.* One group of isolates was examined by the first author at the Federal Meat Research Laboratory, in Kulmbach, Germany, in 1985, and another by the second and third authors at the Institute of Plant Sciences, Knoxfield, Victoria, Australia, in 1987.

Confirmation that *P. solitum* was the correct name for these isolates came from electrophoretic studies of certain isoenzymes (2). The studies included a culture type of *P. solitum.* In consequence, the name *P. solitum* is revived here. A new description is given, based on approximately 20 recent isolates, together with some additional taxonomic information.

Isolates examined in Victoria were obtained during screening tests for Penicillia pathogenic on apples and pears, which showed that *P. solitum* is a pathogen of pome fruit. Studies on the pathogenicity of these isolates and several from meat and cheese, together with temperature relations and sensitivity to benomyl, are described below.

MATERIALS AND METHODS

Sources of isolates. Isolates in West Germany were examined during a major study of the *Penicillium* culture collection at the

Federal Meat Research Laboratory, Kulmbach. They had been isolated over a period of 15 or more years by Dr. L. Leistner and his co-workers from that institute. Nearly all came from processed meat products, and most were isolated in Europe.

Australian isolates were collected in September 1984 and from January through March 1987 at several locations in Victoria. Samples were taken from the surfaces of empty wooden fruit bins, dump tank water, and decaying pear fruits. Samples were diluted with sterile distilled water, then plated on acidifed potato-dextrose agar as described previously (10).

Taxonomy. The taxonomic methodology of Pitt (7) was followed. Cultures were incubated for 7 days on Czapek yeast extract agar (CYA) at 5, 25, and 37 C, and on malt extract agar (MEA) and 25% glycerol nitrate agar (G25N) at 25 C. Identifications were based on colony diameters, colony colors and texture, and microscopic characters and measurements. Capitalized names of colors in the description are taken from the Methuen Handbook of Colour (5).

Electrophoresis of pectic enzymes. The extracellular enzyme polygalacturonase (probably EC 3.2.1.15 with endo action, but not excluding EC 3.2.1.67 with exo action) of several isolates of *P. solitum* was examined by polyacrylamide gel electrophoresis by the method of Cruickshank and Pitt (2). Isolates included were FRR 2575, 3185, 3249, 3416, 3418, 3419, 3420, 3440, and 937 (ex type of *P. solitum*). Cultures were grown on citrus pectin liquid medium at 22 C for 7 days. Wells in the gel were filled with 5 μl from a mixture of 50 μl of culture fluid with 2.5 mg of Sephadex G-150 superfine. Electrophoresis was performed in 10.25% acrylamide gels containing 0.1% low methoxyl citrus pectin at 4 C with constant current and initial potential difference of 5.5 V per cm across the gel. After electrophoresis, gels were incubated for 1 h at room temperature in 0.1 M malic acid, stained overnight in 0.01% ruthenium red solution at 4 C, then washed for 1 h in distilled water. Gels were treated for 30 min in 0.1% ammonium persulphate to increase contrast in photograms.

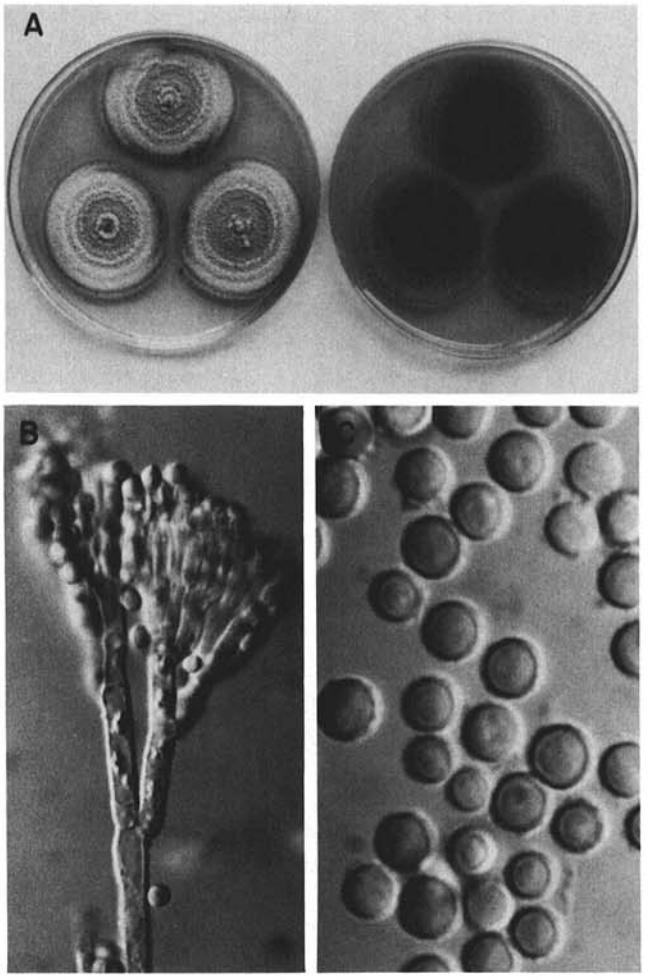

Fig. 1. *Penicillium solitum:* **A,** Colonies on Czapek yeast extract agar (left) and malt extract agar (right) after 7 days at 25 C; **B,** penicillus, ×750; **C,** conidia, ×1,875.

Pathogenicity tests. Mature apple fruits (cultivars Granny Smith, Golden Delicious, and Starking Red Delicious) and pear fruits (cv. Packham's Triumph) were surface-sterilized with 95% ethanol, then puncture-wounded (6 mm diameter, 5 mm deep) at a single location on each fruit. Six Australian isolates of *P. solitum* from fruit or fruit bins and three of *P. expansum* (4-1A, apple storage bin; Pen E, fungicide drench water; AP3, pear fruit), as a reference pathogen, were cultured on MEA. After 6 days, 5-mm-diameter disks were removed from the margins of the colonies and inserted, mycelium inward, into the wounds with a sterile spatula. Six fruits of each cultivar were inoculated with each *Penicillium* isolate. Control fruits were inoculated with sterile disks of MEA. Inoculated fruits were placed in polyethylene bags and incubated at 20 ± 2 C for 7 days. Lesion diameters were measured and recorded as radial measurements from the edge of the wounds.

Pathogenicity to apples and pear of six isolates of *P. solitum* from meat and cheese was evaluated as above. *P. expansum* (Pen E) from fungicide drench water and *P. solitum* (FRR 3418) from a fruit bin were included as reference pathogens.

Effect of temperature on growth. The effect of temperature was assessed on the growth of the six isolates of *P. solitum* from fruit or fruit bins and three reference isolates of *P. expansum* mentioned previously. Five-millimeter-diameter disks were cut from marginal areas of each culture and placed at the center of plates of MEA. Inoculated plates were sealed in polyethylene bags and incubated at 5 C intervals from 5 to 30 C, and at 37 C, all maintained ±0.5 C. Three replicate plates were used at each temperature. Colony radii from the edge of the colonies to the edge of the inoculum were measured after 7 days of incubation.

Sensitivity to benomyl. Isolates were those used in the above tests, i.e., six of *P. solitum* from fruit or fruit bins and three of *P. expansum*. In addition, three isolates of *P. solitum* from meat and one from cheese were evaluated for benomyl sensitivity. Conidia were washed from 6-day-old cultures with sterile distilled water, and the total conidial count adjusted to 4×10^5 conidia per milliliter with the aid of a hemacytometer. MEA plates were inoculated with 100 μl of conidial suspension spread over the surface of each plate. Disks of Whatman No. 1 filter paper, 5 mm in diameter, were soaked in benomyl suspension (100 μg benomyl per milliliter of water), and three disks placed on each MEA plate. Plates were incubated at 20 ± 2 C and zones of inhibition measured after 3 days.

RESULTS AND DISCUSSION

The new description and related taxonomic information for *P. solitum* (Fig. 1) is as follows:

Penicillium solitum Westling
Arkiv fur Botanik 11: 52, 1911
Penicillium psittacinum Thom 1930
Penicillium casei var. *compactum* Abe 1956 (*nom. inval.*)
Penicillium verrucosum var. *melanochlorum* Samson et al 1976
= *Penicillium melanochlorum* (Samson et al) Frisvad 1985
Penicillium mali Novobranova 1972 (*nom. inval.*)
Penicillium mali Gorlenko & Novobranova 1983 (*nom. inval.*)

Colonies on CYA at 25 C were 22–28 mm in diameter, usually lightly radially sulcate, less commonly plicate or plane; low to moderately deep, very dense, with surface texture velutinous or less commonly granular or fasciculate; mycelium white, usually visible only at the margins, narrow in velutinous isolates, wider in those showing fasciculation; conidia usually produced abundantly, dark bluish green or Dark Green (25-26E-F4-7); exudate usually absent or inconspicuous, occasionally abundant or even dominating colony appearance, clear; soluble pigment

absent; reverse usually pale or uncolored, uncommonly yellow or brown or with the light salmon to orange pigmentation characteristic of colonies on MEA.

Colonies on MEA were 20–28 mm in diameter, plane, low with velutinous to distinctly granular texture, or less commonly floccose; mycelium white or occasionally yellow, inconspicuous in the presence of uniform conidial production, in uncommon isolates visible over much of the colony surface; conidia abundant in most isolates, colored as on CYA, rarely confined to central areas or absent; exudate and soluble pigment absent; reverse usually characteristically colored Greyish Orange to Brownish Orange (5-6B-C4-6)

Colonies on G25N were usually 16–22 mm in diameter, closely resembling colonies on CYA; conidia dark green; exudate and soluble pigment absent; reverse pale to pale yellow.

At 5 C on CYA, microcolonies or colonies up to 4 mm in diameter produced. No growth on CYA at 37 C.

Conidiophores borne singly or in definite fascicles, usually from subsurface hyphae, stipes commonly 150–250 × 3–5 μm, but of indeterminate length when in fascicles, with walls smooth to finely roughened or, rarely, rough; penicilli predominantly terverticillate, but biverticillate or quaterverticillate types present in appreciable numbers in some isolates; rami usually one per penicillus, occasionally more, (8–) 12–15 (–18) × 3–4 (–5) μm, sometimes rough walled; metulae in verticils of 3 to 5, 9–15 (–20) × 3–4 (–5) μm, cylindrical or sometimes slightly enlarged; phialides 5 to 8 per metula, ampulliform but tending to acerose or cylindroidal in some isolates, commonly 9–10 (–12) × 3–3.5 μm, with short collula; conidia spherical to subspheroidal, uncommonly broadly ellipsoidal, 3–4 (–4.5) μm diameter or in length, with walls smooth to very finely roughened, borne in disordered chains.

Distinctive features. Dark bluish green to green conidial colors are the most useful features distinguishing *P. solitum* from other closely related species: *P. aurantiogriseum* produces blue conidia on CYA, *P. commune,* grey blue to grey green, and *P. verrucosum,* yellow green. On MEA, the greyish to brownish orange reverse is also characteristic of the species.

Fig. 2. Electrophoresis of extracellular pectic enzymes of isolates of *Penicillium solitum* in 10.25% acrylamide gel containing 0.1% pectin, stained with ruthenium red. Culture fluids of isolates applied to each lane were as follows: 1, blank; 2, FRR 3416; 3, FRR 3418; 4, FRR 3419; 5, FRR 3420; 6, FRR 3440; 7, FRR 937 (ex type of *P. solitum*).

Taxonomy. Based on examination of available, poor quality cultures, Pitt (7) reduced *P. solitum* to synonymy with *P. aurantiogriseum.* However, morphological differences found in examination of newer isolates from meats and fruit and distinctive isoenzyme patterns (2) formed the basis for subsequent revival of this species (8).

Variation. Typical isolates are dense, velutinous with narrow, white margins, and without exudate on CYA. Variations seen include granular or fasciculate texture and copious exudate production. On MEA, weak conidial production and floccose texture are seen occasionally.

Affinities. *P. solitum* appears to be closely related to *P. aurantiogriseum, P. commune,* and *P. verrucosum.* It has been mistaken for each of these species in recent literature (7,11).

Habitats. Due to confusion over its identity, *P. solitum* has frequently been overlooked. It appears to be common on European processed meats (L. Leistner and J. I. Pitt, *unpublished*). Recently, it has been isolated frequently from decaying apples and pears and apple bins in Victoria, Australia, and from orchard soil and packinghouse dump tank water in Oregon.

Isolates examined. FRR 937 (NRRL 937), ex type of *P. solitum,* from an unrecorded source, R. Westling; FRR 957 (NRRL 957), from rotting pear fruit, Pullman, WA, 1939, F. D. Heald; FRR 2575, from rotting apple fruit, Sydney, N.S.W., Australia, 1983, J. Walker; FRR 3174, from country cured ham, MO, about 1966, L. Leistner; FRR 3175, from experimental country cured ham, Ames, IA, about 1966, L. Leistner; FRR 3176, from country cured ham, Harper, KY, about 1966, L. Leistner; FRR 3177, from rohwurst, Valencia, Spain; FRR 3182, from Italian salami, Citterio, Italy; FRR 3185 (Sp 210), from Italian salami, San Francisco, CA, about 1966, L. Leistner; FRR 3249, from

TABLE 1. Virulence of *Penicillium solitum* isolates from fruit and fruit storage bins compared with that of *P. expansum* on apple and pear fruit

		Average radius of lesion (mm) for cultivar			
Species	Isolate	Granny Smith	Golden Delicious	Starking	Packham's Triumph
P. solitum	FRR 3416	1.0	2.0	1.1	2.2
	JB1	0.9	2.8	2.8	1.9
	FRR 3440	2.1	2.7	1.7	2.8
	FRR 3420	1.3	1.9	0.9	2.1
	FRR 3419	1.3	1.1	0.5	1.8
	FRR 3418	1.1	4.1	0.4	1.2
Average		1.3	2.4	1.2	2.0
P. expansum	4-1A	11.6	13.3	3.7	5.6
	Pen E	12.1	9.7	4.9	6.8
	AP3	11.6	12.1	4.5	5.6
Average		11.8	11.7	4.4	6.0
LSD ($P < 0.05$)[a]		0.7	2.2	1.4	1.0

[a] Least significant difference.

TABLE 2. Virulence of *Penicillium solitum* isolates from meat and cheese on apple and pear fruit

			Average radius of lesion (mm) for cultivar			
Species	Isolate	Source	Granny Smith	Golden Delicious	Starking	Packham's Triumph
P. solitum	FRR 3174	ham	2.0	3.6	4.5	3.3
	FRR 3175	ham	2.2	2.0	4.0	3.6
	FRR 3177	rohwurst	1.2	2.7	2.7	1.5
	FRR 3182	salami	1.3	4.9	4.3	2.3
	FRR 3185	salami	0.3	3.5	3.7	2.1
	FRR 3249	cheese	1.7	1.7	2.2	2.2
	FRR 3418	fruit bin	5.3	0.8	3.7	2.3
Average			2.0	2.7	3.6	2.5
P. expansum	Pen E	drench water	15.1	15.5	12.5	10.1
LSD ($P < 0.05$)[a]			0.6	2.2	1.8	1.6

[a] Least significant difference.

refrigerated cheese, Hobart, Tas., Australia, 1986, R. H. Cruickshank; FRR 3416 (Spotts 2-3A), from apple storage bin, Gladysdale, Vic., Australia, 1987, R A. Spotts and R. J. Holmes; FRR 3417 (Spotts 9-11C), from apple storage bin, Harcourt, Vic., Australia, 1987, R. A. Spotts and R. J. Holmes; FRR 3418 (Spotts 8-10A), from pear storage bin, Harcourt, Vic., Australia, 1987, R. A. Spotts and R. J. Holmes; FRR 3419 (Spotts 6-16A) from pear storage bin, Gladysdale, Vic., Australia, 1987, R. A. Spotts and R. J. Holmes; FRR 3420 (Spotts 3-8A), from pear storage bin, Gladysdale, Vic., Australia, 1987, R. A. Spotts and R. J. Holmes; FRR 3440 (Spotts 32T8), from apple storage bin, Gladysdale, Vic., Australia, 1987, R. A. Spotts and R. J. Homes; FRR 3487, from rotting apple fruit, Brisbane, Qld., Australia, 1987, G. I. Johnson.

Electrophoresis of pectin enzymes. The pectic zymograms of the five isolates examines herein all had a banding pattern characteristic of the ex type *P. solitum* FRR 937, with two major isozymes of polygalacturonase and no pectinesterase (Fig. 2). Other isolates listed in the previous section that were examined by electrophoresis and that had a banding pattern characteristic of *P. solitum* included FRR 2575 from apple fruit, FRR 3185 from Italian salami, and FRR 3249 from cheese (zymograms not shown). These results give added support to the identity of the isolates as *P. solitum* and are in agreement with their phenotypic characteristics. Excellent agreement exists between species of terverticillate Penicillia recognized by Pitt (7) and groupings according to pectic zymograms (2).

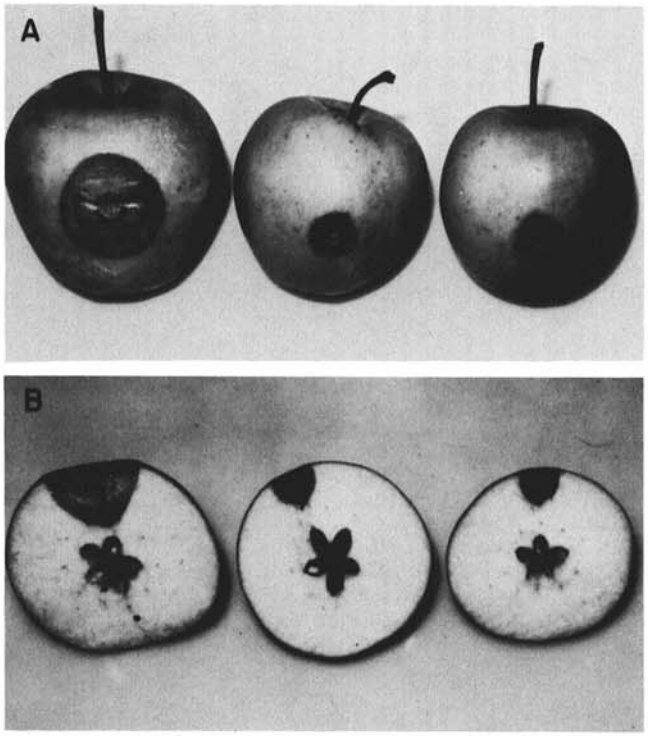

Fig. 3. Lesions formed in apple fruit (cv. Golden Delicious): **A,** intact fruit; **B,** cut fruit. The left fruit was inoculated with *Penicillium expansum* and the other two with *P. solitum* in each case.

TABLE 3. Colony radii of *Penicillium solitum* and *P. expansum* after 7 days growth at various temperatures

Species	Isolate	Temperature (C)						
		5	10	15	20	25	30	37
P. solitum	FRR 3416	3.0	7.8	13.1	13.7	14.0	0.7	0
	JB1	2.8	10.4	12.9	16.1	16.7	2.4	0
	FRR 3440	3.5	9.5	13.7	15.5	15.7	0	0
	FRR 3420	3.0	8.7	12.9	17.5	16.9	0.5	0
	FRR 3419	2.5	7.9	13.1	15.7	15.3	0.6	0
	FRR 3418	2.2	7.5	10.7	13.0	13.7	1.4	0
Average		2.8	8.6	12.7	15.3	15.4	0.9	0
P. expansum	4-1A	4.1	11.7	18.5	24.0	24.0	1.5	0
	Pen E	3.9	15.0	17.1	19.5	24.5	1.7	0
	AP3	4.3	10.3	16.5	22.0	25.1	2.0	0
Average		4.1	12.3	17.4	21.8	24.5	1.7	0
LSD ($P < 0.05$)[a]		0.7	2.7	1.7	3.1	1.9	1.2	...

[a] Least significant difference.

Decay incidence. In a recent study, *P. solitum* was the most frequently isolated pathogenic *Penicillium* from apples and pears in Victoria (3). Of 322 pathogenic isolates, 145 (45%) were *P. solitum,* 75 (23%) were *P. expansum,* 43 (13%) *P. commune,* and 31 (10%) *P. aurantiogriseum* (3).

Pathogenicity. *P. solitum* isolates from fruit or fruit storage bins were significantly less virulent ($P = 0.05$) on all host cultivars than those of *P. expansum* (Table 1). The six isolates of *P. solitum* from meat and cheese were pathogenic on fruit of all apple and pear cultivars (Table 2). Virulence of these isolates was similar to that of an isolate of *P. solitum* from a fruit bin (FRR 3418) but was significantly less ($P = 0.05$) than that of *P. expansum* (Table 2). Both epidermal and flesh color of the decay caused by *P. solitum* were a darker brown than that due to *P. expansum* (Fig. 3). Twelve percent of the control fruit developed decay, primarily from contamination by the species used in the experiment.

Effect of temperature on growth. The influence of temperature on growth of *P. solitum* and *P. expansum* over the range 5–37 C is shown in Table 3. Both species showed similar growth curves, but growth of *P. expansum* was faster at all temperatures. The optimum temperature for growth of *P. expansum* was 25 C, and for *P. solitum,* between 20 and 25 C. The maximum temperature for growth for each species was slightly above 30 C. Results for the various isolates of each species showed good agreement. The growth data for *P. expansum* were similar to a previously published curve (1). These are the first published temperature growth data for *P. solitum.*

Sensitivity to benomyl. All isolates of *P. solitum* and *P. expansum* from fruit and fruit storage bins were insensitive to benomyl and formed no zones of inhibition around the disks treated with benomyl. However, all isolates of *P. solitum* from meat or cheese were sensitive to benomyl; radii of zones of inhibition ranged from 17 to 21 mm.

Insensitivity of *P. expansum* to benomyl was reported previously in Australia (3,4,12) and the United States (6,9). Insensitive isolates were pathogenic to cherry (6), apple (3,4,9,11), and pear (4,11,12) fruits.

This is the first reported evaluation of *P. solitum* for sensitivity to benomyl, and most isolates we tested were insensitive. This appears to be a general phenomenon. Of 145 isolates of *P. solitum* collected from decayed apple and pear fruits in Victoria, only 12 (8%) were sensitive to 250 mg of benomyl per liter in the growth medium (3). Of eight isolates of *P. solitum* recently recovered from packinghouse dump tank water in Hood River, OR, six were insensitive to benomyl (R. A. Spotts, *unpublished*). All Victorian and Hood River isolates of *P. solitum* were collected from environments where benomyl had been used. Sensitivity to benomyl may be a characteristic of the wild type because meat and cheese isolates of *P. solitum* collected from benomyl-free environments were sensitive to benomyl.

Although *P. solitum* is ubiquitous in fruit packinghouses, it is a weak pathogen compared with *P. expansum.* In addition, our pathogenicity tests were conducted at 20 C, but fruit are stored commercially at 5 to −1 C. Thus, additional research is necessary to determine the significance of *P. solitum* as a postharvest pathogen of pome fruit under commercial conditions in the United States.

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