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Antigenic Characterization of *Penicillium camemberti* and Related Common Cheese Contaminants

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Twenty-four isolates of *Penicillium* (including a green-spored mutant from a French Brie cheese, *Penicillium camemberti*) with a proposed relationship to the white cheese mold *P. camemberti* were investigated by immunological procedures. These penicillia, which are representative of species that have caused considerable taxonomic confusion, had common micromorphology (terverticillate penicilli with rough and smooth stipes and smooth ellipsoidal to subglobose [(3 to 5) × $2^{1/2}$ to $4^{1/2}$ μm] conidia); growth rates; good growth on creatine sucrose agar, cheese, and other products with a high amount of protein and lipid as a primary habitat; production (with the exception of *Penicillium solitum*) of cyclopiazonic acid; and the ability to grow at low temperatures and water activities. The isolates that were investigated proved to be strictly antigenically related. Absorbed antiserum of the green-spored mutant of *P. camemberti* showed a specific precipitin band when tested by immunodiffusion either with its homologous reference antigen or with the exoantigens obtained from different isolates. The precipitin band was not present in any *P. camemberti* starter culture but in many unwanted cheese contaminants. The precipitin band can be used in the purity control of *P. camemberti* starter culture spore preparations. Analysis of the exoantigens of all the cultures by reversed phase high-performance liquid chromatography allowed us to subdivide these penicillia into nine groups below the species level. The results indicate that *P. commune* Thom is the wild-type ancestor of *P. camemberti*.

In 1910 C. Thom (21) introduced the taxon *Penicillium commune* for the most common cheese contaminant in the United States. This taxon was accepted by Samson et al. (19) and cited as an uncommon mold, but it was reduced to the synonym of *P. puberulum* Bainier by Pitt (14). These two groups of taxonomists agreed on the synonymy of the important white cheese starter cultures *P. caseicolum* Bainier and *P. camemberti* (14, 18), but they disagreed on the placement of *P. biforme* Thom. The latter was placed in *P. verrucosum* Dierckx var. *cyclopium* (Westling) Samson, Stolk et Hadlok by Samson et al. (19), and Samson (17) proposed that *P. verrucosum* var. *cyclopium* might be the ancestor of *P. camemberti*. Pitt (14) did not indicate an ancestor for *P. camemberti*, but cited a strain from spoiled cheddar cheese (FRR 2160) as a wild-type strain of this species. *P. verrucosum* var. *cyclopium* embraces three distinct groups of fungi, however: (i) *P. crustosum*, a fruit-rotting fungus that produces penitrems, roquefortines, isofumigaclavines, terrestric acid, and viridicatin; (ii) *P. aurantiogriseum*, *P. cyclopium*, and *P. viridicatum*, which are closely related cereal strains that produce penicillic acid, puberulic acid, viridicatin, xanthomegnins, S-toxin, brevianamides, penitrems, and terrestric acid; and (iii) the group of interest here, *P. commune*, *P. palitans*, *P. solitum*, and *P. camemberti*, which are closely related taxa from cheese, meat, and nuts producing the amino acid-related secondary metabolites cyclopiazonic acid, rugulovasines, viridicatin, and the polyketides cyclopaldic acid and palitantin (7). Because these taxa appear nearly alike under a light microscope, other classical taxonomical criteria, including colony texture and conidial color en masse, have been overemphasized, leading to understandable taxonomic confusion and many misidentifications (see Table 1). The cereal strains are easily recognized by their poor growth on creatine-sucrose agar (5) and their yellow obverse and reverse on YES agar (4). *P. crustosum* is recognizable by its ability to rot pomaceous fruits, its high growth rates on solid media, and the production of enormous amounts of conidia on most substrates (6). We wanted to evaluate whether immunological procedures might give an insight into the intricate taxonomical problems in the very important groups of these penicillia (15). Two other important cheese taxa, *P. roqueforti* Thom and *P. verrucosum* Dierckx, were not taken into consideration because they are quite unrelated to the fungi mentioned above and are quite distinctive.

MATERIALS AND METHODS

Cultures. The fungal strains used in this study are listed in Table 1. They represented *P. camemberti* (for synonyms, see references 14 and 18); *P. commune* [= *P. lanosoviride* Thom = *P. ochraceum* Bainier var. *macrosporum* Thom = *P. fuscoglaucum* Biourge = *P. australicum* Sopp ex van Beyma = *P. cyclopium* var. *album* G. Smith = *P. verrucosum* var. *album* (G. Smith) Samson, Stolk, et Hadlok = *P. album* (G. Smith) Stolk et Samson]; *P. palitans* Westling (= *P. majusculum* Westling, *P. flavoglaucum* Biourge = *P. psittacium* Thom = *P. roqueforti* var. *punctatum* Abe); and *P. solitum* Westling [= *P. casei* var. *compactum* Abe = *P. mali* Novobranova = *P. mali* Gorlenko et Novobranova = *P. verrucosum* var. *melanochlorum* Samson, Stolk, et Hadlok = *P. melanochlorum* (Samson, Stolk, et Hadlok) Frisvad]. The strain XX102, which is also known as strains

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TABLE 1. *Penicillium* isolates investigated and their sources and mycotoxin production

Species	Former name	Strain[a]	Source[b]	Mycotoxin(s) produced
P. camemberti	*P. camemberti*	IMI 285506[c]	Brie, DK	Cyclopiazonic acid
	P. camemberti	PD	Brie, F	Cyclopiazonic acid
	P. camemberti	BB	Brie, DK	Cyclopiazonic acid
	P. camemberti	DK	Camembert, DK	Cyclopiazonic acid
	P. camemberti	Grøn Alpe	Contaminated cream cheese, DK	Cyclopiazonic acid
P. commune	*P. camemberti* II	XX102 (IMI 293178)[c]	Brie, F	Cyclopiazonic acid, rugulovasine A, cyclopaldic acid
	P. biforme	NRRL 6497	Canned pear, US	Cyclopiazonic acid, rugulovasine A, cyclopaldic acid
	P. camemberti	FRR 2140	Cheddar cheese, NZ	Cyclopiazonic acid, rugulovasine A, cyclopaldic acid
	P. viridicatum	Quint. 1397	Acorn, E	Cyclopiazonic acid, rugulovasine A, cyclopaldic acid
	P. camemberti II	MRA 55	Early garden turnips, DK	Cyclopiazonic acid, rugulovasine A, cyclopaldic acid
	P. camemberti II	XOO6	Cheese, DK	Cyclopiazonic acid, rugulovasine A, cyclopaldic acid
	P. camemberti II	IMI 285507[c]	Liquorice root, T	Cyclopiazonic acid, rugulovasine A, cyclopaldic acid
P. palitans	*P. palitans*	NRRL 2033	?, N	Cyclopiazonic acid, palitantin, frequentin
	P. camemberti III	IMI 293210	Cheese, DK	Cyclopiazonic acid, palitantin
	P. camemberti III	AMAS 4	House air, DK	Cyclopiazonic acid, palitantin
	P. palitans	MAYO 12	Gravy, DK	Viridicatin, palitantin
P. solitum	*P. mali*	CBS 500.73	Apple, R	Viridicatin (weak)
	P. palitans	NRRL 914	Pear, US	Viridicatin
	P. melanochlorum	JOSC 2	Soil, S	Viridicatin
	P. melanochlorum	FTLS 193	Apple, DK	Viridicatin
	P. melanochlorum	Æble LME_1	Apple, DK	Viridicatin
	P. melanochlorum	G 1, 2 (FRR 3121)	Baker yeast, DK	Viridicatin
	P. melanochlorum	OF13	Paper, DK	Viridicatin
	P. melanochlorum	BE 9, 1	Salami, DK	Viridicatin

[a] Abbreviations: IMI, Commonwealth Mycological Institute, Kew, Great Britain; FRR, Commonwealth Scientific and Industrial Research Organisation, Division of Food Research, Sydney, Australia; NRRL, Northern Regional Research Laboratory, Peoria, Ill.; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; Quint., J. A. Quintanilla, Companias de Agricolas, Valladolid, Spain; all other strains are from the Department of Biotechnology, Technical University of Denmark, Lyngby, Denmark.

[b] Abbreviations: DK, Denmark; F, France; NZ, New Zealand; N, Norway; S, Sweden; E, Spain; R, Russia; T, Thailand; US, United States.

[c] Reference strain.

IMI 296938 and FRR 3112, was obtained from a culture of *P. camemberti* isolated from a French brie cheese. This sector in the white *P. camemberti* culture (IMI 293178, which is the same as ATCC 58609) was only recognizable by its green conidia. The culture is listed as *P. commune* because of its good conidium production and the full profile of secondary metabolites (cyclopiazonic acid, cyclopaldic acid, rugulosavine A) known from the wild-type species.

Mycotoxin production. All strains were examined for production of cyclopiazonic acid, viridicatin, cyclopenin, and cyclopaldic acid on CYA agar, YES agar, or both by the methods described by Frisvad and Filtenborg (8).

Taxonomy. The isolates were characterized by the methods described by Frisvad (5, 6) into *P. camemberti* (*P. camemberti* chemotype I), *P. commune* (*P. camemberti* chemotype II), *P. palitans* (*P. camemberti* chemotype III), and *P. solitum* (*P. mali*) based on differences in conidiation and color on YES agar, conidium color, conidium hydrophobicity (*P. solitum* has hydrophilic conidia), and production of profiles of secondary metabolites.

Control antigen. The procedure used for producing the reference antigens was that recommended by the Centers for Disease Control, Atlanta, Ga., for producing antigens for diagnosing aspergillosis by a microdiffusion test (13). Briefly, the control antigens were obtained from acetone-treated Sabouraud dextrose broth cultures of *P. camemberti* IMI 285506, *P. commune* (the *P. camemberti* green-spored mutant) IMI 296938 (XX102), and *P. commune* IMI 285507. For evaluating optimum activity, the solubilized antigen was adjusted by concentration or dilution with sterile distilled water to contain 1,500 or 4,000 μg of carbohydrates per ml before testing.

Control antisera. The production of reference antisera was carried out by immunizing albino rabbits. The animals were injected subcutaneously with the reference antigens (4,000 μg/ml) in Freund adjuvant on the first 3 days and intravenously at 1 and 2 weeks after the first inoculation. The rabbits were bled from the heart 7 days after the last intravenous injection.

Exoantigen production. Cell-free antigens (exoantigens) of the 24 *Penicillium* isolates were produced by the technique described by Kaufman and Standard (10) for the serological identification of *Coccidioides immitis* and *Histoplasma capsulatum* cultures. Briefly, cultures showing luxuriant growth on Sabouraud dextrose agar (10 days) were covered with 8 ml of an aqueous solution of Merthiolate (thimerosal;

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1:5,000) and kept for 24 h at 25°C. After this period, 5 ml of the extract was concentrated 50 times by lyophilization.

Serological tests. An agar gel double diffusion (ID) test was used for serological tests. The medium and the methodology used were those recommended by the Centers for Disease Control for the serological diagnosis of histoplasmosis by immunodiffusion (13). The exoantigen (1,500 μg/ml) was tested by using the reference antigen and antiserum as controls.

Comparison of the antigenic pattern of each isolate was carried out by using a crossed immunoelectrophoresis technique with intermediate gel (CIEIG) (1). Briefly, each reference antigen (1,500 μg/ml) was first subjected to an electric field in an agarose gel. After the different antigenic determinants migrated, the gel was removed and applied to the bottom of another slide, on which a middle and a top gel were present. In the middle layer, NaCl or heterologous serum diffused, while the homologous serum diffused in the top layer. When an orthogonal electric field was applied, the system with NaCl in the middle layer developed the antigenic pattern of the antigen-antiserum reference system being investigated. The other system, with heterologous serum in the middle gel, revealed the common antigenic determinants that react with the heterologous antiserum and the specific determinants that react with the homologous serum.

Absorption of antisera. Each reference antiserum sample (0.5 ml) was absorbed by adding 0.5 ml of each heterologous reference antigen (1,500 μg/ml) separately and was allowed to stand overnight at 4°C. The antigen-antiserum mixtures were then centrifuged at 12,063 × *g* for 10 min. The supernatants were decanted, and their initial volumes were restored with concentrators (Minicon Macrosolute B15; Amicon Corp., Lexington, Mass.). The absorption procedure was repeated, and the supernatants were tested by immunodiffusion for evidence of absorption.

Reversed phase high-performance liquid chromatography. All common reagents used for reversed phase high-performance liquid chromatography (RPHPLC) were analytical grade. The Acetonitril was Chroma sol grade purchased from Hoechst-Roussel Pharmaceuticals Inc. (Somerville, N.J.); trifluoroacetic acid (TFA) was obtained from Sigma Chemical Co. (St. Louis, Mo.); and the high-performance liquid chromatographic (HPLC) water was double distilled and was filtered (pore size, 0.45 μm; GA6 Metricel membrane filter; Gelman Sciences, Inc., Ann Arbor, Mich.) prior to use. A total of 5 ml of each lyophilized exoantigen was dissolved in 0.10 ml of the solvent A solution (see below) for HPLC; 0.010 ml was then used for each HPLC analysis. The HPLC conditions were as follows. An Aquapore RP300 column (220 by 4.1 mm; Brownlee Labs, Santa Clara, Calif.) and a guard column (30 by 4.1 mm) were used for HPLC. The gradient that was applied was 0% at 0 to 1 min, was increased linearly to 70% at 1 to 18 min, and was held at 70% for 2 min. Solvent A consisted of water-acetonitrile (90:10 vol/vol) with 0.1% (wt/vol) TFA, and solvent B consisted of 100% acetonitrile–0.1% TFA. The UV detector wavelength was 220 nm, and the absorbance scale was 1.280 absorbance units, full scale. The flow rate was set at 1.4 ml/min. A two-pump HPLC apparatus (LC10; The Perkin-Elmer Corp., Norwalk, Conn.) equipped with a spectrophotometric recorder (LC85 UV-Vis) and an autocontrol (LC) connected to a two-channel recorder (LKB Instruments, Inc., Rockville, Md.) was used.

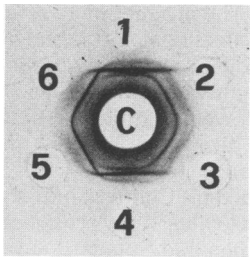

FIG. 1. ID technique determination of *P. camemberti* IMI 285506. Well contents: C, reference antiserum; 1 and 4, reference antigen; 2 and 3, *P. commune* IMI 285507 reference antigen; 5 and 6, *P. commune* (*P. camemberti* mutant) XX102 reference antigen.

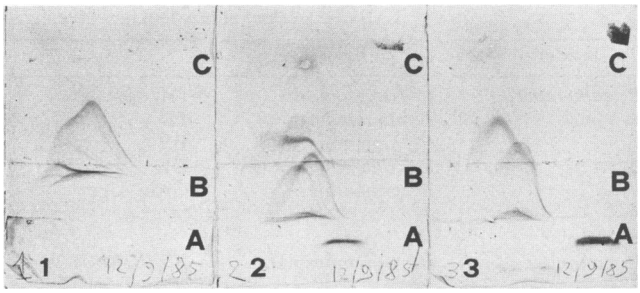

FIG. 2. CIEIG technique determination of *P. camemberti* IMI 285506. Gel contents: A1, A2, and A3, reference antigen; B1, saline; B2, *P. commune* IMI 285507 reference antiserum; B3, *P. commune* (*P. camemberti* mutant) XX102 reference antiserum; C1, C2, and C3, reference antiserum.

RESULTS

Precipitating antibodies were observed in all the serum samples from rabbits that were immunized with the reference antigens. The reference system, represented by each

TABLE 2. Relationships of precipitin bands produced by *P. camemberti* and *P. commune* reference antisera and reference antigens by the immunodiffusion technique

Reference antigen	Reference antiserum precipitin bands for[a]:					
	P. camemberti IMI 285506					
	1	2	3			
P. camemberti IMI 285506	+	+	+			
P. commune IMI 285507	+	±	+			
P. commune XX102	+	+	±			
	P. commune IMI 285507					
	1	2	3	4	5	
P. commune IMI 285507	+	+	+	+	+	
P. camemberti IMI 285506	±	−	+	+	−	
P. commune XX102	+	+	±	+	+	
	P. commune XX102					
	1	2	3	4	5	6
P. commune XX102	+	+	+	+	+	+
P. camemberti IMI 285506	−	−	+	+	−	+
P. commune IMI 285507	±	−	+	+	+	+

[a] Symbols: +, band of identity; ±, band of partial identity; −, no reaction.

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TABLE 3. Relationships of precipitin bands produced by *P. camemberti* and *P. commune* reference antisera and exoantigens by the immunodiffusion technique

Exoantigen	Reference antiserum precipitin bands for[a]:		
	P. camemberti IMI 285506		
	1	2	
P. camemberti IMI 285506	+	+	
P. camemberti Grøn Alpe	+	−	
P. camemberti DK	+	−	
P. camemberti BB	+	+	
P. camemberti PD	+	±	
P. commune FRR 2160	+	−	
P. commune NRRL 6497	+	−	
P. commune 1397	+	−	
P. commune XOO6	+	−	
P. commune MRA 54	+	−	
P. palitans MAYO 12	+	−	
P. palitans IMI 293210	+	±	
P. palitans NRRL 2033	+	±	
P. palitans AMAS 4	+	−	
P. solitum OF13	+	±	
P. solitum G 1, 2	+	−	
P. solitum Æble LME_1	+	−	
P. solitum CBS 500.73	+	±	
P. solitum NRRL 914	+	±	
P. solitum JOSC 2	+	±	
P. solitum FTLS 193	+	−	
P. solitum BE 9, 1	+	−	
	P. commune IMI 285507		
	1	2	3
P. commune IMI 285507	+	+	+
P. camemberti Grøn Alpe	+	±	+
P. camemberti DK	+	−	+
P. camemberti BB	+	±	+
P. camemberti PD	+	−	+
P. commune FRR 2160	+	±	+
P. commune NRRL 6497	+	−	+
P. commune 1397	+	−	+
P. commune XOO6	+	±	+
P. commune MRA 54	+	−	+
P. palitans MAYO 12	+	−	+
P. palitans IMI 293210	+	−	+
P. palitans NRRL 2033	+	−	+
P. palitans AMAS 4	+	−	+
P. solitum OF13	+	±	+
P. solitum G 1, 2	+	−	+
P. solitum Æble LME_1	+	−	+
P. solitum CBS 500.73	+	±	+
P. solitum NRRL 914	+	−	+
P. solitum JOSC 2	+	−	+
P. solitum FTLS 193	+	−	+
P. solitum BE 9, 1	+	±	+
	P. commune XX102		
	1	2	
P. commune XX102	+	+	
P. camemberti Grøn Alpe	+	±	
P. camemberti DK	+	−	
P. camemberti BB	+	−	
P. camemberti PD	+	−	
P. commune FRR 2160	+	+	
P. commune NRRL 6497	+	−	
P. commune 1397	+	−	
P. commune XOO6	+	+	
P. commune MRA 54	−	+	
P. palitans MAYO 12	±	+	
P. palitans IMI 293210	−	+	
P. palitans NRRL 2033	+	−	
P. palitans AMAS 4	±	+	
P. solitum OF13	+	±	
P. solitum G 1, 2	+	−	
P. solitum Æble LME_1	+	−	
P. solitum CBS 500.73	±	+	
P. solitum NRRL 914	±	+	
P. solitum JOSC 2	−	+	
P. solitum FTLS 193	+	±	
P. solitum BE 9, 1	±	+	

[a] Symbols: +, band of identity; ±, band of partial identity; −, no reaction.

reference antigen and homologous antiserum sample, was tested either with the heterologous antigens by ID (Fig. 1) or with heterologous antisera by CIEIG (Fig. 2). The CIEIG technique was used only for the reference systems to better visualize common and specific antigenic determinants, while the ID technique was extended to the exoantigens obtained from all the strains studied. The three reference strains (cheese isolates from Denmark and France and a soil [licorice root] isolate from Thailand) proved to be antigenically related on the basis of numerous cross-reactions when tested reciprocally (Table 2). Strict antigenic relationships were also observed by the reaction of all the exoantigens of the isolates considered in this study with each reference antiserum sample (Table 3). After absorption of each antiserum sample with the heterologous reference antigens, only *P. commune* XX102-absorbed antiserum reacted with its homologous antigen by producing a specific precipitating band (Fig. 3). The absorbed (specific) reference antiserum sample was used for testing the exoantigens obtained from all the isolates investigated. By the exoantigen technique (Fig. 4), 11 different isolates (wild-type isolates of *P. commune*, *P. palitans*, and *P. solitum* but not *P. camemberti*) could be ascribed to *P. commune* XX102 (Table 4).

The exoantigens of all the isolates investigated by the RPHPLC technique (Fig. 5) were grouped into nine different

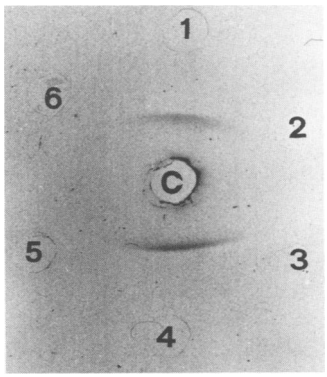

FIG. 3. Specificity of the ID technique for *P. commune* (*P. camemberti* mutant) XX102. Well contents: C, absorbed reference antiserum; 1 and 4, reference antigen; 2 and 3, *P. camemberti* IMI 285506 reference antigen; 5 and 6, *P. commune* IMI 285507 reference antigen.

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classes (Table 5) based on qualitative similarities of the chromatographic peaks, but quantitative differences were not considered. Cyclopiazonic acid producers were placed in classes A, D, E, F, and H, while viridicatin producers were placed in classes B, C, G, and I.

The four taxa distinguished here were closely related and were difficult to separate by classical taxonomic characteristics. *P. camemberti* isolates had a lanose colony texture, a high proportion of irregular penicilli with smooth stipes, rather poor conidium production, and white conidia (in two isolates, they became light grayish green with age); and they only occurred on cheese. All or some of these characteristics have been found in isolates of all the other taxa as well, however (O. Filtenborg and J. C. Frisvad, unpublished observations), especially in cheese isolates. *P. commune* had a lanose or often finely granular colony texture, hydrophobic conidia were produced (in large amounts in most cases) that were a greenish gray to olivaceous gray or occasionally dull green color, conidium production was poor on YES agar, and a brown to black soluble pigment was produced in YES agar. *P. palitans* was characterized by a lanose or, more often, finely granular colony texture; dark green to dull green conidia; hydrophobic conidia produced in moderate amounts; a cadmium yellow reverse; and good conidium production on YES agar. *P. solitum* had a lanose, funiculose, or most often, velutinous colony texture; hydrophilic and very dark green (sometimes with a bluish element, especially on YES agar) conidia; mostly good sporulation on YES agar; and a cadmium yellow to orange obverse and reverse on YES agar. The last three species had a rather high proportion of rough-walled stipes (expecially in good sporulating strains) and occasionally had finely roughened conidia.

The four taxa were also closely related with regard to their production of secondary metabolites (Table 1). Cyclopiazonic acid was produced in all taxa except for *P. solitum* and one strain of *P. palitans*, while viridicatin was produced by *P. solitum* and *P. palitans* MAYO 12. Rugulovasine and cyclopaldic acid were specific for *P. commune*, while palitantin was specific for *P. palitans*. *P. camemberti* was characterized by its consistent production of cyclopiazonic acid and no other secondary metabolites, and *P. solitum* was characterized by its production of few secondary metabolites, while *P. palitans* and especially *P. commune* produced a large number of different secondary metabolites (thin-layer chromatographic results were reported here; HPLC results are to be published by J. C. Frisvad).

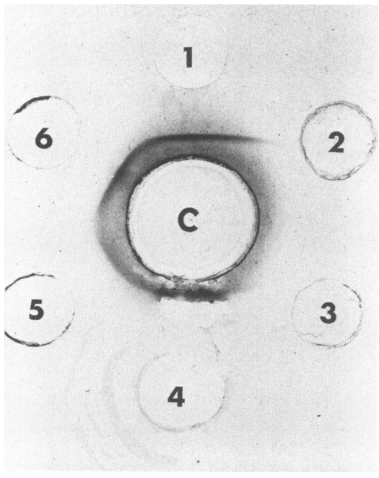

FIG. 4. Specificity of ID exoantigen technique for *P. commune* (*P. camemberti* mutant) XX102. Well contents: C, absorbed antiserum; 1 and 4, reference antigen; 2 and 3, *P. camemberti* PD exoantigen; 5 and 6, *P. commune* FRR 2160 exoantigen.

TABLE 4. Relationships of the precipitin band produced by *P. commune* XX102 absorbed antiserum and exoantigens by the immunodiffusion technique

Exoantigen	Reaction[a]
P. commune XX102	+
P. camemberti IMI 285506	−
P. commune IMI 285507	−
P. camemberti Grøn Alpe	−
P. camemberti DK	−
P. camemberti BB	−
P. camemberti PD	−
P. commune FRR 2160	+
P. commune NRRL 6497	−
P. commune 1397	−
P. commune XOO6	+
P. commune MRA 54	+
P. palitans MAYO 12	+
P. palitans IMI 293210	+
P. palitans NRRL 2033	−
P. palitans AMAS 4	+
P. solitum OF13	−
P. solitum G 1, 2	−
P. solitum Æble LME$_1$	−
P. solitum CBS 500.73	+
P. solitum NRRL 914	+
P. solitum JOSC 2	+
P. solitum FTLS 193	−
P. solitum BE 9, 1	+

[a] Symbols: +, band of identity; −, no reaction.

DISCUSSION

The results obtained in this study show that the isolates that were investigated are strictly antigenically related. By absorption of reference antiserum with the heterologous antigens, it was possible to differentiate the wild-type back mutant from French brie cheese *P. commune* XX102 from *P. camemberti* starter cultures. This is very important, as the wild type produced several secondary metabolites with different pharmacological and toxicological effects (cyclopaldic acid, cyclopolic acid, rugulovasines A and B), which are undesirable in cheese. Two precipitin bands (bands 1 and 2) were produced differently when *P. commune* XX102 reference antiserum reacted with the exoantigens obtained from the *P. camemberti* isolates investigated in this study (Table 3). Precipitinogen 2 proved to be specific for isolates referred to as *P. commune* XX102, as it was the one observed after absorption (Table 4). The more detailed grouping obtained by RPHPLC analysis of the exoantigens correlates perfectly with the ID results. All the exoantigens that reacted with the absorbed *P. commune* XX102-specific antiserum grouped into homogeneous chromatographic classes (classes C, D, E, G, H, and I). Even though the XX102-specific antigen was not present in *P. camemberti*, indicating the possibility of detecting undesirable back mutations in white cheese starter cultures, it was only present in some isolates of the wild-type species (*P. commune*, *P. palitans*, and *P. solitum*). All strains from cheese (FRR 2160, XOO6, IMI 293210, and XX102) contained the XX102 antigen, however.

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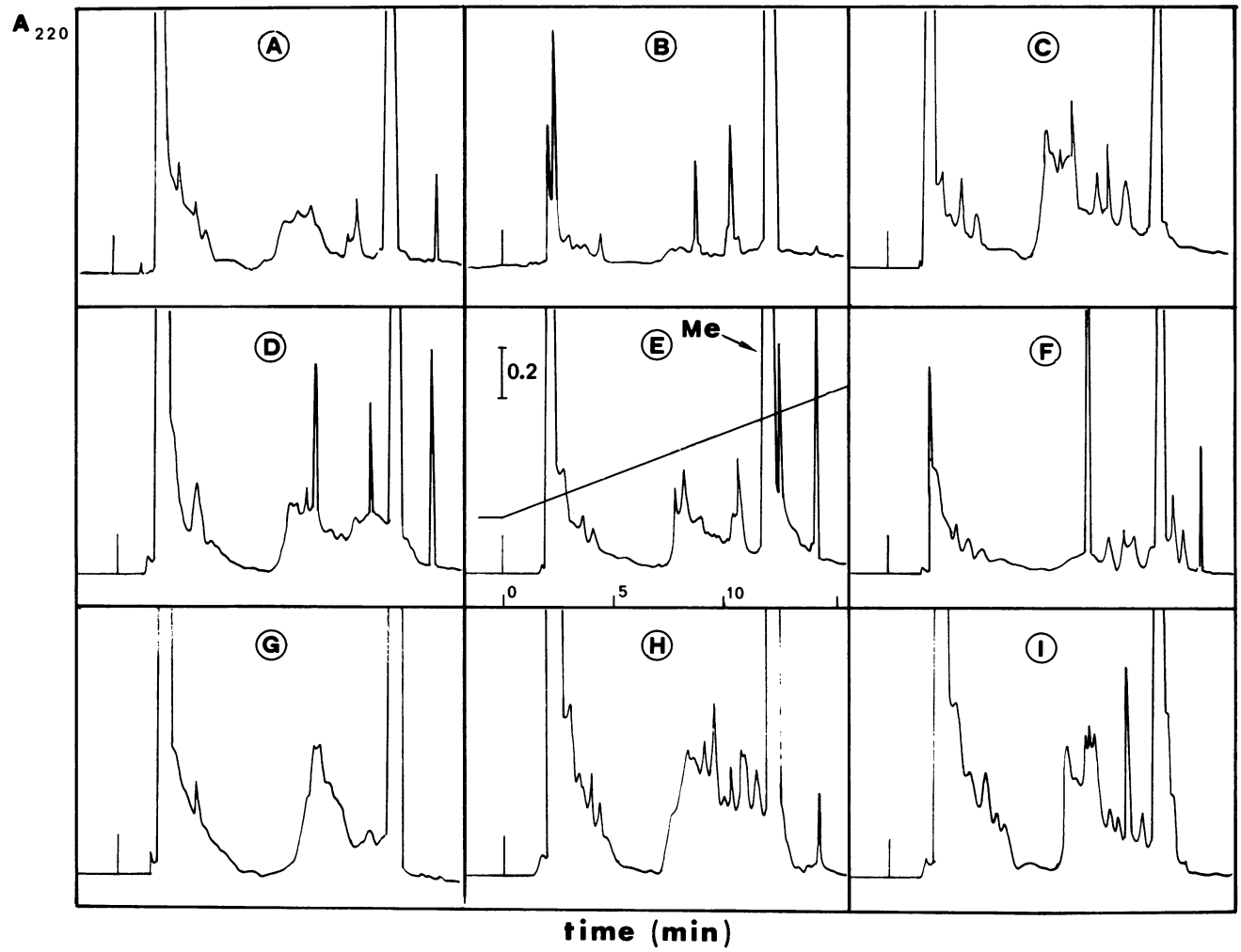

FIG. 5. Reversed phase high-pressure liquid chromatograms of exoantigens within the *P. camemberti, P. commune, P. palitans,* and *P. solitum* complex (see Table 5 for the member strains of each group) subdivided into nine groups, A to I. In panel E, the bar indicates the absorbance unit (0.2); the abscissa indicates the minutes of analysis, and the diagonal line represents the chromatographic gradient applied. Me, Merthiolate.

Even though the isolates treated in this study were collected from widely different sources and geographic regions, they were all antigenically related, indicating that *P. commune* is the wild-type species of the domesticated form of *P. camemberti* and has a close relationship with *P. palitans* and *P. solitum*, as proposed by Frisvad (6). Hermansen et al. (9) included both *P. commune* and *P. palitans* (as *P. camemberti* chemotype II) in one species and an isolate of *P. commune* (*P. terrestre* ATCC 32028) clustered with *P. solitum* (*P. melanochlorum*); they were clearly separated from *P. crustosum* in a pyrolysis gas chromatographic study (20). The cyclopiazonic acid producers in this group of cheese molds are all very closely related and could be regarded as one species. This is not to be recommended, however, as they are significantly different toxicologically. A very similar situation exists in the subgenus *Circumdati* section *Flavi* of the genus *Aspergillus*. Even though *A. flavus* and *A. parasiticus* and their respective domesticated forms *A. oryzae* and *A. sojae* can be regarded as one species based on their high nucleic acid relatedness (72 to 100% DNA-DNA homology) (12), it is important to keep these taxa separated (11): *A. flavus* and *A. parasiticus* are highly toxigenic forms, while *A. oryzae* and *A. sojae* are used in food fermentations. A high serological relatedness among these species was evident from the results of studies of Polonelli et al. (16), but each species had specific exoantigens as well. Klich and Pitt (11) showed that it is indeed possible to differentiate these species by classical morphological criteria. Curiously, the ability to produce cyclopiazonic acid is maintained in the domesticated forms of *A. flavus* and *P. commune* (*A. oryzae* and *P. camemberti*, respectively), while the ability to produce other toxins is apparently completely lost in the domesticated forms.

It is important to differentiate not only species but also individual strains, populations, or very related groups of strains when dealing with fermentation cultures like *P. camemberti* and *P. roqueforti*. Engel and Teuber (3) showed that *P. roqueforti* strains can be differentiated by the thin-layer chromatographic patterns of their secondary metabolites. This is not possible in *P. camemberti*, as cyclopiazonic acid is the only detectable secondary metabolite in that species. The results of this study have shown that *P. camemberti* and *P. commune* strains or groups of related strains can be differentiated by their profile of exoantigens,

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TABLE 5. Grouping of cheese penicillia according to RPHPLC of exoantigens[a]

Group	Species	Strain(s)
A	*P. camemberti*	IMI 285506 Grøn Alpe DK BB PD
	P. commune	XX102 IMI 285507 NRRL 6497
	P. palitans	NRRL 2033
B	*P. solitum*	OF13 G 1, 2 Æble LME_1 FTLS 193
C	*P. solitum*	CBS 500.73 JOSC 2 BE 9, 1
D	*P. commune*	XOO6 MRA 54
	P. palitans	IMI 293210
E	*P. commune*	FR 2160
F	*P. commune*	Quint. 1397
G	*P. palitans*	MAYO 12
H	*P. palitans*	AMAS 4
I	*P. solitum*	NRRL 914

[a] See Fig. 5.

as determined by RPHPLC. Serological methods can be used in the control of conidium starter culture preparations of *P. camemberti* and *P. roqueforti*. *P. commune* and *P. palitans* are the most common contaminants in these conidium preparations (Filtenborg and Frisvad, unpublished observations). Contaminating conidia may be difficult to detect in starter culture conidium preparations, as they occur in small concentrations (2, 22), but methods based on specific antibodies (enzyme-linked immunosorbent assay) could be developed for this purpose, based on the results reported here.

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