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Identification of Fungal Contamination and Determination of Mycotoxigenic Molds by Micellar Electrokinetic Capillary Chromatography in Smoked Paprika

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ABSTRACT

The purpose of this work was to analyze the fungal contamination in smoked and unsmoked paprika processed from different cultivars of pepper and to investigate the ability of these and other mycotoxigenic molds to grow and synthesize mycotoxins in smoked paprika. Eighteen mycotoxins were evaluated using micellar electrokinetic capillary chromatography. No relevant differences were found in fungal contamination between smoked and unsmoked paprika. The number of yeasts obtained was low, ranging from 0.4 to 3.29 log CFU g^{-1}; most of the yeast strains were identified as *Cryptococcus* spp. followed by *Candida* spp. All mold counts were <4 log CFU g^{-1}. *Aspergillus, Cladosporium, Penicillium,* and *Fusarium* were the predominant hyphomycete genera. Six mycotoxins were identified in the extracts of several strains isolated from paprika and incubated on malt extract agar. *Penicillium expansum* followed by *Penicillium citrinum* and *Penicillium raistrickii* were the dominant mycotoxigenic fungi isolated. Most of the mycotoxin-producing fungi produced detectable amounts of mycotoxins when grown on paprika agar.

Smoked paprika is a high-quality product obtained by drying the fruits of autochthonous varieties of pepper (*Capsicum annum* L. and *Capsicum longum* L.) from the region of La Vera in central-west Spain *(20).* Oak logs are used as the heat source to dry this product. The smoke gives this paprika product a flavor preferred for use in the processing of chorizo, a Spanish pork sausage, compared with paprika obtained from sun-dried or hot-air-dried peppers. The drying and storage stages are critical steps because spoilage of the paprika can occur due to the proliferation of molds and yeast. Molds may produce mycotoxins under favorable environmental conditions. Various genera of molds such as *Rhizopus* spp. or *Aspergillus* spp. and yeasts such as *Saccharomyces* spp. have been found in paprika. *Aspergillus flavus* was the dominant fungus isolated from sun-dried mature peppers *(1).* This mold, *Aspergillus parasiticus*, and *Aspergillus nominus* are the main aflatoxin producers. Aflatoxin B_1 is known as a potent liver carcinogen for a wide variety of animals, including humans *(33).* Isolates of *A. flavus* are capable of synthesizing aflatoxins on hot and sweet paprika *(3),* and aflatoxins also have been found in other kinds of paprika *(14, 21, 25, 30).* Both *Penicillium* and *Fusarium* also are known mycotoxigenic fungi found in the human food chain. However, investigations of the incidence of these fungi and their mycotoxins in paprika are scarce. El-Kady et al. *(11)* investigated the presence of ochratoxin A, citrinin, zearalenone, and sterigmatocystin in samples of paprika from Egypt. Sterigmatocystin concentration ranged from 10 to 13 μg/kg.

Various analytical methods such as thin-layer chromatography or high-performance liquid chromatography (HPLC) have been used to detect mycotoxins *(12, 13).* These conventional methods involve an extensive process for extraction of mycotoxin, cleanup for good chromatographic separation, complex gradient mobile phases, and large quantities of organic solvents. Capillary electrophoresis (CE) may be used as an alternative method and offers several advantages over chromatographic techniques, such as more rapid analysis, reduced amount of organic solvents, smaller sample volume, and increased efficiency and resolution *(6, 36).* A particular type of CE, micellar electrokinetic capillary electrophoresis or micellar electrokinetic capillary chromatography (MECC), has been applied to analyze mycotoxins, including aflatoxin, cyclopiazonic acid, ochratoxin, citrinin, penicillic acid, and sterigmatocystin *(5, 16, 17, 26, 27, 34, 35).* MECC operates by adding micelle-forming compounds, such as sodium dodecyl sulfate (SDS), in the run buffer at a concentration higher than the critical micelle concentration *(36, 38).* This method might be useful for detecting mycotoxigenic molds in paprika.

The aim of this work was to analyze the fungal contamination in smoked paprika and investigate the toxigenic potential of the mold strains isolated from this product. The ability of these and other strains from the Spanish Culture Collection (CECT) to grow and synthesize mycotoxins on smoked paprika was also evaluated.

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TABLE 1. *Physical-chemical parameters of the paprika batches studied*[a]

Pepper variety	Fruit form	Moisture (%)	Water activity	pH
Smoked				
Jaranda	Elongated	9.0 A	0.42 AB	5.2 B
Jariza	Elongated	9.4 A	0.48 A	5.0 B
Bola 1	Rounded	9.3 A	0.38 AB	5.1 B
Bola 2	Rounded	10.4 A	0.48 A	5.0 B
Unsmoked				
PapriQueen	Elongated	6.6 B	0.31 B	5.6 A

[a] For a given column, values with different letters are significantly different ($P < 0.05$).

MATERIALS AND METHODS

Sample collection. Pepper varieties from species *C. annum* L. and *C. longum* L. were used in this study. Peppers from three autochthonous varieties of pepper (Jaranda, Jariza, and Bola) were smoked dried for 15 days. We also used sun-dried peppers of the variety PapriQueen from outside the La Vera region. The different varieties of pepper were separated in batches and taken to the processing plant to be powdered. The Bola variety was separated into two batches. The paprika of batch Bola 1 was processed with peduncled fruits. In batch Bola 2, the peduncles were removed from the peppers. Paprika samples were collected from five different processing lines. The samples (approximately 50 g) were placed in plastic bags and kept under refrigeration in dry and dark conditions so that their fungal load would not change during storage. The samples were processed in the laboratory within 1 to 2 days following collection. Five samples of each batch were analyzed. Physical-chemical parameters of the paprika batches were as specified in Table 1.

Yeast and mold isolates and counts. Ten grams of each paprika sample was used for isolation of yeasts and molds. Appropriate dilutions were made in 1% peptone water (Pronadisa, Alcobendas, Madrid, Spain). All of these dilutions were subsequently plated on rose bengal chloramphenicol agar (RBC), dichloran glycerol 18% agar (DG18; Oxoid, Unipath, Basingstoke, UK), and acidified potato dextrose agar (PDA; Scharlab, Barcelona, Spain) at pH 4 adjusted with tartaric acid. All plates were incubated at 25°C for 5 days. Approximately 20% of the yeast and mold colonies were randomly selected from plates with 30 to 300 colonies. The selected colonies were subcultured on the same isolation medium. The yeast isolates were identified by the API 20C yeast identification system (bioMérieux, Marcy l'Etoile, France). The mold colonies were identified to genus using macroscopic and microscopic characteristics *(28, 29, 31).* Strains of mycotoxigenic genera *Aspergillus, Penicillium,* and *Fusarium* were cultured on malt extract agar (MEA; Scharlab) at 25°C for 21 days. These isolates were identified to species level according to their secondary profiles of metabolites analyzed by MECC *(23).*

Mycotoxin extracts. Mold isolates were transferred to MEA and incubated at 25°C for 21 days in the dark. The mycotoxins were extracted from the plates with chloroform *(10).* Residues obtained after evaporating the extract to dryness were redissolved with 0.1 ml water-acetonitrile (1:1) per plate. To determine whether mycotoxins produced on MEA could be produced on paprika, strains with the potential to produce on MEA any of the investigated mycotoxins were plated out on paprika agar (PA; 20 g of sterile paprika, 20 g of agar, and 1 liter of water) incubated at 25°C for 21 days. The following mold strains were used in the study and were acquired from the CECT: *A. flavus* CECT 2687 (aflatoxin and cyclopiazonic acid producing), *A. parasiticus* CECT 2688 (aflatoxin producing), *Penicillium brevicompactum* CECT 2316 (mycophenolic acid producing), and *Penicillium aurantiogriseum* CECT 2264 (penicillic acid producing). These mold species are frequently isolated from spices *(31).* The protocol used for the extraction of the mycotoxins from PA medium was the same as used for the other media.

MECC analysis. Prior to being filtered through a 0.2-μm-pore-size filter, the mycotoxin extracts were analyzed by MECC using an automated PACE 5500 (Beckman Instruments, Palo Alto, Calif.), 75 μm diameter by 57 cm total length (50 cm until window detector), and an uncoated fused silica capillary (Supelco, Tecknocroma, Barcelona, Spain). The capillary was rinsed initially with 100 mM NaOH for 10 min and subsequently with deionized water for 5 min. The capillary column was conditioned after each run by flushing with 100 mM NaOH for 3 min and with deionized water for 3 min. The separation buffer used was 25 mM sodium tetraborate and 50 mM SDS (pH 9) *(34).* Samples were injected by 3-s pressure injection. Electrophoretic separations were performed at 263 V/cm (15 kV) for 30 min and 23°C. The mycotoxins spectra were monitored from 190 to 600 nm with a PACE diode array detector. Mycotoxins were identified based on retention times, retention indices, and UV absorbance spectra. The patterns of the fungal metabolites cyclopiazonic acid, penicillic acid, mycophenolic acid, secalonic acid, aflatoxin B_1, citrinin, sterigmatocystin, fumitremorgin B, griseofulvin, ochratoxin A, patulin, paxillin, penitrem A, roquefortine C, citreoviridin, viridicatumtoxin, deoxynivalenol, and zearalenone (Sigma Chemical Co., St. Louis, Mo.; and Federal Centre for Meat Research, Kulmbach, Germany) were used to confirm the initial identification.

Statistical analysis. Data were analyzed using a one-way analysis of variance. The means were separated by the Tukey honest significant difference test and the Newman-Keuls test using a SPSS software package (version 10.0.6, SPSS Inc., Chicago, Ill.).

RESULTS AND DISCUSSION

Yeast and mold counts. The number of yeasts obtained was low in the four varieties of paprika, ranging from 0.4 log CFU g^{-1} (Bola 1 in medium DG18) to 3.29 log CFU g^{-1} (Jariza in medium RBC) (Table 2). Differences found between physical-chemical parameters of unsmoked and smoked paprika (Table 1) did not seem to influence the yeast counts of the different batches studied. The lowest mean count was obtained in the samples from Bola 1. This result could be explained by the rounded fruit form of the Bola variety, which has a surface/volume ratio lower than that of elongated fruit varieties, or by the fact that fruits in this batch required less manipulation than did the nonpeduncled fruits of the Bola 2 batch.

Only a few studies have been conducted on yeast populations in paprika. Adegoke et al. *(1)* found that *Saccharomyces* spp. was the most abundant yeast on sun-dried *Capsicum* peppers but did not specify the counts. Deak et al. *(8)* found average yeast counts in unspecified spices ranging from 5.30 to 5.67 log CFU g^{-1}.

RBC was significantly better than PDA for recovering yeast (Table 2). Media supplemented with chloramphenicol perform better than media with a reduced water activity or

TABLE 2. *Population (log CFU g^{-1}) of yeast and molds recovered from paprika samples cultured on three media*[a]

Pepper variety	Yeast				Molds			
	DG18	PDA	RBC	Mean	DG18	PDA	RBC	Mean
Smoked paprika								
Jaranda	0.86	1.26	3.04	1.72 AB	0.40	1.88	2.30	1.52 BC
Jariza	1.97	1.26	3.29	2.17 A	2.94	3.39	3.02	3.12 A
Bola 1	0.40	0.51	0.47	0.46 B	0.40	0.86	0.99	0.75 C
Bola 2	2.18	1.77	2.87	2.27 A	1.51	1.89	1.99	1.80 B
Unsmoked paprika								
PapriQueen	3.09	1.92	2.49	2.50 A	2.94	3.50	2.93	3.12 A
Mean	1.70 AB	1.34 B	2.44 A		1.64	2.30	2.25	

[a] DG18, dichloran glycerol 18% agar; PDA, acidified potato dextrose agar; RBC, rose bengal chloramphenicol agar. Means in the same column or same row that are followed by the same letter are not significantly different ($P \leq 0.05$).

pH for enumerating food spoilage yeast *(4).* These conditions represent a wide range of physiological characteristics and test the tolerance of yeasts to extrinsic stress factors, such as drying or smoking.

Mold counts were less than 4 log CFU g^{-1} and were not different with respect to the media used (Table 2). Similar to yeast counts, the lowest mold count was obtained in the samples of Bola 1. PapriQueen and Jariza had the highest counts, about 3 log CFU g^{-1}. Similar mold counts were obtained from samples of sun-dried *C. annum* and *Capsicum frutescens (1).* Red chili powder (from *C. frutescens*) collected from different areas in India had a contamination level of less than 4 log CFU g^{-1} in 67% of the samples analyzed *(2).*

Identification of fungal isolates. From a total of 318 strains, 126 were identified as yeast. No relevant differences were found among the yeast populations isolated from the different batches of paprika. The highest number of yeast isolates were *Cryptococcus* spp. (44.4%) followed by *Candida* spp. (36.5%) in all batches (Table 3). *Saccharomyces* spp. and *Rhodotorula* spp. were also found but at lower percentages (8.7%). Occasionally, isolates from Bola 2 were identified as *Trichosporon* spp. The genus *Cryptococcus* has been reported as the most prevalent yeast in the air and on plant leaves *(31).* The genera *Rhodotorula* and *Trichosporon* also are usually associated with plant material *(19),* indicating that their presence in paprika is the result of their naturally occurrence on fresh fruit. *Saccharomyces* spp. are among the dominant fungi isolated from mature red peppers *(1).*

The yeast species *Cryptococcus laurentii, Cryptococcus albidus,* and *Candida inconspicua* had an isolation frequency greater than 10% (Table 3). In plant material dried as natural infusions, *C. laurentii* was the predominant yeast species *(24).* However, species of *Cryptococcus* and *Candida* also have been isolated from deteriorating fruits such as tomato *(9). C. laurentii* and *Candida humicola* were isolated as spoilage yeasts from mixed lettuce and demonstrated fast and intense growth on mixed bell peppers *(18).*

A total of 192 strains of hyphomycete molds were isolated and identified (Table 4). The majority of the isolates corresponded to the genera *Aspergillus* (22.4%), *Cladospo-*

TABLE 3. *Yeasts isolated from paprika samples*

Yeast	No. of isolates from:					Total	Isolation frequency (%)
	Jariza	Bola 1	Bola 2	Jaranda	PapriQueen		
Cryptococcus	13	7	12	13	11	56	44.4
C. laurentii	7	4	12	7	6	36	28.6
C. albidus	6	3	0	6	5	20	15.9
Candida	14	6	13	8	5	56	36.5
C. inconspicua	2	0	8	5	2	17	13.5
C. colliculosa	4	6	0	0	0	10	7.9
C. famata	8	0	0	0	0	8	6.3
C. glabrata	0	0	0	3	3	6	4.8
C. guilliermondii	0	0	5	0	0	5	4.0
Rhodotorula	3	3	2	3	0	11	8.7
R. glutinis	3	1	2	3	0	9	7.1
R. rubra	0	2	0	0	0	2	1.6
Saccharomyces cerevisiae	1	0	2	4	4	11	8.7
Trichosporon spp.	0	0	2	0	0	2	1.6
Total	31	16	31	28	20	126	

TABLE 4. *Molds isolated from paprika samples*

Mold	No. of isolates from:					Total	Isolation frequency (%)
	Jariza	Bola 1	Bola 2	Jaranda	PapriQueen		
Aspergillus	16	5	5	11	6	43	22.4
A. niger	10	4	3	6	6	29	15.1
A. oryzae	6	1	2	3	0	12	6.3
A. sydowii	0	0	0	2	0	2	1.0
Penicillium	1	11	9	6	6	33	17.2
P. expansum	0	4	8	0	3	15	7.8
P. citrinum	1	3	0	2	0	6	3.1
P. raistrickii	0	0	0	4	1	5	2.6
P. griseofulvum	0	0	0	0	2	2	1.0
Other species	0	4	1	0	0	5	2.6
Fusarium	7	1	4	2	8	22	11.5
F. verticillioides	7	1	4	2	7	21	10.9
F. graminearum	0	0	0	0	1	1	0.5
Cladosporium spp.	0	15	8	6	6	35	18.2
Alternaria spp.	2	5	0	5	1	13	6.8
Mucor spp.	4	0	0	2	6	12	6.3
Rhizopus spp.	2	1	1	1	5	10	5.2
Others	10	5	3	1	5	24	12.5
Total	42	43	30	34	43	192	

rium (18.2%), *Penicillium* (17.2%), and *Fusarium* (11.5%). The predominant strains in the batches of PapriQueen, Jariza, and Jaranda were species of the genera *Aspergillus* and *Fusarium.* However, the majority of strains in the Bola batches were *Cladosporium* and *Penicillium.* Isolates of *Alternaria* (6.8%) were obtained in four of the five batches analyzed. In a previous study of paprika made from sun-dried *C. annum* and *C. frutescens, Aspergillus* was the predominant genus isolated together with *Rhizopus (1). Rhizopus* and *Mucor* strains also were isolated in our survey, with a prevalence greater than 5% (Table 4). Other genera isolated with a prevalence of less than 5% were *Rhizomucor, Phoma,* and *Monascus.* Diseases in fruit, such as rot, are caused by species of *Cladosporium, Alternaria,* and *Fusarium. Penicillium* and *Aspergillus* also are usually associated with deterioration of fruit and vegetables *(7).* Therefore, the use of damaged pepper fruits might be an important source of fungal contamination for paprika.

Strains of the mycotoxigenic genera *Aspergillus, Penicillium,* and *Fusarium* were identified to species according to their secondary metabolite profiles on MEA. This method of chemotaxonomic characterization has been applied

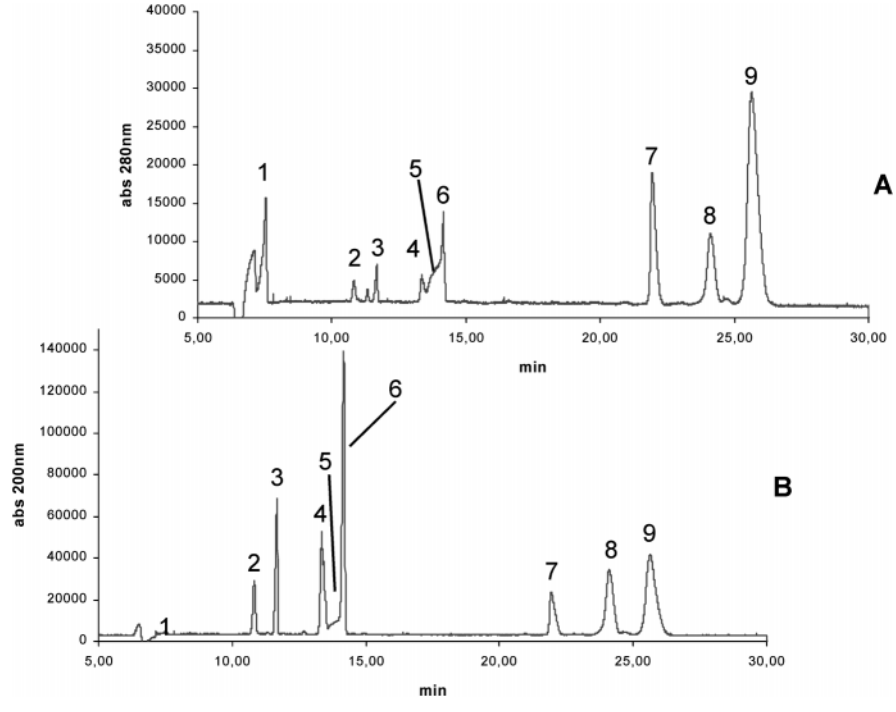

FIGURE 1. *Electropherograms at 280 nm (A) and 200 nm (B) of mycotoxin standards (100 ppm). Lane 1, patulin; lane 2, citrinin; lane 3, penicillic acid; lane 4, ochratoxin A; lane 5, cyclopiazonic acid; lane 6, mycophenolic acid; lane 7, zearalenone; lane 8, aflatoxin B_1; lane 9, griseofulvin + citreoviridin.*

TABLE 5. *Fungal secondary metabolites listed according to main toxin-producing genus and retention time*

Mycotoxins	Retention time (min)	UV maximum (nm)	Function	λ[a]	Range (ppm)	R^2	Detection limit (ppm)	Theoretical plates	Recovery rate (%)[b]
Penicillium spp.									
Patulin	7.24	280[c] 560	$y = 777x - 4386$	280	10–100	0.971	1.41	103366	138.8
Secalonic acid D	9.84	246 343 363[c]	$y = 840x - 60634$	363	30–500	0.988	1.80	66158	111.1
Citrinin	10.60	219[c] (255) 321	$y = 272x - 1862$	219	10–100	0.998	1.71	53712	82.0
Penicillic acid	11.20	222	$y = 4020x - 6647$	222	10–100	0.984	0.49	143711	144.8
Viridicatumtoxin	13.38	247 279[c] 408	$y = 1845x + 1452$	279	10–100	0.989	1.97	24738	102.4
Mycophenolic acid	13.88	228[c] 342 349	$y = 1759x + 1149$	349	10–100	0.999	1.63	143721	95.2
Fumitremorgin B	21.15	e[c] 230 295	$y = 2556x - 3791$	230	50–500	0.967	2.37	19877	95.3
Griseofulvin peak 1	23.08	218 (258) 294[c]							
Citreoviridin	23.05	202 297 397[c]	$y = 3373x - 4596$	398	10–100	0.986	1.34	26648	166.7
Penitrem A	24.57	e[c] 235 300	$y = 3333x - 40501$	235	50–500	0.958	3.04	15380	88.7
Roquefortine C	25.13	206[c] 244 329	$y = 4656x - 54925$	206	50–500	0.994	2.95	23452	70.6
Paxillin	25.20	233[c] 287	$y = 2001x - 45601$	233	50–500	0.969	4.70	21101	90.2
Griseofulvin peak 2	25.80	220 299[c]	$y = 3360x - 27005$	299	25–500	0.999	2.01	46896	179.2
Aspergillus spp.									
Ochratoxin A	13.09	e[c] 217 381	$y = 867x + 126$	381	10–100	0.999	0.36	134654	138.5
Cycloplazonic acid	13.73	225[c] 262 283	$y = 792x - 1878$	283	10–100	0.987	0.59	38775	138.8
Aflatoxin B_1	24.14	e[c] (232) (272) 366	$y = 3448x - 20962$	366	10–100	0.985	1.52	28773	86.8
Sterigmatocystin	26.60	250[c] 335	$y = 3758x - 31547$	250	10–100	0.989	2.10	19544	89.1
Fusarium spp.									
Deoxynivalenol	7.49	222	$y = 356x + 977$	222	50–500	0.998	2.75	15697	128.3
Zearalenone	21.05	243[c] 280 (317)	$y = 8434x - 4596$	243	10–100	0.980	0.53	81455	73.2

[a] Wavelengths for calculating the functions.
[b] Recovery rate in paprika agar with respect to malt extract agar.
[c] Wavelength for the maximum absorbance. Shoulders are represented in parentheses; e, end absorption (below 195 nm).

TABLE 6. *Determination of mycotoxins from extracts of toxigenic molds grown on MEA and PA*

Strain	Mycotoxin	Amount (μg/plate)		$P <$
		MEA	PA	
Isolates from paprika				
Penicillium expansum P23	Patulin	277	31	0.05
	Citrinin	15	4	0.05
Penicillium citrinum R9	Citrinin	184	7	0.01
Penicillium raistrickii D76	Penicillic acid	3,852	10	0.01
	Griseofulvin	41	ND[a]	0.01
P. raistrickii R35	Penicillic acid	403	tr[b]	0.01
Penicillium griseofulvum P63	Patulin	3,171	203	0.05
	Griseofulvin	232	54	0.1
Aspergillus sydowii R92	Griseofulvin	175	ND	0.01
Aspergillus oryzae D58	Cyplopiazionic acid	tr	ND	
Fusarium graminearum P60	Zearalenone	21	ND	0.05
Type strains				
Aspergillus flavus CECT 2687	Mycophenolic acid	77	ND	0.01
	Aflatoxin B_1	3	ND	0.1
Aspergillus parasiticus CECT 2688	Aflatoxin B_1	57	tr	0.05
Penicillium brevicompactum CECT 2316	Mycophenolic acid	304	7	0.01
Penicillium aurantiogreseum CECT 2264	Penicillic acid	8	ND	0.05

[a] ND, not detected.
[b] tr, trace.

with molecular biology techniques to differentiate among species of molds isolated from dry-cured ham *(23).* We isolated *Aspergillus niger* (15.1%) and *Fusarium verticillioides* (10.9%) from each of the five batches of paprika. *Penicillium expansum* (7.8%), *Aspergillus oryzae* (6.3%), and *Penicillium citrinum* (3.1%) were present in three or four of the five batches analyzed, and *Aspergillus sydowii* and *Penicillium raistrickii* were isolated at frequencies less than 3%. Other *Penicillium* strains were found at frequencies less than 1%.

MECC analysis of mycotoxins. An efficient resolution of the investigated mycotoxins was obtained by using MECC (Fig. 1 and Table 5). For griseofulvin, the standard had two peaks, and the resolution by HPLC showed two peaks *(13).* The elution time for patulin and metabolites possessing carboxyl groups, such as citrinin, secalonic acid, penicillic acid, ochratoxin A, cyclopiazinic acid, and mycophenolic acid, was less than 20 min. Aflatoxin B_1, sterigmatocystin, roquefortine C, tremorgenic mycotoxins (fumitremorgin B, citreoviridin, penitrem A, and paxillin),

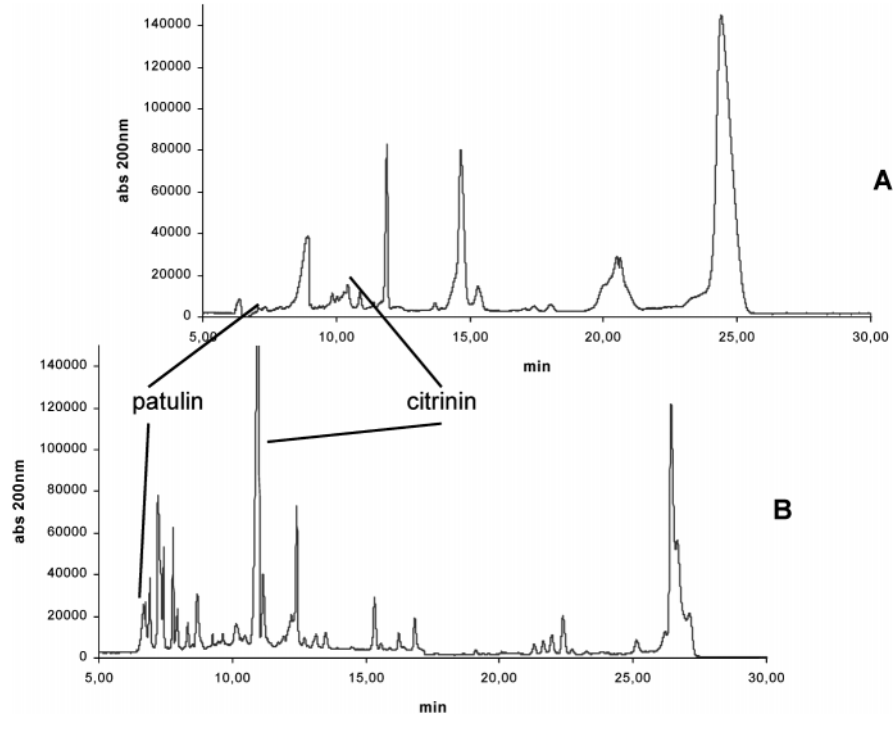

FIGURE 2. *Electropherograms of chloroform extracts from* P. expansum *P23 grown on paprika agar (PA) (A) and malt extract agar (MEA) (B).*

zearalenone, and griseofulvin were eluted between 20 and 30 min. Viridicatumtoxin and patulin had discrete absorbance at 200 nm (wavelength representing the electropherograms) (Fig. 1). Standard curves were constructed by injecting standard solutions at 10 to 100 or 50 to 500 μg ml^{-1}. Those regression equations corresponding to determination of mycotoxins and other characteristic parameters of the method are shown in Table 5. The detection limit for the mycotoxins was less than 10 μg ml^{-1} (range, 0.5 to 5 μg ml^{-1}). These values are similar to those obtained by other authors with HPLC or CE equipped with UV, diode array, light diffusion, or mass spectrometer detectors *(15, 27, 37).* Therefore, the use of the MECC method described here was adequate for determination of mycotoxin-producing fungi isolated from paprika.

Determination of mycotoxin-producing fungi. Six mycotoxins were identified in the extracts of several strains isolated from paprika and incubated on MEA (Table 6). Unlike in other studies, we did not detect any aflatoxin-producing fungi. *P. expansum* was the dominant mycotoxigenic fungus isolated (Table 4). Strains of this mold were found in the paprika batches of both Bola and PapriQueen pepper varieties and produced large amounts of patulin and citrinin during growth on MEA (Table 6 and Fig. 2). *P. expansum* is a common contaminant of damaged fruit and the most important producer of patulin *(32).* Citrinin was also produced by the strains of *P. citrinum* isolated from three of the five batches of paprika (Jariza, Bola 1, and Jaranda). *P. citrinum* commonly contaminates spices and nuts *(31).* Strains of *P. raistrickii* were isolated from the batches of Jaranda and PapriQueen and produced penicillic acid and griseofulvin on MEA. Griseofulvin also was produced by the isolates of *A. sydowii* and *Penicillium griseofulvum,* which in addition produced patulin (Table 6). Both molds were isolated from Jaranda and PapriQueen batches, respectively (Table 4). *P. raistrickii* and *P. griseofulvum* are frequently isolated from plant material such as corn or other cereals *(31).* Some isolates of *A. oryzae* produced cyclopiazionic acid on MEA but only in trace amounts. A total of 22 *Fusarium* isolates were obtained. A wide variety of *Fusarium* species are producers of zearalenone and trichothecenes such as deoxynivalenol *(22).* However, only one isolate, identified as *F. graminearum,* produced zearalenone on MEA.

Production of mycotoxins on PA. The production of mycotoxins decreased when the isolates were grown on PA (Table 6), possibly because of the small amount of nutrients in this culture medium. However, this medium allowed us to find mycotoxigenic fungi well adapted to paprika. All the *Penicillium* strains isolated from paprika were able to produce toxins, especially *P. griseofulvum* P63 and *P. expansum* P23. In addition, *P. raistrickii* D76 and *P. citrinum* R9 also produced small amounts of penicillic acid and citrinin, respectively. The strains *A. sydowii* R92 and *F. graminearum* P60 did not produce any mycotoxins on PA in detectable amounts. With respect to mold types, *P. brevicompactum* CECT 2316 and *A. parasiticus* CECT 2688 were capable of producing toxins, although the latter strain produced only trace amounts of aflatoxin B_1. These observations support the hypothesis that mycotoxins such as patulin, citrinin, griseofulvin, penicillic acid, and mycophenolic acid may be more readily produced in paprika than other mycotoxins such as aflatoxins. These mycotoxins together with aflatoxins and ochratoxins all can be found in paprika. The design of an optimum method for extraction, clean up, and concentration of mycotoxin extracts from paprika will allow the development of a strategy based on MECC for the detection of permissible levels of these mycotoxins in paprika.

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