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Differentiation of Species from the *Penicillium roqueforti* Group by Volatile Metabolite Profiling

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Species from the *Penicillium roqueforti* group were differentiated by volatile metabolite profiling primarily of sesquiterpenes. A total of 24 isolates from species *P. roqueforti*, *Penicillium carneum*, and the recently described species *Penicillium paneum* were inoculated on yeast extract sucrose agar. Volatile metabolites were collected by diffusive sampling onto tubes containing Tenax TA, overnight between the fifth and sixth days of incubation. Volatiles were thermally desorbed and analyzed by gas chromatography coupled to mass spectrometry. The sesquiterpene area of the chromatogram was investigated, and potential sesquiterpenes were tabulated by comparison of their Kovats retention index and mass spectrum. In general, *P. carneum* isolates produced the lowest number of sesquiterpenes, all of which were unique for *P. carneum* within the *P. roqueforti* group. *P. roqueforti* and *P. paneum* produced a larger variety of volatile metabolites, some of which they have in common and some of which are unique for the two species. (+)-Aristolochene was found in samples from *P. paneum* and *P. roqueforti*. Other *Penicillium* species in which (+)-aristolochene was also detected were *P. commune*, *P. glandicola*, and *P. solitum*.

KEYWORDS: ***Penicillium roqueforti* group; *Penicillium roqueforti*; *Penicillium carneum*; *Penicillium paneum*; volatile organic compounds; volatile metabolite profiling; (+)-aristolochene**

INTRODUCTION

Historically *Penicillium roqueforti* has attracted a lot of attention due to its use as a cheese starter culture. This attention was given to a variety of topics such as aroma production and related strain development (*1*−*4*), morphology (*5*), strain differentiation by comparison of secondary metabolite patterns (*6*), mutagenicity testing (*7*), and the investigation of mycotoxin production, in both artificial media and cheese (*8*−*12*).

In 1989 *P. roqueforti* was divided into the two varieties, *P. roqueforti* var. *roqueforti* and *P. roqueforti* var. *carneum* (*13*). At some growth conditions *P. roqueforti* var. *carneum* produces the mycotoxins patulin and cyclopaldic acid (*14*). It is therefore important to distinguish between the two varieties *carneum* and *roqueforti*. A method to differentiate between *P. roqueforti* var. *roqueforti* and *P. roqueforti* var. *carneum* is the analysis of volatile metabolite production profiles (*15*).

The *P. roqueforti* varieties were reclassified as three new species, *P. roqueforti*, *P. carneum*, and *P. paneum*, based on ribosomal DNA analysis and secondary metabolite profiling (*14*). It was also shown that both *P. carneum* and *P. paneum*, given the right growth conditions, produce patulin and cyclopaldic acid (*14*). Thus, it is of importance to be able to distinguish *P. roqueforti* from both *P. carneum* and *P. paneum*.

The major aim of this study was to facilitate complete differentiation of the species in the *P. roqueforti* group by profiling the volatile organic compound (VOC) production mainly of the sesquiterpenes, because volatile production from *P. paneum* has not been investigated previously.

The versatile application of volatile metabolite production analysis by gas chromatography−mass spectrometry (GC-MS) is evident. It has been applied in fields such as chemosystematics (*16*), distinction of cheese-related fungi (*17*), screening of species-specific volatile metabolites from compost associated fungi (*18*), and the distinguishing of toxin-producing isolates from non-toxin-producing isolates in Aspergilli (*19*), *Fusarium sambucinum* (*20*), other *Fusarium* spp. (*21*), and *P. roqueforti* (*22*). Apart from volatile metabolite profile analysis by GC-MS, analysis by electronic nose technology could be of interest as electronic nose technology is increasingly used for analysis in areas such as food quality control, storage, and spoilage by bacteria and fungi (*23*−*30*) of processed as well as nonprocessed food.

It has been shown that *P. roqueforti* strains that produce PR toxin produce the volatile metabolite (+)-aristolochene (*31*), which has been found only in *P. roqueforti* within the genus *Penicillium* (*22*). (+)-Aristolochene is thus considered to be a biomarker for *P. roqueforti* within the genus (*22*, *31*). However, when volatile metabolites from 47 taxa within the genus *Penicillium* were characterized, a volatile metabolite was detected that had a mass spectrum similar to the one of (+)-aristolochene and was found in isolates of *P. commune*, *P. glandicola*, *P. roqueforti*, and *P. solitum* (*15*). The metabolite

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Table 1. Volatile Organic Compound Profiles for the *P. roqueforti* Group, RI 1340–1800[a]

compd	RI	*P. carneum*						*P. roqueforti*											*P. paneum*						
		3474	6884	6885	6888	14042	19478	6754	14408	14412	14420	14425	16401	16403	16404	16407	24729	24748	11839	13929	14356	16402	24721	24723	24728
1	1343																		**X**	**X**	**X**	**X**	**X**	**X**	**X**
2	1350																		**X**	**X**	**X**	**X**	**X**	**X**	**X**
3	1368																		**X**	**X**	**X**	**X**	**X**	**X**	**X**
4	1398																		**X**	**X**	**X**	**X**	**X**	**X**	**X**
5[b]	1400							**X**	**X**		**X**	**X**			**X**	**X**	**X**								
6	1402																		**X**	**X**		**X**	**X**	**X**	**X**
7[b]	1407	**X**	**X**	**X**	**X**	**X**	**X**																		
8	1414																		**X**	**X**	**X**	**X**	**X**		
9[b]	1418								*X*	*X*	*X*		*X*	*X*		*X*	*X*		*X*	*X*			*X*	*X*	*X*
10	1428							*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*		*X*	*X*	*X*
11[b]	1441							**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**								
12	1442																		**X**	**X**	**X**	**X**	**X**	**X**	
13	1461							**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**							
14[b]	1467							*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*			*X*	*X*	*X*	*X*	*X*	*X*	*X*
15[b]	1480							**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**							
16	1485																		**X**	**X**	**X**	**X**	**X**	**X**	**X**
17	1488							**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**								
18	1494							**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**							
19	1494																		**X**	**X**		**X**	**X**	**X**	**X**
20	1498								**X**	**X**	**X**		**X**	**X**		**X**	**X**								
21	1505							**X**	**X**				**X**			**X**	**X**								
22	1511								**X**	**X**	**X**		**X**	**X**		**X**									
23	1514							**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**								
24[b]	1517	**X**	**X**	**X**	**X**	**X**	**X**																		
25	1524							**X**	**X**	**X**	**X**		**X**	**X**		**X**									
26	1527								**X**				**X**			**X**									
27[b]	1532							*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*		*X*	*X*	*X*
28	1535	**X**	**X**	**X**	**X**		**X**																		
29[b]	1540							*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*		*X*	*X*			*X*	*X*	*X*
30	1545							*X*	*X*	*X*	*X*		*X*	*X*	*X*	*X*	*X*		*X*	*X*			*X*	*X*	*X*
31[b]	1547								*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*		*X*	*X*			*X*	*X*	*X*
32[b]	1551								**X**	**X**	**X**		**X**	**X**		**X**	**X**								
33	1567								**X**				**X**	**X**		**X**									
34[b]	1572							**X**	**X**	**X**	**X**					**X**	**X**								
35	1574								*X*	*X*	*X*		*X*	*X*	*X*	*X*	*X*		*X*	*X*			*X*		*X*
36[b]	1578								**X**	**X**	**X**		**X**	**X**		**X**									
37	1598									**X**	**X**	**X**	**X**	**X**		**X**		**X**							
38	1637																		**X**	**X**	**X**	**X**	**X**	**X**	**X**
39	1684							**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**							
40	1686							**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**							
41	1695							**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**							
42	1702																		**X**	**X**	**X**	**X**	**X**	**X**	**X**
43	1758																		**X**	**X**		**X**			
44	1773								**X**	**X**	**X**					**X**									
45	1789																		**X**	**X**		**X**	**X**		**X**
46	1789												**X**	**X**											
47	1790															**X**									
48	1800								**X**	**X**	**X**		**X**	**X**		**X**									

[a] An "X" indicates that the volatile was detected in samples from this isolate. For compounds unique for a species within the group the "X" is given in boldface type (**X**). For compounds produced by more than one of the three species the "X" is given in italic type (*X*). [b] Compound **5**, β-patchoulene; compound **7**, zingiberene; compound **9**, β-elemene; compound **11**, diepi-α-cedrene; compound **14**, β-caryophyllene; compound **15**, β-patchoulene isomer; compound **24**, geosmin; compound **27**, (+)-aristolochene; compount **29**, eremophilene; compound **31**, α-selinene; compound **32**, valencene; compound **34**, β-bisabolene; compound **36**, β-himachalene.

Table 2. Characteristic Ions of the Volatile Compounds, Six Largest Ions from *m/z* 50 to 150 and Three Ions from *m/z* 151 to 272 with Intensities Given in Parentheses

compd	RI	characteristic ions *m/z* 50–150						*m/z* 151–272		
1	1343	91 (100)	133 (87)	105 (79)	106 (62)	147 (54)	148 (51)	189 (49)	204 (31)	162 (12)
2	1350	119 (100)	105 (59)	91 (54)	147 (52)	133 (43)	55 (35)	161 (55)	189 (53)	204 (30)
3	1368	121 (100)	122 (98)	91 (91)	105 (79)	119 (75)	55 (68)	175 (69)	189 (48)	204 (56)
4	1398	122 (100)	91 (82)	147 (78)	121 (69)	105 (67)	119 (65)	175 (99)	204 (69)	189 (24)
5[a]	1400	119 (85)	105 (82)	91 (73)	93 (72)	106 (48)	55 (42)	161 (100)	189 (78)	204 (38)
6	1402	91 (60)	121 (53)	105 (53)	149 (48)	107 (47)	136 (45)	161 (31)	175 (29)	189 (25)
7[a]	1407	93 (100)	119 (89)	91 (47)	69 (39)	77 (34)	105 (17)	161 (4)	204 (1)	189 (1)
8	1414	119 (27)	91 (27)	105 (20)	147 (19)	133 (17)	77 (17)	189 (57)	161 (19)	nd[b]
9[a]	1418	93 (100)	81 (96)	67 (87)	68 (77)	79 (63)	53 (59)	161 (24)	189 (14)	162 (6)
10	1428	81 (100)	93 (98)	67 (90)	68 (83)	79 (67)	53 (62)	161 (20)	189 (15)	162 (6)
11[a]	1441	119 (100)	93 (67)	91 (49)	105 (44)	77 (53)	55 (31)	161 (22)	204 (16)	189 (12)
12	1442	91 (84)	133 (80)	105 (80)	119 (68)	135 (64)	93 (57)	162 (31)	189 (27)	161 (22)
13	1461	133 (100)	91 (23)	119 (16)	105 (15)	134 (14)	55 (11)	161 (16)	204 (4)	189 (3)
14[a]	1467	93 (60)	69 (57)	91 (54)	79 (53)	133 (40)	77 (36)	161 (13)	189 (6)	175 (4)
15[a]	1480	119 (100)	91 (36)	105 (33)	93 (27)	133 (26)	77 (24)	161 (62)	189 (52)	204 (18)
16	1485	91 (100)	148 (68)	105 (63)	133 (56)	79 (47)	77/93 (39)	161 (33)	189 (23)	204 (16)
17	1488	91 (100)	133 (84)	105 (69)	119 (53)	55 (47)	79 (42)	189 (91)	175 (50)	204 (37)
18	1494	55 (74)	57 (52)	69 (45)	83 (43)	56 (41)	70 (35)	189 (3)	nd	nd
19	1494	128 (22)	143 (17)	115 (17)	129 (17)	141 (16)	142 (15)	185 (100)	200 (29)	157 (23)
20	1498	105 (100)	106 (84)	91 (54)	120 (47)	119 (43)	77 (27)	204 (23)	176 (18)	161 (11)
21	1505	91 (98)	93 (68)	133 (61)	81 (61)	105 (60)	79 (58)	189 (22)	161 (17)	nd
22	1511	91 (84)	133 (72)	105 (65)	93 (57)	79 (57)	55 (49)	189 (84)	204 (31)	161 (21)
23	1514	91 (96)	134 (79)	105 (81)	93 (74)	133 (68)	79 (68)	189 (27)	161 (17)	204 (9)
24[a]	1517	112 (100)	55 (35)	69 (16)	97 (15)	126 (15)	83 (14)	182 (2)	nd	nd
25	1524	69 (62)	93 (59)	79 (51)	91 (45)	67 (37)	55 (33)	161 (16)	189 (7)	nd
26	1527	91 (85)	133 (70)	105 (67)	79 (63)	55 (62)	93 (51)	189 (66)	204 (30)	161 (30)
27[a]	1532	105 (100)	91 (71)	93 (51)	107 (46)	79 (40)	121 (39)	189 (57)	161 (16)	204 (6)
28	1535	119 (100)	132 (77)	105 (68)	55 (46)	91 (39)	131 (29)	202 (19)	nd	nd
29[a]	1540	91 (85)	79 (84)	93 (79)	105 (76)	107 (67)	119 (56)	161 (74)	189 (29)	204 (28)
30	1545	93 (77)	79 (72)	105 (72)	91 (69)	107 (65)	67 (62)	161 (28)	189 (23)	204 (21)
31[a]	1547	93 (81)	91 (81)	107 (74)	79 (70)	81 (66)	105 (62)	189 (62)	161 (62)	204 (28)
32[a]	1551	91 (79)	79 (74)	105 (69)	93 (62)	67 (56)	55 (53)	161 (52)	189 (33)	175 (24)
33	1567	91 (100)	105 (86)	131 (65)	77 (56)	145 (52)	55 (52)	202 (52)	187 (33)	159 (21)
34[a]	1572	69 (100)	91 (55)	92 (53)	77 (48)	55 (36)	79 (30)	161 (18)	204 (9)	189 (8)
35	1574	122 (100)	107 (82)	91 (56)	79 (44)	105 (42)	55/93 (40)	161 (91)	204 (14)	162 (9)
36[a]	1578	119 (100)	91 (89)	105 (85)	93 (53)	77 (51)	79 (51)	161 (51)	189 (29)	204 (15)
37	1598	55 (78)	57 (59)	83 (48)	69 (48)	97 (39)	56 (40)	nd	nd	nd
38	1637	123 (100)	151 (75)	55 (63)	97 (59)	111 (42)	110 (37)	151 (74)	166 (61)	165 (53)
39	1684	55 (82)	67 (65)	81 (50)	82 (44)	54 (42)	69 (39)	nd	nd	nd
40	1686	67 (97)	55 (63)	81 (61)	79 (45)	54 (44)	95 (33)	nd	nd	nd
41	1695	55 (84)	57 (62)	69 (49)	83 (49)	56 (42)	97 (40)	nd	nd	nd
42	1702	81 (50)	93 (42)	55 (41)	67 (35)	79 (29)	133 (27)	151 (35)	148 (22)	147 (8)
43	1758	55 (67)	91 (40)	146 (39)	145 (27)	69 (27)	70 (27)	159 (28)	202 (26)	243 (24)
44	1773	81 (46)	55 (41)	67 (38)	71 (33)	95 (24)	69 (24)	204 (10)	189 (9)	161 (9)
45	1789	55 (84)	91 (67)	105 (52)	146 (49)	145 (40)	131 (39)	216 (94)	159 (59)	272 (41)
46	1789	55 (85)	81 (43)	69 (40)	67 (34)	91 (30)	79 (27)	189 (22)	204 (13)	nd
47	1790	55 (80)	91 (41)	69 (33)	105 (30)	67 (29)	79 (27)	216 (38)	159 (26)	229/272 (16)
48	1800	55 (42)	81 (41)	67 (40)	71 (37)	79 (26)	95 (24)	189 (19)	204 (14)	161 (14)

[a] Compound **5**, β-patchoulene; compound **7**, zingiberene; compound **9**, β-elemene; compound **11**, diepi-α-cedrene; compound **14**, β-caryophyllene; compound **15**, β-patchoulene isomer; compound **24**, geosmin; compound **27**, (+)-aristolochene; compound **29**, eremophilene; compound **31**, α-selinene; compound **32**, valencene; compound **34**, β-bisabolene; compound **36**, β-himachalene. [b] Not detected.

was not identified, but referred to by its Kovats retention index (RI), 1521, and its characteristic MS ion fragmentation pattern. Thus, volatile metabolite production of isolates from species *P. commune*, *P. glandicola*, *P. roqueforti*, and *P. solitum* were also investigated in this study, to determine whether the unidentified compound (RI 1521) in each species is in fact (+)-aristolochene.

The overall goal of this study is to differentiate among the three species of the *P. roqueforti* group by volatile metabolite profiling, mainly of sesquiterpenes, as well as to investigate whether (+)-aristolochene is a unique marker for *P. roqueforti* within the genus *Penicillium*.

MATERIALS AND METHODS

Fungi and Media. All isolates used in this study were obtained from the Fungal Culture Collection at BioCentrum-DTU (IBT collection), Technical University of Denmark, Kgs. Lyngby, Denmark. The following strains were investigated (listed by IBT number); *P. carneum*, 3474, 6884, 6885, 6888, 14042, and 19478; *P. commune*, 6373, 10763, 14135, and 21513; *P. glandicola*, 4168, 6592, and 21529; *P. paneum*, 11839, 13929, 14356, 16402, 24721, 24723, and 24728; *P. roqueforti*, 6754, 14408, 14412, 14420, 14425, 16401, 16403, 16404, 16407, 24729, and 24748; *P. solitum*, 10254 and 21545. The strains were center point inoculated from spore suspensions on 9 cm Petri dishes containing yeast extract sucrose agar (YES) medium. The YES medium consisted of yeast extract (Difco, 212750) (2%), sucrose (15%), $MgSO_4 \cdot 7H_2O$

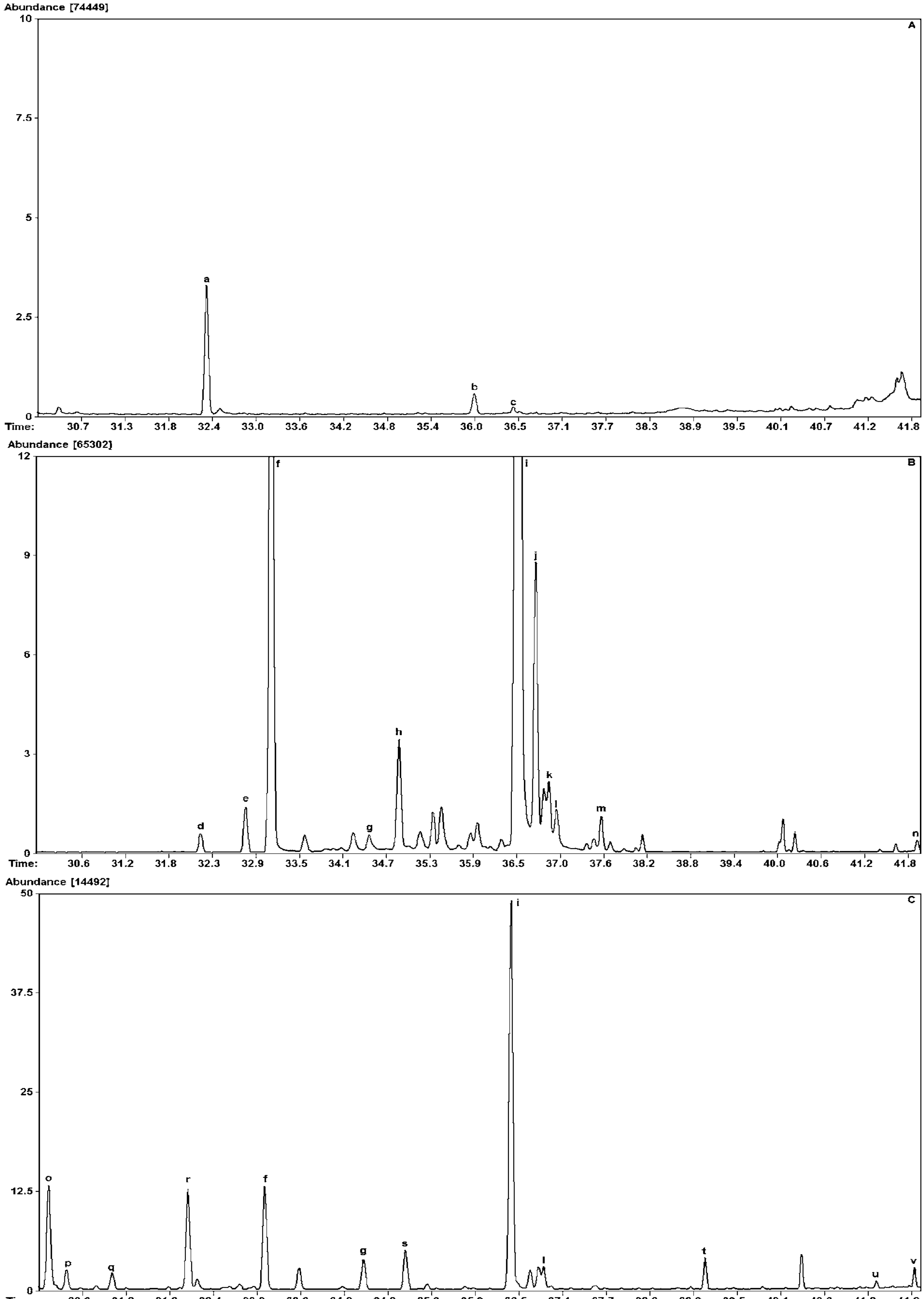

Figure 1. Chromatograms of the RI interval 1340–1800 for (**A**) *P. carneum* (IBT 19478), (**B**) *P. roqueforti* (IBT 16407), and (**C**) *P. paneum* (IBT 11839). The abundance scale is in percentage of the abundance given in the top left corner of each chromatogram. The compounds at the following peaks are noteworthy: (a) zingiberene; (b) geosmin; (c) compound **28**; (d) β-patchoulene; (e) β-elemene; (f) compound **10**; (g) β-caryophyllene; (h) β-patchoulene isomer; (i) (+)-aristolochene; (j) eremophilene; (k) α-selinene; (l) valencene; (m) compound **35**; (n) compound **47**; (o) compound **1**; (p) compound **2**; (q) compound **3**; (r) compound **4**; (s) compound **16**; (t) compound **38**; (u) compound **43**; (v) compound **45**.

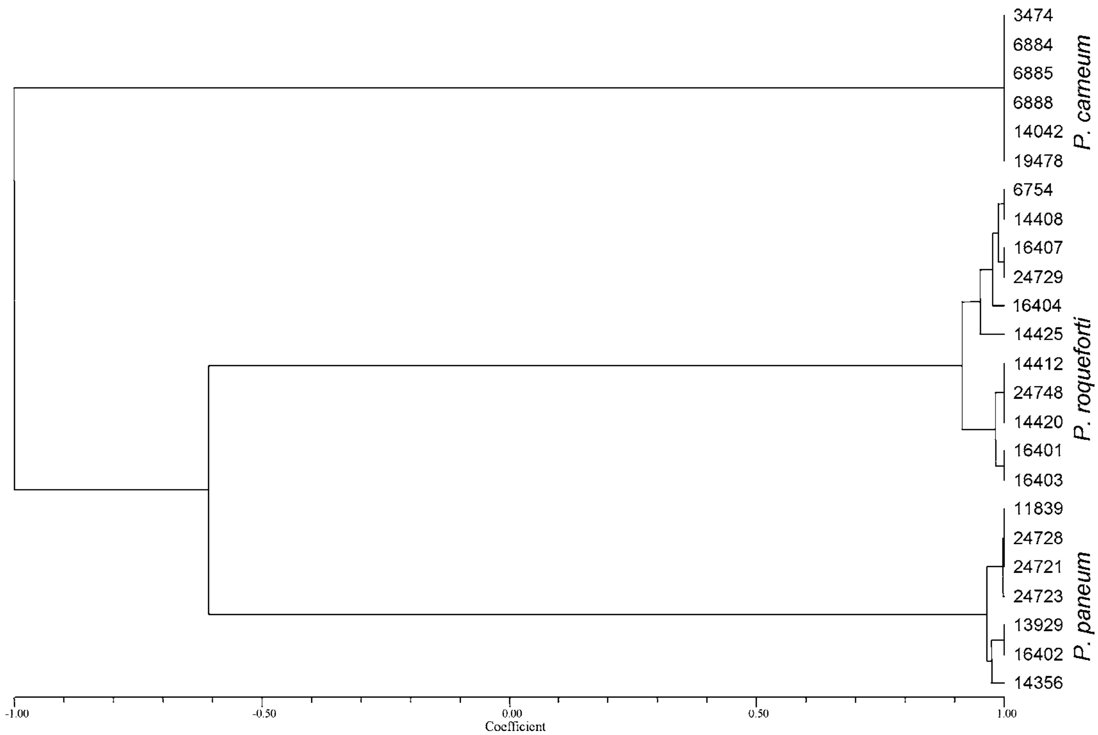

Figure 2. Dendrogram of the isolates from the three species of the *P. roqueforti* group based on volatile metabolite profile of mainly sesquiterpenes. The isolates are clearly separated into three species.

(0.05%), $ZnSO_4 \cdot 7H_2O$ (0.001%), $CuSO_4 \cdot 5H_2O$ (0.0005%), water to 1.0 L, pH 6.5, and agar (2%). The cultures were incubated in the dark at 25 °C for 5 days.

Collection and Analysis of Volatile Metabolites. Volatile metabolites were collected overnight between days five and six at room temperature. The volatiles were collected by diffusive sampling onto Tenax TA adsorption material placed in Perkin-Elmer tubes according to the method described in ref *32*. Volatiles collected were thermally desorbed on a Perkin-Elmer ATD 400 coupled to a Hewlett-Packard 5890 gas chromatograph further coupled to an HP 5972 mass selective detector. Separation of the volatiles was done on a DB-1701 (J&W) capillary column (30 m, 0.25 mm, 1.0 μm) using He as carrier gas. Initial pressure was 13 psi, and the He flow was 1 mL/min. The system was run at a 1:75 split, and the injection temperature was set to 250 °C. Chromatographic conditions were as follows: initial temperature, 35 °C for 1 min, raised at 4 °C min^{-1} to 175 °C and then at 10 °C min^{-1} to 260 °C. Separated compounds were characterized by their mass spectra generated by electron ionization (EI) at 70 eV at a scan range from *m/z* 33 to 330.

Data Analysis. Mass spectra from compounds with identical retention indices were compared to account for similarity. The identity of the compounds was established by comparison of mass spectra and volatile metabolite profiles with data from refs *15*, *22*, and *31*.

Cluster analysis of the volatile metabolite data was carried out with NTSYSpc (version 2.11N, Exeter software), with the data matrix set up as a qualitative, binary (1, 0) matrix of the volatiles listed in **Table 2**. The data were analyzed by UPGMA using the Yule distance coefficient to minimize the influence of biological variety between isolates from the same species as suggested by Frisvad (personal communication, 2004).

RESULTS AND DISCUSSION

The growth medium was chosen on the basis of the knowledge that it induces high production as well as high diversity in the production of both volatile and nonvolatile secondary metabolites when used for incubation of species from the genus *Penicillium* (*15*). By center point inoculating the isolates and incubating them for 5 days, an age gradient was achieved within the colony, and thus the colony produced volatile metabolites corresponding to all growth phases.

In the chromatograms of the 24 isolates of *P. roqueforti*, *P. carneum*, and *P. paneum*, a total of 48 different compounds, mainly sesquiterpenes, were detected in the interval between RI 1340 and 1800. The volatile metabolite profiles are given by species and isolate number as well as RI in **Table 1**. They are also characterized by their six tallest peaks in the interval *m/z* 50−150 and the three tallest in the interval *m/z* 151−272 (shown in **Table 2**). As reported in ref *15*, *P. carneum* produced significantly fewer volatile metabolites than the other species, namely, the three volatile metabolites zingiberene, geosmin, and compound **28**, all of which apparently are unique within the *P. roqueforti* group at the given experimental conditions. *P. roqueforti* and *P. paneum* produced up to 32 and 21 volatile metabolites, respectively. Eight of the compounds, β-elemene, compound **10**, β-caryophyllene, eremophilene, compound **30**, α-selinene, compound **35**, and (+)-aristolochene, were detected from both species. Unique markers for *P. roqueforti*, within the *P. roqueforti* group, are β-patchoulene, diepi-α-cedrene, compound **13**, β-patchoulene isomer, compounds **17**, **18**, **20**−**23**, **25**, and **26**, valencene, compound **33**, β-bisabolene, β-himachalene, and compounds **37**, **39**−**41**, **44**, and **46**−**48**, whereas the unique markers for *P. paneum* are compounds **1**−**4**, **6**, **8**, **12**, **16**, **19**, **38**, **42**, **43**, and **45**. This difference in volatile metabolite profile is visualized in **Figure 1**. The volatile metabolite profile of *P. paneum* exhibits more VOCs in the RI intervals 1340−1400 and 1700−1800 and fewer VOCs in the interval of RI 1401−1699, compared to the profile for *P. roqueforti*.

There is a clear difference in pattern within the volatile metabolite profile from the three species; thus, even with biological variation within the species, it is possible to differentiate among the three species. This is in agreement with the results from ref *15*, which showed that *P. roqueforti* and *P. carneum* are distinguishable on the basis of their VOC profiles.

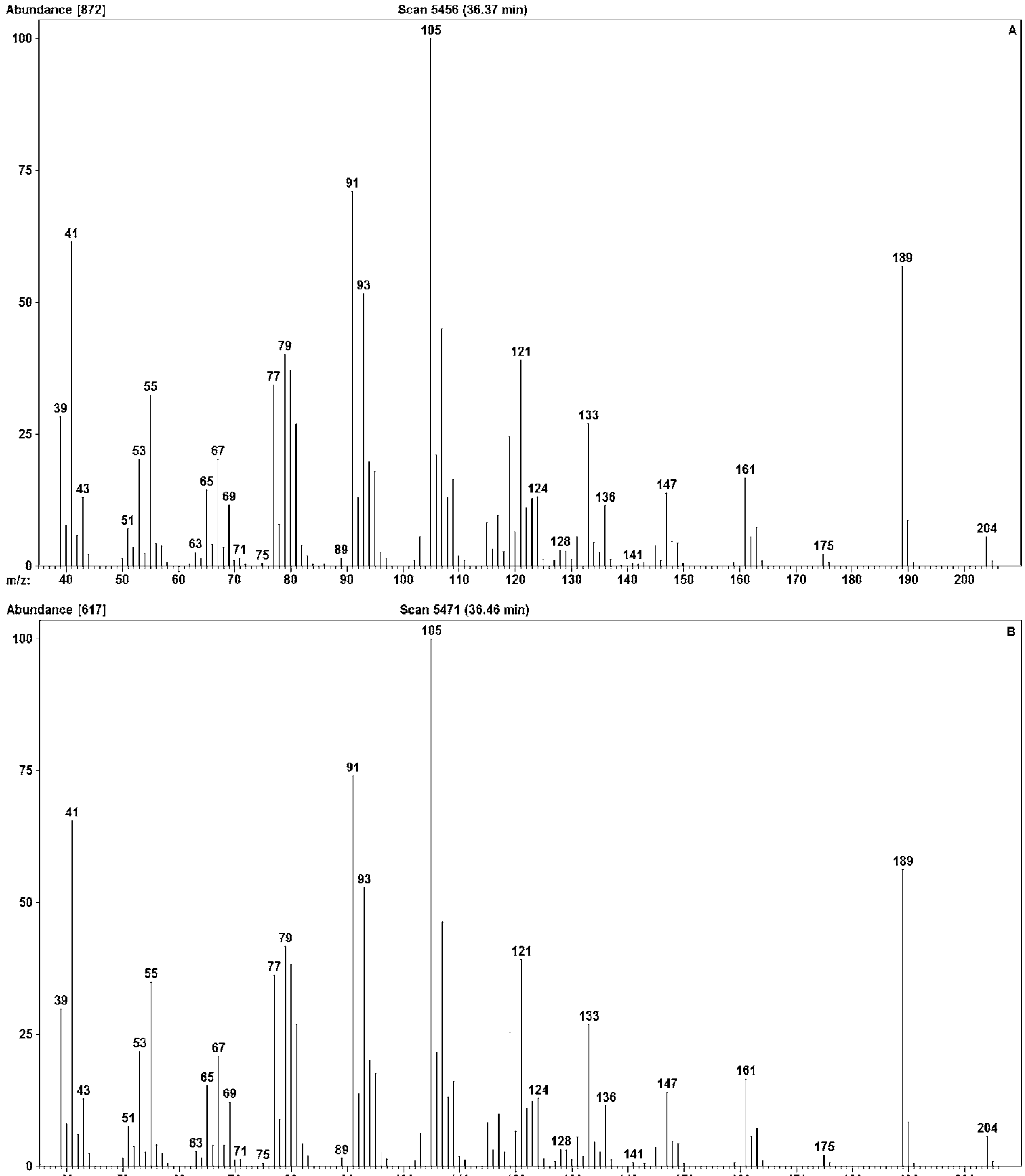

Figure 3. Mass spectrum comparison matching spectra from (**A**) (+)-aristolochene from the authentic sample and a compound of similar RI from (**B**) a sample of *P. paneum* (IBT 11839).

The three species were further investigated by hierarchical cluster analysis of the VOC data. This was desired, as it was the distinction of species rather than variety within the species, which was the objective of this study. The resulting dendrogram (**Figure 2**) shows clear separation of the three species, with *P. paneum* and *P. roqueforti* showing a closer relationship with each other than with *P. carneum*.

Because *P. paneum* and *P. roqueforti* had fairly similar volatile metabolite profiles, distinguishing them by use of an electronic nose might be more difficult than distinguishing any of the two from *P. carneum*. This of course is valid only if the sesquiterpenes play an important role in the overall volatile metabolite profiles as perceived by the electronic nose sensors. Work is in progress in our laboratory to investigate whether it is possible to distinguish the species in the *P. roqueforti* group by electronic nose as an instrument to predict mycotoxin production by species differentiation.

The presence of (+)-aristolochene was determined by comparison of RI and mass spectrum, including deconvolution of the peaks and relative peak intensities, with an authentic sample. **Figure 3** shows a comparison of matching mass spectra of (+)-aristolochene from the authentic sample and a compound with the same RI from a *P. paneum* sample (IBT 11839). Detection of (+)-aristolochene in *P. roqueforti* matches the results published in ref *31*.

Among the 48 VOCs detected, three diterpenes, all unidentified, were found. The diterpenes were compounds **43** and **45**, found in samples from *P. paneum*, and compound **47**, found in samples from *P. roqueforti*. Volatile diterpene hydrocarbons have rarely been reported from fungi as done in ref *33*. Within the genus *Penicillium* only volatile mono- and sesquiterpenes have been described (*15*−*18*, *22*, *31*, *34*−*36*).

In conclusion, it has been demonstrated that *P. paneum* has a unique volatile metabolite profile when compared to *P. roqueforti* and *P. carneum*. This clearly supports *P. paneum* being a separate species within the *P. roqueforti* group. Second, (+)-aristolochene turned out not to be a unique biomarker for *P. roqueforti* because it was detected in volatile samples from *P. commune*, *P. glandicola*, *P. paneum*, and *P. solitum*. The fact that this important group of fungi can be distinguished by GC-MS makes it feasible that electronic nose technology can also be applied for quality control purposes in the food industry. This, of course, is of particular interest in the cheese industry during both production and storage.

ABBREVIATIONS USED

YES, yeast extract sucrose agar; GC-MS, gas chromatography−mass spectrometry; RI, Kovats retention index; VOC, volatile organic compound; EI, electron ionization.

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LITERATURE CITED

(1) Dolezalek, J.; Hoza, I. Effect of *Penicillium roqueforti* strains on the formation of aldehydes and methyl ketones in cream. *Prum. Potravin* **1969**, *20*, 171−175.

(2) Bianchi-Salvadori, B. The production of *Penicillium roqueforti* and its use in making Gorgonzola cheese. *Dtsch. Molk.−Ztg.* **1987**, *108*, 1237−1240.

(3) Larroche, C.; Tallu, B.; Gros, J. B. Aroma production by spores of *Penicillium roqueforti* on a synthetic medium. *J. Ind. Microbiol.* **1988**, 1−8.

(4) Fan, T. Y.; Hwang, D. H.; Kinsella, J. E. Methyl ketone formation during germination of *Penicillium roqueforti*. *J. Agric. Food Chem.* **1976**, *24*, 443−448.

(5) Moreau, C. *Penicillium roqueforti*, its morphology, physiology, use in cheesemaking, and mycotoxins. *Lait* **1980**, *60*, 254−271.

(6) Engel, G.; Teuber, M. Differentiation of *Penicillium roqueforti* strains by thin-layer chromatography of metabolites. *Milchwissenschaft* **1983**, *38*, 513−516.

(7) Schoch, U.; Luethy, J.; Schlatter, C. Mutagenicity testing of commercial strains of *Penicillium camemberti* and *P. roqueforti*. *Z. Lebensm. Unters. Forsch.* **1984**, *178*, 351−355.

(8) Schoch, U.; Luethy, J.; Schlatter, C. Mycotoxins from *Penicillium roqueforti* and *P. camemberti* in cheese. I. Occurrence of chemically-identified mycotoxins. *Milchwissenschaft* **1984**, *39*, 76−80.

(9) Engel, G.; Prokopek, D. No detection of *Penicillium roqueforti* toxin in cheese. *Milchwissenschaft* **1979**, *34*, 272−274.

(10) Szoltysek, K. Toxic metabolites of *Penicillium roqueforti*. *Rocz. Panstw. Zakl. Hig.* **1982**, *33*, 39−43.

(11) Harwig, J.; Blanchfield, B. J.; Scott, P. M. Patulin production by *Penicillium roqueforti* Thom from grape. *Can. Inst. Food Sci. Technol. J.* **1978**, *11*, 149−151.

(12) Medina, M.; Gaya, P.; Nuñez, M. Production of PR toxin and roquefortine by *Penicillium roqueforti* isolates from Cabrales blue cheese. *J. Food Prot.* **1985**, *48*, 118−121.

(13) Frisvad, J. C.; Filtenborg, O. Terverticillate Penicillia: Chemotaxonomy and Mycotoxin Production. *Mycologia* **1989**, *81*, 837−861.

(14) Boysen, M.; Skouboe, P.; Frisvad, J.; Rossen, L. Reclassification of the *Penicillium roqueforti* group into three species on the basis of molecular genetic and biochemical profiles. *Microbiology* **1996**, *142*, 541−549.

(15) Larsen, T. O.; Frisvad, J. C. Characterization of Volatile Metabolites from 47 *Penicillium*-Taxa. *Mycol. Res.* **1995**, *99*, 1153−1166.

(16) Larsen, T. O.; Frisvad, J. C. Chemosystematics of *Penicillium* Based on Profiles of Volatile Metabolites. *Mycol. Res.* **1995**, *99*, 1167−1174.

(17) Larsen, T. O. Identification of cheese-associated fungi using selected ion monitoring of volatile terpenes. *Lett. Appl. Microbiol.* **1997**, *24*, 463−466.

(18) Fischer, G.; Schwalbe, R.; Möller, M.; Ostrowski, R.; Dott, W. Species-specific production of microbial volatile organic compounds (MVOC) by airborne fungi from a compost facility. *Chemosphere* **1999**, *39*, 795−810.

(19) Zeringue, H. J., Jr.; Bhatnagar, D.; Cleveland, T. E. $C_{15}H_{24}$ volatile compounds unique to aflatoxigenic strains of *Aspergillus flavus*. *Appl. Environ. Microbiol.* **1993**, *59*, 2264−2270.

(20) Jeleń, H. H.; Mirocha, C. J.; Wasowicz, E.; Kaminski, E. Production of volatile sesquiterpenes by *Fusarium sambucinum* strains with different abilities to synthesize trichothecenes. *Appl. Environ. Microbiol.* **1995**, *61*, 3815−3820.

(21) Demyttenaere, J. C. R.; Moriña, R. M.; De Kimpe, N.; Sandra, P. Use of headspace solid-phase microextraction and headspace sorptive extraction for the detection of the volatile metabolites produced by toxigenic *Fusarium* species. *J. Chromatogr. A* **2004**, *1027*, 147−154.

(22) Jeleń, H. H. Volatile Sesquiterpene Hydrocarbons Characteristic for *Penicillium roqueforti* Strains Producing PR Toxin. *J. Agric. Food Chem.* **2002**, *50*, 6569−6574.

(23) Olsson, J.; Börjesson, T.; Lundstedt, T.; Schnürer, J. Detection and quantification of ochratoxin A and deoxynivalenol in barley grains by GC-MS and electronic nose. *Int. J. Food Microbiol.* **2002**, *72*, 203−214.

(24) Börjesson, T.; Eklöv, T.; Jonsson, A.; Sundgren, H.; Schnürer, J. Electronic nose for odor classification of grains. *Cereal Chem.* **1996**, *73*, 457−461.

(25) Jonsson, A.; Winquist, F.; Schnürer, J.; Sundgren, H.; Lundström, I. Electronic nose for microbial quality classification of grains. *Int. J. Food Microbiol.* **1997**, *35*, 187−193.

(26) Berna, A. Z.; Lammertyn, J.; Saevels, S.; Natale, C. D.; Nicolai, B. M. Electronic nose systems to study shelf life and cultivar effect on tomato aroma profile. *Sens. Actuators, B* **2004**, *97*, 324−333.

(27) Korel, F.; Balaban, M. O. Microbial and sensory assessment of milk with an electronic nose. *J. Food Sci.* **2002**, *67*, 758−764.

(28) Magan, N.; Pavlou, A.; Chrysanthakis, I. Milk-sense: a volatile sensing system recognises spoilage bacteria and yeasts in milk. *Sens. Actuators, B* **2001**, *72*, 28−34.

(29) Keshri, G.; Magan, N.; Voysey, P. Use of an electronic nose for the early detection and differentiation between spoilage fungi. *Lett. Appl. Microbiol.* **1998**, *27*, 261−264.

(30) Magan, N.; Evans, P. Volatiles as an indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grain spoilage. *J. Stored Prod. Res.* **1999**, *36*, 319−340.

(31) Demyttenaere, J. C. R.; Adams, A.; van Belleghem, K.; De Kimpe, N.; König, W. A.; Tkachev, A. V. De novo production of (+)-aristolochene by sporulated surface cultures of *Penicillium roqueforti*. *Phytochemistry* **2001**, *59*, 597−602.

(32) Larsen, T. O.; Frisvad, J. C. A simple method for collection of volatile metabolites from fungi based on diffusive sampling from Petri dishes. *J. Microbiol. Methods* **1993**, *19*, 297−305.

(33) Oikawa, H.; Toshima, H.; Ohashi, S.; König, W. A.; Kenmoku, H.; Sassa, T. Diversity of diterpene hydrocarbons in fungus *Phoma betae*. *Tetrahedron Lett.* **2001**, *42*, 2329−2332.

(34) Demyttenaere, J. C. R.; Moriña, R. M.; Sandra, P. Monitoring and fast detection of mycotoxin-producing fungi based on headspace solid-phase microextraction and headspace sorptive extraction of the volatile metabolites. *J. Chromatogr.* **2003**, *985*, 127−135.

(35) Schnürer, J.; Olsson, J.; Börjesson, T. Fungal Volatiles as Indicators of Food and Feeds Spoilage. *Fungal Genet. Biol.* **1999**, *27*, 209−217.

(36) Nilsson, T.; Larsen, T. O.; Montanarella, L.; Madsen, J. Ø. Application of headspace solid-phase microextraction for the analysis of volatile metabolites emitted by *Penicillium* species. *J. Microbiol. Methods* **1996**, *25*, 245−255.

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