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Volatile Sesquiterpene Hydrocarbons Characteristic for *Penicillium roqueforti* Strains Producing PR Toxin

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Volatile metabolites that might accompany production of PR toxin by *Penicillium roqueforti* were investigated. Volatiles and PR toxin were evaluated for 16 strains of *P. roqueforti*. Solid phase microextraction was used for isolation of volatiles. Thirteen strains produced PR toxin, and all of them produced a specific set of sesquiterpene hydrocarbons including (+)-aristolochene—an intermediate in PR toxin biosynthesis, β-bisabolene, α-chamigrene, diepi-α-cedrene, β-elemene isomer, β-elemene, β-gurjunene, β-himachalene, α-panasinsene, β-patchoulene, β-patchoulene isomer, α-selinene, and valencene. Aristolochene and the remainder of the sesquiterpene hydrocarbon profile were unique for *P. roqueforti* producing PR toxin. They were absent in nontoxigenic *P. roqueforti* and in 40 strains of other *Penicillium* species. Volatile compounds, sesquiterpene hydrocarbons, and aristolochene paralleled PR toxin synthesis. Incubation temperature (20, 24, or 27 °C) and water content in the medium (20, 30, or 40%) influenced the amount of produced sesquiterpenes, but not their profile, suggesting it is species specific. The sesquiterpene hydrocarbon pattern and especially aristolochene can be used as volatile markers for detecting the process of undergoing biosynthesis of PR toxin by *P. roqueforti*.

KEYWORDS: ***Penicillium roqueforti*; PR toxin; sesquiterpene hydrocarbons; aristolochene**

INTRODUCTION

Penicillium roqueforti is used for the production of a blue-veined Roquefort, Stilton, Gorgonzola, and other cheeses of this type, contributing to their characteristic flavor, mainly due to its ability to convert medium-chain fatty acids to their corresponding methyl ketones with one fewer carbon atom (*1*, *2*). However, this fungus is also a widespread contaminant of various food products and feeds, as it can grow at low temperatures, in an atmosphere containing as much as 90% CO_2, and in the presence of sorbate, 5% lactic acid, or 0.5% acetic acid (*3*−*7*).

In 1983 two subspecies of *P. roqueforti* were identified, and in 1996 three subspecies were described on the basis of secondary metabolites and genetic variation (*8*−*11*). *P. roqueforti* var. *roqueforti* used for the production of blue-veined cheeses produces characteristic dark green to blackish pigments, PR toxin, roquefortine C, mycophenolic acid, and isofumiclavin A. *P. roqueforti* var. *carneum* and *P. roqueforti* var. *paneum* have creamy, beige, or light brownish colonies. *P. roqueforti* var. *carneum* produces roquefortin C, mycophenolic acid, penitrem A, and patulin, whereas *P. roqueforti* var. *paneum* forms patulin and botrydiploidin. Strains that produce PR toxin do not produce patulin and vice versa (*10*).

PR toxin [7-acetoxy-5,6-epoxy-3,5,6,7,8,8a-hexahydro-3′,8,-8a-trimethyl-3-oxaspiro[naphthalene-2(1*H*,2′oxirane)]-3′-carboxyaldehyde] is a sesquiterpenoid toxic metabolite of which the aldehyde group is responsible for the toxic character (*12*, *13*). PR toxin is a derivative of the sesquiterpene hydrocarbon aristolochene, and the crucial enzyme involved in biosynthesis of this compound is aristolochene syntase (*14*, *15*). The simplified scheme of PR toxin and related metabolites biosynthesis in *P. roqueforti* is presented in **Figure 1**.

Use of volatile compounds produced by various *Penicillium* species has been proposed in the chemotaxonomy of this genus, and classification of different strains based on this character has been demonstrated (*16*). However, no data have been presented on the relationship of the biosynthesis of specific volatile compounds to the formation of PR toxin.

The objectives in this study were (i) to identify characteristic volatile compounds of *P. roqueforti* that produce PR toxin and compare them with volatiles produced by nontoxigenic *P. roqueforti* and other *Penicillium* species, (ii) to monitor formation of these metabolites during fungal growth on solid medium, and (iii) to evaluate the variations in culture conditions (temperature and water content) on the amount and profile of produced volatiles and PR toxin synthesis. Identification of volatile metabolites of toxigenic fungi, which are species specific and not prone to environmental variations, is a first step in developing detection methods for these microorganisms invading stored food products or feeds based on volatile compound analysis.

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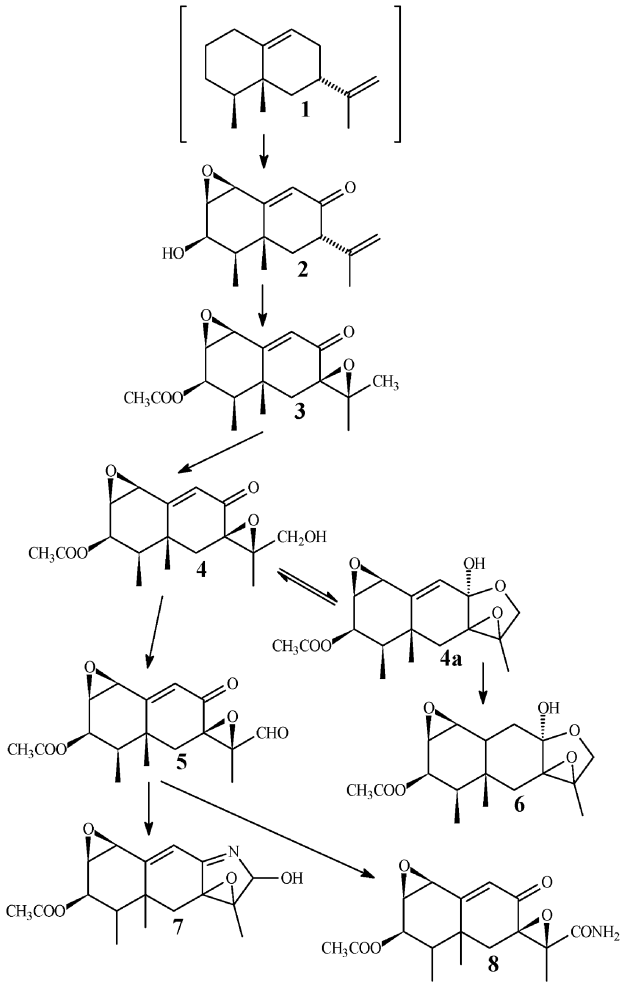

Figure 1. Simplified scheme of PR toxin and related compounds biosynthesis [modified after Moreau et al. (*21*)]: **1**, aristolochene; **2**, eremofortin B; **3**, eremofortin A; **4**, eremofortin C; **4a**, eremofortin C (acetal form); **5**, PR toxin; **6**, eremofortin D; **7**, PR imine; **8**, PR amide.

MATERIALS AND METHODS

Fungal Isolates, Media, and Incubation. Sixteen strains of *P. roqueforti* were obtained from the following fungal collections: American Type Culture Collection (ATCC, Manassas, VA); Department of Biotechnology, Denmark Technical University, Copenhagen, Denmark (IBT); and Institute of Food Technology, Agricultural University of Poznań, Poland (KP). Other *Penicillium* species originated from Agricultural University of Poznań (KP). These were *P. albicans* (KP-68), *P. biforme* (KP-78), *P. citrinum* (KP-94, KP-239, KP-241), *P. expansum* (KP-64, KP-92), *P. terrestre* (KP-53), *P. albidum* (KP-72), *P. nigricans* (KP-29, KP-11), *P. brevicompactum* (KP-44), *P. verrucosum* (KP-2, KP-36, KP-18), *P. viridicatum* (KP-77, KP-30, KP-73, KP-55, KP-17), *P. oxalicum* (KP-48), *P. crustosum* (KP-33), *P. janthinellum* (KP-54, KP-47), *P. funiculosum* (KP-235), *P. rugulosum* (KP-52, KP-238), and *Penicillium* spp. (12 strains). Isolates were grown on PDA agar slants (*17*) for 10 days and then washed with sterile water, and the spore concentration was determined by using a hemacytometer. For the comparison of different isolates spore concentrations ranged from 1.66×10^7/mL to 4.79×10^7/mL, whereas for the dynamics of metabolite formation by strain IBT-19404 and influence of cultivation conditions on the profile of metabolites, spore concentrations were 1.0×10^6/mL and 5.6×10^5/mL, respectively. Incubation was performed in triplicates, in 100 mL bottles filled with 20 g of wheat kernels, moistened to 40% water content, and autoclaved at 121 °C for 20 min.

Bottles were capped during incubation with cotton plugs, which were replaced with crimp-top caps of silicon rubber with a Teflon layer at the volatile sampling time. After inoculation, bottles were placed at 20, 24, or 27 °C for up to 17 days depending upon the experiment. When different strains were compared, they were incubated at 20 °C for 14 days, after which time volatile compounds were collected and analyzed and the amount of PR toxin produced was determined. When the dynamics of volatile metabolites and PR toxin formation was monitored of 18 bottles inoculated with spore suspension, three bottles were taken for analysis on each sampling day.

Analysis of Volatile Compounds. Volatile compounds were isolated from medium by using a solid phase microextraction technique (SPME). Fiber coated with 1 cm PDMS phase (Supelco, Bellefonte, PA) was used for the extraction. After incubation, 5 μg of tridecane in pentane was added to each bottle, which was subsequently capped. Bottles were placed in a water bath (40 °C), and the membrane was pierced with an SPME syringe to expose the fiber to the headspace of the fungal culture. Volatiles were collected for 20 min. Afterward, the fiber was retracted into the SPME syringe and the sample transferred to an injection port of a gas chromatograph coupled to a mass spectrometer, where compounds were desorbed at 260 °C for 5 min. A Hewlett-Packard 5890 II gas chromatograph with an HP 5971 mass detector was used, and compounds were resolved on an MDN-5 column (30 m length × 0.25 mm i.d. × 0.25 μm film, Supelco). Analysis was performed over programmed temperature: 40 °C for 3 min, then raised at 8 °C/min to 280 °C. The temperature of the GC-MS interface was 280 °C. Identification of volatile compounds was based on a comparison of their mass spectra with those present in the NBS 75K library and by matching retention indices with data available in the literature (*18*−*20*). Aristolochene identity and its optical configuration in strain IBT 16404 were confirmed by comparison with authentic standard. On this basis, the remaining *P. roqueforti* cultures were cochromatographed with IBT-16404 on two chiral columns: Restek β-Dex sm and β-Dex sa (both 30 m × 0.32 mm × 0.25 μm) at 2 °C/min from 40 to 160 °C with hydrogen as a carrier gas at 69 cm/s to confirm the optical identity of aristolochene in all strains.

For comparison of volatile production in the performed experiments peak areas were used. To minimize sampling and injection inconsistencies and mass detector response changes over a time period, tridecane was added to cultures prior to the SPME sampling process and peak areas were normalized to the area of added tridecane. Cluster analysis of volatile compounds was performed using Statgraphics Plus 5.0 International Professional software (Mangustics, Inc., Rockville, MD).

Analysis of PR Toxin. After the volatiles were collected, 20 g of inoculated wheat medium was ground with a mortar and pestle in a mixture of 50 mL of acetone and 100 mL of chloroform at room temperature until a homogeneous mass was obtained. The sample was transferred to an Erlenmeyer flask and shaken at room temperature (20−22 °C) for 1 h at 150 rpm. After that, the sample was filtered under reduced pressure and transferred to a separatory funnel, where the organic and aqueous layers were separated. The organic layer was evaporated to dryness under reduced pressure in a rotary evaporator (at 40 °C) and transferred with three portions of chloroform to a vial, where it was dried further under a stream of nitrogen and finally redissolved in 0.5 mL of chloroform. Extracts (5 or 10 μL) were placed on a TLC plate (5553 type, Merck, Darmstadt, Germany), which was run in two directions. As a first solvent a mixture of toluene/ethyl acetate (7:3 v:v) was used and as a second one, chloroform/methanol/25% aqueous ammonia (90:10:1 v/v/v). Dried plates were placed in a chamber saturated with ammonia for 3 min. R_f values and intensities of spots were compared with those of PR standard at UV 366 nm after exposure of plates to UV 254 nm for 0.5 min. The recovery of PR toxin spiked into the wheat matrix at 1 mg/kg was 95%, whereas the limit of detection was 50 ng/spot.

RESULTS AND DISCUSSION

Identification of Volatile Compounds Characteristic for *P. roqueforti* Strains Producing PR Toxin. Of the 16 strains examined 13 produced PR toxin and 3 did not. The first group consisted of strains producing from 2.0 to 867 mg/kg of PR toxin: ATCC-48778 (2.0 mg/kg), ATCC-48779 (2.3 mg/kg), ATCC-48936 (13.3 mg/kg), ATCC-6989 (16.6 mg/kg), IBT-16404 (867 mg/kg), IBT-19482 (6.3 mg/kg), IBT-19480 (2.3

Table 1. EI Mass Spectra (Eight Most Abundant Ions) of Sesquiterpene Hydrocarbons Detected in *P. roqueforti* Cultures Producing PR Toxin

no.	compound	RI[a]	hit[b]	most abundant ions (*m*/*z*) and their intensities (%)
1	unidentified	1367		123 (100), 119 (55), 105 (54), 204 (53), 161 (49), 41 (46), 107 (39), 91 (31)
2	β-patchoulene	1374	99	161 (100), 189 (95), 119 (70), 105 (68), 93 (58), 204 (45), 91 (45), 41 (43)
3	β-elemene isomer	1384		93 (100), 81 (87), 41 (78), 67 (78), 68 (72), 79 (56), 107 (39), 53 (38)
4	β-elemene	1389	99	81 (100), 93 (96), 67 (90), 41 (84), 79 (67), 53 (60), 107 (59), 69 (57)
5	diepi-α-cedrene	1399	91	119 (100), 93 (43), 41 (43), 105 (33), 91 (30), 69 (22), 55 (19), 204 (17)
6	unidentified	1421		133 (100), 91 (18), 119 (14), 41 (13), 105 (12), 161 (12), 55 (7), 204 (6)
7	β-gurjunene	1434	83	161 (100), 105 (68), 41 (56), 91 (52), 119 (37), 107 (34), 204 (34), 55 (32)
8	β-patchoulene isomer	1441		119 (100), 189 (72), 161 (72), 91 (36), 133 (35), 105 (33), 204 (29), 41 (28)
9	unidentified	1446		189 (100), 133 (68), 91 (58), 175 (54), 105 (52), 119 (50), 41 (44), 204 (42)
10	unidentified	1450		105 (100), 106 (88), 120 (51), 91 (49), 119 (46), 204 (41), 41 (40), 176 (24)
11	(+)-aristolochene	1481	*c*	105 (100), 189 (72), 91 (68), 41 (62), 93 (51), 107 (46), 80 (43), 121 (41)
12	valencene	1484	99	161 (100), 91 (89), 79 (88), 41 (81), 105 (68), 93 (80), 107 (67), 119 (59)
13	α-selinene	1489	96	41 (100), 189 (96), 93 (92), 91 (79), 81 (73), 79 (70), 133 (69), 55 (59)
14	β-himachalene	1493	92	119 (100), 41 (82), 93 (66), 91 (60), 105 (51), 55 (35), 79 (26), 204 (24)
15	α-chamigrene	1507	81	121 (100), 136 (62), 91 (20), 77 (19), 41 (19) 79 (16), 105 (16), 93 (15)
16	β-bisabolene	1513	93	69 (100), 41 (73), 93 (52), 91 (37), 77 (37), 55 (30), 105 (28), 133 (22)
17	α-panasinsen	1518	97	161 (100), 122 (82), 107 (61), 41 (41), 91 (35), 105 (32), 79 (29), 204 (24)

[a] Detection indices on MDN-5 column. [b] Hit quality when spectrum was compared to NBS 75K mass spectra database (%); compounds identified tentatively by RI and GC-MS. [c] Identification made by comparison with authentic standard.

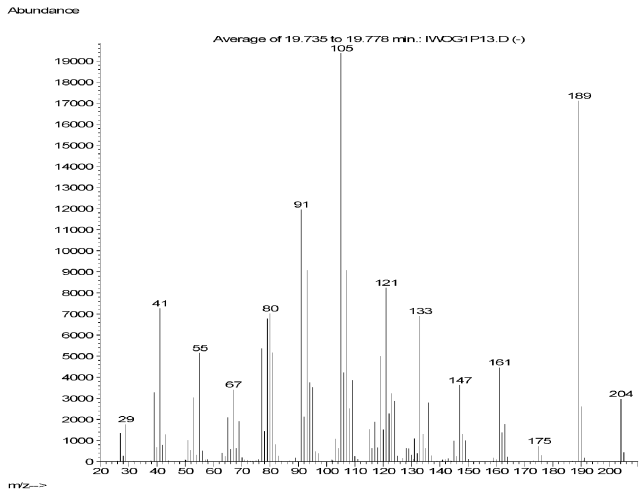

Figure 2. Mass spectrum of aristolochene isolated from *P. roqueforti* strain IBT-16404. Spectrum was acquired on a quadrupole instrument by using electron impact ionization at 70 eV.

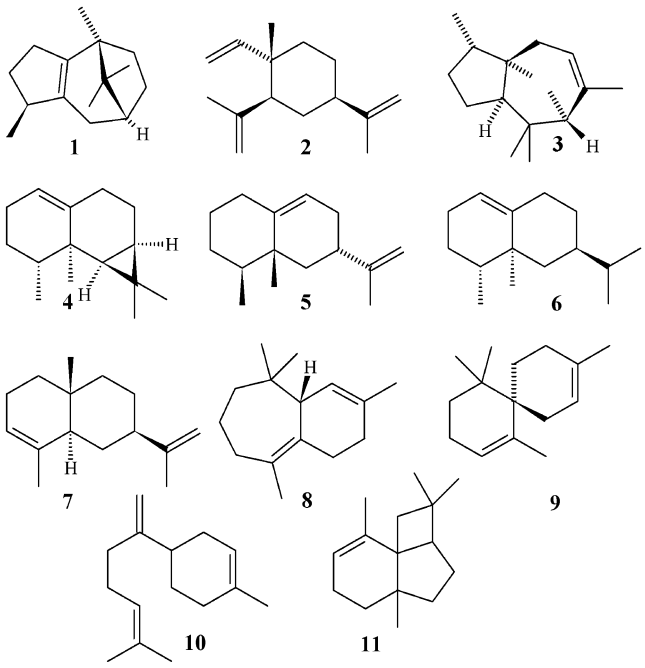

Figure 3. Structures of volatile sesquiterpene hydrocarbons of *P. roqueforti* strains producing PR toxin: **1**, β-patchoulene; **2**, β-elemene; **3**, diepi-α-cedrene; **4**, β-gurjunene; **5**, (+)-aristolochene; **6**, valencene; **7**, α-selinene; **8**, β-himachalene; **9**, α-chamigrene; **10**, β-bisabolene; **11**, α-panasinsen.

mg/kg), IBT-5299 (9.3 mg/kg), KP-S1 (4.0 mg/kg), KP-S2 (3.0 mg/kg), KP-S3 (5.3 mg/kg), KP-S4 (4.7 mg/kg), and KP-S5 (4.3 mg/kg). The three nontoxigenic strains were ATCC-10422, ATCC-42294, and KP-243.

All strains producing PR toxin showed similar profiles of volatile compounds. The largest fraction of volatiles produced by strains capable of synthesizing PR toxin was made up of sesquiterpene hydrocarbons. Differences between toxigenic strains were noted mainly in the amounts of sesquiterpenes produced, not in their profile. The majority of 17 sesquiterpenes were identified on the basis of their retention indices and mass spectra. Data enabling their identification are summarized in **Table 1**. Retention indices agreed well with data in the literature, and this was the main criterion for identification of compounds when mass spectra were very alike. Although the sesquiterpene fraction consisted of additional compounds, it was impossible to identify them all due to the overlapping spectra of the unresolved peaks. Prevailing compounds were aristolochene, β-elemene, valencene, and α-selinene. The presence of aristolochene (**Figure 2**) is the most characteristic feature of PR-toxin-producing *P. roqueforti*. Aristolochene present in investigated *P. roqueforti* cultures was identified as the (+)-isomer. Moreau et al. (*21*) proposed the presence of a sesquiterpene with an aristolochene-like structure as the starting point in PR toxin biosynthesis. Fungal production of aristolochene from *Aspergillus terreus* was first reported by Cane et al. (*22*), and only recently it has been reported as a *P. roqueforti* volatile metabolite together with α-elemene, γ-patchoulene, *E*-β-caryophyllene, valencene, α-selinene, and α-panasinsene (*23*). Larsen and Frisvad (*16*) investigated volatiles of *P. roqueforti* var. *roqueforti* and identified β-elemene, γ-patchoulene, β-caryophyllene, patchoulene isomer, eremophilene, and α-selinene as the most unique sesquiterpene hydrocarbons. Moreover, the unidentified compound RI 1521 they found might be aristolochene as its mass spectrum is very similar to the mass spectrum of aristolochene. Chalier and Crouzet identified β-elemene as a compound produced at high levels by *P. roqueforti* (*24*). The presence of β-elemene as one of the prevailing sesquiterpenes in the 13 strains producing PR toxin

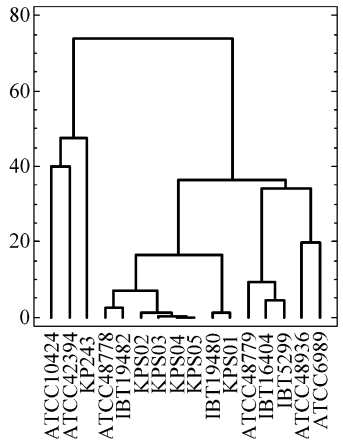

Figure 4. Dendrogram of 16 isolates of *P. roqueforti* with content of volatile compounds as variables.

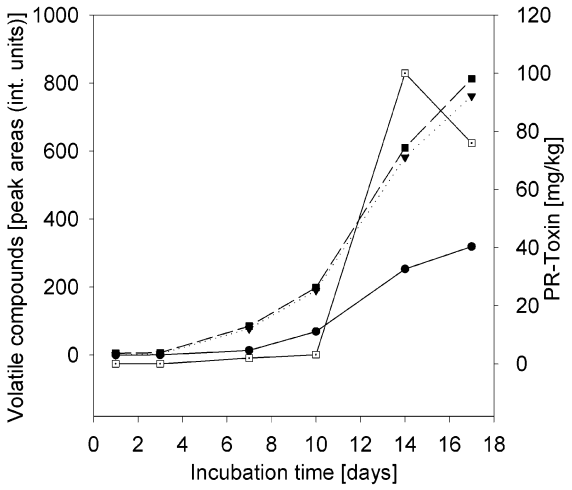

Figure 5. Dynamics of the formation of volatile compounds, including sesquiterpenes and aristolochene, and PR toxin by *P. roqueforti* strain IBT-16404 during 17 days of incubation on autoclaved wheat kernels medium: (●) aristolochene; (▼) sesquiterpene hydrocarbons; (■) total volatiles; (⊡) PR toxin.

is confirmatory of these results. The compounds diepi-α-cedrene, β-gurjunene, β-himachalene, and α-chamigrene are reported as *P. roqueforti* metabolites for the first time in this study (**Figure 3**).

Apart from sesquiterpene hydrocarbons, other compounds known as fungal metabolites were isolated from vapors of toxigenic strains: isoamyl alcohol, 1-octene-3-ol, 3-octanol, 3-octanone, 3-cyclohepten-1-one, β-myrcene, limonene and (+)-2-carene.

To determine the relationship between the amount of PR toxin and the amount of specific volatile metabolite production after 14 days of growth, correlation coefficients were calculated between PR toxin and total volatiles (0.8639), between PR toxin and sesquiterpene hydrocarbons (0.8946), and between PR toxin and aristolochene (0.9750, all at $P = 0.01$).

Only three of the analyzed *P. roqueforti* strains did not produce PR toxin. Although classified as *P. roqueforti*i Thom, they differed from toxigenic strains as the color of the formed colonies was not dark green but beige (suggesting classification of strains as *P. roqueforti* var. *carneum* or *paneum*, not *P. roqueforti* var. *roqueforti*). For these strains the sesquiterpene hydrocarbons were generally absent. All strains differed significantly among themselves. The most abundant volatiles of nontoxigenic strains KP-243 and ATCC-42294 were compounds with a molecular weight of 218 amu—unsaturated sesquiterpene alcohols or ketones—and diterpenes with a molecular weight of 272 amu. Strain ATCC-10422 produced few volatiles compared to the others—toxigenic and nontoxigenic. In nontoxigenic strains the same alcohols and ketones were identified as in the toxigenic ones, but the only monoterpene detected was limonene.

The similarity between *P. roqueforti* strains was evaluated by constructing a dendrogram based on the amounts of volatile compounds eluting from 17 to 21 min (total of 41 compounds for all examined strains). The unweighted pair group method with arithmetic mean algorithm (UPGMA) with squared Euclidean distances was used for the cluster formation. All *P. roqueforti* strains were found to cluster into two groups of 13 toxigenic and 3 nontoxigenic ones, with highest variation between nontoxigenic strains observed (**Figure 4**). The variation between toxigenic strains would be minimized or eliminated if the cluster analysis had been based on a binary matrix (presence or absence of a compound) instead of relative amount of compounds.

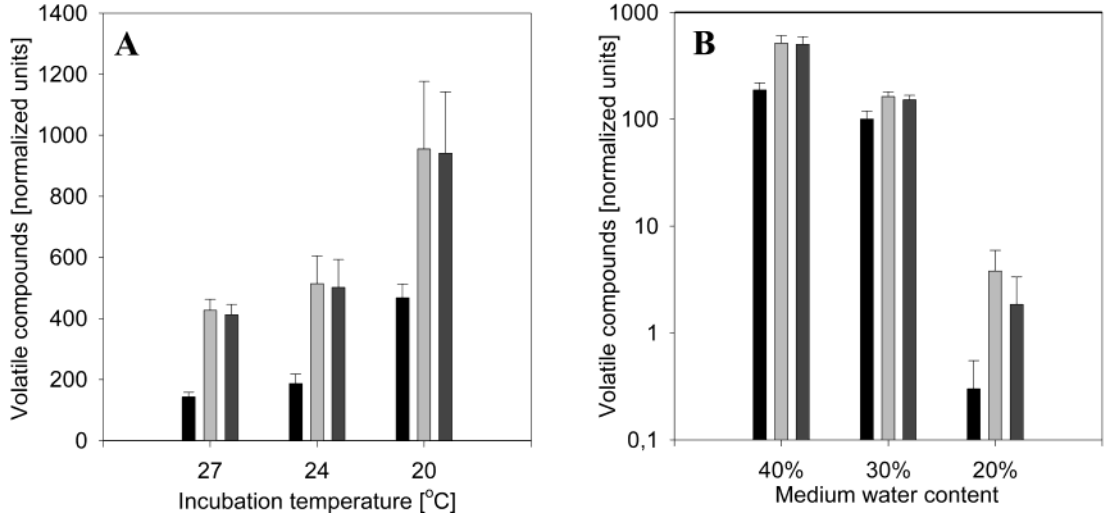

Figure 6. Influence of incubation temperature (A) and medium water content (B) on the formation of volatile compounds by *P. roqueforti* strain IBT-16404. In (A) volatile compounds were analyzed after 14 days of incubation on medium containing 40% water; in (B) volatiles were analyzed after 14 days of incubation at 20 °C. In each group, bars represent, from left to right, aristolochene, total volatiles, and sesquiterpene hydrocarbons.

Comparison of the Volatile Sesquiterpene Profile of *P. roquefortii* with Those of Other *Penicillium* Species. To establish whether the presence of aristolochene and the unique profile of sesquiterpene hydrocarbons are characteristic only for *P. roquefortii*, 40 strains representing different species were checked for the presence of aristolochene and other sesquiterpene hydrocarbons characteristic for toxigenic *P. roqueforti* strains. No aristolochene was detected in any of the examined strains. Moreover, the pattern of volatile sesquiterpenes characteristic for toxigenic *P. roqueforti* was not found in any of 40 examined strains.

Dynamics of PR Toxin and Volatile Compound Formation in *P. roqueforti* IBT-16404. The most efficient producer of PR toxin was chosen for monitoring the dynamics of formation of this compound and volatiles. After the first day, mostly monoterpenes were produced, whereas after 3 days seven sesquiterpenes were detected with aristoloche, α-elemene being the most abundant. At the seventh day of incubation 29 compounds were detected, among which 21 were sesquiterpene hydrocarbons. These compounds comprised 52% of the volatiles after the third day, and after day 7, they comprised 91% of volatiles. PR toxin was detected in the culture of IBT-16404 after 7 days of incubation (2 mg/kg). Its concentration increased to 100 mg/kg after 14 days and then decreased to 76.4 mg/kg on the last day of incubation (**Figure 5**), probably due to its conversion to one of derivatives—PR imine, PR amide, or PR acid (*25*, *26*).

The concentration of volatiles including aristolochene increased throughout the whole incubation period. The emission of aristolochene paralleled PR toxin production, and aristolochene could be detected in vapors prior to the formation of PR toxin. The amount of aristolochene increased throughout the entire incubation period.

Influence of Some Growth Conditions on the Production of Volatile Metabolites and PR Toxin by *P. roqueforti* IBT-16404. An increase in the formation of volatile compounds was observed as the incubation temperature decreased (**Figure 6A**). At 20 °C the total amount of volatiles was twice the amount of volatiles produced at 27 °C. Despite the differences in amounts of produced metabolites their profile remained unchanged. The most abundant fraction of volatile metabolites was sesquiterpene hydrocarbons. The highest ratio of aristolochene compared to other volatiles was noted for 20 °C. As one of the most important factors affecting fungal growth is the water availability, incubation was performed at 20, 30, and 40% wc. The water content in a medium influenced the amount of produced volatiles much more than the incubation temperature did (**Figure 6B**). Although the profiles of produced volatile sesquiterpenes were identical for media containing 40 and 30% water, respectively, decreasing the water contents to 20% resulted in a dramatic decrease in the amounts of produced volatiles (associated with slower fungal growth). As a result, among detectable compounds were mainly sesquiterpene hydrocarbons present in the highest amounts: aristolochene, β-elemene, and β-patchoulene isomer. In all examined conditions the profile of volatile sesquiterpenes remained unchanged. The formation of PR toxin was highly influenced by incubation conditions: from 0 mg/kg (sample A, inoculated on medium containing 20% water and incubated at 24 °C) to 25 μg/g (sample E, 40% water content and incubated at 20 °C). Data shown in **Figure 7** reveal that changing incubation parameters influenced both PR toxin biosynthesis and production of volatile compounds. These processes were highly correlated. The correlation coefficient between total volatiles and PR toxin was 0.9836 ($P = 0.01$), and that between aristolochene and PR toxin was 0.9376 ($P = 0.05$). This indicates, similarly as in dynamics experiment, that formation of volatile compounds parallels the production of PR toxin by *P. roqueforti*.

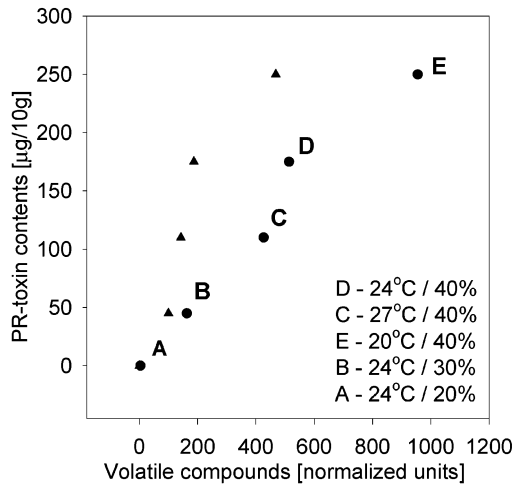

Figure 7. Relationship between amount of produced PR toxin and total amount of biosynthesized volatile compounds and aristolochene for various medium temperature and water contents for *P. roqueforti* strain IBT-16404: (●) total volatile compounds; (▲) aristolochene.

Volatile compounds synthesized by *Penicillium* strains have been examined before (*27*−*32*). Larsen and Frisvad (*16*) characterized 132 *Penicillium* isolates from 25 taxa, noting that strains of the same species have similar profiles of volatile compounds and that these profiles can easily be distinguished. They also noted a similar profile of low-boiling compounds.

The most important volatile compound of *P. roqueforti* from the point of PR toxin biosynthesis was aristolochene. However, one must remember that aristolochene is a secondary metabolite which does not have to be transformed into PR toxin in other fungal genera or species. Data presented for *P. roqueforti* indicate that increased ability to form volatiles, especially sesquiterpenes, accompanies the high potential to produce PR toxin. The presence of aristolochene, together with the specific profile of all sesquiterpenes, is the most reliable indicator that this fungus is undergoing PR toxin biosynthesis. This feature may be utilized in the detection of toxigenic *P. roqueforti* based on headspace analysis.

Aristolochene is probably the last detectable volatile compound in the pathway of PR toxin formation. A similar relationship was found in *Fusarium*, which produced trichothecenes, where all toxigenic strains produced trichodiene as a volatile marker during their biosynthesis (*33*, *34*). Therefore, it may be concluded that the biosynthesis of sesquiterpenoid mycotoxins triggers the formation of accompanying volatile sesquiterpenes, characteristic for the type of toxin and producing species or genera.

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