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Characterization of *Stachybotrys* from water-damaged buildings based on morphology, growth, and metabolite production

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Abstract: *Stachybotrys* was found to be associated with idiopathic pulmonary hemorrhage in infants in Cleveland, Ohio. Since that time, considerable effort has been put into finding the toxic components responsible for the disease. The name *Stachybotrys chartarum* has been applied to most of these isolates, but inconsistent toxicity results and taxonomic confusion prompted the present study. In this study, 122 *Stachybotrys* isolates, mainly from water-damaged buildings, were characterized and identified by combining three different approaches: morphology, colony characteristics, and metabolite production. Two different *Stachybotrys* taxa, *S. chartarum* and one undescribed species, were found in water-damaged buildings regardless of whether the buildings were in Denmark, Finland, or the USA. Furthermore, two chemotypes could be distinguished in *S. chartarum*. One chemotype produced atranones, whereas the other was a macrocyclic trichothecene-producer. The second undescribed taxon produced atranones and could be differentiated from *S. chartarum* by its growth characteristics and pigment production. Our results correlate with different inflammatory and toxicological properties reported for these same isolates and show that the three taxa/chemotypes should be treated separately. The co-occurrence of these three taxa/chemotypes in water-damaged buildings explains the inconsistent results in the literature concerning toxicity of *Stachybotrys* isolated from that environment.

Key Words: Atranones, colony diameter, identification, macrocyclic trichothecenes, pigment production, *Stachybotrys chartarum*

INTRODUCTION

There is an increasing concern about the adverse health effects associated with fungal growth in indoor environments (Johanning et al 1996, Gravesen et al 1999), and *Stachybotrys* has become the focus of attention after reports of its association with idiopathic pulmonary hemorrhage (IPH) in infants in Cleveland, Ohio (Dearborn et al 1999, Vesper et al 2000b). Stachybotryo-toxicoses have long been known in agriculture when wet, *Stachybotrys*-infected hay was consumed by farm animals (Forgacs and Carll 1962). The strong cellulolytic ability of species of *Stachybotrys* (Udagawa 1984) also allows it to grow on water-damaged building materials such as gypsum board, wallpaper and insulation (Gravesen et al 1999). Macrocyclic trichothecenes (e.g., satratoxins, roridins and verrucarins) seem to be the causative components of toxicoses for farm animals eating contaminated hay (Jarvis et al 1986). Considerable effort is now being put into finding the toxic components associated with IPH.

In addition to the macrocyclic trichothecenes, species of *Stachybotrys* are able to produce a number of other components or secondary metabolites: simple trichothecenes, such as trichodermol and trichodermol acetate (trichodermin), (Nielsen et al 1998a, Hinkley and Jarvis 2000); atranones, including their dolabellane precursors (Hinkley et al 2000); spirocyclic drimanes (Ayer and Miao 1993, Jarvis et al 1995); trichoverroids (Croft et al 1986) and the hemolytic protein stachylysin (Vesper et al 2001). Some of these metabolites can cause skin irritation (Gravesen et al 1994) or have immunosuppressant effects (Fung et al 1998). Others are cytotoxic and can induce inflammatory responses (Routsalainen et al 1998) and may be a cause of IPH (Vesper et al 2001). Both in vitro and in vivo studies have revealed novel components that induce inflammation (Routsalainen et al 1998), altered surfactant production in lung tissue (Mason et al 2001), and membrane damage (Pel-

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tola et al 1999). Finally, it has been shown that *Stachybotrys* can produce some of its metabolites during growth on water-damaged building materials in the laboratory (Croft et al 1986, Nielsen et al 1998a,b, Vesper et al 2000b).

Because *Stachybotrys* is considered to be responsible for toxicoses in humans (Flannigan and Miller 1994), it is of great importance that this fungus is detected (Andersen and Nissen 2000) and identified quickly and correctly. The list of synonyms for *S. chartarum* (Ehrenb. ex Link) Hughes, however, is long and the inconsistent use of *S. chartarum* and the synonyms, *S. atra* Corda and *S. alternans* Bon., has contributed to the taxonomic confusion.

In addition to morphology, other approaches have been applied in order to facilitate correct identification of fungi. The production of different metabolites in different species has been used as a means of species characterization and identification in *Aspergillus*, *Fusarium* and *Penicillium* (Frisvad and Filtenborg 1990, Frisvad et al 1998) and *Alternaria* (Andersen et al 2001), but only few papers dealing with species specific metabolites produced by *Stachybotrys* exist (El-Maghraby et al 1991). Physiological criteria, such as growth characteristics on different media and at different water activities and temperatures, have also been used to aid identification of species in *Penicillium* (Pitt 1980) and *Alternaria* (Andersen et al 2001), but such approaches have never been used systematically in the characterization and identification of *Stachybotrys*. Jong and Davis (1976) did, however, record pigmentation and colony diameter.

The purpose of this study was to characterize *Stachybotrys* isolates from water-damaged buildings by combining morphology and growth characteristics with metabolite production in order to determine which taxa are present and to facilitate future identification efforts. The study included 28 *Stachybotrys* isolates from the USA, 30 from Finland, and 15 from Denmark, all isolated from water-damaged buildings. An additional 49 *Stachybotrys* isolates from substrata other than building material and from culture collections were included.

MATERIALS AND METHODS

Fungi, media and growth conditions.—One hundred twenty two isolates of *Stachybotrys* were studied. The identity, other numbers, origin and source of all isolates are shown in TABLE I. All isolates are held at the culture collection at BioCentrum-DTU, Denmark and maintained as soil cultures. Each isolate was inoculated as three-point inoculations onto the following six media: Alkaloid forming agar (ALK) (Reshetilova et al 1992), Czapek yeast extract agar (CYA) (Samson et al 2000), Potato sucrose agar (PSA) (Samson et al 2000), Sigma yeast extract sucrose agar (SYES) (Filtenborg et al 1990), V8 juice agar (V8) (Simmons 1992) and Cornmeal agar (CMA) (DIFCO Manual 1969). The inoculated plates were put in perforated plastic bags and incubated in darkness at 25 C for 7 d.

Characterization of fungal isolates.—After 7 d of incubation, macro-morphological characteristics, such as colony diameter and pigment production, were described from ALK, CYA, PSA, SYES and V8. Micro-morphological characterization (e.g., conidial shape and texture) and identification were done from isolates grown on CMA according to Jong and Davis (1976). The isolates were mounted in lactophenol using tape preparations (Butler and Mann 1959) and examined at 400 × and 1000 × magnification. After examination, all isolates on PSA were re-incubated for 7 d in darkness at 25 C before extraction.

Statistical data analysis.—A data matrix, consisting of 78 objects (*Stachybotrys* isolates) and 10 variables (colony diameters and pigmentation on the five media), was constructed. The matrix was standardized (the variable was divided by the maximum value of each variable) and analyzed using Manhattan and UPGMA in NTSYS (Applied Biostatistics Inc., New York. Version 2.02j for Windows).

Chemicals and metabolite standards.—All chemicals used were either analytical or HPLC grade. Heptafluorobuturylimidazol (HFBI) and verrucarol (VER) were obtained from Sigma (St. Louis, Missouri, USA) and 1,12-dodecanediol was obtained from Fluka (Buchs, Switzerland). Trichodermin was provided by Dr. Jytte Hansen, Løvens Kemiske Fabrik A/S (Ballerup, Denmark) and trichodermol was derived by hydrolyzing trichodermin. Standards of satratoxins H, G and iso-F, roridin E, verrucarins B and J, atranones A to H, griseofulvin, dechlorofulvin and iso-dechlorogriseofulvin were available from previous studies in the laboratory of BBJ. Other compounds were compared by their retention indices and UV-spectra to the Mycology Group database containing about 450 fungal secondary metabolites (Frisvad and Thrane 1987, Smedsgaard 1997).

Preparation of extracts.—From 14-d colonies on PSA, ten agar plugs (6 mm in diam) were cut from each isolate and transferred to a 2 mL vial. 1.5 mL methanol was then added and the vials were left overnight. The next day the methanol was transferred to a clean 2 mL vial and evaporated to dryness in a rotary vacuum concentrator (Christ, Gefriertrocknungsanlagen GmbH, Germany) at 1 mbar, 1300 rpm and 25 C. Polyethylene imine (PEI) silica columns (Jarvis 1992) were prepared in 2-mL disposable syringes by packing 1 mL PEI tightly between 2 discs of porous polyethylene filter support material. The columns were preconditioned with 6 mL methanol followed by 6 mL dichloromethane. The dried methanol extract was re-dissolved in 1 mL dichloromethane, loaded onto the column and eluded with 10 mL dichloromethane. The extracts were evaporated to dryness and re-dissolved in 100 μL methanol and filtered through a 0.45 μm syringe filter (Titan 44513-PL, SRI, Eaton town, NJ, USA) into clean 2 mL vials.

TABLE I. Identity, origin and metabolite production of the 122 *Stachybotrys* isolates listed according to metabolite production and chemotype and taxon

Identity #	Substratum[a]/origin	Species[b]	M[c]	T[d]	A[e]	Other #
ba 157	Build/San Diego, USA	*chartarum*	+	+	−	Sp 2630
ba 158	Build/New Jersey, USA	*chartarum*	+	+	−	Sp 2675
ba 160	Build/Finland	*chartarum*	+	+	−	TAA 95/126
ba 165	Build/Belgium	*chartarum*	+	+	−	IHEM 1413
IBT 7709	Build/Denmark	*chartarum*	+	+	−	—
IBT 7711	Build/Denmark	*chartarum*	+	+	−	—
IBT 8935	Soil/Arizona, USA	*chartarum*	+	+	−	—
IBT 9460	-/Finland	*chartarum*	+	+	−	CBS 414.95
IBT 9631	Build/Cleveland, USA	*chartarum*	+	+	−	JS58-03
IBT 9632	Build/Cleveland, USA	*chartarum*	+	+	−	JS58-05
IBT 9696	Build/Finland	*chartarum*	+	+	−	HT-165†
IBT 9698	Build/Finland	*chartarum*	+	+	−	HT-248†
IBT 9700	Build/Finland	*chartarum*	+	+	−	HT-471†
IBT 9702	Build/Finland	*chartarum*	+	+	−	HT-569†
IBT 9703	Build/Finland	*chartarum*	+	+	−	HT-072†
IBT 9709	Build/Finland	*chartarum*	+	+	−	HT-391†
IBT 9710	Build/Finland	*chartarum*	+	+	−	HT-522†
IBT 9713	Build/Finland	*chartarum*	+	+	−	HT-530†
IBT 9731	Build/Belgium	*chartarum*	+	+	−	IHEM 16707
IBT 9768	Cotton fabric/England	*chartarum*	+	+	−	ATCC 16026§
IBT 9769	Cardboard/Finland	*chartarum*	+	+	−	ATCC 26303§
IBT 14916	Build/Denmark	*chartarum*	+	+	−	—
IBT 9690	Build/Finland	*chartarum*	+	−	−	HT-387†
IBT 9697	Build/Finland	*chartarum*	+	−	−	HT-296†
IBT 9704	Build/Finland	*chartarum*	+	−	−	HT-386†
IBT 9807	Build/Cleveland, USA	*chartarum*	+	−	−	JS58-01
IBT 9808	Build/Cleveland, USA	*chartarum*	+	−	−	JS51-11§
IBT 9809	Build/Cleveland, USA	*chartarum*	+	−	−	JS51-20
IBT 9816	Build/Cleveland, USA	*chartarum*	+	−	−	JS51-18
IBT 9817	Build/Cleveland, USA	*chartarum*	+	−	−	JS51-22
IBT 9819	Build/Cleveland, USA	*chartarum*	+	−	−	JS58-18§
IBT 9820	Build/Cleveland, USA	*chartarum*	+	−	−	JS58-02§
IBT 9821	Build/Cleveland, USA	*chartarum*	+	−	−	JS58-17§
ba 162	Human/Finland	*chartarum*	−	+	+	S 8
ba 164	Build/Cleveland, USA	*chartarum*	−	+	+	Sp 2678
ba 170	Build/Cleveland, USA	*chartarum*	−	+	+	Sp 2680
IBT 7617	-/Denmark	*chartarum*	−	+	+	—
IBT 9290	Build/Denmark	*chartarum*	−	+	+	—
IBT 9292	Build/Denmark	*chartarum*	−	+	+	—
IBT 9466	Build/Denmark	*chartarum*	−	+	+	—
IBT 9469	Build/Denmark	*chartarum*	−	+	+	—
IBT 9472	-/Finland	*chartarum*	−	+	+	CBS 413.95
IBT 9633	Build/Cleveland, USA	*chartarum*	−	+	+	BBJ-22
IBT 9634	Build/Cleveland, USA	*chartarum*	−	+	+	BBJ-23
IBT 9692	Build/Finland	*chartarum*	−	+	+	HT-587
IBT 9693	Build/Finland	*chartarum*	−	+	+	HT-401†
IBT 9694	Build/Finland	*chartarum*	−	+	+	HT-513†
IBT 9699	Build/Finland	*chartarum*	−	+	+	HT-584
IBT 9701	Build/Finland	*chartarum*	−	+	+	HT-520†
IBT 9706	Build/Finland	*chartarum*	−	+	+	HT-580
IBT 9712	Build/Finland	*chartarum*	−	+	+	HT-586
IBT 9730	Build/Belgium	*chartarum*	−	+	+	IHEM 16705
IBT 9732	Build/Belgium	*chartarum*	−	+	+	IHEM 16706
IBT 9733	Build/Belgium	*chartarum*	−	+	+	IHEM 16701
IBT 9735	Build/Belgium	*chartarum*	−	+	+	IHEM 16703

TABLE I. Continued

Identity #	Substratum[a]/origin	Species[b]	M[c]	T[d]	A[e]	Other #
IBT 9736	Build/Belgium	*chartarum*	−	+	+	IHEM 16702
IBT 9766	Build/The Netherlands	*chartarum*	−	+	+	CBS 324.65
IBT 14915	Build/Denmark	*chartarum*	−	+	+	—
ba 155	Build/Finland	*chartarum*	−	−	+	S 7
ba 156	Build/Finland	*chartarum*	−	−	+	VTT D-96622
ba 163	Build/Cleveland, USA	*chartarum*	−	−	+	Sp 2676
ba 167	Build/New York, USA	*chartarum*	−	−	+	Sp 2674
ba 172	Build/Finland	*chartarum*	−	−	+	HMRF-4
IBT 9691	Build/Finland	*chartarum*	−	−	+	HT-435†
IBT 9705	Build/Finland	*chartarum*	−	−	+	HT-503†
IBT 9707	Build/Finland	*chartarum*	−	−	+	HT-502†
IBT 9708	Build/Finland	*chartarum*	−	−	+	HT-523†
IBT 9734	Build/Belgium	*chartarum*	−	−	+	IHEM 16704
IBT 9753	Grain/Finland	*chartarum*	−	−	+	VTT D-83222
IBT 9765	Paper/Italy	*chartarum*	−	−	+	CBS 330.37
IBT 9810	Build/USA	*chartarum*	−	−	+	JS58-15§
IBT 9812	Build/USA	*chartarum*	−	−	+	JS51-05§
IBT 9814	Build/USA	*chartarum*	−	−	+	JS63-01§
IBT 9818	Build/USA	*chartarum*	−	−	+	JS58-32
IBT 9822	Build/USA	*chartarum*	−	−	+	JS58-07§
ba 154	Build/Germany	*chartarum*	−	NT[f]	+	Sp 2683
ba 161	Build/Cleveland, USA	*chartarum*	−	NT	+	Sp 2677
ba 166	Build/Germany	*chartarum*	−	NT	+	Sp 2682
ba 171	Build/Cleveland, USA	*chartarum*	−	NT	+	Sp 2679
IBT 9291	Build/Denmark	*chartarum*	−	NT	+	—
IBT 9306	Build/Denmark	*chartarum*	−	NT	+	—
IBT 9307	Build/Denmark	*chartarum*	−	NT	+	—
IBT 9695	Build/Finland	*chartarum*	−	NT	+	HT-518†
ba 168	Build/Finland	*chartarum*	−	−	NT	HMRB-10
ba 159	Build/Cleveland, USA	sp. Group A	−	+	+	Sp 2681
IBT 9299	Build/Denmark	sp. Group A	−	+	+	—
IBT 9467	Build/Denmark	sp. Group A	−	+	+	—
IBT 9825	Build/USA	sp. Group A	−	+	+	JS58-06§
IBT 10219	Soil/Spain	sp. Group A	−	+	+	—
IBT 9226	Seaweed/Denmark	sp. Group A	−	−	+	—
IBT 9293	Build/Denmark	sp. Group A	−	−	+	—
IBT 9714	Build/Finland	sp. Group A	−	−	+	HT-016†
IBT 9755	Paper/Finland	sp. Group A	−	−	+	VTT D-96593
IBT 9756	Grain/Finland	sp. Group A	−	−	+	VTT D-83220
IBT 9757	Build/Finland	sp. Group A	−	−	+	S12
IBT 9767	Fabric/New Guinea	sp. Group A	−	−	+	NRRL 29940
IBT 9823	Build/Cleveland, USA	sp. Group A	−	−	+	JS51-08§
IBT 9824	Build/Cleveland, USA	sp. Group A	−	−	+	JS63-12
IBT 9826	Build/Cleveland, USA	sp. Group A	−	−	+	JS58-26
IBT 9827	Build/Cleveland, USA	sp. Group A	−	−	+	JS58-30
IBT 9225	Seaweed/Denmark	sp. Group A	−	NT	+	—
IBT 9294	Build/Denmark	sp. Group A	−	NT	+	—
IBT 9754	Build/Belgium	sp. Group A	−	−	−	IHEM 9905
ba 169	Hay/Finland	sp. Group A	−	−	NT	69
ba 173	Soil/Iraq	sp. Group A	−	−	NT	IHEM 2248
IBT 9761	Wood/The Netherlands	*albipes*	−	−	−	CBS 365.94
IBT 9762	Wood/Japan	*albipes*	−	−	−	CBS 100343
IBT 9763	Plant/Spain	*bisbyi*	−	−	−	CBS 142.97
IBT 9764	Plant/Taiwan	*bisbyi*	−	−	−	*CBS 268.76*
IBT 9770	-/-	*cylindrospora*	−	−	−	IHEM 17451
IBT 9771	-/-	*cylindrospora*	−	−	−	IHEM 17450

TABLE I. Continued

Identity #	Substratum[a]/origin	Species[b]	M[c]	T[d]	A[e]	Other #
IBT 9773	Plant/England	*dichroa*	+	+	−	CBS 526.50
IBT 9774	-/-	*dichroa*	+	+	−	IHEM 17452
IBT 9775	Soil/Sudan	*microspora*	−	−	+	CBS 186.79
IBT 9458	Soil/Papua New Guinea	*nephrospora*	−	+	−	CBS 769.95
IBT 9776	-/-	*nilagirica*	−	−	−	IHEM 17453
IBT 9473	Plant/Cuba	*oenanthes*	−	+	−	CBS 252.76
IBT 9777	-/-	*oenanthes*	−	−	−	IHEM 17454
IBT 9778	Plant/Cuba	*parvispora*	−	−	−	CBS 100155
IBT 9779	Plant/Spain	*parvispora*	−	−	−	CBS 173.97
IBT 9780	-/-	*parvispora*	−	−	−	IHEM 17455
IBT 9781	-/-	*theobromae*	−	−	−	IHEM 17456

[a] Build: Building material; Plant: Plant material.
[b] Identified on CMA according to Jong and Davis (1976).
[c] M: Macrocyclic trichothecens.
[d] T: Trichodermol.
[e] A: Atranones.
[f] NT: Not tested.
† Isolates used in Routsalainen et al (1998).
§ Isolates used in Jarvis et al (1998) and Vesper et al (1999, 2000a).

HPLC analysis.—Extracts were analyzed on an HP 1090 Series II HPLC equipped with a diode array detector and a C_{18} column using water-acetonitrile gradient system as described by Smedsgaard (1997). The 260 nm signal was used as the detection wavelength, and retention times and UV-spectra were compared with reference standards of trichothecenes, atranones and griseofulvins and with standards from the Mycology Group metabolite database.

GC-MS-MS analysis.—After HPLC analysis the vials with extract were prepared for GC analysis. 100 μL internal standard (0.8 μg 1,12-dodecanediol/mL methanol) was added to each vial and then evaporated to dryness. The samples were redissolved in 200 μL 0.2 M NaOH in methanol and hydrolyzed overnight. The parent trichothecene alcohols, trichodermol (partly originating from trichodermin) and verrucarol (originating from macrocyclic trichothecenes) were derivatized to their heptafluorobuturyl esters and detected using simultaneous GC-MS and GC-MS-MS analysis on a GCQ (Finnigan Corporation, Austin, Texas, USA) as described by Nielsen and Thrane (2001).

RESULTS

Morphological identification.—All 122 isolates were studied on CMA according to Jong and Davis (1976) and the results are shown in TABLE I. All of the 18 cultures from various culture collections that arrived as *S. albipes*, *S. bisbyi*, *S. cylindrospora*, *S. dichroa*, *S. microspora*, *S. nephrospora*, *S. oenanthes*, *S. parvispora*, *S. theobromae* and *S. nilagirica* fit the respective descriptions by Jong and Davis (1976) and Subramanian (1957) well.

Of the 17 cultures from culture collections that had been deposited as *S. chartarum*, 14 fit the description of *S. chartarum* by Jong and Davis (1976) with branched conidiophores and ellipsoidal conidia with a banded or ridged surface. Of the 88 isolates originating from Danish, Finnish, and American building-related material, 70 were identified as *S. chartarum*. The three remaining cultures from culture collections (ba 173 [= IHEM 2248], IBT 9754 [= IHEM 9905] and IBT 9767 [= NRRL 29940 = QM 94d]), were morphologically similar to each other and had the same distinct appearance as the remaining 18 isolates from building materials. These 21 isolates, labeled ''*Stachybotrys* sp. Group A'' (Group A) in TABLE I, did not fit any described species of *Stachybotrys* treated by Subramanian (1957), Barron (1961), Ellis (1971, 1976), Jong and Davis (1976), Domsch et al (1980), Dorai and Vittal (1986) or by McKenzie (1991).

The conidiophores and phialides of these Group A isolates looked similar on CMA to those of *S. chartarum*, while the conidia resembled those of *S. albipes*. The Group A isolates had dark, branched, tuberculate conidiophores and obovate phialides as compared to the *S. albipes* isolates that had simple, smooth, and hyaline conidiophores and ellipsoidal phialides. The conidia of the Group A isolates were ovate (9 × 5 μm) with a smooth surface and a scar at the base, in contrast to the *S. chartarum* isolates that had ellipsoidal conidia (11 × 4 μm) with a rough surface.

Of the 28 *Stachybotrys* isolates from Cleveland, Ohio, 22 isolates were identified as *S. chartarum* and six as Group A, while 28 isolates from Finnish build-

ing materials were identified as *S. chartarum* and only two as Group A. Eleven of the 15 isolates from Danish buildings were *S. chartarum* and four were Group A.

Metabolite production.—Initially, crude extracts of 40 American and Finnish isolates were made from cultures grown on each of the five media (ALK, CYA, PSA, SYES, and V8) and analyzed by HPLC-DAD with no prior cleanup. The spirocyclic drimanes overshadowed all other compounds produced in the extracts (Compare HPLC chromatograms in FIG. 1A AND 1B), and no correlation between the two morphologically distinguishable groups and drimane production was found. However, the Group A isolates generally produced fewer of the drimane components resembling the 'Mer-NF5003' (FIG. 1A) described by Kaneto et al (1994) albeit in much higher quantities than the *S. chartarum* isolates. This type of drimane has not been detected in rice cultures previously. On ALK, lactones and lactams (also spirocyclic drimanes) were the most dominant components. The *S. chartarum* isolates that were able to produce macrocyclic trichothecenes did so consistently on all five media.

PSA was chosen as the general medium for screening for trichothecenes and atranones, because this medium gave the best ratio between interfering components versus trichothecenes or atranones after a PEI column cleanup. The results from the HPLC-DAD and GC-MS analyzes of PSA extracts of the 122 *Stachybotrys* isolates are shown in TABLE I. It can be seen that the 84 isolates identified as *S. chartarum* could be divided into two groups on the basis of metabolite production. One group (33 isolates) produced macrocyclic trichothecenes (M), always including roridin E and satratoxins G and H, whereas isosatratoxin F and verrucarin J were occasionally detected. The other group (51 isolates) produced no macrocyclic trichothecenes, but instead produced atranones (A) including the dolabellane precursors. All the atranone-producing isolates consistently produced 6-hydroxydolabella-3E, 7E, 12-trien-14-one, and often the corresponding 3,4-epoxydolabellane, but did not usually produce the atranones A-J. Trichodermol (T) could be detected in the hydrolyzed extracts of some isolates in both groups. The presence of trichodermol shows that trichodermol and/or its acetyl ester, trichodermin, originally was produced by these isolates. FIG. 1B AND 1C show HPLC chromatograms of the PEI purified extracts of two *S. chartarum* isolates producing macrocyclic trichothecenes and atranones, respectively. None of the 122 *Stachybotrys* isolates was found to produce both macrocyclic trichothecenes and atranones. All the 21 isolates of *Stachybotrys* sp. Group A were atranone producers and five were also able to produce trichodermol. The two isolates of *S. dichroa* were also able to produce macrocyclic trichothecenes as roridin E and the single isolate of *S. microspora* was found to produce atranones as indicated by the detection of 6-hydroxydolabella-3E, 7E, 12-trien-14-one. Neither macrocyclic trichothecenes nor atranones were detected in any of the isolates of *S. albipes*, *S. bisbyi*, *S. cylindrospora*, *S. parvispora* and *S. theobromae*. Trichodermol was, however, found in the cultures of *S. oenanthes* and in one of the two *S. nephrospora* isolates (TABLE I).

Ten of the 22 *S. chartarum* isolates from Cleveland and 12 of 28 *S. chartarum* from Finland produced macrocyclic trichothecenes. Only three of 11 Danish *S. chartarum* isolates produced these compounds.

Growth characterization.—Colony appearance varied in both size and color among the 12 different *Stachybotrys* species or taxa, but was stable among isolates within each species or taxa. The media ALK, SYES, and V8 supported only sparse pigment production for most of the 122 *Stachybotrys* isolates. Only *S. chartarum* and Group A produced extracellular pigment on both CYA and PSA, as did the single isolate of *S. microspora*, which produced a greenish yellow pigment. TABLE II lists the colors of the pigments and the mean colony diameters (±twice the standard deviation) for all isolates identified as either *S. chartarum* or Group A. There was no obvious difference in either pigmentation or size of colonies between the *S. chartarum* isolates that produced macrocyclic trichothecenes (M) and those that produces atranones (A). In general, the atranone-producing isolates had slightly larger colonies and did not produce the yellow pigment on CYA. The production of yellow pigment by the two *S. chartarum* groups was not consistent, in contrast to pigment production by *Stachybotrys* sp. Group A isolates. All 21 Group A isolates produced a halo of jade green extracellular pigment around each colony on CYA and PSA. Their colony diameters were markedly smaller that those of the *S. chartarum* isolates (TABLE II).

Data on colony diameter and pigment production were analyzed using cluster analysis. FIG. 2 shows a dendrogram based on the 21 *Stachybotrys* sp. Group A isolates, 32 isolates of *S. chartarum* that produced macrocyclic trichothecenes (M) and 25 randomly selected atranone-producing *S. chartarum* isolates (A). The UPGMA dendrogram shows two clusters beyond the 0.25 similarity line. All the 21 Group A isolates segregated in the bottom cluster (gray hatched area in FIG. 2). The other, larger cluster contains all the isolates identified as *Stachybotrys chartarum*. The *S. chartarum* isolates were randomly distributed into minor sub-clusters without any evident segregation of

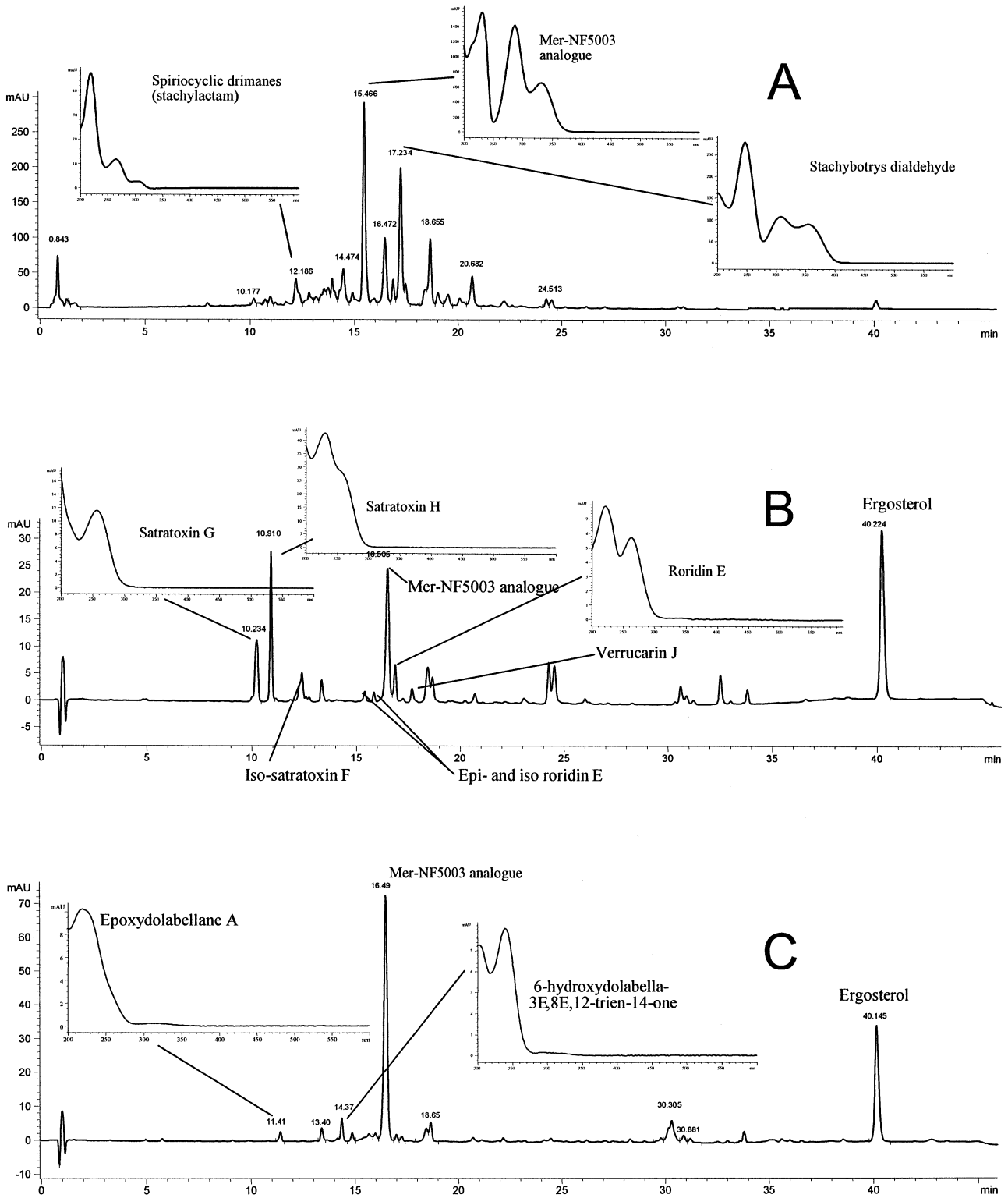


FIG. 1. HPLC chromatograms (260 nm) of *Stachybotrys chartarum* extracts from PSA agar: A. crude extract without clean up of *S. chartarum* (IBT 7711); B. PEI cleaned up extract of a macrocyclic trichothecenes-producing *S. chartarum* (IBT 7711); C. PEI cleaned up extract of an atranone-producing *S. chartarum* (IBT 9466).

TABLE II. Diameter and pigmentation in the three *Stachybotrys* taxa/chemotypes: the macrocyclic trichothecene-producing (M) and the atranone-producing (A) *S. chartarum* and *Stachybotrys* sp. Group A

Stachybotrys spp. (number of isolates)	Mean diameter ± 2SD (mm) after 7 days					Pigmentation	
	ALK	CYA	PSA	SYES	V8	CYA	PSA
chartarum-M (33)	22 ± 7	20 ± 5	25 ± 7	21 ± 8	25 ± 11	Yellow/none	Yellow/none
chartarum-A (51)	25 ± 8	22 ± 5	27 ± 7	23 ± 6	27 ± 11	None	Yellow/none
sp. Group A (21)	14 ± 6	14 ± 3	16 ± 4	15 ± 4	19 ± 9	Green	Green

atranone-producing (A) and macrocyclic trichothecenes-producing (M) isolates. There was no geographic division among the isolates. *S. chartarum* isolates from Cleveland were located in most sub-clusters together with both Finnish and Danish *S. chartarum* isolates. The same was the case for the 21 Group A isolates in the bottom cluster.

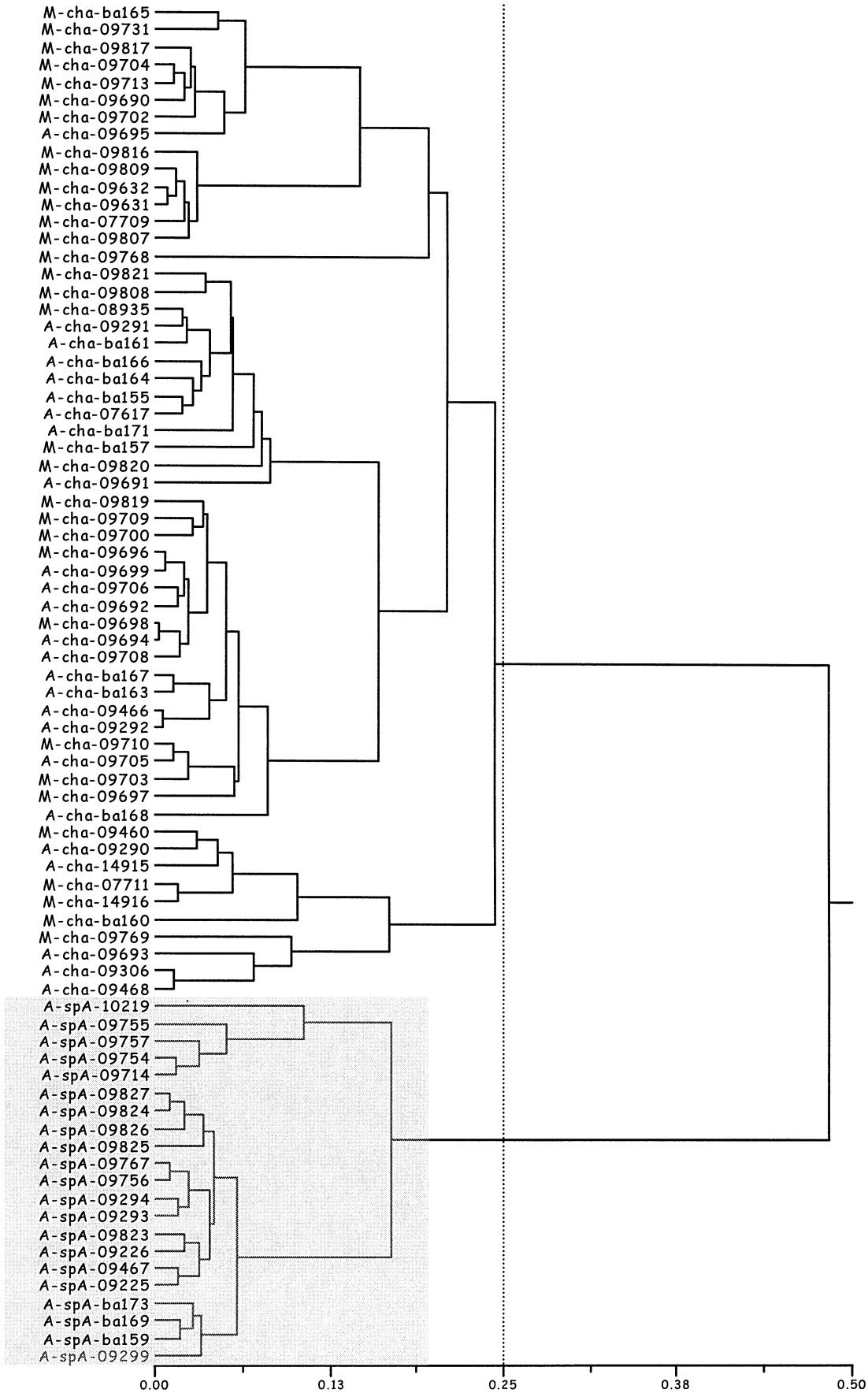
DISCUSSION

The results from this study indicate that *Stachybotrys* isolates found in water-damaged buildings consist of three distinct taxa/chemotypes with different chemical or morphological characteristics. The two chemotypes had the morphological appearance of *S. chartarum*, while the second taxon, *Stachybotrys* sp. Group A, may be a new, undescribed *Stachybotrys* species. The two *S. chartarum* chemotypes could be differentiated by their production of metabolites. One *S. chartarum* chemotype produced macrocyclic trichothecenes but no atranones, while the other produced atranones and no macrocyclic trichothecenes. However, some isolates in both chemotypes did produce the simple trichothecenes, trichodermol and/or trichodermin. All members of the second taxon, *Stachybotrys* sp. Group A, produced atranones. All three taxa/chemotypes were found, in varying ratios, in water-damaged buildings in Cleveland as well as in Finland and Denmark.

A preliminary RAPD-PCR experiment showed that two *Stachybotrys* sp. Group A isolates (IBT 9299 and IBT 9467) had identical RAPD profiles that were different from the profiles of five *S. chartarum* isolates (results not shown). Among the *S. chartarum* isolates in this experiment there were no differences between RAPD-profiles of macrocyclic trichothecene-producing isolates (IBT 9631 and IBT 9632) and atranone-producing isolates (IBT 9292, IBT 9633 and IBT 9634). They could, however, be distinguished by AFLP analysis (results not shown).The two chemotypes in *S. chartarum*, the atranone producers and the macrocyclic trichothecene producers, need to be examined in order to establish if there is more subtle morphological evidence to suggest that they should be two separate sibling species.

Three *Stachybotrys* isolates [IBT 9768 (= ATCC 16026 = NRRL 29942 = NC-1), IBT 9769 (= ATCC 26303 = NRRL 29941 = NC-4) and IBT 9767 (= ATCC 11695 = NRRL 29940)] that had been included in the study of Jong and Davis (1976) as *S. chartarum* were also examined in this study. Two of these isolates, IBT 9768 and IBT 9769, produced rough-surfaced conidia when mature and were identified as *S. chartarum*, while the third isolate, IBT 9767, produced smooth conidia regardless of age and was identified as *Stachybotrys* sp. Group A. *Stachybotrys atra* was depicted as having smooth conidia by Corda (as reproduced in Bisby 1943), but based on three new cultures Bisby (1943) re-described *S. atra* as having both smooth and rough conidia. The three isolates used by Bisby are not available and no neotype was designated. Accepting Bisby and Hughes' arguments, Jong and Davis (1976: 435) describe the mature conidia of *S. chartarum* as ". . . dark olive gray, more or less opaque, smooth-walled or showing banded or ridged,. . . " and later (1976: 439) write that "The distinguishing feature of this species is the mature phialoconidia showing a ridged or banded surface and their size.". It may be incorrect that *S. atra* Corda is synonymous with *S. chartarum* (Ehrenberg) S. (Ehrenb. ex Link) Hughes. The question of whether *Stachybotrys* sp. Group A is a new, undescribed species or actually *S. atra* as Corda described it cannot be resolved until neotypes of the two species have been designated.

Ten of the 16 *Stachybotrys* isolates from Cleveland, analyzed by both Jarvis et al (1998) and Vesper et al (1999, 2000a), were examined in this study (marked with § in TABLE I). The results of Jarvis et al (1998) showed that cytotoxicity (expressed as inhibition of cell proliferation) correlated with the total production of trichothecenes (both simple and macrocyclic). The three most toxic isolates in Jarvis et al (1998) [JS58–02 (= IBT 9820), JS58–17 (= IBT 9821) and JS58–18 (= IBT 9819)] and one of medium toxicity [JS51–11 (= IBT 9808)] were all identified as *S. chartarum* and produced macrocyclic trichothecenes in our study, whereas the four less toxic isolates [JS58–07 (= IBT 9822), JS58–15 (= IBT


M-cha-ba165
M-cha-09731
M-cha-09817
M-cha-09704
M-cha-09713
M-cha-09690
M-cha-09702
A-cha-09695
M-cha-09816
M-cha-09809
M-cha-09632
M-cha-09631
M-cha-07709
M-cha-09807
M-cha-09768
M-cha-09821
M-cha-09808
M-cha-08935
A-cha-09291
A-cha-ba161
A-cha-ba166
A-cha-ba164
A-cha-ba155
A-cha-07617
A-cha-ba171
M-cha-ba157
M-cha-09820
A-cha-09691
M-cha-09819
M-cha-09709
M-cha-09700
M-cha-09696
A-cha-09699
A-cha-09706
A-cha-09692
M-cha-09698
A-cha-09694
A-cha-09708
A-cha-ba167
A-cha-ba163
A-cha-09466
A-cha-09292
M-cha-09710
A-cha-09705
M-cha-09703
M-cha-09697
A-cha-ba168
M-cha-09460
A-cha-09290
A-cha-14915
M-cha-07711
M-cha-14916
M-cha-ba160
M-cha-09769
A-cha-09693
A-cha-09306
A-cha-09468
A-spA-10219
A-spA-09755
A-spA-09757
A-spA-09754
A-spA-09714
A-spA-09827
A-spA-09824
A-spA-09826
A-spA-09825
A-spA-09767
A-spA-09756
A-spA-09294
A-spA-09293
A-spA-09823
A-spA-09226
A-spA-09467
A-spA-09225
A-spA-ba173
A-spA-ba169
A-spA-ba159
A-spA-09299
0.00
0.13
0.25
0.38
0.50

9810), JS51–05 (= IBT 9812) and JS63–01 (= IBT 9814)] were identified as atranone-producing *S. chartarum*. The last two isolates [JS51–08 (= IBT 98230) and JS58–06 (= IBT 9825)], showing low toxicity in Jarvis et al (1998) were identified in this study as *Stachybotrys* sp. Group A and produced atranones. This suggests that the three *Stachybotrys* taxa/chemotypes have different inhibitory properties towards cell proliferation and agrees very well with our findings.

The same ten *Stachybotrys* isolates from Cleveland were also examined by Vesper et al (1999, 2000a) (marked with § in TABLE I), in addition to two isolates from a culture collection [NC-1 (= ATCC 16026) and NC-4 (= ATCC 26303)]. They found that six [JS58–02 (= IBT 9820), JS58–17 (= IBT 9821), JS58–18 (= IBT 9819), JS51–11 (= IBT 9808), NC-1 (= IBT 9768) and NC-4 (= IBT 9769)] out of the above-mentioned 12 isolates were either high or intermediate in toxicity expressed as protein synthesis inhibition. In our study, all six were identified as macrocyclic trichothecene-producing *S. chartarum*. Isolates JS58–07 (= IBT 9822), JS58–15 (= IBT 9810), JS51–05 (= IBT 9812) and JS63–01 (= IBT 9814), identified as atranone-producing *S. chartarum* in our study, were found by Vesper et al (1999) to have low toxicity. The two *Stachybotrys* sp. Group A isolates in our study, JS51–08 (= IBT 9823) and JS58–06 (= IBT 9825), were also low in toxicity according to Vesper et al (1999). Their results on toxicity are parallel to ours, but their results on production of a hemolysin, later identified as stachylysin (Vesper et al 2001) do not correspond with either the toxicity or taxonomic groupings.

A set of 20 Finnish *Stachybotrys* isolates (HT isolates marked with † in TABLE I) was examined by Routsalainen et al (1998). They reported that seven *Stachybotrys* isolates (HT-401, HT-435, HT-502, HT-503, HT-513, HT-518 and HT-520) were able to induce TNF-α and IL-6 in macrophages, but did not induce ROS or cause cytotoxic effects on the macrophages. These seven were identified as atranone-producing *S. chartarum* isolates in our study. They also reported that isolate HT-016 was the only one that did not induce ROS, TNF-α and IL-6 nor cause a cytotoxic effect in macrophages (Routsalainen et al 1998). Results in our study show that HT-016 (= IBT 9714) belonged to *Stachybotrys* sp. Group A. The remaining 12 *Stachybotrys* isolates (HT-072, HT-165, HT-248, HT-296, HT-386, HT-387, HT-391, HT-471, HT-522, HT-523, HT-530, and HT-569) in the study of Routsalainen et al (1998) were able to cause cytotoxic effects, but did not induce TNF-α and IL-6 production in macrophages. All but one of these isolates [HT-523 (= IBT 9708)] belonged to the macrocyclic trichothecene-producing *S. chartarum*. This indicates that the three *Stachybotrys* taxa/chemotypes have different cytotoxic and inflammatory properties towards macrophages and corresponds very well with our findings.

The existence of three different taxa/chemotypes of *Stachybotrys* in water-damaged buildings can explain the variations or inconsistencies in toxicity and inflammation that have been reported in recent papers (Jarvis et al 1998, Routsalainen et al 1998, Vesper et al 1999, Vesper et al 2001). The growth characteristics and the metabolite profiles of isolates within the different taxa/chemotypes of *Stachybotrys* were the same whether they came from Cleveland, Ohio, Finland or Denmark. If it is assumed that this differentiation into three distinct *Stachybotrys* taxa/chemotypes is correct and that they all are present in the same water-damaged building, they should be addressed separately with respect to their toxic and inflammatory potentials. The taxon identified as *Stachybotrys* sp. Group A is a low inhibitor of protein synthesis and does not induce inflamation or toxicity towards macrophages. The atranone-producing chemotype of *S. chartarum* does not induce toxicity to macrophages, but instead induces inflamation and exhibits moderate inhibition of protein synthesis. The macrocyclic trichothecene-producing chemotype of *S. chartarum*, does not induce any inflammatory response in macrophages, but is highly toxic to macrophages and is a strong protein synthesis inhibitor. The different modes of action suggest that the three *Stachybotrys* taxa/chemotypes also have unique metabolite profiles containing different specific components. It is possible that the production of spirocyclic drimanes, common to all three taxa/chemotypes, cover up some unknown component in one of the taxa/chemotypes, that alone or in combination with known metabolites, is responsible for idiopathic pulmonary hemorrhage in infants.

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FIG. 2. Dendrogram produced by UPGMA cluster analysis and the Manhattan coefficient based on colony diameters and colors of extracellular pigment of 78 *Stachybotrys* isolates.

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LITERATURE CITED

Andersen B, Krøger E, Roberts RG. 2001. Chemical and morphological segregation of *Alternaria alternata*, *A. gaisen* and *A. longipes*. Mycol Res 105:291–299.

Andersen B, Nissen, N. 2000. Evaluation of media for detection of *Stachybotrys* and *Chaetomium* species associated with water-damaged buildings. Int Biodet Biodegr 46:111–116.

Ayer WA, Miao S. 1993. Secondary metabolites of the aspen fungus *Stachybotrys cylindrospora*. Can J Chem 71:487–493.

Barron GL. 1961. Studies on species of *Oidiodendron*, *Helicodendron* and *Stachybotrys* from soil. Can J Microbiol 39:1563–1571.

Bisby GR. 1943. *Stachybotrys*. Trans Brit Mycol Soc 26:133–143

Butler EE, Mann MP. 1959. Use of cellophane tape for mounting and photographing phytopathogenic fungi. Phytopathology 49:231–232.

Croft WA, Jarvis BB, Yatawara CS. 1986. Airborne outbreak of trichothecene mycotoxicosis. Atmos Env 20:549–552.

Dearborn DG, Yike I, Sorenson WG, Miller MJ, Etzel RA. 1999. Overview of investigations into pulmonary hemorrhage among infants in Cleveland, Ohio. Env Health Persp 107:S495–S499.

DIFCO Manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures. 1969. Detroit: DIFCO Laboratories Inc.

Domsch KH, Gams W, Anderson T-H. 1980. Compendium of soil fungi. London: Academic Press.

Dorai M, Vittal BPR. 1986. A new *Stachybotrys* from eucalyptus litter. Trans Brit Myc Soc 87:642–644.

El-Maghraby OMO, Bean GA, Jarvis BB, Aboul-Nasr MB. 1991. Macrocyclic trichothecenes produced by *Stachybotrys* isolated from Egypt and Eastern Europe. Mycopath 113:109–115.

Ellis MB. 1971. Dematiaceous Hyphomycetes. CAB Commonwealth Mycological Institute, Surrey.

Ellis MB. 1976. More Dematiaceous Hyphomycetes. CAB Commonwealth Mycological Institute, Surrey.

Filtenborg O, Frisvad JC, Thrane U. 1990. The significance of yeast extract composition on metabolite production in *Penicillium*. In: Samson RA, Pitt JI, eds. Modern concepts in *Penicillium* and *Aspergillus* Classification, New York: Plenum Press.

Flannigan B, Miller JD. 1994. Health implications of fungi in indoor environments—An overview. In: Samson RA, Flannigan B, Flannigan ME, Verhoeff AP, Adan OCG, eds. Health implications of fungi in indoor air environment. 1th ed. Elsevier, Amsterdam. p 3–26.

Forgacs J, Carll WT. 1962. Mycotoxicosis. Adv Vet Sci 7:273–293.

Frisvad JC, Filtenborg O. 1990. Secondary metabolites as consistent criteria in *Penicillium* taxonomy and a synoptic *Penicillium* subgenus *Penicillium*. In: Samson RA, Pitt JI, eds. Modern Concepts in *Penicillium* and *Aspergillus* Classification. New York: Plenum Press. p 373–384.

Frisvad JC, Thrane U. 1987. Standardised High-Performance Liquid Chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (Diode Array Detection). J Chrom 404:195–214.

Frisvad JC, Thrane U, Filtenborg O. 1998. Role and use of secondary metabolites in fungal taxonomy. In: Frisvad JC, Bridge PD, Arora DK, eds. Chemical fungal taxonomy. New York: Marcel Dekker. p 289–319.

Fung F, Clark R, Williams S. 1998. *Stachybotrys*, a mycotoxin-producing fungus of increasing toxicologic importance. Clinical Toxicology 36:79–86.

Gravesen S, Frisvad JC, Samson RA. 1994. Microfungi. Copenhagen: Munksgaard.

Gravesen S, Nielsen PA, Iversen R, Nielsen KF. 1999. Microfungal contamination of damp buildings—examples of risk constructions and risk materials. Env Health Persp 107:Supplement 3. p 505–508.

Hinkley SF, Jarvis BB. 2000. Chromatographic method for *Stachybotrys* toxins. In: Pohland A, Trucksess MW, eds. Methods Molecular Biology 157. Mycotoxin Protocols. Totowa: Humana Press. p 173–194.

Hinkley SF, Mazzola EP, Fettinger JC, Lam YKT, Jarvis BB. 2000. Atranones A-G, from the toxigenic mold *Stachybotrys chartarum*. Phytochem 55:663–673.

Hughes SJ. 1958. Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis. Can J Bot 36:727–836.

Jarvis BB. 1992. Macrocyclic trichothecenes from Brazilian *Baccharis* species: from microanalysis to large-scale isolation. Phytochem Anal 3:241–249.

Jarvis BB, Lee Y-W, Comezoglu SN, Yatawara CS. 1986. Trichothecenes produced by *Stachybotrys atra* from Eastern Europe. Appl Env Microb 51:915–918.

Jarvis BB, Salemme J, Morais A. 1995. *Stachybotrys* toxins 1. Nat Toxins 3:10–16.

Jarvis BB, Sorenson WG, Hintikka E-L, Nikulin M, Zhou Y, Jiang J, Wang S, Hinkley SF, Etzel RA, Dearborn DG. 1998. Study of toxin production by isolates of *Stachybotrys chartarum* and *Memnoniella echinata* isolated during a study of pulmonary hemosiderosis in infants. Appl Env Microb 64:3620–3625.

Johanning E, Biagini RE, Hull D, Morey PR, Jarvis BB, Landsbergis P. 1996. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. Int Arch Occup Health 68:207–218.

Jong SC, Davis EE. 1976. Contribution to the knowledge of *Stachybotrys* and *Memnoniella* in culture. Mycotaxon 3: 409–485.

Kaneto R, Dobashi K, Kojima I, Sakai K, Shibamoto N, Yoshioka T, Nishid AH, Okamoto R, Akagawa H, Mizuno S. 1994. Mer-nf5003b, mer-nf5003e and mer-nf5003f, novel sesquiterpenoids as avian-myeloblastosis virus protease inhibitors produced by *Stachybotrys* sp. J Antib 47: 727–730.

Mason CD, Rand TG, Oulton M, MacDonald J, Anthes M. 2001. Effects of *Stachybotrys chartarum* on surfactant convertase activity in juvenile mice. Toxicol Appl Pharmacol 172:21–28.

McKenzie EHC, 1991. Dematiaceous hyphomycetes on *Freycinetia* (Pandanaceae) 1 *Stachybotrys*. Mycotaxon 41: 179–188.

Nielsen KF, Hansen MØ, Larsen TO, Thrane U. 1998a. Production of trichothecene mycotoxins on water damaged gypsum boards in Danish buildings. Int Biodet Biodegr 42:1–7.

Nielsen KF, Thrane U. 2001. Fast method for screening of trichothecenes in fungal cultures using gas chromatography-tandem mass spectrometry. J Chrom A 929:75–87.

Nielsen KF, Thrane U, Larsen TO, Nielsen PA, Gravesen S. 1998b. Production of mycotoxins on artificially inoculated building materials. Int Biodet Biodegr 42:8–17.

Peltola J, Anderson MA, Raimo M, Mussalo-Rauhamaa H, Salkinoja-Salonen M. 1999. Membrane toxic substances in water-damaged construction materials and fungal pure cultures. In: Johanning E. Bioaerosols, Fungi, Mycotoxins: Health effects, Assessment, Prevention and Control 1. New York: Eastern New York Occupational & Environmental Health Center. p 432–443.

Pitt JI. 1980. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London: Academic Press.

Reshetilova TA, Soloveva TF, Baskunov BP, Kozlovskii AG. 1992. Investigation of alkaloid formation by certain species of fungi of the *Penicillium* genus. Mikrobiologiya 61:873–879.

Ruotsalainen M, Hirvonen M-R, Nevalainen A, Meklin T, Savolainen K. 1998. Cytotoxicity, production of reactive oxygen species and cytokines induced by different strains of *Stachybotrys* sp. from mouldy buildings in RAW2647 macrophages. Env Tox Pharm 6:193–199.

Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O, eds. 2000. Introduction to Food- and Air Borne Fungi. 6th Ed. Utrecht: Centraalbureau voor Schimmelcultures.

Simmons EG, 1992. *Alternaria* taxonomy: current status, viewpoint, challenge. In: Chelkowski J, Visconti A, eds. *Alternaria*: biology, plant diseases and metabolites. Amsterdam: Elsevier. p 1–35.

Smedsgaard J. 1997. Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures. J Chrom A. 760:264–270.

Subramanian CV. 1957. Hyphomycetes—IV. Proceedings of the Indian Academy of Sciences 46,no. 5, Sec. B:324–335.

Udagawa S. 1984. Taxonomy of mycotoxin-producing *Chaetomium*. In: Kurata H, Ueno Y, eds. Toxigenic Fungi—Their toxins and health hazards. Amsterdam: Elsevier. p 139–147.

Vesper SJ, Dearborn DG, Elidermir O, Haugland RA. 2000a. Quantification of siderophore and hemolysin from *Stachybotrys chartarum* strains, including a strain isolated from the lung of a child with pulmonary hemorrhage and hemosiderosis. Appl Env Microb 66:2678–2681.

Vesper SJ, Dearborn DG, Yike I, Allen T, Sobolewski J, Hinkley SF, Jarvis BB, Haugland RA. 2000b. Evaluation of *Stachybotrys chartarum* in the house of an infant with pulmonary hemmorrhage: Quantitative Assessment before, during, and after remediation. J Urb Health 77: 68–85.

Vesper SJ, Dearborn DG, Yike I, Sorenson WG, Haugland RA. 1999. Hemolysis, toxicity, and randomly amplified polymorphic DNA analysis of *Stachybotrys chartarum* strains. Appl Env Microb 65:3175–3181.

Vesper SJ, Magnuson ML, Dearborn DG, Yike I, Haugland RA. 2001. Initial characterization of the hemolysin Stachylysin from *Stachybotrys chartarum*. Infect Immun 69: 912–916.