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Fungal colonization of automobile air conditioning systems

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Air samples and swab samples of the air conditioning vents were collected from 29 automobiles in the metropolitan region of Atlanta, GA, and cultured for fungi. Among the fungi observed, species of *Acremonium*, *Aspergillus*, *Alternaria*, *Aureobasidium*, *Cladosporium*, and *Penicillium* were in the highest densities. Transparent adhesive tape imprints, SEM observations, and enrichment culture of components of five systems demonstrated fungal hyphae on the metal surfaces and within the matrix of various insulation materials. The evaporator removed from one automobile because of a series of complaints of noxious odors was densely colonized by *Penicillium viridicatum*. The amplification of known allergenic and odor-producing fungi occurred within the automobile air conditioning systems.

Keywords: automobiles; air conditioners, fungi; fungal colonization

Introduction

In recent years, fungi have been implicated as quantitatively the most important bioaerosol component of unhealthy indoor air [5]. Fungi commonly isolated from indoor environments include species of *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium* and *Penicillium*. These fungi are usually recovered from sources such as water-damaged wood, ceiling tiles and wallboard, wallcoverings, house dust, pets and houseplants, but some species have been associated with contaminated heating, ventilating, and air conditioning (HVAC) systems [1,2,11]. Rarely, indoor fungi may cause human infections; more commonly conidia and fragments of hyphae may produce allergenic responses ranging from pneumonitis to asthma-like symptoms [9].

Compounds produced by fungi may be toxic or have immunomodulating activity, thus contributing to hyperallergenic responses or rendering those exposed more susceptible to other microorganisms [5]. In addition, numerous fungi are known to produce noxious volatiles [3,5]. The indirect health effects of such volatiles have not been extensively studied [3].

The enclosed environment of the automobile is a microcosm of the indoor environment and is thus potentially susceptible to fungal-mediated allergy problems similar to those reported for sick buildings. Kumar *et al* [6,7] associated allergic rhinitis and hypersensitivity pneumonitis with fungi, including *Aspergillus*, *Cladosporium* and *Penicillium* emanating from automobile air conditioning vents. In later studies [8,10], automobile air conditioning systems, particularly those with filters, were noted to reduce the densities of fungi in the ambient air. Aside from these reports and our preliminary study [12], we have found no additional studies of fungi associated with automobiles. This report examines the incidence of fungi associated with air conditioning systems in automobiles whose owners have complained of periodic unpleasant odors.

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Materials and methods

Automobiles in the metropolitan area of Atlanta, GA, USA, were selected on the basis of owner complaints of noxious odors emanating from the air conditioning systems. All but two of the automobiles were manufactured after 1990 and automobiles which had known water leaks to the interior were excluded from the study. Air samples were collected on malt extract agar (BBL, Cockeysville, MD, USA) and Czapek-40% sucrose agar (Difco, Detroit, MI, USA) with a Graseby-Andersen single stage air sampler (Graseby-Andersen Inc, Atlanta, GA, USA). The sampler was positioned under the air conditioning vents on the dashboard after the system had been running for 2 min. Swab samples were collected from the air conditioning vents and plated on the same media. Plates were incubated at room temperature (22–24°C) for 7–10 days. Fungi were isolated and identified as described previously [11].

Used evaporator units were removed from five automobiles from outside of Atlanta, GA, and shipped in plastic bags to Georgia State University. Two new (unused) evaporator systems were also examined. The metal heat exchanger vanes and end plates of the units were sampled directly by swab culture on malt extract agar and by adhesive tape mounts for light microscopy. Additional samples of case insulation foam and neoprene-like insulation were removed from the evaporator units, mounted on aluminum stubs and examined directly with scanning electron microscopy (SEM) for evidence of fungal colonization [11]. Samples of used case insulation foam and neoprene-like insulation material were placed on malt extract agar plates and incubated at room temperature for 7–10 days. Other samples of case insulation foam and neoprene-like insulation were removed from the used evaporator units and placed in 98% relative humidity chambers for 7–10 days before examination by the microscopic procedures [4,11]. Control sections of unused insulation materials of the same types as in the used units were secured from manufacturers and examined for fungi as described above.

Table 1 Incidence of fungi isolated from 29 automobile air conditioning systems[a]. Automobiles were selected on the basis of complaints of noxious odors emanating from the air conditioning systems

Fungi isolated[b]	No. of automobiles with genus[a]
Acremonium sp	14
Alternaria sp	1
Aspergillus spp	14
Aureobasidium sp	17
Chaetomium sp	2
Chrysonilia sp	1
Cladosporium spp	25
Epicoccum nigrum	14
Fusarium sp	2
Mucor sp	1
Paecilomyces sp	3
Penicillium spp	20
Rhizopus sp	1
Rhodotorula spp	2
Syncephalastrum sp	2

[a]Obtained from year models 1985–86 ($n = 2$), 1991–96 ($n = 27$), all from metropolitan Atlanta, GA.
[b]Air samples or swabs of vents.

Results

Fungi representing 15 genera were isolated from air samples or swab cultures of automobile air conditioning systems (Table 1). All automobiles yielded molds, densities recovered from air ranged from 200–650 CFU m^{-3}, mainly species of *Acremonium*, *Aspergillus* (*Aspergillus niger*, *A. clavatus*, *A. versicolor*), *Cladosporium*, *Penicillium* (*P. oxalicum*, *P. citrinum*), and *Epicoccum nigrum*. Typically, *Cladosporium* and *Penicillium* spp were in the highest densities. Repeat examinations of eight of the automobiles after 2–4 weeks demonstrated similar density ranges and spectra of species in six of the automobiles.

Table 2 Incidence of fungal genera associated with automobile air conditioning evaporators[a]

Fungi	Incidence among evaporators
Acremonium[b]	1
Alternaria[b]	1
Aspergillus	3
Cladosporium[b]	4
Curvularia	1
Dichobotrys	1
Epicoccum	2
Paecilomyces	4
Penicillium[b]	3
Phoma	1
Pithomyces	1

[a]$n = 5$, model years 1990–1995.
[b]Fungi isolated both in culture and detected with light microscopy of adhesive tape preparations from evaporators.

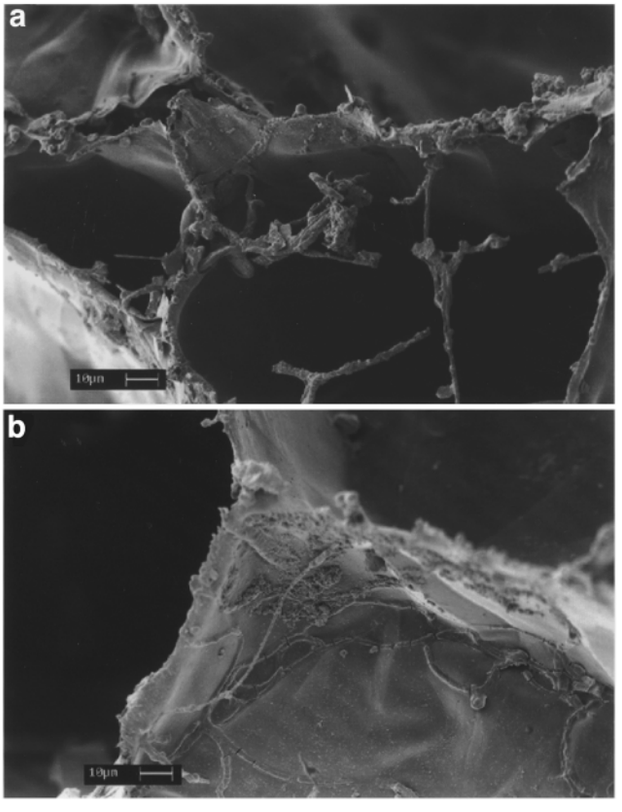

Figure 1 Hyphae of fungi (a) in cells of foam insulation and (b) on surfaces of insulation removed from an evaporator system of an automobile (SEM). *Penicillim viridicatum* was the predominant fungus recovered upon culture of the insulation.

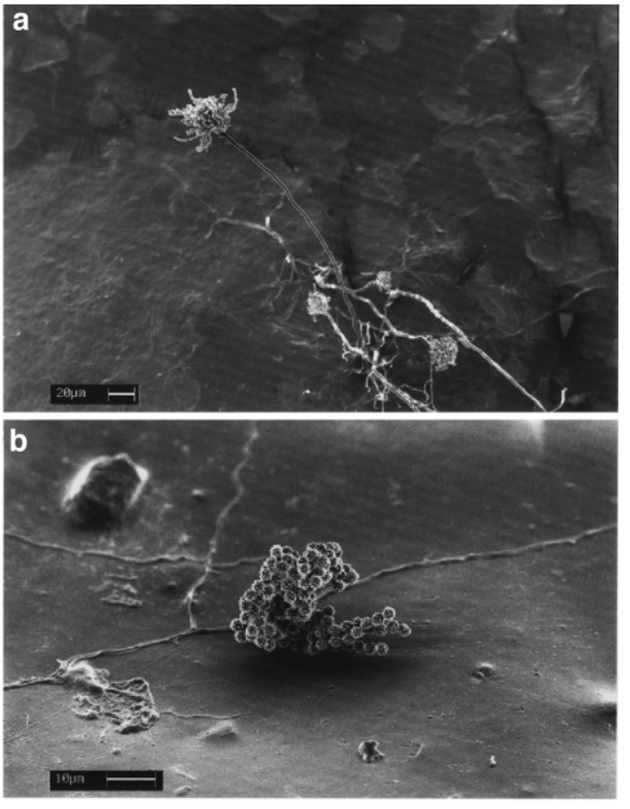

Figure 2 Hyphae and conidia of (a) *Aspergillus* sp and (b) *Penicillium* spp on case insulation foam from a used automobile air conditioning condenser. The foam had been incubated in a high humidity (98% RH) chamber for 7 days prior to SEM examination. *Aspergillus versicolor* and *Penicillium viridicatum* were identified in culture from these materials.

Eleven genera were identified in close association with the used air conditioning evaporator units taken from automobiles in service (Table 2). Adhesive tape mounts from insulation materials (and from metal surfaces of three units) demonstrated the presence of bundles of hyphae and conidiophores with conidia, representative of *Penicillium* spp, *Cladosporium* spp, and *Alternaria* spp.

Foam insulation from one used unit showed evidence of extensive colonization by fungi (Figure 1). When other sections of this foam and of the neoprene-like insulation material were placed at 98% RH, conidiogenesis was observed (Figures 2 and 3) and a sharp musty odor was detected. *Aspergillus versicolor* and *Penicillium viridicatum* were recovered from cultures of this insulation. Unused case insulation foam on initial examination also yielded *P. viridicatum* on culture (Figure 4).

Discussion

All the automobiles in this study yielded fungi from air streams from their air conditioning systems. Whether these systems were colonized with actively growing fungi or whether the fungi originated from entrapped dormant conidia was not established. Nevertheless, microscopic examination of evaporator systems from additional automobiles

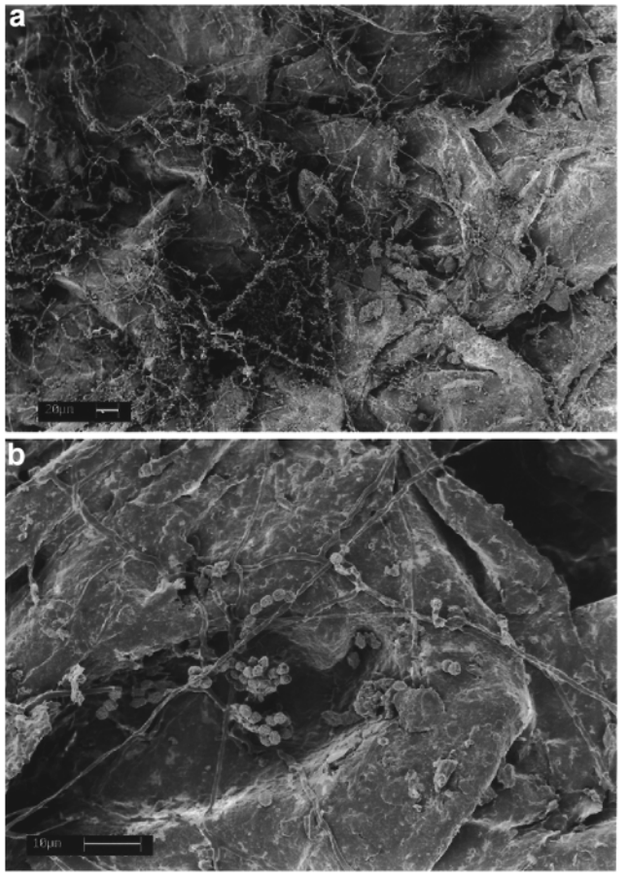

Figure 3 (a) Extensive hyphae and (b) conidiogenesis of fungi on the neoprene-like insulation from the heat sensing tube of the expansion valve of a used automobile air conditioning unit. The neoprene was held in a high humidity (98% RH) chamber for 7 days prior to SEM examination.

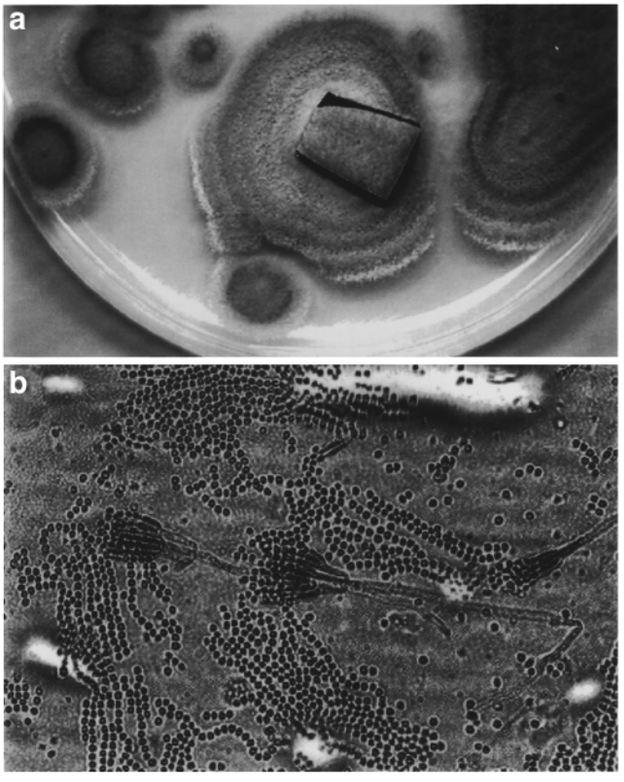

Figure 4 (a) Colony development of *Penicillium viridicatum* on malt extract agar inoculated with new case insulation foam for automobile air conditioning units. (b) Light micrograph of *P. viridicatum* isolated from the unused case insulation foam.

demonstrated that components of these systems, particularly insulation materials, were colonized with fungi of the same species predominating in the air and vents in the automobiles. Glue layers of certain insulations were found to be colonized in use, or, in laboratory challenges of unused material, to be susceptible to colonization by fungi isolated from the automobiles.

Insulation materals that absorb moisture and volatile organics appear to provide suitable substrates for fungal colonization [1,3]. Climatic conditions (high humidity) and airborne fungal populations undoubtedly are major factors in the incidence and severity of fungal colonization. Automobiles once colonized by fungi may continually or sporadically emit noxious odors or sensitizing products that affect the occupants of the automobile. Automobile engines and ventilation fans are seldom kept running after the air conditioning system is inactivated. Running the engine and ventilation fans for a short time would reduce the moisture residues that are necessary for the growth of the fungi. It is not generally recognized that certain individuals complaining of Sick Building Syndrome may be exposed to the hypersensitizing microorganisms during their travel in personal automobiles. Onset of the syndrome may be immediate or be delayed until after arrival at the work site. Studies are now in progress to determine the prevalence of fungal colonization of automobile air conditioning systems and automobile-related fungal hypersensitivities and to develop remedial procedures.

Acknowledgements

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