

Identification and Possible Disease Mechanisms of an Under-Recognized Fungus, *Aureobasidium pullulans*

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Key Words

Aureobasidium pullulans · Respirable-sized conidia · Yeast-like fungi

Abstract

Background: Investigations into the occurrence and health effects of yeast-like fungi in the outdoor air in the US have been limited. We sought to identify a respirable-sized fungus common in the Pasadena air, locate a major source for the emissions and investigate its relevance to allergic disease. **Methods:** Yeast-like fungi sampled from the environment were isolated, microscopically examined and sequenced. Pasadena allergy patients were skin tested with commercially available fungal extracts. Patient serum was immunoanalyzed for specific IgE reactivity. Nearby vegetation was analyzed in a controlled emission chamber to find a major source for the aerosols. **Results:** Hyaline unicellular conidia comprised up to 90% (41,250 m⁻³ of air) of total fungal counts and generally peaked at night and during periods of rainfall and ensuing winds throughout the fall and winter. Flowers were determined to be a major source of the emissions. The cellular and colonial morphology of isolates were consistent with *Aureobasidium* species. The sequence of the D1/D2 region of the 26S ribosomal subunit of isolates from flowers showed identity to two strains of *Aureobasidium pullulans* (black yeast). Seventeen percent

(16/94) of atopic individuals had positive skin testing with *A. pullulans* extract. Patient sera IgE identified several high molecular weight allergens in *Aureobasidium* extracts. **Conclusions:** Respirable-sized conidia of *A. pullulans* are emitted from flowers and form high concentrations in the air. They are associated with immediate reactivity on skin tests, bind to patient sera IgE, and might be relevant in allergic upper and lower airway diseases.

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Introduction

Hyaline conidia emitted from yeasts and yeast-like molds are among the most common fungal organisms detected in air samples in Asia [1], Australia [2], Europe [3–5], the Middle East [6, 7] and South America [8]. These fungi were reported to be less abundant in the outdoor air in the US and, in the few investigations to date, allergy patients showed low rates of clinical symptoms and skin reactivity to the small variety of yeast extracts tested [9]. In San Diego, California, yeasts were reported to be virtually absent from homes as well as the outdoor air [10, 11].

Descriptions and counts of yeast-like fungi are generally absent from pollen and spore atlases and are rarely included in pollen and fungi textbooks used for identify-

ing bioaerosols. A yeast, or yeast-like category is effectively absent from pollen and fungal spore reports of the US National Allergy Bureau [12]. Orange, California, was the only site to report hyaline conidia, and their occurrence was restricted to October through December with counts not exceeding $1,000\text{ m}^{-3}$ of air, averaged for 24 h. However, air sampling over the past 4 years at Santa Barbara, 160 km North-West of Pasadena, showed abundant hyaline conidia, especially after rainfall [Z. Dyer, pers. commun.]. For most published fungal counts that include a yeast category, identification was based on general cell or colony morphology and all yeast-like hyaline conidia were often pooled into the total yeast count. Reasons for failing to identify these fungi included their small size, lack of specific morphological characters and the necessity to isolate, culture and immunologically assay with specific antisera. *Aureobasidium* spp. are likely to be included in these yeast counts. *Aureobasidium* spp. are the so-called black yeasts that lack a known sexual state and produce both yeast-like hyaline conidia and a filamentous growth form. Furthermore, the source of these organisms was nearly always unidentified in air sampling studies, thus limiting our understanding of factors involved in controlling abundance [5].

Exposure to fungal spores correlates with increased pulmonary complaints and emergency department visits for asthma [13]. In California, foehn winds (i.e. Santa Ana winds) that are prevalent in the fall and winter are associated with asthma exacerbations [14] and likely contribute to peaks in respirable allergen exposure; however, direct observations are lacking. We have been monitoring both coarse and respirable-sized biological particles in the Pasadena air as an indicator of the prevalence of allergens. Aerosols containing conidia less than $5\text{ }\mu\text{m}$ in diameter can reach the peripheral lung tissues and may have a greater impact on allergic disease, especially asthma, than the larger fungal spores commonly detected in the air. We identified and quantified a previously unrecognized but abundant yeast-like bioaerosol in Pasadena, found a source for the emissions, and began to investigate the role it plays in episodes of allergic disease.

Methods

Outdoor Sampling

Air samples were collected with a Burkard 7-day volumetric spore trap (Burkard Manufacturing Co., Hertfordshire, UK) located on top of a 3-story building at Caltech. Sampling was compared between three sites arranged in a triangulated pattern with approximately 50 m distance between each site. The nearest veg-

etation was situated 3, 30 and 53 m away from the sampling sites, respectively. A rain gauge was located on an adjacent building. Fungal spores and unicellular hyaline conidia were morphologically identified using a Nikon Eclipse 80i light microscope with bright-field optics. Images were recorded with a Nikon DXM1200 digital camera using the ACT-1 application program (Nikon Instruments Inc., N.Y., USA). Counts, per cubic meter of air, averaged for 24 h, were recorded for hyaline conidia and total fungi. On several days, 2-hour averaged counts were collated. An AGI-30 (Ace Glass Inc., N.J., USA) all-glass liquid impinger was located adjacent to the spore trap. At 5 select times during the peak fungal periods of November and December each year, the sampler was run for 5-min periods to impinge particles smaller than $15\text{ }\mu\text{m}$ into 20 ml of water as previously described [15]. Washings from leaves, stems and flowers were collected at the same time as the liquid-impinged air samples and examined microscopically. Collections were also plated onto potato dextrose agar culture medium (BD Diagnostic Systems, N.J., USA) and yeast-like colonies isolated and incubated at $20\text{--}25^\circ\text{C}$ for 14 days.

Sequencing

Yeast-like fungi isolated from flowers were chosen for further analysis. The D1/D2 region of the large (26S) ribosomal subunit was sequenced using primers NL1 and NL4, as previously described [16]. The ribosomal sequence of isolates was compared with those of published yeast species available in public gene databases, using a BLAST search of all GenBank + EMBL + DDBJ + PDB sequences, on the NCBI website (URL: <http://www.ncbi.nlm.nih.gov/blast>).

Skin Test Reactivity and Asthma Prevalence in Pasadena

To determine the allergenicity of *Aureobasidium pullulans*, skin testing (epicutaneous and intradermal) with commercially available extracts (Greer Laboratories, N.C., USA) was performed on 94 patients at the Asthma and Allergy Center in Pasadena, according to established techniques [17]. A positive test included prick wheal size $\geq 8\text{ mm}$ in diameter for the epicutaneous test and $\geq 10\text{ mm}$ in diameter for the intradermal tests when measured with vernier calipers. Additional tests were performed with a panel of 51 major allergen extracts (Greer Laboratories). A negative control was performed with sterile saline, and a positive control was performed with histamine phosphate (1 mg/ml for epicutaneous, 0.1 mg/ml for intradermal testing). Total monthly asthma admissions and evaluations at the Huntington Memorial Hospital from 1996 to 2003 were collated.

Immunoanalysis

To confirm the recognition of the yeast-like colonies, Ouchterlony immunodiffusion analysis was performed using aqueous extracts prepared from two separate yeast-like colonies isolated from flowers, a reference extract prepared from *A. pullulans* (No. 9348, ATCC, Va., USA), and a reference rabbit anti-*A. pullulans* serum (No. RAK7, Greer Laboratories). Specific IgE in vitro tests for *A. pullulans* were performed using the Pharmacia ImmunoCAP System (Pharmacia Diagnostics DB, Uppsala, Sweden) on sera from 124 consecutively determined clinically unknown patients (Quest Diagnostics, Calif., USA) and sera from 2 patients and 1 control that were previously skin tested with *A. pullulans* extract. Specific IgE recognition of bound *A. pullulans* extracts was detected and quantified using a fluorescein-labeled anti-IgE enzyme immunoCAP assay (Pharmacia Diagnostics, Mich., USA).

The major allergens of *A. pullulans* were identified using SDS-PAGE gels and Western blots that were prepared as previously described [18]. Briefly, approximately 4 µg protein of *A. pullulans* extracts (GM21-64B-2AX4, Greer Laboratories) and 1 µg isolated from a flower were fractionated by SDS-PAGE (12% polyacrylamide) under either reducing or non-reducing conditions on a Bio-Rad Mini-Protein II Slab Cell system (Bio-Rad, Calif., USA). Proteins and molecular weight markers were transferred onto polyvinylidene difluoride membranes (Immobilon-PSQ, Millipore, Mass., USA) according to the manufacturer's instructions. After washing and blocking, the membranes were incubated with (1) colloidal gold stain (Bio-Rad) to visualize the protein bands, (2) sera from 3 patients (1:20) previously skin tested with *A. pullulans* extract, (3) a human mold-allergic serum pool (ZM-P1; 1:20), or (4) rabbit anti-*A. pullulans* serum pool, ZA7-1L (Greer Laboratories; 1:100,000) diluted with TBS buffer pH 8.0 (10 mM Tris, 0.15 M NaCl) with 0.05% Tween-20. After washing, the sera-incubated membranes were incubated with 1:1,000 alkaline phosphatase-labeled anti-human IgE (Sigma, Mo., USA) or 1:4,000 alkaline phosphatase-labeled anti-rabbit IgG (Sigma) in TBS buffer, pH 8.0. Immunoreactive bands were visualized by 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium solution (Amresco, Ohio, USA). Skin testing and IgE analysis of human subjects were approved by the Institutional Review Boards of Huntington Memorial Hospital and Caltech.

Controlled Aerosol Emission

Factors controlling the generation and dispersal into the air of hyaline conidia of *A. pullulans* were evaluated using a controlled emission chamber as previously described [19]. Six flowering stems, 15 cm long, were harvested from a *Corymbia* tree and placed inside a chamber. They were kept at 90% relative humidity (RH) and 25°C for 3 h, and then dry air was passed through the chamber (4 l/min) for 1 h. Next, a wind disturbance was applied from a small fan and the emission of particles counted using an aerodynamic particle sizer (1 l/min flow rate; TSI, Minn., USA), collected onto glass cover slips housed within a cascade impactor (3.0 l/min flow rate; Sierra Instruments Inc., Calif., USA) and examined microscopically.

Results

Over the past 2 years we have noticed that up to 90% of the total mold counts consisted of unicellular hyaline conidia, averaged for 24 h (fig. 1). During and immediately after heavy rain, up to 99% of the total fungal count consisted of hyaline conidia, averaged for 2 h, especially at night when few other particles larger than 1 µm were present (fig. 2a). Counts also peaked with ensuing strong winds. At the start of air sampling in January 2002, daily counts up to 15,140 m⁻³ of air, averaged for 24 h, occurred, and these were associated with moist weather. For the remainder of that spring and summer, few hyaline conidia were detected in air samples and there was an absence of rainfall. After arrival of the first heavy rains in

November, counts peaked again (22,690). The highest daily count for hyaline conidia (41,250) was observed on February 15, 2003. High counts persisted until March 2003. Conidia were present in lower numbers throughout the rest of the spring and summer of 2003, but peaked again (12,630) in November, coincident with the first fall rains. Counts from all three sampling sites at Caltech were similar.

Samples collected outdoors with an AGI-30 air sampler, as well as directly from *Corymbia* flowers, and cultivated onto potato dextrose agar produced either cream- or pink-colored, pasty to leathery colonies. Only unicellular budding conidia were observed in young colonies. The initial cellular and colonial morphology of isolates was similar to *Candida*, except that the colonies turned velvety and brown to black with age. Thus, they were identified as *Aureobasidium* spp. In aged cultures, slimy masses, or tufts, of conidia were located intercalary or terminally on septate hyphae (fig. 2b). Hyphae ranged in diameter from 2 to 20 µm and frequently became dark-brown with age, undergoing homothallic transformation to form chains of 1- to 2-celled, thick-walled, darkly pigmented arthroconidia. Conidia were produced synchronously in dense groups. Single-celled conidia were hyaline with smooth, thin walls and an ellipsoidal shape. In culture, the conidial diameter ranged from 1.5 to 5.0 µm. These conidia continued to multiply on agar culture by budding and formed secondary conidia. *Aureobasidium* was the only culturable fungus detected with hyaline conidia. The only difference in morphology between the two strains of *Aureobasidium* was the time at which the colony turned color and started displaying hyphal growth.

Sequencing

A BLAST search of the ribosomal sequence of isolates showed 100% identity with two strains of *A. pullulans* (DeBary) Arnaud (= *Pullularia pullulans* Berkout) (Accession AY185811 and AJ507454) and one strain of *Disco-sphaerina fagi* (Accession AY016359) (fig. 3). *D. fagi* can be eliminated from consideration because of the yeast-like growth of the isolates.

Asthma Prevalence and Skin Test Reactivity

Seventeen percent (16/94) of atopic individuals had a positive skin test reactivity with *A. pullulans*. All patients who were skin test-positive also showed sensitivity to other allergens. Of the 16 patients who tested positive for *A. pullulans*, 9 (56%) had bronchitis and/or bronchial asthma by history and/or pulmonary function. During each fall and winter from 1996 to 2003 there was an approxi-

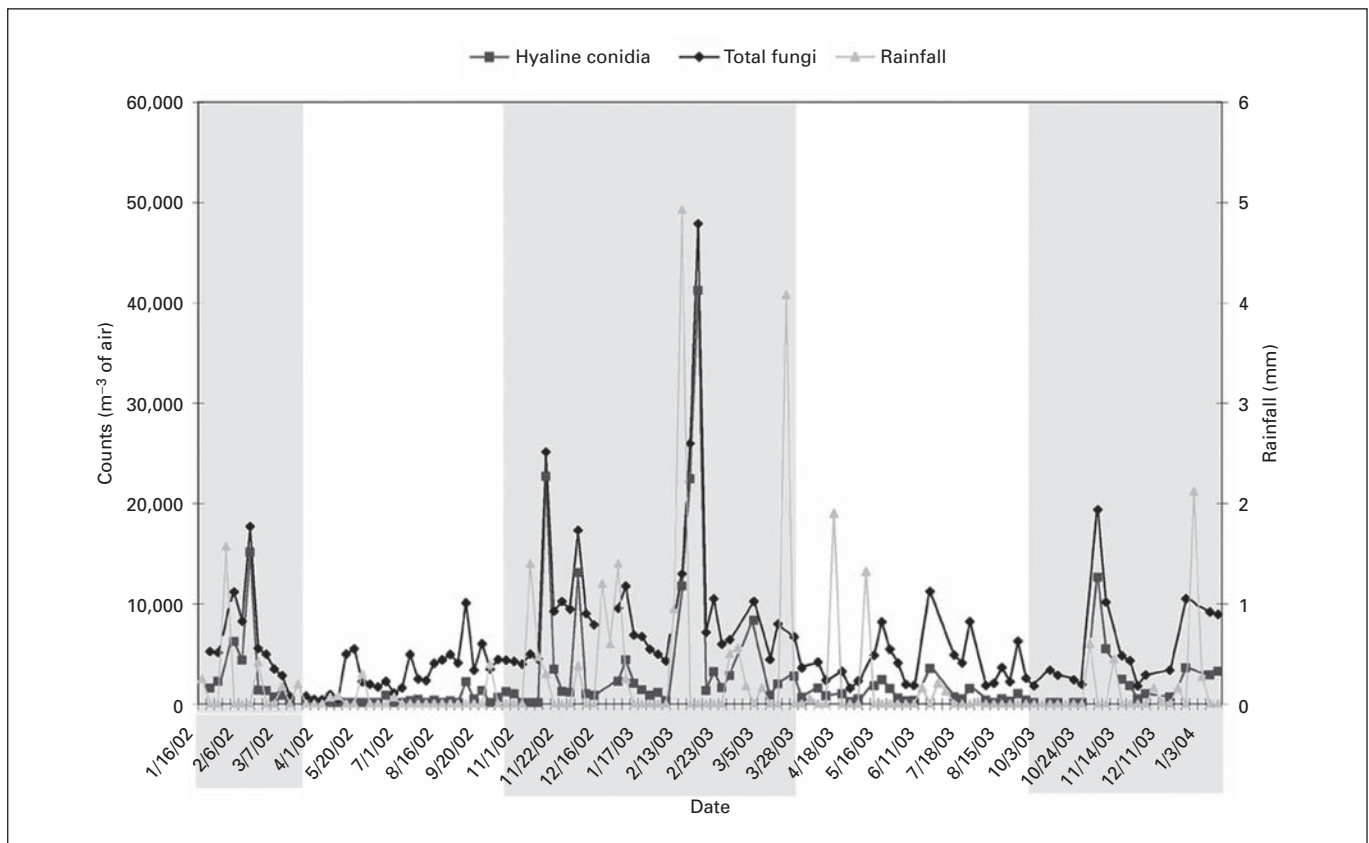


Fig. 1. Counts of hyaline conidia, total fungi, and daily rainfall over a 2-year period. Fall/winter seasons are shaded.

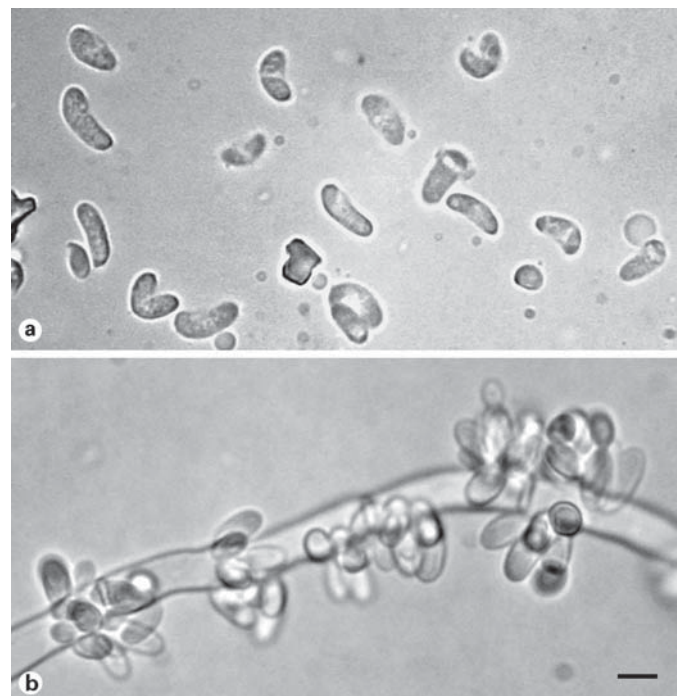


Fig. 2. **a** Unicellular hyaline conidia sampled from the outdoor air during rainfall and at night on 2/13/2003. **b** *A. pullulans* isolated from *C. citriodora* flowers and cultured in vitro. Bar = 3 μ m.

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ATTACGCCAGCATCCTAGCCGAAGCGCGGACCTCAGTCCCGGCTGGCCGTAT
TACACCCTGGGCTATAACACCTCCACCGAAGTGGAGGCCACATTCCCAGAGC
CTTTATCCGGCCGCGGAACTGATGCTGGCCTGTCCAACTGAGTAAACCGG
TCAGAAGACCGGCGCGAACAGTTTAAACAAGTCTGATTGCAAGCGCTTCCCTT
TCAACAATTTCACGTGCTTTTTAACTCTCTTTCCAAAGTGCTTTTCATCTTTG
ATCACTCTACTTGTGCGCTATCGGTCTCTCGCCAATATTTAGCTTTAGAAGAA
ATTTACCTCCCATTTAGAGCTGCATTCCCAAACTCGACTCGTCAAGGAG
ATTTACATAGGGTGCCATTTCTCTGTCACATACGGGATTCTACCCTCTATGAC
GTCCTGTTCCAAGGAACTTAGACAGGCGGCTTCCCAATCATCTCTACAA
ATTACAATGCGAACCCGAAGGCTAGCTTTCAAATTTGAGCTGTTGCCGCTTCA
CTCGCGTTACTAGGGCAATCCCTGTTGGTTTCTTTCTCCGTT

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Fig. 3. RNA sequence of the 571-base pair D1/D2 region of the 26S ribosomal subunit.

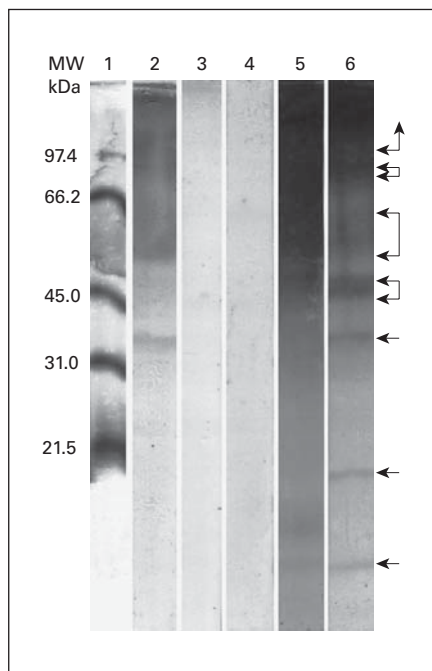


Fig. 4. Western blot analysis of *A. pullulans* isolated from flowers, fractionated under reducing conditions, and incubated with human and rabbit sera, as detailed for each lane, followed by IgE and IgG-linked alkaline phosphatase, respectively. Lane 1: molecular weight (MW) markers. Lane 2: human sera from a skin test- and IgE-positive patient. Lane 3: human sera from a skin test-positive but IgE-negative patient. Lane 4: human sera from a skin test- and IgE-negative patient. Lane 5: pooled sera from mold-allergic patients. Lane 6: pooled rabbit anti-*A. pullulans* sera, with bands indicated by arrows.

mately 1.6-fold increase in asthma admissions and evaluations at the Huntington Memorial Hospital compared with the spring and summer. Minimum admissions for asthma occurred during the hot, dry summer months of July and August.

Immunoanalysis

Following immunodiffusion, precipitin lines of identity were observed between the two isolate extracts (from both the cream- and pink-colored colonies isolated from flowers) and a reference extract prepared from *A. pullulans*. ImmunoCAP analysis of 124 clinically unknown patients showed 6 with IgE that bound *A. pullulans* extracts. Three of the 6 were class 1, i.e. $0.36\text{--}0.7\text{ kU}\cdot\text{l}^{-1}$ of specific IgE reactivity. The other 3 patients were class 2, i.e. $0.71\text{--}3.5\text{ kU}\cdot\text{l}^{-1}$. One of the 2 skin test-positive patients was class 2, with $1.4\text{ kU}\cdot\text{l}^{-1}$. The skin test-negative patient and 118 of the clinically unknown patients tested negative for specific IgE (less than $0.35\text{ kU}\cdot\text{l}^{-1}$). Western blot immunoanalysis of *A. pullulans*, extracted from flower isolates, fractionated under reducing conditions and incubated with either human IgE or rabbit IgG antibodies, showed multiple bands (fig. 4). IgG from rabbit anti-*A. pullulans* sera detected bands at approximately 10 and 18 kDa, major bands at 35 and 45–48 kDa, and darkly stained but diffuse bands from 53–65, 80–90 and above 100 kDa. IgE from pooled sera from mold-allergic patients detected bands at approximately 10 and 15 kDa, a major band at 35 and 45–50 kDa, and darkly stained but diffuse bands above 53 kDa. The IgE-positive sera from the skin test-positive patient, as detected using immunoCAP analysis, showed a band at approximately 35 kDa and diffuse bands at 53–65 and 80 to above 100 kDa. Neither of the IgE negative sera stained the *A. pullulans* extracts. Results were replicated in multiple gels and with low molecular weight markers.

Controlled Chamber Emission of Aerosols

Hyaline conidia occurred in the air in a predominant unicellular state. In controlled chamber emission experiments, *A. pullulans* readily formed an aerosol of respirable-sized conidia from *Corymbia citriodora* flowers. Aerosolized conidia measured less than $5\text{ }\mu\text{m}$ in diameter and *C. citriodora* pollen was $15\text{--}24\text{ }\mu\text{m}$ (fig. 5). With old-

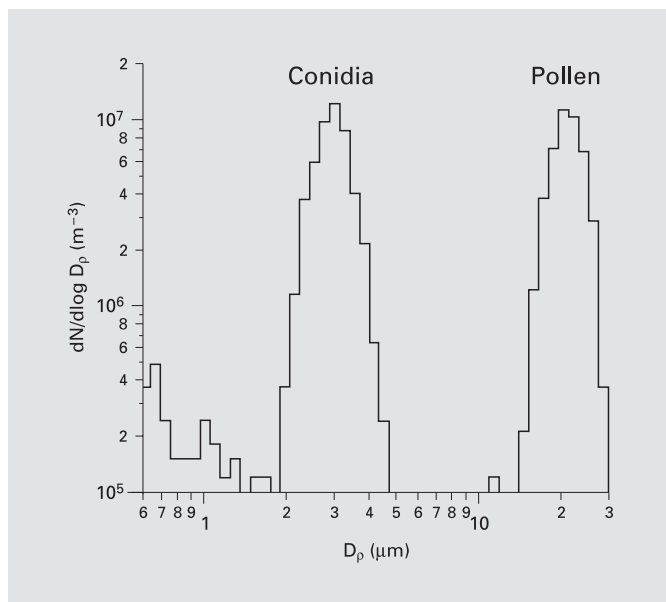


Fig. 5. Emissions from *C. citriodora* flowers housed within a controlled emission chamber showing the number and size distribution of individual *Aureobasidium* conidia and pollen grains released into each cubic meter of sampled air. D_p = Particle diameter; N = number of particles.

er flowers, only *A. pullulans* conidia were emitted, with little or no pollen released. Hyaline conidia were identified on cover slips removed from the cascade impactor that sampled air from the chamber. No emissions were detected from cultures grown on agar plates where conidia had coalesced into mucilage drops on the surface of the colonies. *A. pullulans* was isolated from leaf litter that had collected at the base of trees over summer. Few colonies were isolated from leaves or bark remaining on the tree and few particles were emitted from these sources during controlled emission experiments.

Discussion

High concentrations of unicellular hyaline conidia were detected in the air at night and during rainfall and ensuing wind disturbances. The identity of the fungal isolates was established by cell and colony morphology, reactivity toward a reference anti-*A. pullulans* antiserum, and ribosomal sequence data analysis. *A. pullulans* was previously isolated from air samples in Thailand using internal transcribed spacer sequences of the rDNA [1]. Researchers reported on the difficulty of isolating *Aureo-*

basidium from plant leaves, stems and bark. However, we observed flowers that were coated in filamentous hypha and hyaline unicellular conidia. We also experimentally observed that flowers were a major source of emissions of respirable-sized conidia of *Aureobasidium* that are often found in abundance in the outdoor air. High RH that generally occurred at night throughout the fall and winter encouraged their growth, which increased their presentation to the environment. Moist days also achieved this effect.

Aureobasidium occurs in the air in temperate zones throughout Europe [4], in the tropics [1] and has also been detected in the Arctic air [20], but is rarely reported in high concentrations in the outdoor air in the US [9]. The few US reports for *A. pullulans* describe outdoor levels fluctuating both seasonally and inter-yearly. It was one of the predominant fungi in Washington DC in 1980 but only accounted for less than 4% of total fungal counts in 1982 [21]. It was recorded as one of the predominant fungi in the outdoor air in Tulsa, Oklahoma, in the spring and summer of 1976 [22] but was not recorded by other researchers in a separate study in Tulsa in the same year [23]. *Aureobasidium* was recovered in different locations according to the season, correlating somewhat with the cabbage harvest as well as with the harvest of strawberries in an agricultural community in the Salinas Valley, California [24]. Recently, culturable *Aureobasidium* was identified in air samples from the homes of asthmatic children in seven US cities [25]. An average of 17% of samples collected outdoors contained *Aureobasidium*, but only 10.6% of indoor samples. Yeasts were identified on average in 29.2% (outdoors) and 21.7% (indoors) of samples. However, the main focus of this inner-city asthma study dealt with the occurrence of *Alternaria*, and the role of hyaline conidia was not further pursued.

Source of Aerosol Emission

Aureobasidium is a cosmopolitan fungus that has been isolated from plant debris, soil, wood and textiles. *Aureobasidium* occurs indoors in areas of free water, such as condensate pans, caulk or damp window frames in bathrooms or as a primary colonist of broadloom following a flood. Outdoors, *Aureobasidium* is a saprophytic phylloplane fungus and is one of the most abundant inhabitants of the surface of young green leaves in the UK [26], colonizes the veins and wounds on apple leaves in Wisconsin, US [27], and has been isolated from the leaves of eight different plant species in San Diego [28]. High densities of *Aureobasidium* have been detected in the air conditioning system of automobiles in Atlanta, US [29]. We ob-

served that *Aureobasidium* predominantly inhabited flower surfaces. A variety of yeasts have previously been isolated from insects collected from flowers; however, flowers were not reported as inhabited by *Aureobasidium*. Fungi such as *Aureobasidium* are being applied to agricultural crops for the bio-control of saprophytic and pathogenic fungi [30]. The predominance of *Aureobasidium* in Pasadena might explain the absence of other culturable hyaline fungi.

Climate Effects on Aerosol Distribution

RH and rainfall primarily govern changes in the yeast aerosol in a given habitat [5]. Most yeast-like organisms lack active mechanisms for launching viable propagules. The transfer of yeasts from flower to flower was thought to happen through insect vectors [31]. However, they are more likely dispersed by rain splash and wind disturbance [4]. We induced an aerosol of unicellular conidia from flowers, housed within a controlled emission chamber, with a wind disturbance generated by a fan. Quiescent conditions, high humidity, or a very light wind alone did not generate an aerosol. Large *Alternaria* spores are dispersed with a wind disturbance of 2–3 m·s⁻¹ [32], but even lower wind speeds are likely to disperse small hyaline conidia. Once in the air, the low inertia of these small conidia would allow them to be swept around a raindrop [33]. They are likely collected with very low efficiency by typical raindrops or thunderstorm drops, and not at all by small drizzle drops. There is a 4-time increase in the survival rate of yeast in the air at 70% RH, or higher, compared with 20–60% RH [15]. Thus, flowers are a likely source for the transmission, via the air, of viable *A. pullulans* conidia with the potential to colonize the next host.

Allergy and Pathology of Aureobasidium

Aureobasidium is implicated as a causative agent in allergies [34]. Elevated mean concentrations of *Aureobasidium* in Kansas homes were associated with allergy symptoms [35]. Extracts of *Aureobasidium* have elicited skin reactivity in 8–12% of atopic persons in one study in the Midwest USA [9]. *A. pullulans* also causes hypersensitive pneumonitis such as ‘sauna taker’s lung’ [36]. It has been isolated from air conditioning units coincident with antibodies to this organism being found in the blood of patients in Colorado [37], New York [38] and Germany [39]. Although we recognized an increased incidence of asthma evaluations and admissions during times of increased *Aureobasidium*, identified individuals with skin test reactivity to extracts of *A. pullulans*, and detect-

ed high molecular weight allergens in *Aureobasidium* extracts using patient sera IgE, direct provocation testing has yet to be accomplished. The high proportion (56%) of patients who tested positive for *A. pullulans* and had bronchitis and/or bronchial asthma suggests that *A. pullulans* might be involved in triggering a hyperreactive response. Historically, individuals sensitive to allergenic extracts develop bronchial reactivity when exposed to these extracts by inhalation.

We also observed peaks in the abundance of other allergenic, respirable-sized fungi, including *Cladosporium* and *Aspergillus*, at specific times throughout the fall and winter. Respirable-sized pollen fragments from plants such as *Alnus*, *Fraxinus* and *Ulmus* are also likely to contribute to the allergen load. The flu season peaks in December and January, after the peak in asthma evaluations and admissions. The correlation between viral infections and asthma incidence in 2002 was studied using prospective questionnaires. Similar percentages of patients had symptoms of viral upper respiratory infection in spring (April to June) compared with fall (October to December). Numerous other causes could contribute to the peak in asthma exacerbations in the fall/winter. Here, we have identified another potential trigger that was not previously recognized.

A. pullulans is not a primary human pathogen nor is it recognized as a producer of significant mycotoxins; however, it has been implicated as a causative agent of keratitis, pulmonary, systemic and cutaneous infections, peritonitis in continuous ambulatory peritoneal dialysis patients, as well as invasive mycosis [40, 41]. Infections occurred mostly by inoculation of immunosuppressed patients. Although the pathogenicity of *Aureobasidium* was reported as limited and uncommon [42], there has been an increased incidence of invasive fungal infections in the past 20 years [43]. Currently, we do not know the risk for immunosuppressed patients of exposure to high concentrations of airborne *Aureobasidium* conidia.

Acknowledgments

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