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Isolation and identification of air transported fungus collected in Toluca Valley, México.

Galindo-Martínez A[1], Rivera-Pérez Z[3], Romero-Martínez N[1], Núñez-Cardona MT[2], Falcón-Bárcenas T[3], Díaz-Godoy RV[3], Castellanos-Moguel J[1]*.

1 Universidad Autónoma Metropolitana-Xochimilco. Depto. El Hombre y su Ambiente. Laboratorio de Micología. Calzada del Hueso 1100, Col. Villa Quietud. México, 04960, D.F. Del. Coyoacán. Tel.: +52 5483 7226. Fax: +5254837469. E-mail: mjmoguel@correo.xoc.uam.mx

2 Universidad Autónoma Metropolitana-Xochimilco. Departamento El Hombre y su Ambiente. Laboratorio de Ecología Microbiana. Calzada del Hueso 1100, Col. Villa Quietud. México, 04960, D.F. Del. Coyoacán. Tel.: +52 5483 7226. Fax: +5254837469.

3 Instituto Nacional de Investigaciones Nucleares (ININ), Carretera México Toluca S/N, La Marquesa Ocoyoacac, México. C.P. 52750 Tel.: +52 53 29 72 00 ext. 2304, Fax: +5253297332.

Email responsable: mjmoguel@correo.xoc.uam.mx

ABSTRACT

Fungal spores are transient in the atmosphere, yin urban areas, fungi have diverse effects on human health including allergic respiratory or skin reactions, asthma and different invasive diseases. Toluca City, Mexico, currently has a monitoring system to determine the chemical origin of atmospheric pollutants, but the composition of airborne fungal particles with allergenic potential remains essentially unknown. The aim of this study was to isolate AND identification fungal genera from filters used to determine chemical contaminants. Samples were obtained during the autumn of 2009 (September, October, and November) using a TCR-TECORA sampler operated by the Automatic Network of Atmospheric Monitoring of the Metropolitan Zone of Toluca Valley (Red de Monitoreo Atmosférico de la Zona Metropolitana del Valle de Toluca, RAMAT-ZMVT). The TCR-TECORA sampler is used to collect chemical particulate matter with an aerodynamic diameter of less than or equal to 2.5 □m (PM2.5) and determine the gravimetric concentration of inorganic contaminants. For this study, the samples used for chemical analysis were also used to quantify the number and type of fungal pollutants present in the PM2.5 fractions. Sampler filters were washed to obtain fungal conidia, which were then quantified before aliquots from these washes were placed in Petri dishes with selective media. Twenty fungal genera were isolated, with *Penicillium*, *Epicoccum*, *Cladosporium* and *Aureobasidium* being the most abundant. To the best of our knowledge, this is the first report of fungal genera obtained using a TCR-TECORA sampler in the Metropolitan Zone of Toluca Valley, Mexico.

Keywords: PM2.5, health effects, asthma, allergenic fungi

RESUMEN

Las esporas fúngicas son transitorias en la atmósfera, en áreas urbanas los hongos tienen diversos efectos en la salud humana incluyendo alergias respiratorias, cutáneas, asma y diversas enfermedades invasivas. La Ciudad de Toluca, México, actualmente cuenta con un eficiente sistema de monitoreo para determinar los contaminantes químicos atmosféricos y su origen, pero la composición de partículas fúngicas con potencial alérgeno permanece esencialmente desconocida en la mayor parte de las fracciones de los contaminantes. El objetivo del estudio fue el aislamiento e identificación de géneros fúngicos a partir de los filtros utilizados para medir los contaminantes químicos. Las muestras se obtuvieron durante el otoño de 2009 (septiembre, octubre, y noviembre), usando un muestreador TCR-TECORA operado por la Red Automática de Monitoreo Atmosférico de la Zona Metropolitana del Valle de Toluca (RAMAT-ZMVT). El muestreador TCR-TECORA es utilizado para colectar material particulado con un diámetro aerodinámico menor o igual a los 2.5 mm (PM2.5) y determinar la concentración gravimétrica de los contaminantes inorgánicos. Para este estudio las muestras también se utilizaron para cuantificar el número y tipo de contaminantes fúngicos presentes en las fracciones de PM2.5. Los filtros fueron lavados para obtener conidios fúngicos, los cuales fueron cuantificados antes de que alícuotas de dichos lavados fueran colocadas en cajas de Petri con medio selectivo. Se aislaron veinte géneros fúngicos, siendo *Penicillium*, *Epicoccum*, *Cladosporium* y *Aureobasidium* los más abundantes. En nuestro conocimiento, este es el primer reporte de

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identificación de géneros fúngicos usando un muestreador TCR-TECORA en la Zona Metropolitana del Valle de Toluca, México.

Palabras clave: PM2.5, efectos a la salud, asma, hongos alérgenos.

INTRODUCTION

The atmosphere from urban zones contains a high level of particulate matter, and routine measurements of vehicle emissions and industrial pollutants are collected (Demerjian 2000; Brunekeef and Fosberg 2005). The composition of biological particles in the environment is poorly known, but it is inferred that biological particulates play an important role in public health, either alone or in combination with ozone and inorganic particulate matter (PM) (Sousa et al. 2008). Bioaerosols are conglomerates of biological particles such as bacterial and fungal propagules (Hass et al. 2010) as well as their fragments or metabolites that are present in the atmosphere. Several microorganisms are adapted to air transport and can resist environmental tension (Caballero-Segura et al. 2005). Specifically, fungi have been related to several respiratory problems, including bronchial asthma, oculorhinitis and skin conditions such as eczema. During the last 20 to 30 years, the incidence of these conditions in the population has risen considerably (Oliveria et al. 2009).

According to Lacey and West (2006), spores of several sizes can penetrate the lungs and cause different types of illness in susceptible individuals. Spores that are 10 □m or larger are deposited in the nose and cause rhinitis, while particles from 10 to 4 □m are deposited in the bronchia causing asthma. The smaller spores (optimum 2-4 □m) cause alveolitis upon reaching the lower respiratory tract.

Worldwide, studies have shown that *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Fusarium*, *Epicoccum*, *Aureobasidium* and *Rhizopus* are airborne fungal genera with allergenic potential (Rosas et al. 1990; Rosas et al. 1993; Calderón et al. 1997; Al-Suwaine et al. 1999; Ibañez-Hernandez et al. 2001; Al-Subai 2002; Esquivel et al. 2003; Fang et al. 2005; Grinn-Gofron and Mika 2008; Hasnain et al. 2005; Lee and Jo 2005; Adhikari et al. 2006; Damialis and Gioulekas 2006; El-Morsy 2006; Kasprzyk and Worek 2006; Abdel-Hameed et al. 2007; Griffin et al. 2007; Negrin et al. 2007; Piontelli and Vivar 2007; Fierer et al. 2008; O'Gorman and Fuller 2008; Potoglu-Erkara et al. 2009; Oliveira et al. 2009; Stepalska and Wolek 2009; Degobbi et al. 2011).

Aerobiological studies use several types of samplers (Andersen sampler for example) or gravity sedimentation on Petri dishes with selective media (Esquivel et al. 2003; Al-Subai 2002) to culture viable fungi. Traps are used for the microscopic observation of fungal spores and pollen (Tsai et al. 2007; Stepalska and Wolek 2009; Oliveira et al. 2009; Grinn-Gofrón and Mika 2009). The analysis of culturable fungi (with impactation of fungal propagule on culture media and gravity sedimentation) as well as the use of molecular techniques provide information regarding the composition of airborne fungi or unviable particles that contain allergens (Calderón et al. 2002; Wu et al. 2003; Yamamoto et al., 2010). When performing direct spore counts, the spores are loosened from the conidiophore, making it almost impossible to identify the genera and species of the fungus accurately (Kaspryzyk 2008). Currently, there is no universal sampling method for fungal spores (Kasprzyk and Worek 2006). PM and chemical elemental composition studies use samplers with filters. One such sampler, used for PM with an aerodynamic diameter of 2.5 □m or less, is the TCR-TECORA (User's Manual ECHO PM 2003) sampler, which uses quartz filters to capture PM and components (Demerjian 2000). The particle size of the sample may include biological particles such as fungi; thus, the filter could be used for simultaneous sampling of chemical and biological pollutants.

In the Metropolitan Zone of Toluca Valley, aerobiological studies are scarce. The most representative study was conducted by Alarcón-

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Valdez and Albores-Bernal (1985) who used gravity sedimentation over the course of one year to define the airborne fungal composition of Toluca. Presently, it is necessary to determine which fungal particles are suspended in Toluca's atmosphere and if they have any health effects on the population. Knowing the type and concentration of spores in the environment could allow the prediction of airborne fungal propagules to be made, along with a strategy for aiding the allergic population (Angelosante-Bruno et al. 2007). The objective of this study was to isolate and identify the genera of culturable airborne fungal spores with allergenic potential in Toluca, and to quantify the concentration of fungal propagules from samples obtained with the filters used for $PM_{2.5}$ measurements from the Automatic Environmental Monitoring Network. This study combines culture methods and microscopic analysis. Health damage resulting from fungal exposure is caused by viable spores, fungal fragments following spore death and from mycelia (Kauffman and Van der Heide 2003); thus, it is important to know viable and unviable particle composition. We propose using the $PM_{2.5}$ samplers simultaneously to measure the inorganic and fungal pollutants, optimizing the device for use and sampling time. To the best of our knowledge, this is the first report of fungi isolated from $PM_{2.5}$ sampler filters in Toluca, Mexico.

MATERIAL AND METHODS

Sampling site location

The sampling site was located in the Metropolitan Zone of Toluca Valley, Estado de México, at the San Cristobal Huichochitlan (Primary School Manuel Hinojosa Giles, Guadalupe Victoria Street, on the ancient road to la Magdalena, Col. San Cristobal Huichochitlan; coordinates 19°, 19′38″ E and 99°38′0.33″; elevation, 2692 mosl) sampling station of the Automatic Network of Atmospheric Monitoring of the Metropolitan Zone of Toluca Valley (Red de Monitoreo Atmosférico de la Zona Metropolitana del Valle de Toluca, RAMAT-ZMVT).

Toluca's climate is classified as humid temperate with a summer rainy season. The annual temperature average is 13.7°C. During the cold season, frosts occur from 80 to 140 days (INEGI). The city is associated with vegetal communities, mainly pine, oak, mixed pine oak forests and grasslands.

Sample collection

A PM TCR-TECORA sampling device was used to collect $PM_{2.5}$ samples on a 47 mm diameter quartz fiber filter. Samples were collected according to Gerald (2003) and the International Atomic Energy Agency (IAEA, 1995). Filters were changed every 24 hrs, and the sampling period extended from September through November of 2009 with a sampling frequency of three days (Table 1). Filters were transported as biological material and maintained at 4°C until use.

Processing and analysis of samples

Filters were managed as biological specimens. Under sterile conditions, a piece of filter of approximately 2 cm^2 was placed in sterile 0.05% Tween 80 and stirred by vortex for 1 minute to wash. The wash was repeated three times (Durand et al. 2002), and the resulting suspension was placed on Petri dishes. SPYE medium (in grams per liter: sucrose, 10; glucose, 5; peptone, 0.5; yeast extract, 5; agar, 23) containing chloramphenicol was used to isolate and preserve culturable filamentous fungi (Castellanos-Moguel et al. 2007). Agar plates and slant tubes were immediately placed into incubators at 28°C.

For fungal identification, the macromorphological appearance of the colonies was described, and direct microscopic observation of the cultures was made at 40x using a phase-contrast Olympus 5640 microscope. Riddell microcultures of

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Potato-Dextrose agar (Mier et al. 2002) were prepared and observed as well. Identification was based on Barron (1968), Barnett and Hunter (1972) and Von Arx (1981).

Propagule quantification

From the suspension described previously, a 2 ml aliquot was centrifuged for 30 minutes at 4000 rpm. The supernatant was discarded and the sediment was suspended in 100 □l of cotton blue to stain fungal structures. Ten aliquots of the suspension were counted using a hemocytometer, and the number of propagules/filter/m^3 of filtrated air was calculated. Structures with fungal morphology according to Lacey and West (2006) and cotton blue staining were considered fungal propagules.

Statistical analysis

Statistical analysis was performed using JMP version 8.0 (SAS Institute). Descriptive data analysis and non-parametric statistical tests (Kruskal-Wallis) were used.

RESULTS AND DISCUSSION

Determination of airborne fungal propagules using a TCR-TECORA sampler for $PM_{2.5}$

Air quality is an important factor in environmental health, and this quality is partially related to human activities associated with automobile traffic, farming and stables. Vegetation and animal facilities are related to the amount of spores in the air (Esquivel et al. 2003; Kasprzyk 2008). In this study, the sampling station is in a rural area near a slaughterhouse, the wholesale produce market of Toluca City (Central de Abastos de Toluca), an industrial zone and the Toluca airport; all of these places could be a potential source of fungal spores.

Fungal spores are well adapted to airborne transportation even though the atmosphere is a transient habitat for them. Fungal spores must therefore be considered a part of the $PM_{2.5}$ fraction of pollutants, even when they are considered bigger than 2.5 µm. In this study, the TCR-TECORA $PM_{2.5}$ sampler used allowed the collection of fungal propagules from the filters routinely used to collect chemical and automobile particles. One of the disadvantages of personal samplers is that they have short collection times (Borchers et al. 2006); however, the TCR-TECORA $PM_{2.5}$ sampler allows sampling for a 24 hr collection period. Some fungal spores were viable, and a total quantification of the fungal propagules/m^3 was made (Table 1). It can be observed that the spore concentrations vary, reaching higher values on October 6th (10.1 propagules/m^3) and October 24th (8.5 fungal propagules/m^3), but a lower value on October 8th (0.6 propagules/m^3). This

Table 1. Sampling calendar and concentration of fungal propagules/m^3 of filtrated air. Samples were taken every three days over a 24 hr period during the autumn months (September, October and November) of 2009.

Filter	Sampling day	Fungal propagules/m^3
1	15-Sept-09	1.9
2	24-Sept-09	0.7
3	26-Sept-09	0.9
4	01-Oct-09	1.8
5	03-Oct-09	0.9
6	06-Oct-09	10.1
7	08-Oct-09	0.6
8	13-Oct-09	2.5
9	17-Oct-09	1.4
10	22-Oct-09	0.7
11	24-Oct-09	8.5
12	29-Oct-09	0.8
13	31-Oct-09	2.8
14	5-Nov-09	1.9
15	07-Nov-09	0.8
16	10-Nov-09	1.6
17	17-Nov-09	2.5
18	22-Nov-09	2.7
19	24-Nov-09	1.6
20	28-Nov-09	1.2

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observed fluctuation in filter fungal propagule concentration was significant according to the Kruskall-Wallis test (significance level = 0.05). Degobbi et al. (2011) suggest that chemical PM affects fungal dispersion properties. In our study, the sampling point was at the convergence zone of an airport, slaughterhouse, industrial zone and the wholesale produce market of Toluca City where winds and the presence of fungi could be expected to be high. The analysis of filters from other types of samplers were performed by Griffin et al. (2007) and Degobbi et al. (2011); they found potentially allergenic fungal spores of *Alternaria*, *Acremonium*, *Cladosporium*, *Penicillium*, basidiospores and ascospores. In our study, 85 fungal colonies belonging to 20 genera were isolated from the Toluca filters, and 14 of the 20 genera identified are

potentially allergenic or pathogenic (Table 2). Seven sterile mycelia isolates (8.75% of the total isolates) were registered. Although these last genera had no reproductive structures to allow their morphological identification, they retained allergenic potential because fungal mycelia contain proteins that can cause immune reactions. The major allergenic fungal manifestations are asthma, rhinitis, bronchopulmonary allergic mycoses and hypersensitivity pneumonitis. These disorders can be induced by fungal spores, vegetative cells or metabolic excretions. Spore aggregates can be found in the larger PM fraction and can settle in the upper airways. Smaller, individual conidia, such as those found in the $PM_{2.5}$ fraction can easily reach the lungs (Kurup et al. 2000). The data obtained could be useful for estimating fungal illness and allergy symptoms, the duration of infection and progression (Stepalska and Wolek 2009).

Fungal genera

The fungal genera isolated from the $PM_{2.5}$ TCR-TECORA sampler and their possible health effects are listed in Table 2. The isolation and identification of viable spores and cultivable fungi is very important because the toughness of the fungal cell wall means that the spores generally do not release their allergens until germination or after cell death (Kauffman and Van der Heide 2003).

Penicillium was the most abundant genera found with 34 isolates (44.7% of the fungal genera identified), and this genus along with *Aspergillus* produced a high concentration of airborne conidia. In the atmosphere of Mexico City for example, these genera are second in terms of abundance (Rosas et al. 1993; Calderon et al. 1997). These fungi in relatively low concentrations (6×10^4 spores/m^3 of air) can cause respiratory symptoms (Lee and Jo 2005). For Toluca Valley, Alarcon-Valdez and Albores-Bernal (1985) isolated 26 genera and cultured *Penicillium*, *Cladosporium*, *Alternaria*, *Aspergillus* and *Geotrichum*. We isolated 12 fungal genera for the first time in Toluca Valley even though these genera could be considered ubiquitous fungi because they are reported in other cities worldwide (Lee and Jo 2005; Kasprzyk and Worek 2006; El-Morsy 2006; Abdel-Hameed et al. 2007; Griffin et al. 2007; Negrin et al. 2007; O'Gorman and Fuller 2008). According to Hameed et al. (2007), the airborne spores from any country are essentially the same, with the only differences being quantitative, not qualitative. Most of the fungal genera isolated are potentially allergenic or pathogenic in individuals with transplants, chemotherapy, stress and HIV/AIDS.

Alternaria, *Aspergillus* and *Cladosporium* all produce allergens with high prevalence and are frequently isolated outdoors. These genera can cause skin reactions in allergic patients (Dixit et al. 2000) and have been isolated from the nasal and pharyngeal mucous membranes of patients with allergic rhinitis in Michoacán, México (Rodríguez-Orozco et al. 2007).

Among the isolated genera, most (*Cladosporium*, *Epicoccum*, *Penicillium*, *Alternaria*, *Aspergillus*, *Bipolaris* and *Geotrichum*) present with dark colonies; dark pigments allow the fungi to tolerate UV and solar radiation. Dark spores are

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Table 2. Number and percentage of fungal genera isolated from the TCR-TECORA filters and their potential health effects.

Fungal genera	Number of isolates	Percentage of total identified genera	Health effect of species from this genera	References
Penicillium	34	44.7	Keratitis, outer ear infections, respiratory and urinary tract infections, eczematous dermatitis, suberosis, cheese workers lung, farmers lung, salami workers lung, peat moss processor lung	Rimac et al. 2010; Pettigrew et al. 2010; Nordness et al. 2003
Cladosporium	8	10.3	Type I allergy, type III hypersensitivity pneumonitis; rare keratomycosis, sauna taker's lung	Borchers et al. 2006; Chew et al. 2009; Pettigrew et al. 2010; Nordness et al. 2003;
Epicoccum	8	10.3	Type I allergy, type III hypersensitivity pneumonitis, extrinsic allergic alveolitis	Pettigrew et al. 2010
Aureobasidium	6	8.3	Type I allergy, type III hypersensitivity pneumonitis, extrinsic allergic alveolitis, Phaeohyphomycosis, air conditioner lung	Pettigrew et al. 2010; Nordness et al. 2003
Acladium	2	2.7	We found no reports of health damage	
Alternaria	2	2.7	Type I allergy, type III hypersensitivity pneumonitis, nasal and subcutaneous lesions in patients with AIDS, wood trimmers disease	Borchers et al. 2006; Nordness et al. 2003; Pettigrew et al. 2010
Chrysonilia	2	2.7	Type I allergy, endophthalmitis	Cartier 2010; Rainer et al. 2000
Stachybotrys	2	2.7	Dermatitis, cough, rhinitis, itching or burning in the oral cavity or nasal passages, toxin-related illness (sick building disease)	Pettigrew et al. 2010; Yike et al. 2001
Acremonium	1	1.3	Type I allergy, asthma, type III hypersensitivity pneumonitis, "humidifier lung" mycetoma, maduromycosis, eye infections	Saijo et al. 2005; Piontelli and Vivar 2007
Aspergillus	1	1.3	Type I allergy, type III hypersensitivity pneumonitis, invasive aspergillosis, opportunistic pathogen (may cause lung and brain injuries), sinusitis, stipatosis, malt workers lung, compost lung, tobacco workers disease	Rimac et al. 2010; Borchers et al. 2006; Pettigrew et al. 2010; Nordness et al. 2003
Bipolaris	1	1.3	Type I allergy, fungal sinusitis, eye infections, bone, aorta, lungs, brain, skin, queratitis, osteomyelitis, Phaeohyphomycosis	Borchers et al. 2006; Pettigrew et al. 2010
Botryoderma	1	1.3	We found no reports of health damage	
Chaetophoma	1	1.3	Type I allergy, Phaeohyphomycosis	Borchers et al. 2006
Geotrichum	1	1.3	Bronchial, oral and vaginal lesions; scattered skin lesions; occasional bloodstream infection with sepsis	Rainer et al. 2000
Mammaria	1	1.3	We found no reports of health damage	
Memnoniella	1	1.3	Type I allergy, produce mycotoxins, congestion, sinusitis	Borchers et al. 2006; Wilkins et al. 2003
Varicosporium	1	1.3	We found no reports of health damage	
Monascus	1	1.3	We found no reports of health damage	
Sepedonium	1	1.3	Neutropenia and fever in immunocompromised patients	Arellano-Galindo et al. 2008
Paecilomyces	1	1.3	Hyalohyphomycosis, keratomycosis and endophthalmitis, fungemia in patients with indwelling vascular catheters, cutaneous infections, maxillary sinusitis, peritonitis, suppurative otitis media, cerebrospinal fluid shunt obstruction, allergic alveolitis	Rainer et al. 2000; Nordness et al. 2003

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more abundant in rural areas; this is coincident with the location of the sampling station at a rural school Kasprzyk and Worek 2006). Although the exact origin of the fungal spores is unknown, the isolated genera are fungi capable of growth on a great diversity of substrates in temperate or semitropical regions (Lee and Jo 2005; Kasprzyk and Worek 2006; El-Morsy 2006; Abdel-Hameed et al. 2007; Griffin et al. 2007; Negrin et al. 2007; O'Gorman and Fuller 2008).

Toluca Valley is surrounded by forests and certain areas are considered rural even within the Metropolitan Zone. These characteristics imply a great amount of vegetation that could be a local source of spores.

CONCLUSION

This study of outdoor air provides data on airborne spore levels of allergenic and potentially pathogenic fungi in Toluca Valley, Mexico, as measured in the autumn of 2009. The combined methods of analysis used here provide greater information on the genera and concentration of fungal propagules present. Fungi can be found at $PM_{2.5}$ fraction, allowing them to reach the lower respiratory tract region.

Knowledge of local inhalable allergens helps facilitate the diagnosis and treatment of related pathologies. Although the genera that were isolated are considered ubiquitous, 12 genera in Toluca were isolated here for the first time and most of them have allergenic or pathogenic potential. The fungal genera isolated in Toluca Valley in the autumn could be considered among the allergens tested for in patients with clinical manifestations. To the best of our knowledge, this is the first report of the collection of fungal genera using a TCR-TECORA sampler in the Metropolitan Zone of Toluca Valley.

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