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PENICILLIUM AND *ASPERGILLUS* DOMINATE A COLLECTION OF CULTURABLE MOLDS FROM A TANNERY IN INDIANA

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ABSTRACT. Moser Leather Tannery in New Albany, Indiana operated for over 140 years as a major producer of leather for products such as saddles and shoelaces. After production halted in 2002, the tannery complex was vacant for several years with plans for redevelopment as modern living space and a tannery museum. Visual inspection of the tannery buildings showed mold colonization on walls, floors and walkways, especially during warm and humid summer months. The objective of this study was to conduct a survey of culturable molds in the tannery. Samples were collected from four locations at the tannery yielding 14 purified isolates. Using both standard phenotypic and DNA-based phylogenetic methods, eight species of mold were identified. The two most common molds at the tannery were *Penicillium* followed by *Aspergillus*. *Penicillium brocae* was identified from two tannery locations making this the first report of this mold in Indiana.

Keywords: Moser Leather Tannery, indoor mold, *Penicillium*, *Penicillium brocae*, *Aspergillus*

Fungi are a wide ranging and diverse group of organisms whose members act as efficient decomposers, crop pathogens, opportunistic human pathogens, and important producers of metabolites (Deacon 2006). When fungi move indoors to colonize living space, health effects such as allergies, asthma, irritations and infections can occur (Bush et al. 2001; Etzl et al. 1998; Fisk et al. 2007; Hodgson et al. 1998; Johanning et al. 1996; Mazur et al. 2006). These problems may worsen with water damage, as was observed following hurricanes Katrina and Rita (Rao et al. 2007). Even buildings free of water damage and without obvious fungal growth maintain a unique and detectable mycoflora (Horner et al. 2004) suggesting that the type of fungi and the degree of contamination often dictate whether health problems will arise.

Moser Leather Tannery operated in New Albany, Indiana for over 140 years as a major producer of leather for products such as shoelaces and saddles. The tannery used a vegetable tanning process that relied on tree bark extract rather than toxic heavy metals more common in tanneries (Al Goodman per. comm.). The tannery halted production in 2002 and in the following years some buildings were vandalized, damaged by weather events and subjected to fluctuating temperature, humidity and water damage. Plans are currently underway to renovate some of the tannery complex for apartments. The 0.19 km^2 grounds adjoining the tannery have been converted to a public area known as Loop Island Wetlands.

Given its long history and unique tanning practices, Moser Leather Tannery offers an intriguing environment for microbiological studies. Visual inspection of the tannery showed that floors, walls and equipment were often heavily colonized by molds, especially in the humid summer months. Numerous tanning pits, initially drained when the tannery closed, had filled with rainwater which combined with tanning sludge to offer a rich nutrient source for microbial growth.

The objective of this study was to isolate and characterize molds commonly found colonizing surfaces at the Moser Leather Tannery in New Albany, Indiana. Of the 14 isolates, nine were identified as *Penicillium* and three were identified as *Aspergillus*. *Penicillium brocae*, which was recently found associated with coffee berry borers in Chiapas, Mexico (Peterson et al. 2003), was isolated from two locations at Moser Leather Tannery, making this the first report of this mold in Indiana.

METHODS

Sampling, growth and isolation.—Samples were collected with sterile swabs from the Moser Leather Tannery in New Albany,

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Indiana. Sampling locations included a scrap of leather, an interior brick wall next to a freight elevator, a tannery cart and a support beam next to the tannery's stuffing mill. The swabs were transported to IU Southeast immediately and stored at 4°C. Samples from the swabs were grown on potato dextrose agar (PDA) at 25°C for 3 to 5 days. Purification of fungal isolates was done by several transfers to new PDA plates incubated under identical conditions.

Microscopic evaluation.—Microscopic evaluation was done by slide culture (Benson 2002). Briefly, a PDA agar plug was inoculated with a fungal isolate and then placed onto a sterile slide. A sterile cover slip was placed on top of a second face of the inoculated agar plug. The slide/coverslip combination was placed on a glass rod support in a sterile Petri dish with moisture provided by a filter disc saturated with sterile distilled water. Isolates grown by slide culture were incubated for 3 to 5 days at 25°C after which the agar plug was discarded and a drop of 95% alcohol was added to the glass slide and coverslip for 1 min followed by addition of lactophenol cotton blue. Isolates prepared by slide culture were examined under 40x, 100x and 1000x.

Genomic DNA extraction.—Fungal isolates were grown on PDA for 2–3 days after which samples for extraction were collected by removing agar plugs from the plate or by suspension of fungal mycelia and spores in 2 ml sterile water. DNA extraction was completed using MO BIO's Soil DNA Extraction Kit (MO BIO Laboratories, Carlsbad, California). DNA extracts were stored at −20°C.

PCR conditions.—PCR fragments for sequence analysis were amplified with primers ITS4 and ITS5 which cover the internal transcribed spacer 1 (ITS1), 5.8S and ITS 2 (White et al. 1990). Amplifications were carried out in a Flexigene thermal cycler (Techne Inc., Princeton, New Jersey) using Promega PCR Master Mix (Promega Corporation, Madison, Wisconsin).

DNA sequencing.—PCR fragments were purified using MOBIO's PCR Purification Kit (MO BIO Laboratories, Carlsbad, California), diluted to a final concentration of 50 ng/µl and then sequenced using an Applied Biosystems 3100 Genetic Analyzer at the Indiana University DNA sequencing core facility.

RESULTS

Fourteen fungal isolates were obtained from Moser Leather Tannery in New Albany, Indiana. The genus *Penicillum* was isolated exclusively from three out of four tannery locations (a leather scrap, a tannery cart and the stuffing mill) (Table 1). Overall, *Penicillium* dominated the collection comprising nine out of 14 isolates (Table 1). The most common isolate was *Penicillium spinulosum*, which was isolated four times from two separate tannery locations. *Penicillium steckii* was isolated twice from the stuffing mill, and *Penicillium brocae* was isolated from both the stuffing mill and the tannery cart.

Aspergillus was the next most common isolate from the tannery, with all three isolates originating from the elevator entrance. Of the four locations sampled, the elevator entrance yielded the greatest diversity with four unique fungal species and one unidentified Ascomycete.

DISCUSSION

Penicillium is widely distributed and its spores are common in air samples (Webster 1970). Members of the genus *Aspergillus* are also common in the environment and often found contaminating other cultures (Larone 1995). Both *Penicillium* and *Aspergillus* are frequently encountered in microbiological studies of buildings. For example, the EPA BASE study ranks both genera in their top five most commonly found fungal groups in indoor and outdoor air samples (Womble et al. 1999). In a large study of buildings and outdoor environments in the United States *Penicillium* and *Aspergillus* ranked among the most common culturable airborne fungi in all seasons and across all regions tested (Shelton et al. 2002). These two molds were also predominant in university rooms in Poland (Stryjakowski-Sekulska 2007), abundant in indoor and outdoor samples from homes free of water damage (Horner et al. 2004), and common in homes that experienced differing levels of water damage from hurricanes Katrina and Rita (Rao et al. 2007).

Penicillium and *Aspergillus* are so widespread in outdoor and indoor environments that they are generally considered contaminants (Larone 1995). This is not to say that species of these genera are completely innocuous. *Penicillium*

Table 1.—Description and identification of fungal isolates from Moser Tannery.

Location	Top	Reverse	Match	Closest relative
Leather scrap				
	Olive grey/ white margins	Creamy yellow	520/522 (99%)	*Penicillium variabile*
Elevator entrance				
	Blue-green gray/ white margins	Cream	500/500 (100%)	*Penicillium spinulosum*
	Grey/black-wooly texture	Black/grey margins	516/518 (99%)	*Ascomycete sp.*
	Green/ white margins	Cream, green tinge	507/508 (99%)	*Aspergillus unguis*
	Mint green/ white margins	Cream, grey	487/487 (100%)	*Aspergillus unguis*
	Grey-brown/cottony texture	White	159/160 (99%)	*Syncephalastrum racemosum*
	Black	Grey-black	529/530 (99%)	*Aspergillus niger*
Tannery cart				
	Light green-yellow	Tan	509/509 (100%)	*Penicillium spinulosum*
	Green-grey, white margins	Cream	520/520 (100%)	*Penicillium spinulosum*
	Dark olive green/ white margins	Cream, yellow agar	559/559 (100%)	*Penicillium brocae*
	Forest green/ white margins	White	526/527 (99%)	*Penicillium spinulosum*
Stuffing mill				
	Olive green/ white margins	Creamy tan	240/241 (99%)	*Penicillium steckii*
	Dark olive green/ white margins	Cream, yellow agar	559/559 (100%)	*Penicillium brocae*
	Forest green/ white margins	Rust	365/365 (100%)	*Penicillium steckii*

spp. may cause a variety of infections and, *Aspergillus spp.* are associated with a range of health problems from allergies to invasive infections (Larone 1995). However, the findings in this study indicate that Moser Leather Tannery is colonized primarily by commonly occurring molds (except for *Penicillium brocae*, see below for discussion) that would likely be detected in a variety of outdoor and indoor environments.

Our survey of culturable molds at Moser Leather Tannery fits with previous studies on indoor molds with one clear exception. The isolation of *Penicillium brocae* from the tannery cart and stuffing mill at Moser Leather Tannery was unexpected. Peterson et al. (2003) first described *P. brocae* in association with coffee berry borers and its galleries in Chipas, Mexico. *Penicillium brocae* was subsequently isolated from a Fijian marine sponge and found to produce novel polyketides (Bugni et al. 2003). It appears that ours is the first report of *P. brocae* from Indiana and suggests that expansion of the range of *P. brocae* should be considered.

The question of how *Penicillium brocae* arrived at Moser Leather Tannery is intriguing. One hypothesis is that the range of *P. brocae* is simply broader than previously known. If this is the case, future fungal surveys should readily document this species. An alternative hypothesis is that tree bark extract used in the tanning process at Moser Tannery served as the source of *P. brocae*. The bark extract was made from Quebracho trees which are represented by three species in South America. Quebracho bark is susceptible to bark beetles (Porter 2004), many of which have mutualistic associations with fungi (Booth et al. 1990). Thus, perhaps *P. brocae* made its way to Moser Tannery by way of previously colonized tree bark. Further speculation is difficult because the association of *P. brocae* with multiple species of bark beetles has not been determined. At Moser

Leather Tannery, powdered Quebracho tree bark extract was stored in large quantities and some bark-liquor solutions still remain in the facility. If colonized by *P. brocae*, this abundant material would make an ideal starting point for further colonization of the tannery.

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