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LABORATORY EXPERIMENTS

The graying of cedar shingles in a maritime climate—a fungal basis?

LA Cronin[1], WN Tiffney Jr[1] and DE Eveleigh[2]

[1]*Nantucket Field Station, University of Massachusetts, Boston, Nantucket, MA 02554;* [2]*Department of Biochemistry and Microbiology, Cook College, Rutgers University, New Brunswick, NJ 08901, USA*

Cedar shingling is used extensively on the exterior of buildings of Nantucket Island because it is resistant to damage and decay by the elements. Although often structurally sound for up to 30 years, cedar shingles weather to a characteristic gray in this maritime clime within weeks of their installation. In contrast, cedar shingles when weathered in more inland locations yield a brownish hue. A survey was conducted of fungal populations inhabiting cedar shingles from maritime and inland locales to assess whether fungi contributed to these differences in coloring. *Aureobasidium pullulans*, a black yeast, was recovered consistently in all weathered samples, both maritime and inland. No major differences were seen between the fungal populations from these distinct geographic sites. It was concluded that *A. pullulans* and other blue stain fungi from the shingles could be responsible in part for the coloration of weathered shingles, but did not apparently account for the differences in coloring of gray (maritime) and brownish (inland) shingles. This exercise demonstrates identifiable common deteriogens, and is readily adapted to general microbiology laboratory classes. The isolates are from common microbial niches, wooden posts, window pane putty, tile grout and paint surfaces, and give the student a feel for the ubiquitous influence of microbes. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 319–322.

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Introduction

'*Nantucket! Take out your map and look at it . . . a mere hillock, and elbow of sand; all beach, without a background*.'

Herman Melville in Moby Dick

Nantucket is a small, gently rolling island, and having been stripped bare of tall vegetation is subject to the vagaries of ocean storms being 30 miles off the Massachusetts mainland. Nantucket is subject to strong south-westerly winds that carry sand and salt across the island, which periodically blast onto the buildings. Such elements initiate surface modification of the house shingles, and could indirectly affect the growth of fungi within the shingles. The physical parameters for fungal colonization of the shingles are optimal, being slightly acidic, combined with long warm summers, and high moisture. Fungi generally require a fiber saturation point of 28–40% within wood to grow, a range comparable to 90–95% relative humidity (RH) in the ambient wood fiber environment. Such a moisture regime occurs on Nantucket as it is continually bathed in humid, relatively warm air, being close to the Gulf Stream, and this readily translates to the high relative humidity that is optimal for fungal growth. Although the wood contains a microenvironmental niche ready for exploitation by microbes, cedar has a reputation for being rot-resistant. Cedar shingles are protected by such antimicrobials as α- and γ-thujaplicins, and indeed, they can remain structurally sound for an average of 30 years (Stanley 'Buzz' Polchinski, PO Box 2019, Nantucket, MA, personal communication). An additional benefit of cedar shingling in this severe environment is the ability to swell, thus essentially sealing cracks and protecting the underlying structure from wind and water. Initially Nantucket houses were faced with clap-board, and were often painted. Later, cedar shingles presented a less expensive, low-maintenance practical house exterior that likely appealed to the island's founding Quaker, Baptist, and whaling communities, and they remain an integral component of historic Nantucket [9,11].

Under optimal conditions, wood is principally degraded by brown rots that preferentially attack cellulose, or by white rot fungi that attack both cellulose and lignin leaving a white thin shell [3,5,18]. A third group of cellulose degraders are the soft rots that develop especially in continually damp wood, a sub-optimal condition for brown and white rots. These include sap-stain fungi defined as growing on freshly-felled timber, and also blue-stain molds developing on processed wood [3]. Yet these terms have varied interpretation, with blue-stain being incorporated within the term sap-stain [12]. We use blue-stain *sensu* Eaton & Hale [3] as molds that attack processed wood. Common examples of blue-stain molds include soft rot fungi that develop under water (cooling towers) and besides those evidenced in the spongy zones of window frames continually subject to condensed water running from glass panes. They are an aesthetic nuisance yet also insidious cellulose degraders. On drying, affected zones crack and

Correspondence: DE Eveleigh, Dept of Biochemistry and Microbiology, Cook College, Rutgers University, New Brunswick, NS 08901, USA
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darken. The blue-stains can be subdivided into those associated with wood-boring beetles and bark beetles (*Ceratocystis* and *Ophiostoma*), and those which are general saprophytes often having abundant aerially dispersed spores [3,5,12,18]. These latter blue-stainers include *Alternaria* spp and *Cladosporium* spp, and also the black yeast, *Aureobasidium pullulans*. It is this latter group of blue-stain molds that develops in cedar shingles [7,14].

Cedar shingles in a maritime environment weather to a characteristic gray color within weeks, while the shingles of inland houses weather to a light brown color rather than gray. The photodegradation products of lignocellulose support the growth of *A. pullulans* [13]. To account for the differences in color, it was proposed that different fungi develop within the shingles of Nantucket and inland buildings, and that they could be responsible for the variation in shingle color upon weathering. To test this hypothesis, a comparison of fungi isolated from shingles from a maritime source (Nantucket, MA) and an inland source (Princeton area, NJ) was made.

Materials and methods

White cedar shingles (*Thuja occidentalis*) were obtained from the following sites: southwest face of the Nantucket Post Office (Pleasant Street branch); the west face of the UMASS-Boston Nantucket Field Station laboratory; and Western red cedar (*T. plicata*) from a private residence, Princeton, NJ; and controls of new, unweathered white and red cedar shingles. Comparative wood rots included a white rot from the UMASS-Boston Nantucket Field Station and continuously water-sprayed pinewood in a marine fish tank from the Nantucket Research and Education Foundation Hatchery, Brant Point, Nantucket, MA.

Microscopy and staining [10]

Thin sections were obtained by slicing the wood's surface with a razor blade. The resulting thin sections were placed in vials and heated for 25 min at 90°C in 3% KOH to clear the cell cytoplasm [10]. These sections were rinsed twice with water. Sections were then acidified by soaking them in 1% HCl for 1–4 h in order to facilitate stain absorption. The sections were placed in vials containing 5–8 ml of acidic glycerol Trypan blue-stain and heated for 30–60 min at 90°C. The sections were not rinsed after removal from the HCl. The stain was 0.5% Trypan Blue in 50 ml glycerol, 45 ml water, and 5 ml 1% HCl. The destaining solution consisted of 50 ml glycerol, 45 ml water, and 5 ml 1% HCl. Stained sections were viewed using a Leitz S-M phase-contrast microscope.

Culture methods

The operation was in undergraduate field station style, and included sterilization of culture media in a kitchen pressure cooker, and the development of the concepts of aseptic technique in a sometimes-exposed laboratory setting. Culture media included potato dextrose agar (Difco) and malt extract agar (Difco) and were prepared according to the manufacturer's instructions. In order to prevent bacterial over-growth, penicillin (170 μg ml^{-1} final concentration), streptomycin (500 μg ml^{-1}) and chloramphenicol (400 μg ml^{-1}) from Sigma Chemical Company, St Louis, MO, USA, from concentrated stocks were incorporated into the cooled but molten media. The antibiotics at hand were outdated and thus purposely high concentrations were used to ensure an effective antibacterial dosage.

Small wood slivers were plated directly onto the agar, and the cultures were incubated at room temperature. Identification was based on Domsch and Gams [2], Fergus [8] and Wang and Zabel [16].

Results

Microscopy (400× magnification)

All cedar shingle specimens showed evidence, often considerable, of fungal growth: penetration pegs, hyphal invasion through bordered pit cells, anastomosing hyphae, clamp connections, and occasional asci and ascospores.

The intact wood structure of 'new,' unweathered white and red cedar shingles, contrasted with the extensive 'surface fraying' of tracheids from weathered samples. However, the graying in the weathered samples was limited to the outer surface—perhaps a maximum of 1 mm deep. This outer gray layer tended to disintegrate during the staining process, although the remainder of the shingle maintained its structural soundness.

Cultures

A general observation was that malt extract medium resulted in much more luxuriant growth than potato dextrose agar, though on each medium most fungal species were morphologically distinct.

Culturing from the Nantucket white cedar shingles and the New Jersey red cedar shingles, and even the marine-soaked pine sample, yielded a dominant black yeast, later characterized as *Aureobasidium pullulans* (Figure 1). It was dominant on both potato dextrose and malt extract agars, regardless of the presence or absence of antibiotics. *Alternaria* sp, *Penicillium* spp and *Cladosporium* sp (Figure 1), all common blue-staining fungi, were also routinely recovered from Nantucket cedar shingles and the marine-soaked sample. Airborne fungi, recovered by exposing the agar plates at local sites, yielded distinct, yet generally different molds, as evidenced from colony morphology. A red yeast (*Rhodotorula*) was regularly recovered from the ocean-drenched pine samples. With these aquatic samples, bacteria dominated the recovery plates and fungi were recovered only on media containing antibiotics. In contrast, plating of shingle slivers always yielded fungi. Indeed, rapidly growing fungi could dominate the recovery plates. For instance, pieces of a *Polystictus* fruit body when plated, invariably yielded such rapidly growing cultures, and in this instance *Trichoderma* sp out-competed other fungi. Additionally in the recovery of fungi from cedar shingles, *Trichoderma* sp often became dominant. Thus, one has reservations in interpreting the results with regard to exactly which fungi were actually growing on the shingles and which simply arose from contaminant spores of rapidly-growing species. First, *Trichoderma* spp are known to develop on the freshly felled timber, yet here they were consistently isolated from 'service' wood—older cured material. We considered them true colonizers of the cedar

Figure 1 Recovery of fungi from cedar shingles—comparison of isolates from maritime (laboratory) and inland (New Jersey) locations. Cultures were grown on potato dextrose agar. No major distinction was found between the isolates from either location, and *Aureobasidium pullulans*, the black slimy yeast, was always isolated from both sites.

shingles based on their routine isolation. In contrast, some blue-stain fungi are very slow growing and may not have been recorded, for instance *Endophragmiella*, *Leptodontium* and *Ramichloridium*, while the presence of fruiting bodies could indicate such other classical staining genera as *Ceratocystis* [12].

Discussion

The fungi routinely recovered from within the cedar shingles (*Alternaria alternata*, *Aspergillus* sp, *Aureobasidium pullulans*, *Cladosporium* sp, *Penicillium* spp, and *Trichoderma* sp) are typically classified as 'non-decaying' blue-stains [3,18]. Although accorded a classical 'non-decaying' status, these fungi are a major economic problem, as they disfigure wood in what is termed in wooden joinery as 'blue-stain in-service' [13]. Even so, the preemptive colonization of shingles by these 'non-decaying' blue-stains could be fortuitous as they may prevent attack by true cellulose degraders. For instance although *Trichoderma reesei* is an extremely cellulolytic mold [4], in the lumber industry it is considered to be a mildly cellulolytic blue-stain mold. It has been proposed for use as a biological control agent to prevent attack by the *Lentinus lepideus* of timber including creosoted telephone poles [1].

The most definitive observation was the consistent recovery of *Aureobasidium* from the wide variety of samples. Such growth demonstrates the hardiness and adaptability of *Aureobasidium*. Some of the red cedar samples had been creosoted a few years earlier, yet the *Aureobasidium* was routinely recovered from such older shingles, almost to the point of exclusion of other fungi. *A. pullulans* is known to be hardy; it has been 'isolated from a large variety of marine and terrestrial environments,' and is capable of growing 'over a range of salinities and temperatures' [15]. Furthermore, *A. pullulans* strains can survive at 12% moisture for a year—4 months longer than seven other common sapstains survived at similar conditions [18]. Its melanoid pigment gives resistance to UV radiation. From this perspective, it is not surprising that other workers have found *A. pullulans* is common on shingles [14], besides being a dominant colonist on weathered wood, paint and plastic surfaces and the phyllosphere of many plants [13,17,18].

The dominance of *A. pullulans* in both inland and maritime cedar shingles suggests that the difference in the weathered color of the wood is not due solely to the presence of this blue-staining fungus. Then again even though *A. pullulans* occurs in cedar shingles at both sites studied (Princeton, NJ and Nantucket, MA), it could form different amounts of melanin or other pigments under different environmental conditions. There is also the possibility that other fungi were responsible for the color of the shingles, but were simply not recovered, for instance the very slow growing species already noted—*Endophragmiella*, *Leptodontium* and *Ramichloridium*. In this sense a more detailed study of the shingles is merited, as is a study of sequential colonization of the shingles. Of the other probable determinants of cedar's weathered color is sun exposure, a salty environment, and mechanical damage by blowing sands. This study has shown that one dominant black yeast occurs widely on shingles. Definitive answers to this intriguing question of the ultimate cause of the graying of Nantucket shingles—sun, moisture, salt, wind, abiotic damage, or fungus—are difficult to provide.

The isolation of the dramatic black yeast, *Aureobasidium pullulans* from scrapings of cedar shingles, has been performed periodically by undergraduate students (juniors and seniors) in Microbial Ecology classes at Rutgers. This particular study by LAC, a biology major without a microbiology background, was an independent project from the UMASS course, Maritime Ecology 346N. The study offers a facile manner to illustrate common deteriogens. The student is brought into the project at the beginning by selecting interesting microbial niches such as blackened bathroom grout, and also in recovering black yeasts that are different from the classic text book examples of molds. *Aureobasidium pullulans* is of educational relevance being distinctive as it forms readily identifiable black slimy colonies

(Figure 1), and when observed under the microscope it illustrates both yeast and fragmented mycelial (dimorphic) phases. It is readily recovered from shingles, wooden fences, the grout of showers and also from paint surfaces under the eaves of houses [6,13,17,19].

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