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Yeasts and Yeast-Like Fungi in Stored Timber

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ABSTRACT

A microbiological study of four series of wood samples taken from three areas (heartwood, sapwood and bark) of six species of timber logs (American basswood, black cherry, black locust, red oak, soft maple, and white poplar) during the summer, fall, winter and spring of 1987–8 has previously been reported. The samples were analyzed for aerobic bacteria, Gram-negative bacteria, fungi and bacterial endotoxin. The present paper focuses on the yeasts and yeast-like fungi (e.g. Aureobasidium pullulans*) isolated from these samples. The predominant species varied with the season, tree, and location.* Pichia (Hansenula) *sp. predominated, particularly in the August samples and especially from the sapwood of basswood, locust and cherry.* Candida sake *and other members of group 7 of the genus* Candida *were especially common in the samples taken in May from the sapwood and heartwood of maple and oak logs.* Cryptococcus laurentii *and* Rh. glutinis *were the predominant members of* Cryptococcus *and* Rhodotorula, *respectively, isolated from these samples.*

INTRODUCTION

Early microbial colonization of stored timber is accomplished by both bacteria (Liese & Karnop, 1968; Greaves, 1971; Rossell *et al.*, 1973) and

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fungi (Greaves & Levy, 1968; Käärik, 1975; Levy, 1975), including many yeasts and yeast-like fungi. Advanced colonization of wood culminates in abundant growth of wood-rot fungi, leading to rapid decay of wood tissue (Käärik, 1975; Levy, 1975). In a companion study from this laboratory, the bacteria, endotoxin, and fungi were reported from the heartwood, sapwood and bark of six species of timber trees from a sawmill in West Virginia (Dutkiewicz *et al.*, in press). The species examined were American basswood, black cherry, black locust, red oak, soft maple, and white poplar. Logs examined showed no visible signs of decay. Concentrations of microorganisms and endotoxin were significantly higher during spring and summer than during fall and winter and were highest in American basswood and black locust, often exceeding levels of 10^7 colony-forming unit $gram^{-1}$ (CFU g^{-1}) of sample. Gram-negative bacteria and yeasts predominated among organisms recovered from inner wood while bacilli and filamentous fungi were most common in the bark. Yeasts were the predominant fungi, particularly in the samples taken from heartwood and sapwood and were often in excess of 90% of the fungi observed.

The present paper provides more detailed information on the composition of the yeast mycobiota in these stored timber logs scheduled for processing in the sawmill. This information will provide a better understanding of the microbial exposure to workers in sawmills. Evidence is accumulating to show that exposure to dusts generated during wood processing may result in significant pulmonary distress/injury such as hypersensitivity pneumonitis (HP), asthma, organic dust toxic syndrome (ODTS) and chronic bronchitis (Wimander & Belin, 1980; Rosenhall *et al.*, 1982; Jagels, 1985; Asmussen *et al.*, 1986; Kolmodin-Hedman *et al.*, 1987).

MATERIALS AND METHODS

Samples were taken from timber logs stored on the lumber yard at a sawmill in Kingwood, West Virginia during August and October of 1987 and February and May of 1988, corresponding to summer, fall, winter, and spring periods. They had been stored before sampling for periods of 4–6 weeks and showed no visible signs of decay. The air temperatures were 26·5°C, 5·5°C, −4°C, and 20·5°C, respectively. The following tree species were sampled: American basswood (*Tilia americana* L.), black cherry (*Prunus serotina* Ehrh.), black locust (*Robinia pseudoacacia* L.), red oak (*Quercus coccinea* Muensch.), soft maple (*Acer saccharinum* L.), and white poplar (*Populus alba* L.). The samples were collected with a novel

'drill and collect' device (model #2) for quantification of microorganisms in wood (Dutkiewicz *et al.*, 1989). The surface to be sampled was first disinfected by wiping with 70% propanol and a 5·25% sodium hypochlorite solution and an average sample was taken by multiple boring (5–7 times) in a circle up to 3 cm in diameter. Samples of each log were taken from the heartwood and from the sapwood (by boring from the transverse section), and from the bark (by centripetal boring).

Subsamples of 200 mg were suspended in 20 ml of sterile phosphate buffered saline (Sigma Chemical Co., St Louis, MO) containing 0·1% (v/v) Tween 80 (Fisher Scientific Co., Fairlawn, NJ). After vigorous shaking, serial 10-fold dilutions were made up to 10^{-6}. Aliquots (0·1 ml) were spread on duplicate plates of yeast malt agar (Kreger-van Rij, 1984) containing penicillin (100 units ml^{-1}) and streptomycin sulfate (100 μg ml^{-1}). The plates were incubated for 96 h at 28°C. Following incubation, colonies were counted and differentiated on the basis of colony morphology. Representative colonies of each type were selected for isolation and lyophilization. Isolates were identified on the basis of colony and cellular morphology, assimiliation of carbon and nitrogen compounds, fermentation of sugars, production of extracellular urease, and the reaction with Diazonium Blue B (DBB; Kreger van Rij, 1984). The API System 20°C was used as a supplementary tool even though it is intended for the identification of clinical, rather than environmental, yeasts.

RESULTS

Because the investigation was aimed at providing a view of the yeasts to which workers might be exposed during processing operations rather than to provide a definitive estimate of the size of yeasts populations, samples were collected from a single tree of each species at each sampling time. Least squares means (LSM) for the concentrations of total yeasts for each date, tree species, and location were determined by the General Linear Models procedure of the Statistical Analysis System (SAS). Because the data were not distributed in a normal Gaussian distribution, a square root transformation was performed for analysis of variance and least squares analysis. This analysis generated mean CFU g^{-1} values for each of the various factors, i.e. date, tree species and location for the various levels of each factor (e.g. basswood, cherry, locust, maple, oak and poplar for the factor 'tree species'). These values (represented in Fig. 1 as the log of CFU g^{-1}) are intended to describe only this particular set of trees, i.e. it is not suggested that the data are

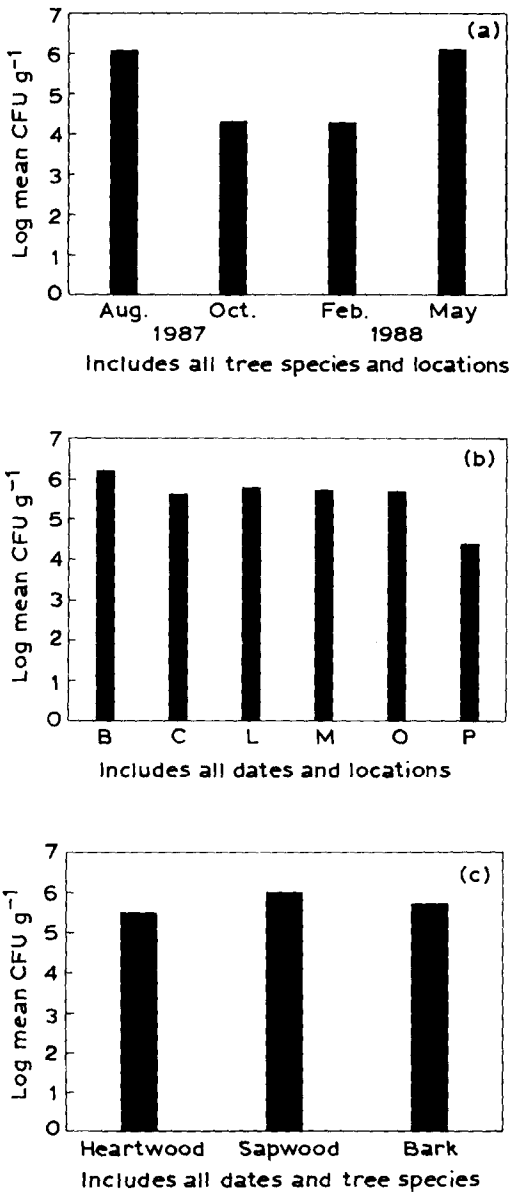

Fig. 1. The numbers of total yeasts by (a) season, (b) tree species, and (c) location within the tree. Values represent the log of mean colony-forming units g^{-1} including all tree species and locations (a), all dates and locations (b), and all dates and tree species (c). Basswood, cherry, locust, maple, oak, and poplar are represented by the letters B, C, L, M, O, and P, respectively, in Fig. 1(b).

necessarily typical of each of these date/tree/location combinations as they would occur in nature. Standard deviations and/or standard errors were not included in Fig. 1 because variance tended to be large owing to the large differences between samples, e.g. the number of colonies from samples collected in the summer were usually very different from samples collected during the winter and occasionally the standard deviation equalled the mean. The Friedman analysis of variance by rank (non-parametric) was employed to test the statistical significance of combination effects for each factor and the Wilcoxon rank sum test was used for comparison of paired samples. The numbers of yeasts ranged up to 10^7 CFU g^{-1} of wood although the mean number averaged over all four seasons was never much higher than 10^6 CFU g^{-1} (Fig. 1(a)–(c)) and varied widely between different sampling dates, tree species and sampling locations. Colony counts for the samples collected in August and May were approximately 100-fold higher than those collected in October or February, suggesting that the lower temperatures during October and February suppressed yeast growth. There was no significant difference between the May and August samples or between the February and October samples ($P > 0{\cdot}05$), but the numbers of colonies from the May (and August) samples were significantly higher than those from February (and October) ($P \leqslant 0{\cdot}005$). No yeasts were recovered from the heartwood of any log species from the February samples.

There was no consistent trend among date–location combinations of the various tree species and one-way ANOVA (Friedman) on tree species was not significant ($P > 0{\cdot}05$). Mean CFU g^{-1} values can be misleading because they are easily inflated by unusually high counts in specific samples. However, when all combinations were ranked and compared in pairs, there were significant differences in certain cases, e.g. both basswood and locust were significantly higher than cherry ($P < 0{\cdot}05$).

Samples collected from sapwood yielded higher concentrations of yeasts ($3{\cdot}3 \times 10^5$ CFU g^{-1}) than samples collected from either bark or heartwood ($1{\cdot}7 \times 10^5$ and $9{\cdot}6 \times 10^4$ CFU g^{-1}, respectively). When the different locations were compared by the Wilcoxon rank sum test, there was no significant difference between heartwood and sapwood ($P > 0{\cdot}05$) or between bark and sapwood ($P > 0{\cdot}05$), but the difference between bark and heartwood was significant ($P < 0{\cdot}01$).

The yeasts observed were assigned to the following categories: *A. pullulans*, *Cryptococcus* spp., *Candida* group 1, *Candida* group 2, *Candida* group 7, *Candida* groups 9–10, other groups of *Candida*, *Pichia* spp. and *Rhodotorula* spp. The predominant yeasts observed in these samples were *Pichia* (*Hansenula*) spp., particularly in the August samples and especially from the sapwood of basswood, locust and cherry. Three

species of *Pichia* were obtained: *P. fabianii*, *P. holstii*, and *P. silvicola*. *Pichia fabianii* was confirmed by obtaining characteristic hat-shaped ascospores upon mating putative *P. fabianii* isolates; *P. silvicola* is homothallic and characteristic ascospores were produced in single cell cultures without mating, and *P. holstii* was observed as the anamorph only (*Candida silvicola*). Wickerham (1960) reported that most isolates of *H. holstii* are not sexually capable and cannot be used in mating tests. The second most predominant group were species of *Candida* (group 7) which were especially prevalent in the samples taken in May from the sapwood and heartwood of maple and oak logs. *Candida sake* was the most common species isolated in this group, with fewer isolates assignable to *C. tropicalis* and possibly also *C. freyschussii* and/or *C. maritima*. Included in *Candida* group 9–10 were isolates identified as *C. zeylanoides* on the basis of the API 20C system, which, however, assimilated xylose and therefore could not be assigned to that species. Other species of *Candida* observed were *C. edax*, *C. famata*, *C. scottii*, and *C. tenuis*. *Rhodotorula glutinis* was the most common of the *Rhodotorula* species observed, with smaller numbers of isolates of *Rh. rubra* and *Rh. minuta*. *Rhodotorula* spp. were common but never the predominant yeasts in any of the samples studied. The majority of the *Cryptococcus* isolates were assignable to *Cr. laurentii* or *Cr. albidus* with *Cr. laurentii* being the more common.

The percentage composition of the yeast mycobiota in the stored timber samples studied is shown in Fig. 2. Values shown represent average percentage composition over the four seasons. The results clearly demonstrate the prevalence of *Pichia* spp. and *Candida* (group 7) species in these samples.

DISCUSSION

In general, the levels of yeasts in these samples closely parallel the numbers of bacteria reported previously (Dutkiewicz *et al.*, in press). There was considerable variation in the numbers of yeasts, depending on the season, the species of log and the location within the tree. Yeasts were significantly higher during the late spring and summer; suggesting the possibility of greater risk for sawmill workers processing logs during warmer seasons of the year.

The numbers of yeasts in the wood of American basswood and black locust were often in the range of 10^4–10^7 CFU g^{-1} and were usually higher than in the wood of the remaining species examined (black cherry, red oak, soft maple, white poplar), which were either lower or more variable. The levels of all categories of microorganisms studied in the most

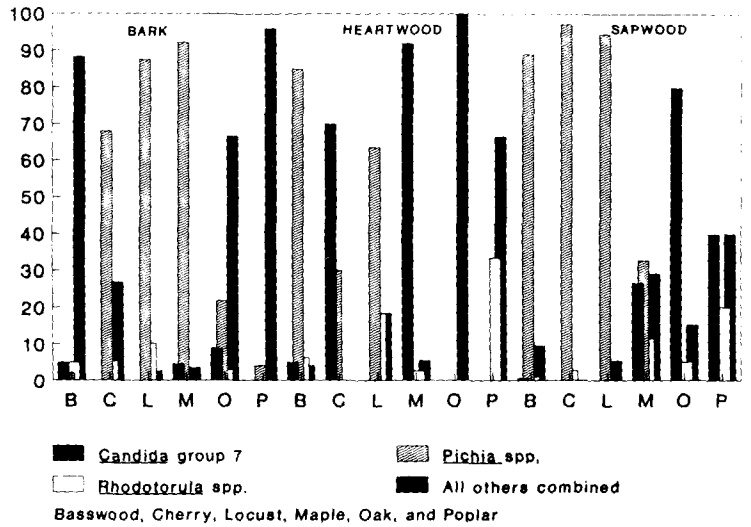

Fig. 2. The percentage composition of the yeast mycobiota in the various tree species. Values shown represent average percentage composition over the four sampling times, i.e. seasons.

contaminated wood samples were comparable to the values reported for certain organic dusts related to harmful respiratory effects in workers (Dutkiewicz, 1978).

Most of the species reported here from West Virginia logs are common environmental species. For example, *C. tropicalis* and *Rh. glutinis* were isolated from *Drosophila* flies (Shehata *et al.*, 1955) and ensiled corn (Burmeister & Hartman, 1966). *C. famata* was reported to be one of the predominant yeasts in aerobically deteriorated ensiled maize treated with antibacterial agents (Woolford *et al.*, 1978), and several of the species isolated from West Virginia trees were observed in the gut of green June beetles feeding on peach slices (Vishniac & Johnson, 1990). *Aureobasidium pullulans, C. sake, C. tropicalis, Cr. albidus, Debaryomyces hansenii* (=*C. famata*), and *Rh. glutinis* were predominant organisms in both West Virginia logs and June beetle gut. Phaff *et al.* (1972) reported that *C. sake, P. holstii, Cr. laurentii*, and *Cr. albidus* were especially common among the yeast populations associated with trees in the Japanese islands and in the US Pacific Northwest and that *D. hansenii* was somewhat less common. *Pichia holstii, Rh. rubra, Rh. glutinis*, and *Cr. albidus* were among the most common yeasts isolated from effluent disposal basins of a pulp mill in Saskatchewan (Spencer *et al.*, 1974*b*) and these species, as well as *C. sake* and *Cr. laurentii*, were isolated from other lakes and rivers of Saskatchewan (Spencer *et al.*, 1974*a*). *Rh. glutinis* was isolated almost

twice as frequently as the second most frequent species in a survey of yeasts from the St. Lawrence river (Simard & Blackwood, 1971). These authors also obtained frequent isolates of *Cr. albidus*, *P. holstii*, and *C. sake*.

Lachance *et al.* (1982) have shown that host tree specificity is an important ecological factor. These authors reported *C. sake*, *C. famata* (=*D. hansenii*), *Cr. laurentii*, and *Rh. glutinis* from *Quercus rubra* (red oak) but not *Populus tremuloides* (trembling aspen).

The sole distinction between *Hansenula* and *Pichia* lies in the ability of *Hansenula* species to assimilate nitrate, whereas species of *Pichia* are unable to do so (Kreger van Rij, 1984). *Pichia fabianii*, *P. holstii*, and *P. silvicola* all assimilate nitrate and therefore fit the traditional concept of *Hansenula*. Kurtzman (1984) compared DNA relatedness among phenotypically similar species of *Hansenula* and *Pichia* and concluded that the ability to assimilate nitrate is not of sufficient taxonomic value for the reliable separation of either species or genera and proposed that all species of *Hansenula* producing hat-shaped ascospores be transferred to *Pichia*. Therefore, these species were listed here as *Pichia* (*Hansenula*).

Although filamentous fungi represent a known respiratory risk for sawmill workers, the significance of wood-borne yeasts as potential hazardous factors is less well known. *Aureobasidium pullulans* has been reported as a potentially hazardous species (Cohen *et al.*, 1967). In the light of the recent reports on the potential role of fungal glucans as inducers of a chronic pulmonary disease (Rylander & Goto, 1989), the possibility of both non-specific and specific effects of yeasts must be considered.

The data presented confirm the views of earlier authors (Greaves & Levy, 1968; Rossell *et al.*, 1973) on the diversity of the microflora of wood. The composition of the microflora of logs studied, characterized by the prevalence of yeasts and Gram-negative bacteria, indicates that it was in an early stage of colonization prior to wood decay by brown rot and white rot fungi (Shigo & Hills, 1973; Käärik, 1975). These results indicate that some kinds of apparently undecayed timber logs stored for processing in sawmills may contain very high numbers of microorganisms (Dutkiewicz *et al.*, in press) and that yeasts can be the predominant fungi. Microorganisms may be the potential cause of respiratory disorders in the woodworkers if inhaled with the sawdust during debarking and sawing operations, e.g. *Cryptostroma corticale* produces a disease known as maple stripper's disease (Towey *et al.*, 1932). There is a need for further characterization of the microbial burden during various job activities within a sawmill and it is suggested that yeasts should be taken into consideration.

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