Polish Journal of Microbiology
2004, Vol. 53, No 4, 283–286

Characteristics of Bacterial Strains Inhabiting the Wood of Coniferous Trees

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Received 10 August 2004

Abstract

The presented studies embraced samples of wood chips from coniferous trees which contained layers of duramen, alburnum and bark. Microbiological analysis involved qualitative and quantitative determination of bacterial flora inhabiting the studied wood material. The wood chips were found to contain primarily species belonging to the genera *Bacillus* and *Pseudomonas*. The presence of the potentially pathogenic species *Bacillus cereus* 1, *Sphingomonas paucimobilis*, *Aeromonas salmonicida* and *Chryseomonas luteola* was also demonstrated.

Key words: wood chips, *Sphingomonas*, *Aeromonas*, *Chryseomonas*, *Bacillus cereus*

Intensive studies on microflora residing in wood were carried out in the ‘60s and ‘70s of the 20th century. Because of the lesser involvement of bacteria in the degradation of wood, greater attention was focused on fungi. However, wood protection issues and utilization of wood waste have recently stimulated interest in wood-degrading bacteria.

Bacteria residing in wood can indirectly affect the degradation of wood or actively participate in these processes. Initially it was thought that bacteria only cooperate as secondary organisms in the breakdown of wood since they were identified in the presence of fungal hyphae. However, Liese (1950) in his studies on wood that had been under water for an extended period demonstrated that changes in the structure of the wood were solely due to the activity of bacteria. The results of consecutive studies showed that wood-degrading bacteria grow in conditions of high moisture and partial or complete lack of oxygen. The most important species of bacteria isolated from wood include: *Bacillus asterosporus*, *B. cereus*, *B. macerans*, *B. megaterium*, *B. mycoides*, *B. polymyxa*, *B. pumilus*, *B. subtilis*, *Bacillus* ssp., *Bacterium xylinum*, *Cellulomonas omelianski*, *Cellulomonas* ssp., *Cellvibrio* ssp., *Clostridium* ssp., *Corynebacterium* ssp., *Erwinia carotovora*, *Flavobacterium* ssp., *Micromonospora* ssp., *Pseudomonas fluorescens*, *P. maltophilia*, *P. pickettii*, *Streptomyces xanthochromagenus*, *Streptomyces* ssp. (*e.g.* Ważny, 2001). Greaves (1971) classified these bacteria into four groups, depending on their role in the decomposition of wood, namely those that: affect: i. the permeability of wood, but cause no strength loss, ii. attack wood structure, iii. work synergistically with other bacteria, causing damage to wood, and iv. passively colonize wood and may be antagonistic towards other species of bacteria.

Microorganisms inhabiting wood cause structural and chemical changes in it and affect its durability. The best studied are structural changes. Three forms of the degradation of cell walls are distinguished: erosion, tunneling and cavitation (Kundzewicz *et al*., 1994; Erikson *et al*., 1990; Daniel *et al*., 1985; Gajewska, 1993; Ważny, 2002). The nature of the degradation of the cell walls is enzymatic and occurs as a result of the immediate contact between bacterial cells and the walls. The best known bacterial hydrolytic enzymes degrading cellulose are cellobiohydrolase, endoglucanase and β-glucosidase (Schimz, 1991). The greatest damage to wood is caused by anaerobic cellulolytic bacteria belonging to the genus *Clostridium*. Initially these microorganisms were linked only to the degradation of wood under anaerobic conditions. However, in-depth studies showed that anaerobic microniches can form within the structure of the wood due to the presence of aerobes, which use up oxygen and consequently create optimum conditions for the growth of anaerobic bacteria (Gajewska, 1993).

Currently, most studies focus on bacteria causing the degradation of preserved wood. The reasons for this are two-fold. The first is an attempt to find agents ensuring the best protection of wood against microorganisms and therefore allow the longer time of its use. The second is the amount of wood waste that is increasing from year to year and whose utilization is causing serious problems. On the other hand, wood waste can be used as a valuable energy source. In view of the growing interest in this material greater focus is also on the possibility of the colonizing of wood by potentially pathogenic, toxin-producing microorganisms (Prażmo *et al.*, 2000). Large-scale growth of bacteria and fungi may be an allergy-inducing factor and toxins produced by the microorganisms may pose a grave threat to the personnel handling raw wood materials (Dutkiewicz *et al.*, 2001).

The objective of this study was to determine the total number of bacteria inhabiting the wood of coniferous trees and to isolate the strains in pure form prior to their characterization. In further studies we intend to check the tolerance of the individual isolates to selected wood protecting preparations, in particular to creosote oil. The studies embraced samples of wood from coniferous trees, which contained layers of duramen, alburnum and bark. Analysis involved qualitative and quantitative determination of bacterial flora inhabiting the studied wood chips. Quantitative determinations were made using the following media: Dubos medium for mesophilic cellulolytic bacteria, CM3 according to Weimer-Zeikus for both meso- and thermophilic cellulolytic bacteria, nutrient agar for mesophilic heterotrophic aerobic bacteria, Bunt-Rovira medium for mesophilic heterotrophic aerobic bacteria, King B medium for bacteria belonging to the genus *Pseudomonas*, McConkey medium for bacteria belonging to the Enterobacteriaceae. Quantitative analysis in liquid media was made using the MPN method. The results from three repetitions were elaborated with the use of McCrady's tables. Qualitative determinations were carried out using nutrient agar, McConkey's medium and King B medium. Cultures were incubated for the various groups of microorganisms at 28, 37 and 65°C for 24 to 48 hours. Following incubation, the number of cells in the above-mentioned groups was determined and selected colonies were picked and streaked out. The obtained quantitative results were calculated per gram dry weight of wood. Preliminary identification of the isolated strains was carried out based on macroscopic observations of colonies (appearance of colonies and production of characteristic pigments) and microscopic observations of the cells, involving Gram stain. The isolates were identified according to Bergey's Manual of Systematic Bacteriology and in addition selected strains were further identified with the use of API 50 CHB and 20 NE tests in a computer-aided system. All isolated bacterial strains were maintained on nutrient agar slants at 4°C.

The numbers of the microorganisms inhabiting the studied wood samples are given in Table I and are a mean of three repetitions. The obtained number of mesophilic, heterotrophic aerobic bacteria growing on nutrient agar is probably underestimated as a result of the antagonistic activity of the microorganisms towards each other. In this connection it seems purposeful to use such selective media as King B medium or McConkey's medium. This is confirmed by the overall number of heterotrophic bacteria growing on Bunt-Rovira medium. The number of gram-negative bacteria determined in this study approximated that observed by Prażmo and Dutkiewicz (1996) for the occurrence of these microorganisms in pine and spruce wood. They found the numbers of bacteria to be the highest in samples of bark, especially spruce bark.

The studied material was found to primarily contain bacteria belonging to the gram-positive genera *Bacillus* and *Pseudomonas*, which included the following species: *Bacillus stearothermophilus*, *B. mega-*

Table I

Number of bacteria inhabiting the wood of coniferous trees growing on the media used, calculated per gram of dry weight of wood

Bacteria	Number (cfu g^{-1} dry weight)
Heterotrophic, mesophilic (28°C) growing on nutrient agar	2.4×10^5
Heterotrophic, mesophilic (28°C) growing on Bunt-Rovira medium	1.5×10^6
Gram-negative rods growing on King B medium	7.9×10^5
Lactose-positive growing on McConkey's medium	6.4×10^5
Mesophilic, cellulolytic aerobes	1.5×10^2
Mesophilic, cellulolytic anaerobes	2.6×10^2
Thermophilic, cellulolytic anaerobes	7.7×10^1

terium, *B. cereus* 1 and *B. cereus* var. *mycoides*, and the gram-negative species *Pseudomonas fluorescens*, *Sphingomonas paucimobilis* (*Pseudomonas paucimobilis*), *Aeromonas salmonicida* and *Chryseomonas luteola* (*Pseudomonas luteola*, *Flavimonas oryzihabitans*). All of the listed gram-negative rods as well as *Bacillus cereus* 1 are potentially pathogenic species. *B. cereus* is a gram-positive rod that mainly occurs in food products and may cause food poisoning. *Aeromonas salmonicida* usually occurs in sea water where it is the causative agent of diseases of fish belonging to the salmon family – furunculosis. *Pseudomonas fluorescens, Sphingomonas paucimobilis* and *Chryseomonas luteola* are opportunistic pathogens of humans, causing various types of infections, including wound infections, septicemia, bacteremia. Prażmo and Dutkiewicz (1996) in their studies mentioned above found that the presence of large numbers of gram-negative rods in the wood of coniferous trees may threaten with the occurrence of diseases of the respiratory tract in wood processing workers. This is connected with biologically active endotoxins produced by these microorganisms.

A particularly interesting strain isolated in this study is *Sphingomonas paucimobilis.* The species belongs to the genus *Novosphinghobium* (*Sphingomonas*) that has only fairly recently been identified and described. The species was described by Yabuuchi *et al*. (1990) and later characterized in detail by Takeuchi *et al*. (1993). Many of the first *S. paucimobilis* isolates were from sick people and samples of water collected at hospitals (Miyazaki *et al*. 1995). Otherwise than other typical gram-negative bacteria, bacteria belonging to the genus *Sphingomonas* instead of lipopolysaccharide contain glycosphingolipids that are usually a component of eukaryotic membranes. Moreover, the microorganisms belonging to this genus are able to degrade a broad spectrum of aromatic hydrocarbons.

All of the isolated bacterial strains will be used in further studies on the tolerance of wood-inhabiting microorganisms to creosote oil used for the preservation of wood. Particularly high hopes are linked with the isolates belonging to the genus *Pseudomonas* and with the two strains described above: *Sphingomonas paucimobilis* and *Chryseomonas luteola*, for which the detailed biochemical characteristics obtained using Api 20NE tests are given in Table II.

Table II
Biochemical characteristics of *Sphingomonas paucimobilis* and *Chryseomonas luteola* strains isolated from wood chips from coniferous trees

Characteristic	*Chryseomonas luteola*	*Sphingomonas paucimobilis*
Reduction of nitrates to nitrites	–	–
Reduction of nitrates to nitrogen	–	–
Production of indole	–	–
Production of acid from glucose	–	–
Hydrolysis of arginine	–	–
Hydrolysis of urea	–	–
Hydrolysis of esculin	+	+
Hydrolysis of gelatin	+	+
Hydrolysis of pNPG*	+	+
Assimilation of glucose	+	+
Assimilation of arabinose	+	+
Assimilation of mannose	–	+
Assimilation of mannitol	+	+
Assimilation of *N*-acetyl-glucosamine	+	+
Assimilation of maltose	+	+
Assimilation of gluconate	–	+
Assimilation of decanate	–	–
Assimilation of malate	+	+
Assimilation of citrate	+	+
Assimilation of phenyl acetate	+	–
Presence of cytochrome oxidase	+	–

* p-nitrophenol-beta-D-glucopyranoside

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