ELSEVIER

International Biodeterioration & Biodegradation 61 (2008) 106–116

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

www.elsevier.com/locate/ibiod

Bacterial diversity associated with archaeological waterlogged wood: Ribosomal RNA clone libraries and denaturing gradient gel electrophoresis (DGGE)

Eleanor T. Landy[a,b,*], Julian I. Mitchell[a], Sarah Hotchkiss[a], Rod A. Eaton[a]

[a]*School of Biological Sciences, University of Portsmouth, King Henry Building, King Henry I Street, Portsmouth, Hants, P01 2DY, UK*
[b]*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK*

Received 8 February 2006; received in revised form 2 October 2006; accepted 23 July 2007
Available online 24 October 2007

Abstract

The identity of bacteria in waterlogged archaeological wood responsible for degradation was investigated using novel isolation techniques and molecular DNA technology. Wood in cultural heritage and archaeological sites is vulnerable to bacterial attack, with bacterially driven wood-decay predominating when wood is buried in sediments. In the research presented, DNA was extracted directly from wood samples and the diversity of bacterial species was determined. Using cultures isolated by project partners from archaeological wood of the same origin at 19 different European sites, a comparison of the DNA of nine culturable isolates and DNA from species commonly appearing in 56 independent molecular fingerprints was made. Results show that sequences representing bacteria from the *Cytophaga-Flavobacterium*-Bacteroides (CFB) complex and the *Pseudomonas* group were commonly recovered, with relatives of the *Cellvibrio* and *Brevundimonas* groups also present. These observations are the first to provide a molecular link between environmental samples where degradation had occurred and cultured organisms, although the precise role and capability of the bacterial strains remains obscure.

Keywords: Denaturing gradient gel electrophoresis (DGGE); Archaeological; Waterlogged wood; Bacteria; Diversity

1. Introduction

Wood in cultural heritage and archaeological sites is vulnerable to bacterial degradation, especially in saline and brackish water environments where lignolytic freshwater microorganisms and marine organisms are active (Clausen, 1996; Blanchette, 2000; Tiano, 2002). Such vulnerability is prominent when wood is submerged in water at low oxygen concentrations (Levy et al., 1974). Microbial decay in aquatic environments is slow and progressive, resulting in timescales extending over centuries for degradation of the polysaccharide components of wood cell walls in large timber structures. The rate of decay is dependent on the natural durability of different wood species, water temperature, oxygen availability, and salinity. In an effort to understand more about processes of wood decay by bacteria in pilings and archaeological timbers, an EU-funded project—BACPOLES, involving researchers from the Netherlands, Germany, Italy, Sweden, and the United Kingdom—began in 2003.

Waterlogged wood is rapidly colonised and subjected to decay by bacteria and soft-rot fungi. However, colonisation by basidiomycetes is less common, as these microbes are less tolerant of low oxygen conditions present in saturated wood. Bacterially driven wood-decay tends to predominate when wood is buried in sediments. Structurally, wood is a suitable substrate for enzymatic attack by invading lignolytic microorganisms because of the open network of microscopic tubes and galleries as well as lumina of axial and radial elements comprising different cell types in hardwoods and soft woods.

*Corresponding author. Tel.: +44 1483 686425; fax: +44 1483 01483686401.
E-mail address: e.landy@surrey.ac.uk (E.T. Landy).

0964-8305/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.ibiod.2007.07.007

Unicellular bacteria are reported to have at least survived in the environment of waterlogged wood in foundation pilings and shipwreck timbers, with the three major components of wood cell walls (cellulose, hemicellulose, and lignin) as the major carbon source (Boutelje and Kiessling, 1964; Harmsen and Nissen, 1965; Boutelje and Bravery, 1968). According to Björdal et al. (1999, 2000), Blanchette (2000), Kim and Singh (2000), and Powell et al. (2001), bacteria can be classified into three decay types. These are erosion bacteria (forming erosion troughs or grooves on the lumen surface of wood cell walls), tunnelling bacteria (removing the polysaccharide components of wood cell walls and some of the lignin, with the appearance of the decayed wood being soft and dark), and cavitation bacteria (resulting in cell wall cavities, the main distinction from the tunnelling type being whether cavities are oriented with the axial direction of wood cells or perpendicular to the cell axis bacteria).

It is generally recognised that single-celled tunnelling bacteria can be seen at the ends of tunnels inside the wood cell walls and that granulation zones develop in the older regions of attack from a loose, irregular network of fine tunnels following bacterial penetration into the cell wall. Bacterial attack is initiated by adhesion to the lumen surface via an extracellular glycocalyx. Areas of cell wall damage by erosion bacteria are associated with single-celled bacteria that are typically rod-shaped, and where the decay is progressive from the lumen surface toward the compound middle lamella of the cell wall. Attachment of erosion bacteria to the lumen surface is via the formation of a glycocalyx where progressive breakdown occurs and the secondary cell wall is converted into amorphous material mixed with bacterial cells and bacterial slime (Björdal et al., 1999). Daniel and Nilsson (1986) further stated that extracellular mucilage was important in erosion bacterial cell adhesion, motility, and enzymatic degradation of the cell wall. Finally, cavitation bacteria are responsible for initiating attack at the lumen surface where bacteria attach themselves via extracellular slime and post-penetration of the S_3 layer, followed by attack of the underlying S_2 as a result of the formation of small cavities. In recent years, wood-decay by erosion bacteria has been regularly recorded in Europe. The bacteria that are more commonly reported associated with wood-decay areas are either cellulolytic aerobes, such as Cytophaga and Cellvibrio, anaerobic species, such as Clostridium, or ubiquitous species such as Bacillus, Pseudomonas, Arthrobacter, Flavobacterium, and Spirillum (Daniel et al.,. 1987; Tiano, 2002). However, little has been reported about the process of bacterial degradation in wood and until now it has not been possible to isolate or identify wood-attacking bacteria (Eslyn and Moore, 1984; Daniel et al., 1987; Line, 1997; Fuhrman and Campbell, 1998; Björdal et al., 1999, 2000; Blanchette, 2000; Theron and Cloete, 2000; Bjordal and Nilsson, 2002). This is due mainly to limitations in, for example, the culturability of isolates and difficulties in successful reinfection of sterile wood (Fuhrman and Campbell, 1998).

Molecular techniques for studying microbial community diversity are well documented (MacGregor, 1999; Amann et al., 2001; Giraffa and Nevianai, 2001; Shafer and Muyzer, 2001; Helms et al., 2004; Landy et al., 2004) using the 16S rRNA or its encoding gene (rDNA) as molecular markers for bacteria. In recent years, sequencing of rRNA genes from DNA obtained directly from various environments, ranging from terrestrial soil to marine locations, has increased our knowledge of bacterial diversity in these systems (Vainio and Hantula, 2000). In 2004, Helms et al. and Landy et al. used molecular techniques to identify groups of bacteria from archaeological wood samples. While in both studies the identification of any bacteria present in the wood was based on molecular analysis of extracted DNA, Helms et al. (2004) still used traditional culture techniques to cultivate bacteria from the wood before any molecular analysis was carried out. The clones identified by Helms et al. (2004) belonged to bacterial groups that are known to exist in soil or bog environments and utilise cellulose as an energy source, e.g., *Spirochaeta, α-Proteobacteria, β-Proteobacteria, δ-Proteobacteria,* and *Geobacteriacea.* Landy et al. (2004), however, extracted DNA directly from wood samples, and using cultures isolated by project partners, adopted a process of elimination to identify species of uncertain identity. Landy et al. (2004) recorded 56 independent molecular fingerprints from samples of different archaeological wood across Europe. These were predominantly affiliated with the bacterial groups *Flavobacterium-Cytophaga, Janthinobacterium-Pseudomonas, Oxalobacteriaceae, Sphingomonas,* and *Cellvibrio*.

As little is known about the process of degradation or the identity or physiology of wood-degrading bacteria, a crucial aim of the BACPOLES project has been to identify the bacteria in waterlogged archaeological wood using novel isolation techniques in combination with molecular DNA technology. The two main approaches used to document bacterial diversity associated with decayed wood in this study were denaturant gradient gel electrophoresis (DGGE) and construction of a clone library representing environmental sequences. The profiles and sequence types so generated provided a framework to assess the identification of bacterial isolates derived from decayed wood and uncultured colonies observed in situ. In this paper, we report the molecular bacterial diversity associated with waterlogged wood from different environments.

2. Materials and methods

2.1. Description of sites

One hundred and eight wood samples from European archaeological sites were provided for this research. Information on sample location (England, Germany, Italy, Norway, Sweden, and the Netherlands), wood type (oak, poplar, Scot's pine, silver fir, and spruce), age (75–1900 years), and extra details, where available, is given in Table 1.

Samples were collected and treated according to Landy et al. (2004). DNA was extracted from four different wood species from 19 European archaeological sites of interest, including piling timbers. Sites and samples are listed in Table 1.