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SHORT COMMUNICATION

Phylogenetic composition and properties of bacteria coexisting with the fungus *Hypholoma fasciculare* in decaying wood

Vendula Valášková[1], Wietse de Boer[2], Paulien JA Klein Gunnewiek[2], Martin Pospíšek[3] and Petr Baldrian[1]

[1]*Laboratory of Environmental Microbiology, Institute of Microbiology of the ASCR, v.v.i., Prague, Czech Republic;* [2]*Department of Terrestrial Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Heteren, The Netherlands and* [3]*Department of Genetics and Microbiology, Charles University, Prague, Czech Republic*

White-rot fungi are major degraders of woody materials in terrestrial environments because of their ability to decompose lignin. However, little is known on the possible associations of white-rot fungi with other microorganisms during wood decay. We investigated the numbers, community composition and functional traits of bacteria present in natural wood samples under advanced decay by the white-rot basidiomycete *Hypholoma fasciculare*. The wood samples contained high numbers of cultivable bacteria (0.2–8 $\times$ 10^9 colony forming units (CFU) per g of dry wood). Most cultivable bacteria belonged to *Proteobacteria* and *Acidobacteria* (75% and 23% of sequences, respectively). The same phyla were also found to be dominant (59% and 23%, respectively) using a non-culturable quantification technique, namely, direct cloning and sequencing of 16sRNA genes extracted from wood. Bacteria that could be subcultured consisted of acid-tolerant strains that seemed to rely on substrates released by lignocellulolytic enzyme activities of the fungus. There were no indications for antagonism (antibiosis) of the bacteria against the fungus.
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White-rot basidiomycetes are important decomposers of woody materials in terrestrial ecosystems and have the unique ability to decompose all major wood polymers, namely, cellulose, hemicellulose and lignin. This complete decomposition relies on the production of lignocellulose-degrading enzymes (Martínez *et al.*, 2005; Baldrian and Valášková, 2008). As opposed to fungi, bacteria have only limited ability to degrade wood because their size limits penetration of woody cell walls and, moreover, they have restricted abilities to modify lignin (Hatakka, 2001; De Boer and van der Wal, 2008). In principle, fungal decay activities do provide opportunities for access and growth of bacteria, as the lignin barrier is weakened and easily degradable oligomers are released from wood polymers by fungal enzymes (De Boer *et al.*, 2005; Baldrian, 2008). The oligomers do, however, also form the energy substrates for the fungus. Consequently, fungi may try to avoid proliferation of bacteria in wood, for example, by producing toxic secondary metabolites. Indeed, Folman *et al.* (2008) showed that two white-rot fungal species, *Hypholoma fasciculare* and *Resinicium bicolor*, had strong bactericidal effects when they colonized beech woodblocks, and basidiomycetes were shown to affect the community composition of bacteria in soils (Tornberg *et al.*, 2003; Warmink and van Elsas, 2008). As some recent reports demonstrate a high bacterial diversity in samples in natural decaying wood in forests (Mikluscak and Dawson-Andoh, 2004; Murray and Woodward, 2007; Zhang *et al.*, 2008), it is not clear whether the bactericidal activities of white-rot fungi do also continue during prolonged decay.

The aim of this study was to investigate the abundance, composition and properties of bacteria in natural wood samples that are in a stage of advanced decay by the white-rot basidiomycete *H. fasciculare*. Wood samples were taken adjacent to fruiting bodies of *H. fasciculare* from deciduous

Correspondence: P Baldrian, Laboratory of Environmental Microbiology, Institute of Microbiology ASCR, v.v.i, Vídeňská 1083, Prague 4 14220, Czech Republic.
E-mail: baldrian@biomed.cas.cz
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Table 1 Abundance of main taxonomical groups of bacteria among the isolated strains and in the clone sequence library of the bacterial community in *Hypholoma fasciculare*-colonized wood

Phylum	*Clones (%)*	*Isolates (%)*
Proteobacteria	59.3	75.0
Alphaproteobacteria	33.3	20.8
Betaproteobacteria	14.1	27.1
Gammaproteobacteria	10.4	27.1
Deltaproteobacteria	0.7	—
Unclassified proteobacteria	0.7	—
Acidobacteria	23.0	22.9
Firmicutes	6.7	—
Bacteroidetes	2.2	2.1
Verrumicrobia	3.0	—
Planctomycetes	3.0	—
Actinobacteria	1.5	—
TM7	1.5	—

wood resources as described in the Supplementary information. Procedures for analysis of wood characteristics, fungal biomass (ergosterol), lignocelluloytic enzyme activities, enumeration of bacteria, cultivable and total bacterial community composition and physiological characteristics of bacterial isolates are given as Supplementary information.

Ergosterol content of the wood samples ranged from 80 to 314 μg per g of wood dry mass, corresponding approximately to 15–65 mg g^{-1} of fungal biomass (Bååth, 2001). The dominance of *H. fasciculare* in the fungal community of the wood samples was confirmed by DGGE analysis of the ITS region of fungal DNA. The activity of extracellular lignocellulose-degrading enzymes was between 0–0.68 mU g^{-1} for laccase, 0.33–1.30 mU g^{-1} for Mn-peroxidase, 1.4–5.7 mU g^{-1} for endoglucanase and 0.9–10.3 mU g^{-1} for endoxylanase demonstrating the active decomposition of all wood polymers by the fungus. All wood samples were strongly acidic, pH ranging from 3.6 to 4.3, and contained 0.2–7.8 $\times 10^9$ bacterial CFU per g of wood dry mass. This is several orders of magnitude higher than the numbers of bacteria that survive in wood after colonization by *H. fasciculare* (Folman *et al.*, 2008). Numbers of CFU were slightly, but significantly, higher on water-yeast agar (pH 5) than on TSB-agar (pH 5 or pH 6.5).

A total of 134 partial ribosomal genes directly amplified from DNA extracted from the wood we sequenced. These ribosomal gene fragments were composed of 63 operational taxonomic units, defined as sequences with >97% identity. The calculated values of the abundance-based richness estimators were 111 for S_{CHAO1} and 123 for S_A w_{CE} (see Supplementary information). The phylogenetic relationships of clones with known bacterial isolates or environmental rDNA sequences are shown in Supplementary Figure 1 and Supplementary Table 1. The community was dominated (59% of the clones) by *Proteobacteria*, of which approximately half consisted of *Alphaproteobacteria* (Table 1). *Alphaproteobacteria* clones were affiliated with the orders *Sphingomonadales*, *Rhizobiales*, *Caulobacterales* and *Rhodospiralles. Betaproteobacteria* clones were largely represented by the genus *Burkholderia*, whereas *Gammaproteobacteria* clones fell into the orders *Legionellales* and *Xanthomonadales.* The second most abundant group of clones (23%) clustered within the phylum *Acidobacteria* and the third (7%) belonged to the phylum *Firmicutes.* Other phyla detected included *Verrumicrobia*, *Bacteroidetes, Planctomycetes* and *TM7*. The phylum *Actinobacteria,* whose members are known for their cellulolytic and lignin-modifying activities (Kirby, 2006), were only present at low numbers which was the main contrast to the study of Zhang *et al.* (2008).

Identification of cultivable members of the wood bacterial community showed a similar dominance of *Proteobacteria* and *Acidobacteria* as for the clones (Table 1). Multiple identical sequences were observed in *Burkholderia* sp. (13 sequences representing eight phylotypes), *Xanthobacteriaceae* (11 sequences representing eight phylotypes) and *Acidobacteria* isolates (11 representing four phylotypes). Of the isolates that could be successfully cultured, all except one (*Pedobacter* sp. WH4) were able to grow at pH 4.0. The *Alpha-* and *Betaproteobacteria* tested seemed to be metabolically versatile and could use several low molecular weight sugars that are released by extracellular fungal cellulases and hemicellulases. None of the isolates, however, was able to hydrolyze colloidal cellulose. Small aromatic compounds, which are released on degradation of lignin, were less commonly used. Interestingly, none of the isolates grew on oxalic acid, which is exuded by *H. fasciculare.* Trehalose, a disaccharide used for carbon storage by fungi, and chitin, the component of fungal cell wall were not utilized either, although *N*-acetyl-D-glucosamine, the monomeric component of chitin, was used as a growth substrate by many strains (Table 2). Most *Gammaproteobacteria* failed to utilize any of the supplied substrates as a sole carbon source. The isolates of *Acetobacteraceae*, *Acidobacteriaceae bacterium*, *Burkholderia glathei*, and one strain of each *Caulobacteraceae*, *Rhodospirillaceae* and *Xanthomonadaceae* failed to grow even when the medium was supplemented with yeast extract, pointing to specific nutritional requirements. None of the isolates exhibited *in vitro* antagonism against *H. fasciculare*. This is in line with a dependency of the bacteria on lignocellulolytic activities of the fungus. The relatively high abundance and diversity of bacteria in this study compared with that of Folman *et al.* (2008) could indicate that selective effects of the white-rot basidiomycete *H. fasciculare* on the bacterial community are stronger during initial decay than in its later phases. Another possibility is that bacteria, which are adapted to the harmful conditions created by the fungus, proliferate during prolonged decay.

Table 2 Physiological properties of bacteria isolated from *Hypholoma fasciculare*-colonized wood

Isolate	D-glucose	D-mannose	D-galactose	L-rhamnose	L-fucose	D-xylose	D-arabinose	N-acetylglucosamine	D-mannitol	D-cellobiose	D-trehalose	Na-acetate	Na-citrate	Oxalic acid	Na-benzoate	Ferulic acid	*p*-Coumaric acid	Sinapic acid	Vanillic acid	Syringic acid	*p*-Hydroxybenzaldehyde
Alphaproteobacteria																					
Sphingomonas sp. WH5	+	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	+	0	0
Sphingomonas sp. WH6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphingomonas sp. WH29	0	+	+	+	0	0	0	0	0	+	0	+	+	0	0	+	+	0	0	0	0
Acetobacteraceae b. WH150	0	0	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0
Betaproteobacteria																					
Burkholderia sp. WH27	+	+	+	+	+	+	+	+	+	+	0	+	+	0	0	0	0	+	0	0	0
Burkholderia sp. WH10	+	+	+	+	+	+	+	+	+	+	0	+	+	0	0	0	0	0	0	0	0
Burkholderia sp. WH11	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	0	0	0	0	0	0
Burkholderia sp. WH12	+	+	+	+	+	+	0	+	+	+	0	+	0	0	0	0	+	0	0	0	0
Burkholderia sp. WH20	+	+	+	0	+	+	0	+	+	+	0	+	+	0	0	+	0	0	+	+	0
Burkholderia sp. WH22	0	+	+	+	+	+	+	+	+	0	0	+	+	0	0	+	+	0	+	+	0
Burkholderia sp. WH24	+	+	+	+	+	+	+	0	+	0	0	+	+	0	0	0	0	0	0	0	0
Burkholderia sp. WH25	+	+	+	+	+	+	+	+	0	+	0	+	+	0	0	0	0	0	+	+	0
Burkholderia sp. WH26	+	+	+	+	+	+	+	+	+	+	0	+	+	0	0	+	+	0	+	+	0
Burkholderia sp. WH8	+	+	+	+	+	+	0	+	+	+	0	+	+	0	+	0	0	0	0	0	+
Gammaproteobacteria																					
Dyella sp. WH3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dyella sp. WH32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dyella sp. WH33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dyella sp. WH34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dyella sp. WH35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthomonadaceae b. WH1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0
Xanthomonadaceae b. WH2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthomonadaceae b. WH7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthomonadaceae b. WH30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xanthomonadaceae b. WH38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rahnella sp. WH9	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0
Rahnella sp. WH28	+	+	+	+	+	+	0	+	+	+	0	0	+	0	+	0	0	0	0	0	0
Bacteroidetes																					
Pedobacter sp. WH4	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0

The ability to grow on a specific substrate as a single carbon source is marked with a plus sign.

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