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ORIGINAL PAPER

Ecology of coarse wood decomposition by the saprotrophic fungus *Fomes fomentarius*

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Abstract Saprotrophic wood-inhabiting basidiomycetes are the most important decomposers of lignin and cellulose in dead wood and as such they attracted considerable attention. The aims of this work were to quantify the activity and spatial distribution of extracellular enzymes in coarse wood colonised by the white-rot basidiomycete *Fomes fomentarius* and in adjacent fruitbodies of the fungus and to analyse the diversity of the fungal and bacterial community in a fungus-colonised wood and its potential effect on enzyme production by *F. fomentarius*. Fungus-colonised wood and fruitbodies were collected in low management intensity forests in the Czech Republic. There were significant differences in enzyme production by *F. fomentarius* between *Betula pendula* and *Fagus sylvatica* wood, the activity of cellulose and xylan-degrading enzymes was significantly higher in beech wood than in birch wood. Spatial analysis of a sample *B. pendula* log segment proved that *F. fomentarius* was the single fungal representative found in the log. There was a high level of spatial variability in the amount of fungal biomass detected, but no effects on enzyme activities were observed. Samples from the fruiting body showed high β-glucosidase and chitinase activities compared to wood samples. Significantly higher levels of xylanase and cellobiohydrolase were found in samples located near the fruitbody (proximal), and higher laccase and Mn-peroxidase activities were found in the distal ones. The microbial community in wood was dominated by the fungus (fungal to bacterial DNA ratio of 62-111). Bacterial abundance composition was lower in proximal than distal parts of wood by a factor of 24. These results show a significant level of spatial heterogeneity in coarse wood. One of the explanations may be the successive colonization of wood by the fungus: due to differential enzyme production, the rates of biodegradation of coarse wood are also spatially inhomogeneous.

Keywords Cellulose · Lignin · Microbial ecology · Saprotrophic basidiomycetes · Wood degradation

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Introduction

Saprotrophic wood-inhabiting basidiomycetes are the most important decomposers of lignin and cellulose in dead wood and as such attracted considerable attention. These studies also included a thorough study of their wood decomposition (Ruel et al. 1994) and ultimately resulted in a detailed knowledge of the enzymology of their lignocellulose-decomposing extracellular enzymes that reflect either the brown-

rot mode of decay where wood polysaccharides are decomposed or the white-rot mode where lignin and polysaccharides are both utilized (Baldrian 2008; Baldrian and Valášková 2008; Arora and Sharma 2010; Lundell et al. 2010).

Although growth in wood and mycelial differentiation accompanied with the production of fungal fruitbodies are the most ecologically relevant modes of existence of wood-rotting fungi, relatively little attention has been paid to the production of enzymes under these conditions. This is striking since both the understanding of the decay process in fungus-colonised wood and the factors affecting fruitbody formation are essential for the understanding of the physiology of fungi causing biodeterioration of wood and may help both to develop preventive measures to reduce fungus-induced wood losses and to undertake measures for the conservation of endangered wood-associated fungi (Dahlberg et al. 2010). In previous papers, enzyme activity in wood was mostly studied under artificial laboratory conditions (e.g., Ruel et al. 1994; Lekounougou et al. 2008). Only recently, papers on enzyme activities in the environmental samples of wood colonised by brown-rot and white-rot fungi appeared, demonstrating high activities of several hydrolytic and oxidative enzymes in this environment (Valášková and Baldrian 2006; Valášková et al. 2009).

Fomes fomentarius is a common and economically important wood-rotting fungus in deciduous forests of Central Europe (Gabriel et al. 1997). It is a white-rot fungus causing heart rot of wood of several tree species including *Fagus sylvatica* and *Betula pendula* (Crockatt et al. 2008). The fungus often arrives at dead wood relatively early or colonizes already a living tree as a parasite but contrary to other early arriving fungi it exhibits better combative abilities and is not easily replaced (Niemela et al. 1995; Hiscox et al. 2010). The ecology of this fungus as early colonizer was confirmed by the study of Heilmann-Clausen and Boddy (2005) who found that wood pre-colonised by this species has a stimulatory effect on several secondary colonisers. As such, it can be presumed that large volumes of wood may be dominantly colonised by this fungus (although the cohabitation with other fungal species cannot be excluded) and enzyme activities measured in wood closely located to fungal fruitbodies are likely to reflect the activity of this single species. *F. fomentarius* is also ecologically relevant as a host of *Coleoptera* and other invertebrates and its antibiotic properties have found its use in medicine (Roussel et al. 2002; Teichert & Bondrup-Nielsen 2005).

The aim of this work was to quantify the activity and spatial distribution of extracellular enzymes in coarse wood colonised by the white-rot basidiomycete *F. fomentarius* and in adjacent fruitbodies of the fungus and to analyse the spatial diversity of enzymatic activities and the fungal and bacterial community in a fungus-colonised wood. We hypothesize that fungus-colonised wood which represents a nutritional base and fruitbodies as organs of reproduction would differ in the activity of extracellular enzymes, especially those acting on plant cell wall polymers. Since the fungus-colonised wood is likely not homogeneous in terms of the development of the fungal colony and the stage of fungal decay, we expected that the activity of extracellular enzymes would reflect this heterogeneity. Since the decay process changes the properties of wood significantly, we also analysed how this is reflected in the composition of microbial communities in wood.

Materials and methods

Sample collection

Fruitbodies of *Fomes fomentarius* and wood immediately adjacent to the fruitbodies were collected form beech (*Fagus sylvatica*) and birch (*Betula pendula*) trees in Jizerske hory Mts, Czech Republic, at 700–900 m a.s.l. in low management forests in a good health situation. Samples were used for the analysis of enzyme activities.

For the study of spatial variation of enzyme activity in wood colonised by *Fomes fomentarius*, a single birch log (8 cm diameter, collected from a standing tree) with a fruitbody was divided into 30 sections, approximately 16 cm^3 each (Fig. 1). Samples were homogenised and used for the analysis of enzyme activities, fungal biomass quantification and DNA extraction immediately after collection in August, 2008. Samples were described as "proximal" or "distal" based on their relative distance from the fruitbody (Fig. 1). For comparative molecular analyses, *Fomes fomentarius* CCBAS534 from the Culture Collection of Basidiomycetes of the Institute of

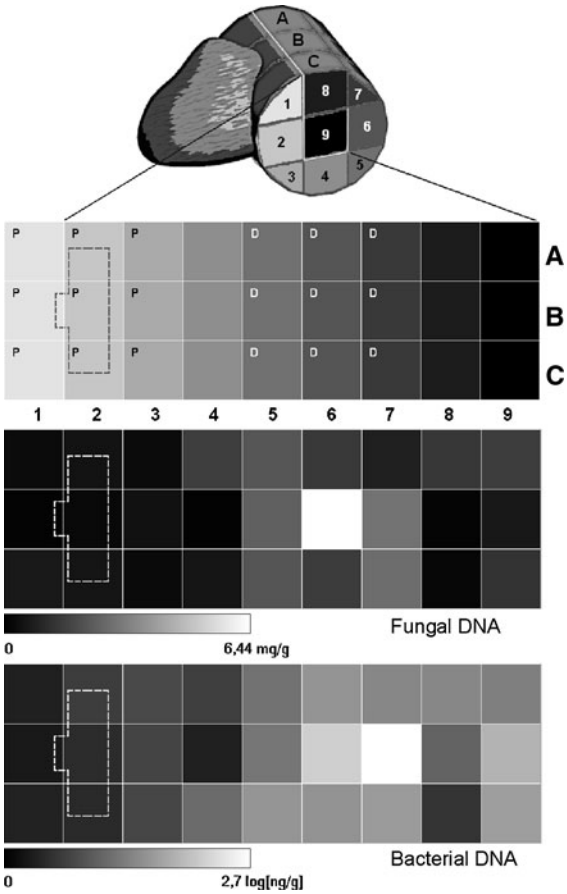

Fig. 1 Graphical representation of the sampling of birch log sample and the distribution of fungal and bacterial biomass in *Betula pendula* log segment colonised by *Fomes fomentarius*. *P* and *D* denote proximal and distal wood samples, fruitbody localisation on the map is indicated by a *dashed line*

Microbiology ASCR, Prague, was maintained on ME agar slants (20 g l^{-1} malt extract, 15 g l^{-1} agar).

Enzyme extraction and assays

Homogenized samples of colonized wood or fruiting bodies were extracted at 4°C for 2 h on an orbital shaker (100 rpm) with distilled water not to affect the enzyme stability during the extraction process, filtered through Whatman #5 filter paper and kept frozen at −18°C until enzyme activity analysis (Valášková and Baldrian 2006). Laccase (EC 1.10.3.2) activity was measured by monitoring the oxidation of ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) in citrate–phosphate (100 mM citrate, 200 mM phosphate) buffer (pH 5.0) at 420 nm (Bourbonnais and Paice 1990). Manganese peroxidase (MnP, EC 1.11.1.13) was assayed using succinate-lactate buffer (100 mM, pH 4.5). MBTH (3-methyl-2-benzothiazolinone hydrazone) and DMAB (3,3-dimethylaminobenzoic acid) were oxidatively coupled by the enzymes, and the resulting purple indamine dye was detected spectrophotometrically at 595 nm (Ngo and Lenhoff 1980). The results were corrected by the activities of the samples without manganese (for MnP)—the addition of manganese sulfate was substituted by an equimolar amount of ethylenediaminetetraacetate (EDTA).

Endo-1,4-β-glucanase (endocellulase; EC 3.2.1.4) and endo-1,4-β-xylanase (EC 3.2.1.8), were routinely measured with azo-dyed carbohydrate substrates (carboxymethyl cellulose and birchwood xylan, respectively) using the protocol of the supplier (Megazyme, Ireland). The reaction mixture contained 0.2 ml of 2% dyed substrate in 200 mM sodium acetate buffer (pH 5.0), and 0.2 ml sample. The reaction mixture was incubated at 40°C for 60 min and the reaction was stopped by adding 1 ml of ethanol followed by 10 s vortexing and 10 min centrifugation (10,000×g) (Valášková et al. 2007). The amount of released dye was measured at 595 nm and the enzyme activity was calculated according to standard curves correlating the dye release with the release of reducing sugars.

Cellobiohydrolase (exocellulase, EC 3.2.1.91) was assayed in microplates using *p*-nitrophenyl-β-D-cellobioside (PNPC).The reaction mixture contained 0.16 ml of 1.2 mM PNPC in 50 mM sodium acetate buffer (pH 5.0) and 0.04 ml sample. Reaction mixtures were incubated at 40°C for 90–120 min. The reaction was stopped by adding 0.1 ml of 0.5 M sodium carbonate, and absorbance was read at 400 nm. 1,4-β-glucosidase (EC 3.2.1.21), 1,4-β-xylosidase (EC 3.2.1.37) and 1,4-β-*N*-acetylglucosaminidase (EC 3.2.1.52) were assayed using *p*-nitrophenyl-β-D-glucoside, *p*-nitrophenyl-β-D-xyloside and *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, respectively, using the same method (Valášková et al., 2007). All spectrophotometric measurements were done in a microplate reader (Infinite, Tecan) or a UV–VIS spectrophotometer (Lambda 11, Perkin-Elmer) and expressed per g dry mass of wood. One unit of enzyme activity was defined as the amount of enzyme forming 1 µmol of reaction product per min.

Quantification of fungal biomass

Total ergosterol was extracted and analyzed as described previously (Šnajdr et al. 2008). Samples (0.5 g) were sonicated (200 Hz) with 3 ml of 10% KOH in methanol at 70°C for 90 min. Distilled water (1 ml) was added and the samples were extracted three times with 2 ml of cyclohexane, evaporated under nitrogen, redissolved in methanol and analyzed isocratically using a Waters Alliance HPLC system (Waters, USA) with methanol as a mobile phase at a flow rate of 1 ml min^{-1}. Ergosterol was quantified by UV detection at 282 nm.

Statistics

Statistical tests were conducted using the software package Statistica 7 (StatSoft, USA). Differences between groups were tested by a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. Principal component analysis was used to identify the main sources of variability in the activities of extracellular enzymes in different parts of the birch log colonised by *Fomes fomentarius*. In all cases, differences at $P < 0.05$ were regarded as statistically significant.

Analysis of microbial communities

DNA from either birch log samples, adjacent fruit-body samples or the cultures of *Fomes fomentarius* CCBAS534 was isolated using modified Miller-SK method according to Sagova-Mareckova et al. (2008). Microbial community analysis was performed using denaturing gradient gel electrophoresis (DGGE) as described previously (Valášková and Baldrian 2009). Primers used for bacterial community analysis were 968-gc and 1378 targeting the V6-V8 hypervariable region of bacterial 16S rDNA (Table 1). For fungal community analysis, nested PCR was used for the amplification of the ITS1 region of fungal rDNA. A fragment comprising both ITS1 and ITS2 was amplified in the first PCR reaction using the primer pair ITS1f/ITS4. After the purification of the PCR product, the ITS1 region was specifically amplified in the second PCR reaction using the ITS1f-gc/ITS2 primers (Valášková and Baldrian 2009). The gel solution for bacterial community analysis consisted of 6% (w/v) acrylamide/bisacrylamide (37.5:1), in 0.5× TAE buffer, pH 8.3, containing 55 to 61% of the denaturant (100% denaturant corresponding to 7 M urea and 40% formamide). The gel solution for fungal community analysis consisted of 9% (w/v) of acrylamide/bisacrylamide (37.5:1) with a linear gradient of denaturant between 36 and 44%. The time of separation was around 17 h at 200 V in 0.5× TAE buffer (60°C). The gels were stained with ethidium bromide. In DGGE of fungal ITS amplicons, DNA isolated from *F. fomentarius* CCBAS534 was used for comparison. To confirm the identity of fungi in wood samples, dominant bands from the fungal community DGGE were cut off from lanes containing DNA extracted from wood and the lane of *F. fomentarius* CCBAS534. DNA was reamplified using the primers ITS1f and ITS2 (Table 1) in 50 µl reaction mixture. Each 50 µl reaction mixture contained 5 µl 10× buffer for DyNAzyme DNA Polymerase, 3 µl of bovine serum albumin (10 mg/ml), 2 µl of each

Table 1 PCR primers used in this work

Target	Primer	Sequence	Reference
Fungal ITS	ITS1	TCCGTAGGTGAACCTGCGG	White et al. (1990)
	ITS1f	CTTGGTCATTTAGAGGAAGTAA	Gardes and Bruns (1993)
	ITS1F-gc	CGCCCGCCGCGCGCGGCGGGCGGGGCGGG GGCACGGGGGGCTTGGTCATTTAGAGGAAGTAA	Valášková and Baldrian (2009)
	ITS2	GCTGCGTTCTTCATCGATGC	White et al. (1990)
	ITS2*	TTYGCTGYGTTCTTCATCG	Wakelin et al. (2007)
	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)
Bacterial 16S rDNA	1108F	ATGGYTGTCGTCAGCTCGTG	Amann et al. (1995)
	1132R	GGGTTGCGCTCGTTGC	Wilmotte et al. (1993)
	1378	TCCTCCGCTTATTGATATGC	Heuer et al. (1997)

primer (0.01 mM), 1.6 µl of PCR Nucleotide Mix (10 mM each), 2 µl polymerase (2U μl^{-1}, DyNAZyme II DNA Polymerase) and 1 µl of the DNA template. PCR cycling conditions were 1× (95°C 3 min, 55°C 30 s, 72°C 1 min), 30× (95°C 30 s, 55°C 30 s, 72°C 1 min), 1× (95°C 30 s, 55°C 30 s, 72°C 10 min) (Valášková and Baldrian 2009). PCRs were run on TGradient thermocycler (Biometra, Germany), PCR products were sequenced as a single extension with primer ITS1f by Macrogen Inc. (Korea) using an ABI 3730 XL DNA Analyzer (Applied Biosystems). Sequences were manually edited and corrected prior to BLAST (blastn) search against the nucleotide database at NCBI (http://www.ncbi.nlm.nih.gov/blast).

Real-time PCR

Two sets of specific PCR primers were used to quantify the relative amounts of fungal and bacterial DNA, ITS1 and ITS2* for fungi (Table 1) and 1108F/1132R for bacteria, in real-time PCR assays based on the method of Leigh et al. (2007). Amplifications were performed on a StepOnePlus cycler (Applied Biosystems) using optical grade 96-well plates. Each 20 µl reaction mixture contained 10 µl SYBR Green Master Mix (Applied Biosystems), 0.9 µl BSA (10 mg/ml), 1.35 µl of each primer, 1.5 µl of template and 6.1 µl of water. The PCR cycling protocol was the same for fungal and bacterial DNA quantification: 56°C for 2 min; 95°C for 10 min; 95°C for 15 s and 60°C for 1 min (40 cycles). *Streptomyces lincolnensis* DNS 40335 and *Hypholoma fasciculare* CCBAS281 genomic DNA were used as standards, DNA content was expressed in nanogram of DNA per gram of wood dry mass. The ratio of fungal to bacterial DNA was calculated by dividing the content of fungal and bacterial DNA in individual samples.

Results

The comparison of extracellular enzyme distribution in wood and fruiting bodies showed that higher activity of the ligninolytic enzyme Mn-peroxidase was present in wood, while the concentrations in fruitbodies were low (Table 2). In *F. sylvatica*, also laccase activity was significantly higher in wood than in the fruitbody. *F. sylvatica* wood colonised by *F. fomentarius* exhibited significantly higher activities of Mn-peroxidase, endocellulase, cellobiohydrolase and especially of the xylanolytic enzymes endoxylanase and β-xylosidase where the differences between the two tree species were four to sevenfold (Table 2).

For the analysis of spatial variability of enzyme activity and microbial biomass content, *B. pendula* log in a medium stage of fungal decay was selected and a disc closely adjacent to a fruitbody of *F. fomentarius* was cut into approximately 2.5 × 2.5 cm samples (Fig. 1). While the fungal mycelium in the wood samples closely located to the fruitbody was apparent by the presence of fungal hyphal cords and mycelial sheaths, in the more distant samples, fungal biomass was less visually apparent. Significantly higher activities of both ligninolytic enzymes, laccase and Mn-peroxidase were found in distal samples, while the proximal samples exhibited higher activity of cellobiohydrolase (exocellulase) and endoxylanase. When enzyme activities were expressed in relative values,

Table 2 Enzyme activities in *Fagus sylvatica* and *Betula pendula* wood colonised by *Fomes fomentarius* and in its fruitbodies

	F. sylvatica wood	*F. sylvatica* fruitbodies	*B. pendula* wood	*B. pendula* fruitbodies
Laccase	0.39 ± 0.17a	0.09 ± 0.06b	0.21 ± 0.09ab	0.13 ± 0.09b
Mn-peroxidase	1.62 ± 0.45a	0.59 ± 0.18b	0.61 ± 0.26b	0.09 ± 0.05c
Endocellulase	9.46 ± 2.93a	7.39 ± 2.28a	2.28 ± 0.59b	1.11 ± 0.76b
Cellobiohydrolase	5.01 ± 1.01a	7.70 ± 1.91a	2.49 ± 0.80b	1.42 ± 0.52b
β-Glucosidase	12.9 ± 3.7b	38.7 ± 7.4a	19.9 ± 5.3ab	13.2 ± 1.6b
Endoxylanase	4.5 ± 2.0a	15.8 ± 7.0a	0.6 ± 0.2b	0.1 ± 0.1c
β-Xylosidase	18.1 ± 3.0a	19.0 ± 2.1a	4.1 ± 1.5b	1.5 ± 0.4b
N-Acetylglucosaminidase	62 ± 19b	140 ± 29a	86 ± 4b	14 ± 5c

Activities are expressed in U g^{-1} dry mass and represent averages and standard errors of means from 15 replicates per treatment
Different letters indicate statistically significant differences between sample types at $P < 0.05$

Table 3 Enzyme activities, ergosterol and DNA content in the *Betula pendula* log segment colonised by *Fomes fomentarius*

		Fruitbody (n = 3)	Proximal wood (n = 9)	Distal wood (n = 9)
Laccase*	(U g^{-1})	0.20 ± 0.20	0.03 ± 0.01	0.20 ± 0.05
Mn-peroxidase*	(U g^{-1})	0.00 ± 0.00	2.47 ± 1.00	5.63 ± 1.02
Endocellulase	(U g^{-1})	0.0 ± 0.0	13.1 ± 5.8	6.4 ± 3.8
Cellobiohydrolase*	(U g^{-1})	10.2 ± 4.2	5.8 ± 1.1	2.8 ± 0.9
β-Glucosidase	(U g^{-1})	119 ± 32	34 ± 6	22 ± 6
Endoxylanase*	(U g^{-1})	1.2 ± 1.2	9.7 ± 1.8	5.0 ± 1.4
β-Xylosidase	(U g^{-1})	4.99 ± 3.02	0.94 ± 0.56	3.14 ± 1.97
N-Acetylglucosaminidase	(U g^{-1})	369 ± 33	18 ± 5	22 ± 3
Ergosterol	(μg g^{-1})	n.d.	84 ± 13	53 ± 4
Fungal DNA*	(ng g^{-1})	n.d.	353 ± 58	2512 ± 539
Bacterial DNA*	(ng g^{-1})	n.d.	4 ± 1	95 ± 51
F/B ratio*		n.d.	111 ± 25	62 ± 15

Data represent averages and standard errors. Asterisks indicate statistically significant differences between samples from proximal and distal wood at $P < 0.05$

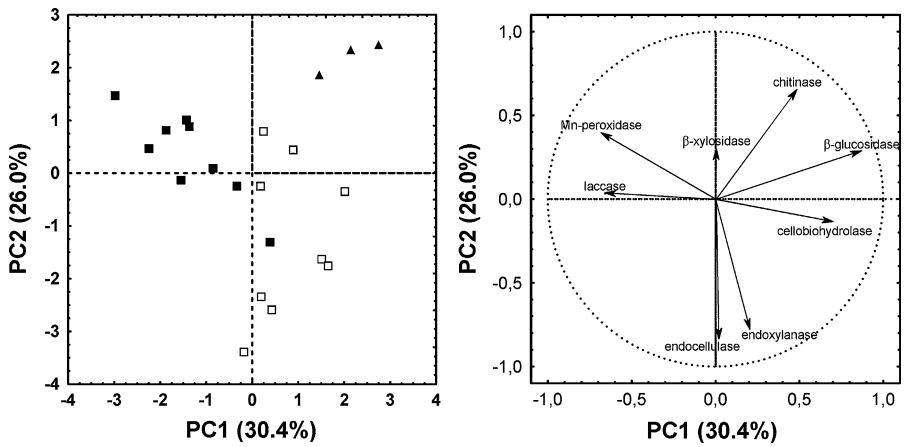

Fig. 2 Principal component analysis of the relative activities of extracellular enzymes in fruitbodies and *Betula pendula* log segment colonised by *Fomes fomentarius*. **a** PC loads of individual samples: fruitbodies (*triangles*), distal samples (*filled squares*), proximal samples (*open squares*); **b** PC loads of individual enzymes

proximal samples showed preferential occurrence of xylanase, β-glucosidase and cellobiohydrolase, while higher laccase and Mn-peroxidase were more important in the distal ones (Table 3). Samples from fruiting body showed high activity of β-glucosidase and chitinase. Principal component analysis of the relative activities of individual enzymes clearly separated proximal, distal and fruitbody-related samples (Fig. 2). Statistically significant differences between proximal and distal samples were found in the PC loads along the first ordinary axis. Fruitbody samples were separated from wood samples along the second PC axis and from the distal samples also along the first PC axis. The results show that the mycelial colony of the fungus in wood exhibits local differences in physiology. While the enzyme activities in wood localised close to fruitbody where the fungal colony is likely older and at a later decay stage produces preferentially polysaccharide hydrolases while in the more distant parts of wood, ligninolytic activity prevails. These observations are corroborated by the fact that endocellulase and endoxylanase activities correlated strongly positively ($P < 0.01$, r = 0.746) as well as cellobiohydrolase and β-glucosidase ($P < 0.01$, r = 0.702). Mn-peroxidase negatively correlated with both endocellulase and endoxylanase. *N*-acetylglucosaminidase and laccase correlated positively with each other ($P < 0.05$, r = 0.439).

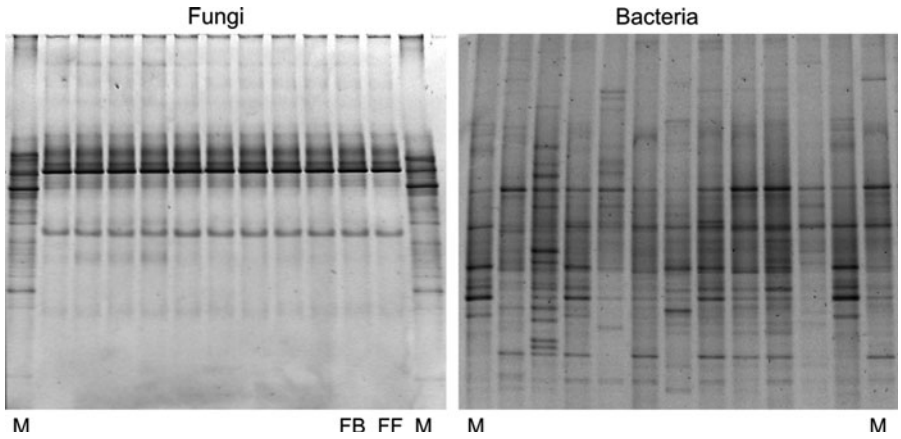

Fig. 3 DGGE analysis of fungal and bacterial community in the *Betula pendula* log segment colonised by *Fomes fomentarius*. DGGE analyses were performed with DNA of the fungal ITS 1 region (primers ITS1f/ITS4 nested with ITS1f-gc/ITS2) and of the bacterial V4-V6 region of 16S rDNA (primers 968-gc/1378). *M* marker, *FB* DNA isolated from *F. fomentarius* fruitbody, *FF* DNA extracted from the pure culture of *Fomes fomentarius* CCBAS534, *all other lanes* DNA extracted from wood

Ergosterol content in wood varied between 28 and 145 μg g^{-1} and did not significantly differ between proximal and distal samples of wood (84 ± 13 μg g^{-1} and 53 ± 4 μg g^{-1}, respectively; Table 3). Ergosterol content did not significantly correlate with any of the enzyme activities. When fungal biomass was analysed based on DNA content per gram of dry wood, its amount was significantly higher in the distal samples (Fig. 1). Also the amount of bacterial DNA in distal samples was significantly higher than in the proximal ones, despite high level of spatial variation. Fungal biomass was highly dominant in the substrate with fungal/bacterial DNA ratio of 111 in the proximal wood and 62 in the distal wood.

Analysis of DGGE gels showed that the banding pattern derived from *F. fomentarius* CCBAS 534 is similar to this obtained from the *F. fomentarius* fruitbody as well as those from the fungus-colonised *B. pendula* log (Fig. 3). Reamplification and sequencing of major DGGE bands derived from wood/fruitbody showed that these sequences share highest similarity (>99%) with the *Fomes fomentarius* strain sequence deposited in GenBank (gi 980706.7; data not shown). As a consequence, *F. fomentarius* represented the only dominant fungal species found in wood. In contrast, there was a rich bacterial community whose composition varied between samples (Fig. 3).

Discussion

The range of enzymes detected in *F. fomentarius*-colonised wood corresponded to the enzymes produced by other ligninolytic basidiomycetes in culture (Baldrian et al. 2010). Significant effect of wood type on the enzyme production was observed: the activity of cellulose and xylan-degrading enzymes was significantly higher in beech wood than in birch wood. Ligninolytic enzymes were found mainly in colonised wood while the activities in fruitbodies were low. In the brown-rot fungus *Piptoporus betulinus*, higher β-glucosidase activity was found in fungal fruitbodies while endocellulase was preferentially produced in wood colonised by the fungus (Valášková and Baldrian 2006). Similar differences were not observed in *F. fomentarius*.

During lignocellulose transformation by white-rot fungi (including *F. fomentarius*), preferential removal of lignin (selective delignification) is performed before extensive utilization of wood polysaccharides (Hatakka 2001). This corresponds well with our findings where wood adjacent to fungal fruitbodies (which is likely at the centre of the fungal colony and at a later stage of decomposition) exhibited higher activity of xylanase, β-glucosidase and cellobiohydrolase while more distant wood parts (in the earlier decomposition stages) showed higher activity of

laccase and Mn-peroxidase. Activities of *N*-acetylglucosaminidase and laccase, enzymes potentially involved in the interactions among microorganisms (Rast et al. 2003), exhibited positive correlation. Due to its participation in chitin degradation, *N*-acetylglucosaminidase may be also an indicator of active rearrangement of fungal mycelia (Lindahl and Finlay 2006).

In wood, primary wood-colonizing fungi spread to occupy the largest available volume of substrate. At places where different strains meet, coloured interaction zones develop. Within these domains, primary colonizers prevent the entry of other competing fungi until late decay (Boddy and Watkinson 1995). Interaction zones were not present in the analysed wood segment and the presence of a single dominant fungal species—*F. fomentarius*—was confirmed by the molecular methods. Due to a large volume of colonised wood, the fungal colony was able to recruit resources sufficient for fruitbody formation (Urcelay and Robledo 2009). The colonization of wood by a single fungal species is often found during initial decay (Vainio and Hantula 2000; Valášková et al. 2009), but in later phases a relatively rich fungal community may develop (Lonsdale et al. 2008; Zhang et al. 2008b).

Fungal biomass content in wood was relatively high, approximately twice as high as in spruce wood inoculated with *Phlebia radiata* or *Phanerochaete chrysosporium* (Niemenmaa et al. 2008). If we consider that 1 g of fungal biomass contains between 0.7 and 2.1 mg ergosterol, this would correspond to 10–150 mg of fungal biomass per g wood dry mass, which roughly corresponds to values in plant litter colonised by a single fungal species (unpublished data) and shows high efficiency of wood assimilation by *F. fomentarius*. Ergosterol content did not differ between proximal and distal wood while the qPCR showed higher amount of fungal DNA in distal wood. This bias may indicate the fact that distal wood contains more actively proliferating mycelium where the relative DNA content is higher than in older parts of mycelia.

Compared to fungal biomass, the biomass of bacteria in wood was very low. Indeed, bacteria are not supposed to be efficient decomposers of bulky lignocellulose like wood (de Boer et al. 2005) except water-saturated wood where fungi are limited by oxygen tension (Clausen 1996; Przybyl 2001). As a consequence, it is most likely that the measured enzyme activities reflected the degradative activity of the fungus. Previous studies on wood colonised by another white-rot basidiomycete, *Hypholoma fasciculare*, showed that bacterial counts were low in wood during initial colonization by the fungus while the abundance and diversity of bacteria was high in wood during later stages of decay (Folman et al. 2008; Valášková et al. 2009). This seems to reflect the fact that the originally high C/N and C/P content of wood decreases during decomposition which makes this substrate more suitable for bacteria (Boddy and Watkinson 1995). Our finding that polysaccharide hydrolysis is higher in proximal samples would suggest that there is more available substrate in the form of oligosaccharides available to bacteria. However, bacterial biomass and the F/B ratio in the studied sample were higher in distal than in the proximal samples.

The bacterial community in *F. fomentarius*-colonised wood was relatively rich and diverse among samples and contrasting with the extremely low diversity of fungi. This is in accordance to previous studies on bacteria associated with decaying wood (Folman et al. 2008; Zhang et al. 2008a; Valášková et al. 2009) and may indicate the fact that bacteria and fungi actually occupy different decomposition niches and do not directly compete while the establishment of other fungi in wood is prevented by the primary coloniser occupying the substrate.

Our results show that in addition to the substrate effect, a significant level of spatial heterogeneity in terms of enzyme activity, fungal and bacterial biomass is present in decaying wood homogeneously colonised by a single fungal species. Based on the differences between proximal and distal samples in a wood log, the wood decay stage may be one of the important driving factors of the variation in wood properties.

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