FUNGI AND BACTERIA ASSOCIATED WITH THE WET AND BROWN WOOD IN TRUNK OF *BETULA PENDULA* TREES

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ABSTRACT

Bacteria and fungi were isolated from external and internal zone of brown and water saturated wood of trunk of *Betula pendula* trees aged 44-46. Quantitative and qualitative differences in the bacterial and fungal populations were found between both the zones. Populations of bacteria increased towards the internal sapwood, contrary to the fungi which more frequently colonised the external zone.

The most common bacteria were *Pseudomonas* spp. (mainly *P. fluorescens* biovar I) whereas *Bacillus macerans*, *B. alvei* and *Erwinia herbicola* were able to degrade the polygalacturonic acid and pectin gels. In case of the fungi population, the most common (more than 3%) colonising the external zone were successively: *Phialophora fastigiata*, *Trichoderma harzianum*, *Alternaria alternata*, *Mortierella isabellina*, *Cladosporium herbarum*, *T. viride*, *C. cladosporioides* and *Melanconium betulinum*. In the community of fungi occurring in the internal zone, the most common (more than 6.5%) were: *Cladosporium herbarum*, *Phialophora lagerbergii* and *Ph. fastigiata*.

KEY WORDS: silver birch, trunk, wet and brown wood, bacteria, fungi.

INTRODUCTION

An increased decline in *Betula pendula* Rothr. stands was observed in Poland since 1992. The decline of silver birch trees was accompanied by a group of symptoms. The most visible symptoms occurring in above-ground organs were the following: premature yellowing of leaves, dying of twigs and branches, wounds resembling frost cracks on the trunk, brown sapwood inside branches and trunk. The brown stained wood in trunks of some trees showing these symptoms exhibited a water saturated appearance.

In case of brown stained wood of paper birch and yellow birch, detailed bacteriological and mycological studies were carried out many years ago (Shigo 1966; Cosenza et al. 1970). However, in case of silver birch not many studies were carried out on bacteria and the so called non-decay fungi associated with brown wood inside the trunk. Recently some information on bacteria inhabiting the wet and brown discoloured trunk wood of silver birch were done by Prażmo et al. (1996) and Hallaksela and Niemistö (1998). The most frequently isolated bacteria were identified as *Pseudomonas cepacia*, *P. maltophila*, *P. putida*, *P. syringae*, *P. fluorescens* and *P. chlororaphis*. Hallaksela and Niemistö (1998) emphasised that *Pseudomonas* spp. and *Enterobacter* spp. more frequently colonised discoloured brown wood than control wood of *B. pendula* trunk. In case of fungi the principal species isolated from discoloured trunk wood were *Phialophora* sp. and *Phialemonium* sp. (Hallaksela and Niemistö 1998).

The aim of this paper was to recognise fungi and bacteria colonising the external and internal zones of brown and water-saturated wood (wetwood) in trunks of *B. pendula* showing decline symptoms.

MATERIAL AND METHODS

Characteristics of silver birch trees selected for the study

The research was conducted on silver birch trees aged 44-46. The trees grew on 3 experimental plots situated in the western and central part of Poland (Łopuchówko, Lipinki Łużyckie and Choszczno Forest District). The studies were carried out on trees that had been removed, which showed the following symptoms: premature yellowing of leaves over all the tree crown, dying of twigs and single branches on top of the crown, occurrence of major wounds on the trunk consisting of branch stubs and wounds resembling frost cracks, damages caused by beetles, most often by *Trypodendron domesticum* (Przybył et al. 1998), outflow of slime on the surface of the bark, occurrence of zones of water saturated wood inside the trunk which were rather irregular and in various shades of brown, seen from the base to a height of 7-10 m.

All *Betula pendula* trees (2 from each experimental plot) were cut into 1-m fragments, so that changes inside the trunk wood could be observed in transverse- and in cross-section. Disks of the trunk were taken at a height of 2 m. The material was transported to the laboratory in portable iceboxes.

Isolation of bacteria and fungi

From the sampled discs of wood, small sections were cut out in three spots within a distance of about 1 cm from the pith (internal zone) and from the peripheral zone (external zone) of brown discoloration for isolation of bacteria and fungi. Thereafter, the pieces of wood were divided into smaller fragments and then placed in Petri dishes. In case of bacteria, the following media were used: nutrient agar (Difco; pH 6.9) and King B medium (proteose pepton Difco 2%, glycerol 1%, H_2HPO_4 0.15%, $MgSO_4$ 0.15%, agar 1.5%, distilled H_2O; pH 6.9) (Schaad (1988). The bacteria cultures from 50 inocula were suspended in 1 ml of sterile water and then serial 10-fold dilutions were made up 10^{-10}. A 0.1 ml aliquot of each dilution was spread on 90 mm Petri disches containing 20 ml nutrient agar and King B medium. The number of colony – forming units (CFU/ml) was determined and then spread onto fresh King B medium. The bacteria were identified using Bergey's Manual of Systematic Bacteriology (Holt et al. 1984, 1994).

In case of isolation of fungi, fragments (ca. 5 × 5 mm) were cut out from trunk disks and then sterilized with 0.5% sublimate (1 min) and 70% ethyl alcohol (1 min). Together 700 inocula (500 from external zone and 200 from internal zone) were placed in Petri dishes containing malt agar (Merck; pH 5.5). The dishes were incubated at room temperature (20-22°C). Fragments of the medium with mycelium were transferred onto malt agar in test tubes.

Cellulase and pectinase assay

The ability of bacteria to produce cellulolytic and pectolytic enzymes was studied using the following solid media;

- for cellulase assay: medium consisting of 5% carboxymethyl cellulose (Sigma C – 5678; sodium salt, low viscosity) in malt extract (200 g fresh per 1l), incorporated in 1% agar (Wong and Preece 1978). A 48-hour old cultures of bacteria were inoculated onto the medium and incubated at 38°C for 3-5 weeks,
- pectinase assay: 1) a basal medium consisting (per 1l) of 6 ml 10% $CaCl_2$. $2H_2O$, 22 g polygalacturonic acid (Sigma P-3850; sodium salt) and 100 ml sterile 4% agar solution: the medium was adjusted to the following pH levels: 4.9-5.1 (medium A), 6.9-7.1 (medium B) and 8.3-8.5 (medium C) (Hildebrand 1971). The inoculated dishes were kept incubated for 1-7 days at 28°C, 2) medium consisting of 60 g pectin gel (Sigma P 9135) per 1 l sterile water (near boiling); the medium was adjusted to pH 4.8-5.0 (medium D) and pH 6.8-7.0 (medium E). To these media 100 ml of sterile 4% agar solution was added. In case of fungi, 2% glucose also was added to the mentioned media (Hildebrand 1971). The dishes were kept incubated for 48 hours at 28°C.

Additionally, the bacteria were tested on soft rot of potato tissue. Washed potatoes were surface sterilized with ethyl alcohol, peeled aseptically and sliced into sections ca. 1 cm thick, and then a slit was made 3-4 mm deep in the center of the upper surface. The place of the slit was inocullated with 5 drops of a water suspension containing bacteria ca. 10^8 CFU/ml. Then these were placed in sterile Petri dishes containing moist filter paper and incubated at 25°C for 24 and 48 hours (Bradbury 1970).

RESULTS

Bacteria isolated from trunk wood and their cellulolytic and pectolytic activity

Altogether from 0.1 ml of the bacterial suspension (10^{10}/ml) 15 bacterial colonies (CFU) from the internal zone and one colony (CFU) from the external zone of brown wood were obtained (Table 1). The following bacteria species were identified: *Bacillus macerans*, *B. alvei*, *Lactobacillus* sp., *Erwinia herbicola*, *Pseudomonas aureofaciens*, *P. fluorescens* biovar I, *P. fluorescens* biovar III, *P. fluorescens* biovar V and *Serratia liquefaciens*. Species belonging to the genus *Pseudomonas* clearly prevailed among bacteria isolated from the wood of trunks of. *Betula pendula*. The most common species was *P. fluorescens* biovar I.

None of the isolated strains of bacteria degraded the carboxymethyl cellulose. Whereas, as is seen in Table 1, *Bacillus macerans* (isolated from the internal zone) produced moderate to large sized pits on media A (pH 4.9-5.1) and B (pH 6.9-7.1) containing polygalacturonic acid, and on media D (pH 4.8-5.0) and E (pH 6.8-7.0) containing pectin gels. On these media smaller pits were caused also by one strain of *B. alvei* and *Erwinia herbicola*.

TABLE 1. Bacteria isolated from trunk wood of *Betula pendula* trees and their pectolytic activity.

Species	Isolation No. strains		Soft rot of potato tissue	No. of strains showing pectolytic activity				
				Medium with polygalacturonic acid			Medium with pectin gels	
	I	E		4.9-5.1 (medium A)	6.9-7.1 (medium B)	8.3-8.5 (medium C)	4.8-5.0 (medium D)	6.8-7.0 (medium E)
Species								
Bacillus macerans	2	0	2	2 h	2 h	2 s	2 h	2 h
Bacillus alvei	1	0	1	1 s	1 s	0	1s	1s
Lactobacillus sp.	1	0	0	0	0	0	0	0
Erwinia herbicola	1	0	0	1 s	1 s	0	1 s	1 s
Pseudomonas aureofaciens	2	0	0	0	0	0	0	0
P. fluorescens I	5	1	0	0	0	0	0	0
P. fluorescens III	2	0	0	0	0	0	0	0
P. fluorescens V	0	0	0	0	0	0	0	0
Serratia liquefaciens	1	0	0	0	0	0	0	0
Total number	15	1	3	4	4	2	4	4

Activity rated according to degree of pitting produced; h – „moderate to hight" indicates a pit with radius more then 1 mm from colony margin, s – „slight" indicates a pit with radius less than 1 mm from colony margin. I – internal zone of brown wood. E – external zone of brown wood

TABLE 2. Fungi isolated from external (E) and internal (I) zone of brown wood in *Betula pendula* trunk.

Fungal species	Frequency %	
	E	I
Absidia sp.	0.7	–
Alternaria alternata (Fr.) Keissler	3.9	–
Alternaria tenuissima (Kunze ex Pers.) Wilts.	2.6	–
Aposphaeria sp.	0.4	2.6
Aureobasidium pullulans (de Bary) Arnaud	1.3	5.2
Botrytis cinerea Pers.	2.2	–
Chaetosphaeria innumera Tul.	2.6	5.7
Cladosporium britannicum M. B. Ellis	2.4	5.9
Cladosporium cladosporioides (Fresen.) de Vries	3.3	7.2
Cladosporium herbarum (Pers.) Link ex Gray	3.5	11.2
Coleophoma empetri (Rostr.) Petrak	1.5	–
Coniothyrium fuckelii Sacc.	1.3	3.9
Coryneum sp.	1.7	–
Epicoccum nigrum Link	1.5	–
Fusarium lateritium Nees	–	1.3
Fusicladium sp.	–	1.3
Hormonema sp.	0.7	–
Melanconium betulinum Kunze et Schm.	3.0	–
Melanconium stromaaticum Corda	0.9	–
Mortierella isabellina Oud. et Koning	3.9	–
Mortierella verticillata Linnem.	1.1	1.3
Mortierella vinaceae Dixon-Steward	1.9	–
Mucor circinelloides van Tiegh.	1.1	–
Mucor mucedo Mich. Ex st.-Am.	0.9	–
Mucor racemosus Fres.	1.5	–
Mucor spp.	2.4	–
Penicillium sp.	4.8	6.5
Penicillium citrinum Thom	1.1	–
Phialocephala cf. *dimorphospora* Kendrick	0.4	4.8
Phialophora fastigiata (Lagerb. et Melin) Conant.	9.9	16.3
Phialophora lagerbergii (Melin et Nannf.) Conant.	1.5	14.3
Phoma sp.	–	4.6
Rhizoctonia sp.	0.9	–
Sordaria fimicola (Rob.) Ces.et de Not.	0.7	–
Seimatosporium sp.	0.2	–
Sporothrix sp.	0.2	–
Trichoderma harzianum Rifai	5.5	3.3
Trichoderma viride Pers. ex Gray	3.5	–
Trimmatostroma cf. *betulae* (Corda) Hughes		2.6
Ulocladium sp.	0.9	–
Not sporulating and not identified	20.4	6.5
Number of examined isolates	452	153

Massive areas of potato rot after 24 hours were observed in the case of *B. macerans* (2 strains) and *B. alvei* (1 strain).

Fungi isolated from brown trunk wood

From among 500 fragments taken from the external zone of brown wood and 200 fragments taken from the internal zone of brown wood of the trunk of *Betula pendula*, 90.4% (452) and 76.5% (153) isolates were obtained, respectively. In the first case, the community was represented by fungi identified as 36 species, whereas in the second one, the population consisted of 17 species. Certain isolated fungi were classified only by genus. The percentages of frequency of fungal isolates are given in Table 2.

The most common fungi isolated from the external zone belonged to the genera *Phialophora* (11.4%), *Cladosporium* (9.2%), *Trichoderma* (9%), *Mortierella* (6.9%), *Mucor* (5.9%) and *Penicillium* (5.9%). Twenty seven fungi occurred in less than 3% of the total number of isolates. On the other hand, the following fungi most frequently occurred: *Phialophora fastigiata* (9.9%), *Trichoderma harzianum* (5.5%), an unidentified taxon from *Penicillium* sp. (4.8%), *Alternaria alternata* (3.9%), *Mortierella isabellina* (3.9%), *Cladosporium herbarum* (3.5%), *Trichoderma viride* (3.5%), *Cladosporium cladosporioides* (3.3%), and *Melanconium betulinum* (3.0%). The frequency of nonsporulating fungi was 20.4%. Among these group (92) isolates, one isolate exhibited clamp connections.

In the community of fungi colonising the internal zone of the brown trunk wood, the most common were fungi belonging to the genera: *Phialophora* (30.6%), *Cladosporium* (24.3%) and *Penicillium* (6.5%). The sporulation was not found in 6.5% of fungi. The most frequent species, isolated in more than ten specimens (6.5%) included: *Cladosporium herbarum* (11.2%), *Phialophora lagerbergii* (14.3%) and *Ph. fastigiata* (16.3%). The *Mortierella* sp. rarely colonised this wood in comparison with the external zone of the brown wood, where this group of fungi were larger and more represented.

The following fungi were common for both zones of wood: *Aureobasidium pullulans*, *Chaetosphaeria innumera*, *Cladosporium britannicum*, *C. cladosporioides*, *C. herbarum*, *Coniothyrium fuckelii*, *Mortierella verticillata*, *Phialocephala* cf. *dimorphospora*, *Phialophora fastigiata*, *Ph. lagerbergii* and *Trichoderma harzianum*. Among these fungi, only *T. harzianum* was frequently isolated from the external zone of the brown wood.

DISCUSSION

The trunk sapwood of the considered in this paper *Betula pendula* trees was brown and more wet in comparison with normal sapwood. The brown and water-saturated wood of the trunks from which the micro-organisms were isolated contained significantly more K, Ca, Mg and Zn (Zn: excluding Lipinki Łużyckie), compared with concentration of these elements in control sapwood (Przybył and Mańka 2000). Accumulation of some nutrients has been previously indicated in the water-soaked condition of the wood (wetwood), in the vicinity of wounds and of wood decay in deciduous and coniferous trees (Shortle and Shigo 1973; Murdoch and Campana 1981).

Phialophora fastigiata was most often isolated from the internal (16.3%) and external zone (9.9%). Similarly, Hallaksela and Niemistö (1998) found that *Ph. fastigiata* belonged to the most common microbial community occurring in the discoloured wood of silver birch. Some authors (Cole and Kendrick 1973; Shortle and Cowling 1978; Domsch et al. 1980; Przybył 1996) indicate that *Phialophora* spp. are common on brown stained and decaying wood of both broadleaves and coniferous trees. *Mucor* spp. and *Mortierella* spp., usually occurring in wood in an advanced stage of decomposition or water saturated (Swift and Boddy 1984; Rayner and Boddy 1988), were isolated in this work in greater numbers from the external zone than from internal one. Some of the isolated fungi in this investigation are common endophytic mycobiota of the trunk and branches of birch and other broadleaved or coniferous trees e.g. *Melanconium betulinum*, *Coryneum brachyurum*, *Phialocephala* cf. *dimorphospora* (Kowalski and Kehr 1992; Kowalski and Gajosek 1998).

The referred literature (Domsch et al. 1980) indicates that nonhymenomycetous fungi can affect cellulose and pectin degradation e.g. *Phialophora fastigiata*, *Trichoderma harzianum* and *Cladosporium herbarum*. Some fungi e.g *Ph. fastigiata*, causing discoloration of wood may also be responsible for soft rot under appropriate circumstances (Käärik 1974).

The isolated fungi were closely associated with bacteria. Generally, moist wood increases the bacteria population (Goodman et al. 1986, Prażmo et al. 1996). Results of this study indicate that the most common bacterial strains were aerobic pseudomonads in which *Pseudomonas fluorescens* biovar I occurred most frequently, particularly inside the trunk. Of all bacterial species, *Bacillus macerans* (two isolates) strongly degraded polygalacturonic acid and pectin gels in vitro. Earlier, Przybył and Żłobińska-Podejma (2000) found positive reactions in degrading the pectin and cellulose with some *Serratia* spp. and *Lactobacillus* spp.

Finaly, the bacteria and nonhymenomycetous fungi isolated from brown sapwood with higher moisture than the normal sapwood inside trunk of *Betula pendula* can participate in degradation of woody cell walls. The most important seem to be *Bacillus macerans* and *B. alvei* in the case of bacteria, and *Phialophora fastigiata* in the case of fungi. We can suppose that some fungi and bacteria cause a type of soft rot in declining trees of the studied birches. Soft rot can be a major cause of decay of wood where some conditions inhibit the growth of white- and brown-rot fungi e.g. high levels of moisture or presence of bacteria inhibiting growth of decay fungi. In the last case, some bacteria of *Pseudomonas* spp. inhibited the growth of *Piptoporus betulinus* in vitro (Przybył and Żłobińska 2000).

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GRZYBY I BAKTERIE WYSTĘPUJĄCE W BRUNATNO PRZEBARWIONYM I WILGOTNYM DREWNIE PNIA DRZEW *BETULA PENDULA*

STRESZCZENIE

Bakterie i grzyby izolowano z zewnętrznej i wewnętrznej strefy wilgotnego i przebarwionego na kolor brunatny drewna pnia 44-46 letnich drzew *Betula pendula*. Między wymienionymi strefami drewna stwierdzono ilościowe i jakościowe różnice w populacji zarówno bakterii jak i grzybów. Większą liczbę bakterii stwierdzono w wewnętrznej strefie drewna, podczas gdy grzyby częściej kolonizowały strefę zewnętrzną.

Bakterie z rodzaju *Pseudomonas* (głównie *P. fluorescens* biovar I) należały do najczęściej występujących, natomiast zdolnością do rozkładu kwasu poligalakturonowego i pektyny charakteryzowały się *Bacillus macerans*, *B. alvei* i *Erwinia herbicola*. W przypadku grzybów, do najczęściej izolowanych (powyżej 3%) z zewnętrznej strefy brunatno przebarwionego drewna należały następujące gatunki: *Alternaria alternata*, *Cladosporium cladosporioides*, *C. herbarum*, *Melanconium betulinum*, *Mortierella isabellina*, *Phialophora fastigiata*, *Trichoderma harzianum* i *T. viride*. Natomiast w populacji grzybów uzyskanych ze strefy wewnętrznej drewna największą częstotliwość (powyżej 6.5%) wykazywały: *Cladosporium herbarum*, *Phialophora fastigiata* i *Ph. lagerbergii*.

SŁOWA KLUCZOWE: brzoza brodawkowata, pień, wilgotna i brązowa strefa drewna, bakterie, grzyby.