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BIODEGRADABILITY OF EXTRACTIVES IN SOUND AND BIOLOGICALLY DECAYED BEECH BY VARIOUS YEAST SPECIES

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ABSTRACT

The yeast-degradation of lipophilic extractives in sound and biologically decayed beech was examined. All tested yeast strains *Sporobolomyces salmonicolor*, *Geotrichum capitatum* and *Geotrichum klebahnii* removed higher amount extractives from sound wood than from decayed wood. The degradation of extractives was examined by HPLC and FTIR spectroscopy of acetone extracts obtained by the extraction of beech wood samples before and after yeast treatment. It was revealed that the used yeast microorganisms remove from 40 to 60 % of extractives. *Sp. salmonicolor* was the most efficient yeast strain in degrading extractive constituents. The obtained results are contribution to development of new biological approaches for controlling pitch problems in pulp and paper mills.

KEY WORDS: decayed beech wood, extractives, *Sporobolomyces salmonicolor, Geotrichum capitatum, Geotrichum klebahnii*, FTIR, HPLC

INTRODUCTION

Wood extractives cause pitch problems in pulp and paper manufacture. Extractives are low-molecular weight lipophilic component in wood consisting mainly of triglycerides waxes, steryl esters, sterols, free long chain fatty acids and resin acids (Fengel and Wegener 1989). Fungal removal of extractives from wood is a promising technology to control pitch formation (Brush et al. 1994, Gutierrez et al. 1999). Recently, sapstain and white rot fungi have been reported to reduce the extractive content in pain sapwood and heartwood (Martinez-Inigo et al. 1999). In our previous research some yeast organisms as *Sporobolomyces roseus* were used for biotransformation of lignin polymers (Košíková and Sláviková 2004). In the present study three yeast strains were examined for their ability to remove extractives from sound and from naturally decayed beech.

MATERIAL AND METHODS

Wood samples

The sound and decayed beech trees (*Fagus sylvatica*) were obtained from East Slovakia. Chemical analysis of wood was performed by methods described in our previous paper (Košíková et al. 1979).

Cultivation and yeast microorganisms

The yeast strains: *Sporobolomyces salmonicolor* 19-4-21, *Geotrichum capitatum* 30-6-6 and *Geotrichum klebahnii* 74-6-2, and were maintained on malt agar slants at 5^{o} C in the Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Bratislava. They were cultured in a medium containing 6.7 g Yeast Nitrogen Base (Difco), and beech sap or heart sawdust (2 g) per liter of solution in distilled water. The pH was adjusted to 6.5. The medium was sterilized by autoclaving at 121 ^{o}C for 15 min. The strains were cultivated in flasks (500 ml) containing 250 ml of medium. The inoculation was performed with 4 ml of cells suspension (10^6 cells per ml). Incubation was proceeded on rotary shakers at 28 ^{o}C for 28 days. All experiments were done in triplicate.

The growth of the yeasts was determined by the nitrogen content of dry solid product separated from the cultivation medium by centrifugation. Protein was calculated from the nitrogen content (% N x 6.25) assayed on an elemental analyser (Model 240 Perkin-Elmer).

Extraction

The wood samples before and after treatment with yeast strains were extracted in Soxhlet with acetone for 6 hour. The extractives dissolved in the cultivation medium were separated by acetone extraction of dry solid products.

FTIR spectroscopy

The IR measurements were performed with FTIR spectrometer NICOLET Magna 750 operating at 4 cm resolution using KBr pellets.

GC analysis

A Fisons 8310 DPFC gas chromatograph was used for determination of lipophilic extractive compounds by the method described before (Gutierrez et al. 1998). The injector and detector temperatures were set at 300 ^{o}C and 330 ^{o}C, respectively. Sample volumes of 1 μL were injected in the splitless mode. A mixture of standard compounds (palmitic acid, abietic acid, β-sitosterol, cholesteryl oleate, and triheptadecanoin) was used for calibration of the column at concentrations ranging from 0.1 to 1.0 mg/mL for quantitation of wood extractives. The corelation coeficient was greater than 0.99 in all cases. All peaks were quantified by peak area.

GPC analysis

Lignin samples were analyzed by gel permeation chromatography on a column separon HEMA S-300 (250 x 8 mm TESSEK) using N,N dimethylformamide (Merck) containing 0.005 M LiBr at 35 ^{o}C with flow rate 1 $mL.min^{-1}$ (Kačík et al. 1992).

RESULTS AND DISCUSSION

A naturally decayed beech stem was investigated from the viewpoint of chemical composition of sound and biologically decayed wood (Tab. 1). The total amount of extractives and lignin was higher in sound as compared to decayed wood in agreement with literature data (Hortling et al. 1992). The decayed beech showed an increase in the yield of dioxane lignin of 17 % related to sound wood. The molecular characteristics of both lignin samples are summarized in Tab. 2. The obtained values indicate that lignin isolated from decayed wood has a slightly lower molecular mass and polydispersity than the lignin from sound wood. This may be explained by assuming a slight depolymerization of the lignin or dissolution of low molecular mass in the beech during the decay.

Since the conditions of lignin isolation from sound and decayed samples were essentially identical, the differences between the molecular characteristics reflect the effect of biological degradation of the lignin wood component. The higher yield and lower molecular weight of lignin fraction obtained by dioxanolysis from decayed wood sample indicate that removal of lignin in the pulping of decayed beech will be more effective related to sound wood.

Tab. 1: Chemical analysis of sound and biologically decayed beech

Sample	Extractives %	Lignin %	Elemental analysis %			Dioxane lignin %
			C	H	OCH_3	
Sound wood	1.78	19.75	48.06	6.35	6.23	18.63
Decayed wood	0.98	20.82	47.25	6.12	6.11	22.40

Tab. 2: Molecular characteristics of lignins isolated from sound and decayed beech

Sample	Mw	Mn	Polydispersity
Sound wood	17582	1759	10.00
Decayed wood	16215	1921	8.44

As it results from the comparison of FTIR spectra of dioxane lignins from both types of wood (Fig. 1), the lignin sample isolated from decayed wood contains relatively higher amount of hydroxyl and carboxyl structures and the decrease of aromaticity. The structural modification of lignin is in agreement with our previous results indicating biotransformation of lignin polymers by *Sporobolomyces roseus* (Košíková and Sláviková 2004). The observed structural differences could explain higher extractability of lignin with dioxane–water (9:1) from decayed beech compared to that of sound.

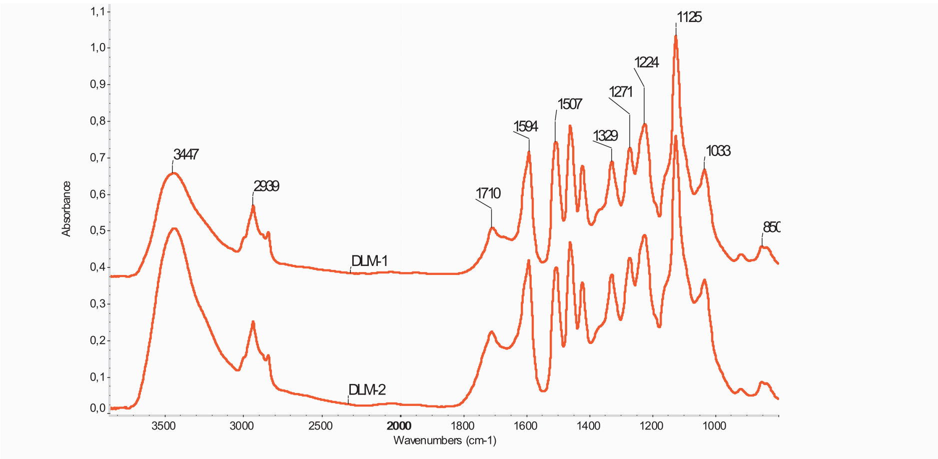

Fig. 1: FTIR spectra dioxane lignins isolated from sound (DLM-1) and decayed beech (DLM-2)

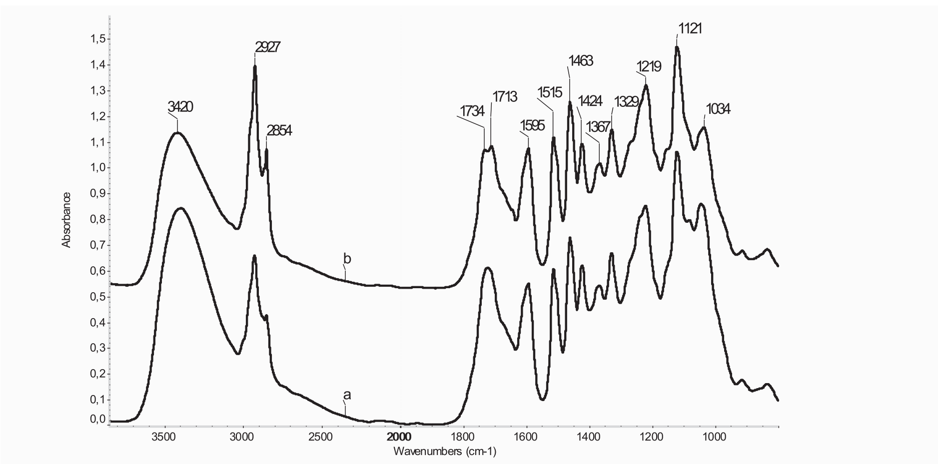

Fig. 2: FTIR spectra of the extractives from beech
a – acetone extract of sound wood
b – acetone extract of decayed wood

The data summarized in the Tab. 3 show the effect of yeast microorganisms: *Sporobolomyces salmonicolor*, *Geotrichum capitatum* and *Geotrichum klebahnii* on removal lipophilic extractives from both beech wood samples. The obtained results clearly demonstrated that treatment with the yeast tested results in the significant reduction of lipophilic wood extractives. The most effective yeast strain *Sporobolomyces salmonicolor* removed about 60 % extractives from both sound and decayed wood.

Tab. 3: The growth of biomass and removal of extractives from sound and decayed beech after a four week treatment with various yeast strains

Species	Biomass g/L		Residual extractives %	
	Sound wood	Decayed wood	Sound wood	Decayed wood
Sporobolomyces salmonicolor	0.95 ± 0.09	0.89 ± 0.06	58	62
Geotrichum capitatum	1.15 ± 0.07	1.21 ± 0.10	52	58
Geotrichum klebahnii	1.28 ± 0.08	1.39 ± 0.12	42	45

The corresponding acetone extracts of sound and decayed wood of beech were characterized by FTIR spectroscopy (Fig. 2). The assignment of the individual absorption bands in the spectra was performed by comparison with the spectra of models for different type of extractives: palmitic acid (resin acid), cholestyroleat (steryl esters) and stigmasterol (sterols). Absorption bands at 3400 cm^{-1} corresponds to δ OH aliphatic carboxyl acids; at 2927 and 2854 cm^{-1} asymmetric and symmetric C-H stretching, respectively; 1743 cm^{-1} C=O stretching vibration of esters and 1700 cm^{-1} C=O stretching vibration of acids; 1460 and 1382 cm^{-1} $-CH_2$ and $–CH_3$ deformation; 1163 cm^{-1} C-O frequency from ester groups; 1159 and 960 cm^{-1} C-O stretching of ester groups trans and cis; 840 and 700 cm^{-1} alkene cis isomers; 824 cm^{-1} C-H bending vibration.

The spectra of acetone extracts of the wood samples treated with *Sporobolomyces salmonicolor* are illustrated in Fig. 3. The corresponding extractives separated from lyophilized cultivation medium by acetone extraction are shown in Fig. 4.

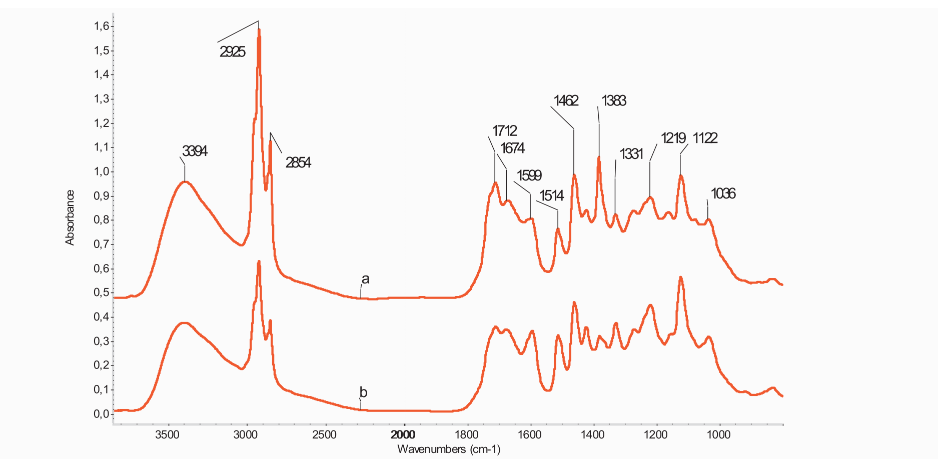

Fig. 3: FTIR spectra of the acetone extracts of the sound (a) and decayed wood (b) treated with Sporobolomyces salmonicolor

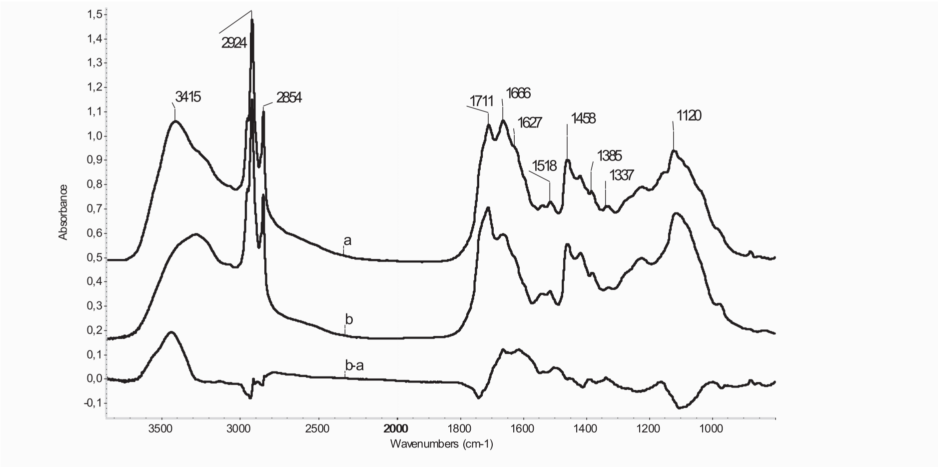

Fig. 4: FTIR spectra of extractives from beech
a – isolated from cultivation medium of sound wood treated with S. salmonicolor
b – isolated from cultivation medium of decayed wood treated with S. salmonicolor difference spectrum (b-a)

A high efficiency of used strains from the viewpoint of fatty and resin acids degradation as well as steryl esters removal was revealed. The results obtained by FTIR were confirmed by gas chromatography analysis. The values summarized in Tab. 4 show that *Sp. salmonicolor* decreases significantly the content of extractives, mainly fatty and resin acids in sound and decayed beech.

Tab. 4: Chemical composition of the acetone-extractives from sound and naturally decayed beech (mg/g (o.d.) wood) before and after treatment with Sporobolomyces salmonicolor

Extractive constituents	Sound wood		Decayed wood	
	before yeast treatment	after yeast treatment	before yeast treatment	after yeast treatment
Fatty and resin acids	2.14	0.36	2.44	0.22
Sterols	0.98	0.20	1.05	0.15
Steryl esters	0.13	0.02	0.05	0.02
Triglycerides	0.04	0	0.02	0

CONCLUSIONS

Three different yeast strains were used to reduce the extractive content in biologically decayed beech wood. The removal of lipophilic extractives by all used yeast strains was higher in sound wood as compared to decayed wood. The extractives in decayed wood mainly consist of resin acids. The most efficient yeast strain *Sporobolomyces salmonicolor* 19-4-21 should be considered as potential agent for pitch control in pulp and paper manufacture. Based on the structural and molecular characteristics of dioxane lignins isolated, it can be suggested that the removal of lignin in the pulping of decayed beech will be more effective related to sound wood.

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