Journal of Molecular Catalysis B: Enzymatic 61 (2009) 143–149

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Coupling of aromatic amines onto syringylglycerol β-guaiacylether using *Bacillus* SF spore laccase: A model for functionalization of lignin-based materials

Tukayi Kudanga[a], Endry Nugroho Prasetyo[a], Jussi Sipilä[b], Anita Eberl[a], Gibson S. Nyanhongo[a,*], Georg M. Guebitz[a]

[a] *Graz University of Technology, Institute of Environmental Biotechnology, Petersgasse 12/1, A-8010, Graz, Austria*
[b] *University of Helsinki, Department of Chemistry, P.O. Box 55 (A. I. Virtasen aukio 1), 00140, Helsinki, Finland*

ARTICLE INFO

Article history:
Received 20 April 2009
Received in revised form 22 May 2009
Accepted 2 June 2009
Available online 10 June 2009

Keywords:
Bacillus SF spore laccase
Coupling
Syringylglycerol β-guaiacylether

ABSTRACT

The potential of *Bacillus* SF spore laccase for coupling aromatic amines to lignin model molecules as a way of creating a stable reactive surface was investigated. The *Bacillus* spore laccase was shown to be active within the neutral to alkaline conditions (pH 7–8.5) and was more resistant to common laccase inhibitors than fungal laccases. Using this enzyme, tyramine was successfully covalently coupled onto syringylglycerol β-guaiacylether via a 4-O-5 bond, leaving the $-NH_2$ group free for further attachment of functional molecules. This study demonstrates the potential of *Bacillus* SF spore laccase for application in lignocellulose surface functionalization and other coupling reactions which can be carried out at neutral to alkaline pH under extreme conditions which normally inhibit fungal laccases.

1. Introduction

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are multi-copper containing enzymes which reduce molecular oxygen to water and simultaneously perform one electron oxidation of various aromatic substrates such as diphenols, methoxy substituted monophenols and aromatic amines [1,2]. Although most of the known and used laccases are of fungal origin, reports of laccases in bacterial systems are increasing [3–7]. Bacterial laccases have properties different from the fungal laccases given the different physiological and environmental conditions which support them [7]. Among the bacterial systems, the discovery of laccases especially in spores of *Bacillus* has attracted a lot of scientific and industrial interest. Since *Bacillus* spores are naturally designed to resist a wide range of extreme physico-chemical conditions such as dry heat, desiccation, radiation, UV light and oxidising agents [8], it is speculated that this enzyme may also posses these properties. It has already been favourably established that *Bacillus subtilis* spore laccases are thermally stable at 80 °C with a half-life of about 2 h and have optimum temperature around 75 °C [8], as compared to fungal laccases which are optimally active below 60 °C. *Bacillus licheniformis* spore laccase was also reported to oxidize ABTS at 85 °C [9]. *Bacillus* spore laccase (CotA) is a component of the spore coat involved in formation of a brown pigment that protects spores against UV radiation and H_2O_2 [5,10]. The crystallographic structure of CotA has been elucidated by Enguita et al. [11].

Apart from oxidation of the traditional laccase substrates [2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonate] (ABTS), syringaldazine (SGZ) and 2,6-dimethoxyphenol (DMP), recent reports show the ability of *Bacillus* spore laccases to oxidize dyes [6] and a few phenolic molecules [9,12]. However, their ability to oxidize a wide range of structurally diverse lignin molecules has not been investigated while existing knowledge on coupling of low molecular weight molecules onto lignin and lignocellulose materials is exclusively based on fungal laccases which are traditionally applied under acidic conditions [13–17]. In our earlier study, we demonstrated that grafted phenolic amines can form a stable reactive wood surface, counteracting negative effects of quenching of unstable radicals [18]. This study demonstrates for the first time the ability of *Bacillus* SF spore laccase to mediate the coupling of these aromatic amines onto lignin model compound (syringylglycerol β-guaiacylether) under different pH conditions. The stability of the laccase against common laccase inhibitors was also studied. Further, the ability of *Bacillus* SF spore laccase to oxidize a wide variety of structurally different lignin molecules (simple phenolics, hydroxycinnamic acids, hydroxybenzoic acids and phenolic amines) is investigated by monitoring both spectrophotometrical changes and oxygen consumption.

* Corresponding author. Tel.: +43 3168738404; fax: +43 3168738819.
E-mail addresses: gnyanhongo@yahoo.com, g.nyanhongo@tugraz.at (G.S. Nyanhongo).

1381-1177/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.molcatb.2009.06.003

Table 1
Oxidation of phenolic molecules and syringylglycerol β-guaiacylether (lignin model compound) by *Bacillus* SF spore laccase after 30 min incubation time.

Monomer	Absorption maxima of substrate (nm)	Absorption maxima of laccase oxidation products (nm)	Oxygen consumed by 1.0 mM substrate (mM)[a]
trans-4-Hydroxycinnamic acid	289; 310	–	0.00
4-Hydroxyphenoxyacetic acid	286; 331	–	0.00
4-Hydroxy-3-methoxybenzylamin hydrochloride	285	330	0.23 ± 0.021
4-Hydroxy-3,5-dimethoxy-cinnamic acid	314	510	0.26 ± 0.024
3,4-Dihydroxycinnamic acid	314	398	0.22 ± 0.019
trans-4-Hydroxy-3-methoxycinnamic acid	319	+	0.28 ± 0.030
4-Hydroxybenzoic acid	286	–	0.00
3-Hydroxy-4-methoxycinnamic acid (pred. trans)	312–315	+	0.17 ± 0.024
Tyramine 2-(4-hydroxyphenyl)ethylamine	285	–	0.00
1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrobromide	284	331	0.25 ± 0.033
3-Hydroxytyramine hydrochloride	280	475	0.24 ± 0.029
1,2,3-Trihydroxybenzene	225; 267	320, 380, 236 (broad peaks)	0.26 ± 0.024
3-(3,4-Dihydroxyphenyl)-DL-alanine	226; 281	312	0.28 ± 0.034
3,4,5-Trihydroxybenzoic acid	264	242.5; 396	0.17 ± 0.012
3,5-Dimethoxy-4-hydroxybenzoic acid	266	250; 289	0.23 ± 0.026
4-O-Methyldopamine hydrochloride	228; 281	475	0.20 ± 0.020
2-Methoxyphenol	287; 290	497	0.29 ± 0.028
1,2-Benzenediol	289; 295	386	0.28 ± 0.024
2,6-Dimethoxyphenol	300	469	0.24 ± 0.020
4-Hydroxy-3,5-dimethoxybenzaldehyde azine	357	530	0.23 ± 0.021
4-Hydroxy-3-methoxybenzaldehyde	279; 309	+	0.22 ± 0.020
4-Hydroxy-3-methoxybenzoic acid	256; 289	+	0.13 ± 0.014
Syringylglycerol β-guaiacylether	274	300	0.26 ± 0.014

(+) oxidized by *Bacillus* SF spore laccase (no product peak detected, substrate consumption was used as an indicator of a positive laccase reaction).
(–) not oxidized by *Bacillus* SF spore laccase.
[a] All values are means of three replicates ± standard deviation.

2. Materials and methods

2.1. Chemicals

The lignin model compound syringylglycerol β-guaiacylether was synthesized as previously described by Sipilä and Syrjänen [19]. All other chemicals were purchased from Sigma–Aldrich.

2.2. Laccase activity assay

The *Bacillus* SF spore laccase was produced as previously reported [6]. The activity of the laccase was determined spectrophotometrically by monitoring the oxidation of 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulphonate (ABTS) (ε_{436} = 29,200 M^{-1} cm^{-1}) as the substrate at 436 nm in 50 mM citrate buffer at pH 7.0 and 37 °C as described by Niku-Paavola et al. [20] with some modifications. The effect of six different known fungal laccase inhibitors namely thymine, thiourea, NaF, H_2O_2, NaN_3 and KCN on the oxidation of ABTS was investigated. Inhibitors were preincubated with the laccase in 50 mM citrate buffer at pH 7.0 and 37 °C for 60 min and residual laccase activity measured using the assay above.

2.3. Oxidation of phenolic molecules and lignin model compounds

The reaction mixture contained 2.0 nkat ml^{-1} laccase (as determined using ABTS as substrate) and 0.1 mM phenolic molecules. Incubation was carried out at different pH levels ranging from 2.2 to 9.0. Citrate buffer (0.05 M) was used in the range 2.2–5.5, phosphate buffer (0.1 M) in the range 6.0–8.0 while 0.025 M borate buffer was used in the range 8.5–9.0. Oxidation was monitored by means of wavelength scans in the range 200–800 nm and by oxygen consumption using a Rank Brothers oxygen meter (Dual Digital Model 20, England). Actual oxygen consumption values were computed by reference to the oxygen saturation concentration of the control reaction mixture (enzyme replaced by buffer) as determined by the Winkler method [21].

2.4. Coupling reactions in solution

Tyramine (1.0 mM) or 3-hydroxytyramine (1.0 mM) was coupled onto the lignin model compound syringylglycerol β-guaiacylether (2.0 mM) in 0.1 M ammonium acetate buffer pH 4.5–7.0 and in 0.1 mM ammonium bicarbonate buffer pH 7.5–8.5. Reactions were carried out at 37 °C while shaking at 650 rpm. Syringylglycerol β-guaiacylether was initially incubated with the laccase for 30 min; after which 3-hydroxytyramine was added and the reaction continued for a further 75 min. In the case of tyramine (non-laccase substrate), the monomer and model were mixed simultaneously and the reaction was run for 1 h 45 min.

2.5. Chromatographic analysis of oxidation and coupling products

Analysis of lignin molecules, their oxidation and coupling products was performed using HPLC. An equal volume of ice cold methanol was added to the incubation mixtures to stop the reaction and to precipitate proteins. The mixture was centrifuged at 0 °C for 15 min at 14,000 × *g* and 700 μl transferred into vials. Analysis was performed by an HPLC-UV system from Dionex with a P580 pump,

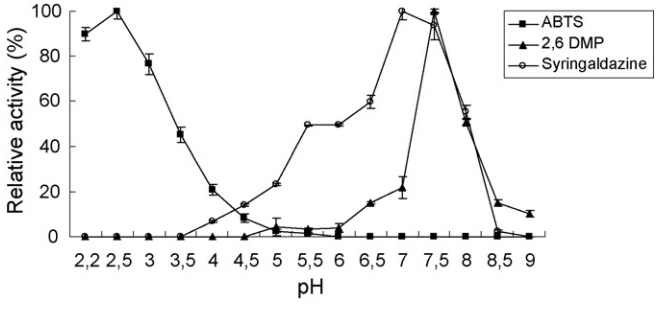

Fig. 1. Effect of pH on oxidation of common laccase substrates by *Bacillus* SF spore laccase. *All values are means of three replicates ± standard deviation and were obtained by monitoring product formation (ABTS at 415 nm; 2,6-DMP at 469 and syringaldazine at 530 nm).

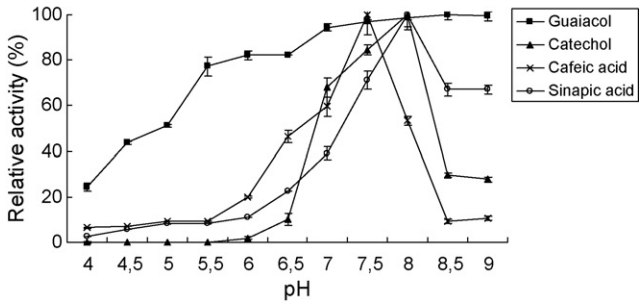

Fig. 2. Effect of pH on oxidation of selected lignin model compounds by *Bacillus* SF spore laccase. *All values are means of three replicates ± standard deviation obtained by monitoring product formation (guaiacol at 497 nm; catechol at 386 nm; caffeic acid at 398 nm and sinapic acid at 510 nm).

an ASI-100 autosampler and a PDA-100 photodiode array detector. Identification and quantitative determination of products was done by reversed phase HPLC, on a Discovery HS C18 column (5 μm; 15 cm × 4.6 mm, Supelco, Bellefonte, USA) using acetonitrile, 10 mM sulphuric acid and deionized water (20:15:65) as solvent with isocratic elution at a flow rate of 1 ml min^{-1} and an oven temperature of 25 °C.

Table 2
Inhibition of *Bacillus* SF spore laccase by putative laccase inhibitors.

Inhibitor	[a]I_{50} *Bacillus* SF spore laccase	I_{50} fungal laccases	References
Thymine	2.8 mM	0.01–0.95 mM	[37,38]
Thiourea	23.4 μM	1.5 mM	[17]
NaF	458.9 μM	12 μM–10 mM	[17,36,38]
H_2O_2	24.7 mM	54 mM	[39]
NaN_3	38.8 μM	9 μM–10 mM	[17,35,36,38]
KCN	1.4 mM	130 μM–0.2 mM	[17,36]

[a] I_{50} is the concentration of inhibitor required to achieve a 50% drop in laccase activity.

Laccase oxidation of phenolic amines was monitored using acetonitrile and 50 mM potassium phosphate buffer pH 4.6 (2:98) as solvent (isocratic elution) at a flow rate of 1 ml min^{-1} and an oven temperature of 25 °C.

The MS spectra were acquired with an Agilent Ion Trap SL (Palo Alto, CA, USA) equipped with electrospray ionization coupled to the Dionex HPLC-UVD-system described above and using the same protocol except that 0.1% formic acid was used instead of 10 mM sulphuric acid. The coupling products were measured in positive ion mode and the electrospray voltage was set to +3500 V. Dry gas flow was set to 12 l min^{-1} with a temperature of 350 °C, nebulizer to 70 psi. Maximal accumulation time was fixed to 300 ms and the

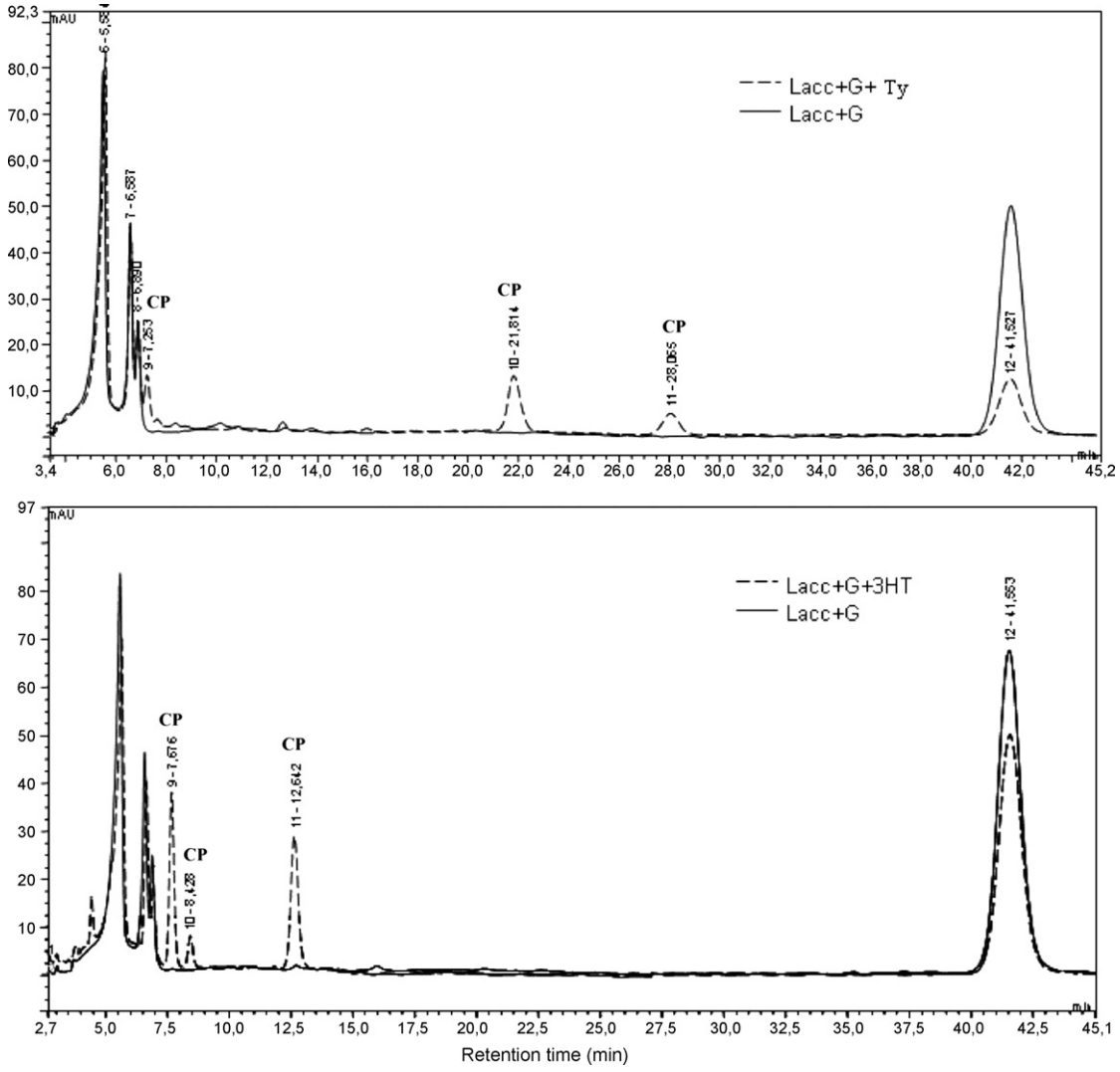

Fig. 3. Coupling of tyramine (Ty) and of 3-hydroxytyramine (3HT) onto syringylglycerol β-ether (G) using *Bacillus* SF laccase. CP—coupling product.

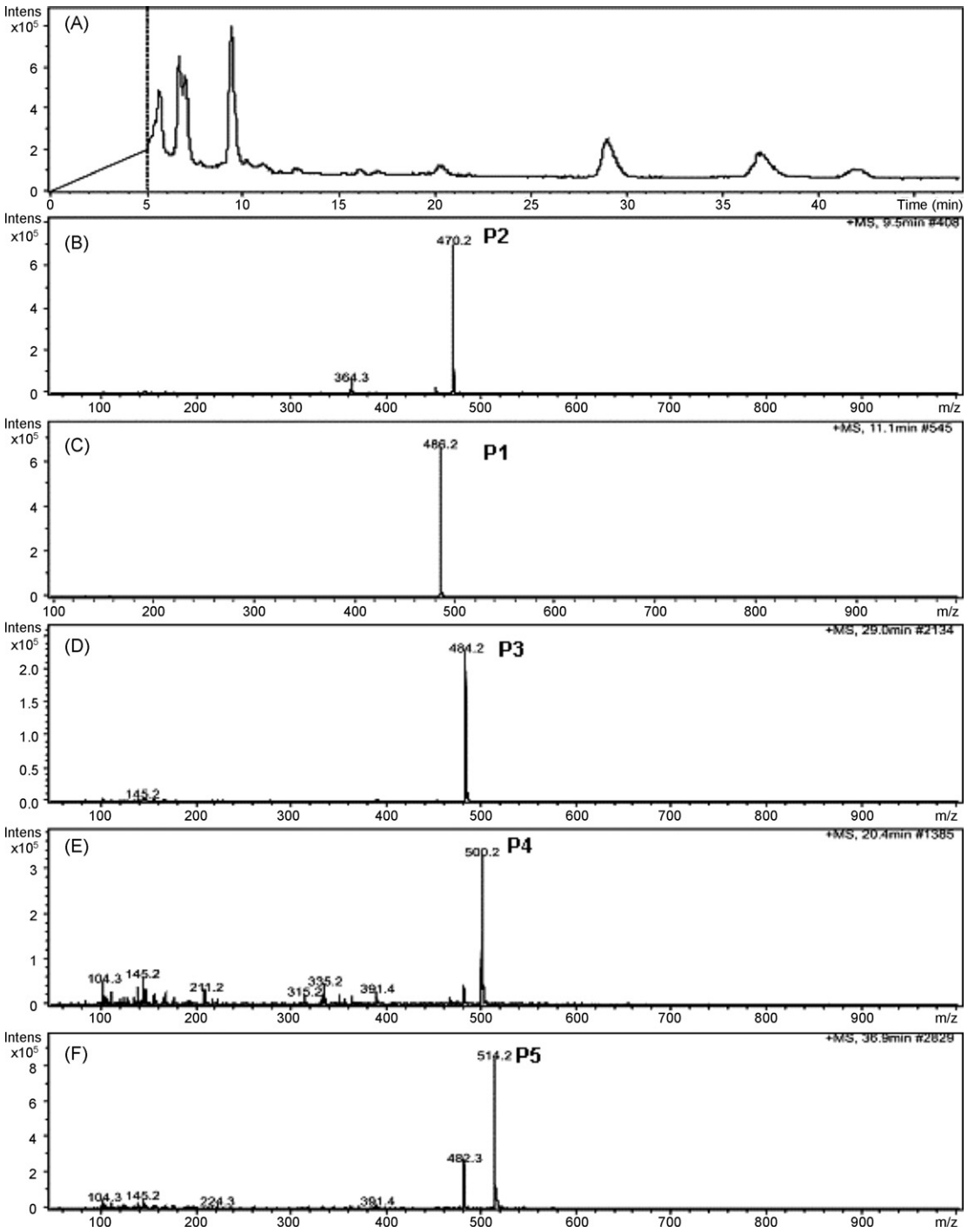

Fig. 4. (A) Total ion chromatogram for coupling products between tyramine and syringylglycerol β-guaiacylether. (B–F) Mass spectra of coupling products between tyramine and syringylglycerol β-guaiacylether (G).

loading of the trap was controlled by the instrument with an ICC of 30,000.

3. Results and discussion

3.1. Oxidation of phenolic molecules and aromatic amines

The substrate specificity of fungal laccases on phenolic molecules and lignin model substrates is well established. However, there is only little known in this respect about bacterial laccases which show a lower redox potential [22]. The spore laccase from *Bacillus* SF oxidized a wide range of phenolic molecules according to UV–vis spectrophotometry and oxygen consumption (Table 1). Approximately 1 mole oxygen was consumed for every 4 moles of substrates upon complete oxidation which is typical for laccase catalyzed reactions [23,24]. However, oxidation of 3,4,5-trihydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid and 3-hydroxy-4-methoxycinnamic acid (predominantly trans) was not complete within the given reaction time. The influence of substituents on position 3 and 5 on the benzene ring with respect to a hydroxyl group on position 4 was quite evident. The presence of methoxy and –hydroxyl substituents on position 3 and 5 makes the phenolic molecules laccase substrates (Table 1). However, the presence of only a hydroxyl

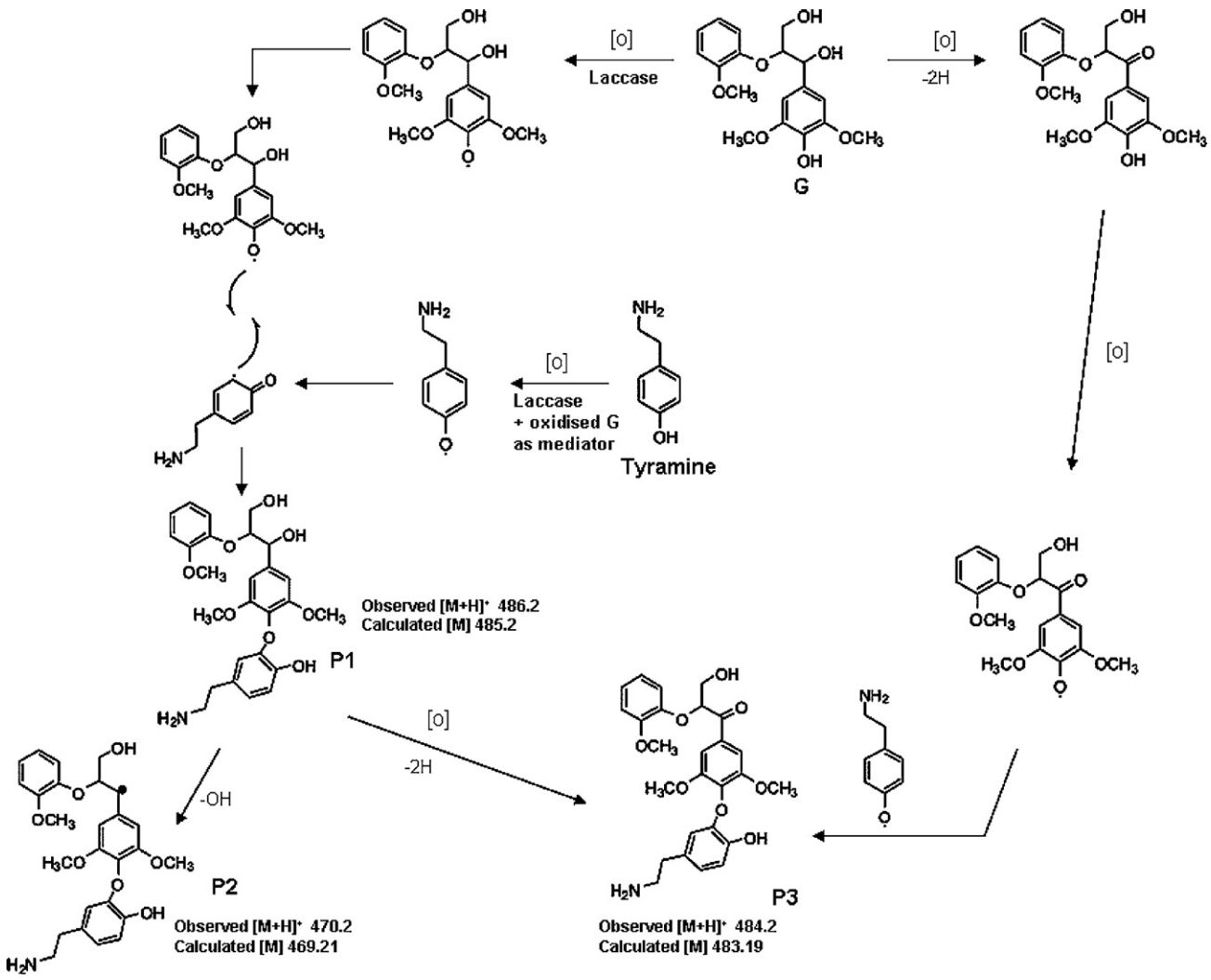

Fig. 5. Proposed reaction pathway scheme for the laccase-mediated coupling of tyramine onto syringylglycerol β-guaiacylether (G).

group on position 4 is not sufficient to make the phenolic molecule a substrate of the *Bacillus* SF spore laccase as evidenced by the respective four molecules that were not oxidized (Table 1). A similar observation has been reported in previous studies using *Bacillus licheniformis* spore laccase [9], although Takahama reported oxidation of coumaric acid with *Pyricularia oryzae* laccase [25]. This is not surprising since bacterial laccases have been reported to exhibit a lower redox potential of about 0.5 V [22] compared to 0.5–0.8 V for fungal laccases [26,27]. Another interesting observation is that carboxyl groups which are electron-withdrawing tend to make the molecules poor substrates when attached directly to the benzene ring as exemplified by the lower turnover (i.e. oxygen consumption) for 4-hydroxy-3-methoxybenzoic acid (Table 1). This is also quite evident when comparing 3,4,5-trihydroxybenzoic acid and 1,2,3-trihydroxybenzene, where the latter was oxidized to higher extent for the given time of the reaction. Indeed Koschorreck et al. reported that 4-hydroxy-3-methoxybenzoic acid (which was one of the poor substrates for our *Bacillus* SF spore laccase) was not oxidized by *Bacillus licheniformis* spore laccase [9]. Nevertheless, electron-donating methoxy groups seem to counteract the effect of electron with-drawing groups (compare for example 3,5-dimethoxy-4-hydroxybenzoic acid and 4-hydroxy-3-methoxybenzoic acid). Despite the narrower substrate range, generally the observed influence of the nature and position of functional groups on the benzene ring of various molecules, on *Bacillus* SF spore laccase oxidation is consistent with that reported for fungal laccases [17,28–34].

3.2. Effect of pH on oxidation of selected common laccase substrates

There was a wide variability among different substrates in terms of the pH optimum for oxidation with ABTS having a very low optimum pH of 2.5 while guaiacol requires pH 8.5 (Figs. 1 and 2). The optimal pH required to oxidize ABTS is similar to that of most fungal laccases [35] and our results for ABTS and syringaldazine are consistent with the findings for a *Bacillus subtilis* spore laccase [9]. In addition to standard substrates, in this study we have determined the pH optima for oxidation of typical lignin model substrates guaiacol, sinapic acid, catechol and caffeic acid by the spore laccase (Fig. 2). It was observed that the common lignin model substrates generally require neutral to alkaline pH for effective oxidation with *Bacillus* SF spore laccase.

3.3. Inhibition of Bacillus SF spore laccase by putative laccase inhibitors

Like all known laccases, the *Bacillus* SF spore laccase was inhibited by the common laccase inhibitors thymine, thiourea, NaF, H_2O_2,

NaN_3 and KCN (Table 2). Of particular interest is the inhibition by cyanide and azide which confirms the presence of a metal (i.e. copper) in the catalytic center while inhibition by fluoride has been reported to be typical for laccases [36]. However, it is worth noting that while the spore laccase has some IC_{50} values within the range of fungal laccases (Table 2), it is three times more resistant to thymine and seven times more resistant to KCN which suggests that it may tolerate harsher conditions than free fungal and bacterial laccases.

3.4. Coupling reactions

In our earlier study, an increase in coupling of fungicides onto amine functionalized wood using *Trametes hirsuta* laccase was reported [18]. Here, due to its higher pH-optimum and stability, we used the *Bacillus* SF spore laccase for a mechanistic study to elucidate the possibility of coupling and the nature of the coupling products between phenolic amines and syringylglycerol β-guaiacylether (mimicking lignin). It was observed that the yield of coupling product is much higher when syringylglycerol β-guaiacylether was pre-oxidized by the laccase before addition of the 3-hydroxytyramine. This reduced chances of formation of homo-oligomeric products. Consequently, in the case of tyramine (non-laccase substrate), there was no need for this preoxidation step. According to HPLC analyses, new peaks indicated coupling products between syringylglycerol β-guaiacylether and the phenolic amines, tyramine and 3-hydroxytyramine (Fig. 3). New peaks were observed in tyramine coupling reactions with retention times t_R = 7.3 min t_R = 21.8 min and t_R = 28.1 min (there was a slight shift in retention times to t_R = 9.5 min, t_R = 20.3 and t_R = 29.0 min, respectively, in LC/MS when 0.1% formic acid was used instead of 10 mM H_2SO_4, Fig. 4). New peaks with retention times t_R = 12.6 min, t_R = 8.4 and t_R = 7.7 were observed when 3-hydroxytyramine was used.

LC-MS studies were carried out to confirm coupling between tyramine and syringylglycerol β-guaiacylether and to elucidate the structure of the coupling products. The molecular weight of syringylglycerol β-guaiacylether is 350.36 while that of tyramine is 137.18. LC-MS showed three main $[M+H]^+$ ions at m/z 486.2 (t_R 11.1 min), m/z 484.2 (t_R = 29.0 min) and m/z 470.2 (t_R 9.5 min) (Fig. 4). The hydroxyl group on the benzene ring is known to be *ortho* or *para* directing [40–42] and in the absence of an unsaturated side chain, syringyl lignin moieties usually undergo 4-O-5 coupling with other molecules [43]. We therefore hypothesize that the electrophilic oxidized syringylglycerol β-guaiacylether cation radical preferentially attacks the sterically unhindered electron-rich *ortho* position on the tyramine molecule forming a 4-O-5 linkage as shown in Fig. 5. For this reaction to occur tyramine is probably oxidized in the presence of oxidized syringylglycerol β-guaiacylether radical acting as mediator. This results in the coupling product (**P1**) with the observed $[M+H]^+$ molecular ion m/z 486.2 (exact mass 485.2). This product was observed in all coupling reactions from pH 4.5–8.5. However, the acidic conditions used, seemed to facilitate cleavage of the benzyl α-hydroxyl group resulting in the coupling product (**P2**) with $[M+H]^+$ molecular ion m/z 470.2 (exact mass 469.21). This is further supported by the fact that this adduct was only observed in acidic conditions and was absent in neutral to alkaline conditions (pH 7–8.5). The product (**P3**) with molecular ion m/z 484.2 (exact mass 483.19) can be explained by the α-oxidation of the benzyl hydroxyl group to a keto group which may be quite a facile reaction.

The other coupling products; **P4**, m/z 500.2, t_R = 20.3 min; and **P5**, m/z 514.2, t_R = 36.9 min (observed on total ion chromatogram) are probably a result of nucleophilic attack by OH groups of water (calculated 499.2) and methanol (calculated 513.2), respectively. The laccase created radicals or their quinone form have been shown to undergo nucleophilic attack by substances having nucleophilic groups like water or methanol present in the reaction mixture [44]. Similar reactions have also been previously reported [45–47]. It has been observed that hydroxylation is a transition step in laccase-mediated demethylation of lignin molecules [45]. Fig. 5 summarizes the possible reaction pathway for the laccase-mediated coupling of tyramine onto syringylglycerol β-guaiacylether.

4. Conclusions

Previously we have shown that laccase-catalyzed amino-functionalization of lignocellulosic material creates a reactive surface for coupling of functional molecules such as antifungal agents [18] and possibly dyes, flame retardants, etc. Here we present the mechanistic evidence that demonstrates the ability of laccases to mediate the covalent bonding of aromatic amines to a lignin model substrate via 4-O-5 coupling leaving the $-NH_2$ group free for further functionalization. In addition, the ability of *Bacillus* SF spore laccase to oxidize a wide range of lignin molecules, work at relatively high pH and tolerate high laccase inhibitor concentrations as shown in this study, coupled with the already known ability to tolerate high temperatures, makes it an attractive alternative to fungal laccases for application in lignocellulosic material functionalization or modification processes.

Acknowledgements

Acknowledgment for support of this work is made to Austrian Academic Exchange Service (ÖAD) and European Union Biorenew Project [Sixth Framework Programme (FP6-2004-NMP-NI-4)].

References

[1] F. Xu, Biochemistry 35 (1996) 7608–7614.
[2] A.M. Mayer, R.C. Staples, Phytochemistry 60 (2002) 551–565.
[3] H. Claus, Micron 35 (2004) 93–96.
[4] H. Claus, Arch. Microbiol. 179 (2003) 145–150.
[5] P. Sharma, R. Goel, N. Capalash, World J. Microbiol. Biotechnol. 23 (2007) 823–832.
[6] H. Held, A. Kandelbauer, M. Schroeder, A. Cavaco-Paulo, G.M. Guebitz, Environ. Chem. Lett. 3 (2005) 74–77.
[7] C. Wang, X. Wei, M. Zhao, J. Biotechnol. 136S (2008) S326.
[8] L.O. Martins, C.M. Soares, M.M. Pereira, M. Teixeira, T. Costa, G.H. Jones, A.O. Henriques, J. Biol. Chem. 277 (2002) 18849–18859.
[9] K. Koschorreck, S. Richter, A. Ene, E. Roduner, R. Schmid, V. Urlacher, Appl. Microbiol. Biotechnol. 79 (2008) 217–224.
[10] M.F. Hullo, I. Moszer, A. Danchin, I. Martin-Verstraete, J. Bacteriol. 183 (2001) 5426–5430.
[11] F.J. Enguita, O.L. Martins, A.O. Henriques, M.A. Carrondo, J. Biol. Chem. 278 (2003) 19416–19425.
[12] H. Hirose, M. Nasu, H. Yokoi, Biotechnol. Lett. 25 (2003) 1609–1612.
[13] R. Chandra, A.J. Ragauskas, 11th International Symposium on Wood and Pulping Chemistry, vol. 2, Nice, France, II, Tappi Press, Atlanta, 2001, pp. 39–43.
[14] R.P. Chandra, A.J. Ragauskas, Enz. Microb. Technol. 30 (2002) 855–861.
[15] C. Felby, R. Chandra, A.J. Ragauskas, J. Wood Chem. Technol. 24 (2004) 69–81.
[16] R.P. Chandra, L.R. Lehtonen, A.J. Ragauskas, Biotechnol. Prog. 20 (2004) 255–261.
[17] G.S. Nyanhongo, S. Rodríguez Couto, G.M. Guebitz, Chemosphere 64 (2006) 359–370.
[18] T. Kudanga, E. Nugroho Prasetyo, J. Sipilä, P. Nousiainen, P. Widsten, A. Kandelbauer, G.S. Nyanhongo, G.M. Guebitz, Eng. Life Sci. 8 (2008) 297–302.
[19] J. Sipilä, K. Syrjänen, Holzforschung 49 (1995) 325–331.
[20] M.L. Niku-Paavola, E. Karhunen, P. Salola, V. Raunio, Biochem. J. 254 (1988) 877–883.
[21] L. Winkler, Berichte der Deutschen Chemischen Gesellschaft 21 (1888) 2843–2855.
[22] E.P. Melo, A.T. Fernandes, P. Dura, L.O. Martins, Biochem. Soc. Trans. 35 (2007) 1579–1582.
[23] E. Nugroho Prasetyo, T. Kudanga, W. Steiner, M. Murkovic, G.S. Nyanhongo, G.M. Guebitz, Anal. Bioanal. Chem. 393 (2009) 679–687.
[24] S. Riva, Trends Biotechnol. 24 (2006) 219–226.
[25] U. Takahama, Physiol. Plantarum 93 (1) (1995) 61–68.
[26] S.V.S. Kumar, P.S. Phale, S. Durani, P.P. Wangikar, Biotechnol. Bioeng. 83 (2003) 386–394.
[27] K.J. Kersten, B. Kalyanaraman, K.E. Hammel, B. Reinhammar, T.K. Kirk, Biochem. J. 268 (1990) 475–480.
[28] D. Litthauer, M.J. van Vuuren, A. van Tonder, W.F. Wolfaardt, Enz. Microb. Technol. 40 (2007) 563–568.

[29] S.D. Varfolomeev, A. Naki, A.L. Yaropolov, I.V. Berezin, Biokhimiya 50 (1985) 1411–1420.
[30] F. Xu, J.J. Kulys, K. Duke, K. Li, K. Krikstopaitis, H.J.W. Deussen, E. Abbate, V. Galinyte, P. Schneider, Appl. Environ. Microbiol. 66 (2000) 2052–2056.
[31] K. Li, F. Xu, K.E.L. Eriksson, Appl. Environ. Microbiol. 65 (1999) 2654–2660.
[32] A. Robbles, R. Lucas, M. Martinez-Canamero, N.B. Omar, P. Perez, A. Galvez, Enz. Microb. Technol. 31 (2002) 516–522.
[33] C. Liers, R. Ullrich, M. Pecyn, D. Schlosser, M. Hofrichter, Enz. Microb. Technol. 41 (2007) 785–793.
[34] T. Saito, P. Hong, K. Kato, M. Okazaki, H. Inagaki, M. Maeda, Y. Yokogawa, Enz. Microb. Technol. 33 (2003) 520–526.
[35] P. Baldrian, FEMS Microbiol. Rev. 30 (2006) 215–242.
[36] M. Scherer, R. Fischer, Arch. Microbiol. 70 (1998) 78–84.
[37] G. Iyer, B.B. Chattoo, FEMS Microbiol. Lett. 227 (2003) 121–126.
[38] S. Ryan, W. Schnitzhofer, T. Tzanov, A. Cavaco-Paulo, G.M. Gübitz, Enz. Microb. Technol. 33 (2003) 766–774.
[39] H. Palonen, M. Saloheimo, L. Viikari, K. Kruus, Enz. Microb. Technol. 33 (2003) 854–862.
[40] O. Suparno, A.D. Covington, P.S. Phillips, C.S. Evans, Resour. Conserv. Recyling 45 (2005) 114–127.
[41] A. Schultz, U. Jonas, E. Hammer, F. Schauer, Appl. Environ. Microbiol. 67 (2001) 4377–4381.
[42] U. Jonas, E. Hammer, E.T.K. Haupt, F. Schauer, Arch. Microbiol. 174 (2000) 393–398.
[43] J. Ralph, Proceedings of the 13th ISWFPC, vol. 1, Auckland, New Zealand, 2005, pp. 3–13.
[44] K. Manda, D. Gördes, M. Mikolasch, E. Hammer, E. Schmidt, K. Thurow, F. Schauer, Appl. Microbiol. Biotechnol. 76 (2007) 407–416.
[45] A. Leonowicz, N.S. Cho, J. Luterek, A. Wilkolazka, M. Wojtas–Wasilewska, A. Matuszewska, M. Hofrichter, D. Wesenberg, J. Rogalski, J. Basic Microbiol. 41 (2001) 185–227.
[46] S. Witayakran, A.J. Ragauskas, Eur. J. Org. Chem. 3 (2009) 358–363.
[47] S. Witayakran, A.J. Ragauskas, Enz. Microb. Technol. 44 (2009) 176–181.