

Additional file 1

Bacterial conversion of depolymerized Kraft lignin

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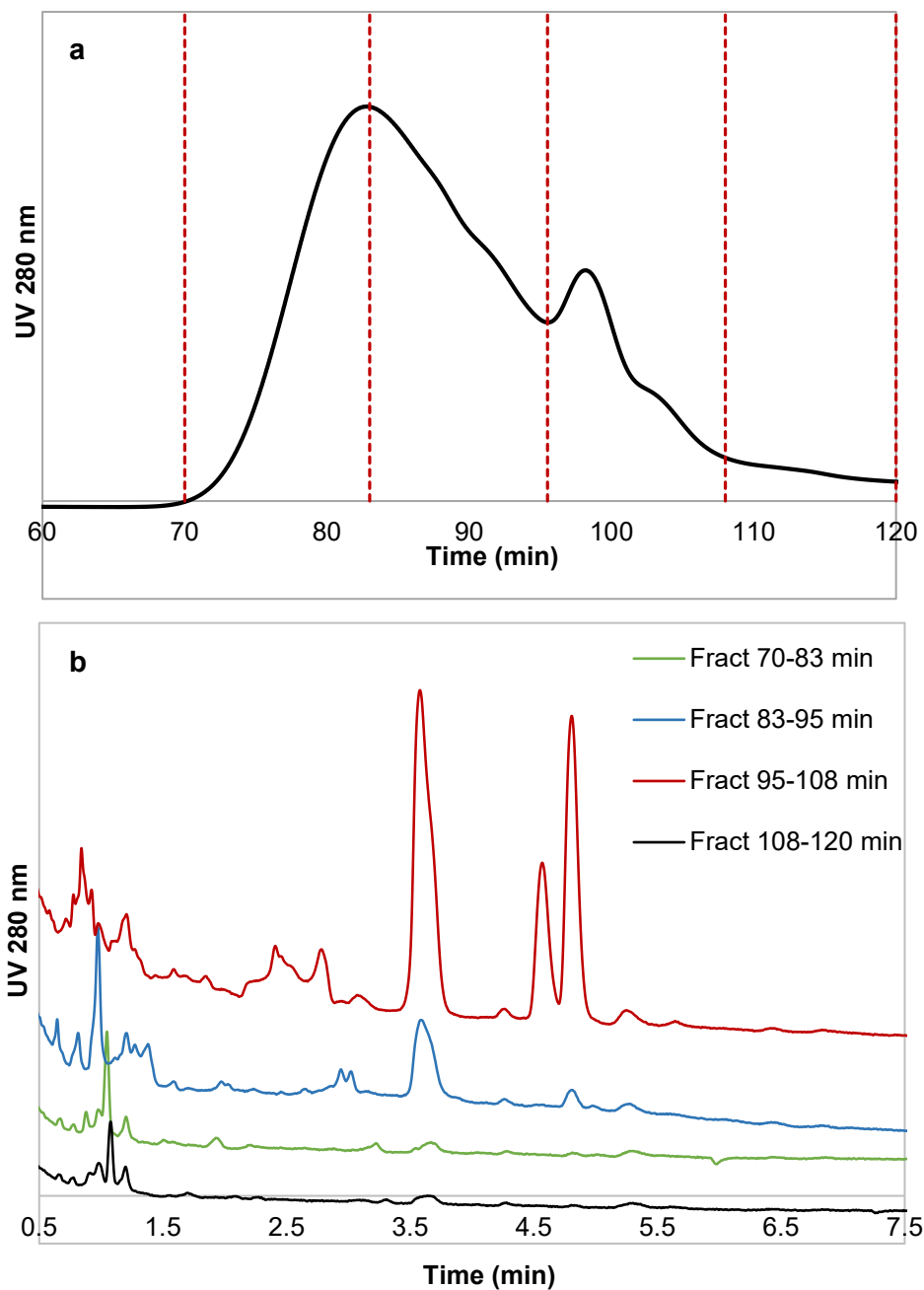


Figure S1: (a) The SEC chromatogram of 1 g/L depolymerized (at 220 °C, 5 mL/min) lignin. Red dotted lines represent the fractions collected. The collected fraction at 95-108 minutes corresponds to the 0.2-0.4 kDa peak in the SEC chromatograms calibrated with PEG standards. (b) UHPLC chromatograms of the fractions obtained from SEC. The peaks in the fraction 95-108 minutes correspond to aromatic monomers (Vanillin-3.5 min; guaiacol-4.6 min; acetovanillone-4.7 min).

Table S1: Results of homology BLAST with the previously well-characterized DyP proteins against the genome of the organisms used in this study. *P. putida* EM42 strain used in this study is the modified version of KT2440 and hence the genome of KT2440 (parental strain) was used for BLAST searches. Proteins with identity more than 75 % are emphasized in green. Proteins that were found absent and the ones with less than 30 % query are highlighted in red. *P. fluorescens* highlighted in blue is the only organism with proteins similar to DyPC and DyPD.

Well characterized DyP proteins	DyP subfamily/ aa length	Blasted against the genome of organisms used in this study	Resultant protein name	Query cover (%)	Identity (%)	Accession number
Dyp-type peroxidase [<i>R. jostii</i> RHA1] UniProtKB: Q0S4I5	A/428	<i>P. fluorescens</i> (taxid: 294)	deferrochelata-se/ peroxidase EfeB	97	36	WP_015635791.1
		<i>P. putida</i> KT2440	Amidohydro-lase	8	35	WP_010952751.1
		<i>R. opacus</i> (taxid: 37919)	deferrochelata-se /peroxidase EfeB	100	98	WP_054246035.1
Dyp-type peroxidase [<i>P. fluorescens</i> Pf-5] UniProtKB: Q4KBM1	A/436	<i>P. fluorescens</i> (taxid: 294)	deferrochelata-se /peroxidase EfeB	100	99	WP_015635791.1
		<i>P. putida</i> KT2440	--			
		<i>R. opacus</i> (taxid: 37919)	deferrochelata-se/ peroxidase EfeB	84	37	WP_020970278.1
Dyp-type peroxidase [<i>R. jostii</i> RHA1] UniProtKB: Q0SE24	B/350	<i>P. fluorescens</i> (taxid: 294)	Peroxidase	90	55	WP_060755404.1
		<i>P. putida</i> KT2440	Peroxidase	63	32	WP_010954130.1
		<i>R. opacus</i> (taxid: 37919)	Peroxidase	100	97	WP_065492214.1
Dyp-type peroxidase [<i>P. fluorescens</i> Pf-5] UniProtKB: Q4KAC6	1B/295	<i>P. fluorescens</i> (taxid: 294)	Peroxidase	100	99	WP_015636122.1
		<i>P. putida</i> KT2440	Peroxidase	97	62	WP_010954130.1
		<i>R. opacus</i> (taxid: 37919)	Peroxidase	79	30	WP_012689337.1
Dyp-type peroxidase [<i>P. fluorescens</i> Pf-5] UniProtKB: Q4KA97	2B/324	<i>P. fluorescens</i> (taxid: 294)	Hypothetical protein	100	99	WP_015636148.1
		<i>P. putida</i> KT2440	Peroxidase	67	32	WP_010954130.1
		<i>R. opacus</i> (taxid: 37919)	Peroxidase	95	59	WP_044480584.1
Multifunctional dye peroxidase [<i>Amycolatopsis</i> sp. 75iv2] UniProtKB: K7N5M8	C/464	<i>P. fluorescens</i> (taxid: 294)	Hypothetical protein	48	30	WP_054597259.1
		<i>P. putida</i> KT2440	--			
		<i>R. opacus</i> (taxid: 37919)	--			
Dye-decolorizing peroxidase [<i>Bjerkandera adusta</i>] UniProtKB: W8ZBJ5	D/496	<i>P. fluorescens</i> (taxid: 294)	Hypothetical protein	72	25	WP_054597259.1
		<i>P. putida</i> KT2440	--			
		<i>R. opacus</i> (taxid: 37919)	--			

Table S2: Results of homology BLAST with laccases against the genome of the organisms used in this study. *P. putida* EM42 strain used in this study is the modified version of KT2440 (parental strain) and hence the genome of KT2440 was used for BLAST searches. Proteins with identity more than 75 % are emphasized in green.

Laccases	aa length	Blasted against the genome of organisms used in this study	Resultant protein name	Query cover (%)	Identity (%)	Accession number
Laccase-2 [<i>R. opacus</i> PD630] UniProtKB: W8HL51	494	<i>P. fluorescens</i> (taxid: 294)	copper resistance system multicopper oxidase	84	34	WP_003193107.1
		<i>P. putida</i> KT2440	copper resistance system multicopper oxidase	87	34	WP_010953188.1
		<i>R. opacus</i> (taxid: 37919)	multicopper oxidase family protein	100	100	WP_005251383.1
Multi-copper polyphenol oxidase [<i>P. fluorescens</i>] UniProtKB: A0A293QHC5	239	<i>P. fluorescens</i> (taxid: 294)	peptidoglycan editing factor PgeF	100	100	WP_057713252.1
		<i>P. putida</i> KT2440	multi-copper polyphenol oxidoreductase	98	76	WP_010951886.1
		<i>R. opacus</i> (taxid: 37919)	peptidoglycan editing factor PgeF	94	37	WP_020969856.1

Table S3: Some studies reporting guaiacol, vanillin and related compounds in depolymerized lignin

Substrate	Depolymerization method	Major monomers	Reference
Steam treated softwood and hemp lignin	Base-catalyzed depolymerization	Guaiacol, vanillin, catechol	[1]
Organosolv lignin	Base-catalyzed depolymerization	Guaiacol, Syringol, syringaldehyde,	[2]
Spruce lignin	Hydrogenolysis/Extraction	Ethylguaiacol, propylguaiacol,	[3]
Dealkaline lignin	Solid acid catalysts	Vanillin, guaiacyl acetone, homovanillic acid	[4]
Birch wood lignin	Hydrogenolysis with Ni-based catalysts	Propyl guaiacol and propyl syringol	[5]
Organosolv switchgrass lignin	Depolymerization and hydrodeoxygenation with formic acid and Pt/C	Guaiacol, 4-methylguaiacol, homovanillyl alcohol	[6]
Organosolv hardwood lignin	Depolymerization in supercritical carbon dioxide/acetone/water fluid	Guaiacol, 2-methoxy-4-methylphenol, syringol	[7]
Kraft lignin	oxidation in aqueous methanol at acidic pH	Vanillin, methyl vanillate	[8]
Kraft lignin (Indulin AT)	Base (NaOH) catalyzed depolymerization at 220 °C	Guaiacol, vanillin, acetovanillone, vanillate, 4-hydroxybenzoate	This study

Table S4: Growth of bacterial strains on agar plates with 5 mM vanillin/guaiacol as the only source of carbon in M9 medium, incubated for 2 weeks at 30 °C. The isolates were previously tentatively reported as *Pseudomonas* sp. (B); *P. plecoglossicida* (C); *P. deceptionensis* (9.1); and *Rhodococcus erythropolis* (19). Duplicate experiments were performed and the growth results were similar (++ abundant growth; + growth; - no growth). *R. opacus* was the only organism to show growth on guaiacol (highlighted).

Organisms	5 mM vanillin	5 mM guaiacol
<i>R. opacus</i>	+	+
<i>R. erythropolis</i>	-	-
<i>P. fluorescens</i>	++	-
<i>P. putida</i> KT2440	++	-
<i>P. putida</i> EM42	++	-
Isolate B	++	-
Isolate C	-	-
Isolate 9.1	+	-
Isolate 19	-	-

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