Electronic Supplementary Material (ESI) for Green Chemistry.

Towards Lignin Consolidated Bioprocessing: Simultaneous lignin depolymerization and product generation by bacteria

Davinia Salvachúa, Eric M. Karp, Claire T. Nimlos, Derek R. Vardon, Gregg T. Beckham

Electronic Supplementary Information

Supplementary Figures

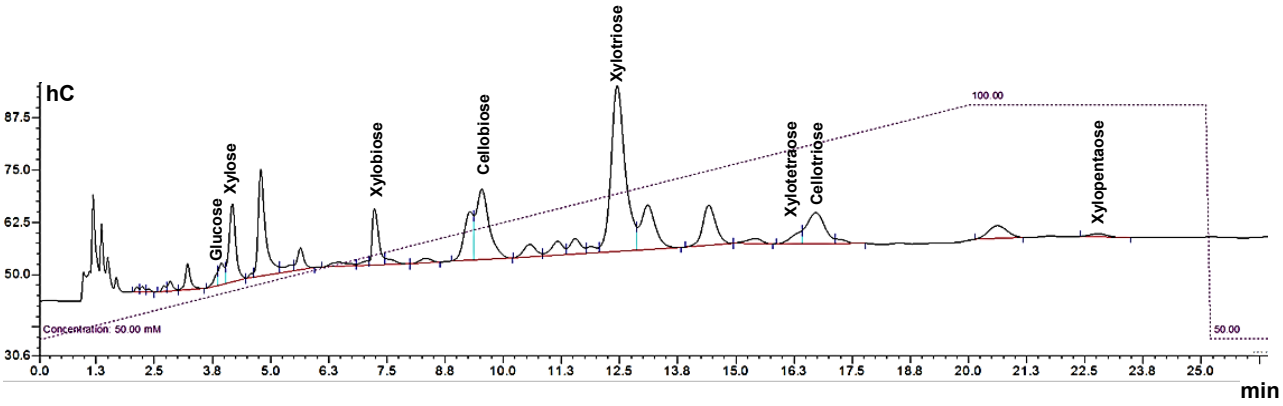

Fig. S1. Detection of monosaccharides and cello- and xylo- oligosaccharides by High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) in alkaline pretreated liquor without bacterial treatment.

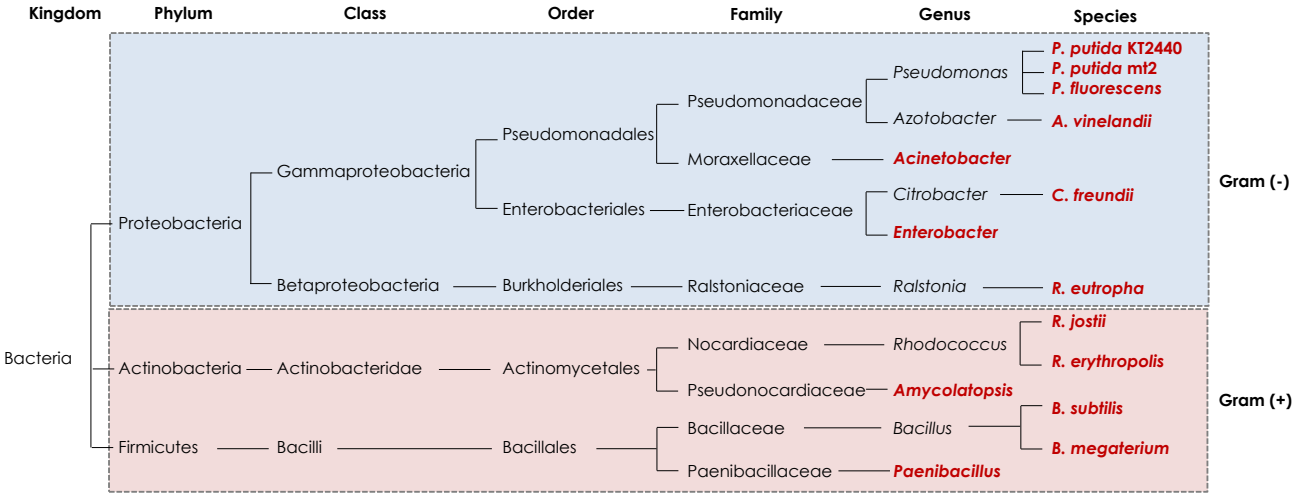

Fig. S2: Taxonomic tree of the bacterial species screened in the current study. The taxonomic tree also delineates Gram (-) and Gram (+) bacteria.

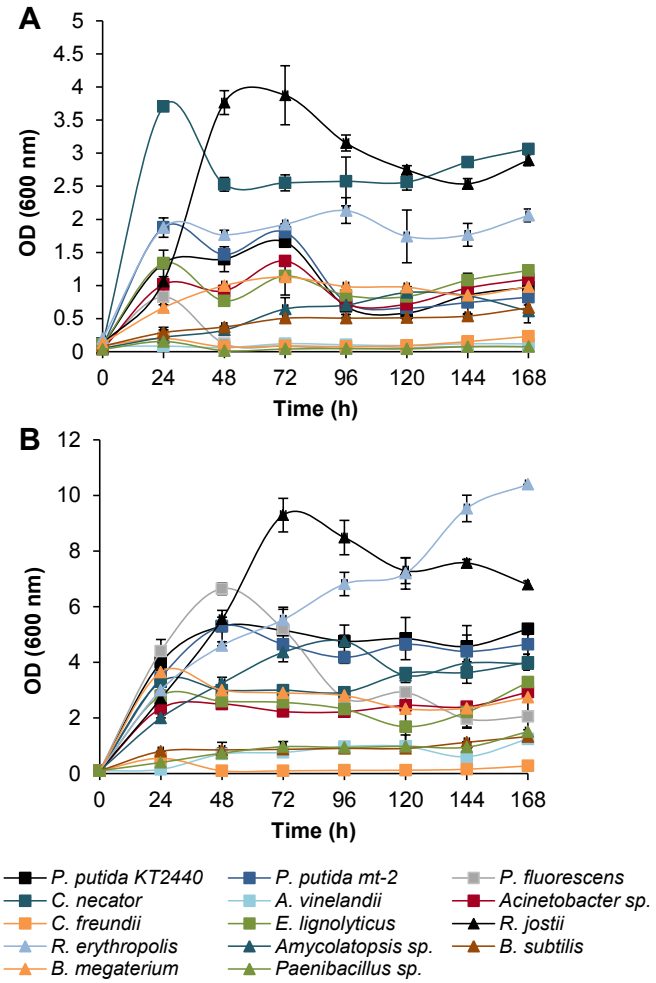

Fig. S3. Growth curves as a function of time (measured by OD600) of 14 bacterial strains on alkaline pretreated liquor over 7 days of incubation in (A) nitrogen-limiting and (B) nutrient-rich conditions.

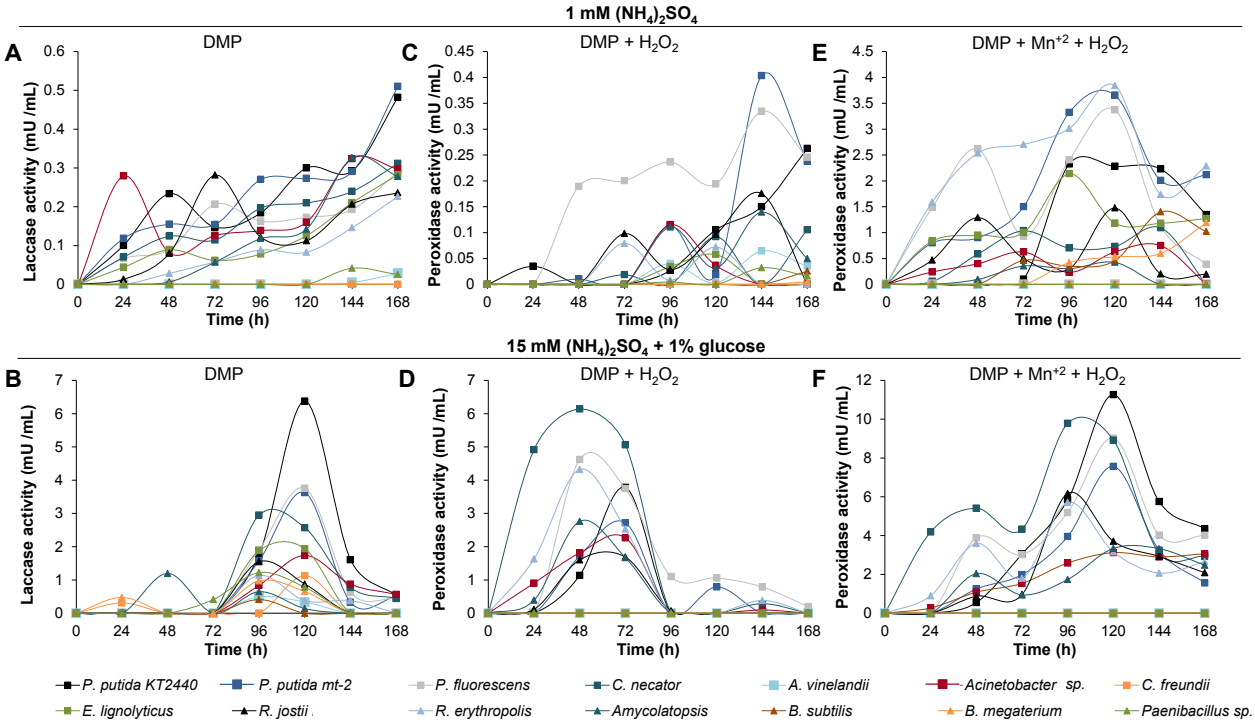

Fig. S4. Profiles of ligninolytic enzyme secretion by the 14 bacteria in nitrogen-limiting (A, C, E) and nutrient-rich (B, D, F) conditions. (A,B) Laccase activity was followed by the oxidation of DMP, (C,D) Mn^{2+}-independent peroxidase activity by the oxidation of DMP in the presence of H_2O_2, and (E,F) Mn^{2+}-oxidizing peroxidase activity with DMP, H_2O_2 and Mn^{2+}.

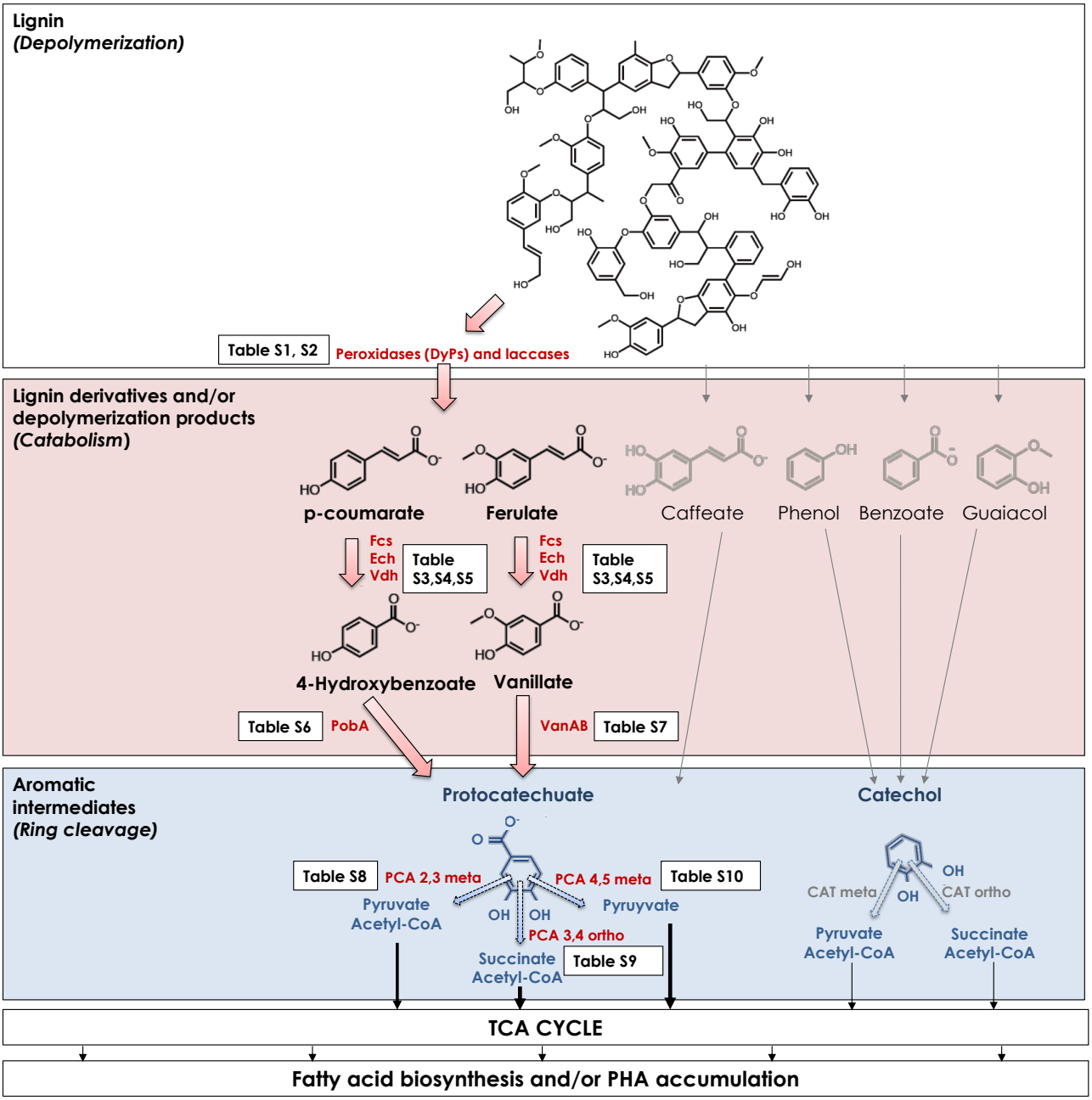

Fig. S5. Diagram of the biological process from lignin depolymerization to fatty acid/PHA production. Molecules found in APL and/or after the bacterial treatments in the current study are drawn in black. Potential enzymes involved in the conversion of those molecules are in red. Although protocatechuate has not been tracked in the present study, the enzymes involved in the ring cleavage are also highlighted (adapted from Johnson *et al.*, 2015[1]). A protein search in all the bacterial genomes has been also conducted for the enzymes, for which the results are detailed in the corresponding tables below (Table S2-S8). Enzymes: DyP = dye-decolorizing peroxidase; Fcs = Feruloyl-CoA synthase; Ech = p-hydroxycinnamoyl CoA hydratase/lyase; Vdh = vanillin dehydrogenase; PobA = 4-hydroxybenzoate-3-monooxygenase; VanAB = vanillate demethylase; PCA 2,3 meta = Protocatechuate 2,3-dioxygenase; PCA 3,4 ortho = protocatechuate 3,4-dioxygenase; ligAB = protocatechuate 4,5-dioxygenase.

Supplementary Tables

Table S1 (A-D): Putative dye-decolorizing peroxidases (DyP) that may be involved in lignin depolymerization

To search for ligninolytic peroxidases (dye decolorizing peroxidases (DyPs), manganese peroxidases (MnPs), and lignin peroxidases (LiPs)) in bacterial genomes, two different strategies were used:

- First, a search was done for each bacterial species in NCBI, using the key words: DyP, MnP, and LiP. No matches with MnP and LiP were found in any of the genomes. All discovered DyPs (**Table S1-A**) contained a conserved region for DyPs (GXXDG).
- Second, three characterized bacterial DyPs were selected to conduct a BLAST search with the genome of each species (with the specific strains or "taxid" detailed in "Bacteria" column; "taxids" were used when the genome of the exact strain was not available). Selected DyPs were (1) from *Amycolatopsis* sp. 75iv2[2] **(Table S1-B)**, (2) from *Pseudomonas putida*[3] **(Table S1-C)**, and (3) from *Bacillus subtilis*[3] **(Table S1-D)**. These enzymes were selected as reference because their previous characterizations revealed typical features of DyPs. Data included in the table correspond to the proteins with higher homologies to the reference protein. We also note that *P. putida* mt-2 contains one plasmid that is absent in *P. putida* KT2440, and as such, every protein in the latter will be also in the former.

Results. Potential DyPs have been found in the genome of all the bacterial strains studied here excluding in *B. megaterium*. As seen in the tables below, DyP2 from *Amycolatopsis* sp. 75iv2 is the most different (low homologies with DyPs from other bacteria) as was explained previously based on the DyP phylogenetic tree presented by Brown et al.[2] Compared to our enzyme assays, there are some bacteria which present DyP in the genome but did not show activity (especially those bacteria with very poor growth such as *C. freundii*, *A. vinelandii*, and *Paenibacillus* sp. (Fig. S3)).

Regarding other peroxidases, there are some reports about LiPs from *Streptomyces viridosporus* T7A,[4] but there is not characterization with further annotation.

Table S1-A: Putative DyPs found in the genome of the 14 screened bacteria by direct NCBI searches. Proteins with a "Query" lower than 70% are highlighted in red to denote lower homology to the reference protein.

Table S1-A Bacteria	Search of peroxidases in NCBI	#Accession
P. putida KT2440	Dyp-type peroxidase [*Pseudomonas putida* KT2440]	NP_745391.1
P. putida mt-2	Dyp-type peroxidase [*Pseudomonas putida* KT2440]	NP_745391.1
P. fluorescens pf-5	Dyp-type peroxidase family protein [*Pseudomonas protegens* Pf-5]	AAY92971.1
	Dyp-type peroxidase family protein [*Pseudomonas protegens* Pf-5]	AAY93000.2
C. necator H16	Predicted iron-dependent peroxidase [*Ralstonia eutropha* H16]	CAJ95737.1
A. vinelandii	Dyp-type peroxidase protein [*Azotobacter vinelandii* DJ]	ACO76274.1
	Peroxidase [*Azotobacter vinelandii*]	WP_012698702.1
Acinetobacter sp. ADP1	Conserved hypothetical protein; putative dyp-type peroxidase [*Acinetobacter* sp. ADP1]	CAG67144.1
C. freundii	Peroxidase [*Citrobacter freundii*]	KJC07926.1
E. lignolyticus SCF1	Dyp-type peroxidase family [*Enterobacter lignolyticus* SCF1]	ADO47592.1
	Dyp-type peroxidase family [*Enterobacter lignolyticus* SCF1]	ADO49007.1
R. jostii RHA1	Chain A, *Rhodococcus jostii* Rha1 Dypb R244l Variant In Complex With Heme	3VEG_A
R. erythropolis	Peroxidase [*Rhodococcus erythropolis*]	WP_029256131.1
	Peroxidase [*Rhodococcus erythropolis*]	WP_029255867.1
	Peroxidase [*Rhodococcus erythropolis*]	WP_029254613.1
Amycolatopsis sp. 75iv2	Chain A, Dyp2 From *Amycolatopsis* sp. ATCC 39116	4G2C_A
	Peroxidase [*Amycolatopsis* sp. ATCC 39116]	WP_020419401.1
	Peroxidase [*Amycolatopsis* sp. ATCC 39116]	WP_039791543.1
	Peroxidase [*Amycolatopsis* sp. ATCC 39116]	WP_020421762.1
B. subtilis	Dyp-type peroxidase family protein [*Bacillus subtilis*]	KFK78021.1
B. megaterium	--	
Paenibacillus sp.	Deferrochelatase [*Paenibacillus polymyxa* E681]	ADM71569.1
	Dyp-type peroxidase family [*Paenibacillus* sp. JDR-2]	ACS99675.1

Table S1-B: Putative DyPs in the genomes of the 14 screened bacteria from homology to *Amycolatopsis sp. 75iv2* DyP. Proteins with a "Query" lower than 70% are highlighted in red to denote lower homology to the reference protein.

Table S1-B	Blast against DyP from *Amycolatopsis* sp. 75iv2 Sequence ID: ref|WP_020421762.1|			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	---	--	--	--
P. putida mt-2 (plasmid)	---	--	--	--
P. fluorescens pf-5	Heme-containing peroxidase/deferrochelatase EfeB [*Pseudomonas protegens* Pf-5]	24	27	AAY92526.1
C. necator H16	---	--	--	--
A. vinelandii(taxid:354)	---	--	--	--
Acinetobacter sp. ADP1	---	--	--	--
C. freundii(taxid:546)	Peroxidase [*Citrobacter freundii*]	23	28	WP_038639565.1
E. lignolyticus SCF1	Dyp-type peroxidase family [*Enterobacter lignolyticus* SCF1]	23	28	ADO49007.1
R. jostii RHA1	--	--	--	--
R. erythropolis(taxid:1833)	--	--	--	--
Amycolatopsis sp. 75iv2	Peroxidase [*Amycolatopsis* sp. ATCC 39116]	100	100	WP_020421762.1
B. subtilis (taxid:1423)	--	--	--	--
B. megaterium (taxid:1404)	--	--	--	--
Paenibacillus (taxid:44249)	Hypothetical protein [*Paenibacillus* sp. FSL R5-192]	95	26	WP_036673677.1

Table S1-C: Putative DyPs in the genomes of the 14 screened bacteria from homology with *P. putida* DyP. Proteins with a "Query" lower than 70% are highlighted in red to denote lower homology to the reference protein.

Table S1-C	Blast against DyP from *P. putida* Sequence ID: ref|NP_745391.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	Dyp-type peroxidase [*Pseudomonas putida* KT2440]	100	100	NP_745391.1
P. putida mt-2 (plasmid)	Dyp-type peroxidase [*Pseudomonas putida* KT2440]	100*	100*	NP_745391.1
P. fluorescens pf-5	Dyp-type peroxidase family protein [*Pseudomonas protegens* Pf-5]	98	61	AAY93000.2
	Dyp-type peroxidase family protein [*Pseudomonas protegens* Pf-5]	71	32	AAY92971.1
C. necator H16	Predicted iron-dependent peroxidase [*Ralstonia eutropha* H16]	62	30	CAJ95737.1
A. vinelandii	Peroxidase [*Azotobacter vinelandii*]	98	59	WP_012698702.1
Acinetobacter sp. ADP1	Conserved hypothetical protein; putative dyp-type peroxidase [*Acinetobacter* sp. ADP1]	91	32	CAG67144.1
C. freundii	Dyp-type peroxidase family protein [*Citrobacter freundii*]	79	31	WP_003847283.1
E. lignolyticus SCF1	Dyp-type peroxidase family [*Enterobacter lignolyticus* SCF1]	79	31	ADO47592.1
R. jostii RHA1	Chain A, Dypb From *Rhodococcus jostii* Rha1, Crystal Form 1 [*Rhodococcus jostii* RHA1]	72	32	3QNR_A
R. erythropolis(taxid:1833)	Peroxidase [*Rhodococcus erythropolis*]	92	29	WP_042953479.1
Amycolatopsis sp. 75iv2	Hypothetical protein [*Amycolatopsis* sp. ATCC 39116]	84	24	WP_020420726.1
B. subtilis (taxid:1423)	Iron-dependent peroxidase convert ferric iron into ferrous iron [*Bacillus subtilis* Miyagi-4]	56	24	GAK81147.1
B. megaterium (taxid:1404)	--	--	--	--
Paenibacillus sp. (taxid:44249)	--	--	--	--

Table S1-D: Putative DyPs in the genomes of the 14 screened bacteria from homology with *B. subtilis* DyP. Proteins with a "Query" lower than 70% are highlighted in red to denote lower homology to the reference protein.

Table S1-D	Blast against DyP from *B. subtilis* Sequence ID: ref|NP_391705.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	Dyp-type peroxidase [*Pseudomonas putida* KT2440]	44	24	NP_745391.1
P. putida mt-2 (plasmid)	Dyp-type peroxidase [*Pseudomonas putida* KT2440]	44	24	NP_745391.1
P. fluorescens pf-5	Heme-containing peroxidase/deferrochelatase EfeB [Pseudomonas protegens Pf-5]	99	33	AAY92526.1
	Dyp-type peroxidase family protein [*Pseudomonas protegens* Pf-5]	52	23	AAY93000.2
C. necator H16	Predicted iron-dependent peroxidase [*Ralstonia eutropha* H16]	63	21	CAJ95737.1
A. vinelandii	Peroxidase [*Azotobacter vinelandii*]	40	26	WP_012698702.1
Acinetobacter sp. ADP1	Conserved hypothetical protein; putative dyp-type peroxidase [*Acinetobacter* sp. ADP1]	47	25	CAG67144.1
	Conserved hypothetical protein; putative iron-dependent peroxidase [*Acinetobacter* sp. ADP1]	61	21	CAG68259.1
C. freundii	Peroxidase [*Citrobacter freundii*]	90	39	WP_003832075.1
E. lignolyticus SCF1	Dyp-type peroxidase family [*Enterobacter lignolyticus* SCF1]	97	38	ADO49007.1
	Dyp-type peroxidase family [*Enterobacter lignolyticus* SCF1]	40	25	ADO47592.1
R. jostii RHA1	Conserved hypothetical protein [*Rhodococcus jostii* RHA1]	89	44	ABG97551.1
R. erythropolis(taxid:1833)	Peroxidase [*Rhodococcus erythropolis*]	89	46	WP_003946328.1
	Putative peroxidase [*Rhodococcus erythropolis*]	85	47	WP_020908546.1
Amycolatopsis sp. 75iv2	Peroxidase [*Amycolatopsis* sp. ATCC 39116]	88	45	WP_020419401.1
B. subtilis (taxid:1423)	Deferrochelatase/peroxidase EfeN [*Bacillus subtilis* subsp. subtilis str. 168]	100	100	NP_391705.1
B. megaterium (taxid:1404)	---			
Paenibacillus sp. (taxid:44249)	MULTISPECIES: deferrochelatase [*Paenibacillus*]	99	48	WP_024629905.1

Table S2 (A-B): Putative laccases that might be involved in lignin depolymerization.
Similar to the DyPs above, two strategies were used to search for laccases in the bacterial genomes:

- First, a search was conducted for each bacterial species in NCBI, using as key words "laccase" and "multi-copper polyphenol oxidoreductase". All discovered laccases contained a conserved domain for multi-copper polyphenol oxidoreductases/laccases (**Table S2-A**).
- Second, a characterized laccase from *Bacillus subtilis*[5] was selected to do a protein BLAST with the genome of each species (with the specific strains or "taxid" detailed in "Bacteria" column. "Taxids" were used when the genome of the exact strain was not available). This enzyme was selected as reference because its previous characterization revealed typical features of laccases.[5] Data included in the table correspond to proteins with high homologies to the reference protein. We note that *P. putida* mt-2 contains one plasmid which is absent in *P. putida* KT2440, thus, every protein in the latter will be also in the former.

Results. All 14 bacteria possess at least one putative enzyme with high homology to laccases. Considering these results and the previous detailed for DyPs, it is likely that the screened bacteria could all depolymerize lignin, but this will certainly also depend on enzyme expression and secretion. The laccase from *B. subtilis*, CotA, has a relative homology with some small laccases from *Streptomyces*, the latter of which have been related with lignin degradation[6] (CotA → homology with small laccase from *Streptomyces ipomoea*, ID: ABH10611.1, Query= 75, Identity= 34%).

Table S2-A: Putative laccases found in the genome of the 14 screened bacteria by direct NCBI searches. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S2-A	**Search of laccases in NCBI**		
Bacteria		**Accession number**	**#Aminoacids**
P. putida KT2440	Hypothetical protein PP_0624 [*Pseudomonas putida* KT2440]	NP_742785.1	246
P. putida mt-2	Hypothetical protein PP_0624 [*Pseudomonas putida* KT2440]	NP_742785.1	246
P. fluorescens Pf-5	Conserved hypothetical protein [*Pseudomonas protegens* Pf-5]	AAY94409.2	238
C. necator H16	Uncharacterized conserved protein [*Ralstonia eutropha* H16]	CAJ92570.1	267
A. vinelandii	Laccase [*Azotobacter vinelandii*]	WP_012699843.1	242
Acinetobacter sp. ADP1	Conserved hypothetical protein [*Acinetobacter* sp. ADP1]	CAG69618.1	246
C. freundii	-------	----	----
E. lignolyticus SCF1	Protein of unknown function DUF152 [*Enterobacter lignolyticus* SCF1]	ADO48396.1	244
R. jostii RHA1	Conserved hypothetical protein [*Rhodococcus jostii* RHA1]	ABG92911.1	247
R. erythropolis	Laccase [*Rhodococcus erythropolis*]	WP_029255552.1	250
Amycolatopsis sp.ATCC39116	Laccase [*Amycolatopsis* sp. ATCC 39116]	WP_027936637.1	233
B. subtilis (taxid:1423)	Laccase [*Bacillus subtilis*]	AIW29756.1	278
B. megaterium (taxid:1404)	Laccase [*Bacillus megaterium*]	WP_016765444.1	273
Paenibacillus sp. (taxid:44249)	Multicopper polyphenol oxidase [*Paenibacillus* sp. 1-18]	WP_025715629.1	294

Table S2-B: Putative laccases found in the genome of the 14 screened bacteria by homology BLAST with *B. subtilis* laccase. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S2-B	**Blast against laccase from *B. subtilis* Sequence ID: ref| AID81987.1**				
Bacteria	**Name**	**Query**	**Identity**	**# Accession**	**# Aminoacids**
P. putida KT2440	Hypothetical protein PP_3184 [*Pseudomonas putida* KT2440].	89	22	NP_745328.1	1131
	Multicopper oxidase [*Pseudomonas putida* KT2440]	90	22	NP_743195.1	468
P. putida mt-2	Hypothetical protein PP_3184 [*Pseudomonas putida* KT2440].	89	22	NP_745328.1	1131
	Multicopper oxidase [*Pseudomonas putida* KT2440]	90	22	NP_743195.1	468
P. fluorescens Pf-5	Multicopper oxidase, CumA [*Pseudomonas protegens* Pf-5]	90	22	AAY94158.1	458
	Copper resistance protein A [*Pseudomonas protegens* Pf-5]	72	25	AAY92165.1	579
C. necator H16	Copper resistance protein A, multi-copper oxidase [*Ralstonia eutropha* H16]	53	21	CAJ96967.1	614
A. vinelandii	Copper oxidase [*Azotobacter vinelandii*]	90	23	WP_012702483.1	456
	Copper oxidase [*Azotobacter vinelandii*]	69	23	WP_012698721.1	621
Acinetobacter sp. ADP1	Copper resistance protein A precursor [*Acinetobacter* sp. ADP1]	77	22	CAG67602.1	586
C. freundii	Multicopper oxidase [*Citrobacter freundii*]	96	25	WP_032942382.1	538
E. lignolyticus SCF1	Multicopper oxidase type 3 [*Enterobacter lignolyticus* SCF1]	88	26	ADO49844.1	526
	Multicopper oxidase type 3 [*Enterobacter lignolyticus* SCF1]	60	28	ADO47010.1	470
R. jostii RHA1	Possible oxidase [*Rhodococcus jostii* RHA1]	92	35	ABG96093.1	597
	Possible phenoxazinone synthase [*Rhodococcus jostii* RHA1]	97	34	ABG93393.1	851
	Multicopper oxidase [*Rhodococcus jostii* RHA1]	93	24	ABG94182.1	494
R. erythropolis	Copper oxidase [*Rhodococcus erythropolis*]	98	33	WP_042447759.1	603
	Copper oxidase [*Rhodococcus erythropolis*]	91	28	WP_003942354.1	512
Amycolatopsis sp.ATCC39116	Copper oxidase [*Amycolatopsis* sp. ATCC 39116]	92	37	WP_020417527.1	486
	Multicopper oxidase [*Amycolatopsis* sp. ATCC 39116]	79	36	WP_027936097.1	573
	Chain A, Small Laccase From *Amycolatopsis* sp. ATCC 39116 [*Amycolatopsis* sp. ATCC 39116]	22	28	3T9W_A	299
B. subtilis (taxid:1423)	Spore coat protein A [*Bacillus subtilis* XF-1]	100	100	AGE62493.1	515
B. megaterium (taxid:1404)	Copper oxidase [*Bacillus megaterium*]	99	58	WP_026682197.1	505
	Multicopper oxidase [*Bacillus megaterium*]	93	24	WP_026682364.1	512
Paenibacillus sp.(taxid:44249)	Spore coat protein A [*Paenibacillus* sp. P22]	100	52	CDN43325.1	524

Table S3, S4, S5. Enzymes involved in the conversion of p-coumarate and ferulate to 4-hydroxybenzoate and vanillate respectively.

Feruloyl-CoA synthases (Fcs), p-hydroxycinnamoyl CoA hydratase/lyases (Ech), and vanillin dehydrogenases (Vdh) are the three enzymes necessary to convert both p-coumarate and ferulate to 4-hydroxybenzoate and vanillate, respectively (Fig. S5). To search for these enzymes in bacterial genomes, Fcs, Ech, and Vdh from *P. putida* KT2440[7, 8] were selected to conduct a protein BLAST against the genome of each bacterial species (with the specific strains or "taxid" detailed in "Bacteria" column. "Taxids" were used when the genome of the exact strain was not available). Data included in the table correspond to those proteins with higher homologies to the reference protein. We also note that *P. putida* mt-2 contains one plasmid which is absent in *P. putida* KT2440, thus, every protein in the latter will be also in the former.

Results and discussion. Fcs, Ech, and Vdh were selected from *P. putida* KT2440 since their function have been physiologically confirmed[8]. Considering the data presented in Table 2 in the main manuscript, all bacteria were able to convert ferulate at least in one of the culture conditions, excluding *Paenibacillus* sp. However, there were more cases where bacteria were not able to catabolize p-coumarate. Ech and Vdh have been found with high homologies in all bacteria (Queries > 78%) (Table S3 and S4). However, Fcs presented cases where "Queries" were as low as 56% (Table S3). As an example, we highlight *P. fluorescens* and *E. lignolyticus* data and compare them with Table 2. The lowest queries are correlated with the incapacity of catabolizing p-coumarate (but not ferulate). This result also suggests that Fcs could be more efficient converting ferulate than p-coumarate.

Table S3: Putative feruloyl-CoA synthases (Fcs) in the genome of the 14 screened bacteria. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein. Bacteria in blue and red also correspond to those cases where bacteria did not catabolize ferulate (according to Table 2, main manuscript).

Table S3	Blast against Feruloyl-CoA synthase (Fcs) from *P. putida* KT2440 Sequence ID: ref| NP_745496.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	Feruloyl-CoA synthase [*Pseudomonas putida* KT2440]	100	100	NP_745496.1
P. putida mt-2	Feruloyl-CoA synthase [*Pseudomonas putida* KT2440]	100	100	NP_745496.1
P. fluorescens Pf-5	Long-chain-fatty-acid--CoA ligase [*Pseudomonas protegens* Pf-5]	56	29	AAY93846.1
C. necator H16	Long-chain-fatty-acid-CoA ligase [*Ralstonia eutropha* H16]	64	24	CAJ94362.1
	4-Coumarate-CoA ligase [*Ralstonia eutropha* H16]	78	25	CAJ95511.1
A. vinelandii	Feruloyl-CoA synthase [*Azotobacter vinelandii*]	98	69	WP_012700060.1
Acinetobacter sp.ADP1	Coenzyme A ligase [*Acinetobacter* sp. ADP1]	99	52	CAG68566.1
C. freundii	Long-chain fatty acid--CoA ligase [*Citrobacter freundii*]	63	24	WP_032943755.1
E. lignolyticus SCF1	AMP-dependent synthetase and ligase [*Enterobacter lignolyticus* SCF1]	60	23	ADO48242.1
R. jostii RHA1	Probable long-chain-fatty-acid--CoA ligase [*Rhodococcus jostii* RHA1]	97	25	ABG95547.1
R. erythropolis	Hypothetical protein [*Rhodococcus erythropolis*]	79	36	WP_019749606.1
Amycolatopsis sp. ATCC39116	AMP-binding protein [*Amycolatopsis* sp. ATCC 39116]	96	24	WP_020416350.1
B. subtilis (taxid:1423)	Long-chain fatty acid--CoA ligase [*Bacillus subtilis*]	78	25	KFF57369.1
B. megaterium (taxid:1404)	Long-chain fatty acid--CoA ligase [*Bacillus megaterium*]	76	25	WP_014461652.1
Paenibacillus sp. (taxid:44249)	MULTISPECIES: AMP-dependent synthetase/ligase [*Paenibacillus*]	74	24	WP_021252282.1

Table S4: Putative p-hydroxycinnamoyl CoA hydratase/lyases (Ech) in the genome of the 14 screened bacteria. Bacteria in blue correspond to those cases where bacteria did not catabolize ferulate (according to Table 2, main manuscript).

Table S4	Blast against p-hydroxycinnamoyl CoA hydratase/lyase (Ech) from *P. putida* KT2440 Sequence ID: ref| NP_745498.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	p-hydroxycinnamoyl CoA hydratase/lyase [*Pseudomonas putida* KT2440]	100	100	NP_745498.1
P. putida mt-2	p-hydroxycinnamoyl CoA hydratase/lyase [*Pseudomonas putida* KT2440]	100	100	NP_745498.1
P. fluorescens Pf-5	Putative 3-methylglutaconyl-CoA hydratase LiuC [*Pseudomonas protegens* Pf-5]	73	32	AAY93202.1
	Enoyl-CoA hydratase/isomerase family protein [*Pseudomonas protegens* Pf-5]	86	29	AAY92536.1
C. necator H16	Enoyl-CoA hydratase/carnithine racemase [*Ralstonia eutropha* H16]	81	31	CAJ92012.1
A. vinelandii	Crotonase [*Azotobacter vinelandii*]	100	88	WP_012700058.1
Acinetobacter sp.ADP1	4-hydroxycinnamoyl CoA hydratase/lyase [*Acinetobacter* sp. ADP1]	98	79	AAP78947.1
C. freundii	2,3-dehydroadipyl-CoA hydratase [*Citrobacter freundii*]	89	31	WP_044711773.1
E. lignolyticus SCF1	Enoyl-CoA hydratase/isomerase [*Enterobacter lignolyticus* SCF1]	90	32	ADO48668.1
R. jostii RHA1	Probable enoyl-CoA hydratase [*Rhodococcus jostii* RHA1]	93	30	ABG93351.1
R. erythropolis	Dihydroxynaphthoic acid synthetase [*Rhodococcus erythropolis*]	87	33	WP_042446607.1
	Enoyl-CoA hydratase [*Rhodococcus erythropolis*]	82	33	WP_042447410.1
Amycolatopsis sp. ATCC39116	Crotonase [*Amycolatopsis* sp. ATCC 39116]	96	65	WP_020422605.1
B. subtilis (taxid:1423)	Dihydroxynaphthoic acid synthetase [*Bacillus subtilis*]	80	34	KFK82123.1
	Enoyl-CoA hydratase [*Bacillus subtilis*]	73	33	WP_003238132.1
B. megaterium (taxid:1404)	Dihydroxynaphthoic acid synthetase [*Bacillus megaterium*]	74	37	WP_026681510.1
	Enoyl-CoA hydratase [*Bacillus megaterium*]	78	32	WP_034654455.1
Paenibacillus sp. (taxid:44249)	MULTISPECIES: enoyl-CoA hydratase [*Paenibacillus*]	85	29	WP_028531354.1

Table S5: Putative vanillin dehydrogenases (Vdh) in the genome of the 14 screened bacteria. Bacteria in blue correspond to those cases where bacteria did not catabolize ferulate (according to Table 2, main manuscript).

Table S5	Blast against Vanillin dehydrogenase (Vdh) from *P. putida* KT2440 Sequence ID: ref | NP_745497.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	Vanillin dehydrogenase [*Pseudomonas putida* KT2440]	100	100	NP_745497.1
P. putida mt-2	Vanillin dehydrogenase [*Pseudomonas putida* KT2440]	100	100	NP_745497.1
P. fluorescens Pf-5	Benzaldehyde dehydrogenase [*Pseudomonas protegens* Pf-5]	98	40	AAY92742.1
C. necator H16	NAD-dependent aldehyde dehydrogenase [*Ralstonia eutropha* H16]	100	60	CAJ92255.1
A. vinelandii	Salicylaldehyde dehydrogenase [*Azotobacter vinelandii*]	100	81	WP_012700059.1
*Acinetobacter sp.*ADP1	Vanillin dehydrogenase [*Acinetobacter* sp. ADP1]	100	63	AAP78946.1
C. freundii	Succinate-semialdehyde dehydrogenase [*Citrobacter freundii*]	96	33	WP_038632937.1
E. lignolyticus SCF1	Succinic semialdehyde dehydrogenase [*Enterobacter lignolyticus* SCF1]	96	35	ADO49058.1
R. jostii RHA1	Benzaldehyde dehydrogenase [*Rhodococcus jostii* RHA1]	96	38	ABG94789.1
R. erythropolis	Salicylaldehyde dehydrogenase [*Rhodococcus erythropolis*]	98	54	WP_029256919.1
Amycolatopsis sp. ATCC39116	Hypothetical protein [*Amycolatopsis* sp. ATCC 39116]	96	46	WP_020419187.1
B. subtilis (taxid:1423)	Aldehyde dehydrogenase [*Bacillus subtilis*]	98	35	WP_044444941.1
B. megaterium (taxid:1404)	Aldehyde dehydrogenase [*Bacillus megaterium*]	98	35	WP_034678430.1
Paenibacillus sp. (taxid:44249)	MULTISPECIES: aldehyde dehydrogenase [*Paenibacillus*]	98	38	WP_024630322.1

Table S6: Enzyme involved in the catabolism of 4-hydroxybenzoate to protocatechuate

4-hydroxybenzoate-3-monooxygenase (PobA) converts 4-hydroxybenzoate to protocatechuate (Fig. S5). To search for this enzyme in bacterial genomes, PobA from *P. putida* KT2440[7] was selected to perform a protein BLAST with the genome of each species (specific strains or "taxid" detailed in "Bacteria" column (Table S6). "Taxids" were used when the genome of the exact strain was not available). Data included in the table correspond to proteins with higher homology to the reference protein. We also note that *P. putida* mt-2 contains one plasmid that is absent in *P. putida* KT2440, thus, every protein in the latter will be also in the former.

Results. PobA is involved in the conversion of 4-hydroxybenzoate to protocatechuate.[7] The lack of this enzyme would produce an accumulation of 4-hydroxybenzoate, especially if the bacterium is able to catabolize p-coumarate. Table S6 shows only one case with low homology for PobA, *E. lignolyticus*, but this bacterium is also able to consume 4-hydroxybenzoate (~60%, Table 2), which may be a result of the protein in Table S6 or low residual activity from another monooxygenase in the genome. Comparing the data in Table S6 and Table 2, it seems that all of the studied strains exhibit this enzyme activity, albeit at different levels.

Table S6: Putative enzyme 4-hydroxybenzoate-3-monooxygenases (PobA) in the genome of the 14 screened bacteria. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S6	Blast against 4-hydroxybenzoate-3-monooxygenase (PobA) from *P. putida* KT2440 Sequence ID: ref | NP_745674.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	4-hydroxybenzoate 3-monooxygenase [*Pseudomonas putida* KT2440]	100	100	NP_745674.1
P. putida mt-2	4-hydroxybenzoate 3-monooxygenase [*Pseudomonas putida* KT2440]	100	100	NP_745674.1
P. fluorescens Pf-5	4-hydroxybenzoate 3-monooxygenase [*Pseudomonas protegens* Pf-5]	100	85	AAY94390.1
C. necator H16	p-Hydroxybenzoate hydroxylase, FAD dependent monooxygenase [*Ralstonia eutropha* H16]	98	61	CAJ97068.1
A. vinelandii	4-hydroxybenzoate 3-monooxygenase [*Azotobacter vinelandii*]	100	79	WP_012700074.1
Acinetobacter sp.ADP1	RecName: Full=p-hydroxybenzoate hydroxylase; AltName: Full=4-hydroxybenzoate 3-monooxygenase [*Acinetobacter* sp. ADP1]	99	60	Q03298.1
C. freundii	3-(3-hydroxyphenyl)propionate hydroxylase [*Citrobacter freundii*]	82	20	WP_003847656.1
E. lignolyticus SCF1	2-polyprenyl-6-methoxyphenol 4-hydroxylase [Enterobacter lignolyticus SCF1]	24	34	ADO47119.1
R. jostii RHA1	4-hydroxybenzoate 3-monooxygenase [*Rhodococcus jostii* RHA1]	98	46	ABG94344.1
R. erythropolis	4-hydroxybenzoate 3-monooxygenase [*Rhodococcus erythropolis*]	98	46	WP_003943079.1
Amycolatopsis sp. ATCC39116	4-hydroxybenzoate 3-monooxygenase [*Amycolatopsis* sp. ATCC 39116]	98	52	WP_020418261.1
B. subtilis (taxid:1423)	Hypothetical protein [*Bacillus subtilis*]	78	27	WP_038430003.1
B. megaterium (taxid:1404)	FAD-binding protein [*Bacillus megaterium*]	81	26	WP_014460247.1
Paenibacillus sp. (taxid:44249)	4-hydroxybenzoate 3-hydroxylase [*Paenibacillus* sp. JJ-1b]*	99	53	BAH79107.1

Table S7: Enzymes involved in the conversion of ferulate to vanillate: Vanillate demethylase A, VanA, and Vanillate-O-demethylase oxidoreductase, VanB

VanAB, consisting of both a vanillate demethylase A (VanA) and Vanillate-O-demethylase oxidoreductase (Van B), is the enzyme pair necessary for ferulate metabolism to vanillate (Fig. S5). To search for this enzyme in bacterial genomes, VanA and VanB from *P. putida* KT2440[7] were selected to perform a protein BLAST with the genome of each species (specific strains or "taxid" detailed in "Bacteria" column (Table S7). "Taxids" were used when the genome of the exact strain was not available). Data included in the table correspond to the proteins with higher homologies to the reference protein. We also note that *P. putida* mt-2 contains one plasmid which is absent in *P. putida* KT2440, thus, every protein in the latter will be also in the former.

Results and discussion: VanAB converts of ferulate into vanillate. Those cases where queries were lower than 70% (Table S7A-B) corresponded with those bacteria that accumulate vanillate (Table 2). The GCxGC/TOF-MS shown in Table 2 very closely reflect the data obtained in this genomic study. Some examples that show this clear correlation between Table S7 and Table 2 are:

- *C. necator* consumes ferulic but it is not able to consume vanillate (low homology with VanA). As a result, vanillate is accumulated (++).
- *A. vinelandii* does not have VanA and it does not consume ferulate efficiently. Thus, vanillate accumulation is lower (+).
- *R. erythropolis* consumes the ferulate, but does not have VanA, thus it accumulates vanillate.

Table S7-A: Putative vanillate demethylases A (VanA) in the genome of the 14 screened bacteria. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S7-A	Blast against Vanillate demethylase A (VanA) from *P. putida* KT2440 Sequence ID: ref|AAN69332.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	Rieske (2Fe-2S) domain-containing protein [*Pseudomonas putida* KT2440]	100	100	NP_745868.1
P. putida mt-2	Rieske (2Fe-2S) domain-containing protein [*Pseudomonas putida* KT2440]	100	100	NP_745868.1
P. fluorescens Pf-5	Vanillate O-demethylase, oxygenase subunit [*Pseudomonas protegens* Pf-5]	98	87	AAY92738.1
C. necator H16	Ring-hydroxylating dioxygenase [*Ralstonia eutropha* H16]	45	37	CAJ95527.1
A. vinelandii	Rieske (2Fe-2S) protein [*Azotobacter vinelandii*]	19	33	WP_012702752.1
Acinetobacter sp.ADP1	Vanillate O-demethylase oxygenase subunit (4-hydroxy-3-methoxybenzoate demethylase) [*Acinetobacter* sp. ADP1]	97	75	CAG67872.1
C. freundii	Dioxygenase [*Citrobacter freundii*]	28	32	WP_043016054.1
E. lignolyticus SCF1	Rieske (2Fe-2S) iron-sulfur domain protein [*Enterobacter lignolyticus* SCF1]	42	28	ADO49059.1
R. jostii RHA1	Possible vanillate monooxygenase oxygenase subunit [*Rhodococcus jostii* RHA1]	95	34	ABG95958.1
R. erythropolis	3-ketosteroid-9-alpha-hydroxylase [*Rhodococcus erythropolis*]	40	28	WP_042448430.1
Amycolatopsis sp. ATCC39116	Vanillate monooxygenase [*Amycolatopsis* sp. ATCC 39116]	95	34	WP_020422153.1
B. subtilis (taxid:1423)	3-chlorobenzoate-3,4-dioxygenase [*Synechocystis* sp. PCC 6803] [Bacillus subtilis BEST7613]	95	25	BAM55063.1
B. megaterium (taxid:1404)	Rieske (2Fe-2S) domain-containing protein [*Bacillus megaterium*]	92	23	WP_013058614.1
Paenibacillus sp. (taxid:44249)	Rieske (2Fe-2S) protein [*Paenibacillus* sp. 1-18]	92	27	WP_025717394.1

Table S7-B: Putative vanillate-O-demethylase oxidoreductases (Van B) in the genome of the 14 screened bacteria. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S7-B	Blast against Vanillate O demethylase oxidoreductase (VanB) from *P .putida* KT2440 Sequence ID: ref| AAN69333.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	Ferredoxin [*Pseudomonas putida* KT2440]	100	100	NP_745869.1
P. putida mt-2	Ferredoxin [*Pseudomonas putida* KT2440]	100	100	NP_745869.1
P. fluorescens Pf-5	Vanillate O-demethylase oxidoreductase [*Pseudomonas protegens* Pf-5]	100	74	AAY92739.1
C. necator H16	Vanillate O-demethylase oxidoreductase [*Ralstonia eutropha* H16]	96	41	CAJ96917.1
A. vinelandii	NADH oxidase [*Azotobacter vinelandii*]	78	28	WP_012699529.1
Acinetobacter sp. ADP1	RecName: Full=Vanillate O-demethylase oxidoreductase; AltName: Full=Vanillate degradation ferredoxin-like protein [*Acinetobacter* sp. ADP1]	99	51	O24840.1
C. freundii	Dioxygenase [*Citrobacter freundii*]	94	39	WP_032943752.1
E. lignolyticus SCF1	Ferredoxin [*Enterobacter lignolyticus* SCF1]	100	37	ADO49057.1
R. jostii RHA1	Probable vanillate O-demethylase oxidoreductase [*Rhodococcus jostii* RHA1]	100	42	ABG94650.1
R. erythropolis	Vanillate O-demethylase oxidoreductase [*Rhodococcus erythropolis*]	99	47	WP_029256917.1
Amycolatopsis sp. ATCC39116	Ferredoxin [*Amycolatopsis* sp. ATCC 39116]	98	46	WP_027936050.1
B. subtilis (taxid:1423)	Dihydropteridine reductase [*Bacillus subtilis*]	67	28	AIW34871.1
B. megaterium (taxid:1404)	Dihydropteridine reductase [*Bacillus megaterium*]	67	28	WP_013081515.1
Paenibacillus sp. (taxid:44249)	Dihydropteridine reductase [*Paenibacillus* sp. 1-49]	68	32	WP_025684658.1

Table S8, S9, S10. Enzymes involved in the ring cleavage of protocatechuate.

Protocatechuate 2,3-dioxygenase, protocatechuate 3,4-dioxygenase (alpha and beta) and, protocatechuate 4,5-dioxygenase (ligAB) are the enzymes involved in the ring cleavage of protocatechuate (Fig. S5). To search for protocatechuate 2,3-dioxygenases, protocatechuate 3,4-dioxygenases (alpha and beta), and ligAB, the bacteria *Paenibacillus* sp. JJ-1b[9], *P. putida* KT2440[1], and *Sphyngobium* SYK-6 [10] (the only ligAB structurally characterized) were selected, respectively, to perform a protein BLAST with the genome of each species (specific strains or "taxid" detailed in "Bacteria" column (Table S8,9,10). "Taxids" were used when the genome of the exact strain was not available). Data included in the table correspond to those proteins with higher homologies to the reference protein. We note that *P. putida* mt-2 contains one plasmid which is absent in *P. putida* KT2440, thus, every protein in the latter will be also in the former.

Results. There are only 2 organisms which present very low homologies to the 3 of the enzymes involved in the ring cleavage of protocatechuate, *B. subtillis* and *B. megaterium*. This would mean, at genomic level, that although these organisms are able to initiate the metabolism of p-coumarate (via 4-hydroxybenzoate) to protocatechuate (but not vanillate to protocatechuate), the bacteria would not be able to further use the protocatechuate as a carbon source. Table 2 also shows how these organisms are more efficient catabolizing p-coumarate and ferulate than 4-hydroxybenzoate and that vanillate is accumulated in most cases.

Table S8: Putative protocatechuate 2,3-dioxygenases in the genome of the 14 screened bacteria. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S8	**Blast against protocatechuate 2,3-dioxygenase from *Paenibacillus* sp. JJ-1b Sequence ID: ref| BAH79099.1**			
Bacteria	**Name**	**Query**	**Identity**	**# Accession**
P. putida KT2440	Hypothetical protein PP_1869 [*Pseudomonas putida* KT2440]	39	29	NP_744024.1
P. putida mt-2	Hypothetical protein PP_1869 [*Pseudomonas putida* KT2440]	39	29	NP_744024.1
P. fluorescens Pf-5	3,4-dihydroxyphenylacetate 2,3-dioxygenase [*Pseudomonas protegens* Pf-5]	88	27	AAY92642.1
C. necator H16	Catalytic subunit of aromatic ring-opening dioxygenase [*Ralstonia eutropha* H16]	36	31	CAJ94613.1
A. vinelandii	Aromatic ring-cleaving dioxygenase [*Azotobacter vinelandii*]	36	27	WP_012699800.1
Acinetobacter sp.ADP1	Conserved hypothetical protein; putative enzyme with aromatic-ring-opening dioxygenase domain [*Acinetobacter* sp. ADP1]	39	26	CAG67994.1
C. freundii	3,4-dihydroxyphenylacetate 2,3-dioxygenase [*Citrobacter freundii*]	89	26	KGZ30116.1
E. lignolyticus SCF1	3,4-dihydroxyphenylacetate 2,3-dioxygenase [*Enterobacter lignolyticus* SCF1]	89	26	ADO50039.1
R. jostii RHA1	conserved hypothetical protein [*Rhodococcus jostii* RHA1]	62	26	ABG92845.1
R. erythropolis	Extradiol ring-cleavage dioxygenase [*Rhodococcus erythropolis*]	77	20	WP_042445069.1
Amycolatopsis sp. ATCC39116	Extradiol ring-cleavage dioxygenase [*Amycolatopsis* sp. ATCC 39116]	88	21	WP_020418881.1
B. subtilis (taxid:1423)	XRE family transcriptional regulator [*Bacillus subtilis*]	30	23	WP_019257879.1
B. megaterium (taxid:1404)	Hypothetical protein [*Bacillus megaterium*]	61	20	WP_034652901.1
Paenibacillus sp. (taxid:44249)	Protocatechuate 2,3-dioxygenase [*Paenibacillus* sp. JJ-1b]	100	100	BAH79099.1

Table S9-A: Putative protocatechuate 3,4-dioxygenases (alpha) in the genome of the 14 screened bacteria. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S9-A	**Blast against alpha protocatechuate 3,4-dioxygenase from *P. putida* KT2440 Sequence ID: ref| NP_746764.1**			
Bacteria	**Name**	**Query**	**Identity**	**# Accession**
P. putida KT2440	Protocatechuate 3,4-dioxygenase subunit alpha [*Pseudomonas putida* KT2440]	100	100	NP_746764.1
P. putida mt-2	Protocatechuate 3,4-dioxygenase subunit alpha [*Pseudomonas putida* KT2440]	100	100	NP_746764.1
P. fluorescens Pf-5	Protocatechuate 3,4-dioxygenase, alpha subunit [*Pseudomonas protegens* Pf-5]	100	82	AAY94605.1
C. necator H16	Protocatechuate 3,4-dioxygenase alpha chain [*Ralstonia eutropha* H16]	98	43	CAJ97072.1
A. vinelandii	Protocatechuate 3,4-dioxygenase subunit alpha [*Azotobacter vinelandii*]	100	80	WP_012702342.1
Acinetobacter sp.ADP1	RecName: Full=Protocatechuate 3,4-dioxygenase alpha chain; AltName: Full=3,4-PCD [*Acinetobacter* sp. ADP1]	97	53	P20371.3
C. freundii	Lactate dehydrogenase [*Citrobacter freundii*]	25	35	WP_043016938.1
E. lignolyticus SCF1	Acyl-CoA dehydrogenase domain-containing protein [*Enterobacter lignolyticus* SCF1]	53	25	ADO50214.1
R. jostii RHA1	Protocatechuate dioxygenase alpha subunit [*Rhodococcus jostii* RHA1]	95	37	ABG93160.1
R. erythropolis	Protocatechuate 3,4-dioxygenase [*Rhodococcus erythropolis*]	96	37	WP_042446987.1
Amycolatopsis sp. ATCC39116	Protocatechuate 3,4-dioxygenase subunit alpha [*Amycolatopsis* sp. ATCC 39116]	95	42	WP_020421708.1
B. subtilis (taxid:1423)	Zinc protease [*Bacillus subtilis*]	23	33	WP_009967270.1
B. megaterium (taxid:1404)	MFS transporter [*Bacillus megaterium*]	51	30	WP_013057197.1
Paenibacillus sp. (taxid:44249)	Hypothetical protein [*Paenibacillus alginolyticus*]	90	27	WP_029198242.1

Table S9-B: Putative protocatechuate 3,4-dioxygenases (beta) in the genome of the 14 screened bacteria. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S9-B	Blast against beta protocatechuate 3,4-dioxygenase from *P. putida* KT2440 Sequence ID: ref| NP_746765.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	Protocatechuate 3,4-dioxygenase subunit beta [*Pseudomonas putida* KT2440]	100	100	*NP_746765.1*
P. putida mt-2	Protocatechuate 3,4-dioxygenase subunit beta [*Pseudomonas putida* KT2440]	100	100	*NP_746765.1*
P. fluorescens Pf-5	Protocatechuate 3,4-dioxygenase, beta subunit [*Pseudomonas protegens* Pf-5]	100	90	AAY94606.1
C. necator H16	Protocatechuate 3,4-dioxygenase beta chain [*Ralstonia eutropha* H16]	89	54	CAJ97073.1
A. vinelandii	Protocatechuate 3,4-dioxygenase subunit beta [*Azotobacter vinelandii*]	99	79	WP_012702343.1
Acinetobacter sp.ADP1	RecName: Full=Protocatechuate 3,4-dioxygenase beta chain; AltName: Full=3,4-PCD [*Acinetobacter* sp. ADP1]	96	59	P20372.2
C. freundii	Hypothetical protein [*Citrobacter freundii*]	39	29	WP_043018002.1
E. lignolyticus SCF1	Peptidase U62 modulator of DNA gyrase [*Enterobacter lignolyticus* SCF1]	22	32	ADO50168.1
R. jostii RHA1	Protocatechuate dioxygenase beta subunit [*Rhodococcus jostii* RHA1]	91	50	ABG93159.1
R. erythropolis	Protocatechuate 3,4-dioxygenase beta subunit [*Rhodococcus erythropolis*]	89	51	WP_020909279.1
Amycolatopsis sp. ATCC39116	Protocatechuate 3,4-dioxygenase subunit beta [*Amycolatopsis* sp. ATCC 39116]	92	48	WP_020421709.1
B. subtilis (taxid:1423)	MFS transporter [*Bacillus subtilis*]	21	31	WP_019713883.1
B. megaterium (taxid:1404)	Hypothetical protein [*Bacillus megaterium*]	79	27	WP_034649490.1
Paenibacillus sp.(taxid:44249)	Hypothetical protein [*Paenibacillus alginolyticus*]	44	33	WP_029198242.1

Table S10-A: Putative protocatechuate 4,5-dioxygenases (ligA) in the genome of the 14 screened bacteria. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S10-A	Blast against alpha ligA from *Sphingobium* sp. SKY-6 Sequence ID: ref| BAK65926.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	Protocatechuate 4,5-dioxygenase [*Pseudomonas putida* KT2440]*	84	26	NP_744666.2
P. putida mt-2	Protocatechuate 4,5-dioxygenase [*Pseudomonas putida* KT2440]*	84	26	NP_744666.2
P. fluorescens Pf-5	5-carboxymethyl-2-hydroxymuconate semialdehyde dehydrogenase [*Pseudomonas protegens* Pf-5]	26	41	AAY92641.1
C. necator H16	Transcriptional regulator, GntR-family [*Ralstonia eutropha* H16]	58	27	CAJ91461.1
A. vinelandii	Protocatechuate 4,5-dioxygenase subunit alpha [*Azotobacter vinelandii*]	83	30	WP_012701674.1
Acinetobacter sp.ADP1	Conserved hypothetical protein [*Acinetobacter* sp. ADP1]	45	29	CAG69838.1
C. freundii	Dehydrogenase [*Citrobacter freundii*]	54	27	WP_044712770.1
E. lignolyticus SCF1	2-deoxy-D-gluconate 3-dehydrogenase [*Enterobacter lignolyticus* SCF1]	46	28	ADO47157.1
R. jostii RHA1	Conserved hypothetical protein [*Rhodococcus jostii* RHA1]	55	28	ABG93280.1
R. erythropolis	LuxR family transcriptional regulator [*Rhodococcus erythropolis*]	49	25	WP_042453392.1
Amycolatopsis sp. ATCC39116	Protocatechuate 3,4-dioxygenase [*Amycolatopsis* sp. ATCC 39116]	69	48	WP_020423187.1
B. subtilis (taxid:1423)	Peptide synthetase [*Bacillus subtilis*]	47	24	WP_033881991.1
B. megaterium (taxid:1404)	Peptide ABC transporter ATP-binding protein [*Bacillus megaterium*]	46	25	WP_014461584.1
Paenibacillus sp. (taxid:44249)	MULTISPECIES: 3-ketoacyl-ACP reductase [*Paenibacillus*]	46	31	WP_024631398.1

* These enzymes are not correctly annotated. They correspond to gallate dioxygenase.[11]

Table S10-B: Putative protocatechuate 4,5-dioxygenases (lig B) in the genome of the 14 screened bacteria. Proteins with a "Query" lower than 70% are highlighted in red as means of lower homology to the reference protein.

Table S10-B	Blast against beta ligB *Sphingobium* sp. SYK-6 Sequence ID: ref| BAK65925.1			
Bacteria	Name	Query	Identity	# Accession
P. putida KT2440	Protocatechuate 4,5-dioxygenase [*Pseudomonas putida* KT2440]*	94*	39*	NP_744666.2
P. putida mt-2	Protocatechuate 4,5-dioxygenase [*Pseudomonas putida* KT2440]*	94*	39*	NP_744666.2
P. fluorescens Pf-5	Catalytic LigB subunit of aromatic ring-opening dioxygenase [*Pseudomonas protegens* Pf-5]	40	28	AAY93506.1
C. necator H16	Two domain protein: Adenylate cyclase, family protein [*Ralstonia eutropha* H16]	27	32	CAJ96942.1
A. vinelandii	Protocatechuate 4,5-dioxygenase subunit alpha [*Azotobacter vinelandii*]	92	41	WP_012701674.1
	Protocatechuate 4,5-dioxygenase subunit beta [*Azotobacter vinelandii*]	77	29	WP_012702716.1
Acinetobacter sp. ADP1	Putative Cyanophycinase (CphI) [*Acinetobacter* sp. ADP1]	15	31	CAG68153.1
C. freundii	3-(2,3-dihydroxyphenyl)propionate dioxygenase [*Citrobacter freundii*]	61	30	WP_003830921.1
E. lignolyticus SCF1	Cysteine synthase A [*Enterobacter lignolyticus* SCF1]	8	44	ADO47605.1
R. jostii RHA1	RecName: Full=2,3-dihydroxyphenylpropionate/2,3-dihydroxicinnamic acid 1,2-dioxygenase; AltName: Full=3-carboxyethylcatechol 2,3-dioxygenase [*Rhodococcus jostii* RHA1]	76	25	Q0SJD2.1
R. erythropolis	3-(2,3-dihydroxyphenyl)propionate dioxygenase [*Rhodococcus erythropolis*]	48	33	WP_042447421.1
Amycolatopsis sp. ATCC39116	Protocatechuate 3,4-dioxygenase [*Amycolatopsis* sp. ATCC 39116]	92	51	WP_039792090.1
B. subtilis (taxid:1423)	DNA polymerase I [*Bacillus subtilis*]	47	22	AIW30799.1
B. megaterium (taxid:1404)	Orotidine 5'-phosphate decarboxylase [*Bacillus megaterium*]	20	25	WP_026680829.1
Paenibacillus sp. (taxid:44249)	MFS transporter [*Paenibacillus alginolyticus*]	34	28	WP_029198730.1

* These enzymes are not correctly annotated. They correspond to gallate dioxygenase.[11]

General discussion from Figure S5 and Tables S2-S11

Lignin depolymerization and aromatic catabolism is driven by the action of highly specific enzymes (detailed in Fig. S5). In the current study, ferulate, p-coumarate, 4-hydroxybenzoate, and vanillate were the main aromatic acids detected and identified in APL before and after the bacterial treatments. These aromatics can come from the performed pretreatment but also from the depolymerization of lignin. We have demonstrated by genetic analysis how all the screened bacteria contain putative enzymes involved in lignin depolymerization (laccases and/or DyPs). However, through enzyme assays, we have not detected all of them in the bacterial supernatants (Fig. 5 and Fig. S4). That might happen due to (1) a lack of gene expression, (2) inefficiency during the secretion, or (3) low enzyme levels due to non-optimal assayed conditions or very low bacterial biomass (thus, producing less enzymes and being difficult to detect). To avoid the last issue, the most appropriate approach to ascertain if enzymes are in the supernatant or the mechanisms for their secretion, will be a proteomic approach. These analyses are being already done in our group with the selected bacteria, constituting a really high effort in terms of economy and time, due to the elevated amount of samples and information generated by these analyses as well as their novelty.

Regarding aromatics catabolism, feruloyl-CoA synthases (Fcs), p-hydroxycinnamoyl CoA hydratase/lyases (Ech), and vanillin dehydrogenases (Vdh) are the three enzymes necessary to convert both p-coumarate and ferulate in 4-hydroxybenzoate and vanillate respectively (Fig. S5). Thus, a homology search was also performed with *Fcs*, *Ech*, and *Vdh* from *P. putida* KT2440[7, 8] against all the bacterial genomes. At genomic level, high homologies were found with Ech and Vdh in all bacterial genomes (Table S4,56), however lower homologies with the enzyme Fcs (<63%) were found in some cases (Table S3). Results suggest that p-coumarate and ferulate could not be metabolized (or efficiently metabolized) by ***P. fluorescens***, ***C. freundii***, and ***E. lignolyticus***. Comparing these results with the observed in Table 2, we can see how these three organisms are not metabolizing p-coumarate as much as ferulate. However, ferulate is also being consumed at low level in all cases (*P. fluorescens* converted aproximately 15% in lean and reach conditions). These results show that high correlation between the experimental results and the genomic analysis. However, there are also some other cases, where although homologies are high, but ferulate conversion is not efficient either (*R. erythropolis*). We also note that not all the genomes from the bacterial strains used in this study are available, what can derive in some variations between experimental and genomic analyses.

The aromatics 4-hydroxybenzoate and vanillate are already present in the initial APL, but they can be also a product from the conversion of p-coumarate and ferulate by PobA (4-hydroxybenzoate-3-monooxygenase) and VanAB (vanillate demethylase A (VanA) and Vanillate-O-demethylase oxidoreductase (Van B)) respectively (Fig. S5). Firstly, ***E. lignolyticus*** was the only bacterium presenting low homologies with the selected PobA (24%) (Table S6). Thus, at genomic level, it could be suggested that 4-hydroxybenzoate cannot be converted to the intermediate protocatechuate by this bacterium. However, some conversion is observed (Table 2), presenting again that although homologies are low, there can also be other enzymes with low activities on that substrate. Secondly, ***C. necator, A. vinelandii, C. freundii, E. lignolyticus,*** **and** ***R. erythropolis*** (homologies < 45%) and ***B. subtilis*** **and** ***B. megaterium*** **and** ***Paenibacillus*** (homologies <68%) presented low homologies with the reference protein VanAB, suggesting that vanillate could be accumulated and no converted to protocatechuate. Most of these cases were highly correlated with the experimental data in Table 2. ***E. ligninolyticus*** was the only case with low homologies for all the previous checked enzymes, suggesting that its enzymes could be "phylogenetically far" ior that protocatechuate pathway is not its preferred. In general, these results also show that the bacteria utilized in this screening are more efficient via 4-hydroxibenzoic acid than vanillic acid to protocatechuate.

Lately, considering that all the bacteria (excluding *B. subtilis* and *B. megaterium*) have at least one enzyme to cleavage protocatechuate, we can state that they have the potential of metabolizing aromatics and lead them to central metabolism (Fig. S5). Then, separately, bacteria can have the capability of accumulating PHA, fatty acids, or none of them, but that is related with other metabolism pathways and out of the scope of lignin depolymerization and aromatics catabolism thus, it is not being detailed in the current study.

In brief, we have demostrated how all these bacteria contain putative enzymes involved in lignin depolymerization and we have described which bacteria are able to catabolize aromatics to central metabolism. Moreover, as seen from all these results, enzyme assays and GC-GC/MS analysis are highly correlated to genomic approaches.

References

1. C. W. Johnson and G. T. Beckham, *Metab. Eng.*, 2015, **28**, 240-247.
2. M. E. Brown, T. Barros and M. C. Chang, *ACS Chem. Biol.*, 2012, **7**, 2074-2081.
3. A. Santos, S. Mendes, V. Brissos and L. O. Martins, *Appl. Microbiol. Biotech.*, 2014, **98**, 2053-2065.
4. L. F. Gottschalk, E. S. Bon and R. Nobrega, *Appl. Biochem. Biotech.*, 2008, **147**, 23-32.
5. M. F. Hullo, I. Moszer, A. Danchin and I. Martin-Verstraete, *J. Bacteriol.*, 2001, **183**, 5426-5430.
6. S. Majumdar, T. Lukk, J. O. Solbiati, S. Bauer, S. K. Nair, J. E. Cronan and J. A. Gerlt, *Biochemistry*, 2014, **53**, 4047-4058.
7. J. I. Jimenez, B. Minambres, J. L. Garcia and E. Diaz, *Environ. Microbiol.*, 2002, **4**, 824-841.
8. R. Plaggenborg, J. Overhage, A. Steinbuchel and H. Priefert, *Appl. Microbiol. Biotech.*, 2003, **61**, 528-535.
9. D. Kasai, T. Fujinami, T. Abe, K. Mase, Y. Katayama, M. Fukuda and E. Masai, *J. Bacteriol.*, 2009, **191**, 6758-6768.
10. K. P. Barry and E. A. Taylor, *Biochemistry*, 2013, **52**, 6724-6736.
11. J. Nogales, A. Canales, J. Jimenez-Barbero, J. L. Garcia and E. Diaz, *J. Biol. Chem.*, 2005, **280**, 35382-35390.