Termite gut microbiota contribution to wheat straw delignification in anaerobic bioreactors

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Supporting table 1. Peak assignments for 2D HSQC NMR spectra of wheat cell walls.

Supporting table 2: Identity, structural classification and relative abundance of lignin-derived pyrolysis products by quantitative ^{13}C-IS py-GC-MS. Average relative abundance of analytical triplicates.

Supplementary Material and Methods. Detailed description of the experimental methodology.

Number of pages: 9 (S1-S9)

Number of tables: 2

Supporting Table S1. Peak assignments for 2D HSQC NMR spectra of wheat cell walls.

Labels	δ_C/δ_H (ppm)	Assignment
Lignin and cinnamate aromatic signals		
$S_{2/6}$	104.2/6.77	C2–H2 and C6–H6 in syringyl units
$S'_{2/6}$	106.8/7.23, 106.6/7.07	C2–H2 and C6–H6 in Cα-oxidized syringyl units
G_2	111.2/7.06	C2–H2 in guaiacyl units
$G_{5/6}$	119.3/6.88, 114.9/6.78	C5–H5 and C6–H6 in guaiacyl units
G'_2	112.5/7.32, 111.6/7.53	C2–H2 in Cα-oxidized guaiacyl units
$H_{2/6}$	128.0/7.23	C2–H2 and C6–H6 in *p*-hydroxyphenyl units
$H_{3/5}$	114.9/6.74	C3–H3 and C5–H5 in *p*-hydroxyphenyl units
T_3	104.9/7.06	C3–H3 in tricin residues
T_6	98.9/6.31	C6–H6 in tricin residues
T_8	94.2/6.63	C8–H8 in tricin residues
$T_{2'/6'}$	104.3/7.36	C2′–H2′ and C6′–H6′ in tricin residues
$P_{2/6}$	130.1/7.50	C2–H2 and C6–H6 in *p*-coumarate residues
$P_{3/5}$	115.6/6.85	C3–H3 and C5–H5 in *p*-coumarate residues
P_8	113.8/6.36	C8–H8 in *p*-coumarate residues

F_2	111.0/7.36	C2–H2 in ferulate residues
F_6	123.2/7.12	C6–H6 in ferulate residues
Lignin inter-monomeric linkage and end-unit signals		
I_α	71.5/4.87	Cα–Hα in β–*O*–4 units
I_β	86.2/4.22, 83.9/4.38	Cβ–Hβ in β–*O*–4 units
I_γ (γ-free)	60.5/3.86	Cγ–H γ in γ-free β–*O*–4 units
I_γ (γ-acylated)	64.6/4.30	Cγ–H γ in γ-acylated β–*O*–4 units
II_α	87.2/5.50	Cα–Hα in β–5 substructures
II_β	53.6/3.49	Cβ–Hβ in β–5 substructures
III_α	85.0/4.67	Cα–Hα in resinol-type β–β substructures
III'_α	82.9/4.98	Cα–Hα in tetrahydrofuran-type β–β substructures
IV_γ	61.8/4.14	Cγ–Hγ in cinnamyl alcohol end-units
IV'_γ	193.9/9.61	Cγ–Hγ in cinnamylaldehyde end-units
IV''_α	190.8/9.81	Cα–Hα in benzaldehyde end-units
IV'''_β	41.2/3.13	Cβ–Hβ in HPV/HPS end-units
IV'''_γ	57.3/3.83	Cγ–Hγ in HPV/HPS end-units

Supporting Table S1 (continued)

Lignin methoxyl signals		
OMe	55.7/3.74	C–H in aromatic methoxyl groups
Polysaccharide anomeric signals		
Gl_1	103.5/4.44	C1–H1 in (1→4)-β-D-glucopyranosyl units
X_1	102.1/4.28	C1–H1 in (1→4)-β-D-xylopyranosyl units
X'_1	99.9/4.58	C1–H1 in 2-*O*-acetyl-β-D-xylopyranosyl units
X''_1	101.8/4.40	C1–H1 in 3-*O*-acetyl-β-D-xylopyranosyl units
X'''_1	99.1/4.78	C1–H1 in 2,3-di-*O*-acetyl-β-D-xylopyranosyl units
A_1	108.2/4.90	C1–H1 in α-L-arabinofuranosyl units
U_1	97.7/5.27	C1–H1 in 4-*O*-methyl-α-D-glucuronopyranosyl units

Measured in DMSO-d_6/pyridine-d_5 (4:1, v/v). Signal assignment was based on comparison with NMR data in literature.[1-7]

Supporting Table S2. Identity, structural classification and relative abundance of lignin-derived pyrolysis products by quantitative ^{13}C-IS py-GC-MS. Average relative abundance of analytical triplicates.

#	Compound	CAS	Retention time (min)	Structural feature	Sidechain length	M_w ^{12}C (g•mol^{-1})	Quan ion ^{12}C [M-*e*]	Original	*N. lujae*	*M. parvus*	*T. hospes*
1	phenol	108952	9.79	H, unsub.	0	94	94.04132	0.7	0.7	0.7	0.7
2	guaiacol	90051	10.03	G, unsub.	0	124	124.05188	2.8	3.5	3.8	3.6
3	2-methylphenol	95487	11.03	H, methyl	C_α	108	108.05698	0.1	0.1	0.1	0.2
4	4-methylphenol (+3-MP)	106445	12.00	H, methyl	C_α	108	107.04914	0.4	0.4	0.3	0.5
5	4-methylguaiacol	93516	12.71	G, methyl	C_α	138	138.06753	1.6	1.7	1.7	1.9
6	2,4-dimethylphenol	105679	13.18	H, methyl	C_α	122	107.04914	0.1	0.1	0.1	0.1
7	4-ethylphenol	123079	14.25	H, misc.	C_β	122	107.04914	0.1	0.1	0.1	0.1
8	4-ethylguaiacol	2785899	14.83	G, misc.	C_β	152	137.05971	0.2	0.2	0.2	0.2
9	4-vinylguaiacol	7786610	16.29	G, vinyl	C_β	150	150.06753	26.7	23.7	23.4	23.3
10	4-vinylphenol	2628173	16.46	H, vinyl	C_β	120	120.05697	8.1	6.2	6.5	6.6
11	eugenol	97530	16.89	G, misc.	C_γ	164	164.08318	0.2	0.2	0.2	0.3
12	4-propylguaiacol	2785877	16.99	G, misc.	C_γ	166	137.05971	0.1	0.1	0.1	0.2
13	syringol	91101	17.64	S, unsub.	0	154	154.06245	1.7	1.9	2.0	1.9
14	*cis*-isoeugenol	97541	18.25	G, misc.	C_γ	164	164.08318	0.1	0.1	0.1	0.1
15	4-propenylphenol	539128	19.24	H, misc.	C_γ	134	133.06479	0.1	0.1	0.1	0.1
16	*trans*-isoeugenol	97541	19.50	G, misc.	C_γ	164	164.08318	0.8	1.0	0.9	1.0
17	4-methylsyringol	6638057	19.86	S, methyl	C_α	168	168.07810	0.8	0.8	0.8	0.8
18	vanillin	121335	19.99	G, C_α-O	C_α	152	151.03897	1.2	1.2	1.1	1.2
19	4-propyneguaiacol	-	20.23	G, misc.	C_γ	162	162.06753	0.1	0.1	0.1	0.1
20	4-alleneguaiacol	-	20.49	G, misc.	C_γ	162	162.06753	0.1	0.1	0.1	0.1
21	homovanillin	5603242	21.44	G, C_β-O	C_β	166	137.05971	0.4	0.5	0.4	0.5
22	4-ethylsyringol	14059928	21.58	S, misc.	C_β	182	167.07022	0.1	0.1	0.1	0.1
23	vanillic acid methyl ester	3943746	21.82	G, C_α-O	C_α	182	182.05736	0.01	0.01	0.01	0.01
24	acetovanillone	498022	21.89	G, C_α-O	C_β	166	151.03897	0.4	0.4	0.4	0.4
25	4-hydroxybenzaldehyde	123080	22.76	H, C_α-O	C_α	122	121.02848	0.1	0.1	0.1	0.1
26	4-vinylsyringol	28343228	22.90	S, vinyl	C_β	180	180.07810	4.8	4.6	4.5	4.4
27	guaiacylacetone	2503460	23.10	G, C_β-O	C_γ	180	137.05971	0.3	0.4	0.4	0.4
28	4-allylsyringol	6627889	23.31	S, misc.	C_γ	194	194.09373	0.3	0.3	0.2	0.3
29	propiovanillone	1835149	23.79	S, C_α-O	C_γ	180	151.03897	0.03	0.03	0.03	0.03
30	guaiacyl vinyl ketone	-	24.09	G, C_α-O	C_γ	178	151.03897	0.2	0.2	0.2	0.2
31	guaiacyl diketone	2034608	24.32	G, C_α-O, C_β-O	C_γ	194	151.03897	0.3	0.3	0.3	0.3
32	cis-4-propenylsyringol	26624135	24.43	S, misc.	C_γ	194	194.09373	0.2	0.2	0.1	0.2
33	4-propynesyringol	-	25.06	S, misc.	C_γ	192	192.07810	0.2	0.2	0.1	0.2
34	4-allenesyringol	-	25.27	S, misc.	C_γ	192	192.07810	0.1	0.1	0.1	0.1
35	trans-4-propenylsyringol	26624135	25.72	S, misc.	C_γ	194	194.09373	1.2	1.2	1.2	1.3
36	dihydroconiferyl alcohol	2305137	25.81	S, C_γ-O	C_γ	182	137.05971	0.1	0.2	0.2	0.2
37	syringaldehyde	134963	26.34	S, C_α-O	C_α	182	182.05736	0.9	0.9	0.9	0.9
38	cis-coniferyl-alcohol	458355	26.42	G, C_γ-O	C_γ	180	137.05971	1.1	1.2	1.2	1.2
39	homosyringaldehyde	-	27.32	S, C_β-O	C_β	196	167.07027	1.1	1.1	0.9	1.0
40	syringic acid methyl ester	884355	27.66	S, C_α-O	C_α	212	212.06793	0.02	0.02	0.02	0.02
41	acetosyringone	2478388	27.76	S, C_α-O	C_β	196	181.04954	0.7	0.7	0.6	0.7
42	trans-coniferyl alcohol	458355	28.11	G, C_γ-O	C_γ	180	137.05971	21.1	24.8	25.8	25.6
43	trans-coniferaldehyde	458366	28.50	G, C_γ-O	C_γ	178	147.04406	1.4	1.4	1.4	1.4
44	syringylacetone	19037582	28.68	S, C_β-O	C_γ	210	167.07027	0.4	0.4	0.4	0.4
45	propiosyringone	5650431	29.29	S, C_α-O	C_γ	210	181.04954	0.05	0.05	0.05	0.05
46	syringyl diketone	6925651	29.43	S, C_α-O, C_β-O	C_γ	224	181.04954	0.3	0.3	0.2	0.3
47	syringyl vinyl ketone	-	29.57	S, C_α-O	C_γ	208	181.04954	0.03	0.03	0.03	0.03
48	dihydrosinapyl alcohol	20736258	31.13	G, C_γ-O	C_γ	212	168.07841	0.1	0.1	0.1	0.1
49	cis-sinapyl alcohol	537337	31.63	S, C_γ-O	C_γ	210	167.07027	1.0	1.0	1.0	0.9

50	trans-sinapyl alcohol	537337	33.31	S, C_γ-O	C_γ	210	167.07027	15.6	15.9	15.3	14.5
51	trans-sinapaldehyde	4206580	33.54	S, C_γ-O	C_γ	208	208.07301	1.3	1.3	1.1	1.2

Supplementary Material and Methods

Lignocellulose substrate and termite gut inocula

Wheat straw from the winter wheat variety Koreli was collected at an experimental farm (INRAE, Boissy-le-Repos, France) in August 2011. After harvesting, the straw was milled to 2 mm and stored at room temperature (20–25°C). As described in our previous work,[8] four different species of higher termites (Termitidae family) *Microcerotermes parvus, Termes hospes, Nasutitermes ephratae*, and an undescribed species closely related to *N. lujae* (herein after *N. lujae*) were selected as inocula. The initial termite gut inoculum (500 dissected guts) from each termite was provided by IRD (Institute for Research and Development, Bondy, France).

Anaerobic bioreactors

The lignocellulose degradation capacity of the different gut microbiomes were assessed in two replicate anaerobic bioreactors (Applikon MiniBio 500) for each termite species as previously described.[8] Briefly, following centrifugation (7,197 × g, 10 min, 4°C) and elimination of the saline PBS solution, termite guts (500 guts) were used to inoculate 400 mL of mineral media

(MM),[1] supplemented with 250 µL of V7 vitamin solution[9] and 1 mL of sterilized (0.2 µm filtration) trace elements solution.[8] Milled wheat straw (2 mm) was autoclaved (120°C, 20 min and 1.2 bars) and added to the medium (20 $g.L^{-1}$) as the sole carbon source. Stirred bioreactors (400 rpm) were operated under strict anaerobic conditions. The absence of dissolved oxygen was ensured by nitrogen flushing after inoculation and continuous monitoring with a polarographic dissolved oxygen probe (AppliSens). The temperature was set to 35°C and pH was maintained at 6.15 by adding a 2 M NaOH solution. During the incubation, methane production was monitored and, if necessary, inhibited by the addition of 2-bromoethanesulfonate (BES), a methanogenesis inhibitor, until a maximum concentration of 10 mM. At the end of the 20 days of incubation, VFA and gas production were determined and the whole culture broth was snap frozen in liquid nitrogen and kept at -80°C until further use. For chemical characterization replicate samples were pooled together.

Volatile solid quantification

Wheat straw concentration was determined at the beginning and at the end of the 20-day incubation by measuring the total (TS) and volatile (VS) solids. TS were determined using 10 mL samples that were first centrifuged (7,197 × g, 10 min), rinsed twice with distilled water and dried for 48 h at 55°C. The mineral fraction (MF) was estimated by mineralization of the

samples at 500°C for 2 h, and VS were determined by subtracting MF from TS. Wheat straw degradation was reported as percentage of VS (%, w/w) related to the initial VS mass.

Cell wall extraction and grinding

Extractive-free cell wall residues (CWR) were obtained by sonication-solvent extraction.[5] Two g of wheat straw samples with 40 ml of ultrapure water were sonicated for 20 min at room temperature then centrifuged (5 min, 7197g, 20°C). The water was removed and replaced by 80% ethanol:water and the sonication/centrifugation cycle was repeated twice and then washed with 100% acetone. CWR was obtained after drying at room temperature overnight and for 24 h at 55°C. CWR was then milled in a ball mill MM 400 (Retsch) by two successive steps (15s mill at 30 $Hz.s^{-1}$) with a 2 cm diameter metal ball.

Polysaccharide analysis and Klason lignin determination

Wheat straw composition was determined on the original wheat straw and the digested samples collected from the bioreactors at the end of the incubation period kept at -80°C. These last were thawed, the replicates were mixed together and centrifuged (7,197 g, 10 min); the solid fraction was then washed with distilled water and dried at 55°C (48h). Raw wheat straw

was incubated in the culture medium for 4 hours, and then treated as the digested samples. Their chemical composition was determined using the sulfuric acid hydrolysis method described by de Souza et al.[10] and modified by Lazuka et al.[11] on triplicate 80 mg samples, using a the first hydrolysis step of 1h, 30°C and a second step for 1h at 120°C in an oil bath. The insoluble residue was washed with distilled water and dried at 105 °C overnight to determine Klason lignin content. The soluble fraction was filtered and monomeric sugar composition was determined on an Ultimate 3000 Dionex HPLC with refractive index detector (Thermo Scientific) equipped with a BioRad Aminex HPX 87H affinity column, as described previously.[12]

Thioacidolysis

Thiacidolysis of the samples was done according to Méchin et al.[13] The thioacidolysis solution was prepared by adding 20ml ethanethiol and 5ml BF_3 etherate in 40 ml dioxane and adjusted to 200ml with dioxane. 10 ml thioacidolysis solution with and 100µl of tetracosane 1.25 mg/ml was added to 10 mg of dry CWR. An aliquot of this solution (10 µL) was dried and trimethylsilylated (TMS) with 50 µL of *N,O*-bis(trimethylsilyl)trifluoroacetamide and 5 µL of ACS-grade pyridine for 1 h at room temperature. The TMS sample was injected (1 µL) onto a Trace1300 GC/MS (Thermoscientific) equipped with a Triple Quadripole-Ion trap in electronic

impact mode with a source at 220 °C, an interface at 280 °C, and a 50 to 650 m/z scanning range. The samples were analyzed on an Agilent DB-5 column (Agilent Technologies) operated in the temperature program mode (from 50 to 110 °C at +30 °C/min, then 110 to 320°C at +6 °C/min), with helium carrier gas at a 1.5 mL/min flow rate.[14]

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