Supplementary Information (SI) for Green Chemistry.

Comparison of microbial strains as candidate hosts and genetic reservoirs for the valorization of lignin streams

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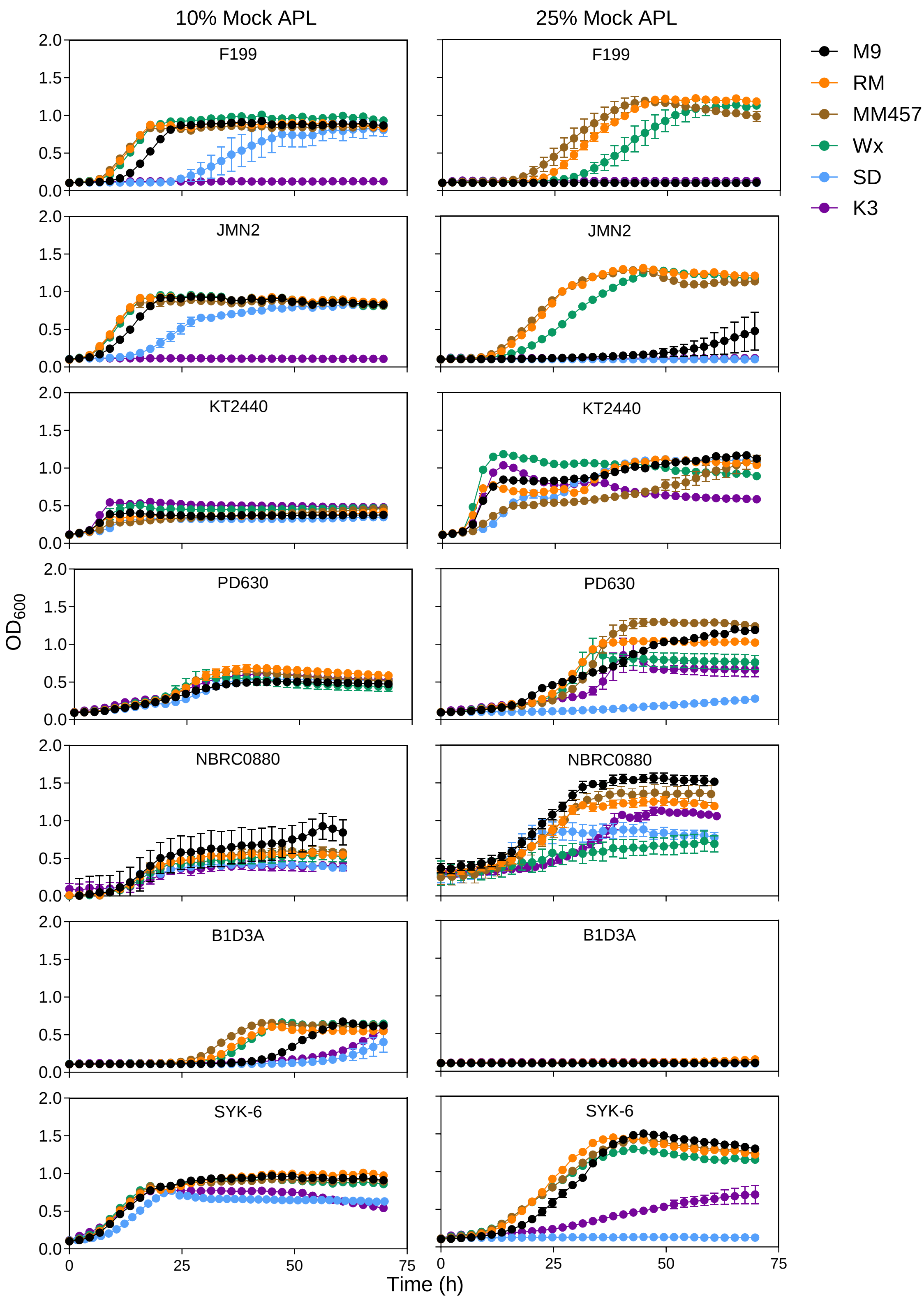


Fig. S1. Growth dynamics of all seven microbial strains grown on 10% or 25% mock APL and the six tested minimal media. Data represents the mean ± the standard error of the mean of three biological replicates.

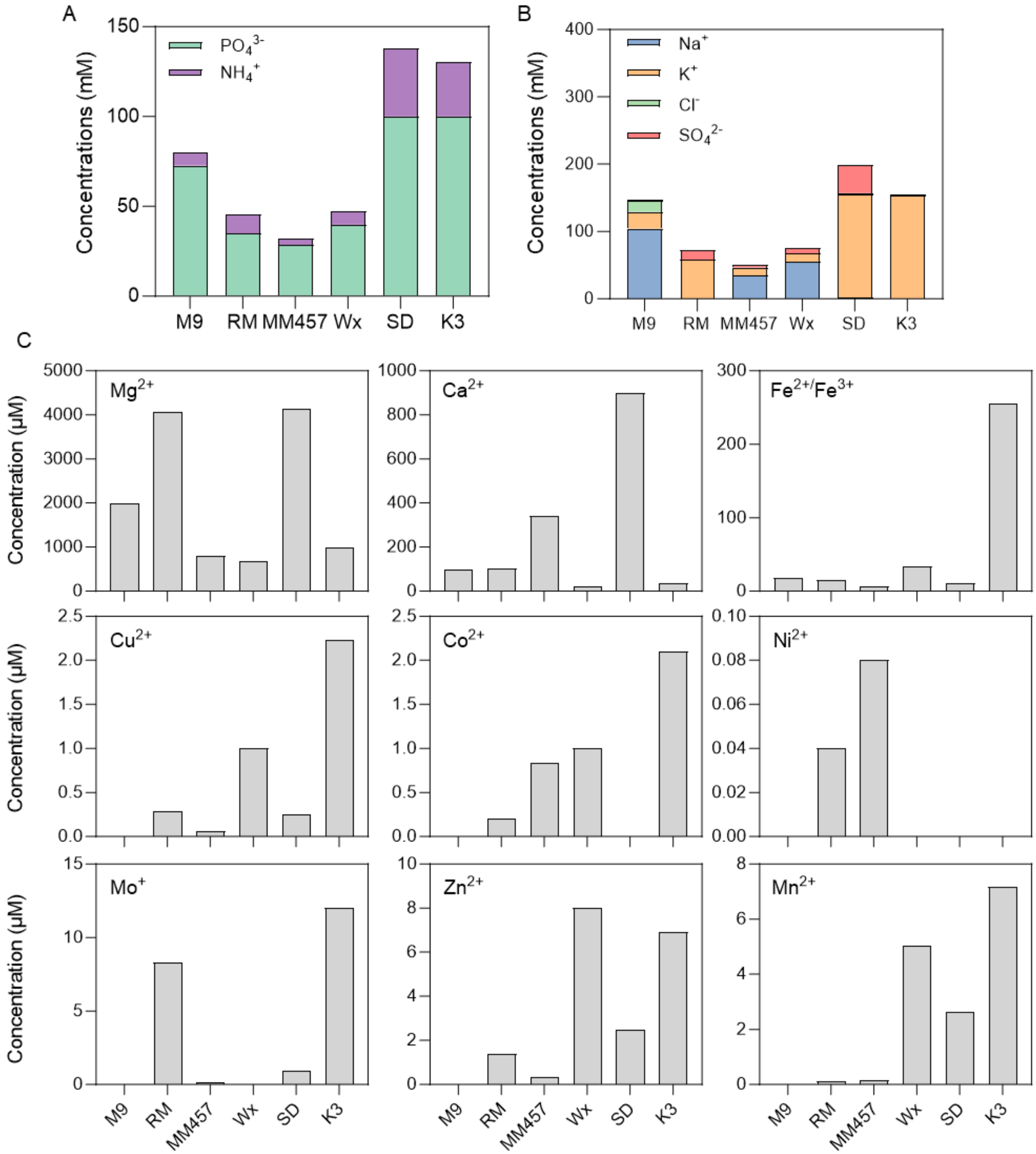
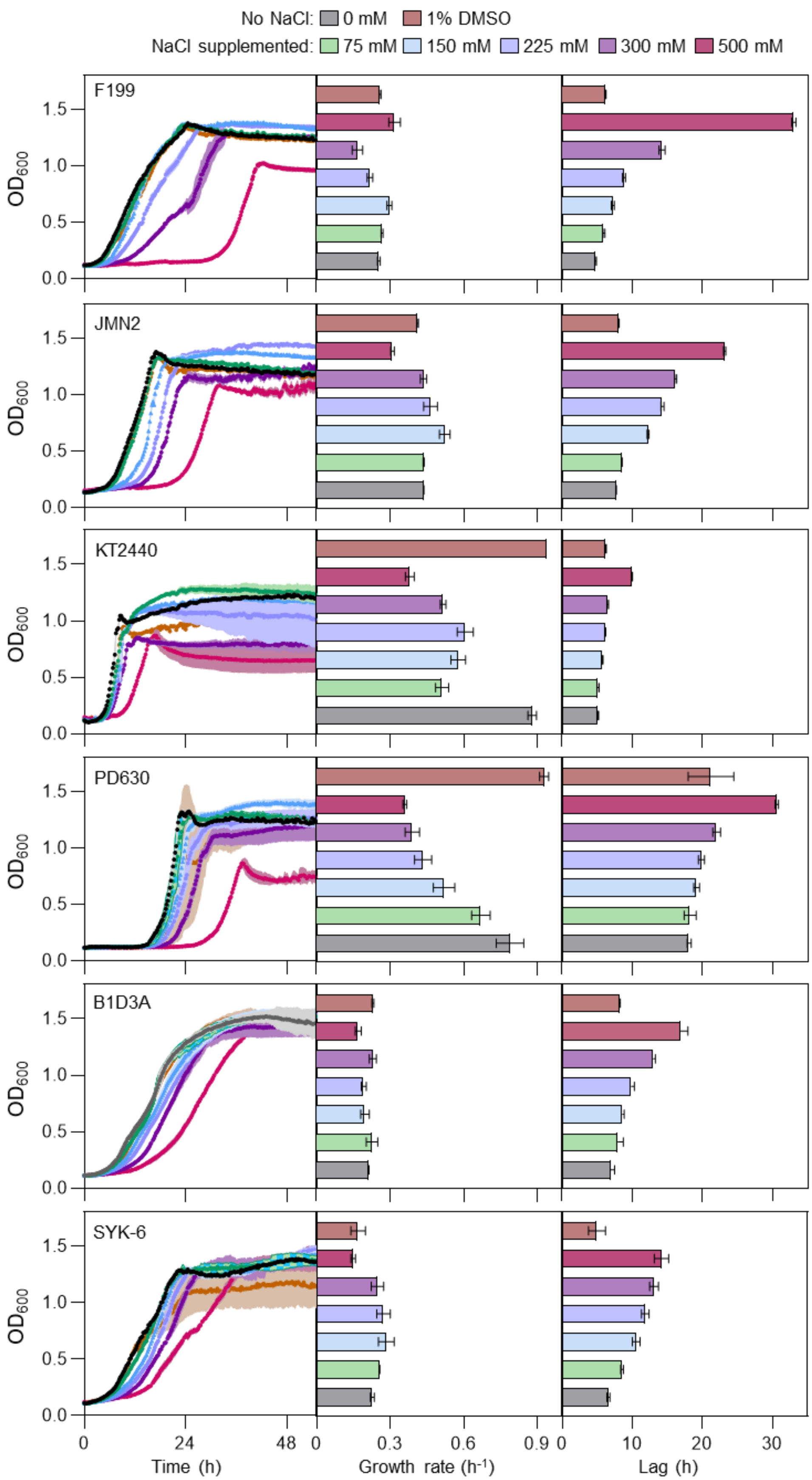


Fig. S2. Comparison of (A) essential nutrients, (B) cumulative salt additives, and (C) other trace metals in the six different media recipes.



No NaCl: 0 mM 1% DMSO
NaCl supplemented: 75 mM 150 mM 225 mM 300 mM 500 mM
F199
JMN2
KT2440
PD630
B1D3A
SYK-6
OD600
0.0
0.5
1.0
1.5
0
24
48
Time (h)
0
0.3
0.6
0.9
Growth rate (h-1)
0
10
20
30
Lag (h)

Fig. S3. Growth dynamics, maximum absolute growth rates (growth rate), and lag times for the six bacterial strains grown on favorable carbon sources: 20 mM glucose for F199, JMN2, KT2440, and PD630 or SEMP for SYK-6 and B1D3A. Addition of DMSO or NaCl was used to assess toxicity tolerance. Bacterial growth in 96-well plates containing 200 µL of growth medium was monitored on a BioscreenC Pro instrument. The data are the means of the maximum absolute growth rate or lag time measurements determined from three replicates and error bars denote the standard deviation of the mean values.

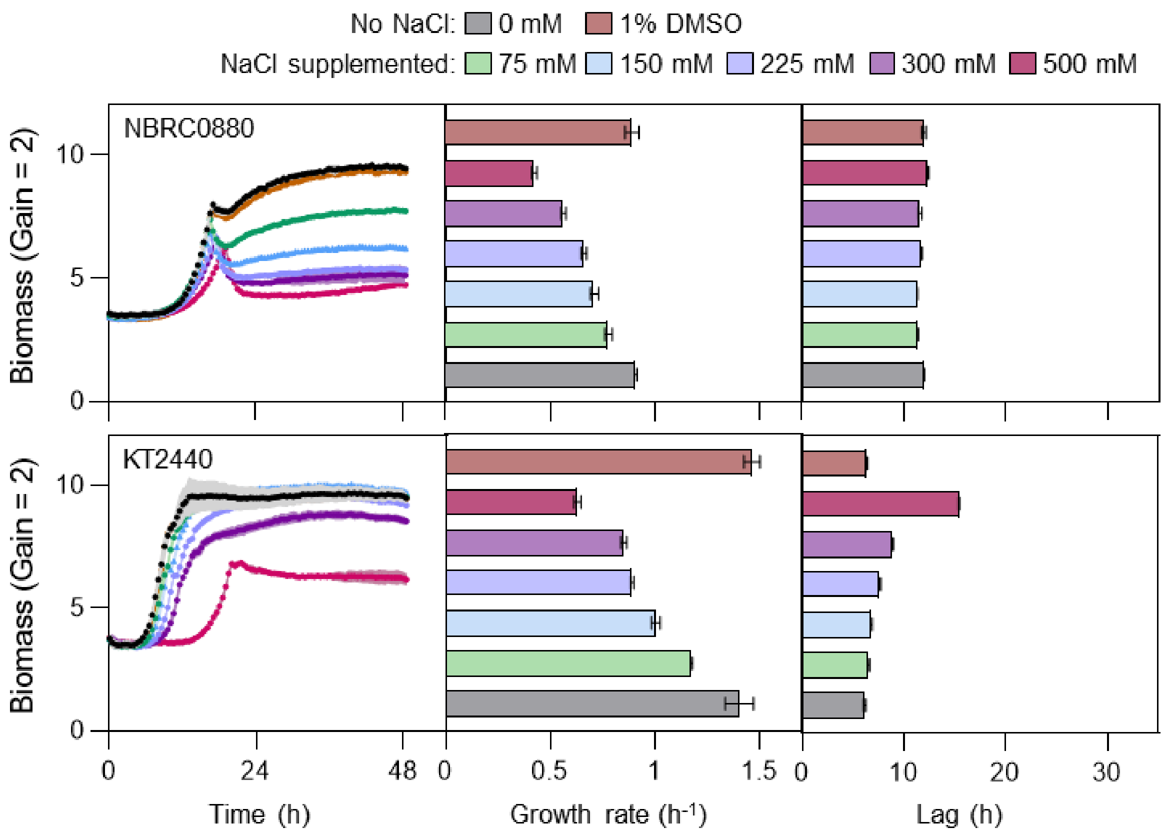
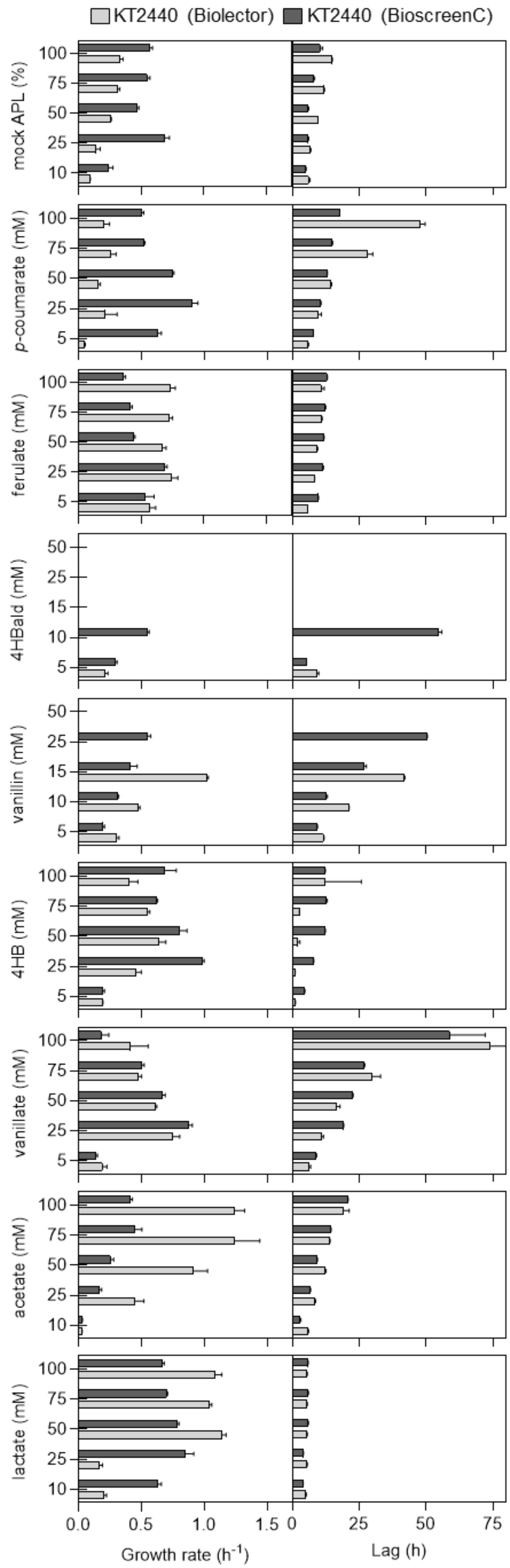


Fig. S4. Growth dynamics, maximum absolute growth rates (growth rate), and lag times for the NBRC0880 compared to KT2440 grown on 20 mM glucose plus increasing concentrations of NaCl or with 1% DMSO. Growth in 48-well plates containing 1 mL of growth medium was monitored using a BioLector® automated growth curve analysis system. The data are the means of the maximum absolute growth rate or lag time measurements determined from three replicates and error bars denote the standard deviation of the mean values.



KT2440 (Biolector)
KT2440 (BioscreenC)
mock APL (%)
p-coumarate (mM)
ferulate (mM)
4HBald (mM)
vanillin (mM)
4HB (mM)
vanillate (mM)
acetate (mM)
lactate (mM)
Growth rate (h⁻¹)
Lag (h)

Fig. S5. Growth comparison of KT2440 in 1 mL culture volumes (light grey) compared to KT2440 in 0.2 mL culture volumes (dark grey). Maximum absolute growth rates and lags were calculated from the change in biomass over time monitored using BioLector® automated growth curve analysis system or a BioscreenC Pro instrument. RM minimal media supplemented with increasing concentrations of mock APL, aromatic acids, aldehydes, or aliphatic acids as sole carbon sources were used to culture strains. If no measurement of growth occurred after 80 h, the strains were considered to not grow on that substrate concentration. All data represent the mean ± the standard deviation of the calculated growth rates and lags determined from three biological replicates.

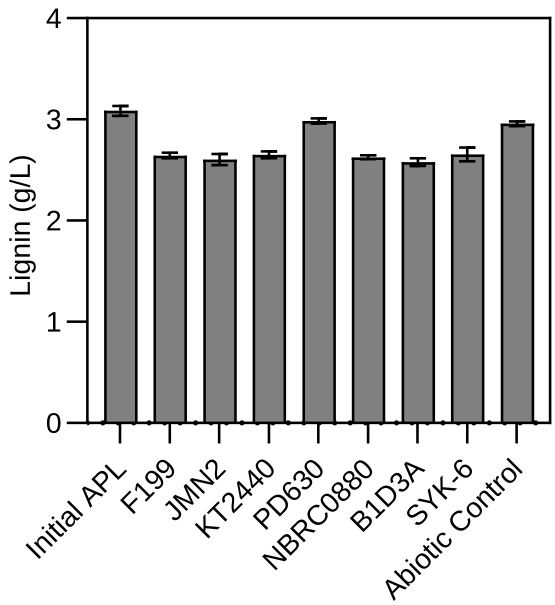


Fig. S6. The concentration of lignin (g/L) measured in the lyophilized supernatant of the initial APL versus the concentration after inoculation with the seven different microbial strains or no inoculation (abiotic control) after 120 h. The concentration was corrected for the amount of evaporation that occurred at the end of 120 h. Data represents the mean ± the standard deviation of three replicates.

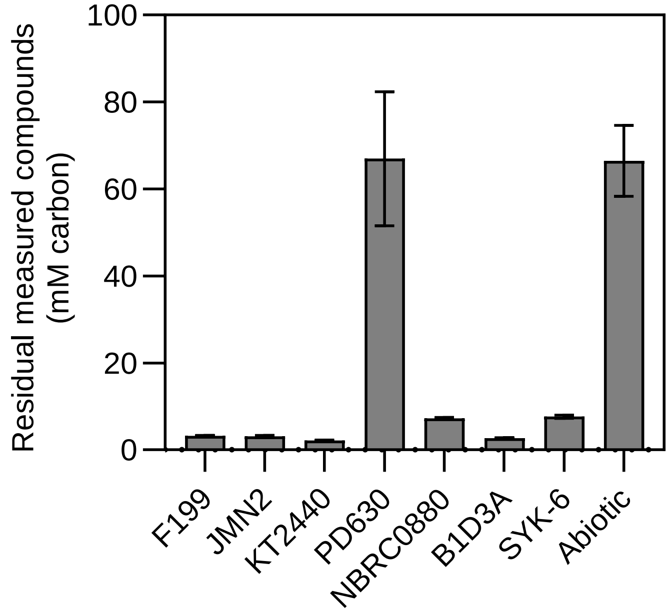


Fig. S7. The sum, in mM of carbon, of all measured aromatic and aliphatic acids present in the supernatant after 120 h. The concentration was corrected to account for the evaporation that occurred at the end of 120 h. Data represents the mean ± the standard deviation of three replicates.

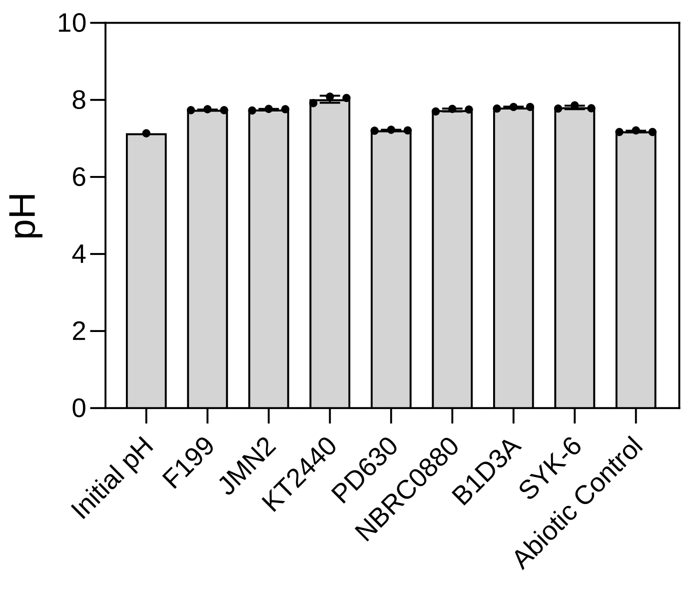


Fig. S8. Measured pH of APL media after 120 h of inoculation with the different microbial strains. Data represents the mean ± the standard deviation of three biological replicates.

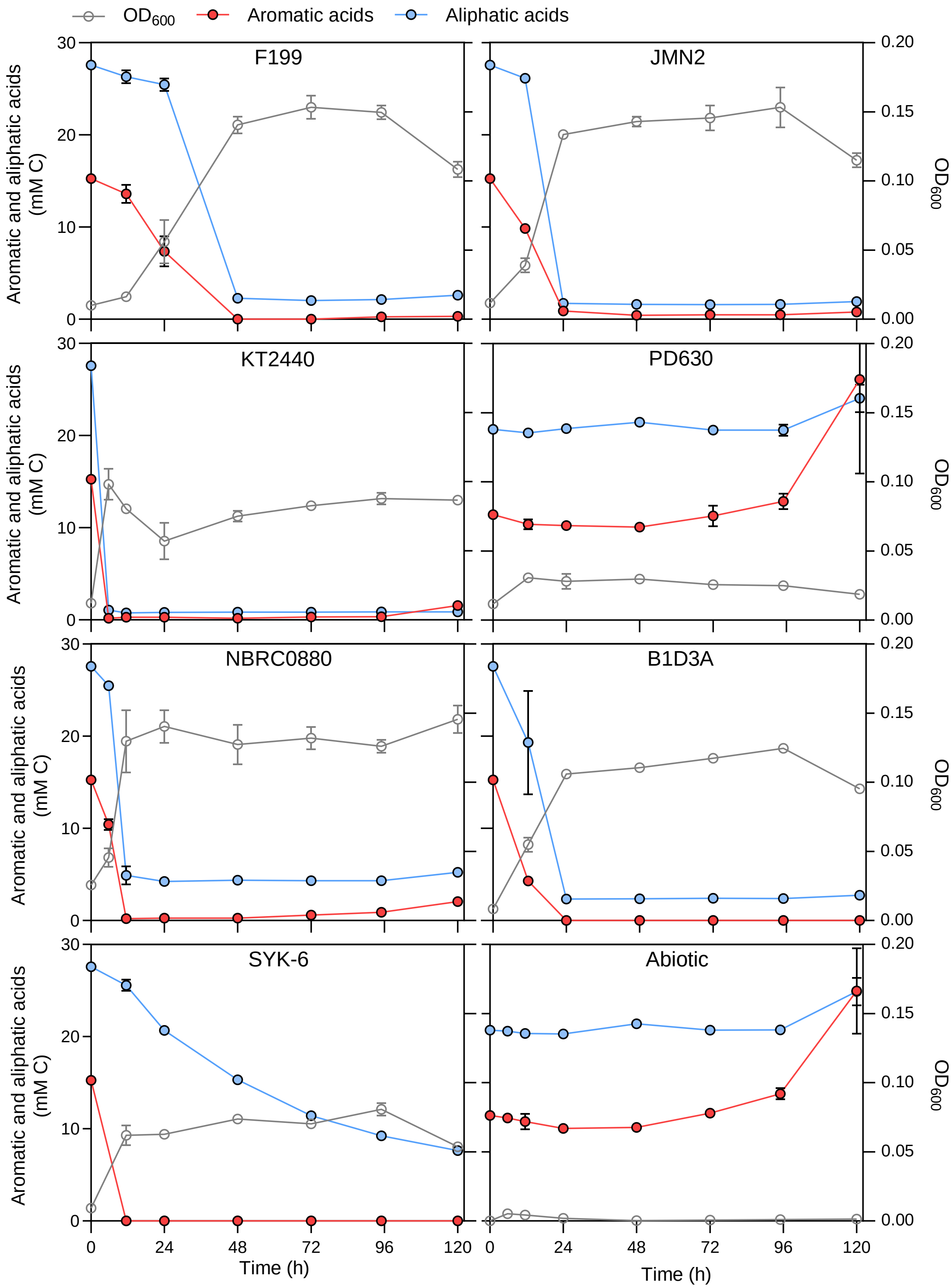


Fig. S9. Depletion profiles of the summed total of measured aromatic acids (red) and aliphatic acids (blue) over time for each strain. Data represents the mean ± the standard deviation of three biological replicates.

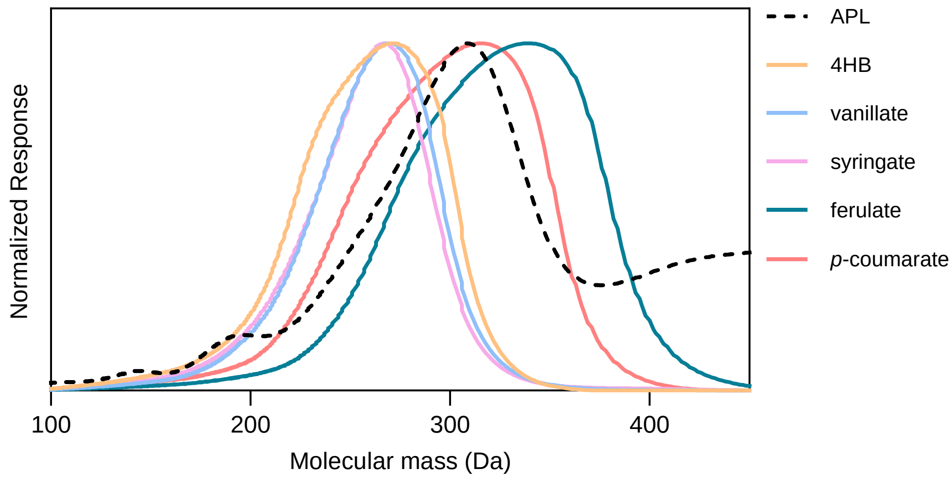


Fig. S10. Low molecular mass distribution of APL abiotic control compared to aromatic compound standards.

Table S1. Chemical components of all minimal media recipes

Chemical Name	Chemical Formula	M9	RM	MM 457	Wx	SD	K3
	mM						
Sodium phosphate dibasic dodecahydrate	$Na_2HPO_4 \cdot 12H_2O$	47.8	-	17.2	27.4	-	-
Potassium phosphate monobasic	KH_2PO_4	25.0	12.2	11.2	12.5	46.4	46.4
Potassium phosphate dibasic	K_2HPO_4	-	22.8	-	-	53.6	53.6
Sodium chloride	NaCl	8.56	-	-	-	1.71	-
Ammonium chloride	NH_4Cl	7.57	-	-	-	-	-
Ammonium sulfate	$(NH_4)_2SO_4$	-	10.6	3.78	7.57	37.8	-
Ammonium phosphate dibasic	$(NH_4)_2HPO_4$	-	-	-	-	-	30.3
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	2.00	4.06	0.81	0.406	4.15	1.00
	µM						
Magnesium oxide	MgO	-	-	-	267	-	-
Ferrous sulfate heptahydrate	$FeSO_4 \cdot 7H_2O$	18.0	1.80	7.19	34.2	10.0	5.00
Calcium chloride dihydrate	$CaCl_2 \cdot 2H2O$	100	102	340	-	901	34.0
Calcium carbonate	$CaCO_3$	-	-	-	20.0	-	-
Zinc sulfate heptahydrate	$ZnSO_4 \cdot 7H_2O$	-	1.39	0.350	8.02	2.48	-
Zinc chloride	$ZnCl_2$	-	-	-	-	-	6.90
Manganese sulfate monohydrate	$MnSO_4 \cdot H_2O$	-	0.120	-	5.02	2.65	7.17
Manganese chloride tetrahydrate	$MnCl_2 \cdot 4H_2O$	-	-	0.150	-	-	-
Copper sulfate pentahydrate	$CuSO_4 \cdot 5H_2O$	-	-	-	1.00	0.250	-
Copper chloride dihydrate	$CuCl_2 \cdot 2H_2O$	-	0.290	0.0600	-	-	2.23
Cobalt sulfate heptahydrate	$CoSO_4 \cdot 7H_2O$	-	0.210	-	1.00	-	-
Cobalt chloride hexahydrate	$CoCl_2 \cdot 6H_2O$	-	-	0.840	-	-	2.10
Boric acid	H_3BO_3	-	0.240	4.85	0.970	8.09	0.501
Ethylenediaminetetraacetic acid (EDTA) disodium salt	Na_2EDTA	-	0.860	17.1	-	-	1.19
Ethylenediaminetetraacetic acid (EDTA) ferric-sodium salt	$FeNa \cdot EDTA$	-	13.6	-	-	-	-
Nickel chloride hexahydrate	$NiCl_2 \cdot 6H_2O$	-	0.0400	0.0800	-	-	-
Sodium molybdate dihydrate	$NaMoO_2 \cdot 2H_2O$	-	8.26	0.120	-	0.970	12.00
Ferric citrate	$C_6H_5FeO_7$	-	-	-	-	-	250

Thiamine HCl	$C_{12}H_{18}Cl_2N_4OS$	-	-	-	-	-	13.3
Ferric chloride	$FeCl_3$	-	-	-	-	1.23	-
Potassium iodide	KI	-	-	-	-	0.600	-
	nM						
Biotin		-	-	-	-	8.19	-
Calcium pantothenate		-	-	-	-	839.4	-
Folate		-	-	-	-	4.53	-
Inositol		-	-	-	-	11101	-
Niacin		-	-	-	-	3249	-
p-Aminobenzoic acid		-	-	-	-	1458	-
Pyridoxine HCl		-	-	-	-	1945	-
Riboflavin		-	-	-	-	531	-
Thiamine HCl		-	-	-	-	1185	-

Table S2. Total ionic content of each minimal media recipe

	M9	RM	MM457	Wx	SD	K3
Essential nutrients (mM)						
PO_4^{3-}	72.8	34.99	28.4	39.9	100	100
NH_4^+	7.57	10.6	3.78	7.57	37.8	30.3
Mg^{2+}	2.00	4.06	0.81	0.67	4.15	0.997
Ca^{2+}	0.10	0.102	0.34	0.0199	0.901	0.0340
Supplemental salts (mM)						
Na^+	104	0.0153	34.4	54.7	1.71	0.00238
K^+	25.0	57.8	11.2	12.50	153	153
Cl^-	16.3	0.205	0.682	0	1.71	0.0767
SO_4^{2-}	2.02	14.7	4.60	8.03	42.0	1.00
Trace metal components (µM)						
Fe^{2+}/Fe^{3+}	18	15.4	7.19	34.2	11.2	255
Zn^{2+}	0	1.39	0.35	8.02	2.48	6.90
Mn^{2+}	0	0.12	0.15	5.02	2.65	7.17
Cu^{2+}	0	0.29	0.06	1	0.25	2.23
Co^{2+}	0	0.21	0.84	1	0	2.10
Ni^{2+}	0	0.04	0.08	0	0	0
Mo^+	-	8.26	0.12	-	0.97	12.0

Table S3. APL compositional analysis.

Sample Description	Composition in freeze-dried APL (%)
Ash	55.3 ± 0.2
Lignin	19.7 ± 0.2
Glucan	1.63 ± 0.03
Xylan	6.16 ± 0.13
Galactan	1.88 ± 0.2
Arabinan	2.88 ± 0.05
Fructan	0.00 ± 0.00
Acetyl	1.41 ± 0.06
Total %	88.9 ± 0.3