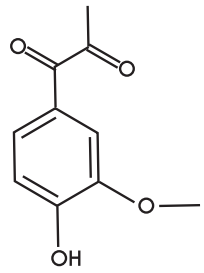
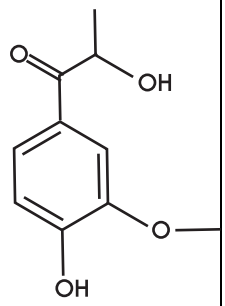
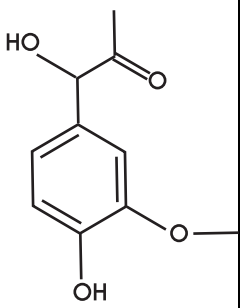
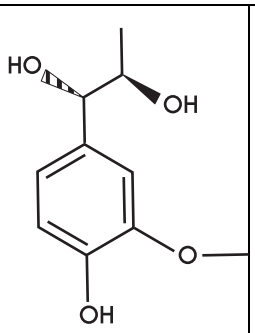
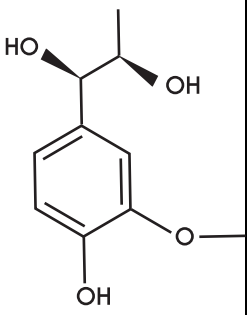
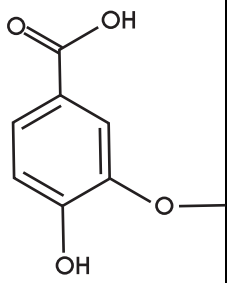
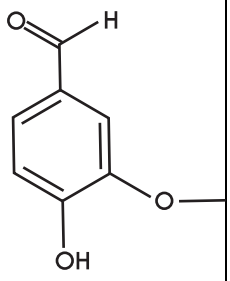
Table S1. Characteristics of G-diketone and other aromatics analyzed by chromatography

Short Name	Structure	IUPAC Name	Molecular Formula	Molecular Weight (g/mol)	Retention time in HPLC (min)	Retention time in GC (min)*
G-diketone		1-(4-hydroxy-3-methoxyphenyl) propane-1,2-dione	$C_{10}H_{10}O_4$	194.180	4.20	14.0
GP-1		2-hydroxy-1-(4-hydroxy-3-methoxyphenyl) propan-1-one	$C_{10}H_{12}O_4$	196.200	2.86	15.8
GP-2		1-hydroxy-1-(4-hydroxy-3-methoxyphenyl) propan-2-one	$C_{10}H_{12}O_4$	196.200	Unknown	14.8

threo-GD		threo-1-(4-hydroxy-3-methoxyphenyl) propane-1,2-diol	$C_{10}H_{14}O_4$	198.220	2.08	15.4
erythro-GD		erythro-1-(4-hydroxy-3-methoxyphenyl) propane-1,2-diol	$C_{10}H_{14}O_4$	198.220	Unknown	15.2
Vanillic acid		4-hydroxy-3-methoxybenzoic acid	$C_8H_8O_4$	168.150	2.54	14.4
Vanillin		4-hydroxy-3-methoxybenzaldehyde	$C_8H_8O_3$	152.150	2.84	12.0

* Retention time in GC is for TMS derivatized compound.

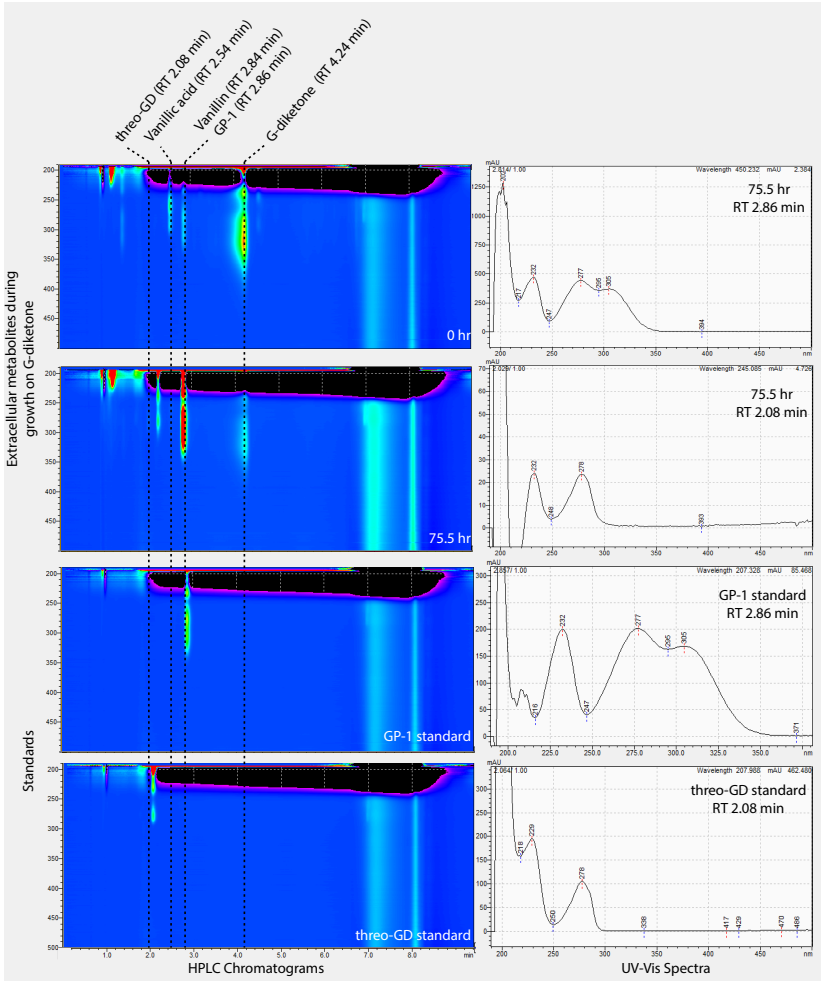


Figure S1. HPLC-UV analysis of extracellular compounds found in the media of cultures grown on glucose plus G-diketone at timepoints 0 hours and 75.5 hours and comparison to standards.

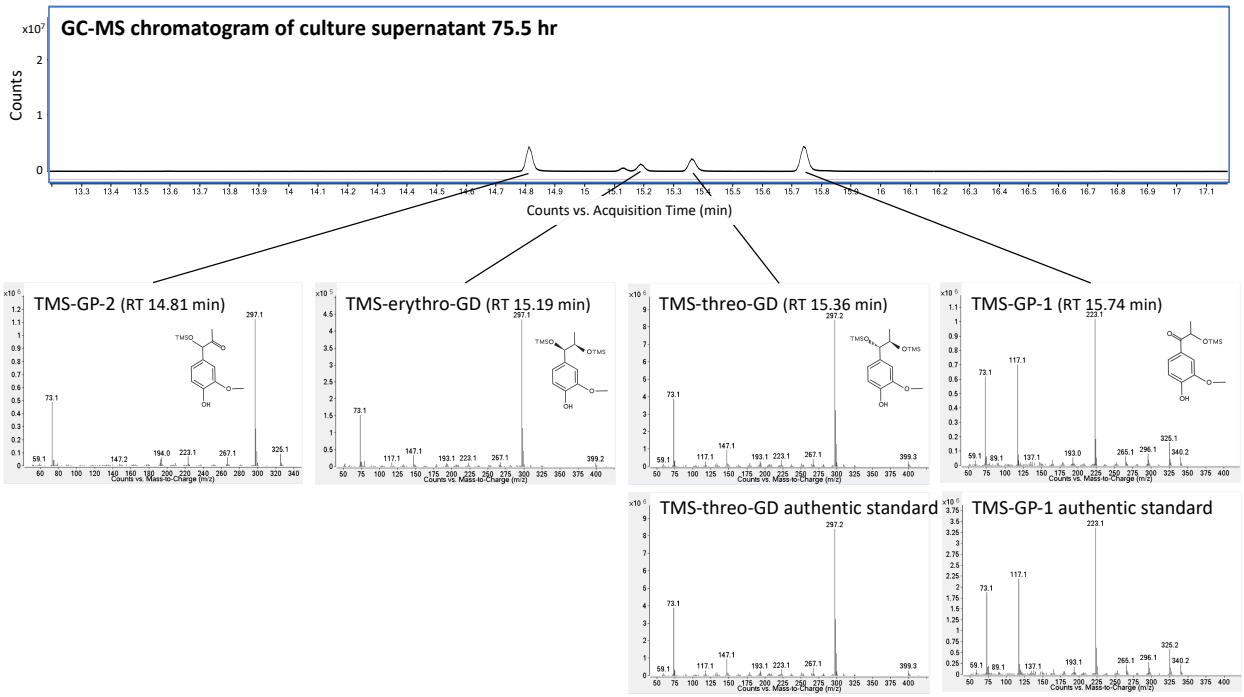


Figure S2. Mass spectra of extracellular compounds identified in the media of cultures grown on glucose plus G-diketone at timepoint 75.5 hours analyzed via GC-MS and comparison to GP-1 and threo-GD standards.

Table S2. Quantification of extracellular vanillic acid and vanillin in cultures grown with the indicated aromatic substrates

Substrates*	Vanillic acid (mM)**	Vanillin (mM)**
G-diketone plus glucose	0.021 ± 0.001	None detected
GP-1 plus glucose	0.028 ± 0.004	0.003 ± 0.000

* Substrates were normalized to having 0.5 gCOD/L of the aromatic compound plus 0.5 g COD/L of glucose.

** Reported concentrations are average and standard deviations of six separate cultures after overnight incubation.

Table S3. Quantification of vanillin and vanillic acid in G-diketone and GP-1 preparations

Contaminant/Substrate ratio (HPLC peak area)	G-diketone (custom synthesized)	GP-1 (Key Organics)
Vanillin	1:77	None detected
Vanillic acid	1:1765	None detected

Dataset S1. RPKM data, differential expression testing, and gene location and annotation information from RNA-Seq analysis of cultures grown in the presence of glucose and G-type aromatics.

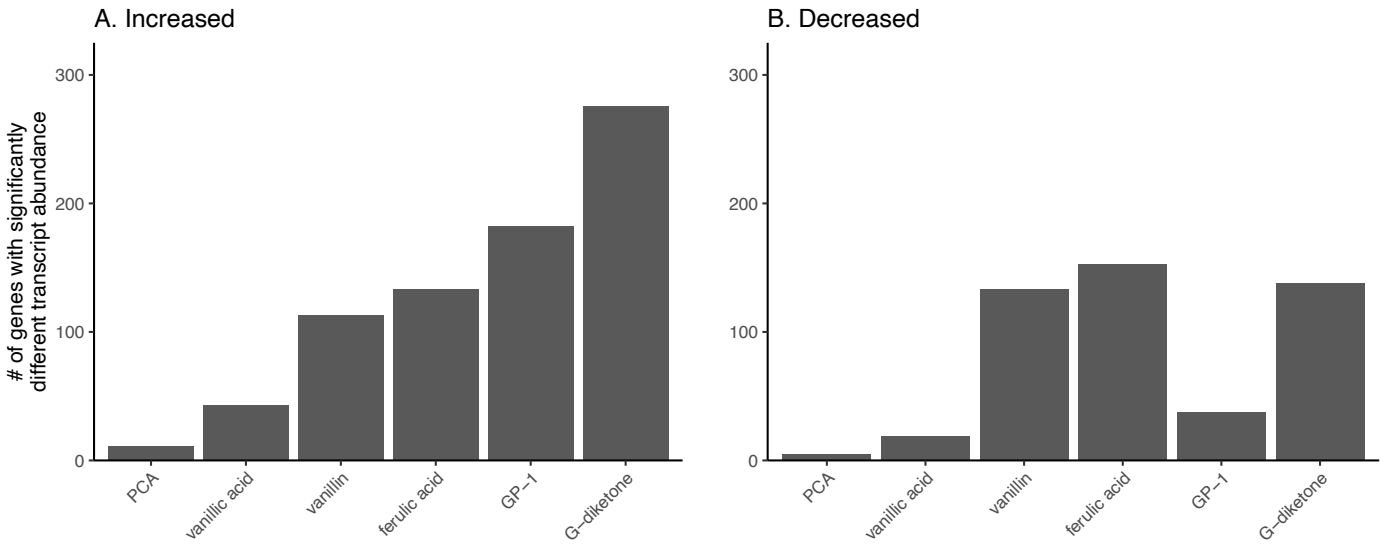


Figure S3. Number of genes that significantly differ in transcript abundance during growth in the presence of glucose alone compared to glucose plus each indicated aromatic substrate. Genes with an FDR < 0.01 are considered significant. Panel A displays counts of genes with increased transcript abundance compared to the glucose control, while Panel B displays counts of genes with decreased transcript abundance.

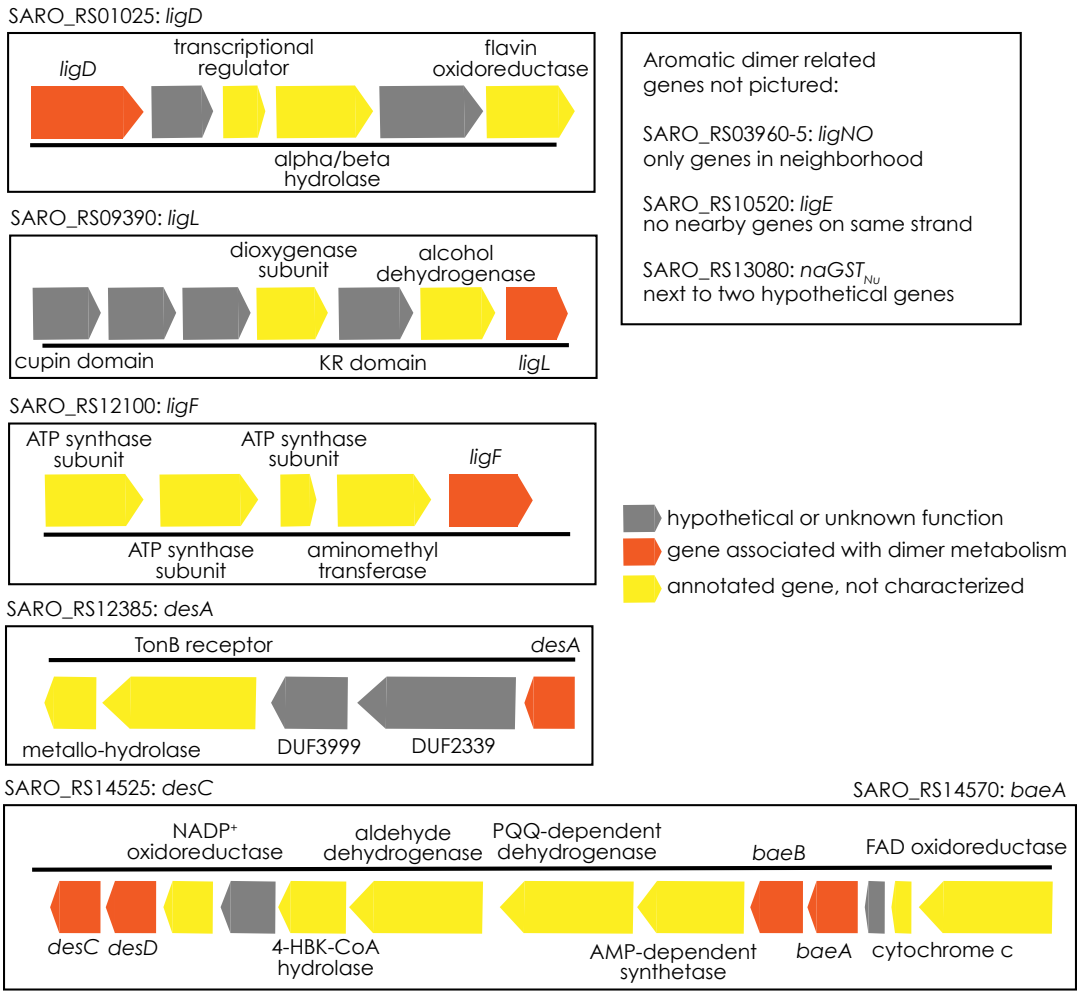


Figure S4. Genomic neighborhoods of *N. aromaticivorans* genes associated with β-O-4 linked aromatic dimer degradation. Shown are position and genes linked to transcripts with increased abundance when cells were grown in the presence of G-diketone and glucose compared to glucose alone.

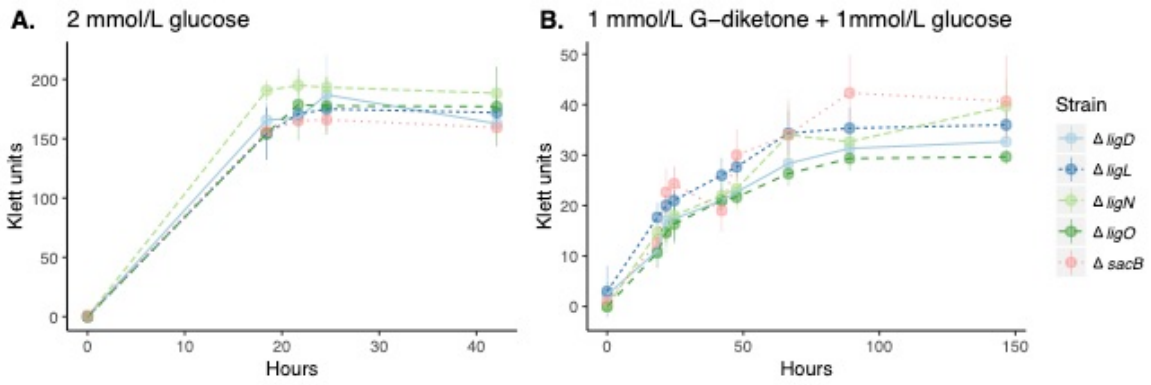


Figure S5. Growth of individual *ΔligLNDO* deletion strains on glucose and glucose plus G-diketone compared to that of a *ΔsacB* parent strain.

Table S4. Strains used in this study.

Strain	Parent strain	Description
Δ*sacB*	Wild type (DSM 12444)	Sucrose sensitivity gene deleted (1)
Δ*ligL*	Δ*sacB*	Markerless deletion of *ligL*
Δ*ligN*	Δ*sacB*	Markerless deletion of *ligN*
Δ*ligD*	Δ*sacB*	Markerless deletion of *ligD*
Δ*ligO*	Δ*sacB*	Markerless deletion of *ligO*

Table S5. Plasmids used to inactivate indicated *lig* genes in this study (2).

Plasmid Name	Base Plasmid	Gene deletion
pJM307	pAK405 (3)	ligD
pJM311	pAK405 (3)	ligO
pJM312	pAK405 (3)	ligN
pJM323	pAK405 (3)	ligL

Table S6. Primers for confirming *lig* deletion mutants (2).

Name	Forward Primer	Reverse Primer	Target
01025	TCAGGTCCACCAGTTCGCCATC	GTCTCTATCGCGTTGACCGACTGG	*ligD*
03960	ACAAGAACTTCGGCCTCTATCGTGAC	GTGAAGCTCGACGTGACCAATCG	*ligO*
03965	CGCGAACTTGGTGGTATTGTAGATGC	CGAAAAGGCGCGAGTGATCTTCTTC	*ligN*
09390	GCTATGCCGAATTTGCCCTGAC	CTGTCGGGATATGCCATCTACATCTGG	*ligL*

Table S7. Multiple-reaction monitoring (MRM) of compounds quantified using HPLC-MS in this study.

Compound	MW (g/mol)	Parent (-) m/z	Transition 1	Transition 2	Transition 3
G-diketone	194.19	193.1	193.1 -> 136.1 CE22	193.1 -> 107.1 CE30	193.1 -> 122.1 CE25
GP-1	196.2	195.2	195.1 -> 180.1 CE15	195.2 -> 136.0 CE22	195.2 -> 108.0 CE25
Vanillic acid	168.15	167	167.0 -> 152.1 CE19	167.0 -> 107.9 CE19	167.0 -> 123.0 CE14

References:

1. Cecil JH, Garcia DC, Giannone RJ, Michener JK. 2018. Rapid, Parallel Identification of Catabolism Pathways of Lignin-Derived Aromatic Compounds in *Novosphingobium aromaticivorans*. Appl Environ Microbiol 84:e01185-18.
2. Kaczmarczyk A, Vorholt JA, Francez-Charlot A. 2012. Markerless gene deletion system for sphingomonads. Appl Environ Microbiol 78:3774–3777.
3. Kontur WS, Bingman CA, Olmsted CN, Wassarman DR, Ulbrich A, Gall DL, Smith RW, Yusko LM, Fox BG, Noguera DR, Coon JJ, Donohue TJ. 2018. *Novosphingobium aromaticivorans* uses a Nu-class glutathione S-transferase as a glutathione lyase in breaking the -aryl ether bond of lignin. J Biol Chem 293:4955–4968.