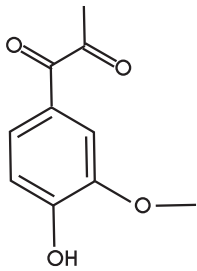
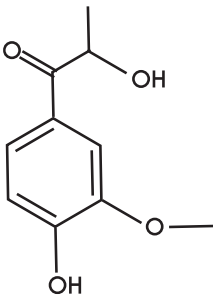
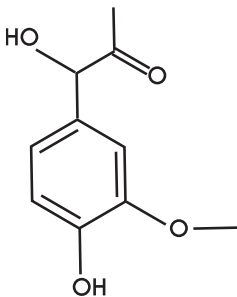
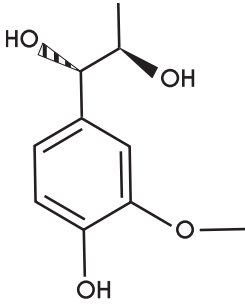
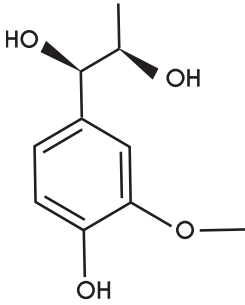
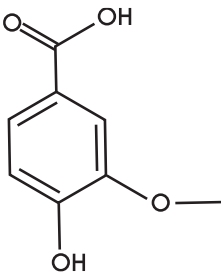
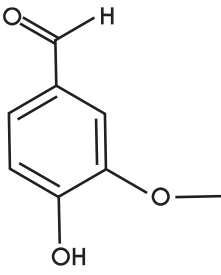
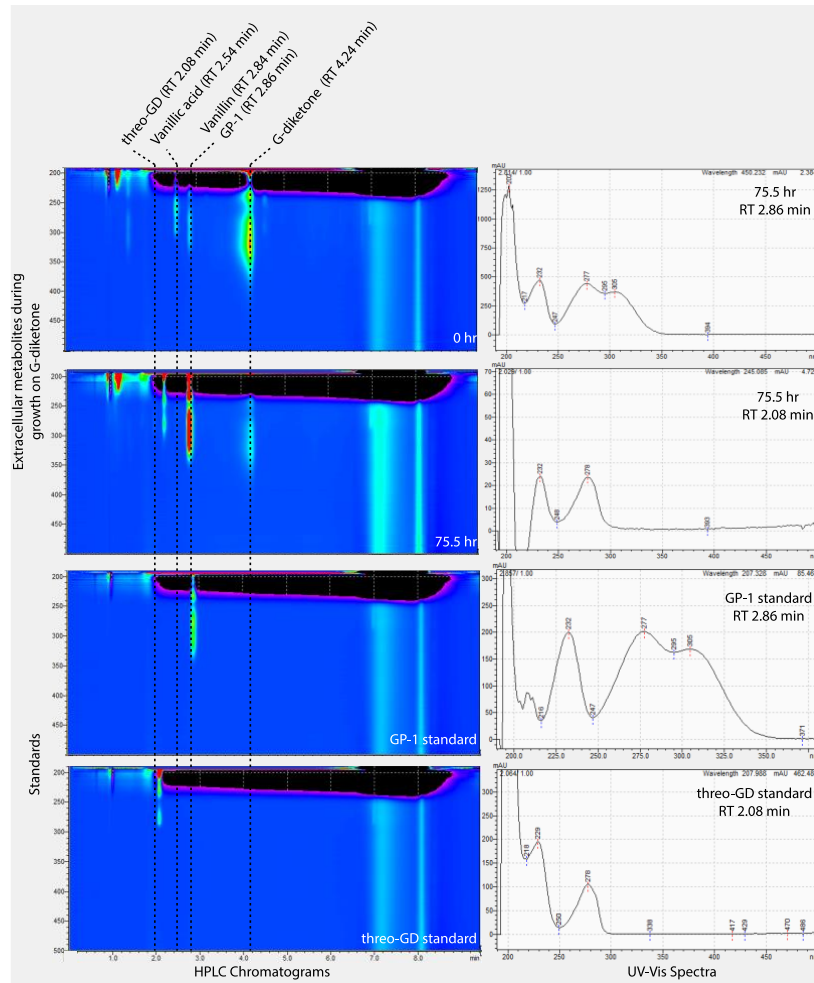


**Table S1. Characteristics of G-diketone and other aromatics analyzed by chromatography**

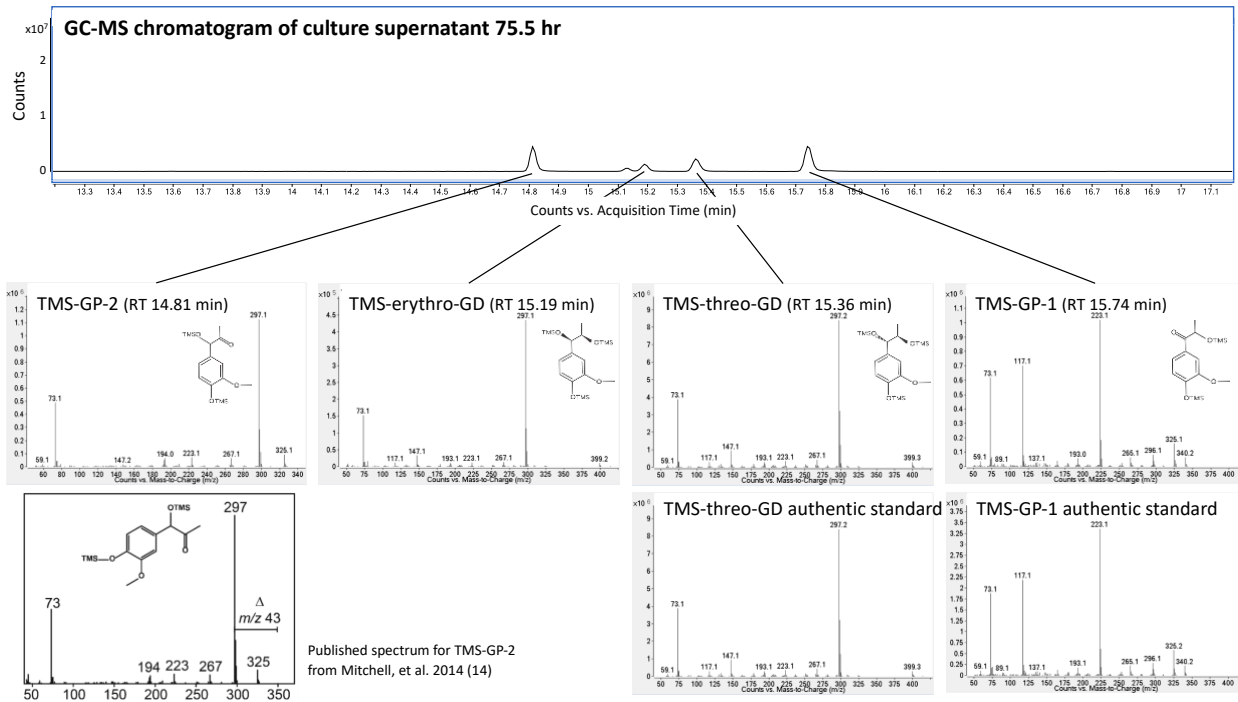
| Short Name | Structure   | IUPAC Name  | Molecular Formula                              | Molecular Weight (g/mol) | Retention time in HPLC (min) | Retention time in GC (min)* |
|------------|---|---|--|--------------------------|------------------------------|-----------------------------|
| G-diketone |    | 1-(4-hydroxy-3-methoxyphenyl)propane-1,2-dione      | C <sub>10</sub> H <sub>10</sub> O <sub>4</sub> | 194.180                  | 4.20                         | 14.0                        |
| GP-1       |   | 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propan-1-one | C <sub>10</sub> H <sub>12</sub> O <sub>4</sub> | 196.200                  | 2.86                         | 15.8                        |
| GP-2       |  | 1-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propan-2-one | C <sub>10</sub> H <sub>12</sub> O <sub>4</sub> | 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. We have also included the published spectrum for GP-2 from Mitchell (2014) (1) for reference to our proposed identification of GP-2.**

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

| <b>Substrates*</b>             | <b>Vanillic acid (<math>\mu\text{M}</math>)**</b> | <b>Vanillin (<math>\mu\text{M}</math>)**</b> |
|--------------------------------|---|--|
| <b>G-diketone plus glucose</b> | 21 $\pm$ 1  | None detected                                |
| <b>GP-1 plus glucose</b>       | 28 $\pm$ 4  | 3 $\pm$ 0                                    |
| <b>Glucose only</b>            | None detected                                     | None detected                                |

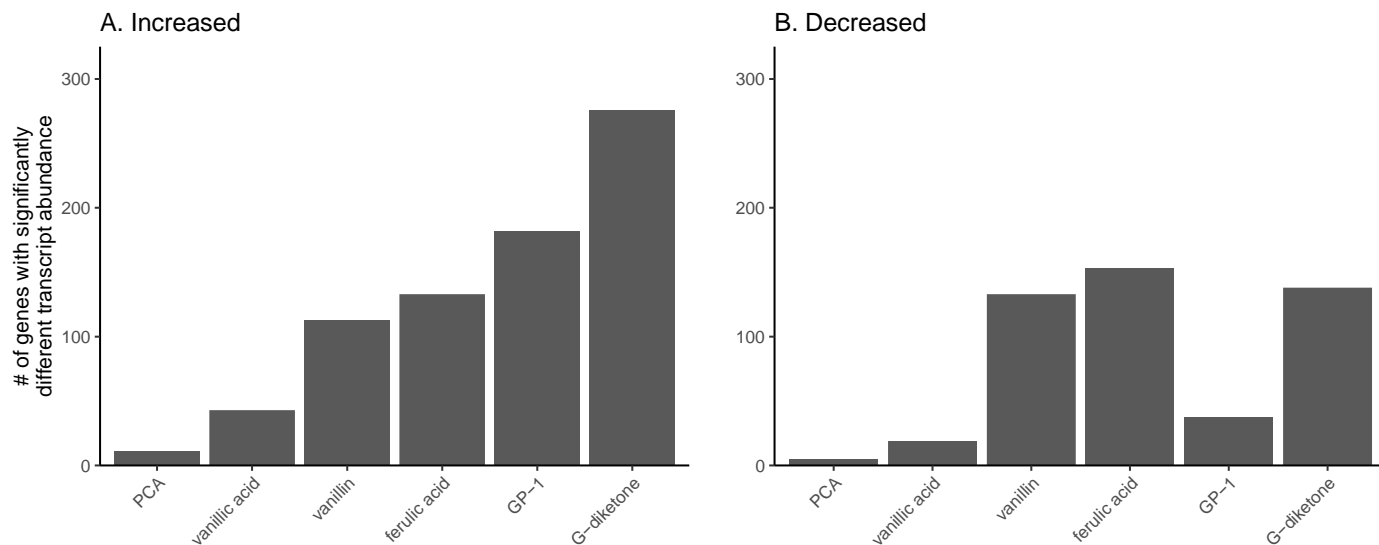
\* Substrates were normalized to having 0.5 gCOD/L of the aromatic compound plus 0.5 g COD/L of glucose.

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

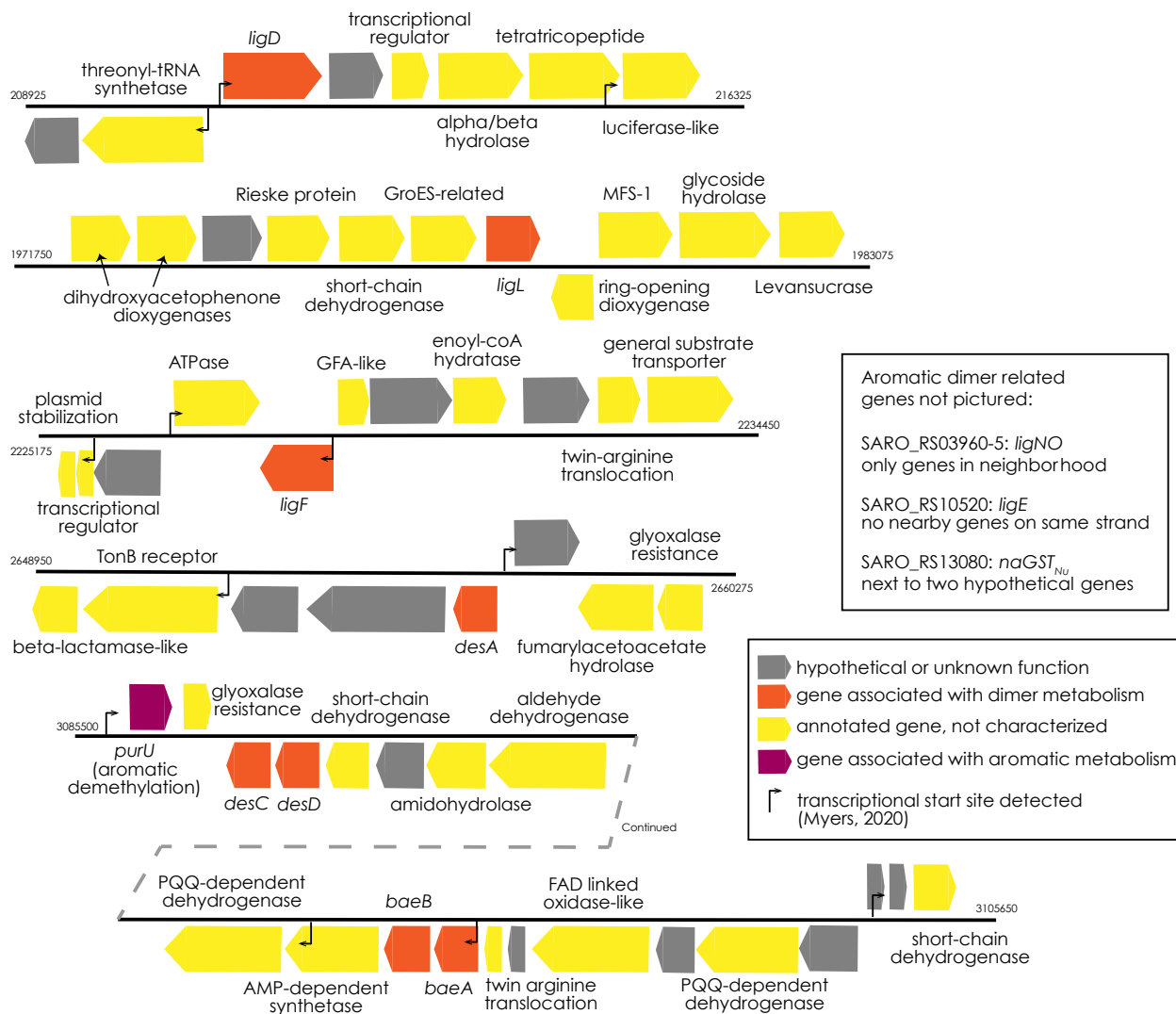
**Table S3.** Quantification of vanillin and vanillic acid in G-diketone and GP-1 preparations

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

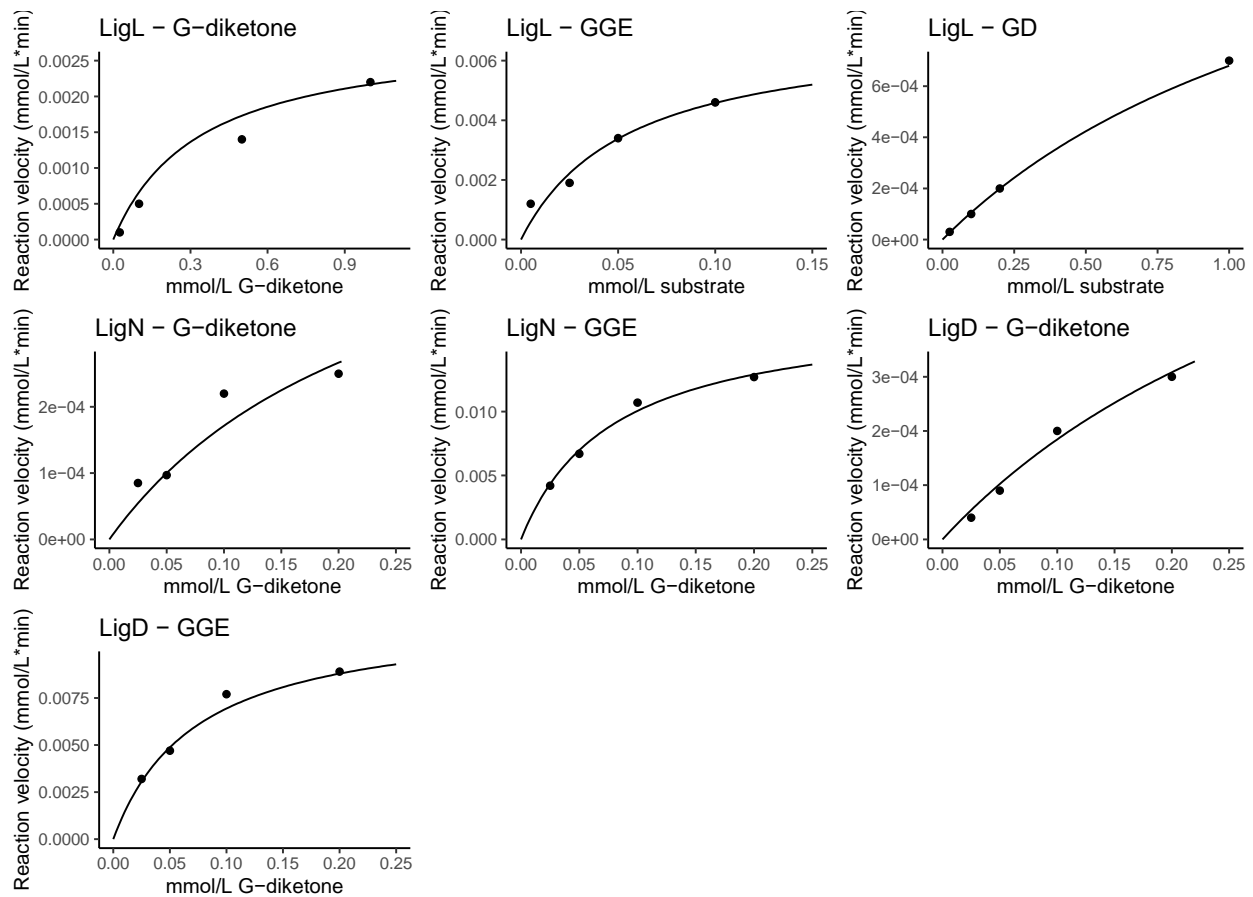
**Dataset S1.** RPKM data, differential expression testing, and gene location and annotation information from RNA-Seq analysis of cultures grown in the presence of glucose and G-type aromatics.



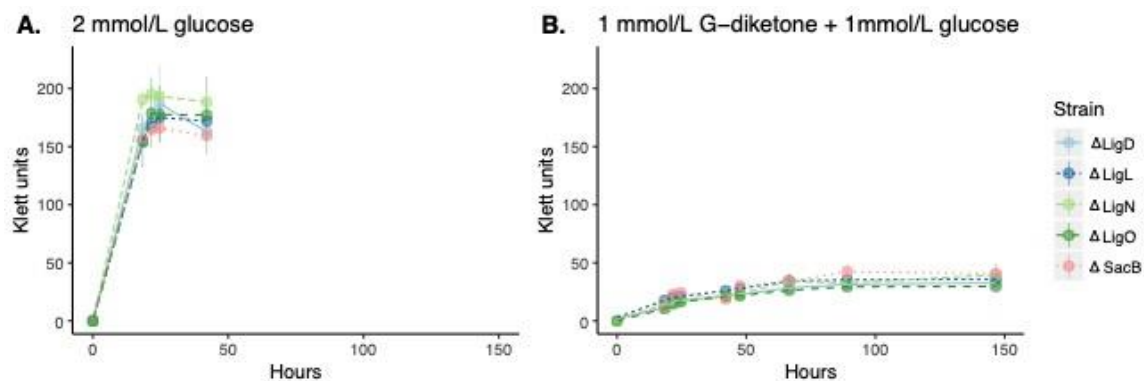
**Figure S3. Number of genes that significantly differ in transcript abundance during growth in the presence of glucose alone compared to glucose plus each indicated aromatic substrate.** Genes with a q-value < 0.01 are considered significant. Panel A displays counts of genes with increased transcript 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  $\beta$ -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. Reaction velocity vs. substrate concentration used to calculate  $K_m$  and  $k_{cat}$  values for LigL, LigN, and LigD on GGE, G-diketone, and GD.**



**Figure S6. Growth of individual 12444 $\Delta$ LigLNDO deletion strains on glucose and glucose plus G-diketone compared to that of the 12444 $\Delta$ SacB parent strain.**



**Table S4. 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<br>CE22 | 193.1 -> 107.1<br>CE30 | 193.1 -> 122.1<br>CE25 |
| GP-1          | 196.2      | 195.2          | 195.1 -> 180.1<br>CE15 | 195.2 -> 136.0<br>CE22 | 195.2 -> 108.0<br>CE25 |
| Vanillic acid | 168.15     | 167            | 167.0 -> 152.1<br>CE19 | 167.0 -> 107.9<br>CE19 | 167.0 -> 123.0<br>CE14 |

**References:**

Myers, KS, Vera, JM, Lemmer, KC, Linz, AM, Landick, R, Noguera, DR, Donohue, TJ. 2020. Genome-wide identification of transcription start sites in two Alphaproteobacteria, *Rhodobacter sphaeroides* 2.4. 1 and *Novosphingobium aromaticivorans* DSM 12444. Microbiology Resource Announcements 9(36) e00880-20.