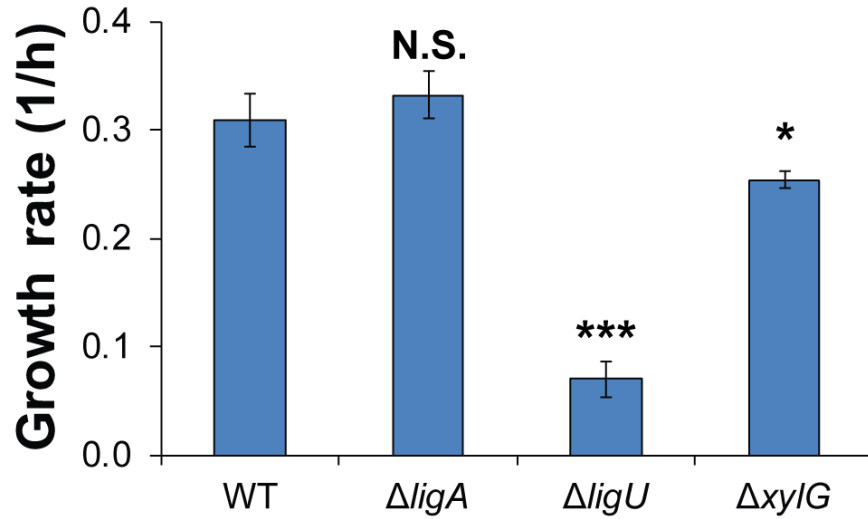
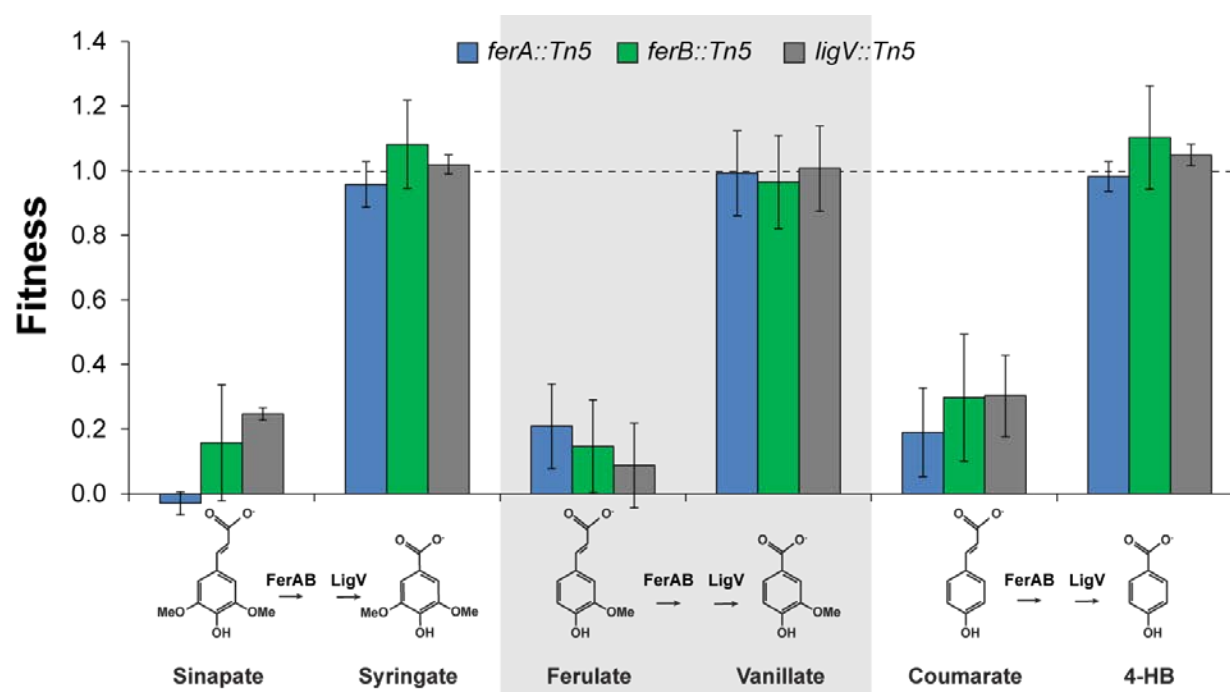


Supplementary Figure 1: Construction of deletion mutants in DSM12444. Deletion plasmids were synthesized *de novo*, with 450 bp regions of homology (orange and purple) flanking the gene to be deleted (green). Approximately 15 bp of the 5' and 3' ends of the gene were retained to minimize polar effects, expanding as necessary to avoid disrupting adjacent genes. Plasmids were introduced into DSM12444 through biparental mating with DAP-auxotrophic *E. coli* containing the appropriate deletion plasmid. Strains with a single crossover integrating the plasmid into the genome are kanamycin-resistant and streptomycin-sensitive (due to a sensitive

rpsL allele). Sequential selection for kanamycin resistance and then streptomycin resistance selects for integration and then loss of the plasmid, either reverting to the WT genotype or producing the desired deletion. The deletion strains can then be identified by colony PCR.

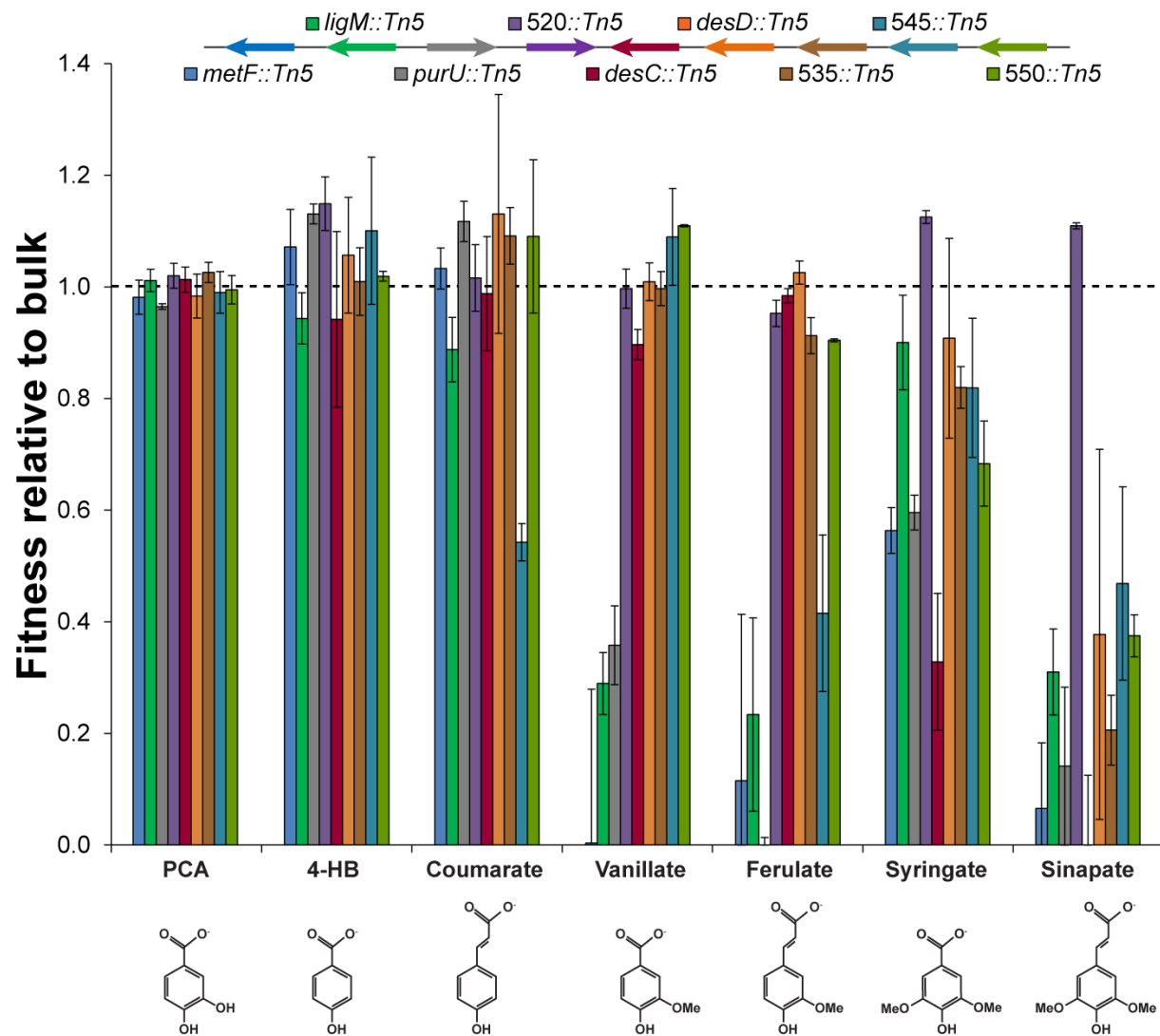


Supplementary Figure 2: Gene deletions affect growth with PCA. Clean deletions were constructed in *ligA*, *ligU*, and *xylG*, and growth with 1 g/L PCA as the sole source of carbon and energy. As predicted from the pooled fitness data, deletion of *ligA* did not affect growth with PCA, while deletion of *ligU* and *xylG* reduced growth. Error bars show one standard deviation, calculated from three biological replicates. * : $p < 0.05$. ***: $p < 0.001$.

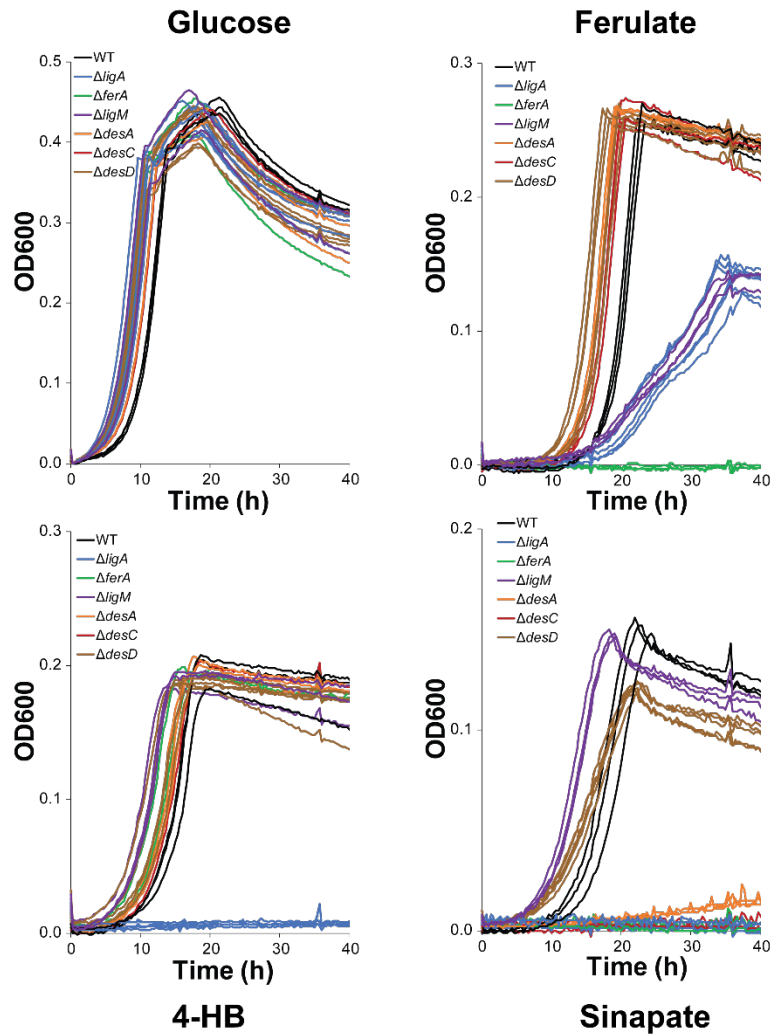


Supplementary Figure 3: Genes involved in deacetylation of sinapate to syringate.

Phenylpropanoids can be deacetylated through the combined action of a CoA-ligase and hydratase/lyase, FerAB, and then oxidized by LigV. Disruption of any of the associated genes significantly diminishes fitness with the phenylpropanoid but not with the associated benzoate derivative. Error bars show one standard deviation, calculated from three biological replicates.



Supplementary Figure 4: Gene disruptions do not affect growth with other phenylpropanoids. Transposon insertions in *desC* and *desD* diminish fitness with sinapate, but not with ferulate or coumarate. The operon structure of the genetic loci is shown above the graph. Error bars show one standard deviation, calculated from three biological replicates.



Supplementary Figure 5: Deletion mutants show growth defects for specific substrates.

Wild-type and deletion mutants were grown with the indicated carbon source. Growth rates extracted from these curves are summarized in Figure 6A.

Supplementary Table 1: Genes referenced in this study

Gene name	Locus	Annotation	SYK-6 homolog	SYK-6 %AA identity
<i>ligA</i>	SARO_RS14270	Protocatechuate 4 5-dioxygenase subunit alpha	SLG_RS06125	68
<i>ligB</i>	SARO_RS14265	Protocatechuate 3 4-dioxygenase subunit beta	SLG_RS06120	70
<i>ligC</i>	SARO_RS14260	Oxidoreductase	SLG_RS06115	87
<i>ligI</i>	SARO_RS14300	2-pyrone-4 6-dicarboxylate hydrolase	SLG_RS06155	83
<i>ligU</i>	SARO_RS14295	4-oxalomesaconate tautomerase	SLG_RS06150	78
<i>ligJ</i>	SARO_RS14275	4-oxalomesaconate hydratase	SLG_RS06130	91
<i>ligK</i>	SARO_RS14290	4-carboxy-4-hydroxy-2-oxoadipate aldolase/oxaloacetate decarboxylase	SLG_RS06145	91
	SARO_RS12260	4-hydroxybenzoate 3-monooxygenase	SLG_RS08320	72
<i>ligM</i>	SARO_RS14510	Vanillate demethylase	SLG_RS06245	78
<i>metF</i>	SARO_RS14505	5,10-methylenetetrahydrofolate reductase	SLG_RS06250	51
<i>purU</i>	SARO_RS14515	Formyltetrahydrofolate deformylase		<40
<i>folD</i>	SARO_RS19830	bifunctional 5 10-methylene-tetrahydrofolate dehydrogenase/5 10-methylene-tetrahydrofolate cyclohydrolase (folD)	SLG_RS01620	54
<i>ferA</i>	SARO_RS03365	Feruloyl-CoA synthetase	SLG_RS12395	67
<i>ferB</i>	SARO_RS08365	p-hydroxycinnamoyl CoA hydratase/lyase	SLG_RS12400	61
<i>ligV</i>	SARO_RS08360	Salicylaldehyde dehydrogenase	SLG_RS03480	87
<i>desA</i>	SARO_RS12095	Syringate O-demethylase	SLG_RS12385	71
<i>desC</i>	SARO_RS14525	Alpha/beta hydrolase	SLG_RS06235	47
<i>desD</i>	SARO_RS14530	Glutathione S-transferase		<40
	SARO_RS14535	Short chain dehydrogenase	SLG_RS06215	59
	SARO_RS14545	4-hydroxyphenyl-beta-ketoacyl-CoA hydrolase	SLG_RS06210	83
	SARO_RS14550	Aldehyde dehydrogenase	SLG_RS04675	50
<i>xylE</i>	SARO_RS17090	Catechol 2,3-dioxygenase		<40
<i>xylG</i>	SARO_RS17080	2-hydroxymuconic semialdehyde dehydrogenase		<40
<i>xylH</i>	SARO_RS17055	4-oxalocrotonate tautomerase		<40

<i>xylI</i>	SARO_RS17060	4-oxalocrotonate decarboxylase	<40
<i>xylJ</i>	SARO_RS17075	2-keto-4-pentenoate hydratase	<40
<i>xylK</i>	SARO_RS17065	4-hydroxy-2-oxovalerate aldolase	<40
<i>xylQ</i>	SARO_RS17070	Acetaldehyde dehydrogenase	<40