Title: Genome-wide identification of tomato xylem sap fitness factors for *Ralstonia pseudosolanacearum* and *Ralstonia syzygii*

Authors: Stratton Georgoulis[a] †, Katie E. Shalvarjian[b] †, Tyler C. Helmann[b,c], Corri D. Hamilton[d], Hans K. Carlson[e], Adam M. Deutschbauer[b,e], and Tiffany M. Lowe-Power[a,b]#

[a]Dept Plant Pathology, UC Davis, Davis, California, USA

[b]Dept Plant and Microbial Biology, UC Berkeley, Berkeley, California, USA

[c]Emerging Pests and Pathogens Research Unit, Robert W. Holley Center, Agricultural Research Service, U.S. Department of Agriculture, Ithaca, NY, USA

[d]Dept Plant Pathology, UW-Madison, Madison, Wisconsin, USA

[e]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA

† Indicates equal contributions as co-first authors. Author order was decided alphabetically by last name.

Running Title: *Ralstonia* fitness factors for xylem sap growth

 # Corresponding author: Tiffany M. Lowe-Power, tlowepower@ucdavis.edu

Abstract Word Count: 214

Text word count: 4218

Abstract

Plant pathogenic *Ralstonia* spp. colonize plant xylem and cause wilt diseases on a broad range of host plants. To identify genes that promote growth of diverse *Ralstonia* strains in xylem sap from tomato plants, we performed genome-scale genetic screens (random barcoded transposon mutant sequencing screens; RB-TnSeq) in *Ralstonia pseudosolanacearum* GMI1000 and *R. syzygii* PSI07. Contrasting mutant fitness phenotypes in culture media versus in xylem sap suggest that *Ralstonia* strains are adapted to sap and that culture media impose foreign selective pressures. Although wild-type *Ralstonia* grew in sap and in rich medium with similar doubling times and to a similar carrying capacity, more genes were essential for growth in sap than in rich medium. Multiple mutants lacking amino acid biosynthesis and central metabolism functions had fitness defects in xylem sap and minimal medium. Our screen identified > 26 genes in each strain that contributed to growth in xylem sap but were dispensable for growth in culture media. Many sap-specific fitness factors are associated with bacterial stress responses: envelope remodeling and repair processes such as peptidoglycan peptide formation (*murI* and RSc1177), LPS O-antigen biosynthesis (RSc0684), periplasmic protein folding (*dsbA*), drug efflux (*tolA* and *tolR*), and stress responses (*cspD3*). Our genome-scale genetic screen identified *Ralstonia* fitness factors that promote growth in xylem sap, an ecologically relevant condition.

Importance

Traditional transposon mutagenesis genetic screens pioneered molecular plant pathology and identified core virulence traits like the type III secretion system. TnSeq approaches that leverage next-generation sequencing to rapidly quantify transposon mutant phenotypes are ushering in a new wave of biological discovery. Here we have adapted a genome-scale approach, random barcoded transposon mutant sequencing (RB-TnSeq), to discover fitness factors that

promote growth of two related bacterial strains in a common niche, tomato xylem sap. Fitness of wild-type and mutants show that *Ralstonia* spp. are adapted to grow well in xylem sap from their natural host plant, tomato. Our screen identified multiple sap-specific fitness factors with roles in maintaining the bacterial envelope. These factors are putative adaptations to resist plant defenses, including antimicrobial proteins and specialized metabolites that damage bacterial membranes.

Introduction

Ralstonia pseudosolanacearum, *R. syzygii*, and *R. solanacearum* (hereafter, "*Ralstonia*") comprise a monophyletic but diverse species complex of plant wilt pathogens (1, 2). *Ralstonia* strains are adapted to numerous plant hosts belonging to over 50 botanical families and are distributed worldwide in in warm tropics and temperate subtropical highlands with year-round precipitation (3–7).

Most *Ralstonia* strains invade plant roots, gain entry to the water-transporting xylem vasculature, spread systemically, disrupt xylem function, and fatally wilt the host plant (8). Key molecular pathogenesis, virulence, and fitness traits have been identified by studying model strains like *R. pseudosolanacearum* GMI1000. However, comparative genomics shows that the *Ralstonia* species complex is genetically heterogeneous (1, 9–11). An average *Ralstonia* genome contains approximately 5,000 genes and the pangenome exceeds 16,700 genes, but less than 1,940 core genes are shared amongst all *Ralstonia* strains (11). Accordingly, ecological niches, host ranges, and physiological traits vary widely among *Ralstonia* strains (1, 12–14). Developing and studying new model strains that capture the phylogenetic diversity of the *Ralstonia* species complex will provide a more complete view of these pathogens' biology.

We hypothesized that diverse *Ralstonia* strains have genetically encoded fitness factors that contribute to their growth in xylem sap of tomato plants, a common host. In this study, we

used a barcoded *mariner* transposon library developed for RB-TnSeq (15) to create genome-wide transposon insertion mutant libraries of three tomato-pathogenic *Ralstonia* species: *R. pseudosolanacearum* GMI1000, and *R. syzygii* PSI07, and *R. solanacearum* IBSBF1503. We mapped the transposon insertion sites and predicted core essential genes. We performed screens to identify genes that contribute to growth of GMI1000 and PSI07 in xylem sap from healthy susceptible tomato. Comparison of genes' fitness contributions between conditions revealed sap-specific fitness factors and genes that contribute to fitness in both sap and in culture media.

Results

Adapting RB-TnSeq for plant pathogenic *Ralstonia* isolates

To identify genes required for *Ralstonia* growth in diverse conditions, we constructed barcoded transposon insertion mutant libraries of three tomato-pathogenic *Ralstonia* isolates: *R. pseudosolanacearum* GMI1000, *R. syzygii* PSI07, and *R. solanacearum* IBSBF1503. We chose these strains because they span the genetic diversity of plant pathogenic *Ralstonia* and because there are high-quality, complete genomes are available for each strain.

The mutant libraries were created by conjugation with the pKMW3 *E. coli* donor library, which carries a pool of *mariner* transposon delivery vectors in which each transposon is marked by a unique 20 base pair sequence. These unique sequences function as DNA "barcodes". The pooled *Ralstonia* mutant libraries contain about 10^5 transposon insertion mutants marked with unique barcodes. We mapped the insertion site of each barcoded transposon. Table 1 shows detailed summary statistics of each mutant library. For barcode sequencing (BarSeq) mutant fitness assays, we calculate fitness contributions using transposons that had inserted into the central 80% of the open reading frame (ORF). Insertions at gene's N- and C-terminus are less

likely to impair protein function. The barcoded mutant libraries cover 79-86% of the protein coding genes of each strain.

Predicting genes essential for *Ralstonia* growth in rich medium

A subset of the 14-21% of genes without transposon insertions would be essential. Other genes not represented in the mutant libraries either lack TA dinucleotide insertion sites for the *mariner* transposon or were missed by chance. Table S1 lists genes that lack fitness data and the number of TA sites in the central 80% of each gene. Additionally, it is not possible to uniquely map transposon insertions in repetitive, indistinguishable genomic regions like the 31 kb tandem duplication in the GMI1000 genome. Even if these genes contribute to fitness in a condition, they would likely be functionally redundant with their paralogs, mitigating any potential impact on fitness.

We predicted essentiality of genes in each strain (Table S1) using the standard Bio::TraDIS pipeline (16) and our previously described analysis pipeline (17). Briefly, both methods predict gene essentiality based on genes that lacked transposon insertions in the central 80% of the coding region. After setting cut-offs to exclude short genes, which are more likely to lack transposon insertions, 450-500 genes were predicted to be essential per strain by at least one pipeline, while 393-396 genes per strain were predicted to be essential by both pipelines (Table S1). To compare results between strains, we identified orthologous genes in GMI1000, PSI07, and IBSBF1503 using the JGI-IMG (18) Genome Gene Best Homologs tool. A core set of 244 genes was predicted to be essential by both pipelines in all three strains (Fig S1) (19). We inspected the predicted putative essential gene set for a positive control, the *speC* ornithine decarboxylase. We previously showed that a GMI1000 *speC* mutant (RSc2365) is a putrescine

auxotroph that cannot not grow in unamended CPG medium (8), and both pipelines predicted *speC* was essential.

To visualize which cellular processes are predicted essential for growth in rich CPG medium, we used KEGG Mapper (20) to query the KEGG database with the GMI1000 locus tags of the 244 genes predicted to be essential in all three strains. As expected, many of these genes are involved in central cellular functions, such as DNA replication, transcription, translation, mRNA degradation, and lipid and peptidoglycan biosynthesis. Several genes from central carbon metabolism and respiration are predicted to be essential, including components of glycolysis/gluconeogenesis, the pentose phosphate pathway, the TCA cycle, NADH dehydrogenase, and ATP synthase. Genes involved in cofactor and vitamin biosynthesis (ubiquinone, coenzyme A, thiamine, heme, and folate) are putatively essential. Genes that encode biosynthetic steps of several amino acids, including threonine, arginine, lysine, and histidine, also appear to be essential.

Growth of *Ralstonia* species representatives in xylem sap

Before conducting an RB-TnSeq genome-wide mutant fitness screen in xylem sap, we empirically determined the carrying capacity of xylem sap to design a screen with a sufficient number of cell doublings to quantify mutant fitness defects. We compared growth of *R. pseudosolanacearum* GMI1000 in xylem sap harvested from greenhouse-grown Moneymaker tomato plants to growth in rich and minimal media (Fig S2A-B). The carrying capacities of xylem sap and minimal medium were similar (3.4-4.8 × 10^8 CFU/ml in sap and 2.2-4.6 × 10^8 CFU/ml in minimal medium), while the carrying capacity of rich media was higher (2.4-2.6 × 10^9 CFU/ml). Both xylem sap and rich medium cultures exceeded 1 × 10^8 CFU within 24 hours while minimal medium cultures had an extended lag time. We were surprised by the high

carrying capacity supported by xylem sap based on our previous experience growing diverse *Ralstonia* in xylem sap from a different susceptible tomato (Bonny Best) grown in environmentally controlled growth chambers (8, 21). To determine whether the growing conditions or plant cultivar influenced the saps' carrying capacity, we collaborated with researchers at University of Wisconsin—Madison to repeat this experiment with sap from growth chamber-grown Moneymaker and Bonny Best tomato. Each *Ralstonia* isolate grew to a higher carrying capacity in xylem sap from Moneymaker plants (3.3-6.6 × 10^9 CFU/ml) than sap from Bonny Best plants (3.62 × 10^7-5.36 × 10^8 CFU/ml) (Fig S2C-E).

To estimate the doubling rate in xylem sap and rich and minimal culture media, we compared growth of GMI1000, PSI07, and IBSBF1503 with frequent sampling intervals (every 4 hours, Fig 1A). All strains grew to a high final density in xylem sap in this trial (over 10^9 CFU / ml). Strains grew slowest in minimal medium (Fig 1B). The maximum doubling rate in xylem sap was 120-131% higher than in rich medium ($P<0.005$ by ANOVA with Tukey's multiple comparison's test).

RB-TnSeq experiments identify mutants with altered fitness in xylem sap and minimal medium.

We used RB-TnSeq to identify genes that affect fitness in *ex vivo* xylem sap, minimal medium, and rich medium. We profiled the GMI1000 and PSI07 mutant libraries in all three conditions and profiled the IBSBF1503 mutant library only in rich and minimal culture. Mutant abundance before and after selective competitive growth was measured by BarSeq. In BarSeq analysis, each gene is assigned a fitness score ("Fit" score), which is a weighted average of the $\log_2$ ratios of each barcoded mutant's abundance after growth in the condition vs. its initial abundance (15). In total, our BarSeq assays measured fitness contributions for approximately

4,000 genes per strain. Each gene's Fit score is derived from fitness measurements from multiple, independent transposon mutants (see Table 1). Table S2 contains the Fit scores and t-like test statistics for each gene in each condition. This table also includes previously published transcriptomic data sets available for GMI1000 showing PhcA quorum sensing-induced and *in planta*-induced genes (22, 23). This data is not available for PSI07 or IBSBF1503. Fitness data is also publicly available on the interactive Fitness Browser (http://fit.genomics.lbl.gov/).

Fit scores varied between strains and culture conditions (Fig 2A-C). Because Fit scores are on a $\log_2$ scale, a score of -5 theoretically shows that those mutants replicated for 5 less generations than the population. In practice, we found that the strongest Fit scores sometimes exceeded the number of generations that we measured by dilution plating. For example, RSc1956 had a mean Fit score of –8.6 in xylem sap even though the population grew for only 7 generations. However, the majority of Fit scores were in the expected ranges for each replicate.

There were more mutants with fitness defects in the xylem sap than culture media conditions (MM and CPG). Disruption of 69 PSI07 and 80 GMI1000 genes led to fitness defects in xylem sap compared to 34-41 and 63-69 genes in CPG and MM, respectively. Similarly, more mutants had increased fitness in culture media than in xylem sap. Disruptions of dozens of PSI07 genes and a few GMI1000 genes improved growth only in culture media.

Many PSI07 and GMI1000 genes had intersecting fitness contributions in 2 or more conditions as well as condition-specific fitness effects (strong fitness: Fit > 2 or <-2; moderate fitness: Fit > 1 or < -1) (Fig 2D-E). Although most genes contributed to fitness in only one condition, 12 PSI07 and 15 GMI1000 genes strongly contributed to growth in both MM and xylem sap. Genes with shared phenotypes between CPG and either xylem sap or MM were less

common, likely because genes required in CPG are inherently excluded during library preparation.

Mutants with improved fitness

Most mutants that with increased fitness had transposon insertions in regulatory genes (Fig 3). Consistent with previous results showing that the PhcA quorum sensing regulator mediates a trade-off between fast growth at low cell density and expression of *in planta* fitness traits at high cell density (23, 24), GMI1000 and IBSBF1503 *phcA* mutants had improved growth in all tested conditions (Fig 3A). Unexpectedly, PSI07 *phcA* mutants behaved differently. Although PSI07 *phcA* mutants had increased fitness in minimal medium (Fit: 1.9), they had strongly reduced growth in xylem sap (Fit: -2.8). At high cell densities, PhcA positively regulates production of an energetically costly extracellular polysaccharide (EPS) (24, 25). Transposon insertion in other regulatory genes that activate EPS production (*phcSR*, *vsrAD*, and *rpoS*) (26, 27) also increased fitness in one or more strains (Fig 3A). Mutating two unstudied regulators increased fitness of PSI07: the RSp0224 MarR regulator nested in an HCA degradation cluster (13) and the RSp1579 regulator that is constitutively expressed *in planta* and co-localized with an ABC transport system (28) (Fig 3B). PSI07 may have more EPS production than

Several non-regulatory mutants also gained fitness. These include a HipA toxin from a type II toxin-antitoxin system (RSc2508), a LysM peptidoglycan binding protein (RSc1206; PSI07 only), a PhcA-regulated polyhydroxybutyrate metabolism phasin (RSc1605; PSI07 only) (23), and a phosphoenolpyruvate-protein phosphotransferase (RSc0348; PSI07 and IBSBF1503).

Amino acid biosynthesis genes are required for fitness in xylem sap and minimal media

Overall fitness patterns for amino acid biosynthesis mutants were similar between the strains (Fig 4, Fig S3). As expected, many amino acid biosynthetic mutants had strong fitness defects in minimal medium. Most of these same mutants also had moderate to strong fitness defects in xylem sap. However, several PSI07 mutants had strong defects in minimal medium but weak to neutral Fit scores in xylem sap: Glu and branched chain amino acid (Leu and Ile) biosynthesis mutants of PSI07 lacked defects in sap (*leuB1, leuC, gltB*). The orthologous mutants in GMI1000 had defects in sap. This might reflect batch-to-batch variation in sap metabolite composition or strain-to-strain differences.

Envelope and metabolism genes are required for growth in xylem sap

We investigated genes that had stronger contributions to fitness in sap than in culture media (Fig 5). In GMI1000, 26 genes contributed to fitness specifically in sap (Fit < -1). In PSI07, 32 genes contributed to fitness specifically in sap (Fit < -1). Fitness patterns were generally congruent between strains. Of the top ten GMI1000 genes strongly and specifically required in sap, PSI07 required six of the homologs specifically in sap and one homolog in both sap and CPG.

Many of the sap-specific fitness genes are involved in bacterial envelope biology: LPS biosynthesis (*rfbA* RSc0684), peptidoglycan remodeling (*murI* RSc1956 and a peptidoglycan endopeptidase RSc1177), folding of periplasmic proteins (*dbsA* RSc0285), or drug efflux (*tolA* RSc0734 and *tolR* RSc0733, a presumed *tolA* activator) (Fig 5A). The *cspD3* gene (RSp0002), encoding a cold shock family protein, also contributed to fitness in xylem sap (Fig 5C). In *Ralstonia pseudosolanacearum* CQPS-1, *cspD3* negatively regulates swimming motility and contributes to virulence on tobacco, but does not influence cold adaptations (29). Recent

informatic and functional studies reveal that this gene is a microbial-associated molecular pattern recognized by a plant pattern recognition receptor (30, 31).

Genes involved in biosynthesis of the cofactors thiamine (*thiC* RSc0113), pantothenate (*panD* RSc2731), and tetrahydrofolate (*purU* RSc1873) were required for fitness in xylem sap (Fig 5B). Because these mutants had neutral fitness or dramatically less severe fitness in minimal medium which also lacks these cofactors, the defects might be due to secondary metabolite salvage functions of these enzymes. In contrast *nadD* mutants (RSp1176), lacking a NAD biosynthesis step, had similar fitness defects in all conditions. Mutations in two metabolism genes reduced fitness specifically in sap: the fructose-bisphosphate aldolase *fbaA* gene (RSc0573) involved in glycolysis, gluconeogenesis, and the Calvin cycle and *gabD* (RSc0028). GabD encodes a succinate-semialdehyde dehydrogenase that contributes to GABA utilization via a two-step pathway (GabT and GabD). A recent study suggested that GABA utilization contributes to *Ralstonia*'s fitness in tomato stem by studying a "*gabT*" mutant with a polar gentamicin cassette inserted into the promoter of the putative *gabTD* operon (32). In our screen, *gabT* mutants had neutral fitness in xylem sap (Fit: -0.2) while *gabD* mutants had strong defects (Fit: -2.05), suggesting that some of the previously described phenotypes of the promoter mutant could be due to polar effects on *gabD* in addition to *gabT*.

Discussion

Xylem sap is widely considered to be nutrient poor, and yet *Ralstonia* and other vascular pathogens grow prolifically in the xylem. We previously found that tomato xylem sap contains over 100 metabolites and that amending minimal medium with sap increases the growth rate of *Ralstonia* GMI1000 (8). To identify the genetic underpinnings of *Ralstonia* fitness in xylem sap,

we performed a TnSeq screen in two *Ralstonia* species representatives in tomato sap. Although wild-type *Ralstonia* grew similarly in tomato xylem sap and rich medium, phenotypes of amino acid and vitamin biosynthesis mutants confirmed that sap is an incomplete medium (28, 33–35). In contrast, an RB-TnSeq study using *Caulobacter crescentus* shows that lake water, the natural environment of *Caulobacter*, is a dilute rich medium (36). Amino acid and vitamin auxotrophic *Caulobacter* mutants had less severe fitness defects in lake water than in minimal medium. The restriction of certain amino in xylem sap is consistent with "nutritional immunity" in the xylem (37, 38).

TnSeq approaches are an efficient way to rapidly screen for genes with non-redundant contributions to competitive fitness. Here we identified multiple stress- and envelope-related gene that promote growth of diverse *Ralstonia* strains in xylem sap from susceptible tomato. These factors may be adaptations to resist "phytoanticipin" antimicrobial proteins (39) and specialized metabolites (40) that function as innate plant immune defenses. Our results suggest that *Ralstonia* uses TolA drug efflux protein to tolerate preformed plant defenses in xylem sap from healthy plants in addition to requiring AcrA/DinF efflux proteins to cause full wilt disease on tomato (41).

Novel environments provide strong selective pressures for bacterial evolution (42). We suggest that the number of gain-of-fitness mutations in a TnSeq screen may be an indicator of how well the test conditions match the environment to which the bacterial isolate is adapted. For instance, transposon insertions in over two dozen *E. coli* increased its growth on cheese agar (43), a foreign growth medium. Similarly, serial passage of *Ralstonia* GMI1000 in *Medicago* nodules (a foreign environment) selected for more gain-of-fitness mutations than serial passage in tomato stems (44, 45). In our study, few mutations increased fitness in sap, but many mutants

increased fitness in artificial culture media, suggesting that the *Ralstonia* isolates are well-adapted to grow in tomato xylem sap. Meta-analyses of TnSeq studies across multiple bacteria in novel and naturalistic conditions can test the hypothesis that gain-of-fitness phenotypes are more common when mutants grow in novel conditions.

Identifying absolutely essential genes of human pathogens aids in antibacterial drug development research (46), but precision antimicrobial treatment for most plant pathogens is not financially viable and poses environmental concerns. Nonetheless, identifying putative essential genes has fundamental value, particularly to the *Ralstonia* research community. Creating mutants lacking these genes might be impossible or require specialized selection media as was required for a Δ*speC::*Sm mutant that had a putrescine auxotrophy (8). Here we identified sets of ~400 essential genes in three *Ralstonia* strains. As expected, many of the putative essential genes are involved in central dogma and biosynthesis processes. The essentiality of multiple amino acid biosynthesis genes in the peptone- and casamino acid-containing CPG rich medium suggests *Ralstonia* may lack effective transporters for some amino acids. Recently, Su *et al.* predicted 464 essential genes in *Ralstonia* GMI1000 (47) using a TnSeq approach, but our libraries contain mutants with transposon insertions in 126 of these genes. Of these 126 genes, only three genes contributed to fitness more than two-fold in any tested condition. These discrepancies highlight the limitations of using randomly generated mutant libraries to predict gene essentiality. Targeted experiments such as CRISPRi (48) may test essentiality more robustly.

Traditional transposon mutagenesis genetic screens pioneered molecular plant pathology and identified core virulence traits like the type III secretion system (49–51). TnSeq approaches leverage next-generation sequencing to rapidly quantify mutant phenotypes. These genome-wide fitness assays are a powerful approach to rapidly investigate basic bacterial biology and identify

pathogen, commensal, and mutualist fitness factors. TnSeq studies have identified genes that promote bacterial fitness in their native environments including plant hosts (52–58) animal hosts (59–61), soft-rind cheese (43), and lake water (36). The RB-TnSeq technique that we use here is a powerful TnSeq methodology that has a low cost and technical barrier per sample, which facilitates the profiling of the same mutant library across dozens of conditions (15, 62). Future studies will profile fitness of *Ralstonia* mutants in tomato plants to test whether sap fitness factors also contribute to fitness in the host.

Methods:

Bacterial Strains and Growth Conditions

This study uses three *Ralstonia* strains, one representative per plant pathogenic species: *R. pseudosolanacearum* GMI1000 (phylotype I sequevar 18), *R. syzygii* PSI07 (phylotype IV sequevar 10), and *R. solanacearum* IBSBF1503 (phylotype IIB sequevar 4). All isolates are pathogenic on tomato (11), and the strains have variable carbon utilization profiles (Table S3). All strains have a pH optimum between 5 and 7 (Fig S4).

Ralstonia was routinely grown in CPG rich medium (per L: 1 g casamino acids, 10 g Bacto-peptone, 5 g glucose, 1 g yeast extract) at 28°C. Agar plates were supplemented with 1% tetrazolium chloride to confirm colony morphology. For minimal medium growth curves and fitness assays, strains were grown in quarter-strength M63 (per L: 3.4 g KH_2PO_4, 0.5 g $(NH_4)_2SO_4$, 10 µl of 1.25 mg/ml $FeSO_4 \bullet 7H_2O$, 51.7 µl of 1 M $MgSO_4$; adjusted to pH 7) with 10 mM glucose. To profile carbon utilization patterns with 95 substrates, strains were inoculated at OD_{600} = 0.02 into minimal media with 10 mM test carbon sources (see Table S3) in a 384-well plate.

Construction of barcoded transposon mutant libraries

Barcoded *mariner* transposon mutant libraries were created in three wild-type strain backgrounds: GMI1000, PSI07, and IBSBF1503. The barcoded transposons were introduced via conjugation. Recipient strains (*Ralstonia* isolates) were grown overnight in 5 ml CPG at 28°C with shaking at 200 rpm. A 2 ml portion of the overnight culture was sub-cultured into 25 ml CPG and grown for 2 hours at 28°C. The donor *E. coli* strain (an auxotroph for diaminopimenlate [DAP]) carrying the pKMW3 (Kan^R) barcoded *Mariner* transposon vector library (15) was thawed on ice and 1 ml was inoculated into 20 ml LB with 25 µg / ml kanamycin and 300 µM DAP and grown to mid-log phase at 37°C with shaking. Conjugations were carried out as previously described (63). Donor and recipient strains were centrifuged at 5000 x*g* for 10 min and resuspended in CPG + 300 µM DAP. Recipient strains and the donor strain were adjusted to OD_{600} of 3.0 and 1.0, respectively, and mixed in equal volumes. In total, 1.2 ml of the strain mixture was spotted onto 12 nitrocellulose filters (82 mm discs with 0.45 µM pore size cut into sixths) overlaid on four plates of CPG with 300 µM DAP and without glucose. Mating was allowed to occur for 3-4 hours at 28°C. Filters were pooled in 20 ml CPG broth and vortexed in a 50 ml tube for 30 s to dislodge bacteria. The suspension was adjusted to 80 ml and evenly spread over 400 CPG plates (200 µl per plate) with 25 µg / ml kanamycin. To calculate transformation efficiencies, a portion of each suspension was dilution plated on CPG with kanamycin (to quantify transformants) and without kanamycin (to quantify total *Ralstonia* cells). Transformation efficiencies were: GMI1000, 3.9×10^{-5}; PSI07, 8.6×10^{-3}; IBSBF1503, 1.5×10^{-4}. Selection plates were incubated for 2 days at 28°C. Cells were harvested by scraping and pooling approximately 2×10^6 colonies in CPG broth with kanamycin. Density was adjusted to

OD_{600} = 0.25 in 200 ml and grown at 28°C with shaking until OD_{600} reached 1.0. Individual 1 ml aliquots were preserved in 20% v/v glycerol (final concentration) at -80°C.

Mapping transposon insertion sites.

Genomic DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit and prepped for sequencing and mapping using the adapter ligation protocol described in (15). Samples were sequenced on a HiSeq4000 at QB3 Berkeley Genomics Center. TnSeq reads were analyzed with a custom Perl script, MapTnSeq.pl, which assigns each unique barcode sequence to a corresponding location in the genome. Each barcode was mapped to a single insertion site by identifying barcodes that consistently map to a unique location in the genome using DesignRandomPool.pl. All Perl scripts used are available at https://bitbucket.org/berkeleylab/feba/src/master/bin/ (15, 62).

Essential gene calculations

Based on the TnSeq data, we used standard computational methods (62) to predict which genes are likely essential for growth in CPG. Briefly, this analysis predicts gene essentiality based on genes that lacked transposon insertions in the central 80% of the coding region. We excluded two categories of genes from the essentiality calculation. Genes sharing high nucleotide identity with other genes in the genome (measured via BLAT) (64) were excluded because we could not map transposon insertions to highly similar genes. Second, we excluded short genes because they are less likely to be disrupted. Due to differences in insertion mutant coverage depth between the transposon libraries, the minimum gene length considered was 450 bp for GMI1000, 325 bp for PSI07, and 400 bp for IBSBF1503. For comparison, we also used the Bio-Tradis pipeline (github.com/sanger-pathogens/Bio-Tradis) (16) to predict essential genes in these

strains, using the same minimum gene length cutoffs and excluding insertions in the first and last 10% of the coding region from analysis.

Orthologous gene predictions

Orthologous genes were predicted using the "Genome Gene Best Homologs" tool from the JGI IMG database (18). We searched for homologs in PSI07 and IBSBF1503 against GMI1000 as the reference genome with a 60% identity threshold. The results, originally reported with IMG locus tags, were matched to the corresponding NCBI locus tags by gene sequence.

Tomato xylem sap harvesting

Xylem sap was harvested from susceptible tomato plants (cv. Moneymaker and Bonny Best). Most experiments were performed with sap from Moneymaker tomato plants grown in the Oxford Tract Greenhouse in Berkeley, CA. Bacterial growth curves were independently replicated with sap from tomato plants grown in a growth chamber (28°C with a 12 hour day/night cycle) at UW-Madison.

Xylem sap was harvested by de-topping each plant at the cotyledon juncture with a razor blade and allowing sap to pool on the stump (65). To reduce cytoplasmic contamination, the sap that accumulated in the first few minutes was discarded; the stump was washed with diH_2O and gently blotted dry. Sap was then pipetted and pooled into a 50 ml conical over a collection period of 3 hours. Pooled sap was centrifuged at 5,000 *xg* RT for 10 minutes and the supernatant was filter sterilized using filters with 0.22 μm pores (Thermo Scientific #725-2520). Sap was aliquoted and stored at -20°C until use. Each batched pool of xylem sap was collected from approximately 70 plants.

R. solanacearum growth in tomato xylem sap

Colonies of each strain were inoculated into 6 ml of CPG and grown overnight at 28°C with shaking at 200 rpm for a total of three biological replicates. Cells from stationary phase cultures were pelleted at 13,000 *xg* and washed twice in 1 ml of ddH_2O. The washed pellet was resuspended in ddH_2O and adjusted to a final OD_{600} of 0.02. Each growth condition was inoculated with 7 µl of each culture at a starting cell density of ~10^5 CFU/ml in 48-well plates (Corning #353078), for a total of two technical replicates per biological replicate. Growth was measured by dilution plating at 0-, 4-, 8-, 24-, 32-, and 48-hour time points. In parallel, measurements for 12-, 16-, and 20-hour time points were taken from independent cultures that were started 12 hours after the first, using the same cultures, growth media, and technique described above.

Fitness experiments with transposon libraries

For genome wide fitness experiments, we adapted established methods (15). Per condition (minimal medium, rich medium, or Moneymaker tomato xylem sap), three 1 ml aliquots of each transposon library (GMI1000, PSI07, and IBSBF1503) were thawed on ice and revived in separate 100 ml flasks of CPG with 12.5 mg/ml Kanamycin with shaking incubation at 28°C for 16-20 hours. Once cultures reached an OD_{600} of 0.2 to 0.5, cells were pelleted by centrifuging for 10 min at 5000 *xg* RT and resuspended in 2 ml of media or sterile H_2O (xylem sap experiments). Cells were washed 3 times in media or xylem sap. An aliquot of the washed cells (>10^9 cells) was retained (pelleted and frozen) for a "time 0" control. For rich and minimal medium experiments, cells were seeded into 5 ml at OD_{600} = 0.02 (~2-5x10^7 total cells). Rich medium cultures were grown to saturation (20-24 hours; 5-6 cell doublings) and minimal medium cultures were grown for over 48 hours (5-6 cell doublings for PSI07 and GMI1000 and

4 doublings for IBSBF1503). For xylem sap experiments, cells were resuspended in 20 ml of xylem sap at a starting cell density of 10^6 cells/ml (2×10^7 cells total) and grown for 25 hours (7-8 doublings for GMI1000 and 8-9 doublings for PSI07). Genomic DNA was extracted (Qiagen DNeasy Blood and Tissue Kit) from the "time 0" and cells harvested after growing in differential media.

BarSeq and Fitness Calculations

Barcodes were PCR amplified from each sample using the previously described reaction protocol, cycling conditions, and multiplex primers (15). Amplified barcodes were sequenced on a HiSeq4000 at QB3 Berkeley Genomics Center with 96 samples multiplexed (50 base pair reads, single end). Sequencing data was analyzed using the BarSeq pipeline, available at https://bitbucket.org/berkeleylab/feba/src/master/bin/. For each competitive fitness assay, fitness score ("Fit scores") for each gene were calculated as the $\log_2$ ratio between barcode abundance after outgrowth in a condition vs. its abundance in the time 0 sample. Each Fit score is the weighted average of fitness values for all mutant strains with transposon insertions in a given gene. These Fit scores are normalized across the genome such that a gene with neither a fitness cost nor benefit has a value of 0. Significance was determined based on an absolute t-like test statistic using a > 2.5 threshold. The t-like test statistic considers the consistency of the fitness scores for all barcoded mutants for each gene in the experiment as previously described in detail (15).

Data availability.

The raw reads used for TnSeq mapping is available in the NCBI SRA under accession PRJNA629015. The fitness browser (http://fit.genomics.lbl.gov) offers a graphical user interface for exploring the fitness data from these experiments, orthology of the genes between these

strains and other Gram negative bacteria, and cross-references to Kegg, Paperblast, and NCBI databases.

Acknowledgements

We thank Steve Lindow, Caitilyn Allen, and Jeff Flynn for useful discussions. This work used the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, supported by NIH S10 OD018174 Instrumentation Grant. Oxford Tract Greenhouse Staff and the QB3 Berkeley Genomics Center provided technical assistance.

This work was supported by USDA NIFA #2018-67012-31497 awarded to TLP; partial support was provided by a UC Berkeley SURF Rose Hills Independent Fellowship awarded to KES. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

References

1. Prior P, Ailloud F, Dalsing BL, Remenant B, Sanchez B, Allen C. 2016. Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. BMC Genomics 17:1–11.
2. Safni I, Cleenwerck I, De Vos P, Fegan M, Sly L, Kappler U. 2014. Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex. Int J Syst Evol Microbiol 64:3087–3103.

3. Hayward AC. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu rev Phytopathol 29:65–87.

4. Safni I, Subandiyah S, Fegan M. 2018. Ecology, epidemiology and disease management of *Ralstonia syzygii* in Indonesia. Front Microbiol 9:1–11.

5. Lopes CA, Rossato M. 2018. History and status of selected hosts of the *Ralstonia solanacearum* species complex causing bacterial wilt in Brazil. Front Microbiol 9:1–6.

6. Jiang G, Wei Z, Xu J, Chen H, Zhang Y, She X, Macho AP, Ding W, Liao B. 2017. Bacterial wilt in China: history, current status, and future perspectives. Front Plant Sci 8:1–10.

7. Lowe-Power T, Chipman K. 2020. A meta-analysis of the known global distribution and host range of the *Ralstonia* species complex. bioRxiv 1–7.

8. Lowe-Power TM, Hendrich CG, von Roepenack-Lahaye E, Li B, Wu D, Mitra R, Dalsing BL, Ricca P, Naidoo J, Cook D, Jancewicz A, Masson P, Thomma B, Lahaye T, Michael AJ, Allen C. 2018. Metabolomics of tomato xylem sap during bacterial wilt reveals *Ralstonia solanacearum* produces abundant putrescine, a metabolite that accelerates wilt disease. Environ Microbiol 20:1330–1349.

9. Remenant B, Coupat-Goutaland B, Guidot A, Cellier G, Wicker E, Allen C, Fegan M, Pruvost O, Elbaz M, Calteau A, Salvignol G, Mornico D, Mangenot S, Barbe V, Médigue C, Prior P. 2010. Genomes of three tomato pathogens within the *Ralstonia solanacearum* species complex reveal significant evolutionary divergence. BMC Genomics 11:1–16.

10. Remenant B, de Cambiaire JC, Cellier G, Jacobs JM, Mangenot S, Barbe V, Lajus A, Vallenet D, Medigue C, Fegan M, Allen C, Prior P. 2011. Ralstonia syzygii, the blood disease bacterium and some Asian R. solanacearum strains form a single genomic species

despite divergent lifestyles. PLoS One 6:1–10.

11. Ailloud F, Lowe T, Cellier G, Roche D, Allen C, Prior P. 2015. Comparative genomic analysis of *Ralstonia solanacearum* reveals candidate genes for host specificity. BMC Genomics 16:1–11.

12. Hayward AC. 1964. Characteristics of *Pseudomonas solanacearum*. J Appl Bacteriol 27:265–277.

13. Lowe TM, Ailloud F, Allen C. 2015. Hydroxycinnamic acid degradation, a broadly conserved trait, protects *Ralstonia solanacearum* from chemical plant defenses and contributes to root colonization and virulence. Mol Plant-Microbe Interact 28:286–297.

14. Lowe-Power TM, Jacobs JM, Ailloud F, Fochs B, Prior P, Allen C. 2016. Degradation of the plant defense signal salicylic acid protects *Ralstonia solanacearum* from toxicity and enhances virulence on tobacco. MBio 7:1–12.

15. Wetmore KM, Price MN, Waters RJ, Lamson JS, He J, Hoover CA, Blow MJ, Bristow J, Butland G, Arkin AP, Deutschbauer A. 2015. Rapid quantification of mutant fitness in diverse bacteria by sequencing randomly bar-coded transposons. MBio 6:1–15.

16. Barquist L, Mayho M, Cummins C, Cain AK, Boinett CJ, Page AJ, Langridge GC, Quail MA, Keane JA, Parkhill J. 2016. The TraDIS toolkit: sequencing and analysis for dense transposon mutant libraries. Bioinformatics 32:1109–1111.

17. Rubin BE, Wetmore KM, Price MN, Diamond S, Shultzaberger RK, Lowe LC, Curtin G, Arkin AP, Deutschbauer A, Golden SS. 2015. The essential gene set of a photosynthetic organism. Proc Natl Acad Sci U S A 112:e6634–e6643.

18. Chen I-MA, Chu K, Palaniappan K, Pillay M, Ratner A, Huang J, Huntemann M, Varghese N, White JR, Seshadri R, Smirnova T, Kirton E, Jungbluth SP, Woyke T, Eloe-

Fadrosh EA, Ivanova NN, Kyrpides NC. 2019. IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. Nucleic Acids Res 47:D666–D677.

19. Hulsen T, Vlieg J De, Alkema W. 2008. BioVenn–a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams 9:1–6.

20. Kanehisa M, Goto S. 2000. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 28:27–30.

21. Dalsing BL, Allen C. 2014. Nitrate assimilation contributes to *Ralstonia solanacearum* root attachment, stem colonization, and virulence. J Bacteriol 196:949–960.

22. Jacobs JM, Babujee L, Meng F, Milling A, Allen C. 2012. The *in planta* transcriptome of *Ralstonia solanacearum*: conserved physiological and virulence strategies during bacterial wilt of tomato. MBio 3:1–11.

23. Khokhani D, Lowe-Power TM, Tran TM, Allen C. 2017. A single regulator mediates strategic switching between attachment/spread and growth/virulence in the plant pathogen *Ralstonia solanacearum*. MBio 8:1–20.

24. Peyraud R, Cottret L, Marmiesse L, Gouzy J, Genin S. 2016. A resource allocation trade-off between virulence and proliferation drives metabolic versatility in the plant pathogen *Ralstonia solanacearum*. PLOS Pathog 12:1–25.

25. Brumbley SM, Denny TP. 1990. Cloning of wild-type *Pseudomonas solanacearum phcA*, a gene that when mutated alters expression of multiple traits that contribute to virulence. J Bacteriol 172:5677–5685.

26. Huang J, Carney BF, Denny TP, Weissinger AK, Schell MA. 1995. A complex network regulates expression of *eps* and other virulence genes of *Pseudomonas solanacearum*. J

Bacteriol 177:1259–1267.

27. Flavier AB, Schell MA, Denny TP. 1998. An RpoS (σS) homologue regulates acylhomoserine lactone-dependent autoinduction in *Ralstonia solanacearum*. Mol Microbiol 28:475–486.

28. Brown DG, Allen C. 2004. *Ralstonia solanacearum* genes induced during growth in tomato: an inside view of bacterial wilt. Mol Microbiol 53:1641–1660.

29. Liu Y, Tan X, Cheng H, Gong J, Zhang Y, Wang D, Ding W. 2020. The cold shock family gene *cspD3* is involved in the pathogenicity of *Ralstonia solanacearum* CQPS-1 to tobacco. Microb Pathog 142:104091.

30. Eckshtain-Levi N, Weisberg AJ, Vinatzer BA. 2018. The population genetic test Tajima's D identifies genes encoding pathogen-associated molecular patterns and other virulence-related genes in *Ralstonia solanacearum*. Mol Plant Pathol 19:2187–2192.

31. Wei Y, Caceres-Moreno C, Jimenez-Gongora T, Wang K, Sang Y, Lozano-Duran R, Macho AP. 2018. The *Ralstonia solanacearum* csp22 peptide, but not flagellin-derived peptides, is perceived by plants from the *Solanaceae* family. Plant Biotechnol 2018:1349–1362.

32. Xian L, Yu G, Wei Y, Li Y, Zhang H, Xue H, Morcillo RJL, Macho AP. 2020. A bacterial effector protein hijacks plant metabolism to support bacterial nutrition. Cell Host Microbe 28:1–10.

33. Coplin DL, Sequeira L, Hanson RS. 1974. *Pseudomonas solanacearum*: virulence of biochemical mutants. Can J Microbiol 20:519–529.

34. Lowe-Power TM, Khokhani D, Allen C. 2018. How *Ralstonia solanacearum* exploits and thrives in the flowing plant xylem environment. Trends Microbiol 26:929–942.

35. Plener L, Boistard P, González A, Boucher C, Genin S. 2012. Metabolic adaptation of *Ralstonia solanacearum* during plant infection: a methionine biosynthesis case study. PLoS One 7.

36. Hentchel KL, Ruiz LMR, Curtis PD, Fiebig A, Coleman ML, Crosson S. 2019. Genome-scale fitness profile of *Caulobacter crescentus* grown in natural freshwater. ISME J 13:523–536.

37. Hood MI, Skaar EP. 2012. Nutritional immunity: transition metals at the pathogen-host interface. Nat Rev Microbiol 10:525–537.

38. Zhang YJ, Rubin EJ. 2013. Feast or famine: the host-pathogen battle over amino acids. Cell Microbiol 15:1079–1087.

39. Campos ML, De Souza CM, De Oliveira KBS, Dias SC, Franco OL. 2018. The role of antimicrobial peptides in plant immunity. J Exp Bot 69:4997–5011.

40. Harris V, Jiranek V, Ford CM, Grbin PR. 2010. Inhibitory effect of hydroxycinnamic acids on *Dekkera* spp. Appl Microbiol Biotechnol 86:721–729.

41. Brown DG, Swanson JK, Allen C. 2007. Two host-induced *Ralstonia solanacearum* genes, *acrA* and *dinF*, encode multidrug efflux pumps and contribute to bacterial wilt virulence. Appl Environ Microbiol 73:2777–2786.

42. Lenski RE, Rose MR, Simpson SC, Tadler SC. 1991. Long-term experimental evolution in *Escherichia coli*. I . Adaptation and divergence during 2,000 generations. Am Nat 138:1315–1341.

43. Morin M, Pierce EC, Dutton RJ. 2018. Changes in the genetic requirements for microbial interactions with increasing community complexity. Elife 7:1–26.

44. Capela D, Marchetti M, Clérissi C, Perrier A, Guetta D, Gris C, Valls M, Jauneau A,

Cruveiller S, Rocha EPC, Masson-Boivin C. 2017. Recruitment of a lineage-specific virulence regulatory pathway promotes intracellular infection by a plant pathogen experimentally evolved into a legume symbiont. Mol Biol Evol 34:2503–2521.

45. Guidot A, Jiang W, Ferdy J, Thébaud C, Barberis P, Gouzy J, Genin S. 2014. Multihost experimental evolution of the pathogen *Ralstonia solanacearum* unveils genes involved in adaptation to plants. Mol Biol Evol 31:2913–2928.

46. Poulsen BE, Yang R, Clatworthy AE, White T, Osmulski SJ, Li L, Penaranda C, Lander ES, Shoresh N, Hung DT. 2019. Defining the core essential genome of *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 116:10072–10080.

47. Su Y, Xu Y, Li Q, Yuan G, Zheng D. 2020. The essential genome of *Ralstonia solanacearum*. Microbiol Res 238:1–6.

48. Larson MH, Gilbert LA, Wang X, Lim WA, Weissman JS, Qi LS. 2013. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. Nat Protoc 8:2180–2196.

49. Arlat M, Gough CL, Zischek C, Barberis PA, Trigalet A, Boucher CA. 1992. Transcriptional organization and expression of the large *hrp* gene cluster of *Pseudomonas solanacearum*. Mol Plant-Microbe Interact 5:187–193.

50. Boucher CA, Barberis PA, Trigalet APH, Demery DA. 1985. Transposon mutagenesis of *Pseudomonas solanacearum*: isolation of Tn5-induced avirulent mutants. J Gen Microbiol 131:2449–2457.

51. Staskawicz BJ, Dahlbeck D, Keent NT. 1984. Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr. PNAS 81:6024–6028.

52. Cole BJ, Feltcher ME, Waters RJ, Wetmore KM, Mucyn TS, Ryan EM, Wang G, Ul-Hasan S, McDonald M, Yoshikuni Y, Malmstrom RR, Deutschbauer AM, Dangl JL, Visel A. 2017. Genome-wide identification of bacterial plant colonization genes. PLoS Biol 15:1–24.

53. Duong DA, Jensen R V., Stevens AM. 2018. Discovery of *Pantoea stewartii* ssp. *stewartii* genes important for survival in corn xylem through a Tn-Seq analysis. Mol Plant Pathol 19:1929–1941.

54. Gonzalez-Mula A, Lachat J, Mathias L, Naquin D, Lamouche F, Mergaert P, Faure D. 2019. The biotroph *Agrobacterium tumefaciens* thrives in tumors by exploiting a wide spectrum of plant host metabolites. New Phytol 222:455–467.

55. Helmann TC, Deutschbauer AM, Lindow SE. 2019. Genome-wide identification of *Pseudomonas syringae* genes required for fitness during colonization of the leaf surface and apoplast. PNAS 116:18900–18910.

56. Helmann TC, Ongsarte CL, Lam J, Deutschbauer AM, Lindow SE. 2019. Genome-wide transposon screen of a *Pseudomonas syringae mexB* mutant reveals the substrates of efflux transporters. MBio 10:1–15.

57. Liu Z, Beskrovnaya P, Melnyk RA, Hossain SS, Khorasani S, O'Sullivan LR, Wiesmann CL, Bush J, Richard JD, Haney CH. 2018. A genome-wide screen identifies genes in rhizosphere-associated *Pseudomonas* required to evade plant defenses. MBio 9:1–17.

58. Royet K, Parisot N, Rodrigue A, Gueguen E, Condemine G. 2019. Identification by Tn-seq of *Dickeya dadantii* genes required for survival in chicory plants. Mol Plant Pathol 20:287–306.

59. Brooks, John F I, Gyllborg MC, Cronin DC, Quillin SJ, Mallama CA, Foxall R, Whistler

C, Goodman AL, Mandel MJ. 2014. Global discovery of colonization determinants in the squid symbiont *Vibrio fischeri*. PNAS 111:17284–17289.

60. Hava DL, Camilli A. 2002. Large-scale identification of serotype 4 *Streptococcus pneumoniae* virulence factors. Mol Mic 45:1389–1406.

61. Lewin GR, Stacy A, Michie KL, Lamont RJ, Whiteley M. 2019. Large-scale identification of pathogen essential genes during coinfection with sympatric and allopatric microbes. PNAS 116:19685–19694.

62. Price MN, Wetmore KM, Waters RJ, Callaghan M, Ray J, Liu H, Keuhl J V, Melnyk RA, Lamson JS, Suh Y, Carlson HK, Esquivel Z, Sadeeshkumar H, Chakraborty R, Zane GM, Rubin BE, Wall JD, Visel A, Bristow J, Blow MJ, Arkin AP, Deutschbauer AM. 2018. Mutant phenotypes for thousands of bacterial genes of unknown function. Nature 557:503–509.

63. Perrier A, Barberis P, Genin S. 2018. Introduction of genetic material in *Ralstonia solanacearum* through natural transformation and conjugation, p. 201–207. *In* Methods in Molecular Biology. Humana Press, New York, NY.

64. Kent WJ. 2002. BLAT—the BLAST-like alignment tool. Genome Res 12:656–664.

65. Khokhani D, Tran TM, Lowe-Power TM, Allen C. 2018. Plant assays for quantifying *Ralstonia solanacearum* virulence. Bio-protocol 8:1–19.

66. Lex A, Gehlenborg N, Strobelt H, Vuillemot R, Pfister H. 2014. UpSet : visualization of intersecting sets. IEEE Trans Vis Comput Graph 20:1983–1992.

Figures and Tables

Table 1: Properties of barcoded *mariner* mutant libraries

Mutant Library	Total genes in genome	Genes with Fit scores: # (% total)	Library Size[a]: # mutants	Mutants per gene in fitness calculations: Median (Mean)	Estimated Essential Genes[b]: # (%)
GMI1000	5,204	4,003 (77%)	90,081	11 (17.1)	456-470 (8.7-9.0%)
PSI07	4,968	4,057 (82%)	190,509	22 (35.7)	454-487 (9.5-9.8%)
IBSBF1503	4,897	3,809 (78%)	104,888	12 (20.4)	454-487 (9.3-10.0%)

[a]Measured by # of unique DNA barcodes used in BarSeq runs.

[b] Two pipelines (described in Methods) were used to predict gene essentiality based on the distribution of transposon insertions in open reading frames.

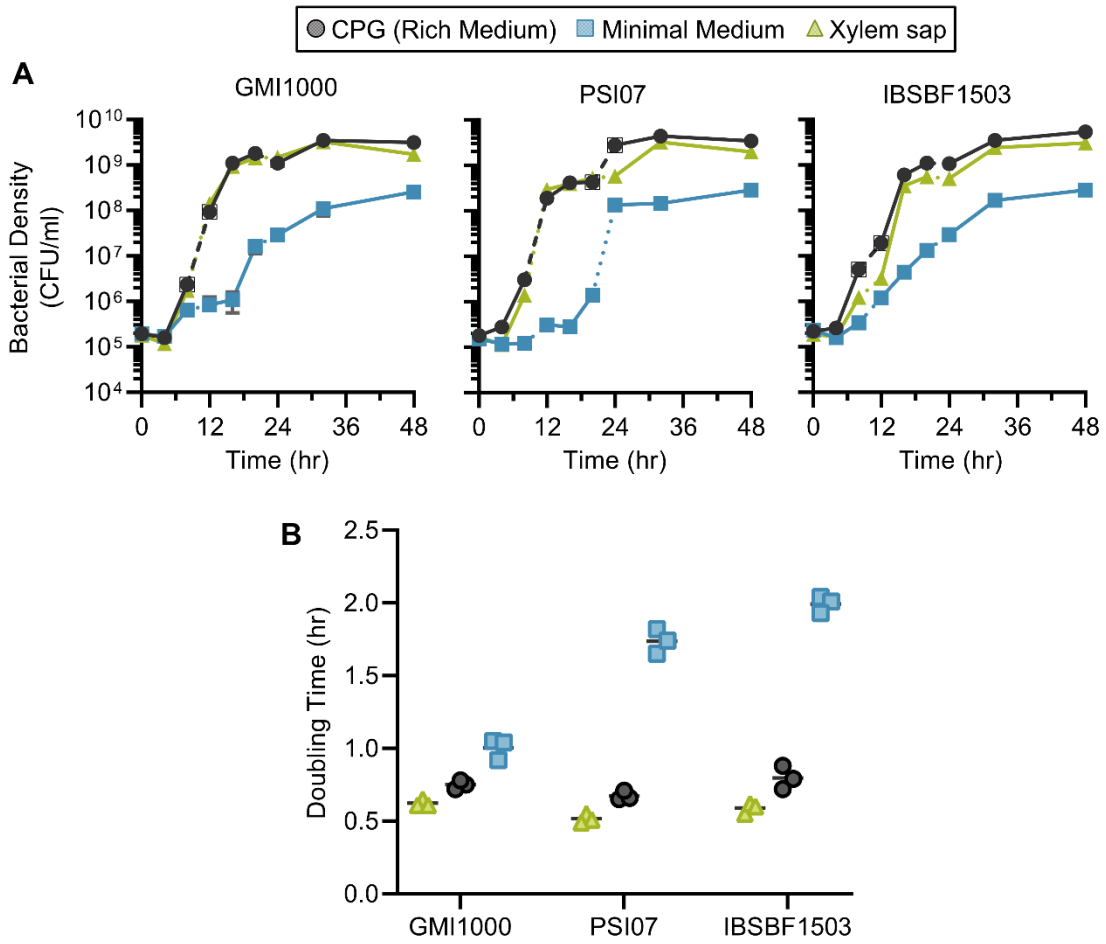
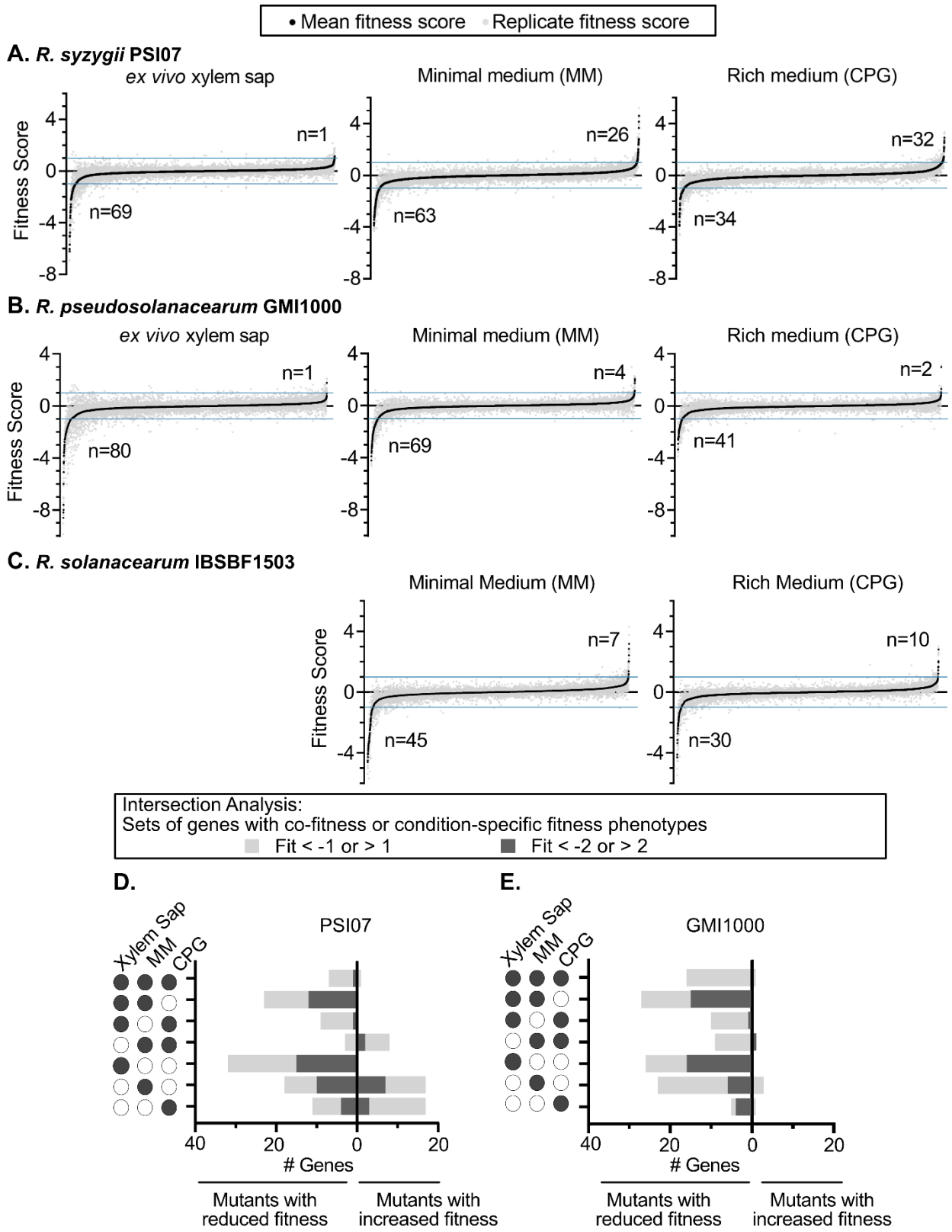


Fig 1. Growth of *Ralstonia* strains in xylem sap is similar to growth in rich medium. *Ralstonia* strain GMI1000, strain PSI07, or strain IBSBF1503 were grown in rich medium (CPG), quarter-strength M63 minimal medium (MM) or xylem sap from Moneymaker tomato plants. (**A**) Strains were inoculated at 10^5 CFU/ml into media or xylem sap. Solid lines indicate time points sampled from the same culture; the 12-24 hour timepoints were from an independent culture, but 0-8 and 24-48 hour timepoints were taken from the same culture. All cultures were set up with 3 biological replicates (individual overnight cultures and individual batches of xylem sap) and were performed in technical duplicate. Error bars indicate standard deviation. (**B**) The minimum doubling time between contiguous 4 hour measurements was calculated per biological

replicate. Lines indicate mean. Per strain, the doubling times in all conditions were significantly different from each other (adjusted $P<0.05$ by ANOVA with Tukey's multiple comparisons).


• Mean fitness score
Replicate fitness score
A. R. syzygii PSI07
ex vivo xylem sap
Minimal medium (MM)
Rich medium (CPG)
Fitness Score
n=1
n=69
n=26
n=63
n=32
n=34
B. R. pseudosolanacearum GMI1000
ex vivo xylem sap
Minimal medium (MM)
Rich medium (CPG)
n=1
n=80
n=4
n=69
n=2
n=41
C. R. solanacearum IBSBF1503
Minimal Medium (MM)
Rich Medium (CPG)
n=7
n=45
n=10
n=30
Intersection Analysis:
Sets of genes with co-fitness or condition-specific fitness phenotypes
Fit < -1 or > 1
Fit < -2 or > 2
D.
PSI07
Xylem Sap
MM
CPG
Genes
Mutants with reduced fitness
Mutants with increased fitness
E.
GMI1000
Xylem Sap
MM
CPG
Genes
Mutants with reduced fitness
Mutants with increased fitness

651

Fig 2. Summary of RB-TnSeq data after growth of mutant libraries in xylem sap, minimal medium (MM), or rich medium (CPG). (A-C) Ranked Fit scores for all measured genes in CPG, MM, and xylem sap for (A) *R. syzygii* PSI07, (B) *R. pseudosolanacearum* GMI1000, and (C) *R. solanacearum* IBSBF1503. The number of genes that influence fitness for each condition is indicated (Fit > 1 or <-1 and t > 2.5 or < -2.5 in at least 2 replicates). (D-E) Intersection analysis quantifying number of genes with condition-specific or co-fitness in multiple conditions. Moderate Fit >1 or <-1 and Strong Fit > 2 or <-2 cut-offs are shown in light and dark grey, respectively. The intersection visualization is modeled on UpSet Plots (66), an alternative to Venn Diagrams.

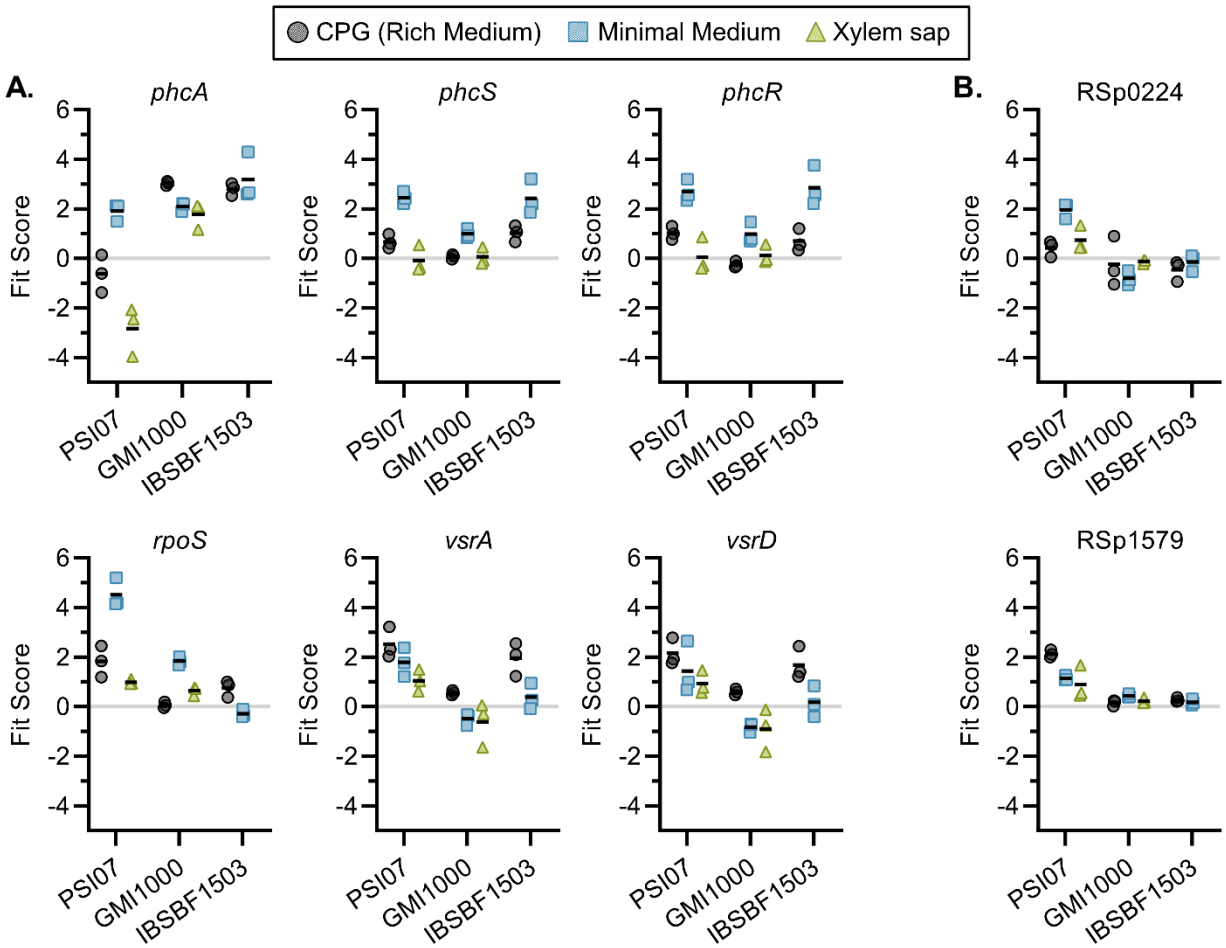


Fig 3. Regulatory mutants with increased fitness in one-or-more strain and condition. (A) Mutants with known defects in extracellular polysaccharide (EPS) production relative to wild type. (B) Transcriptional regulator mutants that have not been studied previously. Full Fit scores and t-like statistics are shown in Table S2.

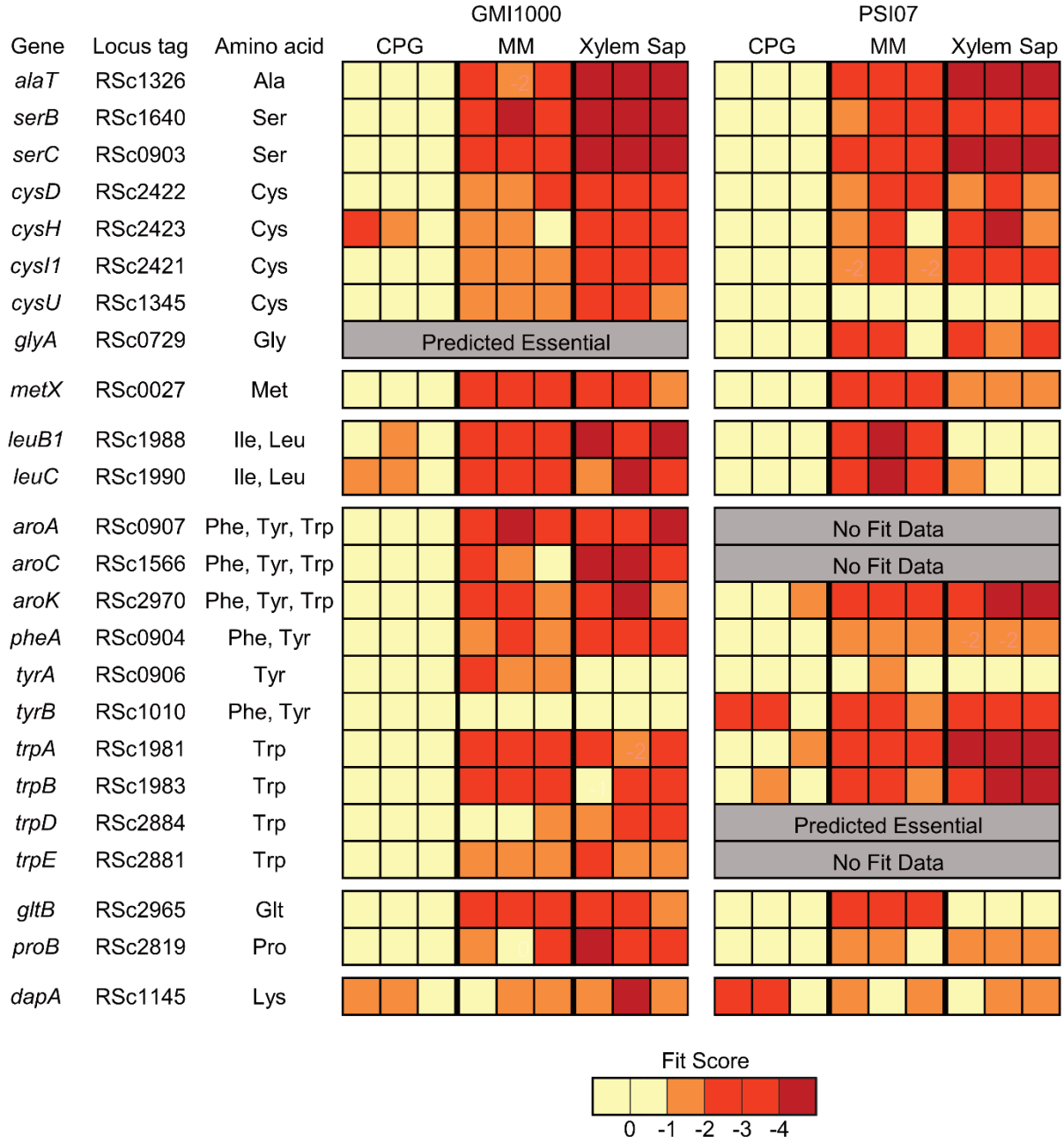


Fig 4. Amino acid auxotroph mutants have fitness defects in minimal medium (MM) and/or xylem sap. Fit scores of GMI1000 and PSI07 amino acid biosynthesis mutants with reduced fitness in sap, MM, or both, with three replicates per strain per condition. Gene name and locus

tag for the GMI1000 gene are displayed. Fit scores for the homologous IBSBF1503 mutants are shown in Fig S4. Full Fit scores and t-like statistics are shown in Table S2.

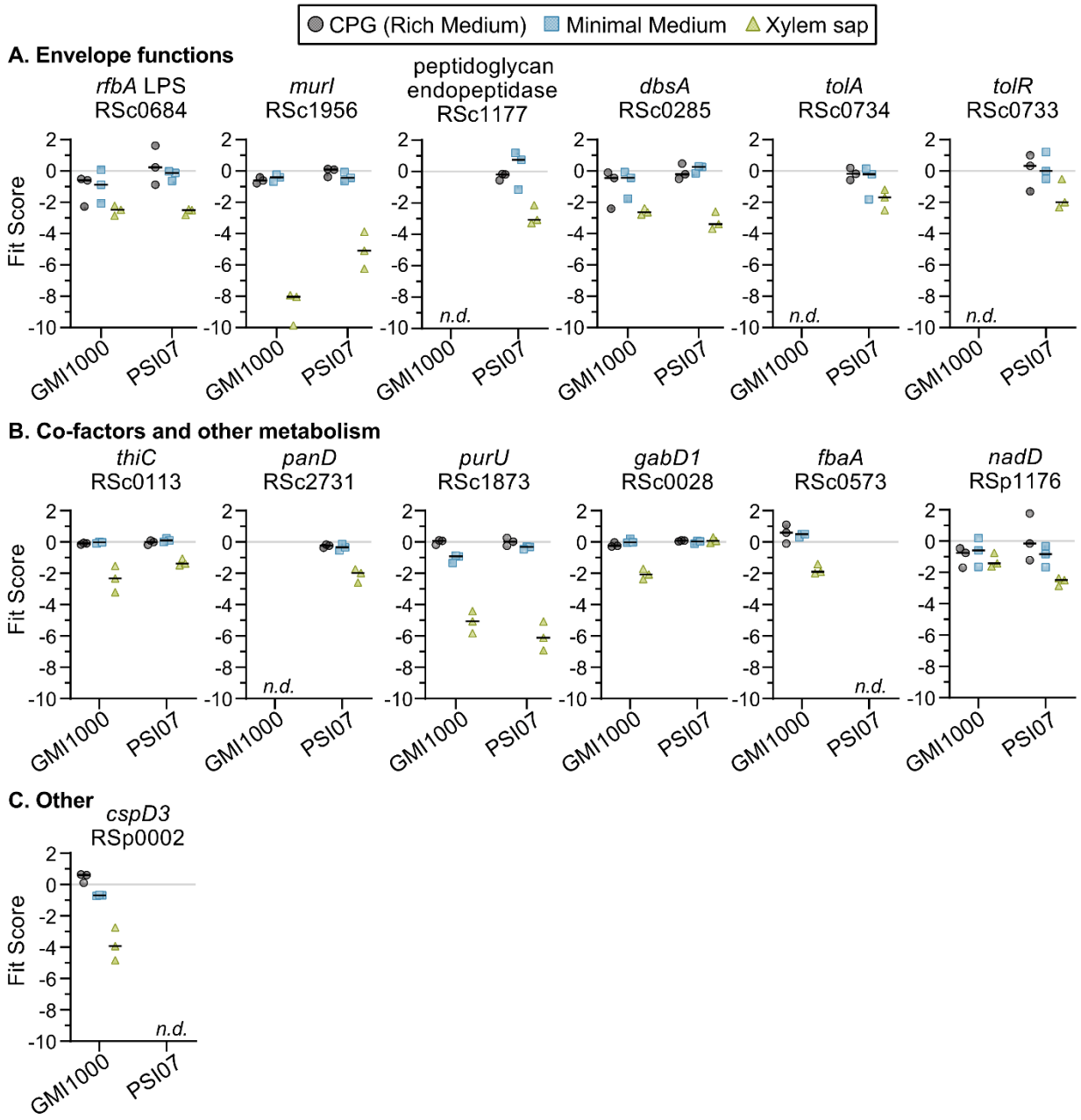


Fig 5. Mutants with specific fitness defects in xylem sap. Fit scores of GMI1000 and/or PSI07 mutants with specific fitness defects in sap: Mutants with (A) bacterial envelope functions, (B)

cofactor biosynthesis and other metabolic functions, and (C) other: *cspD3* a cold-shock protein. Full Fit scores and t-like statistics are shown in Table S2.

Supplementary Data

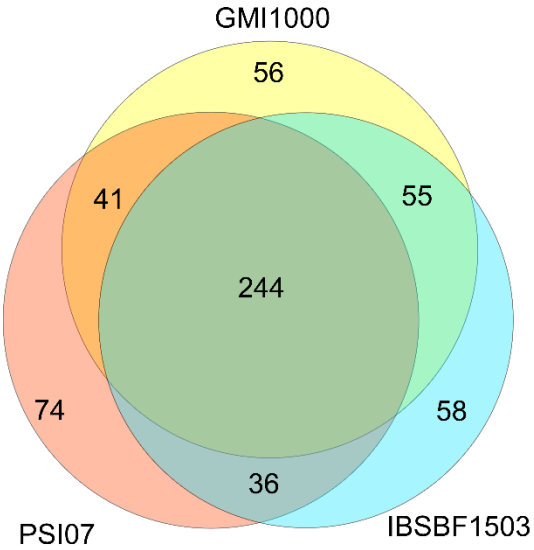


Fig S1. Venn diagram of putative essential genes in *Ralstonia* strains GMI1000, IBSBF1503, and PSI07. Orthologous predicted essential genes are represented proportionally in Venn diagram intersections.

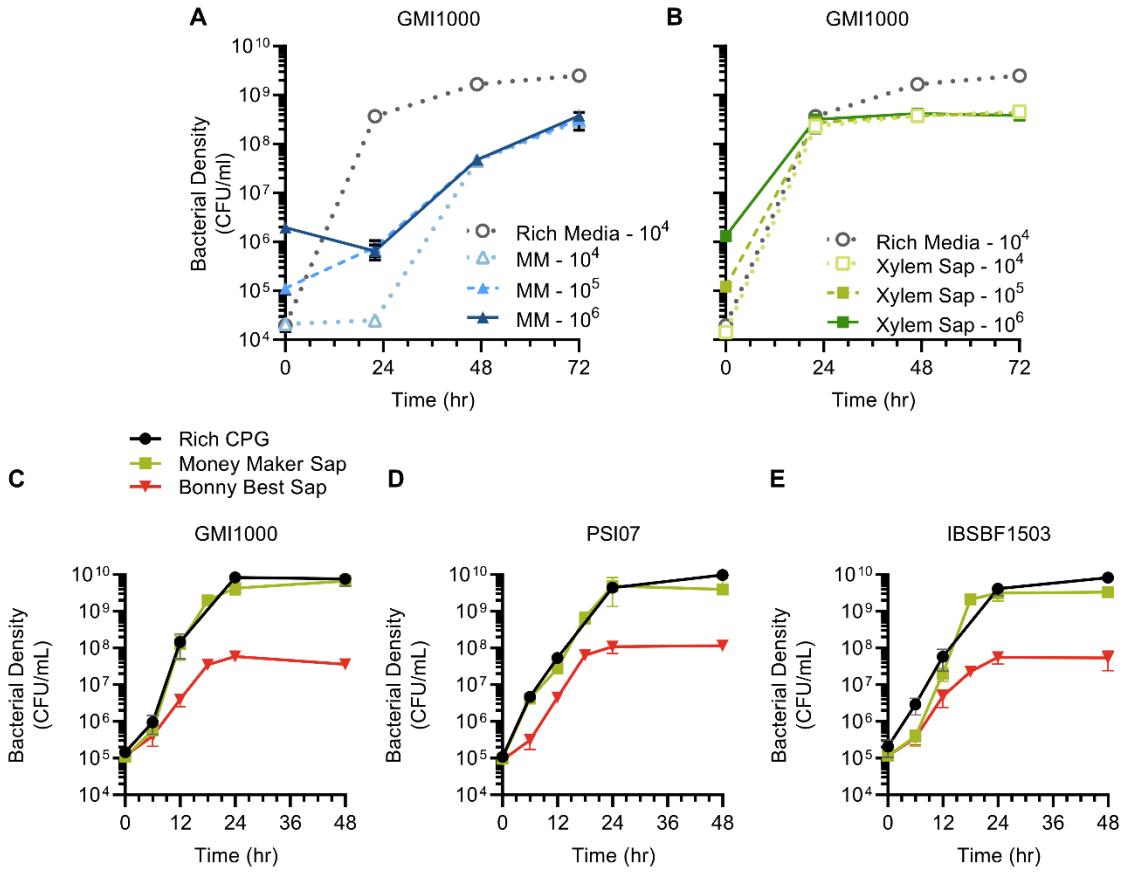


Fig S2. Growth of *Ralstonia* in rich medium, minimal medium, and xylem sap from multiple susceptible tomato cultivars. (A-B) To determine the carrying capacity of sap and culture media, *Ralstonia* strain GMI1000 was inoculated into rich CPG medium, quarter strength M63 medium, or xylem sap from Moneymaker tomato at 10^4, 10^5, or 10^6 CFU/ml starting density. (C-E). Wildtype *Ralstonia* strains GMI1000, PSI07, and IBSBF1503 were grown in sap from Moneymaker and Bonny Best tomato. All strains were inoculated in biological triplicate into three pools of xylem sap incubated at 28°C with shaking. Cell density was measured by dilution plating.

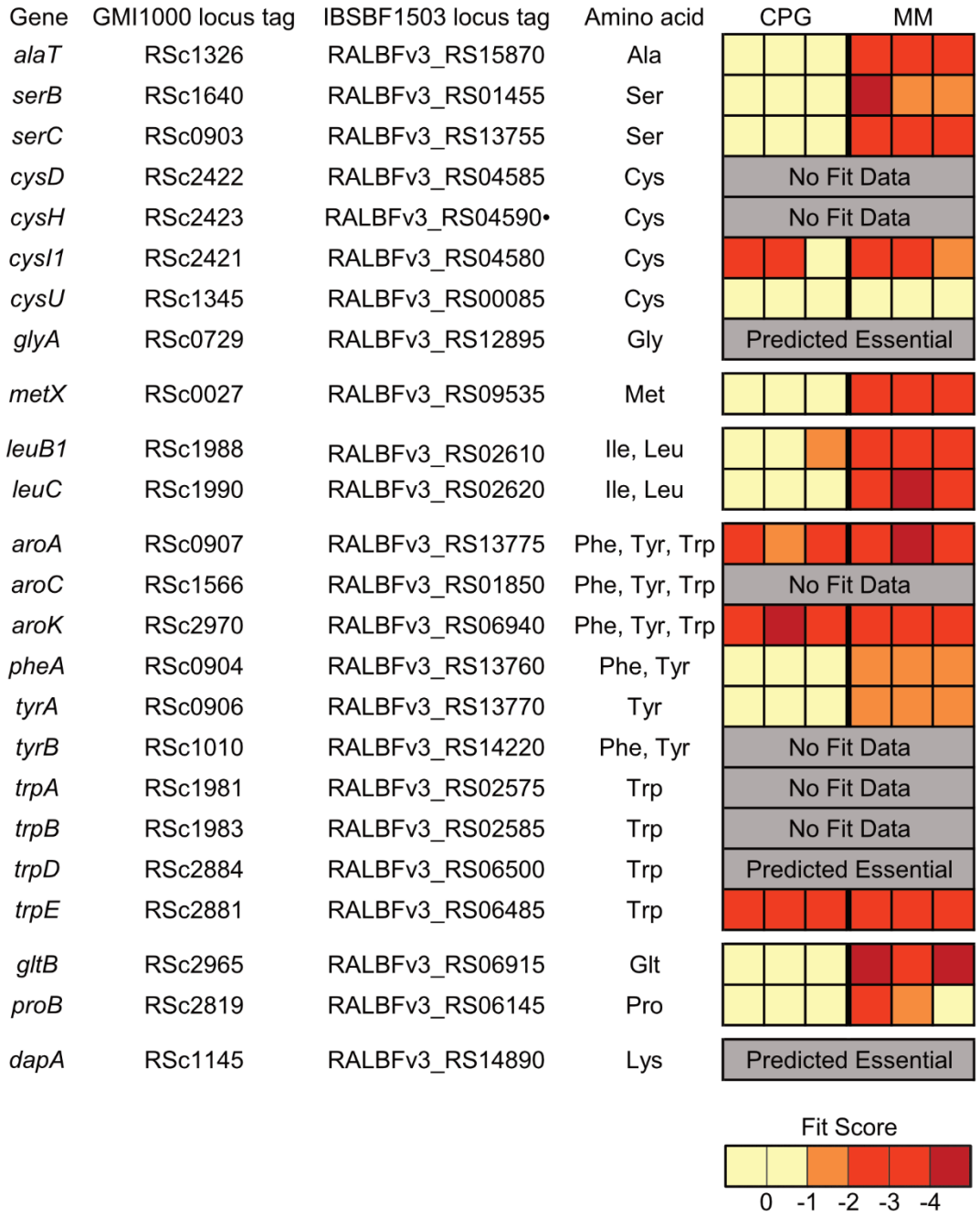


Fig S3. Fit scores of IBSBF1503 amino acid biosynthesis mutants. Grey indicates "data not determined" because mutants for these genes were not represented in the library. Gene name and locus tag for the GMI1000 gene are displayed. Fit scores for the orthologous GMI1000 and PSI07 genes are shown in Fig 4. Full Fit scores and t-like statistics are shown in Table S2.

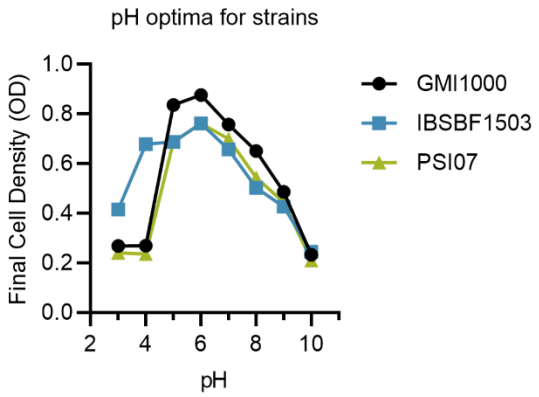
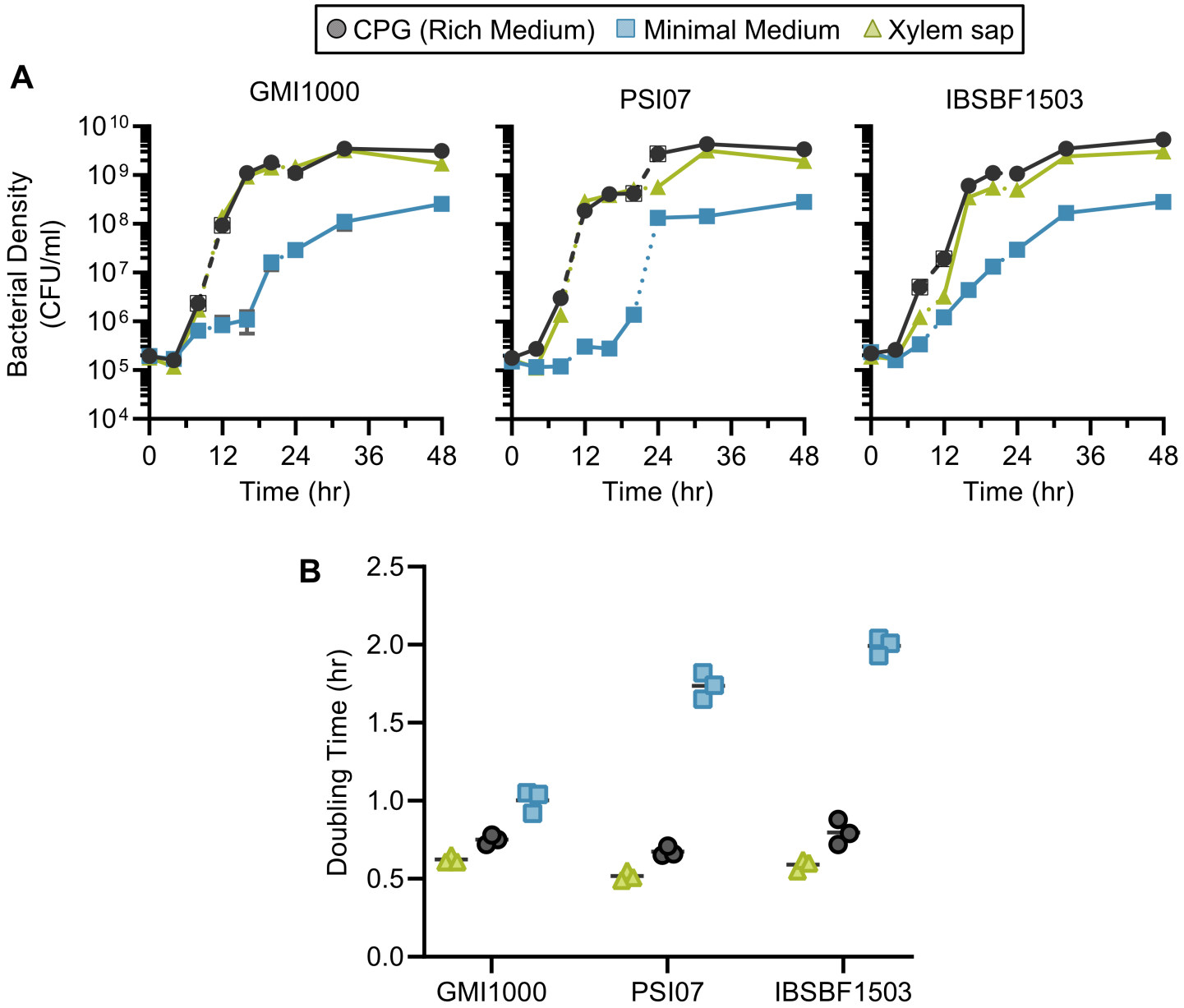


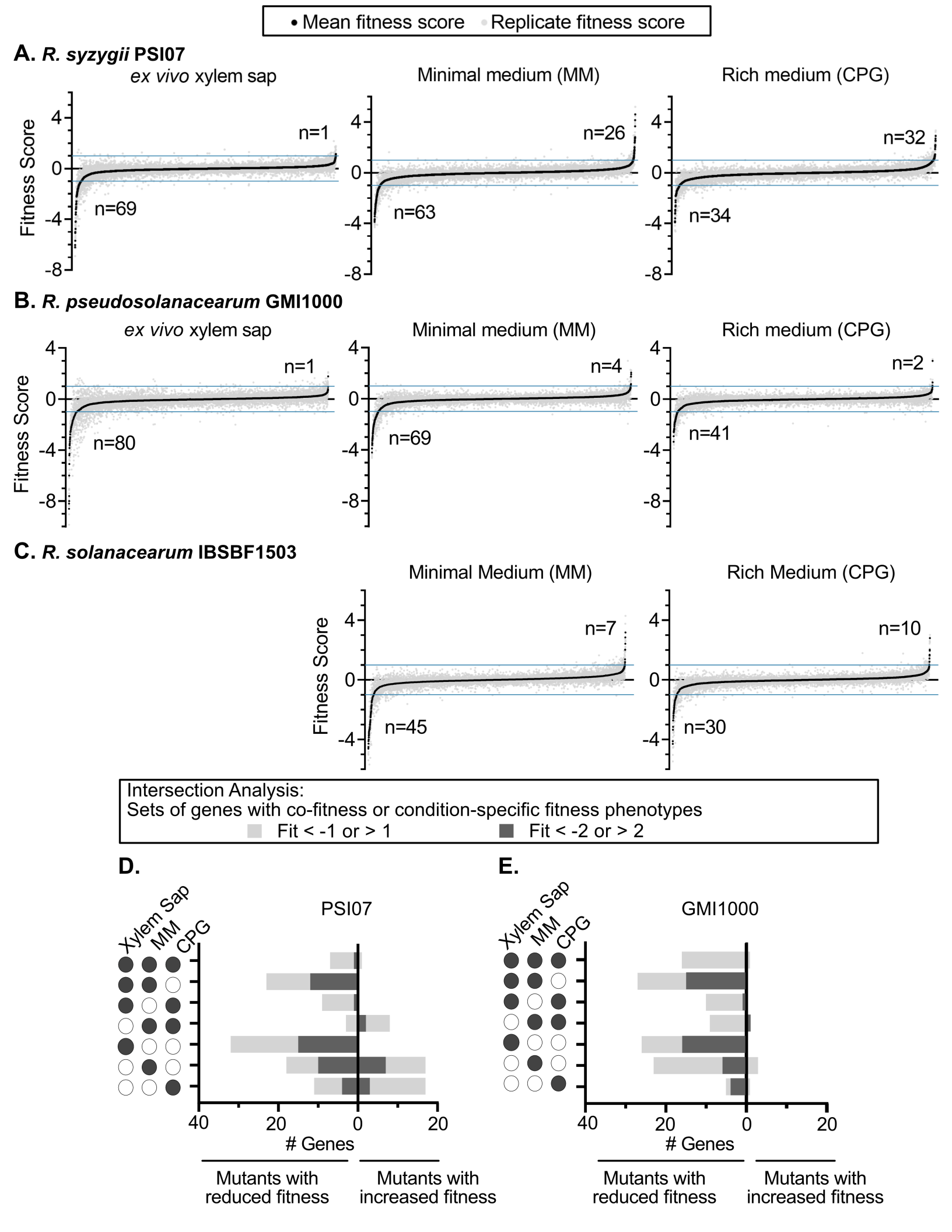
Fig S4. pH optima for *R. syzygii* PSI07, *R. pseudosolanacearum* PSI07, and *R. solanacearum* IBSBF1503. Growth of *Ralstonia* in glucose minimal medium buffered to 30 mM with Good's buffers: pH 3-4, HOMOPIPES (homopiperazine-N,N′-bis(2-ethanesulfonic acid); pH 5-6, MES (2-(*N*-morpholino)ethanesulfonic acid); pH 7, PIPES (piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); pH 8, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); pH 9-10 CHES (*N*-Cyclohexyl-2-aminoethanesulfonic acid).

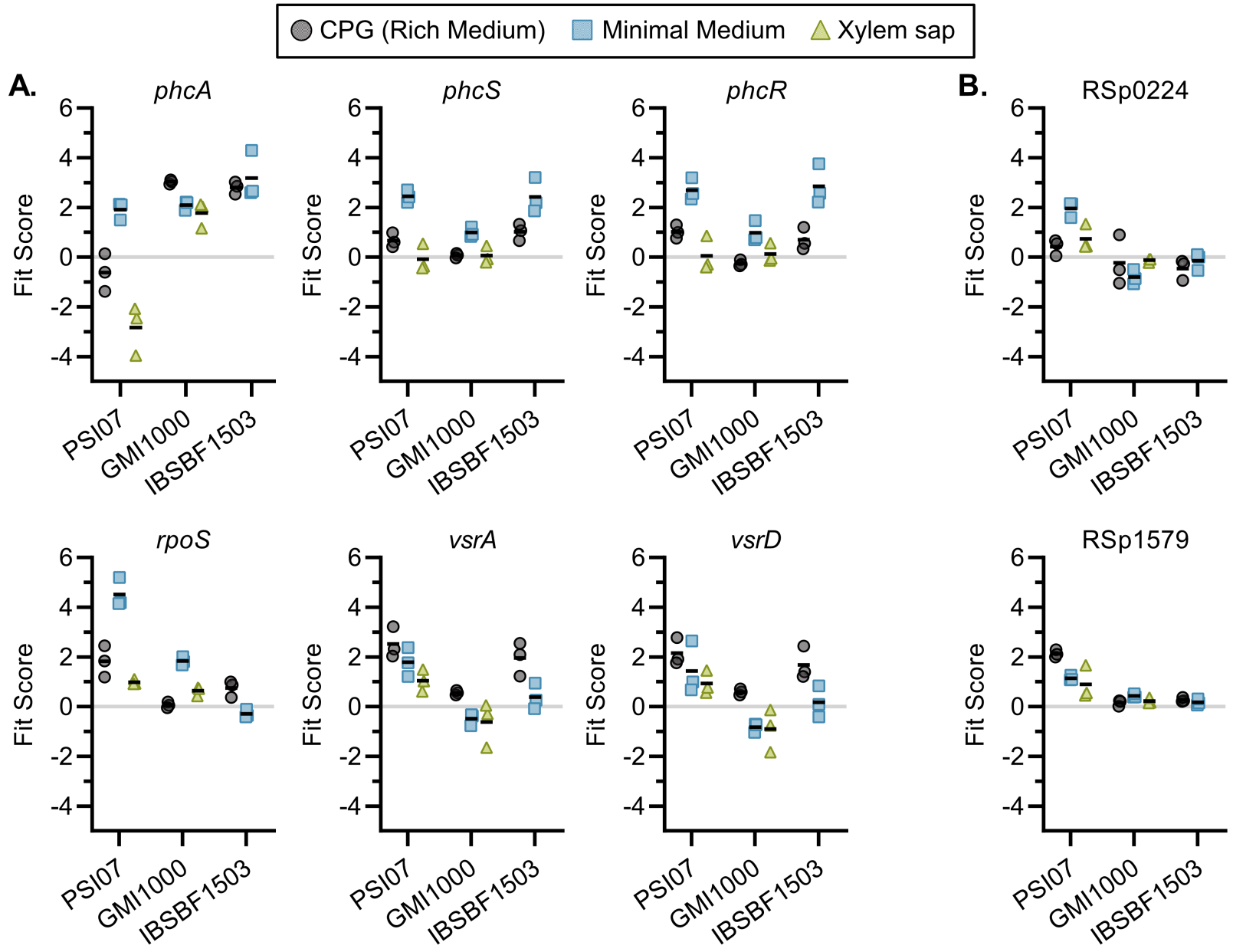
Table S1: Gene essentiality predictions for GMI1000, PSI07, and IBSBF1503

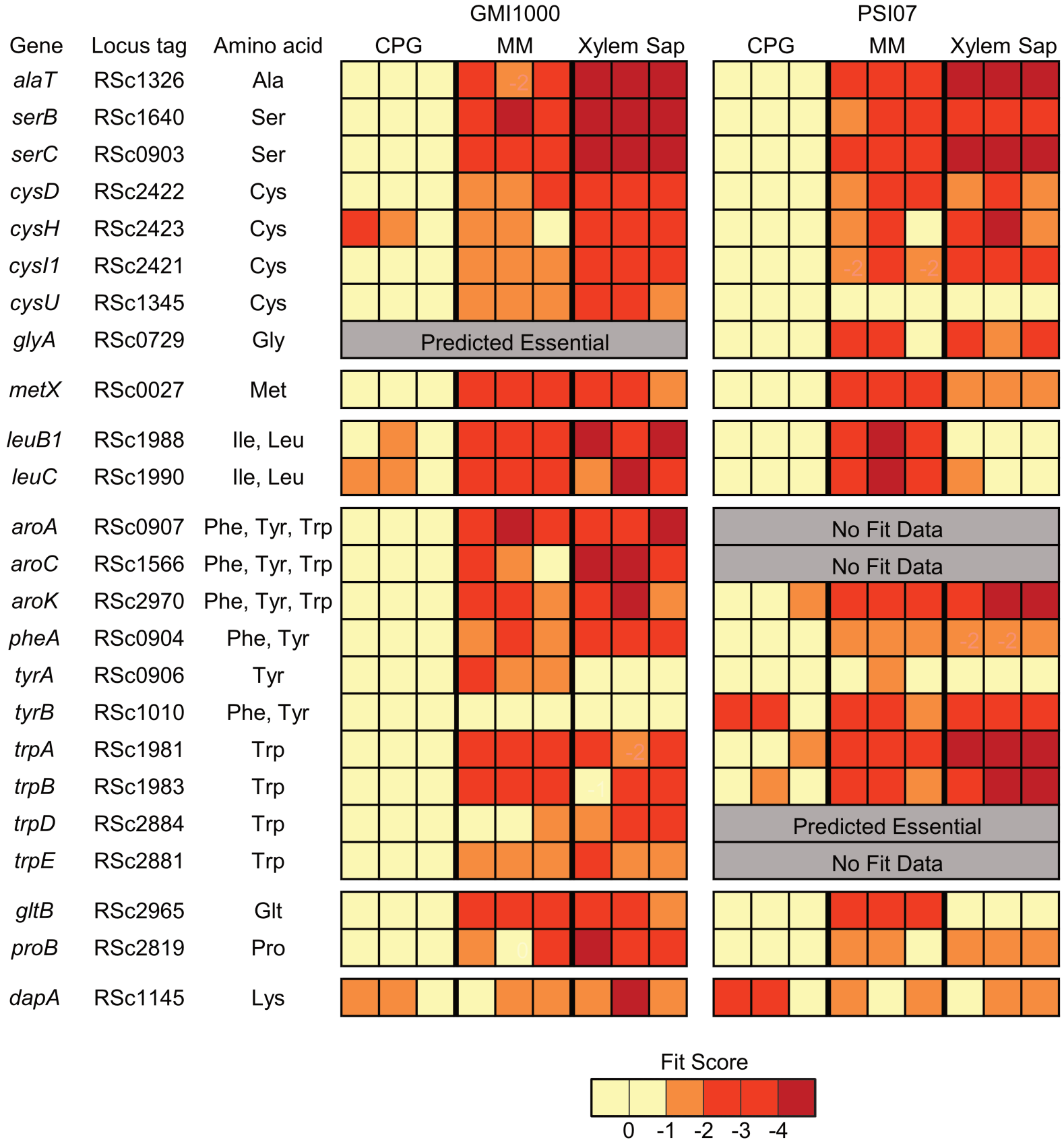
Table S2: Genome-wide Fit scores for GMI1000, PSI07, and IBSBF1503

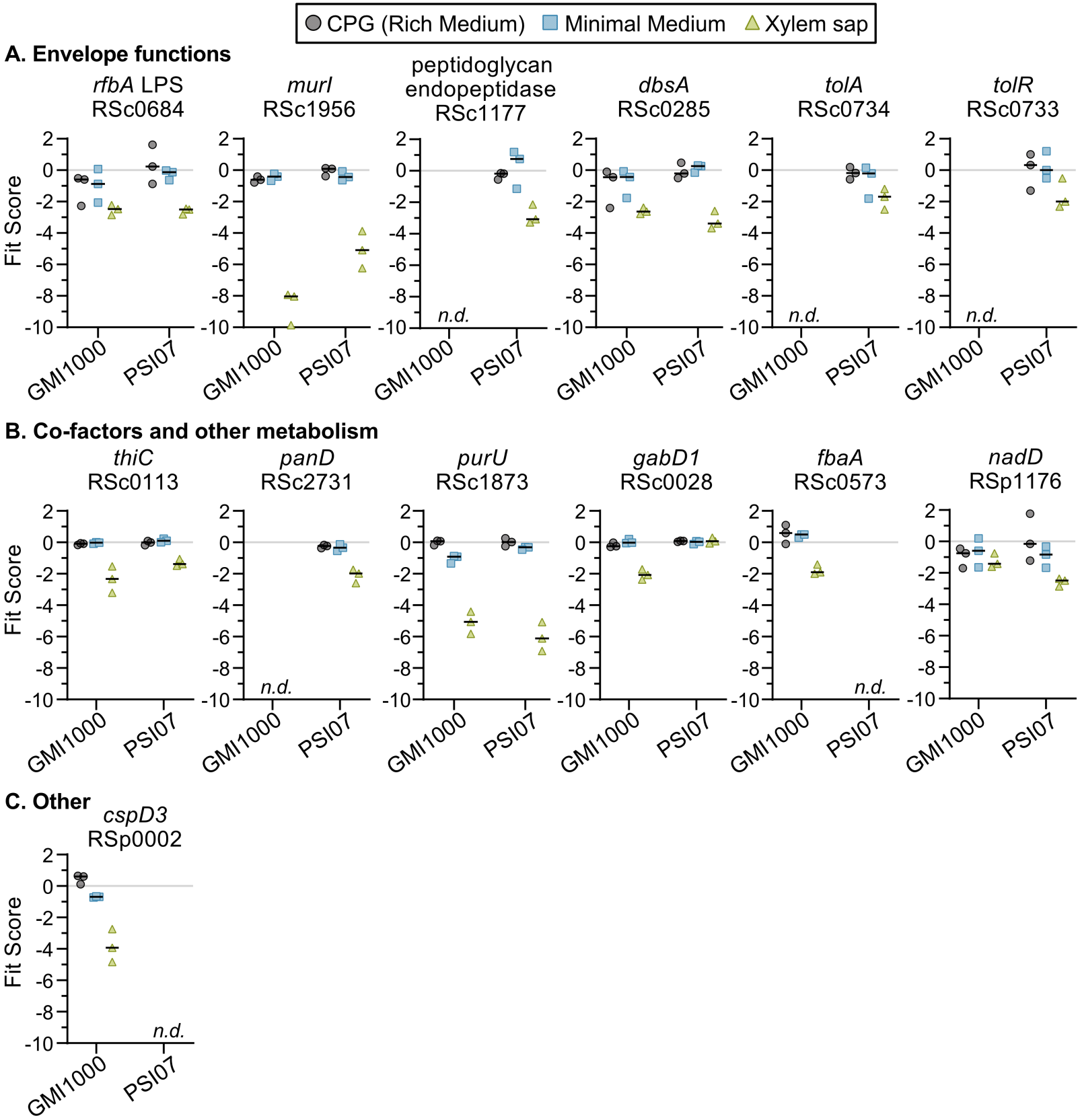
Table S3: Carbon source utilization patterns of GMI1000, PSI07, and IBSBF1503


CPG (Rich Medium)
Minimal Medium
Xylem sap
A
GMI1000
PSI07
IBSBF1503
Bacterial Density (CFU/ml)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
0
12
24
36
48
Time (hr)
B
Doubling Time (hr)
2.5
2.0
1.5
1.0
0.5
0.0
GMI1000
PSI07
IBSBF1503


• Mean fitness score
Replicate fitness score
A. R. syzygii PSI07
ex vivo xylem sap
Minimal medium (MM)
Rich medium (CPG)
Fitness Score
n=1
n=69
n=26
n=63
n=32
n=34
B. R. pseudosolanacearum GMI1000
ex vivo xylem sap
Minimal medium (MM)
Rich medium (CPG)
n=1
n=80
n=4
n=69
n=2
n=41
C. R. solanacearum IBSBF1503
Minimal Medium (MM)
Rich Medium (CPG)
n=7
n=45
n=10
n=30
Intersection Analysis:
Sets of genes with co-fitness or condition-specific fitness phenotypes
Fit < -1 or > 1
Fit < -2 or > 2
D.
PSI07
Xylem Sap
MM
CPG
Genes
Mutants with reduced fitness
Mutants with increased fitness
E.
GMI1000
Xylem Sap
MM
CPG
Genes
Mutants with reduced fitness
Mutants with increased fitness


CPG (Rich Medium)
Minimal Medium
Xylem sap
A.
B.
phcA
phcS
phcR
RSp0224
rpoS
vsrA
vsrD
RSp1579
Fit Score
6
4
2
0
-2
-4
PSI07
GMI1000
IBSBF1503


GMI1000
PSI07
Gene
Locus tag
Amino acid
CPG
MM
Xylem Sap
CPG
MM
Xylem Sap
alaT RSc1326 Ala
serB RSc1640 Ser
serC RSc0903 Ser
cysD RSc2422 Cys
cysH RSc2423 Cys
cysI1 RSc2421 Cys
cysU RSc1345 Cys
glyA RSc0729 Gly
Predicted Essential
metX RSc0027 Met
leuB1 RSc1988 Ile, Leu
leuC RSc1990 Ile, Leu
aroA RSc0907 Phe, Tyr, Trp
aroC RSc1566 Phe, Tyr, Trp
aroK RSc2970 Phe, Tyr, Trp
pheA RSc0904 Phe, Tyr
tyrA RSc0906 Tyr
tyrB RSc1010 Phe, Tyr
trpA RSc1981 Trp
trpB RSc1983 Trp
trpD RSc2884 Trp
trpE RSc2881 Trp
No Fit Data
No Fit Data
Predicted Essential
No Fit Data
gltB RSc2965 Glt
proB RSc2819 Pro
dapA RSc1145 Lys
Fit Score
0 -1 -2 -3 -4


CPG (Rich Medium)
Minimal Medium
Xylem sap
A. Envelope functions
rfbA LPS RSc0684
murI RSc1956
peptidoglycan endopeptidase RSc1177
dbsA RSc0285
tolA RSc0734
tolR RSc0733
B. Co-factors and other metabolism
thiC RSc0113
panD RSc2731
purU RSc1873
gabD1 RSc0028
fbaA RSc0573
nadD RSp1176
C. Other
cspD3 RSp0002
Fit Score
2
0
-2
-4
-6
-8
-10
n.d.
GMI1000
PSI07