Supplemental Information

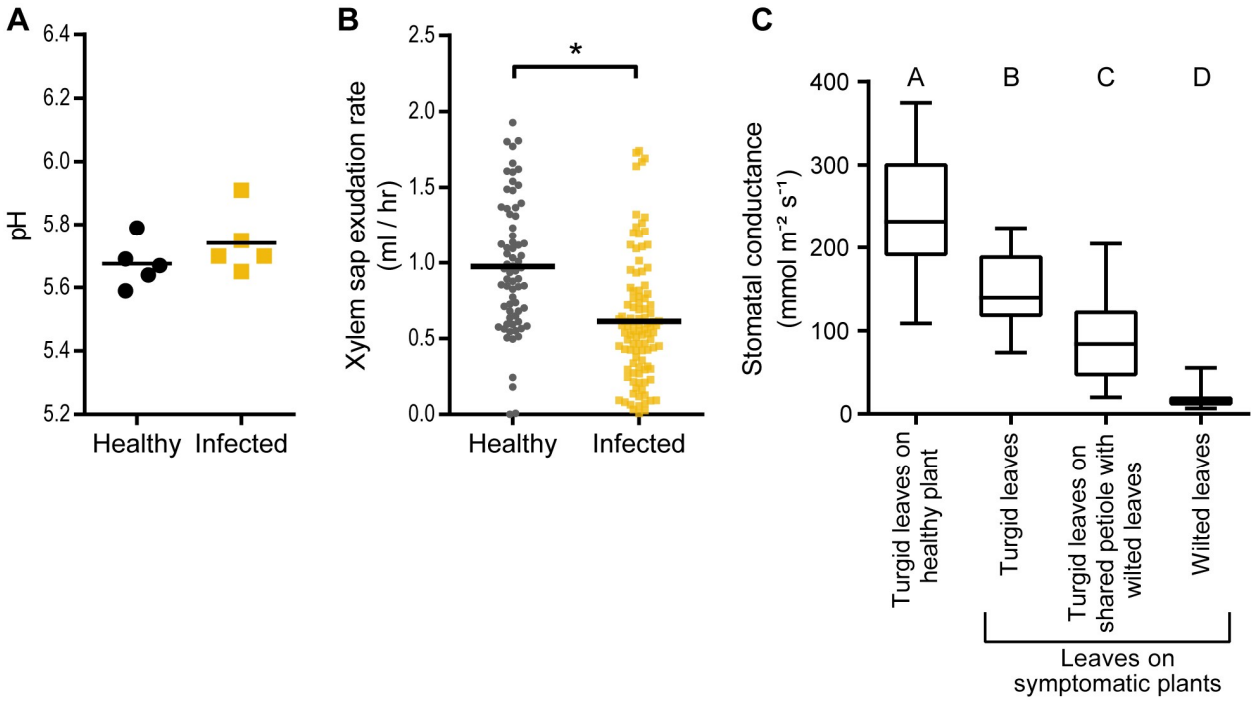

Figure S1. Effect of bacterial wilt disease on tomato xylem sap pH and xylem physiology. (A) Xylem sap pH (n=5) and (B) rate of xylem sap exudation from de-topped tomato stems (*P<0.0001 t-test; n≥69 plants). Samples are from cv. Bonny Best tomato plants soil-soak inoculated with *R. solanacearum* GMI1000 (Infected) or water (Healthy). Sap was harvested when plants displayed first wilting symptoms. (C) Stomatal conductance of Bonny Best tomato leaves was measured with a Licor Photosynthesis instrument. Turgid leaves from healthy plants and apparently turgid and wilted plants from symptomatic plants soil-soak inoculated with *R. solanacearum* GMI1000 were analyzed; n≥18, letters indicate P<0.05 by ANOVA with Tukey's multiple comparison test.

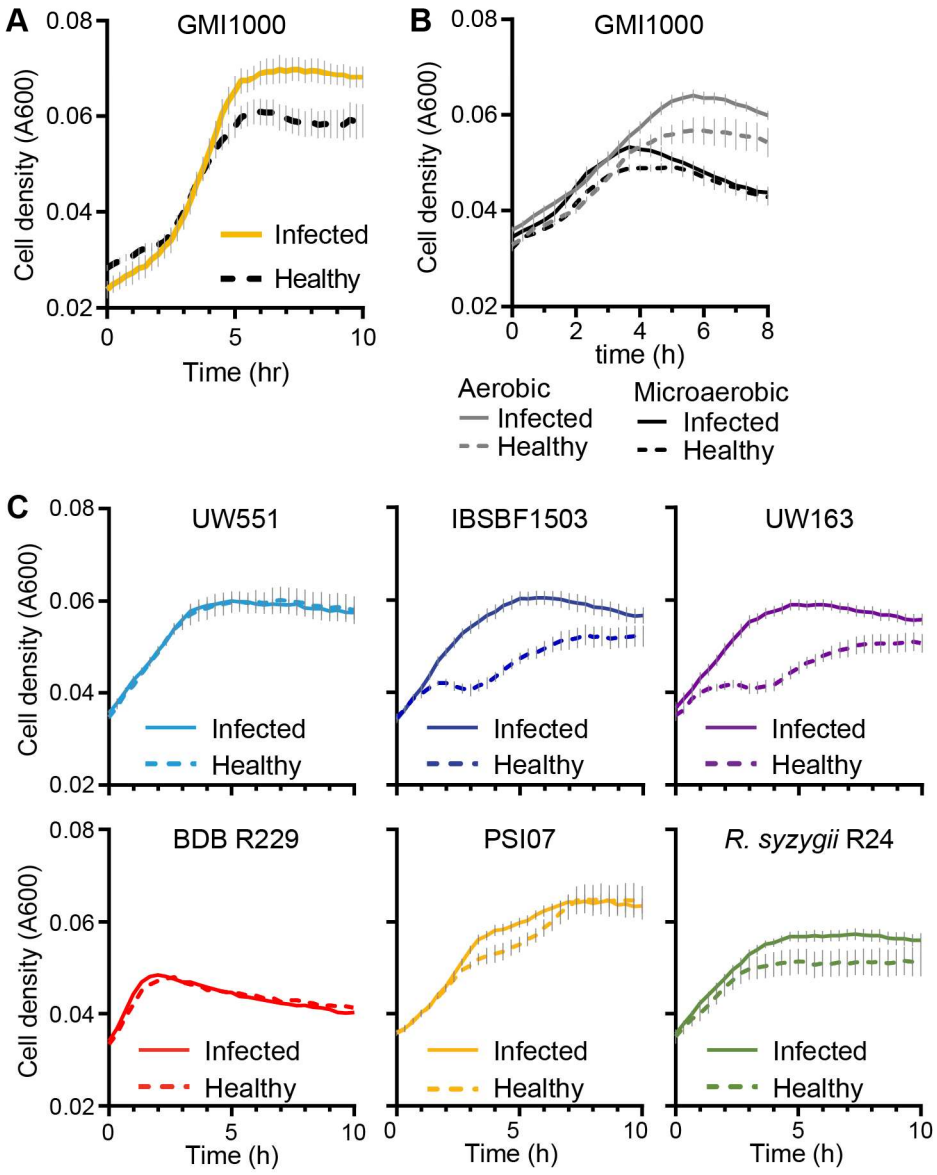

Figure S2. Growth of diverse *R. solanacearum* strains in *ex vivo* xylem sap from healthy tomato plants or from plants infected with *R. solanacearum* GMI1000. (A) Growth of *R. solanacearum* GMI1000 in sap from tomato cv. Money Maker plants resembles growth in sap from GMI1000-infected tomato cv. Bonny Best plants shown in Fig. 1. (B) Aerobic and microaerobic (0.1% O_2) growth of *R. solanacearum* GMI1000 in xylem sap from tomato cv. Bonny Best tomato plants. (C) Growth of diverse *R. solanacearum* strains (phylotype IIB UW551, phylotype IIB IBSBF1503, phylotype IIB UW163, phylotype IV Blood disease bacterium (BDB) R229, phylotype IV PSI07, phylotype IV *R. syzygii* R24) in xylem sap from tomato cv. Bonny Best plants. n≥3 pools of xylem sap for all growth experiments. Data are mean ± SEM.

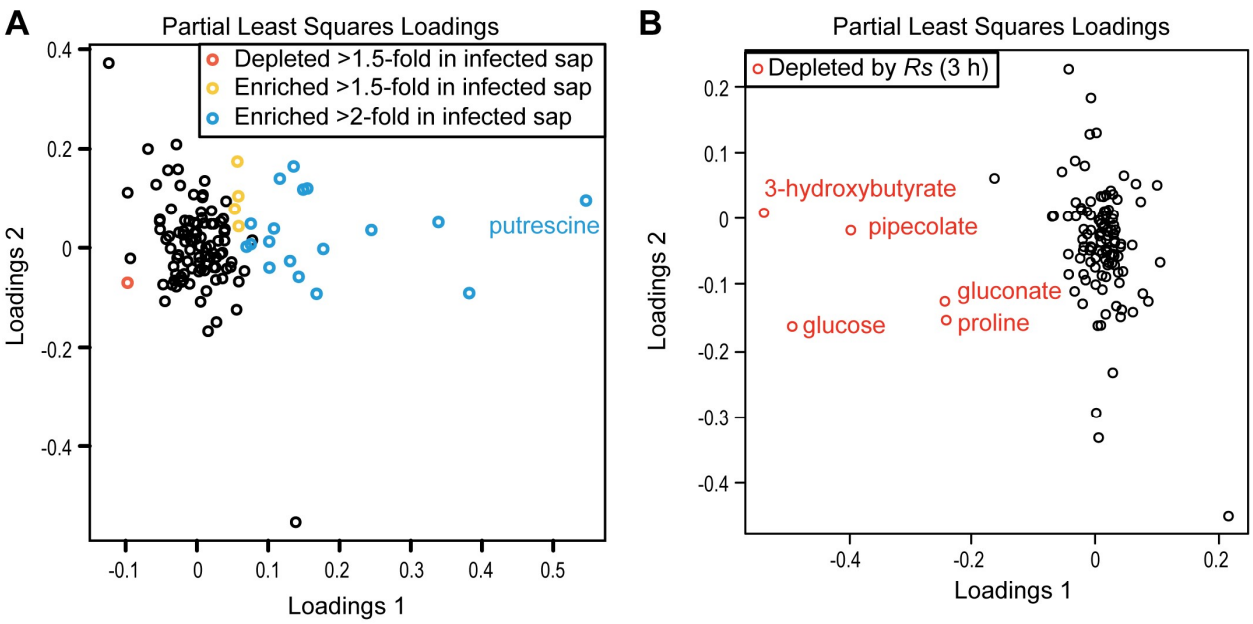

Figure S3. Loadings for partial least squares analyses of metabolomics data. (A) Relative amounts of metabolites from healthy tomato sap vs. *R. solanacearum*-infected sap as detected by untargeted GC-MS analysis. (B) Comparison of metabolomic changes in *ex vivo* sap after 3 h incubation with *R. solanacearum*.

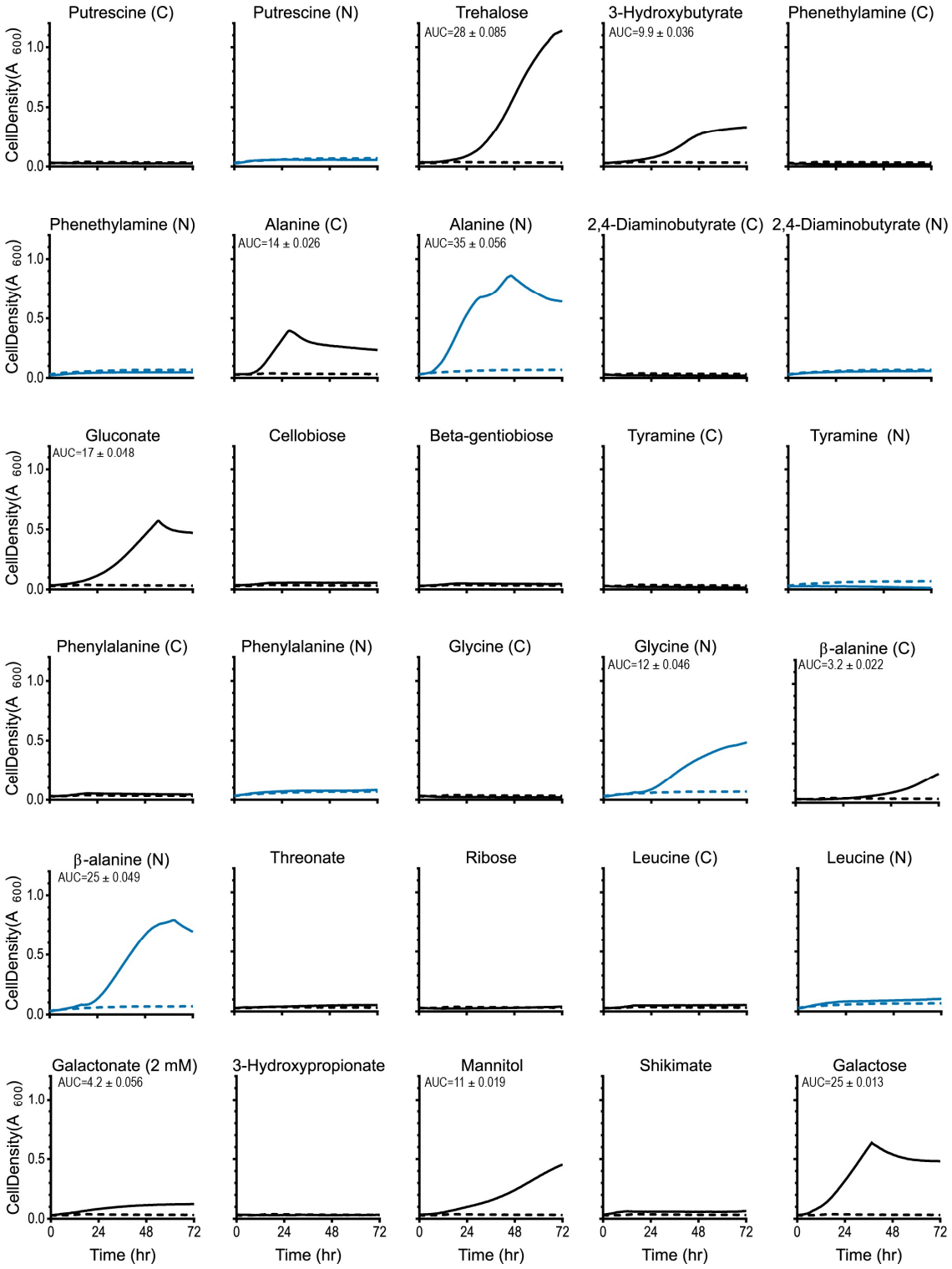

Figure S4. Growth of *R. solanacearum* GMI1000 on metabolites enriched or depleted in xylem sap from *R. solanacearum* GMI1000-infected tomato plants vs. healthy plants. Metabolites (identified as shown in Fig 1) were tested as sole carbon sources (black) and sole nitrogen sources (blue) at 10 mM unless otherwise indicated. Dashed lines show negative controls lacking either carbon or nitrogen sources and solid lines show growth on test metabolites. Area under curve (AUC ± standard error) is indicated for metabolites that supported growth. N=3.

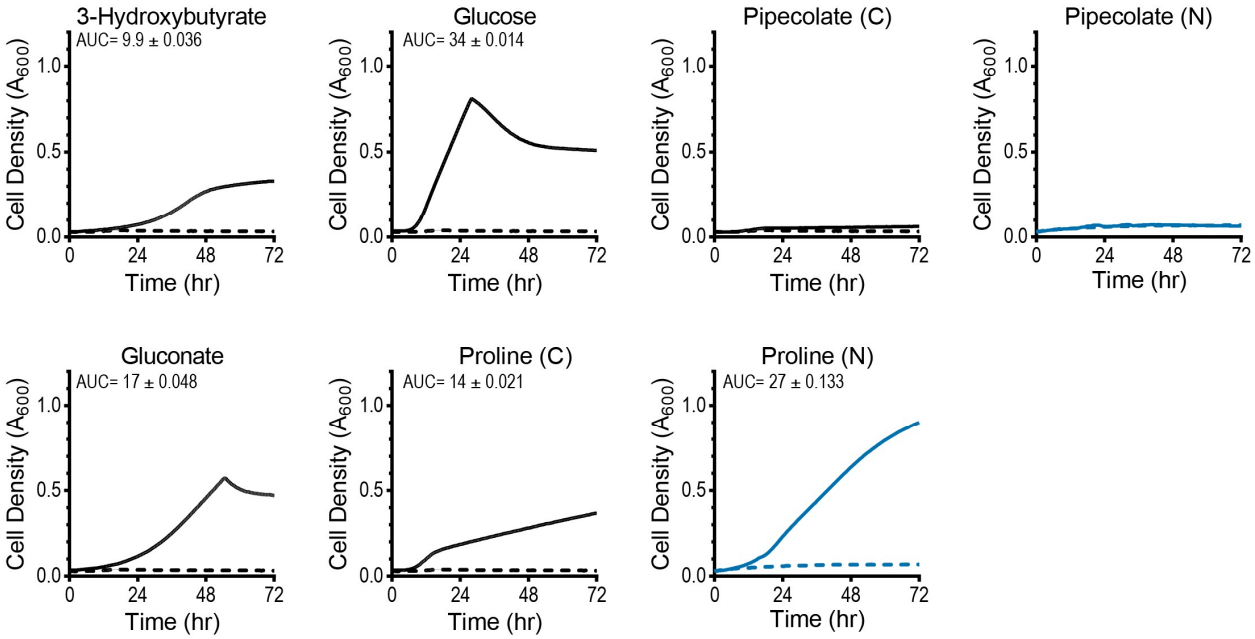

Figure S5. Growth of *R. solanacearum* GMI1000 on metabolites that were depleted in *ex vivo* sap incubated for 3 h with *R. solanacearum*. Metabolites (identified as shown in Fig 2 and S3B) were tested as sole carbon sources (black) and nitrogen sources (blue) at 10 mM in minimal medium unless otherwise indicated. Area under growth curve (AUC ± standard error) is indicated for metabolites that supported growth. N=3.

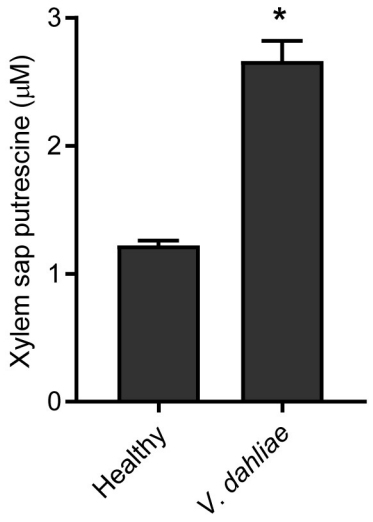

Figure S6. Xylem sap from tomato plants infected with *V. dahliae* has enriched putrescine. Xylem sap was harvested at symptom onset from tomato plants (cv. Money Maker) infected wilt fungus *Verticillium dahliae*; water-inoculated plants served as controls. Putrescine was measured by LC-MS. Values are mean ± SEM. (**P*<0.05 vs. healthy, t-test, *n*≥3 pools).

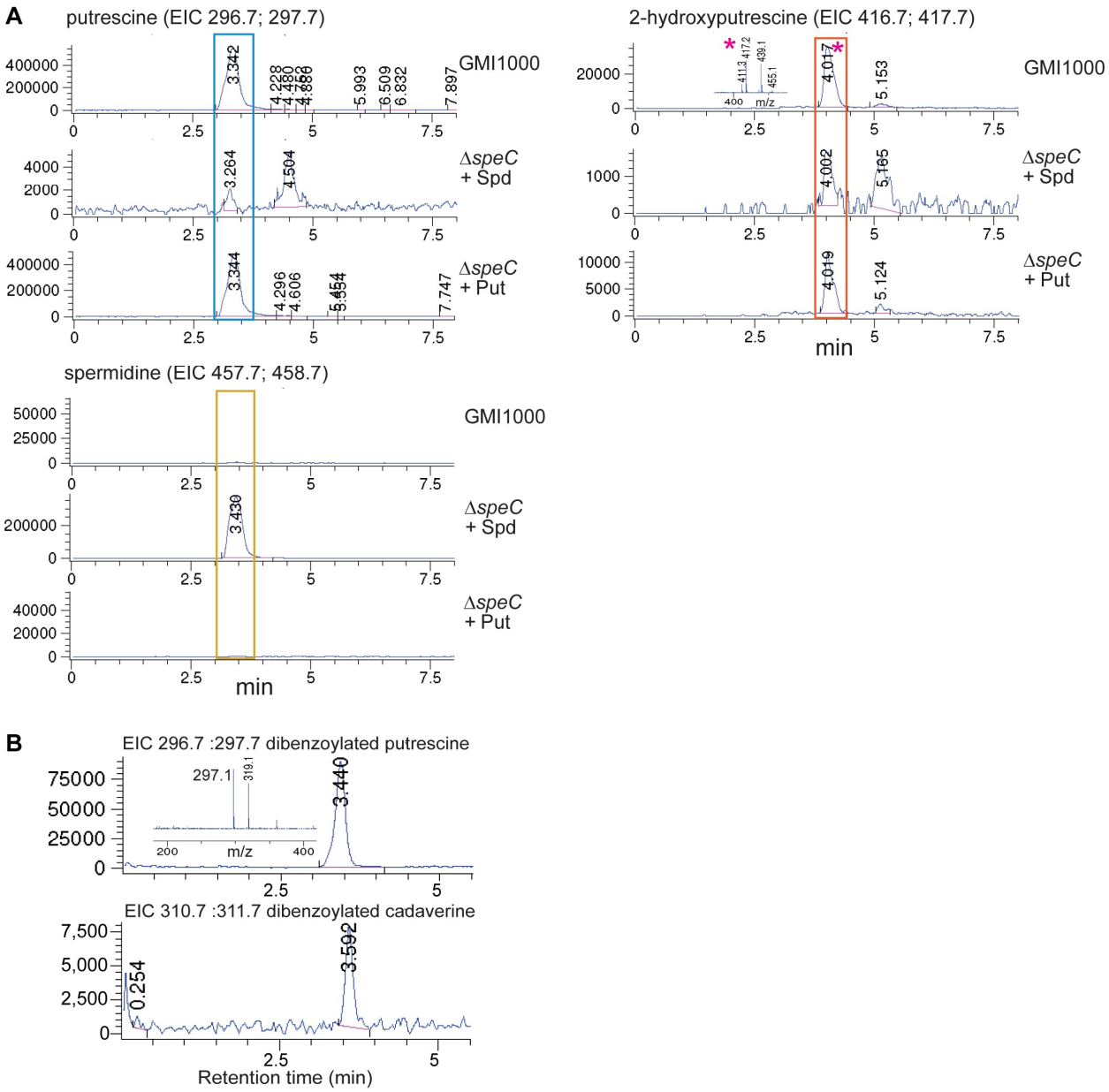

Figure S7. Polyamine profile of *R. solanacearum* cells and substrate specificity of RSc2365 SpeC.
(A) Polyamine profile of *R. solanacearum* cell pellets grown in defined medium as determined by LC-MS analysis of the cellular benzoylated polyamines. *R. solanacearum* GMI1000 and Δ*speC* mutant strains were grown in minimal medium with or without 500 µM putrescine (Put) or spermidine (Spd). Extracted ion chromatograms are shown for dibenzoylated putrescine (296.7; 297.7); tribenzoylated 2-hydroxyputrescine (416.7; 417.7); tribenzoylated spermidine (457.7; 458.7). The mass spectrum for the 2-hydroxyputrescine peak at 4.017 min is indicated by an asterisk in the GMI1000 sample; a peak of 417.2 (m/z) for 2-hydroxyputrescine is found, along with the sodium adduct at +22 (m/z 439.1). Boxes indicate the peaks corresponding to putrescine, 2-hydroxyputrescine, and spermidine. Representative results from lysates analyzed in triplicate are shown. (B) Substrate specificity of RSc2365 decarboxylase was determined by a ornithine/lysine substrate competition *in vitro* assay using recombinant SpeC. Purified SpeC protein (2 µM) was assayed for 1 h at 22°C in the presence of equal amounts (10 mM) of both L-lysine and L-ornithine. After benzoylation of the products, the resulting diamines (putrescine and cadaverine) were detected by LC-MS. Extracted Ion Chromatograms (EIC) for the mass of dibenzoylated putrescine (296.7; 297.7) and dibenozylated cadaverine (310.7; 311.7) are shown. Identity of the peak for dibenzoylated putrescine (3.440 min) was confirmed by the presence of the corresponding mass (m/z 297.1 and sodium adducted form at m/z 319.1) in the mass spectrum (inset).

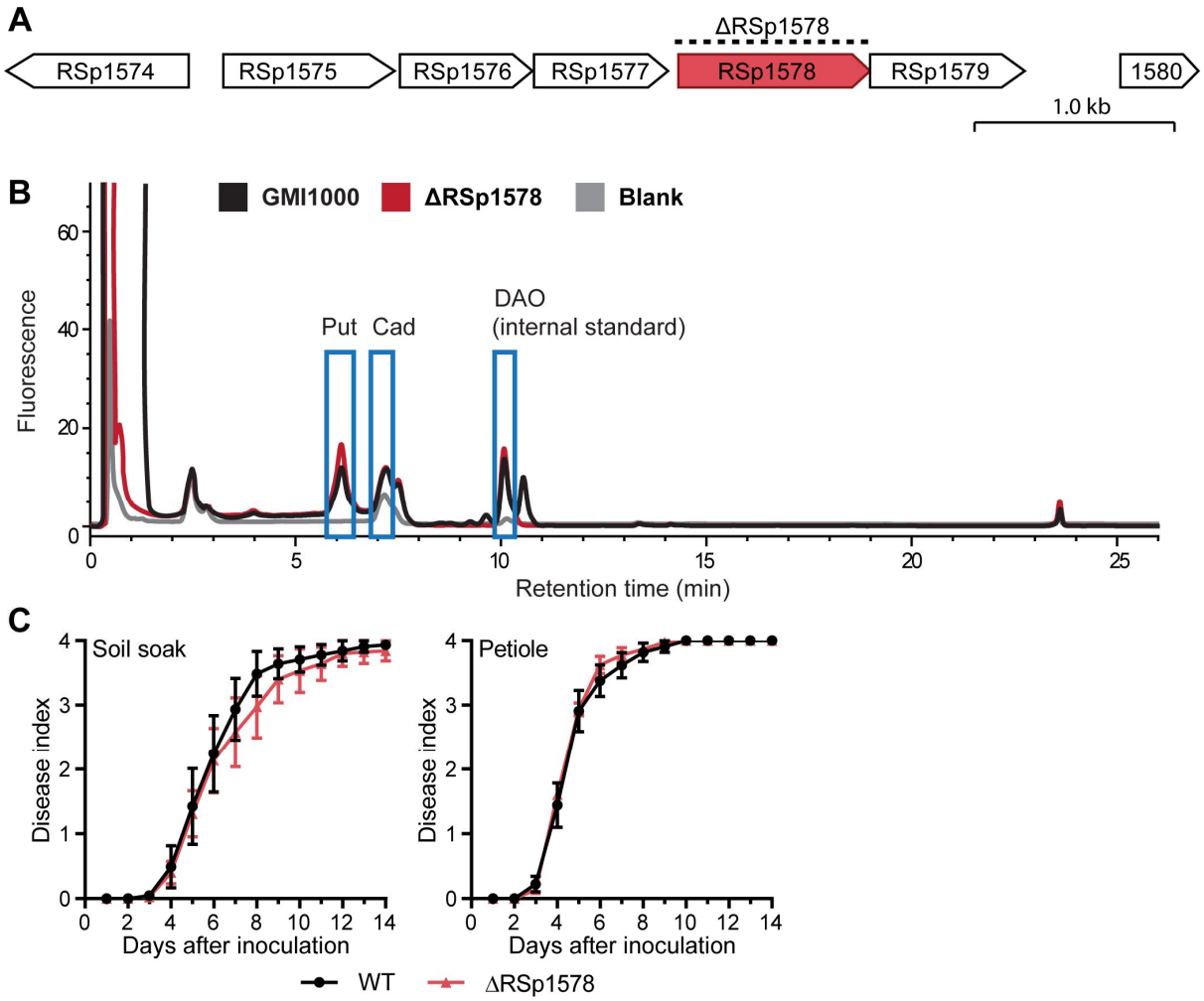

Figure S8. The RSp1578 locus (encoding a putative agmatinase) does not contribute to *R. solanacearum* putrescine production or *R. solanacearum* virulence. (A) Genomic context of RSp1578 in the *R. solanacearum* strain GMI1000 genome. Dashed line indicates region deleted ΔRSp1578 mutant. (B) Polyamine profile of GMI1000 and ΔRSp1578 mutant. Cell lysate polyamines were derivatized with dansyl chloride. HPLC trace (fluorescence excitation: 333 nm; emission: 518 nm) of representative samples. Peaks corresponding to putrescine, cadaverine, and internal standard 1,8-diaminooctane (DAO). Representative results are shown for lysates analyzed in triplicate. (C) Virulence of the ΔRSp1578 mutant on wilt-susceptible tomato cv. Bonny Best after soil soak inoculation ($5x10^8$ CFU g^{-1} soil) or stem inoculation (10^3 CFU). Values are mean ± SEM (*n*=45 plants/treatment).

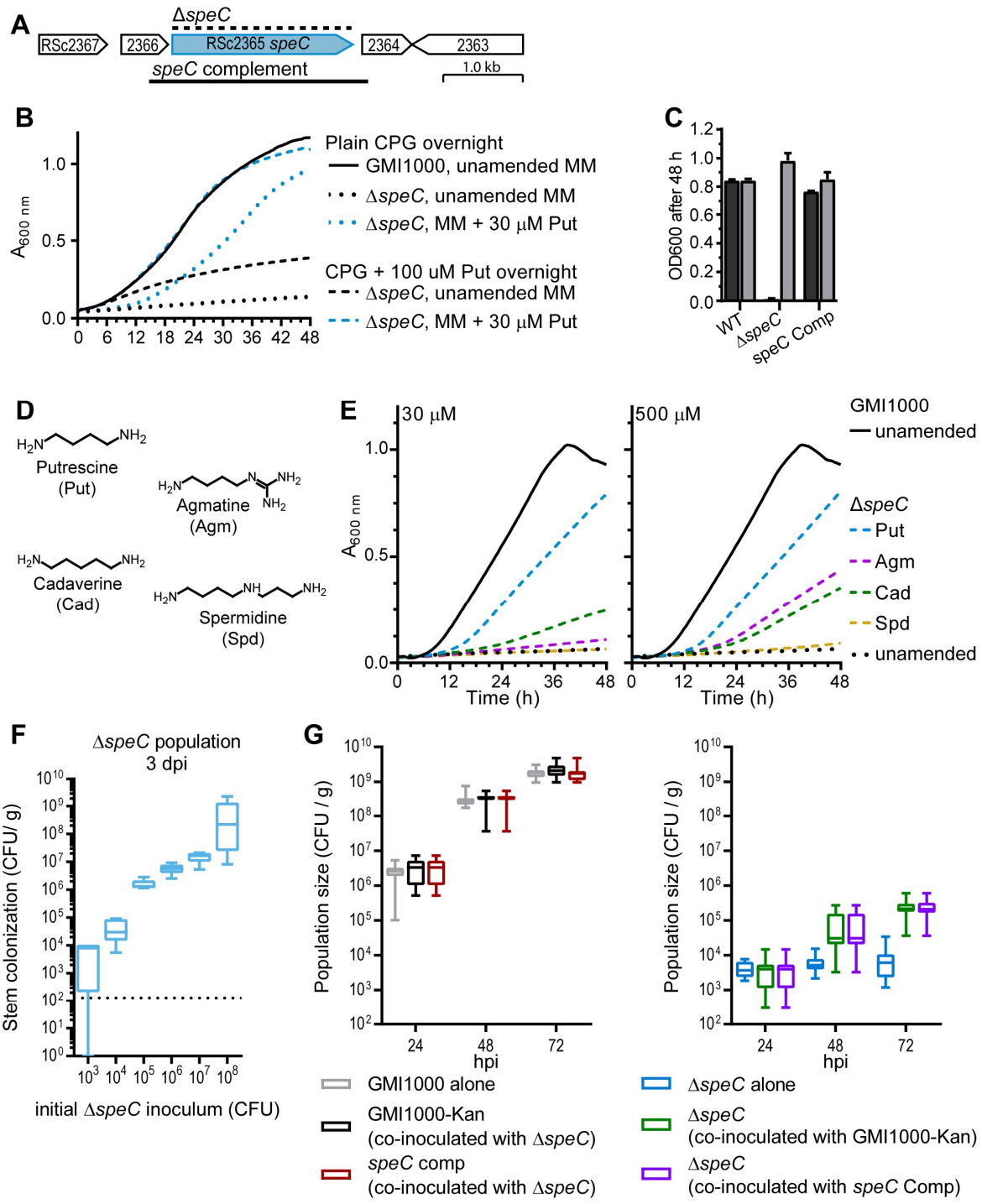

Figure S9. *In vitro* and *in planta* growth of Δ*speC* mutant and its genetic complement. (A) Genomic context of *speC* gene (RSc2365). Dashed line indicates the region replaced by the spectinomycin cassette in the Δ*speC* mutant. Solid line indicates the region used to complement the Δ*speC* mutant using the predicted native promoter. (B) Effect of overnight culture conditions on growth of Δ*speC* mutant in minimal medium (MM). Strains were incubated overnight in CPG or CPG with 100 μM putrescine, washed, and resuspended in media as indicated in figure (*n*=3). (C) Growth of GMI1000 (WT), Δ*speC* mutant, and the complemented Δ*speC* mutant (*speC* Comp) in MM with (grey bars) or without 30 μM putrescine (black bars). (D) Structures of putrescine, spermidine, cadaverine, and agmatine, and (E) ability of 30 μM or 100 μM of these compounds to restore growth of Δ*speC* mutant when added to MM (*n*=3). (F) Stem population sizes of Δ*speC* mutant in tomato cv. Money Maker plants was measured 3 d after stem inoculation with 10^3 to 10^8 CFU (*n*=5 plants per condition). (G)

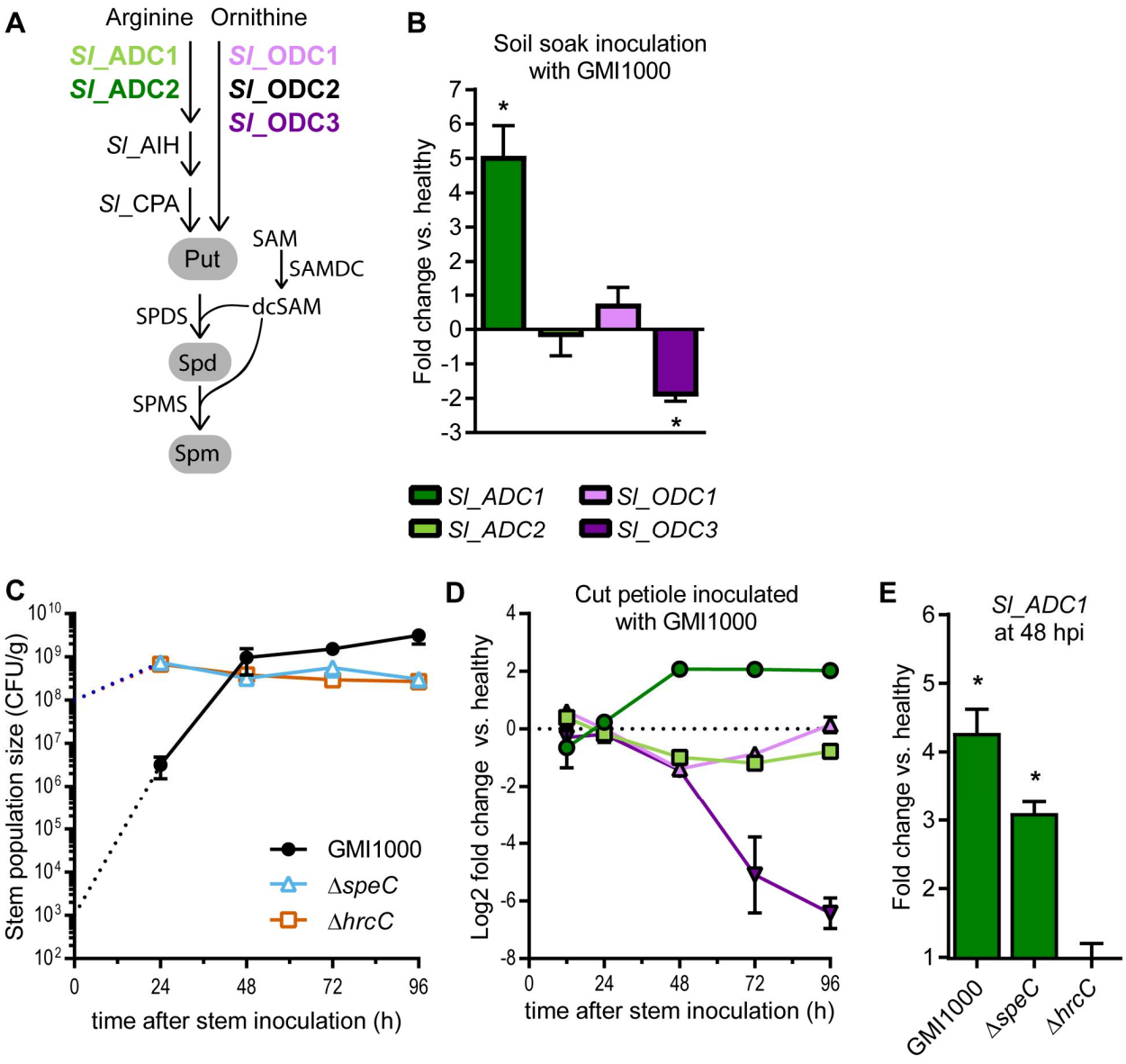

Figure S10. Expression of tomato putrescine biosynthesis genes during bacterial wilt disease. (A) Tomato putrescine biosynthesis pathway. (B) Gene expression of tomato cv. Money Maker after naturalistic soil-soak inoculation with *R. solanacearum* ($5x10^8$ CFU g^{-1} soil) or water. Stem RNA was extracted from healthy or symptomatic infected plants and analyzed by RT-qPCR with normalization to *ACTIN*. Values are mean ± SEM (n=5) * indicates expression levels differ from healthy plants at P<0.005, one-sample t-test. (C-E) Tomato polyamine biosynthesis gene expression after stem inoculation. Polyamine biosynthesis genes are identified by color as indicated. Tomato plants (cv. Money Maker) were cut-petiole inoculated with 10^3 CFU *R. solanacearum* GMI1000, 10^8 CFU Δ*speC* (Put^-), or 10^8 CFU Δ*hrcC* ($T3SS^-$). (C) Population sizes of the three bacterial strains over time in stems directly below the inoculation site. Dashed lines indicate inoculum density at t=0. Values are geometric mean ± SEM (n=3). (D) Time-course expression of polyamine biosynthesis genes in tomato stems infected with *R. solanacearum* GMI1000. (E) Expression of *SI_ADC1* at 48 hpi in tomato stem tissue with equal bacterial burden of *R. solanacearum* GMI1000, Δ*speC* (Put^-), Δ*hrcC* ($T3SS^-$), relative to gene expression in healthy plants. Values are mean ± SEM (n=3).

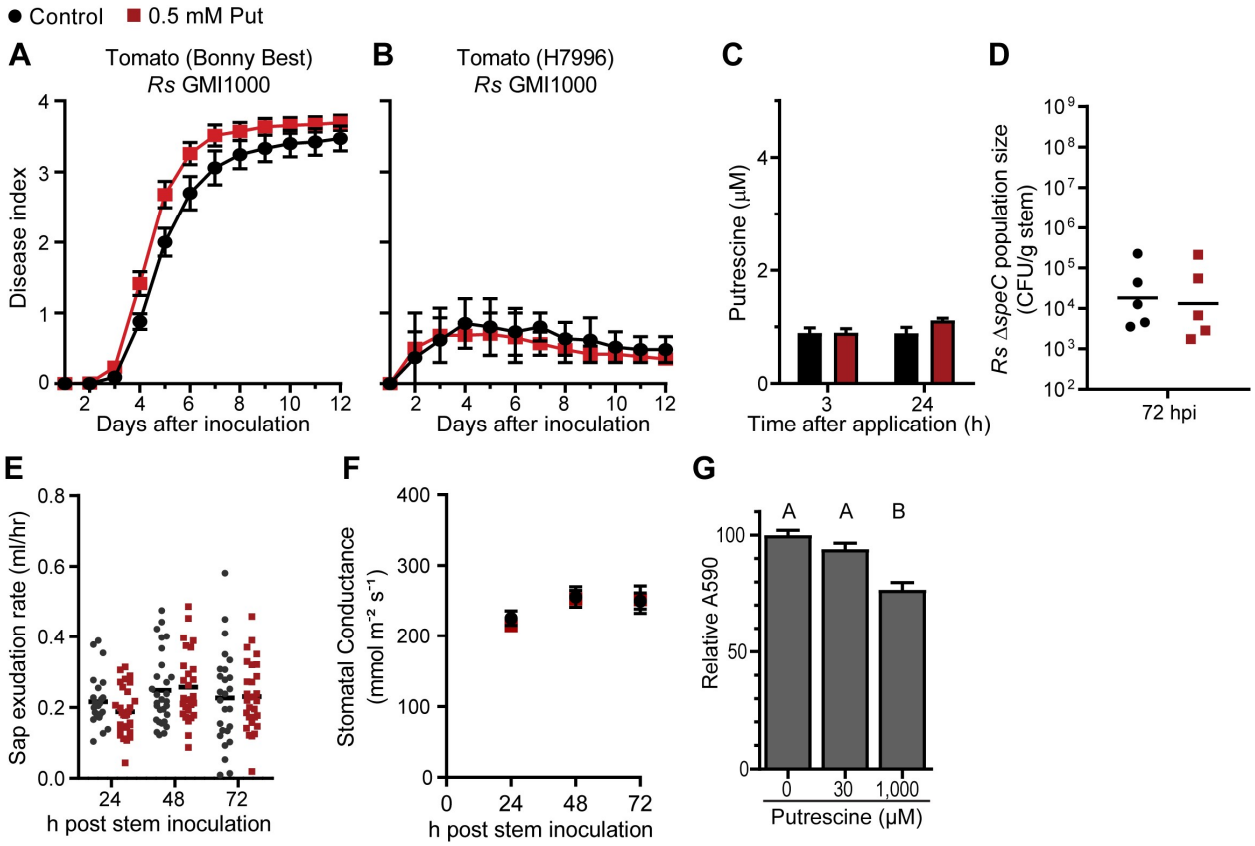

Figure S11. Effects of putrescine treatment on healthy and *R. solanacearum*-infected tomato plants and *R. solanacearum* attachment to polyvyinylchloride. Putrescine (0.5 mM) or water (control) was applied to tomato plants by foliar spray and soil soak. (A-B) At 3 h post putrescine treatment, tomato (A) susceptible Bonny Best (P=0.0151 repeated measures ANOVA; n=180 plants/treatment) or (B) resistant Hawaii7996 (H7996) (P=0.8121; n=30 plants/treatment) were stem inoculated with *(*A) 50 or (B) 50,000 CFU *R. solanacearum* GMI1000. Symptom development was measured using a disease index corresponding to percent of wilted leaflets. Values are means ± SEM. (C-D) Effect of putrescine treatment on putrescine levels in xylem sap as measured by (*C*) LC-MS quantification and (D) growth of *R. solanacearum* Δ*speC* putrescine biosensor strain in xylem vessels. (C) Xylem sap of non-infected plants was harvested at 3 and 24 h after putrescine treatment, and putrescine was measured with LC-MS. Values are means ± SEM (n=3). (D) Leaves and soil of tomato plants were treated with 0.5 mM putrescine or water (control) every 24 h for three treatments. At 3 h after first treatment, 10^4 CFU Δ*speC* were inoculated into the stem. Population size was determined at 72 h post inoculation by dilution plating ground stem sections. Line shows geometric mean (n=5). (E) Effect of putrescine on root pressure-driven sap exudation of *R. solanacearum*-infected tomato cv. Money Maker plants. Plants were stem inoculated with 50 CFU *R. solanacearum* GMI1000. Sap exudation rate of detopped plants was measured for 30 min (n≥26). (F) Leaf stomatal conductance of control or putrescine-treated tomato cv. Money Maker plants was measured by Licor 6400 XT portable photosynthesis system at 24 to 72 h after stem inoculation with 50 CFU *R. solanacearum* GMI1000; (n≥30 plants/treatment). (G) Effect of putrescine on *R. solanacearum* GMI1000 in crystal violet polyvinylchloride attachment assay. *R. solanacearum* was grown in CPG with 0, 30 μM, or 1 mM putrescine. Letters indicate P<0.05 by ANOVA with Tukey's test for multiple comparisons. Values are means ± SEM (n=60).

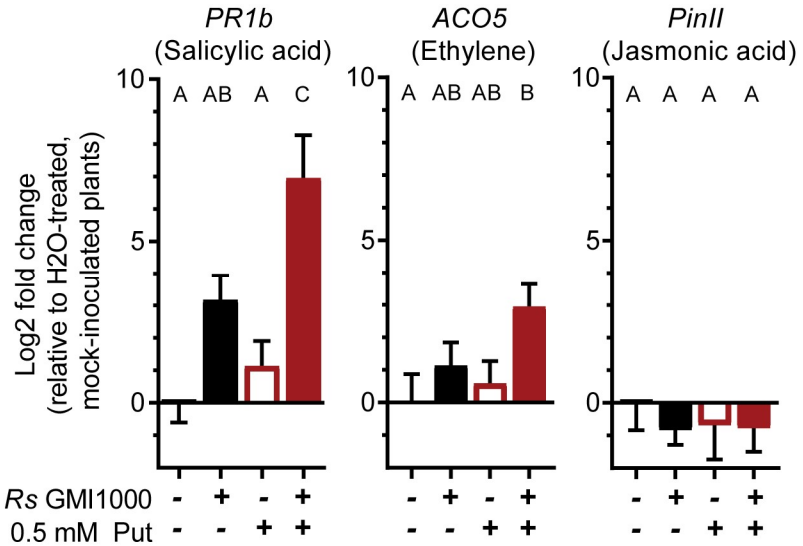

Figure S12. Effect of putrescine treatment and *R. solanacearum* infection on expression of tomato defense genes. Plants were stem inoculated with 50 CFU and RNA was extracted at 48 hpi. Expression was normalized to *ACTIN* transcript levels. Values are mean ± SEM. Letters indicate $P<0.05$ by ANOVA with Tukey test for multiple comparisons ($n=7$ plants/treatment).

Table S1: Expression of tomato polyamine metabolism genes in seedling roots of healthy and GMI1000-infected tomato (cv. Bonny Best)

Enzyme Class	Locus tag	Gene name	Differential expression in Bonny Best seedling roots[a]
Polyamine Biosynthesis Enzymes			
Arginine decarboxylase (ADC)	Solyc10g054440.1.1	Sl_ADC1	+3.4
	Solyc01g110440.2.1	Sl_ADC2	
Ornithine decarboxylase (ODC)	Solyc04g082030.1.1	Sl_ODC1	
	Solyc03g098300.1.1	Sl_ODC2	+6.8
	Solyc03g098310.1.1	Sl_ODC3	
Agmatine deiminase (AIH)	Solyc12g038970.1.1		
N-Carbamoylputrescine amidohydrolase (CPA)	Solyc11g068540.1.1		
Spermidine synthase (SPDS)	Solyc05g005710.2.1		
	Solyc04g026030.2.1		
Spermine synthase (SPMS)	Solyc03g007240.2.1	Sl_SPMS1	
S-Adenosylmethionine decarboxylase (SAMDC)	Solyc05g010420.1.1	Sl_SAMDC	
	Solyc02g089610.1.1	Sl_SAMDC2	+1.8
	Solyc01g010050.2.1	Sl_SAMDC3	
Thermospermine synthase (tSPMS)	Solyc08g061970.2.1		
	Solyc09g075900.2.1		
	Solyc07g041300.1.1		
Polyamine Catabolism			
Diamine oxidase (DAO)	Solyc03g005160.2.1		
	Solyc09g075930.1.1		
	Solyc09g075940.2.1		-4.4
	Solyc09g090490.1.1		
Polyamine oxidase (PAO)	Solyc05g018880.1.1		+1.9
	Solyc07g039310.1.1		
	Solyc01g087590.2.1		
	Solyc07g043590.2.1		+1.5
	Solyc12g006370.1.1		

	Solyc02g081390.2.1		
	Solyc03g031880.2.1		
Putrescine *N*-methyltransferase (PMT)	Solyc08g014310.2.1		
	Solyc06g053510.2.1		
Putrescine N-Acetyltransferase (NATA)	Solyc10g084640.1.1	Sl_NATA1	
Spermidine hydroxycinnamoyl transferase (HCT/SHT)	Solyc07g015960.1.1		
	Solyc03g117600.2.1		+1.6
	Solyc07g005760.2.1		

[a] Differential gene expression in tomato seedling roots with or without GMI1000 at 24 hpi; Differentially expressed genes with q < 0.05 were identified using Cuffdiff (Tuxedo Suite). Genes up- and down-regulated are labeled in yellow and blue, respectively.

Table S2: Strains, plasmids, and primers used in this study

	Description[a]	Reference
***R. solanacearum* species complex strains**		
GMI1000	Phylotype I sequevar 18 strain, isolated from tomato in French Guyana	(Boucher et al., 1985)
ΔspeC	GMI1000 with RSc2365-encoding SpeC ornithine decarboxylase gene replaced with Ω cassette; Sm^R Put^-	This study
speC complement	Δ*speC* complemented with pRCT-speC_com integrated into the chromosome; Sm^R Tet^R	This study
ΔhrcC	Unmarked GMI1000 Δ*hrcC* mutant; lacks type III secretion activity	This study
GMI1000-Kan	GMI1000 with pRCK-GWY integrated into chromosome; Km^R	(Lowe et al., 2015)
K60	Phylotype IIA sequevar 7 strain isolated from tomato in USA	(Kelman, 1954)
UW551	Phylotype IIB sequevar 1 strain isolated from geranium in Kenya	(Gabriel et al., 2006)
IBSBF1503	Phylotype IIB sequevar 4 (NPB "Not pathogenic to banana") strain isolated from cucumber in Brazil	(Ailloud et al., 2015)
UW163	Phylotype IIB sequevar 4 strain isolated from plantain in Peru	(Ailloud et al., 2015)
BDB R229	Phylotype IV sequevar 10 Blood Disease Bacterium strain isolated from banana in Indonesia	(Remenant et al., 2011)
PSI07	Phylotype IV sequevar 9 strain isolated from tomato in Indonesia	(Remenant et al., 2010)
R. syzygii R24	Phylotype IV sequevar 9 strain isolated from clove tree in Indonesia	(Remenant et al., 2011)
***V. dahliae* strains**		
JR2	Strain isolated from tomato in Canada	(Faino et al., 2015)
***E. coli* strains**		
TOP10	Cloning strain with genotype: F- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ*ΔM15 ΔlacX74 r*ecA1 araD139* Δ(*araleu*)7697 *galU galK rpsL* (StrR) *endA1 nupG*	ThermoFisher Scientific
BL21(DE3)	Protein expression strain: fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5	
Plasmids		
pST-Blue	Suicide vector for marked *R. solanacearum* mutants; Kan^R	Novagen
pCR8	PCR template for Sm resistance gene (Ω cassette); Sm^R	ThermoFisher Scientific

pKO-speC::Sm	Vector used to generate Sm-marked *R. solanacearum* Δ*speC* mutant; Kan^R Sm^R	This study
pRCT-GWY	Vector that integrates into the selectively neutral *att* site of strain GMI1000 chromosome; Tet^R	(Monteiro et al., 2012)
pRCT-speC_com	Contains the RSc2365 native promoter and ORF; Tet^R	This study
pRCK-GWY	Vector that integrates into the selectively neutral *att* site of strain GMI1000 chromosome; Kan^R	(Monteiro et al., 2012)
pUFR80	Positive selection (*sacB*) suicide vector; Suc^S, Kan^R	(Castañeda et al., 2005)
pUFR80-hrcC	Vector used to generate unmarked *R. solanacearum* Δ*hrcC* mutant; Suc^S (*sacB*), Kan^R	This study
pET28b-TEV	Overexpression vector for 6xHis-tagged proteins, Kan^R	Margeret Phillips, UT-Southwestern
pET28b-TEV-speC	SpeC overexpression vector for with *E. coli*-optimized codons, Kan^R	This study
Cloning Primers[b]		
RSc2365upF	5`-gatatctgaattcgtcgacaCAAGGTCTTCTACACCACCGG target locus: RSc2365	This study
RSc2365upR	5`-ccagagctgcCGGGATTCCTTGACTGATGAAACAAAAG target locus: RSc2365	This study
omega(c2365)F	5`- aggaatcccgGCAGCTCTGGCCCGTGTC	This study
omega(c2365)R	5`- gcaatcacctAAGGGATTTTGGTCATGGGTGGC	This study
RSc2365dwnF	5`-aaaatcccttAGGTGATTGCCGCAACAGG target locus: RSc2365	This study
RSc2365dwnR	5`-gagctagcctaggctcgagaTGGGTGACAAGGCGAACC target locus: RSc2365	This study
speC_comF	5`-ctagggttaacggtaCATCGTCAACGTCACACCG target locus: RSc2365	This study
speC_comR	5`-ccctagtctaagatcttAAAAGACGCTGATGGGGC target locus: RSc2365	This study
hrcCupF	5'-cgacggccagtgccaCATCTACGAATTCGCCGTG target locus: RSp0874	This study
hrcCupR	5'-gcggtccgGATGTTGTCCAGGAGATG target locus: RSp0874	This study
hrcCdwnF	5'-gacaacatcCGGACCGCGCATTCTGTC target locus: RSp0874	This study
hrcCdwnR	5'-acctgcaggcatgcaCGATGGCCTTCCATGCCAAA target locus: RSp0874	This study
1578upF	5'-cgacggccagtgccaACTTCGTGATCCTGTTCTG target locus: RSp1578	This study
1578upR	5'-ccgcttcaCGCAGTGGTATAGGTGCTC target locus: RSp1578	This study
1578dwnF	5'-ccactgcgTGAAGCGGCCATGCTGAC target locus: RSp1578	This study
1578dwnR	5'-acctgcaggcatgcaCCATCTTGGGCCTCTCTG target locus: RSp1578	This study

RT-qPCR primers[c]		
SlADC1-F	5`-AGGTATCGTACTCTCCGCGA target locus: Solyc10g054440.1.1	This study
SlADC1-R	5`-TAGCGAAAGTGGCAGAGCAA target locus: Solyc10g054440.1.1	This study
SlADC2-F	5`-TTGGTCGCAAGAAAGCTCCT target locus: Solyc01g110440.2.1	This study
SlADC2-R	5`-TGGCCAGAATGCTTTGTCCT target locus: Solyc01g110440.2.1	This study
SlODC1-F	5`-TGCCGATATGGAAGGACACG target locus: Solyc04g082030.1.1	This study
SlODC1-R	5`-GAGATGCCCAATGGGTCCAA target locus: Solyc04g082030.1.1	This study
SlODC3-F	5`-GGACCAAGTTGTGACTGCCT target locus: Solyc03g098310.1.1	This study
SlODC3-R	5`-AGGTAGCTGATCCAAGTTTTAGGT target locus: Solyc03g098310.1.1	This study
SlActin-F	5`-TCAGCAACTGGGATGATATG target locus: BT013524	(Milling et al., 2011)
SlActin-R	5`-TTAGGGTTGAGAGGTGCTTC target locus: BT013524	(Milling et al., 2011)
Sl_PR1bM-F	5`-CAAGACATAGGCCCGACTCC target locus: Solyc00g174340.1	Milling and Allen unpublished
Sl_PR1bM-R	5`-AGGCCCAAAATTCACCCCAA target locus: Solyc00g174340.1	Milling and Allen unpublished
Sl_ACO5-F	5`-AGATGGGCATTGGGTGAACA target locus: Solyc07g026650.2	Milling and Allen unpublished
Sl_ACO5-R	5`-TTCAGCCATCACTCGGTGTC target locus: Solyc07g026650.2	Milling and Allen unpublished
Sl_Pin2-F	5`-TGATGCCAAGGCTTGTACTAGAGA target locus: AY129402	(Milling et al., 2011)
Sl_Pin2-R	5`-AGCGGACTTCCTTCTGAACGT target locus: AY129402	(Milling et al., 2011)

[a] Abbreviations: Sm^R, spectinomycin and streptomycin resistance; Put^-, Put auxotrophy; Tet^R, tetracycline resistance; Kan^R; kanamycin resistance.

[b] lowercase bases show the region that overlaps the adjacent fragment and uppercase bases indicate the region specific for the product of interest.

[c] qPCR primers demonstrated amplification efficiencies between 90-110%.

References for Table S2

Ailloud, F., Lowe, T., Cellier, G., Roche, D., Allen, C., and Prior, P. (2015) Comparative genomic analysis of *Ralstonia solanacearum* reveals candidate genes for host specificity. *BMC genomics* **16**: 270.

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Dataset S1: Relative quantification of metabolites in tomato xylem sap.

(A) Comparison of metabolites in healthy vs *R. solanacearum*-infected sap. (B) Comparison of metabolites in *R. solanacearum*-infected tomato sap with or without 3 h incubation with *R. solanacearum* GMI1000.