Table S1. GC/MS analysis of GBL metabolism *in vitro*.

Substrate[a]	Extract[c]				
	control	AttM	AttL [e]	AttK [e]	AttK +AttL [e]
GBL	GBL, 95%[b] GHB, 5%	GBL, 6% GHB, 94%	ND	ND	ND
GHB	GHB, 100% SSA, 0%	ND[d]	GHB, 97% SSA, 3%	GHB, 95% SSA, 5%	GHB, 9% SA, 91%
SSA	SSA, 94% SA, 6%	ND	SSA, 95% SA, 5%	SSA, 8% SA, 92%	SSA, 6% SA, 94%

a. Substrates (the left-most column) were added at a final concentration of 100 μM. In this and all experiments, GBL, GHB, SSA, and SA were obtained from Sigma-Aldrich. The last three compounds were added as sodium salts.

b. The data show the percentage of the added substrate that was converted to the indicated product.

c. To overexpress AttK, AttL, AttM, or AttK plus AttL derivatives of *E. coli* strain BL21/DE3 containing pYC142, pYC161, pYC145, or pYC162, respectively, were cultured in LB medium supplemented with 400 μg ml^{-1} ampicillin. Cells were cultured to an $O.D._{600}$ of approximately 0.4. IPTG (0.5 mM) was then added to

the cultures. Cells were harvested at an $O.D._{600}$ of approximately 1.0, resuspended in 2 ml of phosphate buffer (50 mM, pH 7.4, 100 mM NaCl, and 10% glycerol), and disrupted using a French Pressure minicell. Cell lysates were clarified by ultracentrifugation at 45,000 x g for 30 min (Beckman). Soluble fractions were dialyzed against 500 ml phosphate buffer overnight at $4^{O}C$. Extracts were diluted with phosphate buffer (50 mM phosphate, pH 7.0, 200 mM NaCl, 10 mM $MgCl_2$) to a final concentration of approximately 20 µg/ml of total protein and were mixed with 100 µM of GBL, GHB, or SSA. Where indicated, NAD^+ was added to the mixtures at a final concentration of 10 mM. Reaction samples were incubated at $28^{O}C$. Samples were collected after 4 h, acidified by addition of 1 N HCl, and extracted twice with equal volumes of ethyl acetate (HPLC grade, Fisher). Ethyl acetate phases were pooled and traces of water were removed by addition of anhydrous sodium sulfate. Samples were dried at room temperature and the pellets were dissolved in a final volume of 10 µl ethyl acetate, mixed with 10 µl BSTFA, and incubated at room temperature for 2 h. GC/MS was performed by introducing 0.2 µl of each sample into a GC/MS spectrometer (Hewlett-Packard Model 5890 Series II) by loop injection. All assays were conducted in triplicate, with an experimental error of approximately 5%.

d. ND: not determined.

e. NAD^+ was provided as an electron acceptor.

Table S2. Strains, plasmids, and oligonucleotides used in this study.

Strains	Genotype	Source or Reference
DH5α	*E. coli*, α-complementation	Stratagene
S17-1/λpir	*pro*$^-$, *res*$^-$, *mod*$^+$, integrated copy of RP4, *mob*+, *pir*+	(10)
NTL4	*A. tumefaciens* C58 strain, Ti plasmid-less	(5)
YC1	*attM-lacZ* (in pYC120) integrated into At plasmid of NTL4, Km [a]	This study
YC5	*attL* non-polar deletion in NTL4 [b]	This study
YC6	*attK* non-polar deletion in NTL4 [b]	This study
HC151	*atu4247-lacZ* (in pHC305) integrated into At plasmid of YC6, Km [a]	This study
HC152	*atu3403-lacZ* (in pHC306) integrated into At plasmid of YC6, Km [a]	This study
HC153	*atu4762-lacZ* (in pHC307) integrated into At plasmid of YC6, Km [a]	This study
HC154	*atu3498-lacZ* (in pHC308) integrated into At plasmid of YC6, Km [a]	This study

Plasmids	Properties	
pBluescriptSK+	Cloning vector, Amp^R, ColE1 ori,	Stratagene
pKP302	Promoter-less *lacZ* cloned into pPZP201, Sp^R	(9)
pRSETA	T7 promoter cloning vector, Amp^R	Stratagene
pVIK112	Suicide vector, ATG-less *lacZ* for translational fusion, Km^R	(4)
pPZP201	Broad-host-range cloning vector	(3)
pMW91	Suicide vector, *bla*, *sacB*, Suc^R,	(6)
pYC118	5' and 3'ends of *attL* cloned into pMW91	This study
pYC119	5' and 3' ends of *attK* cloned into pMW91	This study
pYC120	An internal fragment of C58 *attM* cloned into pVIK112	This study
pYC142	C58 *attK* gene cloned into the BamHI and EcoRI sites of pRSETA[c]	This study

pYC145	C58 *attM* gene cloned into the NdeI and HindIII sites of pRSETA[c]	This study
pYC146	P*tac* promoter introduced into pHC12	This study
pYC160	C58 *attJ* gene cloned into the NheI and EcoRI sites of pRSETA[c]	This study
pYC161	C58 *attL* gene cloned into the NdeI and XhoI sites of pRSETA[c]	This study
pYC162	C58 *attK* gene cloned into the EcoRI and XhoI sites of pYC161[d]	This study
pYC174	*attKLM* promoter cloned into the EcoRI site of pKP302 [e]	This study
pHC305-308	An internal fragment of *atu4247*, *atu3403*, *atu4762*, or *atu3498* cloned into suicide plasmid pVIK112	This study
pCST324	*attK* promoter from nucleotides -63 to +24 introduced into pKP302	This study
pCST325	*attK* promoter from nucleotides -63 to +36 introduced into pKP302	This study
pCST326	*attK* promoter from nucleotides -63 to +51 introduced into pKP302	This study
pHis-AttJ	NdeI-EcoRI fragment from pYC160 containing His-6-AttJ cloned into pYC146	This study
pHC305	PCR fragment of *atu4247* made using oligonucleotides K1F and K1R, cloned into pVIK112	This study
pHC306	PCR fragment of *atu3403* made using oligonucleotides K2F and K2R, cloned into pVIK112	This study
pHC307	PCR fragment of *atu4762* made using oligonucleotides K3F and K3R, cloned into pVIK112	This study
pHC308	PCR fragment of *atu3498* made using oligonucleotides K4F and K4R, cloned into pVIK112	This study

Oligonucleotides	DNA Sequence
*attM*C58F2	5’-GATC<u>CATATG</u>ACCGATATCAGACTTTAC-3’
*attM*C58R1	5'-GCT<u>GGTACC</u>TGTTTGGCAGTCGCACCAGCAA-3'
*attM*C58R2	5’-GATC<u>AAGCTT</u>ACGCGTAAAATTCGGGAGC-3’
*attL*C58F1	5'-GCT<u>GGATCC</u>GGAGGATCAGATGACCATCAAC-3'
*attL*C58F2	5’-TGC<u>AAGCTT</u>TTGGGTGCGACATTGGCA-3'

*attL*C58F3 5'-GCTCATATGACCATCAACCCTTTCGAAT-3'

*attL*C58F4 5'-GCTGAATTCGGAGGATCAGATGACCATCAAC-3'

*attL*C58R1 5'-GCTAAGCTTTGTGACTTCCGAACCGGTAC-3'

*attL*C58R2 5’-GCTCTCGAGAGAAATGGGTCAGAACGCCTC-3'

*attK*C58F1 5’-GCTGGATCCGGGAGAGGTCGATGATCAAG-3’

*attK*C58F2 5’-TGCGAATTCAAAGGTCGAGGATCACGTTGC-3'

*attK*C58R1 5’-GCTGAATTCAGGATGGTAATGCGCTTG-3'

*attK*C58R2 5’-GTCCTCGAGGTTGATGGTCATCTGATCCTC-3'

*attJ*C58R1 5’-GACGCTAGCATGGCTGAAGATCAACAAT-3'

P*attKLM*-F1 5’-GACGAATTCCTAGTCTTTCTGCGATCGGT-3'

P*attKLM*-R1 5’-GTCGAATTCATCTTCAGCCATGCACCTTCC-3'

*attJ*C58F1 5'-GTCGAATTCTTCCACCGCAATCGTCTTGCC-3'

K1F 5'-GCTGAATTCTGAATGATCCTGACGCTGGAACAG-3'

K1R 5'-GCTTCTAGAGCGTCATTAAATACGATGAAG-3'

K2F 5'-GCTCCCGGGTGAAAATGGTTTGCGCTGATGATC-3'

K2R 5'-GCTTCTAGAGCGGAATTTCGAAACGATTG-3'

K3F 5'-GCTGAATTCTGAGATATGCTGCCTGTTGATC-3'

K3R 5'-GCTTCTAGACGAACCCGTAAAGGAAAATCG-3'

K4F 5'-GCTGAATTCTGACTGCGTGAGCGCAAGGACAAG-3'

K4R 5'-GCTTCTAGACCATCGTGGCGCGCTTCATG-3'

a: To construct plasmids pYC142, pYC145, pYC160, and pYC161, genomic DNA of *A. tumefaciens* strain C58 was amplified by PCR using oligonucleotides *attK*C58F1 and *attK*C58R1 (for *attK*), *attM*C58F2 and *attM*C58R2 (for *attM*), *attJ*C58F1 and *attJ*C58R1 (for *attJ*), and *attL*C58F3 and *attL*C58R2 (for *attL*). PCR products were cloned into appropriately digested plasmid pRSETA.

b: To construct plasmid pYC162, the C58 *attL* gene was amplified by PCR using oligonucleotides *attL*C58F4 and *attL*C58R2, and was cloned into the EcoRI and XhoI sites of pYC161.

c: Plasmid pYC174 was constructed by PCR amplification of the *attKLM* promoter using oligonucleotides P*attKLM*-F1 and P*attKLM*-R1. The PCR product was cloned into the EcoRI site of pKP302 (8) to make a P*attK-lacZ* transcriptional fusion.

d: An *attM-lacZ* chromosomal fusion was constructed in strain NTL4 by Campbell integration. An internal region of *attM* was amplified by PCR using oligonucleotides *attM*C58F1 and *attM*C58R1, and was cloned into the EcoRI and KpnI sites of the suicide plasmid pVIK112 (4). The resulting plasmid (pYC120) was first introduced into *E. coli* S17-1/λpir strain (10) by electroporation and then into strain NTL4 by conjugation (2). Integrants were selected on AB agar medium supplemented with 500 μg ml^{-1} of kanamycin and 40 μg ml^{-1} of X-gal. Homologous integration into *attM* was confirmed by PCR amplification. *atu4247*, *atu3403*, *atu4762*, and *atu3498* in YC6 were also disrupted by Campbell integration after cloning the internal gene segments generated by PCR

with *attK1-4F* and *attK1-4R* oligonucleotides, were introduced into pVIK112. The resulting plasmids were introduced into *A. tumefaciens* strain YC6 as described above.

e: To make an in-frame deletion in the *attL* gene of NTL4, 5' end and 3' end DNA fragments of *attL* were amplified by PCR using oligonucleotides *attL*C58F1 and *attL*C58R1, and *attL*C58F2 and *attL*C58R2, respectively. The resulting PCR products were introduced into the suicide plasmid pWM91 (6). The resulting plasmid (pYC118) was first introduced into *E. coli* S17-1/λpir by electroporation and then into NTL4 by conjugation. Cointegrates were selected on AB agar medium supplemented with 100 μg ml^{-1} of carbenicillin. Homologous integration of pYC118 was confirmed by PCR amplification. Cointegrates were then counter-selected on AB agar medium containing 5% sucrose (6). Deletion of *attL* was confirmed by PCR amplification. An in-frame deletion of *attK* was created using similar procedures with oligonucletides *attK*C58F1, *attK*C58R1, *attK*C58F2, and *attK*C58R2.

6. Plasmids pCST324, pCST325, and pCST326 were constructed by PCR amplification, using oligonucleotides pCST324R, pCST325R, and pCST326R, each combined with pAttKLM-F1. The resulting PCR fragments were digested with EcoRI and KpnI, and ligated to appropriately digested pKP302.

Table S3. GC/MS analysis of GBL metabolism *in vivo*.

	Time (hr)	G BL	GHB	SSA	SA
WT[a]	0	+	-	-	-
	3	-	+	-	-
	6	-	+	-	-
	17	-	-	-	-
attM	0	+	-	-	-
	3	+	-	-	-
	6	+	-	-	-
	17	+	-	-	-
attL	0	+	-	-	-
	3	-	+	-	-
	6	-	+	-	-
	17	-	+	-	-
attK	0	+	-	-	-
	3	-	+	trace	-
	6	-	+	trace	-
	17	-	-	-	-
attK, atu3403	0	+	-	-	-
	3	-	+	trace	-
	6	-	+	trace	-
	17	-	-	-	-
attK, atu3498	0	+	-	-	-
	3	-	+	trace	-
	6	-	+	trace	-
	17	-	+	-	-
attK, atu4247	0	+	-	-	-
	3	-	+	trace	-
	6	-	+	trace	-
	17	-	-	-	-
attK, atu4762	0	+	-	-	-
	3	-	+	trace	-
	6	-	+	trace	-
	17	-	-	-	-

a. Strains were cultured in AT minimal medium. GBL was added to the cultures at a final concentration of 1 mM. One ml samples were collected at the indicated time points, acidified by the addition of 1 N HCl and analyzed by GC/MS as described in Table S2.

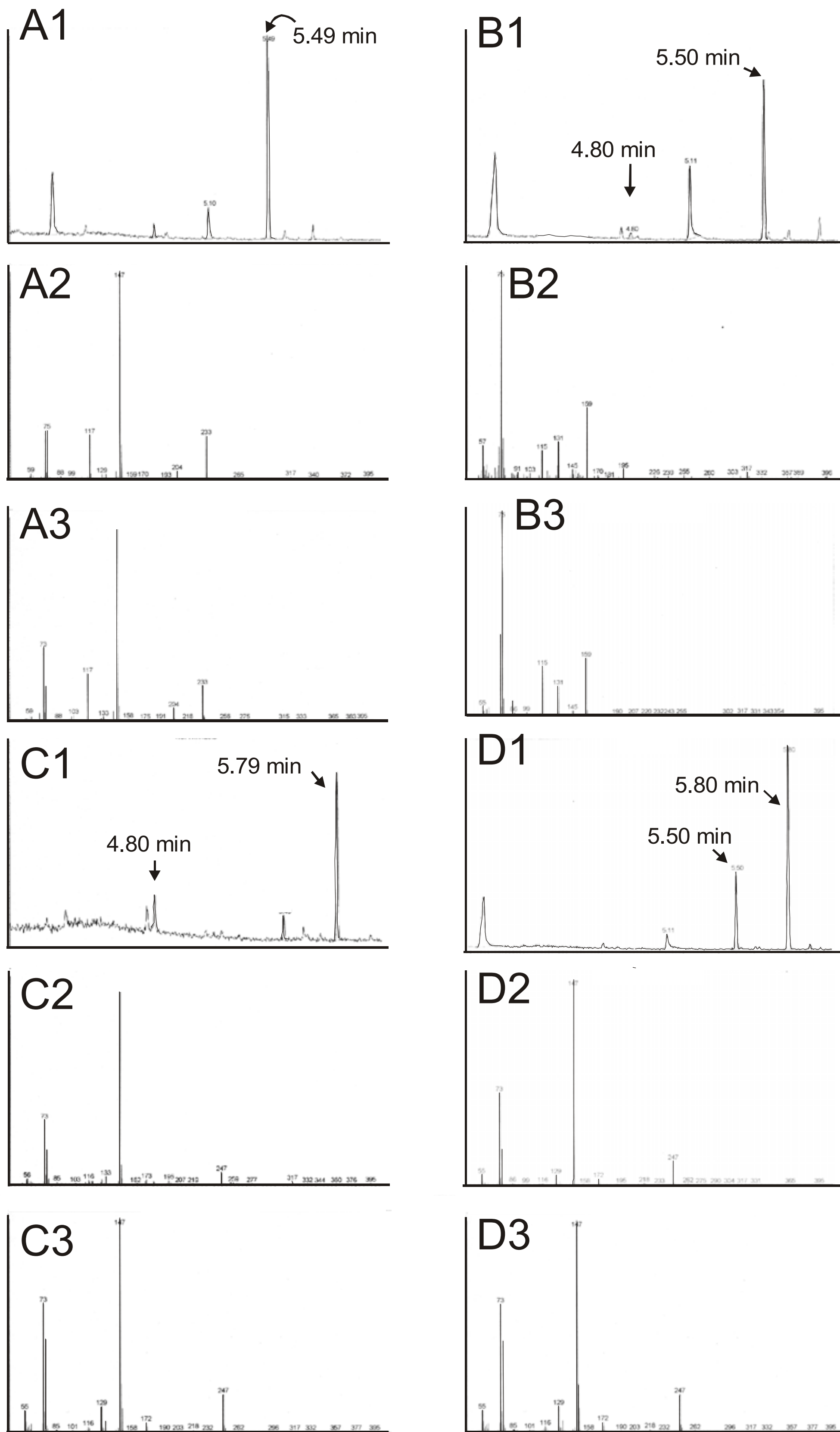
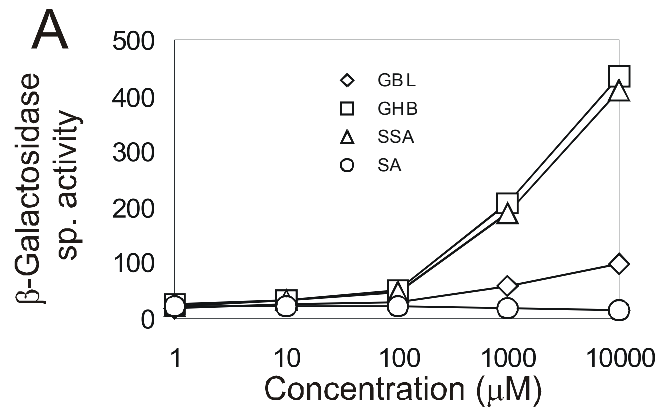


Figure S1 (Chai et al.)



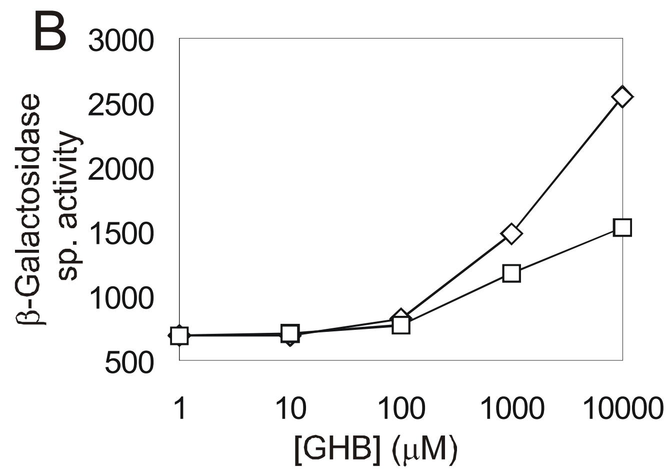


Figure S2 (Chai et al.)

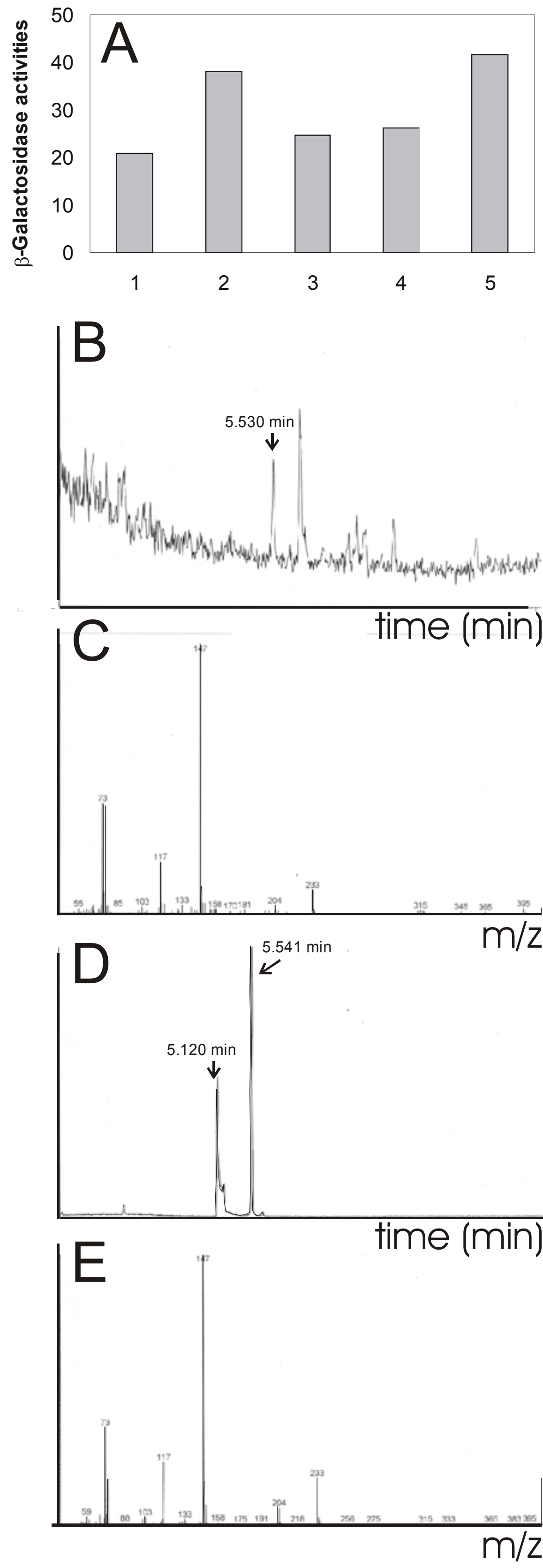


Figure S3 (Chai et al.)

Fig. S1. GC/MS analysis of GBL metabolism by *in vitro* assays. Fig. S1A1 shows the product of a reaction containing GBL and AttM. GBL is converted to a compound (A2) that is identical to reagent GHB (A3). Panels B1-B3 are similar to A1-A3, except that they show the product made when GHB is incubated with AttL. The product (B2) is identical to reagent SSA (B3). Note the poor yield of this product (eluting at 4.80 min in B1). Panels C1-C3 represents the results of incubating SSA with AttK protein. The product (C2) is identical to reagent SA (C3). Panels D1-D3 represents the results of the *in vitro* reaction from GHB to SA by incubation with both AttL and AttK. In all figures, peaks at about 5.50 min represent GHB (after bi-methylation by incubating with BSTFA). Peaks at about 4.80 min represent SSA (after mono-methylation). Peaks at about 5.80 min represent SA (after bi-methylation).

Fig. S2. (A) Induction of the *attM-lacZ* fusion in strain YC1 by GBL, GHB, SSA, and SA. (B) Induction of the *attK-lacZ* fusion (in pYC174) in the presence of GHB in either strain NTL4 (wt, diamonds) or strain YC5(Δ*attL*, squares). Mid log phase cultures of strain YC1 were inoculated into fresh AB broth containing the indicated compounds and grown at 28° C. After culturing for 3 hours, 3 ml of the culture was assayed for β-galactosidase specific activity (7). All assays were conducted in triplicate.

Fig. S3. (A) Induction of the *attM-lacZ* fusion in strain YC1 by incubation with various plant seedling exudates. 1: control; 2: *Arabidopsis*; 3: tomato; 4: tobacco; 5: squash. (B) GC/MS analysis of extracted exudates from *Arabidopsis* seedlings. (C) Mass spectrum of the peak (5.530 min) in Fig. 5B. (D) GC/MS analysis of reagent GHB. The

peak (5.541 min) and the peak (5.120 min) represent bi- and mono-methylated GHB, respectively, after treatment with BSTFA. (E) Spectrum of the GHB peak (5.541 min) from Fig. 5D. Seedlings exudates were made using a procedure described elsewhere (1). Approximately 20 ml of each exudate were acidified by addition of 1 N HCl, and extracted twice using equal volumes of ethyl acetate (HPLC grade, Fisher). Ethyl acetate phases were pooled, and traces of water were removed by addition of anhydrous sodium sulfate. Samples were dried at room temperature, and the resulting pellets were dissolved using 200 µl of ethyl acetate and stored at -80°C for analysis. GC/MS analysis of seedling exudate extractions were performed as described above.

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