SUPPLEMENTAL MATERIALS

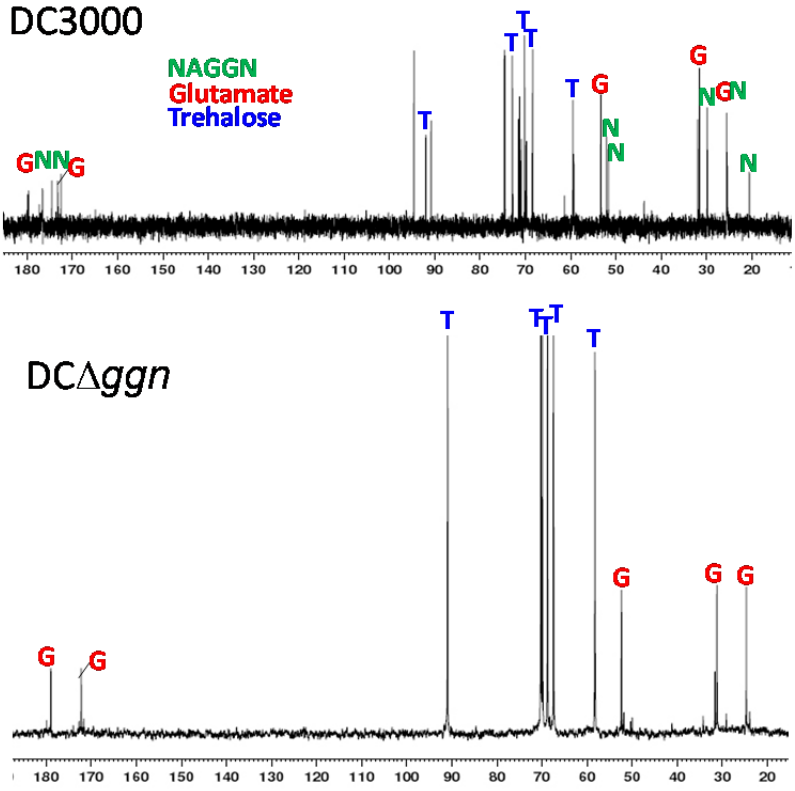


FIG S1. ^{13}C-NMR spectra of the compatible solutes released in ethanolic extracts of osmotically stressed cells of *P. syringae* strains DC3000 and DCΔ*ggn*. Cells were grown in MinAS amended with 0.3M NaCl and NMR spectra were generated as described in Freeman, Chen and Beattie (2010) *Environ. Microbiol.* 12:1486-1497.

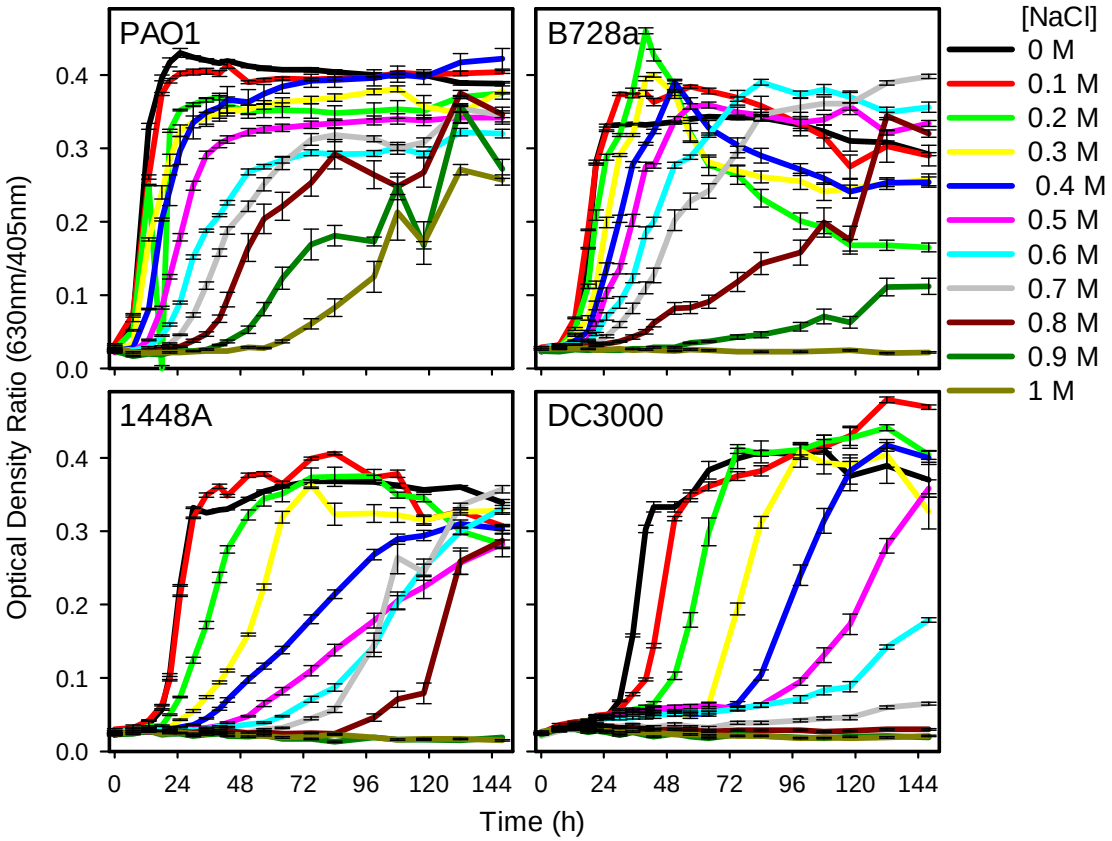


FIG S2. Impact of NaCl on the growth of three *P. syringae* strains, B728a, 1448A, and DC3000, and one *P. aeruginosa* strain, PAOl. Strains were grown in ½-21C medium amended with 0 to 1M NaCl at 0.1M intervals in 96-well microtiter plates on a shaker. The optical densities at 630 nm and 450 nm were measured with a Bio Kinetics Reader EL340 (Bio-Tek Instruments, Winooski, VT). These results are representative of six independent experiments performed with various minimal media.

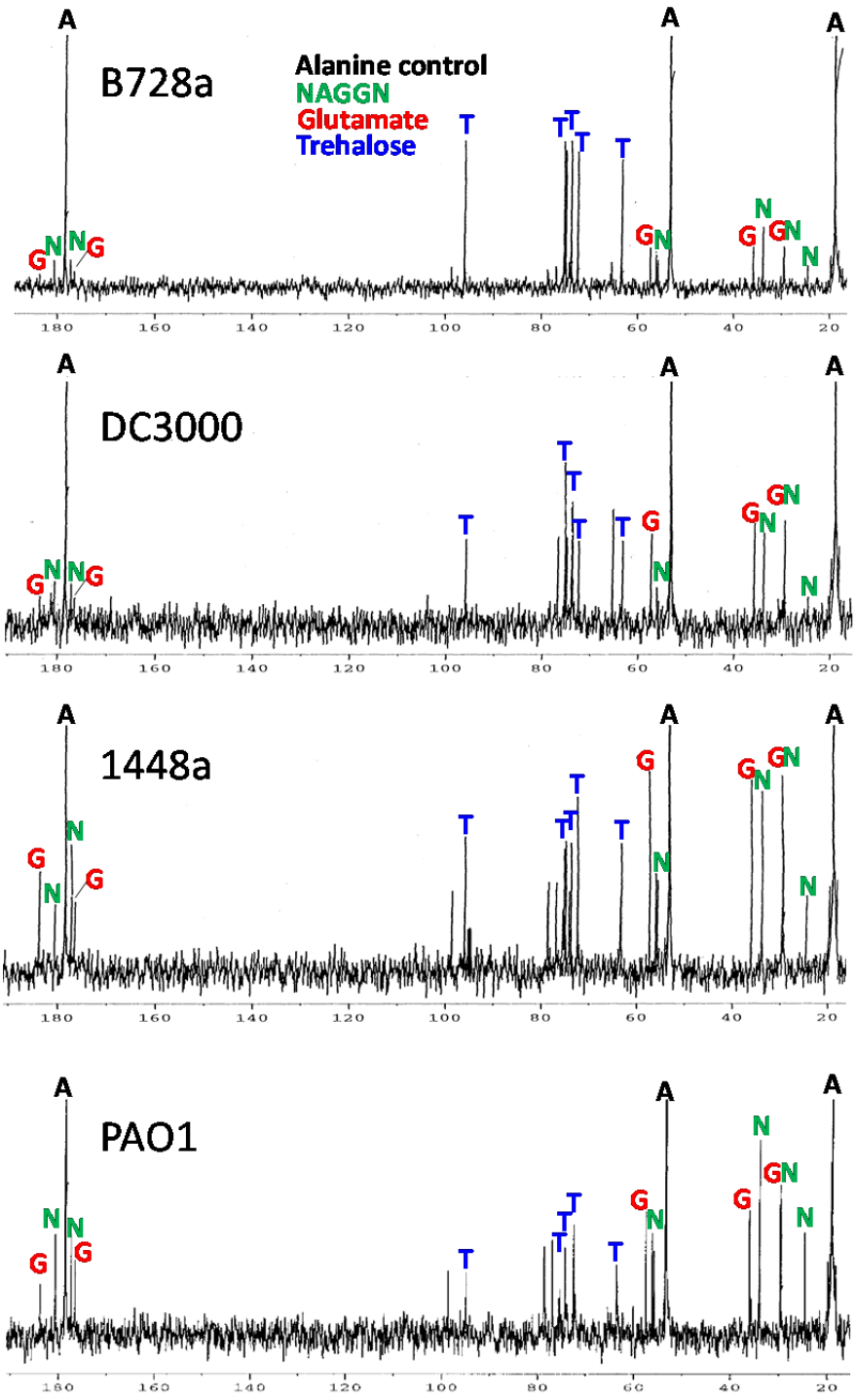


FIG S3. ^{13}C-NMR spectra of the compatible solutes released in ethanolic extracts of osmotically stressed cells of *P. syringae* strains B728a, DC3000, and 1448a and *P. aeruginosa* strain PAO1. Cells were grown in ½-21C medium containing 2 mM succinate and either 0.6M NaCl (B728a), 0.3M NaCl (DC3000), 0.4M NaCl (1448a) or 0.75M NaCl (PAO1). Cells were harvested at an $OD_{600} \approx 0.2$, and prepared and subjected to NMR spectroscopy as described in Freeman, Chen and Beattie (2010) *Environ. Microbiol.* 12:1486-1497.

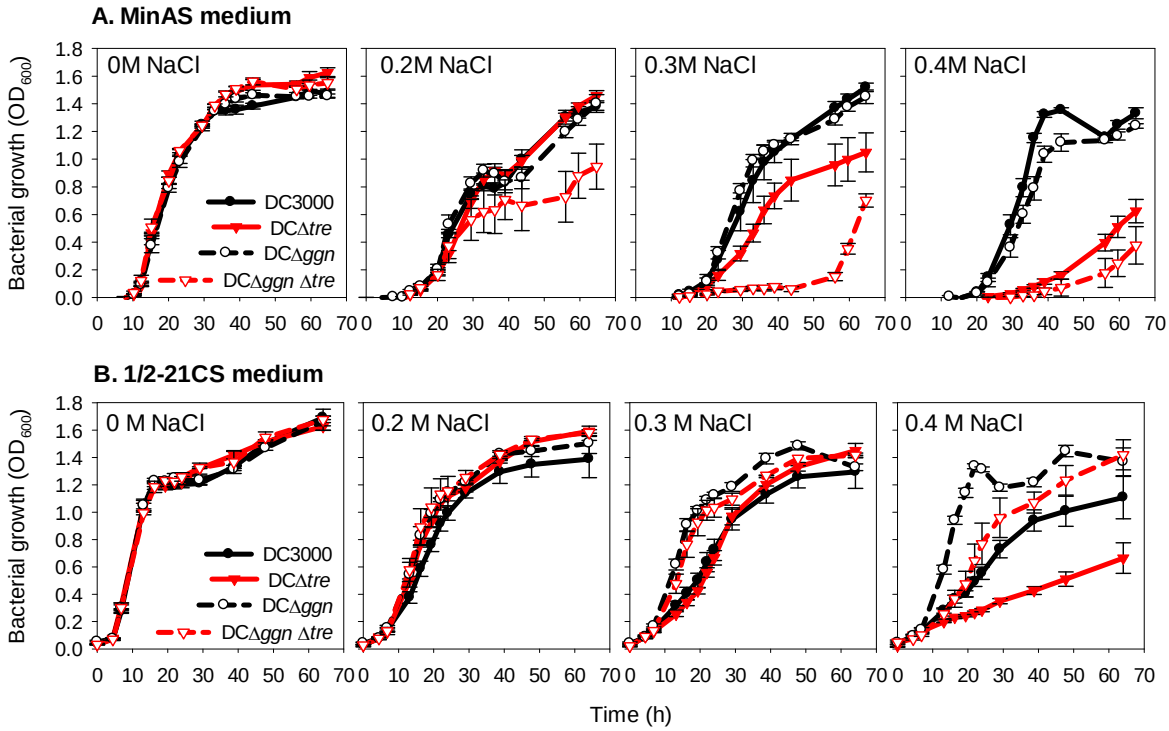


FIG S4. Contribution of NAGGN and trehalose biosynthetic loci to growth of DC3000 and DC3000 compatible solute-deficient mutants at each of various levels of osmotic stress. Strains were grown in MinAS (A) and ½-21CS (B) media as described in Fig S2, with the OD_{630}:OD_{450} ratio converted to OD_{600} using a standard curve. The identities of the constructs in each culture were verified by PCR at the end of the growth study. These results are representative of two independent replicate experiments. The last panel of (A) show the same data as in Fig 4C.

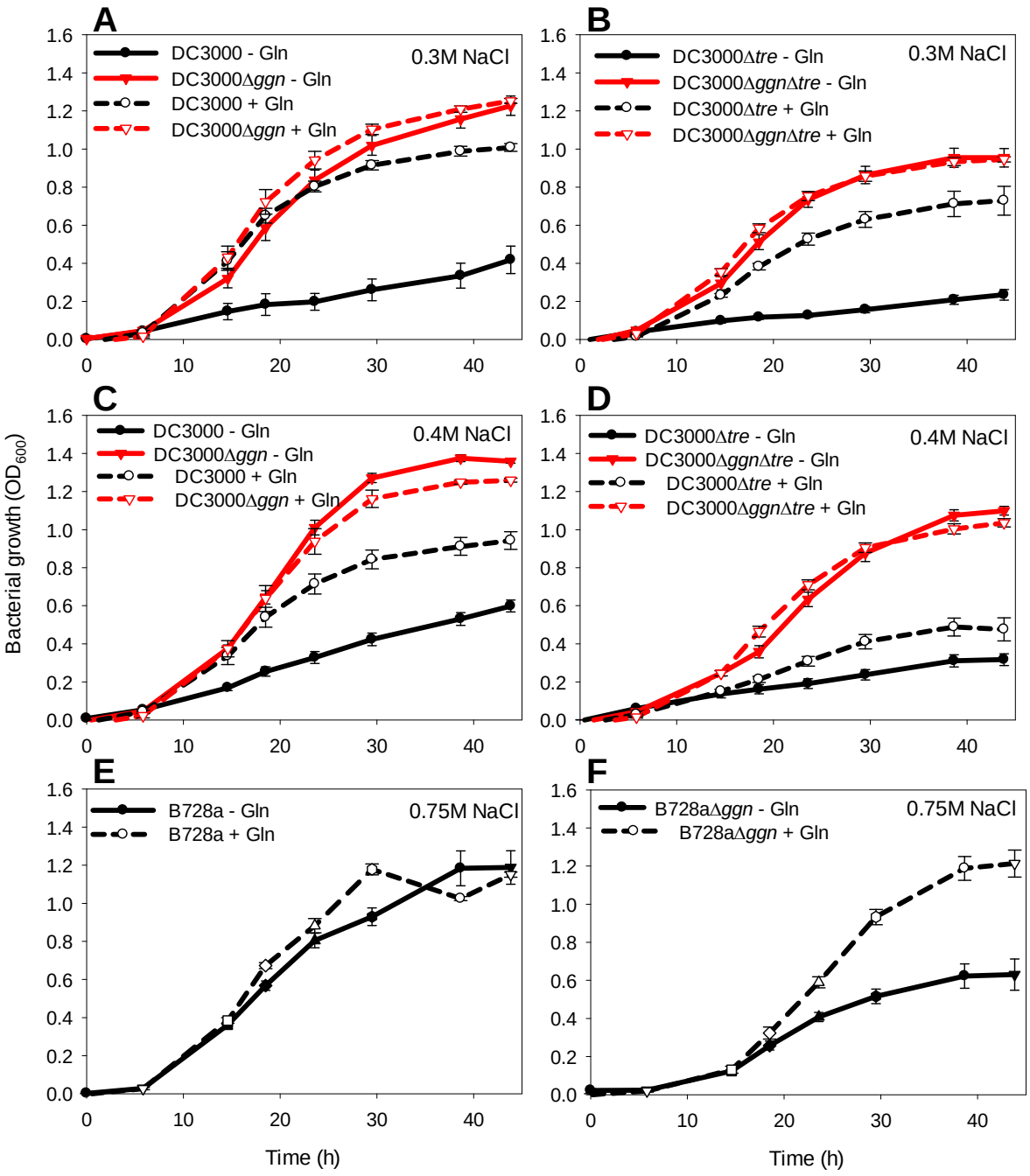


FIG S5. Effect of exogenous glutamine on osmotolerance. DC3000 and its derivatives were grown at 0.3M NaCl (A,B) or 0.4M NaCl (C,D) and B728a and B7Δ*ggn* were grown at 0.75M NaCl (E,F), all in ½-21CS medium with or without 4.7 mM glutamine. Whereas exogenous glutamine may have enhanced the growth of DC3000 by reversing a glutamine limitation incurred by *ggnABC*-mediated NAGGN synthesis, it likely enhanced the growth of B7Δ*ggn* by increasing the internal concentrations of glutamate, which also accumulates as a compatible solute in *P. syringae* (Fig S7).

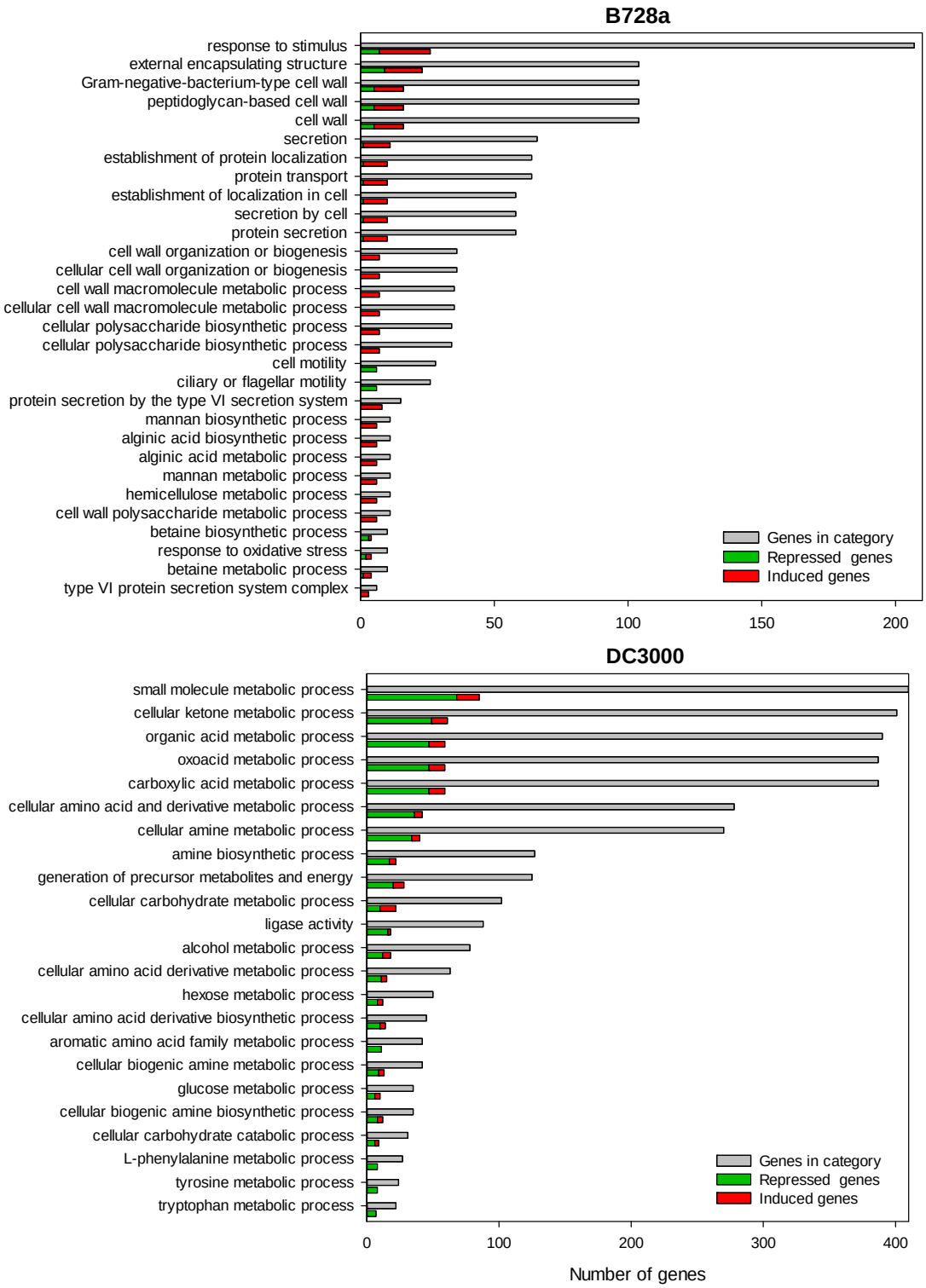
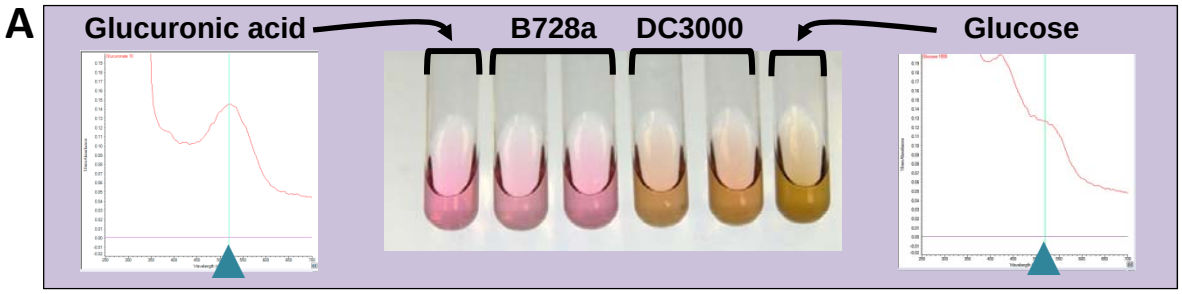
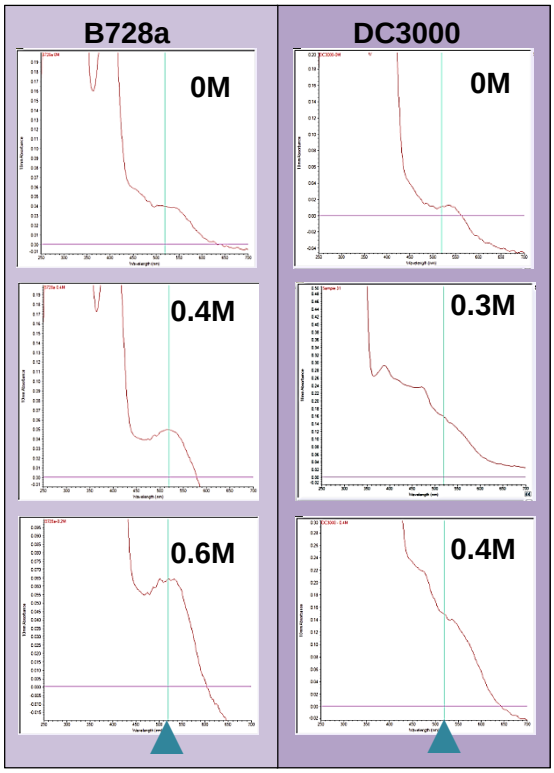
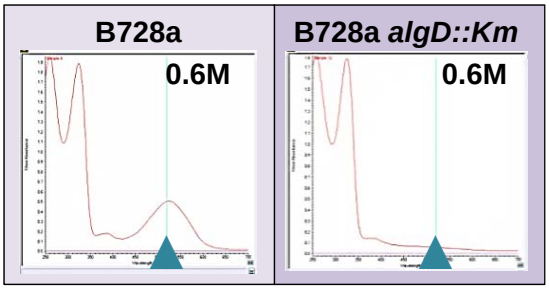
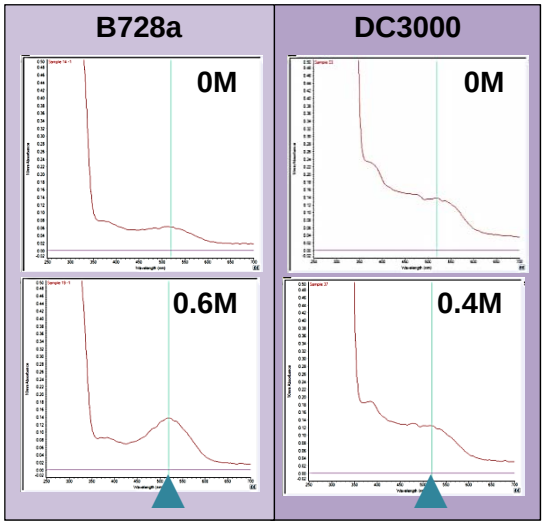


FIG S6. Gene ontology categories that were over-represented among the genes in the NaCl-responsive transcriptomes as compared to in the genome. Individual genes could belong to multiple GO categories. Over-representation was determined using a two-tail Fisher's exact test (FDR < 0.05) and Blast2Go (www.blast2go.org).


A
Glucuronic acid
B728a
DC3000
Glucose


B
B728a
DC3000
0M
0M
0.4M
0.3M
0.6M
0.4M


C
B728a
B728a algD::Km
0.6M
0.6M


D
B728a
DC3000
0M
0M
0.6M
0.4M

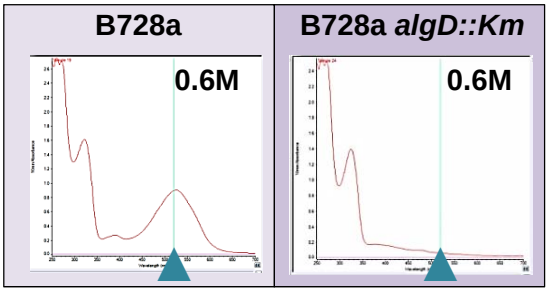

E
B728a
B728a algD::Km
0.6M
0.6M

FIG S7. Absorption spectra of polysaccharides isolated from B728a and DC3000 following colorization with the *meta*-hydroxydiphenyl assay for uronic acids. (A) Glucuronic acid (50 μg/ml), glucose (1,000 μg/ml), and B728a and DC3000 polysaccharides were subjected to the assay. The pink color of the B728a polysaccharides was similar to that of the uronic acid standard, whereas the color of the DC3000 polysaccharides resembled the brown that results from abundant neutral sugars. Strains were grown in ½-21CS (A,B,D) and MinAS (C,E) media that were supplemented with NaCl to the indicated concentrations. The graphs show the wavelengths from 250 to 700 nm on the *x*-axis, with 520 nm indicated with a triangle, and the absorbance at each wavelength on the *y*-axis, with varying scales on the *y*-axis. B728a produced a uronic acid-containing polysaccharide that resembled alginate based on its absorption at 520 nm, its similarity in color to glucuronic acid (A) and galacturonic acid (not shown), and its absence in a B728a *algD::Km* mutant (C,E). In contrast, the polysaccharide recovered from DC3000 was similar in spectra and color to those of glucose (A) and trehalose (not shown), which are both neutral sugars and thus exhibit browning during the heating steps of the *meta*-hydroxydiphenyl assay (A,B,D).

TABLE S1. Primers used in this study

Target genes	Primer sequence
Primers for constructing the DC∆ggn mutant	
∆*ggn* in DC3000 (PSPTO_1630-1633)	F1: 5'-CATTGTGTTCGTTCTGCTTGGCG-3' R1: 5'-GAAGCAGCTCCAGCCTACACAATCAGAATCTGCACGGTGA-TGTCATC-3' F2: 5'-GAGGATATTCATATGGACCATGGGCAGGCAAGTACAGTTG-TCGAGT-3' R2: 5'-GTTACAGGGCGAACAAGGCTAC-3'
kan cassette in pKD4	FRT-F: 5′-ATTGTGTAGGCTGGAGCTGCTTC-3′ FRT-R: 5′-CCATGGTCCATATGAATATCCTCC-3′
Primers for qRT-PCR	
hemD (PSPTO_0129)	F: 5'-TCAGCAGCAGTCTGCCTTTA-3' R: 5'-GTTGCTGAACCCACACTGAA-3'
glnA-1 (PSPTO_0359)	F: 5'-TCACGGACACTAAAGGTACGC-3' R: 5'-TGATGTCGCACACCAGAATC-3'
cspC (PSPTO_1274)	F: 5'- AATGGCTGAACGTCAGAGCG-3' R: 5'-CCTTCGGCGTGTACTTCG-3'
hfnA (PSPTO_1596)	F: 5'-AGTGGGCAACGTCAAGCAAG-3' R: 5'-CGTCTTTCACTTCGCCTTTAGC-3'
ggnA (PSPTO_1633)	F: 5'-CGCTCAATCATTACCTCAACTTC-3' R: 5'-TGCTGTCCAGTACACGGTCG-3'
glgA (PSPTO_3125)	F: 5'-TCGTGACCTCCGAACTGG-3' R: 5'-TCCATACGCCCAACCTTG-3'
opuCD (PSPTO_4578)	F: 5'-AACACCGTTCCGCCACTC-3' R: 5'-GACACGCACGCCACCAATA-3'
PSPTO_4906	F: 5'-GGCAACACGCTCAAAGAAATG-3' R: 5'-GCCGATGTACTGAATGATCTCGC-3'

Sequences that are underlined are complementary to the *kan* cassette primers

TABLE S2. Osmotic stress-induced changes in the transcript levels of selected genes in *P. syringae* strains B728a and DC3000.

		B728a		DC3000	
Predicted Function	**Gene**	**Locus**	**Fold-change[1]**	**Locus**	**Fold-change[1]**
QAC transport & metabolism					
Betaine/choline/carnitine (BCC) transporter ATPase	*opuCA*	Psyr_4249	**8.67**	PSPTO_4575	**2.99**
BCC transporter permease	*opuCB*	Psyr_4250	**12.73**	PSPTO_4576	**3.32**
BCC transporter binding protein	*opuCC*	Psyr_4251	**23.26**	PSPTO_4577	**4.27**
BCC transporter permease	*opuCD*	Psyr_4252	**18.65**	PSPTO_4578	**4.68**
BCCT-family choline transporter	*betT*	Psyr_4827	1.10	PSPTO_5269	**1.57**
Choline dehydrogenase	*betA*	Psyr_4732	**1.72**	PSPTO_0443	**1.58**
Betaine aldehyde dehydrogenase	*betB*	Psyr_4733	**2.34**	PSPTO_0441	**1.78**
Transcriptional regulator	*betI*	Psyr_4734	**2.26**	PSPTO_0440	**1.40**
Compatible solute synthesis: NAGGN					
Amidotransferase	*ggnA*	Psyr_3747	**3.80**	PSPTO_1633	**5.24**
N-acetyltransferase	*ggnB*	Psyr_3748	**17.13**	PSPTO_1632	**4.85**
Gene in the *ggnAB* operon		Psyr_3749	**6.36**	PSPTO_1631	**3.29**
Gene in the *ggnAB* operon		Psyr_3750	**2.19**	PSPTO_1630	**2.86**
Compatible solute synthesis: trehalose					
Alpha amylase, catalytic region		Psyr_2489	1.70	PSPTO_2760	**2.15**
Alpha amylase, catalytic region	*treS*	Psyr_2490	1.67	PSPTO_2761	**1.47**
Glycogen branching enzyme	*glgB*	Psyr_2491	1.73	PSPTO_2762	(1.22)
Glycogen synthase	*glgA*	Psyr_2992	1.56	PSPTO_3125	**2.16**
Alpha amylase	*treZ*	Psyr_2993	1.80	PSPTO_3126	**1.83**
Glycoside hydrolase protein	*malQ*	Psyr_2994	**2.48**	PSPTO_3127	**1.72**
Alpha amylase	*treY*	Psyr_2995	1.62	PSPTO_3128	(1.28)
Glycoside hydrolase	*treX*	Psyr_2997	**2.43**	PSPTO_3130	**1.58**
Methyltransferase, putative		Psyr_3000	1.57	PSPTO_3133	(1.17)
Outer membrane proteins					
Porin	*oprB*	Psyr_1117	**-2.13**	PSPTO_1296	**-1.85**
Porin	*oprD*	Psyr_1400	**-2.70**	PSPTO_3987	**-2.52**
Porin		Psyr_4237	**-2.14**	PSPTO_4560	**-1.53**
Porin		Psyr_4807	(1.00)	PSPTO_0369	**-1.27**
Porin	*oprE*	Psyr_4878	**-7.27**	PSPTO_5318	**-1.41**
Porin	*oprQ*	Psyr_4930	(-1.35)	PSPTO_5391	**-1.45**
Outer membrane protein		Psyr_1005	**1.85**	PSPTO_1164	**2.22**
Outer membrane protein		Psyr_1316	**4.39**	PSPTO_1506	**3.98**
Outer membrane protein	*ompP*	Psyr_3669	**-2.54**	PSPTO_1720	(-1.50)
Outer membrane protein		Psyr_3854	-1.50	PSPTO_4117	(-1.26)
Lipoprotein carrier protein	*lolA*	Psyr_3178	**1.70**	PSPTO_3348	(1.27)
Pili synthesis and regulation	*pilF*	Psyr_1246	**2.52**	PSPTO_1432	**1.94**
Peptidoglycan-associated lipoprotein	*oprL*	Psyr_1416	**-2.15**	PSPTO_3971	**-1.89**

TABLE S2. (continued)

Predicted Function	Gene	B728a Locus	B728a Fold-change[1]	DC3000 Locus	DC3000 Fold-change[1]
Flagellar Synthesis and Motility					
Flagellar motor switch protein	*fliN*	Psyr_3446	**-1.74**	PSPTO_1970	(-1.20)
Flagellar basal bodyd protein	*fliL*	Psyr_3448	-1.59	PSPTO_1968	(-1.19)
Hypothetical protein	*fleP*	Psyr_3462	**-1.88**	PSPTO_1953	(-1.43)
Flagellar protein	*fliS*	Psyr_3463	**-2.18**	PSPTO_1952	**-1.81**
Flagellar hook-associated protein	*fliD*	Psyr_3464	-1.74	PSPTO_1951	**-1.57**
Flagellar protein	*flaG*	Psyr_3465	**-2.02**	PSPTO_1950	**-2.53**
Flagellin	*fliC*	Psyr_3466	**-1.99**	PSPTO_1949	**-2.8**
Flagellar hook-associated protein	*flgL*	Psyr_3470	-1.69	PSPTO_1945	(-1.33)
Flagellar hook-associated protein	*flgK*	Psyr_3471	**-1.89**	PSPTO_1944	(-1.38)
Flagellar basal body rod protein	*flgG*	Psyr_3475	-1.53	PSPTO_1940	(-1.69)
Flagellar hook protein	*flgE-1*	Psyr_3478	**-2.4**	PSPTO_1936	(-1.61)
Flagellar basal body rod modification protein	*flgD*	Psyr_3479	**-1.73**	PSPTO_1935	**-2.21**
Flagellar basal body rod protein	*flgC*	Psyr_3480	**-2.27**	PSPTO_1934	**-2.75**
Flagellar basal body rod protein	*flgB*	Psyr_3481	**-2.24**	PSPTO_1933	**-2.49**
Alginate synthesis & regulation					
Transcriptional regulator	*mucB*	Psyr_3956	**4.34**	PSPTO_4222	**3.69**
Alginate regulatory protein	*algR*	Psyr_0063	**1.80**	PSPTO_0127	**1.72**
Alginate and motility regulator	*algZ*	Psyr_3551	**4.39**	PSPTO_1847	**1.88**
Phosphomannomutase	*algC*	Psyr_0219	**1.99**	PSPTO_0083	**1.97**
Mannose-6-phosphate isomerase	*algA-2*	Psyr_1052	1.54	PSPTO_1232	(1.09)
Alginate biosynthetic protein	*algF*	Psyr_1053	2.07	PSPTO_1233	(1.10)
Alginate biosynthetic protein	*algJ*	Psyr_1054	**3.54**	PSPTO_1234	(1.08)
Membrane bound O-acyl transferase	*algI*	Psyr_1055	**7.21**	PSPTO_1235	(1.13)
Poly(beta-D-mannuronate) lyase	*algL*	Psyr_1056	**10.21**	PSPTO_1236	(1.33)
Alginate biosynthetic protein	*algX*	Psyr_1057	**15.72**	PSPTO_1237	(1.20)
Alginate biosynthetic protein	*algG*	Psyr_1058	**19.20**	PSPTO_1238	(1.47)
Alginate biosynthetic protein	*algE*	Psyr_1059	**8.92**	PSPTO_1239	(1.22)
Alginate biosynthetic protein	*algK*	Psyr_1060	**6.86**	PSPTO_1240	(1.25)
Alginate biosynthetic protein	*alg44*	Psyr_1061	**8.56**	PSPTO_1241	**1.70**
Alginate biosynthetic protein	*alg8*	Psyr_1062	**6.28**	PSPTO_1242	(1.27)
GDP-mannose 6-dehydrogenase	*algD*	Psyr_1063	**18.54**	PSPTO_1243	**2.34**
Type VI secretion system (T6SS)					
T6SS protein	*hsiC*	Psyr_4953	**4.52**	PSPTO_2543	(1.01)
T6SS protein	*hsiF*	Psyr_4954	**6.27**	PSPTO_5432	(-1.35)
T6SS protein	*hsiH*	Psyr_4955	**7.25**	PSPTO_5431	(-1.28)
T6SS protein	*hsiG*	Psyr_4956	**6.22**	PSPTO_2546	(-1.02)
T6SS protein	*hsiH*	Psyr_4957	**3.22**	PSPTO_5426	(-1.06)
T6SS, ATPase ClpV	*clpV*	Psyr_4958	**4.05**	PSPTO_5425	(-1.02)
T6SS protein	*virB*	Psyr_4959	**3.92**	PSPTO_5421	(-1.15)
T6SS protein	*hsiJ*	Psyr_4960	**5.21**	PSPTO_5420	(-1.06)
T6SS protein	*impK*	Psyr_4961	**3.41**	PSPTO_5419	(-1.04)
T6SS protein	*icmF*	Psyr_4962	**2.08**	N	

TABLE S2. (continued)

Predicted Function	Gene	B728a Locus	B728a Fold-change[1]	DC3000 Locus	DC3000 Fold-change[1]
Type III secretion system (T3SS)					
Transcriptional regulator	*hrpL*	Psyr_1217	(1.06)	PSPTO_1404	**-1.93**
T3SS secretion system	*hrpB*	Psyr_1194	(-1.01)	PSPTO_1383	**-1.73**
T3SS secretion system	*hrpF*	Psyr_1198	(1.08)	PSPTO_1387	**-1.64**
T3SS secretion system	*hrpG*	Psyr_1199	(-1.21)	PSPTO_1388	**-1.39**
T3SS helper protein (harpin)	*hrpW1*	Psyr_1184	(-1.19)	PSPTO_1373	**-1.99**
T3SS helper protein (harpin)	*hrpZ1*	Psyr_1193	(1.10)	PSPTO_1382	**-2.26**
T3SS helper protein (harpin)	*hopAK1*	Psyr_3839	(1.02)	PSPTO_4101	**-1.35**
T3SS helper protein	*hrpK1*	Psyr_1218	(-1.06)	PSPTO_1405	**-1.29**
T3SS effector protein	*hopI1*	Psyr_4326	(1.07)	PSPTO_4776	**-1.43**
T3SS effector protein	*hopH1*	Psyr_1889	M	PSPTO_0588	**-3.55**
T3SS effector protein	*hopAB1*	Psyr_4659	M	PSPTO_3087	**-1.49**
T3SS effector protein	*avrPto1*	Psyr_4919	M	PSPTO_4001	**-2.32**
T3SS helper protein (harpin)	*hrpA1*	N		PSPTO_1381	**-2.16**
T3SS chaperone	*shcF*	N		PSPTO_0503	**-1.47**
T3SS chaperone	*shcN*	N		PSPTO_1369	**-1.96**
T3SS chaperone	*shcS1*	N		PSPTO_4599	**-1.34**
T3SS chaperone	*shcV*	N		PSPTO_4721	**-1.43**
T3SS chaperone	*shc01*	N		PSPTO_A0017	**-1.94**
T3SS effector protein	*hopC1*	N		PSPTO_0589	**-1.87**
T3SS effector protein	*hopAM1-1*	N		PSPTO_1022	**-3.38**
T3SS effector protein	*hopAH2-1*	N		PSPTO_3292	**-1.38**
T3SS effector protein	*hopE1*	N		PSPTO_4331	**-2.32**
T3SS effector protein	*hopAQ1*	N		PSPTO_4703	**-1.44**
T3SS effector protein	*hopV1*	N		PSPTO_4720	**-1.30**
T3SS effector protein	*hopAO1*	N		PSPTO_4722	**-1.69**
T3SS effector protein	*hopAM1-2*	N		PSPTO_A0005	**-3.57**
T3SS effector protein	*hopO1-1*	N		PSPTO_A0018	**-2.30**
Nitrogen metabolism					
Glutamine amidotransferase		Psyr_2706	**2.22**	PSPTO_2902	(1.20)
Glutamine synthetase, type I	*glnA-1*	Psyr_4817	**-5.00**	PSPTO_0359	**-2.94**
Nitrogen regulatory protein P-II	*glnK*	Psyr_0190	**-5.04**	PSPTO_0217	**-1.97**
Ammonium transporter	*amtB-1*	Psyr_0189	**-2.38**	PSPTO_0218	(-1.11)
NAD Glutamate dehydrogenase	*gdhB*	Psyr_1724	(-1.21)	PSPTO_3757	**-1.38**
Glutamate synthase subunit alpha	*gltB-1*	Psyr_0411	**-1.72**	N	
Two-component response regulator	*ntrC*	Psyr_4822	**-1.73**	N	
Urease accessory protein	*ureF-2*	Psyr_4452	**-1.88**	N	
Urease accessory protein	*ureG-2*	Psyr_4453	**-1.72**	N	
Urease accessory protein	*ureE-2*	Psyr_4451	**-1.58**	N	

[1]The genes were selected among the 3,861 genes with orthologs in both genomes. All values in bold had significantly different transcript levels in the presence of NaCl versus in its absence based on an FDR of 1%. For B728a, the fold-changes not in bold had significantly different transcript levels based on an FDR of 10%. Values in parentheses did not show significant differences in their transcript levels in response to NaCl and are included for comparison. M, the gene is missing in the B728a microarray; N, the gene has no known ortholog in B728a or DC3000.