TABLE S1. Real-time RT-PCR data showing all normalised transcript values

Data supporting Fig. 1		average transcript levels[a] (3 biological replicates)		stdev (3 biological replicates)		fold change	fold change at each time point related to T_0 (T_X / T_0)	
		in wt	in Δ*sigB*	in wt	in Δ*sigB*	wt / Δ*sigB*	in wt	in Δ*sigB*
gene	**time point**							
opuCA	0 min	0.1189	0.0303	0.0606	0.0055	3.93	-	-
opuCA	5 min	1.1783	0.0287	1.1413	0.0257	**41.10**[b]	**9.91**	0.95
opuCA	10 min	0.9771	0.0260	0.6924	0.0313	**37.52**	**8.22**	0.86
opuCA	15 min	4.1246	0.0368	1.3373	0.0245	**112.22**	**34.70**	1.21
opuCA	20 min	2.6104	0.2294	0.5501	0.0149	**11.38**	**21.96**	**7.58**
opuCA	25 min	1.0373	0.1088	0.2208	0.0136	**9.53**	**8.73**	3.59
lmo2230	0 min	0.1624	0.0207	0.1452	0.0228	7.84	-	-
lmo2230	5 min	0.6022	0.0880	0.5224	0.1460	6.84	3.71	4.25
lmo2230	10 min	6.9458	0.1330	6.4046	0.1666	**52.24**	**42.77**	6.42
lmo2230	15 min	26.0690	0.0547	4.8488	0.0007	**476.61**	**160.51**	2.64
lmo2230	20 min	11.4261	0.1721	2.7008	0.0014	**66.40**	**70.35**	8.31
lmo2230	25 min	3.6372	0.2467	1.8090	0.0031	**14.75**	**22.40**	11.91
lmo2085	0 min	0.0392	0.0105	0.0365	0.0009	3.75	-	-
lmo2085	5 min	0.0730	0.0184	0.0834	0.0010	3.98	1.86	1.77
lmo2085	10 min	0.4494	0.0286	0.2508	0.0905	**15.72**	**11.46**	2.75
lmo2085	15 min	1.9242	0.0106	0.7677	0.0045	**181.35**	**49.07**	1.02
lmo2085	20 min	1.0577	0.0538	0.2440	0.0014	**19.66**	**26.97**	5.18
lmo2085	25 min	0.6695	0.0192	0.0060	0.0002	**34.91**	**17.07**	1.85
sigB	0 min	1.0900	1.5629	0.4020	0.3783	0.70	-	-
sigB	5 min	1.8587	1.3063	1.6710	0.9865	1.42	1.71	0.84
sigB	10 min	3.2143	1.4493	2.5782	1.0993	**2.22**	**2.95**	0.93
sigB	15 min	7.2985[c]	0.9436	2.1507	0.6635	**7.73**	**6.70**	0.60
sigB	20 min	2.6652	4.4280	0.9523	1.0793	0.60	**2.45**	2.83
sigB	25 min	2.8324	2.1037	0.5501	0.3910	1.35	**2.60**	1.35

Data supporting Fig. 2		average transcript levels (3 biological replicates)		stdev (3 biological replicates)		fold change	fold change at each NaCl concentration related to no NaCl (+ NaCl / no NaCl)	
		in wt	in Δ*sigB*	in wt	in Δ*sigB*	wt / Δ*sigB*	in wt	in Δ*sigB*
gene	**condition**							
opuCA	no NaCl	0.0560	0.0098	0.0108	0.0006	**5.71**	**-**	-
opuCA	0.3M NaCl	0.1295	0.0115	0.0157	0.0001	**11.27**	**2.31**	1.17
opuCA	0.6M NaCl	0.4768	0.0241	0.1879	0.0021	**19.78**	**8.51**	2.46
opuCA	0.9M NaCl	1.6546	0.0436	0.3515	0.0025	**37.91**	**29.52**	4.45
lmo2230	no NaCl	0.0383	0.0144	0.0014	0.0047	2.65	**-**	-
lmo2230	0.3M NaCl	0.8843	0.0169	0.0557	0.0016	**52.34**	**23.10**	1.17
lmo2230	0.6M NaCl	1.2342	0.0394	0.3635	0.0037	**31.34**	**32.24**	2.73
lmo2230	0.9M NaCl	3.7359	0.0713	0.5559	0.0450	**52.41**	**97.59**	4.94
lmo2085	no NaCl	0.0185	0.0049	0.0022	0.0043	**3.79**	**-**	-
lmo2085	0.3M NaCl	0.0501	0.0067	0.0059	0.0020	**7.53**	**2.71**	1.36
lmo2085	0.6M NaCl	0.2963	0.0061	0.1200	0.0023	**48.21**	**16.02**	1.26
lmo2085	0.9M NaCl	1.0280	0.0111	0.2517	0.0090	**92.42**	**55.60**	2.28
sigB	no NaCl	0.5139	0.7491	0.1015	0.1568	0.69	-	-
sigB	0.3M NaCl	0.9949	0.7045	0.3466	0.1207	1.41	**1.94**	0.94
sigB	0.6M NaCl	1.5493	0.9884	0.1992	0.2015	1.57	**3.01**	1.32
sigB	0.9M NaCl	4.8051	1.4234	0.3135	0.2562	**3.38**	**9.35**	1.90

a The relative transcript levels are shown for each gene. The values presented are the means of three independent replicates and normalised to the 16S RNA levels in the corresponding sample. In addition the numbers are arbitrarily multiplied by 10^4.

b Numbers in bold indicate statistically significant differences ($p < 0.05$) between treated and untreated conditions, between strains or between time points, as determined with a Student's *t* test.

c Values highlighted in grey were used as the 100% values for calculating the data presented in figure 1 and figure 2.

Fig. S1

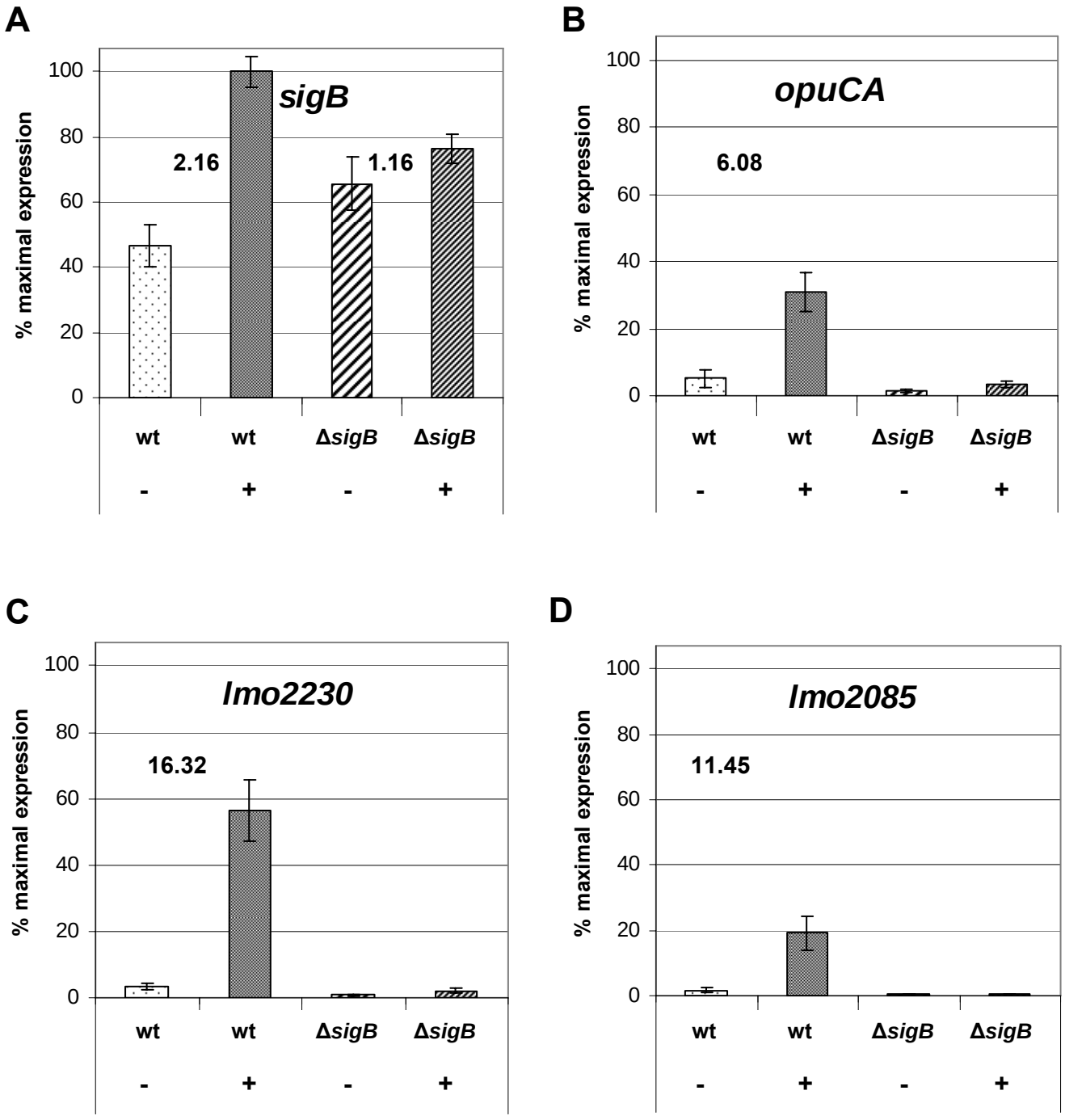

FIG. S1. Induction of *opuCA, lmo2230, lmo2085* and *sigB* transcription in response to osmotic stress is σ^B-dependent. Relative transcript levels of three σ^B-dependent genes (*opuCA, lmo2230, lmo2085*) and *sigB* in exponential-phase cultures (OD_{600} = 0.6) of the

wild-type and the Δ*sigB* mutant, grown in BHI with (+) or without (-) 0.5 M NaCl. RNA extracts and cDNA were prepared as previously described (14). All transcript levels were first normalised to the corresponding 16S RNA levels and then expressed as a percentage of the maximal level of *sigB* transcript detected in the experiment (thereby allowing the relative transcript levels of the 4 genes to be compared). The values presented on the graphs are the means of three independent experiments and error bars indicate the standard deviations (n=3). Numbers shown above the bars indicate statistically significant differences in relative gene expression (fold-change) between the stressed (+NaCl) and non-stressed (-NaCl) conditions ($P<0.05$ in Student's *t* test).

Fig. S2

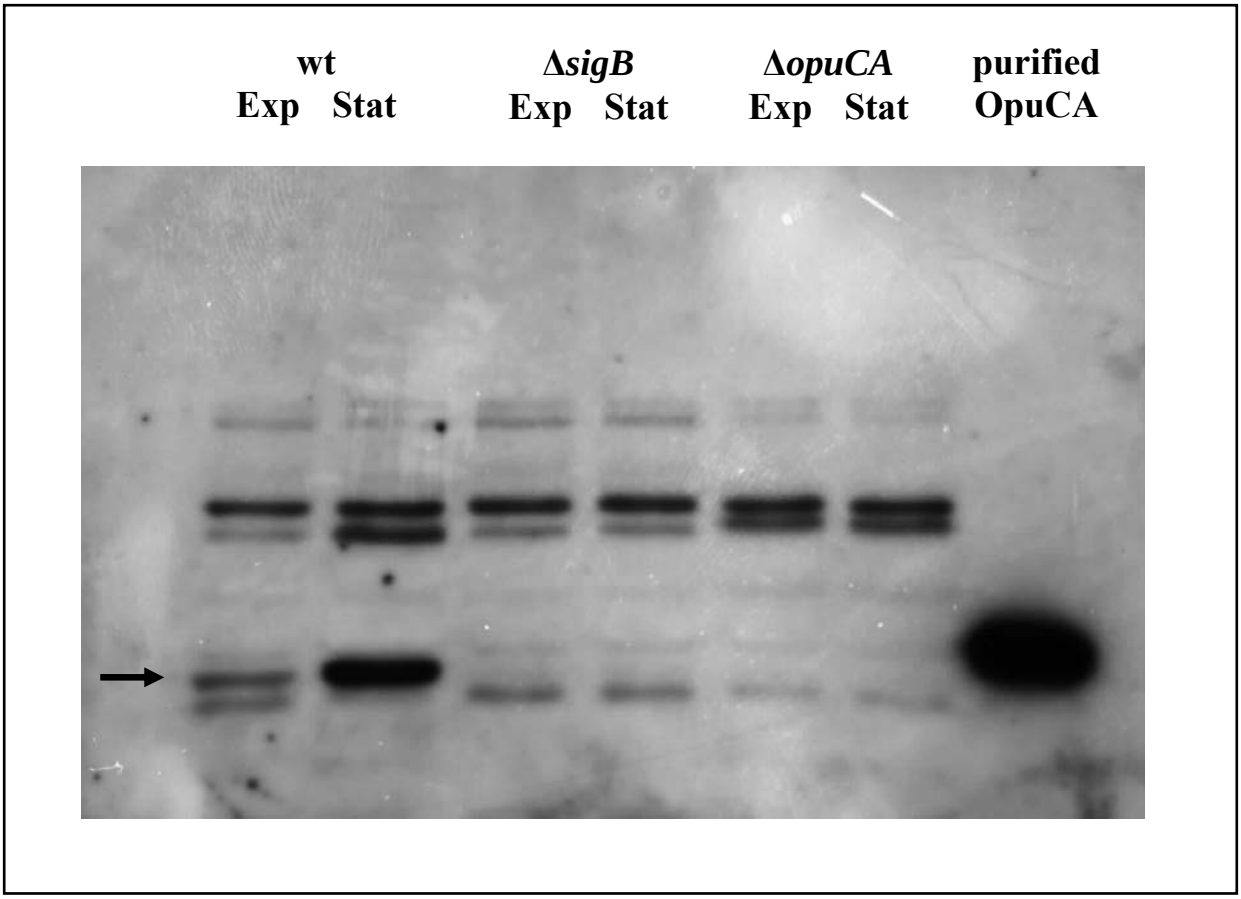

FIG. S2. OpuCA expression is σ^B-dependent. The OpuCA protein was detected in crude cell extracts by Western blotting using anti-OpuCA-His polyclonal antibodies, which were produced in chickens. Histidine-tagged OpuCA was first synthesized (*lmo1428* sequence EMBL accession AL591979.1) and purified by Fusion Antibodies (Belfast). Two leghorn-type chickens (*Gallus gallus domesticus*) were immunised three times with purified His-tagged OpuCA (1.2 mg ml^{-1}) over a nine week period. At each immunisation antigen was injected intramuscularly in four separate sites into the breast muscle in a volume of 150 µl per immunisation site (with 50 µg of antigen in each). The first immunisation consisted of the OpuCA mixed 1:1 by volume with Freunds Complete Adjuvant (Difco), each subsequent immunisation consisted of the OpuCA mixed with an equal volume of Freunds Incomplete Adjuvant (Difco). Seven days after the third immunisation, each chicken was bled of 1 ml

from the wing vein to determine serum antibody response. After a specificity test an additional boost for each chicken was performed in week 12. Then a final bleed of 30 ml from each chicken was taken. Blood was allowed to clot and retract at 4°C overnight before the serum was clarified by centrifugation at 2,500 rpm for 20 min. Stocks of polyclonal antibody were stored in aliquots at -80°C. Protein extracts were prepared from mid-exponential phase (Exp) or stationary phase (Stat) cultures of the wild-type EGD (wt), or the Δ*sigB* and Δ*opuCA* mutant strains grown at 37°C in BHI as previously described (2). Total protein concentrations were normalized to 5 mg ml^{-1} and 10 µl of each protein extract was loaded. Ten µg of purified OpuCA was used as a positive control. 3% w/v skim milk in dH_2O was used for blocking the membrane and for diluting the primary antibody (chicken polyclonal antiserum) 1:2,000, and the secondary antibody (HRP conjugated to goat Anti-Chicken IgY, Promega) 1:20,000. PBS and PBS with 1% (v/v) Tween20 (Promega) was used for the washing steps. The chemiluminescent light, produced due to the HRP enzymatic reaction with a chemiluminescent substrate (SuperSignal® West Pico Chemiluminescent Kit, Pierce), was captured in a dark room using a light sensitive film (Amersham Hyperfilm ECL, GE Healthcare). SDS-PAGE conditions were further standardised for an optimal separation of non-specific bands and washing conditions together with multiple exposures (ranging from 30 s to 180 s) of film carried out to obtain an exposure with reduced non-specific binding. The arrow indicates the position of the OpuCA protein, while the other bands represent non specific binding of the antibodies. A CCD camera (Syngene G:BOX iChemi) and ImageJ software (http://rsbweb.nih.gov/ij/) was used for digital analysis of the acquired nonsaturated films. All findings were reproducible for three biological replicates of protein extractions.