Supporting Information

Table S1. Muropeptide composition[a] of PG from *V. cholerae* LMW PBP mutants

	wt	Δ*dacA-1*	Δ*dacA-2*	Δ*dacB*	Δ*pbpG*	ΔA2ΔG	ΔBΔG	Δ3
Monomers	40.62	55.19	43.40	44.97	43.27	40.33	39.12	43.97
Dimers	26.10	21.12	25.36	24.24	24.96	26.59	26.04	24.43
Trimers	2.39	0.86	1.96	2.18	2.27	2.16	2.93	2.39
Tripeptides	5.78	2.71	3.80	6.06	5.12	4.66	3.85	4.76
Tetrapeptides	60.31	56.00	62.26	60.95	62.28	61.18	59.66	59.39
Pentapeptides	2.52	17.83	3.50	3.60	2.58	2.83	3.54	5.37
Anhydro peptides	10.38	6.35	10.20	12.28	10.13	9.83	14.14	11.59
Average chain length	9.64	15.74	9.80	8.14	9.87	10.17	7.07	8.63
Crosslink	27.30	21.55	26.34	25.33	26.10	27.68	27.51	25.63
LD-crosslink	1.57	0.71	1.68	1.46	1.96	1.60	1.23	1.21
DD-crosslink	25.73	20.84	24.66	23.87	24.14	26.07	26.28	24.41

[a] Relative amounts of muropeptides were calculated as described by Glauner (Glauner, 1988). The values are the means of two independent experiments.

Table S2. Strains used in this study

Strain name		Strain #	genotype/description	reference/source
C6706			*V. cholerae* wild-type strain C6706 El Tor	(Thelin and Taylor, 1996)
C6706 *lacZ*-			*lacZ*-negative derivative of C6706	(Cameron et al., 2008)
E. coli DH5α λpir			cloning strain	(Kolter et al., 1978)
E. coli SM10 λpir			conjugation strain	(Simon et al., 1983)
E. coli MFD pir			DAP auxotroph conjugation strain	(Ferrieres et al., 2010)
ΔdacA-1		AM647	C6706 ΔVC0947	This work
ΔdacB		AM698	C6706 ΔVC0632	This work
ΔpbpG		AM715	C6706 ΔVCA0870	This work
ΔdacA-2 dacA-1 cond		AM735	N16961 ΔVCA0270 $P_{VC0947P}$::P_{BAD}:VC0947	This work
dacA-1 cond		AM738	C6706 $P_{VCA0270P}$::P_{BAD}:VCA0270	This work
ΔdacA-1 dacA-2 cond		AM740	C6706 ΔVC0947 $P_{VCA0270}$::P_{BAD}:VCA0270	This work
ΔdacB ΔpbpG		AM770	C6706 ΔVC0632 ΔVCA0870	This work
ΔdacB ΔpbpG ΔdacA-2	*aka Δ3*	AM783	C6706 ΔVC0632 ΔVCA0870 ΔVCA0270	This work
dacA-2P-lacZ		AM817	C6706 *lacZ*- $P_{VCA0270P}$:$lacZ_{EC}$	This work
dacA-1P-lacZ		AM818	C6706 *lacZ*- $P_{VC0947P}$:$lacZ_{EC}$	This work
ΔdacA-2		AM1065	C6706 ΔVCA0270	This work

Table S3. Plasmids used in this study

Plasmid	description	reference/source
pDS132	*sacB*-containing suicide vector for double homologous recombination, camR	(Philippe et al., 2004)
pBAD18kan	replicating plasmid used for expressing constructs under control of the arabinose-inducible P_{BAD} promoter, kanR	(Guzman et al., 1995)
pAM224	integrating plasmid, R6K, kanR	This work
pAM233	pDS132::VC0947 flanking regions	This work
pAM270	pDS132::VCA0270 flanking regions	This work
pAM290	pDS132::VC0632 flanking regions	This work
pAM291	pDS132::VCA0870 flanking regions	This work
pAM298	pAM299-derived plasmid to generate $P_{dacA\text{-}2}$::P_{BAD}:*dacA-2*	This work
pAM299	pAM224-derived integrating plasmid containing pBAD, kanR	This work
pAM312	pAM299-derived plasmid to generate $P_{dacA\text{-}1}$::P_{BAD}:*dacA-1*	This work
pAM325	pAM224-derived integrating plasmid containing $lacZ_{EC}$, kanR	This work
pAM326	pAM325-derived plasmid to generate $P_{dacA\text{-}1}$::$P_{dacA\text{-}1}$:$lacZ_{EC}$ (merodiploid)	This work
pAM327	pAM325-derived plasmid to generate $P_{dacA\text{-}2}$::$P_{dacA\text{-}2}$:$lacZ_{EC}$ (merodiploid)	This work
pAM332	pBAD18kan::VC0947	This work
pAM333	pBAD18kan::VCA0270	This work

Supplementary Fig legends

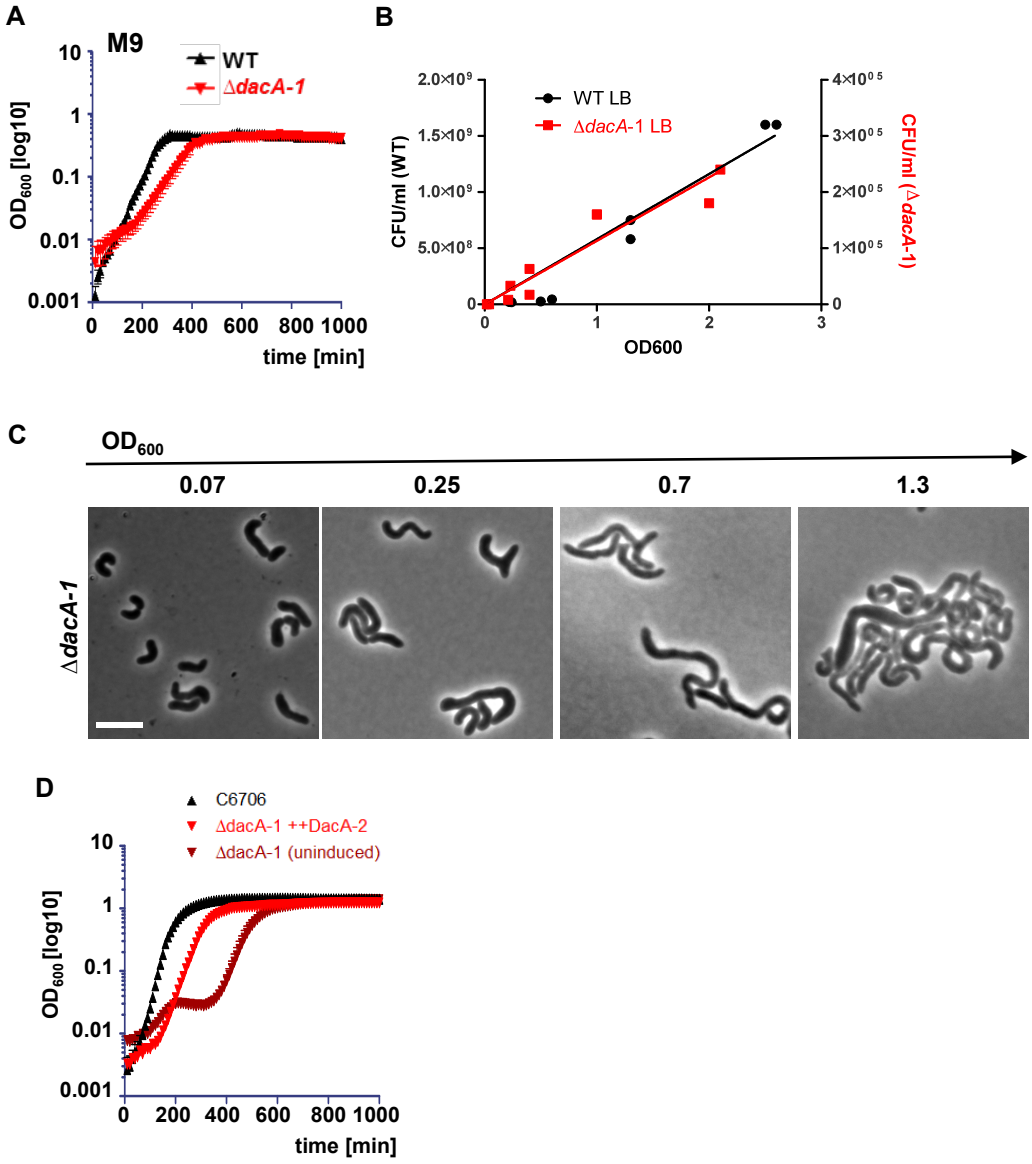
Fig. S1. Alignment of *V. cholerae dacA*-1 and *dacA*-2 and *E. coli dacA* protein sequences. Active site residues are shaded in light red.

Fig. S2. A. Growth of Δ*dacA*-1 and wild type *V. cholerae* in M9 medium. B. Comparison of CFU/ml (based on plating on LBSF agar) and OD600 during growth of Δ*dacA*-1 and wild type cells. Note the different scale bars. C. Phase contrast images of *ΔdacA-1* cells from different culture densities. D. Growth of Δ*dacA*-1 complemented by DacA-2 overproduction. Shown is the growth of wild-type cells in LB medium, and Δ*dacA*-1 cells expressing ectopic *dacA*-2 under control of the P_{BAD} promoter grown in LB and LB supplemented with 0.4% arabinose.

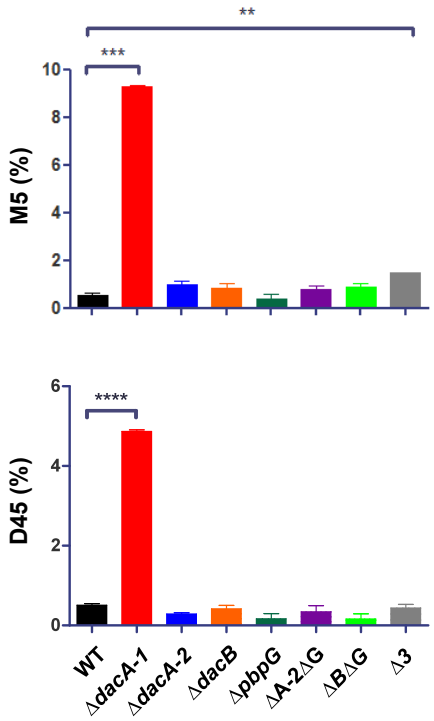
Fig. S3. Comparisons of PG composition for *V. cholerae* LMW PBP mutants. The relative molar abundance of total M5 monomers and D45 dimers (A), total tripepeptides (B), and total anhydro muropeptides (C) are shown. Stars indicate statistically significant differences based on unpaired t-test (*p≤ 0.1, **p≤ 0.01, ***p≤ 0.001).

Fig. S4. TnSeq analysis shows that *dacA-1* is not essential in salt free LB. Red and green vertical bars show the relative abundance in TnSeq data of reads corresponding to forward and reverse strand insertions within the indicated genes. Reads are shown for *V. cholerae* libraries culture in LBSF or in LB. Black lines (TA sites) show all possible transposon insertion sites.

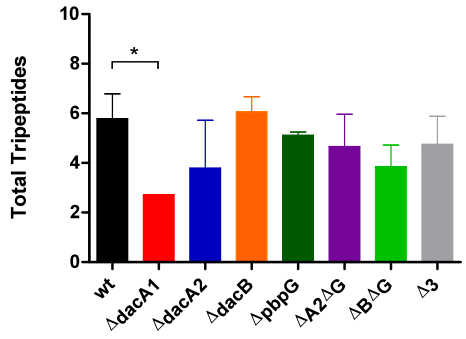
Fig. S5. A. Growth curves of wild type and Δ*dacA-1* strains grown in salt-free LB (LBSF), then transferred LBSF or LB containing 500 mM NaCl (LBHS). B. Phase contrast images of Δ*dacA*-2 *dacA-1* cond grown with and without inducer in salt-free LB medium.


A
M9
WT
ΔdacA-1
OD600 [log10]
time [min]
B
WT LB
ΔdacA-1 LB
CFU/ml (WT)
CFU/ml (ΔdacA-1)
OD600
C
OD600
0.07
0.25
0.7
1.3
ΔdacA-1
D
C6706
ΔdacA-1 ++DacA-2
ΔdacA-1 (uninduced)
OD600 [log10]
time [min]

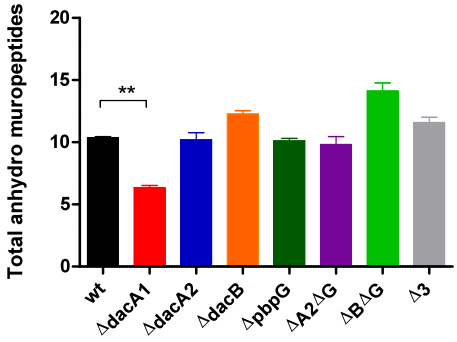
A

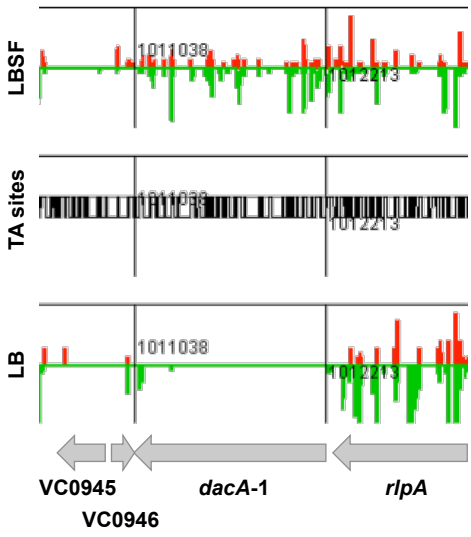

**
***
M5 (%)
10
8
6
4
2
0
****
D45 (%)
6
4
2
0
WT
ΔdacA-1
ΔdacA-2
ΔdacB
ΔpbpG
ΔA-2ΔG
ΔBΔG
Δ3

B

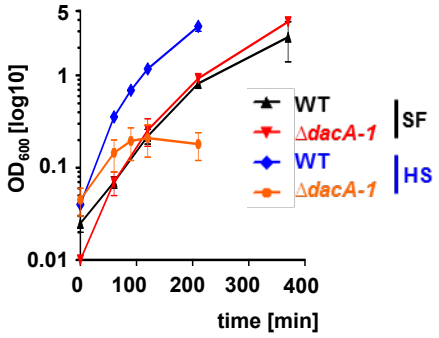

Total Tripeptides
10
8
6
4
2
0
*
wt
ΔdacA1
ΔdacA2
ΔdacB
ΔpbpG
ΔA2ΔG
ΔBΔG
Δ3

C

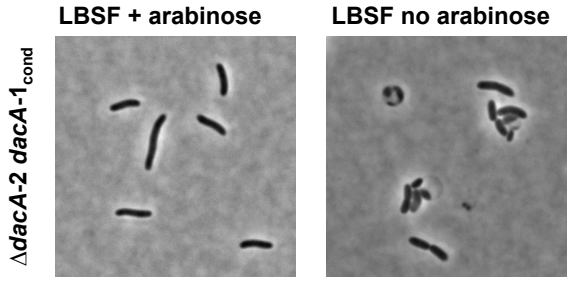

Total anhydro muropeptides
20
15
10
5
0
**
wt
ΔdacA1
ΔdacA2
ΔdacB
ΔpbpG
ΔA2ΔG
ΔBΔG
Δ3


LBSF
1011038
1012213
TA sites
1012213
LB
1011038
VC0945
VC0946
dacA-1
rlpA

A


5
1
0.1
0.01
OD600 [log10]
0
100
200
300
400
time [min]
WT
ΔdacA-1
WT
ΔdacA-1
SF
HS

B


LBSF + arabinose
LBSF no arabinose
ΔdacA-2 dacA-1cond

References

Cameron, D.E., Urbach, J.M., and Mekalanos, J.J. (2008) A defined transposon mutant library and its use in identifying motility genes in *Vibrio cholerae*. *Proceedings of the National Academy of Sciences* **105**: 8736–8741.

Ferrieres, L., Hemery, G., Nham, T., Guerout, A.M., Mazel, D., Beloin, C., and Ghigo, J.M. (2010) Silent Mischief: Bacteriophage Mu Insertions Contaminate Products of *Escherichia coli* Random Mutagenesis Performed Using Suicidal Transposon Delivery Plasmids Mobilized by Broad-Host-Range RP4 Conjugative Machinery. *J. Bacteriol.* **192**: 6418–6427.

Glauner, B. (1988) Separation and quantification of muropeptides with high-performance liquid chromatography. *Anal Biochem* **172**: 451–464.

Guzman, L.M., Belin, D., Carson, M.J., and Beckwith, J. (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J. Bacteriol.* **177**: 4121–4130.

Kolter, R., Inuzuka, M., and Helinski, D.R. (1978) Trans-complementation-dependent replication of a low molecular weight origin fragment from plasmid R6K. *Cell* **15**: 1199–1208.

Philippe, N., Alcaraz, J.-P., Coursange, E., Geiselmann, J., and Schneider, D. (2004) Improvement of pCVD442, a suicide plasmid for gene allele exchange in bacteria. *Plasmid* **51**: 246–255.

Simon, R., Priefer, U., and Pühler, A. (1983) A Broad Host Range Mobilization System for In Vivo Genetic Engineering: Transposon Mutagenesis in Gram Negative Bacteria. *Nat. Biotechnol.* **1**: 784–791.

Thelin, K.H. and Taylor, R.K. (1996) Toxin-coregulated pilus, but not mannose-sensitive hemagglutinin, is required for colonization by *Vibrio cholerae* O1 El Tor biotype and O139

strains. *Infect. Immun.* **64**: 2853–2856.