Supplementary material
to
Structural and functional characterization of the BcsG subunit of the cellulose synthase in *Salmonella typhimurium*

by

Lei Sun[1]*, Peter Vella[2]*, Robert Schnell[2], Anna Polyakova[3], Gleb Bourenkov[3], Fengyang Li[1], Annika Cimdins[1], Thomas Schneider[3], Ylva Lindqvist[2], Michael Y. Galperin[4], Gunter Schneider[2]**, and Ute Römling[1]****

[1]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, S-171 77, Stockholm, Sweden;

[2]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77, Stockholm, Sweden;

[3]European Molecular Biology Laboratory, D-22607 Hamburg, Germany;

[4]National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Maryland 20894, USA

This Supplementary Material includes 11 figures and 3 tables

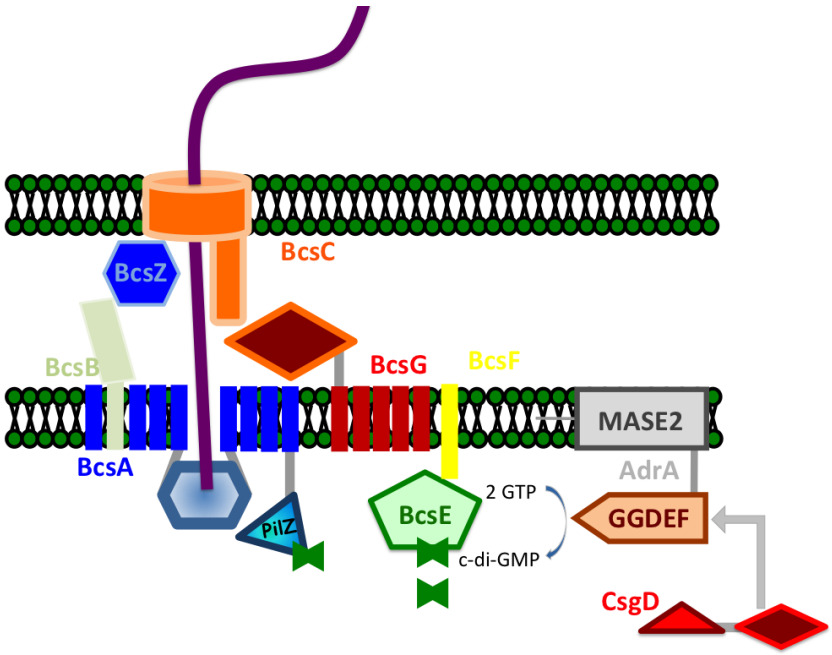

Figure S1. Proteins involved in cellulose biosynthesis and its regulation as mentioned in this work.

BcsA is the catalytic subunit of the cellulose synthase, which contains a C-terminal c-di-GMP binding PilZ domain and BcsB is a periplasmic protein with a C-terminal transmembrane helix required for in vitro cellulose biosynthesis. These two proteins constitute the active cellulose synthase [1]. BcsC is the predicted outer membrane pore and BcsZ is a periplasmic glycosyl hydrolase family 8 cellulase that reduces cellulose production [2, 3]. Accessory proteins are BcsE, a cytoplasmic c-di-GMP receptor [4], the short transmembrane protein BcsF and the alkaline phosphatase-related protein BcsG (this work, [5]). On agar plates, the orphan response regulator CsgD is required for amyloid curli and cellulose biosynthesis through activation of transcription of the diguanylate cyclase AdrA, which produces the c-di-GMP required for cellulose biosynthesis [6].

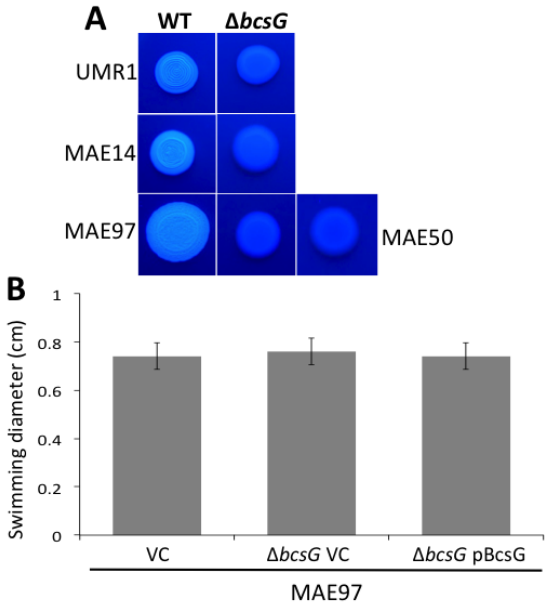

Figure S2. Calcofluor binding and motility of *S. typhimurium* ATCC14028-1s derivatives.

A. Calcofluor binding phenotypes of *S. typhimurium* strains UMR1 (cellulose$^+$/curli$^+$, 28 °C), MAE14 (cellulose$^+$/curli$^-$, 28 °C) and MAE97 (cellulose$^+$/curli$^-$, 28 °C/37 °C) and their respective Δ*bcsG* deletion mutants, observed using Calcofluor white fluorescence. Strain MAE50 (Δ*csgD*) served as a negative control. Cells were grown on salt-free LB agar plates with 50 µg/ml Calcofluor at 28 °C for 48 h. Wild type and mutant colonies are from the same plate.

B. Swimming motility of MAE97, its Δ*bcsG* mutant and the Δ*bcsG* mutant complemented with BcsG expressed from a plasmid (pBcsG). The swimming diameter was measured after 5 h of incubation on an LB agar plate with 0.3% agar at 28 °C.

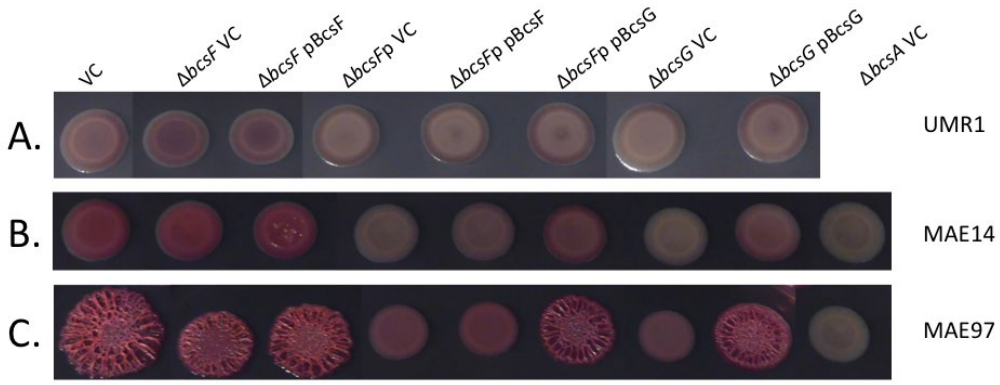

Figure S3. Colony morphotypes of polar and non-polar *bcsF* deletion mutants in *S. typhimurium* ATCC14028-1s derivatives.

Colony morphotypes of *S. typhimurium* strains UMR1 (cellulose$^+$/curli$^+$, 28 °C) (**A),** MAE14 (cellulose$^+$, 28 °C) (**B)** and MAE97 (cellulose$^+$, 28/37 °C) (**C)**. The non-polar (Δ*bcsF*) deletion mutants show a slightly reduced colony morphotype, which can be rescued by the expression of plasmid-borne *bcsF*. The polar (Δ*bcsF*p) deletion mutants show a severely reduced colony morphotype resembling a *bcsG* mutant, which cannot be complemented by *bcsF* plasmid expression, but can be overcome by *bcsG* expression.

Vector control (VC) is plasmid pBAD30. pBcsF is wild-type *bcsF* with a C-terminal 6xHis-tag cloned in pBAD30. pBcsG is wild-type *bcsG* with a C-terminal 8xHis-tag cloned in pBAD30. Δ*bcsA* strain was used as negative control. Cells were grown on salt-free LB agar plates containing Congo red for 24 h at 28 °C. See Table S2 for the complete genotypes.

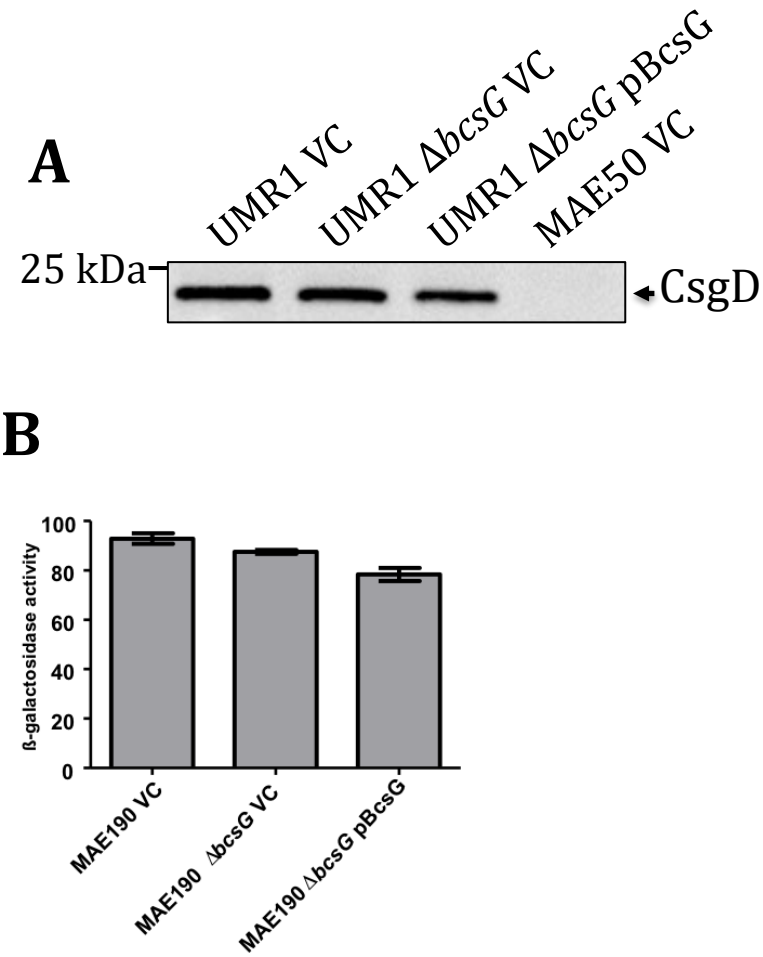

Figure S4. Production of the *rdar* biofilm activator CsgD and expression of the cellulose synthase BcsA are not regulated by BcsG.

A. Expression of CsgD in *S. typhimurium* UMR1 upon deletion or overexpression of *bcsG*. Cells were grown on salt-free LB agar for 24 h at 28 °C. VC indicates vector control pBAD30; pBcsG, wild-type *bcsG* cloned in pBAD30 with a C-terminal 8xHis tag. MAE50 is a *csgD* deletion mutant used as negative control. Western blot analysis with anti-CsgD antiserum (1:5000 dilution) has been performed as described [6].

B. Transcription of the *bcsA* gene encoding the cellulose synthase catalytic subunit is not affected by deletion or overexpression of *bcsG* as measured by β-galactosidase activity expressed from a *bcsA*::MudJ fusion construct. Cells were grown on salt-free LB agar for 24 h at 28 °C and resuspended in cold working buffer adjusted to OD_{600}=0.1. MAE190 contains a *bcsA101::*MudJ insertion in strain MAE97. VC and pBcsG constructs are as in panel A. See Table S2 for the complete genotypes.

A

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BcsG_Yersinia     1  -MNAKNKQQDSQSPWRYWRGLGAWNYYFLLKFALLWFGYLNFHPLANLVFLAFLLFPIPAYRVHRWRHWIAIPIGIGLLYHDTWLPGINSIMSQGSQVTGFSLSY  104
D8MLA0_Erwini     1  ----MTQRPVMPATRFVWRGLGGWNFYFLIKFALLWFGYLNFHPLSNLVLLAGLLLPIRSDRLHRLRNWIAIPVGIGLFWHDTWLPGPESIASQGSQLTGFSASY  101
A6TFE1_Klebsi     1  -MTNKKTTAAPLPLWQYWRGLGGWNFYFLVKFALLWAGYLNFHPMLNLVFLAFLLVPIPRFKLHRIRHWIAIPLGFALFWHDTWLPGPESIFSQGSQIAGFSASY  104
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Q3IER9_Pseuda     1  ---------------MKLSGLGIWNLYFLVKFVLFYYGAIKFDFLSNAALAALFALTFSNSQVDKLKHFIGAVFAIVLLYKDSWLPPIDRLTKQAGNIQDFSLGY   91
D4ZDI3_Shevan     1  -----MTNKNITPNVFMLSGLGWWNVYFIIKIALYLKGSIDFHPVENFAFLALLLFPLASKRLAIARTLIAVPIGLWLMHFDSYLPPLNRLWSQMGQLMQFETSY  100
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H8L5Y4_Frateu     1  -------MTPAAPSMQRVQGLGIWNIYFCIVLMLDWQGRILLQPVANALLAAALLVPLASIRARRLRQCVAIPAGTALLYHETWLPPISRLRDESS-VFHFSADY   97
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Q88JL7_Pseudo     1  ----MTNTTITLPVLARWPGLGGWNLYFFAKFLLVALGMLDFQALPNLLFAAFLLVPLPGKWLRIARQLLAVPIGIALFYQDTWLPPFSRLLAQPG-VLDFSWDY  100
Q7NUM4_Chromo     1  --MTERNPPPPAPMPGPSTGLGGWSLYFFILKLLLAWRGALSAHPAPDLAFALVLLLPLRRRWLRLARDALAWPAAAILLYYDSWLPPAALWRELSELKGFSLSY  103
R5PKS4_Sutte     23  SPPTQKTPRAAAELKLPWRGLGVWNVYFFILKFALAYFSYLNLDVLWNALLLVFLLIPIPYRWLSALRGLAAVAAGAALAYSESWLPPIASLTTNAAAVQGFSLNY  127
B1Y241_Leptot     1  --MGAAPLGAPHPHPLIGRVMGSWSLYFFLVKLGLHVAGLIQLDVPLNLLFAVALAWPWAHPGWRRAWRFLAWPVAVALLYHDSFWPPATRILSQWQAISGFSFAY   85
Q63JZ0_Burkho     1  ---------------------MTFWNLYFVLKLYLFAAGHLKPLWIANLGFALALALSAPARRRSLLRHALALALAVPLMYREADVPPLARLVETLGGLRAFSAGY   87
Q1LL40_Cupria     1  ---------------------MGLWNLYFLAKIYLFHTGQMQPIWLLNLVFALLLVIPLESRILRVLRQIIAIGAGAALAWRESTLPPFTRLLTEFSNIRAFTPGY   85
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D8MLA0_Erwini   102  LLELANRFINWEMVGTAFVMLVLYLFISQWVRITVLVSLALLWLNVVTIAGPSVNLLPGSSTPVAASTSA----------PAAKVAADGLDQSAPPTSANLTAYL  196
A6TFE1_Klebsi   105  IWDLIVRFINWSMVGAFFVLLVLWLFISQWLRVTVFVSAMVVWLAVSPLLPAFTLWPSGQPTTAAATTAQA2GANAAAGAASSPANSDIPPQTEPPTSANLTNWL  210
B4F1A6_Proteu   103  LIELAVNFINLKMIGVAFILLVGYLFIEQWVRVSVFIIAGVVWLNISGFTHFSGAMASAVSANSFTQQTN14STAQTRAPNNNVLSSPYPPQKQPANNKVLDEWL  219
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Q7NUM4_Chromo   104  LMELAGRILTPTLLIGFTVVLAGYLLLSRFLRLGTLVMATLLALSAHELWQQRAPAASASSASAFGGAPSAQ-----------------------------TPDQRL  179
R5PKS4_Sutter   128  VIDFALDFINWQMVGWAALIFCLWYLLRNCVRITFITICYFAVMVTMPYLDAFFAPAAREAAAGGTETAE------------GGPPAEGGPAGAAADSKTIEEWY  220
B1Y241_Leptot    86  LVELVGRVINVQLLVAVAMGAALWWVLKQRLRLATWFVGLVAVAALPSQHGGVTDLAQAATGADGTADR----------------AADRATAPLLDAGQLDQAL  174
Q63JZ0_Burkho    87  WMELVPRFVPPMLALAALGVVIGYLIVNRWLRVATFVLLALIALPVWQAGSAALARLDAAAAAVPGPAGT---------------------GRAVQPQDHNAAL  170
Q1LL40_Cupria    86  LVELFQRFVPVQMVVAVVLVVLVYLLINRWIRTTTLVLLALIAMPIWYGTGITLPGAKAQQATAQSAGSA---------------------RADINATNNYDAIL  169
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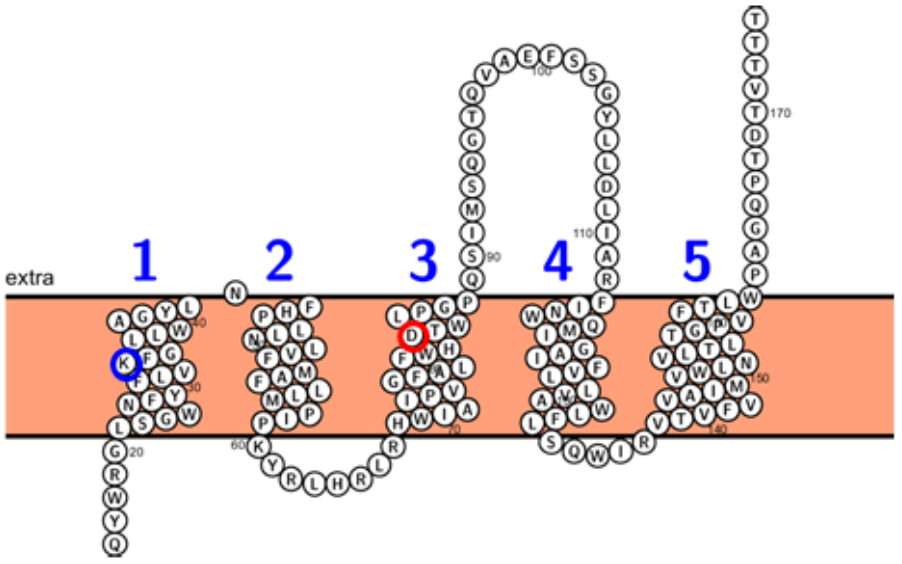

C

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5FGN_Nm   1 MIKPNLRPKLGSSALIAFLSLYSSLVLNYAFFAKVVELHPFNGTGADIFLYTMPVVLFFLSNFVFHVIAL-PFVHKVLIPLILVISAAVSYQEIF  94
ss_dssp     ---------EE---HHHHHHHHHHH---HHHHHHHHHH-------TTHHHHHHHHHHHHHHHHHHHHHCC-TTTHHHHHHHHHHHHHHHHHHHH
Identity                  +    +  **   +++* +++           * + + +*++++  + ++ + *  + * ++**+ +++ +   +   +

BcsG_topo     <---------OUT---------->.....................<--IN-->.........................<--OUT-->
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5FGN_Nm    95 -FNIYFNKSMLNNVLQTTAAESARLITPGYVLWIVCLGVLPALAYIAVKVKYRVWYKEFLTRLVLAAVSFLCALGIAMLQYQDYASFFRN  183
ss_dssp       -TCCCCSHHHHHHHHHCCHHHHHTTCCHHHHHHHCCCCCHHHHHHHHCEECCCCHHHHHHHHHHHHHHHHHHHHHHHTTHHHHHHHHH
Identity         +     +++      +   ****   ++ +++** *+++++       +** +  ++  +++ ++++   +  ++

Sequence identity: 18/147 = 12.2%
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Figure S5. Prediction of the membrane topology of *S. typhimurium* BcsG.

A. Alignment of the N-terminal membrane portion and the linker region of BcsG proteins from *S. typhimurium* and other bacteria. Uncharged residues are shaded yellow, conserved aromatic residues forming a predicted 'aromatic belt' along the membrane surface [7] are in bold, Arg and Lys are in blue, Asp and Glu are in red. Conserved Lys31 and Asp82 in the hydrophobic core are marked with asterisks. The proteins are listed under their UniProt accession codes and abbreviated names of the respective genera. The source sequences are as follows: *Yersinia enterocolitica* LC20_00134 (GenBank accession number AHM71390); *Erwinia billingiae* EbC_43970 (CAX61928); *Klebsiella pneumoniae* KPN_03888 (ABR79275); *Proteus mirabilis* PMI2096 (CAR44189); *Serratia proteamaculans* Spro_0143 (ABV39253); *Pseudoalteromonas haloplanktis* PSHAa2158 (CAI87214); *Shewanella violacea* SVI_0134 (BAJ00105); *Vibrio fischeri* VF_A0887 (AAW87957); *Photobacterium profundum* PBPRA1716 (CAG20123); *Frateuria aurantia* Fraau_2360 (AFC86727); *Pseudomonas stutzeri* PST_0283 (ABP77989); *Pseudomonas putida* PP_2632 (AAN68240); *Chromobacterium violaceum* CV_2673 (AAQ60343); *Sutterella wadsworthensis* BN489_01704 (CCZ17195); *Leptothrix cholodnii* Lcho_2073 (ACB34340); *Burkholderia pseudomallei* BPSS1576 (CAH39049), and *Cupriavidus metallidurans* Rmet_2257 (ABF09136).

B. Predicted membrane topology of BcsG drawn by Protter (http://wlab.ethz.ch/protter/, [8]. Positions of the intramembrane Lys31 and Asp82 are marked with blue and red circles, respectively.

C. Alignment of the membrane portions of *S. typhimurium* BcsG protein and lipid A phosphoethanolamine transferase from *Neisseria meningitidis* (*Nm*EptA, PDB entry 5FGN [7]). The alignment was constructed based on the results of an HHpred [9] search and reconciling the predicted secondary structures and membrane topologies of the two proteins. Coloring is as in panel A, secondary structure elements (predicted for BcsG and derived from the 5FGN structure using the DSSP algorithm [10]) are as follows: H, α-helix; E, β-strand; C, coil; T, turn. Identical residues are marked with asterisk, similar residues with the plus signs.

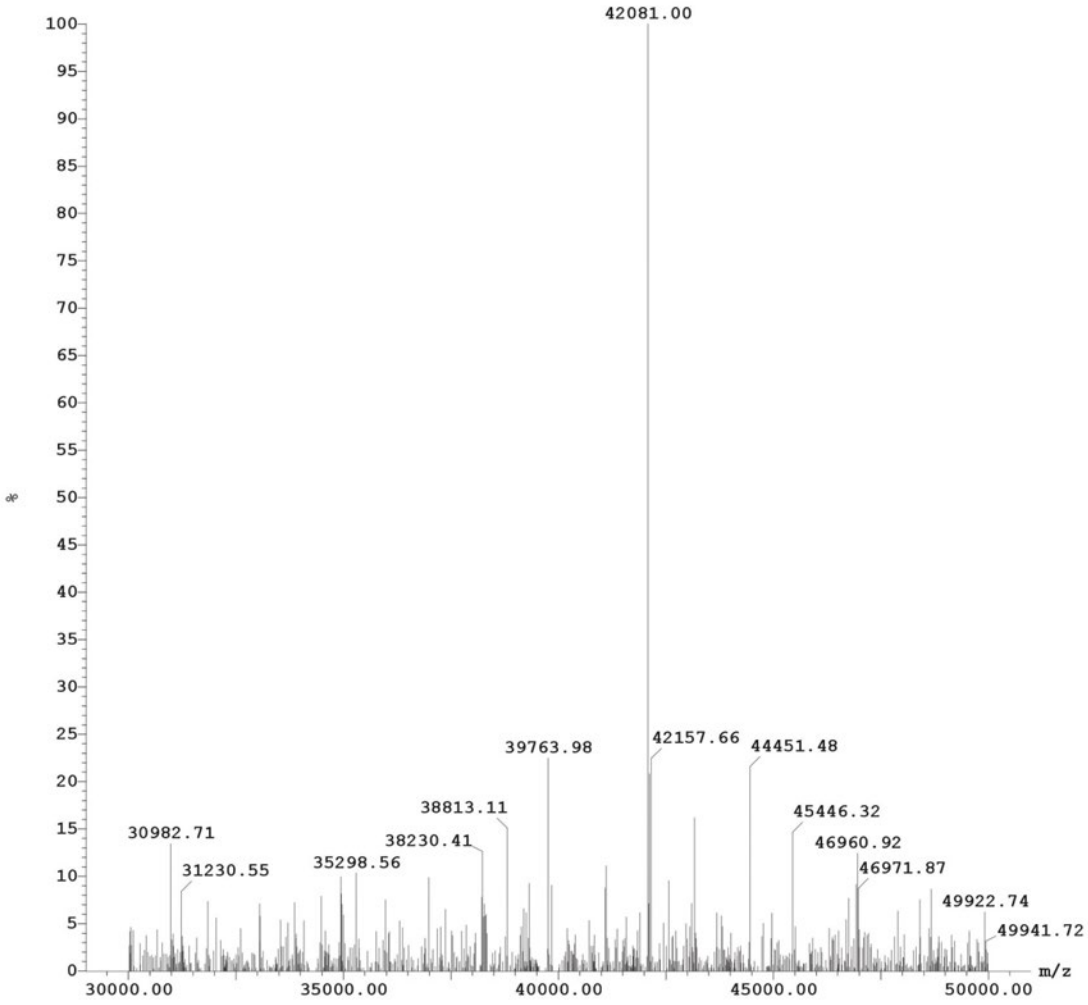
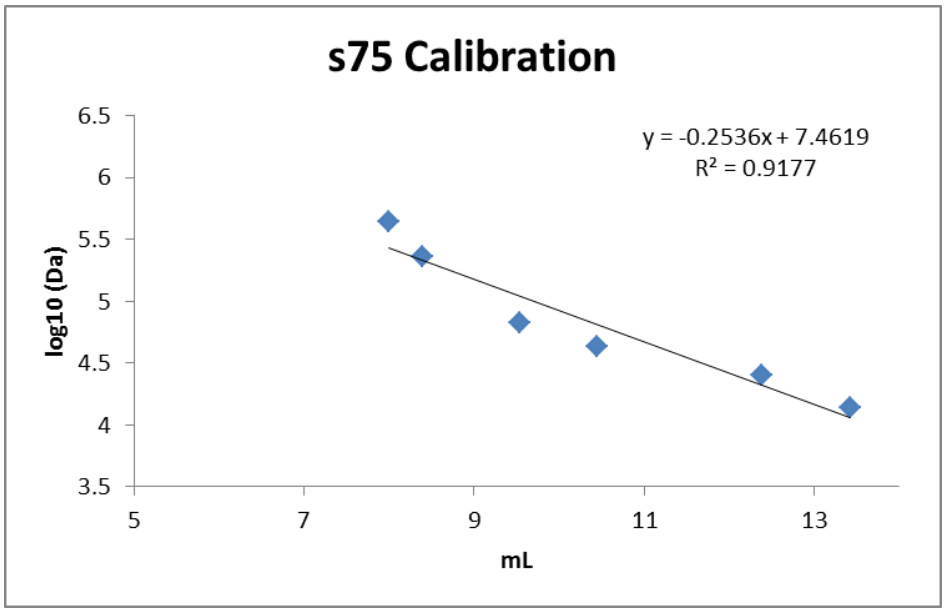


Figure S6. Mass-spectrum of the purified BcsG construct after cleavage with factor Xa.

A



B

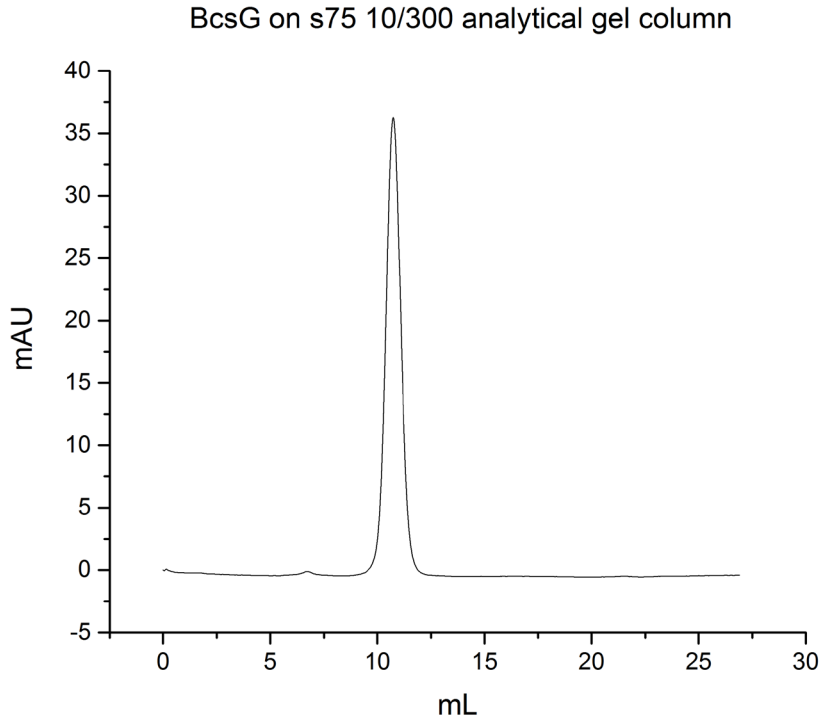


Figure S7. Analytical gel chromatography of the purified BcsG construct after tag cleavage used in the crystallization experiments.

A. Calibration curve obtained with ribonuclease-A (13.7 kDa), chymotrypsinogen-A (25 kDa), ovalbumin (43 kDa), albumin (67 kDa), catalase (232 kDa), ferritin (440 kDa) and Blue Dextran (2 MDa).

B. Elution profile of purified BcsG. BcsG elutes as a monomer.

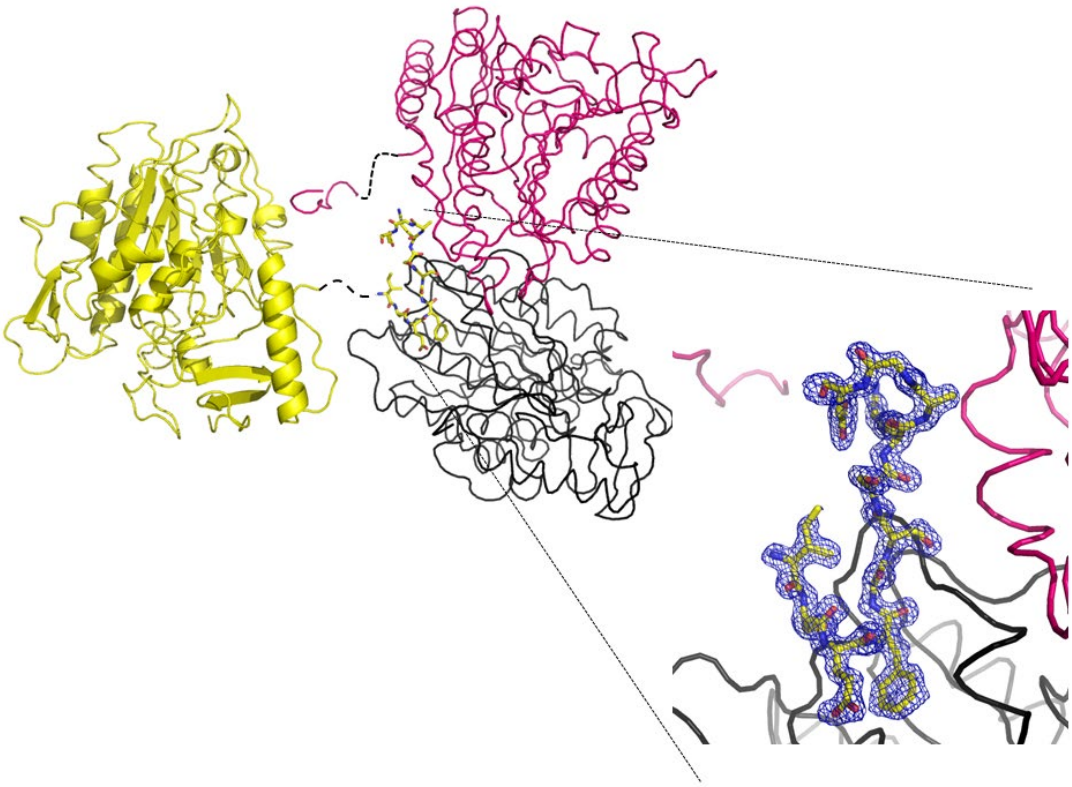


Figure S8. Crystal packing interactions of the tag-derived linker peptide with a neighboring BcsG molecule in the crystal lattice. Part of the 2Fo-Fc electron density map, contoured at 1.5 σ, at the position of the bound peptide derived from the maltose binding protein tag is shown. The side chain of linker residue Phe4 is buried into a small pocket of the neighboring BcsG molecule and forms van-der-Waals interactions with the aliphatic parts of the side chains of Arg503 and Lys519. Other important interactions are made through hydrogen bonds of the carbonyl oxygen atom of Phe4 with the peptide amide group of Asn516, the amide nitrogen and side chain hydroxyl group of the linker Ser6 residue with the side chain of Glu512, and the amide nitrogen atom of linker Ser2 residue with the carbonyl oxygen atom of Ser514. In total, this packing interface contributes with 300 $Å^2$ of buried surface area to the packing interactions between two symmetry related molecules.

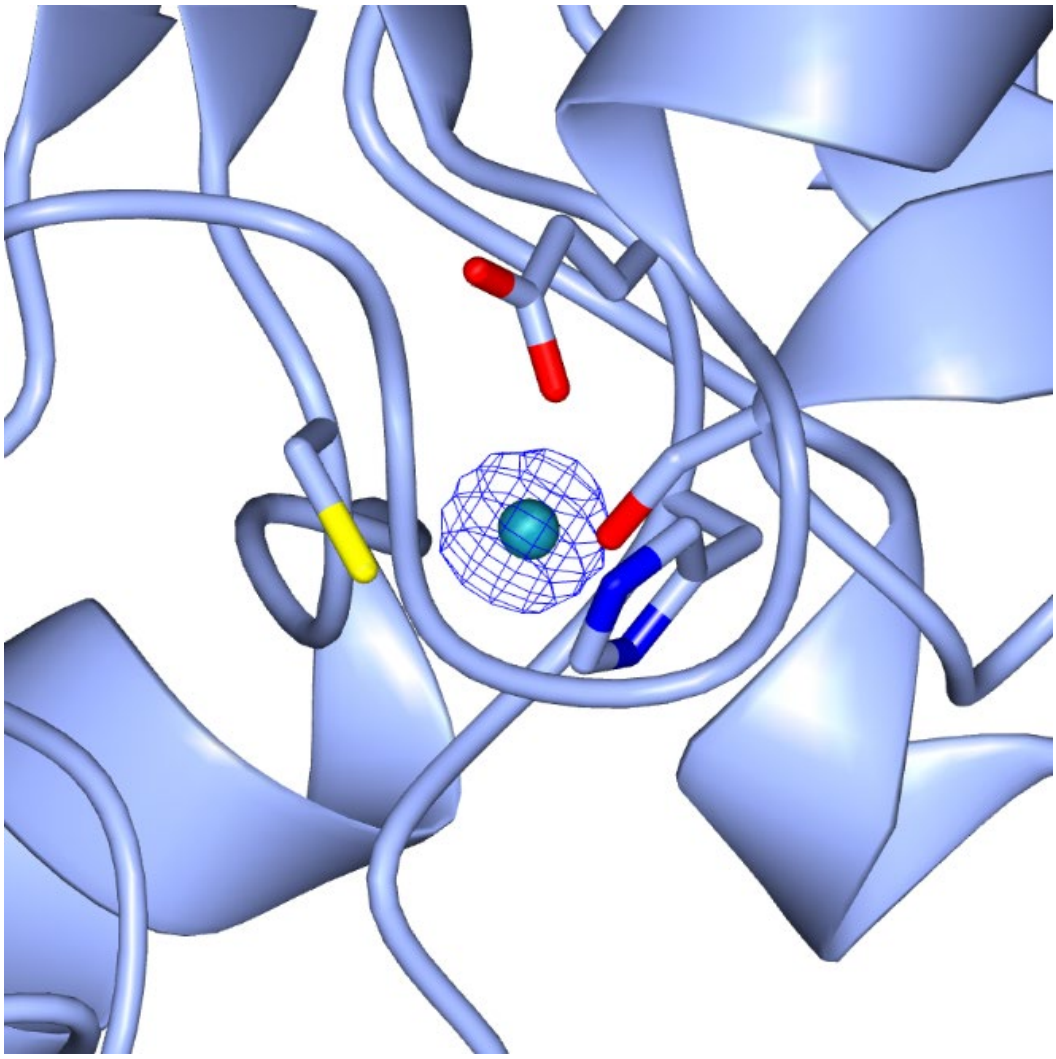
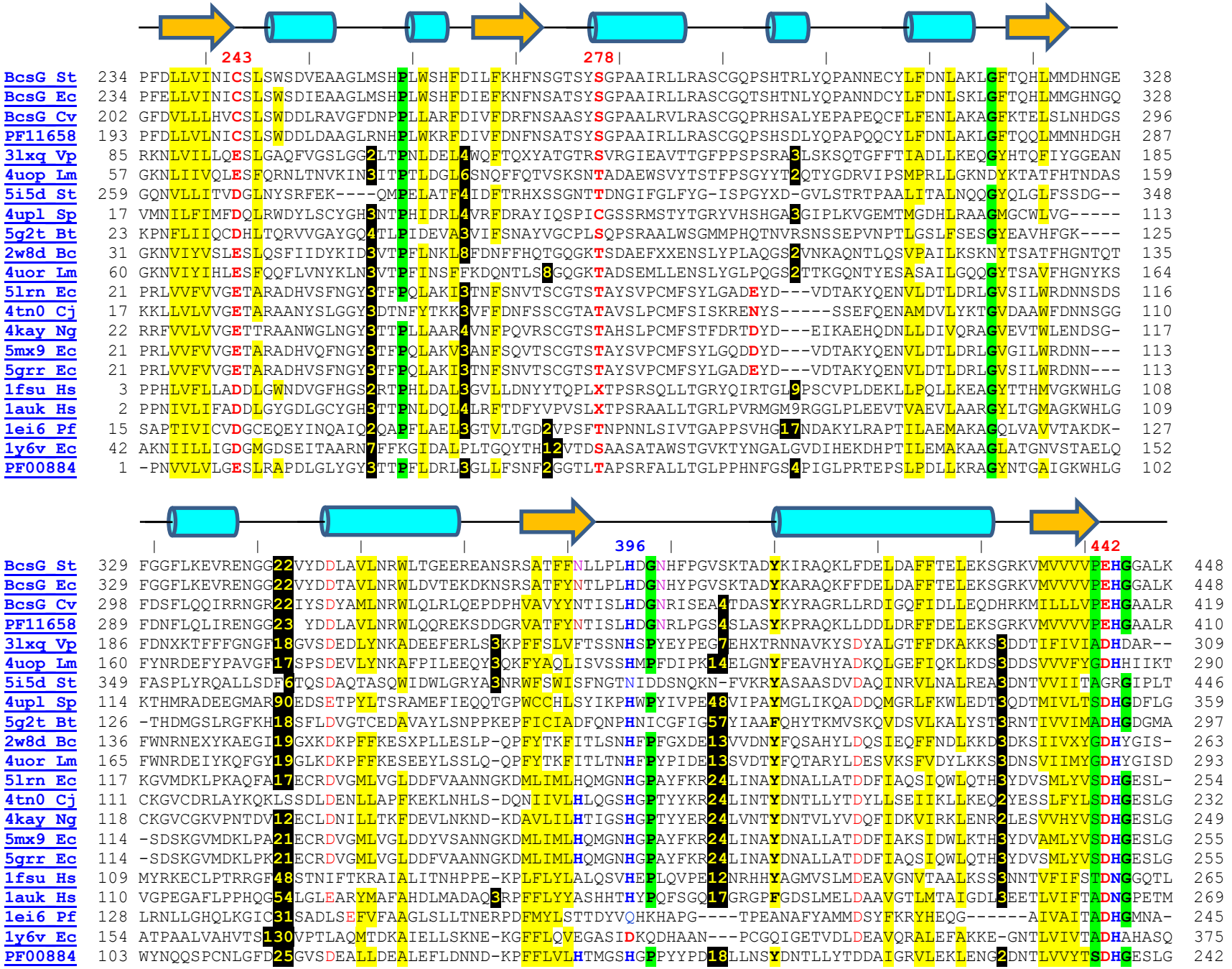


Figure S9. Anomalous difference electron density map at the metal binding site of BcsG contoured at 15σ, demonstrating that the bound metal in this site is a Zn^{2+} ion**.** The electron density map was calculated based on the X-ray data collected at the absorption edge of Zn^{2+} (λ = 1.278 Å).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278
396
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BcsG Cv 202 GFDVLLLHVCSLSWDDLRAVGFDNPPLLARFDIVFDRFNSAASYSGPAALRVLRASCGQPRHSALYEPAPEQCFLFENLAKAGFKTELSLNHDGS 296
PF11658 193 PFDLLVLNICSLSWDDLDAAGLRNHPLWKRFDIVFDNFNSATSYSGPAAIRLLRASCGQPSHSDLYQPAPQQCYLFDNLAKLGFTQQLMMNHDGH 287
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4tn0 Cj 17 KKLLVLVVGETARAANYSLGGY3DTNFYTKK3VFFDNFSSCGTATAVSLPCMFSISKRENYS-----SSEFQENAMDVLYKTGVDAAWFDNNSGG 110
4kay Ng 22 RRFVVLVVGETTRAANWGLNGY3TTPLLAAR4VNFPQVRSCGTSTAHSLPCMFSTFDRTDYD---EIKAEHQDNLLDIVQRAGVEVTWLENDSG- 117
5mx9 Ec 21 PRLVVFVVGETARADHVQFNGY3TFPQLAKV3ANFSQVTSCGTSTAYSVPCMFSYLGQDDYD---VDTAKYQENVLDTLDRLGVGILWRDNN--- 113
5grr Ec 21 PRLVVFVVGETARADHVSFNGY3TFPQLAKI3TNFSNVTSCGTSTAYSVPCMFSYLGADEYD---VDTAKYQENVLDTLDRLGVSILWRDNN--- 113
1fsu Hs 3 PPHLVFLLADDLGWNDVGFHGS2RTPHLDAL3GVLLDNYYTQPLXTPSRSQLLTGRYQIRTGL9PSCVPLDEKLLPQLLKEAGYTTHMVGKWHLG 108
1auk Hs 2 PPNIVLIFADDLGYGDLGCYGH3TTPNLDQL4LRFTDFYVPVSLXTPSRAALLTGRLPVRMGM9RGGLPLEEVTVAEVLAARGYLTGMAGKWHLG 109
1ei6 Pf 15 SAPTIVICVDGCEQEYINQAIQ2QAPFLAEL3GTVLTGD2VPSFTNPNNLSIVTGAPPSVHG17NDAKYLRAPTILAEMAKAGQLVAVVTAKDK- 127
1y6v Ec 42 AKNIILLIGDGMGDSEITAARN7FFKGIDALPLTGQYTH12VTDSAASATAWSTGVKTYNGALGVDIHEKDHPTILEMAKAAGLATGNVSTAELQ 152
PF00884 1 -PNVVLVLGESLRAPDLGLYGY3TTPFLDRL3GLLFSNF2GGTLTAPSRFALLTGLPPHNFGS4PIGLPRTEPSLPDLLKRAGYNTGAIGKWHLG 102
BcsG St 329 FGGFLKEVRENGG22VYDDLAVLNRWLTGEEREANSRSATFFNLLPLHDGNHFPGVSKTADYKIRAQKLFDELDAFFTELEKSGRKVMVVVPEHGGALK 448
BcsG Ec 329 FGGFLKEVRENGG22VYDDTAVLNRWLDVTEKDKNSRSATFYNTLPLHDGNHYPGVSKTADYKARAQKFFDELDAFFTELEKSGRKVMVVVPEHGGALK 448
BcsG Cv 298 FDSFLQQIRRNGR22IYSDYAMLNRWLQLRLQEPDPHVAVYYNTISLHDGNRISEA4TDASYKYRAGRLLRDIGQFIDLLEQDHRKMILLLVPEHGAALR 419
PF11658 289 FDNFLQLIRENGG23 YDDLAVLNRWLQQREKSDDGRVATFYNTISLHDGNRLPGS4SLASYKPRAQKLLDDLDRFFDELEKSGRKVMVVVPEHGAALR 410
3lxq Vp 186 FDNXKTFFFGNGF18GVSDEDLYNKADEEFERLS3KPFFSLVFTSSNHSPYEYPEG7EHXTRNNAVKYSDYALGTFFDKAKKS3DDTIFIVIADHDAR-- 309
4uop Lm 160 FYNRDEFYPAVGF17SPSDEVLYNKAFPILEEQY3QKFYAQLISVSSHMPFDIPK14ELGNYFEAVHYADKQLGEFIQKLKDS3DDSVVVFYGDHHIIKT 290
5i5d St 349 FASPLYRQALLSDF6TQSDAQTASQWIDWLGRYA3NRWFSWISFNGTNIDDSNQKN-FVKRYASAASDVDAQINRVLNALREA3DNTVVIITAGRGIPLT 446
4upl Sp 114 KTHMRADEEGMAR90EDSETPYLTSRAMEFIEQQTGPWCCHLSYIKPHWPYIVPE48VIPAYMGLIKQADDQMGRLFKWLEDT3QDTMIVLTSDHGDFLG 359
5g2t Bt 126 -THDMGSLRGFKH18SFLDVGTCEDAVAYLSNPPKEPFICIADFQNPHNICGFIG57YIAAFQHYTKMVSKQVDSVLKALYST3RNTIVVIMADHGDGMA 297
2w8d Bc 136 FWNRNEXYKAEGI19GXKDKPFFKESXPLLESLP-QPFYTKFITLSNHFPFGXDE13VVDNYFQSAHYLDQSIEQFFNDLKKD3DKSIIVXYGDHYGIS- 263
4uor Lm 165 FWNRDEIYKQFGY19GLKDKPFFKESEEYLSSLQ-QPFYTKFITLTNHFPYPIDE13SVDTYFQTARYLDESVKSFVDYLKKS3DNSVIIMYGDHYGISD 293
5lrn Ec 117 KGVMDKLPKAQFA17ECRDVGMLVGLDDFVAANNGKDMLIMLHQMGNHGPAYFKR24LINAYDNALLATDDFIAQSIQWLQTH3YDVSMLYVSDHGESL- 254
4tn0 Cj 111 CKGVCDRLAYKQKLSSDLDENLLAPFKEKLNHLS-DQNIIVLHLQGSHGPTYYKR24LINTYDNTLLYTDYLLSEIIKLLKEQ2YESSLFYLSDHGESLG 232
4kay Ng 118 CKGVCGKVPNTDV12ECLDNILLTKFDEVLNKND-KDAVLILHTIGSHGPTYYER24LVNTYDNTVLYVDQFIDKVIRKLENR2LESVVHYVSDHGESLG 249
5mx9 Ec 114 -SDSKGVMDKLPA21ECRDVGMLVGLDDYVSANNGKDMLIMLHQMGNHGPAYFKR24LINAYDNALLATDDFIAKSIDWLKTH3YDVAMLYVSDHGESLG 255
5grr Ec 114 -SDSKGVMDKLPK21ECRDVGMLVGLDDFVAANNGKDMLIMLHQMGNHGPAYFKR24LINAYDNALLATDDFIAQSIQWLQTH3YDVSMLYVSDHGESLG 255
1fsu Hs 109 MYRKECLPTRRGF48STNIFTKRAIALITNHPPE-KPLFLYLALQSVHEPLQVPE12NRHHYAGMVSLMDEAVGNVTAALKSS3NNTVFIFSTDNGGQTL 265
1auk Hs 110 VGPEGAFLPPHQG54LGLEARYMAFAHDLMADAQ3RPFFLYYASHHTHYPQFSGQ17GRGPFGDSLMELDAAVGTLMTAIGDL3EETLVIFTADNGPETM 269
1ei6 Pf 128 LRNLLGHQLKGIC31SADLSEFVFAAGLSLLTNERPDFMYLSTTDYVQHKHAPG----TPEANAFYAMMDSYFKRYHEQG------AIVAITADHGMNA- 245
1y6v Ec 154 ATPAALVAHVTS130VPTLAQMTDKAIELLSKNE-KGFFLQVEGASIDKQDHAAN---PCGQIGETVDLDEAVQRALEFAKKE-GNTLVIVTADHAHASQ 375
PF00884 103 WYNQQSPCNLGFD25GVSDEALLDEALEFLDNND-KPFFLVLHTMGSHGPYYPD18LLNSYDNTLLYTDDAIGRVLEKLENG2DNTLVVYTSDHGESLG 242

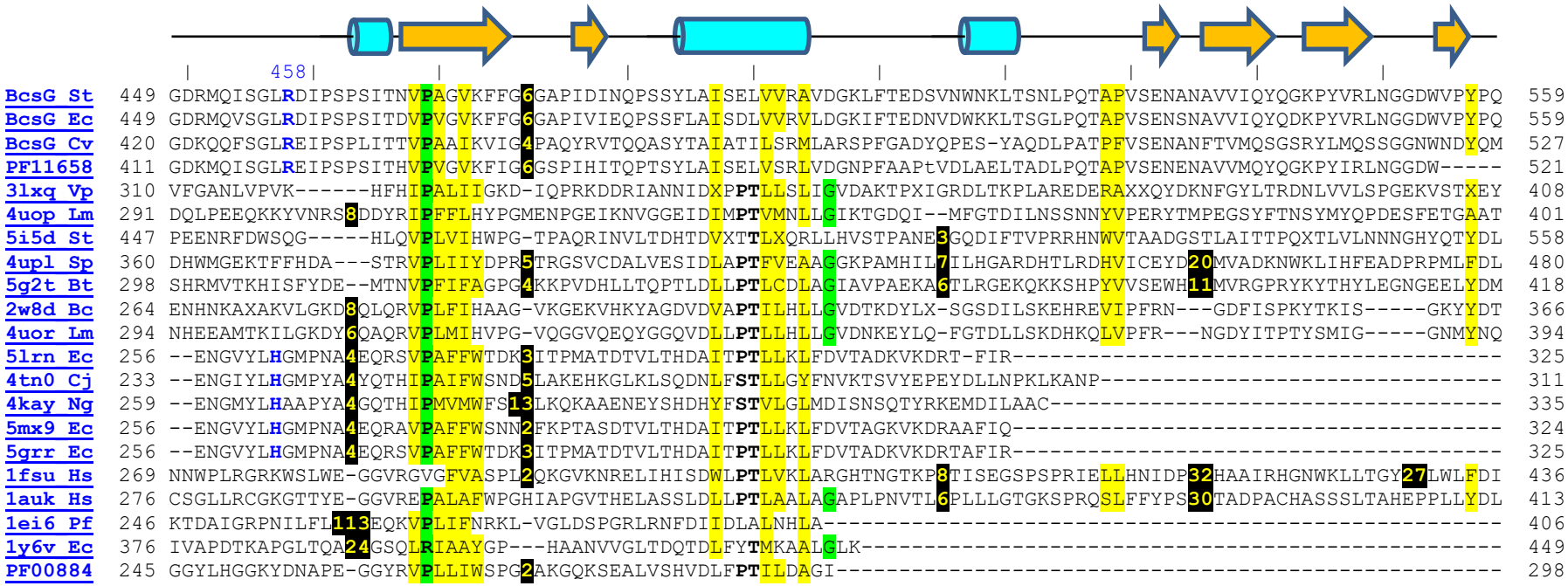

Figure S10. Structure-based sequence alignment of the C-terminal soluble domain of BcsG and related members of the alkaline phosphatase superfamily.

The top four rows include BcsG sequences from *S. typhimurium*, *E. coli*, and *Chromobacterium violaceum*, and from the Pfam domain PF11658, respectively. Subsequent rows include sequences of proteins with known structures, listed under their PDB identifiers and first letters of the genus and species names. All names are hyperlinked to the respective entries in the NCBI protein database. The last row shows the sequence of the Pfam domain Sulfatase (PF00884). The cylinders and arrows on the top indicate α-helices and β-strands, respectively, of the BcsG structure calculated using the DSSP algorithm [10], vertical bars mark each 10th residue. The active site residues are in bold and colored red (Asp, Glu) or blue (His, Asn). Conserved hydrophobic residues are shaded yellow, conserved turn residues (Gly, Pro) are shaded green. The alignment was constructed by reconciling structural alignments generated by the DALI [11] and VAST [12] tools.

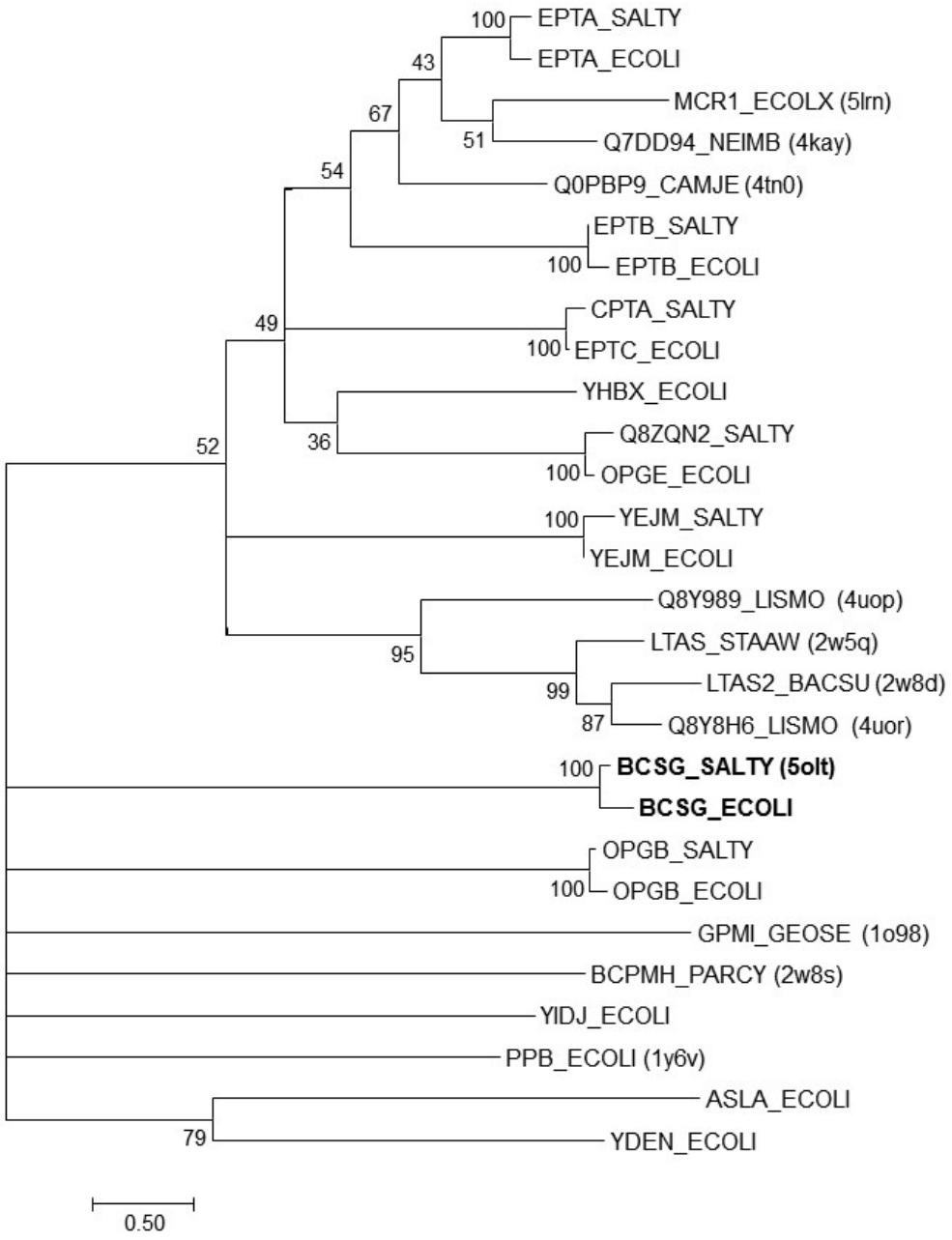

Figure S11. Maximum likelihood tree of phosphoethanolamine transferases from *E. coli*, *S. typhimurium* and related members of the alkaline phosphatase superfamily. Proteins are listed under their UniProt identifiers; PDB entries, where available, are shown in parentheses, see Tables 2 and S3 for details. The tree was constructed in MEGA7 [13] using the maximum likelihood method based on the JTT matrix-based model [14] with default parameters from an alignment built with MUSCLE [15]. All positions containing gaps were eliminated, leaving a total of 264 positions in the final dataset. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The percentage of trees in which the associated taxa clustered together is shown next to the branches; those branches with <30% support have been collapsed.

Table S1. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or description	Reference/ Source
***E. coli* K-12**		
DH5α	λφ80d*lacZ* ΔM15 Δ(*lacZYA*-argF)U169 *recA1 endA1 hsdR17*(rK - mK -) *supE44 thi-1 gyrA relA1*	New England Biolabs
***S. Typhimurium* ATCC 14028 derivatives**		
UMR1	ATCC14028-1s Nal^r , cellulose/curli fimbriae (rdar) 28 °C	[16]
MAE14	UMR1 Δ*csgBA101*::Km^r , cellulose (pdar) 28°C	[6]
MAE50	UMR1 Δ*csgD*	[6]
MAE52	UMR1 p*csgD1*; cellulose/curli fimbriae (rdar) 28/37 °C	[17]
MAE97	UMR1 p*csgD1* Δ*csgBA102*, cellulose (pdar) 28/37 °C	[6]
MAE190	MAE97 *bcsA101::*MudJ cellulose/curli negative, (saw) 28/37 °C	[18]
MAE1264	MAE97 *bcsA*-3xFLAG	[19]
MAE1873	UMR1 *bcsC-3xFLAG Km*	[19]
MAE2201	UMR1 Δ*bcsF::Cm*	This study
MAE2202	UMR1 Δ*bcsF::tetRA*	This study
MAE2204	MAE14 Δ*bcsF::Cm*	This study
MAE2205	MAE14 Δ*bcsF::tetRA*	This study
MAE2207	MAE97 Δ*bcsF::Cm*	This study
MAE2207	MAE97 Δ*bcsF::tetRA*	This work
MAE2203	UMR1 Δ*bcsG101*	This study
MAE2206	MAE14 Δ*bcsG101*	This study
MAE2209	MAE97 Δ*bcsG101*	This study
MAE2211	MAE1264 Δ*bcsG101*	This study
MAE2215	UMR1 Δ*bcsG-3xFLAG Km*	This study
MAE2217	UMR1 Δ*bcsG101 bcsC-3xFLAG Km*	This study
Plasmids		
pBAD30	pACYC184 ori; L-arabinose regulated araC; PBAD promoter; Amp^r	[20, 21]
pLAFR3	IncP Tc^r *cos⁺ rlx⁺*	[22]
pKD3	FRT-cat-FRT; Cm^r , Amp^r	[23]
pKD46	ParaBαβγ; Amp^r	[23]
pSUB11	template for 3xFLAG-Km	[24]
pBAD-BcsF	*bcsF* cloned in XbaI/HindIII sites in pBAD30 with a 8xHis-Tag	This study
pBAD-BcsG	*bcsG* cloned in *Xba*I/*Hind*III sites in pBAD30 with a 8x His-Tag	This study

pBAD-BcsG$_{C243S}$	pBAD30-BcsG$_{C243S}$-8xHis	This study
pBAD-BcsG$_{E442A}$	pBAD30-BcsG$_{E442A}$-8xHis	This study
pBAD-BcsG$_{S278A}$	pBAD30-BcsG$_{S278A}$-8xHis	This study
pBAD-BcsG$_{H396A}$	pBAD30-BcsG$_{H396A}$-8xHis	This study
pBAD-BcsG$_{H443A}$	pBAD30-BcsG$_{H443A}$-8xHis	This study
pBAD-BcsG$_{S493A}$	pBAD30-BcsG$_{S493A}$-8xHis	This study
pBAD-BcsG$_{1-165}$	pBAD30-BcsG (1–165 aa)-8xHis	This study
pBAD-BcsG$_{1-210}$	pBAD30-BcsG (1–210 aa)-8xHis	This study
pMAL-c2x	MBP fusion overexpression vector; plac,Amp^r	New England Biolabs
pMAL-BcsG1	pMAL-c2x-BcsG1 (185-559 aa)	This study
pMAL-BcsG2	pMAL-BcsG1$_{S278A}$	This study
pWJB9 (pAdrA)	pLAFR3*::araC PBAD adrA*	[25]

Table S2. Primers used in this study

Primer name	Sequence (5'-3')
Chromosomal non-polar *bcsF* deletion mutant	
np_mut_bcsF_F	ATGATGACCATCAGCGATATCGTGCAAATTATTCTTTTTTTAAGACCCA CTTTCACATTTAAG
np_mut_bcsF_R	TCATTTTTTGTCTGCCTTGACTTTCGTAGCGCGGCGCAGACTAAGCACTT GTCTCCTGTTTACTC
Chromosomal polar *bcsF* deletion mutant	
mut_bcsF_F	ATGATGACCATCAGCGATATCGTGCAAATTATTCTTTTTGTGTAGGCTG GAGCTGCTTC
mut_bcsF_R	TCATTTTTTGTCTGCCTTGACTTTCGTAGCGCGGCGCAGCATATGAATA TCCTCCTTAGT
Chromosomal *bcsG* deletion mutant	
mut_bcsG_F	ATGACTCAGC ATACTCAAACTCCTTCAATGCCTTCTCCGC GTGTAGGCTGGAGCTGCTTC
mut_bcsG_R	TTACTGCGGGTAAGGCACCCAGTCGCCGCCATTCAGACGA CATATGAATATCCTCCTTAGT
Control primers for chromosomal deletion mutants	
bcsFGcontrolF	GGTAATAAATGCCGAACACG
bcsFGcontrolR	GGCTAACTTGAACCCAAACACT
Cloning of *bcsF* in pBAD30	
com_bcsF_F	TGCTCTAGATAAGGAGGTTGCATCATGATGACCATCAGCGA
com_bcsF_R	CCCAAGCTTTCAGTGATGGTGATGGTGATG TTTTTTGTCTGCCTTGACTT
Cloning of *bcsG* in pBAD30	
bcsG-comF	GTATCTAGAGTCAAGGCAGACAAAAAATGACTCAGCATACTC
bcsG-comR	TACAAGCTTTTAATGATGATGATGATGATGCTGCGGGTAAGGCAC
Construction of *bcsG* variants in pBAD30	
BcsG1-165_R	TACAAGCTT TTAATGATGATGATGATGATGGCCTGCCGGCCACAGCG
BcsG1-210_R	TAC AAGCTTTTAATGATGATGATGATGATGCAACCAGGCGTTCAGAT
BcsG S278A_F	CGGTACGTCTTAC GCG GGCCCGGCGGCC
BcsG S278A_R	GGCCGCCGGGCC CGC GTAAGACGTACCG
BcsG H443A_F	GGTAGTCGTCGTACCGGAG GCG GGCGGCGCGCTGAAGGGCG
BcsG H443A_R	CGCCCTTCAGCGCGCCGCC CGC CTCCGGTACGACGACTACC
BcsG H396A_F	CTTTAACCTGCTGCCGCTG GCG GATGGCAACCACTTCCCCG
BcsG H396A_R	CGGGGAAGTGGTTGCCATC CGC CAGCGGCAGCAGGTTAAAG
BcsG S493A_F	GATATTAATCAGCCGAGC GCG TACCTGGCGATTTCCGAAC
BcsG S493A_R	GTTCGGAAATCGCCAGGTA CGC GCTCGGCTGATTAATATC
BcsG E442A_F	GTAGTCGTCGTACCG GCG CACGGCGGCGCGCTG
BcsG E442A_R	CAGCGCGCCGCCGTG CGC CGGTACGACGACTAC
BcsG C243S_F	CCTATTGGTCATCAATATC AGC TCGCTCTCCTGGTCGGATG
BcsG C243S_R	CATCCGACCAGGAGAGCGA GCT GATATTGATGACCAATAGG
BcsGR458A_F	GCAGATCTCAGGCCTGGCGGATATTCCCAGCCCCTC
BcsGR458A_R	GAGGGGCTGGGAATATCCGCCAGGCCTGAGATCTGC

BcsGR458M_F	GAATGCAGATCTCAGGCCTGATGGATATTCCCAGCCCCTCCATC
BcsGR458M_R	GATGGAGGGGCTGGGAATATCCATCAGGCCTGAGATCTGCATTC
BcsGR458H_F	GCAGATCTCAGGCCTGCATGATATTCCCAGCCCCTC
BcsGR458H_R	GAGGGGCTGGGAATATCATGCAGGCCTGAGATCTGC
Cloning of *bcsG* in pMAL-c2 expression vector	
bcsGpMAL2-XbaIF	GTATCTAGA GCGGGCGATAAGCCGG
bcsGpMAL2-HindIIIR	TACAAGCTTTTACTGCGGGTAAGGCAC

Table S3. Phosphoethanolamine transferase family enzymes in *S. typhimurium*

Protein name	UniProt entry, accession	*S. typhimurium* LT2 locus tag	TM domain length[b]	Active site residue	Reference
Phosphoethanolamine transferase					
Cellulose biosynthesis protein BcsG	BCSG_SALTY, Q7CPI7	STM3624	162 aa, 5 TM	Ser	[5]; this work
Phosphoethanolamine transferase EptA	EPTA_SALTY, P36555	STM4293	175 aa, 5 TM	Thr	[26, 27]
Kdo_2-lipid A phospho-ethanolamine 7''-transferase EptB	EPTB_SALTY, P43666	STM3635	180 aa, 5 TM	Thr	[28]
Phosphoethanolamine transferase CptA	CPTA_SALTY, Q7CPC0	STM4118	174 aa, 5 TM	Thr	[29]
Integral membrane protein OpgE/YbiP	Q8ZQN2_SALTY, Q8ZQN2	STM0834	158 aa, 4 TM	Thr	[30]
Phosphoethanolamine transferase MCR-1[a]	MCR1_ECOLX, A0A0R6L508	N/A[a]	178 aa, 5 TM	Thr	[31, 32]
Phosphoglycerol transferase					
Phosphoglycerol transferase OpgB	OPGB_SALTY, Q8ZJX6	STM4541	132 aa, 4 TM	Thr	[31-33]
No known enzymatic activity					
Cardiolipin transfer protein PbgA	YEJM_SALTY, P40709	STM2228	190 aa, 5 TM	N/A	[34]

[a] – This protein and its Arg536->His variant have been detected in recent environmental isolates of *S. typhimurium* (not in strain LT2 or its derivatives) [31, 32].

[b] – Transmembrane domain length and helices predictions are taken from UniProt and/or calculated using MEMSAT-SVM [35].

References

[1] J.L. Morgan, J. Strumillo, J. Zimmer. Crystallographic snapshot of cellulose synthesis and membrane translocation. Nature 493 (2013) 181-186.

[2] I. Ahmad, S.F. Rouf, L. Sun, A. Cimdins, S. Shafeeq, S. Le Guyon, M. Schottkowski, M. Rhen, U. Romling. BcsZ inhibits biofilm phenotypes and promotes virulence by blocking cellulose production in Salmonella enterica serovar Typhimurium. Microb Cell Fact 15 (2016) 177.

[3] U. Romling, M.Y. Galperin. Bacterial cellulose biosynthesis: diversity of operons, subunits, products, and functions. Trends Microbiol (2015).

[4] X. Fang, I. Ahmad, A. Blanka, M. Schottkowski, A. Cimdins, M.Y. Galperin, U. Romling, M. Gomelsky. GIL, a new c-di-GMP-binding protein domain involved in regulation of cellulose synthesis in enterobacteria. Mol Microbiol 93 (2014) 439-452.

[5] W. Thongsomboon, D.O. Serra, A. Possling, C. Hadjineophytou, R. Hengge, L. Cegelski. Phosphoethanolamine cellulose: A naturally produced chemically modified cellulose. Science 359 (2018) 334-338.

[6] U. Römling, M. Rohde, A. Olsen, S. Normark, J. Reinkoster. AgfD, the checkpoint of multicellular and aggregative behaviour in *Salmonella typhimurium* regulates at least two independent pathways. Mol. Microbiol. 36 (2000) 10-23.

[7] A. Anandan, G.L. Evans, K. Condic-Jurkic, M.L. O'Mara, C.M. John, N.J. Phillips, G.A. Jarvis, S.S. Wills, K.A. Stubbs, I. Moraes, C.M. Kahler, A. Vrielink. Structure of a lipid A phosphoethanolamine transferase suggests how conformational changes govern substrate binding. Proc. Natl. Acad. Sci. USA 114 (2017) 2218-2223.

[8] U. Omasits, C.H. Ahrens, S. Muller, B. Wollscheid. Protter: interactive protein feature visualization and integration with experimental proteomic data. Bioinformatics 30 (2014) 884-886.

[9] L. Zimmermann, A. Stephens, S.Z. Nam, D. Rau, J. Kübler, M. Lozajic, F. Gabler, J. Söding, A.N. Lupas, V. Alva. A completely reimplemented MPI Bioinformatics toolkit with a new HHpred server at its core. J. Mol. Biol. 430 (2018) 2237-2243.

[10] W.G. Touw, C. Baakman, J. Black, T.A. te Beek, E. Krieger, R.P. Joosten, G. Vriend. A series of PDB-related databanks for everyday needs. Nucleic Acids Res. 43 (2015) D364-D368.

[11] L. Holm, L.M. Laakso. Dali server update. Nucleic Acids Res. 44 (2016) W351-W355.

[12] T. Madej, C.J. Lanczycki, D. Zhang, P.A. Thiessen, R.C. Geer, A. Marchler-Bauer, S.H. Bryant. MMDB and VAST+: tracking structural similarities between macromolecular complexes. Nucleic Acids Res. 42 (2014) D297-D303.

[13] S. Kumar, G. Stecher, K. Tamura. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33 (2016) 1870-1874.

[14] D.T. Jones, W.R. Taylor, J.M. Thornton. The rapid generation of mutation data matrices from protein sequences. Comput. Appl. Biosci. 8 (1992) 275-282.

[15] R.C. Edgar. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32 (2004) 1792-1797.

[16] U. Römling, Z. Bian, M. Hammar, W.D. Sierralta, S. Normark. Curli fibers are highly conserved between *Salmonella typhimurium* and *Escherichia coli* with respect to operon structure and regulation. J. Bacteriol. 180 (1998) 722-731.

[17] U. Römling, W.D. Sierralta, K. Eriksson, S. Normark. Multicellular and aggregative behaviour of *Salmonella typhimurium* strains is controlled by mutations in the *agfD* promoter. Mol. Microbiol. 28 (1998) 249-264.

[18] X. Zogaj, M. Nimtz, M. Rohde, W. Bokranz, U. Römling. The multicellular morphotypes of *Salmonella typhimurium* and *Escherichia coli* produce cellulose as the second component of the extracellular matrix. Mol. Microbiol. 39 (2001) 1452-1463.

[19] I. Ahmad, S.F. Rouf, L. Sun, A. Cimdins, S. Shafeeq, S. Le Guyon, M. Schottkowski, M. Rhen, U. Römling. BcsZ inhibits biofilm phenotypes and promotes virulence by blocking cellulose production in *Salmonella enterica* serovar Typhimurium. Microb. Cell Fact. 15 (2016) 177.

[20] L.M. Guzman, D. Belin, M.J. Carson, J. Beckwith. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. J. Bacteriol. 177 (1995) 4121-4130.

[21] I. Ahmad, A. Cimdins, T. Beske, U. Römling. Detailed analysis of c-di-GMP mediated regulation of *csgD* expression in *Salmonella typhimurium*. BMC Microbiol. 17 (2017) 27.

[22] B. Staskawicz, D. Dahlbeck, N. Keen, C. Napoli. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 169 (1987) 5789-5794.

[23] K.A. Datsenko, B.L. Wanner. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA 97 (2000) 6640-6645.

[24] S. Uzzau, N. Figueroa-Bossi, S. Rubino, L. Bossi. Epitope tagging of chromosomal genes in *Salmonella*. Proc. Natl. Acad. Sci. USA 98 (2001) 15264-15269.

[25] R. Simm, M. Morr, A. Kader, M. Nimtz, U. Römling. GGDEF and EAL domains inversely regulate cyclic di-GMP levels and transition from sessility to motility. Mol. Microbiol. 53 (2004) 1123-1134.

[26] Z. Zhou, A.A. Ribeiro, S. Lin, R.J. Cotter, S.I. Miller, C.R. Raetz. Lipid A modifications in polymyxin-resistant *Salmonella typhimurium*: PmrA-dependent 4-amino-4-deoxy-L-arabinose, and phosphoethanolamine incorporation. J. Biol. Chem. 276 (2001) 43111-43121.

[27] H. Lee, F.F. Hsu, J. Turk, E.A. Groisman. The PmrA-regulated *pmrC* gene mediates phosphoethanolamine modification of lipid A and polymyxin resistance in *Salmonella enterica*. J. Bacteriol. 186 (2004) 4124-4133.

[28] C.M. Reynolds, S.R. Kalb, R.J. Cotter, C.R. Raetz. A phosphoethanolamine transferase specific for the outer 3-deoxy-D-manno-octulosonic acid residue of *Escherichia coli* lipopolysaccharide. Identification of the *eptB* gene and Ca^{2+} hypersensitivity of an *eptB* deletion mutant. J. Biol. Chem. 280 (2005) 21202-21211.

[29] R. Tamayo, B. Choudhury, A. Septer, M. Merighi, R. Carlson, J.S. Gunn. Identification of *cptA*, a PmrA-regulated locus required for phosphoethanolamine modification of the *Salmonella enterica* serovar *typhimurium* lipopolysaccharide core. J. Bacteriol. 187 (2005) 3391-3399.

[30] S. Bontemps-Gallo, V. Cogez, C. Robbe-Masselot, K. Quintard, J. Dondeyne, E. Madec, J.M. Lacroix. Biosynthesis of osmoregulated periplasmic glucans in *Escherichia coli*: the phosphoethanolamine transferase is encoded by *opgE*. Biomed. Res. Int. 2013 (2013) 371429.

[31] M. Doumith, G. Godbole, P. Ashton, L. Larkin, T. Dallman, M. Day, B. Muller-Pebody, M.J. Ellington, E. de Pinna, A.P. Johnson, K.L. Hopkins, N. Woodford. Detection of the plasmid-mediated *mcr-1* gene conferring colistin resistance in

human and food isolates of *Salmonella enterica* and *Escherichia coli* in England and Wales. J. Antimicrob. Chemother. 71 (2016) 2300-2305.
[32] X. Lu, Y. Hu, M. Luo, H. Zhou, X. Wang, Y. Du, Z. Li, J. Xu, B. Zhu, X. Xu, B. Kan. MCR-1.6, a new MCR variant carried by an IncP plasmid in a colistin-resistant *Salmonella enterica* serovar Typhimurium isolate from a healthy individual. Antimicrob. Agents Chemother. 61 (2017) e02632-02616.
[33] A.A. Bhagwat, P. Kannan, Y.N. Leow, M. Dharne, A. Smith. Role of anionic charges of osmoregulated periplasmic glucans of *Salmonella enterica* serovar Typhimurium SL1344 in mice virulence. Arch. Microbiol. 194 (2012) 541-548.
[34] H. Dong, Z. Zhang, X. Tang, S. Huang, H. Li, B. Peng, C. Dong. Structural insights into cardiolipin transfer from the Inner membrane to the outer membrane by PbgA in Gram-negative bacteria. Sci. Rep. 6 (2016) 30815.
[35] T. Nugent, D.T. Jones. Detecting pore-lining regions in transmembrane protein sequences. BMC Bioinform. 13 (2012) 169.