

## SUPPLEMENTARY MATERIAL

**Table 1.**

Plasmids and strains used in this study.

<b>Plasmid</b>	<b>Relevant genotype, features, markers</b>	<b>Reference / construction</b>
pQE30-YtvA	pQE30 <i>Pspac::ytvA</i> , Amp	This study
pDG148- <i>Stu</i>	pDG148- <i>Stu</i> , Km	Bacillus Genetic Stock Center
pYtvA	pDG148- <i>Stu Pspac::ytvA</i> , Km	Avila-Perez <i>et al</i> , 2006
pYtvA-Y52F	pDG148- <i>Stu Pspac::ytvA-Y52F</i> , Km	This study
pYtvA-E56Q	pDG148- <i>Stu Pspac::ytvA-E56Q</i> , Km	This study
pYtvA-C62S	pDG148- <i>Stu Pspac::ytvA-C62S</i> , Km	This study
pYtvA-E105L	pDG148- <i>Stu Pspac::ytvA-E105L</i> , Km	This study
pYtvA-D109L	pDG148- <i>Stu Pspac::ytvA-D109L</i> , Km	This study
pYtvA-Q123A	pDG148- <i>Stu Pspac::ytvA-Q123A</i> , Km	This study
pYtvA-Q123N	pDG148- <i>Stu Pspac::ytvA-Q123N</i> , Km	This study
pYtvA-T167A	pDG148- <i>Stu Pspac::ytvA-T167A</i> , Km	This study
pYtvA-T167D	pDG148- <i>Stu Pspac::ytvA-T167D</i> , Km	This study
pYtvA-E168A	pDG148- <i>Stu Pspac::ytvA-E168A</i> , Km	This study
pYtvA-D193N	pDG148- <i>Stu Pspac::ytvA-D193N</i> , Km	This study
pYtvA-S195A	pDG148- <i>Stu Pspac::ytvA-S195A</i> , Km	This study
pYtvA-S195D	pDG148- <i>Stu Pspac::ytvA-S195D</i> , Km	This study
pYtvA-E202A	pDG148- <i>Stu Pspac::ytvA-E202A</i> , Km	This study
pYtvA-T204A	pDG148- <i>Stu Pspac::ytvA-T204A</i> , Km	This study
<b><i>B. subtilis</i> strains</b>		
PB565	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> host strain	Akbar <i>et al</i> 2001
PB565 pDG148- <i>stu</i>	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pDG148- <i>Stu</i> , Km	This study
PB565 pYtvA	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA, Km	This study
PB565 pYtvA-Y52F	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-Y52F, Km	This study
PB565 pYtvA-E56Q	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-E56Q, Km	This study
PB565 pYtvA-C62S	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-C62S, Km	This study
PB565 pYtvA-E105L	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-E105L, Km	This study
PB565 pYtvA-D109L	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-D109L, Km	This study
PB565 pYtvA-Q123A	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-Q123A, Km	This study
PB565 pYtvA-Q123N	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-Q123N, Km	This study
PB565 pYtvA-T167A	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-T167A, Km	This study
PB565 pYtvA-T167D	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-T167D, Km	This study
PB565 pYtvA-E168A	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-E168A, Km	This study
PB565 pYtvA-D193N	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-D193N, Km	This study
PB565 pYtvA-S195A	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-S195A, Km	This study
PB565 pYtvA-S195D	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-S195D, Km	This study
PB565 pYtvA-E202A	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-E202A, Km	This study
PB565 pYtvA-T204A	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-T204A, Km	This study
PB565 pYtvA-T204D	<i>ytvAΔI::ery amyE::Pctc-lacZ Cm trpC2</i> , pYtvA-T204D, Km	This study

**Primers:**

**Y52F**

FW: 5' – CTT TGT TCA AAT GAC CGG CTT CGA GAC CGA GGA AAT TTT AG – 3'

RV: 5' – CTA AAA TTT CCT CGG TCT CGA AGC CGG TCA TTT GAA CAA AG – 3'

**E56Q**

FW: 5' – CAA ATG ACC GGC TAC GAG ACC GAG CAA ATT TTA GGA AAG AAC TGT CGC – 3'

RV: 5' – GAG ACA GTT CTT TCC TAA AAT TTG CTC GGT CTC GTA GCC GGT CAT TTG – 3'

**C62S**

FW: 5' – GAG GAA ATT TTA GGA AAG AAC AGT CGC TTC TTA CAG GGG AAA C – 3'

RV: 5' – GTT TCC CCT GTA AGA AGC GAC TGT TCT TTC CTA AAA TTT CCT C – 3'

**E105L**

FW: 5' – GGA ACG ATG TTC TGG AAT CTA TTA AAT ATT GAT CCA ATG GAA ATA GAG – 3'

RV: 5' – CTC TAT TTC CAT TGG ATC AAT ATT TAA TAG ATT CCA GAA CAT CGT TCC – 3'

**D109L**

FW: 5' – TGG AAT GAA TTA AAT ATT CTT CCA ATG GAA ATA GAG GAT AAA ACG – 3'

RV: 5' – CGT TTT ATC CTC TAT TTC CAT TGG AAG AAT ATT TAA TTC ATT CCA – 3'

**Q123A**

FW: 5' – CGT ATT TTG TCG GAA TTG CGA ATG ATA TCA CCA AGC – 3'

RV: 5' – GCT TGG TGA TAT CAT TCG CAA TTC CGA CAA AAT ACG – 3'

**Q123N**

FW: 5' – CGT ATT TTG TCG GAA TTA ATA ATG ATA TCA CCA AGC – 3'

RV: 5' – CGT ATT TTG TCG GAA TTA ATA ATG ATA TCA CCA AGC – 3'

**T167A**

FW: 5' – CCG CTA GTC GGA AAC CTG GCA GAG GAG CGA TTT AAT TC – 3'

RV: 5' – GAA TTA AAT CGC TCC TCT GCC AGG TTT CCG ACT AGC GG – 3'

**T167D**

FW: 5' – CTT CCG CTA GTC GGA AAC CTG GAT GAG GAG CGA TTT AAT TCC AC – 3'

RV: 5' – GTG GAA TTA AAT CGC TCC TCA TCC AGG TTT CCG ACT AGC GGA AG – 3'

**E168A**

FW: 5' – GCT AGT CGG AAA CCT GAC AGC AGA GCG ATT TAA TTC CAC TG – 3'

RV: 5' – CAG TGG AAT TAA ATC GCT CTG CTG TCA GGT TTC CGA CTA GC – 3'

**D193N**

FW: 5' – GAT GAT TAT TTG ATC ATT AAT TTA TCC GGA TTG GCC C – 3'

RV: 5' – GGG CCA ATC CGG ATA AAT TAA TGA TCA AAT AAT CAT C – 3'

**S195A**

FW: 5' – GAT TAT TTG ATC ATT GAT TTA GCC GGA TTG GCC CAA GTG AAC G – 3'

RV: 5' – CGT TCA CTT GGG CCA ATC CGG CTA AAT CAA TGA TCA AAT AAT C – 3'

**S195D**

FW: 5' – GAT CAT TGA TTT AGA CGG ATT GGC CCA AG – 3'

RV: 5' – CTT GGGCCA ATC CGT CTA AAT CAA TGA TC – 3'

**E202A**

FW: 5' – GCC CAA GTG AAC GCA CAA ACG GCC GAC C – 3'

RV: 5' – GGT CGG CCG TTT GTG CGT TCA CTT GGG C – 3'

**T204A**

FW: 5' – GCC CAA GTG AAC GAA CAA GCG GCC GAC CAA ATT TTC – 3'

RV: 5' – GAA AAT TTG GTC GGC CGC TTG TTC GTT CAC TTG GGC – 3'

**T204D**

FW: 5' – GGC CCA AGT GAA CGA ACA AGA CGC CGA CCA AAT TTT CAA GC – 3'

RV: 5' – GCT TGA AAA TTT GGT CGG CGT CTT GTT CGT TCA CTT GGG CC – 3'

**pQE30YtvA**

FW: 5' – GGG GTC GAC TTA CAT AAT CGG AAG CAC TTT AAC G – 3'

RV: 5' – CCC GGA TCC ATG GCT AGT TTT CAA TCA TTT GGG – 3'

**Figure 1.** Alignment of YtvA<sub>148-261</sub> and *MtRsbS*<sub>6-123</sub> (PBD 2VY9) chain A.

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2VY9A      - PILKVDD-YWVVAI EETLHDQSVI QFKEELLHNI TGVAGKGLV IDISALEVVDSFVTRV 63
YtvA       TPIVP IRNGI SALPL VGNLT EERFNS I VCTLTN I LSTSKDDYLI IDLSGLAQVNEQTADQ 206
           ** : : : . : : . * : : . : * : : : . . * : * : * . * * : . :

2VY9A      L I E I S R L A E L L G L P F V L T G I K P A V A I T L T E X G L D L R G X A T A L N L Q K G L D K L K N L   A R X E Q R   123
YtvA       I F K L S H L L K L T G T E L I I T G I K P E L A M K M N K L D A N F S S L K T Y S N V K D A V K V L P I M ----- 261
           : : : * : * : * * : : * * * * : * : : : . : : . * * : : : . * : :
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**Figure 2.** Alignment of YtvA, RsbS and RsbR paralogs. Underline residues are conserved Threonines. Bold residues negative charges residues.

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YkoB      EPGEKQKWADLIVKVFDFTIYTFVDHAEMNAKQQLNAQREMILELSSPVITLSKSTALLP 179
Yo jH     KSEEAEDLYHALIKAFDLVIHIFIEEMYKNTSLQLQAQKDMITELSAPVIVLFHVSGLLP 179
YqhA      SIQEFYEWRSRILNQNIIDEIIEVFTEEYHQVTMIQLNAQKEMINELSAPIMPITDGIGILP 174
RsbR      PDQESTELIWQIDRFFSPINTEIFNQYSISWEKTVSLQKIALQELSAPLIPVFENITVMP 164
RsbS      -----MRHPKIPILKLYN-CLLVS 18
YtvA      EIEDKTYFVGIQNDITK-----QKEYEKLEDSLTEITALSTPIVPIRNGISALP 161
           : . * : : . : .

YkoB      LVGDIDTERAKFILENTLQACAKRRVEHLLIDLSGVVVDTMVAHQIFKLI EALNLIGVR 239
Yo jH     LIGDIDTVRAKLIMENTLHQCAKKKVTQLYIDLSGVAVIDTMVAHQFLSLIEALRLIGVS 239
YqhA      LVGEIDTHRARTILES VLEQCSALKLSYLFLDISGVP IVDTMVAYQIFKVIDSTKLLGIE 234
RsbR      LVGTIDTERAKRIMENLLNGVVKHRSQVVLIDITGVPVVDTMVAHHIIQASEAVRLVGAK 224
RsbS      IQWELDDQTALTFQEDLLNKIYETGANGVVIDLTSVDMIDSFIAKVLGDVITMSKLMGAK 78
YtvA      LVGNLTEERFNSIVCTLTN I LSTSKDDYLI IDLSGLAQVNEEQTADQIFKLSHLLKLTGTE 221
           : : : : : : : * : . . * *

YkoB      STLSGIRPEIAQTAVQLGIDFSNITIKTNLAQALNYHQ----- 277
Yo jH     STLSGIRPEIAQTAVQLGLSFEGISLRSTLASAIASDLKLV----- 282
YqhA      TIISGIRPEIAQTVVKLGLDFSNVKTEQSLAKALANKGFKIKEC----- 278
RsbR      CLLAGIRPEIAQTIVNLGIDLSQVITKNTLQKGIQTALEMTDRKIVSLGE 274
RsbS      VVLTGIQPAVAVTLIELGIALEEIETALDLEQGLET LKRELGE----- 121
YtvA      LIITGIKPELAMKMNKLDANFSSLKTYSNVKDAVKVLPIM----- 261
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