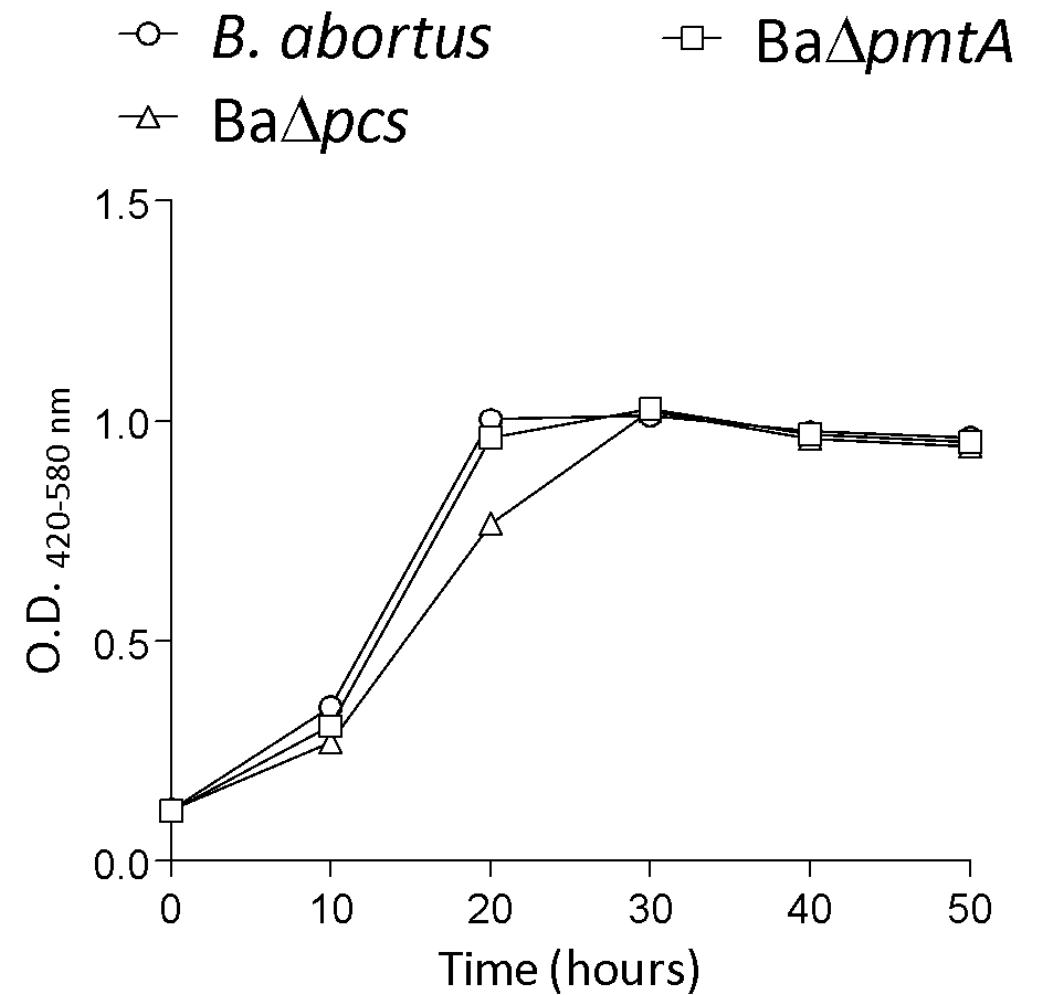
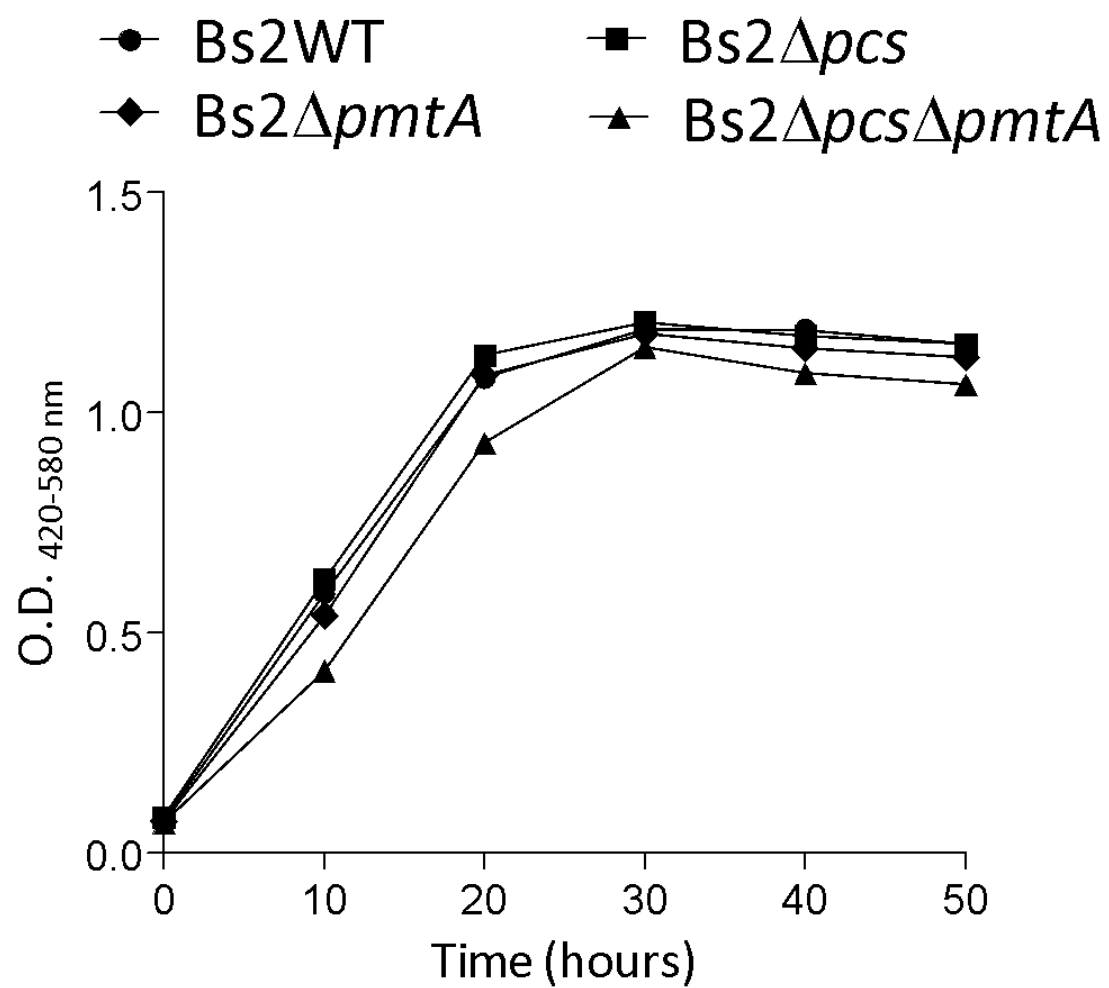


**Supplementary Figure S1. HPTLC analysis of the free lipids of *Bs2ΔpcsΔpmtA\_pcs* and *Bs2ΔpcsΔpmtA\_pmtA* grown in TSB.** OL, ornithine lipids; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; MMPE, monomethyl-phosphatidylethanolamine; DMPE, dimethyl-phosphatidylethanolamine; PC, phosphatidylcholine.



**Supplementary Figure S2.** Left panel: growth curves of Bs2WT and indicated mutants grown in TSB. Right panel: growth curves of *B. abortus* and mutant grown in TSB (taken from [Conde-Álvarez et al., 2006]).

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B. melitensis --MAGQLGRKLAAKFDEEIRFFKGWIDGPKAVGAILPTSSITARRMASVIDVNSGLPVLVLE
B. abortus --MAGQLGRKLAAKFDEEIRFFKGWIDGPKAVGAILPTSSITARRMASVIDVNSGLPVLVLE
B. suis Thomsen --MAGQLGRKLAAKFDEEIRFFKGWIDGPKAVGAILPTSSITARRMASVIDVNSGLPVLVLE
Bs2WT --MAGQLGRKLAAKFDEEIRFFKGWIDGPKAVGAILPTSSITARRMASVIDVNSGLPVLVLE
S. meliloti MSLRLRVKEKFGKRFDEEIRFFKGWMSNTRAVGAILPTSAITARRMASVVDPESEGLPVLVLE
      :  :: .*:. *****:.. :*****:*****:* :*****

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B. melitensis FGPGTGVITKAILKHGPKADLYSIEYSHDFVEHLNKTFFPDVNIIEGDVFDLDTALGDRK
B. abortus FGPD■TGVITKAILKHGPKADLYSIEYSHDFVEHLNKTFFPDVNIIEGDVFDLDTALGDRK
B. suis Thomsen LGPGTGVITKAILKHGPKADLYSIEYSHDFVEHLNKTFFPDVNIIEGDVFDLDTALGDRK
Bs2WT LGPGTGVITKAILKHGPKADLYSIEYSHDFVEHLNKTFFPDVNIIEGDVFDLDTALGDRK
S. meliloti LGPGTGVITKAILERGIPEKLVSI EYSTDFYKQLKAHFDGVHFINGDAFDLSRTLGAFK
      :**.******:~::~* . * ***** * * ::~: * .*~::~**.*** . ** *

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B. melitensis GQKFDCIISAVPMLNFPMDRRVELVESLLTHIPHGRPLMQITYGPLPPVPAGRGNVYVQH
B. abortus GQKFDCIISAVPMLNFPMDRRVELVESLLTHIPHGRPLMQITYGPLPPVPAGRGNVYVQH
B. suis Thomsen GQKFDCIISAVPMLNFPMDRRVELVESLLTHIPHGRPLMQITYGPLPPVPAGRGNVYVQH
Bs2WT GQKFDCIISAVPMLNFPMDRRVELVESLLTHIPHGRPLMQITYGPLPPVPAGRGNVYVQH
S. meliloti DQQFDSVISA VPLLNFPMHRRVELIEDLLSRI PFGRPVVQISYGPMSPVVAMPDRYRIQH
      .*:**.*****:*****.*****:~::~* * ***** * * ::~: * .*~::~**.*** . ** *

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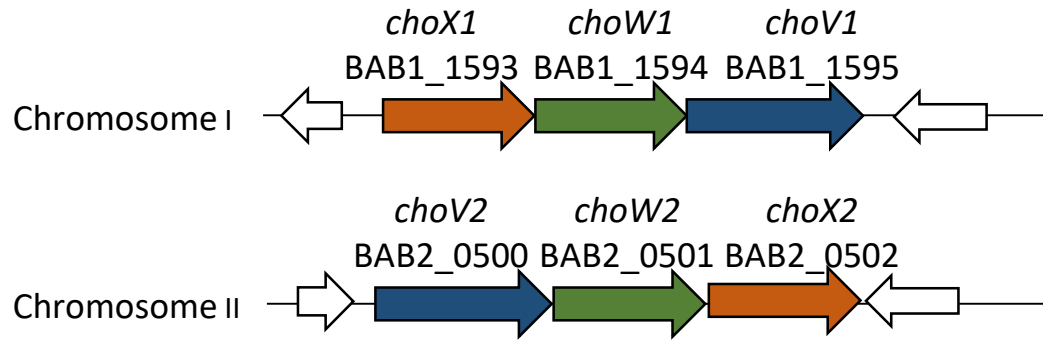
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B. melitensis YDFVVRNVPPAQLWVYRSPLV
B. abortus YDFVVRNVPPAQLWVYRSPLV
B. suis Thomsen YDFVVRNVPPAQLWVYRSPLV
Bs2WT YDFVVRNVPPAQLWVYRSPLV
S. meliloti FDFVVRNIPPAQLWIYRKTH-
      :*****:*****:***.

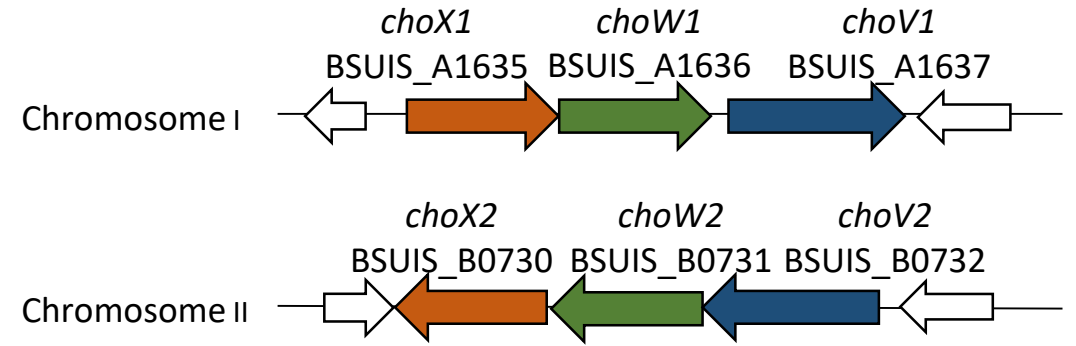
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**Supplementary Figure S3.** Sequence alignment of the PmtA enzymes encoded in *B. melitensis* 16M BMEI2000, *B. abortus* 2308 BAB1\_2131, *B. suis* bv2 Thomsen BSUIS\_A1967, Bs2WT (ORF not available) and *S. meliloti* 1021 SMc00414. The consensus motifs of SAM-utilizing methyltransferases (VL[E/D]XGXXG) are boxed and the changes in **them** are signaled in grey.

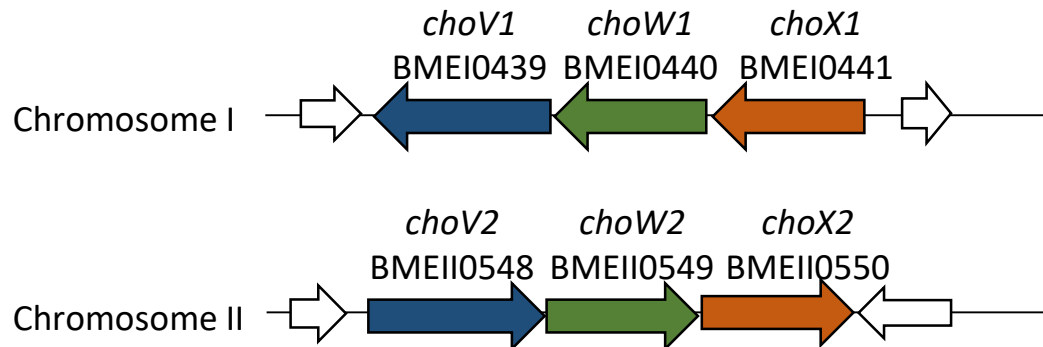
***B. abortus* 2308**



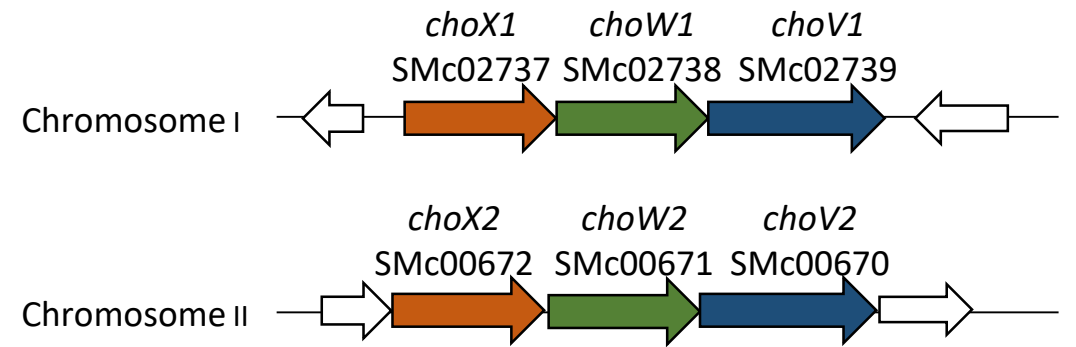
***B. suis* bv2 Thomsen**



***B. melitensis* 16M**



***S. meliloti***



**Supplementary Figure S4.** Schematic representation of the ChoXWV operon in *B. abortus* 2308, *B. melitensis* 16M, *B. suis* bv2 Thomsen and *S. meliloti* 1021.

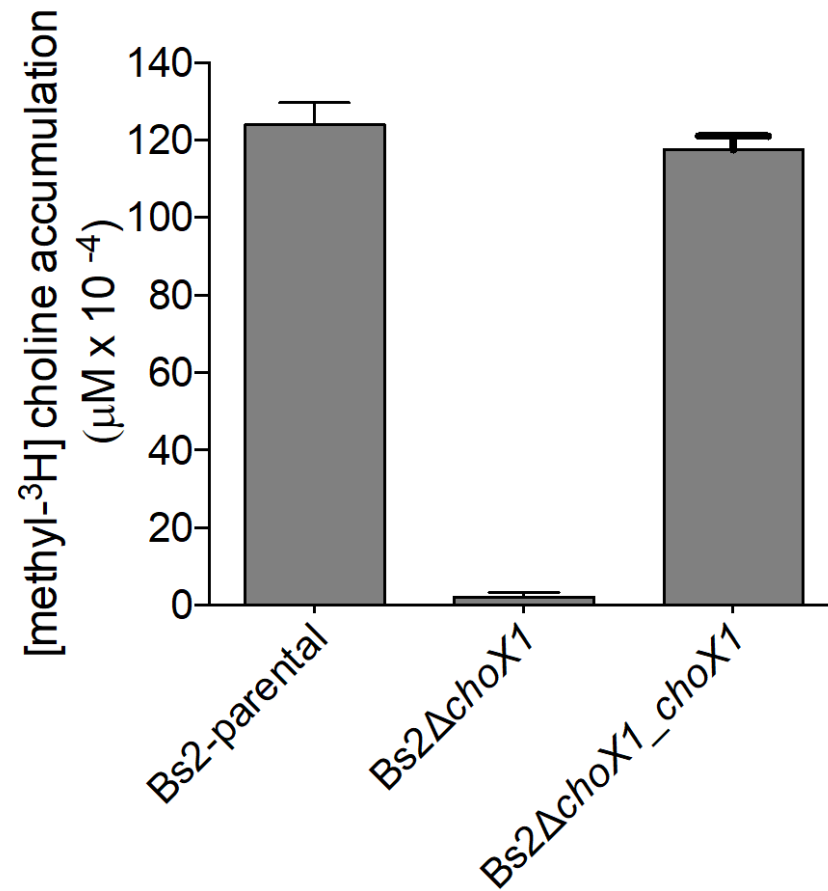
## ChoX1

<i>S. meliloti</i>	41 46 VGWTDI	88 93 GNWMPT	117 122 AKYTLA	154 159 PGNDGN	202 207 FLGWEP
<i>B. melitensis</i> 16M	45 50 VGWTDI	92 97 GYWNPS	121 126 AKYTLA	158 163 PGNDGN	206 211 FLAWEP
<i>B. abortus</i> 2308	VGWTDI	GYWNPS	AKYTLA	PGNDGN	FLAWEP
<i>B. suis</i> bv2 Thomsen	VGWTDI	GYWNPS	AKYTLA	PGNDGN	FLAWEP
Bs2WT	VGWTDI	GYWNPS	AKYTLA	PGNDGN	FLAWEP

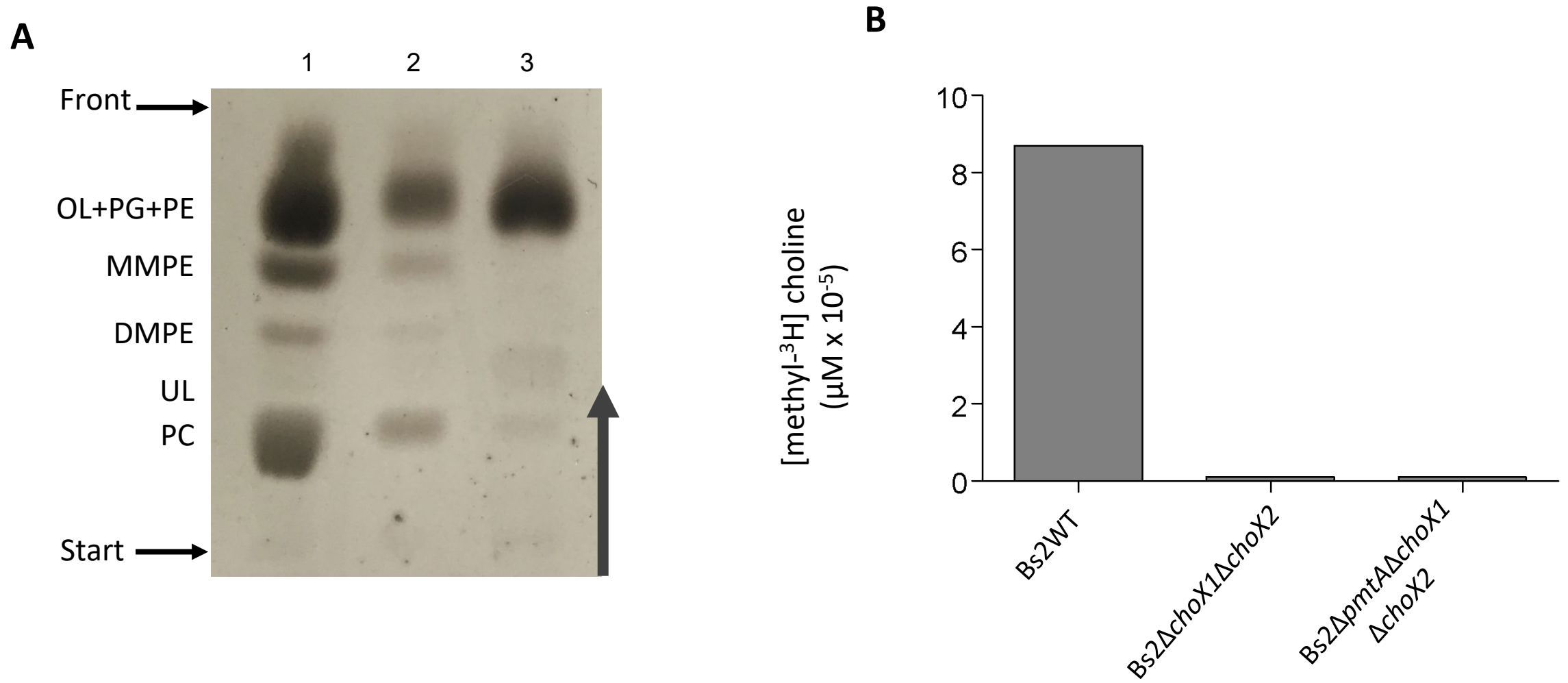
## ChoX2

<i>S. meliloti</i>	40 45 IDWESG	88 93 EEWLGR	123 128 PDYVVH	221 226 FYYWSP
<i>B. melitensis</i> 16M	36 41 APWSDA	84 89 MAWLPE	107 112 PLYEGA	196 201 ATSWSP
<i>B. abortus</i> 2308	APWSDA	MAWLPE	PLYEGA	ATSWSP
<i>B. suis</i> bv2 Thomsen	APWSDA	MAWLPE	PLYEGA	ATSWSP
Bs2WT	APWSDA	MAWLPE	PLYEGA	ATSWSP

**Supplementary Figure S5. Sequence alignment of relevant sections of ChoX1 and ChoX2 of *S. meliloti* (SMc02737 and SMc00672), *B. melitensis* 16M (BMEI0441 and BMEII0550), *B. abortus* 2308 (BAB1\_1593 and BAB2\_0502), *B. suis* bv2 Thomsen (BSUIS\_A1635 and BSUIS\_B0730) and the Bs2WT orthologues. The four aromatic amino acids (W47, W94, Y123, and W209) that form the hydrophobic pocket required for choline binding in *S. meliloti* (Oswald et al., 2008) are boxed.**



**Supplementary Figure S6. Choline accumulation in Bs2WT, Bs2ΔchoX1 and Bs2ΔchoX1\_choX1 grown in mGSM.** Values are the mean ± standard error of technical duplicates of a representative experiment, repeated at least two times with similar results.



**Supplementary Figure S7. Analysis of Bs2WT, Bs2 $\Delta$ choX1 $\Delta$ choX2 and Bs2 $\Delta$ pmtA $\Delta$ choX1 $\Delta$ choX2 after *in vivo* labelling with [methyl-<sup>3</sup>H] choline in mGSM. HPTLC revealed by charring (Panel A) 1: Bs2WT, 2: Bs2 $\Delta$ choX1 $\Delta$ choX2 and 3: Bs2 $\Delta$ pmtA $\Delta$ choX1 $\Delta$ choX2. Quantification of [methyl-<sup>3</sup>H] choline-labelled lipids in the PC positions in the HPTLC plate (Panel B). OL, ornithine lipids; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; MMPE, monomethyl-phosphatidylethanolamine; DMPE, dimethyl-phosphatidylethanolamine; PC, phosphatidylcholine; UL, unknown lipid.**