Supplementary Material

Characterization of the exopolysaccharide biosynthesis pathway in *Myxococcus xanthus*

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WbaP_Se  ----------  ----------  ---MDNIDNK  YNPQLCKIFL  AISDLIFFNL  ALWFSLGCVY  FIFDQVQRFI  PQDQLDTRVI  57
EpsZ     MDTMSGATAS  AAVVGAPGSA  NAQGQVIEEV  QGPPKLAPGS  AAKLNLTVDL  VLLSSVLVGS  AWLSGQLSAE  SGWKVSGLVL  80

WbaP_Se  THFILSVVCV  GWFWIRLRHY  TYRKPFWYEL  KEIFRTIVIF  AIFDLALIAF  TKWQFSRYVW  VFCWTFALIL  VPFFRALTKH  137
EpsZ     AAWVVWIVTG  TALCLYDSRF  AERSKLDHVA  LVSVTTLAVV  TVLTLGSVAV  PTVVTSPVVG  PLLFIFWPVT  LLLRLFVFRP  160

WbaP_Se  LLNKLGIWKK  KTIILGSGQN  ARGAYSALQS  EEMMGFDVIA  FFDTDASDAE  INMLP--VIK  DTEIIWDLNR  --TGDVHYIL  213
EpsZ     VASQE---RP  MDAVLIVGTG  AMGRYTGEDL  ANRGRRQILG  YVRFHDDNGS  VGELPGPVMG  SVDDLEHILR  NTAVDEVYIA  237

WbaP_Se  AYEYTELEKT  HFWLRELSKH  HCRSVTVVPS  FRGLPLYNTD  MSFIFSHEVM  LLRIQNNLAK  RSSRFLKRTF  DIVCSIMILI  293
EpsZ     GNTLKQGESM  QAAIKLAERF  GVPFALPAHS  FRLDRARPVE  RRAVADGFLH  FAAVS---PK  PHQMAMKRLF  DICVSAAALW  314

WbaP_Se  IASPLMIYLW  YKVTRDG-GP  AIYGHQRVGR  HGKLFPCYKF  RSMVMNSQEV  LKELLANDPI  ARAEWEKDFK  LKNDPRITAV  372
EpsZ     ALLPLLGMVA  LAVKFTSKGP  IFFKQLRVGQ  NGKPFYMLKF  RSMVVNAEEL  KEKLAALN--  --EQTGPVFK  MKHDPRITGI  390

WbaP_Se  GRFIRKTSLD  ELPQLFNVLK  GDMSLVGPRP  IVSDELERYC  DDVDYYLMAK  PGMTGLWQVS  GRNDVDYDTR  VYFDSWYVKN  452
EpsZ     GRFIRKFSID  ELPQFINVLR  GEMSIVGPRP  PVPTEVAKYE  TWQRRRLSVR  PGLTCIWQVS  GRNQISFEEW  MYLDMQYIDH  470

WbaP_Se  WTLWNDIAIL  FKTAKVVLRR  DGAY  476
EpsZ     WSLTSDLRLL  LQTVPVVLTG  RGAS  494
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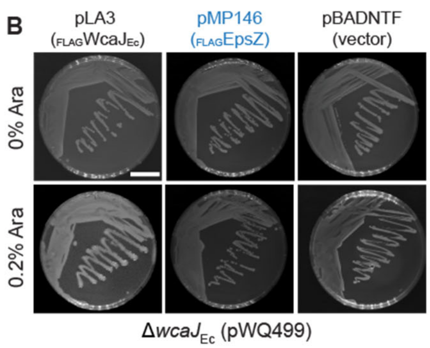

Figure S1. Characterization of MXAN_7415.
(A) Sequence alignment of EpsZ (MXAN_715) and $WbaP_{Se}$ (Accession number: NP_461027.1) showing the Pro and Asp (orange) residues in the motif $DX_{12}P$ and the conserved amino acids essential for catalytic activity (red). (B) Complementation of colanic acid synthesis in *E. coli* K-12 W3110 (Δ*wcaJ*$_{Ec}$) by plasmids encoding the indicated PHPT proteins as in Fig. 5C. Scale bar: 2 cm.

Supplementary Experimental Procedures

Plasmid construction. All oligonucleotides used are listed in Table S3. All constructed plasmids were verified by DNA sequencing.

pMP001 (for generation of in-frame deletion of *MXAN_7415*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 7415_A/7415_B and 7415_C/7415_D respectively, as described in (1). Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 7415_A/7415_D to generate the AD fragment. The AD fragment was digested with KpnI/XbaI and cloned in pBJ114.

pMP012 (for generation of in-frame deletion of *MXAN_7421*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 7421_A/7421_B and 7421_C/7421_D respectively. Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 7421_A/7421_D to generate the AD fragment. The AD fragment was digested with KpnI/XbaI and cloned in pBJ114.

pMP015 (for generation of in-frame deletion of *MXAN_7442*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 7442_A/7442_B and 7442_C/7442_D respectively. Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 7442_A/7442_D to generate the AD fragment. The AD fragment was digested with KpnI/XbaI and cloned in pBJ114.

pMP016 (for generation of in-frame deletion of *MXAN_7416*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 7416_A/7416_B and 7416_C/7416_D respectively. Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 7416_A/7416_D to generate the AD fragment. The AD fragment was digested with KpnI/XbaI and cloned in pBJ114.

pMP018 (for generation of in-frame deletion of *MXAN_7417*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 7417_A/7417_B and 7417_C/7417_D respectively. Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 7417_A/7417_D to generate the AD fragment. The AD fragment was digested with EcoRI/XbaI and cloned in pBJ114.

pMP021 (expression of P_{nat} *MXAN_7415* from the *attB* site): P_{nat} *MXAN_7415* was amplified with the primer combination 7415 Pnat900 forw2/7415 Pnat rev and genomic DNA from *M. xanthus*

DK1622 as a template. The fragment was digested with KpnI and XbaI, cloned into pSWU30 and sequenced.

pMP024 (expression of P_{nat} *MXAN_7416* from the *attB* site): P_{nat} *MXAN_7416* was amplified with the primer combination 7416_Pnat forw2/7416_Pnat rev and genomic DNA from *M. xanthus* DK1622 as a template. The fragment was digested with HindIII and XbaI, cloned into pSWU30 and sequenced.

pMP030 (expression of P_{pilA} *MXAN_7417* from the *attB* site): *MXAN_7417* was amplified with the primer combination 7417_PpilA forw/7417_PpilA rev and genomic DNA from *M. xanthus* DK1622 as a template. The fragment was digested with XbaI and HindIII, cloned into pSW105 and sequenced.

pMP032 (expression of P_{pilA} *MXAN_7421* from the *attB* site): *MXAN_7421* was amplified with the primer combination 7421_PpilA forw/7421_PpilA rev and genomic DNA from *M. xanthus* DK1622 as a template. The fragment was digested with XbaI and HindIII, cloned into pSW105 and sequenced.

pMP091 (expression of P_{nat} *MXAN_7442* from the *attB* site): P_{nat} *MXAN_7442* was amplified with the primer combination 7442_Pnat 600 up /7442_Pnat rev and genomic DNA from *M. xanthus* DK1622 as a template. The fragment was digested with HindIII and XbaI, cloned into pSWU30 and sequenced.

pMP124 (for generation of in-frame deletion of *MXAN_1043*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 1043_A/1043_B and 1043_C/1043_D respectively. Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 1043_A/1043_D to generate the AD fragment. The AD fragment was digested with EcoRI/HindIII and cloned in pBJ114.

pMP146 (expression of *MXAN_7415* under the control of an arabinose promoter): *MXAN_7415* was amplified with the primer combination 7415 fw +1/ 7415 rev new (pilA) and genomic DNA from *M. xanthus* DK1622 as a template. The fragment was digested with XbaI and HindIII, cloned into pBADNTF and sequenced.

pJJ1 (for generation of in-frame deletion of *MXAN_1035*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 1035_A/1035_B and 1035_C/1035_D respectively. Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 1035_A/1035_D to generate the AD fragment. The AD fragment was digested with KpnI/XbaI and cloned in pBJ114.

pJJ2 (for generation of in-frame deletion of *MXAN_1025*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 1025_A/1025_B and 1025_C/1025_D respectively. Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 1025_A/1025_D to generate the AD fragment. The AD fragment was digested with KpnI/XbaI and cloned in pBJ114.

pJJ3 (for generation of in-frame deletion of *MXAN_1052*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 1025_A/1025_B and 1052_C/1052_D respectively. Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 1052_A/1052_D to generate the AD fragment. The AD fragment was digested with KpnI/BamHI and cloned in pBJ114.

pJJ4 (for generation of in-frame deletion of *MXAN_1915*): up- and downstream fragments were amplified from genomic DNA of DK1622 using the primer pairs 1915_A/1915_B and 1915_C/1915_D respectively. Subsequently, the AB and CD fragments were used as templates to perform an overlapping PCR with the primer pair 1915_A/1915_D to generate the AD fragment. The AD fragment was digested with KpnI/XbaI and cloned in pBJ114.

pMAT150 (for generation of in-frame deletion of *pilT*): up- and downstream fragments were amplified from genomic DNA of DK10409 (Δ*pilT*) using the primer pairs pilT-A EcoRI/ pilT-D HindIII. The AD fragment was digested with EcoRI/HindIII and cloned in pBJ114.

Table S1. Analysis of the *eps* locus

Locus tag MXAN	Gene name	(Putative) function of encoded protein	Reference[1]
7415	*epsZ*	Polyprenyl glycosylphosphotransferase **New annotation: polyisoprenyl-phosphate hexose-1-phosphate transferase**	(2)
7416	***wzx***$_{\mathbf{EPS}}$	Wzx flippase	(3)
7417	*epsY*	Polysaccharide biosynthesis/export protein **New annotation: OPX protein**	Uniprot, KEGG
7418	*epsX*	Hypothetical protein	
7420	*epsW*	Response regulator	(4)
7421	*epsV*	Chain length determinant protein **New annotation: Wzz protein**	Uniprot, KEGG
7422	*epsU*	Glycosyltransferase	
7423		Hypothetical protein	
7424		Hypothetical protein	
7425		Hypothetical protein	
7426		Hypothetical protein	
7430		Transposase orfB, IS5 family	Uniprot, KEGG
7431	*epsP*	Transposase orfA, IS5 family	
7433	*epsO*	von Willebrand factor type A domain protein, Ca-activated chloride channel homolog	
7435	*epsN*	Hydrolase	
7436	*epsM*	Outer membrane efflux protein, cobalt-zinc-cadmium efflux system	
7437	*epsL*	Heavy metal efflux pump, CzcA family, cobalt-zinc-cadmium resistance protein	Uniprot, KEGG
7438	*epsK*	putative cobalt-zinc-cadmium resistance protein	Uniprot, KEGG
7439	*epsJ*	Sensor histidine kinase	
7440	*epsI* *nla24*	Sigma-54 dependent DNA-binding response regulator	(5)
7441	*epsH*	Glycosyltransferase	
7442	*sgnF*, ***wzy***$_{\mathbf{EPS}}$	Putative membrane protein, Wzy_C domain **New annotation: Wzy polymerase**	(6)
7443	*epsG*	Magnesium transporter	
7444	*epsF*	Response regulator/sensory box histidine kinase	
7445	*epsE*	Glycosyltransferase	
7447		Hypothetical protein	Uniprot, KEGG
7448	*epsD*	Glycosyltransferase	
7449	*epsC*	Serine O-acetyltransferase	
7450	*epsB*	Glycosyl hydrolase	Uniprot, KEGG
7451	*epsA*	Glycosyltransferase	Uniprot, KEGG

[1]Based on (7) unless indicated otherwise.

Table S2. Analysis of the *MXAN_1025-MXAN_1052* and *MXAN_1915* loci

Locus tag MXAN	Gene name	(Putative) function of encoded protein	Reference[1]
1025		Bacterial tyrosine kinase, Capsular exopolysaccharide family protein **New annotation: Wzc**	(8)
1026		Glycosyltransferase	
1027		Glycosyltransferase	
1028		Putative membrane protein	
1029		Glycosyltransferase	
1030		Glycosyltransferase	
1031		Glycosyltransferase	
1032		Glycosyltransferase	
1033		Glyco_trans_4-like_N domain-containing protein	
1034		Conserved domain protein	
1035		Putative membrane protein, PST family **New annotation: Wzx flippase**	
1036		Glycosyltransferase	
1037		Glycosyltransferase	
1038		Hypothetical protein	
1039	*glkA*	Glucokinase	
1040		Sulfatase family protein	
1041		Acyltransferase family protein	
1042		Glycosyltransferase	
1043		Glycosyltransferase **New annotation: polyisoprenyl-phosphate *N*-acetylhexosamine-1-phosphate transferase**	
1045		Hypothetical protein	
1046		FG-GAP repeat/HVR domain protein	
1047		Hypothetical protein	
1048		UDP-glucose 6-dehydrogenase	
1049		Acyltransferase family protein	
1050		Hypothetical protein	
1051		Hypothetical protein	
1052		O-antigen polymerase family protein **New annotation: Wzy polymerase**	
1914	*suhB*	Inositol-1-monophosphatase	
1915		Polysaccharide biosynthesis/export protein **New annotation: OPX protein**	
1916		Hypothetical protein	
1917		Hypothetical protein	

[1]Based on Uniprot and KEGG, unless indicated otherwise.

Table S3. Oligonucleotides used in this work[1]

Primer name	Sequence 5'-3'	Brief descrption
7415_A	ATCGGGTACCGTGGTGCTCGCCGTCAGTGG	For Δ*MXAN_7415*
7415_B	CACCGGCACCGGGGCCAGCTTGGGCGG	For Δ*MXAN_7415*
7415_C	CTGGCCCCGGTGCCGGTGGTGCTCACG	For Δ*MXAN_7415*
7415_D	ATCGTCTAGACCCCCGCCCCACACCAGCTT	For Δ*MXAN_7415*
7415_E	ACCTCCTGGCCGCCCATGAG	For Δ*MXAN_7415*
7415_F	CTTCACCGCCTCGGACGCCA	For Δ*MXAN_7415*
7415_G	CATCTTCTGGCCGGTGACGC	For Δ*MXAN_7415*
7415_H	GCATGTAGAAGGGCTTGCCG	For Δ*MXAN_7415*
7415 Pnat900 forw2	ATCGGGTACCTGAGCCTTCCTCGACGTGGAGCG	For complementation fw
7415 Pnat rev	ATCGTCTAGACTAGCTGGCGCCGCGGCCCG	For complementation rev
7415 fw +1	ATCGTCTAGAGGTGGACACGATGAGCGGCGC	For protein expression under an arabinose inducible promoter fw.
7415 rev new	ATCGAAGCTTCTAGCTGGCGCCGCGGCCCG	For protein expression under an arabinose inducible promoter rev.
7416_A	ATCGGGTACCGCAGCTTCGCGTGGGGCAGA	For Δ*MXAN_7416*
7416_B	TTCGGGCGCTTGGAGCCCGTTGCGCAC	For Δ*MXAN_7416*
7416_C	GGGCTCCAAGCGCCCGAAGCCGCGCCC	For Δ*MXAN_7416*
7416_D	ATCGTCTAGAGCGCCCCTCGGCGTGGATGA	For Δ*MXAN_7416*
7416_E	TGAAGTTCACCTCCAAGGGC	For Δ*MXAN_7416*
7416_F	TCCACCACCACCACGTCACC	For Δ*MXAN_7416*
7416_G	CGAGGTGCGCCAGCTCGTCT	For Δ*MXAN_7416*
7416_H	CCCATCAGCCCCACCCACAG	For Δ*MXAN_7416*
7416_Pnat forw2	ATCGAAGCTTTGACAAGCCTCCAGGCAACCCAA	For complementation fw
7416_Pnat rev	ATCGTCTAGATCACGGGGTGGGCGCGGCTT	For complementation rev
7417_A	ATCGGAATTCGATGATGCTCATCGTCCTGG	For Δ*MXAN_7417*
7417_B	GTCACCGGGGCGGTGCGTGGACGGCAT	For Δ*MXAN_7417*
7417_C	ACGCACCGCCCCGGTGACGTGGTGGTG	For Δ*MXAN_7417*
7417_D	ATCGTCTAGAGCCGCTGATGGAGAAGCCGC	For Δ*MXAN_7417*
7417_E	GCGCCGCTCGCTGGAGGGCA	For Δ*MXAN_7417*
7417_F	CGCGGGCGGGCCCGTCCAGG	For Δ*MXAN_7417*
7417_G	CGTCCCTGGCGCTCGTTCGC	For Δ*MXAN_7417*
7417_H	CGCAGGCGGAAGGTGGGCGC	For Δ*MXAN_7417*
7417_PpilA forw	ATCGTCTAGAGTGAGGAGAGTTCCACCGCT	For complementation fw
7417_PpilA rev	ATCGAAGCTTTTATTCCACCACCACCACGT	For complementation rev
7421_A	ATCGGGTACCCCCTGCCAGCCAAGGCGGCG	For Δ*MXAN_7421*

7421_B	CGCCAGCACGGGAGCCCCGGGCGCGGG	For Δ*MXAN_7421*
7421_C	GGGGCTCCCGTGCTGGCGGAGCTGGAG	For Δ*MXAN_7421*
7421_D	ATCGTCTAGAGCCAGGACGCCGTGGGGTTC	For Δ*MXAN_7421*
7421_E	CGTGCGGCAAACTGGTATTC	For Δ*MXAN_7421*
7421_F	AGGGCAATGGTCATCAGCCG	For Δ*MXAN_7421*
7421_G	GAGCGCCCGGAGACGAACGC	For Δ*MXAN_7421*
7421_H	CGCGAAGATGCCCATGCCGA	For Δ*MXAN_7421*
7421_PpilA forw	ATCGTCTAGAGTGACGGTCCCCGCGCCCGG	For complementation fw
7421_PpilA rev	ATCGAAGCTTTCAGCGCCGCTCCAGCTCCG	For complementation rev
7442_A	ATCGGGTACCGGGCAGACCGCCATTGAGCG	For Δ*MXAN_7421*
7442_B	GGAGGATGGGGCCAACGCGACCACGGG	For Δ*MXAN_7421*
7442_C	GCGTTGGCCCCATCCTCCGCCGCGAAC	For Δ*MXAN_7421*
7442_D	ATCGTCTAGACCAGAAGGTGGGTGGCACGG	For Δ*MXAN_7421*
7442_E	CCACTCCTTCTCCCGCCGCC	For Δ*MXAN_7421*
7442_F	ATCGTCAGCGTGGTGCAGGC	For Δ*MXAN_7421*
7442_G	CGTGGGGGTGGTGTGGGTCA	For Δ*MXAN_7421*
7442_H	CCTCGTTGGGGTAGGTGATG	For Δ*MXAN_7421*
7442_Pnat 600 up	ATCGAAGCTTTGAGCCCTTGGGCCAGGGCAGA C	For complementation fw
7442_Pnat rev	ATCGTCTAGATCAGCGCGGGTTCGCGGCGG	For complementation rev
1025_A	ATAGGGTACCGTGACGGAGCGCAGCGCCTC	For Δ*MXAN_1025*
1025_B	ATCCTTGGAGGCCGGGTCGAAACCGGT	For Δ*MXAN_1025*
1025_C	GACCCGGCCTCCAAGGATGGGGTGGCG	For Δ*MXAN_1025*
1025_D	ACTGTCTAGAAGTAGACGAGCCGCCCCACC	For Δ*MXAN_1025*
1025_E	CTGCGCGGCCAGCTTCACCA	For Δ*MXAN_1025*
1025_F	ACCCGGCGCAGGGCCTGTAG	For Δ*MXAN_1025*
1025_G	GACGCGGTGGCGCTGGTCCA	For Δ*MXAN_1025*
1025_H	ACGAAGAGGCCGGGCACCTC	For Δ*MXAN_1025*
1035_A	ATCGGGTACCAGCCGGAGCGGTGCACCTGG	For Δ*MXAN_1035*
1035_B	CGCCGGAGTGGCTTCGGGCGCTGGGGT	For Δ*MXAN_1035*
1035_C	CCCGAAGCCACTCCGGCGAGTCCGGCG	For Δ*MXAN_1035*
1035_D	ATCGTCTAGACTTCCAGGCCCGCACGCACC	For Δ*MXAN_1035*
1035_E	GCTTCCAGCCGCTCATGCCG	For Δ*MXAN_1035*
1035_F	TAATCACCGCCTCCGGGCAG	For Δ*MXAN_1035*
1035_G	GCGGCCTCCCTGGGCGTGTT	For Δ*MXAN_1035*
1035_H	AGCGCCTGCGCCACCACACC	For Δ*MXAN_1035*
1043_A	ATCGGAATTCCATGCCGAAGGCGTGTCCTT	For Δ*MXAN_1043*
1043_B	CAGGCGTCCGAAGAAGGCGACCAGAAG	For Δ*MXAN_1043*
1043_C	GCCTTCTTCGGACGCCTGGTCGCGATG	For Δ*MXAN_1043*
1043_D	ATCGAAGCTTCCCTCCAGCGCCTCGCCCCA	For Δ*MXAN_1043*
1043_E	CGCGGAGATGGTGGCCGTCG	For Δ*MXAN_1043*
1043_F	CGTCTCCGCCCGCGCCAGCA	For Δ*MXAN_1043*
1043_G	TGGGGATGGCTGGACCAGGC	For Δ*MXAN_1043*

1043_H	TGTTGGCGAAGTTCAGCGCC	For Δ*MXAN_1043*
1052_A	ATCGGGTACCTTGATGGAGCAGTCGCACAG	For Δ*MXAN_1052*
1052_B	CTCGCCATCAGAGGCACTGCGGGAAGC	For Δ*MXAN_1052*
1052_C	AGTGCCTCTGATGGCGAGGCTCGGGAC	For Δ*MXAN_1052*
1052_D	ATCGGGATCCCTCGAACCCCTGTACGGCGC	For Δ*MXAN_1052*
1052_E	GCGAAGGCACAGCTCCTTCT	For Δ*MXAN_1052*
1052_F	GGATGAAGACGGGAGAGCGC	For Δ*MXAN_1052*
1052_G	GCTCGTCGTGGTGTGTCCGC	For Δ*MXAN_1052*
1052_H	CGAAGAACGCATCCGCGCCA	For Δ*MXAN_1052*
1915_A	ATCGGGTACCGCTTCCTGCCGAAGACGGCG	For Δ*MXAN_1915*
1915_B	CACGAAGACGGTGAGGGCGGCGCGGAA	For Δ*MXAN_1915*
1915_C	GCCCTCACCGTCTTCGTGCCGGAGAGC	For Δ*MXAN_1915*
1915_D	ATCGTCTAGACAGGGCACCGACAGCCTGCG	For Δ*MXAN_1915*
1915_E	GACTACGGCAACCTGCGAGT	For Δ*MXAN_1915*
1915_F	CCCCGACCACGCCATCCTCG	For Δ*MXAN_1915*
1915_G	TGACGCTGCCCGCCTGCTTC	For Δ*MXAN_1915*
1915_H	TCGCCGGGCTGGAGCATGAA	For Δ*MXAN_1915*
pilT-A EcoRI	GCGCGAATTCCGCGACTTCGAGACGGCGG	For Δ*pilT*
pilT-D HindIII	GCGCAAGCTTGAGCTTCTCGTTCTTCTCC	For Δ*pilT*
pilT_E	CTCCGCCAGGACCCGGACATC	For Δ*pilT*
pilT_F	TATCGAGGCACTGCACCA	For Δ*pilT*
pilT_G	CTTGAAGACGGCGCCGCTGA	For Δ*pilT*
pilT_H	CGCGCTGATTCACGAGGCAG	For Δ*pilT*

[1] Underlined sequences indicate restriction sites.

Supplementary References

1. Shi X, Wegener-Feldbrugge S, Huntley S, Hamann N, Hedderich R, Søgaard-Andersen L. 2008. Bioinformatics and experimental analysis of proteins of two-component systems in *Myxococcus xanthus*. J Bacteriol 190:613-24.

2. Berleman JE, Zemla M, Remis JP, Liu H, Davis AE, Worth AN, West Z, Zhang A, Park H, Bosneaga E, van Leer B, Tsai W, Zusman DR, Auer M. 2016. Exopolysaccharide microchannels direct bacterial motility and organize multicellular behavior. ISME J 10:2620-2632.

3. Holkenbrink C, Hoiczyk E, Kahnt J, Higgs PI. 2014. Synthesis and assembly of a novel glycan layer in *Myxococcus xanthus* spores. J Biol Chem 289:32364-78.

4. Black WP, Wang L, Davis MY, Yang Z. 2015. The orphan response regulator EpsW is a substrate of the DifE kinase and it regulates exopolysaccharide in *Myxococcus xanthus*. Sci Rep 5:17831.

5. Lancero H, Caberoy NB, Castaneda S, Li YN, Lu A, Dutton D, Duan XY, Kaplan HB, Shi WY, Garza AG. 2004. Characterization of a *Myxococcus xanthus* mutant that is defective for adventurous motility and social motility. Microbiology-Sgm 150:4085-4093.

6. Youderian P, Burke N, White DJ, Hartzell PL. 2003. Identification of genes required for adventurous gliding motility in *Myxococcus xanthus* with the transposable element mariner. Mol Microbiol 49:555-70.

7. Lu A, Cho K, Black WP, Duan XY, Lux R, Yang Z, Kaplan HB, Zusman DR, Shi W. 2005. Exopolysaccharide biosynthesis genes required for social motility in *Myxococcus xanthus*. Mol Microbiol 55:206-20.

8. Kimura Y, Kato T, Mori Y. 2012. Function analysis of a bacterial tyrosine kinase, BtkB, in Myxococcus xanthus. FEMS Microbiol Lett 336:45-51.