

E206

Staphylococcus aureus Lys-PG-MprF
Listeria monocytogenes Lys-PG-MprF
Clostridium perfringens Lys-PG-MprF
Bacillus anthracis Lys-PG-MprF
Agrobacterium tumefaciens Lys-PG-MprF
Rhizobium tropici Lys-PG-MprF
Bacillus subtilis Lys+Ala-PG-MprF
Enterococcus faecalis Lys+Ala-PG MprF2
Enterococcus faecalis MprF1
Methanosarcina barkeri Ala-PG-MprF
Paenibacillus polymyxa Ala-PG-MprF
Kineococcus radiotolerans Ala-PG-MprF
Pseudomonas aeruginosa Ala-PG-MprF
Pseudomonas putida Ala-PG-MprF
Burkholderia phytatum Ala-PG-MprF

315
-I-Y-S-M-----V-R-P--P-D-K--
-IT-K-W-K-S-----KS-L-F-V-D-L
-L-Y-S-A-Y-----KY-S-K-T-K-D
-GFSK-LK-NRKT-----K-QA-E-GQ-D-E-G
-GR-N-G-K-A-----I-RLGK-WR-L-R-L
-GR-N-G-R-E-----V-RIGP-VA-V-R-L
-VS-K-I-K-D-----RK-A-GD-E-E-N
-IL-H-F-Q-K-----N-SS-F-GN-L-D--
-FT-Y-L-L-K-R-----RT-L-FK-T-F-F
-FF-S-F-R-T-----LNFKA-KGYQ-I-R-V
-I-M-Q-R-S-----K-RY-SIWF-N-K--
-A-A-G-R-S-----VGAR-R-WR-VAK--
-AR-HRLPDERPTPDSLLI--RLGR--RSV-R--L
-R-RRLA-EQPAANNLLV--QLGR--RTL-R--L
CGK-R-T-R-Q-----I-RT-R-WNWL-SFDI
-P-A-R-RDFIEQ-I-L-LAV--L-D-V-VAAGLA-

P247 D254

Staphylococcus aureus Lys-PG-MprF
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Kineococcus radiotolerans Ala-PG-MprF
Pseudomonas aeruginosa Ala-PG-MprF
Pseudomonas putida Ala-PG-MprF
Burkholderia phytatum Ala-PG-MprF

385
-L-Y-FCG-V--I--V--D-A---HVSFMS-F
-A-I-I-GT-L-M--G--EPV--D-IFKV-F
-I-YET---LR-I--T--G-A---SISVLN-F
-M-Y-V-I--L-L-FGI-D-I---E--FQK-F
-L-Y-V--L-L-P--E--THLWPF-TF--F
-L-Y-V--L-Q--P--E--TSIGWP-GF--F
-I-Y-F-A--L-FAMGHA-D-I---R--YV--F
-F-ISI-G--Y-L--L--D-V---PIPLID-I
-FL-M-I-GR-L-M--Q-V---PVSMSVSY-
-I-Y-F--L-L-P--S-N---P-HLTL-LH
-FAW-I-GSHL--L-H--E--L---PMHAV--F
-L-H-V--L-L--PAGD-R-L--P--FLP-F
-L-Y-L--L-L-P--E-T---P-PFAA-F
-L-Y-L--L-L-P--E-A---P-PFGA-F
-L-W-A--L-L-P--H-AH--V-GFVS-F

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Enterococcus faecalis Lys+Ala-PG MprF2
Enterococcus faecalis MprF1
Methanosarcina barkeri Ala-PG-MprF
Paenibacillus polymyxa Ala-PG-MprF
Kineococcus radiotolerans Ala-PG-MprF
Pseudomonas aeruginosa Ala-PG-MprF
Pseudomonas putida Ala-PG-MprF
Burkholderia phytatum Ala-PG-MprF

490
FKTL-G--VPE-EKVLLM-LLLYRFAFYFVVI
LSQL-G--V-SQE-LALAWMLFYRIFYIIFV
LEAL-G--I-PIEQTLV-IILYRISYIVBALI
--QY-G--I-DTGVLPA-MLLYRLVYILFCL
-NAI-S--V-D-Q-LLGS-LVLYRVIYHVLV
-SSV-N--E-D-A-VLSS-LVLYRVIYHVLV
MEQL-GYHQ-E-A-IVTS-IVLYRLAISPFI
LSNL-G--V-DRE-IIVLWLLLYRFLFYIIFLI
LSQL-G--I-DRS-QAVVWLLYRFLFYVTFMT
-PYF-G--T-V-D-IKPL-LLFRTIYFVFLI
-LQIAG--ANS-DRALAV-LLVRFVYIIFWLI
-STT-S--P-A-G-LAVA-LVAYRLCYFVLL
-GQL-G--A-A-P-LAAA-LLLYRLIYVVL
-DQL-G--A-A-P-LAAA-LLLYRLIYVVL
-GSV-Q--T-H-S-MVAA-LLAYRAIYFGLR
A-LI-LS-S--F-E--F-GTSA-K-KY-IEGS-K
G-LL-FF-V--Q-K-----A-G-KK-V--N-D
G-VL-LF-V--H-D--F--G-G-K--I--N-K
G-LI-FA-A--F-E--MTGV-AIK-KFED--K-P
A-IL-VMIV--S-ETKQF-A--A-K-PV-V--S-D
A-IA-AISV--A-D-----C-A-----V--S-S
G-LF-FA-AGDLTE---N-T--M-K-RLLET--N-P
GCLF-FT-K--H-L-----S-Q-KL-D--T-H
G-VI-LF-L--Q-Q-----A-GMK--V--N-Q
G-LLALM-F--Y-E---Y-Q--E-RDKF-L--R-K
G-LV-LA-A--L-E--M-I--P-RNER-L--N-Q
AAVL-LV-A--H-E-----A-R-RG-A--T-A
ACLL-LL-F--L-E-----A-R-RLWV--TRQ
ACVL-LL-A--N-E-----A-R-RLLF--AQQ
AGLL-AG-F--E-----GR-ALK-KH-L--P--

Staphylococcus aureus Lys-PG-MprF
Listeria monocytogenes Lys-PG-MprF
Clostridium perfringens Lys-PG-MprF
Bacillus anthracis Lys-PG-MprF
Agrobacterium tumefaciens Lys-PG-MprF
Rhizobium tropici Lys-PG-MprF
Bacillus subtilis Lys+Ala-PG-MprF
Enterococcus faecalis Lys+Ala-PG MprF2
Enterococcus faecalis MprF1
Methanosarcina barkeri Ala-PG-MprF
Paenibacillus polymyxa Ala-PG-MprF
Kineococcus radiotolerans Ala-PG-MprF
Pseudomonas aeruginosa Ala-PG-MprF
Pseudomonas putida Ala-PG-MprF
Burkholderia phytatum Ala-PG-MprF

525
YFIP-A--KDV--T-SPLM-SYQKDIIAKIP--S
-FL--EGLP-L--L-F-L--Q-K--V-A--H--R
-KF--N--G-LP-Y--E-IV-S-K--V-A--Y--K
-FI--A--P-A--L-E-T--T-GV-IWT-LQ--R
-I--S--Q-L-----A--V-R--L-A--P--P
-I--T--RWL--R-ACAASA-R--L-M--P--Q
-RI--A--P-A--I-E-T--T-NV-LLV-VQ--R
AVLVRILOQS-LS--L-I-V--FVAGLI-V-LASV
--Y--R--Q-L--L-K-QI-TLE--I-A--H--K
-FF--D--N-L--P-R-LF-S-QK-V-A--H--F
--I--E--K-A--TYS-SL-S-E--L-T--P--R
--L--A--E-TGWDS-LN-AWK-VWG-WPGQFR
-LGAA--R-T-----A--A--G--TPG--P--
-AI-----R-V-----S-G--F-A-AP--
-AI-----K-A-----S-G--L-A-AP--
--L--H--H-A-----A-TV-S-Q--L-A--P--

525

560

	595	630
Staphylococcus aureus Lys-PG-MprF	LTIVYDALYD--GNH---L-TYY-ILLAIHTSACL	L---L-LLN-V-V--G-IYKQSRRAIFAMISIL-
Listeria monocytogenes Lys-PG-MprF	AVPNAIYHVPF-LYK-I-M-PFN-FLFTSQITIVA	F-G-F-LLLGL-A-RG-IECKTKKAYIITVI-VL-
Clostridium perfringens Lys-PG-MprF	IAPQYLLKIKL-LKE-I-L-GKO-VLGLSIGMSVV	L-G-F-LIM-L-A-ALMLKYRAKSIYKASMV-LP-
Bacillus anthracis Lys-PG-MprF	ILPTSINR-AH-ALH-I-LAPKH-LIQFS-FSLSL	TFGIL-LLI-L-S-RG-IYYGTRKRSYMTII-SL-
Agrobacterium tumefaciens Lys-PG-MprF	VTPTPDSNLDL-LSN-F-V-PLP-IIIAAHFLTSI	L-G-L-GLV-V-ASRG-LGQRLDGAWWIALI-AAS
Rhizobium tropici Lys-PG-MprF	VTRTPDHNLIIV-LSL-D-Y-L-PLS-LVESAHFLSSL	L-G-L-AII-V-AARG-LSQRLDGAWWVSTP-SA-
Bacillus subtilis Lys+Ala-PG-MprF	SLPI-D-RITV-IPH---I-PRPALLFNGLSLSS	A-L-I-LLI-L-P-IE-LYKRTKRSYMTAIT-AL-
Enterococcus faecalis Lys+Ala-PG MprF2	TIPFAFTQ-VHWLRD---INPFR-SHIIQIPSIIV	L-G-FALLI-M-G-RG-IADRVKRAYYPTII-LL-
Enterococcus faecalis MprF1	VT-N-LSNVSR-LFQVL-L-PFS-FNFLDQ-TLNL	FVG-F-LLLGL-A-RG-ISMKVKKAYWPTII-LL-
Methanosarcina barkeri Ala-PG-MprF	ALPS-DPRYLHDLN--YLI-PLP-LIEVSRLLPGSI	M-GVL-LLL-L-A-NG-LWKRIDGAYISLS-VL-
Paenibacillus polymyxa Ala-PG-MprF	ATPGLLYRLRF-ABH-L-L-SLP-VMRLSHBISVI	I-G-I-MLV-VLS-HG-ISLRIRRALRLTLI-LL-
Kineococcus radiotolerans Ala-PG-MprF	VAGA-GVFMVV-VGD---L-PGR-ALDVSAVTISL	A-G-LSALL-V-A-RG-LHLRLRRAW-VATV-V-
Pseudomonas aeruginosa Ala-PG-MprF	ATPAIDTRLEH-LG--FLI-PHR-LIDASHLVASL	I-GVL-CLL-L-A-QG-LRRRLSAAWALTLV-LL-
Pseudomonas putida Ala-PG-MprF	ATPEIDTRLEH-MG--FLV-PHR-LIDASHFGASL	I-GVL-CLL-L-A-QG-LRRRLSAAWLLTTV-LL-
Burkholderia phytatum Ala-PG-MprF	ATPAFLQRIAI-LRD-L-V-PLW-VLESSQMLCSI	V-G-V-ALLFV-A-RG-LRRRLDAAWLTLG-LA-
	665	700
Staphylococcus aureus Lys-PG-MprF	L-I-TV-A-TFP-TYASYILITW-L-A-IIPVL-L	--IV--A--FRARR-LKRP--VRM-RNI-V-A
Listeria monocytogenes Lys-PG-MprF	G-C-AI-FNTLARVFS-MKQAI-F-L-GIVLLCL-F	-IAR--NE--FYREKLVYWS-K-VIIDS-T-I-F
Clostridium perfringens Lys-PG-MprF	I-LGII-L-SLTKGIN-PYELV-L-IIVAYLL-Y	-LSK--RM-FYRDSFVVSCK-N-TLIDS-G-I-L
Bacillus anthracis Lys-PG-MprF	IGA-AV-F-NLTKGID-IEETFI-L-LIVLAVL-Y	-MLR--KR--FVREKMEVLSLSDI-VKV-F-IFL-L
Agrobacterium tumefaciens Lys-PG-MprF	L---ALIL-SILKATA-VFEAVF-L-GIFIAAL-A	-LNM--RS-FNREHASLVKQA-L-GPS-W-L-A-A
Rhizobium tropici Lys-PG-MprF	L-F-ALFF-SLLKATA-IVEAGL-L-APFVFSL-V	-VSR--RL-FKRPASLLNQT-L-TAG-W-L-T-A
Bacillus subtilis Lys+Ala-PG-MprF	V-GGFV-F-SPLKGLN-ISAIFV-L-PMIIVLL-V	-LLK--KQ--FVREQASYTLG-Q-L-I-F-A-V-A
Enterococcus faecalis Lys+Ala-PG MprF2	I-G-AILY-SFVVDPS-MFSIFY-L-AILLFIV-I	-PSK--SE--LYREQLVYSWEMWTDIG-F-I-F-G
Enterococcus faecalis MprF1	F-C-IV-N-TVARTTS-WQLIAY-Y-AVILLAV-I	-LAR--KE--FYREKPVYSWG-A-LTVDSD-T-L-F
Methanosarcina barkeri Ala-PG-MprF	L-MGGI-F-ALLKNFD-YHEAAT-LFTLFFLLP-P	--CR--KY-FYREKSSLMHQS-F-SRS-N-V-I-A
Paenibacillus polymyxa Ala-PG-MprF	L-AGAI-F-TPIKGLD-FEEALF-L-L-FVALM-L	WISR--SR--FYREVSAPLNRH-N-ILI-W-G-G-L
Kineococcus radiotolerans Ala-PG-MprF	L----L-T-GVT-AVA-AAHGDLVL-SAVTAGL-A	-LLLVPARTAFHREGAP-FALP-R-NPV-WPV-T-A
Pseudomonas aeruginosa Ala-PG-MprF	L-VGAL-L-SLLKGF-D-WEASL-L-S-LTAALLA	-LFR--RS-FYRPSRLLEVP-F-SPL-Y--VGA
Pseudomonas putida Ala-PG-MprF	L-VGAL-L-SLLKGF-D-WEACL-L-T-PTAALLA	-LFR--RS-FYRPSRLLELP-F-SPV-F-L-V-A
Burkholderia phytatum Ala-PG-MprF	V-V-SLAL-SLTKGLA-FVEAGV-L-TTLIVLLIA	--TR--SR--FNRESSLFAEP-F-TAG-W-L-V-S
	735	770
Staphylococcus aureus Lys-PG-MprF	MLLF-S-LF-ILY-VNHI-F----I--AGTL-YA	LDIYTIEMHT--S--VL--RYY-FWLT-IL-I--
Listeria monocytogenes Lys-PG-MprF	IVCL-A-GY-I-V-I-GI-Y----NS-PNIK-HS	KEIP-DYLR-I--A--SE--H-L-W-LV-GFVG--
Clostridium perfringens Lys-PG-MprF	IASFSL-YFFI---L-I-T-F----GT-H-LK-YV	G-IV-RKMPY-KM--AY--K-F-G-FT-AP-A--
Bacillus anthracis Lys-PG-MprF	ITLY-L-YK-N--LGLI-F----AG-AKEA-FK	PDFV-VR-NI-TQ--VK--RSA-L-AA-AP-F--
Agrobacterium tumefaciens Lys-PG-MprF	MAVI-IAGA-AT--I-LL-F----VY-RDTE-YS	HALW-WEPEF-SEE-AP--R-G-L-RA-LL-G--
Rhizobium tropici Lys-PG-MprF	IAVVC-I-GA-IV-V-LF-F----VY-RDVG-YS	NELW-WQFEF-ADE-AP--R-G-L-RA-AL-G--
Bacillus subtilis Lys+Ala-PG-MprF	LFTV-A-LF-N-YNL-IAGF----IWRMCKVLR	HEYF-VH-ST-SH--IT--H-ATT-MA-II-I--
Enterococcus faecalis Lys+Ala-PG MprF2	LLTL-L-YL-V---I-GV-Y----NL-PNPF-HH	RHHF-VEFFL-FP--SE--R-I-W-LS-GF-I--
Enterococcus faecalis MprF1	GCLF-I-GY-A--VAGY-Y----AA-RPAG--G	NQVI-NHFLL-FP--SD--D-V-W-FN-GLIG--
Methanosarcina barkeri Ala-PG-MprF	ITLVFV-SF-A-W-L-GF-F----SY-HNVK-YS	NELW-WQFGINSQ--AS--S-F-L-RA-TV-G-A
Paenibacillus polymyxa Ala-PG-MprF	SLLV-T-G--A-Y-F-II-G----AG-SNTP-FM	RHLH-AKVHL--D--F--F-M-N-RS-DY-G--
Kineococcus radiotolerans Ala-PG-MprF	AALV---GA-AVW-W-HE-SGGSGGAV--DAP-L-	--LV-ASVS--GD--TPGADRLA-M-AAGAV-G--
Pseudomonas aeruginosa Ala-PG-MprF	SICV-I-GA-SVW-L-LL-F----AN-QDVH-YS	NQLW-WQFALDAD--AP--R-A-L-RA-AL-GSC
Pseudomonas putida Ala-PG-MprF	SACT-V-GA-SVW-L-LL-F----AY-QDVP-YS	HQLW-WQFTLDAD--AP--R-G-L-RA-AM-GSA
Burkholderia phytatum Ala-PG-MprF	VAMV-L-ML-AVW-V-ML-F----AF-RDVP-YT	RDLW-WQFAP--DERAP--R-A-L-RA-TL-A--
	805	840
Staphylococcus aureus Lys-PG-MprF	I-A-I-I--IGMI-A-W-L-F-.....
Listeria monocytogenes Lys-PG-MprF	VFIIV-V--SLVI-I---Y-I-.....
Clostridium perfringens Lys-PG-MprF	L-V-T-V--IYVA-I---Y-FL-.....
Bacillus anthracis Lys-PG-MprF	V-P-T-F--LLIG-S--L-I-.....
Agrobacterium tumefaciens Lys-PG-MprF	L-V-L-ASSTIAIFS--L-M-.....
Rhizobium tropici Lys-PG-MprF	I-S-I-VSSAIAIFS--L-L-.....
Bacillus subtilis Lys+Ala-PG-MprF	V-P-L-F--FLIF-T--V-.....
Enterococcus faecalis Lys+Ala-PG MprF2	A-I-L-L--VMSF-N-F-L-FV-.....
Enterococcus faecalis MprF1	L-S-ISL--IGLF-F--L-Y-.....
Methanosarcina barkeri Ala-PG-MprF	F-I-L-L--L-VL-G--M-A-.....
Paenibacillus polymyxa Ala-PG-MprF	V-TAV-F--SLIL-A-W-VFLT-.....
Kineococcus radiotolerans Ala-PG-MprF	I---L-I--G-GR-R--V-L-.....
Pseudomonas aeruginosa Ala-PG-MprF	L-L-L-L--ALAL-G-W-L-L-.....
Pseudomonas putida Ala-PG-MprF	L-L-L-A--AVAL-T-W-L-L-.....
Burkholderia phytatum Ala-PG-MprF	A-S-L-F--A-ATPCFWQL-L-.....

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Supplemental Figure 1: Amino acid sequence alignment of the hydrophobic N-terminal parts of MprF proteins of various bacterial species, known to synthesize Lys-PG, Ala-PG, or both. Conserved charged amino acid and proline positions are indicated. *Staphylococcus aureus* (Q2G2M2), *Listeria monocytogenes* (Q71YX2), *Clostridium perfringens* MprF2 (Q0STHJ7), *Bacillus anthracis* (B0AWU), *Agrobacterium tumefaciens* (A9CHP8), *Rhizobium tropici* (Q8GQE5), *Bacillus subtilis* (C0H3X7), *Enterococcus faecalis* MprF2 (S0L1V5), *Enterococcus faecalis* MprF1 (Q839W9), *Methanosarcina barkeri* (Q469T9), *Paenibacillus polymyxa* (E0RDN6), *Kineococcus radiotolerans* (A6W5P4), *Pseudomonas aeruginosa* (A6VA53), *Pseudomonas putida* (Q88NK5), *Burkholderia phymatum* (B2JPD9).