Supplementary materials

The Mla pathway plays an essential role in the intrinsic resistance of *Burkholderia cepacia* complex species to antimicrobials and host innate components

Steve P. Bernier[1*], Susie Son[1`], and Michael G. Surette[1,2*]

[1]*Department of Medicine, Farncombe Family Digestive Health Research Institute, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada.*

[2]*Department of Biochemistry and Biomedical Sciences, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada.*

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Corresponding authors: Steve P. Bernier (*sbernier19@gmail.com*)
Michael G. Surette (*surette@mcmaster.ca*)

Supplementary Tables

Table S1. Antibiotic resistance profile of *B. cenocepacia* K56-2 transposon mutants selected for their sensitivity to *P. aeruginosa* spent medium on agar surface

		Antibiotic[b]			
Strain/mutant ID	Gene[a]	AZM 15	FEP 30	IPM 10	TOB 10
WT K56-2	-	6	27	9	6
15G6	BCAL0179	6	25	10	6
36D7	BCAL0179	6	21	8	6
37A8	BCAL0182	6	23	8	6
25E5	BCAL0302	23	24	10	6
30G10	BCAL0305	29	26	6	6
2C5	BCAL0305	26	26	13	6
12C7	BCAL0499	6	26	14	6
14C3	BCAL1048	6	26	9	6
37B7	BCAL1172	6	22	7	6
28H12	BCAL1246	6	22	7	6
37H7	BCAL3225	6	21	8	6
2D10	BCAL3252	6	26	10	6
35H10	BCAL3252	6	22	8	6
4E2	BCAL3336	6	27	11	6
8H11	BCAM0275a	6	24	11	6
13F4	BCAM0324	6	26	8	6
16H4	BCAM1948	6	24	8	6
24C3	BCAM2426	6	26	11	6
17F3	BCAM2618	6	26	11	6
33C6	BCAM2620	6	22	7	6

[a] Gene affected by the transposon insertion as described in Table 1.

[b] Zone of clearing in millimeters (mm) around the disk after 24 h. When no zone was present, the size of the disk (6 mm) was recorded. Abbreviations of the antibiotics tested with their concentration in μg on each disk in parentheses are as follow: azithromycin (AZM 15), cefepime (FEP 30), imipenem (IPM 10), and tobramycin (TOB 10).

Table S2. Antibiotic resistance profiles of *B. cenocepacia* K56-2 mutants

Antibiotic class/Antibiotic	[ug]	WT	*mlaE*	*mlaC*	BCAL0499
Penicillins					
Ampicillin (AMP)	2	6	N.D.	6	N.D.
	10	6	N.D.	6	N.D.
	25	6	6	6	6
Amoxicillin-clavulanate (AMC)	30	6	6	6	6
Penicillin (P)	10	6	N.D.	6	N.D.
Cephalosporins					
Cefepime (FEP)	30	28	29	28	28
Cefoxitin (FOX)	30	6	6	6	6
Ceftazidime (CAZ)	30	39	39	37	40
Ceftriaxone (CRO)	30	27	29	26	27
Carbapenems					
Imipenem (IPM)	10	12	13	13	13
Meropenem (MEM)	10	37	37	33	38
Monobactams					
Aztreonam (ATM)	30	25	24	23	25
Aminoglycosides					
Tobramycin (TOB)	10	6	6	6	6
Amikacin (AK)	30	6	6	6	N.D.
Tetracyclines					
Tetracycline (TE)	30	25	32	32	28
Doxycycline (DO)	30	34	50	51	37
Tigecycline (TGC)	15	20	34	34	17
Oxazolidonones					
Linezolid (LZD)	10	6	6	6	N.D.
	30	6	6	6	6
Chloramphenicol (C)	10	9	19	22	N.D.
	30	20	33	32	25
	50	28	35	39	N.D.
Macrolides					
Azythromycin (AZM)	15	6	26	26	6
Clarithromycin (CLR)	2	6	N.D.	7	N.D.
	5	6	N.D.	15	N.D.
	15	6	25	22	6
Erythromycin (E)	15	6	21	19	6
Lincosamides					
Clindamycin (DA)	2	6	6	6	6
Fluoroquinolones					
Ciprofloxacin (CIP)	5	24	37	38	25
Levofloxacin (LEV)	5	22	38	42	N.D.
Rifampicin (RD)	5	6	26	27	6
Others					
Piperacillin/tazobactam (TZP)	110	40	42	40	42
Fosfomycin (FOS)	50	6	6	6	6

Zone of clearing in millimeters (mm) around the disk after 24 h are reported. When no zone was present, the size of the disk (6 mm) was recorded. Gray zones represent antibiotics for which *mlaE* and *mlaC* insertional mutants are more sensitive than their WT parent.

Table S3. The Mla pathway and antibiotic resistance in *E. coli* K-12

		Zone of clearing (mm)[a]						
Antibiotic	**[ug]**	**WT**	**Δ*mlaA***	**Δ*mlaC***	**Δ*mlaD***	**Δ*mlaE***	**Δ*yadG***	**Δ*yadH***
Azythromycin	15	17	17	16	16	15	16	16
Erythromycin	15	10	11	11	10	11	10	10
Clarithromycin	5	6	6	6	6	6	6	6
Rifampicin	5	13	13	14	13	13	12	15
Ciprofloxacin	5	37	37	36	38	35	37	37
Levofloxacin	5	35	34	34	38	35	35	35
Chloramphenicol	30	28	30	31	29	30	28	28
Tetracycline	30	32	32	31	31	31	33	32
Tigecycline	15	30	30	30	31	30	30	31
Doxycycline	30	29	30	30	30	30	30	31

[a]Zone of clearing in millimeters (mm) around the disk after 24 h are reported. When no zone was present, the size of the disk (6 mm) was recorded.
Deletion mutants of *E. coli* strain BW25113 (WT) are from the Keio collection (1) and described in Table S5.

Table S4. The Mla/VacJ pathway and antibiotic resistance in *P. aeruginosa*

		Zone of clearing (mm)[a]							
Antibiotic	**[ug]**	**WT**	***vacJ***	***mlaC***	***mlaD***	***mlaE***	***mlaF***	**PA2811**	**PA2812**
Azythromycin	15	6	6	6	6	6	6	6	6
Erythromycin	15	6	6	6	6	6	6	6	6
Clarithromycin	5	6	6	6	6	6	6	6	6
Rifampicin	5	6	6	6	6	6	6	6	6
Ciprofloxacin	5	43	44	44	44	44	44	42	41
Levofloxacin	5	38	40	40	40	40	40	37	37
Chloramphenicol	30	26	26	28	27	27	27	15	13
Tetracycline	30	34	37	35	35	36	34	31	32
Tigecycline	15	31	35	34	34	34	33	32	32
Doxycycline	30	32	36	35	35	35	36	31	32

[a]Zone of clearing in millimeters (mm) around the disk after 24 h. When no zone was present, the size of the disk (6 mm) was recorded.

Transposon mutants of *P. aeruginosa* strain PA14 (WT) are from (2) and described in Table S5.

Table S5. Microbial strains and plasmids used in this study.

Strain or Plasmid	Relevant characteristics - genotype[a]	Reference or Source
Strains		
E. coli		
DH5α	F$^-$ *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20* Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(r_K^- m_K^+), λ–	Invitrogen
HB101	F$^-$ mcrB mrr hsdS20(r_B^- m_B^-) recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20(Sm^R) glnV44 λ$^-$	Lab collection
BW25113	*E. coli* K-12 derivative	(1)
Δ*mlaF*	BW25113Δ*mlaF*::KmFRT; Km^R; mutant ID (JW3162)	(1)
Δ*mlaE*	BW25113Δ*mlaE*::KmFRT; Km^R; mutant ID (JW3161)	(1)
Δ*mlaD*	BW25113Δ*mlaD*::KmFRT; Km^R; mutant ID (JW3160)	(1)
Δ*mlaC*	BW25113Δ*mlaC*::KmFRT; Km^R; mutant ID (JW3159)	(1)
Δ*mlaA*	BW25113Δ*mlaA*::KmFRT; Km^R; mutant ID (JW2343)	(1)
Δ*yadG*	BW25113Δ*yadG*::KmFRT; Km^R; mutant ID (JW0123)	(1)
Δ*yadH*	BW25113Δ*yadH*::KmFRT; Km^R; mutant ID (JW0124)	(1)
S. cerevisiae INV*Sc*1	*MATa his3Δ1 leu2 trp1-289 ura3-52*	Invitrogen
P. aeruginosa		
PA14	Wild-type (WT); Burn patient isolate	(3)
Δ*lasR*	PA14Δ*lasR*; In-frame deletion of *lasR*	(4)
rhlR	PA14*rhlR*::Tet; Disruption of *rhlR*; Tet^R	(4, 5)
Δ*pqsR*	PA14Δ*pqsR*; In-frame deletion of *pqsR*	(6)
Δ*phz*	PA14Δ*phzA1-G1*Δ*phzA2-G2*; In-frame deletion of the two *phzA-G* operons	(7)
Δ*rhlA*	PA14*rhlA*::Gm; Internal deletion of *rhlA*; Gm^R	(8-10)
Δ*hcnABC*	PA14Δ*hcnABC*	(11)
mlaC; (*mlaC:*:Tn*M*)	PA14_PA14_57840::Tn*M*; mutant ID (41806); PA4453	(2)
mlaD; (*mlaD:*:Tn*M*)	PA14_PA14_57850::Tn*M*; mutant ID (41030); PA4454	(2)
mlaE; (*mlaE:*:Tn*M*)	PA14_PA14_57870::Tn*M*; mutant ID (40312); PA4455	(2)
mlaF; (*mlaF:*:Tn*M*)	PA14_PA14_57880::Tn*M*; mutant ID (27857); PA4456	(2)
vacJ; (*vacJ:*:Tn*M*)	PA14_PA14_27920::Tn*M*; mutant ID (52739); PA2800	(2)
PA2811; PA2811::Tn*M*	PA14_PA14_27780::Tn*M*; mutant ID (35140); PA2811	(2)
PA2812; PA2812::Tn*M*	PA14_PA14_27770::Tn*M*; mutant ID (34523); PA2812	(2)
B. cenocepacia		
K56-2	Wild-type; CF sputum isolate (Canada)	(12, 13)
mlaE; (*mlaE*::Tp)	K56-2 with pMQ87Tp inserted into *mlaE* (BCAL0302); Tp^R	This study
mlaC; (*mlaC*::Tp)	K56-2 with pMQ87Tp inserted into *mlaC* (BCAL0305); Tp^R	This study
BCAL0499; (BCAL0499::Tp)	K56-2 with pMQ87Tp inserted into BCAL0499; Tp^R	This study
K56-2-15G6	BCAL0179::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-36D7	BCAL0179::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-37A8	BCAL0182::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-25E5	BCAL0302::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-30G10	BCAL0305::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-2C5	BCAL0305::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-12C7	BCAL0499::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-14C3	BCAL1048::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-37B7	BCAL1172::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-28H12	BCAL1246::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-37H7	(BCAL3225)::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-2D10	(BCAL3252)::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-35H10	(BCAL3252)::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-4E2	BCAL3336::Tn*rhaB*out derivative of K56-2; Tp^R	This study

K56-2-8H11	(BCAM0275a)::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-13F4	(BCAM0324)::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-16H4	BCAM1948::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-24C3	(BCAM2426)::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-17F3	BCAM2618::Tn*rhaB*out derivative of K56-2; Tp^R	This study
K56-2-33C6	BCAM2620::Tn*rhaB*out derivative of K56-2; Tp^R	This study
vacJ2; 36D10	*vacJ2*::Tn*rhaB*out derivative of K56-2; BCAM2829; Tp^R	This study
B. dolosa		
PC543	CF sputum isolate (USA)	(14, 15)
mlaC; (*mlaC*::Tp)	PC543 with pMQ87Tp inserted into *mlaC* (BDSB_RS14790); Tp^R	This study
vacJ; 2A6	*vacJ*_(BDSB_RS14795)::Tn*rhaB*out derivative of PC543; Tp^R	(16)
bamC; 1A2	*bamC*_(BDSB_RS14795)::Tn*rhaB*out derivative of PC543; Tp^R	(16)
Plasmids		
pSC*rhaB*out	pTn*Mod*-OTp', *rhaR rhaS* P_{rhaB}; Tp^R	(17)
pRK2013	Mobilizing vector, ColE1 Tra (RK2); Km^R	(18)
pMQ87Tp	Suicide vector, *URA3 CEN6/ARSH4 lacZ*α; Gm^R, Tp^R	(16)
pMQ87Tp-*mlaE*	*mlaE* (BCAL0302) insertional mutant construct in pMQ87Tp	This study
pMQ87Tp-*mlaC*	*mlaC* (BCAL0305) insertional mutant construct in pMQ87Tp	This study
pMQ87Tp-BCAL0499	BCAL0499 insertional mutant construct in pMQ87Tp	This study
pCR®2.1	Cloning vector; Km^R, Amp^R	Invitrogen
pCR®2.1-*mlaCB*	pCR®2.1 with 1367-bp PCR fragment containing *mlaCB* and native promoter region; Km^R, Amp^R	This study
pHERD26T	Broad-host-range shuttle vector; Tet^R	(19)
pHERD26T-*mlaCB*	pHERD26T with 1321-bp KpnI-EcoRI fragment from pCR®2.1-*mlaCB*; Tet^R	This study

[a] Tn mutants with genes in parentheses indicate that the insertion was in an intergenic region and that the gene is likely the one affected by the Tn insertion. Tp, trimethoprim; Tet, tetracycline; Gm, gentamicin; Km, kanamycin; Amp, ampicillin.

Table S6. Primers used in this study.

Primer	Sequence (5'- 3')	Reference
M13F	GTAAAACGACGGCCAGT	Lab collection
M13R	CAGGAAACAGCTATGAC	Lab collection
824	GCCCATTTTCCTGTCAGTAACGAGA	(17)
BCAL0302-5L	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGAATTCTTCCCGCTGCTGCG	This study
BCAL0302-3L	AACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCCAGCGCAATGAAGGTCACGG	This study
BCAL0305-5L	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCAGTCGAACCCGCAGGCGCTG	This study
BCAL0305-3L	AACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCCAAGTTGCTGGTTGCGCTGC	This study
BCAL0499-5L	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCCCTGGTGATCGACGACAACG	This study
BCAL0499-3L	AACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCTGCGGCGGTGCATGTTCAGC	This study
mlaCB-For	GTACGACCTGCCGGAAGACG	This study
mlaCB-Rev	TTGGCTGCCCCGCAAAACGC	This study

Supplementary Figures

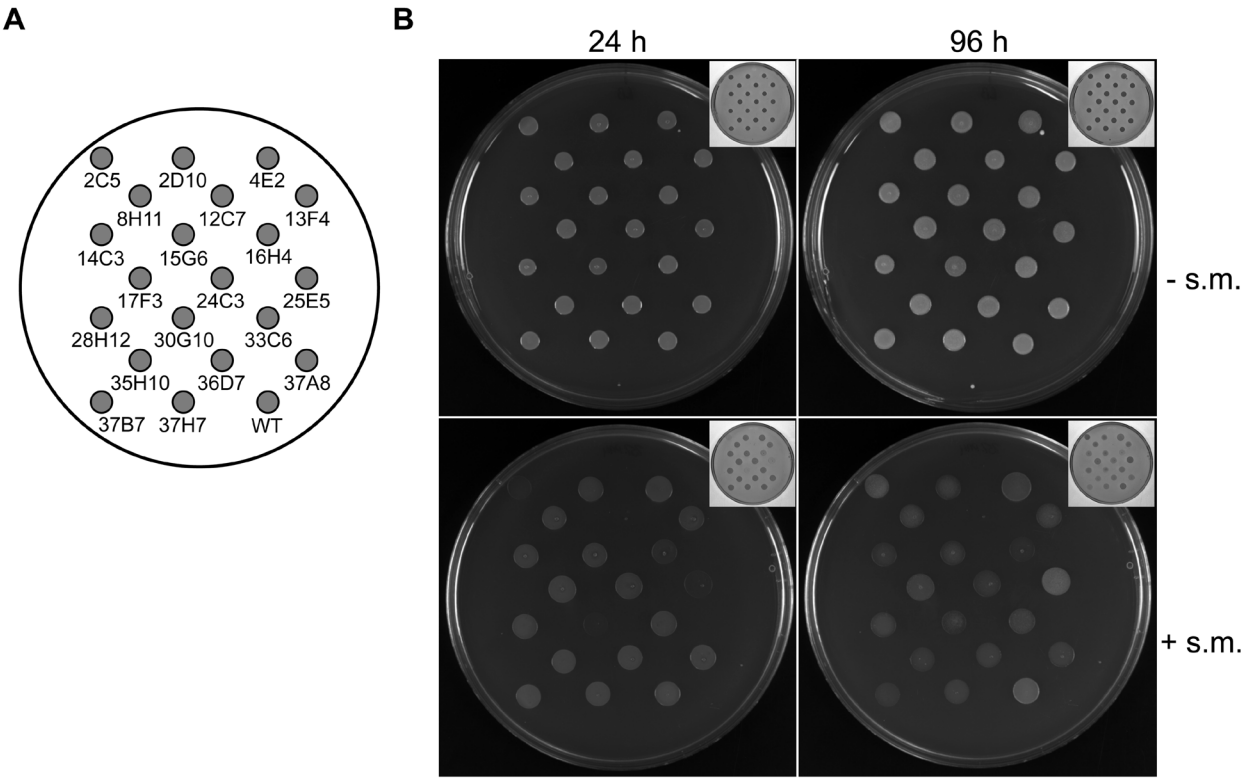

FIG S1. Selection of hypersensitive transposon mutants. (A) The selected transposon mutants of *B. cenocepacia* K56-2 (B) were grown for 24 h at 37°C and 72 h at room temperature with the addition of 25% spent medium (+s.m.) extracted from planktonic cultures of *P. aeruginosa* PA14 and compared to growth in absence of spent medium (- s.m.).

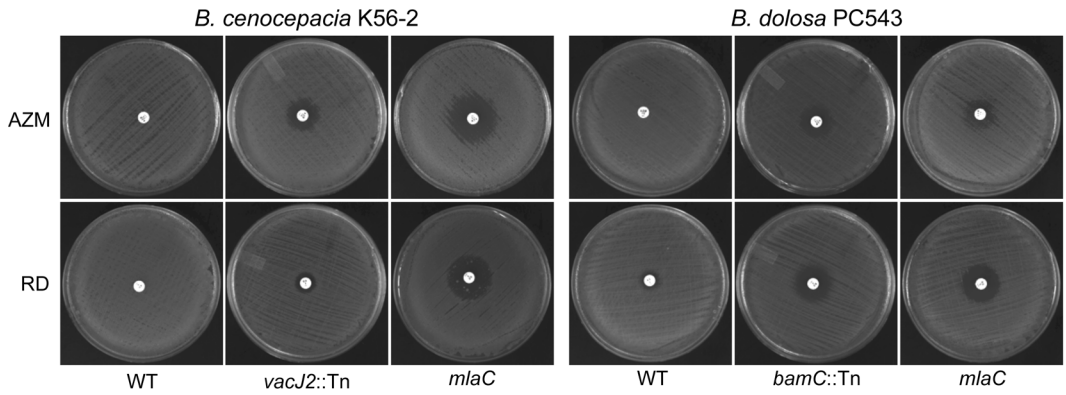

FIG S2. Susceptibility to Gram-positive antibiotics. Resistance profile of transposon mutants (*vacJ2* and *bamC*) selected for their susceptibility to azithromycin (AZM) and rifampicin (RD) in comparison to WT and *mlaC* mutants in *B. cenocepacia* K56-2 and *B. dolosa* PC543.

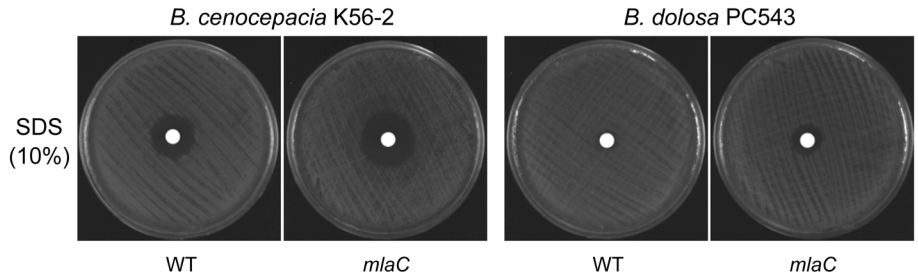

FIG S3. Susceptibility to the SDS detergent. The sensitivity of WT and *mlaC* mutants of *B. cenocepacia* K56-2 and *B. dolosa* PC543 was assessed on agar using 10 µl of a 10% SDS solution on a sterile disk. Zones of inhibition are greater in *mlaC* mutants compared to WT, but WT PC543 is more resistant than WT K56-2.

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