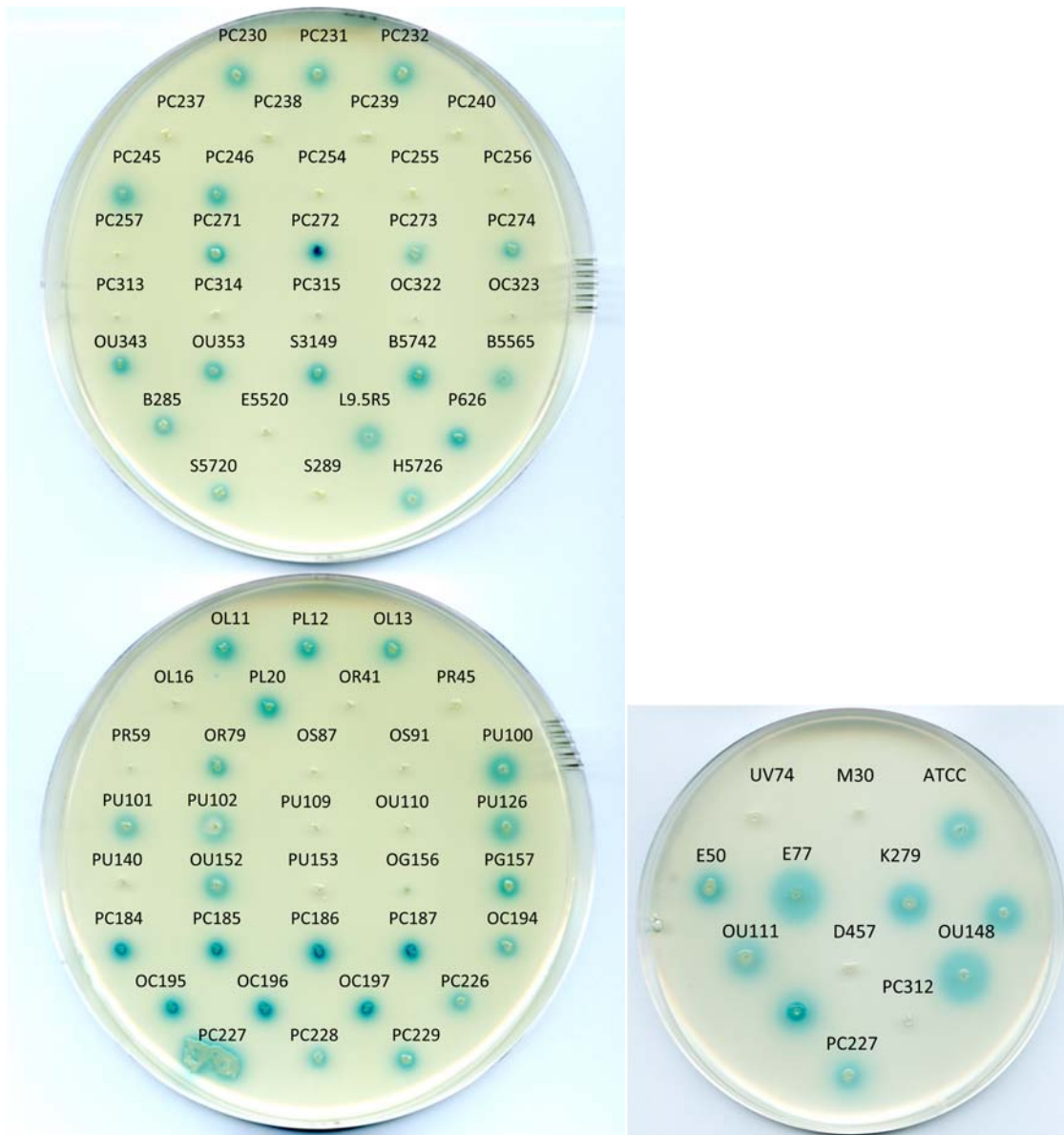**FIG S1** Colony DSF bioassay of 78 *S. maltophilia* strains. Blue halo around the colony indicates DSF activity and corresponded to RpfF-1 variant strains. Colonies without DSF production corresponded to RpfF-2 variant strains.
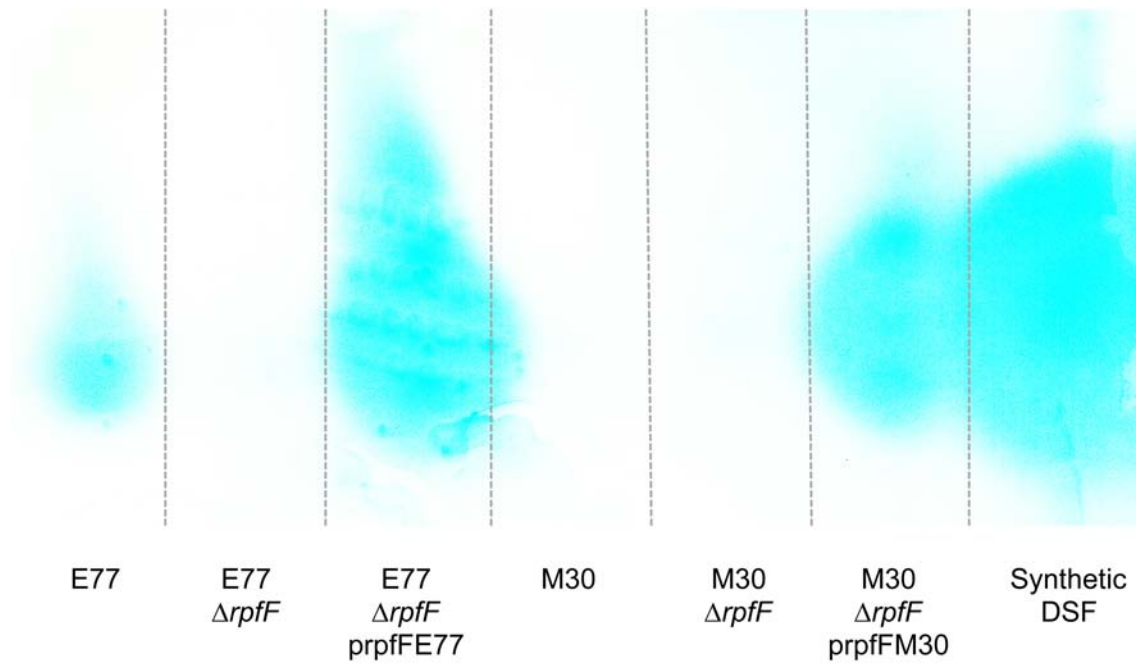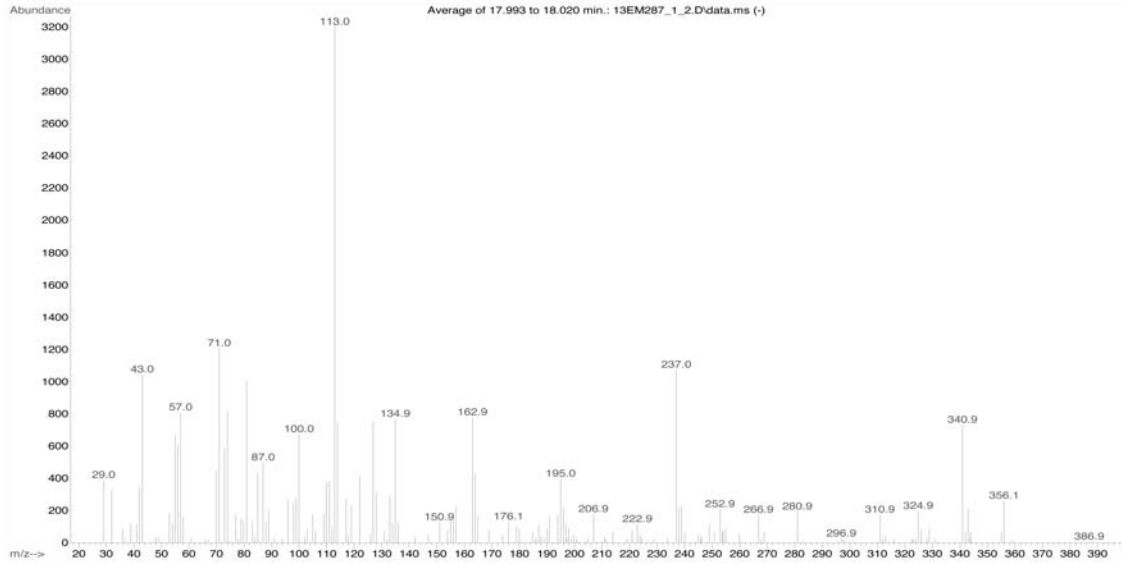
FIG S2 TLC coupled to DSF bioassay of culture supernatants from E77 and M30 with their respective *rpfF* mutants and complemented strains.
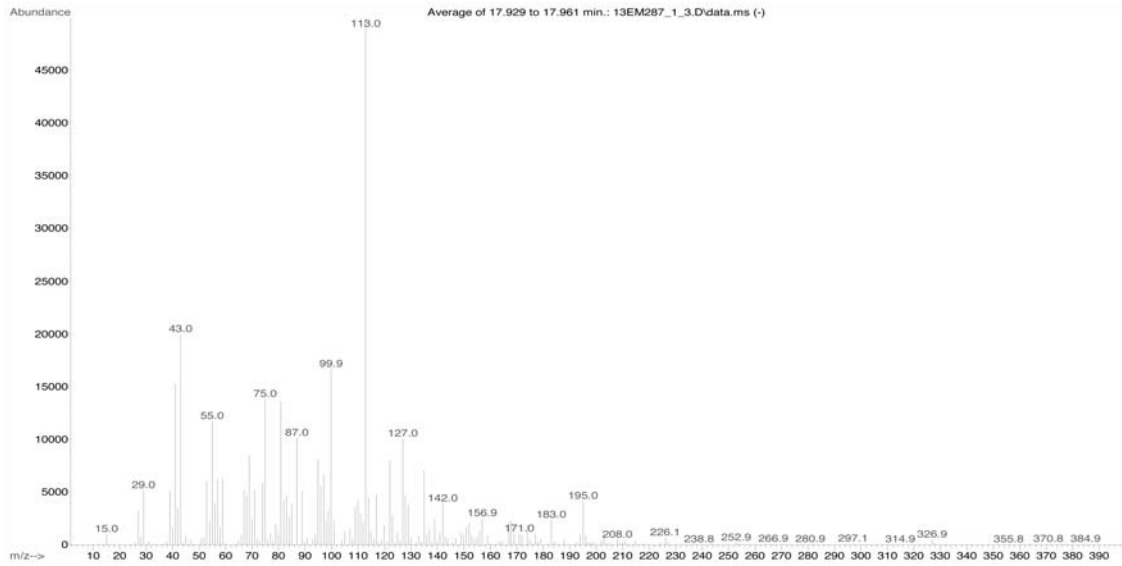
A

File   :\\5973ms\D\data\13EM287 - Huedo\13EM287_1_2.D
Operator   : MBR/Huedo
Acquired   : 19 Nov 2013 17:08   using AcqMethod 13EM287-1.M
Instrument :   5973N
Sample Name: 13EM287-2
Misc Info :
Vial Number: 2

Average of 17.993 to 18.020 min.: 13EM287_1_2.D\data.ms (-)

B

File   :\\5973ms\D\data\13EM287 - Huedo\13EM287_1_3.D
Operator   : MBR/Huedo
Acquired   : 19 Nov 2013 18:06   using AcqMethod 13EM287-1.M
Instrument :   5973N
Sample Name: 13EM287-3
Misc Info :
Vial Number: 3

Average of 17.929 to 17.961 min.: 13EM287_1_3.D\data.ms (-)

C

File   :\\5973ms\D\data\13EM287 - Huedo\13EM287_1_6.D
Operator : MBR/Huedo
Acquired : 19 Nov 2013 21:00   using AcqMethod 13EM287-1.M
Instrument :   5973N
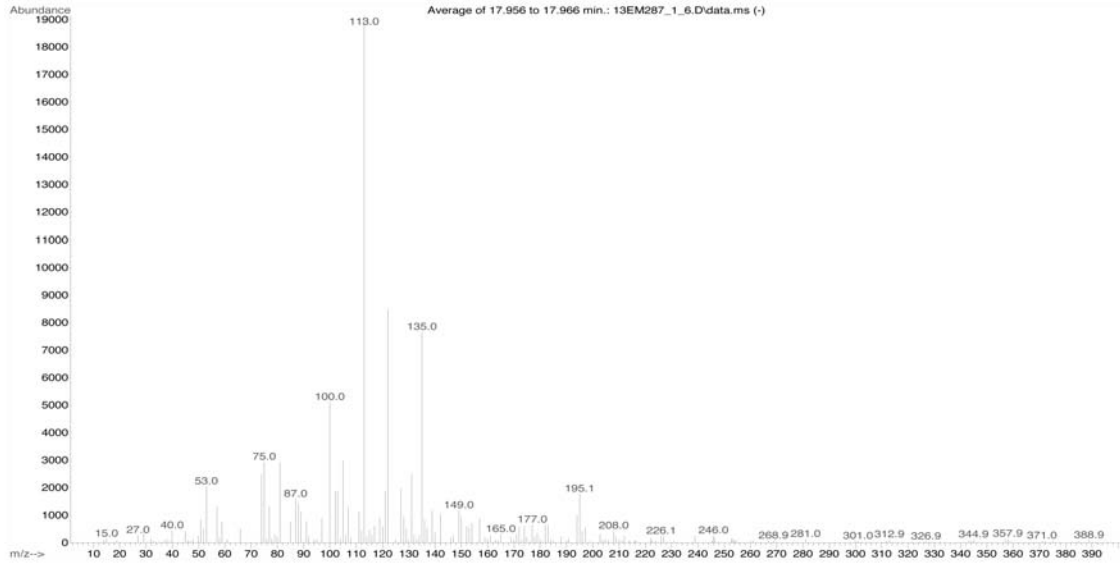Sample Name: 13EM287-6
Misc Info :
Vial Number: 6

D

File   :\\5973ms\D\data\13EM287 - Huedo\13EM287_1_7.D
Operator : MBR/Huedo
Acquired : 19 Nov 2013 21:58   using AcqMethod 13EM287-1.M
Instrument :   5973N
Sample Name: 13EM287-7
Misc Info :
Vial Number: 7
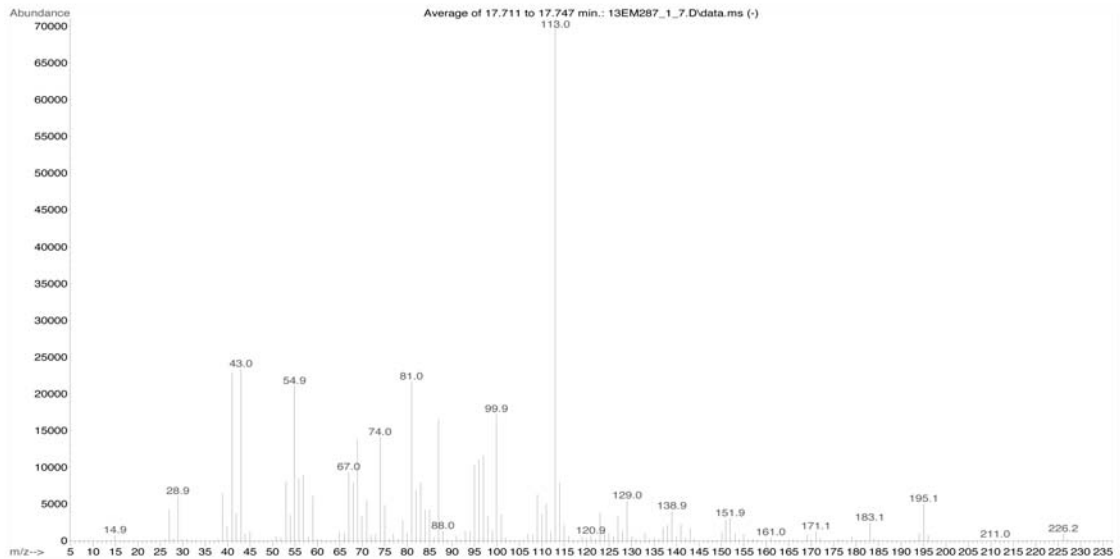
FIG S3 Mass spectra of the Gas Chromatography peaks with DSF activity (17,9 min.) (see also Fig. 4). (A) E77, (B) E77 Δ*rpfF* prpfFE77, (C) M30 Δ*rpfF* prpfFM30 and (D) Synthetic DSF.
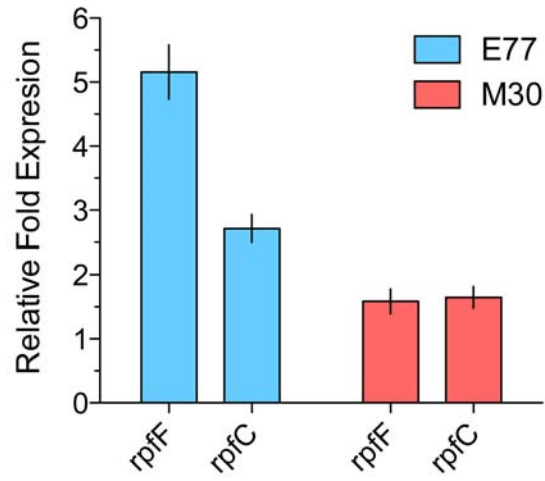
FIG S4 Quantification of gene expression of *rpfF* and *rpfC* from strains E77 and M30 normalized with the gene expression of the housekeeping gene *gyrA*, using the $2^{-\Delta\Delta Ct}$ method.

TABLE S1. *S. maltophilia* clinical isolates used in this study

Strain	RpfF Variant	Source	Hospital	Country
OL11	1	Oropharinx	Ljubljana Medical Center	SLO
PL12	1	Perineum	Ljubljana Medical Center	SLO
OL13	1	Oropharinx	Ljubljana Medical Center	SLO
OL16	2	Oropharinx	Ljubljana Medical Center	SLO
PL20	1	Perineum	Ljubljana Medical Center	SLO
OR41	2	Oropharinx	Hopital Raymond Poincare, Paris	FRA
PR45	2	Perineum	Hopital Raymond Poincare, Paris	FRA
PR59	2	Perineum	Hopital Raymond Poincare, Paris	FRA
OR79	1	Oropharinx	Hopital Raymond Poincare, Paris	FRA
OS87	2	Oropharinx	Hopital St Joseph, Paris	FRA
OS91	2	Oropharinx	Hopital St Joseph, Paris	FRA
PU100	1	Perineum	University of Antwerp	BEL
PU101	1	Perineum	University of Antwerp	BEL
PU102	1	Oropharinx	University of Antwerp	BEL
PU109	2	Perineum	University of Antwerp	BEL
OU110	2	Oropharinx	University of Antwerp	BEL
OU111	1	Oropharinx	University of Antwerp	BEL
PU140	2	Perineum	University of Antwerp	BEL
OU141	2	Oropharinx	University of Antwerp	BEL
OU148	1	Oropharinx	University of Antwerp	BEL
OU152	1	Oropharinx	University of Antwerp	BEL
PU153	2	Perineum	University of Antwerp	BEL
OG156	2	Oropharinx	University Clinic of Respiratory and Allergic Disease, Golnik	SLO
PG157	1	Perineum	University Clinic of Respiratory and Allergic Disease, Golnik	SLO
PC184	1	Perineum	ChariteClinic, Berlin	GER
PC185	1	Perineum	ChariteClinic, Berlin	GER
PC186	1	Perineum	ChariteClinic, Berlin	GER
PC187	1	Perineum	ChariteClinic, Berlin	GER
OC194	1	Oropharinx	ChariteClinic, Berlin	GER
OC195	1	Oropharinx	ChariteClinic, Berlin	GER
OC196	1	Oropharinx	ChariteClinic, Berlin	GER
OC197	1	Oropharinx	ChariteClinic, Berlin	GER
PC226	1	Perineum	ChariteClinic, Berlin	GER
PC227	1	Perineum	ChariteClinic, Berlin	GER
PC228	1	Perineum	ChariteClinic, Berlin	GER
PC229	1	Perineum	ChariteClinic, Berlin	GER
PC230	1	Perineum	ChariteClinic, Berlin	GER
PC231	1	Perineum	ChariteClinic, Berlin	GER
PC232	1	Perineum	ChariteClinic, Berlin	GER
PC237	2	Perineum	ChariteClinic, Berlin	GER
PC238	2	Perineum	ChariteClinic, Berlin	GER
PC239	2	Perineum	ChariteClinic, Berlin	GER
PC240	2	Perineum	ChariteClinic, Berlin	GER
PC245	1	Perineum	ChariteClinic, Berlin	GER
PC246	1	Perineum	ChariteClinic, Berlin	GER
PC254	2	Perineum	ChariteClinic, Berlin	GER
PC255	2	Perineum	ChariteClinic, Berlin	GER
PC256	2	Perineum	ChariteClinic, Berlin	GER
PC257	2	Perineum	ChariteClinic, Berlin	GER
PC271	1	Perineum	ChariteClinic, Berlin	GER
PC272	1	Perineum	ChariteClinic, Berlin	GER
PC273	1	Perineum	ChariteClinic, Berlin	GER
PC274	1	Perineum	ChariteClinic, Berlin	GER
PC312	2	Perineum	ChariteClinic, Berlin	GER
PC313	2	Perineum	ChariteClinic, Berlin	GER
PC314	2	Perineum	ChariteClinic, Berlin	GER
PC315	2	Perineum	ChariteClinic, Berlin	GER
OC322	2	Oropharinx	ChariteClinic, Berlin	GER
OC323	2	Oropharinx	ChariteClinic, Berlin	GER

OU343	1	Oropharinx	University of Antwerp	BEL
OU353	1	Oropharinx	University of Antwerp	BEL
ATCC13637	1	Collection	ATCC	USA
UV74	2	Vascular Ulcera	Hospital Municipal de Badalona	ESP
L9.5R5	1	(1)	Hospital Clínic de Barcelona	ESP
4834-R	2	(2)	Hospital Clínic de Barcelona	ESP
D457	2	(3)	(3)	ESP
K279	1	Blood Infection	(4)	GBR
E77	1	Sputum	Hospital Municipal Badalona	ESP
E50	1	Sputum	Hospital Municipal Badalona	ESP
M30	2	Decubitus ulcer	Hospital Municipal Badalona	ESP
S3149	1	Surgical wound	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP
P626	1	Oropharinx	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP
P815	1	Oropharinx	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP
E5520	2	Environmental control	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP
B5565	1	Bronchitis	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP
S5720	1	Sputum	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP
H5726	1	Hematologic neoplasia	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP
S289	2	Sputum	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP
B285	1	Bronchitis exudate	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP
B5742	1	Bronchitis exudate	Hospital Universitari Germans Trias i Pujol, Barcelona	ESP

TABLE S2. Strains used in mutant generation, complementation and DSF bioassay

Strains	Relevant characteristics	Reference
Stenotrophomonas maltophilia		
E77 Δ*rpfF*	E77 Δ*rpfF* mutant, *Erm*^{r}	This work
E77 prpfFE77	E77 wild type harboring prpfFE77, *Cm*^{r}	This work
E77 pBBR1MCS1-Cm	E77 wild type harboring pBBR1MCS1-Cm *Cm*^{r}	This work
E77 Δ*rpfF* pBBR1MCS1-Cm	E77 Δ*rpfF* harboring pBBR1MCS1 *Erm*^{r} *Cm*^{r}	This work
E77 Δ*rpfF* prpfFE77	E77 Δ*rpfF* harboring prpfFE77 *Erm*^{r} *Cm*^{r}	This work
E77 Δ*rpfF* prpfFM30	E77 Δ*rpfF* harboring prpfFM30 *Erm*^{r} *Cm*^{r}	This work
E77 prpfGCE77	E77 wild type harboring prpfGCE77, *Cm*^{r}	This work
E77 prpfGCM30	E77 wild type harboring prpfGCM30, *Cm*^{r}	This work
M30 Δ*rpfF*	M30 Δ*rpfF* mutant, *Erm*^{r}	This work
M30 prpfFM30	M30 wild type harboring prpfFM30, *Cm*^{r}	This work
M30 pBBR1MCS1-Cm	M30 wild type harboring pBBR1MCS1-Cm *Cm*^{r}	This work
M30 Δ*rpfF* pBBR1MCS1-Cm	M30 Δ*rpfF* harboring pBBR1MCS1 *Erm*^{r} *Cm*^{r}	This work
M30 Δ*rpfF* prpfM30	M30 Δ*rpfF* harboring prpfFM30 *Erm*^{r} *Cm*^{r}	This work
M30 Δ*rpfF* prpfFE77	M30 Δ*rpfF* harboring prpfFE77 *Erm*^{r} *Cm*^{r}	This work
M30 Δ*rpfC*	M30 Δ*rpfC* mutant, *Erm*^{r}	This work
M30 Δ*rpfC* prpfGCE77	M30 Δ*rpfC* wild type harboring prpfGCE77, *Cm*^{r}	This work
M30 Δ*rpfC* prpfGCM30	M30 Δ*rpfC* wild type harboring prpfGCM30, *Cm*^{r}	This work
Escherichia coli		
DH5α	*fhuA2 lac(del)U169 phoA glnV44Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	Lab collection
DH5α pEX18Tc	DH5α harboring suicide vector pEX18Tc, *Tc*^{r}	Lab collection
DH5α pGEM-*rpfF*E77UP	DH5α harboring pGEM-*rpfF*E77UP, *Ap*^{r}	This work
DH5α pGEM-*rpfF*E77DW	DH5α harboring pGEM-*rpfF*E77DW, *Ap*^{r}	This work
DH5α pEXE77*rpfF*	DH5α harboring suicide vector pEXE77*rpfF Tc*^{r}, *Erm*^{r}	This work
DH5α prpfFE77	DH5α harboring prpfFE77	This work
DH5α pGEM-*rpfF*M30UP	DH5α harboring pGEM-*rpfF*M30UP	This work
DH5α pGEM-*rpfF*M30DW	DH5α harboring pGEM-*rpfF*M30DW	This work
DH5α prpfFM30	DH5α harboring prpfFM30	This work
DH5α prpfFE77	DH5α harboring prpfFME77	This work
DH5α pEXM30*rpfC*	DH5α harboring suicide vector pEXM30*rpfC Tc*^{r}, *Erm*^{r}	This work
DH5α prpfGCM30	DH5α harboring prpfGCM30	This work
DH5α prpfGCE77	DH5α harboring prpfGCE77	This work
DH5a pEXM30*rpfF*	DH5a harboring pEXM30*rpfF*	This work
OP50	Non-pathogenic strain, *C. elegans* maintenance	C.G.C.
Xanthomonas campestris pv. *campestris*		
Xcc 8523 pL6engGUS	*rpfF* mutant, DSF reporter strain, carring plasmid pL6engGUS, *Tc*^{r}, *Kan*^{r}	(5)

TABLE S3. Primers used in this study

Primer	Sequence 5'-3'	Restriction Site
Erythromycin resistance gene		
P5Erm	GGATCCGAAACGTAAAAGAAGTTATG	BamHI
P3Erm	GGATCCTACAAATTCCCCGTAGGC	BamHI
P5Ermrev	GATACTGCACTATCAACACAC	-
P3Ermrev	CTTCCAAGGAGCTAAAGAGGT	-
rpfF determination		
PrpfFtypeUp	GCAGAAGACCAACGTCGGCAAG	-
PrpfFtypeDw	CTTCCTAGGCGACGATGGTGTG	-
qRT-PCR		
P1rpfC-RT	GTTCCGCACGCCGTTGAA	-
P2rpfC-RT	CGAGGCCTGGATGGTGTT	-
P1rpfF-RT	CTGAGCTGCCACACCATC	-
P2rpfF-RT	GAACAGCACCTCCGGCAG	-
P1gyrA-RT	GTCGACGGCCAGGGTAAC	-
P2gyrA-RT	GCCTCGGTGTATCGCATT	-
E77		
P1E77rpfFUp	GGATCCAGTTCTCCGTGTGACCGTCC	HindIII
P2E77rpfFUp	AAGCTTCGAGGACGTCATCGCGATGAT	BamHI
P3E77rpfFDw	GAATTCTGGGCATGGGCGATGGCTTC	BamHI
P4E77rpfFDw	GGATCCTCGTCTGGCGGGTCAGGACG	EcoRI
P1DemRpfFE77	TCGACCGCAAGAAGGACATGA	-
P2DemRpfFE77	TCGGCGATGGCGTGCTCGATA	-
ProrpfFE77FUp	AAGCTTCGTGAAGGTCGTGATCGTGAAGAA	HindIII
ProrpfFE77FDw	TCTAGAACGATCAGGCCGGGTCGCCAT	XbaI
P1RpfFE77FInt	TGGTCGACATTCGTTGATACAC	-
P2RpfFE77FInt	GCACGTCCTGTCACCGCAT	-
P1rpfGCE77	ATCAAGCTTTGCTGCTGTTCCCGTACAT	HindIII
P2rpfGCE77	CATCTAGATCTGGGTAGACACTGCGATG	XbaI
M30		
P1M30rpfFUp	GCGGATCCAACGGTCCACACACGCGG	XbaI
P2M30rpfFUp	CATCTAGATGGTGTCCGGCTTCAACG	BamHI
P3M30rpfFDw	CTGAATTCACTGGCTTCGCTGGGCAT	BamHI
P4M30rpfFDw	CCGGATCCCAGCGCGCTTCACCATCA	EcoRI
P1DemRpfFM30	ATCCAGCGCTGTACTCAG	-
P2DemRpfFM30	CAGGTGATGAAGGGCTACT	-
ProrpfFM30Up	GCCAAGCTTGGGTTTGCGGCAATCTGGACAG	HindIII
ProrpfFM30Dw	GGTCTAGACGATGATGGTGAAGCGCGCTGA	XbaI
P1RpfFM30Int	GCCATGCGTCTGACAT	-
P2RpfFM30Int	AGACAATACTTGCTCAT	-
P1M30rpfCUp	AGGAATTCCCAGAATCGCTTCATCCAGGT	EcoRI
P2M30rpfCUp	GCGGATCCAGCTGCGACAACAGGCGTTTC	BamHI
P3M30rpfCDw	ACGGATCCTGATGGTGAAGCGCGCTGA	BamHI
P4M30rpfCDw	GCTCTAGACCCCATCTGGCCGAGAAGA	XbaI
P1DemRpfCM30	GAGGAAGAGCTGGCGATCAT	-

P2DemRpfCM30	GCGACCTCCTGCTGAACTAC	-
P1prpfGCM30	GTCAAGCTTGATCCGCGATGTACTGCTGT	HindIII
P2rpfGCM30	TCGTCTAGAAGTCTCGACGCGGCCTGATT	XbaI
P1rpfCM30int	ATCGGTCTGATCCTGTTGCCA	-
P2rpfCM30int	TTCCAGATAGCCGATGTCACC	-

TABLE S4. Plasmids used in this study

Plasmids	Relevant Characteristics	Reference
pL6engGUS	DSF reporter plasmid, transcriptional phusion *engXCA::gus, Tc^r, Kan^r	(5)
pEX18Tc	Suicide allelic exchange vector; *Tc^r*	(6)
pBBR1MCS1Cm	Broad-host-range cloning vector, *Cm^r*	(7)
pGEM-T Easy Vector	Cloning vector, *Ap^r*	PROMEGA
pEX18TcErm	pEX18Tc carring *Erm* resistance gene, *Erm^r, Tc^τ*	Lab. collection
pGEM-Erm	pGEM-T carring *Erm* resistance gene *Erm^r, Tc^r*	Lab. collection
pGEM-*rpfF*E77FUP	Cloning vector, carring E77F *rpfF* upstream flanking region, *Ap^r*	This work
pGEM-*rpfF*E77FDW	Cloning vector, carring E77F *rpfF* downstream flanking region, *Ap^r*	This work
pEXE77*rpfF*	pEX18Tc suicide vector, carring E77F *rpfF* flanking regions interrumpted with *Erm* gene, *Tc^r, Erm^r*	This work
prpfFE77F	Complementation vector, pBBR1MCS1Cm carring *rpfF* promoter and its ORF from E77F strain, *Cm^r*	This work
pGEM-*rpfF*M30UP	Cloning vector, carring M30 *rpfF* upstream flanking region, *Ap^r*	This work
pGEM-*rpfF*M30DW	Cloning vector, carring M30 *rpfF* downstream flanking region, *Ap^r*	This work
pEXM30*rpfF*	pEX18Tc suicide vector, carring M30 *rpfF* flanking regions interrumpted with *Erm* gene, *Tc^r, Erm^r*	This work
prpfFM30	Complementation vector, pBBR1MCS1Cm carring *rpfF* promoter and its ORF from M30 strain, *Cm^r*	This work
pEXM30*rpfC*	pEX18Tc suicide vector, carring M30 *rpfC* flanking regions interrumpted with *Erm* gene, *Tc^r, Erm^r*	This work
prpfGCM30	Complementation vector, pBBR1MCS1Cm carring *rpfGC* operon from M30 strain, *Cm^r*	This work
prpfGCE77	Complementation vector, pBBR1MCS1Cm carring *rpfGC* operon from E77 strain, *Cm^r*	This work

REFERENCES

1. **Ribera A, Ruiz J, Jiminez de Anta MT, Vila J**. 2002. Effect of an efflux pump inhibitor on the MIC of nalidixic acid for Acinetobacter baumannii and Stenotrophomonas maltophilia clinical isolates. J. Antimicrob. Chemother. **49**:697–698.
2. **Ribera A, Jurado A, Ruiz J, Marco F, Del Valle O, Mensa J, Chaves J, Hernández G, Jiménez de Anta MT, Vila J**. 2002. In vitro activity of clinafloxacin in comparison with other quinolones against Stenotrophomonas maltophilia clinical isolates in the presence and absence of reserpine. Diagn. Microbiol. Infect. Dis. **42**:123–128.
3. **Alonso A, Martinez JL**. 1997. Multiple antibiotic resistance in Stenotrophomonas maltophilia. Antimicrob. Agents Chemother. **41**:1140–1142.
4. **Fouhy Y, Scanlon K, Schouest K, Spillane C, Crossman L, Avison MB, Ryan RP, Dow JM**. 2007. Diffusible signal factor-dependent cell-cell signaling and virulence in the nosocomial pathogen Stenotrophomonas maltophilia. J. Bacteriol. **189**:4964–4968.
5. **Slater H, Alvarez-Morales A, Barber CE, Daniels MJ, Dow JM**. 2000. A two-component system involving an HD-GYP domain protein links cell-cell signalling to pathogenicity gene expression in Xanthomonas campestris. Mol. Microbiol. **38**:986–1003.
6. **Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP**. 1998. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa mutants. Gene **212**:77–86.
7. **Kovach ME, Phillips RW, Elzer PH, Roop RM 2nd, Peterson KM**. 1994. pBBR1MCS: a broad-host-range cloning vector. BioTechniques **16**:800–802.