Running title: Genome-wide mutational analysis of *S. maltophilia* biofilm

Supplementary Information

Genome-Wide Identification of Genes Necessary for Biofilm Formation of Nosocomial Pathogen *Stenotrophomonas maltophilia* Reveals Orphan Response Regulator FsnR is a Critical Modulator

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Table S1. TAIL-PCR identification of genes associated with biofilm formation of *Stenotrophomonas maltophilia*

Library ID	**Gene name**	**Gene code**[1]	**Insertion site**	**Putative function or description**	**Relative production of biofilm**[2]
Flagellar and pili					
SMA21-B18	*flgE*	Smlt2314	2356638	flagellar hook protein FlgE	0.09 ± 0.02
SMA24-K12	*flgE*	Smlt2314	2357167	flagellar hook protein FlgE	0.06 ± 0.02
SMA23-F7	*flgK*	Smlt2308	2351423	flagellar hook-associated protein FlgK	0.20 ± 0.02
SMA22-P5	*flgK*	Smlt2308	2350254	flagellar hook-associated protein FlgK	0.24 ± 0.02
SMA7-D19	*flgG*	Smlt2312	2355626	flagellar basal body rod protein FlgG	0.16 ± 0.02
SMA3-A22	*flgH*	Smlt2311	2354349	flagellar basal body L-ring protein	0.29 ± 0.02
SMA12-E14	*flhF*	Smlt2272	2314389	flagellar biosynthesis regulator FlhF	0.10 ± 0.02
SMA33-M8	*flgI*	Smlt2310	2354014	flagellar basal body P-ring protein	0.09 ± 0.02
SMA36-A20	*flhA*	Smlt2273	2315325	flagellar biosynthesis protein FlhA	0.04 ± 0.02
SMA24-L1	*fliI*	Smlt2286	2329126	flagellum-specific ATP synthase	0.04 ± 0.02
SMA5-N23	*fliI*	Smlt2286	2329375	flagellum-specific ATP synthase	0.15 ± 0.02
SMA1-O11	*fliN*	Smlt2281	2325341	flagellar motor switch protein	0.02 ± 0.02
SMA7-L17	*fliN*	Smlt2281	2325099	flagellar motor switch protein	0.04 ± 0.02
SMA5-B13	*fliD*	Smlt2303	2344144	flagellar hook-associated protein 2	0.29 ± 0.02
SMA8-H1	*fliD*	Smlt2303	2343369	flagellar hook-associated protein 2	0.22 ± 0.02
SMA20-O1	*fliD*	Smlt2303	2343922	flagellar hook-associated protein 2	0.11 ± 0.02
SMA29-J9	*fliO*	Smlt2280	2324844	flagellar protein FliO	0.03 ± 0.02
SMA27-G19	*fliF*	Smlt2289	2332513	flagellar MS-ring protein	0.48 ± 0.02
SMA22-G16	*fliF*	Smlt2289	2332351	flagellar MS-ring protein	0.13 ± 0.02
SMA33-D16	*fliM*	Smlt2282	2325508	flagellar motor switch protein FliM	0.08 ± 0.02
SMA29-N19	*fliK*	Smlt2284	2327270	flagellar hook-length control protein	0.06 ± 0.02
SMA37-A8		Smlt0709	739626	fimbria adhesin protein	0.06 ± 0.02

SMA10-I4		Smlt0707	736153	pili chaperone protein	0.00 ± 0.02
SMA26-D10		Smlt0707	736381	pili chaperone protein	0.05 ± 0.02
SMA21-J12		Smlt0707	735772	pili chaperone protein	0.03 ± 0.02
SMA1-D14	*fimV*	Smlt3426	3467204	fimV protein	0.40 ± 0.02
Polysaccharide biosynthesis and carbohydrate metabolism					
SMA11-N24	*xanA*	Smlt0653	670657	phosphohexosemutase	0.00 ± 0.02
SMA24-K24[3]		Smlt3804	3885560	glyceraldehyde 3-phosphate dehydrogenase	1.19 ± 0.02
SMA6-A2		Smlt4259	4372983	uracil-DNA glycosylase	1.21 ± 0.02
SMA26-B22		Smlt0490	500892	pyruvate dehydrogenase subunit E1	2.08 ± 0.02
Purine biosynthesis					
SMA34-L13	*purE*	Smlt1613	1676463	phosphoribosylaminoimidazole carboxylase	0.47 ± 0.02
SMA2-D12	*purK*	Smlt1614	1677627	phosphoribosylaminoimidazole carboxylase ATPase subunit	0.40 ± 0.02
SMA5-E20	*purK*	Smlt1614	1677333	phosphoribosylaminoimidazole carboxylase ATPase subunit	0.56 ± 0.02
SMA28-I22	*purD*	Smlt4254	4369733	phosphoribosylamine--glycine ligase	0.41 ± 0.02
SMA28-E14	*purC*	Smlt4318	4434102	phosphoribosylaminoimidazolesuccinocarboxamide synthase	0.52 ± 0.02
SMA25-F12	*purC*	Smlt4318	4434385	phosphoribosylaminoimidazolesuccinocarboxamide synthase	0.52 ± 0.02
SMA34-D21	*purL*	Smlt0684	708539	phosphoribosylformylglycinamidine synthase	0.28 ± 0.02
SMA14-N11	*guaA*	Smlt2072	2098217	GMP synthase	0.01 ± 0.02
Transportation					
SMA21-I12	*mrkC*	Smlt0708	738300	outer membrane usher protein mrkc precursor	0.06 ± 0.02
SMA25-J1	*mrkC*	Smlt0708	738264	outer membrane usher protein mrkc precursor	0.05 ± 0.02
SMA1-M13	*mrkC*	Smlt0708	737324	outer membrane usher protein mrkc precursor	0.03 ± 0.02
SMA23-L17	*mrkC*	Smlt0708	739000	outer membrane usher protein mrkc precursor	0.04 ± 0.02
SMA2-C16	*mrkC*	Smlt0708	737190	outer membrane usher protein mrkc precursor	0.13 ± 0.02
SMA20-H21		Smlt4552	4672328	glutathione-regulated cation-efflux system	0.06 ± 0.02
SMA26-E2		Smlt0845	887435	transmembrane transport protein	0.12 ± 0.02
Gene regulation					
SMA4-B6		Smlt2299	2340975	response regulator protein LuxR family	0.03 ± 0.02

SMA7-F19		Smlt2297	2340048	RNA polymerase sigma-54 factor (sigma-N)	0.03 ± 0.02
SMA31-L10	*fliA*	Smlt2270	2311509	RNA polymerase sigma factor for flagellar	0.04 ± 0.02
SMA32-N20	*ravS*	Smlt2324	2366132	PAS domain sensor histidine kinase	0.16 ± 0.02
SMA27-H15	*glnL*	Smlt0158	172801	nitrogen regulation protein NR(II)	0.55 ± 0.02
Peptide and lipid biosynthesis					
SMA26-C23		Smlt0208	222379	peptide synthase	0.35 ± 0.02
SMA11-F15		Smlt0208	222773	peptide synthase	0.39 ± 0.02
SMA26-F10		Smlt0208	223044	peptide synthase	0.56 ± 0.02
SMA23-C6		Smlt0208	222148	peptide synthase	0.56 ± 0.02
SMA15-G14		Smlt0208	221659	peptide synthase	0.56± 0.02
SMA27-O8		Smlt0208	222499	peptide synthase	0.51 ± 0.02
SMA18-J16		Smlt0418	427771	aminopeptidase	0.16 ± 0.02
SMA30-B20		Smlt0625	641485	lipase	1.32 ± 0.02
Oxidoreduction					
SMA25-G12		Smlt0209	223554	NAD(P)H dehydrogenase	0.40 ± 0.02
SMA36-C6		Smlt0209	223158	NAD(P)H dehydrogenase	0.38 ± 0.02
SMA20-E12		Smlt0209	223345	NAD(P)H dehydrogenase	0.59 ± 0.02
SMA3-K17	*pntB*	Smlt0857	897800	NAD(P) transhydrogenase subunit beta PntB	0.32 ± 0.02
SMA14-L15		Smlt3167	3208008	NAD-dependent glutamate dehydrogenase	0.51 ± 0.02
Function unknown					
SMA30-E7		Smlt0276	293008	hypothetical protein	0.27 ± 0.02
SMA31-C20		Smlt3195	3236474	hypothetical protein	0.44 ± 0.02
SMA28-M23		Smlt0416	393052	hypothetical protein	1.44 ± 0.02
SMA34-E19		Smlt0639	656017	hypothetical protein	0.10 ± 0.02
Transposable elements					
SMA14-I22		Smlt0518	526451	transposase	0.47 ± 0.02
Intergenic region					
SMA10-P7		Smlt0704-Smlt0706		Intergenic region	0.12 ± 0.02

SMA13-I9	Smlt0417-Smlt0418		Intergenic region	0.50 ± 0.02
SMA35-A20	Smlt0722-Smlt0723		Intergenic region	0.42 ± 0.02
SMA37-D2	Smlt4645-Smlt4647		Intergenic region	0.06 ± 0.02
Undefined (inserted in multi-copy locus)				
SMA31-H5		416255、421919、4792803、4787139	ND	0.10 ± 0.02
SMA21-O22		416470、422134、4786924、4792588	ND	1.70 ± 0.02

1. Gene code and names are according to the genomic annotation of *S. maltophilia* K279a
2. Relative production of biofilm of mutants are compared to that of the wild-type (set as unit). Standard deviations are shown (n = 3)
3. Mutants in red highlighted that mutation of these genes caused increase in biofilm formation.

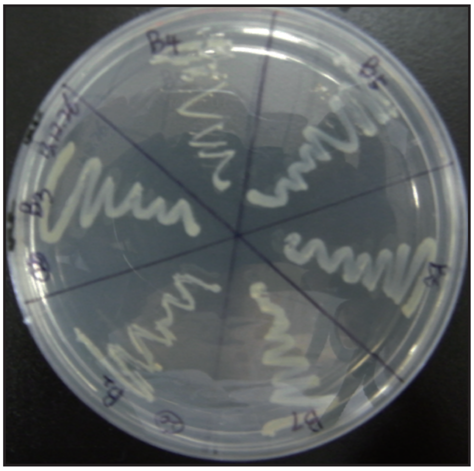

FIG S1. Stability of the EZ::TN transposon inserted mutants. Six transformants from the mutant library were randomly selected. The bacterial progenies were grown in NYG agar without any antibiotics for 20 rounds of subcultures under 28°C. After subculture, bacterial strains were inoculated onto a NYG agar containing kanamycin (50 µg/ml) and cultured for 48 hours under 28°C.

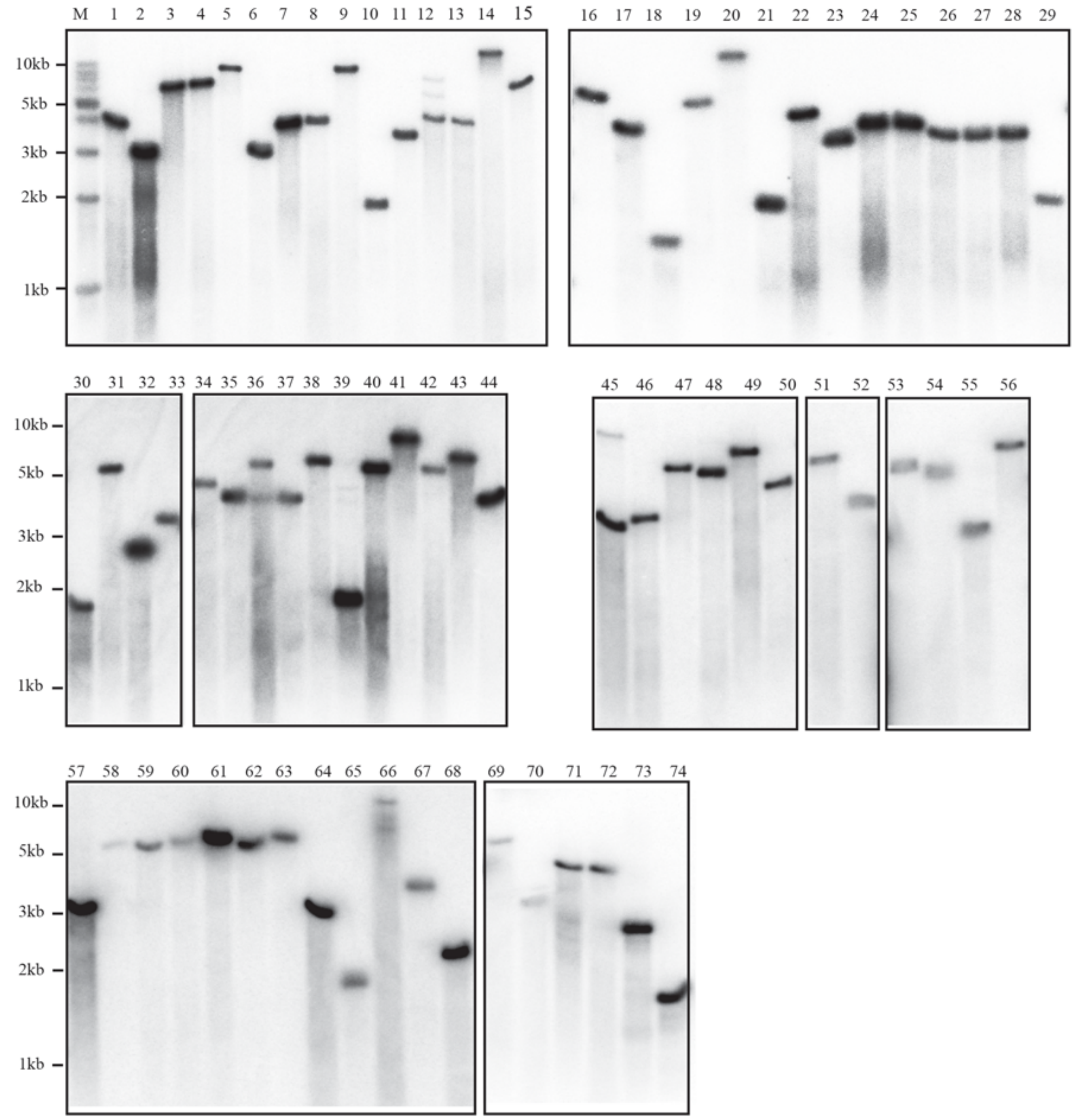

FIG. S2. Determination of insertional copy numbers of identified mutants. Transposon insertion numbers were analyzed by Southern blotting. Total DNA of the bacterial strains were digested by SmaI and separated in 1% agarose gel. A PCR product of EZ::TN transposon was labeled by [α-32P]-dCTP and used as DNA probe in hybridazation. M: 1 kb DNA ladder. The numbers of lanes are according to the codes of mutant library and Table S1, as follows:

1: SMA11-F15, 2: SMA7-F19, 3: SMA5-N23, 4: SMA33-M8, 5: SMA31-L10, 6: SMA4-B6
7: SMA15-G14,8: SMA20-E12, 9: SMA12-E14, 10: SMA3-A22, 11: SMA23-C6, 12: SMA8-H1
13: SMA20-O1, 14: SMA28-I22, 15: SMA24-L1, 16: SMA33-D16, 17: SMA25-G12, 18: SMA34-D21
19: SMA24-K12, 20: SMA31-H5, 21: SMA28-E14, 22: SMA5-B13, 23: SMA23-L17, 24: SMA27-O8
25: SMA26-F10, 26: SMA1-M13,27: SMA25-J1, 28: SMA21-I12, 29: SMA27-H15, 30: SMA25-F12
31: SMA5-E20, 32: SMA10-P7, 33: SMA35-A20, 34: SMA21-B18, 35: SMA26-D10, 36: SMA18-J16
37: SMA21-J12, 38: SMA3-K17, 39: SMA13-I9, 40: SMA14-I22, 41: SMA1-D14, 42: SMA37-A8
43: SMA34-E19, 44: SMA36-C6, 45: SMA2-C16, 46: SMA26-C23, 47: SMA32-N20, 48: SMA29-N19
49: SMA36-A20, 50: SMA29-J9, 51: SMA27-G19, 52: SMA10-I4, 53: SMA34-L13, 54: SMA22-G16
55: SMA7-D19, 56: SMA14-L15, 57: SMA14-N11, 58: SMA37-D2, 59: SMA7-L17, 60: SMA23-F7
61: SMA22-P5, 62: SMA2-D12, 63: SMA1-O11, 64: SMA11-N24, 65: SMA31-C20, 66: SMA 26-E2
67: SMA30-E7, 68: SMA20-H21, 69: SMA30-B20, 70: SMA26-B22, 71: SMA6-A2, 72: SMA28-M23
73: SMA21-O22, 74: SMA24-K24