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Supporting information for article:

Crystal structures of PigF, an *O*-methyltransferase involved in the prodigiosin synthetic pathway, reveal an induced-fit substrate-recognition mechanism

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Figure S2 The maximum absorption wavelength of the prodigiosin from the different complementary strains. A-D represents the FS14-pMTKQS, the FS14ΔpigF-pMTKQS, the complementary strains FS14ΔpigF-pMTKQS-*pigF* and the overexpress strain FS14-pMTKQS-*pigF*; E-N represents the H98A, H247A, D248A, E275A, N294A, E304A, H247AD248A, F148A, S298A and W131A point mutant complementary strains, respectively.

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Figure S3 Sequence alignment of PigF from different strains. The conserved residues are highlighted with a red background, residues (His98, His247, Asp248, Glu275 and Glu304) are represented with star.

Strains and plasmids	Relevant genotype or description	References or source
Strains		
S. marcescens FS14	wild type strain	(Li et al., 2015)
FS14∆PigF	In-frame deletion mutant of $pigF$ gene	This study
Plasmids		
pWDF	Suicide plasmid for construction deletion	(Wu et al., 2016)
	mutants	
pMTKQS	plasmid for complementary assay	This study
pMTKQS-pigF	<i>pigF</i> gene in pMTKQS	This study
pMTKQS-pigF ^{H98A}	<i>pigF^{H98A}</i> gene in pMTKQS	This study
pMTKQS- <i>pigF</i> ^{H247A}	<i>pigF^{H247A}</i> gene in pMTKQS	This study
pMTKQS-pigF ^{H248A}	<i>pigF^{H248A}</i> gene in pMTKQS	This study
pMTKQS-pigF ^{E275A}	<i>pigF^{E275A}</i> gene in pMTKQS	This study
pMTKQS- <i>pigF</i> ^{N294A}	<i>pigF^{N294A}</i> gene in pMTKQS	This study
pMTKQS-pigFE304A	<i>pigF^{E304A}</i> gene in pMTKQS	This study
pMTKQS- <i>pigF</i> ^{H247AD248A}	<i>pigF^{H247AD248A}</i> gene in pMTKQS	This study
pMTKQS-pigF ^{F148A}	<i>pigF^{F148A}</i> gene in pMTKQS	This study
pMTKQS-pigF ^{S298A}	pigF ^{S298A} gene in pMTKQS	This study
pMTKQS- <i>pigF^{W131A}</i>	<i>pigF^{WI31A}</i> gene in pMTKQS	This study

Primers	Amplified regions	Sequence (5'-3')
1	Forward primer for amplifying the upstream	ATTAGGGCCCATGGGCGCCTACTTTA
	homologous arm of <i>pigF</i>	AGCAG
2	Reverse primer for amplifying the upstream	GGCAATTTTCATGAAAAAGCCCATCA
	homologous arm of <i>pigF</i>	TCTGATTGAC
3	Forward primer for amplifying the downstream	ATGGGCTTTTTCATGAAAATTGCCGG
	homologous arm of <i>pigF</i>	CCCA
4	Reverse primer for amplifying the downstream	CCTGGATCTCCAGCGGCG
	homologous arm of <i>pigF</i>	
5	Forward primer for identification of mutant	GATCGCTTCCTGGTGCACAG
	FS14∆PigF	
6	Forward primer for the construction of	CGGATATAGTTCCTCCTTTCAGC
	complementation plasmid of <i>pigF</i>	
7	Reverse primer for the construction of	AGT <u>CTCGAG</u> TTATTTTTCGCCGACGA
	complementation plasmid of <i>pigF</i>	TCAGG
8	Forward primer for amplifying the downstream	GCTGGGCCGCGCCATCGATACCTTC
	homologous arm of $pigF^{H98A}$	
9	Reverse primer for amplifying the upstream	AGGAAGGTATCGATGGCGCGGCCCA
	homologous arm of $pigF^{H98A}$	GCCATC
10	Forward primer for amplifying the downstream	GGATGCTGGCCGACTACG
	homologous arm of $pigF^{H247A}$	
11	Reverse primer for amplifying the upstream	CGTAGTCGGCCAGCATCC
	homologous arm of $pigF^{H247A}$	
12	Forward primer for amplifying the downstream	ATGCTGCACGCCTACGCCC
	homologous arm of <i>pigF</i> ^{D248A}	
13	Reverse primer for amplifying the upstream	GGGCGTAGGCGTGCAGCATC
	homologous arm of $pigF^{D248A}$	
14	Forward primer for amplifying the downstream	TCGCTTCCGCAACGCCGC
	homologous arm of $pigF^{E275A}$	
15	Reverse primer for amplifying the upstream	GCGGCGTTGCGGAAGCGAT
	homologous arm of $pigF^{E275A}$	
16	Forward primer for amplifying the downstream	TGCTGTCGCGCCATGCTGGTCTCCAC
	homologous arm of $pigF^{N294A}$	
17	Reverse primer for amplifying the upstream	GTGGAGACCAGCATGGCCAGCGACA
	homologous arm of $pigF^{N294A}$	GCAG
18	Forward primer for amplifying the downstream	GTGGCATAGCGAGCAGCGC
	homologous arm of $pigF^{E304A}$	

Table S2Primers used in this study.

19	Reverse primer for amplifying the upstream	GCGCTGCTCGCTATGCCACC
	homologous arm of <i>pigF^{E304A}</i>	
20	Forward primer for amplifying the downstream	ATGCTGGCCGCCTACG
	homologous arm of $pigF^{H247AD248A}$	
21	Reverse primer for amplifying the upstream	CGTAGGCGGCCAGCATC
	homologous arm of $pigF^{H247AD248A}$	
22	Forward primer for amplifying the downstream	ACTTCCAGGAGCGTCTCGGTAAATTC
	homologous arm of <i>pigF^{F148A}</i>	G
23	Reverse primer for amplifying the upstream	CGAATTTACCGAGACGCTCCTGGAA
	homologous arm of <i>pigF^{F148A}</i>	G
24	Forward primer for amplifying the downstream	GAACATGCTGGTCGCCACCGACGGT
	homologous arm of <i>pigF</i> ^{S298A}	G
25	Reverse primer for amplifying the upstream	CACCGTCGGTGGCGACCAGCATGTTC
	homologous arm of <i>pigF^{S298A}</i>	
26	Forward primer for amplifying the downstream	ATGACCGCAGTGCGTTCGACATCCTG
	homologous arm of <i>pigF^{W131A}</i>	
27	Reverse primer for amplifying the upstream	CAGGATGTCGAACGCACTGCGGTCA
	homologous arm of <i>pigF^{W131A}</i>	Т

Underlines represent the restriction sites.

Table S3Data-collection and structure refinement statistics.

Values in parentheses are for the highest resolution shell.

Parameter	apo PigF (PDB:7CLU)	PigF+SAH (PDB:7CLF)
Data collection:		
Wavelength (Å)	0.9796	1.0088
Temperature (K)	100	100
Crystal-to-detector distance (mm)	220	250
Rotation range per image (°)	1.0	1.0
Total rotation range (°)	180	180
Space group	<i>P12</i> ₁ 1	$P12_{1}1$
Unit-cell parameters (Å)	a = 69.10,	a = 43.9,
	b = 52.36,	b = 109.2,
	c = 92.83,	c = 63.9,
	$\beta = 97.35$	$\beta = 93.1$
Resolution range (Å)	19.57–1.90 (1.94–1.90)	19.65–1.97 (2.02–1.97)
Observed reflections	105469 (7133)	159034 (11129)
Unique reflections	48618 (3366)	42182 (2963)
Multiplicity	2.2 (2.1)	3.8 (3.8)
Rpim (%)	0.052 (0.624)	0.078(0.567)
Completeness (%)	93.6 (96.5)	99.6 (99.8)
$\langle I \mid \delta(I) \rangle$	7.9 (1.2)	9.9 (1.5)
CC (1/2)	0.996 (0.506)	0.995 (0.628)
Structure refinement		
Total number of atoms	5273	5682
Number of reflections used	48590	41496
R work (%)	19.87	18.53
R free (%)	25.06	25.34
RMSD bonds (Å)	0.01	0.007
RMSD angles (°)	0.85	0.891
Ramachandran plot (%)		
Favoured	98.42	98.96
Allowed	1.42	1.04
Outlier	0.16	0

PDB ID	Z-score ^a	RMSD ^b	Residues	Indentity	% Protein	Reference
4A6D	32.9	2.7	346	27	Human N-acetylserotonin methyltransferase ASMT	Ref. (Botros et al., 2013)
3GWZ	30.8	2.5	340	23	Streptomyces lavendulae: mitomycin-7-O- methyltransferase MmcR	Ref. (Singh et al., 2011)
1TW3	30.6	2.7	340	23	Streptomyces peucetius: carminomycin 4- O-methyltransferase DnrK	Ref. (Jansson et al., 2004)
3158	30.0	2.9	329	21	<i>Streptomyces carzinostaticus</i> : neocarzinostatin O-methyltransferase NcsB1	Ref. (Cooke et al., 2009)
1XDS	28.6	3.0	336	24	Aclacinomycin 10-Hydroxylase RdmB	Ref. (Jansson et al., 2005)
1FP2	27.9	3.7	346	18	Medicago sativa: isoflavone O- methyltransferase IOMT	Ref. (Zubieta et al., 2001)
3P9I	27.7	3.7	358	23	Perennial Ryegrass: caffeic acid O- methyltransferase LpOMT1	Ref. (Louie et al., 2010)
2IP2	27.6	3.8	330	20	<i>Pseudomonas aeruginosa</i> : pyocyanin biosynthetic protein PhzM	Ref. (Parsons et al., 2007)
1KYW	27.4	3.6	361	22	Alfalfa: caffeic acid/5-hydroxyferulic acid 3/5-O-methyltransferase COMT	Ref. (Zubieta et al., 2002)
4EVI	27.0	3.4	361	20	<i>Linum nodiflorum</i> : coniferyl alcohol 9-O- methyltransferase Ca9OMT	Ref. (Wolters et al., 2013)
3LST	26.6	3.1	334	21	Orsellinic acid C3 O-methyltransferase CalO1	Ref. (Chang et al., 2011)
1FP1	25.6	4.3	341	22	Medicago sativa: chalcone O- methyltransferase ChOMT	Ref. (Zubieta et al., 2001)

Table S4 DALI Structural Similarity	Search.
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^a Z-score represents the statistical significance of the best domain-domain alignment.

^b RMSD represents the root mean square deviation of C atoms in rigid body superimposition.