## Fluoroacetate from the marine-derived bacterium Streptomyces

## xinghaiensis NRRL B-24674

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Materials and Methods

Medium and strains used in this study

*E. coli* DH10B and *E. coli* ET12567 (pUZ8002) were cultured in L Broth or L agar at 37 °C. *Streptomyces xinghaiensis* NRRL B-24674 was cultured on ISP4 which contains 18% (w/v) sea salt for spores formation, and cultured in liquid TSBY (which contains yeast extract 5g/L and tryptone soya broth 30g/L) for mycelium growth. DNA isolation and *Streptomyces* genetic manipulation were performed according to standard methods.<sup>1</sup>

In-frame deletion of *fl*K4

To inactivate *fl*K4, a 2325 bp upstream fragment and a 2169 bp downstream fragment were amplified from genomic DNA of *Streptomyces xinghaiensis* NRRL B-24674 by high fidelity PCR using the primers Flk-F1/Flk-R1 and Flk-R2/Flk-F2, respectively (Table S1). PCR was performed in 20  $\mu$ L of volume with 5% DMSO and KOD DNA polymerase (TOYOBO). The amplification conditions were: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 68 °C for 2.5 min; and gap infilling at 68 °C for 10 min. The obtained fragments were digested with XbaI/EcoRV and EcoRV/HindIII respectively, and cloned into the XbaI/HindIII site of pKC1139 to give the in-frame deletion construct pWDY40, which was then transferred into *Streptomyces xinghaiensis* NRRL B-24674 via *E. coli-Streptomyces* conjugation. Following the procedure described previously <sup>2</sup>, the *fl*K4 in-frame deletion mutant strains were screened out through PCR using primers Flk-id-F/Flk-id-R (955 bp for wild type strain, and 604 bp for mutant strain) and designated as WDY40.

To complement  $\Delta f/K4$ , a 441 bp fragment which contains the whole f/K4 gene was amplified from genomic DNA of *Streptomyces xinghaiensis* NRRL B-24674 by high fidelity PCR using the primers Flk-HB-F/Flk-HB-R (Table S1). The amplication conditions were: initial denaturation at 95

 $^\circ$ C for 5 min; 30 cycles of denaturation at 95  $^\circ$ C for 30 s, annealing at 55  $^\circ$ C for 30 s, and extension

at 68 °C for 1 min; and gap infilling at 68 °C for 10 min. The obtained fragment was cloned into the NdeI site of pIB139 by using In-fusion<sup>R</sup> HD Cloning Kit (Clontech) to give the complementation

construct pWDY41, which was then transferred into the  $\Delta fl$ K4 mutant via *E. coli-Streptomyces* conjugation. Following the procedure described previously [2], the  $\Delta fl$ K4 complementation mutant strains were screened out through PCR and designated as WDY41.

Primer	Sequence <sup>a</sup>	Function
Flk-F1	TTTAAGCTTCCTCCCGGGCCAATATCTAC	flK4 in-frame
		deletion
Flk-R1	AAAGATATCTGGAGAAGAAGAAGATCGGCCGT	flK4 in-frame
		deletion
Flk-F2	TTTGATATCGAGTGAACTTCTCTCCGGT	flK4 in-frame
		deletion
Flk-R2	AAATCTAGACAACCTCACCATCAACAATG	flK4 in-frame
		deletion
Flk-id-F	GGGTGTCCATCTCCGAGACGAA	WDY40 verification
Flk-id-R	TCGCACTCCAGCACATGGCA	WDY40 verification
Flk-HB-F	AGGATCCCCAACATATGTCAGCTCTCCCCGGTCTCC	ΔflK4
	A	complementation
Flk-HB-R	GGTAGGATCCACATATGCGGGAAGGCCTGCTCAC	$\Delta flK4$
		complementation

<sup>a</sup> Restriction sites for HindIII (AAGCTT), EcoRV (GATATC), XbaI (TCTAGA), and NdeI (CATATG) are underlined.

**Table S1** Primer used in this study.



**Figure S1**.Scheme showing the in-frame deletion of flK4 and success of construction was confirmed by PCR amplification. For the wild-type gene, the PCR product is 955 bp, while for the mutant the PCR product is 604 bp.

				10	D				<b>-</b> 2	0					3(	C				4	0													
MA37	1	MAA	NG	SQ	RP	11	ΑF	MS	DL	G	ГΤ	DD	S \	/ A (	QC	ΚG	LM	ΗS	I C	PG	۷T	VV	D)	CI	1SI	ИΤ	PW	D٧	E	EGA	R	٢T	/DI	P R
NBRA	1	MAA	NS	ΤR	RΡ	11	ΑF	MS	DL	G	ΓТ	DD	S \	/ A (	QC	ΚG	LM	ΥS	I C	PD	VΤ	VV	٢D١	(CH	1 S I	ЯΤ	PW	D٧	E	EGA	R	٢T	/ <mark>D</mark> I	P R
SCATT	1	MAA	NS	ΤR	RP	11	ΑF	MS	DL	G	ΓТ	DD	S \	/ A (	QC	ΚG	LM	ΥS	I C	PD	۷T	VV	٢D١	CI	1SI	МT	PW	D٧	EE	EGA	R	٢T	/ <mark>D</mark> I	P R
SXING	1	MSA	DP	ΤQ	RP	11	GF	MS	DL	G	ΓТ	DD	S \	/ A (	QC	ΚG	LM	ΗS	I C	PG	VΤ	VI	D١	CH	HS I	ЯΤ	PW	D٧	E	EGA	R	٢T	/ <mark>D</mark> I	P R
AspN902	1	MPA	NG	N –	- P	11	AF	MS	DL	G	ΓТ	DD	S \	/ A (	QC	ΚG	LM	LS	I C	ΡG	VΤ	ΊV	٢D١	/NH	1SI	ЯΤ	PW	D٧	EE	EGA	R	٢T	/ <mark>D</mark> I	P R
Sall	1			MQ	ΗN	LΙ	ΑF	LS	DV	G	SΑ	DE	A٢	ΗA	LC	ΚG	٧ <mark>M</mark>	ΥG	٧A	ΡA	ΑT	ΊV	D	T	ID'	٧A	ΡF	D٧	R	EGA	LI	FL/	٩D١	/ <mark>P</mark> H
PH0463	1					ΜL	ΤL	ТΤ	DF	G١	LΚ	GΡ	ΥN	/G	EМ	KV.	А <mark>М</mark>	LR	I N	ΡN	ΑK	ΞT Ν	D)	/TH	HS I	VΤ	RΗ	SΙ	LE	EGS	۶F۱	/M/	EQ۱	/VK
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MA37	65	FFF	EG	ΤV	FΑ	ΤT	ΤY	PA	TG	T	ГΤ	RS	VA	١٧	RI	RQ	AΑ	KG	GΑ	RG	QW	AC	S S (	D	GF	E R.	AD	GS	ΥI	IYI	A	N N	١G١	LT
NBRA	65	FFF	EG	τv	FΑ	ΤТ	ΤY	ΡA	TG	Т	ГΤ	<mark>r</mark> S	V A	٩V	RI	KQ	AΑ	К <mark>С</mark>	GΑ	RG	QW	AC	s	GA (	G F	E R.	ΑE	GS	YI	I Y I	A	N N	١GI	LT
SCATT	65	FFF	EG	τv	FΑ	ΤТ	ΤY	ΡA	TG	Т	ΤТ	<mark>r</mark> S	V A	٩V	RI	KQ	AΑ	К <mark>С</mark>	GΑ	RG	QW	AC	s (	GA (	G F I	E R.	ΑE	GS	YI	I Y I	A	N N	١D٧	LT
SXING	65	FFF	EG	τv	FΑ	ΤТ	ΤY	ΡA	TG	T	ЕΤ	<mark>r</mark> S	٧Z	٩V	RI	KQ	AΑ	К <mark>С</mark>	GΑ	RG	QW	AC	S S A	١G	GF	E R.	ΑE	GS	YI	١Y	/ A I	N N	۱GI	LT
AspN902	63	FFF	EG	τv	FΑ	ΤТ	ΤY	ΡA	TG	T/	AΤ	<mark>r</mark> S	V A	A L I	RI	KQ	AΑ	QG	GΑ	RG	QW	AC	SS (	GA (	G F	E R.	ΑE	GS	YI	IYI	A	N N	١D٧	LT
Sall	60	SFF	ΑH	ΤV	ΙC	ΑY	٧Y	ΡE	ΤG	T/	AΤ	ΗТ	T A	١V	R -				ΝE	К <mark>С</mark>	Q-								- l	LV	/G	N N	١GI	LS
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NBRA	129	ΤVL	ΕE	ΗG	ΥL	ΕA	ΥE	VT	'S P	K١	V E	ΡE		(	QΡ	ΕP	ΤF	Y S	RΕ	M۷	ΑI	P S	Ał	1L/	۱A	GF	ΡL	SE	V C	G R F	۶LГ	EDł	H E I	I V R
SCATT	129	ΤVL	ΕE	НG	ΥL	ΕA	ΥE	V T	S P	K١	V E	ΡE		(	QΡ	ΕP	ΤF	ΥS	R E	M۷	ΑI	ΡS	Ał	1L/	۱A	GF	ΡL	SE	EV C	G R F	۲ L	EDł	H E I	I V R
SXING	129	ΤVL	ΕE	НG	ΥĪ	ΕA	ΥE	VS	ST	K١	7 F	ΡE			R P	ΕP	ΤF	Y S	RΕ	M۷	ΑI	ΡA	Ał	1LA	۱A	GF	ΡL	SE	EV C	G R F	۲ <mark>۱ د</mark>	E D S	S E I	I V R
AspN902	127	ΤVΙ	ΕE	НG	ΥI	ΕA	ΥE	VS	NT	K١	1 1	ΡA			ΕP	ΕP	ΤF	YS	RE	M۷	ΑI	P S	A	1L/	AA)	GF	ΡL	NE	VC	G R A	ALS	S D I	DE	I V R
Sall	102	FAL	DA.	S P.	ΑV	EC	ΗE	V L	S P	D	ИN	NQ			ΡV	ΤP	τw	YG	ΚD	ΙV	ΑA	СA	A	1L/	AA)	GТ	DL	ΑA	VC	PF	11	DPI	(Q	I V R
PH0463	96	LPL	КΗ	ΙK	VК	SΥ	ΥE	11	ΡD	K	I R	ΚF	ТС	GW	ΕI	SS	ΤF	НG	<mark>r</mark> D	ΙF	GΡ	AC	λI	_   [	ΞK	GI	ΗP	EE	FC	G R E	EIR	PVE	DS	I V K

Figure S2 Amino acid alignment among the fluorinases and other related protein generated by Jalview. [REF] The red and green stars indicated that the key residues in the active sites of the fluorinases are highly conserved. SXING is the fluorinase from Streptomyces xinghaiensis NRRL B24674; MA37 is the fluorinase from *Streptomyces sp.* MA37;[Ref] NBRA is the fluorinase (YP\_006809254) from *Nocardia brasiliensis* HUJEG-1 (ATCC 700358); SCATT is the fluorinase from *Streptomyces cattleya*; AspN902 is the putative fluorinase (YP\_007949809) from the rare actinomycete *Actinoplanes sp.* N902-109; SalI is the chlorinase from the marine actinomycete *Salinospora tropica*<sup>3</sup>; PH0463 is the SAM hydroxide adenosyltransferase from *Pyrococcus horikoshii*<sup>4</sup>.

			10	2	20	30	)	40		50
FIK	1	MKD <mark>G</mark> MR V	GERFTH	DF <mark>V V P</mark> H	H <mark>KTVRH</mark>	LYPESI	PEFAE	FPEVFA	TGFMVG	LMEWACV
FIK1	1	MRE <mark>G</mark> LVV	′ <mark>gtkyth</mark>	RYV <mark>VP</mark> P[	OKTVRH	LYAESI	PEFAT	FPEVFA	TGFMVG	LMEWTCV
FIK4	1	MRE <mark>G</mark> LLT	GEKFTH	RYR <mark>VP</mark> R[	р <mark>кту</mark> рн	LYRESI	PEF <mark>S</mark> T	FPEVFA	TGFMVG	LMEWTOV
		60		70		80		90	100	)
	55		EPGEGS		THTAA	TPPGL	TVTVT	I AFLRSV		
	55		APGEGS	GTAIS	VTHSAA	TPPGL	TVTAT	VELLEA		WOVTAHD
FIK4	55	RAMEPYL	EAGEGS	LGTAIC	VAHTAA	TPPGF	τντντ	AELLGI	EGRRLK	WQVTAHD
		110	120	)	130		140			
FIK	109	GVDEIGS	GTHERA	VIHLEK	- NAK <mark>V</mark> R	Q <mark>K</mark>	<b>T</b>	PAG		
FIK1	109	<mark>gldeig</mark> s	GTHERA		F T QGVE	e <mark>k</mark> lrr,	ATAV-	- PE		
FIK4	109	GVH <mark>EIG</mark> A	GTHERA	VIDVER	TTSLE	k <mark>k</mark> ig <mark>r</mark>	ASVE <mark>T</mark>	GES		

**Figure S3** Amino acid alignment among the fluoroacetyl-CoA thioesterase generated by Jalview. [REF] FlK is from *Streptomyces cattleya*; FlK1 is from *Streptomyces sp.* MA37; FlK4 is from *Streptomyces xinghaiensis* NRRL B24674;



**Figure S4**, <sup>19</sup>F NMR spectra: **A**. 2 mM synthetic 5'fluoro-5'-deoxyadenosine (5'-FDA); **B**. the supernatant of *S. xinghaiensis* culture spiking with 2 mM 5'-FDA.



**Figure S4,** <sup>19</sup>F NMR spectra of the culture supernatants from **A**. the wild type strain of S. xinghainesis; **B**.the mutant WDY40 and **C** the mutant WDY41.

ORF	Proposed functions	AA	Identity(%)	Homologue in <i>S.</i>
				Calleya
FlK4	Fluoroacetyl CoA thioesterase	147	73%	FlK (SCATT_41470)
Orf1	Hypothetical protein	64		
Orf2	Hypothetical protein	99		
OrfA4	Carboxylate/Amino Acid/Amine	80	76%	SCATT_p11750
	Transporter			
OrfB4	YbaK/prolyl-tRNAsynthetase	191	81%	SCATT_p11770
FlB4	5'-FDA phosphorylase	299	66%	FlB (SCATT_41550)
FlA4	5'-FDA synthase	300	88%	FlA (SCATT_41540)
FIFT4	4-fluorothreonine transaldolase	625	72%	FlFT(SCATT_p11780)
FlF4	DNA binding regulatory protein	119	72%	FlF (SCATT_41530)
FlG4	DNA binding regulatory protein	226	61%	FIG (SCATT_41520)
FlH4	Na+/H+ antiporter	451	57%	FlH (SCATT_41500)
FII4	adenosylhomocysteine hydrolase	487	82%	FlI (SCATT_41490)
Orf5	Adenine phosphoribosyltransferase	181		
Orf6	Hypothetical protein	116		
Orf7	S-adenosine-L-methionine synthetase	398		

**Table S2**. Deduced functions of ORFs in the putative fluorometabolite biosynthetic gene cluster in *Streptomyces xinghaiensis* NRRL B24674 compared with the homologs in *S. cattleya*.

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