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 ºC. *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. [1]

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 μL 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 [2], 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 *Δfl*K4, a 441 bp fragment which contains the whole *fl*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 ℃ for 5 min; 30 cycles of denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, and extension at 68 ℃ for 1 min; and gap infilling at 68 ℃ for 10 min. The obtained fragment was cloned into the NdeI site of pIB139 by using In-fusion® HD Cloning Kit (Clontech) to give the complementation

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

Primer	Sequence [a]	Function
Flk-F1	TTTAAGCTTCCTCCCGGGCCAATATCTAC	flK4 in-frame deletion
Flk-R1	AAAGATATCTGGAGAAGAAGATCGGCCGT	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	AGGATCCCCAACATATGTCAGCTCTCCCCGGTCTCCA	ΔflK4 complementation
Flk-HB-R	GGTAGGATCCACATATGCGGGAAGGCCTGCTCAC	ΔflK4 complementation

[a] Restriction sites for HindIII (AAGCTT), EcoRV (GATATC), XbaI (TCTAGA), and NdeI (CATATG) are underlined.

Table S1 Primer used in this study.

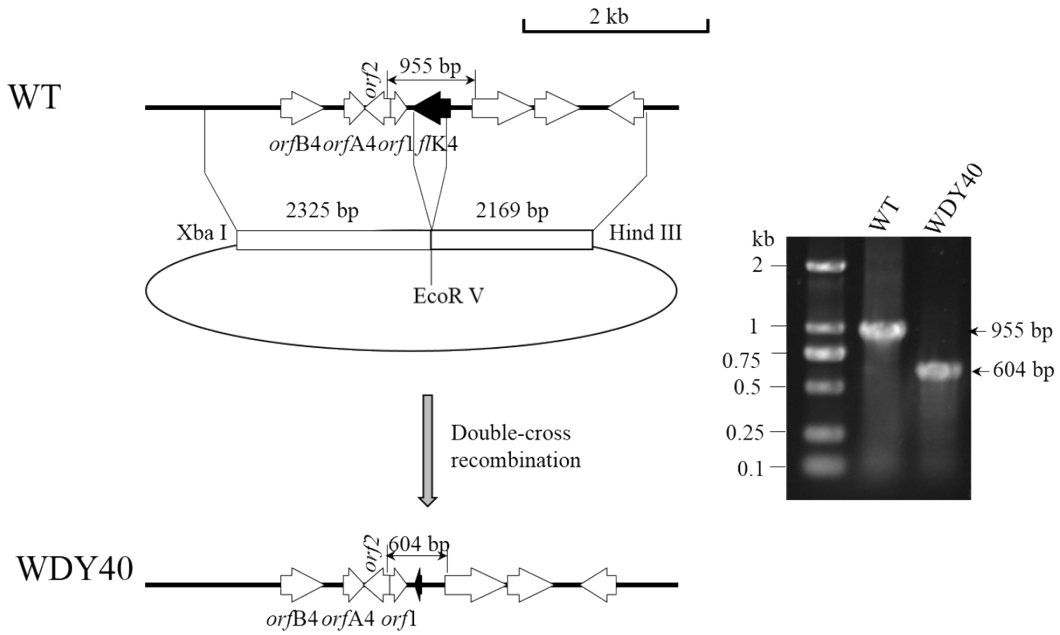

Figure S1.Scheme showing the in-frame deletion of flK4 and success of construction was confirmed by PCR amplification. For the wild-type gene, the PCR product is 955 bp, while for the mutant the PCR product is 604 bp.

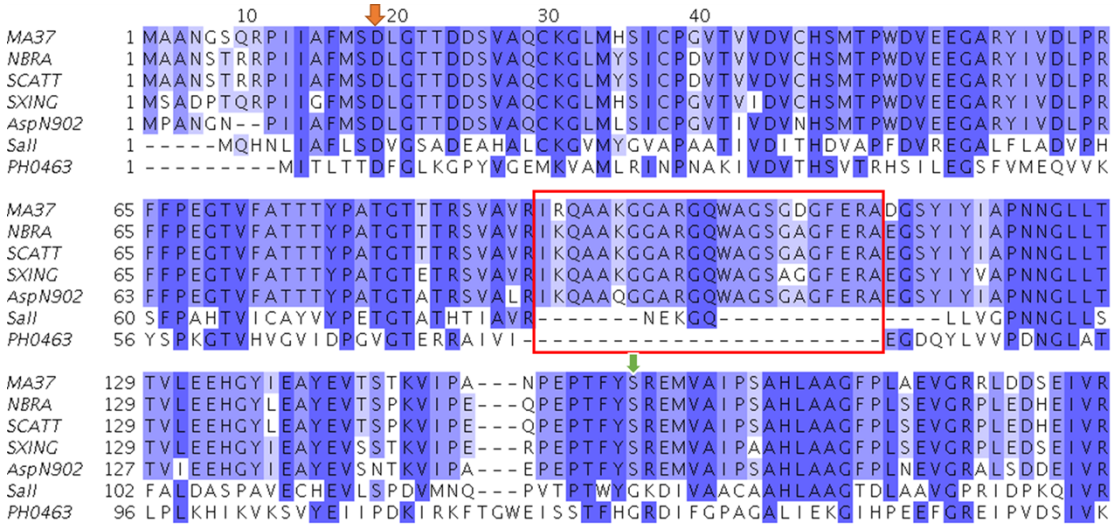

Figure S2 Amino acid alignment among the fluorinases and other related protein generated by Jalview. [REF] The red and green stars indicated that the key residues in the active sites of the fluorinases are highly conserved. SXING is the fluorinase from Streptomyces xinghaiensis NRRL B24674; MA37 is the 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*[3]; PH0463 is the SAM hydroxide adenosyltransferase from *Pyrococcus horikoshii*[4].

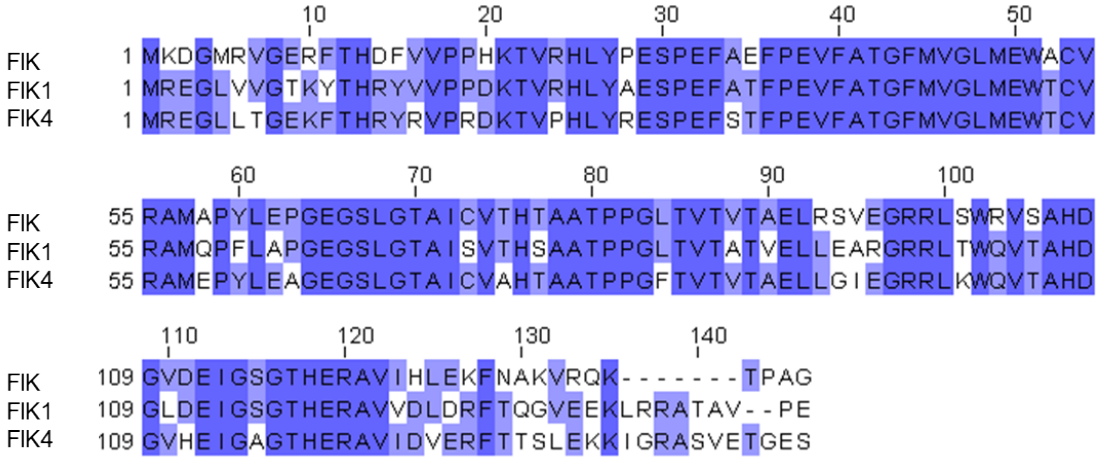

Figure S3 Amino acid alignment among the fluoroacetyl-CoA thioesterase generated by Jalview. [REF] FlK is from *Streptomyces cattleya*; FlK1 is from *Streptomyces sp*. MA37; FlK4 is from *Streptomyces xinghaiensis* NRRL B24674;

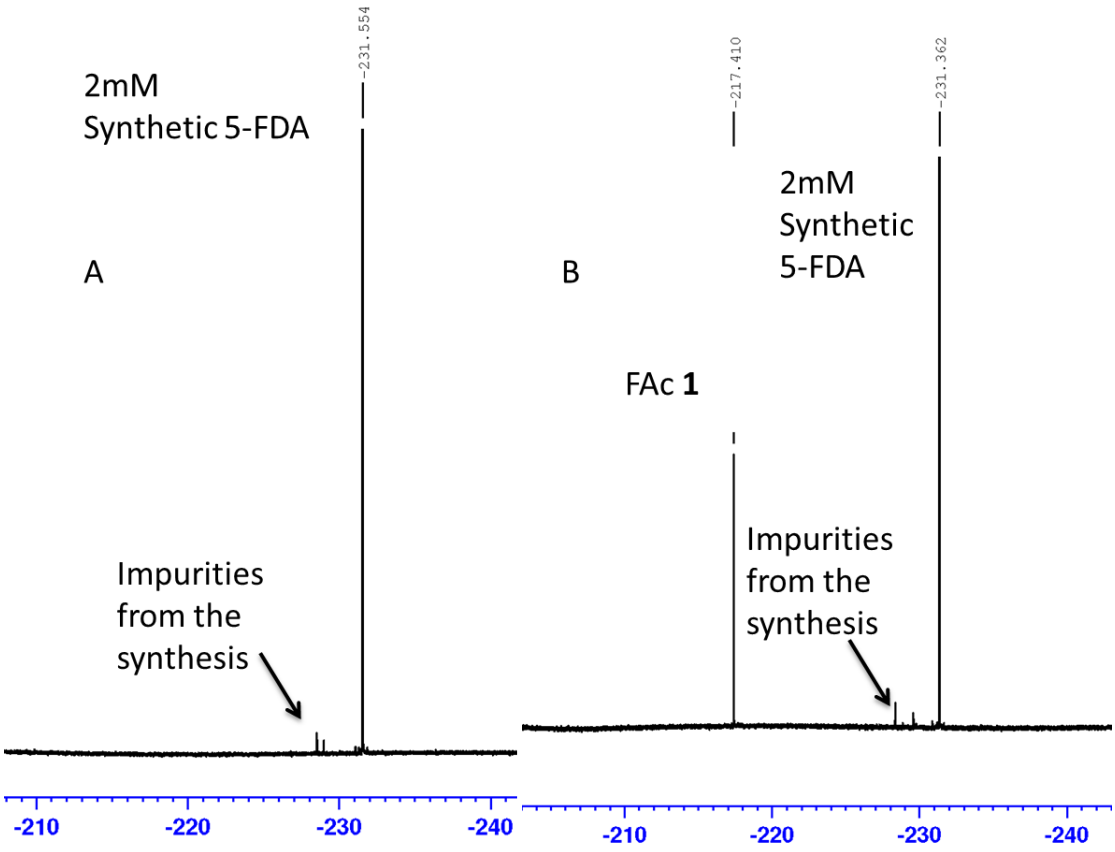

Figure S4, ^{19}F NMR spectra: **A**. 2 mM synthetic 5'fluoro-5'-deoxyadenosine (5'-FDA); **B**. the supernatant of *S. xinghaiensis* culture spiking with 2 mM 5'-FDA.

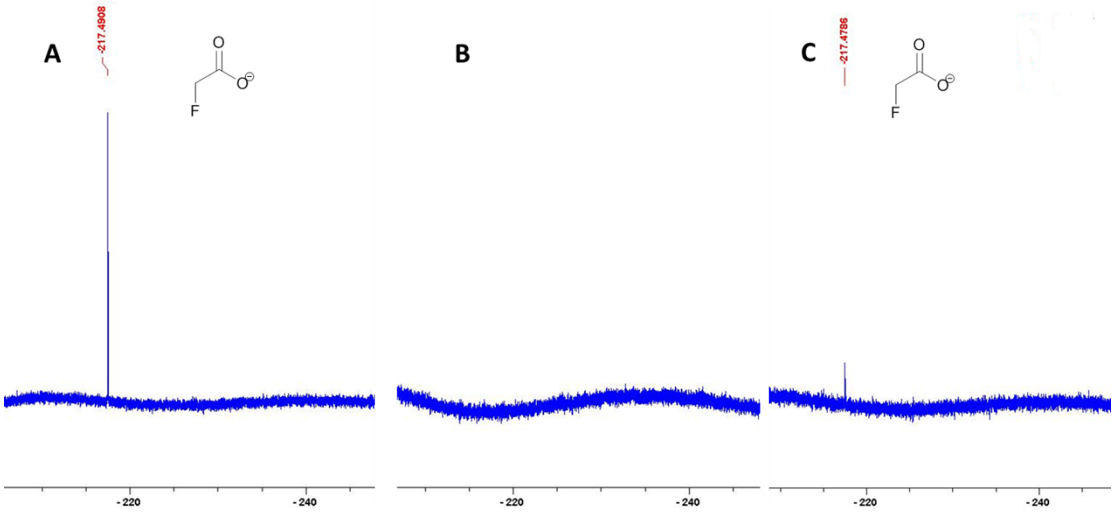

Figure S4, ^{19}F NMR spectra of the culture supernatants from **A**. the wild type strain of S. xinghainesis; **B**.the mutant WDY40 and **C** the mutant WDY41.

ORF	Proposed functions	AA	Identity(%)	Homologue in *S. cattleya*
FlK4	Fluoroacetyl CoA thioesterase	147	73%	FlK (SCATT_41470)
Orf1	Hypothetical protein	64		
Orf2	Hypothetical protein	99		
OrfA4	Carboxylate/Amino Acid/Amine Transporter	80	76%	SCATT_p11750
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)
FlFT4	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%	FlG (SCATT_41520)
FlH4	Na+/H+ antiporter	451	57%	FlH (SCATT_41500)
Fl**I4**	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*.

References:

1 T. Kieser, *Practical streptomyces genetics*. 2000.

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3 A. Eustáquio, F. Pojer, J. P. Noel, B. S. Moore, *Nat. Chem. Biol.,*2008, **4**, 69.

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