Supporting Information

Activation and enhancement of Fredericamycin A production in deepsea-derived *Streptomyces somaliensis* SCSIO ZH66 by using ribosome engineering and response surface methodology

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Table S1 Fermentation media used for detecting FDM A production by strain ZH66-RIF1.

Name	Recipe
1.medium-1	peptone 2 g/L, yeast extract 5 g/L, soluble starch 2 g/L, soy flour 15 g/L, NaCl 4 g/L, $CaCO_3$ 4 g/L, sea salt 33 g/L, pH=7.0
2.medium-2 (APM)	yeast extract 8 g/L, malt extract 20 g/L, NaCl 2g/L, MOPS sodium salt 10 g/L in 500 mL of tap water and 380 mL of Milli-Q water. After autoclaving, 1 mL of 10% $MgSO_4$, 1 mL of 1% $FeSO_4$, 0.1 mL of 10% $ZnSO_4$ and 120 mL of 50% glucose were added [1]
3.medium-3	soluble starch 10 g/L, glucose 20 g/L, corn syrup 4 g/L, yeast extract 10 g/L, beef extract 3 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, KH_2PO_4 0.5 g/L, $CaCO_3$ 2 g/L, sea salt 30 g/L，pH=7.0
4.medium-4	soluble starch 20 g/L, soy flour 5 g/L, yeast extract 5 g/L, peptone 2 g/L, NaCl 4 g/L, $CaCO_3$ 2 g/L, sea salt 33 g/L，pH=7.2~7.4
5.medium-5	malt extraction powder 40 g/L, yeast extract powder 4 g/L, glucose 20 g/L, $(NH_4)_2HPO_4$ 1 g/L，pH=7.0

Table S2 ^{1}H and ^{13}C NMR data of FDM A in DMS0-d_6.

Position	δ_C	δ_H (multiplicity, *J*, Hz)
1	167.17	
2	111.11	
3	156.35	
4	124.31	
5	64.65	
6	198.79	
7	134.80	
8	152.08	
9	118.87	
10	183.42	
11	162.02	
OCH$_3$-11	57.92	3.99 (3H)
12	112.26	
13	189.93	
14	109.18	
15	151.68	
16	134.20	
17	198.82	
18	34.86	2.47(t, *J* = 7.5, 2H)
19	32.69	3.15(t, *J* = 7.5, 2H)
20	154.61	
21	112.26	6.99 (s, 1H)
22	140.34	
23	106.55	6.68 (s, 1H)
24	138.30	
-NH		11.58 (s, 1H)
25	122.62	6.20 (ddq, *J* = 15.4, 8.9 Hz, 1H)
26	133.29	7.13 (dd, *J* = 15.8, 10.4 Hz, 1H)
27	131.69	6.23(d, *J* = 15.4, 1H)
28	138.30	5.91 (dd, *J* = 14.8, 7.2 Hz, 1H)
29	18.60	1.79 (d, *J* = 6.8 Hz, 3H)
OH-3		13.13 (s, 1H)

Table S3. FDM A titers from different producing strains

Strain	Culture conditions		Time (days)	Titer (mg/L)
	Seed medium	Production medium		
S. chattanoogensis ISP 5002 [2]	galactose -glycerol-corn steep	L-phenylalanine 5 g/L, $(NH_4)_2SO_4$ 1.5 g/L, K_2HPO_4 0.5 g/L, KH_2PO_4 0.5 g/L, $CaCO_3$ 2 g/L, pH=7.0	6	10
S. griseus ATCC49344 [3]			9-10	162
S. griseus SB4012 [3]	R2YE	APM (medium-2)	11-12	997
			8-9	400
S. *somaliensis* SCSIO ZH66-RIF1	-	medium-3	7	220.9
	-	optimized medium-3	7	679.5

Table S4 Proposed functions of proteins encoded by the *frd* biosynthetic gene cluster from *S. somaliensis* SCSIO ZH66 and its comparison with other FDM A gene clusters.

	SCSIO ZH66		Comparison of ant gene cluster from other strains					
			Streptomyces griseus			*Streptomyces* sp. SANK 61196		
Protein	Size (aa)	Proposed function	protein	Accession number	Identity (%) /Similarity	protein	Accession number	Identity (%) /Similarity
FrdC	246	putative 3-ketoacyl-ACP reductase	FmdC	AAQ08912	99/99	SanC	ADG86311	83/90
FrdT	493	putative peptide transporter	FmdT	AAQ08913	99/99	SanT	ADG86312	82/89
FrdD	106	putative polyketide cyclase	FmdD	AAQ08914	100/100	SanD	ADG86313	89/94
FrdE	141	putative polyketide cyclase	FmdE	AAQ08915	100/100	SanE	ADG86314	83/88
FrdF	416	3-ketoacyl-ACP synthase	FmdF	AAQ08916	99/99	SanF	ADG86315	92/96
FrdG	403	chain length factor	FmdG	AAQ08917	99/99	SanG	ADG86316	84/90
FrdH	84	ACP	FmdH	AAQ08918	100/100	SanH	ADG86317	80/89
FrdI	155	putative polyketide cyclase	FmdI	AAQ08919	99/100	SanI	ADG86318	83/89
FrdJ	101	putative monooxygenase	FmdJ	AAQ08920	99/100	SanJ	ADG86319	73/81
FrdK	142	putative oxygenase	FmdK	AAQ08921	99/100	SanK	ADG86320	80/87
FrdL	246	putative monooxygenase	FmdL	AAQ08922	100/100	SanL	ADG86321	78/87
FrdM	148	putative monooxygenase	FmdM	AAQ08923	99/100	SanM	ADG86322	83/89
FrdM1	154	putative monooxygenase	FmdM1	AAQ08924	99/99	SanM1	ADG86323	81/87
FrdN	320	putative o-methyltransferase	FmdN	AAQ08925	98/99	SanN	ADG86324	78/84
FrdO	249	putative 3-ketoacyl-ACP reductase	FmdO	AAQ08926	99/100	SanO	ADG86325	88/94
FrdP	98	putative monooxygenase	FmdP	AAQ08927	99/100	SanP	ADG86326	81/92
FrdQ	105	putative monooxygenase	FmdQ	AAQ08928	99/100	SanQ	ADG86327	74/83
FrdS	333	putative 3-keto-acyl-ACP synthase	FmdS	AAQ08929	99/99	SanS	ADG86328	86/92
FrdT1	302	putative membrane transporter	FmdT1	AAQ08930	100/100	SanT1	ADG86329	87/93
-	-	-	-	-	-	SanX	ADG86330	-
FrdR	151	transcriptional regulator	FmdR	AAQ08931	100/100	SanR	ADG86331	80/90
FrdU	141	unknown	FmdU	AAQ08932	99/99	SanU	ADG86332	62/76
FrdV	622	asparagine synthetase	FmdV	AAQ08933	99/99	SanV	ADG86333	78/83
FrdR1	580	regulatory protein	FmdR1	AAQ08934	99/100	SanR1	ADG86334	73/81
FrdT2	531	putative membrane transporter	FmdT2	AAQ08935	99/99	SanT2	ADG86335	78/83
FrdW	159	holo-ACP synthase	FmdW	AAQ08936	99/99	SanW	ADG86336	69/75
FrdR2	170	transcriptional regulator	FmdR2	AAQ08937	99/99	-	-	-
-	-	-	FmdX	AAQ08938	-	-	-	-
FrdT3	525	putative membrane transporter	FmdT3	AAQ08939	99/99	-	-	-

-: means not available.

Table S5. The primer pairs used in this study.

Name	Sequence (5'-3') [a]
16S-FP	AGAGTTTGATCCTGGCTCAG
16S-RP	AAGGAGGTGATCCAGCCGCA
rpoB-FP[a]	CG<u>GGATCC</u>TTGGCCGCCTCGCGCACT
rpoB-RP[a]	CCC<u>AAGCTT</u>TCAGACCTCTTCGACGCT
hrdB-FP	CGACTACACCAAGGGCTACAA
hrdB-RP	GGAGCATCTGACGCTGGAC
frdD-FP	GGAGCCGGGTGTTGATGT
frdD-RP	GACGACGCCGACAAGGTG
frdR1-FP	GCACCGAAGGTCCGACAAGTT
frdR1-RP	CGCAGAGTCCGGGCAGTTT

[a]Underlined letters represent restriction sites.

Table S6 Screening of significant variables for FDM A production using Plackett-Burman Design (PBD)

Treatments	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	FDM A titer (mg/L)
1	-1	1	1	-1	-1	-1	1	1	1	436.57±11.85
2	-1	-1	-1	-1	-1	-1	-1	-1	-1	522.02±6.24
3	1	1	1	-1	1	1	-1	1	-1	308.29±24.23
4	-1	-1	-1	1	1	1	-1	1	1	373.82±13.66
5	-1	1	-1	-1	1	1	1	-1	1	481.57±26.23
6	-1	-1	1	1	1	-1	1	1	-1	385.65±15.51
7	1	-1	1	-1	1	-1	-1	-1	1	252.64±4.92
8	-1	1	1	1	-1	1	-1	-1	-1	563.54±11.04
9	1	1	-1	1	1	-1	1	-1	-1	507.76±14.34
10	1	1	-1	1	-1	-1	-1	1	1	346.15±5.80
11	1	-1	1	1	-1	1	1	-1	1	242.87±16.44
12	1	-1	-1	-1	-1	1	1	1	-1	174.59±13.76

X_1, soluble starch at a low level (-1) of 8 g/L and a high level (+1) of 12 g/L; X_2, glucose at a low level (-1) of 16 g/L and a high level (+1) of 24 g/L; X_3, at a low level (-1) of 3.2 g/L and a high level (+1) of 4.8 g /L; X_4, at a low level (-1) of 8 g/L and a high level (+1) of 12 g/L; X_5, at a low level (-1) of 2.4 g/L and a high level (+1) of 3.6 g/L; X_6, at a low level (-1) of 2.4 g/L and a high level (+1) of 3.6 g/L; X_7, at a low level (-1) of 0.4 g/L and a high level (+1) of 0.6 g/L; X_8, at a low level (-1) of 0.4 g/L and a high level (+1) of 0.6 g/L; X_9, at a low level (-1) of 24 g/L and a high level (+1) of 36 g/L.

Table S7 The effects of each factor on FDM A production.

Factor signs	Factor	Effect	Coefficient	t Value	p Value
X_1	Starch soluble	-148.64	-74.32	-4.62	0.044
X_2	Glucose	128.88	64.44	4.00	0.057
X_3	Corn syrup	-42.89	-21.45	-1.33	0.314
X_4	Yeast extract	17.50	8.75	0.54	0.641
X_5	Beef extract	53.85	26.93	1.67	0.236
X_6	$CaCO_3$	-64.18	-32.09	-1.99	0.184
X_7	$MgSO_4 \cdot 7H_2O$	-16.07	-8.04	-0.50	0.667
X_8	KH_2PO_4	-104.39	-52.19	-3.24	0.083
X_9	Sea salt	-61.20	-30.60	-1.90	0.198

$R^2 = 96.80\%$; $R^2adj = 95.42\%$.

Table S8 The dose of important factors in response surface analysis.

Level	Glucose (g/L)	Starch soluble (g/L)	KH_2PO_4 (g/L)
-1	22	5	0.28
0	26	7	0.35
1	30	9	0.42

Table S9 Analysis of variance (ANOVA) for the second-order polynomial model.

| Source	DF	SS	F-value	p-value
Model	9	174647	62.72	0.000
Linear	3	139635	150.43	0.000
Quadratic	3	31432	33.86	0.001
Cross product	3	3581	3.86	0.090
Residual	5	1547		
Lack of Fit	3	1353	4.63	0.183
Pure Error	2	195		
Total	14	176194		

DF: degree of freedom; SS: sum of squares.

Figure S1 Phylogenetic tree of *S. somaliensis* SCSIO ZH66 based on 16S rRNA sequences.
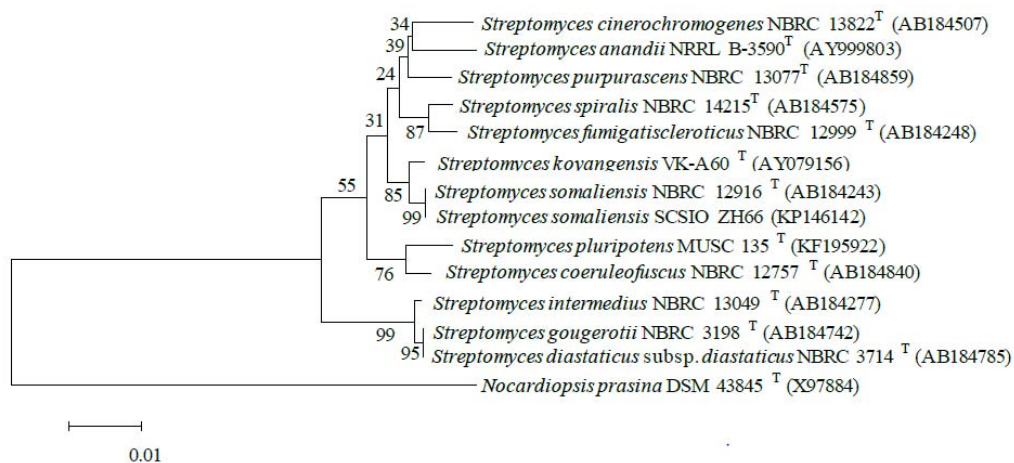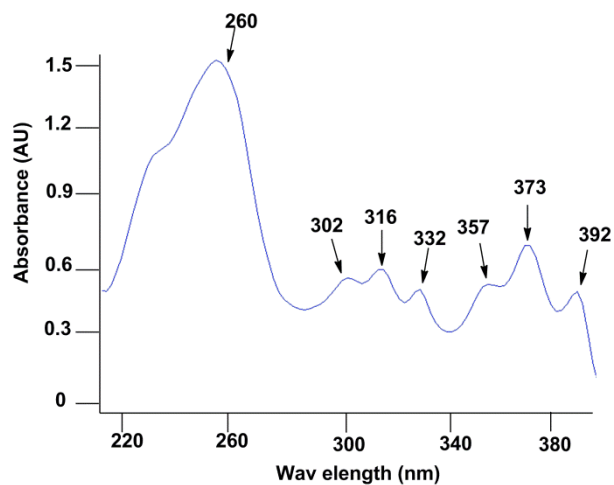

Figure S2 Phenotypes of strain ZH66-WT and strain ZH66-RIF1. (A, B) surface view; (C, D) microscopic analysis. Strains were incubated on Gauze's No. 1 medium for 72 h at 30 ℃. Bar, 5 μm.
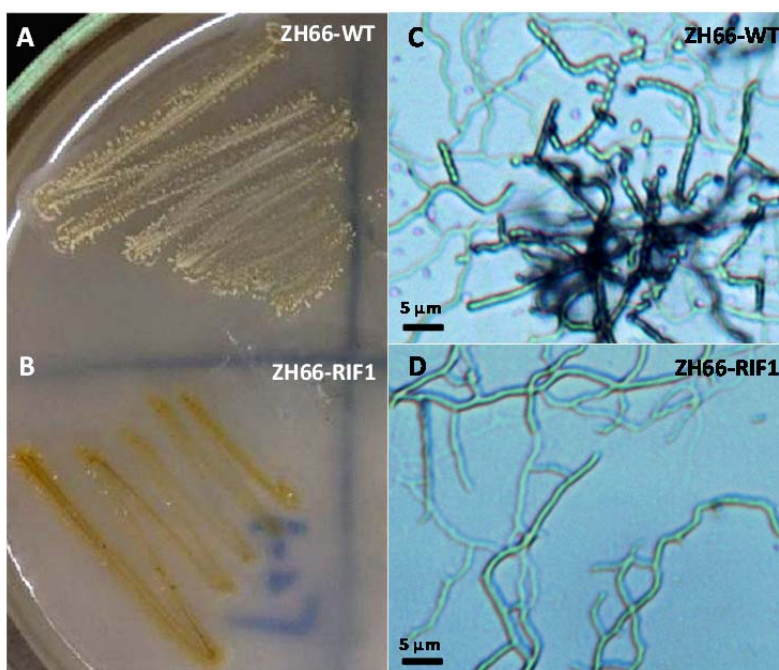
Figure S3 HPLC traces of fermentation products of strain ZH66-RIF1 in different medium.
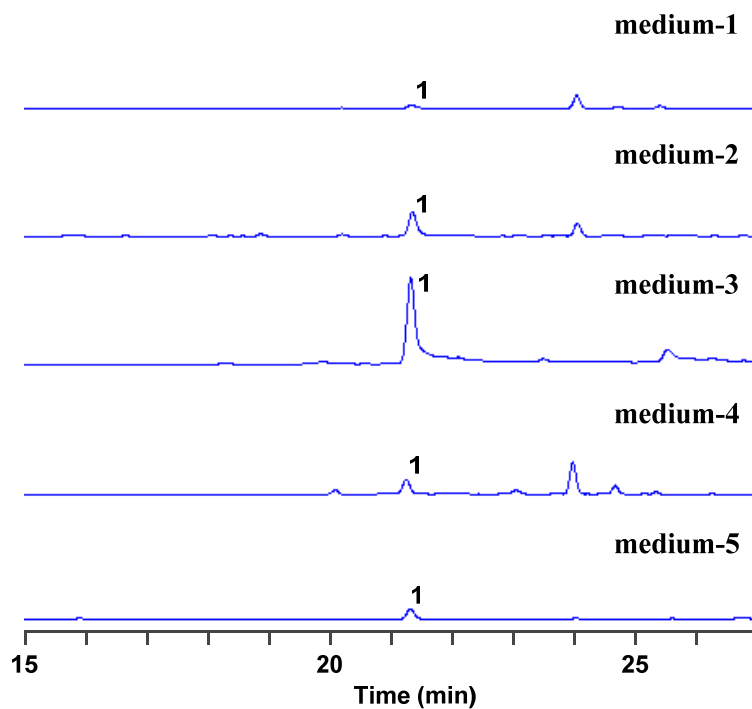
Figure S4 UV spectrum of FDM A.

References

1. Wendt-Pienkowski E, Huang Y, Zhang J, Li B, Jiang H, Kwon H, et al. Cloning, sequencing, analysis, and heterologous expression of the fredericamycin biosynthetic gene cluster from *Streptomyces griseus*. J Am Chem Soc. 2005; 127:16442-16452.

2. Hosoya Y, Okamoto S, Muramatsu H, Ochi K. Acquisition of certain streptomycin-resistant (*str*) mutations enhances antibiotic production in bacteria. Antimicrob Agents Chemother. 1998; 42(8):2041-2047.

3. Chen Y, Wendt-Pienkowski E, Shen B. Identification and utility of FdmR1 as a *Streptomyces* antibiotic regulatory protein activator for fredericamycin production in *Streptomyces griseus* ATCC 49344 and heterologous hosts. J Bacteriol. 2008; 190**:**5587-5596.