Supplementary Material (ESI) for Chemical Communications

Supporting information for:

Mupirocin H, a novel metabolite resulting from mutation of the HMG-CoA synthase analogue, *mupH* in *Pseudomonas fluorescens*

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Preparation of *Pseudomonas fluorescens ΔmupH* mutant strain.

An in frame deletion of *mupH* was created as part of a larger study of tailoring region genes[17] by amplifying two DNA fragments, mupH1 and mupH2, which flank the region to be deleted. These were ligated into the suicide vector pAKE604 to create an in frame deletion of 984bp. The resulting plasmid (pSCΔH) was mobilised to *P. fluorescens* NCIMB 10586 by conjugal mating with *Escherichia coli* S17-1 (pSCΔH), where integration into the chromosome occurs by homologous recombination in either mupH1 or mupH2, and was selected by the kanamycin resistance marker on the plasmid. After purification of the transconjugants to ensure absence of non-integrant bacteria, removal of kanamycin enabled growth of cells in which the suicide vector had excised from the chromosome by recombination in mupH1 or H2 again leaving either WT or mutant DNA. Excisants were selected by growth on sucrose since the integrated plasmid carries the *sacB* gene whose product, levan sucrase, polymerises sucrose in the periplasm leading to cell death. Isolates with *mupH* deleted were identified by PCR with ΔmupH1F and ⊗mupH2R primers. Bioassay[8] of three Δ*mupH* mutants revealed a consistent reduction in antibacterial activity against *Bacillus subtilis* 1064 to *ca* 14% of wild-type levels. The *mupH* ORF was amplified by PCR (primers CmupHF/CmupHR) and cloned into the IncQ *tac* promoter expression vector pJH10, giving pSCCH. The resulting plasmid was introduced into the Δ*mupH* strain and bioassay showed activity being fully restored to wild-type levels, confirming that the defect in PA production must be due to loss of *mupH* function and not due to a polar effect on downstream genes.

Fermentation and isolation.

The *Δmup*H mutant strain of *Pseudomonas fluorescens* NCIMB 10586 were was grown at 25 ºC on L agar for 24 hours. Single colonies were used to inoculate L broth (50 ml in a 250 ml conical flask) supplemented with carbonicillin (100 mgmL^{-1}) and cultured at 25 ºC for overnight to prepare the seed culture. 1000 ml secondary stage medium (20 gL^{-1} soya flour, 6.25 g^{L-1} $CaCO_3$, 5.0 gL^{-1} $(NH_4)_2SO_4$, 2.5 gL^{-1} spray dried corn liquor, 1.5 gL^{-1} KH_2PO_4, 1.0 gL^{-1} Na_2HPO_4, 1.0 gL^{-1} KCl, 0.5 gL^{-1} $MgSO_4$ and 4% glucose) in 10 x 500 ml conical flasks, was inoculated with 5% seed culture and grown at 22 ºC, 250 rpm for 50 hours. Cells were removed by centrifugation at 7500 rpm for 30 minutes. The supernatant was acidified to pH 4.5 with dilute HCl and extracted by ethyl acetate (0.6 volume) twice. After ethyl acetate was removed by rotary evaporation, the residue was subjected to gel filtration chromatography on Sephadex LH-20 with elution by MeOH. Fractions were analysed by NMR which showed the presence of mupirocin H (**5**) in the earlier fractions Further purification was carried on by gradient flash chromatography on normal-phase silica gel eluted by MeOH in $CHCl_3$ from (0:100 to 15:85). Mupirocin H (**5**), $[a]_D^{20}$ = +30.5 (*c* = 1.3 in $CHCl_3$) was isolated as a colourless viscous oil. IR (in $CHCl_3$): ν_{max} 3614 3503, 3018, 1793, 1643, 1466 (s), 1382, 1096 cm^{-1}.

Table 1: Proposed roles of *mupH* and cognate genes in PA-A biosynthesis, and possible roles of homologous genes in related HCS-containing clusters.

	ACP	**KS_S**	**HCS**	**CR**[a]	**CR**[b]	**CR**[c]
Pseudomonic acid A (**1**)	*macpC*	*mupG*	*mupH*	*mupJ*	*mupK*	
Myxovirescin A (**6**)	*taB/E*	*taK*	*taC/F*	*taX*	*taY*	
Pederin (**7**)	*pedN*	*pedM*	*pedP*	*pedL*	*pedI* [d]	*pedI* [d]
Jamaicamide A (**8**)	*jamF*	*jamG*	*jamH*	*jamI*	*jamJ* [d]	
Curacin A (**9**)	*curB*	*curC*	*curD*	*curE*	*curF* [d]	
Bacillaene (PksX)	*acpK*	*pksF*	*pksG*	*pksH*	*PksI*	

[a] Dehydratase; [b] decarboxylase; [c] isomerase; [d] initial domain(s) of associated modular PKS.

Table 2: NMR data for mupirocin H (**5**) in $CDCl_3$

	^{1}H (*J* in Hz)	^{13}C	HMBC	COSY	NOESY
1		175.8			
2	a: 2.54, dd, 18.3, 4.4	38.1	C-1, C-3	H-2b, H-3	H-2b, H-3
	b: 2.94, dd, 18.3, 7.6		C-1, C-4	H-2a, H-3	H-2a, H-3
3	4.61, ddd, 7.6, 4.4, 3.2	68.6		H-2a & b, H-4	H-2a & b, H-5, H-6
4	4.44, dd, 5.8, 3.2	87.5	C-1, C-3	H-3, H-5	H-5, H-13
5	3.60, dd, 6.6, 5.8	75.6	C-3, C-4, C-6, C-13	H-4, H-6	H-3, H-4, H-6, H-13
6	1.92, dqdd, 7.1,6.8, 6.6, 6.6	35.3	C-5, C-7, C-8, C-13	H-5, H-7a & b, H-13	H-3, H-5, H-13
7	a: 2.25, m	34.6	C-5, C-6, C-8, C-9, C-13	H-6, H-7b, H-8	H-4, H-5, H-8, H-9, H-13
	b: 2.27, m			H-6, H-7a, H-8	
8	5.63, ddd, 15.4, 8.6, 6.1	129.7	C-7, C-9, C-10	H-7a & b, H-9	H-7a & b, H-10, H-13
9	5.40, dd, 15.4, 8.6	134.8	C-7, C-8, C-10, C-14	H-8, H-10	H-7a & b, H-10, H-11, H-14
10	2.07, ddq, 8.6, 7.3, 6.8,	45.3	C-8, C-9, C-11, C-14	H-9, H-11, H-14	H-8, H-9, H-12, H-14
11	3.53, dq, 7.3, 6.4	71.5	C-9, C-12	H-10, H-12	H-12, H-14
12	1.20, d, 6.4	20.6	C-10, C-11	H-11	H-11, H-14
13	1.05, d, 6.8	16.9	C-5, C-6, C-7	H-6	H-4, H-5, H-6, H-7
14	1.00, d, 6.8	16.0	C-9, C-10, C-11	H-10	H-10, H-11, H-12