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Supplementary Material

Genome-guided discovery of natural products and biosynthetic pathways from Australia's untapped microbial megadiversity

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General Experimental

NMR and mass spectrometry data were recorded at the Mark Wainwright Analytical Centre, UNSW. NMR data (^{1}H, COSY, HSQC and HMBC) was acquired on either a Bruker Tesla 500 or 600 MHz spectrometer (operating at 500 or 600 MHz for ^{1}H and 125 or 150 MHz for ^{13}C) in DMSO-d_6 or MeOH-d_4 and referenced to δ 2.49 and 39.51 ppm or δ 3.31 and 49.15 ppm respectively. The high resolution mass spectrometry data were obtained using a Thermo LTQ Orbitrap XL spectrometer operating in positive, ESI or nano ESI modes.

Methods describing plant endophyte isolation, DNA extraction, PCR, and taxonomy determinations have been published previously.[1] Sequences of PCR primers used in this study have also been published previously.[2-4] Sequencing of PCR amplicons and genomic DNA was performed at the Ramaciotti Center for Gene Analysis (UNSW).

Isolation of altenusin from the isolate*Penicillium* sp. PS1-3.

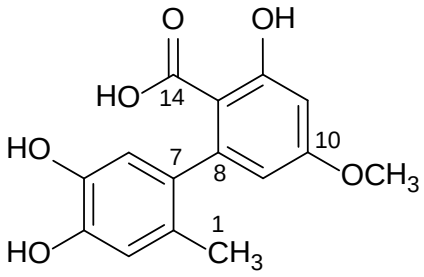
The taxonomy of the isolate PS1-3 was determined using PCR by amplifying the 18S rRNA gene with the primer pair nu-ssu-0817/nu-ssu-1536,[4] followed by gene sequencing and a BLASTN search of the NCBI database. The isolate PS1-3 was assigned as a member of the *Penicillium* genus.

The *Penicillium* sp. was cultured in 1.2 L (3 x 400 mL) of potato dextrose broth (BD Difco) from biomass grown on potato dextrose agar plates. The cultures were grown for 21 days at 25ºC with occasional agitation, and exhaustively extracted with equal volumes of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 (Ajax) and then evaporated to dryness *in vacuo*.

The crude extract was dissolved in 5% methanol/dichloromethane and fractionated using a Reveleris flash chromatography system (Grace) equipped with a 4 g silica cartridge. The extract was separated using normal phase stepwise gradient elution (flow rate 18 mL/min) from 1% methanol/dichloromethane to 6% methanol/dichloromethane over 2.8 min, held for 2.1 min, then ramped up to 100% methanol over 0.3 min and held for a further 5 min. Fractions were collected on the basis of time and UV absorbance, and combined based on silica thin layer chromatography (Merck). Material eluting between 36-54 secs was combined

(~130mg) and then separated on a silica SPE cartridge (Waters) with the 100% ethyl acetate fraction subjected to semi-preparative HPLC.

Semi-preparative HPLC was performed using a HP 1100 series equipped with a 1100 series diode array detector (Hewlett Packard) operating at 254 and 280 nm. Compounds were purified using a Discovery BIO Wide Pore RP-C18, (10 x 250 mm, 5 μm; Supelco), HPLC column at a flow rate of 3.5 mL/min employing a solvent gradient of 5% acetonitrile/water (0.5% TFA) for 10 min, which was then ramped to 95% acetonitrile/ water (0.5% TFA) over 30 min and then to 100% acetonitrile over 5 min. Altenusin (1.3 mg, *m/z* 313.06747 $[C_{15}H_{14}O_6Na]^+$, *calc.* 313.06826, Δ –2.52 ppm) eluted at 15 min under these chromatographic conditions.



NMR data for altenusin

^{1}H NMR (CD_3OD) δ (multiplicity, assignment, coupling constants in Hz, integration, HMBCs): 1.91 (s, H1, 3H, C2, C3, C7), 3.81 (s, 10-OC<u>H</u>$_3$, 3H, C10), 6.17 (d, H9, 2.5 Hz, 1H, C7, C11, C13), 6.43 (d, H11, 2.5 Hz, 1H, C9, C12, C13), 6.48 (s, H6, 1H, C2, C4, C5, C8), 6.58 (s, H3, 1H, C1, C4, C5, C7). ^{13}C NMR (CD_3OD) δ 19.4 (C1), 56.1 (10-OCH_3), 100.7 (C11), 107.1 (C13)*, 111.6 (C9), 116.8 (C6), 117.5 (C3), 127.5 (C2), 135.5 (C7), 143.5 (C5), 145.1 (C4), 148.2 (C8), 165.2 (C10), 165.9 (C12), 174.5 (C14). *observed in HMBC only.

Isolation of desferrioxamine E from the isolate *Marinobacter* sp. SB12.

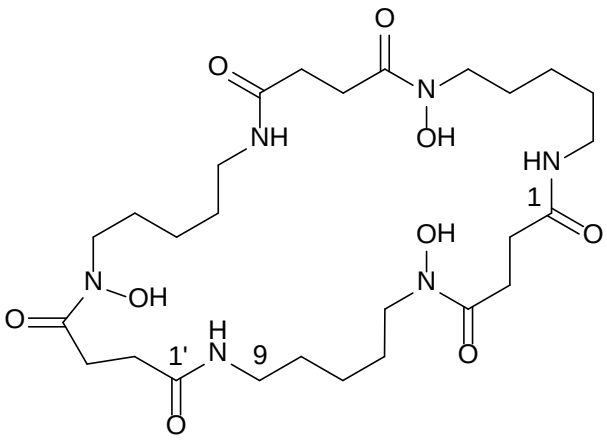
The isolation of *Marinobacter* sp. SB12 was reported previously.[5] *Marinobacter* sp. SB12 maintained on solid HP-LB 50% (pH 8.2) was used to seed 30ml ASG-Fe (HP) liquid media for use as a starter culture for subsequent scale-up. The 3 day old starter culture was used to inoculate 500ml fresh ASG-Fe (HP) for production, and this culture was incubated at 30°C for 7 days with shaking (180 rpm).

1 L of solid HP-LB 50% contains yeast extract (2.5 g), tryptone (5 g), NaCl (50.7 g), KCl (1.4 g), $CaCl_2.2H_2O$ (2.7 g) $MgCl_2.6H_2O$ (7.23 g), $MgSO_4.7H_2O$ (13.3 g), agar (15 g), and was made to 1 L with MilliQ water then sterilised by autoclaving.

1 L of ASG-Fe (HP) contains NH_4Cl (1 g), glycerol (3 ml), casamino acids (10 g), NaCl (50.7 g), KCl (1.4 g), $CaCl_2.2H_2O$ (2.7 g) $MgCl_2.6H_2O$ (7.23 g), $MgSO_4.7H_2O$ (13.3 g), and was made to 1 L with MilliQ water then sterilised by autoclaving. After autoclaving, 10 ml of filter sterilised HEPES buffer (1.0 M, pH 7.4), and 2 ml of filter sterilised $NaHCO_3$ (1.0 M) was added.

The culture was extracted by adding 10g Amberlite XAD7HP resin (Sigma) and shaken for 4–6 hours after which time the resin was filtered from the culture medium and washed twice with 200 ml milliQ water. The resin was then washed (extracted) with 3 x 80 ml methanol to yield the organic extract. Extracts were dried over anhydrous $MgSO_4$ (Ajax) and then evaporated to dryness *in vacuo*.

The crude methanol extract was fractionated on Sephadex LH-20 (flow rate: 2.4 ml/min, length: 44 cm, diameter: 2.87 cm, Pharmacia) in methanol. All 16 fractions (10 ml) collected were analysed by normal phase thin layer chromatography (TLC) using Silicagel-60 plates (Merck) and developed with 1% v/v acetic acid in $CHCl_3$:CH_3OH:H_2O (65:15:2) and visualised under UV_{254} and UV_{365} light. The TLC plate was treated with a 1% $FeCl_3$ in 0.05 N HCl staining reagent [6] with fractions 2 and 3 displaying a positive test indicative of the presence of iron-chelating siderophores. These fractions were combined and ^{1}H NMR of this material (467.4 mg) revealed that the major compound **2** was sufficiently pure for further analysis and found to be desferrioxamine E.



NMR data for desferrioxamine E

^{1}H NMR (DMSO-d_6) δ (multiplicity, assignment, *COSY*s, HMBCs)*: 1.19 (m, H7, 2H, *H6, H8*, C5, C6, C8, C9), 1.36 (m, H8, 2H, *H7*, *H9*, C6, C7, C9), 1.46 (m, H6, 2H, *H5*, *H7*, C5, C7, C8), 2.27 (m, H2, 2H, *H3*, C3, C4), 2.57 (m, H3, 2H, *H2*, C1, C2), 2.98 (m, H9, 2H, *H8*, *9-NH*, C7, C8, C1'), 3.46 (m, H5, 2H, *H6*, C4, C6, C7), 7.70 (brs, 9-NH, 1H, *H9*, C9, C1'), 9.73 (brs, N-OH, 1H). ^{13}C NMR (DMSO-d_6) δ 23.4 (C7), 26.0 (C6), 27.8 (C3), 28.8 (C8), 31.3 (C2), 38.6 (C9), 47.1 (C5), 171.8 (C1), 172.3 (C4). * NMR assignments refer to positions 1-9 of the trimer.

Genome sequencing and biosynthesis cluster identification.

Total DNA was extracted using MoBio kit. Total DNA yield was determined using the Qubit fluorometer double-stranded DNA BR Kit (Invitrogen). Genome sequencing was performed using Genome AnalyzerIIx, with the TruSeq SBS v4 GA kit.*De novo* genome assembly was performed with SOAPdenovo using k-mer a values of 49. These k-mer values represent the minimum read overlap required to during the assembly of contigs. Contiguous DNA sequences (contigs) shorter than 200 bp were discarded from the final assembly.[7] Gene prediction and annotation was performed using the best assembly produced by the software. The Rapid Annotation using Subsystem Technology (RAST) web application server was used for gene prediction and draft annotation.[8] Secondary metabolite biosynthesis clusters were identified using a combination of 2metDB[9] and antiSMASH.[10] Both these software used profile Hidden Markov Models (pHMMs) of known biosynthesis gene domains to identify secondary metabolite genes and their domain architecture in query sequences. All secondary metabolite gene clusters retrieved were manually checked and further confirmation of domain architecture was performed using NCBI Conserved Domain Database (CDD) Search. [11]Functions were assigned based on BLASTP comparisons and homologous gene clusters (antiSMASH).

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Supplementary Table 1. Putative functions of desferrioxamine biosynthesis proteins in *Marinobacter* sp. SB12.

Protein	Length (aa)	Putative Function	Sequence Similarity	Identity (%)/ Similarity (%)	Accession No.
DesA	525	decarboxylation	PLP-dependent glutamate decarboxylase *Marinobacterlipolyticus*SM19	90/95	EON92027.1
DesB	445	N-hydroxylation	Lysine/ornithine N-monooyxgenase *Marinobacterlipolyticus*SM19	91/94	WP_012138100.1
15_318	392	transporter	MFS transporter *Marinobacterlipolyticus*SM19	94/97	WP_012138101.1
DesCD	817	acyl transferase/ siderophore synthase	IucA/IucC *Marinobacterlipolyticus*SM19	94/98	WP_012138102.1