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BRIEF COMMUNICATION

Structure and biosynthesis of mayamycin B, a new polyketide with antibacterial activity from *Streptomyces* sp. 120454

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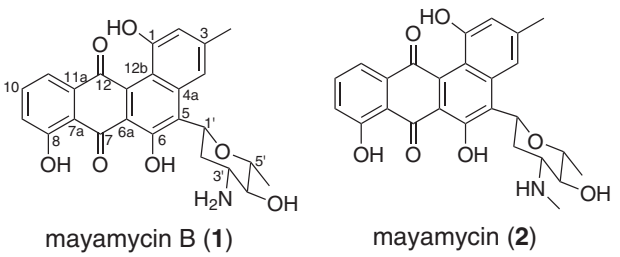
Abstract

Mayamycin B, a new antibacterial type II polyketide, together with its known congener mayamycin A, were isolated from *Streptomyces* sp. 120454. The structure of new compound was elucidated by extensive spectroscopic analysis and comparison with literature data. Sequencing and bioinformatics analysis revealed the biosynthetic gene cluster for mayamycins A and B.

Natural products biosynthesized by type II polyketide synthases (PKSs) comprise many important clinical drugs or drug candidates [1]. The angucycline-type polycyclic compounds, representing one of the largest family of type II polyketides, features a characteristic tetracyclic benz[a] anthracene scaffold, which are derived via successive decarboxylative Claisen condensations of an acetyl-CoA starter unit and nine methylmalonyl-CoA extender units [2–4]. The glycosylation using a combination of diverse sugar units at different angucyline aglycone position creates a great structural diversity for angucycline-type compounds [2, 5, 6]. These include landomycins, urdamycins, and saquayamycin, which are well known for their unusual structural features and potent antibacterial or antitumor bioactivities [7–9].

During our effort to discover new/bioactive natural products from microbes, we found that *S*. sp. 120454 strain mainly produce two major peaks based on chemical profiling when using B medium (dextrin 40 g, tomato paste 7.5 g, NZ Amine 2.5 g, primary yeast 5 g in 1 L distilled water) as fermentation medium. The large-scale fermentation was carried out at 30 °C for 7 days. The broth was harvested, and extracted by ethyl acetate, yielding 3.62 g brown crude extract, which was then fractionated and purified to afford compounds **1** and **2** (Fig. 1).

Compound **1** was isolated as a brown amorphous solid with the molecular formula of $C_{25}H_{23}NO_7$, as determined by high-resolution ESIMS ($[M+H]^+$ $m/z = 450.1497$), indicating 15° of unsaturation. Initial interpretation of its MS, ^1H, ^{13}C NMR spectra (Table 1) indicated the structure of **1** was highly similar to mayamycin (**2**), an aguacycline-type compound firstly isolated from a marine *Streptomyces* strain [10], with the exception of lacking an *N*-methyl group on its aminosugar moiety. Further elucidation of the 1D and 2D NMR spectra confirmed the presence of an identical anguacycline aglycone to that in mayamycin (**2**). An aminosugar moiety was evident by ^1H-^1H COSY correlations of H-1′/H-2′/H-3′/H-4′/H-5′/H-6′ from its ^1H-^1H COSY spectrum, and HMBC correlation of H-5′ (δ_H 3.56) with C-1′ (δ_C 72.5); as well as the NMR data comparison with those in **2**.



mayamycin B (**1**) mayamycin (**2**)

Fig. 1 Structures of compounds **1** and **2**

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Table 1 ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR data of **1** and **2** [10] in methanol-d_4

	1		**2**	
Position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	156.5,C		156.5,C	
2	114.3,CH	6.71,s	114.4,CH	6.74,s
3	143.5,C		143.4,C	
3-CH_3	22.5,CH_3	2.43,s	22.6,CH_3	2.45,s
4	117.2,CH	7.97,s	117.6,CH	8.00,s
4a	139.7,C		139.9,C	
5	126.2,C		126.5,C	
6	156.4,C		154.4,C	
6a	138.7,C		138.4,C	
7	194.2,C		194.2,C	
7a	116.3,C		116.3,C	
8	162.7,C		162.9,C	
9	124.6,CH	7.25,d (8.3)	124.9,CH	7.29,dd (1.2,8.5)
10	138.5,CH	7.71,t (7.7)	138.8,CH	7.75,dd (7.5,8.5)
11	120.1,CH	7.55,d (7.3)	120.4,CH	7.58,dd (1.2,7.5)
11a	137.7,C		137.9,C	
12	187.7,C		188.0,C	
12a	119.2,C		119.4,C	
12b	117.6,C		117.8,C	
1′	72.5,CH	5.69,dd (10.4,1.8)	72.8,CH	5.70,dd (11.7,2.0)
2′a	34.7,CH_2	2.49,m	32.9,CH_2	2.35,ddd (13.0,11.7)
2′b	34.7,CH_2	2.19,m	32.9,CH_2	2.27,ddd (13.0,2.0,4.8)
3′	55.3,CH	3.41,m	63.0,CH	3.23,br ddd (4.8,11.7,9.0)
3′-*N*-CH_3			31.4,CH_3	2.63,s
4′	74.5,CH	3.42,m	74.5,CH	3.41,t (9.0)
5′	79.1,CH	3.56,m	79.2,CH	3.56,dq (9.0,6.1)
5′-CH_3	18.4,CH_3	1.42,d (6.1)	18.7,CH_3	1.42,d (6.1)

The linkage of this aminosugar moiety to angucycline was through a rare C–C bond according to the HMBC correlations of H-1′ with C-4a and C-6.

The stereochemistry of the sugar moiety was determined by interpretation of its NOESY spectrum and J-coupling constant. A large coupling constant (10.2 Hz) between H-1′ and H-2′a indicated an axial configuration of H-1′. The strong NOE correlations of H-1′ with H-3′ and H-5′ revealed that all of these three protons are in axial positions. Furthermore, H-4′ showed NOE correlations to H-2′a suggested that H-4′ and H-2′a are also in axial position. Thus, the relative configuration of the deduced aminosugar is 1′*R**, 3′*R**, 4′*S*, and 5′*R*. The result indicates that compound **1** is an *N*-desmethyl derivative of mayamycin (**2**), accordingly, compound **1** is designated as mayamycin B (Fig. 2).

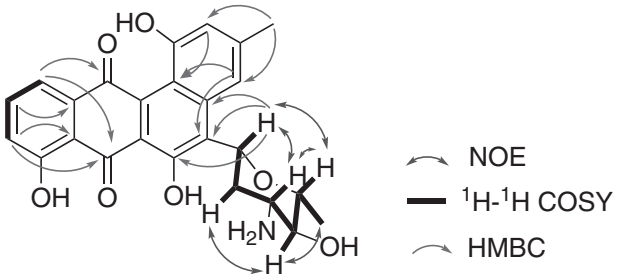

Fig. 2 Key 2D NMR correlations of **1**

Table 2 Antibacterial activities of **1**–**2** (MIC, μM)

Pathogens[a]	**1**	**2**	Rifampicin
S. aureus	64.0	64.0	1.0
MRSA	>128.0	>128.0	4.0
S. pyogenes	64.0	64.0	0.5
B. subtilis	64.0	64.0	1.0
P. aeruginosa	64.0	64.0	0.5
M. luteus	2.0	8.0	0.5

[a]*S. aureus*, MRSA, *B. subtilis*, *S. pyogenes*, *P. aeruginosa*, and *M. luteus* represent *Staphylococcus aureus* CMCC(B)26003, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, *Bacillus subtilis* CICC10283, *Streptococcus pyogenes* ATCC19615, *Pseudomonas aeruginosa* CICC10351, and *Micrococcus luteus* CMCC(B) 28001, respectively

Compound **2** was also isolated as a brown amorphous solid, its structure is identified as mayamycin based on the comparison of its NMR and HRESIMS data with the published data [10, 11].

Mayamycin has been reported to show antibacterial activities against a panel of pathogenic bacteria [10, 11]. Therefore, the antimicrobial activities of compounds **1** and **2** were tested against six pathogenic bacteria as shown in Table 2. The result showed mayamycin B (**1**) showed potent bioactivity against *Micrococcus luteus* with MIC value of 2.0 μM, whereas, its congener mayamycin A (**2**) only has MIC value of 8.0 μM, suggesting the *N*-methyl group is important to its activity.

We next aimed to identify mayamycin biosynthetic gene cluster. To facilitate the process, the strain of *Streptomyces* sp. 120454 was subjected to whole-genome sequencing by Pacbio, yielding a 7.8 Mb linear chromosome with 71.5% GC content. AntiSMASH analysis indicated at least thirty one distinct secondary metabolite gene clusters were encoded in its genome with only one type II polyketide synthetic gene cluster, whose gene products show high similarity to proteins involved in other angucycline biosynthesis [2]. The putative *may* gene cluster spans a

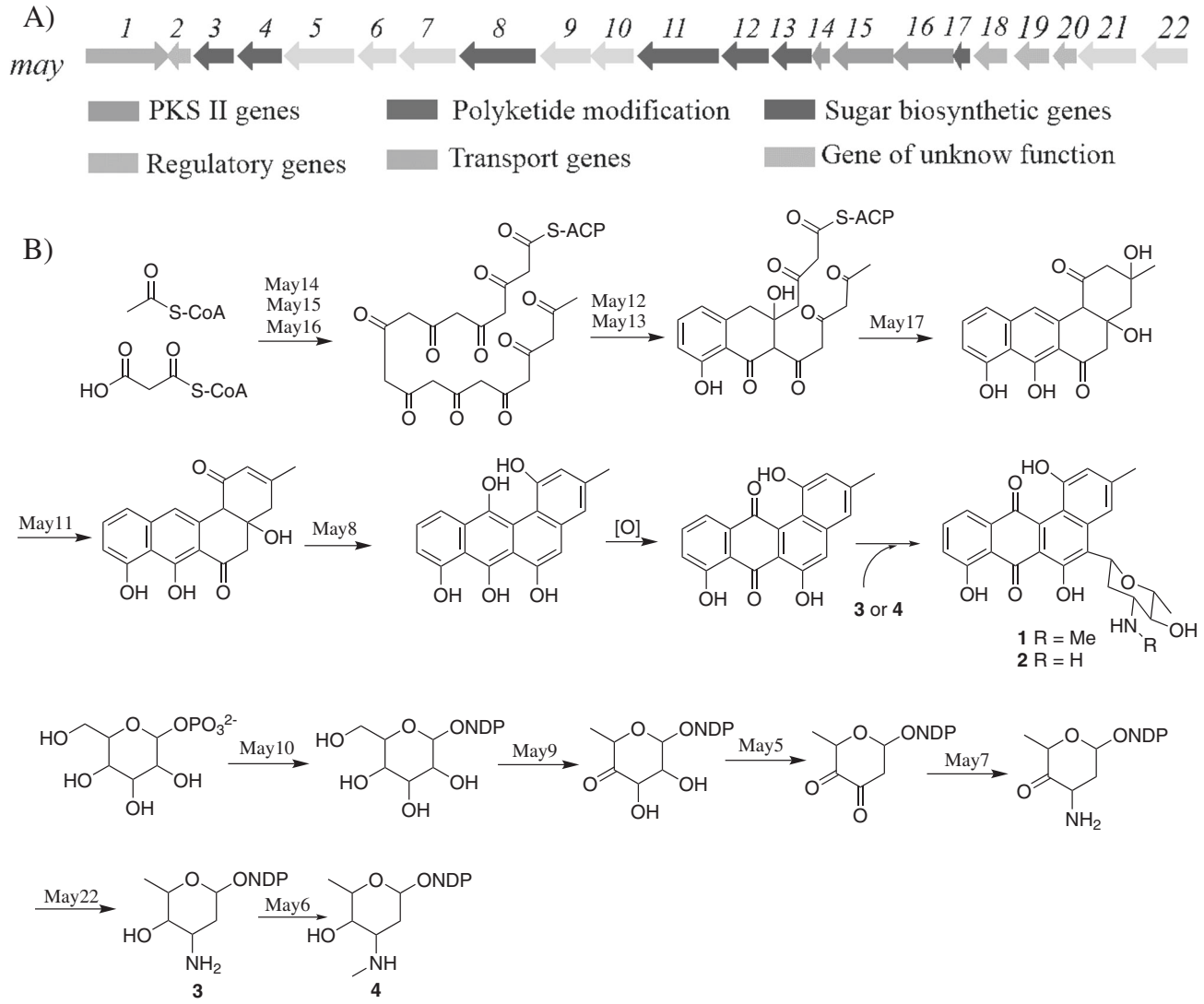

Fig. 3 Putative biosynthetic gene cluster for mayamycins

23.2 kb contiguous DNA region consisting of 20 genes responsible for biosynthesis, regulation, and transporter. The nucleotide sequence have been deposited in the Genbank under accession number MG601230.

Functional assignments for the gene products within *may* gene cluster were made by sequence analysis through BLAST comparison. Biosynthesis of angucycline aglycone was initiated by the minimal type II PKS cassette, May16 (KS_α), May15 (KS_β), and May14 (ACP) which utilize one acetyl CoA and nine malonyl-CoA to form a linear polyketide backbone. May12, May13, and May17 are homologues to bifunctional cyclase/dehydrase JadD, ketoreductase JadE, and polyketide cyclase JadI, respectively, in jadomycin biosynthesis. It is thus anticipated that the products of *may12*, *may13*, and *may17* are sufficient for the formation of tetracyclic ring [12–15]. The subsequent two steps of dehydration followed by oxidation led to the formation of desired aglycone (Fig. 3).

Six genes including *may5*, *may6*, *may7*, *may9*, *may10*, and *may22* encode proteins similar to Med-ORF16, Med-ORF15, Med-ORF20, Med-ORF17, Med-ORF18, Med-ORF14, which are essential for the biosynthesis of aminosugar moiety of medemycin (Table 3) [16], suggesting these proteins were responsible for synthesizing aminosugar moiety in compounds **1** and **2**. A *C*-glycosyltransferase, May21, catalyzes the final *C*-glycosylation step using either **3** or **4** as aminosugar donors to finally form **1** and **2** [16–19].

In summary, a new type II polyketide, mayamycin B (**1**), together with its known congener mayamycin (**2**) were isolated and characterized from *Streptomyces* sp. 120454. Compound **1** showed potent antibacterial bioactivity against *Micrococcus luteus*. Sequencing and bioinformatics

Table 3 Biosynthetic gene clusters of aminosugar moiety in mayamycins

Gene	Size (aa)	Protein homolog	Accession number	Similarity/identity (%)
may1	550	Transporter	OKI73663	92/99
may2	158	JadX: unknown	AAK01935	94/54
may3	266	JadM: phosphopantetheinyl transferase-like protein	AAO65355	80/46
may4	295	Thioesterase	KIA66745	93/60
may5	470	Med-ORF16: NDP-deoxyglucose-2,3-dehydratase	BAC79031	96/66
may6	253	Med-ORF15: N-Methyl transferase	BAC79032	98/48
may7	373	Med-ORF20: NDP-deoxyhexose 3-aminotransferase	BAC79028	98/77
may8	504	JadH: bifunctional monooxygenase-dehydratase	AAV52248	95/68
may9	331	Med-ORF17: NDP-hexose 4,6-dehydratase	BAC79030	96/77
may10	288	Med-ORF18: NDP-glucose phosphate nucleotidyltransferase	BAC79029	62/36
may11	536	JadF: bifunctional monooxygenase-dehydratase	AAV52246	91/64
may12	313	JadD: bifunctional cyclase/dehydrase	AAB36566	99/78
may13	262	JadE: ketoreductase	ACP19356	96/74
may14	117	JadC: acyl carrier protein	ACP19355	68/63
may15	404	JadB: chain length factor	ACP19354	99/70
may16	423	JadA: ketosynthase alpha	ACP19353	98/80
may17	109	JadI: polyketide synthase cyclase	AF126429_2	100/83
may18	224	JadR1: DNA-binding response regulator	AJE85517	100/67
may19	235	Monooxygenase	AF425988_1	78/33
may20	159	Hypothetical protein	SFL52321	91/32
may21	379	Med-ORF8: *C*-glycosyltransferase	BAC79040	100/52
may22	308	Med-ORF18: NDP-4-keto-6-deoxyhexose reductase	BAC79033	94/56

analysis of *S*. sp. 120454 allow us to propose the biosynthetic pathway of mayamycin for the first time.

Mayamycin B (1): dark brown amorphous solid; UV (MeOH) λ_{max} (lgε) 440 (2.83), 328 (3.02), 298 (2.98), 236 (3.26); IR (KBr) ν_{max} cm^{-1}: 3250, 2922, 2857, 2360, 1608, 1420; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data see Table 1; HRESIMS *m/z*: 450.1497 $[M+H]^+$ (calcd. for $C_{25}H_{24}NO_7$, 450.1508).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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