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Genome Report

Whole-genome sequence of *Streptomyces* sp. MUM 163J with a putative plasmid-associated contig

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Abstract: Genome sequencing facilitates the taxonomic identification and characterisation of *Streptomyces* isolates and provides insights into their biosynthetic potential. Here, we report the whole-genome sequence of *Streptomyces* sp. MUM 163J generated using PacBio Revio high-fidelity (HiFi) sequencing. A total of 77,748 HiFi reads, comprising 1,112,147,514 bp, were assembled using Flye v2.9.6, yielding three contigs with a combined length of 12,405,764 bp, an N50 of 12,188,866 bp and a GC content of 70.87%. Genome completeness assessments using BUSCO and CheckM2 yielded estimates of 99.0% and 100.0%, respectively, with CheckM2 predicting 1.13% contamination. Genome-based taxonomic analyses identified strain MUM 163J as *Streptomyces malaysiensis*, supported by an average nucleotide identity of 98.84% and a digital DNA–DNA hybridisation value of 88.2% relative to the type-strain genome. Bakta annotation identified 10,042 coding-sequence features, including two small open reading frames, along with 74 tRNA and 18 rRNA genes. Analysis using antiSMASH predicted 51 biosynthetic regions associated with various classes of specialised metabolites, including polyketides, non-ribosomal peptides and terpenes. The smallest contig (64,833 bp), containing 77 coding-sequence features, exhibited partial nucleotide similarity to previously reported *Streptomyces* plasmids. Among the selected plasmid references, the highest BLASTn query coverage was 51.07%, distributed across multiple local alignments. These findings suggest a possible plasmid association, although the contig's structural organisation and physical topology remain unresolved. The genome sequence provides a resource for further comparative genomic studies and investigations into the biosynthetic potential of strain MUM 163J.

Keywords: *Streptomyces malaysiensis*; whole-genome sequencing; genome assembly; genome annotation; plasmids; SDG 3 Good health and well-being

1. Introduction

Studies of mangrove-associated *Streptomyces* have contributed to the understanding of actinobacterial diversity and their potential biological applications [1]. Studies of mangrove-derived *Streptomyces* isolates from East and West Malaysia have characterised previously undescribed species using polyphasic taxonomic approaches [2–4]. Beyond taxonomic investigations, these *Streptomyces* isolates have exhibited antioxidant, cytotoxic and antibacterial activities, including inhibitory effects against *Vibrio parahaemolyticus*, an important pathogen in aquaculture [5–9]. Genome sequencing has complemented these findings by providing insights into the genetic repertoire and predicted biosynthetic pathways of mangrove-derived strains [10–12]. More recently, comparative genomic approaches have enabled broader investigations of biosynthetic diversity, as demonstrated by the pangenomic analysis of the *Streptomyces* genus and species [13,14]. Collectively, these studies highlight the value of examining individual isolates at the genomic level to expand available sequence resources and facilitate further investigations of their metabolic potential.

The genomic characterisation of individual *Streptomyces* strains remains relevant given the considerable variation in genome organisation and gene content within the genus. The genome sequence of *Streptomyces coelicolor* A3(2) established an important reference for investigating the genomic complexity of these filamentous bacteria [15], while comparative studies have revealed differences in gene repertoires among related species [16]. Such variation is particularly relevant to investigations of specialised metabolism and the identification of biosynthetic pathways with potential biotechnological applications [17,18]. Genome sequence resources also support broader investigations of actinobacterial physiology, genetic diversity and evolutionary relationships [19,20].

In this study, we report the genome sequence of *Streptomyces* sp. MUM 163J, an isolate originating from mangrove soil in East Malaysia, generated using PacBio Revio high-fidelity sequencing. The Flye-derived assembly was subjected to genome annotation, genome-based taxonomic analysis and prediction of biosynthetic gene clusters. An additional contig was examined through sequence similarity searches to investigate its possible relationship to previously characterised *Streptomyces* extrachromosomal elements. These analyses provide a genomic resource for strain MUM 163J and a foundation for subsequent investigations of its genomic organisation and biosynthetic potential.

2. Data Description

Streptomyces sp. MUM 163J was isolated from mangrove soil collected from Trombol, Sarawak, in East Malaysia. The strain was cultured using the International *Streptomyces* Project (ISP) 2 medium at 28 °C. The genomic DNA extraction was performed using the MasterPure Complete DNA and RNA Purification Kit (Biosearch Technologies, Inc., US). The integrity and concentration of the extracted DNA were evaluated using agarose gel electrophoresis and Qubit fluorometry, respectively. Whole-genome sequencing was performed using the PacBio Revio platform with high-fidelity single-molecule real-time sequencing technology by Beijing Novogene Bioinformatics Technology Co., Ltd. The raw sequencing data were provided in a HiFi BAM file and converted to compressed FASTQ format using bam2fastq from the PacBio BAM toolkit (pbtk) version 3.5.0 (https://github.com/pacificbiosciences/pbtk). Inspection of the BAM header using samtools view -H identified circular consensus sequencing software v26.1.0, pbtrim v26.1.0 and lima v26.1.1 [21].

The quality of the FASTQ dataset was assessed using Seqkit stats v2.13.0 [22]. The dataset comprised 77,748 reads, totalling 1,112,147,514 bp, with a mean read length of 14,305 bp. De novo genome assembly was performed using Flye v2.9.6 with the --pacbio-hifi option [23]. Flye was run with the default settings, which include one polishing iteration. The resulting assembly

consisted of three contigs with a combined length of 12,405,764 bp and an N50 of 12,188,866 bp. The largest contig (contig_1) measured 12,188,866 bp, accounting for approximately 98.3% of the assembled sequence. The two remaining contigs, contig_2 and contig_3, measured 152,065 bp and 64,833 bp, respectively. The assembly had an overall GC content of 70.87% and contained no ambiguous N bases. Flye also reported read length N50 and N90 values of 14,442 bp and 11,590 bp, respectively. The mean sequencing depths for the three contigs were 89.09×, 131.04× and 26.69×, respectively, as determined using samtools coverage in SAMtools v1.23. Assembly quality statistics were also assessed using QUAST version 5.3.0 [24]. Genome completeness was evaluated with BUSCO v6.1.0 using the *streptomyces_odb12.2* dataset of 1,438 marker groups [25]. Of these, 1,423 (99%) were complete, including 1,419 single-copy and four duplicated markers; eight were fragmented and seven were missing. CheckM2 v.1.1.0 provided estimates of 100% completeness and 1.13% contamination [26]. The assembly statistics and genome features of strain MUM 163J are summarised in Table 1. Figure 1 illustrates the genomic organisation of MUM 163J using pyCirclize, incorporating gene annotations generated by Bakta, Clusters of Orthologous Groups (COG) functional classifications (COGclassifier v2.0.0) [27] and biosynthetic gene cluster predictions obtained from antiSMASH, which were described in greater detail below.

Table 1. The assembly statistics and genome features of *Streptomyces* sp. MUM 163J.

Genome features	Value
Number of HiFi reads	77,748
Total sequencing bases	1,112,147,514 bp
Genome size	12,405,764 bp
Number of contigs	3
Contig N50	12,188,866 bp
auN	11977962.9
GC content	70.87%
Weighted mean sequencing depth	89.28×
Complete BUSCO markers	1,423 / 1,438 (99%)
CheckM2 completeness	100%
CheckM2 contamination	1.13%
Bakta CDS features (including two small ORFs)	10,042
tRNA / rRNA / tmRNA genes	74 / 18 / 2
ncRNAs / regulatory ncRNA regions	175 / 27
CRISPR arrays	4
antiSMASH predicted biosynthetic regions	51 (strict detection setting)
GenBank nucleotide accession number	JCGPDY000000000.1

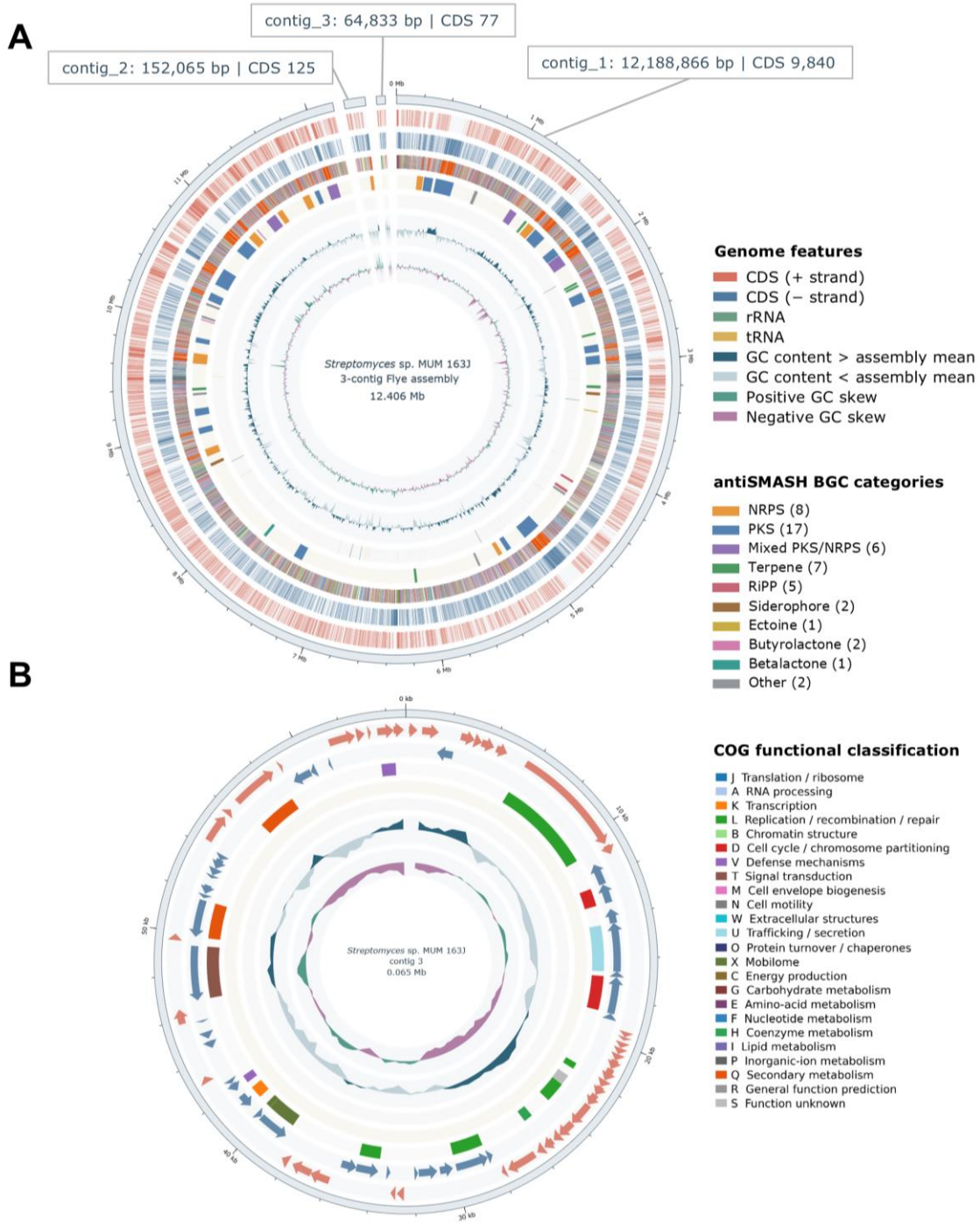


Figure 1. Overview of the MUM 163J genome assembly. (A) Circular representation of the whole-genome assembly of *Streptomyces* sp. MUM 163J. The genome map was generated using pyCirclize v1.10.1 (https://github.com/moshi4/pyCirclize). The outermost track indicates genomic coordinates in megabases (Mb), followed inward by coding sequences (CDSs) on the forward (salmon) and reverse (blue) strands, COG functional classification, predicted biosynthetic gene cluster regions identified by antiSMASH, and annotated rRNA and tRNA genes. The innermost tracks represent variations in GC content relative to the assembly-wide mean and GC skew, with positive and negative deviations shown in contrasting colours. (B) Genome organisation of contig_3 of *Streptomyces* sp. MUM 163J. A 64,833-bp contig flagged as circular by Flye contains 77 annotated CDSs.

Taxonomic placement was examined using the Type (Strain) Genome Server (TYGS) [28] and FastANI v1.34 [29]. Pairwise FastANI analysis showed an average nucleotide identity (ANI) of 98.84% between strain MUM 163J and the *Streptomyces malaysiensis* type strain JCM 10672^T (reference assembly GCA_039525135.1), with a reciprocal ANI of 98.86%. TYGS analysis yielded digital DNA-DNA hybridisation (dDDH) values calculated using d4 of 88.2% (95% confidence interval (CI), 85.7 – 90.3%) against *S. malaysiensis* JCM 10672 [30] and 88.3% (95% CI, 85.9 – 90.4%) against *S. solisilvae* HNM0141^T. Figure 2 illustrates strain MUM 163J clustered with *Streptomyces malaysiensis* JCM 10672, *Streptomyces solisilvae* HNM0141^T and *Streptomyces malaysiensis* subsp. *samsunensis* DSM 42010, supporting its close phylogenomic relationship with *S. malaysiensis*. These genome-based comparisons support assignment of strain MUM 163J to *S. malaysiensis*. The previous designation *Streptomyces solisilvae* has been treated as a later heterotypic synonym of *S. malaysiensis*, consistent with the close relationship observed among the reference genomes [31].

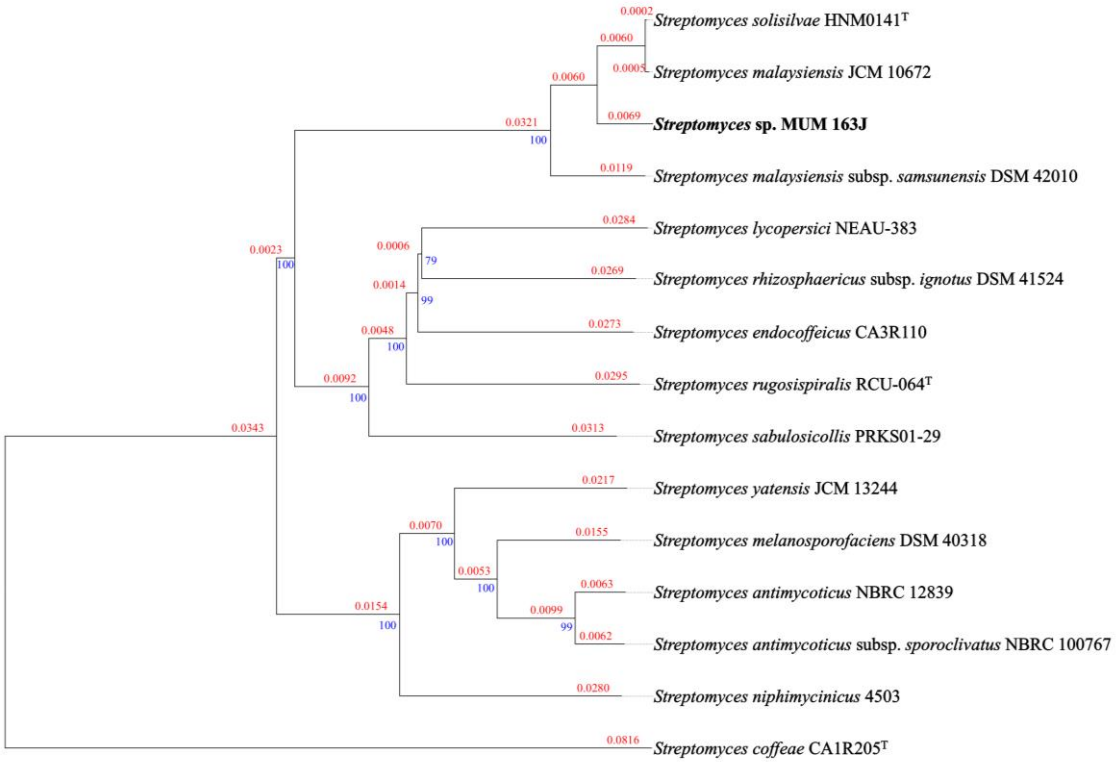


Figure 2. Whole-genome phylogenetic tree illustrating the relationships between *Streptomyces* sp. MUM 163J and selected *Streptomyces* reference strains, generated using the Type (Strain) Genome Server (TYGS). Phylogenetic reconstruction was performed using FastME v2.1.61 [32], based on Genome BLAST Distance Phylogeny (GBDP) distances calculated using formula d5. Red numerical labels represent branch lengths expressed as GBDP distances, while blue labels at the nodes indicate GBDP pseudo-bootstrap support values above 60%, obtained from 100 replications.

Structural and functional annotation was carried out using Bakta v1.9.4 with the full database v5.1 [33]. The annotated assembly contained 10,042 coding-sequence (CDS) features,

comprising 10,040 CDSs and two small open reading frames reported separately in the Bakta summary. Of these, 9,840 features were assigned to contig_1, 125 to contig_2 and 77 to contig_3. Bakta also predicted 74 tRNA genes, 18 rRNA genes, two transfer-messenger RNA genes, 175 non-coding RNAs, 27 regulatory non-coding RNA regions and four CRISPR arrays. Putative biosynthetic gene clusters were predicted using antiSMASH v8.0.4 with default parameters and the "strict" detection strictness [34]. The antiSMASH analysis predicted 51 biosynthetic regions, with 50 located on contig_1 and one on contig_2; none was reported for contig_3. The predicted regions were grouped into broad biosynthetic categories; multi-product regions were classified according to the precedence Mixed PKS/NRPS > PKS > NRPS > RiPP > Terpene > other named categories. The Mixed PKS/NRPS category included regions with NRPS labels together with either explicit PKS or arylpolyene labels and does not establish a chemically hybrid biosynthetic pathway. These regions comprise 17 PKS-associated regions, 8 NRPS-associated regions, 6 regions containing both PKS- and NRPS- associated biosynthetic types, 7 terpene regions, 5 RiPP regions, 2 siderophore regions, 2 butyrolactone regions, and one region each associated with ectoine and betalactone biosynthesis. The remaining 2 regions were classified under other biosynthetic types (Figures 1 and 3). The biosynthetic regions identified in strain MUM 163J are computational predictions and do not establish whether the corresponding pathways are expressed or their metabolites are produced under a given culture condition. Phenotypic heterogeneity within *Streptomyces* populations may also influence metabolic activity and specialised metabolite production. Single-cell approaches have been proposed to investigate this variation [35] and could complement future studies of strain MUM 163J. Further experimental work, including gene-expression and metabolite analyses, will be needed to assess the activities associated with the predicted biosynthetic regions.

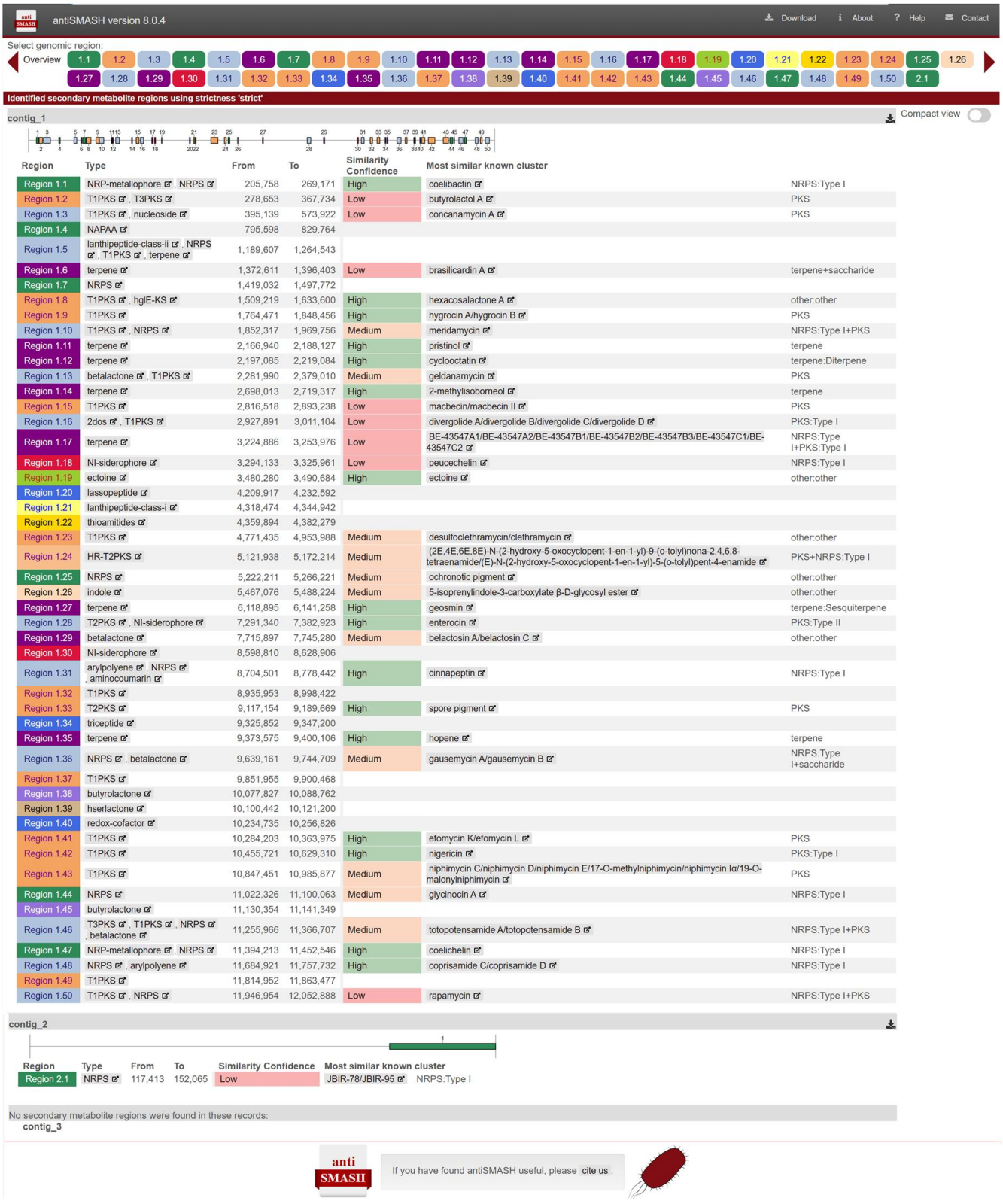
Contig_3 was examined separately because of its size and its circularity flag in the Flye output. This 64,833-bp sequence had a GC content of 70.46% and contained 77 predicted CDS features. Among the annotated products on contig_3 were a TraM recognition domain-containing protein, a transposase and putative tyrosine recombinase/integrase proteins. TraM-recognition domains occur in proteins associated with bacterial conjugative DNA transfer, whereas transposases and tyrosine recombinases are involved in DNA transposition and site-specific recombination, respectively [36–38]. The annotations suggest that contig_3 may harbour genes associated with DNA mobility or rearrangement, although their functions remain unverified. Further investigation could focus on characterising the predicted protein domains, identifying neighbouring genes involved in DNA replication and transfer, and comparing the genetic organisation of contig_3 with characterised *Streptomyces* plasmids. Experimental investigation of DNA transfer and recombination could subsequently help establish whether these genes contribute to the mobility of the element [39,40].

Nucleotide similarity searches of contig_3 were performed using BLASTn v2.17.0+ against the NCBI *core_nt* database, with an E-value threshold of 0.05 [41]. Several matches to annotated *Streptomyces* plasmid sequences were identified (Table 2). The highest-scoring selected match was to plasmid punnamed3 of *Streptomyces* sp. HUAS TT7 (CP196178.1), with 14 high-scoring segment pairs (HSPs) covering 33,108 bp (51.07%) of contig_3. The best individual alignment was 9,936 bp long, with 86.101% nucleotide identity. Other plasmid sequences, CP108099.1, CP109289.1 and CP117711.1, showed combined query coverage values of 41.21%, 30.20% and 23.98%, respectively. Query coverage was calculated from the non-overlapping aligned regions, while alignment length and nucleotide identity were based on the best individual HSP for each reference sequence. The sequence similarity to known *Streptomyces* plasmids, together with the presence of predicted mobility-associated proteins, suggests that contig_3 may be plasmid-associated. Flye also classified contig_3 as circular in its assembly information file. However, these findings do not confirm that it exists as an independently replicating circular plasmid. Therefore, contig_3 was provisionally considered a putative plasmid-associated contig.

Table 2. Selected BLASTn comparisons between MUM 163J contig_3 and plasmid-annotated *Streptomyces* sequences.

Accession number; strain, plasmid	Reference sequence length (bp)	HSPs	Combined query coverage (%)	Best HSP identity (%)	Best HSP length (columns)	Best HSP E-value	Best HSP bit score
CP196178.1; *Streptomyces* sp. HUAS TT7, punnamed3	53,343	14	51.07	86.101	9,936	0.0	10,512.3
CP117711.1; *Streptomyces* sp. MMBL 11-1, unnamed2	53,102	13	23.98	88.587	3,347	0.0	4,021.28
CP108099.1; *Streptomyces* sp. NBC_00239, unnamed4	53,565	17	41.21	90.302	3,011	0.0	3,921.56
CP109289.1; *S. anulatus* NBC 01625, unnamed2	54,177	11	30.20	89.077	3,012	0.0	3,722.12

Note: HSP, high-scoring segment pair. Combined query coverage represents the percentage of contig_3 covered by one or more HSPs against each reference sequence, with overlapping query regions counted only once. Percentage identity, alignment length, E-value and bit score refer only to the highest-scoring HSP for each reference sequence, rather than the combined alignment. An E-value of 0.0 indicates a significant match.



Region	Type	From	To	Similarity Confidence	Most similar known cluster	
Region 1.1	NRP-metallophore, NRPS	205,758	269,171	High	coelibactin	NRPS:Type I
Region 1.2	T1PKS, T3PKS	278,653	367,734	Low	butyrolactol A	PKS
Region 1.3	T1PKS, nucleoside	395,139	573,922	Low	concanamycin A	PKS
Region 1.4	NAPAA	795,598	829,764			
Region 1.5	lanthipeptide-class-ii, NRPS, T1PKS, terpene	1,189,607	1,264,543			
Region 1.6	terpene	1,372,611	1,396,403	Low	brasilicardin A	terpene+saccharide
Region 1.7	NRPS	1,419,032	1,497,772			
Region 1.8	T1PKS, hglE-KS	1,509,219	1,633,600	High	hexacosalactone A	other:other
Region 1.9	T1PKS	1,764,471	1,848,456	High	hygrocin A/hygrocin B	PKS
Region 1.10	T1PKS, NRPS	1,852,317	1,969,756	Medium	meridamycin	NRPS:Type I+PKS
Region 1.11	terpene	2,166,940	2,188,127	High	pristinol	terpene
Region 1.12	terpene	2,197,085	2,219,084	High	cyclooctatin	terpene:Diterpene
Region 1.13	betalactone, T1PKS	2,281,990	2,379,010	Medium	geldanamycin	PKS
Region 1.14	terpene	2,698,013	2,719,317	High	2-methylisoborneol	terpene
Region 1.15	T1PKS	2,816,518	2,893,238	Low	macbecin/macbecin II	PKS
Region 1.16	2dos, T1PKS	2,927,891	3,011,104	Low	divergolide A/divergolide B/divergolide C/divergolide D	PKS:Type I
Region 1.17	terpene	3,224,886	3,253,976	Low	BE-43547A1/BE-43547A2/BE-43547B1/BE-43547B2/BE-43547B3/BE-43547C1/BE-43547C2	NRPS:Type I+PKS:Type I
Region 1.18	NI-siderophore	3,294,133	3,325,961	Low	peucechelin	NRPS:Type I
Region 1.19	ectoine	3,480,280	3,490,684	High	ectoine	other:other
Region 1.20	lassopeptide	4,209,917	4,232,592			
Region 1.21	lanthipeptide-class-i	4,318,474	4,344,942			
Region 1.22	thioamitides	4,359,894	4,382,279			
Region 1.23	T1PKS	4,771,435	4,953,988	Medium	desulfoclethramycin/clethramycin	other:other
Region 1.24	HR-T2PKS	5,121,938	5,172,214	Medium	(2E,4E,6E,8E)-N-(2-hydroxy-5-oxocyclopent-1-en-1-yl)-9-(o-tolyl)nona-2,4,6,8-tetraenamide/(E)-N-(2-hydroxy-5-oxocyclopent-1-en-1-yl)-5-(o-tolyl)pent-4-enamide	PKS+NRPS:Type I
Region 1.25	NRPS	5,222,211	5,266,221	Medium	ochronotic pigment	other:other
Region 1.26	indole	5,467,076	5,488,224	Medium	5-isoprenylindole-3-carboxylate β-D-glycosyl ester	other:other
Region 1.27	terpene	6,118,895	6,141,258	High	geosmin	terpene:Sesquiterpene
Region 1.28	T2PKS, NI-siderophore	7,291,340	7,382,923	High	enterocin	PKS:Type II
Region 1.29	betalactone	7,715,897	7,745,280	Medium	belactosin A/belactosin C	other:other
Region 1.30	NI-siderophore	8,598,810	8,628,906			
Region 1.31	arylpolyene, NRPS, aminocoumarin	8,704,501	8,778,442	High	cinnapeptin	NRPS:Type I
Region 1.32	T1PKS	8,935,953	8,998,422			
Region 1.33	T2PKS	9,117,154	9,189,669	High	spore pigment	PKS
Region 1.34	triceptide	9,325,852	9,347,200			
Region 1.35	terpene	9,373,575	9,400,106	High	hopene	terpene
Region 1.36	NRPS, betalactone	9,639,161	9,744,709	Medium	gausemycin A/gausemycin B	NRPS:Type I+saccharide
Region 1.37	T1PKS	9,851,955	9,900,468			
Region 1.38	butyrolactone	10,077,827	10,088,762			
Region 1.39	hserlactone	10,100,442	10,121,200			
Region 1.40	redox-cofactor	10,234,735	10,256,826			
Region 1.41	T1PKS	10,284,203	10,363,975	High	efomycin K/efomycin L	PKS
Region 1.42	T1PKS	10,455,721	10,629,310	High	nigericin	PKS:Type I
Region 1.43	T1PKS	10,847,451	10,985,877	Medium	niphimycin C/niphimycin D/niphimycin E/17-O-methylniphimycin/niphimycin Iα/19-O-malonylniphimycin	PKS
Region 1.44	NRPS	11,022,326	11,100,063	Medium	glycinocin A	NRPS:Type I
Region 1.45	butyrolactone	11,130,354	11,141,349			
Region 1.46	T3PKS, T1PKS, NRPS, betalactone	11,255,966	11,366,707	Medium	totopotensamide A/totopotensamide B	NRPS:Type I+PKS
Region 1.47	NRP-metallophore, NRPS	11,394,213	11,452,546	High	coelichelin	NRPS:Type I
Region 1.48	NRPS, arylpolyene	11,684,921	11,757,732	High	coprisamide C/coprisamide D	NRPS:Type I
Region 1.49	T1PKS	11,814,952	11,863,477			
Region 1.50	T1PKS, NRPS	11,946,954	12,052,888	Low	rapamycin	NRPS:Type I+PKS

Region	Type	From	To	Similarity Confidence	Most similar known cluster	
Region 2.1	NRPS	117,413	152,065	Low	JBIR-78/JBIR-95	NRPS:Type I

Figure 3. Detailed biosynthetic region coordinates and classifications predicted in the whole genome of strain MUM 163J by antiSMASH version 8.0.4.

The genome sequence of *Streptomyces* sp. MUM 163J provides a reference for subsequent investigations into its genomic diversity, functional characteristics and specialised metabolism. The availability of the assembled sequences, including the putative plasmid-associated contig, facilitates further comparisons with related *Streptomyces* genomes and may support future studies of the biological activities and genetic potential of this strain. The genome sequence of MUM 163J has been deposited in the DDBJ/ENA/GenBank under the accession number JCGPDY000000000. The version described in this paper is version JCGPDY010000000. The data are publicly available at NCBI GenBank under the BioProject accession number PRJNA1556056 and the BioSample accession number SAMN64621036.

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Conflicts of Interest: The authors declare no conflict of interest.

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