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Genome Report

Whole-genome sequencing of *Streptomyces* sp. MUM 161J from mangrove soil in East Malaysia

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Abstract: Whole-genome sequencing provides an essential foundation for investigating the genetic diversity and biosynthetic potential of *Streptomyces*, a genus widely recognised for its production of structurally diverse secondary metabolites. Here, we report the genome sequence of *Streptomyces* sp. MUM 161J using PacBio Revio high-fidelity whole-genome sequencing. A total of 117,499 reads comprising 1,656,239,452 bases, with a read N50 of 14,515 bp, were assembled using Hifiasm version 0.25.0-r726. The resulting assembly comprised two contigs totaling 11,071,069 bp, with a contig N50 of 10,651,607 bp and a G+C content of 71.03%. The length-weighted mean mapped depth was 149.2×, and 99.99% of primary reads mapped to the assembly. BUSCO recovered 98.9% complete lineage

markers, while CheckM2 estimated 100.0% completeness and 0.56% contamination. Genome annotation using Bakta identified 8,894 standard coding-sequence predictions, including 24 pseudogene calls, plus three separately reported short open reading frames. The annotation also included 70 tRNA features, 18 rRNA genes and two transfer-messenger RNA features. Genome-based comparisons revealed close relatedness to *Streptomyces malaysiensis* JCM 10672^{T}, with an average nucleotide identity of 98.84% and digital DNA–DNA hybridisation of 87.5%. AntiSMASH analysis predicted 46 biosynthetic regions, highlighting the potential for diverse specialised metabolite biosynthesis. The genome sequence provides a valuable resource for subsequent taxonomic, comparative and functional investigations, while further analysis of the contig relationships and chromosomal termini will be needed to resolve its physical genome organisation.

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

1. Introduction

The genus *Streptomyces* encompasses filamentous bacteria with extensive genomic diversity and a well-established role in the production of specialised metabolites [1, 2]. The genome of *Streptomyces coelicolor* A3(2) provided an early framework for understanding its large gene repertoire and complex development, while comparative analyses have demonstrated substantial variation in gene content among members of the genus [1–3]. This diversity has sustained interest in individual isolates for biotechnology and natural-product research [4, 5]. Broader studies of actinobacterial physiology and bacterial diversity also emphasise the value of strain-level sequence resources [6, 7].

In Malaysia, mangrove ecosystems have been investigated as reservoirs of taxonomically diverse *Streptomyces* isolates with potentially valuable biological characteristics. Malaysian mangrove environments have yielded *Streptomyces* isolates investigated through taxonomic, genomic and biological approaches. Reports on *Streptomyces griseiviridis* MUM 136J^{T} and *Streptomyces learnhanii* MUM 203J^{T} illustrate the use of polyphasic characterisation to describe novel species from environmental samples [8, 9]. Genome reports on *Streptomyces pluripotens* MUM 16J and *Streptomyces* sp. MUM 178J have provided sequence resources for subsequent biosynthetic investigations [10, 11]. Separate studies of *Streptomyces* sp. MUM 178J and MUM 195J examined antagonism against *Vibrio parahaemolyticus* and potential applications in aquaculture [12, 13]. These observations establish the broader rationale for characterising MUM isolates, while the biological properties of one strain cannot be assigned to another from their collection

designation. More recently, a pangenomic study of mangrove-derived *Streptomyces albidoflavus* MUM256 integrated genome-based taxonomic assignment with comparisons of gene content and biosynthetic diversity [14]. *Streptomyces* sp. MUM 161J was obtained from a collection of isolates originally recovered from mangrove soil samples in East Malaysia. Here, we describe its PacBio Revio HiFi whole-genome assembly, read support, gene annotation, genome-based taxonomic identification and predicted biosynthetic regions.

2. Data Description

Streptomyces sp. MUM 161J was revived on International *Streptomyces* Project medium 2 (ISP2). For genomic DNA extraction, the strain was cultivated overnight in tryptic soy broth at 28°C before harvesting the bacterial biomass. Genomic DNA was extracted using the MasterPure Complete DNA and RNA Purification Kit (Biosearch Technologies, Inc., US). DNA integrity and concentration were assessed using agarose gel electrophoresis and Qubit fluorometry, respectively.

Whole-genome sequencing was performed using the PacBio Revio platform to generate high-fidelity (HiFi) long reads through circular consensus sequencing at Beijing Novogene Bioinformatics Technology Co., Ltd. [15]. The sequencing records identified CCS software version 26.1.0, with lima version 26.1.1 and pbtrim version 26.1.0 recorded in the merged BAM header. The vendor-supplied HiFi BAM file was converted to compressed FASTQ format using bam2fastq from the PacBio BAM toolkit (pbtk) version 3.5.0. The resulting FASTQ dataset used for de novo assembly contained 117,499 reads comprising 1,656,239,452 bases. The mean and median read lengths were 14,095.8 bp and 13,598 bp, respectively, with a read N50 of 14,515 bp and a length range of 230–37,655 bp. De novo genome assembly was performed using Hifiasm version 0.25.0-r726 [16]. The resulting assembly consisted of two contigs, ptg000001l (10,651,607 bp) and ptg000002l (419,462 bp), totaling 11,071,069 bp. Assembly quality statistics were assessed using QUAST version 5.3.0 [17]. The Hifiasm assembly had a G+C content of 71.03%, a contig N50 of 10,651,607 bp and no ambiguous nucleotide bases. The larger contig accounted for 96.21% of the assembled sequence.

Read alignment was performed using minimap2 version 2.31-r1302 with the map-hifi preset [18]. Sequencing depth and coverage breadth were calculated using SAMtools, excluding secondary and supplementary alignments [19]. Of the 117,499 HiFi reads, 117,484 (99.99%) mapped to the selected assembly. The length-weighted mean mapped depth was 149.19×, while 11,071,067 of the 11,071,069 assembled bases (99.99998%) were covered by

at least one aligned read. Mean mapped depths were 147.03× for the larger contig and 204.08× for the smaller contig (Table 1). Although the primary assembly graph retained links between the two contigs, their physical organisation has not been established. *Streptomyces* chromosomes are commonly linear and contain terminal structures that require particular attention during genome reconstruction [20, 21]. The smaller contig has not been classified as a separate chromosome or plasmid.

Genome completeness was evaluated using BUSCO version 6.1.0 with the streptomyces_odb12.2 dataset containing 1,438 lineage-specific markers [22]. Of these, 1,422 markers (98.9%) were complete, including 1,419 single-copy and three duplicated markers, while eight were fragmented and eight were missing. CheckM2 version 1.1.0 estimated genome completeness at 100.0% and contamination at 0.56% [23].

Genome annotation was performed using Bakta version 1.9.4 with the full database version 5.1 and bacterial translation Table 11 [24]. Bakta identified 8,894 standard coding-sequence (CDS) predictions, including 24 pseudogene calls, together with three additional short open reading frames. The annotation also contained 70 tRNA features, 18 rRNA genes comprising six copies each of 5S, 16S and 23S rRNA, and two transfer-messenger RNA features. Additional features included 166 non-coding RNA annotations, 27 ncRNA regions and four predicted CRISPR arrays. The genome had a predicted coding density of 88.5%, with 514 CDS annotations classified as hypothetical. Supporting annotation using Prokka version 1.14.6 identified 8,883 CDS features, 84 tRNAs, 18 rRNAs and two tmRNAs [25]. Of the Prokka CDS predictions, 8,881 shared identical genomic coordinates and strand assignments with Bakta CDS-type features. Bakta was retained as the primary annotation source for the genomic features summarised in Table 2.

Table 1. Contig-level sequence characteristics and mapped-read coverage.

Contig	Length (bp)	G+C (%)	Coverage breadth (%)	Mean mapped depth (×)
ptg000001l	10,651,607	71.11	100.0000	147.031
ptg000002l	419,462	68.85	99.9995	204.076

Mapped-read coverage was calculated using minimap2 and SAMtools. Coverage breadth refers to the percentage of contig bases covered by at least one aligned read.

Table 2. General genomic features of *Streptomyces* sp. MUM 161J.

Genomic feature	Value
Number of HiFi reads	117,499
Total sequencing bases	1,656,239,452
Read N50 (bp)	14,515
Total assembled sequence (bp)	11,071,069
Number of contigs	2
Largest contig (bp)	10,651,607
Contig N50 (bp)	10,651,607
Contig N90 (bp)	10,651,607
L50 / L90	1 / 1
auN (bp)	10,263,930.3
G+C content (%)	71.03
Length-weighted mean mapped depth (×)	149.19
Primary reads mapped (%)	99.99
Standard CDS predictions	8,894
CDS pseudogene calls	24
Additional short ORFs	3
tRNA annotations	70
rRNA genes	18
tmRNA features	2
Other ncRNA features	166
ncRNA regions	27
Predicted CRISPR arrays	4
Coding density (%)	88.5
BUSCO completeness (%)	98.9
CheckM2 completeness (%)	100.0
CheckM2 contamination (%)	0.56
Predicted biosynthetic regions	46
GenBank Nucleotide Accession Number	JCFUEL000000000.1

Genome-based taxonomic identification was conducted using the Type (Strain) Genome Server (TYGS) and FastANI. TYGS analysis indicated affiliation of strain MUM 161J with *Streptomyces malaysiensis*, a species originally described from a Malaysian soil isolate [26], with digital DNA–DNA hybridisation (dDDH) values of 87.5% against *S. malaysiensis* JCM 10672^{T} and 87.6% against strain HNM0141^{T}, formerly classified as *Streptomyces solisilvae* [27]. Figure 1 illustrates strain MUM 161J clustered with *Streptomyces malaysiensis* JCM 10672 and *Streptomyces solisilvae* HNM0141^{T}, supporting its close phylogenomic relationship with *S. malaysiensis*. Pairwise average nucleotide identity (ANI) was calculated using FastANI version 1.34, with each genome pair compared

in both query-to-reference and reference-to-query orientations [28]. Genome-wide ANI and dDDH are established criteria in genome-based prokaryotic taxonomy [29, 30]. The mean bidirectional ANI between strain MUM 161J and *S. malaysiensis* JCM 10672^T (GCA_039525135.1) was 98.84%. Comparable ANI values were obtained against *S. solisilvae* HNM0141^T (98.83%), whereas comparison with *S. malaysiensis* subsp. *samsunensis* DSM 42010^T yielded 97.54% ANI and 74.6% dDDH (Table 3). The close genomic relationships with JCM 10672^T and HNM0141^T are consistent with the reclassification of *S. solisilvae* as a later heterotypic synonym of *S. malaysiensis* [31]. These results support the assignment of strain MUM 161J to *S. malaysiensis*.

Table 3. Closest reference-strain comparisons for strain MUM 161J.

Reference strain	Mean of bidirectional ANI (%)	dDDH (%)
S. malaysiensis JCM 10672^T	98.84	87.5
S. solisilvae HNM0141^T	98.83	87.6
S. malaysiensis subsp. *samsunensis* DSM 42010^T	97.54	74.6

ANI values represent the mean of bidirectional FastANI calculations. dDDH values were obtained independently through TYGS.

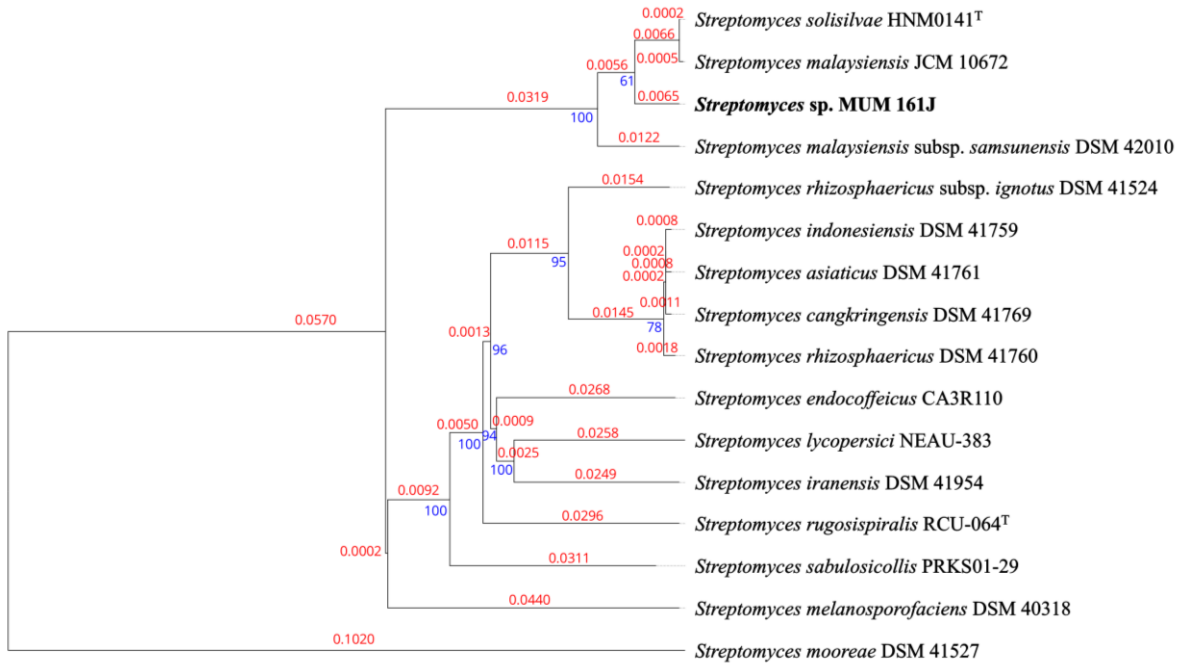


Figure 1. Whole-genome phylogenetic tree showing the relationship of *Streptomyces* sp. MUM 161J to selected *Streptomyces* reference strains, inferred using the Type (Strain) Genome Server (TYGS). The tree was constructed with FastME version 2.1.61. [32]. Red labels indicate the branch length values, which are scaled in terms of the Genome BLAST Distance Phylogeny (GBDP) distance formula d5. Blue node labels indicate GBDP pseudo-bootstrap support values above 60% from 100 replications.

The biosynthetic potential of strain MUM 161J was investigated using antiSMASH version 8.0.4 with strict detection settings [33]. A total of 46 biosynthetic regions were predicted, comprising 43 on the larger contig and three on the smaller contig. These regions contained 61 protoclusters and 63 candidate-cluster features and collectively spanned 2,792,030 bp, representing 25.22% of the assembled sequence when surrounding sequences within predicted region boundaries were included. The identified biosynthetic classes included polyketide synthases, nonribosomal peptide synthetases, ribosomally synthesised and post-translationally modified peptides, terpenes, siderophores and ectoine-associated pathways. Such predicted regions provide an initial survey of biosynthetic repertoire; comparative studies have documented extensive strain-level variation in *Streptomyces* biosynthetic gene clusters [34–36], and their presence does not by itself establish metabolite production. Detailed biosynthetic region coordinates and classifications are provided in Figure 2.

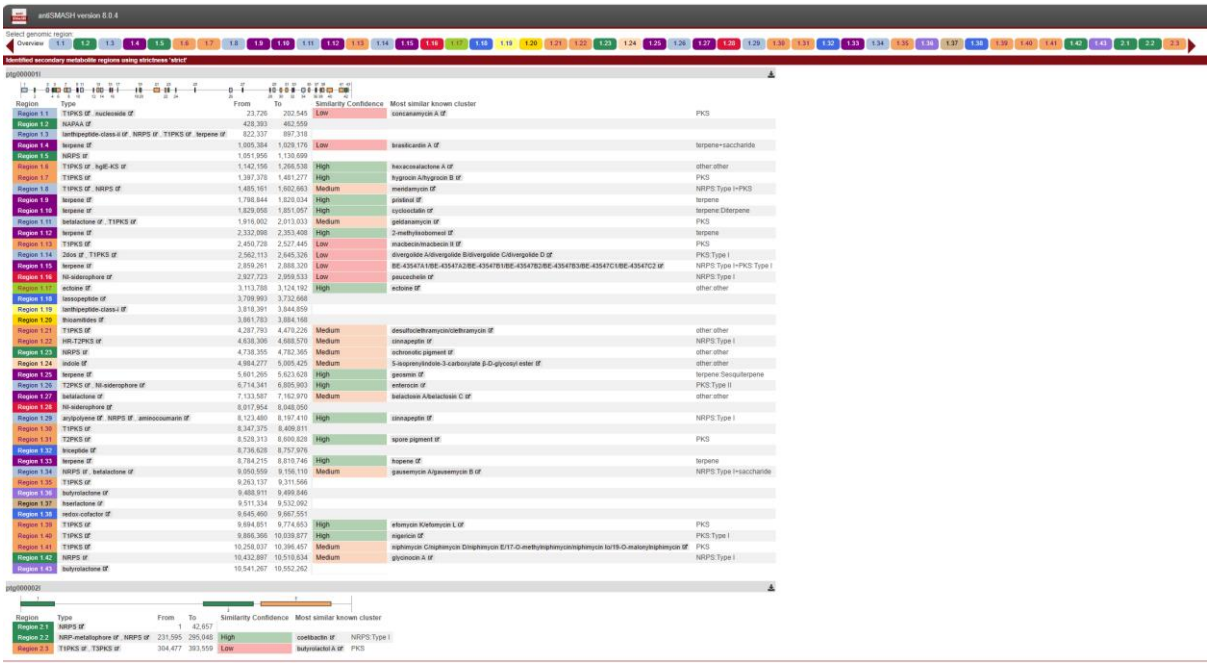


Figure 2. Detailed biosynthetic region coordinates and classifications predicted in the whole genome of strain MUM 161J by antiSMASH version 8.0.4.

The genome sequence of MUM 161J has been deposited in the DDBJ/ENA/GenBank under the accession number JCFUEL000000000. The version described in this paper is version JCFUEL010000000. The data are publicly available at NCBI GenBank under the BioProject accession number PRJNA1548874 and the BioSample accession number SAMN64369642.

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

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