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Streptomyces learnhanii sp. nov., unveiling a Mangrove-Derived Novel "Modern *Actinobacteria*" in Malaysia

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Abstract: *Streptomyces learnhanii* sp. nov. MUM 203J^T was isolated from Malaysia mangrove soil. This Gram-positive bacterium produces pale greenish-yellow aerial and greyish-yellow substrate mycelia on ISP 2 agar. The taxonomy status of strain MUM 203J^T was determined via a polyphasic approach comprising phenotypic observations, genomic and phylogenetic analyses, and chemotaxonomic analyses. The strain demonstrated typical *Streptomyces* features based on a series of phenotypic and chemotaxonomic evaluations. In particular, the cell wall peptidoglycan contained LL-diaminopimelic acid, and the predominant menaquinones detected include MK9(H_8). Analysis of whole-cell sugars revealed the presence of glucose, ribose and mannose. The polar lipid profile of the strain comprised lipid, glycolipid, phospholipid, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphoglycolipid, and diphosphatidylglycerol. The major cellular fatty acids (>10.0 %) were anteiso-$C_{15:0}$ (24.7 %), anteiso-$C_{17:0}$ (16.4 %), iso-$C_{16:0}$ (15.7 %), iso-$C_{15:0}$ (11.5 %), and $C_{16:0}$ (11.1 %). The closely related type strains for strain MUM 203J^T, as determined by phylogenetic analysis, include *Streptomyces coeruleorubidus* JCM 4359^T (98.8 %), *Streptomyces coeruleoprunus* JCM 6919^T (98.5 %), *Streptomyces thermocarboxydovorans* NBRC 16324^T (98.1 %). The DNA–DNA relatedness values between strain MUM 203J^T and closely related type strains ranged from 10.7 ± 0.6 % to 23.3 ± 4.7 %. Strain MUM 203J^T has a genome size of 6,446,886 bp, with DNA G + C content of 72.26 mol%. Based on the polyphasic study of strain MUM 203J^T, it can be concluded that this strain represents a novel species, for which the name *Streptomyces learnhanii* sp. nov. is proposed. The type strain is MUM 203J^T (= NBRC 114250^T = MCCC 1K04200^T).

Keywords: *Streptomyces learnhanii*; actinobacterium; streptomycete; mangrove; MOD-ACTINO; SDG 15 Life on Land

1. Introduction

"Modern *Actinobacteria*" (MOD-ACTINO) has become the recent spotlight, referring to the novel or bioactive microbes from the phylum *Actinobacteria* discovered from distinct environments [1, 2]. The remarkable ability of *Actinobacteria*, particularly its largest genus — *Streptomyces*, to synthesize bioactive secondary metabolites continues to captivate scientists around the world [3, 4]. These microorganisms exhibit unique morphological, physiological, and genetic features that differentiate them from other bacterial genera [5, 6]. Their complex life cycle enables them to grow in diverse environments, such as soils, oceans, and extreme habitats [7-14].

The advent of next-generation sequencing technology and bioinformatics tools reveal that *Streptomyces* spp. have a large genome (> 6Mbp) that could contain over 20 biosynthetic gene clusters account for secondary metabolites production [15-20]. They also possess cryptic gene clusters that are not activated under standard laboratory conditions [21]. The presence of these biosynthetic gene clusters contributes to their prolific production of compounds with great structural diversity [19, 22]. The compounds produced by *Streptomyces* spp. include

bioactivities like antibacterial [23, 24], antifungal [25-27], anticancer [28-30], antioxidant [31-33], neuroprotection [34, 35], and plant growth promotion [36-38].

Streptomyces spp. are notably known for their production of antibiotics that are commercially available [39-41]. Recent studies have explored its capacity to combat deadly pathogens, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) [42-46], and SARS-CoV-2 virus [47-49]. COVID-19 pandemic caused by the SARS-CoV-2 virus had resulted in a substantial loss of human lives worldwide and impacted our global economy [50-57]. The emergence of COVID-19 variants of concern over time remains a matter of public health issue [58-60]. Other efforts that have been taken to treat COVID-19 infection, including the repurposing of Ivermectin (derivative of *Streptomyces*-derived Avermectin) [61, 62]. Apart from these, the probiotics properties of *Streptomyces* have also been explored, especially in aquaculture sector [63-66]. Numerous studies demonstrated that *Streptomyces* spp. exhibited promising antimicrobial activity against *Vibrio* spp. [67-69], which are the pathogens causing vibriosis in fishes, shrimps, and prawns [70-73]. *Vibrio* spp. are also among the main culprits of foodborne disease outbreaks [74-78]. Therefore, the antimicrobial effect produced by *Streptomyces* spp. is connected to their probiotic function, serving to offer protection against infection in aquatic organisms [79-81].

One of the approaches to discover new MOD-ACTINO is to explore the special environments such as mangroves. Mangroves constitute highly specialized ecosystems that thrive in the intertidal areas of lagoon and estuaries at tropical and subtropical areas. Mangroves are characterized by their harsh coastal environmental conditions (e.g., fluctuating salinity, high UV exposure and temperature, and low nutrients) [82, 83]. There are bacteria that can adapt and proliferate in such harsh environments, and thus, mangrove forests pose as a rich source for novel bioactive *Streptomyces* species discovery [84-89]. This study aims to isolate, identify, and characterize a novel *Streptomyces* strain, MUM 203J^T, from mangrove soils collected in Malaysia. A series of phenotypic, genomic, and chemotaxonomic assays have been conducted to understand the strain from different perspectives. The whole genome of strain MUM 203J^T has been sequenced, and bioinformatic analyses have been performed to investigate the strain's potential for bioactive compound production.

2. Materials and Methods

2.1. Sampling and Isolation of strain MUM 203J^T

In June 2015, the soil samples were obtained from a mangrove situated at the East of Malaysia. Strain MUM 203J^T was discovered from mangrove soil sample originated at the mangrove site labelled as KTTAS 7 (1º41'48.08"N 110º11'15.14"E). Soil samples were air-dried and selectively pretreated via wet-heat at 50 ºC for 15 minutes [84]. Strain MUM 203J^T was isolated from a peptone yeast extract iron agar (ISP 6) plate supplemented with cycloheximide (50 mg/L) and nalidixic acid (20 mg/L), and purified on ISP 2 media. Pure cultures were maintained on ISP 2 agar slants and tryptic soy broth (TSB) glycerol suspensions (20 %, v/v) at -20 °C.

2.2. Phenotypic Tests

The growth of strain MUM 203J^T was tested at varying pH ranges (pH 2–10) and salinity (0–10 % NaCl) in TSB, incubated at 28 °C at 200rpm for 14 days. Strain MUM 203J^T was cultured on ISP 2 agar plates and incubated at different temperatures ranging 4–50 °C to observe the growth for up to 14 days. Enzymatic activities of strain MUM 203J^T, including hemolytic, amylolytic, cellulase, chitinase, catalase, protease, and xylanase were tested [87].

A total of 12 media: yeast malt agar (ISP 2), oat meal agar (ISP 3), inorganic salt starch agar (ISP 4), glycerol asparagine agar base (ISP 5), peptone yeast extract iron agar (ISP 6), tyrosine agar base (ISP 7), actinomycetes isolation agar (AIA), *Streptomyces* agar (SA), starch casein agar (SCA), nutrient agar (NA), Luria-Bertani agar (LBA), and Mueller Hinton agar (MHA), were used to culture strain MUM 203J^T, incubated at 28 °C and 14 days for colony morphology observations [86, 90]. The colony colours were given based on ISCC-NBS colour charts. The detection of melanoid pigments was carried out on ISP 6 and ISP 7 agar plates [91]. The cells of strain MUM 203J^T was observed under Light microscopy (80i, Nikon) and scanning electron microscopy (JEOL-JSM 6400) after growing on ISP 2 agar plate at 28 °C for 7–14 days.

2.3. Genotypic and Phylogenetic Examinations

DNA extraction and PCR amplification of the 16S rRNA gene for the strain MUM 203J^T were conducted [86, 87, 90], followed by the determination of sequence similarities of the acquired sequence with its related type strains via BLAST search on the EzBioCloud database (http://www.ezbiocloud.net/). Manual alignment of strain MUM 203J^T 16S rRNA gene sequence with other representative sequences of related *Streptomyces* type strains retrieved from the GenBank/EMBL/DDBJ databases using CLUSTAL-X software was carried out. Phylogenetic analysis was performed with neighbour-joining [92, 93] and maximum likelihood [94] algorithms using MEGA version 7.0. Bootstrap analysis with 1000 resamplings was performed according to Felsenstein [95].

Strain MUM 203J^T and its closely related type strains determined upon 16S rRNA gene sequence similarities and phylogenetic analysis: *Streptomyces coeruleoprunus* JCM 6919^T, *Streptomyces coeruleorubidus* JCM 4359^T, and *Streptomyces thermocarboxydovorans* NBRC 16324^T, were sent to Identification Service of the DSMZ, Braunschweig, Germany for DNA-DNA hybridization analysis [96, 97].

2.4. Whole Genome Sequencing and Bioinformatic Analysis of Strain MUM 203J^T

Total genomic DNA of strain MUM 203J was extracted using MasterPure™ Gram Positive DNA Purification Kit (Lucigen/Epicentre, USA). The extracted DNA quality was checked using NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). Library preparation was constructed using NEXTERA DNA Flex Library Prep Kit (Nextera, USA). Libraries were sequenced on Illumina MiSeq platform with MiSeq Reagent Kit v3 (Illumina Inc., Madison,

WI, USA). FastQC (version 0.11.9) [98] was utilized for verification of the quality of obtained sequencing reads. Trimming of adapters and raw reads were conducted using BBDuk of BBTools (v36) and then assembled using St. Petersburg genome assembler (SPAdes) (v3.14.1) [99].

The assembled genomic sequence was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and analyzed Rapid Annotation using Subsystem Technology (RAST) database (https://rast.nmpdr.org/), set at default pipeline for RASTtk, domain bacteria, and automatically fixed error options turned on [100, 101]. The genome of strain MUM 203J^T was compared with genomes of closely related *Streptomyces* species (retrieved from NCBI database) using FastANI (version 1.33) [102]. Phylogenomic analysis of strain MUM 203J^T was done by Type Strain Genome Server (https://tygs.dsmz.de) [103]. Biosynthetic gene clusters related to secondary metabolite production were detected and analyzed using antiSMASH (version 7.0) [104].

2.5. Chemotaxonomic Properties

Chemotaxonomic investigations of MUM203J^T were conducted by Dr. Brian Tindall, Identification Service of the DSMZ, Braunschweig, Germany, which include the analyses of cell wall peptidoglycan, respiratory quinones, whole cell sugars, fatty acids, and polar lipids [86, 87, 90, 105].

3. Results

3.1. Phenotypic Features of Strain MUM 203J^T

Strain MUM 203J^T grew at 26 – 37 °C (optimum 26 – 32 °C) and optimally at pH 6.0-8.0, with 0 – 2 % NaCl tolerance. The strain exhibited positive catalase and amylolytic activity. The strain also exerted alpha hemolysis. Furthermore, strain MUM 203J^T showed good growth on ISP 6, SA, LBA, and MHA; moderately good growth on ISP 2, SCA, and NA; poor growth on ISP 5, ISP 7, and AIA (Table 1). No growth was detected on ISP 3 and ISP 4 (Table 1). The colours of aerial and substrate mycelia of strain MUM 203J^T shown on different media were presented in Table 1. Melanoid pigment was formed on ISP 6 agar only. Phenotypic experiments were conducted simultaneously on strain MUM 203J^T, *S. coeruleoprunus* JCM 6919^T, *S. coeruleorubidus* JCM 4359^T, and *S. thermocarboxydovorans* NBRC 16324^T (supplementary Table S1). Strain MUM 203J^T displayed the typical features of the genus *Streptomyces* under scanning electron microscopy (Figure 1).

Table 1. The growth and colony colour of *Streptomyces learnhanii* sp. nov. MUM 203J^T on different culture media.

Medium	Growth	Colony colour	
		Aerial mycelium	Substrate mycelium
Yeast malt agar (ISP 2)	Moderate	Pale greenish yellow	Greyish yellow
Oat Meal agar (ISP 3)	No growth	-	-
Inorganic Salt Starch agar (ISP 4)	No growth	-	-
Glycerol Asparagine Agar Base (ISP 5)	Poor	Yellowish white	Yellowish white
Peptone Yeast Extract Iron agar (ISP 6)	Good	Light greyish olive	Dark greyish olive
Tyrosine agar base (ISP 7)	Poor	Yellowish white	Pale yellow
Actinomycete isolation agar (AIA)	Poor	Yellowish white	Pale yellow
Streptomyces agar (SA)	Good	Moderate yellow	Greyish yellow
Starch casein agar (SCA)	Moderate	Light olive brown	Deep yellow
Nutrient agar (NA)	Moderate	Pale yellow	Light yellow
Luria bertani agar (LBA)	Good	Pale yellow	Light yellow
Mueller Hinton agar (MHA)	Good	Yellowish grey	Deep yellow

-, Not detected

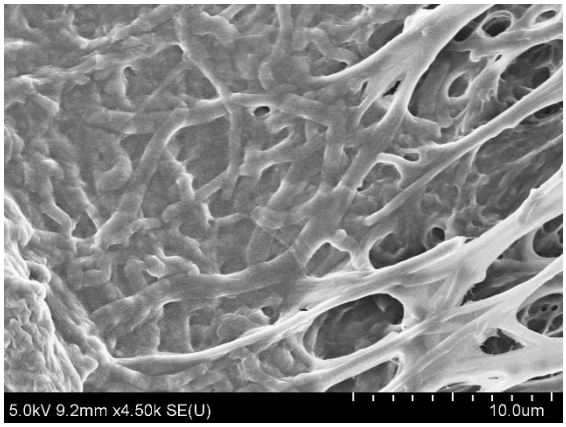

Figure 1. *Streptomyces learnhanii* sp. nov. MUM 203J^T, image obtained by scanning electron microscopy.

3.2. Genotypic and Phylogenetic Outcomes Based on 16S rRNA Gene Sequences

The almost complete 16S rRNA gene sequence of strain MUM 203J^T was obtained (1490 bp; GenBank/EMBL/DDBJ accession number MK368443). Phylogenetic analyses based on neighbour-joining (Figure 2) and maximum-likelihood algorithms (supplementary Figure S1) in combination with 16S rRNA gene sequence analysis revealed that the closely related strains are *S. thermocarboxydovorans* NBRC 16324^T (98.1% sequence similarity), *S. coeruleorubidus* JCM 4359^T (98.8 %), and *S. coeruleoprunus* JCM 6919^T (98.5 %). According to Figure 2, strain MUM 203J^T appeared closely related to *S. thermocarboxydovorans* NBRC 16324^T (DSM 44296^T), forming a distinct clade.

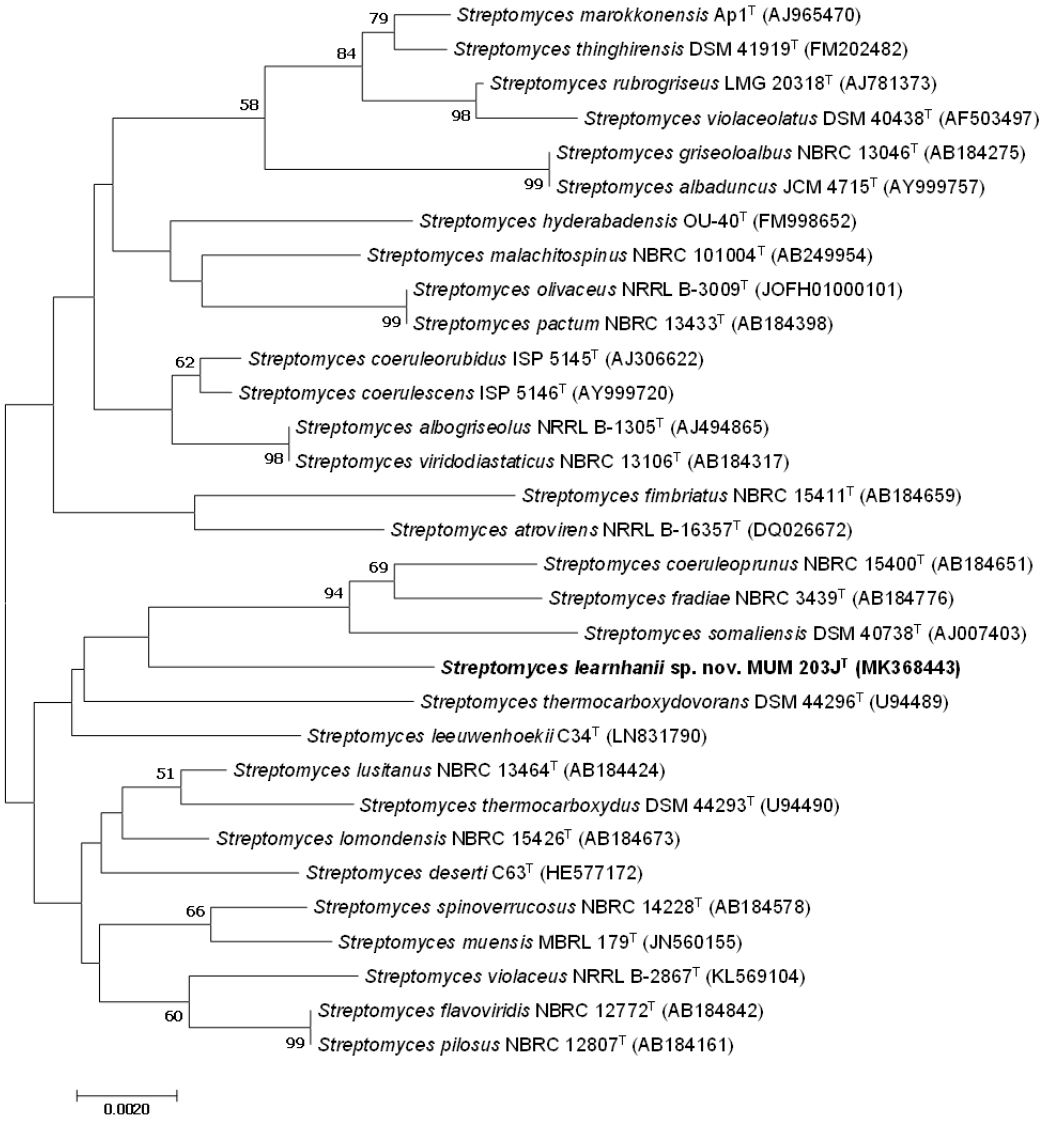

Figure 2. Neighbour-joining phylogenetic tree based on 1490 nucleotides of 16S rRNA gene sequence showing the relationship between *Streptomyces learnhanii* sp. nov. MUM 203J^T and representatives of related taxa. Numbers and nodes indicate percentages (> 50 %) of 1000 bootstrap re-sampling. Bar, 0.002 substitutions per site.

The DNA-DNA relatedness levels between strain MUM 203J^T and respective closely related type strains were 23.3 ± 4.7 % with *S. thermocarboxydovorans* NBRC 16324^T, 10.7 ± 0.6 % with *S. coeruleorubidus* JCM 4359^T (98.8 %), and 15.3 ± 3.8 % with *S. coeruleoprunus* JCM 6919^T. These levels were significantly below the 70 % DNA-DNA similarity cut-off point for defining bacterial species [106].

3.3. Whole Genome Sequence of Strain MUM 203J^T

Strain MUM 203J^T has a genome size of 6,446,886 bp, with DNA G + C content of 72.26 mol%, and average coverage of 125.71-times (Table 2). There were 5731 coding sequences with 67 tRNAs and 7 rRNAs predicted. The genome sequence of strain MUM

203J^T has been deposited at DDBJ/EMBL/GenBank with accession number JADWYO000000000.

Table 2. *Streptomyces learnhanii* sp. nov. MUM 203J^T genome information.

	***Streptomyces learnhanii* sp. nov. MUM203J^T**
Genome size (bp)	6,446,886
Contigs	147
Contigs N_{50} (bp)	84,552
G + C content	72.26 %
Genome coverage	125.71x
CDS	5731
tRNA	67
rRNA	4(5S), 1(16S),2(23S)

Furthermore, a total of 1185 subsystems have been determined by RAST (Figure 3). Most of the genes are amino acids and derivatives metabolism (6.4 %), carbohydrates metabolism (4.0 %), and protein metabolism (3.9 %). The antiSMASH predicted biosynthetic gene clusters responsible for production of ectoine (100 % deduced amino acid sequence similarity), geosmin (100 %), venezuelin (100 %), antipain (100 %), and hopene (76 %) in the genome of strain MUM 203J^T.

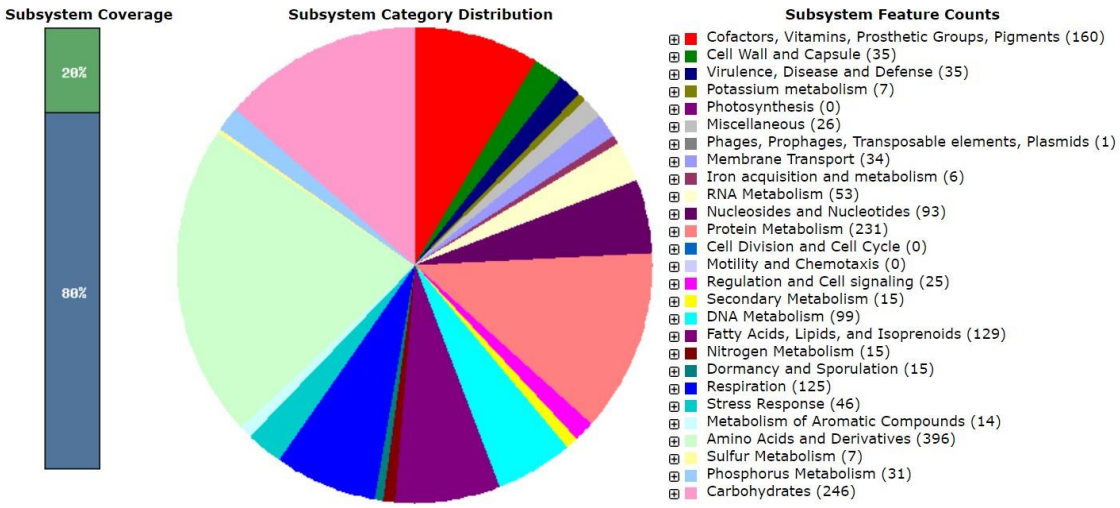

Figure 3. The subsystem category distribution of *Streptomyces learnhanii* sp. nov. MUM 203J^T according to RAST.

The comparison of whole genome sequences between strain MUM 203J^T and its closely related type strain *S. coeruleorubidus* JCM 4359^T based on FastANI analysis estimated an ANI value of 81.32 %. TYGS analysis further supported that strain MUM 203J^T is a potential novel species with digital DDH (dDDH) of < 27.3 % for all closely related type strains.

3.4. Chemotaxonomic Characteristics of Strain MUM 203J^T

Strain MUM 203J^T possessed LL-diaminopimelic acid in the cell wall. Major menaquinones identified were MK9(H_8) (approximately 70 %), and others include MK9(H_4), MK9(H_6), and MK9(H_{12}). The whole cell sugars of MUM 203J^T were glucose, ribose, and mannose. Fatty acids of strain MUM 203J^T were majority comprised of anteiso-$C_{15:0}$ (24.7 %), anteiso-$C_{17:0}$ (16.4 %), iso-$C_{16:0}$ (15.7 %), iso-$C_{15:0}$ (11.5 %) and $C_{16:0}$ (11.1 %) (Table 3). Some similarities in fatty acids composition between strain MUM 203J^T, *S. thermocarboxydovorans* NBRC 16324^T, *S. coeruleorubidus* JCM 4359^T, and *S. coeruleoprunus* JCM 6919^T can be observed. For instance, all of them had anteiso-$C_{15:0}$ and iso-$C_{16:0}$ as major fatty acids, but with quantifiable differences (Table 3). The polar lipid profile of strain MUM 203J^T is shown in Figure 4, in which the presence of lipid, glycolipid, phospholipid, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphoglycolipid, and diphosphatidylglycerol were detected.

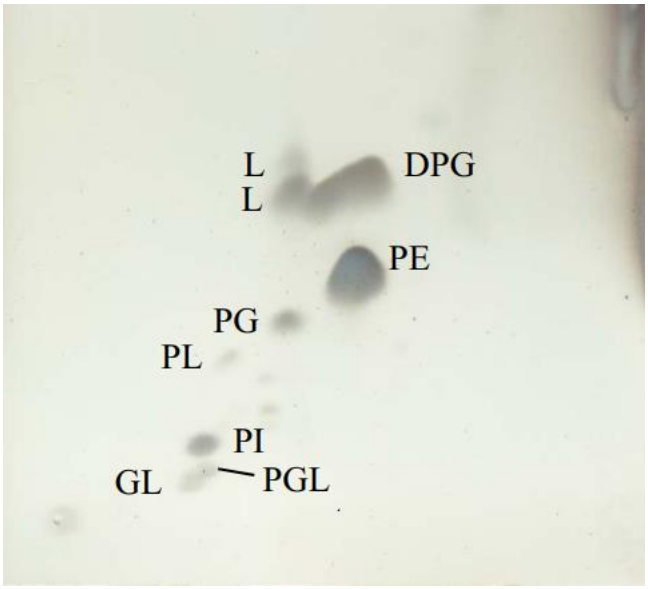

Figure 4. Total lipid profile of *Streptomyces learnhanii* sp. nov. MUM 203J^T. L, lipid; GL, glycolipid; PL, phospholipid; PI, phosphatidylinositol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PGL, phosphoglycolipid; DPG, diphosphatidylglycerol.

Table 3. Fatty acids detected in *Streptomyces learnhanii* sp. nov. MUM 203J^T and three closely related type strains, *Streptomyces coeruleoprunus* JCM 6919^T, *Streptomyces coeruleorubidus* JCM 4359^T, and *Streptomyces thermocarboxydovorans* NBRC 16324^T.

Fatty acid	*Streptomyces learnhanii* MUM 203J^T	*Streptomyces coeruleoprunus* JCM 6919^T	*Streptomyces coeruleorubidus* JCM 4359^T	*Streptomyces thermocarboxydovorans* NBRC 16324^T
iso-C$_{13:0}$	-	-	0.3	0.5
iso-C$_{14:0}$	2.6	2.7	4.6	5.8
C$_{14:0}$	0.5	0.3	0.3	0.3
iso-C$_{15:0}$	11.5	7.9	16.3	20.6
anteiso-C$_{15:0}$	24.7	29.1	14.9	14.5
C$_{15:1}$ B	-	-	-	0.4
C$_{15:0}$	1.4	1.1	0.9	1.7
iso-C$_{16:1}$ H	1.0	0.3	0.8	2.1
iso-C$_{16:0}$	15.7	17.6	17.2	24.1
C$_{16:1}$ Cis 9	2.4	0.3	2.9	1.3
C$_{16:0}$	11.1	6.1	8.6	7.0
C$_{16:0}$ 9Methyl	2.4	0.5	4.0	0.8
anteiso-C$_{17:1}$ C	1.9	1.2	2.0	0.3
iso-C$_{17:0}$	6.7	4.8	11.1	9.6
anteiso-C$_{17:0}$	16.4	24.8	14.4	8.7
C$_{17:1}$ Cis 9	-	-	0.5	-
C$_{17:0}$ Cyclo	0.8	0.9	0.4	0.5
C$_{17:0}$	0.9	0.6	0.8	1.4
iso-C$_{18:0}$	-	-	-	0.3

-, <0.1% or not detected. All data are obtained concurrently from this study.

4. Discussion

The discovery of novel *Streptomyces* species poses an important strategy that holds the potential to unveil untapped reservoirs of bioactive compounds. Identifying novel *Streptomyces* species involves a polyphasic approach to study, understand, and characterize the strain [107-109]. Numerous techniques are applied in this identification and characterization process, which include the conventional culture methods, rapid polymerase chain reaction (PCR) molecular-based method, chromatography, and advanced next-generation sequencing [4, 110]. Notwithstanding the substantial count of recognized *Streptomyces* species, which is about 1179 validated species to date, this study further highlights that mangrove serves as an untapped source for novel *Streptomyces* species discovery. Strain MUM 203J^T isolated from a Malaysian mangrove forest has been proven to be a novel *Streptomyces* species in the present investigation.

Strain MUM 203J^T exhibited the typical phenotypes of the genus *Streptomyces*, for instance, the development of aerial and substrate mycelia that can be observed through its

growth on different culture media (Table 1) and SEM imaging (Figure 1). The chemotaxonomic findings offer supplementary validation that strain MUM 203J^T is indeed a member of the *Streptomyces* genus, with the presence of LL-diaminopimelic acid in cell wall peptidoglycan and prevailing menaquinone MK9(H_8) that are typically observed within the genus [86, 90, 111-115]. Strain MUM 203J^T can grow optimally at 26 – 32 °C, pH 6.0-8.0, and in the presence of up to 2 % NaCl. It falls within the range of similar growth parameters as exhibited by other *Streptomyces* spp. originated from mangrove environments, which have been demonstrated by previous studies [86, 90, 116].

Further analyses were conducted to confirm the novelty of strain MUM 203J^T. The results of 16S rRNA gene sequence similarity and phylogenetic analyses showed that the closest related type strains of strain MUM 203J^T were *S. thermocarboxydovorans* NBRC 16324^T, *S. coeruleorubidus* JCM 4359^T, and *S. coeruleoprunus* JCM 6919^T (98.5 %). As illustrated in Figure 2 phylogenetic tree, a considerable evolutionary distance exists between strain MUM 203J^T and other type strains, highlighting strain MUM 203J^T's substantial potential as a novel species. The novelty of strain MUM 203J^T is validated by DDH and ANI measurements, both of which are acknowledged methods to delineate bacterial species, with threshold values set at DDH < 70 % and ANI < 95 % [117, 118]. Wet-lab DDH analysis, which is a gold standard for bacterial species delineation, was conducted and the whole-genome comparison between strain MUM 203J^T and its closest related type strains that resulted in DDH values of less than 23.3 %. Besides, TYGS analysis was also performed for strain MUM 203J^T. TYGS is an alternative, least laborious genome-based computational method for prokaryote taxonomy and classification [103]. Results from TYGS demonstrated that comparison between strain MUM 203J^T and all closely related type strains presented < 27.3% dDDH values. Both methods emphasized that the DDH values were significantly below 70 %, thus, affirming the proposition that strain MUM 203J^T indeed constitutes a novel species [103, 106]. Additionally, the output of FastANI complements the DDH results. The estimated ANI values were significantly < 95 % between strain MUM 203J^T and its closely related type strain *S. coeruleorubidus* JCM 4359^T. Therefore, the evidence strongly supports that strain MUM 203J^T is a novel species within the genus *Streptomyces*.

Strain MUM 203J^T has a large genome size of 6,446,886 bp, with a high DNA G + C content of 72.26 mol%. Based on antiSMASH prediction, strain MUM 203J^T harbored biosynthetic gene clusters encoding compounds that are frequently produced by various *Streptomyces* spp., for instance, geosmin, ectoine, antipain, and hopene [112, 119, 120]. Antipain is a protease inhibitor that has been reportedly found in actinobacteria such as *Streptomyces* [120]and *Planomonospora* [121]. Hopene is a common precursor in the metabolic pathway of hopanoids and a component of cytoplasmic membrane, which could aid in the defence against water loss across the plasma membrane in the aerial mycelium [122-124]. Besides, *Streptomyces* spp. are known for their "earthy odour", and this is due to the production of geosmin [125, 126]. Geosmin is a volatile compound that contributes to the distinct "earthy odour" or "fragrance of moist soil" produced by microorganisms [127]. Studies have reported that ectoine aids bacterial adaptation in extreme environments, including mangroves distinguished by their

constant changes in salinity and high temperatures [82, 128-131]. In addition, geosmin and ectoine are capable of exerting bioactivities [119]. Khoshakhlagh et al. [132] investigated on the secondary metabolites of *Streptomyces* spp. and they discovered that ectoine and geosmin both exhibited significant antimicrobial against *Staphylococcus aureus* and anticancer activity towards A549 lung adenocarcinoma cells. The anticancer activity of ectoine has been pointed out by several other studies [119, 133, 134]. Furthermore, ectoine has anti-inflammatory properties [135, 136]. Based on these predictions, the novelty of strain MUM 203J^T is accompanied by bioactive potential and it is a promising MOD-ACTINO that is worthwhile to be further explored for the production of medically valuable compounds.

5. Conclusion and Description of *Streptomyces learnhanii* sp. nov. MUM 203J^T

Streptomyces learnhanii sp. nov. (learn.ha'ni.i. N.L. gen. n. learnhanii, of Professor Ts Dr. Lee Learn-Han, a molecular microbiologist in the field of microbial systematics and multidrug resistant pathogens), the type strain is MUM 203J^T (= NBRC 114250^T = MCCC 1K04200^T), and it is isolated from a mangrove forest at East Malaysia. The 16S rRNA gene sequence of strain MUM 203J^T has been deposited in GenBank/EMBL/DDBJ under the accession number MK368443. The genome of strain MUM 203J^T consists of 6,446,886 bp and DNA G + C content is 72.26 mol%. Genome sequence of the strain can be found at DDBJ/EMBL/GenBank under the accession number JADWYO000000000.

S. learnhanii sp. nov. MUM 203J^T is a Gram-positive and aerobic actinobacterium that forms pale greenish-yellow aerial and greyish-yellow substrate mycelia on ISP 2 agar. The strain grows well on ISP 6, SA, LBA, and MHA, with melanoid pigment formed on ISP 6. The strain can grow at 26 – 37 °C, pH 6.0 – 8.0, and with 0 – 2 % NaCl. The cells are positive for catalase, amylolytic and alpha-hemolytic activities. The cell wall peptidoglycan contains LL-diaminopimelic acid and the predominant menaquinone is MK9(H_8). The whole-cell sugars are glucose, ribose and mannose. Polar lipids of strain MUM 203J^T consist of lipid, glycolipid, phospholipid, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphoglycolipid, and diphosphatidylglycerol. The major cellular fatty acids (>10.0 %) are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$, iso-$C_{15:0}$, and $C_{16:0}$.

Supplementary Materials: The files are available online at the journal website.

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References

1. Law JW-F, Letchumanan V, Tan LT-H, *et al.* The rising of "modern actinobacteria" era. Prog Microbes Mol Biol 2020; 3(1): a0000064.
2. Thye AY-K, Letchumanan V, Tan LT-H, *et al.* Malaysia's Breakthrough in Modern *Actinobacteria* (MOD-ACTINO) Drug Discovery Research. Prog Microbes Mol Biol 2022; 5(1): a0000275.
3. Watve MG, Tickoo R, Jog MM, *et al.* How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol 2001; 176: 386-390.
4. Komaki H. Recent progress of reclassification of the genus *Streptomyces*. Microorganisms 2023; 11(4): 831.
5. Claessen D, De Jong W, Dijkhuizen L, *et al.* Regulation of *Streptomyces* development: reach for the sky! Trends Microbiol 2006; 14(7): 313-319.
6. Paradkar A, Trefzer A, Chakraburtty R, *et al. Streptomyces* genetics: a genomic perspective. Crit Rev Biotechnol 2003; 23(1): 1-27.
7. Abirami, Mani and Kannabiran K. Antibiotic potency of extract from *Streptomyces* isolated from terrestrial soil of Amirthi forest, India. Walailak J Sci & Tech 2017; 14(9): 711-721.
8. Yang Z, He J, Wei X, *et al.* Exploration and genome mining of natural products from marine *Streptomyces*. Appl Microbiol Biotechnol 2020; 104(1): 67-76.
9. Ayuningrum D, Sabdaningsih A, and Jati OE. The potential of phylogenetically diverse culturable *Actinobacteria* from *Litopenaeus vannamei* pond sediment as extracellular proteolytic and lipolytic enzyme producers. Trop Life Sci Res 2022; 33(3): 165.
10. Maciejewska M, Adam D, Naômé A, *et al.* Assessment of the potential role of *Streptomyces* in cave moonmilk formation. Front Microbiol 2017; 8: 1181.
11. Zhang B, Wu X, Zhang G, *et al.* The diversity and biogeography of the communities of *Actinobacteria* in the forelands of glaciers at a continental scale. Environ Res Lett 2016; 11(5): 054012.
12. Duan Y-Y, Ming H, Dong L, *et al. Streptomyces calidiresistens* sp. nov., isolated from a hot spring sediment. Antonie Van Leeuwenhoek 2014; 106(2): 189-196.
13. Lee L-H, Cheah Y-K, Mohd Sidik S, *et al.* Molecular characterization of Antarctic actinobacteria and screening for antimicrobial metabolite production. World J Microbiol Biotechnol 2012; 28: 2125-2137.
14. Law JW-F, Pusparajah P, Ab Mutalib N-S, *et al.* A review on mangrove actinobacterial diversity: the roles of *Streptomyces* and novel species discovery. Prog Microbes Mol Biol 2019; 2(1).
15. Ser H-L, Tan LT-H, Tan W-S, *et al.* Whole-genome sequence of bioactive streptomycete derived from mangrove forest in Malaysia, *Streptomyces* sp. MUSC 14. Prog Microbes Mol Biol 2021; 4(1): a0000195.
16. Ser H-L, Law JW-F, Tan W-S, *et al.* Whole genome sequence of *Streptomyces colonosanans* strain MUSC 93J^{T} isolated from mangrove forest in Malaysia. Prog Microbes Mol Biol 2020; 3(1): a0000061.
17. Ser H-L, Law JW-F, Tan W-S, *et al.* Genome sequence of bioactive streptomycete isolated from mangrove forest in East Malaysia, *Streptomyces monashensis* MUSC 1J^{T}. Prog Drug Discov Biomed Sci 2019; 2(1): a0000045.
18. Pusparajah P, Law JW-F, Chan K-G, *et al.* Whole-genome sequence of *Streptomyces pluripotens* strain MUM 16J, a potential resource of glycopeptide antibiotic and biocontrol agent against biofilm-forming bacteria. Prog Microbes Mol Biol 2023; 6(1): a0000330.
19. Lee N, Hwang S, Kim J, *et al.* Mini review: Genome mining approaches for the identification of secondary metabolite biosynthetic gene clusters in *Streptomyces*. Comput Struct Biotechnol J 2020; 18: 1548-1556.

20. Ikeda H, Shin-ya K, and Omura S. Genome mining of the *Streptomyces avermitilis* genome and development of genome-minimized hosts for heterologous expression of biosynthetic gene clusters. J Ind Microbiol Biot 2014; 41(2): 233-250.
21. Choudoir MJ, Pepe-Ranney C, and Buckley DH. Diversification of secondary metabolite biosynthetic gene clusters coincides with lineage divergence in *Streptomyces*. Antibiotics 2018; 7(1): 12.
22. Ab Mutalib N-S, Wong SH, Ser H-L, *et al.* Bioprospecting of microbes for valuable compounds to mankind. Prog Microbes Mol Biol 2020; 3(1): a0000088.
23. Elsalam RM, Goh KW, Mahadi M, *et al.* The antibacterial activities of secondary metabolites derived from *Streptomyces* sp. Prog Microbes Mol Biol 2022; 5(1): a0000281.
24. Jaroszewicz W, Bielańska P, Lubomska D, *et al.* Antibacterial, antifungal and anticancer activities of compounds produced by newly isolated *Streptomyces* strains from the Szczelina Chochołowska cave (Tatra Mountains, Poland). Antibiotics 2021; 10(10): 1212.
25. Law JW-F, Ser H-L, Khan TM, *et al.* The potential of *Streptomyces* as biocontrol agents against the rice blast fungus, *Magnaporthe oryzae* (*Pyricularia oryzae*). Front Microbiol 2017; 8: 3.
26. Gong Y, Liu J-Q, Xu M-J, *et al.* Antifungal volatile organic compounds from *Streptomyces setonii* WY228 control black spot disease of sweet potato. Appl Environ Microbiol 2022; 88(6): e02317-21.
27. Qi D, Zou L, Zhou D, *et al.* Taxonomy and broad-spectrum antifungal activity of *Streptomyces* sp. SCA3-4 isolated from rhizosphere soil of Opuntia stricta. Front Microbiol 2019; 10: 1390.
28. Law JW-F, Law LN-S, Letchumanan V, *et al.* Anticancer drug discovery from microbial sources: The unique mangrove streptomycetes. Molecules 2020; 25(22): 5365.
29. Abd-Elnaby H, Abo-Elala G, Abdel-Raouf U, *et al.* Antibacterial and anticancer activity of marine *Streptomyces parvus*: optimization and application. Biotechnol Biotechnol Equip 2016; 30(1): 180-191.
30. Lee B, Lee G-E, Hwang GJ, *et al.* Rubiflavin G, photorubiflavin G, and photorubiflavin E: Novel pluramycin derivatives from *Streptomyces* sp. W2061 and their anticancer activity against breast cancer cells. J Antibiot 2023: 1-7.
31. Ser H-L, Tan LT-H, Law JW-F, *et al.* Focused review: cytotoxic and antioxidant potentials of mangrove-derived *Streptomyces*. Front Microbiol 2017; 8: 2065.
32. Kemung HM, Tan LT-H, Chan K-G, *et al. Streptomyces* sp. strain MUSC 5 from mangrove forest in Malaysia: Identification, antioxidant potential and chemical profiling of its methanolic extract. Prog Microbes Mol Biol 2020; 3(1): 0000087.
33. Rammali S, Hilali L, Dari K, *et al.* Antimicrobial and antioxidant activities of *Streptomyces* species from soils of three different cold sites in the Fez-Meknes region Morocco. Sci Rep 2022; 12(1): 17233.
34. Duangupama T, Pratuangdejkul J, Chongruchiroj S, *et al.* New insights into the neuroprotective and beta-secretase1 inhibitor profiles of tirandamycin B isolated from a newly found *Streptomyces composti* sp. nov. Sci Rep 2023; 13(1): 4825.
35. Ibusuki Y, Kimata S, and Hayakawa Y. 13-Deoxo-13-iminodutomycin, a new neuroprotective compound from *Streptomyces* sp. RAP78. J Antibiot 2022; 75(1): 40-43.
36. Liu D, Yan R, Fu Y, *et al.* Antifungal, plant growth-promoting, and genomic properties of an endophytic actinobacterium *Streptomyces* sp. NEAU-S7GS2. Front Microbiol 2019; 10: 2077.
37. Kaur T, Rani R, and Manhas RK. Biocontrol and plant growth promoting potential of phylogenetically new *Streptomyces* sp. MR14 of rhizospheric origin. AMB Express 2019; 9: 1-14.

38. Chen J, Hu L, Chen N, *et al.* The biocontrol and plant growth-promoting properties of *Streptomyces alfalfae* XN-04 revealed by functional and genomic analysis. Front Microbiol 2021; 12: 745766.
39. de Lima Procópio RE, da Silva IR, Martins MK, *et al.* Antibiotics produced by *Streptomyces*. Braz J Infect Dis 2012; 16(5): 466-471.
40. Watve MG, Tickoo R, Jog MM, *et al.* How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol 2001; 176(5): 386-390.
41. Quinn GA, Banat AM, Abdelhameed AM, *et al. Streptomyces* from traditional medicine: Sources of new innovations in antibiotic discovery. J Med Microbiol 2020; 69(8): 1040.
42. Kemung HM, Tan LT-H, Chan K-G, *et al. Streptomyces* sp. strain MUSC 125 from mangrove soil in Malaysia with anti-MRSA, anti-biofilm and antioxidant activities. Molecules 2020; 25(15): 3545.
43. Pusparajah P, Letchumanan V, Law JW-F, *et al. Streptomyces* sp.—A treasure trove of weapons to combat methicillin-resistant *Staphylococcus aureus* biofilm associated with biomedical devices. Int J Mol Sci 2021; 22(17): 9360.
44. Jiao W-H, Yuan W, Li Z-Y, *et al.* Anti-MRSA actinomycins D1-D4 from the marine sponge-associated *Streptomyces* sp. LHW52447. Tetrahedron 2018; 74(40): 5914-5919.
45. Qureshi KA, Bholay AD, Rai PK, *et al.* Isolation, characterization, anti-MRSA evaluation, and in-silico multi-target anti-microbial validations of actinomycin X2 and actinomycin D produced by novel *Streptomyces smyrnaeus* UKAQ_23. Sci Rep 2021; 11(1): 1-21.
46. Chen Z, Ou P, Liu L, *et al.* Anti-MRSA activity of actinomycin X2 and collismycin a produced by *Streptomyces globisporus* WA5-2-37 from the intestinal tract of American cockroach (*Periplaneta americana*). Front Microbiol 2020; 11: 555.
47. Melinda YN, Widada J, Wahyuningsih TD, *et al.* Metabologenomics approach to the discovery of novel compounds from *Streptomyces* sp. GMR22 as anti-SARS-CoV-2 drugs. Heliyon 2021; 7(11): e08308.
48. Kory P, Meduri GU, Varon J, *et al.* Review of the emerging evidence demonstrating the efficacy of ivermectin in the prophylaxis and treatment of COVID-19. Am J Ther 2021; 28(3): e299.
49. Gupta D, Sahoo AK, and Singh A. Ivermectin: potential candidate for the treatment of Covid 19. Braz J Infect Dis 2020; 24: 369-371.
50. Johnson D, Ren SEC, Johnson HD, *et al.* COVID-19: Are Malaysians embracing or suffering the new normality? Prog Microbes Mol Biol 2020; 3(1).
51. Loo K-Y and Letchumanan V. COVID-19: Malaysia's fight against this deadly virus. Prog Microbes Mol Biol 2021; 4(1): a0000204.
52. Joseph RJ and Ser H-L. Stories from the East: COVID-19 situation in India. Prog Microbes Mol Biol 2021; 4(1): a0000213.
53. Loo KY, Law JW-F, Tan LTH, *et al.* South Africa’s battle against COVID-19 pandemic. Prog Microbes Mol Biol 2022; 5(1): a0000264.
54. Loo K-Y, Letchumanan V, Tan LT-H, *et al.* Updated COVID-19 condition in Australia. Prog Microbes Mol Biol 2021; 4(1): a0000250.
55. Loo K-Y, Thye AY-K, Law LN-S, *et al.* COVID-19: An updated situation from Singapore. Prog Microbes Mol Biol 2021; 4(1): a0000246.
56. Ang WS, Law JW-F, Letchumanan V, *et al.* COVID-19 pandemic in Brunei Darussalam. Prog Microbes Mol Biol 2023; 6(1): a0000326.

57. Hoo HE, Loh HC, Ch'ng ASH, *et al.* Positive impacts of the COVID-19 pandemic and public health measures on healthcare. Prog Microbes Mol Biol 2021; 4(1): a0000221.
58. Thye AY-K and Law JW-F. A Variant of Concern (VOC) Omicron: Characteristics, transmissibility, and impact on vaccine effectiveness. Prog Microbes Mol Biol 2022; 5(1): a0000280.
59. Thye AY-K and Law JW-F. Focused review: Insight and updates on COVID-19 from Progress in Microbes and Molecular Biology. Prog Microbes Mol Biol 2023; 6(1): a0000333.
60. Law LN-S, Loo K-Y, Goh JXH, *et al.* Omicron: The rising fear for another wave in Malaysia. Prog Microbes Mol Biol 2021; 4(1): a0000261.
61. Chakraborty C, Sharma AR, Bhattacharya M, *et al.* The drug repurposing for COVID-19 clinical trials provide very effective therapeutic combinations: lessons learned from major clinical studies. Front Pharmacol 2021; 12: 704205.
62. Deng Q, Xiao L, Liu Y, *et al. Streptomyces avermitilis* industrial strain as cell factory for Ivermectin B1a production. Synth Syst Biotechnol 2019; 4(1): 34-39.
63. Tan LT-H, Lee L-H, and Goh B-H. The bioprospecting of anti-*Vibrio Streptomyces* species: Prevalence and applications. Prog Microbes Mol Biol 2019; 2(1): a0000034.
64. Cuozzo S, de LeBlanc AdM, LeBlanc J, *et al. Streptomyces* genus as a source of probiotics and its potential for its use in health. Microbiol Res 2022: 127248.
65. Das S, Ward LR, and Burke C. Screening of marine *Streptomyce*s spp. for potential use as probiotics in aquaculture. Aquaculture 2010; 305(1-4): 32-41.
66. Loo K-Y, Tan LT-H, Law JW-F, *et al. Vibrio parahaemolyticus*: Exploring its incidence in Malaysia and the potential of *Streptomyces* sp. as an anti-*Vibrio* agent. Prog Microbes Mol Biol 2023; 6(1): a0000334.
67. Bi Y, Liu G, Yu Q, *et al.* Anti-*Vibrio* dibutyl phthalate from marine-derived *Streptomyces* sp. S073. Res Vet Sci 2021; 140: 198-202.
68. Yang M, Zhang J, Liang Q, *et al.* Antagonistic activity of marine *Streptomyces* sp. S073 on pathogenic *Vibrio parahaemolyticus*. Fish Sci 2019; 85(3): 533-543.
69. Tan LT-H, Lee L-H, and Goh B-H. Critical review of fermentation and extraction of anti-*Vibrio* compounds from *Streptomyces*. Prog Microbes Mol Biol 2020; 3(1): a0000051.
70. Jun L and Woo NY. Pathogenicity of vibrios in fish: an overview. Journal of Ocean University of Qingdao 2003; 2: 117-128.
71. de la Peña LD, Tamaki T, Momoyama K, *et al.* Characteristics of the causative bacterium of vibriosis in the kuruma prawn, *Penaeus japonicus*. Aquaculture 1993; 115(1-2): 1-12.
72. Chandrakala N and Priya S. Vibriosis in shrimp aquaculture a review. Int J Sci Res Sci Eng Technol 2017; 3(2): 27-33.
73. Letchumanan V, Tan W-S, Yin W-F, *et al.* Genome sequence of *Vibrio* sp. OULL4 isolated from shellfish. Prog Microbes Mol Biol 2020; 3(1): a0000066.
74. Letchumanan V, Loo K-Y, Law JW-F, *et al. Vibrio parahaemolyticus*: The protagonist of foodborne diseases. Prog Microbes Mol Biol 2019; 2(1): a0000029.
75. Letchumanan V, Ab Mutalib N-S, Wong SH, *et al.* Determination of antibiotic resistance patterns of *Vibrio parahaemolyticus* from shrimp and shellfish in Selangor, Malaysia. Prog Microbes Mol Biol 2019; 2(1): a0000019.
76. Venggadasamy V, Tan LT-H, Law JW-F, *et al.* Incidence, antibiotic susceptibility and characterization of *Vibrio parahaemolyticus* isolated from seafood in Selangor, Malaysia. Prog Microbes Mol Biol 2021; 4(1): a0000233.

77. Wu Y, Wen J, Ma Y, *et al.* Epidemiology of foodborne disease outbreaks caused by *Vibrio parahaemolyticus*, China, 2003–2008. Food Control 2014; 46: 197-202.
78. Loo K-Y, Law JW-F, Tan LT-H, *et al.* The burden of *Vibrio* sp. infections–A scoping review. Prog Microbes Mol Biol 2023; 6(1): a0000340.
79. Mazón-Suástegui JM, Salas-Leiva JS, Medina-Marrero R, *et al.* Effect of *Streptomyces* probiotics on the gut microbiota of Litopenaeus vannamei challenged with Vibrio parahaemolyticus. Microbiologyopen 2020; 9(2): e967.
80. Bernal MG, Marrero RM, Campa-Córdova ÁI, *et al.* Probiotic effect of *Streptomyces* strains alone or in combination with *Bacillu*s and *Lactobacillus* in juveniles of the white shrimp *Litopenaeus vannamei*. Aquacult Int 2017; 25(2): 927-939.
81. García-Bernal M, Medina-Marrero R, Rodríguez-Jaramillo C, *et al.* Probiotic effect of *Streptomyces* spp. on shrimp (*Litopenaeus vannamei*) postlarvae challenged with *Vibrio parahaemolyticus*. Aquacult Nutr 2018; 24(2): 865-871.
82. Kodikara KAS, Jayatissa LP, Huxham M, *et al.* The effects of salinity on growth and survival of mangrove seedlings changes with age. Acta Botanica Brasilica 2017; 32: 37-46.
83. Miryeganeh M, Marlétaz F, Gavriouchkina D, *et al.* De novo genome assembly and in natura epigenomics reveal salinity-induced DNA methylation in the mangrove tree *Bruguiera gymnorhiza*. New Phytol 2022; 233(5): 2094-2110.
84. Law JW-F, Chan K-G, He Y-W, *et al.* Diversity of *Streptomyces* spp. from mangrove forest of Sarawak (Malaysia) and screening of their antioxidant and cytotoxic activities. Sci Rep 2019; 9(1): 1-15.
85. Lee L-H, Law JW-F, Khan TM, *et al.* IDDF2019-ABS-0323 Unveiling the anti-colon cancer potential of Sarawak mangrove-derived novel streptomycetes. Gut 2019; 68: A42-A43.
86. Law JW-F, Ser H-L, Duangjai A, *et al. Streptomyces colonosanans* sp. nov., a novel actinobacterium isolated from Malaysia mangrove soil exhibiting antioxidative activity and cytotoxic potential against human colon cancer cell lines. Front Microbiol 2017: 877.
87. Lee L-H, Zainal N, Azman A-S, *et al. Streptomyces pluripotens* sp. nov., a bacteriocin-producing streptomycete that inhibits meticillin-resistant Staphylococcus aureus. Int J Syst Evol Microbiol 2014; 64(Pt_9): 3297-3306.
88. Ser H-L, Tan LT-H, Palanisamy UD, *et al. Streptomyces antioxidans* sp. nov., a novel mangrove soil actinobacterium with antioxidative and neuroprotective potentials. Front Microbiol 2016; 7: 899.
89. Chen C, Ye Y, Wang R, *et al. Streptomyces nigra* sp. nov. is a novel actinobacterium isolated from mangrove soil and exerts a potent antitumor activity in vitro. Front Microbiol 2018; 9: 1587.
90. Law JW-F, Ser H-L, Ab Mutalib N-S, *et al. Streptomyces monashensis* sp. nov., a novel mangrove soil actinobacterium from East Malaysia with antioxidative potential. Sci Rep 2019; 9(1): 1-18.
91. Huang X, Kong F, Zhou S, *et al. Streptomyces tirandamycinicus* sp. nov., a novel marine sponge-derived actinobacterium with antibacterial potential against *Streptococcus agalactiae*. Front Microbiol 2019; 10: 482.
92. Saitou N and Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987; 4(4): 406-425.
93. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980; 16(2): 111-120.
94. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 1981; 17(6): 368-376.

95. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985; 39(4): 783-791.
96. Ley Jd, Cattoir H, and Reynaerts A. The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 1970; 12(1): 133-142.
97. Huss VA, Festl H, and Schleifer KH. Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Syst Appl Microbiol 1983; 4(2): 184-192.
98. Brown J, Pirrung M, and McCue LA. FQC Dashboard: integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. Bioinformatics 2017; 33(19): 3137-3139.
99. Bankevich A, Nurk S, Antipov D, *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012; 19(5): 455-477.
100. Brettin T, Davis JJ, Disz T, *et al.* RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 2015; 5(1): 1-6.
101. Cho G and Kwak Y-S. Evolution of antibiotic synthesis gene clusters in the *Streptomyces globisporus* TFH56, isolated from tomato flower. Genes Genomes Genet 2019; 9(6): 1807-1813.
102. Jain C, Rodriguez-R LM, Phillippy AM, *et al.* High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 2018; 9(1): 1-8.
103. Meier-Kolthoff JP and Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 2019; 10(1): 1-10.
104. Weber T, Blin K, Duddela S, *et al.* antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 2015; 43(W1): W237-W243.
105. Ser H-L, Palanisamy UD, Yin W-F, *et al. Streptomyces malaysiense* sp. nov.: a novel Malaysian mangrove soil actinobacterium with antioxidative activity and cytotoxic potential against human cancer cell lines. Sci Rep 2016; 6(1): 1-12.
106. Wayne L, Brenner D, Colwell R, *et al.* Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Evol Microbiol 1987; 37(4): 463-464.
107. Anderson AS and Wellington E. The taxonomy of *Streptomyces* and related genera. Int J Syst Evol Microbiol 2001; 51(3): 797-814.
108. Law JW-F, Tan K-X, Wong SH, *et al.* Taxonomic and characterization methods of *Streptomyces*: A review. Prog Microbes Mol Biol 2018; 1(1): a0000009.
109. Raina V, Nayak T, Ray L, *et al.* Chapter 9 - A polyphasic taxonomic approach for designation and description of novel microbial species. In: *Microbial Diversity in the Genomic Era*.S Das and HR DashAcademic Press; 2019: 137-152.
110. Law JW-F, Ab Mutalib N-S, Chan K-G, *et al.* Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Front Microbiol 2015; 5: 770.
111. Kim SB, Lonsdale J, Seong C-N, *et al. Streptacidiphilus* gen. nov., acidophilic actinomycetes with wall chemotype I and emendation of the family *Streptomycetaceae* (Waksman and Henrici (1943) AL) emend. Rainey et al. 1997. Antonie Van Leeuwenhoek 2003; 83(2): 107-116.
112. Law JWF, Tan LT-H, Letchumanan V, *et al. Streptomyces griseiviridis* sp. nov., a novel "Modern *Actinobacteria*" isolated from Malaysia mangrove soil. Prog Microbes Mol Biol 2023; 6(1): a0000270.
113. Risdian C, Landwehr W, Rohde M, *et al. Streptomyces bathyalis* sp. nov., an actinobacterium isolated from the sponge in a deep sea. Antonie Van Leeuwenhoek 2021; 114: 425-435.

114. Lechevalier MP and Lechevalier H. Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Syst Evol Microbiol 1970; 20(4): 435-443.
115. Zhao J, Han L, Yu M, *et al.* Characterization of *Streptomyces sporangiiformans* sp. nov., a novel soil actinomycete with antibacterial activity against *Ralstonia solanacearum*. Microorganisms 2019; 7(9): 360.
116. Kemung HM, Tan LT-H, Chan K-G, *et al.* Antioxidant activities of *Streptomyces* sp. strain MUSC 14 from mangrove forest soil in Malaysia. Biomed Res Int 2020; 2020: 6402607.
117. Goris J, Konstantinidis KT, Klappenbach JA, *et al.* DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 2007; 57(1): 81-91.
118. Kim M, Oh H-S, Park S-C, *et al.* Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 2014; 64(Pt_2): 346-351.
119. Nguyen QD, Truong PM, Vo TNT, *et al.* Draft genome sequence data of *Streptomyces* sp. SS1-1, an endophytic strain showing cytotoxicity against the human lung cancer A549 cell line. Data in brief 2020; 30: 105497.
120. Suda H, AOYAGI T, HAMADA M, *et al.* Antipain, a new protease inhibitor isolated from agtinomycetes. J Antibiot 1972; 25(4): 263-265.
121. Zdouc MM, Iorio M, Maffioli SI, *et al. Planomonospora*: A metabolomics perspective on an underexplored *Actinobacteria* genus. J Nat Prod 2021; 84(2): 204-219.
122. Bentley SD, Chater KF, Cerdeño-Tárraga A-M, *et al.* Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3 (2). Nature 2002; 417(6885): 141-147.
123. Liu Z, Zhang Y, Sun J, *et al.* A novel soluble squalene-hopene cyclase and its application in efficient synthesis of hopene. Frontiers in Bioengineering and Biotechnology 2020; 8: 426.
124. Poralla K, Muth G, and Härtner T. Hopanoids are formed during transition from substrate to aerial hyphae in *Streptomyces coelicolor* A3 (2). FEMS Microbiol Lett 2000; 189(1): 93-95.
125. Zhi Y, Wu Q, Du H, *et al.* Biocontrol of geosmin-producing *Streptomyces* spp. by two Bacillus strains from Chinese liquor. Int J Food Microbiol 2016; 231: 1-9.
126. Gerber N and Lechevalier H. Geosmin, an earthy-smelling substance isolated from actinomycetes. Appl Microbiol 1965; 13(6): 935-938.
127. Jiang J, He X, and Cane DE. Biosynthesis of the earthy odorant geosmin by a bifunctional *Streptomyces coelicolor* enzyme. Nat Chem Biol 2007; 3(11): 711-715.
128. Handayani I, Saad H, Ratnakomala S, *et al.* Mining indonesian microbial biodiversity for novel natural compounds by a combined genome mining and molecular networking approach. Mar Drugs 2021; 19(6): 316.
129. Bilstein A, Heinrich A, Rybachuk A, *et al.* Ectoine in the treatment of irritations and inflammations of the eye surface. Biomed Res Int 2021; 2021.
130. Bownik A and Stępniewska Z. Ectoine as a promising protective agent in humans and animals. Arh Hig Rada Toksikol 2016; 67(4): 260-264.
131. Richter AA, Mais C-N, Czech L, *et al.* Biosynthesis of the stress-protectant and chemical chaperon ectoine: biochemistry of the transaminase EctB. Front Microbiol 2019; 10: 2811.
132. Khoshakhlagh A, Aghaei SS, Abroun S, *et al.* Investigation of diverse biosynthetic secondary metabolites gene clusters using genome mining of indigenous *Streptomyces* strains isolated from saline soils in Iran. Iran J Microbiol 2022; 14(6): 881.
133. Wittmar J, Meyer S, Sieling T, *et al.* What does ectoine do to DNA? A molecular-scale picture of compatible solute–biopolymer interactions. J Phys Chem B 2020; 124(37): 7999-8011.

134. Yao C-L, Lin Y-M, Mohamed MS, *et al.* Inhibitory effect of ectoine on melanogenesis in B16-F0 and A2058 melanoma cell lines. Biochem Eng J 2013; 78: 163-169.

135. Bethlehem L and van Echten-Deckert G. Ectoines as novel anti-inflammatory and tissue protective lead compounds with special focus on inflammatory bowel disease and lung inflammation. Pharmacol Res 2021; 164: 105389.

136. Casale M, Moffa A, Carbone S, *et al.* Topical Ectoine: a promising molecule in the upper airways inflammation—a systematic review. Biomed Res Int 2019; 2019.