

*Original Research Article*

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

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**Abstract:** *Streptomyces learnhanii* sp. nov. MUM 203J<sup>T</sup> 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<sup>T</sup> 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<sub>8</sub>). 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<sub>15:0</sub> (24.7 %), anteiso-C<sub>17:0</sub> (16.4 %), iso-C<sub>16:0</sub> (15.7 %), iso-C<sub>15:0</sub> (11.5 %), and C<sub>16:0</sub> (11.1 %). The closely related type strains for strain MUM 203J<sup>T</sup>, as determined by phylogenetic analysis, include *Streptomyces coeruleorubidus* JCM 4359<sup>T</sup> (98.8 %), *Streptomyces coeruleoprunus* JCM 6919<sup>T</sup> (98.5 %), *Streptomyces thermocarboxydovorans* NBRC 16324<sup>T</sup> (98.1 %). The DNA–DNA relatedness values between strain MUM 203J<sup>T</sup> and closely related type strains ranged from 10.7 ± 0.6 % to 23.3 ± 4.7 %. Strain MUM 203J<sup>T</sup> 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<sup>T</sup>, 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<sup>T</sup> (= NBRC 114250<sup>T</sup> = MCCC 1K04200<sup>T</sup>).

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

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## 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<sup>T</sup>, 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<sup>T</sup> 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<sup>T</sup>

In June 2015, the soil samples were obtained from a mangrove situated at the East of Malaysia. Strain MUM 203J<sup>T</sup> was discovered from mangrove soil sample originated at the mangrove site labelled as KTTAS 7 (1°41'48.08"N 110°11'15.14"E). Soil samples were air-dried and selectively pretreated via wet-heat at 50 °C for 15 minutes [84]. Strain MUM 203J<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup>, 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<sup>T</sup>, 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 16S rRNA gene sequence with other representative sequences of related *Streptomyces* type strains retrieved from the GenBank/EMBL/DBJ 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<sup>T</sup> and its closely related type strains determined upon 16S rRNA gene sequence similarities and phylogenetic analysis: *Streptomyces coeruleoprunus* JCM 6919<sup>T</sup>, *Streptomyces coeruleorubidus* JCM 4359<sup>T</sup>, and *Streptomyces thermocarboxydovorans* NBRC 16324<sup>T</sup>, 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<sup>T</sup>

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) <sup>[98]</sup> 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) <sup>[99]</sup>.

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 <sup>[100, 101]</sup>. The genome of strain MUM 203J<sup>T</sup> was compared with genomes of closely related *Streptomyces* species (retrieved from NCBI database) using FastANI (version 1.33) <sup>[102]</sup>. Phylogenomic analysis of strain MUM 203J<sup>T</sup> was done by Type Strain Genome Server (<https://tygs.dsmz.de>) <sup>[103]</sup>. Biosynthetic gene clusters related to secondary metabolite production were detected and analyzed using antiSMASH (version 7.0) <sup>[104]</sup>.

### 2.5. Chemotaxonomic Properties

Chemotaxonomic investigations of MUM203J<sup>T</sup> 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 <sup>[86, 87, 90, 105]</sup>.

## 3. Results

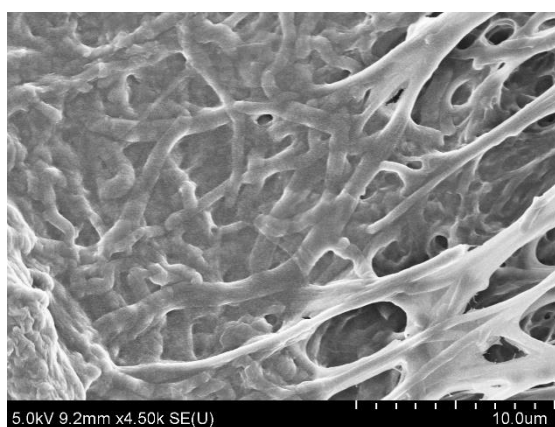
### 3.1. Phenotypic Features of Strain MUM 203J<sup>T</sup>

Strain MUM 203J<sup>T</sup> 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 amyolytic activity. The strain also exerted alpha hemolysis. Furthermore, strain MUM 203J<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup>, *S. coeruleoprunus* JCM 6919<sup>T</sup>, *S. coeruleorubidus* JCM 4359<sup>T</sup>, and *S. thermocarboxydovorans* NBRC 16324<sup>T</sup> (supplementary Table S1). Strain MUM 203J<sup>T</sup> 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<sup>T</sup> on different culture media.

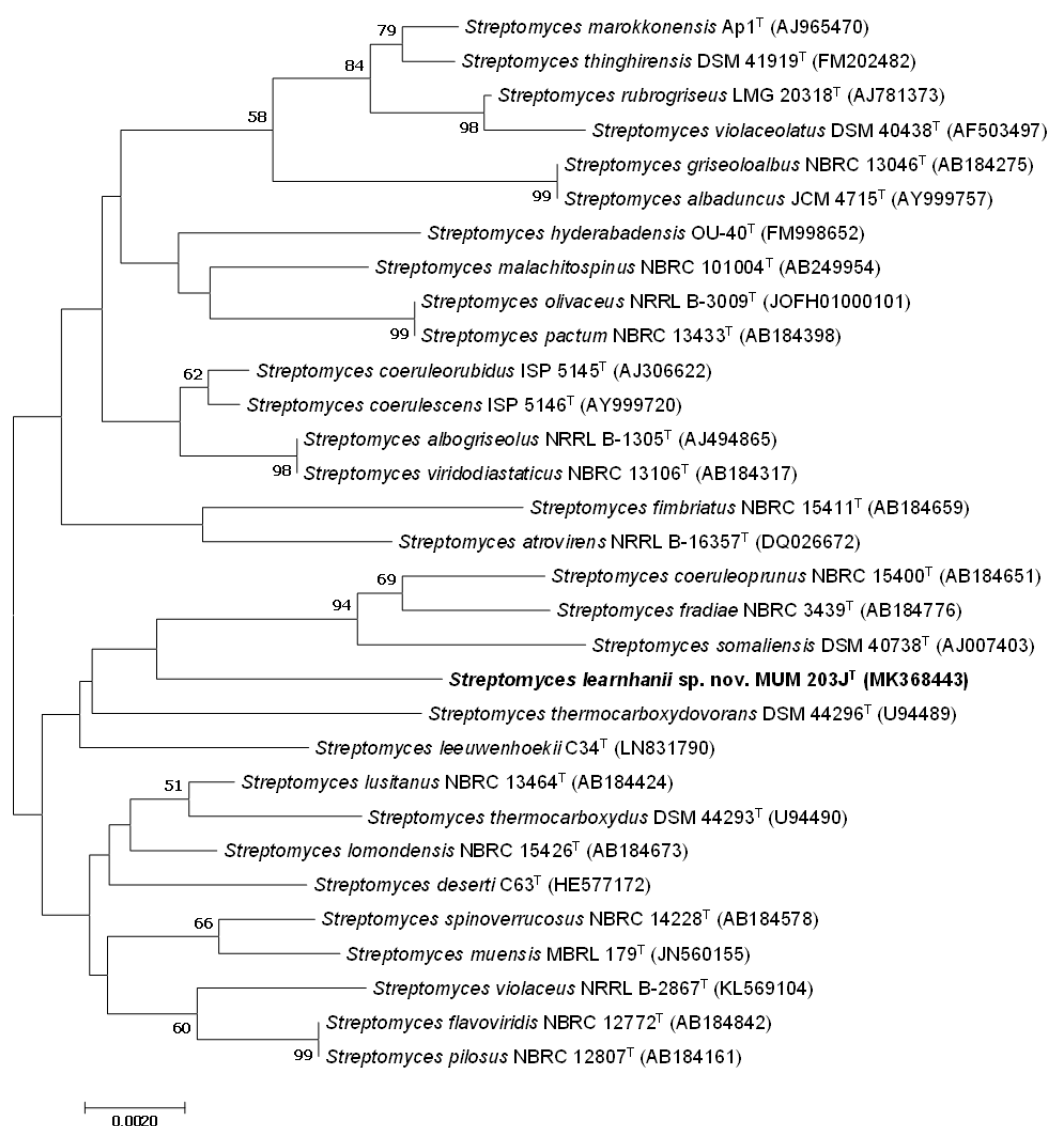
Medium	Growth	Colony colour	
		Aerial mycelium	Substrate mycelium
Yeast malt agar (ISP 2)	Moderate	Pale greenish yellow	Greyish yellow
Oat Meal agar (ISP 3)	No growth	-	-
Inorganic Salt Starch agar (ISP 4)	No growth	-	-
Glycerol Asparagine Agar Base (ISP 5)	Poor	Yellowish white	Yellowish white
Peptone 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
<i>Streptomyces</i> 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<sup>T</sup>, 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<sup>T</sup> 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<sup>T</sup> (98.1% sequence similarity), *S. coeruleorubidus* JCM 4359<sup>T</sup> (98.8 %), and *S. coeruleoprunus* JCM 6919<sup>T</sup> (98.5 %). According to Figure 2, strain MUM 203J<sup>T</sup> appeared closely related to *S. thermocarboxydovorans* NBRC 16324<sup>T</sup> (DSM 44296<sup>T</sup>), 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<sup>T</sup> 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<sup>T</sup> and respective closely related type strains were  $23.3 \pm 4.7$  % with *S. thermocarboxydovorans* NBRC 16324<sup>T</sup>,  $10.7 \pm 0.6$  % with *S. coeruleorubidus* JCM 4359<sup>T</sup> (98.8 %), and  $15.3 \pm 3.8$  % with *S. coeruleoprunus* JCM 6919<sup>T</sup>. 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<sup>T</sup>

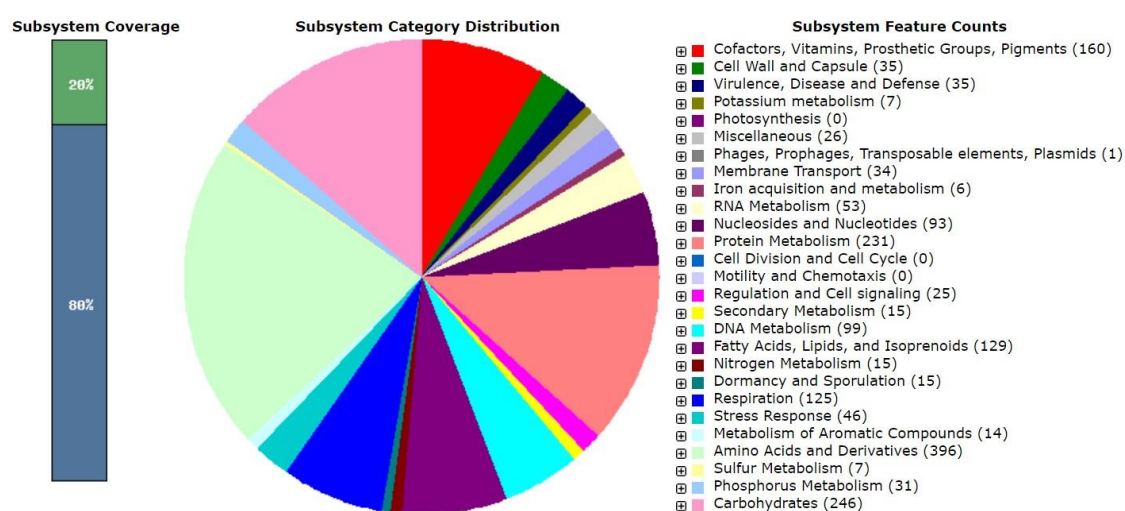
Strain MUM 203J<sup>T</sup> has a genome size of 6,446,886 bp, with DNA G + C content of 72.26 mol%, and average coverage of 125.71-times (Table 2). There were 5731 coding sequences with 67 tRNAs and 7 rRNAs predicted. The genome sequence of strain MUM

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

**Table 2.** *Streptomyces learnhaniae* sp. nov. MUM 203J<sup>T</sup> genome information.

<i>Streptomyces learnhaniae</i> sp. nov. MUM203J <sup>T</sup>	
Genome size (bp)	6,446,886
Contigs	147
Contigs N <sub>50</sub> (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<sup>T</sup>.



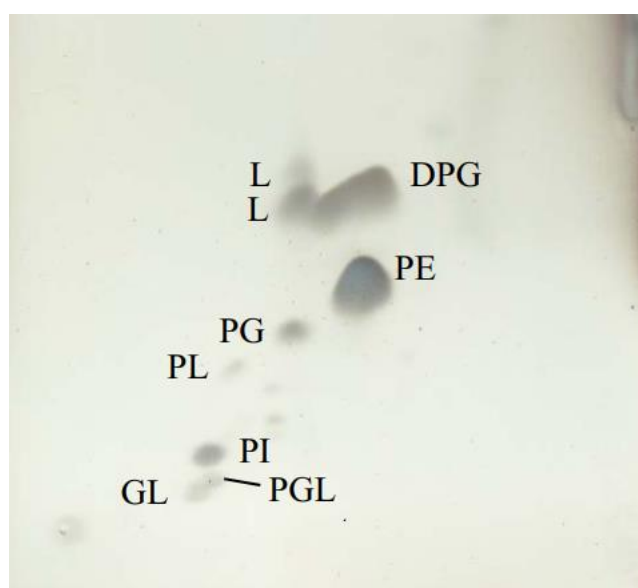
**Figure 3.** The subsystem category distribution of *Streptomyces learnhaniae* sp. nov. MUM 203J<sup>T</sup> according to RAST.



The comparison of whole genome sequences between strain MUM 203J<sup>T</sup> and its closely related type strain *S. coeruleorubidus* JCM 4359<sup>T</sup> based on FastANI analysis estimated an ANI value of 81.32 %. TYGS analysis further supported that strain MUM 203J<sup>T</sup> 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<sup>T</sup>

Strain MUM 203J<sup>T</sup> possessed LL-diaminopimelic acid in the cell wall. Major menaquinones identified were MK9(H<sub>8</sub>) (approximately 70 %), and others include MK9(H<sub>4</sub>), MK9(H<sub>6</sub>), and MK9(H<sub>12</sub>). The whole cell sugars of MUM 203J<sup>T</sup> were glucose, ribose, and mannose. Fatty acids of strain MUM 203J<sup>T</sup> were majority comprised of anteiso-C<sub>15:0</sub> (24.7 %), anteiso-C<sub>17:0</sub> (16.4 %), iso-C<sub>16:0</sub> (15.7 %), iso-C<sub>15:0</sub> (11.5 %) and C<sub>16:0</sub> (11.1 %) (Table 3). Some similarities in fatty acids composition between strain MUM 203J<sup>T</sup>, *S. thermocarboxydovorans* NBRC 16324<sup>T</sup>, *S. coeruleorubidus* JCM 4359<sup>T</sup>, and *S. coeruleoprunus* JCM 6919<sup>T</sup> can be observed. For instance, all of them had anteiso-C<sub>15:0</sub> and iso-C<sub>16:0</sub> as major fatty acids, but with quantifiable differences (Table 3). The polar lipid profile of strain MUM 203J<sup>T</sup> 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<sup>T</sup>. L, lipid; GL, glycolipid; PL, phospholipid; PI, phosphatidylinositol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PGL, phosphoglycolipid; DPG, diphosphatidylglycerol.

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

Fatty acid	<i>Streptomyces learnhaniae</i> MUM 203J <sup>T</sup>	<i>Streptomyces coeruleoprunus</i> JCM 6919 <sup>T</sup>	<i>Streptomyces coeruleorubidus</i> JCM 4359 <sup>T</sup>	<i>Streptomyces thermocarboxydovorans</i> NBRC 16324 <sup>T</sup>
iso-C <sub>13:0</sub>	-	-	0.3	0.5
iso-C <sub>14:0</sub>	2.6	2.7	4.6	5.8
C <sub>14:0</sub>	0.5	0.3	0.3	0.3
iso-C <sub>15:0</sub>	11.5	7.9	16.3	20.6
anteiso-C <sub>15:0</sub>	24.7	29.1	14.9	14.5
C <sub>15:1</sub> B	-	-	-	0.4
C <sub>15:0</sub>	1.4	1.1	0.9	1.7
iso-C <sub>16:1</sub> H	1.0	0.3	0.8	2.1
iso-C <sub>16:0</sub>	15.7	17.6	17.2	24.1
C <sub>16:1</sub> Cis 9	2.4	0.3	2.9	1.3
C <sub>16:0</sub>	11.1	6.1	8.6	7.0
C <sub>16:0</sub> 9Methyl	2.4	0.5	4.0	0.8
anteiso-C <sub>17:1</sub> C	1.9	1.2	2.0	0.3
iso-C <sub>17:0</sub>	6.7	4.8	11.1	9.6
anteiso-C <sub>17:0</sub>	16.4	24.8	14.4	8.7
C <sub>17:1</sub> Cis 9	-	-	0.5	-
C <sub>17:0</sub> Cyclo	0.8	0.9	0.4	0.5
C <sub>17:0</sub>	0.9	0.6	0.8	1.4
iso-C <sub>18:0</sub>	-	-	-	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<sup>T</sup> isolated from a Malaysian mangrove forest has been proven to be a novel *Streptomyces* species in the present investigation.

Strain MUM 203J<sup>T</sup> 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<sup>T</sup> is indeed a member of the *Streptomyces* genus, with the presence of LL-diaminopimelic acid in cell wall peptidoglycan and prevailing menaquinone MK9(H<sub>8</sub>) that are typically observed within the genus [86, 90, 111–115]. Strain MUM 203J<sup>T</sup> 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<sup>T</sup>. The results of 16S rRNA gene sequence similarity and phylogenetic analyses showed that the closest related type strains of strain MUM 203J<sup>T</sup> were *S. thermocarboxydovorans* NBRC 16324<sup>T</sup>, *S. coeruleorubidus* JCM 4359<sup>T</sup>, and *S. coeruleoprunus* JCM 6919<sup>T</sup> (98.5 %). As illustrated in Figure 2 phylogenetic tree, a considerable evolutionary distance exists between strain MUM 203J<sup>T</sup> and other type strains, highlighting strain MUM 203J<sup>T</sup>'s substantial potential as a novel species. The novelty of strain MUM 203J<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup>. 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> and its closely related type strain *S. coeruleorubidus* JCM 4359<sup>T</sup>. Therefore, the evidence strongly supports that strain MUM 203J<sup>T</sup> is a novel species within the genus *Streptomyces*.

Strain MUM 203J<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup>

*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<sup>T</sup> (= NBRC 114250<sup>T</sup> = MCCC 1K04200<sup>T</sup>) and it is isolated from a mangrove forest at East Malaysia. The 16S rRNA gene sequence of strain MUM 203J<sup>T</sup> has been deposited in GenBank/EMBL/DDBJ under the accession number MK368443. The genome of strain MUM 203J<sup>T</sup> 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<sup>T</sup> 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<sub>8</sub>). The whole-cell sugars are glucose, ribose and mannose. Polar lipids of strain MUM 203J<sup>T</sup> consist of lipid, glycolipid, phospholipid, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphoglycolipid, and diphosphatidylglycerol. The major cellular fatty acids (>10.0 %) are anteiso-C<sub>15:0</sub>, anteiso-C<sub>17:0</sub>, iso-C<sub>16:0</sub>, iso-C<sub>15:0</sub>, and C<sub>16:0</sub>.

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

**Author Contributions:** Writing—original draft preparation, JW-FL; conceptualization, JW-FL and LT-HT; methodology and data analysis, JW-FL, LT-HT, and K-WH; validation, NSAM and Y-WH.; review and editing, VL and SHW; resources K-GC

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