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Streptomyces griseiviridis sp. nov., a Novel "Modern *Actinobacteria*" isolated from Malaysia Mangrove Soil

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Abstract: A novel strain, *Streptomyces griseiviridis* MUM 136J^T was recovered from a mangrove forest soil in Malaysia. The Gram-positive bacterium forms strong yellow aerial mycelium and moderate yellow substrate mycelium on ISP 2 agar. A polyphasic approach was used to determine the taxonomy status of strain MUM 136J^T. The strain showed a spectrum of phylogenetic and chemotaxonomic properties consistent with those of the members of the genus *Streptomyces*. The cell wall peptidoglycan was determined to contain

LL-diaminopimelic acid. The predominant menaquinones were identified as MK-9(H_8) and MK-9(H_6), while the identified polar lipids consisted of lipid, aminolipid, phospholipid, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylinositolmannoside. The cell wall sugars consist of ribose, mannose, and galactose. The predominant cellular fatty acids (>10.0 %) were identified as iso-$C_{16:0}$ (31.6 %), anteiso-$C_{15:0}$ (14.8 %), iso-$C_{15:0}$ (12.0 %), and anteiso-$C_{17:0}$ (11.1 %). Phylogenetic analysis identified that closely related strains for MUM 136J^T are *Streptomyces leeuwenhoekii* DSM 42122^T (98.9 %), *Streptomyces erythrogriseus* JCM 9650^T (98.4 %), *Streptomyces griseoincarnatus* JCM 4381^T (98.5 %). The DNA-DNA relatedness values between MUM 136 J^T and closely related type strains ranged from 13.3 ± 1.5 % to 17.4 ± 2.0 %. The name *Streptomyces griseiviridis* sp. nov. is proposed, and the type strain is MUM 136J^T (= NBRC 114249^T = MCCC 1K04199^T).

Keywords: *Streptomyces griseiviridis*; actinobacterial; mangrove; antioxidative; polyphasic taxonomy; MOD-ACTINO

1. Introduction

Actinobacteria have never ceased to gain the attention of researchers worldwide due to their astonishing abilities to produce valuable biologically active compounds. The "Modern *Actinobacteria*" (MOD-ACTINO) has been the focus lately to uncover *Actinobacteria* from unique sources with bioactive potentials [1, 2]. *Actinobacteria* are present in diverse habitats such as terrestrial soil [3, 4], marine [5, 6], pond [7, 8], desert [9-11], cave [12-14], glacier [15, 16], hot spring [17, 18], Artic and Antarctic zones [19-22], and mangrove [23-26]. This phylum of bacteria can survive in a wide range of environmental conditions through their complex multicellular life cycle and the development of unique defence mechanisms, notably observed in the genus *Streptomyces* [27-29].

The largest genus in the phylum *Actinobacteria* is *Streptomyces*, which has largely contributed to improving our health and well-being. This genus consists of bacteria that are producers of important antibiotics currently being used for treating infections in animals and humans [30-32]. *Streptomyces* is well-known for their antimicrobial activity, and many studies have investigated their antimicrobial effect against various pathogens in hopes of searching for new effective antibiotics [33-36]. For instance, the increasing morbidity and mortality burden of life-threatening infection caused by Methicillin-resistant *Staphylococcus aureus* (MRSA) has urged the need for more effective antibiotics. Studies have reported that *Streptomyces* spp. could produce compounds with promising anti-MRSA activity [37-41]. Another example of a recently targeted pathogen is the SARS-CoV-2 virus — a causal pathogen of coronavirus disease (COVID-19) [42-45]. The COVID-19 pandemic has caused a tremendous loss of human life and reduced the population's quality of life globally [46-51]. Ivermectin, refined from avermectin (a *Streptomyces*-derived antiparasitic drug), is proposed as a potential drug candidate for treating (COVID-19) [52, 53]. Efforts taken into repurposing existing drug agents (e.g., ivermectin, hydroxychloroquine, etc.) aim to offer treatment

options for COVID-19 [54-56]. Additionally, a genomic and metabolomic study conducted by Melinda et al. [57] reported the production of antiviral agents, echoside A and echoside B, against SARS-CoV-2 by *Streptomyces* sp. GMR22.

Furthermore, the emerging roles of *Streptomyces* as probiotics for aquaculture applications have been discussed [58-60]. Marine fishes and shrimps are prone to infection caused by *Vibrio* spp. pathogens (e.g. *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio alginolyticus*), resulting in an illness known as vibriosis with a high mortality rate [61]. Consequently, the occurrence of these pathogens in seafood has led to foodborne diseases [62-65]. Several studies have provided evidence that *Streptomyces* spp. exerted significant anti-*Vibrio* activity [58, 66-68]. The antibacterial property of *Streptomyces* spp. is closely associated with their role as probiotics that protect fishes and shrimps against infectious diseases such as vibriosis [69-71].

It is crucial to continue finding new sources of these beneficial *Actinobacteria*. Mangrove forests have unique and dynamic environmental characteristics as they are located in intertidal zones. The mangroves in Malaysia remain largely unexplored, and they are excellent sources for discovering novel and bioactive *Streptomyces* species. Numerous novel *Streptomyces* species have been found in mangroves, and they are capable of producing bioactive compounds associated with antimicrobial, anticancer, antioxidant, and neuroprotection activities [1, 27, 72, 73]. Some examples of these strains are *Streptomyces colonosanans* (anticancer and antioxidant) [74], *Streptomyces pluripotens* (antibacterial) [75], *Streptomyces antioxidans* (antioxidant and neuroprotection) [76], and *Streptomyces nigra* (antitumor) [77]. The *Streptomyces* bacteria possess large genome sizes, typically ranging from 7 to 10 Mbp, which account for their secondary metabolites production capabilities [78-80]. Therefore, research exploring novel species of *Streptomyces* remains worthwhile, considering the great benefits these bacteria can offer and the chances of finding new or valuable compounds.

This study aims to identify and characterize a novel *Streptomyces* strain, MUM 136J^T, isolated from the soil of a Malaysian mangrove forest. A polyphasic approach was carried out to investigate the genotypic, genomic, phenotypic, and chemotaxonomic features of the strain. Next-generation sequencing technique was applied to determine the whole genome sequence of strain MUM 136J^T for further bioinformatic analyses. *Streptomyces griseiviridis* sp. nov. MUM 136J^T is a Malaysian MOD-ACTINO that can serve as a new microbial source of bioactive agents.

2. Materials and Methods

2.1. Soil Sampling, Isolation, and Maintenance of the Strain

Soil samples were collected from a mangrove forest located on the East coast of Malaysia, in June 2015. Strain MUM 136J^T was isolated from a soil sample collected at the mangrove site labelled as KTTAS 4 (1º41’48.48”N 110º11’13.40”E). The soil samples were

processed by air-drying, followed by mixing and selective pretreatment through wet heat for 15 min at 50 ºC [81]. Strain MUM 136J^T was isolated from a nutrient agar (NA) plate supplemented with cycloheximide (50 mg/L) and nalidixic acid (20 mg/L). The strain was purified on ISP 2 plate. Pure cultures of strain MUM 136J^T were maintained on ISP 2 agar slants at 28 °C and kept in glycerol suspensions (20 %, v/v) at -20 °C for long-term storage.

2.2. Genotypic Identification and Phylogenetic Analysis

Genomic DNA extraction and 16S rRNA gene PCR amplification were conducted according to established protocols [74, 75, 82]. The 16S rRNA gene sequence of strain MUM 136J^T was obtained and manually aligned with representative sequences of related type strains of *Streptomyces* genus retrieved from GenBank/EMBL/DDBJ databases using CLUSTAL-X software. Phylogenetic trees were reconstructed with neighbour-joining [83] and maximum likelihood [84] algorithms using MEGA version 7.0. Kimura's two-parameter model [85] was applied for the computation of evolutionary distances in the neighbour-joining phylogenetic tree, and Felsenstein's method [86] of bootstrap analysis based on 1000 resamplings was applied to evaluate the tree topologies. The sequence similarities of strain MUM 136J^T and related type strains were analyzed by EzBioCloud server (http://www.ezbiocloud.net/).

DNA-DNA hybridization (DDH) was conducted by the Identification Service of the DSMZ, Braunschweig, Germany [87, 88], on strain MUM 136J^T and its closely related type strains *Streptomyces leeuwenhoekii* DSM 42122^T, *Streptomyces erythrogriseus* JCM 9650^T, and *Streptomyces griseoincarnatus* JCM 4381^T.

2.3. Next Generation Sequencing and Bioinformatic Analysis

Genomic DNA extraction was done using MasterPure™ Gram Positive DNA Purification Kit (Epicentre, Illumina Inc., Madison, WI, USA). The quality of the bacterial DNA was verified using NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). DNA library construction was carried out using NEXTERA DNA Flex Library Prep Kit (Nextera, USA), and the constructed sequencing libraries were loaded into Illumina MiSeq platform with MiSeq Reagent Kit v3 (Illumina Inc., Madison, WI, USA). The sequencing quality was evaluated using FastQC (version 0.11.9) [89]. Subsequently, along with the adapter sequences, the raw reads were trimmed using BBDuk of BBTools (v36). The trimmed raw reads were then assembled using St. Petersburg genome assembler (SPAdes) (v3.14.1) [90]. The assembled genomic sequence was submitted to Rapid Annotation using Subsystem Technology (RAST) database (https://rast.nmpdr.org/) for annotation, with the following settings: default pipeline for RASTtk, domain bacteria, and automatically fixed error options turned on [91, 92]. The genome assembly was compared with genomes of closely related *Streptomyces* species (retrieved from NCBI database) using FastANI (version 1.33) [93]. The genome assembly was uploaded to Type Strain Genome Server (https://tygs.dsmz.de) for

phylogenomic analysis [94]. The detection of biosynthetic gene clusters related to secondary metabolite production was analyzed using antiSMASH (version 6.1.1) [95].

2.4. Phenotypic Analysis

The cultural morphology of strain MUM 136J^T was observed on 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), at 28 °C for 14 days [74, 82]. The colony colors were determined according to ISCC-NBS color charts. The cellular morphology of strain MUM 136J^T was observed under Light microscopy (80i, Nikon) and scanning electron microscopy (JEOL-JSM 6400) after growing on ISP 2 plate at 28 °C for 7–14 days.

The growth of strain MUM 136J^T at different pH ranges (pH 2–10) and NaCl concentration (0–10 %) were examined using tryptic soy broth (TSB) incubated at 28 °C for 14 days. The effect of different temperatures (4–50 °C) on the growth of strain MUM 136J^T was tested using ISP 2 agar plates, and the responses were recorded for 14 days. Melanoid pigments were produced using ISP 7 medium after incubation at 28 °C for 7–14 days, and hemolytic activity was observed using horse blood agar medium after incubation at 32 °C for 7–14 days [74, 75]. Enzymatic activities of strain MUM 136J^T including amylolytic, cellulase, chitinase, catalase, protease, and xylanase were investigated according to the previously described protocol [75]. All phenotypic tests were conducted simultaneously on strain MUM 136J^T, *S. leeuwenhoekii* DSM 42122^T, *S. erythrogriseus* JCM 9650^T, and *S. griseoincarnatus* JCM 4381^T.

2.5. Chemotaxonomic Evaluation

The evaluation of cell wall peptidoglycan, whole cell sugars, respiratory quinones, fatty acids, and polar lipids were done by the Identification Service of the DSMZ, Braunschweig based on established protocols [74, 75, 82, 96].

3. Results

3.1. Genotypic Identification and Phylogenetic Analysis of Strain MUM 136J^T

The nearly full-length 16S rRNA gene sequence was attained for strain MUM 136J^T (1488 bp; GenBank/EMBL/DDBJ accession number MK368433). The alignment of the sequence with the corresponding partial 16S rRNA gene sequences of the type strains of representative members of the genus *Streptomyces* retrieved from GenBank/EMBL/DDBJ databases was conducted manually. Based on the 16S rRNA gene sequences, phylogenetic trees were reconstructed to determine the phylogenetic position of this strain (Figure 1 and Supplementary Figure S1). The phylogenetic analysis demonstrated that the most closely

related strain is *Streptomyces leeuwenhoekii* DSM 42122^T (98.9 % sequence similarity) with shortest evolutionary distance (Fig. 1). The 16S rRNA gene sequence analysis for strain MUM 136J^T revealed that this strain exhibited the highest similarity to strain *S. leeuwenhoekii* DSM 42122^T (98.9 %), *S. erythrogriseus* JCM 9650^T (98.4 % similarity), and *S. griseoincarnatus* JCM 4381^T (98.5 %).

Furthermore, the results of DDH revealed that the DNA–DNA relatedness levels between strain MUM 136J^T, *S. leeuwenhoekii* DSM 42122^T (17.4 ± 2.0 %), *S. erythrogriseus* JCM 9650^T (15.7 ± 1.8 %), and *S. griseoincarnatus* JCM 4381^T (13.3 ± 14.8 %) were significantly below 70 % which has been designated as the threshold value for the delineation of bacterial species [97].

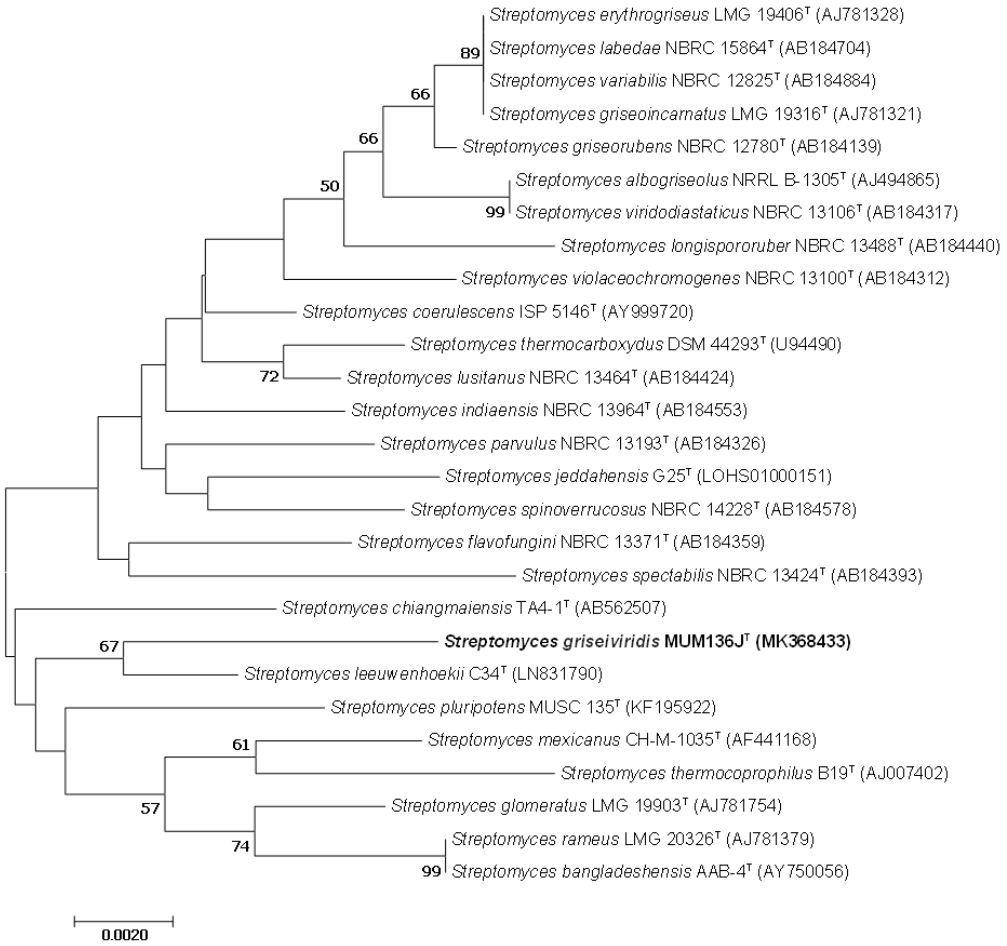

Figure 1. Neighbour-joining phylogenetic tree based on 1488 nucleotides of 16S rRNA gene sequence showing the relationship between strain MUM 136J^T and representatives of related taxa. Numbers and nodes indicate percentages (> 50 %) of 1000 bootstrap re-sampling. Bar, 0.002 substitutions per site.

3.2. Whole Genome Sequence and Bioinformatic Analysis of Strain MUM 136J^T

A total of 6,798,218 reads have been generated from the sequencing experiment. After adapter trimming and trimming the sequencing raw reads, the whole genome sequence was assembled using SPAdes, resulting in a total of 169 contigs and N_{50} of 100,013 bp. The total genome size of strain MUM 136J^T is 7,180,176 bp, with a G+C content of 72.32 % and the calculated sequencing coverage of 144.75-times (Table 1). The genome sequence of *Streptomyces griseiviridis* MUM 136J^T has been deposited at DDBJ/EMBL/GenBank under accession number JADWYP000000000.

Table 1. General features of *Streptomyces griseiviridis* MUM 136J^T genome.

	***Streptomyces griseiviridis* MUM 136J^T**
Genome size (bp)	7,180,176
Contigs	169
Contigs N_{50} (bp)	100,013
G + C content	72.32 %
Genome coverage	144.75x
CDS	6637
tRNA	66
rRNA	2(5S), 1(16S), 1(23S)

Based on the RAST system, a total of 6637 coding sequences assigned to the 1252 subsystem, 66 tRNA, and 4 rRNA, were detected in the genome of strain MUM 136J^T (Table 1). The majority of the genes are involved in amino acids and derivatives metabolism (6.03 %), carbohydrate metabolism (4.81 %), and protein metabolism (3.30 %) (Figure 2). Besides, antiSMASH analysis had identified the presence of gene clusters account for the biosynthesis of compounds such as ectoine (100 % known gene cluster similarity), albaflavenone (100 % known gene cluster similarity), γ-butyrolactone (100 % known gene cluster similarity) and paenibactin (83 % known gene cluster similarity).

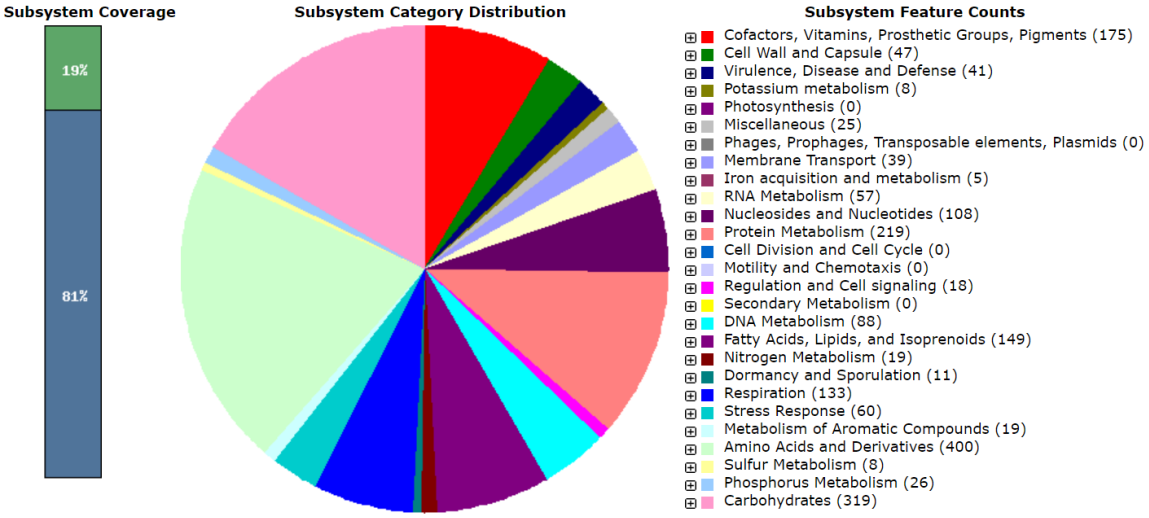

Figure 2. RAST output on subsystem category distribution of *Streptomyces griseiviridis* MUM 136J^T.

FastANI demonstrated that comparing whole genome sequences between strain MUM 136J^T and its closely related type strain *Streptomyces griseosporeus* JCM 4766^T resulted in ANI value of 87.36 % (Table S1). The ANI value between strain MUM 136J^T and *S. leeuwenhoekii* DSM 42122^T was also calculated and resulted in 84.54 %. The phylogenomic analysis of the whole genome sequences with TYGS showed that strain MUM 136J^T is closely related to *S. griseosporeus* JCM 4766^T and *S. leeuwenhoekii* DSM 42122^T, with digital DDH (dDDH) values (formula d_4) of 31.6 % and 27.2 % respectively (Figure 3).

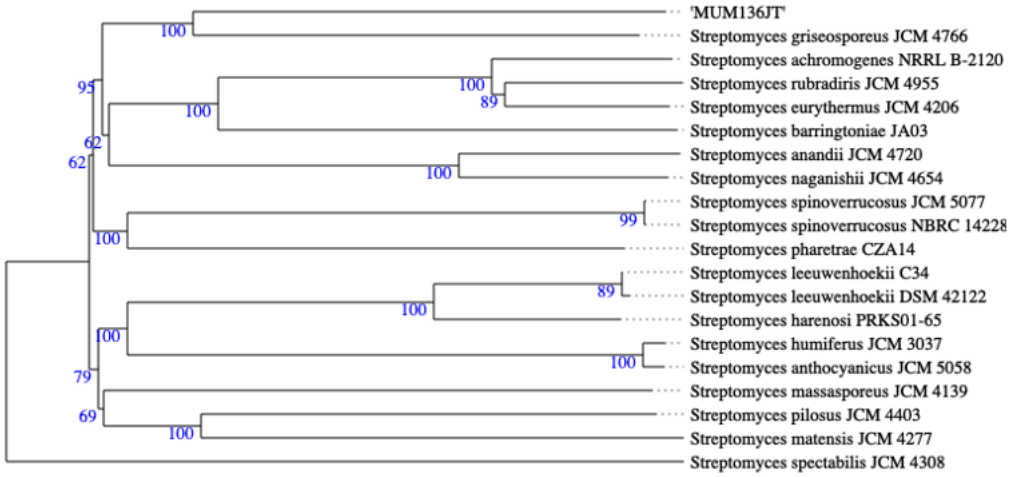

Figure 3. The whole genome sequence tree constructed using TYGS web server for *Streptomyces griseiviridis* MUM 136J^T and closely related type strains. Tree inferred with FastME 2.1.6.1 [98] from Genome BLAST Distance Phylogeny (GBDP) distances calculated from genome sequences. The branch lengths are scaled in terms of the GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values > 60 % from 100 replications, with an average branch support of 96.5 %. The tree was rooted at the midpoint.

3.3. Phenotypic Characteristics of Strain MUM 136J^T

Based on the growth observation of strain MUM 136J^T on different media after incubation at 28 °C, for 7–14 days, strain MUM 136J^T was able to grow well on ISP 2, ISP 6, SA, NA, LBA, and MHA; grow moderately on ISP 5, ISP 7, AIA, and SCA; but unable to grow on ISP 3 and ISP 4. The aerial and substrate mycelium colors were media dependent, as shown in Table 2. The cellular morphology of strain MUM 136J^T matched the typical features observed in genus *Streptomyces* (Figure 4). For the effects of temperature, pH, and NaCl tolerance on the growth of strain MUM 136J^T, the results demonstrated that growth was found to occur at 26–32 °C (optimum 26–28 °C), at pH 6.0-8.0 (optimum pH 8.0), and with 0-6 % NaCl tolerance (optimum 0-2 %). Cells were positive for catalase activity, but no hemolytic activity was observed. Moreover, the cells were capable of hydrolyzing casein.

Table 2. The colony appearance of *Streptomyces griseiviridis* MUM 136J^T after growing on different culture media.

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

-, Not detected

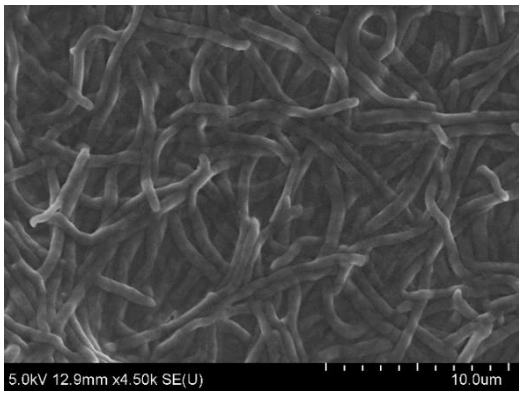

Figure 4. Morphology of *Streptomyces griseiviridis* MUM 136J^T as observed by scanning electron microscopy.

3.4. Chemotaxonomic Characteristics of Strain MUM 136J^T

The cell wall peptidoglycan analysis of strain MUM 136J^T showed that it presented a type I cell-wall as it contained LL-diaminopimelic acid. The predominant menaquinones of strain MUM 136J^T were identified as MK-9(H_8) (68 %) and MK-9(H_6) (10 %), with other minor menaquinones identified as MK9(H_{10}) (5%), MK10 (4%), MK10(H_2) (3%), and MK9(H_4) (2%). The whole-cell sugars detected were ribose, mannose, and galactose.

Fatty acid profiles of strain MUM 136J^T and its closely related type strains are compiled in Table 3. The major cellular fatty acids in strain MUM 136J^T were identified as iso-$C_{16:0}$ (31.6 %), anteiso-$C_{15:0}$ (14.8 %), iso-$C_{15:0}$ (12.0 %), and anteiso-$C_{17:0}$ (11.1 %). The fatty acid profile of strain MUM 136J^T displayed high levels of similarities with those of closely related phylogenetic neighbors, *S. leeuwenhoekii* DSM 42122^T, *S. erythrogriseus* JCM 9650^T, and *S. griseoincarnatus* JCM 4381^T, as they also contain iso-$C_{16:0}$ (21.9–35.3 %) as their major fatty acid (Table 3). However, quantitative differences can be observed in the fatty acid profiles of strain MUM 136J^T and its closely related type strains. For instance, iso-$C_{16:0}$ (31.6 %) was found to be predominant in strain MUM 136J^T, but the quantity of the same fatty acid was higher in *Streptomyces leeuwenhoekii* DSM 42122^T (35.3 %). As for polar lipids analysis, there were lipid, aminolipid, phospholipid, phophatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylinositolmannoside present in strain MUM 136J^T (Figure 5).

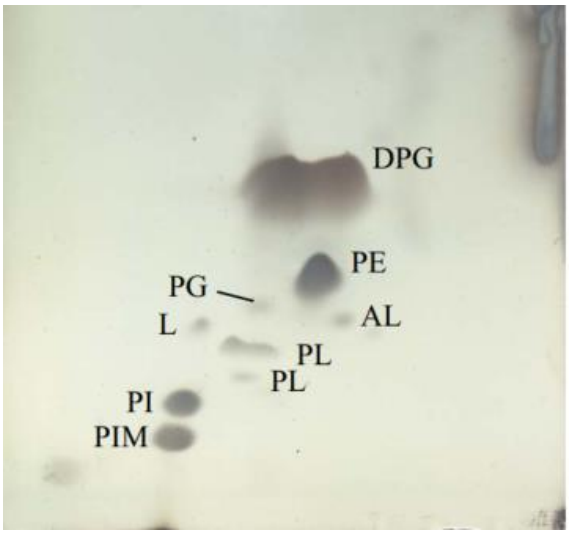

Figure 5. Total lipid profile of *Streptomyces griseiviridis* MUM 136J^T. L, lipid; AL, aminolipid; PL, phospholipid; PG, phophatidylglycerol; PI, phosphatidylinositol; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol; PIM, phosphatidylinositolmannoside.

Table 3. Fatty acid profiles of *Streptomyces griseiviridis* MUM 136J^T and closely related type strains *Streptomyces leeuwenhoekii* DSM 42122^T, *Streptomyces erythrogriseus* JCM 9650^T, and *Streptomyces griseoincarnatus* JCM 4381^T.

Fatty acid	*Streptomyces griseiviridis* MUM 136J^T	*Streptomyces leeuwenhoekii* DSM 42122^T	*Streptomyces erythrogriseus* JCM 9650^T	*Streptomyces griseoincarnatus* JCM 4381^T
iso-$C_{12:0}$	-	-	0.1	-
iso-$C_{13:0}$	0.1	-	0.2	-
anteiso-$C_{13:0}$	0.1	-	0.1	-
iso-$C_{14:0}$	3.5	6.5	8.2	3.1
$C_{14:0}$	0.2	-	0.2	0.2
iso-$C_{15:0}$	12.0	3.7	7.8	8.1
anteiso-$C_{15:0}$	14.8	19.6	20.4	17.2
$C_{15:1}$ B	0.1	-	0.1	-
$C_{15:0}$	1.7	0.3	0.7	0.9
anteiso-$C_{15:0}$ 2OH	-	-	0.1	0.2
iso-$C_{16:1}$ H	4.4	1.4	2.3	2.0
iso-$C_{16:0}$	31.6	35.3	26.2	21.9
$C_{16:1}$ Cis 9	0.5	0.1	2.4	2.2
$C_{16:0}$	4.7	2.8	7.1	5.9
$C_{16:0}$ 9Methyl	2.3	0.7	1.7	4.2
anteiso-$C_{17:1}$ C	4.2	2.8	1.5	4.1
iso-$C_{17:0}$	5.2	5.2	6.8	8.1
anteiso-$C_{17:0}$	11.1	15.7	12.4	15.8
$C_{17:1}$ Cis 9	0.2	-	0.3	0.4
$C_{17:0}$ Cyclo	0.7	1.7	-	1.8
$C_{17:0}$	0.4	0.3	0.7	0.7
$C_{17:0}$ 10Methyl	0.1	0.2	0.2	0.2
iso-$C_{18:1}$ H	-	-	0.2	0.6
iso-$C_{18:0}$	-	0.5	0.4	-
$C_{18:1}$ Cis 9	0.1	-	0.1	1.2
iso-$C_{17:0}$ 2OH	0.2	0.4	-	-
$C_{18:0}$	0.2	-	0.2	-

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

4. Discussion

Due to the tremendous number of existing *Streptomyces* species (approximately 1140 validly identified species), determining a novel strain in this genus is more challenging and complicated. Nonetheless, the standard process for identifying and characterizing a novel strain required a polyphasic approach involving a combination of genotypic, phylogenetic,

and phenotypic tests [99, 100]. The advances in molecular techniques, such as next-generation sequencing, have allowed higher efficiency and better characterization of bacterial species. In this study, the novelty of strain MUM 136J^T is firmly proven and supported by a series of phylogenetic, phylogenomic, genomic, phenotypic, and chemotaxonomic analyses.

Based on 16S rRNA gene similarity according to the EzBioCloud server, strain MUM 136J^T and *S. leeuwenhoekii* DSM 42122^T exhibited the highest similarity of 98.9 %. The outcomes of phylogenetic analysis based on neighbour-joining and maximum likelihood algorithms were consistent, illustrating that the strain MUM 136J^T formed a clade with *S. leeuwenhoekii* DSM 42122^T. Nevertheless, the DDH value of laboratory-based genome-wide comparison between strain MUM 136J^T and its closest related type strain *S. leeuwenhoekii* DSM 42122^T was 17.4 ± 2.0 %, significantly below the 70 % threshold of DNA-DNA relatedness. DDH has been the gold standard for the taxonomic evaluation of strain, and if the value is less than 70 %, the two strains can be categorized as two different species [97, 100, 101]. Furthermore, phylogenomic analysis by TYGS illustrated that the closely related type strain for strain MUM 136J^T is *S. griseosporeus* JCM 4766^T with a dDDH value of 31.6 %. Therefore, strain MUM 136J^T is a distinct species from *S. leeuwenhoekii* DSM 42122^T and *S. griseosporeus* JCM 4766^T.

Average nucleotide identity (ANI) analysis was performed in this study by comparing the genome sequences of the strain MUM 136J^T and closely related type strains to determine their genetic relatedness. ANI technique has become increasingly popular as whole genome sequencing is now more accessible, and it is a promising substitute for the labour-intensive DDH technique [102]. FastANI is a new method that can be applied to both complete and draft genomes to calculate ANI using alignment-free approximate sequence mapping [93]. The estimated ANI values between MUM 136J^T and its closely related type strains *S. griseosporeus* JCM 4766^T, and *S. leeuwenhoekii* DSM 42122^T were significantly below 95 %. Goris et al. [103] reported that ANI values of 95 % and 69 % conserved DNA are equivalent to 70 % DDH cut-off for species delineation. Thus, strain MUM 136J^T is a novel species of the genus *Streptomyces*, as supported by the data obtained from laboratory-based DDH, TYGS (dDDH), and FastANI.

The phenotypic and chemotaxonomic information serve as important supplementary data to confirm that MUM 136J^T belongs to the genus *Streptomyces*. Strain MUM 136J^T exhibits typical colony and cellular morphology of *Streptomyces*, for example, the formation of aerial and substrate mycelia. Moreover, the detection of LL-diaminopimelic acid in cell wall peptidoglycan, accompanied by MK-9(H_8) and MK-9(H_6) predominant menaquinones of strain MUM 136J^T further corroborates the strain as *Streptomyces* species. LL-diaminopimelic acid, MK-9(H_8) and MK-9(H_6) menaquinones are typically found in the genus *Streptomyces*, and similar findings have been reported by many relevant studies [76, 104-109].

Meanwhile, antiSMASH detected the presence of biosynthetic gene clusters accounting for compounds such as ectoine and albaflavenon. Ectoine is an extremolyte

naturally found in bacteria that thrive in extreme environmental conditions such as high salinity, irradiation, drought, extreme pH, and temperature [110, 111]. Ectoine confers protection to the bacteria by regulating osmotic stress, stabilizing lipid bilayers, preventing DNA and protein damage, and offering hydroxyl radical scavenging activity [112]. The presence of ectoine biosynthetic gene cluster in MUM 136J^T could be explained by the need for this bacterium to survive in a dynamic mangrove environment consisting of constant changes in salinity and tidal gradient. Ectoine is a compound commonly used in cosmetic products to promote anti-aging and whitening effects and prevent skin dehydration [113, 114]. Studies have reported anti-inflammation and cell protection properties exhibited by ectoine [113, 115-117]. Another compound, albaflavenon, is a tricycle sesquiterpene antibiotic initially discovered from *Streptomyces albidoflavus* [118]. The biosynthetic gene clusters for albaflavenon was also detected in strain MUMM 136J^T. Given the availability of the whole genome sequence of MUM 136J^T, the genomic information obtained revealed the potential of this mangrove-derived novel strain and its role as MOD-ACTINO. Further studies on the production of compounds such as ectoine and albaflavenone could provide better insights into strain MUM 136J^T as a valuable source of cosmeceutical or pharmaceutical agents.

5. Conclusion and Description of *Streptomyces griseiviridis* sp. nov. MUM 136J^T

Streptomyces griseiviridis sp. nov. (gri.se. i.vi'ri.dis. L. adj. griseus, grey; L. adj. viridis, green; N.L. masc. adj. griseiviridis, grey-green, referring to the color of the mycelia).

The type strain is MUM 136J^T (=NBRC 114249^T = MCCC 1K04199^T) isolated from soil sample collected from the Malaysia mangrove forest. The 16S rRNA gene sequence of strain MUM 136J^T has been deposited in GenBank/EMBL/DDBJ under the accession number MK368433. The cells of strain MUM 136J^T appear with strong yellow aerial mycelium and moderate yellow substrate mycelium on ISP 2 agar plate. The cells grow well on ISP 2, ISP 6, SA, NA, LBA, and MHA media. The strain is capable of growing at 26–32 °C (optimum 26–28 °C), pH 6.0-8.0 (optimum pH 8.0), and 0-6 % NaCl (optimum 0-2 %), with positive casein hydrolysis. The cell wall peptidoglycan consists of LL-diaminopimelic acid. Major menaquinones of strain MUM 136J^T includes MK-9(H_8) and MK-9(H_6), and the major cellular fatty acids (>10 %) are iso-$C_{16:0}$, anteiso-$C_{15:0}$, iso-$C_{15:0}$, and anteiso-$C_{17:0}$. Strain MUM 136J^T has ribose, mannose and galactose as its whole cell sugars. The polar lipids comprise lipid, aminolipid, phospholipid, phophatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylinositolmannoside.

The genome size of strain MUM 136J^T is 7,180,176 bp, with G+C content of 72.32 % and coverage of 144.75-times. The genome has been deposited at DDBJ/EMBL/GenBank under accession number JADWYP000000000. RAST system predicted a total of 6637 coding sequences assigned to 1252 subsystem, 66 tRNA, and 4 rRNA in the genome of strain MUM 136J^T. Most of the genes are involved in amino acids and derivatives metabolism, carbohydrates metabolism, and protein metabolism.

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