Genome Report

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Genome sequence of bioactive streptomycete isolated from mangrove forest in East Malaysia, *Streptomyces monashensis* MUSC 1J^T

Hooi-Leng Ser[1,2*†], Jodi Woan-Fei Law[1,2†], Wen-Si Tan[3], Wai-Fong Yin[4], Kok-Gan Chan[4,5], Learn-Han Lee[2*]

[1]Institute of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou 510006, PR China.
[2]Novel Bacteria and Drug Discovery (NBDD) Research Group, Microbiome and Bioresource Research Strength (MBRS), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, 47500, Bandar Sunway, Selangor Darul Ehsan, Malaysia
[3]Illumina Singapore Pte Ltd, Woodlands Industrial Park E1, Singapore
[4]Division of Genetics and Molecular Biology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[5]Vice Chancellor Office, Jiangsu University, Zhenjiang 212013, PR China
[†]These authors contributed equally to the work.

Abstract : Members of *Streptomyces* are known to be prolific producers of bioactive compounds, including antibacterials, antioxidants, neuroprotective agents as well as immunomodulatory compounds. Isolated from East Malaysia, *Streptomyces monashensis* MUSC 1J^T (=DSM 103626^T=MCCC 1K03221^T) was initially described as a novel streptomycete using a polyphasic approach. Based on phylogenetic analyses, the closely strains related to MUSC 1J^T were identified as *Streptomyces corchorusii* DSM 40340^T (98.7 %), *Streptomyces olivaceoviridis* NBRC 3066^T (98.7 %), *Streptomyces canarius* NBRC 13431^T (98.6 %), and *Streptomyces coacervatus* AS-0823^T (98.4 %). Seven day fermentative extracts of MUSC 1J^T exhibited potent antioxidant activity and significant cytotoxic activity against colon cancer cell lines, thus the strain was selected for whole genome sequencing. The genome size of MUSC 1J^T is described to be 10.3 Mbps with G + C content of 71.50%. Based on antiSMASH analysis, the strain possessess great genomic potential, having fifty nine biosynthetic gene clusters related to production of secondary metabolites and antibiotics. Therefore, these results serve a foundation for further in-depth investigation to harness its bioactive potential for the development of highly valuable pharmaceutical products.

Keywords: *Streptomyces monashensis*; cytotoxic; mangrove; Actinobacteria; Malaysia

***Correspondence:** Hooi-Leng Ser, Institute of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou 510006, PR China; hooileng_ser@y7mail.com; Learn-Han Lee, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, 47500, Bandar Sunway, Selangor Darul Ehsan, Malaysia; lee.learn.han@monash.edu

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Introduction

Actinobacteria consists of a group of Gram-positive bacteria with high GC content which are ubiquitous in nature[1–12]. As the largest genus under *Actinobacteria* phylum, *Streptomyces* species present a complicated life cycle which grant them the ability to colonize and shrive in different environments including both terrestrial and marine regions[13–22]. In fact, streptomycetes begins with spore germination and outgrowth of substrate (vegetative) mycelium which grow radially with frequent branching and subsequently form a young colony[23–26]. When nutrients become scarce and the habitat is unfavourable for growth, streptomycetes will undergo morphological differentiation, in which aerial (reproductive) mycelium formation and spores formation can be observed via cell division processes. Besides having a unique lifecycle, the ability of *Streptomyces* sp. to produce

various bioactive compounds granted them the ability to adapt well in their habitat and attracted much interest from the scientific community as candidates for drug discovery studies[27–36]. Mangrove forest in Asia represents an ideal location for the hunt of bioactive strains as this continent has the largest coverage of mangrove forests, contributing 42% of the global total[37–38]. In Malaysia alone, there are 505,386 hectars of mangrove forest which equates to 3.7% of global total. As a matter of fact, several novel strains belonging to the genus *Streptomyces* have been previously isolated from different parts of Malaysia[39–41]. *Streptomyces monashensis* MUSC 1J^T was isolated from mangrove soil in the state of Sarawak, East Malaysia in June 2015[19]. Thorough polyphasic studies were conducted and results showed that MUSC 1J^T represent a novel bioactive strains belonging to the *Streptomyces* genus. The type strain of MUSC 1J^T is available at two culture collection centres with accession of DSM 103626^T=MCCC 1K03221^T. *In vitro* screening assay using human colon cancer cell lines demonstrated that MUSC 1J^T extract significantly reduced their survival (with cell viability ranging from 17.8±5.3% to 18.3±4.0%). Furthermore, the strain is capable of producing antioxidant(s) which reduced the amount of superoxide anion radical displayed in SOD activity assay (83.80±4.80% at the concentration of 2 mg/mL). Therefore, the strain was selected for whole genome sequencing to explore its genomic potential before allowing further genetic manipulation for the production of pharmaceutically important compounds.

Data description

Genomic DNA of MUSC 1J^T was extracted using Masterpure™ DNA purification kit (Epicentre, Illumina Inc., Madison, WI, USA) before subjected to RNase (Qiagen, USA) treatment[42–43]. Before DNA library preparation with Nextera ™ DNA Sample Preparation kit (Nextera, USA), genomic DNA quality was checked with NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Qubit version 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). Subsequently, DNA library quality was examined with Bioanalyzer 2100 high sensitivity DNA kit (Agilent Technologies, Palo Alto, CA)[44–46]. Whole genome shotgun project of MUSC 1J^T was conducted on an Illumina MiSeq platform with MiSeq Reagent Kit 2 (2 × 250 bp; Illumina Inc., Madison, WI, USA). The assembly of trimmed sequence was carried out on CLC Genomic Workbench version 5.1 (CLC Bio, Denmark), resulted in 218 contigs and an N_{50} contig size of approximately 159,229 bp. The assembled genome size comprised 10,254,857 bp, with an average coverage of 170.0-fold and G + C content of 71.50%. The genome sequence of *Streptomyces monashensis* MUSC 1J^T has been deposited at DDBJ/EMBL/GenBank under accession of MLYO00000000. The version described here is the first version.

Table 1. General genomic features of *Streptomyces monashensis* strain MUSC 1J^T.

	Streptomyces monashensis strain MUSC 1J^T
Genome size (bp)	10,254,857
Contigs	218
Contigs N_{50} (bp)	159,229
G + C content %	71.50
Genome coverage	170.0x
Protein coding genes	8,550
rRNAs	2, 1, 1 (5S, 16S, 23S)

The assembled genome was annotated using Rapid Annotation using Subsystem Technology (RAST) and NCBI Prokaryotic Genome Annotation Pipeline (released 2013)[47]. Gene prediction was performed using Prodigal version 2.6.1, while ribosomal RNA (rRNA) and transfer RNA (tRNA) were predicted using RNAmmer and tRNAscan SE version 1.21, respectively[48–50]. A total of 8,550 protein coding genes were found in the genome of MUSC 1J^T. Based on RAST system, most of the protein-coding genes were involved in primary metabolism, including amino acids metabolism (8.1%), followed by carbohydrate metabolism (7.5%) and production of cofactors, vitamins, prosthetic groups, pigments (4.2%) (Figure 1). The web-based bioinformatics tool, antibiotics & Secondary Metabolite Analysis SHell (antiSMASH)[51] revealed the presence of biosynthetic gene clusters in MUSC 1J^T genome. In fact, more than half of them were predicted as protein kinase synthases and non-ribosomal protein synthetases, potentially producing compounds like indigoidine (100% known gene cluster similarities) and micromonolactams (100% known gene cluster similarities). Indigoidine, a naturally occurring blue pigment has been isolated from several microorganisms and appears to have different bioactivities including antibacterial and antioxidant activity[52,53]. As previous study by Law et al. has indicated the antioxidant activity of MUSC 1J^T extract, the detection of indigoidine biosynthetic gene cluster further emphasizes its potential in scavenging free radicals. On top of that, one gene cluster associated with biosynthesis of desferrioxamine B (83% known gene cluster similarities) was detected in MUSC 1J^T genome. Desferrioxamine B as a siderophore is capable of chelating iron and has been used clinically to treat iron overdose. Additionally, desferrioxamine can prevent oxidative stress by preventing accumulation of free radicals[54–61]. These actions, in turn enabled, desferrioxamine to be able to reduce occurrence of cancer and neurodegenerative diseases like Parkinson's disease and Alzheimer's disease by preventing inflammation including regulating activation of microglial cells which are capable of producing pro-inflammatory cytokine.

In summary, the availability of whole genome sequence of MUSC 1J^T revealed genomic potential of mangrove forest-derived streptomycete and accentuate the importance of novel strain discovery from this underexplored area around the world. Comprehensive investigation into biosynthetic genes responsible for production of interesting bioactive compounds like indigoidine and desferrioxamine would be important to allow scale-up production

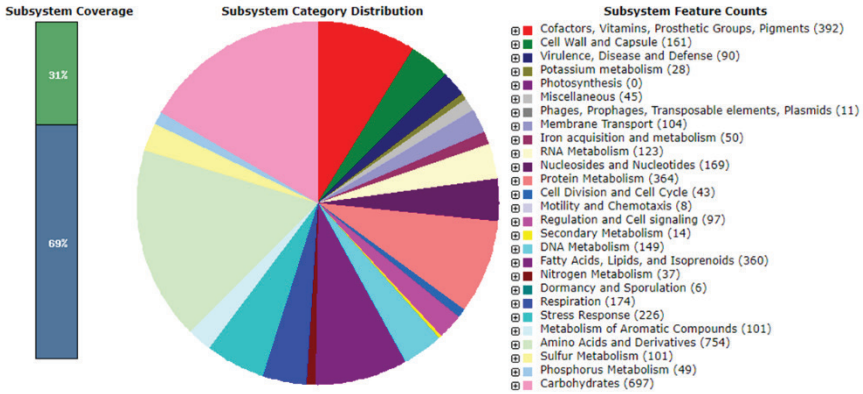

Figure 1. Subsystem category distribution of *Streptomyces monashensis* MUSC 1J^{T} (based on RAST annotation server).

of these compounds as highly valuable pharmaceutical agents.

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