Genome Report

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Complete genome of mangrove-derived anti-MRSA streptomycete, *Streptomyces pluripotens* MUSC 135^T

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Abstract : Microorganisms serve as attractive resources, owing to their ability to synthesize structurally-diverse substances with various bioactivities. Within the Bacteria domain, members of the genus *Streptomyces* have demonstrated remarkable ability to produce clinically useful, secondary metabolites such as anticancer, antioxidants, antivirals and antibacterials. *Streptomyces pluripotens* MUSC 135^T was isolated as novel strain from mangrove forest in Malaysia. This strain exhibited broad spectrum bacteriocin against several pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) strain ATCC BAA-44, *Salmonella typhi* ATCC 19430^T and *Aeromonas hydrophila* ATCC 7966^T. Thus, the strain was selected for whole genome sequencing as an attempt to explore its bioactive potential. Here we report the first complete genome of *S. pluripotens* MUSC 135^T genome which comprise of 7.35 Mbp with G+C content of 69.9 %. A total of 6,404 open reading frames (ORFs) were predicted, along with 18 rRNA and 69 tRNA genes. Using bacteriocin mining tool, BAGEL detected eights gene clusters associated with bacteriocin production including lanthipeptides and linear azol(in)e-containing peptides (LAPs). Members of *Streptomyces* have contributed greatly towards improving lives, particularly against deadly infections and chronic diseases. The availability of *S. pluripotens* MUSC 135^T genome sequence has opened new window for drug discovery, particularly for effective drugs against harmful pathogens such as MRSA and certainly deserves further detailed study.

Keywords: *Streptomyces;* MRSA; mangrove; bioinformatics; genome

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Short Introduction

Members of genus *Streptomyces* are known to produce various types of compounds with bioactivities including anticancer, antioxidant, antifungal and antibacterial properties[1-7]. The unique life cycle of streptomycetes have enhanced their ability to persist in nature and to survive in harsh environmental conditions[8-13]. *Streptomyces pluripotens* MUSC 135^T was firstly isolated as

novel strain from mangrove forest in Peninsular Malaysia during a screening program for antibiotic producers[14,15]. This strain exhibited broad spectrum bacteriocin against several pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) strain ATCC BAA-44, *Salmonella typhi* ATCC 19430^T and *Aeromonas hydrophila* ATCC 7966^T. In order to gain insights on its bioactive potential, strain MUSC 135^T was subjected to complete genome sequencing and annotation to identify presence of biosynthetic gene clusters.

Data Description

Streptomyces pluripotens MUSC 135^T was isolated from Tanjung Lumpur mangrove forest located in the city of Kuantan, State of Pahang, in December of the year 2012[14,15]. Purified cultures were maintained on ISP medium 2 slants at room temperature for short-term storage and as glycerol suspensions (20%, v/v) at −80°C for long-term storage[16]. The general features of the strain are summarized in Table 1[17]. The strain is available from culture collection centers under accession number of MCCC 1K00252^T = DSM 42140^T.

Table 1: General features of *Streptomyces pluripotens* MUSC 135^T and MIGS mandatory information.

Property	Description
Classification	Domain *Bacteria*
	Phylum *Actinobacteria*
	Class *Actinobacteria*
	Order *Actinomycetales*
	Family Streptomycetaceae
	Genus *Streptomyces*
	Species *pluripotens*
	strain: MUSC 135^T
Gram stain	Positive
Cell shape	Branched mycelia
Motility	Dispersion of spores
Sporulation	Yes
Temperature range	20–40°C
Optimum temperature	28–32°C
pH range; Optimum	5.0–9.0; 5.0–8.0
Carbon source	Varied
Oxygen requirement	Aerobic
Pathogenicity	Non-pathogenic
Geographic location	Tanjung Lumpur, Pahang, Malaysia
Latitude	3° 48’ 3.2”N
Longitude	103° 20’ 22.7”E
Altitude	0 above sea level

The genomic DNA of MUSC 135^T was extracted with Wizard® Genomic DNA Purification Kit (Promega) prior to cleanup process using AMPure® PB beads. DNA quality was examined using NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Qubit version 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA)[18]. SMRTbell DNA libraries (Pacific Biosciences) were constructed according to the PacBio standard protocol with the BluePippin size-selection system (Sage Science). Whole genome sequencing of MUSC 135^T was performed with PacBio RSII sequencing technology using P6-C4 chemistry, yielding output data with an average genome coverage of 217.22-fold (Table 2).

Table 2: Genome statistics of *Streptomyces pluripotens* MUSC 135^T

Sequencing platform	PacBio RSII
Assembly	HGAP3
Number of replicon	1
Accession number	CP021080
Genome size (bp)	7,346,075
G + C content %	69.9
Protein coding genes	6,404
tRNA	69
rRNA	6, 6, 6 (5S, 16S, 23S)

Upon sequencing, the raw reads were assembled using HGAP3. *De novo* assembly of the insert reads was performed with the Hierarchical Genome Assembly Process (HGAP) algorithm. The genome sequence of MUSC 135^T was assembled into a single GC-rich (69.9 %) contig with genome size of 7,346,075 bp. rRNA and tRNA predictions were performed using ARAGORN and RNAmmer, respectively[19,20]. Using these tools, a total of 77 tRNA genes and 18 rRNA operons was predicted in MUSC 135^T genome. Gene prediction was conducted using Prodigal (version 1.20), which 6,404 open reading frames (ORFs) were predicted in MUSC 135^T genome[21]. The whole genome project of MUSC 135^T was deposited at DDBJ/EMBL/GenBank under accession number CP021080.

The assembly was uploaded for annotation to Rapid Annotation using Subsystem Technology (RAST)[22]. From RAST annotation system, most of the genes in MUSC 135^T were involved in amino acids and derivatives metabolism, followed by carbohydrates metabolism and protein metabolism subsystems (Figure 1).

Interestingly, one of the genes belonging to virulence, disease and defense subsystem was predicted to encode for colicin E2 tolerance protein CbrC-like protein. The detection of this gene suggests ability of the strain to survive against action of DNA endonuclease, colicin E2 and persist in the environment[23,24]. In addition to that, a web-based bacteriocin mining tool, BAGEL identified eights gene clusters associated with bacteriocin production[25]. Among these gene clusters, one of them was found to be responsible for production of linear azol(in)e-containing peptides (LAPs). The members of LAPs family, such as goadsporin and plantazolicin have previously been detected from microbes have shown potent antibacterial activities against pathogens including *Bacillus anthracis* and MRSA[26-28]. In conclusion, we report the complete

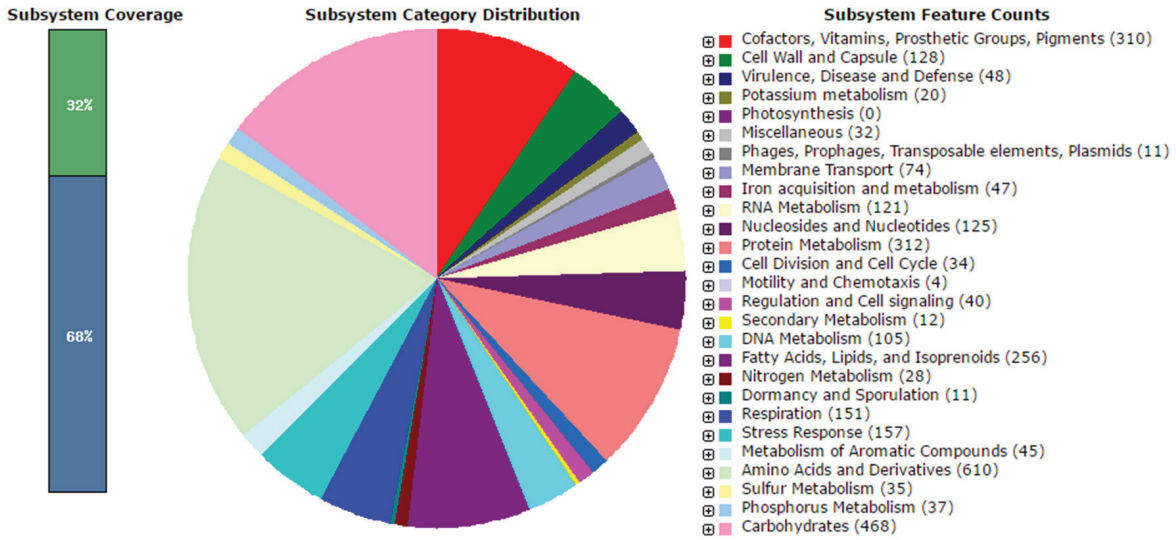

Figure 1. Subsystem category distribution of MUSC 135T based on RAST server.

sequence of *S. pluripotens* MUSC 135^{T}. The availability of its genome sequence has suggested potential exploitation of the strain for potentially useful compounds, particularly against pathogens such as MRSA and certainly deserves further detailed study.

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Conflict of Interest

The authours declared that there is no conflict of interest.

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