Genome Report

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Whole genome sequence of *Streptomyces colonosanans* strain MUSC 93J^T isolated from mangrove forest in Malaysia

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Abstract: Under the family *Actinobacteria*, streptomycetes are ubiquitous in nature, producing a wide spectrum of bioactive compounds including antibacterial, antioxidant, anticancer and immunomodulatory properties. During a screening programme in Malaysia, *Streptomyces colonosanans* MUSC 93J^T was isolated as a novel *Streptomyces* sp. from the mangrove soil in Sarawak. The strain exhibited potent antioxidant activities and cytotoxic activity against several human cancer cell lines. Due to these data, the strain was subjected to whole genome sequencing to uncover its genomic potential and further improve the understanding of the strain. The genome of MUSC 93J^T consists of 7,015,076 bp (G + C content of 69.90%), carrying a total of 5,859 protein coding genes. Analysis using a bioinformatics tool, antiSMASH predicted a total of four biosynthetic gene clusters which displayed similarity of more than 70% to known gene clusters and one of which was associated with the production of a natural protectant, ectoine. Displaying selective toxicity that kills only cancer cells, ectoine has showed its potential to be developed as therapeutic agents for humans. Altogether, the current project clearly highlights the importance of under-explored environment like mangrove in natural product discovery. The availability of whole genome sequence MUSC 93J^T warrants subsequent in-depth investigation and optimization for the production of bioactive compounds which can be exploited for the health and wellbeing of mankind.

Keywords: *Streptomyces*; anti-cancer; mangrove; genome; MUSC 93J^T; actinobacteria

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Short Introduction

Streptomycetes are filamentous bacteria that can be found in various ecosystems and most well-known for their ability to produce secondary metabolites which can be exploited for the benefits of mankind[1–7]. For instance, the isolation of streptomycin from *Streptomyces griseus* described by Professor Waksman and his team was a major breakthrough back in the 1950s, being the first effective treatment against the causative agent of the great white plague, *Mycobacterium tuberculosis*[8,9]. Even though more than 60 years have passed, drug discovery studies investigating bioactive potential of *Streptomyces* sp. from various habitat did not regress, but more efforts are now being poured into the investigation of their genomic potential[10–19]. *Streptomyces colonosanans* MUSC 93J^T was recovered from mangrove forest soil located at Sarawak, Malaysia during a screening programme for bioactive streptomycetes[10,20]. Forming light yellow aerial and vivid yellow substrate mycelium on ISP 2 agar which is a typical trait of streptomycetes, MUSC 93J^T was designated as novel species of genus *Streptomyces* which is closely related to *Streptomyces malachitofuscus* NBRC 13059^T (99.2% sequence similarity), *Strep-*

tomyces misionensis NBRC 13063^T (99.1%)*, and Streptomyces phaeoluteichromatogenes* NRRL 5799^T (99.1%) based on phylogenetic analysis using their 16S rRNA genes. Nonetheless, fermentative extracts of MUSC 93J^T displayed potent antioxidant activity and anticancer activity against several human colon cancer cell lines without significant cytotoxic effect against human normal colon cells. The type strain for MUSC 93J^T is available at two culture collection centres with accession of (= DSM 102042^T = MCCC 1K02298^T). Based on the biosystematics study using a polyphasic approach, the strain was selected for whole genome sequencing to explore its genomic potential, particularly the production of bioactive compounds that are responsible for its anticancer and antioxidant activities[10,21,22].

Data description

Genomic DNA of MUSC 93J^T was obtained using Masterpure™ DNA purification kit (Epicentre, Illumina Inc., Madison, WI, USA) and subjected to RNase (Qiagen, USA) treatment[23–25]. Following that, DNA quality check was conducted with NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Qubit version 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). Construction of DNA library was done using Nextera™ DNA Sample Preparation kit (Nextera, USA) and the library quality was checked by Bioanalyzer 2100 high sensitivity DNA kit (Agilent Technologies, Palo Alto, CA). Paired-end sequencing was performed on MiSeq platform with MiSeq Reagent Kit 2 (2 × 250 bp; Illumina Inc., Madison, WI, USA)[26,27]. After trimming, the paired-end reads were *de novo* assembled on CLC Genomics Workbench version 7 (CLC bio, Denmark), which resulted in 166 contigs and an N_{50} contig size of approximately 99,963 bp. The genome size of MUSC 93J^T comprised 7,015,076 bp, with an average coverage of 53.0-fold and G + C content of 69.90 %. The genome sequence of MUSC 93J^T has been deposited at DDBJ/EMBL/GenBank under accession of MLYP00000000.

Table 1. General genomic features of *Streptomyces colonasanans* MUSC 93J^T.

	Streptomyces colonasanans MUSC 93J^T
Genome size (bp)	7,015,076
Contigs	166
Contigs N_{50} (bp)	99,963
G + C content %	69.90
Genome coverage	53.0x
Protein coding genes	5,859
tRNA	66
rRNA (5S, 16S, 23S)	3, 1, 1

The assembled genome was annotated using Rapid Annotation using Subsystem Technology (RAST)[28]. Gene prediction was performed using Prodigal version 2.6, while ribosomal RNA (rRNA) and transfer RNA (tRNA) were predicted using RNAmmer and tRNAscan SE version 1.21, respectively[29–31]. The analysis from RAST revealed 5,859 protein-coding genes, along with a total 71 RNA genes (Figure 1). Based on RAST system, most of the protein-coding genes were shown to be involved in amino acids and derivatives metabolism (9.18%), followed by carbohydrates metabolism (6.21%) and protein metabolism subsystems (4.91%). Further analysis on antibiotics & Secondary Metabolite Analysis SHell (antiSMASH) detected presence of 23 biosynthetic gene clusters in MUSC 93J^T genome using "strict" detection settings (version 5.1.1)[32,33]. Among the four biosynthetic gene clusters which displayed similarity of more than 70% to known gene clusters, one cluster was associated with the production of ectoine (75 % gene similarities). Ectoine is commonly expressed by bacteria to survive in harsh environments, protecting these microorganisms against extreme osmotic stress[34–38]. As a compatible solute, ectoine has been shown to be safe as it does not interfere with the host's metabolism while offering some beneficial effects including antioxidant and protection against ionizing radiation[39–42]. Apart from that, a recent study by Sheikhpour *et al*. (2019) showed that ectoine induced apoptosis in lung cancer cells without affecting normal cells. As a natural protectant, ectoine seems to be a promising protective agent to be developed for human use, particularly against chronic inflammatory diseases and cancer[43,44]. On top of that, there has been many studies reported ectoine-based spray or lozenges showed superior efficacy in treating acute pharyngitis and/or laryngitis, proposing its potential use as adjuvant treatment for anti-inflammatory or anti-infective drugs[45,46]. The detection of this biosynthesis gene cluster within the genome of MUSC 93J^T reflects the bioactive potential of mangrove-derived actinobacteria (including rare actinomycetes and streptomycetes and further highlighting the possible development of this strain as "mini-factories" for the production of protective molecule like ectoine[47–49]. With the emerging role of probiotics in regulating human diseases caused by gut dysbiosis (i.e. imbalance in gut microbial population), ectoine as a osmoprotectant could potentially increase the viability of probiotics in food and prolong its shelf life[50–60]. With the availability of the whole genome sequence of MUSC 93J^T, these data would greatly accelerate the medium optimization process and allow genomic manipulations to maximize the production of bioactive compounds including ectoine.

Conflict of interest

The authors declare that there is no conflict of interest in this work.

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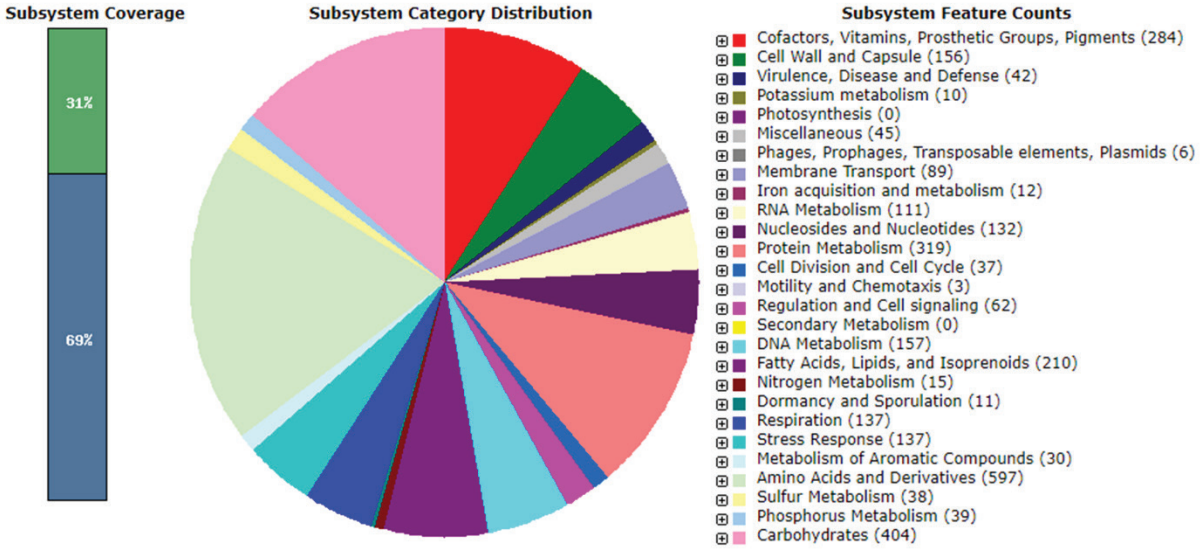

Figure 1. Subsystem category distribution of *Streptomyces colonosanans* MUSC 93J^{T} (based on RAST annotation server).

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