

Genome Report

Whole-genome sequence of bioactive streptomycete derived from mangrove forest in Malaysia, *Streptomyces* sp. MUSC 14

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Abstract: The contribution of streptomycetes to human health is undeniably important and significant, given that these filamentous microbes can produce interesting compounds that can be used to cure deadly infections and even cancer. Isolated from the east coast of Peninsular Malaysia, *Streptomyces* sp. MUSC 14 has shown significant antioxidant capacity. The current study explores the genomic potential of MUSC 14 via a genome mining approach. The genome size of MUSC 14 is 10,274,825 bp with G + C content of 71.3 %. AntiSMASH analysis revealed a total of nine biosynthetic gene clusters (with more than 80 % similarities to known gene clusters). This information serves as an important foundation for subsequent studies, particularly the purification and isolation of bioactive compounds by genetic manipulation techniques.

Keywords: *Streptomyces*, antioxidant, mangrove, genome, MUSC 14, actinobacteria

1. Short Introduction

Gifted with the ability to form spores, streptomycetes are ubiquitous in nature and produce a diverse array of secondary metabolites that can be exploited for the benefit of humanity^[1-16]. One of the significant breakthroughs in streptomycetes research is the discovery of streptomycin (from the soil bacterium, *Streptomyces griseus*) by Professor

Waksman and his team – which subsequently led him to the Nobel Award in Medicine later in 1960^[13, 17-21]. As a matter of fact, the continuous search for pharmaceutically important compounds led to another (one half jointly) Nobel Prize in Physiology or Medicine in 2015 awarded to Professor William C. Campbell and Professor Satoshi Ōmura for the discovery of avermectin, which was isolated from a soil streptomycete, *Streptomyces avermitilis*^[22-27]. The search for bioactive compounds from this prolific genus seems like a never-ending journey, with researchers now hunt for them in unique environments such as hot springs, volcanic soils, deserts, deep-sea, and the mangrove forest^[28-42]. The hypothesis behind searching these special habitats is that microbes may come up with adaptation strategies to ensure their survival in the harsh condition like drastic temperature changes, salinity, and oxygen availability, which in turn may lead to the production of interesting compounds and drive the emergence of a novel bacterium^[43-50]. *Streptomyces* sp. MUSC 14 was isolated from the mangrove forest on the west coast of Peninsular Malaysia during a screening program for bioactive actinobacteria^[4, 51, 52]. A thorough investigation conducted on the strain has reflected the antioxidant potential of MUSC 14, attributed to its production of bioactive secondary metabolites, including pyrrolopyrazines and fatty acid esters^[51, 53-55]. The current study aims to obtain the genome sequence of *Streptomyces* sp. MUSC 14 before exploring the genomic potential of this strain.

2. Data description

The whole genomic DNA extraction of MUSC 14 was carried out using Masterpure™ DNA purification kit (Epicentre, Illumina Inc., Madison, WI, USA), preceding RNase treatment (Qiagen, USA)^[56-58]. Subsequently, the DNA library was constructed with Nextera™ DNA Sample Preparation kit (Nextera, USA). The library quality was evaluated by Bioanalyzer 2100 high sensitivity DNA kit (Agilent Technologies, Palo Alto, CA)^[59, 60]. Paired-end sequencing was performed on the Illumina MiSeq platform with MiSeq Reagent Kit 2 (2 × 250 bp; Illumina Inc., Madison, WI, USA)^[16, 61, 62]. Post-trimming step, *de novo* assembly of the paired-end reads on CLC Genomics Workbench version 7 (CLC bio, Denmark) resulted in 174 contigs and an N₅₀ contig size of 162,032 bp. The genome size of MUSC 14 is 10,274,825 bp, with an average coverage of 211.0-fold and G + C content of 71.3% (**Table 1**). The genome sequence of MUSC 14 has been deposited at DDBJ/EMBL/GenBank under accession of MLYN00000000. The version described in this paper is the first version.

Following genome assembly, MUSC 14 genome was annotated on Rapid Annotation using Subsystem Technology (RAST) and NCBI Prokaryotic Genome Annotation Pipeline (PGAP)^[63-66]. Gene prediction was performed using Prodigal (version 2.6)^[67], while ribosomal RNA (rRNA) and transfer RNA (tRNA) were predicted using RNAmmer^[68] and tRNAscan SE version 1.21^[69], respectively. The whole genome of MUSC 14 consists of 8,799 protein-coding genes and 81 RNA genes (tRNA: 74, rRNA:4).

Table 1. General genomic features of *Streptomyces* sp. MUSC 14.

| Properties | <i>Streptomyces</i> sp. MUSC 14 |
|------------------------------|---------------------------------|
| Genome size (bp) | 10,274,825 |
| Contigs | 174 |
| Contigs N ₅₀ (bp) | 162,032 |
| G + C content % | 71.3 |

| | |
|----------------------|-------------|
| Genome coverage | 211.0 |
| Protein coding genes | 8,799 |
| tRNA | 74 |
| rRNA (5S, 16S, 23S) | 4 (2, 1, 1) |

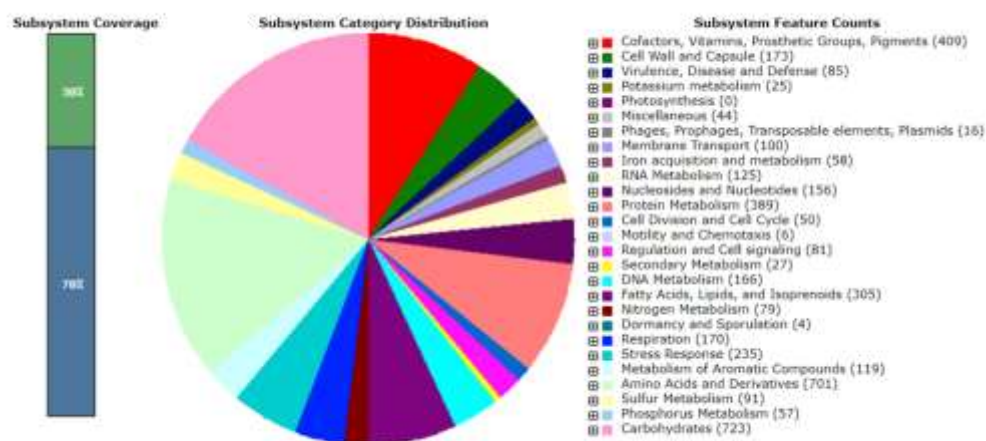


Figure 1. Annotation of MUSC 14^T genome using Rapid Annotation using Subsystem Technology (RAST).

The annotation on RAST showed that most of the protein-coding genes were involved in metabolic processes, with the highest number of genes involved in carbohydrates metabolism (7.60%) (**Figure 1**). Subsequent analysis to investigate the genomic potential of MUSC 14 on antibiotics & Secondary Metabolite Analysis SHell (antiSMASH)^[70-74] revealed a total of nine biosynthetic gene clusters displaying more than 80% similarities to known biosynthetic gene clusters. Besides detecting the gene cluster responsible for the production of the earthy odorant geosmin (100% gene similarities), there is one biosynthetic gene cluster related to nonribosomal peptides production and reflects 100% gene similarities with thioholgamide A/thioholgamide B biosynthesis. Thioholgamide A and B were initially isolated from a mangrove-derived streptomycete, *Streptomyces malaysiense* MUSC 136^T, and possess cytotoxic activities against cancer cell lines^[75, 76]. Recently, a research group in Germany has published an article studying the mechanisms involved behind the cytotoxic and anti-proliferative activities of thioholgamide A^[75, 77]. The team showed that thioholgamide A induces apoptosis via caspase 3 and PARP cleavage (at concentrations comparable to staurosporine)^[77]. Furthermore, it appears that thioholgamide A inhibits oxidative phosphorylation in tumor cells without displaying much toxicity towards non-tumorigenic cells and zebrafish embryos, thus making the compound an appealing candidate that to be developed as anti-cancer therapeutics. Harnessing the genomic potential of microbes, including *Streptomyces* sp. is indeed one way forward in drug discovery^[78-80]. Combined with the conventional methods, including optimization of fermentation media and improvement in extraction processes, the activation of biosynthetic gene clusters via heterologous expression or introduction of promoters in the native host can potentially yield bioactive compound(s) of interest in high purity and quantity, allowing its large-scale production for pharmaceutical use^[81-85].

Author Contributions: HLS, LTHT, and WST carried out the experiments and analyzed the data. WFY and KGC provided vital guidance, technical support, and proofreading for the work. All authors approved the final draft.

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