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Genome report

Whole genome sequence of *Peribacillus* sp. MUM 13 derived from mangrove forest in Malaysia

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Abstract: Acting like mini-factories, microorganisms are a valuable source of natural bioactive compounds of unique chemical structures. *Peribacillus* sp. MUM 13 was recovered from the mangrove forest in Malaysia during a screening program for bioactive microbes. Whole genome analysis revealed that the genome size of MUM 13 as 4,649,225 bp (with G + C content of 40.8 %). Bioinformatic analysis predicted the presence of lassopeptide biosynthetic gene clusters within the genome of MUM 13, which indicates the bioactive potential of the strain and calls for further experiments to explore the strain characteristics, particularly in combatting against pathogenic microbes.

Keywords: *Peribacillus*, genome, bioinformatics, bioactive, biosystematics

1. Short Introduction

Microorganisms continue to strive as a vital source of pharmaceutically essential compounds, and researchers are still heavily exploring different biomes to seek potent molecules that can combat deadly infections and chronic diseases in humans and livestock[1–7]. Among the Bacteria kingdom, those under the *Actinobacteria* are known as prolific producers and valuable enzymes, particularly *Streptomyces* species[8–12]. Despite that, another group of Gram-positive bacteria, *Bacillus* sp. has also significant contribution in producing industrially important enzymes, including protease, amylase, and uricase[13–16].

Outtrup and Jørgensen summarized that almost all protease used in detergents were derived from *Bacillus* sp. and accounted for more than one-quarter of total enzyme sales of the industrial enzyme supplier, Novo Nordisk A/S[16,17]. Majority of the protease in high pH detergents are produced by the alkaliphilic *Bacillus clausii*, before undergoing further modification to increase washing performance under specific conditions (e.g., low temperature and water hardness). Nonetheless, this species has demonstrated its potential beyond industrial applications, particularly in the medical field[18–22]. Due to the ability to produce spores, *B. clausii* can survive through the stomach's acidic environment, allowing it to colonize the intestine, even in the presence of antibiotics[23]. As a result, several studies have suggested the potential use of *B. clausii* as a probiotic in humans against acute diarrhoea, recurrent respiratory infections, or even allergies[19,24–26]. During a screening program for bioactive microorganisms, MUM 13 was recovered from a mangrove forest in Malaysia. The 16S rRNA analysis showed that the strain displayed 98.44% similarities with *Peribacillus muralis*[27–29]. The strain was subjected to whole-genome sequencing to further explore its bioactive potential and specific phenotypic characteristics (e.g., antibiotic resistance).

2. Data Description

During a screening program for bioactive microbes, MUM 13 was initially isolated from a mangrove forest located at Kuala Selangor, Malaysia[30–38]. The whole genomic DNA of MUM 13 was extracted using Masterpure™ DNA purification kit (Epicentre, Illumina Inc., Madison, WI, USA) before RNase treatment[39–41]. The construction of the DNA library was done using Nextera™ DNA Sample Preparation kit (Nextera, USA), while its quality was accessed with Bioanalyzer 2100 high sensitivity DNA kit (Agilent Technologies, Palo Alto, CA)[42–44]. Whole-genome sequencing (paired-end) was done on the Illumina MiSeq platform with MiSeq Reagent Kit 2 (2 × 250 bp; Illumina Inc., Madison, WI, USA)[44–47]. Trimmed sequences were subjected to de novo assembly on CLC Genomics Workbench version 7, which resulted in 186 contigs and an N_{50} contig size of 51,696 bp. The genome size of MUM 13 is 4,649,225 bp, with an average coverage of 59.0-fold and G + C content of 40.8 % (Table 1). The genome sequence of MUM 13 has been deposited at DDBJ/EMBL/GenBank under accession of MLYQ00000000. The version described in this paper is the first version.

Gene prediction was performed using Prodigal (version 2.6)[48], while ribosomal RNA (rRNA) and transfer RNA (tRNA) were predicted using RNAmmer[49] and tRNAscan SE version 1.21[50], respectively. Based on the annotation on Rapid Annotation using Subsystem Technology (RAST) and NCBI Prokaryotic Genome Annotation Pipeline (PGAP)[51–53], MUM 13 genome contains 4,336 protein-coding genes, with most genes involved in amino acids and derivatives processes (9.05 %) (Figure 1). A total of 77 tRNA genes and 10 rRNA genes were detected in MUM 13 genome. The 16S rRNA gene sequences obtained from paired-end Sanger sequencing match the sequence obtained from WGS, leading to the identification of a closely related strain as *P. muralis* with gene similarity recorded as 98.44 %[28,54].

Table 1. Genome properties of *Peribacillus* sp. MUM 13.

Properties	***Peribacillus* sp. MUM 13**
Genome size (bp)	4,649,225
Contigs	186
Contigs N50 (bp)	51,696
G + C content %	40.8
Genome coverage	59.0x
Protein-coding genes	4,336
tRNA	77
rRNA (5S, 16S, 23S)	10 (7, 1, 2)

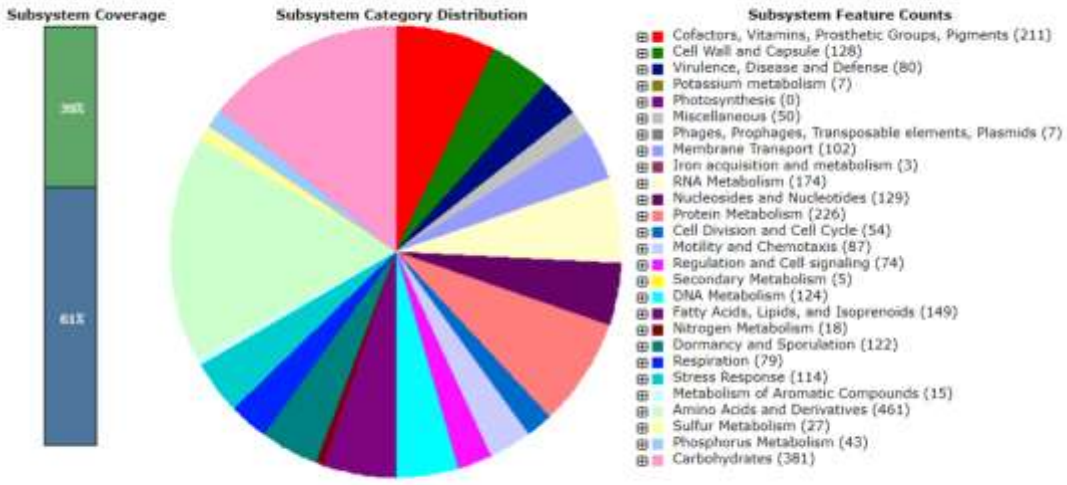

Figure 1. Subsystem classifications for *Peribacillus* sp. MUM 13 based on RAST annotation.

The identification of biosynthetic gene clusters was studied on antibiotics & Secondary Metabolite Analysis Shell (antiSMASH) on strict settings[55–58]. One gene cluster reflected more than 80 % similarities to known gene clusters responsible for the biosynthesis of lassopeptide, paeninodin. The antimicrobial lassopeptide paeninodin was initially described by Zhu and the team via genomic analysis of *Paenibacillus dendritiformis C454*[59]. Even though compounds belonging to the lassopeptide class has been described since 1991, increasing attention is drawn onto this class of compounds as revealed to be useful therapeutics given that these compounds generally have excellent stability against chemical, thermal and proteolytic degradation[60–62]. In 2019, Zyubko and the team have successfully obtained lassopeptide pseudomycoidin encoded by *Bacillus pseudomycoides* DSM 12442 through *in vivo* synthesis method[63,64]. At the time of writing, even though the bioactivity potential of pseudomycoidin remains unknown, the study highlights that lassopeptide,

including pseudomycoidin could serve as a lead compound to develop highly potent bioactive compounds. The antimicrobial resistance (AMR) genes of MUM 13 was predicted using the Resistance Gene Identifier (RGI) from the Comprehensive Antibiotic Resistance Database (CARD)[65–71]. Using strict option as RGI screening criteria, only one AMR gene was identified belonging to ampC-type beta-lactamase gene family, and implied that MUM 13 potentially display resistance towards cephalosporins and penicillins. Nonetheless, the detection of the biosynthetic gene cluster responsible for the production of lassopeptide in the genome of MUM 13 further warrants subsequent analysis, particularly to investigate the antimicrobial activities and possibilities for the strain to be utilized as a probiotic in agriculture[72].

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