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Whole genome sequence of MUM116, a *Bacillus* species isolated from intertidal soil

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Abstract: Over the past few years, mangrove-derived *Bacillus* sp. have been characterized frequently for their bioactive potential. *Bacillus* sp. MUM 116 was isolated from mangrove forest in Kuala Selangor which is located on the west coast of Peninsular Malaysia. In order to obtain better understanding of the strain, the genome sequence of MUM 116 was acquired through Illumina MiSeq sequencing platform and yielded 5,720,395 bp along with 165 tRNA and 25 rRNA genes. Based on antiSMASH and RAST annotation, there was one cluster associated with production of bacteriocin. A deeper analysis into the genome sequence of MUM 116 would be essential to exploit the strain for production of bioactive compounds, which could potentially be developed as potent antibacterial agent.

Keywords: Bacillus; antibiotics; mangrove; secondary metabolite; genome

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Short Introduction

As a unique ecosystem, the mangrove forest are habitat for many plants as well as microbial populations that highly capable of adapting to fluctuations in temperatures, organic matter content, salinities and oxygen conditions^[1,2]. Owing to these factors, some strains came up with adaptation strategies to survive and persist in the environment; one of which is by modifying metabolic pathway by scavenging nutrients available in the environment before converting them into useful, bioactive compounds that improve their survivability (i.e. antibacterials and antifungal)^[3–5]. With reference to mangrove forest, Asia represents an ideal "hunting zone" for bioactive microbial strains as this continent has got the largest coverage of mangrove forests, contributing 42 % of the global total^[6,7].

Several studies have shown that *Bacillus* sp. derived from mangrove forest have great potential in producing bioac-

tive compounds^[8-13]. *Bacillus* sp. MUM 116 was isolated from the west coast of Peninsular Malaysia during a screening program for bioactive microbes^[14-19]. 16S rRNA analysis showed that MUM116 showed high similarities (<90%) to some bioactive type strains including *Bacillus ginsengisoli*, *Bacillus niacini* and *Bacillus mesonae*^[20]. Given that mangrove-derived *Bacillus* sp. have been demonstrated to possess potential bioactive potential and MUM 116 displayed high 16S rRNA gene similarities with bioactive type strains, the strain was subjected to genome sequencing to uncover its genomic potential.

Data description

The genomic DNA of MUM 116 was extracted using Masterpure[™] DNA purification kit (Epicentre, Illumina Inc., Madison, WI, USA) before subjected to RNase

(Qiagen, USA) treatment^[21,22]. Genomic DNA quality was evaluated using NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Oubit version 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA)^[23,24]. Nextera[™] DNA Sample Preparation kit (Nextera, USA) was used to generate DNA library and its quality was examined with Bioanalyzer 2100 high sensitivity DNA kit (Agilent Technologies, Palo Alto, CA) prior to sequencing^[25,26]. Whole genome sequence of MUM 116 was obtained via paired-end sequencing on Illumina MiSeq platform with MiSeq Reagent Kit 2 (2 \times 250 bp; Illumina Inc., Madison, WI, USA)[27]. The assembly of trimmed sequence was done with CLC Genomic Workbench version 5.1 (CLC Bio, Denmark), resulting in 208 contigs and an N_{50} contig size of approximately 52,003 bp. The assembled genome size of MUM 116 consists 5,720,395 bp, with an average coverage of 74.0fold and G + C content of 38.4%. The genome sequence of Bacillus sp. MUM 116 has been deposited at DDBJ/ EMBL/GenBank under accession of MLYR00000000.

Table 1. General genomic features of Bacillus sp. strain MUM 116.

	Bacillus sp. MUM116
Genome size (bp)	5,720,395
Contigs	208
Contigs N ₅₀ (bp)	52,003
G + C content %	38.4
Protein coding genes	5,273
tRNA	165
rRNA	25

Annotation of MUM 116 genome was carried out using Rapid Annotation using Subsystem Technology (RAST) ^[28] while gene prediction was performed using Prodigal version 2.6. The detection of ribosomal RNA (rRNA) and transfer RNA (tRNA) was done using RNAmmer and tRNAscan SE version 1.21, respectively^[29–31]. Based on RAST analysis, more than one-quarter of the proteincoding genes were associated with primary metabolism and highest number of genes were related with metabolism of amino acid and derivatives (12%). Furthermore, both RAST and another bioinformatics tools, antibiotics & Secondary Metabolite Analysis SHell (antiSMASH) revealed potential of MUM 116 in producing bacteriocin under the thiazole/oxazole-modified microcins (TOM-Ms) class^[32,33]. Several *Bacillus* sp. have been described to have the potential of synthesizing TOMMs^[34,35]. For instance, Bacillus amvloliquefaciens FZB42 isolated from plant-pathogen-infested soil was capable of compounds producing not just plant-promoting activity, the strain produced a novel TOMMs - plantazolicin which can suppress growth of bacterial and fungal plant pathogens^[35]. Even though Bacillus sp. isolated from terrestrial region showed great potential in producing bioactive compounds, several studies have hinted that genomes of Bacillus sp. from special environment like mangrove area are generally more "enriched" than those from terrestrial area, as the dynamic environment imposes selective pressure on genomic region associated with adaptation which then promotes production of unique secondary metabolites^[36,37]. Altogether, the availability of MUM 116 genome sequences enabled further investigation into its genomic potential, particularly for the production of bacteriocin(s). In future work, more experimental testing is required to optimize production medium and culture conditions for Bacillus sp. before exhaustively examine all potential antimicrobials.

Conflict of Interest

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

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Reference

- Kathiresan K, Bingham BL. Biology of mangroves and mangrove ecosystems. Adv Mar Biol 2001; 40: 81–251.
- Luther DA, Greenberg R. Mangroves: A global perspective on the evolution and conservation of their terrestrial vertebrates. BioSci 2009; 59(7): 602– 612.



Figure 1. Subsystem category distribution of Bacillus sp. MUM 116 (based on RAST annotation server).

- Ball M. Mangrove species richness in relation to salinity and waterlogging: a case study along the Adelaide River floodplain, northern Australia. Global Ecol Biogeo Lett 1998; 7(1): 73–82.
- Liu S, Ren H, Shen L, et al. pH levels drive bacterial community structure in sediments of the Qiantang River as determined by 454 pyrosequencing. Front Microbiol 2015; 6: 285.
- Azman AS, Othman I, Fang CM, et al. Antibacterial, anticancer and neuroprotective activities of rare *Actinobacteria* from mangrove forest soils. Ind J Microbiol 2017; 57(2): 177–187.
- 6. ITTO. Mangrove ecosystem. Trop Forest Update 2014; 21: 3–15.
- Giri C, Ochieng E, Tieszen LL, *et al.* Status and distribution of mangrove forests of the world using earth observation satellite data. Global Ecol Biogeography 2011; 20(1): 154–159.
- Hu HQ, Li XS, He H. Characterization of an antimicrobial material from a newly isolated *Bacillus amyloliquefaciens* from mangrove for biocontrol of Capsicum bacterial wilt. Biol Control 2010; 54(3): 359–365.
- Geetha I, Manonmani AM, Prabakaran G. Bacillus amyloliquefaciens: a mosquitocidal bacterium from mangrove forests of Andaman & Nicobar islands, India. Acta Tropica 2011; 120(3): 155–159.
- Ramasubburayan R, Sumathi S, Bercy DM, et al. Antimicrobial, antioxidant and anticancer activities of mangrove associated bacterium Bacillus subtilis subsp. subtilis RG. Biocat Agri Biotech 2015; 4(2): 158–165.
- Tabao NS, Monsalud RG. Screening and optimization of cellulase production of *Bacillus* strains isolated from Philippine mangroves. Phil J Syst Biol 2010; 4: 79–87.
- Rajesh P, Athiappan M, Paul R, et al. Bioremediation of cadmium by Bacillus safensis (JX126862), a marine bacterium isolated from mangrove sediments. Int J Curr Microbiol Appl Sci 2014; 3(12): 326–335.
- Mondol MA, Shin HJ, Islam MT. Diversity of secondary metabolites from marine *Bacillus* species: chemistry and biological activity. Mar Drugs 2013; 11(8): 2846–2872.
- Lee LH, Zainal N, Azman AS, et al. Diversity and antimicrobial activities of actinobacteria isolated from tropical mangrove sediments in Malaysia. Sci World J 2014; 2014.
- Tan LT, Chan KG, Pusparajah P, et al. Mangrove derived Streptomyces sp. MUM265 as a potential source of antioxidant and anticolon-cancer agents. BMC Microbiol 2019; 19(1): 38.
- Tan LT, Ser HL, Yin WF, et al. Investigation of antioxidative and anticancer potentials of *Streptomyces* sp. MUM256 isolated from Malaysia mangrove soil. Front Microbiol 2015; 6: 1316.
- Kemung HM, Tan LT, Chan KG, et al. Investigating the antioxidant potential of *Streptomyces* sp. MUSC 11 from mangrove soil in Malaysia. Prog Drug Dis Biomed Sci 2019; 2(1).
- Law JW, Chan KG, He YW, *et al.* Diversity of *Streptomyces* spp. from mangrove forest of Sarawak (Malaysia) and screening of their antioxidant and cytotoxic activities. Sci Rep 2019; 9(1): 1–5.
- Ser HL, Yin WF, Chan KG, et al. Antioxidant and cytotoxic potentials of *Streptomyces gilvigriseus* MUSC 26^T isolated from mangrove soil in Malaysia. Prog Microbes Mol Biol 2018;1(1).
- Harvey ZH, Snider MJ. Draft genome sequence of the nicotinate-metabolizing soil bacterium *Bacillus niacini* DSM 2923. Genome Announc 2014; 2(6): e01251–14.

- Ser HL, Tan WS, Ab Mutalib NS, et al. Genome sequence of Streptomyces pluripotens MUSC 135⁺ exhibiting antibacterial and antioxidant activity. Mar Gen 2015; 24:281–283.
- Letchumanan V, Ser HL, Tan WS, et al. Genome sequence of Vibrio parahaemolyticus VP152 strain isolated from Penaeus indicus in Malaysia. Front Microbiol 2016; 7: 1410.
- Ser HL, Law JWF, Tan WS, et al. Genome sequence of bioactive streptomycete isolated from mangrove forest in East Malaysia, *Streptomyces monashensis* MUSC 1st. Prog Drug Dis Biomed Sci 2019, 2(1): a0000045.
- Letchumanan V, Ser HL, Chan KG, et al. Genome sequence of Vibrio parahaemolyticus VP103 strain isolated from shrimp in Malaysia. Front Microbiol 2016; 7: 1496.
- Ser HL, Tan WS, Cheng HJ, et al. Draft genome of amylolytic actinobacterium, *Sinomonas humi* MUSC 117⁺ isolated from intertidal soil. Mar Gen 2015; 24: 209–210.
- Letchumanan L, Ser HL, Tan WS, et al. Genome sequence of Vibrio sp. SALL 6 isolated from shellfish. Prog Microbes Mol Biol 2019; 2(1): a0000044.
- Ser HL, Tan WS, Ab Mutalib NS, *et al.* Draft genome sequence of mangrove-derived *Streptomyces* sp. MUSC 125 with antioxidant potential. Front Microbiol 2016; 7: 1470.
- Aziz RK, Bartels D, Best AA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008, 9:75.
- Lowe TM, Eddy SR. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nuc Acids Res 1997; 25: 955–964.
- Lagesen K, Hallin P, Rodland EA, et al. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nuc Acids Res 2007; 35: 3100–3108.
- Hyatt D, Chen GL, Locascio PF, et al. Prodigal: Prokaryotic gene recognition and translation initiation site identification. BMC Bioinform 2010; 11: 119.
- Blin K, Medema MH, Kazempour D, et al. antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res 2013; 41(W1): W204–212.
- Blin K, Wolf T, Chevrette MG, et al. antiSMASH 4.0 improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res 2017; 45(W1): W36–41.
- Idriss EE, Makarewicz O, Farouk A, et al. Extracellular phytase activity of Bacillus amyloliquefaciens FZB45 contributes to its plantgrowth-promoting effect. Microbiol 2002; 148(7): 2097–2109.
- Scholz R, Molohon KJ, Nachtigall J, et al. Plantazolicin, a novel microcin B17/streptolysin S-like natural product from Bacillus amyloliquefaciens FZB42. J Bacteriol 2011; 193(1): 215–224.
- Othoum G, Bougouffa S, Razali R, *et al.* In silico exploration of Red Sea *Bacillus* genomes for natural product biosynthetic gene clusters. BMC Gen 2018; 19(1): 382.
- Ser HL, Ab Mutalib NS, Yin WF, et al. Genome sequence of Streptomyces antioxidans MUSC 164^T isolated from mangrove forest. Prog Microbes Mol Biol 2018; 1(1).