Draft genome sequence of root-associated sugarcane growth promoting *Microbispora* sp. GKU 823

Worarat Kruasuwan,[a,b] Paul A. Hoskisson,[b] Arinthip Thamchaipenet[a,c*]

[a] Department of Genetics, Faculty of Science, Kasetsart University, Chatuchak, Bangkok, Thailand

[b] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom

[c] Center for Advanced Studies in Tropical Natural Resources, NRU–KU, Kasetsart University, Chatuchak, Bangkok, Thailand

[*]Correspondence authors:

Dr. Arinthip Thamchaipenet

Department of Genetics, Faculty of Science, Kasetsart University, 50 Ngamwongwan Road, Chatuchak, Bangkok 10900, Thailand.

E-mail: arinthip.t@ku.ac.th

Abstract

The endophytic plant growth promoting *Microbispora* sp. GKU 823 was isolated from the roots of sugarcane cultivated in Thailand. It has an estimated 9.4 Mbp genome and a G+C content of 71.3%. The genome sequence reveals several genes associated with plant growth-promoting traits and extensive secondary metabolite biosyntheses.

Endophytic actinomycetes are free-living filamentous bacteria that mutually colonize inside plants and faciliate plant growth through direct and indirect mechanisms employing plant growth-promoting (PGP) traits (1). Endophytic *Microbispora* sp. GKU 823 was isolated from roots of sugarcane cultivated in Thailand and has been shown to enhance sugarcane growth (2). This strain exhibits PGP traits including production of indole-3-acetic acid (IAA) and siderophores, solubilization of phosphate, and suppression of *Aspergillus niger* ATCC 6275 (2). Based on 16S rRNA gene sequence analysis, *Microbispora* sp. GKU 823 closely related to *Microbispora hainanensis* 211020^{T} (99.09% similarity, GenBank accession no. KR560040). The whole genome sequence of *Microbispora* sp. GKU 823 was obtained in which displays genes associated with plant growth promotion and secondary metabolite biosynthesis.

Total genomic DNA of *Microbispora* sp. GKU 823 was extracted using ISOLATE II genomic DNA extraction kit (BIOLINE, UK) according to manufacturer's instruction. The genome was sequenced using the platform Ion PGM system generating 1,230,781 reads (with approximately 30× coverage) with an average read length of 225 bp. The genome was assembled using SPAdes version 3.9 (3) and evaluated by QUAST version 3.2 (4), the reads were assembled into 262 contigs (coverage ≥10 and length ≥1000 bp) with an N_{50} of 69,483. The largest contig obtained is 321,219 bp in length. The draft genome is estimated to be 9,430,099 bp with a G+C content of 71.3%.

Functional gene annotation of the assembled genome was carried out by the Rapid Annotations using Subsystems Technology (RAST) server (5). rRNA and tRNA genes were determined by RNAmmer (6) and tRNAscan-SE (7), respectively. The annotation predicted a total 9,248 coding sequences, 58 tRNA and 3 rRNA genes. The average nucleotide identity values of the genome were calculated using BlastN (ANIb) in JSpeciesWS (8). The genome comparison revealed that *Microbispora* sp. GKU 823 had an ANIb value of 92.47% similar to *Microbispora rosea* NRRLB-2630, 90.70% to *M. rosea* NRRL B-2631 and 81.20% *Microbispora* sp. ATCC PTA-5024.

Genes related to PGP traits including phosphate solubilization (alkaline phosphatase, and isocitrate dehydrogenase; 9, 10); IAA production (tryptophan 2-monooxygenase; 11) and genes involved in fungal cell wall degradation (family 18 chitinase; 12) were detected in the genome of *Microbispora* sp. GKU 823. Moreover, genes involved in stress tolerance (betaine aldehyde dehydrogenase, proline dehydrogenase, superoxide dismutase and trehalose synthase; 13) were also present. These genes sustain the capability of *Microbispora* sp. GKU 823 to promote growth of sugarcane (2). AntiSMASH version 3.0 (14) predicted 23 seondary metabolite gene clusters in the genome of *Microbispora* sp. GKU 823 including seven gene clusters of nonribosomal peptide synthetase (NRPS), four gene clusters of Type I polyketide synthase (T1PKS) and terpene, three gene clusters of bacteriocin, two gene clusters of siderophore (including desferrioxamine E), and a single gene cluster encoding a lanthipeptide. These secondary metabolite gene clusters indicate that endophytic *Microbispora* species are potential sources of novel specialized metabolites.

Nucleotide sequence accession numbers

The draft genome sequence of *Microbispora* sp. GKU 823 has been deposited in the DDBJ/ENA/GenBank databases under the accession number MWJN00000000.

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References

1. **Glick BR.** 2012. Plant-growth promoting bacteria: machanisms and applications. Scientifica (Cairo) **2012:** 963401.

2. **Kruasuwan W, Thamchaipenet A.** 2016. Diversity of culturable plant growth-promoting bacterial endophytes associated with sugarcane roots and their effect of growth by co-inoculation of diazotrophs and actinomycetes. J Plant Growth Regul **35:**1074–1087.

3. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA.** 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comp Biol **19:** 455–477.

4. **Gurevich A, Saveliev V, Vyahhi N, Tesler G.** 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics **29:**1072–1075.

5. **Aziz R, Bartels D, Best A, DeJongh M, Disz T, Edwards R, Formsma K, Gerdes S, Glass E, Kubal M, Meyer F, Olsen G, Olson R, Osterman A, Overbeek R, McNeil**

L, Paarmann D, Paczian T, Parrello B, Pusch G, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics **9:**75.

6. **Lagesen K, Hallin P, Rødland EA, Stærfeldt H-H, Rognes T, Ussery DW.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res **35:**3100–3108.

7. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res **25:**955–964.

8. **Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J.** 2015. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics **32:**929–931.

9. **Sola-Landa A, Rodríguez-García A, Franco-Domínguez E, Martín JF.** 2005. Binding of PhoP to promoters of phosphate-regulated genes in *Streptomyces coelicolor*: identification of PHO boxes. Mol Microbiol **56:**1373–1385.

10. **Jog R, Pandya M, Nareshkumar G, Rajkumar S.** 2014. Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology **160:**778–788.

11. **Spaepen S, Vanderleyden. J.** 2011. Auxin and plant–microbe interactions., vol 3. Cold Spring Harbor Perspectives in Biology

12. **Kawase T, Saito A, Sato T, Kanai R, Fujii T, Nikaidou N, Miyashita K, Watanabe T.** 2004. Distribution and phylogenetic analysis of family 19 chitinases in actinobacteria. Appl Environ Microbiol **70:**1135–1144.

13. **Liu W, Wang Q, Hou J, Tu C, Luo Y, Christie P.** 2016. Whole genome analysis of halotolerant and alkalotolerant plant growth-promoting rhizobacterium *Klebsiella* sp. D5A. Sci Rep **6:**26710.

14. **Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH.** 2015. antiSMASH 3.0–a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res**:**W237–W243.