**GENOME SEQUENCES**





## **Draft Genome Sequences of Seven Strains of Paenibacillus spp. (Phylum Firmicutes) Inhabiting the Seeds of Cucumis melo L. (Cantaloupe) and Exhibiting Plant Probiotic Traits**

**[Eman M. Khalaf,a](https://orcid.org/0000-0001-7844-8946),b Manish N. Raizadaa**

aDepartment of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada <sup>b</sup>Department of Microbiology and Immunology, Faculty of Pharmacy, Damanhour University, Damanhour, Egypt

**ABSTRACT** Here, we report the draft genome sequences of seven Paenibacillus sp. strains (EKM202P, EKM205P, EKM206P, EKM207P, EKM208P, EKM211P, and EKM212P) that were previously isolated from cultivated surface-sterilized seeds of Cucumis melo L. (cantaloupe). These candidate Paenibacillus plant probiotics displayed in vitro growth-promoting traits and suppressive activity against root-associated fungal/oomycete pathogens.

In recent decades, the genus *Paenibacillus* has been described (1), and many strains<br>
are commercially exploited [\(2\)](#page-1-1). Paenibacilli are recognized as plant symbionts, par-n recent decades, the genus Paenibacillus has been described [\(1\)](#page-1-0), and many strains ticularly root associated [\(2,](#page-1-1) [3\)](#page-1-2); however, their sporulation capacity enables dormancy within seeds [\(4\)](#page-1-3). High-throughput 16S rRNA gene sequencing showed that the genus Paenibacillus dominated cucurbit seeds, including melons [\(5\)](#page-1-4), consistent with their cultivated microbiota [\(6\)](#page-1-5). Seven unique bacterial colonies were selected from surfacesterilized seeds of Cucumis melo L. (cantaloupe), classified using the 16S primer pair 799F/1492R as Paenibacillus spp. (EKM202P, EKM205P, EKM206P, EKM207P, EKM208P, EKM211P, and EKM212P), and then submitted to GenBank (accession numbers [KT281420.1,](https://www.ncbi.nlm.nih.gov/nuccore/KT281420.1) [KT281430.1,](https://www.ncbi.nlm.nih.gov/nuccore/KT281430.1) [KT281426.1,](https://www.ncbi.nlm.nih.gov/nuccore/KT281426.1) [KT281421.1,](https://www.ncbi.nlm.nih.gov/nuccore/KT281421.1) [KT281431.1,](https://www.ncbi.nlm.nih.gov/nuccore/KT281431.1) [KT281427.1,](https://www.ncbi.nlm.nih.gov/nuccore/KT281427.1) and [KT281423.1,](https://www.ncbi.nlm.nih.gov/nuccore/KT281423.1) respectively) [\(6\)](#page-1-5). These microbes were assessed in vitro for their biostimulant/biocontrol potential. All strains demonstrated exoenzymatic activity (cellulase, pectinase, and protease [\[6\]](#page-1-5) and RNase [\[7\]](#page-1-6)) and acetoin/diacetyl (volatile) production and suppressed the plant pathogens Fusarium graminearum, Rhizoctonia solani, and Phytophthora capsici in vitro [\(7\)](#page-1-6) (EKM212P was negative for pectinase [\[6\]](#page-1-5) and RNase [\[7\]](#page-1-6) activities). Exclusively, EKM202P and EKM212P suppressed Pythium aphanidermatum [\(7\)](#page-1-6), while EKM202P and EKM205P grew on a nitrogen-free medium (LGI medium) [\(6\)](#page-1-5).

With the use of  $-80^{\circ}$ C glycerol stocks, the strains were cultured on LB agar. Single colonies were inoculated into lysogeny broth and incubated overnight at 37°C at 250 rpm. Genomic DNA was isolated from pellets using DNeasy UltraClean microbial kits (product number 12224-50; Qiagen) and then adjusted to 50 ng/ $\mu$ l. Libraries were constructed using TruSeq DNA Nano library preparation kits (KAPA HyperPrep kit KK8504) and then sequenced using the Illumina NovaSeq 6000 platform, which delivered 2,850,454 (EKM202P), 2,568,261 (EKM205P), 2,970,533 (EKM206P), 1,587,495 (EKM207P), 3,274,658 (EKM208P), 2,260,644 (EKM211P), and 2,754,662 (EKM212P) raw reads of the 150-bp paired-end format. De novo assembly of clean reads (quality score threshold, 30) was completed using the Evogene Clustering/Assembly Toolbox (Evo-CAT) pipeline, and then the assembled contigs were taxonomically identified using KmerFinder v3.2 [\(8\)](#page-1-7) leading to 115, 100, 118, 65, 136, 96, and 116-fold sequence coverage compared to that of Paenibacillus sp. strain M-152 (GenBank accession number [NZ\\_CP034141.1\)](https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP034141.1) (EKM202P and EKM207P), Paenibacillus polymyxa strain YC0573 [\(NZ\\_CP017968.3\)](https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP017968.3) [\(9\)](#page-1-8) (EKM205P and EKM206P), Paenibacillus polymyxa strain J

**Citation** Khalaf EM, Raizada MN. 2020. Draft genome sequences of seven strains of Paenibacillus spp. (phylum Firmicutes) inhabiting the seeds of Cucumis melo L. (cantaloupe) and exhibiting plant probiotic traits. Microbiol Resour Announc 9:e00715-20. [https://doi.org/10.1128/MRA.00715-20.](https://doi.org/10.1128/MRA.00715-20)

**Editor** David A. Baltrus, University of Arizona

**Copyright** © 2020 Khalaf and Raizada. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0](https://creativecommons.org/licenses/by/4.0/) [International license.](https://creativecommons.org/licenses/by/4.0/)

Address correspondence to Eman M. Khalaf, [emankhalaf@pharm.dmu.edu.eg.](mailto:emankhalaf@pharm.dmu.edu.eg)

**Received** 18 June 2020 **Accepted** 29 July 2020 **Published** 20 August 2020

			No. of		No. of	$G+C$		
<b>Isolate</b>	Bacterial species <sup>a</sup>	Genome size (bp)	contigs	$N_{50}$ (bp)	genes	content (%)	SRA accession no.	<b>GenBank accession no.</b>
EKM202P	Paenibacillus sp.	5,917,862	170	245,720	4,677	50	SRR11051668	JAAMNO000000000
EKM205P	P. polymyxa	5,731,588	208	460,107	4,600	52	SRR11051660	JAAMNU000000000
EKM206P	P. polymyxa	5,726,213	162	393,822	4,595	52	SRR11051647	JAAMNO000000000
EKM207P	Paenibacillus sp.	5,907,745	170	204,300	4,682	49.5	SRR11051667	JAAMNP000000000
EKM208P	P. polymyxa	6,751,337	2.164	371,116	6,210	45	SRR11051674	JAAMNT000000000
EKM211P	P. polymyxa	5,764,313	119	221.247	4,529	48	SRR11051664	JAAMNS000000000
EKM212P	P. polymyxa	5,625,291	102	670,972	4,485	51.5	SRR11051658	JAAMNR000000000

<span id="page-1-9"></span>**TABLE 1** Characteristics and accession numbers of Paenibacillus genomes

<sup>a</sup> The taxonomy of these bacterial species is according to the updated GenBank databases.

[\(NZ\\_CP015423.1\)](https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP015423.1) (EKM208P and EKM212P), and Paenibacillus polymyxa strain HY96-2 [\(NZ\\_CP025957.1\)](https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP025957.1) [\(10\)](#page-2-0) (EKM211P), respectively. Predicted proteins were defined using Prodigal [\(11\)](#page-2-1) and matched against the NCBI nonredundant protein database using BLASTp [\(12\)](#page-2-2), and then peptide domains were identified using InterProScan v5.32-71.0 [\(13\)](#page-2-3). Assembly statistics and accession numbers are presented in [Table 1.](#page-1-9)

All of the Paenibacillus genomes encode candidate proteins implicated in the aforementioned in vitro activities and additional traits, including tryptophan synthase (auxin production), carbon-nitrogen hydrolase, nitrogen regulatory protein PII, nitrogen assimilation/fixation (nif), phytase, alkaline phosphatase, and trehalose-6-phosphate hydrolase [\(2,](#page-1-1) [14,](#page-2-4) [15\)](#page-2-5), cytokinin riboside 5'-monophosphate phosphoribohydrolase LOG [\(16,](#page-2-6) [17\)](#page-2-7), and 1-aminocyclopropane-1-carboxylate synthase (growth/stress regulation) [\(18\)](#page-2-8). Biocontrol/immunomodulation genes were identified for hydrolytic exoenzymes (chitinases,  $\beta$ -glucanases, lipases, proteases, pectin/pectate lyases, and ribonucleases) [\(2,](#page-1-1) [16,](#page-2-6) [19,](#page-2-9) [20\)](#page-2-10), butanediol dehydrogenase-like enzymes (acetoin production) [\(1\)](#page-1-0), iron siderophore-like compounds (aerobactin siderophore biosynthesis and IucA/IucC [exclusively EKM208P and EKM212P]) [\(2,](#page-1-1) [21\)](#page-2-11), bacteriocins (thiopeptide type) [\(22\)](#page-2-12), polyketide synthase and nonribosomal peptide synthase (lipopeptide synthesis) [\(15,](#page-2-5) [23\)](#page-2-13), phenazine biosynthesis PhzF protein (except EKM211P) [\(24\)](#page-2-14), alkyl hydroperoxide reductase (antioxidative enzyme) [\(25\)](#page-2-15), and biomolecules for biofilm formation and quorum sensing [\(10,](#page-2-0) [14,](#page-2-4) [26,](#page-2-16) [27\)](#page-2-17). This fundamental analysis may support future experiments to formulate new agricultural bioproducts.

**Data availability.** The whole-genome shotgun project and raw Illumina reads were deposited in DDBJ/EMBL/GenBank and the SRA, respectively, under the accession numbers provided in [Table 1.](#page-1-9)

## **ACKNOWLEDGMENTS**

We thank Ada Viterbo and collaborators at Lavie Bio Ltd. for genome sequencing and bioinformatic support.

This research was supported by grants from the Ontario Ministry of Agriculture, Food, and Rural Affairs and the Natural Sciences and Engineering Research Council of Canada.

## <span id="page-1-0"></span>**REFERENCES**

- 1. Rybakova D, Cernava T, Köberl M, Liebminger S, Etemadi M, Berg G. 2016. Endophytes-assisted biocontrol: novel insights in ecology and the mode of action of Paenibacillus. Plant Soil 405:125–140. [https://doi.org/](https://doi.org/10.1007/s11104-015-2526-1) [10.1007/s11104-015-2526-1.](https://doi.org/10.1007/s11104-015-2526-1)
- <span id="page-1-2"></span><span id="page-1-1"></span>2. Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC. 2016. Current knowledge and perspectives of Paenibacillus: a review. Microb Cell Fact 15:203. [https://doi.org/10.1186/s12934-016-0603-7.](https://doi.org/10.1186/s12934-016-0603-7)
- <span id="page-1-3"></span>3. Park KY, Seo SY, Oh BR, Seo JW, Kim YJ. 2018. 2,3-Butanediol induces systemic acquired resistance in the plant immune response. J Plant Biol 61:424 – 434. [https://doi.org/10.1007/s12374-018-0421-z.](https://doi.org/10.1007/s12374-018-0421-z)
- <span id="page-1-4"></span>4. Truyens S, Weyens N, Cuypers A, Vangronsveld J. 2015. Bacterial seed endophytes: genera, vertical transmission and interaction with plants. Environ Microbiol Rep 7:40 –50. [https://doi.org/10.1111/1758-2229.12181.](https://doi.org/10.1111/1758-2229.12181)
- 5. Khalaf EM, Raizada MN. 2019. Cucurbit seeds: reservoirs of functional

Ontario, Canada, 7 to 10 July 2019.

<span id="page-1-6"></span>7. Khalaf EM, Raizada MN. 2018. Bacterial seed endophytes of domesticated cucurbits antagonize fungal and oomycete pathogens including powdery mildew. Front Microbiol 9:42. [https://doi.org/10.3389/fmicb](https://doi.org/10.3389/fmicb.2018.00042) [.2018.00042.](https://doi.org/10.3389/fmicb.2018.00042)

<span id="page-1-5"></span>and antagonistic microbiomes. Presented at Plant Canada 2019, Guelph,

- <span id="page-1-7"></span>8. Deng X, den Bakker HC, Hendriksen RS. 2016. Genomic epidemiology: whole-genome-sequencing–powered surveillance and outbreak investigation of foodborne bacterial pathogens. Annu Rev Food Sci Technol 7:353–374. [https://doi.org/10.1146/annurev-food-041715-033259.](https://doi.org/10.1146/annurev-food-041715-033259)
- <span id="page-1-8"></span>9. Liu H, Liu K, Li Y, Wang C, Hou Q, Xu W, Fan L, Zhao J, Gou J, Du B, Ding Y.

2017. Complete genome sequence of Paenibacillus polymyxa YC0573, a plant growth-promoting rhizobacterium with antimicrobial activity. Genome Announc 5:e01636-16. [https://doi.org/10.1128/genomeA.01636-16.](https://doi.org/10.1128/genomeA.01636-16)

- <span id="page-2-0"></span>10. Luo Y, Cheng Y, Yi J, Zhang Z, Luo Q, Zhang D, Li Y. 2018. Complete genome sequence of industrial biocontrol strain Paenibacillus polymyxa HY96-2 and further analysis of its biocontrol mechanism. Front Microbiol 9:1520. [https://doi.org/10.3389/fmicb.2018.01520.](https://doi.org/10.3389/fmicb.2018.01520)
- <span id="page-2-1"></span>11. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. [https://doi.org/10.1186/1471](https://doi.org/10.1186/1471-2105-11-119) [-2105-11-119.](https://doi.org/10.1186/1471-2105-11-119)
- <span id="page-2-2"></span>12. Pruitt KD, Tatusova T, Maglott DR. 2007. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res 35:D61–D65. [https://doi.org/](https://doi.org/10.1093/nar/gkl842) [10.1093/nar/gkl842.](https://doi.org/10.1093/nar/gkl842)
- <span id="page-2-3"></span>13. Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R. 2005. InterProScan: protein domains identifier. Nucleic Acids Res 33:W116 –W120. [https://doi.org/10.1093/nar/gki442.](https://doi.org/10.1093/nar/gki442)
- <span id="page-2-4"></span>14. Chen L, Heng J, Qin S, Bian K. 2018. A comprehensive understanding of the biocontrol potential of Bacillus velezensis LM2303 against Fusarium head blight. PLoS One 13:e0198560. [https://doi.org/10.1371/journal](https://doi.org/10.1371/journal.pone.0198560) [.pone.0198560.](https://doi.org/10.1371/journal.pone.0198560)
- <span id="page-2-5"></span>15. Xie J, Shi H, Du Z, Wang T, Liu X, Chen S. 2016. Comparative genomic and functional analysis reveal conservation of plant growth promoting traits in Paenibacillus polymyxa and its closely related species. Sci Rep 6:21329 –21312. [https://doi.org/10.1038/srep21329.](https://doi.org/10.1038/srep21329)
- <span id="page-2-6"></span>16. McSpadden Gardener BB. 2004. Ecology of Bacillus and Paenibacillus spp. in agricultural systems. Phytopathology 94:1252-1258. [https://doi.org/](https://doi.org/10.1094/PHYTO.2004.94.11.1252) [10.1094/PHYTO.2004.94.11.1252.](https://doi.org/10.1094/PHYTO.2004.94.11.1252)
- <span id="page-2-7"></span>17. Naseem M, Bencurova E, Dandekar T. 2018. The cytokinin-activating LOG-family proteins are not lysine decarboxylases. Trends Biochem Sci 43:232–236. [https://doi.org/10.1016/j.tibs.2018.01.002.](https://doi.org/10.1016/j.tibs.2018.01.002)
- <span id="page-2-8"></span>18. Van de Poel B, Van Der Straeten D. 2014. 1-Aminocyclopropane-1 carboxylic acid (ACC) in plants: more than just the precursor of ethylene! Front Plant Sci 5:640. [https://doi.org/10.3389/fpls.2014.00640.](https://doi.org/10.3389/fpls.2014.00640)
- <span id="page-2-9"></span>19. Veliz EA, Martínez-Hidalgo P, Hirsch AM. 2017. Chitinase-producing bacteria and their role in biocontrol. AIMS Microbiol 3:689 –705. [https://doi](https://doi.org/10.3934/microbiol.2017.3.689) [.org/10.3934/microbiol.2017.3.689.](https://doi.org/10.3934/microbiol.2017.3.689)
- <span id="page-2-10"></span>20. Neeraja C, Anil K, Purushotham P, Suma K, Sarma P, Moerschbacher BM, Podile AR. 2010. Biotechnological approaches to develop bacterial chitinases as a bioshield against fungal diseases of plants. Crit Rev Biotechnol 30:231–241. [https://doi.org/10.3109/07388551.2010.487258.](https://doi.org/10.3109/07388551.2010.487258)
- <span id="page-2-11"></span>21. Zawadzka AM, Abergel RJ, Nichiporuk R, Andersen UN, Raymond KN. 2009. Siderophore-mediated iron acquisition systems in Bacillus cereus: identification of receptors for anthrax virulence- associated petrobactin. Bone 48:3645–3657. [https://doi.org/10.1021/bi8018674.](https://doi.org/10.1021/bi8018674)
- <span id="page-2-12"></span>22. Cotter PD, Ross RP, Hill C. 2013. Bacteriocins: a viable alternative to antibiotics? Nat Rev Microbiol 11:95–105. [https://doi.org/10.1038/](https://doi.org/10.1038/nrmicro2937) [nrmicro2937.](https://doi.org/10.1038/nrmicro2937)
- <span id="page-2-13"></span>23. Cochrane SA, Vederas JC. 2016. Lipopeptides from Bacillus and Paenibacillus spp.: a gold mine of antibiotic candidates. Med Res Rev 36:4 –31. [https://doi.org/10.1002/med.21321.](https://doi.org/10.1002/med.21321)
- <span id="page-2-14"></span>24. Tupinambá G, Da Silva AJR, Alviano CS, Souto-Padron T, Seldin L, Alviano DS. 2008. Antimicrobial activity of Paenibacillus polymyxa SCE2 against some mycotoxin-producing fungi. J Appl Microbiol 105:1044 –1053. [https://doi.org/10.1111/j.1365-2672.2008.03844.x.](https://doi.org/10.1111/j.1365-2672.2008.03844.x)
- <span id="page-2-15"></span>25. Jiang G, Yang J, Li X, Cao Y, Liu X, Ling J, Wang H, Zhong Z, Zhu J. 2019. Alkyl hydroperoxide reductase is important for oxidative stress resistance and symbiosis in Azorhizobium caulinodans. FEMS Microbiol Lett 366:fnz014. [https://doi.org/10.1093/femsle/fnz014.](https://doi.org/10.1093/femsle/fnz014)
- <span id="page-2-16"></span>26. Timmusk S, Van West P, Gow NAR, Paul Huffstutler R. 2009. Paenibacillus polymyxa antagonizes oomycete plant pathogens Phytophthora palmivora and Pythium aphanidermatum. J Appl Microbiol 106: 1473–1481. [https://doi.org/10.1111/j.1365-2672.2009.04123.x.](https://doi.org/10.1111/j.1365-2672.2009.04123.x)
- <span id="page-2-17"></span>27. Yi J, Zhang D, Cheng Y, Tan J, Luo Y. 2019. The impact of Paenibacillus polymyxa HY96-2 luxS on biofilm formation and control of tomato bacterial wilt. Appl Microbiol Biotechnol 103:9643–9657. [https://doi.org/](https://doi.org/10.1007/s00253-019-10162-0) [10.1007/s00253-019-10162-0.](https://doi.org/10.1007/s00253-019-10162-0)