

GENOME ANNOUNCEMENT

Draft Genome Sequence of *Paenibacillus polymyxa* OSY-DF, Which Coproduces a Lantibiotic, Paenibacillin, and Polymyxin E1

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Paenibacillus polymyxa OSY-DF is a Gram-positive rod-shaped bacterium isolated from a fermented vegetable food. This bacterial strain displays potent antimicrobial activities against Gram-positive and Gram-negative pathogenic bacteria, attributed to the production of the lantibiotic paenibacillin and the colistin peptide polymyxin E1. Here we report the draft genome sequence of *Paenibacillus polymyxa* OSY-DF.

here is a perpetual need to discover or develop new antimicrobial agents to combat emerging antibiotic-resistant bacterial pathogens (5). Paenibacillus polymyxa OSY-DF, an isolate from a fermented food, coproduces a lantibiotic (paenibacillin) and polymyxin E1 (7). Paenibacillin is a unique lantibiotic with an N-terminal acetyl group (8). Paenibacillin shows potency against Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus, Listeria monocytogenes, and Mycobacterium smegmatis. Recently, there has been renewed interest in using polymyxin to treat infections caused by multidrug-resistant (MDR) Gram-negative bacteria (4, 12, 14, 19). Therefore, paenibacillin and polymyxin E1 from P. polymyxa OSY-DF have promising applications in treating bacterial infections or protecting foods as natural preservatives. To understand the biosynthesis of these antimicrobial agents, we determined the whole genome sequence of the producer strain, P. polymyxa OSY-DF.

Genomic DNA of P. polymyxa OSY-DF was isolated using a DNA extraction kit (DNeasy blood and tissue kit; Qiagen, Valencia, CA). RNase-treated genomic DNA in Tris-Cl buffer (pH 8.5) was used for construction of a paired-end library with a Truseq DNA sample preparation kit (Illumina, San Diego, CA) according to the manufacturer's instructions. The constructed library was sequenced (76 cycles, paired-end runs) in a flow cell lane using an Illumina genome analyzer II. De novo assembly of the short reads with a commercial software (CLC Genomics Workbench 4.7.2; CLCBio, Cambridge, MA) yielded 139 contigs (>200 bp), with a maximum contig size of 685,087 bp. The resulting draft genome of P. polymyxa OSY-DF consists of 5,695,430 bases; the overall GC content of the genome was calculated as 45.35% by the software Artemis (18). Automatic genome annotation was performed using RAST (Rapid Annotation using Subsystem Technology) (1). Among the 5,139 protein-coding sequences (CDSs), 72% have been assigned a putative function by RSAT. The chromosome has 1 rRNA operon and 42 tRNAs, as predicted by RNAmmer (11) and tRNAscan-SE (15), respectively.

In the draft genome of *P. polymyxa* OSY-DF, two complete lantibiotic gene clusters were identified. One of the lantibiotic gene clusters (11.7 kb) is responsible for paenibacillin biosynthesis, while the other may encode a new lantibiotic as predicted by the bacteriocin mining tool Bagel2 (3). Many nonribosomal peptide synthetase (NRPS) genes were identified in the draft genome. For instance, genes for polymyxin E1 production were found in four nonoverlapping contigs. The complete fusaricidin gene clusters and the synthetic synth

ter was found in a large contig, whose predicted protein shows 93% identity to its homologues in *P. polymyxa* PKB1 and *P. polymyxa* E681 (2, 13).

The average nucleotide identity (ANI) between the draft genome of *P. polymyxa* OSY-DF and the four published *P. polymyxa* genomes of SC2 (16), M-1 (17), E681 (10), and ATCC 842^{T} (9) was determined using the *in silico* DNA-DNA hybridization (DDH) method implemented in the software JSpecies (6). The results indicated that *P. polymyxa* OSY-DF has the closest genetic relatedness to strain ATCC 842^{T} (97.50% ANI), followed by strains SC2 (94.52% ANI), M-1 (94.52% ANI), and E681 (91.78% ANI).

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/ GenBank under accession no. AIPP000000000. The version described in this article is the first version, AIPP01000000.

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REFERENCES

- 1. Aziz R, et al. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75. doi:10.1186/1471-2164-9-75.
- Choi SK, et al. 2008. Identification and functional analysis of the fusaricidin biosynthetic gene of *Paenibacillus polymyxa* E681. Biochem. Biophys. Res. Commun. 365:89–95.
- 3. de Jong A, Van Heel AJ, Kok J, Kuipers OP. 2010. BAGEL2: mining for bacteriocins in genomic data. Nucleic Acids Res. 38:W647–W651.
- Falagas ME, Kasiakou SK, Saravolatz LD. 2005. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin. Infect. Dis. 40:1333–1341.
- Fischbach MA, Walsh CT. 2009. Antibiotics for emerging pathogens. Science 325:1089–1093.
- Goris J, et al. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int. J. Syst. Evol. Microbiol. 57:81–91.
- 7. He Z, et al. 2007. Isolation and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and polymyxin. Appl. Environ. Microbiol. 73:168–178.

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- He Z, Yuan C, Zhang L, Yousef AE. 2008. N-terminal acetylation in paenibacillin, a novel lantibiotic. FEBS Lett. 582:2787–2792.
- Jeong H, et al. 2011. Draft genome sequence of the *Paenibacillus polymyxa* type strain (ATCC 842^T), a plant growth-promoting bacterium. J. Bacteriol. 193:5026–5027.
- Kim JF, et al. 2010. Genome sequence of the polymyxin-producing plantprobiotic rhizobacterium *Paenibacillus polymyxa* E681. J. Bacteriol. 192: 6103–6104.
- Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100–3108.
- Landman D, Georgescu C, Martin DA, Quale J. 2008. Polymyxins revisited. Clin. Microbiol. Rev. 21:449-465.
- Li J, Beatty PK, Shah S, Jensen SE. 2007. Use of PCR-targeted mutagenesis to disrupt production of fusaricidin-type antifungal antibiotics in *Paenibacillus polymyxa*. Appl. Environ. Microbiol. 73:3480–3489.
- Li J, et al. 2006. Colistin: the re-emerging antibiotic for multidrugresistant Gram-negative bacterial infections. Lancet Infect. Dis. 6:589– 601.

- 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.
- Ma M, et al. 2011. Complete genome sequence of *Paenibacillus polymyxa* SC2, a strain of plant growth-promoting rhizobacterium with broadspectrum antimicrobial activity. J. Bacteriol. 193:311–312.
- 17. Niu B, Rueckert C, Blom J, Wang Q, Borriss R. 2011. The genome of the plant growth-promoting rhizobacterium *Paenibacillus polymyxa* M-1 contains nine sites dedicated to nonribosomal synthesis of lipopeptides and polyketides. J. Bacteriol. **193**:5862–5863.
- Rutherford K, et al. 2000. Artemis: sequence visualization and annotation. Bioinformatics 16:944–945.
- Walkty A, et al. 2009. *In vitro* activity of colistin (polymyxin E) against 3,480 isolates of Gram-negative bacilli obtained from patients in Canadian hospitals in the CANWARD Study, 2007–2008. Antimicrob. Agents Chemother. 53:4924–4926.