MPMI Vol. 35, No. 4, 2022, pp. 364–367, https://doi.org/10.1094/MPMI-12-21-0310-A

RESOURCE ANNOUNCEMENT

Genome Sequence Resource of a Quorum-Quenching Biocontrol Agent, *Pseudomonas nitroreducens* HS-18

Huishan Wang,[1,3] Lisheng Liao,[1,3] Xiaofan Zhou,[1,3] Lingling Dong,[1,3] Xin Lin,[2] and Lianhui Zhang[1,3,†]

[1] Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou 510642, China
[2] Institute of Microbiology, Meizhou Academy of Agricultural and Forestry Sciences, Meizhou, Guangdong Province 514071, China
[3] Guangdong Province Key Laboratory of Microbial Signals and Disease Control, Integrative Microbiology Research Centre, South China Agricultural University, Guangzhou 510642, China

Funding
This work was supported by the grants from the Key Realm R&D Program of Guangdong Province (2020B0202090001; 2018B020205003), Guangdong Forestry Science and Technology Innovation Project (2018KJCX009; 2020KJCX009), Key Projects of Guangzhou Science and Technology Plan (201804020066), National Natural Science Foundation of China (31900076) and Basic Research and Applied Basic Research Program of Guangdong Province (2020A1515110111).

Keywords
AHL, DSF, genome sequence resource, quorum quenching

Genome Announcement

Pseudomonas nitroreducens HS-18 isolated from oil contaminated-soil samples in Guangzhou, China was shown to produce multiple substrate-inducible quorum-quenching (QQ) enzymes for effective degradation of *N*-acyl-homoserine lactones (AHL) and diffusible signal factor (DSF) molecules, which represent two families of widely conserved bacterial quorum sensing (QS) signals. HS-18 showed a promising potential in biocontrol of the DSF or AHL-dependent phytopathogens due to its remarkable QQ ability. Here, we report the whole-genome sequence of strain HS-18. Genomic analysis revealed that strain HS-18 contains one circular chromosome of 6,493,503 bp in length and one circle plasmid of 101,450 bp. The confirmed DSF QQ genes *digA*, *digB*, *digC*, *digD* encoding fatty acyl-CoA ligases and confirmed AHL QQ genes *aigA*, *aigB*, *aigC* encoding AHL acylases were all found located in the chromosome. The findings from this study will provide valuable information for further exploitation of this useful biocontrol agent and for unveiling the detailed QQ regulatory mechanisms.

QS is a cell-to-cell communication mechanism utilized by microorganism to regulate their adaptability to changing environmental conditions and pathogenicity on various host organisms (Mukherjee and Bassler 2019). Bacterial cells produce, release, and perceive QS signals to coordinate community activities through modulation of the transcriptional expression of a large set of target genes. AHL and DSF represent two families of widely conserved QS signals (Zhang and Dong 2004; Zhou et al. 2017). Given its important role in regulation of bacterial virulence, a strategy known as QQ aimed at blocking bacterial QS communications was proposed and shown to be an efficient therapy to control QS-dependent bacterial infections (Ahator and Zhang 2019; Zhang 2003). *Pseudomonas nitroreducens* HS-18 was recently identified with a highly efficient DSF degradation capacity (Wang et al. 2020), which has also been found to have AHL signal-degrading properties. The complete genome analysis confirmed the multi-QQ properties of this strain. To uncover the regulatory mechanisms that govern AHL- or DSF-inducible QQ and to further explore the application potential of this useful biocontrol agent, the genome sequence information of strain HS-18 would be of critical importance.

[†]Corresponding author: L. Zhang; lhzhang01@scau.edu.cn

Huishan Wang and Lisheng Liao contributed equally.

The author(s) declare no conflict of interest.

Accepted for publication 23 January 2022.

Table 1. Genome statistics of *Pseudomonas nitroreducens* HS-18

Feature	Chromosome
Genome size (bp)	6,594,953
G+C content (%)	65.47
Total genes	6090
Plasmid	1
Transfer RNAs	67
Ribosome RNAs	16
Small RNAs	16
Prophage	5
Genomic islands	14
CRISPRs	1
GenBank accession	CP084413-CP084414

Here, we report the complete genome sequence information of strain HS-18. Strain HS-18 was cultured overnight at 30°C in 50 ml of Luria Bertani broth, with shaking at 200 rpm. The cells were harvested by centrifugation and genomic DNA of strain HS-18 was extracted, using the EasyPure bacteria genomic DNA kit (Transgene Biotech). A Qubit 2.0 fluorometer (Life Technologies) and NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific) were applied to determine the quantity and quality of the extracted genomic DNA sample. Then, the DNA sample was subjected to complete genome sequencing at Beijing Novogene Bioinformatic Technology Co., Ltd., using Illumina PE150 platform sequencing technologies with 350-bp small-fragment library of strain HS-18 genomic DNA and using PacBio sequencing technologies with a 10 Kb single molecule real-time (SMRT) Bell library of strain HS-18 genome DNA. The short reads from Illumina PE150 were obtained to counter the error-prone long reads produced by PacBio.

Sequencing yielded 1,362,105,628 bp of high-quality long-read sequence (clean data) (sequence depth ≥200×) comprising 190,859 reads with a mean read length of 7,137 bp. The genome was assembled using SMRT Link v5.0.1 and was optimized, using arrow software of SMRT Link v5.0.1, to gain a high-quality contig without gaps (Ardui et al. 2018). The optimized assembly was taken as the reference genome, and Illumina sequencing reads were used to align to the reference genome, using bwa-0.7.8 for further correction. Illumina sequencing reads were quality-filtered (quality score ≥20, 4 ≤ read depth ≤1,000). Circularization corrections were performed by searching the overlap of the beginning and the end sequences of the corrected assembly results. The coding genes were predicted using GeneMarkS (version 4.17) (Besemer et al. 2001). Repeat sequences were analyzed by RepeatMasker (version open 4.0.5) and Tandem Repeats Finder (version 4.07b) (Benson 1999; Saha et al. 2008). For noncoding RNA genes, transfer RNA (tRNA) genes were analyzed by tRNAscan-SE (version 1.3.1), ribosome RNA (rRNA) genes were predicted by rRNAmmer (version 1.2), small RNA (sRNA) were predicted by blasting against the Rfam database and cmsearch (version 1.1rc4) (Gardner et al. 2009; Lagesen et al. 2007; Lowe and Eddy 1997). Genomic islands were predicted by IslandPath-DIOMB (version 0.2), and the CRISPR recognition tool was analyzed by CRISPRdigger (version 1.0) (Grissa et al. 2007; Hsiao et al. 2003). The prophage genes were predicted by phiSpy (version 2.3) (Zhou et al. 2011). The protein functional analysis was conducted by comparing the peptide sequences of strain HS-18 with bacterial protein sequences in the Gene Ontology, Kyoto Encyclopedia of Genes and Genomes (KEGG), Cluster of Orthologous Groups of proteins, Non-Redundant Protein databases, Pfam, the Transporter Classification and Carbohydrate-Active Enzymes databases, and Swiss-Prot. The average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values between strain HS-18 and its closely related strains were calculated on ANI Calculator (Yoon et al. 2017) and Genome-to-Genome Distance Calculator 2.1 (Meier-Kolthoff et al. 2013), respectively.

Genome assembly showed that strain HS-18 contains a single circular DNA chromosome with a length of 6,493,503 bp with G+C content of 64.74% and one single circular plasmid of 101,450 bp with G+C content of 55.01% (Table 1). The overall genome features are presented in Table 1. Strain HS-18 consists of 6,090 genes accounting for 87.81% of the whole genome, which includes 67 tRNA genes, 16 rRNA genes, and 16 sRNA genes (Table 1). HS-18 resulted in one contig and one scaffold, and the contig N_{50} was 6,493,503 bp and L_{50} was 1 (Table 2). The genome assembly statistics of strain HS-18 were

Table 2. Overview of the genome assembly statistics of HS-18 and other *Pseudomonas nitroreducens* strains[a]

Genomic features	HS-18	DSM 14399	NBRC 12694	PSA00705	WZBFD3-5A2	Aramco J	HBP1
ANI value (%)	100	98.9355	98.9139	98.8491	98.8128	98.8081	98.7566
dDDH (%)	100	90.30	90.10	91.60	87.30	88.40	89.20
GenBank assembly accession	GCA_020401845.1	GCA_012986245.1	GCA_002091755.1	GCA_015763095.1	GCA_010994165.1	GCA_000807755.1	GCA_011044415.1
Genome size (bp)	6,594,953	6,171,316	6,105,137	6,787,054	6,397,936	7,333,675	7,427,551
Scaffolds number	1	NA	NA	NA	NA	NA	NA
Contigs number	1	50	40	206	12	91	NA
Contig N_{50} (bp)	6,493,503	855,214	537,643	56,451	932,199	322,424	NA
Contig L_{50}	1	NA	4	37	3	7	NA
Assembly method	SMRT Link v. v5.1.0	SPAdes v. 2.5.1	newbler v. 3.0	SPAdes v. 3.14.1	Newbler v. 2.3	Newbler v. 2.8	HGAP v. 3
Genome coverage	200×	138×	80×	43×	12×	72×	83×
Sequencing technology	PacBio; Illumina	Illumina MiSeq	Illumina HiSeq 1000; 454 GS-FLX Titanium	Illumina NextSeq	Illumina HiSeq	IonTorrent	PacBio

[a] ANI = average nucleotide identity; dDDH = digital DNA-DNA hybridization; NA = not available or not applicable.

compared with a number of sequenced genomes of *P. nitroreducens* from the National Center for Biotechnology Information database with ANI and dDDH. The ANI values between strain HS-18 and these type strains ranged from 98.63 to 98.93%, and the dDDH values ranged from 87.30 to 91.60%. These values are above the threshold ANI value of 95% and dDDH value of 70%, respectively, suggesting that strain HS-18 and the *P. nitroreducens* strains occupied the same taxonomic position.

The genome assembly statistics of strain HS-18 showed high completeness (>98%) without contamination, indicating the higher-quality of the complete genome sequences of strain HS-18 than other *P. nitroreducens* strains. It might provide better resources for comparative genomic studies and for analyzing the function mechanisms presented in *P. nitroreducens* species.

Multiple genes were identified that may contribute to the QQ activity of *P. nitroreducens* HS-18 against other bacteria. Besides *digA*, *digB*, *digC*, and *digD* (Wang et al. 2020), at least another four homologous DSF QQ genes (locus_tags LDJ84_10985, LDJ84_14290, LDJ84_18380, and LDJ84_19965) were found in the strain HS-18 genome. Furthermore, we also found multiple genes, *aigA*, *aigB*, and *aigC* (GenBank accession numbers MW619635, MW619636, and MW619637, respectively), encoding putative AHL acylases, based on KEGG annotation and function BLAST. In the metabolism category, 115 genes in strain HS-18 were predicted to be involved in lipid metabolism, which might be related to the metabolism of fatty acid derivative DSF. The QQ capacity and potentials of strain HS-18 deserve further investigation. The complete genome sequence presented in this study will contribute to further understanding of the genetic and genomic basis of multi-QQ mechanisms of *P. nitroreducens* HS-18 and provide a valuable genome resource to explore other biological functions of this useful bacterial isolate.

Data Availability

The data obtained in this study have been deposited in GenBank under accession numbers CP084413 to CP084414 (BioProject: PRJNA766597, BioSample: SAMN21854795).

Literature Cited

Ahator, S. D., and Zhang, L. 2019. Small is mighty-chemical communication systems in *Pseudomonas aeruginosa.* Annu. Rev. Microbiol. 73:559-578.

Ardui, S., Ameur, A., Vermeesch, J. R., and Hestand, M. S. 2018. Single molecule real-time (SMRT) sequencing comes of age: Applications and utilities for medical diagnostics. Nucleic Acids Res. 46:2159-2168.

Benson, G. 1999. Tandem repeats finder: A program to analyze DNA sequences. Nucleic Acids Res. 27:573-580.

Besemer, J., Lomsadze, A., and Borodovsky, M. 2001. GeneMarkS: A self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. 29:2607-2618.

Gardner, P. P., Daub, J., Tate, J. G., Nawrocki, E. P., Kolbe, D. L., Lindgreen, S., Wilkinson, A. C., Finn, R. D., Griffiths-Jones, S., Eddy, S. R., and Bateman, A. 2009. Rfam: Updates to the RNA families database. Nucleic Acids Res. 37 (Database):D136-D140.

Grissa, I., Vergnaud, G., and Pourcel, C. 2007. CRISPRFinder: A web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res. 35:W52-W57.

Hsiao, W., Wan, I., Jones, S. J., and Brinkman, F. S. 2003. IslandPath: Aiding detection of genomic islands in prokaryotes. Bioinformatics 19:418-420.

Lagesen, K., Hallin, P., Rødland, E. A., Staerfeldt, H. H., Rognes, T., and Ussery, D. W. 2007. RNAmmer: Consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100-3108.

Lowe, T. M., and Eddy, S. R. 1997. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25:0955-0964.

Meier-Kolthoff, J. P., Auch, A. F., Klenk, H.-P., and Göker, M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60.

Mukherjee, S., and Bassler, B. L. 2019. Bacterial quorum sensing in complex and dynamically changing environments. Nat. Rev. Microbiol. 17:371-382.

Saha, S., Bridges, S., Magbanua, Z. V., and Peterson, D. G. 2008. Empirical comparison of ab initio repeat finding programs. Nucleic Acids Res. 36:2284-2294.

Wang, H., Liao, L., Chen, S., and Zhang, L. H. 2020. A quorum quenching bacterial isolate contains multiple substrate-inducible genes conferring degradation of diffusible signal factor. Appl. Environ. Microbiol. 86: e02930-19.

Yoon, S. H., Ha, S. M., Lim, J., Kwon, S., and Chun, J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie van Leeuwenhoek 110:1281-1286.

Zhang, L. H. 2003. Quorum quenching and proactive host defense. Trends Plant Sci. 8:238-244.

Zhang, L. H., and Dong, Y. H. 2004. Quorum sensing and signal interference: Diverse implications. Mol. Microbiol. 53:1563-1571.

Zhou, L., Zhang, L. H., Cámara, M., and He, Y. W. 2017. The DSF family of quorum sensing signals: Diversity, biosynthesis, and turnover. Trends Microbiol. 25:293-303.

Zhou, Y., Liang, Y., Lynch, K. H., Dennis, J. J., and Wishart, D. S. 2011. PHAST: A fast phage search tool. Nucleic Acids Res. 39:W347-W352.