Genome Sequence Resource of *Pseudomonas fulva* HARBPS9.1—Candidate Biocontrol Agent

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Abstract

The genus *Pseudomonas* contains a variety of genomic robust strains and species, well known for their beneficial use in a variety of applications, hence the vast amount of research done on this organism to date. We report here the draft genome sequence of an anti-*Fusarium* rhizospheric *Pseudomonas fulva* HARBPS9.1 strain from South Africa. This genome analysis identified clusters of genes responsible for the synthesis of pyoverdine and rhizomide in HARBPS9.1; these compounds should confer a competitive advantage on the pseudomonad.

The biotechnological benefits ascribed to some genera of bacteria (such as *Agrobacterium*, *Bacillus*, *Paenobacillus*, and *Pseudomonas*) and their metabolites (antibiotics, lytic enzymes, volatiles, and biosurfactants) are well documented (Berg 2009; Kalia et al. 2017; Meena and Kanwar 2015; Singh et al. 2007). Studies on the pseudomonads specifically, which span over 3 decades, have identified various biomolecules (e.g., amphisin, hydrogen cyanide [HCN] phenazines, 2, 4-diacetylphloroglucinol, pyrrolnitrin, siderophores, tensin, lytic enzymes, rhamnolipids, and viscosinamide) and associated these with many of the beneficial traits for which pseudomonads are well known (Götze and Stallforth 2020; Gross and Loper 2009; Loeschcke and Thies 2015; Zhang et al. 2019). Some members of the genus *Pseudomonas*, including *Pseudomonas chlororaphis*, *P. fluorescens*, *P. fulva*, *P. parafulva*, *P. putida*, and *P. mosselii*, are particularly well known for their antiphytopathogenic (biocontrol and plant growth promoting) properties (Kakembo and Lee 2019; Nielsen et al. 1999; Omoboye et al. 2019; Wu et al. 2018; Zhang et al. 2019), and have comprehensively been investigated for use as possible alternatives to chemical pesticides and synthetic fertilizers used in agriculture (Babalola 2010).

Previously, we identified the anti-*Fusarium* candidate *P. fulva* HARBPS9.1, and described its possible use in the in planta management of fusariosis in South Africa (Adeniji et al. 2020). Fusariosis, caused by toxicogenic *Fusarium* strains (e.g., *F. verticillioides*, *F. oxysporum*, *F. culmorum*, and *F. graminearum*), affects the distribution of major cereals (such as wheat, barley, and maize) on a global scale (Adeniji and Babalola 2018). In the previous study (Adeniji et al. 2020), *P. fulva* HARBPS9.1 pure culture was obtained from a distinct colony after a selective isolation was carried out from 5 g of maize farm rhizospheric soil using *Pseudomonas* agar (P1852; Sigma-Aldrich) plates with a cetrinix supplement (C8721; Sigma-Aldrich) following the manufacturer's instructions. The in vitro antagonistic property of *P. fulva* HARBPS9.1 (determined on potato dextrose agar; Oxoid) (28°C for 7 days) is attributed to the production of bioactive metabolites (HCN, pyrrolnitrin, and rhamnolipids) and, hence, the isolate was

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Keywords

anti-*Fusarium*, bioactive metabolites, biocontrol, biological control, disease control and pest management, food safety, fungal pathogens, genome, genomics, microbe-genome sequencing, *Pseudomonas*, sequencing

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Fig. 1. Circular atlas of the distribution and subsystem summary of the *Pseudomonas fulva* HARBPS9.1 genome annotations. The subsystem features from the outer to inner rings include contigs, coding sequence (CDS) (forward strand and reverse strand), RNA genes, CDS for antimicrobial resistance genes and virulence factors, GC content, and GC skew. Genomic features are depicted by the CDS shading on both the forward and reverse strands (Wattam et al. 2017).

molecularly and phylogenetically characterized (16S ribosomal RNA [rRNA] GenBank accession number MF098600.1). Given that very little is currently known about the beneficial properties of the *P. fulva* sp., we sequenced the genome of the isolate *P. fulva* HARBPS9.1 to further identify any novel beneficial traits which it may have.

The genomic DNA of the *P. fulva* HARBPS9.1 strain was extracted using the Zymo Research ZR Soil Microbe DNA Miniprep Genomic Isolation Kit. The DNA was sequenced with the Illumina MiSeq Reagent Kit v2 microsystem at Molecular Research (MR) DNA, Shallowater, TX, U.S.A., following their in-house protocols. The library was constructed with the Nextera DNA sample preparation kit (Illumina), after which the Qubit double-stranded DNA high-sensitivity assay kit (Life Technologies, Inc.) was used for the final library concentration. The average library size was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies), followed by library pooling and dilution to 12.0 pM. The paired-ends sequencing was done using a 600-cycle v.3 reagent kit (Illumina), with an average coverage of 50.0-fold. The Kbase platform (Arkin et al. 2016) was used for checking reads quality (FastQC v.1.0.1), reads trimming (Trimmomatic v0.32), gaps closing, and adaptor sequences removal (Cutadapt v1.0.1). The reads were assembled into contigs using SPAdes v3.13.0 on the Kbase platform. The Kbase contigs were then uploaded on the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP v4.2) (Pruitt et al. 2011), PATRICK online server (v3.3.15) (Wattam et al. 2017), and RAST and SEED online server (version 2.0) (Aziz et al. 2008; Overbeek et al. 2014) for automated annotation and comparison. Default settings were used for all of the bioinformatics analysis.

The sequencing yielded an average read length of 151 bp and 13,137,854 paired-end reads in total. The size of the *P. fulva* HARBPS9.1 genome and number of contigs were 4,822,642 bp and 47, respectively, with a G+C content of 61.6%. The N_{50} value of the raw sequences was 203,762 bp and, in total, 4,508 coding genes were annotated, including 7 rRNAs, 63 tRNAs, 4 ncRNAs, and 54 pseudo genes. The subsystem analysis for this genome is shown in Figure 1. Using the antiSMASH (v.5.2) (Blin et al. 2019) default parameters, the biosynthetic clusters (seven clusters) and bioproducts (e.g., pyoverdine and rhizomide) present in *P. fulva* HARBPS9.1 were predicted. The nonribosomal peptide synthetase secondary metabolite rhizomide predicted in the HARBPS9.1 genome sequence makes it a unique pseudomonad and, hence, of interest for further study as to (i) rhizomide's possible beneficial role in HARBPS9.1 antimicrobial activities and (ii) the possible application of HARBPS9.1 for biocontrol.

This Whole Genome Shotgun project has been deposited at DNA Data Bank of Japan/ European Nucleotide Archive/GenBank under the accession JAATHC000000000; GCA_011777485.1 (https://www.ncbi.nlm.nih.gov/assembly/GCA_011777485.1). The version described in this article is version JAASGY010000000. The BioProject and BioSample designations for this project are PRJNA544897 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA544897>) and SAMN11866291 (<https://www.ncbi.nlm.nih.gov/biosample/SAMN11866291>), respectively.

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