

# Draft Genome Sequences of *Pseudomonas fluorescens* Strains SF39a and SF4c, Potential Plant Growth Promotion and Biocontrol Agents

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***Pseudomonas fluorescens* SF4c and SF39a, strains isolated from wheat rhizosphere, have potential applications in plant growth promotion and biocontrol of fungal diseases of crop plants. We report the draft genome sequences of SF4c and SF39a with estimated sizes of 6.5 Mb and 5.9 Mb, respectively.**

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*Pseudomonas* spp. produce a variety of metabolites involved in biocontrol of plant diseases (1, 2). For example, *Pseudomonas protegens* (formerly *Pseudomonas fluorescens*) Pf-5 produces 2,4-diacetylphloroglucinol (2,4-DAPG), pyrrolnitrin, pyoluteorin, hydrogen cyanide, the siderophores pyochelin and pyoverdine, the cyclic lipopeptide orfamide, and rhizoxin derivatives (3–6).

*P. fluorescens* strains SF4c and 39a, isolated from the rhizosphere of wheat in Argentina (7), both promote wheat growth (7). SF4c also promotes tomato growth and inhibits plant-pathogenic *Rhizoctonia solani* and *Sclerotinia minor* (8), indicating biocontrol and plant growth-promoting potential. SF4c produces a bacteriocin similar to a phage-like pyocin of *P. aeruginosa*, which has antibacterial activity against some phytopathogenic pseudomonads, and is induced by DNA damage, suggesting involvement of the SOS response (9).

Genomic DNA of *P. fluorescens* SF39a and SF4c was extracted using Promega's Wizard Genomic DNA purification kit. Libraries were generated using Illumina's Nextera XT sequencing preparation kit and sequenced at the Tufts University Genomics Core using an Illumina MiSeq. For SF4c we obtained 3,382,840 2 × 250-bp reads (117-fold coverage), and for SF39a there were 3,439,664 2 × 250-bp reads (134-fold coverage). Genomes were assembled using CLC Genomics Workbench version 7.5. The SF4c assembly comprises 47 contigs (from 764 to 757,140 bp), and SF39a has 50 contigs (from 697 to 764,320 bp).

The draft genome sequence of SF4c comprises 6,507,013 bp (60.5% G+C content), while the SF39a sequence comprises 5,884,230 bp (60% G+C content). Both genomes were annotated using NCBI's PGAP pipeline. For SF4c, 5,673 protein-coding sequences, 55 tRNA genes, and 16S, 23S, and 5S (two copies) rRNA genes were predicted. For SF39a, 5,045 protein-coding sequences, 54 tRNA genes, and 16S, 23S, and 5S (two copies) rRNA genes were predicted.

The *hcnABC* operon for hydrogen cyanide (10) was detected in

SF4c (contig 7) and SF39a (contig 15), confirming previous work in SF4c (8). Although genes specifying pyrrolnitrin (11), phenazine (12), or 2,4-DAPG (13) have been characterized in related species, previous work indicated that SF4c does not have these genes (8). These results were confirmed, as was the absence of these genes in SF39a. Genes for pyoluteorin (14) are also absent from both strains.

In *P. fluorescens* Pf0-1, SBW25, and Q8r1-96, and *P. protegens* Pf-5, pyocin-like prophages are integrated between *mutS* and *recA-recX* (15). The genome sequence confirms that previously identified genes encoding a lytic system (*hol* and *lys* genes), the repressor gene (*prtR*), and a structural gene from R-type pyocin (9) are associated with a pyocin-like prophage integrated in the same locus (between QS95\_21670 and QS95\_21900 in contig 18). The presence of another phage-like bacteriocin (F-type pyocin) cluster was also identified in the same region of contig 18. Similar results were found in the strain SF39a (contig 17).

These strains expand the arsenal of bacteria, which may allow reduced fertilizer and chemical pesticide use. The genome sequences will enhance analysis of mechanisms of biocontrol and plant growth promotion, and deepen knowledge of *P. fluorescens* genomics.

**Nucleotide sequence accession numbers.** These whole-genome shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers [JTGG00000000](https://www.ncbi.nlm.nih.gov/nuccore/JTGG00000000) (SF39a) and [JTGH00000000](https://www.ncbi.nlm.nih.gov/nuccore/JTGH00000000) (SF4c). The versions described in this paper are versions [JTGG01000000](https://www.ncbi.nlm.nih.gov/nuccore/JTGG01000000) and [JTGH01000000](https://www.ncbi.nlm.nih.gov/nuccore/JTGH01000000), respectively.

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## REFERENCES

- Gross H, Loper JE. 2009. Genomics of secondary metabolite production by *Pseudomonas* spp. Nat Prod Rep 26:1408–1446. <http://dx.doi.org/10.1039/b817075b>.
- Fischer S, Principe A, Alvarez F, Cordero P, Castro M, Godino A, Jofré E, Mori G. 2013. Fighting plant diseases through the application of *Bacillus* and *Pseudomonas* strains, p 165–193. In Aroca R (ed), Symbiotic endophytes: soil biology, vol. 37. Springer, Berlin, Germany.
- Paulsen IT, Press CM, Ravel J, Kobayashi DY, Myers GS, Mavrodi DV, DeBoy RT, Seshadri R, Ren Q, Madupu R, Dodson RJ, Durkin AS, Brinkac LM, Daugherty SC, Sullivan SA, Rosovitz MJ, Gwinn ML, Zhou L, Schneider DJ, Cartinhour SW, Nelson WC, Weidman J, Watkins K, Tran K, Khouri H, Pierson EA, Pierson LS, Thomashow LS, Loper JE. 2005. Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. Nat Biotechnol 23:873–878. <http://dx.doi.org/10.1038/nbt1110>.
- Brendel N, Partida-Martinez LP, Scherlach K, Hertweck C. 2007. A cryptic PKS-NRPS gene locus in the plant commensal *Pseudomonas fluorescens* Pf-5 codes for the biosynthesis of an antimitotic rhizoxin complex. Org Biomol Chem 5:2211–2213. <http://dx.doi.org/10.1039/b707762a>.
- Gross H, Stockwell VO, Henkels MD, Nowak-Thompson B, Loper JE, Gerwick WH. 2007. The genomisotopic approach: a systematic method to isolate products of orphan biosynthetic gene clusters. Chem Biol 14: 53–63. <http://dx.doi.org/10.1016/j.chembiol.2006.11.007>.
- Loper JE, Henkels MD, Shaffer BT, Valeriote FA, Gross H. 2008. Isolation and identification of rhizoxin analogs from *Pseudomonas fluorescens* Pf-5 by using a genomic mining strategy. Appl Environ Microbiol 74:3085–3093. <http://dx.doi.org/10.1128/AEM.02848-07>.
- Fischer SE, Fischer SI, Magris S, Mori GB. 2007. Isolation and characterization of bacteria from the rhizosphere of wheat. World J Microbiol Biotechnol 23:895–903. <http://dx.doi.org/10.1007/s11274-006-9312-4>.
- Fischer SE, Jofré EC, Cordero PV, Gutiérrez Mañero FJ, Mori GB. 2010. Survival of native *Pseudomonas* in soil and wheat rhizosphere and antagonist activity against plant pathogenic fungi. Antonie Van Leeuwenhoek 97:241–251. <http://dx.doi.org/10.1007/s10482-009-9405-9>.
- Fischer S, Godino A, Quesada JM, Cordero P, Jofré E, Mori G, Espinosa-Urgel M. 2012. Characterization of a phage-like pyocin from the plant growth-promoting rhizobacterium *Pseudomonas fluorescens* SF4c. Microbiology 158:1493–1503. <http://dx.doi.org/10.1099/mic.0.056002-0>.
- Laville J, Blumer C, Von Schroetter C, Gaia V, Défago G, Keel C, Haas D. 1998. Characterization of the *hcnABC* gene cluster encoding hydrogen cyanide synthase and anaerobic regulation by ANR in the strictly aerobic biocontrol agent *Pseudomonas fluorescens* CHA0. J Bacteriol 180: 3187–3196.
- Hammer PE, Hill DS, Lam ST, Van Pée K-H, Ligon JM. 1997. Four genes from *Pseudomonas fluorescens* that encode the biosynthesis of pyrrolnitrin. Appl Environ Microbiol 63:2147–2154.
- Mavrodi DV, Ksenzenko VN, Bonsall RF, Cook RJ, Boronin AM, Thomashow LS. 1998. A seven-gene locus for synthesis of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2–79. J Bacteriol 180: 2541–2548.
- Bangera MG, Thomashow LS. 1996. Characterization of a genomic locus required for synthesis of the antibiotic 2,4-diacetylphloroglucinol by the biological control agent *Pseudomonas fluorescens* Q2-87. Mol Plant Microbe Interact 9:83–90. <http://dx.doi.org/10.1094/MPMI-9-0083>.
- Nowak-Thompson B, Gould SJ, Loper JE. 1997. Identification and sequence analysis of the genes encoding a polyketide synthase required for pyoluteorin biosynthesis in *Pseudomonas fluorescens* Pf-5. Gene 204: 17–24. [http://dx.doi.org/10.1016/S0378-1119\(97\)00501-5](http://dx.doi.org/10.1016/S0378-1119(97)00501-5).
- Mavrodi DV, Loper JE, Paulsen IT, Thomashow LS. 2009. Mobile genetic elements in the genome of the beneficial rhizobacterium *Pseudomonas fluorescens* Pf-5. BMC Microbiol 9:8. <http://dx.doi.org/10.1186/1471-2180-9-8>.