High-Quality Complete Genome Resource of Plant-Pathogenic Bacterium *Dickeya solani* IPO 2019, Isolated from *Hyacinthus orientalis*

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Abstract

Dickeya solani is an emerging plant-pathogenic bacterium causing disease symptoms in a variety of agriculturally relevant crop species worldwide. To date, a number of *D. solani* genomes have been sequenced and characterized; the great majority of these genomes have, however, come from *D. solani* strains isolated from potato (*Solanum tuberosum* L.) and not from other plant hosts. Herewith, we present the first complete, high-quality genome of *D. solani* IPO 2019 (LMG 25990), isolated from the ornamental plant *Hyacinthus orientalis*. The genome of *D. solani* IPO 2019 consists of one chromosome of 4,919,542 bp, with a GC content of 56.2% and no plasmids. The genome contains 4,502 annotated features, 22 ribosomal RNA genes, 73 transfer RNA genes, and one CRISPR. We believe that the information on this high-quality, complete, closed genome of *D. solani* strain isolated from a host plant different from potato (i.e. hyacinth) will provide resources for comparative genomic studies and for analyses targeting adaptation and ecological fitness mechanisms present in *Dickeya solani* species.

Genome Announcement

Dickeya solani (former *Dickeya* spp. biovar 3) is an emerging plant-pathogenic bacterium, causing disease symptoms in a variety of agricultural plant species worldwide (Toth et al. 2011; van der Wolf et al. 2014). The pathogen was first reported in potato in the early 2000s as a distinct genetic clade of *Dickeya* spp. biovar 3, causing severe outbreaks in potato cultivation in different European countries (Czajkowski et al. 2009; Laurila et al. 2010; Slawiak et al. 2009). Until now, the pathogen has been repeatably isolated from symptomatic as well as from symptomless potato plants grown in agricultural fields across Europe and worldwide (van der Wolf et al. 2014). As *D. solani* was reported to cause severe disease symptoms under hot climatic conditions and because there is an observed relationship between *D. solani* high disease incidences in crops and the presence of hot growing seasons in temperate countries (Degefu et al. 2013; Tsror (Lahkim) et al. 2013), it has been speculated that the bacterium has been introduced from one or more subtropical and tropical agricultural regions outside Europe to Europe (Slawiak et al. 2009; Toth et al. 2011). Likewise, it has been suggested

Funding

The work was financially supported by the Polish Ministry of Higher Education (Ministerstwo Nauki i Szkolnictwa Wyższego, Polska) funds DS 531-N104-D800-21 to R. Czajkowski and by the Polish Ministry of Science and Higher Education (Ministerstwo Nauki i Szkolnictwa Wyższego, Polska) funds DS 531-M105-D786-21 to S. Jafra.

Keywords

adaptation, blackleg, *Erwinia chrysanthemi*, flower bulb, genomics, plant pathogen, potato, soft rot

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The author(s) declare no conflict of interest.

Accepted for publication 15 April 2021.

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that *D. solani* might have first appeared either in ornamental plants cultivated commercially outside Europe, in plants grown in Europe in glasshouses under elevated temperature regime, or both and from there spread to the potato ecosystem in temperate regions (Slawiak et al. 2009). At this time, due to the limited number of *D. solani* strains isolated from different (e.g., tropical, subtropical, ornamental) plant hosts, there is not enough data supporting either of these hypotheses. However, it is well-accepted that the pathogen has been only recently introduced to the potato ecosystem, as most of the strains of *D. solani* analyzed belong to the same haplotype and express only a low number of genetic variations (Khayati et al. 2016).

Since the establishment of the *D. solani* species in 2014 (van der Wolf et al. 2014), strain IPO 2222 (DSM 28711, LMG 25993, NCPPB 4479) is recognized as a type strain. A complete, closed, high-quality genome sequence of *D. solani* IPO 2222 isolated from potato together with the corresponding phylogenetic and phenotypic information about IPO 2222 has been published (Khayati et al. 2016). Likewise, to date, a number of *D. solani* genomes have been sequenced and characterized (National Center for Biotechnology Information [NCBI] database). At the time of manuscript preparation (February 2021), the great majority of these (completed and draft) genome sequences, however, have come from *D. solani* strains isolated exclusively from potato and not from other plant hosts.

D. solani IPO 2019 was isolated from *Hyacinthus orientalis* (common name: garden hyacinth, Dutch hyacinth) in 2009 in the Netherlands. The strain has been deposited in the Belgian Coordinated Collection of Microorganisms (BCCM) by J. van Vaerenbergh (Institute for Agricultural, Fisheries and Food Research, Ostend, Belgium) under collection number LMG 25990. In our comparative phenotypic studies involving *D. solani* strains IPO 2019 and IPO 2222, we detected that IPO 2019 but not IPO 2222 was able to utilize a unique panel of saccharides including dextrin, D-maltose, D-trehalose, gentobiose, D-turanose, L-rhamnose, and sorbitol as carbon sources (P. Bartnik and R. Czajkowski, unpublished). To further explore the hypothesis that IPO 2019 isolated from hyacinth may show distinct phenotypic and genetic characteristics from *D. solani* type strain IPO 2222 isolated from potato and to link the preliminarily observed phenotypes with the genomic data, the genome of IPO 2019 strain has been sequenced and annotated.

For the isolation of genomic DNA, *D. solani* IPO 2019 was grown at 28°C in tryptone soya broth (Oxoid) for 16 h with shaking (150 rpm). Bacterial DNA was isolated using a Wizard Genomic DNA purification kit (Promega) according to the protocol provided by the manufacturer. The IPO 2019 genome was sequenced by a hybrid approach with long reads generated using Oxford Nanopore Technologies (ONT) and short reads generated using Illumina technology. DNA sequencing libraries were prepared according to the manufacturer (for ONT, it was LSK kit and, for Illumina, it was a Nextera XT kit). Long reads were generated by a single Flongle run on MinION, and short reads were generated during the Midoutput run on Illumina Miniseq. *De novo* assembly of long reads was performed using Flye 2.8.2, with final mean coverage $\times 28$. Initial genome polishing was conducted by a tool integrated into the assembler. Pilon 1.23 was used for further error corrections. The final mean coverage of short reads to the polished genome was 748.9. The combined procedure produced a single contig of 4,919,542 bp (Table 1), which was further manually inspected and curated. Repetitive genetic elements were searched using Geneious (Kearse et al. 2012). Finally, BLAST, InterProScan, and HMMER (phmmer, UniProtKB) (Finn et al. 2011) were used to annotate the IPO 2019 genome. The obtained structural and functional annotations were retested using RAST (rapid annotation using subsystem technology) (Aziz et al. 2008) and DFAST (Tanizawa et al. 2018). Preliminary comparative genomics analyses (IPO 2019 vs. IPO 2222) were done using EDGAR (Blom et al. 2009, 2016). The presence of prophages (bacteriophage sequences) in the genome of IPO 2019 was predicted using PHASTER (Arndt et al. 2016) and the presence of gene clusters involved in the biosynthesis of secondary metabolites was predicted using anitSMASH 5.0 database (Blin et al. 2019).

The complete, closed, high-quality genome of *D. solani* IPO 2019 has been uploaded to NCBI GenBank under accession number CP071062 (Table 1). Upon submission to NCBI GenBank, the genome was also annotated by the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016). The genome (Fig. 1) is a double-stranded 4,919,542 bp DNA sequence, possessing 4,502 annotated features and an average GC content of 56.2% with no plasmids. The average gene length was predicted to be approximately 1,200 bp, the average protein length was predicted to be approximately 333 amino acids, and 85.8% of the genome consists

Table 1. General features of the *Dickeya solani* IPO 2019 complete genome

Parameters	IPO 2019 genome
Genome size (bp)	4,919,542
GC content (%)	56.2
No. contigs	1
N ₅₀	1
Coding ratio (%)	85.8
No. of total coding sequences (features)	4502
Average gene length (bp)	1200
Presence of plasmids	No
Ribosomal RNAs	22
Transfer RNAs	73
CRISPRs	1
No. of intact prophage genomes (according to PHASTER)	0
GenBank accession number	CP071062

Fig. 1. Features of the *Dickeya solani* IPO 2019 genome and its comparison with the IPO 2222 genome. **A**, The circular plot of the IPO 2019 and IPO 2222 genomes and **B**, the core genome shared between *D. solani* strains IPO 2019 and IPO 2222. The comparative analyses were done using EDGAR, an enhanced software platform for comparative gene content analyses (Blom et al. 2016).

of coding regions. The *D. solani* IPO 2019 genome encodes 22 ribosomal RNAs, 73 transfer RNAs, and one CRISPR. We also identified 11 gene clusters responsible for the synthesis of secondary metabolites. Six of these matched known clusters for the biosynthesis of non-ribosomal peptide synthases, batalactone, cyanobacin, arylpolyene, and thiopeptide. These clusters are conserved in genomes of the other *D. solani* strains analyzed for production of secondary metabolites, i.e., strain 3337 (RNS 08.23.3.1.A) (Pédron et al. 2014), strain D s0432-1 (Garlant et al. 2013), and type strain IPO 2222 (Khayati et al. 2016). Although the core genome, shared between IPO 2019 and IPO 2222, contains 4,470 open reading frames (ORFs), the genome of IPO 2019 contains 32 ORFs absent in the genome of IPO 2222 (Fig. 1). However, these genes were characterized as coding for hypothetical proteins with unknown functions, showing no homology with genes present in the NCBI database.

The investigation of the IPO 2019 genome for the presence of viral sequences performed with PHASTER did not result in the prediction of regions containing intact (complete) viral genomes; three regions in the genome were, however, found containing incomplete prophage genomes.

The first, completed, high-quality genome of *D. solani* strain isolated from the hyacinth plant may prove helpful in (comparative) adaptation studies and ecological fitness analyses done to understand better the origin, spread, and virulence traits of this new, emerging *Dickeya* species.

Acknowledgements

The authors thank J. M. van der Wolf (Plant Research International, Wageningen University and Research Center, Wageningen, The Netherlands) for providing *D. solani* IPO 2019 for this study.

Author-Recommended Internet Resources

BLAST: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>

DFAST: <http://dfast.ddbj.nig.ac.jp>

Flye 2.8.2: <https://github.com/fenderglass/Flye>

HMMER: <http://hmmer.org>

InterProScan: <http://www.ebi.ac.uk/Tools/pfa/iprscan>

NCBI *Dickeya solani* database: <https://www.ncbi.nlm.nih.gov/genome/14829>

NCBI Prokaryotic Genome Annotation Pipeline: https://www.ncbi.nlm.nih.gov/genome/annotation_prok

Pilon 1.23: <https://github.com/broadinstitute/pilon>

RAST: <http://rast.nmpdr.org>

Literature Cited

- Arndt, D., Grant, J. R., Marcu, A., Sajed, T., Pon, A., Liang, Y., and Wishart, D. S. 2016. PHASTER: A better, faster version of the PHAST phage search tool. *Nucleic Acids Res.* 44 (W1):W16-W21.
- Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E. M., Kubal, M., Meyer, F., Olsen, G. J., Olson, R., Osterman, A. L., Overbeek, R. A., McNeil, L. K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G. D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., and Zagnitko, O. 2008. The RAST server: Rapid annotations using subsystems technology. *BMC Genomics* 9:75.
- Blin, K., Shaw, S., Steinke, K., Villebro, R., Ziemert, N., Lee, S. Y., Medema, M. H., and Weber, T. 2019. antiSMASH 5.0: Updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res.* 47 (W1):W81-W87.
- Blom, J., Albaum, S., Doppmeier, D., Puhler, A., Vorholter, F.-J., Zakrzewski, M., and Goesmann, A. 2009. EDGAR: A software framework for the comparative analysis of prokaryotic genomes. *BMC Bioinformatics* 10:154.
- Blom, J., Kreis, J., Spänig, S., Juhre, T., Bertelli, C., Ernst, C., and Goesmann, A. 2016. EDGAR 2.0: An enhanced software platform for comparative gene content analyses. *Nucleic Acids Res.* 44 (W1):W22-W28.
- Czajkowski, R., van Veen, J. A., and van der Wolf, J. M. 2009. New biovar 3 *Dickeya* spp. strain (syn. *Erwinia chrysanthemi*) as a causative agent of blackleg in seed potato in Europe. (Abstr.) *Phytopathology* 99:S6.27.
- Degefu, Y., Potrykus, M., Golanowska, M., Virtanen, E., and Lojkowska, E. 2013. A new clade of *Dickeya* spp. plays a major role in potato blackleg outbreaks in North Finland. *Ann. Appl. Biol.* 162:231-241.
- Finn, R. D., Clements, J., and Eddy, S. R. 2011. HMMER web server: Interactive sequence similarity searching. *Nucleic Acids Res.* 39 (Suppl):W29-W37.
- Garlant, L., Koskinen, P., Rouhiainen, L., Laine, P., Paulin, L., Auvinen, P., Holm, L., and Pirhonen, M. 2013. Genome sequence of *Dickeya solani*, a new soft rot pathogen of potato, suggests its emergence may be related to a novel combination of non-ribosomal peptide/polyketide synthetase clusters. *Diversity (Basel)* 5:824-842.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., and Drummond, A. 2012. Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647-1649.
- Khayati, S., Blin, P., Chong, T. M., Chan, K.-G., and Faure, D. 2016. Complete genome anatomy of the emerging potato pathogen *Dickeya solani* type strain IPO 2222(T). *Stand. Genomic Sci.* 11:87.
- Laurila, J., Hannukkala, A., Nykyri, J., Pasanen, M., Hélias, V., Garland, L., and Pirhonen, M. 2010. Symptoms and yield reduction caused by *Dickeya* spp. strains isolated from potato and river water in Finland. *Eur. J. Plant Pathol.* 126:249-262.
- Pédron, J., Mondy, S., Raoul des Essarts, Y., Van Gijsegem, F., and Faure, D. 2014. Genomic and metabolic comparison with *Dickeya dadantii* 3937 reveals the emerging *Dickeya solani* potato pathogen to display distinctive metabolic activities and T5SS/T6SS-related toxin repertoire. *BMC Genomics* 15:283-295.
- Slawiak, M., van Beckhoven, J. R. C. M., Speksnijder, A. G. C. L., Czajkowski, R., Grabe, G., and van der Wolf, J. M. 2009. Biochemical and genetical analysis

- reveal a new clade of biovar 3 *Dickeya* spp. strains isolated from potato in Europe. *Eur. J. Plant Pathol.* 125:245-261.
- Tanizawa, Y., Fujisawa, T., and Nakamura, Y. 2018. DFAST: A flexible prokaryotic genome annotation pipeline for faster genome publication. *Bioinformatics* 34: 1037-1039.
- Tatusova, T., DiCuccio, M., Badretdin, A., Chetvermin, V., Nawrocki, E. P., Zaslavsky, L., Lomsadze, A., Pruitt, K. D., Borodovsky, M., and Ostell, J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* 44:6614-6624.
- Toth, I. K., van der Wolf, J. M., Saddler, G., Lojkowska, E., Hélias, V., Pirhonen, M., Tsrör (Lahkim), L., and Elphinstone, J. G. 2011. *Dickeya* species: An emerging problem for potato production in Europe. *Plant Pathol.* 60:385-399.
- Tsrör (Lahkim), L., Ben-Daniel, B., Chalupowicz, L., van der Wolf, J., Lebiush, S., Erlich, O., Dror, O., Barel, V., Nijhuis, E., and Manulis-Sasson, S. 2013. Characterization of *Dickeya* strains isolated from potato grown under hot-climate conditions. *Plant Pathol.* 62:1097-1105.
- van der Wolf, J. M., Nijhuis, E. H., Kowalewska, M. J., Saddler, G. S., Parkinson, N., Elphinstone, J. G., Pritchard, L., Toth, I. K., Lojkowska, E., Potrykus, M., Waleron, M., de Vos, P., Cleenwerck, I., Pirhonen, M., Garland, L., Hélias, V., Pothier, J. F., Pflüger, V., Duffy, B., Tsrör, L., and Manulis, S. 2014. *Dickeya solani* sp. nov., a pectinolytic plant pathogenic bacterium isolated from potato (*Solanum tuberosum*). *Int. J. Syst. Evol. Microbiol.* 64:768-774.