High-Quality Complete Genome Resource of Plant-Pathogenic Bacterium *Pectobacterium atrosepticum* Strain Green1 Isolated from Potato (*Solanum tuberosum* L.) in Greenland

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Abstract

Pectobacterium atrosepticum is a narrow-host-range, pectinolytic, plant-pathogenic bacterium causing blackleg of potato (*Solanum tuberosum* L.) worldwide. Till present, several *P. atrosepticum* genomes have been sequenced and characterized in detail; however, all of these genomes have come from *P. atrosepticum* isolates from plants grown in temperate zones, not from hosts cultivated under different climatic conditions. Herewith, we present the first complete, high-quality genome of the *P. atrosepticum* strain Green1 isolated from potato plants grown under a subarctic climate in Greenland. The genome of *P. atrosepticum* strain Green1 consists of one chromosome of 4,959,719 bp, with a GC content of 51% and no plasmids. The genome contains 4,531 annotated features, including 4,179 protein-coding genes, 22 ribosomal RNA genes, 70 transfer RNA genes, 8 non-coding RNA genes, 2 CRISPRs, and 126 pseudogenes. We believe that the information in this first high-quality, complete, closed genome of *P. atrosepticum* strains isolated from host plants grown in a subarctic agricultural region will provide resources for comparative genomic studies and for analyses targeting climatic adaptation and ecological fitness mechanisms present in *P. atrosepticum*.

Genome Announcement

Pectobacterium atrosepticum (formerly *Erwinia carotovora* subsp. *atroseptica*), belonging to the soft-rot *Pectobacteriaceae* family (SRP; formerly pectinolytic *Erwinia* spp.), is a plant-pathogenic bacterium with a narrow host range primarily limited to potato (*Solanum tuberosum* L.) cultivated in temperate agricultural regions (Harris and Lapwood 1977; Pérombelon 2000).

In the past, particularly in Europe and North America, *P. atrosepticum* was regarded as the dominant causative agent of potato blackleg, repeatably isolated from both symptomatic and symptomless potato plants grown in agricultural regions across Europe and worldwide (Pérombelon 1991, 2002). In the 1970s, Molina and Harrison (1977) reported that the high blackleg incidences in potato crops in Colorado, United States, were almost exclusively

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Keywords

adaptation, blackleg, *Erwinia carotovora* subsp. *atroseptica*, genomics, microbial ecology, population biology, plant pathogen, potato

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caused by *P. atrosepticum* (Molina and Harrison 1977). Pérombelon (1972) in Scotland showed that up to 80% of progeny tubers were contaminated with *P. atrosepticum*, although the great majority of infected plants did not show any disease symptoms (Pérombelon 1972). Today, the bacterium still remains the prevalent SRP species causing blackleg in several temperate areas of the Northern Hemisphere, including the United Kingdom, Norway, and Canada (van der Wolf et al. 2021). Results from the seed potato surveys performed recently in England, Wales, and Scotland indicated that *P. atrosepticum* still represented the majority (over 89%) of all samples containing pectinolytic bacteria (van der Wolf et al. 2021).

Similarly, *P. atrosepticum* was also one of the most frequently detected *Pectobacterium* spp. in surveys done between 2015 and 2017 in Northern Ireland (Zaczek-Moczyłowska et al. 2019). Likewise, some SRP bacteria are also known to live as epiphytes and endophytes on plants and as saprophytes in soil and groundwater (Bell et al. 2004). However, these types of *P. atrosepticum* lifestyles remain largely unexplored (Bell et al. 2004). Therefore, it cannot be excluded that the pathogen is more widely distributed in the environment outside agricultural fields in Europe and worldwide than expected.

P. atrosepticum is known to be found in cool temperate regions worldwide, causing symptoms at average temperatures below 25°C (Kaczynska et al. 2021; Pérombelon 2002). Temperature plays an essential role in the pathogenicity of SRP bacteria (du Raan et al. 2015; Lebecka 2013). It may act as a trigger that activates the expression of specific virulence factors during infection (Smirnova et al. 2001). It is widely acknowledged that different SRP species possess different growth temperature optima (du Raan et al. 2015; Kaczynska et al. 2021; Lebecka 2013), under which they can prevail in the environment and cause disease symptoms in host plants. Comparing the optimal growth temperatures of all SRP bacterial species, *P. atrosepticum* isolates are known to grow and cause symptoms in plants at the lowest environmental temperatures (18°C) observed during crop cultivation (du Raan et al. 2015; Lebecka et al. 2018).

The *P. atrosepticum* strain NCPPB 549 (CFBP 1526, CIP 105192, G/39, ICMP 1526, ICPB EA 172, LMG 2386, PDDCC 1526, and genome GenBank accession number JQHK000000000.1) isolated in 1957 in Scotland, United Kingdom is recognized as a type strain of this species (Gardan et al. 2003). In contrast, strain SCRI 1043 (ATCC BAA-672, genome GenBank accession number NC_004547.2) isolated in 1985 in the United Kingdom has been widely used as a reference for the majority of the (molecular) work done to characterize the virulence determinants of the pathogen and *P. atrosepticum* interactions with host plants (Bell et al. 2004; Hinton et al. 1989; Kaczynska et al. 2021; Mulholland et al. 1993; Toth et al. 1997, 1999b). To date, several other *P. atrosepticum* genomes have been sequenced and characterized. However, at the moment of manuscript preparation for this article (May 2021), all of these genome sequences have come from *P. atrosepticum* strains isolated exclusively from plants grown in temperate climate zones.

P. atrosepticum strain Green1 was isolated from a potato (*S. tuberosum* L.) plant in 2019 in southern Greenland (de Neergaard et al. 2020). During the blackleg and soft rot surveys done in the potato fields in agricultural regions, samples of plants and tubers with typical blackleg and soft rot symptoms were collected at six areas and tested for the presence of SRP bacteria, including *P. atrosepticum*. Ten strains, named Green1 to Green10, representing SRP isolates from symptomatic plants from different locations in Greenland, were all classified as *P. atrosepticum* based on 16S ribosomal RNA (rRNA), *recA*, and detailed biochemical tests (de Neergaard et al. 2020).

It is generally accepted that *P. atrosepticum* strains isolated thus far demonstrate a high degree of similarity (De Boer et al. 1979; Ślędź et al. 2000; Toth et al. 1999a). Therefore, to assess the genetic diversity of the Green1 to Green 10 isolates collected in the survey, the repetitive element sequence-based PCR (rep-PCR) (Versalovic et al. 1994), executed as described by Czajkowski et al. (2010), was used. rep-PCR analysis showed that the fingerprints of all 10 isolates were identical (data not shown).

In our comparative phenotypic studies involving *P. atrosepticum* strains Green1 to Green10 and the reference strain SCRI 1043, we detected that Greenlandic strains but not SCRI 1043 showed the ability to utilize pyruvic acid methyl ester, L-asparagine, myo-inositol, and acetic acid. In contrast, SCRI 1043 but not Green1-Green10 isolates used D-galacturonic acid, D-maltose, D-turanose, and D-glucuronic acid as the sole carbon source (R. Czajkowski, unpublished).

Subarctic and arctic agricultural areas represent a unique environment characterized by extreme temperature, elevated UV radiation, and low nutrient and low water content (Altdorff

et al. 2021; McCartney and Lefsrud 2018). This environment may be a source of microbes, including adapted plant pathogens, with unique features. The southern Greenland agricultural regions (60 to 61° 15' N) are characterized by the average temperatures from -7 to -4°C in January and from 9 to 11°C in July and annual precipitations varying between 260 and 815 mm, depending on the location. To further explore the hypothesis that Greenlandic isolates of *P. atrosepticum* may show distinct phenotypic and genetic characteristics from *P. atrosepticum* reference strain SCRI 1043 and to link the preliminarily observed phenotypes with the genomic data, the genome of Green1 strain has been sequenced and annotated.

For the isolation of genomic DNA, *P. atrosepticum* strain Green1 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 Corp.) according to the protocol provided by the manufacturer. The Green1 genome was sequenced by a hybrid approach with long reads generated using Oxford Nanopore Technologies (ONT) and short reads generated using Illumina technology, as described earlier (Czajkowski et al. in press). DNA sequencing libraries were prepared according to the manufacturer (for ONT, it was the 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 Mini-seq. De novo assembly of long reads was performed and validated using Pomoxis, Medaka, and Unicycler, with final mean coverage of 230x. Initial genome polishing was conducted by a tool integrated into the assembler. Pilon 1.23 was used to further error corrections. The combined procedure produced a single contig of 4,959,719 bp 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 *P. atrosepticum* Green1 genome. The obtained structural and functional annotations were retested using Rapid Annotation using Subsystem Technology (Aziz et al. 2008) DFAST (Tanizawa et al. 2018). Preliminary comparative genomics analyses (Green1 versus SCRI 1043 [reference strain]) were done using EDGAR 3.0 (Blom et al. 2009, 2016). The presence of prophages (bacteriophage sequences) in the genome of Green1 was predicted using Prophage-Hunter (Song et al. 2019) and PHASTER (Arndt et al. 2016). The presence of gene clusters involved in the biosynthesis of secondary metabolites was predicted using the antiSMASH 5.0 database (Blin et al. 2019).

The complete, closed, high-quality genome of *P. atrosepticum* strain Green1 has been uploaded to NCBI GenBank and received the accession number CP055224. 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 4,959,719-bp DNA sequence possessing 4,531 annotated features, including 4,179 protein-coding genes and an average GC content of 51% with no plasmids (Table 1). The average gene length was predicted to be approximately 1,100 bp and the average protein length was approximately 366 amino acids. In all, 85.3% of the genome consists of coding regions. The *P. atrosepticum* Green1 genome encodes 22 rRNAs, 70 transfer RNAs, 8 noncoding RNA genes, 2 CRISPRs, and 126 pseudogenes.

Using antiSMASH, we also identified nine gene clusters responsible for the synthesis of secondary metabolites in the genome of *P. atrosepticum* strain Green1. They matched the secondary metabolite clusters described previously in *Enerobacteria* (Mohite et al. 2019) and *Pectobacteria* (Jonkheer et al. 2021). Seven of these (namely, siderophore, betalactone, phenazine, oronofacic acid, amonabactin, hserlactone, and arylpolyene) were present in the genome of both the reference *P. atrosepticum* strain SCRI1043 and Greenlandic strain Green1. In contrast, the other two (anikasin and thiopeptide) were found only in the genome of strain Green1. One of the secondary metabolites predicted to be encoded by the Green1 genome (anikasin) is a cyclic lipopeptide, enabling swarming motility of *Pseudomonas fluorescence* strain HKIO770, protecting the bacterium from protozoal grazing (Götze et al. 2017). The role of anikasin in the ecology of *Pectobacterium* spp., including *Pectobacterium atrosepticum*, has not been assessed yet; however, it can be speculated that the metabolite may protect the Green1 strain from predators found in soil. The second metabolite (thiopeptide) is an antibiotic known to be produced by microbial dwellers present in soil (Just-Baringo et al. 2014). Likewise, its role in the ecology of *P. atrosepticum* strain Green1 remains unknown. Still, based on literature data, it can be speculated that this antibiotic may be crucial for the competitive survival of the bacterium in the soil environment.

Fig. 1. Features of the *Pectobacterium atrosepticum* strain Green1 genome and its comparison with the SCRI 1043 genome. **A**, Circular plot of the Green1 and SCRI 1043 genomes and **B**, core genome shared between *P. atrosepticum* strains Green1 and SCRI 1043. Comparative analyses were done using EDGAR 3.0, an enhanced software platform for comparative gene content analyses (Blom et al. 2009, 2016).

Although the core genome shared between Green1 and SCRI 1043 calculated with EDGAR 3.0 contains 4,312 open reading frames, the genome of Green1 contains 205 singletons, absent in the genome of *P. atrosepticum* strain SCRI 1043. The great majority of these singletons (168 genes) were predicted to encode hypothetical proteins for which no homology with known proteins could be found. However, the genome of strain Green1 also contained singletons coding for, among others, toxin or antitoxin proteins (CcdA or CcdB), conjugal transfer protein TraA, transcriptional factors, site-specific recombinases, mobile genetic elements, and restriction or modification systems not found in the genome of the reference *P. atrosepticum* strain SCRI 1043. These genes may give the bacterium ecological advantages over SCRI 1043 when both species are present in the same niche. Still, more work is needed to (experimentally) assess the (comparative) ecology of *P. atrosepticum* strain Green1 in detail.

The investigation of the Green1 genome for the presence of viral sequences done with Prophage-Hunter and PHASTER did not, in both cases, result in the prediction of regions containing intact (complete) viral genomes. However, several regions in the genome of Green1 were found, harboring incomplete prophage genomes or individual phage-derived genes. For example, the gene coding for Tin protein present in the genome of Green1 strain may provide the bacterium with resistance against T-even phages in the environment (Christie and Calendar 2016), which would have an ecological relevance in the phage-full settings.

Table 1. General features of the *Pectobacterium atrosepticum* strain Green1 complete genome

Features	Green1
Genome size (bp)	4,959,719
GC content (%)	51
Number of contigs	1
N ₅₀	1
Coding ratio (%)	85.3
Number of total annotated sequences (features)	4,531
Average gene length (bp)	1,100
Presence of plasmids	No
Ribosomal RNAs	22
Transfer RNAs	70
CRISPRs	2
Number of intact prophage genomes ^a	0
GenBank accession number	CP055224

^a According to PHASTER and Prophage-Hunter.

The first, completed, high-quality genome of *P. atrosepticum* strain Green1 isolated from potato grown in a subarctic agricultural area may prove helpful in (comparative) climatic adaptation studies and ecological fitness analyses done to better understand the origin, spread, and virulence traits of *Pectobacterium* spp.

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Author-Recommended Internet Resources

antiSMASH 5.0 database: <https://antismash.secondarymetabolites.org/#/start>
BLAST: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>
DFAST: <https://dfast.ddbj.nig.ac.jp/>
EDGAR 3.0: https://edgar3.computational.bio.uni-giessen.de/cgi-bin/edgar_login.cgi?cookie_test=1
InterProScan: <https://www.ebi.ac.uk/interpro/search/sequence/>
Medaka: <https://github.com/nanoporetech/medaka>
NCBI Prokaryotic Genome Annotation Pipeline:
https://www.ncbi.nlm.nih.gov/genome/annotation_prok/
Pectobacterium atrosepticum genomes: <https://www.ncbi.nlm.nih.gov/genome/1088>
PHASTER: <https://phaster.ca/>
Pilon 1.23: <https://github.com/broadinstitute/pilon>
Pomoxis: <https://nanoporetech.github.io/pomoxis/>
Preprint version of this article: <https://www.biorxiv.org/content/10.1101/2021.06.01.446529v1>
Prophage-Hunter: <https://pro-hunter.genomics.cn/>
Unicycler: <https://github.com/rwrick/Unicycler>

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