Abstract As a part of a blackleg and soft rot survey on potato plants cultivated in the agricultural region of Southern Greenland during the growing season of 2019 diseased potato plants and tubers were collected at six localities. Samples of plants and tubers with typical blackleg and soft rot symptoms were cut into parts weighing ca. 10–15 g each and incubated in potato enrichment broth (PEB) under anaerobic conditions for 4–7 days at ca. 20–22 °C (conditions during shipment of the samples from Greenland to Poland). After enrichment plant extracts were plated on crystal violet pectate (CVP) medium to isolate individual cavity-forming bacterial colonies. The inoculated plates were incubated at 22, 25 and 28 °C to maximize the chance of finding pectinolytic bacteria with different growth temperature optima. Cavity-forming isolates were collected and purified to pure cultures on tryptone soya agar (TSA). Ten cavity-forming isolates, named Green1 – Green10, representing isolates from symptomatic plants from different locations, were selected for analyses. The isolates gave a 434 bp. product in *Pectobacterium* spp.-specific PCR and a 439 bp. product in *P. atrosepticum* (Pba) - specific PCR. These isolates were Gram (−) rods, facultative anaerobic, catalase positive, oxidase and indole negative, grew in TSB + 5% NaCl, produced acid from lactose, maltose and raffinose. BlastN analyses of the ca. 1300 bp. 16S rDNA sequences of all 10 strains indicated a 99–100% similarity to the 16S rDNA of *Pectobacterium atrosepticum*. All 10 isolates caused soft rot of potato tuber slices after 72 h at 28 °C. Phylogenetic analysis based on the recA gene sequence grouped the isolates together with *P. atrosepticum* strains CFBP1526T and SCRI1043. This is the first report on isolation of *P. atrosepticum* from blackleg-diseased potato plants in Greenland. The presence of *P. atrosepticum* and its possible impact on potato cultivation in Greenland is discussed.

Keywords *Erwinia atroseptica* · *Solanum tuberosum* · Wilting · Soft rot · Chlorosis · Climate change

Potato (*Solanum tuberosum* L.) is a worldwide cultivated tuberous plant and the fourth main food crop in the world after rice, maize and wheat both when counting area used for crop cultivation and its total production (Bishop et al. 2012; Bradshaw and Ramsay 2009, FAOSTAT 2020 [http://www.fao.org]). The area of potato cultivation is rapidly increasing especially in developing regions. This is due to the fact that commercial potato cultivars may be selected to grow under a broad range of soil and temperature conditions. Likewise, potato has been for a long time a major field crop in temperate and progressively also in warmer
European regions (Czajkowski et al. 2011, Pawelzik and Möller 2014).

With the increasing temperatures due to climate change, the agricultural production in subarctic and boreal regions in Greenland is expected to expand in the near future (Barbeau et al. 2015; Lehmann et al. 2016; Munk et al. 2009). In Greenland, potato tubers are produced mainly within the agricultural area in the southwestern part of the island, mainly along the fjords on the west coast between 60 °N and 61.5 °N. Potato plants are cultivated on a commercial scale by a limited number of sheep holders. Furthermore, a small scale production of potato tubers for private consumption takes place at many locations in Greenland (de Neergaard and Harding 2020).

In Greenland, no production of certified seed potato tubers takes place. Seed potato tubers are imported from the European Union, primarily from Denmark, Norway and the Netherlands (de Neergaard, personal communication). Alternatively, home-saved potatoes are used as a planting material in the following season (Munk et al. 2009). Likewise, potatoes for consumption imported from outside Europe (e.g. Egypt) and sold in local supermarkets are occasionally used as seed material. Planting of potato tubers takes place between late May and mid-June, and the crop is harvested from mid-August to September. Due to steadily decreasing rainfall, reported during the recent decades, irrigation is frequently applied throughout the entire growing season (potato growers, personal communication). It is worth noting that Greenlandic potato production is carried out without the use of pesticides and that crop rotation is not practiced at the majority of potato production sites (potato growers, personal communication).

The majority of potato diseases present in Europe and worldwide (excluding late blight) has been already reported in Greenland (de Neergaard et al. 2014; de Neergaard and Harding 2020). In years with humid weather conditions suitable for pathogen spread, potato diseases develop often as vigorously as in the other parts of continental Europe (de Neergaard and Harding 2020). This is somehow remarkable since agriculture in Greenland is characterized by: (i) isolated cultivation sites often situated far from each other, (ii) very cold winter season, (iii) a very low number or absence of alternative hosts, as well as (iv) the absence of volunteers to transmit the pathogens to the following season.

In surveys undertaken in the period of 2007–2010 plants with symptoms of blackleg and soft rot were recorded in 11 out of 15 commercial potato fields screened (de Neergaard and Harding 2020). Blackleg and soft rot field assessments done in 2009 resulted in the incidence (measured as a percentage of symptomatic plants present) values of 2 to 7% (Munk and de Neergaard, personal observations). The accepted limit of disease incidence in seed potato tubers in Europe differs per tuber class and the respective EU member state regulations (Toth et al. 2011) but, in general, the incidence in certified seed tubers used as a planting material should remain zero or (in some cases) as low as possible. In Denmark the upper accepted limit of soft rot in certified seed potato tubers is 0.2% (Ministry of

![Fig. 1](image)

Fig. 1 Locations of potato fields in South Greenland from where diseased potato samples were obtained. 1: Eqaluit Ilua Sermilik, 61° 07’ N, 45° 58’ W, 2: Sarlia, Alanngorsup Nuna, 60° 50’ N, 46° 08’ W, 3: Qaqortoq, 60° 43’ N, 46° 02’ W, 4: Ammassivik 60° 36’ N, 45° 23’ W, 5: Saputit Tasia, 60° 14’ N, 44° 45’ W, 6: Narsarsuaq, 61° 10’ N, 45° 26’ W. No *P. atroseptica* isolates were found in samples collected from symptomatic plants at Saputit Tasia.
A follow-up survey for potato blackleg and soft rot was undertaken in the potato production region in South Greenland from August to September 2019. All commercial potato growers (six) were included in the survey. Furthermore, the plants from potato cultivar trials at the Agricultural Experiment Station in Upernaviarsuk together with the plants in 12 smallholder sites with potato cultivation were evaluated for the presence of blackleg and soft rot. At six localities (Fig. 1) symptoms including blackleg, chlorosis, wilting, stem rot, pre-emergence tuber rot and soft rot of (seed and progeny) tubers in field-growing potato plants were observed (Fig. 2). These symptoms resembled typical potato blackleg symptoms caused by soft rot Pectobacteriaceae (SRP: Pectobacterium spp. and Dickeya spp.) previously described on potatoes in Europe and worldwide (Pérombelon 2002; Charkowski 2007).

Stem parts with typical blackleg symptoms and potato tubers expressing soft rot were collected and transported to the field laboratory in Greenland. Samples were incubated in potato enrichment broth (PEB) medium (Pérombelon and van der Wolf 2002) in 50 ml Falcon tubes for 4–7 days at 20–22 °C to increase the size of SRP bacterial populations. The tubes were tightly
closed to provide low oxygen conditions during incubation. The enriched samples were used for isolation of SRP. In the laboratory in Poland, 100 μl each of the enriched samples was serially diluted and separately plated on crystal violet pectate (CVP) (Hélias et al. 2011) plates and incubated at 22, 25 and 28 °C for up to 5 days. Three different incubation temperatures were chosen to maximize the chances of finding SRP with different growth temperature optima (du Raan et al. 2016). Pectinolytic bacterial isolates producing typical cavities (pits) on CVP plates were collected and purified to pure cultures on tryptone soya agar (TSA) as previously described by Czajkowski et al. (2015). Ten cavity-forming isolates, named Green1–Green10 (Table 1) and representing isolates collected from symptomatic plants at different locations, were selected for further analysis.

Gram staining of the isolates was done as described by Coico (2006). Biochemical assays, including growth at 37 °C, growth in tryptone soya broth (TSB) supplemented with 5% NaCl, growth under anaerobic conditions, activity of catalase and oxidase, production of indole, production of acid from lactose, maltose and raffinose were done as described by Pérombelon and van der Wolf (2002) and Czajkowski et al. (2009). The Green1 – Green10 isolates were Gram(−) rods, facultative anaerobic, catalase positive, oxidase and indole negative, did not grow at 37 °C but grew in TSB + 5% NaCl and under anaerobic conditions and produced acid from lactose, maltose and raffinose. These tests allowed classifying the Green1-Green10 isolates to P. atrosepticum species.

For molecular characterization of pectinolytic bacterial isolates a colony PCR procedure was used as described in Czajkowski et al. (2009). PCR detection of Dickeya spp. was performed according to Nassar et al. (1996) using ADE1/ADE2 primers (ADE1: 5′GATCATAAAGCCCGCAGCAT 3′, ADE2: 5′CTGTGGCCGATCAGGATGG 3′). Detection of Pectobacterium spp. was performed according to Darrasse et al. (1994) using Y1/Y2 primers (Y1: 5′TTACCAGGACGACGCTGTCG 3′, Y2: 5′CAGGAAGATGTCGTTATCGCAG 3′). PCR detection of P. atrosepticum was performed according to Frechon et al. (1998) using Y45/Y46 (Y45: 5′TCACCAGGACGCGCCGAGCTGTCG 3′, Y46: 5′TCGCCAACGTTTCGAGCAACAGT 3′) primers. In all cases, amplified DNA was detected by electrophoresis in a 1.5% agarose gel in 0.5 × TBE buffer and stained with 5 mg ml⁻¹ of GelRed (Biotium). The

### Table 1 Bacterial isolates characterized in this study

| No. | Isolate name | Location | Potato cultivar | Cultivation history | Bacterial species present |
|-----|--------------|----------|-----------------|--------------------|--------------------------|
| 1   | Green1       | Qaqortoq, (60°43'N, 46°02'W) | unknown           | continuous potato cultivation, soil pH 4.5 | Pectobacterium atrosepticum |
| 2   | Green2       | Qaqortoq, (60°43'N, 46°02'W) | unknown           | continuous potato cultivation, soil pH 4.5 | P. atrosepticum |
| 3   | Green3       | Ammassivik, (60°36'N, 45°23'W) | Folva            | continuous potato cultivation, soil pH 4.0 | P. atrosepticum |
| 4   | Green4       | Ammassivik, (60°36'N, 45°23'W) | Folva            | continuous potato cultivation, soil pH 4.0 | P. atrosepticum |
| 5   | Green5       | Eqaluit Ilua, Sermilik, (61°07'N, 45°58'W) | Folva            | crop rotation, soil pH 5.0 | P. atrosepticum |
| 6   | Green6       | Sarlia, Alamngorsup Nunaa, (60°50'N, 46°08'W) | Folva            | potato cultivation for the first time on this location, soil pH 4.0 | P. atrosepticum |
| 7   | Green7       | Sarlia, Alamngorsup Nunaa, (60°50'N, 46°08'W) | Folva            | potato cultivation for the first time on this location, soil pH 4.0 | P. atrosepticum |
| 8   | Green8       | Qaqortoq, (60°43'N, 46°02'W) | unknown           | continuous potato cultivation, soil pH 4.5 | P. atrosepticum |
| 9   | Green9       | Narsarsuaq, (61°10'N, 45°26'W) | Asparges         | continuous potato cultivation, soil pH 5.5 | P. atrosepticum |
| 10  | Green10      | Narsarsuaq, (61°10'N, 45°26'W) | Asparges         | continuous potato cultivation, soil pH 5.5 | P. atrosepticum |
Green1 – Green10 bacterial isolates, gave a 434 bp product in Pectobacterium spp. specific PCR and a 439 bp product in P. atrosepticum (Pba) specific PCR but no product was detected after a PCR with ADE1/ADE2 primers. The results of the PCR assays indicated that all 10 isolates belonged to P. atrosepticum.

The genomes of Green1–Green10 were sequenced at the Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, Poland and annotated using RAST (Aziz et al. 2008). Identification of Green1 – Green10 isolates by 16S rDNA sequence analysis was done according to Czajkowski et al. (2012). NCBI BlastN analyses of the ca. 1200–1300 bp. 16S rDNA sequences of Green1 – Green10 strains (Genbank accession no.: MT318667-MT318676) derived from the bacterial genomes indicated a 99–100% similarity to the 16S rDNA of P. atrosepticum. Multiple sequence alignment of recA gene and phylogenetic analyses were performed using Phylogenetic Pipeline of Information Génomique et Structurale, CNRS-AMU, France (http://www.phylogeny.fr/) with bootstrap support for 1000 replicates. The bar indicates the number of substitutions per sequence position. The cutoff for separating the clades was the bootstrap support for particular branch (n) of at least 70% together with the bootstrap support for particular predecessor branch (n-1) of at least 70%. recA gene sequences of P. atrosepticum strain IPO1943, SCR1043 and CFBP1526T, P. carotovorum Subsp. brasiliense CFBP6617, P. carotovorum subsp. carotovorum strain ATTC15713, P. parmentieri strain SCC3193, P. carotovorum strain NCPPB3839, P. carotovorum subsp. odoriferum strain ICMP11533 and D. dadantii strain NCPPB3459 were all retrieved from NCBI Genbank (https://www.ncbi.nlm.nih.gov/genbank/). The recA gene sequence from D. dadantii NCPPB3459 was used as an outgroup.

None of the pectinolytic isolates characterized in this study was classified as Dickeya spp., which may suggest that these bacteria have not yet been introduced to Greenland and/or that the environmental conditions in agricultural fields there (low temperatures during and between growing seasons, low precipitation) do not support buildup of Dickeya spp. populations (Toth et al. 2011). For a long time, Dickeya spp. have been associated with blackleg in tropical and subtropical regions only (Pérombelon 2002), however the so-called
cold tolerant strains of *D. dianthicola* were occasionally found in potato in Western and Northern Europe including Denmark in the last four decades (Janse and Ruissen 1988). Indeed, *D. dianthicola* is now commonly reported in Danish seed potato fields (Nielsen et al. 2011). Since 2000, a new aggressive species of *Dickeya* named *D. solani* has spread in Europe (van der Wolf et al. 2014) including countries with temperate climate: Poland, Sweden, Denmark, the Netherlands and Finland, and the new species seems to replace (competitively) *D. dianthicola* in potato in Danish agricultural fields (cabi.org/isc/datasheet/120278). At the moment *D. solani* remains one of the major threats to potato cultivation in many locations in Europe (van der Wolf et al. 2014). In Denmark, both *D. dianthicola* and *D. solani* have been frequently reported and the isolation of bacteria from the same locations supports the theory that two or three different SRP species may co-occur in the same plant (Nielsen et al. 2011).

Likewise, this survey has not resulted in the isolation of the emerging SRP pathogens currently limiting potato production under European climatic conditions (van der Wolf et al. 2017) viz. *P. parmentieri* and *P. brasiiliense*. It can be assumed that these pathogens have not yet been transferred to Greenland with seed potato tubers originating in Europe and/or that, similarly, as in case of *Dickeya* spp., they require higher temperatures during the growing season to survive and cause symptoms in developing potato plants (van der Wolf et al. 2017).

In conclusion, this is the first report demonstrating the presence of the pectinolytic blackleg pathogen *P. atrosepticum* in potato cultivation in Greenland. Observations made during the growing season and isolation of the bacteria from symptomatic plants and tubers at different locations indicate that the pathogen may be widespread in Greenland. Blackleg and/or soft rot outbreaks in potato may become more severe in the coming years, especially if the climate becomes more suitable for pathogens belonging to *Pectobacteriaceae* (Skelsey et al. 2018). Furthermore, as disease symptoms were frequently observed at production localities where no crop rotation was practiced, the role of pathogen survival in soil should be more deeply investigated (Pérombelon 2002). Precautions, including efficient pathogen detection in potato seed tubers before planting, rotation of crops to decrease the survival of *P. atrosepticum* in field soils, monitoring of surface water for the presence of SRP and compulsory use of certified seed potato tubers should be considered.

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**Compliance with ethical standards** The results presented in this manuscript did not involve any protected and/or endangered species, human participants, specimens or tissue samples, or vertebrate animals, embryos or tissues.

**Conflict of interest** The authors declare that no conflict of interest exists.

**Informed consent** All authors consent to this submission.

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