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First detection of *Pectobacterium carotovorum* associated with potato soft rot in Georgia

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Summary. Potato (*Solanum tuberosum* L.) is an important food crop in Georgia; but use of poor-quality seed potatoes and presence of harmful pathogens are important contributors to low crop productivity. Potato soft rot, caused by *Pectobacterium carotovorum*, had not been previously identified in potato crops in Georgia. During field surveys, potato tubers exhibiting typical soft rot symptoms, including water-soaked lesions, tissue maceration, and discolouration, were collected from farmers' fields, storage facilities, and experimental plots. The sampled potato varieties included Marfona, Jelly, Picasso, Slovyanka, Khortytsa, and Nevsky. Research using cultural, morphological, biochemical, and molecular analyses showed that *Dickeya solani* and *Pectobacterium carotovorum* causing potato soft rot are common in the studied regions of Georgia. These results, together with the 100% sequence identity of the *gapA* gene, provide the first record *P. carotovorum* in Georgia.

Keywords. Bacterial disease, isolate, *gap*A gene, sequence.

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INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are important food crops in Georgia, where annual per capita consumption reaches approx. 55 kg. However, the total area planted with potatoes (14,000–25,000 ha) and the average yields (12.2–15.6 t ha^{-1}) during 2016–2024 were low compared with other European and neighboring countries, including Armenia, Azerbaijan, and Turkey (Geostat, 2024). "Seed degeneration", defined as yield losses in vegetatively propagated crops due to pathogen accumulation over successive propagation cycles, is among the major constraints to potato productivity (Thomas-Sharma *et al*., 2015).

In Georgia, seed potatoes are predominantly self-saved by growers or obtained from local sources. For smallholder farmers, who are the majority

of potato producers in Georgia, certified seed potatoes are often unavailable or prohibitively expensive (Kelsey *et al*., 2021). Consequently, the use of poor-quality seed potatoes and the presence of particularly harmful pathogens are among the main factors contributing to low productivity.

The major potato diseases in Georgia include late blight, caused by *Phytophthora infestans*, and early blight, caused by *Alternaria solani*. In addition, several quarantine potato diseases occur in this country, including brown rot (caused by *Ralstonia solanacearum*; Muradashvili *et al*., 2014), wart (*Synchytrium endobioticum*; Gorgiladze *et al*., 2014), and ring rot (*Clavibacter sepedonicus*; Sadunishvili *et al*., 2020). During 2010 to 2012, outbreaks of blackleg caused by *Dickeya* spp. were recorded in the main potato-producing region of Samtskhe–Javakheti (Tsror *et al.,* 2011). Subsequent molecular studies showed that the majority of soft rot cases in Georgia were caused by *Dickeya solani* (Muradashvili *et al*., 2023). However, *Pectobacterium carotovorum* had not been previously identified in Georgian potato crops.

Bacterial diseases, including soft rot caused by *Pectobacterium carotovorum*, lead to significant losses in international potato production by causing plant wilting and tuber rot under both field and storage conditions (Vand der Wolf, *el al*., 2021). The aim of the present study was to characterize bacterial isolates obtained in Georgia in 2022 to 2023 from potato tubers showing soft rot symptoms. Cultural and biochemical tests, and molecular phylogenetic analyses, were used to determine isolate identity to *Pectobacterium*, potential pathogens not previously reported in association with soft rot of potato tubers in Georgia.

MATERIALS AND METHODS

Field surveys and sample collection

Field surveys for potato soft rot were carried out during the 2022–2023 growing and harvesting seasons in major potato-producing regions of Georgia, including Marneuli (village of Maradis), Bolnisi (village of Nakhiduri), Dmanisi (villages of Sarkineti and Gomareti), Tsalka (village of Artsivani), and Ninotsminda, Adigeni, Oni, and Ambrolauri.

Potato tubers exhibiting typical soft rot symptoms, including water-soaked lesions, tissue maceration, and discolouration, were collected from farmers' fields, storage facilities, and from experimental plots of the Institute of Phytopathology and Biodiversity. Potato varieties sampled included Marfona, Jelly, Picasso, Slovyanka, Khortytsa, and Nevsky.

Isolation and culturing of bacteria

Tuber samples were washed under running tap water, surface-sterilized in 0.3% sodium hypochlorite, rinsed with sterile distilled water, and air-dried in a laminar flow cabinet. Tissue from lesion margins was aseptically excised, macerated in sterile water, and plated onto nutrient agar. After incubation at 28 °C for 24 h, single colonies were transferred to crystal violet pectate (CVP) medium (Perombelon and Van Der Wolf, 2002). The CVP plates were incubated at 28 °C for 48 h, and then examined for cavity formation.

Phenotypic and biochemical characterization of isolated bacteria

Gram reaction, fluorescence on King's B medium, growth at 37 °C, and pigmentation on yeast dextrose calcium carbonate (YDC) and nutrient glycerol manganese (NGM) media were assessed for isolates obtained. Standard biochemical tests, including oxidase, catalase, urease, indole production, carbohydrate utilization, and citrate and malonate metabolism, were carried out, according to recommended and validated protocols (EPPO, 2023).

Pathogenicity tests

Pathogenicity of bacterial isolates was assessed using whole potato tubers. Bacterial suspensions (10^8 CFU mL^{-1}) were inoculated into the tuber tissues and incubated at 27 to 30 °C in moist chambers. Symptom development was monitored for 24 to 72 h. Control samples were inoculated with sterile distilled water. Re-isolation of bacteria from symptomatic tissues was performed to determine fulfilment of Koch's postulates.

Molecular identification of bacteria

Total genomic DNA was extracted from selected isolates using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. The housekeeping gene *gapA* was amplified by PCR using the primer pair gapA-7-F (5′-ATGGCAGGTTGTTGGTAA-3′) and gapA-938-R (5′-TTAGCCGTTGACCTTGTTG-3′) (Cigna *et al*., 2017). PCR products were visualized on 1.5% agarose gels stained with ethidium bromide. Amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN), and were sequenced by Macrogen Europe using the Sanger method with forward and reverse primers.

Raw sequence reads were assembled and edited to obtain high-quality consensus sequences using Geneious

Prime 2026.0.2 (Biomatters Ltd). Multiple sequence alignment of *gapA* gene sequences was performed using MUSCLE (Edgar, 2004).

Sequence similarity searches against the NCBI GenBank database were conducted using BLASTn (Altschul *et al*., 1990) to identify closely related taxa. BLASTn analysis showed that 11 Georgian isolates (PP060483 to PP060493) shared 100% nucleotide identity and full query coverage with *Pectobacterium carotovorum* strains MBr2 (OR651974) and ZB18121 (OP793220), indicating close genetic relationships.

Phylogenetic analysis was performed in MEGA X (Kumar *et al*., 2018). *gapA* sequences were aligned using ClustalW, and a Maximum Likelihood (ML) tree was constructed based on the best-fit nucleotide substitution model selected using the Bayesian Information Criterion (BIC). Bootstrap analysis with 1,000 replicates was used to assess tree robustness, with values ≥70% considered significant (Felsenstein, 1985).

Bacterial isolates and reference sequences

11 Georgian isolates (PP060483 to PP060493) were included in the phylogenetic analysis together with reference sequences of *Pectobacterium* species retrieved from the NCBI database, (NCBI, 2026). including *Pectobacterium carotovorum* (OP793220), *Pectobacterium atrosepticum* (NC_004547/ NZ_CP009125), *Pectobacterium basiliense* (NZ_CP020350), *Pectobacterium versatile* (NZ_CP021894), *Pectobacterium polare* (NZ_CP017482), *Pectobacterium parmentieri* (CP003415 / CP001790), *Pectobacterium punjabense* (SAMN11162174) and *Pectobacterium aroidearum* (SAMN47853470). These reference taxa were selected to represent the major phylogenetic lineages within *Pectobacterium*.

RESULTS AND DISCUSSION

The field surveys in Georgia revealed that potato tubers with typical soft rot symptoms were widespread in major potato-producing regions of Georgia. A total of 28 symptomatic samples were collected. Nineteen bacterial isolates produced characteristic cavities on CVP medium, indicating their pectolytic activity typical of soft rot bacteria (Figure 1 A). All isolates were Gram-negative, facultatively anaerobic, and non-fluorescent on King's B medium, suggesting affiliation with *Pectobacterium* spp. or *Dickeya* spp. Based on strong pectolytic activity, 11 isolates were selected for further characterization. These isolates did not produce blue pigmentation on yeast dextrose calcium carbonate (YDC) or nutrient glycerol medium (NGM), distinguishing them from five other isolates that displayed phenotypic traits consistent with *Dickeya* spp. (Figure 1 B).

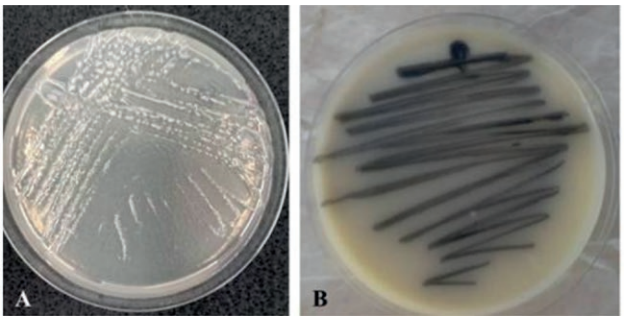

Figure 1. (A) A pure culture of *Pectobacterium carotovorum* grown on crystal violet pectate (CVP) medium. (B) Dark blue pigmentation produced by *Dickeya* spp. on yeast dextrose calcium carbonate (YDC) medium.

The 12 selected isolates were oxidase-, urease-, and indole-negative, unable to utilize citrate or malonate, and did not produce acid from dulcitol or sorbitol. All isolates were catalase-positive, fermented lactose, rhamnose, and trehalose, and were capable of growth at 37 °C.

Pathogenicity assays conducted on whole potato tubers showed that all tested isolates induced typical soft rot symptoms in the tuber tissues, including rapid maceration and decay, comparable to those observed in naturally infected samples. The bacterial isolates obtained from inoculated tubers matched the inoculation isolates, thereby fulfilling Koch's postulates for the respective bacteria.

The PCR assay resulted in amplification in all 12 Georgian isolates as well as in the reference isolate *Pectobacterium carotovorum* (43_0332), which was from National plant pathogen cultural collection of Wageningen University and Research. Agarose gel electrophoresis gave a single, distinct amplicon of approx. 932 bp in each positive reaction (Figure 2), corresponding to the expected fragment size. No amplification was detected in the negative control, confirming absence of contamination and specificity of the PCR assay. The uniformity of amplicon size among all isolates indicated conservation of the *gapA* gene region within the studied population. Minor variations in band intensity were observed, but are attributed to differences in DNA template concentrations or PCR efficiency, rather than nonspecific amplifications. The clear and consistent amplification products obtained provided high-quality templates for downstream sequencing and subsequent comparative sequence analysis.

BLASTn analysis showed that the Georgian isolates (deposited in GenBank under accession numbers: PP060483, PP060484, PP060485, PP060486, PP060487,

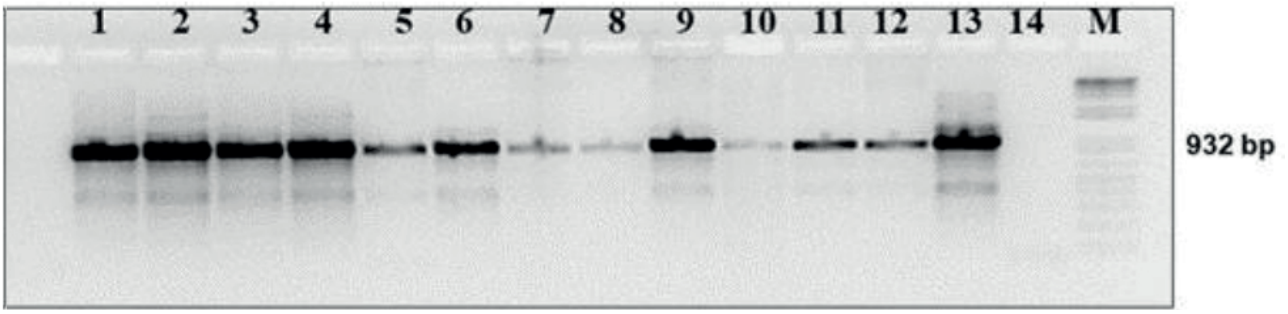

Figure 2. PCR amplification products using primer pair gapA-7-F/gapA-938-R: Lanes 1, Kob.4.22; 2, Tsk 1.22; 3, Kob 5.22; 4, Kob 3.22; 5, Tsk 3.22; 6, Mar 5.22; 7, Akh 2.22; 8, Mar 4.22; 9, Mar 1.22; 10, Mar 9.22; 11, Ats 1.22; 12, Mar 2.22; 13, Reference strain 43_0332; 14, Negative control.

PP060488, PP060489, PP060490, PP060491, PP060492, PP060493) shared 100% nucleotide identity and 100% query coverage with *Pectobacterium carotovorum* reference strains MBr2 (OR651974, Taiwan) and ZB18121 (OP793220, China), with maximum similarity scores (bit score = 1,000), confirming very close genetic relationships.

Phylogenetic analysis based on the *gapA* gene confirmed the taxonomic placement of the isolates. Both Maximum Likelihood and Neighbor-Joining analyses consistently grouped all Georgian isolates within the *Pectobacterium carotovorum* clade (Figure 3).

Phylogenetic relationships inferred from *gapA* gene sequences showed that all Georgian isolates (PP060483 to PP060493) clustered within the *Pectobacterium carotovorum* clade, together with reference strains MBr2 (OR651974) originating from Taiwan, and ZB18121 (OP793220) originating from China. This grouping was strongly supported by a high bootstrap value (98%), indicating a robust and reliable phylogenetic placement. The Georgian isolates formed a compact and well-defined subcluster characterized by short branch lengths, suggesting low intra-group genetic diversity.

In contrast, other *Pectobacterium* species, including *P. aroidearum*, *P. parmentieri*, and *P. punjabense*, were resolved in a separate lineage with moderate bootstrap support (77%). A distinct and well-supported clade (bootstrap = 96) comprised *P. atrosepticum*, *P. brasiliense*, *P. versatile*, and *P. polare*, clearly differentiated from the *P. carotovorum* cluster.

Overall, the topology of the tree (Figure 3) demonstrates a clear separation among species within *Pectobacterium*, and confirms the assignment of all the Georgian isolates to *P. carotovorum*, highlighting the close genetic relatedness and low sequence divergence within the studied population (Table 1).

The present study represents the first confirmed report of *Pectobacterium carotovorum* as a causal agent

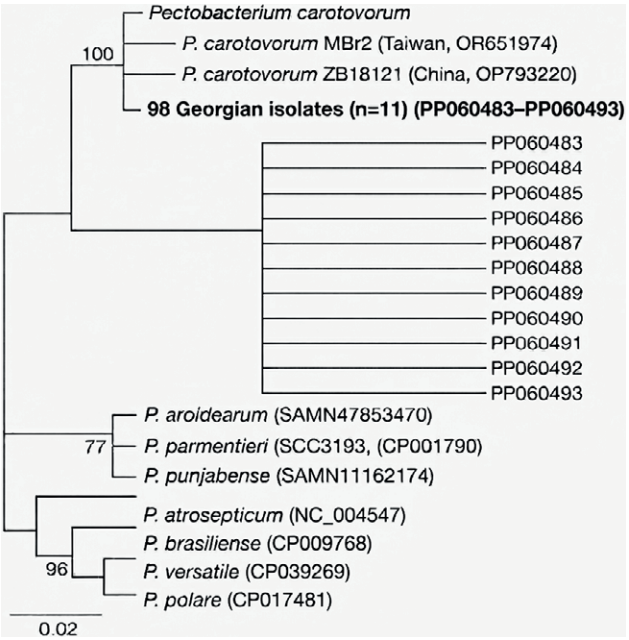

Figure 3. Phylogenetic tree based on *gapA* gene sequences, showing that Georgian isolates (PP060483 to PP060493) cluster within the *Pectobacterium carotovorum* clade with high bootstrap support (98%). Other *Pectobacterium* species form distinct, well-supported lineages. Bootstrap values (1,000 replicates) are indicated at nodes. The scale bar represents 0.02 substitutions per site.

of potato soft rot in Georgia. Previously, *Dickeya solani* had been identified as the predominant soft rot pathogen in this country, particularly in the Samtskhe–Javakheti region and adjacent areas (Muradashvili *et al*., 2023). However, *D. solani* was also detected in samples collected during the 2022–2023 field surveys. The present research demonstrates that two bacterial populations causing potato soft rot, *D. solani* and *P. carotovorum*, are predominantly circulating in the studied regions.

Table 1. List of identified *Pectobacterium carotovorum* isolates from Georgia.

No.	BioSample accession No.	Georgian isolate No.	Host potato variety	Year isolated	Sampling location
1	PP060483	Geo_Kob.4.22	Khortytsa	2023	Kobuleti
2	PP060484	Geo_Tsk.1.22	Marfona	2023	Tsalka
3	PP060485	Geo_Kob.5.22	Slovyanka	2022	Kobuleti
4	PP060486	Geo_Kob.3.22	Nevsky	2022	Kobuleti
5	PP060487	Geo_Tsk. 3.22	Picasso	2022	Tsalka
6	PP060488	Geo_Mar.5.22	Unknown	2022	Marneuli, v. Maradisi
7	PP060489	Geo_Akh.2.22	Marfona	2022	Akhalkalaki
8	PP060490	Geo_Mar.1.22	Unknown	2022	Marneuli
9	PP060491	Geo_Mar.2.22	Unknown	2023	Marneuli
10	PP060492	Geo_Mar.9.22	Unknown	2022	Marneuli
11	PP060493	Geo_Ats.1.22	Jelly	2022	Akhaltsikhe

The coexistence of *P. carotovorum* and *D. solani* within the same potato production areas may complicate disease management, as these pathogens differ in aggressiveness, environmental adaptation, and responses to control measures. The recovery of numerous isolates from seed tubers from unknown origins suggests that seed-borne dissemination is probably a major pathway for pathogen introduction, which is consistent with international reports on the epidemiology of *P. carotovorum* (Van Der Wolf *et al*., 2021). These results highlight the importance of implementing strict phytosanitary measures, including the use of certified seed potatoes and systematic monitoring of seed potato lots. Future research should focus on comprehensive epidemiological surveys, population genetic analyses, and evaluation of disease control strategies to mitigate the impact of these pathogens on potato production in Georgia.

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