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TAXONOMIC DESCRIPTION

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Pectobacterium parvum sp. nov., having a *Salmonella* SPI-1-like Type III secretion system and low virulence

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Abstract

Pectobacterium strains isolated from potato stems in Finland, Poland and the Netherlands were subjected to polyphasic analyses to characterize their genomic and phenotypic features. Phylogenetic analysis based on 382 core proteins showed that the isolates clustered closest to *Pectobacterium polaris* but could be divided into two clades. Average nucleotide identity (ANI) analysis revealed that the isolates in one of the clades included the *P. polaris* type strain, whereas the second clade was at the border of the species *P. polaris* with a 96 % ANI value. *In silico* genome-to-genome comparisons between the isolates revealed values below 70%, patristic distances based on 1294 core proteins were at the level observed between closely related *Pectobacterium* species, and the two groups of bacteria differed in genome size, G+C content and results of amplified fragment length polymorphism and Biolog analyses. Comparisons between the genomes revealed that the isolates of the atypical group contained SPI-1-type Type III secretion island and genes coding for proteins known for toxic effects on nematodes or insects, and lacked many genes coding for previously characterized virulence determinants affecting rotting of plant tissue by soft rot bacteria. Furthermore, the atypical isolates could be differentiated from *P. polaris* by their low virulence, production of antibacterial metabolites and a citrate-negative phenotype. Based on the results of a polyphasic approach including genome-to-genome comparisons, biochemical and virulence assays, presented in this report, we propose delineation of the atypical isolates as a novel species *Pectobacterium parvum*, for which the isolate s0421^T (CFBP 8630^T=LMG 30828^T) is suggested as a type strain.

Plant pathogenic bacteria in the genera *Pectobacterium* and *Dickeya* belonging to the soft rot *Pectobacteriaceae* [1] family are causing disease problems in about half of the plant orders worldwide [2]. They are important plant pathogens that cause significant yield losses in storage and field [3]. The taxonomy of the genus *Pectobacterium* has been re-evaluated several times and new species have been designated. Recently, subspecies of *Pectobacterium carotovorum* have been elevated to the species level as *Pectobacterium carotovorum*, *Pectobacterium odoriferium*, *Pectobacterium actinidiae* and *Pectobacterium brasiliense* [4]. *Pectobacterium polonicum* isolated from groundwater collected from vegetable fields was recently characterized in Poland [5], *Pectobacterium punjabense* was isolated in Pakistan from potato plants showing blackleg symptoms [6] and *Pectobacterium aquaticum* was isolated from waterways in France [7]. *Pectobacterium polaris* was described as a new species with high tuber maceration capacity after its isolation from potato tubers in Norway [8]. The novel species *Pectobacterium peruviense* was proposed for misclassified *P. carotovorum* strains isolated from potato

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Keywords: *Pectobacterium*; *Pectobacterium parvum*; *Salmonella* SPI-1 T3SS; potato; soft rot; virulence.
Abbreviations: AFLP, amplified fragment length polymorphism; ANIm, average nucleotide identity counted using MUMmer; GGDC, Genome-to-Genome Distance Calculator; *is*DDH, *in silico* DNA–DNA hybridization; T3SS, type III secretion system; T6SS, type VI secretion system; UPGMA, unweighted pair-group method with arithmetic mean.
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Previously published 16S sequences of the following strains are available with the following accession numbers: s0421^T, KC337335; IFB5220, MH166802; IFB5222, KU510098; IFB5223, MH166801; IFB5252, KU510101.
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Eight supplementary tables and four supplementary figures are available with the online version of this article.

tubers at high altitudes in Peru, based on data from additional analyses [9]. The species classification *Pectobacterium parmentieri* was created for potato isolates that were originally classified as *P. carotovorum* subsp. *carotovorum*, then as *Pectobacterium wasabiae* [10, 11], and finally identified as a separate species after comparison of the strains from wasabi root and potato [12]. Furthermore, *Pectobacterium* strains isolated from infected potato and cabbage plants in Russia were proposed as *Candidatus Pectobacterium maceratum*, with the Finnish *Pectobacterium* strain SCC1 [13] as the type strain [14]. Recently this taxon was renamed as *Pectobacterium versatile* and given an official status [4].

Some atypical *Pectobacterium* isolates showing low production of homoserine lactones and small colony size were identified in a survey conducted in Finland 2004–2005 and later published as atypical *P. carotovorum* subsp. *carotovorum* [15]. Although the isolates originated from rotten potato stems, subsequent field experiments conducted with artificially inoculated seed tubers suggested that they were not able to cause blackleg [15]. When these strains were tested with *Pectobacterium* and *Dickeya* primers (Table S1, available in the online version of this article) used for diagnostics of soft rot bacteria in the Potato Disease Laboratory at Natural Resources Institute in Oulu, Finland, no amplification was obtained. This suggested that the tested strains do not belong to any of the tested *Pectobacteriaceae* species. New BLASTN analyses of the sequences published previously [15] showed the highest identity to sequences of *P. polaris*, suggesting that the Finnish isolates belonged to this species. In order to classify the atypical Finnish strains, genomic sequences of two Finnish isolates, s0416 and s0421, were obtained and compared to genomes of verified and tentative *P. polaris* isolates. A set of verified and tentative *P. polaris* isolates, obtained from culture collections or from collaborators, were subsequently compared through a polyphasic approach. The strains and/or their genomes used in these polyphasic analyses correspond to isolates collected from potato in Norway (NIBIO1006^T and NIBIO1392), Finland (s0416, s0417, s0421, s0424 and s0425), Poland (IFB220, IFB222 and IFB5252), The Netherlands (NCBBP 3395, IPO1606, IPO3720, IPO3841 and IPO3842), Morocco (S4.16.03.2B) and Pakistan (SS28). In addition, strain IFB5223, isolated from *Solanum dulcamara* in Poland, and Y1, isolated from diseased *Brassica rapa* subsp. *chinensis* in PR China, were included in the comparisons. Used isolates and/or their genome sequences are listed in Table S2, including the genome sequences of *P. parvum* sp. nov. isolates s0421^T (OANP00000000), s0416 (OANO0000000) and IFB5220 (PHSZ00000000), and *P. polaris* isolates IFB5222 (PHSV00000000), IFB5223 (PHSY00000000), IFB5252 (PHSX00000000), IPO1606 (CABFUY010000), IPO1948 (CABHLY010000), IPO3720 (CABFUV010000), IPO3841 (CABFUX010000) and IPO3842 (CABFUW01000) generated in this work.

Verified and tentative *P. polaris* isolates for which genomic sequences were available were compared in a phylogenomic analysis based on 382 core proteins in a PhyloPhlAn analysis (https://huttenhower.sph.harvard.edu/phylophlan) designed to assign microbial taxonomy based on proteins optimized from among 3737 genomes [16]. The results suggested that most of the analysed *P. polaris*-like isolates clustered with the *P. polaris* type strain suggesting they belonged clearly to this species. However, five isolates, s0416, s0421, IFB5220, NCPPB3395 and Y1, could be separated from *P. polaris* in the PhyloPhlAn analysis with 100 % bootstrap support indicating they could form a new taxon (Fig. 1). In the present work, a new taxon named *P. parvum* is proposed for these atypical isolates.

In average nucleotide analysis counted using MUMmer (ANIm) calculated with Pyani (https://github.com/widdowquinn/pyani) (Table 1 and S3), the values between *P. polaris* and *P. parvum* ranged between 96.0–96.2 %, which is just above the generally recommended cut-off for species delineation (95–96 %) [17]. However, these values were based on low alignment coverages, 81.8–86.5 % of *P. parvum* genomes and 77.2–81.9 % of *P. polaris* genomes, suggesting a considerable difference between these two groups of bacteria (Table 1, Table S4). Furthermore, high similarity among the *P. parvum* genomes, more than 99.3 % in ANIm, and an alignment coverage of 96.4 % or higher, showed that they are more similar to each other than to the *P. polaris* isolates. *In silico* DNA–DNA hybridizations (*is*DDH), performed with the Genome-to-Genome Distance Calculator (GGDC 2.1; http://ggdc.dsmz.de/distcalc2.php) using the BLAST+ alignment and formula 2 (identities/HSP length) [18], showed values ranging from 66.1–68.4 % between *P. polaris* and *P. parvum* isolates (Table 1 and S5), which is just below the generally recommended cut off for species delineation (70%) [19]. Because of the discrepancy between the ANIm results and GGDC results, the former suggesting identification of the atypical isolates as *P. polaris* and the latter suggesting they are not members of *P. polaris*, the isolates and their genomes where studied further to clarify their taxonomic position. The genome sizes of the five *P. parvum* isolates appeared somewhat shorter, 4.5–4.6 Mb, which is 0.1–0.2 Mb shorter when compared to most of the other *Pectobacterium* strains. This suggests a reduced genome size of the *P. parvum* isolates. Furthermore, the genome size of Y1 is even smaller, 3.9 Mb, which according to NCBI is too small to be acceptable and thus it is excluded from RefSeq, suggesting that it may lack part of the genome rendering it unsuitable for genomic comparisons. G+C contents of the isolates showed that the *P. parvum* isolates had lower G+C content, 51%, when compared to *P. polaris* strains that had a G+C content at or close to 52 % (Table S2). When inferred from genome sequences, within-species differences in the G+C content are most often below 1 % [20]. The G+C differences between the *P. polaris* type strain NIBIO1006^T and the *P. parvum* isolates is 1.0 % and close to 1 % in the other comparisons, which places *P. polaris* and *P. parvum* in the borderline of belonging to separate species.

Patristic distances between and within the core genomes of *Pectobacterium* species (Table S6) were calculated with PATRISTIC version 1.0 software [21]. The core genome was calculated with BPGA version 1.3 software and consisted of 1294 core proteins of 63 genomes representing all known

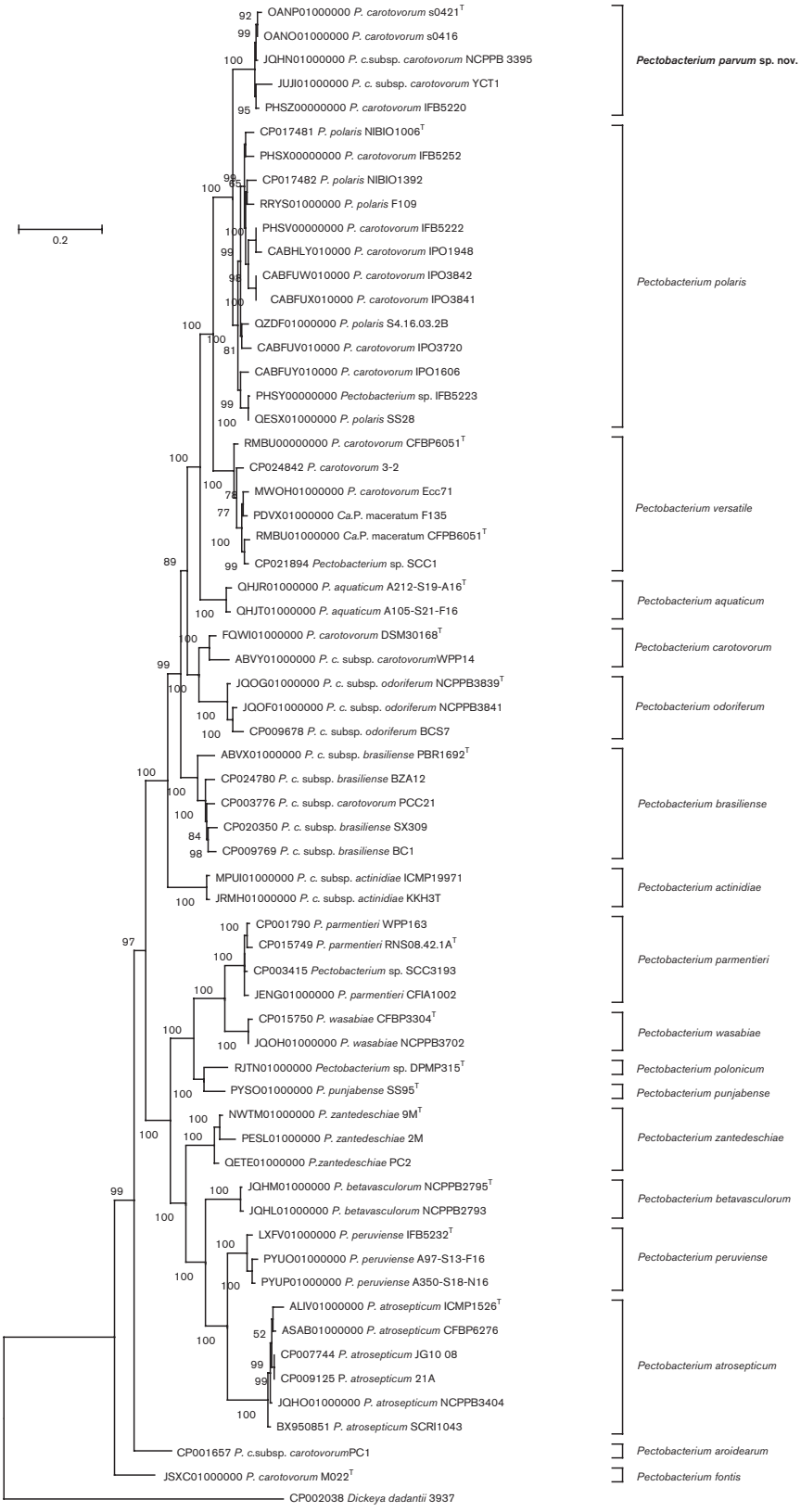

Fig. 1. Phylogenomic analysis of Pectobacterium *parvum* sp. nov. strains s0416, s0421, IFB5220, NCPPB3395 and Y1 and members of the genus *Pectobacterium*, based on 382 core proteins: The maximum-likelihood tree was reconstructed using the PhyloPhlAn computational pipeline (https://huttenhower.sph.harvard.edu/phylophlan).

Table 1. Genomic comparisons of Pectobacterium *parvum* strains to *Pectobacterium polaris* strains and type strains of known *Pectobacterium* species

The number of strains used in each comparison is shown in the parentheses. The individual values are presented as supplementary information.

	GGDC result (%)	ANIm identity (%)	ANIm coverage (%)
Species	*P. parvum*	*P. parvum*	*P. parvum**
P. parvum (5)	95.2–99.6	99.3–99.8	96.3–100
P. polaris (13)	66.1–68.4	96.0–96.2	81.8–86.5
Pectobacterium sp^T (15)	35.3–55.6	89.0–94.1	65.9–81.8

*JUJI01000000 (Y1) was not included in the coverage comparison because the sequence is short and may lack a substantial part of the genome.

Pectobacterium species and *P. parvum* isolates. The results showed that the patristic distances among *P. parvum* isolates were 0.003–0.010 and among *P. polaris* isolates 0.010–0.016, which are in line with the patristic distances observed within the other *Pectobacterium* species. When patristic distances were compared between *P. parvum* and *P. polaris* isolates, higher values of 0.017–0.026 were observed. Patristic values in *P. polaris*/*P. parvum* comparisons are in line with the distances observed between closely related *Pectobacterium* species, such as between *P. carotovorum*/*P. odoriferum* (0.020–0.025), *P. carotovorum*/*P. versatile* (0.021–0.027), *P. polonicum*/*P. punjabense* (0.020) and *P. wasabiae*/*P. parmentieri* (0.024–0.026) (Table S6). Thus, the patristic distances between *P. parvum* and *P. polaris* exceed the distances observed within the other *Pectobacterium* species, and coincide with the distances observed in comparisons between closely related *Pectobacterium* species, which suggests that *P. parvum* and *P. polaris* are closely related species rather than representatives of the same species.

Comparison of the 16S sequences of the *P. parvum* isolates to each other showed that they had identical 16S sequences, which were 99.55 % similar to the 16S sequence of the *P. polaris* type strain, and 99.22, 99.09, 99.03, 98.96, 98.83, 98.83 and 98.70% similar to 16S sequences of type strains of *P. versatile*, *P. carotovorum*, *P. brasiliense*, *P. aquaticum*, *P. odoriferum*, *P. actinidiae* and *P. wasabiae,* respectively.

To further characterize the differences between the *P. parvum* and *P. polaris* isolates, they were subjected to amplified fragment length polymorphism (AFLP) DNA fingerprinting (Keygene NV) along with the type strains of the most closely related *Pectobacterium* species, as observed in ANI and 16S rRNA gene sequence analysis. DNA for this application, was extracted using a Maxwell 16 Tissue DNA Purification Kit and a Maxwell 16 instrument. AFLP DNA fingerprinting was performed as reported previously [22], except that the restriction enzymes EcoRI and TaqI and the primer combination E01-6-carboxyfluorescein (6-FAM) and T11 [23] were used. The Gene Mapper 4.0 software (Applera Co.) was used to normalize the resulting electrophoretic patterns and convert part of the patterns (namely the fragments of 20 to 600 bp) into text files that were subsequently used as input files for the BioNumerics 7.6.3 software package (Applied Maths). Peak-based data analysis of the AFLP DNA fingerprints was performed using the Dice coefficient (tolerance value of 0.15 %) and the unweighted pair-group method with arithmetic mean (UPGMA) cluster algorithm. For numerical analysis, the zone from 40 to 580 bp was used. AFLP DNA fingerprinting revealed that the *P. parvum* isolates tested formed a single cluster separate from the related species including *P. polaris*, which confirms their unique taxonomic position (Fig. 2). In addition, a detailed comparison of the AFLP DNA fingerprints of the *P. parvum* isolates revealed six distinct DNA fingerprint types (NCPPB 3395 and s0416 have identical profiles), indicating that they represent at least six different strains.

Genome comparisons between the *P. parvum* and *P. polaris* isolates performed with BRIG analysis [24] showed differences between the genomes. The results showed that genome of Y1 appeared fragmented, possibly due to missing sequence data. Furthermore, BRIG analysis suggested that *P. parvum* isolates harbour genomic islands. The largest identified island was 0.1 Mb and present only in s0421 (Fig. 3). Similarity search with BLASTX suggested it codes mainly for replication proteins, transposases and conjugative DNA transfer proteins. Of its sequence, 36 % had 98 % identity to *P. parmentieri* strain IFB5427 plasmid pPAR01, and 28–38 % of its length had 73–74% identity to plasmids in *Erwinia amylovora*, *Pantoea ananatis*, *P. vagans* and *Rahnella* sp., but also to genomic sequences of several *E. amylovora* strains. Mash screen search in PLSDB plasmid sequence database [25], with maximum *p*-value 0.1 and minimum identity 0.90, revealed similarity of s0421 sequence to 89 plasmids, mostly in *Enterobacteriacae* isolates, the best hit being pPAR01. No plasmids were identified in the other *P. parvum* isolates with the same settings.

Genes that are present in *P. parvum* but missing from *P. polaris*, and vice versa, were identified using OrthoMCL [26] with a BLAST E-value cut-off of 1.0 e-6 and an inflation parameter of 1.5 as described by Lara-Ramirez *et al.* [27] (Table S7). A second analysis was performed with GET_HOMO-LOGUES version 07112016 (https://github.com/eead-csic-compbio/get_homologues) determining orthology based on all-versus-all Best Bidirectional BLASTP Hits, using the COGOMCL and COG algorithms (Table S8). Y1 was omitted from both analyses because some genes may be absent due to issues with sequencing, and are thus not biologically informative in comparisons. The results of the comparisons were verified with BLASTP and BLASTN analyses to identify the corresponding genes and their genomic neighbours. The analysis revealed that isolates s0416, s0421, IFB5220 and NCPPB3395 had a large gene cluster coding for Type III secretion (T3SS) machinery that is similar in sequence and in gene organization with *Salmonella* SPI-1-like Inv-Mxi-Spa T3SS present in some *Pantoea ananatis*, *P. stewartii* subsp. *stewartii*, *Erwinia amylovora*, *E. pyrifolia* and *E. tasmaniensis* isolates (Fig. S1). *Salmonella enterica* has been shown to need SPI-1 T3SS to be able to persist inside leaf hopper [28], *P. stewartii*

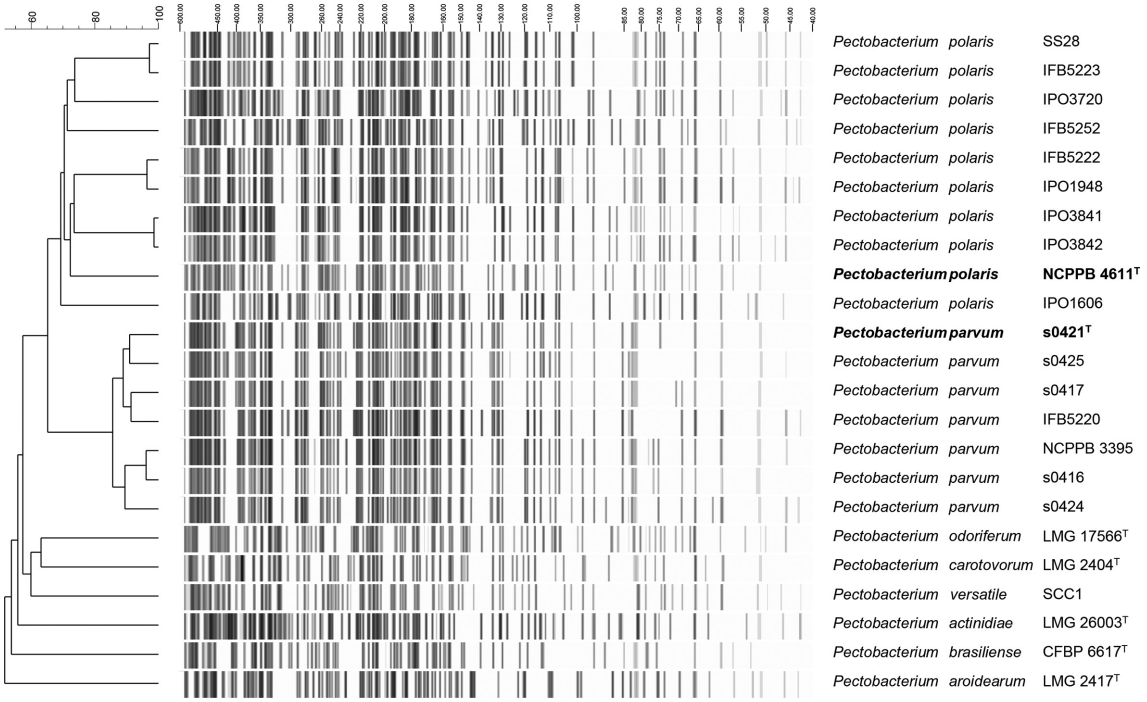

Fig. 2. AFLP fingerprints of Pectobacterium *parvum* sp. nov. strains and their closest phylogenetic relatives. The dendrogram is derived from unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis of the fingerprints with levels of linkage expressed as Dice similarity coefficients.

subsp. *stewartii* has been shown to use the SPI-1-like T3SS to colonize its insect vector [29], and also in other bacterial pathogens this type of T3SS has been linked to persistence in insects [30]. *P. parvum* is the first example among *Pectobacterium* species that harbours SPI-1-like T3SS. Among the *P. parvum*-specific proteins were also nematicidal protein 2 that was 59 % identical and 70 % similar to *Serratia proteomaculans* antifeeding protein Afp18 lethal to beetle larva [31]. Also a small protein similar to *Burkholderia cenocepasia* AidA that is required for slow killing of nematodes [32] was identified in *P. parvum* isolates. OmpT outer membrane protease similar to protein that is involved in killing of nematodes by *Yersinia pestis* [33] was identified as *P. parvum*-specific protein. Also proteins annotated as chitinase and chitin binding protein were among the identified *P. parvum*-specific proteins. Soft rot *Pectobacteriaceae* have been identified in numerous insect species [34] and in slugs [35], and they can also be vectored by free living [36] and root-knot nematodes [37]. The characteristics of the *P. parvum*-specific genes may suggest that it forms a more intimate relationship with a vector when compared to *P. polaris* or other *Pectobacteriaceae* species.

P. parvum isolates harboured a gene cluster that is similar in gene organization and sequence to the gene cluster coding for proteins involved in production of phenazine antibiotic D-alanylgriseoluteic acid by *Enterobacter agglomerans* (syn. *Erwinia herbicola*, *Pantoea agglomerans*) Eh1087 [38]. The corresponding cluster was not identified in *P. polaris* isolates or any other *Pectobacterium* strains by BLASTN analysis. Phenazines are involved in toxicity of bacteria to their animal and plant hosts and bacterial and fungal competitors, and contribute to biofilm formation and gene regulation in bacteria [39]. *P. parvum*, but not the *P. polaris* isolates, produce a small, diffusible molecule that is toxic to *Dickeya solani* s0432-1 (Fig. S2), suggesting that *P. parvum* produces an antibacterial metabolite that may enhance its ecological fitness in plant or vector tissues.

Many proteins were identified as missing from *P. parvum* but present in *P. polaris* in the genome comparisons, many of them known virulence determinants in plant tissue. All but two *P. polaris* isolates, IPO3720 and 16.3.2B, had a typical *Pectobacterium hrp/hrc* T3SS (Fig. S1). *Pectobacterium hrp/hrc* T3SS has been shown to be necessary in the early phase of the infection in leaf tissue, probably due to secretion of DspE effector [40]. The ability of most *P. polaris* isolates but not the *P. parvum* isolates to cause HR response in *Nicotiana benthamiana* leaf tissue was verified with infiltration of the bacterial cells into leaf tissue (data not shown). Also Type VI secretion system (T6SS) genes needed for virulence in tuber tissue [11] and a neighbouring lipase gene were identified in *P. polaris* but not in *P. parvum*. A large *phn* operon, needed for utilization of phosphonates as phosphorous source, and previously shown to be upregulated in *P. atrosepticum* by host extracts [41], was absent from *P. parvum* genomes. Several genes, involved in utilization of citrate (*citDEFXG*) or uptake of iron–dicitrate complex (*fecCDE*) were absent from *P. parvum*, suggesting that

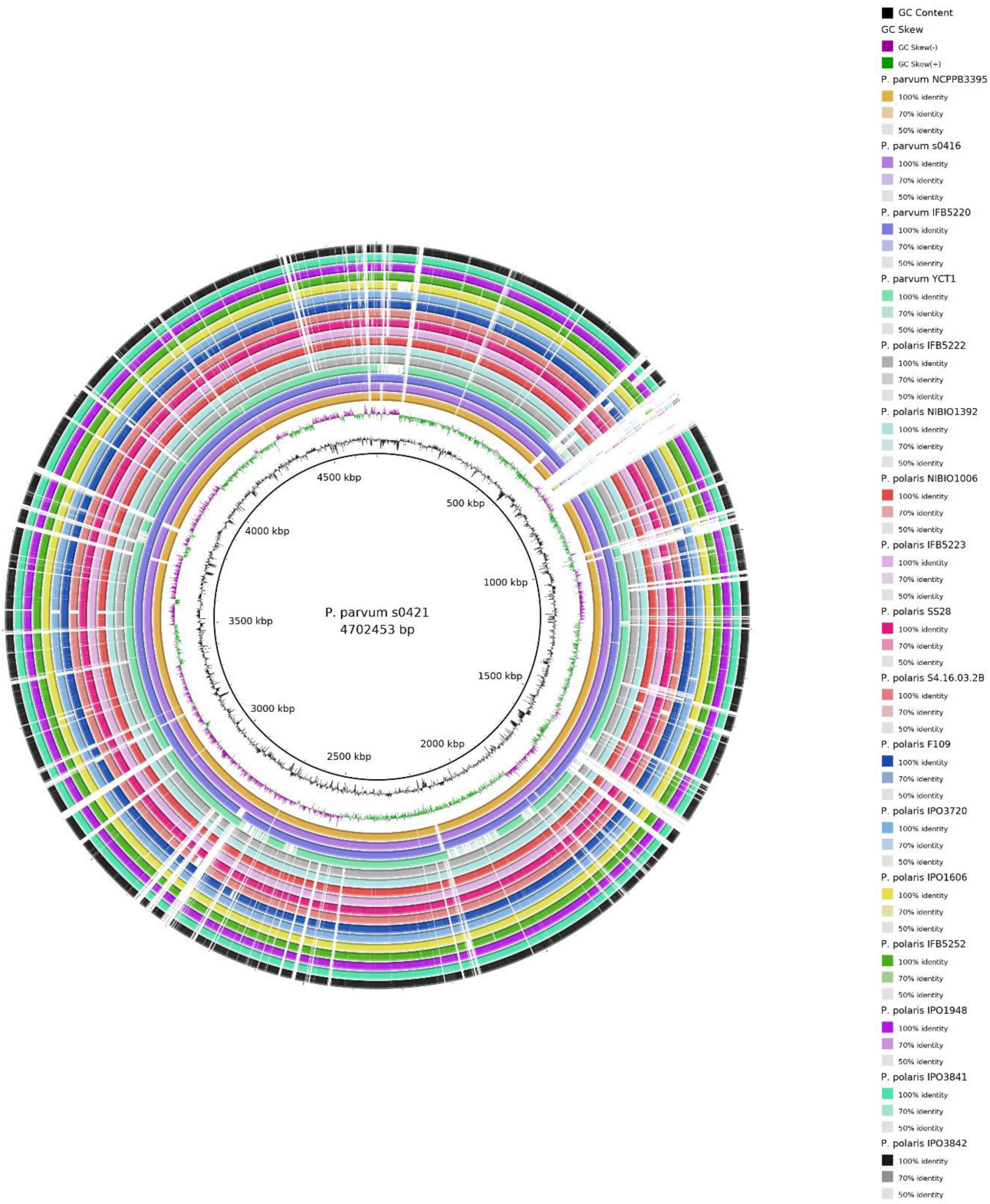

Fig. 3. Circular representation of genome sequences of Pectobacterium *parvum* and *Pectobacterium polaris* isolates. The inner ring portrays the reference *P. parvum* s0421^{T} genome with corresponding genetic coordinates. The coloured rings (from inner to outer ring) portray: G+C% skew, G+C content skew and whole-genome sequences of *P. parvum* strains NCPPB 3395, S0416, IFB5220 and Y1, and *P. polaris* strains, IFB5222, NIBIO1392, NIBIO1006^{T}, IFB5223, SS28, S4.16.03.2B, F109, IPO3720, IPO1606, IFB5252, IPO1948, IPO3841 and IPO3842. Comparison created using the BRIG platform application [24].

Table 2. Phenotypic characters that differentiate Pectobacterium *parvum*, *Pectobacterium polaris* and *Pectobacterium* species commonly present in potatoes in Central and Northern Europe

Ppar (*P. parvum,* four strains, s0416, s0417, s0421 and NCPPB 3395), *Ppol* (*P. polaris* NIBIO1006^T), *Pv* (*P. versatile* SCC1), *Pc* (*P. carotovorum* CFBP 2046^T), *Pb* (*P. brasiliense* CFBP 6617^T), *Pa* (*P. atrosepticum* ICMP 1526^T), *Pprm* (*P. parmentieri* SCC3193) and *Ds* (*D. solani* s0432-1)

	***Pectobacterium* species**							
Test	***Ppar***	***Ppol***	***Pv***	***Pc***	***Pb***	***Pa***	***Pprm***	***Ds***
Growth at 37 °C	+	+	+	+	+	−	−	+
Growth in 5 % NaCl	+	+	+	+	+	−	−	−
Utilization of α-methyl glucoside	−	−	−	−	−	+	−	−
Reducing sugars from sucrose	−	−	−	−	+	+	−	−
Utilization of:								
Citrate	−	+	+	+	+	+	+*	+
Lactose	+	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+

*Slow reaction for citrate utilization by *P. parmentieri* strains.

P. parvum isolates are not able to utilize citrate. Citrate-negative phenotype has been linked previously to low virulence in soft rot bacteria [42]. Furthermore, *P. polaris* isolates seem to lack several small operons containing genes involved in stress responses in various bacteria. Among them were *ter* operon coding for tellurite resistance genes [43], *potABC* involved in spermidine/putrescine polyamine uptake [44], *kdpBCDE* coding for two-component regulators and ATPases involved in potassium uptake [45] and pectin lyase *pnlA* gene activated during UV stress and cell lysis [46]. Pectin lyase production [47] and potassium [48] and putrescine uptake [49] have been linked previously to virulence or cell–cell communication in soft rot bacteria. In addition, *P. parvum* lacked several small operons coding for PTS transporters with adjacent aldolase or sugar kinase, suggesting that *P. parvum* may not be able to fully utilize all sugars and polysaccharides present in plant tissues.

Further comparison of *P. parvum* and *P. polaris* isolates was performed with Biolog analysis using GEN III plates. The results showed that all tested *P. parvum* isolates included in the analysis were unable to utilize citrate (Table 2, Fig. S3). The negative citrate phenotype of *P. parvum* was verified with Simmons citrate agar test (Merck) according to the supplier's instructions. In these tests, all seven tested *P. parvum* isolates were citrate negative and the 12 tested *P. polaris* isolates, including the type strain, we found citrate positive. Furthermore, growth at +37 °C and in 5 % NaCl, utilization of α-methyl glucoside, reducing sugars from sucrose and utilization of lactose, melibiose and raffinose were manually compared between *P. parvum* isolates s0416, s0417, S0417 and NCPPB 3395 and *P. polaris* type strain NIBIO1006^T and type strains of *Pectobacterium* species commonly present in potatoes in Europe using standard tests and conditions used for the characterization of *Pectobacterium* and *Dickeya* species [50, 51], but no additional differences were observed between the tested species (Table 2). Because *P. parvum* isolates appeared to lack several known virulence determinants needed for successful colonization of plant tissues, virulence phenotypes of all available *P. parvum* and *P. polaris* strains were compared in potato tuber maceration assay. Several *P. polaris* isolates had high ability to macerate potato tuber tissue, as originally published [8], whereas the *P. parvum* isolates had low virulence in the potato maceration test (Fig. S4).

The *P. parvum* isolates characterized in this work originate from four countries, the Netherlands, Finland, Poland and China. NCPPB 3395 was isolated in the Netherlands from *Solanum tuberosum* by H. Maas-Geesteranus, supposedly sometime during 1970s as the first publication mentioning it (as strain 196) was published in 1979 [52]. Finnish isolates s0416, s0417, s0421, s0424 and s0425 were isolated 2004 from diseased potato stems of five cultivars originating from three locations in Finland, Y1 was isolated in China 2013 from *Brassica rapa* subsp. *chinensis*, and IFB5220 was isolated in 1996 in Poland from potato stem. After completion of the experiments described in the present work, LMG 2402 isolated from rotten *Helianthus annuus* stalk in former Yugoslavia in 1969 was tentatively identified as *P. parvum* at the BCCM/LMG culture collection by AFLP analysis (Ilse Cleenwerck, personal communication), making it the ninth known isolate and third host plant species. In spite of the different host plants and wide geographical and time span between the isolation of *P. parvum* strains, they appear very similar on phenotypic and genomic levels, which may suggest a narrow ecological niche or an interaction with a vector. To conclude, based on the presented genomic and phenotypic data, we propose that these isolates form a separate species named *P. parvum*.

DESCRIPTION OF *PECTOBACTERIUM PARVUM* SP. NOV.

Pectobacterium parvum (par′vum L. neut. adj. *parvum* small).

Cells are Gram-negative, rod-shaped and facultatively anaerobic. They form small, round-shaped, white, opaque and flat colonies on nutrient agar with 0.5–1 mm in diameter after 17 h. Like other *Pectobacterium* species, they are catalase-positive, oxidase-negative and pectinolytic. They grow at +37 °C and on Luria–Broth with 5 % NaCl. They can utilize sucrose, lactose, melibiose and raffinose as well as many other sugars, but are negative on citrate and many amino acids and sugars on Biolog plates. *Pectobacterium parvum* isolates have a low maceration ability on potato tuber tissue and inhibit growth of *D. solani* in dual culture. The *Pectobacterium parvum* type strain is s0421^T (CFBP 8630=LMG 30828).

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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