Diversity of Pectobacteriaceae Species in Potato Growing Regions in Northern Morocco

Saïd Oulghazi 1,2, Mohieddine Moumni 1, Slimane Khayi 3, Kévin Robic 2,4, Sohaib Sarfraz 5, Céline Lopez-Roques 6, Céline Vandecasteele 6 and Denis Faure 2,*

1 Department of Biology, Faculty of Sciences, Moulay Ismaïl University, 50000 Meknes, Morocco; s.oulghazi@yahoo.fr (S.O.); mmoumni02@yahoo.fr (M.M.)
2 Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, 91198 Gif-sur-Yvette, France; Kevin.ROBIC@i2bc.paris-saclay.fr
3 Biotechnology Research Unit, CRRA-Rabat, National Institut for Agricultural Research (INRA), 10101 Rabat, Morocco; slimane.khayi@inra.ma
4 National Federation of Seed Potato Growers (FN3PT-RD3PT), 75008 Paris, France
5 Department of Plant Pathology, University of Agriculture Faisalabad Sub-Campus Depalpur, 38000 Okara, Pakistan; sohaib002@gmail.com
6 INRA, US 1426, GeT-PlaGe, Genotoul, 31320 Castanet-Tolosan, France; celine.lopez-roques@inra.fr (C.L.-R.); celine.vandecasteele@inra.fr (C.V.)
* Correspondence: denis.faure@i2bc.paris-saclay.fr

Received: 28 April 2020; Accepted: 9 June 2020; Published: 13 June 2020

Abstract: Dickeya and Pectobacterium pathogens are causative agents of several diseases that affect many crops worldwide. This work investigated the species diversity of these pathogens in Morocco, where Dickeya pathogens have only been isolated from potato fields recently. To this end, samplings were conducted in three major potato growing areas over a three-year period (2015–2017). Pathogens were characterized by sequence determination of both the gapA gene marker and genomes using Illumina and Oxford Nanopore technologies. We isolated 119 pathogens belonging to P. versatile (19%), P. carotovorum (3%), P. polaris (5%), P. brasiliense (56%) and D. dianthicola (17%). Their taxonomic assignation was confirmed by draft genome analyses of 10 representative strains of the collected species. D. dianthicola were isolated from a unique area where a wide species diversity of pectinolytic pathogens was observed. In tuber rotting assays, D. dianthicola isolates were more aggressive than Pectobacterium isolates. The complete genome sequence of D. dianthicola LAR.16.03.LID was obtained and compared with other D. dianthicola genomes from public databases. Overall, this study highlighted the ecological context from which some Dickeya and Pectobacterium species emerged in Morocco, and reported the first complete genome of a D. dianthicola strain isolated in Morocco that will be suitable for further epidemiological studies.

Keywords: Pectobacterium; Dickeya; plant pathogen; potato tuber; genome; field sampling

1. Introduction

Pectinolytic Pectobacterium and Dickeya spp. are causative agents of severe diseases in a wide range of plants of high economic value [1,2]. On potato tubers and stems, the diseases caused by pectinolytic pathogens are soft rot and blackleg, respectively. These pathogens produce a large set of extracellular enzymes that degrade the plant cell wall, resulting in plant tissue decay and maceration. This rotting process causes losses in the production of potato tubers sold both on the food market and as certified seed tubers [3]. The pathogens may be acquired by the host plants from soil and/or from contaminated seed tubers [4]. On plants, pathogen populations remain at a low level in asymptomatic
plant tissues, and may become particularly destructive when environmental conditions favor their proliferation and the expression of virulence factors.

*P. atrosepticum* was considered as the primary pathogen responsible for the rotting of stored potato tubers and wilting of potato plants under temperate climates [4]. Other *Pectobacterium* species frequently associated with damage of potato crops are *P. carotovorum*, *P. brasiliense*, *P. parmentieri* and *P. polaris* [5–9]. Some *Pectobacterium* species have also been characterized in some specific areas, such as *P. peruvianum* strains isolated from tubers in Peru, and *P. punjabense* species from symptomatic potato plants in Pakistan [10,11]. *P. odoriferum* exhibits a very wide host range, including potato plants [12], while some other species were characterized by a more restricted host range, at least in the fields. Thus, *P. wasabiae* was isolated from symptomatic Japanese horseradish [13]; *P. betavasculorum* was reported almost exclusively on sugar beet [14]; *P. aroidearum* exhibits a preference for some monocotyledonous plants [15]; *P. zantedeschiae* strains were isolated from *Zantedeschia* spp. (Calla lily) [16]; and *P. actiniae* from symptomatic *Actinidia chinensis* (kiwi fruit) [17]. Recently, some other species, isolated from surface waters, have also been described: *P. fontis*, *P. aquaticum* and *P. versatile* [7,18,19]. Altogether, 16 *Pectobacterium* species have been described so far [7].

A limited number of *Dickeya* species, i.e., *D. dianthicola*, *D. dadantii* and *D. solani*, have been associated with symptoms on potatoes. *D. dianthicola* was first reported on potatoes in the Netherlands in the 1970s, and has been detected since then in many other European countries [20]. *D. dadantii* causes soft rot disease in several members of the *Solanaceae* family, including the potato [21]. Another virulent species, namely *D. solani*, spread rapidly throughout Western Europe [22] and in Russia [23], and into other countries such as Turkey [24], Georgia [25] and Brazil [26]. During the past decade, the taxonomy of *Dickeya* and *Pectobacterium* species was revisited following genomic studies bearing on international culture collections and diverse ecosystems around the world [27,28]. By now, the genus *Dickeya* encompasses 10 species: *D. aquatica*, *D. chrysanthemi*, *D. dadantii*, *D. dianthicola*, *D. fangzhongdai*, *D. lacustris*, *D. paradisiaca*, *D. solani*, *D. undicola*, and *D. zeae* [22,29–34]. Bacteria belonging to this genus cause plant diseases in temperate, tropical and subtropical climates [35].

The unambiguous identification of *Dickeya* and *Pectobacterium* species is crucial for epidemiological purposes, to develop appropriate prophylactic approaches and quality controls in national and international trade exchanges. Multi-Locus Sequence Analysis (MLSA) provides relevant information for a better understanding of speciation, and hence for proposing pertinent taxa delineations [15,36]. MLSA may exploit gene sequences, obtained by PCR-sequencing of several loci or by whole genome sequencing. Among the loci commonly included in MLSA, the *rrs* sequence is poorly informative at a species level, while the *gapA* gene appeared as an appropriate marker to discriminate the different *Dickeya* and *Pectobacterium* species [10,18,19,31,32,37]. Taxonomy of *Dickeya* and *Pectobacterium* gained precision and robustness with additional genome analyses, such as average nucleotide identity (ANI) and in silico DNA–DNA hybridization (isDDH) [38]. Comparative genomics is also used to identify species-specific DNA regions. Analysis of the functions encoded by these DNA regions allows the prediction of species-specific metabolic traits. This knowledge contributes to the understanding of both the taxonomy and ecology of the *Dickeya* and *Pectobacterium* pathogens [22,30–32,39].

*P. atrosepticum*, *P. carotovorum* and *P. brasiliense* were described in Morocco as the main causative agents of blackleg and soft rot diseases in potato crop [40–43]. In 2016, *D. dianthicola* was described for the first time in the North of Morocco [44]. In this respect, the main objectives of this study were: (i) to investigate the species composition of the Moroccan *Dickeya* and *Pectobacterium* populations, collected between 2015 and 2017 from diseased potato tubers and stems, (ii) to compare the aggressiveness of some identified pathogens belonging to different species, and (iii) to propose a complete genome of the emerging pathogen *D. dianthicola* in Morocco, that could be used for further studies as a reference genome. This work represents the most important sampling effort of the *Pectobacterium* and *Dickeya* potato pathogens in Morocco over the past decade.
2. Materials and Methods

2.1. Sampling and Isolation of Pectinolytic Bacteria

In 2015, 2016 and 2017, blackleg symptoms were searched for in potato fields in four regions (Meknes, Guigo, Boumia and Larache) in Northern Morocco. Pectinolytic bacteria were isolated from symptomatic plant tissues using crystal violet pectate (CVP) medium as described previously \[45\]. The CVP plates were incubated at 28 °C for 3 days and colonies that had formed pits were re-streaked onto Tryptone (5 g/L) yeast extract (3 g/L) agar medium (TY). The purified isolates were spotted again on CVP to confirm the pectinolytic activity. The obtained cultures from single colonies were stored in 25% glycerol at −80 °C.

2.2. Molecular Characterization of Pectobacterium and Dickeya Isolates

The primer couples Y1/Y2 and ADE1/ADE2 (Table S1) were used for the identification of isolates belonging to Pectobacterium and Dickeya genera \[46,47\]. The reaction was carried out in a final volume of 25 µL, containing 1 µL of bacterial DNA (50 ng/µL), 2.5 µL of PCR buffer (10×), 2 µL of MgCl2 (25 mM), 2.5 µL of dNTPs (1 mM), 1U Taq polymerase and 1 µL of each primer (1 µM) and water. The temperature settings for PCR were the same as described before \[46,47\]. The analysis of PCR products was done by electrophoresis on 2% agarose gels, using PCR products of \*P. atrosepticum\* CFBP1526\(^T\) and \*D. solani\* IPO2222\(^T\) as control along with the 1 Kb DNA ladder.

Positive strains for either Y1/Y2 or ADE1/ADE2 PCR were further characterized using the gapA barcode procedure \[37\]. All the gapA PCR products obtained with gapA-F/gapA-R primers (Table S1) were sequenced using Sanger technology (GATC Biotech, Konstanz, Germany). The sequences were trimmed using the CLC genomic workbench (V10.1.1, Aarhus, Denmark) and aligned using ClustalW. The phylogenetic analysis of the gapA gene was performed as follows: the evolutionary distances were computed using the maximum composite likelihood method (Mega7 software, Pennsylvania State University Park, PA, USA) with 1000 bootstrap. The obtained sequences were deposited in GenBank (Table S2).

2.3. Genome Sequencing

A total of 10 strains representing 5 species identified with gapA sequencing were selected for genome sequencing (Table 1). DNA of the 10 isolates (listed in Table 1) was extracted from overnight cultures in TY medium using the MasterPure™ Complete DNA and RNA Purification Kit (Epicenter, Madison, WI, USA) followed by an ethanol precipitation. The quantity and quality control of the DNA was completed using a NanoDrop (Wilmington, DE, USA) device and 1.0% agarose gel electrophoresis. Paired-end libraries (500 bp in insert size) were constructed for each strain, and DNA sequencing was performed by Illumina NextSeq technology. Sequencing of the library was carried out using the 2 × 75 bp paired-end read module. Illumina sequencing was performed at the I2BC sequencing platform (Gif-sur-Yvette, France).

In the case of the \*D. dianthicola\* strain LAR.16.03.LID, Nanopore sequencing was also performed. Library preparation and sequencing were performed at the GeT-PlaGe core facility, INRA Toulouse, using the “1D Native barcoding genomic DNA kit (EXP-NBD103 and SQK-LSK108)”, according to the manufacturer’s instructions. At each step, DNA was quantified using the Qubit dsDNA HS Assay Kit (Life Technologies). DNA purity was tested using a NanoDrop device (Thermofisher, Waltham, MA, USA) and size distribution and degradation was assessed using the fragment analyzer (AATI) High Sensitivity DNA Fragment Analysis Kit. Purification steps were performed using AMPure XP beads (Beckman Coulter). Quantities of 5 µg of each DNA (five samples) were purified then sheared at 20 kb using the Megaruptor1 system (Diagenode, Seraing, Belgium). A DNA damage repair step was performed on 3 µg of sample. Then END-repair and dA-tailing of double stranded DNA fragments were performed on 1 µg of each sample. Then, a specific index was ligated to each sample. The library was generated by an equimolar pooling of these barcoded samples. Then adapters were ligated to the
library. The library was loaded on a R9.4.1 flowcell and sequenced on MinION instrument at 0.15 pmol within 48 h.

### Table 1. Draft genome sequences of Pectobacterium and Dickeya strains isolated from Northern Morocco.

| Organism                  | Accession Number | Genome Size (bp) | N50 (bp) | Number of Contigs | Coverage | Number of CDS | Number of tRNAs |
|---------------------------|------------------|------------------|----------|-------------------|----------|---------------|-----------------|
| *Pectobacterium polaris*  | QZDF00000000      | 4,862,009        | 155,865  | 65                | 41       | 4355          | 54              |
| *Pectobacterium brasiliense* S1.16.03.2B | QZDG00000000     | 4,946,598        | 146,844  | 74                | 410      | 4337          | 36              |
| *Pectobacterium brasiliense* S1.16.01.3k | QZDI00000000     | 4,818,836        | 99,392   | 91                | 420      | 4206          | 35              |
| *Pectobacterium brasiliense* S4.16.03.1C | QZDJ00000000     | 4,944,722        | 139,665  | 74                | 467      | 4336          | 37              |
| *Pectobacterium carotovorum* S1-A16 | QZDJ00000000     | 4,835,633        | 255,206  | 37                | 55       | 4261          | 63              |
| *Pectobacterium versatile* S4.16.03.3I | QZDK00000000     | 4,854,084        | 8262     | 108               | 246      | 4247          | 34              |
| *Pectobacterium versatile* S4.16.03.3K | QZDL00000000     | 4,870,940        | 90,195   | 106               | 237      | 4262          | 34              |
| *Pectobacterium versatile* S4.16.03.3F | QZDM00000000     | 4,852,595        | 89,731   | 114               | 143      | 4244          | 40              |
| *Dickeya dianthicola* S4.16.03.P2.4 | QZDN00000000     | 4,865,147        | 92,028   | 101               | 415      | 4238          | 37              |
| *Dickeya dianthicola* LAR.16.03.LID | QZDO00000000     | 4,854,084        | 71,707   | 108               | 344      | 4238          | 37              |

2.4. Genome Assembly

Assembly of the Illumina reads was performed using the CLC Genomics Workbench v10.1.1 software (CLCInc, Aarhus, Denmark). After quality (quality score threshold 0.05) and length (above 40 nucleotides) trimming of the reads, contigs were generated by de novo assembly (CLC parameters: automatic determination of the word and bubble sizes with no scaffolding). The draft genome sequences of each strain were deposited at NCBI and annotated using the NCBI Prokaryotic Genome Annotation Pipeline. Statistics of all the ten draft genomes are presented in Table 1.

Assembly of the Nanopore reads was performed as follows. Fast5s from Nanopore sequencing were obtained with MinKNOW version 1.10.23 and were basecalled with ONT Albacore Sequencing Pipeline Software version 2.1.10 and reads passing the internal test were used for subsequent analysis. Porechop 0.2.1 (https://github.com/rrwick/Porechop) was used for adaptor trimming. Illumina paired-end reads were processed with trim_galore 0.4.0 (https://github.com/FelixKrueger/TrimGalore), to trim adaptor sequences. Nanopore reads were assembled using Canu 1.7 [48] with the “genomeSize = 5 m” and “minReadLength = 3000” options. For Nanopore-only assembly, one output contig was obtained, then polished three times using Pilon 1.22 (https://github.com/broadinstitute/pilon), with the “–mindepth 25” option. The contig was finally circularized using Circlator 1.5.1 (https://github.com/sanger-pathogens/circlator).

2.5. Genome Analysis

Phylogenetic and molecular evolutionary analyses were conducted using MEGA, version 7. An MLSA was performed using 13 concatenated housekeeping genes (fisA, rpoD, acnA, purA, gyrB, recA, mdh, mtlD, groEL, secY, glyA, gapA, rplB) retrieved from all the Pectobacterium spp. and Dickeya spp. strains to confirm their phylogenetic position within the reference strains *P. atrosepticum* ICMP1526T, *P. betavasculorum* NCPPB2795T, *P. parmentieri* RNS 08-42.1A1, *P. wasabiae* CFBP 3304T, *P. actinidiae* KKH3, *P. brasiliense* LMG21371T, *P. odoriferum* BCS7, *P. aroidearum* PC1, *D. dianthicola* NCPPB 453T, *D. dadantii* NCPPB 898T, and *D. solani* IPO2222T. The average nucleotide identity (ANI) value was calculated as previously proposed using the ANI calculator (http://enveomics.ce.gatech.edu/ani/, Atlanta, GA, USA). The in-silico DNA–DNA hybridization (isDDH) was evaluated using genome sequence-based species delineation (http://ggdc.dsmz.de/, Braunschweig, Germany) (Table 2).
Table 2. Pairwise Average Nucleotide Identity (ANI) and in-silico DNA-DNA Hybridization (is-DDH) values of Pectobacterium and Dickeya strains isolated from Northern Morocco.

| Strains | 1-Pp NIBIO 1006<sup>T</sup> | 2-Pp S4.16.03.2B | 3-Pc ICMP5702<sup>T</sup> | 4-Pc S1-A16 | 5-Pb LMG 21371<sup>T</sup> | 6-Pb S4.16.03.1C | 7-Pb S1.15.11.2D | 8-Pb S1.16.03.1K | 9-Pv SCC1 | 10-Pv S4.16.03.3F | 11-Pv S4.16.03.3k | 12-Pv S4.16.03.3I | 13-Po BCS7 | 14-Ddi NCPPB 453<sup>T</sup> | 15-Ddi S4.16.03.P2.4 | 16-Ddi LAR.16.03.LID |
|---------|-----------------|-----------------|-----------------|------------|-----------------|-----------------|-----------------|-----------------|---------|-----------------|-----------------|-----------------|------------|-----------------|-----------------|-----------------|
| ANI Values | 96.8 | 92.9 | 92.9 | 93.5 | 93.7 | 93.5 | 93.7 | 93.4 | 93.4 | 93.5 | 93.5 | 92.2 | 79.3 | 79 | 79.1 |
| 2-Pp S4.16.03.2B | 73.30 | 93 | 92.9 | 93.5 | 93.7 | 93.5 | 93.4 | 93.4 | 93.5 | 93.5 | 93.4 | 92.2 | 78.9 | 79 | 79 |
| 3-Pc ICMP5702<sup>T</sup> | 52.3 | 52.1 | 97.2 | 92.6 | 92.8 | 92.8 | 95.1 | 95.2 | 95.2 | 94.8 | 78.9 | 78.9 | 78.9 | 78.9 |
| 4-Pc S1-A16 | 52.2 | 51.90 | 92.6 | 92.9 | 92.7 | 92.9 | 95.3 | 95.3 | 95.3 | 94.8 | 78.8 | 79.6 | 79.6 | 79.6 |
| 5-Pb LMG 21371<sup>T</sup> | 54.4 | 54.5 | 50.9 | 50.9 | 96.1 | 95.9 | 96.1 | 92.2 | 92.2 | 92.3 | 93.00 | 78.6 | 79.5 | 79.5 |
| 6-Pb S4.16.03.1C | 56 | 55.7 | 52 | 51.7 | 68.5 | 96.3 | 100 | 92.3 | 91.2 | 91.1 | 91.2 | 91.9 | 78.7 | 79.6 | 79.6 |
| 7-Pb S1.15.11.2D | 54.7 | 54.2 | 51.3 | 51 | 67.1 | 69.4 | 96.3 | 92.3 | 92.2 | 92.3 | 91.8 | 79.1 | 79.1 | 79.1 |
| 8-Pb S1.16.03.1K | 56 | 55.7 | 76 | 51.7 | 68.5 | 100 | 71.4 | 92.4 | 92.4 | 92.4 | 92.3 | 91.9 | 79.6 | 79.7 |
| 9-Pv SCC1 | 54.3 | 54.3 | 63.5 | 63.9 | 48.9 | 49.8 | 49.5 | 49.3 | 99.5 | 99.5 | 99.5 | 94.7 | 79.2 | 79.1 | 79.3 |
| 10-Pv S4.16.03.3F | 54.4 | 54.3 | 63.9 | 64.3 | 49.1 | 49.9 | 49.5 | 49.9 | 96.6 | 100 | 100 | 94.7 | 79.2 | 79.2 | 79.2 |
| 11-Pv S4.16.03.3k | 54.4 | 54.3 | 63.8 | 64.2 | 49.2 | 49.9 | 49.5 | 49.9 | 96.6 | 99.3 | 100 | 94.7 | 79.3 | 79.3 | 79.4 |
| 12-Pv S4.16.03.3I | 54.4 | 54.3 | 63.8 | 64.2 | 49.1 | 49.9 | 49.5 | 49.8 | 96.5 | 100 | 99.3 | 94.7 | 79.3 | 79.3 | 79.3 |
| 13-Po BCS7 | 49.2 | 49 | 61.3 | 60.6 | 47.4 | 47.8 | 47.6 | 47.6 | 60.4 | 60.7 | 60.6 | 60.6 | 79.1 | 79.1 | 79.1 |
| 14-Ddi NCPPB 453<sup>T</sup> | 21.1 | 20.7 | 20.5 | 20.7 | 20.9 | 21 | 20.6 | 21 | 21.2 | 21 | 21 | 21 | 21 | 21 | 21.1 | 99.5 | 99.5 |
| 15-Ddi S4.16.03.P2.4 | 20.8 | 20.6 | 20.4 | 20.9 | 21.1 | 20.9 | 20.7 | 20.9 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 20.8 | 95.6 | 100 |
| 16-Ddi LAR.16.03.LID | 20.8 | 20.6 | 20.4 | 21 | 21.1 | 20.9 | 20.6 | 20.9 | 21 | 21 | 21 | 21 | 21 | 20.8 | 95.6 | 100 |

Strains: 1, P. polaris NIBIO1006<sup>T</sup>; 2, P. polaris S4.16.03.2B; 3, P. carotovorum ICMP5702<sup>T</sup>; 4, P. carotovorum S1-A16; 5, P. brasiliense LMG21371<sup>T</sup>; 6, P. brasiliense S4.16.03.1C; 7, P. brasiliense S1.15.11.2D; 8, P. brasiliense S1.16.01.3K; 9, P. versatile SCC1; 10, P. versatile S4.16.03.3F; 11, P. versatile S4.16.03.3k; 12, P. versatile S4.16.03.3I; 13, P. odoriferum BCS7; 14, D. dianthicola NCPPB 453<sup>T</sup>; 15, D. dianthicola S4.16.03.P2.4; 16, D. dianthicola LAR.16.03.LID.
The genome map of the *D. dianthicola* strain LAR.16.03.LID was generated using CGView Server [48]. Synteny analysis of the complete genomes of *D. dianthicola* LAR.16.03.LID, *D. dianthicola* ME23 and *D. dianthicola* RNS049 was performed using the MAUVE software [49]. Paired end reads for the strain LAR.16.03.LID were mapped against the two complete genome sequences of *D. dianthicola* strains ME23 and RNS049 with threshold set as 0.8 of identity on 0.5 of read length using CLC Genomics Workbench version 10.1.1 software. The mappings were used for detection of variations (SNPs and InDels) using the basic variant calling tool from the CLC genomic workbench version 10.1.1.

The presence of clustered regularly interspaced short palindromic repeats (CRISPRs) was determined using CRISPRfinder (http://crispr.i2bc.paris-saclay.fr/Server/, Orsay, France) [50]. The prophage identification tool PHAge Search Tool—Enhanced Release (PHASTER) was used to check for the regions containing prophage-like elements in bacterial genomes (http://phaster.ca/, Edmonton, AB, Canada) [51]. The Predicted resistome was checked using Resistance Gene Identifier tool (https://card.mcmaster.ca/analyze/rgi, Hamilton, ON, Canada). Finally, genomic regions containing secondary metabolite biosynthesis gene clusters were identified using the AntiSMASH server (version 4.1.0, https://doi.org/10.1093/nar/gkv437, Hørsholm, Denmark).

To investigate the phylogenetic position of the Moroccan *D. dianthicola* strain against the available genomes of this species in NCBI, an MLSA was generated using 15 housekeeping genes (*fusA*, *rpoD*, *leuS*, *rpoS*, *purA*, *infB*, *gyrB*, *recA*, *groEL*, *secY*, *glyA*, *gapA*, *rplB*, *dnaX*, *gyrA*) with the MEGA7 software.

### 2.6. Potato Tuber Rotting Assays

Bacterial strains from Morocco were cultivated in TY broth for 24 h at 28 °C in a rotary shaker set at 125 rpm. Bacterial cultures were washed twice, resuspended in 0.8% NaCl, and the optical density was adjusted to OD$_{600}$ = 1.0. Potato tubers (cv. Bintje) were surface-disinfected by submerging them into a 5% sodium hypochlorite solution for 5 min. They were subsequently rinsed twice in distilled water and air dried at room temperature one day before inoculation. A total of 10 potato tubers were infected with 10 µL of cell suspension of each strain, along with 10 tubers with NaCl 0.8% alone as a negative control. After 5 days of incubation at 24 °C, the tubers were cut vertically through the inoculation points. Disease symptoms were evaluated to define five aggressiveness classes [52]. Significance of the observed differences was assessed using a Kruskal–Wallis test ($p < 0.05$).

### 3. Results

#### 3.1. Diversity of the Pectinolytic *Dickeya* and *Pectobacterium* in Northern Morocco

From 2015 to 2017, our field inspections revealed the occurrence of blackleg symptoms in several potato growing areas, located in many townships distributed in three regions (Meknes, S1; Guigo, S2; Larache, S4) in northern Morocco (Figure 1). No symptoms were found in fields in the Boumia region (S3). Out of 200 strains isolated from plant symptoms, 140 provoked cavities on the pectate-containing medium. These were tested by PCR to evaluate whether they belonged to the *Pectobacterium* and *Dickeya* genera: 119 isolates generated amplification signals for either the *Y1/Y2* or *ADE1/ADE2* primer couples. Most of the isolates (83%) generated a signal with the *Pectobacterium* primers *Y1/Y2*, while the others (17%) did so with the *Dickeya*-specific *ADE1/ADE2* primers.

All these PCR-positive *Pectobacterium/Dickeya* isolates were further characterized at species level based on their *gapA* gene sequence. Phylogenetic analyses using the Neighbor-Joining method (Mega7) of the *gapA* sequences are presented in the Figure S1. The regional diversity of the *Dickeya* and *Pectobacterium* isolates is summarized in Figure 1 (a detailed list is given in Table S2). The samples of the Larache region (S4) showed the highest diversity of taxons with the presence of *D. dianthicola* (20 isolates), *P. polaris* (6 isolates), *P. brasiliense* (5 isolates) and *P. carotovorum* (23 isolates). On the other hand, our investigations revealed the presence of only two species, *P. brasiliense* (53 isolates) and *P. carotovorum* (3 isolates), in the Meknes region (S1), and only a single one, *P. brasiliense* (9 isolates), in the Guigo region (S2).
On the other hand, our investigations revealed the occurrence of blackleg symptoms in several potato growing areas located in many townships of the seed tubers (local production or importation), irrigation mode (surface water from a dam or underground water from wells), and geography. No symptoms were found in fields in the Boumia (S3) region. Legend: Ddi, Dickeya dianthicola; Pb, Pectobacterium brasiliense; Pc, Pectobacterium carotovorum; Pp, Pectobacterium polaris; Pv, Pectobacterium versatile.

Important information was also collected from the farmers regarding the potato variety, origin of the seed tubers (local production or importation), irrigation mode (surface water from a dam or underground water from wells) and geography (the sampled regions). We tested whether a correlation between these different parameters and the diversity of pathogens (the combination of Dickeya and Pectobacterium species) existed. Statistical analysis with the SAS (Statistical Analysis System, version 9.00, SAS Institute, 2002, Cary, NC, USA) software (Qui2 test with p < 0.05) revealed that the higher diversity of pathogens was associated with three confounding factors: geography (the unique Larache region), surface water irrigation and imported seed tubers (Table S1).

3.2. Draft Genomes of 10 Pectinolytic Bacteria from Northern Morocco

A draft genome (Illumina technology) was used to consolidate the taxonomic position of 10 isolates belonging to the collected taxons. Between 705,755 and 17,016,482 trimmed reads were used for the contigs assembly of each of the 10 genomes. Characteristics of the draft genomes are presented in Table 1. Genome data were exploited to retrieve 13 housekeeping genes from each genome using BLAST. The concatenated genes were used for MLSA. The MLSA tree (Figure 2) showed a similar topology to the one generated by the gapA analysis (Figure S1).

Genomic data were also used to calculate ANI and isDDH values. Most of the Moroccan strains exhibited an ANI value higher than 95%, and an isDDH value higher than 70% with the closest type strains, confirming their taxonomic assignation. Strains belonging to the P. versatile clade showed an isDDH lower than 70%, but an ANI value higher than 95% with the strain SCC1. Recently, another study confirmed the classification of the strains S4.16.03.3I (= CFBP8660), S4.16.03.3F (= CFBP8659) and SCC1 into the P. versatile species [7].
that the higher diversity of pathogens was associated with three confounding factors: geography (the unique Larache region), surface water irrigation and imported seed tubers (Table S1).

3.2. Draft genomes of 10 Pectinolytic Bacteria from Northern Morocco

A draft genome (Illumina technology) was used to consolidate the taxonomic position of 10 isolates belonging to the collected taxons. Between 705,755 and 17,016,482 trimmed reads were used for the contigs assembly of each of the 10 genomes. Characteristics of the draft genomes are presented in Table 1. Genome data were exploited to retrieve 13 housekeeping genes from each genome using BLAST. The concatenated genes were used for MLSA. The MLSA tree (Figure 2) showed a similar topology to the one generated by the gapA analysis (Figure S1).

Figure 2. Phylogeny of the Moroccan strains based on MLSA. The phylogenetic trees were generated separately (A) for Pectobacterium and (B) for Dickeya strains. The alignment of the concatenated genes fusA, rpoD, rpoS, acnA, purA, recA, mdh, mtlD, groEL, secY, glyA, gapA and rplB was generated using ClustalW; the evolutionary history was inferred using the Neighbor-Joining method and the evolutionary distances were computed using the Maximum Composite Likelihood method. Phylogenetic analyses were conducted using MEGA7 software. The name of the Moroccan isolates is underlined.

3.3. Aggressiveness of the Pectinolytic Bacteria from Northern Morocco

Of the 10 Moroccan strains whose genome sequence is available (Table 1), all but 1 (P. brasiliense S4.16.03.1C) were tested for aggressiveness on potato tubers. P. brasiliense strain S4.16.03.1C was isolated from the same field as P. brasiliense strain S1.16.01.3K, and they showed 100% identify by ANI (Table 2). Hence, we retained one of the two for tuber maceration assays. For each of the nine strains, 10 tubers were inoculated, and the resulting maceration symptoms were classified into five symptomatic classes (Figure 3). In addition, 10 tubers were used as uninfected control. The aggressiveness was compared between all the strains using a Kruskal–Wallis test. All the pathogens provoked symptoms that are different to the control condition. No significant difference was observed between strains belonging to the same species. In contrast, D. dianthicola strains showed a higher aggressiveness when compared with Pectobacterium strains (p value < 0.05).
For each of the nine D. dianthicola–D. dianthicola (Figure 6). These regions contained strain-specific genes with no counterpart in the other D. dianthicola genomes as they provide acquired immunity against viruses and plasmids [53]. The three intact prophages (Figure 6 and Table S4). The CRISPR elements are very important features of bacterial also contain mobile elements (Figure 6 and Table S3-1–6). Phaster analysis suggested the presence of regions 1 and 2 are presented in Figure 6. Additional information about these strain-specific regions contained some mobile elements, such as genes from transposons and prophages; strain-specific regions 1 and 2 are presented in Figure 6. The typology of these classes was illustrated by a picture of an example. Data were statistically analyzed by a Kruskal–Wallis test (α = 5%). Lower case letters on the right of the graph indicate statistical differences between the different inoculated pathogens.

3.4. Complete Genome of D. dianthicola LAR.16.03.LID

The genome of D. dianthicola LAR.16.03.LID was the first complete genome of a D. dianthicola strain collected in Morocco, and the third D. dianthicola genome in the NCBI database that already hosted those of strains ME23 and RNS04.9 (Figure 4). Phylogenetic relationships between all D. dianthicola genomes available in NCBI were determined using MLSA. In the phylogenetic tree, the two Moroccan strains LAR.16.03.LID and S4.16.03.P2.4 appeared to be highly related. This could be explained by the close isolate locations, that were two potato fields separated only by a road. The two Moroccan D. dianthicola strains clustered with D. dianthicola strain ME23, which has been recently collected in a potato field in USA (Figure 4). The genomic relationship between D. dianthicola ME23 and D. dianthicola LAR.16.03.LID was confirmed using SNP/InDels calling. The SNP and InDel number in the LAR.16.03.LID genome reached 12,259 and 16,335, using the ME23 and D. dianthicola RNS04.9 genomes as a reference, respectively. The LAR.16.03.LID genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline. A graphical genome map is provided in Figure 5. The D. dianthicola LAR.16.03.LID genome exhibited a high synteny with that of D. dianthicola strains ME23 and RNS049, with the exception of some large insertion/deletions scattered in the genomes (Figure 6). These regions contained strain-specific genes with no counterpart in the other D. dianthicola genomes. In strains LAR.16.03.LID and ME23, the analyses evidenced one strain-specific region that contained some mobile elements, such as genes from transposons and prophages; strain-specific regions 1 and 2 are presented in Figure 6. Additional information about these strain-specific regions are available in Table S3-1 and Table S3-2. Strain RNS049 exhibited four strain-specific regions which also contain mobile elements (Figure 6 and Table S3-1–6). Phaster analysis suggested the presence of intact prophages (Figure 6 and Table S4). The CRISPR elements are very important features of bacterial genomes as they provide acquired immunity against viruses and plasmids [53]. The three D. dianthicola genomes hosted three or four CRISPR loci (Figure 6 and Table S5).

![Symptomatic class of each of the 10 tubers](image-url)
ME23 and RNS049, with the exception of some large insertion/deletions scattered in the genomes (Figure 6). These regions contained strain-specific genes with no counterpart in the other *D. dianthicola* genomes. In strains LAR.16.03LID and ME23, the analyses evidenced one strain-specific region that contained some mobile elements, such as genes from transposons and prophages; strain-specific regions 1 and 2 are presented in Figure 6. Additional information about these strain-specific regions are available in Table S3-1 and Table S3-2. Strain RNS049 exhibited four strain-specific regions which also contain mobile elements (Figure 6 and Table S3-1–6). Phaster analysis suggested the presence of intact prophages (Figure 6 and Table S4).

The CRISPR elements are very important features of bacterial genomes as they provide acquired immunity against viruses and plasmids [53]. The three *D. dianthicola* genomes hosted three or four CRISPR loci (Figure 6 and Table S5).

**Figure 4.** Phylogenetic analysis and characteristics of the Moroccan and NCBI *Dickeya* genomes. The genes *fusA*, *rpoD*, *leuS*, *rpoS*, *purA*, *infB*, *gyrB*, *recA*, *groEL*, *secY*, *glyA*, *gapA*, *rplB*, *dnaX* and *gyrA* were concatenated. The alignment was generated using ClustalW; the evolutionary history was inferred using the Neighbor-Joining method and the evolutionary distances were computed using the Maximum Composite Likelihood method. Phylogenetic analyses were conducted using MEGA7 software. The Moroccan *D. dianthicola* isolates are indicated in bold face.

**Figure 5.** Circular map of the genome of *Dickeya dianthicola* LAR.16.03.LID. The genome size is 4,976,211 bp with 4223 predicted protein-coding genes. The GC content and GC skew are represented on the distance scale (in kbp) on the inner map. The arrows around the map indicate the deduced Coding DNA Sequences (CDS) and their orientation.
Figure 5. Circular map of the genome of Dickeya dianthicola LAR.16.03.LID. The genome size is 4,976,211 bp with 4223 predicted protein-coding genes. The GC content and GC skew are represented on the distance scale (in kbp) on the inner map. The arrows around the map indicate the deduced Coding DNA Sequences (CDS) and their orientation.

Figure 6. Synteny between the complete genomes of D. dianthicola LAR.16.03.LID, RNS049 and ME23 strains. Synteny analysis was performed using MAUVE software. The numbers indicate the position of strain specific genomic regions. The secondary pathway gene clusters were searched using AntiSMASH, the prophages were identified using PHASTER, and CRISPER loci were localized using CRISPER finder.

The D. dianthicola LAR.16.03.LID genome exhibited an arsenal of virulence genes similar to that described for D. dianthicola RNS049 [39]. All the pectinase-encoding genes described in the model strain D. dadantii 3937 [54] were conserved in the three D. dianthicola genomes, with the noticeable exceptions of the lacking pehK gene (which encodes a predicted polygalacturonase) and the presence of a truncated form of the pelA gene (which encodes Pectate lyase A). Aside macerating enzymes determinants, other genes implicated in different stages of the host infection were conserved in the three D. dianthicola strains, including those involved in the resistance to oxidative stress, acidic pH (cfA, asr) and antimicrobial peptides (arrB-T, sapABCDF), synthesis of cell envelope components (such as bscABCD and wza-wzb-wzc), and siderophore synthesis and uptake (acsF-A and cbrABCDE for achronomobactin and fct-cbsCEBA for chrysobactin) [54].

We strengthened this analysis using the resistance gene identifier (RGI) and AntiSMASH softwares. We searched for genes involved in the resistance to different families of antimicrobial compounds. No differences were observed between the three D. dianthicola genomes (Table S6). The AntiSMASH analysis identified many secondary metabolite biogenesis clusters in D. dianthicola genomes that were already described in several Dickeya species, like those responsible for the synthesis of siderophores, cyanobactin with cytotoxic activity, bacteriocin, nonribosomal peptide-synthetase (NRPS) and arylpolyene (Figure 6 and Table S7).

4. Discussion

The main objective of this study was to characterize the pectinolytic populations isolated from symptomatic potato plants in Morocco between 2015 and 2017. A set of 119 pectinolytic bacteria, belonging to the genus Pectobacterium or Dickeya, were isolated and characterized using the gapA gene marker in combination with MLSA and ANI. Most of the isolates (83%) belonged to the Pectobacterium genus: the P. brasiliense species dominated in the Meknes and Guigo regions, while D. dianthicola was identified in the Larache region only.

P. brasiliense caused major economic losses to several crops (potato, cucumber, paprika, etc.) in many countries, including Canada, USA, South Africa, China, Korea and New Zealand [55–60]. The
wide host range of this pathogen could facilitate its survival even in harsh environments, by parasitizing many alternative host plants. In several studies, *P. brasiilense* isolates have been shown to be more aggressive than other *Pectobacterium* spp., except in the case of three Canadian strains exhibiting low aggressiveness [61]. In our study, the *P. brasiilense* isolates were as virulent as the other *Pectobacterium* strains. *P. brasiilense* was described in Morocco in 2012, and by now is the dominant species in two regions (S1 and S2). In the S2 region, the farmers use seed tubers produced in the S1 region, confirming the effective adaptation of this pathogen to the northern parts of Morocco.

The northern region (S4) exhibited the highest diversity of the pathogens. *P. versatile* and *P. Polaris*, described for the first time in Morocco, along with *P. brasiilense* and *D. dianthicola*, were isolated in the region S4. In this region, the majority of seed tubers were imported, and the irrigation water was derived from a dam. Either one or both agronomic practices could contribute to the wider diversity of pathogens in the Larache (S4) region than in the other investigated regions. While previous studies identified the *P. carotovorum* species as the most prevalent soft rot pathogens in Morocco [41,42], our study extended the diversity to other *Pectobacterium* and *Dickeyea* species, including the recently described species *P. versatile* [7]. This species encompasses isolates, including the *P. versatile* strain SCC1 isolated in 1980 in Finland [62], which had been collected from a wide diversity of environments (host plants, surface waters) and geographic areas [7]. The presence of members of this clade in the potato field could be linked to irrigation, as *P. versatile* is also able to survive in this environment. This hypothesis remains to be investigated by sampling water from the dam. In addition, our study extended the worldwide distribution of this species to Morocco.

The international distribution of the genus *Pectobacterium* increases concerns about the economic losses caused by this bacterium to the potato growers. A study in the neighboring country Algeria, carried out between 2014 and 2015, revealed the presence of pectinolytic bacteria causing soft rot in potatoes that belonged to *P. brasiilense* and *P. carotovorum*, as judged by MLSA [63]. Ozturk et al. reported the presence of *P. atrosepticum*, *P. brasiilense*, *P. carotovorum* and *P. parmentieri* species in Turkey [64]. In Europe, the prevalence of different species belonging to the genera *Pectobacterium* and *Dickeyea* detected in diseased potato plants differs from year to year and between countries, five bacterial species being the main causative agents of blackleg, namely *P. atrosepticum*, *P. parmentieri*, *P. brasiilense*, *D. solani* and *D. dianthicola* [65].

The *D. dianthicola* species has been detected in Morocco in 2017 [44], and described as the main species causing losses in potato in North America [66]. More studies are needed for monitoring the spread of this highly aggressive pathogen. To reach this objective, we used Illumina and ONT sequencing technologies to assemble a complete genome of one *D. dianthicola* isolate that could be used, in the future, as a reference for studying the clonal variability of *D. dianthicola* populations in Morocco and elsewhere. Comparison of the three complete genomes available indicated the presence of several clusters that encode the biosynthesis of a number of secondary metabolites implicated in stress defense, possibly playing an important role during plant–bacteria interactions. For instance, bacteriocins are small molecules with bactericidal activity usually restricted to closely related species, increasing the competition during infection, while the production of arylpolyene, implicated in the protection against reactive oxygen species [67], has been recently described in *D. fangzhongdai* genomes [68].

5. Conclusions

This study revealed a wide diversity of *Pectobacterium* and *Dickeyea* pathogens in northern Morocco, including *P. polaris* and *P. versatile*, that are reported for the first time in this country. In tuber maceration assays, the tested isolates of the emerging pathogen *D. dianthicola* were more aggressive than the *Pectobacterium* isolates. This feature should alert stakeholders to the threat that this pathogen poses to potato tuber production in northern Morocco. The nucleotide sequence data of the *Dickeyea* and *Pectobacterium* Moroccan isolates, including a complete genome of *D. dianthicola*, 10 draft genomes and 119 partial sequences of the *gapA* gene, were deposited in a public database (NCBI GenBank).
to be used as genetic resources for monitoring the spread of these pathogens in Northern Africa and elsewhere.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-2607/8/6/895/s1.

Figure S1: Phylogenetic analysis of the gapA barcode, Table S1: Primers used in this study, Table S2: Strains of Pectobacterium and Dickeya isolated from different regions in Morocco, Table S3-1: Strain-specific region number 1 identified by Mauve, Table S3-2: Strain-specific region number 2 identified by Mauve, Table S3-3: Strain-specific region number 3 identified by Mauve, Table S3-4: Strain-specific region number 4 identified by Mauve, Table S3-5: Strain-specific region number 5 identified by Mauve, Table S3-6: Strain-specific region number 6 identified by Mauve, Table S4: Secondary metabolite gene clusters identified with antismath, Table S5: Prophage in Dickeya dianthicola identified using Phaster, Table S6: CRISPR identification in Dickeya dianthicola, Table S7: Resistome genes identified in Dickeya dianthicola genomes.

**Author Contributions:** D.F. and M.M. supervised the data production and analysis, S.O. carried out isolation, taxonomical characterization and genome assembly and analysis, S.K. participated in Sanger sequence analysis, S.O. and S.S. conducted potato maceration assays, K.R. and S.K. conducted statistical analysis, C.L.-R. and C.V. carried out the ONT sequencing and assembly of the complete genome, S.O. and D.F. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by The French National Agency for Research (ANR-15-CE21-0003) and a cooperative project between France and Morocco (PRAD 14-02, Campus France n°30229ZK). Research in the I2BC was supported by the LabEx Saclay Plant Sciences-SPS (ANR-10-LABX-0040-SPS).

**Acknowledgments:** Yves Dessaux and Peter Mergaert (I2BC) are thanked for their advice during the preparation of the manuscript of which an uncorrected preprint was deposited (04 Feb, 2020) in Research Square database (https://www.researchsquare.com/article/rs-13222/v1; DOI 10.21203/rs.2.22648/v1) before a submission to Microorganisms Journal.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Czajkowski, R.; Pérombelon, M.C.M.; Van Veen, J.A.; Van der Wolf, J.M. Control of blackleg and tuber soft rot of potato caused by Pectobacterium and Dickeya species: A review. *Plant Pathol.* **2011**, *60*, 999–1013. [CrossRef]

2. Mansfield, J.; Genin, S.; Magori, S.; Citovsky, V.; Sriorianum, M.; Ronald, P.; Dow, M.; Verdier, V.; Beer, S.V.; Machado, M.A.; et al. Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.* **2012**, *13*, 614–629. [CrossRef] [PubMed]

3. Leonard, S.; Hommais, F.; Nasser, W.; Reverchon, S. Plant–phytopathogen interactions: Bacterial responses to environmental and plant stimuli. *Environ. Microbiol.* **2017**, *19*, 1689–1716. [CrossRef] [PubMed]

4. Pérombelon, M.C.M. Potato diseases caused by soft rot erwinias: An overview of pathogenesis. *Plant Pathol.* **2002**, *51*, 1–12. [CrossRef]

5. De Boer, S.H.; Verdonck, L.; Vrugtink, H.; Harju, P.; Bång, H.O.; Ley, J. De Serological and biochemical variation among potato strains of *Erwinia carotovora* subsp. atroseptica and their taxonomic relationship to other *E. carotovora* strains. *J. Appl. Bacteriol.* **1987**, *63*, 487–495. [CrossRef]

6. Khayi, S.; Cigna, J.; Chong, T.M.; Quié 1/2 tu-Laurent, A.; Chan, K.G.; Helias, V.; Faure, D. Transfer of the potato plant isolates of *Pectobacterium wasabiae* to *Pectobacterium parmentieri* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 5379–5383. [CrossRef]

7. Portier, P.; Pédron, J.; Taghouti, G.; Fischer-Le Saux, M.; Caulineau, E.; Bertrand, C.; Laurent, A.; Chawki, K.; Oulghazi, S.; Mounmi, M.; et al. Elevation of *Pectobacterium carotovorum* subsp. odoriferum to species level as *Pectobacterium odoriferum* sp. nov., proposal of *Pectobacterium brasiliense* sp. nov. and *Pectobacterium actiniae* sp. nov., emended description of *Pectobacterium carotovorum*. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 3207–3216. [CrossRef]

8. Smith, C. Variation in the Pathogenicity and Aggressiveness of Strains of *Erwinia carotovora* subsp. carotovora Isolated from Different Hosts. *Plant Dis.* **2007**, *74*, 505. [CrossRef]

9. Dees, M.W.; Lysøe, E.; Rossmann, S.; Perminow, J.; Brurberg, M.B. *Pectobacterium polaris* sp. nov., isolated from potato (*Solanum tuberosum*). *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 5222–5229. [CrossRef]

10. Sarfraz, S.; Riaz, K.; Oulghazi, S.; Cigna, J.; Sahi, S.T.; Khan, S.H.; Faure, D. *Pectobacterium punjabense* sp. nov., isolated from blackleg symptoms of potato plants in Pakistan. *Int. J. Syst. Evol. Microbiol.* **2018**, *68*, 3551–3556. [CrossRef]
11. Waleron, M.; Misztak, A.; Waleron, M.; Franczuk, M.; Wielgomas, B.; Waleron, K. Transfer of *Pectobacterium carotovorum* subsp. carotovorum strains isolated from potatoes grown at high altitudes to *Pectobacterium peruvianum* sp. nov. *Syst. Appl. Microbiol.* 2018, 41, 85–93. [CrossRef] [PubMed]

12. Waleron, M.; Waleron, K.; Lojkowska, E. Characterization of *Pectobacterium carotovorum* subsp. odoriferum causing soft rot of stored vegetables. *Eur. J. Plant Pathol.* 2014, 139, 457–469. [CrossRef]

13. Goto, M.; Matsumoto, K. Erwinia carotovora subsp. wasabiae subsp. nov. Isolated from Diseased Rhizomes and Fibrous Roots of Japanese Horseradish (*Eutrema wasabi* Maxim.). *Int. J. Syst. Bacteriol.* 1987, 37, 130–135. [CrossRef]

14. Gardan, L.; Gouy, C.; Christen, R.; Samson, R. Elevation of three subspecies of Pectobacterium carotovorum to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. *Int. J. Syst. Evol. Microbiol.* 2003, 53, 381–391. [CrossRef] [PubMed]

15. Nabhan, S.; de Boer, S.H.; Maiss, E.; Wydra, K. *Pectobacterium aroidearum* sp. nov., a soft rot pathogen with preference for monocotyledonous plants. *Int. J. Syst. Evol. Microbiol.* 2013, 63, 2520–2525. [CrossRef]

16. Waleron, M.; Misztak, A.; Waleron, M.; Franczuk, M.; Jorfa, J.; Wielgomas, B.; Mikiciński, A.; Popović, T.; Waleron, K. *Pectobacterium zantedeschiae* sp. nov. a new species of a soft rot pathogen isolated from Calla lily (*Zantedeschia* spp.). *Syst. Appl. Microbiol.* 2019, 42, 275–283. [CrossRef]

17. Koh, Y.J.; Kim, G.H.; Lee, Y.S.; Sohn, S.H.; Koh, H.S.; Kwon, S.; Heu, S.; Jung, J.S. *Pectobacterium carotovorum* subsp. actinidiae subsp. nov., a new bacterial pathogen causing canker-like symptoms in yellow kiwifruit, *Actinidia chinensis*. N. Z. J. Crop Hortic. Sci. 2012, 40, 269–279. [CrossRef]

18. Oulghazi, S.; Cigna, J.; Lau, Y.Y.; Mouroni, M.; Chan, K.G.; Faure, D. Transfer of the waterfall source isolate *Pectobacterium carotovorum* M022 to *Pectobacterium fontis* sp. nov., a deep-branching species within the genus Pectobacterium. *Int. J. Syst. Evol. Microbiol.* 2019, 69, 470–475. [CrossRef]

19. Péron, J.; Bertrand, C.; Taghouti, G.; Portier, P.; Barny, M.-A. *Pectobacterium aquaticum* sp. nov., isolated from waterways. *Int. J. Syst. Evol. Microbiol.* 2019, 69, 745–751. [CrossRef]

20. Toth, I.K.; van der Wolf, J.M.; Saddler, G.; Lojkowska, E.; Hélia, V.; Pirhonen, M.; Tsror (Lahkim), L.; Elphinstone, J.G. Dickeya species: An emerging problem for potato production in Europe. *Plant Pathol.* 2011, 60, 385–399. [CrossRef]

21. Tsror, L.; Erlich, O.; Lebiush, S.; Hazanovsky, M.; Zìg, U.; Slawiak, M.; Van Der Haar, J.J. Assessment of recent outbreaks of Dickeya sp. (syn. *Erwinia chrysanthemi*) slow wilt in potato crops in Israel. *Eur. J. Plant Pathol.* 2009, 123, 311–320.

22. 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.; et al. Dickeya solani sp. nov., a pectinolytic plant-pathogenic bacterium isolated from potato (*Solanum tuberosum*)*. *Int. J. Syst. Evol. Microbiol.* 2014, 64, 768–774. [CrossRef] [PubMed]

23. Ignatov, A.; Karlov, A.; Dzhalilov, F. Spreading of the blackleg of potatoes in Russia caused by bacteria of Dickeya genus. *Zaschita i Karantin Rastenij* 2014, 11, 41–43.

24. Ozturk, M.; Aksoy, H.M. First report of *Dickeya solani* associated with potato blackleg and soft rot in Turkey. *J. Plant Pathol.* 2017, 99, 298.

25. Tsror (Lahkim), L.; Erlich, O.; Lebiush, S.; van der Wolf, J.; Czajkowski, R.; Mozes, G.; Sikharulidze, Z.; Ben Daniel, B. First report of potato blackleg caused by a biovar 3 Dickeya sp. in Georgia. *New Dis. Rep.* 2011, 23, 1. [CrossRef]

26. Cardoza, Y.F.; Duarte, V.; Lopes, C.A. First report of blackleg of potato caused by *Dickeya solani* in Brazil. *Plant Dis.* 2017, 101, 243. [CrossRef]

27. Nykyri, J.; Niemi, O.; Koskinen, P.; Nakso-Koivisto, J.; Pasanen, M.; Broberg, M.; Pylusnin, I.; Törönen, P.; Holm, L.; Pirhonen, M.; et al. Revised Phylogeny and Novel Horizontally Acquired Virulence Determinants of the Model Soft Rot Phytopathogen Pectobacterium wasabiae SCC3193. *PLoS Pathog.* 2012, 8, e1003013. [CrossRef]

28. Zhang, Y.; Fan, Q.; Loria, R. A re-evaluation of the taxonomy of phytopathogenic genera Dickeya and Pectobacterium using whole-genome sequencing data. *Syst. Appl. Microbiol.* 2016, 39, 252–259. [CrossRef]

29. Brady, C.L.; Cleenwerck, I.; Denman, S.; Venter, S.N.; Rodriguez-Palenzuela, P.; Coutinho, T.A.; De Vos, P. Proposal to reclassify Brenneria quercina (Hildebrand and Schrho 1967) Hauben et al. 1999 into a new genus, Lonsdalea gen. nov., as *Lonsdalea quercina* comb. nov., descriptions of *Lonsdalea quercina* subsp. *quercina* comb. nov., *Lonsdalea quercina* subsp. *ib.* *Int. J. Syst. Evol. Microbiol.* 2012, 62, 1592–1602. [CrossRef]
30. Tian, Y.; Zhao, Y.; Yuan, X.; Yi, J.; Fan, J.; Xu, Z.; Hu, B.; De Boer, S.H.; Li, X. Dickeya fangzhongdai sp. nov., a plant-pathogenic bacterium isolated from pear trees (Pyrus pyrifolia). Int. J. Syst. Evol. Microbiol. 2016, 66, 2831–2835. [CrossRef] [PubMed]

31. Samson, R.; Legendre, J.; Richard, C.; Fischer-Le Saux, M.; Achouak, W.; Gardan, L. Transfer of Pectobacterium carotovorum Val, F.; Bouteau, F.; et al. Occurrence of Erwinia carotovora pectate media for isolation of Pectobacterium and Dickeya. Plant Pathol. 2015, 64, 2264–2266. [CrossRef] [PubMed]

32. Hugouvieux-Cotte-Pattat, N.; Jacot-Des-Combes, C.; Briolay, J. Dickeya aquatica sp. nov., isolated from waterways. Int. J. Syst. Evol. Microbiol. 2014, 64, 3613–3619. [CrossRef]

33. Parkinson, N.; DeVos, P.; Pirhonen, M.; Elphinstone, J. Characterization of a pectinolytic bacterium isolated from lakes in France. Int. J. Syst. Evol. Microbiol. 2019, 69, 721–726. [CrossRef] [PubMed]

34. Hanage, W.P.; Spratt, B.G.; Turner, K.M.E.; Fraser, C. Modelling bacterial speciation. Philos. Trans. R. Soc. B Biol. Sci. 2006, 361, 2039–2044. [CrossRef]

35. Golanowska, M.; Galardini, M.; Bazzicalupo, M.; Hugouvieux-Cotte-Pattat, N.; Mengoni, A.; Potrykus, M.; Slawiak, M.; Lojkowska, E. Draft Genome Sequence of a Highly Virulent Strain of the Plant Pathogen Dickeya solani, IFB0099. Genome Announc. 2015, 3, e00109-15. [CrossRef] [PubMed]

36. Grant, J.R.; Stothard, P. The CGView Server: A comparative genomics tool for circular genomes. Nucleic Acids Res. 2008, 36, W181–W184. [CrossRef]
