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Saïd Oulghazi, Mohieddine Moumni, Slimane Khayi, Kévin Robic, Sohaib Sarfraz, et al.. Diversity of Pectobacteriaceae Species in Potato Growing Regions in Northern Morocco. *Microorganisms*, 2020, 8 (6), pp.895. 10.3390/microorganisms8060895 . hal-02877933

HAL Id: hal-02877933

<https://hal.science/hal-02877933>

Submitted on 22 Nov 2021

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Diversity of *Pectobacteriaceae* species in potato growing regions in Northern Morocco

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21

22 **Abstract**

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

40

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

42

1. Introduction

Pectinolytic *Pectobacterium* and *Dickeya* spp. are causative agents of severe diseases on 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 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 rotting of stored potato tubers and wilting of potato plants under temperate climates [4]. Other *Pectobacterium* species frequently associated with damage on 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. peruvienne* 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. betavascularum* was reported almost exclusively on sugar beet [14]; *P. aroidearum* exhibits preference for some monocotyledonous plants [15]; *P. zantedeschiae* strains were isolated from *Zantedeschia* spp. (Calla lily) [16]; and, *P. actinidiae* from symptomatic *Actinidia*

67 *chinensis* (kiwi fruit) [17]. Recently, some other species, isolated from surface waters, have
68 also been described: *P. fontis*, *P. aquaticum* and *P. versatile* [7,18,19]. Altogether, 16
69 *Pectobacterium* species are described so far [7].

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

83 The unambiguous identification of *Dickeya* and *Pectobacterium* species is crucial for
84 epidemiological purposes, to develop appropriate prophylactic approaches and quality
85 controls in national and international trade exchanges. Multi-Locus Sequence Analysis
86 (MLSA) provides relevant information for a better understanding of speciation, hence for
87 proposing pertinent taxa delineations [15,36]. MLSA may exploit gene sequences obtained
88 by PCR-sequencing of several loci or by whole genome sequencing. Among the loci
89 commonly included in MLSA, the *rrs* sequence is poorly informative at a species level while
90 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 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 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, Germany). The sequences were trimmed using the CLC genomic workbench (V10.1.1) and aligned using ClustalW. The phylogenetic analysis of the *gapA* gene was performed as follow: the evolutionary distances were computed using the maximum composite Likelihood method (Mega7 software) with 1000 bootstrap. The obtained sequences were deposited in GenBank (TableS2).

2.3. Genome sequencing

138 Ten strains representing five species identified with *gapA* sequencing were selected for
139 genome sequencing (Table 1). DNA of the ten isolates (listed in Table 1) was extracted from
140 overnight cultures in TY medium using the MasterPure™ Complete DNA and RNA Purification
141 Kit (Epicenter, Madison, WI, USA) followed by an ethanol precipitation. The quantity and
142 quality control of the DNA was completed using a NanoDrop device and 1.0 % agarose gel
143 electrophoresis.

144 Paired-end libraries (500 bp in insert size) were constructed for each strain, and DNA
145 sequencing was performed by Illumina NextSeq technology. Sequencing of the library was
146 carried out using the 2×75 bp paired-end read module. Illumina sequencing was performed
147 at the I2BC sequencing platform (Gif-sur-Yvette, France).

148 In the case of the *D. dianthicola* strain LAR.16.03.LID, Nanopore sequencing was also
149 performed. Library preparation and sequencing were performed at the GeT-PlaGe core
150 facility, INRA Toulouse, using the “1D Native barcoding genomic DNA kit (EXP-NBD103 and
151 SQK-LSK108)”, according to the manufacturer’s instructions. At each step, DNA was
152 quantified using the Qubit dsDNA HS Assay Kit (Life Technologies). DNA purity was tested
153 using a NanoDrop device (Thermofisher) and size distribution and degradation was assessed
154 using the fragment analyzer (AATI) High Sensitivity DNA Fragment Analysis Kit. Purification
155 steps were performed using AMPure XP beads (Beckman Coulter). Five micrograms of each
156 DNA (five samples) were purified then sheared at 20 kb using the Megaruptor1 system
157 (Diagenode). A DNA damage repair step was performed on 3 µg of sample. Then END-repair
158 and dA tailing of double stranded DNA fragments were performed on 1 µg of each sample.
159 Then, a specific index was ligated to each sample. The library was generated by an equimolar
160 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 hours.

2.4. Genome assembly

Assembly of the Illumina reads was performed using the CLC Genomics Workbench v10.1.1 software (CLC Inc, 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

184 Phylogenetic and molecular evolutionary analyses were conducted using MEGA, version
185 7. An MLSA was performed using 13 concatenated housekeeping genes (*fusA*, *rpoD*, *acnA*,
186 *purA*, *gyrB*, *recA*, *mdh*, *mtlD*, *groEL*, *secY*, *glyA*, *gapA*, *rplB*) retrieved from all the
187 *Pectobacterium spp.* and *Dickeya spp.* strains to confirm their phylogenetic position within
188 the reference strains *P. atrosepticum* ICMP1526^T, *P. betavasculorum* NCPPB2795^T, *P.*
189 *parmentieri* RNS 08-42.1A^T, *P. wasabiae* CFBP 3304^T, *P. actinidiae* KKH3, *P. brasiliense*
190 LMG21371^T, *P. odoriferum* BCS7, *P. aroidearum* PC1, *D. dianthicola* NCPPB 453^T, *D. dadantii*
191 NCPPB 898^T, *D. solani* IPO2222^T. The average nucleotide identity (ANI) value was calculated
192 as previously proposed using the ANI calculator (<http://enveomics.ce.gatech.edu/ani/>). The *in-*
193 *silico* DNA-DNA hybridization (*isDDH*) was evaluated using genome sequence-based species
194 delineation (<http://ggdc.dsmz.de/>) (Table2).

195 The genome map of the *D. dianthicola* strain LAR.16.03.LID was generated using CGView
196 Server [44]. Synteny analysis of the complete genomes of *D. dianthicola* LAR.16.03.LID, *D.*
197 *dianthicola* ME23 and *D. dianthicola* RNS049 was performed using the MAUVE software [45].
198 Paired end reads for the strain LAR.16.03.LID were mapped against the two complete
199 genome sequences of *D. dianthicola* strains ME23 and RNS049 with threshold set as 0.8 of
200 identity on 0.5 of read length using CLC Genomics Workbench version 10.1.1 software. The
201 mappings were used for detection of variations (SNPs and InDels) using the basic variant
202 calling tool from the CLC genomic workbench version 10.1.1.

203 The presence of clustered regularly interspaced short palindromic repeats (CRISPRs) was
204 determined using CRISPRfinder (<http://crispr.i2bc.paris-saclay.fr/Server/>) [46]. The prophage
205 identification tool PHAge Search Tool – Enhanced Release (PHASTER) was used to check for
206 the regions containing prophage-like elements in bacterial genomes (<http://phaster.ca/>)
207 [47]. The Predicted resistome was checked using Resistance Gene Identifier tool

(<https://card.mcmaster.ca/analyze/rgi>). 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>).

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 fifteen 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 24h 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 OD₆₀₀ = 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. Ten 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 the Northern Morocco

From 2015-2017, our field inspections revealed the occurrence of blackleg symptoms in several potato growing areas located in many townships distributed in three regions

232 (Meknes, S1; Guigo, S2; Larache, S4) in Northern Morocco (Fig. 1). No symptoms were found
233 in fields in the Boumia region (S3). Out of 200 strains isolated from plant symptoms, 140
234 provoked cavities on pectate-containing medium. These were tested by PCR to evaluate
235 whether they belong to *Pectobacterium* and *Dickeya* genera: 119 isolates generated
236 amplification signals for either the Y1/Y2 or ADE1/ADE2 primer couples. Most of the isolates
237 (83%) generated a signal with the *Pectobacterium* primers Y1/Y2, while the others (17%) did
238 so with the *Dickeya*-specific ADE1/ADE2 primers.

239 All these PCR-positive *Pectobacterium*/*Dickeya* isolates were further characterized at
240 species level based on their *gapA* gene sequence. Phylogenetic analyses using Neighbor-
241 Joining method (Mega7) of the *gapA* sequences are presented in the Figure S1. The regional
242 diversity of the *Dickeya* and *Pectobacterium* isolates is summarized in Figure1 (a detailed list
243 is given in Table S2). The samples of the Larache region (S4) showed the highest diversity of
244 taxons with the presence of *D. dianthicola* (20 isolates), *P. polaris* (6 isolates), *P. brasiliense*
245 (5 isolates) and *P. carotovorum* (23 isolates). On the other hand, our investigations revealed
246 the presence of only two species, *P. brasiliense* (53 isolates) and *P. carotovorum* (3 isolates)
247 in the Meknes region (S1), and that of a single one, *P. brasiliense* (9 isolates), in the Guigo
248 region (S2).

249 Important information was also collected from the farmers including the potato variety,
250 origin of the seed tubers (local production or importation), irrigation mode (surface water
251 from a dam or underground water from wells) and geography (the sampled regions). We
252 tested whether a correlation between these different parameters and the diversity of
253 pathogens (the combination of *Dickeya* and *Pectobacterium* species) existed. Statistical
254 analysis with the SAS (Statistical Analysis System, version 9.00, SAS Institute, 2002, Cary,
255 USA) software (Qui2 test with $p < 0.05$) revealed that the higher diversity of pathogens was

associated to 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 ten 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 ten genomes. Characteristics of the draft genomes are presented in the 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 (Fig. 2) showed a similar topology as the one generated by the *gapA* analysis (Fig. 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].

3.3. Aggressiveness of the pectinolytic bacteria from Northern Morocco

Of the ten Moroccan strains whose genome sequence is available (Table 1), all but one (*P. brasiliense* S4.16.03.1C) were tested for aggressiveness on potato tubers. *P. brasiliense* strain S4.16.03.1C has been 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, ten tubers were inoculated and the resulting maceration symptoms were classified into five symptomatic classes (Fig. 3). In addition, ten 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).

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 (Fig. 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 that has been recently collected in a potato field in USA (Fig. 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 12259 and 16335 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 Fig. 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 (Fig. 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 Fig. 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 (Fig. 6 and TableS3). Phaster analysis suggested the presence of intact prophages (Fig. 6 and TableS5). The CRISPR elements are very important features of bacterial genomes as they provide acquired immunity against viruses and plasmids [49]. The three *D. dianthicola* genomes hosted three or four CRISPR loci (Fig6 and TableS6).

The *D. dianthicola* LAR.16.03.LID genome exhibited a 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 [48] were conserved in the three *D. dianthicola* genomes, with the noticeable exceptions of the lacking *pehK* gene (that encodes a predicted polygalacturonase) and the presence of a truncated form of the *peIA* gene (that 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 (*arnB-T*, *sapABCDF*), synthesis of cell envelope components (such as *bscABCD* and *wza-wzb-wzc*) and siderophore synthesis and uptake (*acsF-A* and *cbrABCDE* for achromobactin and *fct-cbsCEBA* for chrysobactin) [48].

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 (TableS7). 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, NRPS (nonribosomal peptide-synthetase) and arylpolyene (Fig. 6 and Table S4).

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: *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...) in many countries including Canada, USA, South Africa, China, Korea and New Zealand [50–55]. 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. brasiliense* isolates have been shown to be more aggressive than other *Pectobacterium* spp., except in the case of three Canadian strains exhibiting low aggressiveness [56]. In our study, the *P. brasiliense* isolates were as virulent as the other *Pectobacterium* strains. *P. brasiliense* was described in Morocco in 2012 and by now was 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.

352 The Northern region (S4) exhibited the highest diversity of the pathogens. *P. versatile*
353 and *P. Polaris*, described for the first time in Morocco, along with *P. brasiliense* and *D.*
354 *dianthicola* were isolated in the region S4. In this region the majority of seed tubers were
355 imported and the irrigation water was derived from a dam. Either one or both agronomic
356 practices could contribute to the wider diversity of pathogens in the Larache (S4) region than
357 in the other investigated regions. While previous studies identified *P. carotovorum* species as
358 the most prevalent soft rot pathogens in Morocco [41,42], our study extended the diversity
359 to other *Pectobacterium* and *Dickeya* species, including the recently described species *P.*
360 *versatile* [7]. This species encompasses isolates, including *P. versatile* strain SCC1 isolated in
361 1980 in Finland [57], which had been collected from a wide diversity of environments (host
362 plants, surface waters) and geographic areas [7]. The presence of members of this clade in
363 the potato field could be linked to irrigation, as *P. versatile* is also able to survive in this
364 environment. This hypothesis remains to be investigated by sampling water from the dam. In
365 addition, our study extended the worldwide distribution of this species to Morocco.

366 The international distribution of the genus *Pectobacterium* increases concerns about the
367 economic losses caused by this bacterium to the potato growers. A study in the neighboring
368 country Algeria carried out between 2014 and 2015 revealed the presence of pectinolytic
369 bacteria causing soft rot on potato that belonged to *P. brasiliense* and *P. carotovorum* as
370 judged by MLSA [58]. Ozturk *et al.* reported the presence of *P. atrosepticum*, *P. brasiliense*,
371 *P. carotovorum* and *P. parmentieri* species in Turkey [59]. In Europe, prevalence of different
372 species belonging to the genus *Pectobacterium* and *Dickeya* detected in diseased potato
373 plants differs from year to year and countries, five bacterial species being the main causative
374 agents of blackleg, namely *P. atrosepticum*, *P. parmentieri*, *P. brasiliense*, *D. solani* and *D.*
375 *dianthicola* [60].

D. dianthicola species has been detected in Morocco in 2017 [44] and described as the main species causing losses in potato in North America [61]. 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 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, playing a possible 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 [63].

5. Conclusion

This study revealed a wide diversity of *Pectobacterium* and *Dickeya* 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 puts on potato tuber production in Northern Morocco. The nucleotide sequence data of the *Dickeya* 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: Fig.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* using CRISPER Finder, Table S7: Resistome genes identified in *Dickeya dianthicola* genomes.

Acknowledgments: 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). 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](https://doi.org/10.21203/rs.2.22648/v1)) before a submission to Microorganisms Journal.

Author Contributions: DF and MM supervised the data production and analysis, SO carried out isolation, taxonomical characterization and genome assembly and analysis, SK participated in Sanger sequence analysis, SO and SS conducted potato maceration assays, KR and SK conducted statistical analysis, CLR and CV carried out the ONT sequencing and assembly of the complete genome, SO and DF wrote the manuscript. All authors have read and agreed on the published version of the manuscript.

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

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Figure legends

Fig. 1: Species diversity of the *Pectobacterium* and *Dickeya* pathogens in the sampling areas of Northern Morocco.

Sampling of pathogens was performed in four potato growing areas in the Northern Morocco: Meknes (S1), Guigo (S2), Boumia (S3) and Laraache (S4). The map of Morocco was generated by a free and open source geographic information system (<https://www.qgis.org/fr/site/index.html>). The pie charts represent diversity of the isolated pathogens. None of them was collected from Boumia (S3) region. Legend: Ddi, *Dickeya dianthicola*; Pb, *Pectobacterium brasiliense*; Pc, *Pectobacterium carotovorum*; Pp, *Pectobacterium polaris*; Pv, *Pectobacterium versatile*.

Fig. 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.

Fig. 3: Virulence test of *Pectobacterium* and *Dickeya* on potato tubers.

The symptoms provoked by each strain were compared by infecting ten tubers per strain. Ten non-inoculated tubers were used as control. Symptoms were classified into five classes

(CLA0, CLA1, CLA2, CLA3, CLA4 according an increasing severity). The typology of these classes was illustrated by a picture of an example. Data were statistically analyzed by a Kruskal-Wallis test ($\alpha = 5\%$). Lower case letters on the right of the graph indicate statistical differences between the different inoculated pathogens.

Fig. 4: Phylogenetic analysis and characteristics of the Moroccan and NCBI *Dickeya* genomes

The gene *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.

Fig. 5: Circular map of the genome of *Dickeya dianthicola* LAR.16.03.LID.

The genome size is 4,976,211 bp with 4,223 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 deduced ORFs and their orientation.

Fig. 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

717 using AntiSMASH, the prophages were identified using PHASTER and CRISPER loci were
718 localized using CRISPER finder.

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722 **Table1: Draft genome sequences of *Pectobacterium* and *Dickeya* strains isolated from Northern Morocco.**

Organism	Accession N°	Genome size	N50 (pb)	No. of contigs	Coverage	No. of CDS	No. of tRNAs
<i>Pectobacterium polaris</i> S4.16.03.2B	QZDF000000000	4,862,009	155,865	65	41	4,355	54
<i>Pectobacterium brasiliense</i> S1.16.01.3k	QZDG000000000	4,946,598	146,844	74	410	4,337	36
<i>Pectobacterium brasiliense</i> S1.15.11.2D	QZDH000000000	4,818,836	99,392	91	420	4,206	35
<i>Pectobacterium brasiliense</i> S4.16.03.1C	QZDI000000000	4,944,722	139,665	74	467	4,336	37
<i>Pectobacterium carotovorum</i> S1-A16	QZDJ000000000	4,835,633	255,206	37	55	4,261	63
<i>Pectobacterium versatile</i> S4.16.03.3I	QZDK000000000	4,854,084	82,62	108	246	4,247	34
<i>Pectobacterium versatile</i> S4.16.03.3K	QZDL000000000	4,870,940	90,195	106	237	4,262	34
<i>Pectobacterium versatile</i> S4.16.03.3F	QZDM000000000	4,852,595	89,731	114	143	4,244	40
<i>Dickeya dianthicola</i> S4.16.03.P2.4	QZDN000000000	4,865,147	92,028	101	415	4,238	37
<i>Dickeya dianthicola</i> LAR.16.03.LID	QZDO000000000	4,863,939	71,707	108	344	4,238	37

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728 **Table 2. Pairwise ANI and isDDH values of *Pectobacterium* and *Dickeya* strains isolated from Northern Morocco.**

Strains	ANI values															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1-Pp NIBIO 1006^T		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	93.7	93.4	93.5	93.5	93.4	92.2	78.9	79	79
3-Pc ICMP5702^T	52.3	52.10		97.2	92.6	92.9	92.8	92.8	95.1	95.2	95.2	95.2	94.8	78.9	78.9	78.9
4-Pc S1-A16	52.2	51.90	76.30		92.6	92.9	92.7	92.9	95.3	95.3	95.3	95.3	94.8	78.8	79.6	79.6
5-Pb LMG 21371^T	54.4	54.5	50.9	50.9		96.1	95.9	96.1	92.2	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	92.3	91.8	79.1	79.1	79.1
8-Pb S1.16.01.3K	56	55.7	76	51.7	68.5	100	71.4		92.4	92.4	92.4	92.3	91.9	79.6	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	47.8	60.4	60.7	60.6	60.6		79.1	79.1	79.1
14-Ddi NCPPB453^T	21.1	20.7	20.5	20.7	20.9	21	20.6	21	21.2	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	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	20.8	95.6	100	
	is-DDH															

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730 Strains: **1**, *P. polaris* NIBIO1006^T ; **2**, *P. polaris* S4.16.03.2B **3**, *P. carotovorum* ICMP5702^T; **4**, *P. carotovorum* S1-A16; **5**, *P. brasiliense* LMG21371^T; **6**, *P.*
731 *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*
732 S4.16.03.3k **12**, *P. versatile* S4.16.03.3I; **13**, *P. odoriferum* BCS7; **14**, *D. dianthicola* NCPPB 453T, **15**, *D. dianthicola* S4.16.03.P2.4; **16**, *D. dianthicola*
733 LAR.16.03.LID.