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► **To cite this version:**

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

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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

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## 44 **1. Introduction**

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

55 *P. atrosepticum* was considered as the primary pathogen responsible for rotting of  
56 stored potato tubers and wilting of potato plants under temperate climates [4]. Other  
57 *Pectobacterium* species frequently associated with damage on potato crops are *P.*  
58 *carotovorum*, *P. brasiliense*, *P. parmentieri* and *P. polaris* [5–9]. Some *Pectobacterium*  
59 species have also been characterized in some specific areas such as *P. peruviense* strains  
60 isolated from tubers in Peru and *P. punjabense* species, from symptomatic potato plants in  
61 Pakistan [10,11]. *P. odoriferum* exhibits a very wide host range including potato plants [12],  
62 while some other species were characterized by a more restricted host range at least in the  
63 fields. Thus, *P. wasabiae* was isolated from symptomatic Japanese horseradish [13]; *P.*  
64 *betavasculorum* was reported almost exclusively on sugar beet [14]; *P. aroidearum* exhibits  
65 preference for some monocotyledonous plants [15]; *P. zantedeschiae* strains were isolated  
66 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. zae* [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

91 *Pectobacterium* species [10,18,19,31,32,37]. Taxonomy of *Dickeya* and *Pectobacterium*  
92 gained precision and robustness with additional genome analyses such as average  
93 nucleotide identity (ANI) and *in silico* DNA-DNA hybridization (*isDDH*) [38]. Comparative  
94 genomics is also used to identify species-specific DNA regions. Analysis of the functions  
95 encoded by these DNA regions allows the prediction of species-specific metabolic traits. This  
96 knowledge contributes to the understanding of both the taxonomy and ecology of the  
97 *Dickeya* and *Pectobacterium* pathogens [22,30–32,39].

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

108

## 109 **2. Materials and Methods**

### 110 *2.1. Sampling and isolation of pectinolytic bacteria*

111 In 2015, 2016 and 2017, blackleg symptoms were searched in potato fields in four  
112 regions (Meknes, Guigo, Boumia and Larache) in Northern Morocco. Pectinolytic bacteria  
113 were isolated from symptomatic plant tissues using crystal violet pectate (CVP) medium as  
114 described previously [45]. The CVP plates were incubated at 28°C for 3 days and colonies

115 that had formed pits were re-streaked onto Tryptone (5 g/L) Yeast extract (3 g/L) agar  
116 medium (TY). The purified isolates were spotted again on CVP to confirm the pectinolytic  
117 activity. The obtained cultures from single colonies were stored in 25% glycerol at -80°C.

118

## 119 2.2. *Molecular characterization of Pectobacterium and Dickeya isolates*

120 The primer couples Y1/Y2 and ADE1/ADE2 (Table S1) were used for the identification of  
121 isolates belonging to *Pectobacterium* and *Dickeya* genera [46,47]. The reaction was carried  
122 out in a final volume of 25 µl, containing 1 µl of bacterial DNA (50 ng/µl), 2.5 µl of PCR buffer  
123 (10×), 2 µl of MgCl<sub>2</sub> (25 mM), 2.5 µl of dNTPs (1 mM), 1U Taq polymerase and 1 µl of each  
124 primer (1 µM) and water. The temperature settings for PCR were the same as described  
125 before [46,47]. The analysis of PCR products was done by electrophoresis on 2% agarose  
126 gels, using PCR products of *P. atrosepticum* CFBP1526<sup>T</sup> and *D. solani* IPO2222<sup>T</sup> as control  
127 along with the 1 Kb DNA ladder.

128 Positive strains for either Y1/Y2 or ADE1/ADE2 PCR were further characterized using the  
129 *gapA* barcode procedure [37]. All the *gapA* PCR products obtained with gapA-F/gapA-R  
130 primers (Table S1) were sequenced using Sanger technology (GATC Biotech, Germany). The  
131 sequences were trimmed using the CLC genomic workbench (V10.1.1) and aligned using  
132 ClustalW. The phylogenetic analysis of the *gapA* gene was performed as follow: the  
133 evolutionary distances were computed using the maximum composite Likelihood method  
134 (Mega7 software) with 1000 bootstrap. The obtained sequences were deposited in GenBank  
135 (TableS2).

136

## 137 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



161 was loaded on a R9.4.1 flowcell and sequenced on MinION instrument at 0.15 pmol within  
162 48 hours.

163

#### 164 2.4. Genome assembly

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

172 Assembly of the Nanopore reads was performed as follows. Fast5s from Nanopore  
173 sequencing were obtained with MinKNOW version 1.10.23 and were basecalled with ONT  
174 Albacore Sequencing Pipeline Software version 2.1.10 and reads passing the internal test  
175 were used for subsequent analysis. Porechop 0.2.1 (<https://github.com/rrwick/Porechop>)  
176 was used for adaptor trimming. Illumina paired-end reads were processed with trim\_galore  
177 0.4.0 (<https://github.com/FelixKrueger/TrimGalore>) to trim adaptor sequences. Nanopore  
178 reads were assembled using Canu 1.7 [48] with the “genomeSize=5 m” and  
179 “minReadLength=3000” options. For Nanopore-only assembly, one output contig was  
180 obtained, then polished three times using Pilon 1.22  
181 (<https://github.com/broadinstitute/pilon>) with the “--mindepth 25” option. The contig was  
182 finally circularized using Circlator 1.5.1 (<https://github.com/sanger-pathogens/circlator>).

#### 183 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<sup>T</sup>, *P. betavascolorum* NCPPB2795<sup>T</sup>, *P.*  
189 *parmentieri* RNS 08-42.1A<sup>T</sup>, *P. wasabiae* CFBP 3304<sup>T</sup>, *P. actinidiae* KKH3, *P. brasiliense*  
190 LMG21371<sup>T</sup>, *P. odoriferum* BCS7, *P. aroidearum* PC1, *D. dianthicola* NCPPB 453<sup>T</sup>, *D. dadantii*  
191 NCPPB 898<sup>T</sup>, *D. solani* IPO2222<sup>T</sup>. 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 (*is*DDH) 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

208 (<https://card.mcmaster.ca/analyze/rgi>). Finally, genomic regions containing secondary  
209 metabolite biosynthesis gene clusters were identified using the AntiSMASH server (version  
210 4.1.0, <https://doi.org/10.1093/nar/gkv437>).

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

215

## 216 2.6. *Potato tuber rotting assays*

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

227

## 228 3. Results

### 229 3.1. *Diversity of the pectinolytic Dickeya and Pectobacterium in the Northern Morocco*

230 From 2015-2017, our field inspections revealed the occurrence of blackleg symptoms in  
231 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

256 associated to three confounding factors, geography (the unique Larache region), surface  
257 water irrigation and imported seed tubers (Table S1).

258

### 259 3.2. Draft genomes of 10 pectinolytic bacteria from Northern Morocco

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

267 Genomic data were also used to calculate ANI and *isDDH* values. Most of the Moroccan  
268 strains exhibited an ANI value higher than 95% and an *isDDH* value higher than 70% with the  
269 closest type strains, confirming their taxonomic assignation. Strains belonging to the  
270 *P. versatile* clade showed an *isDDH* lower than 70% but an ANI value higher than 95% with  
271 the strain SCC1. Recently, another study confirmed the classification of the strains  
272 S4.16.03.3I (= CFBP8660), S4.16.03.3F (= CFBP8659) and SCC1 into the *P. versatile* species  
273 [7].

274

### 275 3.3. Aggressiveness of the pectinolytic bacteria from Northern Morocco

276 Of the ten Moroccan strains whose genome sequence is available (Table 1), all but one  
277 (*P. brasiliense* S4.16.03.1C) were tested for aggressiveness on potato tubers. *P. brasiliense*  
278 strain S4.16.03.1C has been isolated from the same field as *P. brasiliense* strain S1.16.01.3K  
279 and they showed 100% identify by ANI (Table 2). Hence, we retained one of the two for

280 tuber maceration assays. For each of the nine strains, ten tubers were inoculated and the  
281 resulting maceration symptoms were classified into five symptomatic classes (Fig. 3). In  
282 addition, ten tubers were used as uninfected control. The aggressiveness was compared  
283 between all the strains using a Kruskal-Wallis test. All the pathogens provoked symptoms  
284 that are different to the control condition. No significant difference was observed between  
285 strains belonging to the same species. In contrast, *D. dianthicola* strains showed a higher  
286 aggressiveness when compared with *Pectobacterium* strains ( $P$  value < 0.05).

287

### 288 3.4. Complete genome of *D. dianthicola* LAR.16.03.LID

289 The genome of *D. dianthicola* LAR.16.03.LID was the first complete genome of a *D.*  
290 *dianthicola* strain collected in Morocco and the third *D. dianthicola* genome in the NCBI  
291 database that already hosted those of strains ME23 and RNS04.9 (Fig. 4). Phylogenetic  
292 relationships between all *D. dianthicola* genomes available in NCBI were determined using  
293 MLSA. In the phylogenetic tree, the two Moroccan strains LAR.16.03.LID and S4.16.03.P2.4  
294 appeared to be highly related. This could be explained by the close isolate locations that  
295 were two potato fields separated only by a road. The two Moroccan *D. dianthicola* strains  
296 clustered with *D. dianthicola* strain ME23 that has been recently collected in a potato field in  
297 USA (Fig. 4). The genomic relationship between *D. dianthicola* ME23 and *D. dianthicola*  
298 LAR.16.03.LID was confirmed using SNP/InDels calling. The SNP and InDel number in the  
299 LAR.16.03.LID genome reached 12259 and 16335 using the ME23 and *D. dianthicola* RNS04.9  
300 genomes as a reference, respectively. The LAR.16.03.LID genome was annotated using the  
301 NCBI Prokaryotic Genome Annotation Pipeline. A graphical genome map is provided in Fig. 5.  
302 The *D. dianthicola* LAR.16.03.LID genome exhibited a high synteny with that of *D. dianthicola*  
303 strains ME23 and RNS049, with the exception of some large insertion/deletions scattered in

304 the genomes (Fig. 6). These regions contained strain-specific genes with no counterpart in  
305 the other *D. dianthicola* genomes. In strains LAR.16.03LID and ME23, the analyses evidenced  
306 one strain-specific region that contained some mobile elements such as genes from  
307 transposons and prophages: strain-specific regions 1 and 2 are presented in Fig. 6. Additional  
308 information about these strain-specific regions are available in Table S3-1 and Table S3-2.  
309 Strain RNS049 exhibited four strain-specific regions which also contain mobile elements (Fig.  
310 6 and TableS3). Phaster analysis suggested the presence of intact prophages (Fig. 6 and  
311 TableS5). The CRISPR elements are very important features of bacterial genomes as they  
312 provide acquired immunity against viruses and plasmids [49]. The three *D. dianthicola*  
313 genomes hosted three or four CRISPR loci (Fig6 and TableS6).

314 The *D. dianthicola* LAR.16.03.LID genome exhibited a arsenal of virulence genes similar to  
315 that described for *D. dianthicola* RNS049 [39]. All the pectinase-encoding genes described in  
316 the model strain *D. dadantii* 3937 [48] were conserved in the three *D. dianthicola* genomes,  
317 with the noticeable exceptions of the lacking *pehK* gene (that encodes a predicted  
318 polygalacturonase) and the presence of a truncated form of the *peIA* gene (that encodes  
319 Pectate lyase A). Aside macerating enzymes determinants, other genes implicated in  
320 different stages of the host infection were conserved in the three *D. dianthicola* strains,  
321 including those involved in the resistance to oxidative stress, acidic pH (*cfa*, *asr*) and  
322 antimicrobial peptides (*arnB-T*, *sapABCDF*), synthesis of cell envelope components (such as  
323 *bscABCD* and *wza-wzb-wzc*) and siderophore synthesis and uptake (*acsF-A* and *cbrABCDE* for  
324 achromobactin and *fct-cbsCEBA* for chrysobactin) [48].

325 We strengthened this analysis using the resistance gene identifier (RGI) and AntiSMASH  
326 softwares. We searched for genes involved in the resistance to different families of  
327 antimicrobial compounds. No differences were observed between the three *D. dianthicola*

328 genomes (TableS7). The AntiSMASH analysis identified many secondary metabolite  
329 biogenesis clusters in *D. dianthicola* genomes that were already described in several *Dickeya*  
330 species like those responsible for the synthesis of siderophores, cyanobactin with cytotoxic  
331 activity, bacteriocin, NRPS (nonribosomal peptide-synthetase) and arylpolyene (Fig. 6 and  
332 Table S4).

333

#### 334 **4. Discussion**

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

342 *P. brasiliense* caused major economic losses to several crops (potato, cucumber,  
343 paprika...) in many countries including Canada, USA, South Africa, China, Korea and New  
344 Zealand [50–55]. The wide host range of this pathogen could facilitate its survival even in  
345 harsh environments by parasitizing many alternative host plants. In several studies, *P.*  
346 *brasiliense* isolates have been shown to be more aggressive than other *Pectobacterium* spp.,  
347 except in the case of three Canadian strains exhibiting low aggressiveness [56]. In our study,  
348 the *P. brasiliense* isolates were as virulent as the other *Pectobacterium* strains. *P. brasiliense*  
349 was described in Morocco in 2012 and by now was the dominant species in two regions (S1  
350 and S2). In the S2 region, the farmers use seed tubers produced in the S1 region, confirming  
351 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].

376 *D. dianthicola* species has been detected in Morocco in 2017 [44] and described as the  
377 main species causing losses in potato in North America [61]. More studies are needed for  
378 monitoring the spread of this highly aggressive pathogen. To reach this objective, we used  
379 Illumina and ONT sequencing technologies to assemble a complete genome of one *D.*  
380 *dianthicola* isolate that could be used, in the future, as a reference for studying clonal  
381 variability of *D. dianthicola* populations in Morocco and elsewhere. Comparison of the three  
382 complete genomes available indicated the presence of several clusters that encode the  
383 biosynthesis of a number of secondary metabolites implicated in stress defense, playing a  
384 possible important role during plant-bacteria interactions. For instance, bacteriocins are  
385 small molecules with bactericidal activity usually restricted to closely related species,  
386 increasing the competition during infection, while the production of arylpolyene implicated  
387 in the protection against reactive oxygen species [67] has been recently described in *D.*  
388 *fangzhongdai* genomes [63].

389

## 390 **5. Conclusion**

391 This study revealed a wide diversity of *Pectobacterium* and *Dickeya* pathogens in  
392 northern Morocco including *P. polaris* and *P. versatile* that are reported for the first time in  
393 this country. In tuber maceration assays, the tested isolates of the emerging pathogen *D.*  
394 *dianthicola* were more aggressive than the *Pectobacterium* isolates. This feature should alert  
395 stake holders to the threat that this pathogen puts on potato tuber production in Northern  
396 Morocco. The nucleotide sequence data of the *Dickeya* and *Pectobacterium* Moroccan  
397 isolates, including a complete genome of *D. dianthicola*, 10 draft genomes and 119 partial  
398 sequences of the *gapA* gene were deposited in a public database (NCBI GenBank) to be used

399 as genetic resources for monitoring the spread of these pathogens in Northern Africa and  
400 elsewhere.

401

402 **Supplementary Materials:** Fig.S1: Phylogenetic analysis of the *gapA* barcode, Table S1:  
403 Primers used in this study, Table S2: Strains of *Pectobacterium* and *Dickeya* isolated from  
404 different regions in Morocco, Table S3-1: Strain-specific region number 1 identified by  
405 Mauve, Table S3-2: Strain-specific region number 2 identified by Mauve, Table S3-3: Strain-  
406 specific region number 3 identified by Mauve, Table S3-4: Strain-specific region number 4  
407 identified by Mauve, Table S3-5: Strain-specific region number 5 identified by Mauve, Table  
408 S3-6: Strain-specific region number 6 identified by Mauve, Table S4: Secondary metabolite  
409 gene clusters identified with antismath, Table S5: Prophage in *Dickeya dianthicola*  
410 identified using Phaster, Table S6: CRISPR identification in *Dickeya dianthicola* using CRISPER  
411 Finder, Table S7: Resistome genes identified in *Dickeya dianthicola* genomes.

412

413 **Acknowledgments:** This work was supported by The French National Agency for Research  
414 (ANR-15-CE21-0003) and a cooperative project between France and Morocco (PRAD 14-02,  
415 Campus France n°30229ZK). Research in the I2BC was supported by the LabEx Saclay Plant  
416 Sciences-SPS (ANR-10-LABX-0040-SPS). Yves Dessaux and Peter Mergaert (I2BC) are thanked  
417 for their advice during the preparation of the manuscript of which an uncorrected preprint  
418 was deposited (04 Feb, 2020) in Research Square database  
419 (<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  
420 a submission to Microorganisms Journal.

421

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

428

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

430

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669

670 **Figure legends**

671

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

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

681

682 **Fig. 2: Phylogeny of the Moroccan strains based on MLSA.**

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

690

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

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

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

698

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

701 The gene *fusA*, *rpoD*, *leuS*, *rpoS*, *purA*, *infB*, *gyrB*, *recA*, *groEL*, *secY*, *glyA*, *gapA*, *rplB*, *dnaX*  
702 and *gyrA* were concatenated. The alignment was generated using ClustalW; the evolutionary  
703 history was inferred using the Neighbor-Joining method and the evolutionary distances were  
704 computed using the Maximum Composite Likelihood method. Phylogenetic analyses were  
705 conducted using MEGA7 software. The Moroccan *D. dianthicola* isolates are indicated in  
706 bold face.

707

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

709 The genome size is 4,976,211 bp with 4,223 predicted protein-coding genes. The GC content  
710 and GC skew are represented on the distance scale (in kbp) on the inner map. The arrows  
711 around the map indicate deduced ORFs and their orientation.

712

713 **Fig. 6: Synteny between the complete genomes of *D. dianthicola* LAR.16.03.LID, RNS049**  
714 **and ME23 strains.**

715 Synteny analysis was performed using MAUVE software. The numbers indicate the position  
716 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	QZDF00000000	4,862,009	155,865	65	41	4,355	54
<i>Pectobacterium brasiliense</i> S1.16.01.3k	QZDG00000000	4,946,598	146,844	74	410	4,337	36
<i>Pectobacterium brasiliense</i> S1.15.11.2D	QZDH00000000	4,818,836	99,392	91	420	4,206	35
<i>Pectobacterium brasiliense</i> S4.16.03.1C	QZDI00000000	4,944,722	139,665	74	467	4,336	37
<i>Pectobacterium carotovorum</i> S1-A16	QZDJ00000000	4,835,633	255,206	37	55	4,261	63
<i>Pectobacterium versatile</i> S4.16.03.3I	QZDK00000000	4,854,084	82,62	108	246	4,247	34
<i>Pectobacterium versatile</i> S4.16.03.3K	QZDL00000000	4,870,940	90,195	106	237	4,262	34
<i>Pectobacterium versatile</i> S4.16.03.3F	QZDM00000000	4,852,595	89,731	114	143	4,244	40
<i>Dickeya dianthicola</i> S4.16.03.P2.4	QZDN00000000	4,865,147	92,028	101	415	4,238	37
<i>Dickeya dianthicola</i> LAR.16.03.LID	QZDO00000000	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
<b>1-Pp NIBIO 1006<sup>T</sup></b>		<b>96.8</b>	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
<b>2-Pp S4.16.03.2B</b>	<b>73.30</b>		<b>93</b>	<b>92.9</b>	<b>93.5</b>	<b>93.7</b>	<b>93.5</b>	<b>93.7</b>	<b>93.4</b>	<b>93.5</b>	<b>93.5</b>	<b>93.4</b>	<b>92.2</b>	<b>78.9</b>	<b>79</b>	<b>79</b>
<b>3-Pc ICMP5702<sup>T</sup></b>	52.3	52.10		<b>97.2</b>	92.6	92.9	92.8	92.8	95.1	95.2	95.2	95.2	94.8	78.9	78.9	78.9
<b>4-Pc S1-A16</b>	<b>52.2</b>	<b>51.90</b>	<b>76.30</b>		<b>92.6</b>	<b>92.9</b>	<b>92.7</b>	<b>92.9</b>	<b>95.3</b>	<b>95.3</b>	<b>95.3</b>	<b>95.3</b>	<b>94.8</b>	<b>78.8</b>	<b>79.6</b>	<b>79.6</b>
<b>5-Pb LMG 21371<sup>T</sup></b>	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
<b>6-Pb S4.16.03.1C</b>	<b>56</b>	<b>55.7</b>	<b>52</b>	<b>51.7</b>	<b>68.5</b>		<b>96.3</b>	<b>100</b>	<b>92.3</b>	<b>91.2</b>	<b>91.1</b>	<b>91.2</b>	<b>91.9</b>	<b>78.7</b>	<b>79.6</b>	<b>79.6</b>
<b>7-Pb S1.15.11.2D</b>	<b>54.7</b>	<b>54.2</b>	<b>51.3</b>	<b>51</b>	<b>67.1</b>	<b>69.4</b>		<b>96.3</b>	<b>92.3</b>	<b>92.2</b>	<b>92.3</b>	<b>92.3</b>	<b>91.8</b>	<b>79.1</b>	<b>79.1</b>	<b>79.1</b>
<b>8-Pb S1.16.01.3K</b>	<b>56</b>	<b>55.7</b>	<b>76</b>	<b>51.7</b>	<b>68.5</b>	<b>100</b>	<b>71.4</b>		<b>92.4</b>	<b>92.4</b>	<b>92.4</b>	<b>92.3</b>	<b>91.9</b>	<b>79.6</b>	<b>79.6</b>	<b>79.7</b>
<b>9-Pv SCC1</b>	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
<b>10-Pv S4.16.03.3F</b>	<b>54.4</b>	<b>54.3</b>	<b>63.9</b>	<b>64.3</b>	<b>49.1</b>	<b>49.9</b>	<b>49.5</b>	<b>49.9</b>	<b>96.6</b>		<b>100</b>	<b>100</b>	<b>94.7</b>	<b>79.2</b>	<b>79.2</b>	<b>79.2</b>
<b>11-Pv S4.16.03.3k</b>	<b>54.4</b>	<b>54.3</b>	<b>63.8</b>	<b>64.2</b>	<b>49.2</b>	<b>49.9</b>	<b>49.5</b>	<b>49.9</b>	<b>96.6</b>	<b>99.3</b>		<b>100</b>	<b>94.7</b>	<b>79.3</b>	<b>79.3</b>	<b>79.4</b>
<b>12-Pv S4.16.03.3I</b>	<b>54.4</b>	<b>54.3</b>	<b>63.8</b>	<b>64.2</b>	<b>49.1</b>	<b>49.9</b>	<b>49.5</b>	<b>49.8</b>	<b>96.5</b>	<b>100</b>	<b>99.3</b>		<b>94.7</b>	<b>79.3</b>	<b>79.3</b>	<b>79.3</b>
<b>13-Po BCS7</b>	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
<b>14-Ddi NCPPB453<sup>T</sup></b>	21.1	20.7	20.5	20.7	20.9	21	20.6	21	21.2	21	21	21	21.1		<b>99.5</b>	<b>99.5</b>
<b>15-Ddi S4.16.03.P2.4</b>	<b>20.8</b>	<b>20.6</b>	<b>20.4</b>	<b>20.9</b>	<b>21.1</b>	<b>20.9</b>	<b>20.7</b>	<b>20.9</b>	<b>21</b>	<b>21</b>	<b>21</b>	<b>21</b>	<b>20.8</b>	<b>95.6</b>		<b>100</b>
<b>16-Ddi LAR.16.03.LID</b>	<b>20.8</b>	<b>20.6</b>	<b>20.4</b>	<b>21</b>	<b>21.1</b>	<b>20.9</b>	<b>20.6</b>	<b>20.9</b>	<b>21</b>	<b>21</b>	<b>21</b>	<b>21</b>	<b>20.8</b>	<b>95.6</b>	<b>100</b>	
	is-DDH															

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730 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.*  
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.