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The phytopathogenic nature of *Dickeya aquatica* 174/2 and the dynamic early evolution of *Dickeya* pathogenicity

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► **To cite this version:**

Alexandre Duprey, Najwa Taïb, Simon Leonard, Tiffany Garin, Jean-Pierre Flandrois, et al.. The phytopathogenic nature of *Dickeya aquatica* 174/2 and the dynamic early evolution of *Dickeya* pathogenicity. *Environmental Microbiology*, 2019, 21 (8), pp.2809-2835. 10.1111/1462-2920.14627 . hal-02099020

HAL Id: hal-02099020

<https://hal.science/hal-02099020>

Submitted on 10 Nov 2021

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25 **Originality-Significance statement**

26 Although the reach of large-scale comparative studies has spread exponentially over the years, the
27 phytopathogenic *Dickeya* group remains overlooked. In this work, we sequence the complete
28 genome of *Dickeya aquatica* type strain, a species isolated from water that was first assumed to be
29 non-phytopathogenic. We show that the proteome of *D. aquatica* contains a wide number of
30 proteins involved in *Dickeya* virulence, including plant cell wall degrading enzymes, suggesting that
31 this species could be in fact pathogenic. Using experimental approaches, we confirm this
32 prediction and uncover the particular affinity of *D. aquatica* for acidic fruits. In-depth phylogenomic
33 analyses reveal that *Dickeya* species display a great degree of genetic plasticity in the
34 pathogenicity determinants, explaining how this bacterial group was able to colonize a wide variety
35 of plants growing in different climates. These observations greatly advance our understanding of
36 how bacteria adapt to new ecological niches.

37

38 **Summary**

39 *Dickeya* is a genus of phytopathogenic enterobacterales causing soft rot in a variety of plants (e.g.
40 potato, chicory, maize). Among the species affiliated to this genus, *Dickeya aquatica*, described in
41 2014, remained particularly mysterious because it had no known host. Furthermore, while *D.*
42 *aquatica* was proposed to represent a deep-branching species among *Dickeya* genus, its precise
43 phylogenetic position remained elusive.

44 Here, we report the complete genome sequence of the *D. aquatica* type strain 174/2. We
45 demonstrate the affinity of *D. aquatica* strain 174/2 for acidic fruits such as tomato and cucumber,
46 and show that exposure of this bacterium to acidic pH induces twitching motility. An in-depth
47 phylogenomic analysis of all available *Dickeya* proteomes pinpoints *D. aquatica* as the second
48 deepest branching lineage within this genus and reclassifies two lineages that likely correspond to
49 new genomospecies (gs.): *Dickeya* gs. poaceaephila (*Dickeya* sp NCPPB 569) and *Dickeya* gs.
50 undicola (*Dickeya* sp 2B12), together with a new putative genus, tentatively named *Prodigosinella*.
51 Finally, from comparative analyses of *Dickeya* proteomes we infer the complex evolutionary history
52 of this genus, paving the way to study the adaptive patterns and processes of *Dickeya* to different

53 environmental niches and hosts. In particular, we hypothesize that the lack of xylanases and xylose
54 degradation pathways in *D. aquatica* could reflect adaptation to aquatic charophyte hosts which,
55 in contrast to land plants, do not contain xyloglucans.

56

57 **Introduction**

58 *Enterobacterales* represent one of the most studied orders of *Gammaproteobacteria*. According to
59 current systematics, *Enterobacterales* are divided into eight families: *Enterobacteriaceae*,
60 *Yersiniaceae*, *Thorselliaceae*, *Hafniaceae*, *Morganellaceae*, *Budviciaceae*, *Erwiniaceae*, and
61 *Pectobacteriaceae*. *Enterobacterales* are widespread, being found in very different environments
62 such as soils, fresh water, ocean, sediments, and many of them are associated with plants and
63 animals, including insects and humans (Brenner and Farmer III, 2005). *Enterobacterales* include
64 also important model organisms such as *Escherichia coli*, human pathogens such as *Salmonella*,
65 *Shigella*, and *Yersinia* (Dekker and Frank, 2015), and plant pathogens such as *Erwiniaceae* (e.g.
66 *Erwinia*, *Pantoea*, *Phaseolibacter*) and *Pectobacteriaceae* (e.g. *Pectobacterium*, *Dickeya*,
67 *Brenneria*, *Lonsdalea*) (Hauben et al., 1998; Samson et al., 2005). These phytopathogens share
68 virulence genes with zoopathogens such as type III secretion systems (T3SS) that inject effector
69 proteins in eukaryotic cells to suppress the host innate immune defence (Buttner, 2016). They also
70 produce specialized plant virulence factors such as pectinases found in pectinolytic bacteria
71 (Hugouvieux-Cotte-Pattat et al., 2014).

72 Among pectinolytic enterobacterales, *Dickeya* is the causative agent of soft rot in a wide variety of
73 plants including economically important crops (e.g. potato, chicory, maize, rice, tomato, sugar beet,
74 pineapple, banana) and many ornamental plants (Ma et al., 2007). This causes substantial
75 production losses amounting, for instance for potato, to tens of millions of Euros/year in Europe
76 (Toth et al., 2011). The *Dickeya* genus was first described by Samson et al. (2005), who initially
77 distinguished six species: *Dickeya dadantii*, *Dickeya dieffenbachiae*, *Dickeya chrysanthemi*,
78 *Dickeya paradisiaca*, *Dickeya zaeae*, and *Dickeya dianthicola*. Subsequently, *Dickeya*
79 *dieffenbachiae* has been reclassified as *D. dadantii* subsp. *dieffenbachiae* (Brady et al., 2012).
80 More recently, three additional *Dickeya* species have been described: *D. solani*, a species isolated

81 from potatoes and hyacinth (Van der Wolf et al., 2014; Slawiak et al., 2009), *Dickeya aquatica* from
82 freshwater rivers (Parkinson et al., 2014), and *Dickeya fangzhongdai* from pear trees displaying
83 symptoms of bleeding canker in China (Tian et al., 2016). Thus, the *Dickeya* genus now comprises
84 eight species with distinctive phenotypic features (Supplementary Table S1). Virulence
85 mechanisms have been extensively studied in *Dickeya dadantii*. During infection, the bacterium
86 enters the host using natural openings and multiplies in intercellular spaces without causing major
87 damage. Then, it suddenly induces the production of aggression factors, such as pectinases, that
88 break down the plant cell wall pectin, causing the macroscopic symptom called soft rot (Reverchon
89 and Nasser, 2013). *Dickeya* populations were initially considered to be restricted to tropical and
90 subtropical plant hosts and areas (Perombelon, 1990). This assumption was called into question
91 following the identification of *D. dianthicola* strains from potato plants in Western Europe (Janse
92 and Ruissen, 1988) and the first isolation of *D. solani* strains from potato plants in France, Finland,
93 Poland, the Netherlands, and Israel (Czajkowski et al., 2011; van der Wolf et al., 2014). Strains of
94 *D. solani* have also been isolated from hyacinth in Netherlands and it was proposed a possible
95 transfer from hyacinth to potato, via contaminated irrigation waters (Sławiak et al., 2009). Finally, it
96 was postulated that the dissemination of the pathogen in Europe has occurred via the international
97 trade of potato seeds (van der Wolf et al., 2014). This highlights the high capacity of adaptation
98 and dissemination of *Dickeya* to new geographic areas and to new hosts.

99 According to a large phylogenomic analysis of 895 single copy gene families, *D. paradisiaca* was
100 pinpointed as the first diverging species within *Dickeya*, while other species formed two groups,
101 referred hereafter as to clusters I and II (Zhang et al., 2016). Cluster I encompassed *D. zea* and
102 *D. chrysanthemi*, while in cluster II, *D. solani* and *D. dadantii* formed the sister-lineage of *D.*
103 *dianthicola* (Zhang et al., 2016). Species corresponding to these two clusters display different
104 behaviours. For example, in temperate climate, *D. chrysanthemi* and *D. zea* were frequently
105 isolated from water and were rarely associated to potato infections, contrarily to *D. dianthicola* and
106 *D. solani* (Potrykus et al. 2016). It is noteworthy, that in this study, strains M074 and M005,
107 annotated as *D. chrysanthemi* and *D. solani*, respectively, branch in-between *D. dianthicola* and
108 the *D. solani* / *D. dadantii* group within Cluster II, thus far away from the type strains of their

109 species (Zhang et al., 2016). *D. fangzhongdai* branched at the base of the Cluster II in a tree
110 based on seven housekeeping genes (Tian et al., 2016). Finally, according to a tree based on six
111 housekeeping genes, *D. aquatica* emerge just after the divergence of *D. paradisiaca* but before the
112 divergence of Cluster I and Cluster II (Parkinson et al., 2014). Yet, the associated bootstrap values
113 were weak (Parkinson et al., 2014), meaning that the relative order of emergence of *D. paradisiaca*
114 and *D. aquatica* remained to determine. Among *Dickeya*, *D. aquatica* is remarkable because all
115 the three known strains 174/2^T, 181/2, and Dw0440 have been isolated from waterways and, in
116 contrast to other species, have no known vegetal host (Supplementary Table S1) (Parkinson et al.,
117 2014; Ma et al., 2007). These features and its early-branching position within *Dickeya* make *D.*
118 *aquatica* very interesting to study the origin and the early steps of the diversification of *Dickeya*,
119 and in particular, the emergence of their virulence factors and capacity to infect plants.

120 These important questions were the focus of this study. For this purpose, we sequence the
121 complete genome of the *D. aquatica* 174/2 type strain. Based on the phylogenetic analysis of
122 1,341 single copy core protein families, we show that *D. aquatica* represents the second deepest
123 branching lineage within *Dickeya*, reclassify two lineages that likely correspond to new
124 genomospecies (gs.): *Dickeya* gs. *poaceaephila* and *Dickeya* gs. *undicola*, and identify a new
125 lineage, tentatively called *Prodigiosinella*, that likely represents the closest relative of *Dickeya*. We
126 also highlight the presence of different virulence factors, suggesting that *D. aquatica* 174/2 could
127 be pathogenic. Stimulated by these findings, we explore the pathogenic potential of *D. aquatica*
128 174/2. Surprisingly, we identify the acidic fruits tomato (pH 4.8) and cucumber (pH 5.1) as potential
129 hosts for *D. aquatica*, reflecting the potential of this aquatic species to flexibly adapt to a new
130 ecological niche provided by acidic fruits with a high water content. Accordingly, we show that this
131 strain displays a specific induction of twitching motility under acidic conditions. Finally, using
132 phylogenomic approaches, we trace back the origin and evolution of key factors associated with
133 virulence and host specificity in *Dickeya*, including *D. aquatica*. From this study we infer the
134 evolution of gene repertoires along the diversification of *Dickeya* and highlight a remarkable
135 general tendency toward proteome reduction in all *Dickeya* species.

136

137 **Results and discussion**

138 *General genomic features of D. aquatica 174/2 type strain*

139 The *D. aquatica* 174/2^T genome consists in a single circular chromosome of 4,501,560 base pairs
140 in size, with a GC content of 54.6%. The sequence was submitted to European Nucleotide Archive
141 (accession GCA_900095885). This genome contains a total of 4,202 genes including 4,080
142 protein-coding DNA sequences (CDS), 22 ribosomal RNA-coding genes organized into seven
143 operons, 76 tRNA-coding genes, and 23 non-coding RNA genes identified by sequence similarity
144 with known functional RNAs entries in the RFAM database (Daub et al., 2015) (Figure 1). These
145 features are typical of *Dickeya* species (Supplementary Table S2). The origin of chromosome
146 replication (*oriC* position 1-8 bp) was predicted between *mnmG/gidA* and *mioC* as observed in
147 *Escherichia coli* (Wolanski et al., 2014) and other enterobacterales, including *Dickeya* (Glasner et
148 al., 2011; Zhou et al., 2015; Khayi et al., 2016). The terminus of replication is predicted between
149 positions 2,220,802 and 2,220,829 bp with the *D. aquatica* 174/2^T Dif site
150 (GGTTCGCATAATGTATATTATGTTAAAT) differing from the *E. coli* K-12 Dif site by only one
151 nucleotide substitution (GGTGCGCATAATGTATATTATGTTAAAT). The distance from *oriC* to the
152 predicted terminus was nearly equal for the two halves of the chromosome. It corresponds to a
153 region near an inflection point in a slight strand-specific nucleotide compositional bias (Figure 1).
154 The protein coding density is 85% of the genome with a slight preference for the leading strand
155 utilization (57%). This density of predicted ORFs (slightly less than one per kilobase) is typical for
156 *Enterobacterales*, including *Dickeya* species (Supplementary Table S2) (Glasner et al., 2011; Zhou
157 et al., 2015; Khayi et al., 2016). Thus, despite its different ecological niche, *D. aquatica* shows
158 genomic organisation very similar to other species of the *Dickeya* genus.

159

160 ***Deciphering the early evolution of Dickeya***

161 The massive release of *Dickeya* genomic sequences (7 complete genomes and 41 draft genomes)
162 in public databases (Supplementary Table S3), in addition to the complete genome of *D. aquatica*
163 174/2^T reported in this study, provided an interesting resource to explore the evolutionary history of
164 this genus. We investigated the phylogeny of *Dickeya* using 51 ribosomal proteins (rprots), as

165 these were shown to be well suited to study the systematics of prokaryotes, especially
166 *Proteobacteria* (Yutin et al., 2012; Ramulu et al., 2014), on the one hand, and 1,341 core protein
167 families (core-pf), on the other hand. The maximum likelihood rprots tree, rooted with
168 *Pectobacterium* (*Pectobacteriaceae*) and *Serratia* (*Yersiniaceae*), recovered the monophyly of
169 *Dickeya* (Bootstrap Value (BV) = 100%, Supplementary Figure S1). Surprisingly, *Serratia* strains,
170 used as outgroup to root the phylogeny of *Dickeya* together with *Pectobacterium*, did not form a
171 monophyletic group, due to the robust clustering of *Serratia* sp. ATCC 39006 with *Dickeya* (BV =
172 99%). A similar grouping was also observed in trees based on the RNA components of the small
173 and large subunits of the ribosome (SSU and LSU rRNA, respectively) (Supplementary Figure S2),
174 suggesting that strain ATCC 39006 represents the sister-lineage of *Dickeya*, and was wrongly
175 affiliated to the *Serratia*, based on its capacity to synthesize prodigiosin, a red pigment secondary
176 metabolite with antimicrobial, anticancer, and immunosuppressant properties, characteristic of
177 *Serratia* species (Thomson et al., 2000). The sister-relationship of ATCC 39006 and *Dickeya* is
178 consistent with the close evolutionary link between the pectinolytic gene clusters (*pel*, *paeY*, *pemA*)
179 shared by these taxa (Duprey et al., 2016b), and with the presence, in *Serratia* sp. ATCC 39006, of
180 an homologue of the Vfm quorum sensing system previously reported as specific to *Dickeya*
181 species (Supplementary Table S4 and Figure S3) (Nasser et al., 2013). The phylogenetic analysis
182 of the genes involved in the biosynthesis of the prodigiosin showed that strain ATCC 39006 genes
183 emerge in a cluster gathering sequences from unrelated taxa: *Serratia* (*Yersiniaceae*), *Hahella*
184 (*Hahellaceae*), and *Streptomyces* (*Actinobacteria*), suggesting that the prodigiosin gene cluster
185 spread among these lineages (including ATCC 39006 strain) through horizontal gene transfer
186 (HGT) (Supplementary Figure S3). Interestingly, strain ATCC 39006, like the early-diverging *D.*
187 *paradisiaca* species, was deprived of the *indABC* gene cluster responsible for the characteristic
188 production of blue-pigmented indigoidine in *Dickeya* spp. (Supplementary Table S4) (Reverchon et
189 al., 2002; Lee and Yu, 2006). Phylogenetic analyses showed that *Dickeya* IndA, IndB, and IndC
190 proteins are closed to sequences from unrelated bacteria (Supplementary Figure S3), suggesting
191 that indigoidine genes underwent HGT and have been secondarily acquired in *Dickeya* after the
192 divergence with *D. paradisiaca*. The evolutionary link between strain ATCC 39006 and *Dickeya* is

193 also consistent with comparison of their proteomes. In fact, strain ATCC 39006 share in average
194 more proteins families with *Dickeya* (2,554/4,221 – 60.5%), compared to *Pectobacterium*
195 (2,387/4,221 - 56.5%) or other *Serratia* species (2,162/4,221 - 51.2%) (Supplementary Table S5).
196 Yet, the large evolutionary distance between ATCC 39006 strain and *Dickeya* (Supplementary
197 Figure S1), its lower GC content (49.2% vs 52.6 - 56.9% in *Dickeya*) (Supplementary Table S3),
198 and the lower number of protein families shared between ATCC 39006 and *Dickeya* (60.5%)
199 compared to between *Dickeya* species (64.7% - 89.6%, Supplementary Table S5), suggested that
200 strain ATCC 39006 does not belong to *Dickeya* and represents rather a close but distinct lineage,
201 possibly a new genus, that we propose to call *Prodigiosinella*, with strain ATCC 39006 being
202 reclassified as *Prodigiosinella confusarubida*. By using leBIBI^{QBPP} phylogenetic SSU rRNA-based
203 positioning tool (Flandrois et al., 2015), we detected two *Erwinia* sp. Strains: MK01 (SSU
204 accession number at the NCBI: AY690711.1) and MK09 (SSU accession number at the NCBI:
205 AY690717.1), isolated from the rhizosphere of *Phragmites communis*, that were very closely
206 related to ATCC 39006 and could belong to *P. confusarubida*.

207 According to these results and to decrease computation time, the core-pf phylogeny was inferred
208 using *P. confusarubida* ATCC 39006 as outgroup. The maximum likelihood core-pf and rprots trees
209 displayed similar topologies (Figure 2 and Supplementary Figure S1), excepted regarding *D.*
210 *chrysanthemii*, *D. sp.* NCPPB 569, and *D. solani* (see below). Worth to note, the rprots tree was
211 overall less resolved than the core-pf tree (i.e. the former displayed lower BV than the latter). Both
212 trees supported the monophyly of *D. paradisiaca*, *D. aquatica*, *D. zea*, *D. dianthicola*, *D. dadantii*,
213 and *D. solani* (BV \geq 84%). The monophyly of *D. chrysanthemii* was recovered with core-pf (BV =
214 100%) but not with rprots. In fact, while the relationships among *D. chrysanthemii* strains were
215 strongly supported in the core-pf tree, they were mostly unresolved (i.e. associated to weak BV)
216 with rprots, meaning that these markers did not contain enough phylogenetic signal to resolved the
217 *D. chrysanthemii* phylogeny. A composite group composed of strains annotated as *D. sp.*, *D.*
218 *solani*, and *D. chrysanthemii*: M074, M005, B16, MK7, S1, NCPPB 3274, and ND14b (formerly
219 misidentified as *Cedecea neteri* and transferred recently to *D. solani*) was present in both trees (BV
220 \geq 95%). By using leBIBI^{QBPP} tool (Flandrois et al., 2015), we identified strains B16, MK7, and S1 as

221 *D. fangzhongdai*, suggesting that the whole clade could correspond to *D. fangzhongdai* (Alič et al.,
222 2018).

223 Cluster I (*D. zea* and *D. chrysanthemi*) and cluster II (*D. dianthicola*, *D. solani*, *D. dadantii*, *D.*
224 *fangzhongdai* and *D. undicola*) were monophyletic (core-pf: both BV \geq 100% and rprots: both BV \geq
225 80%, respectively). Within cluster II, *D. fangzhongdai* diverged first (BV = 100%), while *D. solani*
226 represented either the sister-group of *D. dadantii* (core-pf: BV = 100%) or *D. dianthicola* (rprots: BV
227 = 98%). The conflicting position of *D. solani* was supported by high BV in both trees, indicating a
228 real inconsistency between the two trees. To go further, approximately unbiased tests (Shimodaira
229 2002) performed on individual core-pf and rprots by using the core-pf and the rprots maximum
230 likelihood topologies (Supplementary Table S6). These tests showed that 1,063 out of 1,341
231 (79.3%) core-pf protein families reject the rprots topology, while 853 out of 1,341 (63.6%) core-pf
232 protein families do not reject the core-pf topology. In contrast, 31.4% of the rprots do not reject the
233 core-pf topology and 29.4% do not reject the rprots topology. Thus, a large majority of core-pf
234 protein families favour the core-pf topology, while an equal number of rprots supports either one or
235 the other topology. Accordingly, the rprots topology and in particular the grouping of *D. solani* with
236 *D. dianthicola* could be questioned.

237 Regarding the first divergences within *Dickeya*, both trees pinpointed *D. paradisiaca* and *D.*
238 *aquatica*, as early diverging lineages (core-pf: BV = 100%, rprots: BV = 86%), with *D. paradisiaca*
239 emerging first (core-pf: BV = 100%, rprots: BV = 65%). Finally, the core-pf placed robustly *D. sp.*
240 NCPPB 569, a strain isolated from sugarcane in Australia (Supplementary Table S3), at the base
241 of Cluster I (BV = 100%), while its position was unresolved in the rprots tree (i.e. unsupported
242 grouping with *D. aquatica*, BV = 46%). Irrespective of its position, the evolutionary distances
243 between strain NCPPB 569 and the eight *Dickeya* species were roughly similar, suggesting
244 NCPPB 569 could correspond to an independent lineage within *Dickeya*. This observation was in
245 agreement with a previous work based on the phylogenetic analysis of *recA* (Parkinson et al.
246 2009), and led us to propose that strain NCPPB 569 could represent a distinct genomospecies, we
247 proposed to name *Dickeya* gs. *poaceaephila*. This hypothesis was strengthened by the fact that

248 strain NCPPB 569 could be distinguished from other species according to shared protein families
249 (Figure 2 and Supplementary Figure S4). Among cluster II, *Dickeya* sp. 2B12 deserved attention.
250 In fact, this strain branched as a distant sister-lineage of *D. fangzhongdai* (core-pf: BV = 100% and
251 rprots: BV = 95%). This suggested that this strain could also represent a new genomospecies that
252 we have tentatively called *Dickeya* gs. *undicola*.
253 Altogether, the analysis of more than one thousand core protein families and ribosomal proteins
254 allows to resolve most of the speciation events within *Dickeya* and provides a solid framework to
255 investigate the origin and the evolution of important *Dickeya* biological features. As expected, both
256 trees were largely consistent, even if the rprots tree was globally less resolved, in particular
257 regarding *D. chrysanthemi* and *Dickeya* gs. *poaceaephila*. The main inconsistency concerned the
258 relative order of divergence of *D. dadantii*, *D. dianthicola*, and *D. solani* within cluster II.
259 Approximately unbiased tests suggested that rprots could contain a mix phylogenetic signal, and
260 thus that the rprots tree could be less reliable than the core-pf tree. Determining the origin of the
261 conflicting signal in rprots would require more investigations that are beyond the scope of the
262 study. Because *D. solani* is a relatively late diverging lineage within *Dickeya*, we anticipated that
263 this will not impact significantly inferences on the ancient *Dickeya* evolution. Nevertheless, in all
264 analyses (e.g. evolution of virulence related systems, inference of ancestral gene repertoires, see
265 below), we used the species tree based on core-pf, but by considering both possible placements
266 for *D. solani*: either as the sister-group of *D. dadantii* or *D. dianthicola*. Regarding *D. aquatica*, the
267 core-pf tree suggested that this species represent the second diverging lineage with *Dickeya*, while
268 its position was unresolved according to rprots. Our analyses also reclassified strain ATCC 39006
269 as *Prodigiosinella confusarubida*, which could represent the closest relative of *Dickeya* genus.
270 Better characterization of the biology and diversity of *Prodigiosinella* will provide important clues
271 about their evolutionary links with *Dickeya* and the emergence of *Dickeya* as a genus.

272

273 ***Virulence-associated phenotypes of D. aquatica***

274 While most *Dickeya* species have known vegetal hosts, all the identified strains of *D. aquatica*
275 (174/2^T, 181/2 and Dw0440) have been isolated from waterways (Supplementary Table S1)

276 (Parkinson et al., 2014). To our knowledge, their pathogenicity has never been demonstrated. The
277 *in silico* survey of the *D. aquatica* 174/2^T genome for transcriptional regulator binding sites revealed
278 the presence of conserved key regulators of virulence (Supplementary Table S7). More precisely,
279 all the regulators (KdgR, PecS, PecT, CRP, Fis, H-NS, GacA-GacS, RsmA-RsmB, MfbR)
280 controlling virulence gene expression in *D. dadantii* (Reverchon et al., 2016) are present in *D.*
281 *aquatica* 174/2^T. The global regulators FNR and CRP displayed the highest number (approximately
282 80) of targets, followed by Fur, CpxR, ArcA, Lrp and KdgR each with more than 30 targets. The
283 activator CRP and the repressor KdgR have a key role in *Dickeya* virulence (Duprey et al., 2016a),
284 as they tightly control the pectin degradation pathway and are involved in coupling central
285 metabolism to pectinase gene expression (Nasser et al., 1997), while Fur acts as a repressor of
286 pectinase genes (Franza et al., 2002). In addition, Fur represses genes involved in the metabolism
287 of iron, a metal that plays an important role as a virulence regulatory signal in *Dickeya* (Franza et
288 al., 2002).

289 The presence of regulators controlling the virulence in *D. aquatica* 174/2^T was puzzling and
290 prompted us to investigate its pathogenicity. For this, we compared the pathogenic potential of *D.*
291 *aquatica* 174/2^T and *D. dadantii* 3937 on various infection models such as chicory leaves, potato
292 tubers, cucumbers and tomato fruits (Figure 3). *D. aquatica* showed little efficiency in rotting potato
293 and chicory. Indeed, only a small rotten area was observed near the entry point, opposed to *D.*
294 *dadantii*, which spread inside the tuber or the leaf (Figure 3). Surprisingly, *D. aquatica* appeared
295 particularly efficient on tomatoes and cucumbers, showing a generalised infection and fruit opening
296 after 42 hours. By contrast, *D. dadantii* was much less efficient in infecting tomatoes and
297 cucumbers. Importantly, tomatoes and cucumbers are acidic fruits (pH 4.8 and pH 5.1,
298 respectively), whereas potato tubers and chicory leaves display higher pH (around 6.0 and 6.5,
299 respectively), suggesting that *D. aquatica* could be more efficient on acidic fruits. Yet, neither *D.*
300 *aquatica* nor *D. dadantii* are able to induce pathogenic symptoms on very acidic fruits such as
301 pineapple (pH 4) or kiwi (pH 3) (Figure 3B). Previous studies have shown that some unrelated
302 *Dickeya* species (i.e. *D. chrysanthemi*, *D. dianthicola*) are also capable of infecting tomatoes
303 (Supplementary Table S1), supporting the hypothesis that recurrent adaptations to similar hosts

304 occurred independently during the evolution of *Dickeya* genus.

305

306 ***Stress resistance phenotypes of D. aquatica***

307 **Osmotic stress**

308 Depending on the plant host, *Dickeya* species encounter various stresses during the infectious
309 process (Reverchon et al., 2016). We therefore assessed stress resistance of *D. aquatica* 174/2^T
310 (Figure 4). This strain proved to be very sensitive to osmotic stress, as it displayed a 50% growth
311 rate reduction on 0.3 M NaCl while *D. dadantii* was only slightly affected (20% growth rate
312 reduction). This effect was even more pronounced on 0.5 M NaCl with a growth rate reduction of
313 90 % for *D. aquatica* and 43% for *D. dadantii* (Figure 4A). This sensitivity to osmotic stress could
314 be linked to the lack of two osmoprotectant biosynthetic pathways in *D. aquatica*: glycine betaine
315 biosynthesis (*betA-betB* gene cluster) and phosphoglycosyl glycerate biosynthesis (*pggS-pggP*
316 gene cluster) (Jiang et al., 2016) (Supplementary Table S4). The *pggSP* gene cluster was present
317 in *P. confusarubida* and all *Dickeya* species except *D. aquatica*. The phylogeny of the two genes
318 was consistent with the phylogeny of species (Supplementary Figure S3), indicating that their
319 absence in *D. aquatica* results from a specific and secondary loss in this taxon. In contrast, the
320 *betABI* gene cluster was absent in *D. paradisiaca*, *D. aquatica*, *D. dianthicola* and Cluster I species
321 (Supplementary Table S4). Their phylogenies suggested that the *betABI* gene cluster could have
322 been present in the ancestor of *P. confusarubida* and *Dickeya*, and secondarily and independently
323 lost in the *Dickeya* lineages mentioned above (Supplementary Figure S3). Finally, the *ousA* gene
324 encoding the major osmoprotectant uptake system in *D. dadantii* is missing in *D. aquatica*, as well
325 as in most other *Dickeya* species, suggesting either a recent acquisition by a few species or
326 multiple losses of an ancestral system (Supplementary Table S4). Interestingly, *D. solani* and *D.*
327 *dianthicola*, both lacking the *ousA* gene, are also more sensitive to osmotic stress than *D. dadantii*
328 (Figure 4B).

329 **Oxidative stress**

330 *D. aquatica* 174/2^T was also very sensitive to oxidative stress, displaying longer lag time than *D.*
331 *dadantii* in presence of 75 μM H₂O₂ (Figure 4C). This is consistent with the lack of the periplasmic

332 superoxide dismutase SodC, whose gene was likely acquired in *Dickeya* after the divergence of *D.*
333 *paradisiaca* and *D. aquatica* (Supplementary Figure S3), and the lack of the Fe-S cluster assembly
334 SUF system (Supplementary Table S4). Iron-sulfur (Fe-S) clusters are fundamental to numerous
335 biological processes in most organisms, but these protein cofactors can be prone to damage by
336 various oxidants (e.g., O₂, reactive oxygen species, and reactive nitrogen species) (Roche et al.,
337 2013). In addition, the release of free iron in the bacterial cytoplasm amplifies the oxidative stress
338 through the Fenton reaction, producing the highly toxic and reactive hydroxyl radical OH[•]. Most
339 gammaproteobacteria have two Fe-S cluster biogenesis systems SUF and ISC (Roche et al.,
340 2013). For example, *Escherichia coli* cells switch from the ISC to the SUF system under oxidative
341 stress as OxyR, a sensor of oxidative stress, acts as an activator of *suf* operon expression (Lee et
342 al., 2004). Both the SUF and ISC systems were shown to be required for *D. dadantii* growth in the
343 plant host environment that is continually changing in terms of iron availability and redox conditions
344 (Nachin et al., 2001; Expert et al., 2008; Rincon-Enriquez et al., 2008). While both the ISC and
345 SUF systems were likely present in the ancestor of *Dickeya* and *P. confusarubida*, phylogeny and
346 taxonomic distribution suggested that SUF genes were secondarily lost in *D. paradisiaca*, *D.*
347 *aquatica*, *D. gs. poaceaephila*, and *D. zaeae* (Supplementary Figure S3). The absence of both the
348 SUF system and SodC in *D. aquatica* could be an important factor limiting its host range
349 (Supplementary Figure S3). Finally, the presence of the DNA-binding protein Dps, which has a
350 protective function against a wide range of stresses, in *P. confusarubida* and all *Dickeya* excepted
351 *D. paradisiaca* suggested a specific loss in this latter lineage (Supplementary Figure S3).

352 Regarding other oxidative stress sources, the NorWVR system responsible for nitrite oxide
353 detoxification was absent in the first diverging *Dickeya* species (Supplementary Table S4). The
354 phylogenies of the corresponding proteins suggested secondary losses in *P. confusarubida*, *D.*
355 *paradisiaca*, and *D. aquatica*, as these proteins were present in *Pectobacterium*, a closely related
356 genus of *Dickeya* (Supplementary Figure S3). Finally, the ascorbate degradation pathway encoded
357 by the *ula* genes was present in the *D. paradisiaca*, *D. aquatica*, *D. zaeae*, and *D. chrysanthemi*
358 species, suggesting that the pathway was also present in the ancestor of *Dickeya* and secondarily
359 lost in *D. gs. poaceaephila* and in the ancestor of Cluster II (Supplementary Figure S3). Ascorbic

360 acid, the major and probably the only antioxidant buffer in the plant apoplast, becomes oxidised
361 during pathogen attack (Pignocchi and Foyer, 2003). Modification of the apoplastic redox state
362 modulates receptor activity and signal transduction to regulate plant defence and growth
363 (Pignocchi and Foyer, 2003). The capacity of some *Dickeya* species to catabolize ascorbate could
364 be a strategy to weaken the plant defence.

365 **Acidic stress**

366 Regarding pH sensitivity, both *D. aquatica* 174/2^T and *D. dadantii* showed optimum growth at pH
367 7.0. *D. aquatica* 174/2^T displayed no significant growth rate reduction down to pH 5.3 while *D.*
368 *dadantii* was slightly affected (20% growth rate reduction at pH 5.3 compared to optimum pH 7.0)
369 (Figure 4D). Below pH 4.9, the growth of *D. aquatica* abruptly diminished compared to that of *D.*
370 *dadantii* indicating that *D. aquatica* growth was much more impacted by low pH (Figure 4D).
371 Bacterial response to acid stress involves evasion of cell damage and adaptation of the enzymatic
372 profile by reducing reactions producing protons and promoting those consuming protons (Bearson
373 et al., 1997). Concerning the protection of proteins against pH damage, *Dickeya* strains, including
374 *D. aquatica* strain 174/2^T, encode the lysine-rich protein Asr acid-inducible periplasmic chaperone,
375 known to protect the proteins by sequestering protons due to its high basic amino acids
376 composition (Seputiene et al., 2003). In *Dickeya*, the corresponding gene is located downstream
377 the *rstAB* gene cluster, known to control the *asr* gene expression (Ogasawara et al., 2007). Yet,
378 due to their atypical amino acid composition these proteins were often wrongly annotated as
379 histone proteins (e.g. in *D. dadantii* NCPPB 898, *D. dianthicola* NCPPB 3534, *D. dianthicola*
380 NCPPB 453, *D. dianthicola* GBBC 2039, and *D. chrysanthemi* ATCC 11663). In terms of metabolic
381 adaptation, a major strategy for bacterial pH homeostasis consists in using decarboxylases to
382 remove cytoplasmic protons (Krulwich et al., 2011). The glutamate-, arginine-, and lysine-inducible
383 decarboxylases classically found in enteric bacteria (Foster, 2004) are absent in *Dickeya* species.
384 However they contain some organic acid decarboxylases such as oxalate and malonate
385 decarboxylases (Supplementary Table S4). Some *Dickeya* species, including *D. dadantii*, have two
386 oxalate decarboxylation pathways: the *frc-oxc* pathway, being involved in the acid tolerance
387 response in *E. coli* (Fontenot et al., 2013) and the *OxdD* decarboxylase pathway. Most *Dickeya*

388 species harbour at least one of these oxalate decarboxylation pathways, except *D. aquatica*, *D. gs.*
389 *poaceaephila*, *D. chrysanthemi*, and a few *D. zea* strains, which were deprived of both pathways
390 (Supplementary Table S4 and Supplementary Figure S3). The lack of the two oxalate-
391 decarboxylation pathways in *D. aquatica* could contribute to its sensitivity to acidic pH. Finally, in
392 contrast with *P. confusarubida* and most *Dickeya* species, *D. aquatica* and a few *D. zea* strains
393 were also devoid of malonate decarboxylation pathway (mdcABCDEFGHR) (Maderbocus *et al.*,
394 2017), which consumes protons (Supplementary Table S4), suggesting secondary gene losses in
395 these strains, a hypothesis that was confirmed by phylogenetic analyses (Supplementary Figure
396 S3).

397 **Twitching motility**

398 Interestingly, colony shape changes were observed when *D. aquatica* was grown at low pH in
399 presence of malic acid (Figure 5). More precisely, colonies became wider (2-3 folds increase in
400 diameter compared to unstressed *D. aquatica*) with a morphology characteristic of twitching motility
401 (Henrichsen, 1972). Correspondingly, we detected genes encoding a complete type IV pilus
402 assembly responsible for twitching motility in *D. aquatica* genome and named them *pil* genes
403 according the *Pseudomonas aeruginosa* nomenclature (Maier and Wong, 2015). Among the
404 collection of twenty-six *Dickeya* strains representing different species, only eight showed twitching
405 at acidic pH (Figure 5). Interestingly, this phenotype appeared strain-dependent rather than
406 species-dependent, as *D. solani*, strain RNS07.7.3B showed twitching motility at acidic pH, while
407 *D. solani* strain PP09019 did not (Figure 5). As the *pil* genes, inherited by *Dickeya* from the
408 common ancestor shared with *P. confusarubida*, were conserved in all species (Supplementary
409 Table S4 and Figure S3), twitching was probably linked to strain-specific induction of these genes
410 under acidic pH. It is noteworthy that twitching motility is strongly influenced by changes in the
411 environment (Henrichsen, 1975). In plant pathogens, the contributions of type IV pilus to virulence
412 have been investigated mainly in vascular pathogens, such as *Ralstonia* and *Xylella*, where they
413 were proposed to contribute to bacterial colonization and spread in the xylem through cell
414 attachment, biofilm formation, and twitching motility (Burdman *et al.*, 2011). However, type IV
415 piliation was also shown to be important for initial adhesion and colonization of leaves in a few non-

416 vascular bacteria such as *Xanthomonas oryzae* pv. *oryzicola*, *Pseudomonas syringae* pv. *tabaci*
417 and *Pseudomonas syringae* pv. *syringae* (Burdman et al., 2011). The importance of type IV pilus in
418 *Dickeya* pathogenicity is therefore an interesting question for the future studies.

419 **Antimicrobial peptides**

420 In response to infection, plants produce antimicrobial peptides (AMPs) to limit pathogen
421 propagation. To overcome AMPs, bacteria remodel their envelope, and more precisely, they
422 modify their LPS to decrease interaction with positively charged AMPs. Among the genes involved
423 in LPS modification, the operons *arnABCDEFT* and *dltXABCD* turned out to be ancestral and
424 conserved in all *Dickeya* species (Supplementary Figure S3), while other genes (i.e. *eptAB*, *pagP*,
425 *lpxO* and *lpxT*) displayed different taxonomic distributions and evolutionary histories
426 (Supplementary Table S4 and Figure S3). For example, *D. aquatica* 174/2^T is lacking *pagP*, *lpxO*
427 and *lpxT* genes possibly contributing to a greater sensitivity to AMPs.

428 To conclude, our data indicate that, *D. aquatica* 174/2^T has an unanticipated phytopathogenic
429 capacity similar to that of other *Dickeya* species. Particular stress resistance profiles and induction
430 of twitching at acidic pH may contribute to its restricted host range. Based on this observation, we
431 decided to compare the *D. aquatica* 174/2^T and other *Dickeya* species proteomes with a special
432 focus on the virulence determinants including plant cell wall degrading enzymes, secretion
433 systems, iron metabolism, plant adhesion elements and secondary metabolism.

434

435 ***Distribution of plant cell wall degrading enzymes in Dickeya***

436 The plant cell wall is a complex and dynamic meshwork of polymers (cellulose, hemicellulose,
437 pectin, structural glycoproteins) (Pauly and Keegstra, 2016). Among these polymers, pectin is the
438 most complex and includes both linear regions composed of polygalacturonan and ramified regions
439 (RGI and RGII, respectively). RGI contains a rhamnogalacturonan backbone and various lateral
440 chains such as galactan, arabinan and galacturonan (Caffall and Mohnen, 2009). RGII contains a
441 short galacturonan backbone, carrying four side chains, with a diversity of rare monosaccharides
442 (O'Neill et al., 2004). The carboxylic groups of D-galacturonate residues are methyl-esterified to
443 various degrees (up to 80%) and these residues are, to a lesser extent, acetylated at the C2 and/or

444 C3 positions. Feruloyl esters are a type of modification commonly found in arabinan and galactan
445 chains of ramified regions (Ishii, 1997). The virulence of *Dickeya* is correlated with their ability to
446 synthesize and secrete plant cell wall degrading enzymes, including a full set of pectinases
447 (Hugouvieux-Cotte-Pattat et al., 2014), xylanases and xylosidases (Keen et al., 1996) proteases
448 PrtA, PrtB, PrtC, PrtG (Wandersman et al., 1987), and the cellulase Cel5Z (Py et al., 1991). The
449 presence of these virulence factors varies depending on the species (Matsumoto et al., 2003;
450 Duprey et al., 2016b). To obtain a comprehensive view of this phenomenon, we explored the “Plant
451 cell wall degradosome” of *Dickeya* species including *D. aquatica* (**Figure 6**).

452

453 **The pectinasome**

454 The *Dickeya* pectinasome includes multiple pectate lyases (PelA, PelB, PelC, PelD, PelE, Pell,
455 PelL, PelN, PelW, PelX, PelZ, Pel10), pectin lyases (PnlG, PnlH), polygalacturonases (PehK,
456 PehN, PehV, PehW, PehX), pectin methyl esterases (PemA, PemB), pectin acetyl esterases
457 (PaeX, PaeY), feruloyl esterases (FaeD, FaeT), rhamnogalacturonate lyases (RhiE, RhiF), and
458 one periplasmic endogalactanase (GanA) (Figure 6) (Hugouvieux-Cotte-Pattat et al., 2014). *D. gs.*
459 *poaceaephila* NCPPB 569 was the genospecies with the poorest pectinase content. According to
460 their taxonomic distribution and phylogeny, most of these proteins could be inferred in the ancestor
461 of *Dickeya* and in its sister lineage *P. confusarubida*. Regarding the *pelAED* cluster, while *P.*
462 *confusarubida* has a single gene, the ancestor of *Dickeya* had two copies, *pelA* and an
463 undifferentiated *pelDE* pectate lyase-coding gene (Duprey et al., 2016b), suggesting that a
464 duplication event occurred in the stem of *Dickeya*. This undifferentiated *pelDE* has undergone a
465 duplication event after the emergence of *D. paradisiaca*, giving rise to *pelD* and *pelE*
466 (Supplementary Figure S5) (Duprey et al., 2016b). *D. aquatica* has then lost *pelE*, while *D.*
467 *poaceaephila* has lost both *pelA* and *pelE*. The *pelA* gene was also lost in *D. dianthicola*, while
468 *pelE* was lost in some *D. chrysanthemi* strains (Figure 6, Supplementary Figure S5). The *pelBC*
469 cluster was also likely present in the *Dickeya* ancestor, and conserved in most *Dickeya* species.
470 However, *pelB* was lost in *D. gs. poaceaephila*, and *D. dadantii subsp. dieffenbachiae* NCPPB
471 2976 (Supplementary Figure S3). The pectate lyase Pell was present in *Pectobacterium* and in all

472 *Dickeya*, except *D. paradisiaca*, and in *P. confusarubida*, suggesting that it could be ancestral in
473 *Dickeya*. Similarly, the phylogeny of pectin methyl esterase PemB indicated that the protein was
474 present in *P. confusarubida* and *Pectobacterium*, suggesting an ancestral presence in *Dickeya*.
475 Accordingly, its absence in *D. paradisiaca*, *D. aquatica*, *D. gs. poaceaephila*, and *D. zea* strains
476 likely reflected secondary losses. The phylogeny of this protein also suggested that the *pemB* gene
477 found in *D. chrysanthemi* was acquired by HGT from Cluster II species (Supplementary Figure S3).
478 Regarding the polygalacturonase PehN, a similar scenario could be inferred except that the
479 corresponding gene was also lost in *P. confusarubida*, and that a few *D. zea* strains seemed to
480 have reacquired a PehN from Cluster II species by HGT (Supplementary Figure S3). Regarding
481 the *pehVWX* cluster, the three genes were derived from a single *pehX* gene that was present in the
482 ancestor of *P. confusarubida* and *Dickeya*. A gene duplication event occurred in the ancestor of *D.*
483 *dianthicola*, *D. solani* and *D. dadantii* leading to PehV. A second event led to the divergence of
484 PehW in the ancestor of *D. solani* and *D. dadantii* (Supplementary Figure S5). The
485 polygalacturonase PehK was likely present in the ancestor of all *Dickeya* and secondarily lost in *D.*
486 *gs. poaceaephila*, *D. solani*, and *D. dianthicola* (Supplementary Figure S3). The pectin lyase PnlG
487 was likely present in the ancestor shared by *Dickeya* and *P. confusarubida*, and secondarily lost in
488 *D. aquatica*, *D. gs. poaceaephila*, *D. chrysanthemi*, *D. dianthicola* and in some *D. zea* strains
489 (Supplementary Figure S3). Finally, the rare pectate lyase Pel10 and pectin lyase PnlH displayed
490 patchy taxonomic distributions (Figure 6). Their phylogenies suggested that they spread through
491 HGT in *Dickeya* (Supplementary Figure S3).

492 The saturated and unsaturated digalacturonates resulting from pectin degradation by pectinases
493 are converted into monogalacturonate and 5-keto-4-deoxyuronate by the oligogalacturonate lyase
494 Ogl, which is present in all *Dickeya* species (Figure 6). The phylogenies of the *gan* gene cluster
495 responsible for degradation of galactan chains in pectin-ramified regions and the
496 rhamnogalacturonate lyase RhiE involved in degradation of RGI pectin-ramified regions indicated
497 they were likely present in the ancestor of *P. confusarubida* and *Dickeya*, and secondarily lost in
498 the basal *D. paradisiaca* species, in *D. gs. poaceaephila*, and in a few other strains (Figure 6). The
499 ferulate esterases FaeT and FaeD were absent in *P. confusarubida* and were acquired in the basal

500 *D. paradisiaca* and *D. aquatica* species. The FaeT enzyme was then secondarily lost in *D. gs.*
501 *poaceaephila*, and *D. dadantii* subsp *dieffenbachiae* (Supplementary Figure S3). Altogether, the
502 variability observed in the pectinasome among *Dickeya* species and even among different strains
503 likely reflects the dynamic evolution of the corresponding gene families involving gene acquisitions
504 and losses. More compellingly, this variability indicates that the pectinasome cannot be used to
505 distinguish among various species.

506

507 **Cellulases and Xylanases**

508 Two cellulases Cel5Z and CelY were likely ancestral in *Dickeya* species except in *D. gs.*
509 *poaceaephila*, which has lost Cel5Z. While Cel5Z was involved in cellulose degradation, CelY
510 belonged to the *bcs* gene cluster responsible for cellulose fiber formation (Prigent-Combaret et al.,
511 2012). Xylanases (XynA, XynB) and xylosidases (XynC, XynD) cleave xylan and xyloglucan, which
512 belong to the hemicelluloses. The β 1,4 xylan is mainly present in plant cell wall of monocots (Pena
513 et al., 2016) and is further decorated, often by acetyl, arabinosyl, and glucuronosyl side-chain
514 substitutions. Notably, the xylan substitution patterns depend on the plant species and are distinct
515 in gymnosperms and angiosperms (Busse-Wicher et al., 2016). Xyloglucan is a β -1,4 glucan that
516 can be substituted with a diverse array of glycosyl and nonglycosyl residues. The type and order of
517 xyloglycan substituents depend on the plant species (Pauly and Keegstra, 2016). Xyloglucan
518 polymers fall into one of two general types. In one type, three out of four backbone glucosyl
519 residues are xylosylated, leading to an XXXG-type xyloglycan, which is predominant in most
520 dicots. Another type of xyloglycan exhibits reduced xylosylation in that only two out of the four or
521 more backbone glucosyl residues are xylosylated, resulting in the XXGGn-type xyloglucan, which
522 is present in early land plants such as liverworts, mosses, lycophytes, and ferns of the order
523 Polypodiales. This type of xyloglucan seems to be absent from the gymnosperms and
524 angiosperms, with the exception of the grasses (Poales) and plants from the order Solanales, such
525 as potato, tobacco and tomato (Pauly and Keegstra, 2016). Thus, it is tempting to hypothesize that
526 the xylanase and xylosidase content of *Dickeya* species could be correlated with their plant host.
527 Interestingly, all *Dickeya* species as well as *P. confusarubida* contained at least one XynA, XynB,

528 XynC, or XynD coding gene (Figure 6). The phylogeny of XynA indicated that the corresponding
529 gene was likely present in the ancestor of *P. confusarubida* and *Dickeya*, and secondarily lost in *D.*
530 *dianthicola*, *D. gs. undicola*, *D. chrysanthemi*, *D. gs. poaceaephila*, and *D. aquatica*
531 (Supplementary Figure S3). The XynB phylogeny suggested a secondary acquisition in *Dickeya*
532 after the divergence of *P. confusarubida*, *D. paradisiaca*, and *D. aquatica*, followed by losses in *D.*
533 *dianthicola*, *D. chrysanthemi*, and *D. gs. undicola* (Supplementary Figure S3). While the mode of
534 action of the xylanase XynB was not studied, XynA is a glucuronoxylanase (CAZy family GH30)
535 hydrolyzing the xylan backbone adjacent to each glucuronosyl side-chain (Urbanikova et al., 2011).
536 *D. aquatica*, *D. chrysanthemi*, *D. gs. undicola*, and *D. dianthicola* were deprived of both XynA and
537 XynB (Figure 6) suggesting that these species would preferentially infect dicots as xylan is mainly
538 present in plant cell wall of monocots. However, this is probably a tendency since *D. chrysanthemi*
539 strain Ech1591 was isolated from maize, which is a monocot. The phylogeny of Xylosidase XynD
540 suggested that the corresponding gene has been acquired by HGT in *D. aquatica* and *D.*
541 *chrysanthemi*, (Supplementary Figure S3). A secondary acquisition via HGT could also be
542 hypothesized for XynC (Supplementary Figure S3), as it is absent in *P. confusarubida* and the
543 basal *Dickeya* species (i.e. *D. paradisiaca*, and *D. aquatica*), as well as in *D. gs. poaceaephila*, *D.*
544 *zeae* and some *D. chrysanthemi* strains. Strikingly, the xylose degradation pathway (XylABFGHR),
545 present in *P. confusarubida* and in all *Dickeya*, was absent in *D. aquatica*, indicating clearly a
546 specific loss in this species (Supplementary Figure S3). The absence of the xylanases XynA and
547 XynB, and xylosidase XynC as well as the xylose degradation pathway in *D. aquatica* could
548 contribute to its restricted host range.

549

550 **Proteases**

551 Finally, the proteases PrtA, PrtB, PrtC, and PrtG, resulting from specific duplications that occurred
552 during the diversification of *Dickeya*, and the associated type I protease secretion system PrtDEF
553 were absent in *P. confusarubida* and the basal branching *D. paradisiaca*. Yet, *Pectobacterium*
554 harbour closely related homologues of PrtDEF and a single protease-coding gene closely related
555 to *Dickeya* PrtA, PrtB, PrtC and PrtG. This suggested that the whole system could have been

556 present in the common ancestor they shared with *Dickeya*, and then secondarily lost in *P.*
557 *confusarubida* and *D. paradisiaca* (Supplementary Figure S3). PrtG was specifically lost in *D.*
558 *chrysanthemi*, *D. dianthicola*, *D. gs. undicola* and *D. gs. poaceaephila*. This later genomospecies
559 conserved only one protease PrtC whereas *D. dianthicola* strains RNS04-9 and NCPPB453
560 contained a *prtA* pseudogene and retained two proteases PrtC, PrtB (Figure 6).

561

562 **Other factors**

563 In addition to plant cell wall degrading enzymes, *Dickeya* use several other factors to colonize plant
564 tissue and enhance the progression of disease. Such factors include the extracellular necrosis
565 inducing protein NipE and the two paralogous proteins AvrL and AvrM. NipE and AvrL are
566 conserved in *Pectobacterium* and in most *Dickeya* species, except in *D. paradisiaca*, *D. gs.*
567 *poaceaephila*, and *P. confusarubida*, suggesting secondary losses in these lineages
568 (Supplementary Figure S3). AvrM was also absent in *D. zea*, some *D. chrysanthemi*, *D. gs.*
569 *undicola*, and *D. dianthicola*. Interestingly *D. zea* strains isolated from rice were the only *Dickeya*
570 to be devoid of Avr proteins (Supplementary Table S4). Altogether, our data indicate that the host
571 range specificity of the various *Dickeya* species is probably linked to the particular combination of
572 plant cell wall degrading enzymes and accessory toxins they produce.

573

574 **Distribution of secretion systems in *Dickeya* species**

575 For all *Dickeya* species, possession of secretion systems allowing them to actively secrete
576 virulence factors is of crucial importance. Unsurprisingly, different protein secretion systems (T1SS
577 to T6SS) are present in *Dickeya* species (Supplementary Table S4). As previously mentioned, the
578 type I protease secretion system PrtDEF was present in the ancestor of all *Dickeya* species and
579 lost in the basal *D. paradisiaca* species and in *P. confusarubida*. All *Dickeya* species are equipped
580 with the Out specific T2SS responsible for the secretion of most pectinases and the cellulase
581 Cel5Z. The phylogeny of the components of the Out system indicated it was likely present in the
582 ancestor shared by *Pectobacterium*, *P. confusarubida*, and *Dickeya*, and was conserved during the
583 diversification of *Dickeya* (Supplementary Figure S3). A second Stt specific T2SS, allows secretion

584 of the pectin lyase PnlH (Ferrandez and Condemine, 2008). Accordingly, the taxonomic
585 distributions of the pectin lyase PnlH and the Stt T2SS components were similar, being present in
586 *D. dianthicola*, *D. chrysanthemi*, and some *D. dadantii* strains, (Supplementary Table S4 and
587 Figure 6). In *D. gs. poaceaephila*, the Stt T2SS was also present but not associated with PnlH,
588 thus its function in this strain remains to be determined (Supplementary Table S4 and Figure 6).
589 Interestingly, a T3SS is present in *Pectobacterium*, *P. confusarubida* and in all *Dickeya* species,
590 except *D. paradisiaca* and *D. gs. poaceaephila* deprived of the T3SS and the associated DspE
591 effector, and thus suggesting secondary losses (Supplementary Table S4). Therefore, these latter
592 species are probably unable to suppress the plant immune response (see below). Interestingly,
593 phylogenies of these proteins disclosed a close relationship with two bacterial phytopathogens,
594 *Erwinia* and *Pseudomonas syringae*, suggesting that HGT occurred among these lineages
595 (Supplementary Figure S3). In fact, the effector DspE belongs to the AvrE superfamily of Type III
596 effectors (T3Es) (Degrave et al., 2015). The AvrE family is the only family of T3Es present in all
597 type III-dependent, agriculturally important phytobacterial lineages that belong to the unrelated
598 *Enterobacteriales*, *Xanthomonadales*, *Pseudomonadales* and *Ralstonia* taxa. This indicates that
599 HGT of these effectors occurred in the ancestors of these important plant pathogen lineages
600 (Jacobs et al., 2013). Recent studies indicated that AvrE-type effectors alter the sphingolipid
601 pathway *in planta* by inhibiting the serine palmitoyl transferase (Siamer et al., 2014). The
602 sphingolipid biosynthetic pathway is induced during the plant hypersensitive response that blocks
603 pathogen attack at the site of infection (Berkey et al., 2012). Therefore, inhibition of this pathway
604 delays hypersensitive response-dependent cell death and allows bacterial development *in planta*
605 (Degrave et al., 2015). In *Dickeya* species, the T3SS genes are in synteny with the *plcA* gene
606 encoding a phospholipase. These genes were probably acquired during the same event since *D.*
607 *paradisiaca* and *D. gs. poaceaephila* that were deprived of the T3SS are also deprived of PlcA
608 (Figure 6).

609 All *Dickeya* species and *P. confusarubida* were found to possess a two-partner secretion system
610 (T5SS) CdiB-CdiA mediating bacterial intercellular competition. Their phylogenies clearly indicate
611 an ancestral presence in both lineages (Supplementary Figure S3). CdiB is a transport protein that

612 exports and presents CdiA proteins on the cell surface (Willett et al., 2015). The Cdi system is
613 involved in contact-dependent growth inhibition (CDI) by delivering the C-terminal toxin domain of
614 CdiA (CdiA-CT) to target bacteria (Aoki et al., 2010). Some *Dickeya* strains are equipped with two
615 CdiA proteins, for example, *D. dadantii* 3937, which produces two different CdiA-CT toxins: the first
616 one being a tRNase and the second one harbouring DNase activity (Aoki et al., 2010; Ruhe et al.,
617 2013). Each Cdi system also encodes a specific CdiI antitoxin that interacts with the cognate CdiA-
618 CT toxin and prevents auto-inhibition (Willett et al., 2015).

619 The most striking feature in *D. aquatica* was the absence of both type IV (T4SS) and type VI
620 (T6SS) secretion systems, a trait that was shared with *D. paradisiaca*, *D. gs. poaceaephila* and *P.*
621 *confusarubida*. Yet, the presence of closely related T4SS and T6SS in *Pectobacterium* and other
622 *Dickeya* species suggests that both systems were present in the ancestor of *Dickeya* and
623 *Prodigiosinella*, and secondarily lost in the three mentioned species (Supplementary Figure S3).

624 T4SS systems were used to transport a variety of biomolecules (DNA or proteins) across the
625 bacterial envelope (Chandran Darbari and Waksman, 2015). Most T4SS detected in *Dickeya*
626 species are associated with conjugal transfer proteins and thus, correspond likely to conjugative
627 T4SS that transferred DNA (de la Cruz et al., 2010; Ilangovan et al., 2015). This process is
628 instrumental in bacterial adaptation to environmental changes (Thomas and Nielsen, 2005). T6SS
629 is used for interaction with the host and for inter-bacterial competition (Poole et al., 2011).

630 Unfortunately, while effectors associated to the *Dickeya* T6SS carry C-terminal nuclease domains
631 that degrade target cell DNA, little is known concerning their function in virulence (Ryu, 2015),
632 limiting the interpretation of its absence in *D. aquatica*.

633

634 ***Distribution of plant adherence elements in Dickeya species***

635 A chaperone–usher pilus assembly pathway, associated with type I fimbriae (*fimEAICDFGHB*),
636 was present in *D. aquatica* but not in any other *Dickeya* species (Supplementary Table S4).

637 Phylogenetic analyses suggested an acquisition from *Morganellaceae* or *Enterobacteriaceae*,
638 through HGT (Supplementary Figure S3). The adhesin FimH, a two-domain protein at the tip of
639 type I fimbriae is known to recognize mannoside structures and to be responsible for adhesion to

640 both animal epithelial cells and plant surface (Haahtela et al., 1985; Sauer et al., 2000). Therefore,
641 we can hypothesize that type I fimbriae could be involved in adherence of *D. aquatica* to plant
642 surface. Interestingly, in *Xylella fastidiosa*, the Fim system is known to be an antagonist of
643 twitching motility caused by type IV pili (De La Fuente et al., 2007). In *D. aquatica*, the mutually
644 exclusive production of type I fimbriae and type IV pilus could be linked to the specific pH
645 regulation of type IV pilus. When *D. aquatica* penetrates into the intercellular apoplast, which is an
646 acidic compartment, twitching motility would be induced to favour bacteria dissemination in plant
647 tissues, while the type I fimbriae would be no longer required during the colonization. This
648 regulation of twitching would thus contribute to the efficiency of *D. aquatica* for infecting tomatoes,
649 cucumbers, and probably other acidic fruits.

650 While the presence of type I fimbriae is a specific feature of *D. aquatica* among *Dickeya*, the
651 Flp/Tad pilus, involved in plant surface adherence (Nykryri et al., 2013), is restricted to *D.*
652 *chrysanthemi* likely as the consequence of a HGT from another proteobacterium (Supplementary
653 Table S4 and Supplementary Figure S3). An operon encoding a multi-repeat adhesin
654 (Dda3937_01477) associated to a T1SS secretion pathway was found in the genome of *D. dadantii*
655 (Supplementary Table S4). This protein contains multiple cadherin-homologous domains and is
656 likely involved in plant adhesion. Indeed, in *Pectobacterium atrosepticum*, such a multi-repeat
657 adhesin secreted by a type I pathway was shown to be required for binding to the host plant
658 (Perez-Mendoza et al., 2011). This adhesion and its secretion system were absent in the basal
659 *Dickeya* species as well as in *D. zeae* and *D. chrysanthemi*. Phylogenetic analyses indicated an
660 acquisition in the ancestor of Cluster II, followed by a secondary loss in *D. dianthicola*
661 (Supplementary Figure S3).

662 From this analysis, it appears that the different *Dickeya* species retained distinct strategies to
663 adhere to plant surfaces and that HGT played an essential role in the acquisition of the involved
664 genes.

665

666 ***Distribution of iron assimilation systems in Dickeya species***

667 Iron acquisition by *Dickeya* is required for the systemic progression of maceration symptoms in the

668 plant hosts (Enard et al., 1988, Dellagi et al., 2005, Franza et al., 2005). To chelate iron from the
669 surroundings, most *Dickeya* species synthesize and excrete two siderophores: the
670 hydroxycarboxylate achromobactin encoded by the *acsABCDEF* cluster (Munzinger et al., 2000),
671 and the catecholate chrysobactin encoded by the *cbsABCEFHP* genes (Persmark et al., 1989).
672 The *Dickeya* strain EC16 produces dichrysobactin and linear/cyclic trichrysobactin in addition to
673 the monomeric siderophore chrysobactin (Sandy and Butler, 2011). These siderophores form a
674 complex with Fe(III) designated as ferric-siderophore (Franza and Expert, 2013). The ferric-
675 siderophores are specifically recognized by outer membrane transporters (Acr for ferric-
676 achromobactin; Fct for ferric-chrysobactin). These transporters are gated-channels energized by
677 the cytoplasmic membrane-generated proton motive force transduced by the TonB protein and its
678 auxiliary proteins ExbB and ExbD (Franza and Expert, 2013). Two pairs of ExbB and ExbD
679 proteins are present in most *Dickeya* species. Transport of a ferric-siderophore across the inner
680 membrane involves a specific ABC permease (CbrABCD for ferric achromobactin; CbuBCDG for
681 ferric chrysobactin). Interestingly, the achromobactin genes (*acs* gene cluster) and related
682 transport system (*cbr* gene cluster) were absent in *P. confusarubida* and *D. paradisiaca*,
683 suggesting they were acquired by HGT in *Dickeya* after the divergence of these two lineages.
684 *Dickeya* genes are closely related to *Pseudomonas fulva* and *P. syringae* sequences, suggesting
685 an HGT between these plant-associated bacteria (Supplementary Figure S3). By contrast the
686 chrysobactin genes (*cbs* gene cluster) and related transport system (*cbu* gene cluster) were likely
687 present in the ancestor of *Dickeya* and *P. confusarubida* (Supplementary Figure S3), and then
688 specifically lost in *D. dadantii* subspecies *dieffenbachiae*. In addition to ferric-siderophores, various
689 other iron uptake systems are present in *Dickeya*. The ferrous iron transport systems FeoAB and
690 EfeUOB can be inferred as ancestral in all *Dickeya* species and *P. confusarubida* (Supplementary
691 Figure S3). In contrast, the taxonomic distribution and the phylogeny of the YfeABCD permease
692 that can import both iron and manganese, suggested an acquisition through HGT by *D.*
693 *chrysanthemi* and Cluster II species, except *D. gs. undicola* (Supplementary Table S4 and
694 Supplementary Figure S3). The haem transport Hmu system was present in most *Dickeya*. Its
695 phylogeny suggested an ancestral presence in *Dickeya*, followed by secondary losses in *D.*

696 *dianthicola*, *D. gs. undicola*, *D. zea*, and *D. aquatica* (Supplementary Figure S3, Supplementary
697 Table S4). Although variable combinations of iron assimilation systems exist in *Dickeya* species, at
698 least four systems were present in each species. This multiplicity underscores the fact that
699 competition for this essential metal is critical for the outcome of the plant-*Dickeya* interaction.

700

701 ***Biosynthesis of secondary metabolites in Dickeya species***

702 In addition to siderophores, some *Dickeya* species produce secondary metabolites such as the
703 phytotoxin zeamine and the antifungal compound oocydin via non-ribosomal peptide synthases
704 (NRPS) and polyketide synthases (PKS) (Zhou et al., 2011a; Matilla et al., 2012). To evaluate the
705 diversity of secondary metabolites produced by the *Dickeya* genus, we screened the eight
706 complete genomes (*D. paradisiaca* Ech703, *D. aquatica* 174/2, *D. zea* EC1, *D. zea* Ech586, *D.*
707 *chrysanthemi* Ech1591, *D. solani* IPO2222, *D. fangzhongdai* N14b, *D. dadantii* 3937) and the three
708 partial genomes (*D. dianthicola* RNS04.9, *D. gs. poaceaephila* Ech569, *D. gs. undicola* 2B12) for
709 gene clusters encoding NRPS or/and PKS. Then, we analysed the evolutionary history of these
710 genes clusters among the 49 *Dickeya* genomes. The *oocBCDEFGJKLMNQRSTUUVW* gene
711 cluster coding for oocydin biosynthesis proteins was present in *D. paradisiaca*, *D. zea* strains
712 isolated from rice, *D. chrysanthemi* subspecies *chrysanthemi*, *D. solani*, *D. dianthicola*, and *D.*
713 *fangzhongdai* strain NCPPB 3274 (Supplementary Table S4). This could suggest an ancestral
714 presence in *Dickeya* accompanied by losses in *D. aquatica*, *D. gs. poaceaephila*, *D. dadantii*, *D.*
715 *gs. undicola*, most *D. fangzhongdai* strains, and some Cluster I strains (Supplementary Figure S3).
716 However, the hypothesis of acquisition and spreading through HGT within *Dickeya* could not be
717 excluded. The *zmsABCDEFGHIJKLMNPQRS* gene cluster directing zeamine biosynthesis was
718 restricted to *D. zea* strains isolated from rice, *D. fangzhongdai* and *D. solani* (Supplementary
719 Table S4), suggesting secondary acquisition by HGT (Supplementary Figure S3). In addition,
720 genes involved in coronafic acid biosynthesis, a phytotoxin classically produced by *Pseudomonas*
721 *syringae* (Bender et al., 1999), were present only in *D. gs. poaceaephila* and *D. dadantii*
722 subspecies *dieffenbachiae* (Supplementary Table S4), suggesting specific acquisition via HGT. We
723 detected four additional gene clusters encoding NRPS and PKS, (i) cluster 1 was specific to *D.*

724 *paradisiaca* and *P. confusarubida*, (ii) cluster 2 was specific to *D. paradisiaca*, (iii) cluster 3 was
725 specific to *D. aquatica*, suggesting recent acquisitions by these species, while (iv) cluster 4 was
726 more widely distributed, being detected in *D. aquatica*, *D. gs. poaceaephila*, *D. fangzhongdai*, *D.*
727 *solani*, some *D. dadantii* strains, and *D. zea* except the strains isolated from rice (Supplementary
728 Table S4). To conclude, each *Dickeya* species was characterized by a specific combination of
729 large gene clusters possibly involved in the production and secretion of toxic secondary
730 metabolites. These clusters were likely acquired from unrelated bacteria through HGT and could
731 have been selected based on the constraints imposed by host or environmental factors. For
732 example, the *D. zea* strains can be subdivided in two groups, the strains isolated from rice, which
733 produce both zeamine and oocydin, while the other strains infecting other crops produce the
734 fourth-type metabolite encoded by cluster 4. This difference between *D. zea* strains was used by
735 Zhou et al. (2015) to define the distinct pathovar linked to rice as *D. zea* subsp. *oryzae*.

736

737 ***Evolution of Dickeya gene repertoires***

738 The 49 *Dickeya* proteomes used in this study contained in average 4,022 proteins, ranging in size
739 from 3,533 (*D. gs. poaceaephila*) up-to 4,352 (*D. fangzhongdai* NCPPB 3274) proteins,
740 representing a difference of 819 proteins (Supplementary Table S3). Comparison of the 197,073
741 proteins contained in the 49 *Dickeya* proteomes led to the delineation of 11,566 protein families
742 (Figure 7). These protein families correspond to the pan-proteome of *Dickeya*. Among these
743 protein families, 13.9% (1,604) were present at least in one copy in all *Dickeya* proteomes (Figure
744 7) and defined the core-proteome of this genus. Yet, the size of both pan- and core-proteomes
745 could be slightly underestimated because the proteomes of some strains were deduced from draft
746 genomes. Nevertheless, this meant that in average, ~39.9% of the proteins of any *Dickeya*
747 proteome belonged to the core proteome, while a given *Dickeya* proteome encompassed only
748 ~34.8% of the pan-proteome of this genus. Random taxonomic sampling-based rarefaction curves
749 indicated that sequencing more *Dickeya* genomes will probably not change significantly the
750 estimated size of the core-proteome, while it appears that the pan-proteome is far from being fully
751 disclosed (Figure 7B). This highlights the high diversity and plasticity of the gene repertoires in

752 *Dickeya*. This observation coupled to the great diversity of the virulence, stress resistance,
753 metabolism, and secretion systems imply that none of the *Dickeya* strains could be regarded as a
754 representative model for this genus. Core protein families could be punctually lost in a given strain,
755 while some protein families can be present transiently in a few strains. Thus, it is also relevant to
756 consider the persistent (i.e. protein families present in more than 90% of the strains) and volatile
757 proteomes (i.e. protein families present in less than 10% of the strains) (Touchon et al., 2009). In
758 *Dickeya*, most protein families could be classified either as persistent (2,714 protein families,
759 23.5%) or volatile (6,426 protein families, 55.6%) (Figure 7A). Unsurprisingly, the persistent
760 proteome was enriched in proteins with known functions, while volatile proteome encompassed
761 mostly hypothetical proteins, prophage elements, and transposases. It is tempting to consider the
762 genes encoding for the volatile proteome as a reservoir of functional innovations, yet the adaptive
763 potential of these genes remains a matter of debate (Touchon et al., 2009).

764 Among the 11,566 protein families inferred in *Dickeya*, 3,452 corresponded to strain specific
765 families (i.e. being present in a single proteome) (Figure 7A and Supplementary Table S8). The
766 number of strain specific protein families ranged from 336 in *D. aquatica* 174/2^T and 304 in *D. gs.*
767 *Poaceaephila*, down to zero in *Dickeya solani* strains MK16, PPO9134 and RNS0773B
768 (Supplementary Table S8). To determine the origin of these strain specific protein families, we
769 used them as seeds to query with BLASTP (e-value cut-off 10⁻⁴) a local database gathering 3,104
770 complete prokaryotic proteomes, including the 49 *Dickeya* and *P. confusarubida* ATCC 39006
771 proteomes. Results indicated that 1,051 (30.4%) *Dickeya* strain specific protein families displayed
772 best hit in one of the other 48 *Dickeya* strains, meaning that those sequences were wrongly
773 considered as strain specific because they did not satisfy the coverage and identity parameters
774 used to delineate the protein families. This was not surprising because some protein families could
775 be fast-evolving, meaning that applying uniform parameters can fail to delineate correctly these
776 protein families and lead to an overestimation of the strain specific protein families. In contrast,
777 1,625 (47.1%) *Dickeya* strain specific protein families displayed best hits in non-*Dickeya*
778 proteomes, meaning that the corresponding genes were likely acquired by HGT from non-*Dickeya*
779 donors. The taxonomic distribution of the corresponding sequences pinpointed *Proteobacteria*

780 (especially *Enterobacteriales*), and to a less extent *Firmicutes* as major donors (Supplementary
781 Table S8 B-C). Yet, the contribution of these two phyla is likely overestimated due to their
782 overrepresentation in sequence databases compared to other lineages. Accordingly, these results
783 should be interpreted as general trends but additional data would be required to precisely estimate
784 the real contribution of *Firmicutes* and *Proteobacteria*. Finally, 776 (22.5%) *Dickeya* strain specific
785 protein families displayed no significant hits or no hits at all, indicating that the corresponding
786 genes were truly strain specific or corresponded to annotation errors (false positives).

787 At the species level proteome size variation ranged from 435 (*D. aquatica*) to 120 (*D. paradisiaca*)
788 proteins (Supplementary Table S8). The taxonomic distribution of the species-specific protein
789 families displayed overall similar pictures, with most of them being present in all strains of the
790 species (Supplementary Figure S6). This revealed a relative homogeneity of proteomes within
791 species. Interestingly, a few strains diverged from this general trend, such as strain NCPBB 3274
792 within *D. fangzhongdai*, *D. aquatica* 174/2^T, *D. chrysanthemi* NCPPB 402, *D. dadantii* NCPPB
793 2976, and *D. solani* RNS 0512A. This is consistent with some previous studies. For instance, *D.*
794 *dadantii* NCPPB 2976 is part of the *dieffenbachiae* subspecies and has been shown to be clearly
795 different from the other *D. dadantii* subsp *dadantii* strains based on ANI values (Zhang et al.,
796 2016). Among, *D. solani*, strain RNS 0512A was proposed to define a novel *D. solani* sub-group
797 based on the high number and wide distribution of nucleotide variations compared to other *D.*
798 *solani* strains (Khayati et al., 2015).

799 Using COUNT (Csuros, 2010) on the 12,660 protein families built with SILIX and the topology of
800 the core-pf tree, we inferred the ancestral protein repertoires at each node of the *Dickeya* core-pf
801 phylogeny (Figure 8). COUNT provided similar results for different *Dickeya* species, irrespectively
802 of the position of *D. solani*, as either the sister-group of *D. dadantii* or *D. dianthicola*
803 (Supplementary Table S9), as suggested by core-pf and rprotS phylogenetic analyses (see above).
804 The main difference lies in the numbers of gains and losses at the base of *Dickeya*.

805 We inferred 4,627 protein families in the ancestor of *Dickeya*, while in average 3,921 protein
806 families are contained in present-day *Dickeya* proteomes. This corresponds to a global loss of 18%
807 of the protein families. Interestingly, loss of protein families dominated over gains and affected all

808 *Dickeya* species (Figure 8). Highest protein losses were observed on the stems leading to *D. gs.*
809 *poaceaephila* and *D. aquatica* and to a lesser extent in *D. paradisiaca*, *D. gs. undicola*, and *D.*
810 *dianthicola*. These losses were only partially compensated by protein family gains. Surprisingly,
811 more gains were observed in *D. aquatica* 174/2^T, compared to the two other *D. aquatica* strains
812 (Figure 8). We assume that this was not due to biases in the annotation process by RAST,
813 because very similar results were obtained when using PROKKA (Seeman, 2014). In fact, this may
814 reflect the fact that the genomes of DW 0440 and CSL RW240 strains were not completed, being
815 reported as draft genomes. The general trends observed were robust irrespectly of the postion of
816 *D. solani* relatively to *D. dadantii* and *D. dianthicola* (Supplementary Table S9).

817

818 *Characterization of the D. aquatica mobilome*

819 Mobile Genetic Elements (MGEs) are the main actors of the HGT and include plasmids, viruses
820 (phages and prophages) and transposons (Jackson et al., 2011). They are often localised within
821 genomic islands on chromosomes. The mobilome of a strain is the repertoire of all the genes
822 associated with MGEs. Using both PHAST and IslandViewer, we detected seven phage elements
823 and ten genomic islands in *D. aquatica* 174/2^T (Figure 1, Supplementary Table S10). Most of the
824 genomic islands contained genes of transposases, integrases or mobile elements that were likely
825 remnants of HGT. They also contain 105 of the 336 ORFAN genes detected in *D. aquatica* 174/2^T
826 strain. Among the seven detected prophages, P2, P6 and P7 were related to transposable Mu-
827 phage. These were also present in some *D. zeae* strains isolated from river as well as *D.*
828 *dianthicola* strains (Supplementary Table S10). P3 and P5 were specific to some *D. aquatica*
829 strains, while P1, a defective prophage with only few conserved genes, and P4 were widespread in
830 all *Dickeya* species (Supplementary Table S10). Among the ten genomic islands detected in *D.*
831 *aquatica* 174/2^T, seven (GI1, GI2, GI4, GI5, GI6, GI7, GI9) were mainly composed of mobile
832 elements and small hypothetical proteins, GI2 also contained a type III restriction-modification
833 system, whereas GI4 included a type I restriction-modification system and a toxin-antitoxin system
834 (Supplementary Table S10). Similarly, GI6 contained a toxin-antitoxin system as well as an
835 isolated non-ribosomal peptide synthase, which was also found in *D. gs. poaceaephila* and *D. zeae*

836 (Supplementary Table S10). GI7 contained some metabolic proteins, including the previously
837 mentioned cluster 3 encoding NRPS and PKS, as well as transporters, notably a cobalt/nickel ABC
838 transporter (Supplementary Table S10). Excluding the mobile elements, GI1, GI2, GI4, GI5, GI6
839 GI7, and GI9 were specific to *D. aquatica* strains, even if a few genes composing these GIs can be
840 punctually detected in some others strains (Supplementary Table S10). The three other genomic
841 islands (GI3, GI8, GI10) were metabolic islands (Supplementary Table S10). GI3 has been laterally
842 transferred between *Erwinia pyrofolia* and *D. aquatica* (Supplementary Figure S3). GI8 that
843 included proteins related to fatty acid metabolism, was conserved in *D. zeeae* strains isolated from
844 rice or originated from China (Supplementary Table S10). GI10 contained proteins related to the
845 complete carbapenem biosynthetic pathway CarABCDE and the associated resistance proteins
846 CarFG (Supplementary Table S10). *P. confusarubida* contained genes coding for CarABCDE as
847 well as CarFG, in agreement with its capacity to synthesize the carbapenem antibiotic (carbapen-
848 2-em-3-carboxylic acid) (Thomson et al., 2000; Coulthurst et al., 2005). This cluster was conserved
849 in *D. undicola*, *D. dadantii* subspecies *dieffenbachiae*, some *D. chrysanthemi* strains and in the *D.*
850 *zeeae* CSL-RW192 strain isolated from water (Supplementary Table S10). *D. paradisiaca* only
851 contained the *carFG* resistance genes but was deprived of the biosynthetic genes (Supplementary
852 Table S9). The phylogeny of CarABCDE and CarFG shows that relationships among the *Dickeya*
853 sequences are inconsistent with the species phylogeny, suggesting a secondary acquisition
854 through HGT in this genus (Supplementary Figure S3).

855 Overall, phages and genomic islands from *D. aquatica* are mostly species specific suggesting a
856 wide genomic plasticity in the *Dickeya* genus.

857

858 *Concluding remarks*

859 In this work, we sequenced the complete genome of *D. aquatica* 174/2 and showed that unlike
860 initially supposed, this bacterium, similarly to other *Dickeya* species, is a phytopathogen. Who is
861 the natural host of *D. aquatica* and why is this bacterium found in aquatic environments rather than
862 associated with plants as are its close relatives? Although the data at hand are not sufficient to
863 answer these questions, we speculate that *D. aquatica* can be pathogenic for aquatic plants such

864 as charophytes, which are a family of complex-structured algae living in a variety of wetland and
865 freshwater habitats including those, from which the *D. aquatica* strains have been isolated.
866 Charophytes are thought to be the closest ancestor of land plants. The cells of these algae are
867 surrounded by polysaccharide-based cell walls. However, their cell walls are thin and cannot be
868 distinguished as primary or secondary cell walls. Furthermore, they lack xyloglucans, which are
869 common in most land plants (Sarkar et al., 2009). Intriguingly, *D. aquatica* lacks the xylanases and
870 xylose degradation pathways and we hypothesize that this could reflect an evolutionary adaptation
871 to charophyte hosts.

872 The comparison of *D. aquatica* 174/2 and *Dickeya* strain proteomes available in public databases
873 showed that the *Dickeya* genus displays a remarkable diversity featuring many unique protein
874 families and emphasizing that our knowledge of this genus is still limited. The real size of the pan-
875 proteome is an open question encouraging further exploratory studies on these bacteria, the
876 success of which will depend on the collection of new strains isolated from various environments
877 and the sequencing of the genomes of newly identified representatives of the genus. Remarkably,
878 we observed an enormous degree of genetic plasticity in the pathogenicity determinants that
879 enable various *Dickeya* species to colonize a wide range of plant hosts. Furthermore, in this work,
880 we have postulated the existence of a sister genus of *Dickeya*, *Prodigiosinella*, and characterized
881 two new *Dickeya* genomospecies. The reconstruction of ancestral genomes allowed us to gain
882 new insights into the evolutionary history of this genus and highlighted an evolutionary trajectory
883 dominated by the loss of protein families.

884

885 **Experimental procedures**

886 *Genome sequencing, assembly and annotation*

887 DNA for sequencing of the *D. aquatica* type strain (174/2) genome was extracted from overnight
888 broth culture using Promega bacterial genomic DNA kit. PacBio sequencing to > 350X coverage
889 was performed by Eurofins Genomics (<https://www.eurofinsgenomics.eu/>). Reads were assembled
890 using CANU (Koren et al., 2017). The annotation was performed automatically with RAST (Aziz et
891 al., 2008), then expertly reviewed using MAGE (Vallenet et al., 2013) and literature data. The

892 expert review allowed to assign 2,457 gene names, correct 547 annotations and add 5 CDS
893 missed by RAST. The replication origin (oriC) was predicted by OriFinder
894 (<http://tubic.tju.edu.cn/Ori-Finder>) (Gao and Zhang, 2008). The presence of mobile genetic
895 elements in the *D. aquatica* 174/2 genome was investigated by the following online tools:
896 IslandViewer (<http://pathogenomics.sfu.ca/islandviewer>) (Dhillon et al., 2015) for the GI regions,
897 CRISPRDetect (http://brownlabtools.otago.ac.nz/CRISPRDetect/predict_crispr_array.html) (Biswas
898 et al., 2016) for CRISPR arrays, whereas putative prophage sequences were identified by PHAST
899 and PHASTER analysis (<http://phast.wishartlab.com/>) (Zhou et al., 2011b; Arndt et al., 2016). The
900 genome sequence has been submitted to EMBL database under accession number
901 GCA_900095885 (<http://www.ebi.ac.uk/>).

902

903 *Virulence assays on various hosts*

904 Bacterial cultures were grown in M63G minimal medium (M63 + 0.2% w/v glucose) (Miller 1972)
905 and diluted to a given OD₆₀₀ depending on the host: 0.2 (chicory) or 1 (potato, cucumber, tomato,
906 pineapple and kiwi). For chicory, 5 µL of bacterial suspension were injected into a 2 cm incision at
907 the center of the leaf. For potato, cucumber and tomato, 5, 100 and 200 µL of bacterial suspension
908 were injected into the vegetable, respectively. 200 µL of bacterial suspension were also injected
909 into pineapple and kiwi fruits. Plants were incubated at 30°C with 100% humidity for 18 h (chicory)
910 or 42 h (potato, cucumber and tomato) or 78 h (pineapple and kiwi). The soft rot mass is used to
911 quantify virulence.

912

913 *Stress resistance assays*

914 Bacteria were cultured at 30°C in 96 well plates using M63G (M63 + 0.2% w/v glucose) pH 7.0 as
915 minimal medium. Bacterial growth (OD_{600nm}) was monitored for 48 h using an Infinite® 200 PRO -
916 Tecan instrument. Resistance to osmotic stress was analysed using M63G enriched in 0.05 to 0.5
917 M NaCl. Resistance to oxidative stress was analysed in the same medium by adding H₂O₂
918 concentrations ranging from 25 to 200 µM. The pH effect was analyzed using the same M63G
919 medium buffered with malic acid at different pH ranging from 3.7 to 7.0.

920

921 *Proteome database construction*

922 We built a local database (DickeyaDB) gathering the proteomes of *Serratia* sp. ATCC 39006, 48
923 *Dickeya* available at the NCBI (by January 2017), and *D. aquatica* type strain 174/2
924 (Supplementary Table S3). A second database (prokaDB) containing 3,104 proteomes of
925 prokaryotes, including the 50 proteomes of DickeyaDB, was also built.

926

927 *Assembly of Dickeya and Serratia ATCC 39006 protein families*

928 *Dickeya* and *Serratia* ATCC 39006 protein families were assembled with SILIX version 1.2.9 (Miele
929 et al., 2011). More precisely, pairwise comparisons of protein sequences contained in DickeyaDB
930 were performed using the BLASTP program version 2.2.26 with default parameters (Altschul et al.,
931 1997). Proteins in a pair providing HSP (High-scoring Segment Pairs) with identity over 60% and
932 covering at least 80% of the protein lengths were gathered in the same family. This led to the
933 assembly of 12,660 protein families, among which 1,493 were present in at least one copy in all
934 DickeyaDB proteomes. In contrast, considering the 49 *Dickeya* proteomes without taking into
935 account *Serratia* ATCC 39006 led to the assembly of 11,566 protein families, among which 1,604
936 were present at least in one copy in all *Dickeya* and *Serratia* ATCC 39006 proteomes, and 1,420 in
937 exactly one copy.

938

939 *Inference of reference phylogenies of Dickeya*

940 Reference phylogenies of *Dickeya* were inferred using ribosomal proteins (rprots) on the one hand
941 and core protein families (core-pf) on the other hand. The rprots phylogenetic tree was rooted with
942 sequences from *Serratia* ATCC 39006, together with three *Pectobacterium* species
943 (*Pectobacterium carotovorum* PC1, *Pectobacterium atrosepticum* SCRI1043, and *Pectobacterium*
944 *wasabia* WPP163) and five additional *Serratia* species (*Serratia marcescens* FGI94, *Serratia*
945 *fonticola* DSMZ4576, *Serratia liquefaciens* ATCC27592, *Serratia proteamaculans* 568, and
946 *Serratia plymuthica* AS9), while the core-pf phylogenetic tree was rooted with *Serratia* ATCC
947 39006 (see results).

948 Rprots sequences were extracted from the DickeyaDB using the engine of the riboDB database
949 (Jauffrit et al., 2016). Briefly, the riboDB engine allows retrieving rprots sequences through a
950 double approach combining reciprocal best-blast-hits and hidden Markov model (HMM) profiles
951 searches.

952 Starting from the 1,420 core-pf protein families containing exactly one copy in each proteome of
953 the DickeyaDB and 51 rprots, we applied several quality controls. First, within protein families
954 extremely short sequences (<30% of the median length of the family) were discarded. Then, we
955 used FastTreeMP (Price et al., 2010), and PhyloMCOA (de Vienne et al., 2012) to detect and
956 discard outlier sequences, on the basis of nodal and patristic distances. At the end of the quality
957 control process, 1,341 protein families present in more than 35 (70%) out of the 50 considered
958 proteomes and 51 rprots were kept. For each of these protein families, multiple alignments were
959 built using the CLUSTAL-Omega-1.1.0 program (Sievers et al., 2011) and trimmed using
960 GBLOCKS (Castresana, 2000) with parameters set to a minimal trimming. The trimmed multiple
961 alignments corresponding to 1,341 core-pf on the one hand and the 51 rprots on the other hand
962 have been combined using Seaview-4.5.4 (Gouy et al., 2010) to build the core-pf and the rprots
963 supermatrices containing 414,696 and 6,295 amino acid positions, respectively.

964 Maximum likelihood phylogenies of these supermatrices have been inferred with IQ-TREE-1.5.3
965 (Nguyen *et al.*, 2015) with a C60 profile mixture of the Le and Gascuel evolutionary model (Le and
966 Gascuel 2008) and a gamma distribution with four site categories (Γ_4) to model the heterogeneity
967 of evolutionary rates across sites, as proposed by the model testing tool (Kalyaanamoorthy *et al.*,
968 2017) available in IQ-TREE. The robustness of the inferred maximum likelihood trees was
969 estimated with the non-parametric bootstrap procedure implemented in IQ-TREE-1.5.3 for the
970 rprots supermatrix (100 replicates of the original alignments) and the ultrafast bootstrap approach
971 for the core-pf supermatrix (1,000 replicates).

972

973 *Phylogenetic analysis of single markers*

974 947 SSU rRNA and 125 LSU rRNA complete sequences from *Dickeya*, *Serratia* (including strain
975 ATCC 39006), and *Pectobacterium* available in public databases were retrieved and aligned with

976 MAFFT v7.222. The resulting multiple alignments were trimmed using BMGE-1.1 (default
977 parameters). The phylogeny of the 947 SSU rRNA sequences was inferred using FastTree-2.1.9
978 (Price et al., 2010), with the GTR + gamma + cat 4 model, while the LSU rRNA tree was inferred
979 with IQ-TREE with the TIM3+F+I+Γ4 model according to the BIC criterion, as suggested by the
980 propose model tool available in IQ-TREE.

981 Homologues of proteins of interest were identified in the prokaDB with BLASTP. The first 75 HSP
982 hits with evaluate smaller than 10^{-4} were kept. The retrieved sequences, together with the seed were
983 aligned using MAFFT v7.222, alignments were trimmed using BMGE-1.1 (default parameters).
984 Maximum likelihood phylogenies were built using IQTREE-1.5.3 (Nguyen *et al.*, 2015) with the
985 LG+Γ4+I+F model. In order to identify the origin and evolution of these proteins in *Dickeya*, their
986 phylogenies were compared and reconciliated with the *Dickeya* species phylogenies, by
987 considering the two alternative positions of *D. solani*: as either sister-group of *D. dadantii* or *D.*
988 *dianthicola*.

989

990 *Identification of the D. fangzhongdai phylogenetic cluster*

991 To determine whether some sequenced strains are related to *D. fangzhongdai*, we used all the *D.*
992 *fangzhongdai* genes available in public databases SSU-rDNA (KT992690.1), *dnaX* (KT992713.1),
993 *fusA* (KT992697.1), *purA* (KT992705.1), *recA* (KT992693.1), *gapA* (KT992701.1) and *rplB*
994 (KT992709.1) genes, we extracted the corresponding genes from the DickeyaDB database and
995 then used these sequences as input for phylogenetic analyses using leBIBI^{QBPP} phylogenetic
996 positioning tool (Flandrois et al., 2015). We found that the strains B16, MK7 and S1 were
997 systematically affiliated with *D. fangzhongdai*.

998

999 *Ancestral gene content*

1000 The program COUNT (Csuros, 2010) was used for gene families evolutionary reconstruction in
1001 *Dickeya* species using the topology of the core-pf tree as reference. All the generated 12,660
1002 families were submitted to COUNT, which can perform ancestral genome reconstruction by
1003 posterior probabilities in a phylogenetic birth-and-death model. Rates were optimized using a gain–

1004 loss–duplication model and three discrete gamma categories capturing rate variation across
1005 families, with other parameters set at default and allowing different gain–loss and duplication–loss
1006 rates for different branches. One hundred rounds of optimization were computed. COUNT was run
1007 twice: first with *D. solani* as sister-group of *D. dadantii* and then as sister-group of *D. dianthicola*.

1008

1009 **Acknowledgements**

1010 This work was supported by the Investissement d'Avenir grant (ANR-10-BINF-01-01), by the ANR
1011 Combicontrol grant (ANR-15-CE21-0003-01), by a grant from the FR BioEnviS and using the
1012 computing facilities of the Computing Cluster of the LBBE/PRABI. We would like to thank Georgi
1013 Muskhelishvili for critical reading the manuscript and Frederic Jauffrit as well as Mailys Dumet for
1014 providing the extraction of ribosomal proteins.

1015

1016 **Conflict of interest statement**

1017 The authors declare that no conflicting interests exist.

1018

1019 **References**

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1051 *Lonsdalea quercina* subsp. *britannica* subsp. nov., emendation of the description of the genus *Brenneria*,
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1381 **Figure legends**

1382

1383 **Figure 1. Genomic organisation of *Dickeya aquatica* 174/2^T chromosome**

1384 The chromosome is represented as a wheel and the origin of replication (OriC) and terminus (Ter)
1385 are indicated. The circles from outside to inside represent protein-coding sequences (CDS) on the
1386 forward strand, CDS on the reverse strand, distribution of ribosomal RNA operons (four on the right
1387 replichore and three on the left replichore), distribution of tRNA genes, and then distribution of
1388 ncRNA. The blue areas correspond to phage elements detected using PHAST. The thin black lines
1389 correspond to four CRISPR arrays and the red areas represent the ten genomic islands predicted
1390 using IslandViewer. The next circle (black) indicates the GC content and the central circle
1391 (green/purple) shows the GC-skew. The window size of the GC content and GC-skew is 100
1392 nucleotides. Figure 1 was drawn using Gview <https://server.gview.ca/>.

1393

1394 **Figure 2. Phylogeny and proteome comparison of *Dickeya***

1395 Maximum likelihood phylogeny (left) of 1,341 *Dickeya* single copy protein families (core protein
1396 families: 50 sequences, 414,696 amino acid positions). The tree was computed with IQ-TREE with
1397 the LG+C60 model and rooted using *Serratia* ATCC 39006. Numbers associated to branches
1398 correspond to ultrafast bootstrap values. The scale bar corresponds to evolutionary distance (i.e.
1399 the average number of the substitutions inferred per site). The table (right) corresponds to the S_{AB}
1400 association coefficient computed for each pair of strains as $S_{AB} = (100 \times 2N_{AB}) / (N_A + N_B)$, in which
1401 N_A is the number of protein families present in strain A, N_B is the number of protein families present
1402 in strain B and N_{AB} is the number of protein families shared by strain A and strain B. This
1403 coefficient ranged from 0 when both strains do not share any gene family to 100 when all the
1404 families present in strain A were also present in strain B. The figure was generated using Evolvview
1405 (He et al., 2016).

1406

1407 **Figure 3. Virulence of *D. aquatica* on potato, chicory, cucumber and tomato.** Bacterial

1408 cultures were grown in M63G minimal medium (M63 + 0.2% w/v glucose) and diluted to a given

1409 OD₆₀₀ depending on the host: 0.2 (chicory) or 1 (potato, cucumber and tomato). For chicory, 5 µL
1410 of bacterial suspension were injected into a 2 cm incision at the center of the leaf. For potato,
1411 cucumber and tomato, 5, 100 or 200 µL of bacterial suspension were injected into the vegetable,
1412 respectively. Plants were incubated at 30°C with 100% humidity for 18 h (chicory) or 42 h (potato,
1413 cucumber and tomato). **A)** Picture of representative specimens of infected plants after incubation.
1414 Note that the rotten area was removed for potato. **B)** Quantification of the soft rot mass. Data is
1415 represented as mean +/- SD of 6 replicates. No soft rot symptoms were detected after 78 h for
1416 pineapple and kiwi fruits infected with 200 µL of bacterial suspension at OD₆₀₀ 1.

1417

1418 **Figure 4. *D. aquatica* stress resistance.** Bacteria were cultured at 30°C in 96 well plates using
1419 M63G (M63 + 0.2% w/v glucose) pH 7.0 as minimal medium. Bacterial growth (OD_{600nm}) was
1420 monitored for 48 h using an Infinite® 200 PRO - Tecan instrument. A and B) Resistance to
1421 osmotic stress was analysed using M63G enriched in 0.05 to 0.5 M NaCl (abscissa) and growth
1422 rates (ordinate) were determined. C) Resistance to oxidative stress was analysed in the same
1423 medium by adding H₂O₂ concentrations ranging from 25 to 200 µM (abscissa). The lag time
1424 (ordinate) is represented instead of the growth rate because after the degradation of H₂O₂ by
1425 bacterial catalases, the growth rates are similar. D) The pH effect on growth rate (ordinate) was
1426 analyzed using the same M63G medium buffered with malic acid at different pH ranging from 3.7
1427 to 7.0 (abscissa).

1428

1429 **Figure 5. Twitching motility induced under acidic condition in *Dickeya* species.** Colony
1430 morphologies of various *Dickeya* strains grown on M63G pH 7.0 and M63G buffered with malic
1431 acid at pH 5 in agar plates. The strains corresponding to colony numbers and their twitching
1432 phenotypes are indicated on the right.

1433

1434 **Figure 6. Distribution of plant cell wall degrading enzymes in *Dickeya*.** The type of cell wall
1435 degrading enzyme and the number of homologues of each enzyme are indicated for each of the

1436 strains. The phylogeny on the left corresponds the core-pf maximum likelihood tree as described in
1437 figure 2.

1438

1439 **Figure 7: The core-, pan-, versatile-, and persistent-proteomes of *Dickeya* genus**

1440 The delineation of core-, pan-, versatile-, and persistent-proteomes was based on the taxonomic
1441 distribution of the 11,566 proteins families identified by analyzing the 49 proteomes of *Dickeya*.

1442 The core-proteome is defined by the protein families present at least in one copy in all 49 *Dickeya*
1443 proteomes, the pan-proteome is defined by the 11,566 protein families, while the versatile- and the
1444 persistent-proteomes are defined as the protein families present in less than 10% and in more than
1445 90% of the 49 *Dickeya* proteomes, respectively.

1446 **(A) Distribution 11,566 protein families across the 49 *Dickeya* proteomes.**

1447 The 3,452 protein families present in a single *Dickeya* proteome (i.e. strain specific families) are on
1448 the left of the x-axis, while the 1,604 protein families defining the core-proteome are on the right of
1449 the x-axis.

1450 **(B) Estimation of the *Dickeya* core and pan-genomes**

1451 The graph shows the estimated sizes of the core- and pan-proteomes of *Dickeya* according to the
1452 number of considered strains. The curves were computed by calculating the core- and the pan-
1453 proteomes for an increasing number of strains randomly selected among the 49 *Dickeya* strains
1454 (100 replicates at each point). When all the 49 *Dickeya* strains were considered, the core- and the
1455 pan-proteomes encompass 1,604 and 11,566 protein families.

1456

1457 **Figure 8. Evolution of protein family repertoires along the *Dickeya* phylogeny**

1458 The number of protein family repertoires, gains, and losses inferred by COUNT are mapped on the
1459 topology of the core-pf reference phylogeny of *Dickeya*. Values mapped above the branches
1460 correspond to gains / losses, while values below branches correspond to the number of protein
1461 families inferred. Considering the alternative placement of *D. solani* as sister-group of *D.*
1462 *dianthicola* provided very similar results (see Supplementary Table S9).

1463

1464 **Supplementary materials**

1465

1466 **Supplementary Figure S1: Maximum likelihood phylogenetic tree of *Dickeya* strains based**
1467 **on the Fr-protein supermatrix gathering 51 ribosomal proteins** (LG+C60, 58 sequences, 6,295
1468 amino acid positions).

1469 Numbers at branch correspond to bootstrap values (100 replicates of the original data set). The
1470 scale bar indicates the average number of substitutions per site. The figure was generated using
1471 Evolview (He et al., 2016).

1472

1473 **Supplementary Figure S2: Phylogenetic position of “*Serratia* sp. ATCC 39006” based on**
1474 **LSU and SSU rDNA trees**

1475 Trees of the *Dickeya* genus inferred with a collection of **(A)** 947 SSU rDNA and **(B)** 125 LSU rDNA
1476 sequences retrieved from public databases. The trees were rooted using *Pectobacterium* and
1477 *Serratia* sequences. The scale bars represent the estimated average number of substitution per
1478 site. Numbers at nodes represent ultrafast bootstrap values **(A)** and bootstrap values **(B)**.
1479 *Pectobacterium* sequences are in pink, *Serratia* sequences in purple, and *Dickeya* sequences in
1480 black. Worth to note, LSU and SSU rDNA sequences from *Serratia* sp. 39006 robustly branch with
1481 *Dickeya* sequences and not within the *Serratia* genus. The trees were drawn using the iTOL
1482 webserver (Letunic and Bork, 2016).

1483

1484 **Supplementary Figure S3 - Single gene phylogenies**

1485 Maximum likelihood phylogenetic trees of 912 proteins of interest. Numbers associated with each
1486 branch correspond to ultrafast bootstrap values (1000 replicates of the original data set). The scale
1487 bars indicate the average number of substitutions per site. For each tree, the name and the
1488 annotation of the seed is provided in red.

1489

1490 **Supplementary Figure S4 – Non-metric multi-dimensional scaling (NMDS) plot of *Dickeya***
1491 **genomes according to their gene content.** A Bray distance similarity matrix was calculated

1492 based on the presence/absence profiles of 8,115 gene families (present in at least 2 genomes) for
1493 the 49 analyzed genomes and used to generate NMDS coordinates for each strain. The shorter
1494 distance linking two genomes indicates higher similarities between these genomes. Genomes from
1495 different species are indicated in different colors.

1496

1497 **Supplementary Figure S5: Phylogenies and genetic organisation of *peIAED* and *pehVWX***
1498 **clusters.**

1499 Maximum likelihood trees of *peIAED* and *pehVWX* clusters together with genetic organisation of
1500 these clusters are shown. Numbers associated with each branch correspond to ultrafast bootstrap
1501 values (1000 replicates of the original data set). The scale bar indicates the average number of
1502 substitutions per site. For each tree, the name and the annotation of the seed is provided in red.

1503

1504 **Supplementary Figure S6: The number of shared protein families between and within**
1505 ***Dickeya* species.** Figures were generated using the package UpSetR (Conway et al. 2017).

1506

1507 **Supplementary Table S1: Phenotypic differentiation of species within the genus *Dickeya*.**

1508 Table showing the phenotypic features of *Dickeya* species based on Samson et al. (2005),
1509 Parkinson et al. (2014), van der Wolf et al. (2014) and Tian et al. (2016).

1510

1511 **Supplementary Table S2: Genomic features of the *Dickeya* strains with completely**
1512 **sequenced genomes**

1513

1514 **Supplementary Table S3: List of the 49 *Dickeya* genomes used in this study indicating the**
1515 **plant host or habitat and the geographical origin of each strain**

1516

1517 **Supplementary Table S4: Distribution of genes with a role in pathogenicity or in adaptation**
1518 **to plant niche in *Dickeya* species**

1519 Genes of *Dickeya* species were considered as present if identity of the encoded protein was higher

1520 than 60% of full-length amino acids sequence. If local alignments were too short with regard to the
1521 length of similar sequences, we performed a nucleotide BLAST on full-length DNA sequences with
1522 similar thresholds (10^{-5} e-value, 80% identity of full length sequence). This allowed us to eliminate
1523 false genes. Note that because some genomes represent only drafts, false negatives may occur.

1524

1525 **Supplementary Table S5: Protein family distribution within proteomes from *Dickeya*,**
1526 ***Pectobacterium* and *Serratia*.**

1527 Considered strains were *Dickeya aquatica* 174/2, *Dickeya dadantii* 3937, *Dickeya paradisiaca*
1528 NCPPB 2511, *Dickeya solani* IPO 2222, *Dickeya zeae* EC1, *Pectobacterium atrosepticum*
1529 SCRI1043, *Pectobacterium carotovorum* subsp *carotovorum* PC1, *Pectobacterium wasabiae*
1530 WPP163, *Serratia fonticola*, *Serratia liquefaciens* ATCC27592, *Serratia marcescens* FGI94,
1531 *Serratia plymuthica* AS9, *Serratia proteamaculans* 568 and *Serratia* sp ATCC39006. Families were
1532 built using SILIX version 1.2.9 with 60% of sequence identity and 80% of sequence coverage.

1533

1534 **Supplementary Table S6: Results of the approximately unbiased tests performed on core-pf**
1535 **and rprot topologies using the 1,341 core-pf and the 51 rprot.**

1536

1537 **Supplementary Table S7: Distribution of Transcription factor-binding sites in *D. aquatica***
1538 **174/2 genome.** Binding sites for 56 transcriptional regulators were predicted in *D. aquatica* using
1539 nhmmer (Wheeler and Eddy, 2013).

1540

1541 **Supplementary Table S8: Protein family distribution within the proteomes of the 49 *Dickeya***
1542 **strains.**

1543 Families were computed with SILIX version 1.2.9 using 60% of sequence identity and 80% of
1544 sequence coverage.

1545

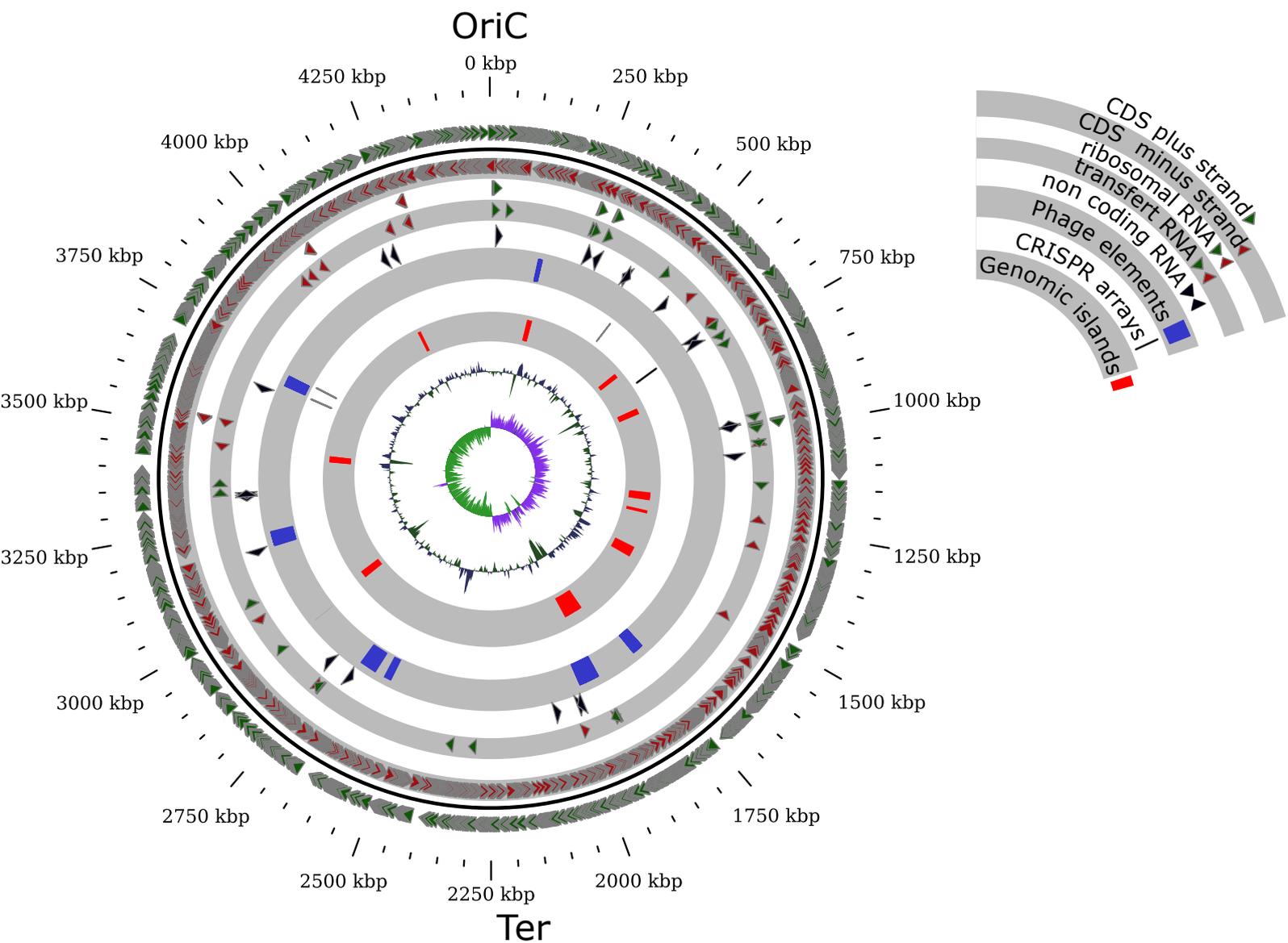
1546 **Supplementary Table S9: Comparison of inferences of ancestral gene repertoires in *Dickeya***
1547 **considering the two possible alternative positions for *D. solani***

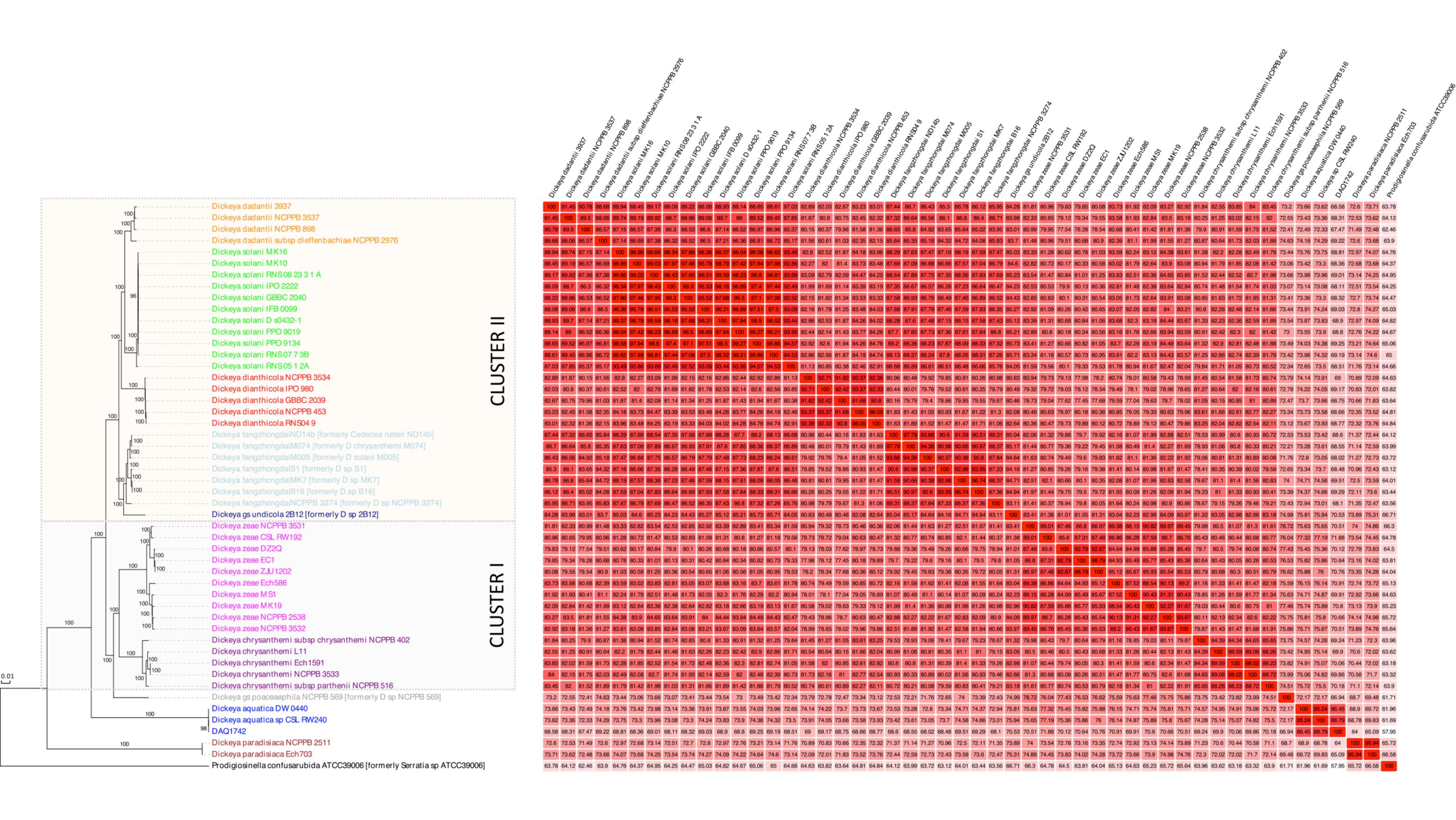
1548

1549 **Supplementary Table S10: Characterization of the mobilome of *D. aquatica* 174/2 and its**
1550 **conservation in other *Dickeya* species**

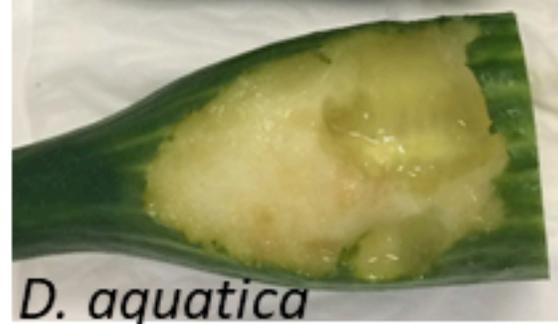
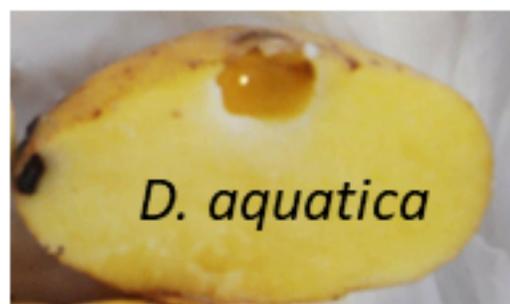
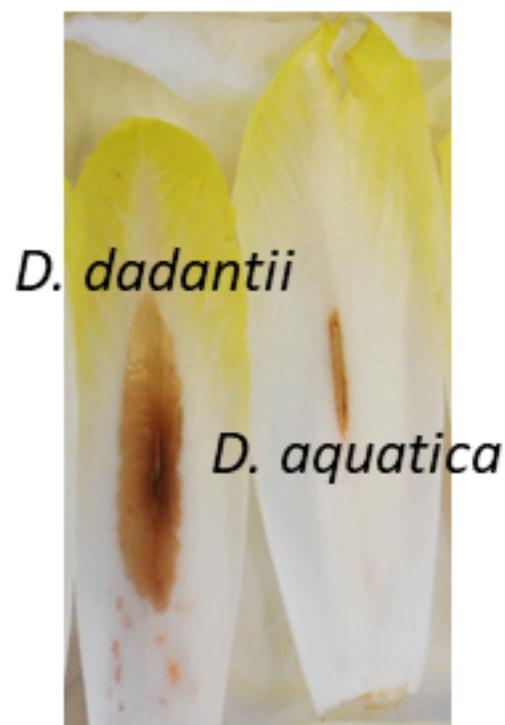
1551 Genes of *Dickeya* species were considered as present if identity of the encoded protein was higher
1552 than 60% of full-length amino acids sequence. If local alignments were too short with regard to the
1553 length of similar sequences, we performed a nucleotide BLAST on full-length DNA sequences with
1554 similar thresholds (10^{-5} e-value, 80% identity of full length sequence). This allowed us to eliminate
1555 false genes. Note that because some genomes represent drafts, false negatives may occur.

Dickeya aquatica 174/2 genome



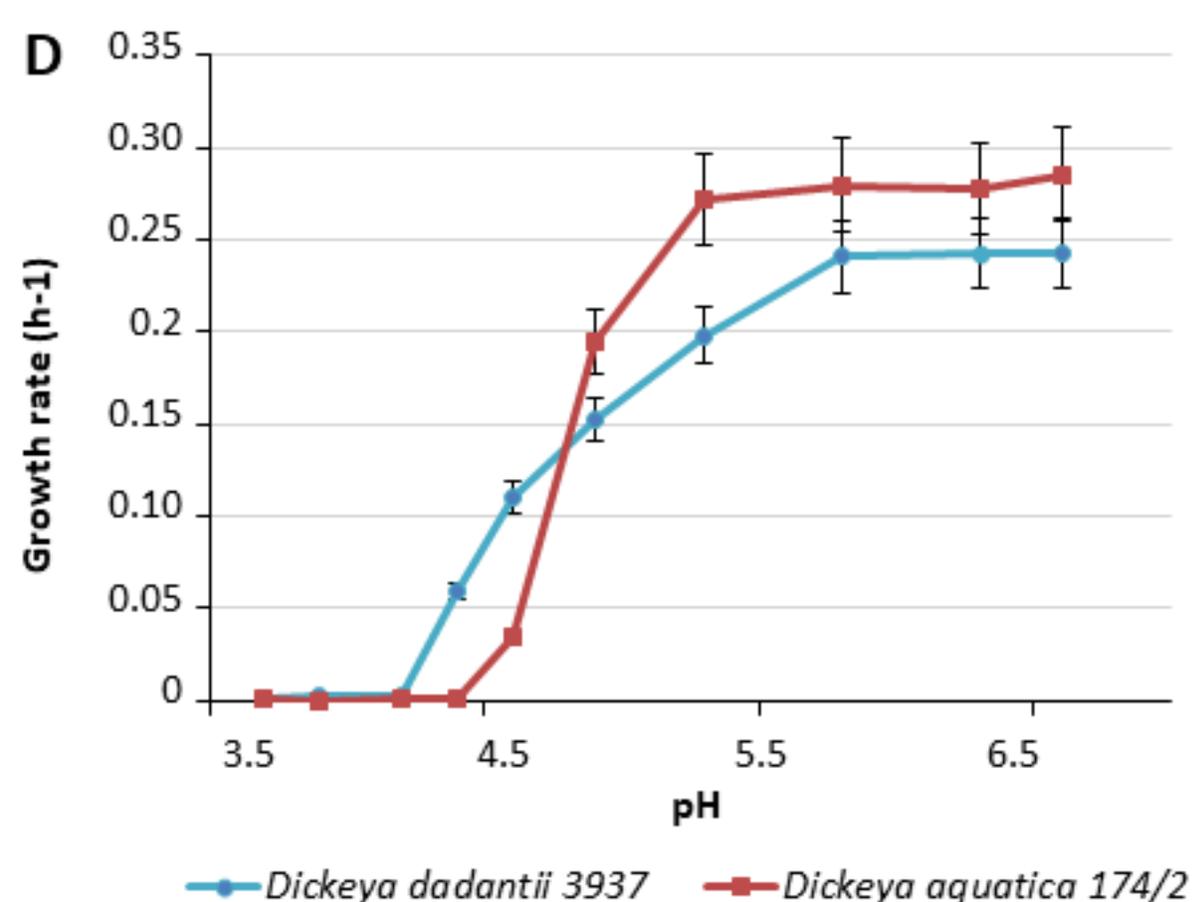
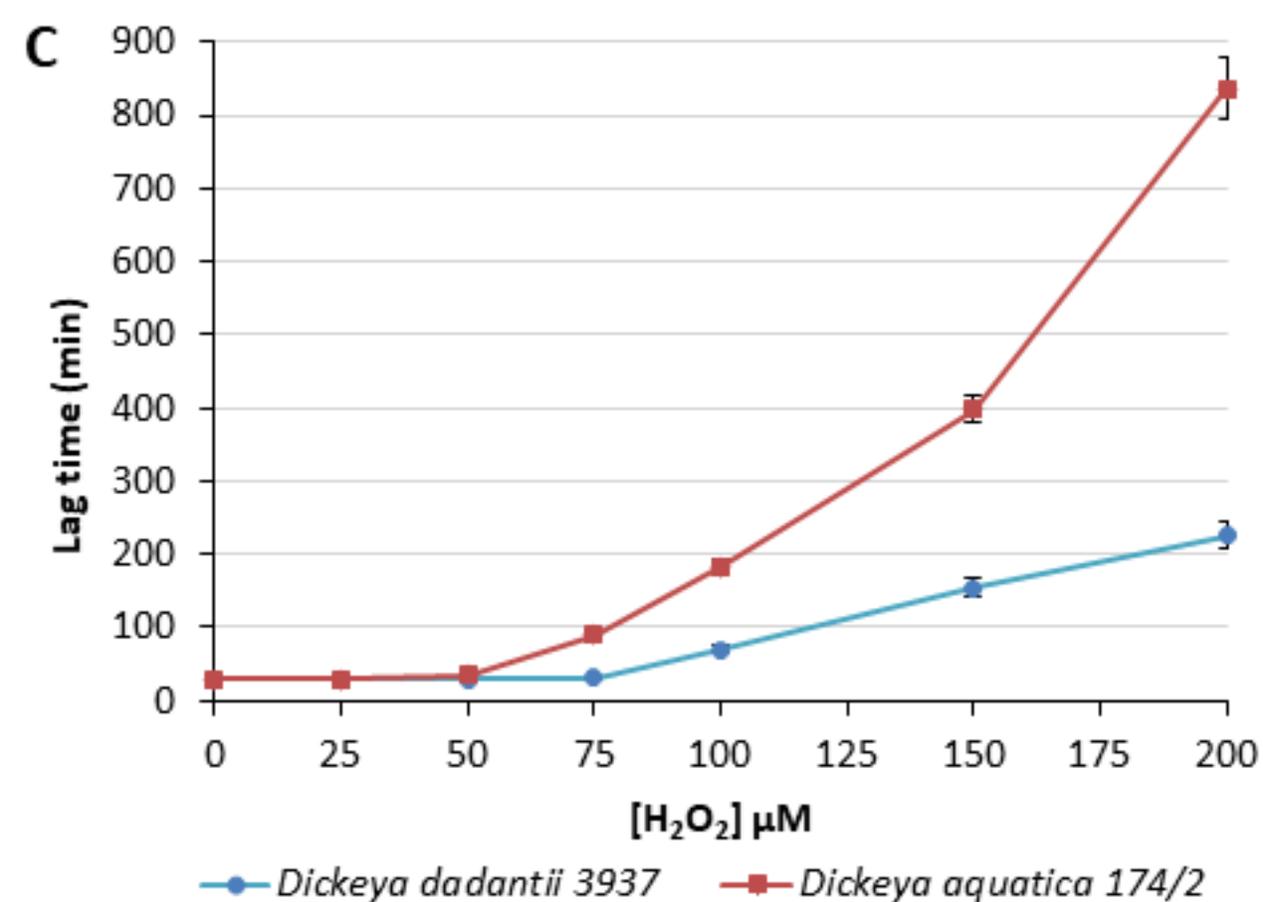
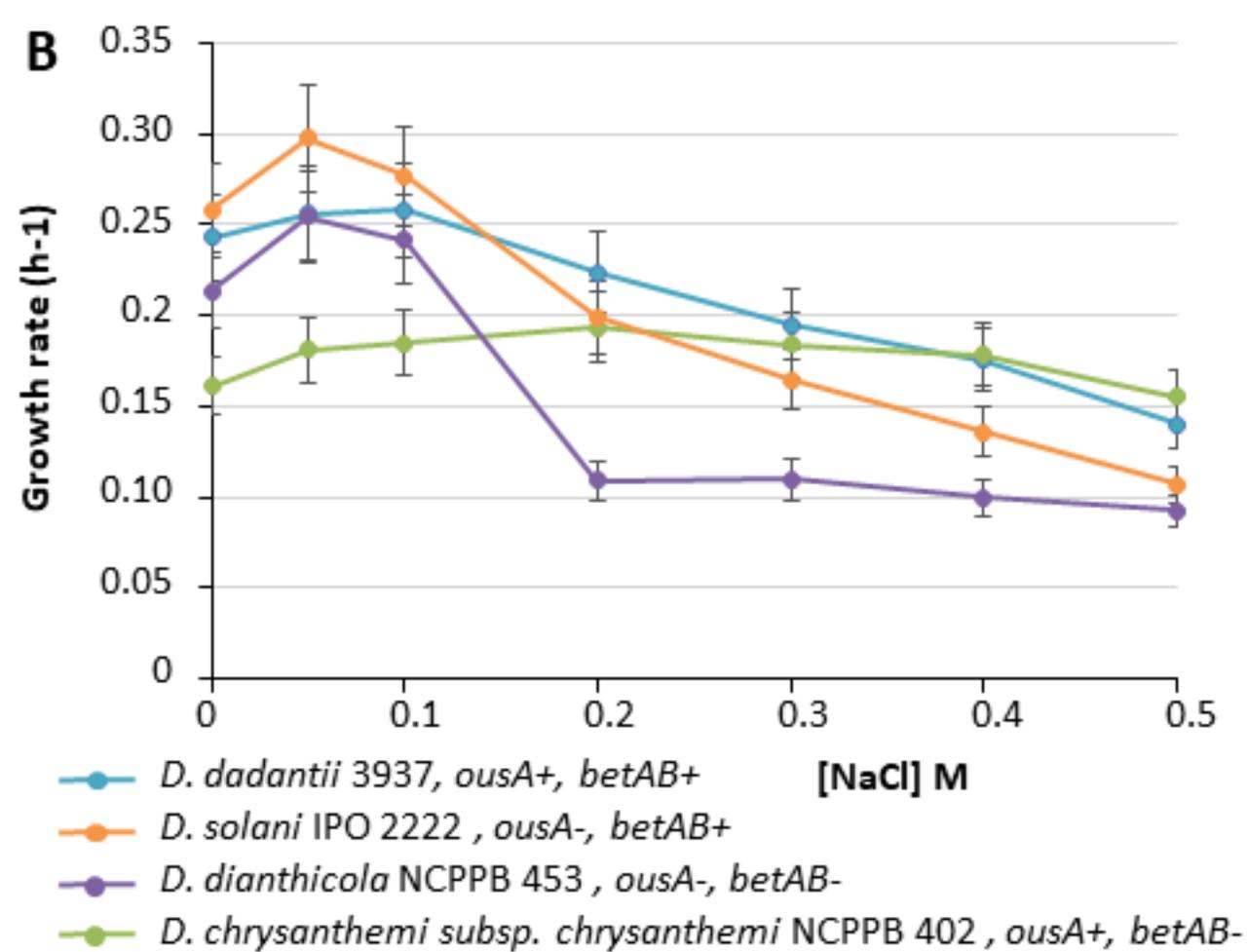
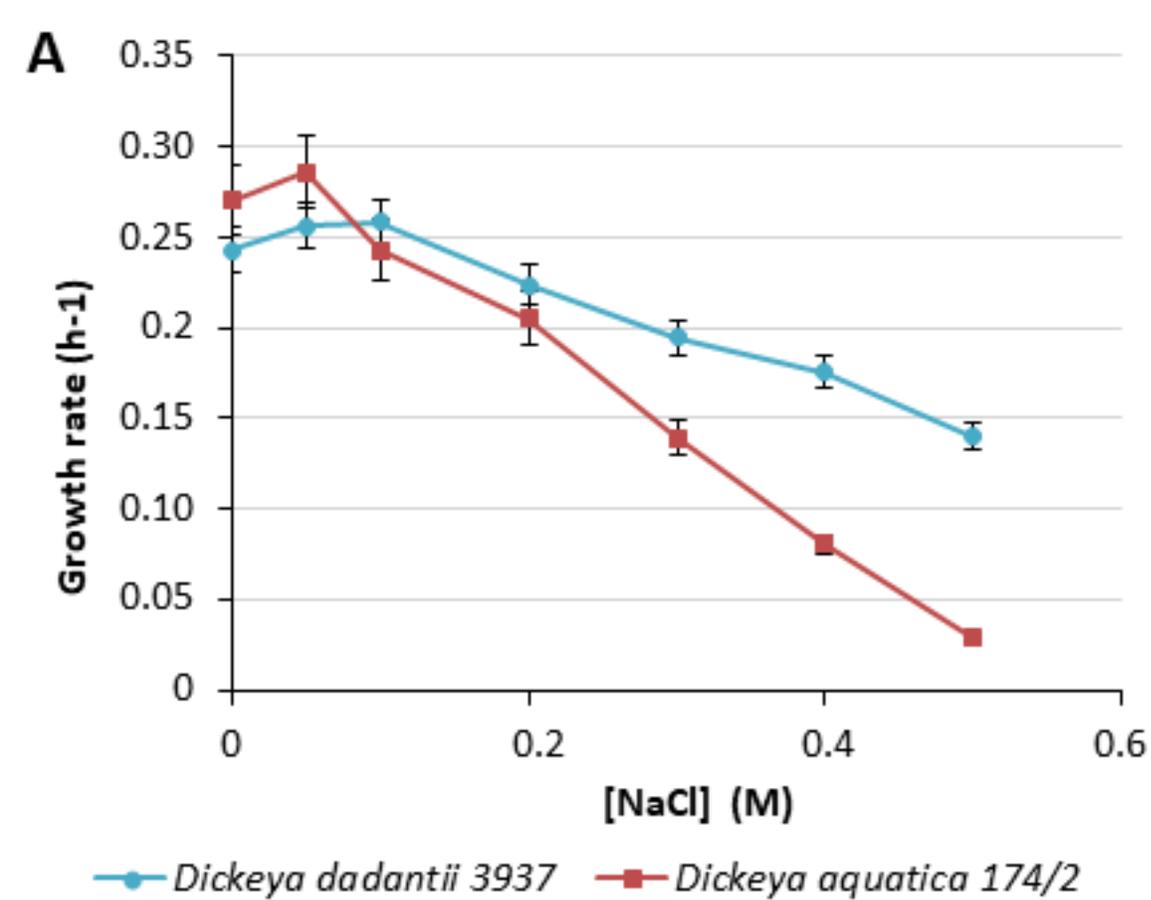


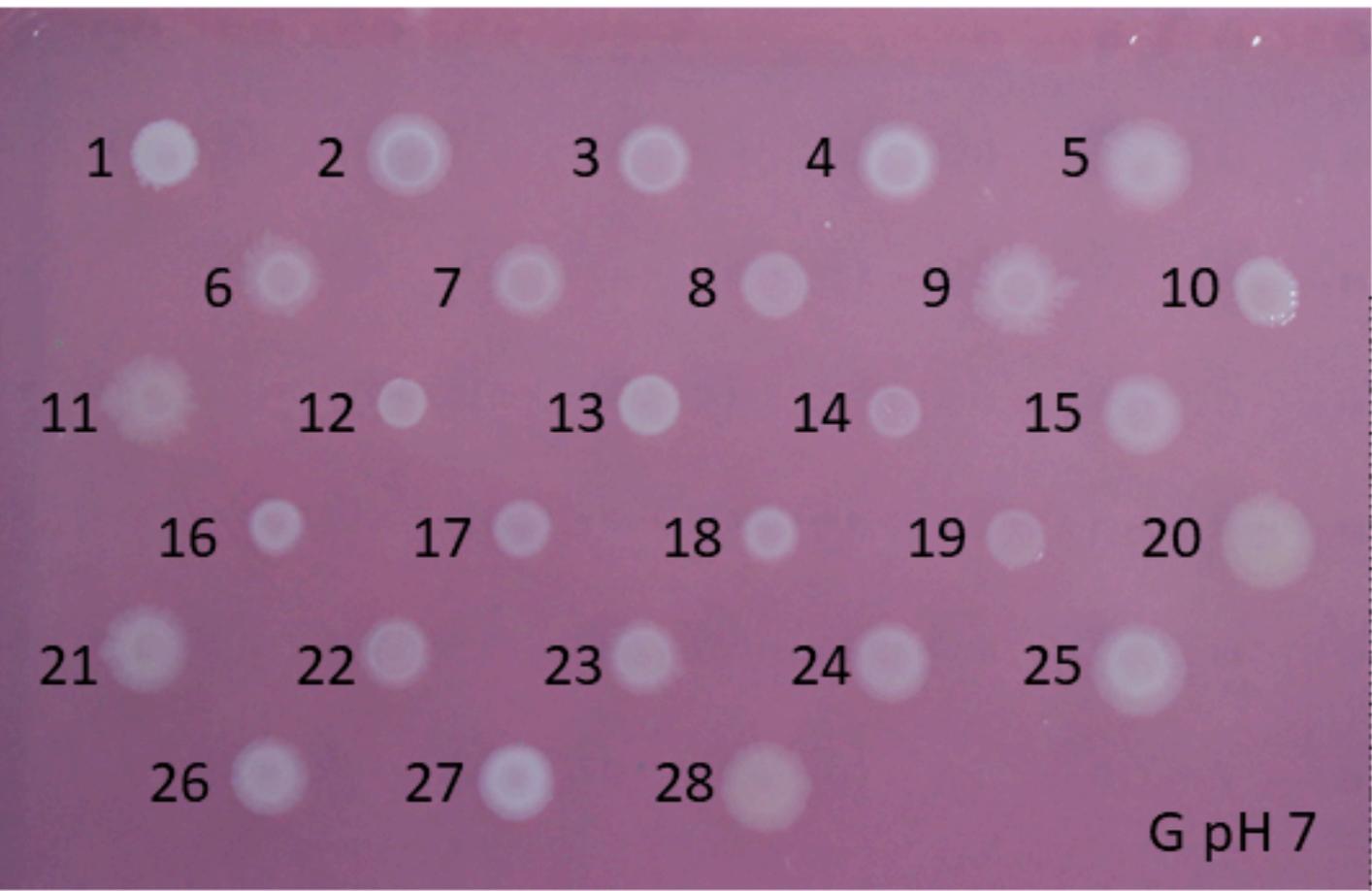
A Chicory (pH 6.5) Potato (pH 6.0) Cucumber (pH 5.1) Tomato (pH 4.8)



B

Rotten mass (g)	<i>D. dadantii</i>	<i>D. aquatica</i>
Chicory (leaf)	0.9 ± 0.5	0.02 ± 0.04
Potato (tuber)	5.7 ± 2.4	1.7 ± 0.2
Cucumber (fruit)	0.38 ± 0.49	11.6 ± 10.6
Tomato (fruit)	8.0 ± 4.6	36 ± 16
Pineapple (fruit)	0.0 ± 0.0	0.0 ± 0.0
Kiwi (fruit)	0.0 ± 0.0	0.0 ± 0.0





Colony	Strain	Twitching
1	<i>D. dadantii</i> 3937	-
2	<i>D. dadantii</i> 3937	-
3	<i>D. dadantii</i> NCPPB 3537	-
4	<i>D. dadantii</i> NCPPB 898	-
5	<i>D. aquatica</i> 174/2	+
6	<i>D. fangzhongdai</i> NCPPB 3274	+
7	<i>D. fangzhongdai</i> B16	+
8	<i>D. fangzhongdai</i> NCPPB 3274	+
9	<i>D. undicola</i> 2B12	+
10	<i>D. poaceaphila</i> NCPPB 569	-
11	<i>D. zeae</i> NCPPB 3532	+
12	<i>D. zeae</i> NCPPB 2547	-
13	<i>D. zeae</i> NCPPB 2538	-
14	<i>D. chrysanthemi</i> NCPPB 402	-
15	<i>D. chrysanthemi</i> NCPPB 3533	+
16	<i>D. dianthicola</i> RNS04.9	-
17	<i>D. dianthicola</i> CFBP 1888	-
18	<i>D. dianthicola</i> CFBP 2982	-
19	<i>D. dianthicola</i> NCPPB 453	-
20	<i>D. paradisiaca</i> NCPPB 2511	-
21	<i>D. solani</i> IPO2222	-
22	<i>D. solani</i> PP09019	-
23	<i>D. solani</i> RNS07.7.3B	+
24	<i>D. solani</i> GBBC2040	-
25	<i>D. solani</i> Ds0432-1	+
26	<i>D. solani</i> RNS05.1.2A	-
27	<i>D. dadantii</i> subsp. <i>dieffenbachiae</i> CFBP3694	-
28	<i>D. dadantii</i> subsp. <i>dieffenbachiae</i> NCPPB 2976	-

