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Diversity within the *Dickeya zeae* complex, identification of *Dickeya zeae* and *Dickeya oryzae* members, proposal of the novel species *Dickeya parazeae* sp. nov.

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1 **Diversity within the *Dickeya zea* complex, identification of *Dickeya zea* and**
2 ***Dickeya oryzae* members, proposal of the novel species *Dickeya parazeae* sp.**
3 **nov.**

4

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16

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19

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26 project has been deposited at DDBJ/ENA/GenBank under the accession
27 JAGJWU000000000. Whole Genome Shotgun project of *D. oryzae* strains S20, FVG03 and
28 FVG08 has been deposited at DDBJ/ENA/GenBank under the accessions
29 JAGJWV000000000, JAGJWX000000000, and JAGJWW000000000, respectively.

30

31 **ABSTRACT**

32 The genus *Dickeya* comprises plant pathogens that provoke diseases on a large range of
33 economically important crops and ornamentals. Strains previously assigned to the species
34 *Dickeya zeae* are major pathogens attacking vital crops such as maize or rice. They are also
35 frequently isolated from surface waters. The newly described species *Dickeya oryzae* is
36 closely related to *D. zeae* members, so that the limit between the two species can be
37 difficult to define. In order to clearly distinguish the two species, globally described by the
38 term “*D. zeae* complex”, we sequenced the genome of four new water isolates and
39 compared them to 14 genomes available in databases. Calculation of average nucleotide
40 identity (ANI) and digital DNA-DNA hybridization (dDDH) values confirmed the phylogenomic
41 classification into the two species *D. zeae* and *D. oryzae*. It also allowed us to propose a new
42 species, *Dickeya parazeae* sp. nov., to characterize a clade distinct from those containing the
43 *D. zeae* type strain NCPPB2538^T. The strain S31^T (CFBP 8716^T, LMG 32070^T) isolated from
44 water in France is proposed as the type strain of the new species. Phenotypic analysis of
45 eight publically available strains revealed traits common to the five tested *D. oryzae*
46 members but apparently not shared by the *D. oryzae* type strain. Genomic analyses
47 indicated that a simple distinction between the species *D. zeae*, *D. parazeae* and *D. oryzae*
48 can be obtained on the basis of the *recA* sequence. *D. oryzae* can be distinguished from the
49 two other species by the growth on L-tartaric acid. Based on the *recA* marker, several strains
50 previously identified as *D. zeae* were re-assigned to the species *D. parazeae* or *D. oryzae*.
51 This study also highlighted the broad host range diversity of these three species.

52

53 INTRODUCTION

54 The genus *Dickeya* comprises enterobacterial plant pathogens that provoke diseases on a
55 large range of economically important crops and ornamentals but also environmental strains
56 isolated from water [1, 2]. This genus was defined in 2005 by the reclassification of former
57 *Erwinia chrysanthemi* into six species: *Dickeya chrysanthemi*, *Dickeya dadantii*, *Dickeya*
58 *diefenbachiae*, *Dickeya dianthicola*, *Dickeya zeae* and *Dickeya paradisiaca* [3]. Subsequently,
59 *D. dieffenbachiae* was reclassified as a subspecies of *D. dadantii* [4]. More recently, six new
60 *Dickeya* species have been described: *D. solani* isolated from potato and ornamentals [5], *D.*
61 *fangzhongdai* isolated from water, pear tree and several monocots [6, 7], *D. poaceiphila*
62 isolated from sugarcane [8], and three species isolated from water *D. aquatica* [9], *D.*
63 *lacustris* [10] and *D. undicola* [11]. In the genus *Dickeya*, the intra-specific diversity greatly

64 varies depending on the species. Indeed, members of some species are highly homogenous
65 like *D. aquatica*, *D. dianthicola* and *D. undicola* that group bacteria sharing at least 99%
66 average nucleotide identity (ANI) values between them. In contrast, strains classified in the
67 species *D. fangzhongdai*, *D. dadantii* or *D. chrysanthemi* are more diverse with some isolates
68 being close to the limits of species definition now generally recognized as 95-96% for ANI
69 and 70% for digitally derived DNA-DNA hybridization (dDDH) [12, 13, 14]. This is particularly
70 true for the species *D. zeae* that comprises different isolates clearly forming two distinct
71 clades in phylogenetic analyses [14, 15, 16]. To address this observation the new species
72 *Dickeya oryzae*, which is closely related to *D. zeae*, was recently described for a rice isolate
73 and five strains previously classified as *D. zeae* were re-identified as *D. oryzae* [17]. For
74 clarity, we used the term “*D. zeae* complex” to describe all the strains previously classified as
75 *D. zeae*.

76 Members of the *D. zeae* complex are major pathogens responsible for the maize stalk rot
77 and rice foot rot diseases [18]. They have the ability to infect monocot but also dicot plants
78 like potato [19, 20, 2] and they are commonly found in waterways [21, 22]. Fourteen
79 genomes of strains belonging to the *D. zeae* complex were present in public databases in
80 June 2020. However most of these strains are not publically available for phenotypic
81 analyses. To be able to perform phenotypic comparisons, we increased the panel of strains
82 clustering with *D. zeae* in preliminary phylogenetic tests, taking advantage of recent surveys
83 on the diversity of *Dickeya* isolates in surface waters. To potentially maximize strain
84 diversity, we chose to sequence new genomes including four strains isolated in two types of
85 aquatic environments in the South East of France: running water from the river Durance
86 catchment and calm water from small lakes of the French region of La Dombes.

87

88 **ISOLATION OF NEW STRAINS AND GENOME SEQUENCING**

89 Strain FVG03 was isolated in October 2017 from an irrigation channel (Montfavet), which is
90 one of the six branches of the Crillon Canal connected to the river Durance. FVG08 was
91 isolated in August 2017 from another channel, Canal de l'île (Les Taillades), also connected
92 to the river Durance. For their isolation, 500 ml of water were filtered using 0.22 µm filters.
93 The bacteria retained on the filters were suspended in water and serially diluted onto crystal
94 violet pectate (CVP) medium, a semi-selective medium containing pectin that is widely used
95 for the isolation of pectinolytic bacteria of the genera *Pectobacterium* and *Dickeya* [23].

96 Colonies forming pits on CVP plates were grown overnight in liquid medium (LB without
97 NaCl) and qPCR amplifications were performed out of bacterial cell lysates with primers
98 *pelD*1118d-F (VRC BTA CAA ACC SAC TCT G) and *pelD*1200d1-R (TGC GTT GYT RTT GAT GCT
99 G), derived from the sequence of the gene *pelD* that is specific of the genus *Dickeya*. The
100 *Dickeya* candidates were further purified on CVP plates and then on LB⁻ plates (LB medium
101 without added NaCl).

102 The two strains S20 and S31 were isolated in September 2017 from lake Boufflers (28 ha)
103 situated in a conservation site that is protected from direct agricultural inputs (Foundation
104 Pierre Vérots, Saint-Jean de Thurigneux). For their isolation, a pectate enrichment broth [24]
105 was inoculated with 0.1 ml of a 30-fold concentrated sample to favour growth of bacteria
106 able to use pectin as the sole carbon source. After incubating for 48 h at 30°C, pectinolytic
107 bacteria were isolated by plating serial dilutions onto CVP plates. Colonies forming pits on
108 CVP were further purified twice by streaking isolation on LB plates. PCR amplifications using
109 the primers *pelADE* [25] were performed on isolated bacterial colonies to identify strains of
110 the genus *Dickeya*.

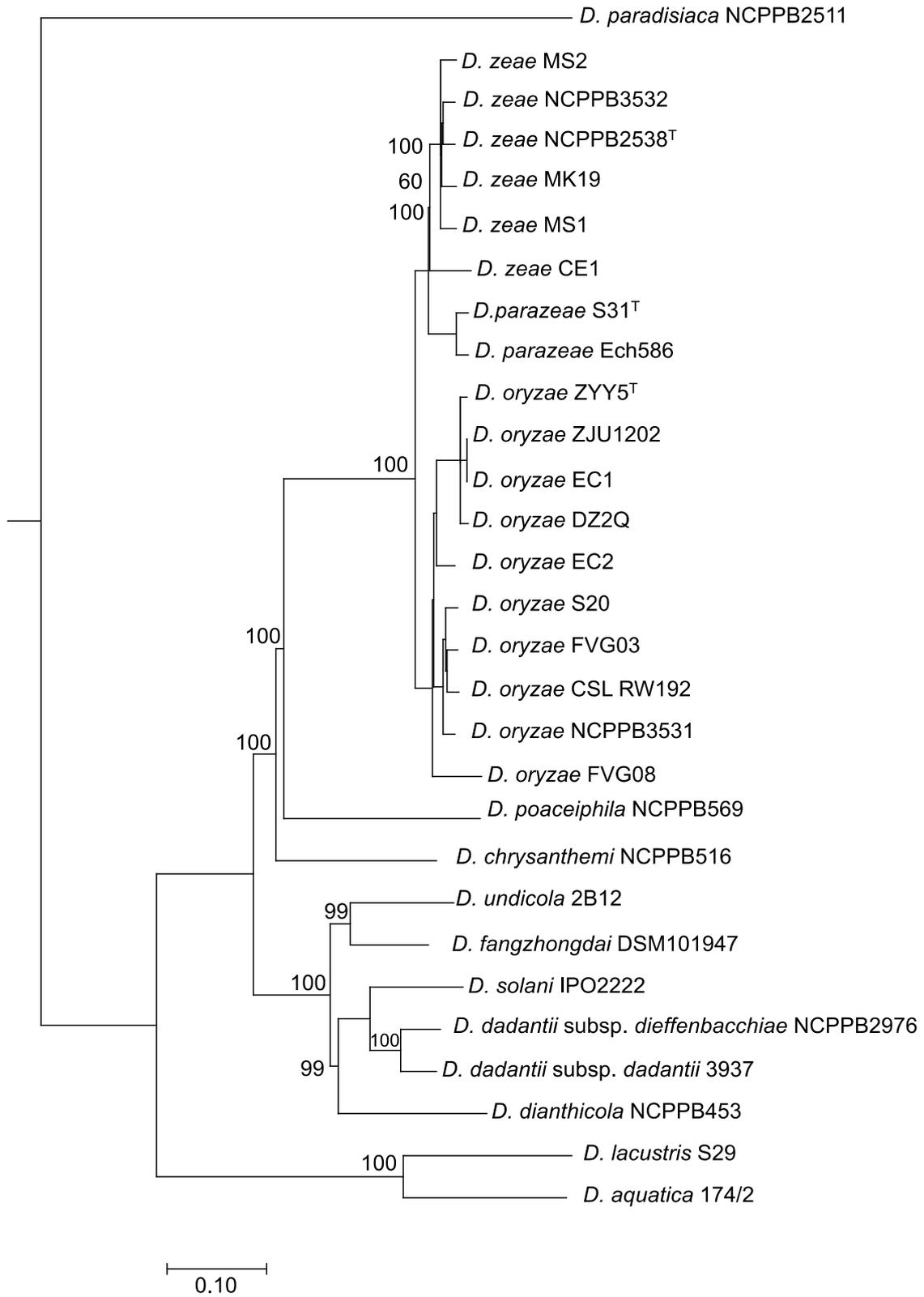
111 The accurate *Dickeya* species of isolates from both surveys was then determined by PCR
112 amplification of the housekeeping gene *gapA* using the *gapA*-7-F and *gapA*-938-R primer set
113 and sequencing of the *gapA* amplicon [26]. Two strains from each survey clustering with
114 strains of the *D. zeae* complex were chosen for whole genome sequencing. The total DNA of
115 strains FVG03 and FVG08 was extracted using the Wizard genomic DNA purification kit
116 (Promega) and that of strains S20 and S31 was extracted using the NucleoSpinR bacterial
117 DNA purification kit (Macherey-Nagel). Genome sequencing was performed by Illumina
118 technology either at the next generation sequencing core facilities of the Institute for
119 Integrative Biology of the Cell (I2BC Gif-sur-Yvette France) or at the Illumina platform
120 Biofidal (Vaux-en-Velin, France). The reads were assembled using CLC Genomics Workbench
121 (version 9.5.2, Qiagen Bioinformatics). CDS prediction and automatic annotation were
122 performed using the RAST server [27] with the Glimmer 3 prediction tool [28]. The genomic
123 characteristics of the four strains are summarized in Table S1.

124

125 **GENOME COMPARISON**

126 The four new genomes were compared to the 14 genomes of the *D. zeae* complex available
127 in databases, including the *D. zeae* type strain NCPPB2538 isolated from maize in USA, the *D.*

128 *oryzae* type strain ZYY5^T isolated from rice roots in China, four strains isolated from rice
129 either in Italy (DZ2Q) or in China (EC1, EC2, ZJU1202), two strains isolated from banana in
130 China (MS1, MS2), two strains isolated from potato in Australia (NCPBP 3531, NCPBP 3532),
131 a strain isolated in USA from Philodendron (Ech586), a strain isolated from Canna lily in
132 China (CE1), and two strains isolated from rivers in UK (CSL_RW192, MK19) (Table 1).
133 A phylogenomic tree was constructed from concatenated sequences of 2079 homologous
134 core proteins retrieved from the 18 analysed genomes and genomes of type strains of the
135 other *Dickeya* species (Fig. 1). This tree confirmed that the 18 analysed genomes clearly split
136 in two major clades: five genomes (NCPBP 2538, NCPBP 3532, MK19, MS1, MS2, and CE1)
137 cluster with *D. zeae* NCPBP2538^T while ten genomes (NCPBP 3531, DZ2Q, CSL_RW192, ZJU
138 1202, EC1, EC2, FVG03, FVG08 and S20) are separated into a second clade including the *D.*
139 *oryzae* type strain ZYY5^T. The present work including new genomic data clearly confirms the
140 separation of the *D. zeae* complex in two species *D. zeae* and *D. oryzae*. Furthermore, the
141 two strains Ech586 and S31 form a separate branch close to the *D. zeae* clade, supported by
142 a 100% bootstrap value (Fig. 1).



143

144

145 To further analyze the strain diversity in the *D. zeae* complex, pairwise comparison of the 18

146 available genomes was used to calculate ANI (Average Nucleotide Identity) and pairwise
147 dDDH (digital DNA–DNA hybridization) values (Table 2, Fig. S1). Considering a threshold for
148 belonging to the same species of 96% for ANI values, the species *D. oryzae* clearly form a
149 separate clade with ANI values of 94.1-95.1% with other strains (Table 2, Fig. S1). The ten
150 members of the species *D. oryzae* showed ANI values of 96.0-99.2% with the *D. oryzae* type
151 strain. However, these genome comparisons showed that a few strains are somewhat
152 divergent from the core strains (Table 2, Fig. S1). The strains can be separated in different
153 branches also highlighted in the genomic tree (Fig. 1). Among the species *D.oryzae*, one
154 branch comprises the five strains isolated from rice either in China or in Italy. Four of them,
155 ZYY5^T, DZ2Q, ZJU 1202 and EC1, appear to be very closely related as they share ANI and
156 dDDH values of 99.1-100% and 92.2-99.8%, respectively (Fig. S1). The rice strain EC2 is more
157 distant, showing ANI and dDDH values of 97.2-97.3% and 76.0-76.2%, respectively, with the
158 other strains isolated from rice. Another *D. oryzae* branch regroups strains isolated from
159 water and potato that share ANI and dDDH values of 98.2-98.7 and 84.5-88.6%, respectively,
160 between them. The strain FVG08 isolated from water is at the limit of belonging to the
161 species *D. oryzae* with ANI values of 96.0-96.6% with other *D. oryzae* strains but dDDH
162 values below 70% with most *D. oryzae* strains (67.1-71.2%).

163 Among the eight other strains of the *D. zea* complex, the two strains Ech586 and S31 show
164 ANI values of 96-96.1% with the *D. zea* type strain and 95.7-96% with the other *D. zea*
165 strains (Table 2, Fig. S1). These two strains are closely related as they share ANI and dDDH
166 values of 98.5 and 88.1%, respectively. These strains are at the limit of the 96% threshold
167 with the type strain recommended for species delineation but they are below the
168 recommended cut-off for species delineation (70%) with dDDH values of 65.6-67.3% with
169 other *D. zea* members including the type strain. These values support the proposal of a new
170 species within the *D. zea* complex. This new classification of strains Ech586 and S31 is
171 clearly confirmed by the phylogenetic tree (Fig. 1) and the analysis of virulence gene content
172 (see below, Table 3). We thus propose the description of a novel species, namely *D.*
173 *parazeae* sp. nov. with strain S31^T (CFBP 8716^T, LMG 32070^T) as the type strain.

174 Strains NCPPB 2538^T, MK19, NCPPB 3532, MS1 and MS2 remain classified as *D. zea*
175 members. These five strains are homogeneous as they share ANI and dDDH values of 98.0-
176 98.4% and 81.7-86%, respectively. Strain CE1 shows ANI values of 96.3% with the *D. zea*
177 type strain NCPPB2538^T and 96.1-96.2% with other *D. zea* strains. Since this strain is not

178 available in any public collection, we cannot analyze its phenotypic features. In absence of
179 additional data, we propose to leave it in the species *D. zea*.

180

181 **DIVERSITY IN GENE CONTENT**

182 Comparative genomic analyses were conducted to identify distinctive genes between the
183 species *D. zea*, *D. parazea* and *D. oryzae*. We performed genome-to-genome comparisons
184 by bi-directional protein–protein BLAST sequence comparison of translated open reading
185 frames (ORFs) with a 10^{-5} e-value threshold. Orthologous sequences were then clustered
186 into homologous families using the SiLix software package [29]. Proteins were classified as
187 homologous to another in a given family if the amino acid identity was above 70% with at
188 least 80% overlap. The core genome of *D. oryzae* encodes 2909 protein families,. Only nine
189 protein families are present in all *D. oryzae* members and absent in *D. zea* or *D. parazea*
190 strains (Table S2). One of them is predicted to be involved in D-allose metabolism as it
191 encodes a step of the D-allose pathway described in *Escherichia coli* [30]. Twenty seven gene
192 families are absent in all *D. oryzae* strains and present in all other members of the *D. zea*
193 complex (Table S2). Notably, among them is a cluster of five genes that encodes a
194 transcriptional regulator, a transporter, and three enzymes annotated as involved in the
195 metabolism of L-tartarate. This metabolic pathway was described in different bacteria, such
196 as *Salmonella typhimurium* and *E. coli* [31]. The pathway encoded by *D. zea* and *D.*
197 *parazea* strains may have a similar function but it showed differences at the protein level.
198 The genes encoding the two subunits of tartrate dehydratase are homologous to the *E. coli*
199 genes *ttdA* and *ttdB* with 60% and 69% identity, respectively. However the regulator, the
200 transporter and the second enzyme of the pathway (oxaloacetate decarboxylase) may have
201 activities equivalent to the *E. coli* products but they belong to different protein families (Fig.
202 S2).

203 The core genome of *D. zea* encodes 3426 protein families. Twenty five protein families are
204 present in all *D. zea* members and absent in other strains of the *D. zea* complex (Table S2).
205 These gene families include the gene *rhiE* encoding a rhamnogalacturonase, *pehN* encoding
206 an exo-polygalacturonase and two genes encoding putative transcriptional regulators. The
207 two *D. parazea* genomes share 4029 protein families of which 208 are not present in other
208 members of the *D. zea* cluster (Table S2). These numbers could be however over-estimated
209 due to the low amount of genomes analysed for this species.

210 We also compared the repertoire of virulence genes among strains of the *D. zea* complex.
211 As reported for other *Dickeya* species, the maceration symptom is mainly linked to a set of
212 pectate lyase genes present in all three species (*pelADE*, *pelBCZ*, *pell*, *pell*, *pelN*, *pelX*, and
213 *pelW*), along with genes encoding accessory pectinases (*pemA/B*, *pnIG/H*, *rhiE/F*, *paeY*, *faeD*,
214 *pehK*, and *pehN*) and other plant cell wall degrading enzymes (*celZ*, *xynA*, *plcA*, and
215 *prtABCG*). However, some differences in the pectinase repertoire were observed between
216 isolates (Table 3). As stated before, the genes encoding the rhamnogalacturonate lyase RhiE
217 and the exo-polygalacturonase PehN are present in *D. zea* members but neither in *D.*
218 *parazeae* nor in *D. oryzae*. It could be noticed that all members of the *D. zea* complex
219 possess another gene encoding a potential rhamnogalacturonate lyase RhiF that is 50%
220 identical to RhiE. The pectin lyase gene *pnIG* is present in *D. zea* but absent in *D. parazeae*
221 and strain EC1. In *D. oryzae*, *pnIG* is present only in the five strains rice strains and in a water
222 isolate. Since the gene *pnIH* is absent in all *D. zea* and *D. oryzae* members, several strains of
223 these two species do not encode any pectin lyase. The two adjacent genes *pell* and *celZ* are
224 present in all genomes except in *D. zea* MS1. The genome of all strains contains T1SS, T2SS,
225 T3SS and T6SS gene clusters. At least one type 4 protein secretion system (T4SS) is encoded
226 by all strains isolated from water but it is diversely distributed in the other strains (Table 3).
227 Some differences in their virulence equipment disclose particularities of rice strains in
228 comparison to other *D. oryzae* members, with some exceptions for strain EC2. The toxin
229 zeamine was shown to be involved in rice strain virulence [32]. The gene cluster involved in
230 the biosynthesis of zeamine is not largely distributed in the *D. zea* complex as it is absent in
231 all *D. zea* and *D. parazeae* strains and restricted to four *D. oryzae* rice strains, including the
232 type strain ZYY5^T. However, this cluster is absent in strain EC2 isolated from diseased rice,
233 suggesting that the toxin zeamine is not always essential for causing rice disease. Most *D.*
234 *oryzae* rice strains do not possess the avirulence related gene *avrL* that is present in all other
235 strains of the *D. zea* complex including the rice strain EC2 (Table 3). Three rice strains
236 possess the genes *cyt* encoding entomotoxins, a property shared only by another *D. oryzae*
237 strain, NCPPB3531. While most *D. oryzae* rice strains possess *zms* and *cyt* but not *avrL*, the
238 *D. oryzae* water strains and the *D. zea* or *D. parazeae* members possess the gene *avrL* but
239 not the cluster *zms* and rarely the genes *cyt*.

240

241 PHENOTYPIC ANALYSIS

242 We performed a phenotypic characterization of the nine available strains (Table 1), including
243 two *D. zea*, two *D. parazeae* and five *D. oryzae* strains. We first tested properties directly
244 linked with the bacterial virulence, such as the secretion of plant cell wall degrading
245 enzymes, motility and maceration capacity. When production of extracellular enzymes was
246 tested on specific media [33], the nine strains showed a high secretion of pectinase,
247 protease and cellulase activities (Table 4). They showed swimming and swarming motilities
248 [34], except the *D. zea* type strain NCPPB 2538^T that was not able to swim (Table 4). The
249 nine strains had the capacity to macerate potato tubers and chicory leaves although with
250 variable efficiencies (Table 4). Thus, no significant difference between the three species was
251 observed for these characteristics.

252 An exhaustive biochemical characterization was performed using Biolog plates PM1 and
253 PM2A for two strains of *D. oryzae*, *D. zea*, and *D. parazeae* (Table S3). Out of 190 carbon
254 sources, 45 appeared to be metabolized by the six strains and 17 compounds gave variable
255 results depending on the strains. Most of the differences were observed for compounds
256 giving weak responses, without any correlation with the species appurtenance. Only one
257 difference could be clearly correlated with species, namely assimilation of L-tartaric acid.
258 Since furthermore, genomic data revealed the conservation of a cluster of genes potentially
259 involved in L-tartarate utilization in all *D. zea* and *D. parazeae* sequenced strains (Table S2),
260 we tested the growth of the nine available strains on minimal medium M63 containing L-
261 tartaric acid as the sole carbon source. The *D. zea* and *D. parazeae* strains were able to
262 grow in this medium while the *D. oryzae* strains could not (Table 5).

263 Tartaric acid exists in three forms: L-tartaric, D-tartaric and m-tartaric acid. L-tartaric acid is
264 the natural form that is abundant in many fruits. Further analysis shows that *D.*
265 *chrysanthemi*, *D. dadantii*, *D. dianthicola*, *D. solani* and *D. paradisiaca* are not able to use L-
266 tartaric acid as the sole carbon source. In contrast, *D. aquatica*, *D. fangzhongdai*, *D. lacustris*
267 and *D. undicola* are able to grow with L-tartarate [7 and data not shown]. These four species
268 contain the same cluster *ttd* as *D. zea* and *D. parazeae* (Fig. S2) confirming the correlation
269 between phenotypic and genomic data, i. e., the presence of the cluster *ttd* in the genomes
270 of *Dickeya* strains able to grow with L-tartarate as the sole carbon source.

271 The growth of the nine strains was also tested on minimal medium containing diverse
272 compounds as the sole carbon source (Table 5). All strains gave similar results for D-
273 arabinose, m-inositol, melibiose, D-xylose, malate and L-rhamnose. The ability to grow with

274 D- glucosaminic acid, gluconic acid, glucuronic acid, glutamic acid and glutamine was variably
275 distributed among strains of the *D. zea* complex. In a previous analysis [17], the *D. oryzae*
276 type strain ZYY5^T was found to be unable to assimilate mannitol, gluconic acid and N-acetyl
277 glucosamine, this is not the case for the five *D. oryzae* strains tested in this study (Table 5
278 and Table S3). This discrepancy could be due to methodologic differences or it could indicate
279 that the type strain has an atypical phenotype in comparison with other *D. oryzae* strains.
280 Indeed, contrarily to other *D. oryzae*, the cluster *mt/ADR* involved in mannitol catabolism is
281 absent in the genome of strain ZYY5^T. In contrast, the genes *nagABE*, *nagK* and *nagZ*
282 encoding N-acetyl glucosamine catabolism, as well as *gntR*, *gntK* and *gntT* encoding gluconic
283 acid catabolism, are present in the genome of strain ZYY5^T.

284 As noticed before (Table S2), the gene *asl*, present in all *D. oryzae* members, is predicted to
285 be involved in D-allose metabolism. However, none of the six strains tested in Biolog plates
286 was able to assimilate this sugar. D-allose is a rare aldohexose found in tissues of some
287 plants; it is the C3 epimer of glucose. Further analysis of the *asl* gene cluster suggests that it
288 is incomplete for D-allose catabolism since, in comparison to *E. coli*, it lacks the gene *alsI*
289 encoding an isomerase essential for D-allose assimilation (Fig. S3). The absence of this gene
290 in the *Dickeya* genomes explains the incapacity of these bacteria to utilize D-allose. While it
291 is incomplete for allose catabolism, the *D. oryzae* cluster could be sufficient for the
292 assimilation of a related sugar, D-psicose, by a not yet characterized pathway that may join
293 the allose pathway (Fig. S3). D-psicose (or D-allulose) is a rare ketose, sparsely present in
294 plants; it is the C3 epimer of D-fructose. Both D-allose and D-psicose are able to induce some
295 plant defence genes and to confer resistance to plant diseases [34]. Interestingly, the gene
296 cluster including *asl* is also found in the genome of *D. fangzhongdai* and *D. poaceiphila*, the
297 two *Dickeya* species able to utilize D-psicose [7, 8]. Only one *D. oryzae* strain was able to
298 weakly assimilate D-psicose when tested in Biolog plates, suggesting that this pathway is not
299 very efficient or expressed at a low level in *D. oryzae*. Thus, this phenotypic analysis indicates
300 that the growth on L-tartaric acid is the best phenotypic marker found to discriminate
301 between the species *D. oryzae* and the two species *D. zea* and *D. parazeae*.

302

303 IDENTIFICATION OF MEMBERS OF THE SPECIES *D. ZEA*, *D. PARAZEA* AND *D. ORYZAE*

304 Examination of the phylogenetic trees obtained for individual genes showed that *recA* is a
305 good marker to differentiate the three species, *D. zea*, *D. parazeae* and *D. oryzae* (Fig S4).

306 The *recA* sequence was already shown to be a good reflect of the *Dickeya* classification [15].
307 In comparison, the gene *gapA* often used for strain classification in the genus *Dickeya* [26]
308 appears to be less efficient to differentiate the three species of the *D. zeae* complex (Fig S4).
309 To identify new members of these species, we took advantage of previous phylogenetic
310 analyses performed on large sets of strains using the *recA* sequence [15, 16]. We extracted
311 from databases the *recA* sequences of 68 strains belonging to the *D. zeae* complex and
312 compared them with the *recA* sequences of the six *D. zeae*, two *D. parazeae* and ten *D.*
313 *oryzae* genomes (Fig. S5). The resulting tree clearly shows the division of these strains in the
314 three clades corresponding to *D. zeae*, *D. parazeae* and *D. oryzae*. This clear differentiation
315 allows the classification of the 68 strains in the three species. Remarkably, since the *recA*
316 sequence of 75% of these strains clustered with the *D. oryzae* type strain (Fig. S5), they have
317 to be reclassified in the novel species, *D. oryzae*. In addition, the *recA* phylogeny allows
318 identification of five new members of the species *D. parazeae* (Fig. S5).

319 The origin of the 18 strains analysed in this study (Table 1) shows that the hosts of strains of
320 the *D. zeae* complex are predominantly monocots but that they may also infect dicots like
321 potato. This is confirmed on a larger set of 115 strains (Fig. 2). Among 31 strains isolated
322 from maize, 18 belong to the species *D. oryzae*, nine to *D. zeae* and four to *D. parazeae*.
323 Water strains also mostly belong to the species *D. oryzae* (9) and only few to the species *D.*
324 *zeae* (1) or *D. parazeae* (1). All strains isolated from rice in different countries (China, Japan,
325 or Italy) belong to the species *D. oryzae* (Fig. 2). In the *recA* tree, rice strains, except EC2,
326 form a very homogeneous leaf also including a millet strain (Fig. S5).

327 Surprisingly, most of the 51 *D. oryzae* isolates also come from maize (35%, 18 strains out of
328 51); they are commonly found in water (17%, 9 strains), rice (14%, 7 strains) and potato
329 (14%, 7 strains), and less frequently found in pineapple (3), banana (2) and some other crops
330 or ornamental plants (Fig. 2). Most of the 25 *D. zeae* isolates originate from maize (36%, 9
331 strains out of 25) and they are also found in banana (5) potato (3), water (1) and diverse
332 ornamental plants (Fig. 2). Similarly, the seven *D. parazeae* strains have different origins,
333 mostly maize (4/7), but also *Philodendron*, *Aechmea* and water (Fig. S5).

334

335 IDENTIFICATION OF SPECIES IN THE D. ZEAE COMPLEX

336 In conclusion, phylogenetic analyses show that the genome sequences of 18 members of the
337 *D. zeae* complex form three distinct clades corresponding to the species *D. zeae*, *D. oryzae*

338 and the new species *D. parazeae*. Genetic and phenotypic analyses indicated that a simple
339 distinction between the three species can be obtained on the basis of the *recA* sequence.
340 The absence of growth on L-tartaric acid can differentiate *D. oryzae* from the two other
341 species.

342

343

344 **DESCRIPTION OF *DICKEYA PARAZEAE* SP. NOV.**

345 *Dickeya parazeae* (pa.ra.ze'ae, L. pref. *para-* next to; N.L. gen. n. *zeae* of the plant genus *Zea*;
346 N.L. gen. n. *parazeae* close to (*Dickeya*) *zeae*].

347 *Dickeya parazeae* is a motile facultatively anaerobic pectinolytic bacterium that grows on LB
348 medium (5 g.l⁻¹ tryptone, 3 g.l⁻¹ yeast extract, 5 g.l⁻¹ NaCl and 15 g.l⁻¹ agar). After 48 h at
349 30°C, bacteria form colonies of 1-2 mm in diameter with whitish translucent appearance,
350 shiny surface and regular margin. On crystal violet pectate medium, individual colonies
351 produce large and deep pits. They also show cellulolytic and proteolytic activities. Cells are
352 gram-negative and rod shaped approx. 0.5 mm in width and 2 mm in length. The *parazeae*
353 type strain is able to utilize D-arabinose, L-arabinose, N-acetyl-D-glucosamine, D-fructose, D-
354 fructose-6-phosphate, D-galactose, D-galactaric acid, D-galacturonic acid, D-glucosamine, D-
355 glucose, D-glucose-1-phosphate, D-glucose-6-phosphate, D-glucaric acid, D-mannose, D-
356 mannitol, D-melibiose, D-raffinose, D-ribose, sucrose, D-xylose, pectin, glycerol, *m*-inositol,
357 arbutin, salicin, acetic acid, citric acid, formic acid, fumaric acid, L-malic acid, L-lactic acid,
358 pyruvic acid, succinic acid and L-tartaric acid. This species include strain Ech586 and S31^T
359 identified on the basis of their genome sequence, and five strains identified on the basis of
360 their *recA* sequence, NCPPB 2540, NCPPB 3731 (CFBP 1536), MAFF311098, PD1619 and
361 SUPP27. Characterized members of this species were isolated mostly from *Zea mays*, but
362 also from *Philodendron*, *Aechmea*, and surface water.

363 The type strain of *Dickeya parazeae* is S31^T (=CFBP8716^T, LMG 32070^T); its G+C content is
364 56.4mol% based on the draft genome sequence.

365 Genome EMBL/GenBank accession: JAGJWU000000000

366 EMBL/GenBank accession (16S rRNA gene): MW947273

367

368

369

370 **AUTHORS' STATEMENTS**

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376

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385

386 **Conflicts of interest**

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

388

389 **ABBREVIATIONS**

390 ANI, average nucleotide identity; CDS, coding DNA sequence; CFBP, Collection Française de
391 Bactéries Phytopathogènes; CVP, crystal violet pectate; dDDH, digital DNA-DNA
392 hybridization; MLSA, multilocus sequence analysis; LMG, Laboratory of Microbiology Ghent
393 University; MCP, methyl-accepting chemotaxis protein; ORF, open reading frame; RAST,
394 rapid annotations using subsystems technology; TxSS: Type x protein Secretion System.

395

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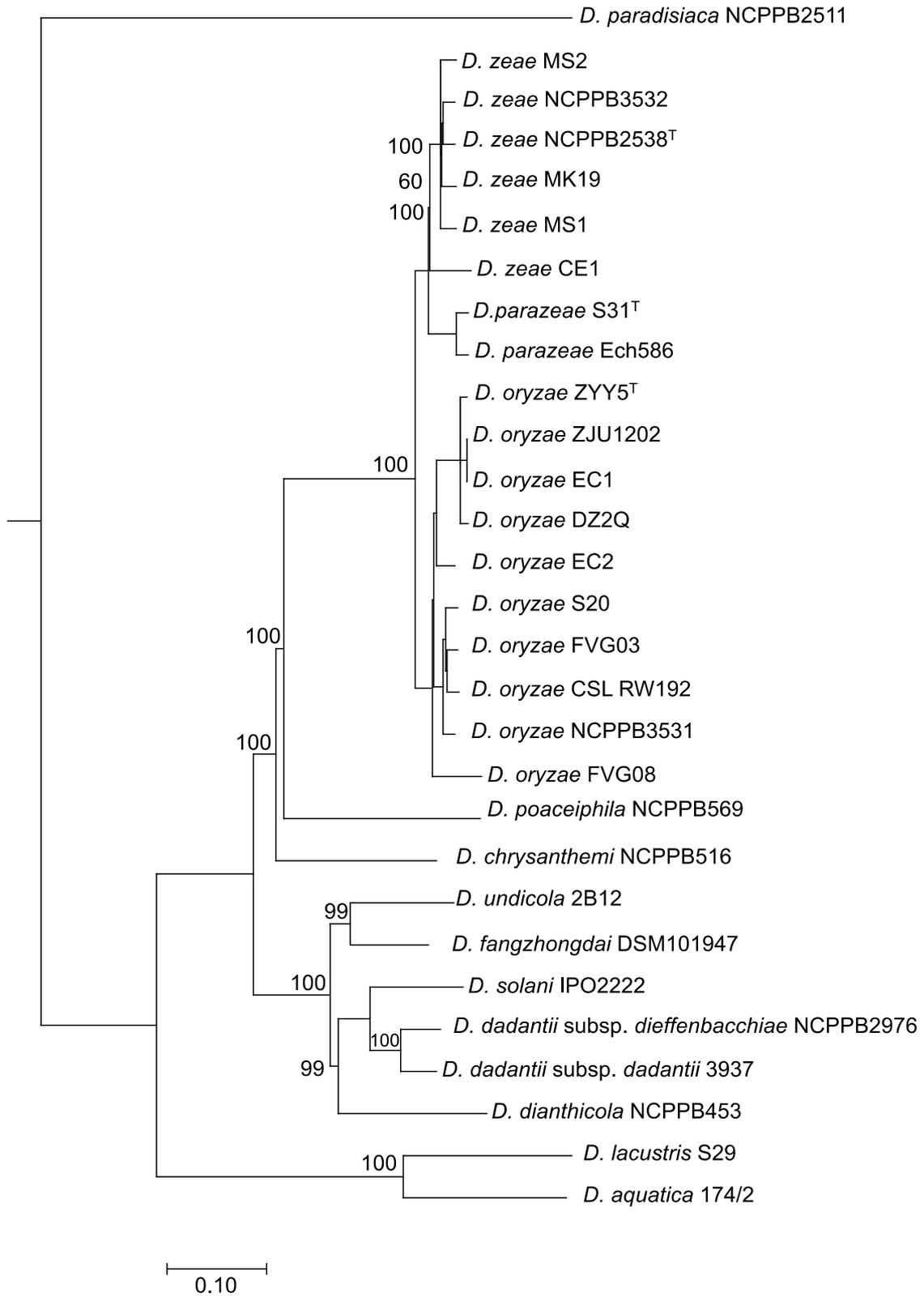
494

495 **FIGURES AND TABLES**

496

497 **Fig. 1.** Phylogenomic analysis of *D. oryzae*, *D. zeae* and *D. parazeae* strains using 2079
498 homologous core proteins

499 The phylogenetic tree, constructed from concatenated amino acid sequences of 2079
500 homologous proteins (660657 sites), was computed using the BioNJ distance method. One
501 hundred bootstrap replicates were performed to assess the statistical support of each node,
502 all values are 100% except when indicated.



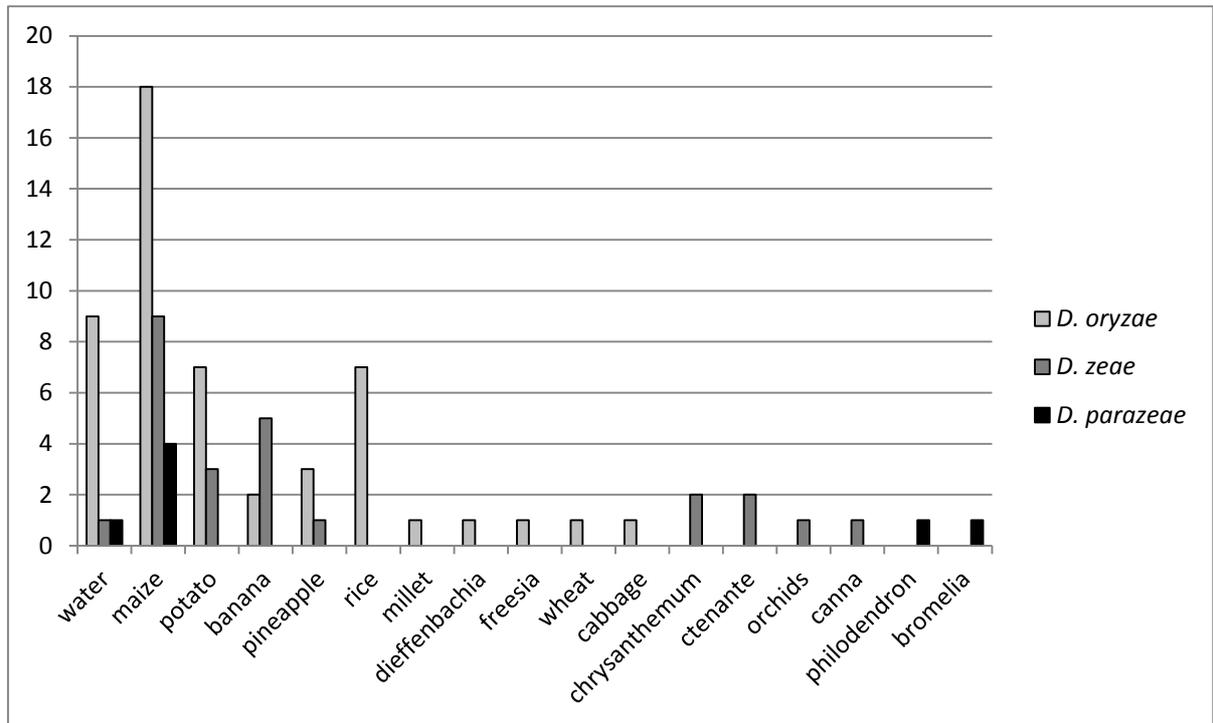
503

504

505 **Fig. 2.** Origin of the 83 *D. oryzae* (51), *D. zeae* (25) and *D. parazeae* (7) isolates

506 Whole data are given in Fig. S5.

507



508

509

510 **Table 1** General features of the different *Dickeya* strains/genomes analyzed in this study.

511

Strains*	Species	Genome length	# scaffolds	# CDS	# of Isolated from	Geographic origin	Year of isolation
NCPBP2538^T	<i>D. zeae</i>	4.56	7	4380	<i>Zea mays</i>	USA	1970
CFBP 1596	<i>D. zeae</i>	-	-		<i>Zea mays</i>	France	1974
MK19	<i>D. zeae</i>	4.67	4	4494	river water	UK	
NCPBP3532	<i>D. zeae</i>	4.56	1	4390	<i>Solanum tuberosum</i>	Australia	
MS1	<i>D. zeae</i>	4.75	58	4589	<i>Musa paradisiaca</i>	China	2009
MS2	<i>D. zeae</i>	4.74	complete	4529	<i>Musa paradisiaca</i>	China	2014
CE1	<i>D. zeae</i>	4.71	complete		<i>Canna indica</i>	China	2017
S31	<i>D. parazeae</i>	4.71	54	4098	lake water	France	2017
Ech586	<i>D. parazeae</i>	4.82	complete	4515	<i>Philodendron</i>	USA	
CSL_RW192	<i>D. oryzae</i>	4.70	4	4587	river water	UK	
DZ2Q	<i>D. oryzae</i>	4.65	26	4456	<i>Oryza sativa</i>	Italy	
NCPBP3531	<i>D. oryzae</i>	4.63	2	4354	<i>Solanum tuberosum</i>	Australia	
EC1	<i>D. oryzae</i>	4.53	complete	4260	<i>Oryza sativa</i>	China	1997
ZJU1202	<i>D. oryzae</i>	4.59	188	4417	<i>Oryza sativa</i>	China	2002
EC2	<i>D. oryzae</i>	4.58	complete	4371	<i>Oryza sativa</i>	China	2016
ZYY5 ^T	<i>D. oryzae</i>	4.59	27	4356	<i>Oryza sativa</i>	China	2011
FVG03	<i>D. oryzae</i>	4.75	81	4111	river water	France	2017
FVG08	<i>D. oryzae</i>	4.53	94	4026	river water	France	2017
S20	<i>D. oryzae</i>	4.67	70	4110	lake water	France	2017

512

513 * Strains available for phenotypic studies are shown in bold letters.

514

515

516 **Table 2.** Genomic relatedness between *D. zea*, *D. parazeae* and *D. oryzae* strains

517 ANI values were calculated using the Pyani python module
 518 (<https://github.com/widowquinn/pyani>) with the BLAST algorithm (ANiB) [35]. dDDH values
 519 were calculated using a dedicated pipeline (<http://ggdc.dsmz.de/>) from formula 2 (sum of all
 520 identities found in high-scoring segment pairs (HSPs) divided by overall HSP length and
 521 normalized to genome length to take into account incomplete draft genomes).
 522 The type strains (^T) were first compared to all other members of each species, *D. oryzae*, *D.*
 523 *zea* and *D. parazeae*. Then, all members of each species except the type strain, were
 524 compared two by two.
 525 Additional data are given in Fig. S1.
 526

	ANI %		
	<i>D. oryzae</i>	<i>D. zea</i>	<i>D. parazeae</i>
<i>D. oryzae</i> ^T	96.0-99.2	94.2-94.8	94.1
<i>D. oryzae</i>	96.0-100	94.0-95.1	93.9-94.5
<i>D. zea</i> ^T	94.4-95.0	96.3-98.1	96.0-96.1
<i>D. zea</i>	94.0-95.0	96.1-98.4	95.7-96.0
<i>D. parazeae</i> ^T	94.0-94.5	95.8-96.0	98.5
<i>D. parazeae</i>	93.9-94.5	95.7-96.1	98.5
	dDDH %		
	<i>D. oryzae</i>	<i>D. zea</i>	<i>D. parazeae</i>
<i>D. oryzae</i> ^T	73.7-99.8	56.2-59.2	55.9-56.0
<i>D. oryzae</i>	67.1-99.8	55.2-61.2	54.9-57.5
<i>D. zea</i> ^T	56.8-60.4	68.6-86.0	67.1-67.3
<i>D. zea</i>	55.2-61.2	67.4-84.9	65.6-67.1
<i>D. parazeae</i> ^T	55.1-58.1	65.6-67.3	88.1
<i>D. parazeae</i>	54.9-57.8	65.0-67.1	88.1

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 528

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530

Table 3. Differences in virulence gene repartition among *D. zea*, *D. parazeae* and *D. oryzae* isolates

	Origin	<i>pehN</i>	<i>rhiE</i>	<i>rhiF</i>	<i>pnIG*</i>	<i>pelL</i>	<i>celZ</i>	<i>avrL*</i>	<i>zms</i>	<i>cyt</i>	T4SS VirB
<i>D. zea</i> NCPPB 2538 ^T	Maize	+	+	+	+	+	+	+	-	-	-
<i>D. zea</i> MS1	Banana	+	+	+	+	-	-	+	-	-	+
<i>D. zea</i> MS2	Banana	+	+	+	+	+	+	+	-	-	-
<i>D. zea</i> MK19	Water	+	+	+	+	+	+	+	-	-	+
<i>D. zea</i> NCPPB 3532	Potato	+	+	+	+	+	+	+	-	-	-
<i>D. zea</i> CE1	Canna	+	+	+	-	+	+	+	-	-	-
<i>D. parazeae</i> Ech586	Philodendron	-	-	+	-	+	+	+	-	-	+
<i>D. parazeae</i> S31 ^T	Water	-	-	+	-	+	+	+	-	-	+
<i>D. oryzae</i> ZYY5 ^T	Rice	-	-	+	+	+	+	-	+	-	+
<i>D. oryzae</i> DZ2Q	Rice	-	-	+	+	+	+	-	+	+	+
<i>D. oryzae</i> EC1	Rice	-	-	+	+	+	+	-	+	+	+
<i>D. oryzae</i> EC2	Rice	-	-	+	+	+	+	+	-	-	-

<i>D. oryzae</i> ZJU1202	Rice	-	-	+	+	+	+	-	+	+	+
<i>D. oryzae</i> NCPPB 3531	Potato	-	-	+	-	+	+	+	-	+	-
<i>D. oryzae</i> CSL_RW192	Water	-	-	+	+	+	+	+	-	-	2
<i>D. oryzae</i> S20	Water	-	-	+	-	+	+	+	-	-	+
<i>D. oryzae</i> FVG03	Water	-	-	+	-	+	+	+	-	-	+
<i>D. oryzae</i> FVG08	Water	-	-	+	-	+	+	+	-	-	+

* *pnIH*, *avrM*, and *pemB* are absent in all *D. zeae*, *D. parazeae* and *D. oryzae* strains

Table 4. Main phenotypes of *D. zea*, *D. parazeae* and *D. oryzae* strains.

The model strain *Dickeya dadantii* 3937 was used for comparison. Protease (Prt), pectinase (Pel) and cellulase (Cel) activities were detected on specific media [33]. Methods to measure the swimming and swarming motilities as well as the maceration capacity on potato tubers and chicory leaves were previously described [33].

	Extracellular activities			Motility		Maceration	
	Pectate lyase	Cellulase	Protease	Swimming	Swarming	Chicory leaves	Potato tubers
<i>Dickeya</i> strains	mm	mm	mm	mm	mm	mm	g
<i>D. dadantii</i> 3937	15±2	8±1	12±1	19±1	54±5	51±16	1.68 ±0.82
<i>D. oryzae</i> DZ2Q	13±3	12±1	14±1	6±1	24±2	17±10	1.77 ±0.67
<i>D. oryzae</i> NCPPB3531	13±2	12±1	13±1	28±2	32±5	37±9	2.31 ±0.89
<i>D. oryzae</i> S20	15±1	14±1	13±1	22±1	11±1	43±16	2.30 ±1.41
<i>D. oryzae</i> FVG3	14±0,5	16±1	13±1	23±1	40±5	84±9	2.75 ±0.91
<i>D. oryzae</i> FVG8	12±2	10±1	11±2	17±1	7±2	69±14	2.21 ±1.33
<i>D. zea</i> NCPPB 2538 ^T	16±0.5	16±2	13±2	2±0.5	7±1	15±8	1.39 ±0.39
<i>D. zea</i> NCPPB 3532	16±0.5	16±1	14±1	32±1	61±8	39±15	2.87 ±0.73
<i>D. parazeae</i> S31 ^T	13±2	11±1	11±1	29±1	50±9	68±10	1.99 ±0.48
<i>D. parazeae</i> CFBP 1536	12±2	13±1	14±1	25±1	35±5	47±8	2.19 ±0.37

Table 5. Growth of *D. zea*, *D. parazeae* and *D. oryzae* strains with different carbon sources

Solidified M63 minimal medium supplemented with a carbon source at 2 g l⁻¹ (Ltart, L-tartaric acid; Gma, D-glucosaminic acid; Dara, D-arabinose; Mtl, mannitol; Mel, melibiose; Xyl, D-xylose; Ino, myo-inositol; Glu, glutamic acid; Gln glutamine; O, gluconic acid; U glucuronic acid, Rha, L-rhamnose) was used to test bacterial growth after 24 to 72 hours at 30°C.

<i>Dickeya</i> strains	Ltart	U	Dara	Mtl	Mel	Xyl	Ino	Rha	O	GmA	Glu	Gln
<i>D. dadantii</i> 3937	–	–	w	+	+	w	+	–	+	–	–	–
<i>D. oryzae</i> DZ2Q	–	w	w	+	+	w	+	–	w	w	w	w
<i>D. oryzae</i> NCPPB 3531	–	w	w	+	+	w	w	–	+	w	+	+
<i>D. oryzae</i> S20	–	–	w	+	+	w	+	–	w	–	w	w
<i>D. oryzae</i> FVG3	–	–	w	+	+	w	+	–	w	+	–	–
<i>D. oryzae</i> FVG8	–	–	w	+	+	w	+	–	+	–	–	–
<i>D. zea</i> NCPPB 2538 ^T	w	–	w	+	+	w	+	–	+	+	+	+
<i>D. zea</i> NCPPB 3532	w	–	w	+	+	w	+	–	+	+	+	+
<i>D. parazeae</i> S31 ^T	w	w	w	+	+	w	+	–	+	–	+	+
<i>D. parazeae</i> CFBP 1536	w	w	w	+	+	w	+	–	+	+	+	+