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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The type strain *D. parazeae* S31^T (CFBP 8716^T, LMG 32070^T) Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAGJWU000000000. Whole Genome Shotgun project of *D. oryzae* strains S20, FVG03 and FVG08 has been deposited at DDBJ/ENA/GenBank under the accessions JAGJWV000000000, JAGJWX000000000, and JAGJWW000000000, respectively.

ABSTRACT

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

INTRODUCTION

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

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

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

ISOLATION OF NEW STRAINS AND GENOME SEQUENCING

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

Colonies forming pits on CVP plates were grown overnight in liquid medium (LB without NaCl) and qPCR amplifications were performed out of bacterial cell lysates with primers pelD1118d-F (VRC BTA CAA ACC SAC TCT G) and pelD1200d1-R (TGC GTT GYT RTT GAT GCT G), derived from the sequence of the gene *pelD* that is specific of the genus *Dickeya*. The *Dickeya* candidates were further purified on CVP plates and then on LB^- plates (LB medium without added NaCl).

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

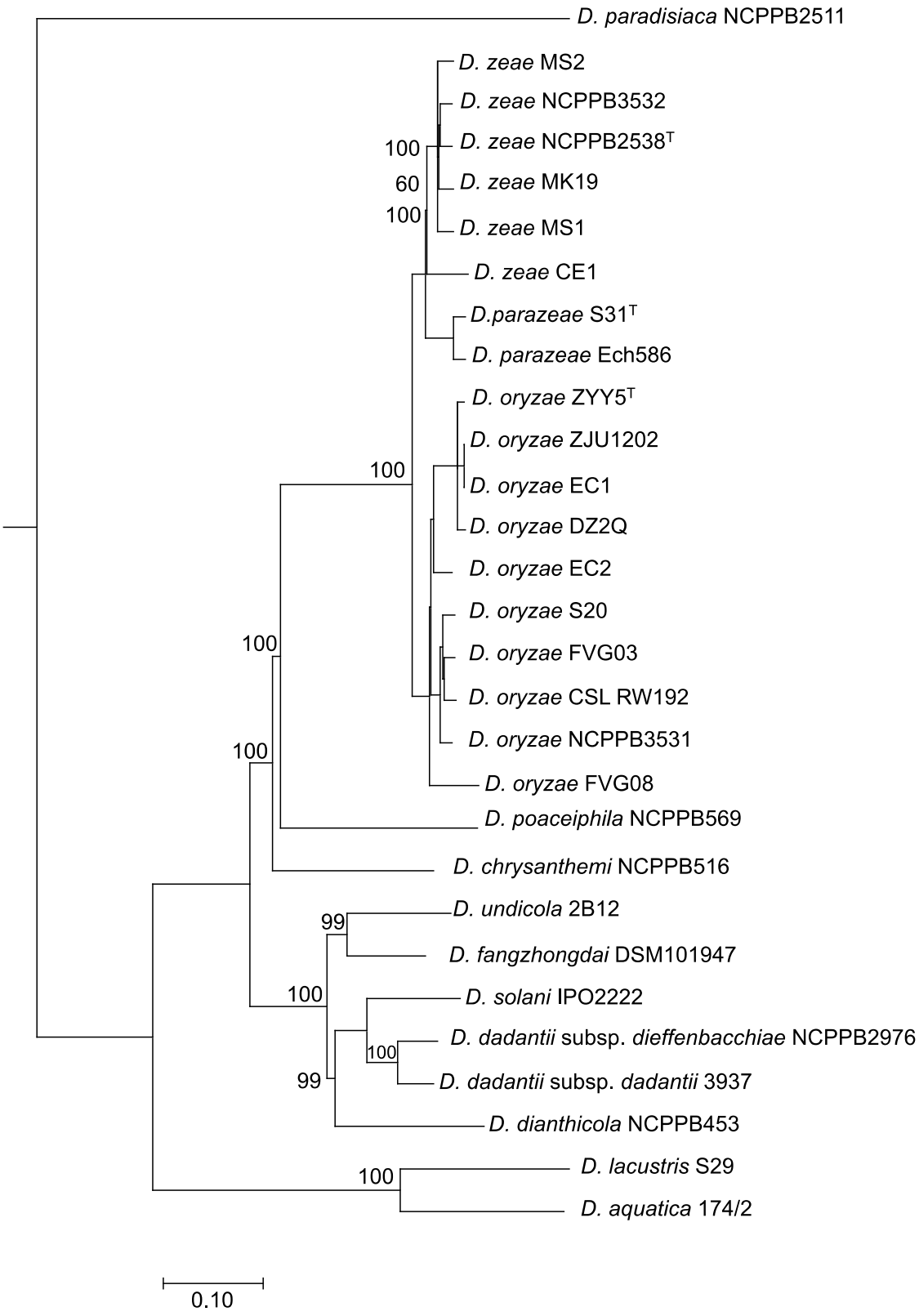
The accurate *Dickeya* species of isolates from both surveys was then determined by PCR amplification of the housekeeping gene *gapA* using the gapA-7-F and gapA-938-R primer set and sequencing of the *gapA* amplicon [26]. Two strains from each survey clustering with strains of the *D. zeae* complex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

GENOME COMPARISON

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

oryzae type strain ZYY5^{T} isolated from rice roots in China, four strains isolated from rice either in Italy (DZ2Q) or in China (EC1, EC2, ZJU1202), two strains isolated from banana in China (MS1, MS2), two strains isolated from potato in Australia (NCPPB 3531, NCPPB 3532), a strain isolated in USA from Philodendron (Ech586), a strain isolated from Canna lily in China (CE1),and two strains isolated from rivers in UK (CSL_RW192, MK19) (Table 1).

A phylogenomic tree was constructed from concatenated sequences of 2079 homologous core proteins retrieved from the 18 analysed genomes and genomes of type strains of the other *Dickeya* species (Fig. 1). This tree confirmed that the 18 analysed genomes clearly split in two major clades: five genomes (NCPPB 2538, NCPPB 3532, MK19, MS1, MS2, and CE1) cluster with *D. zeae* NCPP2538^{T} while ten genomes (NCPPB 3531, DZ2Q, CSL_RW192, ZJU 1202, EC1, EC2, FVG03, FVG08 and S20) are separated into a second clade including the *D. oryzae* type strain ZYY5 ^{T}. The present work including new genomic data clearly confirms the separation of the *D. zeae* complex in two species *D. zeae* and *D. oryzae*. Furthermore, the two strains Ech586 and S31 form a separate branch close to the *D. zeae* clade, supported by a 100% bootstrap value (Fig. 1).



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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. zeae* complex, the two strains Ech586 and S31 show
164 ANI values of 96-96.1% with the *D. zeae* type strain and 95.7-96% with the other *D. zeae*
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. zeae* members including the type strain. These values support the proposal of a new
170 species within the *D. zeae* 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. zeae*
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. zeae*
177 type strain NCPPB2538 ^{T} and 96.1-96.2% with other *D. zeae* 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. zeae*.
180
181 **DIVERSITY IN GENE CONTENT**
182 Comparative genomic analyses were conducted to identify distinctive genes between the
183 species *D. zeae*, *D. parazeae* 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. zeae* or *D. parazeae*
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. zeae*
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. zeae* and *D.*
197 *parazeae* 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. zeae* encodes 3426 protein families. Twenty five protein families are
204 present in all *D. zeae* members and absent in other strains of the *D. zeae* 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. parazeae* genomes share 4029 protein families of which 208 are not present in other
208 members of the *D. zeae* cluster (Table S2). These numbers could be however over-estimated
209 due to the low amount of genomes analysed for this species.

We also compared the repertoire of virulence genes among strains of the *D. zeae* complex. As reported for other *Dickeya* species, the maceration symptom is mainly linked to a set of pectate lyase genes present in all three species (*pelADE, pelBCZ, pelI, pelL, pelN, pelX,* and *pelW*), along with genes encoding accessory pectinases (*pemA/B, pnlG/H*, *rhiE/F*, *paeY, faeD, pehK,* and *pehN*) and other plant cell wall degrading enzymes (*celZ*, *xynA, plcA*, and *prtABCG*). However, some differences in the pectinase repertoire were observed between isolates (Table 3). As stated before, the genes encoding the rhamnogalacturonate lyase RhiE and the exo-polygalacturonase PehN are present in *D. zeae* members but neither in *D. parazeae* nor in *D. oryzae*. It could be noticed that all members of the *D. zeae* complex possess another gene encoding a potential rhamnogalacturonate lyase RhiF that is 50% identical to RhiE. The pectin lyase gene *pnlG* is present in *D. zeae* but absent in *D. parazeae* and strain EC1. In *D. oryzae, pnlG* is present only in the five strains rice strains and in a water isolate. Since the gene *pnlH* is absent in all *D. zeae* and *D. oryzae* members, several strains of these two species do not encode any pectin lyase. The two adjacent genes *pelL* and *celZ* are present in all genomes except in *D. zeae* MS1. The genome of all strains contains T1SS, T2SS, T3SS and T6SS gene clusters. At least one type 4 protein secretion system (T4SS) is encoded by all strains isolated from water but it is diversely distributed in the other strains (Table 3).

Some differences in their virulence equipment disclose particularities of rice strains in comparison to other *D. oryzae* members, with some exceptions for strain EC2. The toxin zeamine was shown to be involved in rice strain virulence [32]. The gene cluster involved in the biosynthesis of zeamine is not largely distributed in the *D. zeae* complex as it is absent in all *D. zeae* and *D. parazeae* strains and restricted to four *D. oryzae* rice strains, including the type strain ZYY5^{T}. However, this cluster is absent in strain EC2 isolated from diseased rice, suggesting that the toxin zeamine is not always essential for causing rice disease. Most *D. oryzae* rice strains do not possess the avirulence related gene *avrL* that is present in all other strains of the *D. zeae* complex including the rice strain EC2 (Table 3). Three rice strains possess the genes *cyt* encoding entomotoxins, a property shared only by another *D. oryzae* strain, NCPPB3531. While most *D. oryzae* rice strains possess *zms* and *cyt* but not *avrL*, the *D. oryzae* water strains and the *D. zeae* or *D. parazeae* members possess the gene *avrL* but not the cluster *zms* and rarely the genes *cyt*.

PHENOTYPIC ANALYSIS

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

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

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

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

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

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

IDENTIFICATION OF MEMBERS OF THE SPECIES *D. ZEAE*, *D. PARAZEAE* AND *D. ORYZAE*

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

The *recA* sequence was already shown to be a good reflect of the *Dickeya* classification [15]. In comparison, the gene *gapA* often used for strain classification in the genus *Dickeya* [26] appears to be less efficient to differentiate the three species of the *D. zeae* complex (Fig S4).

To identify new members of these species, we took advantage of previous phylogenetic analyses performed on large sets of strains using the *recA* sequence [15, 16]. We extracted from databases the *recA* sequences of 68 strains belonging to the *D. zeae* complex and compared them with the *recA* sequences of the six *D. zeae*, two *D. parazeae* and ten *D. oryzae* genomes (Fig. S5). The resulting tree clearly shows the division of these strains in the three clades corresponding to *D. zeae*, *D. parazeae* and *D. oryzae*. This clear differentiation allows the classification of the 68 strains in the three species. Remarkably, since the *recA* sequence of 75% of these strains clustered with the *D. oryzae* type strain (Fig. S5), they have to be reclassified in the novel species, *D. oryzae*. In addition, the *recA* phylogeny allows identification of five new members of the species *D. parazeae* (Fig. S5).

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

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

IDENTIFICATION OF SPECIES IN THE D. ZEAE COMPLEX

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

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

DESCRIPTION OF *DICKEYA PARAZEAE* SP. NOV.

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

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

The type strain of *Dickeya parazeae* is $S31^T$ (=$CFBP8716^T$, LMG 32070^T); its G+C content is 56·4mol% based on the draft genome sequence.

Genome EMBL/GenBank accession: JAGJWU000000000

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

AUTHORS' STATEMENTS

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Conflicts of interest

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

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

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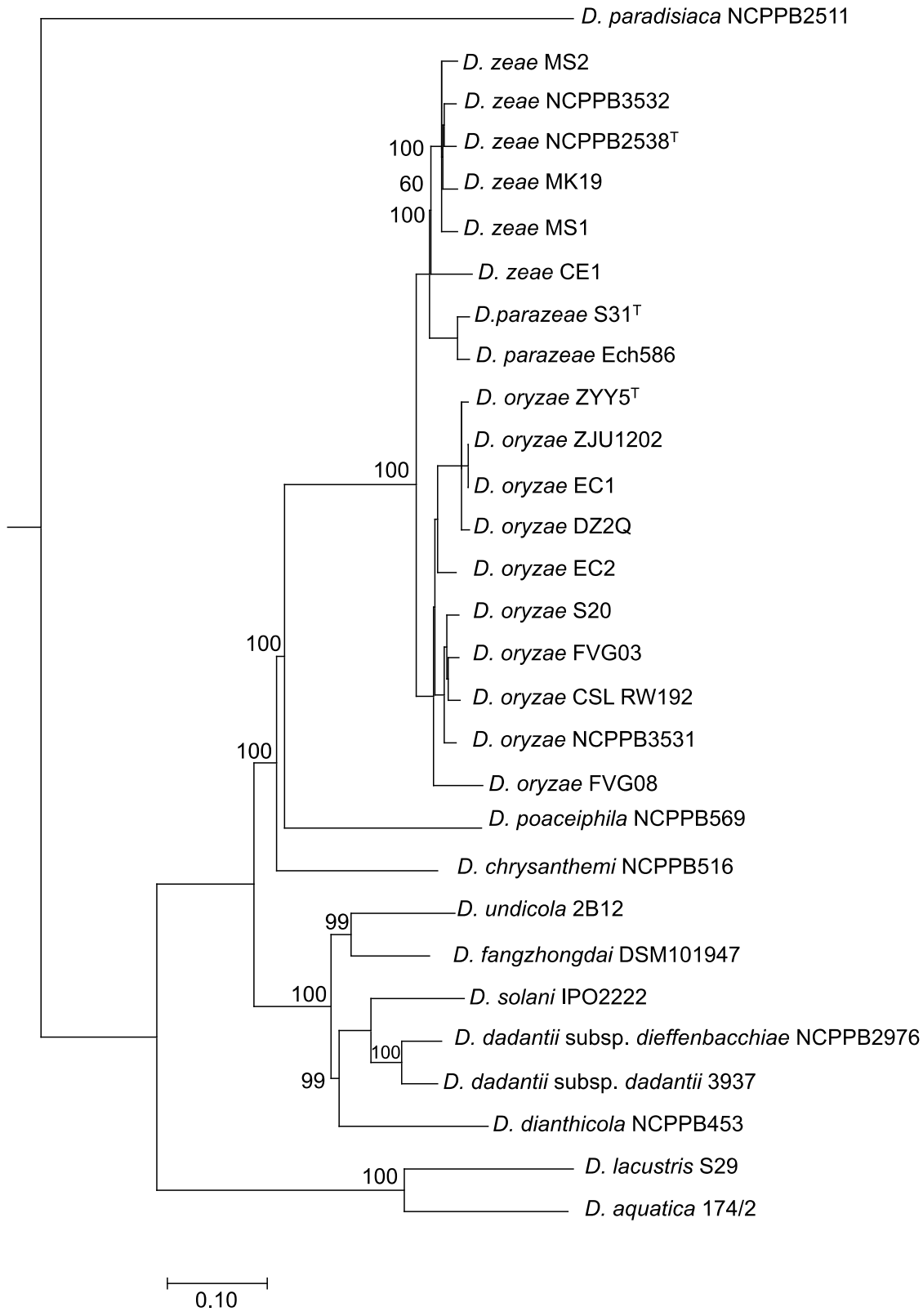
FIGURES AND TABLES

Fig. 1. Phylogenomic analysis of *D. oryzae*, *D. zeae* and *D. parazeae* strains using 2079 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.



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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.

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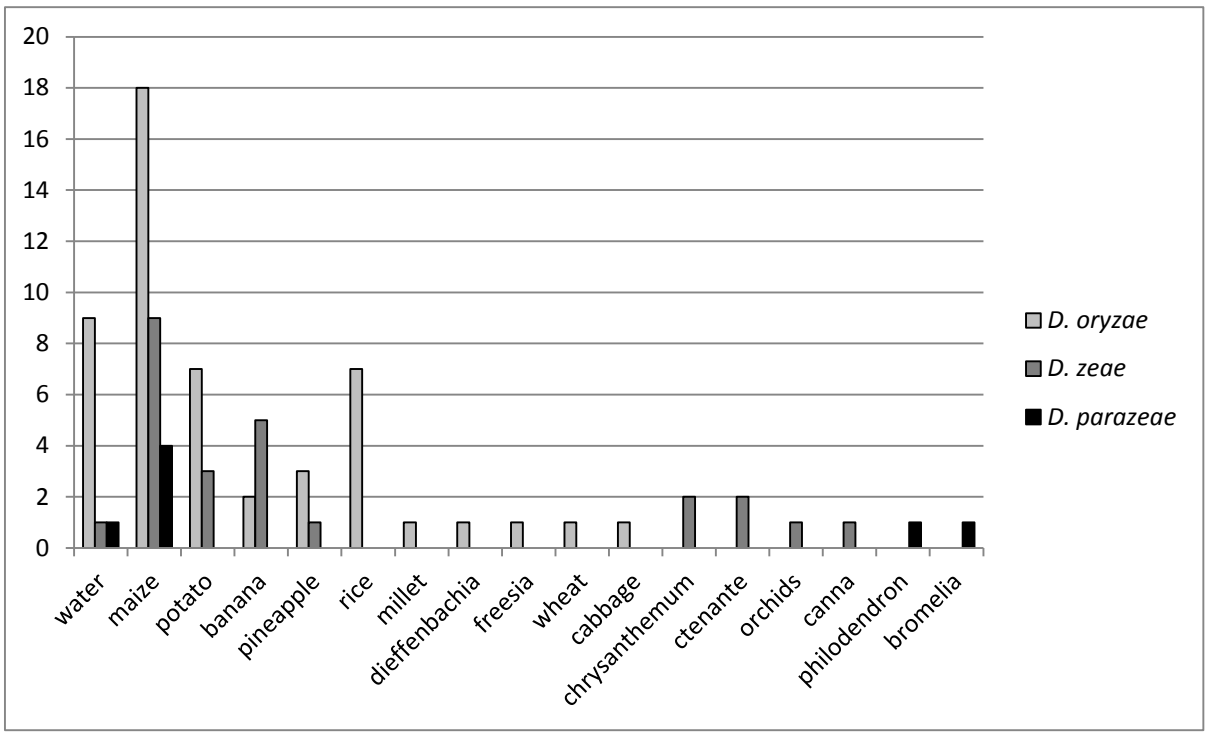

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Table 1 General features of the different *Dickeya* strains/genomes analyzed in this study.

Strains*	Species	Genome length	# scaffolds	# of CDS	Isolated from	Geographic origin	Year of isolation
NCPPB2538^T	***D. zeae***	**4.56**	**7**	**4380**	***Zea mays***	**USA**	**1970**
CFBP 1596	***D. zeae***	**-**	**-**		***Zea may***	**France**	**1974**
MK19	*D. zeae*	4.67	4	4494	river water	UK	
NCPPB3532	***D. zeae***	**4.56**	**1**	**4390**	***Solanum tuberosum***	**Australia**	
MS1	*D. zeae*	4.75	58	4589	*Musa paradisiaca*	China	2009
MS2	*D. zeae*	4.74	complete	4529	*Musa paradisiaca*	China	2014
CE1	*D. zeae*	4.71	complete		*Canna indica*	China	2017
S31	***D. parazeae***	**4.71**	**54**	**4098**	**lake water**	**France**	**2017**
Ech586	*D. parazeae*	4.82	complete	4515	*Philodendron*	USA	
CSL_RW192	*D. oryzae*	4.70	4	4587	river water	UK	
DZ2Q	***D. oryzae***	**4.65**	**26**	**4456**	***Oryza sativa***	**Italy**	
NCPPB3531	***D. oryzae***	**4.63**	**2**	**4354**	***Solanum tuberosum***	**Australia**	
EC1	*D. oryzae*	4.53	complete	4260	*Oryza sativa*	China	1997
ZJU1202	*D. oryzae*	4.59	188	4417	*Oryza sativa*	China	2002
EC2	*D. oryzae*	4.58	complete	4371	*Oryza sativa*	China	2016
ZYY5^T	*D. oryzae*	4.59	27	4356	*Oryza sativa*	China	2011
FVG03	***D. oryzae***	**4.75**	**81**	**4111**	**river water**	**France**	**2017**
FVG08	***D. oryzae***	**4.53**	**94**	**4026**	**river water**	**France**	**2017**
S20	***D. oryzae***	**4.67**	**70**	**4110**	**lake water**	**France**	**2017**

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

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

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

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

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

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

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

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

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

The model strain D*ickeya. dadan*tii 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**
***Dickeya* strains**	mm	mm	mm	**mm**	**mm**	**mm**	**g**
D. dadantii 3937	15±2	8±1	12±1	19±1	54±5	51±16	1.68 ±0.82
D. oryzae DZ2Q	13±3	12±1	14±1	6±1	24±2	17±10	1.77 ±0.67
D. oryzae NCPPB3531	13±2	12±1	13±1	28±2	32±5	37±9	2.31 ±0.89
D. oryzae S20	15±1	14±1	13±1	22±1	11±1	43±16	2.30 ±1.41
D. oryzae FVG3	14±0,5	16±1	13±1	23±1	40±5	84±9	2.75 ±0.91
D. oryzae FVG8	12±2	10±1	11±2	17±1	7±2	69±14	2.21 ±1.33
D. zeae NCPPB 2538^T	16±0.5	16±2	13±2	2±0.5	7±1	15±8	1.39 ±0.39
D. zeae NCPPB 3532	16±0.5	16±1	14±1	32±1	61±8	39±15	2.87 ±0.73
D. parazeae S31^T	13±2	11±1	11±1	29±1	50±9	68±10	1.99 ±0.48
D. parazeae CFBP 1536	12±2	13±1	14±1	25±1	35±5	47±8	2.19 ±0.37

Table 5. Growth of *D. zeae*, *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-tartarate; 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.

	Ltart	**U**	**Dara**	**Mtl**	**Mel**	**Xyl**	**Ino**	**Rha**	**O**	**GmA**	**Glu**	**Gln**
***Dickeya* strains**												
D. dadantii 3937	_	_	w	+	+	w	+	_	+	_	_	_
D. oryzae DZ2Q	_	w	w	+	+	w	+	_	w	w	w	w
D. oryzae NCPPB 3531	_	w	w	+	+	w	w	_	+	w	+	+
D. oryzae S20	_	_	w	+	+	w	+	_	w	_	w	w
D. oryzae FVG3	_	_	w	+	+	w	+	_	w	+	_	_
D. oryzae FVG8	_	_	w	+	+	w	+	_	+	_	_	_
D. zeae NCPPB 2538^T	**w**	_	w	+	+	w	+	_	+	+	+	+
D. zeae NCPPB 3532	**w**	_	w	+	+	w	+	_	+	+	+	+
D. parazeae S31^T	**w**	**w**	w	+	+	w	+	_	+	_	+	+
D. parazeae CFBP 1536	**w**	**w**	w	+	+	w	+	_	+	+	+	+