Ecology and Diversity

SciEPublish

Article

Surveys of *Pectobacteriaceae* in Lake Water over Consecutive Years Show That Lake-Specific Populations Survive the Winter

Nicole Hugouvieux-Cotte-Pattat * and Véronique Utzinger *

Microbiologie Adaptation et Pathogénie, Unité Mixte de Recherche 5240, Centre National de la Recherche Scientifique, Institut National des Sciences Appliquées de Lyon, Université Lyon 1, 69622 Villeurbanne, France

* Corresponding author. E-mail: nicole.cottepattat@gmail.com (N.H.-C.-P.); veronique.utzinger@univ-lyon1.fr (V.U.)

Received: 16 September 2026; Revised: 19 October 2026; Accepted: 5 November 2026; Available online: 18 November 2026

ABSTRACT: The *Pectobacteriaceae* family includes plant pathogenic bacteria of the genera *Pectobacterium* and *Dickeya* that cause plant maceration due to their pectinolytic activity. To gain information on their presence in surface water, four surveys were performed to collect water samples in three lakes included in a nature reserve. Most water samples contained pectinolytic bacteria, including six *Pectobacteriaceae* species. While *Pectobacterium* isolates dominated at the end of winter, their proportion decreased in early summer, and *Dickeya* reached up to 100% in late summer. The *Pectobacterium* isolates mostly consisted of the species *P. quasiaquaticum*, with a low proportion of *P. aquaticum* and *P. versatile*. The *Dickeya* isolates mostly belonged to the species *D. oryzae*, with a low occurrence of *D. chrysanthemi* and *D. aquatica*. Phenotypic and phylogenetic analysis disclosed the presence of *D. oryzae* subgroups differently distributed in the three lakes, each lake containing a specific subpopulation. The same subpopulation was recovered in the same lake in surveys conducted in two successive years, showing the recovery of the lake-specific population after the winter season. These data indicate that *D. oryzae* populations can survive the annual cold period and resume multiplication in the same lake the following year.

Keywords: Plant pathogens; *Dickeya*; *Pectobacterium*; Lake water; Winter survival; Bacterial population; Seasonal distribution

1. Introduction

The *Pectobacteriaceae* family contains several pectinolytic bacteria, including members of the genera *Dickeya* and *Pectobacterium,* which contain several plant pathogenic species [1]. After several reclassifications and the recent descriptions of new species, the List of Prokaryotic Names with Standing in Nomenclature (LPSN) currently recognizes 13 *Dickeya* species and 22 *Pectobacterium* species [2].

Several *Dickeya* and *Pectobacterium* species are able to infect a wide range of plants, including major crops of economic importance [3]. Typical symptoms in infected plants include soft rot caused by maceration of plant tissues by pectinolytic enzymes. Phytopathogenic *Pectobacteriaceae* were mostly isolated from diseased plants and less frequently from non-host environments [4]. Recent reports relate their repetitive presence in surface water [5–7]. The exploration of aquatic ecosystems disclosed the

© 2026 The authors. This is an open access article under the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/).

existence of seven new species associated with water, namely *D. aquatica* isolated in Scotland and Finland [8], *D. lacustris* in France [9], *D. undicola* in Malaysia and France [10], *P. fontis* in Malaysia [11], *P. polonicum* in Poland [12], *P. aquaticum* and *P. quasiaquaticum* in France [13,14]. Aside from these water-associated species, surface waters contain potential plant-pathogenic species, mostly *D. oryzae* observed in Poland and France [5–7], and *P. versatile* in France [6]. In these three surveys of surface waters, bacteria were selected on the basis of their pectinolytic activity, using a semi-selective medium previously developed to isolate phytopathogens from symptomatic plants [15].

The objective of this new study was to explore the diversity of *Pectobacteriaceae* in surface water of temperate wetlands located near the Rhone River and to compare these data with other recent studies. Water samples were collected from three neighboring lakes supplied with rainwater and groundwater. Pectinolytic bacteria collected in lake water were mostly affected to the genera *Dickeya* or *Pectobacterium*. Surveys carried out at different dates disclosed a different proportion of each genus according to the sampling season. The phenotypic and phylogenetic analysis of the isolates allowed an in-depth comparison of the populations present during successive years, showing the presence of lake-specific populations and their maintenance for consecutive years.

2. Materials and Methods

2.1. Collection of Water Samples

The nature reserve of Mepieu covers 162 ha and is located 2.4 to 3 km from the Rhone River in France (45°44′34″ N, 5°26′22″ E, altitude 246 m) (www.reserves-naturelles.org/etangs-de-mepieu, accessed on 16 September 2026) (Figure S1). It includes three lakes, Grand Etang (31 ha), Barral (7 ha) and Fulye (1.5 ha), surrounded by marshlands, forests and meadows. These lakes are fed by rainwater and underground waters. Surveys were carried out in June 2020, September 2020, March 2021 and August 2021. Water samples were collected approximately 1 m from the lake edge and at ~0.5 m depth. Each sample was filtrated on pleated paper filters to remove particles such as algae, protists, and filamentous fungi.

2.2. Selection and Preliminary Characterization of Pectinolytic Isolates

Bacterial media, and culture conditions were similar to those described in recent surveys [6,7]. Bacteria present in 15 mL of filtered water were recovered as a pellet after centrifugation for 10 min at 7000× *g*. The pellet was suspended in 100 mL of sterile water, and 50 mL was spread on a semi-selective medium, crystal violet pectate (CVP) containing both pectin (polygalacturonate) and citrate as carbon sources [15]. The remaining 50 mL were added to 2 mL of a pectate enrichment broth (PEB), which contains only polygalacturonate as a carbon source [16]. After incubation for 48 h at 30 °C, the PEB cultures were serially diluted before spreading on CVP medium. The method was repeated twice for winter samples, which contain less bacteria. Under these experimental conditions, the detection threshold of pectinolytic bacteria was about 66 bacteria per liter of water (1 isolate in 15 mL) for summer samples and 22 bacteria per liter for winter samples (1 isolate in 45 mL).

The CVP plates were incubated at 30 °C and examined for the presence of colonies forming a typical pit due to degradation of the pectin gel after 48 h and 72 h. Each pit-forming colony was picked up in 50 µL of water, and appropriate dilutions were spread on CVP medium to obtain isolated colonies. After a second step of purification by isolation on LB plates, the phenotype of the isolates was determined to evaluate the production of degrading enzymes (pectinase, cellulase, and protease) and the utilization of potential carbon sources (glycerol, D-arabinose, D-xylose, mannitol, melibiose, sucrose, and trehalose). For the pectinase plate assay, bacteria were grown for 24 h on plates containing M63 medium supplemented with glycerol (2 g/L) and polygalacturonate (4 g/L). After flooding the plates with a saturated solution of copper acetate, a clear halo appears around colonies that can degrade the pectic polysaccharide [17].

2.3. PCR Amplification, Sequencing and Phylogenetic Analysis

Sequencing of the housekeeping genes *gapA* or *recA* is routinely used to refine interspecific phylogenetic positions within *Pectobacteriaceae* [18,19]. The purified isolates were subjected to PCR on colonies using primers specific for the gene *gapA* (gapAF, AAGTGAAAGACGGTCACCTGGT, and gapAR, CGATCAGGTCCAGAACCTTGTT, 806 nt amplicon). In some cases, PCR was also performed using the primers designed for partial amplification of the gene *recA* (DrecA1, GGTAAAGGGTCTATCATGCG, and DrecA2, CCCTCGCCATACAGAATCTGGA, 734 nt amplicon). Sequencing of the PCR products was performed in both directions, with the same primer sets, using a commercial sequencing service (Microsynth France SAS, Lyon, France).

Phylogenetic trees were generated with a ready-to-use pipeline operating with programs MUSCLE 3.7 for multiple alignments, PhyML 3.0 for tree building, and TreeDyn 198.3 for tree rendering [20] (https://phylogeny.fr, accessed on 1 June 2026).

3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Water Sampling and Selection of Pectinolytic Bacteria

Samples were collected from three neighboring lakes, namely Grand Etang, Barral, and Fulye (Figure S1). Surface water was collected in June 2020, September 2020, March 2021, and August 2021 (Table 1, Table S1). During the first survey of June 2020, a water sample was also collected from the marsh Marterin, but this site could not be sampled subsequently as it was almost dry in late summer.

In total, 28 water samples were recovered (Tables 1 and S1). The presence of cultivable bacteria was assessed on LB plates. Since LB medium tends to favor fast growing copiotrophic bacteria, such as *Pectobacteriaceae*, these CFU values may not reflect the total cultivable bacterial community. In the 6 winter samples, at water temperatures of 11.4 to 12 °C, the bacterial populations varied from 1.6×10^2 to 5.8×10^2 CFU·mL^{-1} (Table 1). The 22 summer samples, with water temperatures ranging from 16.7 to 22.2 °C, showed high concentrations of cultivable bacteria, with values fluctuating from 7×10^2 to 3.5×10^3 CFU·mL^{-1}. The presence of pectinolytic bacteria was assessed on CVP medium. The concentrations of pectinolytic bacteria were highly variable depending on the samples, they reached up to 155 and 7100 bacteria per liter of water in winter and summer, respectively (Table 1). They were below the detection threshold for only 2 of the 28 water samples, one in summer and one in winter. The low concentration of pectinolytic bacteria observed in winter suggests they are generally disadvantaged at low temperatures (Table 1). Among the summer samples, the range of water temperatures (16.7 to 22.2 °C) was too limited to observe a potential effect of temperature.

Table 1. Information on the sampling surveys. The species distribution, based on phenotypic profiles and *gapA* sequence, is given with the indication of the phenotypic classes (D1, D2, D3, D4, P1 P2) corresponding to each species. ND, not detected.

Sampling Day	**23 June**	**15 September**	**18 March**	**31 August**	**Total**	**Total**
	2020	2020	2021	2021	including	for the
Season	Early summer	Late summer	Winter	Summer	Marterin	3 lakes
Number of samples	4 (J1–J18)	10 (S1–S10)	6 (M1–M6)	8 (A1–A8)	28	27
Water temperature	17–21 °C	19.2–22.2 °C	11.4–12 °C	16.7–18.8 °C		
Cultivable bacteria (CFU/mL)	8×10^2–2.6×10^3	1.5×10^3–3.5×10^3	1.6×10^2–5.8×10^2	7×10^2–2.2×10^3		
Pectinolytic colonies per liter	3.7×10^2–2.7×10^3	ND–7.1×10^3	ND–1.6×10^2	11×10^3–7×10^3		
Samples containing pectinolytic bacteria	4	9	5	8	26	25

Number of pectinolytic isolates	52	66	21	64	203	190
Pectobacteriaceae selected by phenotypic analyses	47	55	17	57	176	163
Pectobacteriaceae identified by *gapA* phylogeny	19	18	17	26	80	77
Species distribution						
Dickeya						130
Class D1 *(D. oryzae*)	13	29	4	25	71	71
Class D2 *(D. oryzae*)	3	16	0	18	37	37
Class D3 *(D. chrysanthemi*)	5	1	0	11	17	12
Class D4 *(D. aquatica*)	0	9	1	0	10	10
Pectobacterium						33
Class P1 *(P. aquaticum* or *P. quasiaquaticum*)	25	0	12	3	40	32
Class P2 *(P. versatile*)	1	0	0	0	1	1

3.2. Diversity of Pectinolytic Isolates Revealed by Phenotypic Analysis

In total, 203 pectinolytic isolates were recovered, including 52 isolates in June 2020, 66 isolates in September 2020, 21 isolates in March 2021, and 64 isolates in August 2021 (Tables 1 and S1). After purification using successive cultures on CVP and LB media, pure isolates were submitted to a phenotypic analysis, including the secretion of degradative enzymes and the assimilation of diverse carbon sources (Tables 2 and S2). About 13% of them were rejected as not *Pectobacteriaceae,* as they gave a negative response to the pectinase test, associated with an abnormal colony appearance (color, size, *etc.*). The other isolates produced extracellular pectinases using either the CVP medium or the pectinase plate assay. These 176 isolates were retained as potential *Pectobacteriaceae,* including 47 isolates recovered in June 2020, 55 isolates in September 2020, 17 isolates in March 2021, and 57 isolates in August 2021 (Tables 1 and S2). Most of them produced extracellular cellulase and protease activities (Tables 2 and S2).

Analysis of the pectinolytic isolates for their potential assimilation of a set of sugars revealed different profiles, classified into six classes (Tables 2 and S2). Among the 176 potential *Pectobacteriaceae* members isolated, 71 isolates (40%) belonged to class D1, corresponding to Dickeya biovar 3/8, characterized by the ability to assimilate D-arabinose; this biovar has been described for several species, such as *D. oryzae, D. parazeae,* and *D. zeae* [21,22]. 37 isolates (20%) belonged to class D2, which did not correspond to any previously described *Dickeya* biovar, but they were able to assimilate D-arabinose. In comparison to biovar 3/8, they were unable to assimilate melibiose and to secrete proteases (phenotype Mel- Prt-). Notably, these atypical isolates exclusively originated from Lake Fulye (Tables 2 and S2). 17 pectinolytic isolates (10%) were members of class D3 similar to the biovar 5/6 described for *D. chrysanthemi* [21]. 10 isolates (6%) belonged to class D4 similar to the *Dickeya* biovar 10, differing by the inability to assimilate xylose and corresponding to the species *D. aquatica* or *D. lacustris* [9]. 40 isolates (23%) belonged to class P1, whose profile corresponded to the species *P. aquaticum* or *P. quasiaquaticum*, unable to assimilate melibiose. One isolate (0.6%) belonged to class P2, able to assimilate trehalose, a characteristic of several *Pectobacterium* species (Tables 2 and S2). Among them, 163 isolates originated from lake water and 13 isolates from the marsh Marterin, which contained only isolates of class D3 (5 isolates) or class P1 (8 isolates) (Tables 2 and S2).

Table 2. Phenotypic analysis of the 80 isolates also submitted to phylogenetic analyses. The phenotypic analysis included the production of extracellular enzymes (Pel, pectinase; Cel, cellulase; Prt, protease) and the assimilation of carbon sources (Mtl, mannitol; Mel, melibiose; Dara, D-arabinose; Xyl, xylose; Tre, trehalose). xx, strongly positive, x, positive; w, weakly positive; -, negative. For the selection method, cc and enrich mean concentration and enrichment, respectively.

Site, Date	Sample	Water Temp.	Isolate Name	Select Method	CVP Pits	Phenotypic Analysis								Class	Identification
						Pel	Cel	Prt	Mtl	Mel	Dara	Xyl	Tre		
Grand	J1	19.5 °C	IC1	cc	xx	w	xx	x	x	-	-	x	-	P1	*P. quasiaquaticum*
Etang			IC17	cc	w	w	xx	x	x	-	-	x	-	P1	*P. quasiaquaticum*
6/2020			IC18	cc	w	w	x	x	x	-	-	x	-	P1	*P. quasiaquaticum*
			IC190	cc	xx	xx	xx	w	x	w	w	x	-	D1	*D. oryzae*
			IE11	enrich	x	xx	xx	w	x	x	w	x	-	D1	*D. oryzae*
			IE14	enrich	w	xx	xx	xx	x	x	w	x	-	D1	*D. oryzae*
Barral	J2	18.8 °C	IC20	cc	xx	xx	xx	xx	x	x	w	x	-	D1	*D. oryzae*
6/2020			IC25	cc	w	w	xx	w	x	-	-	x	-	P1	*P. quasiaquaticum*
			IC26	cc	w	w	xx	xx	x	-	-	x	-	P1	*P. quasiaquaticum*
			IC27	cc	w	w	xx	xx	x	-	-	x	-	P1	*P. quasiaquaticum*
			IC202	cc	w	w	xx	w	x	-	-	x	-	P1	*P. quasiaquaticum*
			IE21	enrich	x	w	xx	w	x	x	w	x	-	D1	*D. oryzae*
			IE22	enrich	x	xx	xx	x	x	x	w	x	-	D1	*D. oryzae*
Fulye	J3	17 °C	IC3	cc	w	xx	xx	x	x	x	-	x	w	P2	*P. versatile*
6/2020			IC30	cc	w	xx	xx	-	x	-	w	x	-	D2	*D. oryzae* Mel-Prt-
			IE30	enrich	x	x	w	-	w	-	w	x	-	D2	*D. oryzae* Mel-Prt-
Marterin 6/2020	J4	17.2 °C	IC41	cc	xx	w	xx	x	x	-	-	x	-	P1	*P. quasiaquaticum*
			IC49	cc	x	w	xx	xx	x	w	-	x	-	D3	*D. chrysanthemi*
			IE40	enrich	w	w	w	w	w	w	-	x	-	D3	*D. chrysanthemi*
Grand	S1	22 °C	IS10	cc	xx	x	xx	x	x	x	w	x	-	D1	*D. oryzae*
Etang			ISE22	enrich	x	x	x	x	x	x	w	x	-	D1	*D. oryzae*
9/2020	S3	22.1 °C	IS30	cc	x	x	x	x	x	x	w	x	-	D1	*D. oryzae*
			IS31	cc	xx	x	x	x	x	x	w	x	-	D1	*D. oryzae*
	S9	21.2 °C	IS90	cc	x	x	xx	x	x	x	w	x	-	D1	*D. oryzae*
			IS92	cc	x	x	x	x	-	w	-	-	-	D4	*D. aquatica*
	S10	21.6 °C	IS101	cc	xx	x	xx	x	x	x	w	x	-	D1	*D. oryzae*
Barral	S7	19.2 °C	IS70	cc	xx	xx	xx	x	x	x	w	x	-	D1	*D. oryzae*
9/2020			IS71	cc	x	x	x	xx	x	x	w	x	-	D1	*D. oryzae*
			ISE7	enrich	w	x	x	x	x	x	w	x	-	D1	*D. oryzae*
	S8	19.9 °C	IS80	cc	x	x	x	x	-	x	-	-	-	D4	*D. aquatica*
			IS81	cc	x	x	x	x	-	x	-	-	-	D4	*D. aquatica*
			IS84	cc	xx	xx	xx	x	x	w	w	x	-	D1	*D. oryzae*
			ISE8	enrich	x	x	xx	x	-	w	-	-	-	D4	*D. aquatica*
Fulye	S4	20.6 °C	IS42	cc	x	x	xx	-	x	-	w	x	-	D2	*D. oryzae* Mel-Prt-
9/2020	S5	20.6 °C	IS50	cc	x	x	w	w	x	x	-	x	-	D3	*D. chrysanthemi*
			IS51	cc	x	x	xx	-	x	-	w	x	-	D2	*D. oryzae* Mel-Prt-
			IS54	cc	x	x	x	-	w	-	w	x	-	D2	*D. oryzae* Mel-Prt-
Grand	M1	12 °C	I11	cc	xx	w	xx	x	x	-	-	x	-	P1	*P. aquaticum*
Etang			I12	cc	xx	w	xx	x	x	-	-	x	-	P1	*P. quasiaquaticum*
3/2021			I13	cc	xx	xx	xx	x	x	-	w	x	-	D1	*D. oryzae*
			I14	cc	xx	xx	xx	x	x	-	w	x	-	D1	*D. oryzae*
			I15	cc	xx	xx	xx	x	-	x	-	-	-	D4	*D. aquatica*
			IP191	enrich	w	w	xx	x	x	-	-	x	-	P1	*P. quasiaquaticum*
			IP193	enrich	w	w	xx	x	x	-	-	x	-	P1	*P. quasiaquaticum*

	M2	11.9 °C	I21	cc	x	x	x	x	x	-	-	x	-	P1	*P. quasiaquaticum*
			I22	cc	x	w	x	w	x	-	-	x	-	P1	*P. quasiaquaticum*
Barral	M6	11.4 °C	IP63	enrich	w	w	x	x	x	-	-	x	-	P1	*P. aquaticum*
3/2021			IP66	enrich	x	xx	xx	x	x	x	w	x	-	D1	*D. oryzae*
			IP67	enrich	x	w	xx	x	x	-	-	x	-	P1	*P. aquaticum*
			IP694	enrich	xx	xx	xx	x	x	x	w	x	-	D1	*D. oryzae*
Fulye	M4	11.8 °C	I42	cc	x	w	xx	xx	x	-	-	x	-	P1	*P. quasiaquaticum*
3/2021			IP45	enrich	x	w	xx	x	x	-	-	x	-	P1	*P. quasiaquaticum*
			IP48	enrich	x	w	xx	x	x	-	-	x	-	P1	*P. quasiaquaticum*
	M5	11.5 °C	I51	cc	x	w	xx	xx	x	-	-	x	-	P1	*P. quasiaquaticum*
Grand	A1	17.7 °C	M11	cc	xx	xx	xx	x	x	w	w	x	-	D1	*D. oryzae*
Etang			M15	cc	x	xx	xx	w	x	x	w	x	-	D1	*D. oryzae*
8/2021	A2	18.1 °C	M21	cc	x	xx	xx	x	x	x	w	x	-	D1	*D. oryzae*
			M24	cc	xx	xx	x	x	x	x	w	x	-	D1	*D. oryzae*
Barral	A3	17 °C	M33	cc	w	xx	xx	xx	x	w	-	x	-	D3	*D. chrysanthemi*
8/2021			M35	cc	x	x	x	w	x	x	-	x	-	D3	*D. chrysanthemi*
			M36	cc	w	w	x	w	x	x	-	x	-	D3	*D. chrysanthemi*
	A7	18.8 °C	M71	cc	xx	xx	w	x	w	w	w	x	-	D1	*D. oryzae*
			M72	cc	x	x	x	w	w	w	w	x	-	D1	*D. oryzae*
			M76	cc	x	x	x	w	w	w	-	x	-	D3	*D. chrysanthemi*
			M77	cc	x	x	x	w	w	w	-	x	-	D3	*D. chrysanthemi*
	A8	18.5 °C	M81	cc	x	xx	x	w	w	w	w	x	-	D1	*D. oryzae*
			M83	cc	xx	xx	w	w	w	w	w	x	-	D1	*D. oryzae*
			M85	cc	x	w	x	xx	xx	-	-	x	-	P1	*P. quasiaquaticum*
			M86	cc	xx	xx	x	x	x	x	-	x	-	D3	*D. chrysanthemi*
			M87	cc	xx	xx	x	x	x	x	-	x	-	D3	*D. chrysanthemi*
			M813	cc	x	w	x	xx	xx	-	-	x	-	D1	*P. quasiaquaticum*
Fulye	A4	16.7 °C	M42	cc	xx	xx	xx	-	w	-	w	x	-	D2	*D. oryzae* Mel-Prt-
8/2021			M45	cc	xx	xx	xx	-	w	-	w	x	-	D2	*D. oryzae* Mel-Prt-
	A5	17.4 °C	M52	cc	xx	xx	xx	x	w	w	w	x	-	D1	*D. oryzae*
			M53	cc	xx	x	x	xx	w	w	w	x	-	D1	*D. oryzae*
			M54	cc	xx	x	x	-	w	-	w	x	-	D2	*D. oryzae* Mel-Prt-
			M55	cc	xx	x	x	xx	w	w	w	x	-	D1	*D. oryzae*
			M57	cc	x	x	x	w	x	w	-	x	-	D3	*D. chrysanthemi*
	A6	17 °C	M61	cc	xx	xx	xx	-	w	-	w	x	-	D2	*D. oryzae* Mel-Prt-
			M68	cc	w	x	x	-	w	-	w	x	-	D2	*D. oryzae* Mel-Prt-

3.3. Assignment of Pectobacteriaceae at the Species Level Using GapA Sequencing

In order to precise the species of the pectinolytic isolates, we used the partial sequence of the moderately evolving gene *gapA*, shown to be a reliable phylogenetic marker for *Pectobacteriaceae* members from plant or environmental origin [6,18]. Since only a few isolates were recovered in March, they were all included in this analysis. After PCR amplification, high quality *gapA* sequences were obtained for 77 isolates from lake water and 3 isolates from the marsh Marterin (Table 2, sequence data in Supplementary Materials). Phylogenetic analysis based on *gapA* sequences showed that these isolates are distributed into different clades, each comprising the type strain of a *Dickeya* or *Pectobacterium* species (Figures 1 and 2). This phylogenetic analysis was consistent with the phenotypic classification, allowing the affectation of species to each phenotypic class (Figures 1 and 2, Tables 2 and S1). Class D1 strains were *D. oryzae* members. Class D2 strains, showing an atypical phenotype, form a subclade of the *D. oryzae* clade, showing the existence of a particular *D. oryzae* subpopulation unable to assimilate melibiose and to secrete proteases. This assignment was verified by the determination of the *recA* sequence of ten D2 isolates,

showing their genetic homogeneity and classification within the species *D. oryzae* (sequence data in Supplementary Materials).

Class D3 strains belonged to the species *D. chrysanthemi*. Class D4 members belonged to the species *D. aquatica*. Class P1 strains were either *P. aquaticum* or *P. quasiaquaticum* members, two close species that could not be differentiated by phenotypic analysis. The class P2 isolate was identified as *P. versatile.*

Based on this *gapA* phylogenetic analysis, the 77 isolates from lake water were distributed into 55 *Dickeya* strains (71%) and 22 *Pectobacterium* strains (29%). The *Dickeya* isolates belonged to the species *D. oryzae* (53%), *D. chrysanthemi* (12%), and *D. aquatica* (6%). The *Pectobacterium* isolates were assigned to the species *P. quasiaquaticum* (23%), *P. aquaticum* (4%) and *P. versatile* (1%) (Tables 2 and S3).

When considering the classification by phenotypic analysis of the 163 lake isolates, they were distributed in 130 *Dickeya* isolates (80%) and 33 *Pectobacterium* isolates (20%) (Table S3). At the species level, *D. oryzae*, *D. chrysanthemi*, and *D. aquatica* represented 66%, 7%, and 6% of the *Pectobacteriaceae* population, respectively (Table S3 and Figure 3). *P. quasiaquaticum*, *P. aquaticum*, and *P. versatile* represented 18%, 2%, and 0.6% of the water isolates, respectively (Table S3 and Figure 3). In contrast to the lakes, the marsh Marterin contained only two species, *P. quasiaquaticum* (62%) and *D. chrysanthemi* (38%) (Tables 2 and S2).

About 43% of the water samples (12 out of 28) included diverse species of *Pectobacteriaceae* (Table 3). Members of both genera *Dickeya* and *Pectobacterium* were recovered in seven samples. The highest diversity was observed in a sample from Grand Etang (M1), including four species: *P. quasiaquaticum*, *P. aquaticum*, *D. oryzae*, and *D. aquatica* (Table 3). A sample from Barral (A8) contained three species, *P. quasiaquaticum*, *D. oryzae*, and *D. chrysanthemi.* In five samples, *P. quasiaquaticum* cohabited with either *D. oryzae* (J1 and J2) or *D. chrysanthemi* (J4), *P. aquaticum* coexisted with *D. oryzae* (M6), and *P. versatile* with *D. oryzae* (J3). Five other samples enclosed two *Dickeya* species, *D. oryzae* cohabiting along with either *D. chrysanthemi* (S5, A5 and A7) or *D. aquatica* (S8 and S9). Thus, various species, or even two genera of *Pectobacteriaceae*, frequently coexist in small volumes of lake water (15 to 45 mL).

Table 3. Co-occurrence in a sample of several *Pectobacteriaceae* species and/or genera.

Sample Information				***Pectobacteriaceae* Identified in the Sample**	
Number	**Lake**	**Date**	**Temperature**	***Dickeya* Species**	***Pectobacterium* Species**
M1	Grand Etang	March 2021	12 °C	*D. oryzae*, *D. aquatica*	*P. quasiaquaticum*, *P. aquaticum*
A8	Barral	August 2021	18.5 °C	*D. oryzae*, *D. chrysanthemi*	*P. quasiaquaticum*
J1	Grand Etang	June 2020	19.5 °C	*D. oryzae*	*P. quasiaquaticum*
J2	Barral	June 2020	18.8 °C	*D. oryzae*	*P. quasiaquaticum*
J4	Marterin	June 2020	17.2 °C	*D. chrysanthemi*	*P. quasiaquaticum*
M6	Barral	March 2021	11.4 °C	*D. oryzae*	*P. aquaticum*
J3	Fulye	June 2020	17 °C	*D. oryzae*	*P. versatile*
S5	Fulye	September 2020	21.2 °C	*D. oryzae*, *D. chrysanthemi*	
A5	Fulye	August 2021	17.4 °C	*D. oryzae*, *D. chrysanthemi*	
A7	Barral	August 2021	18.8 °C	*D. oryzae*, *D. chrysanthemi*	
S8	Barral	September 2020	19.9 °C	*D. oryzae*, *D. aquatica*	
S9	Grand Etang	September 2020	20.6 °C	*D. oryzae*, *D. aquatica*	

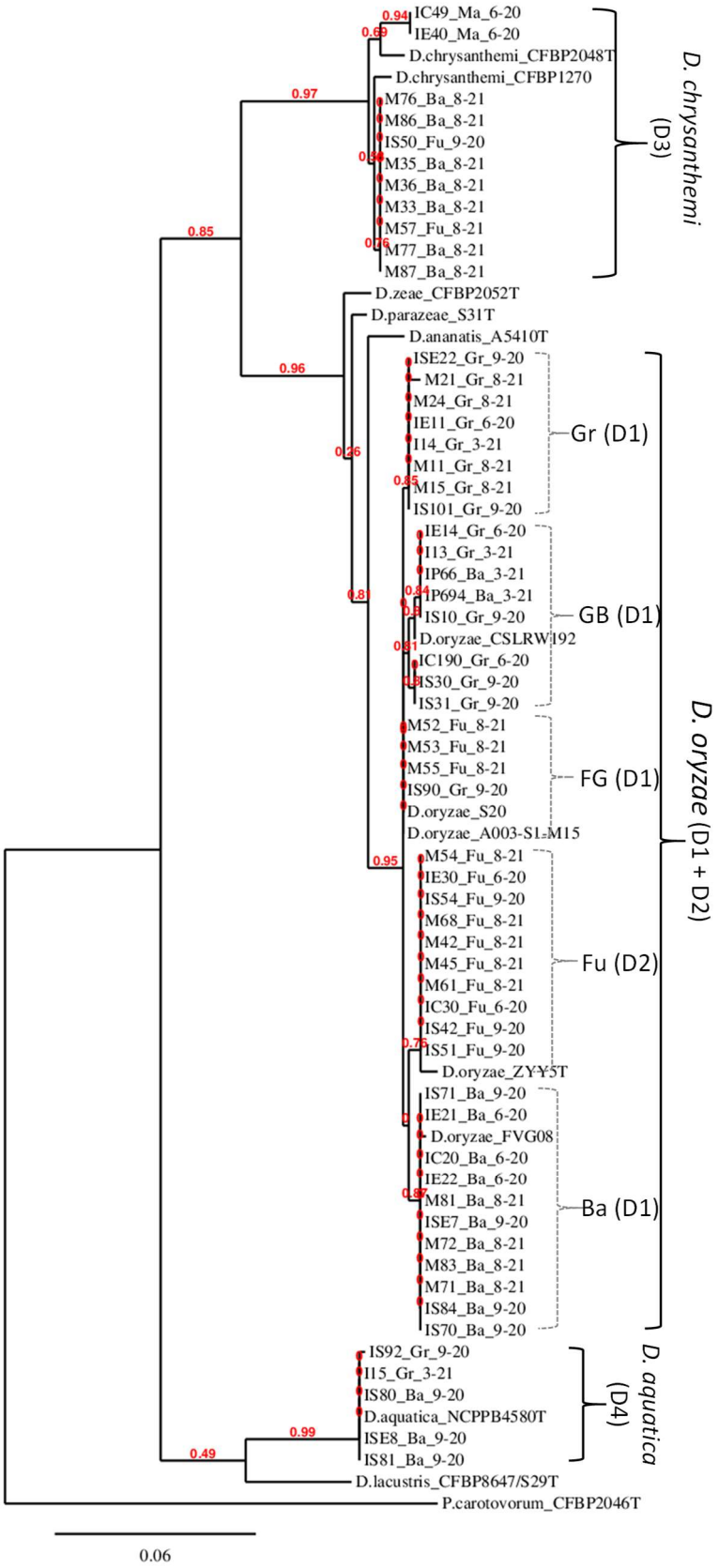

Figure 1. The *gapA* phylogenetic tree of the *Dickeya* isolates. This analysis was performed using sequences of the *gapA* PCR product of the water isolates and from some reference strains, including the type strain of each reported species. The phenotypic class (D1 to D4) is indicated for each species and for each *D. oryzae* phylogroup (PGr, PGB, PFG, PFu and PBa). The phylogenetic tree was generated with PhyML, branch support values were calculated using Ultrafast Bootstrap (5000 replicates), and they are shown in red above each branch (range 0 to 1).

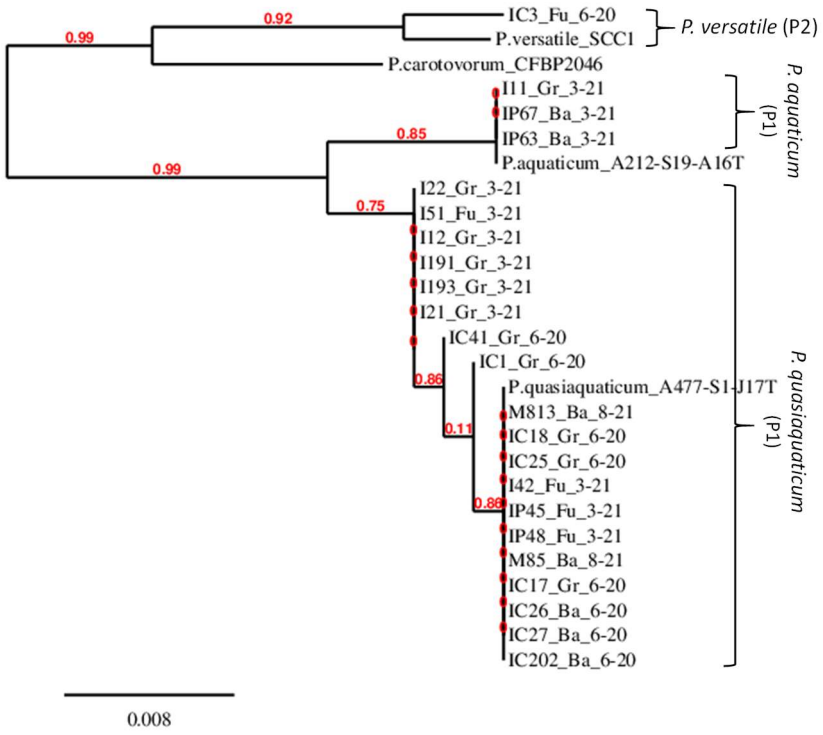

Figure 2. The *gapA* phylogenetic tree of the *Pectobacterium* isolates. This analysis was performed using sequences of the *gapA* PCR product of the water isolates and some reference strains. The phenotypic class (P1 or P2) is indicated for each species.

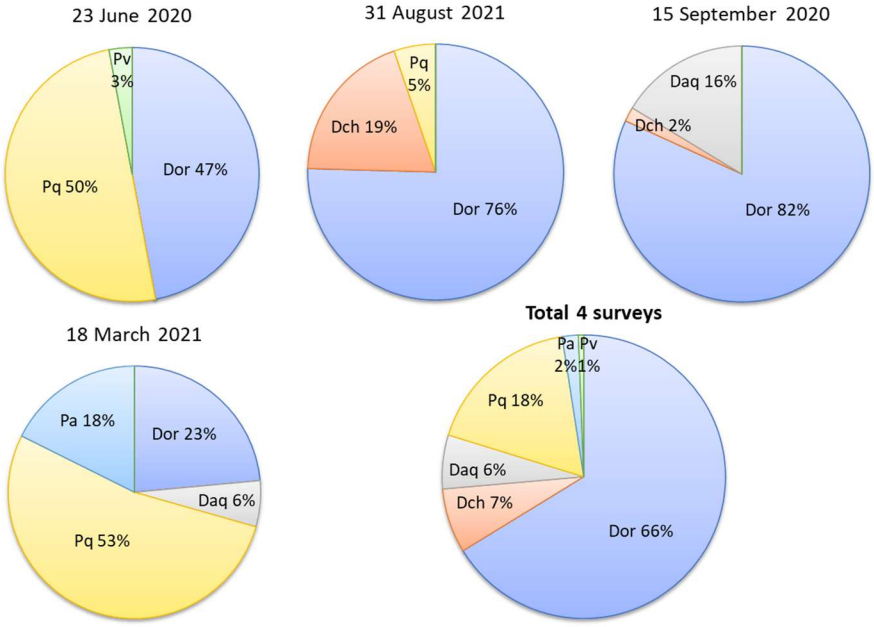

Figure 3. Seasonal distribution of the six *Dickeya* or *Pectobacterium* species. The distribution of the different species is given for each survey and for the total of the four surveys. This figure includes the isolates from the three lakes but not those from the marsh Marterin. Dor, *D. oryzae*; Dch, *D. chrysanthemi*; Daq, *D. aquatica*; Pa, *P. aquaticum*, Pq, *P. quasiaquaticum*, Pv, *P. versatile*.

3.4. Seasonal Repartition of the Two Pectobacteriaceae Genera

Considering the global data of the four surveys, lake water contained more *Dickeya* than *Pectobacterium* but notable differences were observed depending on the sampling season, showing population evolution during the year (Figure 3). At the end of winter, in March, the *Pectobacteriaceae* population contained mostly *Pectobacterium* (71%) consisting of *P. quasiaquaticum* (53%) and *P. aquaticum* (18%), and a minority of

Dickeya members (29%) consisting of *D. oryzae* (23%) and *D. aquatica* (6%) (Figure 3). At the beginning of summer, in June, nearly equilibrium was observed between the *Dickeya* and *Pectobacterium* populations, with ratios of 53% and 47%, respectively (Figure 3). The dominant species were *D. oryzae* and *P. quasiaquaticum.* The *Pectobacterium* proportion fell to 5% in August, and none were isolated in September (Figure 3). In late summer, *D. oryzae* was undoubtedly the major *Pectobacteriaceae* species, reaching 76% and 82% of the isolates in August and September, respectively.

3.5. Species Distribution According to Each Lake

The *Pectobacteriaceae* populations varied depending on the lake considered, each lake containing four to five species (Figure 4). Grand Etang, which is a large and deep lake, contained a large majority of *D. oryzae* (74%), a significant proportion of *P. quasiaquaticum* (21%), a few *P. aquaticum* (3%), and a few *D. aquatica* (2%). Fulye, which is a small and deep lake, also contained a large majority of *D. oryzae* (85%) together with some *P. quasiaquaticum* (9%), a few *D. chrysanthemi* (4%), and the sole *P. versatile* isolate found in this study (2%). The *Dickeya* population of Barral, which is very shallow and naturally eutrophic, was quite different, as the *D. oryzae* proportion decreased to 42% concomitantly with increased proportions of *D. chrysanthemi* (18%) and *D. aquatica* (14%). Lake Barral also contained *P. quasiaquaticum* (22%) and *P. aquaticum* (4%).

The differences in the distribution of *Pectobacteriaceae* species in each lake (Figure 4) could be linked to specific characteristics. Grand Etang or Fulye are deeper than Barral, and their clear water contrasts with the entropic aspect of Lake Barral, which supports dense aquatic vegetation. In the three lakes, *D. oryzae* was the predominant species, but with a higher abundance in Grand Etang or Fulye (74% and 85%, respectively) than in Barral (42%). Notably, no *D. oryzae* isolates were found in the marsh Marterin, but the frequency of *D. chrysanthemi* was clearly higher in the marsh Marterin (38%) than in the lakes Barral, Fulye and Grand Etang (18%, 4% and 0%, respectively). Moreover, the *D. chrysanthemi* isolates from Marterin or lakes formed two subclades in the *gapA* phylogenetic tree, suggesting two different *D. chrysanthemi* populations in marsh and lakes. While isolates from marsh grouped with the *D. chrysanthemi* type strain CFBP2048 (Figure 1), those from lakes Fulye or Barral grouped with strain CFBP1270, previously proposed as the reference strain of the *D. chrysanthemi* pathovar "*parthenii*" whose members were initially isolated from guayule, *Parthenium argentatum* [21].

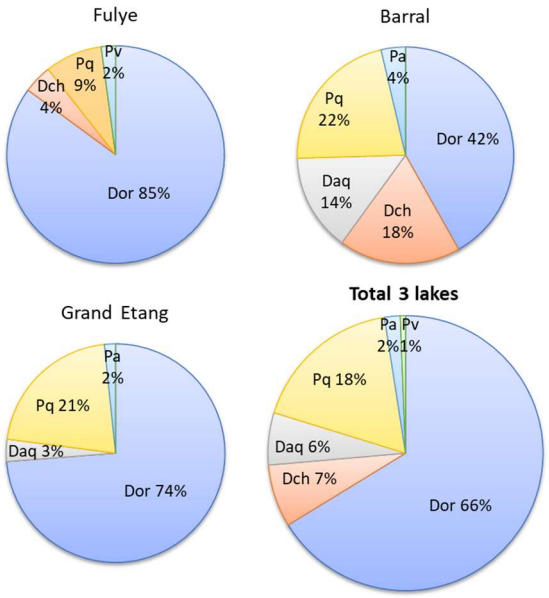

Figure 4. Distribution of the six *Dickeya* or *Pectobacterium* species according to the lake. The distribution of the different species is given for each lake and for the total of the three lakes. Grand Etang (31 ha) is the largest lake, Barral (7 ha) is shallow

(2 m maximum depth) and rich in aquatic plants, and Fulye, the smallest lake (1.5 ha), is mainly fed by an underground spring. Dor, *D. oryzae*; Dch, *D. chrysanthemi*; Daq, *D. aquatica*; Pa, *P. aquaticum*, Pq, *P. quasiaquaticum*, Pv, *P. versatile*.

3.6. Identification of D. oryzae Subpopulations by Phenotypic and Phylogenetic Analyses

D. oryzae was the major *Pectobacteriaceae* species isolated from each lake in summer (Figures 3 and 4). Most *D. oryzae* isolates (66%) possessed the expected phenotype of class D1 (Table 2). The *gapA* phylogeny showed that the class D2 isolates also belong to the species *D. oryzae* (Figure 1 and Table 2). Thus, the atypical population of class D2 was homogeneous at the genetic level, and it corresponded to a *D. oryzae* subclade exclusively found in Lake Fulye.

Further examination of the *gapA* phylogenetic tree revealed the presence of five *D. oryzae* phylogroups, which were designed by PFu, PGr, PBa, PFG, and PGB (Figures 1 and 5). Notably, three of these phylogroups appeared to be lake-specific. The phylogroup PFu strictly corresponded to the *D. oryzae* atypical subpopulation of class D2 found in lake Fulye. The phylogroup PBa was found only in lake Barral, and all the PGr members originated from Grand Etang (Figure 1). Each of these lake-specific groups composed the major population of its hosting lake (Figure 5). The two other phylogroups were present in two lakes. The group PFG contained a minor *D. oryzae* subpopulation of lake Fulye and one isolate from Grand Etang (Figure 5). The group PGB contained a minor *D. oryzae* subpopulation of Grand Etang and a few members of lake Barral. Despite the global observation that the three lakes hosted a majority of *D. oryzae*, the distribution of each *D. oryzae* phylogroup largely differed in each lake (Figure 5).

The phylogenetic analysis was extended to the *gapA* gene of all *D. oryzae* strains whose genome has been previously sequenced, allowing the identification of additional members of some phylogroups (Figure 1). The *D. oryzae* type strain ZYY5, isolated from rice in China [23], was associated with phylogroup PFu. The group PBa included strain FVG08 isolated from a water channel connected to the Durance River in France [24]. The group PFG contained strain A003-S1-M15 isolated from the Durance River [6]. The group PGB included strain CSLRW192 isolated from water in England [25]. Thus, the phylogroups observed in this study are not specific of the Mepieu site as other members were previously reported in other regions or countries, from either water or rice.

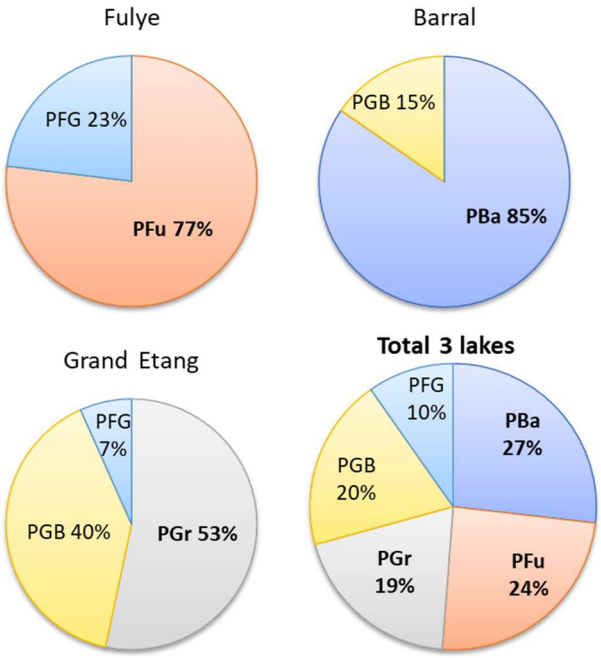

Figure 5. Distribution of the five *D. oryzae* phylogroups in each lake. The *gapA* phylogeny showed the presence of five *D. oryzae* phylogroups: PFu, population of the phenotypic class D2 found in lake Fulye; PBa, phylogroup specific to Barral; PGr,

phylogroup specific to Grand Etang; PFG, minor subpopulation of lake Fulye and one isolate from Grand Etang; PGB, subpopulation of Grand Etang and a few members of lake Barral. The three lake-specific populations are shown in bold letters.

The *D. oryzae* atypical isolates corresponding to the phenotypic class D2 and phylogroup PFu were collected in lake Fulye both in summer 2020 and 2021 (Figure 1, Tables 2 and S2). Similarly, the *D. oryzae* phylogroups PBa and PGr, specific of lakes Barral and Grand Etang, respectively, were observed in surveys performed during the two successive years, in summers 2020 and 2021 (Figure 1). These observations showed that each lake maintained its *D. oryzae* specific population in two successive years.

4. Discussion

The affinity of *Dickeya* and *Pectobacterium* members for aquatic environments is quite well documented. While in older studies, the identification at the species level is largely outdated [26], recent surveys have disclosed the diversity of *Pectobacteriaceae* species in surface water [5–7]. In this study performed in three lakes of the nature reserve of Mepieu, pectinolytic isolates were observed in most water samples (93%). The two genera *Dickeya* and *Pectobacterium* represented 80% and 20%, respectively, of the *Pectobacteriaceae* isolates, and six species were identified. Moreover, both *Dickeya* and *Pectobacterium* members were frequently isolated from the same water sample, indicating their ability to coexist in small volumes of lake water (Table 3).

Out of 163 *Pectobacteriaceae* isolates, *Dickeya* members were assigned to the species *D. oryzae* (67%), *D. chrysanthemi* (7%), and *D. aquatica* (6%) (Figure 3). The genus *Pectobacterium* included *P. quasiaquaticum* (18%) *P. aquaticum* (2%), and *P. versatile* (0.6%) (Figure 3). All these species were also observed in other recent studies of lake or river waters. The surveys of surface waters performed in Poland showed the presence of two species, *D. oryzae* (strains initially classified as *D. zeae*) and *D. chrysanthemi,* representing 85% and 15% of the *Dickeya* isolates, respectively [5]. In the surveys along the Durance River, *Dickeya* accounted for only 6% of the *Pectobacteriaceae* isolates, including mainly *D. oryzae* and *D. chrysanthemi* (69% and 16% of the *Dickeya* isolates, respectively) [6]. In the surveys of lakes in La Dombes, the genus *Dickeya* constituted 82% of the *Pectobacteriaceae* isolates, distributed among six species, *D. oryzae* (47%), *D. lacustris*, *D. parazeae*, *D. chrysanthemi*, *D. aquatica* and *D. zeae* [7]. In our study of the three Mepieu lakes, the species *D. oryzae* also dominated, representing 67% of the *Pectobacteriaceae* and 84% of the *Dickeya* isolates from lake water (Figures 1 and 3). Thus, considering these four recent studies, *D. oryzae* was the predominant *Dickeya* species in surface waters. Given that the species *D. oryzae* is a plant pathogen primarily isolated from maize, rice, or potato [22], the risk of crop contamination by water should be taken into account.

In the survey of the Durance River, *Pectobacterium* accounted for 94% of the isolates assigned to nine species, including the most abundant species *P. versatile*, and *P. aquaticum,* but also *P. carotovorum* and *P. quasiaquaticum* (47%, 40%, 6%, and 2% of the *Pectobacterium* isolates, respectively) [6]. Four *Pectobacterium* species were recovered from lake water of La Dombes, including *P. quasiaquaticum*, *P. aquaticum*, and *P. brasiliense* (58%, 25%, and 17% of the *Pectobacterium* isolates, respectively) [7]. During our study of the Mepieu lakes, *P. quasiaquaticum* was clearly the most abundant *Pectobacterium* species (83%), alongside some isolates of *P. aquaticum* and *P. versatile* (Figures 2 and 3). Considering these recent studies, *P. versatile* and *P. aquaticum* were the dominant *Pectobacterium* species in the Durance River, but *P. quasiaquaticum* dominated in lakes waters. Additional data will be necessary to evaluate the potential preference of each *Pectobacterium* species for the different types of aquatic systems.

In the Mepieu surveys, we observed notable differences in the ratio between the two genera *Dickeya* and *Pectobacterium* depending on the sampling date, with a clear population evolution over the course of the year. While *Pectobacterium* members were dominant at the end of winter, their proportion decreased in early summer until becoming barely or undetectable by the end of summer, when the genus *Dickeya*

became largely dominant (Figure 3). This evolution in the population may result from seasonal temperature changes. Under laboratory conditions, *Pectobacterium* members were shown to grow better than *Dickeya* members at a low temperature (8 °C) [6] and, conversely, *Dickeya* members better tolerate high temperatures (41–43 °C) than *Pectobacterium* members [7]. In the Durance River, members of the genus *Pectobacterium* were isolated at temperatures of 3.6 to 22.6 °C from February to November, and *Dickeya* members were isolated only at temperatures above 19 °C, from May to August [6]. In the region La Dombes, both *Dickeya* and *Pectobacterium* were isolated between 15 and 25 °C and only *Dickeya* species were found above 25 °C [7]. In the Mepieu lakes, the genus *Pectobacterium* was dominant (71%) in March, at temperatures of 11.4 to 12 °C; the two genera were almost equivalent in June, at 17 to 19.5 °C; the genus *Dickeya* became largely predominant (95%) in August, at 16.7 to 18.6 °C, and only *Dickeya* species were recovered in September, at 19.2 to 22.2 °C (Figure 3). These studies show a disadvantage for the genus *Dickeya* at low temperatures and for the genus *Pectobacterium* at high temperatures in natural environments. In our study, the temperature ranges for June 2020 (17.0–19.5 °C), September 2020 (19.2–22.2 °C) and August 2021 (16.7–18.8 °C) overlapped, but the proportion of *Dickeya* increased from 47% in June to 76–82% in August and September (Table 1 and Figure 3). 23 June falls at the very beginning of summer, while 31 August and 15 September, towards the end of summer, follow the warmest weeks of that season. Therefore, an overall effect of seasonality could be observed rather than an effect related to the temperatures recorded at the sampling time. These observations highlight the importance not only of the temperature at the time of sampling, but also of the temperatures of the preceding days and weeks, which influenced bacterial growth and led to the recorded results. Thus, this study suggests that the *Pectobacterium*/*Dickeya* population shift could result from an overall effect of seasonal temperature variations.

While the species *D. aquatica* or *P. aquaticum* appeared to be very homogeneous in the *gapA* phylogenetic tree, several subgroups were observed in the *D. chrysanthemi*, *D. oryzae*, and *P. quasiaquaticum* clades, suggesting intraspecies diversity (Figures 1 and 2). Two phylogroups *D. chrysanthemi*, were observed, corresponding to two different aquatic systems, marsh or lake (Figure 1). While the marsh isolates grouped with the *D. chrysanthemi* type strain, the lake isolates grouped with strain CFBP1270, proposed as the reference strain of the *D. chrysanthemi* pathovar "*parthenii*" [21].

The *D. oryzae* isolates were distributed into five phylogroups, and interestingly, some of them appeared to be lake-specific. Notably, other members of these phylogroups were previously isolated in other regions or countries, either from water (CSLRW192, A003-S1-M15 and FVG08) or from rice for the *D. oryzae* type strain ZYY5 (Figure 1). The *D. oryzae* phylogroup PFu, observed only in Lake Fulye, corresponds to the subpopulation of the phenotypic class D2, noticed for its atypical profile. The *D. oryzae* phylogroup PBa included isolates originating only from Barral, and the phylogroup PGr was specific to Grand Etang (Figure 1). The existence of specific subpopulations of *D. oryzae* in each of these three neighboring lakes suggests that there are limited exchanges between them, whereas bacteria could have been transported by water runoff, wind, insects, or animals. While the lake-specific phylogroups represented the major population of each lake, two other phylogroups were present as minor populations in two lakes (PGF in Grand Etang and Fulye; PGB in Grand Etang and Barral, Figure 5).

Interestingly, the *D. oryzae* specific population for each lake was found in the same lake during surveys conducted over two consecutive years. As first observed for the atypical phylogroup PFu specific to Fulye, the phylogroup PBa specific to Barral, and the phylogroup PGr specific to Grand Etang, each was observed in both 2020 and 2021 (Figures 1 and 2), indicating that the different D. oryzae populations inhabiting each lake persisted across consecutive years. The fact that each specific population was recovered the following year strongly suggested that it did not originate from an external source, but rather from the maintenance of resident hibernating bacteria. The *D. oryzae* populations specific to each lake would spend the winter within their host lake, and this resident population would begin to multiply again when the temperature increases the following year. Although their means of resisting the winter period remain poorly understood,

this study indicates that *D. oryzae* populations present in lakes survive the winter season to renew in the same place the following year.

Supplementary Materials

The following supporting information can be found at: https://www.sciepublish.com/article/pii/1334, Figure S1: View of the three lakes of the nature reserve of Mepieu; Table S1: Detailed information, for each lake, on the samples taken in the Mepieu reserve; Table S2: Phenotypic analysis of the 176 SRP isolates from the four surveys in the Mepieu site. Table S3. Classification by phenotypic and phylogenetic analysis of 163 isolates from lake water. List of the *gapA* sequence of the *Dickeya* isolates and reference strains. List of the *gapA* sequence of the *Pectobacterium* isolates and reference strains. List of the *recA* sequence of ten D2 isolates from Lake Fulye and *D. oryzae* reference strains, including the corresponding phylogenetic tree.

Acknowledgments

We thank Raphael Quesada of the association Lo Parvi for introducing us to the Réserve Naturelle des Etangs de Mepieu, Marie-Anne Barny for helpful discussions on water-associated pectinolytic bacteria, and our team members (MTSB MAP) for their interest in this work. We also acknowledge Jihane Mennana of the platform DTAMB of FR3728 (UCBL, CNRS) for technical assistance and Sebastien Santini (CNRS/AMU IGS UMR7256) and the PACA Bioinfo platform for the availability and management of the website https://phylogeny.fr (accessed on 1 June 2026).

Author Contributions

Conceptualization, N.H.-C.-P. and V.U.; Methodology, V.U.; Validation, N.H.-C.-P. and V.U.; Formal Analysis, N.H.-C.-P. and V.U.; Investigation, V.U.; Writing—Original Draft Preparation, N.H.-C.-P.; Writing—Review & Editing, N.H.-C.-P. and V.U.; Supervision, N.H.-C.-P.

Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The datasets supporting the conclusions are included in the article supplementary material.

Funding

This work was funded by core grants from the CNRS, Université Lyon 1 and INSA Lyon allocated to the MAP laboratory (UMR5240). It received no external funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Ma B, Hibbing ME, Kim HS, Reedy RM, Yedidia I, Breuer J, et al. Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya*. *Phytopathology* **2007**, *97*, 1150–1163. DOI:10.1094/PHYTO-97-9-1150
2. Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 5607–5612. DOI:10.1099/ijsem.0.004332
3. van der Wolf JM, Acuña I, De Boer SH, Brurberg MB, Cahill G, Charkowski AO, et al. Diseases caused by *Pectobacterium* and *Dickeya* species around the world. In *Plant Diseases Caused by Dickeya and Pectobacterium Species*; Van Gijsegem F, van der Wolf JM, Toth IK, Eds.; Springer Nature: Cham, Switzerland, 2021; pp. 215–261.
4. Broders K, Aspin A, Bailey J, Chapman T, Portier P, Weir BS. Building more resilient culture collections: A call for increased deposits of plant-associated bacteria. *Microorganisms* **2022**, *10*, 741. DOI:10.3390/microorganisms10040741
5. Potrykus M, Golanowska M, Sledz W, Zoledowska S, Motyka A, Kolodziejska A, et al. Biodiversity of *Dickeya* spp. isolated from potato plants and water sources in temperate climate. *Plant Dis.* **2016**, *100*, 408–417. DOI:10.1094/PDIS-04-15-0439-RE
6. Ben Moussa H, Bertrand C, Rochelle-Newall E, Fiorini S, Pédron J, Barny MA. The diversity and abundance of soft rot *Pectobacteriaceae* along the Durance River stream in the Southeast of France revealed by multiple seasonal surveys. *Phytopathology* **2022**, *112*, 1676–1685. DOI:10.1094/PHYTO-12-21-0515-R
7. Hugouvieux-Cotte-Pattat N, Utzinger V. Occurrence of *Dickeya* and *Pectobacterium* in lake water and the rhizosphere of waterside plants collected in the French region La Dombes. *Microorganisms* **2026**, *14*, 1459. DOI:10.3390/microorganisms14071459
8. Parkinson N, DeVos P, Pirhonen M, Elphinstone J. *Dickeya aquatica* sp. nov., isolated from waterways. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 2264–2266. DOI:10.1099/ijs.0.058693-0
9. Hugouvieux-Cotte-Pattat N, Jacot-des-Combes C, Briolay J. *Dickeya lacustris* sp. nov., a water-living pectinolytic bacterium isolated from lakes in France. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 721–726. DOI:10.1099/ijsem.0.003208
10. Oulghazi S, Pédron J, Cigna J, Lau YY, Moumni M, Van Gijsegem F, et al. *Dickeya undicola* sp. nov., a novel species for pectinolytic isolates from surface waters in Europe and Asia. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 2440–2444. DOI:10.1099/ijsem.0.003497
11. Oulghazi S, Cigna J, Lau YY, Moumni M, Chan KG, Faure D. Transfer of the waterfall source isolate *Pectobacterium carotovorum* M022 to *Pectobacterium fontis* sp. nov., a deep-branching species within the genus *Pectobacterium*. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 470–475. DOI:10.1099/ijsem.0.003180
12. Waleron M, Misztak A, Waleron M, Jonca J, Furmaniak M, Waleron K. *Pectobacterium polonicum* sp. nov. isolated from vegetable fields. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 1751–1759. DOI:10.1099/ijsem.0.003387
13. Pédron J, Bertrand C, Taghouti G, Portier P, Barny MA. *Pectobacterium aquaticum* sp. nov., isolated from waterways. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 745–751. DOI:10.1099/ijsem.0.003229
14. Ben Moussa H, Pédron J, Bertrand C, Hecquet A, Barny MA. *Pectobacterium quasiaquaticum* sp. nov., isolated from waterways. *Int. J. Syst. Evol. Microbiol.* **2021**, *71*, 005042. DOI:10.1099/ijsem.0.005042
15. Helias V, Hamon P, Huchet E, Wolf JVD, Andrivon D. Two new effective semiselective crystal violet pectate media for isolation of *Pectobacterium* and *Dickeya*. *Plant Pathol.* **2012**, *61*, 339–345. DOI:10.1111/j.1365-3059.2011.02508.x
16. Meneley JC, Stanghellini ME. Isolation of soft-rot *Erwinia* spp. from agricultural soils using an enrichment technique. *Phytopathology* **1976**, *66*, 367–370. DOI:10.1094/PHYTO-66-367
17. Ben Moussa H, Pédron J, Hugouvieux-Cotte-Pattat N, Barny MA. Two species with a peculiar evolution within the genus *Pectobacterium* suggest adaptation to a new environmental niche. *Environ. Microbiol.* **2023**, *25*, 2465–2480. DOI:10.1111/1462-2920.16479
18. Cigna J, Dewaegeneire P, Beury A, Gobert V, Faure D. A *gapA* PCR-sequencing assay for identifying the *Dickeya* and *Pectobacterium* potato pathogens. *Plant Dis.* **2017**, *101*, 1278–1282. DOI:10.1094/PDIS-12-16-1810-RE
19. Waleron M, Waleron K, Podhajska AJ, Łojkowska E. Genotyping of bacteria belonging to the former *Erwinia* genus by PCR-RFLP analysis of a *recA* gene fragment. *Microbiology* **2002**, *148*, 583–595. DOI:10.1099/00221287-148-2-583
20. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, et al. Phylogeny.fr: Robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* **2008**, *36*, W465–W469. DOI:10.1093/nar/gkn180
21. Samson R, Legendre JB, Christen R, Saux MF, Achouak W, Gardan L. Transfer of *Pectobacterium chrysanthemi* (Burkholder et al. 1953) Brenner et al. 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp.

nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 1415–1427. DOI:10.1099/ijs.0.02791-0

22. Hugouvieux-Cotte-Pattat N, Pédron J, Van Gijsegem F. Insight into biodiversity of the recently rearranged genus *Dickeya*. *Front. Plant Sci.* **2023**, *14*, 1168480. DOI:10.3389/fpls.2023.1168480
23. Wang X, He SW, Guo HB, Han JG, Thin KK, Gao JS, et al. *Dickeya oryzae* sp. nov., isolated from the roots of rice. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 4171–4178. DOI:10.1099/ijsem.0.004265
24. Hugouvieux-Cotte-Pattat N, Van Gijsegem F. Diversity within the *Dickeya zeae* complex, identification of *Dickeya zeae* and *Dickeya oryzae* members, proposal of the novel species *Dickeya parazeae* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2021**, *71*, 005059. DOI:10.1099/ijsem.0.005059
25. Pritchard L, Humphris S, Saddler GS, Elphinstone JG, Pirhonen M, Toth IK. Draft genome sequences of 17 isolates of the plant pathogenic bacterium *Dickeya*. *Genome Announc.* **2013**, *1*, e00978-13. DOI:10.1128/genomeA.00978-13
26. Toth IK, Barny M, Brurberg MB, Condemine G, Czajkowski R, Elphinstone JG, et al. *Pectobacterium* and *Dickeya*: Taxonomy and evolution. In *Plant Diseases Caused by Dickeya and Pectobacterium Species*; Van Gijsegem F, van der Wolf JM, Toth IK, Eds.; Springer Nature: Cham, Switzerland, 2021; pp. 13–37.