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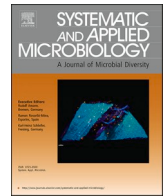
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## Description of a new genus of the *Pectobacteriaceae* family isolated from water in coastal brackish wetlands of the French Camargue region, *Prodigiosinella* gen. nov., including the new species *Prodigiosinella aquatilis* sp. nov

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### ABSTRACT

The *Pectobacteriaceae* family comprises plant pathogens able to provoke diverse diseases, including plant maceration due to the production of pectinases disrupting the plant cell wall. To better understand their diversity, a survey of pectinolytic bacteria was performed in brackish lakes of the French region La Camargue near the Mediterranean Sea. The genome of six atypical isolates was sequenced; their size is around 4.8 to 5.0 Mb, including a plasmid of 59 to 61 kb; their G+C values range from 49.1 to 49.3 mol%. Phylogenetic analyses indicated that the novel strains form a new clade of *Pectobacteriaceae* that branches at the basis of the group encompassing the genera *Lonsdalea*, *Musicola*, and *Dickeya*. Based on phenotypic, genomic and phylogenetic characteristics, we propose the creation of a new genus with the name *Prodigiosinella* gen. nov. Both the phenotypic and phylogenetic analyses separated the strains into two distinct subgroups, G1 and G2. The type strain LS101<sup>T</sup> (CFBP 8826<sup>T</sup> = LMG 32072<sup>T</sup>) and strain CE70 (CFBP 9054 = LMG 32867) are representative G1 and G2 members, respectively. Three genomic methods were used to analyze DNA–DNA relatedness: digital DNA–DNA hybridization (isDDH), average nucleotide identity (ANI), and genome alignment fraction (AF). They revealed a close relationship between genomes of the two groups, supporting their appurtenance to a same species for which we propose the name *Prodigiosinella aquatilis* sp. nov. Four strains previously designated as *Serratia* sp. (ATCC 39006), *Brenneria* “*ulupoensis*” (K61) or *Erwinia* sp. (MK01 and MK09) belong to the new genus *Prodigiosinella*.

### Introduction

The bacterial order *Enterobacterales* has been created in 2016 and is currently divided into eight recognized families: *Budviciaceae*, *Enterobacteriaceae*, *Erwiniaceae*, *Hafniaceae*, *Morganellaceae*, *Pectobacteriaceae*, *Thorselliaceae*, and *Yersiniaceae* (Adeolu et al., 2016; Kampf et al., 2015). A ninth family was more recently proposed with the not yet

validated name “*Bruguierivoracaceae*” (Li et al., 2021). Among them, *Pectobacteriaceae* currently comprises the eight genera: *Acerihabitans* (Lee et al., 2021), *Affinibrenneria* (Bian et al., 2021), *Brenneria* (Brady et al., 2012), *Dickeya* (Samson et al., 2005), *Lonsdalea* (Brady et al., 2012), *Musicola* (Hugouvieux-Cotte-Pattat et al., 2021), *Pectobacterium* (Hauben et al., 1998), and *Samsonia* (Sutra et al., 2001), some of them being major plant pathogens. In addition, the genus

**Abbreviations:** ANI, average nucleotide identity; uBV, ultrafast bootstrap value; CAZY, Carbohydrate-Active enZYmes; CE, carbohydrate esterase; CVP medium, crystal violet pectate medium; dDDH, digital DNA–DNA hybridization; GH, glycoside hydrolase; JV, Jackknife value; LB, Luria-Bertani medium; ML tree, maximum likelihood tree; NCBI, National Center for Biotechnology Information; OD<sub>600</sub>, optical density at 600 nm; PGAP, prokaryotic genome annotation pipeline; PL, polysaccharide lyase; T2SS, type II secretion system, T3SS, type III secretion system.

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*Symbiopectobacterium* was recently described as being closely related to *Pectobacterium* (Nadal-Jimenez et al., 2022). Moreover, the genera *Biostraticola* and *Sodalis*, previously affected to *Pectobacteriaceae*, should be transferred to “*Bruguierivoracaceae*” (Li et al., 2021). While members of *Brenneria* and *Lonsdalea* are responsible for tree diseases (Brady et al., 2012), *Dickeya*, *Musicola*, and *Pectobacterium* affect a wide range of hosts, provoking soft-rot diseases due to the action of extracellular pectinases that degrade the plant cell wall (Hugouvieux-Cotte-Pattat et al., 2020; Van Gijsegem et al., 2021). Most of the characterized strains of these three soft-rot genera were isolated from diseased crops or ornamental plants, but some strains have also been isolated from surface water (Parkinson et al., 2014; Hugouvieux-Cotte-Pattat et al., 2019a; Oulghazi et al., 2019a; Oulghazi et al., 2019b; Waleron et al., 2019; Pédrón et al., 2019; Ben Moussa et al., 2021). A recent analysis of bacterial diversity in river water suggested that the *Pectobacteriaceae* members correspond to rare taxa in freshwater (Pédrón et al., 2020). While no *Pectobacteriaceae* were detected through direct 16S barcoding of water samples, they were recovered by a culture-based approach using a semi-selective medium (Pédrón et al., 2020). This selection method was recently used to evaluate the diversity and abundance of *Pectobacterium* and *Dickeya* species along a river stream, confirming that these phytopathogens are present in water but at low concentrations (Ben Moussa et al., 2022).

In this study, we describe sixteen new bacterial strains isolated from brackish water of lakes in the French region of La Camargue, near the Mediterranean Sea. These strains were selected on the basis of their pectinolytic activity using a semi-selective solid medium commonly used to isolate members of the genera *Dickeya* or *Pectobacterium* (Helias et al., 2012). However, the isolates showed atypical phenotypes in comparison to the known *Dickeya* or *Pectobacterium* species. The genome sequence of six of these new strains was determined. Altogether, phenotypic, genomic and phylogenetic analyses show that these isolates belong to a new genus within *Pectobacteriaceae*, for which we propose the name *Prodigosinella* gen. nov. Furthermore, phylogenetic analysis disclosed other members of this genus, including the misidentified strain *Serratia* sp. ATCC 39006 recently shown to be a member of the *Pectobacteriaceae* family (Duprey et al., 2019).

## Material and methods

### Strain isolation and ecology

Water samples were collected in the south-east of Camargue, in a conservation site “La Tour du Valat” (43.5089° N, 4.66757° E, altitude ~1 m, <https://tourduvalat.org/>) dedicated to the study and preservation of wetlands (Fraixedas et al., 2019). La Camargue is situated in the delta of the Rhone River in France, and bordered by the Mediterranean Sea. This region contains multiple lakes of various sizes, filled with either fresh or brackish water. Water samples (50 ml) were taken approximately 1 m from the lake shore and at ~0.5 m depth. Each sample was filtrated on pleated paper filters to remove particles. Serial dilutions of the sampled water were spread on Luria agar plates and, after incubation 48 h at 30 °C, colony counts were used to determine the number of colony forming units (CFU) per ml. Spreading on the semi selective medium Cristal Violet Pectate (CVP) was performed to detect pectinolytic bacteria, after concentration of the water samples. Bacteria present in 15 ml water were recovered in the pellet after centrifugation 10 min at 7000g. They were spread on CVP plates which were incubated at 30 °C and examined each day during one week for the presence of cavity-forming colonies. Each pectinolytic colony was picked up in 50 µl water, appropriately diluted in water and spread on CVP and LB media, to obtain isolated colonies. A second step of purification was performed on LB plates before phenotypic analysis.

### Growth conditions and phenotypic analysis

Growth was performed on Luria broth (LB), tryptic soy agar (TSA) and M63 medium (Miller, 1972). Colony appearance was assessed on plates solidified with 0.8 % agar. Semiaerobic conditions were obtained in LB liquid cultures layered with paraffin oil and incubated without shaking. Anaerobic conditions were obtained by incubation of LB plates in anaerobic jars (BBL GasPak® System). Cell morphology was observed by light microscopy (Zeiss) at 1000 magnification. Motility assay was performed in 0.3 % Luria agar as previously described (Hugouvieux-Cotte-Pattat et al., 2019b).

The isolates were analysed by replica plating to evaluate the utilization of different carbon sources and the production of extracellular enzymes. M63 minimal medium plates supplemented with a sole carbon source at the final concentration of 2 g l<sup>-1</sup> (D-arabinose, glycerol, D-glucose, D-mannitol, melibiose, raffinose, saccharose, D-trehalose, or D-xylose) were used to assess the growth with each carbon source. Bacterial growth was observed after 24 to 72 h at 30 °C.

Biolog plates PM1 and PM2A were used to test the assimilation of 190 potential carbon sources (Bochner, 2009). Inoculations were performed according to the manufacturer instructions and the lecture was made after 48 h at 30 °C. Rather than bacterial growth, the Biolog system detects the metabolic activity of the cells due to substrate assimilation; this activity is visualized by the color change of an oxidoreduction indicator (Bochner, 2009).

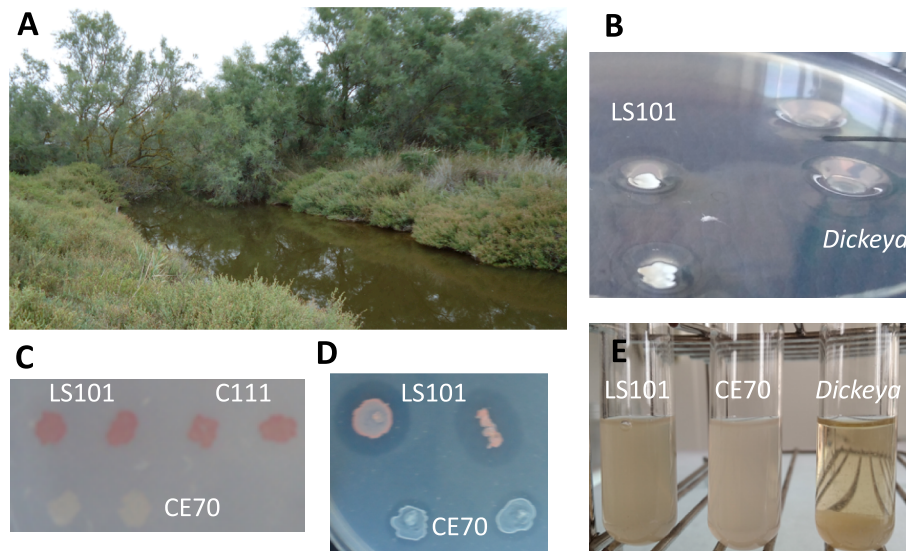
Pectinase, cellulase and protease activities were detected on substrate-containing agar plates as described in Hugouvieux-Cotte-Pattat et al., 2019b. Pectinase activity was assessed on M63 agar plates containing 4 g l<sup>-1</sup> polygalacturonic acid and glycerol 2 g l<sup>-1</sup>. After incubation for 24 h at 30 °C, plates were flooded with a saturated solution of copper acetate; clear haloes appear around colonies secreting pectate-lyases. Cellulase activity was detected on M63 agar plates supplemented with 10 g l<sup>-1</sup> carboxymethylcellulose and glycerol 2 g l<sup>-1</sup>. After incubation for 24 h at 30 °C, the plates were overflowed with 10 mg ml<sup>-1</sup> Congo red solution for 10 min and washed for 5 min with 1 M NaCl. Clear haloes surround colonies secreting cellulases. Protease production was tested on LB plates supplemented with skim milk (12.5 g l<sup>-1</sup>). After incubation at 30 °C for 24 to 48 h, clear haloes are visible around colonies secreting proteases.

Production of antibacterial compounds was tested against the *E. coli* strain BW25113 ( $\Delta tolC::Kan$ ) as described in Slater et al. 2008. Bacteria to be tested were spotted onto agar containing the *E. coli* BW25113 culture and plates were incubated at 30 °C for 24 h. Clear zone of antibiosis are visible around colonies producing antibacterial compounds.

The effect of temperature on the bacterial growth was analyzed in LB medium by incubations at temperatures ranging from 25 to 41 °C. Cell density was estimated by measuring optical density at 600 nm (OD600) at 24 h. The effect of salt concentration on the bacterial growth was analyzed at 30 °C in LB medium containing NaCl concentrations ranging from 0 to 1 M. Cell density was estimated by measuring OD600 at 24 h.

To analyse the flotation ability of the bacterial cells, the bacteria were first grown in liquid medium 24 h at 30 °C with shaking and then left for seven days at room temperature without shaking. Cells of bacterial species producing gas vesicles remain in suspension throughout the liquid, while other bacteria settle at the bottom of the tube (Tashiro et al., 2016).

Ability to macerate plant tissues was evaluated by measuring the percentage of inoculations leading to maceration and the quantity of macerated tissue obtained 48 h after inoculation on either chicory leaves or potato tubers (Hugouvieux-Cotte-Pattat et al., 2019b). *D. lacustris* and *D. dadantii* were used for comparison during analysis of these phenotypic characters.



**Fig. 1.** Isolation and characteristics of *Prodigiosinella aquatilis* strains. A, view of the Lake Poutrague (Camargue, France) where the type strain LS101<sup>T</sup> was collected. B, aspect of *Prodigiosinella* isolates on CVP medium (left) and comparison with *D. dadantii* (right). C, aspect of *Prodigiosinella* colonies on LB plates (3 µl drops). D, production of antibacterial compound(s) by strains LS101<sup>T</sup> and CE70 shown on a lawn of *E. coli* BW25113 ( $\Delta$ tolC::Kan). E, cultures in M63 liquid medium showing the flotation ability of strains LS101<sup>T</sup> and CE70; *D. dadantii* 3937 was used as a negative control (after growth, cultures were allowed to stand for 7 days).

#### PCR and amplicon sequencing

PCR amplifications were performed on bacterial cell lysates using an Illustra PuReTaq Ready-To-Go kit (GE Healthcare), using primers gapAF and gapAR specific for the gene *gapA* (0.8 kb amplicon) (Cigna et al., 2017) and primers 27f and 1492r specific for the 16S rRNA gene (Lane et al., 1985). The amplicon sequences were determined by Sanger sequencing using the same sets of primers (Microsynth France, Vaulx-en-Velin France). These sequences were used for the initial strain strain identification using BlastN comparison and construction of phylogenetic trees (see below).

#### Genome sequencing and comparative genomic analysis

DNA extraction was performed using a New England Biolabs kit (<https://international.neb.com/monarch/high-molecular-weight-dna-extraction>). Genomic DNA was sequenced by combining both the Illumina technique (Microbes NG, UK) and a Nanopore Minion apparatus in the platform DTAMB (Federation BioEEnViS, FR3728 UCBL-CNRS). Nanopore reads were filtered by using FiltLong (<https://github.com/rwrick/Filtlong>), removing the 20 % low quality reads and reads of length less than 5,000 bp. Retained reads were then assembled by using Flye v2.9.1 (Kolmogorov et al., 2019). The circularity of the assembly was verified with Bandage v0.9.0 (Wick et al., 2015). The Illumina reads were aligned on the assembly with Bowtie 2 v2.5.0 (Langmead et al., 2009), and indexed and sorted with Samtools v1.17 (Li et al., 2009), and used to polish the Nanopore assembly with Pilon v1.23 (Walker et al., 2014). The six genomic sequences were annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.2022-12-13.build6494 (Li et al., 2021).

For dDDH calculation, the genomes sequences were uploaded to the Type (Strain) Genome Server (TYGS) and dDDH values were calculated using this webserver (<https://tygs.dsmz.de>) using the formula d4 (Meier-Kolthoff et al., 2013). For genus delineation, genome relatedness was determined using genome alignment fraction (AF) of protein coding genes based on the MUMmer algorithm (Barco et al., 2020). The percentage alignment and the Average Nucleotide Identity (ANIm) values between genome pairs were calculated using the web-service at JSpeciesWS (<https://jspecies.ribohost.com/jspeciesws/>) (Richter et al., 2015).

#### Phylogenetic analysis

All maximum likelihood (ML) trees were inferred using IQ-TREE v2.1.2 (Minh et al., 2020) with the best suited model according to ModelFinder (Kalyaanamoorthy et al., 2017). For the *gapA* tree, sequences were aligned at the codon level using MUSCLE v3.8.1551 (Edgar, 2004) implemented in SEAVIEW 5.0.5 (Gouy et al., 2010) and the ML tree was inferred with the SYM+R6 model. For the 16S rRNA gene tree, sequences were aligned using MAFFT v7.453 with the accurate option L-INSI (Katoh, 2013), trimmed with BMGE v.2.0 (Criscuolo and Gribaldo, 2010) and the ML tree was inferred with the TN+F+I+G4 model. For both *gapA* and 16S rRNA trees, branch supports were estimated with ultrafast bootstrap (1,000 replicates).

To specify the phylogenetic position of the isolates within *Pectobacteriaceae*, phylogenetic analyses of core protein families and ribosomal proteins (rprots) were performed. The two datasets encompassed 87 strains: the six Camargue isolates, strain *Serratia* sp. ATCC 39006, 50 strains representative of the eight *Pectobacteriaceae* genera, and 30 representatives of other *Enterobacterales* families used as outgroup. To avoid annotation biases, the genome sequences of these 87 strains were reannotated using PGAP v.2022-12-13.build6494 (Li et al., 2021). Gene families were delineated according to information from the annots.gff files generated by PGAP (i.e. gene name, function, and hmm profile number when available). The core protein families are defined as the set of 737 protein families present in a single copy in at least 95 % of the 87 genomes. For rprots, we considered the 51 rprot families present in a single copy in more of 80 % of the 87 genomes.

Amino acid sequences of core protein families were aligned using MAFFT v7.453 with the accurate option L-INSI (Katoh, 2013), while nucleotide sequences of rprots genes were aligned at the codon level with MUSCLE v3.8.1551 (Edgar, 2004) implemented in SEAVIEW 5.0.5 (Gouy et al., 2010). The resulting multiple alignments were trimmed using BMGE v.2.0 (Criscuolo and Gribaldo, 2010). Rprots gene alignments on the one hand and core protein alignments on the other hand were then combined to build two large supermatrices containing, respectively, 19,311 nucleotide and 221,882 amino acid positions. These supermatrices were used to infer ML trees with the SYM+I+I+R5 model (rprots) and the Q.insect+F+I+I+R9 model (core protein families). Branch supports of the rprots ML tree were estimated with ultrafast bootstrap (1,000 replicates), while supports of the core protein

**Table 1**

Description of the samples containing members of the new genus *Prodigiosinella*. This table shows the origin (date and lake) of the seven samples, the water temperature and salinity, the number of cultivable bacteria (CFU, colony forming units, observed on LB plates), the number of pectinolytic colonies (PC, pectinolytic colonies, observed on CVP plates), the percentage of pectinolytic bacteria among cultivable bacteria, the number of isolates identified as *Prodigiosinella* and the name of the 16 corresponding isolates. The water salinity was determined by conductimetry (ND, not determined).

Sampling date	June 23, 2020		October 6, 2020				
	Poutrague		Grande Palun	Piemanon		Poutrague	
Lake							
Sample name	J10	J11	O5	O7	O8	O9	O11
Water temperature	21.8 °C	20.1 °C	15.3 °C	14.9 °C	15 °C	15.1 °C	15.5 °C
Water salinity (NaCl)	ND	ND	1.93 g/l	4.38 g/l	3.62 g/l	6.55 g/l	11.22 g/l
<b>Bacterial concentration in the water samples</b>							
CFU/ml	1.5 10 <sup>3</sup>	2.5 10 <sup>3</sup>	4.4 10 <sup>2</sup>	6 10 <sup>2</sup>	3.4 10 <sup>2</sup>	1.5 10 <sup>3</sup>	1 10 <sup>3</sup>
PC/ml	0.14	0.07	0.42	0.35	0.14	0.07	0.14
Ratio PC/CFU	0.009 %	0.003 %	0.095 %	0.058 %	0.041 %	0.005 %	0.014 %
<b>Number of pectinolytic isolates analysed</b>							
Isolates identified as <i>Prodigiosinella</i>	2	2	1	4	4	1	2
Isolates belonging to other genera	0	0	4	0	3	0	0
<i>Prodigiosinella</i> isolates	LS101 <sup>T</sup> , LS102	LS111, LS112	C52	CE70, C73, C76, C77	C80, C81, C83, C84	CE90	C110, C111

**Table 2**

Phenotypic analysis of the *Prodigiosinella* isolates. The salinity of the water samples was determined by conductimetry (ND = not done). Sugar assimilation was analyzed using strains belonging to the two *Prodigiosinella* groups G1 and G2; a few SRP and *Lonsdalea* strains were used for comparison. The sign +, indicates growth; - indicates no growth after 72 h at 30 °C. Dara, D-arabinose; Mel, D-melibiose, Mtl, mannitol; Raf, raffinose; Tre, trehalose; Xyl, D-xylose; PGA, polygalacturonate.

Strain	Collection names	Classification	Sample salinity g.l <sup>-1</sup>	Sugar assimilation							Colony colour
				Mtl	D-ara	Xyl	Tre	Mel	Raf	PGA	
LS101 <sup>T</sup>	A6420, CFBP8826, LMG32072	<i>Prodigiosinella</i> G1	ND	+	+	+	+	-	-	+	opaque light pink
LS111	A6421	<i>Prodigiosinella</i> G1	ND	+	+	+	+	-	-	+	opaque bright pink
C111	A6483, CFBP9052	<i>Prodigiosinella</i> G1	6.31	+	+	+	+	-	-	+	opaque pink
CE90	A6482, CFBP9051	<i>Prodigiosinella</i> G1	6.55	+	+	+	+	-	-	+	opaque bright pink
C52	A6477, CFBP9053	<i>Prodigiosinella</i> G2	1.93	+	+	+	+	+	+	+	opaque white
CE70	A6479, CFBP9054, LMG32867	<i>Prodigiosinella</i> G2	4.38	+	+	+	+	+	+	+	opaque light pink
C77	A6478	<i>Prodigiosinella</i> G2	4.38	+	+	+	+	+	+	+	opaque white
C80	A6480, CFBP9055	<i>Prodigiosinella</i> G2	3.62	+	+	+	+	+	+	+	opaque light pink
C84	A6481	<i>Prodigiosinella</i> G2	3.62	+	+	+	+	+	+	+	opaque light pink
3937	A4922, CFBP3855, NCPPB4426	<i>Dickeya dadantii</i>		+	+	+	-	+	+	+	translucent cream
S29 <sup>T</sup>	A6067, CFBP8647, LMG30899	<i>Dickeya lacustris</i>		+	-	-	-	-	-	+	translucent cream
NCPPB2511 <sup>T</sup>	A6065, CFBP4178	<i>Musicola paradisiaca</i>		-	+	+	+	+	+	+	translucent pale cream
A3967 <sup>T</sup>	CFBP8732 <sup>T</sup> , LMG31880 <sup>T</sup>	<i>Musicola keenii</i>		-	+	+	+	-	-	+	translucent pale cream
CFBP 8637 <sup>T</sup>	A6647 <sup>T</sup>	<i>Pectobacterium aquaticum</i>		+	-	+	-	+	+	+	translucent cream
CFBP 6051 <sup>T</sup>	A6644 <sup>T</sup>	<i>Pectobacterium versatile</i>		+	-	+	+	+	+	+	translucent cream
A6242 <sup>T</sup>	CFBP3617 <sup>T</sup>	<i>Lonsdalea quercinia</i>		+	-	-	+	-	-	+	translucent pale cream

families ML tree were estimated using a jackknife sampling of 7.5 % of the supermatrix sites (1,000 replicates). Trees were drawn using iTOL v6 (Letunic and Bork, 2021).

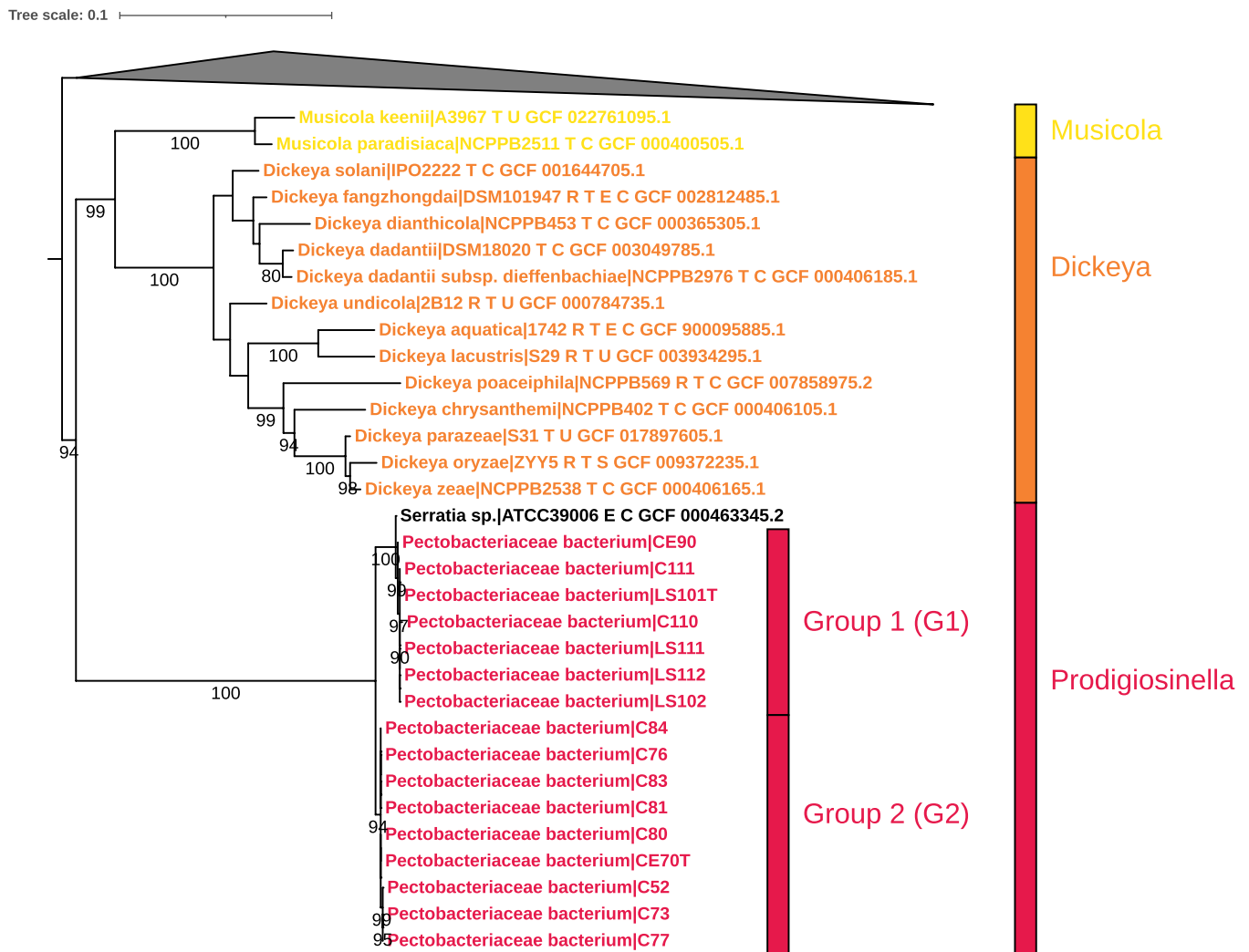
## Results and discussion

### Strain isolation and preliminary characterization of the new genus

A survey of pectinolytic bacteria present in lakes of the wetland region La Camargue (Fig. 1A), near the Mediterranean Sea, in France, was performed using the CVP medium, commonly used to isolate members of the genera *Dickeya* and *Pectobacterium* (Helias et al., 2012). In some water samples, we observed atypical isolates corresponding to white or light pink colonies (Fig. 1C). Our first observation was in June 2020 with four isolates (LS101<sup>T</sup>, LS102, LS111, and LS112) showing, after three days, small deep cavities onto CVP medium, indicating that the colonies were able to degrade the pectate gel (Fig. 1B, Table 1). The ratio of pectinolytic bacteria compared to cultivable bacteria was low in the

water samples (0.003 % to 0.095 %) (Table 1), showing that pectinolytic *Pectobacteriaceae* are among minor taxa in lake water.

Preliminary analyses based on a few phenotypic tests showed that the four pectinolytic strains constituted an original phenotypic group that clearly differed from members of the pectinolytic genera *Dickeya*, *Musicola*, or *Pectobacterium* (Table 2). Furthermore, LS101<sup>T</sup>, LS102, LS111, and LS112 colonies appeared strongly opaque when grown on solid media, with white to light pink color while *Dickeya*, *Musicola*, or *Pectobacterium* colonies were translucent and cream (Fig. 1C, Table 2). A second survey of brackish lakes was performed in October 2020, allowing us to isolate twelve additional strains corresponding to this new phenotypic group (CE90, C110, C111, CE70, C52, C73, C76, C77, C80, C81, C83, C84) (Table 1). The salinity of the corresponding water samples was determined by conductimetry and ranged between 1.93 g.l<sup>-1</sup> and 6.55 g.l<sup>-1</sup> in the samples containing the novel type of bacteria (Table 1, Table 2). After 48 h at 30 °C on LB agar, the colonies of all these strains were opaque, white to pink-orange and small, with diameters of 0.8–1 mm. These bacteria grow in liquid culture both in aerobic and



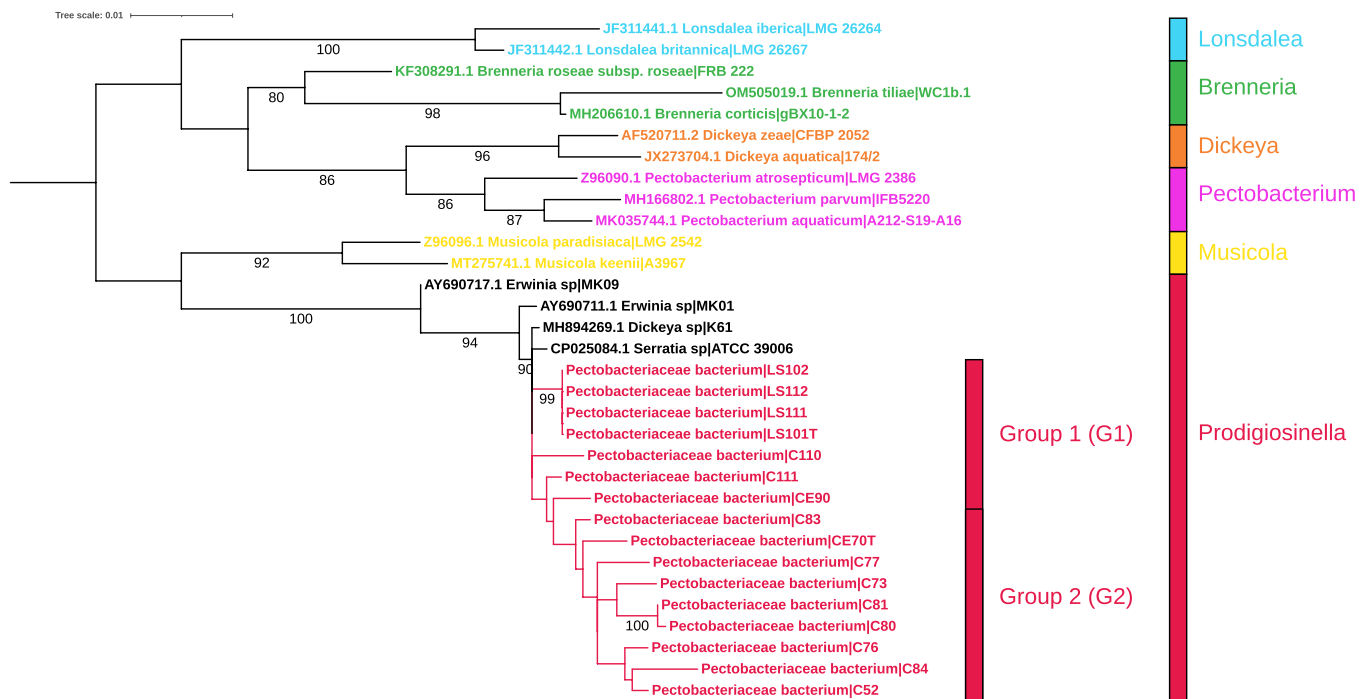
**Fig. 2.** Maximum likelihood phylogeny of the *gapA* gene showing the relationships between the sixteen isolates, strain ATCC 39006, and their closest relatives within *Pectobacteriaceae* (97 sequences, 996 nucleotide positions). The tree was inferred with IQ-TREE (SYM+R6 model). For clarity, the part of the tree encompassing the sequences of the 16 isolates (in red), strain ATCC 39006 (in black), and their closest relatives (*Dickeya* in orange and *Musicola* in yellow) is detailed, while other *Pectobacteriaceae* sequences have been collapsed (grey triangle). Numbers at branch correspond to ultrafast bootstrap values computed by IQ-TREE (1,000 replicates). For clarity, values <80 % are not shown. The scale bar represents the average number of substitutions per nucleotide site. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

semi-aerobic conditions. In anaerobic condition, colonies became visible only after two weeks, showing a low growth in anaerobiosis.

The *gapA* gene is commonly used for preliminary identification in *Pectobacteriaceae* (Cigna et al., 2017). After PCR amplifications, the *gapA* amplicon sequences of the sixteen selected isolates were determined. A BLASTN search against the nr/nt NCBI database gave the *gapA* sequence of strain *Serratia* sp. ATCC 39006 (GCF\_000463345.2) as best hit. Recent phylogenomic analyses showed that this strain was misidentified as it robustly groups close to the genus *Dickeya* within *Pectobacteriaceae* and not with the *Serratia* genus within *Yersiniaceae* (Duprey et al., 2019). A phylogenetic tree of the *gapA* gene was constructed for a preliminary classification of the sixteen isolates; it includes 97 strains: the 16 Camargue isolates, strain *Serratia* sp. ATCC 39006, one strain of each currently recognized *Pectobacteriaceae* species and 18 additional strains from other *Enterobacteriales* families. The resulting *gapA* phylogenetic tree shows that the sixteen Camargue strains and strain *Serratia* sp. ATCC 39006 form a monophyletic group (ultrafast bootstrap value (uBV) = 100 %), well separated from other *Pectobacteriaceae* lineages (Fig. 2). The sixteen isolates can be further subdivided into two closely related groups, referred hereafter as to G1 and G2 (Fig. 2). G1 contains seven strains (LS101<sup>T</sup>, LS102, LS111, LS112, CE90, C110, and C111) (uBV =

100 %), while G2 encompasses the nine other strains (C52, C73, C76, C77, CE70, C80, C81, C83, C84) (uBV = 94 %) (Fig. 2). At the phenotypic level, G1 and G2 strains differ in their ability to assimilate some carbon sources (Table 2).

The 16S rRNA genes of each of the sixteen strains were amplified by PCR and sequenced. A BLASTN search against the nr/nt NCBI database showed best-hits with sequences from four strains classified as unidentified species of three distant genera, namely *Serratia* sp. ATCC 39006, *Dickeya* sp. K61, and *Erwinia* sp. MK01 and MK09, clearly denoting a problem of classification of these strains. The strain *Serratia* sp. ATCC 39006 was isolated in 1982 from channel water in Cheesequake Salt Marsh (New Jersey, USA), after selection for the production of antibiotics of the carbapenem family (Parker et al., 1982) and its genome was sequenced in 2013 (GCF\_000463345.2) (Fineran et al., 2013). Strains *Erwinia* sp. MK01 and MK09 were isolated in 2004 from the rhizosphere of reeds growing in salt marshes in Korea (16S rRNA sequence accession numbers AY690711 and AY690717, respectively). Strain *Dickeya* sp. K61 was isolated in 2018 from flooded fields in Hawaii (USA) (16S rRNA sequence accession number MH894269); it was recently reassigned to a new species *Brenneria "ulupoensis"* (Prescott et al., 2023).



**Fig. 3.** Maximum likelihood phylogeny of *Prodigiosinella* strains based on the 16S rRNA gene sequences (32 sequences, 1,468 nucleotide positions). The tree was inferred with IQ-TREE (TN+I+G4 model) using sequences of the 16S rRNA PCR products of sixteen strains isolated in Camargue and the available 16S rRNA gene sequences of *Serratia* sp. ATCC 39006 (CP025084.1), *Erwinia* sp. MK01 and MK09 (AY690711 and AY690717), *Dickeya* sp. K61 (MH894269), and a set of *Pectobacteriaceae* type strains. The scale bar represents the average number of substitutions per site. Numbers at branch represent ultrafast bootstrap values computed with IQ-TREE (1,000 replicates). For clarity values <80 % are not shown.

The 16S rRNA gene sequences of the sixteen Camargue isolates, *Serratia* sp. ATCC 39006, *Dickeya* sp. K61, *Erwinia* sp. MK01, *Erwinia* sp. MK09, and a representative set of *Pectobacteriaceae* type strains were used to construct a ML tree. The resulting tree strongly supports the grouping of the Camargue isolates with strains *Serratia* sp. ATCC 39006, *Dickeya* sp. K61, *Erwinia* sp. MK01, and *Erwinia* sp. MK09 (uBV = 100 %, Fig. 3). This group forms a distinct lineage, distant from the other *Pectobacteriaceae* genera, suggesting it represents a novel genus within *Pectobacteriaceae*.

Both the *gapA* and 16S rRNA gene trees of the Camargue isolates (Fig. 2 and Fig. 3) are consistent with previous data suggesting that *Serratia* sp. ATCC 39006 is the member of a new genus within *Pectobacteriaceae* (Duprey et al., 2019). According to this study, we propose the name *Prodigiosinella* gen. nov. for this novel genus. To confirm this new classification, we performed in-depth phenotypic, genomic, and phylogenetic analyses of the Camargue isolates.

#### Phenotypic analysis of the Camargue isolates

A biochemical characterization was performed using nine Camargue isolates, including four G1 strains (LS101<sup>T</sup>, LS111, C111, CE90) and five G2 strains (C52, C77, CE70, C80, C84) (Table 2). Minimal medium supplemented with the appropriate carbon source was used to assess their growth with different carbon sources. All the strains are able to grow in the presence of D-arabinose, D-mannitol, D-trehalose, and D-xylose. These biochemical characteristics are sufficient to distinguish *Prodigiosinella* strains from *Dickeya*, *Musicola*, and *Pectobacterium* whose members are unable to assimilate D-trehalose, D-mannitol, and D-arabinose, respectively (Table 2) (Hugouvieux-Cotte-Pattat et al., 2021; Coutinho et al., 2021a; 2021b). *Prodigiosinella* strains also differ by several characters from the close genus *Lonsdalea*, such as for the assimilation of D-arabinose and D-xylose (Table 2). The *Prodigiosinella* strains belonging to the groups G1 and G2 differ by their capacity to assimilate melibiose and raffinose (Table 2).

To complete this phenotypic characterization, we analyzed three *Prodigiosinella* strains of each group (for G1 strains LS101<sup>T</sup>, C111, and CE90; for G2 strains C52, C80, and CE70) using Biolog plates PM1 and PM2A which contain 190 potential carbon sources (Bochner, 2009) (Table S1). The six *Prodigiosinella* strains assimilate the following sugars: N-acetyl-D-glucosamine, D-arabinose, L-arabinose, D-fructose, D-galactose, D-galacturonic acid, D-gluconic acid, D-glucosamine, α-D-glucose, glycerol, m-inositol, D-mannitol, D-mannose, mucic acid, β-methyl-D-glucoside, D-ribose, L-rhamnose, D-saccharic acid, D-sorbitol, D-trehalose, and D-xylose. They assimilate different plant-derived glycosides, oligo or polysaccharides such as arbutin, D-cellobiose, gentiobiose, salicin, sucrose, and pectin. They also metabolize several small organic acids: γ-amino butyric acid, citric acid, fumaric acid, D-malic acid, L-malic acid, malonic acid, pyruvic acid, and succinic acid (Table S1). However, differences are observed between G1 and G2 strains. While G1 strains are able to assimilate m-hydroxy phenyl acetic acid, p-hydroxy phenyl acetic acid, tyramine and D,L-octopamine, G2 strains could not. Conversely, only G2 strains are able to use D-melibiose and D-raffinose, and to weakly assimilate D-alanine, L-alanine, thymidine, and D,L-α-glycerol-phosphate (Table S1). This phenotypic analysis shows that G1 and G2 strains represent two closely but distinct phenotypic groups.

The effect of temperature on the growth of six selected *Prodigiosinella* strains was analyzed by incubations 24 h at temperatures ranging from 25 to 41 °C and measure of cell density at OD<sub>600</sub>. Growth of the *Prodigiosinella* strains is observed in a large range, from 25 to 36 °C, but not at 39 °C (Fig. S1). The effect of salt concentration on the growth of the six *Prodigiosinella* strains was analyzed in LB medium containing a range of NaCl concentrations (Fig. S2). Growth of the *Prodigiosinella* strains is observed in a large range up to 0.7 M NaCl (Fig. S2). They are more resistant to salt addition than *D. dadantii* or *D. lacustris* whose growth is inhibited at concentrations of 0.5 and 0.6 M NaCl, respectively (Fig. S2).

The strain *Serratia* sp. ATCC 39006 was originally identified on the basis of its capacity to produce antibiotic (Parker et al., 1982). Consistently, the new *Prodigiosinella* isolates are able to inhibit the growth of

**Table 3**

Enzyme secretion, motility and maceration ability. *D. dadantii* 3937 was used as a reference strain. The enzyme secretion was evaluated after 24 h growth on plates containing an enzyme substrate allowing the detection of protease (Prt), pectinase (Pel) or cellulase (Cel) activity: +, positive; w, weak; -, negative. For virulence tests, the percentage of plants presenting maceration was observed 48 h after the bacterial inoculation. The length of macerated tissue (mm) and the weight of macerated tissue (g) were measured to quantify maceration on chicory leaves and potato tubers, respectively. For each measurement, the mean value was calculated only for plants showing maceration.

Strain	Identification	Secreted enzymes			Motility		Virulence tests			
		Pel	Cel	Prt	Swimming mm	Swarming mm	Chicory leaves % disease	Potato tubers mm	% disease	g
LS101 <sup>T</sup>	<i>Prodigiosinella</i> G1	w	-	-	35	10	10	22	30	0.47
C111	<i>Prodigiosinella</i> G1	w	-	-	39	12	0	0	20	0.15
CE90	<i>Prodigiosinella</i> G1	w	-	-	36	30	0	0	10	0.28
C52	<i>Prodigiosinella</i> G2	w	-	-	23	6	20	8	80	0.66
CE70	<i>Prodigiosinella</i> G2	w	-	-	24	18	20	12	50	0.32
C80	<i>Prodigiosinella</i> G2	w	-	-	19	6	0	0	60	0.43
S29 <sup>T</sup>	<i>D. lacustris</i>	+	+	+	28	9	100	32	100	1.55
3937	<i>D. dadantii</i>	+	+	+	31	85	100	89	100	4.67

the *E. coli* strain BW25113 ( $\Delta tolC::Kan$ ), more efficiently for G1 strains than for G2 strains (Fig. 1D). *Serratia* sp. ATCC 39006 was shown to produce gas vesicles allowing bacterial cells to float in stagnant water (Tashiro et al., 2016). The production of such gas vesicles was responsible for an opaque aspect of ATCC 39006 colonies grown on solid media (Tashiro et al., 2016), a characteristic also observed for all the Camargue isolates (Fig. 1C). To directly assess the flotation ability of the Camargue isolates, cultures were made with shaking and then allowed to stand for seven days at room temperature. While cells of *D. dadantii* and *D. lacustris* settle at the bottom of the tube, cells of the *Prodigiosinella* isolates remain in suspension throughout the liquid, a characteristic due to the production of gas vesicles allowing flotation (Tashiro et al., 2016) (Fig. 1E).

*Dickeya* and *Musicola* are characterized by their ability to secrete several plant cell wall degrading enzymes. Because of their close relationship with *Prodigiosinella*, we investigated these activities in Camargue strains using a range of specific media (Table 3). In each case, *D. dadantii* 3937 was used as a reference strain. The six *Prodigiosinella* strains show pectinase activity on medium containing polygalacturonate, and they are able to grow in the presence of this polysaccharide as a sole carbon source (Table 2). In contrast, no significant cellulase or protease activity was detected (Table 3). The swimming motility of the strains was measured by the growth diameter 24 h after picking colonies in low concentration L agar. Similarly to *D. lacustris* and

*D. dadantii*, the six *Prodigiosinella* strains showed high swimming motility (Table 3). The swarming motility of these strains was also measured by the growth diameter 24 h after inoculation at the surface of L agar plates. While *D. dadantii* showed a very high motility in these conditions, the six *Prodigiosinella* strains and *D. lacustris* showed only moderate swarming motility (Table 3). The ability to macerate plant tissues was evaluated by measuring the percentage of inoculations leading to maceration and the quantity of macerated tissue obtained 48 h after inoculation on either chicory leaves or potato tubers (Hugouvieux-Cotte-Pattat et al., 2019b). Only a small part of the inoculations with a *Prodigiosinella* strain results in plant tissue maceration and, in these cases, the quantity of macerated tissue remains low (Table 3). Thus, in comparison to *Dickeya* strains, the *Prodigiosinella* strains show a very low efficiency to macerate plant tissues, they are probably not phytopathogens. Their presence in lake water suggests that *Prodigiosinella* members could play a role in the degradation of plant debris, quite abundant in lakes of the Camargue region which are surrounded by dense vegetation (Fig. 1A).

#### Genome sequences and genomic relatedness

The genome of the six *Prodigiosinella* strains used for deep phenotypic analysis (for G1: LS101<sup>T</sup>, C111, CE90; for G2: C52, C80, CE70) was sequenced by combining both the Illumina technique (Microbes NG, UK)

**Table 4**

Characteristics and accession numbers of the *Prodigiosinella* genomes.

Strain	<i>Prodigiosinella aquatilis</i> G1			<i>Prodigiosinella aquatilis</i> G2			
	LS101 <sup>T</sup>	C111	CE90	C52	CE70	C80	ATCC 39006
Genome accession	CP128857	CP128859	CP129114	CP128861	CP128863	CP129116	GCF_000463345.2
Plasmid accession	CP128858	CP128860	CP129115	CP128862	CP128864	CP129117	
BioProject	PRJNA905946	PRJNA905946	PRJNA905946	PRJNA905946	PRJNA905946	PRJNA905946	PRJNA224116
BioSample	SAMN-31888678	SAMN-31888679	SAMN-31888680	SAMN-31888681	SAMN-31888682	SAMN-31888683	SAMN-02470747
Genome size	4,918,023 bp	4,918,635 bp	4,898,109 bp	4,941,093 bp	4,892,228 pb	4,858,875 pb	4,971,757 pb
Plasmid size	59,070 bp	59,071 bp	59,832 bp	61,716 pb	61,717 pb	61,716	NA
Genomic GC%	49.1 %	49.1 %	49.2 %	49.1 %	49.1 %	49.1 %	49.3 %
Genes (total)	4,644	4,638	4,574	4,638	4,518	4,509	4,519
Genes (CDS)	4,537	4,531	4,468	4,528	4,408	4,399	4,415
- Coding	4,357	4,362	4,270	4,306	4,291	4,195	4,310
- Pseudogenes	180	169	198	222	117	204	105
Genes (RNA)	107	107	106	110	110	110	104
- rRNAs 5S	8	8	8	8	8	8	8
- rRNAs 16S	7	7	7	7	7	7	7
- rRNAs 23S	7	7	7	7	7	7	7
- tRNAs	75	75	75	75	75	75	74
- ncRNAs	10	10	9	13	13	13	8
CRISPR arrays	1	1	1	1	1	1	4



**Table 5**

Coverage values within and between the genera *Dickeya*, *Muscolia*, *Lonsdalea*, and *Prodigiosinella*. Pairwise AF values (percentage of nucleotide alignment) were calculated together with ANIm values, using the web-service at <https://jspecies.ribohost.com/jspeciesws/>. The dataset encompassed genomes of the seven *Prodigiosinella* strains and the type strain of each species of *Dickeya*, *Muscolia* and *Lonsdalea*. Values for strains inside a given genus are shown in bold numbers. AF and ANIm values for each strain pairs are given in Tables S4A and S4B, respectively.

	<i>Dickeya</i> (12 type strains)	<i>Muscolia</i> (2 type strains)	<i>Lonsdalea</i> (4 type strains)	<i>Prodigiosinella</i> (7 strains)
<i>Dickeya</i>	<b>33–88 %</b>	17–32 %	8–14 %	10–13 %
<i>Muscolia</i>	16–33 %	<b>86–91 %</b>	9–11 %	10–12 %
<i>Lonsdalea</i>	10–18 %	11–13 %	<b>83–89 %</b>	7–11 %
<i>Prodigiosinella</i>	9–13 %	11–12 %	10–11 %	<b>85–90 %</b>

and a Nanopore Minion apparatus in the platform DTAMB (Federation BioEEnViS, FR3728 UCBL-CNRS). Complete genome sequences were obtained for the six strains (Table 4). The six strains contain a circular plasmid. The plasmid of G1 strains, pPrAq1, is slightly smaller (~59 kb) than pPrAq2 found in G2 strains (~61 kb). In contrast, no plasmid was reported in the *Serratia* sp. ATCC 39006 genome available in public databases (Table 4). The plasmids pPrAq1 and pPrAq2 are very similar to each other. Their nucleic sequences are more than 99 % identical and they present similar gene content (Table S2, Table S3). The main difference is the presence in pPrAq2 of two additional genes, encoding an IS21 family transposase and an IS21 ATPase; this gene addition explains the size difference between the two plasmids. Both pPrAq1 and pPrAq2 are conjugative plasmids encoding a type IV conjugative transfer machinery, replication and partition proteins, and a toxin-antitoxin module to ensure the plasmid maintenance (Table S3).

The size of *Prodigiosinella* genomes is around 4.8–5 Mb, in the same range as *Dickeya* or *Muscolia* genomes (4.3–5.35 and 4.4–4.68 Mb, respectively) but higher than the *Lonsdalea* genomes ranging from 3.54 to 4.02 Mb. The *Prodigiosinella* genomes have G+C contents of 49.1 to 49.3 mol%, clearly lower than that of *Dickeya*, *Muscolia* or *Lonsdalea* genomes (52.6 to 56.9 mol%).

The taxonomic relationships between the genomes of *Prodigiosinella* strains and closely related genera were investigated by calculation of pairwise genome alignment fractions (AF) (Table 5, Table S4). The AF values were calculated both within each genus and between the different genera (Table 5, Table S4). More precisely, we used the genome sequence of the seven *Prodigiosinella* strains and the type strain of each species of *Dickeya*, *Muscolia* and *Lonsdalea*. While a high coverage was observed between the *Prodigiosinella* strains (AF > 85 %), a low coverage was observed with members of the genera *Dickeya*, *Muscolia* or *Lonsdalea* (AF < 13 %). Considering an AF value of at least 33 % for the genus demarcation boundaries (Barco et al., 2020), genome comparison also suggests that the six Camargue strains and ATCC 39006 represent a separate genus.

Then, we investigated the taxonomic relationships between the

**Table 6**

ANI and dDDH values for the genomes of the *Prodigiosinella* isolates. This analysis was performed using the six *Prodigiosinella* genomes of groups G1 or G2 and that of the misclassified strain *Serratia* sp. ATCC 39006. ANI values higher than 99% and dDDH values higher than 90% are shown in bold numbers.

		<i>Prodigiosinella</i> G1			<i>Prodigiosinella</i> G2			ATCC 39006	dDDH
		LS101 <sup>T</sup>	CE90	C111	CE70	C52	C80		
<i>Prodigiosinella</i> G1	LS101 <sup>T</sup>	<b>100</b>	<b>91.0</b>	<b>99.9</b>	74.9	74.9	75.0	76.1	
<i>Prodigiosinella</i> G1	CE90	<b>99.0</b>	<b>100</b>	<b>91.2</b>	73.4	73.7	73.5	74.9	
<i>Prodigiosinella</i> G1	C111	<b>100</b>	<b>99.0</b>	<b>100</b>	74.9	74.9	75.0	76.3	
<i>Prodigiosinella</i> G2	CE70	97.2	97.0	97.2	<b>100</b>	<b>98.1</b>	<b>99.9</b>	<b>91.0</b>	
<i>Prodigiosinella</i> G2	C52	97.1	97.0	97.1	<b>99.7</b>	<b>100</b>	<b>98.1</b>	<b>91.0</b>	
<i>Prodigiosinella</i> G2	C80	97.2	97.0	97.2	<b>100</b>	<b>99.7</b>	<b>100</b>	<b>90.9</b>	
<i>Serratia</i> sp.	ATCC 39006	97.3	97.1	97.3	<b>99.1</b>	<b>99.0</b>	<b>99.0</b>	<b>100</b>	
		<b>ANI</b>							

genomes of the seven *Prodigiosinella* strains by calculation of *in silico* DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) (Table 6). dDDH and ANI values among *Prodigiosinella* strains are high (>73 % for dDDH and > 97 % for ANI). Thus, based on a dDDH threshold of 70 % and an ANI threshold of 95 % to separate two species (Goris et al., 2007, Meier-Kolthoff et al., 2013), all the strains of the proposed new genus *Prodigiosinella* belong to the same species for which we propose the name *Prodigiosinella aquatilis*. However, the two clades observed in phylogenetic trees (Fig. 2, Fig. 4, and Fig. S3) show the differentiation of two evolutionary lineages which present both phenotypic and genomic differences. While dDDH values are > 91 % within each group of *Prodigiosinella* strains, they are < 77 % between the two groups G1 and G2. Considering a thresholds dDDH value of 79–80 % for delineating subspecies (Meier-Kolthoff et al., 2014), each group may correspond to a subspecies. However, recent taxonomic reports propose that the separation at the subspecies level should be abandoned as this taxonomic category loose relevance with the development of genomics (Venter et al., 2022).

Recently, the genome sequence of strain K61 became available (CP135917) (Prescott et al., 2023). This strain, previously named *Dickeya* sp. K61 for the deposit of the 16S rRNA gene sequence (MH894269), has been reassigned to the genus *Brenneria* and named *Brenneria “ulupoensis”* (Prescott et al., 2023). Comparison of the *Brenneria “ulupoensis”* K61 genome with those of the seven *Prodigiosinella aquatilis* genomes shows that the AF, ANI and DDH values are in the range 82.86–88.00 %, 96.9–98.6 % and 77.1–85.2 %, respectively (Table S7). Thus, on the basis of these genomic data, the *Brenneria “ulupoensis”* strain K61 should be reclassified as *Prodigiosinella aquatilis*. Moreover, the high DDH values (>82.5 %) with *P. aquatilis* G2 genomes indicates its appurtenance to the group G2 (Table S7). According to current data, G2 strains were isolated from three continents: Europe (this study), North America (strain ATCC 39006) and Oceania (strain K61). On the contrary, the G1 strains were isolated from a single site, lake Poutrague in Camargue (Table 1). The G1 population could therefore have a clonal origin and the limited distribution of G1 strains could reflect a recent evolution of these strains within the species *Prodigiosinella aquatilis*.

#### Phylogenomics supporting establishment of *Prodigiosinella* gen. Nov.

The phylogenetic position of *Prodigiosinella* within the *Pectobacteriaceae* and the relationships between the *Prodigiosinella* members were investigated by analyzing the ribosomal proteins and core protein families. For these analyses, the seven sequenced *Prodigiosinella* strains and a set of 50 strains representative of eight *Pectobacteriaceae* genera (*Acerihabitans*, *Affinibrenneria*, *Brenneria*, *Lonsdalea*, *Dickeya*, *Muscolia*, *Pectobacterium*, *Symbiopectobacterium*, and *Samsonia*) were considered. We also included 30 representatives of other *Enterobacterales* families used as outgroup. ML trees inferred with ribosomal proteins or core protein families support the monophyly of *Pectobacteriaceae*, with the exception of *Acerihabitans* (Fig. 4 and Fig. S3). In fact, this genus robustly branches with *Sodalis* and *Biostraticola* (Jackknife value (JV) and uBV =



Fig. 4. Maximum likelihood phylogeny of the *Pectobacteriaceae* based on core proteins. The tree shows the relationships between the six Camargue isolates and strain ATCC 39006 within *Pectobacteriaceae* (87 genome sequences, 221,882 amino acid positions). The *Pectobacteriaceae* family is rooted with a sample of representatives of other *Enterobacteriales* families. The tree was inferred with IQ-TREE (Q.insect+F+I+I+R7 model) through a jackknife procedure (1,000 replicates, random selection of 7.5 % of the positions). The scale bar represents the average number of substitutions per site. Grey nodes at branch correspond to jackknife values = 100 %. The presence in a genome of the *pig* genes encoding prodigiosin biosynthesis is indicated by a black triangle near the accession number.

100 %). This is consistent with the original publication describing *Acerihabitans* as a new genus of *Pectobacteriaceae*, close to *Sodalis* and *Biostraticola* (Lee et al., 2021). The same year, another study suggested that *Sodalis* and *Biostraticola* are not *Pectobacteriaceae*, but the members of a new family of *Enterobacterales*, tentatively named “*Bruguiervoracaceae*” (Li et al., 2021). However, *Acerihabitans* representatives were not included in their analysis, which likely explains why this genus is still considered as part of *Pectobacteriaceae*. Our results indicate clearly that *Acerihabitans* is close to *Sodalis* and *Biostraticola* and consistently, should be reclassified as a member of “*Bruguiervoracaceae*”. The ML trees of ribosomal genes and core protein families also strongly support the relationship between the *Symbiopectobacterium* genus and *Pectobacteriaceae* (JV and uBV = 100 %, Fig. 4 and Fig. S3), suggesting that *Symbiopectobacterium* is likely part of this family.

Consistently with the *gapA* and 16S rRNA gene trees (Fig. 2 and Fig. 3), in ML trees of core protein families and ribosomal genes (Fig. 4 and Fig. S3), the six *Prodigiosinella* isolates form a well-supported monophyletic group that also includes ATCC 39006 (JV and uBV = 100 %). This group is clearly separated from other *Pectobacteriaceae* genera and branches as the sister-lineage of the large clade encompassing *Lonsdalea*, *Muscolola*, and *Dickeya*, three major phytopathogenic genera (uBV = 78 % and JV = 100 %). Regarding the relationships between the *Prodigiosinella* strains, phylogenetic trees clearly recover the G1 and G2 dichotomy between strains, with ATCC 39006 belonging to the G2 group (uBV and JV = 100 %). The phylogenetic analyses are consistent with phenotypic and genomic analyses and strongly sustain that Camargue isolates and ATCC 39006 represent a new genus within *Pectobacteriaceae*.

*Functional genomics, noticeable features of the Prodigiosinella genomes*

Genome comparisons were used to identify distinctive traits of the *P. aquatilis* strains. First, we looked for the presence of two gene clusters encoding the biosynthesis of secondary metabolites previously described in ATCC 39006. The *pig* cluster contains fourteen genes (*pigABCDEFGHIJKLMN*) allowing the biosynthesis of the red pigment prodigiosin (2-methyl-3-pentyl-6-methoxyprodigiosin) (Harris et al., 2004), and the *car* cluster consists of eight genes (*carABCDEFGH*) involved in the synthesis of the carbapenem antibiotic 1-carbapen-2-em-3-carboxylic acid (McGowan et al., 1999) (Table S5). The six sequenced genomes contain the two complete clusters, explaining the pink color of the colonies and the inhibition of *E. coli* growth, respectively (Fig. 1C and 1D). Among *Enterobacterales*, the *pig* cluster and prodigiosin production were observed only in a few *Serratia* species (Williams et al., 2022) (Fig. 4). In contrast, the *car* cluster is quite widespread and found in some strains of several *Pectobacteriaceae* genera, such as *Brenneria*, *Dickeya*, *Muscolola*, *Pectobacterium*, *Samsonia*, and *Symbiopectobacterium*. The seven *P. aquatilis* genomes also contain the *gvp* gene cluster consisting of nineteen genes (*gvpA<sub>1</sub>CNVF<sub>1</sub>GWA<sub>2</sub>KXA<sub>3</sub>Y-gvrA-gvpHZF<sub>2</sub>F<sub>3</sub>gvrBC*) involved in the production of gas vesicles in ATCC 39006 (Ramsay et al., 2011) and responsible for the capacity of cell flotation in liquid medium and the opaque aspect of the colonies on solid medium (Fig. 1E and 1C, Table S5). This locus includes three genes (*gvpA1*, *gvpA2* and *gvpA3*) encoding isoforms of the primary gas vesicle structural protein GvpA, and three genes (*gvpF1*, *gvpF2* and *gvpF3*) encoding isoforms of the minor structural protein GvpF, required for gas vesicle formation (Tashiro et al., 2016). Cell flotation is a rare property and the *gvp* cluster is not found in any other *Enterobacterales* genome, outside the genus *Prodigiosinella*. Since genomic analyses showed that the well-studied but misidentified strain *Serratia* spp. ATCC 39006 belongs to the genus *Prodigiosinella*, it is important to note that members of the genus *Prodigiosinella* and not the genus *Serratia* are producing gas vesicles.

Some differences between the genomes of the two *P. aquatilis* groups G1 and G2 are observed, in particular the presence of genes *rafAT* involved in both melibiose and raffinose assimilation (Hugouvieux-

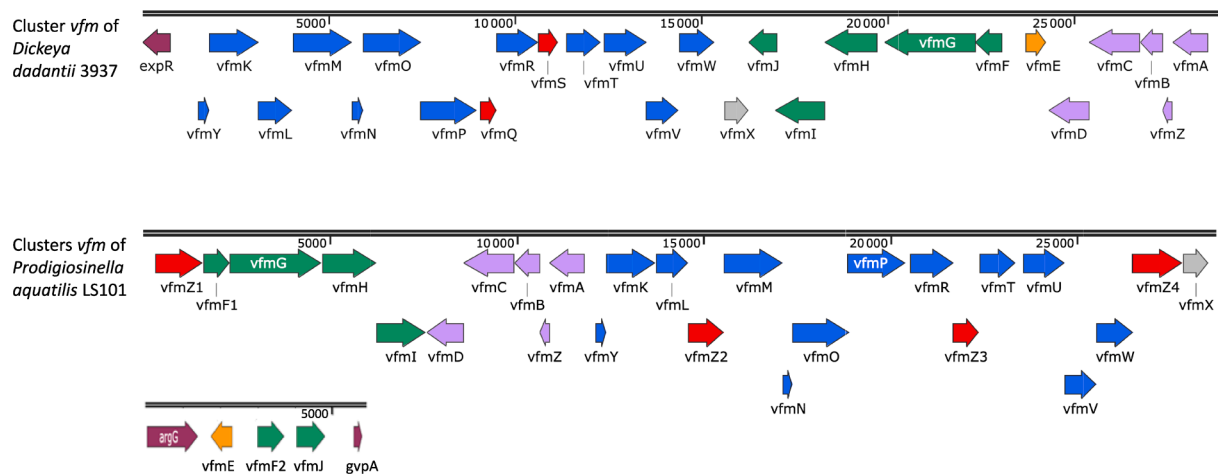
**Table 7**

Genes or clusters encoding plant cell wall degrading enzymes in *Prodigiosinella aquatilis* strains. The enzyme families given below the genes (polysaccharide lyase, PL, glycoside hydrolase, GH, and carbohydrate esterase, CE) are defined on CAZy (<https://www.cazy.org/>).

<i>Dickeya dadantii</i> 3937 genes or clusters encoding plant cell wall degrading enzymes	<i>Prodigiosinella aquatilis</i> G1 Strains LS101 <sup>T</sup> , CE90, C111	<i>Prodigiosinella aquatilis</i> G2 Strains CE70, C52, C80	<i>Prodigiosinella aquatilis</i> G2 Strain ATCC 39006
<i>pelA-pelE-pelD-paeY-pemA</i>	<i>pelD-paeY-pemA</i>		
PL1 PL1 PL1 CE12 CE8			
<i>pelX</i>		<i>pelX</i>	
PL9			
<i>ogl</i>		<i>ogl</i>	
PL22			
<i>rhiN</i>		<i>rhiN</i>	
GH105			
<i>paeX</i>		<i>paeX</i>	
CE12			
<i>ganA-ganB</i>		<i>ganA-ganB</i>	
GH53 GH42			
<i>pelW</i>		<i>pelW</i>	
PL2		(except C80)	
<i>pehV-pehW-pehX</i>		<i>pehV</i>	
GH28 GH28 GH28		(except C52)	
<i>xynA</i>		<i>xynA</i>	
GH30		(except C52)	
<i>pelB-pelC-pelZ</i>	<i>pelB</i>	<i>pelB-pelC-pelZ</i>	
PL1 PL1 PL1			
<i>pelN</i>	-	<i>pelN</i>	
PL9	pseudogene		
<i>rhiE</i>	-	<i>rhiE</i>	
PL4	pseudogene		
<i>pemB</i>	-	<i>pemB</i>	
CE8	pseudogene		
<i>pnlG</i>	-	<i>pnlG</i>	
PL1			
<i>pell-celZ</i>	-		<i>pell-celZ</i>
PL9 GH5			
<i>prtA-prtC prtB-prtF-prtE-prtD-inh-prtG</i>	-		

Cotte-Pattat and Charaoui-Boukerzaza, 2009) in the genomes of G2 strains, including ATCC 39006 (Table S5). This explains the phenotypic differences observed between the G1 and G2 strains for melibiose and raffinose assimilation (Table 2). The differences regarding the assimilation of hydroxyphenyl acetic acids can be explained by the presence in the genome of *P. aquatilis* G1 strains of the *hpa* gene cluster (*hpaR-GEDFJSHIXABC*) involved in the catabolism of these compounds (Diaz et al., 2001, Luengo and Olivera, 2020) (Table S1, Table S5). Compared to the cluster *hpa* of *E. coli* strains degrading hydroxyphenyl acetic acids (Diaz et al., 2001), the *P. aquatilis* cluster encodes the additional protein HpaJ. The *hpa* cluster is also involved in the catabolism of tyramine and D.L-octopamine (Luengo and Olivera, 2020), two substrates which are also assimilated by *P. aquatilis* G1 strains (Table S1).

A survey of *P. aquatilis* gene annotations was performed to search for genes potentially involved in the degradation of plant cell walls, and especially pectate lyases, the main determinants of the soft rot symptoms caused by soft-rot *Pectobacteriaceae* (Hugouvieux-Cotte-Pattat et al., 2014) (Table S5, Table 7). The seven *P. aquatilis* genomes contain two genes encoding pectate lyases of the family PL1 (PelB, PelD), one of family PL9 (PelX), an intracellular oligogalacturonate lyase of the family PL22 (Ogl), an hydrolase of the family GH105 (RhiN), a pectin methyl-esterase of the family CE8 (Pema), two pectin acetyl-esterases of family CE12 (PaeX and PaeY), an endogalactanase of the family GH53 (GanA) and an exogalactanase of the family GH42 (GanB) (PL, GH and CE families are defined on CAZy, <https://www.cazy.org/>) (Drula et al., 2022). Most genomes encode a pectate lyase of the family PL2 (PelW), a polygalacturonase of the family GH28 (PehV) and a glucuronoxylanase



**Fig. 5.** Comparison of the *Dickeya* and *Prodigiosinella* *vfm* clusters. The *Dickeya vfm* cluster consists of 26 contiguous genes. The *Prodigiosinella vfm* genes are located in two separate clusters of 26 and 3 genes, respectively, with a different organization of the transcriptional units which are shown by different colours: blue, *vfmY-vfmW*; green, *vfmF-vfmJ*; purple, *vfmAZBCD*; orange, *vfmE*; grey, *vfmX*. The genes present only in one genus are shown in red. SNAPGENE was used to extract the *vfm* clusters of the 3937 and LS101 genomes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of the family GH30 (XynA) (Table S5, Table 7). The four *P. aquatilis* G2 genomes contain additional genes encoding two PL1 pectate lyase (PelC, PelZ), another PL9 pectate lyase (PelN), a PL1 pectin lyase (PnlG), a rhamnolacturonate lyase of the family PL4 (RhiE), and a periplasmic CE8 pectin methyl esterase (PemB). In *P. aquatilis* G1 genomes, there is only a partial sequence of *pelN*, *rhiE* and *pemB* that are annotated as pseudogenes. Thus, *P. aquatilis* G2 genomes contain more genes encoding plant cell walls degrading enzymes than the *P. aquatilis* G1 genomes (Table 7). In addition, the ATCC 36009 genome contains two adjacent genes encoding a PL9 pectate lyase (*pelL*) and a cellulase of the family GH5 (*celZ*), respectively. Interestingly, the *pelL-celZ* cluster is present in the genome of all *Dickeya* species (such as *D. dadantii*, Table 7).

The seven *P. aquatilis* genomes contain a cluster encoding a type II secretion system (T2SS) which is responsible for the specific secretion of extracellular pectinases and of the cellulase CelZ in the genus *Dickeya* (Pineau et al., 2014). In *P. aquatilis*, it could at least allow the secretion of the pectinases PelB, PelD, PemA, and PaeY (Table 7). In contrast to *Dickeya*, the *P. aquatilis* genomes possess neither genes encoding proteases nor the type I secretion system responsible for their secretion (Table S5). This is consistent with the absence of protease secretion observed in these strains (Table 3). The genomes of most *P. aquatilis* strains, except LS101 and C111, encode a complete type III secretion system (T3SS) including genes encoding the effector HrpW homologous to PL3 enzymes and the effector DspE belonging to the AvrE family (Table S5). T3SSs are currently divided into different families (Kvitko and Collmer, 2023). Both Hrp1 and Hrp2 families are common in phytopathogenic bacteria but they are evolutionary distant from each other. The closest homologs of the T3SS genes belong to the Hrp1 family. Besides *Pseudomonas syringae*, Hrp1 members are found in phytopathogenic *Enterobacterales* of the genus *Brenneria*, *Dickeya*, *Erwinia*, *Pantoea*, and *Pectobacterium*, as well as in the new genus *Prodigiosinella*.

The number of *luxI-luxR* couples encoding quorum sensing systems dependent on signals of the acyl-homoserine lactone family is variable (Table S5, Table S6). All the *P. aquatilis* genomes possess the *smal-smaR* couple described in strain ATCC 39006 (Ramsay et al., 2011). Notably, CE90 has a second *luxI-luxR* couple related to the *expI-expR* couple of *Dickeya* (Nasser et al., 1998) (Table S5). In addition, all the *P. aquatilis* genomes encode the Vfm quorum sensing system previously identified in the genus *Dickeya* which is dependent of the synthesis of non-ribosomal peptides (Nasser et al., 2013, Hugouvieux-Cotte-Pattat et al., 2022). While the *Dickeya vfm* cluster consists of 26 contiguous genes, the *P. aquatilis* Vfm system consists of 29 genes encoded by two distinct loci

(Fig. 5, Table S5, Table S6). The major *vfm* locus of *P. aquatilis* contains 26 genes but, in comparison to the *Dickeya* cluster, it shows a different organization of the transcriptional units. Moreover, it lacks the genes *vfmS* and *vfmQ* but contains four additional genes (Fig. 5). The second *vfm* locus of *P. aquatilis* contains the genes *vfmJ*, a *vfmF* homolog and *vfmE* encoding the major regulator of the Vfm system. This second locus is adjacent to the *gvp* cluster involved in the production of gas vesicles (Fig. 5).

## Conclusions

The isolation of pectinolytic isolates from water samples recovered in coastal saline wetland lakes allowed the identification of a new bacterial genus belonging to the family *Pectobacteriaceae* within the order *Enterobacterales*. Phenotypic, genomic and phylogenetic arguments support the creation of the genus *Prodigiosinella* gen. nov., with *Prodigiosinella aquatilis* sp. nov. as the type species. LS101<sup>T</sup> (CFBP 8826<sup>T</sup>, LMG 32072<sup>T</sup>) is proposed as the type strain for this species. Other members of this genus were isolated from lake water in Camargue and include strains LS102, LS111, LS112, C52 (CFBP 9053), CE70 (CFBP 9054, LMG 32867), C73, C76, C77, C80 (CFBP 9055), C81, C83, C84, CE90 (CFBP 9051), C110, and C111 (CFBP 9052). Characterization of these sixteen Camargue isolates led to the identification of two groups showing genomic and phenotypic differences. G1 members are the type strain LS101<sup>T</sup> (CFBP 8826<sup>T</sup>, LMG 32072<sup>T</sup>) and strains LS102, LS111, LS112, C110, C111 (CFBP 9052), and CE90 (CFBP 9051) isolated from lake water in Camargue. They are able to assimilate m-hydroxyphenyl acetic acid, p-hydroxy phenyl acetic acid, tyramine and D,L-octopamine but unable to assimilate D-melibiose and D-raffinose. On the contrary, members of Group 2 are able to assimilate D-melibiose and D-raffinose, but unable to assimilate hydroxyphenyl acetic acids, tyramine and octopamine. Group G2 includes strains CE70 (CFBP 9054, LMG 32867), C52 (CFBP 9053), C73, C76, C77, C80 (CFBP 9055), C81, C83 and C84 isolated in Camargue and ATCC 39006, isolated in New Jersey (USA).

All the phenotypic, genomic, and phylogenetic evidences presented here support that strain ATCC 39006 belongs to the genus *Prodigiosinella* and showed that its previous classification in the genus *Serratia* sp. was erroneous. Indeed, except for the production of the red pigment prodigiosin, there are clear phenotypic and genomic differences between members of the genus *Prodigiosinella*, which is clearly part of *Pectobacteriaceae*, and the genus *Serratia*, which belongs to *Yersiniaceae*. Thus, the misclassified strain *Serratia* sp. ATCC 39006, used as model in several molecular genetic studies (Slater et al., 2003, Harris et al., 2004,

Table 8

Description of the new genus *Prodigiosinella* including the species *P. aquatilis*.

Guiding Code for Nomenclature	The rules of the ICNP have been followed.	The rules of the ICNP have been followed.
Nature of the type material	Indicate the strain or genome sequence	Indicate the strain or genome sequence
Genus name	<i>Prodigiosinella</i>	<i>Prodigiosinella</i>
Species name		<i>aquatilis</i>
Genus status	gen. nov.	–
Genus etymology	<i>Prodigiosinella</i> (Pro.di.gi.o.si.nel'la. N.I. neut. n. prodigiosinum, prodigiosin, the red pigment produced by these bacteria; L. suff. -ella, diminutive ending; N.I. fem. dim. n. <i>Prodigiosinella</i> , producer of the red pigment prodigiosin).	–
Type species of the genus	<i>Prodigiosinella aquatilis</i>	–
Specific epithet	–	<i>aquatilis</i>
Species status	–	sp. nov.
Species etymology	–	a.qua'ti.lis. l. fem. adj. <i>aquatilis</i> , living in water LS101 <sup>T</sup>
Designation of the Type Strain	–	–
Strain Collection Numbers	–	CFBP 8826 <sup>T</sup> , LMG 32072 <sup>T</sup>
Type Genome, MAG or SAG accession Nr.	–	GCA_030388725.1
Genome status	–	Complete.
Genome size	–	4,918 kbp
GC mol%	–	49.10 %
16S rRNA gene accession nr.	–	OR243667
Description of the new taxon and diagnostic traits	<i>Prodigiosinella</i> members are Gram-negative, motile, non-spore forming, facultatively anaerobic bacteria. Cells have average dimensions of 0.6 by 1.7 µm. They are motile and show flotation ability in liquid medium. After 48 h at 30 °C on LB medium (5 g.l <sup>-1</sup> tryptone, 3 g.l <sup>-1</sup> yeast extract, 5 g.l <sup>-1</sup> NaCl and 15 g.l <sup>-1</sup> agar), they form milky-white or pink-colored colonies of 0.5–2 mm in diameter with opaque appearance. They can grow up to 36 °C but not at 39 °C. They are able to grow in the presence of N-acetyl-D-glucosamine, D-arabinose, L-arabinose, arbutin, D-cellobiose, D-fructose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, D-glucosamine, α-D-glucose, glycerol, m-inositol, D-mannitol, D-mannose, mucic acid, β-methyl-D-glucoside, pectin, D-ribose, L-rhamnose, D-saccharic acid, salicin, D-sorbitol, sucrose, D-trehalose, D-xylose, γ-amino butyric acid, citric acid, fumaric acid, D-malic acid, L-malic acid, malonic acid, pyruvic acid, and succinic acid.	General description as for the genus. <i>P. aquatilis</i> is able to grow in the presence of N-acetyl-D-glucosamine, D-arabinose, L-arabinose, arbutin, D-cellobiose, D-fructose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, D-glucosamine, α-D-glucose, glycerol, m-inositol, D-mannitol, D-mannose, mucic acid, β-methyl-D-glucoside, pectin, D-ribose, L-rhamnose, D-saccharic acid, salicin, D-sorbitol, sucrose, D-trehalose, D-xylose, γ-amino butyric acid, citric acid, fumaric acid, D-malic acid, L-malic acid, malonic acid, pyruvic acid, and succinic acid.

Table 8 (continued)

Guiding Code for Nomenclature	The rules of the ICNP have been followed.	The rules of the ICNP have been followed.
	They secrete extracellular pectinases and they produce the red pigment prodigiosin and antibiotic compounds.	
Country of origin	France	France
Region of origin	Camargue	Camargue
Source of isolation	Brackish water	Brackish water
Sampling date (dd/mm/yyyy)	23/06/2020	23/06/2020
Latitude (xx°xx'xx"N/S)	43.5089° N	43.5089° N
Longitude (xx°xx'xx"E/W)	4.66757° E	4.66757° E
Altitude (meters above sea level)	1 m	1 m
Number of strains in study	16	16
Information related to the Nagoya Protocol	Certificate ABSCH-IRCC-FR-266612-1, Ministère de la Transition Ecologique FRANCE	Certificate ABSCH-IRCC-FR-266612-1, Ministère de la Transition Ecologique FRANCE

Fineran et al., 2013), should be reclassified as *Prodigiosinella aquatilis* ATCC 39006. The genome sequence of *Brenneria "ulupoensis"* K61 (named *Dickeya* sp. K61 for the deposit of its 16S rRNA gene sequence MH894269) became recently available (CP135917); comparison with the *Prodigiosinella* genomes shows that strain K61 should be reclassified as *Prodigiosinella aquatilis*. Regarding their 16S rRNA gene sequence, strains *Erwinia* sp. MK01 (AY690711), and *Erwinia* sp. MK09 (AY690717) should also be classified in the genus *Prodigiosinella* but their appurtenance at the species level cannot firmly be proposed on the basis of the 16S rRNA gene sequence.

The geographical repartition of the genus *Prodigiosinella* is large as members have been isolated in four different continents, North America (*Serratia* sp. ATCC 39006 in New Jersey), Asia (*Erwinia* sp. MK01 and MK09 in Korea), Oceania (*Brenneria "ulupoensis"* K61 in Hawaii), and Europe (Camargue, France). Notably, all the *Prodigiosinella* strains described so far originate from similar ecosystems, i. e. coastal brackish wetlands. The *Brenneria "ulupoensis"* strain K61 was isolated from water samples collected from flooded plots for taro (*Colocasia esculenta*) cultivation at Ulupo on the Oahu Hawaiian Island (Prescott et al., 2023). The *Erwinia* sp. strains MK01 and MK09 were isolated from the rhizosphere of reeds growing in salt marshes on the southwest coast of Korea (GenBank AY690711 and AY690717, respectively) and *Serratia* sp. ATCC 39006 was collected from channel water in the Cheesequake Salt Marsh, New Jersey (Parker et al., 1982). The precise role of members of the genus *Prodigiosinella* in these coastal aquatic ecosystems remain to be clarified. The description of the new genus and species *Prodigiosinella aquatilis* is given in Table 8.

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#### Sequence accession numbers

The GenBank/EMBL/DDBJ accession numbers for the genome sequence of the type strains *Prodigiosinella aquatilis* gen. nov. sp. nov. LS101<sup>T</sup> (CFBP 8826<sup>T</sup>, LMG 32072<sup>T</sup>) are CP128857 and CP128858 for its chromosome and plasmid, respectively. The accession number for the 16S rRNA sequence of the type strain is OR243667.

## CRedit authorship contribution statement

**Nicole Hugouvieux-Cotte-Pattat:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Jean-Pierre Flandrois:** Writing – review & editing, Software, Methodology, Formal analysis. **Jérôme Briolay:** Methodology, Formal analysis. **Sylvie Reverchon:** Writing – review & editing, Visualization, Investigation, Formal analysis, Conceptualization. **Céline Brochier-Armanet:** Writing – review & editing, Visualization, Supervision, Software, Methodology, Investigation, Funding acquisition, Conceptualization.

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.syapm.2024.126497>.

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