Pectobacterium quasiaquaticum sp. nov., isolated from waterways

Hajar Ben Moussa, Jacques Pédron, Claire Bertrand, Amandine Hecquet, Marie-Anne Barny

Pectobacterium quasiaquaticum sp. nov., isolated from waterways

Hajar Ben Moussa, Jacques Pédron, Claire Bertrand, Amandine Hecquet and Marie-Anne Barny

Sorbonne Université, INRAE, Institute of Ecology and Environmental Sciences-Paris, 4 place 7 Jussieu, F-75 252 Paris, France.

for correspondance : marie-anne.barny@sorbonne-universite.fr

The 7 genomes described in this manuscript have been deposited in the GenBank database under the bioproject number PRJNA662694

ABSTRACT

Through this study, we established the taxonomic status of seven strains belonging to the *Pectobacterium* genus (A477-S1-J17^T, A398-S21-F17, A535-S3-A17, A411-S4-F17, A113-S21-F16, FL63-S17 and FL60-S17) collected from four different river streams and an artificial lake in south-east France between 2016 and 2017. Ecological surveys in rivers and lakes pointed out different repartition of strains belonging to this clade compared the closest species, *P. aquaticum.* The main phenotypic difference observed between these strains and the *P. aquaticum* type strain was a strongly impaired growth with rhamnose as sole carbon source. This correlates with three different forms of pseudogenisation of the L-rhamnose/proton symporter gene *rha*T in the genomes of strains belonging to this clade. Phylogenetic analysis using *gapA* gene sequences and MLSA analysis of the core genome showed that these strains formed a distinct clade within the genus *Pectobacterium* closely related to *Pectobacterium aquaticum*. *In silico* DNA-DNA hybridization and average nucleotide identification values showed a clear discontinuity between the new clade and *P. aquaticum*. However, the calculated values are potentially consistent with either splitting or merging of this new clade with *P. aquaticum*. In support of the split, ANI coverages were higher within this new clade than between this new clade and *P. aquaticum*. The split is also consistent with the range of observed ANI or dDDH values that currently separate several accepted species within the *Pectobacterium* genus. On the basis of these data, the strains A477-S1-J17^T, A398-S21-F17, A535-S3-A17, A411-S4-F17, A113-S21-F16, FL63-S17 and FL60-S17 represent a novel species of the genus *Pectobacterium*, for which the name *Pectobacterium quasiaquaticum* sp. nov. is proposed. The type strain is A477-S1-J17^T (=CFBP 8805^T =LMG 32181^T).

INTRODUCTION

The *Pectobacterium* genus belongs to the *Pectobacteriaceae* family of the *Enterobacterales* order [1]. This genus groups bacteria are well known for their ability to secrete a large cocktail of plant cell wall degrading enzymes (PCWDE) inducing soft rot symptoms in a large variety of plants around the world [2] and resulting in reduced yields and significant crop production losses. The *Pectobacterium* genus currently includes 17 described species: *Pectobacterium aquaticum* [3], *Pectobacterium actinidiae* [4], *Pectobacterium aroidearum* [5], *Pectobacterium atrosepticum* [6], *Pectobacterium betavasculorum* [6], *Pectobacterium brasiliense* [4], *Pectobacterium carotovorum* [4], *Pectobacterium cacticidum* [7], *Pectobacterium fontis* [8], *Pectobacterium odoriferum* [4], *Pectobacterium parmentieri* [9], *Pectobacterium polaris* [10], *Pectobacterium polonicum* [11], *Pectobacterium punjabense* [12], *Pectobacterium versatile* [4], *Pectobacterium wasabiae* [6], *Pectobacterium parvum* [13], and two proposed species not yet validated by ad hoc committees: *"Pectobacterium zantedeschiae"* [14] and *"Pectobacterium peruviense"* [15].

Several of the above mentioned species are closely related and were previously grouped within the same species. Notably, the *P. carotovorum* species was previously highly heterogenous and regrouped within the same clade two species *P. aquaticum* and *P. polaris* that were embedded within accepted or proposed subspecies previously named *Pectobacterium carotovorum* subsp. *carotovorum, Pectobacterium carotovorum* subsp. *odoriferum*, '*Pectobacterium carotovorum* subsp. *brasiliense'* and '*Pectobacterium carotovorum* subsp. *actinidiae'*. To avoid incongruity between the taxonomic status of the species and subspecies within this large clade all the subspecies were recently elevated at the species level [4]. This analysis also allowed to distinguish the new species, *P. versatile,* closely related to *P. carotovorum* [4]. Moreover, the *P. polaris* clade was also recently splitted in two closely related species, *P. polaris* and *P. parvum* [13].

To date, most *Pectobacterium* species have been described following sampling and isolation from diseased host plants during outbreaks or sustained epidemics and their descriptions outside the agricultural context are rare [16]. Nevertheless, previous studies have indicated that *Pectobacterium* species could be isolated from a variety of non-host environments, such as water, soil or air [17] [18] [19] [16]. Recently, several species isolated from fresh water have been described. *P. aquaticum* strains were isolated from river streams in France [3], *P. polonicum* strains were isolated from ground water in Poland [11] and the *P. fontis* strain was isolated from waterfall in Malaysia [8]. Here, we described a new *Pectobacterium* species

Pectobacterium quasiaquaticum sp. nov that was recovered at various time in 2016 and 2017 from river and artificial lakes water in France.

ISOLATION AND ECOLOGY

In this study, we established the taxonomic position of seven strains (A477-S1-J17^T, A398-S21-F17, A535-S3-A17, A411-S4-F17, A113-S21-F16, FL63-S17 and FL60-S17) that were collected in 2016 and 2017 from two different freshwater surveys performed along a river stream in south-east France and from the CEREEP Ecotron artificial lakes located in the South of Paris. Water samples (150 or 500 ml) collected from the surface water were filtered through 0.2 μm pore filters (Sartorius cellulose acetate filter). The bacteria retained on the filter were resuspended in 1ml of sterile distilled water and 100 μl were spread over the CVP (Crystal Violet Pectate) plates [20]. The colonies forming pits on CVP medium were isolated and characterized.

As described below, these 7 strains formed a clade distinct from the closely related species *P. aquaticum*. No strain isolated from host plant were described for *P. aquaticum* or the new clade in the recent taxonomy update of 265 strains of *Pectobacterium* spp. hosted at the CIRM-CFBP collection that gathers strains isolated since 1944 from all over the world [21] and potential host plants for each clade are currently unknow. *P. aquaticum* has nevertheless the capacity to macerate potato slices indicating its potential to infect plant or to degrade plant debris [3]. We therefore checked if strains of the new clade were able to macerate potato slices. The strains were grown overnight in LB medium devoid of NaCl (Hereafter LB: 10.g L^{-1} tryptone, 5g.L^{-1} yeast extract, 15 g. L^{-1} agar) under agitation at 27.6°C. 100 µl of the bacterial cultures were spread on a 10% TSA plate (14g.L^{-1} agar, 3g.L^{-1} trypic soy broth) and placed at 27.6°C for 24 hours. The bacteria were then scraped off the plates and resuspended in 50 mM phosphate buffer (pH 6.8), adjusted to an OD_{600nm} of 1, and 10 µl were placed on the surface of the potato slices. As negative control, a potato slice was inoculated with 10 µl of 50 mM phosphate buffer (pH 6.8). This test showed that strains of the new clade were also able to macerate potato tuber slices (Figure S1) indicating that strains of this new clade also potentially infect plant or degrade plant debris. Although both *P. aquaticum* and the new clade were isolated from water, their potential virulence on plant suggests that water may not be their primary habitat. This is reinforced by the fact that *Pectobacterium* spp. remains rare in water and are only isolated from water thanks to a very efficient selective medium [22]. Interestingly, our ecological survey of river and artificial lake water highlighted a differential repartition of *P. aquaticum* and strains the new

clade in surface water. Extensive two years survey of the river Durance watershed that covers 14280 km^2, allowed isolation of 219 *P. aquaticum* strains while only 13 isolates belonging to the new clade were identified. Conversely, during a 2 years survey at the CEREEP Ecotron artificial lakes, 7 strains of the new clades were isolated while only 1 strain of *P. aquaticum* was found. The differential presence of *P. aquaticum* and the new clade in different places suggested different ecological niche for the new clade and *P. aquaticum*. While the exact nature of these ecological niches remains to be determined, one could hypothesize that strains of *P. aquaticum* and strains of the new clade are likely associated with different plants. The differential presence of both clades in water prompted us to evaluate whether this new clade could represent a species distinct from *P. aquaticum*.

16S rRNA AND *gap*A GENE PHYLOGENIES

The 16S rRNA gene phylogeny including the 16S rRNA genes from the 7 studied strains, 6 *P. aquaticum* strains, 16 strains of other *Pectobacterium* spp. type strains and the outgroup 16S rRNA gene sequence of *Dickeya solani* was performed. The 16S rRNA gene nucleotide sequences were aligned using the MUSCLE software [23] and filtered using the GBLOCKS tool [24]. The alignments were used to build a phylogenetic tree using the PhyML algorithm [25] based on Tamura-Nei model [26] with the SeaView software [27], with 200 bootstrap replications. The generated 16S rRNA gene phylogeny separated the 7 studied strains from the species *P. aquaticum* but the bootstrap at the separation node was inferior to 50% (Fig S2). Furthermore, '*P. zantedeschiae*' and *P. fontis* grouped with the 7 studied strains (Fig S2).
The poor discriminative resolution of 16S rRNA phylogeny within the *Enterobacterales* order has been previously noted by Adeolu *et al*. [1]. We therefore decided to use the housekeeping gene *gap*A (glyceraldehyde-3-phosphate dehydrogenase) as an alternative to the 16S rRNA gene phylogeny. The *gap*A gene is present in each genome in a single copy and was described as appropriate for quickly characterizing the different *Pectobacterium* species [28]. The *gap*A genes were aligned using the MUSCLE [23] software and were filtered using the GBLOCKS tool [24]. The alignments were used for building a phylogenetic tree with PhyML algorithm based on Tamura-Nei model [26] with the SeaView software [27], with 200 bootstrap replications. The *gap*A gene phylogeny showed that the seven studied strains grouped together and formed a new clade, close to the clade formed by the *P. aquaticum* species but clearly separated from it and from other *Pectobacterium* spp. (Fig. 1).

GENOME FEATURES

To further characterize this clade, genomes of the 7 studied strains were sequenced. For genome sequencing, the genomic DNA was first prepared by growing the strains overnight at 28°C on solid LB medium. A single colony was then picked up and grown overnight in 2ml of liquid LB medium at 28°C with 120 rpm shaking. Bacterial cells were harvested by centrifugation (5 min at 12,000 rpm) and DNA was extracted with the Genomic DNA Extraction Kit (Promega) according to the supplier's specifications. The DNA was suspended in 100 µl of sterile distilled water and the quantity and quality of the DNA was assessed by nanodrop measurement, spectrophotometric analysis and gel analysis. Nextera DNA libraries were then prepared from 50 ng of high quality genomic DNA. These libraries were sequenced at the next generation sequencing core facilities of the Institute of Integrative Biology of the Cell (Avenue de la Terrasse, 91190 Gif-sur-Yvette, France). Paired end 2 x 75 pb sequencing was performed on an Illumina NextSeq500 instrument, with a High Output 150 cycle kit. The readings were assembled using the CLC Genomics Workbench (version 9.5.2, Qiagen Bioinformatics). Coding sequences were predicted and annotated using the PATRIC RASTtk genome annotation service [29]. Genome assembly statistics are indicated in the Table 1.

A multilocus sequence analysis (MLSA) was performed using the concatenated nucleotide sequences of 265 homologous genes of the core genome (Table S1) of the 7 studied strains as well as those of 6 strains of the *P. aquaticum* species and those 62 *Pectobacterium* genomes representative of the whole *Pectobacterium* genus. Multigenic homologous families were excluded to avoid confusion between orthologs and paralogs. The clustering of homologous nucleotide sequences was performed with SiLix [30] software with a 80% identity threshold. Homologous sequences of each gene were aligned using MUSCLE [23] software then concatenated. The alignments were filtered using the GBLOCKS tool [24] resulting in a data set of 297,907 sites (of which 67,801 are informative). All the scripts used are available online (https://zenodo.org/record/2639652) as described in [4]. The tree was computed with the SeaView software [27] using the BioNJ method [31]. Bootstrap percentages were calculated based on 200 replicates. This analysis confirmed that these seven strains constitute a well-separated clade (Fig. 2 and Fig. S3 for extended tree) supporting the phylogenetic analysis previously performed with the *gap*A housekeeping gene.

To further define the genetic proximity of this new clade to the species *P. aquaticum* and the other species of the *Pectobacterium* genus, the average nucleotide identity (ANI) values were

calculated using the python3 script pyani [32] (https://github.com/widdowquinn/pyani) with the BLAST algorithm (ANIb) (Table 2, for pairwise ANI with all *Pectobacterium* spp. see Table S2). There is a clear discontinuity of ANI values between the new clade and *P. aquaticum* (Table 2). However, ANI values between this new clade and the species *P. aquaticum* remains in the borderline to separate species (Table 2 and Table 3). These observed ANI values are nevertheless in the same range as the one that currently separates *P. versatile* and *P. carotovorum* [4 and Table 3 and Table S2] and are slightly lower than the one that separates *P. parvum* from *P. polaris* [13 and Table 3 and Table S2]. Furthermore, the range of coverage between *P. aquaticum* and the new clade (84.5 - 79.0 %) is lower than the range of coverage within *P. aquaticum* (91.9 - 85.7 %) or within the new clade (99.3 - 88.6 %) further supporting the split rather than the merge of *P. aquaticum* and the new clade (Table 3).

We also calculated digital DNA-DNA hybridization (dDDH) values. The digital DNA-DNA hybridization was proposed to approach wet-lab DDH as close as possible [33]. The lowest dDDH values between the seven studied genomes were 89.5% and dDDH values dropped to 65.9% when comparing these 7 genomes to those of closest species *P. aquaticum* (Table 2). Again, the dDDH values between *P. aquaticum* and the new clade are borderlines to separate species (Table 2). Nevertheless, one could observe that these dDDH values are lower than the one observed between the closely related species *P. parvum* and *P. polaris* [13 ; Table 3 and Table S2] further supporting the discrimination between this new clade and *P. aquaticum*.

PHYSIOLOGY AND CHEMOTAXONOMY

In order to determine the distinctive metabolic traits between *P. aquaticum* and the new clade, biochemical tests were performed with Biolog GENIII plates using the inoculation fluid IF-A following supplier's recommendations. The microplates were incubated at 28°C and optical density at 595nm was read after 24h incubation with a i-Mark Bio-Rad microplate reader. The tested strains were the type strain of *P. aquaticum* A212-S19-A16^{T} and 5 strains of the new clade (A477-S1-J17^{T}, A113-S21-F16, A411-S4-F17, FL63-S17 and FL60-S17). These biochemical tests revealed a few differences (Table 4 and Table S3 for complete results). First, strains FL63-S17 and FL60-S17, both isolated from an artificial lake, were the only tested strains that could not grow in the presence of D-aspartic acid. In addition, *P. aquaticum* type strain grew poorly in the presence of lithium chloride while strains of the new clade grew well in the presence of lithium chloride. Strains of the new clade were also unable or weakly able to use L-rhamnose as the only carbon source while the type strain of *P. aquaticum* can efficiently

198 metabolize L-rhamnose. Further investigation confirmed a strongly impaired growth in M63
199 medium with rhamnose as sole carbon source (rhamnose 0,02%, KH_2PO_4 13,6g.L^{-1}, (NH_4)
200 $2SO_4$ 2g.L^{-1}, $FeSO_4$ 10mM 200µl.L^{-1}, NaCl 10g.L^{-1}; pH adjusted to 7 with KOH 10N) at 28°C,
201 170 rpm (Figure 4A). This phenotypic difference is stronger than the phenotypic difference
202 described between *P. punjabense* and *P. parmentieri* or between *P. polaris* and *P. carotovorum*
203 [10, 12]. This impaired growth with rhamnose as sole carbon source correlates with 3 different
204 forms of pseudogenization of the L-rhamnose/proton symporter gene *rha*T in the genomes of
205 strains belonging to this new clade while this gene was found intact in all the sequenced
206 genomes of *P. aquaticum* (Figure 4B). In the genome of strain NAK:467 recently available in
207 NCBI (accession GCA_016949085.1), the L-rhamnose/proton symporter gene *rha*T was also
208 truncated (Figure 4B). Accordingly, ANIm and MLSA analysis (Figure S4 and Table S4)
209 indicated that the strain NAK:467 belongs to the new clade. Interestingly, in plant, rhamnose is
210 primarily found in the pectic matrix of the plant cell wall and rhamnose accumulation in the
211 cell wall of grasses in significantly smaller than the amount of rhamnose in the cell wall of
212 dicots [34]. As degradation of the plant cell wall is the main pathogenicity factor within the
213 *Pectobacterium* genus, the pseudogenization of the L-rhamnose/proton symporter gene *rha*T
214 for strains of the new clade suggests that strains of the new clade may preferentially infect
215 grasses while *P. aquaticum* retains the ability to infect dicots. This hypothesis remains to be
216 confirmed with identification of plants infected by strains of *P. aquaticum* and strains of the
217 new clade. Given the differential amount of rhamnose in different plants [34] this
218 pseudogenization may be interpretated as a sign of evolutionary divergence between the new
219 clade and *P. aquaticum*.
220

221 DESCRIPTION OF *PECTOBACTERIUM QUASIAQUATICUM* SP. NOV.

222 *Pectobacterium quasiaquaticum* (qua.si.a.qua'ti.cum. L. adv. *quasi* almost, nearly; L. neut. adj.
223 *aquaticum* aquatic, and a specific epithet in the genus *Pectobacterium*; N.L. neut. adj.
224 *quasiaquaticum* referring to the fact that the species is most closely related to *Pectobacterium*
225 *aquaticum*). Gram-negative, motile bacterium, grows optimally at 28 °C in LB medium
226 depleted from NaCl (10 g tryptone, 5 g yeast extract, 15 g agar per litre of medium). Forms pits
227 within 48 h when grown at 28°C on CVP medium [35]. Using GENIII Biolog plates, the *P.*
228 *quasiaquaticum* strains were negative for 3-O-Methyl-D-Glucose, 4% NaCl, 8% NaCl,
229 Acetoacetic Acid, Aztreonam, D-Arabitol, D-Cellobiose, D-Fucose, D-Glucuronic Acid, D-

Lactic Acid Methyl Ester, D-Malic acid, D-Maltose, D-Serine, D-Sorbitol, D-Trehalose, Turanose, Dextrin, Gelatin, Glucuronamide, Gly-Pro, Inosine, L-Alanine, L-Arginine, L-Fucose, L-Histidine, L-Lactic acid, L-Pyroglutamic Acid, Minocycline, N-Acetyl-Neuraminic Acid, N-Acetyl-D-Galactosamine, N-Acetyl-β-D-Mannosamine, Nalidixic Acid, Potassium Tellurite, Propionic Acid, Quinic Acid, Sodium Bromate, Stachyose, Tween 40, α-Hydroxy-Butyric Acid, α-Keto-Butyric Acid, α-Keto-Glutaric Acid, β-Hydroxy-Butyric Acid, p-Hydroxy-Phenylacetic Acid, γ-Amino-n-Butyric Acid, weakly or variably reacting for D-Aspartic Acid, Sodium Formate, L-Glutamic Acid, L-Rhamnose, pH 5 and were positive for 1% NaCl, 1% Sodium Lactate, Acetic acid, Bromo-Succinic acid, Citric acid, D-Fructose, D-Fructose-6-Phosphate, D-Galactose, D-Galacturonic acid, D-Gluconic acid, D-Glucose 6-Phosphate, D-Mannitol, D-Mannose, D-Melibiose, D-Raffinose, D-Saccharic acid, D-Salicin, Fusidic acid, β-Gentiobiose, Glycerol, Guanidine Hydrochloride, L-Aspartic acid, L-Galactonic Acide-γ-Lactone, L-Malic acid, L-Serine, Lincomycin, Methyl Pyruvate, Mucic acid, myo-Inositol, N-Acetyl-D-Glucosamine, Niaproof, Pectin, pH 6, Rifamycin SV, Butyric Acid, Sucrose, Tetrazolium Blue, Tetrazolium Violet, Troleandomycin, Vancomycin, D-Glucose, α-D-Lactose and β-Methyl-D-Glucoside.

The type strain is *P. quasiaquaticum* A477-S1-J17^{T} (=CFBP 8805 ^{T} =LMG 32181 ^{T}), which was isolated in 2017 from the Durance river at Sisteron, France. The GC content of the type strain DNA is 51.68%. A398-S21-F17, A535-S3-A17, A411-S4-F17, A113-S21-F16, FL63-S17 , FL60-S17 and NAK:467 are additional strains of the species. The draft genomes of 7 *Pectobacterium quasiaquaticum* strains sequenced in the course of this study have been deposited in the GenBank database under the bioproject number PRJNA662694 and the GenBank accession number of each genome is listed in Table 1.

AUTHOR STATEMENTS

Funding informations

This work was supported by the Agence Nationale de la Recherche ANR-17-CE32-0004 and the CNRS Ec2Co program (EC2COBiohefect/Ecodyn/Dril/MicrobienCARTOBACTER). The sampling campaigns realized in the CEREEP-Ecotron Ile-De-France benefited from the support received by the infrastructure PLANAQUA under the program “Investissements d'Avenir” launched by the French government and implemented by ANR with the references ANR-10-EQPX-13-01 Planaqua and ANR-11-INBS-0001 AnaEE France.

Acknowledgements

We thank Odile Berge and Frederique Van Gijsegem for their help during river samplings and Ariane Toussaint and Antoine Pourbaix for hosting us during the field samplings. We would also like to thank the "Plateforme expérimentale nationale d'écologie aquatique" (PLANAQUA) of the CEREEP-Ecotron Ile-De-France for the access to the artificial lakes and associated facilities and the expertise of the High-Throughput Sequencing Platform of I2BC, Gif sur Yvette, France for genomes sequencing. The authors wish also to thank the reviewers for their useful comments.

Ethical statement

not applicable

Conflicts of interest

The authors declare that there are no conflicts of interest.

REFERENCES

[1] **Adeolu M, Alnajar S, Naushad S, S. Gupta R.** Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. *Int J Syst Evol Microbiol* 2016;66:5575–99. https://doi.org/10.1099/ijsem.0.001485.

[2] **Ma B, Hibbing ME, Kim H-S, Reedy RM, Yedidia I, Breuer J, et al.** Host Range and Molecular Phylogenies of the Soft Rot Enterobacterial Genera *Pectobacterium* and *Dickeya*. *Phytopathol* 2007;97:1150–63. https://doi.org/10.1094/PHYTO-97-9-1150.

[3] **Pédron J, Bertrand C, Taghouti G, Portier P, Barny M-A.** *Pectobacterium aquaticum* sp. nov., isolated from waterways. *Int J Syst Evol Microbiol* 2019;69:745–51. https://doi.org/10.1099/ijsem.0.003229.

[4] **Portier P, Pédron J, Taghouti G, Fischer-Le Saux M, Caullireau E, Bertrand C, et al.** Elevation of *Pectobacterium carotovorum* subsp. *odoriferum* to species level as *Pectobacterium odoriferum* sp. nov., proposal of *Pectobacterium brasiliense* sp. nov. and *Pectobacterium actinidiae* sp. nov., emended description of *Pectobacterium carotovorum* and description of *Pectobacterium versatile* sp. nov., isolated from streams and symptoms on diverse plants. *Int J Syst Evol Microbiol* 2019. https://doi.org/10.1099/ijsem.0.003611.

[5] **Nabhan S, De Boer SH, Maiss E, Wydra K.** *Pectobacterium aroidearum* sp. nov., a soft rot pathogen with preference for monocotyledonous plants. *Int J Syst Evol Microbiol* 2013;63:2520–5. https://doi.org/10.1099/ijs.0.046011-0.

[6] **Gardan L, Gouy C, Richard C, Samson R.** Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. *Int J Syst Evol Microbio*l 2003;53:381–91. https://doi.org/10.1099/ijs.0.02423-0.

[7] **Alcorn SM, Orum TV, Steigerwalt AG, Foster JLM, Fogleman JC, Brenner DJ.**

Taxonomy and Pathogenicity of *Erwinia cacticida* sp. nov. *Int J Syst Bacteriol* 1991;41:197–212. https://doi.org/10.1099/00207713-41-2-197.

[8] **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;69:470–5. https://doi.org/10.1099/ijsem.0.003180.

[9] **Khayi S, Cigna J, Chong TM, Quêtu-Laurent A, Chan KG, Hélias V, Faure D.** Transfer of the potato plant isolates of *Pectobacterium wasabiae* to *Pectobacterium parmentieri* sp. nov. *Int J Syst Evol Microbiol* 2016;66:5379–83. https://doi.org/10.1099/ijsem.0.001524.

[10] **Dees MW, Lysøe E, Rossmann S, Perminow J, Brurberg MB.** *Pectobacterium polaris* sp. nov., isolated from potato (*Solanum tuberosum*). *Int J Syst Evol Microbiol* 2017;67:5222–9. https://doi.org/10.1099/ijsem.0.002448.

[11] **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;69:1751–9. https://doi.org/10.1099/ijsem.0.003387.

[12] **Sarfraz S, Riaz K, Oulghazi S, Cigna J, Sahi ST, Khan SH, et al.** *Pectobacterium punjabense* sp. nov., isolated from blackleg symptoms of potato plants in Pakistan. *Int J Syst Evol Microbiol* 2018;68:3551–6. https://doi.org/10.1099/ijsem.0.003029.

[13] **Pasanen M, Waleron M, Schott T, Cleenwerck I, Misztak A, Waleron K, et al.** *Pectobacterium parvum* sp. nov., having a *Salmonella* SPI-1-like Type III secretion system and low virulence. *Int J Syst Evol Microbiol* 2020;70:2440–8.

[14] **Waleron M, Misztak A, Waleron M, Franczuk M, Jońca J, Wielgomas B, et al.** *Pectobacterium zantedeschiae* sp. nov. a new species of a soft rot pathogen isolated from Calla lily (*Zantedeschia* spp.). *Syst Appl Microbiol* 2019;42:275–83. https://doi.org/10.1016/j.syapm.2018.08.004.

[15] **Waleron M, Misztak A, Waleron M, Franczuk M, Wielgomas B, Waleron K.** Transfer of *Pectobacterium carotovorum* subsp. *carotovorum* strains isolated from potatoes grown at high altitudes to *Pectobacterium peruviense* sp. nov. *Syst Appl Microbiol* 2018;41:85–93. https://doi.org/10.1016/j.syapm.2017.11.005.

[16] **Perombelon MCM, Kelman A.** Ecology of the Soft Rot *Erwinias*. Annu Rev *Phytopathol* 1980;18:361–87. https://doi.org/10.1146/annurev.py.18.090180.002045.

[17] *Quinn CE, Sells IA, Graham DC.* Soft Rot *Erwinia* Bacteria in the Atmospheric Bacterial Aerosol. *J Appl Bacteriol* 1980;49:175–81. https://doi.org/10.1111/j.1365-2672.1980.tb01055.x.

[18] Burr TJ. Occurrence of Soft-rot Erwinia spp. in Soil and Plant Material. Phytopathology 1977;77:1382. https://doi.org/10.1094/Phyto-67-1382.

[19] **Jorge PE, Harrison MD.** The association of *Erwinia carotovora* with surface water in Northeastern Colorado. I. The presence and population of the bacterium in relation to location, season and water temperature. *Am Potato J* 1986;63:517–31. https://doi.org/10.1007/BF03044052.

[20] **Faye P, Bertrand C, Pédron J, Barny M-A.** Draft genomes of "*Pectobacterium peruviense*" strains isolated from fresh water in France. *Stand Genomic Sci* 2018;13:27. https://doi.org/10.1186/s40793-018-0332-0.

[21] **Portier P, Pédron J, Taghouti G, Dutrieux C, Barny M-A.** Updated Taxonomy of Pectobacterium Genus in the CIRM-CFBP Bacterial Collection: When Newly Described Species Reveal "Old" Endemic Population. *Microorganisms* 2020;8:1441. https://doi.org/10.3390/microorganisms8091441.

[22] **Pédron J, Guyon L, Lecomte A, Blottière L, Chandeysson C, Rochelle-Newall E, et al.** Comparison of Environmental and Culture-Derived Bacterial Communities through 16S Metabarcoding: A Powerful Tool to Assess Media Selectivity and Detect Rare Taxa.

Microorganisms 2020;8:1129. https://doi.org/10.3390/microorganisms8081129.
[23] **Edgar RC.** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–7. https://doi.org/10.1093/nar/gkh340.
[24] **Castresana J.** Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis. *Mol Biol Evol* 2000;17:540–52. https://doi.org/10.1093/oxfordjournals.molbev.a026334.
[25] **Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O.** New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Syst Biol* 2010;59:307–21. https://doi.org/10.1093/sysbio/syq010.
[26] **Tamura K, Nei M.** Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993;10:512–26. https://doi.org/10.1093/oxfordjournals.molbev.a040023.
[27] **Gouy M, Guindon S, Gascuel O.** SeaView Version 4: A Multiplatform Graphical User Interface for Sequence Alignment and Phylogenetic Tree Building. *Mol Biol Evol* 2010;27:221–4. https://doi.org/10.1093/molbev/msp259.
[28] **Cigna J, Dewaegeneire P, Beury A, Gobert V, Faure D.** A *gap*A PCR-sequencing Assay for Identifying the *Dickeya* and *Pectobacterium* Potato Pathogens. *Plant Dis* 2017;101:1278–82. https://doi.org/10.1094/PDIS-12-16-1810-RE.
[29] **Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, et al.** RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep* 2015;5:8365. https://doi.org/10.1038/srep08365.
[30] **Miele V, Penel S, Duret L.** Ultra-fast sequence clustering from similarity networks with SiLiX. *BMC Bioinformatics* 2011;12:116. https://doi.org/10.1186/1471-2105-12-116.
[31] **Gascuel O.** BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Mol Biol Evol* 1997;14:685–95. https://doi.org/10.1093/oxfordjournals.molbev.a025808.
[32] **Pritchard L, Glover RH, Humphris S, Elphinstone JG, Toth IK.** Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. *Anal Methods* 2016;8:12–24. https://doi.org/10.1039/C5AY02550H.
[33] **Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M.** Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60. https://doi.org/10.1186/1471-2105-14-60.
[34] **Jiang N, Dillon FM, Silva A, Gomez-Cano L, Grotewold E.** Rhamnose in plants - from biosynthesis to diverse functions. *Plant Sci* 2021;302:110687. https://doi.org/10.1016/j.plantsci.2020.110687.
[35] **Hélias V, Hamon P, Huchet E, Wolf JVD, Andrivon D.** Two new effective semiselective crystal violet pectate media for isolation of Pectobacterium and Dickeya: Isolating pectolytic bacteria on CVP. *Plant Pathol* 2012;61:339–45. https://doi.org/10.1111/j.1365-3059.2011.02508.x.

ABBREVIATIONS

CVP: Crystal Violet Pectate

ANI: Average Nucleotide Identity

dDDH: digital DNA-DNA hybridization

*gap*A: glyceraldehyde-3-phosphate dehydrogenase A

MLSA: Multi Locus Sequence Analysis

402

403 # FIGURES AND TABLES

404

405 **Figure 1:** *gap*A phylogenetic tree of *P. quasiaquaticum* sp. nov. strains and type strains of other
406 *Pectobacterium* species.
407 Phylogenetic tree reconstructed from the *gap*A nucleotide sequences. Accession numbers are
408 indicated in brackets after the strain name. Only bootstrap support values above 70% are
409 indicated. Bar, 0.05 changes per nucleotide position. *Dickeya solani* IPO2222^T was used as
410 outgroup.
411

412 **Figure 2**: MLSA phylogenetic tree of *P. quasiaquaticum* sp. nov. strains and strains of other
413 *Pectobacterium* species.
414 MLSA Phylogenetic tree reconstructed from concatenated nucleotide sequences of 265
415 homologous gene sequences of 75 *Pectobacterium* genomes. The bootstrap support values are
416 indicated if less than 100%. The number of analysed genomes per species is indicated in bracket
417 after name of the species. *Dickeya solani* IPO2222^T was used as outgroup. Bar, 0.02 changes
418 per nucleotide position. For single strain branches, accession numbers are indicated in brackets
419 after the strain name. Extended version of this phylogenetic tree is provided Fig. S3 with
420 accession numbers of each genome.
421

422 **Figure 3:** Impaired growth of *P. quasiaquaticum* strains in rhamnose M63 medium correlates
423 with pseudogenisation of the L-rhamnose/proton symporter RhaT.
424 A: Growth of *P. aquaticum* A212-S19-A16 (4 replicates) and *P. quasiaquaticum* strains A477-
425 S1-J17^T, A411-S4-F17, A113-S21-F16, FL63-S17 and FL60-S17 in M63-rhamnose medium.
426 B: Alignment of the L-rhamnose/proton symporter RhaT 1: genomes of *P. aquaticum* strains
427 A212-S19-A16, A105-S21-F16, A35-S23_M15, A101-S19-F16, A104-S21-F16 and A127-
428 S21-F16 code for a 344 amino acids long RhaT protein. 2: genomes of strain A398-S21-F17
429 code for a truncated version of 150 amino acids. 3: genomes of strains A113-S21-F16, A411-
430 S4-F17, A535-S3-A17 code for a smaller truncated version of 144 amino acids. 4: genomes of
431 strains FL60-S17, A477-S1-J17, FL63-S17 and NAK:467 code for a further truncated version
432 where a tryptophan is replaced by a stop codon leading to two short open reading frames of 91
433 and 52 amino acids.
434

Table 1: Descriptive table of the seven sequenced genomes *of P. quasiaquaticum*.

Table 2: ANI (lower diagonal) and dDDH (top diagonal) values between *P. quasiaquaticum* strains sp. nov. and *P. aquaticum* strains and *P. brasiliense* 1692^{T} strain.
ANI values ≥96% are indicated in red, ANI values between 96%-95% are indicated in light purple and ANI values ≤95% are indicated in blue, dDDH values ≥70% are indicated in pale red and dDDH values below 70% are indicated in blue.

Table 3: Range of ANIb (Identity/Coverage) and dDDH (excluding near identities -100% and 99.9%) within and between related species.

Table 4: Biolog GENII Main phenotypic differences between *P. quasiaquaticum* and *P. aquaticum*

SUPPLEMENTAL MATERIAL:

Figure S1: Symptoms observed following inoculation of potato slices.
Photo were taken after 48h of incubation at 26°C. The inoculated strains are indicated below each photo.

Figure S2: Phylogenetic tree of 16S rRNA gene of 29 *Pectobacterium* strains. Accession numbers are indicated in brackets after the strain name. Only bootstrap support values above 50% are indicated. Bar, 0.1 changes per nucleotide position. *Dickeya solani* IPO2222T was used as outgroup. The 16S ribosomal rRNA sequences of the 7 analyzed strains have been deposited to NCBI and the accession numbers are the following: MW115912 (strain FL63-S17), MW115908 (strain A477-S1-J17T), MW115910 (strain A535-S3-A17), MW115911 (strain FL60-S17), MW115909 (strain A411-S4-F17), MW115906 (strain A113-S21-F16), MW115907 (strain A398-S21-F17).

Figure S3: Extended MLSA phylogenetic tree of *P. quasiaquaticum* sp. nov. strains and type strains of other *Pectobacterium* species. Accession numbers are indicated in brackets after the strain name. The bootstrap support values are indicated if less than 100%. Bar, 0.020 changes per nucleotide position.

Figure S4: MLSA phylogenetic tree of *P. quasiaquaticum* sp. nov. strains and *P. aquaticum* strains including the reclassified strain NAK:467. The MLSA phylogenetic tree was reconstructed from concatenated nucleotide sequences of 2899 homologous gene sequences of the 15 *Pectobacterium* genomes. The clustering of homologous nucleotide sequences was performed with SiLix [30] software with an 80% identity threshold. Homologous sequences of each gene were aligned using MUSCLE [23] software then concatenated. The alignments were filtered using the GBLOCK tool [24] resulting in a data set of 2,881,708 sites (of which 142,278 are informative). The tree was computed with the SeaView software [27] using the BioNJ method [31]. Bootstrap percentages were calculated based on 200 replicates. *P. brasiliense* 1692T was used as an outgroup. Bar, 0.005 changes per nucleotide position. The position of the NAK:467 genome is highlighted in yellow.

Table S1: genes ID of the 265 genes of strain A477-S1-J17^T used in the MLSA phylogenetic tree Figure 2.

Table S2: ANIb, ANIb coverage and dDDH values between *P. quasiaquaticum* strains sp. nov. and other *Pectobacterium* spp. ANI values ≥96% are indicated in red, ANI values between 96%-95% are indicated in light purple and ANI values ≤95% are indicated in blue. dDDH values ≥70% are indicated in pale red and dDDH values below 70% are indicated in blue.

Table S3: Phenotypic characterization of *P. quasiaquaticum* A477-S1-J17^T, A113-S21-F16, A411-S4-F17, FL63-S17 and FL60-S17 and *P. aquaticum* A212-S19-A16^T using GENIII Biolog plates.

Table S4: ANIm, values between genomes of *P. quasiaquaticum* strains sp. nov., *P. aquaticum* and NAK:467. ANI values ≥96% are indicated in red, ANI values between 96%-95% are indicated in light blue and ANI values ≤95% are indicated in blue. Pq: *P. quasiaquaticum*; Pa: *P. aquaticum*; Pb: *P. brasiliense*. NAK:467 is highlighted in yellow.

File S1.fasta: 16S rRNA gene sequences of 29 *Pectobacterium* strains and *Dickeya solani* IPO222^T used to build the 16S rRNA gene phylogeny provided Figure S2.

File S2.fasta: *gap*A gene sequences of 29 *Pectobacterium* strains and *Dickeya solani* IPO222^T used to build the phylogenetic tree provided Figure 1.

File S3.fasta: *rha*T A: genes and B: protein sequences extracted from 7 *P. quasiaquaticum* and 7 *P. aquaticum* genomes available at NCBI (genomes assemblies GCA_003382565.2, GCA_016949085.1, GCA_003382585.2, GCA_003382645.2, GCA_003382625.2, GCA_003382595.2, GCA_003382655.2, GCA_014946805.1, GCA_014946825.1, GCA_014946845.1, GCA_014946775.1, GCA_014946835.1, GCA_014946865.1, GCA_014946905.1).

FIGURE 1

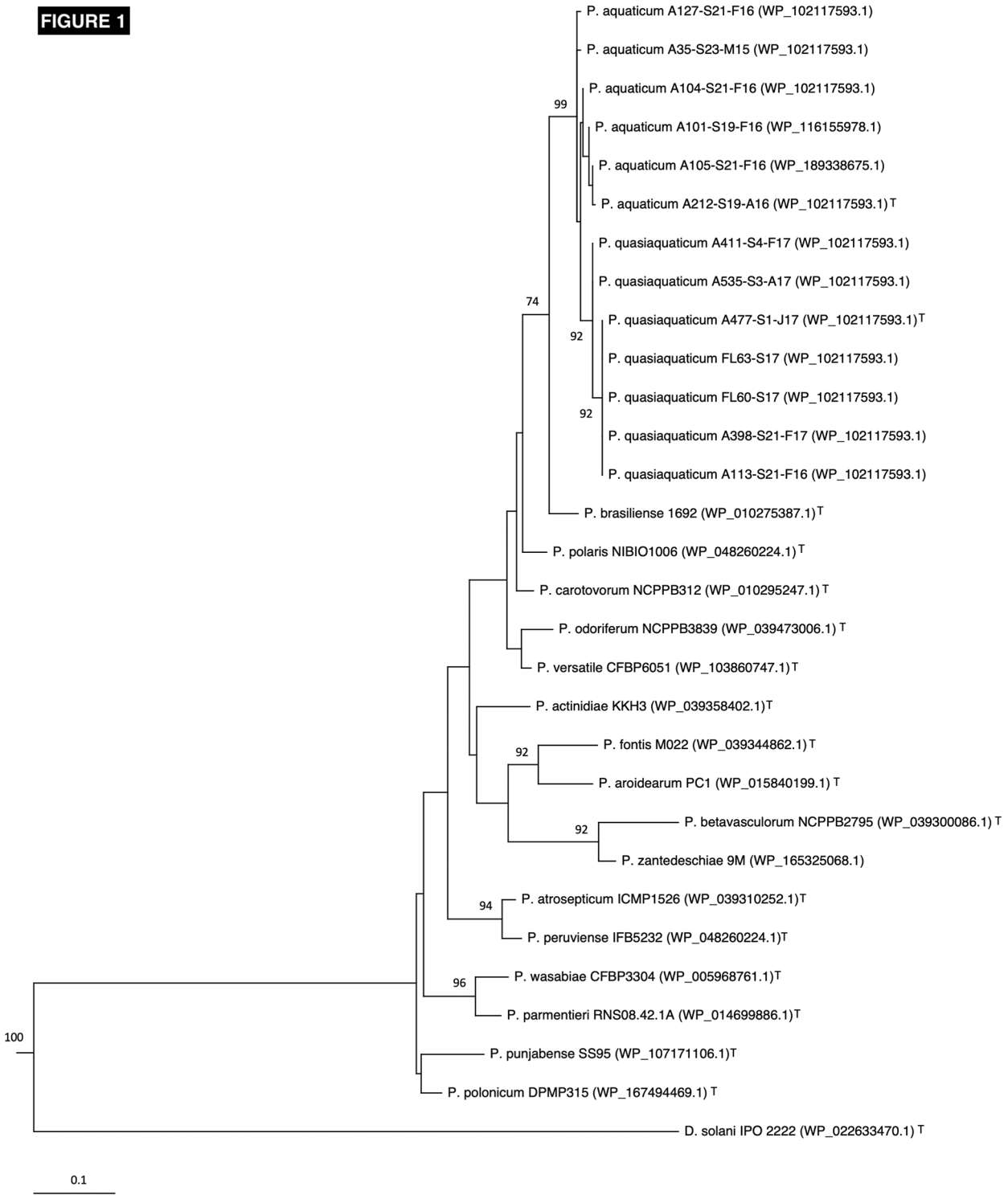

P. aquaticum A127-S21-F16 (WP_102117593.1)
P. aquaticum A35-S23-M15 (WP_102117593.1)
P. aquaticum A104-S21-F16 (WP_102117593.1)
P. aquaticum A101-S19-F16 (WP_116155978.1)
P. aquaticum A105-S21-F16 (WP_189338675.1)
P. aquaticum A212-S19-A16 (WP_102117593.1)T
P. quasiaquaticum A411-S4-F17 (WP_102117593.1)
P. quasiaquaticum A535-S3-A17 (WP_102117593.1)
P. quasiaquaticum A477-S1-J17 (WP_102117593.1)T
P. quasiaquaticum FL63-S17 (WP_102117593.1)
P. quasiaquaticum FL60-S17 (WP_102117593.1)
P. quasiaquaticum A398-S21-F17 (WP_102117593.1)
P. quasiaquaticum A113-S21-F16 (WP_102117593.1)
P. brasiliense 1692 (WP_010275387.1)T
P. polaris NIBIO1006 (WP_048260224.1)T
P. carotovorum NCPPB312 (WP_010295247.1)T
P. odoriferum NCPPB3839 (WP_039473006.1)T
P. versatile CFBP6051 (WP_103860747.1)T
P. actinidiae KKH3 (WP_039358402.1)T
P. fontis M022 (WP_039344862.1)T
P. aroidearum PC1 (WP_015840199.1)T
P. betavasculorum NCPPB2795 (WP_039300086.1)T
P. zantedeschiae 9M (WP_165325068.1)
P. atrosepticum ICMP1526 (WP_039310252.1)T
P. peruviense IFB5232 (WP_048260224.1)T
P. wasabiae CFBP3304 (WP_005968761.1)T
P. parmentieri RNS08.42.1A (WP_014699886.1)T
P. punjabense SS95 (WP_107171106.1)T
P. polonicum DPMP315 (WP_167494469.1)T
D. solani IPO 2222 (WP_022633470.1)T
99
74
92
92
92
92
94
96
100
0.1

FIGURE 2

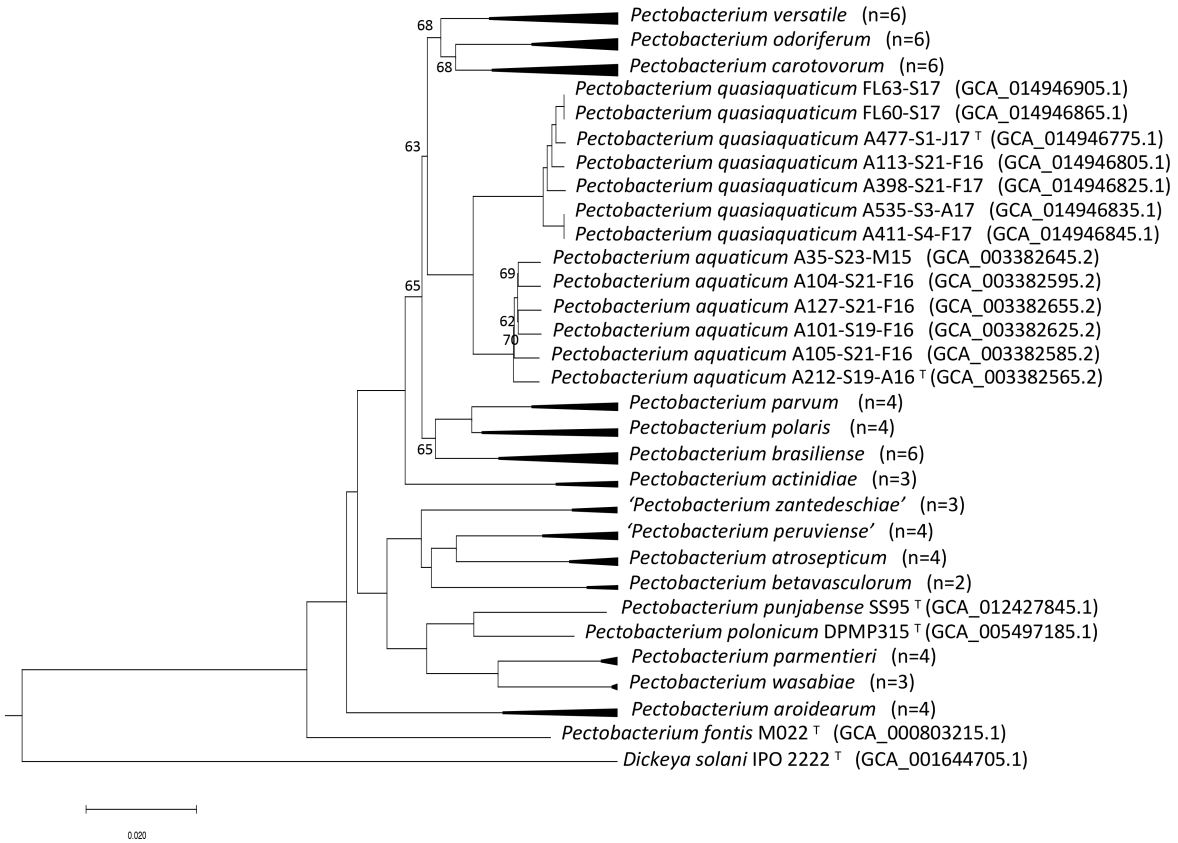
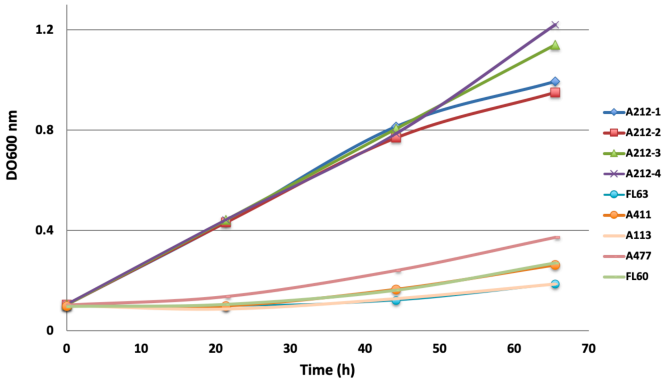

68
68
63
65
69
62
70
65
Pectobacterium versatile (n=6)
Pectobacterium odoriferum (n=6)
Pectobacterium carotovorum (n=6)
Pectobacterium quasiaquaticum FL63-S17 (GCA_014946905.1)
Pectobacterium quasiaquaticum FL60-S17 (GCA_014946865.1)
Pectobacterium quasiaquaticum A477-S1-J17 T (GCA_014946775.1)
Pectobacterium quasiaquaticum A113-S21-F16 (GCA_014946805.1)
Pectobacterium quasiaquaticum A398-S21-F17 (GCA_014946825.1)
Pectobacterium quasiaquaticum A535-S3-A17 (GCA_014946835.1)
Pectobacterium quasiaquaticum A411-S4-F17 (GCA_014946845.1)
Pectobacterium aquaticum A35-S23-M15 (GCA_003382645.2)
Pectobacterium aquaticum A104-S21-F16 (GCA_003382595.2)
Pectobacterium aquaticum A127-S21-F16 (GCA_003382655.2)
Pectobacterium aquaticum A101-S19-F16 (GCA_003382625.2)
Pectobacterium aquaticum A105-S21-F16 (GCA_003382585.2)
Pectobacterium aquaticum A212-S19-A16 T (GCA_003382565.2)
Pectobacterium parvum (n=4)
Pectobacterium polaris (n=4)
Pectobacterium brasiliense (n=6)
Pectobacterium actinidiae (n=3)
'Pectobacterium zantedeschiae' (n=3)
'Pectobacterium peruviense' (n=4)
Pectobacterium atrosepticum (n=4)
Pectobacterium betavasculorum (n=2)
Pectobacterium punjabense SS95 T (GCA_012427845.1)
Pectobacterium polonicum DPMP315 T (GCA_005497185.1)
Pectobacterium parmentieri (n=4)
Pectobacterium wasabiae (n=3)
Pectobacterium aroidearum (n=4)
Pectobacterium fontis M022 T (GCA_000803215.1)
Dickeya solani IPO 2222 T (GCA_001644705.1)
0.020

FIGURE 3

A


1.2
0.8
0.4
0
DO600 nm
0
10
20
30
40
50
60
70
Time (h)
A212-1
A212-2
A212-3
A212-4
FL63
A411
A113
A477
FL60

B

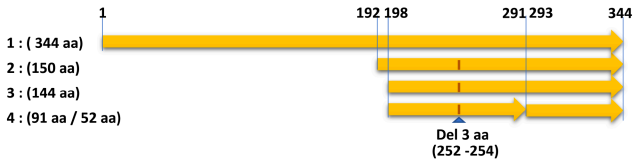

1
192 198
291 293
344
1 : (344 aa)
2 : (150 aa)
3 : (144 aa)
4 : (91 aa / 52 aa)
Del 3 aa
(252 -254)

Table 1 : Descriptive table of the seven genomes *of P. quasiaquaticum*

Strain name	Isolation date	Isolation site	Genome accessions	Genome coverage	Number of Contigs	Genome Size (pb)	G+C (mol%)	Protein coding genes
A113-S21-F16	02-2016	S21	JACYTG010000000	165	85	4,421,657	51.42	4345
A398-S21-F17	02-2017	S21	JACYTH010000000	204	81	4,248,246	51.73	4152
A411-S4-F17	02-2017	S4	JACYTI010000000	208	96	4,301,740	51.4	4210
A477-S1-J17^{T}	06-2017	S1	JACYTJ010000000	186	87	4,398,604	51.68	4293
A535-S3-A17	08-2017	S3	JACYTK010000000	233	95	4,334,305	51.41	4259
FL60-S17	09-2017	Sf	JACYTM010000000	156	141	4,286,319	51.51	4215
FL63-S17	09-2017	Sf	JACYTL010000000	205	92	4,267,445	51.53	4148

Isolation sites: S21, River Grand-Anguillon at Nove; S4, River Durance at Sisteron; S1, Irrigation canal at Logis Neuf; S3, River Durance at Manosque; Sf, CEREEP Ecotron artificial lakes.

TABLE 2

	P. quasiaquaticum A411-S4-F17	*P. quasiaquaticum* A535-S3-A17	*P. quasiaquaticum* A398-S21-F17	*P. quasiaquaticum* A113-S21-F16	*P. quasiaquaticum* A477-S1-J17^{T}	*P. quasiaquaticum* FL60-S17	*P. quasiaquaticum* FL63-S17	*P. aquaticum* A127-S21-F16	*P. aquaticum* A35-S23-M15	*P. aquaticum* A104-S21-F16	*P. aquaticum* A101-S19-F16	*P. aquaticum* A105-S21-F16	*P. aquaticum* A212-S19-A16^{T}	*P. brasiliense* 1692^{T}
P. quasiaquaticum A411-S4-F17	100.0	100.0	90.9	89.8	91.2	90.3	90.2	64.4	64.2	64.0	64.9	63.9	65.3	55.7
P. quasiaquaticum A535-S3-A17	100.0	100.0	90.8	89.8	91.1	90.2	90.2	64.4	64.2	64.1	64.9	63.9	65.3	55.8
P. quasiaquaticum A398-S21-F17	98.9	98.9	100.0	89.5	90.6	89.7	89.7	64.4	63.8	63.9	64.4	63.7	64.8	55.6
P. quasiaquaticum A113-S21-F16	98.8	98.7	98.7	100.0	95.6	95.2	95.2	64.5	64.0	64.1	64.6	63.7	64.1	55.4
P. quasiaquaticum A477-S1-J17^{T}	98.9	98.9	98.9	99.4	100.0	96.5	96.5	64.4	64.2	63.9	64.9	64.0	64.9	55.1
P. quasiaquaticum FL60-S17	98.8	98.8	98.7	99.4	99.5	100.0	99.9	65.5	65.1	65.6	65.7	65.0	65.8	55.3
P. quasiaquaticum FL63-S17	98.8	98.8	98.7	99.3	99.5	99.9	100.0	65.6	65.2	65.6	65.7	65.1	65.9	55.4
P. aquaticum A127-S21-F16	95.5	95.6	95.5	95.6	95.5	95.7	95.8	100.0	90.5	89.4	88.7	87.9	86.2	51.4
P. aquaticum A35-S23-M15	95.4	95.5	95.5	95.5	95.5	95.6	95.7	98.8	100.0	88.5	88.8	87.6	86.8	51.4
P. aquaticum A104-S21-F16	95.4	95.5	95.4	95.5	95.4	95.7	95.8	98.7	98.6	100.0	88.8	87.3	87.7	51.3
P. aquaticum A101-S19-F16	95.6	95.6	95.5	95.6	95.6	95.7	95.8	98.6	98.7	98.7	100.0	88.9	88.1	51.6
P. aquaticum A105-S21-F16	95.5	95.5	95.5	95.5	95.5	95.7	95.7	98.6	98.5	98.5	98.7	100.0	87.8	51.4
P. aquaticum A212-S19-A16^{T}	95.7	95.7	95.6	95.5	95.6	95.7	95.8	98.3	98.4	98.5	98.6	98.5	100.0	51.7
P. brasiliense 1692^{T}	94.0	94.0	94.0	94.0	93.8	93.9	94.0	93.1	93.1	93.1	93.2	93.1	93.2	100.0

Table 3 : Range of ANIb (Identity/Coverage) and dDDH (excluding near identities -100% and 99.9%) within and between related species.

Analyzed genomes *	ANIb Identity (%)	ANIb Coverage (%)	dDDH (%)
P. quasiaquaticum - P. quasiaquaticum (7X7)	99.5 - 98.7	99.8 - 88.6	96.5 - 89.5
P. aquaticum – P. aquaticum (6X6)	98.9 - 98.3	91.9 - 85.7	90.5 - 86.2
P. aquaticum - P.quasiaquaticum (6X7)	95.8 - 95.4	84.5 - 79.0	65.9 - 63.7
P. parvum - P. parvum (3X3)**	99.5 - 99.3	99.5 - 93.2	99.6 - 95.2
P. polaris - P. polaris (4X4)	97.1 - 96.8	87.5 - 82.8	74.4 - 73.3
P. polaris - P. parvum (4X3)**	96.1 - 95.9	79.7 - 73.9	68.4 - 66.4
P. versatile - P. versatile (6X6)	98.2 - 97.4	90.6 - 83.2	83.8 - 78.4
P. carotovorum - P. carotovorum (6X6)	98.1 - 97.1	93.9 - 85.2	83.5 - 75.6
P. versatile - P. carotovorum (6X6)	95.6 - 95.3	88.2 - 79.3	64.6 - 62.6

The number of analyzed genomes is indicated in brackets. Details of pairwise comparisons are presented Fig. S2.**the genome of strain *P. parvum* Y1 used in MLSA (Fig.2) was exclude from this table as sequence is short and may lack substantial part of the genome [13].

Table 3: Biolog GENIII Main phenotypic differences between *P. quasiaquaticum* and *P. aquaticum*

Test	*P. aquaticum* A212-S19-A16^T	*P. quasiaquaticum* A113-S21-F16	*P. quasiaquaticum* A411-S4-F17	*P. quasiaquaticum* A477-S1-J17^T	*P. quasiaquaticum* FL63-S17	*P. quasiaquaticum* FL60-S17
D-Aspartic Acid	+	+	+	+	-	-
L-Rhamnose	+	w	w	w	-	w
4% NaCl	w	-	-	-	-	-
Lithium Chloride	w	+	+	+	+	+

+, Positive; w, weakly positive; -, negative

Supplementals for

Pectobacterium quasiaquaticum sp. nov., isolated from waterways

Hajar Ben Moussa, Jacques Pédron, Claire Bertrand, Amandine Hecquet and Marie-Anne Barny
Sorbonne Université, INRAE, Institute of Ecology and Environmental Sciences-Paris, 4 place
7 Jussieu, F-75 252 Paris, France.
for correspondance : marie-anne.barny@sorbonne-universite.fr

Include Supplementals are in the following order

- **Figure S1 and legend**: Symptoms observed following inoculation of potato slices.
- **Figure S2 and legend** : Phylogenetic tree of 16S rRNA gene of 29 *Pectobacterium* strains.
- **Figure S3 and legend:** Extended MLSA phylogenetic tree of *P. quasiaquaticum* sp. nov. strains and type strains of other *Pectobacterium* species
- **Figure S4 and legend:** MLSA phylogenetic tree of *P. quasiaquaticum* sp. nov. strains and *P. aquaticum* strains including the reclassified strain NAK:467.
- **Table S1**: genes ID of the 265 genes of strain A477-S1-J17^T used in the MLSA phylogenetic tree Figure 2.
- **Table S3**: Phenotypic characterization of *P. quasiaquaticum* A477-S1-J17^T, A113-S21-F16, A411-S4-F17, FL63-S17 and FL60-S17 and *P. aquaticum* A212-S19-A16^T using GENIII Biolog plates.
- **Table S4**: ANIm, values between genomes of *P. quasiaquaticum* strains sp. nov., *P. aquaticum* and NAK:467. ANI values ≥96% are indicated in red, ANI values between 96%-95% are indicated in light blue and ANI values ≤95% are indicted in blue.

Table S2 is not included in this supplemental and is a separate exel file

A398-S21-F17

A411-S4-F17

A477-S1-J17^{T}

FL63-S17

Buffer

Figure S1: Symptoms observed following inoculation of potato slices.
Photo were taken after 48h of incubation at 26°C. The inoculated strains are indicated below each photo.

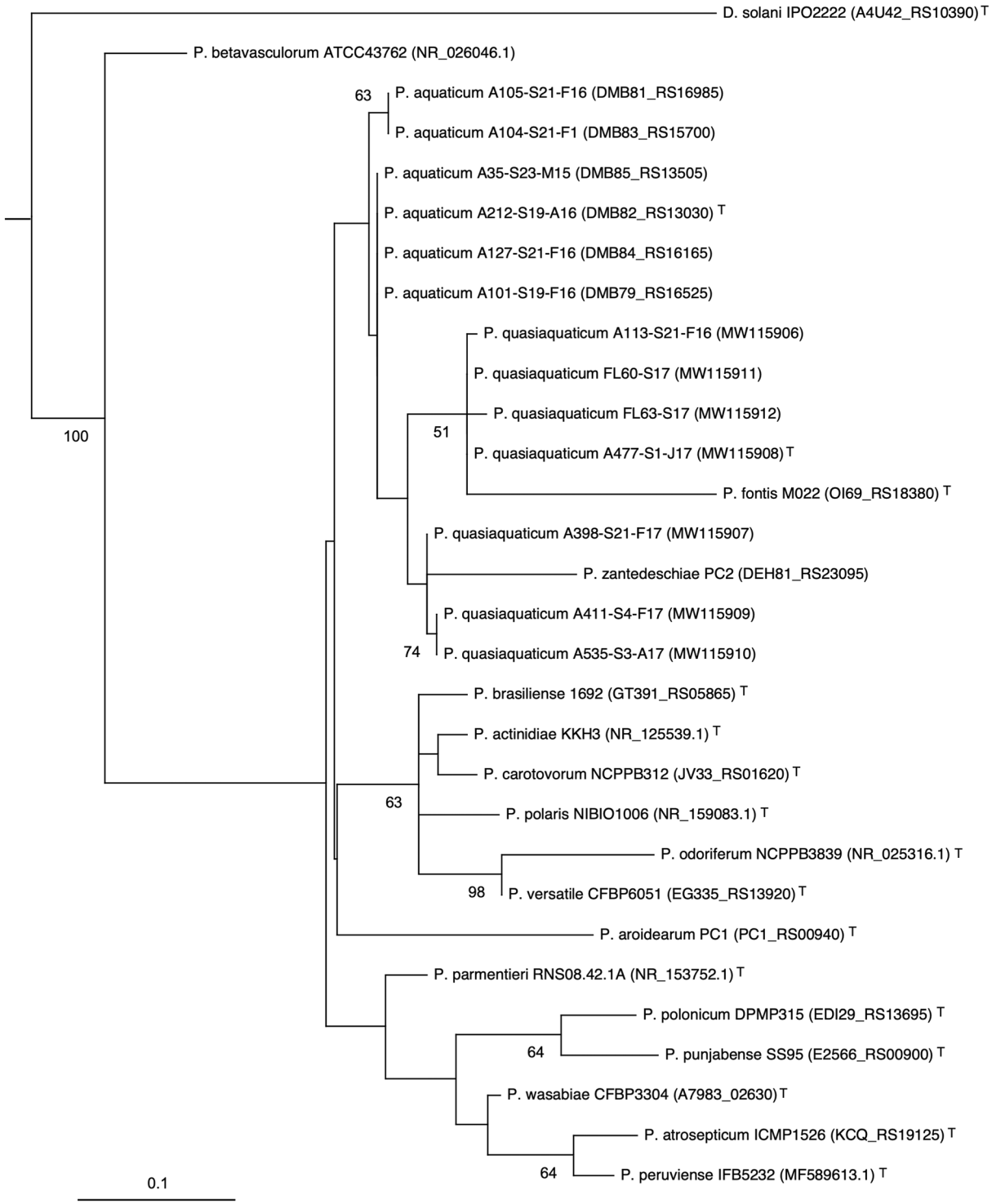

D. solani IPO2222 (A4U42_RS10390) T
P. betavasculorum ATCC43762 (NR_026046.1)
63
P. aquaticum A105-S21-F16 (DMB81_RS16985)
P. aquaticum A104-S21-F1 (DMB83_RS15700)
P. aquaticum A35-S23-M15 (DMB85_RS13505)
P. aquaticum A212-S19-A16 (DMB82_RS13030) T
P. aquaticum A127-S21-F16 (DMB84_RS16165)
P. aquaticum A101-S19-F16 (DMB79_RS16525)
P. quasiaquaticum A113-S21-F16 (MW115906)
P. quasiaquaticum FL60-S17 (MW115911)
P. quasiaquaticum FL63-S17 (MW115912)
51
P. quasiaquaticum A477-S1-J17 (MW115908) T
P. fontis M022 (OI69_RS18380) T
100
P. quasiaquaticum A398-S21-F17 (MW115907)
P. zantedeschiae PC2 (DEH81_RS23095)
P. quasiaquaticum A411-S4-F17 (MW115909)
74
P. quasiaquaticum A535-S3-A17 (MW115910)
P. brasiliense 1692 (GT391_RS05865) T
P. actinidiae KKH3 (NR_125539.1) T
P. carotovorum NCPPB312 (JV33_RS01620) T
63
P. polaris NIBIO1006 (NR_159083.1) T
P. odoriferum NCPPB3839 (NR_025316.1) T
98
P. versatile CFBP6051 (EG335_RS13920) T
P. aroidearum PC1 (PC1_RS00940) T
P. parmentieri RNS08.42.1A (NR_153752.1) T
P. polonicum DPMP315 (EDI29_RS13695) T
64
P. punjabense SS95 (E2566_RS00900) T
P. wasabiae CFBP3304 (A7983_02630) T
P. atrosepticum ICMP1526 (KCQ_RS19125) T
64
P. peruviense IFB5232 (MF589613.1) T
0.1

Figure S2: Phylogenetic tree of 16S rRNA gene of 29 *Pectobacterium* strains. Accession numbers are indicated in brackets after the strain name. Only bootstrap support values above 50% are indicated. Bar, 0.1 changes per nucleotide position. *Dickeya solani* IPO2222T was used as outgroup. The 16S ribosomal rRNA sequences of the 7 analysed strains have been deposited to NCBI and the accession numbers are the following: MW115912 (strain FL63-S17), MW115908 (strain A477-S1-J17T), MW115910 (strain A535-S3-A17), MW115911 (strain FL60-S17), MW115909 (strain A411-S4-F17), MW115906 (strain A113-S21-F16), MW115907 (strain A398-S21-F17).

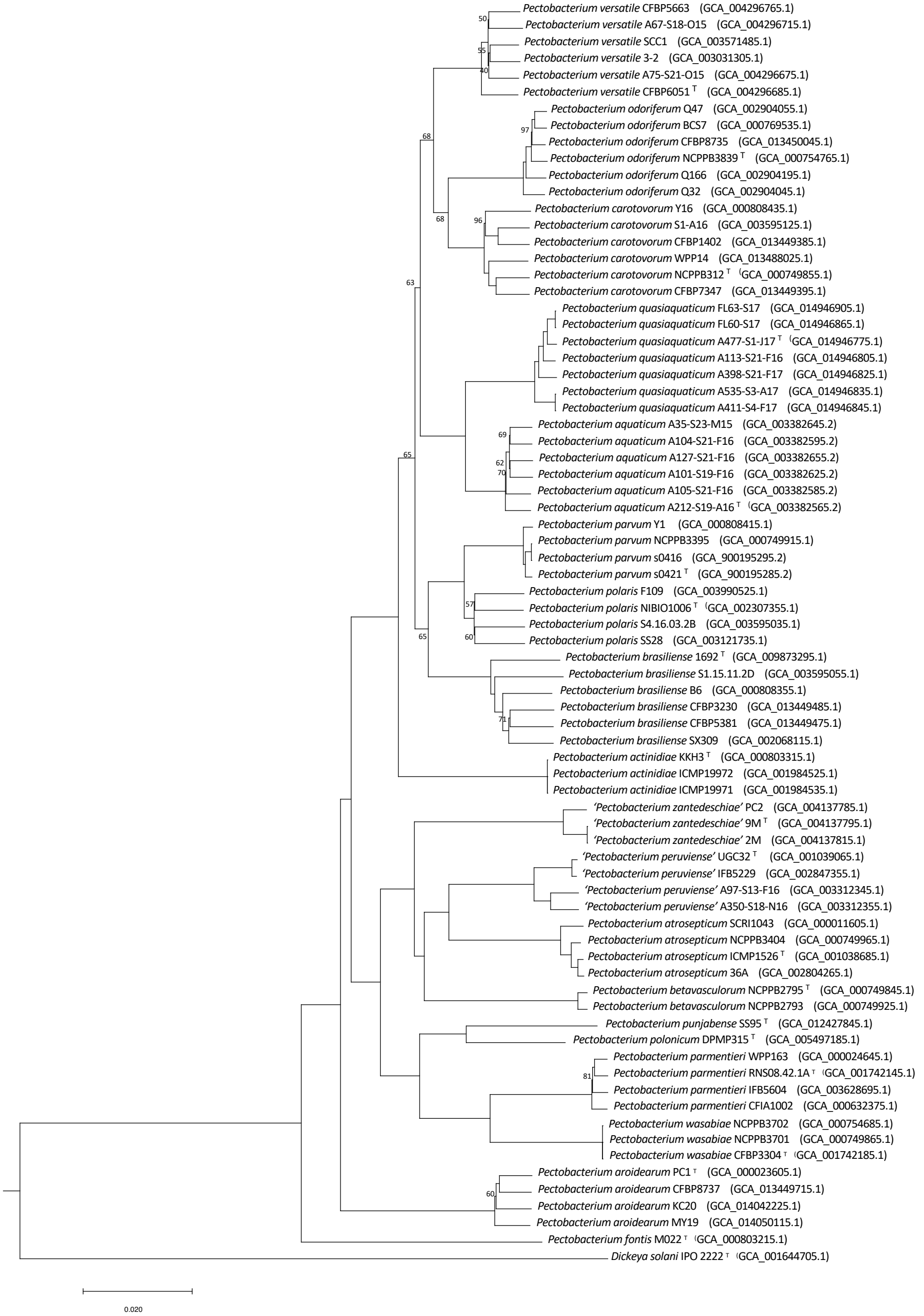

Pectobacterium versatile CFBP5663 (GCA_004296765.1)
Pectobacterium versatile A67-S18-O15 (GCA_004296715.1)
Pectobacterium versatile SCC1 (GCA_003571485.1)
Pectobacterium versatile 3-2 (GCA_003031305.1)
Pectobacterium versatile A75-S21-O15 (GCA_004296675.1)
Pectobacterium versatile CFBP6051 T (GCA_004296685.1)
Pectobacterium odoriferum Q47 (GCA_002904055.1)
Pectobacterium odoriferum BCS7 (GCA_000769535.1)
Pectobacterium odoriferum CFBP8735 (GCA_013450045.1)
Pectobacterium odoriferum NCPPB3839 T (GCA_000754765.1)
Pectobacterium odoriferum Q166 (GCA_002904195.1)
Pectobacterium odoriferum Q32 (GCA_002904045.1)
Pectobacterium carotovorum Y16 (GCA_000808435.1)
Pectobacterium carotovorum S1-A16 (GCA_003595125.1)
Pectobacterium carotovorum CFBP1402 (GCA_013449385.1)
Pectobacterium carotovorum WPP14 (GCA_013488025.1)
Pectobacterium carotovorum NCPPB312 T (GCA_000749855.1)
Pectobacterium carotovorum CFBP7347 (GCA_013449395.1)
Pectobacterium quasiaquaticum FL63-S17 (GCA_014946905.1)
Pectobacterium quasiaquaticum FL60-S17 (GCA_014946865.1)
Pectobacterium quasiaquaticum A477-S1-J17 T (GCA_014946775.1)
Pectobacterium quasiaquaticum A113-S21-F16 (GCA_014946805.1)
Pectobacterium quasiaquaticum A398-S21-F17 (GCA_014946825.1)
Pectobacterium quasiaquaticum A535-S3-A17 (GCA_014946835.1)
Pectobacterium quasiaquaticum A411-S4-F17 (GCA_014946845.1)
Pectobacterium aquaticum A35-S23-M15 (GCA_003382645.2)
Pectobacterium aquaticum A104-S21-F16 (GCA_003382595.2)
Pectobacterium aquaticum A127-S21-F16 (GCA_003382655.2)
Pectobacterium aquaticum A101-S19-F16 (GCA_003382625.2)
Pectobacterium aquaticum A105-S21-F16 (GCA_003382585.2)
Pectobacterium aquaticum A212-S19-A16 T (GCA_003382565.2)
Pectobacterium parvum Y1 (GCA_000808415.1)
Pectobacterium parvum NCPPB3395 (GCA_000749915.1)
Pectobacterium parvum s0416 (GCA_900195295.2)
Pectobacterium parvum s0421 T (GCA_900195285.2)
Pectobacterium polaris F109 (GCA_003990525.1)
Pectobacterium polaris NIBIO1006 T (GCA_002307355.1)
Pectobacterium polaris S4.16.03.2B (GCA_003595035.1)
Pectobacterium polaris SS28 (GCA_003121735.1)
Pectobacterium brasiliense 1692 T (GCA_009873295.1)
Pectobacterium brasiliense S1.15.11.2D (GCA_003595055.1)
Pectobacterium brasiliense B6 (GCA_000808355.1)
Pectobacterium brasiliense CFBP3230 (GCA_013449485.1)
Pectobacterium brasiliense CFBP5381 (GCA_013449475.1)
Pectobacterium brasiliense SX309 (GCA_002068115.1)
Pectobacterium actinidiae KKH3 T (GCA_000803315.1)
Pectobacterium actinidiae ICMP19972 (GCA_001984525.1)
Pectobacterium actinidiae ICMP19971 (GCA_001984535.1)
'Pectobacterium zantedeschiae' PC2 (GCA_004137785.1)
'Pectobacterium zantedeschiae' 9M T (GCA_004137795.1)
'Pectobacterium zantedeschiae' 2M (GCA_004137815.1)
'Pectobacterium peruviense' UGC32 T (GCA_001039065.1)
'Pectobacterium peruviense' IFB5229 (GCA_002847355.1)
'Pectobacterium peruviense' A97-S13-F16 (GCA_003312345.1)
'Pectobacterium peruviense' A350-S18-N16 (GCA_003312355.1)
Pectobacterium atrosepticum SCRI1043 (GCA_000011605.1)
Pectobacterium atrosepticum NCPPB3404 (GCA_000749965.1)
Pectobacterium atrosepticum ICMP1526 T (GCA_001038685.1)
Pectobacterium atrosepticum 36A (GCA_002804265.1)
Pectobacterium betavasculorum NCPPB2795 T (GCA_000749845.1)
Pectobacterium betavasculorum NCPPB2793 (GCA_000749925.1)
Pectobacterium punjabense SS95 T (GCA_012427845.1)
Pectobacterium polonicum DPMP315 T (GCA_005497185.1)
Pectobacterium parmentieri WPP163 (GCA_000024645.1)
Pectobacterium parmentieri RNS08.42.1A T (GCA_001742145.1)
Pectobacterium parmentieri IFB5604 (GCA_003628695.1)
Pectobacterium parmentieri CFIA1002 (GCA_000632375.1)
Pectobacterium wasabiae NCPPB3702 (GCA_000754685.1)
Pectobacterium wasabiae NCPPB3701 (GCA_000749865.1)
Pectobacterium wasabiae CFBP3304 T (GCA_001742185.1)
Pectobacterium aroidearum PC1 T (GCA_000023605.1)
Pectobacterium aroidearum CFBP8737 (GCA_013449715.1)
Pectobacterium aroidearum KC20 (GCA_014042225.1)
Pectobacterium aroidearum MY19 (GCA_014050115.1)
Pectobacterium fontis M022 T (GCA_000803215.1)
Dickeya solani IPO 2222 T (GCA_001644705.1)
50
55
40
68
97
68
96
63
69
62
70
65
65
57
60
71
60
81
60
0.020

Figure S3: Extended MLSA Phylogenetic tree reconstructed from concatenated nucleotide sequences of 265 homologous gene sequences of 75 *Pectobacterium* genomes. Accession numbers are indicated in brackets after the strain name. The bootstrap support values are indicated if less than 100%. Bar, 0.020 changes per nucleotide position.

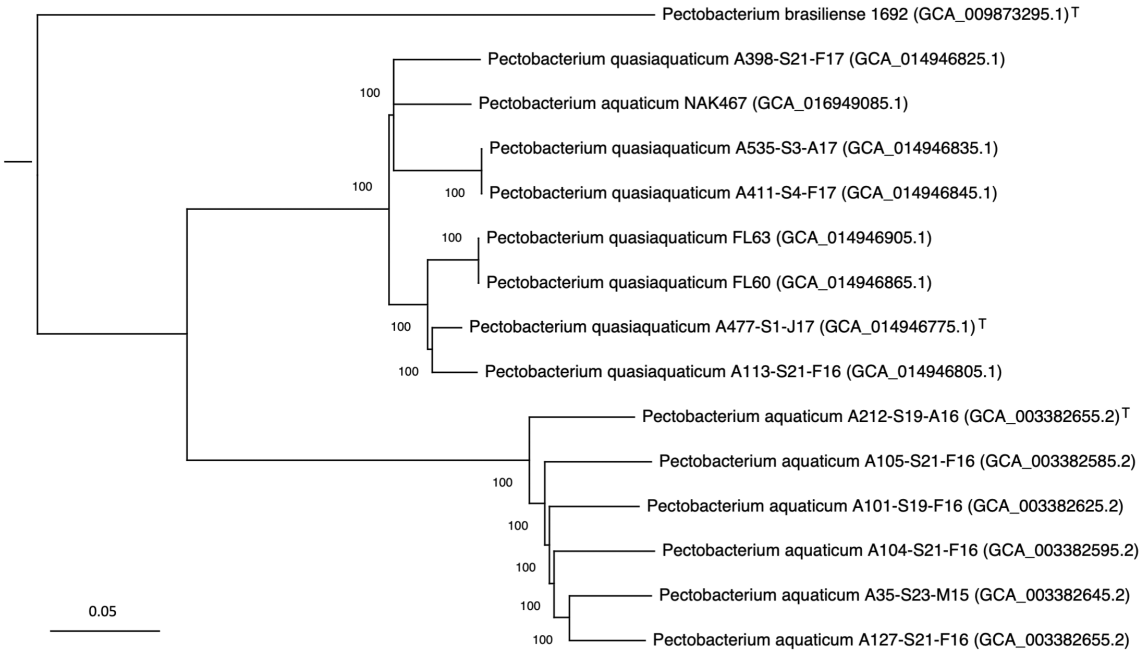

Pectobacterium brasiliense 1692 (GCA_009873295.1)T
Pectobacterium quasiaquaticum A398-S21-F17 (GCA_014946825.1)
100
Pectobacterium aquaticum NAK467 (GCA_016949085.1)
Pectobacterium quasiaquaticum A535-S3-A17 (GCA_014946835.1)
100
100
Pectobacterium quasiaquaticum A411-S4-F17 (GCA_014946845.1)
100
Pectobacterium quasiaquaticum FL63 (GCA_014946905.1)
Pectobacterium quasiaquaticum FL60 (GCA_014946865.1)
100
Pectobacterium quasiaquaticum A477-S1-J17 (GCA_014946775.1)T
100
Pectobacterium quasiaquaticum A113-S21-F16 (GCA_014946805.1)
Pectobacterium aquaticum A212-S19-A16 (GCA_003382655.2)T
Pectobacterium aquaticum A105-S21-F16 (GCA_003382585.2)
100
Pectobacterium aquaticum A101-S19-F16 (GCA_003382625.2)
100
Pectobacterium aquaticum A104-S21-F16 (GCA_003382595.2)
100
Pectobacterium aquaticum A35-S23-M15 (GCA_003382645.2)
100
Pectobacterium aquaticum A127-S21-F16 (GCA_003382655.2)
0.05

Figure S4: MLSA phylogenetic tree of *P. quasiaquaticum* sp. nov. strains and *P. aquaticum* strains including the reclassified strain NAK:467. The MLSA phylogenetic tree was reconstructed from concatenated nucleotide sequences of 2899 homologous gene sequences of the 15 *Pectobacterium* genomes. The clustering of homologous nucleotide sequences was performed with SiLix [30] software with an 80% identity threshold. Homologous sequences of each gene were aligned using MUSCLE [23] software then concatenated. The alignments were filtered using the GBLOCK tool [24] resulting in a data set of 2,881,708 sites (of which 142,278 are informative). The tree was computed with the SeaView software [27] using the BioNJ method [31]. Bootstrap percentages were calculated based on 200 replicates. *P. brasiliense* 1692T was used as an outgroup. Bar, 0.05 changes per nucleotide position.

Table S1: List of homologous genes used for the MLSA phylogeny Fig 2 and Figure S2
Protein ID and annotation were from the genome of the *Pectobacterium quasiaquaticum* A477-S1-J17 type strain (accession JACYTJ010000000)

WP_002208627.1	P-II family nitrogen regulator
WP_002211347.1	translation initiation factor IF-1
WP_002442576.1	50S ribosomal protein L33
WP_005967968.1	50S ribosomal protein L28
WP_005967978.1	nucleoid occlusion factor SlmA
WP_005968491.1	fumarate/nitrate reduction transcriptional regulator Fnr
WP_005968829.1	transcription antiterminator/RNA stability regulator CspE
WP_005968887.1	50S ribosomal protein L20
WP_005969047.1	iron-sulfur cluster insertion protein ErpA
WP_005969846.1	NADH-quinone oxidoreductase subunit B
WP_005970171.1	ISC system 2Fe-2S type ferredoxin
WP_005971295.1	co-chaperone GroES
WP_005971891.1	phosphocarrier protein Hpr
WP_005973551.1	autonomous glycyl radical cofactor GrcA
WP_005975342.1	DNA-binding transcriptional regulator Fis
WP_005975488.1	50S ribosomal protein L13
WP_005975503.1	stringent starvation protein A
WP_005975525.1	DnaA initiator-associating protein DiaA
WP_005975910.1	UMP kinase
WP_005975999.1	transcriptional regulator NrdR
WP_005976031.1	DNA-binding protein HU-beta
WP_005976668.1	50S ribosomal protein L34
WP_010274915.1	ribose-phosphate diphosphokinase
WP_010275594.1	cupin domain-containing protein
WP_010275699.1	50S ribosomal protein L35
WP_010275900.1	YebC/PmpR family DNA-binding transcriptional regulator
WP_010277211.1	leucine-responsive transcriptional regulator Lrp
WP_010278884.1	NADH-quinone oxidoreductase subunit NuoI
WP_010280087.1	Fe-S cluster assembly transcriptional regulator IscR
WP_010280182.1	30S ribosomal protein S9
WP_010280320.1	peptidylprolyl isomerase B
WP_010282093.1	YajQ family cyclic di-GMP-binding protein
WP_010282097.1	protoheme IX farnesyltransferase
WP_010282437.1	outer membrane protein assembly factor BamE
WP_010282899.1	chaperonin GroEL
WP_010284196.1	DJ-1/PfpI family protein
WP_010284552.1	aspartate carbamoyltransferase regulatory subunit
WP_010284628.1	leucyl aminopeptidase
WP_010284845.1	acyl-ACP--UDP-N-acetylglucosamine O-acyltransferase
WP_010286428.1	glycine--tRNA ligase subunit alpha
WP_010287191.1	RpiB/LacA/LacB family sugar-phosphate isomerase
WP_010295235.1	formyltetrahydrofolate deformylase
WP_010296489.1	alkylphosphonate utilization protein
WP_010296630.1	RNase adapter RapZ

WP_010298042.1	division/cell wall cluster transcriptional repressor MraZ
WP_010299049.1	peroxiredoxin C
WP_010299774.1	type 3 dihydrofolate reductase
WP_010300175.1	acyl carrier protein
WP_010300311.1	type II 3-dehydroquinate dehydratase
WP_010300322.1	rod shape-determining protein MreB
WP_010301142.1	serine hydroxymethyltransferase
WP_010301392.1	PTS glucose transporter subunit IIA
WP_010302498.1	ribonuclease III
WP_010303333.1	biotin synthase BioB
WP_010305867.1	fumarate hydratase
WP_010306328.1	pyruvate dehydrogenase complex transcriptional repressor PdhR
WP_010306529.1	high-affinity branched-chain amino acid ABC transporter ATP-binding protein LivF
WP_010681437.1	phosphate ABC transporter ATP-binding protein PstB
WP_012772977.1	rod shape-determining protein MreD
WP_012773638.1	molecular chaperone Skp
WP_012822898.1	GTPase Era
WP_014915263.1	phenylalanine--tRNA ligase subunit alpha
WP_014915875.1	bifunctional 3-hydroxydecanoyl-ACP dehydratase/trans-2-decenoyl-ACP isomerase
WP_014916138.1	5-(carboxyamino)imidazole ribonucleotide mutase
WP_014916217.1	iron-sulfur cluster assembly protein IscA
WP_014916629.1	dihydrolipoyl dehydrogenase
WP_014916699.1	carbamoyl-phosphate synthase large subunit
WP_014917083.1	dipeptide ABC transporter ATP-binding protein
WP_039275834.1	sn-glycerol-3-phosphate ABC transporter permease UgpE
WP_039275909.1	RNA polymerase sigma factor RpoH
WP_039275912.1	high-affinity branched-chain amino acid ABC transporter permease LivH
WP_039277752.1	YeaH/YhbH family protein
WP_039278778.1	argininosuccinate lyase
WP_039280199.1	NADH-quinone oxidoreductase subunit NuoN
WP_039283781.1	D-galactonate dehydratase family protein
WP_039284904.1	oligopeptidase A
WP_039344379.1	bifunctional 4-hydroxy-2-oxoglutarate aldolase/2-dehydro-3-deoxy-phosphogluconate aldolase
WP_039364143.1	serine O-acetyltransferase
WP_039476015.1	elongation factor 4
WP_039484051.1	NADH-quinone oxidoreductase subunit C/D
WP_039484058.1	NADH-quinone oxidoreductase subunit M
WP_039487144.1	acetate uptake transporter
WP_039487851.1	NCS2 family permease
WP_039490396.1	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
WP_039490971.1	UDP-3-O-acyl-N-acetylglucosamine deacetylase
WP_039491522.1	pyridoxal phosphate-dependent aminotransferase
WP_039507167.1	homoserine kinase
WP_039507277.1	FAD-dependent oxidoreductase
WP_039517275.1	threonylcarbamoyl-AMP synthase

WP_095699512.1	peptide chain release factor 1
WP_095993276.1	phosphate signaling complex protein PhoU
WP_102116768.1	endopeptidase La
WP_102116769.1	ATP-dependent protease ATP-binding subunit ClpX
WP_102116770.1	ATP-dependent Clp endopeptidase proteolytic subunit ClpP
WP_102116798.1	phosphate response regulator transcription factor PhoB
WP_102116996.1	rhodanese-like domain-containing protein
WP_102117032.1	YicC family protein
WP_102117081.1	LPS export ABC transporter ATP-binding protein
WP_102117157.1	YhcH/YjgK/YiaL family protein
WP_102117354.1	cell division protein FtsA
WP_102117359.1	cell division protein FtsW
WP_102117369.1	2-isopropylmalate synthase
WP_102117547.1	ABC transporter ATP-binding protein
WP_102117548.1	carbohydrate ABC transporter permease
WP_102117549.1	sugar ABC transporter permease
WP_102117551.1	2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase KduD
WP_102117575.1	septum site-determining protein MinD
WP_102117593.1	glyceraldehyde-3-phosphate dehydrogenase
WP_102117717.1	phosphate acetyltransferase
WP_102117728.1	NADH-quinone oxidoreductase subunit NuoH
WP_102117835.1	glucose-6-phosphate dehydrogenase
WP_102117915.1	translation initiation factor IF-3
WP_102117963.1	DNA topoisomerase (ATP-hydrolyzing) subunit B
WP_102117966.1	chromosomal replication initiator protein DnaA
WP_102118125.1	branched-chain amino acid ABC transporter substrate-binding protein
WP_102118126.1	high-affinity branched-chain amino acid ABC transporter permease LivM
WP_102118188.1	6-phosphofructokinase
WP_102118227.1	phosphoenolpyruvate-protein phosphotransferase PtsI
WP_102118228.1	cysteine synthase A
WP_102118232.1	3,4-dihydroxy-2-butanone-4-phosphate synthase
WP_102118266.1	acetyl-CoA carboxylase carboxyl transferase subunit alpha
WP_102118277.1	30S ribosomal protein S2
WP_102118278.1	type I methionyl aminopeptidase
WP_102118484.1	molecular chaperone DnaJ
WP_102118497.1	energy-dependent translational throttle protein EttA
WP_102118560.1	RNA polymerase sigma factor RpoE
WP_102118774.1	23S rRNA (adenine(2030)-N(6))-methyltransferase RlmJ
WP_102118793.1	universal stress protein UspA
WP_102119376.1	putrescine ABC transporter permease PotI
WP_102119406.1	fumarate reductase (quinol) flavoprotein subunit
WP_102119538.1	ribonuclease G
WP_103860923.1	spermidine/putrescine ABC transporter substrate-binding protein PotF
WP_110162335.1	small ribosomal subunit biogenesis GTPase RsgA
WP_116155383.1	LysR family transcriptional regulator ArgP
WP_116162252.1	arabinose ABC transporter substrate-binding protein
WP_116162312.1	PD40 domain-containing protein
WP_116163731.1	cell division protein FtsZ

WP_116163903.1	dipeptide ABC transporter permease DppC
WP_116164523.1	DNA polymerase III subunit beta
WP_116166183.1	acetate kinase
WP_116186251.1	3-isopropylmalate dehydrogenase
WP_116227065.1	aspartate carbamoyltransferase
WP_129709163.1	ABC transporter permease subunit
WP_137740624.1	serine endoprotease DegQ
WP_170309992.1	3-hydroxyacyl-ACP dehydratase FabZ
WP_174435741.1	acetylglutamate kinase
WP_180774649.1	molecular chaperone HtpG
WP_180784214.1	DNA-binding transcriptional regulator KdgR
WP_180784335.1	tryptophan synthase subunit beta
WP_180784382.1	superoxide dismutase
WP_180785144.1	AMP-binding protein
WP_180785292.1	bifunctional aspartate kinase/homoserine dehydrogenase I
WP_180785294.1	transaldolase
WP_180786002.1	oxaloacetate-decarboxylating malate dehydrogenase
WP_180786186.1	tRNA 4-thiouridine(8) synthase ThiI
WP_180786425.1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
WP_180786596.1	AAA family ATPase
WP_180787217.1	two-component system response regulator DcuR
WP_180787247.1	membrane protein insertase YidC
WP_180787248.1	tRNA uridine-5-carboxymethylaminomethyl(34) synthesis GTPase MnmE
WP_180787263.1	phosphate ABC transporter permease PstA
WP_180787264.1	phosphate ABC transporter permease PstC
WP_180830202.1	threonine synthase
WP_193396996.1	nitrate reductase subunit beta
WP_193396997.1	nitrate reductase subunit alpha
WP_193397180.1	aerobic respiration two-component sensor histidine kinase ArcB
WP_193397190.1	lipid asymmetry maintenance ABC transporter permease subunit MlaE
WP_193397197.1	RNA polymerase factor sigma-54
WP_193397198.1	metalloprotease PmbA
WP_193397202.1	metalloprotease TldD
WP_193397211.1	50S ribosomal protein L11 methyltransferase
WP_193397213.1	tRNA dihydrouridine synthase DusB
WP_193397220.1	transporter substrate-binding domain-containing protein
WP_193397244.1	ABC transporter substrate-binding protein
WP_193397275.1	phosphate ABC transporter substrate-binding protein PstS
WP_193397373.1	NCS2 family permease
WP_193397397.1	aminomethyl-transferring glycine dehydrogenase
WP_193397554.1	trigger factor
WP_193397559.1	cytochrome o ubiquinol oxidase subunit I
WP_193397560.1	cytochrome o ubiquinol oxidase subunit III
WP_193397616.1	porin OmpA
WP_193397705.1	glutamate--tRNA ligase
WP_193397714.1	5\\'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
WP_193397716.1	glutamate-1-semialdehyde 2,1-aminomutase
WP_193397772.1	envelope stress response regulator transcription factor CpxR

WP_193397798.1	cell division ATP-binding protein FtsE
WP_193397817.1	PTS transporter subunit EIIC
WP_193397825.1	anaerobic ribonucleoside-triphosphate reductase
WP_193397837.1	valine--tRNA ligase
WP_193397838.1	DNA polymerase III subunit chi
WP_193397843.1	cupin domain-containing protein
WP_193397890.1	rhamnulose-1-phosphate aldolase
WP_193397989.1	transcriptional regulator GcvA
WP_193397992.1	DUF3412 domain-containing protein
WP_193398006.1	DNA polymerase III subunit alpha
WP_193398130.1	pyruvate kinase
WP_193398170.1	succinate dehydrogenase/fumarate reductase iron-sulfur subunit
WP_193398171.1	elongation factor P--(R)-beta-lysine ligase
WP_193398217.1	phosphoglycerate dehydrogenase
WP_193398229.1	bifunctional riboflavin kinase/FAD synthetase
WP_193398230.1	isoleucine--tRNA ligase
WP_193398232.1	glutamine-hydrolyzing carbamoyl-phosphate synthase small subunit
WP_193398240.1	bifunctional tRNA pseudouridine(32) synthase/23S rRNA pseudouridine(746) synthase RluA
WP_193398241.1	RNA polymerase-associated protein RapA
WP_193398253.1	3-isopropylmalate dehydratase large subunit
WP_193398255.1	acetolactate synthase 3 large subunit
WP_193398257.1	peptidoglycan glycosyltransferase FtsI
WP_193398260.1	phospho-N-acetylmuramoyl-pentapeptide-transferase
WP_193398267.1	preprotein translocase subunit SecA
WP_193398277.1	pyruvate dehydrogenase (acetyl-transferring), homodimeric type
WP_193398366.1	Dyp-type peroxidase
WP_193398508.1	NADH-quinone oxidoreductase subunit NuoG
WP_193398509.1	NADH-quinone oxidoreductase subunit NuoF
WP_193398601.1	HTH-type transcriptional regulator CysB
WP_193398602.1	type I DNA topoisomerase
WP_193398608.1	anthranilate synthase component 1
WP_193398620.1	oligopeptide ABC transporter permease OppC
WP_193398632.1	asparaginase
WP_193398638.1	protein kinase YeaG
WP_193398661.1	L-serine ammonia-lyase
WP_193398664.1	mannose/fructose/sorbose family PTS transporter subunit IIC
WP_193398679.1	ammonia-dependent NAD(+) synthetase
WP_193398682.1	phenylalanine--tRNA ligase subunit beta
WP_193398683.1	threonine--tRNA ligase
WP_193398702.1	polyphosphate kinase 1
WP_193398750.1	Fe-S cluster assembly scaffold IscU
WP_193398751.1	IscS subfamily cysteine desulfurase
WP_193398899.1	DUF1852 family protein
WP_193398900.1	methionine synthase
WP_193399014.1	glutathione-disulfide reductase
WP_193399026.1	glycine--tRNA ligase subunit beta
WP_193399036.1	argininosuccinate synthase

WP_193399097.1	3-deoxy-8-phosphooctulonate synthase
WP_193399099.1	Re/Si-specific NAD(P)(+) transhydrogenase subunit alpha
WP_193399100.1	Re/Si-specific NAD(P)(+) transhydrogenase subunit beta
WP_193399288.1	HTH-type transcriptional regulator MetR
WP_193399295.1	ADP-glyceromanno-heptose 6-epimerase
WP_193399337.1	cysteine--tRNA ligase
WP_193399340.1	HAAAP family serine/threonine permease
WP_193399343.1	membrane-bound O-acyltransferase
WP_193399453.1	excinuclease ABC subunit B
WP_193399986.1	anaerobic C4-dicarboxylate transporter
WP_193400178.1	NADP-dependent oxaloacetate-decarboxylating malate dehydrogenase
WP_193400192.1	pyruvate dehydrogenase complex dihydrolipoyllysine-residue acetyltransferase
WP_193400212.1	glutamine-hydrolyzing GMP synthase
WP_193400215.1	IMP dehydrogenase
WP_193400223.1	5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase
WP_193400225.1	ribosome biogenesis GTPase Der
WP_193400229.1	histidine--tRNA ligase
WP_193400231.1	flavodoxin-dependent (E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase
WP_193400272.1	orotate phosphoribosyltransferase
WP_193400281.1	NADH-quinone oxidoreductase subunit L
WP_193400296.1	alanine transaminase
WP_193400314.1	glutamine--fructose-6-phosphate transaminase (isomerizing)
WP_193400316.1	DNA replication/repair protein RecF
WP_193400330.1	dipeptide ABC transporter permease DppB
WP_193400332.1	dipeptide ABC transporter ATP-binding protein
WP_193400334.1	valine--pyruvate transaminase
WP_193400499.1	aspartate--tRNA ligase
WP_193400501.1	Holliday junction branch migration protein RuvA
WP_193400628.1	glutathione ABC transporter substrate-binding protein
WP_193400663.1	phosphoenolpyruvate carboxylase
WP_193400973.1	DNA topoisomerase IV subunit B
WP_193400982.1	PTS mannitol transporter subunit IICBA
WP_193400992.1	DNA topoisomerase IV subunit A

Table S3 : Phenotypic characterization of *P. quasiaquaticum* (A477-S1-J17T, A113-S21-F16, A411-S4-F17, FL63-S17 and FL60-S17 and *P. aquaticum* A212-S19-A16T using GENIII Biolog plates.

	P. aquaticum	***P. quasiaquaticum***				
Test	**A212-S19-A16*T***	**A113-S21-F16**	**A411-S4-F17**	**A477-S1-J17T**	**FL63-S17**	**FL60-S17**
1% NaCl	+	+	+	+	+	+
1% Sodium Lactate	+	+	+	+	+	+
3-O-Methyl-D-Glucose	-	-	-	-	-	-
4% NaCl	w	-	-	-	-	-
8% NaCl	-	-	-	-	-	-
Acetic Acid	+	+	+	+	+	+
Acetoacetic Acid	-	-	-	-	-	-
Aztreonam	-	-	-	-	-	-
Bromo-Succinic Acid	+	+	+	+	+	+
Citric Acid	+	+	+	+	+	+
D-Arabitol	-	-	-	-	-	-
D-Aspartic Acid	+	+	+	+	-	-
D-Cellobiose	-	-	-	-	-	-
D-Fructose	+	+	+	+	+	+
D-Fructose-6-Phosphate	+	+	+	+	+	+
D-Fucose	-	-	-	-	-	-
D-Galactose	+	+	+	+	+	+
D-Galacturonic Acid	+	+	+	+	+	+
D-Gluconic Acid	+	+	+	+	+	+
D-Glucose-6-Phosphate	+	+	+	+	+	+
D-Glucuronic Acid	-	-	-	-	-	-
D-Lactic Acid Methyl Ester	-	-	-	-	-	-
D-Malic Acid	-	-	-	-	-	-
D-Maltose	-	-	-	-	-	-
D-Mannitol	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+
D-Melibiose	+	+	+	+	+	+
D-Raffinose	+	+	+	+	+	+
D-Saccharic Acid	+	+	+	+	+	+
D-Salicin	+	+	+	+	+	+
D-Serine #1	-	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	-
D-Trehalose	-	-	-	-	-	-
Turanose	-	-	-	-	-	-
Dextrin	-	-	-	-	-	-
Sodium Formate	-	w	w	-	-	-
Fusidic Acid	+	+	+	+	+	+
Gelatin	-	-	-	-	-	-
β-Gentiobiose	+	+	+	+	+	+
Glucuronamide	-	-	-	-	-	-
Glycerol	+	+	+	+	+	+

+ : Positive
- : Negative

Gly-Pro	-	-	-	-	-	-
Guanidine Hydrochloride	+	+	+	+	+	+
Inosine	-	-	-	-	-	-
L-Alanine	-	-	-	-	-	-
L-Arginine	-	-	-	-	-	-
L-Aspartic Acid	+	+	+	+	+	+
L-Fucose	-	-	-	-	-	-
L-Galactonic Acid-γ-Lactone	+	+	+	+	+	+
L-Glutamic Acid	w	W	W	W	W	W
L-Histidine	-	-	-	-	-	-
L-Lactic Acid	-	-	-	-	-	-
L-Malic Acid	+	+	+	+	+	+
L-Pyroglutamic Acid	-	-	-	-	-	-
L-Rhamnose	+	W	W	W	-	W
L-Serine	+	+	+	+	+	+
Lincomycin	+	+	+	+	+	+
Lithium Chloride	w	+	+	+	+	+
Methyl Pyruvate	+	+	+	+	+	+
Minocycline	-	-	-	-	-	-
Mucic Acid	+	+	+	+	+	+
myo-Inositol	+	+	+	+	+	+
N-Acetyl-Neuraminic Acid	-	-	-	-	-	-
N-Acetyl-D-Galactosamine	-	-	-	-	-	-
N-Acetyl-D-Glucosamine	+	+	+	+	+	+
N-Acetyl-β-D-Mannosamine	-	-	-	-	-	-
Nalidixic Acid	-	-	-	-	-	-
Niaproof	+	+	+	+	+	+
Pectin	+	+	+	+	+	+
pH 5	w	W	W	W	W	-
pH 6	+	+	+	+	+	+
Potassium Tellurite	-	-	-	-	-	-
Propionic Acid	-	-	-	-	-	-
Quinic Acid	-	-	-	-	-	-
Rifamycin SV	+	+	+	+	+	+
Sodium Bromate	-	-	-	-	-	-
Butyric Acid	+	+	+	+	+	+
Stachyose	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Tetrazolium Blue	+	+	+	+	+	+
Tetrazolium Violet	+	+	+	+	+	+
Troleandomycin	+	+	+	+	+	+
Tween 40	-	-	-	-	-	-
Vancomycin	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+
α-D-Lactose	+	+	+	+	+	+
α-Hydroxy-Butyric Acid	-	-	-	-	-	-
α-Keto-Butyric Acid	-	-	-	-	-	-
α-Keto-Glutaric Acid	-	-	-	-	-	-

β-Hydroxy-Butyric Acid	-	-	-	-	-	-
p-Hydroxy-Phenylacetic Acid	-	-	-	-	-	-
β-Methyl-D-Glucoside	+	+	+	+	+	+
γ-Amino-n-Butyric Acid	-	-	-	-	-	-

Table S4: ANIm, values between genomes of *P. quasiaquaticum* strains sp. nov., *P. aquaticum* and NAK:467. ANI values ≥96% are indicated in red, ANI values between 96%-95% are indicated in light blue and ANI values ≤95% are indicated in blue. Pq: *P. quasiaquaticum*; Pa: *P. aquaticum*; Pb: *P. brasiliense*. NAK:467 is highlighted in yellow

		A398-S21-F17	NAK:467*	A411-S4-F17	A535-S3-A17	A113-S21-F16	A477-S1-J17 **T**	FL60-S17	FL63-S17	A101-S19-F16	A104-S21-F16	A127-S21-F16	A35-S23-M15	A105-S21-F16	A212-S19-A16 **T**	1692 **T**
Pq	A398-S21-F17	100,00	98,91	98,98	98,99	98,87	99,01	98,88	98,88	95,76	95,67	95,70	95,67	95,62	95,84	94,25
Pa	NAK:467*	98,91	100,00	98,97	98,98	98,82	99,05	98,82	98,83	95,72	95,65	95,67	95,66	95,61	95,80	94,25
Pq	A411-S4-F17	98,98	98,97	100,00	99,99	98,87	99,03	98,88	98,87	95,77	95,68	95,73	95,70	95,66	95,84	94,25
Pq	A535-S3-A17	98,99	98,98	99,99	100,00	98,87	99,03	98,88	98,88	95,77	95,69	95,73	95,70	95,65	95,84	94,24
Pq	A113-S21-F16	98,87	98,82	98,87	98,87	100,00	99,48	99,40	99,39	95,78	95,68	95,74	95,72	95,61	95,73	94,23
Pq	A477-S1-J17 **T**	99,01	99,05	99,03	99,03	99,48	100,00	99,54	99,55	95,76	95,66	95,71	95,68	95,63	95,79	94,22
Pq	FL60-S17	98,88	98,82	98,88	98,88	99,40	99,54	100,00	99,98	95,93	95,88	95,89	95,86	95,80	95,98	94,18
Pq	FL63-S17	98,88	98,83	98,87	98,88	99,39	99,55	99,98	100,00	95,93	95,89	95,89	95,86	95,80	95,98	94,18
Pa	A101-S19-F16	95,76	95,72	95,77	95,77	95,78	95,76	95,93	95,93	100,00	98,84	98,81	98,82	98,80	98,66	93,37
Pa	A104-S21-F16	95,67	95,65	95,68	95,69	95,68	95,66	95,88	95,89	98,84	100,00	98,81	98,79	98,69	98,70	93,36
Pa	A127-S21-F16	95,70	95,67	95,73	95,73	95,74	95,71	95,89	95,89	98,81	98,81	100,00	99,01	98,72	98,57	93,39
Pa	A35-S23-M15	95,67	95,66	95,70	95,70	95,72	95,68	95,86	95,86	98,82	98,79	99,01	100,00	98,70	98,57	93,35
Pa	A105-S21-F16	95,62	95,61	95,66	95,65	95,61	95,63	95,80	95,80	98,80	98,69	98,72	98,70	100,00	98,69	93,37
Pa	A212-S19-A16 **T**	95,84	95,80	95,84	95,84	95,73	95,79	95,98	95,98	98,66	98,70	98,57	98,57	98,69	100,00	93,45
Pb	1692 **T**	94,25	94,25	94,25	94,24	94,23	94,22	94,18	94,18	93,37	93,36	93,39	93,35	93,37	93,45	100,00