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Taxonomic Description

***Pectobacterium aquaticum* sp. nov, isolated from waterways**

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ABSTRACT

This work aimed to establish the taxonomic status of six strains (A212-S19-A16 ; A127-S21-F16 ; A105-S21-F16 ; A104-S21-F16 ; A101-S19-F16 and A35-S23-M15) isolated from three different waterways in 2015 and 2016 in the south east of France. Amplification and sequencing of the *gapA* housekeeping gene clustered these six strains together inside the *Pectobacterium* genus outside of already described or proposed *Pectobacterium* species and subspecies. Phenotypic analysis, using GENIII biologi plates performed with strains A212-S19-A16, A105-S21-F16, A101-S19-F16 and the closely related *P. polaris* (CFBP 1403), *P. carotovorum* subsp *odoriferum* (CFBP 1878^T), "*P. carotovorum* subsp *actinidiae*" (CFBP 7370), *P. carotovorum* subsp *carotovorum* (CFBP 2046^T), "*P. carotovorum* subsp *brasiliense*" (CFBP 6617) or the most distantly related *P. aroidearum* (CFBP 8168^T) failed to identify specific compounds metabolized by these three strains but a weak activity was specifically observed at pH5 with these three strains. Illumina sequencing was used to sequence these six strains. Based on phylogenetic data, ANI, and in silico DNA-DNA hybridization, strains A212-S19-A16 ; A127-S21-F16 ; A105-S21-F16 ; A101-S19-F16 ; A35-S23-M15 ; A104-S21-F16 are suggested to represent a novel species of the genus *Pectobacterium*, for which the name *Pectobacterium aquaticum* sp. nov. is proposed. The type strain is A212-S19-A16^T (=CFBP 8637^T =NCPB 4640^T).

The genus *Pectobacterium* gathers seven recognized species, *Pectobacterium carotovorum*, *P. atrosepticum*, *P. betavasculorum*, *P. wasabiae*, *P. aroidearum*, *P. polaris*, *P. parmentieri*, and the recently proposed "*P. peruvienne*"(1-6). The *P. carotovorum* species is itself highly heterogeneous and several recognized and proposed subspecies have been ascribed to *P. carotovorum* to account for this heterogeneity: *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *odoriferum*, "*P. carotovorum* subsp. *brasiliense*" and "*P. carotovorum* subsp. *actinidiae*" (7-12).

Altogether, bacteria belonging to the *Pectobacterium* genus have a broad host range and cause soft rot and blackleg disease on a wide variety of plants, including important crops

46 and ornamentals (13). As these bacteria collectively cause big losses on agricultural field,
47 *Pectobacterium* species have been mainly described so far following bacterial sampling and
48 isolation from diseased host plants. For example, *P. wasabiae* type strain has been isolated
49 from the diseased rhizomes and fibrous roots of Japanese horseradish (14) and *P.*
50 *parmentieri* type strain from symptomatic potato plants (4).
51 In contrast, the diversity of *Pectobacterium* sp. outside the plant context remains largely
52 unexplored. Early studies showed that *Pectobacterium* sp. could be recovered from various
53 non-host environments, such as water, soil or air (15-18). However, the taxonomic status of
54 most of the isolated strains in these early works was not clearly assigned since most
55 *Pectobacterium* species were described later on. Therefore, is it still unclear which particular
56 *Pectobacterium* species could be recovered from various non-host environments.
57 Furthermore, it remains to be determined if sampling outside diseased plants will allow the
58 discovery of new *Pectobacterium* species.

59
60

61 Isolation and Ecology

62 This study aimed to establish the taxonomic status of six strains isolated from three
63 different fresh water river streams in 2015 and 2016 in the south east of France (Table1).
64 Following sampling, 500 ml of fresh water was filtered through 0.2 µm pore filters (Sartorius
65 cellulose acetate filters), the bacteria present on the filters were suspended in 1 ml sterile
66 distilled water and 100 µl of the suspension were poured onto semiselective modified
67 single-layers CVP_{AG366} plates (same medium as described in (19) except that tryptone was
68 not added to the medium, hereafter described as CVP). After 2 days of growth at 28°C,
69 strains forming pits on CVP medium were further isolated, named A35-S23-M15, A101-S19-
70 F16, A104-S21-F16, A105-S21-F16, A127-S21-F16 and A212-S19-A16 (table 1) and stored in
71 40% /60% glycerol/ LB liquid medium (10 g tryptone, 5 g yeast extract, 10 g NaCl per one
72 liter of medium) at -80°C.

73
74

75 16S RNA and *gapA* phylogeny

76 The 16S rRNA rrs barcode was amplified and sequenced using the universal PCR primers 8F
77 (AGAGTTTGATCCTGGCTCAG) and 1542R (AAGGAGGTGATCCAGCCGCA). The sequenced DNA
78 fragments were identical between strains A35-S23-M15, A101-S19-F16, A127-S21-F16 and
79 A212-S19-A16 and displayed 5 nucleotide changes with the sequences of the strains A104-
80 S21-F16, A105-S21-F16 (Fig. S1). Blast search with complete genome of *Pectobacterium* sp.
81 identified 16S rRNA sequences of related *Pectobacterium* sp. that were used to build up a
82 16S phylogenetic tree (supplemental S1). There was only 33 informative sites along the
83 sequences and the generated rRNA tree is not informative at the specie level as already
84 stated by Aldeolu *et al.* (2016) (20). However, amplification and sequencing of the *gapA*
85 housekeeping gene was recently described to rapidly characterize the different
86 *Pectobacterium* species (21). Therefore, to delineate the taxonomic position of the six
87 isolated strains, amplification and sequencing of the *gapA* housekeeping gene was
88 performed (sequences provided in supplemental S2). This highlighted that these six strains
89 clustered together and formed a new clade outside of the already described *Pectobacterium*
90 species and subspecies (Fig. 1 (a)).

91

92

93 **Genome Features**

94 To further gain insight into the taxonomic status of these six strains, draft illumina
95 sequences were obtained. For preparation of genomic DNA, the strains were first grown
96 overnight at 28°C on solid LB medium. A single colony was then picked up and grown
97 overnight in 2ml of liquid LB medium at 28°C with 120 rpm shaking. Bacterial cells were
98 harvested by centrifugation (5min at 12,000 rpm) and DNA was extracted with the wizard®
99 genomic DNA extraction kit (Promega) following the supplier's specification. DNA was
100 suspended in 100µl of sterile distilled water and the quantity and quality of DNA was
101 assessed by nano-drop measurement, spectrophotometry analysis and agarose gel
102 electrophoresis at 1%.

103 Genome sequencing was performed at the next generation sequencing core facilities of the
104 Institute for Integrative Biology of the Cell, Avenue de la Terrasse 91190 Gif-sur-Yvette
105 France. Nextera DNA libraries were prepared from 50ng of high quality genomic DNA. Paired
106 end 2x75pb sequencing was performed on an Illumina NextSeq500 instrument, with a High
107 Output 150 cycle kit. CLC Genomics Workbench (Version 9.5.2, Qiagen Bioinformatics) was
108 used to assemble reads. Final sequencing coverages were between 60 and 180 (table 1).

109 Coding sequences were predicted using the RAST server (22) with the Glimmer 3 prediction
110 tool (23). Statistics of the six draft genomes are presented in table 1. A phylogenetic tree,
111 constructed from concatenated sequences of 523 homologous genes, confirmed that these
112 six strains clustered together outside of the previously described species or subspecies (Fig.
113 1 (b)).

114 Pairwise comparison of the six genomes and other *Pectobacterium* genomes representative
115 of known and proposed species and subspecies was performed using the average nucleotide
116 identity (ANI) calculator (Table 2 and supplemental S3 for more distantly related
117 *Pectobacterium* sp.) (24). Pairwise ANI values between the six studied genomes were $\geq 98\%$.
118 In contrast, pairwise ANI values of these six genomes with those of known *Pectobacterium*
119 species and subspecies dropped below 94,5%, below the suggested cut-off value of 95–96%
120 to delineate bacterial species. Digital DNA-DNA hybridisation (dDDH) is an *in silico* method
121 to approach the wet-lab DDH method as closely as possible (25). dDDH were calculated
122 between the six genomes and *Pectobacterium* genomes representative of known species
123 and subspecies (Table 2 and Table S1 for more distantly related *Pectobacterium* sp.). dDDH
124 pairwise values between the six studied genomes were above 86%, well above the 70%
125 species boundary. When pairwise calculations were performed between these six genomes
126 with those of known *Pectobacterium* species and subspecies the estimated dDDH values
127 dropped below 57%, well below the species boundary.

128

129

130 **Physiology and Chemotaxonomy**

131 Tests on potato showed that the six studied strains were able to macerate potato tuber
132 slices (supplemental S4). Biochemical tests were performed using BIOLOG GENIII plates with
133 strains A212-S19-A16, A105-S21-F16, A101-S19-F16 and the closely related *P. polaris* (CFBP
134 1403), *P. carotovorum* subsp *odoriferum* (CFBP 1878^T), "*P. carotovorum* subsp *actinidiae*"
135 (CFBP 7370), *P. carotovorum* subsp *carotovorum* (CFBP 2046^T), "*P. carotovorum* subsp

136 *brasilense*“ (CFBP 6617) or the most distantly related *P. aroidearum* (CFBP 8168^T). The
137 plates were treated according to the manufacturer’s instructions using inoculation fluid A
138 except for strains CFBP 1878^T and CFBP 2046^T for which inoculation fluid B was used to
139 better adjust the metabolism rate of these two strains and read with an OmnilogTM
140 apparatus at 48h. With most of the tested compounds (68 out of 93), all the *Pectobacterium*
141 sp. tested showed similar reactions. For 25 compounds or conditions tested, variable
142 reactions were observed depending on the *Pectobacterium* species tested (Table 3). The
143 three tested *Pectobacterium* strains, A212-S19-A16 , A101-S19-F16 and A105-S21-F16
144 always reacted similarly. However, strain A212-S19-A16 and strain A101-S19-F16 showed a
145 weaker activity than strain A105-S21-F16 when tested for lithium chloride and L-Glutamic
146 acid (Table 3). We could not identify specific compounds specifically metabolized by these
147 three strains. With the three studied strains, contrary to the other tested strains, a weak
148 activity was observed at pH5 and the three strains were also specifically unable to use D-
149 Trehalose and D-Cellobiose as carbon source (Table 3). However, strains of *P.*
150 *betavasculorum* not tested in our study, were also described by Gardan *et al* (2) as unable to
151 use D-Cellobiose, therefore this feature is not specific to the three tested strains.
152 In conclusion, DNA-DNA relatedness analysis, whole genome sequences, phylogenetic data,
153 phenotypic characterisation, indicates that this cluster of six strains represent a novel
154 species, included in the *P. carotovorum* complex but distinct from the already described and
155 proposed species and subspecies, for which the name *Pectobacterium aquaticum* sp. nov. is
156 proposed.

157

158 **Protologue**

159 *Pectobacterium aquaticum* (*aquaticum* pertaining to the fresh water river stream from
160 which the type strain was isolated).

161 Gram-negative, motile bacterium. Grows optimally at 28°C in LB medium (10 g tryptone, 5 g
162 yeast extract, 10 g NaCl, 15 g agar per one liter of medium) forming colonies within 24h.
163 Causes maceration of potato tuber slices and, when on grown at 28°C on CVP medium (19),
164 forms pits within 48h. Using GENIII biolog[®] plates, positive for D-Raffinose, α-D-Glucose,
165 Pectin, α-D-Lactose, D-Mannose, D-Mannitol, D-Galacturonic Acid, Methyl pyruvate, D-
166 Melbiose, D-Fructose, β-Methyl-D-Glucoside, D-Galactose, myo-Inositol, D-Gluconic Acid, D-
167 Salicin, Glycerol, L-Aspartic Acid, Gentiobiose, N-Acetyl-D-Glucosamine, D-Glucose-6-PO₄,
168 Sucrose, D-Fructose-6-PO₄, Mucic Acid, L-Rhamnose, D-Aspartic Acid, L-Malic Acid, Acetic
169 Acid, L-Serine, D-Saccharic Acid, Bromo-Succinic Acid, 1% and 4% NaCl, 1% Sodium Lactate,
170 Troleandomycin, Vancomycin, pH6, Rifamycin SV, Tetrazolium violet, Niaproof 4,
171 Tetrazolium blue, L-Galactonic Acid Lactone, Citric Acid, L-Glutamic Acid, Lincomycin, Fusidic
172 Acid, Guanidine HCl, Lithium Chloride, Sodium Butyrate and weakly positive at pH5.

173 *P. aquaticum* strains clusters separately in phylogenetic analyses based on the *gapA*
174 housekeeping gene and whole genome alignment of *Pectobacterium* described or proposed
175 species strains.

176 The type strain is A212-S19-A16^T (=CFBP 8637^T =NCPPB4640^T) and was isolated from fresh
177 water river Verdon, close to the confluence with the river Durance in France in 2016. The
178 DNA G+C content of the DNA of the type strain is 51,2%. A35-S23-M15 (CFBP8632), A101-
179 S19-F16 (CFBP8633), A104-S21-F16 (CFBP8634), A105-S21-F16 (CFBP8635) and A127-S21-
180 F16 (CFBP8636) are additional strains of the specie.

181

182 The whole genome sequence of strains A212-S19-A16^T, A35-S23-M15, A101-S19-F16, A104-
183 S21-F16, A105-S21-F16, and A127-S21-F16 have been deposited at DDBJ/ENA/GenBank
184 under the accession QHJR000000000, QHJW000000000, QHJV000000000, QHJU000000000,
185 QHJT000000000, QHJS000000000. The versions described in this paper are versions
186 QHJR020000000, QHJW020000000, QHJV020000000, QHJU020000000, QHJT020000000,
187 QHJS020000000. The bioproject number is PRJNA473080 and the assembly accession
188 numbers are: GCA_003382565.2 for A212-S19-A16^T, GCA_003382645.2 for A35-S23-M15,
189 GCA_003382625.2 for A101-S19-F16, GCA_003382595.2 for A104-S21-F16,
190 GCA_003382585.2 for A105-S21-F16, and, GCA_003382655.2 for A127-S21-F16. The rRNA
191 16S sequences are available with the following accession numbers : MK035744 for A212-
192 S19-A16^T, MK035739 for A35-S23-M15, MK035740 for A101-S19-F16, MK035741 for A104-
193 S21-F16, MK035742 for A105-S21-F16 and, MK035743 for A127-S21-F16.

194

195 AUTHOR STATEMENTS

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205 Yvette, France.

206 *Ethical statement*

207 not applicable

208 *Conflicts of interest*

209 The authors declare that they have no conflicts of interest

210

211 ABBREVIATIONS

212 ANI: Average Nucleotide Identity

213 dDDH: digital DNA-DNA hybridization

214 gapA: glyceraldehyde-3-phosphate dehydrogenase A

215 MLSA: Multi Locus Sequence Analysis

216 Pcb: *Pectobacterium carotovorum* subsp. *brasiliense*

217 Pco: *Pectobacterium carotovorum* subsp. *odoriferum*

218 Pcc: *Pectobacterium carotovorum* subsp. *carotovorum*

219

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302

303 FIGURES AND TABLES

304

305 Figure 1

306 Phylogenetic trees of environmental *P. aquaticum* strains and other strains representatives
307 of all the currently described or “proposed” species and subspecies of the *Pectobacterium*
308 genus. (a): Phylogenetic tree constructed from the *gapA* nucleotide sequences. Sequences
309 were aligned using the MUSCLE software (26) and the alignments were filtered by using the
310 program GBLOCKS (27). Tree was computed using PHYML (28). One hundred bootstrap
311 replicates were performed to assess the statistical support of each node. Bootstrap support
312 values (percentages) are indicated if superior to 70 %. *gapA* sequences were retrieved from
313 full genome of type strains (accession numbers are indicated in figure 1 B) or obtained from
314 the sequenced *gapA* amplicon for strains A35-S23-M15 (CFBP8632), A101-S19-F16 (CFBP
315 8633), A104-S21-F16 (CFBP 8634), A105-S21-F16 (CFBP 8635), A127-S21-F16 (CFBP 8636),
316 A212-S19-A16 (CFBP 8637^T). All the *gapA* sequences used to construct the phylogenetic tree

317 are provided. (b) : Phylogenetic tree constructed from concatenated sequences of 523
318 homologous gene sequences. Complete procedure is described in the supplemental S5. All
319 scripts and data are available on GitHub (github.com/jacques-pedron/MLSA_scripts). Briefly,
320 clustering of homologous nucleotide sequences was performed with the SiLix software with
321 80% identity threshold (29). Homologous sequences of each gene were aligned using the
322 MUSCLE software (26) then concatenated. SeaView (version 4.6.5), a multiplatform program
323 for molecular phylogeny, was used to implement specified tools and options (30).
324 Alignments were filtered by using the GBLOCK tool (27) resulting 568,204 sites dataset. Tree
325 was computed using PHYML (28). One hundred bootstrap replicates were performed to
326 assess the statistical support of each node. Bootstrap support values (percentages) are
327 shown if less than 100%. The accession number for each genome is indicated inside brackets
328 after the strain name. *Dickeya solani* RNS08.23.3.1.A was used as outgroup. Type strains
329 are marked with T after the strain name. The bars indicated the number of changes per
330 nucleotide position.

331

332 Table 1

333 Isolation sites and genome informations for the six *P. aquaticum* strains

334

335 Table 2

336 Pairwise ANI (below diagonal) and dDDH (above diagonal) between the genomes of novel *P.*
337 *aquaticum* strains and the genomes of closely related *Pectobacterium* described and
338 “proposed” species and subspecies.

339

340 Table 3. Distinctive metabolic traits between *P. aquaticum* (A101-S19-F16, A105-S21-F16,
341 A212-S19-A16) and *P. polaris* (CFBP 1403), “*P. carotovorum* subsp. *brasiliense*” (CFBP 6617),
342 “*P. carotovorum* subsp. *actinidiae*” (CFBP 7370), *P. aroidearum* (CFBP 8168^T), *P.*

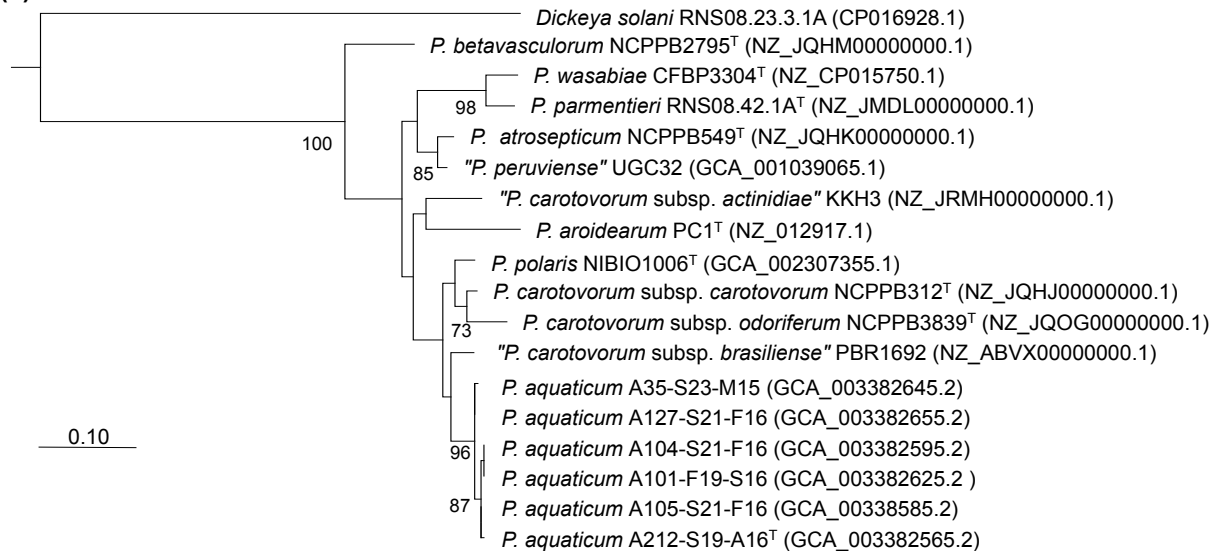
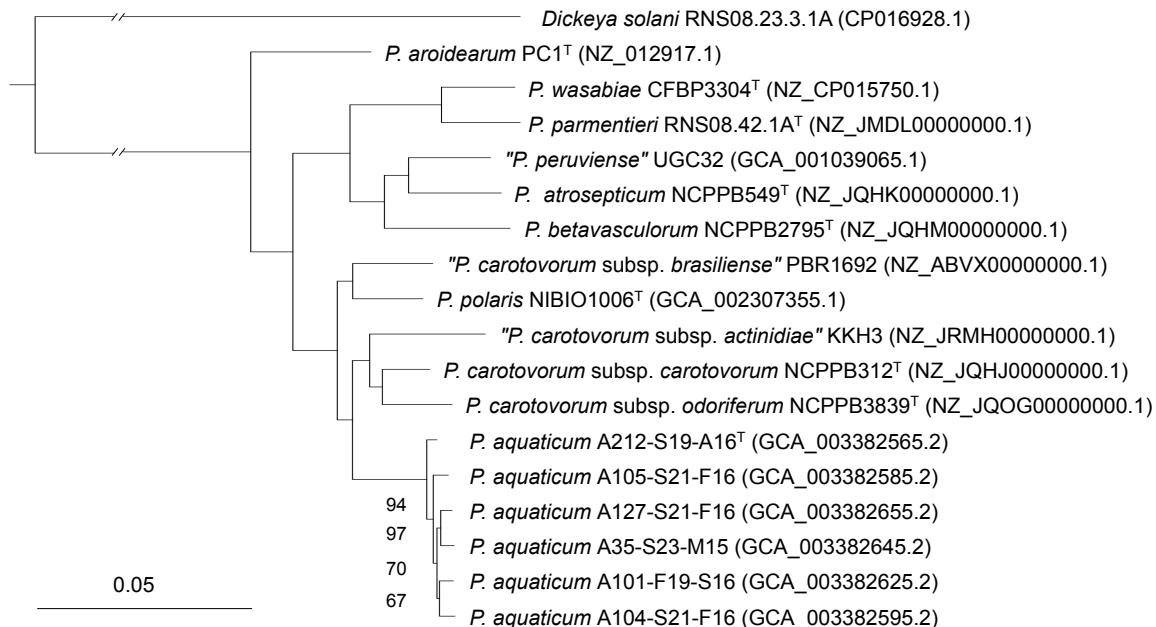
343 *carotovorum* subsp. *odoriferum* (CFBP 1878^T) and *P. carotovorum* subsp. *carotovorum* (CFBP
344 2046^T), using GENIII biolog[®] plates. All plates read were performed on an Omnilog[™]
345 apparatus at 48h. +: positive reaction; -: negative reaction; w: weak reaction.

346 All strains showed positive reactions for D-Raffinose, α-D-Glucose, Pectin, α-D-Lactose, D-
347 Mannose, D-Mannitol, D-Galacturonic Acid, Methyl pyruvate, D-Melbiose, D-Fructose, β-
348 Methyl-D-Glucoside, D-Galactose, myo-Inositol, D-Gluconic Acid, D-Salicin, Glycerol, L-
349 Aspartic Acid, Gentiobiose, N-Acetyl-D-Glucosamine, D-Glucose-6-PO₄, Sucrose, D-Fructose-
350 6-PO₄, Mucic Acid, L-Rhamnose, D-Aspartic Acid, L-Malic Acid, Acetic Acid, L-Serine, D-
351 Saccharic Acid, Bromo-Succinic Acid, 1% NaCl, 1% Sodium Lactate, Troleandomycin,
352 Vancomycin, pH6, 4% NaCl, Rifamycin SV, Tetrazolium violet, Niaproof 4 and Tetrazolium
353 blue. All strains showed negative reaction for Gelatin, β-Hydroxy-Phenylacetic Acid, Tween
354 40, γ-Amino-Butyric-Acid, D-Lactic Acid Methyl Ester, α-Hydroxy-Butyric Acid, L-Arginine, β-
355 Hydroxy-D-L-Butyric Acid, 3-Methyl Glucose, α-Keto-Butyric Acid, Glucuronamide,
356 Acetoacetic Acid, N-Acetyl-β-D-Mannosamine, L-Fucose, L-Histidine, D-Malic Acid, Propionic
357 Acid, N-Acetyl-D-Galactosamine, L-Pyroglutamic Acid, Quinic Acid, N-Acetyl Neuraminic Acid
358 D-Serine, Nalidixic Acid, Aztreonam, 8% NaCl, Minocycline, Potassium tellurite and Sodium
359 Bromat.

360

361

362

(a)**(b)**

Strains	CFBP names	Isolation date	Isolation site	Genome coverage	number of scaffolds	Genome Size (pb)	G+C mol%	Protein coding genes	RNA genes	genebank accession No
A35-S23-M15	CFBP8632	30/05/15	1	102	152	4467243	51.3	4335	50	QHJW01000000
A101-S19-F16	CFBP8633	04/02/16	2	175	104	4274151	51.5	4114	49	QHJV01000000
A104-S21-F16	CFBP8634	17/02/16	3	180	133	4395676	51.4	4297	46	QHJU01000000
A105-S21-F16	CFBP8635	17/02/16	3	167	135	4310812	51.4	4162	50	QHJT01000000
A127-S21-F16	CFBP8636	17/02/16	3	60	209	4465293	51.2	4364	46	QHJS01000000
A212-S19-A16 ^T	CFBP8637 ^T	25/08/16	2	101	119	4348076	51.2	4266	45	QHJR01000000

1 : Canal de Vaucluse ; 2 : Verdon ; 3 Grand anguillon

	<i>P. aquaticum</i> A101-S19-F16	<i>P. aquaticum</i> A104-S21-F16	<i>P. aquaticum</i> A35-S23-M15	<i>P. aquaticum</i> A105-S21-F16	<i>P. aquaticum</i> A127-S21-F16	<i>P. aquaticum</i> A212-S19-A16 ^T	<i>Pcc</i> NCPPB312 ^T	<i>Pco</i> NCPPB3839 ^T	<i>P. polaris</i> NIBIO1006 ^T	"Pcb" PBR1692
<i>P. aquaticum</i> A101-S19-F16	---	88.80	88.80	88.90	88.70	88.10	55.70	53.10	52.90	51.50
<i>P. aquaticum</i> A104-S21-F16	98.76	---	88.50	87.30	89.40	87.70	56.00	53.00	53.00	51.30
<i>P. aquaticum</i> A35-S23-M15	98.72	98.7	---	87.60	90.50	86.80	55.70	52.90	52.90	51.40
<i>P. aquaticum</i> A105-S21-F16	98.73	98.63	98.63	---	87.90	87.80	55.90	53.00	52.90	51.40
<i>P. aquaticum</i> A127-S21-F16	98.70	98.70	98.90	98.63	---	86.20	55.70	52.80	52.80	51.50
<i>P. aquaticum</i> A212-S19-A16 ^T	98.61	98.65	98.53	98.64	98.50	---	55.60	52.70	53.30	51.90
<i>Pcc</i> NCPPB312 ^T	94.23	94.26	94.23	94.30	94.23	94.24	---	61.50	52.40	51.00
<i>Pco</i> NCPPB3839 ^T	93.69	93.68	93.68	93.73	93.66	93.64	95.18	---	49.50	47.40
<i>P. polaris</i> NIBIO1006 ^T	93.66	93.68	93.66	93.67	93.67	93.73	93.52	92.90	---	54.50
"Pcb" PBR1692	93.36	93.34	93.35	93.34	93.37	93.45	93.26	92.40	94.02	---

***Pectobacterium aquaticum* sp. nov, isolated from waterways**

Pédron Jacques¹, Bertrand Claire¹, Taghouti Géraldine², Portier Perrine²^{\$} and Barny Marie-Anne¹^{\$}

¹Sorbonne Université, INRA, Institute of Ecology and Environmental sciences-Paris, 4 place Jussieu, F-75 252 Paris, France

²IRHS, INRA, Université d'Angers, Agrocampus-Ouest, SFR 4207 QuaSaV, CIRM-CFBP, 49071, Beaucouzé, France

\$ co-last author

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

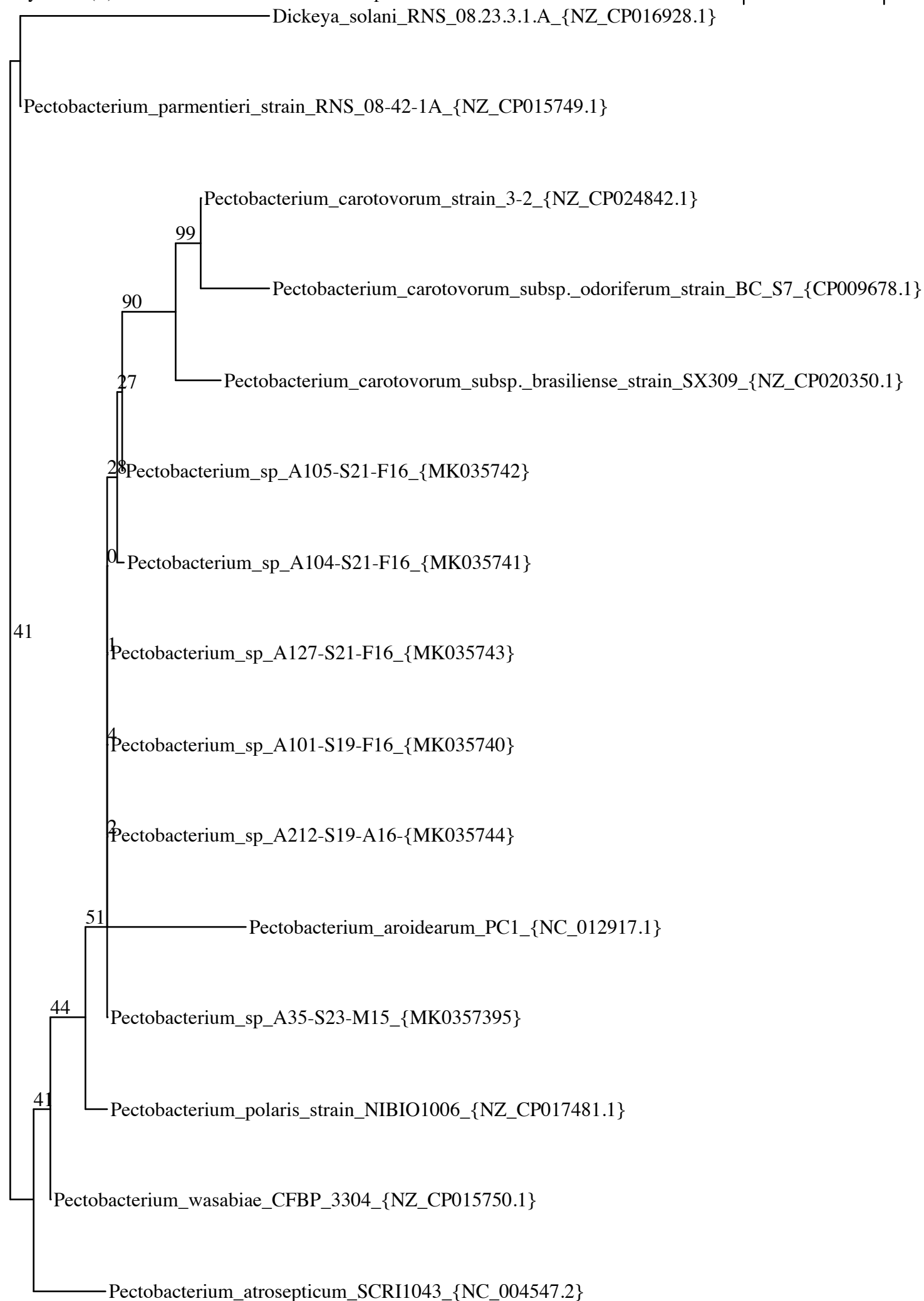
Journal name : International Journal of Systematic and Evolutionary Microbiology

Supplemental data

Supplemental S1 : 16S phylogenetic tree and associated sequences

PhyML ln(L)=-2623.9 1365 sites GTR 100 replic. 4 rate classes

0.1



Legend S1 : Phylogenetic tree constructed from the 16S nucleotide sequences. Sequences were aligned using the MUSCLE software (1) and the alignments were filtered by using the program GBLOCKS (2). Tree was computed using PHYML (3). One hundred bootstrap replicates were performed to assess the statistical support of each node. Bootstrap support values (percentages) are indicated.

1. **Edgar RC.** MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 2004;5:113

2. **Castresana J.** Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000;17:540-52

3. **Guindon S, Gascuel O.** A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol.* 2003;52:696-704

>Pectobacterium_sp_A35-S23-M15_{MK0357395} 16S ribosomal RNA_partial sequence
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>Pectobacterium_sp_A101-S19-F16_{MK035740} 16S ribosomal RNA_partial sequence
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>Pectobacterium_sp_A104-S21-F16_{MK035741} 16S ribosomal RNA_partial sequence
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>Pectobacterium_sp_A105-S21-F16_{MK035742} 16S ribosomal RNA_partial sequence
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>Pectobacterium_sp_A127-S21-F16_{MK035743} 16S ribosomal RNA_partial sequence
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Supplemental S2 : GapA sequences used to construct the
phylogenetic tree presented in fig. 1 (a)

Supplemental S2: GapA sequences used to construct the phylogenetic tree presented in fig. 1 (a)

This includes

- Partial gapA sequences of A35-S23-M15, A101-S19-F16, A104-S21-F16, A105-S21-F16, A127-S21-F16 and A212-S19-A16 obtained following amplification and sequencing of the gapA housekeeping gene as described in Cigna et al 2017
- homologous gapA sequences of Pectobacterium sp. type strains retrieved from NCBI genomes through BLAST analysis

>P.aquaticum_A35-S23-M15 Partial_gapA

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Supplemental S3 : ANI and dDDH values between *Pectobacterium* sp.

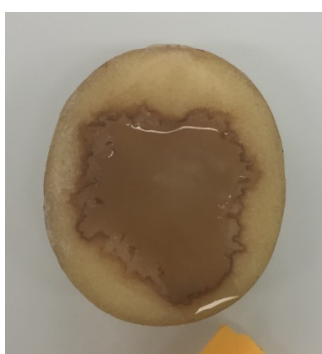
	<i>P. aquaria</i> A101-S19-F16	<i>P. aquaria</i> A104-S21-F16	<i>P. aquaria</i> A35-S23-M15	<i>P. aquaria</i> A105-S21-F16	<i>P. aquaria</i> A127-S21-F16	<i>P. aquaria</i> A212-S19-A16 ^T	<i>P. carotovorum</i> subsp. <i>carotovorum</i> NCPPB312 ^T	<i>P. carotovorum</i> subsp. <i>odoriferum</i> NCPPB3839 ^T	<i>P. polaris</i> NIBIO1006 ^T	" <i>P. carotovorum</i> subsp. <i>brasiliense</i> " PBR1692	" <i>P. carotovorum</i> subsp. <i>actinidiae</i> " KKH3	<i>P. aroidearum</i> PC1 ^T	<i>P. atrosepticum</i> NCPPB549 ^T	" <i>P. peruvienne</i> " UGC32	<i>P. betavasculorum</i> NCPPB2795 ^T	<i>P. wasabiae</i> CFBP3304 ^T	<i>P. parmentieri</i> RNS08.42.1A ^T
<i>P. aquaria</i> A101-S19-F16	--	88.80	88.80	88.90	88.70	88.10	55.70	53.10	52.90	51.50	46.40	40.00	38.00	38.10	37.70	36.60	36.30
<i>P. aquaria</i> A104-S21-F16	98.76	---	88.50	87.30	89.40	87.70	56.00	53.00	53.00	51.30	46.60	40.00	38.40	38.20	37.60	36.60	36.40
<i>P. aquaria</i> A35-S23-M15	98.72	98.7	---	87.60	90.50	86.80	55.70	52.90	52.90	51.40	46.40	40.00	38.00	37.90	37.60	36.70	36.30
<i>P. aquaria</i> A105-S21-F16	98.73	98.63	98.63	---	87.90	87.80	55.90	53.00	52.90	51.40	46.40	40.00	38.30	38.10	37.60	36.60	36.30
<i>P. aquaria</i> A127-S21-F16	98.70	98.70	98.90	98.63	---	86.20	55.70	52.80	52.80	51.50	46.40	39.80	38.20	38.00	37.60	36.70	36.40
<i>P. aquaria</i> A212-S19-A16 ^T	98.61	98.65	98.53	98.64	98.50	---	55.60	52.70	53.30	51.90	46.30	40.20	38.20	38.30	37.70	36.60	36.40
<i>P. carotovorum</i> subsp. <i>carotovorum</i> NCPPB312 ^T	94.23	94.26	94.23	94.30	94.23	94.24	---	61.50	52.40	51.00	51.00	40.30	38.80	38.50	38.10	36.80	36.70
<i>P. carotovorum</i> subsp. <i>odoriferum</i> NCPPB3839 ^T	93.69	93.68	93.68	93.73	93.66	93.64	95.18	---	49.50	47.40	50.10	39.50	38.30	38.20	37.70	36.70	36.50
<i>P. polaris</i> NIBIO1006 ^T	93.66	93.68	93.66	93.67	93.67	93.73	93.52	92.90	---	54.50	45.20	40.40	38.50	38.30	38.30	36.80	36.60
" <i>P. carotovorum</i> subsp. <i>brasiliense</i> " PBR1692	93.36	93.34	93.35	93.34	93.37	93.45	93.26	92.40	94.02	---	43.90	41.30	38.10	37.90	38.10	36.40	36.30
" <i>P. carotovorum</i> subsp. <i>actinidiae</i> " KKH3	92.28	92.32	92.28	92.29	92.27	92.25	93.37	93.15	92.02	91.57	---	38.60	37.40	37.00	36.80	35.80	35.60
<i>P. aroidearum</i> PC1 ^T	90.40	90.43	90.42	90.39	90.37	90.42	90.49	90.22	90.56	90.75	89.96	---	36.60	36.60	36.80	35.60	35.70
<i>P. atrosepticum</i> NCPPB549 ^T	89.81	89.89	89.86	89.9	89.85	89.87	89.97	89.83	89.91	89.74	89.51	89.30	---	53.90	46.80	40.00	39.40
" <i>P. peruvienne</i> " UGC32	89.72	89.74	89.72	89.79	89.73	89.77	89.89	89.77	89.81	89.70	89.38	89.20	93.76	---	47.30	39.90	39.60
<i>P. betavasculorum</i> NCPPB2795 ^T	89.64	89.66	89.67	89.65	89.64	89.67	89.83	89.68	89.81	89.83	89.34	89.28	92.29	92.46	---	38.50	37.80
<i>P. wasabiae</i> CFBP3304 ^T	89.23	89.32	89.30	89.26	89.29	89.26	89.42	89.27	89.49	89.24	89.16	88.94	90.42	90.42	89.96	---	54.70
<i>P. parmentieri</i> RNS08.42.1A ^T	89.20	89.25	89.23	89.20	89.26	89.24	89.36	89.28	89.35	89.20	88.99	88.94	90.24	90.21	89.76	94.14	---

Table S1: Pairwise ANI (below diagonal) and dDDH (above diagonal) between the genomes of the six *P. aquaria* strains and the genomes of all described and "proposed" *Pectobacterium* species and subspecies.

Supplemental S4 : Phenotypic characterization of *P. aquaticum* strains on potato tuber slices



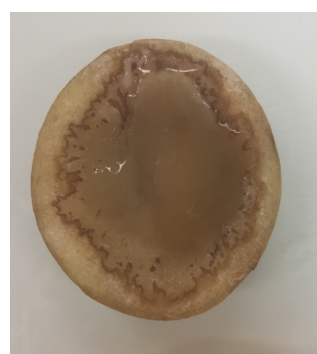
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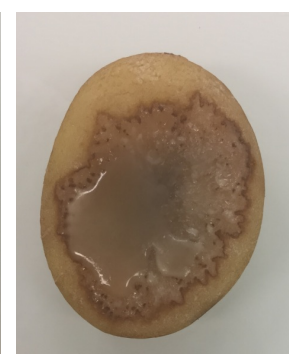
A104-S21-F16



A105-S21-F16



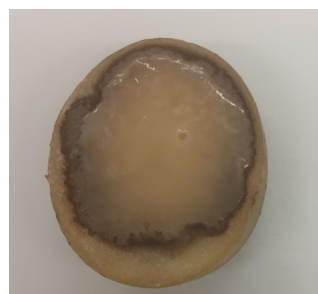
A127-S21-F16



A212-S19-A16^T



buffer



CFBP2046^T
Pectobacterium
carotovorum
subsp.
carotovorum

Legend S4: Phenotypic characterization of *P. aquaticum* strains on potato tuber slices
Bacterial strains A35-S23-M15, A101-S19-F16, A104-S21-F16, A105-S21-F16, A127-S21-F16, A212-S19-A16 were plated on LB medium and cultured for 15h at 28°C. Bacteria were then scrapped off the Petri dish, suspended in 50mM phosphate buffer pH 6,8 and adjusted to 1.0 at OD_{600nm}. Tubers of *S. tuberosum* var. charlotte were cut in slices and 10µl of the cell suspension were deposit in the centre of seven slices. The slices were placed at 28°C on wet paper towel in a plastic box. Typical obtained symptoms are shown.
Positive control; *P. carotovorum* subsp. *carotovorum* CFBP2046_r, negative control: 50mM phosphate buffer pH 6,8.

Supplemental S5

Supplemental S5: Procedure to generate the phylogenetic tree
presented in Fig 1 (b)

Dependencies:

Python version 3.7.1

Blast version 2.6.0. To install:

```
conda install blast
```

SiLix version 1.2.9. Downloading: <http://lbbe.univ-lyon1.fr/Download.html>

To install:

```
tar zxvf silix-1.x.x.tar.gz
cd silix-1.x.x
./configure
make
make check
make install
```

Muscle version 3.8.1551

To install:

```
conda install muscle
```

Predicted nucleotide protein sequences (.fasta) of all genomes located in the sequences/ directory are first concatenated in a single fasta file (sequences.fasta):

```
cat sequences/* | awk '/^>/ {print "\n"$0} /^[a-z]/ {printf $0} /^[A-Z]/ {print $0}' > sequences.fasta
```

The next command generates a names.txt file containing names of genomes:

```
ls sequences/ | sed 's/\.fasta//g' > names.txt
```

The sequences.fasta file is used to create a Blastp database:

```
makeblastdb -in sequences.fasta -dbtype nucl -out dbblastall
```

A Blastn all vs all is then performed:

```
blastn -db dbblastall -query sequences.fasta -outfmt 6 -out blastall.blastn
-num_threads 8 -evaluate 0.00001
```

The blastp output (blastall.blastn file) is then processed by the SiLix software that clusters homologous genes in families with a 80% threshold. SiLix generates the seq.80.fnodes file.

```
silix sequences.fasta blastall.blastn -f FAM -i 0.80 > seq.80.fnodes
```

The seq.80.fnodes is processed with the python script core.py that generates a matrix (output fam.80.txt file) with species in columns and number of homologous genes in rows. The script needs the seq.80.fnodes and names.txt files as arguments:

```
python3 core.py seq.80.fnodes names.txt
```

The homologous.py script then searches for homologous genes present in all genomes (core genome). The core.80.txt output file is a list of homologous families.

```
python3 homologous.py fam.80.txt
```

The align.py script extracts homologous sequences, aligns with Muscle and concatenates aligned sequences. The output file is align.fasta.

```
python3 align.py core.80.txt seq.80.fnodes sequences.fasta names.txt
```

Molecular phylogeny is performed by using the multiplatform GUI Seaview software version 4.6.5. Input file is the align.fasta alignment. First, alignment is curated by Gbloks tool with default parameters to remove all gaps or X positions. Phylogeny is performed with PhyML algorithm with the following settings: substitution model GTR, 100 bootstraps, nucleotide equilibrium frequencies, invariable sites optimized, tree searching NNI, starting tree BioNJ.