Transfer of the potato plant isolates of *Pectobacterium wasabiae* to *Pectobacterium parmentieri* sp. nov.

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Pectobacterium wasabiae was originally isolated from Japanese horseradish (*Eutrema wasabi*), but recently some *Pectobacterium* isolates collected from potato plants and tubers displaying blackleg and soft rot symptoms were also assigned to *P. wasabiae*. Here, combining genomic and phenotypical data, we re-evaluated their taxonomic position. PacBio and Illumina technologies were used to complete the genome sequences of *P. wasabiae* CFBP 3304^T and RNS 08-42-1A. Multi-locus sequence analysis showed that the *P. wasabiae* strains RNS 08-42-1A, SCC3193, CFIA1002 and WPP163, which were collected from potato plant environment, constituted a separate clade from the original Japanese horseradish *P. wasabiae*. The taxonomic position of these strains was also supported by calculation of the *in-silico* DNA–DNA hybridization, genome average nucleotide indentity, alignment fraction and average nucleotide indentity values. In addition, they were phenotypically distinguished from *P. wasabiae* strains by producing acids from (+)-raffinose, α-D(+)-α-lactose, D(+)-galactose and (+)-melibiose but not from methyl α-D-glycopyranoside, (+)-maltose or malonic acid. The name *Pectobacterium parmentieri* sp. nov. is proposed for this taxon; the type strain is RNS 08-42-1A^T (=CFBP 8475^T=LMG 29774^T).

The genus *Pectobacterium* encompasses six recognized species: *Pectobacterium atrosepticum*, *P. carotovorum*, *P. betavasculorum*, *P. cacticida, P. aroidearum* and *P. wasabiae* (Gardan *et al.*, 2003; Hauben *et al.*, 1998; Nabhan *et al.*, 2013). These bacteria cause soft rot and blackleg diseases on a wide range of plants and crops, provoking considerable economic damage (Perombelon & Kelman, 1980; Perombelon, 2002). At least 16 dicotyledonous and 11 monocotyledonous angiosperm

Abbreviations: AF, alignment fraction; DDH, DNA–DNA hybridization; gANI, genome average nucleotide identity; GGD, genome-to-genome distance; MiSI, microbial species identifier; MLSA, multi-locus sequence analysis.

The GenBank/EMBL/DDBJ accession numbers for the genomes of *Pectobacterium* and *Dickeya* species used in multi-locus sequence analysis and genome-based analysis are: CP015749 (*P. parmentieri* RNS 08-42-1A^T), CP003415.1 (*P. parmentieri* SCC3193), CP001790 (*P. parmentieri* WPP163), JENG00000000 (*P. parmentieri* CFIA1002), CP015750 (*P. wasabiae* CFBP 3304^T), JQOH00000000 (*P. wasabiae* NCPPB 3702), BX950851 (*P. atrosepticum* SCRI1043), CP007744 (*P. atrosepticum* JG10-08 *P*), CP001657 (*P. carotovorum* subsp. *carotovorum* PC1), ABVX00000000 ('*P. carotovorum* subsp. *brasiliense*' PBR1692), CP009678 (*P. carotovorum* subsp. *odoriferum* BC S7), CP003776 (*P. carotovorum* subsp. *carotovorum* PCC21) and CP015137 (*Dickeya solani* IPO 2222^T).

One supplementary table is available with the online Supplementary Material.

families are subjected to *Pectobacterium* species threats (Ma *et al.*, 2007).

P. wasabiae has been isolated from the diseased rhizomes and fibrous roots of Japanese horseradish (Goto & Matsumoto, 1987). More recently, several *P. wasabiae* isolates were also recovered from symptomatic potato plants and tubers in Canada, New Zealand, Iran, South Africa, Zimbabwe, Finland and France (Baghaee-Ravari *et al.*, 2011; De Boer *et al.*, 2012; Ma *et al.*, 2007; Moleleki *et al.*, 2013; Nabhan *et al.*, 2013; Pasanen *et al.*, 2013; Pitman *et al.*, 2010). Furthermore, several *P. carotovorum* isolates that were collected up to 40 years ago from potato plants in Ireland, Germany, Poland, Scotland, Serbia, The Netherlands and USA were reclassified as members of *P. wasabiae*, indicating that these *P. wasabiae* strains had been infective on this plant for a long time (Nabhan *et al.*, 2012, 2013; Nykyri *et al.*, 2012; Waleron *et al.*, 2013). However, different studies indicated that potato isolates of *P. wasabiae* are less virulent than *P. carotovorum* and *P. atrosepticum* strains (Kim *et al.*, 2009; Nykyri *et al.*, 2012).

Almost 60 complete and draft genome sequences of *Pectobacterium* species are available in public databases, providing substantial information to be integrated into taxonomic studies (Chun & Rainey, 2014). Using genomic data, several studies have reported incongruences in the positioning of several strains within *Pectobacterium* and *Dickeya* species, and highlighted the need of species reclassification (Khayi *et al.*, 2015; Nykyri *et al.*, 2012; Pritchard *et al.*, 2016; Zhang *et al.*, 2016). In this regard, *P. wasabiae* species can be grouped into two phylogenetic clades: the first groups the Japanese horseradish isolates including the type strain CFBP 3304^T while the second includes all the characterized potato isolates (Khayi *et al.*, 2015; Nykyri *et al.*, 2012; Pritchard *et al.*, 2016; Zhang *et al.*, 2016).

In this study, four strains (RNS 08-42-1A^T, SCC3193, CFIA1002 and WPP163) were included. They were collected from potato plant environment and previously assigned to *P. wasabiae*. Here, we re-established their taxonomic position using molecular methods such as multi-locus sequence analysis (MLSA) (De Vos, 2011; Glaeser & Kämpfer, 2015), genome-to-genome distance (GGD) (Meier-Kolthoff *et al.*, 2013) and microbial species identifier (MiSI) (Varghese *et al.*, 2015). These *in silico* methods were coupled to DNA–DNA hybridization (DDH) and phenotypic characterization. We propose the delineation of a novel species of the genus *Pectobacterium* on this basis, *P. parmentieri* sp. nov.

To initiate this study, we obtained the complete genome sequences of *P. wasabiae* CFBP 3304^T and *P. parmentieri* RNS 08-42-1A^T. DNA extractions were performed from overnight cultures using MasterPure DNA purification kit (Epicentre). Quantification and quality control of the DNA was completed using a NanoDrop (ND 1000) device, Qubit 2.0 fluorometer and agarose gel electrophoresis at 1.0 %. The two genomes were sequenced and assembled using combined sequencing technologies provided by Illumina HiSeq 2000 and PacBio RSII platforms. The complete circular chromosomes for these two strains have been deposited at DDBJ/EMBL/GenBank under accession numbers CP015749 (*P. parmentieri* RNS 08-42-1A^T) and CP015750 (*P. wasabiae* CFBP 3304^T). In addition, we used the complete and draft genomes of *P. wasabiae* and *P. parmentieri* isolates collected from Japanese horseradish (strain NCPPB 3702) or potato (strains SCC3193, CFIA1002 and WPP163) hosts, respectively.

Eleven housekeeping genes (*rpoD*, *gyrB*, *recA*, *rpoS*, *fusA*, *dnaX*, *gyrA*, *purA*, *dnaN*, *gapA*, *rplB*) were retrieved from these six genome sequences, as well as those of *P. atrosepticum* SCRI1043 (BX950851), *P. atrosepticum* JG10-08 (CP007744), *P. carotovorum* subsp. *carotovorum* PC1 (CP001657), '*P. carotovorum* subsp. *brasiliense*' PBR1692 (ABVX00000000), *P. carotovorum* subsp. *odoriferum* BC S7 (CP009678), *P. carotovorum* subsp. *carotovorum* PCC21 (CP003776) and *Dickeya solani* IPO 2222^T (CP015137). The concatenated genes (~17 370 kbp) were aligned using CLUSTAL W (Larkin *et al.*, 2007), evolutionary distances were computed using the maximum-composite-likelihood method (Tamura *et al.*, 2004) and the evolutionary history was then inferred using the neighbour-joining method (Saitou & Nei, 1987). These analyses were conducted with MEGA 7 (Kumar *et al.*, 2016); the MLSA tree is shown in Fig. 1. The *P. parmentieri* clade containing strain RNS 08-42-1A^T was separated from *P. wasabiae* encompassing the horseradish isolates CFBP 3304^T and NCPPB 3702.

At the whole genome scale, several *in silico* methods were available to evaluate assignation to existing species as well as proposition of novel species. GGD was proposed to approach the wet-lab DDH method as closely as possible (Meier-Kolthoff *et al.*, 2013). Using the dedicated pipeline (http://ggdc.dsmz.de/), GGD was calculated between *P. parmentieri* RNS 08-42-1A^T as a reference and other *Pectobacterium* strains as queries (Table 1). Estimated DDH was higher than 90 % between *P. parmentieri* strains while it dropped below 55 % when *P. wasabiae* strains CFBP 3304^T and NCPPB 3702 were used as queries. Reciprocally, when *P. wasabiae* CFBP 3304^T was used as a reference and each of the *P. parmentieri* strains SCC3193, WPP163, CFIA1002 and RNS 08-42-1A^T was used as a query, the DDH value was near 55 % (Table 1). These results were in agreement with DDH experiments that were conducted by the DSMZ laboratory (Braunschweig, Germany). DNA crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DDH was carried out as described by De Ley *et al.* (1970) under consideration of the modifications proposed by Huss *et al.* (1983) using a model Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). The pairings between *P. wasabiae* CFBP 3304^T and two *P. parmentieri* strains (RNS 08-42-1A^T and RNS14.18.2.1A) showed 68.5 and 67.4 % DDH values, respectively. Hence, according to a threshold DDH value of 70 % to separate two species, *P. wasabiae* and *P. parmentieri* were distinguished using DDH estimations.

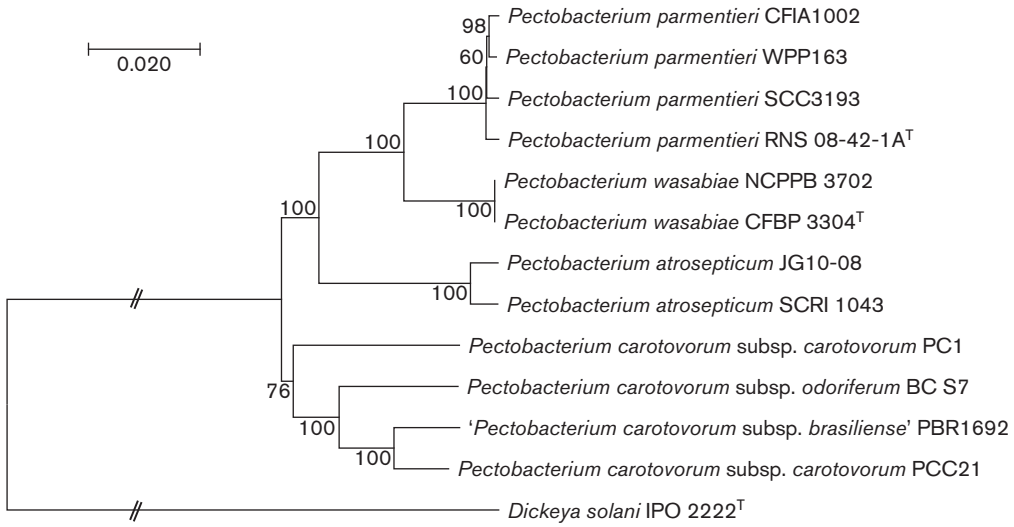

Fig. 1. Distance tree of concatenated sequences of *rpoD*, *gyrB*, *recA*, *rpoS*, *fusA*, *dnaX*, *gyrA*, *purA*, *dnaN*, *gapA* and *rplB* genes. Distances were calculated using the maximum-composite-likelihood method; the tree was inferred using the neighbour-joining method; bootstrap percentages were calculated based on 1000 replicates. *Dickeya solani* IPO 2222^T was used as an outgroup.

Other genomic methods were applied to support the proposition of *P. parmentieri* species. According to the MiSI approach (Varghese *et al.*, 2015), two genomes belong to the same species if they share at least 60 % of common gene content (alignment fraction, AF) with at least 95 % nucleotide sequence similarity (genome average nucleotide identity, gANI). This threshold value matches with species boundaries (Varghese *et al.*, 2015). The gANI and AF values were calculated based on pairwise comparison between *P. parmentieri* RNS 08-42-1A^T and *Pectobacterium* genomes. *P. parmentieri* RNS 08-42-1A^T versus SCC3193 and *P. parmentieri* RNS 08-42-1A^T versus WPP163 comparisons resulted in values of AF$\geq$0.88 and gANI$\geq$99 %, suggesting they belong to the same species (Table 2). In contrast, when *P. parmentieri* RNS 08-42-1A^T and *P. wasabiae* CFBP 3304^T were compared, the AF value was 0.86 and gANI was 94 %, indicating that they belong to different species. These results were in accordance with ANI values (Table 2), which also

Table 1. Genome-to-Genome Distance (GGD) and DDH estimations of *P. parmentieri* RNS 08-42-1A^T and *P. wasabiae* CFBP 3304^T against related *Pectobacterium* strains

Reference genome	Query genome	DDH (%) (formula 2)	Confidence interval	GGD
P. parmentieri RNS 08-42-1A^T	*P. parmentieri* SCC3193	90.40	88.2–92.3	0.0878
P. parmentieri RNS 08-42-1A^T	*P. parmentieri* CFIA1002	90.80	88.6–92.7	0.0726
P. parmentieri RNS 08-42-1A^T	*P. parmentieri* WPP163	90.50	88.2–92.4	0.0829
P. parmentieri RNS 08-42-1A^T	*P. wasabiae* CFBP 3304^T	54.70	52–57.4	0.1821
P. parmentieri RNS 08-42-1A^T	*P. wasabiae* NCPPB 3702	54.60	51.9–57.3	0.1826
P. parmentieri RNS 08-42-1A^T	*P. carotovorum* subsp. *carotovorum* PC1	35.70	33.2–38.2	0.3146
P. parmentieri RNS 08-42-1A^T	*P. carotovorum* subsp. *carotovorum* PCC21	36.60	34.1–39.1	0.3145
P. parmentieri RNS 08-42-1A^T	*P. atrosepticum* SCRI1043	39.40	36.9–42	0.3108
P. wasabiae CFBP 3304^T	*P. wasabiae* NCPPB 3702	97.90	97–98.5	0.0032
P. wasabiae CFBP 3304^T	*P. parmentieri* SCC3193	54.60	51.9–57.3	0.1907
P. wasabiae CFBP 3304^T	*P. parmentieri* WPP163	54.90	52.1–57.6	0.1880
P. wasabiae CFBP 3304^T	*P. parmentieri* CFIA1002	54.60	51.9–57.3	0.1829
P. wasabiae CFBP 3304^T	*P. carotovorum* subsp. *carotovorum* PC1	35.60	33.2–38.1	0.3203
P. wasabiae CFBP 3304^T	*P. carotovorum* subsp. *carotovorum* PCC21	36.80	34.4–39.3	0.3258
P. wasabiae CFBP 3304^T	*P. atrosepticum* SCRI1043	40.20	37.7–42.7	0.3168

Table 2. gANI and AF values calculated for *P. parmentieri* RNS 08-42-1A^T and *P. wasabiae* CFBP 3304^T against related *Pectobacterium* strains

Pairings*	gANI (%)	AF	ANI (%)
Pp RNS 08-42-1A^T vs Pw CFBP 3304^T	94.27	0.86	93.8
Pp RNS 08-42-1A^T vs Pp SCC3193	99.07	0.88	98.8
Pp RNS 08-42-1A^T vs Pp WPP163	99.03	0.90	98.8
Pp RNS 08-42-1A^T vs Pa SCRI1043	90.51	0.75	89.5
Pp RNS 08-42-1A^T vs Pcc PC1	88.88	0.78	88.1
Pp RNS 08-42-1A^T vs Pcc PCC21	89.32	0.78	88.5
Pw CFBP 3304^T vs Pp SCC3193	94.31	0.84	93.8
Pw CFBP 3304^T vs Pp WPP163	94.35	0.85	93.9
Pw CFBP 3304^T vs Pa SCRI1043	90.51	0.75	89.7
Pw CFBP 3304^T vs Pcc PC1	88.89	0.77	88.2
Pw CFBP 3304^T vs Pcc PCC21	89.41	0.76	88.6

*Pp, *P. parmentieri*; Pw, *P. wasabiae*; Pa, *P. atrosepticum*; Pcc, *P. carotovorum* subsp. *carotovorum*.

supported the proposal of *P. parmentieri* according to a threshold of 95 % for delineating species boundaries (Goris *et al.*, 2007).

Comparative genomics analyses were also performed to further identify distinctive traits between *P. wasabiae* and *P. parmentieri*. The complete genome of both type strains was automatically annotated under the RAST server (Aziz *et al.*, 2008). *P. parmentieri* RNS 08-42-1A^T showed 4457 protein coding genes and 22 rRNA genes, while 4472 protein coding genes and 22 rRNA genes were predicted in *P. wasabiae* CFBP 3304^T. Using the function-based comparison tool under RAST, 61 genes were predicted to be specific to *P. parmentieri* RNS 08-42-1A^T while 62 genes were specific to *P. wasabiae* CFBP 3304^T (Table S1, available in the online Supplementary Material). Among *P. parmentieri* RNS 08-42-1A^T specific genes, we found those implicated in maltose/maltodextrin utilization, and α/β-galactosidase and siderophore production. Using these predicted traits in *P. parmentieri* and those used to describe the species *P. wasabiae* (Goto & Matsumoto, 1987), biochemical tests were performed. Indeed, eight carbohydrates and one amino acid were tested in minimum medium 9 (Elbing & Brent, 2002) as sole source of carbon and nitrogen, respectively. The ability to use (+)-raffinose, D(+)-α-lactose, D(+)-galactose and (+)-melibiose as sole source of carbon differentiated *P. parmentieri* strains RNS 08-42-1A^T and SCC3193 from *P. wasabiae* CFBP 3304^T (Table 3).

Table 3. Distinctive metabolic traits between *P. parmentieri* and *P. wasabiae*

ND, No data; V, variable reaction among the strains used.

Utilization of:	Pp*	Pw	Pcc	Pa	Pb	Pco
D(+)-α-Lactose	+	−	+	+	V	+
(+)-Melibiose	+	−	+	+	−	+
(+)-Raffinose	+	−	+	+	+	+
D(+)-Galactose	+	−	+	+	+	ND

*Data were collected from *P. parmentieri* (Pp) RNS08421A^T and SCC3193, *P. wasabiae* (Pw) CFBP 3304^T (=SR91), SR115 and SR36 (Goto & Matsumoto, 1987), *P. carotovorum* subsp. *carotovorum* (Pcc) CFBP2046^T and CFBP2138 (Gardan *et al.*, 2003), *P. atrosepticum* (Pa) CFBP1526^T and CFBP511 (Gardan *et al.*, 2003), *P. betavasculorum* (Pb) NCPPB 2795^T and Ecb173 (Goto & Matsumoto, 1987), and *P. carotovorum* subsp. *odoriferum* (Pco) CFBP1878^T and CFBP2599 (Gardan *et al.*, 2003).

Description of *Pectobacterium parmentieri* sp. nov.

Pectobacterium parmentieri [par.men.ti.e′ri. N.L. gen. neut. n. *parmentieri*, of Antoine Augustin Parmentier (1737–1813), a pharmacist who promoted use of the tuber of the potato plant (*Solanum tuberosum*) as a food source for humans in France and throughout Europe].

Gram-negative, motile bacterium. Grows optimally at 28 °C in TY medium (5 g tryptone l^{-1}, 3 g yeast extract l^{-1} and agar 1.5 %) forming colonies 1–2 mm in diameter within 17 h. Catabolizes (+)-raffinose, α-D(+)-α-lactose, D(+)-galactose and (+)-melibiose but not methyl α-D-glycopyranoside, (+)-maltose or malonic acid. Can provoke soft rot and blackleg symptoms on tubers and stems of potato plants.

The type strain, RNS 08-42-1A^T (=CFBP 8475^T=LMG 29774^T), was recovered in 2008 from a potato plant (cv. Bintje) expressing blackleg symptoms in a greenhouse at the French National Institute for Agricultural Research (INRA) in Le Rheu (Brittany, France). The DNA G+C content of the type strain is 50.40 %.

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