Whole Genome Analysis of Two Pectobacterium Species Isolated from Blackleg Disease of Potato Occurred in Nagasaki Prefecture, Japan

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Whole Genome Analysis of Two *Pectobacterium* Species Isolated from Blackleg Disease of Potato Occurred in Nagasaki Prefecture, Japan

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In Japan, *Dickeya dianthicola, D. chrysanthemi, Pectobacterium atrosepticum, P. parmentieri,* and *P. brasiliense* are known as blackleg disease pathogens of potato. A transient outbreak of the blackleg disease occurred in Nagasaki Prefecture, and the pathogens were identified as *P. parmentieri* and *P. brasiliense*. Because repetitive extragenic palindromic PCR showed clonal relatedness among strains in each species, two Nagasaki strains (NK5 and NK14) were selected as the representative strains, and their whole genome sequences were determined. Average nucleotide identity analysis supported the identification in the previous study. Multilocus sequence analysis with seven housekeeping genes indicated the relationship between NK14 and other strains isolated in Japan, Russia, China, Switzerland, Poland, and Belgium. NK5 was related to the strains from various geographic locations including Syria, China, Canada, South Korea, Netherlands, Algeria, South Africa, Mexico, and Belarus. Subsequently, core genome multilocus sequence typing was performed with publicly available sequences, indicating that NK14 and NK5 were most closely related to the strains Poland/Russian and Netherlands/Belarus/Russia, respectively. This study concerns the first phylogenetic analyses based on the whole genome sequences of Japanese strains of *P. parmentieri* and *P. brasiliense*, and contributes to the molecular epidemiological analysis of the pathogen of potato blackleg.

Key words: Blackleg disease, Potato, *Pectobacterium brasiliense, Pectobacterium parmentieri*, Whole genome sequencing

INTRODUCTION

Potato (*Solanum tuberosum*) is one of the most important crops in the world, and its production (359 million tons) was ranked sixth among produced crops worldwide in 2020 (FAO, 2022).

Bacterial diseases are severe obstacles to potato production, especially in tropical and subtropical regions and some warm temperate regions of the world. Potato crop losses due to bacterial diseases could be direct and indirect. They are short–term impacts like yield loss and unmarketability, and long–term impacts with environmental, economic, and social effects (Charkowski *et al.*, 2020). Several bacterial diseases affect potatoes worldwide and cause serious damage, especially to tubers. Bacterial wilt and blackleg are considered the most important diseases, potato ring rot, pink eye, and common scab are the minor, and zebra chip is extremely rare (Charkowski *et al.*, 2020).

The blackleg disease of potato is caused by six bacterial species including *Pectobacterium atrosepticum, P. brasiliense* (synonym of *P. carotovorum* subsp. *brasiliense*; Portier *et al.*, 2019), *P. parmentieri* (synonym of *P. wasabiae* potato isolate; Khayi *et al.*, 2016), *Dickeya dianthicola, D. solani*, and *D. chrysanthemi*.

In Japan, blackleg disease caused by five of these species other than *D. solani* has been reported, and the recent expansion of outbreak areas has been noted.

The occurrence of potato blackleg disease has increased recently in Japan, and it is pointed out that epidemiological studies of the disease are needed (Fujimoto *et al.*, 2017).

Recently, the blackleg of potato suddenly occurred in Minamishimabara, Shimabara, and Unzen City in Nagasaki Prefecture. The causal agents were identified as *P. brasiliense* and *P. parmentieri* according to bacterial and biochemical characteristics and phylogenetic analysis based on nucleotide sequences of 16S rRNA and housekeeping genes, *recA*, and *dnaX* (Le *et al.*, 2023).

Whole genome sequences of causal agents of blackleg were determined elsewhere, and epidemical, phylogenetic, and evolutional analyses have been performed. However, to our knowledge, the whole genome of Japanese strains is not determined, and analyses based on the whole genome were limited. A study concerning the phylogenetic relationship of Nagasaki strains with other strains from around the world is necessary for plant protection and disease management. Therefore, whole genome analyses of the two representative strains were performed, and based on this information, the rela-

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tionship to other strains isolated in different geographical locations of the world was studied.

MATERIALS AND METHODS

Bacterial strains, culture, and genome extraction

Bacterial strains used in this study are shown in Table 1. The strain was cultured in LB broth (Lennox; 1% tryptone, 0.5% yeast extract, 0.5% sodium chloride, pH 7.0) at 28°C overnight with aerobic conditions. Genomic DNA was extracted by the CTAB method (Wilson, 1987), and stored at –20°C until use.

Repetitive element palindromic (rep) PCR

In the rep–PCR, ERIC1R (5'–ATGTAAGCTCCTGGGGATTCAC–3') and ERIC2 (5'–AAGTAAGTGACTGGGGTGAGCG–3') were used as primers (Versalovic *et al.*, 1991). PCR was performed as previously reported (Kyaw *et al.*, 2019). The PCR products were analyzed with agarose electrophoresis.

Whole genome analysis

Draft genome sequences of *P. brasiliense* NK5 and *P. parmentieri* NK14 were determined as described below. Genome sequencing was carried out on the Illumina NovaSeq platform (Novogene Co., Ltd, Beijing, China). A 350–bp library was prepared and sequenced to obtain 150–bp paired–end reads. Raw reads were trimmed using Trimmomatic v. 0.32 (Bolger *et al.*, 2014), and *de novo* assembly was carried out using SKESA v. 2.3.0 (Souvorov *et al.*, 2018). The resulting draft genome sequences were annotated using the DDBJ Fast Annotation and Submission Tool (DFAST) with default settings (Tanizawa *et al.*, 2018).

Publicly available sequences were downloaded at NCBI and used as reference sequences in the following analyses (Supplementary file 1).

Average nucleotide identity (ANI)

The average nucleotide identity (ANI) between the strains were determined using Pyani v. 0.2.12 (Pritchard *et al.*, 2016). The generated heat maps were modified graphically.

Multilocus sequencing analysis (MLSA)

Seven housekeeping genes including *acnA, gapA, icdA, mdh, mtlD, pgi*, and *proA* (Ma *et al.*, 2007) in the whole genome sequences were used. Partial sequences of these genes were used for the strains whose whole genome sequences are not determined. The sequences were aligned with MUSCLE (Edgar, 2004), sequences of *acnA* (275 bp), *gapA* (255 bp), *icdA* (471 bp), *mdh* (428 bp), *mtlD* (316 bp), *pgi* (466 bp), and *proA* (415 bp) were concatenated (2626 bp). Maximum–likelihood phylogenetic analysis was performed with IQ–Tree (Trifinopoulos *et al.*, 2016). The phylogenetic tree was drawn using iTOL v.6.8.1 (Letunic and Bork, 2021) and then modified graphically.

Core genome multilocus sequence typing (cgMLST)

The sequences were analyzed using chewBBACA v. 2.8.5 (Silva *et al.*, 2018) with a BLAST score ratio of 0.60 for core genome multilocus sequence typing (cgMLST). Minimum spanning trees were constructed based on the allelic profiles obtained by the cgMLST scheme using GrapeTree (Zhou *et al.*, 2018).

RESULTS

Rep–PCR analysis

The uniformity of rep–PCR fingerprinting patterns of *P. brasiliense* Nagasaki strains was confirmed (Fig. 1). In *P. parmentieri*, the patterns of NK13 and NK14 were the same. Similar results were obtained when other primer pairs for BOX and ERIC PCR were used (data not shown). These results indicated that the

Table 1. Bacterial strain used in rep–PCR analysis

Bacterial strain [1)]	Host	Location (isolated year)	Reference
Pectobacterium brasiliense			
KuroAshi 1	*Solanum tuberosum*	Unzen City (2018)	Le *et al.* (2023)
NK5*	*S. tuberosum*	Minamishimabara City (2020)	Le *et al.* (2023)
NK8	*S. tuberosum*	Minamishimabara City (2020)	Le *et al.* (2023)
NK9	*S. tuberosum*	Minamishimabara City (2020)	Le *et al.* (2023)
NK10	*S. tuberosum*	Minamishimabara City (2020)	Le *et al.* (2023)
NK11	*S. tuberosum*	Shimabara City (2020)	Le *et al.* (2023)
NK15	*S. tuberosum*	Unzen City (2020)	Le *et al.* (2023)
NK16	*S. tuberosum*	Unzen City (2020)	Le *et al.* (2023)
NK17	*S. tuberosum*	Unzen City (2020)	Le *et al.* (2023)
NK18	*S. tuberosum*	Unzen City (2020)	Le *et al.* (2023)
NK19	*S. tuberosum*	Unzen City (2020)	Le *et al.* (2023)
NK22	*S. tuberosum*	Unzen City (2020)	Le *et al.* (2023)
P. parmentieri			
NK13	*S. tuberosum*	Shimabara City (2020)	Le *et al.* (2023)
NK14*	*S. tuberosum*	Shimabara City (2020)	Le *et al.* (2023)

1) Asterisks indicate the strains used in the whole genome analysis as representative strains.

Nagasaki strains in both species used here were clonal.

Whole genome analyses

NK5 and NK14 were selected as the representative strains for *P. brasiliense* and *P. parmentieri*, and whole genome analyses were performed. Statistics of genome assembly and annotation are summarized in Table 2. Over 4.6 million clean reads were used for genome assembly in both *P. brasiliense* NK5 and *P. parmentieri* NK14. The numbers of contigs were 40 and 43, and total contig sizes were approximately 4.91 Mb (coverage read depth, ×302) and 4.86 Mb (×289) in NK5 and NK14, respectively.

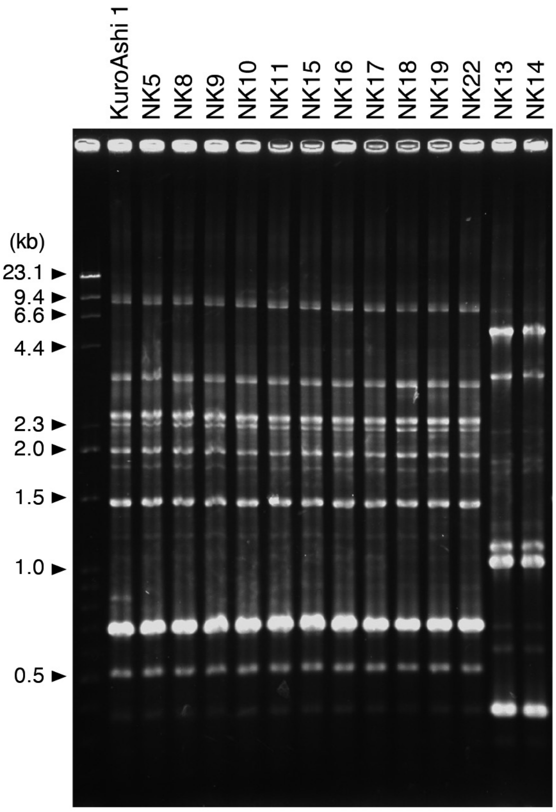

Fig. 1. Comparison of rep–PCR profile fingerprint patterns of genomic DNA from Nagasaki strains of *P. brasiliense* (KuroAshi 1 to NK22) and *P. parmentieri* (NK13 and NK14). ERIC1R and ERIC2 were used as a primer pair.

Nucleotide sequences determined in this study have been deposited at DDBJ/EMBL/GenBank. The accession numbers for *P. brasiliense* NK5 and *P. parmentieri* NK14 are BSWF01000001 to BSWF01000040 and BSWE01000001 to BSWE01000043, respectively.

ANI between NK5/NK14 and the type strains of *Pectobacterium* species were calculated (Fig. 2). ANI values were 0.962 and 0.990 between NK5–*P. brasiliense* IPO 3540^{T} and NK14–*P. parmentieri* RNS 08–42–1A^{T}, respectively. The ANI values between NK5/NK14 and other strains were below 0.95, which is the most used standard cutoff for species demarcation and corresponds to 70% in DNA–DNA hybridization (Richter and Rosselló-Móra, 2009).

MLSA

Branch lengths were short in the phylogenetic tree for *P. parmentieri*, indicating that the nucleotide sequences were highly conserved (Fig. 3). In particular, the concatenated sequences were identical in NK14, PB20, HAFL01, IFB5619, IPO1955, QK–5, and IFB 5485.

On the other hand, the corresponding sequences were relatively diverse in *P. brasiliense* (Fig. 4). P. *brasiliense* NK5 was closely related to CFBP 7357 and formed a clade with kbs–1, pcbm–1, CFBP 5381, A1, and so on. In the tree, *P. brasiliense* strains were divided into three, subgroup I, II, and III. Subgroup I contained only IPO 0590. NK5 and IPO 3540^{T} belonged to different subgroups, III and II, respectively. ANI values between IPO 3540^{T} and other strains were higher than 0.95. These ANI

Table 2. Statistics for the genome assembly and annotation of *P. brasiliense* NK5 and *P. parmentieri* NK14

Statistics	*P. brasiliense* NK5	*P. parmentieri* NK14
Genome assembly		
Number of clean reads	4,951,513 reads	4,683,794 reads
Total nucleotide	1,485,453,900 bp	1,405,138,200 bp
N50/L50	315,539 bp/5 contigs	418,489 bp/4 contigs
N90/L90	69,086 bp/16 contigs	88,942 bp/13 contigs
Longest contig	732,862 bp	939,572 bp
Average length of contig	122,811.27 bp	112,988.86 bp
Number of contigs	40 contigs	43 contigs
Total length	4,912,451 bp	4,858,435 bp
GC content	52.1%	50.5%
Coverage read depth	×302	×289
Genome annotation		
Number of coding sequence	4,262	4,373
Number of rRNA	6	4
Number of tRNA	66	67
Number of CRISPRs	3	4

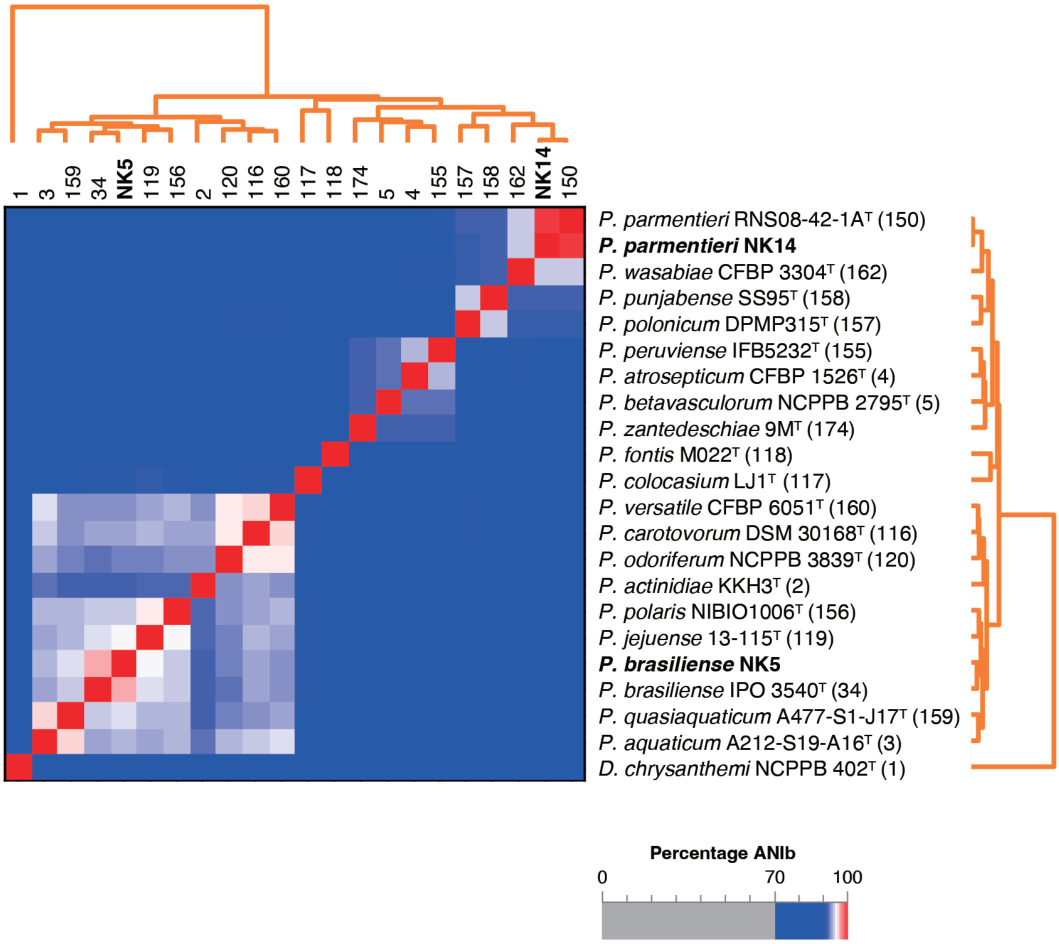

Fig. 2. Average nucleotide identity (ANI) between *P. brasiliense* NK5, *P. parmentieri* NK14, type strains of *Pectobacterium* species, and *Dickeya chrysanthemi*, calculated using the program Pyani. ANIb means identity determined using BLAST+. The dendrogram directly reflects the degree of identity between genomes. Details of strains are shown in Supplementary file 1. The number after strain is also described in the supplementary file.

values suggested that the strains belong to the same species, and subgroups similar to MLSA were shown (Fig. 5).

CgMLST

In cgMLST for *P. parmentieri*, the training file for Prodigal v. 2.6.3 (Hyatt *et al.*, 2010) was made with the complete sequence of the type strain, RNS08–42–1A^{T}. On the other hand, the genome data of *P. brasiliense* IPO 3540^{T} was a draft sequence. Therefore, the finished sequence of strain 1692 which was shown to be closely related to the type strain in MLSA analysis (Fig. 4) was used for training file construction. The number of loci found in at least 95% of *P. brasiliense* genomes was 2048, and that of *P. parmentieri* was 3187.

P. parmentieri NK14 was closely related to a Poland strain (IFB5604) and a Russian strain (PB20), and followed by the strains from China, Belgium (Fig. 6A). In the analysis for *P. brasiliense*, the close relationship between NK5 and the strains from Belarus, Netherlands, and Russian strains was shown (Fig. 6B).

DISCUSSION

Potato blackleg is a common bacterial disease that causes serious losses in potato production worldwide. In Japan, the disease is known to be caused by five pathogens. Fujimoto (2022) examined the pathogen composition of blackleg occurred since 2000 and found that *P. brasiliense*, *P. parmentieri*, and *D. dianthicola* accounted for 44.7%, 42.9%, and 12.4%, respectively; *P. atrosepticum* was not observed; *D. chrysanthemi* was shown to be locally restricted (Fujimoto, 2022). Although seed potato production is strictly managed in accordance with the Plant Protection Law in Japan (Kawakami *et al.*, 2015), the occurrence of potato blackleg has increased recently (Fujimoto *et al.*, 2020).

Under the current circumstances, blackleg caused by *P. brasiliense* and *P. parmentieri* suddenly occurred in Nagasaki Prefecture in 2018 and 2020 (Le *et al.*, 2023). Since the phylogenetic relationship with other strains was not studied, we determined the whole genome sequences of Nagasaki strains (*P. brasiliense* NK5 and *P. parmentieri* NK14) and performed the phylogenetic analyses including ANI, MLSA, and cgMLST in this study.

The number of loci was low in the cgMLST analysis for *P. brasiliense*, suggesting that some assemblies were of low quality. However, we used all assemblies in our analy-

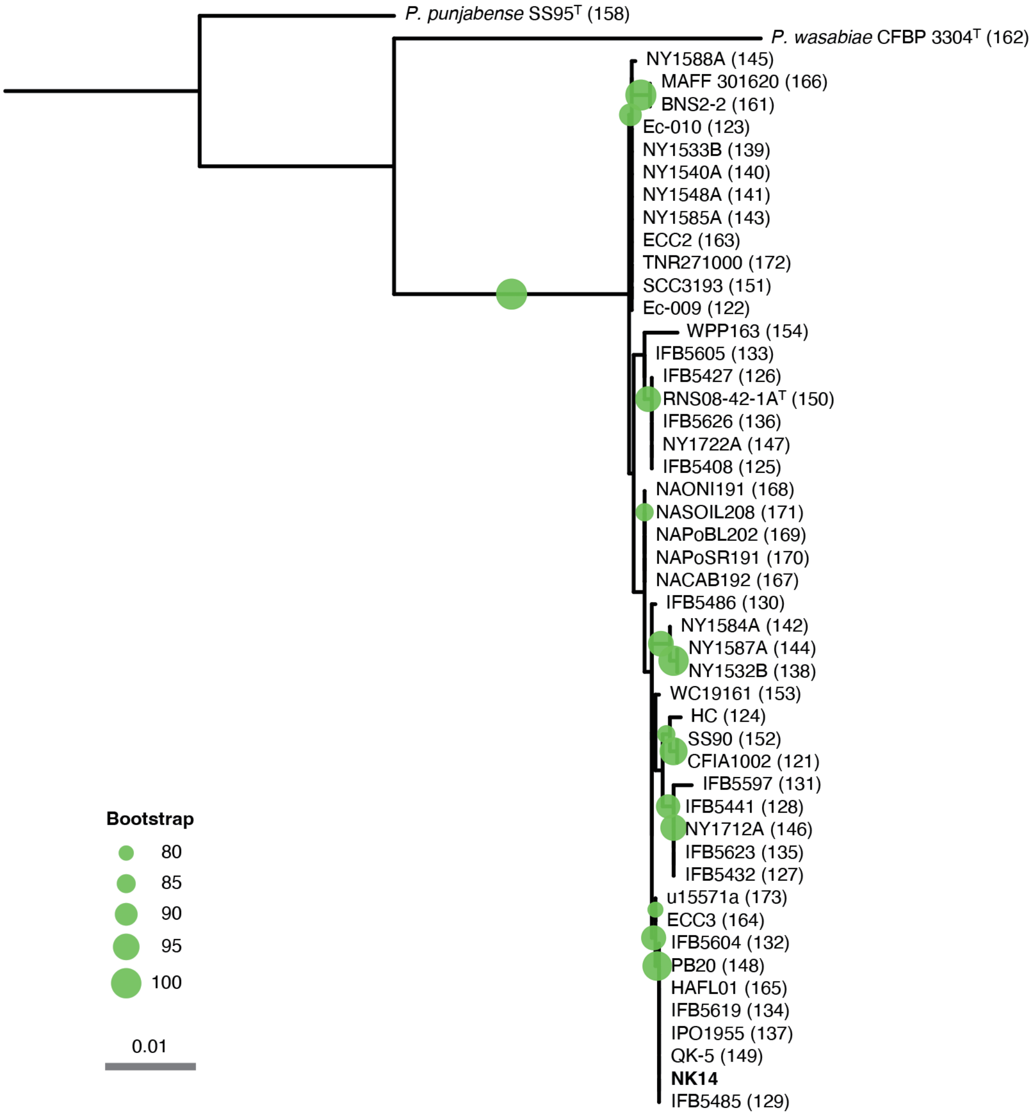

Fig. 3. Maximum–likelihood phylogenetic tree constructed using multilocus sequence analysis based on seven concatenated housekeeping genes (*acnA–gapA–icdA–mdh–mtlD–pgi–proA*) of *P. parmentieri* strains. Circles at the nodes indicate the bootstrap values over 80% (1,000 replications). *P. punjabense* SS95^{T} and *P. wasabiae* CFBP 3304^{T} were used as outgroups. The scale shows substitutions per site.

sis to ensure as much geographic diversity as possible.

It was shown that *P. brasiliense* NK5 was related to the strains isolated in Syria, China, Canada, South Korea, Netherlands, Algeria, South Africa, Mexico, Belarus, and Russia by MLSA, and cgMLST indicated a close relationship with the Netherlands Belarus, and Russia strains. Whereas, *P. parmentieri* MLSA indicated that NK14 was related to Poland, Russia, Switzerland, China, and Belgium strains. In cgMLST, NK14 was shown to be very similar to the Poland, China isolates.

The relationship with other Japanese strains was not determined in cgMLST, because of the lack of whole genome sequence data, but MLSA suggested that some strains (u15571a, ECC3 for NK14, kbs–1, kbs–2, pcbm–1, pcbm–2, pcbm–3 for NK5) were closely related with Nagasaki strains.

MLSA suggested that *P. brasiliense* was divided into three subgroups, but differences among the subgroups remain unknown. Comparative analysis of biological and pathological properties among the subgroups will be needed.

Until now, strain–level genotyping of bacteria has been promoted mainly by fragment analysis, including pulsed–field gel electrophoresis, PCR–based analyses like rep–PCR, and randomly amplified polymorphic DNA–PCR (RAPD–PCR). In the phylogenetic and epidemical analyses of the blackleg pathogens, which are isolated worldwide, it will be necessary to compare strains from different areas of the world. Genome sequence information is available worldwide and can be used for high–resolution and robust analysis. To our knowledge, this is the first report of genome sequences of the two causal agents of blackleg of potato isolated in Japan. The information in the present study contributes to the analysis of the molecular epidemiology of blackleg of potato.

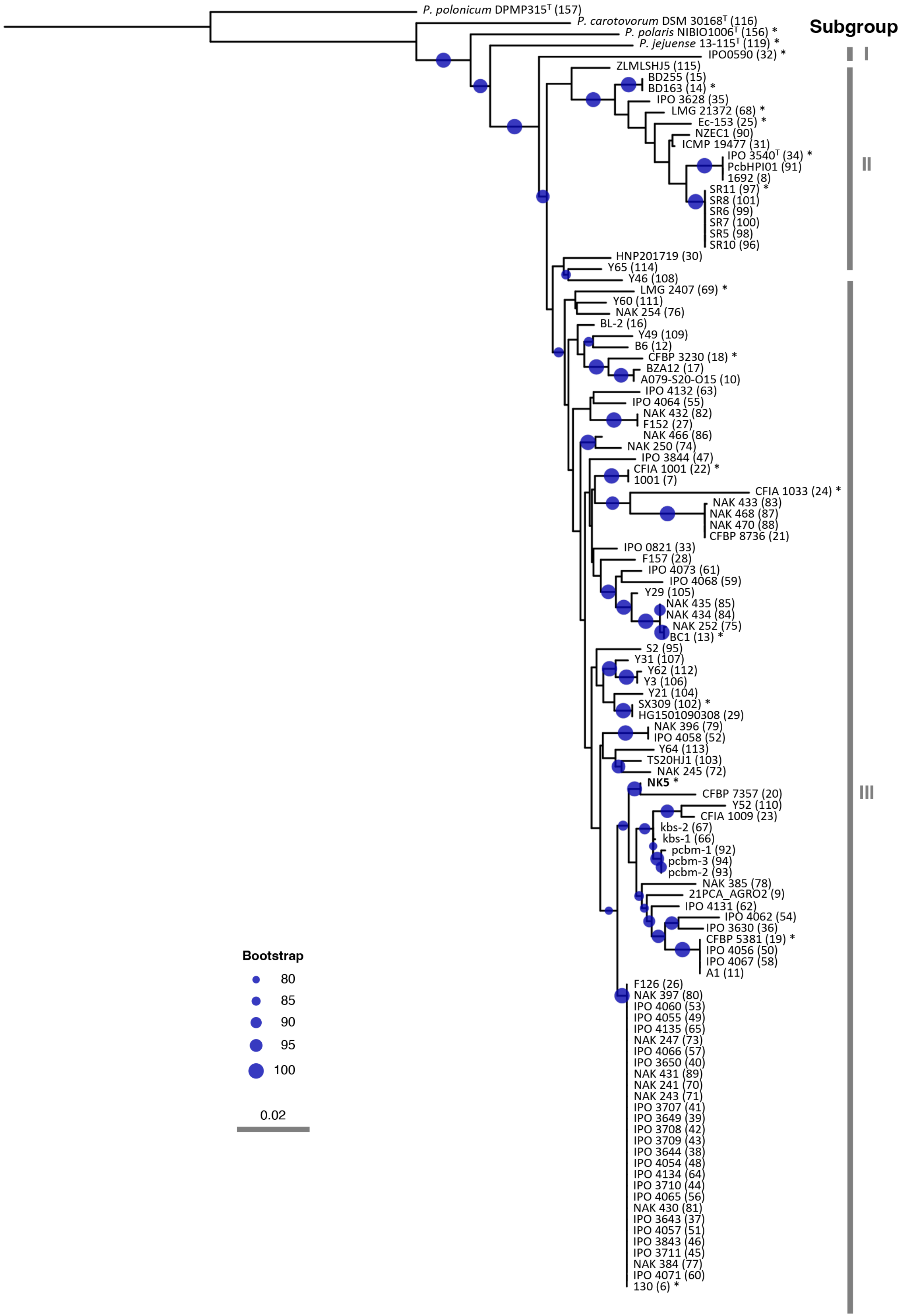

Fig. 4. Maximum–likelihood phylogenetic tree constructed using multilocus sequence analysis based on seven concatenated housekeeping genes (*acnA–gapA–icdA–mdh–mtlD–pgi–proA*) of *P. brasiliense* strains. Circles at the nodes indicate the bootstrap values over 80% (1,000 replications). *P. polonicum* DPMP315^T, *P. carotovorum* DSM 30168^T, *P. polaris* NIBIO1006^T, and *P. jejuense* 13–115^T were used as outgroups. The scale shows substitutions per site. The strains with the asterisks were used in the ANI analysis shown in Figure 5.

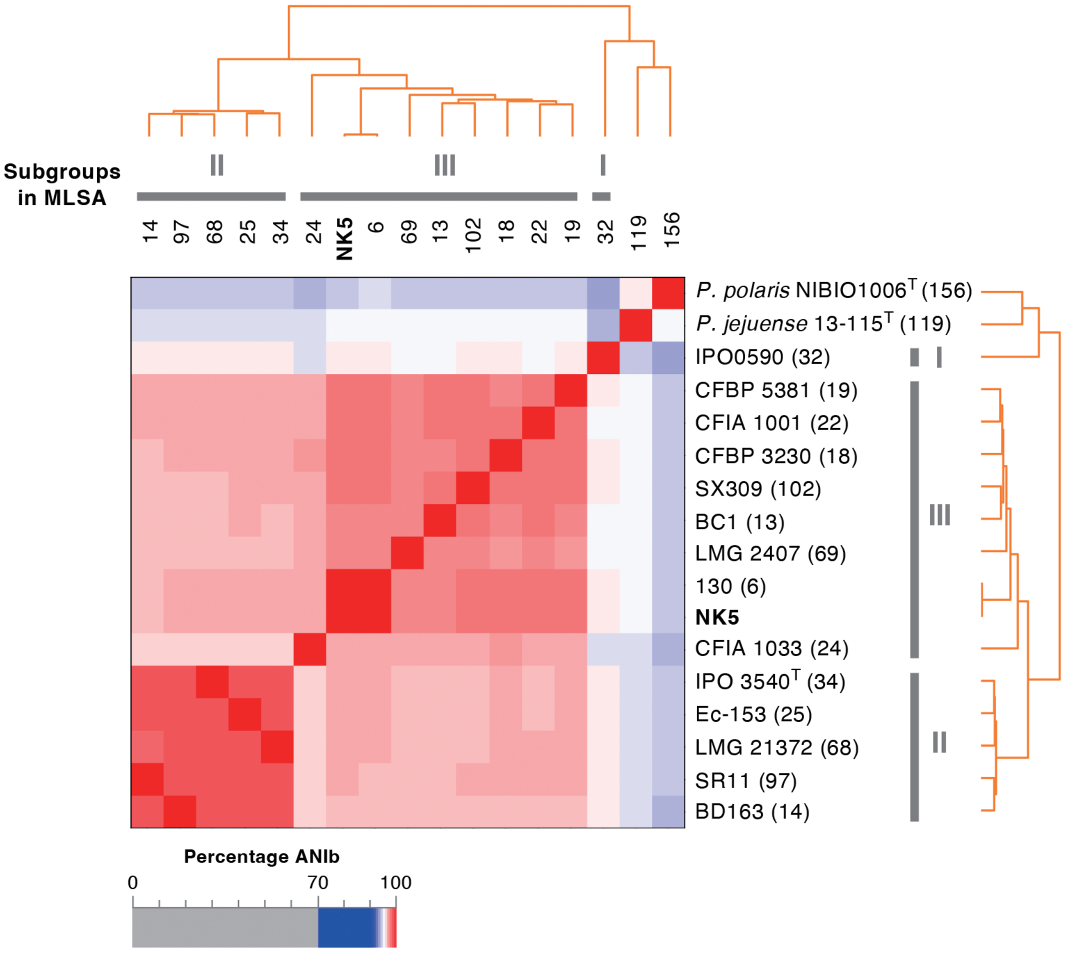

Fig. 5. Average nucleotide identity (ANI) between *P. brasiliense* strains, calculated using the program Pyani. ANIb means identity determined using BLAST+. *P. polaris* NIBIO1006^{T}, and *P. jejuense* 13–115^{T} were used as outgroups. The dendrogram directly reflects the degree of identity between genomes. The gray solid bars represent subgroups in MLSA.

AUTHOR CONTRIBUTIONS

M. Q. Le determined whole genome sequencing, performed rep–PCR, MLSA, and cgMLST analyses. K. Iiyama designed the study, performed data analysis, and wrote the paper. H. Nishiyama performed cgMLST. H. Otofuji performed data analysis, and wrote the paper. Y. Suga collected disease samples and isolated the causal agent. K. Tsuchiya and N. Furuya supervised the work. All authors assisted in editing the manuscript and approved the final version.

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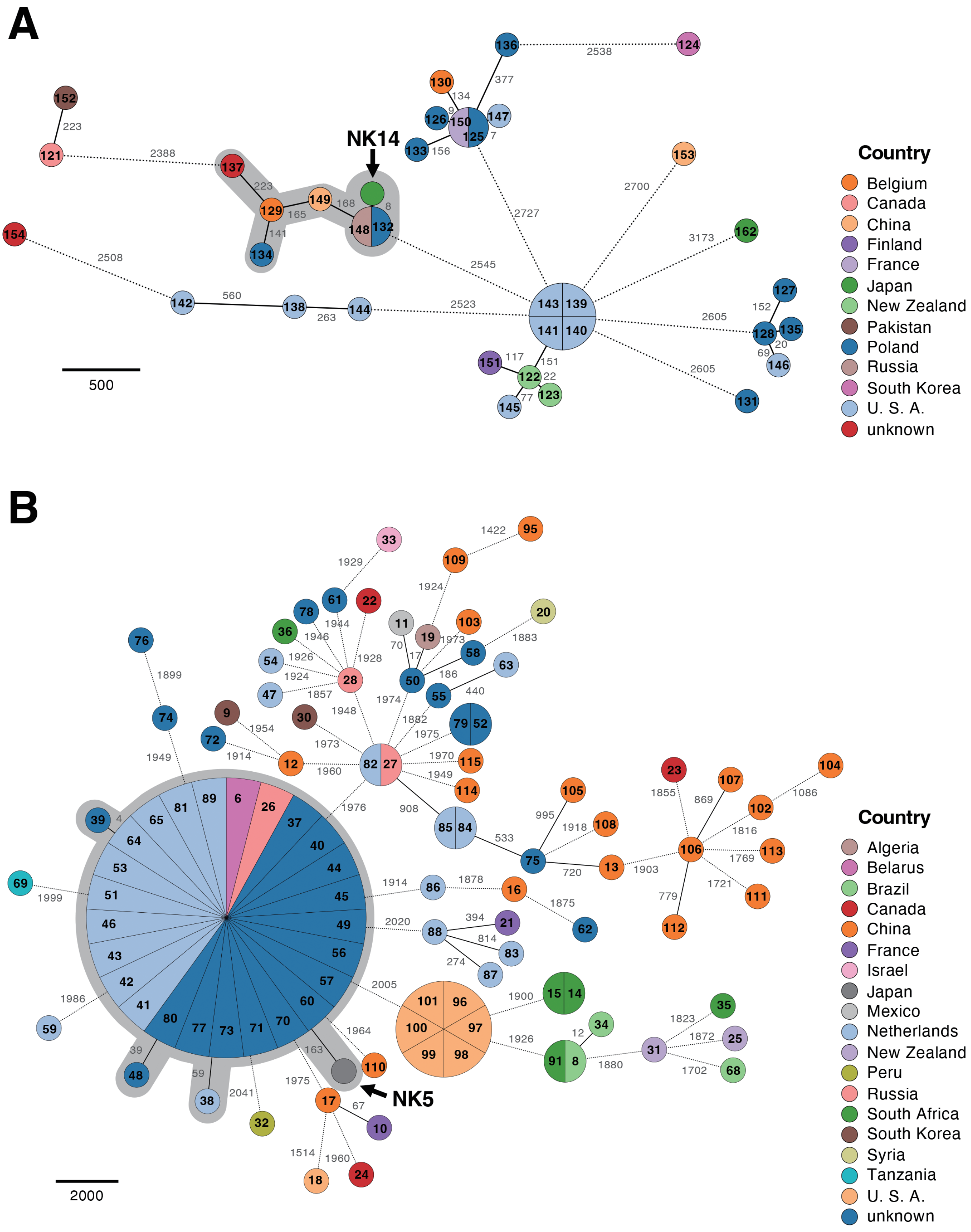

Fig. 6. Minimum spanning tree based on core genome multilocus sequence typing (cgMLST) profiles of 36 *P. parmentieri* (A) and 103 *P. brasiliense* (B) strains. The trees were created using 3,187 loci (A) and 2,048 loci (B) The numbers at the nodes indicate strains, and the details are listed in Supplementary file 1. The numbers of different alleles between pairs of strains are shown near the connecting lines. If the number of different alleles is less than 1000, the lengths are according to scale (logarithmic values) and are indicated by solid lines. When the numbers are greater than 1000, the lengths are indicated as shortened dotted lines. The collapsed node indicates the number of difference alleles is lower than 6 and 4 in the trees for *P. parmentieri* and for *P. brasiliense*, respectively.

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Supplementary file 1. List of reference strains used in this study

No.	Species (*Dickeya* /*Pectobacterium*)	Strain	Source$^{1)}$	Location	Year	Accession number						
						acnA	*gapA*	*icdA*	*mdh*	*mtlD*	*pgi*	*proA*
1	*D. chrysanthemi*	NCPPB 402^{T}	*Chrysanthemum* x *morifolium*	U. S. A.	unknown				GCA_000406105.1			
2	*P. actinidiae*	KKH3^{T}	kiwi fruit	South Korea	2006				GCA_000803315.1			
3	*P. aquaticum*	A212-S19-A16^{T}	fresh water	France	2016				GCA_003382565.3			
4	*P. atrosepticum*	CFBP 1526^{T}	*Solanum tuberosum*	U. K.	1957				GCA_019056595.1			
5	*P. betavasculorum*	NCPPB 2795^{T}	*Beta vulgaris*	U. S. A.	1972				GCA_000749845.1			
6	*P. brasiliense*	130	potato	Belarus	2020				GCA_022220705.1			
7	*P. brasiliense*	1001	*S. tuberosum*	Canada	2007	JF926767	JF926777	JF926787	JF926797	JF926807	JF926817	JF926827
8	*P. brasiliense*	1692	potato	Brazil	2015				GCA_009873295.1			
9	*P. brasiliense*	21PCA_AGRO2	napa cabbage	South Korea	2021				GCA_026723725.1			
10	*P. brasiliense*	A079-S20-O15	river water	France	2016				GCA_020406955.1			
11	*P. brasiliense*	A1	*Cephalocereus tetetzo*	Mexico	2017				GCA_019426325.1			
12	*P. brasiliense*	B6	*Brassica rapa* subsp. *pekinensis*	China	2013				GCA_000808355.1			
13	*P. brasiliense*	BC1	Chinese cabbage	China	2002				GCA_001932635.1			
14	*P. brasiliense*	BD163	*S. tuberosum*	South Africa	1999				GCA_022172285.1			
15	*P. brasiliense*	BD255	potato	South Africa	2011				GCA_001238575.1			
16	*P. brasiliense*	BL-2	potato	China	2018				GCA_017255075.1			
17	*P. brasiliense*	BZA12	cucumber	China	2015				GCA_002764035.1			
18	*P. brasiliense*	CFBP 3230	*Gossypium* sp.	U. S. A.	1964				GCA_013449485.1			
19	*P. brasiliense*	CFBP 5381	*S. tuberosum*	Algeria	1997				GCA_013449475.1			
20	*P. brasiliense*	CFBP 7357	*S. tuberosum*	Syria	2003				GCA_013449535.1			
21	*P. brasiliense*	CFBP 8736	river water	France	2016				GCA_013449685.1			
22	*P. brasiliense*	CFIA 1001	potato	Canada	2007				GCA_000738115.1			
23	*P. brasiliense*	CFIA 1009	potato	Canada	2008				GCA_000738105.1			
24	*P. brasiliense*	CFIA 1033	potato	Canada	2009				GCA_000738125.1			
25	*P. brasiliense*	Ec-153	*S. tuberosum*	New Zealand	2005				GCA_023507985.1			
26	*P. brasiliense*	F126	*S. tuberosum*	Russia	2012				GCA_003990515.2			
27	*P. brasiliense*	F152	*S. tuberosum*	Russia	2014				GCA_002930555.1			
28	*P. brasiliense*	F157	*S. tuberosum*	Russia	2015				GCA_002930535.1			
29	*P. brasiliense*	HG1501090308	cucumber	China	2016	KX010014	KX010023	KX010032	KX010041	KX010050	KX010068	KX010077
30	*P. brasiliense*	HNP201719	potato	South Korea	2017				GCA_009931555.1			
31	*P. brasiliense*	ICMP 19477	potato	New Zealand	2004				GCA_001038675.1			
32	*P. brasiliense*	IPO 0590	*S. tuberosum*	Peru	1979				GCA_016950315.1			
33	*P. brasiliense*	IPO 0821	*S. tuberosum*	Israel	1986				GCA_016950285.1			
34	*P. brasiliense*	IPO 3540^{T}	*S. tuberosum*	Brazil	1999				GCA_016950255.1			
35	*P. brasiliense*	IPO 3628	*S. tuberosum*	South Africa	unknown				GCA_016950195.1			
36	*P. brasiliense*	IPO 3630	*S. tuberosum*	South Africa	unknown				GCA_016950175.1			
37	*P. brasiliense*	IPO 3643	*S. tuberosum*	unknown	unknown				GCA_016950225.1			
38	*P. brasiliense*	IPO 3644	*S. tuberosum*	Netherlands	unknown				GCA_016950185.1			
39	*P. brasiliense*	IPO 3649	*S. tuberosum*	unknown	unknown				GCA_016944615.1			
40	*P. brasiliense*	IPO 3650	*S. tuberosum*	unknown	unknown				GCA_016949525.1			
41	*P. brasiliense*	IPO 3707	*S. tuberosum*	Netherlands	2013				GCA_016949595.1			
42	*P. brasiliense*	IPO 3708	*S. tuberosum*	Netherlands	2013				GCA_016949545.1			
43	*P. brasiliense*	IPO 3709	*S. tuberosum*	Netherlands	2013				GCA_016949435.1			
44	*P. brasiliense*	IPO 3710	*S. tuberosum*	unknown	unknown				GCA_016944595.1			
45	*P. brasiliense*	IPO 3711	*S. tuberosum*	unknown	2013				GCA_016949375.1			
46	*P. brasiliense*	IPO 3843	*S. tuberosum*	Netherlands	2013				GCA_016950125.1			
47	*P. brasiliense*	IPO 3844	*S. tuberosum*	Netherlands	2009				GCA_016950075.1			
48	*P. brasiliense*	IPO 4054	*S. tuberosum*	unknown	unknown				GCA_016950115.1			
49	*P. brasiliense*	IPO 4055	*S. tuberosum*	unknown	unknown				GCA_016949995.1			
50	*P. brasiliense*	IPO 4056	unknown	unknown	unknown				GCA_016949515.1			
51	*P. brasiliense*	IPO 4057	*S. tuberosum*	Netherlands	2017				GCA_016944555.1			
52	*P. brasiliense*	IPO 4058	*S. tuberosum*	unknown	unknown				GCA_016949915.1			
53	*P. brasiliense*	IPO 4060	*S. tuberosum*	Netherlands	2017				GCA_016944435.1			
54	*P. brasiliense*	IPO 4062	*S. tuberosum*	Netherlands	2017				GCA_016944315.1			
55	*P. brasiliense*	IPO 4064	*S. tuberosum*	unknown	unknown				GCA_016950085.1			
56	*P. brasiliense*	IPO 4065	*S. tuberosum*	unknown	unknown				GCA_016944295.1			
57	*P. brasiliense*	IPO 4066	*S. tuberosum*	unknown	unknown				GCA_016949655.1			
58	*P. brasiliense*	IPO 4067	*S. tuberosum*	unknown	unknown				GCA_016950015.1			
59	*P. brasiliense*	IPO 4068	*S. tuberosum*	Netherlands	2017				GCA_016949975.1			
60	*P. brasiliense*	IPO 4071	*S. tuberosum*	unknown	unknown				GCA_016944275.1			
61	*P. brasiliense*	IPO 4073	*S. tuberosum*	unknown	unknown				GCA_016949795.1			
62	*P. brasiliense*	IPO 4131	*S. tuberosum*	unknown	unknown				GCA_016949255.1			
63	*P. brasiliense*	IPO 4132	*S. tuberosum*	Netherlands	2017				GCA_016944255.1			
64	*P. brasiliense*	IPO 4134	*Hyacinthus* sp.	Netherlands	2017				GCA_016944235.1			
65	*P. brasiliense*	IPO 4135	insect	Netherlands	2017				GCA_016949335.1			

66	*P. brasiliense*	kbs-1	potato	Japan	2017	LC145701	LC145702	LC145703	LC145704	LC145705	LC145706	LC145707
67	*P. brasiliense*	kbs-2	potato	Japan	2017	LC145708	LC145709	LC145710	LC145711	LC145712	LC145713	LC145714
68	*P. brasiliense*	LMG 21372	*S. tuberosum*	Brazil	2002				GCA_000754705.1			
69	*P. brasiliense*	LMG 2407	*Lycopersicon esculentum*	Tanzania	1954				GCA_922014665.1			
70	*P. brasiliense*	NAK 241	*S. tuberosum*	unknown	unknown				GCA_016949885.1			
71	*P. brasiliense*	NAK 243	*S. tuberosum*	unknown	unknown				GCA_016949835.1			
72	*P. brasiliense*	NAK 245	*S. tuberosum*	unknown	unknown				GCA_016949855.1			
73	*P. brasiliense*	NAK 247	*S. tuberosum*	unknown	unknown				GCA_016949875.1			
74	*P. brasiliense*	NAK 250	*S. tuberosum*	unknown	unknown				GCA_016949775.1			
75	*P. brasiliense*	NAK 252	*S. tuberosum*	unknown	unknown				GCA_016949735.1			
76	*P. brasiliense*	NAK 254	*S. tuberosum*	unknown	unknown				GCA_016949705.1			
77	*P. brasiliense*	NAK 384	insect	unknown	unknown				GCA_016949355.1			
78	*P. brasiliense*	NAK 385	insect	unknown	unknown				GCA_016949345.1			
79	*P. brasiliense*	NAK 396	hyacint	unknown	unknown				GCA_016949295.1			
80	*P. brasiliense*	NAK 397	gyacint	unknown	unknown				GCA_016949315.1			
81	*P. brasiliense*	NAK 430	insect	Netherlands	2018				GCA_016949235.1			
82	*P. brasiliense*	NAK 432	insect	Netherlands	2018				GCA_016949145.1			
83	*P. brasiliense*	NAK 433	insect	Netherlands	2018				GCA_016949215.1			
84	*P. brasiliense*	NAK 434	insect	Netherlands	2018				GCA_016949135.1			
85	*P. brasiliense*	NAK 435	insect	Netherlands	2018				GCA_016949195.1			
86	*P. brasiliense*	NAK 466	water	Netherlands	2017				GCA_016949155.1			
87	*P. brasiliense*	NAK 468	water	Netherlands	2017				GCA_016949055.1			
88	*P. brasiliense*	NAK 470	water	Netherlands	2017				GCA_016949095.1			
89	*P. brasiliense*	NAK 431	insect	Netherlands	2018				GCA_016949275.1			
90	*P. brasiliense*	NZEC1	*S. tuberosum*	New Zealand	2012	JQ820119	JQ820118	JQ820117	JQ820116	JQ820115	JQ820114	JQ820120
91	*P. brasiliense*	PcbHPI01	cucumber	South Africa	2012				GCA_001429565.2			
92	*P. brasiliense*	pcbm-1	potato	Japan	2017	LC145715	LC145716	LC145717	LC145718	LC145719	LC145720	LC145721
93	*P. brasiliense*	pcbm-2	potato	Japan	2017	LC145722	LC145723	LC145724	LC145725	LC145726	LC145727	LC145728
94	*P. brasiliense*	pcbm-3	potato	Japan	2017	LC145729	LC145730	LC145731	LC145732	LC145733	LC145734	LC145735
95	*P. brasiliense*	S2	*B. rapa* subsp. *pekinensis*	China	2014				GCA_000808375.1			
96	*P. brasiliense*	SR10	squash	U. S. A.	2019				GCA_020423105.1			
97	*P. brasiliense*	SR11	pumpkin	U. S. A.	2019				GCA_020295425.1			
98	*P. brasiliense*	SR5	pepper	U. S. A.	2019				GCA_020295605.1			
99	*P. brasiliense*	SR6	squash	U. S. A.	2019				GCA_020296625.1			
100	*P. brasiliense*	SR7	pumpkin	U. S. A.	2019				GCA_020297385.1			
101	*P. brasiliense*	SR8	squash	U. S. A.	2019				GCA_020295505.1			
102	*P. brasiliense*	SX309	cucumber	China	2015				GCA_002068115.1			
103	*P. brasiliense*	TS20HJ1	*Zingiber officinale*	China	2020				GCA_020683105.1			
104	*P. brasiliense*	Y21	*B. rapa* subsp. *chinensis*	China	2013				GCA_000808235.1			
105	*P. brasiliense*	Y29	*B. rapa* subsp. *pekinensis*	China	2013				GCA_000808245.1			
106	*P. brasiliense*	Y3	*B. rapa* subsp. *chinensis*	China	2013				GCA_000808315.1			
107	*P. brasiliense*	Y31	*B. rapa* subsp. *pekinensis*	China	2013				GCA_000808295.1			
108	*P. brasiliense*	Y46	*B. rapa* subsp. *chinensis*	China	2013				GCA_000808255.1			
109	*P. brasiliense*	Y49	*B. rapa* subsp. *chinensis*	China	2013				GCA_000808115.1			
110	*P. brasiliense*	Y52	*B. rapa* subsp. *chinensis*	China	2013				GCA_000808385.1			
111	*P. brasiliense*	Y60	*B. rapa* subsp. *chinensis*	China	2013				GCA_000808455.1			
112	*P. brasiliense*	Y62	*B. rapa* subsp. *chinensis*	China	2013				GCA_000808145.1			
113	*P. brasiliense*	Y64	*B. rapa* subsp. *chinensis*	China	2013				GCA_000808195.1			
114	*P. brasiliense*	Y65	*B. rapa* subsp. *pekinensis*	China	2013				GCA_000808335.1			
115	*P. brasiliense*	ZLMLSHJ5	*S. tuberosum*	China	2018				GCA_016864975.1			
116	*P. carotovorum*	DSM 30168^{T}	*S. tuberosum*	Denmark	unknown				GCA_900129615.1			
117	*P. colocasium*	LJ1^{T}	potato	China	unknown				GCA_020181655.1			
118	*P. fontis*	M022^{T}	waterfall	Malaysia	2013				GCA_000803215.1			
119	*P. jejuense*	13-115^{T}	*Cucumis sativus*	South Korea	2022				GCA_026800125.1			
120	*P. odoriferum*	NCPPB 3839^{T}	*Cichorium intybus*	France	1978				GCA_000754765.1			
121	*P. parmentieri*	CFIA1002	potato	Canada	2007				GCA_000632375.1			
122	*P. parmentieri*	Ec-009	*S. tuberosum*	New Zealand	2005				GCA_023508125.1			
123	*P. parmentieri*	Ec-010	*S. tuberosum*	New Zealand	2005				GCA_023508065.1			
124	*P. parmentieri*	HC	potato	South Korea	2016				GCA_009931535.1			
125	*P. parmentieri*	IFB5408	*S. tuberosum*	Poland	2013				GCA_003628575.1			
126	*P. parmentieri*	IFB5427	*S. tuberosum*	Poland	2013				GCA_003992745.1			
127	*P. parmentieri*	IFB5432	*S. tuberosum*	Poland	2013				GCA_003628635.1			
128	*P. parmentieri*	IFB5441	*S. tuberosum*	Poland	2013				GCA_003628655.1			
129	*P. parmentieri*	IFB5485	*S. tuberosum*	Belgium	2012				GCA_003628595.1			
130	*P. parmentieri*	IFB5486	*S. tuberosum*	Belgium	2012				GCA_003628675.1			
131	*P. parmentieri*	IFB5597	*S. tuberosum*	Poland	2014				GCA_003628025.1			
132	*P. parmentieri*	IFB5604	*S. tuberosum*	Poland	2014				GCA_003628695.1			
133	*P. parmentieri*	IFB5605	*S. tuberosum*	Poland	2014				GCA_003628715.1			
134	*P. parmentieri*	IFB5619	*S. tuberosum*	Poland	2014				GCA_003628735.1			
135	*P. parmentieri*	IFB5623	*S. tuberosum*	Poland	2014				GCA_003628615.1			
136	*P. parmentieri*	IFB5626	*S. tuberosum*	Poland	2014				GCA_003628015.1			
137	*P. parmentieri*	IPO 1955	unknown	unknown	unknown				GCA_016950275.1			
138	*P. parmentieri*	NY1532B	potato	U. S. A.	2016				GCA_016107185.1			
139	*P. parmentieri*	NY1533B	potato	U. S. A.	2016				GCA_016107195.1			
140	*P. parmentieri*	NY1540A	potato	U. S. A.	2016				GCA_016107165.1			
141	*P. parmentieri*	NY1548A	potato	U. S. A.	2016				GCA_016107125.1			
142	*P. parmentieri*	NY1584A	potato	U. S. A.	2016				GCA_016107135.1			
143	*P. parmentieri*	NY1585A	potato	U. S. A.	2016				GCA_016107095.1			
144	*P. parmentieri*	NY1587A	potato	U. S. A.	2016				GCA_016107085.1			
145	*P. parmentieri*	NY1588A	potato	U. S. A.	2016				GCA_016107065.1			
146	*P. parmentieri*	NY1712A	potato	U. S. A.	2017				GCA_016107025.1			
147	*P. parmentieri*	NY1722A	potato	U. S. A.	2017				GCA_016107035.1			
148	*P. parmentieri*	PB20	sewage water	Russia	2014				GCA_002920045.2			
149	*P. parmentieri*	QK-5	*S. tuberosum*	China	2019				GCA_016864935.1			
150	*P. parmentieri*	RNS08-42-1A^{T}	potato	France	2008				GCA_001742145.1			
151	*P. parmentieri*	SCC3193	*S. tuberosum*	Finland	2012				GCA_000260925.1			
152	*P. parmentieri*	SS90	potato	Pakistan	2017				GCA_003121785.1			
153	*P. parmentieri*	WC19161	*S. tuberosum*	China	2019				GCA_016126895.1			
154	*P. parmentieri*	WPP163	unknown	unknown	unknown				GCA_000024645.1			
155	*P. peruviense*	IFB5232^{T}	potato	U. S. A.	1979				GCA_002847345.1			
156	*P. polaris*	NIBIO1006^{T}	*S. tuberosum*	Norway	2010				GCA_002307355.1			
157	*P. polonicum*	DPMP315^{T}	ground water from potato field	Poland	2016				GCA_005497185.1			
158	*P. punjabense*	SS95^{T}	*S. tuberosum*	Pakistan	2017				GCA_012427845.1			
159	*P. quasiaquaticum*	A477-S1-J17^{T}	fresh water from irrigation canal	France	2017				GCA_014946775.2			
160	*P. versatile*	CFBP 6051^{T}	*S. tuberosum*	Netherlands	2001				GCA_004296685.1			
161	*P. wasabiae*	BNS2-2	potato	Japan	2018	LC258444	LC258452	LC258460	LC258468	LC258476	LC258484	LC258492
162	*P. wasabiae*	CFBP 3304^{T}	*Eutrema japonicum*	Japan	1987				GCA_001742185.1			
163	*P. wasabiae*	ECC2	potato	Japan	2018	LC258445	LC258453	LC258461	LC258469	LC258477	LC258485	LC258493
164	*P. wasabiae*	ECC3	potato	Japan	2018	LC258446	LC258454	LC258462	LC258470	LC258478	LC258486	LC258494
165	*P. wasabiae*	HAFL01	potato	Switzerland	2013	KP027712	KM017545	KP027713	KM017537	KP027714	KP027715	KP027716
166	*P. wasabiae*	MAFF 301620	potato	Japan	2018	LC258451	LC258459	LC258467	LC258475	LC258483	LC258491	LC258499
167	*P. wasabiae*	NACAB192	*Brassica oleracea* var. *capitata*	Japan	2019	LC597924	LC597971	LC597997	LC598023	LC598049	LC598075	LC598080
168	*P. wasabiae*	NAONI191	*Allium cepa*	Japan	2019	LC597925	LC597972	LC597998	LC598024	LC598050	LC598076	LC598099
169	*P. wasabiae*	NAPoBL202	*S. tuberosum*	Japan	2021	LC597939	LC597960	LC597986	LC598012	LC598038	LC598064	LC598092
170	*P. wasabiae*	NAPoSR191	*S. tuberosum*	Japan	2021	LC597946	LC597967	LC597993	LC598019	LC598045	LC598071	LC598102
171	*P. wasabiae*	NASOIL208	soil	Japan	2020	LC597935	LC597956	LC597982	LC598008	LC598034	LC598060	LC598088
172	*P. wasabiae*	TNR271000	*S. tuberosum*	Japan	2018	LC258450	LC258458	LC258466	LC258474	LC258482	LC258490	LC258498
173	*P. wasabiae*	u15571a	*S. tuberosum*	Japan	2018	LC258449	LC258457	LC258465	LC258473	LC258481	LC258489	LC258497
174	*P. zantedeschiae*	9M^{T}	*Zantedeschia aethiopica*	Poland	2005				GCA_004137795.1			

1) The sources followed the description of the submitter that registered the sequence.