# 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.

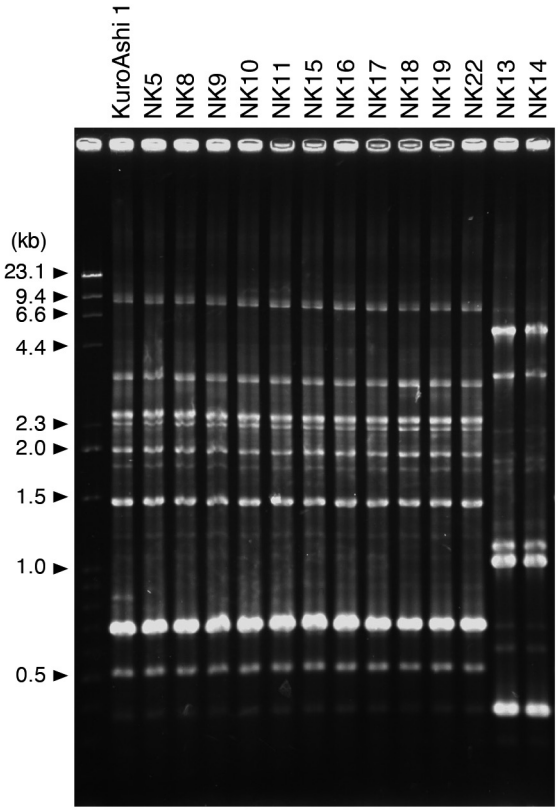


**Fig. 1.** Comparison of rep–PCR profile fingerprint patterns of genomic DNA from Nagasaki strains of *P. brasiliense* (KuroAshi 1 to NK22) and *P. parmentieri* (NK13 and NK14). ERIC1R and ERIC2 were used as a primer pair.

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 |

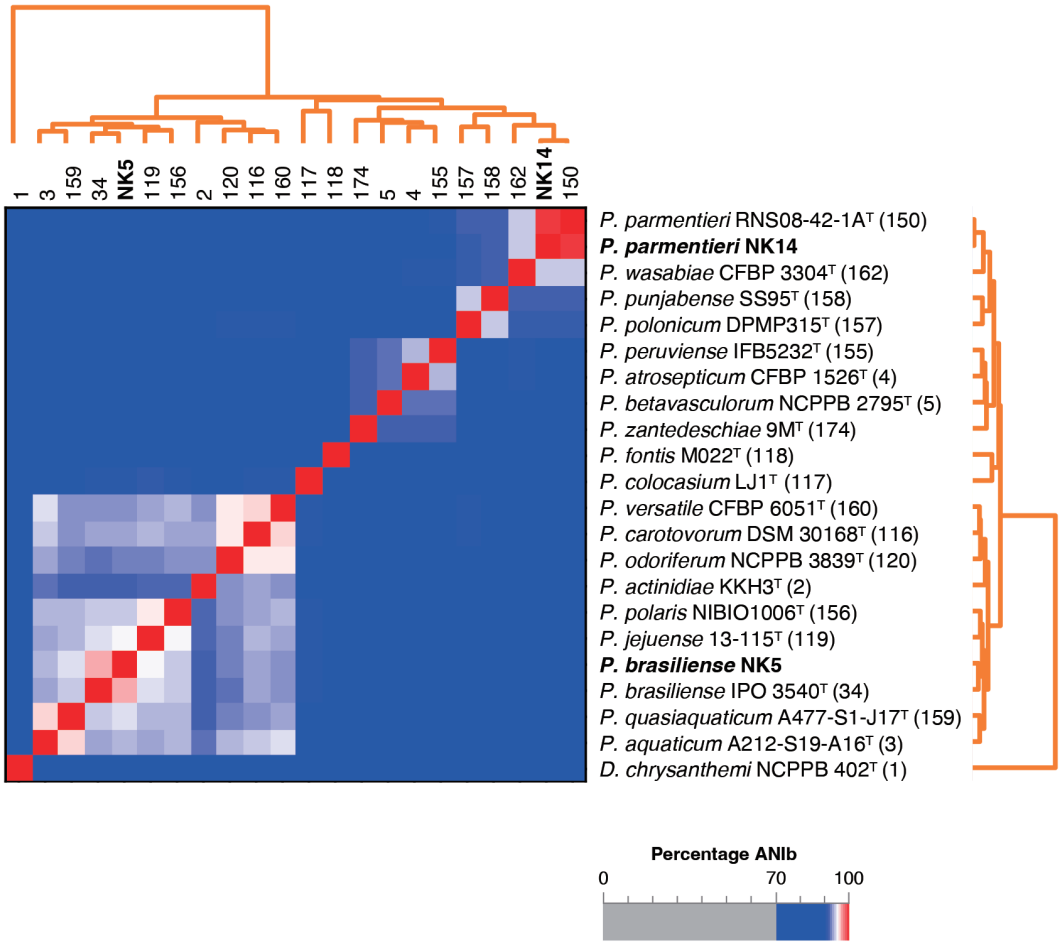


**Fig. 2.** Average nucleotide identity (ANI) between *P. brasiliense* NK5, *P. parmentieri* NK14, type strains of *Pectobacterium* species, and *Dickeya chrysanthemi*, calculated using the program Pyani. ANIb means identity determined using BLAST+. The dendrogram directly reflects the degree of identity 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-

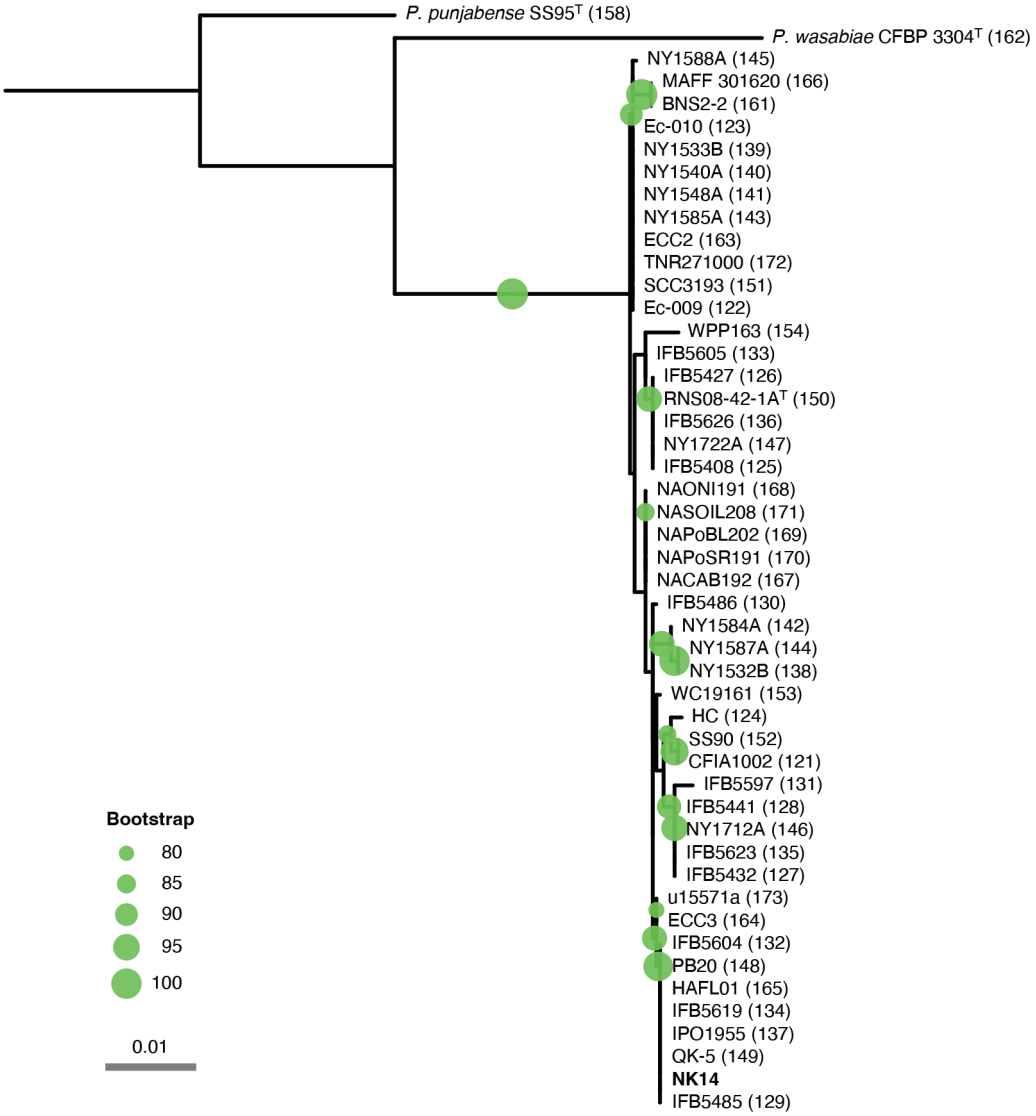


**Fig. 3.** Maximum–likelihood phylogenetic tree constructed using multilocus sequence analysis based on seven concatenated housekeeping genes (*acnA–gapA–icdA–mdh–mtlD–pgi–proA*) of *P. parmentieri* strains. Circles at the nodes indicate the bootstrap values over 80% (1,000 replications). *P. punjabense* 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.

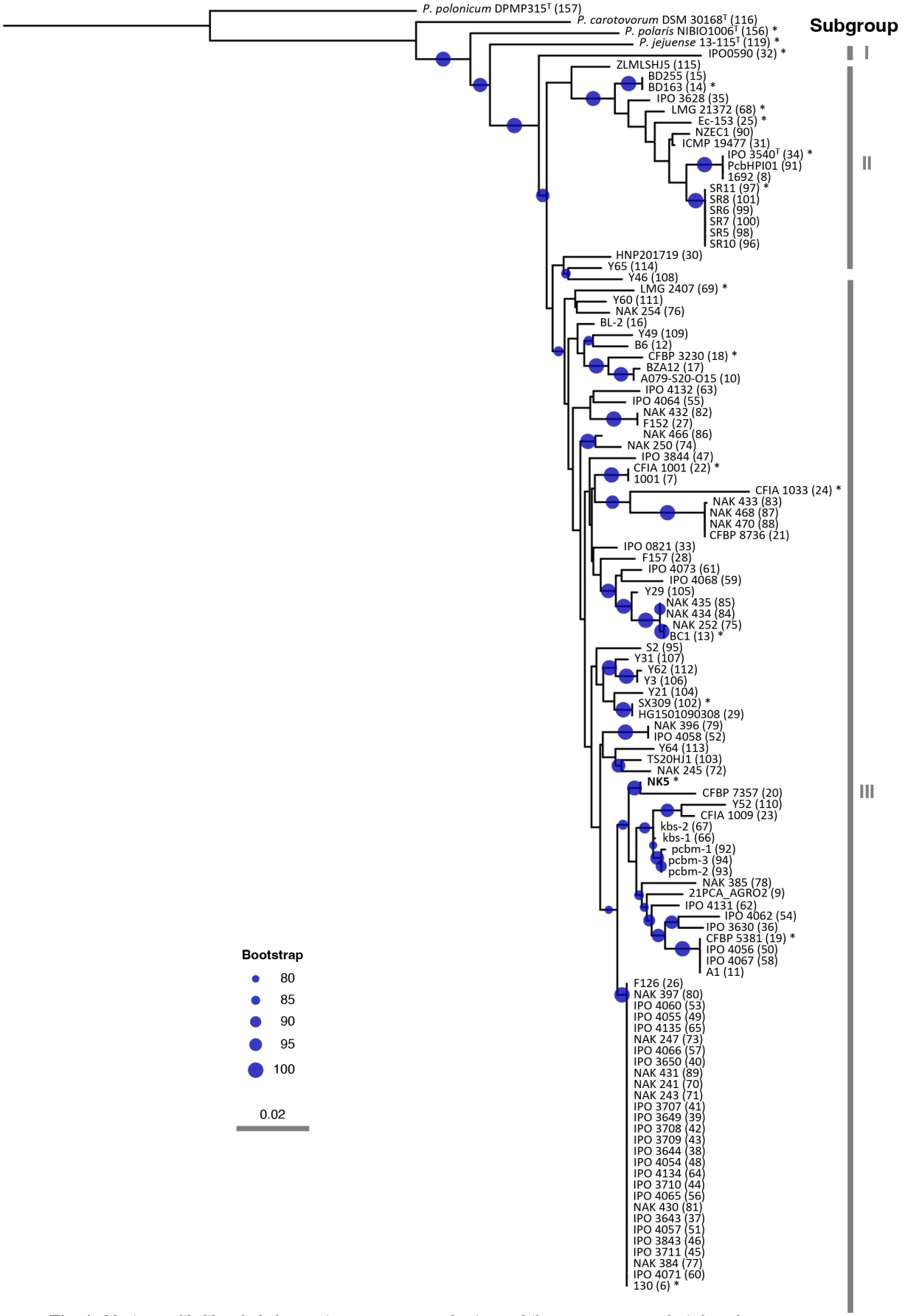


**Fig. 4.** Maximum–likelihood phylogenetic tree constructed using multilocus sequence analysis based on seven concatenated housekeeping genes (*acnA–gapA–icdA–mdh–mtlD–pgi–proA*) of *P. brasiliense* strains. Circles at the nodes indicate the bootstrap values over 80% (1,000 replications). *P. polonicum* 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.

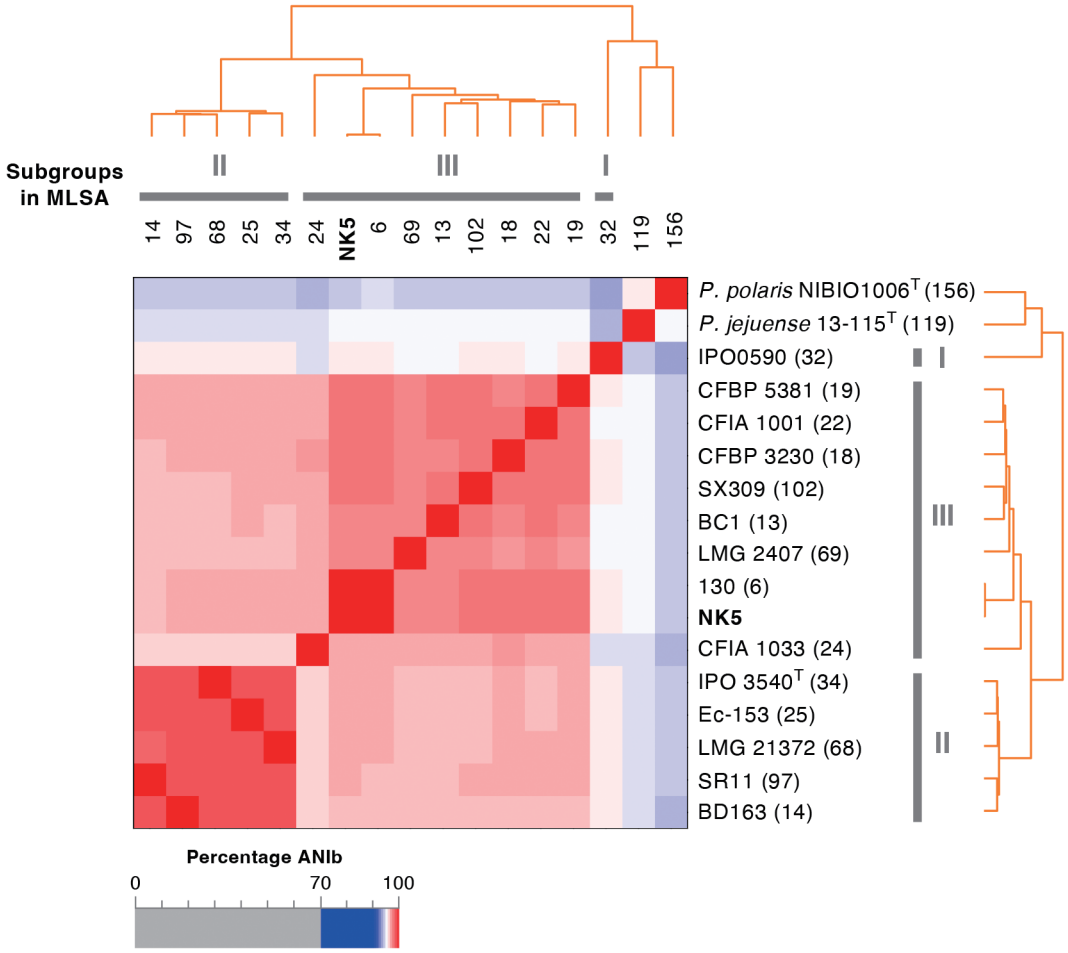


**Fig. 5.** Average nucleotide identity (ANI) between *P. brasiliense* strains, calculated using the program Pyani. ANIb means identity determined using BLAST+. *P. polaris* NIBIO1006$^{T}$, and *P. jejuense* 13–115$^{T}$ were used as outgroups. The dendrogram directly reflects the degree of identity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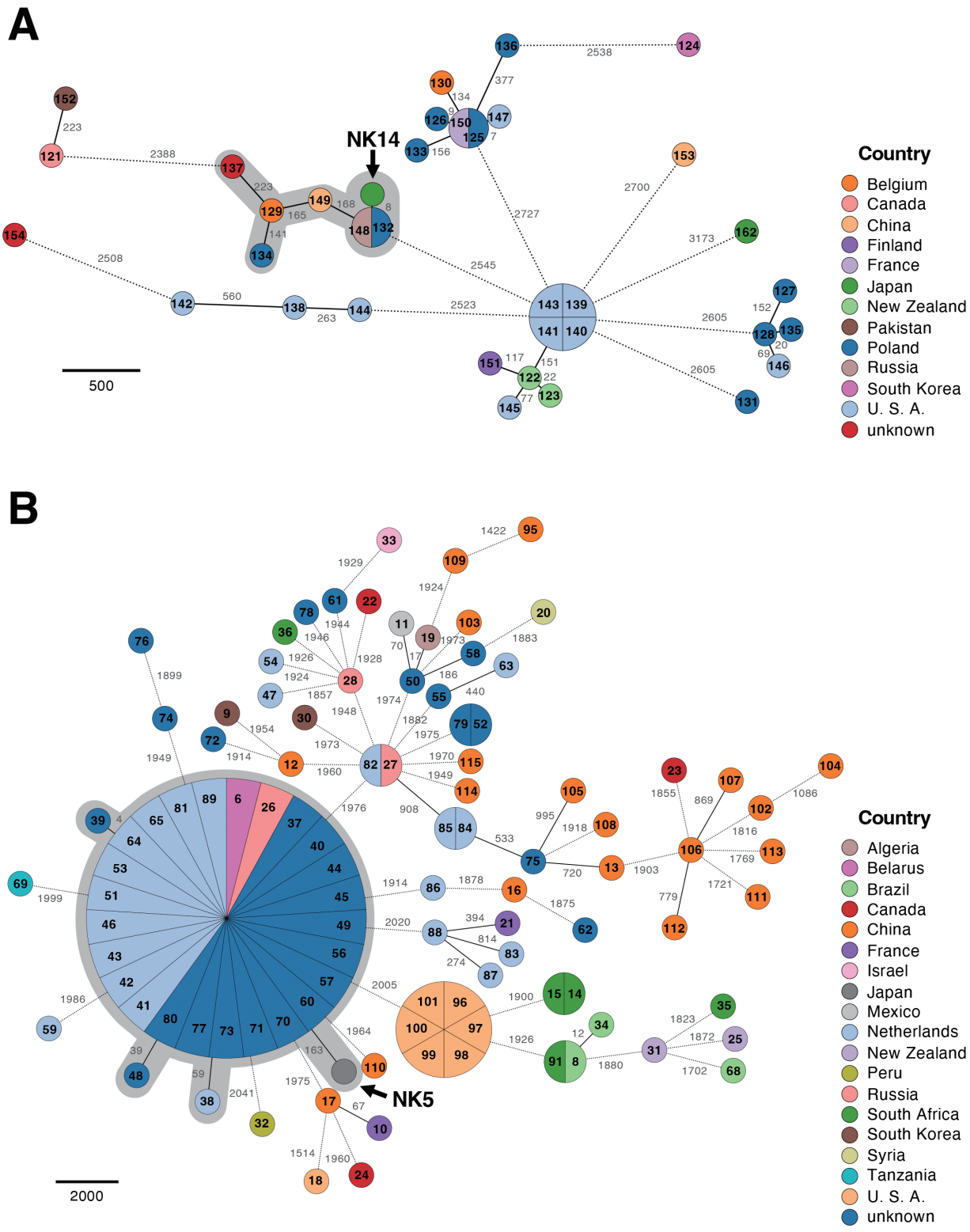


**Fig. 6.** Minimum spanning tree based on core genome multilocus sequence typing (cgMLST) profiles of 36 *P. parmentieri* (A) and 103 *P. brasiliense* (B) strains. The trees were created using 3,187 loci (A) and 2,048 loci (B) The numbers at the nodes indicate strains, and the details are listed in 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.