Affinibrenneria salicis gen. nov. sp. nov. isolated from Salix matsudana bark canker

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Research Article

Abstract

L3-3HA[T], a Gram-negative-staining, facultatively anaerobic, motile bacterial strain, was isolated from the symptomatic bark of *Salix matsudana* canker in China. 16S rRNA gene analysis revealed that the novel strain shares the highest sequence similarity with *Brenneria goodwinii* FRB141[T] (95.5 %). In phylogenetic trees based on four housekeeping genes (*gyr*B, *rpo*B, *atp*D and *inf*B) and the 16S rRNA gene sequence, the novel strain formed a separate branch from the five genera of the family *Pectobacteriaceae* (*Lonsdalea*, *Brenneria*, *Dickeya*, *Pectobacterium* and *Sodalis*), suggesting that the novel strain should belong to a novel species of a novel genus within the family *Pectobacteriaceae*. The result was also supported by phylogenomics, amino acid identity and average nucleotide identity. The major fatty acids were $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ cyclo, and $C_{19:0}$ cyclo $ω8c$. Genome analysis showed that the novel strain has a large genome (5.89 Mb) with 5,052 coding genes, including 181 virulence genes by searching the pathogen-host interactions database (PHI-base), indicating that the novel strain is a potential pathogen of plants and animals. Based on phenotypic and genotypic characteristics, the L3-3HA[T] strain represents a novel species of a novel genus in the *Pectobacteriaceae* family, for which the name *Affinibrenneria salicis* gen nov. sp. nov. is proposed. The strain type is L3-3HA[T] (= CFCC 15588[T] = LMG 31209[T]).

Introduction

Adeolu et al. proposed a novel order, *Enterobacterales* ord. nov., based on phylogenomics and taxonomy that divided the original family of *Enterobacteriaceae* into seven families, *Enterobacteriaceae*, *Erwiniaceae*, *Pectobacteriaceae*, *Yersiniaceae*, *Hafniaceae*, *Morganellaceae*, and *Budviciaceae* (Adeolu et al. 2016). The family *Pectobacteriaceae* contains five genera, *Pectobacterium* (Waldee 1945), *Brenneria* (Hauben et al. 1998), *Dickeya* (Samson et al. 2005), *Lonsdalea* (Brady et al. 2012), and *Sodalis* (Dale et al. 1999). At the time of writing this manuscript, *Pectobacteriaceae* comprises five genera, 36 species, and 15 subspecies with validly published names (https://lpsn.dsmz.de/family/pectobacteriaceae) (Parte 2018).

A novel strain, L3-3HA[T], was isolated from the symptomatic bark of *Salix matsudana* canker. The novel strain belongs to the family *Pectobacteriaceae* based on 16S rRNA gene sequence analysis. In the family *Pectobacteriaceae*, many species are associated with plant disease, such as *Pectobacterium carotovorum*, *Brenneria salicis*, and *Lonsdalea populi*. To explore the possible function of the novel strain, genome comparison among the novel and five reference strains was performed, and virulence genes and carbohydrate-active enzymes of the novel strain were predicted by searching the carbohydrate-active enzyme database (CAZy) and pathogen-host interactions database (PHI-base). To confirm the taxonomy status of the novel strain, polyphasic approaches, including 16S rRNA gene sequence analysis, multilocus sequence analysis (MLSA), phylogenomics, average nucleotide identity (ANI), amino acid identity (AAI), digital DNA-DNA hybridization (dDDH) and phenotypic characterization, were performed in the present study.

Materials And Methods

Isolation and cultivation of the novel strain

The novel strain was isolated from the bark samples of *Salix matsudana* canker as described by Li et al. (2017a). Samples were sterilized in 4 % (v/v) NaClO solution for 3 min and immersed in 75 % alcohol for 30 s. After rinsing three times with sterile water, the sterilized bark samples were cut into 2×2-mm pieces and soaked in sterile water for 30 min. The solutions were diluted with sterile water to a series of $1:10^{-1}$ to 10^{-4} and spread on tryptic soy agar plates (TSA; Difco). Single colonies were obtained after incubating at 30 °C for 48 h. The strain was deposited at the Laboratorium voor Microbiologie, Universiteit Gent (LMG 31209[T]) and China Forestry Culture Collection Center (CFCC 15588[T]).

Sequencing of the 16S rRNA gene and phylogenetic analysis

16S rRNA gene sequences were determined using bacterial universal primers 8F/1525R (5¢-AGAGTTTGATCCTGGCTCAG-3¢ and 5¢-AAGGAGGTGATCCAGCC-3¢) as described by Lane (1991) and Baker et al. (2003). The EzBioCloud identify service (Yoon et al. 2017) was used to analyze 16S rRNA gene sequence similarities with validly published species.

Phylogenetic analysis of the 16S rRNA gene was performed according to Li et al. (2016). 35 almost complete (1344 bp) 16S rRNA gene sequences were aligned and overhangs trimmed using CLUSTAL W in MEGA 7.0 software (Kumar et al. 2016). The trees were reconstructed using the neighbor-joining and maximum likelihood methods and were evaluated with 1000 replicates.

MLSA based on four housekeeping gene partial sequences (*gyr*B, 745 bp; *rpo*B, 637 bp; *atp*D, 642 bp; and *inf*B, 615 bp) was performed on the novel strain and reference type strains (Brady et al. 2013). The reference strain sequences of genera *Lonsdalea*, *Brenneria*, *Dickeya*, *Pectobacterium*, *Sodalis*, *Izhakiella*, *Pantoea* and *Mixta* were downloaded from GenBank (Table S1), and the sequences of the novel strain were retrieved from its genome sequence data. Phylogenetic trees based on the maximum likelihood and neighbor-joining method were reconstructed by Mega 7.0. The bootstrap method with 1,000 replicates was performed.

Whole-genome sequencing

Genome sequencing of the novel strain was performed using Illumina Miseq sequencing strategies as described by Li et al. (2016). The library was constructed with 300- to 500-bp DNA fragmentation using the TruSeq™ Sample Prep Kit. Sequence assembly was performed using SOAPdenovo v2.04. Coding sequences were predicted using Glimmer 3.02 software. Functional annotation of the protein sequences was performed against the nonredundant GenBank database using BLASTp.

An almost full-length 16S rRNA gene sequence obtained by conventional Sanger sequencing was compared with the sequence extracted from whole-genome assembly. Two sequences (1,409 bp and 276 bp) from the genome sequence shared 100 and 99.26 % sequence similarity with the 16S rRNA gene sequence obtained by conventional Sanger sequencing. However, the 276-bp sequence may not be a 16S rRNA gene sequence, as confirmed by two methods. First, the DNA used for genome sequencing was PCR (polymerase chain reaction) amplified and sequenced by conventional Sanger sequencing, and no doublet and impure peaks were found. Second, the K-mer and GC-depth figures were regular and normal in the genome sequence analysis (Fig. S3 and S4).

As recommended by Chun et al. (2018), phylogenomics, DNA-DNA hybridization and ANIb analysis were used for the taxonomy of the novel strain. Phylogenomics was performed on the novel and its closely related reference species. The genomic coding sequences were clustered using CD-HIT v4.6.1 (c 0.5 -n 3 -p 1 -T 4 -g 1 -d 0 -s 0.7 -aL 0.7 -aS 0.7) (Fu et al. 2012). Six hundred eighteen single-copy genes were selected using Perl scripts and aligned using Muscle 3.8.31 (Edgar 2004). A phylogenetic ML tree was constructed based on the resulting multiple alignments using PhyML V3.3 (Guindon et al. 2010) (parameters: phyml -i temp. phy -d aa -b -4 -m LG -a e -c 4 -t e >ml.log). The level of support for internal branches was assessed using the Bayesian test in PhyML.

Average nucleotide identity (ANI) is a similarity measure between two genome sequences that can be used to replace DNA-DNA hybridization (Richter et al. 2009; Goris et al. 2007). The OrthoANI values between the L3-3HA^T strain and five reference type strains of the five genera of the family *Pectobacteriaceae* and other reference strains were calculated using the ANI calculator tool of the EzBioCloud website (https://www.ezbiocloud.net/tools/ani).

dDDH was conducted using the genome-to-genome distance calculator (GGDC) and the free web service at http://ggdc.dsmz.de/ based on genome sequences (Meier-Kolthoff et al. 2013). Amino acid identity (AAI) analysis among the novel and reference strains of genera *Lonsdalea*, *Brenneria*, *Dickeya*, *Pectobacterium*, and *Sodalis* was performed as described by Konstantinidis and Tiedje (2005).

Genome comparison among the novel strain and *D. chrysanthemi* NCPPB 402^T, *S. praecaptivus* HS^T, *P. carotovorum* LMG 2404^T, *B. salicis* DSM 30166^T and *L. quercina* ATCC 29281^T was performed. The genomic coding sequences were clustered using CD-HIT v4.6.1 (Fu et al. 2012).

To understand the potential function of the novel strain, virulence genes and carbohydrate-active enzymes were predicted by searching the Carbohydrate-Active enZYmes Database (CAZy) (Lombard et al. 2014) and Pathogen-Host Interactions database (PHI-base) (Urban et al. 2020) using DIAMOND (Buchfink et al. 2015) based on the genome amino acid sequence of the novel strain (evalue≤1e-5, identity≥50 %, coverage ≥40 %), respectively. PHI-base is a web-accessible database that catalogs experimentally verified pathogenicity, virulence and effector genes from fungal, oomycete and bacterial pathogens (Urban et al. 2020). CAZy is a specialist database dedicated to the display and analysis of genomic, structural and biochemical information on carbohydrate-active enzymes (CAZymes) (Lombard et al. 2014).

Morphological, physiological and biochemical analyses

Colony morphology, motility and growth conditions (temperature and NaCl tolerance) experiments were performed according to the description of Li et al. (2017b). Cell morphology was examined by transmission electron microscopy with cells grown on TSA for 24 h at 30 °C. The motility of the novel isolate was examined by observing cells developed on wet mounts using a phase-contrast microscope (TMS-F; Nikon). The novel strain was inoculated in tryptic soy broth (TSB; Difco) and incubated at different temperatures (4, 10, 30, 37 and 50 °C) as described by Li et al. (2015), different pH values (4.0−11.0, at intervals of 1.0) and various tolerances to NaCl (0 %−10 % [w/v], at intervals of 1 %). As reported by Gomori (1955), the pH values were adjusted using the following buffers: citric acid/sodium citrate (pH 4.0−5.5), Na_2HPO_4/NaH_2PO_4 (pH

6.0–8.0), $Na_2CO_3/NaHCO_3$ (pH 8.0–9.5) and $Na_2HPO_4/NaOH$ (pH 10.0–11.0). Anaerobic growth was examined after the novel strain was incubated on TSA plates at 30 °C for 1 week in anaerobic jars filled with N_2/CO_2 (80: 20, v/v) (Gerhardt et al. 1981). Catalase and oxidase activities were measured as recommended by Smibert and Krieg (1994). The oxidase activity of the novel strain was examined by determining whether a color change to purple occurred after using 1 % (w/v) tetramethyl-p-phenylenediamine. Gram staining was tested according to the description of Jenkins (2003). Other biochemical and physiological tests were performed using API 20E, API 20NE, and API 50CHB/E kits (bioMérieux) and the Biolog GN_2 MicroPlate according to the manufacturer's instructions. The strains tested in these analyses were cultured on TSA at 30 °C for 24 h and then were harvested during the exponential phase.

Chemotaxonomic analyses

The strains were cultured on TSA at 30 °C for 24 h and then were harvested during the exponential phase. Fatty acids were analyzed using the Microbial Identification System, Sherlock version 6.0 (Sasser 1990).

Pathogenicity determination

4 year-old poplar trees were chosen for inoculation at the poplar nursery in Puyang City, Henan Province. The strain was cultured on nutrient agar plates at 30 °C for 24 hours, then were washed and centrifuged to pellet the cells. The collected cells were diluted in SDW to 10^8 CFU/ml. After the bark was surface sterilized with 75 % ethanol, 1 cm-long cross-wounds were cut into the bark with a sterilized knife. A total of 100 µl of bacterial suspension was delivered to the inoculation point. Negative control plants were inoculated with sterile distilled water. The results of pathogenicity tests were recorded 7, 14, and 21 days after inoculation.

Results And Discussion

Morphological and phenotypic characteristics

Transmission electron microscopy showed that the cells were short rods or globose, with peritrichous flagella and 0.5–1.0 × 0.8–2.5 µm in size (Fig. S5). The colonies were white, circular, and approximately 1–2 mm in diameter after 48 h of growth at 30 °C on TSA. The strain is a motile, facultative anaerobic bacterium with positive catalase and negative oxidase activities. The detailed differentiated biochemical and physiological characteristics of the novel strain and close genera are listed in Tables 1.

Phylogenetic analysis

The 16S rRNA gene sequencing of novel strain L3-3HA[T] shares the highest sequence similarity with *Brenneriagoodwinii* FRB141[T] (95.5 %) and lower sequence similarity values (95.4 %) with other validly published species. In ML and NJ phylogenetic trees based on 16S rRNA gene sequencing (Fig. 1, Fig. S1), the novel strain formed an dependent lineage, separated from those of five *Pectobacteriaceae* genera and other family reference species of *Izhakiella*, *Pantoea* and *Mixta*. And in the ML and NJ phylogenetic trees based on MLSA data (Fig. 2, Fig. S2), the novel strain clustered into a distinct branch from other genera in the present

study, suggesting that the novel strain should represent a novel species in a novel genus within the *Pectobacteriaceae* family.

Genomic characteristics

The sequencing depth of coverage was 130 ´. The genome of strain L3-3HA^T is 5.89 Mb in size across 47 contigs (N50 is 322317 bp), including 5,052 coding genes, 10 rRNAs, 74 tRNAs, 9 other RNAs and 197 pseudogenes. The DNA G+C content is 57.1 %. Also, the strain L3-3HA^T formed a distinct branch from the five genera within the *Pectobacteriaceae* family clades in the phylogenomic tree (Fig. 3), demonstrating that the novel strain represents a novel species in a novel genus within the family *Pectobacteriaceae*.

The novel strain showed 72.43-77.56 % ANI values with the reference strains of the family *Pectobacteriaceae* and *Erwiniaceae*, and the detailed data were shown in Table 2. These values were all lower than the proposed species boundary ANI cut-off (95–96 %) (Fu et al. 2012). The dDDH values between L3-3HA^T strain and the same reference strains used in ANI analysis were 13 %–22 %, lower than the 70 % cut-off for species determination (Table 2) (Wayne et al. 1987).

The AAI analysis results revealed that the five reference type strains of the genera *Lonsdalea*, *Brenneria*, *Dickeya*, *Pectobacterium*, *Sodalis* shared 64 %–81 % AAI values with each other. The novel strain showed 64.2 %–74.9 % AAI values with these five reference type strains (Table S3), lower than the AAI values among the five reference type strains. These results supported that the novel isolate represents a novel species in a novel genus in family *Pectobacteriaceae*.

Genome comparison shows that the novel strain (5.89 Mb) has large genome size , comprises 5,052 coding genes, 1,907, 1,617, 990, and 1,045 more genes than the reference strains *L. quercina* ATCC 29281^T, *B. salicis* DSM 30166^T, *P. carotovorum* LMG 2404^T and *D. chrysanthemi* NCPPB 402^T. *L. quercina* and *B. salicis* are pathogens of *Quercus* spp. and *Salix* spp., respectively (Brady et al. 2012; Sakamoto et al. 1999), and they have a small genome size (3.9–4.0 Mb) with a single host compared with *P. carotovorum* and *D. chrysanthemi*. The plant pathogens *P. carotovorum* and *D. chrysanthemi*, with a diverse host range (Toth et al. 2003), have a large genome (4.8 Mb in size), which is seemingly important to adopt diverse hosts for the bacterial pathogen. Thus, the novel strains may be a multihost bacterial plant pathogen.

In the present study, 181 virulence genes were found in the genome of the novel strain, including 15 "loss of pathogenicity" genes, 155 "reduced virulence" genes and 15 "increased virulence" genes. The results indicated that the novel strain is a potential pathogen of plants, although it is not a pathogen of the poplar tree. And in total, 145 carbohydrate-active enzymes were found from the genome sequence of the novel strain. Eleven enzymes were found to be associated with degradation, including 2 cellulases 8 chitinases and 1 pectin lyase, which are important enzymes for the pathogens of plants.

Chemotaxonomic characteristics

The major fatty acids detected in the novel strain were $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ cyclo and $C_{19:0}$ cyclo 8*c*. The percentages of the peak area for fatty acids of the novel and reference strains are listed in Table S2. The

amount of $C_{17:0}$ cyclo and $C_{19:0}$ cyclo ⍵8c can be used to distinguish the novel strain from the five reference strains used in the present study.

Pathogenicity test

The results of pathogenicity experiment showed that the novel strain did not show pathogenicity to poplar trees.

Taxonomic conclusion

In the trees based on the 16S rRNA gene and MLSA data, the strains of the genus *Brenneria* were clustered into two separate clades, indicating that the taxonomy of the *Brenneria* species may be polyphyletic. Although the *Brenneria* strains clustered together in the phylogenomic trees, the *Brenneria* species were still divided into two clades. Therefore, we speculated that *B. alni*, *B. corticis*, *B. goodwinnii*, *B. nigrifluens*, and *B. populi* may represent a novel genus within the family *Pectobacteriaceae.*

The 16S rRNA gene and MLSA analysis revealed that the novel strain belongs to a novel species in a novel genus within the family *Pectobacteriaceae,* which is also supported by AAI, fatty acid and phyologenomic analyses. Moreover, many physiological and biochemical characteristics can be used to distinguish the novel strain from close genera of the family *Pectobacteriaceae* (detailed in Table 2). Based on those data, the L3-3HA^T strain represents a novel species of a novel genus in the *Pectobacteriaceae* family, for which the name *Affinibrenneria salicis* gen nov. sp. nov. is proposed.

Description of *Affinibrenneria* gen. nov.

Affinibrenneria (Af.fi.ni.bren.ne'ri.a. L. masc. adj. *affinis* associated with, adjacent; N.L. fem. n. *Brenneria*, a bacterial genus; N.L. fem. n. *Affinibrenneria*, a genus associated with *Brenneria*).

Gram-negative, facultative anaerobic, motile, oxidase-negative, and catalase-positive. Growth occurs between 4 °C and 50 °C and at pH 6–8 and 0 %–5 % salinity. The cells are also positive for β-galactosidase activity and negative for the activities of urease and gelatinase and the production of H_2S and indole. Citrate is not utilized, and nitrates are not reduced to nitrites. The main fatty acids are $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ cyclo and $C_{19:0}$ cyclo ⍵8c. The DNA G+C content is 57.1 %, and the type species is *Affinibrenneria salicis*. *Affinibrenneria salicis* gen. nov. sp. nov. is a member of the family *Pectobacteriaceae*.

Description of *Affinibrenneria salicis* sp. nov.

Affinibrenneria salicis (sa'li.cis. L. gen. n. *salicis* of *Salix matsudana*)

The cells are Gram-negative, facultative anaerobic, motile, short rod or globose with peritrichous flagella. The colonies are circular, convex with complete margins. The cells show negative activities for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, and gelatinase and are negative for the production of H_2S and indole. The cells do not utilize citrate and do not reduce nitrates to nitrites. The cells are positive for acid production from d-arabinose, d-ribose, d-galactose, d-

glucose, d-fructose, d-mannose, methyl α-d-mannopyranoside, methyl α-d-glucopyranoside, n-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, d-cellobiose, d-maltose, d-lactose, d-melibiose, d-saccharose, d-trehalose, d-raffinose, gentiobiose, d-turanose, d-lyxose and d-arabitol (API 50CH). The cells are also positive for the utilization of α-cyclodextrin, dextrin, glycogen, Tween 80, n-acetyl-d-galactosamine, n-acetyl-d-glucosamine, adonitol, l-arabinose, d-arabitol, d-cellobiose, i-erythritol, d-fructose, l-fucose, d-galactose, gentiobiose, α-d-glucose, m-inositol, α-d-lactose, lactose, maltose, d-mannitol, d-mannose, d-melibiose, β-methyl d-glucoside, d-psicose, d-raffinose, l-rhamnose, d-sorbitol, sucrose, d-trehalose, turanose, xylitol, methyl pyruvate, mono-methyl succinate, cis-aconitic acid, formic acid, d-galacturonic acid, d-gluconic acid, d-glucosaminic acid, d-glucuronic acid, α-hydroxybutyric acid, α-keto butyric acid, α-keto glutaric acid, d,l-lactic acid, propionic acid, succinic acid, succinamic acid, l-asparagine, l-aspartic acid, l-glutamic acid, l-serine, inosine, uridine, thymidine, 2,3-butanediol, glycerol, glucose-1-phosphate and glucose-6-phosphate (Biolog GN$_2$). The main fatty acids are $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ cyclo and $C_{19:0}$ cyclo $\omega 8c$. The DNA G+C content is 57.1 %. And the type strain is L3-3HA^T (=CFCC 15588^T = LMG 31209^T) isolated from the bark of *Salix matsudana* canker. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and whole-genome sequences of L3-3HA^T are MN036523 and NZ VYKJ00000000, respectively.

Declarations

Compliance with ethical standards

Conflicts of interest The authors declare that there are no conflicts of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Table 1. Biochemical and physiological characteristics that distinguish the novel strain from five close genera of the family *Pectobacteriaceae*

Taxa: 1, *Affinibrenneria* gen. nov; 2, *Lonsdalea*; 3, *Brenneria*; 4, *Dickeya*; 5, *Pectobacterium*; 6, *Sodalis*. +, positive; -, negative; v, variable reaction; nd, not determined. [a]Except *B.paradisiaca*; [b]Except *B. salicis* and *B.quercina*; [c]Except *B.nigrifluens* and *B.populi*; [d]Except *D.dadantii* and *D.undicola*; [e]Except *D.dieffenbachiae*;; [f], Type species of the genus, *Pectobacterium carotovorum*, the data were from Brady (2012). [g], the data were from Dale (1999).*, column 2, data were from Li et al. 2017b; Brady et al. 2012; column 3, data were from Hauben et al. 1999; Li et al. 2019; Denman et al. 2012; Li et al. 2015; Brady et al. 2014; column 4, data were from Samson et al. 2005; Parkinson et al. 2014; Tian et al. 2016; Hugouvieux-Cotte-Pattat et al. 2019 and 2020; Wang et al. 2020; van der Wolf et al. 2014; Oulghazi et al. 2019; column 5, data were from Hauben et al. 1999; Gardan et al. 2003; Portier et al. 2019; Khayi et al. 2016; column 6, data were from Santos-Garcia et al. 2017.

Characteristic	1	2	3	4	5[f]	6[g]
β-Galactosidase	+	-	v	nd	+	-
Citrate utilization	-	+	-	nd	+	nd
H_2S production	-	-	-	nd	v	nd
Indole	-	-	-[a]	nd	-	-
Gelatinase	-	-	-	nd	+	-
Acid from:						
Amygdalin	+	-	v	+	+	nd
l-Arabinose	-	-	+[b]	+	+	-
Cellobiose	+	-	v	+	+	nd
Gentiobiose	+	-	v	-[d]	+	nd
Melibiose	+	-	v	+[e]	+	-
Methyl α-d-glucopyranoside	+	+	-[c]	-	-	-
l-Rhamnose	-	-	v	v	+	-
d-Xylose	-	-	v	+	+	-
G+C (%)*	57.1	54.5-55.6	50.1-56.2	53.1-59.5	50.4-56.1	44.5-57.5
Host*	*Salix matsudana*	Oak and *Populus × euramericana*	Oak, *Populus × euramericana*, *Juglans regia*, *Salix* spp, Alnus and Musa paradisiaca	Water, soft rot and wilt of various plants	Water and wide range of host plants, like *Actinidia chinensis*, *Solanum tuberosum*, *Zantedeschia aethiopica* and *Eutrema wasabi*	Insects and human wound

Table 2. Average nucleotide identity (ANI) and genome-to-genome distance calculator (GGDC) from the novel and reference strains of the family *Pectobacteriaceae* and *Erwiniaceae.*

DDBJ/EMBL/GenBank genome accession numbers: *Lonsdalea quercina* ATCC 29281[T], NZFNQS01000000; *Brenneria salicis* ATCC 15712[T], NZMJMA01000000; *Pectobacterium carotovorum* DSM 30168[T], NZFQWI01000025; *Sodalis praecaptivus* HS[T], CP006569; *Dickeya chrysanthemi* NCPPB 402[T], NZCM001974; *Mixta calida* DSM 22759[T], NZCP026378; *Pantoea agglomerans* C410P1[T], NZCP016889; *Pantoea dispersa* CCUG 25232[T], NZVXKA01000001; *Izhakiella capsodis* N6PO6[T], NZFOVC01000030.

| Strain	ANI	GGDC DDH1	GGDC DDH2	GGDC DDH3
Lonsdalea quercina ATCC 29281[T]	75.70	16.5	21.0	16.4
Brenneria salicis ATCC 15712[T]	76.45	17.1	21.3	16.9
Pectobacterium carotovorum DSM 30168[T]	75.92	15.9	20.8	15.9
Sodalis praecaptivus HS[T]	74.60	14.5	20.5	14.7
Dickeya chrysanthemi NCPPB 402[T]	75.93	15.8	21.0	15.9
Mixta calida DSM 22759[T]	77.56	15.6	20.5	15.6
Pantoea agglomerans C410P1[T]	73.91	14.1	20.4	14.4
Pantoea dispersa CCUG 25232[T]	74.75	14.8	20.2	14.9
Izhakiella capsodis N6PO6[T]	72.43	13.4	19.9	13.7

Figures
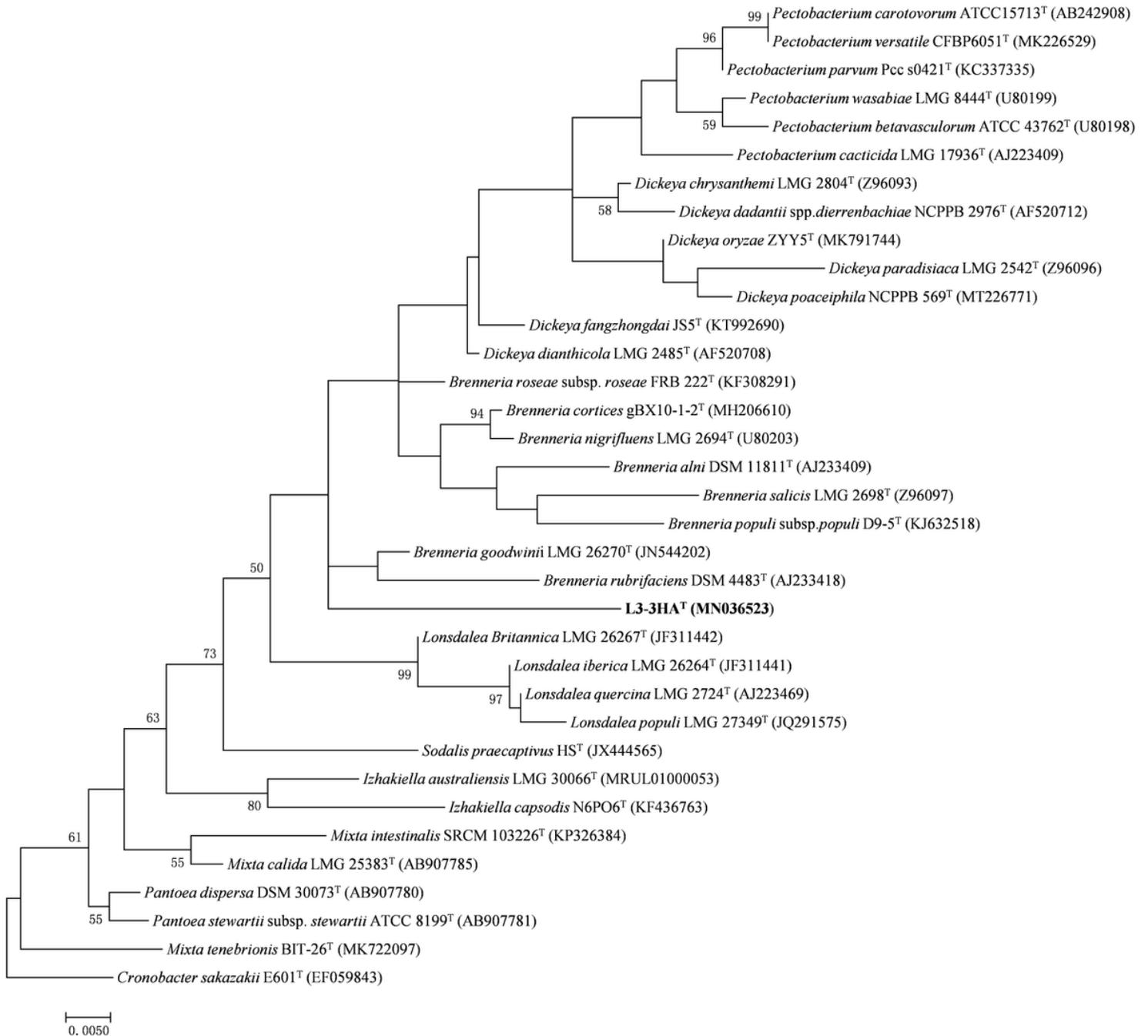
Figure 1

Maximum-likelihood tree based on 16S rRNA gene sequences showing the relationship between the novel species and closely related species. Bootstrap values after 1,000 replicates are expressed as percentages. Cronobacter sakazakii is included as an outgroup. Bar, 0.01 substitutions per site.
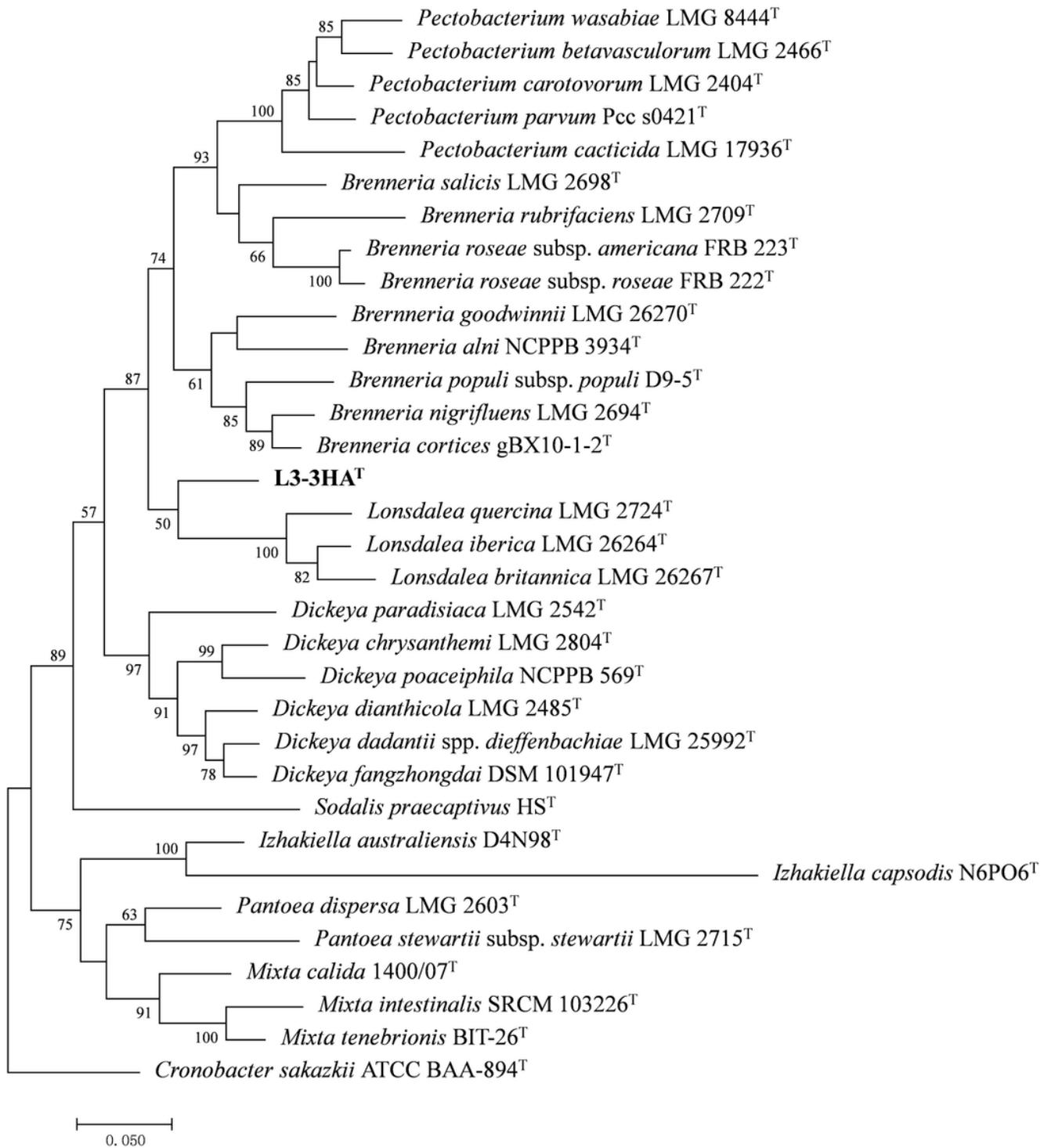
Figure 2

Maximum-likelihood tree based on concatenated partial gyrB, rpoB, atpD, infB gene sequences of the type strains in the genera Lonsdalea, Brenneria, Dickeya, Pectobacterium, Sodalis, Izhakiella, Pantoea and Mixta. Bootstrap values after 1,000 replicates are expressed as percentages. Cronobacter sakazakii is included as an outgroup. Bar, 0.050 substitutions per site.
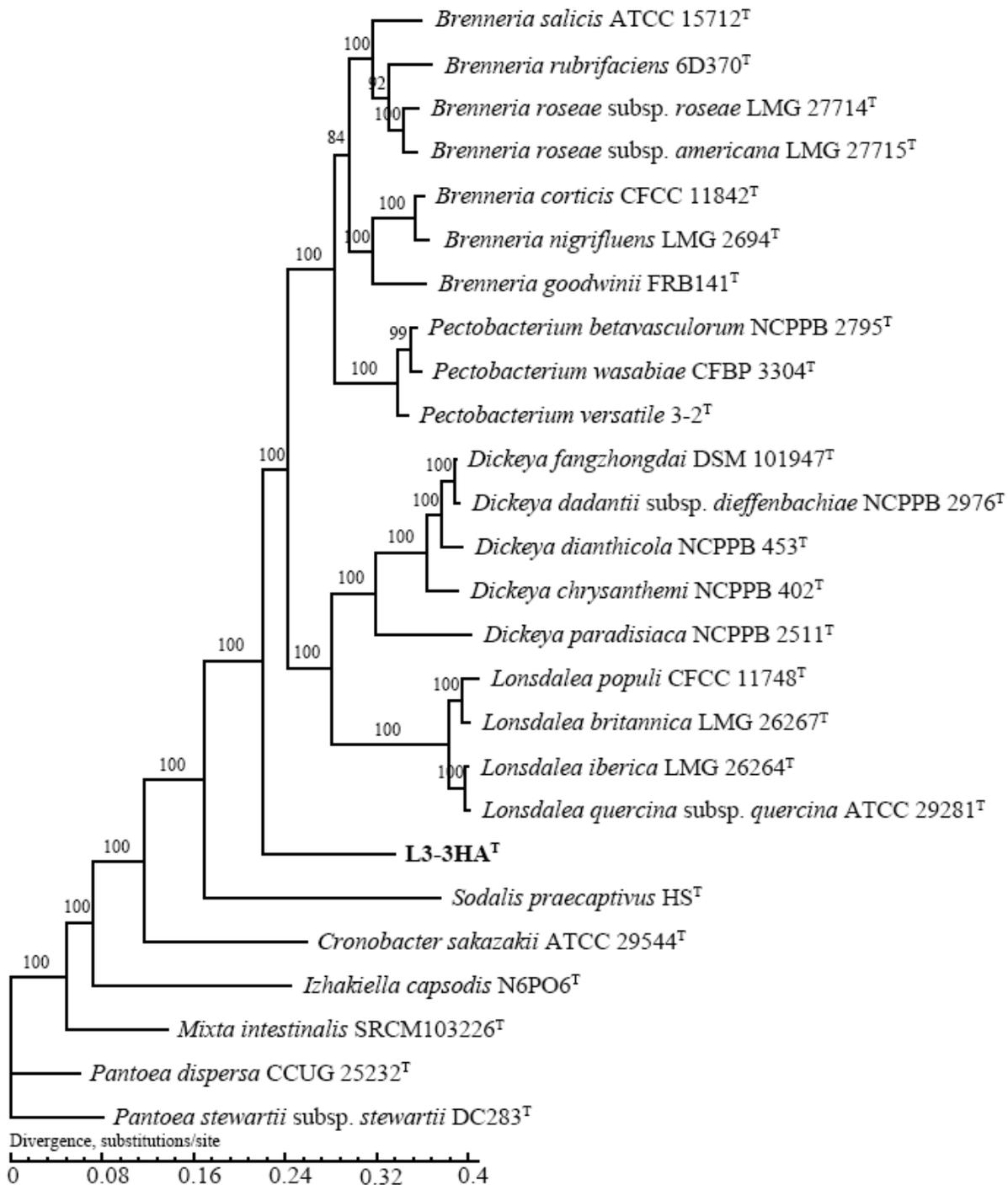
Figure 3

The phylogenetic ML tree was constructed based on the resulting multiple alignments using PhyML V3.3. The genomic coding sequences were clustered using CD-HIT v4.6.1. Four hundred sixteen single-copy genes were selected using Perl scripts and aligned using MUSCLE 3.8.31. The level of support for internal branches was assessed using the Bayesian test in PhyML.

Supplementary Files

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