Comparative Genomics, Pangenome, and Phylogenomic Analyses of *Brenneria* spp., and Delineation of *Brenneria izadpanahii* sp. nov.

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ABSTRACT

Brenneria species are bacterial plant pathogens mainly affecting woody plants. Association of all members with devastating disorders (e.g., acute oak decline in Iran and United Kingdom) are due to adaptation and pathogenic behavior in response to host and environmental factors. Some species, including *B. goodwinii*, *B. salicis*, and *B. nigrifluens*, also show endophytic residence. Here we show that all species including novel *Brenneria* sp. are closely related. Gene-based and genome/pangenome-based phylogeny divide the genus into two distinct lineages, *Brenneria* clades A and B. The two clades were functionally distinct and were consistent with their common and special potential activities as determined via annotation of functional domains. Pangenome analysis demonstrated that the core pathogenicity factors were highly conserved, an *hrp* gene cluster encoding a type III secretion system was found in all species except

B. corticis. An extensive repertoire of candidate virulence factors was identified. Comparative genomics indicated a repertoire of plant cell wall degrading enzymes, metabolites/antibiotics, and numerous prophages providing new insights into *Brenneria*–host interactions and appropriate targets for further characterization. This work not only documented the genetic differentiation of *Brenneria* species but also delineates a more functionally driven understanding of *Brenneria* by comparison with relevant *Pectobacteriaceae* thereby substantially enriching the extent of information available for functional genomic investigations.

Keywords: *Brenneria izadpanahii*, *Brenneria* spp., comparative genomics, pangenome analysis, phylogenomics

The family *Pectobacteriaceae* has become a major threat to trees worldwide. Most species from this genus seem ubiquitous and were isolated from a variety of distinctive forest habitats and urban trees (Brady et al. 2014; Denman et al. 2012; Hauben et al. 1998; Li et al. 2015, 2019; Zheng et al. 2017). The destructive capability and a causal association of *Brenneria* spp. with diseased woody plants have been demonstrated (Broberg et al. 2018; Hauben et al. 1998). Due to their wide spatiotemporal distribution and diverse habitats (Maes et al. 2009; Pettifor et al. 2020), the range of tree species affected and the severe pathogenic activity in the microbiota (e.g., acute oak decline [AOD] microbiome), their impacts can be devastating on forest health (Broberg et al. 2018; Sapp et al. 2016). Research aimed at deciphering their pathogenic behavior within their ecological niches has become crucial to shed light onto their ecological roles and their pathogenic activity.

At the time of writing, the genus *Brenneria* comprises eight species with valid published names (www.bacterio.net/brenneria.html): *B. alni*, *B. salicis*, *B. goodwinii*, *B. nigrifluens*, *B. rubrifaciens*, *B. roseae*, *B. corticis*, and *B. populi* (Brady et al. 2014; Denman et al. 2012; Hauben et al. 1998; Li et al. 2015, 2019). Molecular marker-based taxonomy paradigms have changed with the transition from multilocus sequence analysis and typing (MLSA/MLST) 16S rRNA to genome-based phylogeny, with many *Brenneria* spp. strains being assigned into novel taxa as it was shown that they have been misidentified based on phenotypic-genotypic features. Adeolu et al. (2016) reconstructed phylogenetic and taxonomic relationships in *Enterobacteriales* and introduced the

monophyletic *Dickeya*–*Pectobacterium* (*Brenneria*) clade based on a comprehensive study using genomic data. The relationship among *Brenneria* species is not completely clear because phylogenetic trees were based on MLSA of only four housekeeping genes at most and genome-based phylogenetic analyses were conducted only with a few species (Denman et al. 2012; Zhang et al. 2016). Given their phytopathological importance, the number of available *Brenneria* spp. genome sequences in GenBank has increased recently; however, their taxonomic placement are still controversial and additional investigations are needed to further clarify the taxonomy of the genus.

High throughput sequencing has produced bacterial genome datasets enabling comparative genomic analyses and facilitating wider and more comprehensive assessments of similarities and differences between and within plant and animal pathogens (Toth et al. 2006). Doonan et al. (2019) demonstrated that canonical bacterial species contributed to the AOD syndrome by using comparative genomics and showed that orthologous gene inferences (e.g., identification of virulence gene orthogroups) can be used to evaluate the pathogenic potential of bacterial species and their impacts on host–microbe and microbe–microbe interactions. They identified *B. goodwinii* as a keystone taxa and primary pathogen that contributes vital pathogenicity repertoires in the polymicrobial consortium of AOD causal agents (Doonan et al. 2019). The communications among the community of species associated with the AOD, the interactions with other symbionts in the environment (*B. goodwinii* and *Gibbsiella quercinecans* in AOD syndrome), and the potential mechanisms and effects of *Brenneria* species on related pathobiome are diverse and incompletely understood.

Despite the deep knowledge of the soft rot *Pectobacteriaceae* (*Pectobacterium* spp. and *Dickeya* spp.), genomic information of other members like *Brenneria* and *Lonsdalea* (formerly *Brenneria*) is still scarce. Our objectives were to (i) conduct a comparative genomics investigation among *Brenneria* spp. using all available

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genome sequences; (ii) develop a novel taxonomic understanding of the genus; and (iii) clarify whether we have uncovered a new species of *Brenneria* in the north of Iran, represented by a *Brenneria* sp. isolate obtained from diseased oak trees. To this end, we inferred the phylogeny of the novel isolate and other strains of the family *Pectobacteriaceae*. A pangenome analysis emphasizing the predicted pathogenicity determinants was obtained by considering an increasing number of bacterial species, most of which represent new microflora and lack of a holistic comparison.

MATERIALS AND METHODS

Maintenance of bacterial strain, 16S rRNA/MLSA typing, genomic DNA extraction, library preparation, and genome sequencing. The novel isolate of *Brenneria* sp. (Iran isolate 50) was obtained from diseased oak trees (*Quercus castaneifolia*) in the north of Iran. It was stored in 40% glycerol stocks at -80°C and maintained on nutrient agar at 30°C . Genomic DNA for amplification was extracted by the alkali extraction method (Niemann et al. 1997) and stored at -20°C until use. The 16S rRNA gene and MLSA (for housekeeping genes *gyrB*, *infB*, and *atpD*) sequencing were conducted using primers and sequencing conditions according to Hauben et al. (1998) and Brady et al. (2008), respectively. The 16S rRNA gene and MLSA similarities evaluation were conducted by the EzTaxon-e (<https://www.ezbiocloud.net/identify>) for 16S rRNA gene in special and GenBank similarity engine (Blastn) for both. High-quality genomic DNA was extracted using Qiagen Genomic-tip 500/G following the manufacturer's instructions (Qiagen, Germany). Genome sequencing of the novel isolate was accomplished with single molecule real-time (SMRT) technology of the Pacific Biosciences RSII platform (Macrogen Co., South Korea). The library was reconstructed with approximately 20-kb insert size using the SMRT Cell 8Pac V3, DNA Polymerase Binding Kit P6, DNA Sequencing Reagent 4.0 v2. The quality (DNA integrity) and quantity of the extracted genome and subsequent library were evaluated using 1% agarose gel electrophoresis and spectrophotometry/Nanodrop/DNA Bio-analyzer 12000 chip and DNA QC-Picogreen, respectively.

Genome assembly of pacific biosciences RSII generated data. The de novo sequence assembly was performed using the hierarchical genome assembly 3 (HGAP3) workflow, incorporating the CELERA assembler and finished using the Quiver consensus polisher according to Doonan et al. (2019). Genome estimated completeness and contamination were verified with CheckM version 1.0.7 (Parks et al. 2015). Resultant assembly produced one contig and a $<200\times$ sequencing depth.

Additional whole genome sequence data. Twenty-four draft genome sequences consisting of all valid published genomes from genus *Brenneria* (January 2020) and a few genomes from *Lonsdalea* spp., *P. carotovorum* ssp. *carotovorum*, and *Dickeya* spp. were retrieved from the NCBI GenBank database (Table 1). These genomes were incorporated into the workflow described below.

Genome annotation, identification of orthologs, and pangenome analysis. Aiming to assess the level of discrepancy among the genomes, whole genome sequence identity was computed using indices useful for species delineation; pairwise average nucleotide identity (ANI) according to BLAST (ANIb) in JSpeciesWS (Richter et al. 2016); ANI calculator (<https://www.ezbiocloud.net/tools/ani>) (Yoon et al. 2017) and OrthoANI values with the standalone orthologous average nucleotide identity tool (OAT) in Ezbiocloud (Lee et al. 2016). Also, digital DNA-DNA hybridization (dDDH) percentages were determined in silico with the genome-to-genome distance calculator (dGGDC 2.0) using the BLAST method and recommended formula 2 (Meier-Kolthoff et al. 2013) (<http://ggdc.dsmz.de/ggdc.php#>). Both ANI and dDDH values were below the accepted threshold in the case of the new species (≤ 95 and $\leq 70\%$ for ANI and dDDH, respectively) (Table 2).

Automatic annotation of draft genomes and complete genome of *Brenneria* sp. achieved in this study was conducted using RAST (Overbeek et al. 2014). Also, prokaryotic genome assembly and annotation were performed using KBase, according to Arkin et al. (2018). New raw/annotated genomes (e.g., *B. corticis*) were imported into the KBase. A genome set was made for all genomes and then phylogenetic trees were constructed from the *Brenneria* sp. along with the 29 nearest neighbors using the Insert Genome into Species Tree app (version 2.1.10), which utilizes FastTree 2 (Price et al. 2010). A pangenome was constructed Build Pangenome with OrthoMCL app (Pangenome Orthomcl v0.0.7) using aforementioned genome set. Pangenomes were visualized using the Pangenome Circle Plot app (v1.2.0) and pangenome-based phylogenomic analysis was conducted by the Phylogenetic Pangenome Accumulation (v1.4.0) app. Genomes were further compared by annotating the functional domains in the whole genome set with DomainAnnotation (version 1.03) and viewing the results using the View Function Profile for Genomes app (version 1.0.1) with the domain namespace set to BSEED Roles.

The online bacterial genome analysis service PATRIC (Pathosystems Resource Integration Center) was used for determination of the GC content, number of coding sequence regions (CDS), tRNA genes, pseudogenes, and others features, and the obtained information was used to reconstruct metabolic networks and subsystems. The clusters that were involved in diverse categories such as virulence and pathogenicity determinants, including type secretion systems (TSS), phytotoxins, iron uptake, polysaccharides biosynthesis, flagella encoding genes, and cell attachment were screened and compared across all *Brenneria* species. Circular genome maps were created using the circus software in PATRIC (Wattam et al. 2017). Also, the online service IslandViewer version 4.0 (an integrated interface for computational identification and visualization of genomic islands; <http://www.pathogenomics.sfu.ca/islandviewer>) was used for identification of pathogenicity islands within our bacterial genome (Bertelli et al. 2017).

The CAZy and dbCAN2 meta server databases were used to annotate plant cell wall degrading enzymes (PCWDEs) (Lombard et al. 2014; Zhang et al. 2018). The presence of plasmids was screened using PlasmidFinder 2.0 (<https://cge.cbs.dtu.dk/services/PlasmidFinder/>) (Carattoli et al. 2014) for all the genomic sequences. The online service PHASTER (PHAge Search Tool Enhanced Release; <http://phaster.ca/>) was used for identification of prophage sequences within bacterial genomes (Arndt et al. 2016). The Prokka annotation output was used once as input data for in silico secondary metabolite and antibiotic profiling by searching against the antiSMASH database-bacterial version (<https://antismash.secondarymetabolites.org/#!/start>) (Blin et al. 2019) in addition to the genome wide comparisons and visualization of orthologous clusters that were performed using the online service OrthoVenn2 (<https://orthovenn2.bioinfotoolkits.net/>) (Xu et al. 2019).

RESULTS

Phylogenetic analysis, genomic DNA extraction, and qualification of library preparation. Concatenated phylogenetic trees placed *Brenneria* sp. from this study separately from other *Brenneria* clade members, indicating that the isolate from the north of Iran likely belongs to a novel species in the genus *Brenneria*. Sequence similarity assessments using 16S rRNA gene and MLSA (*gyrB*, *infB*, and *atpD*) showed that the novel *Brenneria* sp. is most similar (97 to 98%) to *B. goodwinii* along with all other *Brenneria* species (Fig. 1A). The quantification and qualification of produced library demonstrated the insert size was almost 17 kb.

Complete genome sequencing of *B. izadpanahii* sp. nov. The sequence data for *B. izadpanahii* (isolated from diseased oak trees at the north of Iran) was assembled into one contig and the genome size was determined to be 5,330,697 bp by using the hierarchical genome assembly 3 (HGAP3) workflow. Annotation

of the complete genome predicted 4,743 CDS transcribed for *B. izadpanahii*. It identified 39 rRNA, 255 tRNA, 153 ribosomal protein, and 79 flagellum and flagellar motility-related genes. Subsystems and their related gene categories (e.g., metabolism), circular view of annotated subsystems within the *B. izadpanahii* genome, and pathogenicity islands screened by using IslandViewer tool are depicted in Figure 2A, B, and C, respectively.

The ANI/ANIb/orthoANI and dDDH values between *B. izadpanahii* and other *Pectobacteriaceae* members were estimated. The ANI values varied from 78 to 90% between different pairs of strains, showing *B. goodwinii* strain 141 with (89.98/90.35/90.57–41.10) identity/similarity lower than the proposed species boundary ANI cut-off value of 95 to 96% and ≤70% for dDDH was the most similar neighbor (Table 2).

Whole genome phylogeny. All *Brenneria* clade members appeared to be closely related and the whole genome phylogeny suggests two distinct lineages (Fig. 1B). Clade A has five taxa including *B. goodwinii*, *B. izadpanahii*, *B. alni*, *B. nigrifluens*, and *B. corticis* that appear to share a recent common ancestor. Clade B includes the three others, *B. roseae*, *B. rubrifaciens* and *B. salicis*, sharing another recent common ancestor. The phylogeny also indicates that the *Brenneria* clades share a common recent ancestor with the *Pectobacterium* spp. and are more distantly related to *Dickeya* spp. and *Lonsdalea* spp. and share least with the *Sodalia* spp. These results are in agreement with the rearrangements of *Enterobacteriales* and creation of the family *Pectobacteriaceae* (Adeolu et al. 2016).

Pangenome analysis. The pangenome comparisons of *Brenneria* clades A and B and other relevant in *Pectobacteriaceae* indicated the distinctive status and genetic makeup of *B. izadpanahii* (Figs. 1C and 3). Summary statistics of the pangenome datasets and shared genes are presented in Table 3. Genomes in *Brenneria* clades (A and B) had a total of 47,402 genes. Of the genes in clade A, 28,745 are in homologous families among the pangenome, and 2,680 are in singleton families; while in clade B, 13,547 genes are in homologous families and 2,430 are in singleton families.

Orthologous protein families and clusters were identified and categorized in both KBase and Orthovenn 2 and were visualized by online web server Orthovenn 2 for genome sets. Comparisons of the orthologous gene clusters within species of clades A and B and other relevant were determined through four versus four, five versus five, and six versus six genome sets (Fig. 4).

Brenneria clade A and B species shared 2,391 and 2,369 proteins in their genome sequences, respectively (Fig. 4A and C). Clade A members (*B. izadpanahii*, *B. goodwinii*, *B. alni*, *B. nigrifluens*, and *B. corticis*) showed 534, 365, 438, 377, and 443 unique proteins among their genome sequences, and clade B (*B. salicis*, *B. rubrifaciens*, *B. roseae* ssp. *americana*, and *B. roseae* ssp. *roseae*) had 526, 292, 275, and 251 unique proteins, respectively. Similarities and relatedness of the orthologous clusters within the genomes were depicted as a heatmap (Fig. 5B). As expected in clades A and B phylogeny, *B. izadpanahii*-*B. goodwinii*, *B. nigrifluens*-*B. corticis*, and *B. roseae* ssp. *americana*-*B. roseae* ssp. *roseae* had most similarities.

TABLE 1. Genome metrics of *Brenneria izadpanahii* sp. nov. and other genomes available in GenBank database used in this study

Organism (accession)	Origin of isolation	Number of contigs	Chromosome size (bp)/GC content (mol %)	Number of coding sequence regions	Reference
<i>B. izadpanahii</i> Iran 50 (CP050854)	Mazandaran, Iran	1	5,330,697 (53.7)	4,743	This study
<i>B. goodwinii</i> FRB141 (CP014137)	Outwood, U.K.	1	5,281,917 (53.2)	4,625	Doonan et al. (2019)
<i>B. goodwinii</i> FRB171 (MJLY000000000)	Gorse Covert, U.K.	128	5,377,922 (53.0)	4,881	Doonan et al. (2019)
<i>B. goodwinii</i> OBR1 (CGIG000000000)	U.K.	1	5,350,059 (53.1)	4,835	Doonan et al. (2019)
<i>B. nigrifluens</i> ATCC 13028 (CP034036)	California, U.S.A.	1	4,891,702 (55.9)	4,392	Poret-Peterson et al. (2019)
<i>B. nigrifluens</i> LMG 2694 (QDKK000000000)	–	84	4,837,857 (55.9)	4,395	Li et al. (2019)
<i>Brenneria</i> sp. EniD312 (NZ_CM001230.1)	–	1	4,943,773 (55.9)	4,540	–
<i>B. alni</i> NCPPB 3934 (MJLZ000000000)	Italy	132	4,127,267 (51.1)	4,013	Doonan et al. (2019)
<i>B. alni</i> DSM11811 (BioProject PRJNA234905)	–	60	4,100,984 (51)	3,803	–
<i>B. salicis</i> DSM30166 (MJMA000000000)	–	106	3,929,937 (52.1)	3,781	Doonan et al. (2019)
<i>B. salicis</i> (NZ_QNRY01000001)	–	93	3,907,359 (52.06)	3,613	–
<i>B. rubrifaciens</i> 6D370 (CP034035)	California, U.S.A.	1	4,028,093 (52.6)	3,521	Poret-Peterson et al. (2019)
<i>B. roseae</i> ssp. <i>americana</i> LMG 27715 (NZ_QDKJ000000000.1)	U.K.	54	4,476,002 (51.7)	4,152	Li et al. (2019)
<i>B. roseae</i> ssp. <i>roseae</i> LMG 27714 (QDKI000000000)	U.K.	58	4,504,465 (51.6)	4,196	Li et al. (2019)
<i>B. corticis</i> gBX10-1-2 (QDKH000000000)	Shandong and Henan, China	56	5,040,874 (56.4)	4,501	Li et al. (2019)
<i>Lonsdalea quercina</i> ssp. <i>quercina</i> ATCC 29281 (NZ_JIBO000000000.1)	U.S.A.	37	3,850,073 (55.1)	3,327	Caballero et al. (2014)
<i>L. quercina</i> ssp. <i>iberica</i> LMG 26265 (NZ_LUTQ000000000.1)	–	150	3,755,411 (55.04)	3,362	–
<i>L. quercina</i> ssp. <i>britannica</i> 477 (CP023009)	Surrey, U.K.	1	4,015,589 (55.1)	3,801	Doonan et al. (2019)
<i>L. quercina</i> ssp. <i>britannica</i> LMG 26267 (NZ_LUTN000000000.1)	–	57	3,882,080 (55.1)	3,475	–
<i>L. quercina</i> ssp. <i>populi</i> CFCC 11196 (NZ_LUTE000000000.1)	–	130	3,784,815 (55.4)	3,381	–
<i>L. quercina</i> ssp. <i>populi</i> CFCC13122 (NZ_LUTI01000001)	–	163	3,648,192 (55.4)	3,304	–
<i>Dickeya dadantii</i> 3937 (NC_014500)	–	1	4,922,802 (56.3)	4,513	Glasner et al. (2011)
<i>D. paradisaica</i> NCPPB 2511 (NZ_CM001857)	Colombia	1	4,631,867 (55.0)	4,016	Pritchard et al. (2013)
<i>Pectobacterium carotovorum</i> ssp. <i>carotovorum</i> PC1 (NC_012917)	–	1	4,862,913 (51.9)	4,461	–

The orthoANI diagram from OTA tool and the distance matrices heatmap of the generated orthologous clusters from Orthovenn 2 showed highly consistent results although they are nucleotide and protein-based pipelines, respectively (Fig. 5A and B).

The clades identified in the phylogenetic analysis were also functionally distinct as determined via annotation of functional domains in the genomes using the SEED database. Genes in all categories (especially in the singletons) that were compared between the genomes included those specifically associated with transcription regulators, potentially associated virulence genes and carbohydrate, amino acid and lipid transport, and metabolism (Fig. 6). In comparison between *B. izadpanahii* and its closest neighbor, *B. goodwinii* 141, unique pathogenicity associated domains was notable such as transcriptional regulator, AraC family, type IV secretory pathway, VirD4 components, and many phage-related domains in *B. izadpanahii* and *B. goodwinii* 141, respectively.

Pathogenicity gene clusters in *Brenneria* clades. Similar to other necrotrophic phytopathogens, pathogenicity determinants

within the genus *Brenneria* including bacterial secretion systems are highly conserved (Doonan et al. 2019). These systems include PCWDEs, which may have a crucial role in disease occurrence, progress and suppression of the plant defense mechanisms. Sequence similarity searches may be employed in order to determine putative pathogenicity mechanisms in novel bacterial pathogens by identifying the core repertoire of virulence genes shared by related organisms (Broberg et al. 2018; Doonan et al. 2019). Significant potential virulence homologs were determined in silico in *Brenneria* clade A and B species and compared with other relevant *Pectobacteriaceae* emphasizing PCWDEs (particularly pectinases), functional secretion systems (in special type III T3SS), and associated effectors and harpins.

We identified PCWDEs by using automated CAZym annotation performed in dbCAN2 meta-server database, which identifies closely related carbohydrate active enzymes including PCWDEs. The annotation results revealed that the family *Pectobacteriaceae* has a core enzyme repertoire in addition to a few specialists that might be involved in interactions with host plants and other

TABLE 2. Definition of the in silico average nucleotide identity and DNA-DNA hybridization values in the form (ANI/ANIb/orthoANI-dDDH) (first column only), ANI index for the rest of columns, of *Brenneria izadpanahii* sp. nov. and representative strains of the other described *Pectobacteriaceae* species^a

Organism (accession number)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1- <i>B. izadpanahii</i> (CP050854)		90.05	81.74	82.33	82.34	79.79	79.57	78.85	79.01	82.27	77.22	74.32	74.51	74.70	74.35	75.26	74.29
2- <i>B. goodwinii</i> FRB141 (CP014137)	89.98/90.35/ 90.57–41.10		82.08	82.15	82.15	80.29	80.14	78.65	79.00	82.16	77.05	74.15	74.15	74.15	74.15	74.93	74.32
3- <i>B. alni</i> NCPPB 3934 (MJLZ000000000)	82.59/83.17/ 83.14–27.30	83.03		83.92	83.92	81.04	80.87	80.05	80.43	83.62	78.07	74.01	74.14	74.18	74.02	75.11	74.40
4- <i>B. nigrifluens</i> ATCC 13028 (CP034036)	82.66/83.38/ 83.44–28.10	82.55	83.44		99.98	80.34	80.15	79.78	80.16	94.22	78.27	75.29	75.47	75.60	75.25	76.49	75.04
5- <i>Brenneria</i> sp. EniD312 (NZ_CM001230.1)	82.65/83.48/ 83.57–28.10	82.59	83.43	99.98		80.43	80.28	79.84	80.16	94.29	78.26	75.27	75.33	75.53	75.26	76.50	75.09
6- <i>B. roseae</i> ssp. <i>roseae</i> LMG 27714 (QDKI000000000)	80.18/80.65/ 80.88–24.80	80.76	80.64	80.50	80.54		96.42	84.82	85.20	80.68	78.80	74.31	74.56	74.45	74.30	75.50	74.59
7- <i>B. roseae</i> ssp. <i>americana</i> LMG 27715 (NZ_QDKJ000000000)	80.05/80.44/ 80.74–24.60	80.53	80.60	80.52	80.56	96.42		84.73	85.13	80.50	78.68	74.22	74.42	74.41	74.16	75.29	74.48
8- <i>B. salicis</i> DSM30166 (MJMA000000000)	79.52/79.81/ 80.08–23.60	79.51	80.00	80.11	80.11	85.20	85.08		83.47	79.90	78.46	74.48	74.59	74.68	74.42	75.77	74.79
9- <i>B. rubrifaciens</i> 6D370 (CP034035)	79.87/80.07/ 80.23–24.10	79.81	80.30	80.53	80.84	85.69	85.45	83.36		80.13	78.48	74.65	74.78	75.05	74.49	75.47	74.52
10- <i>B. corticis</i> gBX10-1-2 (QDKH000000000)	82.43/83.38/ 83.52–27.70	82.48	82.88	94.00	93.95	80.28	80.18	79.39	79.60		77.86	74.83	74.86	74.94	74.85	76.02	74.73
11- <i>Pectobacterium</i> <i>carotovorum</i> ssp. <i>carotovorum</i> (NC_012917)	77.71/78.08/ 78.34–23.00	77.52	78.07	78.26	78.26	78.90	78.78	78.35	78.29	78.25		74.34	74.43	74.45	74.35	75.18	74.25
12- <i>Lonsdalea quercina</i> ssp. <i>quercina</i> ATCC 29281 (NZ_JIBO000000000.1)	75.03/75.59/ 75.61–21.30	74.94	74.26	75.76	75.81	74.66	74.43	74.70	74.56	75.37	74.38		88.37	88.81	91.23	76.48	74.99
13- <i>L. quercina</i> ssp. <i>britannica</i> 477 (CP023009)	75.10/75.98/ 75.78–21.70	74.79	74.34	75.61	75.63	74.76	74.61	74.72	74.53	75.52	74.51	88.38		89.70	88.98	76.49	75.15
14- <i>L. quercina</i> ssp. <i>populi</i> CFCC 11196 (NZ_LUTE000000000.1)	75.15/76.01/ 75.75–22.00	74.76	74.23	75.73	75.73	74.73	74.66	74.50	74.51	75.41	74.44	88.64	89.43		88.36	76.25	75.00
15- <i>L. quercina</i> ssp. <i>iberica</i> LMG 26265 (NZ_LUTQ000000000.1)	74.88/75.48/ 75.41–21.20	74.62	74.26	75.41	75.41	74.60	74.46	74.41	74.28	75.33	74.26	91.10	88.91	88.51		76.01	74.92
16- <i>Dickeya dadanti</i> 3937 (NC_014500)	75.56/76.40/ 76.30–21.60	75.49	75.03	76.60	76.65	75.53	75.27	75.70	75.32	76.48	75.09	76.06	76.25	76.01	76.16		79.09
17- <i>Dickeya paradoxa</i> NCPB 2511 (NZ_CM001857)	76.67/75.45/ 75.32–20.80	74.62	74.22	75.15	75.14	74.54	74.48	74.57	74.25	75.12	88.51	74.71	74.18	74.67	74.79	79.04	

^a The specific threshold value is 95 to 96% for ANI and 70% for DDH. ANI values were calculated using three different algorithms, i.e., ANI calculator, JSpeciesWS (ANIb), and OrthoANI.

symbionts (Table 4). The most common enzymes we identified were annotated as polysaccharide lyase (PL) family, PL22 (oligogalacturonide lyase [EC 4.2.2.6]); glycoside hydrolases (GH) family, GH102 (peptidoglycan lytic transglycosylase [EC

3.2.1.-]); and glycosyl transferase (GT) family, GT2—with variety of activities—were notable. Also, the family carbohydrate esterase (CE) only had three most frequent and top hits for CE1 in *Dickeya dadantii*, *D. paradisaica*, and *P. carotovorum* ssp. *carotovorum*

Fig. 1. **A**, Bayesian 50% majority rule consensus tree based on concatenated partial *16S rRNA*/*gyrB*, *atpD*, and *infB* gene sequences of *Brenneria izadpanahii* sp. nov. and all validly described species of the genus *Brenneria* in addition to phylogenetically related species under the GTR + G + I model. Bayesian posterior probabilities (BPP), maximum likelihood bootstrap (ML BS), and maximum parsimony bootstrap (MP BS) values >50% are given for appropriate clades in the form: BPP/ML/MP BS. *Cronobacter sakazakii* is included as an out-group. The scale bar indicates the fraction of substitutions per site. **B**, Phylogenomic tree of the *Brenneria* clade A and B species along with neighbor *Pectobacteriaceae* members generated by Insert Genome into Species Tree app in KBase using the 29 closest relatives in the public database. Numbers are local support values. **C**, Pangenome-based phylogenomic analysis of *Brenneria* clade A and B species. Orthologous gene set percentages within a pangenome partitioned into three categories: core (blue), singleton (red), and partial pangenome (pink). Pangenome created by OrthoMCL app distinguish which homologous genes are true orthologs and vertically inherited from those with lineage derived paralogous expansions by duplication.

PC1 in genome set. A pectate lyase, PL4 (EC 4.2.2.23), which degrades the plant cell wall component rhamnogalacturonan, was determined uniquely in *B. izadpanahii* and *B. goodwinii* (Table 4).

The T3SS is essential to the virulence of numerous phytopathogens, e.g., *Pseudomonas syringae* pathovars that use the system to enable host manipulation (Tampakaki et al. 2010). In *Brenneria* clade A, complete T3SS was detected in all members but surprisingly typical genes in the core type III nanomachinery were not identified in the *B. corticis* genome; the result was confirmed with the analysis system in PATRIC.

Pathogenicity islands encoding the secretion of effector molecules via type III secretion system have been discovered in several gram-negative pathogens (Winstanley and Hart 2001). The *hrpN* harpin gene is key to the virulence of *E. amylovora* and secretes the DspA/E effector, both *hrpN* and *dspA/E* are encoded within *Brenneria* species. (Doonan et al. 2019). HrpG, HrpD, HrpA (TTSS pilin *hrpA*), HrpF, HrpW (harpin with pectate lyase domain), HrpB, HrpP (annotated as hypothetical protein in some cases), HrpQ, and HrpJ were found in all clade A and B members, except for *B. corticis*, which only shares both ATP-dependent helicase HrpA and ATP-dependent helicase HrpB with other members. Type III secretion protein HrpT (annotated as hypothetical protein in some cases) was found in all members except for *B. alni*, *B. corticis*, and *B. nigrifluens*.

As described previously in AOD pathobiome, the major *B. goodwinii* homologous virulence factors, effector repertoire, were screened and *hopAN1*, *hopX1*, *hopAJ2*, *hopA1*, *hopAW1*, *avrXccB*, *dspA/E*, *dspF*, *mxIE*, and *avrXv*, which are overall key to disease causation in bacterial phytopathogens, were detected (Doonan et al. 2019). We identified potential virulence factors to add to those of Doonan et al. (2019); they include *hopV1* (*B. alni*), virulence factor VirK (*B. goodwinii* and *B. izadpanahii*), and protein of avirulence locus *impE* (*B. izadpanahii*) in *Brenneria* clade A species.

In *Brenneria* clade B, homologous sequences were identified for virulence factors such as type III effector protein AvrE1 (*B. roseae* ssp. *roseae*, *B. roseae* ssp. *americana*, and *B. salicis*, annotated as hypothetical protein in *B. rubrifaciens*) and *hopV1* within the *B. salicis* and *B. rubrifaciens* genomes. Sequences detected in both clades A and B of *Brenneria* included virulence factor MviM (*B. salicis*, *B. goodwinii* 141/171/OBR, *B. izadpanahii*, and *B. roseae* ssp. *americana*), virulence-associated protein VagC (*B. corticis*; *B. roseae* ssp. *roseae*), and virulence-associated protein C (VapC toxin protein) (*B. corticis*-two families, *B. nigrifluens*, *B. roseae* ssp. *roseae*, and *B. roseae* ssp. *americana*).

In silico detection of the plasmids, phages, and secondary metabolites. The genome datasets were scanned for plasmids by the PlasmidFinder online service for *Brenneria* clades A and B and of relevant *Pectobacteriaceae* members, and the results indicated that they carry no detectable plasmid.

Scanning the *Brenneria* spp. genomes investigated in this study for prophage sequences with the PHASTER online service detected several hypothetical prophage groups including PHAGE_Salmon_SEN1_NC_029003(3), PHAGE_Aeromo_phiO18P_NC_009542(16), and PHAGE_Gordon_Schwabeltier_NC_031255(1) (Table 5). We detected a few additional phage regions by using a GenBank formatted file (.gff) as a query that were not found when using FASTA formatted genomes files. Also, in our experience, the number of genomes contigs in .gff format could affect outputs. For example, using FASTA drafted genome of *B. corticis*, three prophage groups were determined, but using .gff format at least seven different prophage families were detected within the genome.

Bacterial metabolite detection online tools were developed reinforcing the importance of their biological and ecological functions. Turnorbactin (up to 46% similarity with the reference data-*Teredinibacter turnerae* T7901-GenBank CP001614.2) and aryl polyene (100% similarity-*Xenorhabdus doucetiae* strain FRM16, reference sequence NZ_F0704550.1) were detected as

Fig. 2. A, Subsystems and their related gene categories (e.g., metabolism). B, Circular view of annotated subsystems of the *Brenneria izadpanahii* sp. nov. genome generated in PATRIC (Pathosystems Resource Integration Center). C, Pathogenicity islands screening using Island Viewer tool.

Fig. 3. Pangenome analysis of *Brenneria* **A**, clade A+B species, **B**, clade A, and **C**, clade B performed using the Build Pangenome with OrthoMCL app (Pangenome Orthomcl version 0.0.7). Red (medium gray) indicates base singletons; and sky blue (light gray) represents noncore genes with respect to *B. izadpanahii* (A and B) and *B. salicis* (C) as based genomes compared with the respective pangenomes. Dark blue (dark gray) represents core genes.

the most encoded metabolites/antibiotic factors within the genome dataset using antiSMASH database-bacterial version (Table 6).

DISCUSSION

Only a few species within the genus *Brenneria* have been described so far despite increasing reports demonstrating that strains of *Brenneria* spp. are ubiquitous and commonly associated with woody plants presenting symptoms including bark canker and stem bleeding as the most visible (Brady et al. 2014; Denman et al. 2012; Hauben et al. 1998; Li et al. 2015, 2019; Zheng et al. 2017). The relatively small number of species analyzed is a likely consequence of the media and culturing conditions used in laboratory procedures and associated isolation biases. Also, screening of the population structure and evolutionary potential in genomic scales could be a useful tool since these bacteria may

share close genetic relationships (e.g., *B. goodwinii* in U.K. [Kaczmarek et al. 2017]). So, combination of genomic indices provides added resolution to effectively identify species within the genus.

A genome-based phylogeny using 50 clusters of orthologous groups (Fig. 1B) proposed rearrangement necessity of the family *Pectobacteriaceae* into two subfamilies, sharing most common recent ancestor in *Brenneria-Pectobacterium* and *Dickeya-Lonsdalea*, respectively. The *B. izadpanahii*-*B. goodwinii* 141, *B. nigrifluens*-*B. corticis*, and *B. roseae* ssp. *roseae*-*B. roseae* ssp. *americana* formed very tight pairs related by almost 90% for *B. izadpanahii*-*B. goodwinii* pair and 95 to 96% for another two pairs, respectively, with all ANI calculations and reciprocal relatedness of the dDDH values. These results are consistent with the 16S rRNA gene/MLSA sequence similarity data (Fig. 1A). All other comparisons between *Brenneria* clades A and B gave even

TABLE 3. Summary statistics of the pangenome datasets and shared genes within *Brenneria* clade A and B species retrieved from KBase

Organism (accession)	Number of genes in homologs	Number of homolog families	Number of genes in singleton
<i>B. izadpanahii</i> (Iran 50) (CP050854)	4,208	4,060	535
<i>B. goodwinii</i> FRB141 (CP014137)	4,423	4,293	201
<i>B. goodwinii</i> FRB171 (MJLY000000000)	4,505	4,374	143
<i>B. goodwinii</i> OBR1 (CGIG000000000)	4,506	4,377	197
<i>B. nigrifluens</i> ATCC 13028 (CP034036)	4,032	3,918	360
<i>B. corticis</i> gBX10-1-2 (QDKH000000000)	4,022	3,895	479
<i>B. alni</i> NCPB 3934 (MJLZ000000000)	3,386	3,312	428
<i>B. salicis</i> DSM30166 (MJMA000000000)	3,246	3,170	376
<i>B. rubrifaciens</i> 6D370 (CP034035)	3,210	3,082	311
<i>B. roseae</i> ssp. <i>americana</i> LMG 27715 (NZ_QDKJ000000000.1)	3,871	3,780	540
<i>B. roseae</i> ssp. <i>roseae</i> LMG 27714 (QDKI000000000)	3,882	3,767	471

Fig. 4. Venn diagrams constructed using the OrthoVenn 2 online service showing the distribution of shared gene families (orthologous clusters) among different sets of strains. A, *Brenneria* clade A; B, *Brenneria* clade B; C, *Brenneria* clade A (– *B. alni* and + *Pectobacterium carotovorum* ssp. *carotovorum*); D, *Brenneria* clade B (+ *P. carotovorum* ssp. *carotovorum*); E, *B. izadpanahii* + *B. goodwinii* + *B. salicis* + *P. carotovorum* ssp. *carotovorum*; and F, *B. izadpanahii* + *B. goodwinii* + *B. salicis* + *Lonsdalea quercina* ssp. *quercina* + *P. carotovorum* ssp. *carotovorum* + *Dickeya dadantii*.

lower similarity levels (below-threshold genomic similarity) and were scattered in several clades. Thus, the novel isolate should also be considered as another new species from a genomic point of view (Fig. 5A).

With advances in high-throughput sequencing technologies bacterial taxonomy is undergoing many changes as a consequence and new innovative tools were developed (Caputo et al. 2019). Using pangenomic analyses, species can be redefined or new species definitions generated. The analysis pipeline in KBase was useful in providing a phylogenetic and functional genomic assessment of many whole bacterial genomes simultaneously. The *Brenneria* species were divided according to their host plants, the disease symptoms they cause, and other phenotypic or genotypic features. The taxonomic classification of *Brenneria* species has been the subject of controversy (Denman et al. 2012; Hauben et al. 1998). In a recent update, *B. quercina* was transferred to the new genus *Lonsdalea* (Brady et al. 2012). Here, we analyzed core/

noncore and singleton genes in pangenomes once for genome-based taxonomy point of view in addition to deciphering their common and special potential activities.

Both the core and dispensable genes are crucial for determining bacterial species diversity that are responsible for fundamental functions including replication, translation, and maintenance of cellular homeostasis and survivability, antimicrobial resistance, virulence traits, and development of novel gene functions, respectively (Adeniji et al. 2019).

A majority of the core genes irrespective of their functions and location in the genome are useful for phylogenomic reassignments among *Brenneria* and elsewhere. This pangenomic analysis enabled us to conclude that *B. izadpanahii*, which exhibit as many differences between them as with other *Brenneria* species, is a distinct *Brenneria* species. The conserved genomic regions of the *Brenneria* strains were probably due to their common ancestry, either by duplication or by speciation of the genes (Fig. 1C). While

Fig. 5. A, Average nucleotide identity (ANI)-based neighbor joining phylogenetic tree of *Brenneria* clade A and B species in addition to *Pectobacterium carotovorum* ssp. *carotovorum* constructed using the OrthoANI calculator software. Different colors represent degree of identity. **B**, Heatmap showing intrastrain similarities of orthologous subsets from the *Brenneria* clade A and B species in addition to *P. carotovorum* ssp. *carotovorum* generated by the OrthoVenn 2 online service.

Fig. 6. Pangenome functional domains annotation of the *Brenneria* clade A and B species identified in the phylogenomic analysis using the SEED database.

Figure 6 KEY

Amino acids and derivatives

- 1-Alanine biosynthesis
- 2-Arginine biosynthesis extended
- 3-Arginine deiminase pathway
- 4-Arginine and ornithine degradation
- 5-Aromatic amino acid degradation
- 6-Aromatic amino acid interconversions with aryl acids
- 7-Branched-chain amino acid biosynthesis
- 8-Chorismate: Intermediate for synthesis of PAPA antibiotics, PABA, anthranilate, 3-hydroxyanthranilate and more
- 9-Chorismate synthesis

- 10-Common pathway for synthesis of aromatic compounds (DAHP synthase to chorismate)
- 11-Cysteine biosynthesis
- 12-Glutamine, glutamate, aspartate, and asparagine biosynthesis
- 13-Glycine and serine utilization
- 14-Glycine cleavage system
- 15-HMG CoA synthesis
- 16-Histidine biosynthesis
- 17-Histidine degradation
- 18-Ketoisovalerate oxidoreductase
- 19-L-2-amino-thiazoline-4-carboxylic acid-L-cysteine conversion
- 20-Lysine biosynthesis DAP pathway

- 21-Lysine degradation
- 22-Methionine biosynthesis
- 23-Methionine degradation
- 24-Methionine salvage
- 25-Phenylalanine and tyrosine branches from chorismate
- 26-Polyamine metabolism
- 27-Proline, 4-hydroxyproline uptake and utilization
- 28-Proline synthesis
- 29-Serine biosynthesis
- 30-Threonine and homoserine biosynthesis
- 31-Threonine degradation
- 32-Urea decomposition
- 33-Valine degradation

Fig. 6. Key (Continued from previous page)

- 34-2-Ketogluconate utilization
- 35-2-Methylcitrate to 2-methylaconitate metabolism cluster
- 36-Acetoin, butanediol metabolism
- 37-Acetyl-CoA fermentation to butyrate
- 38-Alpha-acetolactate operon
- 39-Beta-glucoside metabolism
- 40-Butanol biosynthesis
- 41-CO₂ uptake, carboxysome
- 42-Calvin-Benson cycle
- 43-Carboxysome
- 44-Chitin and N-acetylglucosamine utilization
- 45-D-Galacturonate and D-glucuronate utilization
- 46-D-Sorbitol (D-glucitol) and L-sorbose utilization
- 47-D-Tagatose and galactitol utilization
- 48-D-Allose utilization
- 49-D-Galactarate, D-glucarate, and D-Glycerate catabolism
- 50-D-Galactonate catabolism
- 51-D-Gluconate and ketogluconates metabolism
- 52-D-Ribose utilization
- 53-Deoxyribose and deoxynucleoside catabolism
- 54-Di-Inositol-phosphate biosynthesis
- 55-Dihydroxyacetone kinases
- 56-Entner-Doudoroff pathway
- 57-Ethanolamine utilization
- 58-Fermentations: Mixed acid
- 59-Formaldehyde assimilation: Ribulose monophosphate pathway
- 60-Fructooligosaccharides (FOS) and raffinose utilization
- 61-Fructose utilization
- 62-Glycerol and glycerol-3-phosphate uptake and utilization
- 63-Glycogen metabolism
- 64-Glycolysis and gluconeogenesis
- 65-Glycolysis and gluconeogenesis, including archaeal enzymes
- 66-Hexose phosphate uptake system
- 67-Inositol catabolism
- 68-Isobutyryl-CoA to propionyl-CoA module
- 69-L-Arabinose utilization
- 70-L-Ascorbate utilization (and related gene clusters)
- 71-L-Fucose utilization
- 72-L-Fucose utilization temp
- 73-Lactate utilization
- 74-Lacto-N-biose I and galacto-N-biose metabolic pathway
- 75-Lactose and galactose uptake and utilization
- 76-Lactose utilization
- 77-Maltose and maltodextrin utilization
- 78-Mannitol utilization
- 79-Mannose metabolism
- 80-Methylglyoxal metabolism
- 81-N-Acetyl-galactosamine and galactosamine utilization
- 82-One-carbon metabolism by tetrahydropterines
- 83-Pentose phosphate pathway
- 84-Photorespiration (oxidative C2 cycle)
- 85-Predicted carbohydrate hydrolases
- 86-Propanediol utilization
- 87-Propionate-CoA to succinate module
- 88-Pyruvate/ferredoxin oxidoreductase
- 89-Pyruvate alanine serine interconversions
- 90-Pyruvate metabolism I: anaplerotic reactions, PEP
- 91-Pyruvate metabolism II: acetyl-CoA, acetogenesis from pyruvate
- 92-Ribitol, xylitol, arabitol, mannitol, and sorbitol utilization
- 93-Serine-glyoxylate cycle
- 94-Sucrose utilization
- 95-TCA cycle
- 96-Trehalose biosynthesis
- 97-Trehalose uptake and utilization
- 98-Unknown sugar utilization (cluster yphABCDEF G)
- 99-Xylose utilization
- Cell division and cell cycle**
- 100-Bacterial cytoskeleton
- 101-MukBEF chromosome condensation
- 102-Two cell division clusters relating to chromosome partitioning
- Cell wall and capsule**
- 103-CMP-N-acetylneuraminate biosynthesis
- 104-Capsular heptose biosynthesis
- 105-Colanic acid biosynthesis
- 106-KDO2-lipid A biosynthesis
- 107-LOS core oligosaccharide biosynthesis
- 108-Lipid A-Ara4N pathway (polymyxin resistance)
- 109-Lipid A modifications
- 110-Peptidoglycan biosynthesis
- 111-Rhamnose containing glycans
- 112-Sialic acid metabolism
- 113-Teichuronic acid biosynthesis
- 114-UDP-N-acetylmuramate from fructose-6-phosphate biosynthesis
- 115-dTDP-rhamnose synthesis
- 116-Linker unit-arabinogalactan synthesis
- 117-Mycolic acid synthesis
- Clustering-based subsystems**
- 118-Bacterial cell division
- 119-CBSS-214092.1.peg.3450
- 120-CBSS-262719.3.peg.410
- 121-CBSS-343509.6.peg.2644
- 122-CBSS-562.2.peg.5158 SK3 including
- 123-Cluster-based subsystem grouping hypotheticals-perhaps proteosome related
- 124-Conserved gene cluster associated with Met-tRNA formyltransferase
- 125-LMPTP YwIE cluster
- 126-NusA-TFII Cluster
- 127-Putative hemin transporter
- 128-Putative sulfate assimilation cluster
- 129-Type III secretion systems, extended
- 130-YeiH
- Cofactors, vitamins, prosthetic groups, pigments**
- 131-Biotin biosynthesis
- 132-Coenzyme A biosynthesis
- 133-Coenzyme B12 biosynthesis
- 134-Experimental type
- 135-Flavodoxin
- 136-Folate biosynthesis
- 137-Heme and siroheme biosynthesis
- 138-Lipoic acid metabolism
- 139-Menaquinone and phyloquinone biosynthesis
- 140-Molybdenum cofactor biosynthesis
- 141-NAD and NADP cofactor biosynthesis global
- 142-NAD regulation
- 143-Plastoquinone biosynthesis
- 144-Pyridoxin (vitamin B6) biosynthesis
- 145-Pyrroloquinoline quinone biosynthesis
- 146-Riboflavin, FMN, and FAD metabolism
- 147-Thiamin biosynthesis
- 148-Ubiquinone biosynthesis
- 149-p-Aminobenzoyl-glutamate utilization
- DNA metabolism**
- 150-2-phosphoglycolate salvage
- 151-CRISPRs
- 152-DNA-replication
- 153-DNA repair base excision
- 154-DNA repair, UvrABC system
- 155-DNA repair, bacterial
- 156-DNA repair, bacterial DinG, and relatives
- 157-DNA repair, bacterial MutL-MutS system
- 158-DNA repair, bacterial RecFOR pathway
- 159-DNA repair, bacterial UvrD, and related helicases
- 160-DNA structural proteins, bacterial
- 161-DNA topoisomerases, Type I, ATP-independent
- 162-Plasmid replication
- 163-Restriction-modification system
- 164-RuvABC plus a hypothetical
- 165-YcfH
- Fatty acids, lipids, and isoprenoids**
- 166-Fatty acid biosynthesis FASII
- 167-Glycerolipid and glycerophospholipid metabolism in bacteria
- 168-Isoprenoid biosynthesis
- 169-Polyhydroxybutyrate metabolism
- 170-Triacylglycerol metabolism
- 171-Polyprenyl synthesis
- Iron acquisition and metabolism**
- 172-*Campylobacter* iron metabolism
- 173-Heme, hemin uptake and utilization systems in gram positives
- 174-Hemin transport system
- 175-Iron acquisition in *Vibrio*
- 176-Siderophore aerobactin
- 177-Siderophore enterobactin
- 178-Transport of iron
- Membrane transport**
- 179-ABC transporter alkylphosphonate (TC 3.A.1.9.1)
- 180-ABC transporter branched-chain amino acid (TC 3.A.1.4.1)
- 181-ABC transporter dipeptide (TC 3.A.1.5.2)
- 182-ABC transporter oligopeptide (TC 3.A.1.5.1)
- 183-ABC transporter peptide (TC 3.A.1.5.5)
- 184-Fructose and mannose inducible PTS
- 185-Na(+) H(+) antiporter
- 186-Ton and Tol transport systems
- 187-Transport of manganese
- 188-Transport of nickel and cobalt
- 189-Transport of zinc
- 190-Twin-arginine translocation system
- 191-Type 4 conjugative transfer system, IncI1 type
- 192-Type III secretion system orphans
- 193-Type IV pilus
- 194-Widespread colonization island
- 195-pVir Plasmid of *Campylobacter*
- Metabolism of aromatic compounds**
- 196-Aromatic amin catabolism
- 197-Benzoate degradation
- 198-Biphenyl degradation
- 199-Catechol branch of beta-ketoadipate pathway
- 200-Central meta-cleavage pathway of aromatic compound degradation
- 201-Homogentisate pathway of aromatic compound degradation
- 202-Phenylpropanoid compound degradation
- 203-Protocatechuate branch of beta-ketoadipate pathway
- 204-Quinate degradation
- 205-Salicylate and gentisate catabolism
- Miscellaneous**
- 206-Conserved gene cluster possibly involved in RNA metabolism
- 207-Muconate lactonizing enzyme family
- 208-YaaA
- 209-YbbK
- 210-ZZ gjo need homes
- Motility and chemotaxis**
- 211-Bacterial chemotaxis
- 212-Flagellar motility
- 213-Flagellum
- 214-Flagellum in *Campylobacter*
- Nitrogen metabolism**
- 215-Allantoin utilization
- 216-Amidase clustered with urea and nitrile hydratase functions
- 217-Ammonia assimilation
- 218-Cyanate hydrolysis
- 219-Dissimilatory nitrite reductase
- 220-Nitrate and nitrite ammonification
- 221-Nitric oxide synthase
- 222-Nitrogen fixation

the phylogenomic position of the *Dickeya-Pectobacterium* clade was clarified in these analyses, only further investigations using a larger collection of strains will shed light on the genetic content and taxonomic status of the family *Pectobacteriaceae*.

Although the existence of T3SS cluster within a putative pathogen is typically considered a substantial factor in establishing the causality of disease (Arnold and Jackson 2011), it should be noted that the functional hrp systems as a minor virulence component in the *Pectobacteriaceae* (*Pectobacterium* and *Dickeya*) (Pérombelon 2002) may reflect a hemibiotrophic lifestyle of *Brenneria* ssp. (Glazebrook 2005). While *B. corticis* with high similarity in genome metrics and functional domains with the *B. nigrifluens* (a well-known pathogenic member of the genus) does not carry the T3SS cluster and potential effectors, only further investigations could decipher exact nature of the bacterium in related pathosystem. The whole genome sequence analyses Lee et al. (2020) showed that the nonpathogenic *Xanthomonas campestris* strain nE1 does not contain the entire pathogenicity island (*hrp* gene cluster; type III secretion system) and all type III effector protein genes. Experimental evidence demonstrated that

this nonpathogenic strain (nE1) could acquire the entire pathogenicity island from the endemic *X. campestris* pv. *campestris* and *X. campestris* pv. *raphani* strains by conjugation, while type III effector genes were not cotransferred (Lee et al. 2020). This results in raising questions in the case of symptomatic trees where several bacterial species occasionally are found as complex networks in the microbiome and the possible transfer events of pathogenicity determinants between the species is not impossible.

The analysis of prophage regions within *Brenneria* genomes may be fundamental to understanding their evolution and the potential for transfer for virulence factors between strains or species such as reported for ECA41 and hopAR1 in potato and cherry, respectively (Evans et al. 2010; Hulin et al. 2018), and influencing ecological fitness of their bacterial hosts (e.g., genes encoding metal ion transporters) (Matos et al. 2013; Ohnishi et al. 2001). Thirty-seven intact prophages have been characterized in *Pectobacterium* spp. and *Dickeya* spp. genomes and 6 of 37 were located near genes coding for pectate lyase degrading enzyme (Czajkowski 2019). The relatively high number of intact prophages found in the present analysis may suggest that the interaction of *Brenneria* and

Fig. 6. Key (Continued from previous page)

223-Nitrosative stress	RNA metabolism	Stress response
Nucleosides and nucleotides	265-ATP-dependent RNA helicases, bacterial	307-Bacterial hemoglobins
224-De novo purine biosynthesis	266-Polyadenylation bacterial	308-Choline and betaine uptake and betaine biosynthesis
225-De novo pyrimidine synthesis	267-Queuosine-archaeosine biosynthesis	309-D-trotyl-tRNA(Tyr) deacylase
226-Hydantoin metabolism	268-RNA polymerase bacterial	310-Ectoine biosynthesis and regulation
227-Nudix proteins (nucleoside triphosphate hydrolases)	269-RNA processing and degradation, bacterial	311-Flavohemoglobin
228-Purine utilization	270-Ribonuclease H	312-Glutaredoxins
229-Purine conversions	271-Rrf2 family transcriptional regulators	313-Glutathione-dependent pathway of formaldehyde detoxification
230-Purine nucleotide synthesis regulator	272-Transcription factors bacterial	314-Glutathione: Biosynthesis and gamma-glutamyl cycle
231-Pyrimidine utilization	273-Transcription initiation, bacterial sigma factors	315-Glutathione: Nonredox reactions
232-Ribonucleotide reduction	274-Wyeosine-MimG biosynthesis	316-Glutathione: Redox cycle
233-Xanthosine utilization (xap region)	275-tRNA processing	317-Glutathionylspermidine and trypanothione
Phages, prophages, transposable elements, plasmids	Regulation and cell signaling	318-Heat shock <i>dnaK</i> gene cluster extended
234-Phage capsid proteins	276-CytR regulation	319-Hfl operon
235-Staphylococcal pathogenicity islands SaPI	277-DNA-binding regulatory proteins, strays	320-Oxidative stress
236-Staphylococcal phi-Mu50B-like prophages	278-MazEF toxin-antitoxin (programmed cell death) system	321-Redox-dependent regulation of nucleus processes
Phosphorus metabolism	280-Murein hydrolase regulation and cell death	322-Rubryerthrin
237-Alkylphosphonate utilization	279-Orphan regulatory proteins	323-SigmaB stress response regulation
238-Phosphate metabolism	280-Sex pheromones in <i>Enterococcus faecalis</i> and other firmicutes	324-Sugar-phosphate stress regulation
239-Phosphonate metabolism	281-Stringent response, (p)ppGpp metabolism	325-Universal stress protein family
Potassium metabolism	282-cAMP signaling in bacteria	Sulfur metabolism
240-Glutathione-regulated potassium-efflux system and associated functions	Respiration	326-Alkanesulfonate assimilation
241-Potassium homeostasis Protein metabolism	283-Anaerobic respiratory reductases	327-Alkanesulfonates utilization
242-Glycine reductase, sarcosine reductase, and betaine reductase	284-Biogenesis of c-type cytochromes	328-Sulfate reduction-associated complexes
243-GroEL GroES	285-Biogenesis of cytochrome c oxidases	329-Taurine utilization
244-N-linked glycosylation in bacteria	286-Carbon monoxide induced hydrogenase	330-Thioredoxin-disulfide reductase
245-Peptidyl-prolyl cis-trans isomerase	287-FOF1-type ATP synthase	331-Utilization of glutathione as a sulfur source
246-Periplasmic disulfide interchange	288-Flavocytochrome C	Virulence, disease, and defense
247-Programmed frameshift	289-Formate dehydrogenase	332-Type 4 secretion and conjugative transfer
248-Proteasome bacterial	290-Formate hydrogenase	333-Aminoglycoside adenyltransferases
249-Protein chaperones	291-Hydrogenases	334-Arsenic resistance
250-Protein degradation	292-Membrane-bound Ni, Fe-hydrogenase	335-Bacteriocin-like peptides Blp
251-Protein secretion by ABC-type exporters	293-Na(+)-translocating NADH-quinone	336-Beta-lactamase
252-Proteolysis in bacteria, ATP-dependent	294-NiFe hydrogenase maturation	337-Cobalt-zinc-cadmium resistance
253-Putative TldE-TldD proteolytic complex	295-Respiratory complex I	338-Copper homeostasis
254-Ribosomal protein S12p Asp	296-Respiratory dehydrogenases I	339-Copper homeostasis: Copper tolerance
methylthiotransferase	297-Soluble cytochromes and functionally related electron carriers	340-Fosfomycin resistance
255-Ribosome LSU bacterial	298-Succinate dehydrogenase	341-MLST
256-Ribosome SSU bacterial	299-Terminal cytochrome oxidases	342-Methicillin resistance in staphylococci
257-Ribosome activity modulation	300-Trimethylamine N-oxide (TMAO) reductase	343-Multidrug resistance, 2-protein version found in gram-positive bacteria
258-Ribosome biogenesis bacterial	Secondary metabolism	344-Multidrug resistance, tripartite systems found in gram-negative bacteria
259-Translation elongation factor G family	301-Auxin biosynthesis	345-Multidrug resistance efflux pumps
260-Translation elongation factors eukaryotic and archaeal	302-Biflavanoid biosynthesis	346-Multidrug efflux pump in <i>Campylobacter jejuni</i> (CmeABC operon)
261-Translation initiation factors eukaryotic and archaeal	303-Cinnamic acid degradation	347-Resistance to fluoroquinolones
262-Universal GTPases	304-Phenazine biosynthesis	348- <i>Streptococcus pyogenes</i> recombinatorial zone
263-tRNA aminoacylation, Asp, and Asn	305-Steroid sulfates	349-Zinc resistance
264-tRNA aminoacylation, Glu, and Gln	306-Tannin biosynthesis	

(bacterio) phages at the microbiome scale is underestimated. The potential effects of phages in AOD is undescribed but is suggested by the upregulation of bacteriophages genes in the AOD pathobiome in which *B. goodwinii* was the dominant taxa and was an essential virulence component.

The factors that determine the interactions within phyllosphere microbial communities are poorly understood whether they are between different bacteria or between bacteria and other microorganisms. The production of microbial secondary metabolites and antibiotics might be the principal mechanisms by which endogenous bacteria and fungi antagonize each other (Vorholt 2012).

Aryl polyene are yellow pigments produced by bacteria living in widely varied environments such as soil, human intestines, or other ecological niches. Embedded in the membrane of the bacteria, they protect against oxidative stress or reactive oxygen species. (Grammbitter et al. 2019). The forces of biochemical adaptive evolution operate at the level of genes, manifesting in complex phenotypes and the global biodiversity of proteins and metabolites. Secondary metabolites characterizations aiming to decipher how these factors shape adaptive landscapes is an interesting era of bacterial plant pathogen studies. To our knowledge, the first evidence of derived type II polyketide synthases bacterial pigment across the family *Pectobacteriaceae* was documented in our in silico analysis.

The majority of detectable metabolites within the *Brenneria* genomes were related to the iron acquisition (Table 6). In response to iron limitation, many bacteria and some fungi produce siderophores. Turnerbactin is a triscatecholate siderophore that was first described in *Teredinibacter turnerae*, a shipworm

endosymbiont found in decaying wood floating on water. This compound is crucial in obtaining iron, bacterial competition, and to the survival of the symbiont in nature (Han et al. 2013). The number of gene clusters related to iron acquisition and transfer within the *Brenneria* clades members (of various types) suggests they may be crucial to the successful colonization of their plant host. The in silico evidence presented here suggest that these factors could be important in epiphytic maintenance of bacterial species and that experimental evidence should be sought to discover their role in planta.

Our findings provide the first genome/pangenome-based approach conducted on the genus *Brenneria* and allowed the classification of our new isolate into novel species, whose description is given below. The genetic variations cataloged here provide new insights into the evolutionary history of the genus, generating hypotheses about phylogenetic relationships, common and new pathogenicity scenarios, and how possibly they may synchronize their functions as a dominant complex community of the *Brenneria*. Functionally distinctive domains (in singletons category) within the *Brenneria* species genomes that specifically massive enzymatic potential activity, transcriptional regulatory, and ABC transporters in many types encoded by *B. izadpanahii* and huge number of phage-related genes in *B. goodwinii* are only small examples that show the diversification and adaptive capabilities in the biology of *Brenneria*, and each species may act as a puzzle pieces. Other members encompass a variety batch of pathogenicity repertoires that enable them to settle an offensive front line against the host defense barriers and become dominant taxa in the microbiome. These data are consistent with our observations in the Iranian diseased oak pathosystem that isolation of multiple *Brenneria*

TABLE 4. The carbohydrate-active enzymes database (CAZym) top 10 most frequent and hit repertoires of the *Brenneria* species and their relevant family *Pectobacteriaceae*^a

Carbohydrate-active enzymes family	Polysaccharide lyase	Glycoside hydrolase	Glycosyl transferase
<i>B. izadpanahii</i> (Iran 50) (CP050854)	PL22	GH31-GH102-GH105-GH42-GH4-GH94	GT2-GT9-GT84
<i>B. goodwinii</i> FRB141 (CP014137)	PL22-PL2	GH31-GH102-GH105-GH23-GH94-GH105	GT2-GT84
<i>B. goodwinii</i> FRB171 (MJLY000000000)	PL22-PL2	GH31-GH102-GH105-GH23-GH94-GH105	GT2-GT84
<i>B. goodwinii</i> OBR1 (CGIG000000000)	PL22-PL2	GH31-GH102-GH105-GH23-GH94-GH105	GT2-GT84
<i>B. nigrifluens</i> ATCC 13028 (CP034036)	PL22-PL2	GH31-GH102-GH94-GH105-GH23	GT2-GT84-GT56
<i>B. nigrifluens</i> LMG 2694 (QDKK000000000)	PL22-PL2	GH31-GH102-GH94-GH105-GH23	GT2-GT84-GT56
<i>Brenneria</i> sp. EniD312 (NZ_CM001230.1)	PL22-PL2	GH31-GH102-GH94-GH105-GH23	GT2-GT84-GT56
<i>B. alni</i> NCPPB 3934 (MJLZ000000000)	–	GH102-GH23-GH94-GH4-GH3-GH103	GT2-GT84-GT9-GT65
<i>B. alni</i> DSM11811 (BioProject PRJNA234905)	–	GH102-GH23-GH94-GH4-GH3-GH103	GT2-GT84-GT9-GT65
<i>B. salicis</i> DSM30166 (MJMA000000000)	PL22-PL1	GH102-GH4-GH23-GH94	GT2-GT84-GT9-GT56
<i>B. salicis</i> (NZ_QNRY01000001)	PL22-PL1	GH102-GH4-GH23-GH94	GT2-GT84-GT9-GT56
<i>B. rubrifaciens</i> 6D370 (CP034035)	–	GH31-GH4-GH23-GH102-GH94-GH3-GH105	GT2-GT56-GT9
<i>B. roseae</i> ssp. <i>americana</i> LMG 27715 (NZ_QDKJ000000000.1)	PL22-PL2-PL17	GH31-GH105-GH102-GH23-GH94	GT2-GT84
<i>B. roseae</i> ssp. <i>roseae</i> LMG 27714 (QDKI000000000)	PL22-PL2-PL1	GH31-GH105-GH102-GH23-GH94	GT2-GT84
<i>B. corticis</i> gBX10-1-2 (QDKH000000000)	PL22	GH1-GH31-GH102-GH105-GH94-GH23	GT2-GT84-GT56
<i>L. quercina</i> ssp. <i>quercina</i> ATCC 29281 (NZ_JIBO000000000.1)	–	CH23-CH102-GH94-GH3	GT2-GT4-GT9-GT84-GT26-GT81
<i>L. quercina</i> ssp. <i>iberica</i> LMG 26265 (NZ_LUTQ000000000.1)	–	GH23-GH102-GH94 GH3	GT2-GT4-GT9-GT26-GT84-GT81
<i>L. quercina</i> ssp. <i>britannica</i> 477 (CP023009)	–	GH102-GH23-GH94-GH3	GT2-GT4-GT9-GT26-GT84-GT81
<i>L. quercina</i> ssp. <i>britannica</i> LMG 26267 (NZ_LUTN000000000.1)	–	GH102-GH23-GH94-GH3	GT2-GT4-GT9-GT26-GT84-GT81
<i>L. quercina</i> ssp. <i>populi</i> CFCC 11196 (NZ_LUTE000000000.1)	–	GH102-GH23-GH3-GH94	GT2-GT4-GT9-GT26-GT84-GT81
<i>L. quercina</i> ssp. <i>populi</i> CFCC13122 (NZ_LUTI01000001)	–	GH102-GH23-GH94-GH37	GT2-GT4-GT9-GT26-GT84-GT81
<i>Dickeya dadantii</i> 3937 (NC_014500)	PL22-PL4	GH28-GH1-GH31-GH105-GH102	GT9-GT2
<i>Dickeya paradisaica</i> NCPPB 2511 (NZ_CM001857)	PL22	GH105-GH31-GH102-GH1-GH3-GH23	GT9-GT2
<i>Pectobacterium carotovorum</i> ssp. <i>carotovorum</i> PC1 (NC_012917)	PL1-PL4-PL22-PL2	GH28-GH1-GH31-GH105	GT2

^a The family carbohydrate esterase (CE) only had three most frequent and top hits for CE1 in *Dickeya dadantii*, *D. paradisaica*, and *P. carotovorum* ssp. *carotovorum* PC1. The family carbohydrate-binding (CB) module had no most frequent and top hit within the genomes investigated in this study (PL, polysaccharide lyase family; GH, glycoside hydrolases family; and GT, glycosyl transferase family).

TABLE 5. Prophages within the genome sequences of *Brenneria* spp. and relevant *Pectobacteriaceae* strains detected using the online service PHASTER

Organism (accession)	Region length (kb)	Completeness	Number of proteins	Position	Phage (GenBank accession number)	GC%
<i>B. izadpanahii</i> (Iran 50) (CP050854)**	10.5 kb	Incomplete	14	2164668–2172805	PHAGE_Salmon_SEN1_NC_029003(3)	45.56%
	32.6 kb	Intact	37	4580442–4613088	PHAGE_Aeromo_phiO18P_NC_009542(16)	49.60%
	17.7 kb	Incomplete	10	5308310–5326077	PHAGE_Gordon_Schwabellier_NC_031255(1)	55.40%
<i>B. goodwinii</i> FRB141 (CP014137)**	31.8 kb	Intact	46	5067377–5099204	PHAGE_Vibrio_12B12_NC_021070(27)	52.22%
	47.7 kb	Intact	61	1117565–1165336	PHAGE_EnteromEp237_NC_019704(8)	49.35%
	47.2 kb	Intact	50	5313441–5360697	PHAGE_Shigel_Sfil_NC_021857(8)	49.89%
<i>B. goodwinii</i> FRB171 (MJLY000000000)*	37.5 kb	Intact	51	100426–137944	PHAGE_Pectob_ZF40_NC_019522(20)	49.01%
	26.9 kb	Questionable	15	254–27201	PHAGE_Salini_M1EM_1_NC_042348(1)	46.24%
	12.7 kb	Incomplete	9	56752–69526	PHAGE_Paenib_Fern_NC_028851(1)	49.67%
<i>B. goodwinii</i> OBR1 (CGIG000000000)**	34.6 kb	Intact	45	4675256–4709932	PHAGE_Pectob_ZF40_NC_019522(18)	49.21%
<i>B. nigrifluens</i> ATCC 13028 (CP034036)**	35.7 kb	Intact	43	2091919–2127630	PHAGE_Salmon_118970_sal3_NC_031940(11)	52.93%
	34.4 kb	Intact	46	2352275–2386708	PHAGE_Mannhe_vB_MhM_3927AP2_NC_028766(15)	52.77%
	48.9 kb	Intact	52	2587333–2636302	PHAGE_Pectob_ZF40_NC_019522(32)	52.43%
	46.1 kb	Intact	58	2909769–2955959	PHAGE_Pectob_ZF40_NC_019522(18)	50.91%
	35.2 kb	Intact	48	3485094–3520326	PHAGE_Burkho_BcepMu_NC_005882(34)	54.95%
	34.6 kb	Intact	46	4625062–4659760	PHAGE_Salmon_SEN5_NC_028701(22)	51.13%
	14.8 kb	Incomplete	17	1439155–1453976	PHAGE_Enterom_P4_NC_001609(3)	49.87%
	33.4 kb	Incomplete	13	2066903–2100388	PHAGE_Salmon_118970_sal3_NC_031940(3)	50.32%
	27.8 kb	Incomplete	14	4155884–4183780	PHAGE_Acinet_vB_AbaS_TRS1_NC_031098(1)	49.08%
<i>Brenneria</i> sp. EniD312 (NZ_CM001230.1)**	14.8 kb	Incomplete	17	1433989–1448810	PHAGE_Enterom_P4_NC_001609(3)	49.87%
	62.5 kb	Intact	61	2065618–2128139	PHAGE_Salmon_118970_sal3_NC_031940(14)	52.29%
	34.4 kb	Intact	45	2346736–2381169	PHAGE_Mannhe_vB_MhM_3927AP2_NC_028766(15)	52.77%
	48.9 kb	Intact	51	2581794–2630763	PHAGE_Pectob_ZF40_NC_019522(32)	52.43%
	61.2 kb	Intact	58	2904230–2965489	PHAGE_Pectob_ZF40_NC_019522(18)	50.83%
	35.2 kb	Intact	48	3537380–3572612	PHAGE_Burkho_BcepMu_NC_005882(34)	54.95%
	27.8 kb	Incomplete	14	4208171–4236067	PHAGE_Acinet_vB_AbaS_TRS1_NC_031098(1)	49.08%
	34.6 kb	Intact	44	4677347–4712045	PHAGE_Salmon_SEN5_NC_028701(22)	53.13%
	8.9 kb	Incomplete	11	4225894–4234795	PHAGE_Paenib_Fern_NC_028851(1)	48.76%
<i>B. alni</i> NCPPB 3934 (MJLZ000000000)**	35.9 kb	Intact	52	49840–85804	PHAGE_Vibrio_12B12_NC_021070(29)	51.29%
	61.5 kb	Intact	68	13957–75481	PHAGE_Escher_PA28_NC_041935(14)	50.72%
	26.2 kb	Incomplete	36	59809–86095	PHAGE_Burkho_BcepMu_NC_005882(22)	54.45%
	64.5 kb	Intact	83	28–64615	PHAGE_Pseudo_B3_NC_006548(20)	54.90%
	15.9 kb	Incomplete	21	2479–18388	PHAGE_Enterom_vB_KleM_RaK2_NC_019526(1)	43.98%
<i>B. salicis</i> DSM30166 (MJMA000000000)*	34.9 kb	Intact	49	80150–115135	PHAGE_Vibrio_12B12_NC_021070(29)	51.00%
	11.6 kb	Intact	17	209–11865	PHAGE_Escher_HK75_NC_016160(7)	49.92%
<i>B. rubrifaciens</i> 6D370 (CP034035)**	33.5 kb	Intact	41	3759662–3793182	PHAGE_Salmon_SEN5_NC_028701(20)	51.50%
	21.8 kb	Incomplete	9	2779933–2801797	PHAGE_Escher_phiV10_NC_007804(2)	51.55%
	18.6 kb	Questionable	21	404008–422613	PHAGE_Enterom_P4_NC_001609(1)	51.30%
	23.5 kb	Questionable	22	1352642–1376210	PHAGE_Paenib_Tripp_NC_028930(2)	50.38%
<i>B. roseae</i> ssp. <i>americana</i> LMG 27715 (NZ_QDKJ000000000.1)*	44.6 kb	Intact	64	99117–143778	PHAGE_Pectob_ZF40_NC_019522(28)	50.48%
	37.0 kb	Intact	34	111756–148839	PHAGE_Salmon_SEN5_NC_028701(15)	53.48%
	28.9 kb	Incomplete	17	294796–323702	PHAGE_Pectob_ZF40_NC_019522(3)	52.02%
	5.0 kb	Incomplete	10	182444–187482	PHAGE_Sodali_phiSG1_NC_007902(2)	45.47%
<i>B. roseae</i> ssp. <i>roseae</i> LMG 27714 (QDKI000000000)*	23.4 kb	Incomplete	15	49239–72733	PHAGE_EnteromEp234_NC_019715(2)	48.40%
<i>B. corticis</i> gBX10-1-2 (QDKH000000000)*	29.2 kb	Intact	32	1–29251	PHAGE_Aeromo_phiO18P_NC_009542(14)	49.85%
	14.8 kb	Intact	17	101137–116026	PHAGE_Escher_pro483_NC_028943(9)	52.78%
	30.3 kb	Incomplete	37	1–30367	PHAGE_Escher_PA28_NC_041935(9)	54.11%
<i>L. quercina</i> ssp. <i>quercina</i> ATCC 29281 (NZ_JIBO000000000.1)*	27.7 kb	Questionable	44	99899–127641	PHAGE_Burkho_phiE255_NC_009237(20)	59.37%
	15.4 kb	Questionable	20	142035–157468	PHAGE_Escher_D108_NC_013594(2)	50.12%
	30.4 kb	Incomplete	10	65883–96379	PHAGE_Bacill_Shanette_NC_028983(2)	50.17%
<i>L. quercina</i> ssp. <i>iberica</i> LMG 26265 (NZ_LUTQ000000000)*	12.1 kb	Incomplete	16	111–12306	PHAGE_Burkho_BcepMu_NC_005882(13)	61.31%
	5.3 kb	Incomplete	9	132–5481	PHAGE_Salmon_SEN1_NC_029003(4)	47.51%
<i>L. quercina</i> ssp. <i>britannica</i> 477(CP023009)**	36.2 kb	Intact	49	42746–78945	PHAGE_Burkho_phiE255_NC_009237(22)	58.53%
	17.6 kb	Incomplete	15	256669–274319	PHAGE_Enterom_P4_NC_001609(2)	50.41%
	10.8 kb	Incomplete	11	1666900–1677782	PHAGE_Salisa_1_NC_017983(1)	51.15%
	52.4 kb	Intact	66	2839465–2891943	PHAGE_Cronob_ENT47670_NC_019927(11)	50.66%
	29.3 kb	Intact	42	3985464–4014799	PHAGE_Burkho_BcepMu_NC_005882(20)	59.88%
<i>L. quercina</i> ssp. <i>populi</i> CFCC 11196 (NZ_LUTE000000000.1)*	11.3 kb	Incomplete	13	47358–58710	PHAGE_Escher_D108_NC_013594(2)	51.58%
	10.3 kb	Incomplete	18	3459–13797	PHAGE_Burkho_phiE255_NC_009237(9)	56.03%
	21.0 kb	Intact	31	683–21688	PHAGE_Salmon_Fels_2_NC_010463(22)	56.02%
<i>Dickeya dadantii</i> 3937 (NC_014500)**	32.8 kb	Intact	44	915372–948193	PHAGE_Haemop_HP1_NC_001697(17)	52.11%
	6.9 kb	Incomplete	10	1903506–1910478	PHAGE_Erwin_vB_EamM_RisingSun_NC_042018(2)	50.87%
	23.6 kb	Questionable	19	2024025–2047705	PHAGE_Enterom_P4_NC_001609(3)	50.12%
	27.8 kb	Intact	33	2733074–2760902	PHAGE_Escher_pro483_NC_028943(12)	56.34%
	26.1 kb	Incomplete	22	3123500–3149610	PHAGE_Ralsto_RSY1_NC_025115(2)	52.75%
<i>Dickeya paradisaica</i> NCPPB 2511 (NZ_CM001857)**	31.3 kb	Incomplete	22	3944213–3975565	PHAGE_Paenib_Fern_NC_028851(1)	48.84%
	17.0 kb	Questionable	22	4081106–4098183	PHAGE_Enterom_P4_NC_001609(3)	50.16%
	36.4 kb	Intact	37	4377338–4413753	PHAGE_Salmon_SEN5_NC_028701(18)	55.43%

(Continued on next page)

a **, (.gff and .fasta); and *, (.fasta).

TABLE 5. (Continued from previous page)

Organism (accession)	Region length (kb)	Completeness	Number of proteins	Position	Phage (GenBank accession number)	GC%
<i>Pectobacterium carotovorum</i> ssp. <i>carotovorum</i> PC1 (NC_012917)**	12.9 kb	Incomplete	15	833155–845434	PHAGE_Enterо_P4_NC_001609(5)	46.86%
	20.9 kb	Incomplete	24	1811972–1832962	PHAGE_Burkho_BcepMu_NC_005882(16)	53.15%
	13.8 kb	Incomplete	17	2642964–2656853	PHAGE_Shigel_Ss_VASD_NC_028685(2)	43.54%
	36.4 kb	Intact	48	2986224–3022684	PHAGE_Salmon_SEN4_NC_029015(20)	49.57%
	35.4 kb	Intact	50	3573259–3608691	PHAGE_Enterо_PsP3_NC_005340(18)	52.08%
	12.8 kb	Incomplete	15	3683594–3696452	PHAGE_Enterо_P4_NC_001609(5)	45.98%

TABLE 6. In silico secondary metabolite profiling by searching against the antiSMASH database-bacterial version within the genome sequences of *Brenneria* spp. and relevant *Pectobacteriaceae* strains

Organism (accession)	Type ^a	Position	Most similar known cluster	Similarity (%)
<i>B. izadpanahii</i> (Iran 50) (CP050854)	NRPS-TIPKS	558,967–615,840	Yersiniabactin	8%
	Thiopeptide	2,039,704–2,064,242	O-antigen	14%
	NRPS	2,900,414–2,947,453	Turnerbactin	38%
	Thiopeptide-LAP	3,151,259–3,205,833	Klebsazolicin	60%
<i>B. goodwinii</i> FRB141 (CP014137)	NRPS	1,334,406–1,379,9911	Turnerbactin	23%
	Thiopeptide	2,090,803–2,117,249	O-antigen	14%
	NRPS-TIPKS	3,123,664–3,176,845	–	–
	NRPS-TIPKS	4,483,386–4,507,022	Yersiniabactin	10%
<i>B. goodwinii</i> FRB171 (MJLY00000000)	Thiopeptide	240,768–267,250	O-antigen	14%
	NRPS-TIPKS	43,419–98,435	–	–
	Arylpolyene	16,023–59,618	Arylpolyene	100%
	NRPS	1–31,659	–	–
	NRPS-TIPKS	1–16,865	Microcin	26%
	NRPS	37,233–65,550	Amonabactin	42%
	NRPS	1–6,339	–	–
	NRPS	1–3173	–	–
	NRPS	1–3141	–	–
	NRPS-TIPKS	523,740–575,858	–	–
<i>B. goodwinii</i> OBR1 (CGIG00000000)	Arylpolyene	1,624,347–1,667,942	Arylpolyene	100%
	NRPS	2,367,733–2,410,744	Thailanstatin	10%
	NRPS-TIPKS	3,652,192–3,715,065	N-myristoyl-D-asparagine	8%
	NRPS	4,052,101–4,099,132	Turnerbactin	23%
	Thiopeptide	4,813,399–4,839,881	O-antigen	14%
	Betalactone	749,459–773,280	–	–
	Hserlactone	739,318–805,205	Taxllaid	8%
<i>B. nigrifluens</i> ATCC 13028 (CP034036)	NRPS-TIPKS	2,202,105–2,260,740	Yersiniabactin	14%
	Thiopeptide	2,746,000–2,772,417	O-antigen	14%
	NRPS	2,945,000–3,030,660	Pseudomonine	20%
	NRPS-TIPKS	4,169,935–4,261,339	Entrerobactin	12%
	Arylpolyene	4,786,417–4,829,997	Arylpolyene	100%
	Betalactone	748,456–772,277	–	–
	Hserlactone	1,760,324–1,780,956	Taxllaid	8%
	NRPS-TIPKS	2,196,941–2,255,576	Yersiniabactin	14%
	Thiopeptide	2,740,461–2,766,878	O-antigen	14%
	NRPS	2,932,508–2,979,014	Pyoverdine	1%
<i>Brenneria</i> sp. EniD312 (NZ_CM001230.1)	NRPS	2,995,656–3,082,947	Pseudomonine	20%
	NRPS-TIPKS	4,222,222–4,313,626	Entrerobactin	12%
	Arylpolyene	4,838,702–4,882,282	Arylpolyene	100%
	Betalactone/ siderophore	72,368–100,981	Aerobactin	100%
	Phenaine	99,314–119,772	Fontizine	60%
	NRPS	3,616–59,328	Turnerbactin	30%
	Arylpolyene	1,989–45,584	Arylpolyene	88%
	TIPKS	1–42,595	Filipine	15%
	Ladderane	1–29,330	–	–
	NRPS-TIPKS	9,487–47,918	–	–
<i>B. salicis</i> DSM30166 (MJMA00000000)	Betalactone/ siderophore	137,738–165,465	Aerobactin	100%
	Hserlactone	1–18,735	–	–
	NRPS	64,241–90,327	–	–
	NRPS	517–56,210	Turnerbactin	30%
	Thiopeptide	40,220–60,229	O-antigen	14%
	NRPS-TIPKS	5,785–36,071	–	–
	TIPKS	1–25,905	Oronofac acid	100%
	Arylpolyene	1–17,535	–	–

(Continued on next page)

^a NRPS, nonribosomal peptide synthetase cluster; LAP, linear azol (in)e-containing peptides; TPKS, type (I, II, or III) polyketide synthase; and hserlactone, homoserine lactone cluster.

TABLE 6. (Continued from previous page)

Organism (accession)	Type ^a	Position	Most similar known cluster	Similarity (%)
<i>B. rubrifaciens</i> 6D370 (CP034035)	NRPS	358,508–410,513	Amanobactin	100%
	Butyrolactone	730,919–742,022	–	–
	NRPS	1,654,251–1,702,638	–	–
	Thiopeptide	2,182,699–2,209,220	O-antigen	14%
	NRPS-Hserlactone	2,375,295–2,461,142	Syngomycin	17%
	NRPS	2,469,826–2,516,293	Butenoic acid	30%
<i>B. roseae</i> ssp. <i>americana</i> LMG 27715 (NZ_QDKJ00000000.1)	Siderophore	3,141,150–3,155,590	Aerobactin	100%
	Thiopeptide	94,264–120,787	O-antigen	14%
	NRPS	212,882–267,597	Turnerbactin	46%
	Betalactone	176,245–200,064	–	–
<i>B. roseae</i> ssp. <i>roseae</i> LMG 27714 (QDKI00000000)	Betalactone	243,443–267,262	–	–
	Arylpolyene	226,092–269,720	Arylpolyene	100%
	Thiopeptide	74,157–100,680	O-antigen	14%
<i>B. corticis</i> gBX10-1-2 (QDKH00000000)	NRPS-TIPKS	1–76,023	Oronofacic acid	100%
	NRPS	194,852–238,739	–	–
	NRPS	1–38,204	Turnerbactin	–
	Betalactone	174,393–198,312	–	–
	Siderophore	173,811–185,640	Putrebactin/avarobactin	30%
	Hserlactone	18,131–38,763	TaaxIIIaid	13%
	NRPS	104,640–174,717	Pseudomonine	20%
	Bacteriocin	766–12,355	–	–
	Thiopeptide	104,826–131,262	O-antigen	14%
	Arylpolyene	22,023–65,603	Arylpolyene	100%
	Siderophore	148,709–167,707	Vibrioferin	18%
	NRPS	6,790–64,163	–	–
	NRPS	220,473–281,198	Lipopolysaccharide	22%
	TIPKS	410,032–454,785	Candicidin	9%
	NRPS	411,236–455,102	Butenoic acid	30%
<i>L. quercina</i> ssp. <i>quercina</i> ATCC 29281 (NZ_JIBO00000000.1)	Thiopeptide	192,990–219,448	O-antigen	14%
	NRPS	11,225–67,022	Turnerbactin	30%
	Arylpolyene	21,577–65,157	Arylpolyene	100%
	NRPS	101,472–134,275	Butenoic acid	30%
	TIPKS	1–26,675	Filipin	23%
	NRPS	90,840–118,379	Lipopolysaccharide	22%
	Thiopeptide	5,340–31,799	O-antigen	14%
	NRPS	638–49,560	–	–
	NRPS	1–41,120	Turnerbactin	30%
	Siderophore	1–16,115	–	–
<i>L. quercina</i> ssp. <i>iberica</i> LMG 26265 (NZ_LUTQ00000000.1)	TIPKS	1–19,336	Algamyacin	5%
	NRPS	1–10,512	Ralatomyacin	40%
	NRPS	86,969–142,768	Turnerbactin	30%
	NRPS	284,756–328,613	Idigoidine	60%
	Thiopeptide	38,462–64,920	O-antigen	14%
	TIPKS	95,348–174,919	Filipin	30%
	NRPS	251,738–290,164	Glidiopeptin	37%
	NRPS	100,934–144,800	Butenoic acid	30%
	Siderophore	102,938–121,927	Vibrioferin	18%
	NRPS	45,465–110,621	S56-p	11%
	NRPS	1–3,204	–	–
	NRPS	1–2,567	Rhizomide	100%
	NRPS	67,007–118,316	Turnerbactin	30%
	Thiopeptide	13,924–40,378	O-antigen	14%
	NRPS	22,163–70,982	Glidiopeptin	25%
<i>L. quercina</i> ssp. <i>populi</i> CFCC 11196 (NZ_LUTE00000000.1)	Arylpolyene	13,010–55,217	Arylpolyene	100%
	NRPS	4,665–26,436	–	–
	Siderophore	1–16,128	–	–
	NRPS	1–18,036	–	–
	NRPS	1–13,461	S56-p	9%
	NRPS	1–4,019	Rhizomide A	100%
	NRPS	1–2,677	Rhizomide B	100%
	NRPS	1–2,531	Rhizomide C	100%
	Siderophore	1,737,415–1,756,606	–	–
	NRPS	1,864,721–1,911,445	Rhizomide	100%
	Thiopeptide	2,166,294–2,192,792	O-antigen	14%
	NRPS	3,344,779–3,400,479	Turnerbactin	38%
	Bacteriocin	4,123,513–4,147,303	–	–
	Cyanobactin	4,415,302–4,437,263	–	–
	Hserlactone	4,701,004–4,765,018	Idigoidine	60%
<i>Dickeya paradisaica</i> NCPPB 2511 (NZ_CM001857)	Betalactone	690,792–714,578	–	–
	T3PKS	1,590,366–1,708,048	Oocydlin	86%

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TABLE 6. (Continued from previous page)

Organism (accession)	Type ^a	Position	Most similar known cluster	Similarity (%)
<i>Pectobacterium carotovorum</i> ssp. <i>carotovorum</i> PC1 (NC_012917)	NRPS	1,722,708–1,776,660	Amonabactin	57%
	NRPS	1,944,874–1,999,802	Sulfazecin	11%
	Thiopeptide	2,603,050–2,629,807	O-antigen	14%
	NRPS	3,508,607–3,616,479	S56-p1	15%
	Siderophore	3,649,496–3,664,102	Vibrioferin	36%
	NRPS	508,574–562,953	Amonabactin	100%
	NRPS-T1PKS	639,867–695,982	–	–
	Thiopeptide	1,985,432–2,011,857	O-antigen	14%
	Betalactone	4,056,003–4,079,817	–	–
	Siderophore	4,390,324–4,402,225	–	–
	Hserlactone	4,668,638–4,689,288	–	–

species (*B. goodwinii*, *B. roseae* ssp. *roseae*, *B. nigrifluens*—only two isolates—, and *Brenneria* sp.—now named *B. izadpanahii*) from individual symptomatic oak trees indicated the complexity of the syndromes that *Brenneria* species are involved (Bakhshi ganje et al. 2020). In the light of high-throughput sequencing technologies and improved metagenomics (e.g., metagenomic based- strain-level identification [Mechan Llonet et al. 2020]), metatranscriptomics, and metaproteomics analyses, studies of tree and forest ecosystem microbiomes have increased and consequently lead to developing an understanding of complicated communications between the species.

Description of *B. izadpanahii* sp. nov. *B. izadpanahii* sp. nov. (i.zad.pa.nah'i.i. N.L. gen. n. izadpanahii, named in honor of Professor Keramatollah Izadpanah, for his 6 decades of efforts in plant pathology education and research in Iran). Cells are gram stain-negative and facultatively anaerobic. Colonies are pale cream, circular, opaque, convex with entire margins, smooth and approximately 0.5 to 1.0 mm in diameter after incubation for 2 days at 30°C on nutrient agar. Growth occurs at 10 to 40°C and the optimum growth temperature is 30°C. It is negative for activities of lysine decarboxylase, arbutin (β -galactosidase), arginine dihydrolase, nitrate reductase, ornithine decarboxylase, urease, oxidase, d-arabinose, and l-rhamnose. It is positive for fermentation of inositol, amygdalin, sucrose, glucose, mannitol, and arabinose. Positive for acid production from glycerol, L-arabinose, D-xylose, aesculin ferric citrate, D-glucose, D-fructose, β -d-methyl glycoside, inositol, D-mannitol, raffinose, melibiose, sucrose, trehalose, and D-galactose. It is positive for assimilation of Tween 80, N-acetyl-D-glucosamine, L-arabinose, D-fructose, D-galactose, a-D-glucose, m-inositol, D-mannitol, D-mannose, melibiose, raffinose, sucrose, trehalose, glycerol, glucose-1-phosphate, and glucose-6-phosphate. The DNA G+C content is 53.66 mol%. The type strain is Iran isolate 50 (BioProject PRJNA616088; accession number CP050854 = Iran 52 = Iran 29), isolated from symptomatic bark of *Q. castaneifolia* canker in north of Iran.

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