Drippy Pod of White Lupine: A New Bacterial Disease Caused by a Pathovar of *Brenneria quercina*

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ABSTRACT

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Drippy pod is a unique bacterial disease of Mediterranean white lupine (*Lupinus albus*) that first appeared in commercial fields in Eastern Washington State in the mid-1980s. The disease is most noticeable in the field as water-soaked lesions on lupine pods that produce an abundance of whitish-colored ooze with a sticky and foamy consistency. As the disease progresses, yellowing of lupine plants occurs with ooze characteristically dripping down the infected pods and stems and solidifying. A gram-negative rod-shaped bacterium with facultative anaerobic growth was repeatedly isolated from infected lupine tissues, and subsequently confirmed by Koch's postulates to infect lupines. Physiological and biochemical tests, including the API 20E and 50CHE strip assays, showed a highly uniform phenotype for the lupine strains that was distinctive for the genus *Brenneria* and most closely resembled the oak pathogen *Brenneria quercina.* Furthermore, sequence analyses of the 16S rDNA gene and the 16S-23S intergenic region of lupine strains revealed the highest similarity (>97%) to the corresponding regions of *B. quercina* and less similarity to the next closest species, *B. salicis.* Fatty acid profiling demonstrated that lupine strains were qualitatively similar in composition to *Brenneria* spp., and supported placement of the drippy pod bacterium in the species *B. quercina.* Oak strains of *B. quercina*, however, did not incite drippy pod disease on lupine. Consequently, the lupine strains that cause bacterial drippy pod disease were classified as *B. quercina* pv. *lupinicola* pv. nov.

White lupine (*Lupinus albus* L.) is a shrublike legume plant and has been cultivated more than 3,000 years in the Mediterranean region (13). Lupine seeds have high protein content, up to 40% dry matter, and stalks of the plant are used for silage or hay. The lupine is able to fix nitrogen biologically, and is commonly used as a green manure crop. In the 1980s, white lupines were evaluated in the inland Pacific Northwest as an alternative crop for forage (31).

A previously undescribed disease was discovered on field-grown white lupines in Eastern Washington in the 1980s that caused severe infections that limited the suitability of lupine as an alternative crop. Diseased plants in the fields first showed yellowing around bloom period in late spring when pods were first set. The affected pods had small water-soaked brown lesions, and as the disease progressed lesions exuded frothy, sticky ooze (Fig. 1). Consequently, the common name for the disease was "drippy pod" of lupine. A bacterial etiology was suspected from the beginning due to the consistent observation of bacterial streaming from the infected lupine tissues. An entire pod could be destroyed once infected with the drippy pod disease. The ooze attracted the feeding of phytophagous insects, primarily *Lygus* spp., which rapidly spread disease throughout the lupine field to typically result in nearly all plants becoming infected (26). In some fields disease was extensive, with almost complete destruction of the lupine crop.

In a preliminary report by Regner and Gross (33) in 1988, the drippy pod pathogen was provisionally identified as a member of the genus *Erwinia* and as a possible new species called *E. lupinicola*. The results were based on key physiological and biochemical tests including a gram-negative stain reaction, growth under anaerobic conditions, nonpectolytic activity, and overall growth and colony characteristics (35). Subsequently, the genus *Erwinia* was subdivided into several genera based on 16S rDNA sequence analysis of plant-pathogenic enterobacterial species (15). In particular, the genus *Brenneria* was established in 1998 (15,16) to compose a distinct phylogenetic group with six recognized species, namely *B. alni*, *B. nigrifluens*, *B. paradisiaca*, *B. quercina*, *B. rubrifaciens*, and *B. salicis*. These bacterial species cause blights, cankers, wilts, and necrosis of various trees, such as oaks, willows, and walnuts (15,16). *B. quercina* was of particular interest because of its ability to cause "drippy nut" disease of several species of oaks with symptoms characterized by profuse oozing from bacterial infections (5,19).

Three of the bacterial strains isolated from lupines collected in Eastern Washington (33) were sent to another laboratory where they later were distributed for phylogenetic studies of enterobacterial plant pathogens (7,28). Brown et al. (7) analyzed the *gapDH* gene, which encodes glyceraldehyde-3-phosphate dehydrogenase, and established that the lupine strains were grouped with strains of *B. quercina* in a necrogenic clade distinct from other *Brenneria* species. A follow-up study by Naum et al. (28) of horizontal gene transfer of type III secretion system genes demonstrated that the phylogeny for lupine strains was again closely linked to *B. quercina* based on analyses of the *hrcC* and *hrcR* sequences, which encode structural proteins of the needle complex. However, these taxonomic studies employing lupine strains lacked geographic, pathological, morphological, and physiological context important to establishing a valid taxonomic allocation of these "lupinicola" strains to recognized species of enterobacterial plant pathogens.

The purpose of this study was to describe and characterize the causal agent responsible for the drippy pod disease that was ravaging fields of white lupine. Disease samples were collected from six locations in Eastern Washington, beginning in 1983 and concluding in 1988, and a series of 17 representative strains were evaluated in pathogenicity tests on lupine pods to confirm Koch's postulates. In contrast, we report that none of the related enterobacterial plant pathogens, including *B. quercina*, caused disease infections of lupines. In a numerical taxonomic study by Verdonck et al. (41), the API 20E and 50CHE systems (bioMérieux, Inc., Durham, NC) composed of a battery of standardized biochemical tests were used to phenotypically charac-

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The GenBank accession numbers of the 16S rRNA sequence of *Brenneria quercina* pv. *lupinicola* are HM196336, HM196337, HM196338, HM196339, HM196340, HM196341, and HM196342.

*The *e*-Xtra logo stands for "electronic extra" and indicates that eight supplemental figures are available in the online edition.

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terize plant-pathogenic enterobacterial species. These miniaturized API systems were reported to have the "highest discriminatory potential and reproducibility in *Erwinia*" (41), and therefore were used for phenotypic characterization of the lupine strains to permit direct comparisons of related species. We also analyzed the 16S rDNA and the 16S-23S rDNA intergenic sequences (IGS) of representative lupine strains to infer taxonomic relationships among plant-pathogenic enterobacteria at both the genus and species levels (11,15,22,38). Finally, cellular fatty acid analyses (1,5,9) were used to substantiate the clustering of the lupine strains with related *Brenneria* species. Because the phenotypic and genotypic characteristics of the lupine strains did not differentiate them from strains of *B. quercina*, it is proposed that the lupine strains be placed in a new pathovar called *B. quercina* pv. *lupinicola* pv. nov.

MATERIALS AND METHODS

Reference strains of plant-pathogenic bacteria and culture maintenance. Reference strains of plant-pathogenic enterobacterial species of *Brenneria*, *Erwinia*, *Dickeya*, *Pectobacterium*, and *Pantoea* are listed in Table 1 along with the original sources. All reference strains are named according to the approved list of nomenclature for plant-pathogenic members of the *Enterobacteriaceae* (12). Bacteria were routinely cultured on nutrient broth–yeast extract (NBY) agar medium (42) and incubated at 25°C. Strains were preserved at –80°C in glycerol stocks, and lyophilized cultures were prepared for long-term storage of bacterial strains.

Isolation of the causal agent from diseased lupines collected in Eastern Washington. Symptomatic lupine tissues were collected between 1986 and 1988 from six locations in Eastern Washington (Table 1, Fig. 2). Specimens of wild native lupines, identified as *L. sulphureus* and *L. leucophyllus*, with apparent drippy pod symptoms were scouted and collected in 1988 for isolation of the causal pathogen. In addition, an air-dried diseased herbarium specimen of *L. albus* collected near Warden, WA in 1983 was used for isolation of

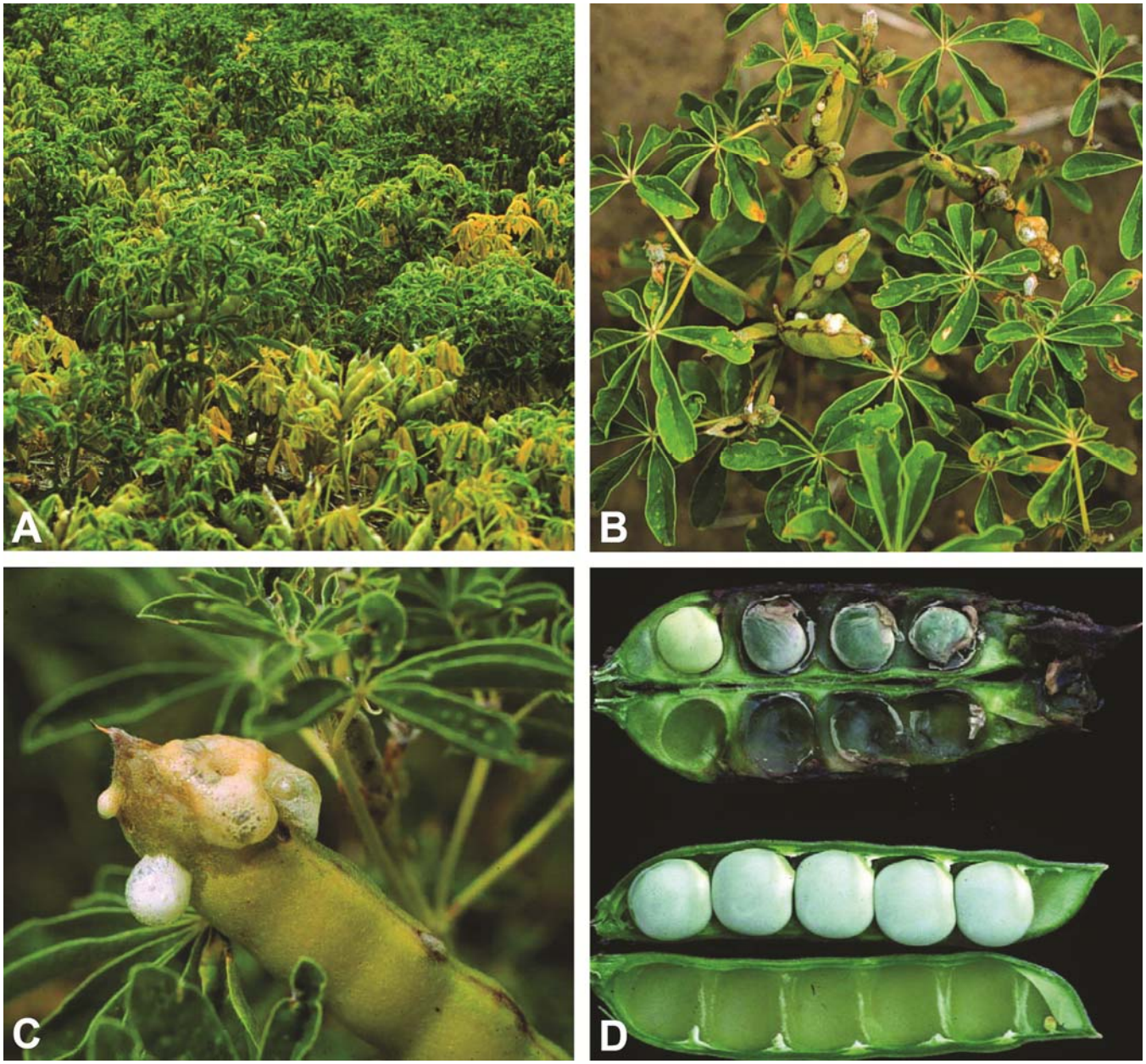

Fig. 1. Symptoms of drippy pod disease of white lupine (*Lupinus albus*) caused by a new pathovar, *Brenneria quercina* pv. *lupinicola*, in Eastern Washington State. **A,** Symptoms and incidence of drippy pod disease in a field near Waitsburg, WA, in July 1987; **B,** drippy pod symptoms observed from above a diseased lupine; **C,** close-up of an infected lupine pod showing profuse foaming from drippy pod infections; **D,** internal drippy pod disease symptoms on a lupine pod (top) compared with a healthy lupine pod (bottom).

the bacterial pathogen. Samples collected in the field were placed in an ice chest and transferred to the laboratory in Pullman, WA for isolation. The diseased tissues from infected lupine pods and stems were surface-sterilized with 0.25% (vol/vol) sodium hypochlorite solution for 2 min, rinsed three times with sterile distilled water (SDW), and then placed on the surface of paper towels. Typically, the tissue sections from advancing margins of the lesions were excised with a razor blade and then macerated with a sterile mortar and pestle in 50 µl of SDW. The resulting suspensions were serially diluted in SDW, plated onto NBY agar medium, and incubated at 25°C for 2 days. Single colonies of the bacterium were selected, streaked onto NBY agar plates for single colonies, and repeated at least twice prior to storage of the pure bacterial cultures. A total of 17 strains from lupine pod or stem lesions collected from different lupine fields or years (Table 1) were preserved for phenotypic and genotypic analyses as described below. All 17 strains were verified by pathogenicity tests to cause typical drippy pod lesions.

Pathogenicity tests in lupine pods and tobacco hypersensitivity tests. Pathogenicity tests were conducted by inoculation of full seed lupine pods (*L. albus* cv. Ultra) grown at the greenhouse facility at Washington State University. Bacterial strains were grown at 25°C in 5 ml of NBY liquid medium shake cultures (250 rpm) to late exponential growth phase. Cultures were centrifuged for 15 min at 11,700 × *g* to pellet cells. The cells were resuspended in SDW (5 ml), pelleted by centrifugation, and the wash step repeated. The bacteria were adjusted to an approximate final concentration of 2×10^8 colony forming units (CFU) per ml in SDW, and cell suspensions were kept on ice prior to inoculation of lupine pods. Inoculations were made with a sterile syringe (25-gauge needle) that delivered ~0.01 ml of the bacterial suspension per injection site. Each strain was tested on pods in triplicate lupine plants. The inoculated plants were returned to the greenhouse, and results of pathogenicity tests were recorded after 3 days. Positive tests for pathogenicity were recorded at day 3 for strains that caused water-soaked brown lesions that characteristically produced sticky ooze; strains testing negative did not form water-soaked lesions, and necrosis was limited to the wound site. Strains of *B. quercina* (strains ATCC 29281^T and NCPPB 1852), *B. salicis* (strain NCPPB 447^T), and *E. amylovora* (strains E9 and Ea225) were included in pathogenicity tests on lupine pods as reference strains to *Brenneria* strains isolated from lupines. Control lupine pods were injected with SDW.

Leaves of *Nicotiana tabacum* (cv. Havana) were used to test strains for the ability to induce a hypersensitive reaction. Strains were tested at a dose of 2×10^8 CFU per ml in SDW as described earlier (14).

Preliminary characterization of lupine strains. The colony characteristics and pigmentation of bacteria isolated from diseased lupines were observed on NBY agar and recorded after 2 days of incubation at 25°C. Initial tests for identification of bacteria, as described by Schaad (35), included the gram stain reaction, flagella stain reaction, ability to grow as a facultative anaerobe, gelatin liquefaction, and ability to form pits on crystal violet-pectate (CVP) medium (8). The lupine strains were compared to reactions observed for representative enterobacterial strains listed in Table 1 to identify species with the greatest similarity to the lupine strains.

Physiological and biochemical analysis. The API 20E and API 50CHE kits (bioMérieux) were used for physiological and biochemical analyses of the lupine strains following the methods described by Mergaert et al. (25). All of the strains were grown at 30°C overnight in 5 ml of NBY liquid medium. Bacterial cells were harvested by centrifugation and washed twice with SDW as described above. For API 20E test, the cells were suspended in SDW to a concentration equivalent to 0.5 McFarland. The test strips were handled as

Table 1. Lupine strains of *Brenneria* and reference bacterial strains

Strain[a]	Host	Origin[b]
Brenneria quercina pv. *lupinicola*		
W3L1	*Lupinus albus*	Waitsburg, Walla Walla Co., WA 1987 (this study)
W3L2	*L. albus*	Waitsburg, Walla Walla Co., WA 1987 (this study)
W3L4	*L. albus*	Waitsburg, Walla Walla Co., WA 1987 (this study)
W3L5	*L. albus*	Pullman, Whitman Co., WA 1987 (this study)
W3L7	*L. albus*	Davenport, Lincoln Co., WA 1987 (this study)
W3L8	*L. albus*	Warden, Warden Co., WA 1983 (this study)
W3L9	*L. albus*	Waitsburg, Walla Walla Co., 1986 (this study)
W3L13	*L. albus*	Waitsburg, Walla Walla Co., 1987 (this study)
W3L14	*L. albus*	Waitsburg, Walla Walla Co., 1987 (this study)
W3L15	*L. albus*	Waitsburg, Walla Walla Co., WA 1987 (this study)
W3L16	*L. albus*	Waitsburg, Walla Walla Co., WA 1987 (this study)
W3L18	*L. albus*	Davenport, Lincoln Co., WA 1986 (this study)
W3L19	*L. albus*	Davenport, Lincoln Co., WA 1986 (this study)
W3L20	*L. albus*	Davenport, Lincoln Co., WA 1986 (this study)
W3L21	*L. sulphureus*	Hanford Reservation, Benton Co., WA 1988 (this study)
W3L22	*L. leucophyllus*	Central Ferry, Whitman Co., WA 1988 (this study)
W3L23	*L. albus*	Waitsburg, Walla Walla Co., WA 1988 (this study)
B. rubrifaciens		
NCPPB 2020^T	*Juglans regia*	PDDCC
632-B	*J. regia*	C. Kado, University of California-Davis
B. salicis		
NCPPB 447^T (synonym = ATCC 15712^T)	*Salix alba*	PDDCC
B. nigrifluens		
ICPB EN104	*J. regia*	PDDCC
NCPPB 564^T	*J. regia*	PDDCC
B. quercina		
ATCC 29281^T (synonym = LMG 2724^T)	*Quercus agrifolia*	ATCC
NCPPB 1852	*Quercus* sp.	PDDCC
Erwinia amylovora		
Ea225	*Malus domestica*	S. Beer, Cornell University
E9	*Pyrus communis*	A. Chatterjee, University of Missouri
E. rhapontici		
NCPPB 1578^T	*Rheum rhaponticum*	PDDCC
Dickeya dadantii		
A310	*Saintpaulia ionantha*	N. Schaad, USDA-ARS
A15	*Ipomoea batatas*	N. Schaad, USDA-ARS
Pectobacterium cypripedii		
NCPPB 752	*Cypripedium* sp.	PDDCC
ATCC 29267^T	*Cypripedium* sp.	ATCC
P. carotovorum subsp. *carotovorum*		
cc306	*Solanum tuberosum*	M. Powelson, Oregon State University
W3C105	*S. tuberosum*	M. Powelson, Oregon State University
Pantoea stewartii		
Missouri	*Zea mays*	A. K. Vidaver, University of Nebraska

[a] Type strains are identified by "T".

[b] Abbreviations: ATCC = American Type Culture Collection; ICPB = International Collection of Phytopathogenic Bacteria; NCPPB = National Collection of Plant Pathogenic Bacteria; PDDCC = Plant Diseases Division Culture Collection of New Zealand.

described by the manufacturer. The inoculated strips were incubated at 30°C for 24 h, and results were recorded according to the instructions provided with the API 20E kit. For the API 50CHE kit, the cells were suspended in 5 ml of API 50CHB/E medium (bioMérieux) to a concentration equivalent to 0.5 McFarland. Tubes of the API 50CHE kits were filled as recommended by the manufacturer, and the inoculated strips were incubated at 30°C for 48 h. The results were recorded according to the instructions of the manufacturer. Although the two strips share several carbohydrates in common, the API 50CHE cupules are covered with sterile mineral oil to create anaerobic conditions, and this can lead to different results from API 20E tests (1). The API assays were repeated three times. The averaged data (72 characteristics per strain) were submitted for numerical analysis using Phylogenetic Analysis Using Parsimony (PAUP) Version 4.0 (Sinauer Associates, Inc., Publishers, Sunderland, MA). Clustering was achieved by using the unweighted pair group average (UPGA) method (37). In addition to the API data of the strains listed in Table 1, the API data previously reported by Verdonck et al. (41) was used in comparative differentiation of related enterobacterial species from the lupine strains (Table 2).

Molecular cloning of 16S rDNA and the 16S-23S intergenic regions. Extraction of genomic DNA was performed using the cetyl trimethyl ammonium bromide protocol (2). Primers of the 16S rRNA genes (16F: 5′-GATCCTGGCTCAGAT TGAAC and 16R: 5′-ACCTTGTTACGA CTTCACCC) were selected based on the 16S rDNA sequence of *B. quercina* strain LMG 2724^T (i.e., ATCC 29281^T) (15). The PrimerSelect program in the Lasergene expert sequence analysis software package (Version 5.0; DNASTAR, Inc., Madison, WI) was used in primer analysis and design. The 16S-23S intergenic regions of lupine strains and related bacteria were polymerase chain reaction (PCR) amplified with two primers (FI4: 5′-CTACACACG TGCTACAATG and 23SR: 5′-GTTGTG AGGTTAAGCGAG) as described by Kwon et al. (22), with the exception that high fidelity Vent DNA polymerase was used (New England BioLabs, Beverly, MD). After adenylation, the PCR products were cloned into pGEM T-Easy vector (Promega Corp., Madison, WI) as recommended by the manufacturer. Routine procedures were used for plasmid preparation (2).

Sequence determination and phylogenetic analysis. The 16S rDNA and 16S-23S intergenic regions were sequenced using universal primers T7 and SP6 (Promega Corp., Madison, WI), and internal regions of 16S rDNA were sequenced using the primers reported by Kwon et al. (22). Sequencing reactions were performed using fluorescence-based dideoxy terminators and Ampli-Tag polymerase (Perkin-Elmer Applied Biosystems, Inc., Norwalk, CT). Products of the sequencing reactions were run on an ABI PRISM 377 DNA Sequencer (Perkin-Elmer Applied Biosystems). The SeqMan program in the Lasergene expert sequence analysis software package (DNASTAR) was used to assemble nucleotide sequences. The resulting DNA sequence data was deposited into the GenBank database with accession numbers HM196336, HM196337, HM196338, HM196339, HM196340, HM196341, and HM196342. The corresponding 16S rDNA sequences of the reference bacteria used in this study were downloaded from the National Center for Biotechnology Information website, and their accession numbers are listed in Figure 3. Phylogenetic analyses were conducted using the program MegAlign of the Lasergene expert sequence analysis software package and the phylogenetic tree was constructed using MEGA4 (39).

Fatty acid analyses. The Sherlock Microbial Identification System (Version 3.0, Microbial ID Inc., Newark, DE) was used for quantitative analyses of cellular fatty acid profiles as described by Alcorn et al. (1). Ten representative lupine strains (W3L1, W3L5, W3L8, W3L9, W3L15, W3L16, W3L18, W3L20, W3L21, and W3L23) and *Brenneria* reference strains (*B. quercina* ATCC 29281^T and NCPPB1852, *B. salicis* NCPPB 447^T, and *B. rubrifaciens* NCPPB 2020^T and 632-B) were cultured for 24 h on trypticase soy agar at 28°C prior to fatty acid determinations. Cell harvesting, saponification, methylation, fatty acid extraction, and base wash were performed (1) as recommended by the manufacturer. Percentages of major fatty acids for lupine strains were calculated to evaluate relative quantitative differences of their compositions compared to those of closely related reference strains of *B. quercina*, *B. salicis*, and *B. rubrifaciens*.

Electron microscopy. Flagella were visualized by electron microscopy using a standard negative staining procedure (36). Log-phase cultures of strains W3L1 and W3L7 were harvested; cells were gently washed twice using SDW and then the bacterial suspension was diluted to 2×10^8 CFU per ml. A drop of the bacterial suspension was placed on a 200 mesh grid. After 5 min on the grid, the bacterial cells were stained for 1 to 2 min with 1% aqueous uranyl acetate. The stain was removed gently by touching the edge of the grids to filter paper. The stained cells were observed for flagella under a transmission electron microscope (36).

DNA base composition. The G+C content of lupine strain W3L1 was determined from the thermal denaturation temperature (23), and was calculated by using the equation of Owen and Lapage (30). *Pseudomonas syringae* pv. *syringae* B728a (G+C content: 59.23 mol%) (10) and *Agrobacterium tumefaciens* C58 (G+C content: 56.00 mol%) (43) were used as controls.

RESULTS

Occurrence and field symptoms of drippy pod bacterial disease of lupines. Drippy pod disease of lupines was first observed in the mid-1980s, and was consistently found in all white lupine fields surveyed throughout Eastern Washington (Fig. 2). The disease symptoms are striking because of the profuse foamy and sticky ooze emanating from infected lupine pod lesions (Fig. 1A to C). The whitish-colored ooze appeared to bubble out of infection sites on pods, especially along the veins. Hence, the common name for the disease was called bacterial "drippy pod" of lupine, a name that is reflective of the bacterial "drippy nut" disease symptoms of oak caused by *B. quercina* (5,19,32). The white lupine pods are borne on erect, shrub-like

Fig. 2. Locations in Eastern Washington State where drippy pod disease of lupine was observed from 1983 to 1988, and where strains of *Brenneria quercina* pv. *lupinicola* were isolated.

plants that bear flowers in late May to June. Early infections of drippy pod disease were observed at, but not before, flowering. Thereafter, disease progressed with pod development in late June into early August. Disease lesions were largely found on primary and secondary lupine pods (Fig. 1D) with occasional lesions developing on stems and flower heads. The infection of lupine pods frequently resulted in disease spread to the developing lupine seeds. Abundant amounts of ooze accumulated that oftentimes was observed to drip down the stems and collect in pools on the soil surface. Insects were attracted to the seeping lesions and bacteria-laden ooze, especially *Lygus* spp. that are associated with the epidemic spread of drippy pod disease in the field as a result of their feeding activity. As also reported by Myhre (26), the feeding of *Lygus* spp. adults and nymphs on bacterial ooze from lupine pods was commonly observed in fields in July and August when disease was at its peak. Although phytophagous *Lygus* species were observed to feed on lupine leaves, no disease development was observed on these tissues.

Native lupine species in noncultivated areas of Eastern Washington were observed to develop typical drippy pod disease symptoms at a low frequency. For example, the sulphur lupine (*L. sulphureus*) and the velvet lupine (*L. leucophyllus*) were collected, respectively, from the Hanford Reservation and Central Ferry regions of Eastern Washington and confirmed to be infected with the drippy pod bacterial pathogen (Table 1).

Isolation of the pathogen and pathogenicity tests. The diseased lupine specimens collected from various geographical locations in Washington State consistently yielded white, translucent bacterial colonies on NBY agar after 2 days incubation. The bacterium was also isolated from ooze associated with a dried, infected herbarium specimen of *L. albus* that was collected in 1983 at Warden, WA. Lupine pods inoculated with representative isolated colonies developed water-soaked lesions that spread several millimeters from the inoculation site with the eventual production of ooze from lesions that resembled closely the symptoms observed in the field (Fig. 4). Regardless of the field of origin (Fig. 2), year of isolation (Table 1), or original lupine species as host (Table 1), the lupine strains were uniform in appearance on NBY agar and produced similar levels of disease in pathogenicity tests of lupine pods (*L. albus* cv. Ultra). Accordingly, pathogenicity of the 17 lupine strains listed in Table 1 was confirmed by fulfilling Koch's postulates on lupine pods (Fig. 4). In contrast, *B. quercina* strains ATCC 29281^T and NCPPB 1852 did not produce disease symptoms on lupine pods at an inoculum dose of about 10^6 CFU per wound site, and after 3 days of incubation the lupine pod reactions were indistinguishable from that of the sterile water control. None of the other reference bacteria (i.e., *E. amylovora* strains E9 and Ea225 and *B. salicis* strain NCPPB 447^T) caused disease symptoms on lupine pods (Fig. 4).

Physiological and biochemical characterization. The phenotypic characteristics of the lupine strains were compared to representative phytopathogenic species of erwiniae listed in Table 1. All of the lupine strains were gram-negative rod-shaped bacteria that exhibited facultative anaerobic growth. The lupine strains were motile based on flagella stain reactions, and visualization of cells of strain W3L1 by electron microscopy revealed peritrichous flagella. Strains on NBY agar medium exhibited circular, translucent, and nonpigmented colonies with entire margins. The lupine strains were negative for gelatin liquefaction, and bacterial colonies failed to form pits on CVP medium to indicate that they do not secrete pectolytic enzymes in these in vitro conditions. The lupine strains produced a typical hypersensitive reaction on tobacco leaves with total collapse of infiltrated tissues within 24 h, which was comparable to hypersensitive reactions elicited by strains of *E. amylovora* in tobacco. Based on these and other characteristics, the lupine strains resembled phytopathogenic members of the family *Enterobacteriaceae* (15,16).

The lupine strains were highly uniform in the API 20E and API 50CHE biochemical and physiological tests, and they most

Table 2. Selected physiological and biochemical characteristics that differentiate lupine strains from related bacteria

Test	Percentage of positive reactions for strains tested			
	***Brenneria rubrifaciens* (13)[a]**	***B. salicis* (18)[a]**	***B. quercina* (4)[a]**	**Lupine strains (17)[b]**
API 20E tests[c]				
Citrate utilization	0	0	100	100
Gelatinase production	0	0	25	0
Acid production from				
L-Rhamnose	0	0	0	12
Melibiose	0	0	0	6
Amygdalin	8	100	100	100
L-Arabinose	100	0	0	0
API 50CHE tests[d]				
Acid production from				
Glycerol	100	94	50	0
D-Arabinose	8	0	0	0
L-Arabinose	100	0	0	0
D-Ribose	100	100	100	41
D-Galactose	100	100	100	0
D-Mannose	100	100	100	88
meso-Inositol	0	94	0	0
D-Mannitol	92	100	100	12
α-Methyl-D-glucoside	92	22	75	0
Amygdalin	54	0	0	0
Arbutin	31	100	100	88
Esculin	62	100	100	100
Salicin	8	100	100	82
Maltose	31	11	0	0
Melibiose	0	94	0	0
Trehalose	15	6	0	53
Raffinose	0	51	0	0
Gentiobiose	92	0	0	0
Turanose	15	11	100	0
D-Fucose	0	0	25	0
D-Arabitol	0	6	0	0
D-Gluconate	0	100	100	100

[a] Differential test results shown were from Verdonck et al. (41) with numbers of strains tested listed in parentheses.

[b] Number of lupine strains tested (in parentheses).

[c] Tests for H_2S production, tryptophan deaminase, oxidase, lysine decarboxylase, urease, β-D-galactosidase, arginine dihydrolase, ornithine decarboxylase, indole reaction, reduction of nitrate to nitrite, reduction of nitrite, and acid production from *meso*-inositol and sorbitol were negative for all lupine strains and for all strains of *B. rubrifaciens*, *B. salicis*, and *B. quercina* (41). Tests for acetoin production and acid production from D-glucose, D-mannitol, and sucrose were positive for all lupine strains and for all strains of *B. rubrifaciens*, *B. salicis*, and *B. quercina* (41).

[d] Tests for acid production from D-xylose, L-xylose, adonitol, L-rhamnose, dulcitol, sorbitol, α-methyl-D-mannoside, cellobiose, lactose, melezitose, starch, *meso*-xylitol, D-lyxose, D-tagatose, 2-keto-D-gluconate, L-sorbose, glycogen, *meso*-erythritol, α-methyl-D-xyloside, inulin, L-fucose, L-arabitol, and 5-keto-D-gluconate were negative for all lupine strains and for all strains of *B. rubrifaciens*, *B. salicis*, and *B. quercina* (41). Tests for acid production from D-glucose, D-fructose, *N*-acetylglucosamine, and sucrose were positive for all lupine strains and for all strains of *B. rubrifaciens*, *B. salicis*, and *B. quercina* (41).

closely resembled the phenotypic characteristics of *B. quercina*, *B. salicis,* and *B. rubrifaciens* (Table 2). The lupine strains had the same biochemical results distinctive for the genus *Brenneria* as described by Hauben et al. (15), except for no acid production from D-galactose. In the API 20E tests, all lupine strains utilized citrate and produced acid from amygdalin but not from L-arabinose. Thus, the API 20E results for the lupine strains were essentially indistinguishable from utilization patterns for *B. quercina* strains. In the API 50CHE tests, the only distinguishing differences between *B. quercina* and the lupine strains were observed for acid production from D-galactose, D-mannitol, α-methyl-D-glucoside, and turanose. In contrast, strains of *B. rubrifaciens* exhibited key differences from lupine strains in utilization of citrate, L-arabinose, glycerol, D-galactose, D-mannitol, α-methyl-D-glucoside, amygdalin, arbutin, salicin, gentiobiose, and D-gluconate. Likewise, strains of *B. salicis* exhibited contrasting differences from lupine strains in utilization of citrate, glycerol, D-galactose, *meso*-inositol, D-mannitol, melibiose, and trehalose. Among the lupine strains, the only notable differences were observed for strains W3L7 and W3L8, which produced acid from L-rhamnose in the API 20E test, and for strains W3L14 and W3L22, which showed weak positive reactions for acid production from D-mannitol in the API 50CHE test. In addition, strain W3L8 was the only lupine strain that produced acid from melibiose in the API 20E test. As an internal measure of the reproducibility of the API 20E and CHE test systems in this study, the biochemical profiles of the reference strains of enterobacterial species listed in Table 1 resembled closely the results reported by Verdonck et al. (41).

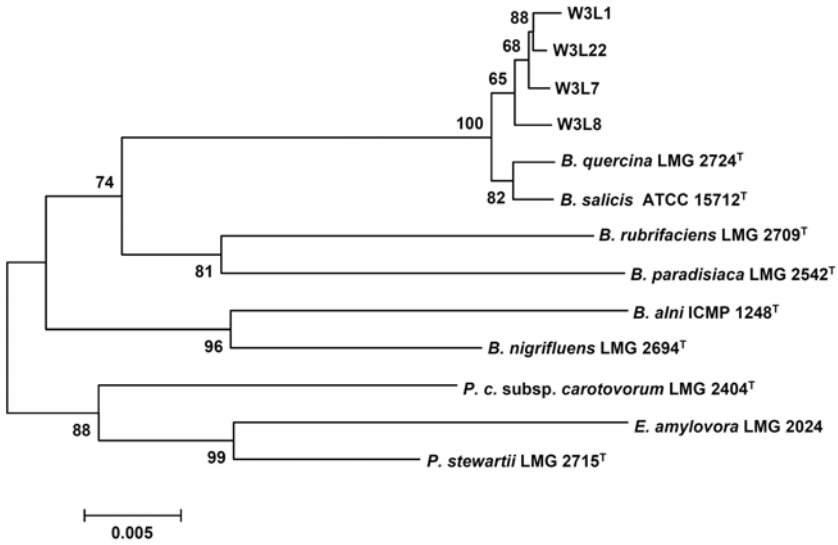

Fig. 3. Phylogenetic dendrogram of the lupine strains (W3L1, W3L7, W3L8, and W3L22) and reference bacteria based on comparative analysis of the nearly complete 16S rDNA sequences using MEGA4 (39). The evolutionary history was inferred using the neighbor-joining method (34). The optimal tree with the sum of branch length = 0.22938113 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Accession numbers for the rDNA sequences are: *Brenneria quercina* LMG 2724^T, gb:AJ223469; *B. salicis* ATCC 15712^T, gb:U80210; *B. rubrifaciens* LMG 2709^T, gb:Z96098; *B. alni* ICMP 12481^T, AJ223468; *B. paradisiaca* LMG 2542^T, gb:Z96096; *Pantoea stewartii* LMG 2715^T, gb:Z96080; *B. nigrifluens* LMG 2694^T, gb: Z96095; *Pectobacterium carotovorum* subsp. *carotovorum* LMG 2404^T, gb:Z96089; *Erwinia amylovora* LMG 2024, gb:Z96088.

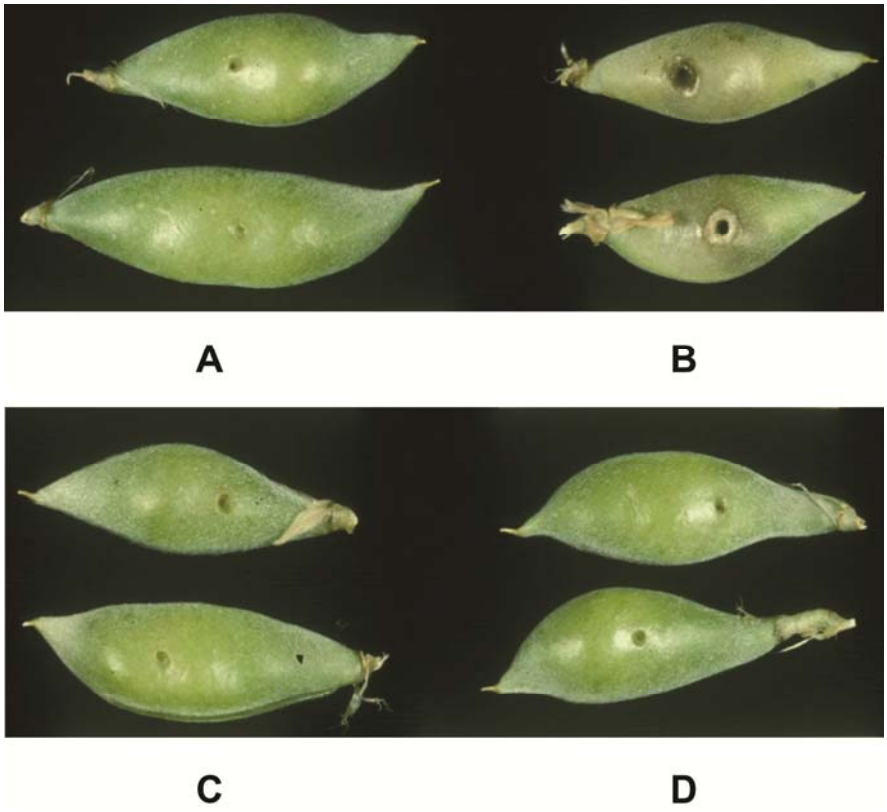

Fig. 4. Pathogenicity tests of the lupine strain and reference bacteria on pods of *Lupinus albus* cv. Ultra. **A,** *Erwinia amylovora* strain E9; **B,** lupine strain W3L8; **C,** *Brenneria quercina* strain ATCC 29281^T; **D,** *B. salicis* strain NCPPB 447^T. Symptoms are shown 3 days after incubation at 25°C.

Computational analyses of the phenotypic data for the 17 lupine strains obtained from the combined API 20E and CHE test systems was conducted by the UPGA method using PAUP. The API test results of Verdonck et al. (41) for various enterobacterial species were included in the analysis to determine the relative clustering of lupine strains relative to recognized phytopathogenic species. All of the lupine strains were clustered in one phenon that also included strains of *B. quercina* (data not shown). The outcome resembled closely the UPGA dendrogram of Verdonck et al. (41) that was generated from API 20 and CHE test results for enterobacterial species. A major observation was the distinct clustering of the lupine and *B. quercina* strains together in a phenon distinct from three phena (41) containing related species of *Brenneria* (i.e., *B. salicis*, *B. rubrifaciens*, and *B. nigrifluens*).

Sequence analyses of the 16S rDNA genes and the 16S-23S intergenic regions. A nearly full length (99.1%) of the 16S rDNA gene sequence was determined for each of lupine strains W3L1, W3L7, W3L8, and W3L22. This 16S rDNA region corresponds to 1,492 bases from position 15 to 1506 according to the numbering of the *E. coli* 16S rDNA sequence (6). The 16S rDNA gene sequence of strain W3L1 had 99.7, 99.4, and 99.8% identities with those of strains W3L7, W3L8, and W3L22,

respectively. The 16S rDNA sequence of strain W3L1 shared 98.9 and 99.3% identities to those of *B. quercina* strain ATCC 29281^T and *B. salicis* strain ATCC 15712^T, respectively. In contrast, the 16S rDNA gene sequence of strain W3L1 had 94.5 to 95.2% identities to those of other type strains of *Brenneria* species. Similarly, the 16S rDNA gene sequence of strain W3L1 showed approximately 93 to 95% identities to those of species of *Pectobacterium, Erwinia*, and *Pantoea*. Nucleotide signatures of the 16S rDNA for the genera *Brenneria*, *Pectobacterium*, and *Erwinia* were proposed in the genus descriptions (15), and were updated by Sutra et al. (38). All six signature loops of the 16S rDNA were identified in the corresponding locations of all four lupine strains. The 16S rDNA sequences of strains W3L1, W3L7, W3L8, and W3L22 possessed all 10 of the signature nucleotides defined for the genus *Brenneria*, 12 of 17 signature nucleotides defined for the genus *Pectobacterium*, and 5 of 14 signature nucleotides defined for the genus *Erwinia* (15,38).

Phylogenetic analysis of the 16S rDNA sequences demonstrated that strains W3L1, W3L7, W3L8, and W3L22 shared the highest similarity to species of *B. quercina* and *B. salicis*. Multiple sequence alignments were performed using three methods: Jotun Hein (17), Clustal V (18), and Clustal W (40), and all three methods showed the lupine strains clustered with strains of *B. quercina* and *B. salicis*. In the phylogenetic tree of the 16S rDNA gene sequences constructed using the neighbor-joining algorithm, the lupine strains formed a distinct cluster with the phytopathogenic enterobacteria *B. quercina* strain LMG 2724^T (i.e., ATCC 29281^T) and *B. salicis* strain ATCC 15712^T (i.e., NCPPB 447^T) (Fig. 3). In contrast, phylogenetic analyses of the 16S rDNA of the lupine strains by the neighbor-joining algorithm indicated that they are dissimilar to several species of *Brenneria*, including type strains (38) for *B. rubrifaciens* (LMG 2709^T), *B. paradisiaca* (LMG 2542^T), *B. alni* (ICMP 1248^T 1), and *B. nigrifluens* (LMG 2694^T) (Fig. 3). Representative species of *Pectobacterium*, *Erwinia,* and *Pantoea* were relatively distantly related to the lupine strains based on 16S rDNA sequence analyses.

Sequence analysis of the 16S-23S IGS regions of four representative lupine strains revealed the highest similarity with that of *B. quercina* (ATCC 29281^T). The 16S-23S IGS regions were PCR amplified using the two primers FI4 and 23SR from lupine strains W3L1, W3L7, W3L8, W3L22, *B. quercina* strain ATCC 29281^T, and *B. salicis* strain NCPPB 447^T. Two PCR products (630 and 670 bp) were obtained from each of the bacterial genomes used in this study. Fessehaie et al. (11) reported that the smaller IGS region was more powerful than the larger IGS region for differentiating species of the erwinias into groups. Therefore, the smaller IGS fragments were cloned and sequenced from the representative lupine strains. After the 16S rDNA portions were deleted from each of the obtained sequences, the resulting 16S-23S IGS sequences were compared by multiple sequence alignments that demonstrated that the small 16S-23S intergenic regions of the four lupine strains shared 99.7% similarity with one another. Similarly, the 16S-23S intergenic regions of these lupine strains showed 97.6% similarity on average with that of *B. quercina* strain ATCC 29281^T (Fig. 5). In contrast, the intergenic sequences of strain W3L1 and *B. quercina* strain ATCC 29281^T only had 50.4 and 57.2% identities, respectively, to that of *B. salicis* strain NCPPB 447^T. Sequence alignment analysis identified two major deletions in the 16S-23S intergenic region of *B. salicis* strain NCPPB 447^T (Fig. 5).

Analysis of fatty acid profiles. The fatty acid profiles of 10 lupine strains showed consistent patterns of fatty acid composition with the major species identified as tetradecanoic acid (14:0), hexadecanoic acid (16:0), *cis*-9-hexadecanoic acid (16:1 *cis* 9), and octadecanoic acid (18:1) (Table 3). Furthermore, no appreciable quantitative differences in fatty acid levels were observed for the 10 lupine strains representing differences in year of isolation and geographic location in Washington State (Table 1, Fig. 2). Although the fatty acid compositions of the lupine strains did not match any known bacterial species in the MIDI database (TSBA Rev: 3.0), direct comparisons to the fatty acid profiles of related *Brenneria* spp. showed that they were qualitatively very similar in fatty acid composition, with the lupine strains most similar to that of *B. quercina* (Table 3). In terms of fatty acid classes, saturated fatty acids predominated (44.1%), with 16:0 comprising the major

Fig. 5. Alignment of the 16S-23S rDNA intergenic regions of *Brenneria quercina* pv. *lupinicola* strain W3L1 (460 bp), *B. quercina* strain ATCC 29281^T (458 bp; synonym LMG 2724^T), and *B. salicis* strain NCPPB 447^T (397 bp; synonym ATCC 15712^T) by MegAlign. The degree of sequence similarity was determined by MegAlign as observed by black boxes (identical), shaded (similar), and no shading (dissimilar). Nucleotide residue numbers for each sequence are presented at the left of each line. The ends of the 16S rDNA genes and the start nucleotides of the 23S rDNA are indicated by an omega (Ω) and a star (*), respectively.

component (31.7%), as was observed for reference strains of *B. quercina* (42.9% for saturated fatty acids and 31.1% for 16:0). In addition, the fatty acid profiles of the lupine strains were highly similar to those of the *B. quercina* strains reported by Biosca et al. (5) to occur on oak species in Spain. The lupine strains were differentiated from the *B. salicis* and *B. rubrifaciens* strains by the occurrence of significantly lower levels of dodecanoic acid (12:0) and higher levels of tetradecanoic acid (14:0) (Table 3); *B. salicis* and *B. rubrifaciens* also exhibited, respectively, higher and lower levels of cyclopropane-hexadecanoic acid (17:0 cyclo) than the lupine strains.

DNA base composition. The DNA G+C content of strain W3L1 was 54.32 mol%. This G+C content is in the range of the genus *Brenneria* (50.1 to 56.1 mol%) (15).

DISCUSSION

White lupine, a crop cultivated for centuries in the Mediterranean region of Europe and Africa (13), was introduced into Eastern Washington State and evaluated in the 1980s as an alternative to wheat-based cropping systems (31). The occurrence of bacterial drippy pod disease of white lupines emerged as a barrier to expanding cultivation of the legume crop despite favorable agronomic and climatic conditions. Although white lupines were cultivated in the Southeastern United States (4) and elsewhere in the world (13,31), this is the first report of bacterial drippy pod disease of lupines. The disease was observed to occur throughout Eastern Washington wherever white lupines were grown and oftentimes caused complete loss of the lupine crop. The epidemic spread of disease can be attributed to the feeding activities of phytophagous *Lygus* spp. and other insects that are attracted to diseased plants and then rapidly disseminate the bacterial pathogen throughout the field (26). Consequently, it was recommended (26) that insecticides be applied during flowering followed by two additional applications at biweekly intervals to manage the drippy pod disease. Windblown and splashing rain also can spread disease from infected lupine plants in the field. Contaminated lupine seeds were not considered to be a significant source of disease in the field (26).

The disease symptoms in the field are impressive due to abundant quantities of foamy ooze emanating from lupine pod infections. In fact, drippy nut, caused by *B. quercina*, on oak trees was the first bacterial disease distinguished by formation of large quantities of ooze that drips down from infected tissues (5,19). Although bacterial diseases of white lupine are limited to only a few reports (3,20,21), symptoms of these diseases are dissimilar from the drippy pod disease occurring in Eastern Washington. Consequently, the drippy pod bacterial pathogen appears to be indigenous to Eastern Washington and has not been reported to occur on lupines grown elsewhere in the world. A local origin for the pathogen is supported by the discovery of sulphur lupine and velvet lupine species with typical bacterial drippy pod disease symptoms. These lupine species are native to the Pacific Northwest region where they grow in diverse mountain, prairie, and plateau habitats. Thus, it appears that native lupine species are the likely source of the bacterial pathogen, which is then readily carried by phytophagous insects to commercial fields of highly susceptible varieties of white lupines.

The causal agent of drippy pod disease of lupine was identified as a member of the genus *Brenneria*, which was erected after 16S rDNA gene sequence analyses by Hauben et al. (15) as a distinct phylogenetic group within the *Enterobacteriaceae*. Major characteristics include rod-shaped cells that stain gram negative with peritrichous flagella. The lupine strains all exhibited facultative anaerobic growth, were negative in assays for extracellular pectolytic enzyme activity, and elicited a hypersensitive reaction in tobacco leaves. The G+C content of 54.32 mol% for lupine strain W3L1 was well within the range reported (15,16) for the genus *Brenneria*. Furthermore, the lupine strains shared almost the same biochemical and physiological traits, including major fatty acids, as described by Hauben et al. (15) for the genus *Brenneria*. One noteworthy exception to the genus description (15) is that the lupine strains did not produce acid from D-galactose, which also was a physiological distinction to oak strains of *B. quercina*. Finally, the 16S rDNA gene sequences for the lupine strains was 98.9 to 99.3% identical to strains of *B. quercina* and *B. salicis*, with no other enterobacterial genera approaching this measure of relatedness based on 16S rDNA sequence analyses. Furthermore, the six sequence signature loops of the 16S rDNA gene sequence together with all 10 signature nucleotides used to differentiate the genus *Brenneria* (15,38) were identified in the lupine strains analyzed.

Regardless of whether the lupine strains were examined by standardized biochemical tests, cellular fatty acid analysis, or comparisons of 16S rDNA and 16S-23S rDNA intergenic sequences, the outcome uniformly showed close relatedness to strains of *B. quercina*, a pathogen causing dippy nut disease of oak trees (5,19,32). The API 20E and 50CHE series of standardized biochemical tests proved to be a reproducible and convenient means of comparing lupine strains to recognized species of enterobacteria. Accordingly, the two API test series have been used successfully in several studies focused on the identification and numerical taxonomy of plant-pathogenic enterobacterial species (1,5,24,25,41). For example, a comprehensive numerical analysis of API phenotypic responses by Verdonck et al. (41) placed four strains of *B. quercina* into subphenon 32A, which was closely linked to subphenon 32B for *B. salicis*. Direct comparisons of the API data of the lupine strains (Table 2) was strongly aligned with the results for *B. quercina* reported by Verdonck et al.

Table 3. Fatty acid methyl ester profiles of the lupine strains compared to strains of related *Brenneria* species

Fatty acid species	Lupine strains[a]	*B. quercina*[b]	*B. salicis*[c]	*B. rubrifaciens*[d]
Straight-chain				
12:00	0.73 (0.18)	0.54 (0.04)	3.34 (0.10)	2.52 (0.13)
14:00	11.26 (1.16)	10.91 (0.08)	5.51 (0.05)	6.35 (0.11)
15:00	0.08 (0.06)	0.07 (0.09)		
16:00	31.72 (0.69)	31.09 (0.34)	29.55 (0.44)	30.07 (0.54)
18:00	0.34 (0.06)	0.29 (0.06)	0.10 (0.09)	0.28 (0.05)
Unsaturated				
16:1 *cis* 9	27.59 (3.87)	26.86 (0.66)	25.51 (1.18)	28.68 (1.02)
Cyclopropane				
17:0 cyclo	5.72 (1.21)	6.92 (1.11)	9.43 (1.83)	3.33 (0.71)
19:0 cyclo	0.99 (0.22)	1.21 (0.18)	0.69 (0.11)	
Hydroxy				
12:0 3OH	0.10 (0.06)	0.03 (0.04)	0.41 (0.06)	
16:0 3OH	0.22 (0.04)	0.06 (0.08)		
Summed feature 3[e]	9.78 (1.41)	9.90 (0.56)	8.26 (0.75)	9.56 (0.23)
Summed feature 7[e]	13.77 (2.04)	11.04 (0.20)	16.30 (0.52)	18.08 (0.39)
Unknown 14.503[f]	0.95 (0.20)	1.09 (0.16)	1.05 (0.12)	1.14 (0.01)

[a] Lupine strains: W3L1, W3L5, W3L8, W3L9, W3L15, W3L16, W3L18, W3L20, W3L21, and W3L23; Mean (%) and standard deviation of 10 strains.
[b] *Brenneria quercina* strains: ATCC 29281^T and NCPPB1852; mean (%) and standard deviation of two strains.
[c] *Brenneria salicis* strain: NCPPB 447^T; mean (%) and standard deviation of three extractions.
[d] *Brenneria rubrifaciens* strains NCPPB 2020^T and 632-B. Mean (%) and standard deviation of two strains.
[e] Summed features represent groups of two or three fatty acids that could not be separated with the MIDI system. Summed feature 3 contained 14:0 3OH, 12:0 alde, and 16:1 iso. Summed feature 7 contained 18:1 *cis* and 18:1 *trans*.
[f] Equivalent chain length relative to lengths of known fatty acids.

(41) and Biosca et al. (5). Subsequent computational analysis using the UPGA method showed formation of a clustering of lupine strains with oak strains of *B. quercina* into a discrete phenon distinct from other species of *Brenneria*. Cellular fatty acid profiles likewise showed no appreciable differences in saturated and unsaturated fatty acid composition for the lupine and oak strains, and yet there was clear differentiation from strains of *B. salicis* and *B. rubrifaciens* (Table 3). Although the nearly complete 16S rDNA gene sequences of four representative lupine strains shared highest identities to both the type strains of *B. quercina* and *B. salicis*, analysis of the 16S-23S intergenic region revealed that the lupine pathogen shared extensive sequence identity to the IGS region of *B. quercina* (over 97%) and not to *B. salicis* (less than 60%) (Fig. 5). Fessehaie et al. (11) observed that the small IGS sequences of enterobacterial species vary considerably and are useful for diagnosis and phylogenetic analyses of closely related bacteria. Consequently, the virtual identity of the 16S-23S IGS regions of the lupine and oak strains is evidence for classification within the species *B. quercina*.

The results of our phenotypic and genotypic characterization and placement of the lupine strains into the species *B. quercina* is consistent with the phylogenetic analyses of the *gapDH* gene by Brown et al. (7). Cladistic analyses of *gapDH* sequences demonstrated that three lupine strains, originally from our collection (33) (Table 1), were grouped along with *B. quercina* strains from oak trees in a distinct necrogenic clade from other necrogenic *Brenneria* and *Erwinia* species. The *gapDH* gene sequence of the lupine strains exhibited the smallest genetic distance (7.2%) from *B. quercina*, which was even less than the genetic distance (7.8%) between *P. carotovorum* subsp. *carotovorum* and *P. carotovorum* subsp. *betavasculorum* (7). A subsequent study by Naum et al. (28) demonstrated that type III secretion genes encoding structural components of the needle complex are phylogenetically informative in enterobacterial plant pathogens. Analyses of the *hrcC* and *hrcR* gene sequences, for example, showed clustering of lupine strains with oak strains of *B. quercina*. Although diverse phenotypic and genotypic data sets support the inclusion of lupine and oak strains into a single species, the taxonomic relationship of *B. quercina* to other species of *Brenneria* is in flux (27–29,44). Young and Park (44) analyzed conserved gene (i.e., *atpD*, *carA*, *recA*, and 16S rDNA genes) sequences of plant-pathogenic enterobacteria and observed that *B. quercina* was unrelated to other *Brenneria* species and may represent a separate genus for the taxon. Additional taxonomic studies of *B. quercina* are necessary to resolve its position among genera within the enterobacterial plant pathogens, recognizing that horizontal gene transfer processes have complicated bacterial systematics and whole genomic data may be vital to delineate taxonomic relationships in the enterobacteria (28,29).

The creation of a pathovar of *B. quercina* called "*lupinicola*" is supported by evidence that: (i) natural infections of bacterial drippy pod occur on at least three species of lupines (i.e., *L. albus*, *L. leucophyllus,* and *L. sulphureus*); (ii) lupine strains of *B. quercina* are highly uniform in virulence to commercial cultivars of white lupine and produce the full range of drippy pod disease symptoms; (iii) oak strains of *B. quercina* fail to cause disease in pathogenicity tests on highly susceptible pods of white lupine; and (iv) other species of *Brenneria* do not infect pods of white lupine. The unavailability of related strains of *Brenneria* and inherent difficulties in testing pathogenicity on their natural woody hosts was an obstacle. Nevertheless, the various phenotypic and genotypic characterizations of the lupine strains of *B. quercina* confirm that they are unique pathogens and a valuable resource for further delineation of enterobacterial plant pathogens.

Description of *Brenneria quercina* pv. *lupinicola* pv. nov. *Brenneria quercina* pv. *lupinicola* pv. nov. (lu.pi.ni'co.la. M.L. n. *Lupinus*, generic name of lupine; L. suff. *-cola* dweller; M.L. n. *lupinicola*, lupine-dweller) causes bacterial drippy pod disease of lupines including commercial varieties of white lupine (*Lupinus albus* L.) and native sulphur lupine (*L. sulphureus*) and velvet lupine (*L. leucophyllus*) of the Pacific Northwest. Phenotypic and genotypic characteristics of the lupine strains resemble those described for strains of *B. quercina* isolated from oak trees.

Cells are nonsporeforming rods (0.5–1.0 μm wide by 1.3–3.0 μm long), gram-negative, and motile with peritrichous flagella. The bacterium exhibits facultative anaerobic growth. Colonies grown on NBY agar medium for 24 to 48 h at 30°C are circular, translucent, glistening, and nonpigmented with entire margins. The bacterium is negative for gelatin liquefaction, and does not form pits on CVP medium due to lack of extracellular pectolytic enzymes. Strains of *B. quercina* pv. *lupinicola* cause a typical hypersensitive reaction on tobacco (*N. tabacum*) leaves.

Strains utilize citrate, produce acetoin, and produce acid from acetylglucosamine, amygdalin, L-arabinose, esculin, D-fructose, D-gluconate, D-glucose, D-mannitol, and sucrose. The majority of strains produce acid from arbutin, D-mannose, salicin, and trehalose. A few strains produce acid from L-rhamnose, D-mannitol, melibiose, and D-ribose. Tests are negative for H_2S production, indole reaction, arginine dihydrolase, β-D-galactosidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, oxidase, urease, reduction of nitrate to nitrite, and reduction of nitrite. Tests are negative for acid production from adonitol, L-arabitol, cellobiose, dulcitol, *meso*-erythritol, L-fucose, D-galactose, gentiobiose, 2-keto-D-gluconate, 5-keto-D-gluconate, α-methyl-D-glucoside, glycerol, glycogen, inulin, *meso*-inositol, lactose, D-lyxose, α-methyl-D-mannoside, melezitose, melibiose, L-rhamnose, sorbitol, L-sorbose, starch, D-tagatose, turanose, *meso*-xylitol, D-xylose, L-xylose, and α-methyl-D-xyloside.

The major fatty acids are tetradecanoic acid (14:0), hexadecanoic acid (16:0), *cis*-9-hexadecanoic acid (16:1 *cis* 9), *cis*- and *trans*-octadecanoic (18:1), and cyclopropane-hexadecanoic acid (17:0 cyclo). Relatively low levels of dodecanoic acid (12:0) are produced.

Strains of *B. quercina* pv. *lupinicola* infect lupine seed pods to cause copious oozing of sap, whereas *B. quercina* strains from oak are nonpathogenic to lupine. Strain W3L1, which was isolated from diseased white lupine pods collected at Waitsburg, WA in 1987, is designated as the type strain. The DNA G+C content of the type strain W3L1 was measured as 54.32 mol%.

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