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Description of *Gibbsiella quercinecans* gen. nov., sp. nov., associated with Acute Oak Decline☆

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ABSTRACT

Gram-negative, facultatively anaerobic bacterial strains were consistently isolated from oak trees displaying symptoms of extensive stem bleeding. In Britain, this disorder is called Acute Oak Decline (AOD). A similar condition has been noted on species of Mediterranean oak in Spain. The identity of bacterial isolates from symptomatic trees in both countries was investigated using molecular techniques and phenotypic assays. 16S rRNA gene sequencing indicated that the strains were most closely related to the genera *Serratia*, *Kluyvera*, *Klebsiella* and *Raoultella* (all > 97%). Phylogenetic analysis revealed that the strains formed a distinct lineage within the family *Enterobacteriaceae*, which was confirmed by both *gyrB*- and *rpoB*-gene sequencing. DNA–DNA hybridization confirmed that the strains belonged to a single taxon which could also be differentiated phenotypically from its closest phylogenetic neighbours. The phylogenetic and phenotypic data both demonstrated that the strains isolated from oak represented a novel genus and species within the family *Enterobacteriaceae* for which the name *Gibbsiella quercinecans* gen. nov., sp. nov. (type strain=FRB 97^T=LMG 25500^T=NCPPB 4470^T) is proposed.

Introduction

Oak decline is a term used to describe ill health of oak trees. Declines are a category of tree diseases which result from an interacting set of factors. Specifically, oak decline is the result of 'a multi-factorial phenomenon that has been attributed to many different causes and often involves a succession of several biotic and abiotic factors' [3]. Two forms of this disorder are currently recognised in Britain: an acute form called Acute Oak Decline (AOD) and a chronic form (COD) [8]. Both forms of decline are usually episodic and with a complex cause but certain agents may play a dominant role. For example, the 1920s outbreak of AOD in Britain was attributed to over-riding effects of successive first flush defoliations by the caterpillar of the oak roller moth (*Tortrix viridana*), followed by damage to summer leaves by powdery mildew (*Erysiphe alphitoides*) [7,19,22].

A new episode of AOD now appears to be taking hold in Britain in which a predominant feature is the attack on oak stems. Symptoms of extensive stem bleeding or oozing of dark sticky exudate from small (5–10 cm) vertical cracks formed between the bark plates are characteristic. Only mature (>50 years, diameter at breast height (dbh) > 30 cm) oak trees native to Britain, for example, *Quercus robur* (pedunculate oak) and *Quercus petraea* (sessile oak), are known to be affected so far in the UK. The disease focus is in the Midlands, England, with an increasing number of reports coming from the south and south-east regions and Wales. Affected trees occur in farmland, parkland, urban and woodland environments. Several thousand trees are estimated to be affected, and surveys are underway to obtain more accurate information about its true extent.

A similar disorder has been observed on several species of oak native to Spain, including *Q. ilex* and *Q. pyrenacia*. It was suggested that *Brenneria quercina*, the causal agent of bark canker and drippy nut disease, is responsible for the oak decline in Spain [1]. A recent study, based on 16S rRNA gene sequencing and rep-PCR, indicated that some of the strains isolated from Mediterranean oaks in Spain do belong to the genus *Brenneria*, but a number of isolates were also shown to be closely related to *Serratia* species [21]. Pathogenicity tests with the two groups of isolates revealed that both could cause a very small lesion when wound inoculated on oak saplings.

Over two seasons (2008–2009) cream-coloured, Gram-negative bacteria were consistently isolated from symptomatic oaks at 13 different sites in Britain. Based on the reports of the condition in

☆ *Note*: The GenBank/EMBL accession numbers for the sequences presented in this study are: GU562337–GU562342 (16S rRNA), GU562317–GU562336 (*gyrB*) and GU562297–GU562316 (*rpoB*).

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Spain [1,21], the consistency of isolation from symptomatic trees in Britain, positive hypersensitivity reactions on *Nicotiana tabacum* (data not shown) and results from preliminary pathogenicity tests (data not shown) it was suspected that these bacteria played a role in the current episode of AOD. Further pathogenicity tests are underway to provide robust evidence of the relationship between this bacterial species and sessile and pedunculate oaks in Britain. Two strains, found to be closely related to *Serratia* species from affected oaks in Spain [21], were compared to those isolated from symptomatic oaks in Britain. Following 16S rRNA gene sequencing, the strains from Britain and Spain displayed high similarity to each other (>98%) as well as to species of *Serratia*, *Kluyvera*, *Klebsiella* and *Raoultella* (all > 97%) placing them firmly in the family *Enterobacteriaceae*. Due to the polyphyletic nature of many genera within the *Enterobacteriaceae*, it is often not possible to resolve taxonomic relationships in this family with confidence. However, protein-encoding genes with known house-keeping functions, specifically *gyrB* and *rpoB*, have been shown to be useful for determining intra- and inter-generic phylogenies of members of the *Enterobacteriaceae* [2,6,16,27]. Consequently, the strains isolated from oak were investigated using both genotypic and phenotypic techniques to determine their true taxonomic position.

Materials and methods

Isolation of strains and DNA extraction

In 2008, samples were taken from ten symptomatic oak trees at five different sites in Britain, with an additional ten trees being sampled from eight new sites in 2009. More than 2000 pieces of tissue were taken from junctions between live and dead tissue in the inner and outer bark, as well as the sapwood of the trees, surface-sterilized and then plated onto peptone yeast glucose agar (PYGA) [17]. Plates were incubated at 22 °C and examined 2, 3, 7 and 10 days after isolation. Selections of developing bacterial colonies were streaked on nutrient agar to obtain single cell colonies. All strains were then subjected to Gram staining, KOH and oxidation–fermentation tests. A representative subset of 18 strains of the most frequently occurring group (which was Gram-negative and facultatively anaerobic) was selected for further study (Table 1). Genomic DNA was extracted using an alkali extraction method [18] and stored at −20 °C.

16S rRNA gene sequencing

The almost complete (1396 bp) 16S rRNA gene sequence was determined for a selection of strains using the primers and conditions determined by Coenye et al. [5]. Sequences were aligned with those of the closest phylogenetic neighbours, as demonstrated by 16S rRNA sequence similarity, using CLUSTAL_X [28] and the overhangs were trimmed. MODELTEST 3.7 [20] was applied to the data set to determine the best-fit evolutionary model. A maximum likelihood tree was constructed using PHYML [12] by applying the models and parameters set by MODELTEST. Bootstrap analysis with 1000 replicates was performed on the tree to assess the reliability of the clusters generated.

gyrB- and rpoB-gene sequencing

Two protein-encoding genes were selected to provide further differentiation of the strains from closely related genera, namely *gyrB* (DNA gyrase) and *rpoB* (RNA polymerase β subunit). Amplification and sequencing of the *gyrB*- and *rpoB*-genes was carried out on the strains listed in Table 1, as described previously [2]. Additional sequences for the most closely related genera were downloaded from GenBank. Sequence analysis and tree construction were performed as for 16S rRNA gene sequencing.

DNA–DNA hybridization and DNA G + C content

DNA–DNA hybridizations were carried out by the BCCM/LMG Bacteria Collection, Ghent University, Belgium using a modified version of the microplate method [4,9]. The hybridization temperature used was 45 ± 1 °C. Reciprocal reactions (A × B and B × A) were performed for each DNA pair and the variation was within the limits of this method [10]. The values presented were based on a minimum of four replicates. The DNA G + C content range was determined using HPLC [15] also by the BCCM/LMG Bacteria Collection.

Phenotypic and chemical characterization

Biochemical and physiological tests were performed on 18 oak strains and the type strains of *Serratia marcescens* ssp. *marcescens*, *S. entomophila*, *S. ficaria*, *S. fonticola*, *S. rubidaea*, *Edwardsiella tarda*, *E.*

Table 1
Strains of *Gibbsiella quercinecans* used in this study.

Strain	Source	Location	16S rRNA[a]	*gyrB*[a]	*rpoB*[a]
FRB 97^T = LMG 25500^T = NCPPB 4470^T	*Quercus petraea*, inner bark	Hoddesdon Park, UK	GU562337	GU562317	GU562297
FRB 24	*Quercus robur*, inner bark	Booth Wood, UK		GU562318	GU562298
FRB 61	*Quercus robur*, excised lesion	Booth Wood, UK		GU562319	GU562299
FRB 82	*Quercus petraea*, outer bark	Hoddesdon Park, UK		GU562320	GU562300
FRB 83	*Quercus petraea*, outer bark	Hoddesdon Park, UK		GU562321	GU562301
FRB 88	*Quercus petraea*, outer bark	Hoddesdon Park, UK		GU562322	GU562302
FRB 89	*Quercus petraea*, outer bark	Hoddesdon Park, UK	GU562338	GU562323	GU562303
FRB 91	*Quercus petraea*, outer bark	Hoddesdon Park, UK		GU562324	GU562304
FRB 92 = LMG 25501 = NCPPB 4471	*Quercus petraea*, inner bark	Hoddesdon Park, UK		GU562325	GU562305
FRB 93	*Quercus petraea*, inner bark	Hoddesdon Park, UK		GU562326	GU562306
FRB 98	*Quercus petraea*, inner bark	Hoddesdon Park, UK		GU562327	GU562307
FRB 115	*Quercus robur*, inner bark	Outwood, UK		GU562328	GU562308
FRB 116	*Quercus robur*, inner bark	Outwood, UK		GU562329	GU562309
FRB 124	*Quercus robur*, inner bark	Outwood, UK		GU562330	GU562310
FRB 147	*Quercus robur*, sapwood	Outwood, UK	GU562339	GU562331	GU562311
FRB 169	*Quercus robur*, outer bark	Gorse Covert, UK		GU562332	GU562312
FRB 185 = LMG 25502 = NCPPB 4472	*Quercus robur*, inner bark	Gorse Covert, UK	GU562340	GU562333	GU562313
FRB 214	*Quercus robur*, inner bark	Spinney Wood, UK		GU562334	GU562314
N78	*Quercus pyrenaica*, canker	Burgos, Spain	GU562341	GU562335	GU562315
N221	*Quercus pyrenaica*, canker	Madrid, Spain	GU562342	GU562336	GU562316

FRB = Forest Research Bacteria Collection, Forest Research, UK; N = local culture reference number, Centro de Biotecnología y Genómica de Plantas, Madrid, Spain; LMG = BCCM/LMG Bacteria Collection, Ghent University, Belgium; NCPPB = National Collection of Plant Pathogenic Bacteria, York, UK; ^{T} = type strain.

[a] GenBank accession number.

hoshinae and *E. ictaluri* (the closest phylogenetic neighbours) using API 20E, API 50CHB/E (bioMérieux) and GN2 MicroPlate (Biolog). The tests were carried out according to the manufacturer's instructions and were incubated for 18 h (API 20E, Biolog) or 48 h (API 50CHB/E). FRB 97^T was included as a positive control in each set of tests and the results from the closest phylogenetic neighbours were compared to phenotypic data available in Bergey's Manual of Systematic Bacteriology [11,23].

Whole-cell fatty acid composition was determined for five selected strains (FRB 97^T, FRB 92, FRB 185, FRB 92, N78) using gas chromatography [14].

Results and discussion

The 16S rRNA gene sequence similarity of strains FRB 97^T, FRB 89, FRB 147, FRB 189, N78 and N221 ranged from 98.2 to 99.7% to each other and was greater than 97% to species of *Serratia*, *Kluyvera*, *Klebsiella*, *Enterobacter* and *Raoultella*. In the 16S rRNA gene phylogenetic tree (Fig. 1), the strains clustered together with high bootstrap support and formed a distinct lineage, suggesting they belonged to a single species within a novel genus. There was a minor sequence divergence between strains from Britain and Spain (<0.2%) which may account for the slight distance observed in the phylogenetic tree. The closest phylogenetic neighbours, based on 16S rRNA gene sequence analysis appeared to be *S. rubidaea* and species of the genus *Edwardsiella*, although the bootstrap support for this relationship was minimal. The remaining *Serratia* species were dispersed throughout the 16S rRNA gene phylogenetic tree which is in keeping with the polyphyletic nature of this genus [31].

In both the *gyrB*- (Fig. 2) and *rpoB*-gene (Suppl. Fig. S1) phylogenetic trees, the strains isolated from oak formed a discrete cluster with bootstrap support of 100%. No sequence divergence could be observed between strains from Britain and Spain based on *gyrB*- and *rpoB*-gene sequence analysis. The cluster was clearly separate from the closest phylogenetic neighbours, providing further support for the proposal of a novel genus. The *Serratia* species were contained in a strongly supported monophyletic group in the *rpoB* phylogenetic tree, whereas in the *gyrB* phylogenetic tree, the genus *Serratia* was polyphyletic. In both the *gyrB*- and *rpoB*-phylogenies, *S. rubidaea* was far removed from the oak strains, in contrast to the 16S rRNA phylogeny where a closer phylogenetic relationship was suggested. Consequently, based on the 16S rRNA- and *gyrB*-gene sequencing data, the taxonomic position of several *Serratia* species should be re-evaluated.

Five oak strains (FRB 97^T, FRB 92, FRB 185, FRB 92, N78), four from Britain and one from Spain, were hybridized amongst each other and exhibited levels of DNA similarity ranging from 78 to 93%. This is well above the species limit of 70%, confirming that the strains belonged to a single species. In the past, it was generally expected that strains sharing more than 97% 16S rRNA gene sequence similarity would exhibit more than 70% DNA similarity [26]. Recently, it was proposed that the 16S rRNA gene sequence similarity requirement of 97% for mandatory DNA–DNA hybridization should be increased to 98.7% [24]. Despite a lower 16S rRNA gene sequence similarity range of 96.8–97.7% between the oak strains and *S. rubidaea* LMG 5019^T, this strain was hybridized to the type strain of the proposed novel genus (FRB 97^T) because of its clustering position in the 16S rRNA gene phylogenetic tree. However, the DNA similarity between LMG 5019^T and FRB 97^T was 36%, confirming that the oak strains did not belong to *S. rubidaea*.

The DNA G + C content of the five selected strains (FRB 97^T, FRB 92, FRB 185, FRB 92, N78), ranged from 56.0 to 56.4 mol%. The complete results of the DNA–DNA hybridizations and DNA G + C content are presented in Table 2.

High correlation was observed between the phenotypic results obtained for the *Serratia* and *Edwardsiella* type strains and the corresponding data in Bergey's Manual of Systematic Bacteriology [11,23]. The strains from oak in both Britain and Spain were found to be fairly biochemically reactive. The majority of strains could utilize 58 out of the 95 substrates tested in the GN2 Microplate, and could produce acid from 29 of the 49 carbohydrates in the API 50 CHB/E tests. Complete results from the phenotypic tests are listed in Table 3 and in the genus and species descriptions below. The oak strains could be distinguished from *Serratia* and *Edwardsiella* by their ability to hydrolyze β-galactosidase (differentiation from *Edwardsiella* spp.), their inability to produce lysine decarboxylase (differentiation from *S. marcescens*, *S. fonticola*, *S. rubidaea* and *Edwardsiella* spp.) or gelatinase (differentiation from *Serratia* spp.), their positive reaction to L-sorbose (differentiation from *Edwardsiella* and *Serratia*) and their negative reaction to glycyl-L-aspartic acid (differentiation from *Edwardsiella* and *Serratia*).

The fatty acid profiles of the oak strains were typical for members of the *Enterobacteriaceae* [30], although slight variation was observed between the compositions of the strains. The major fatty acid components contributed 79.6–84.2% to the whole-cell fatty acid composition and included $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1}\omega7c$, and summed feature 3 ($C_{16:1}$ ω7*c* and/or iso-$C_{15:0}$ 2-OH). FRB 97^T displayed the following fatty acid profile: $C_{12:0}$ (2.7%), $C_{14:0}$ (7.9%), $C_{16:0}$ (28.3%), $C_{18:1}$ ω7*c* (17.1%), summed feature 2 (iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH; 11.5%) and summed feature 3 ($C_{16:1}$ ω7*c* and/or iso-$C_{15:0}$ 2-OH; 27.9%). $C_{13:0}$, $C_{17:0}$, $C_{17:0}$ cyclo, $C_{17:1}$ ω8*c* and an unidentified fatty acid with a chain length of 14.502 were also detected in low amounts. The fatty acid profiles of the five strains differed significantly from that of *B. quercina*, the only reported bacterial pathogen of oak [1].

Currently, there are no minimal standards defined for the characterization of members of the family *Enterobacteriaceae* [13]. Probably because this is a massive undertaking and the family comprises a diverse array of genera, each with varying degrees of 16S rRNA sequence similarity and DNA–DNA hybridization boundary values for species delineation. It is generally required when a novel genus is suspected, that taxa displaying more than 95% 16S rRNA gene pairwise sequence similarity should be examined using other methods [29]. This value should be increased for genera of the family *Enterobacteriaceae*, considering many genera display more than 97% 16S rRNA gene sequence pairwise similarity. Nevertheless, additional methods were performed on the strains isolated from oak and species of *Serratia* and *Edwardsiella*, the closest phylogenetic neighbours as demonstrated by 16S rRNA gene sequence analysis. Both *gyrB*- and *rpoB*-gene sequencing verified that the oak

Table 2
DNA–DNA hybridization values and DNA G + C content *of Gibbsiella quercinecans*.

	1	2	3	4	5	6	DNA G + C content (mol%)
Gibbsiella quercinecans							
1. FRB 97^T	100						56.0
2. FRB 92	88	100					56.2
3. FRB 185	80	84	100				56.0
4. FRB 214	93	91	84	100			56.4
5. N78	86	78	82	88	100		56.0
6. *Serratia rubidaea* LMG 5019^T	36					100	

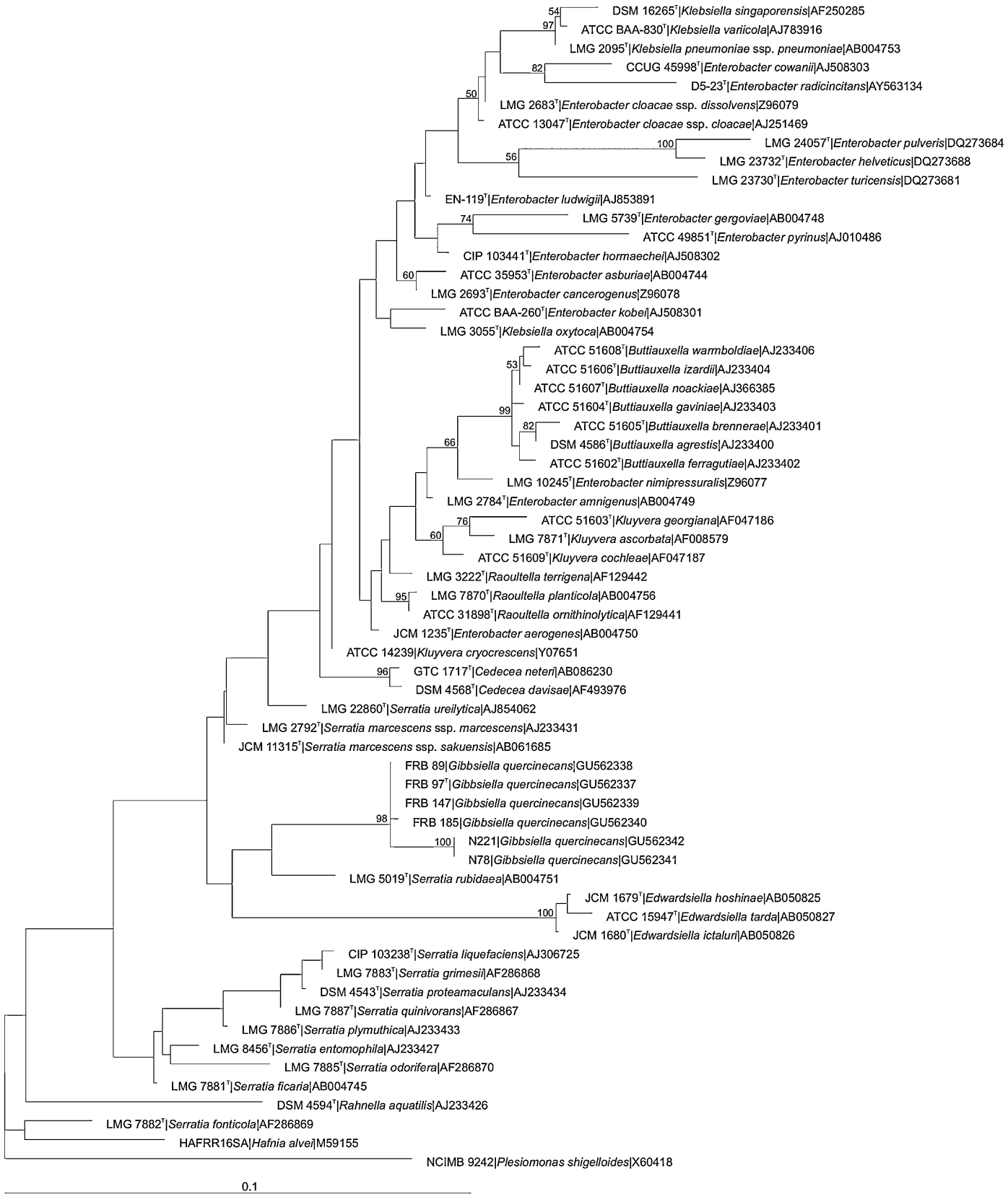

Fig. 1. Maximum likelihood tree based on almost complete 16S rRNA gene sequences of *Gibbsiella quercinecans* and phylogenetically related species. Bootstrap values after 1000 replicates are expressed as percentages. *Plesiomonas shigelloides* is included as an outgroup. The scale bar indicates the fraction of substitutions per site.

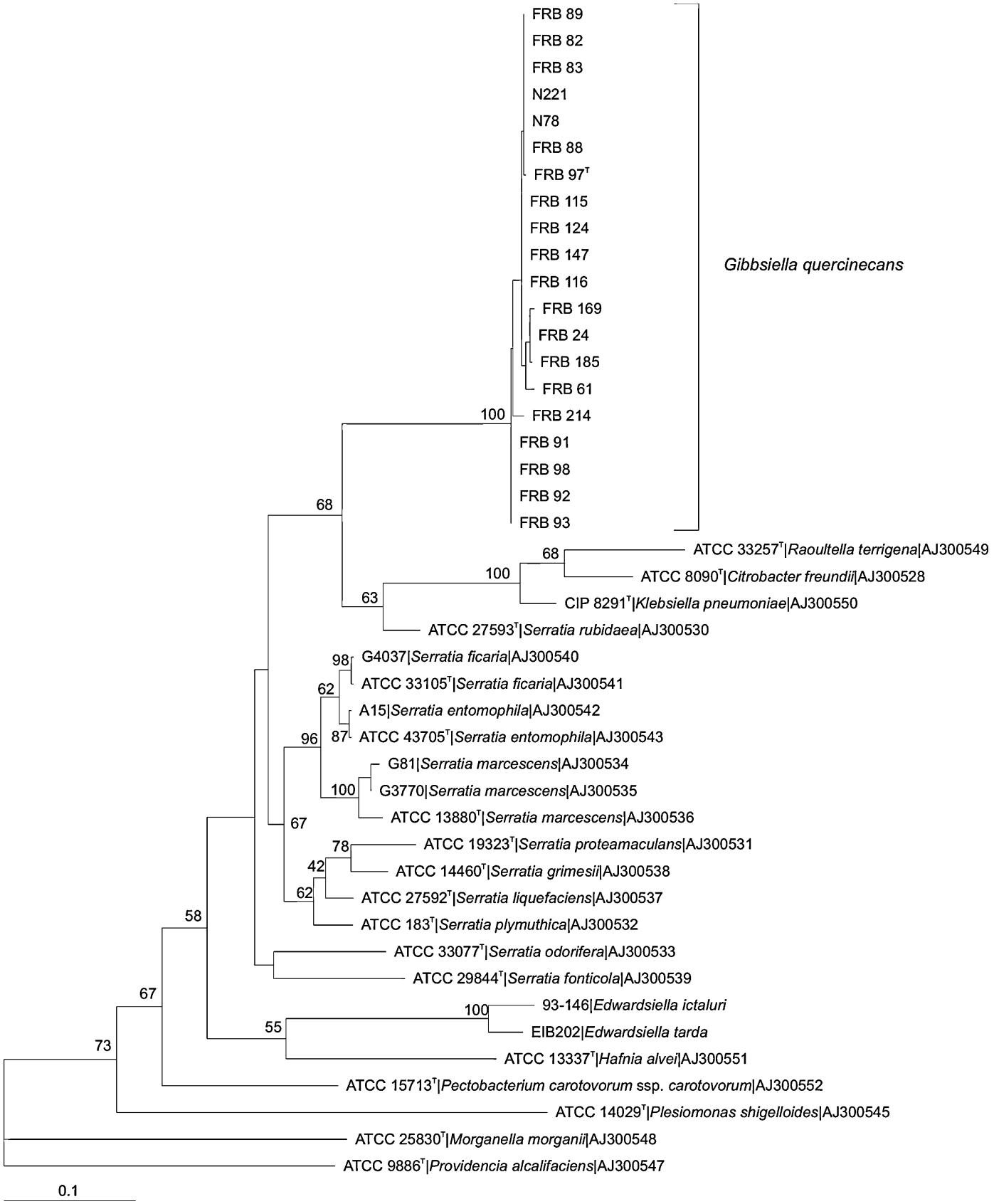

Fig. 2. Maximum likelihood tree based on *gyrB* gene sequences of *Gibbsiella quercinecans*. Bootstrap values after 1000 replicates are expressed as percentages. *Providencia alcalifaciens* is included as an outgroup. The gene sequence for *Edwardsiella ictaluri* was obtained from the genome sequencing database (http://www.ncbi.nlm.nih.gov). Accession numbers are listed in Table 1. The scale bar indicates the fraction of substitutions per site.

Table 3
Phenotypic characteristics distinguishing *Gibbsiella quercinecans* from its closest phylogenetic neighbours. 1 = *Gibbsiella quercinecans* (18 strains), 2 = *Serratia marcescens* ssp. *marcescens* (LMG 2792^T), 3 = *Serratia entomophila* (LMG 8456^T), 4 = *Serratia ficaria* (LMG 7881^T), 5 = *Serratia fonticola* (LMG 7882^T), 6 = *Serratia rubidaea* (LMG 5019^T), 7 = *Edwardsiella tarda* (LMG 2793^T), 8 = *Edwardsiella hoshinae* (LMG 7865^T), and 9 = *Edwardsiella ictaluri* (LMG 7860^T).

Characteristic	1	2	3	4	5	6	7	8	9
β-Galactosidase	+	+	+	+	+	+	–	–	–
Lysine decarboxylase	–	+	–	–	+	+	+	+	+
Gelatinase	–	+	+	+	–	+	–	–	–
Acid from									
D-Sorbitol	+	+	–	+	+	–	–	–	–
D-melibiose	+	–	–	+	+	+	–	–	–
Erythritol	–	+	–	+	+	+	–	–	–
D-Arabinose	+	–	+	+	–	+	–	–	–
D-Adonitol	–	+	+	+	+	+	–	–	–
L-Sorbose	+	–	–	–	–	–	–	–	–
L-Rhamnose	+	–	–	+	+	–	–	–	–
D-Fucose	–	+	+	+	–	+	–	–	–
Potassium gluconate	+	–	–	–	+	–	+	+	+
Utilization of									
N-acetyl-galactosamine	–	+	–	–	+	+	+	+	+
Adonitol	–	+	+	+	+	+	–	–	–
D-Arabitol	+	–	+	+	+	+	–	–	–
Gentiobiose	+	+	+	+	+	+	–	–	–
Turanose	+	+	–	+	–	+	–	–	–
L-Alaninamide	–	+	+	+	+	+	–	–	–
D-Alanine	–	+	+	+	–	+	–	–	–
Glycyl-L-aspartic acid	–	+	+	+	+	+	+	+	+
Putrescine	–	+	+	+	+	+	–	–	–
2,3-Butanediol	+	+	–	–	–	–	–	–	–

strains formed a distinct genetic lineage within the *Enterobacteriaceae*. DNA–DNA hybridization ruled out the possibility that the strains belonged to *S. rubidaea* and several phenotypic differences were noted between the oak strains and species of *Serratia* and *Edwardsiella*.

When describing a novel species, it is recommended that: strains should be hybridized to other strains sharing more than 97% 16S rRNA gene sequence similarity; strains should be delineated by protein-encoding genes and the novel species should be differentiable from other species based on phenotypic and chemotaxonomic markers [25,29]. The DNA–DNA hybridization data, *gyrB*- and *rpoB*-gene sequencing, phenotypic tests and fatty acid profiles indicated that the strains isolated from oak belonged to a single taxon within a novel genus. Therefore, the criteria for describing a novel genus and species were fulfilled and we propose the classification of the oak strains from Britain and Spain as *Gibbsiella quercinecans*.

Description of Gibbsiella gen. nov.

Gibbsiella (Gibb.si'el.la. N.L. fem. n. *Gibbsiella* named in honour of British forest pathologist John N. Gibbs for his contributions to forest pathology).

Gram-negative short rods (0.9 × 1–1.5 μm), facultatively anaerobic, oxidase negative and catalase positive. Cells occur singly, in pairs or in groups of four, and possess very fine fimbriae (Supplementary Fig. S2) but no flagella. Colonies are white to cream on nutrient agar, round, convex and smooth with entire margins. Strains can grow at temperatures between 10 and 40 °C. Positive for β-galactosidase activity but negative for H_2S, urease, indole, acetoin and gelatinase production. Citrate is utilized and nitrate is reduced to nitrite. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activity are all negative. Acid is produced from: glycerol, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, inositol, D-mannitol, D-sorbitol, α-methyl-D-glucoside, *N*-acetyl-D-glucosamine, arbutin, aesculin, ferric citrate, salicin, D-maltose, D-melibiose, D-saccharose, D-trehalose, D-raffinose, D-turanose and D-arabitol. The following carbon sources are utilized at 28 °C: dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, inositol, α-D-lactose, maltose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, pyruvic acid methyl ester, succinic acid mono-methyl ester, acetic acid, citric acid, D-gluconic acid, α-ketoglutaric acid, D,L-lactic acid, succinic acid, bromosuccinic acid, L-aspartic acid, glycerol and α-D-glucose-1-phosphate. The G + C content ranges from 56.0 to 56.4 mol%. The type species is *G. quercinecans*.

Description of G. quercinecans sp. nov.

G. quercinecans (quer.ci.ne'cans. L.n. *quercus*, oak, oaktree; L.v. *necare*, to kill, to destroy; N.L. part. adj. *quercinecans*, oak-destroying (causing necrosis of oak)).

Cell morphology and colony morphology are as given for the genus. Phenotypic properties are as given for the genus. In addition, some strains may produce acid from D-arabinose, D-cellobiose, gentiobiose, L-fucose and potassium gluconate and utilize D-cellobiose, lactulose, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, succinamic acid, L-aspargine, L-glutamic acid, L-proline, D-serine, L-serine, inosine, uridine, thymidine, D,L,α-glycerol phosphate and D-glucose-6-phophate as sole carbon sources. The following carbon sources are not utilized: *N*-acetyl-D-galactosamine, adonitol, erythritol, xylitol, D-galactonic acid lactone, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxy-phenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxyl-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-threonine, D,L-carnitine, γ-aminobutyric acid, urocanic acid, phenylethylamine, putrescine and 2-aminoethanol. The G + C content ranges from 56.0 to 56.4 mol%.

The type strain is FRB 97^T (=LMG 25500^T = NCPPB 4470^T), isolated from *Q. petraea* in Hoddesdon Park Wood, Hertfordshire, England. Strains of this species have been isolated from various oak species exhibiting symptoms of Acute Oak Decline.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.syapm.2010.08.006.

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