

Work Package 4

Assessing pathogenicity of bacteria isolated from stem bleeds on broadleaf trees

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Research question

Do bacteria, in particular *Brenneria* species, play a significant role in tree stem bleeding diseases?

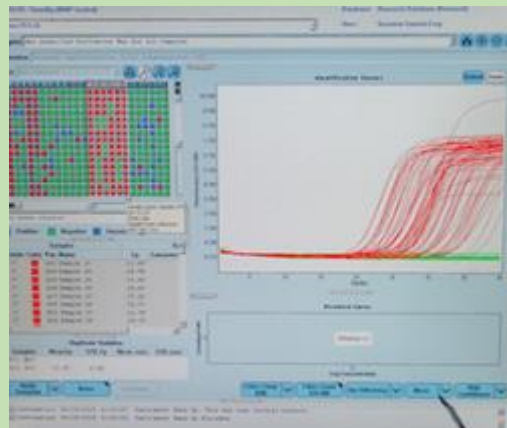
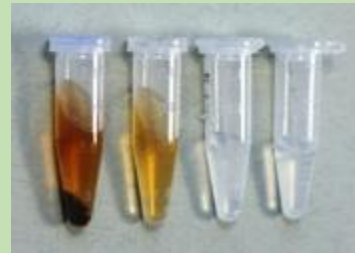
Methods

Sample collection

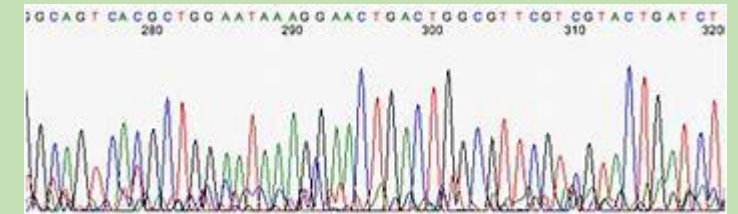
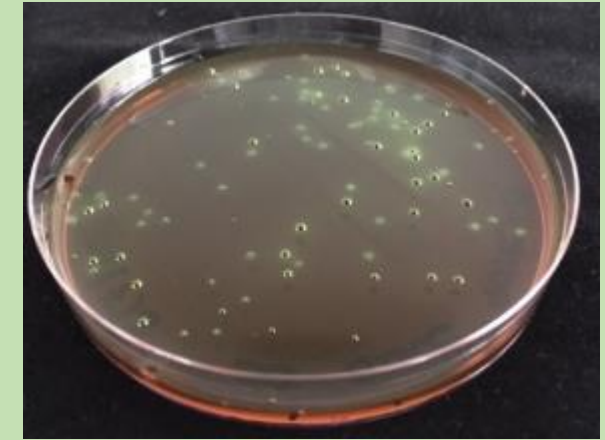
- THADS records
- Site surveys
- Tree Health Officers
- Citizen Science



qPCR detection of AOD associated bacteria

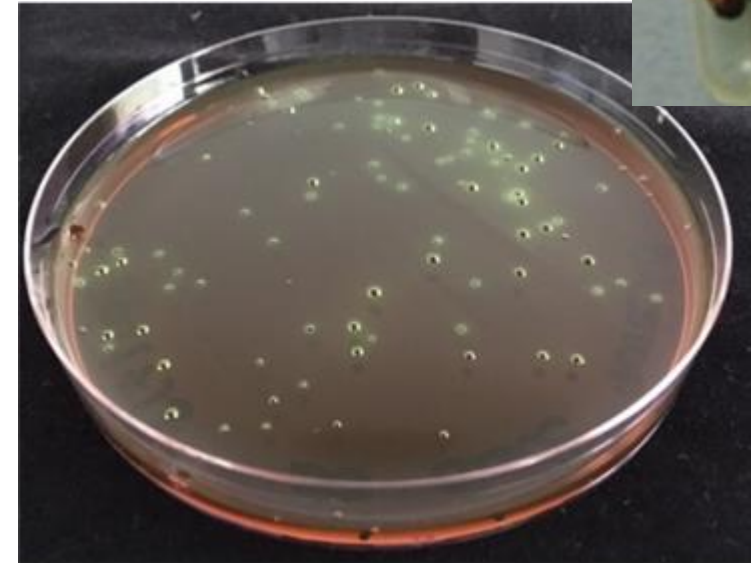
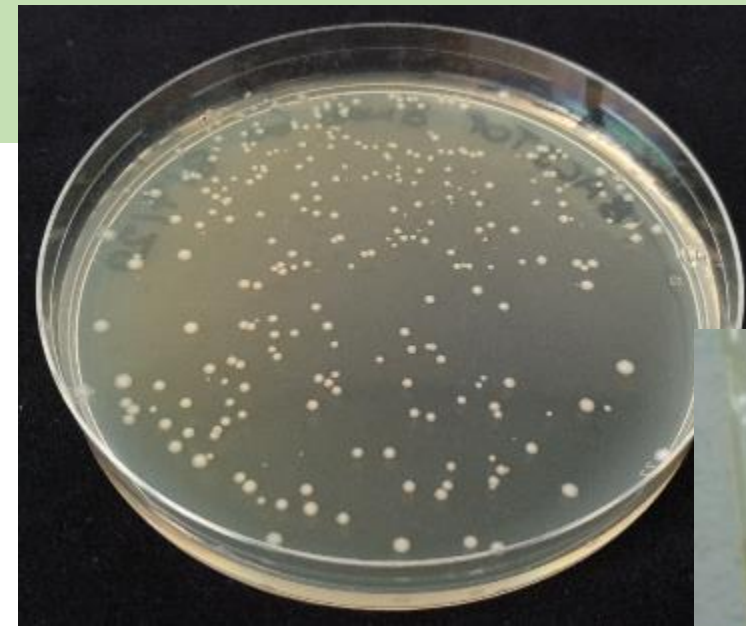


Bacterial isolation and identification



Bacteria on non-oak bleeds

- 121 swabs
- 19 different tree species
- qPCR assay has identified AOD bacteria
 - *Brenneria goodwinii*, *Gibbsiella quercinecans* and *Rahnella victoriana*
 - 50 swabs have tested positive for at least one of the AOD-associated bacteria.
- Bacterial isolations from swabs
 - *Brenneria goodwinii* from *Tilia* (lime)
 - *Rahnella victoriana* from *Tilia*, *Betula* (birch) and *Fagus* (beech).
 - *Gibbsiella quercinecans* from *Tilia*.
- 137 bacteria were isolated from the swabs and bark panels.
- Four new species characterised



Characterisation of new bacterial species

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

Volume 72, Issue 1

Research Article

Rahnella perminowiae sp. nov., *Rahnella bonaserana* sp. nov., *Rahnella rivi* sp. nov. and *Rahnella ecdela* sp. nov., isolated from diverse environmental sources, and emended description of the genus *Rahnella* 📄

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📄 View Affiliations

Published: 20 January 2022 | <https://doi.org/10.1099/ijsem.0.005190>

frontiers | Frontiers in Microbiology

TYPE: Original Research
PUBLISHED: 17 November 2022
DOI: 10.3389/fmicb.2022.1063107

Check for updates

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to
Evolutionary and Genomic
Microbiology,
a section of the journal
Frontiers in Microbiology

RECEIVED 06 October 2022
ACCEPTED 31 October 2022
PUBLISHED 17 November 2022

CITATION
Brady C, Kaur S, Crampton B,
Maddock D, Arnold D and Denman S (2022)

Transfer of *Erwinia toletana* and *Erwinia iniecta* to a novel genus *Winslowiella* gen. nov. as *Winslowiella toletana* comb. nov. and *Winslowiella iniecta* comb. nov. and description of *Winslowiella arboricola* sp. nov., isolated from bleeding cankers on broadleaf hosts

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INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

Volume 72, Issue 10

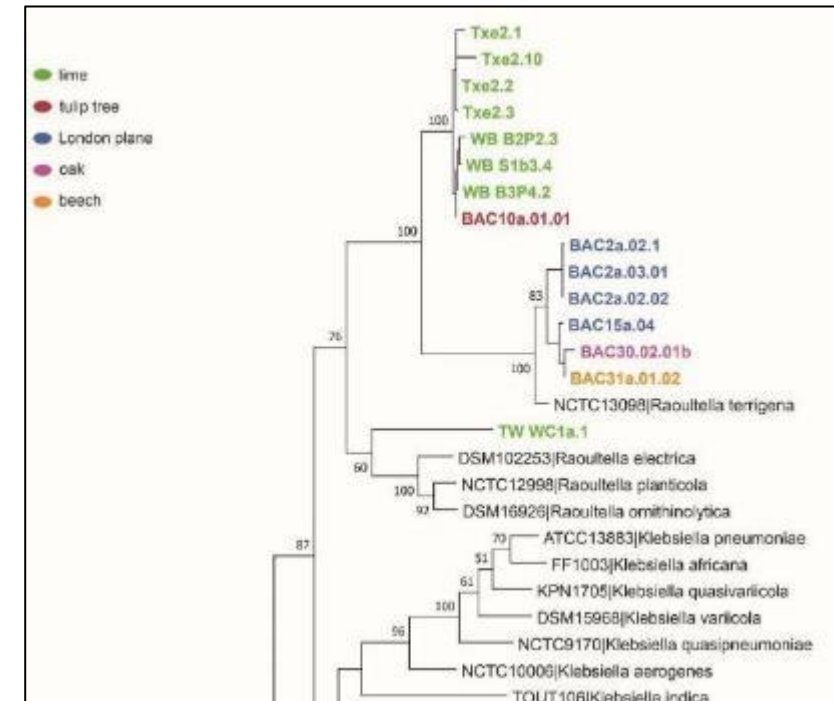
Research Article

Brenneria tiliae sp. nov., isolated from symptomatic *Tilia × moltkei* and *Tilia × europaea* trees in the UK 📄

Helene Kite¹, Dawn Arnold², Joel Allainguillaume¹, Sandra Denman³, Carrie Brady¹ 📧

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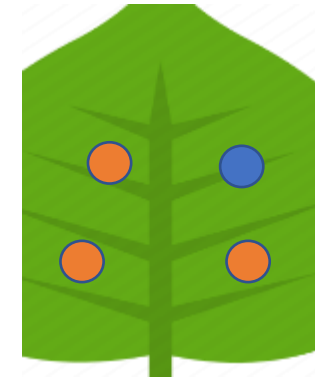
Published: 07 October 2022 | <https://doi.org/10.1099/ijsem.0.005515>



HR assays to test the pathogenicity of bacteria isolated from non-oak stem bleeds

Acinetobacter halotolerans
Bacillus cereus
Bacillus subtilis
Buttiauxella agrestis
Citrobacter freundii
Erwinia billingiae
Escherichia coli
Gibbsiella greigii
Hafnia paralvei
Klebsiella oxytoca
Pseudomonas daroniae
Pseudomonas poae
Rahnella bruchi/variigena
Rahnella victoriana
Raoultella electrica
Raoultella ornithinolytica
Raoultella terrigena
Serratia myotis
Serratia proteamaculans
Shigella flexneri

Staphylococcus warneri
Yersinia nurmii
Erwinia persicina
Serratia liquifaciens
Pseudomonas fluorescens
Klebsiella pasteurii
Brenneria goodwinii (strain FRB141)
Brenneria goodwinii
Gibbsiella quercinecans T97
Serratia fonticola
Pseudomonas aylmerense
Gibbsiella quercinecans
Brenneria goodwinii
Gibbsiella quercinecans
Lelliottia amnigena
Lelliottia amnigena
Lelliottia amnigena
Serratia fonticola



● Inoculation point: *Lonsdalea britannica* control
● Inoculation point: bacteria

P. fluorescens



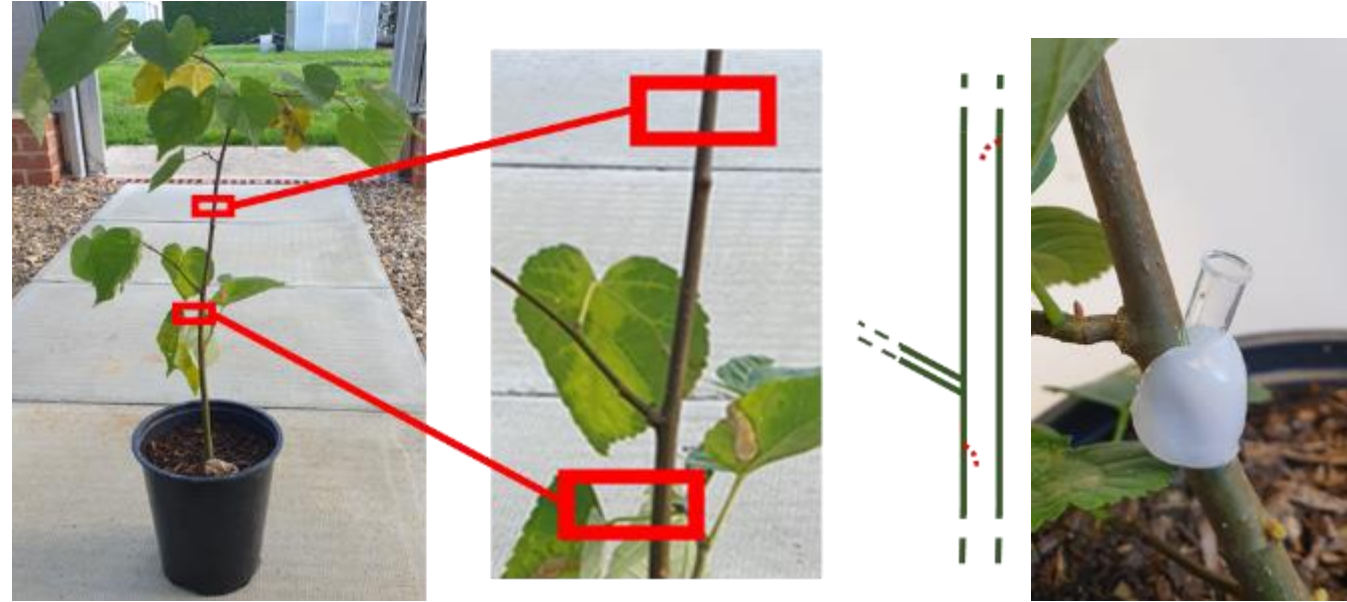
G. quercinecans



Pathogenicity of AOD-associated bacteria isolated from non-oak trees

Sapling trial

- Does *B. goodwinii* cause lesions in lime and oak?
- Does *B. tiliae* cause lesions in lime and oak?
- Does *W. arboricola* cause lesions in lime and oak?
- Is there a difference in lesions formed by *B. goodwinii* isolated from oak vs *B. goodwinii* isolated from lime species?
- Do single strains of *B. goodwinii* produce lesions of the same size compared to mixed strains of *B. goodwinii* when inoculated into lime and oak saplings?



Treatments:

- 4 *B. goodwinii* strains (oak, lime)
- 2 *W. arboricola* (lime, London plane)
- 2 *B. tiliae* (lime)

Pathogenicity of AOD-associated bacteria isolated from non-oak trees – Sapling trial

No lesions on lime after inoculation with any of the bacterial species



Inoculated lime sapling



B. goodwinii from oak



B. goodwinii from lime



Inoculated oak saplings



W. arboricola from London Plane



B. tiliae from lime



Pathogenicity of AOD-associated bacteria isolated from non-oak trees

Billet trial

- Does *B. goodwinii* cause lesion formation in oak and non-oak billets?
- Does a combination of AOD bacteria cause lesion formation in oak and non-oak billets?
- Are there any differences in lesion formation in billets when inoculated with *B. goodwinii* or a combination of AOD-associated bacteria?

Logs inoculated:

Oak, hornbeam, lime, beech, birch

Treatments:

1. *Brenneria goodwinii* (3 strains)
2. Bg (3 strains), Gq (3 strains) and Rvic (3 strains)
3. Ringers solution (Control)



Pathogenicity of AOD-associated bacteria isolated from non-oak trees – Billet trial

Hornbeam



Oak



Lime



Beech



Birch



Preliminary results

- Only oak and hornbeam developed lesions after inoculation with Bg and Bg/Rvic/Gq
- *B. goodwinii* could be back isolated after two months from inoculation points of all species
- Bg, Rvic and Gq back isolated from oak billets after two months
- Oak control inoculations developed lesions from which *B. goodwinii* could be isolated

BACTERIAL TREE DISEASE FACT SHEETS



Bridget Crampton, Carrie Brady and Sandra Denman



DISEASES OF WOODLAND TREES CAUSED BY BRENNERIA SPECIES

Bacteria belonging to the genus *Brenneria* are responsible for causing disease on woody hosts. Species cause a range of cankers, wilts, and necroses on willow, oak, alder and walnut and are also associated with disease-causing species of *Lonsdalea*.



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<https://bacterialplantdiseases.uk/bac-stop>



Bacterial Tree Disease Fact Sheets

- Series of Fact Sheets on Bacterial Tree Diseases
- For use by Stakeholders to identify bacterial tree diseases
- First Fact Sheet on Diseases of Woodland Trees caused by *Brenneria* species
 - *Brenneria salicis* (watermark disease of willow)
 - *Brenneria nigrifluens* (shallow bark canker of walnut)
 - *Brenneria rubrifaciens* (deep bark canker of walnut)
 - *Brenneria alni* (bark canker of alder)
 - *Brenneria goodwinii* (acute oak decline)
- PDF versions available from Sally.Simpson@ForestResearch.gov.uk
- <https://bacterialplantdiseases>



BACTERIAL TREE DISEASE FACT SHEETS



Bridget Crampton, Carrie Brady and Sandra Denman



DISEASES OF WOODLAND TREES CAUSED BY LONSDALEA SPECIES

Bacterial species belonging to the genus *Lonsdalea* have been isolated from diseased trees, specifically oak, poplar and willow. They cause blights and cankers, and are also associated with the pathobiome of acute oak decline (AOD). Some confusion may exist regarding naming, as *Lonsdalea quercina* was called *Brenneria quercina* and was divided into several subspecies (*L. quercina* ssp. *britannica*; *L. quercina* ssp. *iberica* and *L. quercina* ssp. *populi*). These subspecies have been elevated to species level and *Lonsdalea* currently consists of *L. quercina*, *L. britannica*, *L. iberica* and *L. populi*.



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 <https://bacterialplantdiseases.uk/bac-stop>



Bacterial Tree Disease Fact Sheets

- Second Fact Sheet on Diseases of Woodland Trees caused by *Lonsdalea* species
 - *Lonsdalea quercina* (Drippy acorn and drippy shoot blight of oak)
 - *Lonsdalea britannica* (Acute Oak Decline)
 - *Lonsdalea iberica* (Drippy nut and bark canker of oak)
 - *Lonsdalea populi* (Bacterial canker of poplar and willow)
- PDF versions available from Sally.Simpson@ForestResearch.gov.uk
- <https://bacterialplantdiseases.uk>



The way forward

- Continue to receive and process swabs and bark panels.
- Microbiome analysis (single gene community profiling) of cankers from lime trees (Thetford, Tidworth and Westonbirt).
- Comparative population genomics of *Brenneria goodwinii* isolated from different host species and regions.

