



BELGIAN CO-ORDINATED COLLECTIONS OF MICRO-ORGANISMS
BACTERIA COLLECTION

GREAT AT SMALL THINGS

New insights in the taxonomy of plant-pathogenic and plant-associated bacteria in the *Enterobacteriaceae*

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ENTEROBACTERIACEAE

GREAT AT SMALL THINGS

- family within the *Gammaproteobacteria*
- contains many plant-pathogenic and plant-associated bacteria, as well as pathogens to humans & animals



- *Erwinia*
- *Pectobacterium*
- *Pantoea*
- *Brenneria*
- *Samsonia*
- *Dickeya*



- *Escherichia*
- *Salmonella*
- *Klebsiella*
- *Enterobacter* ✦
- *Serratia* ✦

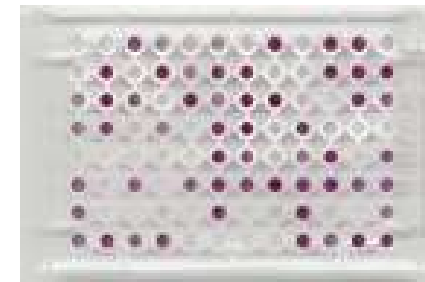
✦ Also plant-associated or plant-pathogenic isolates



ENTEROBACTERIACEAE

GREAT AT SMALL THINGS

- phenotypically similar → difficult to identify some taxa using commercial ID kits
- eg. species of *Pantoea*, *Erwinia* and *Enterobacter* cannot be accurately identified
- incorrect classification of numerous strains
- many taxonomic rearrangements





TAXONOMY OF PP AND PA *ENTEROBACTERIACEAE*

- plant pathologists → species according to disease, not phylogenetic relationship
- eg. the genus *Erwinia* was created in 1920 to unite all G⁻, fermentative, non-sporulating, peritrichously flagellated plant pathogenic bacteria
- end of 1998, most species of this genus were reclassified into new or existing genera



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ERWINIA

→ end of 1998:

E. amylovora
E. mallitovora
E. rhapontici
E. tracheiphila
E. psidii
E. persicinus

~ 20 species on the AL

DDH



16S rRNA



Enterobacter

E. cloaca subsp. *dissolvens*
E. cancerogenus
E. nimipressuralis

Pantoea

P. agglomerans (Br HG XIII)
P. dispersa (Br HG III)
P. ananatis (incl. Br HG VI)
P. stewartii subsp. *stewartii*

Pectobacterium

P. carotovorum subsp. *carotovorum*
P. carotovorum subsp. *odoriferum*
P. atrosepticum
P. betavasculorum
P. wasabiae
P. cacticida
P. cypripedii
P. chrysantemi

Brenneria

B. quercina
B. nigrifluens
B. rubrifaciens
B. salicis
B. alni
B. paradisiaca

Erwinia herbicola
Enterobacter agglomerans
species complex

Brenner *et al.* (1984): 13 HG

Dickeya

in 2005:

D. chrysantemi
D. paradisiaca

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TAXONOMY

- “ ... one of the goals of any taxonomy is to create a classification scheme that reflects the genealogy of the organisms, thus circumscribing all taxa in monophyletic groups” – Ramon Rossello-Mora (Ed. Syst Appl Microbiol)



TAXONOMY OF PP AND PA *ENTEROBACTERIACEAE*

GREAT AT SMALL THINGS

- 16S rRNA gene sequence analysis → many genera not monophyletic
- such as:
 - *Enterobacter*
 - *Serratia*
 - *Pantoea*
 - *Erwinia*
 - *Pectobacterium*
 - *Brenneria*

Enterobacteriaceae

16S rRNA



Pantoea

Erwinia

Enterobacter

Brenneria

Pectobacterium

Serratia



TAXONOMY OF PP AND PA *ENTEROBACTERIACEAE*

- housekeeping gene sequences → phylogenies more compatible with the “accepted classifications”
 - eg. *Serratia* was monophyletic based on *gyrB* (Dauga, 2002)
- ➔ phylogenies should be based on sequences of several genes encoding different functions, as comparisons of a single housekeeping gene can provide misleading results



BCCM/LMG – FABI/UNIV. PRETORIA

- MLSA study of plant-pathogenic and plant-associated *Enterobacteriaceae* based on partial sequences of four house keeping genes:

atpD (ATP synthase β subunit)

gyrB (DNA gyrase)

infB (initiation translation factor 2)

rpoB (RNA polymerase β subunit)



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OUR INTEREST IN *ENTEROBACTERIACEAE*

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- FABI: Forestry and Agricultural Biotechnology Institute, Univ. of Pretoria, SA
- Isolation campaigns/field trips in Africa, Asia and South America
- *Eucalyptus* + bacterial blight and dieback
→ *Pantoea*
- Community studies of diseased vs. healthy *Eucalyptus* seedlings → *Enterobacter* (?)





OUR INTEREST IN *ENTEROBACTERIACEAE*

- Onion + centre rot → *Pantoea*
- Maize + stalk rot → *Pantoea*
- Potato + soft rot → *Dickeya*
- Collaboration with University of Perugia (Italy)
Olive trees + knots → *Pantoea/Erwinia*
- Collaboration with Forest Research (UK)
Oak + Acute Oak Decline (AOD) → close to *Serratia* and *Brenneria*





MLSA STUDY OF PP AND PA *ENTEROBACTERIACEAE*

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- BILA (Belgium-SA), BOF, UGent (2006-2008)
 - focus on *Pantoea* from *Eucalyptus* and onion
- Post Doctoral Fellowship for non-EU researchers (2009)
 - plant pathogenic and plant-associated bacteria in the *Enterobacteriaceae* and their relationships with other representatives of the family



MLSA STUDY OF PP AND PA *ENTEROBACTERIACEAE*

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- included:
- genera such as *Pantoea*, *Erwinia*, *Brenneria*, *Pectobacterium*, *Dickeya*, *Samsonia*, *Serratia* and *Enterobacter*
- strains of closely related genera containing (mainly) isolates from clinical origin, such as *Tatumella*, *Citrobacter*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Raoultella*, *Yokenella*
- unidentified new isolates from trees/plants

Pantoea-Erwinia

atpD, *gyrB*, *infB*, *rpoB*

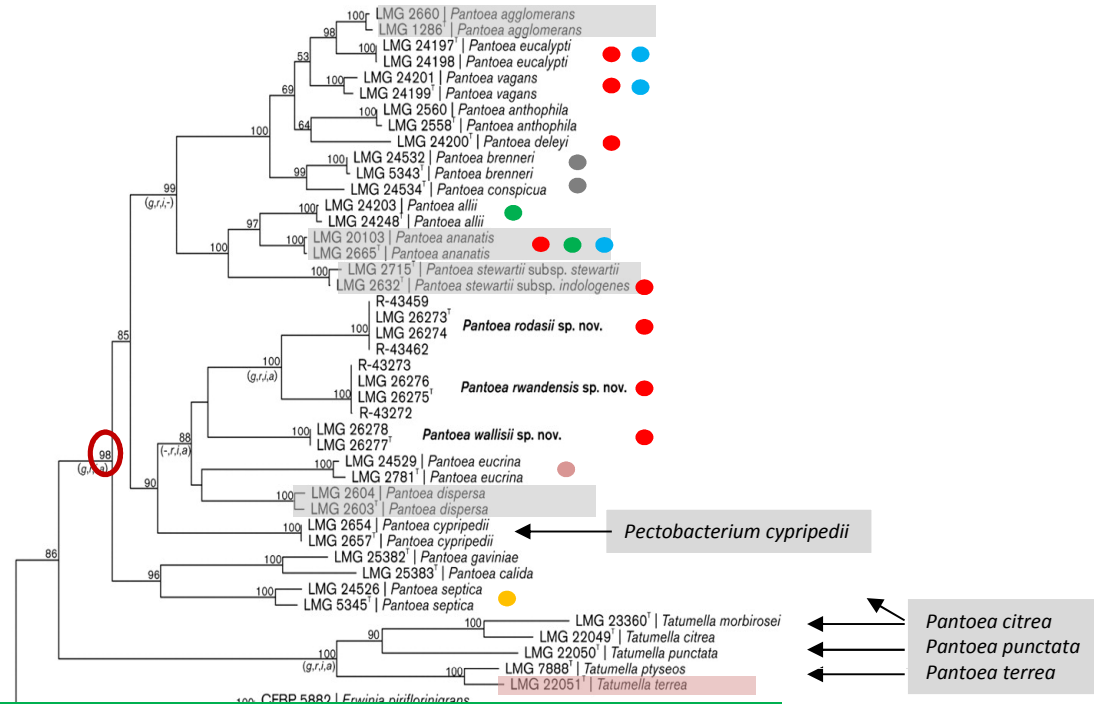
Br HG

- II
- IV
- V

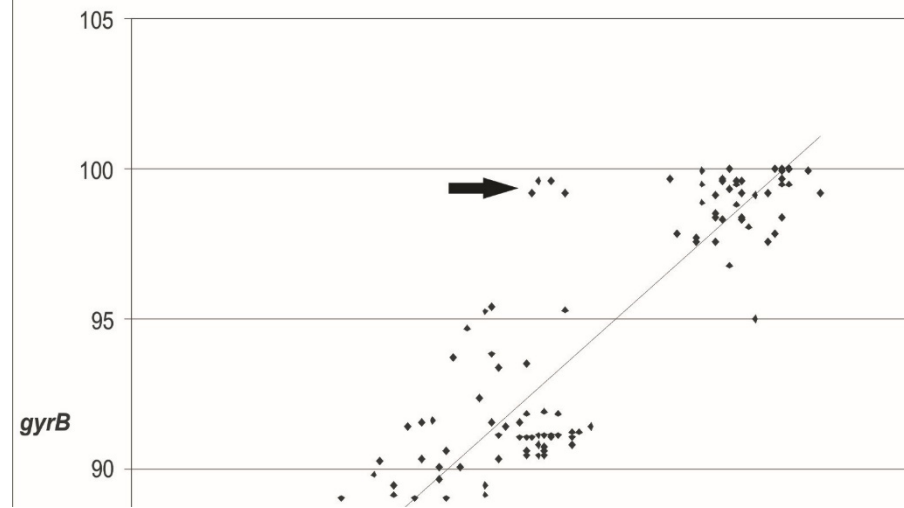
isolates from:

- *Eucalyptus*
- Onion
- Maize
- Olive tree knots

0.1

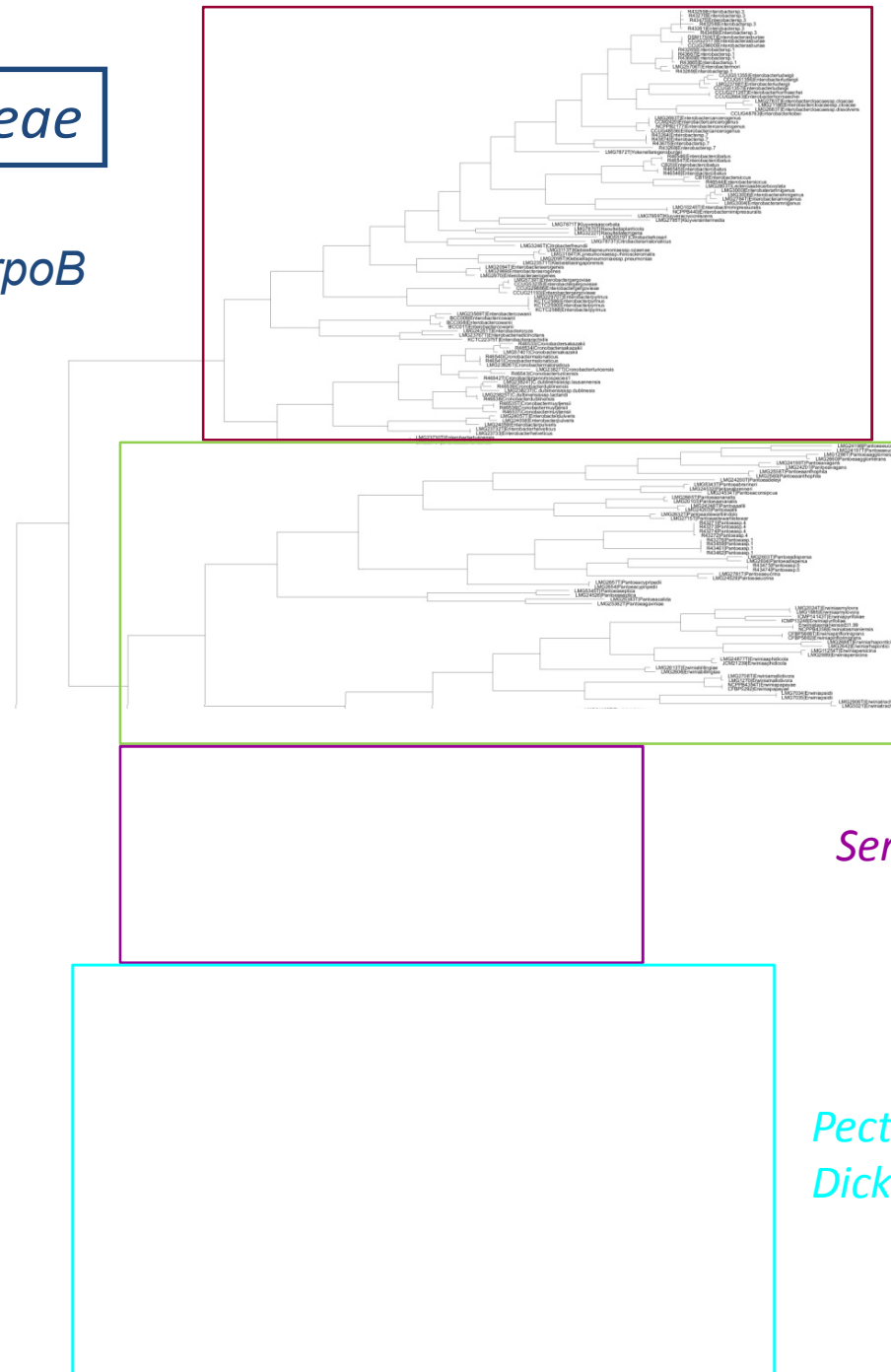


Congruence



Enterobacteriaceae

atpD, *gyrB*, *infB*, *rpoB*



Enterobacter,
Cronobacter &
Mangrovibacter

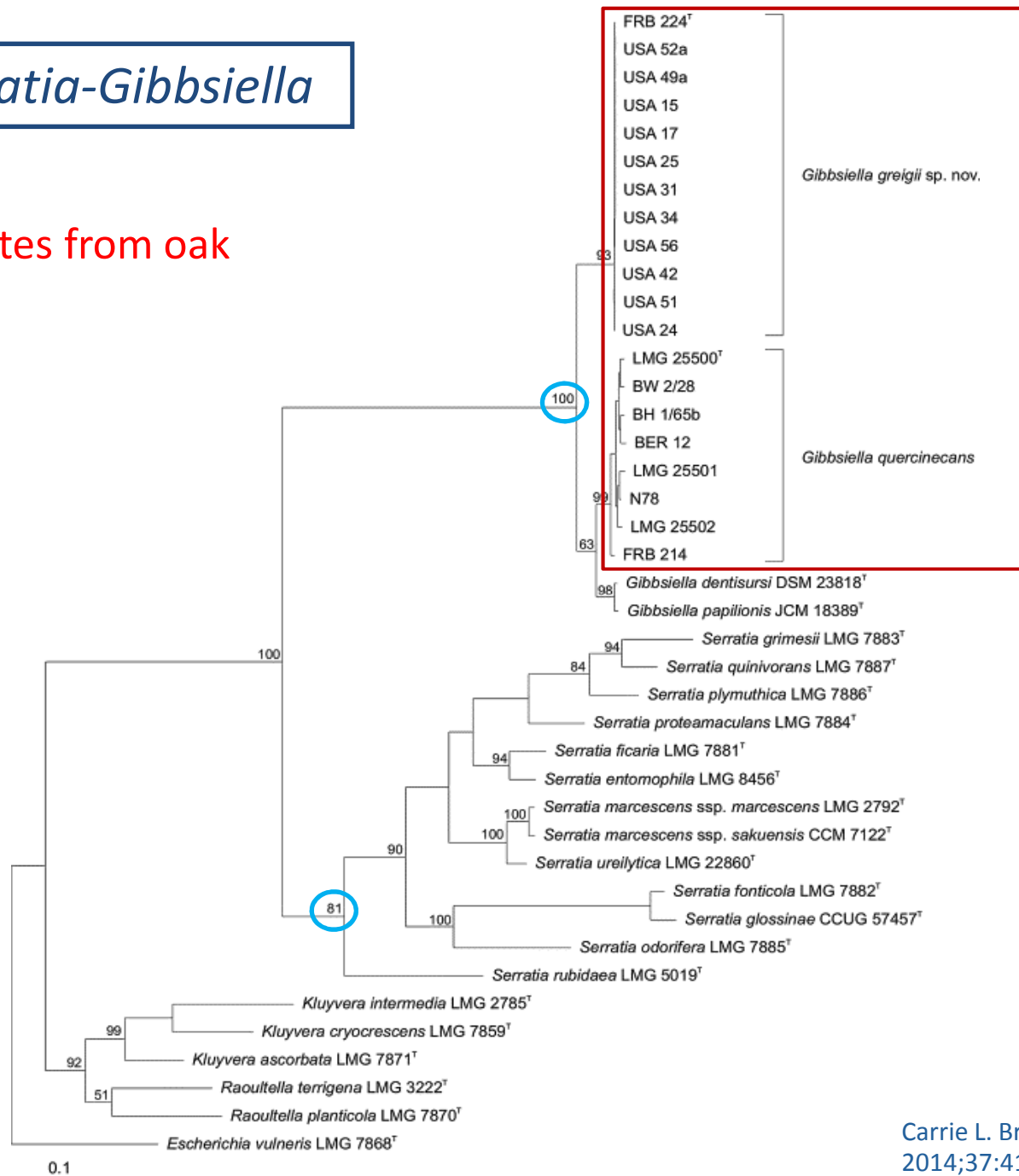
Pantoea, *Erwinia*
& *Tatumella*

Serratia & *Gibbsiella*

Pectobacterium, *Brenneria*,
Dickeya & *Samsonia*

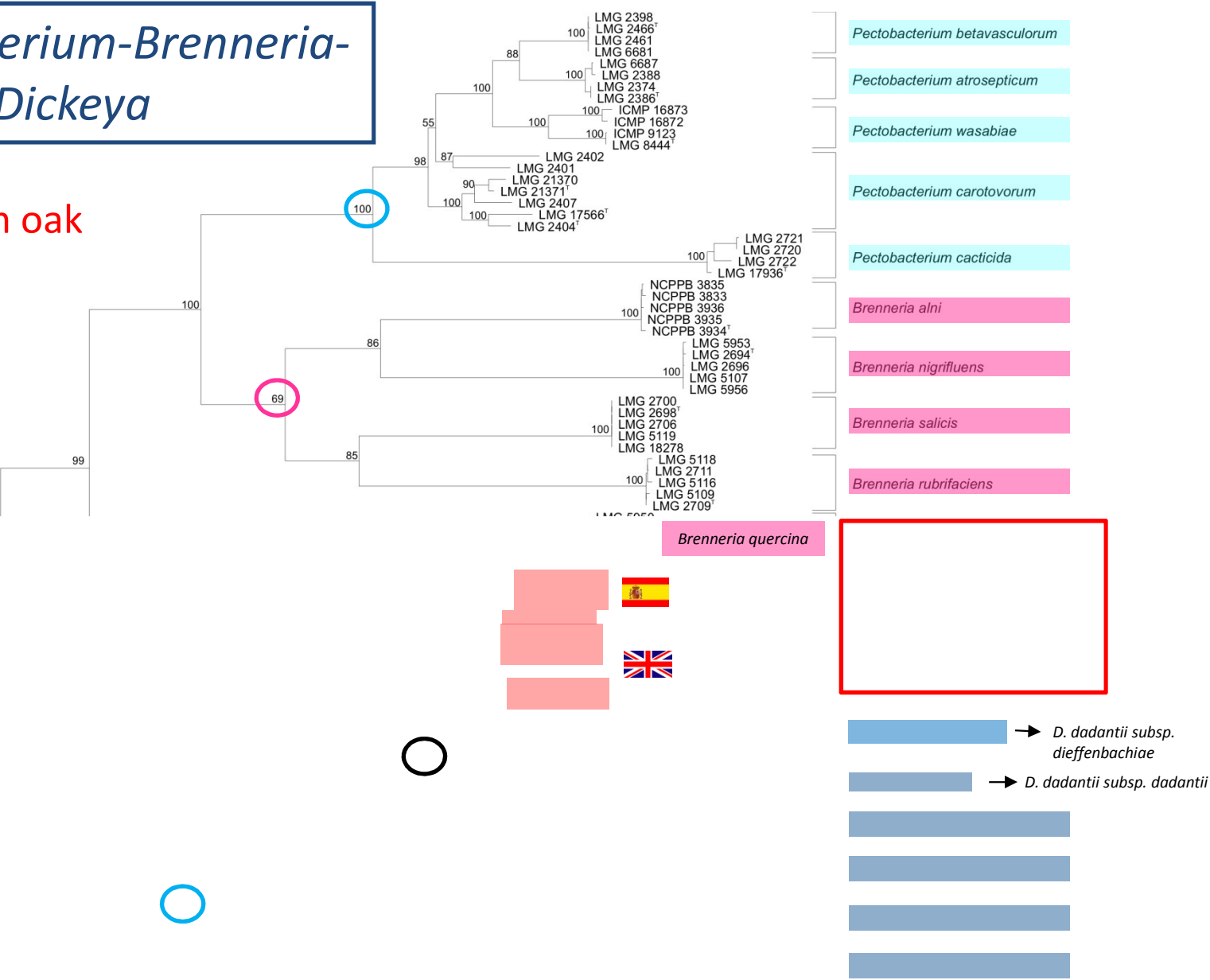
Serratia-Gibbsiella

isolates from oak



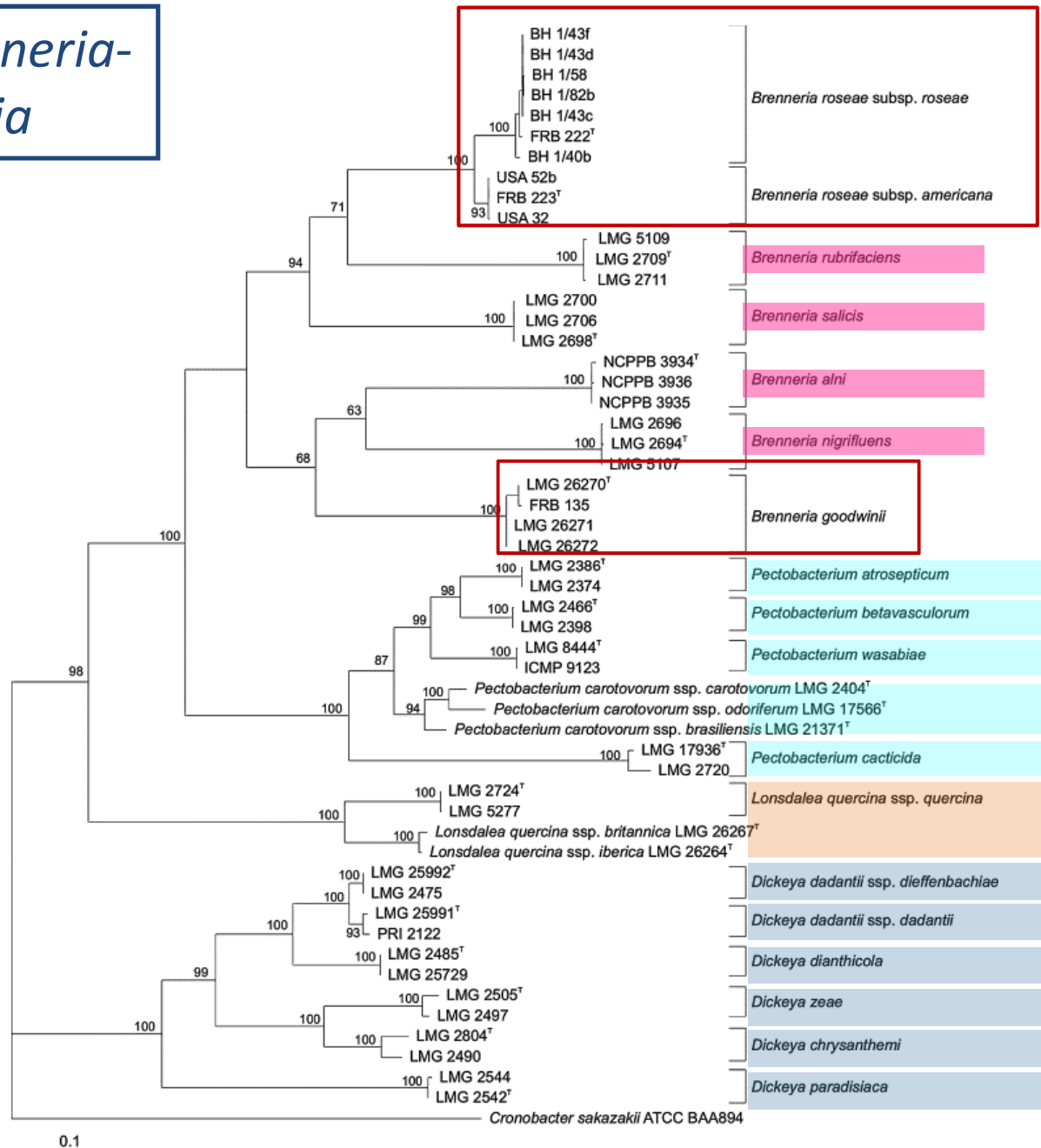
Pectobacterium-Brenneria-Dickeya

isolates from oak



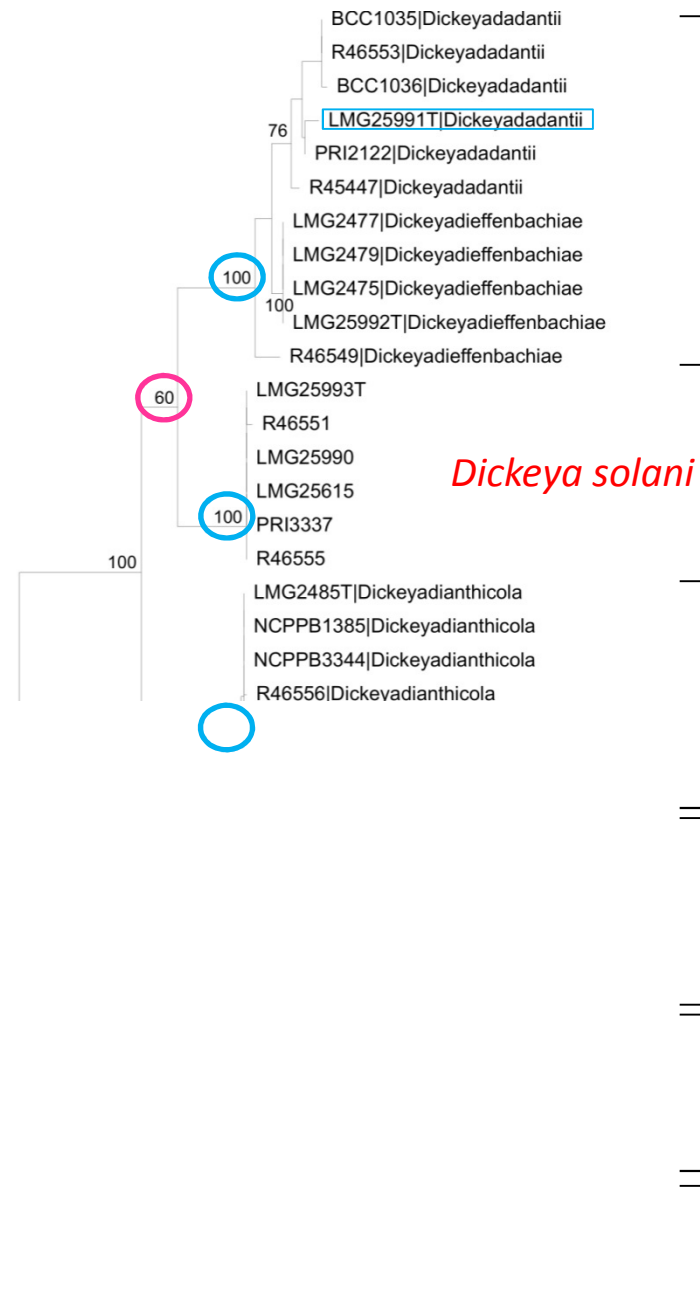
Pectobacterium-Brenneria-Dickeya-Samsonia

isolates from oak



Dickeya

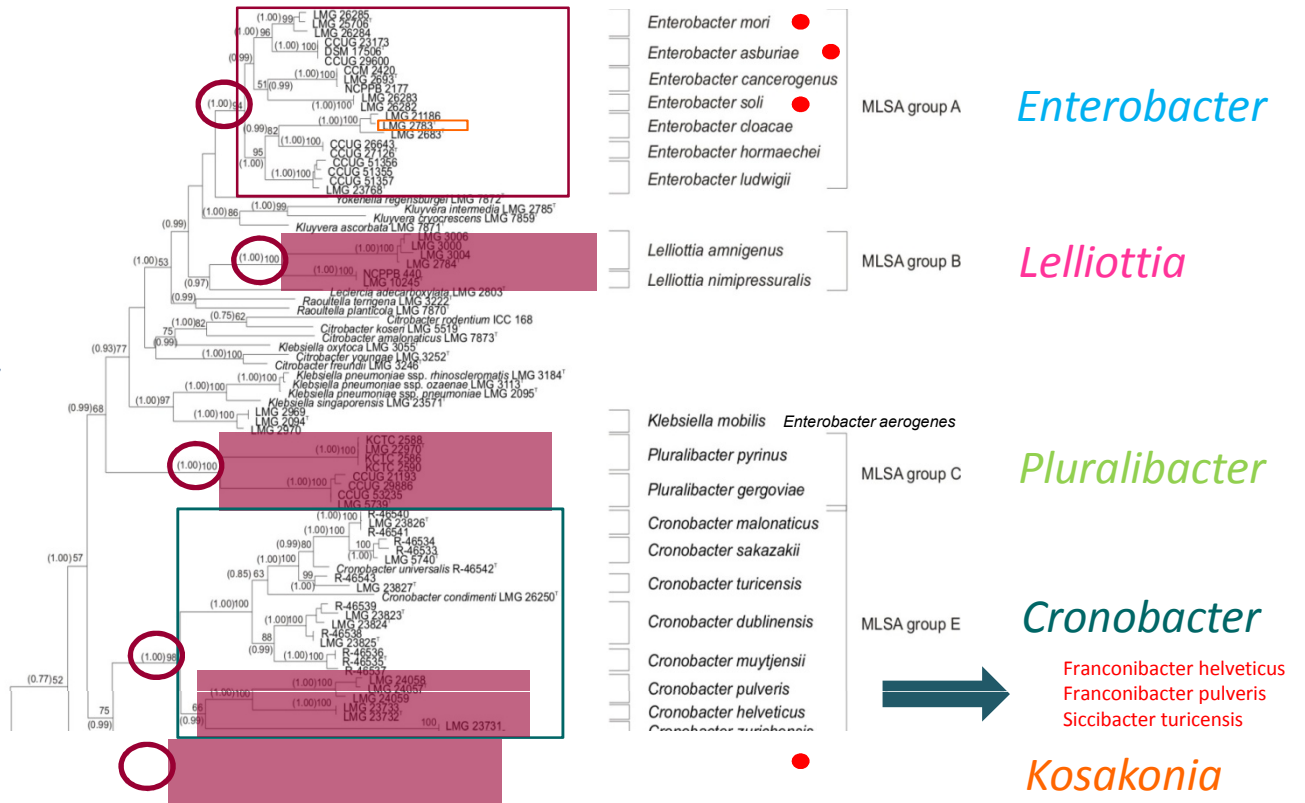
isolates from potato



Enterobacter

Shigella: previously classified as *Enterobacter*

isolates from
eucalyptus





MLSA STUDY OF PP AND PA *ENTEROBACTERIACEAE*

GREAT AT SMALL THINGS

- successfully constructed an MLSA scheme that included all plant-pathogenic and plant-associated genera of the family *Enterobacteriaceae*
- MLSA phylogeny was partially congruent with 16S rRNA phylogeny
- using MLSA data, several genera were less polyphyletic
- MLSA data suggested the reclassification of some established species into existing or new genera
- the proposed taxonomic rearrangements were supported by DDH data, FAMES and phenotypic data



MLSA STUDY OF PP AND PA *ENTEROBACTERIACEAE*

GREAT AT SMALL THINGS

- MLSA is more reliable than 16S rRNA gene sequences for classification of new *Enterobacteriaceae* isolates at the genus and species level
- MLSA of the isolates enabled to identify them at the species level or suggested the existence of new species
- closely related plant-associated and clinical bacteria should not be studied separately



MLSA STUDY OF PP AND PA *ENTEROBACTERIACEAE*

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- distinction between PA and clinical bacteria is becoming more vague

- *Erwinia*
- *Pectobacterium*
- *Enterobacter* ✦
- *Serratia* ✦
- *Rahnella* ✦
- *Tatumella* ✦
- *Pantoea* ✦
- *Saccharobacter*
- *Brenneria*
- *Samsonia*
- *Raoultella*
- *Dickeya*
- *Mangrovibacter*
- *Lonsdalea*
- *Phaseolibacter*
- *Gibbsiella* ✦
- *Rosenbergiella*
- *Kosakonia* ✦
- *Lelliottia*
- *Pluralibacter* ✦
- *Franconibacter*
- *Siccibacter*

✦ Also isolates from human/clinical samples



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GREAT AT SMALL THINGS

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