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NATIONAL INSTITUTE OF **BIOLOGY**

Revealing Diagnostic Gaps and Sequence Diversity in *Xanthomonas* Pathovars of Common Bean Through Molecular Testing and DNA Barcoding

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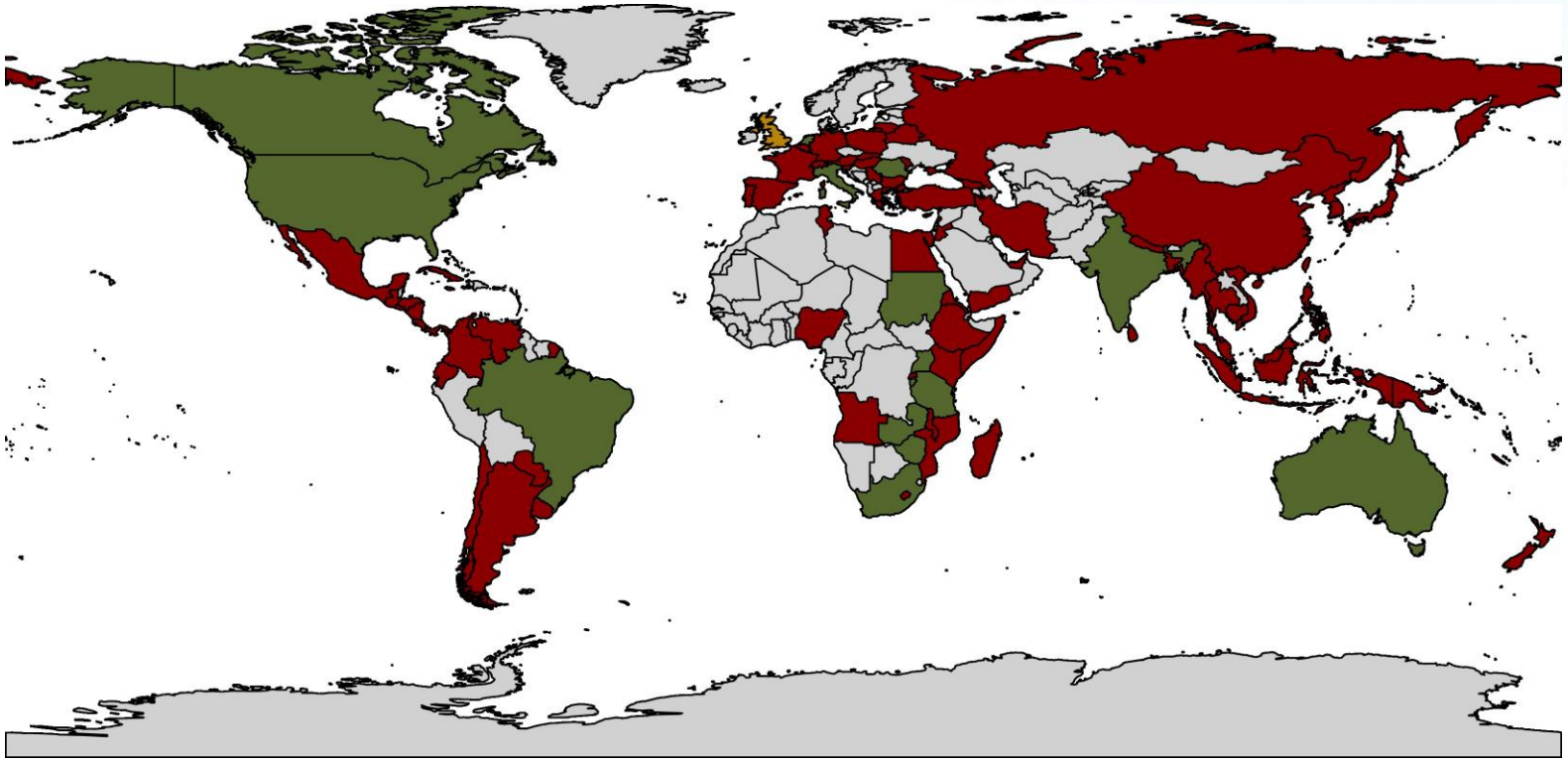
Common bacterial blight of bean and the two pathogens

- CBB is **caused by two closely related pathogens** occupying the same **ecological niche**.
  - *Xanthomonas phaseoli* pv. *phaseoli* (XANTPH)
  - *Xanthomonas citri* pv. *fuscans* (XANTFF)
- **Seed transmission is highly efficient**
- **Different genetic backgrounds, similar disease - a challenge for diagnostics.**

Symptoms caused by Xanthomonas phaseoli pv. phaseoli in common bean (Phaseolus vulgaris). Images: Howard F. Schwartz, Colorado State University (USA, Bugwood.org).

Do diagnostics reflect real diversity?

These pathogens are globally distributed and seedborne, meaning detection must be sensitive and reliable across diverse conditions.



Reported distribution of common bacterial blight (CBB) and availability of isolates in the EPPO-Q-bank database:

- Disease reported; isolates available
- Disease reported; no isolates avaiable
- No information in EPPO Global Database

! **Reference datasets may not represent the diversity of circulating strains.**

To test: diagnostics VS reality

- genetic diversity
- incomplete reference data
- method limitations

Study design

- **34 isolates** from culture collections
- methods:
  - MALDI-TOF
  - PCR, real-time PCR
  - DNA barcoding

Target isolates

X. phaseoli pv. *phaseoli* (XANTPH)

~ Lablab (hyacinth bean) isolates

X. citri pv. *fuscans* (XANTFF)

~ highly-pathogenic isolate CFBP 6988

Non-target isolates

Xanthomonas citri pv. *glycines*

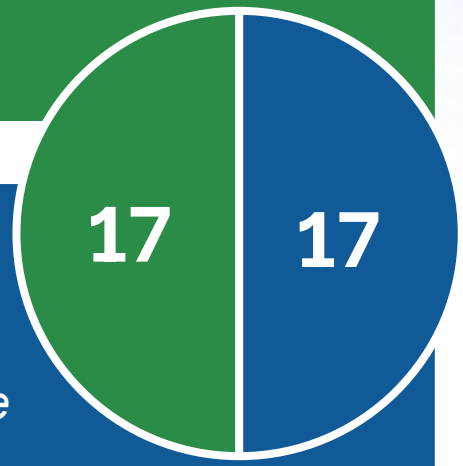
Xanthomonas vesicatoria

Pseudomonas syringae pv. syringae

Pseudomonas viridiflava

Curtobacterium flaccumfaciens pv. *flaccumfaciens*

Stenotrophomonas maltophilia



Diagnostic gaps identified

- MALDI-TOF: **no library entries** for the target organisms
- no cross-reactivity with bacteria of non-Xanthomonas genus
- **conventional PCR:**
  - some isolates **not detected**
  - type/pathotype isolate
  - Lablab XANTPH isolates
- **real-time PCR**
  - Baldwin (XANTPH, XANTFF)
  - **He-ph (XANTPH)** – need for a Cq cut-off of 35
  - **He-ff (XANTFF) –** some isolates **not detected**

When diagnostics meet real-life diversity

CFBP 6988

- **known highly-pathogenic XANTFF isolate**
- ***Phaseolus vulgaris* cv. *Marla***, France, **La Reunion, 2000**
- genomic lineage 2 (GL2)
- **not detected with real-time PCR specific for XANTPH (He-ph)**
- **no exact barcode match in the EPPO Q-BANK gyrB and avrBs2**

Similarity in DNA barcoding analysis can be misleading

When reference datasets are incomplete, **highest similarity does not guarantee correct identification**.

CFBP 6988

Top matches: wrong pathovars

X. citri pv. *vignaeradiatae* (gyrB, 99.3 %)

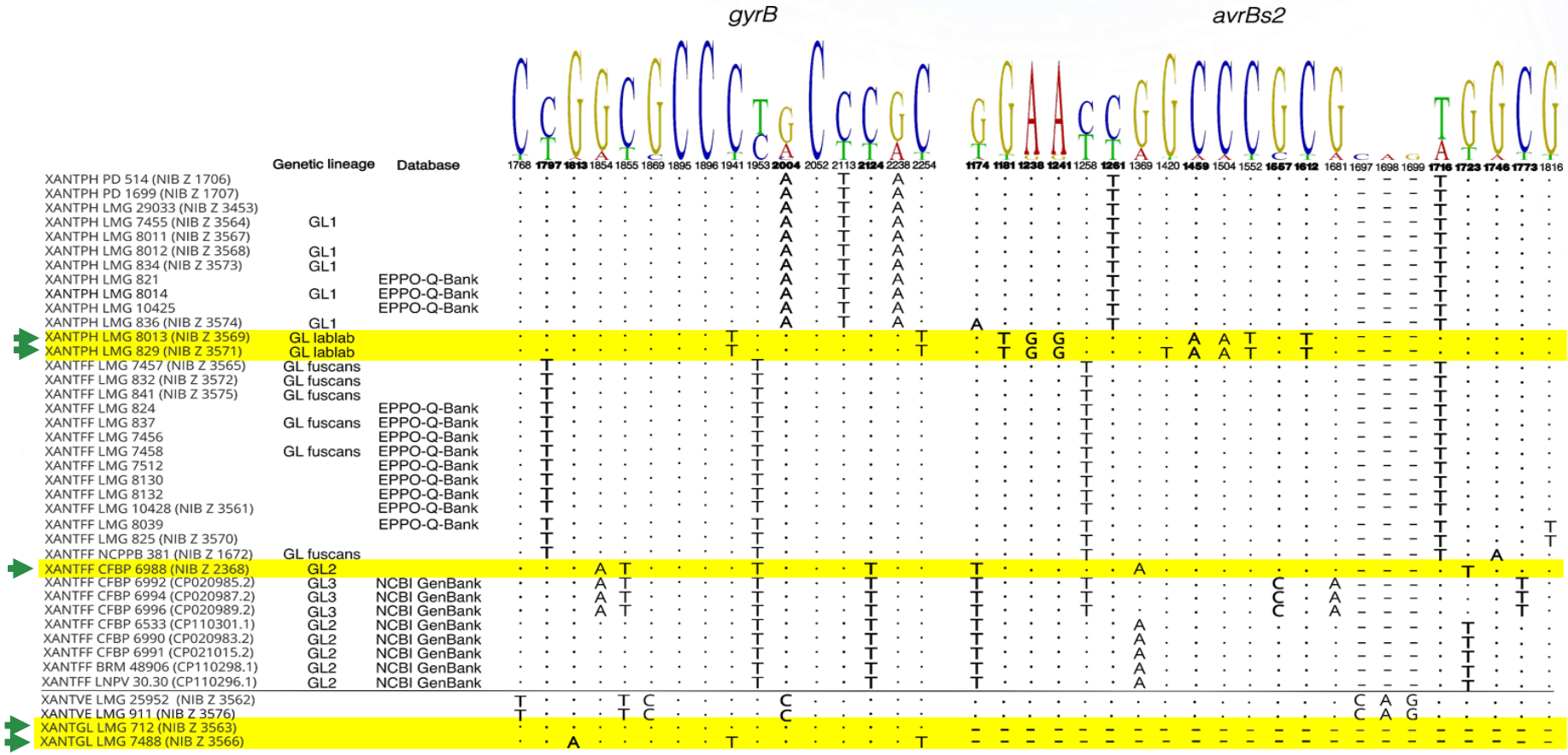
X. citri pv. *thirumalacharii* (avrBs2, 99.4 %)

pathovars not reported to be pathogenic on common bean

risk of incorrect diagnostic conclusion

Going beyond similarity

- **not all positions** are equally **informative**
- diagnostic nucleotide characters – **DNCs** - highlight informative positions
- support interpretation when similarity is ambiguous



Summary

Summary of diagnostic performance parameters for tests evaluated on 34 bacterial isolates for the detection of *Xanthomonas phaseoli* pv. *phaseoli* (XANTPH) and *X. citri* pv. *fuscans* (XANTFF) on common bean (*Phaseolus vulgaris*).

Test	TP	TN	FP	FN	Inclusivity (%)	Exclusivity (%)	Accuracy (%)
MALDI-TOF MS	17	15	2	0	**100.0%**	**88.2%**	**94.1%**
Conventional PCR: Audy	14	17	0	3	**82.4%**	**100.0%**	**91.2%**
Real-time PCR: Baldwin	15	17	0	2	**88.2%**	**100.0%**	**94.1%**
Real-time PCR: He-ph	8	24	0	2	**80.0%**	**100.0%**	**94.1%**
Real-time PCR: He-ff	6	27	0	1	**85.7%**	**100.0%**	**97.1%**
DNA barcoding: *gyrB*	17	16	1	0	**100.0%**	**94.1%**	**97.1%**
DNA barcoding: *avrBs2*	17	17	0	0	**100.0%**	**100.0%**	**100.0%**

target		non-target
CFBP 6988	**Lablab**	**XANTGL**
N = 1	N = 2	N = 2
TP	TP	FP
TP	FN	TN
TP	FN	TN
TN	FN	TN
FN	TN	TN
TP	TP	FP
TP	TP	TN

! **Different tests fail differently - no perfect method.**

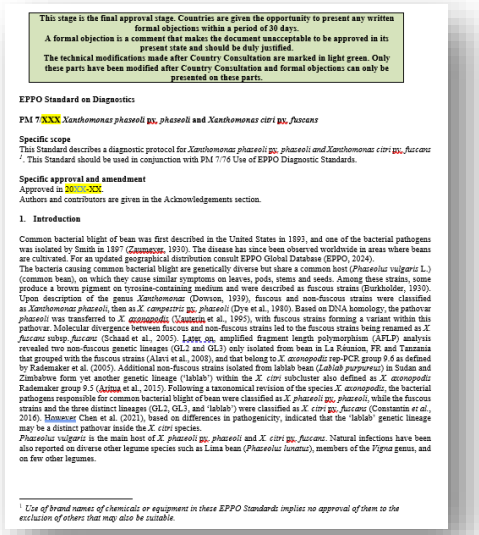
Audy et al. (1994). https://doi.org/10.1094/phyto-84-1185; Baldwin (2017) Unpublished report available from the ISF secretariat.; He (2010). Improved seed health tests for Xanthomonas axonopodis pv. phaseoli in common bean. Master thesis, Iowa, Iowa State University.

Conclusions

- Current diagnostic methods do not fully capture the **diversity** of *Xanthomonas* associated with bean (XANTPH, XANTFF).
- Even well-characterised, **pathogenic isolates** may **escape detection** or be misidentified.
- Similarity-based DNA barcoding can be **misleading** when reference databases are incomplete.
- A **multi-step diagnostic approach** improves reliability.
- Character-based approaches (e.g. DNCs) may provide a more **robust framework** for interpretation.

From study to EPPO diagnostic standard

The study findings contributed to EPPO diagnostic standard on Xanthomonas on beans - currently under „final approval“.



This stage is the final approval stage. Countries are given the opportunity to present any written formal objections within a period of 30 days.
A formal objection is a comment that makes the document unacceptable to be approved in its present state and should be duly justified.
The technical modifications made after Country Consultation are marked in light green. Only these parts have been modified after Country Consultation and formal objections can only be presented on these parts.

EPPO Standard on Diagnostics

PM 7/XXX *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* pv. *fuscans*

Specific scope
This Standard describes a diagnostic protocol for *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* pv. *fuscans* [1]. This Standard should be used in conjunction with PM 7/76 Use of EPPO Diagnostic Standards.

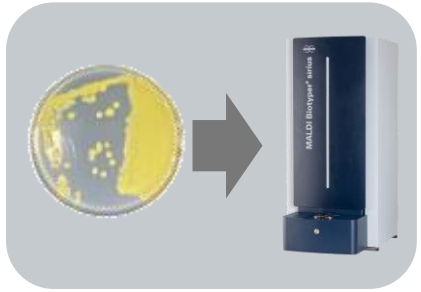
Specific approval and amendment
Approved in 20XX-XX.
Authors and contributors are given in the Acknowledgements section.

1. Introduction

Common bacterial blight of bean was first described in the United States in 1893, and one of the bacterial pathogens was isolated by Smith in 1897 (Zaumeyer, 1930). The disease has since been observed worldwide in areas where beans are cultivated. For an updated geographical distribution consult EPPO Global Database (EPPO, 2024).
The bacteria causing common bacterial blight are genetically diverse but share a common host (*Phaseolus vulgaris* L.) (common bean), on which they cause similar symptoms on leaves, pods, stems and seeds. Among these strains, some produce a brown pigment on tyrosine-containing medium and were described as fuscous strains (Burkholder, 1930). Upon description of the genus *Xanthomonas* (Dowson, 1939), fuscous and non-fuscous strains were classified as *Xanthomonas phaseoli*, then as *X. campestris* pv. *phaseoli* (Dye et al., 1980). Based on DNA homology, the pathovar *phaseoli* was transferred to *X. axonopodis* (Vauterin et al., 1995), with fuscous strains forming a variant within this pathovar. Molecular divergence between fuscous and non-fuscous strains led to the fuscous strains being renamed as *X. fuscans* subsp. *fuscans* (Schaad et al., 2005). Later on, amplified fragment length polymorphism (AFLP) analysis revealed two non-fuscous genetic lineages (GL2 and GL3) only isolated from bean in La Réunion, FR and Tanzania that grouped with the fuscous strains (Alavi et al., 2008), and that belong to *X. axonopodis* rep-PCR group 9.6 as defined by Rademaker et al. (2005). Additional non-fuscous strains isolated from lablab bean (*Lablab purpureus*) in Sudan and Zimbabwe form yet another genetic lineage ('lablab') within the *X. citri* subcluster also defined as *X. axonopodis* Rademaker group 9.5 (Arrua et al., 2015). Following a taxonomical revision of the species *X. axonopodis*, the bacterial pathogens responsible for common bacterial blight of bean were classified as *X. phaseoli* pv. *phaseoli*, while the fuscous strains and the three distinct lineages (GL2, GL3, and 'lablab') were classified as *X. citri* pv. *fuscans* (Constantin *et al.*, 2016). However Chen et al. (2021), based on differences in pathogenicity, indicated that the 'lablab' genetic lineage may be a distinct pathovar inside the *X. citri* species.
Phaseolus vulgaris is the main host of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*. Natural infections have been also reported on diverse other legume species such as Lima bean (*Phaseolus lunatus*), members of the *Vigna* genus, and on few other legumes.

[1] *Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.*

- ✓ MALDI → screening
- ✓ PCR limitations → recognized
- ✓ barcoding → recommended
- ✓ multi-step approach → required



qPCR

Baldwin

DNA barcoding

gyrB, avrBs2
+NCBI, **DNCs**

Future directions

- **Address** diagnostic gaps
- **Improve** sequence interpretation
- **Extend** to multi-locus / EGS
- **Refine** diagnostic strategies

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