## GEVES Expertise & Performance Contact:

# SeqNetectVeg: a major project to develop NGS tools for multi-target detection of bacterial and fungal pathogens transmitted by vegetable seeds

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## Background and context



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Seeds enable genetic resources to be disseminated as part of programmes to select, multiply and market varieties throughout the world. At the same time, seeds are also carriers of a diversity of microorganisms (collectively referred to as the microbiota), which can be beneficial or detrimental to plant health. The availability of accurate and high-throughput methods for identifying seed lots of high sanitary quality is therefore essential for the seed industry.

SeqDetectleg is a French collaborative project (2023-2027) which aims to develop and validate methods for detecting multiple bacterial and fungal pathogens in vegetable seed lots, which will be proposed for addition as a pre-selection step to recognised health analysis methods (e.g. ISTA).

#### Detection process by metabarcoding



## SeqNetectVeg project

Non-exhaustive list of vegetable crops and bacterial and fungal pathogens targeted by the project:

Targets	Bacteria	Fungi
Bean	<ul> <li>Pseudomonas savastanoi pv. phaseolicola</li> <li>P. syringae pv. syringae</li> <li>Xanthomonas spp.</li> </ul>	<ul> <li>Boeremia spp.</li> <li>Colletotrichum lindemuthianum</li> <li>Fusarium oxysporum f. sp. phaseoli</li> <li>Macrophomina phaseolina</li> <li>Pseudocercospora griseola</li> <li>Stagonosporopsis hortensis</li> </ul>
Cabbage	<ul> <li>P. syringae pv. maculicola</li> <li>Xanthomonas spp.</li> </ul>	<ul> <li>Alternaria brassicae and brassicicola</li> <li>Plenodomus lingam</li> </ul>
Carrot	<ul> <li>Candidatus Liberibacter solanacearum (CLso)</li> <li>X. hortorum pv. carotae</li> </ul>	<ul> <li>A. dauci and radicina</li> <li>Cercospora carotae</li> </ul>
Lamb's lettuce	- Acidovorax valerianellae	<ul><li>Peronospora valerianellae</li><li>Phoma valerianellae</li></ul>
Melon	- A. citrulli	- Didymella bryoniae
Soybean	- P. savastanoi pv. glycinae	- Phomopsis complex (Diaporthe)
Tomato	<ul> <li>Clavibacter michiganensis (Cmm)</li> <li>P. corrugata and P. syringae pv. tomato (Pst)</li> <li>Xanthomonas spp.</li> </ul>	<ul> <li>A. solani</li> <li>D. lycopersici</li> <li>F. oxysporum</li> </ul>

### First results: Advances for bacterial targets

*In silico* analysis of the *gyrB* marker:

- Collection of 1000 gyrB sequences representing the diversity of all the targeted bacterial species and related species (*Acidovorax*, *CLso*, *Clavibacter*, *Pseudomonas* and *Xanthomonas*).
- **KI-S tool** (Briand *et al.*, 2021. A rapid and simple method for assessing and representing genome sequence relatedness) for the calculation of the percentage of shared 15-mers between all gyrB sequences.

Key stages in the project to develop and validate methods for detecting pathogens by metabarcoding:



Example of the identification by *in silico* analysis of the target bacterial species in tomato: *Clavibacter* michiganensis (Cm), P. corrugata, P. viridiflava, P. syringae pv. tomato (Pst), X. euvesicatoria pv. euvesicatoria (Xee), X. euvesicatoria pv. perforans (Xep), X. hortorum pv. gardneri (Xhg) and X. vesicatoria.

- 19 Xhg seq. share 100% id. with 10 seq. from various X. *hortorum* pathovars
- 1 *Xhg* seq. shares 100% id. with 1 *X. hortorum* seq. + 1 other *Xhg* seq. shown <u>elsewhere in the figure</u> shares 100% id. with 8 seq. from various *X. hortorum* pathovars
- $\rightarrow$  Either it is not possible to distinguish at pathovar level for X. hortorum
- $\rightarrow$  Or some X. hortorum gyrB seq. (Xhg included) are not correctly assigned
- All *Xee* (10) and *Xep* (28) seq. share at least 50% id. with each other and with all the other *X. euvesicatoria* (27) and some *Xanthomonas* spp. (18) seq.
- All *Xee* seq. (10) share 100% id. with 10 *Xep* and 12 *X*. *euvesicatoria* seq.
- 17 *Xep* seq. share 100% id. with 6 *X. euvesicatoria* and 8 Xanthomonas spp. seq.
- 1 *Xep* seq. shares 100% id. with 9 *X. euvesicatoria* and 9 Xanthomonas spp. seq.
- $\rightarrow$  Either it is not possible to distinguish at pathovar level for X. euvesicatoria
- $\rightarrow$  Or some X. euvesicatoria gyrB seq. (Xee and Xep included) are not

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20 Cm seq. share at least 50% id. with 20 seq. from Clavibacter spp. including: 1 Cm seq. which shares at least 90% id. with 1 Clavibacter spp. seq.

*Cm* seq. which shares 100% id. with 2 *Clavibacter* spp. seq.

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All other Cm seq. (12) shown elsewhere in the figure share less than 50% id. with all other *gyrB* seq.

 $\rightarrow$  Highly likely that the 2 *Cm* and/or the 3 *Clavibacter* spp. *gyrB* seq. sharing at least 90% identity are misassigned

> 7 X. vesicatoria seq. are identical 1 X. vesicatoria seq. shares at least 50% id. with only the 7 others seq.

SeqNetectVeg

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 $\rightarrow$  Definite identification for X *vesicatoria* species

> **Thresholds** (identity percentage) ●<50% ●≥50% ●≥90% ●=100%

**Species** (number of *gyrB* sequences) Acidovorax spp. (16)

CLso (8) gyrB sequences *Cm* (32) of targeted Clavibacter spp. (25) bacterial species P. corrugata (9) for tomato Pst (14)

P. syringae (2) P. syringae pv. other (50) • *P. savastanoi* pv. other (19) • P. viridiflava (8) *Xee* (10) Xep (28) • X. euvesicatoria pv. vesicatoria (1) • *X. euvesicatoria* (15) • X. euvesicatoria pv. other (11) Xhg (21) • X. hortorum (8) • *X. hortorum* pv. other (39)



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