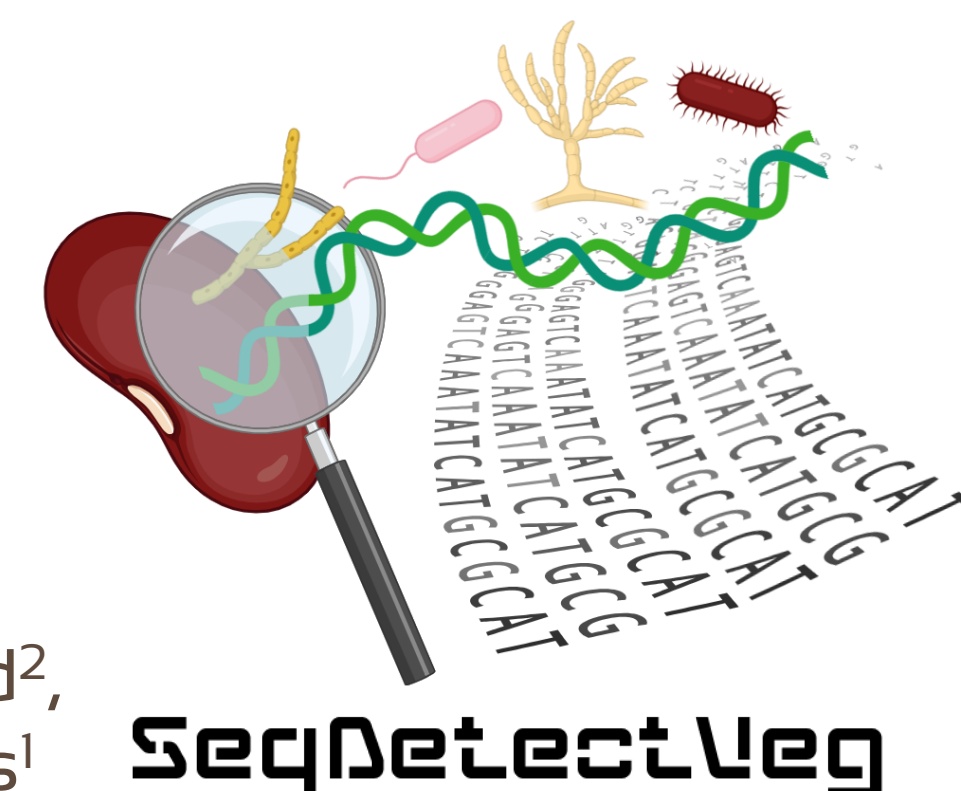


SeqDetectVeg: a major project to develop NGS tools for multi-target detection of bacterial and fungal pathogens transmitted by vegetable seeds

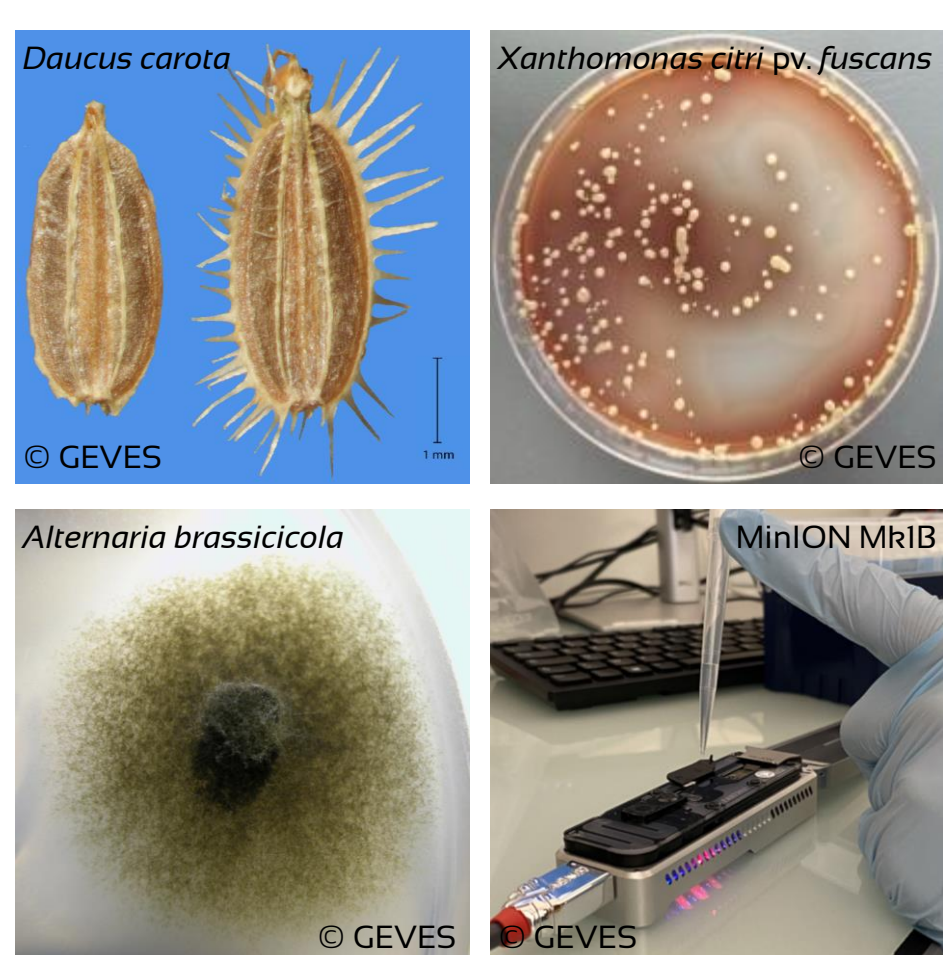


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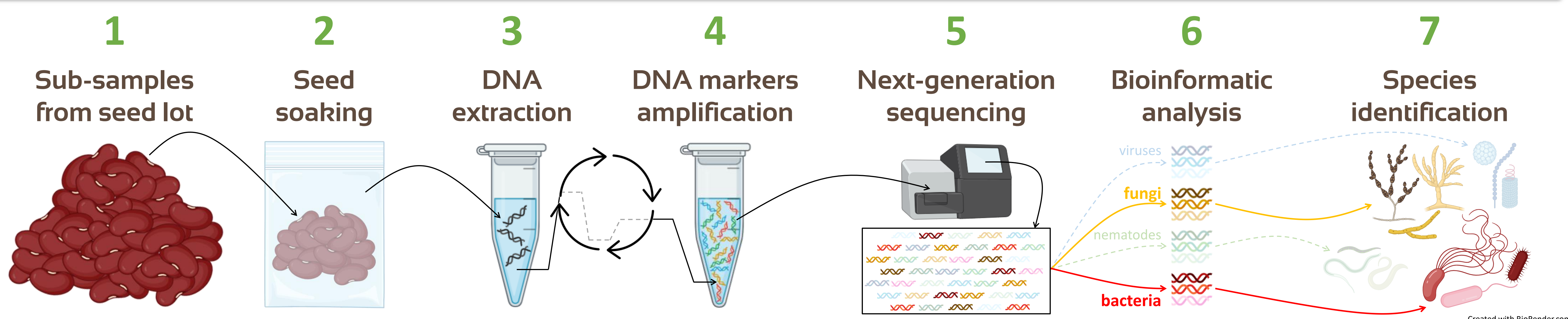
Background and context



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 micro-organisms (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.

SeqDetectVeg 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

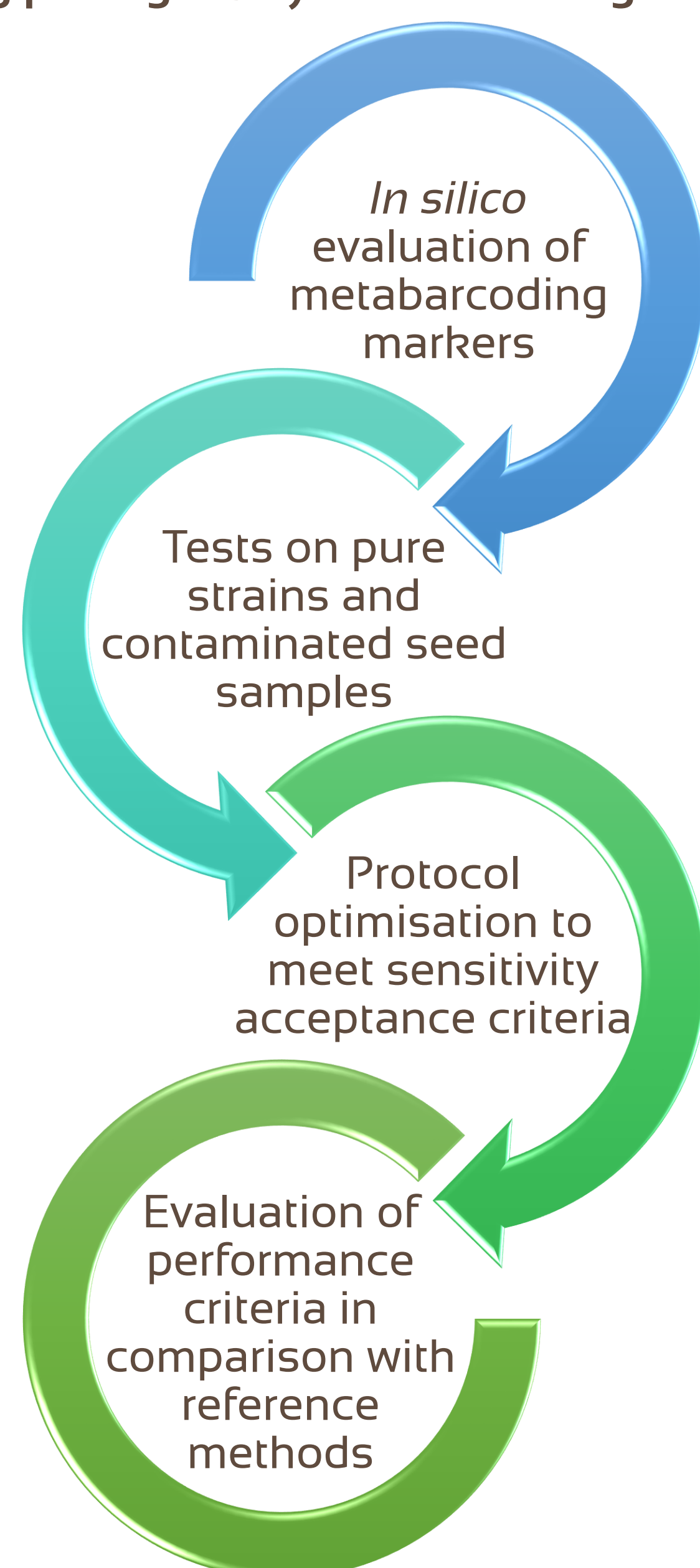


SeqDetectVeg project

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

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

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

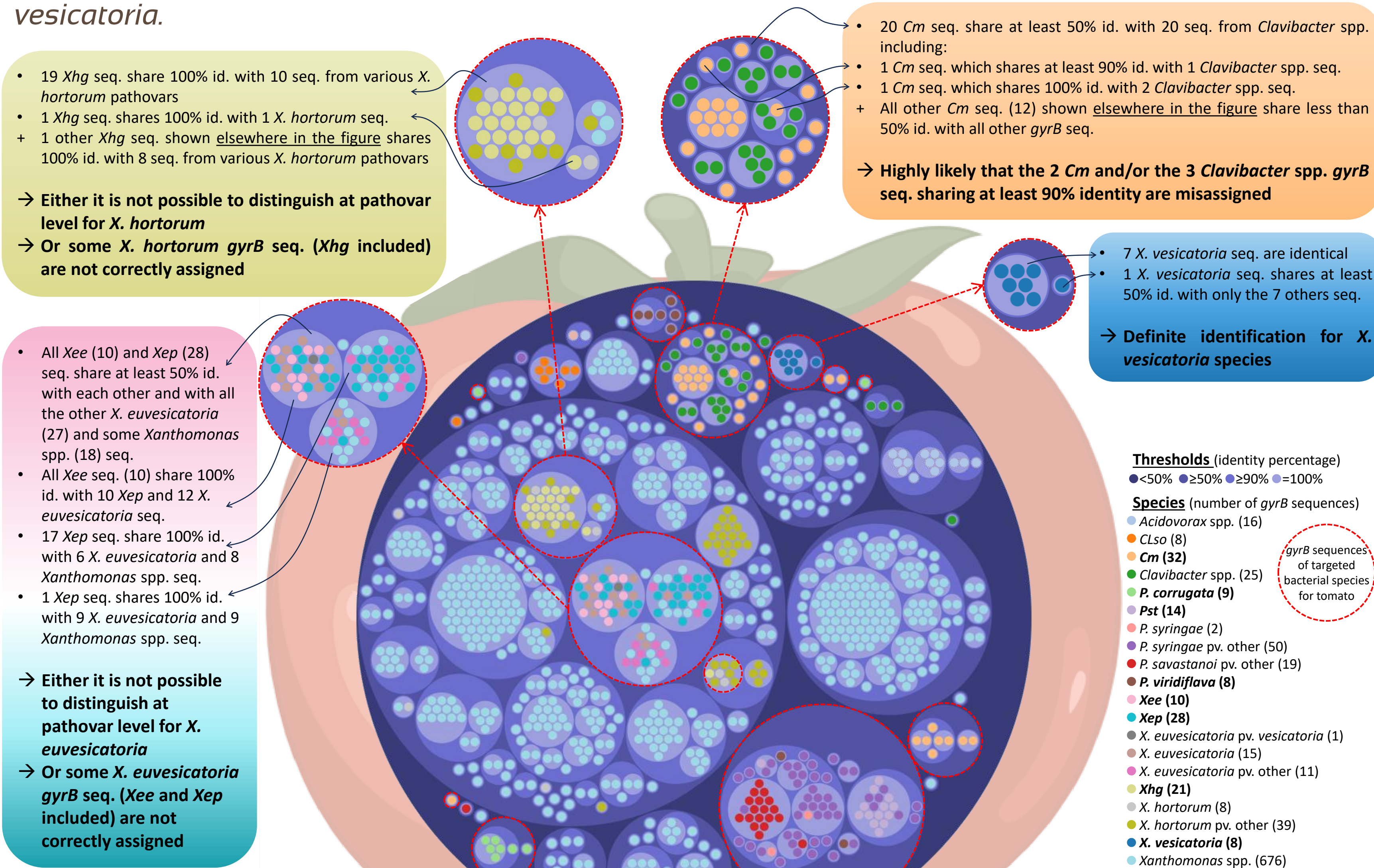


First results: Advances for bacterial targets

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.

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*.



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Conclusion of *in silico* analysis:

- Essential to have a database composed of sequences whose identity is certain and enhanced by a maximum of diversity.
- Identification certain at bacterial genera and species level, but uncertain at pathovar level on the basis of *in silico* analysis of the *gyrB* marker.

In progress:

Confirmation of the identification ability of the *gyrB* marker by sequencing with nearly 300 seed samples characterised by sanitary analysis belonging to 25 vegetable species healthy or contaminated by 1 to 3 bacterial pathogens (16 species)