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Full genome and characterization of a new species of *Chryseobacterium* with phytopathogenic potential on *Vicia faba* (Fabaceae; Papilionoidae)



Yvan Rahbé¹, Michel Droux¹, Philippe Oger¹, Nicole Cotte-Pattat¹

¹) Université de Lyon, MAP UMR 5240 CNRS, Université Lyon 1, INSA de Lyon, F-69622 Villeurbanne, France.

Chryseobacterium is a genus of the Flavobacteriaceae family whose members are usually chemoorganotrophic and form typical yellow-orange colonies due to flexirubin-type pigment. The genus contains more than 100 species from very diverse ecosystems, including freshwater, soil, marine fish, and human hosts. Other sources include lakes, sediments, water cooling systems, drinking water, lactic acid beverages, beer bottling plants, bioreactor sludge, polluted soil, marine sediment and even permafrost. *Chryseobacterium* strains are associated with a multitude of animal guts and feces - mosquitos, cockroaches, millipedes, penguin guano, freshwater copepods-, but also bird feathers, milk, meat and chicken. In agricultural systems, they were recovered from soils, roots, flowers, or decaying plant material, but none was described as phytopathogen so far. *C. indologenes*, mainly found in soil and water, has been reported as uncommon though deadly human pathogen.

Here, we report the complete genome sequence and the phenotype characterization of a new strain and new species of *Chryseobacterium* with a clear though weak phytopathogenic potential on *Vicia faba*. Initially detected in an aphid-associated *V. faba* seedling growth cabinet, it induced typical soft rot in all aphid-harboring plants. The causal agent was identified as *Chryseobacterium* sp., close to *C. indologenes* by 16S amplicon sequencing, but not identical to any characterized species (databases in early 2019). This prompted us to characterize the F3 strain and determine its genome sequence. The genome of strain F3 consists of a circular chromosome of 5,277 Kb without plasmid. The main phenotypic traits of strain F2 are heavy enzyme secretion, among which pectinases and proteases, no direct transmission by aphids at notable frequency but showing increased symptoms on aphid-infected plants, and potential migration through xylem pathway from crown to apex during in planta artificial infection. Sequence analysis reveals 6 complete SS.

Very recently, this strain was shown to belong to the same species as strain JV274.1, a PGPR strain on maize, which was characterized by French colleagues (Vacheron et al. 2017), and soon-to-be published as a novel species, *Chryseobacterium morensis* (Muller et al. in prep).

→ **Genome sequence of the F3 / Priori strain of *Chryseobacterium***
 5,227,721 bp; 36.1%GC; 4,584 predicted proteins; 84 RNA-coding sequences, including 75 tRNAs and 9 rRNAs (23S, 16S, and 7 x 5S), 535 strain-specific genes (vs strains JV274 [459] and JV558 [915]), no plasmid

From Infected faba bean stems



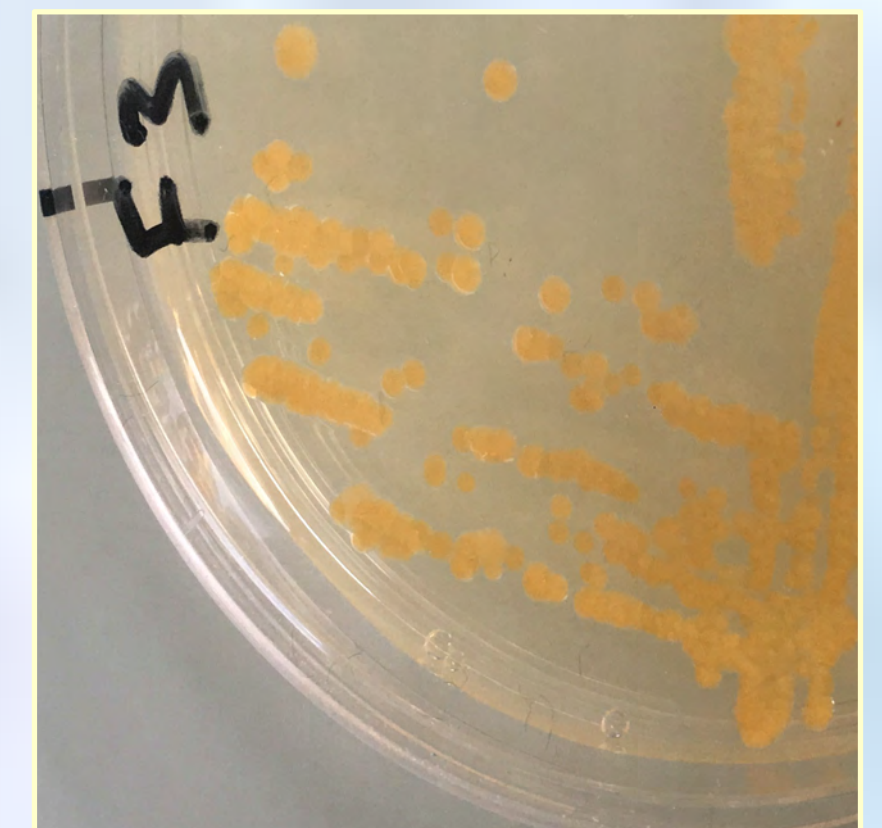
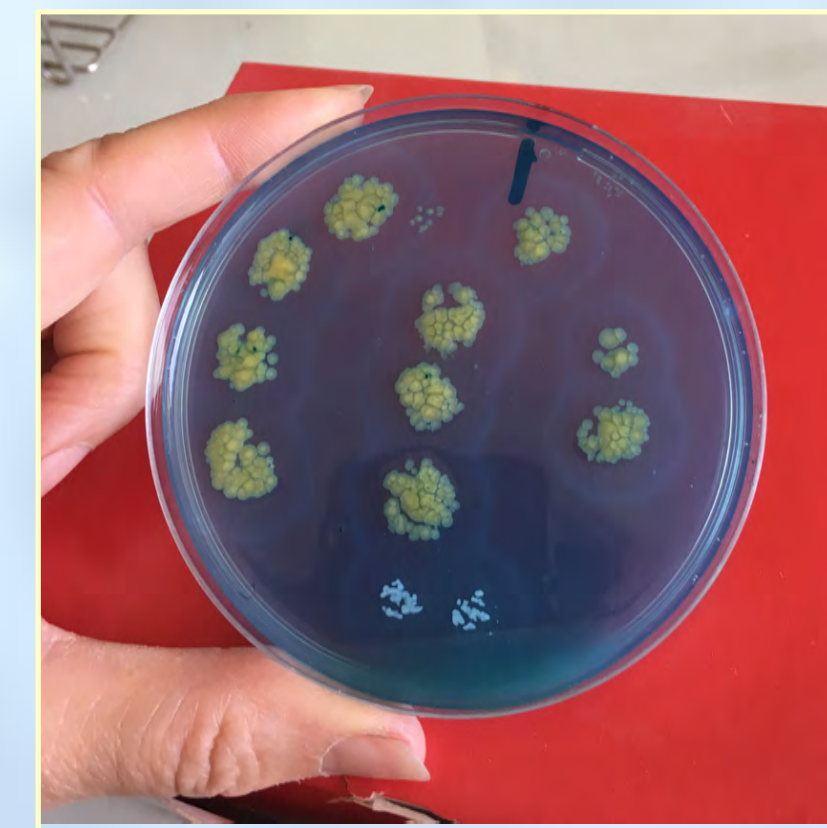
Selective recovery of pectinolytic bacteria on CVP (Cristal Violet Pectate) medium
 Visual inspection from 2 to 7 days

Second isolation by streaking on LB plates

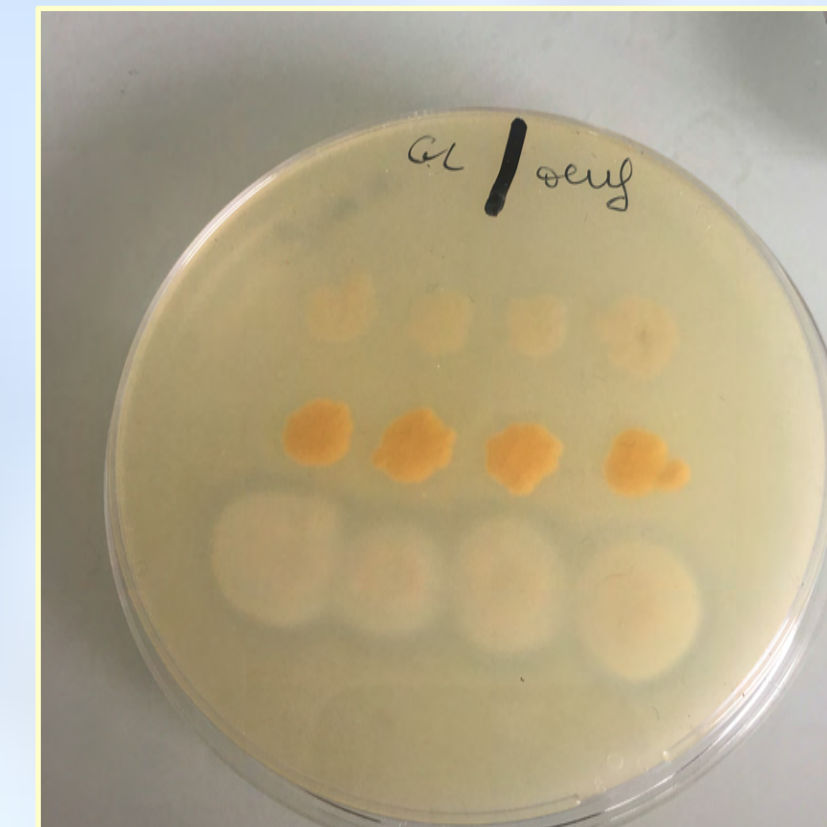
gapA and 16S identification

No gapA amplification (not *Enterobacteriaceae*)
 16S with less than 96% identity match: sequence

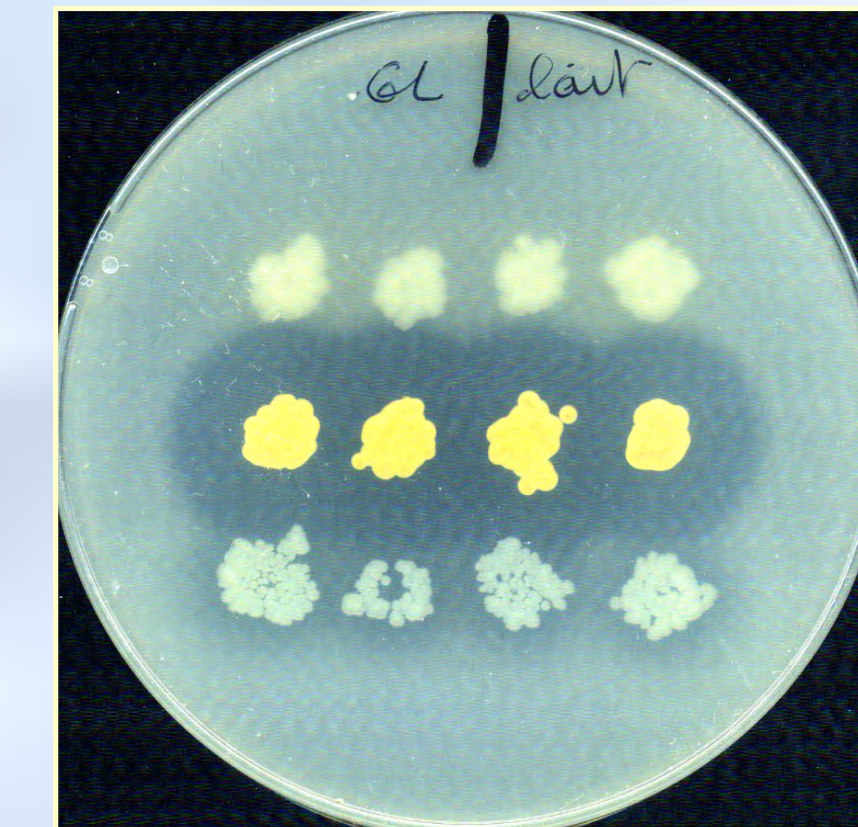
Pectinases



Lipases (egg)



Proteases (milk)



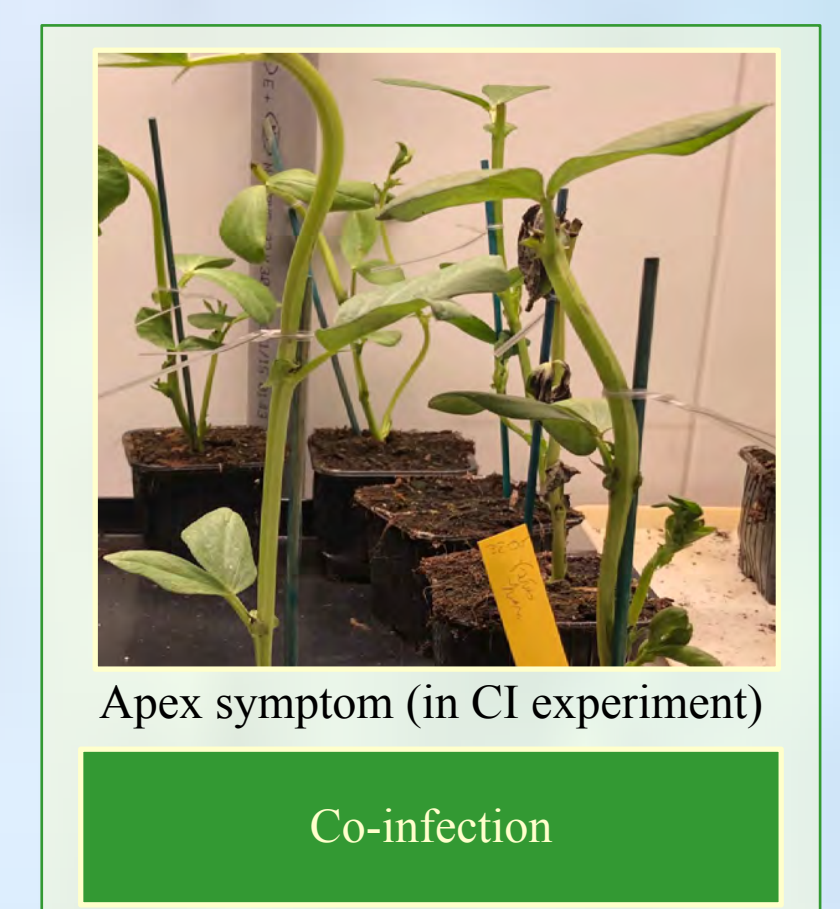
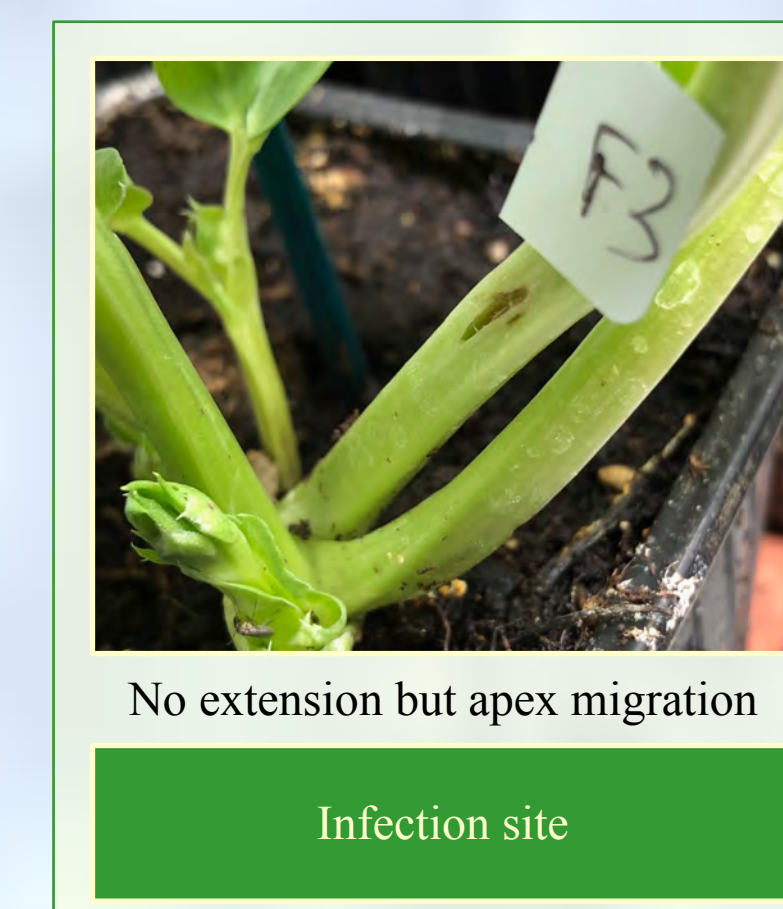
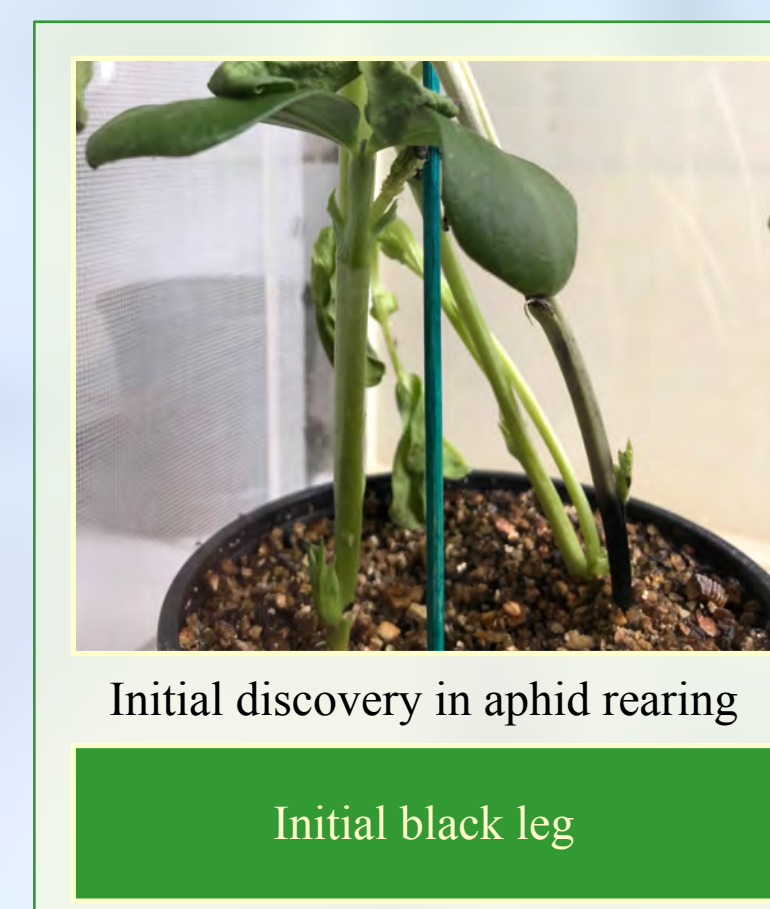
Cellulases (CMC)



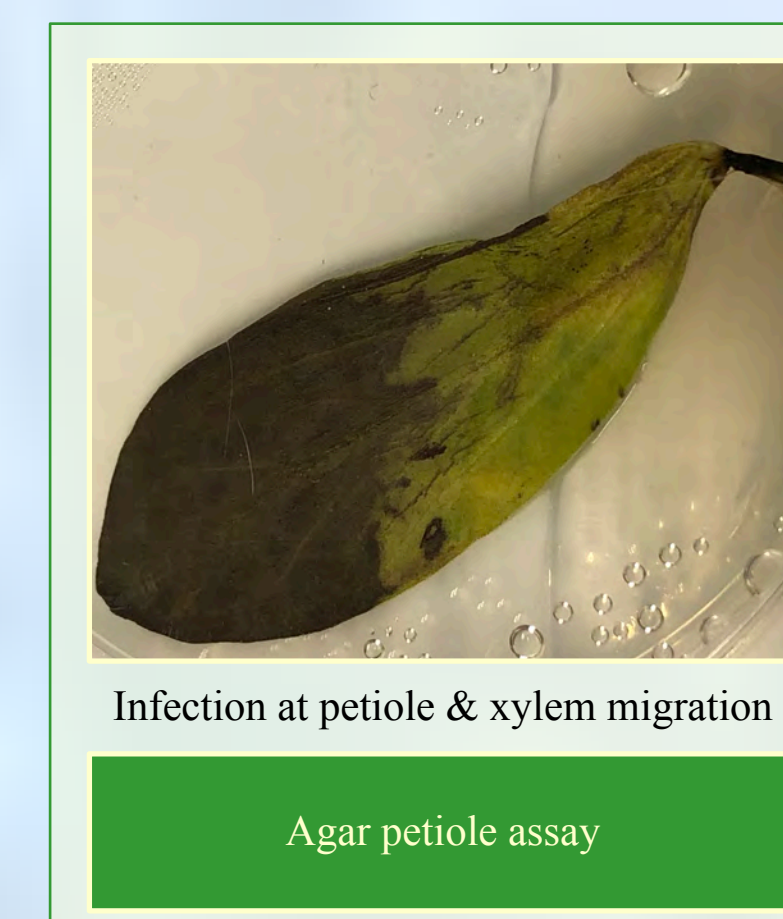
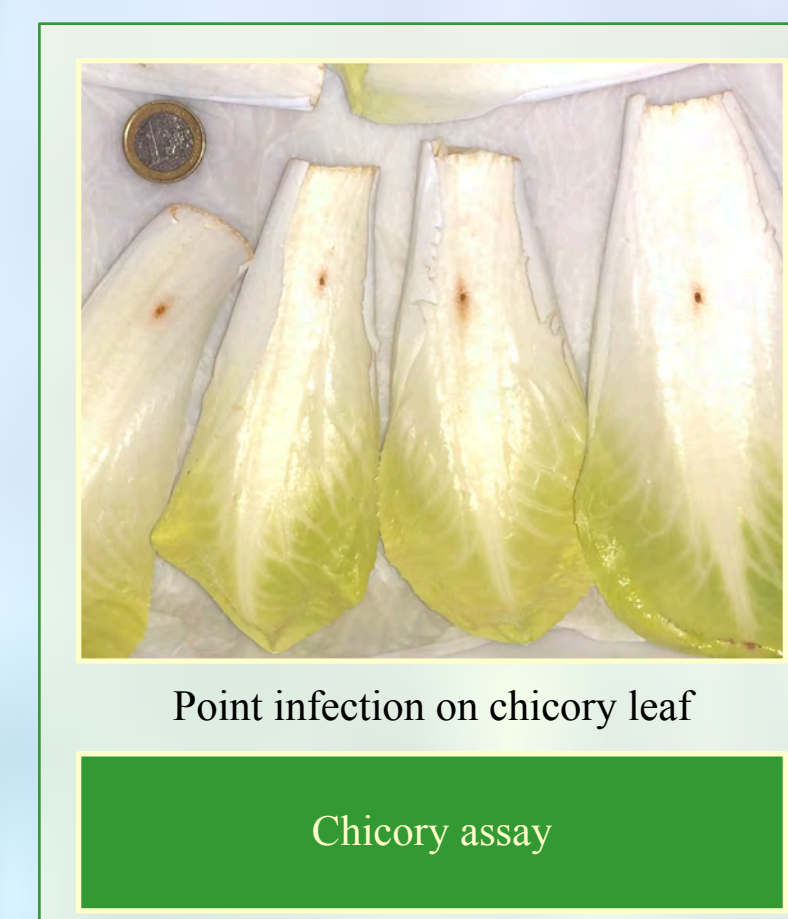
Genome

Secretion systems

Secretion System Type	Number complete	Loci
Flagellum	none	
T1SS	1 complete	Single locus, some scattered
T2SS	none	
T3SS	none	Some scattered
T4SS type B	2 complete	Single locus
T5aSS	1 complete	Single locus
T6SSiii	1 complete	Multiloci, scattered
T9SS	1 complete	Multiloci, scattered



In planta symptoms



In vitro *Vicia faba* (& chicory) assays

Conclusions

A new species of *Chryseobacterium* is described in two papers, with two plant-associated phenotypes. One strain of swiss origin exhibits PGPR phenotype on corn (Muller et al. type species); another of French (greenhouse) origin exhibits black leg symptoms on faba bean.

First intention comparative genomics is ongoing between the two strains with plant-associated phenotypes (~500 strain-specific genes), and versus non plant-associated strains (~1500 strain-specific genes), with special focus on secretion systems and predicted secretome.

- A new strain of *Chryseobacterium* (*morensis*), the first being phytopathogenic in the genus.
- A complete circularized genome with no plasmid
- A moderate phytopathogenicity associated with secretion activities, notably pectinolytic.
- A black leg symptom on *Vicia faba*, with increased severity upon co-infection by aphids (pea aphid, *Acyrtosiphon pisum*)

Référence

[1] Rahbé, Y., Guschinskaya, N., Droux, M., Valentovich, L., Briolay, J., & Hugouvieux-Cotte-Pattat, N. (in prep, hopefully 2020).

Full genome and characterization of *Chryseobacterium* xx, a new phytopathogenic strain with pectinolytic, xylem-association and insect interactions. *Systematic and Applied Microbiology*, In Prep.

[2] Muller et al. in prep...

