

The bad guys did it again...

« What do we know about them ? »

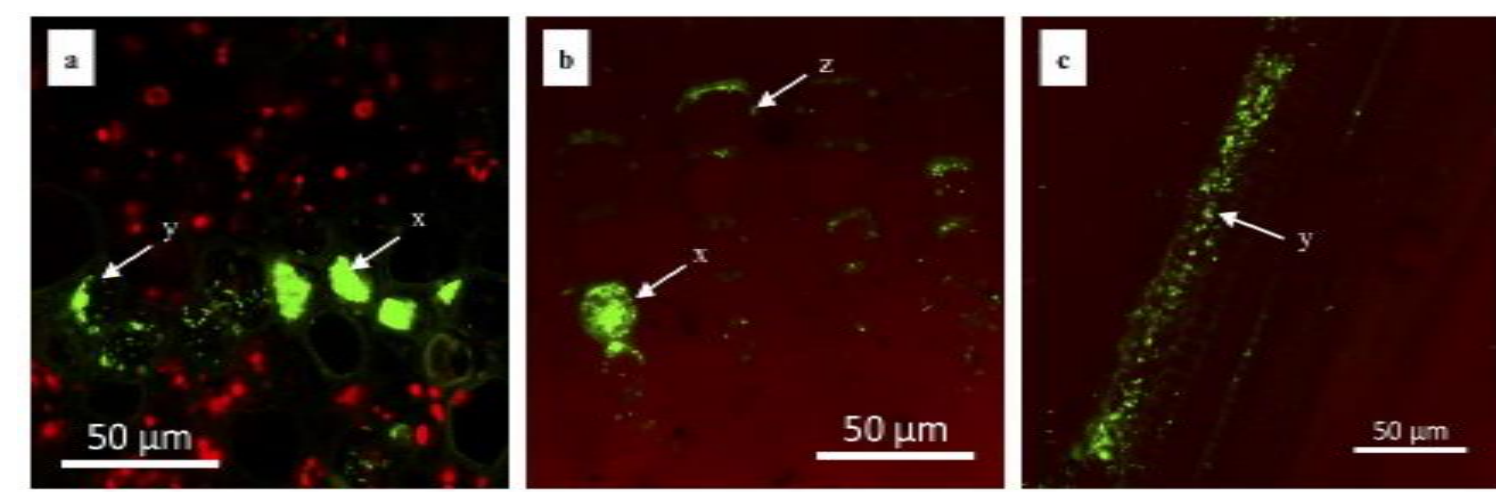
Brenneria salicis is the causal agent of the watermark disease on *Salix* and spreads in UK, Netherlands, Belgium and Japan [1].

Xylella fastidiosa is the causal agent of Pierce disease, Almond leaf scorch, Plum leaf scald, Citrus variegated chlorosis and Olive quick decline syndrome [2].

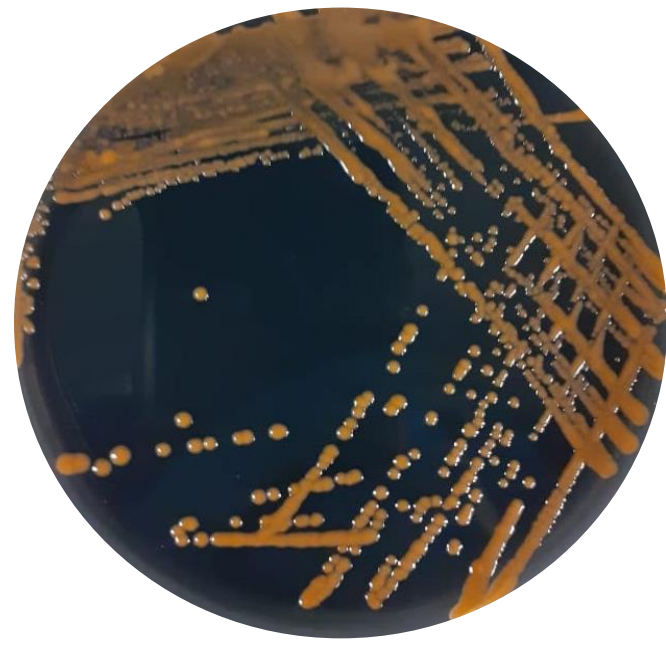
Salicaceae are good - potential - host for *B. salicis* and *X. fastidiosa* in Belgium :

- Along riparian areas forming a closed-mesh network
- Hosts of several potential insect vectors
- Plants can be symptomatic or asymptomatic [3,4,5].

Transversal (a,b) and longitudinal (c) sections of *Catharanthus roseus* stems inoculated with *X. fastidiosa* observed by confocal microscopy. *X. fastidiosa* cells contained in xylem vessels are depicted in green; plant tissues and xylem are depicted in red [5].



Symptomatic reaction induced by *X. fastidiosa* and *B. salicis* on olive trees and willows after infection.



Endophytic bacterial isolate fighting for *Salix* and *Populus* life after infection by *X. fastidiosa* or *B. salicis*.

Good guys to the rescue

« Are endophyte a solution for the future ? »

Some endophytes present in the xylem of *Salix* and *Populus* have been found to interact with xylem pathogen, such as *B. salicis* and *X. fastidiosa*. These interactions can range from inhibition of pathogen growth and colonization to inducing the plant's defense mechanisms against the pathogen.

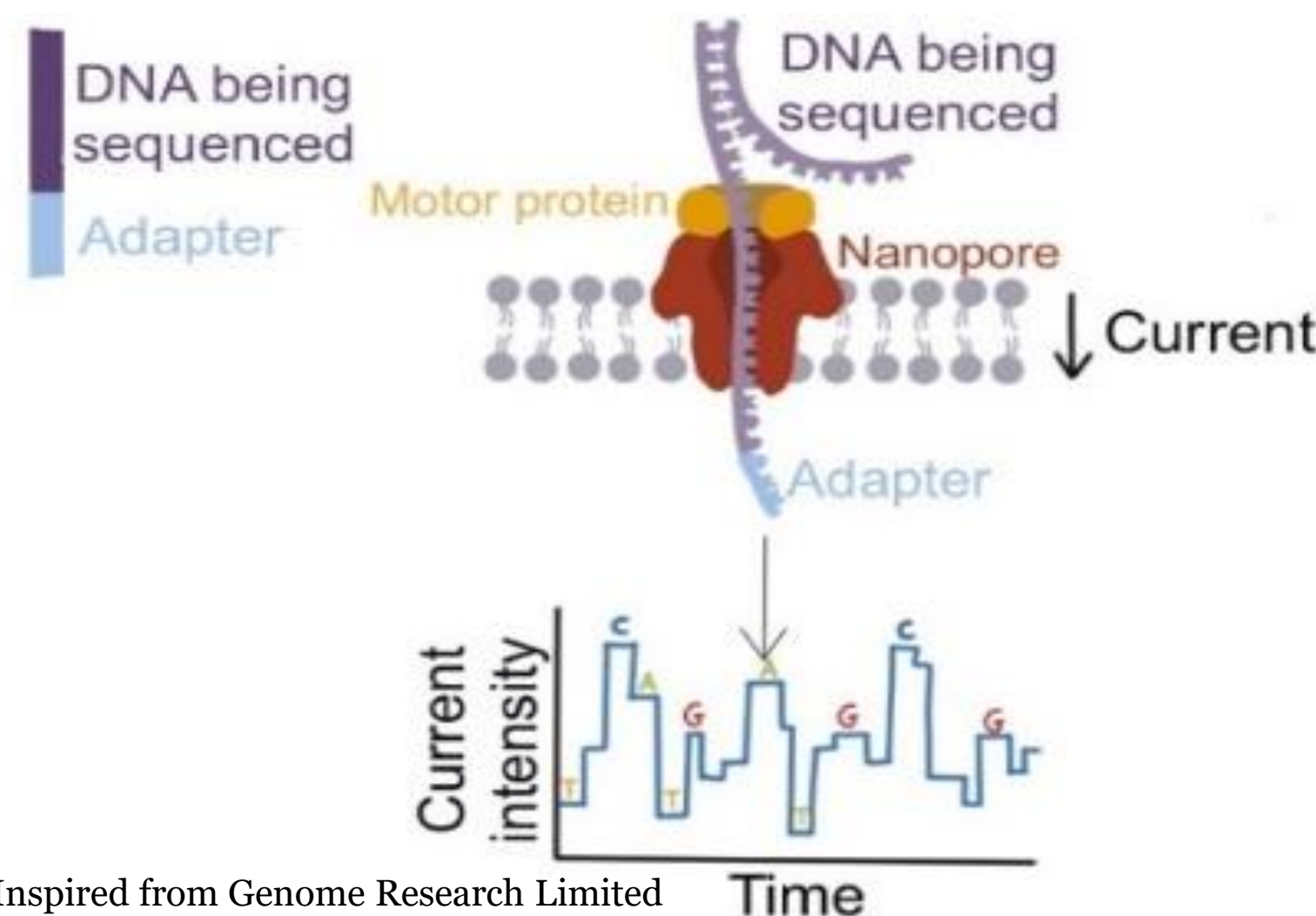
Who are they ?

423 bacterial isolates were cultivated from xylem sap. 207 were selected for further study (113 from *Populus* species and 94 from *Salix* species). A total of four phylla, six classes, 11 orders, 18 families and 39 genera were identified.

Where are they coming from ?

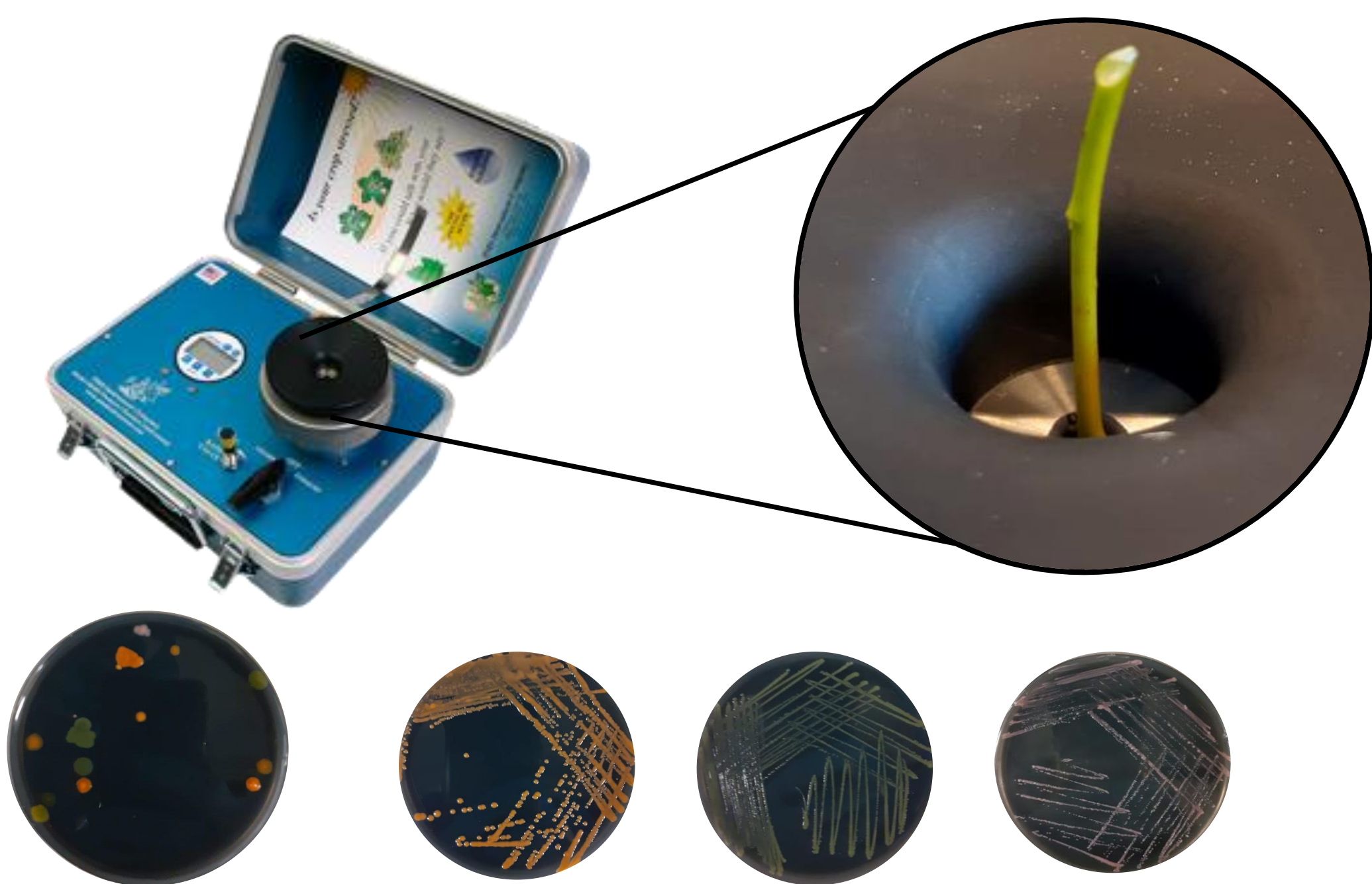
« Culture dependant or independant approach ? »

Culture independant approach – sequencing extracted and amplified DNA with Oxford Nanopore Technologies.



Inspired from Genome Research Limited
DNA extracted with a CTAB protocol then amplified with a two-step PCR (tagging gene and then amplifying tagged gene) and sequenced with Oxford Nanopore technologies.

Culture dependant approach - Xylem sap extraction with Scholander pressure chamber and plating of 50 µL on TSA, KB, BCYE and PDA.

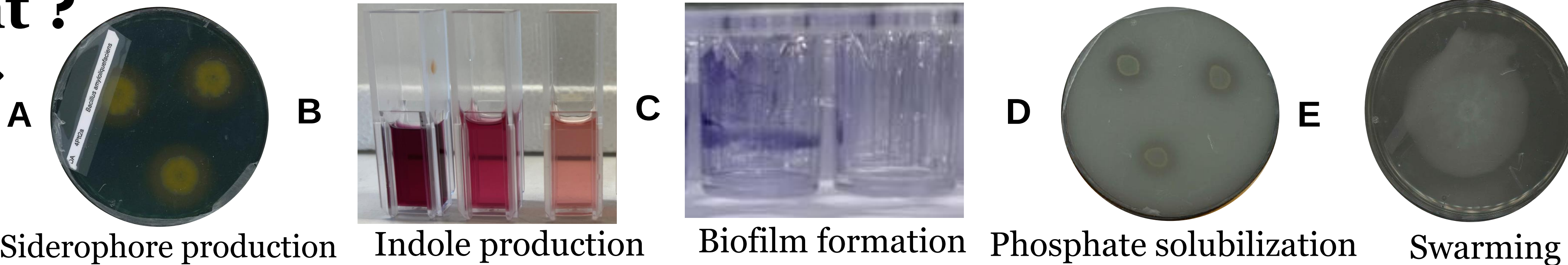
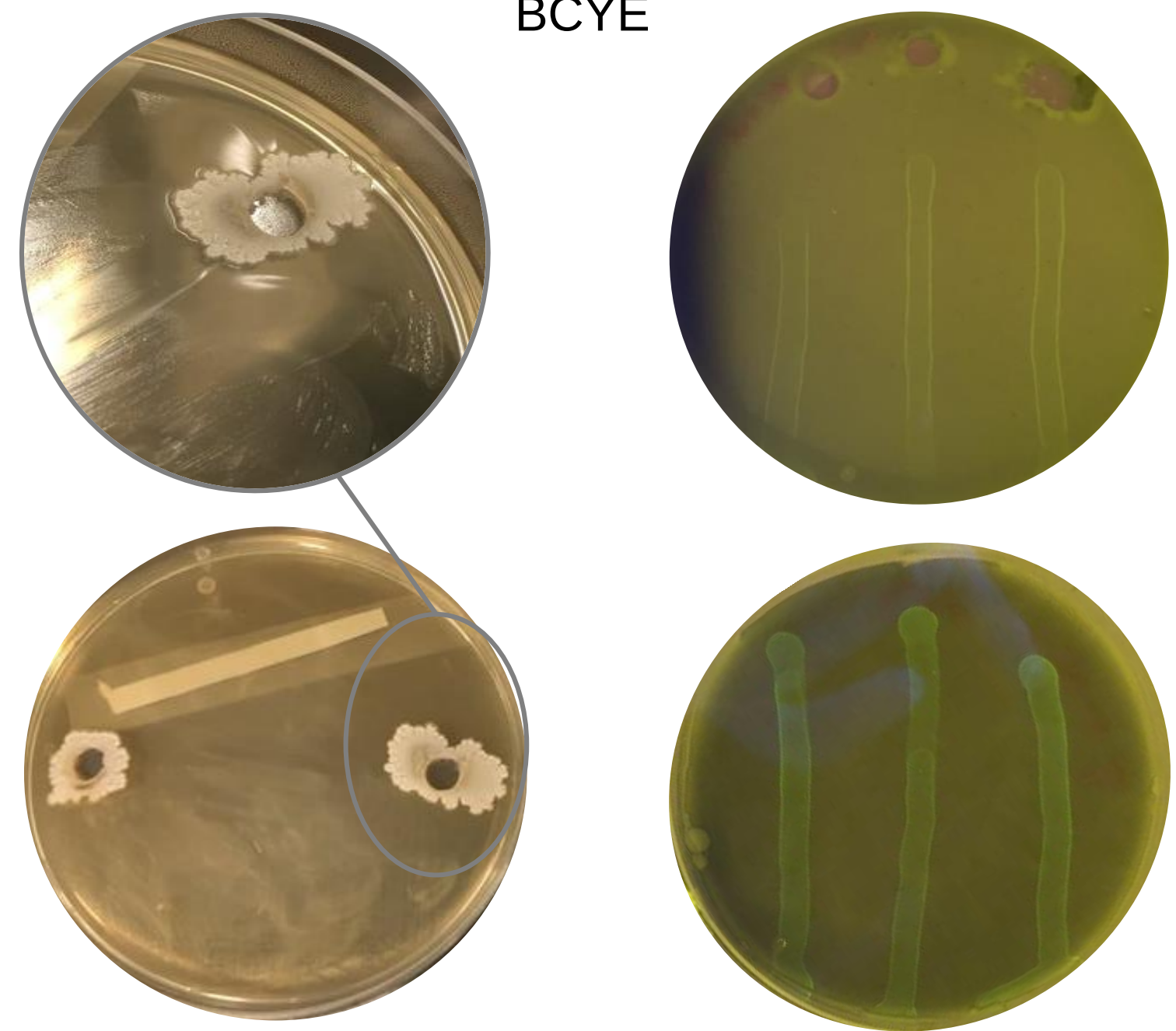
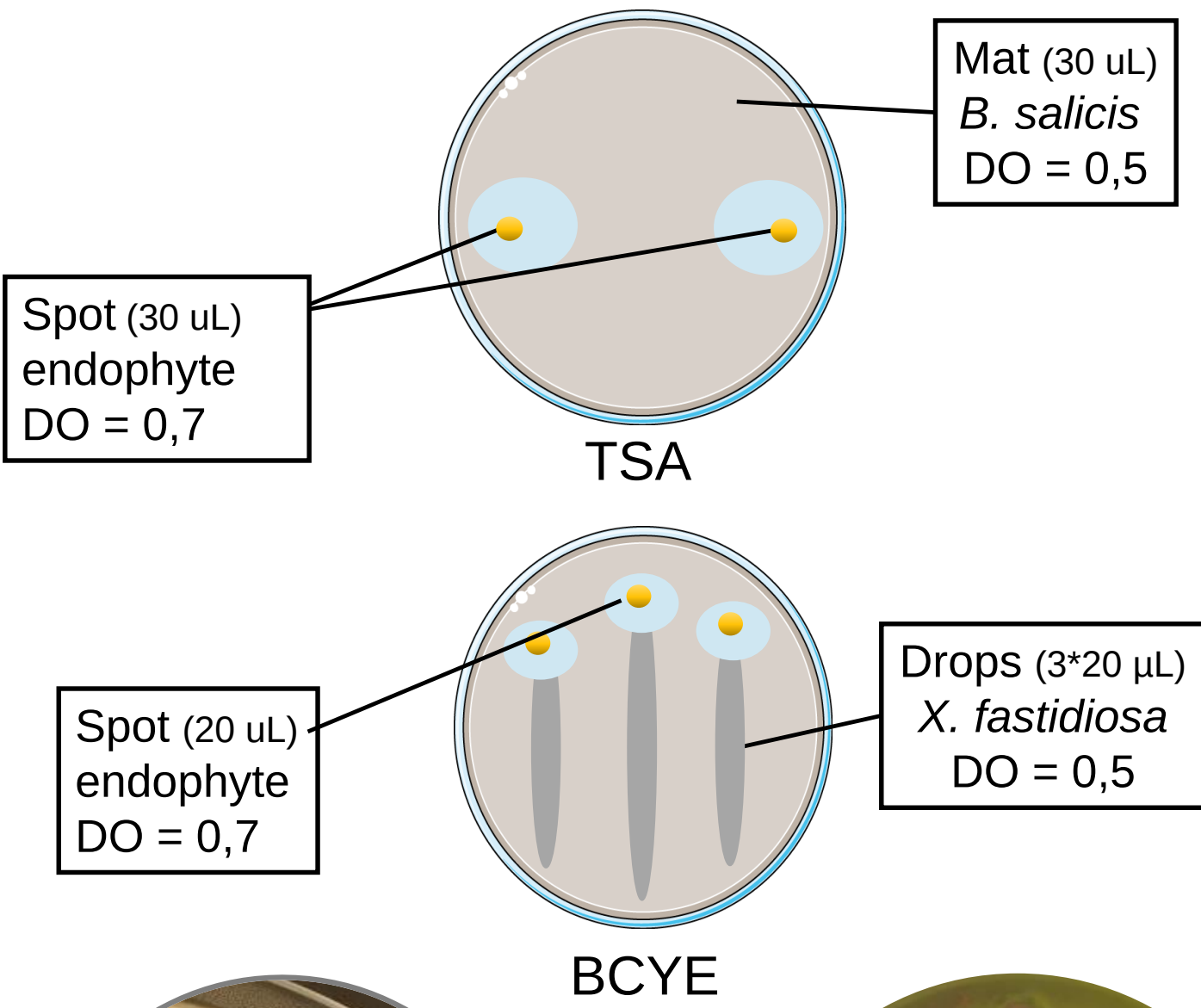


Xylem sap was extracted with a Scholander pressure chamber. Per plant, 50 µL of xylem sap is plated on each medium with two repetitions per medium. Media are incubated at 28°C for 2-3 days. The colonies are then purified by three successive subcultures and identified with a 16S PCR and Sanger sequencing.

What are they doing right ?

« Let's review some mechanisms »

Endophytic bacterial strains doing a good job at inhibiting *B. salicis* and *X. fastidiosa*



Different *in vitro* test studying plant growth promoting traits and environmental fitting traits of endophytic bacterial strains.

The aim is to discover endophytic bacterial strains that can potentially hinder the growth of the pathogenic bacteria *X. fastidiosa* and *B. salicis*. This is achieved through confrontation assays that investigate the interactions between endophytes and pathogenic bacteria. Any endophyte that exhibits inhibitory activity against one or both of the bacteria is subjected to further *in vitro* testing before being inoculated *in vivo*.

Several screening methods are used to evaluate their plant growth promoting and environmental fitting traits:

- siderophore production, which refers to the ability of bacteria to produce iron-chelating compounds that are essential for plant growth
- production of indole, a plant hormone that plays a vital role in promoting plant growth and development
- biofilm formation, which is the ability of bacteria to attach and grow on a surface, as this trait is critical for bacterial colonization in the plant root zone
- phosphate solubilization potential of the bacterial isolates, as this trait enables bacteria to convert insoluble phosphate into a form that plants can uptake
- swarming motility which refers to their ability to move across surfaces and colonize new areas



Symptomatic reaction induced by *X. fastidiosa* and *B. salicis* on *Salix* and *Populus* in Belgium after infection in quarantine greenhouse.