Beyond *Xylella*, Integrated Management Strategies for Mitigating *Xylella fastidiosa* impact in Europe

Practice abstract 8 - Extended Version

Innovative Bacteriophage-Based Strategies to Control *Xylella fastidiosa*



Funded by the European Union

Innovative Bacteriophage-Based Strategies to Control *Xylella fastidiosa*

Xylella fastidiosa is one of the most serious plant pathogens affecting almond trees, olive trees, grapevines and many other crops across Europe. Current control measures are limited and mainly rely on vector control and containment strategies. There is therefore an urgent need for **innovative, sustainable and environmentally friendly solutions** that directly target the bacterium.

The **BeXyl project** is exploring the use of **bacteriophages**, viruses that specifically infect and kill bacteria, as a novel biocontrol tool against ***X. fastidiosa***. Bacteriophages occur naturally in the environment and offer a highly specific approach that does not harm beneficial microorganisms, insects, animals or humans.



How will BeXyl make a difference?

Identifying effective bacteriophages: BeXyl is isolating and characterising bacteriophages active against ***X. fastidiosa*** from different environments, including infected plants, insect vectors, associated ecosystems and wastewater.

Targeted and safe control of the pathogen: selected bacteriophages will be tested for their ability to reduce ***X. fastidiosa*** populations without affecting non-target bacteria. This high specificity makes bacteriophages a promising alternative to broad-spectrum chemical treatments.

Reducing disease spread: by lowering bacterial loads within plants and insect vectors, bacteriophages may contribute to reducing disease transmission and slowing down the spread of ***X. fastidiosa*** in affected areas.

Compatibility with Integrated Pest Management (IPM): bacteriophage-based solutions are fully compatible with existing IPM strategies and can be combined with vector control, resistant plant material and surveillance programs.

Environmentally friendly and sustainable solution: as **natural biological agents**, bacteriophages leave no chemical residues, do not accumulate in the environment and support sustainable agriculture aligned with EU environmental policies.

Funded by the European Union

Towards practical applications: By advancing our understanding of bacteriophage–Xylella fastidiosa interactions, **BeXyl** aims to deliver safe, effective and environmentally friendly biocontrol tools that can be integrated into future management strategies, helping to protect European agriculture and natural ecosystems from this devastating pathogen.



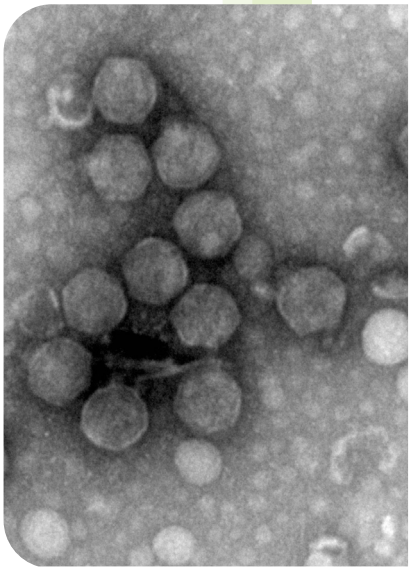
Main Outcomes:

Building a diverse and safe bacteriophage collection: Within the **BeXyl project**, an extensive collection of bacteriophages with activity against Xylella fastidiosa was established and thoroughly characterised. Nineteen selected phages showing high antibacterial activity in laboratory assays were fully sequenced and analysed. Genomic analyses revealed a **high diversity of bacteriophages,** belonging to several viral families and genera, including *Pradovirus*, *Alphatectivirus*, *Bosavirus* and *Salvovirus*, as well as previously unclassified groups. Importantly, all analysed phages showed a **strictly lytic life cycle**, meaning they kill the bacterial cells without integrating their genetic material into the host genome. In addition, no genes associated with bacterial virulence or antibiotic resistance were detected. These characteristics are essential to ensure the **biosafety** of bacteriophages intended for biocontrol applications. Advanced genomic and phylogenetic analyses allowed a precise taxonomic classification of the phages and revealed that several of them likely represent **new phage species or even new genera**. This diversity increases the chances of identifying highly effective phages adapted to different ***X. fastidiosa*** strains and environmental conditions.

Broad and selective host range against plant pathogenic bacteria: the host range of the isolated bacteriophages was carefully evaluated against a wide panel of plant-pathogenic bacteria. Many phages showed lytic activity not only against ***X. fastidiosa*** but also against selected Xanthomonas species, while maintaining a **high level of specificity**. This balance between efficacy and selectivity is a key advantage of bacteriophage-based approaches.

Funded by the European Union

Within ***X. fastidiosa***, most effective phages were active against strains belonging to the subsp. ***fastidiosa***, particularly sequence type ST1, which is responsible for Pierce's disease in grapevine. Several phages consistently inhibited bacterial growth in liquid cultures in a dose-dependent manner, allowing the selection of the most promising candidates for further testing.



Successful reduction of *Xylella fastidiosa* in planta: selected bacteriophages were evaluated in plant infection experiments using tobacco and periwinkle as model hosts. Phage treatments significantly reduced bacterial populations within plant tissues compared to untreated controls. In many cases, ***X. fastidiosa*** could only be detected at very low levels or was not recovered at all from phage-treated plants. In periwinkle plants, both individual phages and phage cocktails effectively prevented or strongly reduced disease symptoms. While untreated plants developed severe chlorosis, wilting and vascular necrosis, phage-treated plants showed either no symptoms or only mild symptoms, with healthy-looking xylem vessels comparable to those of non-infected controls. To better monitor phage behaviour inside plants, a specific molecular method was developed to detect and quantify bacteriophages in plant tissues. This confirmed that phages can persist and move within the plant, reaching relevant tissues where ***X. fastidiosa*** colonises.

Towards field application of phage-based biocontrol: to validate the practical potential of bacteriophages under real agricultural conditions, a pilot field trial was conducted in grapevine plots affected by Pierce's disease in the Balearic Islands, in collaboration with an industrial partner. Phages were applied both as a **preventive treatment** on healthy plants and as a **therapeutic treatment** on infected vines. Preliminary results indicate that preventive phage applications protected healthy plants from developing disease symptoms, while therapeutic treatments halted symptom progression in already infected plants. Although final evaluations are ongoing, these results strongly support the feasibility of bacteriophage-based strategies for the sustainable control of ***X. fastidiosa*** in the field.

Impact: By combining extensive genomic characterisation, laboratory screening, plant experiments and pilot field trials, BeXyl demonstrates that bacteriophages represent a safe, highly specific and environmentally friendly tool to control *Xylella fastidiosa*. These results lay the foundation for the development of tailored phage-based solutions that can be integrated into Integrated Pest Management (IPM) strategies, contributing to long-term, sustainable protection of European crops and landscapes.

Funded by the European Union