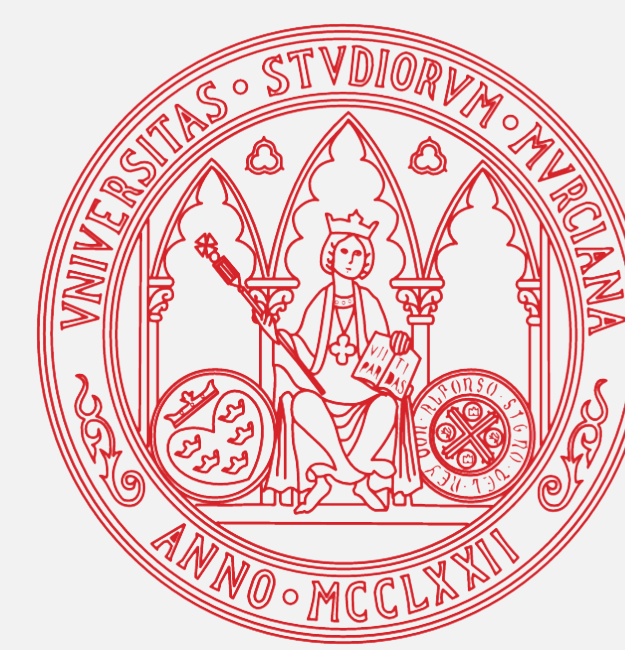


# ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES AS A METHOD OF BIOCONTROL FOR PHYTOPATHOGENIC BACTERIA



**Probelte**

BY AGRONOVA BIOTECH

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## INTRODUCTION

*Erwinia amylovora* is a Gram-negative bacterium and the causal agent of "fire blight," which affects stone and pome fruit trees (such as pear, apple, apricot, and peach, among others) and other ornamental plants, causing significant economic losses. To manage the bacteriosis caused by this species, containment measures are typically used (such as quarantine, removal, and incineration of affected crops), as there are hardly any phytosanitary treatments that can control the disease. The use of conventional products like copper salts does not yield satisfactory results and poses unfavorable environmental impacts.

In recent years, interest in using biocontrol tools in agriculture has increased exponentially, with the use of bacteriophages (viruses capable of specifically infecting and lysing bacteria) being a promising approach to finding effective and environmentally sustainable solutions for specifically affected crops.

## GOALS

This study involves the isolation of *E. amylovora* strains from samples of infected crops, as well as the phages that infect them, as a strategy for the biocontrol of bacteriosis in crops of agro-economic interest.

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(2) Clokie, MRJ, & Kropinski, AM (2009). Bacteriophages: methods and protocols Volume 1: Isolation, Characterization, and Interactions. In *Methods in molecular biology*

(3) International Plant Protection Convention. (2018). *Diagnostic protocols for documented pests. PD 13: Erwinia amylovora.*

(4) Moraru, C., Varsani, A., and Kropinski, AM (2020) VIRIDIC – a novel tool to calculate the intergenomic similarities of prokaryote-infecting viruses. *Viruses* 12(11). <https://doi.org/10.3390/v12111268>

This research project is part of the CDTI project IDI-20200837 "Isolation and characterization of bacteriophages as a method of biocontrol of bacterial pathogens in agriculture".

## RESULTS AND DISCUSSION

- The first step in isolating phages useful for biocontrol was the identification of indigenous pathogenic strains of *Erwinia amylovora*. For this purpose, diseased pear tree tissues were collected from different areas in the Region of Murcia, following an isolation protocol using King B medium supplemented with cycloheximide (a fungal microbiota inhibitor) (Fig. 1). The isolated strains were identified by sequencing their 16S rRNA (Table 1).

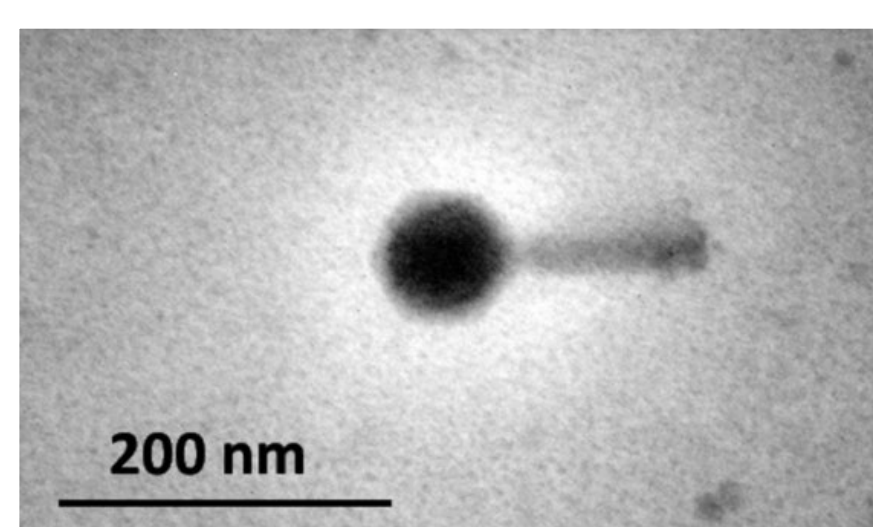


**Figure 1.** A) Wild samples of pear trees affected by "fire blight". B) Isolation from infected samples in King B medium with cycloheximide.

STRAIN	RNA16S IDENTIFICATION	AREA
AB-28	<i>Erwinia amylovora</i>	Jumilla (Murcia)
AB-42	<i>Erwinia amylovora</i>	Calasparra (Murcia)
AB-44a	<i>Erwinia amylovora</i>	Calasparra (Murcia)
AB-44b	<i>Erwinia amylovora</i>	Calasparra (Murcia)
AB-47	<i>Erwinia amylovora</i>	Cieza (Murcia)
AB-48	<i>Erwinia persicina</i>	Cieza (Murcia)
AB-50	<i>Erwinia persicina</i>	Cieza (Murcia)

**Table 1.** Summary of strains isolated from pear trees *Erwinia* affected by "fire blight" in different locations in the Region of Murcia.

- Strains identified as *Erwinia amylovora* were used as hosts for the phage isolation process. For this purpose, enrichment was performed using irrigation water samples from the areas where the bacterial strains had been isolated, as well as wastewater from the wastewater treatment plant (WWTP). The sequencing of the genomes of the isolated phages and their observation by microscopy with negative staining using uranyl acetate (Fig. 2) allowed for the characterization of phages active against the isolated phytopathogenic strains (Table 2).



**Figure 2.** Micrograph obtained by Transmission Electron Microscopy (TEM). Bacteriophage (BS-3) isolated from dry riverbed water. Stained with uranyl acetate.

STRAIN	ISOLATION	GENOME SIZE (BP)	CLASS	FAMILY	GENDER
BS-3	06/11/2021, Rambla Water (Albujón)	84583	Caudoviricetes	Subfamily: Ounavirinae	Kolesnikvirus
BS-4	11/11/2021, Rambla Water (Albujón)	85195	Caudoviricetes	Subfamily: Ounavirinae	Kolesnikvirus
BS-19	11/19/2021, Water Ditch (Aljucer)	40625	Caudoviricetes	Not classified	Not classified
BS-24	09/20/2022, T1 WWTP (Fortuna)	162472	Caudoviricetes	Ackermannviridae	Nezavisimistyvirus

**Table 2.** Summary of phages isolated against *Erwinia amylovora* AB-28. Four different phages were isolated from irrigation and wastewater (WWTP). Data obtained through sequencing and genomic analysis (BLASTn and VIRIDIC) are included.

- In order to explore the efficacy of the isolated phages as biocontrol agents, their lytic capacity was tested on both the isolated wild type bacterial strains and reference strains of *Erwinia amylovora*, as well as on a different species, *E. persicina*. All isolated phages were capable of infecting the tested strains of *E. amylovora*, but not other closely related species such as *E. persicina* (Table 3).

STRAIN	SPECIES	BS3	BS4	BS19	BS24
AB-28 (CONTROL)	<i>Erwinia amylovora</i>	+	+	+	+
AB-42	<i>Erwinia amylovora</i>	+	+	+	+
AB-44a	<i>Erwinia amylovora</i>	+	+	+	+
AB-44b	<i>Erwinia amylovora</i>	+	+	+	+
AB-47	<i>Erwinia amylovora</i>	+	+	+	+
CECT 222	<i>Erwinia amylovora</i>	+	+	+	+
AB-48	<i>Erwinia persicina</i>	-	-	-	-
AB-50	<i>Erwinia persicina</i>	-	-	-	-

**Table 3.** Host range of the isolated phages. Evaluated by SPOT TEST. "+" indicates sensitivity to phage and "-" indicates resistance.

## CONCLUSIONS

- Four specific phages against *Erwinia amylovora* were isolated, including some that had not been previously described.
- The isolated phages have a broad range of activity against various strains of *E. amylovora*, while being harmless to related bacterial species such as *Erwinia persicina*.
- The isolated phages could be used as biocontrol agents against Fire Blight.

