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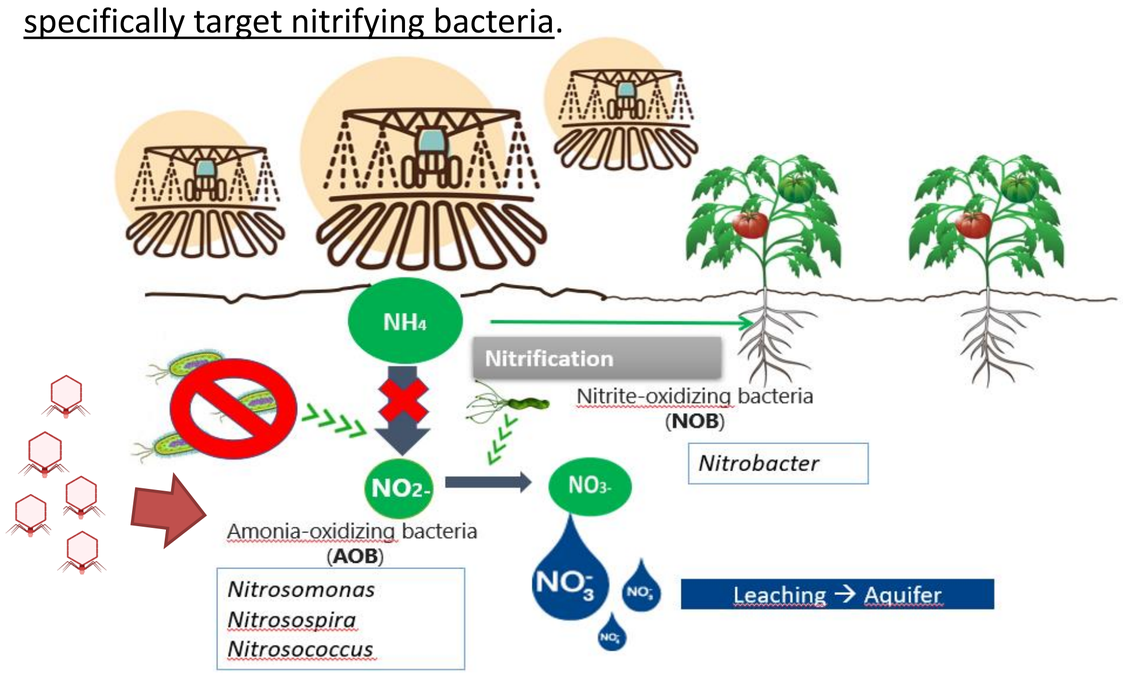
Phage-based biocontrol of nitrification in agricultural soil

Laura Sala-Comorera[1], Pedro Blanco-Picazo[1], Gloria Vique[1], Pablo Quirós[2], María Dolores Ramos-Barbero[1], Clara Gómez-Gómez[1], Lorena Rodríguez-Rubio[1], Tula Yance-Chávez[2], Ignasi Salaet [2], Maite Muniesa[1]

1 - Department of Genetics, Microbiology and Statistics. University of Barcelona, Spain
2 - Departamento of I+D+i Fertinagro Biotech S.L., Teruel, Spain

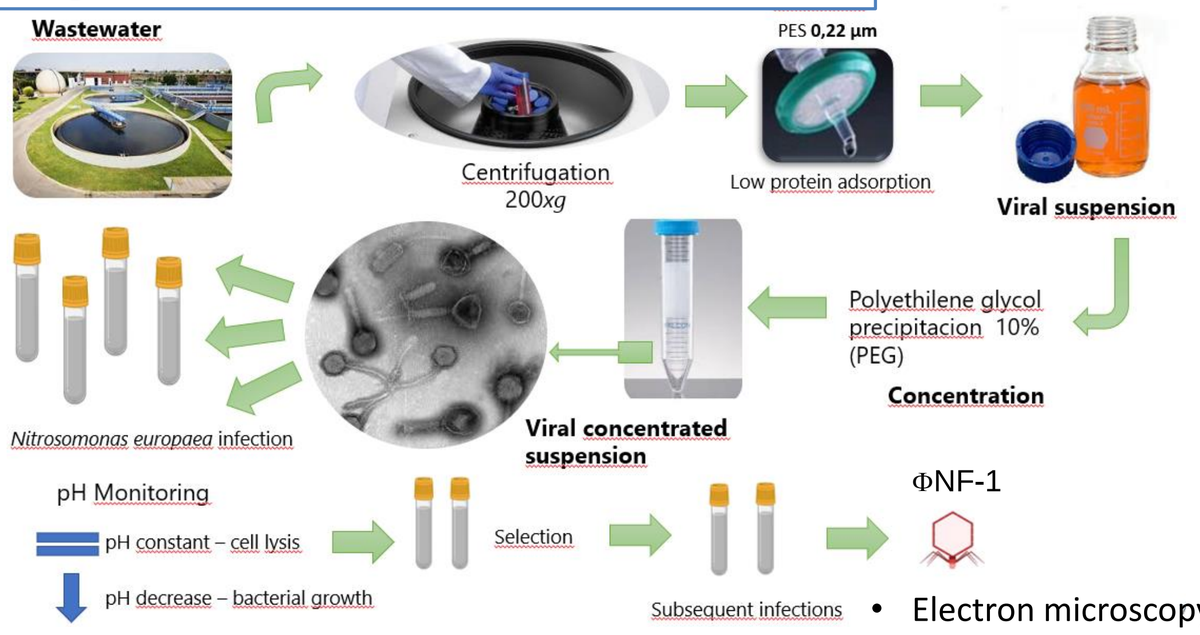
INTRODUCTION & AIM

Nitrogen fertilization boosts crop yields but is **inefficient due to the rapid activity of nitrifying bacteria**, which leads to the loss of useful nitrogen forms. This requires the use of large amounts of fertilizers, leading to **environmental pollution** from compounds like NO_3 and N_2O. Although **chemical inhibitors can improve fertilization, their impact on the environment and human health** remains uncertain. A new strategy is the use of bacteriophages (phages) that specifically target nitrifying bacteria.



METHOD

Phage isolation and characterization



- Electron microscopy
- Illumina MiSeq system

Host spectra and Infectivity

Nitrosomonas and Nitrosospira sp.

ΦNF-1

In vitro:
AOB medium

In vivo:
soil samples

Availability to infect and inhibit nitrification

- No media acidification
- No ammonium reduction (colorimetric assay)
- No nitrite formation (colorimetric assay)
- Increase in bacteriophage particles (qPCR)

Persistence

pHs 3, 5 6, 7, 8,

4, 15, 20, 30 ˚C

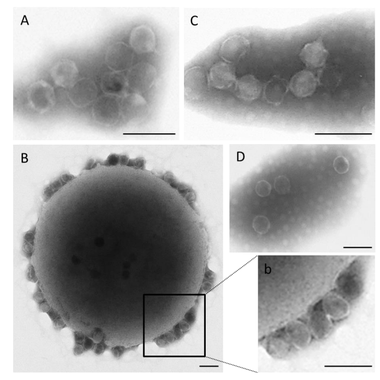
Soil at 20 ˚C

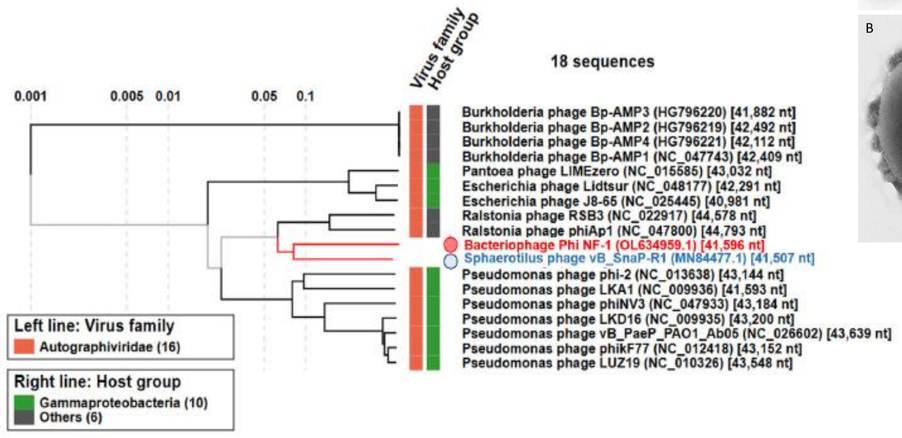
Concentration of infectious bacteriophage particles over 6 months → Propagation in AOB medium

RESULTS & DISCUSSION

Morphological and Genetic characterization

Phage ΦNF1, isolated from wastewater, had an icosahedral capsids of 50 ± 3 nm in diameter and very small tails typical of *Podoviridae* phages.

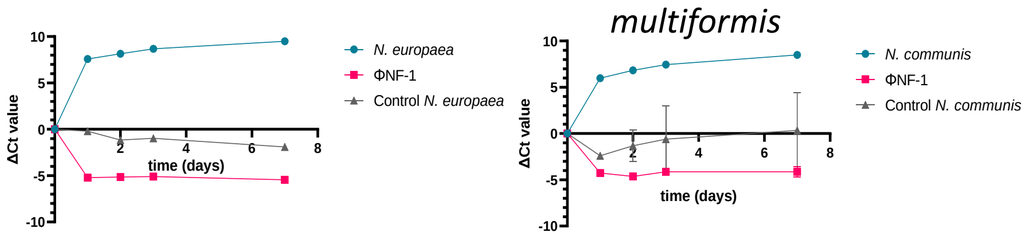




Phage ΦNF-1 had a genome of 41,596 bp and 45.09% GC content and showed 53 predicted ORFs. The genome analysis of ΦNF-1 suggested it was a virulent phage rather than a temperate one, as it lacks genes associated with lysogen. The most closed sequence was from a *Podoviridae* phage infecting *Sphaerotilus natans*.

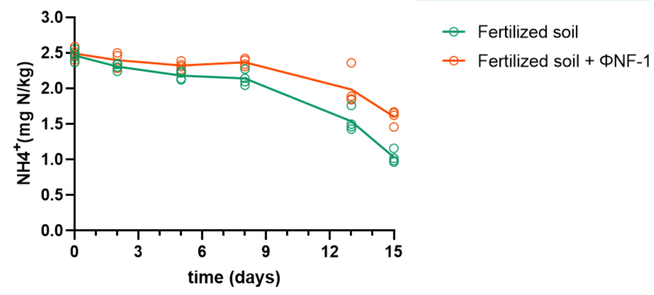
Host spectra and Infectivity

ΦNF-1 was capable of infecting 4 species of *Nitrosomonas* genus and *Nitrosospira multiformis*

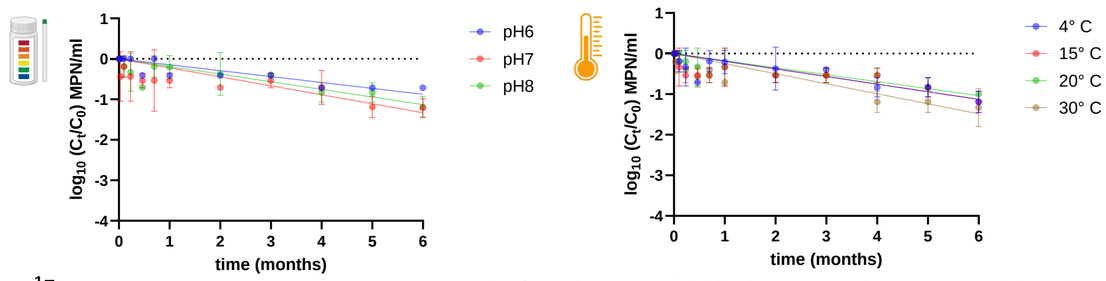


Host spectra-*Nitrosomonas*:
N. europaea
N. communis
N. nitrosa
N. oligotropha

ΦNF-1 inhibited bacterial growth, resulting in a reduction of nitrite formation in phase-treated samples in soils and a lower decrease of NH4+



Persistence



Infectious ΦNF-1 remained stable in water suspensions for 6 months at 4, 15, 20, and 30 ˚C, and at pHs 6, 7 and 8, but was inactivated when exposed to pHs 3 and 5. In soils, ΦNF-1 infectivity showed a 1-log10 reduction over 6 months.

CONCLUSION

This study presents a **novel approach using bacteriophages to eliminate nitrifying bacteria**. Bacteriophages as biocontrol agents offer an effective and **environmentally friendly alternative** to inhibit nitrification while overcoming the limitations of chemical products.

FUTURE WORK

This application shows potential for improving agricultural fertilization, further research is required to scale up and optimize a product.