

Bacteriophage-Based Biocontrol of Psychrotrophic *Pseudomonas* spp. in Low-Sodium Cheese Production

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Background

Problem: Sodium reduction weakens the cheese's natural antimicrobial barrier, increasing spoilage risk.

Spoilage agent: Psychrotrophic *Pseudomonas* spp. (*P. lundensis* and *P. taetrolens*) produce heat-resistant enzymes that cause defects, off-flavours, and shelf-life loss.

Solution: Bacteriophages provide a targeted, safe, and sustainable biocontrol strategy without affecting starter cultures.

Methods

PHAGE ISOLATION AND CHARACTERIZATION

- **Sampling sources:** dairy wastewater and cheese rinds.
- **Target bacteria:** *P. lundensis* and *P. taetrolens*.
- **Host Range:** tested against 84 *Pseudomonas* isolates from dairy environments.

IN VITRO INACTIVATION

- **Assay Conditions:** Liquid medium (TSB) at 25 °C using individual phages against *P. lundensis* and *P. taetrolens*.
- **Phage-to-Bacteria Ratios:** Multiple infection ratios (MOIs of 0.1, 1, 10, and 100).
- **Bacterial Monitoring Timeline:** Samples collected at 0, 12, 18, and 24 h for bacterial survival quantification.

GENOMIC SAFETY

- **Sequencing:** Illumina NovaSeq X Plus platform (2 x 15 bp paired-end reads).
- **Assembly:** *De novo* assembly using Unicycler, with quality checked via CheckV.
- **Safety (In Silico):** Screening via Pharokka, Bakta, and PhageAI to confirm lytic lifestyle and absence of toxins, virulence, or resistance genes.

PHYSICOCHEMICAL STABILITY

- **Parameters:** Tested at 4, 16, and 25 °C; pH 4.0, 5.0, and 7.0; and salinity (NaCl and KCl) at 3%, 12%, and 18%.
- **Matrix:** Phage suspensions (10^7 PFU·mL⁻¹) incubated in PBS.
- **Phage Monitoring Timeline:** Viability monitored over 42 - 56 days.

Results

PHAGES ISOLATED

C7GGH

Broad host range – infecting multiple *P. lundensis* isolates from Portuguese and Spanish cheese dairies.

AR2_7H

High lytic activity against its host strain (*P. lundensis* Ps007) but limited activity against other *Pseudomonas* isolates.

AR1_8

Highly specific to *P. taetrolens* Ps008, with no activity against other tested isolates.

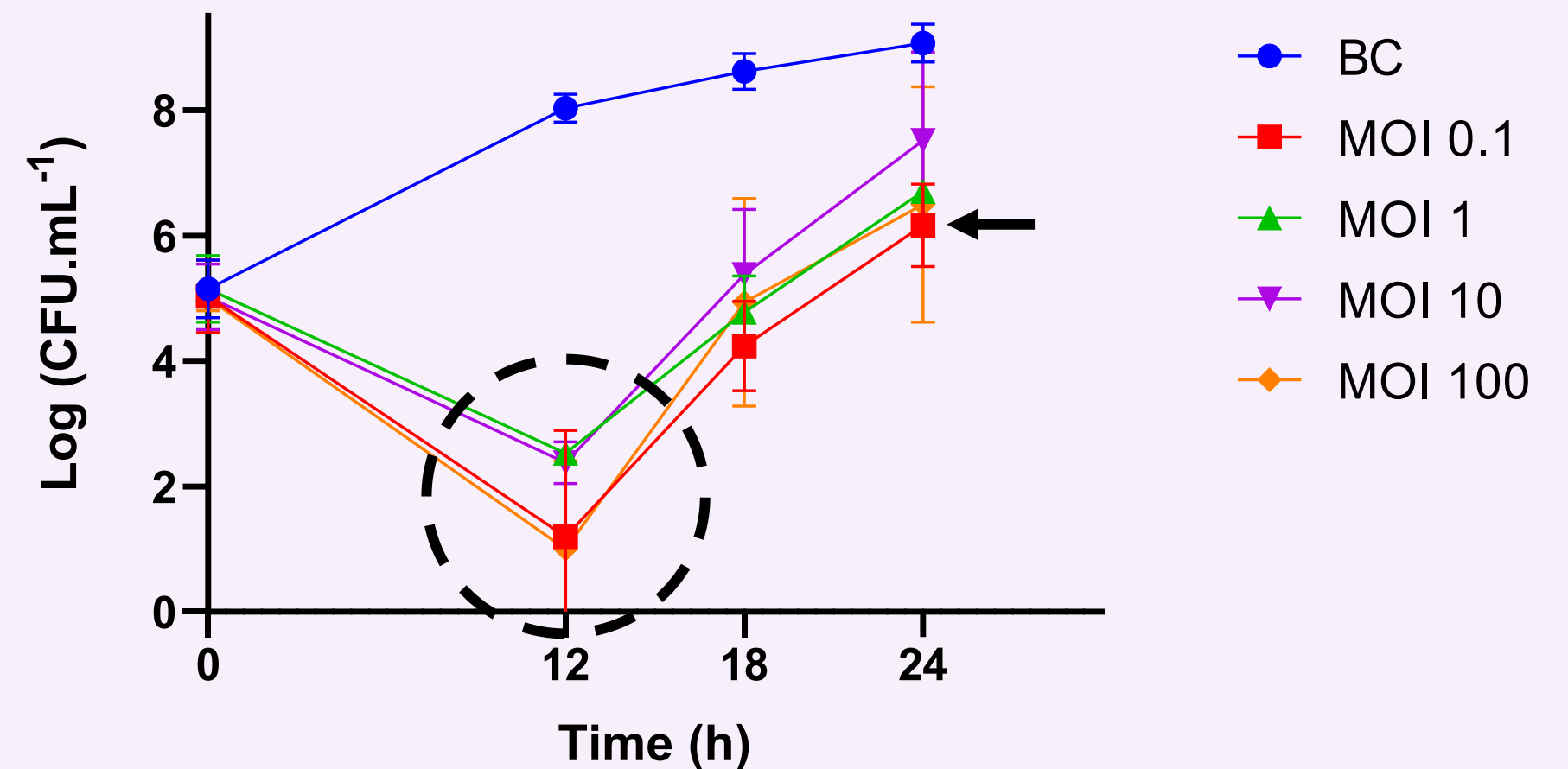
HOST RANGE

Takeaway Message:

These phages provide an effective biocontrol tool for preserving cheese's structural integrity, sensory quality, and shelf life, thereby supporting healthier, low-sodium products that meet strict food safety standards.

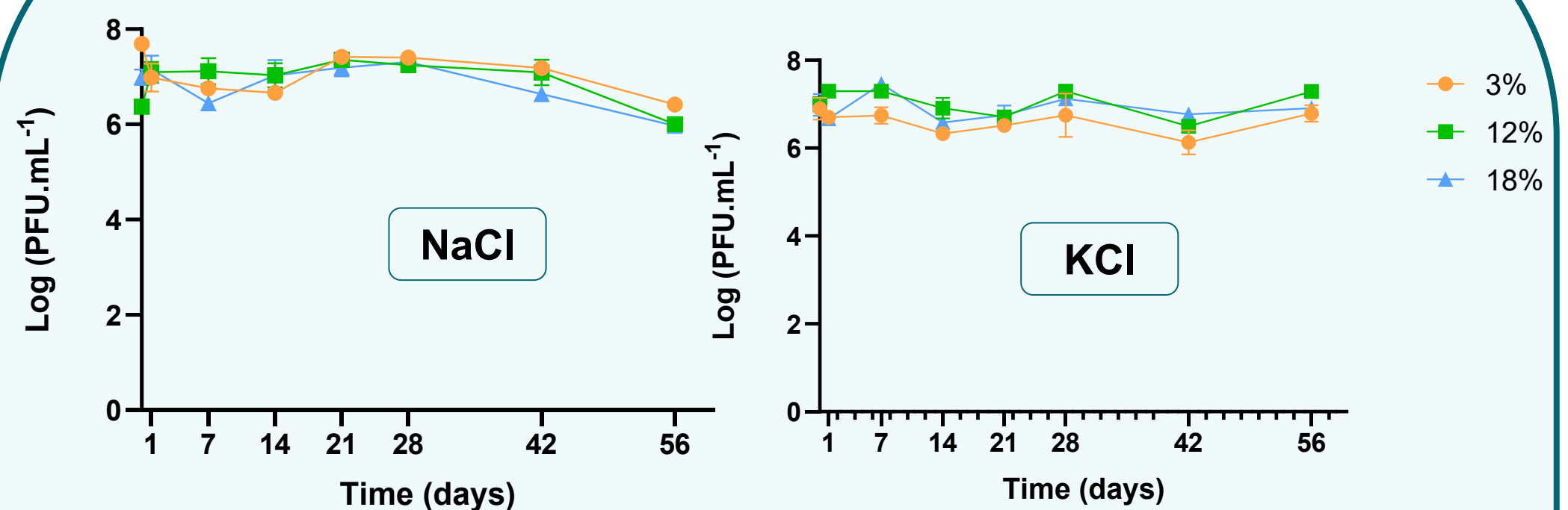
Results

IN VITRO INACTIVATION

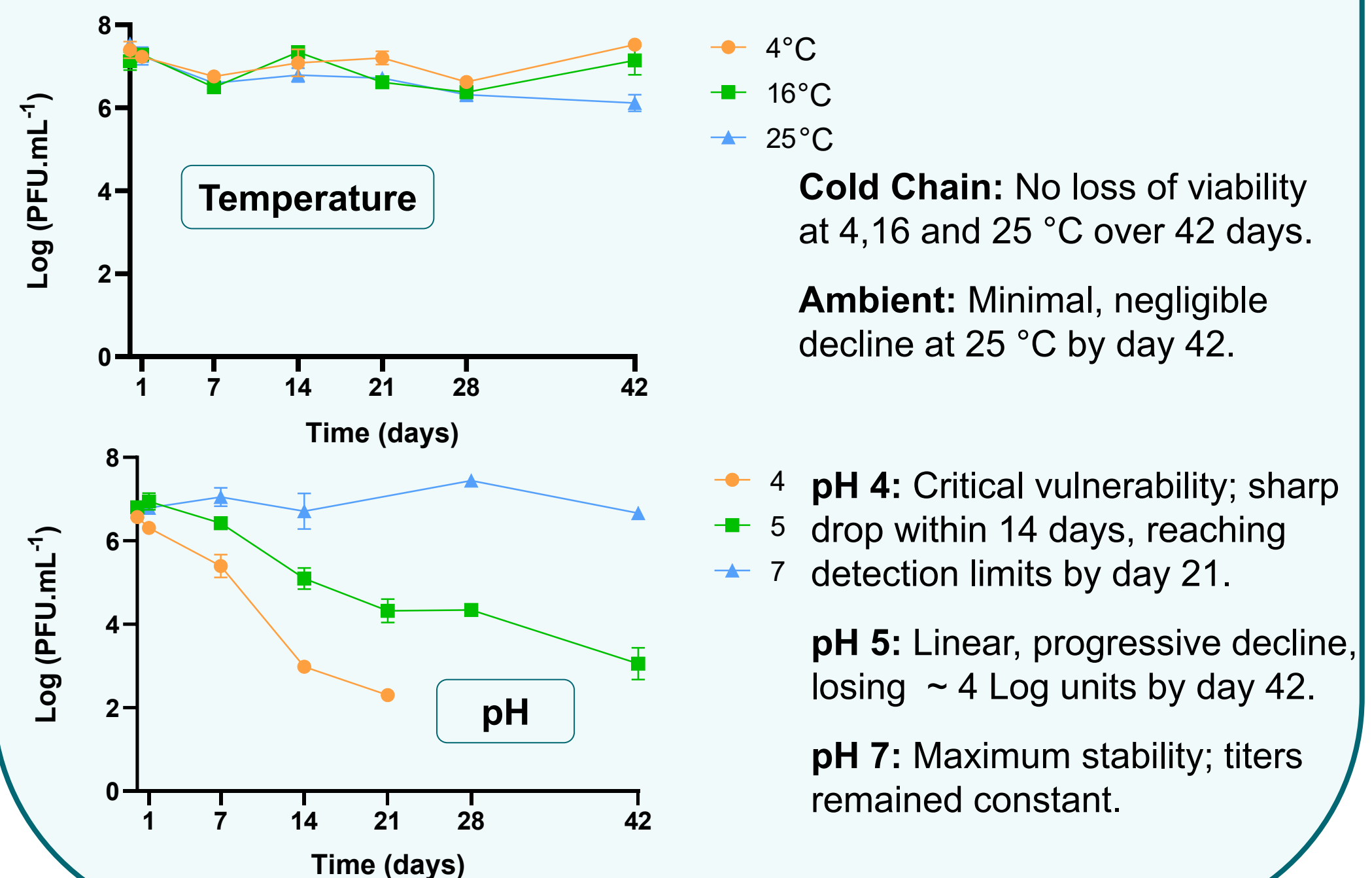


- Phage **AR2_7H** caused the best *in vitro* inactivation of all the phages tested.
- All phage treatments **reduced** bacterial counts by **5.5–7.0 log CFU·mL⁻¹** after 12 h.
- **Phage dose** did not affect inactivation efficacy at 12 h; MOI 0.1 performed as well as MOI 100.
- **After 24 h**, only the lowest phage dose (**MOI 0.1**) maintained a **significant bacterial reduction** of 2.9 log CFU·mL⁻¹ compared with the control.

PHYSICOCHEMICAL STABILITY



- **High Tolerance:** Stable across all concentrations (3%, 12%, 18%) for 56 days.
- **Salt Substitution:** Identical stability in NaCl and KCl; ideal for sodium-reduced formulations.



- **Cold Chain:** No loss of viability at 4, 16 and 25 °C over 42 days.
- **Ambient:** Minimal, negligible decline at 25 °C by day 42.
- **pH 4:** Critical vulnerability; sharp drop within 14 days, reaching detection limits by day 21.
- **pH 5:** Linear, progressive decline, losing ~ 4 Log units by day 42.
- **pH 7:** Maximum stability; titers remained constant.

WHOLE GENOME SEQUENCING

- Strictly Virulent Lifestyle
- Virulence Factors / Toxins
- Antimicrobial Resistance Genes