

Host-specificity and therapeutic potential of novel *Escherichia coli*-targeting bacteriophages for bovine mastitis control.

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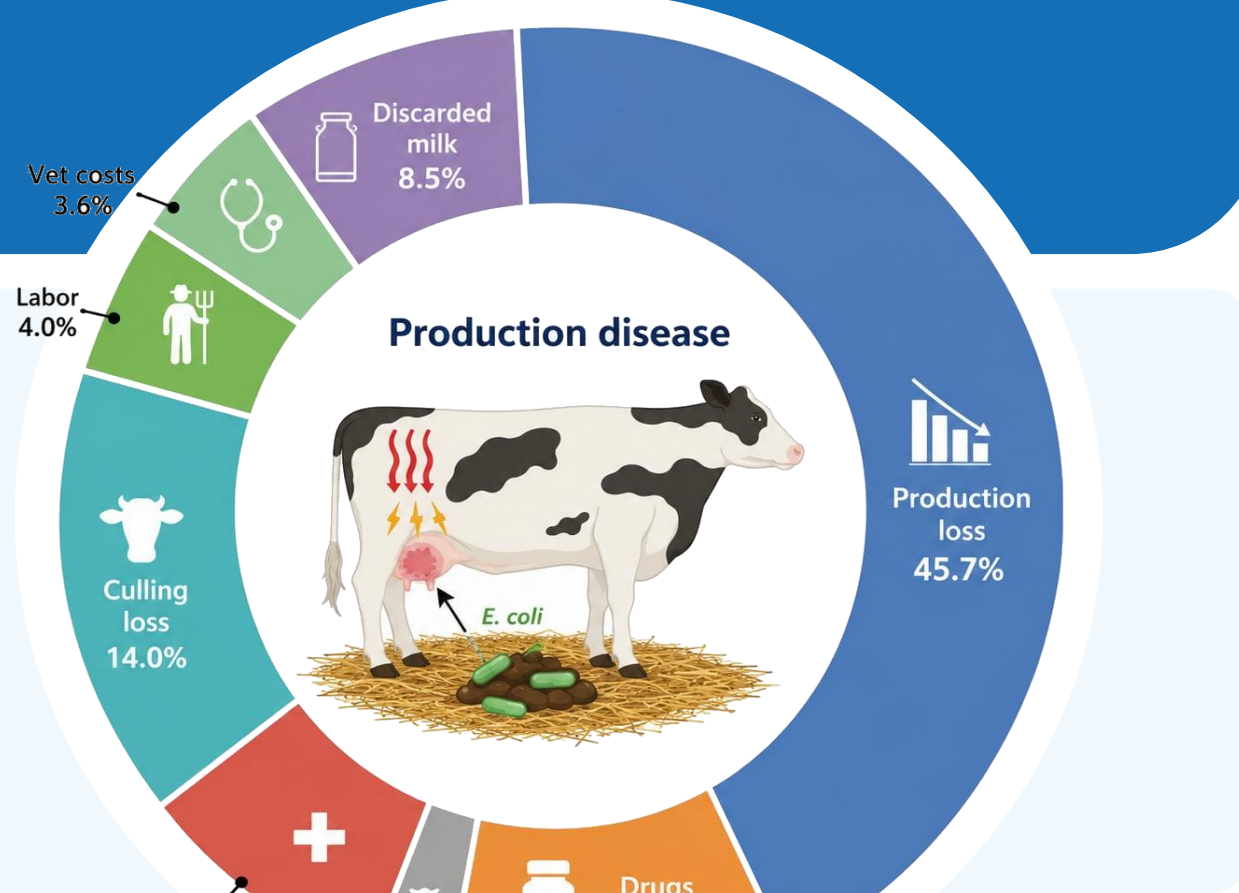


Fig. 1. Mastitis cost

Introduction: *Escherichia coli* (*E. coli*) mastitis is a major production disease (Fig. 1.) in dairy cattle and requires alternative treatments to antibiotics. Phages are of growing interest, but their host specificity remains a challenge in therapy.

Aims: Characterize phages – Investigate their specificity – Assess their efficacy in milk

1 Phage isolation and DNA sequencing

M&M: Enrichment method: wastewater and slurry samples with *E. coli* strains from bovine mastitis + Illumina sequencing.

Res: 8 phages were isolated and sequenced (Fig. 2.).

2 Therapeutic potential

a) Phage stability

M&M:

- Stability in milk: raw and heat-treated (HT) milk + phages, incubation 6h at 37°C.
- Stability across pH (2-12) and T°C (4-60°C) ranges during 1h.

Res: Fig. 3.

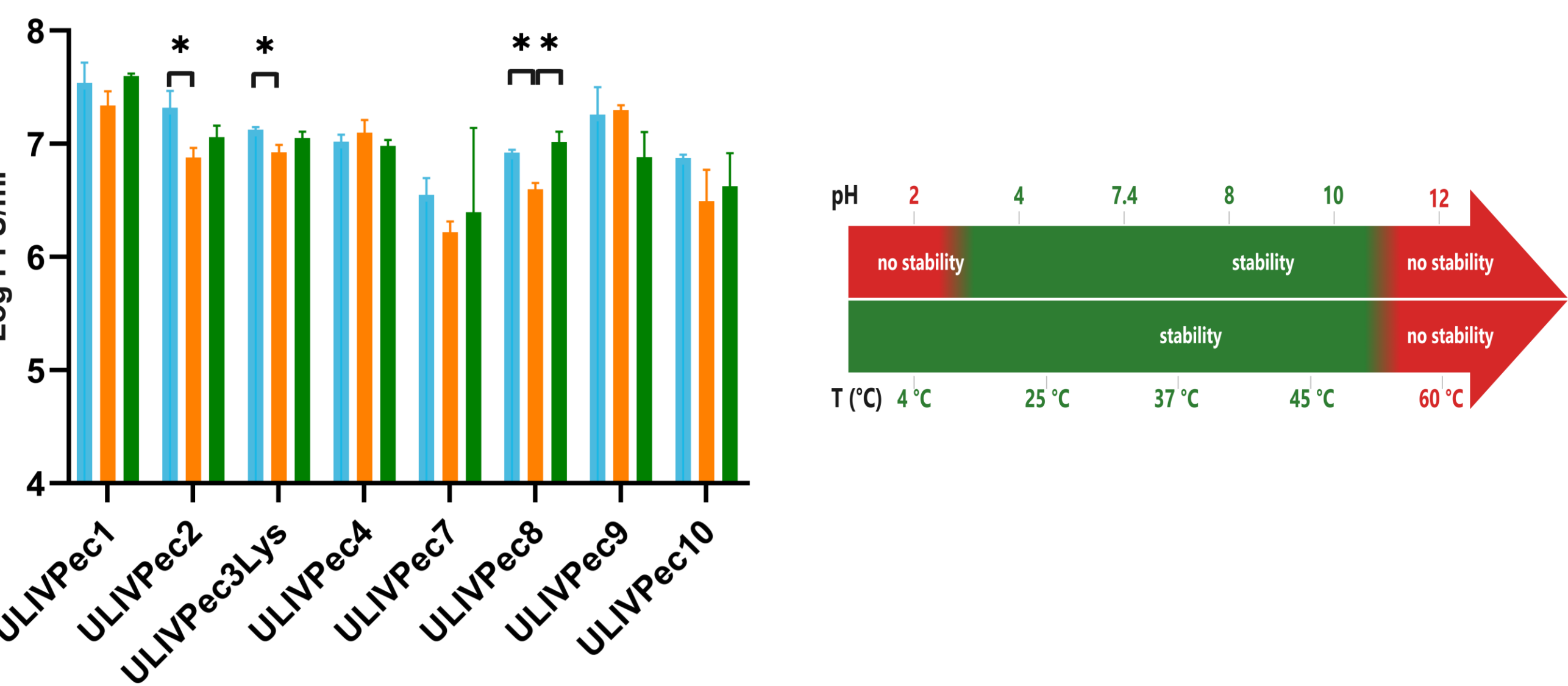


Fig. 3. Stability of phages in milk after 6 h of incubation (left). Stability across pH and T°C ranges (right). * = p < 0.05 ; Blue = inoculum, Orange = HT milk, Green = raw milk.

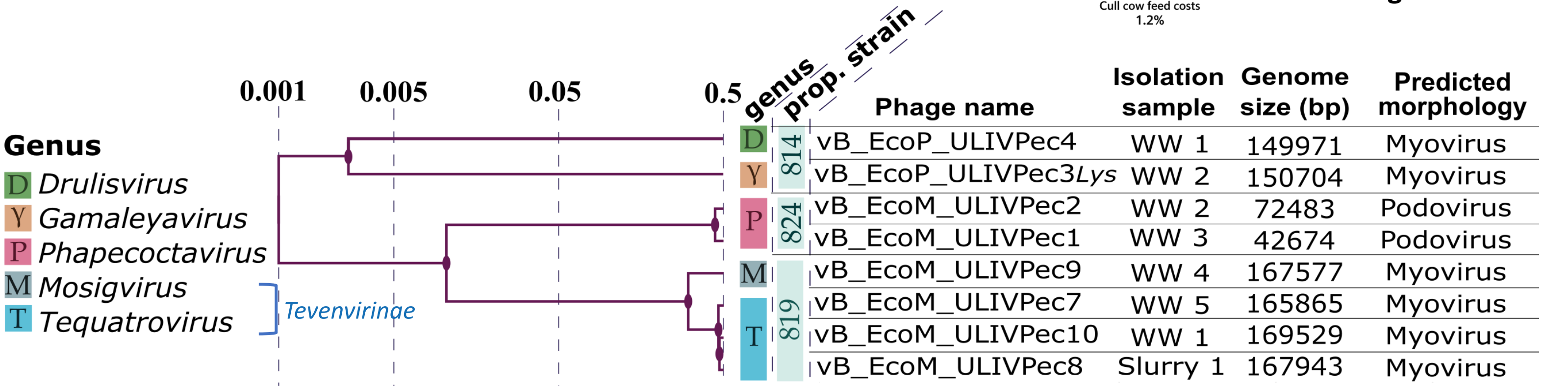


Fig. 2. Viral proteomic tree of the isolated phages and their general information. WW = wastewater.

b) In vitro efficacy in milk

MOI 0.01 > Track 1-3-5h

MM: MOI 0.01 in raw, HT and UHT milk, bacterial tracking.

Res: Significant bacterial drop (p = 6.6e-7) with phages, bacterial regrowth occurred 5h post incubation (Fig. 4.).

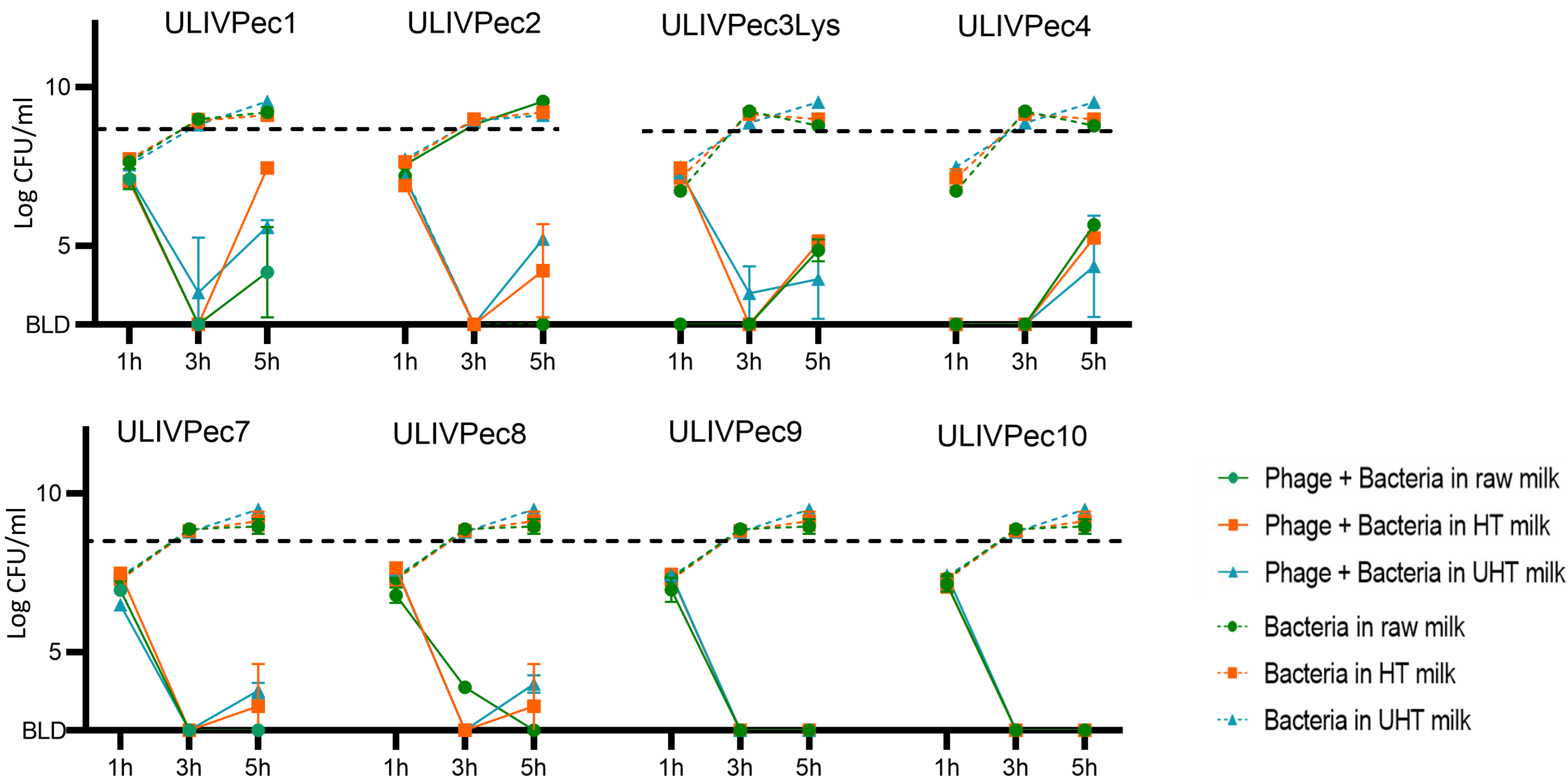


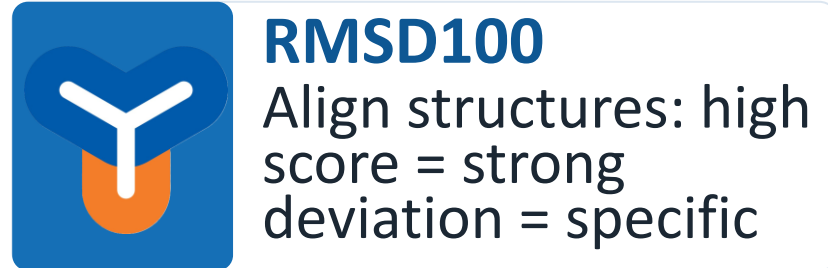
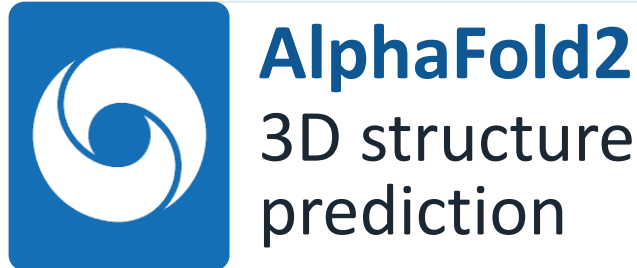
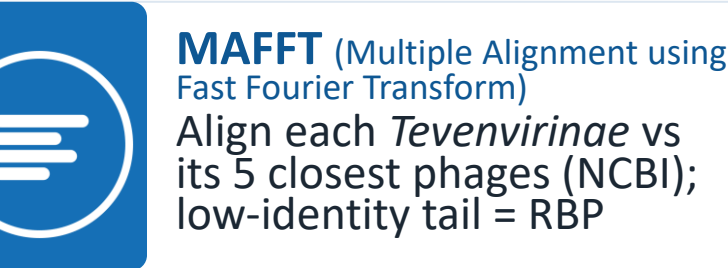
Fig. 4. Lytic activity of phages in raw, HT and UHT milk. BLD = below limit of detection. Dotted line = titer of inoculum.

3 Host specificity

a) Receptor-binding proteins (RBP)

M&M:

“What is the key and what is the lock?”



Res: Fig. 5. and 6.

- Variable needle of the Long Tail Fiber (LTF):** The LTF is responsible for reversible attachment and drives specificity (RMSD100 = 28.1 high).
- Conserved Short Tail Fiber (STF):** The STF is responsible for irreversible attachment and is unspecific (RMSD100 = 10.2 low).

b) Bacterial receptor (perspectives)

M&M: Linking components of the outer membrane to the host range, analyze of resistant strain (loss of receptor).

Res: No link between the host range and the serogroup. Loss of capsule in resistant strains caused by *wcaJ* deletion => colanic capsule = receptor?

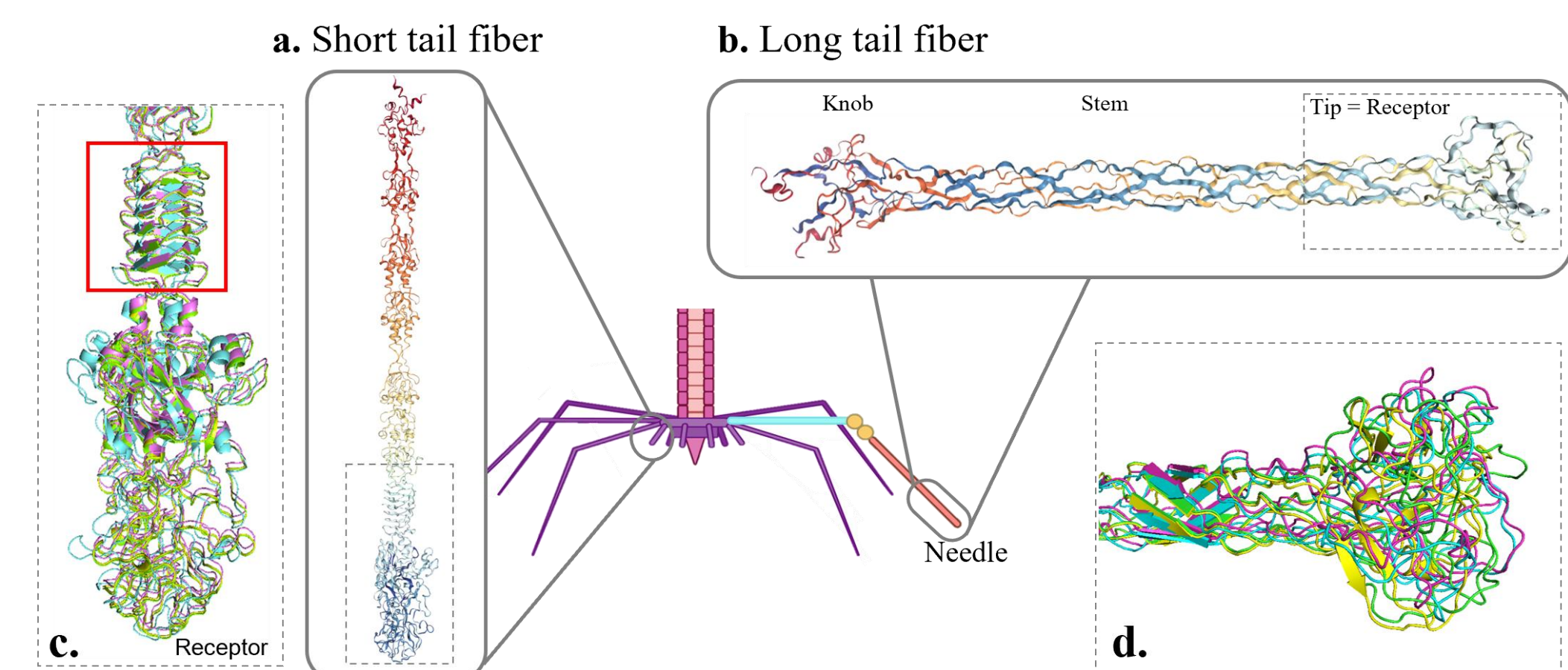


Fig. 5. Structure of RBPs (a and b) and their alignments (c and d). In a and b: the gradient represents the N-terminal (red) to C-terminal (blue) region. In c and d: each colour represents the structure of a distinct homologous phage.

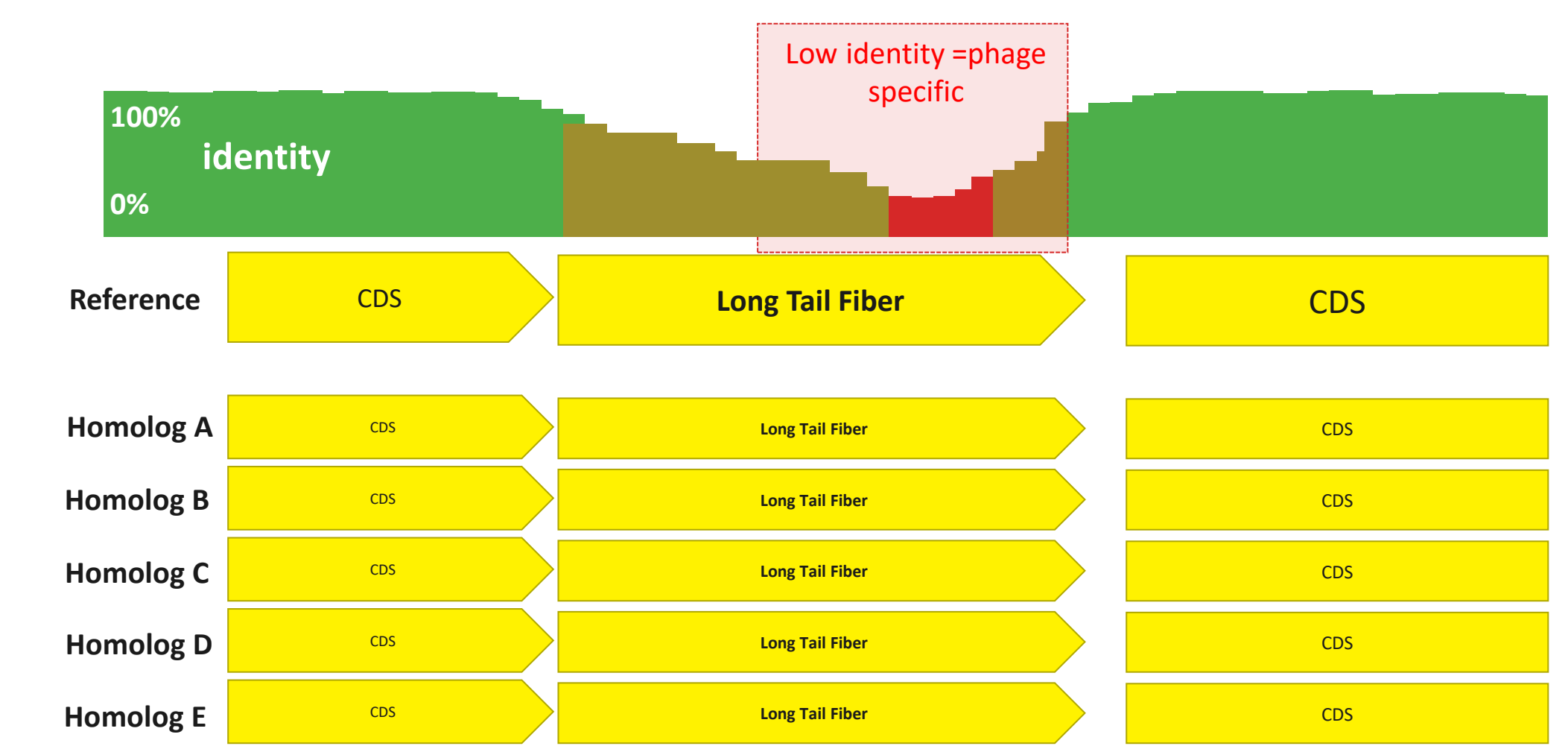


Fig. 6. MAFFT alignment of a reference phage and its 5 closest homologs.

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Conclusion:

- Eight *E. coli* phages effective in raw milk:** Milk components did not impair phage efficacy or stability, and bacterial titer was reduced.
- Quick bacterial regrowth:** could be overcome by higher MOI and phage cocktails, but dynamics still need investigation.
- Two RBPs were identified:** A specific and reversible attachment mediated by LTF is followed by irreversible and less specific attachment by STF.
- Receptor likely tied to the colanic capsule:** resistant-strain characterization and targeted knockouts (*wcaJ*) will clarify the adaptation mechanism and fitness trade-off.

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