Title: Detection of lytic phage infecting flavour-producing strain of *Lacticaseibacillus paracasei* in the dairy effluents of Kerala

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SUPPLEMENTARY FILE

Table S 1. *Lc. paracasei* strains used for phage propagation

***Lc. paracasei* strains**	**Accession No.**	**Isolation location (GPS Coordinates)**
Lc. paracsei ADMT 26	MW 429781	Puzhakkal, Thrissur (10.5262321,76.1657239)
Lc. paracsei ADMH 7	MW 644962	Kuppady Bathery (11.6830477,76.2738919)
Lc. paracasei ADMH 13	MW 644965	Rattakolly, Kalpetta (11.61056,76.08222)

Cultivation media for bacteria and phages

Bacteria were cultured and propagated in MRS broth (HiMedia Laboratories Pvt.Ltd., Mumbai, India) at 37ºC without agitation. MRS broth supplemented with 10 mM $CaCl_2$ was used for phage propagation. Soft agar was prepared with MRS broth supplemented with 0.6% Agarose and 1 % glycine (Lillehaug, 1997).

PCR conditions for 16S rRNA amplification of *Lc.paracasei* strains

PCR amplification was carried out in a thermal cycler (Prima Trio Thermal cycler, HiMedia laboratories Pvt. Ltd., Mumbai, India) using the conditions: Initial denaturation for 3 min at 94

◦C followed by 30 cycles of PCR denaturation at 94 ◦C for 30 s, annealing at 55 ◦C for 45 s, extension at 72 ◦C for 1 min, final extension for 7 min at 72 ◦C (Masumizu *et al*., 2019). Amplification products were separated on a 1.5 % agarose gel and visualized under UV light after staining with ethidium bromide (1 mg/mL). The sequencing of PCR products was done at AgriGenome Labs Pvt. Ltd., Ernakulam, India.

PCR conditions for RAPD analysis

The amplification reactions were carried in a thermal cycler (Prima Trio Thermal cycler, HiMedia laboratories Pvt. Ltd., Mumbai, India) using the conditions: Initial denaturation at 94^{o}C for 5 min, followed by 40 cycles of PCR amplification at 94^{o}C for 1 min, annealing at 37^{o}C for 1 min, 72^{o}C for 2 min and final extension at 72^{o}C for 10 min. Amplification products were separated on a 1.5 % agarose gel and visualized under UV light after staining with ethidium bromide (1 mg/mL).

Phage enrichment

Sterile 40 ml MRS with 10mM $CaCl_2$ was used for the enrichment of phages. *Lc.paracasei* strains were activated by inoculating 1% culture and incubating at 37^{o}C for 18 h. After overnight incubation 100 ul of each host cell was added to the enrichment broth. To this 10 ml of filtered effluent, the sample was added and incubated at 37^{o}C for 18 h. After incubation, the enrichment broth was centrifuged at 4000 rpm for 10 min at 4^{o}C followed by filtration through a 0.45 µm syringe filter. The enrichment was repeated twice and the filtered sample was then tested on three *Lc. paracasei* strains

Spot assay for detection of phages

100ul overnight grown host cells were mixed with 50 µL 1M $CaCl_2$ and 10 ml of 0.6% soft agar. The mixture was overlayed onto MRS agar plate supplemented with 10mM $CaCl_2$. 10 µL of the filtered effluent sample was spotted onto each host cell plate and incubated at 37^{o}C for 18 h and checked for the presence of clear zones.

Enumeration of phages

The filtered effluent sample was serially diluted in SM buffer (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM MgSO4) and a 10 µL volume of the appropriate phage dilution and 200 µL of host cells were added to 10 mL of soft agar supplemented with 10 mM $CaCl_2$ and 1 % glycine. The soft agar with the host cell was uniformly mixed and poured onto an MRS agar plate supplemented with 10 mM $CaCl_2$. The plate was incubated at 37 ºC for 18 h and resulting plaques were enumerated as plaque-forming units (PFU/mL). Phages were purified further by single-plaque isolation using an appropriate *Lc.paracasei* host strain. A single plaque was picked from the bacterial lawn and transferred into 10 mL MRS broth supplemented with 10 mM $CaCl_2$. *Lc.paracasei* host strain was inoculated at the rate of 1% into this broth and incubated at 37 ◦C for 18 h. The phage lysate formed was centrifuged at 4000× g for 10 min at 4 ◦C. The supernatant was filtered using a 0.45 µm mixed cellulose esters membrane syringe filter (Merck Millipore Ltd., Cork, Ireland) and stored at 4 ◦C until required.

References

Lillehaug D (1997) An improved plaque assay for poor plaque-producing temperate lactococcal bacteriophages. *J. Appl. Microbiol*. 83(1), 85-90.

Masumizu Y, Zhou B, Kober AK, Islam M, Iida H, Ikeda-Ohtsubo W, Suda Y, Albarracin L, Nochi T, Aso H and Suzuki K (2019) Isolation and immunocharacterization of *Lactobacillus salivarius* from the intestine of wakame-fed pigs to develop novel “immunosynbiotics”. *Microorganisms.* 7(6):167.