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BACTERIOPHAGE-HOST INTERACTIONS IN LACTOCOCCI

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ABSTRACT

Host range specificities of 60 bacteriophages were determined on 98 lactococcal strain, isolated from raw milk and whey samples. Bacteriophage-host interactions of twenty four bacteriophages which were selected for their wide host range characters and indicator specificities, were analysed. Adsorptions, efficiencies of plaquing, latent periods, rise periods, burst sizes and plaque sizes of these 24 bacteriophages were ranged at the rates of 94.6-99.9 % , 0.96-1.00, 5-15 min, 5-35 min, 44-147 and 0.6-1.5 mm, respectively.

1. INTRODUCTION

Lactococcus lactis is a gram-positive lactic acid bacterium (LAB) used to acidify milk during the manufacture of fermented dairy products (Deveau *et al.* 2002). Bacteriophage infection of dairy lactococcal starter strains represents a significant threat to the efficient performance of starter cultures in dairy fermentations (Platteauw *et al.* 1996; Mills *et al.* 2002). As a result of this constant threat of bacteriophage infections, fermented milk products manufacturers nowadays routinely employ highly bacteriophage resistant strains in defined starter systems. In reality, there is a very limited number of such bacteriophage-resistant strains which process traits rendering them suitable for prolonged fermented milk product manufacture (Coffey *et al.* 1998; Schouler *et al.* 1998; O'Sullivan *et al.* 2001). In response to this predicament, there has been an intensive worldwide research effort focusing on bacteriophage-host interactions in lactococci to understand bacteriophage defense systems which occur naturally in these starter strains (Lillehaug 1997; O'Sullivan *et al.* 1998). Currently, four groups of naturally occurring bacteriophage resistance mechanisms are recognized based on their modes of action: adsorption interference (Akçelik and Tunail 1992), restriction modification systems (Hill 1993), DNA injection inhibition and abortive infection (Dinsmore and Klaenhammer 1995; Garvey *et al.* 1995). More recently, details

revealing how bacteriophages overcome these mechanisms have became available. In order to be successful in long term, future starter culture designs must consider the interactions behind host and bacteriophage evolution and take steps minimize the genetic routes leading to the appearance of new virulent bacteriophages (Dinsmore and Klaenhammer 1995; Allison and Klaenhammer 1998).

The aim of this study was to characterize bacteriophage-host interactions in *Lactococcus lactis* strains, used in the production of traditional cheeses in Turkey.

2. MATERIALS AND METHODS

Bacterial strain, bacteriophages and media

The bacterial strains and the bacteriophages were obtained from Culture Service of Ankara University (AÜZF). Lactococcal bacteriophages and *Lactococcus lactis* strains were propagated at 30 ^{0}C in M17 broth (Terzaghi and Sandine 1975) or on M17 agar plates containing 5 g of glucose (GM17), instead of lactose, per litre medium; for propagation of bacteriophage, $CaCl_2$ was added to the M17 medium to a final concentration of 5 $mmol^{-1}$. Bacteriophage and culture stocks were stored in broth containing 40 % glycerol at – 18 ^{0}C.

Plaque assay

A sample of bacteriophage suspension was either mixed with lawn bacteria suspended in a small volume (2.5 ml) or melted M17 top agar (0.45 % W/V agar) kept at 45 ^{0}C, which immediately was poured onto a 85 mm petri dish containing M17 bottom agar (pour-plate assay), or 10 µl of bacteriophage suspension were dripped onto the bacterial lawn after the top agar containing bacterial cells was hardened (spot-test). Then the plates were sealed with parafilm and placed in an upright position at 30 ^{0}C for 24-36 h before they were examined for plaques (Lillehaug 1997).

Bacteriophage adsorption and one step growth experiment

Bacteriophage adsorption to the cells was conducted as described by Sanders and Klaenhammer (1980). The percentage adsorption was calculated as [(control titer – residual titer) (control titer)$^{-1}$] x 100.

One step growth experiment were done as described by Gautier and Chopin (1987). The latent period and the rise period were estimated at the midpoint and top point of exponential phase of one step growth curve, respectively. Burst size was calculated by dividing the average bacteriophage number of the latent period.

Efficiency of plaquing (EOP) and plaque diameter were measured as described by Jarvis *et al.* (1991).

3. RESULTS AND DISCUSSION

The host range of 60 bacteriophages was determined on 98 lactococcal strains. Sixteen of 98 strains were insensitive to all bacteriophages, used in this study. The most sensitive strain was *Lactococcus lactis* subsp. *cremoris* PLC 79 which lysed by 54 of totally 60 bacteriophages. Other strains showed different degrees of sensitivity patterns to these bacteriophages. Only two (Φplc 61-56 - Φplc 61-58) of 60 lytic bacteriophages were lysed exactly the same host strains. According to the wide host range specificities, (equal or up to 50 % lytic activity against 98 lactococcal strains), dominant bacteriophages in traditional milk fermentations in Turkey were determined as Φpll 35-6, Φpll 35-8, Φpll 36-10, Φpll 36-14, Φpll 36-15, Φpll 98-22, Φpll 98-23, Φpll 98-25, Φpll 98-26, Φpll 98-28, Φpll 98-32, Φpld 64-33, Φpld 67-38, Φpld 67-39, Φpld 67-41, Φpld 67-42, Φpld 67-43 and Φpld 67-44 (Table 1). In preparation of starter cultures, it is desirable to select and combine bacteriophage unrelated lactococcal strains (Lawrence *et al.* 1976). This practice requires extensive knowledge of bacteriophage-host interactions, and attempts have been made to differentiate strains on the basis of their susceptibility to different bacteriophages (Jarvis 1989). Our results, shown in Table 1, and the data presented in literature (Klaenhammer 1987; Sanders 1988; Daly *et al.* 1995; Şanlıbaba and Akçelik 2000) showed similarities with respect to non subspecies specific host range character of lactococcal bacteriophages. Primary causes of the variability of lactococcal strains with respect to bacteriophage sensitivity is bacterial heterogeneity (Declour *et al.* 2000, Barrette *et al.* 2000). In addition to bacterial heterogeneity, changes in bacteriophage-host interactions also occurs because bacteriophages are continually evolving (Jarvis *et al.* 1991; Pillidge *et al.* 2000; Deveau *et al.* 2002).

Totally 24 lactococcal bacteriophages were selected to investigate the bacteriophage-host interactions. Eighteen of these bacteriophages were identified dominant bacteriophages for traditional fermented milk products in Turkey which described above. On the other hand, bacteriophages Φpll 6-2, Φpll 10-5, Φpll 47-21 and Φpld 66-36 were chosen for their different indicator strains. Last two bacteriophages (Φplc 61-56 and Φplc 61-58) used to determine bacteriophage-host interactions were chosen for their similar host range character (Table 1). Bacteriophage adsorption rates were changed between the values of 94.6-99.9 % at 30 ^{0}C for 12 min. In these incubation conditions, latent periods and rise periods of bacteriophages were determined at the ranges of 5-15 min and 5-35 min, respectively. Efficiencies of plaquing, burst sizes and plaque sizes of the bacteriophages, used in bacteriophage-host bioassays, were differed at the ranges 0.96-1.00, 44-147 and 0.6-1.5 mm, respectively (Table 2). Bacteriophage growth characteristics on the indicator strains and the sensitive host were the same (data not

shown) indicating that sensitive host strain did not carry any resistance mechanism. Based on previous reports (Coffey *et al.* 1998; Forde and Fitzgerald 1999; Tükel *et al.* 2002) and the results described above, growth parameters of lytic bacteriophages of lactococci can be said to have high multiplication factors, and they can cause complete failure of the starter culture even when present in low concentrations in the initiation of the milk fermentation processes. However our results were opposite to the data in literature with respect to correlation between latent period and rise period. It is generally accepted that the bacteriophages which have higher burst sizes show shorter latent periods (Petersen *et al.* 2001; Tükel *et al.* 2002).

Analysis of bacteriophage-host interactions will provide an insight into the phylogenetic relatedness of lactoccocal bacteriophages and frequency with which certain species interfere with fermentation process. This knowledge may prove to be extremely useful in designing strategies to minimize the consequences of bacteriophage attack; for example, the introduction of various bacteriophage resistance mechanisms directed against different bacteriophage species into different strains of starter culture.

Table 1. Host specificities of lactococcal bacteriophages

Bacteria	Bacteriophages																													
	Φpll 6-1	Φpll 6-2	Φpll 6-3	Φpll 6-4	Φpll 10-5	Φpll 35-6	Φpll 35-7	Φpll 35-8	Φpll 35-9	Φpll 36-10	Φpll 36-11	Φpll 36-12	Φpll 36-13	Φpll 36-14	Φpll 36-15	Φpll 36-16	Φpll 36-17	Φpll 36-18	Φpll 36-19	Φpll 36-20	Φpll 47-21	Φpll 98-22	Φpll 98-23	Φpll 98-24	Φpll 98-25	Φpll 98-26	Φpll 98-27	Φpll 98-28	Φpll 98-29	Φpll 98-30
PLL1	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+	+	-	+	-	-
PLL2	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+
PLL3	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-
PLL4	-	+	+	+	+	+	+	-	+	-	-	+	-	-	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+
PLL5	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	-
PLL6	+	+	+	+	-	+	+	-	+	+	+	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+
PLL7	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
PLL8	-	+	+	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+
PLL9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
PLL10	+	+	+	+	+	-	-	+	-	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	+
PLL11	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-
PLL12	-	+	+	+	+	-	+	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	-	+	-	+	+	-
PLL13	+	+	+	+	+	+	-	+	-	+	-	-	+	+	+	+	+	-	+	-	-	-	+	+	+	+	+	+	+	+
PLL14	-	+	+	+	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-
PLL15	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
PLL16	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
PLL17	-	-	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+
PLL18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL19	-	+	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-
PLL20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
PLL21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL22	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-

Table 1. Host specificities of lactococcal bacteriophages (continue)

PLL23	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL24	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL27	-	+	-	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
PLL28	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-
PLL30	+	-	-	-	-	+	-	+	+	+	+	-	-	+	+	-	+	+	+	-	+	+	+	-	+	+	-	+	-	+
PLL31	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	-
PLL32	-	-	+	-	-	-	+	-	-	+	+	-	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	-
PLL33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-
PLL34	-	-	+	+	-	-	-	-	-	+	+	-	-	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+
PLL35	-	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+	+	+
PLL36	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	-	+	-
PLL37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL39	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-
PLL40	+	+	-	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+
PLL41	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL42	-	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL43	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
PLL44	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
PLL45	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+
PLL46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1. Host specificities of lactococcal bacteriophages (continue)

PLL47	-	-	-	-	-	+	-	+	-	-	+	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-
PLL48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL49	-	-	+	+	+	-	-	+	+	+	-	+	-	-	-	-	+	+	-	-	+	+	+	+	+	+	-	-	-	+
PLL50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL51	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	+	+	+	+	-	+	-	+	+	+
PLL52	-	+	+	-	+	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	-	+	+	-	+	+	-
PLL53	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
PLL54	+	-	+	+	-	+	-	+	-	+	-	-	-	+	+	-	+	+	-	-	+	+	-	-	+	+	-	+	-	+
PLL55	-	-	+	+	-	+	-	+	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	-	+	-	-	-	+
PLL56	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
PLL57	-	+	+	-	+	-	-	+	-	+	-	+	-	-	+	-	+	+	+	-	+	+	+	-	+	+	-	+	+	+
PLL58	+	+	-	+	+	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	+	-	-	+	+	-	+	-	+	-
PLL59	+	-	+	+	-	-	+	+	+	-	-	-	-	+	-	-	+	-	-	+	+	+	-	+	+	+	+	+	+	-
PLL60	-	+	+	-	+	+	+	+	-	+	-	-	-	+	+	-	-	+	+	+	+	+	-	+	+	+	-	+	+	+
PLL98	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
PLD62	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
PLD63	-	+	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	-	-	+	+	+	+	-	+	+	-	+	-
PLD64	+	-	-	-	-	+	-	+	-	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+
PLD65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLD66	-	+	+	-	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+
PLD67	-	+	-	-	-	-	-	+	-	+	+	-	-	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+
PLD68	+	-	+	-	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PLD69	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
PLD70	-	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-

Table 1. Host specificities of lactococcal bacteriophages (continue)

PLD71	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLD72	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
PLD73	+	-	+	+	-	+	+	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
PLD90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC61	-	+	-	+	-	+	+	+	-	+	-	-	-	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	+	+
PLC74	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
PLC75	-	+	-	-	-	+	+	-	-	+	-	-	-	-	+	-	+	+	+	-	-	+	+	-	+	+	+	+	-	-
PLC76	+	+	-	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
PLC77	+	-	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	+	+	+
PLC78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC79	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
PLC80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC81	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	-	-
PLC82	+	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	+	+	-	+	+	+
PLC83	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	+	+	-	+	+	+	-	+	+	-	-	-	-
PLC84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC85	-	+	-	+	+	+	-	+	-	+	-	-	+	+	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-
PLC86	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	+	+	+	-	+	+	+	+	+
PLC87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC89	+	+	+	+	-	+	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+
PLC91	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+
PLC92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC93	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	+	+	+	+	+	+	-	-	-	-

Table 1. Host specificities of lactococcal bacteriophages (continue)

PLC94	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-	+	+
PLC95	-	+	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
PLC96	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-
PLC97	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-

Table 1. Host specificities of lactococcal bacteriophages (continue)

Bacteria	Bacteriophages																												
	Φpll 98-31	Φpll 98-32	Φpld 64-33	Φpld 64-34	Φpld 64-35	Φpld 66-36	Φpld 67-37	Φpld 67-38	Φpld 67-39	Φpld 67-40	Φpld 67-41	Φpld 67-42	Φpld 67-43	Φpld 67-44	Φpld 67-45	Φpld 67-46	Φplc 61-47	Φplc 61-48	Φplc 61-49	Φplc 61-50	Φplc 61-51	Φplc 61-52	Φplc 61-53	Φplc 61-54	Φplc 61-55	Φplc 61-56	Φplc 61-57	Φplc 61-58	Φplc 61-59	Φplc 61-60
PLL1	-	-	+	-	-	+	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL2	-	+	+	-	+	+	-	+	-	-	+	+	+	+	+	-	-	+	+	-	-	-	+	-	-	-	+	-	-	+
PLL3	+	+	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+
PLL4	-	+	+	-	+	-	-	+	+	-	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+
PLL5	-	+	+	-	+	+	-	+	+	+	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
PLL6	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
PLL7	-	+	-	-	-	-	+	+	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
PLL8	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+
PLL9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL10	-	+	+	-	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+

Table 1. Host specificities of lactococcal bacteriophages (continue)

PLLL11	-	+	+	-	-	+	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL12	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	+	-	-	+
PLL13	-	+	+	-	+	-	-	+	-	-	-	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-
PLL14	-	+	+	-	-	-	-	+	+	-	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
PLL15	-	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-	-	-
PLL16	+	+	+	+	+	-	-	+	+	-	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	+
PLL17	+	-	-	-	-	-	-	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL19	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
PLL20	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL22	+	+	-	-	-	-	-	+	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL23	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
PLL24	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL27	-	+	+	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PLL28	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-
PLL29	-		-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL30	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	-	-	+
PLL31	-	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL32	-	+	+	-	+	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PLL33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL34	-	+	+	-	+	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
PLL35	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+
PLL36	-	+	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1. Host specificities of lactococcal bacteriophages (continue)

PLL37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL39	-	+	+	-	-	-	-	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PLL40	+	+	+	-	-	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-
PLL41	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+
PLL43	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-	+	-	-	+
PLL44	-	-	+	-	-	+	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL45	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	-	-	+	-	-	+
PLL46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL47	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PLL48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL49	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	-
PLL50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLL51	+	-	+	-	-	+	+	+	-	-	-	+	+	+	-	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-
PLL52	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+	-	-	+
PLL53	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	-	+	-
PLL54	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	+	+	-	-	+	-	-	+
PLL55	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+
PLL56	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	+	+	-	-	+	-	-	-
PLL57	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	-	-	+	
PLL58	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	-	+
PLL59	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	-	+	-	-	+
PLL60	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-	-	+
PLL 98	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1. Host specificities of lactococcal bacteriophages (continue)

PLD62	-	-	+	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLD63	-	+	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	+
PLD64	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	-	-	+
PLD65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLD66	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-
PLD67	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PLD68	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+	-	-	-
PLD69	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	-	-	+	-	-	+
PLD70	-	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-	-	+	-	-	+
PLD71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+
PLD72	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	-	-	-	-	+
PLD73	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+
PLD90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC61	+	+	+	-	+	-	-	+	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PLC74	+	+	+	-	-	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+
PLC75	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-
PLC76	+	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
PLC77	+	+	+	-	+	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	-	+	+	+	-	-	+	-	-	+
PLC78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC79	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PLC80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC81	-	+	+	+	-	+	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+
PLC82	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-
PLC83	-	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	+	-	-	-	-	+	+	-	-	+	-	-	-
PLC84	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-

Table 1. Host specificities of lactococcal bacteriophages (continue)

PLC85	-	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-
PLC86	-	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	-	+
PLC87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC89	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	-	-	+
PLC91	+	+	+	-	+	+	-	+	+	-	+	+	+	+	-	-	+	+	-	+	+	+	+	+	-	-	+	-	-	+
PLC92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PLC93	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	-	+	-	-	+
PLC94	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+
PLC95	-	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
PLC96	-	+	+	-	-	+	-	-	-	-	+	+	+	+	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+
PLC97	-	+	+	-	+	-	-	-	+	-	+	+	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-

PLL: *Lactococcus lactis* subsp. *lactis*
PLC: *Lactococcus lactis* subsp. *cremoris*
PLD: *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*
\+ : Sensitive
\- : Resistant

Table 2. Growth parameters of lactococcal bacteriophages

Bacteriophage	Indicator Strain	Latent Period (min)	Rise Period (min)	Plaque Size (mm)	Burst Size	Adsorption Rate (%)	Efficiency of Plaquing (EOP)
Φpll 6-2	PLL 6 *	10	20	0,7	69	95,0	-
	PLC 79 **	10	20	0,7	65	94,6	0,98
Φpll 10-5	PLL 10 *	10	30	1,2	62	96,2	-
	PLC 79 **	10	30	1,2	62	96,8	0,99
Φpll 35-6	PLL 35 *	10	35	0,8	63	99,2	-
	PLC 79 **	10	35	0,8	60	99,8	1,0
Φpll 35-8	PLL 35 *	5	15	1,0	95	99,0	-
	PLC 79 **	5	15	1,0	95	99,9	1,0
Φpll 36-10	PLL 36 *	10	25	0,7	70	96,4	-
	PLC 79 **	10	25	0,7	70	96,0	1,0
Φpll 36-14	PLL 36 *	5	10	1,2	121	96,0	-
	PLC 79 **	5	10	1,2	126	96,2	1,0
Φpll 36-15	PLL 36 *	10	30	1,0	60	98,4	-
	PLC 79 **	10	30	1,0	58	98,6	1,0
Φpll 47-21	PLL 47 *	5	5	0,7	145	99,6	-
	PLC 79 **	5	5	0,7	147	99,8	1,0
Φpll 98-22	PLL 98 *	15	30	0,8	44	99,4	-
	PLC 79 **	15	30	0,8	46	99,4	1,0
Φpll 98-23	PLL 98 *	5	10	1,5	108	97,0	-
	PLC 79 **	5	10	1,5	112	97,6	0,99
Φpll 98-25	PLL 98 *	5	15	0,8	106	98,8	-
	PLC 79 **	5	15	0,8	102	99,4	0,98
Φpll 98-26	PLL 98 *	10	20	1,0	78	98,4	-
	PLC 79 **	10	20	1,0	73	98,0	1,0

Table 2. Growth parameters of lactococcal bacteriophages (continue)

Bacteriophage	Indicator Strain	Latent Period (min)	Rise Period (min)	Plaque Size (mm)	Burst Size	Adsorption Rate (%)	Efficiency of Plaquing (EOP)
Φpll 98-28	PLL 98 *	5	10	1,2	105	97,2	-
	PLC 79 * *	5	10	1,2	109	97,8	1,0
Φpll 98-32	PLL 98 *	10	25	0,9	68	99,2	-
	PLC 79 * *	10	25	0,9	68	99,2	1,0
Φpld 64-33	PLD 64 *	5	20	0,8	104	97,6	-
	PLC 79 **	5	20	0,8	108	97,3	1,0
Φpld 66-36	PLD 66 *	10	15	0,8	70	98,0	-
	PLC 79 **	10	15	0,8	70	98,0	0,97
Φpld 67-38	PLD 67 *	5	10	0,7	98	98,7	-
	PLC 79 **	5	10	0,7	96	99,4	0,96
Φpld 67-39	PLD 67 *	10	10	1,3	77	98,4	-
	PLC 79 **	10	10	1,3	75	98,4	1,0
Φpld 67-41	PLD 67 *	10	15	0,6	63	98,6	-
	PLC 79 **	10	15	0,6	65	98,2	0,98
Φpld 67-42	PLD 67 *	15	25	1,0	50	96,2	-
	PLC 79 **	15	25	1,0	50	96,0	1,0
Φpld 67-43	PLD 67 *	10	25	0,6	70	98,6	-
	PLC 79 **	10	25	0,6	71	98,6	0,97
Φpld 67-44	PLD 67 *	10	10	1,2	88	97,0	-
	PLC 79 **	10	10	1,2	87	96,7	1,0
Φplc 61-56	PLC 61 *	5	10	0,9	105	99,2	-
	PLC 79 **	5	10	0,9	103	99,0	0,99
Φplc 61-58	PLC 61 *	5	10	0,9	115	99,6	-
	PLC 79 **	5	10	0,9	115	99,7	1,0

* : Indicator strain

** : Sensitive strain

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