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CHARACTERIZATION OF BACTERIOPHAGE RESISTANCE MECHANISMS ENCODED BY PLASMIDS IN *LACTOCOCCUS LACTIS* SUBSP. *LACTIS* MLL76

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ABSTRACT

Lactococcus lactis subsp. *lactis* MLL76 exhibits two mechanisms which confer resistance to virulent bacteriophages. These include interference with bacteriophage adsorption (Ads^+) and bacteriophage DNA injection blocking (Phi^+). Curing of plasmid DNA from strain MLL76 has allowed bacteriophage resistance functions assigned to two of its six plasmids. A 43.2 kb plasmid was found to harbour lactose utilization ability (Lac^+) and Ads^+ mechanism for Øml41, Øml80 and Ømlc7 and a 38.0 kb plasmid was found to mediate an Phi^+ mechanism for Ømld22. Conjugal transfer of the 43.2 kb plasmid was demonstrated in two matings between donor strain MLL76 and plasmid free recipient strains P81-1 and MLL76-111 with the frequencies of $6.3x10^{-5}$ and $1.5x10^{-4}$ per donor cell, respectively. But the 38.0 kb plasmid could not be transferred recipient strains via conjugation.

KEYWORDS

Lactococcus lactis subsp. *lactis*, bacteriophage resistance

1. INTRODUCTION

Dairy lactococci are essential to cheese manufacture and the early stages of cheese ripening (Nomura *et al.* 2000). *Lactococcus* species are susceptible to bacteriophage infections that may result in slowed or failed fermentations (Dinsmore and Klaenhammer, 1997). Worldwide, a number of strategies rely upon particular choices of starter strains in order to control bacteriophage. Traditionally, bacteriophage problems have been approached with varying degrees of success by isolation of bacteriophage resistant mutants, which are altered in their fermentative capacities or have short-lived bacteriophage resistance. More recently natural bacteriophage defenses have characterized in prototype bacteriophage-resistant bioprocessing strains and used in starter culture formulations (Durmaz and

Klaenhammer,1995; Djordevic and Klaenhammer, 1997). A great deal of research on lactococci has been focused on identification and characterization of mechanisms that mediate bacteriophage resistance. Detailed characterization should ultimately allow rational construction of strains that exhibit levels of bacteriophage resistance. Naturally occuring bacteriophage resistance mechanisms of lactococci have included abortive infection (Jarvis, 1993; Emond *et al.* 1997), host-controlled restriction and modification (Moineau *et al.* 1993; Su *et al.* 1999), bacteriophage adsorption inhibition (Akçelik and Tunail, 1992) and prevention of bacteriophage DNA penetration (Akçelik *et al.* 2000; Garvey *et al.* 1996). Genetic studies have shown that in lactococci determinants for bacteriophage resistance are frequently encoded by plasmid-located genes, which has proved to be advantageous in term of their characterization and for conjugal transfer to other strains. As these are naturally occuring lactococcal plasmids with GRAS (generally recognized as safe) status, their introduction in to other lactococcal hosts by conjugation does not require regulatory clearance (Forde *et al.* 1999)

The emphasis of this research was on the identification of novel lactococcal bacteriophage resistance plasmids in *L. lactis* subsp. *lactis* MLL76. Additionally, conjugal transfer abilities of bacteriophage resistance plasmids were investigated.

2. MATERIALS AND METHODS

Bacterial strains and bacteriophages

Table 1 lists strains and bacteriophages used in this study. Lactococcal cultures were grown in M17 medium (Terzaghi and Sandine,1975) in which glucose was replaced with lactose when necessary (GM17 medium). The streptomycin (Str), and kanamycin (Km) were added into medium at 200μg/ml and 70 μg/ml, respectively. Bacteriophage and culture stocks were stored in broth containing 40 % glycerol at –80° C.

Table 1. Bacterial strains and bacteriophages used in this study

Strain / bacteriophage	Relevant Characteristics[a]	Plasmid content(Kb)[b]	Source / reference
Strain			
L. lactis* subsp. *lactis			
MLL76	Lac^+, Ads^+, Phi^+, Str^s, Km^s Donor, wild type strain	43.2 38.0, 29.6, 17.5, 6.8	This study
MLL76-12	Lac^-, Ads^-, Phi^+, Str^s, Km^s Plasmid cured mutant of MLL76	38.0	This study
MLL76-72	Lac^-, Ads^-, Phi^-, Str^s, Km^s Plasmid cured mutant of MLL76	29.6	This study
MLL76-90	Lac^-, Ads^-, Phi^-, Str^s, Km^s Plasmid cured mutant of MLL76	17.5, 6.8	This study
MLL76-103	Lac^+, Ads^+, Phi^-, Str^s, Km^s Plasmid cured mutant of MLL76	43.2	This study
MLL76-111	Lac^-, Ads^-, Phi^-, Str^s, Km^s Plasmid free mutant of MLL76	-	This study
P81-1	Lac^-, Ads^-, Phi^-, Str^r, Km^r Plasmid free mutant of P81	-	Akçelik and Tunail,1992
P81-C10	Lac^+, Ads^+, Phi^-, Str^r, Km^r Transconjugant from mating MLL76 x P81-1	43.2	This study
MLLT111-T17	Lac^+, Ads^+, Phi^-, Str^s, Km^s Transconjugant from mating MLL76 x MLL76-111		This study
MLL41	Homologous host of Øml41		AUZF[c]
MLL80	Homologous host of Øml80		AUZF
L. lactis* subsp.*cremoris			
MLC7	Homologous host of Ømlc7		AUZF
L. lactis* subsp. *lactis* biovar. d*iacetylactis			
MLD22	Homologous host of Ømld22		AUZF
Bacteriophages			
Øml41	Small isometric-headed		AUZF
Øml80	Prolate-headed		AUZF
Ømlc7	Large isometric-headed		AUZF
Ømld22	Large isometric-headed		AUZF

[a] Lac^+, Metabolizes lactose; Lac^-, does not metabolize lactose; Ads^+, bacteriophage adsorption inhibition positive; Ads^-, bacteriophage adsorption inhibition negative; Phi^+, bacteriophage DNA injection blocking positive; Phi^-, bacteriophage DNA injection blocking negative; Str^r, streptomycin resistance; Str^s, streptomycin sensitivity; Km^r, kanamycin resistance; Km^s, kanamycin sensitivity

[b] kb, kilobase

[c] AUZF, culture collection of Faculty of Agriculture, Ankara University, Ankara/Turkey

Plasmid and bacteriophage methodology

Plasmid cured mutants were obtained using the protoplast-induced curing method described by Gasson (1983). Lactose positive (Lac^+) and lactose negative (Lac^-) mutants were detected on bromocresol purple lactose indicator agar (McKay *et al.* 1972) and tested for susceptibility to bacteriophages by standard plaque assay (Terzaghi and Sandine,1975). The lysis procedure of Anderson and McKay (1983) was used to isolate plasmid DNA from lactococcal strains. Purification of plasmids in cesium chloride-ethidium bromide density gradients and analysis on agarose gels were done as described previously (Macrina *et al.* 1982). Efficiency of plaquing (EOP) and adsorption assays were performed as described by Sanders and Klaenhammer (1983). Cell survival was assayed by the method of Behnke and McKay (1978) with multiplicity infection of 3 one-step growth and center infection (COI) were performed as described by Moineau *et al.* (1993). The efficiency at which COI formed (ECOI) was obtained by dividing the number of COI from resistant strain by the number of COI from sensitive strain. The burst size was determined as the titer of phage at time 120 min divided by the titer of infecting centers at time 15 min. The latent period was estimated at the midpoint of the exponential phase of one-step growth curve (Behnke and McKay, 1978). The intracellular replication of bacteriophage DNA was also followed at time intervals after infection by the procedure of Hill *et al.* (1991). The production of major capsid proteins of bacteriophages (MCPs) was monitored at time intervals after phage infection with monoclonal antibodies and a sandwich enzyme-linked immunosorbent assay (ELISA) as described previously (Moineau *et al.* 1993). Bacteriophage DNA was isolated by the method of Fitzgerald *et al.* (1982). Electroporation of lactococcal strains was executed according to the procedure of Holo and Nes (1989) with a gene pulser apparatus (Bio-Rad Corp., Richmond, CA, USA).

Conjugal matings

Filter matings were conducted as described by McKay *et al.* (1980). Donor and recipient cells (1,5 mL each) were mixed and collected on sterile membrane filters (pore size 0.45 μm; Sartorius, Goettingen, Germany). Filters were then transferred to M17-glucose agar plates and incubated at 30° C for 24 h. After incubation, the filters were removed into sterile flasks and rinsed with 1.0 mL 0.85 % NaCl to obtain a cell suspension. After that, cells were plated on bromocresol purple lactose indicator agar (McKay *et al.* 1972) supplemented with streptomycin (200 μg/mL) and kanamycin (70 μg/mL) to select Lac^+, Str^r, Km^r recombinants.

3. RESULTS AND DISCUSSION

Bacteriophage resistance mechanisms of *L. lactis* subsp. *lactis* MLL76 were determined by using phages Øml141, Øml180, Ømlc7 and Ømld22 which had been propagated on their homologous hosts; *L. lactis* subsp. *lactis* MLL41 and MLL80, *L. lactis* subsp. *cremoris* MLC7 and *L. lactis* subsp. *lactis* biovar. *diacetylactis* MLD22. Adsorption assays showed that the Ømld22 adsorbed to MLL76 at a rate of 98.8 % whereas adsorptions of Øml141, Øml180 and Ømlc7 were completely inhibited. In spite of high adsorption capacity of Ømld22 to MLL76, no bacteriophage DNA replication, major capsid protein (MCP) production and plaque formation was observed on MLL76 (Table 2), ruling out adsorption inhibition, restriction/modification and abortive infection type resistance mechanisms. After electrotransformation of Ømld22 DNA into MLL76, which permits the bacteriophage to bypass adsorption and DNA injection stages, Ømld22 DNA replication could be determined at 20 min followed by a decrease 35 min. Major capsid protein (MCP) production, latent period, burst size and efficiency of center of infection (ECOI) of Ømld22 were found to be 100 %, 55 min, 65 and 1.0 (Table 3), the same as those obtained on its homologous host (data not shown). Based on current classification of bacteriophage resistance systems in lactococci (de Vos *et al.* 1984; Hill, 1993; Garvey *et al.* 1996; Deng *et al.* 1999; Akçelik and Şanlıbaba, 2000) and the results described above, the resistance mechanisms of *L. lactis* subsp. *lactis* MPL76 were classified as adsorption inhibition (Ads^+) for Øml141, Øml180 and Ømlc7 and DNA injection blocking (Phi^+) for Ømld22.

The relationship between plasmids and bacteriophage resistance mechanisms in MLL76 were established following plasmid curing experiments. Plasmid profiles of MLL76 and its bacteriophage sensitive ($Ø^s$) and resistant ($Ø^r$) mutants were examined. Wild type strain MLL76 harboured plasmids of 43.2, 38.0, 29.6, 17.5 and 6.8 kb (Figure 1). Plasmid free mutant MLL76-111, only the 29.6 kb plasmid carrying mutant MPL76-72 and two plasmids (17.5 and 6.8 kb) carrying mutant MLL76-90 were found to be sensitive to all bacteriophages. Two different mutants showed resistant phenotype. The 43.2 kb plasmid carrying lactose positive (Lac^+) mutant MLL76-103 completely inhibited adsorption of Øml141, Øml180 and Ømlc7 but became sensitive to Ømld22. On the other hand, only the 38.0 kb plasmid carrying lactose negative (Lac^-) mutant MLL76-12 showed full resistance to Ømld22 but it was sensitive against Øml141, Øml180 and Ømlc7 (Figure 1 and Table 2). These results strongly suggested that the 43.2 kb plasmid encoded adsorption inhibition of Øml141, Øml180 and Ømlc7 and the 38.0 kb plasmid encoded blocking of DNA injection of Ømld22 in *L. lactis* subsp. *lactis* MLL76.

Bacteriophage proliferation parameters on sensitive hosts (Table 2) were the same as those obtained on their homologous hosts (data not shown) indicating that there is no additional resistance mechanisms in strain MPL76 for Øml141, Øml180, Ømcl7 and Ømld22.

Table 2. Bacteriophage proliferation parameters of *L. lactis* subsp. *lactis* strains

Bacteriophages And assays	Values for MLL 76	MLL 76-12	MLL 76-72	MLL 76-90	MLL 76-103	MLL 76-111	P81-1
Øml141							
Adsorption (%)[a]	-	94.6	94.2	94.6	-	94.6	98.0
ECOI[b]	-	1.0	1.0	1.0	-	1.0	1.0
Burst size[a]	-	85	85	85	-	85	85
Latent period(min)[a]	-	50	50	50	-	50	50
DNA replication[c]	-	+++	+++	+++	-	+++	+++
MCP production[a]	-	100	100	100	-	100	100
Øml180							
Adsorption (%)	-	99.2	99.2	99.4	-	99.0	96.6
ECOI	-	1.0	1.0	1.0	-	1.0	1.0
Burst size	-	60	60	60	-	60	60
Latent period(min)	-	55	55	55	-	55	55
DNA replication	-	+++	+++	+++	-	+++	+++
MCP production	-	100	100	100	-	100	100
Ømlc7							
Adsorption (%)	-	96.4	96.6	96.6	-	96.6	96.2
ECOI	-	1.0	1.0	1.0	-	1.0	1.0
Burst size		98	98	98	-	98	98
Latent period(min)	-	45	45	45	-	45	45
DNA replication	-	+++	+++	+++	-	+++	+++
MCP production	-	100	100	100	-	100	100
Ømld22							
Adsorption (%)	98.8	98.6	98.8	98.8	98.8	98.4	98.6
ECOI	-	-	1.0	1.0	1.0	1.0	1.0
Burst size	-	-	65	65	65	65	65
Latent period(min)	-	-	55	55	55	55	55
DNA replication	-	-	+++	+++	+++	+++	+++
MCP production	-	-	100	100	100	100	100

[a] Average of five trials, [b] average of ten trials, [c] +++, strong

Table 3. Bacteriophage Ømld22 proliferation parameters of *L. lactis* subsp. *lactis* MLL76 after electroporation of Ømld22 DNA

Bacteriophage and assays	Values for MLL76
Ømld22	
ECOI[a]	1.0
Latent Period (min)[b]	55
Burst Size[b]	65
DNA replication[c]	+++
MCP production[b]	100

[a] Average of ten trials, [b]Average of five trials, [c] +++, strong

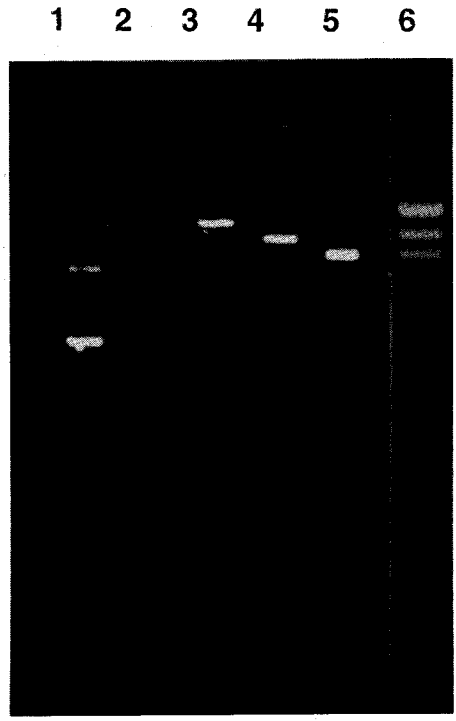

Figure 1. Plasmid profiles of *L. lactis* subsp. *lactis* MLL76 and its Ads^+ (lane 3), Phi^+ (lane 4) and Ads^-/Phi^- (lanes 1,2 and 5) mutants

Lanes; 1·(MLL76-90): 17.5 and 6.8 kb, 2 (MLL76-111): plasmid free mutant, 3 (MLL76-103):43.2 kb, 4 (MLL76-12): 38.0 kb, 5 (MLL76-72): 29.6 kb; 6 (MLL76): 43.2, 38.0, 29.6, 17.5 and 6.8 kb.

The ability of *L. lactis* subsp. *lactis* MLL76 to transfer bacteriophage insensitivity markers in filter matings was assessed. Using *L. lactis* subsp. *lactis* P81-1 and MLL76-111 as recipients , transfer of lactose fermenting ability were observed at the frequencies of $6.3x10^{-5}$ and $1.5x10^{-4}$, respectively. All Lac^+ transconjugants were found to be insensitive to Ømll41, Ømll80 and Ømcl7 but not to Ømld22 (Table 4). To determine the conjugal transfer ability of the 38.0 kb plasmid, encoding DNA injection blocking of Ømld22, whether independent from lactose transfer, a number of Lac^- colonies in mating plates were examined. All Lac^- colonies selected on mating plates, were insensitive against Ømld22 (data not shown) indicating that the 38.0 kb plasmid had no conjugal transfer functions. Plasmid profiles showed that all transconjugants harboured only the 43.2 kb plasmid (Table 4).

Table 4. Conjugal transfer of lactose fermentation ability and bacteriophage adsorption inhibition type resistance from *L. lactis* subsp. *lactis* MLL76 to plasmid-free *L. lactis* subsp. *lactis* recipients

Donor	Recipient	Representative transconjugant	Plasmid content (kb)[a]	Phenotypic characters[b]	Frequency of conjugation (Per donor cell)
MLL76	P81-1	P81-C10	43.2	Lac^+, Ads^+, Phi^-, Str^r, Km^r	$6.3x10^{-5}$
MLL76	MLL76-111	MLLT111-T17	43.2	Lac^+, Ads^+, Phi^-, Str^r, Km^r	$1.5x10^{-4}$

[a] kb, kilobase

[b] Lac^+, Metabolizes lactose; Ads^+, adsorption inhibition positive for Ømll41, Ømll80 and Ømlc7 Phi^-, DNA injection blocking negative for Ømld22; Str^r, streptomycin resistance, Km^r, kanamycin resistance

Many strains that are naturally resistant to bacteriophage contain two to three different bacteriophage resistance mechanisms either on the same plasmid or on seperate plasmids (McLandsborough *et al.* 1998). In some cases a single resistance mechanism may manifest itself phenotypically as adsorption inhibition in one strain, but as DNA injection blocking in other bacteriophage-host combination (Harrington and Hill, 1992; Hill, 1993). But, bacteriophage adsorption inhibition and bacteriophage DNA injection blocking type resistance mechanisms, determined in this study, are supposed to be independent since they were encoded by different plasmids in *L. lactis* subsp. *lactis* MLL76. It has long been recognized that the conjugative properties of many bacteriophage resistance plasmids can be exploited to develop cheese starter cultures for dairy industry with improved bacteriophage resistance (Coakley *et al.* 1997; O'Sullivan *et al.* 1998). The transfer abilities associated with the 43.2 kb plasmid may be particularly useful for dissemination of bacteriophage adsorption inhibition type resistance mechanism in lactococci. If transconjugant strains are to be used in dairy fermentations, it will be necessary to select them without the aid of antibiotic markers.

Because of the powerful antibacteriophage characters of adsorption inhibition and DNA injection blocking type resistance mechanisms in lactococci, attempts are presently being made to isolation and DNA sequence analysis of corresponding genes and characterization of these gene products.

REFERENCES

[1] Akçelik, M., and N. Tunail.1992. A 30 kd cell wall protein produced by plasmid DNA which encodes inhibition of phage adsorption in *Lactococcus lactis* subsp. *lactis* p25. Milchwissenschaft, 47:215-217.

[2] Akçelik, M., and P. Şanlıbaba. 2000. *Lactococcus* genusuna ait starter kültür suşlarında faj dirençlilik sistemleri. Gıda, 25:25-31.

[3] Akçelik, M., P. Şanlıbaba, and Ç. Tükel. 2000. Phage resistance in *L. lactis* subsp. *lactis* isolated from traditional fermented milk products in Turkey. Int. J. Food Sci. Technol. 35:473-481.

[4] Anderson, D.G., and L.L. McKay. 1983. Simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl. Environ. Microbiol. 46:549-552.

[5] Behnke, D., and L.L. McKay. 1978. Bacteriophage interference in *Streptococcus pyogens*. 1. Characterization of prophage-host systems interfering with the virulent phage A25. Virology, 85:118-128.

[6] Coakley, M., G.F. Fitzgerald, and R.P. Ross. 1977. Application and evaluation of phage resistance- and bacteriocin-encoding plasmid pMRC01 for the improvement of dairy starter cultures. Appl. Environ. Microbiol. 63:1434-1440.

[7] Deng, Y., C. Liu, and N.W. Dunn. 1999. Genetic organization and functional analysis of a novel phage abortive system, AbiL, from *Lactococcus lactis*. J. Biotechnol. 67:135-149.

[8] de Vos, W.M., H.M. Underwood, and F.L. Davies.1984. Plasmid encoded bacteriophage resistance in *Streptococcus cremoris* SK11. FEMS Microbiol. Lett. 23:175-178.

[9] Dinsmore, P.K., and T.R. Klaenhammer, 1977. Bacteriophage resistance in *Lactococcus*. Mol. Biotechnol. 4:297-314.

[10] Djordevic, G.M., and T.R. Klaenhammer. 1977.Bacteriophage tiggered defense systems: phage adaptation and design improvements. Appl. Environ. Microbiol. 63:4370-4376.

[11] Durmaz, E., and T.R. Klaenhammer. 1995. A starter rotation strategy incorporating paired restriction/modification and abortive infection bacteriophage defenses in a single *Lactococcus lactis* strain. Appl. Environ. Microbiol. 61:1266-1273.

[12] Emond, E.B., B.J. Hollier, I. Boucher, P.A. Vandenbergh, E.R. Vedamutlu, J.K. Kondo, and S. Moineau. 1977. Phenotypic and genetic characterization of the bacteriophage abortive infection mechanism AbiK from *Lactococcus lactis*. Appl. Environ. Microbiol. 63:1274-1283.

[13] Fitzgerald, G.F., C. Daly, L.R. Brown, and T.R. Gingeras.1982. ScrFI: a new sequence specific endonuclease from *Streptococcus cremoris*. Nucleic Acid Res.10:8171-8178.

[14] Forde, A., C. Daly, and G.F. Fitzgerald. 1999. Identification of four phage resistance plasmids from *Lactococcus lactis* subsp. *cremoris* H02. Appl. Environ. Microbiol. 65:1540-1547.
[15] Garvey, P., C. Hill, and G.F. Fitzgerald. 1996. The lactococcal plasmid pNP40 encodes a third bacteriophage resistance mechanism, one which affect phage DNA penetration. Appl. Environ. Microbiol. 62:676-679.
[16] Gasson, M.J. 1983. Plasmid complements of *Streptococcus lactis* NCDO712 and other lactic streptococci after protoplast induced plasmid curing. J. Bacteriol.154:1-9.
[17] Harrington, A., and C. Hill. 1992. Plasmid involvement in the formation of a spontaneous bacteriophage insensitive mutant of *Lactococcus lactis*. FEMS Microbiol. Lett. 96:135-142.
[18] Hill, C., I.J. Massey, and T.R. Klaenhammer. 1991. Rapid method to characterize lactococcal bacteriophage genomes. Appl. Environ. Microbiol. 57:283-288.
[19] Hill, C. 1993. Bacteriophage and bacteriophage resistance in lactic acid bacteria. FEMS Microbiol. Rev. 12:87-108.
[20] Holo, H., and I.F. Nes.1989. High frequency transformation, by electroparation, of *Lactococcus lactis* subsp. *cremoris* grown with glycine in osmotically stabilized media. Appl. Environ. Microbiol. 55:3119-3123.
[21] Jarvis, A.W. 1993. Analysis of phage resistance mechanisms encoded by lactococcal plasmid pAJ2074. Can. J. Microbiol. 39:252-258.
[22] Macrina, F.L., J.A. Tobian, K.R. Jones, R.P. Evans, and D.B. Clewell. 1982. A cloning vector able to replicate *Escherichia coli* and *Streptococcus sangius*. Gene, 19:345-353.
[23] McKay, L.L., K.A. Baldwin, and E.A. Zottola. 1972. Loss of lactose metabolism in lactic streptococci. Appl. Environ. Microbiol. 23:1090-1096.
[24] McKay, L.L., K.A. Baldwin, and P.M. Walsh. 1980. Conjugal transfer of genetic information in group N streptococci. Appl. Environ. Microbiol. 40:81-84.
[25] McLandsborough, L.A., L. Sechaud, and L.L. McKay. 1998. Synergetic effect of *abiE* or *abiF* from pNP40 when cloned in combination with *abiD* from pBF61. J.Dairy Sci. 81:362-368.
[26] Moineau, S., S. Pandian, and T.R. Klaenhammer. 1993. Restriction/ modification systems and restriction endonucleases are more effective on lactococcal bacteriophage that have emerged recently in the dairy industry. Appl. Environ. Microbiol. 59:197-202.
[27] Nomura, M., M. Kobayashi, S. Ohmomo, and T. Okomato. 2000. Inactivation of the glutamate decarboxylase gene in *Lactococcus lactis* subsp. *cremoris* Appl. Environ. Microbiol. 66:2235-2237.
[28] O'Sullivan, D., A. Coffey, G.F. Fitzgerald, C. Hill, and P. Ross.1998. Design of a phage-insensitive dairy starter via sequential transfer of naturally occuring conjugative plasmids. Appl. Environ. Microbiol. 64:4618-4622.

[29] Sanders, M.E., and T.R. Klaenhammer. 1983. Characterization of phage-sensitive mutants from a phage-insensitive strain of *Streptococcus lactis*: evidence for a plasmid determinant that prevents phage adsorption. Appl. Environ. Microbiol. 46:1125-1133.

[30] Su, P., I.M. Heejdong, H. Hsieh, S. Kang'a, and N.W. Dunn. 1999. LlaFI, a type III restriction and modification system in *Lactococcus lactis*. Appl. Environ. Microbiol. 65:686-693.

[31] Terzaghi, B.E., and W.E. Sandine. 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. 29:807-813.