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Optimization of Electrotransformation Conditions to Improve Genetic Engineering Potential of *Lactobacillus* spp. Isolated from Gastrointestinal Tract of Chickens

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Abstract: The electrotransfromation conditions of *Lb. crispatus* I12, *Lb. brevis* I23, *Lb. fermentum* I25 and *Lb. acidophilus* I16C, which were isolated from the gastrointestinal tract of chickens, were studied. A series of experiments that involved manipulation of growth and recovery media, cell wall weakening agent, cell concentration, concentration of transforming plasmid, washing buffer and field strength were carried out. The effects of these parameters were found to vary among the *Lactobacillus* spp. However, under optimal conditions, the transformation efficiencies were improved from 5.4-66.7 transformants/μg DNA to 10^2-10^3 transformants/μg DNA. The *Lactobacillus* spp. were also amenable to transformation by various plasmids such as pGK12, pLP3537, pLP825 and pSA3. This indicated that these strains could be potential candidates for bioengineering to improve feed degradation and growth performance of chickens.

Key words: Chicken, electroporation, *Lactobacillus*, optimization, transformation

INTRODUCTION

In recent years, an increase in development of genetic expression system for industrial gram positive bacteria with low guanine and cytosine content that belong to the genera *Bacillus* and *Clostridium* has been observed. Food grade microorganisms having Generally Recognized As Safe (GRAS) status such as Lactic Acid Bacteria (LAB) is also regarded as a promising group of microorganism for genetic modification. The LAB which include *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Leuconostoc* and *Carnobacterium* have been used for millennia as fermenting agents for preservation of food and today, they are being widely used in manufacturing of fermented milk products, vegetables and meat. In addition, probiotics, which consists of LAB, have been reported to improve the gastrointestinal health of the host, be it human or animals (Nava *et al*., 2005; Senok *et al*., 2005).

Efforts to genetically engineered the LAB are mainly targeted at strain improvement to allow wider applications. Among them is to develop the LAB into vehicle for heterologous protein expression including oral vaccines and to bioengineer these strains to improve their probiotic functions (Reuter *et al*., 2003; Beasley *et al*., 2004). Various genetic engineering techniques have been applied to transform this group of microorganism. Gene transfer systems using conjugation and protoplast transformation have shown low efficiency and lack of reproducibility (Wei *et al*., 1995). Electroporation has been reported to be one of the easiest and efficient methods of genetic transformation for all microorganisms. The successful application of electroporation to streptococci by Harlander and McKay (1984) had initiated widespread interest in the possibility of bacterial electrotransformation in LAB. Since then, successful electrotransformation in lactobacilli, lactococci and other LAB have been reported (Scheirlinck *et al*., 1989; Mercenier, 1990: Gory *et al*., 2001). The procedures and conditions of electrotransformation were found to vary among the species and strains of LAB. For instance, Mason *et al*. (2005) found that the *Lb. salivarius* and *Lb. crispatus* were not electrocompetent when the electroporation protocols described by Thompson and Collins (1996) and Walker *et al*. (1996) for *Lb. plantarum* and *Lb. acidophilus* Group A1, respectively, were used. Nevertheless, these strains were electrotransformed after the electroporation conditions were optimized.

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Lactobacillus isolated from different sources was also found to have different electrocompetency. Beasly *et al.* (2004) observed that *Lb. crispatus* isolated from the poultry crop and ileum electrotransformed at a low frequency or not at all. Thus, it was recommended that several parameters such as growth phase, cell density, medium composition and electrical conditions should be optimized for each strain in order to ensure the success of the electroporation procedure (Trevors *et al.*, 1992).

Four *Lactobacillus* strains isolated from the gastrointestinal tract of chickens, namely *Lb. crispatus* I12, *Lb. brevis* I23, *Lb. fermentum* I25 and *Lb. acidophilus* I16C which exhibited probiotic properties such as moderate to good adherence ability, acid resistance and bile tolerance (Jin *et al.*, 1996; 1998a, b), are suitable candidates for genetic manipulation. These strains may act as vehicle for heterologous protein expression, especially cellulolytic enzymes, in monogastric animals such as poultry. Successful transformation and expression of the enzyme could assist the birds in feed degradation and improve their growth performance. Thus, in the present study, the optimum electrotransformation condition and amenability of four *Lactobacillus* strains to electrotransformation were studied.

MATERIALS AND METHODS

***Lactobacillus* strains:** The bacterial strains used in this study are *Lb. crispatus* I12, *Lb. brevis* I23, *Lb. fermentum* I25 and *L. acidophilus* I16C. The strains were subcultured three times before use in Man Rogosa Sharpe broth (Oxoid, Unipath Ltd., Basingstoke, UK) at 39°C for 18 h.

Plasmid DNA: The plasmids used are listed in Table 1. These plasmids were maintained in *E. coli JM109* (recA1, endA1, gyrA96, thi, hsdR17, supE44, relA1, D(lac-proAB)/ F'[traD36, proAB+, lacIq, lacZ DM15]) grown at 37°C with agitation in Luria-Bertani medium (Luria *et al.*, 1960) supplemented with 10 μg mL^{-1} of chloromphenicol or erythromycin. The plasmids were extracted from *E. coli* JM109 using the Plasmid Midi Kit according to the protocol supplied by the manufacturer (Qiagen, Germany). The concentration and purity of the plasmid DNA preparations were determined spectrophotometrically (Maniatis *et al.*, 1982). Of these plasmids, plasmid pSA3 was used to optimize the electroporation procedure.

Preparation of electrocompetent cells: A stationary phase (16-18 h) culture of the recipient *Lactobacillus* strain was inoculated (1 [v/v] inoculum) into 100 mL MRS broth (containing 0, 2, 4, 6, 8 and 10% glycine) and incubated at 39°C under anaerobic condition. The cells were harvested at early exponential phase (OD_{600} 0.2-0.3), mid exponential phase (OD_{600} 0.4-0.5) and late exponential phase (OD_{600} 0.7-0.9) by centrifugation at 3,000×g for 10 min at 4°C and washed twice with an equal culture volume of ice-cold sucrose-magnesium chloride electroporation buffer (SMEB) (272 mM sucrose, 1 mM $MgCl_2$, 7 mM sodium phosphate, pH 7.4) or Sucrose Electroporation Buffer (SEB) (272 mM sucrose, pH 7.4). The cells were resuspended in the original culture volume or concentrated 0.5-, 10- or 100-fold of original culture volume in ice-cold SMEB buffer. The electrocompetent cells were then divided into 100 μL aliquots, kept on ice and were used for electroporation within 30 min.

Electroporation: Plasmid DNA (0.1, 0.5, 1.0 and 2.0 μg mL^{-1} dissolved in 10 mM Tris HCl, 1 mM EDTA, pH 8.0) was mixed with 80 μL of ice-cold bacterial suspension. The suspension was transferred into a pre-cooled Gene Pulser™ disposable cuvette (inter-electrode distance 0.2 cm; Bio-Rad Laboratories, Richmond, CA). A single electric pulse was delivered by a Gene Pulser™ (Bio-Rad Laboratories, Richmond, CA) set at 25 μF. The cuvette was connected parallel to a 200 Ω resistor (pulse controller; Bio-Rad) generating a peak field strength of 2.5, 5.0, 7.5, 10.0 or 12.5 kV cm^{-1}. Immediately following the discharge, the suspension was diluted with 900 μL of recovery medium (MRS broth with or without 0.3 M sucrose). The bacteria were incubated for about 2.5 h at 39°C to allow recovery and expression of antibiotic resistance marker and then plated on MRS agar supplemented with 12.5 μg mL^{-1} erythromycin. Transformants were enumerated after 2 days of anaerobic incubation at 39°C. The transformation efficiencies were expressed as transformants per microgram of plasmid DNA. Control experiments were performed by plating

Table 1: Designations and characteristics of plasmids used in this study

Plasmid	Description	Size (kb)	Reference
pGK12	Cm^r, Em^r, broad host range cloning vector	4.4	Kok *et al.* (1984)pLP3537
pIP 3537	Em^r, *E. coli-Lactobacillus* shuttle vector	6.3	Posno *et al.* (1991a)
pLP825	Cm^r, *L. plantarum* origin of replication	7.6	Posno *et al.* (1991a)
pNCKH104	Em^r, *E. coli – L. reuteri* shuttle vector	5.7	Heng *et al.* (1997)
pSA3	Cm^r, Em^r, Tet^r, *E. coli - Streptococcus* shuttle vector	10.2	Dao and Ferretti (1985)

Cm^r = Chloromphenicol resistant; Em^r = Erythromycin resistant; Tet^r = Tetracycline resistant

cells, which had received no electrical pulse or which were electroporated without plasmid DNA, on media without antibiotic.

Electroporation of *Lactobacillus* strains with various plasmids: The *Lactobacillus* strains were electroporated at optimized conditions with the plasmids listed in Table 1. The electroporated strains were plated on MRS agar supplemented with appropriate antibiotics for selection of transformants.

Statistical analysis: For each parameter studied, three transformation assays in triplicate were performed. The data were analyzed using the General Linear Model (GLM) procedure for analysis of variance (SAS Institute, 1997). Significant differences were separated by the Duncan's New Multiple Range Test (Duncan, 1995) at 5% level of probability.

RESULTS

Higher transformation efficiency were observed for all strains when mid- and late-exponential phase (OD_{600} 0.4-0.9) cells were used. The transformation efficiency of cells harvested at early exponential (OD_{600} 0.2-0.3) (5.4-11.7 transformants/μg DNA) and stationary phase (8.3-9.9% transformants/μg DNA) were approximately 82-92 lower than those observed in mid and late exponential phase cells (46.6-62.3 transformants/μg DNA). The transformation efficiencies were improved when glycine was included in the growth medium at a concentration of 6% for *Lb. crispatus* I12, *Lb. brevis* I23 and *Lb. acidophilus* I16C and 8% for *Lb. fermentum* I25. Inclusion of glycine at a concentration higher than 6 or 8% significantly ($p<0.05$) reduced the number of transformants in the corresponding strains. Inclusion of sucrose into MRS broth containing 6 or 8 glycine also enhanced their transformation efficiencies. The transformation efficiencies increased by 2.7-, 1.3-, 1.7- and 2.0-fold for *Lb. crispatus* I12, *Lb. brevis* I23, *Lb. fermentum* I25 and *Lb. acidophilus* I16°C, respectively.

Modification of recovery medium, which was used during recovery and phenotypic expression of electroporated cells, was found to affect the transformation efficiency of *Lactobacillus* strains. Significantly ($p<0.05$) higher number of transformants was obtained for all the strains when the porated cells were recovered in MRS broth containing 0.3 M sucrose. The transformation efficiencies were generally 1.1- to 1.3-fold higher than the rate obtained when the recovery medium was not supplemented with sucrose.

The transformation efficiencies were not significantly ($p>0.05$) different for all the strains when the cells were concentrated 1- or 10-fold of the original culture volume. However, cells concentrated to 0.5-fold and 100-fold resulted in 34-47% and 23-36% reduction, respectively. Transformation efficiencies were significantly ($p<0.05$) higher when *Lb. crispatus* I12, *Lb. brevis* I23 and *Lb. acidophilus* I16C were washed and electroporated in buffer containing $MgCl_2$ (SMEB). Nevertheless, the transformation efficiency of *L. fermentum* I25 was not significantly ($p>0.05$) affected by the types of buffer used.

The number of transformants recovered for *Lb. crispatus* I12, *Lb. brevis* I23 and *Lb. fermentum* I25 increased significantly ($p<0.05$) with increasing plasmid concentrations till the concentration of 1 μg mL^{-1} DNA, after which the increase was not significant. For *Lb. acidophilus* I16C, the number of transformants recovered did not increase further when more than 2 μg mL^{-1} DNA was used.

Lb. crispatus I12 and *Lb. acidophilus* I16C were transformed with the highest efficiency at 12.5 kV cm^{-1}, which was the maximum field strength generated by the apparatus (Table 2). However, high field strength could also result in reduced transformation efficiency. The transformation efficiency of *Lb. brevis* I23 was reduced by 62 and 89% when the field strength was increased from 7.5 to 10 kV cm^{-1} and from 7.5 to 12 kV cm^{-1}, respectively. Similarly, the transformation efficiency of *Lb. fermentum* I25 was reduced by 41% when the field strength was increased from 10.0 to 12.5 kV cm^{-1}. It was also noted that the pulse duration (time constant) was comparatively longer at lower field strengths than at higher field strengths.

Using the optimized electroporation procedure, several plasmids (Table 1) from various sources were electroporated into the *Lactobacillus* strains. All the plasmids, except for pNCKH104, transformed the *Lactobacillus* strains at an efficiency of 10^3-10^4 transformants/μg DNA, regardless of plasmid size which ranged from 4.4-10.2 kb (Table 3). Plasmid pNCKH104 was transformed at a very low frequency (a few or no transformant per μg DNA) in all the strains and the results were not reproducible. All the transformants were selected based on chloromphenicol or erythromycin resistance, depending on the type of plasmid and the *Lactobacillus* strain used. Plasmid such as pGK12 which allowed dual selection (contained chloromphenicol and erythromycin resistant genes) in certain *Lactobacillus* strains (*L. crispatus* I12,

Table 2: Effects of field strengths on the electrotransformation of *Lactobacillus* strains with pSA3

Strain	Field strength (kV cm^{-1})	Time constant(ms)	Transformation efficiency* (Transformants/μg DNA)
L. crispatus I12	2.5	35.1±0.9^{a}	$(3.0\times10^{2})\pm28^{a}$
	5.0	25.8±0.9^{b}	$(1.6\times10^{3})\pm52^{a}$
	7.5	16.8±0.6^{c}	$(2.6\times10^{3})\pm68^{a}$
	10.0	12.9±0.3^{d}	$(2.6\times10^{4})\pm1344^{b}$
	12.5	5.4±0.3^{e}	$(6.1\times10^{4})\pm5168^{c}$
L. brevis I23	2.5	40.4±0.6^{f}	$(1.8\times10^{2})\pm18^{a}$
	5.0	29.6±0.7^{g}	$(1.2\times10^{3})\pm63^{a}$
	7.5	20.7±0.5^{h}	$(2.3\times10^{3})\pm81^{a}$
	10.0	12.3±0.3^{d}	$(8.8\times10^{2})\pm57^{a}$
	12.5	6.7±0.3^{e}	$(2.5\times10^{2})\pm21^{a}$
L. fermentum I25	2.5	42.7±1.2^{i}	$(6.0\times10^{2})\pm30^{a}$
	5.0	22.8±0.6^{j}	$(1.3\times10^{3})\pm83^{a}$
	7.5	16.1±0.3^{c}	$(4.0\times10^{3})\pm101^{a}$
	10.0	10.4±0.6^{k}	$(1.3\times10^{4})\pm587^{d}$
	12.5	5.1±0.3^{e}	$(7.6\times10^{3})\pm381^{e}$
L. acidophilus I16C	2.5	35.2±0.8^{a}	$(1.4\times10^{2})\pm15^{a}$
	5.0	27.9±0.5^{g}	$(1.5\times10^{3})\pm64^{a}$
	7.5	23.9±1.0^{j}	$(2.6\times10^{3})\pm76^{a}$
	10.0	16.4±0.6^{c}	$(1.9\times10^{4})\pm1108^{f}$
	12.5	5.1±0.3^{e}	$(3.4\times10^{4})\pm81^{g}$

*Values represent means±SE (n = 9), $^{a-f}$Means with different superscripts in the same column are significantly different ($p<0.05$)

Table 3: Transformation efficiencies of various plasmids in *Lactobacillus* strains

Strain	Plasmid	Size (kb)	Transformation efficiency* (Transformants/μg DNA)
L. crispatus I12	pGK12	4.4	$(4.4\times10^{4})\pm935^{a}$
	pGK12#	4.4	$(6.4\times10^{4})\pm1231^{b}$
	pLP3537	6.3	$(3.6\times10^{4})\pm1112^{c}$
	pLP825#	7.6	$(7.4\times10^{4})\pm853^{d}$
	pNCKH104	5.7	0^{e}
	pSA3	10.2	$(6.4\times10^{4})\pm951^{b}$
L.brevis I23	pGK12	4.4	$(1.7\times10^{3})\pm151^{f}$
	pGK12#	4.4	$(4.3\times10^{3})\pm151^{gmt}$
	pLP3537	6.3	$(2.4\times10^{3})\pm89^{f}$
	pLP825#	7.6	$(2.7\times10^{3})\pm96^{f}$
	pNCKH104	5.7	0^{e}
	pSA3	10.2	$(2.6\times10^{3})\pm102^{ft}$
L. fermentum I25	pGK12	4.4	$(1.4\times10^{4})\pm559^{j}$
	pGK12#	4.4	$(1.8\times10^{4})\pm641^{lp}$
	pLP3537	6.3	$(1.1\times10^{4})\pm452^{k}$
	pLP825#	7.6	$(1.6\times10^{4})\pm647^{lq}$
	pNCKH104	5.7	0^{e}
	pSA3	10.2	$(1.2\times10^{4})\pm584^{k}$
L. acidophilus I16C	pGK12	4.4	$(2.7\times10^{4})\pm1075^{n}$
	pGK12#	4.4	ND
	pLP3537	6.3	$(4.2\times10^{4})\pm556^{a}$
	pLP825#	7.6	ND
	pNCKH104	5.7	0^{e}
	pSA3	10.2	$(3.4\times10^{4})\pm797^{c}$

* Values represent means±SE (n = 9). $^{a-t}$Means with different superscripts in the same column are significantly different ($p<0.05$). # Selection by chloromphenicol resistance; ND = not determined due to resistance to selective antibiotic.

L. brevis I23 and *L. fermentum* I25) showed that the number of transformants selected through chloromphenicol resistance were significantly ($p<0.05$) higher than those obtained through selection by erythromycin resistance.

DISCUSSION

An important prerequisite for applying genetic techniques for improvement of the industrially important lactic acid bacteria is an efficient and reliable high-frequency transformation system. Although electroporation offers a relatively simple, rapid and reliable alternative to conventional transformation procedures, a number of electrical and biological parameters must be optimized for each strain to be porated, so as to ascertain efficient transformation and to allow the application of other genetic manipulation techniques such as chromosomal integration (McIntyre and Harlander, 1989; Klaenhammer, 1995).

As shown in the present study, the transformation efficiencies of *Lactobacillus* strains increased significantly after various electroporation parameters were optimized. It was observed that *Lactobacillus* strains grown to mid or late exponential phases were most suitable to be used for the procedure. Inclusion of 6% (w/v) or 8% (w/v) glycine into the growth medium could improve the transformation efficiency of *Lb. crispatus* I12, *Lb. brevis* I23 and *Lb. acidophilus* I16C; and *Lb. fermentum* I25, respectively. The cells could be concentrated 1 or 10-fold and higher transformation efficiencies could be obtained for *Lb. crispatus* I12, *Lb. brevis* I23 and *Lb. acidophilus* I16C if the strains were washed with SMEB buffer. Addition of 0.3 M of sucrose to the recovery medium is also an advantage to the cells. The highest transformation efficiency was obtained for *Lb. crispatus* I12, *Lb. brevis* I23 and *Lb. fermentum* I25 when 1 μg mL^{-1} DNA was used. *Lb. acidophilus* I16°C required 2 μg mL^{-1} DNA to obtained highest transformation efficiency. High field strength was found to have adverse effect on some strains. The transformation efficiencies of *Lb. brevis* I23 and *Lb. fermentum* I25 were significantly reduced when field strength above 7.5 and 10 kV cm^{-1} were used, respectively. Nevertheless, highest transformation efficiencies were observed for *Lb. crispatus* I12 and *Lb. acidophilus* I16C when a field strength of 12.5 kV cm^{-1} was used.

The growth phase of cells has been suggested as one of the factors which determined electroporation efficiency. It not only determines the electrocompetency of the cells, but also the survivability of the cells after exposure to electrical pulse (Szostkova *et al*., 1999). Previous studies have indicated that the addition of cell-wall-weakening agent coupled with sucrose into the growth medium enhances the transformation efficiency. Lytic enzymes and other cell-wall-weakening agents such as penicillin, DL-threonine and glycine are primarily used to gently inhibit synthesis and assembly of the cell wall of gram positive bacteria (Buckley *et al*., 1999). For gram positive bacteria such as lactobacilli, the structure and composition of the cell wall is considered to be a major hindrance to successful transformation (Bonnassie *et al*., 1990). Holo and Nes (1989) suggested that the use of cell-wall-weakening agents is better than the use of lytic enzymes as the former ensures the viability of competent cells. Among the cell-wall-weakening agents, glycine is the most commonly used although DL-threonine and penicillin produce similar effects (Posno *et al*., 1991b). The concentration of cell-wall-weakening agent to be included in the growth medium has been reported to be strain dependent. Aymerich *et al*. (1993) reported that the electrotransformation of *Lb. plantarum*, isolated from dry fermented sausages, was enhanced by the addition of 3% (w/v) glycine to the growth medium and Thompson and Collins (1996) observed that the optimum concentration of glycine in the growth medium for the transformation of *Lb. plantarum,* isolated from ensiled vegetable material, was 8%.

It was reported that above the optimum levels, progressive weakening of the cell wall and the consequent osmotic fragility of the cells occur, leading to leakage of cytoplasmic components (Well *et al*., 1993). With the exposure of the vulnerable cells to high electrical pulse, the damage could be irreversible and thus lowers the survivability and transformation efficiencies of the cells. In order to improve the efficiency of transformation, a balance must be achieved between cell membrane accessibility and cell wall integrity (Powell *et al*., 1988). Addition of sucrose into the recovery medium could also prevent lysis of cells rendered electropermeable to plasmid DNA by the glycine pre-treatment (Thompson and Collins, 1996).

An appropriate cell density is required to obtain high transformation efficiency. Cell densities higher than the optimum requirement would alter the threshold pulse intensity or duration required by individual cells for electroporation. This would then deprive the population from experiencing the full effect of the pulse (Luchansky *et al*., 1989). At an appropriate cell density, the cells were not severely damaged by the electrical pulse and the interaction of cells and plasmid DNAs to be transformed was enhanced. Thus, a higher number of transformants was obtained. Thompson and Collins (1996) reported that the effect of cell density on transformation efficiency was more pronounced at low cell densities. They observed that there was no change in the number of transformants when the density of cells to be electroporated exceeded 2×10^8 cfu mL^{-1} but there was a decline when the cell density was below 10^8 cfu mL^{-1}.

Previous studies have also shown that the constituent of medium that contains the cells and DNA during electroporation also has a significant effect on transformation efficiency. In most cases, the electroporation media are usually unbuffered or buffered by phosphate or HEPES buffer and contain osmotic agents such as sucrose, raffinose or glycerol and divalent ions such as Ca^{2+}, Mn^{2+} or Mg^{2+} (Shigekawa and Dower, 1988). All these components have been reported to have different effects on different species of bacteria. In *Lactobacillus* species, sucrose and $MgCl_2$ appear to be the major component of buffers affecting the electroporation efficiency. The *Lactobacillus* strains in the present study were transformed at a higher efficiency in electroporation medium containing 272 mM sucrose and 1 mM $MgCl_2$ (SMEB buffer). Magnesium chloride is basically used to wash off the extracellular polysaccharides of the cells, which may interrupt the transformation procedure (Berthier *et al*., 1996).

The amount of transforming plasmid DNAs could also affect the transformation efficiency. McIntyre and Harlander (1989), Dunny *et al*. (1991) and Serror *et al*. (2002) reported similar trends in their results. They found that the yield of transformants was maintained as DNA approached 1-2 μg mL^{-1}. At higher DNA concentrations, deleterious contaminants such as phenol, SDS and EDTA in DNA preparations are likely to affect the transformation efficiency (Shigekawa and Dower, 1988).

To determine the effect of field strength on transformation efficiency, cells were exposed to field strengths ranging from 2.5-12.5 kV cm^{-1}, with the pulse controller set to 200 Ω and the capacitance to 25 μF. *Lactobacillus brevis* I23 and *Lb. fermentum* I25 showed the highest transformation efficiency at 7.5 and 10.0 kV cm^{-1}, respectively. Meanwhile, 12.5 kV cm^{-1} produced the highest number of transformants for *Lb. crispatus* I12 and *Lb. acidophilus* I16C. Although different field strengths were required to achieve the highest transformation efficiency in the

different *Lactobacillus* strains, the trend of transformation was similar. The transformation efficiency would increase with the field strength to an optimum level, afterwhich the transformation efficiency would decrease. Trevors *et al.* (1992) suggested that the requirement of different field strengths to achieve the highest transformation efficiency was related to the size of the bacterial cells. They suggested that the field strength must be adequate to achieve the 0.5-1.0 V depolarization across the membrane of the bacterial cells that is necessary for transient permeabilization and subsequently providing the force for DNA to drive through the barrier presented by the cell wall.

With the optimized conditions, the electroporation efficiency of *Lb. crispatus* I12, *Lb. brevis* I23, *Lb. fermentum* I25 and *Lb. acidophilus* I16C increased by 39- to 1027-fold when compared to the initial electroporation efficiencies.

Using the respective optimized electroporation procedure, transformation of the different *Lactobacillus* strains with various plasmids was determined. The size of plasmid did not appear to affect the transformation efficiency. The results indicated that plasmids ranging from 4.4 to 10.2 kb could be transformed with comparable efficiency. Ohse *et al.* (1997) revealed that the transformation efficiency might be affected by the topology, rather than the size, of plasmid DNA. The results of the present study also showed that a higher number of transformants was recovered when the transformants were selected based on chlorompenicol resistance rather than erythromycin resistance. This could be observed in pGK12 transformants, which were selected based on these two antibiotics. The expression of erythromycin resistance involves a post-transcriptional regulatory mechanism which causes the inefficiency or delayed expression when compared to chloromphenicol resistance which is rapidly expressed following resumption of bacterial protein synthesis (Wei *et al.*, 1995).

All the plasmids, except for pNCKH104, transformed the *Lactobacillus* strains at an efficiency of 10^3-10^4 transformants/μg DNA. The failure of plasmid pNCKH104, which was derived from a plasmid from *Lb. reuteri* (Heng *et al.*, 1997), to transform the *Lactobacillus* strains could be due to restriction modification of incoming DNA or failure of the replicon of plasmid to establish in the host cell (Serror *et al.*, 2002). Argnani *et al.* (1996) suggested that the failure to recognize the replication functions of the plasmids by the host enzymes would also lead to unsuccessful transformation. Successful transformation as observed by using pGK12, pSA3, pLP825 and pLP3537 in the present study indicated otherwise. Plasmid pGK12, a broad-host-range vector with its replicon originated from *Lactococcus lactis* subsp. *cremoris* plasmid pWVO1, has been reported to replicate in various gram positive and gram negative bacteria (Kok *et al.*, 1984; Argnani *et al.*, 1996). Similarly, transformation by pSA3, which was derived from a plasmid of *Streptococcus sanguis* (Dao and Ferretti, 1985), has been reported in various *Lactobacillus* species such as *Lb. helveticus*, *Lb. fermentum* BR11 and *Lb. casei* (Chassy and Flickinger, 1987; Thompson and Collins, 1991; Wei *et al.*, 1995). Plasmids pLP825 and pLP3537, which were constructed from cryptic plasmid from *Lb. plantarum* and *Lb. pentosus*, respectively, were found to transform *Lb. casei* ATCC 393, *Lb. plantarum* NCDO 1193, *Lb. pentosus* MD353, *Lb. plantarum* ATCC 14917, *Lb. acidophilus* NCK 89 and *Lb. fermentum* NCK127 (Posno *et al.*, 1991a). The capability of the *Lactobacillus* strains used in the present study to recognize the replicon from various sources indicated the possibility of using these plasmids as cloning vectors.

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