African Journal of Biotechnology Vol. 8 (16), pp. 3964-3969, 18 August, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Differentiation of *Lactobacillus*-probiotic strains by visual comparison of random amplified polymorphic DNA (RAPD) profiles

Norlida Abdul Manan[1], Sieo Chin Chin[1,2*], Norhani Abdullah[1,2] and Ho Yin Wan[1]

[1]Institute of Bioscience, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.
[2]Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.

Accepted 3 July, 2009

In the present study, distinctive RAPD fingerprints were generated for 12 *Lactobacillus*-probiotic strains from 5 *Lactobacillus* species (*L. brevis*, *L. reuteri*, *L. gallinarium*, *L. salivarius* and *L. panis*) after optimization of the RAPD parameters such as $MgCl_2$, Taq polymerase, primer concentration and type of primer. The strains were differentiated under the same PCR protocol but different concentration of primer OPM-05 (50 pmole to differentiate the 5 *L. brevis* strains and 75 pmole to differentiate 2 strains of *L. gallinarium*, 3 strains of *L. reuteri*, a strain of *L. panis* and *L. salivarius*). The RAPD fingerprints generated could be differentiated by visual comparison of the profiles, without being analysed by relevant software. This allows specific, rapid, immediate and convenient identification of the *Lactobacillus* strains.

Key words: *Lactobacillus*, RAPD analysis, differentiation.

INTRODUCTION

The random amplified polymorphic DNA (RAPD) technique represents an efficient tool for the study of genetic polymorphism of DNA. The amplification occurs at low stringency, allowing primers to anneal at several locations of the two strands of the DNA, thus, generate unique banding profiles. Several previous reports have indicated that the reproducibility of RAPD fingerprints involved many factors (Gzyl and Augustynowicz, 1999; Tyler et al., 1997). Although RAPD requires only small amounts of template DNA which need not be double stranded or highly purified (Wang et al., 1993), it needs a robust polymerase chain reaction (PCR) protocol as the technique can be sensitive to changes in reaction conditions e.g. concentration of DNA polymerase, primers, $MgCl_2$, template and etc. (Hilton et al., 1997). Several studies have demonstrated that these experimental parameters can affect the amplification profiles causing false bands and non-reproducibility of assay (Quintaes et al., 2004). Results from different laboratories may also vary if there is a lack of standardisation (Deplano et al., 2006). Optimization of the reaction is imperative to eliminate most of the variations that are sometimes observed in duplicate DNA profiles (Quintaes et al., 2004). It is necessary to standardise the PCR parameters as it may lead to a more robust and reliable reaction capable of recognizing related strains and discriminating between unrelated strains (Hilton et al., 1996). Reproducibility of RAPD results could be achieved by appropriate optimisation of the RAPD protocol (Blixt et al., 2003). In addition, in order to investigate the polymorphism in closely related strains, the highest possible complexity of the RAPD patterns is required and this can be obtained by optimising the RAPD conditions. As RAPD is easy and rapid to perform, the applicability of this technique to differentiate 12 *Lactobacillus*-probiotic strains, which were made up of 5 species, under optimised RAPD conditions was evaluated. Several parameters such as concentration of primer, $MgCl_2$ and Taq DNA polymerase were examined to increase pattern complexity to allow visual discrimination of the RAPD profiles of the 12 *Lactobacillus* strains. The most suitable condition for RAPD differentiation of

*Corresponding author. E-mail: ccsieo@biotech.upm.edu.my. Tel.: +603-89466702. Fax: +603-89430913.

Table 1. Primers used in the present study.

Primer	Sequence (5' → 3')	Source
OPA-02	CAGCACCCAC	Roy et al. (2000)
OPA-18	AGGTGACCGT	Roy et al. (2000)
OPL- 07	AGGCGGGAAC	Roy et al. (2000)
OPL-16	AGGTTGCAGG	Roy et al. (2000)
OPM-05	GGGAACGTGT	Roy et al. (2000)

Table 2. *Lactobacillus* strains used in present study.

S/N	Strain
1	*L. brevis* I12
2	*L. brevis* 123
3	*L. brevis* I25
4	*L. brevis* I211
5	*L. brevis* I128
6	*L. gallinarum* I16
7	*L. gallinarums* I26
8	*L. reuteri* C1
9	*L. reuteri* C10
10	*L. reuteri* C16
11	*L. panis* C17
12	*L. salivarius* subsp. *salivarius* I24

the 12 *Lactobacillus* strains was also determined.

MATERIALS AND METHODS

Preparation of genomic DNA

Genomic DNA was extracted from overnight culture by using DNeasy tissue kit (Qiagen), according to the manufacturer's protocol.

RAPD optimization

Primers used in the present study are as listed in Table 1. Primer OPL-07 was used to optimise the RAPD condition of the 12 *Lactobacillus* strains (Table 2). The *Lactobacillus* strains were previously isolated from the gastrointestinal tract of chickens (Jin et al., 1996) and identified based on 16S rRNA gene and 16S-23S rRNA gene intergenic spacer region (ISR) analysis (Lee et al., 2008).

The amplification was carried out with the 2400 GeneAmp PCR thermal cycler (Perkin Elmer, USA) using the following protocol: preincubation at 94°C for 2 min, followed by a 40-cycle amplification at 94°C for 1 min, 30°C for 1 min and 72°C for 1.5 min and a single cycle of primer extension at 74°C for 5 min. The amplification was performed in a final volume of 25 µl containing 1 x PCR reaction buffer (Promega, USA), 200 mM of each dNTP (Promega, USA), 50 pmole of template DNA, 2.5 mM $MgCl_2$, 1.75 U Taq polymerase (Promega, USA) and 50 pmole primer (Invitrogen, USA). Different concentrations of $MgCl_2$ (1.5, 2.0, 2.5, 3.0, 3.5 mM), Taq DNA polymerase (2.0, 2.5, 3.0 U) and primer (75, 100, 125 pmole) were used to optimised the protocol. One parameter was optimised at a time and the optimised condition was used for subsequent optimisation process. The amplification was carried out twice for each parameter tested. Amplified products were separated by electrophoresis in 2% agarose gel (Seakem LE, UK) in 1 x TAE buffer. The gel was visualized and imaged using AlphaImager ^{TM}1220 gel documentation system (CA, United States) after staining with 0.5 mgl^{-1} ethidium bromide. RAPD condition which produced more bands was considered as the optimised condition.

After each parameter was optimised, the condition was used for the amplification of random DNA markers to reveal genetic diversity among the 12 *Lactobacillus* strains using primers OPA-02, OPA-18, OPL-16, OPL-07 and OPM-05 (Table 1).

RESULTS

Optimisation of magnesium chloride concentration

The RAPD profiles of the *Lactobacillus* strains were affected by different concentrations of $MgCl_2$. More bands were generated at higher concentration of $MgCl_2$. The optimum concentration of $MgCl_2$ was 1.5 mM for *L. salivarius* I24; 2.0 mM for *L. brevis* I12, I25 and I218, *L. gallinarum* I16 and I26 and *L. panis* C17; 2.5 mM for *L. brevis* I23 and I211 and 3.0 mM for *L. reuteri* C1, C10 and C16. As constant banding profiles were generated with the optimum or higher concentrations of $MgCl_2$, 3.0 mM $MgCl_2$ was selected for amplification of all strains in sub-sequent studies.

RAPD profilling at optimised magnesium chloride concentration

The RAPD profiles of the 12 *Lactobacillus* strains were obtained at optimised $MgCl_2$ concentration (3.0 mM), 1.75 U Taq polymerase and 50 pmole different primers. It was observed that different banding profiles were generated for different species of *Lactobacillus*, regardless of type of primer used. However, the banding profiles were similar or highly similar among some strains of similar species.

Among the 5 primers, primer OPA-02 generated the most number of unique profiles (Figure 1). Under this PCR condition, profiles of *L. brevis* I12, I23 and I218, *L. gallinarum* I16 and I26, *L. reuteri* C1, C10 and C16, *L. panis* C17 and *L. salivarius* I24 could be differentiated from the other strains. In fact, banding patterns for *L. panis* C17 and *L. salivarius* I24 were always unique regardless of primer used. Primer OPM-05 fail to generate any unique profiles to differentiate the *Lactobacillus* strains except *L. panis* C17 and *L. salivarius* I24. Primers OPL-07 only produced unique profile for *L. reuteri* C16, apart from *L. panis* C17 and *L. salivarius* I24. Different banding profiles were generated for *L. brevis* I12, *L. reuteri* C10 and C16, *L. panis* C17 and *L. salivarius* I24 when primer OPA-18 was used. As for primer OPL-16, three types of banding patterns were generated for the 5 *L. brevis* strains. *L. brevis* I12 and I23 and *L. brevis* I25 and I211 shared similar profiles whereas *L. brevis* I218 profile was slightly different from the others. A unique RAPD profile was also observed for *L. reuteri* C10 when this primer was used.

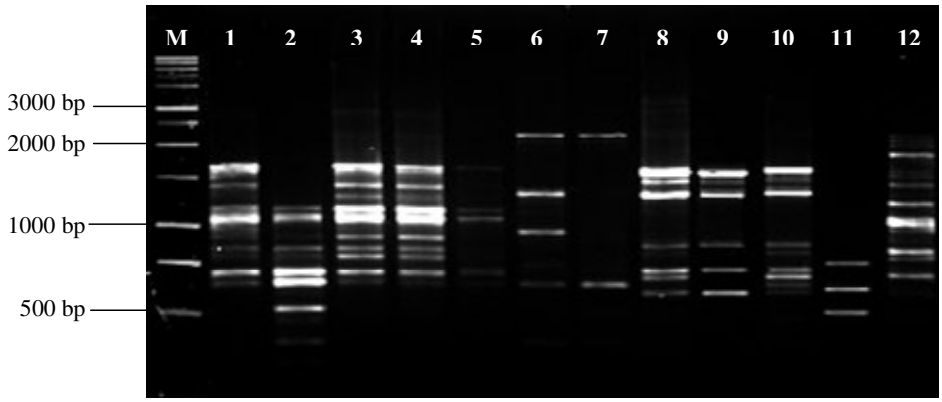

Figure 1. RAPD profiles of *Lactobacillus* strains generated from primer OPA-02 at optimized $MgCl_2$ concentration (3.0 mM). Lanes M; 1 kb DNA ladder; 1: *L. brevis* I12, 2: *L. brevis* I23; 3: *L. brevis* I25; 4: *L. brevis* I211; 5: *L. brevis* I218, 6: *L. gallinarium* I16; 7: *L. gallinarium* I26; 8: *L. reuteri* C1; 9: *L. reuteri* C10; 10: *L. reuteri* C16; 11: *L. panis* C17 and 12: *L. salivarius* I24.

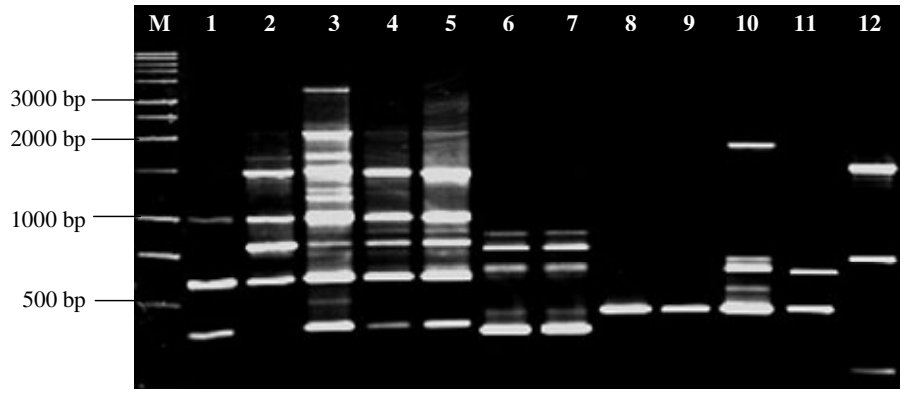

Figure 2. RAPD profiles of *Lactobacillus* strains generated from primer OPM-05 at optimized $MgCl_2$ (3.0 mM) and Taq polymerase (2.0 U) concentrations. Lanes M; 1 kb DNA ladder; 1: *L. brevis* I12, 2: *L. brevis* I23; 3: *L. brevis* I25; 4: *L. brevis* I211; 5: *L. brevis* I218, 6: *L. gallinarium* I16; 7: *L. gallinarium* I26; 8: *L. reuteri* C1; 9: *L. reuteri* C10; 10: *L. reuteri* C16; 11: *L. panis* C17 and 12: *L. salivarius* I24.

Optimisation of Taq polymerase concentration

The RAPD profiles of the 12 *Lactobacillus* strains which were amplified at 3.0 mM $MgCl_2$ and various concentrations of Taq polymerase were not affected by different concentrations (2.0, 2.5 and 3.0 U) of Taq polymerase. Thus, the lowest concentration (2.0 U) was used in subsequent studies.

RAPD profilling at optimised magnesium chloride and Taq polymerase concentrations

RAPD profiles of the 12 *Lactobacillus* strains were obtained at optimised $MgCl_2$ (3.0 mM) and Taq polymerase (2.0 U) concentrations and 50 pmole of different primers. Under this PCR condition, unique profiles were always generated for *L. panis* C17 and *L. salivarius* I24, regardless of primer used. Results also revealed that *L. brevis* strains produced highly similar profile when primers OPA-02 and OPL-07 were used. Slight variation in the banding patterns could be observed for *L. brevis* strains when primer OPA-18 was used in which 3 types of profiles were generated for the 5 *L. brevis* strains. *L. brevis* I25, I211 and I218 shared the same profile but *L. brevis* I12 and I23 generated unique profiles. As for the profiles generated by primer OPM-05, unique banding patterns were produced for the five *L. brevis* strains (Figure 2). *L. reuteri* C16, *L. panis* C17 and *L. salivarius* I24 were also differentiated from the others by using this primer.

The *L. gallinarium* strains could only be differentiated by using primers OPA-02, OPA-18 and OPL-07 under

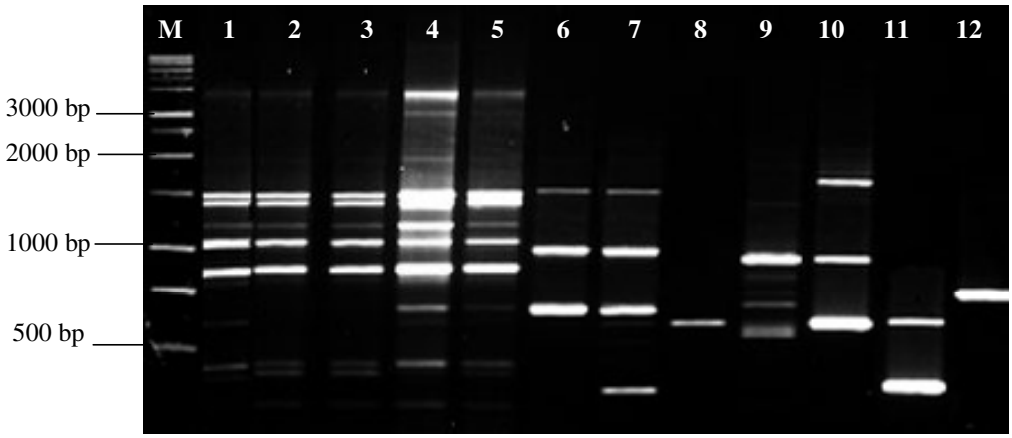

Figure 3. RAPD profiles of *Lactobacillus* strains generated from primer OPM-05 at optimized $MgCl_2$ (3.0 mM), Taq polymerase (2.0 U) and primer (75 pmole) concentrations. Lanes M; 1 kb DNA ladder; 1: *L. brevis* I12, 2: *L. brevis* I23; 3: *L. brevis* I25; 4: *L. brevis* I211; 5: *L. brevis* I218, 6: *L. gallinarium* I16; 7: *L. gallinarium* I26; 8: *L. reuteri* C1; 9: *L. reuteri* C10; 10: *L. reuteri* C16; 11: *L. panis* C17 and 12: *L. salivarius* I24.

this PCR condition. Profiles generated by primers OPA-02 and OPL-07 were more clearly distinguished as compared to those generated by primers OPA-18.

Primers OPA-02, OPA-18 and OPL-07 successfully differentiated the 3 *L. reuteri* strains. Unique profiles which clearly discriminated *L. reuteri* C1, C10 and C16 could be observed. Primer OPL-16 and OPM-05 could only differentiate *L. reuteri* C10 and *L. reuteri* C16, respectively, from the other *L. reuteri* strains.

Optimisation of primer concentration

Different concentrations of primer (75, 100 and 125 pmole) have no effect on the banding profiles of all the strains. Thus, the lowest concentration (75 pmole) was selected as the optimum concentration of primer.

RAPD profilling at optimised $MgCl_2$, Taq polymerase and primer concentrations

As shown in Figure 3, after optimisation of $MgCl_2$ (3.0 mM), Taq polymerase (2.0 U) and primer (75 pmole) concentrations, nine unique profiles for *L. brevis* I211 and I218, *L. gallinarium* I16 and I26, *L. reuteri* C1, C10 and C16, *L. panis* C17 and *L. salivarius* I24 were generated when primer OPM-05 was used. However, *L. brevis* I12, I23 and I25 shared similar profile.

The other 4 primers (OPA-02, OPA-18, OPL-16 and OPL-07) were less effective in discriminating the *Lactobacillus* strains under this PCR condition. Although *L. panis* C17 and *L. salivarius* I24 could always be differentiated regardless of primers, only selected strains were differentiated by these primers under this PCR condition. The three *L. reuteri* strains were differentiated by primer OPL-16. Unique profile was generated for *L. reuteri* C10 with primer OPA-02 and OPA 18. With primer OPL-07, *L. reuteri* C16 could be distinctively distinguished from the other strains.

The other 4 primers (OPA-02, OPA-18, OPL-16 and OPL-07) could not differentiate *L. gallinarium* I16 and I26. Similar profiles were observed for these two strains regardless of primers used. Similarly, none of the primers could differentiate the 5 *L. brevis* strains under this PCR condition.

DISCUSSION

In this study, the optimum RAPD-PCR condition to generate constant and distinctive banding patterns for 12 *Lactobacillus* strains was determined. Precise optimisation of the RAPD-PCR procedure could overcome problem with reproducibility and increase its discriminatory power (Perry et al., 2003; Gzyl and Augustynowicz, 1999). The optimisation process is also necessary in order to reveal limited polymorphism in closely related strains (Diakou and Dovas, 2001). Some of the critical parameters that have been reported to affect RAPD profiles are concentrations of $MgCl_2$, Taq polymerase, primer and template DNA, the annealing temperature and thermal cycling profile (Fraga et al., 2005; Quintaes et al., 2004). Thus, in the present study, a few of these parameters were examined in an effort to increase pattern complexity sufficient for visual differentiation of the profiles to ease future identification of the strains. In many cases, RAPD profiles needs to be processed with software such as TDI software (Technologia para el Diagnóstico y la investigació S. A., Madrid, Spain). Similarities between strains were then estimated using the Dice co-efficient, follow by cluster analysis of the pairwise distance matrix among molecular profiles using the un-

weighted pair group method with average linkage (UPGMA) (Callado and Hernández, 2007). This makes the protocol cumbersome and inconvenient especially when the fingerprint is used for rapid and specific identification of the strains.

Various concentrations of $MgCl_2$ (1.5, 2.0, 2.5, 3.0, 3.5 mM), Taq DNA polymerase (2.0, 2.5, 3.0 U) and primer (75, 100, 125 pmole) were used to optimise the RAPD-PCR condition. Among the 3 parameters evaluated, the concentration of $MgCl_2$ has the highest effect on the banding profiles, whereas in the range of concentration tested for Taq polymerase and primer, no differences in RAPD fingerprint profiles were observed.

By increasing the concentration of $MgCl_2$, a higher number of bands and a better fingerprinting pattern were observed. The optimum $MgCl_2$ concentration for all the *Lactobacillus* strains was determined as 3.0 mM. Several strains produced the highest number of constant bands at a concentration below 3.0 mM. For instance, *L. salivarius* I24 produced highest complexity pattern at 1.5 mM; *L. brevis* I12, I25 and I218, *L. gallinarum* I16 and I26 and *L. panis* C17 at 2.0 mM and *L. brevis* I211 and I23 at 2.5 mM. Nevertheless, a concentration of 3.0 mM was selected as the optimum concentration in order to standardise the PCR condition for all the strains. At this concentration, the patterns produced for all the *Lactobacillus* were constant (not changing with increasing concentration of $MgCl_2$). As reported by Hopkins and Hilton (2001), the function of $MgCl_2$ is to stabilise primer annealing. The concentration of $MgCl_2$ could affect specificity and yield of the reaction. Too little Mg^{2+} would decrease the yield whilst excess Mg^{2+} resulted in non-specific amplification and reduced enzyme fidelity (Cobb and Clarkson, 1994). Riedel et al. (1992) and Benter et al. (1995) showed in their studies that alteration of $MgCl_2$ concentration from lower to higher concentration produced more bands and dramatically reduced non-specific priming and enhanced the specificity. Drastic alterations in fingerprinting profiles produced by variations of $MgCl_2$ were also described by Ellsworth et al. (1993). Benter et al. (1995) recommended lower $MgCl_2$ concentration to avoid undesired annealing. The optimum concentration of $MgCl_2$ also seemed to be species dependent. For instance, the best RAPD performance observed for *Yersinia enterocolitica* strains was at 2.0 mM $MgCl_2$. In contrast, for *S. enteritica* serovar *enteritidis*, no difference in RAPD fingerprint profiles was obtained when the concentration varied from 2.0 to 4.0 mM (Quintaes et al., 2004).

The concentrations of Taq polymerase and primer which have been reported to be critical parameters in determining the RAPD profiles (Sansinforiano et al., 2001) were found to have no effect in the present study. The RAPD profiles did not change within the range of concentration tested (2.0, 2.5, 3.0 U for Taq polymerase and 75, 100, 125 pmole for primer). Thus, the optimum concentration was determined as 2.0 U and 75 pmole for Taq polymerase and primer, respectively.

An optimal concentration of Taq polymerase is required to avoid poor amplification and non-reproducibility of banding profiles (Benter et al., 1995). A recommended concentration range for Taq polymerase is between 1 and 2.5 U per 100 µl reaction when other parameters are optimum. However, enzyme requirements may vary with respect to individual target templates or primers (Landre et al., 1995). Quintaes et al. (2004) reported that the amplification profiles of *Salmonella enterica* serovar Typhi obtained with primer 784 were affected markedly with varying concentrations of Taq polymerase. The optimum concentration of Taq polymerase in their study was 5 U/25 µl. Loss of fragments in the profiles was observed when 1 U/25 µl was used. On contrary, Fraga et al. (2005) observed a general increase in both the number and intensity of detectable bands with increasing Taq DNA polymerase up to 2 U/25 µl. Taq polymerase affects the reproducibility and fidelity of RAPD patterns. If the enzyme concentration is too high, non-specific background product may accumulate and if too low, an insufficient amount of desired product is made.

The number and intensity of bands could also be affected by the primer. At lower concentration, the number and intensity of bands drop. If primer concentration is too low, no amplification or low product yields were observed. But, when the primer concentration is too high, mispriming may occur more frequently and produce greater non-specific amplification (Tyler et al., 1997). Under this condition, intensity of large bands will decrease whereas the intensity of smaller bands will increase (Fraga et al., 2005). Ronimus et al. (1997) found that increasing the concentration of primer from 0.2 to 2.0 µM resulted in increasing number of amplification products which could be used to discriminate or identify thermophilic and mesophilic *Bacillus* species. In general, such differences in amplification may occur because as more primer is available, it binds to more locations on the DNA. Thus, smaller fragments become more prominent compare to larger fragments. On contrary, at low concentrations, the primer may be exhausted before the reaction is completed, resulting in lower yields of amplification bands (Ramírez et al., 2005). The ideal concentration of the primer could be closely related to the species being examined and some bacterial species are more sensitive to changes. As in the present study, varying concentrations of primer (75, 100 and 125 pmole) have no effect on the amplification profiles of the *Lactobacillus* strains. Some primers interact differently with various DNA templates and a primer that works well for one species may be less suited for use with another (Tyler et al., 1997). Alos et al. (1993) suggested that due to the distribution of binding sites, certain primers may amplify regions of the genome that overlap and therefore, amplify from only a small section of the genome, thus possibly not reflecting the true heterogeneity between strains. This explains why some of the primers can be used to differentiate between strains and some primers are not discriminating. Therefore, it is important to try many set of primers in RAPD

analysis. Quintaes et al. (2004) found only one primer applicable from the 16 arbitrary primers used in their study. From the 5 primers examined for suitability in the present study, primer OPM-05 was found to generate the best fingerprint patterns to differentiate the *Lactobacillus* strains. Thus, the following condition was determined for the discrimination of the 12 *Lactobacillus* strains: Preincubation at 94°C for 2 min, followed by a 40-cycle amplification at 94°C for 1 min, 30°C for 1 min, and 72°C for 1.5 min and a single cycle of primer extension at 74°C for 5 min. The amplification was performed in a final volume of 25 µl containing 1 x PCR reaction buffer (Promega, USA), 200 mM of each dNTP (Promega, USA), 50 pmole of template DNA, 3.0 mM $MgCl_2$, 2.0 U Taq polymerase (Promega, USA) and 50 pmole of primer OPM-05 to differentiate the *L. brevis* strains or 75 pmole of primer OPM-05 to differentiate strains of *L. gallinarium*, *L. reuteri*, *L. panis* and *L. salivarius*. Although unique profiles were also produced for *L. brevis* I12, I23 and I218, *L. gallinarium* I16 and I26, *L. reuteri* C1, 10 and C16, *L. panis* C17 and *L. salivarius* I24 with 50 pmole primer OPA-02, 30 mM MgCl2 and 1.75 U Taq polymerase (Figure 1), the profiles for *L. reuteri* strains were less distinctive. In addition, this protocol also successfully differentiated the identity of two strains of *L. gallinarium* (I16 and 26), in which its identity were initially unable to be resolved by 16S rRNA gene sequencing but later identified by 16S-23S rRNA gene ISR analysis (Lee et al., 2008).

In conclusion, optimization of the RAPD condition and the primer are important to successfully produce profiles to differentiate the strains. In the present study, the 12 *Lactobacillus* strains could be differentiated by visual comparison of the profiles produced at optimised condition by using primer OPM-05 at 2 different concentrations (50 or 75 pmole). In fact, the identity of *L. gallinarium* I16 and I26 which were initially unable to be resolved by 16S rRNA gene sequencing but later identified by 16S-23S rRNA gene ISR analysis (Lee et al., 2008), were successfully differentiated by this protocol. These profiles could be used as a fingerprint for the strains and are useful for specific, rapid, immediate and convenient identifycation of the strains.

REFERENCES

Alos JI, Lambert T, Courvalin P (1993). Comparison of two molecular typing methods for tracing nosocomial transmission of *Escherichia coli* K1 in a neonatal unit. J. Clin. Microbiol. 31: 1704-1709.

Benter T, Papadopoulos S, Pape M, Manns M, Poliwoda H (1995). Optimization and reproducibility of random amplified polymorphic DNA in human. Anal. Biochem. 230: 92-100.

Blixt Y, Knutsson R, Borch E, Rådström P (2003). Interlaboratory random amplified polymorphic DNA typing of *Yersinia enterocolitica* and *Y. enterocolitica*-like bacteria. Int. J. Food Microbiol. 83: 15-26.

Cobb BD, Clarkson JM (1994). A simple procedure for optimising the polymerase chain reaction (PCR) using modified Taguchi methods. Nucl. Acids Res. 22: 3801-3805.

Deplano A, De Mendonca R, De Ryck R, Struelens MJ (2006). External quality assessment of molecular typing of *Staphylococcus aureus* isolates by a network of laboratories. J. Clinical Microbiol. 44: 3236-3244.

Diakou A, Dovas CI (2001). Optimisation of random-amplified polymorphic DNA producing amplicons up to 8500 bp and revealing intraspecies polymorphism in *Leishmania infantum* isolates. Anal. Biochem. 228: 195-200.

Ellsworth DL, Rittenhouse KD, Honeycutt RL (1993). Artifactual variation in randomly amplified polymorphic DNA banding patterns. Biotechniques, 14: 214-217.

Fraga J, Rodriguez J, Fuentes O, Fernandez-Calienes A, Castex M (2005). Optimization of random amplified polymorphic DNA techniques for use in genetic studies of Cuban *Triatominae*. Rev. Inst. Med. Trop. S. Paulo, 47: 295-300.

Gzyl A, Augustynowicz E (1999). Technical aspects of random amplified polymorphic DNA (RAPD) technique in genotyping of bacterial strains. Acta Microbiol. Pol. 48: 243-259.

Hilton AC, Banks JG, Penn CW (1996). Random amplification of polymorphic DNA (RAPD) of *Salmonella*: strain differentiation and characterization of amplified sequences. J. Appl. Bacteriol. 81: 575-584.

Hilton AC, Banks JF, Penn CW (1997). Optimization of RAPD fingerprinting *Salmonella*. Lett. Appl. Microbiol. 24: 243-248.

Hopkins KL, Hilton AC (2001). Optimization of random amplification of polymorphic DNA analysis for molecular subtyping of *Escherichia coli* O157. Lett. Appl. Microbiol. 32: 126-130.

Jin LZ, Ho YW, Ali MA, Abdullah N, Ong KB, Jalaludin S (1996). Adhesion of *Lactobacillus* isolates to intestinal epithelial cells of chicken. Lett. Appl. Microbiol., 22: 229-232.

Landre PA, Gelfand DH, Watson RM (1995). The use of cosolvents to enhance amplification by the polymerase chain reaction. In: Innis MA, Gelfand DH, Sninsky JJ (eds) PCR Strategies, Academic Pr. Inc., US, pp. 3-16.

Lee CM, Sieo CC, Wong CMVL, Abdullah N, Ho YW (2008). Sequence analysis of 16S rRNA gene and 16S-23S rRNA gene intergenic spacer region for differentiation of probiotics *Lactobacillus* strains isolated from the gastrointestinal tract of chicken. Annals Microbiol. 58: 133-140.

Perry AL, Worthington T, Hilton AC, Lambert PA, Stirling AJ, Elliott TSJ (2003). Analysis of clinical isolates of *Propionibacterium acnes* by optimised RAPD. FEMS Microbiol. Lett. 228: 51-55.

Quintaes BR, Leal NC, Reis EMF, Hofer E (2004). Optimization of randomly amplified polymorphic DNA-polymerase chain reaction for molecular typing of *Salmonella enterica* serovar Typhi. Rev. Soc. Bras. Med. Trop. 37: 143-147.

Ramírez CJ, Jaramillo CA, Delgado MP (2005). Genetic structure of sylvatic, peridomestic and domestic populations of *Triatoma dimidiate* (Hemiptera: Reduvidae) from an endemic zone of Boyaca, Colombia. Acta Trop. 93: 23-29.

Riedel KHJ, Wingfield BD, Britz TJ (1992). Combined influence of magnesium concentration and polymerase chain reaction specificity enhancers. FEMS Microbiol. Letts. 92: 69-72.

Ronimus RS, Parker LE, Morgan HW (1997). The utilization of RAPD-PCR for identifying thermophilic and mesophilic *Bacillus* species. FEMS Microbiol. Lett. 147: 75-79.

Roy D, Ward P, Vincent D, Mondou F (2000). Molecular identification of potentially probiotic lactobacilli. Curr. Microbiol. 40: 40-46.

Sansinforiano ME, Rabasco A, Martinez-Trancon M, Parejo JC, Hermoso-de-Mendoza M, Padilla JA 2001. Optimizaton of the conditions for RAPD-PCR of *Candida* spp. and *Cryptococcus* spp. Rev. Iberoam Micol. 18: 65-69.

Tyler KW, Wang G, Tyler SD, Johnson WM (1997). Factors affecting reliability and reproducibility of amplification based DNA fingerprinting of representative bacterial pathogens. J. Clin. Microbiol. 35: 339-346.

Wang G, Whittam TS, Berg CM, Berg DE (1993). RAPD (arbitrary primer) PCR is more sensitive than multilocus enzyme electrophoresis for distinguishing related bacterial strains. Nucl. Acid Res. 21: 5930-5933.