

Full Length Research Paper

## The reproducibility of random amplified polymorphic DNA (RAPD) profiles of *Streptococcus thermophilus* strains with XD9, M13 and OPI-02 MOD primers

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We optimized the conditions for generating random amplified polymorphic DNA (RAPD) profiles of *Streptococcus thermophilus* strains by using the polymerase chain reaction (PCR). Several factors can cause the amplification of false and non reproducible bands in the RAPD profiles. We tested three primers, OPI-02 MOD, M13 and XD9 throughout this study. In addition, we tested different concentrations of primer, DNA template and Taq DNA polymerase. We adjusted the ratio of the primer to DNA template. All the three primers yielded reproducible profiles on several days, under optimized concentrations of components and cycling parameters used. The bands of such profiles probably corresponded to perfect annealing sites amplified with good efficacy or present in multiple copies in the genome. Five months later, repeated experiment generated identical bands. However, extra faint bands were detected with M13 and XD9 primers, possibly, corresponding to nonspecific binding resulting from slight variation in temperature or calibration of the thermocycler. Therefore, OPI-02 MOD was determined as the most reliable primer for reproducible profiles of *S. thermophilus* strains.

**Key words:** *Streptococcus thermophilus*, random amplified polymorphic DNA (RAPD), DNA template, Taq DNA polymerase, OPI-02MOD, XD9, M13, optimization, reproducibility.

### INTRODUCTION

*Streptococcus thermophilus* is considered to be the second most important species of industrial lactic acid bacteria, after *Lactococcus lactis*, with a market value of about 40 billion US\$; over  $10^{21}$  live cells are ingested annually by the human population. It is well known as a starter culture component in yoghurt fermentation and cheese making (De Vuyst and Tsakalidou, 2008; Hols et al., 2005). Despite the fact that no natural habitat for this species outside the dairy environment has been identified, considerable inter-strain diversity has been observed (O'Sullivan and Fitzgerald, 1998).

Random amplified polymorphic DNA technique (RAPD)

constitutes a useful technique for the study of genetic polymorphism of DNA. It involves the amplification of random segments of genomic DNA by polymerase chain reaction (PCR), using short single primers of arbitrary sequence (Williams et al., 1990).

This molecular approach has been widely utilized to identify and study the level of biodiversity among *S. thermophilus* strains (Morandi and Brasca, 2012; Lazzi et al., 2009; Rizzotti et al., 2009; Andrightetto et al., 2002; Mora et al., 2002; Giraffa et al., 2001; Moschetti et al., 1998). It is a very sensitive and simple technique and is suggested as a fast tool for characterization of a large

number of lactic acid bacteria isolated from dairy products (Lazzi et al., 2009; Ramos et al., 2008); but, this method is prone to poor reproducibility in the band pattern, due to even small changes in reaction conditions; for this reason, it should be highly standardized to achieve satisfactory reproducibility (Skorić et al., 2012; Singh et al., 2009).

The PCR conditions for RAPD analysis can be optimized by varying the concentrations of the reaction mixture components and other reaction factors such as primer annealing, primer extension, denaturation time (Skorić et al., 2012; Singh et al., 2010; Fraga et al., 2005; Tyler et al., 1997; Wolff et al., 1993). The aim of this study was to optimize the concentrations of Taq DNA polymerase, DNA template and primer of PCR reactions and to study their effect on RAPD profiles and reproducibility. The RAPD-PCR of *S. thermophilus* genomic DNA was performed with XD9, M13 and OPI-02 MOD primers. It is useful to develop a reliable RAPD-PCR fingerprinting method for further study of *S. thermophilus* genetic diversity at strain level and to be able to distinguish between a large number of new isolates of *S. thermophilus* quickly and efficiently.

## MATERIALS AND METHODS

### *Streptococcus thermophilus* strains

Twenty one (21) *S. thermophilus* strains (S1-3, K1-15, N4-3, K1-31, N8-2, S1-3, K1-15, K1-7, N5-4, K1-1, S1-3, N2-1, K1-15, N8-2, N6-1, K1-22, N6-2, S1-1, K1-26, K1-12, N3-1) isolated from Turkish traditional yoghurts, were used in this study (from the collection of Food Analysis Laboratory, Middle East Technical University, Ankara, Turkey). The reference strain *S. thermophilus* LMG18311 and the *S. thermophilus* strain Yo-mix 410-3 (from Danisco Commercial starter culture) were also included. They were grown at 42°C in M17 broth, pH 6.8 and stored at -80°C in M17 glycerol.

### DNA isolation

The DNA of *S. thermophilus* strains was extracted by The Gene JET™ Genomic DNA Purification kit (Fermentas) according to the manufacturer's instructions. DNA concentration and the ratio A260/A280, for checking the purity of DNA, were calculated using UV Spectrophotometer (NanoDrop). All the DNA solutions obtained were stored at -20°C.

### Optimization of RAPD reaction

The following three primers, obtained from Metabion International (Deutschland) were used, separately, for amplification: XD9 primer: 5'-GAAGTCGTCC-3' (Moschetti et al., 1998); OPI-02 MOD primer: 5'-GCTCGGAGGAGAGG-3' (Mora et al., 2002) and M13 primer: 5'-GAGGTGGCGTCT-3' (Huey and Hall, 1989).

PCR amplification was carried out in MJ mini personal Thermal cycler (Bio-Rad). The cycling programs used were those described by Moschetti et al. (1998), for XD9 primer and Mora et al. (2002) for OPI-02 MOD primer. The cycling program with M13 primer consisted of an initial denaturation step at 94°C for 2 min and then 40 cycles of 94°C for 1 min; 42°C for 20 s and 72°C for 2 min. The

final elongation was performed at 72°C for 10 min. Amplification was performed in a final volume of 50 µl RAPD-PCR reaction mixture containing 1X PCR buffer. The optimization of RAPD-PCR reaction conditions was performed by varying concentrations as follows: 40, 60, 80, 100, 150 ng of DNA template, 0.5, 1, 1.25, 1.3, 1.5, 2, 2.5, 3.5 U of Taq DNA polymerase (Fermentas) and 0.1, 0.2, 0.3, 0.4, 0.5, 1 µM of primer.

The concentrations of MgCl<sub>2</sub> (3.5 mmol l<sup>-1</sup> for XD9 primer, 2.5 mmol l<sup>-1</sup> for OPI-02 MOD primer and 3 mmol l<sup>-1</sup> for M13 primer) and dNTPs (200 µmol l<sup>-1</sup> of each dATP, dCTP, dGTP and dTTP) were maintained constant with values as given by Fermentas Manufacturer and the authors above. Reactions without DNA were used as negative controls, which were prepared for each set of reaction mixture and included in all gels.

RAPD products were resolved by electrophoresis at 90 V in 1.5% (w/v) in Basica LE Prona agarose in 1XTBE gels. 100 bp Plus DNA ladder (Fermentas) was used as a molecular size standard. Gels were stained in 0.5 mg of ethidium bromide and 500 ml of distilled water for 20 min in a covered container; they were destained in distilled water for 5 min and images were captured by TIFF files using the Gel Doc XR digital imaging system (Bio-Rad).

### Reproducibility study

DNA extraction and RAPD-PCR amplification were performed at least two times, on several days. The *S. thermophilus* strains used for the optimization were: Yo-mix 410-3, S1-3, K1-15, N4-3, K1-31 with XD9 primer, N8-2, S1-3, K1-15 with OPI-02 MOD primer and K1-7, N5-4, K1-1, S1-3, N2-1, K1-15, N8-2 with M13 primer. Then, the optimum components' concentrations of PCR reaction mixture obtained were applied for amplification of the following *S. thermophilus* strains: N6-1, LMG18311 (DNA extraction was performed three times for *S. thermophilus* LMG 18311), K1-22, N6-2, S1-1, K1-26, K1-12, N3-1. The experiment was repeated three times, with the three primers, on several days.

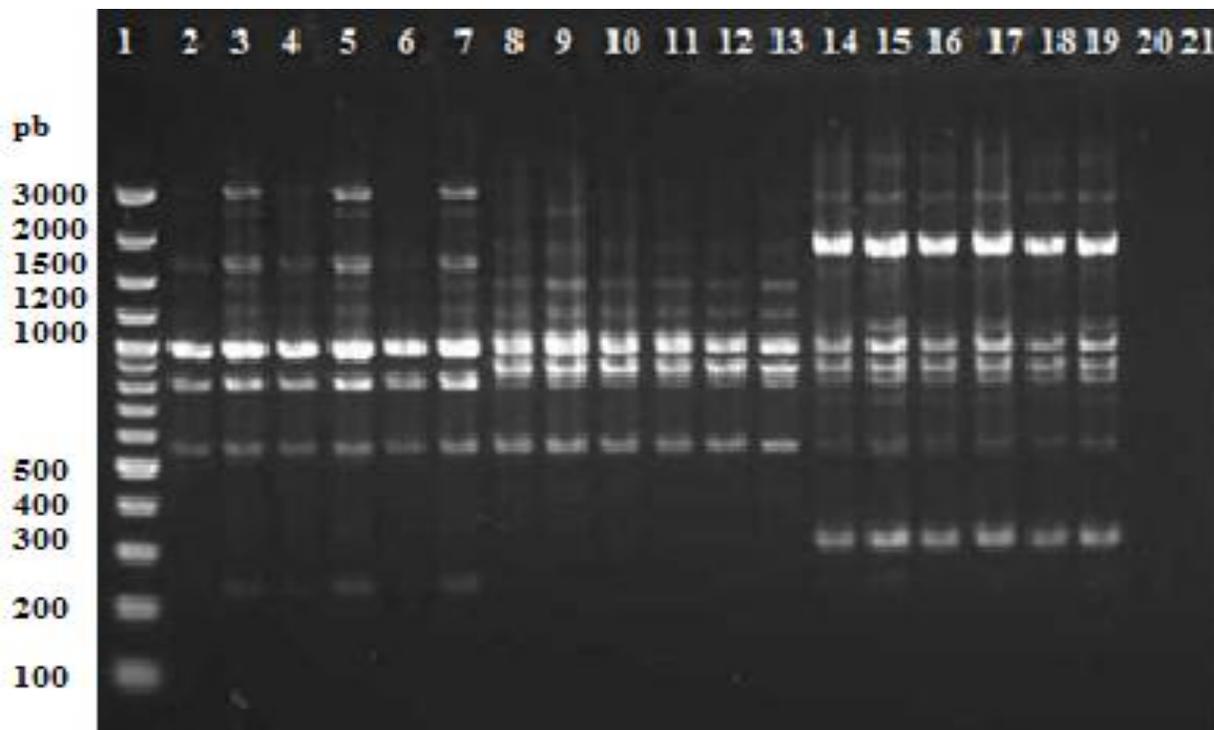
About five months after optimization experiments, the RAPD-PCR amplification was performed with the three primers again. Six strains were tested for each primer as follows: N6-1, LMG18311, K1-22, N6-2, S1-1, K1-26 with OPI-02MOD primer and XD9 primer. The amplification was carried out with *S. thermophilus* strains, LMG18311, K1-12, N3-1, K1-31, N4-3, Yo-mix 410-3, for M13 primer.

## RESULTS

### Optimization of RAPD protocol

In order to optimize RAPD-PCR method, for *S. thermophilus* strains, several concentrations of template DNA, Taq DNA polymerase and primers were tested. The concentrations of PCR mixture selected were optimized according to their relative effects on RAPD amplifications in terms of the highest number and intensity of bands, the generation of clear, scorable and reproducible amplified products.

The ratio of the primer concentration to DNA template concentration is one of the critical factors affecting RAPD profiles and reproducibility (Thangaraj et al., 2011; Tyler et al., 1997; Davin-Regli et al., 1995). The titer of the template DNA concentration should be carefully determined against a fixed primer concentration to obtain ideal conditions (Tyler et al., 1997).



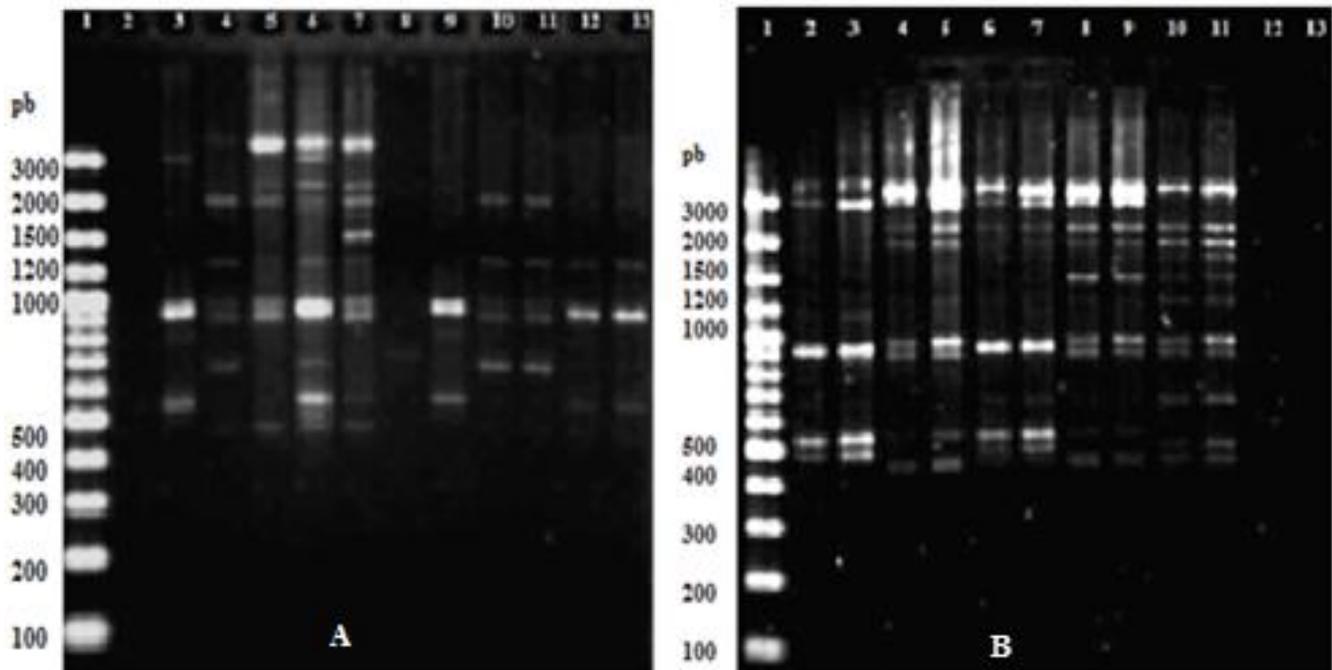
**Figure 1.** RAPD profiles of *S. thermophilus* strains with  $1 \mu\text{mol l}^{-1}$  of OPI-02 MOD primer; 1, Molecular size marker; 2, N8-2 strain, 80 ng of DNA template, 1.3 U of Taq DNA polymerase; 3, N8-2 strain, 80ng of DNA template, 2.5 U of Taq DNA polymerase; 4, N8-2 strain, 100 ng of DNA template, 1.3 U of Taq DNA polymerase; 5, N8-2 strain, 100 ng of DNA template, 2.5 U of Taq DNA polymerase; 6, N8-2 strain, 150 ng of DNA template, 1.3U of Taq DNA polymerase; 7, N8-2 strain, 150 ng of DNA template, 2.5 U of Taq DNA polymerase; 8, S1-3 strain, 80ng of DNA template, 1.3 U of Taq DNA polymerase; 9, S1-3 strain, 80 ng DNA template 2.5 U of Taq DNA polymerase; 10, S1-3 strain, 100 ng of DNA template, 1.3 U of Taq DNA polymerase; 11, S1-3 strain, 100 ng of DNA template, 2.5 U of Taq DNA polymerase; 12, S1-3 strain, 150 ng of DNA template, 1.3 U of Taq DNA polymerase; 13, S1-3 strain, 150 ng of DNA template, 2.5 U of Taq DNA polymerase; 14, K1-15 strain, 80 ng of DNA template, 1.3U of Taq DNA polymerase; 15, K1-15 strain, 80 ng of DNA template, 2.5 U of Taq DNA polymerase; 16, K1-15 strain, 100 ng of DNA template, 1.3 U of Taq DNA polymerase; 17, K1-15 strain, 100 ng of DNA template, 2.5 U of Taq DNA polymerase; 18, K1-15 strain, 150 ng of DNA template, 1.3U of Taq DNA polymerase; 19, K1-15 strain, 150 ng of DNA template, 2.5 U of Taq DNA polymerase; 20, negative control, 1.3 U of Taq DNA polymerase; 21, negative control, 2.5 U of Taq DNA polymerase.

It was observed that the use of 80, 100 and 150 ng of DNA template concentrations, with a fixed concentration ( $1 \mu\text{mol l}^{-1}$ ) of OPI-02MOD primer provided the same result when using 1.3 or 2.5 U of Taq DNA polymerase (Figure 1). Similar RAPD profiles were obtained using 1.3 and 2.5 U of Taq DNA polymerase for S1-3 and K1-15 strains. However, there was an increase in the number of the detectable bands by increasing the concentrations of Taq DNA polymerase from 1.3 to 2.5 U for N8-2 strain. As a result, identical RAPD profiles were obtained using 2.5 and 3.5 U of Taq DNA polymerase with 100 ng of DNA template for the three strains (data not shown). Subsequently, the optimized PCR reaction mixture included:  $1 \mu\text{mol l}^{-1}$  OPI-02 MOD primer, 100 ng of DNA template, 2.5 U of Taq DNA polymerase, 1X PCR buffer,  $2.5 \text{ mmol l}^{-1}$   $\text{MgCl}_2$ ,  $200 \mu\text{mol l}^{-1}$  of each of the four dNTPs in a final volume of 50  $\mu\text{l}$ .

Identical profiles were generated, with a fixed concentration of  $1 \mu\text{mol l}^{-1}$  XD9 primer, using concentra-

tions of 100 and 150 ng DNA template and 2 U of Taq DNA polymerase for the five *S. thermophilus* strains tested (data not shown). Moreover, amplification of 100 ng of DNA template, using 1.5 and 2 U Taq DNA polymerase, produced identical patterns (Figure 2A and B). Subsequently, optimized RAPD-PCR reaction contained 50  $\mu\text{l}$ ; 100 ng of DNA template,  $1 \mu\text{mol l}^{-1}$  of XD9 primer, 2 U of Taq DNA polymerase, 1X PCR buffer,  $3.5 \text{ mmol l}^{-1}$   $\text{MgCl}_2$ , and  $200 \mu\text{mol l}^{-1}$  of each of the four dNTPs.

Amplification bands were observed in the negative controls with ratios of M13 primer to DNA template of  $0.3 \mu\text{mol l}^{-1} / 100 \text{ ng}$ ,  $0.4 \mu\text{mol l}^{-1} / 100 \text{ ng}$ ,  $0.5 \mu\text{mol l}^{-1} / 100 \text{ ng}$  and  $1 \mu\text{mol l}^{-1} / 100 \text{ ng}$  (Figures 3A, 4A and B). However, no bands were detected, in the negative control, with a ratio of  $0.2 \mu\text{mol l}^{-1} / 100 \text{ ng}$  (Figure 3B). Similar RAPD profiles were obtained with ratios of M13 primer to template DNA of  $0.2 \mu\text{mol l}^{-1} / 100 \text{ ng}$ ,  $0.3 \mu\text{mol l}^{-1} / 100 \text{ ng}$  and  $0.4 \mu\text{mol l}^{-1} / 100 \text{ ng}$  (Figure 3A and B).



**Figure 2.** (A) RAPD profiles of *S. thermophilus* strains with  $1 \mu\text{mol l}^{-1}$  of XD9 primer, 100 ng of DNA template, 1.25 U of Taq DNA polymerase. 1, molecular size marker; 2, negative control; 3, 410-3 strain; 4, K1-31 strain; 5, S1-3 strain; 6, K1-15 strain; 7, N4-3 strain; 8, negative control; 9, 410-3 strain; 10, K1-31 strain; 11, K1-31 strain; 12, K1-15 strain; 13, K1-15 strain. (B) RAPD profiles of *Streptococcus thermophilus* strains with  $1 \mu\text{mol l}^{-1}$  of XD9 primer, 100 ng of DNA template; 1, molecular size marker; 2, 410-3 strain, 1.5 U of Taq DNA polymerase; 3, 410-3 strain, 2 U of Taq DNA polymerase; 4, S1-3 strain, 1.5 U of Taq DNA polymerase; 5, S1-3 strain, 2 U of Taq DNA polymerase; 6, K1-15 strain, 1.5 U of Taq DNA polymerase; 7, ) K1-15 strain, 2 U of Taq DNA polymerase; 8, N4-3 strain, 1.5 U of Taq DNA polymerase; 9, N4-3 strain, 2 U of Taq DNA polymerase; 10, K1-31 strain, 1.5 U of Taq DNA polymerase; 11, K1-31 strain, 2 U of Taq DNA polymerase; 12, negative control, 1.5 U of Taq DNA polymerase; 13, Negative control, 2 U of Taq DNA polymerase.

Therefore,  $0.2 \mu\text{mol l}^{-1}$  of M13 primer, 100 ng of bacterial DNA and 1 U Taq DNA polymerase were chosen as optimum conditions since no bands were detected in the negative control under these conditions (Figure 3B). Moreover, using 1 and 1.5 U of Taq DNA polymerase yielded identical patterns for all tested strains (result not shown). As a result, the optimized PCR reaction mixture included 100 ng of bacterial DNA, 1X PCR buffer,  $3 \text{ mmol l}^{-1}$   $\text{MgCl}_2$ ,  $200 \mu\text{mol l}^{-1}$  of each of the four dNTPs,  $0.2 \mu\text{mol l}^{-1}$  of M13 primer and 1 U Taq DNA polymerase.

### The reproducibility of RAPD profiles

The reproducibility of band patterns generated using the optimized parameters was confirmed, in several days, for the three primers, M13, OPI-02 MOD, XD9.

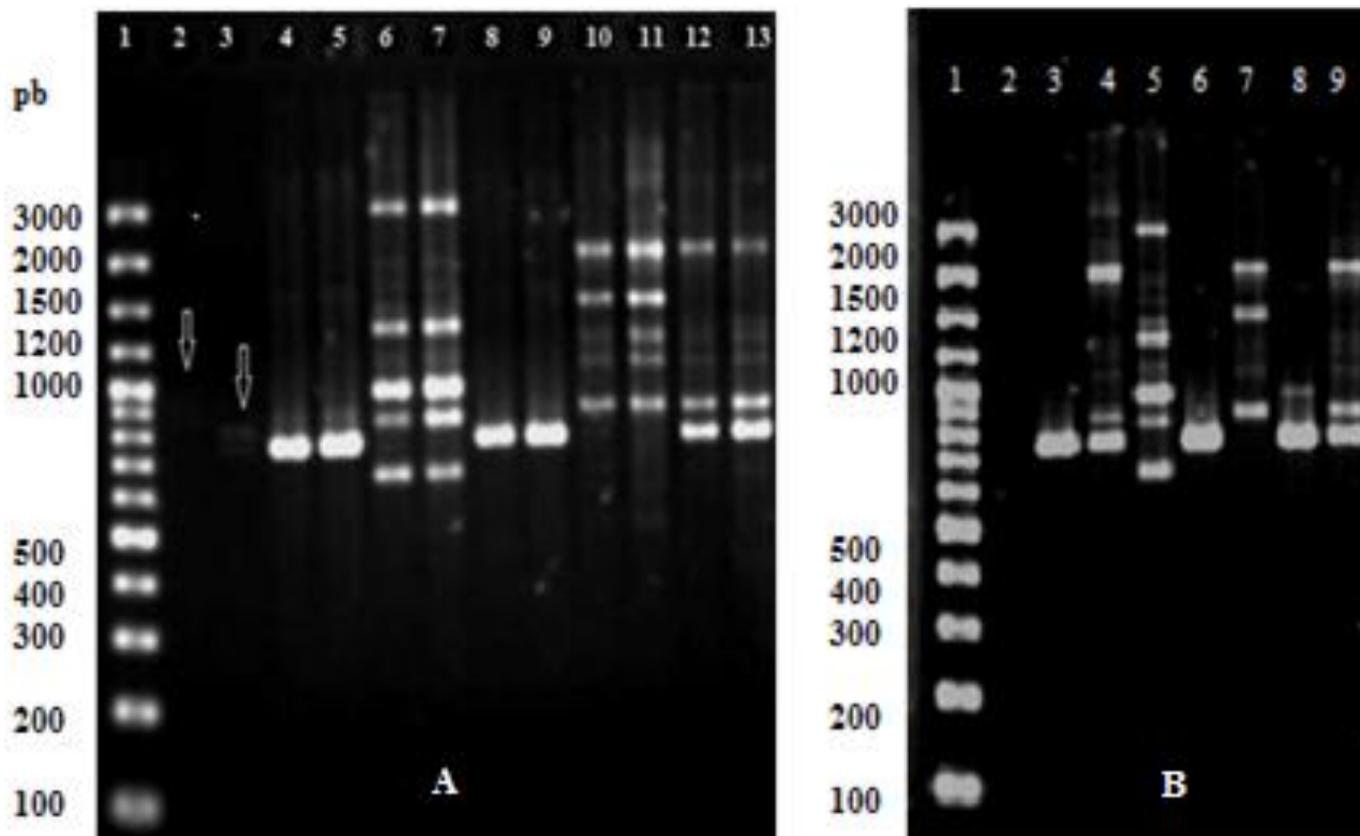
Five months later, results of RAPD PCR amplification performed with the same three primers were compared with those already obtained. Six strains tested produced identical patterns with OPI-02MOD primer, while four from the six strains produced identical patterns with M13 primer and only one was identical with XD9 primer. PAPD profiles of N4-3 strain and Yo-mix 410-3 strain showed

two extra faint bands in their profiles, with M13 primer as compared to those obtained in the former five months. RAPD profiles of LMG 18311, Yo-mix 410-3, N6-2, K1-26 and N6-1 strains showed one, three, one, two and four extra faint bands, respectively with XD9 primer (M13 primer; Figure 5).

### DISCUSSION

The different concentrations of the RAPD mixture tested had different degrees of influence on the RAPD patterns and their reproducibility. An efficient protocol for RAPD analysis should be reasonably resistant to variations in template DNA concentrations (Skorić et al., 2012).

In our experiments, identical profiles were reached over a range of different concentrations of template DNA. Similar observations have been reported by Skorić et al. (2012) and Wolff et al. (1993). For instance, chrysanthemum DNA amplification was relatively constant between a large range of 1 and 500 ng template DNA (Wolff et al., 1993). With regard to the Taq polymerase concentration, a threshold concentration, in which the RAPD profiles were identical, was noticed; same result has been re-



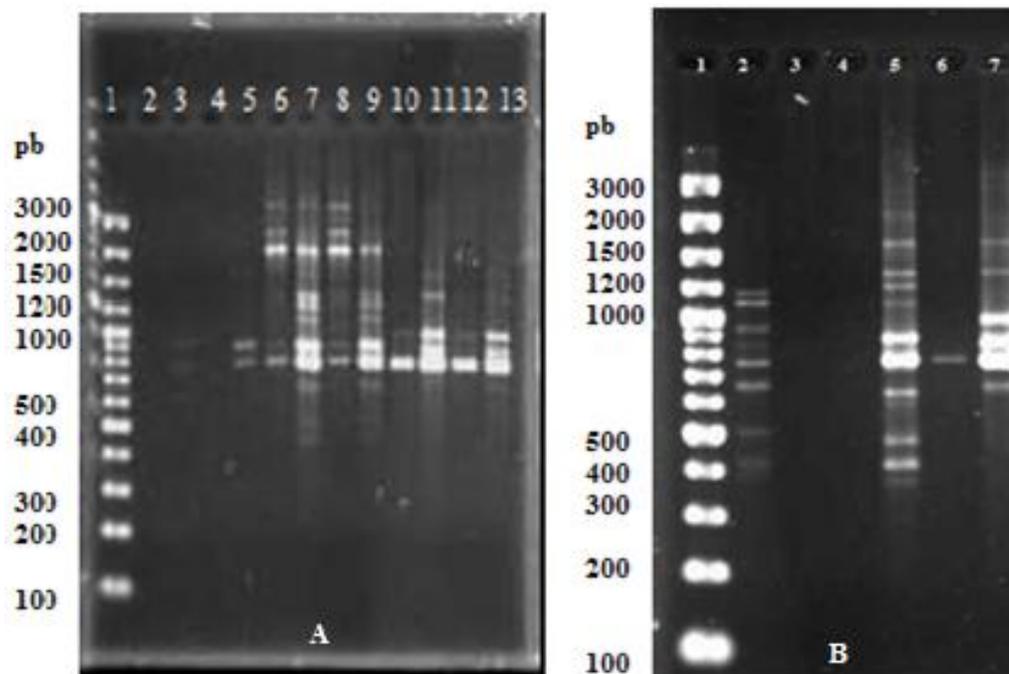
**Figure 3. (A)** RAPD profiles of *S. thermophilus* strains with M13 primer, 100 ng of DNA template, 1 U of Taq DNA polymerase. 1, Molecular size marker; 2, negative control, 0.3  $\mu\text{mol l}^{-1}$  of primer; 3, negative control, 0.4  $\mu\text{mol l}^{-1}$  of primer; 4, K1-7 strain, 0.3  $\mu\text{mol l}^{-1}$  of primer; 5, K1-7 strain, 0.4  $\mu\text{mol l}^{-1}$  of primer; 6, N5-4 strain, 0.3  $\mu\text{mol l}^{-1}$  of primer; 7, N5-4 strain, 0.4  $\mu\text{mol l}^{-1}$  of primer; 8, K1-1 strain, 0.3  $\mu\text{mol l}^{-1}$  of primer; 9, K1-1 strain, 0.4  $\mu\text{mol l}^{-1}$  of primer; 10, S1-3 strain, 0.3  $\mu\text{mol l}^{-1}$  of primer; 11, S1-3 strain, 0.4  $\mu\text{mol l}^{-1}$  of primer; 12, N2-1 strain, 0.3  $\mu\text{mol l}^{-1}$  of primer; 13, N2-1 strain, 0.4  $\mu\text{mol l}^{-1}$  of primer. **(B)** RAPD profiles of *S. thermophilus* strains with M13 primer, 100 ng of DNA template, 1 U of Taq DNA polymerase, 0.2  $\mu\text{mol l}^{-1}$  of primer. 1, Molecular size marker; 2, negative control; 3, K1-7 strain; 4, N2-1 strain; 5, N5-4 strain; 6, K1-1 strain; 7, S1-3 strain; 8, K1-15 strain; 9, N8-2 strain.

ported (Skorić et al., 2012; Fraga et al., 2005). When using lower concentrations of template DNA or Taq DNA polymerase, no visible amplification or lower number and intensity of bands were detected (results not shown).

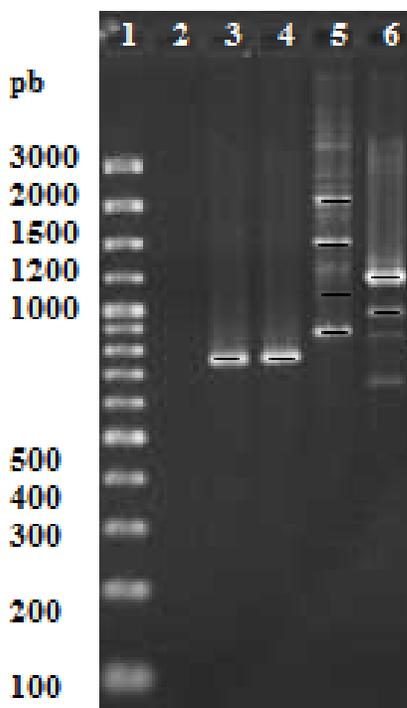
In this work, the effect of primer concentration on the obtained results was noticed, particularly with M13 primer; whereas, identical profiles were observed using a range of ratios of primer to DNA template from 0.2 to 0.4  $\mu\text{mol l}^{-1} / 100 \text{ ng}$ . At lower ratio of 0.1  $\mu\text{mol l}^{-1} / 100 \text{ ng}$ , different RAPD profiles were observed for N5-4, S1-3 and N2-1 strains (result not shown). In addition, at higher ratio of M13 primer to DNA template of 0.5 and of 1  $\mu\text{mol l}^{-1} / 100$ , extra bands were generated. This was clear when comparing profiles of N8-2 strain in Figures 3B (lane 9), 4A (lane 9) and 4B (lane 5). The same observation was demonstrated with profiles of K1-15 strain in Figures 3B (lane 8), 4A (lane 13) and 4B (lane 7). At higher ratio of primer/template, rare or inaccessible sites can be amplified (Davini-Regli et al., 1995). In this case, bands could also result from the increase in weaker mismatch

annealing of the primer to the target (Tyler et al., 1997; Caetano-Anolles et al., 1992). In addition, at higher ratio of primer/DNA template, the amplification of artefactual bands in the control samples without DNA has also been mentioned (William et al., 1990). This phenomenon was observed in the results with ratios of M13 primer to DNA template of 0.3  $\mu\text{mol l}^{-1} / 100 \text{ ng}$ , 0.4  $\mu\text{mol l}^{-1} / 100 \text{ ng}$ , 0.5  $\mu\text{mol l}^{-1} / 100 \text{ ng}$  and 1  $\mu\text{mol l}^{-1} / 100 \text{ ng}$  (Figure 3A, 4A and 4B). This can be explained by the possible contaminants or nonspecific products (primer-dimers) (Singh et al., 2010; Harini et al., 2008; Padmalatha and Prasad, 2006; Raghunathachari et al., 2000; Pan et al., 1997). The first possibility was excluded since it was checked by repeating our experiment with new reagents. Therefore, the second possibility is the most probable. Moreover, the artefactual bands in the negative control and extra bands (of N8-2 and K1-15 strains) increased by raising the concentration of M13 primer.

Therefore, the extra bands of N8-2 K1-15 strains generated with high M13 primer concentration could not



**Figure 4.** (A) RAPD profiles of *S. thermophilus* strains with 0.5  $\mu\text{mol}$  l-1 of M13 primer. (5) Negative control; (9), N8-2 strain; (13), K1-15 strain. (B) RAPD profiles of *S. thermophilus* strains with 1  $\mu\text{mol}$  l-1 of M13 primer. (2), negative control; (5), N8-2 strain; (7), K1-15 strain.



**Figure 5.** RAPD profiles of *S. thermophilus* strains with M13 primer was generated five months later. The black lines indicate the reproducible bands detected five months before. We can see the extra faint bands of strains (5), N4-3 and (6), Yo-mix 410- 3 without black lines.

be only due to the amplification of rare or inaccessible sites on DNA. The primer-dimers phenomenon could also affect RAPD strain's profiles. These nonspecific products could be amplified in the presence of a DNA template, as has been already reported by Pan et al. (1997).

The optimized conditions led to RAPD pattern's bands reproducibility with OPI-02MOD, M13, XD9 primers. Such bands correspond to perfect annealing sites amplified with good efficacy or present in multiple copies in the genome (Davin-Regli et al., 1995). When the experiments were repeated five months later, identical strains patterns were generated with OPI-02 MOD. However, the reproducible bands persisted in the profiles that have resulted from amplification with XD9 and M13 primers with the appearance of extra faint bands. This cannot be due to the contamination or degradation of stocked DNA over time (Black et al., 1992). Whereas, the five month's DNA samples gave identical patterns with OPI-02 MOD, contrary to XD9 primer. Moreover, the amplification of new extracted DNA of *S. thermophilus* LMG 18311, with XD9 primer, generated an extra faint band. However, it yielded identical profiles with OPI-02 MOD and M13 primers.

The study by Saunders et al. (2001) demonstrated that thermal cycler calibration and temperature monitoring have an important effect on RAPD profiles reliability and repeatability. The extra faint bands obtained could be a consequence of slight variation in annealing temperature or calibration of the thermocycler generating the amplifi-

cation of nonspecific DNA target sites.

In conclusion, OPI-02 MOD primer seemed to be the most reproducible, followed by M13 primer. XD9 primer showed the worst performance. Authors noted that, some primers are extremely reliable whereas others gave notoriously inconsistent results (Tyler et al., 1997; Grosberg et al., 1996; Bielawski et al., 1995; Penner et al., 1993). This could be explained by the presence of high amount of specific target sequence for OPI-02 MOD primer on *S. thermophilus* DNA template as compared to nonspecific sequences, and this could have a major effect on its good reproducibility.

One of the purposes of optimizing RAPD-PCR conditions is to increase the specificity and efficiency of primer-template interactions. Higher number of specific sites than nonspecific sites should be reasonably in favor of primer/specific target site interaction. Subsequently, varying slightly the temperature or calibration of thermocycler causes RAPD-PCR intralaboratory or interlaboratory non reproducibility. However, such slight variations may not have a significant consequence on the reproducibility for such primer/DNA template (OPI-02 MOD/*Streptococcus thermophilus* DNA). Ramos et al. (2008) concluded that, at least in part, problems of repeatability attributed to RAPD markers could be due to bias in the selection of loci and primers and not necessarily the RAPD technique per se.

The reproducibility problem of RAPD-PCR can be overcome, in principle, by using rigid laboratory protocols and doing repeatability tests. The purpose of such tests is to repeat the analyses and retain only the bands that appear in both initial and later screenings for further analyses (Telles and Soares, 2007; Santos et al., 2007). Ramos et al. (2008) developed a procedure to optimize repeatability and avoid bias in sampling loci for genetic analyses based on RAPD data.

Using such reproducible OPI-02 MOD primer in studying *S. thermophilus* genetic diversity, at different laboratories or at different time periods within the same laboratories can be efficient. The strict standardization of RAPD-PCR conditions has to be applied using the same brand of Taq DNA polymerase, same model of Thermocycler, controlling the temperature and calibration of the Thermocycler.

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