

Probiotic lactobacilli in fermented dairy products: Selective detection, enumeration and identification scheme

Nasim Farahmand, Labia I. I. Ouoba, Shahram Naghizadeh Raeisi, Jane
Sutherland, Hamid B. Ghoddusi

Microbiology Research Unit, School of Human Sciences, London Metropolitan University, London
N7 8DB, UK

ABSTRACT

A selection of 36 commercial probiotic fermented dairy products from UK and Europe markets were evaluated for the numbers, types and viability of *Lactobacillus* strains against the stated information on their packages. A comparative study was carried out on selectivity of MRS-Clindamycin, MRS-Sorbitol and MRS-IM Maltose, to select the right medium for enumeration of probiotic *Lactobacillus*. Based on selectivity of medium for recovery of the targeted lactobacilli and also simplicity of preparation, MRS-Clindamycin was chosen as the best medium for enumeration of probiotic *Lactobacillus* in fermented milks. The results of enumeration of lactobacilli showed that 22 out of a total 36 tested products contained more than 10^6 colony forming units/g at the end of their shelf-life, which comply with the recommended minimum therapeutic level for probiotics. Rep-PCR using primer GTG-5 was applied for initial discrimination of isolated strains, and isolates, which presented different band profile, were placed in different groups. The isolated *Lactobacillus* spp. were identified mainly as *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus paracasei* by analysis of partial sequences of the 16S ribosomal RNA and *rpoA* genes. In conclusion, it is unknown to recommend the adequate number of probiotic bacteria to be consumed to ensure the beneficial properties.

KEYWORDS: Probiotic, Lactobacilli, Fermented dairy product, Identification, enumeration, and Rep-PCR.

1. INTRODUCTION

Certain dairy products are vehicles by which consumers receive adequate counts of probiotic lactobacilli (Vinderola and Reinheimer, 2000). Probiotic effects are dependent on the number of viable microbial cells that reach the human gut (Leahy et al., 2005). Therefore, their viability in the product is considered as an important prerequisite for achieving health effects.

There are various reports regarding the adequate number of probiotic microorganisms in different products in order to ensure the probiotic effects. The recommended quantity of probiotic lactobacilli that needs to be consumed for a health benefit varies in different studies (Guarner et al., 2008). Some of the suggested minimum levels of viable cells in dairy products are 10^5 CFU/g (Shah et al., 1995), 10^6 CFU/g (Ghoddusi and Robinson, 1996; Talwalkar and Kailasapathy, 2004) and 10^7 CFU/g (Korbekandi et al., 2011). It is not simple to keep a high number of viable probiotic bacteria in fermented milk throughout the shelf life, because their viability in the product matrix is influenced by numerous factors. Such parameters include temperature of storage condition, hydrogen peroxide (H_2O_2) which might be produced by other existing bacteria, dissolved oxygen content due to process conditions, pH of the final product and finally, strain variation that may be considered the most important factor for the survival of probiotic cultures in final product (Vinderola et al., 2002).

Probiotic lactobacilli are incorporated alone or in combination with other commercial cultures into specific dairy products. Interactions between microorganisms in co-cultured products cause difficulties in enumeration.

Lactobacillus acidophilus, *Lactobacillus casei* and *Bifidobacterium lactis* are the most frequently used strains in commercial probiotic products (Tabasco et al., 2007).

In the past few decades, many selective/differential media have been developed for accurate enumeration of *Lactobacillus* spp. in fermented milks. However, due to presence of closely related species of *Lactobacillus* spp. in probiotic products, the differential enumeration seems challenging and relies directly on differences in colonial morphology (Van de Castele et al., 2006).

There are also various instructions regarding the probiotic enumeration, but only a few are official protocols for lactobacilli for example ISO (2006). Enumeration in co-cultured products is more complicated than in products made with single culture. In mixed cultures, inhibitory agents are needed to suppress the interfering species in order to recover the target lactobacilli. However, one real concern is that some culture media that contain antibiotics might also restrict the growth of target lactobacilli and the counts may not be representative of the real number of viable cells present in the product (Ashraf and Shah, 2011). On the other hand, some antibiotics cannot inhibit the growth of all non-target bacteria (Novik et al., 2007). Several reports have revealed the misidentification of a number of strains belonging to some lactobacilli (Bull et al., 2013; Yeung et al., 2002).

The probiotic ability is often strain dependent and therefore accurate detection and identification of probiotic lactobacilli is required. Characteristics including phenotype, physiological and biochemical features and sequence comparisons of

16S rRNA gene have been suggested to make the identification of *Lactobacillus* species more reliable (Kwon et al., 2004). There are however taxonomic dispute and ambiguity among some lactobacilli due to the differences at nucleotide level in the 16S rRNA gene (Singh et al., 2009). It is therefore hard to differentiate between some species and strains of lactobacilli (Song et al., 1999) and some closely related groups of lactobacilli species are indistinguishable based on phenotype. Molecular identification methods on the other hand proven to be consistent, rapid, reliable and reproducible, compared to phenotypic methods. For example, species-specific oligonucleotide probes have been employed to identify various *Lactobacillus* species (Park and Itoh, 2005). Most genetic probes have been designed based on 16S rRNA or 23S rRNA genes (Sghir et al., 1998).

In general, there are some ambiguities in differentiation of specific lactobacilli. According to the study by Singh et al. (2009), there are similarity at nucleotide level in the 16S rRNA gene in some lactobacilli, such as *Lb. acidophilus*, *Lb. casei*, *Lb. plantarum* and *Lb. delbrueckii*, making hard to distinguish them in a mixed culture. It has been reported that sometimes *Lb. gasseri* and *Lb. johnsonii* are difficult to differentiate from each other even by molecular methods (Walter et al., 2000). *Lactobacillus plantarum* and *Lb. pentosus* have greater than 99% similarity with only 0.3% difference in their 16S rRNA sequences (Quere et al., 1997). But some alternative molecular markers have been used for discrimination among these species. Torriani et al. (2001) could differentiate *Lb. plantarum*, *Lb. pentosus*, and *Lb. paraplantarum* through *recA* gene sequence comparison. In their study, the sizes of the amplicons were 318, 218 and 107 bp for *Lb. plantarum*, *Lb. pentosus*

and *Lb. paraplantarum*, respectively. An unambiguous identification of strains belonging to *Lb. plantarum*, *Lb. pentosus*, and *Lb. paraplantarum* becomes possible in a single reaction using this strategy.

Recent research into the relatedness of species in the *Lb. acidophilus* group has used sequence analyses of genes, such as 16S rRNA, *rpoA*, *pheS* (Naser et al., 2007), *groEL* (Claesson et al., 2008) and *tuf* (Ventura et al., 2003).

The aim of the work described in this research was to isolate, enumerate and identify *Lactobacillus* spp. in commercial probiotic dairy products from the UK and European supermarkets using genotyping methods. In addition, accuracy of the label descriptions for fermented milk products was assessed.

2. MATERIALS AND METHODS

2.1 General/ selective/ elective media

MRS agar (CM0361, Oxoid, Basingstoke, UK) was used as general medium. MRS agar supplemented with 0.1 mg l⁻¹ clindamycin (C5269, Sigma, UK) was prepared according to ISO (2006) for enumeration of *Lb. acidophilus*, *Lb. rhamnosus*, *Lb. casei* and *Lb. paracasei*. MRS agar supplemented with 20 g l⁻¹ sorbitol (Ong and Shah, 2009) to replace the original dextrose for elective enumeration for *Lb. acidophilus*. MRS-IM Maltose agar (Dave and Shah, 1996) was used for elective differential enumeration of *Lb. acidophilus* and *Lb. casei*. All elective or selective supplements were purchased from Sigma (Poole, UK).

2.2 Microbial culture

Three commercial cultures (*Lb. acidophilus* La5, *Lb. delbrueckii* subsp. *bulgaricus* Lb12 and *Lb. casei* C431) kindly provided by Chr. Hansen. Type strain *Lb. delbrueckii* subsp. *bulgaricus* 11778, *Lb. acidophilus* 701748, *Lb. casei* subsp. *casei* 11970 and *Lb. paracasei* subsp. *paracasei* 700151 purchased from National Collections of Industrial, Marine and Food Bacteria (NCIMB).

2.3 Commercial probiotic products

Thirty six commercial fermented milks claiming to contain probiotic *Lactobacillus* strains were purchased from UK and European supermarket, transported to the laboratory and stored at 4 °C. Samples from countries outside the UK were purchased and sent to the UK in a cool box. Table 1 shows details of the tested products.

2.4 Measurement of pH value

The pH of the initial and final (on the expiry date) samples of the fermented milks was measured using a Whatman PHA 2000 pH meter.

Table1

2.5 Determination of viable cell count of *Lactobacillus* spp. in the fermented milks

Four pots of each product were purchased. All products were analysed on the day of purchase (2 pots) and again on their expiry date (2 pots) using unopened product each time. One gram of homogenised sample was mixed with 9 ml of Maximum Recovery Diluent (MRD) (CM0733, Oxoid, Basingstoke, UK) and vortexed. Dilutions up to 10^{-8} were made using MRD. Agar plates were divided into 4 sections using a marker and 25 μ l of each dilution was spread on to each quarter of MRS, MRS-IM Maltose, MRS-Sorbitol and MRS-Clindamycin in duplicate. The plates were then incubated for three days at 37 °C in an anaerobic cabinet (Don Whitley, Skipton, UK) using an atmosphere of 80% nitrogen, 10% hydrogen and 10% carbon dioxide.

2.6 Isolation and storage of the isolates

Two to four typical colonies grown on MRS-Clindamycin were randomly harvested from each product and streaked on MRS agar. Following overnight anaerobic incubation at 37°C, the single colonies were streaked on MRS agar for the second time and incubated in the same conditions. One pure isolated colony was picked up and inserted aseptically into a cryovial (Micro bank, Pro-Lab Diagnostics, Neston, UK) following manufacturer's instructions and stored at - 20 °C.

2.7 Grouping and identification of isolates

2.7.1 DNA Extraction

Fresh colonies of isolates were grown from cryovial beads following two consecutive streaks on MRS agar. The DNA was extracted using InstaGene

(BioRad, Hemel Hempstead, UK) according to the manufacturer's instructions and stored at -20 °C.

2.7.2 Differentiation (grouping) of the isolates using rep-PCR

Repetitive element Sequence - Based polymerase chain reaction (Rep-PCR) was applied for differentiation of isolates by the method of Ouoba *et al.* (2008) (Ouoba *et al.*, 2008). Rep-PCR was undertaken in 25 µl of reaction mixture containing 2 µl of DNA template, 2.5 µl of 10 × PCR buffer (Applied Biosystems, UK), 4 µl of dNTP (1.25 mmol l⁻¹; Promega, UK), 2 µl of MgCl₂ (25 mmol l⁻¹; Applied Biosystems, UK), 4 µl of GTG-5 (5 pmol µl⁻¹) primer (GTG-GTG-GTG-GTG-GTG), 2.5 U of Taq polymerase (5 Uµl⁻¹; Applied Biosystems, UK) and 10.25 µl of autoclaved high purity water (Sigma, UK). Amplification consisted of 30 PCR cycles in a thermocycler (GeneAmp PCR 2700 system). The cycling was programmed as follows: initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 45°C for 1 min and elongation at 65°C for 8 min. Also, final extension at 65°C for 16 min ended the rep-PCR and the amplified product was cooled at 4°C. The DNA fragments were separated by applying 10 µl of each PCR product with 2 µl of loading buffer (Sigma, UK) on a 1.5% agarose gel (BioRad, UK). A DNA molecular marker (Sigma, UK) was included as standard for the calculation of the size of the fragments. The gel was run in 1 × Tris-Borate-EDTA (TBE) buffer (Sigma, UK) for 2 h at 120 V and photographed using a UV transilluminator. The DNA profiles obtained were clustered using the Bionumerics system (Bio-Numerics 2.50, UPGMA Pearson Correlation, Applied Maths, Sint-Martens-Latem, Belgium).

2.7.3 Identification of the isolates by sequence analysis of 16S ribosomal RNA gene

Following rep-PCR screening and arranging the isolates into different groups, further identification was carried out using 16S rRNA gene sequencing according to the method described by Ouoba *et al.* (2008).

A search was performed in GenBank database using Blast program (National Center for Biotechnology Information, Bethesda, MD, USA). Sequences of representative isolates from each rep-PCR group were compared with the GenBank/DDBJ Nucleotide Sequence Data Libraries.

2.7.4 Identification of bacteria by *rpoA* gene sequencing

Primarily, all randomly selected isolates were identified by 16S rRNA gene sequencing, however, where it was not possible to distinguish between closely related species (i.e. *Lb. casei* and *Lb. paracasei*), amplification and sequencing of *rpoA* gene was carried out.

The amplification of *rpoA* gene was carried out using the forward primer *rpoA*-21-F (5`ATG ATTC GAGA TTT GAA AAA CC 3`) and reverse primer *rpoA*-23-R (5`ACACT GTGA TTGA ATD CCGAT GCGA CG 3`) (Anyogu *et al.*, 2014).

2.8 Statistical analysis

All data were analysed statistically using SPSS version 20.0 (SPSS Inc. 444 North Michigan Ave., Chicago, Ill., U.S.A.). The two-tailed unpaired Student's t-test was performed to determine differences at levels of significance of $P < 0.05$. Experiments were replicated at least three times.

3. RESULTS

3.1 Enumeration of *Lactobacillus* spp. in commercial fermented milk

In the present study MRS agar, MRS-IM Maltose agar, MRS-Sorbitol agar and MRS-Clindamycin agar were used for enumeration of probiotic lactobacilli in 36 probiotic dairy products (Figure 1 a, b, c, d, e and f). MRS agar was used as a non-selective reference medium. MRS-IM Maltose, MRS-sorbitol and MRS-clindamycin are quite common as selective and elective media for counting of *Lactobacillus* species. The shape and size of colonies of *Lactobacillus* species vary on different media. *Lactobacillus acidophilus* gives star shaped, irregular small colonies, and *Lb. casei* gives larger regular colonies in MRS-IM Maltose agar. *Lactobacillus acidophilus* forms small, rough, brownish, dull colonies of 0.1 to 0.5 mm in MRS-Sorbitol agar.

MRS-IM Maltose agar did not give a good recovery of the lactobacilli even when compared with the control medium (MRS agar) and the other MRS variants. In this medium 19 samples had lower than the estimated detection limit (log 2.7 CFU/g). Therefore, it was not considered as a suitable medium due to low recovery of the lactobacilli.

MRS-Sorbitol showed higher viable counts than MRS-Clindamycin. Recovery of lactobacilli below the noted detection limit (log 2.7 CFU/g) was seen on MRS-sorbitol and MRS-clindamycin in 2 and 3 samples, respectively.

Comparison of the results indicated that in eight products (P8, P9, P11, P13, P14, P31, P32 and P35) the viable counts on MRS-Sorbitol were higher than on MRS-Clindamycin, while in six products (P15, P17, P26, P29, P34 and P36) viable counts on MRS-Clindamycin were higher than on MRS-Sorbitol.

Thirty one out of 36 fermented milks contained more than log 6 CFU/g on at least one medium at the time of purchase (Figures 1 a, b, c, d, e and f). The number of *Lactobacillus* recovered on MRS-Clindamycin agar at the expiry dates compared to the purchase dates are shown in Figures (2 a, b, c and d). The number of *Lactobacillus* spp. declined almost in all samples. The highest decline was log 2.62 CFU/g in products P15 and P18. However, at the end of the shelf life, 22 (61.1%) of the tested samples contained greater than log 6 CFU/g colonies. Out of the remaining 14 with less than log 6 CFU/g, products P3, P4, P15, P18, P21, P22 and P23 contained an initial *Lactobacillus* spp. population of more than log 6 CFU/g, which had significantly decreased to less than log 6 CFU/g by the expiry date ($p < 0.05$). However, products P8, P11, P14, P27 and P32 contained less than log 6 CFU/g viable *Lactobacillus* spp. at the time of purchase. According to the results of the present study MRS-Clindamycin seems to be the most valuable selective culture medium for enumeration of probiotic lactobacilli and was selected for further experiments.

Figure 1 a,b,c,d,e and f

Figure 2 a,b,c and d

3.2 Differentiation of isolates by rep-PCR

A total of 85 isolates were selected from different media based on their shape, size and/or colour. These isolates along with the commercial and type strain *Lactobacillus* were grouped using rep-PCR resulting in 8 groups (Figure 3). Group A, as the major group, contained 51 isolates with the same DNA profile. Other groups included group B (22), C (6), D (5), E (4), F (1), G (1) and H (2) isolates.

In total, 20 isolates (representative of groups, A – H) were randomly selected from the above groups, and identified by partial sequencing of 16S rRNA and *rpoA* genes.

3.3 Identification of isolates by partial sequencing of 16S rRNA and *rpoA* genes

Random representatives of each group; A (6), B (3), C (4), D (2), E (2), F (1), G (1) and H (1) were analysed using the 16S rRNA gene and further experiments with *rpoA* gene sequencing were applied when 16S rRNA gene failed to provide accurate identification. Table 2 presents the results of identification using 16S rRNA and *rpoA* gene sequencing of the tested isolates, compared with the identities claimed on the product labels.

The isolates from group A were all identified as *Lb. acidophilus*, and isolates from group B were identified as *Lb. casei/paracasei*. As the 16S rRNA gene sequencing could not differentiate between *Lb. casei* and *Lb. paracasei*, sequencing of *rpoA* gene was used to discriminate between these two species. However, *rpoA* gene sequencing also could not differentiate between these two closely-related species.

Similarly, isolates from group C were identified as *Lb. casei/paracasei* by both, 16S rRNA and *rpoA* gene sequencing.

Isolates from group D, were identified as *Lb. johnsonii* and group E as *Lb. helveticus/gallinarum/suntoryeus* and *rpoA* gene sequencing could not differentiate between them. The only isolate from group F was identified as *Streptococcus thermophilus*. Groups G and H were identified as *Lb. helveticus/gallinarum/suntoryeus* by both 16S rRNA and *rpoA* gene sequencing.

Sequencing of *rpoA* gene in addition to sequencing of 16S rRNA was not able to discriminate between isolates from groups B, C, E, G and H. Therefore, the DNA profiles of unconfirmed isolates were compared with those of type strains and their identity confirmed according to their similarities with the type strains (Figure 3).

3.4 pH reduction during the shelf life

During storage, the pH in all samples decreased from 4.05 to 3.92. Therefore, the pH dropped constantly between 0.00-0.42 over the period of storage that is an indication of starter cultures activities (Table 2).

Figure 3

Table 2

4. DISCUSSION

The use of food as a carrier for probiotic organisms is of considerable interest to food manufacturers due to the claimed health-associated benefits of probiotics. However, maintaining high numbers of viable probiotics in fermented milks is not easy and a large quantity of probiotic cultures is needed to compensate for the likely losses of probiotics during the shelf life (Wang et al., 2009). Procedures for enumeration of lactobacilli have not been properly defined. Such a situation causes difficulties in quality control of the probiotic products containing *lactobacillus* species using the conventional enumeration technique. The suitability of various media to selectively enumerate lactobacilli has been examined in different studies. Although there are several

elective/selective media for isolation of lactobacilli, the levels of recovery of the lactobacilli are discordant with each other.

Oberg et al. (2011) reported that while MRS-Sorbitol is a medium designed for *Lb. acidophilus* in which sorbitol is the sole sugar, *Lb. casei* can also grow on the medium, although only at elevated incubation temperature (42 °C). At this temperature, the MRS-Sorbitol medium gave higher bacterial counts compared to the *Lb. casei* specific medium (*Lactobacillus casei* agar), indicating that it could be used to obtain the total LAB count at different temperature (Oberg et al., 2011). However, in our study colonies of target strains were recovered at 37 °C on MRS-Sorbitol agar. Due to the high recovery, no other recovery temperatures were employed.

MRS-Sorbitol demonstrated higher viable counts than MRS-Clindamycin, suggesting that MRS-Sorbitol might allow the growth of additional LAB. Shah (2000) stated that MRS-Sorbitol agar could not be used for selective enumeration of *Lb. casei* and *Lb. acidophilus* in products containing both bacteria.

This study also reports that MRS-IM Maltose is not an ideal choice for selective enumeration of lactobacilli since the recovery was low compared with other MRS variants.

MRS-Clindamycin has been proposed for enumeration of lactobacilli in different studies (Ashraf and Shah, 2011; Van de Castele et al., 2006). Furthermore, the International Organization for Standardization (ISO) (2006) recommended MRS-Clindamycin agar for the enumeration of *Lb. acidophilus* in dairy products in the presence of other probiotics including other lactobacilli, streptococci and

bifidobacteria (Ashraf and Shah, 2011). Simplicity of medium preparation and availability of the antibiotic supplement led to its consideration as the preferred medium compared to the other selective media. Hence, in our research, MRS-Clindamycin was chosen as a reliable medium to accurately enumerate *Lactobacillus* spp. in fermented dairy products, but we found that MRS-Clindamycin has limitations, since *S. thermophilus*, which is difficult to distinguish morphologically from *Lactobacillus* spp., will also grow.

Our research shows that on the purchase and the expiry dates respectively, 86% and 61% of tested samples contained the minimum recommended therapeutic level of log 6-7 CFU/g, concordant with the findings of Wang et al. (2010). Other researchers have also reported commercially probiotic dairy products with inadequate amounts of viable probiotics (Gilliland et al., 2002; Iwana et al., 1993; Schillinger et al., 2005), which in some cases may be attributable to disruption of the cold chain (Godward and Kailasapathy, 2003). In this study during cold storage, the number of *Lactobacillus* spp. in some samples decreased considerably. The most important contributing factors for loss of cell viability are decreasing pH during storage, presence of dissolved oxygen and presence of preservatives in the final products (Vinderola et al., 2002). In this study, the pH decline between the purchase and expiry date was in some cases noticeable. It could be due to continued fermentation process by LAB even in low temperatures (post acidification). However, no correlation was found between pH decline of samples and their probiotic counts.

The presence of dissolved oxygen might be the other important reason for drop in viability of cell count in fermented milk (Shah, 2000). The majority of tested products in this study were stirred yoghurts, in which air could have been

incorporated when the yoghurt was mixed with the fruit compote. In addition, some of the commercial fruit products contain preservatives to control contamination and this might affect the viability of the probiotic cells (Kailasapathy, 2008).

Based on results obtained in this research which confirmed lower counts of probiotic cultures approaching the end of shelf life, and supported by the study of Jayamanne and Adams (2006), it is recommended that probiotic fermented products need to be consumed earlier than the expiry date to ingest maximal numbers of probiotic bacteria.

Although there are no universally established standards for microbial content and health claims for probiotic products, the manufacturers should at least clearly express the genus, species and strain of the probiotic microorganism(s) and also the minimum viable count of each probiotic strain at the end of shelf-life (Guarner et al., 2008; Reid et al., 2001). To ensure that the consumers benefit from commercial probiotic products, it is necessary to confirm the identity of the claimed organisms at species/strain level and that they are present in the product in appropriate numbers before consumption. Some of the tested products in this study presented inadequate information on the labels. Microbial investigations of probiotic products by others have indicated that the number and identity of recovered species do not always correspond to those stated on the labels of products (Hamilton-Miller and Shah, 2002; Temmerman et al., 2003).

Identification of probiotic species used in carrier products should be verified in support of claimed health benefits. To obtain accurate and reliable identification

of the probiotic species, molecular techniques should be applied. It has been suggested that DNA profiling by PCR based methods are the best means for identification of probiotic bacteria at strain level (Gueimonde et al., 2004; Tabasco et al., 2007). Many mis-identifications of probiotic microorganisms maybe due to the use of solely phenotypic methods for taxonomic characterization (Ouwehand et al., 2006).

The rep-PCR fingerprinting profile revealed relative genetic differences between the tested isolates. In this study, 85 isolates from fermented milks were grouped based on their DNA patterns by rep-PCR, and 20 isolates out of 85 were selected for identification by sequence analysis of 16S rRNA. Amplification of the 16S rRNA gene often provides a rapid and reliable tool for bacterial identification without the need for phenotypic characterization. However, 16S rRNA sequencing cannot discriminate closely related species. Thus, sequencing of alternative genes such as *rpoA* with more discriminatory power has been proposed (Koo et al., 2003; Santos and Ochman, 2004).

In this research, amplification and sequencing of the *rpoA* gene did not provide enhanced discriminatory information for the tested isolates compared to use of 16S rRNA gene sequences. Sequencing of other genes such as *rpoB* and *pheS* would enhance discriminatory potential, enabling differentiation of strains with close genetic profiles. Anyogu et al. (2014) stated that sequencing of the *pheS*, *rpoA* and *rpoB* genes along with 16S rRNA gene sequencing provide a better identification of LAB and *Bacillus* isolate.

5. CONCLUSION

Evaluation of MRS-IM Maltose, MRS-Sorbitol and MRS-Clindamycin as selective media for enumeration of probiotic *Lactobacillus* spp. in commercial fermented milks indicated that MRS-IM Maltose and MRS-Sorbitol were not appropriate media due to the possible growth of additional LAB. However, the advantage of MRS-Clindamycin is its simplicity and ease of preparation. Our survey of commercial probiotic dairy products in the UK/European market has shown that the most frequent species used in the probiotic products was *Lb. acidophilus* followed by *Lb. casei*. Some other strains were identified which are not popular in fermented dairy products. Commercial use of other useful probiotics such as *Lb. helveticus*, *Lb. plantarum* and *Lb. fermentum* is recommended in dairy producers to provide more diversity amongst probiotic products. Although 16s and *rpoA* gene sequences have been extensively used to classify *Lactobacillus* strains, identification of lactobacilli at species and/or subspecies level using these gene sequences is proven to be difficult. Therefore, analysis of other gene sequences might be helpful as alternative genomic markers to the aforementioned gene sequencing techniques and may have a higher discriminatory power for reliable identification of *Lactobacillus* spp.

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