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REVIEW

Advantages and limitations of potential methods for the analysis of bacteria in milk: a review

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Abstract Contamination concerns in the dairy industry are motivated by outbreaks of disease in humans and the inability of thermal processes to eliminate bacteria completely in processed products. HACCP principles are an important tool used in the food industry to identify and control potential food safety hazards in order to meet customer demands and regulatory requirements. Milk testing is of importance to the milk industry regarding quality assurance and monitoring of processed products by researchers, manufacturers and regulatory agencies. Due to the availability of numerous methods used for analysing the microbial quality of milk in literature and differences in priorities of stakeholders, it is sometimes confusing to choose an appropriate method for a particular analysis. The objective of this paper is to review the advantages and disadvantages of selected techniques that can be used in the analysis of bacteria in milk. SSC, HRMA, REP, and RAPD are the top four techniques which are quick and cost-effective and possess adequate discriminatory power for the detection and profiling of bacteria. The following conclusions were arrived at during this review: HRMA, REP and RFLP are the techniques with the most reproducible results, and the techniques with the most discriminatory power are AFLP, PFGE and Raman Spectroscopy.

Keywords Bacteria · Analysis · Identification · Quantification · Milk · PCR

Research highlight Contamination concerns in the dairy industry are motivated by disease outbreaks.
Testing is of importance to the milk industry regarding quality assurance.
Numerous methods for analysing the microbial quality of milk are available.
Advantages and disadvantages of testing techniques must be considered in choosing.
HRMA, REP and RFLP are the most reproducible discriminatory.

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Introduction

Bacteria from silage are considered to be an important source of contamination of raw milk with spores, and a study on the populations of aerobic spore formers isolated from grass and maize silage has validated this hypothesis (Te Giffel et al. 2002). Bacteria spores have the ability to survive high processing temperatures and proceed to growth in the final product (Tewari and Abdullah 2014). Contamination concerns in the dairy industry are motivated by outbreaks of disease in humans and the inability of thermal processes to eliminate bacteria completely in processed products (Oliver et al. 2005). Hazard Analysis Critical Control Point (HACCP) principles are an important tool used in the food industry to identify and control potential food safety hazards in order to meet customer demands and regulatory requirements (Murphy 2010). Milk testing is of importance to the milk industry regarding quality assurance and monitoring of processed products by researchers, manufacturers and regulatory agencies.

Due to the availability of numerous methods used for analysing the microbial quality of milk in literature and differences in priorities of stakeholders, it is sometimes confusing to choose an appropriate method for a particular analysis (Jasson et al. 2010; Matsui et al. 2011). The objective of this paper is to review the advantages and disadvantages of selected

techniques that can be used in the analysis of bacteria in milk. This paper will assist stakeholders in dairy research, processing and quality control in choosing techniques that will suit their needs.

Molecular techniques used for identification and profiling

Single strand conformation polymorphism

The single strand conformation polymorphism (SSCP) analysis is based on the direct analysis of the 16S rRNA gene pool after polymerase chain reaction (PCR) amplification, separation in non-denaturing electrophoresis and analysis using genescan software. The genescan software analyses the single strand conformation of any gene or DNA segment to generate a profile that is specific to that particular strand. The SSCP method has been used in distinguishing and classifying the bacterial diversity of raw milk (Verdier-Metz et al. 2009). The SSCP method is advantageous in that it is quick, simple and cost-effective (Saubusse et al. 2007). Furthermore, it has been found to be highly reproducible, more discriminatory than RFLP and less discriminatory than AFLP and DNA sequencing (Olivares-Fuster et al. 2007). RFLP coupled with SSCP analysis is a simpler technique with discriminatory power compared to AFLP or DNA sequencing (Olivares-Fuster et al. 2007).

The disadvantage of this technique lies in the fact that there is currently no theoretical model for predicting the exact conformation of a DNA fragment under different parameters such as mutation, size of DNA fragment, G and C content, porosity of gel matrix, DNA concentration, ionic strength and pH. Furthermore, the complexity and variability in the balance of different peaks from one milk sample to another have been observed (Verdier-Metz et al. 2009). However, the standardisation of analytical parameters can lead to reproducibility of results as well as the detection of specific bacteria.

High resolution melt analysis

High resolution melt analysis (HRMA) of DNA fragments is a simple, flexible, low-cost, easy-to-use technique with high specificity used in the screening of bacteria variants by analysing the melting curve of DNA fragments after PCR amplification (Vossen et al. 2009). HRMA has been used as a rapid approach to identify the dominant mesophilic and thermophilic aerobic bacteria in various dairy products by analysing the V3 and V6 variable regions in the 16S rDNA and to match the melting profile with that of known reference bacteria (Chauhan et al. 2013). The advantage of the HRMA analysis is that it can detect small changes in a DNA fragment or sequence, making it suitable for discerning variations between bacteria species (Thomsen et al. 2012). Furthermore, the HRMA analysis has been found to be highly reproducible, with a discriminatory power similar to that of AFLP (Naze et al. 2010). The disadvantage of this technique is that it is possible for different heterozygote genes to produce melting curves that are similar in a situation where genetic homology in DNA segments is being analysed. It is advisable to use housekeeping genes for HRMA analysis (Chauhan et al. 2013).

Repetitive element palindromic PCR

Repetitive element palindromic (REP) PCR is a molecular typing technique based on designing primers to repetitive DNA sequences specific to a particular bacteria species. REP primers such as 5′ III ICG ICG ICA TCI GGC 3′ and 5′ ICG ICT TAT CIG GCC TAC 3′ have been used to distinguish eleven *Bacillus* strains isolated from milk (Guillaume-Gentil et al. 2002).

An advantage of REP is that it can easily be adapted into kit-based semi-automated setup in which a microfluidics chip can be used for fractionation and detection of the DNA amplicons. Furthermore, this technique is rapid and reproducible and can distinguish between related bacteria strains. It is low in cost and easy to use and can generate results in real time (Ross et al. 2005). The REP-PCR has been found to be less discriminatory compared to PFGE, making it suitable for intial screening of bacteria species (Ross et al. 2005). Furthermore, it has been found to have similar discriminative potential compared to RAPD analysis with 3 to 4 primers but is more discriminatory when compared to DGGE (Anderson et al. 2010; Jersek et al. 2008). The disadvantage of REP-PCR lies in the expertise required to conduct analyses (Ross et al. 2005).

Random amplified polymorphic DNA

Random amplified polymorphic DNA (RAPD) is a PCR-based technique which is used to distinguish between intra- and interspecific differentiations of bacteria associated with foods under controlled conditions. The identification of new isolates using this technique can be done by comparing its RAPD fingerprint with a data base of RAPD fingerprints (Rossetti and Giraffa 2005). In this technique, the genomic DNA from milk isolates is used as a template for PCR fingerprinting using a suitable primer such as the M13 primer with sequence 5′-GAGGGTGGCGGTTCT-3′ following an optimised protocol like that used to differentiate between and identify lactic acid bacteria (LAB) isolated from dairy products (Rossetti and Giraffa 2005). The accuracy of species identification by the RAPD technique can be improved by the combination of RAPD with other kinds of genotypic methods such as RFLP (Yang et al. 2013). Isolates of *Escherichia coli* O157:H7 strains have been distinguished by RAPD using single 10-mer oligonucleotides GEN15001 (5′-GTGCAATGAG-3′).

The advantages of RAPD include its appropriateness to analyse anonymous genomes, efficiency, low cost and quickness and its requiring less expertise in conducting analysis (Hadrys et al. 1992). Based on the ease of usage, cost, technical labour, speed and amount of DNA needed, the RAPD technique is much preferred in profiling studies over RFLP. However, based on the degree of polymorphism, the precision of genetic distance estimates and the statistical power of the test, there are insignificant differences between the two (Galal 2009; Garcia et al. 2004). The disadvantage of this method is the poor reproducibility of fingerprints, and it requires strict standardisation of PCR conditions considering that the utilisation of different concentrations of DNA polymerases, DNA template and primer ratios or annealing temperatures can lead to differences in the final results (Thangaraj et al. 2011).

Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) involves the amplication of a DNA segment with primers followed by digestion of the amplified segment with restriction enzymes and analysis by gel electrophoresis. The amplification and restriction of the 16S rDNA gene of 171 strains of milk-related bacteria with *Alu*I, *Hae*III, *Bsma*I, *TspR*I and *Hinf*I have been used to distinguish between *Lactobacillus* species (Yu et al. 2009a). RFLP is advantageous in being a fast, simple and accurate molecular tool for the profiling and identification of population (Martya et al. 2012). This technique can be used to detect single nucleotide polymorphism (SNP), and the analysis of protein-coding genes of *Lactobacillus delbruckii* by RFLP has been found to be an effective tool for distinguishing very closely related strain subpopulations within *L. delbrueckii* (Ota et al. 2009; Giraffa et al. 2003). Like many other fingerprinting techniques, RFLP has lower discriminatory power and is more expensive to run compared to RAPD (Smith et al. 2002). The typing of *Salmonella gallinarum* has also indicated that RFLP has good repeatability (Taddele et al. 2011). Amplified ribosomal DNA restriction analysis (ARDRA) is an RFLP technique which involves the amplification and restriction of the 16S rDNA genes. Gene fragments of 16S rDNA of strains representing specific patterns have been used to profile bacteria species (Carmen Collado and Hernandez 2007).

Terminal restriction fragment length polymorphism

Terminal restriction fragment length polymorphism (T-RFLP) is an extension of RFLP, and in this technique the 5′ end of one or both of the primers used to amplify a gene segment of interest is labelled with a fluorescent dye such as fluorescein amidite (6-FAM) (Liu et al. 1997). The generated PCR product can be restricted with one or more restriction enzymes of choice and fragment separated using a DNA sequencer as in RFLP. The sizes of the different terminal fragments with the fluorescent dye are determined using a fluorescence detector. T-RFLP has been shown to be sensitive in analysing the gut microbiota composition by amplifying 16S rRNA genes using labelled universal primers ENV1 (5′-6-FAM-AGA GTT TGA TII TGG CTC AG −3′, *E. coli* nr. 8–27) and ENV 2 (5′-CGG ITA CCT TGT TAC GAC TT-3′, *E. coli* nr. 1511–1492) (Sjöberg et al. 2013). Most of the advantages and limitations of this technique are similar to those of RFLP. However, this technique has the unique advantage of being able to give the relative amounts of bacteria flora of a sample containing different bacteria and is suitable for the profiling a mixed bacteria culture without prior culturing (Yu et al. 2009b). Furthermore, the more restriction enzyme used the better the resolution of microbial profiles (Liu et al. 1997).

Amplified fragment length polymorphism

Amplified fragment length polymorphism (AFLP) analysis is a PCR-based DNA fingerprinting technique consisting of digesting genomic DNA with restriction enzymes followed by ligation to double-stranded adaptors to create fragments with the same adaptors. Primers specific to the adaptors are then used to amplify individual fragments, which are then analysed by means of electrophoresis (Fry et al. 2009). The advantage of this method lies in its discriminatory power, and it has been found to have a higher discriminatory power than RAPD-PCR in a study conducted on isolates of *L. plantarum* and *Streptococcus thermophiles* (Di Cagno et al. 2010; Lazzia et al. 2009). Further, the AFLP analysis can be completed within 24 h and is less likely to produce inconsistent results, unlike PFGE analysis, which often requires 3 to 4 days to complete and is more likely to generate inconclusive results due to DNA degradation (Klaassen et al. 2002). The disadvantage of this method lies in its variation in precision of fragment sizes, which leads to suboptimal reproducibility (Fry et al. 2009).

Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis (DGGE) is a technique in which DNA fragments are separated with the aid of a denaturing buffer such as urea and formamide during electrophoresis. Primers used in DGGE are designed to ensure that a GC clamp, a high melting domain, is inserted into the amplified product prior to gel separation (Sheikha et al. 2011). Universal primers to the 16S rRNA gene can be used to amplify the segment of any bacteria prior to DGGE analysis. This technique has been used to profile the thermophilic bacteria diversity from a particular geographical area (Delgado et al. 2013). The major

advantage of DGGE over other profiling techniques is that it is possible to excise band from gel for amplification and sequencing (Sheikha et al. 2011) and it is robust and valuable as a first-line test (Tardy et al. 2009). The main disadvantage of DGGE lies in the fact that different DNA sequences of different bacteria species can display the same separation as a result of the same GC contents (Muyzer et al. 2004). Furthermore, there is a possibility that intra-specific and intra-isolate heterogeneity in the genome of bacteria could result in the production of different band patterns for the same species (Nakatsu et al. 2000). DGGE has been found to be less discriminatory than PFGE and RAPD in a study which involves the subtyping of *Campylobacter jejuni* (Nielsen et al. 2000).

Pulsed field gel electrophoresis

Pulsed field gel electrophoresis (PFGE) is a fingerprinting technique whereby large fragments of DNA molecules are separated by applying an electric field that changes direction periodically in a gel matrix. Similar to REP, the PFGE method has been found to discriminate between subtypes of *E. coli* O157:H7 although it is more time consuming and unnecessary if subtyping is not required (Hahm et al. 2003). This technique is similar to but more discriminatory than DGGE and has been used to establish the microbial diversity of lactic acid bacteria as well as the prevalence of *Salmonella* spp. using milk filters from a sub-set of milk-producing commercial dairy herds in the southern region of Ireland (Murphy et al. 2008; Nielsen

Table 1 A summary of the advantages and limitations of potential methods for the identification and profiling of bacteria in milk

Single strand conformation polymorphism (SSCP)	**Advantages:** Quick, simple and cost-effective; highly reproducible; more discriminatory than RFLP; RFLP coupled with SSCP analysis is a simpler technique with discriminatory power compared to AFLP or DNA sequencing.
	Limitations: Unknown parameters (mutation, size of DNA fragment, G and C content, porosity of gel matrix, DNA concentration, ionic strength and pH can affect analysis; less discriminatory than AFLP and DNA sequencing; complexity and variability in the balance of different peaks from one milk sample to another.
High resolution melt analysis (HRMA)	**Advantages:** Simple, flexible, low-cost and easy-to-use; high specificity; it can detect small changes in a DNA fragments; highly reproducible; discriminatory power is similar to that of AFLP.
	Limitation: It is possible for different heterozygote genes to produce melting curves that are similar.
Repetitive element palindromic (REP) PCR	**Advantages:** It can easily be adapted into kit-based semi-automated setup in which a microfluidics chip; it is low-cost and easy to use; quick and reproducible; high specificity; similar discriminative potential compared to RAPD; more discriminatory power compared to DGGE.
	Limitation: Expertise is required to conduct analyses.
Random amplified polymorphic DNA (RAPD)	**Advantages:** Quick, simple and cost effective; high specificity; suitable for the analysis anonymous genomes.
	Limitations: Poor reproducibility of fingerprints; it requires strict standardisation of reaction parameters.
Restriction fragment length polymorphism (RFLP)/ Terminal-RFLP	**Advantages:** High specificity; good reproducibility; T-RFLP is able to give the relative amounts of different bacteria flora in a sample
	-Limitations: Low discriminatory power; more expensive to run compared to RAPD.
Amplified fragment length polymorphism (AFLP)	**Advantage:** Higher discriminatory power than RAPD-PCR.
	Limitations: More likely to generate inconclusive results due to DNA degradation; requires expertise to run analysis.
Denaturing gradient gel electrophoresis (DGGE)/ Pulsed field gel electrophoresis	**Advantages:** Possible to excise band from gel for amplification and sequencing; PFGE has been found to be more discriminatory than AFLP; RAPD and PFGE were found to have similar discriminatory powers.
	Limitations: DNA sequences of different bacteria species can display the same separation; DGGE has been found to be less discriminatory than PFGE and RAPD; PFGE is labour-intensive requiring up to 3 to 4 days for analysis.
DNA microarray	**Advantage:** It is rapid and specific.
	Limitation: Low signal intensity due to improper content of targeted DNA and probe can lead to inaccurate analysis.
Propidium monoazide-PCR	**Advantage:** It detects only live bacteria.
	Limitations: It can display all the inherent PCR limitations; false positive result can be obtained with a high level of dead bacteria cells

Sources: Anderson et al. 2010; Bouvier and Del Giorgio 2003; Cawthorn and Witthuhn 2008; Chauhan et al. 2013; Di Cagno et al. 2010; Fawley and Wilcox 2002; Fry et al. 2009; Galal 2009; Garcia et al. 2004; Giraffa et al. 2003; Hadrys et al. 1992; Jersek et al. 2008; Klaassen et al. 2002; Lazzia et al. 2009; Lin et al. 2005; Martin et al. 2012; Verdier-Metz et al. 2009; Moter and Göbel 2000; Muyzer et al. 2004; Nakatsu et al. 2000; Olivares-Fuster et al. 2007; Ota et al. 2009; Ross et al. 2005; Sheikha et al. 2011; Taddele et al. 2011; Thangaraj et al. 2011; Thomsen et al. 2012; Yu et al. 2009a, b

et al. 2000). Furthermore PFGE has been found to be more discriminatory than AFLP in a study conducted on *Streptococcus pneumoniae* (Ross et al. 2005). RAPD and PFGE were found to have similar discriminatory powers in a study conducted on *Clostridium difficile* (Fawley and Wilcox 2002). The main disadvantage of PFGE is that it is labour-intensive with up to 3 to 4 days required to complete the lengthy PFGE protocol, and it has a high chance of yielding inconclusive results when compared to AFLP (Klaassen et al. 2002).

DNA microarray

DNA microarray is a technique in which several thousand surface-immobilised DNA probes are used as a tool for the identification of microbes in a single hybridisation assay (Fukushima et al. 2003). DNA microarray has been used to detect *Listeria monocytogenes* and distinguish it from other *Listeria* spp. as well as other food-borne pathogens in milk (Bang et al. 2013). The advantage of the DNA microarray is that it is rapid and sensitive, and one protocol can be utilised to identify different targeted bacteria simultaneously on a single array (Lin et al. 2005). The major disadvantages of this technique are due to the fact that low signal intensity due to insufficient penetration and improper contact of the probe with targeted DNA segments and the fading away of fluorochromes upon excitation can lead to inaccurate analysis (Moter and Göbel 2000). Furthermore, variability can occur in the detection of a targeted bacteria cell as a result of methodological factors as well as systematic variation between major ecosystem types and cell physiology (Bouvier and Del Giorgio 2003).

Propidium monoazide-PCR

In this method a DNA-intercalating agent, propidium monoazide (PMA), is used to treat bacteria prior to DNA isolation. It penetrates the membranes of dead cells and forms stable DNA which cannot be amplified by PCR; hence only DNA from viable cells can be amplified and quantified (Yang et al. 2011). The advantage of this technique lies in the fact that it can discriminate between dead and live bacteria cells by selectively allowing the amplification, detection and quantification of DNA from only live cells in a sample (Cawthorn and Witthuhn 2008). This technique has been used to differentiate between viable and non-viable *Escherichia coli* cells killed at different pasteurising temperatures and to detect viable cells of *B. sporothermodurans* in UHT milk (Cattani et al. 2013). In addition to all the inherent PCR limitations, another disadvantage of this method lies in the fact that a false positive result can be obtained with a high level of dead bacteria cells (Martin et al. 2012).

Table 2 A summary of the advantages and limitations of potential molecular methods for the identification and quantification of bacteria in milk

Real-time PCR (RT PCR)	Advantages: All inherent PCR benefits; it is faster than conventional PCR and can detect and quantify bacteria DNA in the same reaction vessel in real time.
	Limitations: All inherent PCR limitations.
PCR-enzyme-linked immunosorbent assay (ELISA)	Advantages: It is accurate in the detection of single nucleotide polymorphism (SNP) of different systems.
	Limitations: It takes relatively longer to complete the analysis and there is a risk of sample contamination after the PCR reaction when compared to real-time PCR; It is costly and labour-intensive; requires expertise to run analysis.

Sources: Espy et al. 2006; Knight et al. 1999; Perelle et al. 2004

Methods used for identification and quantification

Real-time PCR

Real-time (RT) PCR is a quantitative technique in which detection chemistries such as the TaqMan probe and SYBR Green are used to quantify bacteria. The use of SYBR Green and a FAM (6-carboxy-fluorescein)-labelled TaqMan probe specific to *Enterobacter sakazakii* have been used to detect *E. sakazakii* in infant formula and to quantify *C. tyrobutyricum* spores in dairy products (López-Enríquez et al. 2007; Liu et al. 2006). In addition to the inherent PCR benefits, the advantage of RT PCR lies in the fact that it is faster than conventional PCR and can detect and quantify bacteria DNA in the same reaction vessel in real time (Espy et al. 2006).

Table 3 A summary of the advantages and limitations of potential non-molecular methods for the identification and profiling of bacteria in milk

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS)	**Advantages:** Highly specific; it can be used to identify bacteria that are difficult to culture; it is not sensitive to alterations in the growth protocol of microorganisms.
	Limitations: It is expensive to run; the accuracy of detection can be affected by the cultivation time of the bacteria to be analysed.
Raman Spectroscopy	**Advantages:** Highly reproducible; high discriminatory power similar to that of PFGE.
	Limitations: The existence of backgrounds bacteria cells, yeast cells and other organic and inorganic particles of the same size can hamper the detection andidentification of the targeted bacteria.

Sources: Biswas and Rolain 2013; Cobo 2013; Harz et al. 2009; Wenning et al. 2014; Willemse-Erix et al. 2009

PCR- enzyme-linked immunosorbent assay

In the PCR-enzyme-linked immunosorbent assay (ELISA) technique, PCR primers which have been labelled to biotin are used to amplify a target DNA sequence in a normal PCR reaction in the presence of digoxigenin (DIG)-11-dUTP to generate DIG-labelled amplicons. These amplicons can be analysed in *Streptavidin*-coated microplates with the aid of dinitrophenol (DNP)-labelled oligonucleotide and IgG (anti-DNP) horseradish peroxidase conjugate (Daly et al. 2002). This technique has been used to detect and quantify *E. coli* in milk using the *alr* gene sequence and also for the detection of *Salmonella* spp. in milk and meat using the *invA* gene of *Salmonella*. The advantage of this technique is that it is accurate in the detection of SNP of different systems (Knight et al. 1999). On the other hand, the disadvantages of this method are that it takes relatively longer to complete the analysis and there is a risk of sample contamination after the PCR reaction when compared to real-time PCR (Perelle et al. 2004). Furthermore, this technique can be costly and labour-intensive and requires expertise to accomplish (Knight et al. 1999).

Non-molecular methods for identification and profiling

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is widely used for the identification and typing of microorganisms to the subspecies level (Carbonnelle et al. 2011). This method, which is suitable for screening bacteria, has been used to identify *Lactobacilli* isolates from foods with a 93 % success rate in identification to the species level (Dušková et al. 2012). MALDI-TOF MS has also been used for sub-species classification of *L. brevis* strains during which strain-level identification was achieved in 90 % of 204 spectra (Kern et al. 2014). The advantages of using this method lie in the fact that it is precise and sensitive and can be used for the identification of whole bacteria cells. Furthermore, it can also be used to identify bacteria cultures that are difficult to culture, and it is not sensitive to alterations in the growth protocol of microorganisms prior to analysis (Biswas and Rolain 2013; Wenning et al. 2014). The disadvantages of this technique lie in the fact that, it is expensive and the accuracy of detection can be affected by the cultivation time of the bacteria (Wenning et al. 2014; Cobo 2013).

Raman Spectroscopy

This is a technique in which a laser light is directed onto a sample, which leads to excitations in the system, and the scattered light emitted from the sample is measured (Willemse-Erix et al. 2009). This method, which is considered faster and more accurate for identification within 2 h without pre-cultivation, has been used to detect *Brucella* directly from milk (Meisel et al. 2012) as well as two *Bacillus* sub-species: *B. subtilis* and *B. sphaericus* (Rösch et al. 2003). The advantages of this method are that it can be used for the characterisation, discrimination and identification of microorganisms at the genus, species, and strain level. Furthermore, it allows for a non-destructive and reliable online identification of microbial cells as it permits a single-cell analysis in real time, and it is fast as it requires only minimal sample preparation without the need for cell cultivations (Harz et al. 2009). This produces reproducible results with a discriminatory power similar to that of PFGE (Willemse-Erix et al. 2009). The disadvantages of this method are that the existence of complex environmental backgrounds such as the presence of other bacteria cells, yeast cells, and other organic and inorganic particles of the same size can hamper the detection and identification of targeted bacteria (Harz et al. 2009).

A summary of the advantages and limitations of selected molecular and non-molecular techniques that can be used for the analysis of bacteria in milk can be found on Tables 1, 2 and 3.

Conclusions

The purpose of this review has been to explore the advantages and limitations of selected molecular and non-molecular techniques that can be used for the analysis of bacteria in milk. SSC, HRMA, REP and RAPD are the top four techniques which are quick and cost-effective and possess adequate discriminatory power for the detection and profiling of bacteria. HRMA, REP and RFLP are the techniques with the most reproducible results. The techniques with the most discriminatory power are AFLP, PFGE and Raman Spectroscopy, while RT PCR is the quantitative technique which is quick, simple and less labour-intensive. Raman Spectroscopy is the non-molecular technique with the most discriminatory power. The need of a particular identification or profiling PCR-based method will determine which PCR instrument and accessories to use. The cost of the instrument will also be a determining factor in choosing which analytical technique to use.

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