2.1 INTRODUCTION

Antimicrobial susceptibility testing of LAB can be performed by several methods, including agar disc diffusion and agar overlay disc diffusion, Etest, agar dilution and broth macro- and microdilution (Klare et al., 2005). In general, dilution methods and the Etest are preferred over diffusion tests providing inhibition zones, as the former techniques allow determination of MICs of antimicrobials that result in a more reliable indication of the intrinsic or acquired nature of a given resistance phenotype. However, since many LAB require special growth conditions in terms of medium acidity and carbohydrate supplementation, conventional media such as Mueller–Hinton and Iso-Sensitest (IST) agar or broth are often not suitable for susceptibility testing of lactobacilli, pediococci and lactococci. Previously, developed a broth formula referred to as the LAB susceptibility test medium (LSM) for determining MICs of antibacterial agents of all major antibiotic classes for Lactobacillus, Pediococcus and Lactococcus species (Klare et al., 2005).

The present study is to further validate the use of the bacteriocins for susceptibility testing by broth dilution of an extensive collection of 473 well-characterized isolates encompassing 24 species of the genera Lactobacillus, Pediococcus and Lactococcus against 16 antimicrobials. Isolates were of human, animal or food category and also included several cultures that are currently under investigation as probiotic candidates or those are already on the market in commercial probiotic products. Secondly, from the large data set obtained from this study, tentative species- or group-specific epidemiological cut-off (ECOFF) values of MICs were defined for most of the antimicrobial agents tested, to allow better differentiation between wild-type (WT) isolates (lacking acquired antimicrobial resistance traits) and
non-wild-type (NWT) isolates (containing one or more acquired antibiotic resistance traits). Recognition of isolates with acquired antibiotic resistances is very important because of the potential transferability of resistance traits to other bacteria, including pathogenic microbes. Commensal intestinal flora of humans and animals (Vaughan et al., 2005; Zoetendal et al., 2006), Strains of these genera are frequently used on a large-scale as starter cultures in food industries (e.g. in the production of fermented milk products or sausages) or as probiotics (Holzapfel et al., 1998; Temmerman et al., 2004).

The emergence of antibiotic-resistant bacteria is of worldwide concern. Antibiotics have been indiscriminately used and this has contributed to the rise in antibiotic resistance in a wide range of bacteria, using a variety of resistance mechanisms (Van veen et al., 1999). To extend the range of therapeutic options, non-peptide antibiotics could be used in combination with cationic peptides. It is well documented that some mixtures have synergic interactions; nevertheless the mechanisms of these positive interactions appear to be complex and are not fully understood (Giacometti et al., 2000). Bacteriocins are bioactive peptides, and most are cationic at physiological pH. Although they have potential to inhibit some foodborne pathogens, (Cleveland et al., 2001), Continuing attempts are being made to find applications in veterinary science and medicine. Typical examples are nisin and lacticin 3147, which have proved to be effective agents in the treatments of mastitis. (Delves-Broughton et al., 1996; Ryan et al., 1999). Additionally, nisin is currently being considered for the treatment of stomach ulcers caused by Helicobacter pylori (Hancock et al., 1999). Enterocin CRL35 is a bacteriocin produced by Enterococcus mundtii CRL35, a strain isolated from regional Argentinean (Tafi) cheese (Farias et al., 1996). This peptide is highly active against bacteria from the genus Listeria. It
displays a dual mode of action: at high concentrations, it produces localized holes in the cell wall and cellular membrane with leakage of macromolecules such as proteins into the external medium; at lower concentrations, it modifies the ion permeability of the cells, dissipating both components of the proton motive force (Minahk et al., 2000).

Potential biotechnological applications of bacteriocins produced by lactic acid bacteria include their use as food preservatives, as they are natural inhabitants of fermented foods and usually inhibit pathogenic bacteria such as Listeria or Enterococcus (Deegan et al., 2006; Galvez et al., 2007; Settanni et al., 2008).

Antimicrobial peptides of bacterial origin are designated bacteriocins. The sequence, structure and mechanisms of activity of bacteriocins are diverse. (Jack et al., 1995). Much research has focused on utilizing bacteriocins as novel food preservatives Abbe et al., 1995, Cleveland et al., 2000, but there is also interest in using them for the control of bacterial diseases in humans and animals The most widely studied group of bacteriocins are the lantibiotics. Research on the lantibiotic nisin has shown this molecule to be active against Staphylococcus aureus and vancomycin-resistant enterococci when tested in vitro (Brumfitt et al., 2002). Nisin has been used to prevent colonization of chicken skins by Salmonella spp., (Natrajn et al., 2000), whereas other reports have shown the effectiveness of nisin (Sears et al., 1992) and lacticin 3147 (Ryan et al., 1998) (a two-component bacteriocin) at controlling surface-related infections such as mastitis. A related lantibiotic, mersacidin, cured systemic staphylococcal infections (Chatterjee et al., 1992) but was ineffective at controlling listerial infections, (Kretschmar et al., 1993) due to a proposed inability to cross the eukaryotic cell membrane.
Antimicrobial peptides from prokaryotic and eukaryotic origin have attracted much attention due to their favorable properties in contrast to conventional antibiotics (Hancock et al., 1999; Hancock et al., 2000; Preet et al., 2010). One promising alternative is the use of nisin, a ribosomally synthesized and post-translationally modified bacteriocin (Class I bacteriocin) produced by *Lactococcus lactis* subsp. *lactis*. This peptide from prokaryotic origin has been in use for decades in the food industry and has not been reported to induce widespread resistance. It is the only bacteriocin approved as “Generally Regarded as Safe (GRAS) compound for use as food preservative in over 50 countries (Jay et al., 2000). Nisin, though generally successful against Gram-positive organisms, has been found to be effective against gram-negative organisms only in the presence of EDTA (Cutter CN et al., 1995; Murdock et al., 2007) and some other agents like citrate (Stevens et al., 1991), lysozyme (Carneiro et al., 1998) and plant essential oils (Solomakos et al., 2008; Govaris et al., 2010). Its use in clinical trials is restricted due to the limitations on in-vivo use of EDTA. It has been suggested that the sensitization of gram-negative organisms by chelating agents may not work since the concomitant acid production can antagonize the chelation reaction (Boziaris et al., 1999).

Recently, it has been revealed that antimicrobial peptides combined with clinically used antibiotics could be the alternatives to solve the problem of antibiotic-resistance (Hancock et al., 2000; Rishi et al., 2011), no information has been available on the combined activity of nisin and conventional anti-*Salmonella* drugs.

The present study was therefore, aimed at evaluating the potential of bacteriocin from *L. garveiae* to increase the efficacy of conventional antibiotics coupled with the possibility of preventing or at least reducing the chances of emergence of resistance in bovine mastitis pathogens.