3.1 INTRODUCTION

Mastitis, inflammation of the mammary gland, is one of the most costly and complex diseases of the dairy industry. Over 135 different microorganisms have been isolated from bovine intramammary infection. Among them *S. aureus, E. coli, B. subtilis, B. cereus and P. aeruginosa* are some of the pathogens (Bogni et al., 2011). Commensal bacteria, with a broad spectrum of antimicrobial activity, have previously been isolated from healthy bovine udders and suggested as potential anti-mastitis agents. Recently the application of bacteria as potential therapeutic against mastitis has gained interest. LAB are comprised of at least ten genera according to taxonomic revisions representing *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus and Vasococcus* (Axelson et al., 1993). They are widely used as starter cultures in a variety of food fermentations. It is well known that many lactic acid bacteria show antagonistic activities against other bacteria, including food spoilage organisms and food borne pathogens. There are several different mechanisms responsible for this inhibition. In most cases, the inhibition is caused by the production of organic acid, hydrogen peroxide and bacteriocins (Tagg et al., 1976; Klaenhammer et al., 1993).

This present study was addressed to produce bacteriocins from *L. garvieae* and to test it as an antimicrobial agent which may inhibit growth of huge number of microbes. Lactic acid bacteria (LAB) are ubiquitous in nature. They exist in various ecological niches including a wide array of fermented food products. LABS have a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic, and shelf-life of foods (De Vuyst et al., 2007). Among the beneficial attributes of LAB, its ability to produce antimicrobial peptides-bacteriocins have attracted particular attention both in food and pharmaceutical industries due to
its potential use as natural food preservative and therapeutic antibiotics (Cleveland et al., 2001; Riley et al., 2002; Chen et al., 2003). In the last decade, numerous strains of LAB have been isolated which show production of bacteriocins. Since their initial discovery in 1925, numerous bacteriocins have been isolated from LAB. Continuous discovery of new bacteriocins with novel features requires an updating of the classification of bacteriocins. Bacteriocins are ribosomally synthesized antimicrobial peptides, produced by a variety of gram-positive and gram-negative bacteria, and inhibit the development of other related bacteria (Fuqin et al., 2013).

Bacteriocin producing species have now been identified among all the genera that comprise the LAB, including Lactococcus, Streptococcus, Lactobacillus, Leuconostoc, Pediococcus and Carnobacterium as well as several Enterococcus spp. (Jack et al., 1995). Among the Lactococcus genera, L. garvieae originally described from a bovine mastitis (Garvie et al., 1981; Collins et al., 1992) has been isolated from various human and animal sources (Elliott et al., 1991; Teixeira et al., 1996), L. garvieae is found in dairy products produced from raw milk, such as artisanal cheeses (Alegria et al., 2009; Ricci et al., 2012). Hence, L. garvieae has been selected in the present study for the production of bacteriocin. Further, for economical use in food, the bacteriocins have to be produced in large amounts and preferably by growing the strains in media containing food grade ingredients. Production of a bacteriocin in a simple medium can be increased by growing the cells at optimum pH and supplementing with nutrients specific for a species/strain. Conditions that provide high cell density favor high bacteriocin production (Said Ennahar et al., 2000). Several reports have shown that complex media and well controlled physical factors, such as temperature and pH are required to obtain optimal bacteriocin production (De Vuyst et al., 1992; Parente et al., 1994; Moortvedt-Abildgaard et al., 1995).
Bacteriocin production can be influenced by medium composition and growth phase of microorganism (Ganzle *et al.*, 1999). The production of bacteriocins is usually studied on complex rich media and the most currently evaluated parameters are the concentration of the carbon source, complex nitrogen source and Tween 80 (Keren *et al.*, 2004; Mataragas *et al.*, 2004). In the light of the above statements, lactic acid bacteria (probiotics) and their products (bacteriocins) could be eco-friendly antimicrobials for substituting the commercial and synthetic antibiotics in veterinary. However, optimization of culture media for efficient production of bacteriocin that mitigate the growth of bovine mastitis pathogens are under researched.

Thus, in the present investigation bacteriocin production from *L. garvieae* isolated from cow’s milk was studied. The effect of incubation period, incubation temperature and pH, supplements such as enzymes, salts and detergents on the antimicrobial activity of bacteriocin was also studied.