

INTRODUCTION

Milk and dairy products are basic foodstuffs and play an important role in the well being of all age groups. They are rich in nutrients and therefore support growth of a wide variety of microorganisms. Among these, are lactic acid bacteria (LAB), which comprise a heterogeneous group of non-sporulating, gram positive, microaerophilic bacteria, catalase negative cocci (e.g. *Lactococcus*) and rods (e.g. *Lactobacillus*), and produce lactic acid as a major end product during fermentation of carbohydrates (Tailliez, 1999; Rattanachaikunsopon & Phumkhachorn 2000 and Zambou *et al.* 2004).

LAB are industrially important organisms as recognized for their fermentative ability as their health and nutritional benefits. These unique microorganisms are a part of the daily diet of virtually people all over as indigenous contaminants in raw milk (El-Attar *et al.* 2004).

The beneficial role of lactic acid bacteria (LAB) and their safety in food fermentation have been well documented. First and foremost, by their metabolic activities the shelf-life and safety of fermented food products are increased. In addition, the aroma, texture and flavour may be improved (Du Toit *et al.* 2000). Today, an increasing number of healthy foods and so-called functional foods as well as pharmaceutical preparations are promoted with health claims based on the probiotic characteristics of some of these bacteria.

Also, lactic acid bacteria produce a wide many different inhibitory substances that prolong the time scale of preservation of the fermented products. The preservative action of LAB in foods results from the formation of metabolites' with antimicrobial activity, e.g. organic acids (lactic, acetic, formic), hydrogen peroxide (in the presence of oxygen), diacetyl, aldehydes (e.g. β -hydroxypropionaldehyde) and bacteriocins or bactericidal proteins during lactic fermentations, which make them useful in food biopreservation (**Talarico & Dobrogosz 1989; Lindgren & Dobrogosz 1990; Piard & Desmazeaud 1991; Brink Ten *et al.* 1994; De Vuyst & Vandamme 1994a; Holzapfel *et al.* 1995; Anderssen *et al.* 1998; Ouwehand, 1998; Sholeva *et al.* 1998; Zhennai, 2000 and Oyetayo *et al.* 2003).**

Lactic acid bacteria exert strong antagonistic activity against many microorganisms, including food spoilage organisms and pathogens. In addition, some strains may contribute to the preservation of fermented foods by producing bacteriocins (**Brink Ten *et al.* 1994**)

Bacteriocins are antimicrobial proteinaceous compounds or small proteins which inhibit, by a bactericidal or bacteriostatic mode of action, micro-organisms that are inhibitory towards sensitive strains and produced by both Gram-positive and Gram-negative bacteria (**Tagg *et al.* 1976; De Vuyst & Vandamme 1994a; Schillinger and Holzapfel 1996**).

The bacteriocins generally recognized as safe (GRAS) lactic acid bacteria (LAB), have arisen a great deal of attention as a novel approach to control pathogens in foodstuffs and

spoilage organisms (**Tagg et al. 1976; Bhunia et al. 1988; Federal Register, 1988; Ahn & stiles 1990; Harding & Shaw 1990 and Klaenhammer, 1993**).

Bacteriocins are produced by some strains of LAB; which exhibit antimicrobial activity against strains closely related to the producer microorganisms (**Du Toit et al. 2000**). Most of the bacteriocins produced by LAB are active against Gram-positive bacteria including *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis* & *Clostridium spp.*, and a wide range of Gram negative bacteria including *Pseudomonas sp.*, *Escherichia coli*, *Salmonella enteritidis* (**Stiles, 1996 and Carrasco et al. 2002**).

Nisin is the only bacteriocin, has no adverse side effects when ingested and so the **U.S. Food and Drug Administration in 1988** designated nisin with generally recognized as safe status (GRAS) and the World Health Organization has approved its use as a food additive (**Hurst, 1981; Federal Register, 1988 and Liu & Hansen 1990**).

Some bacteriocins are heat-resistant, remained active over a wide range of pH, remained active when treated with pepsin & trypsin and remained active during storage at -20°C & $+4^{\circ}\text{C}$ for 3 months, while partially or completely lost when stored at 25°C .

The increasing consumption of yoghurt in tropical and subtropical countries emphasis the need for a preservative method to meet the microbiological safety and quality parameters of the finished product. Due to the mishandling conditions during storage, and distribution from the suppliers to the retail chain, the shelf life of yoghurt can be affected.

Furthermore, wide temperature fluctuations of the refrigeration system during handling of yoghurt are inadequate to prevent appreciable temperature rises until it reaches the consumer (**Kebary and Kamaly 1991**).

It is important to isolate new species from LAB strains, which produce bacteriocins so they can be used as starter cultures in fermentation of dairy products to control spoilage and foodborne pathogens.

Manufacturing of yoghurt by adding nisin (<100 RU ml⁻¹) could be used effectively for prolonging shelf-life of yoghurt with good organoleptic properties (**Bayoumi, 1991 and Gupta & Prasad (1989 a & b)**).

The present study was planned to isolate LAB (from raw milk samples) able to produce high active amount of bacteriocin. Also, the use of bacteriocins as a natural preservative in yoghurt was evaluated.

Consequently, this study was carried out in three parts:

- 1- Isolation, classification and identification of lactic acid bacteria from raw milk samples.
- 2- Production and characterization of bacteriocins produced by isolated LAB from raw milk samples.
- 3- Use of bacteriocins produced and/or extracted by some LAB as a natural preservative in yoghurt.