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Introduction

Bacteriocins from lactic acid bacteria have attracted much attention in recent years because of their potential as natural food preservatives and as phenotypic markers for the construction of food grade cloning vectors (Klaenhammer 1988; Nettles and Barefoot 1993). The study of bacteriocins began with the discovery by Gratia in 1925 of a highly specific antibiotic (principle V), produced by one strain of *Escherichia coli* and active against another strain of the same species. Gratia's publications described many features of what later turned out to be a general class of antibiotic-like proteins produced by Enterobacteriaceae, for which the generic name 'colicine' was proposed by Gratia and Fredericq (1946). With the discovery that the production of apparently similar agents is not limited to coliform organisms, Jacob *et al.* (1953) proposed the more general term 'bacteriocine' for highly specific antibacterial proteins, produced by certain strains of bacteria and active mainly against some other strains of the same species. Although this definition still holds good, both 'colicine' and 'bacteriocine' are now spelt without the final 'e'.

However, most of the bacteriocins produced by Gram positive bacteria do not fit the classical colicin mould. Rather, they tend to be more broadly active against strains of Gram positive species, with little evidence of their action being mediated by specific receptor molecules or their release from producer cells being enhanced by the action of lysins or bacteriocin release proteins. The absence of an outer membrane in Gram positive bacteria excludes any possibility of a modulating receptor molecule in the manner which applies to the interaction of colicins with sensitive bacteria. Rather, the potentially lethal interaction of bacteriocins of Gram positive bacteria with sensitive cells appears to be dependent upon a more general compatibility between surface charges and hydrophobic domains of the interacting molecules. Another difference is that the level of immunity of the producing strain to its own inhibitory product is generally less strong for bacteriocins of Gram positive bacteria than it is for the colicins (Tagg 1992).

Bacteriocinogeny has been ascribed to several species. However, in many instances the presence of bacteriocins has not been convincingly established, since it has been based solely on a finding of antagonism between two bacterial strains. It is thus important that claims of bacteriocinogeny be supported by determination of protein nature of the antagonist as well as a demonstration that the producing organism is insensitive (immune) to its action. Immunity is not always absolute. In some cases bacteriocinogenic cells have been reported to be sensitive to high concentration of homologous bacteriocins (Ivanovics and Alföldi 1954; Fredericq 1957; Levisohn *et al.* 1968).

The antibacterial activity of lactic acid bacteria is associated with the major end products of their metabolism, such as lactic acid, acetic acid, hydrogen peroxide and bacteriocins (Klaenhammer 1982). Lactic acid bacteria are well recognized for their production of bacteriocins (Klaenhammer 1988; Lindgren and Dobrogosz 1990; Schillinger 1990). Bacteriocins have been found in all genera of lactic acid bacteria,

and most of the bacteriocin-producing strains have been isolated from foods (Schillinger *et al.* 1993).

The ability to produce bacteriocins has technological and scientific importance. Research on bacteriocins from lactic acid bacteria has expanded exponentially during the last decade, as these have several interesting application possibilities. Lactic acid bacteria are used for biological processing of many raw materials to produce acceptable foods which are with improved flavour and consumed for their prophylactic and therapeutic properties (Fernandes and Shahani 1989). Bacteriocins or bacteriocin producers occupy important position in fermented foods. Firstly, bacteriocin-producing starter cultures may result in a more reliable fermentation process preventing growth of spoilage bacteria (Mortvedt and Nes 1990). In mixed starter cultures, bacteriocin producers may dominate and affect the microbiological balance required to obtain products with defined characteristics. Bacteriocin production may offer certain advantages. Bacteriocins from food grade lactic acid bacteria are bactericidal to many Gram positive bacteria associated with food spoilage (Bhunia *et al.* 1987). Several unique properties, such as activity over a wide range of pH and high or low temperature treatment make them suitable as biological preservatives to extend the shelf life of refrigerated semi-preserved and canned foods. Their use in foods has an added advantage, because they are degraded by the proteolytic enzyme of the gastrointestinal tract and are nontoxic and nonantigenic to animals (Biswas *et al.* 1991). The most important compound of this type is nisin, produced by *Lactococcus* ssp. *lactis,* which has commercial importance in food industry. In many foods, higher levels of acids are undesirable. For such products, the inhibition of the undesired organisms must be achieved at least partially by means other than acidification. (Schillinger and Lücke 1989). The second reason for undertaking extensive research on bacteriocins is that the genetic determinants for bacteriocin production and immunity could be used in the construction of food grade vectors for strain improvement using r-DNA technology. (Mortvedt and Nes 1990; ten Brink *et al.* 1994). Thirdly, bacteriocin production or susceptibility could be used in taxonomic studies. (ten Brink *et al.* 1994).

Compared to other antimicrobial agents, studies on distribution of bacteriocin-producing microorganisms in nature, factors affecting bacteriocin production and their characterization are relatively rare. Therefore, the main objectives of the present investigation had been the folowing:

1) Isolation of lactic acid bacteria from natural habitats;
2) Detection and assay of bacteriocin activity;
3) Optimization of bacteriocin production;
4) Purification of bacteriocins;
5) Characterization of bacteriocins; and
6) Characterization of selected bacteriocin-producing isolates with a view to identify their taxonomic status.