2

Review of Literature

2.1. Bacteriocins from lactic acid bacteria

Majority of bacteriocins produced by lactic acid bacteria have been characterized by the initial definition of a proteinaceous inhibitor, crude estimation of molecular weight (via retention in dialysis membranes or ultrafiltration) and determination of susceptible strains. Lactic acid bacteria produce three general classes of antimicrobial proteins : (1) lantibiotics, (2) small hydrophobic heat-stable peptides (<13 kDa) and (3) large heat-labile proteins (>30 kDa) (Klaenhammer *et al.* 1992).

2.1.1. Lantibiotics

The term 'lantibiotics' has been coined for those bacteriocins in which the amino acid lanthionine is a main component (Hurst 1981). Bacteriocins such as nisin (Gross and Morell 1971), subtilin (Gross and Kiltz 1973), epidermin (Allgaier *et al.* 1985), gallidermin (Kellner *et al.* 1988) and PEP5 (Kellner *et al.* 1989) have been shown to contain amino acid lanthionine. Reports of lantibiotics within Lactobacillaceae are rare. However, Mortvedt *et al.* (1991a) reported that the *Lactobacillus sake* bacteriocin, lactocin S, contains lanthionine residue. The presence of lanthionine has also been detected in a bacteriocin of 4.63 kDa (33-35 amino acid residues) produced by *Carnobacterium* sp. isolated from fish. Incorporation of a lanthionine residue introduces a monosulphur bridge which results in unique peptide ring structures among the various lantibiotics. Foremost among the characterized bacteriocins is the lantibiotic nisin, produced by *Lactococcus lactis* subsp. *lactis*. This small 34 amino acid-containing peptide has two sulphur-containing amino acids, lanthionine and β-methyllanthionine. If shows antimicrobial activity against a range of Gram positive bacteria, particularly sporeformers (Delves-Broughton 1990). The nisin molecule is acidic in nature and exhibits greatest stability under acid conditions; it is more soluble in acidic pH (Hurst 1981). Due mainly to its relatively narrow antibacterial spectrum, low solubility in body liquids, susceptibility to digestive proteases and instability at physiological pH (7.0-7.5), it is found to be unsuitable for therapeutic effect for veterinary and clinical uses (Hurst 1983).

2.1.2. Small hydrophobic heat-stable peptides

The first of this class characterized and purified is a lipocarbohydrate-protein macromolecular complex produced by *Lactobacillus fermenti* (de Klerk and Smit 1967). This bacteriocin is relatively heat-stable (96°C for 30 min) and contains a high proportion of glycine (11.1%) and alanine (13.4%) residues. Lactocin 27 produced by *Lactobacillus helveticus* is a small (12.4 kDa) heat-stable glycoprotein, similar to the *Lb. fermenti*

bacteriocin. It was initially isolated as a large molecular weight complex (>200 kDa) while the active peptide was defined only at 12.4 kDa and contained unusually high concentrations of glycine (15.1%) and alanine (18.1%) residues (Upreti and Hinsdill 1973, 1975). Lactacins B and F produced by *Lactobacillus acidophilus* N2 and 11088, respectively are highly heat-stable (121°C for 15 min) (Barefoot and Klaenhammer 1983; Muriana and Klaenhammer 1991a). Lactacin B showed many similarities to lactocin 27 (Barefoot and Klaenhammer 1984). Both were initially isolated as large molecular weight complexes. The initial molecular weight estimates were 100 kDa and >200 kDa for lactacin B and lactocin 27, respectively. Lactacin B was later purified as a 6.3 kDa peptide. The antimicrobial peptides within this group are small, ranging in size between 3.7 and 6.3 kDa. Native lactacin F was sized at approximately 180 kDa by gel filtration. However, the lactacin F, purified by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was identified as only a 2.5 kDa peptide. Compositional analysis indicates that lactacin F contains 56 amino acid residues; the peptide is composed primarily of hydrophobic and polar neutral residues (87.3%) which include glycine (21.6%), alanine (15.8%) and valine (8.8%) (Muriana and Klaenhammer 1991a, 1991b). Lactocin S, produced by *Lactobacillus sake* L45, is yet another bacteriocin in this group. Analysis of the amino acid composition and N-terminal sequencing of lactocin S reveals that 50% of the approximated 33 amino acids are hydrophobic and nonpolar residues of alanine, valine and glycine (Mortvedt *et al.* 1991b). The lower glycine content and hydrophobic propensity of lactocin S compared to lactacin F correlates with their relative heat stabilities (lactacin F7S). A number of additional heat-stable hydrophobic proteins have been purified subsequently. A number of carnobacteriocins produced by *Carnobacterium piscicola* (Ahn and Stiles 1990), brevicin 37 (Rammelsberg and Radler 1990) and gassericin A (Toba *et al.* 1991a) are small (~5 kDa) heat-stable (121°C for 20 min) peptides. Some of the recently reported *Lactobacillus* bacteriocins that can be enumerated within this category are sakacin B, a hydrophobic peptide (6.3 kDa) produced by *Lb. sake* (Samelis *et al.* 1994), acidocin B, a heat-stable, 2.4 kDa hydrophobic peptide produced by *Lb. acidophilus* M46 (ten Brink *et al.* 1994) and brevicin 27, a heat-stable hydrophobic peptide with an apparent molecular weight between 10 and 30 kDa for the crude inhibitory molecule produced by *Lactobacillus brevis* SB 27 (Benoit *et al.* 1994).

Majority of the lactococcal and pediococcal bacteriocins belong to this group. Lactococcins are heat-stable (100°C for 60 min), small hydrophobic peptide bacteriocins. Lactococcin A is produced by *Lactococcus lactis* subsp. *cremoris* LMG2130 (Holo *et al.* 1991) and bacteriocin S50 is produced by *Lactococcus lactis* subsp. *diacetylactis* S50 (Kojic *et al.* 1991). *Lactococcus lactis* CNRZ 481 produces lacticin 481, a 5.5 kDa heat-stable peptide (Piard *et al.* 1990). Parrot *et al.* (1989) reported four bacteriocins (mutacins) produced by different strains of *Streptococcus mutans*. The mutacins produced by the strains C67-1, Ny266 and T8 possess similar properties.

They are thermoresistant (100°C for 30 min) and low molecular weight (<3.5 kDa) peptides. The mutacin produced by the strain Ny 257-S is of lower thermoresistance (80°C for 30 min) but of a slightly higher molecular weight (8-14 kDa).

The pediococcal bacteriocins include pediocin PA-1, a 16.5 kDa peptide produced by *Pediococcus acidilactici* PAC 1.0 (Gonzales and Kunka 1987), pediocin AcH, a 2.7 kDa-peptide produced by *Pc. acidilactici* (Bhunia *et. al.* 1988) and pediocin N5p produced by *Pc. pentosaceus* N5p (Strasser de Saad and Manca de Nadra 1993). Pediocin N5p is somewhat lipophilic in nature and is inactivated by chloroform and ethanol.

2.1.3. Large heat-labile proteins

Recent reports of large heat-labile bacteriocins suggest that there are numerous members of this class. Acidophilucin A, lacticins A and B, and caseicin 80 appear to be large proteins, since it takes 10-15 min to be inactivated at 60°C (Rammelsberg and Radler 1990; Toba *et al.* 1991b, 1991c). To date, only helveticin J (37 kDa) produced by *Lactobacillus helveticus* has been purified and characterized at the genetic level. It is a heat-sensitive protein (inactivated at 100°C within 30 min) which retains activity after treatment with various dissociating agents (Joerger and Klaenhammer 1986). Another recently reported bacteriocin, helveticin V-1829, from *Lb. helveticus* 1829 is heat-labile. A partially purified sample approximates a molecular weight of more than 10 kDa (Vaughan *et al.* 1992). The biochemical properties and mechanism of action of many larger bacteriocins remain to be investigated. Considering their size and heat lability, the bactericidal activities of these proteins are likely to be affected by changes in conformation and secondary structure.

Table I lists bacteriocins reported from different lactic acid bacteria.

2.2. Optimization of bacteriocin production

Optimization of bacteriocin production by regulation of environmental growth parameters is important for effective commercial application.

2.2.1. Medium composition

This is one of the important environmental factors. Complex growth media commonly used for the growth of bacteriocin-producing lactic acid bacteria are MRS, M17, Elliker lactic, *Lactobacillus* selection (LBS), tryptone-yeast extract-Tween (TYT), APT and

Table 1. Bacteriocins from lactic acid bacteria

Producer	Bacteriocin (MW, kDa)	Activity spectrum	Reference
Lactobacillus acidophilus	Acidolin		Hamada *et al.* (1971)
	Acidophilin		Shahani *et al.* (1977)
	Lactacin B (6.3)	*Lactobacillus delbrueckii, Lactobacillus helveticus, Lactobacillus leichmannii*	Barefoot and Klaenhammer (1983, 1984)
	Lactacin F (6.3)	*Lactobacillus fermentum, Lactobacillus delbrueckii, Lactobacillus helveticus, Enterococcus faecalis, Acromonas hydrophila, Staphylococcus aureus*	Muriana and Klaenhammer (1987,1991a, 1991b); Lewus *et al. (1991)*
	Acidophilucin A	*Lactobacillus delbrueckii, Lactobacillus helveticus*	Toba *et al.* (1991b)
	Acidocin B (2.4)	*Lactobacillus, Clostridium sporogenes*	ten Brink *et al.* (1994)
Lactobacillus bavaricus	Bavaricin MN		Kaiser and Montville (1993)
Lactobacillus brevis	Lactobacillin		Wheater *et al.* (1951)
	Lactobrevin		Kavasnikov and Sodenko (1967)
	Brevicin 37	*Pediococcus damnosus, Lactobacillus brevis , Leuconostoc oenos*	Rammelsberg and Radler (1990)
	Brevicin 27 (10-30)	Heterofermentative	Benoit *et al.* (1994)

Contd....

Producer	Bacteriocin (MW, kDa)	Activity spectrum	Reference
		lactobacilli	
Lactobacillus casei	Caseicin 80 (42)	*Lactobacillus casei*	Rammelsberg and Radler (1990); Rammelsberg *et al.* (1990); Müller and Radler (1993)
Lactobacillus carnis	Bacteriocins (4.9)	*Lactobacillus, Carnobacterium, Pediococcus , Enterococcus*	Ahn and Stiles (1990); Schillinger and Holzapfel (1990)
Lactobacillus curvatus	Curvacin a	*Lactobacillus, Enterococcus faecalis, Listeria monocytogenes*	Vogel *et al. (1993)*
Lactobacillus delbrueckii subsp. *bulgaricus*	Bulgarican	*Staphylococcus aureus, Pseudomonas fragi*	Reddy *et al.* (1984)
Lactobacillus delbrueckii	Lacticin A, B	*Lactobacillus delbrueckii* subsp. *lactis, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus delbrueckii* subsp. *delbrueckii*	Toba *et al.* (1991c)
Lactobacillus fermenti	Baccteriocin	*Lactobacillus fermenti*	de Klerk and Smit (1967)
Lactobacillus helveticus	Lactocin 27 (12.4)	*Lactobacillus acidophilus, Lactobacillus helveticus*	Upreti and Hinsdill (1975)

Contd....

Producer	Bacteriocin (MW, kDa)	Activity spectrum	Reference
	Helveticin J (37)	*Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus delbrueckii*, subsp. *bulgaricus*	Joerger and Klaenhammer (1986)
	Helveticin V-1829	*Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*	Vaughan *et al.* (1992)
Lactobacillus plantarum	Plantaricin SIK-83	*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*	Andersson *et al.* (1988)
	Plantaricin A (>8)	*Lactobacillus plantarum*, *Leuconostoc sp.* *Pediococcus sp.*, *Lactococcus lactis*, *Enterococcus faecalis*	Daeschel *et al.* (1990)
	Plantacin B	*Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Pediococcus damnosus*	West and Warner (1988)
	Plantaricin C19 (3.5)	*Listeria* spp.	Atrih *et al.* (1993)
	Plantaricin-149 (2.2)	*Lactobacillus delbrueckii*, *Lactobacillus bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Pediococcus acidilactici*, *Pediococcus cerevisiae*, *Enterococcus hirae*, *Lactococcus lactis*	Kato *et al.* (1994)
	Plantaricin LC74 (<5)		Rekhif *et al.* (1994)

Contd....

Producer	Bacteriocin (MW, kDa)	Activity spectrum	Reference
Lactobacillus sake	Lactolin		Kodama (1952)
	Sakacin A	*Carnobacterium piscicola, Enterococcus sp., Lactobacillus sake, Lactobacillus curvatus, Leuconostoc paramesentederoides, Listeria monocytogenes, Acromonas hydrophila, Staphylococcus aureus*	Schillinger and Lücke (1989); Lewus *et al.* (1991)
	Sakacin B (6.3)	*Lactobacillus sake, Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus minor, Lactobacillus farciminis, Lactobacillus halotolerans, Lactobacillus viridescens, Leuconostoc mesenteroides, Leuconostoc paramesenteroides, Lactococcus sp., Listeria monocytogenes, Listeria innocua*	Samelis *et al.* (1994)
	Lactocin S (3.7)	*Lactobacillus sp., Leuconostoc sp., Pediococcus sp.*	McCormick and Savage (1983); Mortvedt and Nes(1990); Mortvedt *et al.* (1990, 1991a, 1991b)
Lacobacillus salivarius	Salivaricin B	*Listeria monocytogenes, Bacillus cereus, Brocothrix thermosphacta Enterococcus faecalis, Lactobacillus sp.*	ten Brink *et al.* (1994)

Contd....

Producer	Bacteriocin (MW, kDa)	Activity spectrum	Reference
Lactococcus lactis subp. *lactis*	Nisin	*Staphylococcus, Listeria monocytogenes, Streptococcus, Micrococcus, Lactobacillus, Bacillus, Clostridium*	Hirsch (1950); Hirsch *et al.* (1951); Hurst (1981)
Lactococcus lactis subsp. *cremoris*	Lactococcin A	*Lactococcus lactis ssp. cremoris, Lactococcus lactis* subsp. *lactis*	Holo *et al.* (1991)
Lactococcus lactis subsp. *diacetylactis*	Bacteriocin S-50	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *diacetylactis*	Kojic *et al.* (1991)
Lactococcus lactis	Lacticin 481	*Lactococcus sp., Leuconostoc sp., Lactobacillus sp., Clostridium tyrobutyricum*	Piard *et al.* (1992)
Lactococcus mutans	Mutacins (<3.5)	*Neissaria subflava, Flavobacterium capsulatum*	Parrot *et al.* (1989)
Lactococcus cremoris	Diplococcin		Davey (1981)
Leuconostoc carnosum	Carnocin 54 (~4)	*Leuconostoc mesenteroides, Carnobacterium divergens, Enterococcus faecalis, Listeria monocytogenes Listeria innocua*	Keppler *et al.* (1994); Schillinger *et al.* (1995)

Contd....

Producer	Bacteriocin (MW, kDa)	Activity spectrum	Reference
Pediococcus acidilactici	Pediocin PA-1		Gonzales and Kunka (1987)
	Pediocin AcH (2.7)	*Lactobacillus, Leuconostoc, Staphylococcus aureus, Clostridium perfrinngens, Listeria monocytogenes, Pseudomonas putida*	Bhunia *et al.* (1988)
	Pediocin L50		Cintas *et al.* (1995)
Pediococcus pentosaceus	Pediocin N5p	*Pediococcus pentosaceus, Lactobacillus hilgardii, Leuconostoc oenos*	Strasser de Saad and Manca de Nadra (1993)

tomato juice broths. However media components, especially peptides, which are present in relatively high concentration interfere with subsequent bacteriocin purification (Barefoot and Klaenhammer 1984). Dialysates of complex media containing only low molecular weight fractions were found to be effective for the production of pediocin AcH (Bhunia *et al.* 1988), but not for lactococcal bacteriocins (Geis *et al.* 1983). Semidefined media, containing casitone (a pancreatic digest of casein), yeast extract, glucose and several growth factors are used for the production of lactacin B (Barefoot and Klaenhammer 1984), helveticin J (Joerger and Klaenhammer 1986) and helveticin V-1829 (Vaughan *et al.* 1992). Studies on the comparison of different media or the effect of media ingredients for bacteriocin production are scarce. Geis *et al.* (1983) compared bacteriocin production by 16 strains of lactococci in Elliker lactic broth (ELB), M17, brain heart infusion, a synthetic medium and litmus milk. ELB followed by M17 supported the highest bacteriocin production. Biswas *et al.* (1991) studied the effect of the ingredients of a complex medium, trypticase-glucose-yeast extract (TGE) broth on the production of pediocin AcH. Glucose was found to be the best carbon source. Tween 80 and Mn^{2+} allowed optimal biomass and bacteriocin production, which were stabilized by the presence of Mg^{2+}. However, Tween 80 sometimes interferes with subsequent ammonium sulphate precipitation of bacteriocins (Mortvedt *et al.* 1991b). Parente and Hill (1992) studied the effects of tryptone, yeast extract and Tween 80 on the production of enterocin 1146 and lactocin D in TYT medium using factorial experiments and emperical modelling. On the basis of predictions of the models developed, three TYT media (TYT10, TYT11 and TYT30) were designed to maximize bacteriocin production, while minimizing the amount of peptides in the medium. Bacteriocin production in TYT media is comparable with that in M17 and MRS broths which have higher peptide content.

2.2.2. pH of medium

A number of studies have shown that control of the medium pH is a critical factor in bacteriocin production (Goebel *et al.* 1955; Joerger and Klaenhammer 1986). APT broth adjusted at an initial pH of 6.0 - 6.5 allowed optimal production by *Carnobacterium piscicola* (Ahn and Stiles 1990) and *Leuconostoc gelidium* (Hastings and Stiles 1991). Production of pediocin AcH was maximum in TGE broth adjusted at an initial pH of 6.0-6.5. External pHcontrol can provide further improvement of bacteriocin production. The optimal pH varies among strains: lactacin B production was optimal at pH 6.0 (Barefoot and Klaenhammer 1984), helveticin J at pH 5.5 (Joerger and Klaenhammer 1986), lacticin B at pH 5.5 (Piard *et al.* 1990), helveticin V-1829 at pH 5.5 (Vaughan *et al.* 1992) and bavaricin MN at pH 6.0 (Kaiser and Montville 1993). Production of lactacin F is also pH dependent; maximum levels of lactacin F are obtained in MRS broth

maintained at pH 7.0, whereas negligible activity is found in fermentors held at pH 7.5 or 6.5 (Muriana and Klaenhammer 1987).

2.2.3. Duration and temperature of incubation

The period and temperature of incubation needed to achieve maximum yield are usually different for different strains. Studies on the production of bacteriocins with respect to growth of the producer strains are thus common. Most of the bacteriocins of lactic acid bacteria are produced during late exponential phase, indicating that a high amount of biomass was essential for bacteriocin synthesis (Piard and Desmazeaud 1991). About 60% of the pediocin AcH was produced within 8 h, and the final 40% was produced during the next 8 h (stationary phase) at 37°C. Thus, pediocin AcH appears to be a secondary metabolite (Biswas *et al.* 1991) produced by *Pc. acidilactici* H. In *Lb. sake* LB706 producing sakacin A, no activity was detected during the first 8 h of incubation. However, significant activity was detected after 23 h at 25°C when the cells were in the mid or late logarithmic growth phase. A loss of activity of Lb 706 supernatant was observed after 47 h at 25°C (Schillinger and Lücke 1989). The highest activity of *Lactobacillus casei*, producing caseicin 80, is observed when the cells reach the stationary phase usually after 3 d at 30°C (Rammelsberg and Radler 1990). Helveticins J and V-1829 produced by *Lb. helveticus* (Joerger and Klaenhammer 1986; Vaughan *et al.* 1992), sakacin B produced by *Lb. sake* 251 (Samelis *et al.* 1994) and acidocin B produced by *Lb. acidophilus* M46 (ten Brink *et. al* 1994) are produced during late logarithmic and begining of stationary phases. However, excretion during early growth phase has also been reported (Ahn and Stiles 1990; Hastings and Stiles 1991). Production of bacteriocin by *Pc. pentosaceus* N5p occurs early in the growth cycle of the organism, rather than as a secondary metabolite of growth (Strasser de Saad and Manca de Nadra 1993). Thus, the timing of bacteriocin harvest must also be determined emperically for each different organism, method and set of conditions.

2.3. Purification of bacteriocins

Since bacteriocins are proteins or peptides, the methods applied to bacteriocin purification are those which are generally used in protein purification.

2.3.1. Ultrafiltration

Ultrafiltration achieves concentration of large molecules, but not of small ones. This

method is not practical for the concentration of large volumes. Ultrafiltration was carried out with crude lactacin F by sequentially filtering through membranes of decreasing pore sizes (Muriana and Klaenhammer 1991a). To concentrate the antimicrobial activity of *Lb. casei*, culture supernatants were lyophilized and later dissolved in a small volume of water or buffer, followed by ultrafiltration (Rammelsberg and Radler 1990). Cell-free culture supernatant of *Lb. plantarum* SIK83, producing plantaricin SIK83, was concentrated 50 times by ultrafiltration using a 10 kDa molecular weight cut off membrane (Andersson *et al.* 1988). Besides concentration and partial purification, sequential ultrafiltration through membranes of decreasing molecular weight cut off helps to approximate the size of the bacteriocin, as in helveticin J (Joerger and Klaenhammer 1986).

2.3.2. Ammonium sulphate precipitation

This method is frequently used as a preliminary concentration step and also achieves some degree of purification. The reason for the choice of ammonium sulphate is that it is highly soluble, and the solubility does not vary much with temperature. The amount of ammonium sulphate needed to precipitate different bacteriocins varies. Ammonium sulphate precipitation was used as the preliminary purification step by Bhunia *et al.* (1988) for pediocin AcH, Mortvedt *et al.* (1991b) for lactocin S, Muriana and Klaenhammer (1991a) for lactacin F, Vaughan *et al.* (1992) for helveticin V-1829 and Samelis *et al.* (1994) for sakacin B. The pellet that is received after precipitation was dialysed exhaustively against large volumes of buffer or deionized water to remove ammonium sulphate.

2.3.3. Gel chromatography

Molecular exclusion chromatography or gel chromatography is another method that is frequently employed for the purification of bacteriocins. The active fraction may be passed successively through columns with different packing materials. Joerger and Klaenhammer (1986) applied helveticin J concentrate to columns containing Sephadex G-200-120 (a cross-linked dextran). The active fraction from this column was pooled, concentrated and then applied to a Sephadex G-200-50 column for further purification.

2.3.4. Ion exchange chromatography

Ion exchange chromatography of both types i.e. cation and anion is frequently used

for bacteriocin purification. The most widely used materials in this process are DEAE cellulose, Q sepharose, CM cellulose and Mon QHR5/5. Ion exchange chromatography was used as one of the steps for the purification of lactacin B (Barefoot and Klaenhammer 1984), pediocin AcH (Bhunia *et al.* 1988), lactocin S (Mortvedt *et al.* 1991b) and sakacin B (Samelis *et al.* 1994).

2.3.5. Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) is often used as the terminal purification step. SDS is an anionic detergent that disrupts all noncovalent interactions in native protein, and SDS-PAGE is performed mainly for determining the molecular weight of the purified bacteriocin. Direct detection of the peptide subunit responsible for bacteriocin activity is possible (Bhunia *et al.* 1987).

Judicious combination of the methods described above are used for purification of various bacteriocins. Properties of the bacteriocin in question are often exploited for their purification. Acidocin B was purified (>90%) by butanol extraction in a single step from concentrated cell suspensions in a chemically defined medium (ten Brink *et al.* 1994).

2.3.6. Activity assay

Activity of the active fraction is assayed after each purification step, mostly by critical dilution method described by Mayr-Harting *et al.* (1972), and then amount of protein measured. Progress of purification at each step is indicated by drawing up a table which gives the information of the following types: volume (ml), concentration (units/ml), total activity (arbitrary units), protein (mg/ml), specific activity (units/mg), yield (%) and degree of purification (%).

2.4. Mode of action

Although the pioneering work of Fredericq (1946) pointed out a narrow range-killing specificity among the colicins (i.e. they are active on strains of the same or related species), bacteriocins produced by Gram positive organisms are often active on a wide variety of Gram positive bacteria (Hamon and Peron 1963; Hamon 1964).

In most cases, mode of action studies have been carried out with a single susceptible strain. Studies on the mode of action of many lactic acid bacterial bacteriocins implicate bactericidal or bacteriostatic action.

Quite often the killing kinetics or even the designation of a bactericidal versus bacteriostatic effect are dependent upon aspects of the assay system such as the concentration and purity of the inhibitor, the type of buffer or broth, the sensitivity of the indicator species and the density of cell suspension used (Lewus *et al.* 1991). The physiological state of an indicator culture has been shown to have a strong influence on susceptibility to the lethal action of a bacteriocin, with actively multiplying cells being the most sensitive (Tagg *et al.* 1976). Helveticin V-1829 showed more pronounced lethal action on susceptible cells in logarithmic phase than stationary phase of growth (Vaughan *et al.* 1992). Lacticin showed an action similar to helveticin V-1829 (Piard *et al.* 1990).

The pH of a bacteriocin preparation plays an important role on its action on susceptible cells. The killing kinetics of carnocin 54 were affected by the pH. When susceptible cells of *Listeria innocua* WS2257 were treated with carnocin 54, a higher inactivation rate was observed at pH 4.9 than at pH 6.5. The higher killing rate at acidic pH may either be the result of the higher activity of carnocin 54 or may be due to higher susceptibility of the indicator strain at lower pH (Schillinger *et al.* 1995). Similarly, Harris *et al.* (1991) observed an increase in the effectiveness of nisin when the pH of the medium was decreased from 6.5 to 5.5.

The killing of sensitive bacteria is thought to be a two-step process; the initial adsorption of bacteriocin by cell receptors being followed by transfer of a 'lethal' message to specific biochemical targets (Nomura 1974). A number of biochemical targets have been identified with principal effects in energy production, macromolecular synthesis (or membrane transport) and permeability (Reeves 1972).

The common mechanism of action which has been determined for other bacteriocins of lactic acid bacteria is disruption of the electrochemical gradients across the cytoplasmic membrane by pore formation (Klaenhammer 1993). Exposure of sensitive cells to pediocin SJ-1 caused rapid leakage of potassium ions and amino acids, leading to rapid depolarization of the cytoplasmic membrane, finally leading to cell death (Schved *et al.* 1994).

Plantaricin SIK 83 has been shown to bind specifically to sensitive cells, but not to non-sensitive lactic acid bacteria and Gram negative bacteria. Sensitive cells, after exposure to bacteriocin, could be rescued by treatment with proteolytic enzymes. Morphological strains are observed within 2 h after the cells are exposed to bacteriocins. A lethal mode of action appears to be due to damage to the cell membrane, resulting in cell lysis (Andersson *et al.* 1988).

The bactericidal effect of salivaricin B and acidocin B on sensitive lactobacilli in the absence of cell lysis suggests that their mode of action is not impairment of cell wall biosynthesis (ten Brink *et al.* 1994). Also, sakacin B and pediocin N5p exhibit a bactericidal mode of action, without causing cell lysis (Strasser de Saad and Manca de Nadra 1993; Samelis *et al.* 1994). Pediocin AcH is bactericidal to sensitive bacterial cells, and this effect is produced within a few minutes. Cell death is not associated

with lysis or leakage of the cell membrane. The effect of pediocin AcH could be related to inhibition of the synthesis of ATP and/or cellular macromolecules such as proteins and nucleic acid (Bhunia *et al.* 1988).

2.5. Activity spectrum

Antibacterial spectrum is one of the criteria in the characterization of a bacteriocin and determining its novelty. According to the definition of Tagg *et al.* (1976), bacteriocins are proteinaceous compounds with a bactericidal mode of action against a limited range of organisms closely related to the producer. Several different bacteriocins produced by the same species are differentiated on the basis of their antibacterial spectrum. In spite of several similarities, plantaricin 149 produced by *Lb. plantarum* is considered different from plantaricins A, B, S and T based on differences in antibacterial activity, towards *Enterococcus, Lactococcus* and *Micrococcus* strains (Kato *et al.* 1994). Acidocin B, produced by *Lb. acidophilus* M46, is considered unique and atypical when compared to other *Lb. acidophilus* bacteriocins such as lactacin B, lactacin F, acidophilucin A and acidocin 8912, since it combines inhibition of *Clostridium sporogenes* with a narrow activity spectrum within the genus *Lactobacillus* (ten Brink *et al.* 1994). The antimicrobial substance from *Pc. pentosaceus* N4p has specific activity against other strains of *Pediococcus*, whereas the inhibitor produced by *Pc. pentosaceus* N5p has an antagonistic effect on large number of other lactic acid bacteria (Strasser de Saad and Manca de Nadra 1993).

In general, bacteriocins found in lactobacilli display a narrow range of inhibitory activity, towards closely related species within Lactobacillaceae (Klaenhammer 1988). However, *Lactobacillus* bacteriocins showing a broad activity spectrum has been reported. *Lactobacillus salivarius* M7 produces a broad spectrum bacteriocin, salivaricin B, which inhibits the growth of *Listeria monocytogenes, Bacillus cereus, Brocothrix thermosphacta* and *Enterococcus faecalis* (ten Brink *et al.* 1994). Sakacin A, (Schillinger and Lücke 1989) produced by *Lb. sake* possess a relatively broad spectrum activity that includes activity against certain pathogens. *Listeria monocytogenes* is most commonly inhibited, probably because of its taxonomic relation to Lactobacillaceae (Wilkinson and Jones 1977). Pediocins, produced by pediococci, have a wide spectrum of bactericidal activity against Gram positive bacteria (Gonzales and Kunka 1987; Hoover *et al.* 1988; Bhunia *et al.* 1988; Ray *et al.* 1989).