

# Contents

<b>1. Introduction</b>	<b>1</b>
<b>2. Review of literature</b>	<b>3</b>
2.1. Bacteriocins from lactic acid bacteria	3
2.1.1. Lantibiotics	3
2.1.2. Small hydrophobic heat-stable peptides	3
2.1.3. Large heat-labile proteins	5
2.2. Optimization of bacteriocin production	5
2.2.1. Medium composition	5
2.2.2. pH of medium	6
2.2.3. Duration and temperature and period of incubation	7
2.3. Purification of bacteriocins	7
2.3.1. Ultrafiltration	7
2.3.2. Ammonium sulphate precipitation	8
2.3.3. Gel chromatography	8
2.3.4. Ion exchange chromatography	8
2.3.5. Polyacrylamide gel electrophoresis	9

2.3.6.	Activity assay	9
2.4.	Mode of action	9
2.5.	Activity spectrum	11
<b>3.</b>	<b>Materials and methods</b>	<b>12</b>
3.1.	Culture media used	12
3.2.	Reagents used	16
3.3.	Buffers used	18
3.4.	Organisms used	19
3.5.	Experimental	19
3.5.1.	Collection of sample	19
3.5.2.	Isolation of lactic acid bacteria	19
3.5.3.	Characterization of isolates	19
3.5.3.1.	Cell morphology	19
3.5.3.2.	Gram staining	20
3.5.3.3.	Production of catalase	20
3.5.3.4.	Production of gas from glucose	20
3.5.3.5.	Growth in 6.5% NaCl	20
3.5.3.6.	Hydrolysis of casein	21
3.5.3.7.	Production of indole	21
3.5.3.8.	Reduction of nitrate	21
3.5.3.9.	Hydrolysis of starch	21
3.5.3.10.	Hydrolysis of fat	21
3.5.3.11.	Liquefaction of gelatin	21
3.5.3.12.	Utilization of sugars	22
3.5.4.	Effect of anaerobiosis on lactic acid bacterial growth	22
3.5.5.	Detection of antibacterial activity	22
3.5.6.	Preparation of cell-free culture supernatants	22
3.5.7.	Well diffusion assay	22
3.5.8.	Determining antagonism due to phage	23
3.5.9.	Determining antagonism due to hydrogen peroxide	23

3.5.10.	Determining antagonism due to bacteriocin	23
3.5.10.1.	Treatment with pepsin	23
3.5.10.2.	Treatment with trypsin	23
3.5.11.	Determining antagonism due to acids	23
3.5.12.	Elimination of Bac <sup>+</sup> variants	24
3.5.13.	Optimization of bacteriocin production	24
3.5.13.1.	Type of medium	24
3.5.13.2.	pH of medium	24
3.5.13.3.	Period of incubation	24
3.5.13.4.	Temperature of incubation	24
3.5.14.	Characterization of bacteriocins	25
3.5.14.1.	Thermostability	25
3.5.14.2.	pH stability	25
3.5.14.3.	Storage stability	25
3.5.14.4.	Stability against organic solvents	25
3.5.15.	Purification of bacteriocin	26
3.5.15.1.	Concentration	26
3.5.15.2.	Dialysis	26
3.5.15.3.	Gel filtration	26
3.5.15.4.	Ion exchange chromatography	26
3.5.15.5.	Desalting column chromatography	27
3.5.15.6.	Protein estimation	27
3.5.15.7.	Activity assay	27
3.5.16.	Mode of action	27
3.5.17.	Activity spectrum	28
3.5.18.	Statistical analysis	28

#### 4. Results 29

4.1.	Isolation of lactic acid bacteria	29
4.2.	Screening and nature of antimicrobial substance	29
4.3.	Selection of Bac <sup>+</sup> cells from a mixed population	29

4.4. Identification of selected Bac <sup>+</sup> strains	30
4.5. Optimization of bacteriocin production	30
4.5.1. Type of medium	30
4.5.2. pH of medium	31
4.5.3. Temperature of incubation	31
4.5.4. Period of incubation	31
4.6. Characterization of bacteriocins	31
4.6.1. Thermostability	31
4.6.2. pH stability	32
4.6.3. Storage stability	32
4.6.4. Stability against organic solvents	32
4.7. Purification of bacteriocin	32
4.7.1. Dialysis	32
4.7.2. Gel filtration	33
4.7.3. Anion-exchange and desalting column chromatographies	33
4.8. Mode of action	33
4.9. Inhibitory spectra	34
<b>5. Discussion</b>	<b>35</b>
<b>6. Summary</b>	<b>41</b>
<b>7. Bibliography</b>	<b>42</b>