

# Contents

Chapter 1 Introduction .....	1
[1.1]. <i>Listeria</i> spp.....	1
[1.2]. Main genus characteristics .....	1
[1.3]. <i>Listeria</i> infections: clinical description.....	2
[1.4]. <i>Listeria monocytogenes</i> infectious cycle .....	5
[1.5]. Molecular properties of interesting .....	6
[1.6]. Relevant aspects of <i>Listeria monocytogenes</i> concerning food processing environments.....	7
[1.7]. Bibliography .....	15
Chapter 2 <i>Listeria</i> Biofilm formation on abiotic surfaces related to food processing environments .....	21
[2.1]. Introduction.....	21
[2.2]. Defining biofilms and understanding their architectural complexity.....	21
[2.3]. Stages of biofilm formation .....	23
[2.4]. Adherence ability and biofilm formation of <i>Listeria</i> .....	24
[2.5]. Materials and methods .....	25
I. Test materials.....	25
II. Bacterial strains .....	25
III. Static biofilm on stainless steel.....	26
IV. Biofilm formation test under dynamic conditions .....	27
V. Sample preparation for SEM observation.....	28
VI. Microbial adhesion to solvents (MATS) .....	29
VII. Statistical analysis and treatment of data.....	29
[2.6]. Results.....	29
I. Adhesion under static conditions.....	29
II. Bacterial adhesion in turbulent flows .....	32
III. Cell superficial hydrophobicity .....	33
IV. Surface materials .....	34

V.	SEM examination of inoculated wires.....	36
VI.	Temperature.....	38
VII.	Influence of the medium on biofilm formation .....	40
[2.7].	Discussion.....	43
[2.8].	Bibliography .....	46
	Chapter 3 Biocide resistance of <i>Listeria</i> in different physiological state .....	50
[3.1].	Antimicrobials and antimicrobial resistance: general background .....	50
[3.2].	Assessment of bactericidal capacity of a biocide.....	50
[3.3].	Involved resistance mechanisms to biocides .....	52
[3.4].	Biocide resistance of biofilm and involved mechanism .....	53
[3.5].	Quaternary ammonium compounds (QACs) .....	54
[3.6].	Peroxyacetic acid or peracetic acid (PAA) .....	55
[3.7].	Polyphenolic compounds .....	56
[3.8].	Materials and methods .....	58
I.	Strains and growth conditions .....	58
II.	Investigated active substances .....	58
III.	Assessment of inhibitory effect of biocides.....	59
IV.	Bactericidal effect of antimicrobial on <i>Listeria</i> spp. strains.....	59
V.	Resistance of statically grown biofilm to pinosylvin.....	60
VI.	Bactericidal efficiency of disinfectants on biofilm grown under dynamic condition.....	60
[3.9].	Results.....	60
I.	Strains susceptibility to tested biocides .....	60
II.	Resistance of statically grown bacterial biofilm to antimicrobials.....	77
III.	Biocide resistance of biofilms grown under dynamic conditions.....	81
[3.10].	Discussion.....	84
[3.11].	Bibliography .....	89
	Chapter 4 Influence of <i>luxS</i> gene on biofilm formation and biocide resistance of <i>L. innocua</i> UC 8410.....	97
[4.1].	Introduction.....	97
[4.2].	Quorum sensing and bioluminescence in <i>V. fischeri</i> .....	98

[4.3]. Quorum sensing in <i>V. harvey</i> .....	100
[4.4]. Quorum sensing signalling in diverse bacterial species.....	101
[4.5]. Specificity of AI-based signalling.....	102
[4.6]. Role of <i>luxS</i> and AI-2 in cell methabolism .....	104
[4.7]. Biofilm formation and <i>LuxS</i> .....	106
[4.8]. Technical approaches to study of biofilms .....	106
[4.9]. Materials and methods .....	108
I. Test materials.....	108
II. Disinfectants .....	108
III. Bacterial strains and plasmids .....	108
IV. DNA Manipulation.....	108
V. Cloning procedure .....	109
VI. Preparation of electrocompetent cells.....	110
VII. Electroporation into <i>L. innocua</i> .....	110
VIII. Biofilm formation in two different conditions of growth.....	111
IX. Bactericidal efficiency of disinfectants .....	111
X. Monitoring inhibitory effect of selected antimicrobials during growth kinetic.....	111
XI. Microbial adhesion to solvents (MATS) .....	112
XII. AI2 bioluminescence assay.....	112
XIII. Statistical analysis and treatment of data.....	113
[4.10]. Results.....	114
I. <i>luxS</i> gene inactivation in <i>L. innocua</i> .....	114
II. Effect of <i>luxS</i> gene disruption through MATS and bioluminescence.....	117
III. Role of <i>luxS</i> in biofilm formation on abiotic surfaces .....	120
IV. Influence of <i>luxS</i> on biocide resistance .....	124
V. Evaluation of influence of <i>luxS</i> on growth kinetic and antimicrobial susceptibility through continuous monitoring methods.....	126
VI. Susceptibility of statically grown biofilms to polyphenols .....	131
[4.11]. Discussion.....	133
[4.12]. Bibliography .....	136

Chapter 5 Conclusions .....	141
Chapter 6 Acknowledgments .....	145