

Table of Contents

Abstract	1
Chapter 1 Literature review and Introduction	4
1 Introduction	5
1.1 Quorum sensing: an overview	5
1.1.1 Quorum sensing regulated pathogenesis of <i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i> (Pcc)	9
1.1.2 Quorum sensing regulated pathogenesis of <i>Pseudomonas aeruginosa</i>	13
1.2 Quorum quenching	15
1.2.1 Signal synthesis (LuxI) as target for quorum quenching	15
1.2.2 Sensing of signal molecule (AHL-LuxR) as target for quorum quenching	16
1.2.3 AHL as target for quorum quenching	16
1.2.4 Substrate specificity of AHL degrading enzymes	23
1.2.5 Occurrence of AHL degrading enzymes	23
1.3 Quorum quenching as an anti-virulence strategy	24
1.3.1 Expression of a gene coding AHL synthase in the plant tissue	24
1.3.2 Heterologous expression of genes encoding AHL-degrading enzymes in pathogen cell or in plant tissue	25
1.3.3 Employing natural AHL degrading bacteria as biocontrol agents	25
1.4 Biological control	26
1.5 <i>Bacillus</i> as quorum quenching based biocontrol agent	32
Rationale of the present study	33
Chapter 2 Isolation of special strains of <i>Bacillus</i> spp. possessing AHL degrading activity and their identification	35
2.1 Introduction	36
2.2 Materials and methods	37
2.2.1 Bacterial strains and culture conditions	37

2.2.2 Isolation of sporulating bacteria from root	37
2.2.3 Isolation of sporulating bacteria from rhizospheric soil	38
2.2.4 Bioassay for AHL degradation	38
2.2.5 Genomic DNA extraction	39
2.2.6 PCR amplification of 16S rRNA gene of isolates	39
2.2.7 Amplified ribosomal DNA restriction analysis (ARDRA)	40
2.3 Results	40
2.3.1 Isolation of sporulating bacteria	40
2.3.2 Screening of isolates for C6-HSL degrading ability	40
2.3.3 Genotypic characterization of selected isolates	42
2.4 Discussion	44
Chapter 3 Characterization of the selected AHL degrading <i>Bacillus</i> isolates for attenuation of soft rot caused by <i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	47
3.1 Introduction	48
3.2 Materials and method	50
3.2.1 Bacterial strains and culture conditions	50
3.2.2 <i>PccBR1</i> growth inhibition assay	51
3.2.3 Effect of <i>Bacillus</i> isolates on potato host	51
3.2.4 <i>In vitro</i> co-culture assay	51
3.2.5 Effect of <i>Bacillus</i> isolates on virulence factor production	52
3.2.6 <i>In vitro</i> soft rot attenuation assay on different hosts of <i>PccBR1</i>	52
3.2.7 <i>In vitro</i> curative biocontrol assay for soft rot caused by <i>PccBR1</i>	53
3.2.8 <i>In vitro</i> preventive biocontrol assay for soft rot by <i>PccBR1</i>	54
3.2.9 Colonization ability of <i>Bacillus</i> on mung bean roots	54
3.2.10 <i>In planta</i> assay for biocontrol of spoilage of mung bean sprouts	55
3.2.11 Data analysis	55
3.3 Results	56

3.3.1 <i>PccBR1</i> growth inhibition by <i>B. subtilis</i> Pls8	56
3.3.2 Deleterious effect of <i>B. aerius</i> Pls17 on potato	56
3.3.3 Effect of selected <i>Bacillus</i> isolates on 3-oxo-C6HSL accumulation and growth of <i>PccBR1</i>	57
3.3.4 Effect of <i>Bacillus</i> isolates on virulence enzymes production	59
3.3.5 Attenuation of soft rot caused by <i>PccBR1</i> on different host plants	60
3.3.6 Curative biocontrol of soft rot caused by <i>PccBR1</i>	61
3.3.7 Preventive biocontrol of soft rot caused by <i>PccBR1</i>	63
3.3.8 Root colonisation ability of <i>Bacillus</i> isolates	63
3.3.9 Biocontrol of spoilage of bean sprouts caused by <i>PccBR1</i> on susceptible variety of mung bean (<i>Vigna radiata</i>)	64
3.4 Discussion	66
Chapter 4 Characterization of the quorum quenching mechanism of <i>Lysinibacillus</i> sp. Gs50	70
4.1 Introduction	71
4.2 Materials and method	73
4.2.1 Bacterial strains and culture conditions	73
4.2.2 Degradation of different AHLs	73
4.2.3 Localisation of AHL degrading enzyme in <i>Lysinibacillus</i> sp. Gs50	74
4.2.4 AHL restoration assay	75
4.2.5 Carbon source utilisation	75
4.2.6 Genomic DNA extraction	75
4.2.7 Primer design and PCR amplification of putative <i>adeH</i> gene	76
4.2.8 Plasmid isolation	76
4.2.9 Restriction digestion of plasmid	76
4.2.10 Gel elution and purification of DNA by freeze thaw method	76
4.2.11 Cloning and heterologous expression of <i>adeH</i>	77
4.2.12 SDS PAGE and staining	77

4.2.13 Soft rot attenuation by <i>E.coli</i> BL21 (DE3) pET22b(+)/adeH	79
4.2.14 Purification of AHL degrading enzyme (AdeH)	79
4.2.15 Protein estimation	80
4.2.16 Mass spectrometry analysis of the products of AHL degradation by AdeH	80
4.2.17 Agar diffusion bioassay for quantification of AHL	80
4.2.18 Biochemical characterization of AdeH	81
4.2.18.1 Effect of substrate concentration	81
4.2.18.2 Temperature range and temperature optimum	81
4.2.18.3 pH range and pH optimum	81
4.2.18.4 Effect of metal ions	82
4.2.18.5 Determination of <i>KM</i> and <i>Vmax</i>	82
4.2.18.6 Thermal-stability	82
4.2.19 Nucleotide sequence accession number	82
4.3 Results	83
4.3.1 Characterization of AHL degrading <i>Lysinibacillus</i> sp. Gs50	83
4.3.1.1 Localisation of AHL degrading enzyme	83
4.3.1.2 Growth of <i>Lysinibacillus</i> sp. Gs50 on AHL as sole carbon source	84
4.3.1.3 Restoration of AHL degraded by <i>Lysinibacillus</i> sp. Gs50	84
4.3.2 Cloning of the gene responsible for AHL degradation in <i>Lysinibacillus</i> sp. Gs50	85
4.3.2.1 PCR amplification of putative gene coding for AHL degrading enzyme	85
4.3.2.2 Cloning into pTZ57R/T vector in <i>E.coli</i> DH5 α	85
4.3.2.3 Sub-cloning into pET22b(+) vector in <i>E.coli</i> BL21(DE3)	85
4.3.3 Heterologous expression of the gene for AHL degradation from <i>Lysinibacillus</i> sp. Gs50	86
4.3.4 AdeH is an AHL lactonase	89
4.3.5 Quantification of C6-HSL using agar diffusion bioassay	93
4.3.6 Biochemical characterization of AdeH	94

4.3.6.1 Effect of temperature on AdeH activity	94
4.3.6.2 Effect of pH on AdeH activity	94
4.3.6.3 Effect of metal ions on AdeH activity	95
4.3.6.4 Thermal stability of AdeH	95
4.3.6.5 AHL degradation kinetics of AdeH	95
4.4 Discussion	96
Chapter 5 Efficacy of quorum quenching based virulence attenuation of quorum sensing pathogen in situ	99
5.1 Introduction	100
5.2 Materials and Methods	102
5.2.1 Bacterial strains	102
5.2.2 Influence of environmental factors on quorum quenching activity of <i>Lysinibacillus</i> sp. Gs50	103
5.2.2.1 Effect of temperature	103
5.2.2.2 Effect of pH	103
5.2.2.3 Effect of C6-HSL concentration	103
5.2.3 Efficacy of quorum quenching on mung bean roots	103
5.2.3.1 Quorum sensing pathogen <i>P. aeruginosa</i> and Mung bean infection model	104
5.2.3.2 Quorum quenching of <i>P. aeruginosa</i> by <i>Lysinibacillus</i> sp. Gs50	106
5.2.4 Data Analysis	107
5.3 Results	107
5.3.1 Influence of environmental factors on quorum quenching activity of <i>Lysinibacillus</i> sp. Gs50	107
5.3.1.1 Influence of different temperature	107
5.3.1.2 Influence of different pH	108
5.3.1.3 Influence substrate concentration on quorum quenching activity of <i>Lysinibacillus</i> sp. Gs50	109
5.3.2 Efficacy of quorum quenching by <i>Lysinibacillus</i> sp. Gs50 in virulence attenuation of QS pathogen	109

5.3.2.1 Mung bean infection model of <i>P. aeruginosa</i> PAO1	109
5.3.2.2 Role of quorum sensing in infection of <i>P. aeruginosa</i>	112
5.3.3 Quorum quenching of <i>P.aeruginosa</i> PAO1 by <i>Lysinibacillus</i> sp. Gs50	117
5.3.3.1 In vitro AHL degradation by <i>Lysinibacillus</i> sp. Gs50	117
5.3.3.2 In planta virulence attenuation of <i>P. aeruginosa</i> PAO1 by quorum quenching <i>Lysinibacillus</i> sp. Gs50	118
5.3.3.3 Spatial distribution of quorum sensing pathogen <i>P. aeruginosa</i> PAO1 and quorum quenching <i>Lysinibacillus</i> sp. Gs50	120
5.4 Discussion	121
Summary	124
Conclusion	131
References	136
List of poster and oral presentations	151
List of publications	152