

List of figures

Figure 1.1: Microbiota composition Human Gastro-Intestinal tract (GIT)	4
Figure 1.2: Diagrammatic representation of healthy and disrupted commensal microbial community	4
Figure 1.3: Major mode of actions of probiotics	9
Figure 1.4: Cell surface of <i>Lactobacilli</i> with a schematic representation of cell-wall components and membrane-embedded proteins	9
Figure 1.5: Schematic representation of the secretion systems and the surface associated proteins in <i>Lactobacillus</i>	11
Figure 1.6: Bacterial adhesins and their host target	16
Figure 1.7: Schematic representation of bacterial Pili or Pilus-like structure, assembly and their secretion	16
Figure 1.8: Schematic representation of anchorless adhesins	21
Figure 2.1: Schematic representation of the secretome in <i>Lactobacillus</i>	25
Figure 2.2: Flowchart schema of secretome prediction from <i>L. acidophilus</i> genome	30
Figure 2.3: Sequence logo representing conserved LPxTG residues and motif in the alignment of well-known 168 LPxTG protein sequences	34
Figure 2.4: Sequence logo representing conserved lipobox motif in the alignment of well-known 65 prokaryotic Lipoprotein sequences	36
Figure 2.5: Graphical representation of predicted 223 proteins by category-wise	47
Figure 2.6: Distribution of predicted proteins with non-covalent binding domain and MUB domain	50
Figure 3.1: Functional diversity of GAPDH	52
Figure 3.2: Schematic representation of GAPDH affinity to ECM components	53
Figure 3.3: The vector map used in cloning of LaGAPDH	57
Figure 3.4: Agarose electrophoresis gel for genomic DNA and 16s rRNA amplicon	64
Figure 3.5: 16s rRNA sequencing analysis of strain	64
Figure 3.6: Cloning results of LaGAPDH	65
Figure 3.7: Sequence comparison of LaGAPDH nucleotide sequencing result and LBA0698 GAPDH entry from NCBI	66
Figure 3.8: SDS-PAGE of protein expression and purification results of r-LaGAPDH in <i>E. coli</i> Rosetta (DE3)	67
Figure 3.9: MALDI-TOF MS/MS confirmation of r-LaGAPDH	67
Figure 3.10: Results of hemagglutination assay of r-LaGAPDH towards rabbit erythrocytes	69
Figure 3.11: The effect of temperature on the hemagglutination of r-LaGAPDH in the hemagglutination assay	69
Figure 3.12: Representative SDS-PAGE gel showing r-LaGAPDH protein recovered from the coated mucin after the mucin binding assay	70
Figure 3.13: Enzyme kinetics plot of r-LaGAPDH using Michaelis-Menten equation	70
Figure 3.14: r-LaGAPDH molecular mass identification through gel filtration column	72
Figure 3.15: Circular dichroism spectra (CD) of r-LaGAPDH	72
Figure 3.16: Fluorescence spectroscopy studies of r-LaGAPDH with mannose and galactose	73
Figure 3.17: Fluorescence spectroscopy studies of r-LaGAPDH with N-Acetyl-D-	74

galactosamine and N-Acetyl-D-glucosamine	
Figure 4.1: LaGAPDH_C1 crystal and diffraction image	97
Figure 4.2: LaGAPDH_C7 crystal and diffraction image	97
Figure 4.3: LaGAPDH_C13 crystal and diffraction image	97
Figure 4.4: Electron density map	100
Figure 4.5: The ribbon representation of LaGAPDH	108
Figure 4.6: Sequence alignment of LaGAPDH with other known representative GAPDH	109
Figure 4.7: Multiple sequence alignment of GAPDH sequences and highlighting respective positions of three cysteine at 101, 156 and 326	110
Figure 4.8: Multiple sequence alignment and phylogenetic analysis	110
Figure 4.9: Comparison of amino acids in the active site of LaGAPDH	112
Figure 4.10: Structural difference in LaGAPDH model	112
Figure 4.11: Comparison of active site between LaGAPDH and PDB: 4QX6 structures with respect to its surface charge	116
Figure 5.1: Gene amplification and cloning of LaMubP	124
Figure 5.2: Clone confirmation of LaMubP. Sequence comparison of LaMubP nucleotide sequencing result and LBA1018 MubP entry from NCBI	125
Figure 5.3: Gene amplification and cloning of LaMubPtr	126
Figure 5.4: LaMubPtr Clone confirmation through nucleotide sequencing	126
Figure 5.5: Gene amplification and cloning of LaFBP	127
Figure 5.6: Gene amplification and cloning of LaFBPtr	128
Figure 5.7: SDS-PAGE analysis of r-LaMubP (pET15b+LaMubP) in <i>E. coli</i> Rosetta BL21 (DE3)	130
Figure 5.8: SDS-PAGE analysis of r-LaMubP (pET15b+LaMubP) in <i>E. coli</i> Rosetta BL21 (DE3) pLysS using autoinduction at 20°C induction	130
Figure 5.9: SDS-PAGE analysis of r-LaMubP protein purification	131
Figure 5.10: SDS-PAGE analysis of r-LaMubPtr (pET30a+LaMubPtr) in <i>E. coli</i> Rosetta BL21 (DE3) pLysS at 20°C induction	131
Figure 5.11: SDS-PAGE analysis of r-LaMubPtr (pET30a+LaMubPtr) purification	132
Figure 5.12: SDS-PAGE analysis of dialysed and concentrated purified r-LaMubP and r- LaMubPtr protein	132
Figure 5.13: SDS-PAGE analysis of r-LaFBP (pET28a+LaFBP & pET15b+LaFBP) in <i>E.</i> <i>coli</i> Rosetta BL21 (DE3) pLysS at 20°C induction	134
Figure 5.14: SDS-PAGE analysis of r-LaFBPtr (pET28a+LaFBPtr)	134
Figure 5.15: MALDI MS/MS analysis of purified protein	135
Figure 5.16: Far-UV CD spectrum of r-LaMubP	135
Figure 5.17: Multiple sequence alignment (MSA) analysis of LaMubP	137
Figure 5.18: Phylogenetic analysis of LaMubP	137
Figure 5.19: Secondary structure prediction and model generation of LaMubP	138
Figure 5.20: Multiple sequence alignment (MSA) analysis of LaFBP	139
Figure 5.21: Phylogenetic analysis of LaFBP	140
Figure 5.22: Secondary structure prediction and model generation of LaFBP	140

List of tables

Table 1.1: Positive benefits on human health by various strains of <i>Lactobacillus</i> .	5
Table 2.1: List of the 12 putatively identified LPxTG motif-containing proteins.	35
Table 2.2: List of identified 42 Lipoproteins from <i>L. acidophilus</i> .	36
Table 2.3: List of 108 identified proteins as putative N-terminal anchored protein	38
Table 2.4: List of 2 identified proteins as putative C-terminal anchored protein	41
Table 2.5: List of 59 identified putative secreted proteins	41
Table 2.6: List of identified proteins containing cell-wall anchoring/binding domain	43
Table 2.7: List of identified mucus-binding proteins by HMM using MUB profile search.	44
Table 2.8: List of predicted secretome proteins with their functional classification based on the protein domain.	45
Table 3.1: Extracellular GAPDH reported in bacteria and their host targets.	53
Table 3.2: Description of vectors and respective primers used for LaGAPDH cloning.	56
Table 3.3: Summary of expression trials of r-LaGAPDH detected on SDS-PAGE gel in different vectors.	68
Table 3.4: Dissociation constant of different carbohydrates with r-LaGAPDH determined by fluorescence spectroscopy	74
Table 4.1: Preliminary hits obtained with different crystallization screens	94
Table 4.2: Optimization of initial hits from crystallization trials	95
Table 4.3: X-ray diffraction statistics of LaGAPDH_C7 crystals	98
Table 4.4: X-ray diffraction statistics of LaGAPDH_C13 crystals	99
Table 4.5: Refinement statistics of LaGAPDH_C13	101
Table 4.6: Details of crystallization trials and diffraction of r-LaGAPDH complex	102
Table 4.7: X-ray diffraction statistics of LaGAPDH_S12, LaGAPDH_S16, LaGAPDH_S18 and LaGAPDH_S20 crystals	106
Table 5.1: List of primers used for PCR amplification of LaMubP, LaMubPtr, LaFBP and LaFBPtr cloning.	120
Table 5.2: Summary of expression trials of pET15b+LaMubP in <i>E.coli</i> strains.	133
Table 5.3: Sequence search results of LBA1018 against all other 11 mucus binding protein from <i>L. acidophilus</i>	136