

TABLE OF CONTENTS

Metabolic Engineering of <i>Escherichia coli</i> as Probiotics		
Chapters	Title	Page No.
Chapter 1	Review of literature and introduction	1-23
1.	Introduction	2
1.1.	Probiotics	2
1.2.	Current global and national status of Probiotics	2
1.3.	Characteristics of Probiotics	5
1.4	Groups of Probiotics	6
1.4.1	Lactic acid bacteria (LAB)	6
1.4.2	Bifidobacteria	7
1.4.3	Others	7
1.4.3.1	<i>Saccharomyces boulardii</i>	7
1.4.3.2	<i>Streptococcus gordonii</i>	7
1.5	Mechanism of action of Probiotics	8
1.6	Applications of Probiotic	9
1.7	<i>Escherichia coli</i>	11
1.7.1	Intra- and extra-intestinal pathogenic <i>E. coli</i> variants	14
1.7.2	<i>E. coli</i> : Transition between commensalism and pathogenicity	15
1.8	<i>Escherichia coli</i> as probiotic and its clinical significance	17
1.8.1	<i>E. coli</i> Nissle 1917	17
1.8.2	<i>E. coli</i> M-17 (EC-M17)	19
1.8.3	<i>E. coli</i> H22	19
1.9	Significance of <i>E. coli</i> in Genetic engineering	20
1.10	Genetic engineering of probiotics	20
1.10.1	Sequestration of toxins	21
1.10.2	Replacement therapy	21
1.10.3	Strategies that involve antibody production	21
1.10.4	Immune intervention	22
Chapter 2	Isolation and characterization of potential probiotic <i>Escherichia coli</i> strains from rat faecal samples	24-37
2.1	Introduction	25
2.2	Materials and Methods	27-29
2.2.1	Isolation of <i>E. coli</i> from Rats faecal samples	27

2.2.2	Testing for antimicrobial activity	27
2.2.3	Testing for resistance to antibiotics	27
2.2.4	Tolerance to acidic pH values	28
2.2.5	Detection of pathogenic strains	28
2.2.6	Characterization of the antimicrobial agent	29
2.2.7	Detection and identification of Colicin	29
2.3	Results	30-35
2.3.1	Isolation of <i>E. coli</i> strains from rat faecal matter	31
2.3.2	Screening of <i>E. coli</i> for antimicrobial activity	31
2.3.3	Antibiotic susceptibility of rat faecal <i>E. coli</i> isolates	32
2.3.4	Acid tolerance assay	33
2.3.5	Detection of pathogenic strains by Multiplex PCR of indicator genes	35
2.3.6	Polymerase chain reaction (PCR) for identification of colicin gene in <i>E. coli</i> isolates	35
2.4	DISCUSSION	36-37
Chapter 3	<i>In-vivo</i> localization of probiotic <i>Escherichia coli</i> containing <i>Vitreoscilla</i> hemoglobin (<i>vgb</i>) gene in rats and its effects in colonization	38-61
3.1	Introduction	39
3.2	Materials and methods	44-48
3.2.1	Bacterial strains, plasmids and culture conditions	44
3.2.2	Recombinant plasmids construction and transformation in <i>E. coli</i> 16	44
3.2.3	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis	45
3.2.4	Preparation of cells and cell free extracts	46
3.2.5	Catalase assay of cell free extract.	46
3.2.6	Animal Experiments	46
3.2.6.1	Experimental animals	46
3.2.6.2	Colonization experiments	47
3.2.6.3	Animal study to monitor effect of <i>E. coli</i> 16 pUC8-16gfp under oxidative stress	47
3.2.6.3.1	Assessment of liver function	48
3.2.6.3.2	Hepatic lipid peroxidation and catalase assay	48
3.2.6.3.3	Microscopic examination of liver	48
3.2.7	Statistical analysis	48
3.3	Results	49-59
3.3.1	<i>in vitro</i> studies	49
3.3.1.1	Growth profile and SDS PAGE of <i>E. coli</i> isolate 16 harboring GFP and VHb-GFP under microaerophilic condition.	49

3.3.1.2	VHb enhances <i>in vitro</i> catalase activity of <i>E. coli</i> 16 expressing VHb protein	51
3.3.2	<i>In vivo</i> studies	52
3.3.2.1	Colonization of probiotic <i>E. coli</i> 16 <i>vgb-gfp</i> in gastro-intestinal tract of rats exposed with intermittent antibiotic challenge.	52
3.3.2.2	Effects of probiotic <i>E. coli</i> 16 (pUC8-16 <i>gfp</i>) harboring <i>vgb</i> gene on liver function under CCl ₄ induced oxidative stress	54
3.3.2.2.1	SGOT and SGPT activity in plasma sample of rats	54
3.3.2.2.2	Catalase and lipid peroxidation activity in liver	56
3.3.2.2.3	Microscopic examination of liver	56
3.3.2.2.4	<i>In vivo</i> localization of the <i>E. coli</i> 16 tagged with <i>gfp</i>	59
3.4	Discussion	61
Chapters	Title	Page No.
Chapter 4	Molecular Fingerprinting of the faecal microbiota in relation to high fructose induced metabolic disorder.	62-78
4.1	Introduction	63
4.2	Materials and Methods	68-71
4.2.1	Animals and treatment	68
4.2.2	Experimental groups	68
4.2.3	Genomic DNA isolation from fecal samples	69
4.2.4	PCR amplification of V3 region of 16S rDNA from faecal matter	70
4.2.5	Denaturing gradient gel electrophoresis (DGGE)	70
4.2.6	Silver staining	71
4.2.7	Biochemical analyses in serum samples	71
4.2.8	Statistical analysis	71
4.3	Results	72-76
4.3.1	Microbiota of the rat faecal matter fed with starch and fructose in diet	72
4.3.2	Oral glucose tolerance	74
4.3.3	Serum lipid profiles	74
4.4	Discussion	77-78
Chapter 5	Effect of probiotic <i>E. coli</i> 16 strain containing inulosucrase gene in alleviation of sucrose mediated metabolic disorder.	79-127
5.1	Introduction	80

5.2	Materials and Methods	91-98
5.2.1	Bacterial strains, plasmids and culture conditions	91
5.2.2	Construction of plasmids: pGRG- <i>gfp</i> , pGRG 8-16 <i>gfp</i> , pMAL-p2Δ <i>lacI</i> ^Q and pMAL-p2Δ <i>lacI</i> ^Q - <i>inuJ</i>	91
5.2.3	Chromosomal integrations of <i>gfp</i> and <i>vgb-gfp</i> genes in <i>E. coli</i> 16	92
5.2.4	Transformation of pMAL-p2Δ <i>lacI</i> ^Q and pMAL-p2Δ <i>lacI</i> ^Q - <i>inuJ</i> plasmid in <i>E. coli</i> 16 integrants.	93
5.2.5	Preparation of <i>E. coli</i> cell extracts and <i>inuJ</i> activity assay	93
5.2.6	SDS-PAGE and Activity staining of <i>inuJ</i> gene	93
5.2.7	Animal Experiments	94
5.2.7.1	Experimental animals	94
5.2.7.2	<i>In vivo</i> localization of <i>E. coli</i> 16 integrants in Charles Foster rats	94
5.2.7.3	Composition of standard and high-Sucrose diet (%) given to the rats	94
5.2.7.4	Short (28 days) and Long (120 days) term effects of probiotic on 20% sucrose.	96
5.2.8	Biochemical Parameters estimation	97
5.2.8.1	Biochemical analyses in serum samples	97
5.2.8.2	Determination of biochemical parameters in liver	97
5.2.9	Cytokine estimation in intestinal tissue	97
5.2.10	Statistical analysis	98
5.3	Results	99-124
5.3.1	Characterization of integrants of <i>gfp</i> and <i>vgb-gfp</i> in probiotic <i>E. coli</i> 16	99
5.3.2	Constitutive expression periplasmic expression pMAL-p2Δ <i>lacI</i> ^Q - <i>inuJ</i>	101
5.3.3	Inulosucrase enzyme activity in <i>E. coli</i> BL-21 transformants containing pMAL-p2Δ <i>lacI</i> ^Q - <i>inuJ</i> plasmid	104
5.3.4	Activity staining of inulosucrase	108
5.3.5	Determining the effectiveness of <i>E. coli</i> 16 integrant harboring <i>inuJ</i> ge on sucrose fed rats	108
5.3.6	Short term effects (28 days) of probiotic harbouring <i>inuJ</i> gene	110
5.3.6.1	Serum Lipid profile	110
5.3.6.2	Antioxidant status of liver	112
5.3.6.3	Cytokine estimation in intestinal tissue	114
5.3.10	Long term effects (120 days) of probiotic harbouring <i>inuJ</i> gene	117
5.3.10.1	Biochemical analysis of serum samples.	117
5.4	Discussion	125-127