

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

THE EFFECTS OF RICE FIBRE ON PROBIOTIC FERMENTATION

A thesis presented in partial fulfilment of the requirements
for the degree of

Doctor of Philosophy in

Food Technology and Microbiology

at
Massey University, Palmerston North
New Zealand

Warnakulasuriya Mary Ann Dipika Binosha Fernando

2011

ABSTRACT

The role of rice fibre in stimulating the growth and SCFA (Short Chain Fatty Acid) formation by human faecal micro-flora and individual probiotics and co-cultures was investigated. The effects of environmental factors on the adhesion of probiotics on rice fibre were also evaluated.

Fibre fractions of rice enhanced the growth of human colon microflora (*Bifidobacterium* species and *Lactobacillus* species) with a corresponding increase in the quantity of SCFA produced. However, individual microorganisms showed different preferences for different rice varieties and specific fractions of rice fibre. Pure cultures of the genus *Bifidobacterium* and genus *Lactobacillus* fermented rice fibre fractions irrespective of the rice variety. However, the genus *Bifidobacterium* produced more SCFA than genus *Lactobacillus*. Co-cultures of *Bifidobacteria* and *Lactobacilli* showed a greater ability than pure cultures to digest fibre and form SCFA, indicating synergism. Co-cultures used the fibre fractions irrespective of the rice variety. All microflora from mixed faecal inocula, pure and combinations of probiotic cultures showed a preference for total dietary fibre than insoluble and soluble dietary fibre fractions based on fermentation and SCFA production. All cultures tested, including human faecal cultures, pure cultures and co-cultures, produced more acetate than propionate and butyrate.

Pure cultures and co-cultures adhered to rice fibre. Adhesion was influenced by environmental factors and is believed to play a role in the fermentation of rice fibre. Rice fibre is a suitable substrate for probiotic microflora.

ACKNOWLEDGEMENTS

Firstly I would like to thank Almighty God for his indescribable blessings and good health given to me and my family to make this thesis a success.

The writing of a thesis can be an amazing learning experience; however, it is obviously not possible without the personal and practical support and encouragement of numerous people.

My sincere gratitude goes to my supervisors, my husband, children, parents, and all my friends, both in New Zealand and Sri Lanka. I wish to thank Associate Professor Steve Flint, Professor Charles Brennan from Massey University and Manchester University UK, and Professor Bamunuarchichi and Professor Ranaweera from University of Sri Jayawardanapura, Sri Lanka, for inspiring and guiding me through the research.

My research for this dissertation was made more competent, but also much more widespread, through the use of several electronic and library assets. Thus, I gladly convey my gratitude to the IT team at Massey University and the library staff in supporting me in my work.

Many people within the Institute and staff of the Massey University assisted and encouraged me in various ways during my studies. I am especially grateful to my colleagues at Open University, Sri Lanka, for all the support and encouragement during my research.

My postgraduate studies would not have been the same without the financial support given by the ADB – Sri Lanka. I not only studied, but relaxed and enjoyed life well together with our lovely two daughters, Kendrea and Porshia during this period.

This thesis would not have been possible without the perceptive, helpful, and appropriate comments of my esteemed advisors, Associate Professor Steve Flint and Professor Charles Brennan. They were readily available for me and always read and responded to the drafts of each stage of my work more rapidly than I could have expected.

Of course, despite all the assistance provided by my supervisors and others I alone remain responsible for the content of the following, including any errors or omissions, which may unwittingly remain.

PUBLICATIONS

Studies completed during candidature, some of which are reported in this thesis have been presented in books, Journals and in conferences.

Papers

- Fernando, W.M.A.D.B.,** Ranaweera, K.K.D.S., Bamunuarachchi, A., Brennan, S.C. (2007) In vitro fermentative activity of human fecal microflora on rice fiber. **Asia Pacific Journal of Clinical Nutrition** 16 (supplement 3):s83.
- Fernando,, W.M.A.D.B.,** Ranaweera', K.K.D.S., Bamunuarachchi', A., Brennan, C.S. (2008) The influence of fibre fractions in vitro fermentation production of short chain fatty acid using human feacal micro flora. **International journal of food science and technology** 43(12): 2237 – 2244.
- Fernando, W.M.A.D.B.,** Ranaweera, K.K.D.S., Bamunuarachchi, A., Brennan, C.S. (2008) The invitro fermentation of rice dietary fibre: production of short chain fatty acid **In: Proceeding of Nutritional society of New Zealand** (Eds C.S. Brennan), Massey university press ISSN 0110-4187.volume 31:68.
- Fernando, W.M.A.D.B.,** Brennan, C.S., Flint, S.H., Ranaweera, K.K.D.S., Bamunuarachchi, A. (2009) Enhancement of metabolite formation by pure cultures of probiotics on rice fibre. **International Journal of Food Science and Technology** 45(4):690 – 696.
- Fernando, W.M.A.D.B.,** Brennan, C.S., Flint, S.H., Ranaweera, K.K.D.S., Bamunuarachchi, A. (2010) In vitro fermentation of co cultures to rice dietary fibre and its implications in probiotic technologies. **Australasian Medical Journal** 1(1): 107.
- Fernando, W.M.A.D.B.,** Brennan, C.S., Flint, S.H., Ranaweera, K.K.D.S., Bamunuarachchi, A. (2011) The effect of rice fibre fractions on the growth of co-cultures of probiotics. **The journal of food science and technology** 48(1): 14-25.

TABLE OF CONTENT

ABSTRACT	i
ACKNOWLEDGEMENTS.....	ii
PUBLICATIONS	iv
TABLE OF CONTENT.....	v
LIST OF TABLES	xiv
LIST OF FIGURES	xvii
ABBREVIATIONS	xxii
CHAPTER 1	1
General Introduction	1
<i> 1.1 Research Question</i>	<i>4</i>
<i> 1.2 Hypothesis</i>	<i>4</i>
<i> 1.3 Research Objectives</i>	<i>4</i>
<i> 1.4 Research steps</i>	<i>5</i>
CHAPTER 2	8
Literature Review	8
<i> 2.1 Introduction.....</i>	<i>8</i>
<i> 2.2 Concept of fibre</i>	<i>8</i>
<i> 2.3 Development and evolution of fibre determination methods</i>	<i>12</i>
<i> 2.4 Classification of dietary fibre</i>	<i>16</i>
<i> 2.5 Rice</i>	<i>19</i>
<i> 2.6 Soluble and insoluble fibre in rice</i>	<i>27</i>

2.7 Physiological and metabolic effects of dietary fibre on human health.....	28
2.8 Daily intake of dietary fibre	31
2.9 Fermentation of dietary fibre	31
2.10 Why <i>in-vitro</i> fermentation?	31
 2.10.1. Limitations and importance of <i>in-vitro</i> fermentation	32
2.11 <i>In-vitro</i> fermentation models	32
2.12 Products from the fermentation of dietary fibre	34
2.13 Short chain fatty acids and their implications	35
2.14 Factors influencing SCFA formation during fermentation.....	36
2.15 Concept of prebiotic, probiotic, and symbiotic	39
2.16 Colonic food and prebiotics	40
2.17 Current probiotic position and state of the art.....	41
2.18 <i>Lactobacillus</i> Species	46
2.19 <i>Bifidobacterium</i> species	48
2.20 Concept of symbiotic	49
2.21 Strategy for acetate, propionate and butyrate formation by <i>Bifidobacteria</i> and <i>Lactobacillus</i> species	50
2.22 Microbial adhesion	53
2.23 Conclusion.....	54
CHAPTER 3	56
General Materials and methods.....	56
 3.1 Sample collection	56
 3.2 Sample preparation	56
 3.3 Determination and extraction of soluble, insoluble and total dietary fibre	56
 3.3.1 Digesting the original sample with enzymes	57

3.3.2 Determination of insoluble dietary fibre.....	58
3.3.2.1. Protein and ash content of the sample	58
Calculation of the ash weight	59
3.3.3 Determination of soluble dietary fibre	59
3.3.4 Determination of total dietary fibre	59
MES/TRIS buffer preparation	60
3.3.5 Calculation of percentage of dietary fibre in the samples.....	61
3.3.5.1 Filtration	61
3.3.6 Nitrogen Determination by the Kjeldahl method	61
3.3.6.1 Digestion.....	61
3.3.6.2 Distillation.....	62
3.3.6.3 Titration	62
3.3.6.4 Calculation of crude protein in the sample.....	62
3.4. <i>Determination of purity of extracted fibre fractions.....</i>	62
3.4.1 Determination of the presence of starch in extracted fibre.....	62
3.4.2 Determination of moisture content of extracted fibre	62
3.4.3 Determination of the presence of fat in the extracted fibre	63
3.5 <i>In-vitro fermentation of fibre fraction with human inocula</i>	63
3.5.1 Human subjects	63
3.5.2 Diet.....	63
3.5.3 Preparation of inocula	64
3.5.4 Analysis of faecal samples	64
3.5.5 <i>In-vitro</i> fermentation with faecal inocula.....	65
3.6 <i>In-vitro fermentation of dietary fibre with probiotic cultures</i>	66
3.6.1 Bactereial culture media.....	66

3.6.2 Bacterial strains.....	66
3.6.3 Co-cultures.....	67
3.6.4 Preparation of cell suspensions	67
3.6.5 Preparation of growth medium.....	67
3.6.6 <i>In-vitro</i> fermentation with pure cultures or co-cultures.....	68
3.7 <i>A study of in-vitro fermentation with human inocula and pure probiotics cultures</i>	69
 3.7.1 Determination of pH, optical density and viable cell counts	69
 3.7.2 Specific growth rate	69
 3.7.3 Determination of the relative growth yield	69
3.8 <i>Determination of short chain fatty acids produced from fermentation by pure and mixed cultures</i>	70
 3.8.1 Determination of SCFA (short chain fatty acids).....	70
 3.8.2 Preparation of samples and standards for Gas Chromatography	70
 3.8.2.3 Preparation of stock standard solution.....	71
3.9 <i>Determination of indigestible fibre.....</i>	74
 3.9.1 Determination of the indigestible soluble dietary fibre following fermentation of SDF.....	74
 3.9.2 Determination of the indigestible insoluble dietary fibre following fermentation of IDF	74
 3.9.3 Preparation of acid pepsin solution	75
 3.9.4 Determination of indigestible total dietary fibre following fermentation of TDF.....	75
 3.9.5 Determination of the dry matter disappearance in fermented substrate	75
3.10 <i>Statistical analysis</i>	75
CHAPTER 4	77

IDF, SDF and TDF Dietary Fibre Content of Rice.....	77
4.1 Abstract.....	77
4.2 Introduction.....	77
4.3 Materials and Methods	78
4.4 Results.....	78
4.5 Discussion.....	80
4.6 Conclusion.....	82
CHAPTER 5	83
Growth of Fecal Organisms on Rice Fibre during <i>In-vitro</i> Fermentation.....	83
5.1 Abstract.....	83
5.2 Introduction.....	84
5.3 Materials and Methods	85
5.4 Results.....	85
5.5 Discussion.....	91
5.6 Conclusion.....	94
CHAPTER 6	95
Short Chain Fatty Acid Formation from the <i>In-vitro</i> Fermentation of Rice Fibre with Human Inocula.....	95
6.1 Abstract.....	95
6.2 Introduction.....	96
6.3 Materials and methods	98
6.4 Results.....	98
6.5. Discussion.....	104
6.6 Conclusion.....	109

CHAPTER 7	110
Role of Rice Fibre in Stimulating <i>in-vitro</i> Growth of <i>Lactobacillus</i> and <i>Bifidobacterium</i> species	110
7.1 Abstract.....	110
7.1 Introduction.....	111
7.3 Materials and methods.....	112
7.4 Results.....	112
7.5 Discussion.....	122
7.6 Conclusion.....	126
CHAPTER 8	127
Enhancement of Short Chain Fatty Acid (SCFA) Formation by Pure Cultures of Probiotics on Rice Fibre	127
8.1 Abstract.....	127
8.2 Introduction.....	128
8.3 Materials and Methods	129
8.4 Results.....	130
8.5 Discussion.....	138
8.6 Conclusion.....	144
CHAPTER 9	145
The Effect of Rice Fibre Fractions on the Growth of	145
Co-Cultures of Probiotics.....	145
9.1 Abstract.....	145
9.2 Introduction.....	146

<i>9.3 Materials and methods</i>	147
<i>9.4 Results</i>	148
<i>9.5 Discussion</i>	169
<i>9.6 Conclusion</i>	175
CHAPTER 10	176
SCFA Formation by Combinations of Probiotics	176
on Rice Fibre.....	176
<i>10.1 Abstract</i>	176
<i>10.2 Introduction</i>	177
<i>10.3 Materials and Methods</i>	178
<i>10.4 Results</i>	178
<i>10.5 Discussion</i>	191
<i>10.6 Conclusion</i>	197
CHAPTER 11	199
The Influence of Environmental Factors on the Adhesion of Probiotics to Insoluble, Soluble and Total Dietary Fibre of Rice	199
<i>11.1 Abstract</i>	199
<i>11.2 Introduction</i>	199
<i>11.3 Materials and methods</i>	201
<i>11.3.1 General methods</i>	201
<i>11.3.2 Preparation of cell suspensions</i>	201
<i>11.3.3 Adhesion to fibre</i>	201
<i>11.3.5 Study of adhesion mechanisms</i>	203

11.3.6 Adhesion under conditions to simulate to the environment of the human stomach and small intestine	205
11.4 Results.....	206
11.5 Discussion.....	215
11.6 Conclusion.....	220
CHAPTER 12	221
Environmental factors affecting the adhesion of combinations of probiotics to insoluble, soluble and total dietary rice fibre.....	221
12.1 Abstract.....	221
12.2 Introduction.....	222
12.3 Materials and methods.....	223
12.4 Results.....	223
12.5 Discussion.....	245
12.6 Conclusion.....	251
CHAPTER 13	252
General Discussion	252
13.1 Composition of dietary fibre in rice.....	252
13.2 Growth of faecal inocula, pure cultures and combinations on rice fibre	253
13.3 SCFA formation on fibre	256
13.4 Adhesion on fibre	258
13.5 Limitations of the study.....	261
CHAPTER 14	263
Future Developments and Final Conclusions.....	263

<i>14.1 Compositional analysis of IDF, SDF, and TDF</i>	263
<i>14.2 Sampling.....</i>	263
<i>14.3 Microbial characterisation</i>	263
<i>14.4 Physiological changes.....</i>	264
<i>14.5 Adhesion of organisms on fibre.....</i>	264
<i>14.6 Extending studies on dietary fibre.....</i>	264
<i>14.7 Application in medicine</i>	264
<i>14.8 Final Conclusions</i>	265
REFERENCES.....	268
APPENDIX A	323
APPENDIX B	331
APPENDIX C	336
APPENDIX D	343

LIST OF TABLES

Chapter 2

Table 2.1	Fibre classification by degree of fermentation.....	18
Table 2.2	Nutritional composition of selected cereals	21
Table 2.3	Approximate Composition of Rough Rice	25
Table 2.4	Proximate Analyses of Different Rice Types	26
Table 2.5	Different types of probiotic products.....	44
Table 2.6	Common probiotics.....	46

Chapter 4

Table 4.1	Content (%) of dietary fibre in rice varieties as TDF, SDF and IDF	79
Table 4.2	Analysis of purity of extracted fibre	80

Chapter 6

Table 6.1	SCFA molar ratios (%) measured after 24 h fermentation with all rice fibre using specific human fecal inocula.....	98
Table 6.2	SCFA molar ratios (%) measured after 24 h fermentation with human inocula for specific rice varieties	99

Chapter 7

Table 7.1	pH values at different h of incubation times	119
Table 7.2	Relative biomass yields on different fibre extracts compared with growth on glucose	120
Table 7.3	Specific growth rates of microorganisms at different time points	121

Chapter 8

Table 8.1	Fermentation of rice dietary fibre with probiotics leading to formation of short chain fatty acids (SCFA, m moles/100mL) at 24h	131
Table 8.2	Relative percentage of SCFA formation by probiotics at 24h	132
Table 8.3	Percentage of substrate remaining after each time point	133

Chapter 9

Table 9.1	pH value of culture combinations at each time point	162
Table 9.2	Specific growth rates of combinations as individuals/ species	163

Chapter 10

Table 10.1	Fermentation of rice dietary fibre with combinations leads to formation of short chain fatty acids (SCFA, m moles/100mL)	180
Table 10.2	Molar fraction of acetate: propionate: butyrate	182
Table 10.3	Percentage of substrate remaining after each time point	184

Chapter 11

Table 11. 1	Treatments used in the investigation of mechanisms of adhesion	203
Table 11. 2	Treatments used to simulate conditions for adhesion during passage through the upper gastrointestinal tract	205

Appendix B

Table 1-App B	Fermentation of <i>rice</i> dietary fibre with probiotics leads to formation of short chain fatty acids (SCFA, m moles/100mL) (Chapter 8).....	331
---------------	--	-----

Table 2-App B Relative percentage of SCFA formation by probiotics	333
---	-----

Appendix C

Table 1App C Fermentation of rice dietary fibre with combinations leads to formation of short chain fatty acids (SCFA, m moles/100mL) (Chapter10).....	336
Table 2 AppC Molar fraction of acetate: propionate: butyrate	340

Appendix D

Table 1App D Stock solutions	343
Table 2 AppD Standard mix	343

LIST OF FIGURES

Chapter 1

Figure 1.1	Research in simple format.....	7
------------	--------------------------------	---

Chapter 2

Figure 2.1	Dietary fibre classifications	17
Figure 2.2	Fibre classifications according to water solubility	18
Figure 2.3	World cereal production and utilisation <i>Source: FAO, (2009)</i>	19
Figure 2.4	Basic steps for the production of white rice and brown rice	22
Figure 2.5	Rice grain structure.....	24
Figure 2.6	Structures of sugars present in rice.....	27
Figure 2.7	Formation of SCFA and stoichiometry	35
Figure 2.8	Glycolytic path way	50
Figure 2.9	Fermentation of pentoses and hexoses by the phosphoketolase	51
Figure 2.10	Bifidus path way	52

Chapter 4

Figure 4.1	Physical appearances of selected rice varieties.....	78
------------	--	----

Chapter 5

Figure 5.1	Comparison between the faecal bacteria counts before and after consuming rice for 4 months.	85
Figure 5.2	Proportion of <i>Bifidobacterium</i> species and <i>Lactobacillus</i> species from the total bacterial count at each sampling time.	86

Figure 5.3	Growth curve of Total aerobes, Total anaerobe, Genus <i>Bifidobacterium</i> and Genus <i>Lactobacillus</i> on rice varieties.....	88
Figure 5.4	pH values (bars) and the optical density (lines) of rice varieties at different time intervals.....	90

Chapter 6

Figure 6.1	Comparison of SCFA formation from rice fibre fermentation using faecal microflora from individuals taken before the rice diet.....	99
Figure 6.2	Total SCFA (acetate +propionate +butyrate) formation by individual inocula (H1 – H4)	101
Figure 6.3	Total SCFA formation by rice types.....	102
Figure 6.4	Individual SCFA formation by rice varieties during 24 h fermentation	104

Chapter 7

Figure 7.1	Bacterial growth in media containing different dietary fibre isolates and in glucose.	115
Figure 7.2	Growth of four different microorganisms, measured by optical density readings, on three different fractions of dietary fibre and glucose.	118

Chapter 8

Figure 8.1	Total SCFA formation by probiotics on rice varieties.....	135
Figure 8.2	Total SCFA formations on rice varieties	136
Figure 8.3	Total SCFA formation from IDF, SDF and TDF.....	137

Chapter 9

Figure 9.1	Growth of individual organisms in combination on rice variety RR1.....	153
------------	--	-----

Figure 9.2	Growth of individual organism in combinations on rice variety RR2.....	157
Figure 9.3	Optical densities of combinations at each point.....	161
Figure 9.4	Comparative growths of different microbial combinations on fibre.....	168

Chapter 10

Figure 10.1	Total SCFA produced on different substrates.....	188
Figure 10.2	Total SCFA on different rice varieties using nine probiotic combinations.	189
Figure 10.3	Total SCFA on fibre fractions.....	190

Chapter 11

Figure 11.1	Effect of time on adhesion (have considered average of fibre fractions of RR1 and RR2) on bacterial adhesion to different rice fibre fractions.	206
Figure 11.2	Adhesion of bacteria to fibre fractions.....	206
Figure 11.3A	Effect of concentration of substrate from RR1 on bacterial adhesion to different rice fibre fraction.....	207
Figure 11.3B	Effect of concentration of substrate from RR2 on bacterial adhesion to different rice fibre fractions)	207
Figure 11.4	Effect of body temperature (37^0C), heat killed cells and, room temperature on bacterial adhesion to different rice fibre fractions	208
Figure 11.5A	Effect of growth on adhesion of RR1.....	208
Figure 11.5B	Effect of growth phase on adhesion of RR2.....	209
Figure 11.6	Effect of Chemicals NaCl, Tween 80 and Phosphate buffer on bacterial adhesion to different rice fibre fractions.....	209
Figure 11.7A	Effect of pH on fibre from RR1 on bacterial adhesion to different rice fibre fractions.....	210

Figure 11.7B	Effect of pH on fibre from RR2 on bacterial adhesion to different rice fibre fractions.....	210
Figure 11.8	Effect of pepsin and proteinase on bacterial adhesion to different rice fibre fractions.....	211
Figure 11.9	Adhesion of bacteria to rice fibre fractions in the presence of glucose.....	211
Figure 11.10A	Adhesion of bacteria to fibre fractions in the presence of Sucrose, Lactose and Maltose.....	212
Figure 11.10B	Adhesion of fibre fractions in the presence of Cellobiose, Trehalose	212
Figure 11.11	Adhesionof fibre fractions in the presence of Amylopectin, motodextrin, Amylose	213
Figure 11.12	Effect spent medium, fresh medium, Pepsin treated medium on bacterial adhesion to different rice fibre fractions.....	214
Figure 11.13A	Effect of simulated gastrointestinal conditions on fibre from RR1 on bac adhedsion different rice fibre fractions	214
Figure 11.13B	Effect of simulated gastrointestinal conditions on fibre from RR2 on bac terial adhesion to different rice fibre fractions.....	215

Chapter 12

Figure 12.1	Adhesion of bacterial combinations to rice fibre fractions.....	224
Figure 12.2	Adhesion of bacterial combinations to rice fibre fractions in the presence of Glucose.....	224
Figure 12.3	A,B,C- Adhesion of bacterial combinations to rice fibre fractions in the presence of Sucrose, Lactose, and Maltose.....	226
Figure 12.3	D,E,F- Adhesion of bacterial combinations to rice fibre fractions in the presence of Cellobiose, Trehalose	227

Figure 12.4	Adhesion of bacterial combinations to rice fibre fractions in the presence of Amylopectin, moltodextrin, Amylose.....	229
Figure 12.5	Effect of Chemicals	231
Figure 12.6	Effect of medium	232
Figure 12.7	Effect of pepsin and proteinase	234
Figure 12.8	Effect of Temperture	235
Figure 12.9	A, B, C- Effect of pH on fibre from RR1 (on the adhesion of bacterial combinations to rice fibre fractions.	237
Figure 12.9	D, E, F- Effect of pH on fibre from RR2 on the adhesion of bacterial combinations to rice fibre fractions.	238
Figure 12.10	Effect of gastrointestinal conditions on the adhesion of fibre fraction of rice (Acid pepsin solution, Pancreatin, Bile).	240
Figure 12.11	A, B, C- Effect of concentration of substrate on the adhesion of bacterial combinations to rice fibre fractions	241
Figure 12.12	Effect of time on the adhesion of bacterial combinations to rice fibre fractions (average of fibre fractions of RR1 and RR2).....	242
Figure 12.13	A,B,C- Effect of growth phase on the adhesion of bacterial combinations to rice fibre fractions RR1	243
Figure 12.13	D,E,F- Effect of growth phase on the adhesion of bacterial combinations to rice fibre fractions RR2	244

ABBREVIATIONS

ADF	Acid Detergent Fibre
DF	Dietary fibre
DP	Degree of polymerization
GC	Gas chromatography
GLC	Gas liquid chromatography
GIT	Gastrointestinal tract
HPLC	High performance liquid chromatography
NDF	Neutral Detergent Fibre
NDO	Non digestible oligosaccharides
NSP	Non starch polyaccharide
<i>BB/B. breve</i>	<i>Bifidobactrea breve</i>
<i>BL/B. longum</i>	<i>Bifidobactera longum</i>
h	Hours
IDF	Insoluble dietary fibre
<i>LA/L. acidophilus</i>	<i>Lactoabcillus acidophilus</i>
<i>LR/L. rhamnosus</i>	<i>Lactoabcillus rhamnosus</i>
LAB	Lactic acid bacterea
OD	Optical Density
RS	Resistant starch