# OUTLINE OF THE Ph.D. THESIS PRESENTATION

## SYNOPSIS

1.0 INTRODUCTION  
2.0 REVIEW OF LITERATURE  
2.1 Microorganisms and human health  
2.2 Fermented traditional foods of India  
2.3 Nutritional status of Indian traditional foods  
2.4 Prebiotics  
2.5 Probiotics  
2.6 Food associated yeasts  
2.7 Lactic acid bacteria  
2.8 Bifidobacteria  
3.0 OBJECT AND SCOPE OF STUDY  
4.0 EXPERIMENTAL PLAN, RESULTS AND DISCUSSION  
4.1 Isolation and characterization of native cultures of lactic acid bacteria, bifidobacteria and yeasts for desirable attributes by conventional and nucleic acid based detection approaches  
4.2 Optimized phytase, α-D-galactosidase, bile salt hydrolase and antibacterial activities elaborated by potent microbial cultures in soy whey based medium by response surface methodology  
4.3 Functional attributes of microbial phytase and α-D-galactosidase in lowering levels of phytic acid and flatulence causing oligosaccharides in flours of cereals and legumes  
4.4 Beneficial attributes in selected foods through prebiotic and probiotic interactions  
5.0 SUMMARY AND CONCLUSION  
6.0 BIBLIOGRAPHY
2.0 REVIEW OF LITERATURE

2.1 MICROORGANISMS AND HUMAN HEALTH

2.2 FERMENTED TRADITIONAL FOODS OF INDIA

2.2.1 Background scenario

2.2.2 Milk-based foods
2.2.2.1 Dahi
2.2.2.2 Mishti doi
2.2.2.3 Lassi
2.2.2.4 Shrikhand

2.2.3 Cereal and legume-based foods
2.2.3.1 Idli
2.2.3.2 Dosa
2.2.3.3 Kadhi
2.2.3.4 Dhokla
2.2.3.5 Punabi warri

2.2.4 Milk and Cereal / legume-based foods
2.2.4.1 Rabadi
2.2.4.2 Kulcha
2.2.4.3 Naan

2.2.5 Vegetable-based fermented foods

2.3 NUTRITIONAL STATUS OF INDIAN TRADITIONAL FOODS

2.3.1 Bioavailability of minerals, vitamins and proteins as affected by phytate
2.3.1.1 pH
2.3.1.2 Minerals and phytate interactions

2.4 PREBIOTICS

2.4.1 Definition and nomenclature
2.4.2 Non-digestible higher polysaccharides
2.4.3 Significance of prebiotics
2.4.4 Categories of prebiotics

2.4.4.1 Established prebiotic oligosaccharides
2.4.4.2 Emerging prebiotic oligosaccharides
2.4.4.3 Potential prebiotics

2.4.5 Interactions between Prebiotics and microbiota

2.5 PROBIOTICS

2.5.1 Definition and nomenclature
2.5.2 Selection criteria and attributes

2.5.2.1 Source of strains
2.5.2.2 Tolerance to acid and bile
2.5.2.3 Adherence to intestinal cells
2.5.2.4 β-galactosidase activity
2.5.2.5 Stability and viability
2.5.2.6 Beneficial attributes
2.5.3 Protocols for commercial probiotic preparations 51
2.5.4 Health and therapeutic attributes 54
   2.5.4.1 Treatment of diarrhoea 55
   2.5.4.2 Clostridium difficile disease 56
   2.5.4.3 Alleviation of constipation 57
   2.5.4.4 Alleviation of symptoms of lactose malabsorption 57
   2.5.4.5 Enhancement of immune function 58
   2.5.4.6 Suppression of tumorigenesis 58
   2.5.4.7 Cholesterol reduction 59
2.5.5 Safety of probiotics and food applications 59

2.6 FOOD ASSOCIATED YEASTS 63
   2.6.1 Yeasts of common occurrence in foods 64
      2.6.1.1 Saccharomyces cerevisiae 64
      2.6.1.2 Saccharomyces kluuyveri 65
      2.6.1.3 Schizosaccharomyces pombe 65
      2.6.1.4 Candida spp. 66
      2.6.1.5 Kluyveromyces spp. 66
   2.6.2 Role of yeasts in fermented foods 67
      2.6.2.1 Dairy products 67
      2.6.2.2 Sourdough breads 68
      2.6.2.3 Fermented foods 69
      2.6.2.4 Probiotic yeasts 70
   2.6.3 Phytase from yeasts 72
      2.6.3.1 Significance of phytate 72
      2.6.3.2 Phytate in cereals and legumes 73
      2.6.3.3 Phytase 75
      2.6.3.4 Phytate hydrolysis 77
      2.6.3.5 Phytase production and its assay 78
      2.6.3.6 Purification and characterization of phytase 81

2.7 LACTIC ACID BACTERIA  85
   2.7.1 Probiotic LAB 87
      2.7.1.1 Lactobacillus acidophilus 87
      2.7.1.2 Lactobacillus rhamnosus 88
      2.7.1.3 Lactobacillus bulgaricus 89
      2.7.1.4 Lactobacillus plantarum 89
      2.7.1.5 Lactobacillus casei 90
      2.7.1.6 Lactobacillus reuteri 90
      2.7.1.7 Lactobacillus sporogenes 91
      2.7.1.8 Streptococcus thermophilus 92
   2.7.2 Alpha-D-galactosidase 92
      2.7.2.1 Potentiometer 92
      2.7.2.2 Cultural conditions and assays 93
      2.7.2.3 Purification and characterization 97
      2.7.2.4 Molecular characterization 98
2.7.3 Bile salt hydrolase

2.7.3.1 Significance

2.7.3.2 Cultural conditions and assays

2.7.3.3 Purification and characterization

2.7.3.4 Molecular characterization

2.8 BIFIDOBACTERIA

2.8.1 Characteristics

2.8.2 Potential probiotic bifidobacteria

2.8.2.1 Bifidobacterium bifidum

2.8.2.2 Bifidobacterium longum

2.8.2.3 Bifidobacterium infantis

2.8.3 Alpha-D-galactosidase

2.8.4 Bile salt hydrolase
4.1 ISOLATION AND CHARACTERIZATION OF NATIVE CULTURES OF LACTIC ACID BACTERIA, BIFIDOBACTERIA AND YEASTS FOR DESIRABLE ATTRIBUTES BY CONVENTIONAL AND NUCLEIC ACID BASED DETECTION APPROACHES

4.1.1 MATERIALS
4.1.1.1 Food samples 123
4.1.1.2 Reference cultures 124
4.1.1.3 Diluents and reagents 124
4.1.1.4 Stains for microbial cultures 128
4.1.1.5 Microbiological media 129
4.1.1.6 Molecular biology requisites 134

4.1.2 METHODOLOGY
4.1.2.1 Native isolates of LAB and bifidobacteria 136
4.1.2.1.1 Isolation from food samples 136
4.1.2.1.2 Characterization for identification of genus/species 136
4.1.2.1.3 Beneficial attributes (probiotics and prebiotics) associated with native isolates of LAB and bifidobacteria 142
4.1.2.1.4 Molecular characterization of native isolates of LAB and Bifidobacterium spp. for genus specificity and beneficial attributes 146
4.1.2.2 Native isolates of yeasts 151
4.1.2.2.1 Isolation from food samples 151
4.1.2.2.2 Characterization for identification of genus/species 152
4.1.2.2.3 Beneficial attribute associated with native isolates of yeasts 154
4.1.2.2.4 Molecular characterization of identified native yeast isolates for phytase 155

4.1.3 RESULTS
4.1.3.1 Isolation and characterization of LAB and bifidobacteria 156
4.1.3.2 Beneficial attributes associated with native isolates of LAB and bifidobacteria 163
4.1.3.3 Isolation and characterization of yeast cultures 177
4.1.3.4 Phytase activity associated with native yeast isolates 180

4.1.4 DISCUSSION
4.1.4.1 Beneficial attributes among LAB and bifidobacteria 183
4.1.4.2 Native yeast cultures with phytase activity 188
FORMAT OF PRESENTATION

4.2 OPTIMIZED PHYTASE, α-D-GALACTOSIDASE, BILE SALT HYDROLASE AND ANTIBACTERIAL ACTIVITIES ELABORATED BY POTENT MICROBIAL CULTURES IN SOY WHEY BASED MEDIUM BY RESPONSE SURFACE METHODOLOGY

4.2.1 MATERIALS
4.2.1.1 Microbial (test) cultures
4.2.1.2 Normal saline
4.2.1.3 Reagents for assay
4.2.1.4 Microbiological media

4.2.2 METHODOLOGY
4.2.2.1 Preparation of cell suspension (Inoculum) of microbial test cultures
4.2.2.2 Evaluation of broth media for desirable attributes elaborated by microbial cultures
4.2.2.3 Experimental design for desirable attributes in microbial cultures
4.2.2.4 Statistical Analysis

4.2.3 RESULTS
4.2.3.1 Phytase activity in Saccharomyces cerevisiae MTCC 5421
4.2.3.2 α-D-galactosidase and antibacterial activities in Lactobacillus plantarum MTCC 5422
4.2.3.3 Bile salt hydrolase and antibacterial activities in Bifidobacterium adolescentis MTCC 5423

4.2.4 DISCUSSION
4.2.4.1 Phytase activity in Sac. cerevisiae MTCC 5421
4.2.4.2 Desirable attributes in Lb. plantarum MTCC 5422 and Bif. adolescentis MTCC 5423
### 4.3 FUNCTIONAL ATTRIBUTES OF MICROBIAL PHYTASE AND α-D-GALACTOSIDASE IN LOWERING LEVELS OF PHYTIC ACID AND FLATULENCE CAUSING OLIGOSACCHARIDES IN FLOURS OF CEREALS AND LEGUMES

#### 4.3.1 MATERIALS

<table>
<thead>
<tr>
<th>Subcategory</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal and legume substrates</td>
<td>227</td>
</tr>
<tr>
<td>Microbial (test) cultures</td>
<td>227</td>
</tr>
<tr>
<td>Requisites for gel electrophoresis</td>
<td>228</td>
</tr>
<tr>
<td>Solutions for washing of dialysis tubing</td>
<td>231</td>
</tr>
<tr>
<td>Requisites for α-D-galactosidase</td>
<td>231</td>
</tr>
<tr>
<td>Requisites for phytase assay</td>
<td>231</td>
</tr>
<tr>
<td>Bradford reagent</td>
<td>232</td>
</tr>
<tr>
<td>Normal saline</td>
<td>232</td>
</tr>
<tr>
<td>Requisites for Thin Layered Chromatography</td>
<td>232</td>
</tr>
<tr>
<td>Requisites for High Performance Liquid Chromatography</td>
<td>232</td>
</tr>
<tr>
<td>Microbiological media</td>
<td>233</td>
</tr>
</tbody>
</table>

#### 4.3.2 METHODOLOGY

<table>
<thead>
<tr>
<th>Subcategory</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation of cell suspension (Inoculum) of microbial test cultures</td>
<td>234</td>
</tr>
<tr>
<td>Assay for phytase and α-D-galactosidase activities</td>
<td>234</td>
</tr>
<tr>
<td>Determination of total protein</td>
<td>235</td>
</tr>
<tr>
<td>Phytase of <em>Saccharomyces cerevisiae</em></td>
<td>235</td>
</tr>
<tr>
<td>α-D-galactosidase of <em>Lactobacillus plantarum</em></td>
<td>239</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>242</td>
</tr>
</tbody>
</table>

#### 4.3.3 RESULTS

<table>
<thead>
<tr>
<th>Subcategory</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytase of <em>Sac. cerevisiae</em> MTCC 5421 and its action on phytic acid in cereal flours</td>
<td>243</td>
</tr>
<tr>
<td>α-D-galactosidase of <em>Lb. plantarum</em> and its action on FCOs in legume flours</td>
<td>249</td>
</tr>
</tbody>
</table>

#### 4.3.4 DISCUSSION

<table>
<thead>
<tr>
<th>Subcategory</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytase of <em>Sac. cerevisiae</em> MTCC 5421 and its action on phytic acid in cereal flours</td>
<td>261</td>
</tr>
<tr>
<td>α-D-galactosidase of <em>Lb. plantarum</em> and its action on FCOs in legume flours</td>
<td>264</td>
</tr>
</tbody>
</table>
4.4 BENEFICIAL ATTRIBUTES IN SELECTED FOODS THROUGH PREBIOTIC AND PROBIOTIC INTERACTIONS

4.4.1 MATERIALS 269
- 4.4.1.1 Ingredients / Substrates 269
- 4.4.1.2 Microbial (test) cultures 269
- 4.4.1.3 Requisites for estimation of short chain fatty acids (SCFAs) 270
- 4.4.1.4 Requisites for estimation of sugars 270
- 4.4.1.5 Requisites for estimation of phytate 270
- 4.4.1.6 Test for F-6-ppk 271
- 4.4.1.7 Requisites for HPLC 271
- 4.4.1.8 Requisites for alpha-D-galactosidase 271
- 4.4.1.9 Requisites for phytase assay 271
- 4.4.1.10 Normal saline 271
- 4.4.1.11 Microbiological media 271

4.4.2 METHODOLOGY 273
- 4.4.2.1 Preparation of cell suspension (Inoculum) of microbial test cultures 273
- 4.4.2.2 Base materials for product making 274
- 4.4.2.3 Evaluation of functional attributes in prepared products 275
- 4.4.2.4 Evaluation of prepared products for microbial safety and sensory attributes 279

4.4.3 RESULTS 280
- 4.4.3.1 Prepared foods with functional attributes 280
- 4.4.3.2 Microbiological safety and sensory profile 292

4.4.4 DISCUSSION 292
- 4.4.4.1 Prebiotic and probiotic based foods 292