Summary and conclusion

Prebiotic galacto-oligosaccharides (GOS), are a class of non-digestible oligosaccharides, which are promising ‘nutra’ food ingredients. GOS are generally synthesized by the transgalactosylation of lactose using β-galactosidases. GOS are of considerable interest due to their beneficial attributes to the human health. These oligosaccharides are structurally different, but show similar prebiotic and immunomodulatory effects, that mimic the prebiotic effects of human milk and promote a bacterial microflora that closely resembles that of breast-fed infants and hence are popular as additives to infant food formula. They find use in bakery and confectionary.

This thesis titled “Prebiotic Galactooligosaccharides: Enzymatic synthesis, Characterization and Bioactive studies” is a compilation of the research on the various basic, biochemical and technological aspects of GOS production.

The important findings are summarized herewith

1. Based on the preliminary screening trials, probiotic Lactobacillus plantarum MCC2156 was selected as a potent source of β-galactosidase, exhibiting high transgalactosylating activity for GOS production from lactose. The selected organism was thus used for further research.

2. Fermentation and optimization studies on various nutritional parameters and cultural conditions for the production of β-galactosidase from L. plantarum resulted in 1.7 fold increase in an enzyme production. Maximum production was achieved under the optimized conditions; medium containing (g/ 100 ml) galactose (4), yeast extract (2), sodium acetate (3), MnSO4. H2O (0.075 g) at 35°C, pH 7.0 during a period of 14- 18 h of fermentation.
3. β-Galactosidase from *L. plantarum* was purified by affinity chromatography followed by size exclusion chromatography. The purified β-gal was found to be a monomer with the molecular mass of 66 kDa. In addition, the permeabilized whole cells of *L. plantarum* were effectively used as whole cell biocatalyst (intracellular β-gal) for GOS production.

4. Reaction parameters for GOS production from lactose using purified β-gal and permeabilized cells have been optimized. The optimized conditions for the GOS production were found to be: 40% (w/v) initial lactose concentration; pH 7.0 at 50°C with 10 U/ml of either purified β-gal or equivalent amount of permeabilized cells. The maximum GOS yield achieved with permeabilized cells by 12 h of reaction was found to be 33.8 (w/w) and was 34.1% (w/w) with purified β-gal by 10 h of reaction.

5. Under optimized conditions, the composition (w/w) of the final GOS mixture is as follows; 38 - 45% monosaccharides (glucose and galactose), 20 - 28% lactose, 12 - 14% GOS-2 (disaccharides), 15 - 17% GOS-3 (trisaccharides) and 3 - 5% GOS-4 (tetrasaccharides). The ESI/MS characterization confirmed the presence of GOS-2, GOS-3, GOS-4 and also trace amounts of GOS-5 (pentasaccharides). The major trisaccharide component was identified as β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-D-Glucopyranose (4-galactoysl-lactose) from $^{13}$C and 2D-HSQC NMR.

6. The process for GOS production using whey has been developed. Untreated whey and deproteinized whey were spray dried and used as the substrate for the production of GOS under the above mentioned optimized conditions using purified β-gal. The yield attained was 34.7% and 34.4 (w/w) GOS from untreated whey and deproteinized whey, respectively.
7. Further, approaches for the efficient removal of monosaccharides (glucose and galactose) and unreacted lactose for the production of high purity GOS was demonstrated by dialysis and fermentation using yeast. The microbial fermentation process using immobilized yeast was found to be effective for the production of high purity GOS (91-93%) with a recovery of 81-84% from initial GOS used.

8. Efforts have been made for value addition to an abundantly available dairy industry by-product, whey, by using it as a substrate for the synthesis of GOS. GOS and high purity GOS produced from whey also retained the major nutraceutical components of whey proteins such as lactoferrin, BSA, α- and β-casein, α- and β-lactoglobulin.

9. The in-vitro studies on bioactive properties of GOS revealed that it effectively inhibited the adherence of enteropathogenic bacterial cultures such as *E. coli* and *B. cereus* to human intestinal epithelial (HEp-2) cell lines. GOS were found to specifically stimulate the growth of probiotic lactic acid bacteria but not the growth of pathogenic cultures tested.

10. The in-vivo evaluation of GOS supplementation at 10% dietary level to female Wistar rats for four weeks resulted in stimulation of beneficial microbiota such as lactobacilli and bifidobacteria in the gut. GOS supplementation also resulted in increased production of organic acids such as lactic, acetic and butyric acids in the gut.

11. GOS supplementation effectively mobilized the calcium from a complex food matrix, finger millet/ *ragi* flour and enhanced the bioavailability of calcium in experimental female Wistar rats via fermentation though gut microbiota.