

# Development of Synbiotic Formulation Method via Freeze Drying Technique

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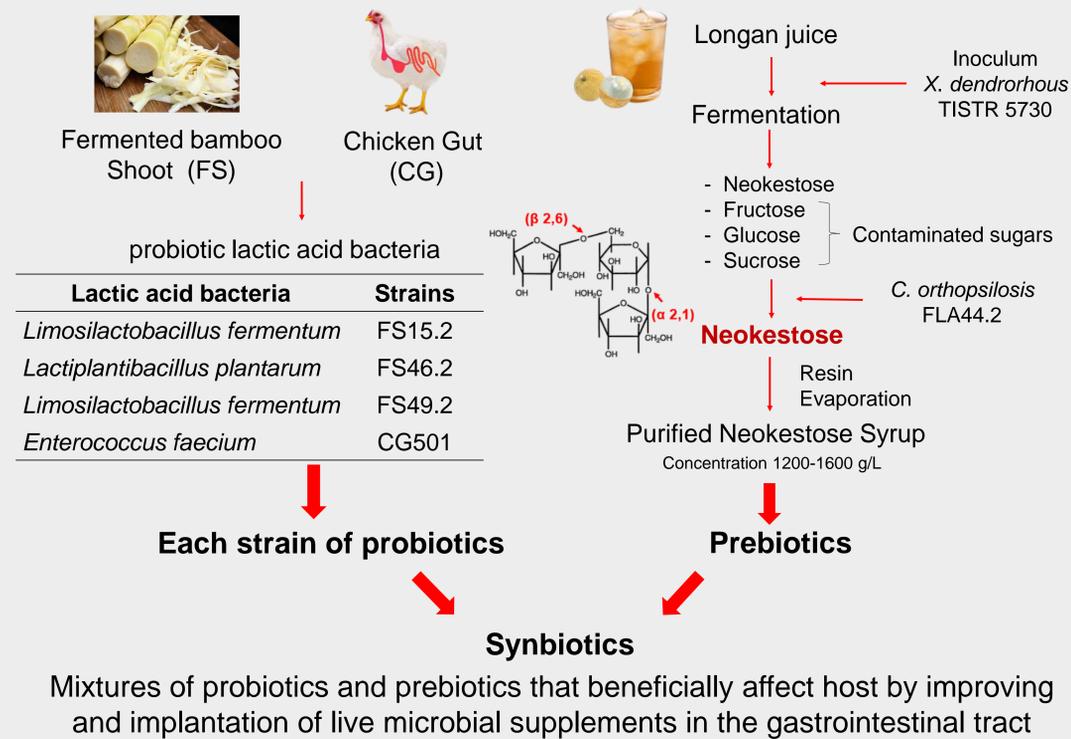
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## ABSTRACT

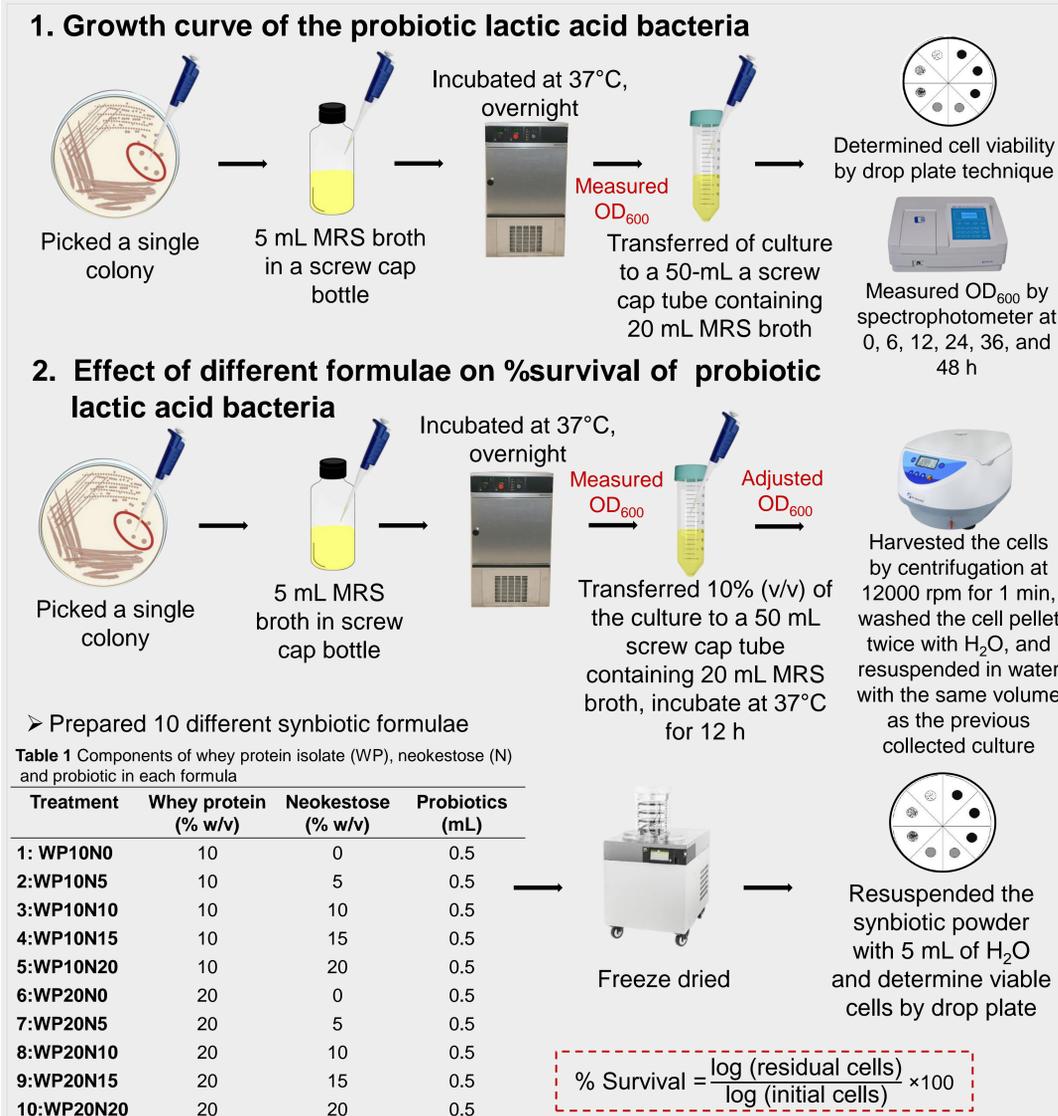
Neokestose is the shortest-chain fructooligosaccharide prebiotic that has different structure from other commercial kestoses such as 1-kestose and 6-kestose, but it is more resistant to acidic and thermal conditions. Neokestose can specifically stimulate the growth of probiotic bacteria, particularly bifidobacteria. The use of neokestose as a prebiotic for stimulation of the human gut microbiota is of considerable attention. Formulation of a synbiotic product containing neokestose and the specific neokestose-fermenting probiotic might broaden neokestose applicability to a wide range of consumers. The purpose of this research was to develop a powdered synbiotic formulation combining neokestose with four individual strains of probiotic lactic acid bacteria including *Limosilactobacillus (Lm.) fermentum* FS15.2, *Lm. fermentum* FS49.2, *Lactiplantibacillus plantarum* FS46.2, and *Enterococcus faecium* CG501. The formulations consisted of three components: whey protein isolate (WP) as a drying aid, neokestose (N), and probiotic lactic acid bacteria ( $10^8$  cells/mL). The concentrations of WP were varied at 10 and 20 (%w/v) and N at 0, 5, 10, 15, and 20 (%w/w), resulting in 10 formulae: WP10N0, WP10N5, WP10N10, WP10N15, WP10N20, WP20N0, WP20N5, WP20N10, WP20N15, and WP20N20. They were freeze-dried to a powder, and the percentage of cell survival was assessed. It was found that WP supported cell survival above by retaining 70% survival and addition of neokestose significantly enhanced cell survival. The principal component analysis (PCA) categorized the formulae WP10N0 and WP20N0 within the same group whereas the Analysis of variance (ANOVA) and multiple comparison of the percentage of cell survival indicated that WP10N20 significantly produced the highest survival percentage for all bacterial strains ( $p < 0.05$ ). This formulation was deemed the most suitable for synbiotic product development. Additionally, neokestose likely possessed cryoprotective properties.

**Keyword:** Neokestose, Synbiotics, Freeze-drying, Probiotic lactic acid bacteria

## BACKGROUND



## METHODS



## RESULTS

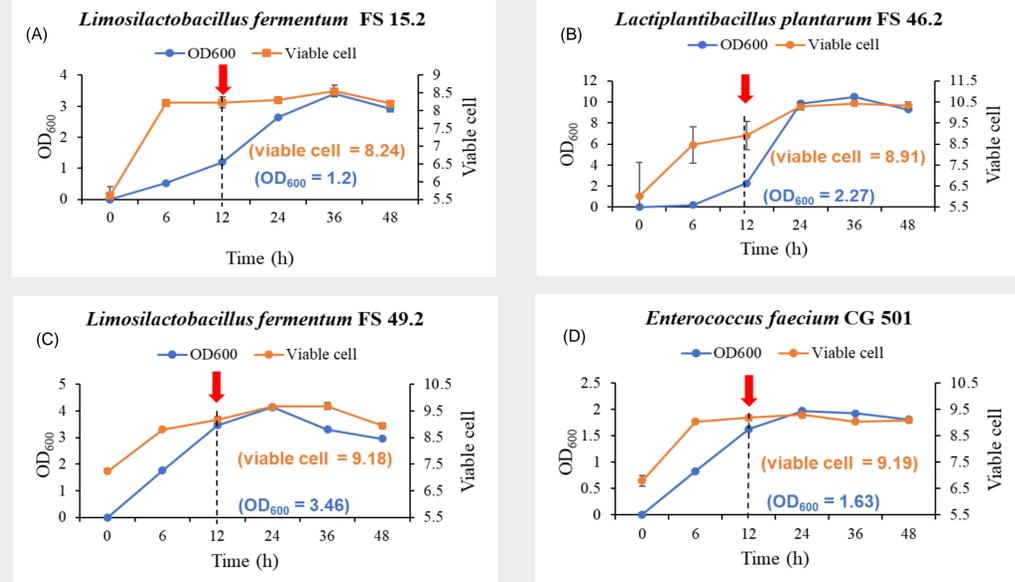
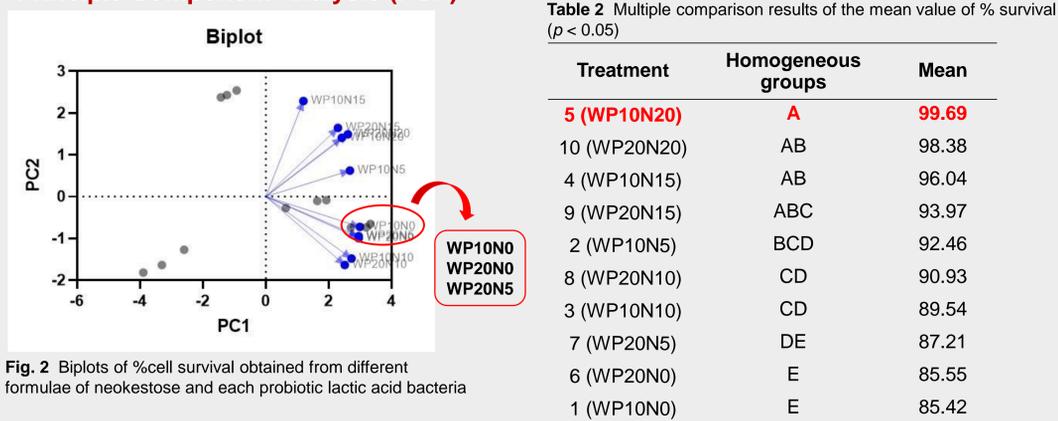


Fig. 1 OD<sub>600</sub> and viable cells of *Limosilactobacillus fermentum* FS15.2 (A), *Lactiplantibacillus plantarum* FS46.2 (B), *Limosilactobacillus fermentum* FS49.2 (C) and *Enterococcus faecium* CG501 (D)

## Principle Component Analysis (PCA) ANOVA & Multiple comparison of the mean value



## CONCLUSION

- The formula WP10N20 exhibited the most suitable composition for formulation of synbiotic powder of probiotic lactic acid bacteria
- Neokestose was likely to possess cryoprotective property

## ACKNOWLEDGMENT

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## References:

- Panday, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics-A review. *Plants*, 52(12), 1-11.
- Kilian S., Kritzinger S., Rycroft C., Gibson G., & Preez J. D. (2002). The effects of the novel bifidogenic trisaccharide, neokestose, on the human colonic microbiota, *Microbiology and Biotechnology*, 18, 637-644.