Effect of Synbiotics based Neokestose on Growth of Some Foodborne Pathogenic Bacteria

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ABSTRACT

In a previous study, eight strains of lactic acid bacteria (LAB) capable of fermenting the shortest-chain fructooligosaccharide, neokestose have been characterized as potential probiotics based on the following properties: tolerance to gastric acid and bile salts, adhesion to the human intestinal epithelium, inhibition of a monoculture of pathogenic microorganisms via antimicrobial activity assay, and the ability to utilize or grow on neokestose. Each of the following probiotics, namely *Limosilactobacillus (Lm.) fermentum* strains FS2.2, FS15.2, FS48.1, and FS49.2, *Lactobacillus (L.) argentoratensis* FS41.1, *Lactiplantibacillus (Lp.) plantarum* FS46.2, and *Enterococcus (E.) faecium* strains CG403 and CG501, has been mixed with neokestose at a certain ratio to produce eight synbiotic formulae. This study aimed to screen synbiotics with potential antibacterial effect against some foodborne pathogens for developing potential multi-strain synbiotics based neokestose. Each synbiotic formula was assessed antagonistic activity against mixed pure culture of *Escherichia (E.) coli* TISTR 527, *Staphylococcus (St.) aureus* TISTR 746, and *Salmonella (S.)* typhimurium TISTR 1472. The results revealed that antagonistic activity of synbiotics was strain dependent. A synbiotic of *Lm. fermentum* FS49.2 completely inhibited *S.* typhimurium within 24 h, while that of *Lm. fermentum* FS48.1 completely inhibited *S.* typhimurium within 12 h. In addition, synbiotics of *Lm. fermentum* FS2.2 and *Lp. plantarum* FS46.2 completely inhibited *S.* typhimurium within 24 h. Notably, the combination of *Lm. fermentum* FS49.2 and *Lp. plantarum* FS46.2 completely inhibited *S.* typhimurium within 12 h and *E. coli* within 24 h, indicating the potential use as a double strain synbiotic

BACKGROUND



Fermented bamboo Shoot (FS)



Chicken Gut (CG)



Neokestose

- Short-chain fructooligosaccharide
- Prebiotic

probiotic lactic acid bacteria

LAB	strains
Limosilactobacillus (Lm.) fermentum	FS2.2
Limosilactobacillus (Lm.) fermentum	FS15.2
Lactobacillus (L.) argentoratensis	FS41.1
Lactiplantibacillus (Lp.) plantarum	FS46.2
Limosilactobacillus (Lm.) fermentum	FS48.1
Limosilactobacillus (Lm.) fermentum	FS49.2
Enterococcus (E.) faecium	CG403
Enterococcus (E.) faecium	CG501

Synbiotics

A mixture probiotics and prebiotics exert beneficial effects on the host health

Antagonistic activity
- nutrient competition, pH reduction, and ecological interference

Foodborne pathogens
- *E. coli* • *S. aureus* • *S.* typhimurium

Multi-strain synbiotics

METHODS

1. Culture preparation: Probiotics

LAB	strains
Limosilactobacillus (Lm.) fermentum	FS2.2
Limosilactobacillus (Lm.) fermentum	FS15.2
Lactobacillus (L.) argentoratensis	FS41.1
Lactiplantibacillus (Lp.) plantarum	FS46.2
Limosilactobacillus (Lm.) fermentum	FS48.1
Limosilactobacillus (Lm.) fermentum	FS49.2
Enterococcus (E.) faecium	CG403
Enterococcus (E.) faecium	CG501



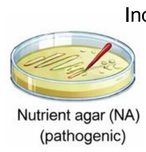
MRS agar (probiotic)

Incubated at 37 °C for 24

Isolation of single colonies by streaking on MRSA plate

2. Cultures preparation: Pathogens

Foodborne Pathogenic Bacteria	strains
Escherichia (E.) coli	TISTR 527
Staphylococcus (St.) aureus	TISTR 746
Salmonella (S.) typhimurium	TISTR 1472

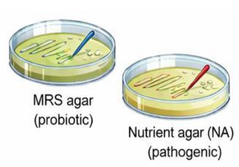


Nutrient agar (NA) (pathogenic)

Incubated at 37 °C for 24

Isolation of single colonies by streaking on NA plate

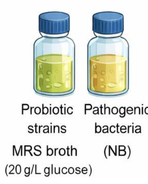
3. Effect of synbiotics on foodborne pathogens



MRS agar (probiotic)

Nutrient agar (NA) (pathogenic)

incubated at 37 °C for 24 h



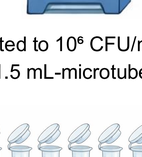
Probiotic strains

Pathogenic bacteria

MRS broth (20 g/L glucose)

(NB)

5 mL



diluted to 10^6 CFU/mL in 1.5 mL-microtubes

10 µL of each culture

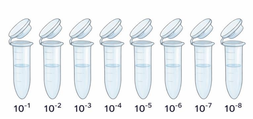


10 mL basal medium (20 g/L neokestose)

- At 0, 6, 12, and 24 h.
- Selective media : MSA, SSA, EMB agar, and MRSA
- Colony counting



Drop Plate Technique



Serial dilution (10^{-1} to 10^{-8}) in NSS

CONCLUSION

- Synbiotic-based neokestose exhibited strain-dependent antagonistic activity against major foodborne pathogens
- The combination of *Lm. fermentum* FS49.2 and *Lp. plantarum* FS46.2 completely inhibited *S.* typhimurium within 12 h and *E. coli* within 24 h, indicating the potential use as a double strain synbiotic

ACKNOWLEDGEMENT

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RESULTS

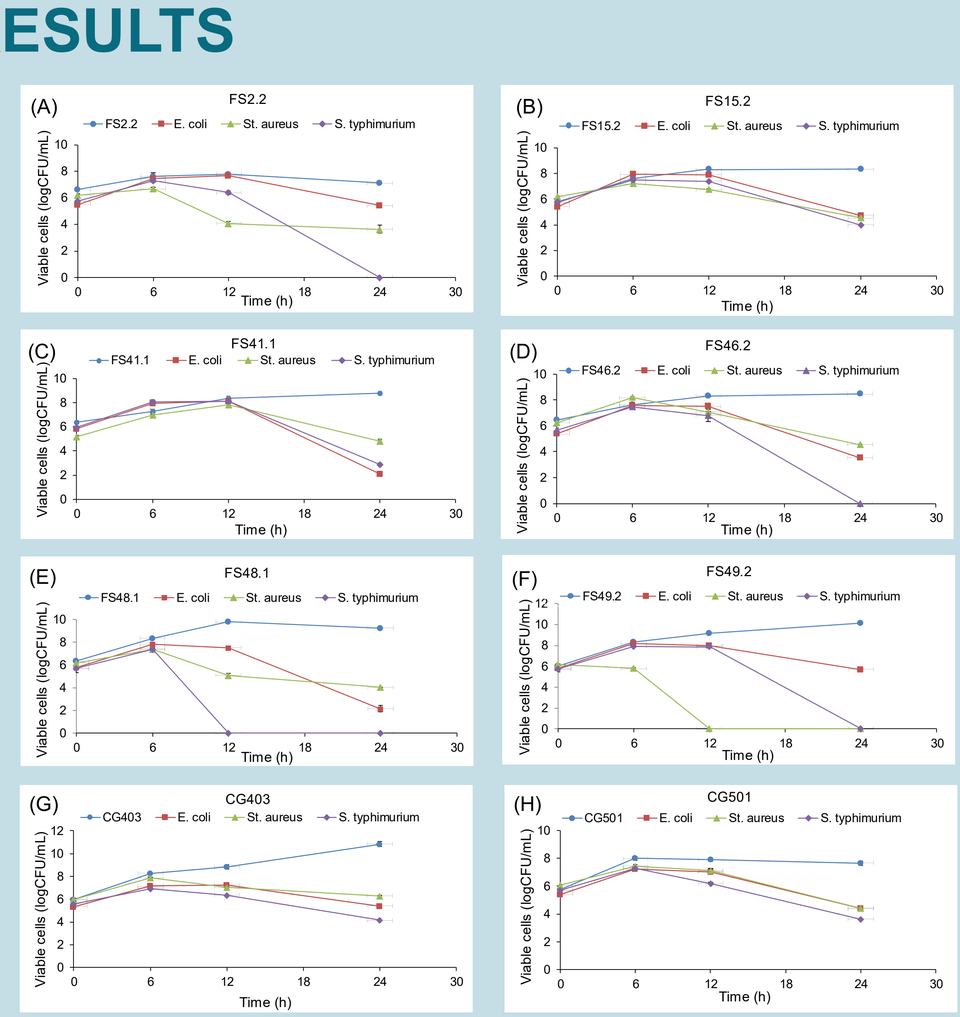


Fig. 1 Growth of each pathogenic bacteria and probiotic *Limosilactobacillus fermentum* FS2.2 (A) *Limosilactobacillus fermentum* FS15.2 (B) *Lactobacillus argentoratensis* FS41.1 (C) *Lactiplantibacillus plantarum* FS46.2 (D) *Limosilactobacillus fermentum* FS48.1 (E) *Limosilactobacillus fermentum* FS49.2 (F) *Enterococcus faecium* CG403 (G) and *Enterococcus faecium* CG501 (H) when cultured in different synbiotic formulae

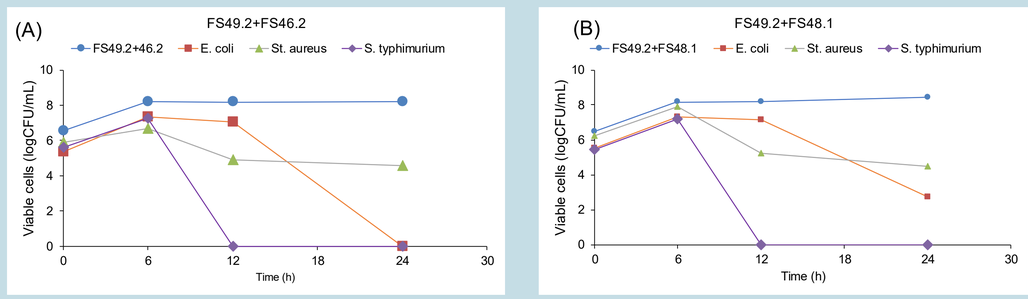


Fig. 2 Growth of each pathogenic bacteria and the combination of *Limosilactobacillus fermentum* FS49.2 with *Lactiplantibacillus plantarum* FS46.2 (A) and the combination of *Limosilactobacillus fermentum* FS49.2 with *Limosilactobacillus fermentum* FS48.1 (B) when cultured in different synbiotic formulae

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- Van der Westhuizen, A. J., Pretorius, I. S., & van Rensburg, P. (2008). Oligosaccharides in foods and beverages: Prebiotic effects and applications. Food Chemistry, 106(3), 765–775.