EFFECT OF TREHALOSE AS SUBSTRATE AND INACTIVATION METHODS ON ANTIMICROBIAL ACTIVITY OF PROBIOTIC-BASED PARABIOTICS

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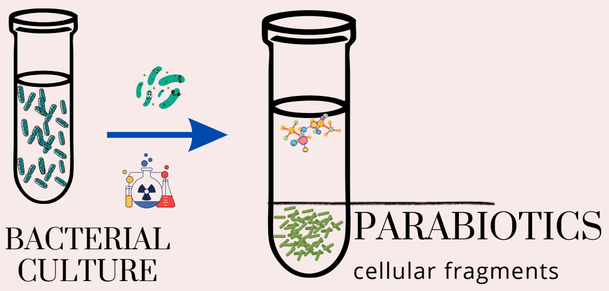
Introduction

Probiotics have many beneficial advantages, however these effects in some cases are due to the cellular substances and/or fragments that can be synthetised separately by fermentation and generally do not need the presence of alive cells. **This study investigated the effect of trehalose and inactivation methods on antimicrobial properties of probiotic-based parabiotics.** These parabiotics show strong antimicrobial activity against food pathogens. Our work points to a natural, safe way to enhance food safety and preservation.



PARABIOTICS

Parabiotics are defined as non-living forms of bacteria that can enhance human health. These can include bacterial cell lysates, structure, components, and bacterial fraction.

Methods

Fermentation on trehalose

Probiotic lactic acid bacteria were grown on MRS medium with different carbohydrate content: glucose, 0.5%, 1%, 2% trehalose. We investigated their effect on the microbial inhibitory property. The sample was taken after 18 hours of fermentation at 37°C.

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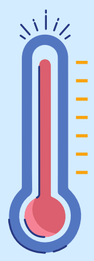
Production of parabiotics

Physical cell lysis

Heat treatment

Application:

- 60 °C
- 80 °C
- 100 °C

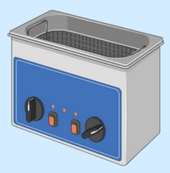


The antimicrobial effect may result from the leakage of intracellular substances and cell wall structure and components, peptides, enzymes, and vitamins.

Sonication

Application:

- 5 seconds
- with 5 second break
- repeated for 10 mins



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This technology induces chemical and physical changes in cell wall structures through intracellular cavitation. Cavitation leads to DNA and cell wall damage.

Freezing- thawing

Application:

- freezing at -18°C
- thawing at ~20°C
- 6× repeated cycle

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This cause ice crystal formation inside the cells that damages cell walls. Upon thawing, the disrupted structures further crack, resulting in cell lysis and release of intracellular contents.

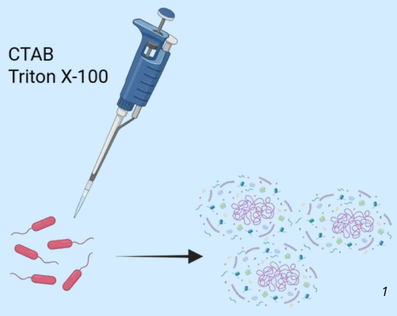
Chemical cell lysis

CTAB

Cetyl trimethylammonium bromide(CTAB) was used to rapidly compromised cell membranes, efficiently releasing bioactive components, including peptides, enzymes that may drive antimicrobial activity.

Triton X-100

Triton X-100 is a non-ionic detergent that forms micelles. Lysis is probably due to the effect of the surfactant on the cytoplasmic membrane-associated components of the autolytic system.



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Discussion

Our research indicates that replacing glucose with trehalose in the fermentation medium triggers a stress response in probiotic lactic acid bacteria, boosting the antimicrobial properties of the resulting parabiotics. Among the cell disruption techniques examined, CTAB treatment and heat shock at 100°C were most effective in inactivating the cells while maintaining their antimicrobial elements. Although approaches like sonication, freeze-thaw cycles Triton X-100 and SDS treatment were less effective, the optimized methods hold significant promise for creating natural and safe food preservation solutions.

Results

Effects of trehalose concentration on the antimicrobial activity

	MRS broth containing glucose	0.5% trehalose MRS broth	1% trehalose MRS broth	2% trehalose MRS broth
Enterococcus faecalis	-	2 mm	3 mm	2 mm
Escherichia coli O157:H7	1 mm	2 mm	2.5 mm	2.5 mm
Listeria innocua	-	2 mm	3 mm	2 mm
Enterobacter cloacae	-	3 mm	3 mm	2 mm

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Inhibition zones using agar diffusion method against foodborne pathogens and test microorganisms.

Effects of cell lysis methods on the antimicrobial activity

Moderate temperatures (60°C and 80°C) did not fully inactivate the cell, however, **heat treatment at 100°C** was the most effective physical method for the inactivation of probiotics cells. Sonication only resulted in antimicrobial activity in some cases, while freezing-thawing was not effective against any tested microorganism.

Out of the detergents tested, **CTAB** stood out for its speed and potency, making it ideal for producing parabiotics with the best antimicrobial effect among the tested methods. Triton X-100 was not suitable for the development of inhibition, while SDS could not inactivate the cells.

***Lactobacillus helveticus* R-52 PARABIOTICS**

METHOD	*Enterococcus faecalis*	*Escherichia coli* 8739	*Escherichia coli* O157:H7	*Listeria innocua*	*Enterobacter cloacae*
Heat treatment	+	+	+	+	+
Sonication	-	-	-	+	-
Freezing-thawing	-	-	-	-	-
CTAB	+	+	+	+	+
Triton X-100	-	-	-	-	-

Methods for the antimicrobial activity development:

- Heat treatment
- CTAB
- Sonication (against *Listeria innocua*)

***Ligilactobacillus salivarius* HA-118 PARABIOTICS**

METHOD	*Enterococcus faecalis*	*Escherichia coli* 8739	*Escherichia coli* O157:H7	*Listeria innocua*	*Enterobacter cloacae*
Heat treatment	+	+	+	+	+
Sonication	-	-	-	-	-
Freezing-thawing	-	-	-	-	-
CTAB	+	+	+	+	+
Triton X-100	-	-	-	-	-

Methods for the antimicrobial activity development:

- Heat treatment
- CTAB

***Lactobacillus crispatus* LCR01 PARABIOTICS**

METHOD	*Enterococcus faecalis*	*Escherichia coli* 8739	*Escherichia coli* O157:H7	*Listeria innocua*	*Enterobacter cloacae*
Heat treatment	+	+	+	+	+
Sonication	+	-	-	-	-
Freezing-thawing	-	-	-	-	-
CTAB	+	+	+	+	+
Triton X-100	-	-	-	-	-

Methods for the antimicrobial activity development:

- Heat treatment
- CTAB
- Sonication (against *Enterococcus faecalis*)

"+": inhibition was detected; "-": no inhibition was detected

Conclusion

Using 1% trehalose in the fermentation medium instead of glucose enhance the antimicrobial activity of parabiotics.

Parabiotics can be produced using different methods, but the best inhibitory effect resulted after CTAB or heat treatment.

Their potential as biological preservatives is emphasized, highlighting their functional benefits and safety for food preservation.

FOOD SAFETY

All probiotic-based parabiotics showed antimicrobial activity against Gram-negative and Gram-positive foodborne pathogenic bacteria using 1% trehalose in the fermentation medium and CTAB or heat treatment cell lysis. This result is new and very promising making parabiotics excellent candidates in food preservation application.

Acknowledgement

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1Graphics created in https://BioRender.com