IOCBE 2024 Conference

The 1st International Online Conference on Bioengineering

16–18 October 2024 | Online

MDPI

Alginate–Polysaccharide Hydrogel Films for Lactic Acid Bacteria Immobilization, Cultivation, and Low-Temperature Storage

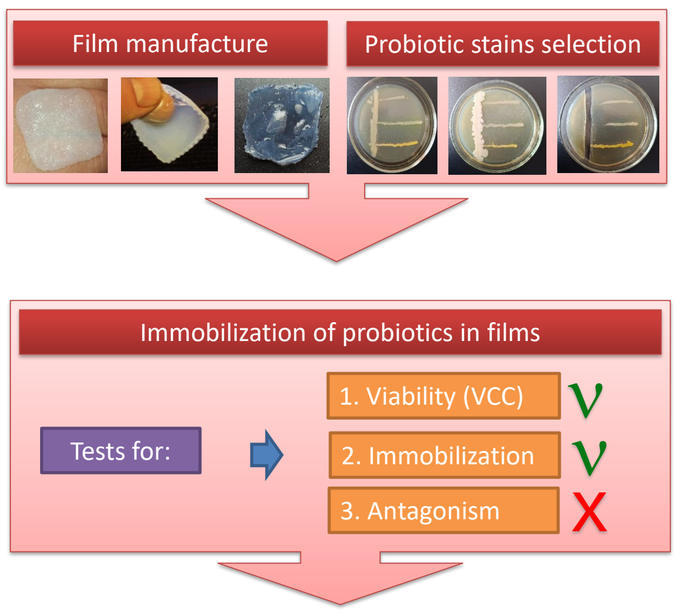
Oleh Trufanov[1], Valentyna Martsenyuk[1], Liudmyla Stepaniuk[1], Hanna Ananina[1], Natalia Trufanova[2]

[1]Department of Cryomicrobiology, [2]Department of Cryobiochemistry, Institute for Problems of Cryobiology and Cryomedicine of National Academy of Sciences of Ukraine, Kharkiv, 61016, Ukraine, olegtrufanov2015@gmail.com

INTRODUCTION & AIM

Alginate-based hydrogel films are widely used as wound dressings. Probiotic lactic acid bacteria, particularly lactobacilli and bifidobacteria, are promising therapeutic agents for wound dressings due to their antagonistic action against wound infection pathogens, potentially through competitive exclusion and the production of antimicrobial compounds. The aim of this study was to address the challenges encountered in the development of probiotic-loaded hydrogel wound dressings, namely, the loss of antibacterial substances during the immobilization, reduced cell viability, and deterioration of the films' mechanical properties during storage.

METHOD

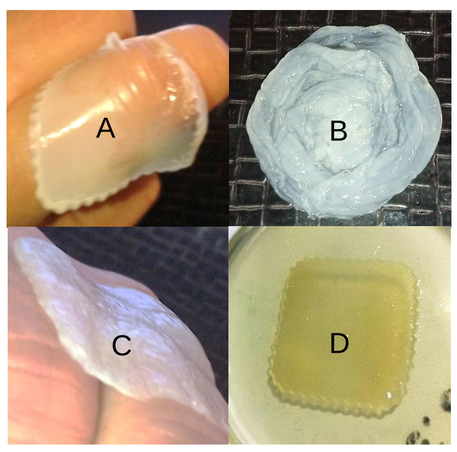


Probiotic lactic acid bacteria (*Lactobacillus bulgaricus* and *Bifidobacterium bifidum*) were immobilized in alginate-based hydrogel, produced by spray method, supplemented with pectin or starch. Films were cultured in Blaurock medium for 2-6 days, saturated with cryoprotectant glycerol at 10-20% concentration, and stored at various temperatures (+25°C to -80°C) for 7 days.

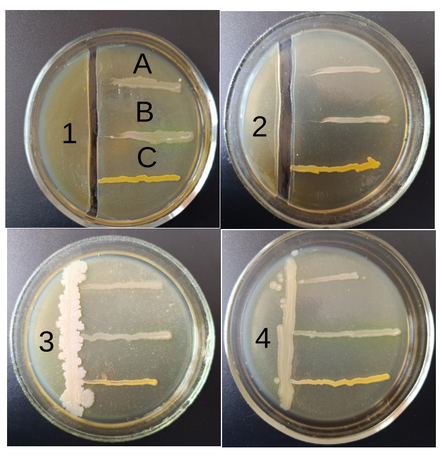
The efficiency of bacterial immobilization, viable cell count, and antagonistic activity against wound infection pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) were evaluated.

RESULTS & DISCUSSION

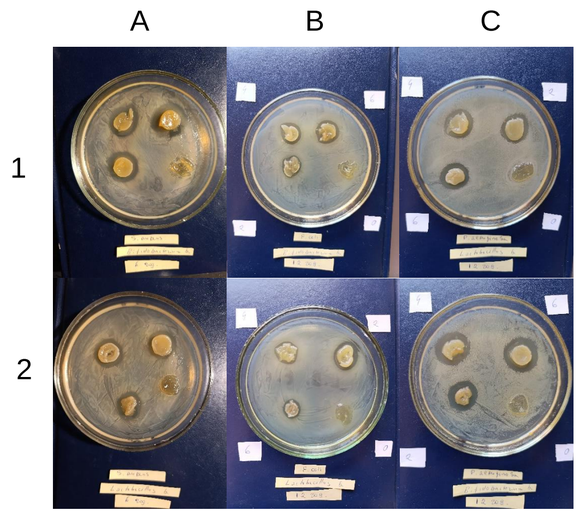
Bacterial cells were immobilized by spraying calcium chloride on a cell suspension in sodium alginate. Pectin or starch addition significantly improved the films' mechanical properties. Culturing films with immobilized bacteria for 2 days resulted in a 100-fold increase in viable cell count and the acquisition of antagonistic properties against wound infection pathogens. Optimal cryoprotectant concentrations were established as 20% of glycerol, and the films with immobilized bacteria maintained their key properties after low-temperature storage.



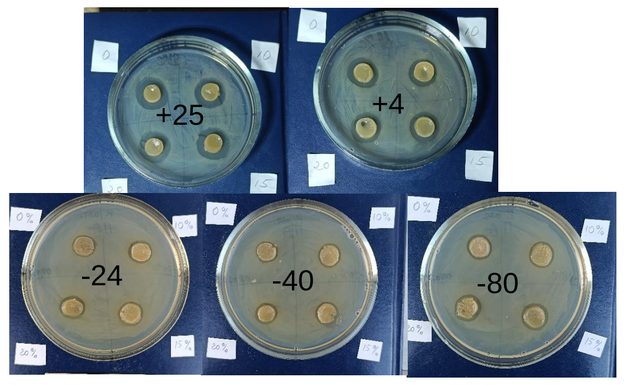
Alginate films produced by:
A – rehydration, B – mixture,
C – cryogelation, D – spray method



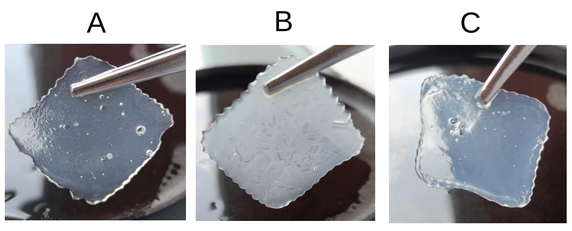
Probiotic strains:
1 – *B. bifidum*, 2 – *L. bulgaricus*,
3 – *B. subtilis*, 4 – *E. coli*
Wound infection pathogens: A – *E. coli*,
B – *P. aeruginosa*, C – *S. aureus*



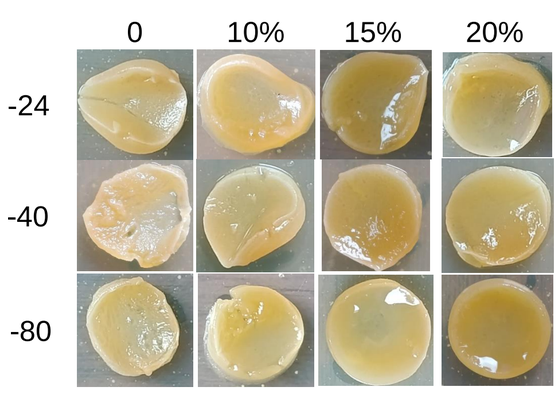
Antagonism of probiotics against pathogens:
1 – *B. bifidum* , 2 – *L. bulgaricus*
Wound infection pathogens: A – *S. aureus*,
B – *E. coli*, C – *P. aeruginosa*
Cultivation: 0 (control), 2, 4, and 6 days



Antagonism of alginate-pectin films with *L. bulgaricus* and various glycerol content against *P. aeruginosa* after low-temperature storage for 7 days



Modification of alginate films (A) by addition of starch (1%, B) and pectin (1%, C)



Preservation of the mechanical properties of films during storage at various temperatures and with different glycerol contents

CONCLUSION

A protocol for obtaining alginate-based hydrogel films supplemented with pectin or starch for the immobilization, cultivation, and low-temperature storage of lactic acid bacteria was developed. The films exhibited high antagonistic activity against major wound pathogens, suggesting their potential application as wound dressings for treating infectious wounds.

FUTURE WORK

In further studies, the antagonistic effect of immobilized probiotic bacteria on the opportunistic microflora of infected wounds will be studied in *in vitro* and *ex vivo* experiments, as well as on models of infected wounds on laboratory animals.