Production of nanofibers containing postbiotics using electrospinning method

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Introduction

Postbiotics are non-living microbial metabolites such as enzymes, peptides and bacteriocins. Although they do not contain live microorganisms, they exert health benefits through mechanisms similar to probiotics while eliminating the risks associated with live microbe intake. They contribute positively to host health by promoting antimicrobial, anti-inflammatory, antiproliferative, and antioxidant activities [1,2]. Sharma et al. (2018) investigated the anti-biofilm potential of bacteriocins and exopolysaccharide-based postbiotics isolated from probiotics against *Pseudomonas aeruginosa* PAO1. Their study demonstrated a significant reduction in biofilm-forming cells on coated surfaces [3]. Similarly, Yordshahi et al. (2020) developed antibacterial nanopaper using bacterial nanocellulose (BNC) infused with lyophilized postbiotics from *Lactobacillus plantarum*. The films exhibited dose-dependent antimicrobial activity against *Listeria monocytogenes* [4]. Nanofibers are solid fibers with theoretically unlimited length and diameters generally below 1000 nm, whose small pore size and high surface area make them attractive for diverse applications [5]. Among production methods, electrospinning is widely preferred due to its low cost, broad polymer compatibility, and ease of implementation. This technique, employing a high-voltage power supply, polymer feed, and collector, enables controlled fabrication of nanoscale fibers [6]. In a 2023 study, PVA nanofibers containing the antimicrobial agent nerolidol (NER) were fabricated via electrospinning, and their morphology and wound dressing potential were evaluated. Increasing NER concentration correlated with higher contact angles, confirming its presence. Antimicrobial assays revealed no inhibition zones for pure PVA, while NER-loaded mats exhibited varying inhibition against both Gram-positive and Gram-negative bacteria [7]. Thus, electrospinning successfully produced PVA nanofibers with confirmed antimicrobial activity.

Research Goal

In this study, the incorporation of postbiotics, obtained from two different bacteria (*Lactobacillus acidophilus* and *Lactobacillus rhamnosus*) via centrifugation following thermal inactivation, into nanofibers was investigated. Accordingly, the produced postbiotics were integrated into polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) using the electrospinning technique, and their antimicrobial activities were evaluated.

Methods

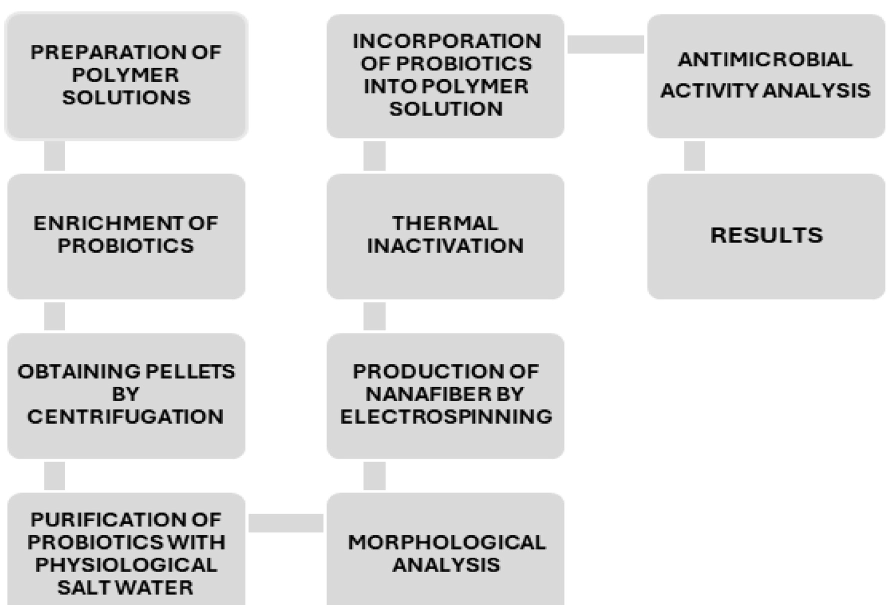

Figure 1. Workflow diagram of the study

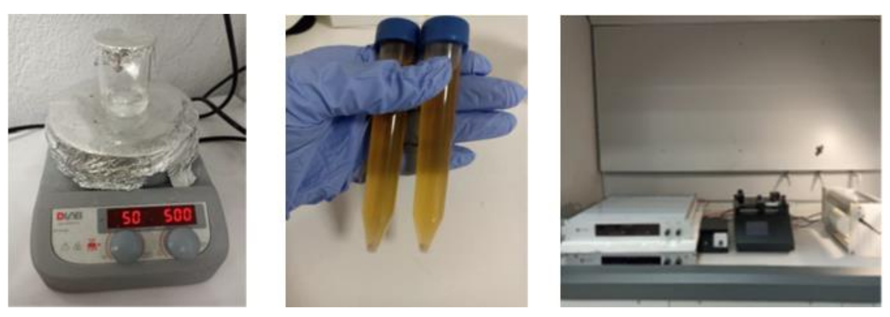

Figure 2. Experimental images of the study

The names and contents of the obtained nanofibers are given in Table 1.

Nanofiber Sample	Polymer	Bacteria
PVA0	PVA	-
PVP0	PVP	-
PVA/LA	PVA	*Lactobacillus acidophilus (ATCC 4356)*
PVA/LR	PVA	*Lactobaciluus rhamnosus (ATCC 7469)*
PVP/LA	PVP	*Lactobacillus acidophilus (ATCC 4356)*
PVP/LR	PVP	*Lactobaciluus rhamnosus (ATCC 7469)*

Table 1. Contents of Nanofiber Samples

Characterization:

a) ***FESEM:*** The morphological analysis of the produced nanofibers was performed using a field emission scanning electron microscope (FESEM). Images were acquired at 20 kV and magnifications of 5000x and 10000x.

b) Antimicrobial Analysis: In this study, the antimicrobial activities of the produced nanofibers against the pathogenic microorganisms listed in Table 2 were evaluated using the agar diffusion antimicrobial test. For this purpose, samples of all nanofibers with a diameter of 25 ± 5 mm were prepared, placed on the agar surface, and incubated at 37 °C for 24 h.

Results & Discussion

Morphological Analysis Results

From the FESEM images, 30 randomly selected fibers were measured for their diameters, and the average fiber diameter was subsequently calculated. This procedure was performed for all samples. Table 3 presents the diameter measurements of PVA nanofibers, while Table 4 presents those of PVP nanofibers, respectively.

Nanofiber Sample	Maximum Diameter (nm)	Minimum Diameter (nm)	Average Diameter (nm)
PVA0	561.637	222.785	335.146
PVA/LA	612.323	317.012	450.338
PVA/LR	320.973	160.486	263.177

Table 3. Average diameters of PVA nanofibers

The FESEM images of PVA nanofibers with and without postbiotics are presented in Figures 2, Figure 3, and Figure 4.

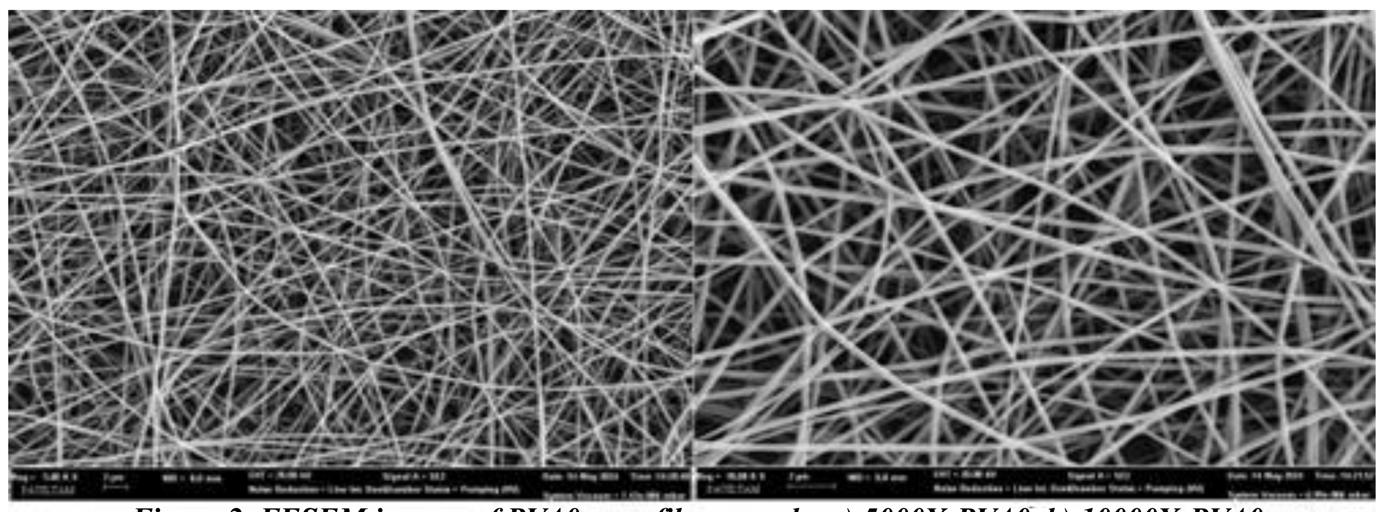

Figure 2. FESEM images of PVA0 nanofiber sample. a) 5000X-PVA0, b) 10000X-PVA0

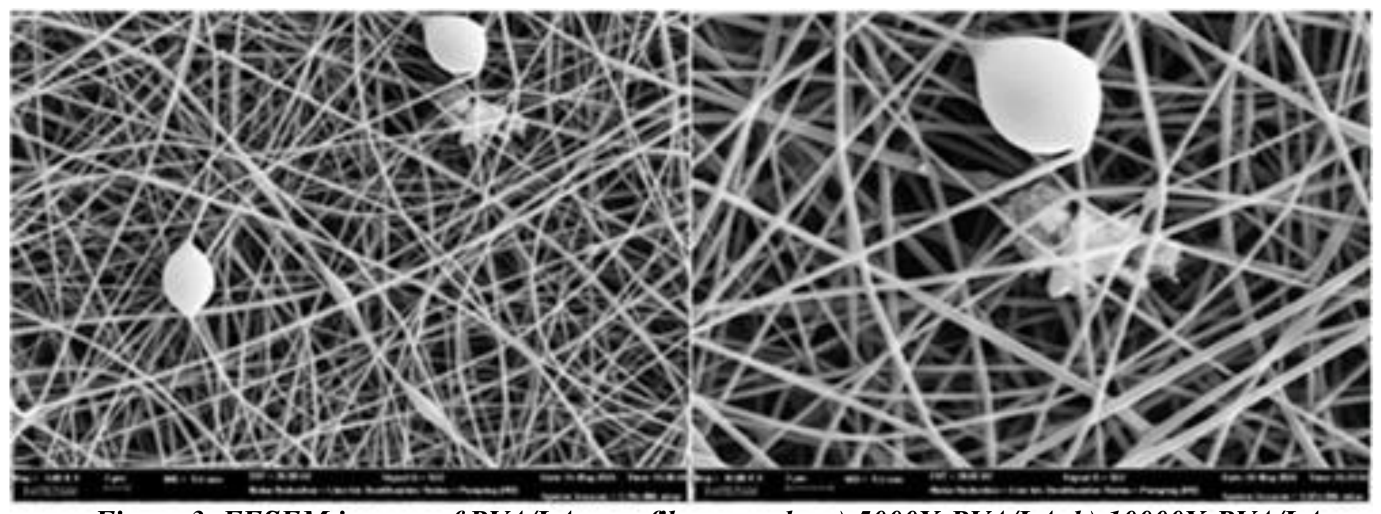

Figure 3. FESEM images of PVA/LA nanofiber sample. a) 5000X-PVA/LA. b) 10000X-PVA/LA

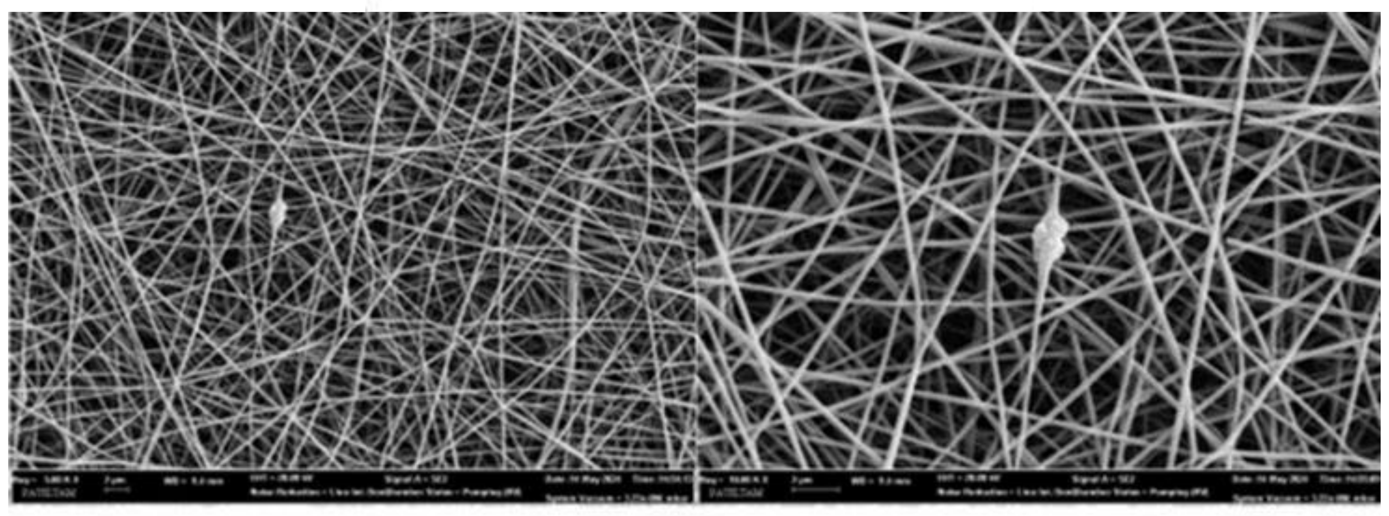

Figure 4. FESEM images of PVA/LR nanofiber sample. a) 5000X-PVA/LR, b) 10000X-PVA/LR

Nanofiber Sample	Maximum Diameter (nm)	Minimum Diameter (nm)	Average Diameter (nm)
PVP0	755.217	500.807	608.069
PVP/LA	781.520	346.678	535.513
PVP/LR	762.056	335.584	490.929

Table 3. Average diameters of PVA nanofibers

The FESEM images of PVP nanofibers with and without postbiotics are presented in Figures 5, Figure 6, and Figure 7.

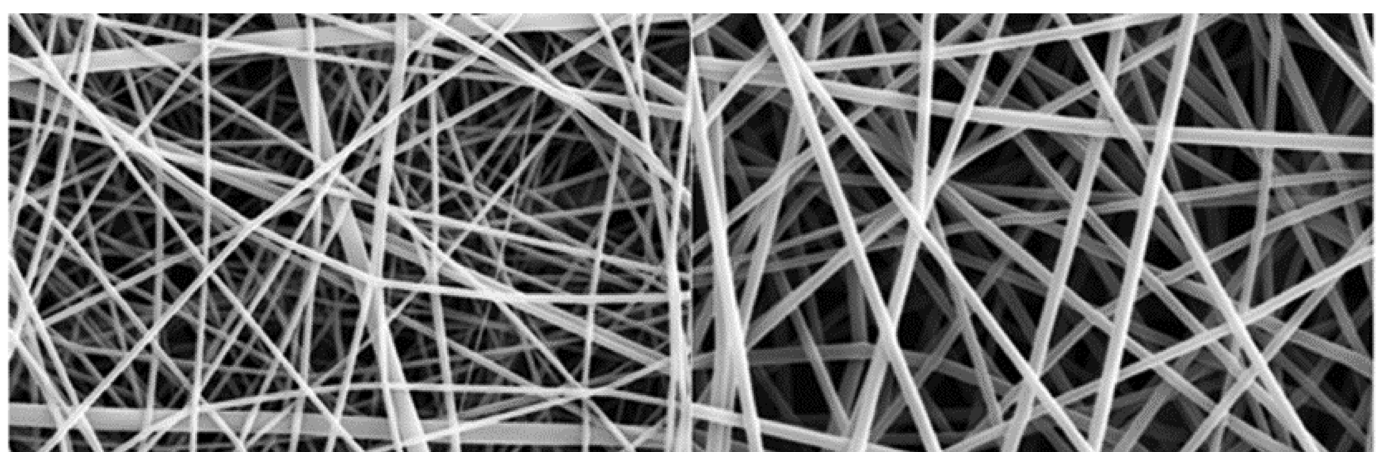

Figure 5. FESEM images of PVP0 nanofiber sample. a) 5000X-PVP0, b) 10000X-PVP0

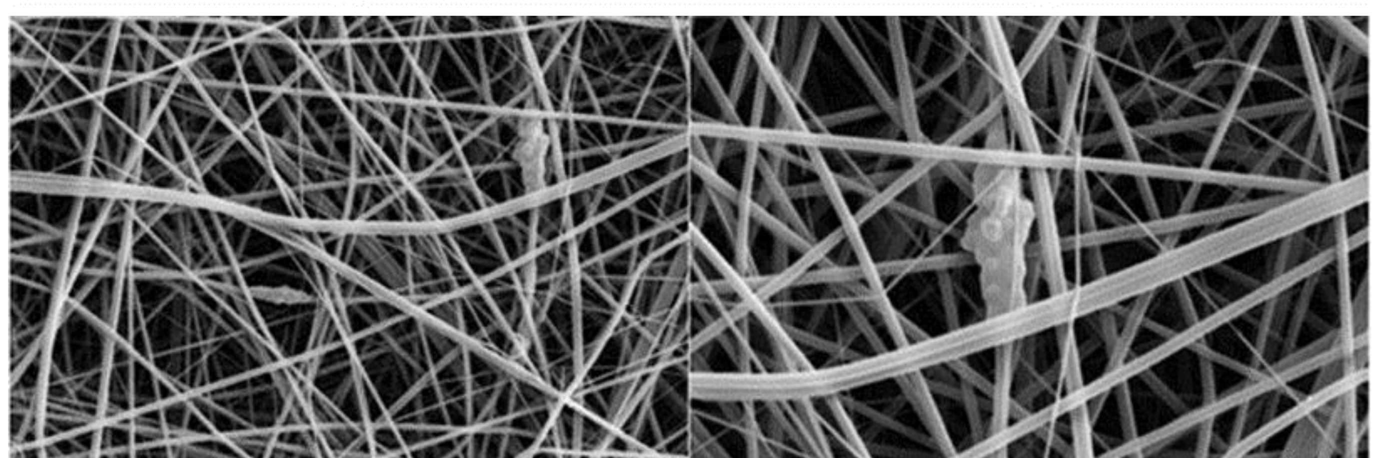

Figure 6. FESEM images of PVP/LA nanofiber sample. a) 5000X-PVP/LA, b) 10000X-PVP/LA

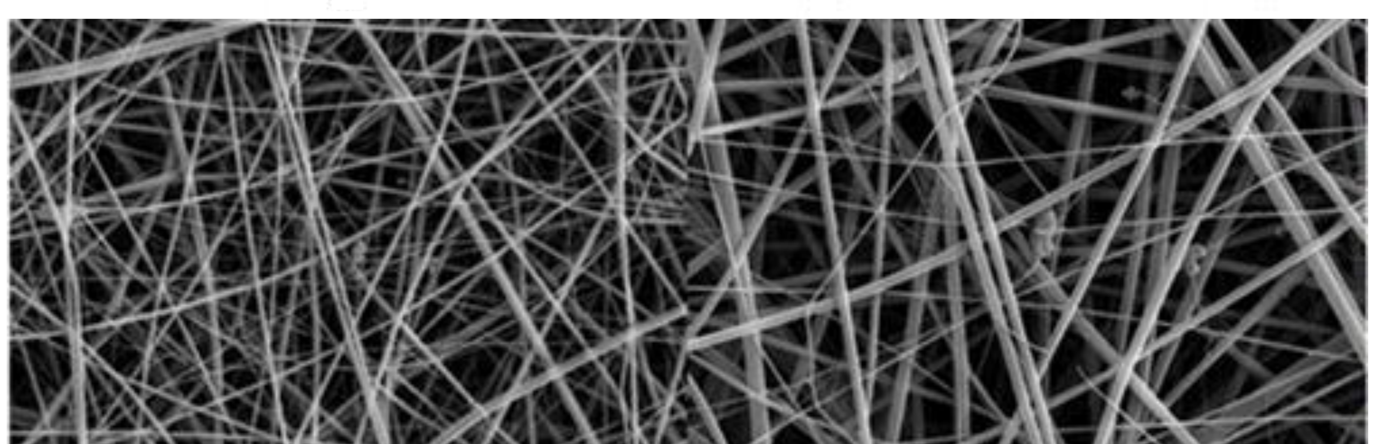

Figure 7. FESEM images of PVP/LR nanofiber sample. a) 5000X-PVP/LR, b) 10000X-PVP/L

Antimicrobial Analysis Results

Since PVA and PVP polymers do not possess antimicrobial properties, microbial growth was observed beneath and around the PVA0 and PVP0 nanofiber samples, as shown in Figure 8.

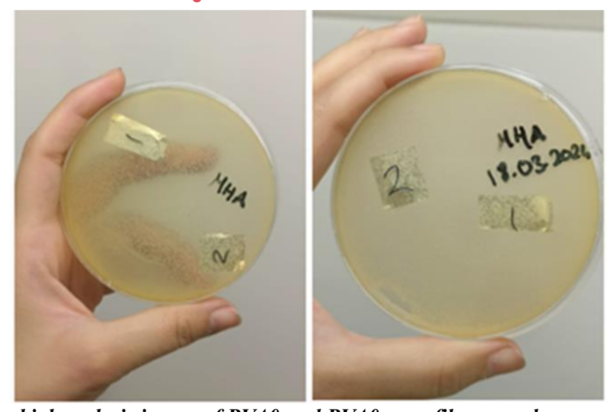

Figure 8. Antimicrobial analysis image of PVA0 and PVA0 nanofiber samples

The PVA/LA nanofiber sample exhibited strong antimicrobial activity against all tested microorganisms except *Listeria monocytogenes* (LM), against which no effect was observed. Both PVP/LA and PVP/LR nanofiber samples demonstrated strong antimicrobial effects against all microorganisms, with no microbial growth observed on the samples. The PVA/LR nanofiber sample showed good antimicrobial activity against all microorganisms and additionally formed an inhibition zone against Salmonella typhimurium (ST).

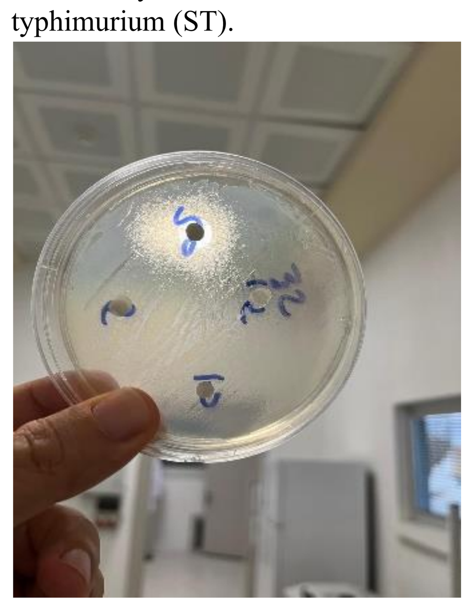

Figure 9 illustrates the antimicrobial effect of nanofiber samples containing postbiotics against the pathogen *Salmonella typhimurium*. The top-left sample corresponds to PVA/LA, the top-right to PVA/LR, the bottom-right to PVP/LA, and the bottom-left to PVP/LR nanofibers. While no microbial growth was observed beneath any of the samples, a distinct inhibition zone is clearly visible around the top-right sample (PVA/LR).

Figure 9. Antimicrobial effect of postbiotic containing nanofibers against Salmonella typhimurium

Figure 10 illustrates the antimicrobial effect of nanofiber samples containing postbiotics against the pathogen *Staphylococcus epidermidis*. The absence of microbial growth beneath the samples indicates that the nanofibers exhibited a strong antimicrobial effect. The top-left sample corresponds to PVA/LA, the top-right to PVA/LR, the bottom-right to PVP/LA, and the bottom-left to PVP/LR nanofibers.

Figure 10. Antimicrobial effect of postbiotic containing nanofibers against Staphylococcus epidermidis

Postbiotics have been shown to positively influence host health through various physiological activities. Jamaran et al. (2021) developed a biodegradable postbiotic/chitosan/PEG film from Lactobacillus reuteri cell-free supernatant using solvent casting and demonstrated, via animal experiments, its ability to inhibit wound pathogens. Similarly, bacteriocins from *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* exhibited strong antimicrobial effects against multidrug-resistant, protease-producing Pseudomonas aeruginosa isolates, with inhibition zones of 32 mm and 25 mm, respectively [8,9]. These findings, consistent with the bacterial strains used in our study, highlight their significant antimicrobial potential. Golkar et al. (2021) evaluated topical cold creams containing postbiotics from Lactobacillus fermentum, L. reuteri, and Bacillus subtilis sp. natto in a rat wound model. Postbiotic creams accelerated healing compared to controls, with B. subtilis natto showing the greatest effect, including highest hydroxyproline levels. After 14 days, complete epithelialization occurred in L. reuteri and B. subtilis natto groups, while controls showed incomplete healing and higher inflammation. The study demonstrated that postbiotic creams enhance wound repair [10].

Conclusion

In this study, the integration of postbiotics into nanofibers using the electrospinning technique and their antimicrobial activity were investigated. Postbiotics derived from *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* strains were incorporated into polymer solutions and converted into nanofiber form. The inclusion of postbiotics in the nanofibers was confirmed through morphological analysis using FESEM. Additionally, the produced nanofiber membranes were tested against five different pathogenic microorganisms, demonstrating strong antimicrobial properties.

References