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Modulating Growth Conditions and Microenvironment to Boost Antimicrobial Efficacy of Probiotics Against Pathogens

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Abstract

The increasing prevalence of antimicrobial resistance and biofilm-associated infections highlights the need for alternative strategies to control pathogenic microorganisms. Probiotics have emerged as promising candidates due to their ability to inhibit pathogen growth. However, their potential ways of application in human medicine remain insufficiently understood. Here we investigate the antimicrobial and antibiofilm activity of selected probiotic strains, *Lactiplantibacillus plantarum* Fibro 1 *and Lacticaseibacillus rhamnosus* Fibro 2, against clinically relevant pathogens, with the aim of generating insights to support the design of improved probiotic-based systems for wound infection treatment. Probiotics were investigated for their growth, lactic acid production, and biofilm formation in different media. A range of *in vitro* antimicrobial activity assays were employed, including growth inhibition and antibiofilm assays. In addition to free probiotics, probiotics incorporated into electrospun fiber mats were investigated in *in vitro* biofilm models. Probiotics exhibit strain- and condition-dependent inhibitory effects, influencing both planktonic growth and biofilm development of pathogens, while metabolic analysis showed marked medium-dependent glucose utilization and lactate production. Probiotics incorporated into fiber mats retain measurable metabolic activity after encapsulation and preserve their antibiofilm activity. Overall, this study provides a deeper understanding of probiotic effects on pathogens and identifies key factors influencing antimicrobial efficacy. These findings support the rational design of enhanced probiotic-based formulations for applications in infection control and wound care.

Keywords: probiotics; antimicrobial; functionality; growth conditions; microenvironment

Introduction

The excessive use of antibiotics and the lack of globally agreed stewardship have resulted in increasing pathogen resistance to common antibiotics. This overuse has turned into a

serious health threat causing significant morbidity and mortality worldwide. According to the World Health Organization, antibiotic resistance is one of the top public health and development threats, and was responsible for 1.27 million global deaths in 2019 [1].

An additional major challenge is that bacteria, especially pathogenic bacteria, have a tendency to form biofilms, which are three-dimensional microbial communities embedded in an extracellular matrix. Biofilm formation significantly increases bacterial tolerance to antibiotics [2, 3] and host immune defenses [4]. Therefore, there is an urgent need for new and more effective antimicrobial agents and their delivery systems that can combat pathogens and their biofilms.

Microorganisms are a natural source of antimicrobial compounds, which they produce as part of competitive survival strategies in complex microbial environments. Probiotics are defined as live microorganisms that, when administered in sufficient quantities, are beneficial to health [5]. Not every bacterium can be considered as probiotic [6]. Most probiotics belong to the highly diverse families *Lactobacillus spp*. and *Bifidobacterium spp*. [7], which are important in human nutrition and biotechnology due to their ability to produce antimicrobial peptides (AMPs) and other biologically active substances, such as organic acids and hydrogen peroxide. It has been established that AMPs produced by probiotics have many benefits, including a broad spectrum of antimicrobial activity, activity against biofilms, and a lower probability of resistance formation [8, 9]. Typically, bacteria produce multiple antimicrobial compounds simultaneously, which broadens their overall antimicrobial spectrum. Probiotics exhibit antimicrobial properties against both Gram (+) and Gram (-) bacteria [10, 11], can promote human dermal fibroblast' migration, and play a major role in wound healing [12]. Although probiotics have demonstrated antimicrobial activity against a range of pathogens, their mechanism of action remains incompletely understood. There is still limited understanding of how wound-relevant environmental conditions (e.g. pH, temperature, nutrients) influence their functionality and therapeutic potential.

The wound environment is very dynamic and differs from standard *in vitro* culture conditions. Chronic wounds typically have elevated pH, reduced oxygen availability, nutrient fluctuations, and polymicrobial biofilms [13, 14]. These conditions promote pathogen

persistence and reduce the effectiveness of antimicrobial therapies [3]. At the same time, wound healing is associated with a shift toward a more acidic and oxygen-rich environment that supports tissue regeneration and limits pathogen growth [15, 16]. Importantly, probiotics have potential to actively influence the wound environment through the production of antimicrobial compounds, including organic acids, hydrogen peroxide, bacteriocins, and other bioactive metabolites. These molecules can inhibit the growth of wound-associated pathogens, disrupt biofilms [17], lower the local pH [18] , thereby creating conditions that may support tissue repair and regeneration. In addition, probiotic-derived metabolites , may influence fibroblast activity and contribute to the improved wound healing [19, 20] by reducing infection while simultaneously promoting conditions that support tissue repair [21] and regeneration [22].

The antimicrobial activity of probiotics is strongly influenced by their cultivation microenvironment. Optimization of factors such as pH, temperature, growth phase, nutrient availability, and oxygen availability can modulate the production of antimicrobial substances and thereby enhance antimicrobial activity [23, 24]. The production of antimicrobial metabolites generally reaches its maximum during the late exponential growth phase and the early stationary phase, although the exact timing is strain and cultivation-dependent, and biomass production does not necessarily correlate with antimicrobial metabolite production [25]. In addition to optimizing cultivation conditions, incorporation of probiotics into advanced delivery systems may improve their stability and therapeutic functionality [26]. Such systems are designed to protect probiotics from harsh environmental conditions encountered during storage and after administration (e.g. in the human body), while preserving biological activity [27–30]. One promising approach is microchip electrospinning technology [31], which allows the production of fiber mats composed of fibers incorporating semi-solid agarose beads encapsulating living probiotics. The system can protect microorganisms while allowing nutrient exchange and release of bioactive compounds. However, encapsulation also creates a distinct local microenvironment around the probiotics, which may alter their metabolism, stress response, and antimicrobial functionality.

The aims of this study are two-fold: (i) to investigate the antimicrobial properties of two different probiotics, namely *Lactiplantibacillus plantarum* Fibro 1 (LP) and *Lacticaseibacillus rhamnosus* Fibro 2 (LR), under varying growth and environmental conditions and (ii) to compare their antimicrobial activity in free form and when incorporated into electrospun fiber mats as an advanced delivery system. To address these aims, we systematically evaluated the effects of selected cultivation and microenvironmental conditions on probiotic growth and antimicrobial activity and assessed whether antimicrobial functionality was retained following incorporation into the electrospun delivery system. This approach provides a framework for understanding how both the cultivation environment and the delivery system influence probiotic functionality, with the aim of supporting the rational development of probiotic-based antimicrobial formulations for future wound-care applications.

Materials and Methods

Bacterial strains and culture conditions

Two probiotic strains, both lactic acid bacteria (LAB), were used in this study: *Lactiplantibacillus plantarum* Fibro 1 *and Lacticaseibacillus rhamnosus* Fibro 2 (Bioprox Healthcare, France). The pathogenic strains *Escherichia coli* DSM 1103*, Staphylococcus aureus* DSM 2569*, Pseudomonas aeruginosa* DSM 1117*, Pseudomonas aeruginosa* PAO1*,* and *Staphylococcus epidermidis* DSM 28319 (DSMZ, Germany), were used to assess the antimicrobial activity of LAB.

All bacterial strains were stored at -80°C (pHcbi, Japan) in a liquid BD Difco™ Luria-Bertani (LB) (BD Bioscience, USA) or De Man–Rogosa–Sharpe (MRS) (BD Bioscience, USA) selected medium supplemented with 20% [v/v] glycerol (AppliChem GmbH, Germany) for pathogens and LAB strains, respectively. They were revived in the respective medium before each experiment as specified below.

Heat-treated (HT) LAB samples as controls were prepared as follows: bacteria were pre-cultured on MRS agar plates at 37°C for 24 h. Bacterial suspensions were made and then adjusted to OD_{600} 0.1 in phosphate-buffered saline (1 × PBS). Suspensions were heated at 100°C for 10 min. The complete loss of viability was confirmed by plating 10 µL of the suspension on MRS agar and incubating at 37°C for 24 h. For the inoculum preparation, LAB strains were pre-cultured on MRS agar plates, and pathogenic bacteria on LB agar plates at 37°C for 24 h prior to each experiment. Both bacterial suspensions were prepared and adjusted to OD_{600} 0.1.

Electrospun fiber mats

The LAB-incorporated fiber mats were prepared as described previously [31], using a solution containing 12% [w/w] poly(L-lactide-co-glycolide) (PLC) and 0.3% [w/w] polyethylene oxide (PEO) in Me_2CO_3 and *in situ* microchip electrospinning. Microcapsules were prepared using a 0.625% [w/w] aqueous agarose solution as the dispersed phase. Polymeric fiber mats with incorporated agarose microcapsules, but without probiotics (pristine fiber mats) were used as controls. In the *in vitro* biofilm inhibition model assays, electrospun gelatine-glucose (Gel-Glu) fiber mats were used as an artificial skin and were prepared as described previously [32, 33].

PDMS substrates

The polydimethylsiloxane (PDMS) disks were used as an abiotic substrate in biofilm formation and inhibition assays. PDMS disks were prepared by mixing the curing agent and base polymer at a mass ratio of 1:10 using a SYLGARD™ 184 Silicone Elastomer Kit. The mixture (18 g) was homogenized and poured into 90 mm diameter Petri dishes, achieving a thickness of 3 mm. The PDMS mix was then cured overnight in a vacuum oven at 60°C, followed by shaping it into disks of 6 mm diameter using biopsy punches. To remove unreacted surface residues, the PDMS disks were immersed in 100% ethanol for 2 h, followed by sterilization in 70% [v/v] ethanol (Sigma-Aldrich, Merck, Germany) for 15 min.

Afterwards, the disks were air-dried in a biosafety cabinet and kept in a sterile plate until further use.

Viability and morphological characterization of probiotics

To determine the viability and morphology of probiotics, an overnight culture of LAB grown in MRS broth at 37°C was stained with a green-fluorescent nucleic acid dyes SYTO 9 and propidium iodide (PI) (Invitrogen, Thermo Fischer, USA) [31]. Next these samples were analyzed with confocal laser scanning microscopy (CLSM) (LSM710, Carl Zeiss, Germany).

To determine the cell morphology in more detail, probiotic aqueous suspensions adjusted to OD_{600} 0.1 were centrifuged at 5,000 × g for 5 min and resuspended in 100 µL of 4% paraformaldehyde, followed by incubation at room temperature for 30 min. The fixative was then removed, and the samples were dehydrated through a graded ethanol series (30%, 60%, and 90% [v/v]), with each step lasting 10 min. Finally, 10 µL of each sample was pipetted onto the scanning electron microscopy (SEM) stub for analysis.

Morphological characterization of the electrospun fiber mats and PDMS substrate

The morphology of electrospun fiber mats (Gel-Glu, LAB-incorporated and pristine fiber mats) and PDMS substrate was characterized by SEM, and mean diameter of the fibers was determined by image analysis of 2-dimensional (2-D) SEM micrographs using ImageJ software, as described previously [31].

Growth kinetics of probiotics in different growth media

Growth kinetics of LAB were tested in different growth media: BD Difco™ M17 (BD Bioscience, USA), MRS, MRS with pH 5.5 (adjusted with 1 M HCl (Sigma-Aldrich, USA)), MRS with the addition of 0.5% and 1% [w/v] of inulin (Thermo Fischer, USA), 1:1 MRS:Mueller-Hinton broth (MHB, Cation-Adjusted) (BD Bioscience, USA), or with the combination of both factors (Table 1). Prepared probiotic inoculum suspensions were diluted 1:100 in the appropriate media,

and 200 µL was dispensed into each well of a 96-well plate. The plates were incubated at 37°C for 24 h with shaking. The OD_{600} was measured every hour in a microplate reader (Clariostar^{Plus}, BMG Labtech, Germany).

Table 1 *Overview of the growth media tested for lactic acid bacteria (LAB) strains. The bacteria were inoculated into 200 µL of the appropriate medium at starting concentration of 10^6 CFU/mL and incubated at 37°C for 24 h*

Strains	**Media (names used in this article)**	**Additives**
L. plantarum *L. rhamnosus*	MRS	
	MRS pH 5.5	
	MRS (0.5% [w/v] inulin)	+0.5% [w/v] inulin
	MRS pH 5.5 (0.5% [w/v] inulin)	+0.5% [w/v] inulin
	MRS (1% [w/v] inulin)	+1% [w/v] inulin
	MRS pH 5.5 (1% [w/v] inulin)	+1% [w/v] inulin
	MRS:MHB	
	M17	
	M17 pH 5.5	
	GMEM	
	GMEM (GMEM + GLU)	+2% [w/v] GLU
	GMEM (GMEM pH 5.5)	
	GMEM (GMEM+GLU pH 5.5)	+2% [w/v] GLU
	DMEM	
	DMEM (DMEM +GLU)	+2% [w/v] GLU
	DMEM (DMEM pH 5.5)	
	DMEM (DMEM+GLU pH 5.5)	+2% [w/v] GLU
	DMEM/F12	
	DMEM/F12 (DMEM/F12 + GLU)	+2% [w/v] GLU
	DMEM/F12 (DMEM/F12 pH 5.5)	
	DMEM/F12 (DMEM/F12+GLU pH 5.5)	+2% [w/v] GLU

Key: DMEM – Dulbecco's Modified Eagle Medium; F12 – Ham's F-12 Nutrient Mixture; GMEM – Glasgow Minimum Essential Medium; GLU – glucose, M17 – basal medium; MHB – Mueller–Hinton Broth; MRS – de Man, Rogosa and Sharpe broth; [w/v] – weight per volume

LAB strains were also cultured in a cell culture media designed to mimic wound fluid – Glasgow Minimum Essential Medium (GMEM), Dulbecco's Modified Eagle Medium (DMEM),

or Dulbecco's Modified Eagle Medium and Ham's F-12 Nutrient Mixture (DMEM F12) (Sigma-Aldrich, USA), with the addition of 10% of fetal calf serum (FCS), with or without 2% [w/v] glucose (Sigma-Aldrich, USA) and/or initial pH adjustment to 5.5 (adjusted with 1 M HCl (Sigma-Aldrich, USA) (Table 1). Prepared probiotic inoculum suspensions were diluted 1:100 in the appropriate media and incubated in the test media at 37°C for 24 h. The procedure was proceeded as described previously. After incubation, serial dilutions were prepared from each sample and plated on MRS agar plates, incubated at 37°C for 24 h. CFUs were determined. The pH of the growth media was measured before and after incubation with pH-meter (Hanna instruments, USA).

Determination and quantification of metabolites produced and substances consumed by probiotics

The concentration of metabolites produced (e.g. production of lactic acid) and substances consumed were determined in samples with free LAB strains or those incorporated into the fibers. For free probiotic samples, LAB inoculum was freshly prepared and diluted 1:100 in 5 mL of appropriate media. For fiber-incorporated LAB samples, 1 x 6.5 cm pieces of fiber mat, containing an average of 10^6 probiotic cells, were cut and incubated in 5 mL of appropriate media at 37°C for 24 h under aerobic or anaerobic conditions (Table 2). The anaerobic conditions were established using AnaeroGen™ anaerobic gas-generating sachets (Oxoid, Thermo Fisher Scientific, UK).

Table 2 *Overview of media and growth conditions tested for lactic acid production by probiotics*

MRS pH 5.5, AE	**MRS pH 5.5, AN**	**DMEM/F12+GLU pH 5.5, AE**
L. plantarum free	*L. plantarum* free	*L. plantarum* free
L. rhamnosus free	*L. rhamnosus* free	*L. rhamnosus* free
L. plantarum in fibers	*L. plantarum* in fibers	*L. plantarum* in fibers
L. rhamnosus in fibers	*L. rhamnosus* in fibers	*L. rhamnosus* in fibers
Control 1 (sterile medium)	Control 1 (sterile medium)	Control 1 (sterile medium)

Control 2 (pristine fiber mat)	Control 2 (pristine fiber mat)	Control 2 (pristine fiber mat)
Control 3 (HT_LP)	Control 3 (HT_LP)	Control 3 (HT_LP)
Control 4 (HT_LR)	Control 4 (HT_LR)	Control 4 (HT_LR)

Key: AE – aerobic conditions, AN – anaerobic conditions, free – planktonic probiotics; GLU – glucose, HT_LP – heat-treated L. plantarum Fibro 1, HT_LR – heat-treated L. rhamnosus Fibro 2; in fibers – probiotics encapsulated into the fibers via microchip electrospinning; pristine fiber mat – control fiber mat not consisting any probiotics and prepared via microchip electrospinning

After incubation, samples were centrifuged for 15 min at 4°C and 4600 × g, and then the supernatants were filtered through 0.22 µm filter into sterile tubes. Samples were at -80°C for 24 h and then lyophilized at -40°C, 0.2 mbar for 24 h. The concentration of substances produced and consumed was revealed using nuclear magnetic resonance (NMR) assay. Lyophilized samples were each reconstituted in 600 µL of a buffer containing, per liter, 100 mL D_2O, 900 mL H_2O, 10.48 g Na_2HPO_4 (73.82 mM), 0.602 g 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (TSP-d4) (3.49 mM) as internal standard and 0.398 g NaN_3 (6.12 mM) and adjusted to pH 7.43 with the addition of 3% HCl. A pooled sample was prepared by combining 10 µL of each sample for metabolite assignment. ^1H NMR spectra were recorded on Bruker 600 MHz Bruker Avance III NMR spectrometer (Bruker BioSpin, Coventry, United Kingdom) equipped with a 5 mm ^1H, ^{13}C, ^{15}N TCI Prodigy Probe and a cooled sample changer with all samples kept at 4°C. The 1D spectra were acquired under automation at a temperature of 25°C using Noesygppr1d which is a standard 1D nuclear Overhauser effect spectroscopy (NOESY) pulse sequence. The parameters of spectra acquisition are 32 transients, a spectral width of 20.83 ppm and 65,536 datapoints. For assignment of metabolite peaks additional spectra, Total correlation spectroscopy (TOCSY), ^1H-^{13}C heteronuclear single quantum correlation spectroscopy and J-resolved spectroscopy (JRES), were acquired from a pooled sample containing a small volume of all prepared samples (s.f. Table 2). All spectra were Fourier transformed (FT) in Bruker software and adjusted using automatic baseline correction and phasing in Bruker TopSpin 4.1.4. Chenomx NMR suite software (Chenomx Inc, Canada) was used for assignment and quantification of metabolites with calibration to the concentration of TSP-d4 present in the

sample and adjusted for dilution by D_2O. NMR assignments were performed as previously reported [34, 35].

L-lactic acid assay kit (Megazyme, Ireland) was used to quantify the lactic acid content in prepared samples (s.f. Table 2). Freeze-dried samples were reconstituted in distilled water to their original volume, and lactic acid content was measured following manufacturer's instructions for microplate assay procedure. The calibration curve for quantitative analysis was prepared with 5 different lactic acid standard solution concentrations. Absorption was measured at 340 nm using CLARIOstar Plus microplate reader (BMG Labtech, Germany).

Characterization of probiotics for biofilm formation

Biofilm cultivation (static conditions) on PDMS substrates in droplet biofilm model

Few LAB colonies of overnight culture were collected and resuspended in MHB or in DMEM/F12+GLU pH 5.5 medium and adjusted to an OD_{600} 0.1. *S. aureus* DSM 2569 was used as a control bacterium in the PDMS droplet biofilm model. Then, 50 µL droplet of bacterial suspension was applied onto the surface of each PDMS disk placed in a 24-well plate. To minimize evaporation during incubation, 200 µL of sterile PBS was added to the bottom of each well. Plates were incubated at 37°C for 24 h. To quantitatively determine colony forming units (CFUs) in the biofilm, after incubation, each PDMS disk was gently washed three times with 500 µL of sterile 1 × PBS to remove loosely attached cells. The wash solutions were pooled and CFUs quantified. The washed PDMS disk was transferred into 500 µL sterile 1 × PBS and sonicated for 5 min to detach adherent biofilm cells, followed by vortexing at maximum speed for 15 s. The resulting suspensions were serially diluted and plated onto LB agar plates for pathogenic bacteria and MRS agar plates for LAB counting. Plates were incubated overnight at 37°C, and CFUs were enumerated to determine the number of viable cells. The experiment was performed in triplicate.

To determine the biomass of the biofilms, at the end of biofilm incubation, PDMS disks were processed by gently washing each disk as described above. Then, 900 µL of 0.5% (w/v)

crystal violet (CV) solution was added to fully cover the disk surface. After 15 min at room temperature, the staining solution was removed, and disks were washed three times with 500 µL of sterile 1× PBS, air-dried on sterile well plate lids, and recorded with images for analysis. To quantify biofilm biomass, the CV dye was eluted in 30% acetic acid followed by absorbance measurement at 590 nm. Additional samples were prepared for CLSM.

Biofilm cultivation in flow-through model

The microfluidic chips were made from PDMS according to the H. Straub *et. al.* method [36]. To evaluate the ability of bacteria to adhere to PDMS under static conditions and in the presence of flow, the glass surface inside the microfluidic chamber was coated with a thin PDMS layer (0.1 mm thickness)[37] . The tubing was connected to the device and sealed with epoxy. Before the experiment, the entire system was sterilized with 70% w/w ethanol. Then, 70% w/w ethanol was carefully replaced with 1 × PBS. The device was mounted onto a microscope. Few LAB colonies of overnight culture were collected and resuspended in DMEM/F12+GLU pH 5.5 medium and adjusted to an OD_{600} 0.1. *S. aureus* DSM 2569 was used as a control bacterium in the PDMS droplet biofilm model. Next, a 10 mL syringe containing the bacterial (probiotics or *S. aureus*) suspension was connected to the central channel and injected into the observation chamber. Bacterial adhesion on the PDMS surface of the observation channel was recorded at 60× magnification every 2 min. An outlet tube was connected to collect excess liquid. Using a syringe pump, the bacterial suspension was introduced into the middle channel at a flow rate of 10 µL/h for 4 h, while the top and bottom inlet channels were sealed with epoxy. All experiments were performed at 37°C and in triplicate. The microfluidic chips were imaged using a fully automated Nikon Ti2E inverted microscope (Japan).

Antimicrobial activity of probiotics in agar overlay assay after different pre-cultivation and under different incubation conditions

The antimicrobial activity of LAB strain probiotics was evaluated using an agar overlay method [31](Fig. S4). The assay was performed in different environmental conditions,

varying the media, temperature, and the presence of oxygen while two different growth agars were utilized – MRS with 1.5% [w/v] agar as a base for probiotics, and the top agar composed of BD Difco™ Mueller-Hinton II medium with 0.5% [w/v] of BD Difco™ Bacto agar (BD Biosciences, USA) and containing 10^6 CFU/mL of pathogenic bacteria. For bacterial dilutions 1 × PBS buffer (pH 7.4) (Corning, USA) was used. Suspension of overnight LAB culture was prepared in 1 × PBS (with final OD_{600} 0.2) and 5 µL of it was spotted onto Petri dishes containing 15 mL of MRS base agar and incubated for 24 h at 30°C, 33°C or 37°C under aerobic or anaerobic conditions. After incubation, suspension of pathogenic bacteria was prepared with OD_{600} 0.1 and diluted 1:100 in soft agar. 100 µL of soft agar with pathogenic bacteria was pipetted on the 24 h pre-cultured probiotics in the middle of the Petri dish, covering the LAB growth spot to prevent bacterial spreading, and let to solidify. After that 10 mL of soft agar with pathogenic bacteria was poured over the base agar and let to solidify. Plates were incubated at 37°C for 24 h, and inhibition zones were measured with ImageJ, the mean and standard deviation (SD) was calculated. All assays were performed in triplicate.

Anaerobic vs aerobic pre-cultivation conditions for LAB strains. To evaluate the influence of oxygen in the environment on the antimicrobial activity, LAB bacteria were pre-cultured on MRS agar plates under different environmental conditions – aerobic and anaerobic – at 37°C for 24 h prior to antimicrobial activity assessment in agar overlay assay.

LAB pre-cultivation at different temperatures. To evaluate the influence of temperature on the antimicrobial activity, LAB strains were pre-cultured on MRS base agar at different temperatures 30°C, 33°C or 37°C for 24 h prior to antimicrobial activity assessment in agar overlay assay.

LAB pre-cultivation at different pH. To evaluate the influence of pH on antimicrobial activity, LAB strains were pre-cultured on MRS agar adjusted to pH 5.5, 6.0, 6.5, 7.0, or 7.5. Agar at pH 5.5 and 6.0 was prepared by adding 1 M HCl (Sigma-Aldrich, USA) to liquid MRS, while pH 7.0 and 7.5 was obtained by adding 1M NaOH (Sigma-Aldrich, Merck, Germany). Unmodified MRS (pH 6.5) was used as the control. Final pH values were verified with a calibrated pH meter (Hanna instruments, USA) before solidification. Plates were inoculated with LAB and

incubated at 37°C under aerobic conditions for 24 h prior to antimicrobial activity assessment in agar overlay assay.

Impact of the inulin addition into the base agar. 1 [w/v] % inulin solution (prebiotic for probiotics) was prepared in sterile water, then filtered through the 0.22 µm filter. 2x concentrated MRS agar was prepared and autoclaved. Then solutions were mixed at 1:1 before MRS solidification to get final concentration of 0.5 [w/v] %. Prepared agar plates were used as base layers in agar overlay assay.

Buffers. To evaluate the buffering capacity of the agar overlay model, different buffers (HEPES (Corning Life Sciences, Mediatech, Inc., USA), and Tris (Sigma-Aldrich, Merck, Germany)) were tested at different concentrations (100 mM, 200 mM and 300 mM). Bromocresol purple (Thermo Fischer, USA) was included as a pH indicator at a 1:50 ratio in the top agar. The presence of a yellow zone in the top agar layer was investigated. Furthermore, to investigate the effect of buffered environmental pH on antimicrobial activity, Tris buffer was incorporated into the top agar layer during the agar overlay assay at varying concentrations (100, 200, and 300 mM) and pH values (7.5, 8.0, and 8.8). As a base layer, MRS agar was used. For each concentration and pH condition, two sample sets were prepared: one containing pathogenic bacteria in the top layer without the pH indicator, and another containing the pH indicator but no pathogen. Plates were incubated at 37°C for 24 h after pipetting the LAB strains and additional 24 h after top layer solidification. The diameters of the pH change zones (yellow zones indicating acid production) and inhibition zones (areas lacking pathogen growth) were measured in millimeters using ImageJ program (Table S1).

Antimicrobial activity in liquid media against planktonic pathogenic bacteria. The inoculum suspensions of probiotic and pathogenic bacteria were freshly prepared. Pathogenic bacteria suspensions were diluted 1:1000 in 1 × PBS. HT probiotics samples were prepared as described previously. 20 µL of probiotic and 20 µL of pathogenic suspensions were added to 500 µL of mixed 1:1 MRS and MHB or to 500 µL of DMEM/F12+GLU pH 5.5. Eppendorf tubes were incubated under static conditions at 37°C for 24 h. Serial dilutions were made and plated on LB and MRS agar plates to evaluate the concentrations of pathogenic bacteria

and LAB strains, respectively, using CFU counting. The pH of the growth media was determined before and after incubation with pH-strips (Macherey-Nagel, Germany).

Antibiofilm activity of probiotics in *in vitro* assays

Different reported biofilm models were set up for analyzing probiotics and used for the characterization to understand the antimicrobial and antibiofilm activity of LAB strains, namely: i) *in vitro* biofilm inhibition and treatment models [38] and ii) PDMS droplet biofilm inhibition and treatment models [39].

In vitro biofilm model - inhibition and mature biofilm disruption by free probiotics

Biofilm inhibition assay. The inoculum suspensions of probiotic and pathogenic bacteria were prepared. Pathogenic suspensions were diluted 1:1000 in 1 x PBS. HT LAB samples as controls were prepared as described above. 10 µL of pathogenic bacterial suspension and 10 µL of LAB suspension were added to 250 µL of DMEM/F12+GLU pH 5.5 into the well containing 3 filter papers and Gel-Glu fiber mat (1 x 1 cm) (according to Lorenz et al. [38]). Samples without LAB were used as controls. Well plate was incubated at 37°C for 24 h. Then Gel-Glu fiber mats were washed in 1x PBS, put into new Eppendorf, containing 1 mL of 1 × PBS and homogenized at 8000 rpm for 20 s (Precellys Evolution homogenizer, Bertin Technologies, France). Serial dilutions were made and plated on LB and MRS agar plates to evaluate the concentrations of pathogenic bacteria and LAB, respectively, using CFU counting.

Biofilm treatment assay. The mature biofilm of *P. aeruginosa* PAO1 and *S. aureus* were pre-cultured according to Lorenz et al. [38]. 24 h later, the Gel-Glu fiber mats were placed into a new well with 250 µL or into an Eppendorf with 500 µL of DMEM/F12+GLU pH 5.5 (adjusted with 1M HCl). LAB inoculum suspensions were prepared and 10 µL was added into the well with pre-grown pathogenic bacteria biofilm or suspensions were directly diluted 1:100 in appropriate media in Eppendorf, respectively. HT LAB samples as controls were prepared as

described above. Samples were incubated for 24 h, then the samples were processed as described above in *Biofilm inhibition assay*.

In vitro PDMS droplet biofilm model - inhibition and mature biofilm disruption by free probiotics

Biofilm inhibition assay. For the PDMS droplet biofilm inhibition assay, LAB and *S. aureus* were incubated simultaneously on the PDMS surface. The LAB inoculums were freshly prepared as described above. The suspension of pathogenic bacteria was adjusted to OD_{600} 0.001. Bacterial suspensions were mixed 10:1. 50 µL of the mixed suspension was applied onto PDMS disks, and the samples were incubated under static conditions at 37°C for 24 h to allow biofilm formation. The CFUs were enumerated as described previously.

Biofilm treatment assay. For the treatment of mature pathogenic bacteria biofilms, *S. aureus* was first incubated on PDMS disks at an initial concentration of OD_{600} 0.0001 under static conditions. After 24 h of incubation at 37°C, non-adherent (planktonic) cells were removed by a washing step. Subsequently, suspensions of both LAB at OD_{600} 0.1 were prepared and 50 µL of each were separately added onto the pre-formed *S. aureus* biofilm. The samples were then further incubated to assess the effect of LAB on the established biofilm at 37°C for 24 h. The CFUs were enumerated as described above

In vitro antimicrobial and antibiofilm activity of probiotic-incorporated fiber mats

Agar overlay assay. A small disk of LAB-incorporated fiber mat (0.6 cm diameter) was cut out, placed on MRS base agar and incubated at 37°C for 24 h. Following procedure was the same as described above.

In vitro biofilm inhibition by probiotics incorporated into the fiber mats. The biofilm inhibition experiment with ES LAB-incorporated fiber mats was set up as described above in *Biofilm inhibition assay*, with modification. 10 µL of the diluted pathogenic bacterial suspension was applied to the Gel-Glu fiber mat. LAB-incorporated fiber mat (1 × 1 cm) was placed on top of the Gel-Glu fiber mat and pathogenic bacteria suspension. Fiber mat without LAB was used

as control. To maintain moisture, a piece of hydrogel matrix (1 × 1 cm; HydroTac® transparent, Hartmann, Germany) was placed over the LAB-incorporated fiber mat. Finally, a cell culture insert was positioned above the system to hold all layers firmly in place within the microplate well. Samples were incubated for 24 h at 37°C. The hydrogel was discarded. Fiber mats with LAB were put in 1 × PBS buffer and gently vortexed. Then the samples were processed as described above in *Biofilm inhibition assay*.

Statistical analysis and creation of visuals

All experiments were performed using at least three independent biological replicates (N ≥ 3), each with three technical replicates. For statistical analysis, the mean of the three technical replicates was treated as a single biological replicate. Results are presented as the arithmetic mean ± standard deviation (SD) of the biological replicates. Parametric analyses were conducted under the assumption of normally distributed data. Unless otherwise stated, statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Bonferroni-adjusted multiple-comparison tests. Statistical significance thresholds were set at $P < 0.05$ (*) and $P < 0.01$ (**). Values below the limit of detection (LOD = 2.3) were assigned a value of 2.3 for statistical analysis. Associations between variables were assessed using Pearson's correlation analysis. The statistical analyses were performed, and graphs constructed using GraphPad Prism version 10.0.3 (GraphPad Software, USA, www.graphpad.com). Images were created with BioRender (www.biorender.com).

Results

Viability and morphology of the planktonic LAB and LAB-incorporated fiber mats

The morphology of the LAB cells pre-grown 24 h in MRS medium was analyzed by SEM, while the size and viability were additionally assessed using CLSM after staining with the

fluorescent dyes SYTO 9 and PI. *L. plantarum* Fibro 1 and *L. rhamnosus* Fibro 2 are Gram (+) rod-shaped bacteria. The morphology in these conditions shows that *L. plantarum* Fibro 1 is slightly larger compared to *L. rhamnosus* Fibro 2, with the lengths of 1.71 ± 0.35 µm and 1.39 ± 0.15 µm, and widths of 0.77 ± 0.21 µm and 0.52 ± 0.11 µm, respectively (Fig. 1a). Living probiotics showed fluorescence only in the green channel, while HT samples exhibited fluorescence in both the green and red channels. Hence, it was confirmed that probiotics are dead in the HT samples. No morphological differences were observed between HT (100 °C, 10 min) and untreated LAB cells in SEM analysis, suggesting that HT did not induce detectable surface structural alterations under the applied conditions (Fig. 1b).

The LAB-incorporated fiber mats exhibit smooth surfaces. SEM micrograph analysis shows that the fibers in both samples are randomly oriented, forming a network (Fig. 1c). The mean thickness of fiber mats was 0.15 mm. The mean fiber diameter was 0.49 ± 0.37 µm for the *L. plantarum*-incorporated fiber mat and 0.61 ± 0.40 µm for the *L. rhamnosus*-incorporated fiber mat. The pore sizes between the fibers appear heterogenous across the fiber mats, with mean pore surface areas of 0.9 ± 1.1 μm^2 and 0.8 ± 1.0 μm^2 for *L. plantarum* and *L. rhamnosus*, respectively, matching with previously reported values [31]. The average number of probiotics within the electrospun fiber mats was 3.0×10^5 per cm^2 for *L. plantarum* and 6.5×10^5 for *L. rhamnosus*, and viability was 28.8% and 50.9%, respectively.

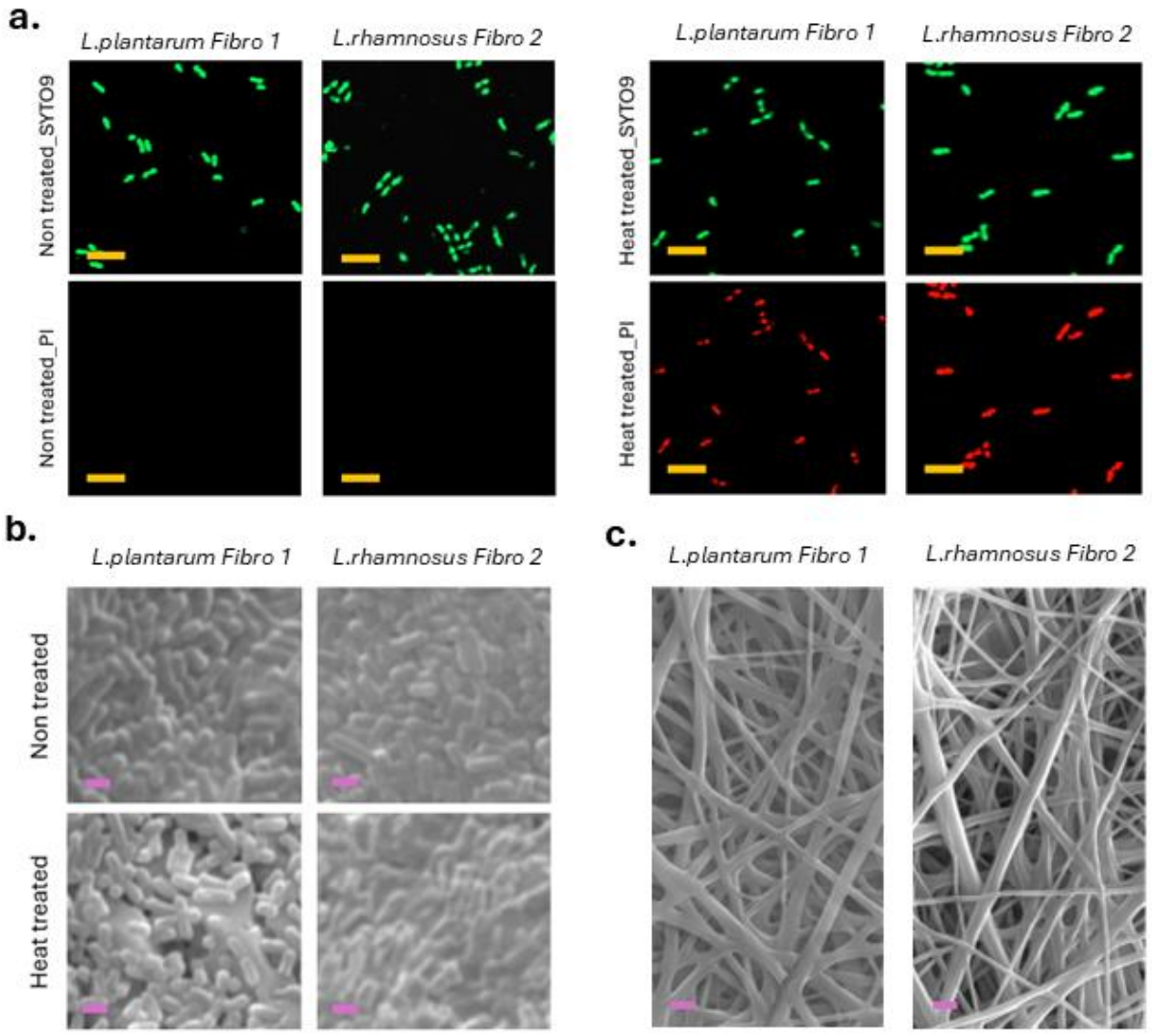
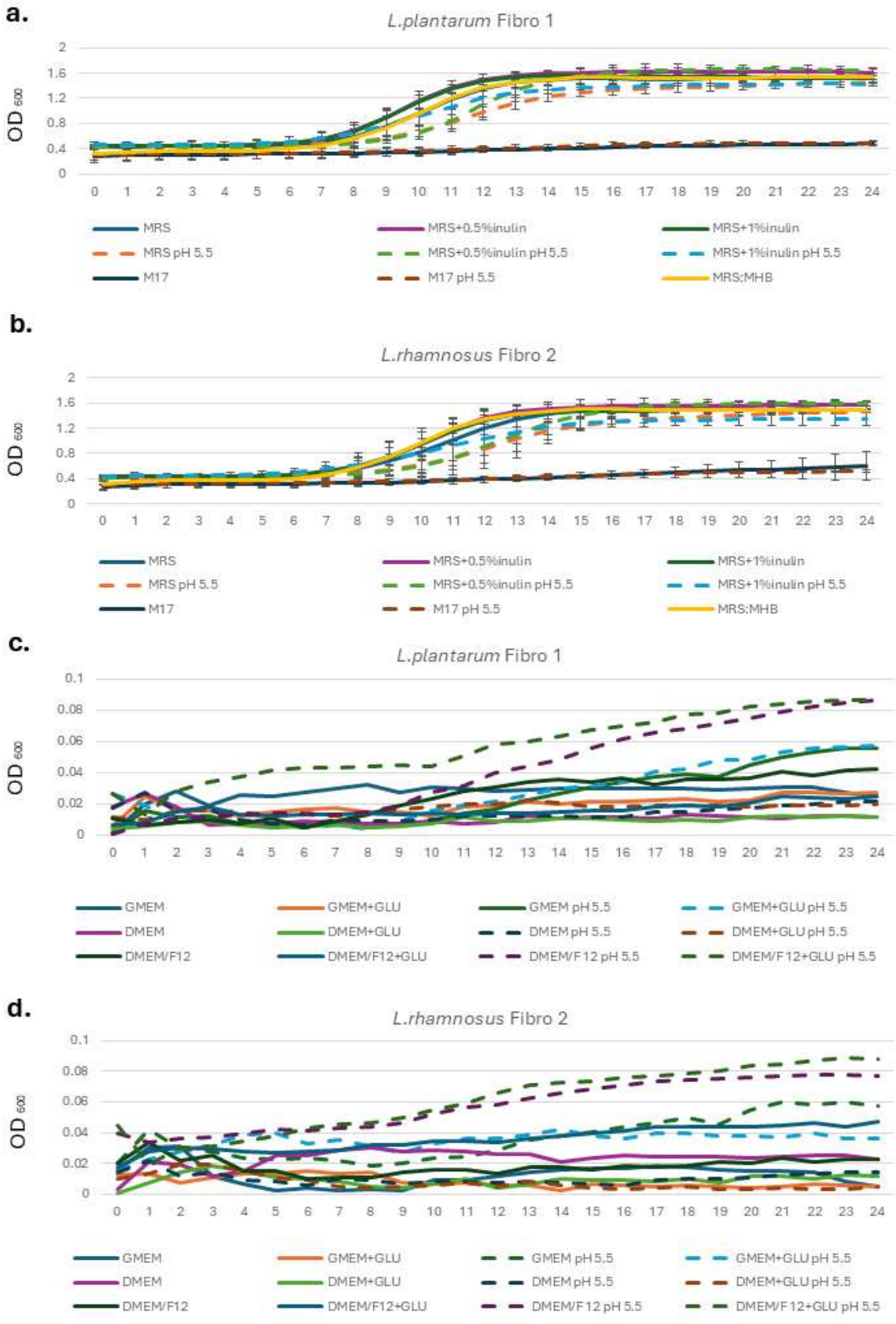


Fig. 1 ***a.*** *The confocal laser scanning microscope (CLSM) and* ***b.*** *scanning electron microscope (SEM) micrographs of heat-treated, HT (control samples), non-treated planktonic LAB strains (L. plantarum Fibro 1, L. rhamnosus Fibro 2) pre-grown in MRS for 24 h at 37°C. For SEM imaging the samples were taken up in distilled water.* ***c.*** *LAB-incorporated (L. plantarum Fibro 1, L. rhamnosus Fibro 2) fiber mats. Key: orange scale bar is 5 µm and purple scale bar is 2 µm*

Evaluation of LAB growth in different media

Before it is possible to evaluate the antimicrobial activity of probiotics in different environments, it is relevant to understand the growth of LAB in these different conditions. The growth kinetics of LAB was investigated in different growth media at 37 °C for 24 h. MRS

476 medium has been developed for the optimal growth of LAB [40], and for this reason it was
477 used as a control. M17 medium is a specialized growth medium developed for the cultivation
478 of *Lactococcus* species, especially *Lactococcus lactis* [41]. It was used as a lower-
479 supporting medium. MRS medium, whether modified or not, indeed supported robust
480 growth of lactobacilli (Figs. 2a and b).


a.
L.plantarum Fibro 1
OD 600
MRS
MRS+0.5%inulin
MRS+1%inulin
MRS pH 5.5
MRS+0.5%inulin pH 5.5
MRS+1%inulin pH 5.5
M17
M17 pH 5.5
MRS:MHB
b.
L.rhamnosus Fibro 2
c.
L.plantarum Fibro 1
GMEM
GMEM+GLU
GMEM pH 5.5
GMEM+GLU pH 5.5
DMEM
DMEM+GLU
DMEM pH 5.5
DMEM+GLU pH 5.5
DMEM/F12
DMEM/F12+GLU
DMEM/F12 pH 5.5
DMEM/F12+GLU pH 5.5
d.
L.rhamnosus Fibro 2

481

Fig. 2 Growth curves of lactic acid bacteria (LAB) in different growth media – ***a***. *L. plantarum Fibro 1 and* ***b.*** *L. rhamnosus Fibro 2 in bacterial growth media****,*** ***c.*** *L. plantarum Fibro 1 and* ***d.*** *L. rhamnosus Fibro 2 in cell culture media - with various modifications. Samples were incubated at 37°C for 24 h. Optical density at 600 nm (OD_{600}) was measured hourly. Experiments were performed in three biological replicates (N=3); the mean values were calculated. Key: GLU – glucose, dashed line – pH-adjusted medium (pH 5.5), solid line – unadjusted medium*

The pH of all growth media was measured before probiotic inoculation and after probiotic growth. All tested probiotics and media combinations, except for M17, demonstrated comparable acidification performance, which is mainly associated with lactic acid production. The initial pH of the growth media was approximately 5.5 (adjusted with HCl) or 6.5, whereas the final pH reached approximately 3.8 following growth of both LAB strains (Table S2). M17 medium proved largely unsuitable as a growth medium for tested probiotics, showing almost no change in optical density (Figs. 2a and b) nor significant pH changes throughout the experiment (Table S2). The pH remained relatively stable for both LAB strains with only minor variations compared to the initial values. Even in modified M17 medium with initial pH of 5.5, only a slight increase in pH was observed (Table S2). MRS 0.5% [w/v] inulin gave slightly higher growth speed for both bacterial strains (Figs. 2a and b). The logarithmic phase of *L. plantarum* Fibro 1 was between 6 and 12 h and *L. rhamnosus* Fibro 2 – between 7 and 13 h from the inoculation moment.

The growth of LAB was assessed not only in the optimal growth media (e.g. MRS) but also in cell culture media that more closely resemble physiological conditions of potential infection sites (Table 3).

Table 3 *The measured pH of different cell culture media with and without modifications and viable cell concentration of lactic acid bacteria (LAB) before and after incubation at 37°C for 24 h, aerobically with an initial inoculum concentration of 10^6 CFU/mL (N=3). Further details on the different media and experimental conditions are provided in the section "Growth kinetics of probiotics in different growth media" (Table 1)*

Cell culture media		***L. plantarum***	***L. rhamnosus***

	Initial pH	Final pH	Final log_{10} (CFU/mL)	Final pH	Final log_{10} (CFU/mL)
GMEM	8.90	9.00 ± 0.35	5.13 ± 0.76	8.95 ± 0.24	5.36 ± 1.08
GMEM+GLU	8.23	8.99 ± 0.32	4.30 ± 0.10	8.96 ± 0.25	5.16 ± 1.22
GMEM pH 5.5	5.59	6.75 ± 2.10	5.73 ± 1.60	6.82 ± 1.80	6.53 ± 0.19
GMEM+GLU pH 5.5	5.58	4.57 ± 0.13	7.59 ± 0.90	8.51 ± 0.64	5.98 ± 0.32
DMEM	8.12	9.32 ± 0.02	5.19 ± 0.31	9.32 ± 0.01	4.51 ± 0.37
DMEM+GLU	7.87	8.90 ± 0.03	5.14 ± 0.66	9.30 ± 0.01	4.95 ± 0.60
DMEM pH 5.5	5.58	8.90 ± 0.03	5.14 ± 0.66	8.90 ± 0.05	5.28 ± 0.87
DMEM+GLU pH 5.5	5.56	8.80 ± 0.15	5.38 ± 1.20	8.74 ± 0.13	5.27 ± 0.82
DMEM/F12	7.54	8.46 ±0.05	4.62 ± 0.45	8.49 ± 0.01	5.50 ± 0.89
DMEM/F12+GLU	7.44	8.47 ± 0.02	4.41 ± 0.16	8.46 ± 0.01	5.02 ± 0.95
DMEM/F12 pH 5.5	5.57	5.45 ± 0.36	8.45 ± 1.42	5.11 ± 0.52	7.97 ± 0.51
DMEM/F12+GLU pH 5.5	5.54	5.40 ± 0.54	7.80 ± 0.14	5.09 ± 0.36	8.36 ± 1.06

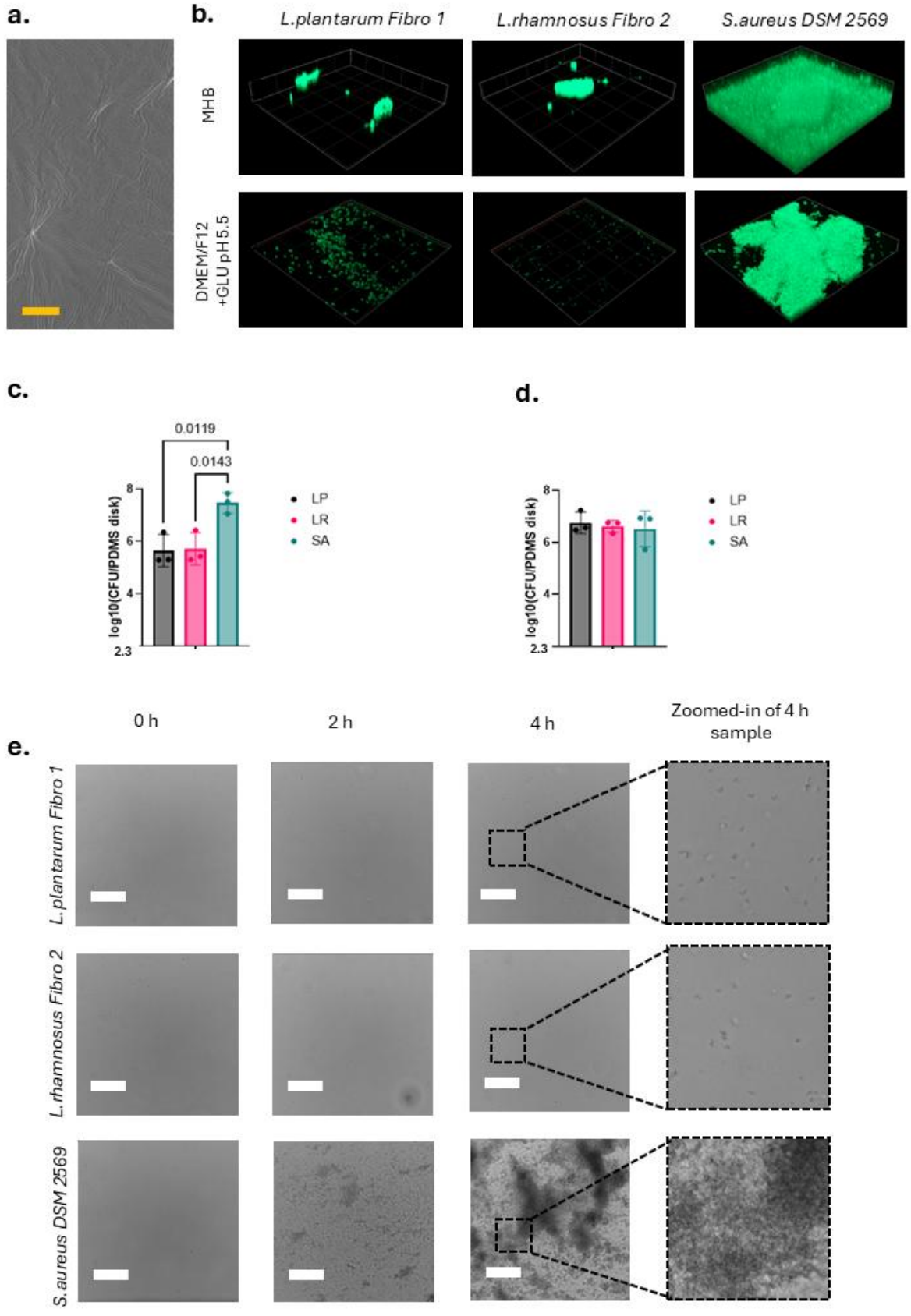
Key: CFU- colony forming unit; GLU- glucose

LAB are fastidious organisms that require complex, nutrient-rich media [42] and do not grow efficiently in media with limited nutrient composition, such as serum-supplemented mammalian cell culture media. The tested media included GMEM, DMEM, and DMEM/F12, along with their respective modifications (s.f. Table 1 for exact names and media compositions). Although these media are not designed to support the growth of LAB strains due to their limited nutrient content, they were selected for their relevance to the wound environment. As expected, the growth in cell culture media was slower compared to MRS, given that it is not optimized for LAB cultivation (Figs. 2c and d). Nonetheless, a measurable increase in both optical density and viable cell count was observed. The growth dynamics of LAB strains in cell culture media were monitored over time using OD_{600} measurements and CFU/mL counts. Growth varied significantly depending on medium composition and initial pH (Figs. 2c and d). The results demonstrate that 24 h later, the highest OD_{600} was detected in DMEM/F12 pH 5.5 and DMEM/F12+GLU pH 5.5 media for both LAB strains. These media

also yielded the highest viable bacterial concentrations for both LAB strains. Viable counts of *L. plantarum* reached 8.45 ± 1.42 and 7.80 ± 0.14 $\log_{10}$(CFU/mL), whereas *L. rhamnosus* reached 7.97 ± 0.57 and 8.36 ± 1.06 $\log_{10}$(CFU/mL) in the respective media. A high viable cell concentration was detected for *L. plantarum* in GMEM+GLU pH 5.5 medium. At the same time, this medium did not support the growth of *L. rhamnosus* (Table 3). The acidification observed in the cell culture media was considerably weaker than in the bacterial growth medium. The pH of DMEM/F12+GLU (initial pH 5.5) changed only slightly after incubation with either LAB strain (Table 3). Moreover, GMEM- and DMEM-based media, with or without supplementation, generally did not support LAB growth, as evidenced by the absence of a substantial decrease in pH and no increase in viable bacterial counts (Table 3). Overall, lowering the initial pH of mammalian cell culture media alone was insufficient to support the growth of LAB, indicating that nutrient composition rather than acidity is the primary determinant of bacterial proliferation. Although, a strong negative correlation was observed between bacterial growth and final pH (Pearson`s r = -0.93, p = 4.23 x 10^{11}), indicating that greater bacterial growth was associated with stronger acidification of the medium.

Biofilm formation capability of LAB on PDMS

The droplet biofilm method was used to assess the intrinsic biofilm-forming ability of the probiotics and its potential contribution to their persistence on surfaces. The PDMS disks used for this model setup displayed smooth and uniform surface morphology. SEM analysis revealed only minor irregularities, suggesting low surface roughness, which could impact initial bacterial adhesion and subsequent biofilm development (Fig. 3a).


a.
b.
L.plantarum Fibro 1
L.rhamnosus Fibro 2
S.aureus DSM 2569
MHB
DMEM/F12 +GLU pH 5.5
c.
0.0119
0.0143
log10(CFU/PDMS disk)
8
6
4
2.3
LP
LR
SA
d.
log10(CFU/PDMS disk)
8
6
4
2.3
LP
LR
SA
e.
0 h
2 h
4 h
Zoomed-in of 4 h sample
L.plantarum Fibro 1
L.rhamnosus Fibro 2
S.aureus DSM 2569

548

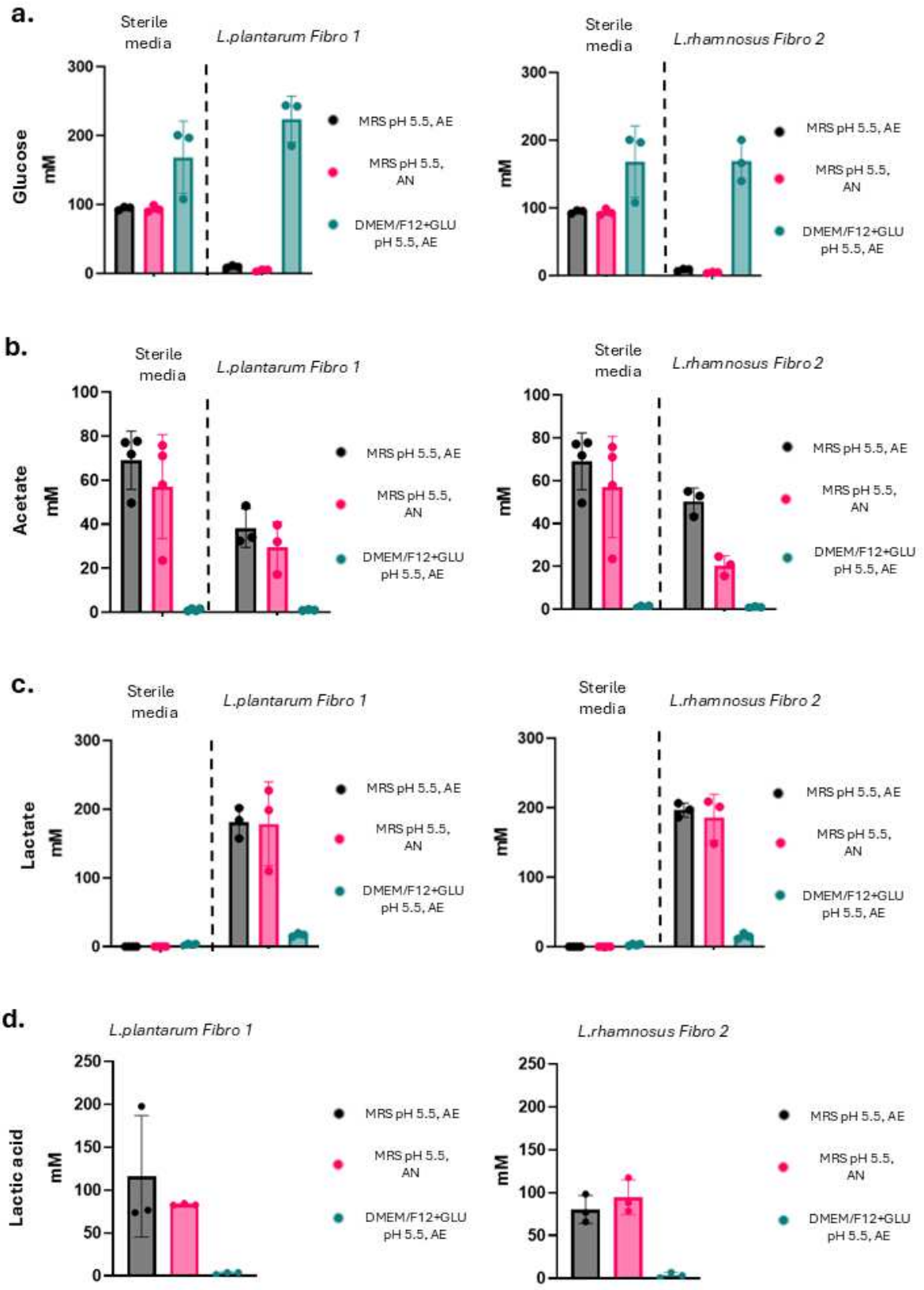
Fig. 3 ***a****. SEM micrograph of the PDMS disk surface.* ***b****. CLSM micrographs of adhered LAB strains and S. aureus on PDMS disk surfaces under static conditions in* DMEM/F12+GLU pH 5.5 medium*, stained with SYTO 9. The concentration of LAB and S. aureus adhered to the PDMS disks in* ***c.*** *MHB (N=3). and* ***d.*** *DMEM/F12+GLU pH 5.5 media (N=3) under static conditions after 24 h.* ***e.*** *The adhesion of bacteria in* DMEM/F12+GLU pH 5.5 *medium in flow-through system at 0, 2 and 4 h. Square inside the dashed lines: zoomed-in view of the microscope micrograph at the 4 h time point. Key: LP – L. plantarum Fibro 1, LR – L. rhamnosus Fibro 2, SA – S. aureus DSM2569. P values for statistically significant differences are indicated above the brackets. Orange scale bar – 2 µm, white scale bar – 100 µm; size of the zoomed-in images 100 µm x 100 µm*

The LAB ability to form biofilm was conducted on an abiotic surface – PDMS disks in two different media – MHB and DMEM/F12+GLU pH 5.5. *S. aureus* was used as a control bacterium, as it is known to form biofilms under various environmental conditions and surfaces [3, 43]. The CLSM results showed that in MHB medium, LAB formed only a few isolated 3D aggregates (Fig. 3b). In contrast, in DMEM/F12+GLU pH 5.5 medium the surface was evenly covered by a monolayer of bacteria, with no substantial aggregate formation. *S. aureus* strain formed much thicker biofilm in both growth media (Fig. 3b). While *L. plantarum* and *L. rhamnosus* exhibited similar adhesion capacities, with $\log_{10}$ CFU values/per disk of 5.65 ± 0.61 and 5.72 ± 0.61, respectively, *S. aureus* reached a substantially higher value of 7.46 ± 0.39 (Fig. 3c). Differences between the strains are statistically significant. In DMEM/F12+GLU pH 5.5 medium, biofilm formation by *S. aureus* and probiotic strains is comparable, indicating that nutrient limitation reduces species-dependent differences (Fig. 3d). Also, differences between microscopy and CFU results may be related to recovery of loosely associated cells during sonication and variability introduced during the washing step. In addition to CFU plating, crystal violet (CV) staining was conducted from PDMS droplet biofilm model (Supplementary information) and these results confirmed the CFU ones obtained (Fig. S1). CV staining (Fig. S1) and CFU measurements consistently showed that *S. aureus* formed markedly more biofilm biomass in MHB medium than the tested probiotic strains.

To further investigate the biofilm-forming ability of the probiotics under dynamic conditions, a flow-through microfluidic chamber model on PDMS surface was used, with *S. aureus* serving as a biofilm-forming control. Bacterial cells resuspended in DMEM/F12+GLU pH 5.5 medium with OD_{600} 0.1 were injected into the microfluidic chip. Both tested probiotic strains exhibited similar surface adhesion; however, the adhesion was minimal and randomly distributed compared to *S. aureus*. Pathogenic *S. aureus* bacteria adhered to the PDMS surface faster than the probiotics (Fig. 3e), suggesting it forms biofilms more rapidly, as adhesion is the initial step in biofilm formation.

Determination and quantification of the produced and consumed metabolites

To investigate how the growth environment affects substrate utilization and metabolic activity, glucose, acetate, and lactate concentrations were measured in MRS and DMEM/F12 + GLU pH 5.5 media. Quantitative NMR analysis showed that both substrate consumption and metabolite production were dependent on the growth medium and the LAB strain (Figs. 4a, b and c). In MRS, glucose concentrations decreased after cultivation with LAB strains compared to the sterile medium, indicating active carbohydrate utilization. By contrast, glucose concentrations remained relatively high in DMEM/F12 +GLU pH 5.5, suggesting considerably lower glucose utilization under these conditions (Fig. 4a). Acetate concentrations were also higher in MRS samples than in DMEM/F12 +GLU pH 5.5. The consumption of acetate by *L. plantarum* was higher than for *L. rhamnosus* (Fig. 4b).


a.
Sterile media
L.plantarum Fibro 1
L.rhamnosus Fibro 2
Glucose
mM
300
200
100
0
MRS pH 5.5, AE
MRS pH 5.5, AN
DMEM/F12+GLU pH 5.5, AE
b.
Acetate
100
80
60
40
20
c.
Lactate
d.
Lactic acid
250
150
50

602

Fig. 4 Quantitative analysis of substrates and metabolites in different growth media with and without lactic acid bacteria (LAB) strains. *NMR results revealed* ***a****. the concentration of glucose and* ***b****. acetate and* ***c****. lactate in different growth media with or without LAB strains.* ***d****. Results of spectrophotometric quantification assay demonstrating the concentration of lactic acid in different growth media after incubation of LAB strains. Experiments were performed in three biological replicates (N=3). For clarity, statistical comparisons are reported separately in Supplementary Table S3*

Glucose consumption from the media reflected the lactate production patterns. Lactate was detected at the highest concentrations in samples grown in MRS medium under aerobic conditions for both *L. plantarum* and *L. rhamnosus* – 181.08 ± 22.20 mM and 196.64 ± 10.02 mM, respectively. Under anaerobic conditions, the concentration of produced lactate was only slightly lower – 178.56 ± 61.24 mM for *L. plantarum* and 186.21± 33.13 mM for *L. rhamnosus*. In contrast, only low amounts of lactate were detected in DMEM/F12 +GLU pH 5.5 – 17.13 ± 2.57 mM for *L. plantarum* and 15.23 ± 4.12 mM for *L. rhamnosus* (Fig. 4c).

Using a spectrophotometric quantification method, it was established that both LAB produce lactic acid in different growth media. Supporting the NMR results, the lactic acid concentration was the highest in MRS medium under aerobic conditions for both *L. plantarum* and *L. rhamnosus* – 112.79 ± 73.70 mM and 80.60 ± 16.43 mM, respectively (Fig. 4d). Under anaerobic conditions, the concentrations were slightly lower. The lowest concentrations were detected in DMEM/F12+GLU pH 5.5 medium – 3.17 ± 1.72 mM for *L. plantarum* and 3.84 ± 3.23 mM for *L. rhamnosus*. It was confirmed that HT LAB samples did not contain any lactic acid.

Antimicrobial activity of probiotics in agar overlay assay

The effect of the LAB pre-growth environment on antimicrobial activity was evaluated using an agar overlay assay against wound-associated pathogens (*Escherichia coli* DSM 1103, *Staphylococcus aureus* DSM 2569, *Pseudomonas aeruginosa* DSM 1117 and PAO1, and *Staphylococcus epidermidis* DSM 28319; Fig. S2). Neither oxygen availability during pre-incubation nor pre-incubation temperature (30–37 °C) significantly affected antimicrobial

activity against *E. coli* or *S. aureus* (Figs. 5a–d), demonstrating that the antimicrobial efficacy of both probiotic strains remained stable under the tested conditions. Longer incubation increased inhibition zone size (Fig. S3), and both strains inhibited multiple wound-associated pathogens (Fig. S4). HT controls showed no antimicrobial activity (Fig. S5), confirming that inhibition depends on viable, metabolically active cells. Addition of the prebiotic inulin to the base agar did not enhance antimicrobial activity (Fig. S6).

LAB typically exhibits optimal growth within a pH range of 5.5 - 6.5 [25, 44–47]. To evaluate the impact of environmental pH, LAB were pre-cultured on base agars adjusted to pH values of 5.5, 6.0, 6.5, 7.0 and 7.5. Lower pH conditions significantly enhanced antimicrobial activity, as evidenced by larger inhibition zones (Figs. 5e and f). The strongest inhibitory effects were observed at pH 5.5 against *E. coli*, *S. aureus*, and *P. aeruginosa*, whereas increasing the pH to 7.5 resulted in a significant reduction in inhibition zone size. The differences observed were statistically significant.

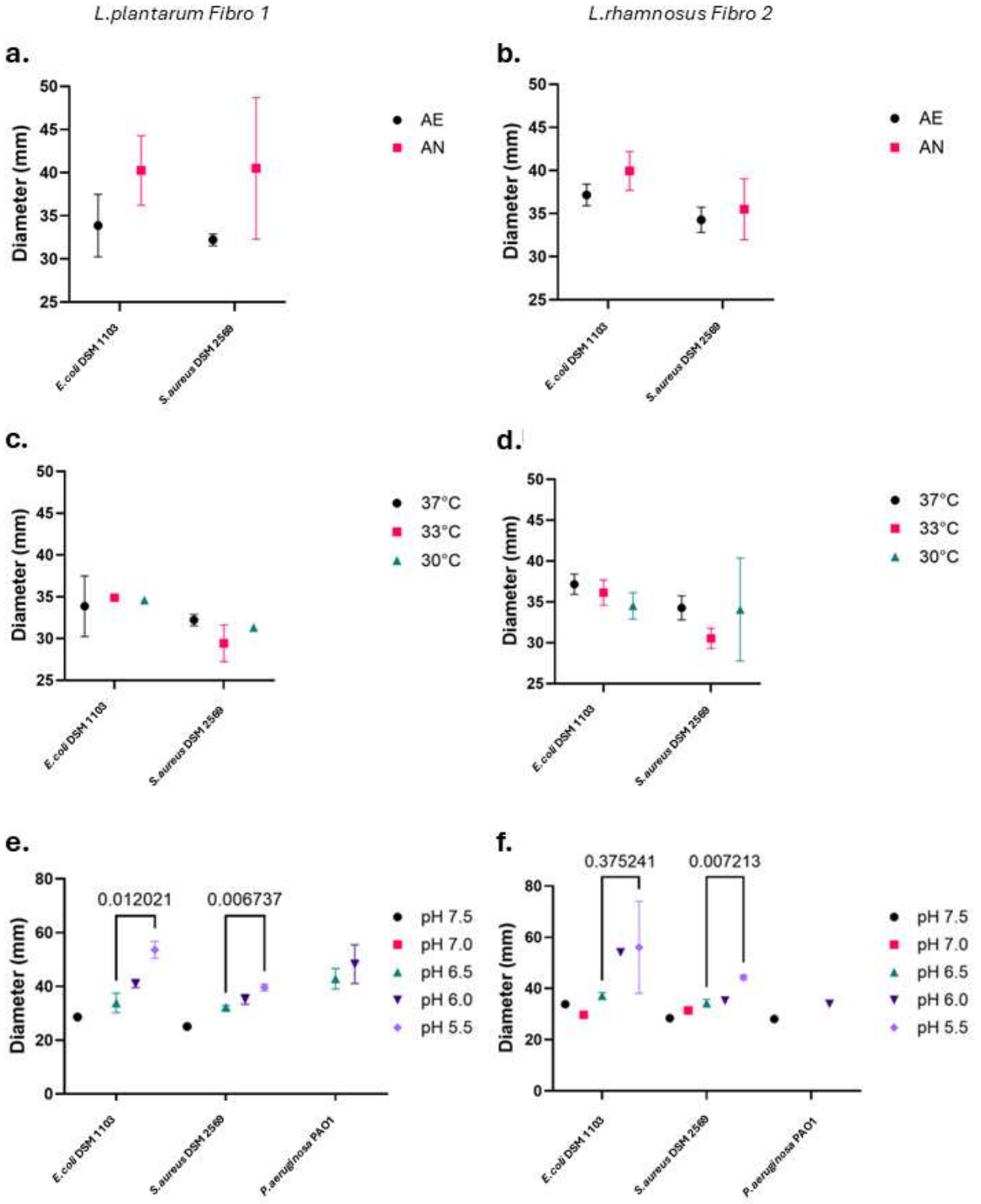
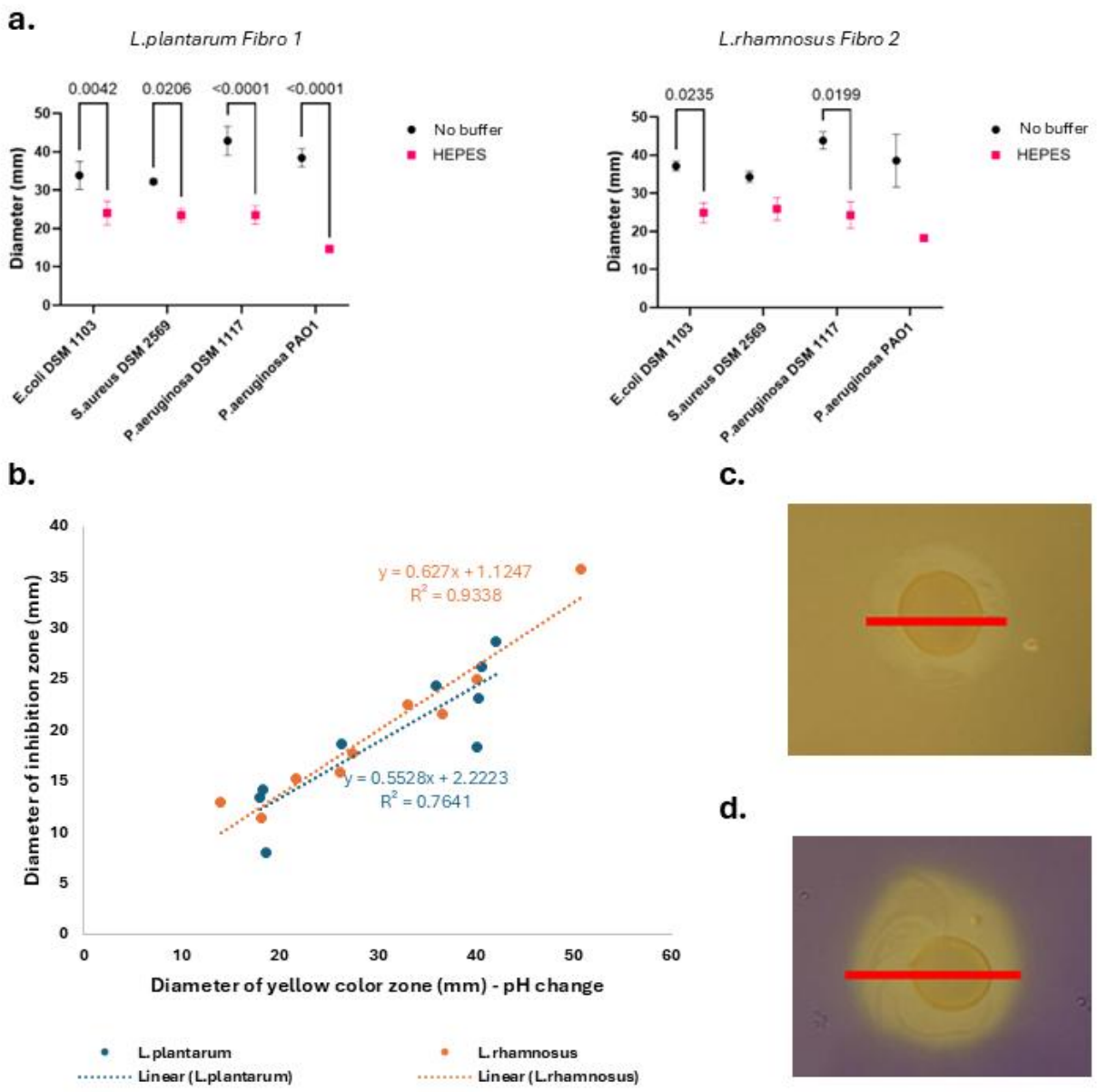


Fig. 5 Effect of pre-incubation and environmental conditions on the antimicrobial activity of probiotic strains grown on MRS base agar. *Effect of aerobic and anaerobic pre-incubation on the antimicrobial activity of* ***a.*** *L. plantarum Fibro 1 (LP) and* ***b.*** *L. rhamnosus*

Fibro 2 (LR) against E. coli DSM 1103 and S. aureus DSM 2569 at 37°C. No statistically significant differences in inhibition zone diameter were observed between aerobic and anaerobic conditions. Effects of pre-incubation temperature on the antimicrobial activity of ***c.*** *LP and* ***d.*** *LR against E. coli DSM 1103 and S. aureus DSM 2569 at aerobic conditions. Effects of MRS base agar pH on antimicrobial activity of* ***e.*** *LP and* ***f.*** *LR against E. coli DSM 1103, S. aureus DSM 2569, and P. aeruginosa DSM 1117 at 37°C under aerobic conditions. Antimicrobial activity increased with decreasing pH. Key: AE – aerobic environment, AN – anaerobic environment. Data are presented as mean ± SD of biological replicates (N ≥ 3). Statistical significance was assessed using two-way ANOVA, followed by Bonferroni corrections. P values for statistically significant differences are indicated above the brackets.*

To determine the mechanisms underlying the observed antimicrobial effect – specifically whether it resulted from acid production by LAB rather than another mechanism of action – HEPES buffer (100 mM, pH 7.5) was added to the soft agar [48]. In addition, the pH of the base agar was varied to identify optimal conditions to produce antimicrobial compounds. The addition of HEPES reduced the antimicrobial activity, supporting the conclusion that at least part of the observed effect was attributable to acidification (Fig. 6a).



669

670 **Fig. 6** ***a.*** *Comparison of antimicrobial activity of L. plantarum Fibro 1 (LP) and L. rhamnosus*
671 *Fibro 2 (LR) at 37°C with and without HEPES buffer (pH 7.5), aerobically. Results confirm the*
672 *partial dependence of antimicrobial activity on acid production.* ***b.*** *Relationship between the*
673 *antimicrobial activity against E. coli DSM 1103 (diameter of inhibition zone, mm) and the*
674 *environmental pH (diameter of yellow color zone, mm). Blue dots represent LP and orange*
675 *dots represent LR. Dotted lines indicate linear regression fits. Stronger correlation was*
676 *observed for LR (R^2 = 0.9338) compared to LP (R^2 = 0.7652).* ***c.*** *Top agar with pathogenic*
677 *bacteria, without the pH indicator (for inhibition zone measurements).* ***d.*** *Top agar without*
678 *pathogenic bacteria, with the pH indicator (for pH change zone measurements). Key: HEPES*
679 *- HEPES buffer in the top agar, red line - inhibition zone size (diameter, mm). Data are*

presented as mean ± SD of three biological replicates (N = 3). Statistical significance was assessed using two-way ANOVA, followed by Bonferroni corrections. P values for statistically significant differences are indicated above the brackets

Initially, HEPES buffer was applied to limit pH changes. However, inhibition zones were still observed, indicating that buffering was insufficient to fully prevent acidification (Fig. S7). To achieve stronger pH control, Tris buffer at different concentrations was used for further experiments. To better understand the impact of environmental pH on probiotic antimicrobial activity, the top agar was supplemented with varying concentrations of Tris buffer (100 mM, 200 mM and 300 mM, pH 7.5, pH 8.0 and pH 8.8). A pH indicator was used to visualize pH changes, indicated by the diameter of the yellow zone. These were then compared with the inhibition zones formed in identical buffer conditions but inoculated with probiotics and pathogenic bacteria (*E. coli* DSM 1103) (Table S1). The incubation took place at 37°C for 24 h aerobically. As shown in Fig. 6b, a clear correlation was observed between the diameter of the yellow zone (indicative of acidification) and the diameter of the inhibition zone. *L. rhamnosus* Fibro 2 exhibited a strong linear relationship $R^2 = 0.9338$, suggesting that acid production plays a relevant role in its antimicrobial mechanism. *L. plantarum* Fibro 1 also demonstrated a positive correlation, although the data had more variation showing $R^2 = 0.7641$. These results support the statement that the antimicrobial effectiveness of LAB strains is closely linked to their ability to acidify the environment. This acidification appears to enhance their inhibitory impact on pathogenic bacteria, indicating the importance of environmental pH in LAB-pathogen interactions.

Antibiofilm activity of probiotics in *in vitro* biofilm assay

The antimicrobial activity of LAB against planktonic pathogens was initially evaluated (Fig. S8). Although LAB strains inhibited planktonic growth under certain conditions, the main objective of this study was to determine their antibiofilm efficacy, including inhibition of biofilm formation and treatment of mature biofilms. Antibiofilm activity of LAB strains was evaluated in two *in vitro* biofilm experiments using DMEM/F12+GLU (pH 5.5), which

prevented degradation of the Gel-Glu matrix by *P. aeruginosa* PAO1 (Fig. S9). First, the ability of probiotics to prevent the formation of *P. aeruginosa* PAO1 and *S. aureus* biofilms was assessed. The formation of *P. aeruginosa* biofilm was inhibited very effectively, resulting in approximately 5 $\log_{10}$ reduction in the presence of LAB ($p < 0.0001$) (Fig. 7a). Both LAB strains also significantly inhibited the biofilm development of *S. aureus*, resulting in a 2.5 $\log_{10}$ reduction compared with the control ($p < 0.0004$) (Fig. 7a). Next, the treatment effect of probiotics on pre-grown biofilm was examined. In a smaller volume of DMEM/F12+GLU pH 5.5 medium (250 µL) the number of pre-grown *P. aeruginosa* biofilm bacteria decreased by 1.5 $\log_{10}$ and 2 $\log_{10}$ in the presence of *L. plantarum* and *L. rhamnosus*, respectively ($p < 0.02$). The treatment of mature biofilm of *S. aureus* with LAB led to 1.5 $\log_{10}$ reduction, which was also statistically significant ($p < 0.0009$) (Fig. 7b). At the same time, the treatment of the mature biofilm in the larger volume of liquid media (500 µL) did not show any significant changes in the number of biofilm bacteria (Fig. S10).

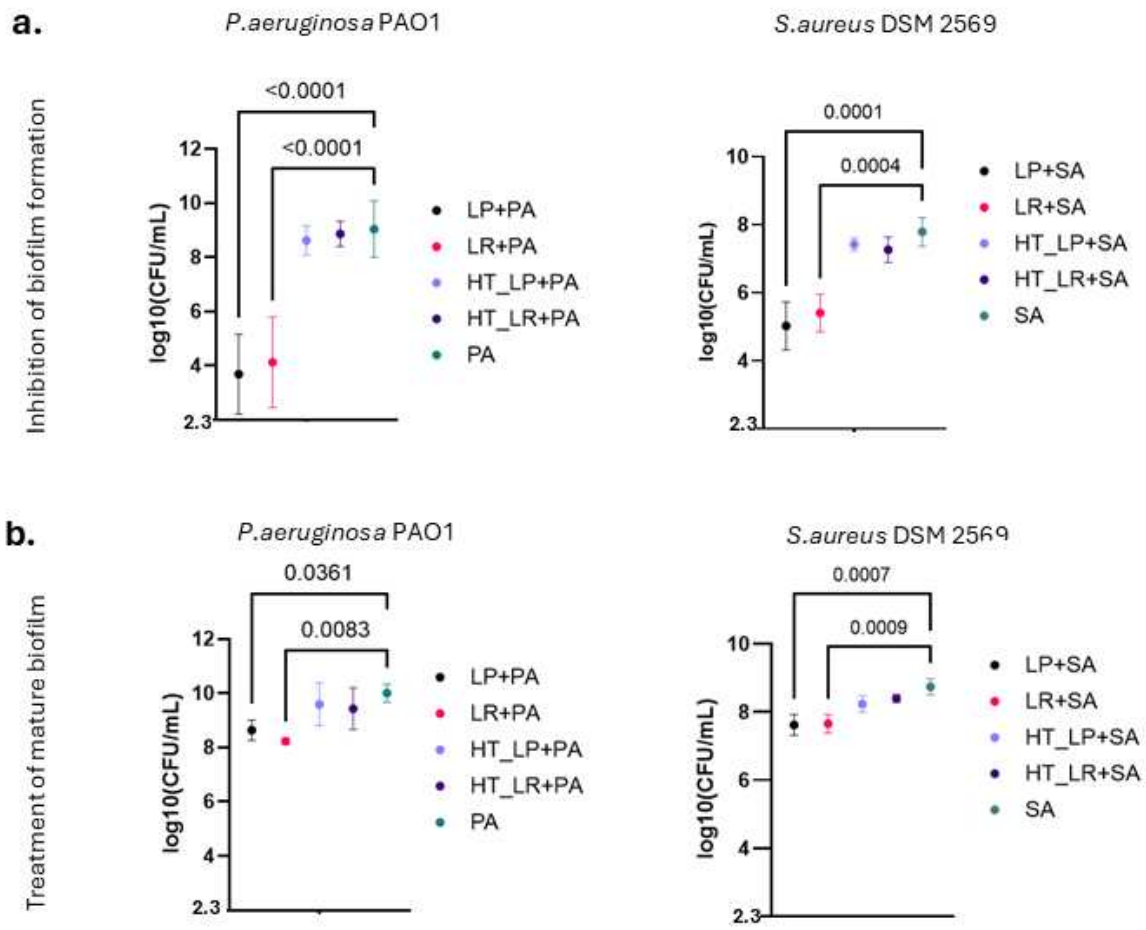


Fig. 7 ***a.*** *The antibiofilm activity of probiotics* in *DMEM/F12+GLU pH 5.5 medium against the biofilm formation and* ***b.*** *mature biofilm of P. aeruginosa PAO1 and S. aureus DSM 2569. Data are presented as mean ± SD of three biological replicates (N = 3). P values for statistically significant differences are indicated above the brackets. Key: HT - heat treated, LP - L. plantarum Fibro 1, LR - L. rhamnosus Fibro 2, PA - P. aeruginosa PAO1, SA - S. aureus DSM 2569. 2.3 log_{10} is the LOD*

Antibiofilm activity of probiotics in PDMS droplet biofilm assay

Similarly to the *in vitro* biofilm assay, two sets of experiments were conducted in PDMS droplet biofilm assay. It was seen in biofilm inhibition experiments that the co-cultivation of pathogenic bacteria with probiotics resulted in a significant reduction of *S. aureus* biomass in MHB (Fig. 8a). *L. rhamnosus* had stronger antimicrobial activity against *S. aureus* with decrease in 1.5 log_{10} CFU, at the same time *L. plantarum* resulted only in 1 log_{10} CFU decrease (Fig. 8A). The inhibitory effect of probiotics was lost in DMEM/F12+GLU pH 5.5

medium, there were no statistically significant differences between incubation of *S. aureus* alone or with probiotics (Fig. 8b).

The treatment of mature *S. aureus* biofilm by probiotics on PDMS surface was conducted in MHB. There was a slight trend towards CFU reduction, but results between different samples do not show any significant differences (Fig. 8c). In DMEM/F12+GLU pH 5.5 medium there were no statistically significant differences between treated and untreated samples (Fig. 8d).

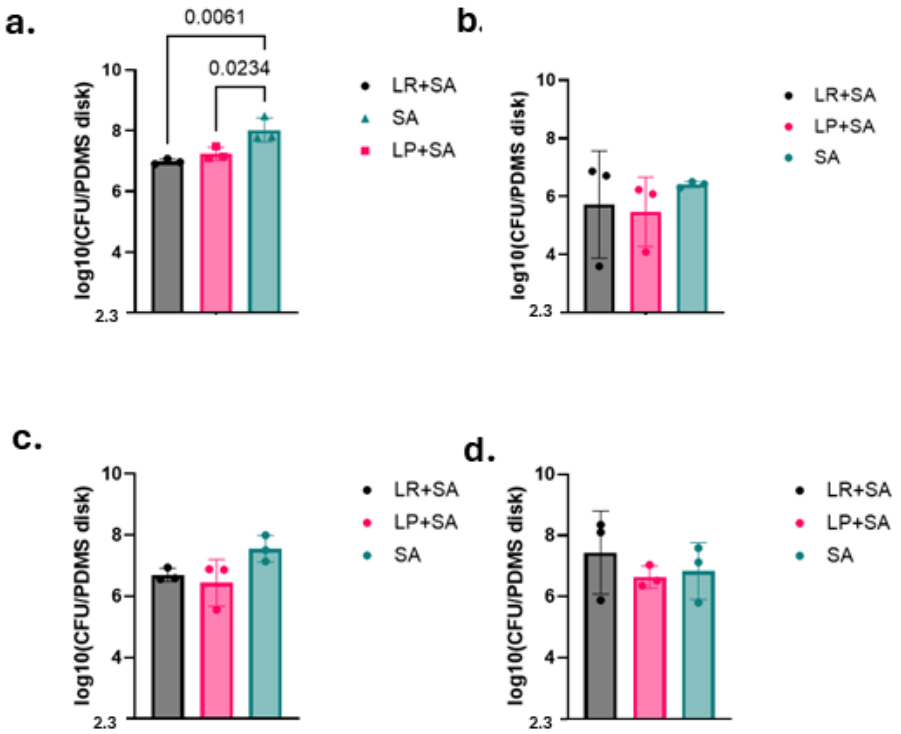


Fig. 8 ***Biofilm inhibition and treatment of probiotics in the PDMS droplet biofilm assay after 24 h in MHB and DMEM/F12+GLU pH 5.5 media.*** *The biofilm inhibition of S. aureus by LAB in* ***a.*** *MHB and* ***b.*** DMEM/F12+GLU pH 5.5. *The antibiofilm activity against mature S. aureus DSM2569 biofilm in* ***c.*** *MHB and* ***d.*** DMEM/F12+GLU pH 5.5 *media. Data are presented as mean ± SD of biological replicates (N = 3). P values for statistically significant differences are indicated above the brackets. 2.3* $\log_{10}$ *is the LOD. Key: LP - L. plantarum Fibro 1, LR - L. rhamnosus Fibro 2, SA - S. aureus DSM 2569*

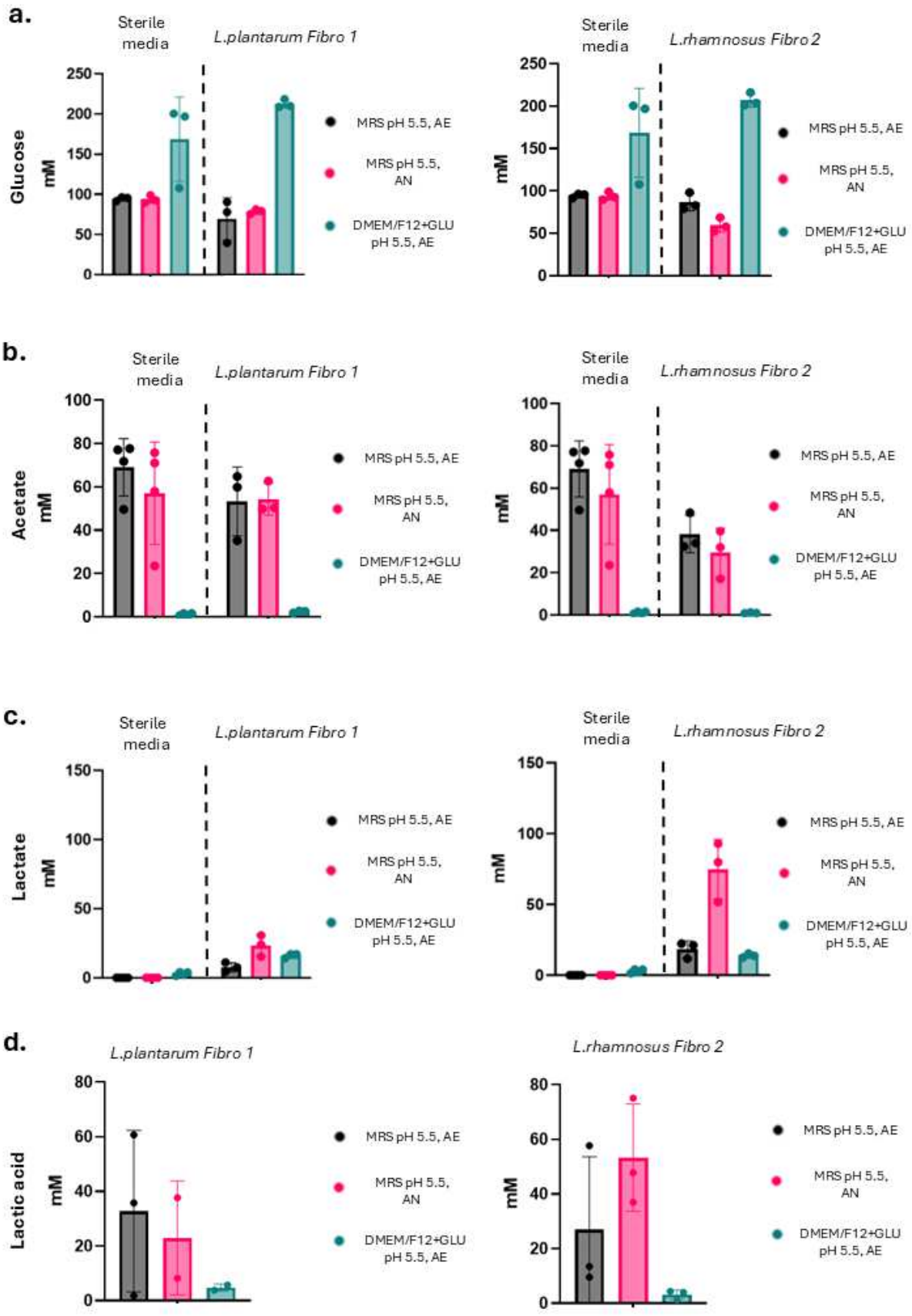
As with the biofilm formation assay in the PDMS droplet biofilm model, CV staining was also employed to evaluate biofilm inhibition and treatment efficacy. However, the results exhibited considerable variability between biological replicates, and no statistically significant differences were observed between the probiotic strains and *S. aureus* with respect to biofilm inhibition or treatment outcomes (Fig. S1).

Electrospun probiotic-incorporated fiber mat antimicrobial and antibiofilm activity *in vitro*

Fiber-incorporated LAB were able to consume substrates from the environment and produce metabolites. The NMR quantification assay revealed that both fiber-incorporated LAB strains were able to consume glucose in MRS medium, which indicates active carbohydrate utilization (Fig. 9a). The acetate consumption was higher by the L. *rhamnosus*, than by *L. plantarum* in MRS medium under both conditions (Fig. 9b). The NMR quantification assay revealed that fiber-incorporated probiotics were also able to produce and release lactate into the surrounding environment. The concentration of lactate in samples containing fiber mats was lower (Fig. 9c) compared to the samples with free (planktonic) bacteria (Fig. 4c). The experimental setup was designed to provide comparable numbers of viable probiotics in the free-probiotic and probiotic-incorporated fiber mat samples (approximately 10^6 CFU). However, variability in viable cell numbers was observed in the fiber mat samples, likely due to the heterogeneous distribution of probiotics within the fiber mats. In MRS medium, fiber-incorporated *L. plantarum* produced lower amounts of lactate under anaerobic conditions compared with fiber-incorporated *L. rhamnosus*, reaching 23.34 mM and 74.79 mM, respectively. Under aerobic conditions, the difference between LAB strains was reduced with lactate concentrations of 7.72 mM and 18.39 mM, respectively. In the DMEM/F12 +GLU pH 5.5 medium, lactate production remained low despite the availability of glucose.

The spectrophotometric quantification assay showed similar trend to NMR analysis confirming specifically lactic acid production by fiber-incorporated LAB strains (Fig. 9d). Lactic acid concentrations were 32.79 ± 29.53 mM and 26.91 ± 26.73 mM under aerobic

781 conditions and 15.30 ± 19.82 mM and 53.29 ± 19.66 mM under anaerobic conditions, for *L*.
782 *plantarum* and *L*. *rhamnosus*, respectively. In DMEM/F12+GLU pH 5.5 medium, no
783 significant difference was detected between LAB samples, the values were 3.36 ± 1.30 mM
784 and 3.84 ± 3.23 mM, respectively.


a.
Sterile media
L.plantarum Fibro 1
L.rhamnosus Fibro 2
Glucose
mM
MRS pH 5.5, AE
MRS pH 5.5, AN
DMEM/F12+GLU pH 5.5, AE
b.
Acetate
c.
Lactate
d.
Lactic acid

785

Fig. 9 Quantitative analysis of metabolites in different growth media with and without lactic acid bacteria (LAB)-incorporated fiber mats. *Results of NMR reveal:* ***a.*** *the concentration of glucose and* ***b****. acetate and* ***c****. lactate in different growth media with or without LAB-incorporated fibers.* ***d.*** *Results of spectrophotometric quantification assay demonstrating the concentration of lactic acid in different growth media after incubation of LAB-incorporated fibers. Data are presented as mean ± SD of three biological replicates (N = 3). For clarity, statistical comparisons are reported separately in Supplementary Table S4. Key: AE- aerobic; AN- anaerobic; DMEM/F12* - Dulbecco's Modified Eagle Medium and Ham's F-12 Nutrient Mixture*; GLU- glucose; MRS* - De Man–Rogosa–Sharpe broth

LAB-incorporated fiber mats were used to evaluate the ability of incorporated probiotics to inhibit the growth of pathogenic bacteria. The results indicate that the fiber mats containing *L. rhamnosus* had greater antimicrobial activity compared to those containing *L. plantarum* (Fig. 10a). However, the variability of the results was observed, which may be attributed to the inhomogeneous distribution of probiotics within the fiber mats which should be considered when developing LAB incorporated fiber mats.

LAB-incorporated fiber mats were used in biofilm inhibition and treatment models similarly as free probiotics to investigate the influence of incorporation process on the antimicrobial activity. Results showed that LAB retain their antimicrobial and antibiofilm activity in the fibers against *P. aeruginosa* PAO1 (Fig. 10b).

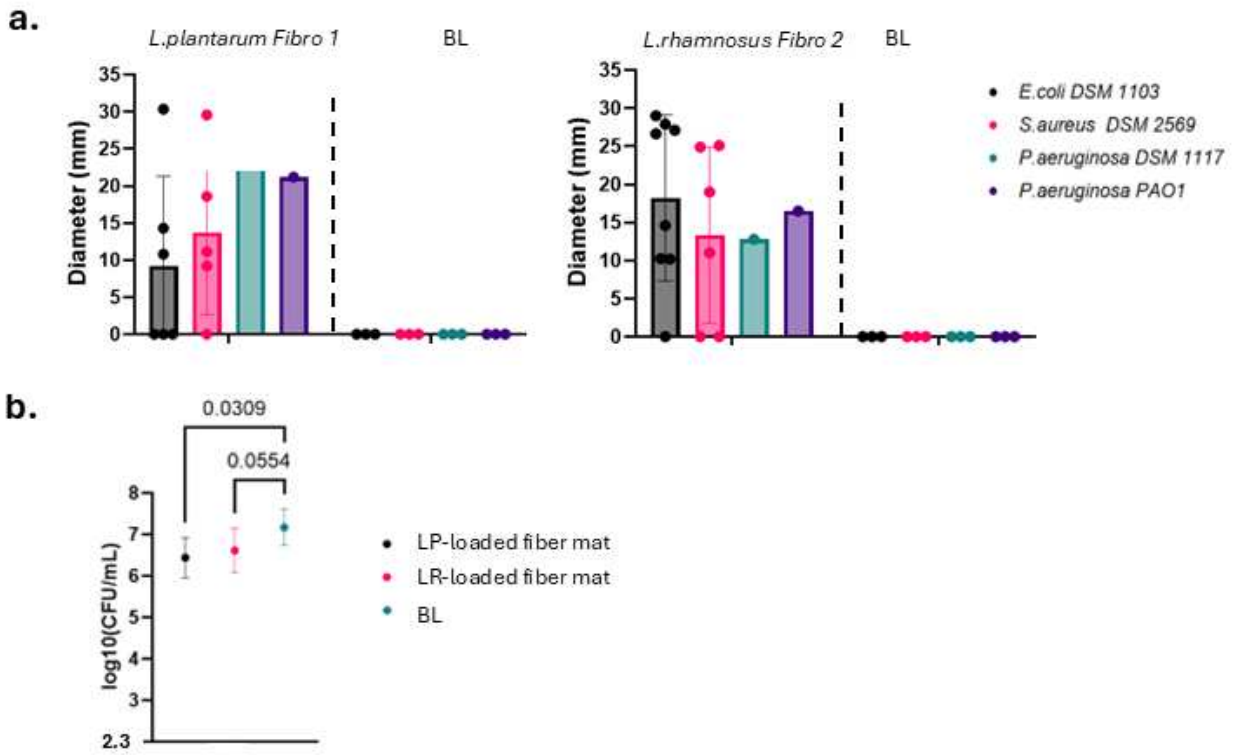


Fig. 10 ***a.*** *Antimicrobial activity of LAB-incorporated fiber mats in agar overlay assay against different pathogens.* ***b.*** *Antibiofilm activity of LAB-incorporated fiber mats against P. aeruginosa PAO1 in* DMEM/F12+GLU pH 5.5 medium. *P values for statistically significant differences are indicated above the brackets. Key: BL – pristine fiber mat, LP – L. plantarum Fibro 1, LR – L. rhamnosus Fibro 2. Data are presented as mean ± SD of biological replicates (N ≥ 3). 2.3 log_{10} is the LOD*

Discussion

The results of this study indicate that the planktonic *L. plantarum* and *L. rhamnosus* exhibit antimicrobial activity against a broad spectrum of wound-associated pathogens. However, this activity is strongly dependent on the surrounding environment, particularly nutrient composition and pH (Figs. 2 and 5, and S4). Among the media tested, MRS agar supports the highest antimicrobial activity, whereas M17 agar supports bacterial growth without detectable inhibition and Mueller-Hinton agar (MHA) does support LAB growth. These findings highlight the importance of optimizing environmental conditions to maximize the therapeutic potential of probiotic wound treatments.

One of the principal findings of this study is that environmental pH strongly influences the antimicrobial activity of both probiotic strains (Figs. 5e and 5f). Antimicrobial efficacy increases with decreasing pH, while buffering reduces the antimicrobial activity of probiotics, resulting in increased pathogen growth (Fig. 6). These findings suggest that environmental acidification is an important contributor to probiotic-mediated pathogen inhibition and that local wound pH may critically influence probiotic performance. This interpretation is supported by previous studies demonstrating that acidic conditions can directly restrict the growth of wound-associated pathogens, including *S. aureus*, *E. coli*, and *P. aeruginosa*, even in the absence of probiotics [49–52]. Furthermore, the antimicrobial activity of *L. plantarum* against wound-associated pathogens is strongly influenced by organic acid production and environmental acidification [18]. However, the contribution of other probiotic-derived antimicrobial compounds and pH-dependent mechanisms cannot be excluded.

NMR analysis and spectrophotometric measurements confirmed active glucose consumption accompanied by lactate production by both free LAB and LAB-incorporated fiber mats (Figs. 4 and 9). The production of the lactate depends on both – the bacterial strain and cultivation medium. *L. plantarum* and *L. rhamnosus* exhibited distinct antimicrobial and antibiofilm activities depending on the experimental conditions (Figs. 6b, 7a and 10b), despite producing comparable amounts of organic acids (Fig. 4). Neither strain consistently outperformed the other, suggesting that their antimicrobial functionality is both strain- and environment-dependent and that different conditions may favor the activity of one strain over the other. This suggests that additional antimicrobial mechanisms, including bacteriocins or other bioactive metabolites, contribute to the enhanced activity of *L. plantarum* [18, 24].

Besides pH, other environmental factors were investigated to determine whether they influence probiotic antimicrobial activity. Supplementation with prebiotic inulin does not enhance antimicrobial activity (Fig. S6), despite previous reports suggesting increased production of lactic acid and bacteriocins in some LAB strains [53]. Chronic wounds are often associated with reduced oxygen availability [54] and alkaline microenvironments [16,

853 54–56], which both favor the growth of anaerobic pathogenic bacteria [57]. Furthermore, the
854 temperature of chronic wounds is dynamic and depends on both the stage of healing and
855 the presence of infection [58]. In this study, neither oxygen availability during pre-incubation
856 nor pre-incubation temperature (30–37°C) significantly altered antimicrobial activity (Fig. 5).
857 These findings indicate that, under the tested conditions, environmental pH is the dominant
858 determinant of probiotic antimicrobial efficacy, whereas oxygen availability, temperature,
859 and inulin have comparatively minor effects. The observed stability across physiologically
860 relevant wound temperatures is encouraging for topical wound applications, where wound-
861 bed temperature varies depending on wound type and healing stages [55].

862 Retention of probiotics within the wound is considered important for sustained production
863 of antimicrobial metabolites and prolonged protection against pathogen colonization.
864 Therefore, the intrinsic biofilm-forming capacity of the LAB strains was investigated.
865 Compared with *S. aureus*, both probiotic strains form significantly less biofilm on PDMS
866 surfaces (Fig. 3), indicating a limited natural capacity to establish dense surface-associated
867 communities. Medium composition influences bacterial attachment, with visible
868 aggregation observed in Mueller–Hinton broth and more uniform monolayer-like surface
869 coverage in DMEM/F12+GLU (pH 5.5). Similar observations were obtained using a
870 microfluidic model that better mimics fluid movement and nutrient exchange in chronic
871 wounds. Under continuous flow, LAB again exhibits substantially lower biofilm formation
872 than *S. aureus*, confirming that prolonged colonization of wound surfaces is unlikely to occur
873 through natural biofilm formation alone.

874 To better simulate wound conditions, biofilm inhibition studies were performed using
875 DMEM/F12+GLU (pH 5.5), a medium selected to maintain the integrity of the Gel-Glu matrix
876 while supporting probiotic viability. The medium was supplemented with glucose, as
877 glucose is the main substrate for lactic acid production by LAB [59]. Although this model
878 does not fully replicate the complexity of the wound environment, it provides a more
879 physiologically relevant system than conventional bacterial growth media. Under these
880 conditions, probiotics effectively inhibit biofilm formation by *S. aureus* and *P. aeruginosa*
881 PAO1, whereas inhibition of pre-established biofilms is less pronounced (Fig. 7). These

findings suggest that probiotic therapy may be particularly effective as a preventive strategy against biofilm formation, while still providing some therapeutic benefit against mature biofilms. Glucose supplementation proved essential for probiotic activity, as *P. aeruginosa* degraded the Gel-Glu matrix in glucose-deficient conditions (Fig. S10), emphasizing the importance of nutrient availability for sustained probiotic metabolism. Biofilm inhibition assays performed on PDMS further demonstrated that LAB retains inhibitory activity (Fig. 8). However, this effect is medium-dependent, as biofilm inhibition was observed in MHB but not in DMEM/F12+GLU pH 5.5 medium, indicating that nutrient composition and environmental conditions strongly influence probiotic-pathogen interactions on surfaces.

The limited biofilm-forming ability of LAB highlights one of the major advantages of electrospun probiotic-incorporated wound dressings. Rather than relying on natural bacterial colonization, fiber mats prepared by microchip electrospinning physically retain viable probiotics within the wound, enabling prolonged production of antimicrobial metabolites directly at the wound site. Fiber-incorporated probiotics retain antimicrobial activity comparable to free bacterial cultures (Fig. 10), demonstrating that the electrospinning process preserves bacterial viability and functionality. Lower lactate concentrations were measured in samples containing probiotic-incorporated fiber mats than in those containing free probiotics (Fig. 9). However, as the preparations were not normalized to viable bacterial numbers and bacterial distribution within the fiber mats was heterogeneous, this difference cannot be attributed to reduced probiotic metabolic activity following incorporation. Importantly, the probiotic-incorporated fiber mats retained their ability to inhibit pathogen biofilm formation. These findings demonstrate that electrospinning effectively overcomes one of the main limitations of free probiotic therapy—poor long-term retention at the wound site—while maintaining antimicrobial functionality.

Overall, this study demonstrates that environmental conditions, especially microenvironment conditions surrounding probiotics, strongly influence the antimicrobial performance of probiotic wound therapies, with acidic conditions representing the primary determinant of efficacy. While the intrinsic biofilm-forming capacity of LAB is limited, electrospun fiber mats compensate for this limitation by retaining viable probiotics at the

wound site and sustaining their antimicrobial activity. These findings support electrospun probiotic-incorporated wound dressings as a promising strategy for localized infection management and provide a strong foundation for future *in vivo* studies and clinical translation.

Conclusions

This study demonstrates that probiotics *L. plantarum* Fibro 1 and *L. rhamnosus* Fibro 2 exhibit antimicrobial activity against broad range of wound-associated pathogens, which efficacy is strongly influenced by environmental conditions. Among the tested factors, acidic pH was the key determinant of antimicrobial activity, primarily through enhanced organic acid production. Both LAB strains inhibited pathogenic bacteria biofilm formation and retained antibiofilm activity after incorporation into the fiber mats. Further studies are needed to optimize the formulation and evaluate utilization in more clinically relevant models.

Author Contributions

- Oksana Gerulis: Writing – original draft, Investigation, Methodology, Formal analysis, Data curation, Visualization, Conceptualization, Writing – review & editing.
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- Vincent Hickl: Investigation, Methodology, Validation, Formal analysis, Data curation, Writing – review & editing.
- Marta Putrinš: Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing.
- Tanel Tenson: Conceptualization, Methodology, Formal analysis, Resources, Supervision, Writing – review & editing.

- Andrija Djuric: Investigation, Methodology, Formal analysis, Data curation, Validation
- James Mason: Methodology, Investigation, Resources, Supervision, Writing – review & editing.
- Qun Ren: Methodology, Investigation, Resources, Supervision, Writing – review & editing.
- Karin Kogermann: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Validation, Resources, Project administration, Funding acquisition, Supervision, Writing – review & editing.

Supporting Information

Supporting Information is available on-line at ..... All relevant data-files are uploaded together with the manuscript and Supplementary file.

Data availability

All data supporting the findings of this study are included in the article and its Supplementary Information. Additional raw data are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Murray CJ, Ikuta KS, Sharara F, et al (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet 399:629–655. https://doi.org/10.1016/s0140-6736(21)02724-0

2. Gupta K, Marques CNH, Petrova OE, Sauer K (2013) Antimicrobial tolerance of pseudomonas aeruginosa biofilms is activated during an early developmental stage and requires the two-component hybrid sagS. J Bacteriol 195:4975–4987. https://doi.org/10.1128/jb.00732-13

3. DeLeon S, Clinton A, Fowler H, et al (2014) Synergistic interactions of Pseudomonas aeruginosa and Staphylococcus aureus in an In vitro wound model. Infect Immun 82:4718–4728. https://doi.org/10.1128/iai.02198-14

4. Domenech M, Ramos-Sevillano E, García E, et al (2013) Biofilm formation avoids complement immunity and phagocytosis of Streptococcus pneumoniae. Infect Immun 81:2606–2615. https://doi.org/10.1128/iai.00491-13

5. Domenech M, Ramos-Sevillano E, García E, et al (2013) Biofilm formation avoids complement immunity and phagocytosis of Streptococcus pneumoniae. Infect Immun 81:2606–2615. https://doi.org/10.1128/iai.00491-13

6. International Committee on Systematics of Prokaryotes. *Scope of the International Code of Nomenclature of Prokaryotes (ICNP) guidelines*. Accessed July 27, 2026. https://www.bacterio.net/

7. Food and Agriculture Organization of the United Nations; World Health Organization. *Probiotics in Food: Health and Nutritional Properties and Guidelines for the Evaluation of Probiotics in Food*. Rome: Food and Agriculture Organization of the United Nations; 2006. FAO Food and Nutrition Paper 85

8. Dobson A, Cotter PD, Paul Ross R, Hill C (2012) Bacteriocin production: A probiotic trait? Appl Environ Microbiol 78:1–6. https://doi.org/10.1128/aem.05576-11

9. Cotter PD, Ross RP, Hill C (2012) Bacteriocins — a viable alternative to antibiotics? Nat Rev Microbiol 11:95–105. https://doi.org/10.1038/nrmicro2937

10. Pereira WA, Piazentin ACM, de Oliveira RC, et al (2022) Bacteriocinogenic probiotic bacteria isolated from an aquatic environment inhibit the growth of food and fish pathogens. Sci Rep 12:5530. https://doi.org/10.1038/s41598-022-09263-0

11. Fotso Techeu UD, Kaktcham PM, Momo HK, et al (2022) Isolation, Characterization, and Effect on Biofilm Formation of Bacteriocin Produced by Lactococcus lactis F01 Isolated from Cyprinus carpio and Application for Biopreservation of Fish Sausage. Biomed Res Int 2022:8437926. https://doi.org/10.1155/2022/8437926

12. Zhang S, Elbs-Glatz Y, Tao S, et al (2025) Probiotics promote cellular wound healing responses by modulating the PI3K and TGF-β/Smad signaling pathways. Cell Commun Signal 23:195. https://doi.org/10.1186/s12964-025-02179-y

13. Argandoña L, Ivanova K, Tzanov T (2026) Engineering in vitro models to replicate the complexity of chronic wounds. Nat Commun 17:6246. https://doi.org/10.1038/s41467-026-75311-2

14. Uberoi A, McCready-Vangi A, Grice EA (2024) The wound microbiota: microbial mechanisms of impaired wound healing and infection. Nat Rev Microbiol 22:507–521. https://doi.org/10.1038/s41579-024-01035-z

15. Mohammedsaeed W, McBain AJ, Cruickshank SM, O'Neill CA (2014) Lactobacillus rhamnosus GG inhibits the toxic effects of Staphylococcus aureus on epidermal keratinocytes. Appl Environ Microbiol 80:5773–5781. https://doi.org/10.1128/AEM.00861-14

16. Sim P, Strudwick XL, Song YM, et al (2022) Influence of Acidic pH on Wound Healing In Vivo: A Novel Perspective for Wound Treatment. Inte J Mol Sci 23:13655. https://doi.org/10.3390/ijms232113655

17. Li Z, Zhang S, Zuber F, et al (2023) Topical application of Lactobacilli successfully eradicates Pseudomonas aeruginosa biofilms and promotes wound healing in chronic wounds. Microbes Infect 25:105176. https://doi.org/10.1016/j.micinf.2023.105176

18. Van Ginneken S, Lories B, Steenackers HP (2026) The probiotic Lactiplantibacillus plantarum ATCC10241 inhibits wound-associated pathogens through the secretion of organic acids. Sci Rep 16:14201. https://doi.org/10.1038/s41598-026-38966-x

19. Kim DY, Lee TS, Lee YJ, et al (2025) Lactobacillus reuteri NCHBL-005 improves wound healing by promoting the activation of fibroblasts through TLR2/MAPK signaling. Inflamm Regen 45:10. https://doi.org/10.1186/S41232-025-00370-9

20. Demir A, Aslim B (2025) Investigation of multifaceted wound healing effect of exopolysaccharide (EPS) produced from probiotic strain Lactiplantibacillus plantarum GD2 as in vitro and in ovo. Sci Rep 15:36512. https://doi.org/10.1038/s41598-025-90682-0

21. Lukic J, Chen V, Strahinic I, et al (2017) Probiotics or pro-healers: the role of beneficial bacteria in tissue repair. Wound Repair Regen 25:912–922. https://doi.org/10.1111/wrr.12607

22. Moghadam SS, Momeni M, Atabaki SM, et al (2022) Topical Treatment of Second-Degree Burn Wounds with Lactobacillus plantarum Supernatant: Phase I Trial. Iran J Pathol 17:460. https://doi.org/10.30699/ijp.2022.551202.2863

23. Nguyen DL, Saha S, Thacharodi A, et al (2025) Environmental pH controls antimicrobial production by human probiotic Streptococcus salivarius. J Bacteriol 207:. https://doi.org/10.1128/jb.00059-25

24. Dai J, Fang L, Zhang M, et al (2022) Isolation and identification of new source of bacteriocin-producing Lactobacillus plantarum C010 and growth kinetics of its batch fermentation. World J Microbiol Biotechnol 38:67. https://doi.org/10.1007/s11274-022-03244-1

25. Parente E, Ricciardi A (1999) Production, recovery and purification of bacteriocins from lactic acid bacteria. Appl Microbiol Biotechnol 52:628–638. https://doi.org/10.1007/s002530051570

26. Tao S, Zhang S, Wei K, et al (2024) An Injectable Living Hydrogel with Embedded Probiotics as a Novel Strategy for Combating Multifaceted Pathogen Wound Infections. Adv Healthc Mater 13:2400921. https://doi.org/10.1002/adhm.202400921

27. Bu W, McClements DJ, Zhang Z, et al (2025) Encapsulation method of probiotic embedded delivery system and its application in food. Food Hydrocoll 159:110625. https://doi.org/10.1016/j.foodhyd.2024.110625

28. Pandey RP, Gunjan, Himanshu, et al (2024) Nanocarrier-mediated probiotic delivery: a systematic meta-analysis assessing the biological effects. Sci Rep 14:631. https://doi.org/10.1038/s41598-023-50972-x

29. Negm El-Dein A, Soliman TN, Ezzat A, et al (2025) Innovative Hydrogel Formulation Combining Phycocyanin and Probiotic for Enhancing Skin Regeneration and Accelerated Wound Healing: A Preclinical Investigation in Wistar Rats. Probiotics Antimicrob Proteins 18:2037–2055. https://doi.org/10.1007/s12602-025-10635-x

30. Shao YX, Wei P, Tu J wei, et al (2025) A probiotic microemulsion with antioxidant activity to promote wound healing. J Drug Deliv Sci Technol 114:107534. https://doi.org/10.1016/j.jddst.2025.107534

31. Gerulis O, Lanno GM, Putrinš M, et al (2025) Living probiotics-loaded wound matrices prepared by microchip electrospinning. Mater Today Bio 35:102403. https://doi.org/10.1016/j.mtbio.2025.102403

32. Siimon K, Reemann P, Põder A, et al (2014) Effect of glucose content on thermally cross-linked fibrous gelatin scaffolds for tissue engineering. Mater Sci Eng: C 42:538–545. https://doi.org/10.1016/j.msec.2014.05.075

33. Siimon K, Siimon H, Järvekülg M (2015) Mechanical characterization of electrospun gelatin scaffolds cross-linked by glucose. J Mater Sci Mater Med 26:37. https://doi.org/10.1007/S10856-014-5375-1

34. Horrocks V, Hind CK, Sutton JM, et al (2025) Metabolism of Lactobacillus and Gardnerella vaginalis in vaginal defined media. Anaerobe 95:102991. https://doi.org/10.1016/j.anaerobe.2025.102991

35. Horrocks V, Hind CK, Wand ME, et al (2022) Nuclear Magnetic Resonance Metabolomics of Symbioses between Bacterial Vaginosis-Associated Bacteria. mSphere 7:. https://doi.org/10.1128/msphere.00166-22

36. Straub H, Eberl L, Zinn M, et al (2020) A microfluidic platform for in situ investigation of biofilm formation and its treatment under controlled conditions. J Nanobiotechnol 18:166. https://doi.org/10.1186/S12951-020-00724-0

37. Hickl V, Khan A, Rossi RM, et al (2025) Segmentation of dense and multi-species bacterial colonies using models trained on synthetic microscopy images. PLoS Comput Biol 21:e1012874. https://doi.org/10.1371/journal.pcbi.1012874

38. Lorenz K, Preem L, Sagor K, et al (2023) Development of In Vitro and Ex Vivo Biofilm Models for the Assessment of Antibacterial Fibrous Electrospun Wound Dressings. Mol Pharm 20:1230–1246. https://doi.org/10.1021/acs.molpharmaceut.2C00902

39. Zhang S, Lee M, Zuber F, et al (2026) A simple, reproducible, and rapid droplet culture method for the formation and characterization of microbial biofilms. Chem Eng J 546:180067. https://doi.org/10.1016/j.cej.2026.180067

40. Ko HI, Yang H Il, Kim SR, et al (2024) Development a modified MRS medium for enhanced growth of psychrotrophic lactic acid bacteria isolated from kimchi. LWT 210:116815. https://doi.org/10.1016/j.lwt.2024.116815

41. Frantzen C, Kleppen HP, Holo H (2016) Use of M17 and a milk-based medium enables isolation of two distinct and diverse populations of Lactococcus lactis strains from undefined mesophilic starter cultures. Int Dairy J 53:45–50. https://doi.org/10.1016/j.idairyj.2015.09.005

42. Hayek SA, Gyawali R, Aljaloud SO, et al (2019) Cultivation media for lactic acid bacteria used in dairy products. Journal of Dairy Research 86:490–502. https://doi.org/10.1017/s002202991900075x

43. Randmäe K, Lorenz K, Putrinš M, et al (2025) Preparing a Dual-Species In Vitro Biofilm Model for Testing Antibiofilm Efficacy. Mol Pharm 22:6862–6873. https://doi.org/10.1021/asc.molpharmaceut.5c00798

44. Gökmen GG, Sarıyıldız S, Cholakov R, et al (2024) A novel Lactiplantibacillus plantarum strain: probiotic properties and optimization of the growth conditions by response surface methodology. World J Microbiol Biotechnol 40:66. https://doi.org/10.1007/S11274-023-03862-3

45. Du Y, Wang L, Xu J, et al (2025) Antimicrobial activity and safety profile of Lactiplantibacillus plantarum LY-21: Genomic and phenotypic insights. Food Biosci 74:108026. https://doi.org/10.1016/j.fbio.2025.108026

46. Adamberg K, Kask S, Laht TM, Paalme T (2003) The effect of temperature and pH on the growth of lactic acid bacteria: a pH-auxostat study. Int J Food Microbiol 85:171–183. https://doi.org/10.1016/s0168-1605(02)00537-8

47. Bisson G, Marino M, Poletti D, et al (2021) Turbidimetric definition of growth limits in probiotic Lactobacillus strains from the perspective of an adaptation strategy. J Dairy Sci 104:12236–12248. https://doi.org/10.3168/jds.2021-20888

48. Hinnu M, Mets T, Kerkez I, et al (2025) Nanomotion technology for testing azithromycin susceptibility of Salmonella enterica. Microbiol Spectr 13:e02385-24. https://doi.org/10.1128/spectrum.02385-24

49. Mosiichuk N, Enggren G, Da Silva ZL, et al (2025) Antibacterial activity of propylene glycol against Staphylococcus aureus and Staphylococcus epidermidis in neutral and mild acidic conditions. Journal of Antimicrobial Chemotherapy 80:1947–1950. https://doi.org/10.1093/jac/dkaf153

50. Presser KA, Ratkowsky DA, Ross T (1997) Modelling the growth rate of Escherichia coli as a function of pH and lactic acid concentration. Appl Environ Microbiol 63:2355–2360. https://doi.org/10.1128/aem.63.6.2355-2360.1997

51. Segura Munoz RR, Sourjik V (2025) Collective dynamics of Escherichia coli growth under near-lethal acid stress. mBio 16:1–15. https://doi.org/10.1128/MBIO.01932-25

52. Alexandre Y, Le Berre R, Barbier G, Le Blay G (2014) Screening of Lactobacillus spp. for the prevention of Pseudomonas aeruginosa pulmonary infections. BMC Microbiol 14:107. https://doi.org/10.1186/1471-2180-14-107

53. da Silva Sabo S, Converti A, Todorov SD, et al (2015) Effect of inulin on growth and bacteriocin production by Lactobacillus plantarum in stationary and shaken cultures. Int J Food Sci Technol 50:864–870. https://doi.org/10.1111/IJFS.12711

54. Schreml S, Meier RJ, Kirschbaum M, et al (2014) Luminescent Dual Sensors Reveal Extracellular pH-Gradients and Hypoxia on Chronic Wounds That Disrupt Epidermal Repair. Theranostics 4:721. https://doi.org/10.7150/thno.9052

55. Rembe JD, Witte M, Ertas N, et al (2026) pH profiling reveals progressive wound acidification during healing and higher pH in chronic non-healing wounds: a prospective, multicenter cohort study. Sci Rep 16:10522. https://doi.org/10.1038/s41598-026-45000-7

56. Rabello Sergio F, Caldas Santos N, Andrade Silveira I, et al (2022) Does pH Influence the Bacterial Profile of Chronic Lesions? An Analysis of Venous Ulcer Samples. Adv Skin Wound Care 35:30–36. https://doi.org/10.1097/01.asw.0000801532.60229.78

57. Dowd SE, Wolcott RD, Sun Y, et al (2008) Polymicrobial Nature of Chronic Diabetic Foot Ulcer Biofilm Infections Determined Using Bacterial Tag Encoded FLX Amplicon Pyrosequencing (bTEFAP). PLoS One 3:e3326. https://doi.org/10.1371/journal.pone.0003326

58. Research progress on the effect of wound microenvironment temperature on chronic wound healing (2025) Wanfang Data Knowledge Service Platform. https://d.wanfangdata.com.cn/ Accessed 6 Aug 2026

59. Senedese ALC, MacIel Filho R, MacIel MRW (2015) L-Lactic Acid Production by Lactobacillus rhamnosus ATCC 10863. The Sci World J 2015:501029. https://doi.org/10.1155/2015/501029

Figure legends

Fig. 1 ***a.*** *The confocal laser scanning microscope (CLSM) and* ***b.*** *scanning electron microscope (SEM) micrographs of heat-treated, HT (control samples), non-treated planktonic LAB strains (L. plantarum Fibro 1, L. rhamnosus Fibro 2) pre-grown in MRS for 24 h at 37°C. For SEM imaging the samples were taken up in distilled water.* ***c.*** *LAB-incorporated (L. plantarum Fibro 1, L. rhamnosus Fibro 2) fiber mats. Key: orange scale bar is 5 µm and purple scale bar is 2 µm*

Fig. 2 Growth curves of lactic acid bacteria (LAB) in different growth media – ***a***. *L. plantarum Fibro 1 and* ***b.*** *L. rhamnosus Fibro 2 in bacterial growth media****,*** ***c.*** *L. plantarum Fibro 1 and* ***d.*** *L. rhamnosus Fibro 2 in cell culture media - with various modifications. Samples were incubated at 37°C for 24 h. Optical density at 600 nm (OD_{600}) was measured hourly. Experiments were performed in three biological replicates (N=3); the mean values were calculated. Key: GLU – glucose, dashed line – pH-adjusted medium (pH 5.5), solid line – unadjusted medium*

Fig. 3 ***a***. *SEM micrograph of the PDMS disk surface.* ***b***. *CLSM micrographs of adhered LAB strains and S. aureus on PDMS disk surfaces under static conditions in* DMEM/F12+GLU pH 5.5 medium*, stained with SYTO 9. The concentration of LAB and S. aureus adhered to the PDMS disks in* ***c.*** *MHB (N=3). and* ***d.*** *DMEM/F12+GLU pH 5.5 media (N=3) under static conditions after 24 h.* ***e.*** *The adhesion of bacteria in* DMEM/F12+GLU pH 5.5 *medium in flow-through system at 0, 2 and 4 h. Square inside the dashed lines: zoomed-in view of the microscope micrograph at the 4 h time point. Key: LP – L. plantarum Fibro 1, LR – L. rhamnosus Fibro 2, SA – S. aureus DSM2569. P values for statistically significant differences are indicated above the brackets. Orange scale bar – 2 µm, white scale bar – 100 µm; size of the zoomed-in images 100 µm x 100 µm*

Fig. 4 Quantitative analysis of substrates and metabolites in different growth media with and without lactic acid bacteria (LAB) strains. *NMR results revealed* ***a.*** *the concentration of glucose and* ***b***. *acetate and* ***c***. *lactate in different growth media with or without LAB strains.* ***d.*** *Results of spectrophotometric quantification assay demonstrating the concentration of lactic acid in different growth media after incubation of LAB strains. Experiments were performed in three biological replicates (N=3). For clarity, statistical comparisons are reported separately in Supplementary Table S3*

Fig. 5 Effect of pre-incubation and environmental conditions on the antimicrobial activity of probiotic strains grown on MRS base agar. *Effect of aerobic and anaerobic pre-*

incubation on the antimicrobial activity of ***a.*** *L. plantarum Fibro 1 (LP) and* ***b.*** *L. rhamnosus Fibro 2 (LR) against E. coli DSM 1103 and S. aureus DSM 2569 at 37°C. No statistically significant differences in inhibition zone diameter were observed between aerobic and anaerobic conditions. Effects of pre-incubation temperature on the antimicrobial activity of* ***c.*** *LP and* ***d.*** *LR against E. coli DSM 1103 and S. aureus DSM 2569 at aerobic conditions. Effects of MRS base agar pH on antimicrobial activity of* ***e.*** *LP and* ***f.*** *LR against E. coli DSM 1103, S. aureus DSM 2569, and P. aeruginosa DSM 1117 at 37°C under aerobic conditions. Antimicrobial activity increased with decreasing pH. Key: AE – aerobic environment, AN – anaerobic environment. Data are presented as mean ± SD of biological replicates (N ≥ 3). Statistical significance was assessed using two-way ANOVA, followed by Bonferroni corrections. P values for statistically significant differences are indicated above the brackets.*

Fig. 6 ***a.*** *Comparison of antimicrobial activity of L. plantarum Fibro 1 (LP) and L. rhamnosus Fibro 2 (LR) at 37°C with and without HEPES buffer (pH 7.5), aerobically. Results confirm the partial dependence of antimicrobial activity on acid production.* ***b.*** *Relationship between the antimicrobial activity against E. coli DSM 1103 (diameter of inhibition zone, mm) and the environmental pH (diameter of yellow color zone, mm). Blue dots represent LP and orange dots represent LR. Dotted lines indicate linear regression fits. Stronger correlation was observed for LR (R^2 = 0.9338) compared to LP (R^2 = 0.7652).* ***c.*** *Top agar with pathogenic bacteria, without the pH indicator (for inhibition zone measurements).* ***d.*** *Top agar without pathogenic bacteria, with the pH indicator (for pH change zone measurements). Key: HEPES - HEPES buffer in the top agar, red line - inhibition zone size (diameter, mm). Data are presented as mean ± SD of three biological replicates (N = 3). Statistical significance was assessed using two-way ANOVA, followed by Bonferroni corrections. P values for statistically significant differences are indicated above the brackets.*

Fig. 7 ***a.*** *The antibiofilm activity of probiotics* in *DMEM/F12+GLU pH 5.5 medium against the biofilm formation and* ***b.*** *mature biofilm of P. aeruginosa PAO1 and S. aureus DSM 2569. Data are presented as mean ± SD of three biological replicates (N = 3). P values for statistically significant differences are indicated above the brackets. Key: HT - heat treated, LP - L. plantarum Fibro 1, LR - L. rhamnosus Fibro 2, PA - P. aeruginosa PAO1, SA - S. aureus DSM 2569. 2.3 log_{10} is the LOD*

Fig. 8 ***Biofilm inhibition and treatment of probiotics in the PDMS droplet biofilm assay after 24 h in MHB and DMEM/F12+GLU pH 5.5 media.*** *The biofilm inhibition of S. aureus by LAB in* ***a.*** *MHB and* ***b.*** DMEM/F12+GLU pH 5.5. *The antibiofilm activity against mature S. aureus DSM2569 biofilm in* ***c.*** *MHB and* ***d.*** DMEM/F12+GLU pH 5.5 *media. Data are presented as mean ± SD of biological replicates (N = 3). P values for statistically significant*

differences are indicated above the brackets. 2.3 log_{10} is the LOD. Key: LP - L. plantarum Fibro 1, LR - L. rhamnosus Fibro 2, SA - S. aureus DSM 2569

Fig. 9 Quantitative analysis of metabolites in different growth media with and without lactic acid bacteria (LAB)-incorporated fiber mats. *Results of NMR reveal:* ***a.*** *the concentration of glucose and* ***b****. acetate and* ***c****. lactate in different growth media with or without LAB-incorporated fibers.* ***d.*** *Results of spectrophotometric quantification assay demonstrating the concentration of lactic acid in different growth media after incubation of LAB-incorporated fibers. Data are presented as mean ± SD of three biological replicates (N = 3). For clarity, statistical comparisons are reported separately in Supplementary Table S4. Key: AE- aerobic; AN- anaerobic; DMEM/F12* - Dulbecco's Modified Eagle Medium and Ham's F-12 Nutrient Mixture*; GLU- glucose; MRS* - De Man–Rogosa–Sharpe broth

Fig. 10 ***a.*** *Antimicrobial activity of LAB-incorporated fiber mats in agar overlay assay against different pathogens.* ***b.*** *Antibiofilm activity of LAB-incorporated fiber mats against P. aeruginosa PAO1 in* DMEM/F12+GLU pH 5.5 medium. *P values for statistically significant differences are indicated above the brackets. Key: BL – pristine fiber mat, LP – L. plantarum Fibro 1, LR – L. rhamnosus Fibro 2. Data are presented as mean ± SD of biological replicates (N ≥ 3). 2.3 log_{10} is the LOD*

Supplementary Files

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