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Pediococcus acidilactici MI129-DERIVED POSTBIOTIC FORMULATIONS: *IN VITRO* INSIGHTS INTO APPLICATIONS FOR HEALTH IMPROVEMENT

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Abstract

The skin microbiome is crucial for maintaining epidermal barrier integrity and immune homeostasis. This study was conducted to assess in vitro the efficacy of postbiotic formulations prepared from the cell-free supernatant (CFS) of Pediococcus acidilactici MI129, formulated as micellar water and dietary supplement capsules. Seven genes associated with bacteriocin biosynthesis (nis, plnN, plnEF, plnS, plnA, plnQ, and plnW) were detected in the MI129 genome. A multiplex PCR method was established and effectively applied to identify key bacteriocin genes (plnQ, plnS, plnEF, plnW). Both postbiotic formulations exhibited notable antimicrobial activity, generating clear zones of inhibition against all pathogenic indicator strains tested, and demonstrated significant antioxidant activity, indicating their potential to enhance cutaneous health. These results suggest that postbiotics from P. acidilactici MI129 may contribute to skin barrier reinforcement and microbial balance, highlighting their applicability along the skin-gut axis. Future in vivo studies are required to validate these findings and explore the dynamics of postbiotic-skin microbiome interactions.

Key words*: Pediococcus acidilactici, multiplex PCR, postbiotic formulations, antimicrobial activity, antioxidant activity.*

INTRODUCTION

Grand View Research, Inc.'s latest report (https://www.grandviewresearch.com/press-release/global-skin-care-products-market) forecasts that the care products sector will achieve a valuation of USD 196.20 billion by 2030, driven by a surge in demand for natural and organic skincare products, prompting the cosmetics industry to enhance its focus on this category (Lew & Liong, 2013; Cinque et al., 2011; Lopes et al., 2017; Majeed et al., 2020; De Pessemier et al., 2021; Duarte et al., 2022; Grigore et al., 2022; Gropoșilă-Constantinescu et al., 2022, 2023; da Silva Vale et al., 2023; Ma et al., 2023). This global shift within the beauty sector stems from growing consumer awareness of probiotics' clinical efficacy and supporting evidence (Cinque et al., 2011; Puebla-Barragan & Reid, 2021; Kiepś, & Dembczyńsk, 2022). On the other hand, formulating live microorganisms into mainstream cosmetics is extremely problematic in terms of technological, economic, and clinical aspects (Lew & Liong, 2013; Lopes et al., 2017; Majeed et al., 2020; De Pessemier et al., 2021; Duarte et al., 2022; Ma et al., 2023; da Silva Vale et al., 2023). After a close inspection of the cosmetics' market, the ingredient lists show that cosmetic products are prepared especially with bacteria of the genera *Lactobacillus, Lactococcus, Bifidobacterium, Leuconostoc,* and *Streptococcus,* as well as *Bacillus* and yeast *Saccharomyces* followed by a term such as ferment lysate, ferment filtrate, extract, etc. than with living microbes (Foo et al., 2019; Wegh et al., 2019; Abbasi et al., 2020; Lew & Liong, 2013; Lopes et al., 2017; Majeed et al., 2020; De Pessemier et al., 2021; Duarte et al, 2022; Pristavu et al., 2022;

Thorkkattu et al., 2022; da Silva Vale et al., 2023). Research corroborates the utility of postbiotic components as effective tools in dermatological care that are applicable for prevention and treatment (Foo et al., 2019; Wegh et al., 2019 Abbasi et al., 2020; Lew & Liong, 2013; Lopes et al., 2017; Majeed et al., 2020; Vallejo-Cordoba et al., 2020; De Pessemier et al., 2021; Duarte et al., 2022; Thorkkattu et al., 2022; Da Silva Vale et al., 2023). The skin microbiome, a complex assemblage of bacteria, yeasts, and parasites, is essential for cutaneous health, with its composition shaped by skin type, hormonal profiles, age, hygiene practices, genetic makeup, and environmental factors (Byrd et al., 2018; Lee et al., 2019; Eisenstein, 2020). The skin-gut axis impacts dermatological integrity, with gut dysbiosis triggered by poor diet, stress, or antibiotics linked to conditions like eczema, psoriasis, and acne (Lee et al., 2019; De Pessemier et al., 2021; Cheruvari & Kammara, 2025). Acne pathogenesis involves *Cutibacterium acnes* (formerly *Propionibacterium acnes*), alongside *Bacillus cereus, Escherichia coli, Staphylococcus, Streptococcus pyogenes*, and *Pseudomonas aeruginosa* (Lee et al., 2019; Majeed et al., 2020; Thorkkattu et al., 2022). The pandemic's reliance on personal protective equipment (PPE), especially facemasks, has markedly altered the facial skin microbiome, particularly in the chin, nasal, and oral regions (Kisielinski et al., 2021). Maskne, a PPE-related dermatosis, results from extended mask use, creating a humid niche that enhances bacterial proliferation due to exhaled air and sweat, affecting 53% of users with acne and 51% with irritations (Kisielinski et al., 2021). Despite therapeutic options like topical and systemic retinoids, antibiotics, and keratolytics for acne, challenges arise from increasing antibiotic resistance and dermal side effects (Oh et al., 2006; Majeed et al., 2017; 2020; Kisielinski et al., 2021). Probiotics have proven effective in infection prevention and wound repair, but topical probiotic research is limited (Cinque et al., 2011; Muizzuddin et al., 2012; Puebla-Barragan & Reid, 2021). The International Scientific Association of Probiotics and Prebiotics (ISAPP) delineates postbiotics as "preparations comprising inactivated microbial cells and/or their structural or metabolic components that provide a beneficial effect on the health of the host" (Salminen et al., 2021; Vinderola et al., 2023).

Probiotics produce bioactive metabilites, such as bacteriocins, carbohydrates enzymes, short-chain fatty acids, hydrogen peroxide, vitamins, alcohols and other low molecular weight metabolites or complex cell-derived components such as peptidoglycan-derived muropeptides, S layer proteins, lipoteic acids, etc. with biotherapeutic potential (Kang et al., 2009; Cicenia et al., 2014; Wegh et al., 2019; Abbasi et al., 2020; Natarj et al., 2020; Teame et al., 2020; Żółkiewicz et al., 2020; Cheruvari & Kammara, 2025; Pristavu et al., 2022; Rafique et al., 2023; Hijová, 2024; Isaac-Bamgboyeet al., 2024). Postbiotics, with defined structures, scalable production, precise dosing, safety, and stability, offer antimicrobial, immunomodulatory, antioxidant, anti-inflammatory, and hepatoprotective benefits (Żółkiewicz et al., 2020; Cheruvari & Kammara, 2025; Pristavu et al., 2022; da Silva Vale et al., 2023; Rafique et al., 2023; Hijová, 2024; Isaac-Bamgboye et al., 2024).

The present study aimed to investigate the antimicrobial and antioxidant efficacy of postbiotic formulations derived from the cell-free supernatant (CFS) obtained from *Pediococcus acidilactici* MI129, delivered as micellar water and capsule-based products, to explore their application in skin health.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Pediococcus acidilactici MI129 strain from the Microorganisms Collection of the Faculty of Biotechnologies (USAMV of Bucharest), stored in a 40% glycerol solution at –20°C, was used in this study. The MI129 strain was initially transferred into a specific culture medium (De Man-Rogosa-Sharpe broth, abbreviated as MRS, procured from Merck, Germany) and incubated at 37°C for 24 hours. Three Gram-positive bacteria (*B. cereus* CP1, *Staphylococcus aureus* ATCC 43300, *Streptococcus pyogenes* ATCC 19615), two Gram-negative bacteria (*E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027) were used to test antibacterial activity of CFS derived from *P. acidilactici* MI129. Reference pathogenic bacteria were cultured on trypticase soy agar (TSA; Alliance Bio Expertise, Guipry Messac, France) at 37°C for 24 hours. A selection of 1-2 colonies was then picked and transferred to the physiological solution, with the concentration adjusted to 10^6 CFU/mL for each pathogen, under the McFarland standard.

Detection of bacteriocin-encoding genes using singleplex and multiplex PCR

P. acidilactici MI129 was cultivated in MRS broth at 37°C for 48 h. The cells were subsequently harvested by centrifugation at 5000 × g for 10 min.

Plasmid DNA extraction was conducted using the E.Z.N.A.® Plasmid DNA Mini Kit (Omega Bio-tek, Inc., Norcross, Georgia, USA), following the manufacturer's protocol.

Bacteriocin-encoding genes were detected using both singleplex and multiplex PCR methods, with the sequences of all gene-specific primer pairs provided in Table 1. For singleplex PCR, each 25 µL reaction mixture included 10 ng of plasmid DNA, 0.5 µM of each forward and reverse primer, 1× DreamTaq™ Green Buffer containing 20 mM $MgCl_2$ (Thermo Fisher Scientific, Baltics, UAB, Vilnius, Lithuania), 0.2 mM of dNTP, and 0.025 U of DreamTaq™ DNA Polymerase, with the final volume adjusted using DNase-free ultrapure water. Amplification was carried out in a MultiGene™ Thermal Cycler (Labnet International, Inc., Cambridge, United Kingdom) with the following conditions: initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at gene-specific temperatures (50°C for *pln423*, 53°C for *nis*, *plnXY*, *plNC8IF*, *plnN*, and *ped*, and 58°C for *plnJK*, *plnS*, *plnW*, *plnA*, *plnEF*, and *plnQ*) for 45 s, and extension at 72°C for 45 s, followed by a final elongation at 72°C for 7 min. A multiplex PCR protocol was established for the simultaneous detection of five target genes (*plnA*, *plnQ*, *plnS*, *plnEF*, and *plnW*) in a single reaction, using the same PCR program. The amplified products were separated by electrophoresis on a 2% (w/v) agarose gel (VWR International BVBA, Leuven, Belgium) at 90 V for 60 min, visualized under UV illumination. The PCR sizes were determined using a 100 bp DNA ladder (GeneRuler™ 100 bp Plus DNA Ladder, Thermo Scientific, USA).

Table 1. Primers for detection of bacteriocin-encoding genes (Chen et al., 2022)

Target genes	**Sequence (5' – 3')**		**PCR product (bp)**	**Temperature, °C**
	Forward	**Reverse**		
pln423	TATGATGAAAAAAATTGAAAAAT	CCAAAGATAATCCCCCCCCAT	197	50
plNC8IF	TTGGCGGAAAAACAAAGACT	TCAGCATGTCATTTCACCATC	114	53
plnN	ATTGCCGGGTTAGGTATCG	CCTAAACCATGCCATGCAC	146	53
plnXY	ATTCAGCGATTAGCATTG	GGAGCCATAAACTCTTCTT	286	53
ped	GGTAAGGCTACCACTTGCAT	CTACTAACGCTTGGCTGGCA	332	53
nis	GGCATAGTTAAAATTCCCCCC	CAGGTTGCCGCAAAAAAAG	428	53
plnA	ATGAAAATTCAAATTAAAGG	TTACCATCCCCATTTTTTA	146	58
plnQ	TGAAATCCTACAATATGAAATTGAACCGCGA	TTATTTTCTCTTACTTGTAAAGGCTCTCAA	188	58
plnS	ATGGCACACTCAAATAAAC	TCAACAATAATGAGCACGA	299	58
plnEF	TGATGGCTTGAACTATCCGTG	CATACAAGGGGGATTATTT	385	58
plnJK	GCCACAAAGAGCACTAACA	CATACAAGGGGGATTATTT	427	58
plnW	ATGTTACAGAAGAATTTACGGT	TTAGCTAGGAACCAACCAG	686	58

Preparation of cell-free supernatants (CFSs) from *P. acidilactici* MI129

Pediococcus acidilactici MI129 was grown on MRS broth at 37°C for 24 hours. Following incubation, the culture was centrifuged at 6000 x g for 5 minutes to separate the bacterial cells. The resulting supernatant was collected and filtered through 0.22 µm pore-size membrane filters to obtain the cell-free supernatant (CFS). The pH of the CFS was then adjusted to 5.0-5.5 using 1 N NaOH solution. The CFS suspension was frozen at –20°C and then freeze-dried using a FreeZone 6 freeze-dryer (Labconco, Kansas City, MO, USA) operating at –55°C and 0.3 mbar. The resulting freeze-dried powder was stored at 4°C in airtight, light-protected containers until further use.

Preparation of the Postbiotic Capsule Supplement

To prepare the capsule supplement, the CFS derived from *P. acidilactici* MI129 was mixed with 10% (w/v) maltodextrin (PanReac AppliChem, Darmstadt, Germany), which served both as carrier and cryoprotectant. The mixture was freeze-dried according to the protocol outlined in section 2.3 and encapsulated in an HPMC capsule (hypromellose (IN), short for hydroxypropyl methylcellulose) (Figure 1).

Figure 1. Formulations Assessed in This Study (Left to Right): Freeze-Dried CFS solution (50 mg/mL), Micellar Water Containing CFS, Micellar Water Base, and Encapsulated Freeze-dried CFS

Preparation of micellar water formulation

The micellar water was formulated using 49.05% distilled water (as a hydrating base), 40.0% rose water, known for its anti-inflammatory and revitalizing properties, and 10% of freeze-dried CFS from *Pediococcus acidilactici* MI129 at a concentration of 50 mg/mL, added for its demonstrated antimicrobial and antioxidant properties. Additional active ingredients included 0.5% Vitamin C functioning as a hydrating, antioxidant, and anti-aging agent; 0.3% sodium benzoate as a preservative; 0.1% Vitamin E for hydration, antioxidant defense, and anti-wrinkle benefits; and 0.05% rose essential oil (*Rosa damascena* flower oil), acting as a hydrophilic excipient with an aromatic profile. All ingredients were mixed under controlled hygienic conditions and homogenized at ambient temperature to ensure uniform distribution of active substances. The final product was stored at 4°C until further analysis (Figure 1).

Antimicrobial activity

The antimicrobial properties of CFS derived from *P. acidilactici* MI129 and postbiotic formulations were assessed using the agar well diffusion method against the aforementioned bacterial pathogens, according to the method described by Balouiri et al. (2006). Petri dishes (90 mm diameter) were inoculated with 2 mL of standardized bacterial suspensions (0.5 McFarland) evenly distributed on Mueller-Hinton agar (Sigma, Germany). After solidification, wells (6 mm diameter) were performed in the agar using a sterile needle. Each well was filled with 100 µL of sample. Plates were initially incubated at 4°C for 4 hours to ensure complete diffusion of the CFS into the agar, followed by incubation at 37°C for 24 hours. The antibacterial activity was determined by measuring the diameter of the inhibition zones (in mm) surrounding the wells using a ruler.

Antioxidant activity

The antioxidant capacity of CFS derived from *P. acidilactici* MI129 and postbiotic products was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazine) assay, adapted from the method described by Brand-Williams et al. (1990) with modifications. Briefly, 1 mL of the sample was thoroughly mixed with 2 mL of a 100 µM DPPH solution (Alfa Aesar, Kandel, Germany) prepared in absolute methanol. The mixture was incubated at room temperature in the dark for 30 minutes, then centrifuged at 10,000 × g for 5 minutes. Distilled water (1 mL) was used as a calibration standard to verify the accuracy and precision of the results, while a blank control was prepared by substituting DPPH with an equivalent volume of absolute methanol. Absorbance was measured at 517 nm using a UV-1800 spectrophotometer (ChromTech, Minneapolis, MN, USA). The radical scavenging activity (RSA) was expressed as a percentage, calculated using the standard equation for DPPH inhibition:

$$\%\ \text{RSA} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \text{ x } 100$$

where: ABS control denotes the absorbance of the DPPH solution in the absence of any sample, and ABS sample refers to the absorbance of the DPPH solution combined with the sample under investigation.

Statistical analysis

All experiments were conducted independently in triplicate (n = 3), with results expressed as mean values ± standard deviation (SD). Statistical significance was assessed using a one-way analysis of variance (ANOVA) performed in GraphPad Prism (version 10.4.1; GraphPad Software, San Diego, CA, USA). Post hoc analysis was carried out with Tukey's multiple comparison test, and differences were deemed statistically significant when $p < 0.05$.

RESULTS AND DISCUSSIONS

Postbiotics, recognised for their well-defined structures, ease of manufacture, and safety, deliver antimicrobial, anti-inflammatory, and antioxidant properties, marking them as a feasible alternative to probiotics for skin health (Foo et al., 2019; Wegh et al., 2019; Abbasi et al., 2020; Lew & Liong, 2013; Lopes et al., 2017; Majeed et al., 2020; Vallejo-Cordoba et al., 2020; De Pessemier et al., 2021; Duarte et al., 2022; Thorkkattu et al., 2022; Da Silva Vale et al., 2023).

Detection of bacteriocin-encoding genes by PCR and multiplex PCR

Bacteriocins are among the most extensively studied postbiotic metabolites, recognized for their potent and well-documented broad antimicrobial activity antimicrobial properties, and pH and thermal stability (Bowe et al., 2006; Oh et al., 2006; De Vuyst & Leroy, 2007; Kang et al., 2009; Wegh et al., 2019; Nataraj et al., 2020; Thorakkattu et al., 2022; Ma et al., 2023; Cheruvari & Kammara, 2025).

In this study, PCR was conducted using genomic DNA extracted from the MI129 strain. Twelve gene-specific primer pairs were employed to screen for commonly reported bacteriocin-encoding genes (Table 1). PCR screening of *P. acidilactici* MI129 revealed the presence of seven bacteriocin-encoding genes (*nis, plnN, plnEF, plnS, plnA, plnQ,* and *plnW*). All amplified fragments matched the expected PCR product sizes, confirming the reliability and specificity of the primers used (Table 1).

A multiplex PCR assay was successfully developed and optimized for the simultaneous detection of bacteriocin-encoding genes in *Pediococcus acidilactici* MI129. The assay targeted five key genes - *plnA, plnQ, plnS, plnEF,* and *plnW* (Figure 2). Primer sets were carefully selected to have closely matched annealing temperatures, effectively minimizing nonspecific amplification and reducing the formation of unintended bands. The reaction conditions were meticulously adjusted to enable efficient and concurrent amplification of all targeted gene sequences.

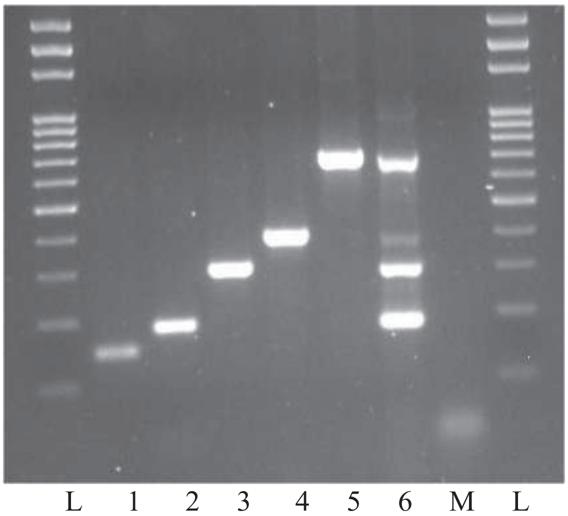

Figure 2. Electrophoretic profile of PCR amplification targeting genes involved in bacteriocin biosynthesis in *P. acidilactici* MI129
Lanes: 1 – *plnA*; 2 – *plnQ*; 3 – *plnS*; 4 – *plnEF*; 5 – *plnW*; 6 – multiplex PCR reaction using all primers; M – negative control (no DNA template); L – GeneRuler™ 100 bp DNA molecular weight marker

The assay demonstrated high specificity and reliability in detecting four bacteriocin-encoding genes (*plnQ, plnS, plnEF,* and *plnW*), highlighting its potential as a rapid, robust, and cost-effective tool for bacteriocin gene profiling in probiotic strains (Figure 2). Notably, while the *plnA* gene was successfully amplified using conventional singleplex PCR, it failed to produce a detectable band in the multiplex assay, possibly due to primer interference or competitive inhibition during multiplex amplification. Bacteriocins, besides their direct antimicrobial effects, could contribute to maintaining skin homeostasis by modulating immune responses, regulating sebaceous gland activity, and supporting a balanced skin microbiota (Bowe et al., 2006; Oh et al., 2006; De Vuyst & Leroy, 2007; Cheruvari & Kammara, 2025). These multifunctional peptides are increasingly recognized for their therapeutic value in the management of resistant skin infections (Bowe et al., 2006; Oh et al., 2006; Kang et al., 2009; Wegh et al., 2019; Nataraj et al., 2020; Thorakkattu et al., 2022; Ma et al., 2023; Cheruvari & Kammara, 2025).

Antimicrobial properties of postbiotic formulations

The skin is a vital protective layer that protects the body against dehydration, infection, and harmful chemicals, whilst also contributing to immune oversight (Byrd et al., 2018; Lee et al., 2019; Eisenstein, 2020).

Common skin disorders include psoriasis, eczema, dermatitis, acne, and dehydration. (Byrd et al., 2018; Lee et al., 2019; Eisenstein, 2020; De Pessemier et al., 2021; Cheruvari & Kammara, 2025). These disruptions may result in infections caused by *Staphylococcus* species such as *S. aureus*, *S. epidermidis*, and *Cutibacterium acnes* (Bowe et al., 2006; Oh et al., 2006; Lee et al., 2019; Majeed et al., 2020; Thorkkattu et al., 2022).

Research on postbiotics sourced from lactic acid bacteria has markedly increased, boosted by their proven antimicrobial and antioxidant properties, particularly organic acids, bacteriocins, and other metabolites, which offer significant potential in the pharmaceutical and food industries for combating microbial resistance, improving food safety, and reducing oxidative damage (Diguță et al., 2020; Voaideș et al., 2022; Pristavu et al., 2022; Badea et al., 2022; 2024 a,b; Zamfir et al., 2022; Ouili et al., 2023; Coulibaly et al., 2023; Kouadio et al., 2024).

Our investigation demonstrated that the crude CFS from *P. acidilactici* MI129 exhibited significant antimicrobial efficacy, with the largest inhibition zones against *Streptococcus pyogenes* (15.67 mm) and *E. coli* (14.58 mm), followed by *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (8.83-11.33 mm) (Figure 3 and Table 2). These findings align with previous studies showing that postbiotics produced by lactic acid bacteria (LAB) possess inhibitory activity against both Gram-positive and Gram-negative pathogens (Kim et al., 2006; Aguilar-Toalá et al., 2018; Coulibaly et al., 2023; Kouadio et al., 2024). *Streptococcus salivarius* targets *P. acnes* with bacteriocin-like inhibitors (Bowe et al., 2006), while *Lactococcus* strains inhibit *S. epidermidis*, *S. aureus, S. pyogenes,* and *P. acnes* via bacteriocins (Oh et al., 2006).

The capsule formulation containing freeze-dried CFS maintained a broad-spectrum and consistent antibacterial profile across all tested pathogens (Figure 3 and Table 2), indicating that freeze-drying effectively preserved the stability and bioactivity of the antimicrobial compounds, in agreement with earlier reports that this method is suitable for stabilizing postbiotic formulations without compromising efficacy (Luca & Oroian, 2020; Żółkiewicz et al., 2020; da Silva et al., 2023).

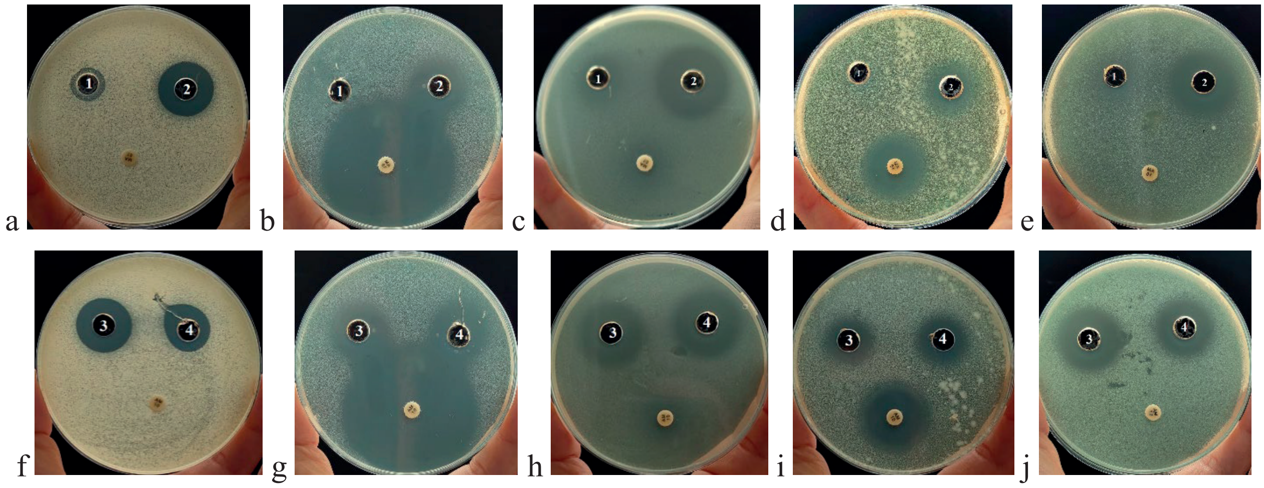

Figure 3. Zone of inhibition illustrating the antibacterial activity of various formulations:
1 - micellar water base (without CFS); **2** - micellar water containing CFS; **3** - unprocessed CFS;
4 - capsules containing CFS; **AM 10** - Ampicillin 10 U, used as a reference antimicrobial control.
Pathogenic bacteria: *Bacillus cereus* CP1 (a and f); *Staphylococcus aureus* ATCC 43300 (b and g);
Streptococcus pyogenes ATCC 19615 (c and h); *Escherichia coli* ATCC 25922 (d and i);
Pseudomonas aeruginosa ATCC 9027 (e and j)

Table 2. *In vitro* evaluation of the antibacterial activity of various formulations against pathogenic bacteria, expressed as the diameter of inhibition zones (mm)

Samples	**Pathogenic bacteria**				
	B. cereus CP1	*Staph. aureus* ATCC 43300	*S. pyogenes* ATCC 19615	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 9027
CFS derived from MI129	8.83±.0.29^{a}	8.83±.0.29^{a}	15.67±.0.29^{c}	14.58±.0.52^{c}	11.33±.0.29^{b}
Capsules prepared with CFS from MI129	13.00±.0.00^{a}	13.00±.0.50^{a}	13.50±.0.29^{a}	14.33±.0.29^{a}	11.17±.0.29^{a}
Micellar water base	07.00±.0.29^{c}	00.00±.0.00^{a}	04.00±.0.00^{b}	00.00±.0.00^{a}	00.00±.0.00a
Micellar water containing CFS from MI129	14.17±.0.29^{c}	12.00±.0.00^{b}	16.67±.1.15^{d}	11.17±.0.29^{a}	11.13±.0.29^{a}
Ampicillin (AM 10)	R	S	R	S	R

Legend: Resistant (R) ≤10 mm; intermediate resistant (IR) 10-19 mm; susceptible (S) ≥ 20 mm

The micellar water formulation enriched with CFS showed significantly enhanced antimicrobial activity, particularly against *S. pyogenes* and *B. cereus*, followed by *P. aeruginosa* and *E. coli* (Figure 3 and Table 2). The improved performance of this formulation may be attributed to the micellar system's ability to facilitate better delivery and surface interaction of postbiotic components with bacterial membranes. However, the micellar water base (without CFS) exhibited limited and variable antimicrobial effects, with statistically significant inhibition observed only against *B. cereus* and *S. pyogenes*, and no activity detected against *S. aureus*, *E. coli*, or *P. aeruginosa* (Figure 3 and Table 2). Capsules, being solid dosage forms, generally seem to provide better stability and a longer shelf life compared to fluid suspensions, like micellar water, for postbiotic formulations. This is because liquids are more susceptible to degradation from factors like oxidation and microbial growth, which can shorten their shelf life.

The micellar water formulation containing CFS from *P. acidilactici* MI129 demonstrated a uniform appearance, with no detectable particulate aggregation. It retained a stable viscous liquid texture, exhibited a distinct brown coloration characteristic of the CFS, and possessed an aromatic fragrance linked to the formulation's ingredients, with a pH value within the range specified for cosmetic applications (Luca & Oroian, 2020; Żółkiewicz et al., 2020; da Silva et al., 2023).

Antioxidant properties of postbiotic formulations

Postbiotics have been shown to contribute to the maintenance of microbiota stability, the strengthening of the skin barrier via antioxidant effects, and the suppression of matrix-degrading enzymes, potentially providing UV protection by preventing cell senescence (Majeed et al., 2020).

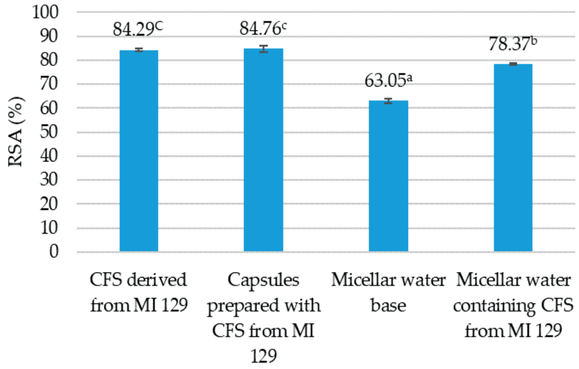

Figure 4. Determination of antioxidant properties through the DPPH method

The antioxidant activity, expressed as a percentage, varied significantly among the tested formulations. The capsule formulation containing freeze-dried CFS from *P. acidilactici* MI129 demonstrated the highest antioxidant potential (84.76%), closely followed by the crude CFS itself (84.29%). The micellar water formulation enriched with CFS exhibited slightly lower, yet still notable, antioxidant activity (78.37%) (Figure 4). Without the addition of postbiotic components, the micellar water base displayed a minimal antioxidant activity of 63.05%, attributable to the inclusion of vitamins C and E as active ingredients (Figure 4). Our investigation into the antioxidant properties of CFS yields results that are in accordance with those previously established by Badea et al. (2024b), Coulibaly et al. (2023), and Kouadio et al. (2024).

CONCLUSIONS

Through regular PCR, seven genes responsible for bacteriocin biosynthesis (*nis*, *plnN*, *plnEF*, *plnS*, *plnA*, *plnQ*, *plnW*) were identified in the *Pediococcus acidilactici* MI129 strain. A multiplex PCR method was established to simultaneously detect target genes (*plnQ*, *plnS*, *plnEF*, and *plnW*), offering a straightforward, rapid, reliable, and affordable alternative to traditional PCR techniques. Postbiotic formulations, such as micellar water and dietary supplement capsules, were developed using the cell-free supernatant (CFS) derived from *P. acidilactici* MI129. Both formulated products containing CFS demonstrated strong antibacterial activity against pathogenic bacteria that may inhabit the skin microbiota, alongside significant antioxidant activity.

To support the multiplex PCR results, it is suggested that the biosynthesized compounds should be characterized using methods such as SDS-PAGE and HPLC.

Future research should involve optimizing the fermentation process at laboratory and micropilot levels, performing *in vivo* studies to demonstrate the beneficial effects of the postbiotic formulations, and verifying the stability of these new functional products under suitable storage conditions.

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