Microb Health Dis 2024; 6: e996

ENCAPSULATION OF A PEDIOCIN PA-1 PRODUCER *PEDIOCOCCUS ACIDILACTICI* AND ITS IMPACT ON ENHANCED SURVIVAL AND GUT MICROBIOTA MODULATION

M. Mokrani[1,2], A.M. Elie[2], C. Jacquot[1,2], F. Kiran[3], M.C. Urdaci[1,2]

[1]University of Bordeaux, CNRS, CBMN, UMR 5248, Pessac, France
[2]Bordeaux Sciences Agro, Gradignan, France
[3]Pharmabiotic Technologies Research Laboratory, Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey

Corresponding Author: Maria C. Urdaci, MD; e-mail: maria.urdaci@agro-bordeaux.fr; probiotics-urdaci@pm.me

Abstract – *Objective:* Bacteriocin production is proposed as an important factor in the beneficial effect of probiotics. The present study used encapsulation technology to improve bacteriocin-producing *Pediococcus acidilactici* HM-2 survivability under *in vitro* gastrointestinal conditions and evaluate its impact *in vivo* on gastrointestinal transit and gut microbiota modulation.

Materials and Methods: Bacteriocin produced by *P. acidilactici* HM-2 was purified by chromatographic techniques and identified according to its molecular mass and gene sequencing. Encapsulation was carried out using alginate, and capsules were then coated with whey proteins using the lyophilized extrusion method. A Scanning Electron Microscope (SEM) was used to study the surface and core structure of capsules. The effect of encapsulation on probiotic viability and transit under either simulated or *in vivo* conditions was evaluated using microbiological and molecular assays. Furthermore, the impact of either encapsulated (E) or non-encapsulated (NE) *P. acidilactici* in mice gut microbiota was determined using quantitative Polymerase Chain Reaction (qPCR) and Denaturing Gradient Gel Electrophoresis (DGGE).

Results: Mass spectrometry analysis and the sequence of the amplified pediocin gene identified the bacteriocin as a pediocin PA-1. Encapsulation improves probiotic viability *in vitro* but does not modify the gut transit time of the probiotic strain in mice. Interestingly, the two probiotic forms (E and NE) significantly increased *Bifidobacterium* spp. In addition, the E form also increased the Bacteroidetes (Bacteroides and Prevotella) and *Lactobacillus murinus* and *Lactobacillus acidophilus* species.

Conclusions: *P. acidilactici* HM-2, a pediocin PA-1 producer, could modify some gut microbiota groups in mice depending on encapsulated or non-encapsulated forms of administration.

Keywords: Pediocin PA-1, Encapsulation, Alginate, Whey, Gastrointestinal transit, Gut microbiota.

INTRODUCTION

Lactobacilli species belonging to the lactic acid bacteria (LAB) display various beneficial effects on host wellbeing with their anti-microbial, anti-inflammatory, anti-cancer, anti-diabetic, anti-allergic, and anti-obesity activities[1]. LAB can prevent and/or improve multiple diseases using different modes of action, such as adhesion to the intestinal tract, host immunomodulation, inhibition

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License

of pathogenic bacteria, and modulation of microbiota[2]. Bacteriocins, the main antimicrobial substances produced LAB, are ribosomally synthesized peptides that inhibit or kill some bacteria, generally by membrane pore-forming[3]. Although the influence of bacteriocins on intestinal pathogens is well understood, their specific effects on the gut microbiota remain little known. Recently, a few studies[4,5] discovered that bacteriocins can modulate microbiota or immunity which is related to several health-promoting functions on the host. However, to exert their health-promoting claims, sufficient concentrations of viable bacteria have to reach the target niches to interact with commensal microbiota[6].

Probiotics are exposed to various environmental conditions that decrease their viability throughout processing, storage, and utilization. Furthermore, loss of probiotic viability is attributed to the high acid and bile salt concentrations in the gastrointestinal (GI) tract[7]. The encapsulation technique has great potential to preserve probiotics. Calcium alginate (Alg), a label-friendly ingredient, is one of the most used compounds for protecting and delivering bacteria[8-10]. To increase bacterial viability in stomach conditions, because of the diffusion of small hydrogen ions into the Alg capsules[11], they can be coated with whey proteins thereby improving cell viability as demonstrated in other studies[12,13].

Although the bacteriocins produced by LAB have a narrower antimicrobial spectrum than antibiotics, and are active principally against Gram-positive bacteria, the producer LABs can play an important role in the competitive exclusion of some pathogens with slight disturbance to the commensal gut microbiota, in contrast to most antibiotics[14]. They can also directly help the producers to survive and establish a niche in the microbial ecosystems, leading to the modulation of microbiota[15]. Various studies[16-18] have described the effect of bacteriocin-producing LAB on changing gut microbiota in animals and humans. Producer strains could change certain bacterial groups but and not the overall gut microbiota structure[5]. Furthermore, proof-of-concept studies based on bacteriocin producer LAB and their respective isogenic mutants point out that, in most cases, only producer strains, but not their mutants, were able to modulate some microbiota groups[5,15,19]. Therefore, bacteriocin-producing probiotics can be a next-generation approach for a finer/more targeted way of modulating the gut microbiota[20].

Pediococcus acidilactici HM-2 isolated from human breast-fed infant faeces was used as a bacteriocin-producing strain, in this study. The main objectives of this study were (1) to identify the bacteriocin produced by the strain HM-2, (2) to investigate the protective effect of encapsulation on the viability and on the gut transit of the strain, and (3) to explore the modulation of gut microbiota comparing the non-encapsulated and the encapsulated *P. acidilactici* cells in mice.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Pediococcus acidilactici HM-2, isolated from human breast-fed infant faeces and identified by 16S rRNA gene sequencing (GenBank: OR532460.1) was used as a bacteriocin producer strain. It was grown in Man, Rogosa and Sharpe (MRS, Difco Laboratories, France) medium at 37°C under aerobic conditions. *Listeria monocytogenes* ATCC 7644 and *Listeria innocua* CIP 8011 were used as indicator microorganisms for evaluating antimicrobial activities and grown in Brain Heart Infusion (BHI, Difco Laboratories, France) medium at 37°C.

Bacteriocin Purification and Identification

Bacteriocin was purified from the supernatant of the 1L culture of *P. acidilactici* HM-2 through cation exchange chromatography followed by hydrophilic-interaction and reverse-phase chromatography (Pharmacia-LKB, Uppsala, Sweden), essentially described by Nissen-Meyer et al[21]. Molecular mass of the purified bacteriocin was determined using Mass spectrometry[22]. A peptide mass similarity search was conducted using the TagIdent tool on the ExPASy database.

The antimicrobial activity of purified bacteriocin was confirmed by agar well-diffusion assay against *L. monocytogenes* and *L. innocua*[23]. The presence of the gene encoding bacteriocin production was also identified by PCR analysis. Briefly, the total genomic DNA of the strain was

extracted using a commercial kit protocol (Macherey-Nagel, Germany), and PCR was carried out using pediocin PA1-specific primers (F:5'AGCAGCTTTCGAGTTTCCCCACT-3' and R:5'TG-CCAGGTTTATGAAGATTCTCTGCAC-3') according to Rodriguez et al[24]. The PCR product was then sequenced using ABI Prism 377 DNA sequencing system (Applied Biosystem, USA) and assembled using the BioEdit software, version 7.0.0. A similarity search was conducted with the BLAST program from NCBI database (version 2.2.15).

Encapsulation

Bacterial cell suspensions (10^{10} CFU/mL) mixed with 1.8% alginic acid sodium salt (Alg) from brown algae (Sigma-Aldricht, Cambridge, UK) were placed in a syringe attached to the pump of the encapsulation system (NISCO, Switzerland). The mixture was then dropped through a 600 μm nozzle into the gelifying solution (0.1 M $CaCl_2$). After rinsing with 1.5% peptone water for 30 min, capsules were coated with a whey protein solution (5% w/v) on an orbital shaker at 100 rpm for 30 min. Finally, capsules were freeze-dried and analyzed under a Scanning Electron Microscope (SEM)[11,12].

Resistance to Gastrointestinal Tract Conditions

Resistance to simulated gastric and intestinal compartments was assessed as described by Lankaputhra and Shah[25], and Truelstrup-Hansen et al[26]. Briefly, non-encapsulated (10^9 CFU/mL) and encapsulated (10^9 CFU/100mg capsule) bacterial suspensions were incubated sequentially in solutions simulating the gastric and intestinal compartments. Then, cell viability was assessed using a plate counting assay.

In vivo Experiments

Six-week-old male Swiss albino mice (Janvier, Le Genest-Saint-Isle, France) were quarantined one week after arrival. They were housed under a controlled environment and standard laboratory conditions with free access to food and water. Mice were then divided into four groups (n=5/group) and fed with 100 μL water for the control group (C), with non-encapsulated bacteria (NE, 10^8 CFU/100 μL), encapsulated bacteria (E, 10^8 CFU/10 mg) and empty capsules (CC) for 21 days. Mice were weighed every two days and their behavior and signs of pain were also analyzed daily. All animal work and procedures complied with the guidelines set forth by the European Economic Community (directive 2010/63/UE). The protocol was approved by both the Animal Research Committee of the Agriculture Ministry and the Ethical Committee C2EA50.

Gastrointestinal Transit and Survival of *P. acidilactici* HM2 in Mice

At the end of the experimental protocol, the GI passage of the strain was explored. Feces of each group were collected before the administration protocol (0 h), every two hours until 16 h, and then every 12 h until 72 h post-feeding, aseptically. Suspensions of fecal samples (0.1 g/mL) were diluted and then plated onto agar selective media (100 μg novobiocin, 10 mg vancomycin, 1 mg ampicillin in 1L MRS). All colonies were counted after the incubation period at 37°C for 48 h. Most were randomly picked up for confirmation using a specific PCR based on the pediocin PA-1 production gene (ped-A gene) (see above). The native strain was used as a control.

Fecal Microbiota Evaluation

After 21 days of probiotic administration, stool samples were collected, and DNA was extracted using the Nucleospin Soil Genomic DNA Isolation kit (Macherey-Nagel, Germany) as previously described[27]. For DGGE profiling, the hypervariable V2-V3 region of the bacterial 16S rRNA gene

region was amplified using universal bacterial primers (HDA1-GC, HDA-2)[28]. DGGE was performed with a DCode Mutation Detection System (Bio-Rad, Hercules, CA, USA) according to the protocol from Carasi et al[29]. Clustering analysis was performed using the UPGMA (unweighted pair group method with arithmetic mean clustering algorithm) linkage GEL-COMPARE (ApplMath Version 4.1).

For quantitative analysis, specific primers for Firmicutes, Bacteroidetes, *Bacteroides fragilis* group, *Prevotella* group, *Bifidobacterium* spp., *Lactobacillus* spp., *Lactobacillus murinus*, *Lactobacillus acidophilus* group, *Escherichia coli*, *Akkermansia muciniphila*, and *Faecalibacterium prausnitzii* were used for PCR reactions which were performed on a CHROMO 4™ System (Bio-Rad) using SYBR Green/ ROX qPCR Master Mix (Bio-Rad, France)[29].

Statistical Analysis

The means of at least three replications for each experiment were analyzed by single factor analysis of variance. Statistical comparisons for significant differences were performed according to GraphPad Prism 6,0 (GraphPad Software, San Diego, CA, USA), using Tukeys' test. A *p*-value <0.05 was considered statistically significant.

RESULTS

During the purification of the bacteriocin, the fraction that displayed the highest antimicrobial activity from the first run on the reverse-phase column was collected and re-chromatographed again. The fraction with a single absorbance pick corresponding to the activity (Figure 1A) was then used for identification. Mass spectrometry analysis confirmed the mono-isotropic molecular mass of the purified bacteriocin to be 4,623 Da (Figure 1B), which was found identical to pediocin PA-1 on the ExPASy database. The activity of the purified bacteriocin against *L. monocytogenes* ATCC 7644 and *L. innocua* CIP 8011 was also confirmed with the inhibition zones as 21±1.8 mm

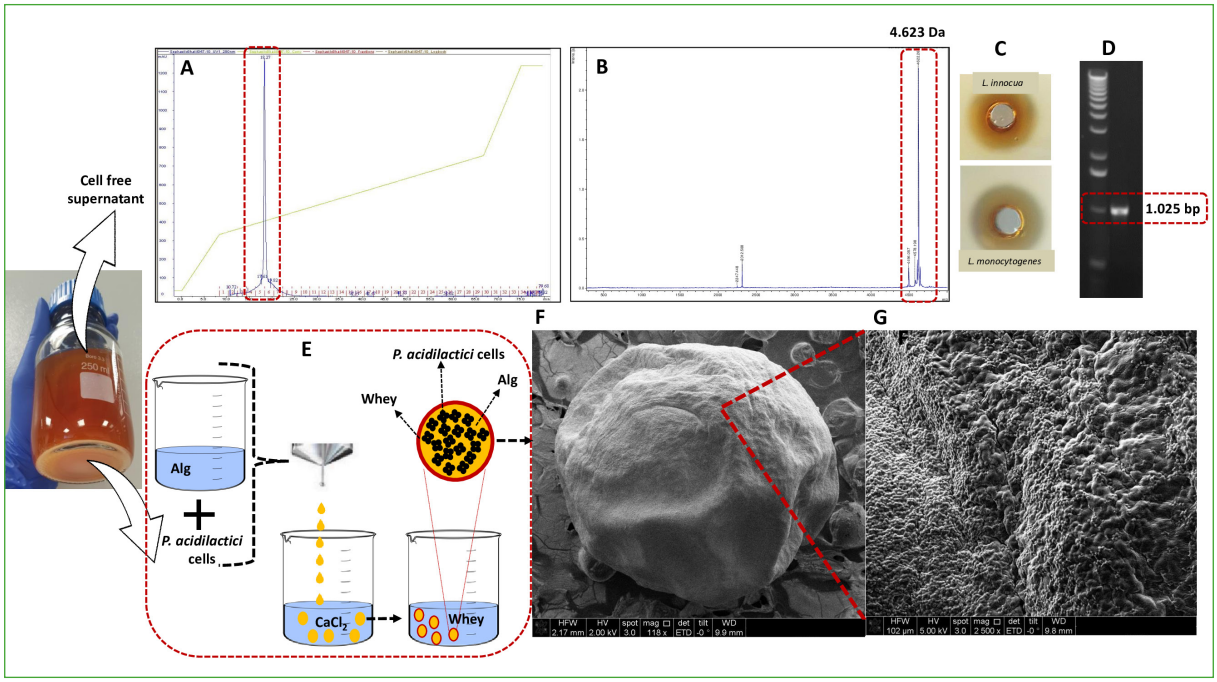

Figure 1. A, Second reversed-phase chromatogram. **B**, Electrospray ionization mass spectrometry analysis of the fraction shown in A. **C**, Inhibition zones of bacteriocin. **D**, Agarose gel electrophoresis of Pediocin PA-1 PCR product. **E**, Schematic diagram of the encapsulation protocol. **F**, General SEM images of capsules. **G**, SEM images of cross sections of the spherical capsules.

and 20±1.2 mm, respectively (Figure 1C). To genetically prove bacteriocin identification, specific PCR primers were used to amplify the pediocin PA-1 peptide region. The PCR fragment found, approximately 1.025 bp by agarose gel electrophoresis (Figure 1D), was then sequenced. The BLAST search on the NCBI database showed that it was 100% identical to the pediocin PA-1 gene of *P. acidilactici* strain MTCC 5101 (GenBank: GQ214404.1).

To increase the cell viability, *P. acidilactici* HM-2 cells were encapsulated using Alg and then coated with whey proteins (Figure 1E). The capsules obtained as a uniformly spherical and white opaque color were about 0.5-0.6 mm in diameter. SEM images also clearly illustrated their shape and wrinkled surface (Figure 1F). When the cross sections of the spherical capsules were analyzed under SEM, it was shown that *P. acidilactici* cells were distributed randomly in the alginate matrix, and whey completely covered the encapsulated cells as a rough surface (Figure 1G).

Following the encapsulation, we investigated the impact of whey protein-coated Alg capsules on the viability of cells when exposed to simulated GI system conditions. Initially, no significant differences between encapsulated and non-encapsulated groups were observed at pH 7 (Figure 2A). Furthermore, the number of viable cells in pH 3 was also found to be similar (Figure 2B). However, non-encapsulated cells completely lost their viability following the exposure to simulated gastric fluid pH 2 after three hours, contrary to the encapsulated cells, presenting more than 7 $\log_{10}$ CFU/mL of viable cells (Figure 2C). No significant differences between encapsulated and non-encapsulated groups were observed after exposure to the simulated small intestine phase, including different concentrations of bile salt (Figure 2D).

In addition to the *in vitro* assays, an *in vivo* mice model was applied to determine the impact of the encapsulation on GI passage of *P. acidilactici* HM-2. Faecal samples were collected at different post-feeding times. Colonies obtained in the selective agar plates were confirmed by a specific PCR, based in the pediocin PA1-gene. Ninety-eight percent of the colonies analyzed were positive for this PCR. The transit study demonstrates that the HM-2 strain was detected after the 6th hour of feeding in the NE and E formulations. The highest amount of the HM-2 strain in faeces was obtained at 10 h for the two formulations. Then it decreased, and no bacteria were detected at the 48th hour and later time points (Figure 3A). Globally, transit time of the

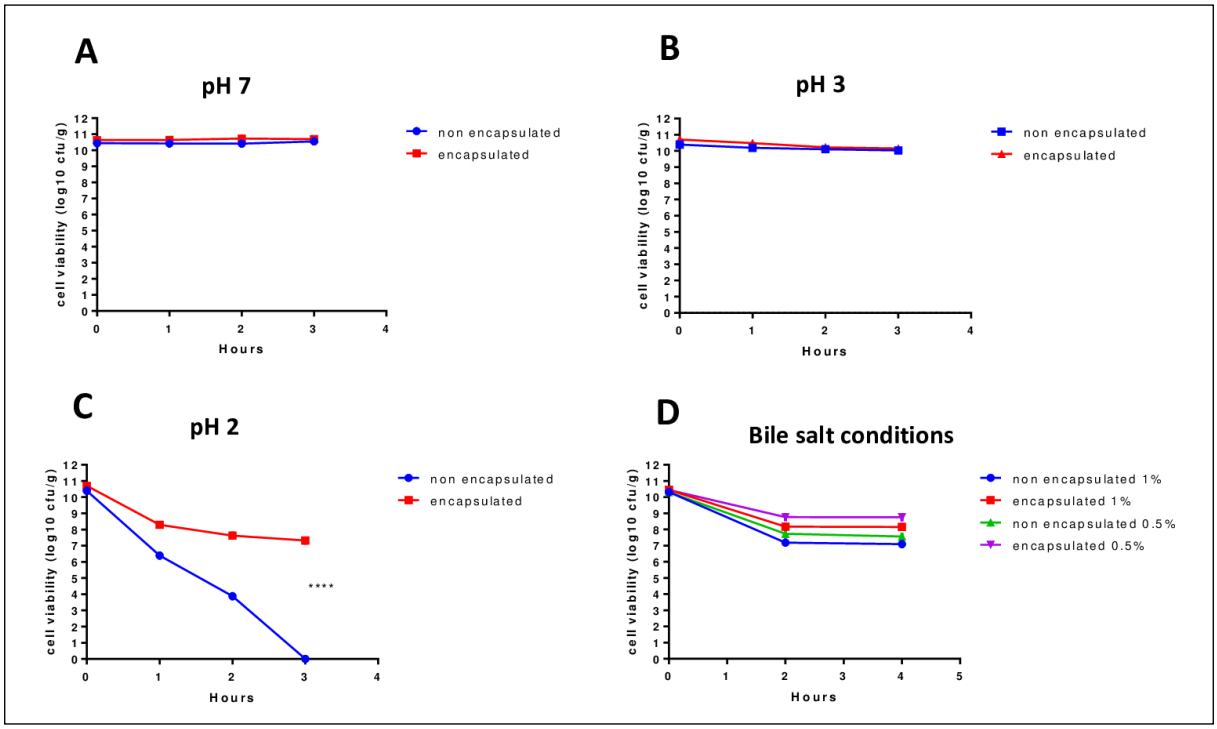

Figure 2. Effect of encapsulation on the viability of *Pediococcus acidilactici* HM-2 cells after simulated gastrointestinal digestion. **A**, pH:7. **B**, Gastric conditions as pH:3 and **C**, pH:2. **D**, Different concentrations of bile salt. ***Indicates 0.001<*p*-value<0.01.

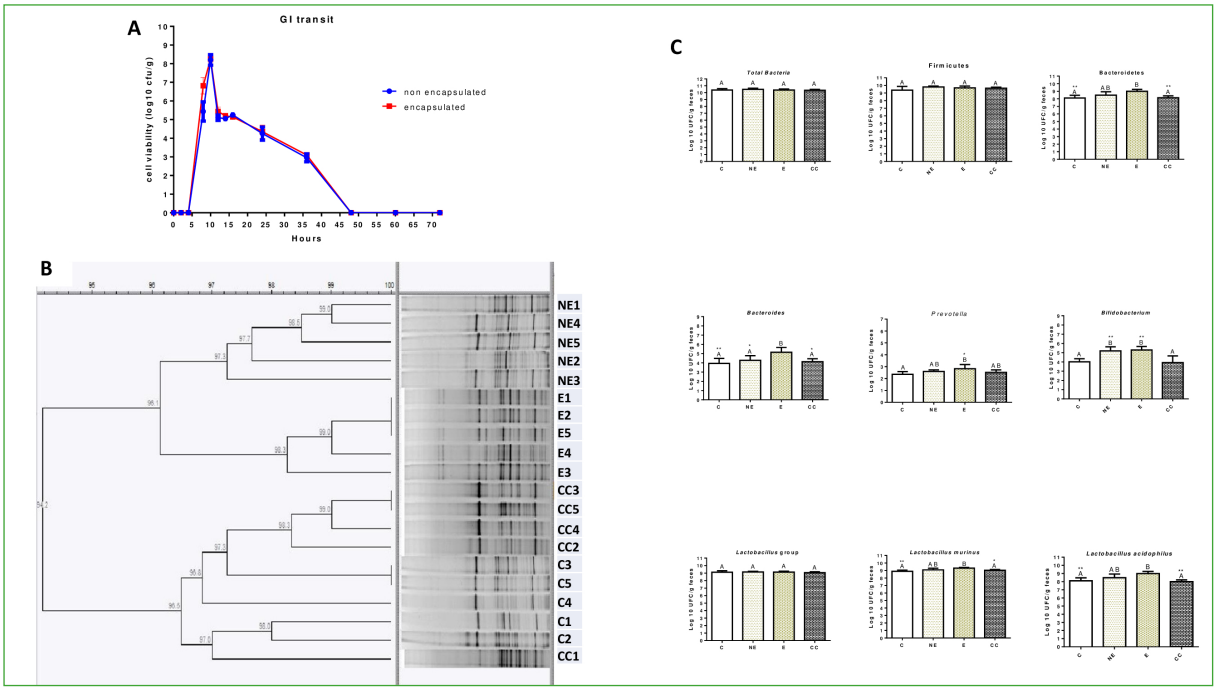

Figure 3. A, Gastrointestinal tolerance of *Pediococcus acidilactici* HM-2 cells after administration to mice. **B** and **C** represent the effect of strain and encapsulation on fecal microbiota. In **B**, DGGE profiles and dendrogram of different groups of treatment (NE, non-encapsulated; E, encapsulated; C, negative control; CC, control capsule not including the viable cells) was shown. In **C**, qPCR quantification of microbiota members in different groups of treatment were given. Data are expressed as mean ± SEM (n=5). On top of each bar, lowercase and capital letters indicate significant differences analyzed by two-way ANOVA followed by Tukey's test ($p<0.05$. The asterisk represents the significant difference ($p<0.05$) ****Indicates $0.001<p$-value<0.01; ***Indicates $0.001<p$-value<0.01; **Indicates $0.001<p$-value<0.01; * $0.01<p$-value<0.05.

free or encapsulated HM-2 strain seemed to be identical. The impact of introducing the bacteriocin producer strain on the gut bacterial populations was investigated using DGGE and qPCR analysis. DGGE analysis generated two principal clusters, one including the encapsulated and non-encapsulated probiotic groups together, with a 96.1% similarity, while the second cluster composed of the negative control and empty capsule groups together, with a 96.5% similarity, indicating probiotic-dependent composition of the microbiota (Figure 3B).

The qPCR analysis of the different groups of microbiota tested revealed some modifications. The two probiotic formulations (NE and E) significantly increased the *Bifidobacterium* spp. Moreover, the E form of *Pediococcus* increased also the Bacteroidetes (*B. fragilis* group and *Prevotella*) and *L. murinus* and *L. acidophilus* species group (Figure 3C).

No significant differences were observed in the other bacterial groups tested (Firmicutes, *Lactobacillus, E. coli*, *Faecalibacterium prausnitzii*, and *Akkermansia muciniphila*). In addition, the empty capsules will not modify the microbial groups tested.

DISCUSSION

An important aspect of a probiotic is that it must remain viable during its use to provide many of its health benefits[30]. Because probiotics can lose viability during gut transit, protection techniques, such as encapsulation could be interesting[9]. In accordance with other authors, the encapsulation of *P. acidilactici* HM-2, using whey-coated Alg capsules, improved its viability at acidic pH, but is not significant in the presence of bile salts, even if we observe a trend[12,13]. Moreover, viable HM-2 cells were recovered in the faeces of mice after their GI transit, in the two formulations (NE and E), with a maximum peak at 10 h, with an average of 8.2×10^8 CFU/g of feces, demonstrating at least a good viability during transit.

Selected LAB can be interesting probiotics[1] and bacteriocin production is considered as an important selection criterion for LAB, however, not all LAB strains harbour bacteriocin genes, even within the same species[20,31]. We demonstrated that the HM-2 strain of *P. acidilactici* produces the pediocin PA-1, a class IIa bacteriocin, presenting a relatively narrow spectrum of inhibition, and presumed to have little effect on the host gut microbiota[5,15]. However, very few *in vivo* studies have investigated the effects of bacteriocin-producing LAB on the gut microbiota, other than studies in which pathogenic bacteria were antagonized[4,15,19] and any studies utilized an encapsulated strain. Our study, using healthy mice, showed that the administration of the *Pediococcus* HM-2 strain increased the *Bifidobacterium*, in the non-encapsulated and encapsulated forms of administration. Interestingly, *Bifidobacterium* is well known to exert positive health benefits in the host, producing important compounds such as B vitamins and healthy short-chain fatty acids (SCFAs)[32].

Furthermore, our study showed that the encapsulated HM-2 strain increased two *Lactobacillus* species, but did overall increase all *Lactobacillus*, indicating an internal competition/reorganization of *Lactobacillus* gender. Besides that, the Bacteroidetes (as *B. fragilis* group and *Prevotella*) were increased, and even without an increase in Firmicutes, the F/B ratio decreased, which appears to be directly related to the form of administration of the probiotic in our study. Bacteroidetes significantly decrease in obese mice, and similarly in patients with metabolic syndrome and type 2 diabetes[33]. The encapsulated HM-2 strain could be a good alternative because it might contribute to a healthy metabolic balance. Jia et al[34] showed an increased level of *Bifidobacterium* and *Bacteroides*, like us, when mice were treated with the bacteriocin nisin; however, nisin can have a much broader spectrum than pediocin. In another study using healthy mice and *L. acidophilus* JCM 1132, despite the narrow antibacterial spectrum of the produced bacteriocin, significant differences in the gut microbiota were observed, as an increase in *Bifidobacterium*, and a reduction in the F/B ratio, implicating preferentially the increase in the Bacteroidete S24-7[35]. Furthermore, a *Pediococcus* producer of pediocin PA-1 increased the *Clostridiaceae* in mice, contrary to the non-producer isogenic mutant[5]. In contrast, the isogenic PA-1 producer or non-producer strain decreased the *Lactobacillus*, *Streptococcus* and *Enterococcaceae*[5]. Results differ slightly from ours, but the authors use a different and non-encapsulated *P. acidilactici* strain. Likewise, Qiao et al[31] screened bacteriocin producer *P. acidilactici* strains and studied their impact on healthy mice microbiota. They observed different variation profiles. One strain increased *Bacteroides* and decreased Firmicutes, but another increased *Bifidobacterium* and *Lactobacillus* principally. The two bacteriocins presented a spectrum of inhibition close to pediocins, but unfortunately, they have not been characterized.

Discrepancies between *in vitro* and *in vivo* spectra of bacteriocin producers on the microbiota can occur due to bacteriocin production, for example, which can be regulated by signal transduction systems (quorum-sensing)[36], as well as feedback through the symbiosis or inhibition of other intestinal bacteria. A bifidobacterial effect, concerning both quantity and functionality, was shown for other microbiota members[37] as the cross-feeding mechanisms between *Bifidobacterium* and *Bacteroides* species[38,39], which could explain some results using bacteriocin producer strains that involved a concomitant increase in both bacteria. *Bacteroides* spp. appear to be critical players in immunomodulation and provide nutrients and vitamin K to the host and other intestinal microbial residents[40].

In addition, our results showed that the encapsulation of the probiotic enhanced the effect of the HM-2 strain on microbiota. This could be explained because the capsules will open in the intestine (at pH 6 to 6.5), releasing probiotic bacteria in its proximity and thus create a punctual cell density, contrary to the non-encapsulated strain. Interestingly, bacteriocins can act as signal peptides, inducing their biosynthesis in a cell density-dependent manner[36] and, in addition to regulating their synthesis, they can engage in interspecies communication or bacterial cross talk[20,] which will create a snowball effect in microbiota modifications.

CONCLUSIONS

In this study, we characterized the bacteriocin produced by the HM-2 strain as pediocin PA-1, a class IIa bacteriocin. Alginate capsules coated with whey proteins enhanced the probiotic strain

viability at acidic pH but did not modify its intestinal transit. Moreover, we showed for the first time that encapsulation of a bacteriocin producer strain could provoke different effects on gut microbiota than the same non-encapsulated strain, probably due to the quorum-sensing and bacterial crosstalk effects. Accordingly, bacteriocin production traits and delivery forms should be considered in applying LAB probiotics.

Conflict of Interest

The authors of this work declared that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Statement

This study was carried out in accordance with the recommendations of the European Economic Community (directive 2010/63/UE). All mice were housed in the Animal Facility of Bordeaux University and all procedures for the care and use of laboratory animals were performed according to the Animal Research Committee of the Agriculture Ministry and the Ethical Committee C2EA50 guidelines.

Acknowledgments

None declared.

Financial Support and Sponsorship

None declared.

Authors' Contributions

Research design and planning was a collaboration between MCU, FK, and MM. Data collection, analysis and interpretation were undertaken predominantly by MM, with guidance and support by both FK and MCU. AME participated in mice experiments and microbiota analysis by DGGE and CJ participated in qPCR microbiota analysis. The manuscript was written by MCU and FK with the support of MM.

ORCID ID

Mohamed Mokrani: https://orcid.org/0000-0001-9942-7853
Claudine Jacquot: https://orcid.org/0000-0002-6927-7986
Fadime Kiran: https://orcid.org/0000-0002-4536-2959
Maria C. Urdaci: https://orcid.org/0000-0002-0868-0050

REFERENCES

1. George Kerry R, Patra JK, Gouda S, Park Y, Shin HS, Das G. Benefaction of probiotics for human health: A review. J Food Drug Anal 2018; 26: 927-939.
2. Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health 2014; 11: 4745-4767.
3. Cavera VL, Arthur TD, Kashtanov D, Chikindas ML. Bacteriocins and their position in the next wave of conventional antibiotics. Int J Antimicrob Agents 2015; 46: 494-501.
4. Anjana, Tiwari SK. Bacteriocin-producing probiotic Lactic Acid Bacteria in controlling dysbiosis of the gut microbiota. Front Cell Infect Microbiol 2022; 12: 851140.
5. Umu ÖC, Bauerl C, Oostindjer M, Pope PB, Hernandez, E, PerezMartinez G. The potential of Class II bacteriocins to modify guť microbiota to improve host health. PloS One 2016; 11: 1-22.
6. Heidebach T, Forst P, Kulozik U. Microencapsulation of probiotic cells for food applications. Crit Rev Food Sci Nutr 2012; 52: 291-311.
7. Cassani L, Gomez-Zavaglia A, Simal-Gandara J. Technological strategies ensuring the safe arrival of beneficial microorganisms to the gut: From food processing and storage to their passage through the gastrointestinal tract. Food Res Int 2020; 129: 108852.
8. Yao M, Xie J, Du H, McClements DJ, Xiao H, Li L. Progress in microencapsulation of probiotics: A review. Compr Rev Food Sci Food Saf 2020; 19: 857-874.

9. Rajam R, Subramanian P. Encapsulation of probiotics: past, present and future. J Basic Appl Sci 2022; 11: 46-64.
10. Zhang Z, Gu M, You X, Sela DA, Xiao H, McClements DJ. Encapsulation of *Bifidobacterium* in alginate microgels improves viability and targeted gut release. Food Hydrocoll 2021; 116: 106634.
11. Kiran F, Mokrani M, Osmanagaoglu O. Effect of encapsulation on viability of *Pediococcus pentosaceus* OZF during its passage through the gastrointestinal tract model. Curr Microbiol 2015; 71: 95-105.
12. Gbassi GK, Vandamme T, Ennahar S, Marchioni E. Microencapsulation of *Lactobacillus plantarum* spp. in an alginate matrix coated with whey proteins. Int J Food Microbiol 2009; 129: 103-105.
13. Doherty SB, Auty MA, Stanton C. Ross RP, Fitzgerald GF, Brodkorb A. Survival of entrapped *Lactobacillus rhamnosus* GG in whey protein micro-beads during simulated ex vivo gastro-intestinal transit. Int Dairy J 2012; 22: 31-43.
14. Zheng J, Ganzle MG, Lin XB, Ruan L, Sun M. Diversity and dynamics of bacteriocins from human microbiome. Environ Microbiol 2015; 17: 2133-2143.
15. Millette M, Cornut G, Dupont C, Shareck F, Archambault D, Lacroix M. Capacity of human nisin- and pediocin-producing lactic acid bacteria to reduce intestinal colonization by vancomycin-resistant enterococci. Appl Environ Microbiol 2008; 74: 1997-2003.
16. Yang SC, Lin CH, Sung CT, Fang JY. Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. Front Microbiol 2014; 5: 241-251.
17. Kwok LY, Guo Z, Zhang J, Wang L, Qiao J, Hou Q. The impact of oral consumption of *Lactobacillus plantarum* P-8 on faecal bacteria revealed by pyrosequencing. Benef Microbes 2015; 64: 405-413.
18. Hernández-González JC, Martínez-Tapia A, Lazcano-Hernández G, García-Pérez BE, Castrejón-Jiménez NS. Bacteriocins from Lactic Acid Bacteria. A powerful alternative as antimicrobials, probiotics, and immunomodulators in veterinary medicine. Animals (Basel) 2021; 11: 979-996.
19. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. Proc Natl Acad Sci U S A 2007; 104: 7617-7621.
20. Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin production: A probiotic trait? Appl Environ Microbiol 2012; 78: 1-6.
21. Nissen-Meyer, J, Holo H, Havarstein LS, Sletten K, Nes IF. A novel lactococcal bacteriocin whose activity depends on the complementary action of two peptides. J Bacteriol 1992; 174: 5686-5692.
22. Diep DB, Godager L, Brede D, Nes IF. Data mining and characterization of a novel pediocin-like bacteriocin system from the genome of *Pediococcus pentosaceus* ATCC 25745. Microbiol 2006; 152: 1649-1659.
23. Cintas LM, Rodriguez JM, Fernandez MF, Sletten K, Nes IF, Hernandez PE, Holo H. Isolation and characterization of pediocin L50, a new bacteriocin from *Pediococcus acidilactici* with a broad inhibitory spectrum. Appl Environ Microbiol 1995; 61: 2643-2648.
24. Rodriguez JM, Cintas LM, Casaus P, Martinez MI, Suarez A, Hernandez PE Detection of pediocin PA-1-producing pediocin by rapid molecular biology techniques. Food Microbiol 1997; 14: 363-371.
25. Lankaputhra WEV, Shah NP. Survival of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in the presence of acid and bile salts. Cult Dairy Prod J 1995; 30: 2-7.
26. Truelstup-Hansen L, Allan-Wojtas PM, Jin YL, Paulson AT. Survival of free and calcium–alginate microencapsulated *Bifidobacterium* spp. in stimulated gastro-intestinal conditions. Food Microbiol 2002; 19: 35-45.
27. Mokrani M, Charradi K, Limam F, Aounani E, Urdaci MC. Grape seed and skin extract, a potential prebiotic with anti-obesity effect through gut microbiota modulation. Gut Pathog 2022; 14: 1-14.
28. Walter J, Tannock GW, Tilsala-Timisjarvi A, Rodtong S, Loach DM, Munro K, Alatossava T. Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. Appl Environ Microbiol 2000; 66: 297-303.
29. Carasi P, Racedo SM, Jacquot C, Romanin DE, Serradell MA, Urdaci MC. Impact of kefir derived *Lactobacillus kefiri* on the mucosal immune response and gut microbiota. J Immunol Res 2015; 2015: 361604.
30. Fuller R. Probiotics in human medicine. Gut 1991; 32: 439-442.
31. Qiao Y, Qiu Z, Tian F, Yu L, Zhao J, Zhang H, Zhai Q, Chen W. Effect of bacteriocin-producing *Pediococcus acidilactici* strains on the immune system and intestinal flora of normal mice. Food Sci Hum Wellness 2022; 11: 238-246.
32. Hills RD, Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut microbiome: Profound implications for diet and disease. Nutrients 2019; 11: 1613-1653.
33. Zhu G, Ma F, Wang G, Wang Y, Zhao J, Zhang H, Chen W. Bifidobacteria attenuate the development of metabolic disorders, with inter-and intra-species differences. Food Funct 2018; 9: 3509-3522.
34. Jia Z, Chen A, Bao F, He M, Gao S, Xu J, Zhang X, Niu P, Wang C. Effect of nisin on microbiome-brain-gut axis neurochemicals by *Escherichia coli*-induced diarrhea in mice. Microb Pathog 2018; 119: 65-71.
35. Wang G, Yu Y, Garcia-Gutierrez E, Jin X, He Y, Wang L, Tian P, Liu Z, Zhao J, Zhang H, Chen W. *Lactobacillus acidophilus* JCM 1132 strain and its mutant with different bacitracin-producing behaviour have various in situ effects on the gut microbiota of healthy mice. Microorganisms 2019; 8: 49-75.
36. Kleerebezem M, Quadri LE, Kuipers OP, de Vos WM. Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. Mol Microbiol 1997; 24: 895-904
37. Sugahara H, Odamaki T, Fukuda S, Kato T, Xiao JZ, Abe F, Kikuchi J, Ohno H. Probiotic *Bifidobacterium longum* alters gut luminal metabolism through modification of the gut microbial community. Sci Rep 2015; 5:13548.
38. Sonnenburg JL, Chen CT, Gordon JI. Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. PLoS Biol 2006; 4: e413.
39. Rios-Covián D, Sánchez B, Salazar N, Martínez N, Redruello B, Gueimonde M, de Los Reyes-Gavilán CG. Different metabolic features of *Bacteroides fragilis* growing in the presence of glucose and exopolysaccharides of bifidobacteria. Front Microbiol 2015; 6: 825-838.
40. Zafar H, Saier MH, Jr. Gut *Bacteroides* species in health and disease. Gut Microbes 2021; 13: 1-20.