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Original Article

Prebiotic Properties and Encapsulation Potential of Chenopodium quinoa Seed Mucilage on *Lactobacillus acidophilus* ATCC 4356

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Abstract

Background & objectives: Growing demand for functional foods and the importance of gut microbiota have highlighted the need for strategies to enhance the survival of probiotic strains during processing and through gastrointestinal tract. Plant-derived mucilages are promising biopolymers that may serve as both prebiotics and protective matrices. This study aimed to investigate the prebiotic effect of Quinoa (*Chenopodium quino*a) seed mucilage on the growth and survival of *Lactobacillus acidophilus* ATCC 4356 and to evaluate its role in alginate–mucilage encapsulation for improved probiotic protection under simulated gastrointestinal (GI) conditions.

Material & Methods: Quinoa mucilage was extracted and incorporated into culture media in different concentrations (up to 1% w/v). Probiotic growth stimulation was compared with controls like chitosan. *Lactobacillus acidophilus* cells were encapsulated in alginate–mucilage beads, and encapsulation efficiency (EE) was determined. Free and encapsulated cells survival was assessed under simulated gastric and bile conditions. Antimicrobial activity against *E. coli*, *S. aureus*, *S. enterica*, and *Candida albicans* was evaluated using the well-diffusion method. Capsule morphology was observed by light microscopy and SEM.

Results: Quinoa mucilage significantly stimulated the growth of *L. acidophilus*, with the highest effect at 1% (w/v). Alginate–mucilage encapsulation provided greater protection under acidic and bile conditions compared with free cells, although encapsulation efficiency was moderate (16.71% ± 5.81). Formulations containing mucilage exhibited measurable antimicrobial activity, most notably against *C. albicans*. Microscopy confirmed the integrity of the capsules and the distribution of cells within the matrix.

Conclusion: Quinoa seed mucilage demonstrates prebiotic potential and protective effects in encapsulation. Findings suggest its applicability in functional food formulations.

Keywords: Quinoa seed, Mucilage, Prebiotic, Probiotic, *Lactobacillus acidophilus*, Encapsulation.

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Introduction

The human gut microbiome is a complex ecosystem composed of trillions of microorganisms that play essential roles in digestion, nutrient absorption, immune modulation, and overall health (1). Disruption of this microbial balance, known as dysbiosis, has been associated with a wide range of diseases, including obesity, diabetes, inflammatory bowel disease, and neurological disorders (2,3). Consequently, strategies to maintain or restore a balanced microbiota have become a central focus in nutrition and health sciences. Among these, probiotics, prebiotics, synbiotic, and, more recently, postbiotics have attracted considerable attention(4,5).

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer health benefits on the host" (6). Species belonging to the genera *Lactobacillus* and *Bifidobacterium* are among the most widely used probiotics in dairy and non-dairy products (7,8). *Lactobacillus acidophilus* is of particular importance due to its ability to survive in acidic conditions, its anti-pathogenic effects, and its capacity to improve host immunity, cholesterol metabolism, and lactose intolerance symptoms (9-12). Despite these benefits, the viability of probiotics during industrial processing, storage, and gastrointestinal transit remains a major limitation (13).

To address this challenge, the incorporation of prebiotics has proven to be an effective strategy. Prebiotics are non-digestible food ingredients that selectively stimulate the growth and activity of beneficial probiotic microorganisms in the gut (14,15). Various compounds, including inulin, fructo-oligosaccharides (FOS), galacto oligosaccharides (GOS), and resistant starches, have been investigated for their prebiotic properties (16). By serving as fermentable substrates, prebiotics increase short-chain fatty acid production, improve gut integrity, regulate metabolism, and strengthen the immune system (17-21). Recent studies also suggest that prebiotics can modulate the gut-brain axis, contributing to improved mental health outcomes such as reduced anxiety and depression (22,23).

The combined use of probiotics and prebiotics, known as synbiotics, has demonstrated synergistic effects in enhancing probiotic viability and health benefits (24-26). However, the effectiveness of these formulations depends on the stability and survival of probiotic strains in adverse environments such as gastric acidity and bile salts (27). In this regard, encapsulation has emerged as a promising approach to improve probiotic delivery. By entrapping cells within protective matrices, encapsulation enhances their survival through processing and gastrointestinal passage, while allowing controlled release at target sites (28-30).

Plant-derived biopolymers, particularly mucilages, have recently gained interest as both prebiotic agents and encapsulation materials. Mucilages are hydrophilic polysaccharides with functional properties such as viscosity enhancement, film formation, and water retention (31). They are biodegradable, biocompatible, and abundant, making them suitable for applications in functional foods and pharmaceuticals. Several mucilages from seeds and fruits have been tested for their ability to support probiotic growth and survival, with promising results (31,32).

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal rich in proteins, essential amino acids, minerals, and bioactive compounds (33, 34). In addition to its nutritional value, quinoa seeds contain a mucilaginous layer with unique rheological and biological properties. Although quinoa has been extensively studied for its health-promoting effects, its mucilage remains underexplored as a potential prebiotic and encapsulation agent. Given its natural abundance and functional versatility, quinoa mucilage may represent an innovative resource for improving probiotic viability in functional foods (35,36).

In light of these considerations, the present study was designed to (I) evaluate the prebiotic effect of quinoa seed mucilage on the growth of *Lactobacillus acidophilus* ATCC 4356, (II) investigate its role in alginate–mucilage encapsulation to enhance probiotic survival under simulated gastrointestinal conditions, and (III) assess the antimicrobial activity of these formulations against selected pathogens. The findings are expected to provide insights into the potential applications of quinoa mucilage in the development of novel synbiotic formulations and functional foods.

Materials and Methods

A) Bacterial strain and culture conditions: *Lactobacillus acidophilus* ATCC 4356 was obtained from the Iranian Genetic Resources Center and maintained in de Man, Rogosa, and Sharpe (MRS, Merck, Germany) broth at 37 °C under microaerophilic conditions. For long-term preservation, bacterial suspensions were stored in tryptic soy broth (TSB, Merck, Germany) supplemented with 20% glycerol at –20 ° Celsius (37, 38). Prior to experiments, Gram staining and biochemical tests catalase and oxidase were performed to ensure the purity (37,39).

B) Extraction of quinoa seed mucilage: *Chenopodium quinoa* seeds were purchased from a local market and thoroughly washed to remove impurities. Mucilage was extracted using the standard aqueous method described in previous studies. Briefly, seeds were soaked in distilled water for 24 hours, and the mucilaginous fraction was separated by filtration and drying at 45 °Celsius. The dried mucilage stored in airtight containers until use. Different concentrations of mucilage solutions (0.2–1.0% w/v) were prepared by dissolving the dried mucilage in sterile distilled water under constant stirring (40,41).

C) Prebiotic Activity Score: The prebiotic potential of quinoa seed mucilage (QSM) was determined using the Prebiotic Activity Score (PAS) with a slight modification. Briefly, 0.5 g of QSM, glucose in 30 milliliters of distilled water and 0.5 g of Chitosan in 30 milliliters of 1% acetic acid were dissolved and a glucose-free culture medium (MRS Modified) was prepared and 300 µl of microbial suspension was added to all of them. Then they incubated for 48 hours at 37°Celsius and then counted the bacteria. PAS was calculated according to the following formula (42).

$$\text{Log viable cell count (CFU/ml)} = \text{Log}\ \frac{\text{Mean number of cells in triplicates}}{\text{Volume plated x dilution factor}}$$

$$\text{Prebiotic scores} = \frac{[\text{log (CFU/ml) on prebiotic at 24 h} - \text{log (CFU/ml) on prebiotic at 0 h}]}{[\text{log (CFU/ml) on glucose at 24 h} - \text{log (CFU/ml) on glucose at 0 h}]}\ \text{X 100}$$

D) Determination of the optimal prebiotic concentration: *Lactobacillus acidophilus* was inoculated into MRS broth containing different concentrations of quinoa mucilage (QSM) (0.2%, 0.5%, 0.8% and 1% of mucilage and a control medium without mucilage) and the number of viable microorganisms was counted at time zero and 36 hours after cultivation at 37°C. The pH and acidity of the culture medium were also measured every 12 hours. The volume required was recorded and Titrable acidity was expressed as a percentage of lactic acid. Different concentrations of quinoa mucilage (QSM) in 40 milliliters of MRS broth was dissolved. 0.5 McFarland concentration of bacterial suspension was prepered and diluted 5 times (to 10^3). For each concentration, 1% of the medium into each separate container was poured (4 different concentrations of mucilage. 1 milliliter of each was taken and diluted it and counted it. pH and acidity was measured. This process were repeated at 12 and 36 hours (42). Finally, the acidity level was calculated from the following formula (42).

Consumption = NaOH acidity × 0.9 / sample volume

E) Encapsulation procedure: Encapsulation of *L. acidophilus* cells was performed by ionic gelation using alginate and quinoa mucilage as wall materials. Freshly harvested bacterial cells were mixed with sterile sodium alginate (2% w/v) containing quinoa mucilage, and the mixture was extruded dropwise into a sterile $CaCl_2$ solution (0.1 M) under constant stirring. The resulting beads were allowed to harden for 30 minutes, washed with sterile saline, and stored at 4 °C until further use. Encapsulation efficiency (EE) was calculated as the percentage of viable cells recovered after encapsulation relative to the initial cell load (43). The encapsulation efficiency was calculated using the following formula (43).

EE = Free bacteria / Encapsulated bacteria × 100

F) Microscopy and morphological analysis: The morphology and surface characteristics of the alginate–mucilage beads were examined using light microscopy and scanning electron microscopy (SEM). SEM images were obtained after dehydration and gold coating of samples, providing insights into bead integrity and bacterial entrapment within the matrix (30).

G) Assessment of probiotic survival under simulated gastrointestinal conditions: The survival of free and encapsulated *L. acidophilus* was evaluated under simulated gastric juice (SGJ, pH 2.0 with pepsin) and simulated bile solution (0.3% oxgall) following standard protocols (44, 45). Samples were incubated at 37 °C, and aliquots were collected at specified time intervals 3 and 8 hours for viable cell enumeration on MRS agar (44,45).

H) Antimicrobial activity assay: The antimicrobial potential of free and encapsulated probiotic formulations was tested against common gastrointestinal pathogens, including *Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 14028, *Staphylococcus aureus* ATCC 25923, and a clinical isolate: *Candida albicans*. The well-diffusion method was used, and inhibition zones were measured after incubation for 24 hours at 37 Celsius degree on Muller-Hinton agar (Merck, Germany) media (46).

I) Stability under storage conditions: This study investigated the survival of encapsulated bacteria at 4 and 25°C on days 0, 20 and 30. Encapsulated bacteria (1 g) and free-cell bacteria (1 milliliter) were suspended in PBS (pH 7.4) at a ratio of 1:10. The mixtures were incubated separately at 4 and 25°C. Samples were collected on days 0, 20 and 30 to count the number of viable probiotic cells (43).

J) Statistical analysis: All experiments were performed in triplicate. Results were expressed as mean ± standard deviation (SD). Statistical differences among treatments were determined using one-way analysis of variance (ANOVA) followed by Tukey's test, with $p < 0.05$ considered significant (13).

Results

A) Prebiotic activity of quinoa seed mucilage: The growth of *Lactobacillus acidophilus* ATCC 4356 was significantly affected by the addition of quinoa seed mucilage. The prebiotic activity score (PAS) averaged 87.98 ($p < 0.05$) (Table 1 ,(and among the concentrations tested, 1% (w/v) mucilage showed the strongest stimulatory effect, consistent with acid production and pH reduction. Lower concentrations (0.25% and 0.5%) also increased bacterial growth, but to a lesser extent (Figure 1 .(These findings suggest that quinoa mucilage can act as an effective substrate for the growth of *L. acidophilus*.

Table 1. The highest average value was observed in the Modified MRS group and the lowest value was observed in the chitosan group. The glucose group was recorded without dispersion (fixed).

Sample group	confidence %95 interval for the mean	Max	Min	Variance	SD	Mean	N
MRS Modified	53.79-70.98	66.38	60.31	11.98	3.46	62.38	3
Glucose	100-100	100	100	0	0	100	3
	114.99-176.63	154.7	131.6	153.92	12.41	145.81	3
Quinoa M	66.57-109.39	97.52	80.75	74.26	8.62	87.98	3

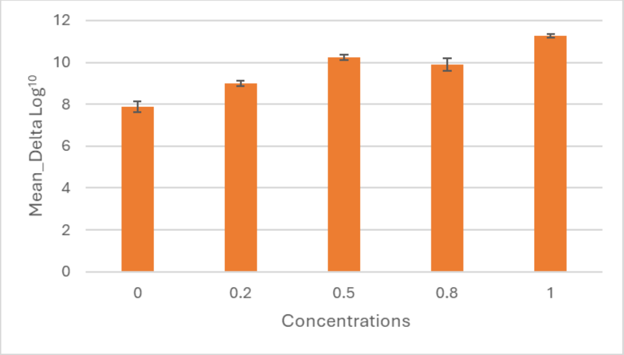

Fig 1. Average logarithmic change of bacterial population at different concentrations (mg/ml).

B) Encapsulation efficiency: Encapsulation of *L. acidophilus* cells using alginate–mucilage beads achieved a mean encapsulation efficiency (EE) of 16.71% ± 5.81. Although the encapsulation yield was moderate, the addition of mucilage improved bead stability and structural integrity compared with alginate alone.

C) Morphological analysis: Light microscopy confirmed the spherical shape and uniform size of the alginate–mucilage beads. SEM images of the encapsulated samples showed the formation of encapsulated particles with diverse and mostly irregular morphology (Fig 2). According to Fig 2. a, microscopic image of encapsulated bacteria with magnificent of 100 has been represented and microcapsule formation is visible. Also, Fig1. part b to e visualized the electron graphs of successful entrapped bacteria in to encapsulated structure by mucilage. The outer surface of the capsules is rough and porous, which is probably due to the presence of mucilage and emulsifiers in the alginate matrix. This feature can both enhance the exchange of substances and improve the mechanical protection of the encapsulated bacteria. The presence of smaller spherical particles around the capsules indicates the entrapment of microemulsion droplets and the effect of rapeseed oil and Tween 80. The aggregation and cohesion of particles in some images could be due to the high concentration of polysaccharides or the high gelation rate. At higher magnifications, protrusions and structures with cellular dimensions were observed, indicating the entrapment of *Lactobacillus acidophilus* cells in the alginate-mucilage matrix, indicating the success of the method in protecting and trapping bacteria.

D) Survival under simulated gastrointestinal conditions: The survival of free and encapsulated cells in simulated gastric (pH 2.0) and bile

(0.3% oxgall) environments was compared. Free cells showed a sharp decrease in viability after 3 h in SGJ and 8 h in bile conditions, whereas encapsulated cells maintained significantly higher numbers ($p<0.05$). A similar protective effect was observed under bile conditions, where encapsulated cells showed better tolerance compared to free cells (Table 2).

E) Antimicrobial activity: The antimicrobial activity of free and encapsulated formulations was evaluated against selected pathogens. Zones of inhibition were measured for *E. coli*, *S. aureus*, *S. enterica*, and *C. albicans*. The strongest inhibitory effect was against *S. aureus* (17 ± 0.9 mm), while inhibition against Gram-negative bacteria was moderate (Fig 3).

F) Stability under storage conditions: Free samples showed a sharp decline at both temperatures until day 30, while encapsulated samples had a smaller decline and, especially at 4 °C, maintained relatively higher values at day 30. table 3 shows the mean change in Log (CFU/ml) of probiotic bacteria in encapsulated (Cap) and free (Free) samples at storage temperatures of 4 °C and 25 °Celsius over 30 days on days 0, 20 and 30.

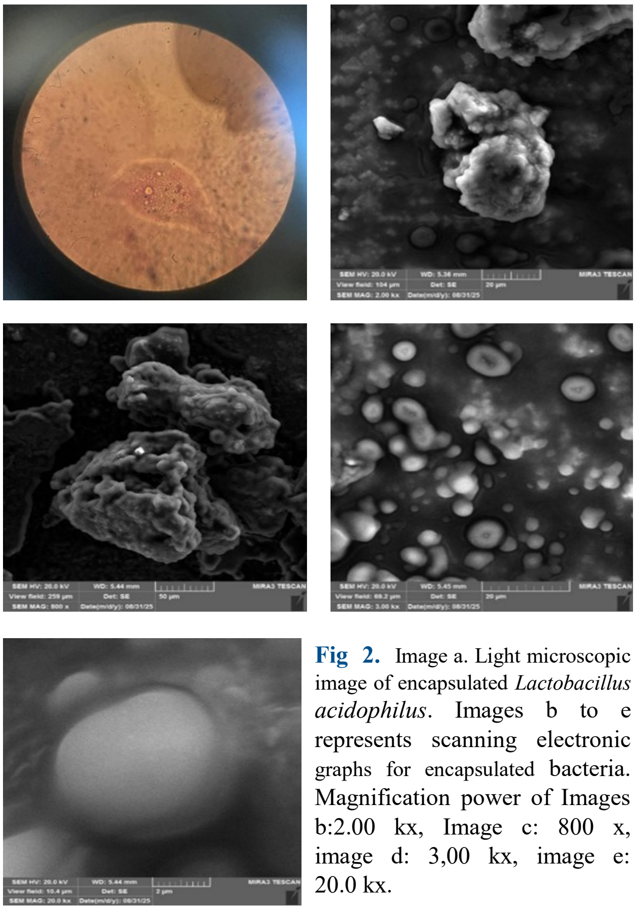

Fig 2. Image a. Light microscopic image of encapsulated *Lactobacillus acidophilus*. Images b to e represents scanning electronic graphs for encapsulated bacteria. Magnification power of Images b:2.00 kx, Image c: 800 x, image d: 3,00 kx, image e: 20.0 kx.

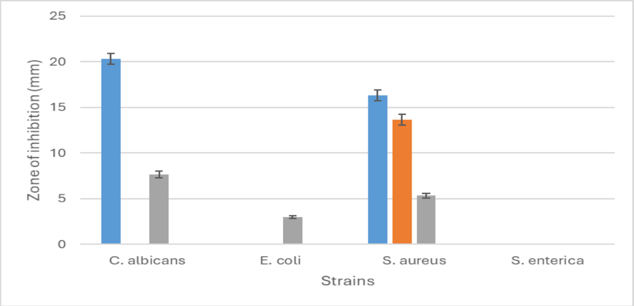

Fig 3. Mean size of the zone of inhibition ± SD), mm (for each strain (*C. albicans*, *E. coli, S. aureus, S. enterica*) in four treatments: Antibiotic (oxacillin), Cap (quinoa mucilage), Ethanol 70% and Water (negative control). (n = 3). (Blue column: Antibiotics, Orange column: capsule, Grey column: Ethanol)

Discussion

This study demonstrated that quinoa seed mucilage (QSM) serves a dual role as both a prebiotic and a protective encapsulation material for *Lactobacillus acidophilus* ATCC 4356. The growth-promoting effect of QSM was most

Table 2. Values represent the mean ± standard deviation of bacterial counts at time points 0 and 3 hours in gastric conditions and Optical Density of samples at time points 0 and 8 hours in biliary conditions in both free and encapsulated groups.

Bacteria group	OD_8 (Mean ± SD)	OD_0 (Mean ± SD)	t=3 (Mean ± SD)	t=0 (Mean ± SD)
Capsulated	0.171 ± 0.003	0.335 ± 0.009	7.55 ± 0.31	9.17 ± 0.45
Free	0.068 ± 0.005	0.264 ± 0.009	7.66 ± 0.90	9.97 ± 0.55

pronounced at 1% (w/v), where the prebiotic activity score (PAS) was significantly higher than that of the control. These findings are consistent with previous reports showing that plant-derived polysaccharides such as inulin, resistant starch, and seed mucilage selectively stimulate lactic acid bacteria (16,47). The polysaccharide-rich structure of QSM, particularly its soluble fibers and arabinogalactan fractions, may provide fermentable substrates for *L. acidophilus* metabolism, thereby enhancing its growth and survival (36). Similar growth stimulation has been reported for flaxseed mucilage, indicating that polysaccharides from diverse plant sources can act as efficient prebiotic agents(41).

The encapsulation efficiency (EE) of *L. acidophilus* in alginate–QSM beads was moderate (16.71% ± 5.81). Although this value is lower than those reported for some alginate-inulin or alginate–chitosan systems (28, 30), the addition of QSM clearly improved bead stability and uniformity compared with alginate alone. The reduced EE may be attributed to structural properties of QSM, such as lower viscosity or limited interactions with alginate molecules during ionic gelation. This limitation suggests that optimization of process variables, including polymer ratio, $CaCl_2$ concentration, and bead hardening time, is necessary to increase encapsulation yield (29). Nonetheless, the enhanced structural stability observed in SEM images highlights QSM’s potential as a complementary encapsulation agent.

The protective role of QSM was evident under simulated gastrointestinal conditions. Encapsulated cells exhibited significantly higher survival rates than free cells when exposed to acidic gastric juice (pH 2.0) and bile salts. This is consistent with earlier findings that encapsulation in polysaccharide matrices enhances probiotic tolerance to GI stress (13,27). The mechanism likely involves the formation of a semi-permeable barrier by mucilage polysaccharides, which reduces the diffusion of hydrogen ions and bile salts into the microenvironment surrounding bacterial cells. Moreover, the hydrophilic and viscous properties of mucilage can provide an additional buffering effect, thereby prolonging cell viability during transit.

A noteworthy observation was the antimicrobial activity exhibited by the encapsulated formulations. Inhibition zones were detected against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, and *Candida albicans*, with the strongest effect observed against *S. aureus*. Previous studies have shown that probiotics produce antimicrobial substances such as lactic acid, acetic acid, hydrogen peroxide, and bacteriocins (48,49). The stronger inhibition observed in encapsulated cells may be attributed to the controlled release of these metabolites, which accumulate around the beads and create a localized antimicrobial environment. This suggests a novel role for QSM in enhancing not only probiotic viability but also their antagonistic activity.

Microscopic and SEM analyses further supported the protective role of QSM. The encapsulated bacteria with Quina granules exhibited a morphology with rough surfaces of entrapped bacterial cells. These results are consistent with previous encapsulation studies using other plant polysaccharides, where the combination of mucilage and gum resulted in improved granule morphology and resistance to environmental stress (30,31). The stability of QSM alginate granules under simulated gastrointestinal conditions suggests their suitability for use in functional foods, nutraceuticals, and potential pharmaceutical probiotic formulations. Quinoa is widely cultivated as a pseudocereal and is recognized for its nutritional and functional properties (33,34). Utilizing its mucilage, often considered a by-product, adds value to quinoa processing and provides a sustainable resource for functional food applications (35,22) Compared with synthetic prebiotics and encapsulating materials, QSM offers the advantages of natural origin, biocompatibility, and consumer acceptability, aligning with the growing demand for clean-label and plant-based ingredients.

Despite the promising results, certain limitations must be acknowledged. First, the encapsulation efficiency was lower than optimal, indicating a need for further process optimization. Second, the antimicrobial activity of QSM–encapsulated probiotics requires mechanistic clarification, as the specific compounds responsible were not identified in this study. Finally, the current research was limited to *in vitro* conditions; *in vivo* studies are necessary to confirm the prebiotic and protective effects of QSM in animal models and human trials.

All in all, the present study highlights quinoa seed mucilage as a promising prebiotic and encapsulation material for *L. acidophilus*. Its ability to stimulate probiotic growth, enhance survival under GI conditions, and exhibit antimicrobial activity positions it as a valuable candidate for synbiotic formulations. Future research should focus on process optimization, molecular characterization of mucilage components, and *in vivo* validation to fully establish its potential for industrial applications.

Conclusion

The present study demonstrated that quinoa seed mucilage (QSM) exhibits both prebiotic properties and protective effects in probiotic encapsulation. Supplementation of the culture medium with QSM significantly promoted the growth of *Lactobacillus acidophilus* ATCC 4356, confirming its potential as a fermentable substrate for beneficial gut microbiota. Furthermore, the incorporation of QSM into alginate beads enhanced probiotic survival under simulated gastrointestinal conditions and increased antimicrobial activity, particularly against *S. aureus*. Morphological analysis confirmed the stability and integrity of the alginate–mucilage capsules. Overall, these findings highlight QSM as a natural, plant-derived biopolymer with promising applications in functional foods and synbiotic formulations. Nevertheless, further studies are warranted to optimize encapsulation efficiency, identify the bioactive compounds responsible for antimicrobial activity, and validate these effects in *in vivo* models.

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Statements and Declarations

I hereby declare the information given above and in the enclosed document is true to the best of my knowledge and belief and nothing has been concealed therein.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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