ARTICLE

Assessment of the functional properties of probiotic-loaded alginate beads and their effects on the growth performance of juvenile Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Probiotics play a crucial role in improving aquaculture productivity, but their integration in aquaculture farming is restricted by environmental and biological factors. To address these limitations, alginate-based encapsulation was explored for improved functionality and efficient probiotic delivery in tilapia aquaculture. Probiotic isolates, including *Lacticaseibacillus* sp. FSPL001, *Saccharomyces* sp. FSPL011, and *Bacillus* sp. FSPL020, were encapsulated within a sodium alginate/soy protein isolate (SA/SPI) polymer matrix coated with carboxymethyl cellulose (CMC) to produce probiotic-loaded alginate beads (PLABs). High encapsulation efficiency was achieved, with encapsulation rates exceeding 95% and viability counts reaching at least 1 × 10^7 CFU/g beads. Furthermore, encapsulation significantly enhanced probiotic tolerance to biological barriers, including low pH and bile, while maintaining stability under high salinity. The SA/SPI polymer matrix displayed pH-sensitive dynamic swelling behavior, enabling a controlled-release mechanism as confirmed by *in vitro* release assays during simulated gastrointestinal digestion. Moreover, the use of the CMC coating notably enhanced the mucoadhesive properties of PLABs, ensuring effective attachment to the mucosal epithelium and minimizing bead wash-off during digestion. The development of PLABs permitted the easy integration of probiotic supplementation into tilapia feeding regimens through direct oral administration. Significant increases in body weight gain

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KEYWORDS

Aquaculture, alginate encapsulation, probiotics, Nile tilapia, *Lacticaseibacillus* sp., *Saccharomyces* sp., *Bacillus* sp., controlled-release mechanism

were observed after 70 days of culture with no adverse effects on feed utilization. These findings highlight the potential of PLABs as an efficient and targeted delivery system for probiotics, offering promising prospects for enhancing aquaculture productivity.

INTRODUCTION

Probiotics have garnered considerable attention in aquaculture systems because of their multi-functional and technological properties in improving growth performance, resisting diseases, enhancing immunity, and improving water quality (Lara et al. 2003, Verschuere et al. 2000). The proposed mechanisms of action for probiotics include competitive exclusion of pathogens, competition for nutrients, adhesion site competition, improvement of digestion, contributions to macro- and micronutrients, immunomodulation, and neurotransmitter production (Latif et al. 2023, Plaza-Diaz et al. 2019). Some of the most studied probiotic microorganisms and their applications in aquaculture include lactic acid bacteria and yeast, which are known growth promoters that maintain the microbial balance in the gut (del Valle et al. 2023, Coulibaly et al. 2023, Ringø et al. 2020). *Bacillus* species have also displayed efficacy in maintaining the aquaculture rearing environment owing to their high capacity for nutrient cycling (Gobi et al. 2018, Kuebutornye et al. 2019, Ringø et al. 2020, Verschuere et al. 2000).

However, the successful incorporation of probiotics into aquaculture farming faces several challenges, primarily related to their susceptibility to adverse environmental conditions such as water temperature, pH, and salinity. This can compromise their functionality and efficacy in eliciting functionality in the host organism (Iribarren et al. 2012). Microencapsulation has emerged as a promising strategy for enhancing probiotic viability and bioavailability in aquaculture species. This process involves encapsulating microbial cells within a colloidal framework using materials such as pectin, sodium alginate (SA), chitosan, and even modified carboxymethylcellulose (CMC). These materials provide robust physical and chemical protection (Zhang et al. 2022). Recent studies reported promising results in improving probiotic stability and survival through encapsulation. For instance, Bevilacqua et al. (2020) successfully encapsulated *Saccharomyces cerevisiae* in an alginate matrix, leading to improved stability for up to 30 days. Similarly, Song et al. (2018) found that the survival of encapsulated probiotics was enhanced under simulated gastrointestinal digestion conditions through chemical modification of the alginate matrix via chitosan coating. Additionally, de Araújo Etchepare et al. (2016) improved the shelf life of *Lactobacillus acidophilus* by adding protein macromolecules such as maize protein hydrolysates into the core of microcapsules.

Considering the environmental challenges inherent in aquaculture farming and the potential benefits of probiotics in enhancing the growth of aquaculture species, the limitations of probiotic integration were addressed in the present study by exploring encapsulation in an appropriate matrix for improved functionality and efficient probiotic delivery in tilapia aquaculture. Specifically, the study evaluated the functional properties of probiotic isolates from *Lacticaseibacillus*, *Bacillus*, and *Saccharomyces* spp. following encapsulation using an SP/soy protein isolate (SPI) polymer matrix. Furthermore, this study elucidated the mucoadhesive properties and controlled-release mechanism of the system during simulated gastrointestinal digestion and evaluated its performance as a potential feed supplement for tilapia.

MATERIALS AND METHODS

Chemicals and culture media

All chemicals used were of analytical grade unless otherwise stated. SA, calcium chloride, CMC, pepsin, bile salts, sodium bicarbonate, sodium citrate, and citric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride and sodium hydroxide were sourced from Techno PharmChem (India). SPI was obtained from a local supplier. Hydrochloric acid (37% w/w) was acquired from RCI Labscan Group (Bangkok, Thailand). Nutrient broth (NB), de Man–Rogosa–Sharpe (MRS) broth, and yeast peptone dextrose (YPD) broth were sourced from HiMedia (Maharashtra, India). Bacteriological-grade agar was obtained from Condalab (Madrid, Spain).

Microorganisms

Strains of *Lacticaseibacillus* sp. FSPL001, *Saccharomyces* sp. FSPL011, and *Bacillus* sp. FSPL020 were obtained from the culture collection of Feeds and Specialty Products Laboratory, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (Laguna, Philippines). These microorganisms were previously identified and confirmed to have desirable probiotic characteristics such as non-hemolytic activity, tolerance to gastrointestinal conditions, and antimicrobial compound production (NG Dumandan, unpublished observations).

Encapsulation of probiotic strains in the SA/SPI matrix

Encapsulation of the probiotic strains was done using an SA/SPI matrix following the method of Solanki and Shah (2016) with modifications. In brief, cells from 24-h old cultures of *Lacticaseibacillus* sp. FSPL001, *Saccharomyces* sp. FSPL011, and *Bacillus* sp. FSPL020 grown in MRS broth, YPD broth, and NB, respectively, were harvested by centrifugation at 4°C at 10,000 rpm for 10 min. The cells were then washed and resuspended in saline solution (0.9% NaCl w/v) and subsequently mixed in a mixture of sterile SA (5.0% w/v) and SPI (3.0% w/v). The resulting mixture was then slowly extruded into $CaCl_2$ solution (3.0% w/v) using a syringe and permitted to harden for 20 min to produce probiotics-loaded alginate beads (PLABs). The produced beads were then coated with CMC solution (0.2% w/v) for 1 min and then stored at 4°C until further analyses.

Encapsulation efficiency, size, and morphological structure

The efficiency of probiotic encapsulation into the SA/SPI matrix was calculated by determining the viable microbial load before and after encapsulation. The viable counts of the beads were determined by dissolving 100 mg of PLABs in 9.90 mL of citrate buffer solution (0.1 M, pH 6.0). The encapsulation efficiency was calculated using Equation 1 as follows:

$$Encapsulation\ Efficiency, \% = \left(\frac{log\ CFU_{M}}{log\ CFU_{Mo}}\right) x\ 100 \qquad Equation\ 1$$

where log CFU_M denotes the number of viable cells (CFU/mL) entrapped in the beads and log CFU_{Mo} is the number of viable cells (CFU/mL) before encapsulation.

Morphology was analyzed via scanning electron microscopy (SEM) performed by the Electron Microscopy Service Laboratory, BIOTECH, UPLB, and beads were imaged at the Bureau of Plant Industry (Malate, Manila). Beads were fixed in glutaraldehyde solution followed by dehydration in a series of increasing concentrations of ethanol. Ion coating was performed to prepare the samples for SEM. Imaging was conducted using the Prisma™ E SEM (Thermo Fisher Scientific, Waltham, MA, USA). The images were taken and saved as TIFF files for document processing.

In vitro tolerance assay against biological barriers

The effect of encapsulation on the probiotic characteristics of the culture strains was evaluated according to their tolerance to biological barriers such as acidic pH, bile, and varying levels of salinity (Ding et al. 2007). The tolerance of PLABs and free-living cells (FLCs) to low pH (2.0), bile (0.3% w/v), and salinity (0%, 1.75%, and 3.50%) was evaluated by determining the microbial load count before and after 4 h of treatment.

Swelling behavior and *in vitro* release assay

The swelling behavior of PLABs was investigated by determining the swelling index as outlined by Solanki and Shah (2016). In total, 100 mg of PLABs were placed in a flask containing 50 mL of simulated gastric fluid (0.90% NaCl, 8.0 mL of 0.10 M HCl, and 4.0 mL of 40 mg/mL pepsin in 0.10 M HCl, pH 1.2). The beads were then weighed every 60 min for 2 h. Afterwards, 50 mL of simulated intestinal fluid (18.0 mL of 2 mg/mL pancreatin and 12 mg/mL bile salts in 0.10 mol/L $NaHCO_3$, adjusted to pH 7.4 using $NaHCO_3$ solution) were added to the flask. The weight of the beads was determined for 6 h at 60-min intervals. The swelling index was calculated using Equation 2 as follows:

$$Swelling\ Index = \frac{m_f - m_i}{m_i} \times 100, \qquad Equiation\ 2$$

where m_f is the weight of the beads after swelling and m_i is the initial weight of the beads.

The *in vitro* release assay was used to evaluate the targeted release mechanism of PLABs in the gastrointestinal tract. For gastric digestion, 100 mg of PLABs were immersed in 50 mL of simulated gastric fluid at 37°C. After 2 h of incubation, 50 mL of simulated intestinal fluid were added to stimulate the intestinal phase. Aliquots of the gastric and intestinal phases were drawn to determine the number of microbes released during each phase.

Ex vivo mucoadhesive assay

The effect of the CMC coat on the mucoadhesive property of PLABs was evaluated by an *ex vivo* wash-off method under simulated gastrointestinal digestion (Lehr et al. 1990). Freshly excised sections of tilapia intestinal mucosa measuring 2 × 5 cm^2 were affixed onto glass slides. Approximately 10 pieces of CMC-coated (0.2% w/v) and uncoated PLABs were evenly dispersed onto the wet rinsed tissue specimen. Then, the prepared slides were immersed in a beaker containing simulated gastric fluid, positioned upright, and subjected to continuous agitation for 2 h. Subsequently, the glass slides were then transferred to a beaker filled with simulated intestinal fluid, followed by further incubation for 2 h. The number of beads adhering to the tissue was counted at hourly intervals during both phases of simulated digestion.

Effect of PLAB supplementation on the growth performance of Nile tilapia (*Oreochromis niloticus*)

The effect of PLAB supplementation on the growth performance of juvenile Nile tilapia was determined through direct feeding application. In total, 225 juvenile Nile tilapia (size 24) were stocked in 80-L tanks, which were randomly divided into five groups with three replicates (15 fish per replicate). Prior to the introduction of PLABs at various levels (0.0%, 0.1%, 0.3%, 0.5%, and 1.0% weight per body weight of fish), the fish underwent a 2-week acclimatization period. Fish were fed commercial diets twice daily at 8:30 am and 4:00 pm. After a feeding period of 70 days, mortality was recorded in each treatment group. Additionally, the average final body weight, average daily growth, feed conversion ratio (FCR), and specific growth rate (SGR) were determined for each treatment.

RESULTS AND DISCUSSION

Encapsulation of probiotic cultures into the SA/SPI polymer matrix

Probiotics cultures were encapsulated into an SA/SPI polymer matrix using an ionic gelation process. The incorporation of macro-polymeric molecules such as SPI in the alginate matrix to form hydrogel beads improves its emulsifying properties and enhances the stability and integrity of the beads (Xiang et al., 2020). The capacity of SPI to form electrostatic interactions and intermolecular forces such as van der Waals forces, hydrogen bonding, and specific interactions with both alginate and cells makes this material biocompatible with the encapsulation of microorganisms for enhanced adsorption efficiency (Cao et al. 2022, Burgain et al. 2013). As presented in Figure 1, the SA/SPI matrix possessed a highly porous structure, which provides a larger surface area and more binding sites for microorganisms to attach, leading to an encapsulation efficiency exceeding 95% with a bead diameter of 1055–1075 µm. This finding agrees with the results of Mokarram et al. (2009) and Khosravi Zanjani et al. (2014), who reported mean encapsulation values of 99.8% and 97.4%, respectively.

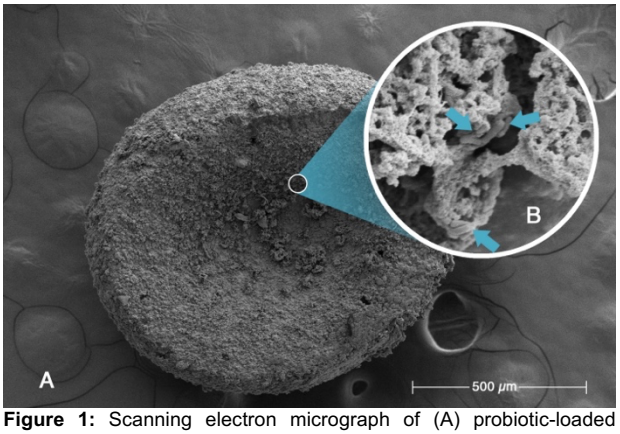

Figure 1: Scanning electron micrograph of (A) probiotic-loaded alginate/soy protein isolate beads at 100x magnification. Micrograph insert (B) is the higher magnification view (8000x) showing encapsulated *Lacticaseibacillus* sp. FSPL001 (pointed by arrows).

Following encapsulation, CMC, which is rich in hydroxyl and ether groups, was utilized to coat the PLABs, thereby introducing an additional protective layer onto the bead surface. This process enhances mucoadhesive properties and fortifies barrier function (Cook et al. 2018, Dafe et al. 2017, Heidebach et al. 2012, Solanki and Shah 2016). This strategy also improves the stability of the core matrix and protects cells by limiting moisture loss, preventing probiotic cell leaching, decreasing permeability, and strengthening the overall mechanical structure of the bead (Sun et al. 2023, Ebrahimi et al. 2018). SEM unveiled discernible differences in surface morphology between uncoated and CMC-coated beads (Figure 2). Uncoated beads exhibited rough, uneven, and irregular surface structures. By contrast, CMC-coated beads displayed relatively smoother surfaces with a notable reduction in exterior pores, a characteristic often observed in coated microcapsules (Călinoiu et al., 2019).

In vitro tolerance assay against biological barriers

In their free-living state, probiotics are vulnerable to biological barriers such as enzymatic attacks, highly acidic pH, solutes, and competition with native microflora during their transit to the intestinal environment (Ding et al. 2007, Song et al. 2018). The impact of encapsulation on the viability of the three probiotic cultures following exposure to low pH and bile is depicted in Figure 3. Despite being an endospore former, *Bacillus* sp. FSPL020 was completely vulnerable in its free-cell form when exposed to pH 2.0 for 4 h. However, upon encapsulation in the

SA/SPI matrix coated with CMC, its survival rate significantly increased to 86.76%. A similar trend was observed for encapsulated *Lacticaseibacillus* sp. FSPL001, with respective increases in its survival rates in the presence of pH 2.0 and 0.3% bile of 4.96% and 36.86%. Conversely, *Saccharomyces* sp. FPSL011 demonstrated complete tolerance to acidic pH and medium containing 0.3% bile in both its free-living and encapsulated forms.

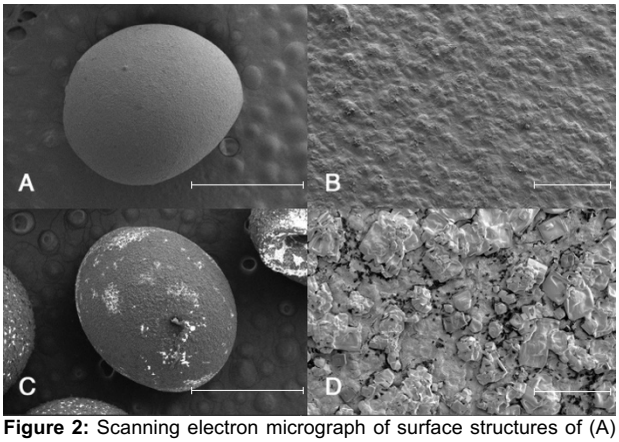

Figure 2: Scanning electron micrograph of surface structures of (A) CMC-coated PLAB at 150x magnification, (B) CMC-coated PLAB at 1000x magnification, (C) uncoated PLAB at 150x magnification, (C) uncoated PLAB at 1000x magnification.

The high solute and acid resistance of *Saccharomyces* sp. FPSL011 could be associated with its known cell wall modification activity (Kapteyn et al. 2001). The induction of cell wall integrity genes along with other stress-response genes also allows this microbe to resist lysis caused by osmotic shock (Levin 2005). These adaptations could explain the high tolerance of *Saccharomyces* sp. FPSL011 to the experimental conditions even in its free-living state. It is worth noting that the probiotic properties of this strain were previously characterized. As such, resistance to specific harsh conditions is to be expected. Furthermore, it should be highlighted that some environmental adaptations are strain-specific, resulting in varied responses to the same stimuli among different strains of the same microbial species (Zhu et al. 2016).

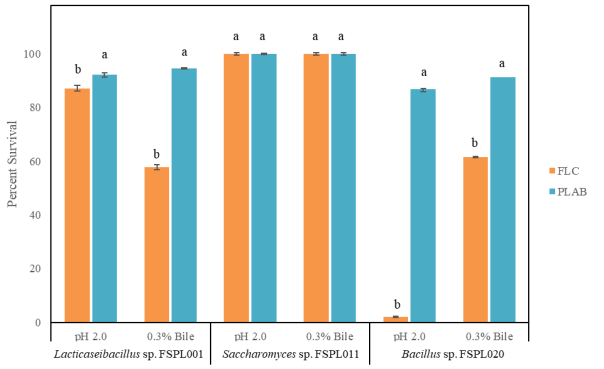

Figure 3: Tolerance of free-living cells (FLC) and probiotic-loaded alginate beads (PLAB) of *Lacticaseibacillus* sp. FSPL001, *Saccharomyces* sp. FSPL011, and *Bacillus* sp. FSPL020 after 4 h treatment at pH 2.0 and 0.3% bile. [a-b]Different letters indicate significant differences between treatments at P <0.05 as analyzed by one-way ANOVA and the Tukey HSD test.

To assess the salinity tolerance of each strain in its FLC and PLAB forms, the microbes were exposure to various salinity levels (0, 17.5, and 35 ppt) to represent freshwater, brackish water, and seawater environments commonly encountered in aquaculture. As depicted in Figure 4, the isolates exhibited high survival rates (>97%) in both their FLC and PLAB forms following exposure to all salinity treatments. This resilience to salinity fluctuations represents a significant advantage for probiotics, particularly in open culture systems in which salinity levels commonly vary. This tolerance ensures that microorganisms can effectively endure osmotic stress, thereby enhancing water quality and providing beneficial health effects (Jahangiri and Esteban, 2018). Notably, consistent observations indicate that encapsulation does not compromise the salinity tolerance of probiotic cultures, rendering them stable and viable across diverse environmental salinity conditions.

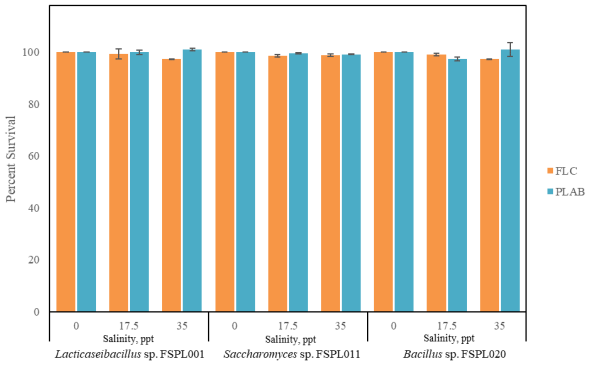

Figure 4: Tolerance of free-living cells (FLC) and probiotic-loaded alginate beads (PLAB) of *Lacticaseibacillus* sp. FSPL001, *Saccharomyces* sp. FSPL011, and *Bacillus* sp. FSPL020 after 4 h treatment at 0, 17.5 and 35 ppt saline solution. [ns]No significant differences were observed between treatments (P>0.05) as analyzed by one-way ANOVA and the Tukey HSD test.

Swelling behavior, *in vitro* release assay, and mucoadhesive properties

The swelling behavior of PLABs under *in vitro* gastrointestinal digestion was examined to evaluate their dynamic swelling properties and breakage point under different digestion phases. As presented in Figure 5, PLABs displayed an extremely low swelling index during gastric digestion. As the digestive process progresses into the intestine, a substantial 30-fold increase in PLAB swelling was recorded. This behavior likely occurs because of the alkaline environment in the small intestine, which is more favorable for dissolution of the alginate matrix. According to Mokarram et al. (2009), the swelling of beads in alkaline solution is favored because of the presence of counterions that neutralize carboxylic groups on alginate. The increased yet gradual swelling in the intestine is beneficial, as it indicates controlled release of the probiotics, thereby promoting their colonization and beneficial effects in the intestinal tract (Anal and Singh, 2007). After 7 h of digestion, complete dissolution or breakage was observed. Complete dissolution is an essential outcome for controlled-release systems, as it ensures the full delivery of the probiotics to the intended site for optimal efficacy (Yan et al. 2019).

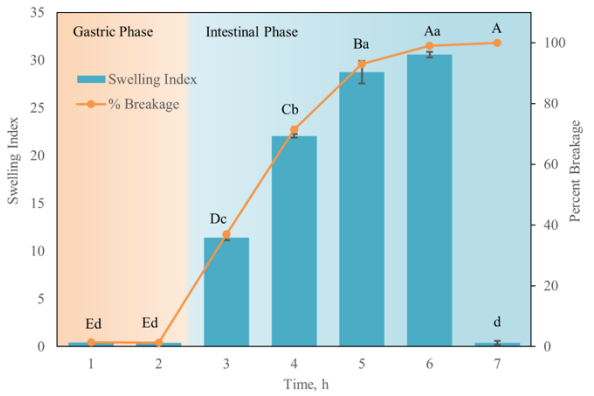

Figure 5: Swelling index and percent breakage of PLAB during simulated gastrointestinal digestion. Different letters indicate significant differences in percent breakage$^{(A-E)}$ and swelling index$^{(a-d)}$ across various digestion times at P <0.05 as analyzed by one-way ANOVA and the Tukey HSD test.

As presented in Figure 6, a comparative assessment was conducted to analyze the *in vitro* release profile of probiotics from two formulations, namely PLABs and a commercially available non-encapsulated probiotic product (COM), under simulated gastrointestinal conditions. The results revealed that although the COM released approximately 58% of its probiotic content during the gastric digestion phase, a mere 3% was released from PLABs. Moreover, the figure highlights a distinctive characteristic of PLABs to completely discharge their probiotic content during the intestinal digestion phase. This observation suggests the operation of a matrix-mediated controlled release mechanism inherent to PLABs, distinguishing it from the commercial counterpart.

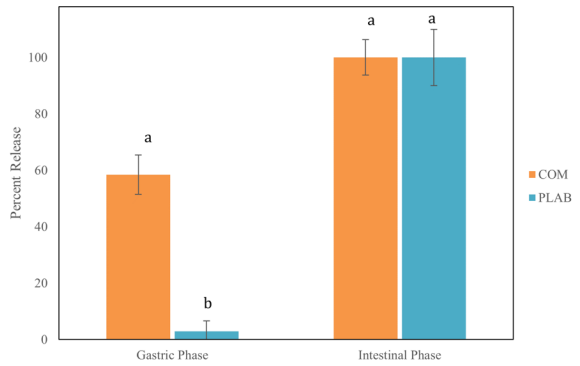

Figure 6: *In vitro* release of PLAB and non-encapsulated commercially available (COM) probiotics during simulated gastrointestinal digestion. $^{a-b}$Different letters indicate significant differences between treatments at P <0.05 as analyzed by one-way ANOVA and the Tukey HSD test.

The mucoadhesive property of PLABs, attributed to the CMC coat, was investigated using *ex vivo* fish intestinal tissues. As illustrated in Figure 7, CMC-coated PLABs demonstrated distinctly superior mucoadhesive characteristics compared to uncoated PLABs during the gastric digestion phase. The surface coating with CMC imparts PLABs with strong hydrophilic properties, facilitating favorable interactions with mucin glycoproteins present in the mucosal epithelium.

As digestion progressed, a notable decrease in mucoadhesion was observed during the intestinal phase, and this decrease was associated with the swelling behavior and controlled-release mechanism of PLABs (Figures 5 and 6). This finding agrees with those of a study conducted by Anselmo et al. (2016), who employed a layer-by-layer approach to encapsulate *B. coagulans* through an alginate–chitosan layering series, leading to enhanced mucoadhesive properties and targeted release upon entering the intestinal phase of digestion.

The combined attributes of controlled release, mucoadhesive properties, and dynamic swelling behavior position PLABs as a promising candidate for the targeted and efficient delivery of probiotics within the gastrointestinal tract. Its low swelling index in the gastric environment helps protect encapsulated probiotics from premature release, and as digestion progresses into the intestine, the controlled-release mechanism permits the gradual release of probiotics through adhesive interactions between PLABs and the mucosal epithelium, ensuring complete release of the probiotics at the intended site.

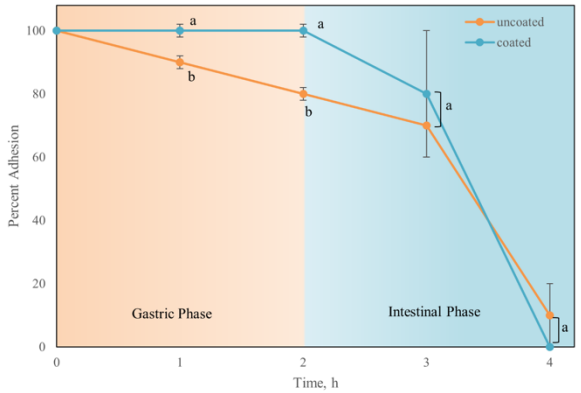

Figure 7: *Ex vivo* mucoadhesive property of uncoated and CMC-coated PLAB during simulated gastrointestinal digestion. $^{a-b}$Different letters indicate significant differences between treatments at P <0.05 as analyzed by one-way ANOVA and the Tukey HSD test.

Growth performance of Nile tilapia

The development of PLABs allowed the easy integration of probiotic supplementation into existing feeding regimens in aquaculture through direct oral administration. After 70 days of culture, tilapia directly fed PLABs at 0.1% body weight exhibited the greatest ($P < 0.05$) average weight gain, followed by those fed 0.3% and 0% PLABs (Table 1). Probiotics are known to modulate the composition and activity of the gut microbiota, thereby maintaining a balanced microbial community that promotes efficient nutrient metabolism and overall health. They can exclude pathogens by competing for nutrients and producing inhibitors such as bacteriocins with potent antibacterial properties (Han et al. 2023). Additionally, probiotics such as *S. cerevisiae* release extracellular digestive enzymes that enhance nutrient synthesis and metabolism, leading to increased weight gain in Nile tilapia (Dawood et al. 2020). Tabassum et al. (2021) also demonstrated that dietary supplementation with commercial gut probiotics containing *B. mesentericus*, *Enterococcus faecalis*, and *Clostridium butyricum* led to significant enhancements in growth performance, feed utilization, hematological parameters, and intestinal microbial load and morphology in Nile tilapia compared to the findings in non-probiotic-treated fish.

PLAB supplementation at higher concentrations of 0.5% and 1.0% body weight resulted in the lowest weight gain during the trial period (Figure 8). One possible hypothesis for this observation is that greater consumption of beads led to satiation in the fish. This suggests that at this level of supplementation, beads occupied a significant portion of the digestive tract, which might have inadvertently affected the feeding behavior and satiety levels of the tilapia.

Although FCR, SGR, and the average daily feed intake did not significantly differ among the treatments, it is noteworthy that PLAB supplementation in tilapia did not adversely affect or

improve feed utilization. This contradicts the findings of Xia et al. (2020), who found that *B. subtilis* and *B. cereus* supplementation improved FCR by 76% compared to the control findings. Similar obaaxservations were noted in studies by Lara-Flores et al. (2010) and Ramos et al. (2013), who recorded improvements in FCR and SGR with probiotic-treated diets.

For this experimental set-up, the absence of observable improvement in feed utilization parameters in tilapia following PLAB supplementation could be attributed to factors such as the scale of the experimental tanks used, inherent individual differences among the fish, and, potentially, the relatively short duration of the study, which might have prevented significant changes from manifesting. Nevertheless, within the limits of this experimental design, the results indicate that daily PLAB supplementation at 0.1%–0.3% body weight positively influenced the average weight gain of tilapia without compromising other performance indicators. However, to fully grasp the potential impact of PLABs on tilapia farming, further investigations on a larger scale, ideally at the farm level, are necessary to validate its efficacy and practical application.

Table 1: Growth performance of tilapia fed with varying levels of PLAB for 70 d.

Parameter	PLAB Application rate, % of BW per day				
	0%	0.1%	0.3%	0.5%	1%
AWG, %	9521.21±287.88^{c}	12888.64±647.72^{a}	11191.67±859.84^{b}	8081.82±1928.47^{d}	7127.27±578.54^{d}
FCR	0.55±0.05	0.52±0.04	0.51±0.02	0.57±0.06	0.60±0.14
SGR	6.60±1.09	7.35±1.05	7.15±0.80	7.05±0.75	7.31±2.36
ADFI, g/fish	0.15±0.02	0.20±0.05	0.19±0.05	0.13±0.05	0.22±0.08

ABW-average body weight; AWG-average weight gain; FCR-feed conversion ratio; SGR-specific growth rate; ADFI-average daily feed intake

$^{a-b}$Different superscript letters indicate significant differences between treatments at P <0.05 as analyzed by one-way ANOVA and the Tukey HSD test.

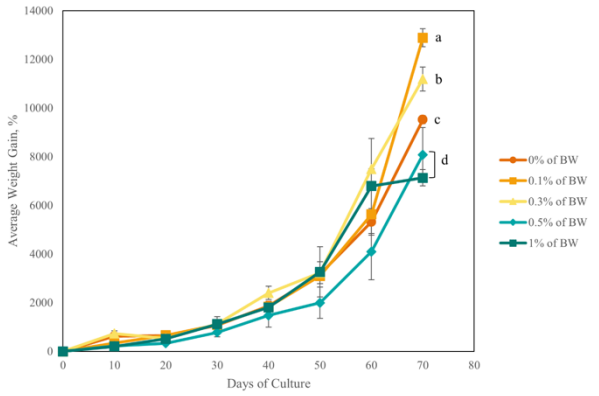

Figure 8: Average body weight gain (%) of tilapia fed with varying levels of PLAB for 70 d. $^{a-d}$Different letters indicate significant differences between treatments at P <0.05 as analyzed by one-way ANOVA and the Tukey HSD test.

CONCLUSION

In summary, encapsulation of probiotic isolates, namely *Lacticaseibacillus* sp. FSPL001, *Saccharomyces* sp. FSPL011, and *Bacillus* sp. FSPL020, within an SA/SPI polymer matrix coated with CMC led to the production of PLABS with high encapsulation rates exceeding 95% and viability counts of at least 1×10^7 CFU/g beads. Encapsulation significantly enhanced probiotic tolerance to biological barriers with enhanced survival rates at pH 2.0 and in the presence of 0.3% bile. Moreover, the encapsulation in the matrix promoted stability of the bead and the maintenance of high microbial loads even when subjected to salinity levels of up to 35 ppt.

The SA/SPI polymer matrix formulation displayed pH-sensitive dynamic swelling behavior, enabling a controlled-release mechanism as demonstrated in the *in vitro* release assay. The encapsulated probiotics remained intact within the beads following gastric digestion, with complete discharge observed during the initial phase of intestinal digestion. Additionally, the incorporation of the 0.2% w/v CMC coat notably enhanced the mucoadhesive properties of PLABs, facilitating effective attachment to the mucosal epithelium and minimizing bead wash-off during digestion.

PLABs were easily integrated into existing feeding regimens in tilapia reared through direct oral administration at 0.1%–0.3% body weight, which led to a significant increase in body weight gain after 70 days of culture with no adverse effect on feed utilization. Thus, the combined attributes of probiotic activity, enhanced stability and mucoadhesive properties, and a controlled-release mechanism during digestion highlight the potential of PLABs as promising candidates for the targeted and efficient delivery of probiotics to boost aquaculture productivity. However, to fully understand the impact of PLABs on tilapia farming, additional large-scale studies, preferably conducted at the farm level, are needed to confirm their effectiveness and practical use. Furthermore, an economic analysis is recommended to assess the cost-effectiveness of PLAB implementation, considering both the potential increase in productivity and any associated costs, to ensure a viable financial benefit to aquaculture operations.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

All authors have no conflict of interest to disclose.

ETHICAL STATEMENT

All the experimental procedures involving animals were conducted in accordance with the University of the Philippines Los Baños Institutional Animal Care and Use Committee (UPLB IACUC) in compliance to the provisions of Philippine Republic Act No. 8485 (Animal Welfare Act of 1998) as amended by RA 10631, and the Department of Agriculture Administrative Order No. 40, Series of 1999.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

All the authors contributed to the conceptualization of the study. NGD supervised and directed the study. CRT, IDFA, and RDPA performed the analyses and feeding trial experiment. All authors processed, analyzed, and provided their expertise to the final version of the manuscript.

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