https://doi.org/10.17221/181/2023-CJFS

A frontier approach for the production of enteric soft capsules containing omega-3 fatty acids and probiotics

*Hayoung Kim[1], Won Yeong Bang[1], Boyoung Choi[1], Han Bin Lee[1], Jungwoo Yang[2]**

[1]Ildong Bioscience, Pyeongtaek-si, Gyeonggi-do, Republic of Korea
[2]Department of Microbiology, College of Medicine, Dongguk University, Gyeongju, Republic of Korea
**Corresponding author: dbl3jwy@dongguk.ac.kr*

Citation: Kim H., Bang W.Y., Choi B., Lee H.B., Yang J. (2024): A frontier approach for the production of enteric soft capsules containing omega-3 fatty acids and probiotics. Czech J. Food Sci., 42: 127–135.

Abstract: The study investigated whether omega-3 fatty acids could promote bacterial growth, acid and bile tolerance, and cell adhesion onto Caco-2 cells to develop a multifunctional enteric soft capsule containing probiotics and omega-3 fatty acids. The probiotic strains used were *Enterococcus faecium* IDCC 2102 and *Bacillus coagulans* IDCC 1201. Supplementation with omega-3 fatty acids did not significantly affect the growth and viability of either strain at lower concentrations (i.e. 0.5–1.0 *w*/*w* %). In comparison, the adhesion ability to Caco-2 cells of both strains was significantly increased (up to 107.5%). Furthermore, *Bacillus coagulans* IDCC 1201 [1×10^{10} CFU (colony forming unit) per capsule] contained together with omega-3 fatty acids (600 mg per capsule) in the enteric soft capsules showed a stability of over 95% during 12 months of storage at room temperature, which was similar to that of unencapsulated lyophilised probiotics. Thus, these results indicate that multifunctional food supplements in the form of enteric soft capsules are feasible.

Keywords: *Bacillus coagulans; Enterococcus faecium*; intestinal adhesion; omega-3 fatty acids; probiotics

Probiotics are live microorganisms that, when consumed in adequate quantities (FAO/WHO 2001), can provide health benefits such as improved digestive function and gut health, enhanced immune system activity, and to prevent pathogenic infection (Kechagia et al. 2013; Suez et al. 2019; Latif et al. 2023). To attain these beneficial effects, probiotics should colonise the intestine and migrate to the gastrointestinal system, where they can be exposed to toxic environments, reducing their viability (Dimidi et al. 2017; Han et al. 2021). Consequently, various methods have been introduced to overcome the challenges associated with probiotics (i.e. the effects of gastric and bile acids and digestive enzymes), one of which involves the encapsulation of probiotics using proteins, carbohydrates, and chemical compounds with conjugation abilities (Rajam and Anandharamakrishnan 2015; Rodrigues et al. 2017; Bang et al. 2022).

Omega-3 fatty acids polyunsaturated fatty acids are mainly composed of eicosapentaenoic acids (EPAs, C_{20}), docosapentaenoic acids (DPAs, C_{22}), and docosahexaenoic acids (DHAs, C_{22}) (Cholewski et al. 2018). These compounds can improve eye and brain health, prevent heart disease, reduce inflammation, and enhance bone and joint health (Yashodhara et al. 2009; Swanson et al. 2012; Luchtman and Song 2013). The human body only synthesises a small amount of omega-3 fatty acids (i.e. EPA and DHA); therefore, additional supplementation is recommended to maintain an optimal level (Surette 2008). Generally, enteric soft capsules are used to produce commercially obtainable omega-3 fatty acids because these compounds are chemically unstable

https://doi.org/10.17221/181/2023-CJFS

(e.g. oxidative), which causes off-flavours or nutritional value losses (Kaushik et al. 2015).

To our knowledge, no enteric soft capsules containing probiotics and omega-3 fatty acids have been commercially developed as synergistic food supplements. This study manufactured enteric soft capsules containing physically and thermally stable probiotics and omega-3 fatty acids using the probiotic strains *Enterococcus faecium* IDCC 2102 and *Bacillus coagulans* IDCC 1201. First, the probiotic strains were mixed with a slurry form of omega-3 fatty acids, which was used to produce the enteric soft capsule and their growth was measured. Second, the viability of the probiotic strains under acid and bile stress and intestinal adhesion were evaluated. Finally, the shelf stability of the probiotics within the enteric soft capsule containing the omega-3 fatty acids was determined at room temperature. The results of this study can contribute to the development of enteric soft capsules for multifunctional synergistic health products.

MATERIAL AND METHODS

Bacterial strains and culture conditions. *E. faecium* IDCC 2102 (ATCC BAA-3146™) was isolated from the faeces of breast milk-fed infants, and *B. coagulans* IDCC 1201 (ATCC BAA-3143™) was isolated from the Green malt, and probiotic strains were cultured in De Man-Rogosa-Sharpe medium (MRS; BD Difco, USA) at 45 °C and 200 revolutions per minute (rpm) for 24 h (*B. coagulans*) or 37 °C for 24 h anaerobically (*E. faecium*). The bacterial strains were manufactured by Ildong Bioscience (South Korea).

Measurements of bacterial growth. To evaluate the effect of omega-3 fatty acids on the viability and growth of *E. faecium* IDCC 2102 and *B. coagulans* IDCC 1201, 1% (*v*/*v*) of an overnight culture of each strain was inoculated onto a 96-well plate that contained MRS broth and 0.5, 1, 2, 8, or 16% (*v*/*v*) omega-3 fatty acids. Bacterial cell growth was monitored every 6 h for 42 h using a plate reader (BioTek, USA) at 600 nm.

Acid and bile tolerance. The acid tolerance assessment was performed according to the method of Kim et al. (2018), with minor modifications. Briefly, 10 mL of *E. faecium* IDCC 2102 overnight cultures and *B. coagulans* IDCC 1201 (1×10^8 CFU·mL^{-1}; CFU – colony forming unit) were centrifuged at 6 000 rpm for 5 min. The cell pellets were washed twice with sterile phosphate-buffered saline (PBS) buffer (pH 7.4) and resuspended in 10 mL of PBS buffer. Subsequently, 100 µL of each sample (1×10^8 CFU·mL^{-1}) was inoculated into 10 mL of MRS broth, which was adjusted to a pH of 2.5 with 4 mol·L^{-1} HCl and incubated for 3 h at 37 °C. After 0 and 3 h, 100 µL of each culture was spread onto an MRS agar plate with appropriate dilutions, and colony counts were performed after 24 h of incubation. Cell viabilities (%) were expressed as cell densities at 3 h of initial cell density. The bile tolerance test used the same experimental procedure as the acid tolerance test, except that the MRS broth contained 0.3% (*w*/*v*) of bile salts (MB-B1653; MB Cell, South Korea). Omega-3 fatty acids was treated with concentrations of 0, 0.5, 1, and 2% in MRS, and both acid tolerance and bile tolerance were examined at each concentration. The omega-3 fatty acids used here, following a specific manufacturing process, exhibits high miscibility, easily blending with MRS.

Cell line and culture conditions. The human colon epithelial cell line Caco-2 was purchased from Korean Cell Line Bank (Seoul, South Korea) and cultured in low-glucose Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, USA) supplemented with 1% (*w*/*v*) penicillin/streptomycin (Sigma-Aldrich, USA) and 10% (*v*/*v*) heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich) in a humidified atmosphere containing 5% CO_2 at 37 °C.

Cell viability. The viability of the Caco-2 cells was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Presti et al. 2015). The Caco-2 cells were seeded at a density of 1×10^5 per cells well in a 96-well plate and incubated for 48 h, after which they were treated with a probiotic strain (1×10^8 CFU·mL^{-1}) and various concentrations of specific manufactured omega-3 fatty acids for 48 h. Subsequently, MTT solution was added to each well and the samples were incubated for 4 h at 37 °C. The excess MTT solution was then removed and dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. Finally, the optical density was measured at 540 nm using a microplate reader (BioTek, USA).

Adhesion assay. *E. faecium* IDCC 2102 and *B. coagulans* IDCC 1201 were cultured in MRS broth for 18 h at 37 and 45 °C, respectively. Subsequently, the cultures were adjusted to a concentration of 1×10^8 CFU·mL^{-1} and bacterial cells were obtained by centrifugation at 7 370 × *g* for 15 min. The bacterial cell pellets were washed in PBS twice and resuspended in low-glucose DMEM without antibiotics. Caco-2 cells (1×10^5 per cells well) were seeded onto 24-well plates and incubated in 5% CO_2 at 37 °C for 48 h. The cell monolayers were then rinsed with ster-

https://doi.org/10.17221/181/2023-CJFS

ile PBS and treated with either a mixture of omega-3 fatty acids and the bacterial strains or the bacterial strains only. The Caco-2 cell monolayers were incubated in 5% CO_2 at 37 °C for 3 h, after which the medium was removed, the cell monolayer was washed twice with PBS to eliminate unattached bacteria, and 100 µL of trypsin-EDTA was added to each well to release adherent bacteria. The collected bacteria were spread onto MRS agar plates and incubated at 37 °C for 48 h. Finally, adhesion was expressed as the percentage of adherent bacteria (CFU·mL^{-1}) compared to the initial inoculum.

Shelf stability of *Bacillus coagulans* IDCC 1201 in enteric soft capsules containing omega-3 fatty acids. Enteric soft capsules containing omega-3 fatty acids and *B. coagulans* IDCC 1201 were manufactured following the production process, as shown in Figure 1. To manufacture the capsule gel, gelatin (Sammi, South Korea), low methoxyl amidated pectin (LMA; DANISCO, USA), plasticisers, and purified water were prepared. The esterification and amide levels in the LMA were 24.5% and 22.8%, respectively. As a plasticiser, glycerin (LG H&H, South Korea) and polyglycerol syrup (LG H&H) were used to increase flexibility and mixed in a weight ratio of 1 : 0.3. The purified water and plasticisers were melted into the tank. When the temperature reached 85 °C, gelatin and LMA were added to the tank and stirred for 2 h to dissolve. After mixing, the solution was cooled to 60 °C and used for encapsulation. The capsule gel consisted of 33.6 wt% gelatin, 3 wt% LMA, and 22 wt% plasticisers. To prepare probiotic compounds of *B. coagulans* IDCC 1201, the omega-3 fatty acids (Wiley companies, USA), which mainly consisted of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and yellow wax (Strahl & Pitsch INC, USA) were melted into the tank at 60 °C. After cooling to 30 °C, soybean lecithin (Berg + Schmidt Asia Pte Ltd, Singapore) was added for emulsification and mixed with *B. coagulans* IDCC 1201. The probiotic compounds were filled in the capsule. The manufactured capsule consisted of 77.5 wt% omega-3 fatty acids (387.5 mg per capsule), 15 wt% *B. coagulans* IDCC 1201 (1×10^{10} CFU per capsule), 6.5 wt% yellow wax, and 1 wt% soybean lecithin.

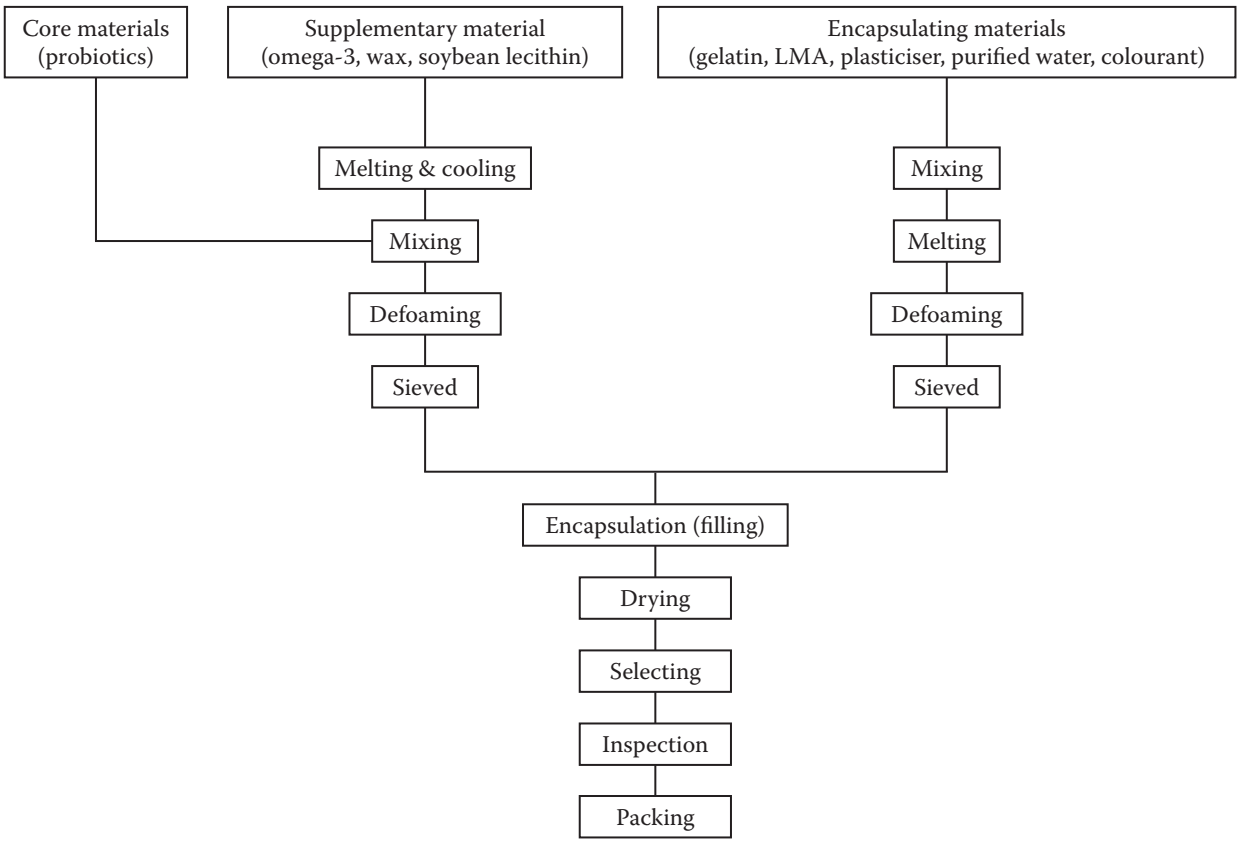

Figure 1. The manufacturing process of enteric soft capsules containing omega-3 fatty acids and *Bacillus coagulans* IDCC 1201

LMA – low methoxyl amidated

https://doi.org/10.17221/181/2023-CJFS

The shelf-stability of *B. coagulans* IDCC 1201 in capsules with omega-3 fatty acids was investigated by storing the samples at room temperature for 12 months. The viability of the probiotic strains was determined at 1, 2, 3, 6, 9, and 12 months of storage. Briefly, samples (10 g) were serially diluted with sterile saline solution, and 1 mL of the diluted solution was added to 15 mL of MRS medium in Petri dishes. The procedure was performed in triplicate. After solidification, the plates were anaerobically incubated at 37 °C for 72 h. The number of cells (CFU·mL^{-1}) was determined by counting the plate's viable bacterial cells.

Statistical analysis. The experiments were performed in triplicate, and the results are presented as means ± standard deviations. Significant differences were determined using an unpaired *t*-test, and *P*-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The effect of omega-3 fatty acids on bacterial growth. To examine the effect of omega-3 fatty acids on bacterial growth, *E. faecium* IDCC 2102 and *B. coagulans* IDCC 1201 were incubated in MRS broth supplemented with one of six concentrations of omega-3 fatty acids (Figure 2). Overall, bacterial cell growth decreased in an omega-3 fatty acids concentration-dependent manner. The highest specific growth rate (μ) occurred in the MRS broth with 0% (*w*/*v*) supplementation, while the lowest rate was achieved with 16% omega-3 fatty acids supplementation. Specifically, *E. faecium* IDCC 2102 attained 0.78 and 0.32 of the specific growth rate (h^{-1}) in the presence of 0% and 16% omega-3 fatty acids, respectively (Figure 2A), while the rates for *B. coagulans* IDCC 1201 were 0.18 and 0.25, respectively (Figure 2B). Therefore, the omega-3 fatty acids concentration range of 0.5–2% was selected for further experiments since these concentrations had no significant effect on the growth of the two probiotic strains.

The effect of omega-3 fatty acids on bacterial tolerance to acid and bile. The survivability of probiotics in acid or bile conditions is an important consideration due to the acidic gastrointestinal environments to which these probiotics are exposed (Han et al. 2021). This study evaluated the viability of *E. faecium* IDCC 2102 and *B. coagulans* IDCC 1201 at a pH of 2.5 and 0.3% (*w*/*v*) of bile acid. The results for *E. faecium* IDCC 2102 indicated an approximately 80% viability with increased omega-3 fatty acids concentrations under acidic stress, whereas that of *B. coagulans* IDCC 1201 decreased with increased omega-3 fatty acids concentrations from 100% to 80% when compared to the control (0%) (Figure 3). The viability of *B. coagulans* IDCC 1201 was superior to that of *E. faecium* IDCC 2102, possibly due to spore formation observed in the *B. coagulans* strain

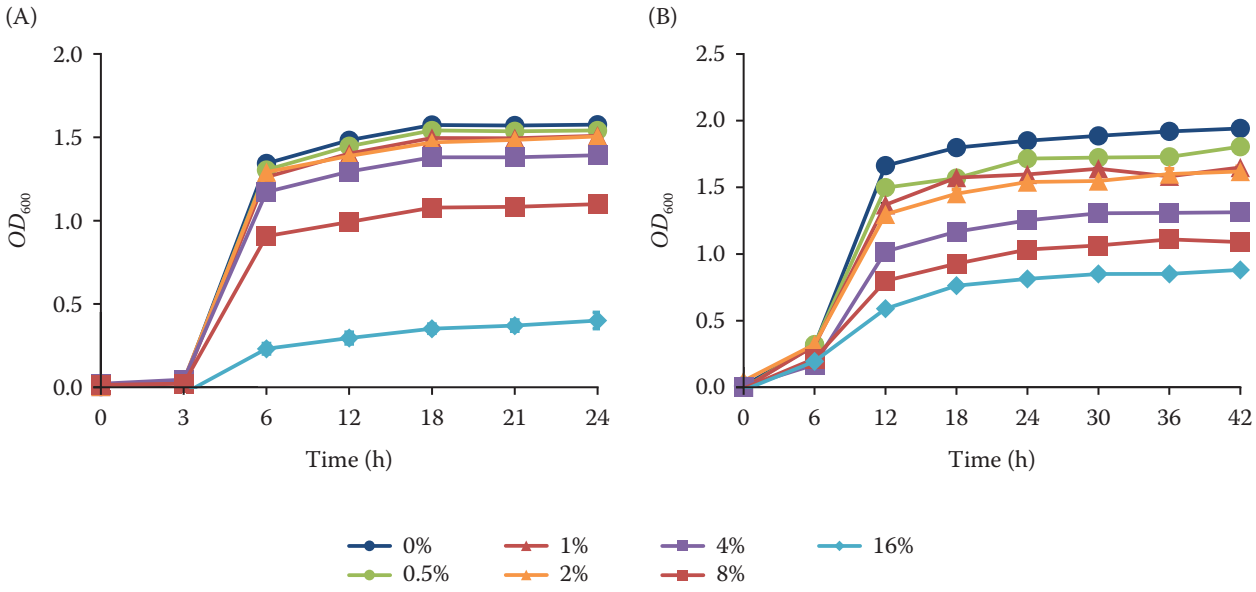

Figure 2. Changes in the optical density (OD_{600}) of (A) *Enterococcus faecium* IDCC 2102 and (B) *Bacillus coagulans* IDCC 1201 in Man-Rogosa-Sharpe medium (MRS) medium with sublethal concentrations (wt%) of omega-3 fatty acids

The number of repeats for each treatment n = 3; the experimental data are represented as three independent experiments' means ± standard deviations

https://doi.org/10.17221/181/2023-CJFS

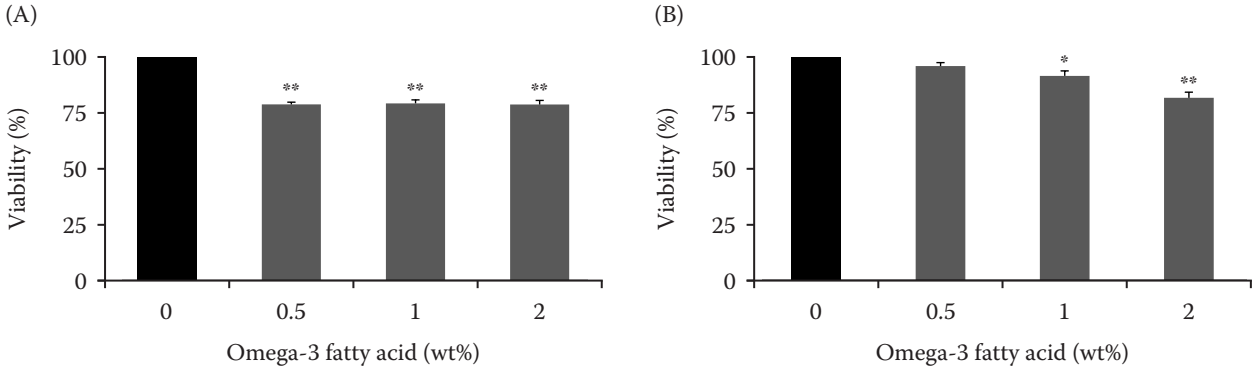

Figure 3. Viability of (A) *Enterococcus faecium* IDCC 2102 and (B) *Bacillus coagulans* IDCC 1201 in gastrointestinal tract related acid stresses

*, ** The values are represented as the means ± standard deviations at significance levels $P < 0.05$ and 0.01, respectively; (A) *E. faecium* IDCC 2102 (1×10^8 CFU·mL^{-1}) and (B) *B. coagulans* IDCC 1201 (1×10^8 CFU·mL^{-1}) were inoculated in acidic medium at 37 °C at 3 h; the acidic medium adjusted pH 2.5 and treated omega-3 fatty acids with concentrations of 0, 0.5, 1, and 2%; the standard error ($n = 3$ independent experiments) is indicated in the error bar; CFU – colony forming unit

(Bader et al. 2012). The bile tolerance test showed that omega-3 fatty acids supplementation did not affect viability at 0.3% (*w*/*v*) bile acid when compared to the control (0%) (Figure 4). In conclusion, supplementation with omega-3 fatty acids had no significant effect on bacterial viability (comparatively 80–100%) under acid and bile stress at the tested concentrations of 0.5% to 2%.

The effect of omega-3 fatty acids on bacteria adhesion to Caco-2 cells. Before the adhesion assay, the viability of Caco-2 cells treated with omega-3 fatty acids and the probiotic strains was investigated using an MTT assay (Figure 5). The omega-3 fatty acids did not statistically affect the viability of Caco-2 cells up to 2% (*w*/*v*) (Figure 5A). However, omega-3 fatty acids significantly decreased the viability of *E. faecium* IDCC 2102 at > 1% and decreased the viability of *B. coagulans* IDCC 1201 at > 2% (Figure 5B and C). Thus, the omega-3 fatty acids concentrations of 0.5% and 1% (*w*/*v*), which had no significant effects on Caco-2 cell viability, were used for the *E. faecium* IDCC 2102 and *B. coagulans* IDCC 1201 adhesion assays.

The Caco-2 cells were then treated with the *E. faecium* IDCC 2102 and *B. coagulans* IDCC 1201 strains at a concentration of 1×10^7 CFU·mL^{-1}, and the adhesion of both strains significantly increased with the

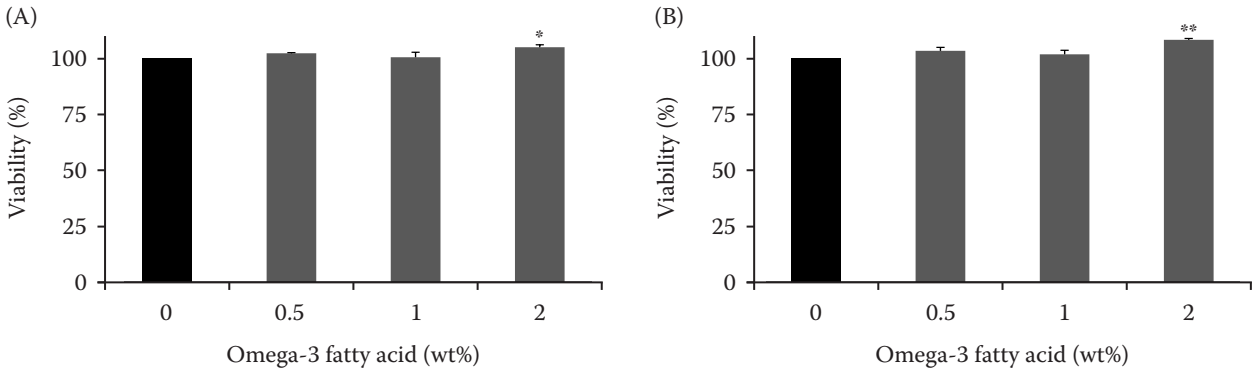

Figure 4. Viability of (A) *Enterococcus faecium* IDCC 2102 and (B) *Bacillus coagulans* IDCC 1201 in gastrointestinal tract related bile stresses

*, ** The values are represented as the means ± standard deviations at significance levels $P < 0.05$ and 0.01, respectively; (A) *E. faecium* IDCC 2102 (1×10^8 CFU·mL^{-1}) and (B) *B. coagulans* IDCC 1201 (1×10^8 CFU·mL^{-1}) in MRS containing 0.3% (*w*/*v*) of bile salts; omega-3 fatty acids were treated with concentrations of 0, 0.5, 1, and 2% in bile medium containing 0.3% (*w*/*v*) of bile salts; the standard error ($n = 3$ independent experiments) is indicated in the error bar; CFU – colony forming unit

https://doi.org/10.17221/181/2023-CJFS

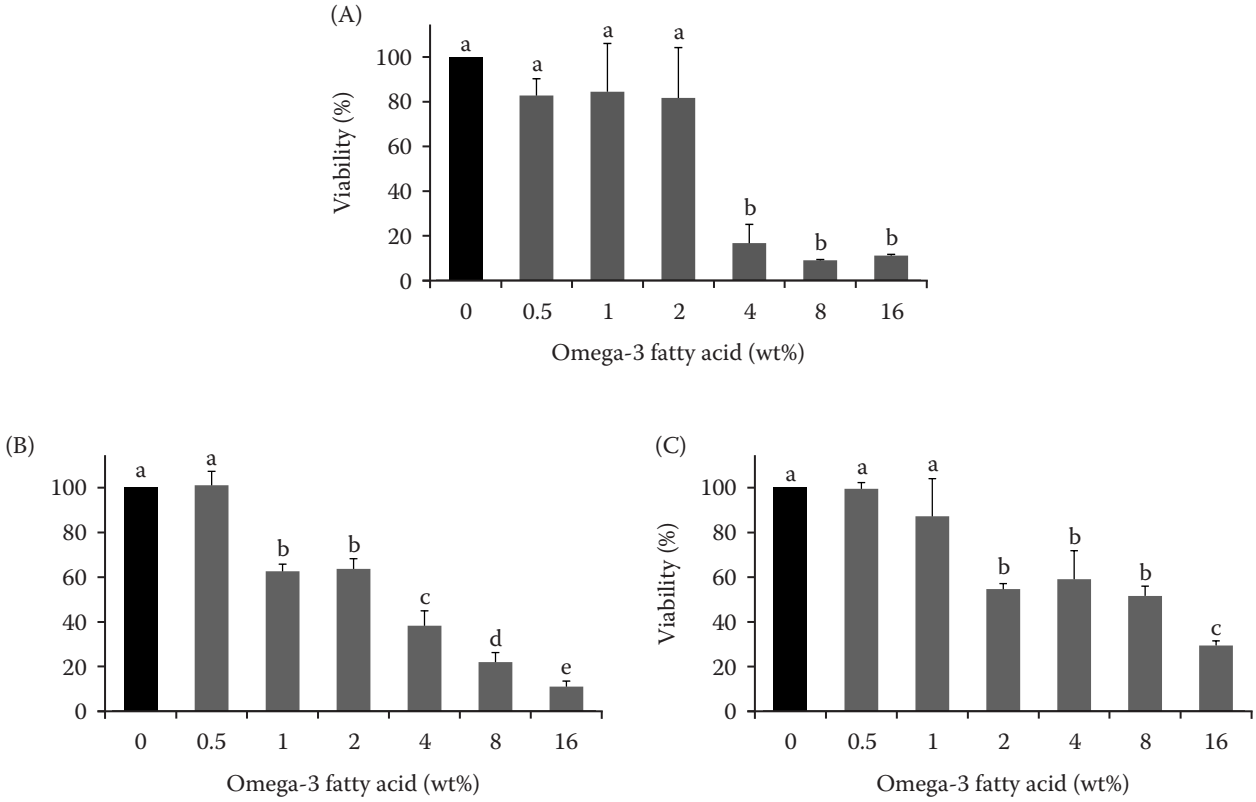

Figure 5. Effect of (A) omega-3 fatty acids only, (B) *Enterococcus faecium* IDCC 2102 (1×10^8 CFU·mL^{-1}), and (C) *Bacillus coagulans* IDCC 1201 (1×10^8 CFU·mL^{-1}) on proliferation of Caco-2 cells

a–e – different letters indicate significant differences at $P < 0.05$ by Duncan's multiple range test; the experimental data are represented as the means ± standard deviations of three independent experiments; CFU – colony forming unit

1% omega-3 fatty acids concentration [(by 48.9% and 107.5% compared to control (0%), respectively; Figure 6]. These results indicate that omega-3 fatty acids could benefit probiotic strain adhesion to the intestinal wall. Polyunsaturated fatty acids (PUFAs) have been reported to promote the settlement of lactic acid bacteria in the digestive tract of the arctic charr, *Salvelinus alpinus* (L.), and it has been suggested that PUFAs, such

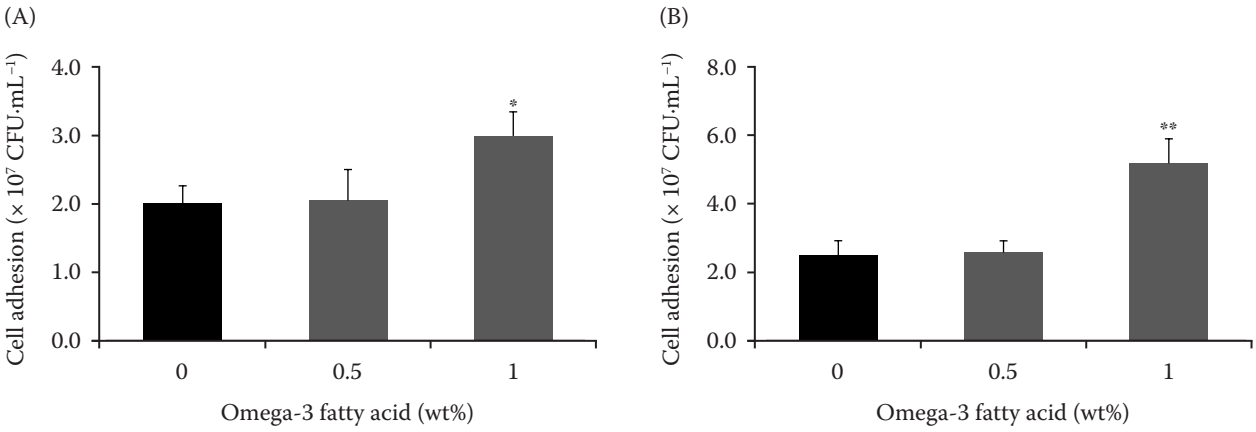

Figure 6. Adhesion of either (A) *Enterococcus faecium* IDCC 2102 or (B) *Bacillus coagulans* IDCC 1201 mixed with omega-3 fatty acids onto Caco-2 cells

*, ** The values are represented as the means ± standard deviations of three independent experiments at significance levels $P < 0.05$ and 0.01, respectively; CFU – colony forming unit

https://doi.org/10.17221/181/2023-CJFS

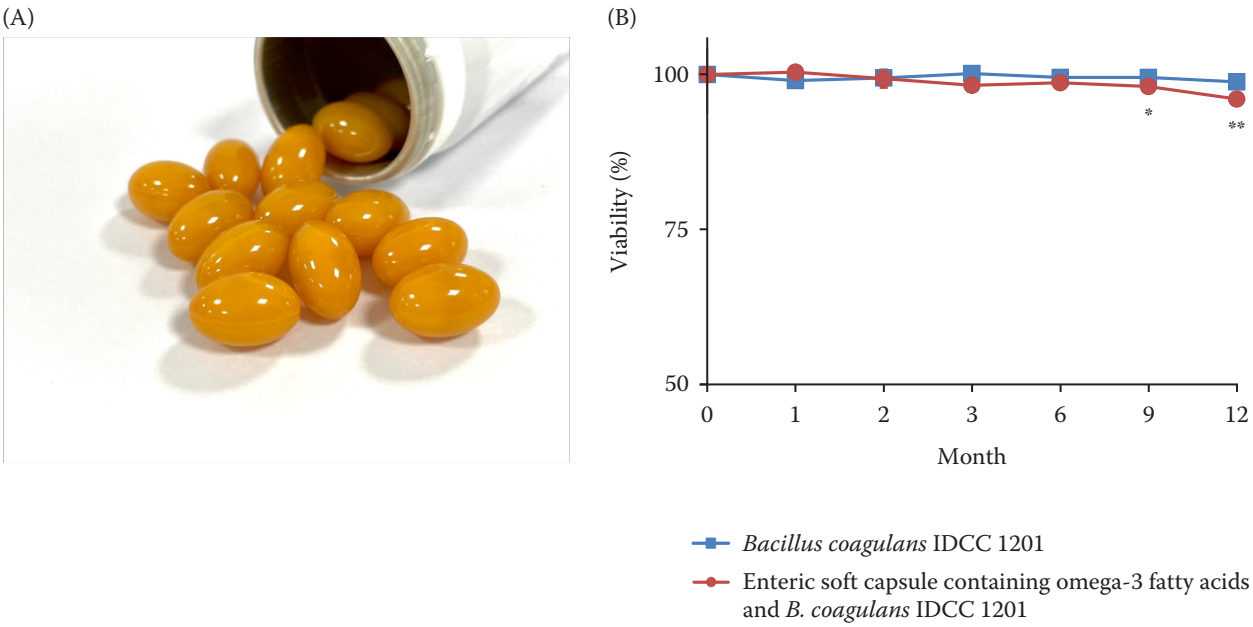

Figure 7. (A) Production of enteric soft capsule containing *Bacillus coagulans* IDCC 1201 (15 wt%, 1×10^{10} CFU per capsule) and omega-3 fatty acids (77.5 wt%, 387.5 mg per capsule) and (B) viability of *B. coagulans* IDCC 1201 within the capsule over a storage period of 12 months

*, ** The values are represented as the means ± standard deviations of three independent experiments at significance levels $P < 0.05$ and 0.01, respectively; CFU – colony forming unit

as omega-3 fatty acids, could modify the membrane fatty acid composition of intestinal epithelial cells for bacterial adhesion (Ringø et al. 1998). Furthermore, low concentrations of PUFAs significantly enhanced the growth and adhesion of probiotic strains (e.g. LGG, *Lactobacillus casei* Shirota, and *Lactobacillus bulgaricus*). In contrast, high concentrations of PUFAs had no effect (Kankaanpää et al. 2001). Thus, supplementation with PUFAs, such as omega-3 fatty acids, can benefit the intestinal adhesion of probiotics.

Shelf stability of probiotics in enteric soft capsules containing omega-3 fatty acids. Probiotic products should meet suitable viable microorganism counts at the end of their shelf life to ensure that the appropriate health benefits are provided to consumers (Hill et al. 2014). In this study, the shelf life of *B. coagulans* IDCC 1201 encapsulated with omega-3 fatty acids (Figure 7A) was investigated during 12 months of storage, and the survival rates of the bacteria were maintained above 95% compared to the initial control at all checkpoints (Figure 7B). The number of viable cells was greater than 1 billion CFU, considered the minimal effective level for health benefits (Settanni and Moschetti 2010). After the 12-month storage period, the survival rate was approximately 96.05% (Figure 7B), while the survival rate of unencapsulated lyophilised cells was approximately 98.81% (Figure 7B). These results showed that ingredients in enteric soft capsules containing omega-3 fatty acids affected the cells' viability. The hydrogels, comprising gelatin and pectin, increase the stability of probiotics due to their capacity to absorb water within their structure (Ishwarya et al. 2022). Furthermore, they protect cells against gastric acid and are easily digested in the intestine by lipases (Ishwarya et al. 2022). In addition, the lipid matrix, such as omega-3 fatty acids, contributes to the stability by protecting bacterial cells from exposure to water and stressors such as H^+ ions (Lahtinen et al. 2007). Thus, these results suggest that probiotic *B. coagulans* IDCC 1201 encapsulated with omega-3 fatty acids can be stored for a minimum of one year under the conditions of this study.

CONCLUSION

Probiotic strains and omega-3 fatty acids are functional ingredients that boost their hosts' immune systems by producing beneficial metabolites such as short-chain fatty acids and modifying the gut barrier composition. In this study, supplementation with lower concentrations of omega-3 fatty acids (i.e. 0.5–1.0 wt%) increased the intestinal adhesion of the two probiotic strains test-

https://doi.org/10.17221/181/2023-CJFS

ed, *E. faecium* IDCC 2102 and *B. coagulans* IDCC 1201. Based on these results, enteric soft capsules containing *B. coagulans* IDCC 1201 (15 wt%, 1×10^{10} CFU per capsule) and omega-3 fatty acids (77.5 wt%, 387.5 mg per capsule) were manufactured, and the viability of the probiotic strains during long-term storage was determined using a shelf-stability test. Thus, the results of this study can provide fundamental data for the future development of multi-functional enteric soft capsules.

Acknowledgement. We are grateful to RP Bio Inc., which generously manufactured and provided enteric soft capsules for this study. We also appreciate the constructive comments of anonymous reviewers, who improved the clarity of this manuscript.

REFERENCES

Bader J., Albin A., Stahl U. (2012): Spore-forming bacteria and their utilisation as probiotics. Beneficial Microbes, 3: 67–75.

Bang W.Y., Kim H., Chae S.A., Yang S.Y., Ban O.H., Kim T.Y., Kwon H.S., Jung Y.H., Yang J. (2022): A quadruple coating of probiotics for enhancing intestinal adhesion and competitive exclusion of *Salmonella typhimurium*. Journal of Medicinal Food, 25: 213–218.

Cholewski M., Tomczykowa M., Tomczyk M. (2018): A comprehensive review of chemistry, sources and bioavailability of omega-3 fatty acids. Nutrients, 10: 1662.

Dimid E., Christodoulides S., Scott S.M., Whelan K. (2017): Mechanisms of action of probiotics and the gastrointestinal microbiota on gut motility and constipation. Advances in Nutrition, 8: 484–494.

FAO/WHO (2001): Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, Córdoba, Oct 1–4, 2001: 1–4.

Han S., Lu Y., Xie J., Fei Y., Zheng G., Wang Z., Liu J., Lv L., Ling Z., Berglund B., Yao M., Li L. (2021): Probiotic gastrointestinal transit and colonization after oral administration: A long journey. Frontiers in cellular and Infection Microbiology, 11: 609722.

Hill C., Guarner F., Reid G., Gibson G.R., Merenstein D.J., Pot B., Morelli L., Canani R.B., Flint H.J., Salminen S., Calder P.C., Sanders M.E. (2014): The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology, 17: 687–701.

Ishwarya S.P., Nisha P. (2022): Advances and prospects in the food applications of pectin hydrogels. Critical Reviews in Food Science and Nutrition, 62: 4393–4417.

Kankaanpää P.E., Salminen S.J., Isolauri E., Lee Y.K. (2001): The influence of polyunsaturated fatty acids on probiotic growth and adhesion. FEMS Microbiology Letters, 194: 149–153.

Kaushik P., Dowling K., Barrow C.J., Adhikari B. (2015): Microencapsulation of omega-3 fatty acids: A review of microencapsulation and characterization methods. Journal of Functional Foods, 19: 868–881.

Kechagia M., Basoulis D., Konstantopoulou S., Dimitriadi D., Gyftopoulou K., Skarmoutsou N., Fakiri E.M. (2013): Health benefits of probiotics: A review. ISRN Nutrition, 2013: 481651.

Kim M., Nam D.G., Kim S.B., Im P., Choe J.S., Choi A.J. (2018): Enhancement of viability, acid, and bile tolerance and accelerated stability in lyophilized *Weissella cibaria* JW15 with protective agents. Food Science & Nutrition, 6: 1904–1913.

Lahtinen S.J., Ouwehand A.C., Salminen S.J., Forssell P., Myllärinen P. (2007): Effect of starch- and lipid-based encapsulation on the culturability of two *Bifidobacterium longum* strains. Letters in Applied Microbiology, 44: 500–505.

Latif A., Shehzad A., Niazi S., Zahid A., Ashraf W., Iqbal M.W., Rehman A., Riaz T., Aadil R. M., Khan I.M., Özogul F., Rocha J.M., Esatbeyoglu T., Korma S.A. (2023): Probiotics: Mechanism of action, health benefits and their application in food industries. Frontiers in Microbiology, 14: 1216674.

Luchtman D.W., Song C. (2013): Cognitive enhancement by omega-3 fatty acids from child-hood to old age: Findings from animal and clinical studies. Neuropharmacology, 64: 550–565.

Presti I., D'orazio G., Labra M., la Ferla B., Mezzasalma V., Bizzaro G., Giardina S., Michelotti A., Tursi F., Vassallo M., di Gennaro P. (2015): Evaluation of the probiotic properties of new *Lactobacillus* and *Bifidobacterium* strains and their *in vitro* effect. Applied Microbiology and Biotechnology, 99: 5613–5626.

Rajam R., Anandharamakrishnan C. (2015): Microencapsulation of *Lactobacillus plantarum* (MTCC 5422) with fructooligosaccharide as wall material by spray drying. LWT – Food Science and Technology, 60: 773–780.

Ringø E., Bendiksen H.R., Gausen S.J., Sundsfjord A., Olsen R.E. (1998): The effect of dietary fatty acids on lactic acid bacteria associated with the epithelial mucosa and from faecalia of Arctic charr, *Salvelinus alpinus* (L.). Journal of Applied Microbiology, 85: 855–864.

Rodrigues F.J., Omura, M H., Cedran M.F., Dekker R.F., Barbosa-Dekker A.M., Garcia S. (2017): Effect of natural polymers on the survival of *Lactobacillus casei* encapsu-

https://doi.org/10.17221/181/2023-CJFS

lated in alginate microspheres. Journal of Microencapsulation, 34: 431–439.

Settanni L., Moschetti G. (2010): Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. Food Microbiology, 27: 691–697.

Suez J., Zmora N., Segal E., Elinav E. (2019): The pros, cons, and many unknowns of probiotics. Nature Medicine, 25: 716–729.

Surette M.E. (2008): The science behind dietary omega-3 fatty acids. Canadian Medical Association Journal, 178: 177–180.

Swanson D., Block R., Mousa S.A. (2012): Omega-3 fatty acids EPA and DHA: Health benefits throughout life. Advances in Nutrition, 3: 1–7.

Yashodhara B.M., Umakanth S., Pappachan J.M., Bhat S.K., Kamath R., Choo B.H. (2009): Omega-3 fatty acids: A comprehensive review of their role in health and disease. Postgraduate Medical Journal, 85: 84–90.

Received: November 3, 2023
Accepted: March 20, 2024
Published online: April 23, 2024