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Research Article

Sustainable Encapsulation of *Limosilactobacillus fermentum* Using Cellulose Microfiber Hydrogel: Antimicrobial and Immunomodulatory Effects Against *Staphylococcus aureus*

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Abstract | Probiotics, live bacteria that confer health benefits when administered in enough quantities, have garnered significant interest for their capacity to modulate the immune system. This study aimed to investigate the effects of challenging *Limosilactobacillus fermentum* InaCC B1295 (LFB1295) with *Staphylococcus aureus* on weight gain, the amount of lactic acid bacteria (LAB) and *S. aureus* in feces, and the immune system in rat blood serum. This research was conducted experimentally *in vivo* using LFB1295 cells encapsulated with cellulose microfiber hydrogel (CMFH) from oil palm solid waste (OPSW) challenged with *S. aureus* and measured weight gain parameters, total faecal LAB and *S. aureus*, and several blood serum immune parameters. The results demonstrated that rats given LFB1295 encapsulated in CMFH had lower *S. aureus* counts by 3.16 to 14.47%, greater body weights by 19.50 to 37.33%, and total LAB counts by 7.80 to 13.15%. Oral administration of CMFH-encapsulated LFB1295 significantly ($p<0.05$) influenced the immune system in the blood serum of rats. Rat blood levels of LFB1295 encapsulated in CMFH could be significantly ($p<0.05$) increased, and IgE level could be lowered by24.04 to 64.94% and IL-10 could be increased by 9.27 to 9.59%; however, IgA and IL-6 levels were not significantly ($p>0.05$) affected. Based on the results of in vitro and in vivo studies, the findings of this study are the first use of CMFH from OPSW that works well as a local probiotic encapsulant. *Lb. fermentum* InaCC B1295 can inhibit the growth of *S. aureus* which is closely related to the formation of immune responses. This probiotic has the potential to be used to maintain the health of the digestive tract and immune system in humans.

Keywords | Probiotics, *Limosilactobacillus fermentum, Staphylococcus aureus,* Immune system, Cellulose microfiber, Oil palm

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INTRODUCTION

Staphylococcus aureus is a prevalent bacterium transmitted by food. It can lead to several diseases, including the lower respiratory tract, skin and soft tissue, bloodstream infections (Kadariya *et al*., 2014; Tong *et al*., 2015), septicemia, meningitis, toxic shock syndrome, and human osteomyelitis (Ramezani *et al*., 2020). Plenty of research indicates that these phenomena significantly impact human health. *Staphylococcus aureus* poses a more significant threat compared to other microbial pathogens due to its ability to produce toxins, thrive in various environments (both non-pathogenic and clinically significant), adjust its metabolic rate to adapt to its surroundings (such as altering the cell wall, population adaptation, and cytoplasmic modulation), and effectively evade the host's immune defences (Chan *et al*., 2021). Probiotics, such as lactic acid bacteria (LAB), inhibit *S. aureus* infection by improving the intestinal barrier and increasing the host's immune response.

Live bacteria called probiotics can modify the immune system and improve health when taken in sufficient concentrations (Salminen and van Loveren, 2012). Common probiotics, such as lactic acid bacteria (LAB) and bifidobacteria (Kechagia *et al*., 2013) as culture or as food adjunct must be tolerant to acid and bile (Naghmouchi *et al*., 2020; Pato *et al*., 2022), have the ability to live, grow, and perform therapeutic functions such as combating mutations and cancer (Kocabay and Çetinkaya, 2020; Wang *et al*., 2015), deconjugating bile salt, and binding cholesterol (Cui *et al*., 2016; García *et al*., 2017; Russo *et al*., 2016), bacteriocin production (Barone *et al*., 2016; Zhao *et al*., 2019), immune modulation (Jang *et al*., 2017; Mazziotta *et al*., 2023) in the intestinal tract. By modulating the gut microbiota and promoting a balanced immune response, probiotics influence the immune system's response to pathogens and immune-related conditions. The human immune system protects the body against pathogens and maintains overall health (Pato *et al*., 2019). According to Risnasari *et al*. (2012) *Lb. fermentum* can help reduce inflammation in animal models whose immune systems are damaged by lipopolysaccharide (LPS), dextran sulphate sodium (DSS), 2,4,6-trinitrobenzene sulfonic acid (TNBS), and pathogen infections. It has a critical role in reducing pro-inflammatory factors like TNF-α, IFN-γ, and IL-6 while boosting anti-inflammatory factors like IL-10. Additionally, by boosting the Th-1 response and virus-neutralizing antibodies, *Lb. fermentum* CECT-5716 protects against recurrent infection and improves the immunologic reaction to an influenza vaccine (12% increase in specific antibodies; 37.5% reduction in the occurrence of an influenza-like illness) (Fung *et al*., 2011; Pato *et al*., 2022).

Manufacturing foods with probiotic claims is difficult due to the challenges associated with the survival and retention of probiotic cells incorporated during production, storage, distribution, and consumption (Min *et al*., 2018). Simultaneously, the application of probiotics in diverse food matrices is increasingly gaining popularity due to their cell encapsulation (Rodrigues *et al*., 2020). Proteins like milk gluten, casein, gelatin, and albumin are common encapsulant materials, as well as lipids like wax, paraffin, mono- and diglycerides, and carbohydrates like dextrins, pectins, cellulose, chitosan, and carrageenan (de la Cruz Pech-Canul *et al*., 2020). In recent times, cellulose microfiber (CMF) have been produced using oil palm solid waste (OPSW). CMF is cellulose that has been ground into microfibrils with a diameter of 10–100 μm. CMF is lightweight, renewable, and biodegradable. It also has a high specific surface area, strength, and stiffness (Risnasari *et al*., 2012). The innovative use of CMFH from OPSW enhances probiotic viability and promotes sustainable resource utilization, aligning with global waste management goals. Cellulose microfiber hydrogel (CMFH) made from oil palm solid waste (OPSW), oil palm leaves (OPL), oil palm trunk (OPT), oil palm fronds (OPF), and oil palm empty fruit bunches (OPEFB), can improve probiotic survival (Pato *et al*., 2019; Steinberg *et al*., 2014; Xing *et al*., 2017). In 2022, Indonesia was the leading global producer of palm oil, generating 46.729 million tons of crude palm oil (CPO) from 14.99 million hectares (Directorate General of Estates, 2022). Cellulose microfiber (CMF) derived from OPSW biomass is beneficial in the healthcare, medical, filtration, and sensor sectors (Dea-Ayuela *et al*., 2008; Galdeano *et al*., 2019). The characteristics of cellulose microfiber include its specific surface area, high strength and stiffness, low weight, biodegradability, and renewable nature (Wojdasiewicz *et al*., 2014).

In previous studies, the viability of encapsulation of *Limosilactobacillus fermentum* InaCC B1295 (LFB1295) was studied at room and cold temperatures for 28 days, as well as its potential as a probiotic due to its ability to lower carcinogenic enzymes (Pato *et al*., 2019), inhibit the growth of multiple food-borne pathogens, such as *Staphylococcus aureus*, and be resistant to antibiotics (Xing *et al*., 2017), also resistance to bile acids and stomach acids (Steinberg *et al*., 2014), The function of applied encapsulation to LAB used as probiotics is to enhance their survival during transit through the harsh conditions of the digestive tract, ensuring their delivery to the gut in an active form where they can exert their beneficial effects (Steinberg *et al*., 2014; Tempelmans Plat-Sinnige *et al*., 2009; Ren *et al*., 2017). The current study aimed to provide valuable insights into the potential of encapsulated *Lb. fermentum* InaCC B1295 with CMFH-OPSW as immunomodulatory agents and their role in maintaining or improving immune function challenged with *S. aureus*. The results of this study could provide valuable information on the immunoregulatory effects of encapsulated *Lb. fermentum* with CMFH-OPSW

in modulating the microbiota and immune response in the presence of *S. aureus* infection with animal studies that may promote recovery from inflammatory disease. These studies set the stage for a comprehensive exploration of the effects of encapsulated *Lb. fermentum* on the immune system through in vivo studies, offering a glimpse into the potential applications of these probiotics for promoting immune health and preventing immune-related disorders (Frossard *et al*., 2007; Pato *et al*., 2023).

MATERIALS AND METHODS

Materials

Oil palm plantations in Riau, Indonesia, generate oil palm solid waste (OPSW) from oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunches (OPEFB), and oil palm leaves (OPL) for the production of cellulose microfiber (CMF). The LFB1295 strains were obtain from the Indonesian Culture Collection (InaCC) at National Research and Innovation Agency (BRIN), Indonesia. We procured the polyvinyl alcohol (Sigma-Aldrich in Steinheim, Germany), MRS broth (MRSB), Columbia blood agar (CBA), brain heart infusion agar (BHIA), and decarboxylases agar (DA) as microbiological media. We also used different chemicals and reagents from Merck and Oxoid (Figure 1).

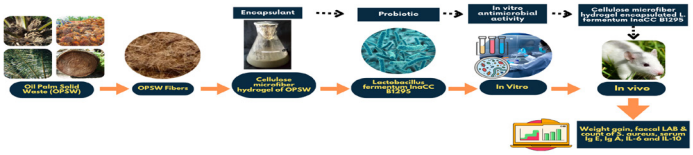

Figure 1: Flowchart of the research methodology.

Propagation of Cells Bacterial

The propagation of LFB1295 was described by Pato *et al*. (2022). Active cultures of LFB1295 were individually inoculated into sterile MRSB medium and incubated for 24 hours at 37°C.

Separation of Cells Bacterial

The separation of LFB1295 cells were referred to by Pato *et al*. (2022). The active culture was centrifuged for 15 minutes at 4°C at 4500 rpm following incubation to segregate the cells from the supernatant. The harvested cells were subsequently rinsed twice with sterile distilled water until they were free from the medium. Additionally, the cells were eliminated by the addition of phosphate buffer at a pH of 7. A phosphate buffer in a 1:1 ratio with the cells was prepared, transferred to a sterile Erlenmeyer flask, and stored at refrigeration temperature.

Preparation of Cellulose Microfiber

The preparation of cellulose microfiber (CMF) from OPSW was referred to by Pato *et al*. (2022). The OPSW was segmented into small pieces measuring approximately 0.5–1 cm in length, followed by drying in an oven at 60°C for 4 hours. The dried OPSW was immersed in 10 L of a 6% KOH solution, using 2.5 kg, and left at room temperature for 12 hours. Subsequently, the OPSW underwent three rinses with water. Additionally, the washed OPSW was immersed in a hypochlorite solution for 5 hours. Next, we filtered the OPSW fiber and rinsed it with water until a neutral pH of 7 was achieved. The OPSW fiber was then dried in an oven set at 60°C for 4 hours, followed by pulverization with a blender and filtration through an 80-mesh sieve. The sample was processed to yield cellulose microfiber (CMF) by grinding in a planetary ball mill for 60 minutes at a speed of 8,000 rpm. The CMF was obtained by sieving the milling output through a 100-mesh sieve.

Preparation of Sterile Cellulose Microfiber Hydrogel

Sterile CMF hydrogel (CMFH) was prepared according to Fung *et al*. (2011). CMF derived from OPSW (OPT, OPF, OPEFB, and OPL) was combined with 8% PVA in a 1:10 ratio. The mixture was heated on a hot plate and homogenized with a magnetic stirrer until fully dissolved, followed by sterilization in an autoclave at 121°C for 15 minutes to create the CMY hydrogel.

Preparation of Lactic Acid Bacteria Encapsulation

Encapsulated LFB1295 was prepared according to Pato *et al*. (2022). by adding 40 mL of cell biomass LFB1295 to 40 mL of sterile CMFH-OPSW (OPT, OPF, OPEFB, and OPL), then stirring using a stir bar until well mixed, and the encapsulated LAB is ready for use.

Preparation of *S. Aureus* Cell Suspension

S. aureus cell suspensions were prepared according to Pato *et al*. (2022). *S. aureus* was activated by inoculating 0.1 mL of a bacterial suspension into 5 mL of nutrient broth, thoroughly shaking the mixture, and incubating it aerobically for 18 hours at 37°C. The cell biomass was then separated from the supernatant through centrifugation at 4,000 rpm for 15 minutes at 4°C. The cell biomass was washed three times with distilled water. Finally, phosphate buffer solution was added to the cell biomass until the concentration reached 10^5 CFU/mL.

In Vitro Antimicrobial Activity

This experiment employed the paper disc diffusion technique as described by (Saranya and Hemashenpagam, 2011). Nutrient agar (NA) is a fundamental, all-purpose microbiological medium designed for cultivating a variety of non-fastidious microorganisms, primarily bacteria. Its formulation consists of peptone (5 g/L), beef extract or yeast extract (3 g/L), and agar (15 g/L). The NA medium was sterilized and poured into a petri dish to solidify at

room temperature. A 0.1 mL sample of the test bacteria was evenly spread using a hockey stick and allowed to dry for 15 minutes. The paper disc technique involved immersing a paper disc in the supernatant until fully saturated, followed by placement onto the nutrient agar that had been inoculated with the test bacteria. The samples were incubated at 37°C for 24 hours. The diameter of the clear zone was measured three times at each location, and the mean of these measurements was calculated.

Animal Test

The probiotic activity of LFB1295 in the form of free cells or cells encapsulated with CMFH challenged with *S. aureus* on the number of faecal lactic acid bacteria and *S. aureus* as well as modulation of the immune system *in vivo* was carried out according to Pato *et al.* (2019); Xing *et al.* (2017) with slight modifications. This research was conducted based on the Code of Ethics and Guidelines for Research using Animals issued by the Faculty of Nursing, Universitas Riau, Pekanbaru, Indonesia. The in vivo test used 30 male Sprague Dawley (SD) rats with weights ranging from 120–130 g. The treatment was divided into six groups (Figure 2). After 5 days, consume probiotic LFB1295 at 10^8 CFU/mL, then group 3–6 was administered at a concentration of 10^5 CFU/mL with *S. aureus* for 4 days. The flowchart of *in vivo* animal test using rats applied in this study is shown in Figure 2.

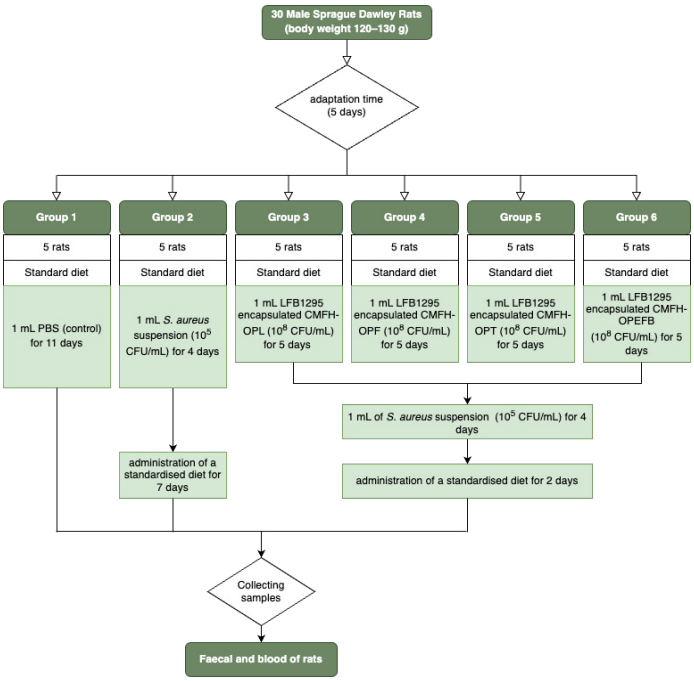

Figure 2: Flowchart of *in vivo* animal test using rats.

Microbial Analysis of Faeces

Fresh fecal samples were collected from each rat by gently pressing the rectal area. The stool samples were placed in test tubes, securely sealed, and analyzed within 30 to 60 minutes of collection. The samples were then homogenized and diluted with sterile phosphate buffer. The count of lactic acid bacteria (LAB) was determined using the spread plate method on MRS agar medium, while the number of *S. aureus* was assessed using Blair-Parker medium. All plates were incubated at 37°C for 48 hours.

Measuring Body Weight and Collecting Faeces and Blood Samples

Body weight data were recorded at both the beginning and the end of the experiment. Stool samples were collected and stored in a freezer. Blood was drawn from the inferior vena cava, allowed to stand at room temperature for 1 hour, and then centrifuged at 3,000 rpm for 10 minutes at 4°C to obtain serum samples. Body weight gain was calculated by subtracting the initial body weight from the terminal body weight and expressed in grams.

Serum Immunoglobulin and Interleukin Measurements

Serum immunoglobulin and interleukin concentrations were measured using an Elisa Kit according to the manufacturer's instructions, Bioassay Technology Laboratory (228 Ningguo Rd, Yangpu District, Shanghai 200090, China). The types of cytokines evaluated were interleukins (IL), namely IL-6 and IL-10, and immunoglobulins (Ig), namely Ig-A and Ig-E. The amount of Interleukins 6 and 10 were analyzed using Rat IL-6 ELISA Kit and Rat IL-10 ELISA Kit, respectively. Immunoglobulin A and E were analyzed using the Rat Ig-A ELISA Kit and Rat Ig-E ELISA Kit, respectively. Each serum parameter's levels were determined according to procedures determined by the Bioassay Technology Laboratory (228 Ningguo Rd, Yangpu District, Shanghai 200090, China). Then, each well's optical density (OD value) was determined using a microplate reader set to 450 nm within 10 minutes after adding the stop solution. The concentrations of the immunoglobulin and interleukin were determined using purified rat IgA, IgE, IL-6, and IL-10 standards (228 Ningguo Rd, Yangpu District, Shanghai 200090, China). The results are expressed as ng/mL for IgA and IL-6, µg/mL for IgE and pg/mL for IL-10.

Data Analysis

Data on weight gain, faecal microflora (total LAB and *S. aureus* count), immunoglobulin serum level (Ig-A and Ig-E), and serum cytokine (IL-6 and IL-10) were statistically analyzed using analysis of variance (ANOVA) using IBM SPSS version 23 software. If Fcount ≥ Ftable, the analysis was continued with the Duncan multiple range test (DNMRT) at 5%.

RESULTS AND DISCUSSION

Body Weight Gain

Body weight gain is a parameter that needs to be observed in vivo research to determine the effect of treatment.

The results showed that administration of LFB1295 cells encapsulated with CMFH of various OPSW significantly influenced the body weight gain of rats (Table 1).

Table 1: Body weight gain of rats fed LFB1295 encapsulated with CMFH from oil palm solid waste (CMFH-OPSW) challenged with *S. Aureus.*

Treatments	Weight gain of rats (g) ± SD
Group 1	25.66^{ab} ± 11.41
Group 2	23.20^{a} ± 3.51
Group 3	26.92^{ab} ± 12.34
Group 4	28.82^{b} ± 10.98
Group 5	30.40^{b} ± 13.23
Group 6	37.02^{b} ± 19.24

^{a,b}Means in the same column followed by different superscript letters indicate significant ($p<0.05$).

An analysis of variance showed that treatments with LFB1295 encapsulated with CMFH from OPSW had an impact on the rats' weight gains. The numbers show that there was a statistically significant difference between the treatment groups (Groups 4, 5, and 6—with probiotic) and the control group (Group 2—without probiotic) ($p < 0.05$), with a 95% confidence interval spanning from 23.20 g to 37.02 g. Table 1 demonstrates that administered with *S. aureus* and LFB1295 encapsulated with CMFH derived from oil palm fronds (Group 4), oil palm trunks (Group 5), and empty oil palm fruit bunches (Group 6) exhibited a rise in body weight (26.92–37.02 g) compared to rats that were solely administered with *S. aureus* without LFB1295 about 23.20 g (Group 2). This discovery is attributed to the ability of *S. aureus* to induce disruptions in the gastrointestinal system, thus interfering with the assimilation of nutrients and leading to a decline in body weight. The administration of LFB1295 can inhibit the growth of *S. aureus*, as evidenced by the findings presented in Table 3. The LFB1295 leads to maintaining a healthy digestive system and facilitates the smooth absorption of food matrix. Additionally, it contributes to the augmentation of rat's body weight. The findings of this study corroborate prior research indicating the introduction of *Lactobacillus acidophilus* L36, *Lactobacillus salivarius* L38, and *Salmonella enterica* ser. *typhimurium* to a group of rats increased body weight compared to a group of rats solely exposed to *Salmonella enterica* ser. *typhimurium* (Steinberg *et al*., 2014). Moreover, according to Ren *et al*. (2017), rats infected with pathogenic bacteria had more significant body weight loss. However, rats that were fed probiotics while infected showed minimal weight loss.

Total Lactic Acid Bacteria

Lactic acid bacteria (LAB) are prokaryotic microorganisms that produce lactic acid as the main product of their metabolism. Lactic acid is an organic compound with acidic properties found in various fermented foods, such as yoghurt, kefir, kimchi, and dadih. LAB have an essential role in the food industry. They are also present in the human digestive system, where LAB helps maintain the balance of the gut microbiota and plays a role in maintaining gut health. Most of the microbes used as probiotics are LAB, especially the genus Lactobacillus. for example. The assay results showed that the administration of LFB1295 cells encapsulated with CMFH of various OPSW significantly affected the total LAB in the faeces of rats (Table 2).

Table 2: Fecal total lactic acid bacteria in rats fed LFB1295 encapsulated with CMFH from oil palm solid waste (CMFH-OPSW) challenged with *S. Aureus.*

Treatments	Total LAB (log CFU/g)	Total S. aureus (log CFU/g)
Group 1	9.28^{b} ± 0.12	8.76^{bc} ± 0.18
Group 2	8.39^{a}± 0.26	9.19^{e} ± 0.23
Group 3	9.10^{b} ±0.34	9.11^{de} ± 0.28
Group 4	9.33^{bc} ± 0.19	8.90^{cd} ± 0.15
Group 5	9.59^{cd}± 0.19	7.86^{a} ± 0.24
Group 6	9.66^{d} ± 0.10	8.61^{b} ± 0.11

^{a,b}Means in the same column followed by different superscript letters indicate significant ($p<0.05$).

Table 3: *In vitro* antimicrobial activity of *Lb. fermentum* InaCC B1295 without encapsulation and encapsulated CMFH from oil palm solid waste (CMFH-OPSW) against *Staphylococcus aureus.*

Treatments	Clear zones (cm)
Free cells of LFB1295	0.56^{c}±0.12
Cells of LFB1295 encapsulated with CMFH from OPF	0.49^{b}±0.09
Cells of LFB1295 encapsulated with CMFH from OPL	0.59^{c}±0.12
Cells of LFB1295 encapsulated with CMFH from OPT	0.46^{b}±0.06
Cells of LFB1295 encapsulated with CMFH from OPEFB	0.39^{a}±0.06

^{a,b,c}Means in the same column followed by different superscript letters indicate significant ($p<0.05$).

The probiotic-treated groups (Groups 3, 4, 5, and 6) exhibited a significant change in total LAB compared to the control group (Groups 1 and 2). The p-value is less than 0.05, and the 95% confidence interval ranges from 8.39 to 9.66 log CFU/g. The findings indicate that encapsulating LFB1295 with CMFH-OPSW enhances the viability of probiotics in the gastrointestinal system. Table 2 displays the total LAB and *S. aureus* for each group in this investigation. Group 1 had a higher total LAB count than Group 2 when introducing *S. aureus* alone (Group 2). Nevertheless, the administration of LFB1295 cells encapsulated with CMFH derived from OPT and OPEFB to rats in Groups 5 and 6, respectively, resulted in a greater abundan-

ce of total faecal LAB (9.59–9.66 log CFU/g) compared to the treatments given to rats in Group 1 (9.28 log CFU/g) and Group 2 (8.39 log CFU/g). The finding is potentially due to the combined effects of CMFH originating from both forms of OPWS. The oil palm solid waste sustains the survival of LFB1295 while it crosses the digestive system of rats and enhances the presence of total faecal LAB.

According to Pato *et al*. (2022), standard biopolymer-based encapsulation approaches have inherent limitations in providing stable probiotics against the effects of gastrointestinal fluids, including in the intestines of rats. Surprisingly, cellulose-based hydrogels, whether used alone or in conjunction with other biopolymers, have recently shown promising capabilities in surpassing the limitations of traditional biopolymer-based encapsulation methods. In the current study, the encapsulation of LFB1295 with CMFH-OPEFB and CMFH-OPT contributes significantly to the widespread appeal and use of these materials. Thus, CMF improves the functionality and durability of the hydrogel due to its strong hydrogen bonding and entanglement, even at low concentrations, to protect LFB1295 through the gastrointestinal of rats. The hydrogel demonstrates improved compression resistance, cross-linking density, and interfacial adhesion due to the efficient dispersion of CMF. Hydrogels from CMF are highly effective for encapsulation, as they can reliably release probiotic microorganisms in the gut.

One of the limitations and challenges associated with encapsulated probiotics is colonization resistance. This phenomenon occurs when the normal gut microbiota establishes a stable bacterial community that prevents the invasion of foreign bacteria and the proliferation of pathogens. Colonization resistance involves both direct and indirect mechanisms. Direct colonization resistance stops foreign microbes from colonizing based on the gut microbiota alone, without any host interactions. It includes both blocking mechanisms and resource competition mechanisms. Indirect colonization resistance is mediated by host-derived mechanisms, such as the production of antimicrobial peptides, the maintenance of the epithelial barrier, and the modification of bile acids through host interactions. Additionally, ribosomes in both Gram-positive and Gram-negative bacteria produce bacteriocins, which are protein-based compounds that inhibit closely related species or those that compete for the same resources or environments (He *et al*., 2023). *Enterococcus faecalis* that produces bacteriocin inhibits the colonization of vancomycin-resistant enterococci (Kommineni *et al*., 2015). Commensal gut microbiota colonization resistance impedes probiotics. Certain studies indicate that humans excrete probiotics in feces at the time of ingestion and shortly thereafter (He *et al*., 2023). According to Zmora *et al*. (2018), probiotics do not modify the structure or diversity of gut microbial communities. Colonization resistance might shorten the long-lasting effects of giving 11 types of probiotics to adult male mice that are specific pathogen-free (SPF) and germ-free (GF) after 28 days of supplementation. Analysis of the stool samples from the GI tract showed that GF mice had higher numbers of viable bacteria than SPF groups. Probiotics may exhibit greater mucosal colonization resistance in specific pathogen-free (SPF) mice compared to germ-free (GF) animals. A further compelling investigation revealed that probiotic colonization operates differently across individuals. Volunteers exhibited either a "permissive" or "resistant" disposition. The permissive group exhibited a significant increase in probiotic strains within their intestinal mucus barrier, whereas the "resistant" group did not.

Staphylococcus Aureus Count

Staphylococcus aureus is both a commensal and pathogenic bacterium in humans, with approximately 30% of the population being colonized by it. Typically, this bacterium resides on the skin, in the respiratory tract, and within the digestive tract without causing any health issues. However, *S. aureus* can become problematic when an infection occurs and can be transmitted from one individual to another through direct contact or contaminated objects. As an invasive pathogenic bacterium, *S. aureus* can lead to local skin infections, such as impetigo, as well as life-threatening systemic conditions, including osteomyelitis and infective endocarditis. Variance analysis results indicated that the administration of lactic acid bacteria (LAB) cells encapsulated with CMFH from OPSW significantly influenced the number of *S. aureus* in the feces of rats (Table 2). Furthermore, oral administration of *S. aureus* (Group 2) resulted in a significant increase in the number of *S. aureus* in the feces of the rats compared to the control treatment (Group 1).

However, the administration of LFB1295 encapsulated with CMFH caused a significant reduction in the number of *S. aureus* in Group 5 (CMFH from oil palm trunk) and Group 6 (CMFH from empty oil palm fruit bunches), respectively, 7.86 log CFU/g and 8.61 log CFU/g. This finding shows that OPT and OPEFB as the encapsulant material of LFB1295 have a significant effect on the protection of LFB1295 in the digestive tract of rats. Then, the colonisation of LFB1295 successfully inhibits the growth of *S. aureus* as pathogenic bacteria. The inhibitory effect of LFB1295 on the growth of *S. aureus* resulted in a reduction in its number. LFB1295 significantly produces lactic acid and other organic acids in the digestive tract of rats, which effectively lowers the pH of the digestive tract and inhibits the growth of *S. aureus*. LFB1295 significantly produces lactic acid and other organic acids in the digestive tract of rats, which effectively lowers the pH of the digestive tract and inhibits the growth of *S. aureus*. The inhibition of *S. aureus* growth by free cells or encapsulated cells of LFB1295 in vitro as presented in Table 3.

Table 4: IgA, IgE, IL-6, and IL-10 level in rats fed LFB1295 encapsulated with CMFH from oil palm solid waste (CMFH-OPSW) challenged with *S. Aureus*.

Treatments	IgA level (ng/mL)	IgE level (ng/mL)	IL-6 level (ng/mL)	IL-10 level (ng/mL)
Group 1	340.58^{a}±55.93	4.49^{b}±0.91	5.59^{a}±0.47	33.79^{abc}±1.93
Group 2	499.38^{b}±109.13	5.99^{d}±0.53	4.52^{a}±0.82	33.28^{ab}±4.26
Group 3	271.36^{a}±92.93	5.19^{cd}±0.51	4.80^{a}±0.30	36.42^{bc}±2.33
Group 4	295.84^{a}±78.51	4.13^{b}±0.55	4.77^{a}±0.57	36.81^{c}±1.73
Group 5	348.16^{a}±71.42	4.55^{bc}±1.04	4.71^{a}±0.64	36.68^{c}±1.92
Group 6	371.48^{a}±61.49	2.10^{a}±0.69	4.74^{a}±0.42	32.54^{a}±0.48

^{a,b}Means in the same column followed by different superscript letters indicate significant ($p<0.05$).

Table 3 demonstrated the antibacterial efficacy of each group in this study. The in vitro antimicrobial activity of the groups treated with probiotics and encapsulated with CMFH-OPSW (OPF, OPL, OPT, and OPEFB) significantly differed from that of the control group (free cell). The p-value is less than 0.05, and the 95% confidence interval clearly ranges from 0.39 to 0.59 cm. The findings indicate that the incorporation of LFB1295 into CMFH-OPSW enhances the antibacterial efficacy of probiotics against *S. aureus*. Previous finding also reported *L. fermentum* strains isolated from fermented foods and the oral mucosa of healthy individuals demonstrated inhibitory effects on multiple pathogens, including *S. aureus* (Hossain and Mozumder, 2022; Mourad *et al*., 2023). These findings support its potential application in food preservation and as a therapeutic probiotic for controlling antibiotic-resistant pathogens. Hence, *Limosilactobacillus fermentum* exhibits inhibitory effects on the growth rate and downregulates the expression of the gene responsible for biofilm formation in methicillin-resistant *Staphylococcus aureus* (MRSA) by decreasing the expression of the *tst*, *sae*, and *sea* genes, as well as the *agr* quorum-sensing system, in MRSA, without impacting its growth. The *agr* A system is an essential worldwide regulatory system that controls the expression of multiple genes associated with the secretion of proteins and virulence factors, such as protease and collagenase in *S. aureus* (Ramezani *et al*., 2020).

Rat Serum Immunoglobulin and Interleukin Levels

Immunoglobulin A (IgA) is one of the human immune system's five primary immunoglobulins (Ig) or antibodies. IgA has a vital role in the body's defense against infection and acts as the main component of the immune system, which is found in the respiratory tract, digestive tract, urinary tract, and various mucosal surfaces of the body. IgA plays a role in the body's defense by binding to pathogens (such as bacteria, viruses, and fungi) and preventing them from attaching to mucosal cells. It helps prevent infections by blocking the invasion of pathogens into the body. IgA also plays a role in the secretory immune system by helping to protect mucosal surfaces from infection. The results of variance analysis showed that administration of LAB cells encapsulated with CMFH from OPSW had no significant effect on the amount of IgA in rat serum (Table 4).

Table 4 illustrated the levels of immunoglobulins (IgA and IgE) and interleukins (IL-6 and IL-10) for each group in this investigation. The ANOVA test indicated that treatments with LFB1295 encapsulated with CMFH from OPSW significantly influenced IgA, IgE, and IL-10 levels ($p < 0.05$), however IL-6 levels were not significantly affected ($p > 0.05$) in comparison to Group 1 (control) and Group 2 (*S. aureus* without LFB125). Data in Table 4 shows that *S. aureus* (Group 2) orally stimulated a significant increase in IgA. These findings are supported by previous studies showing increased IgA in cattle immunized with non-active *S. aureus* strains (Tempelmans Plat-Sinnige *et al*., 2009). However, administration of *S. aureus* and LFB1295 encapsulated with CMFH from OPSW (Groups 3, 4, 5, and 6) can reduce production back to levels observed in Group 1 (without *S. aureus* and LFB1295) by about 25–45%. This finding is because LFB1295 can reduce the number of *S. aureus* (Table 2) so that IgA production decreases. IgA is the primary class of antibodies present in mucosal secretions. In most mammals, IgA represents the first line of defense against invasion by inhalation, ingestion, or oral administration of pathogens on susceptible mucosal surfaces. IgA produced by the mucosa is secreted primarily in secretory IgA (sIgA), an IgA dimer complex with secretory protein components (Tempelmans Plat-Sinnige *et al*., 2009). This study showed that the administration of LFB1295 encapsulated with CMFH from OPSW (Groups 3, 4, 5, and 6) restored IgA levels to the same level as the control group (Group 1) about 271.36–371.48 ng/mL. These findings contrast with those of study (Cox *et al*., 2010), which showed that immunization might induce a rise in Th-1 cytokines and that, following the administration of 10^{7} CFU/mL of *Lb. fermentum* CECT-5, the proportion of T-helpers and T-cytotoxics was much higher. Surprisingly, the probiotic group showed antibody induction with a notable rise in antigen-specific IgA. Immunoglobulin A protects against the attachment and infiltration of toxic substances, intestinal bacteria, and intestinal epithelial cells. Immunoglobulin A suppresses the growth of allergic and pathogenic microorganisms and protects the gastrointestinal mucosa from bacterial infection (Ren *et al*., 2017). Furthermore, according to Dea-Ayuela *et al*. (2008), the presence of *L. casei* decreases the concentrations of cytokines (IFN-γ, TNF-α, IL-4, and Il-13) and antibodies (faecal IgA) that target *Trichuris muris*, hence increasing the susceptibility to *T. muris* infection.

Immunoglobulin E (IgE) is one of the five significant antibodies in the human immune system. IgE is unique in the

immune response to allergies and parasitic infections. Here is some vital information about IgE. IgE is an antibody primarily involved in allergic responses. When the body is exposed to allergens (substances that cause allergies), such as pollen, animal dander, or specific food proteins, mast cells, and basophils release histamine and other mediators. Histamine and other mediators cause allergy symptoms, such as itching, sneezing, watery eyes, and swelling. IgE helps initiate and amplify the allergic response. The results of variance analysis showed that administration of BAL cells encapsulated with CMFH from OPSW did not significantly affect the amount of IgE in rats blood serum (Table 4).

Table 4 shows that administration of LFB1295 cells encapsulated with CMFH from various OPSW tended to decrease IgE level range from 5.19 to 2.10 ng/mL, especially cells encapsulated with CMF from OPEFB. The OPSW as an encapsulant of LFB1295 shows a significant effect in suppressing the IgE level of blood serum (Group 3, 4, 5, and 6) compared to Group 2. This result is likely because LFB1295 cells administered orally increase the number of Th2 cells, which reduces the production of IL-4 and IL-13 (not analyzed), reducing IgE production. This statement is supported by Galdeano *et al*. (2019), who reported a decrease in IgE and an increase in IgG triggered by an increase in CD4, IFN-γ, and Th2. Increasing IL-10 levels increases the amount of IgG during oral probiotic treatment. The OPSW as an encapsulant of LFB1295 showed a significant effect on suppressing blood serum IgE levels (Groups 3, 4, 5, and 6) compared to Group 2. The result indicates that the types of OPSW as an encapsulant material for LFB1295 bacteria were able to protect bacterial cells in the digestive tract of rats and have an impact on preventing respiratory tract and skin infections caused by *S. aureus*. High IgE values indicate allergy symptoms, such as itching and sneezing. Based on Table 4, it was found that the lowest IgE value, where LFB1295 was encapsulated with CMFH-OPEFB. This data shows that OPEFB as an encapsulant is the most effective material in protecting LFB1295 in the gastrointestinal tract of rats and inducing inhibition of *S. aureus*, as demonstrated by a decrease in IgE values.

Interleukin-6 (IL-6) is a cytokine or protein that plays a role in the immune system. Cytokines are molecules used by immune cells to communicate with each other. IL-6 has a vital role in various biological processes and the immune system, and it is an example of an interleukin, a group of cytokines that mediate the body's immune response. IL-6 is an anti-inflammatory cytokine produced by macrophage cells, T cells, B cells, and endothelial cells, which are involved in the immune system and inflammatory response. Various stimuli, including infection, inflammation, injury, stress, and diseases, can trigger IL-6 production. Table 4 demonstrates that the administration of *S. aureus* alone (Group 2), *S. aureus* with continued administration of LFB1295 (Groups 3, 4, 5, and 6), or no administration of pathogenic bacteria and LAB (Group 1) had no impact on IL-6 levels. ANOVA analysis revealed that IL-6 levels observed in this study (ranging from 4.52–5.59 ng/L) do not significantly ($P>0.05$) influence encapsulated LFB1295 with OPSW to produce IL-6 as an anti-inflammatory cytokine in the immune system. Rather than inducing broad immunological changes, LFB1295 may selectively exert its effects, targeting specific cytokines or immune pathways. For instance, the modulation of TNF-α or IL-10 levels, also measured in this study, may provide more direct evidence of its immune-modulatory properties. In rats, IL-6 induction occurs 24 hours after administration of *S. aureus* and returns to normal after 60 hours (Tempelmans Plat-Sinnige *et al*., 2009). Zhao *et al*. (2019) found that *Lb. fermentum* reduced inflammation by increasing the production of mediators involved in colitis, including PGE2, IL-4, IL-6, IL-10, IL-17, TNF-α, IFN-γ, and NO. Subsequent investigation confirmed that the crude exopolysaccharide (EPS) obtained from *Lb. fermentum* Lf2 when added to yogurt or milk, offered defense against Salmonella Sp. infection. Additionally, it modified the levels of secretory immunoglobulin A (s-IgA) and interleukin-6 (IL-6) in the small intestine of rats used in the study.

Interleukin-10 (IL-10) is a cytokine protein that plays a crucial role in the immune system. As a member of the interleukin family, which comprises a large group of molecules utilized by the body's immune cells for communication, IL-10 functions as an anti-inflammatory cytokine that mitigates inflammation within the body. This action is essential for preventing excessive or prolonged inflammatory responses that can lead to tissue damage. IL-10 is produced by various immune cell types, including T lymphocytes, macrophages, dendritic cells, and B cells. Additionally, some non-immune cells, such as epithelial cells, are also capable of producing IL-10. The anti-inflammatory effects of IL-10 are mediated by the inhibition of pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), and nuclear factor kappa B (NF-κB). Consequently, IL-10 plays a significant role in reducing inflammatory reactions and minimizing cellular damage. The results of variance analysis (ANOVA) showed that administration of LFB1295 cells encapsulated with CMFH from OPF (Group 4) and OPT (Group 5) significantly influenced ($P<0.05$) the amount of IL-10 in rat serum (Table 4). Data in Table 4 shows that administration of LFB1295 cells encapsulated with CMFH from various OPSW can increase IL-10 levels ranging from 32.54 to 36.81 ng/mL. This finding is possible because LFB1295 cells can increase the amount of IL-12, CD4, IFN-γ, and Th2, stimulating an increase in IL-10 levels in experiments. This statement is supported by Maldonado Galdeano *et al*. (2019) regar-

ding the increase in IL-10 during oral administration of probiotic cells. According to Wojdasiewicz *et al*. (2014), the significant impact of IL-10 on chondrocyte proliferation is demonstrated. IL-10 was found to activate the kinase pathways SMAD1/SMAD5/SMAD8 and ERK1/2 MAP, inducing the expression of bone morphogenetic proteins 2 and 6 (BMP-2, BMP-6) and revealing additional signalling pathways in a mouse model. Various probiotics exhibit distinct methods of action in the context of pathogenic diseases. They mitigate problems, regulate cytokine synthesis, and promote the generation of anti-parasitic antibodies. Subsequent analysis has revealed that the oral administration of a strain of *Lactococcus lactis* that secretes IL-10 could significantly inhibit the progression of food-induced IgE sensitization in a mouse model of food allergy. This research confirms that IL-10-secreting LAB can be utilized in treating several inflammatory diseases, where this cytokine acts as a modulating agent (Frossard *et al*., 2007). Encapsulation using methylcellulose, hydroxypropyl methylcellulose and carboxymethyl cellulose improves the stability and delivery of probiotics in the gastrointestinal tract, enhancing their immunomodulatory effects (Sun *et al*., 2023). Encapsulated *Bifidobacterium animalis* subsp. *lactis* strain IU100 significantly reversed immunosuppression in cyclophosphamide (CTX)-treated rats. It increased serum levels of immunoglobulins (IgA, IgG, and IgM), promoted cytokine production (IL-1β, IL-2, IL-6, TNF-α), and restored gut microbiota diversity, leading to improved immune function (Zhou *et al*., 2024). Encapsulated Lactobacillus strains have demonstrated the ability to modulate immune responses by reducing systemic inflammation and increasing anti-inflammatory cytokine production (e.g., IL-10). Clinical trials have shown encapsulated probiotics to be effective in reducing symptoms of inflammatory bowel diseases and in improving gut-barrier integrity, which plays a crucial role in immune system regulation (Thoda and Touraki, 2023). Despite advancements in *in vivo* study using rats, there are still a number of obstacles to overcome, such as the requirement to create effective and repeatable encapsulation techniques that preserve probiotic viability and activity. Additionally, the clinical effecacy of *L. fermentum* InaCC B1295 encapsulated with OPSW needs to be studied.

CONCLUSIONS AND RECOMMENDATIONS

Rats fed LFB1295 encapsulated with CMFH showed increased body weight and total LAB count and decreased *S. aureus*. Oral administration of LFB1295 encapsulated significantly affected the immune system in the blood serum of rats. Administration of LFB1295 encapsulated with CMFH significantly increased IL-10 levels and reduced IgA and IgE levels but did not significantly influence IL-6 levels in the rat serum. These results suggest that LFB1295 may be taken as a probiotic to strengthen human immunity and restore the proper balance of intestinal microbiota. The present findings documented the first use of CMFH from OPSW as a local probiotic encapsulant for *in vivo* study. This study is limited by its reliance on a single animal model and the lack of human clinical trials to validate findings. Future studies should explore CMFH with LFB1295 to evaluate their efficacy in human infection models.

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NOVELTY STATEMENTS

This is the first study to use male SD rats to examine in vivo the effects of probiotic supplementation encapsulated with CMFH from OPSW on the immune system.

AUTHOR'S CONTRIBUTIONS

Research on the main theme was initiated by Usman Pato, who also oversaw various stages of the project. Usman Pato, Yusmarini and Agrina carried out the following research stages in this study: designing the experiments and supervising several phases of the work. Usman Pato, Yusmarini, Agrina and Evy Rossi are the main contributors to research and writing articles. This experiment was planned by Usman Pato and Emma Riftyan, who also proofread the article. Article writing and editing were done in collaboration with Usman Pato and Emma Riftyan.

Conflict of Interest

None of the authors has any conflict of interest to declare.

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