Antioxidant activity, GABA (γ-aminobutyric acid) production, and potential for colonization of *Lactobacillus fermentum* InaCC B1295 encapsulated with cellulose microfiber hydrogel from oil palm solid waste during storage

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Abstract. *Lactobacillus fermentum* InaCC B1295 is a probiotic bacterium that improves intestinal and immune systems, positively impacting human health. It produces various bioactive compounds, including bacteriocin, antioxidant enzymes, and gamma-aminobutyric acid (GABA), and colonizes the human gastrointestinal system. This study aimed to evaluate the antioxidant activity (IC_{50}), GABA production, and colonization potential of *L. fermentum* InaCC B1295 with encapsulation by cellulose microfiber from oil palm solid waste (trunk, frond, leaf, and empty bunches) at room and refrigeration temperature with storage time 0, 14, and 28 days. The strain InaCC B1295 showed a high activity to scavenging ability against DPPH (1,1-diphenyl-2-picrylhydrazyl) for the treatment with encapsulation with oil palm empty bunches at refrigeration temperature after storage 14 days, which reached 88.56 ppm. Furthermore, *L. fermentum* InaCC B1295 produced GABA ranging from 0.435 mg/L to 2.215 mg/L. *Lactobacillus fermentum* InaCC B1295 encapsulated with oil palm leaf produced the most GABA at 0-day storage with a concentration of 2.215 mg/L and decreased during storage for all treatments. *L. fermentum* InaCC B1295 cells encapsulated in CMF hydrogel from empty oil palm fruit bunches had a lower autoaggregation value than free cells. The cells encapsulated with CMF hydrogel from oil palm empty bunches, on the other hand, had higher coaggregation and hydrophilicity values than the free cells (unencapsulated cells).

1 Introduction

Indonesia ranks among the world's top palm oil producers. Statistics Indonesia estimates that the oil palm plantation produces approximately 8,579,000 hectares in 2021. Oil palm plantations generated 22 million tonnes of crude palm oil (CPO) in 2021 and 23.5 million tonnes in 2022. Indonesia aims to expand its oil palm industry and double its CPO production

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to 40 million tonnes annually by 2023 [1]. The process generates oil palm solid waste such as oil palm leaves (OPL), oil palm trunk (OPT), oil palm fronds (OPF), and oil palm empty fruit bunches (OPEFB), respectively [2]. It is considered a substantial renewable energy source potential. Oil palm solid waste, which is produced from oil palm plantations, is acknowledged as a potentially valuable lignocellulosic resource. The chemical composition of oil palm solid waste includes crude fiber (cellulose, hemicellulose, and lignin), carbohydrate, protein, water, fat, and ash [3–5]. Furthermore, the biomass of OPT, OPEFB, OPL, and OPF consists of lignin (13–37%), cellulose (29–65%), and hemicellulose (12–38%) [2, 3, 6].

Cellulose microfibers (CMF) are abundant in cellulose content. Cellulose microfiber (CMF), also known as microfibrillated cellulose, is cellulose that has been treated to separate its fibres into microfibrils [3], which have a diameter ranging from 0.02–287 nm and a length of several micrometres [6]. Furthermore, The SEM micrographs CMF of oil palm solid waste showed a micrometric scale, rough surface, and adequate homogeneity [3]–[6]. Encapsulation techniques for probiotics using cellulose microfiber hydrogel derived from oil palm solid waste have demonstrated promising results in enhancing the viability of acid and bile tolerance [3–6]. Cellulosic microfibre hydrogels (CMFHs) are hydrophilic polymers cross-linked to form 3D networks. Thus, CMFHs are highly hydrated materials. In addition, CMFHs have a smooth texture, exhibit highly porous structures upon incorporation, and result in minimal protein adsorption [6].

Lactobacillus fermentum InaCC B1295, a strain of lactic acid bacteria, was obtained from dadih, a traditional fermented buffalo milk originating from the Indonesian provinces of Riau and West Sumatra [7]. *Lactobacillus fermentum* is well recognized for its potential as a probiotic, demonstrating probiotic effects, antiviral activity, bio-preservative qualities, and immunobiotic capabilities. Additionally, it has been categorized as a GRAS (generally recognized as safe) bacterium. [8]. Extensive research has demonstrated the beneficial impact of probiotics on health [9, 10]. The safety of probiotics needs to be assessed before new strains of probiotics are introduced into human food, especially those for which the safety data needs to be established [11]. Recent studies are being conducted on lactic acid bacteria (LAB) due to the growing interest in their antioxidant potential and ability to produce GABA (γ-aminobutyric acid).

Lactic acid bacteria produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) due to their metabolic processes. These substances are crucial in the immunological response to invading pathogens and regulating cell communication. However, excessive formation of ROS/RNS can lead to oxidative stress, which damages DNA, proteins, and lipids [12]. DPPH radical scavenging activity of LAB was 2.55–59.4% [12–15]. LAB strains have the potential to enhance the cellular antioxidant defense mechanism through the secretion of enzymes such as superoxide dismutase (SOD) or by inducing the synthesis of glutathione (GSH), the principal non-enzymatic antioxidant and free radical scavenger. Gamma-aminobutyric acid (GABA) is an L-glutamate-derived compound [16]. The enzyme glutamate decarboxylase (GAD) catalyzes the decarboxylation of L-glutamic acid. It has several physiological functions, including neurotransmission, induction of hypotension, and diuretic and sedative effects [17]. According to [17], among the LABs that were examined, only a few strains were able to produce significant quantities of GABA in vitro, exceeding 300 mg/L. The identified bacterial species are *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus otakiensis*, *Lactobacillus namurensis, Lactobacillus futsaii,* and *Lactobacillus fermentum* [16,18,19]

Hydrophobicity, autoaggregation, and coaggregation properties of LAB are prerequisites for probiotics. The first step in determining LAB's capacity to adhere to the digestive system is to assess its hydrophobicity and autoaggregation potential in vitro [20]. According to [21], the bacterial genus, growth medium, and surface structure affect microbial hydrophobicity.

The hydrophobicity of each cell will impact the bacteria's capacity for coaggregation and autoaggregation. Tests for autoaggregation demonstrate that LAB may colonize and adhere to the digestive tract, particularly the colon and small intestine, and that coaggregation with pathogenic bacteria is a crucial characteristic in halting the spread of pathogenic bacteria.

This study aimed to investigate the potential of using cellulose microfiber hydrogel from oil palm solid waste as an encapsulation material for *Lactobacillus fermentum* InaCC B1295 in order to maintain its antioxidant activity and γ-aminobutyric acid production during storage and to evaluate its autoaggregation, coaggregation, and hydrophobicity activities. Recent research in food science and nutrition has brought attention to the potential health benefits of incorporating functional foods to enhance human health. Specifically, using waste materials from the oil palm industry, such as cellulose microfiber hydrogel, for probiotic encapsulation presents a novel and sustainable solution to enhance the functional properties of probiotics. The findings from this study could provide valuable insights into the application of waste-derived materials for the development of functional foods with improved health-promoting properties.

2 Material and method

2.1 Material

Oil palm solid waste utilized in the production of CMF consists of empty fruit clusters (OPEFB), oil palm trunks (OPT), oil palm fronds (OPF), and oil palm leaves (OPL) acquired from PT. Multi Plasma Sejahtera, located in the Bandar Sikijang District of Pelalawan Regency, Riau Province. *Lactobacillus fermentum* InaCC B1295 was acquired from the Research Centre for Biology, Indonesian Institute of Sciences (LIPI), located in West Java, Indonesia, as part of the Indonesian Culture Collection (InaCC). A purchase was made of polyvinyl alcohol (PVA) from Sigma-Aldrich in Steinheim, Germany.

2.2 Preparation of CMF from palm oil solid waste

The solid waste from palm oil (OPT, OPF, OPEFB, and OPL) was cut into smaller pieces of 0.5–1 cm in length. These pieces were then washed with water and boiled at a temperature of 100ºC for 1 hour. Subsequently, they underwent filtration. After being boiled, the OPT, OPF, OPEFB, and OPL were washed extensively with water and then dried for four hours at a temperature of 60ºC. The fibre was immersed in a beaker containing 1000 cc of a 6% (w/v) potassium hydroxide (KOH) solution and allowed to soak for 12 hours at ambient temperature. The fibres are thereafter rinsed with water thrice. In addition, the fibres are soaked in a hypochlorite solution for 5 hours after being washed. Subsequently, they are filtered and rinsed with water at a pH level of 7. OPT was dried and pulverized in a blender till achieving a smooth consistency, and then it underwent filtration using an 80-mesh sieve. To prevent damage to the samples caused by the heat generated during milling, the cellulose flour (CMF) was milled at a speed of 8,000 rpm for 60 minutes. Each milling run lasted for 15 seconds, followed by a rest period of 2 minutes. The milled product was subsequently passed through a sieve with a screen size of 100 to get CMF [5].

2.3 CMF hydrogel preparation

The PVA was measured to weigh 96 g. It was then mixed with 1104 mL of distilled water and heated to a temperature of 100°C using a hot magnetic stirrer until it completely dissolved. The solution was let to reach ambient temperature. Subsequently, a solution

containing 250 g of PVA with a concentration of 8% was combined with 250 mL of CMF palm oil solid waste (OPT, OPF, OPEFB, and OPL). The mixture was then heated to 60°C until complete dissolution of the CMF occurred, leading to the formation of a CMF hydrogel (CMFH). Subsequently, the pH and viscosity of the CMFH were assessed utilising a pH meter and a viscometer. The hydrogel underwent autoclaving at a temperature of 121°C for 15 minutes. Once the CMFH had cooled to the temperature of the surrounding room, it was in a sterile state and could be utilised as an encapsulant for LAB [4].

2.4 Activation of Lactobacillus fermentum InaCC B1295

Lactobacillus fermentum InaCC B1295 culture was inoculated into a 5 mL MRSB medium test tube and then agitated by the vortex. Incubate the medium for 24 hours at 37°C in an incubator to obtain the active culture, as indicated by a change in the colour of the medium to cloudy. Store the active cultures in the refrigerator until use [22].

2.5 Preparation of encapsulated Lactobacillus fermentum InaCC B129

For the encapsulation procedure, 40 mL of cell biomass InaCC B129 is combined with 40 mL of sterile CMF hydrogel derived from palm oil solid waste (OPT, OPF, OPEFB, and OPL). An agitating rod is utilized to ensure that the components are thoroughly combined. The procedure for storing *L. fermentum* InaCC B129 involved the insertion of 2 mL of each encapsulated LAB into a 5 mL cryovial. The cryovial was then subjected to storage at ambient temperature and refrigeration (4°C) for durations of 0, 14, and 28 days. The probiotic properties of the encapsulated L. fermentum InaCC B129 were subsequently evaluated [4].

2.6 Scavenging ability of DPPH Radicals

The ability of DPPH radicals to scavenge was assessed using the methods described by [23]. In 100 ml of methanol, dissolve 0.1 g of DPPH to produce the DPPH solution. Furthermore, 1.3 ml of culture supernatant was utilized from *L. fermentum* InaCC B129. Twenty-four hours were spent incubating the cells at ambient temperature. In the dark, 1.3 ml of culture supernatant was transferred from CMFH-encapsulated L. fermentum InaCC B129 cells (OPT, OPF, OPEFB, and OPL) to a test tube containing 5 ml of DPPH solution at concentrations of 500 ppm, 250 ppm, 125 ppm, and 62.5 ppm. The DPPH solution was homogenized using a vortex mixer. Following homogenization with a vortex mixer, the mixture was incubated in the dark for thirty minutes. At 517 nanometers, the absorbance of the solution was quantified utilizing a spectrophotometer [24]. The DPPH radical inhibition was then calculated using the following formula:

$$\text{Inhibition}(\%) = 100 - \left(\frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100\right) \quad (1)$$

2.7 Measurement of γ-aminobutyric acid (GABA)

The ability of *Lactobacillus fermentum* InaCC B1295 encapsulated in CMF hydrogel from palm oil solid waste (OPT, OPF, OPEFB, and OPL) to produce γ-aminobutyric acid (GABA) by consuming L-glutamate were assessed by [25]. The enclosed strain of L. fermentum InaCC B129 was introduced into a medium of MRS broth and subjected to incubation at a temperature of 37°C for 12 hours in order to acquire a thriving culture. The strain B1295 was cultivated on a modified de Man, Rogosa, and Sharpe medium (MRS) that included 0.05% (w/v) L-cysteine-HCl and 1% (w/v) sodium glutamate. The culture was subsequently

cultivated at a temperature of 37°C for a duration of 48 hours. Following incubation, cells were isolated from the supernatant using centrifugation. The supernatant, devoid of cells, was passed through a filter with a molecular weight cut-off (MWCO) of 3 kDa. Ultimately, the samples underwent derivatization, and the quantities of GABA were analysed using HPLC.

2.8 Autoaggregation activity

Autoaggregation of *Lactobacillus fermentum* InaCC B1295 encapsulated with CMF hydrogel of empty oil palm fruit bunches refers to the method of [26]. *Lactobacillus fermentum* InaCC B1295 was inoculated as much as 0.5 ml into 50 ml MRSB and incubated at 37°C for 20 hours. The cells were centrifuged at 4500 rpm for 15 minutes at 4°C to obtain cell biomass. The cell biomass was washed twice with phosphate-buffered saline (PBS) pH 7.2 and resuspended in 60 ml of PBS. The cell suspension was mixed using a vortex for 10 seconds and evaluated at 0 and 5 hours of incubation at 37°C. A total of 0.1 ml of the upper suspension was transferred to a tube containing 3.9 ml of PBS, and the absorbance was measured using a spectrophotometer at an absorbance of 600 nm. The percentage of autoaggregation is calculated using the following equation:

$$\text{Autoaggregation (\%)} = \left(1 \quad \frac{\text{At}}{\text{A0}}\right) \text{x100} \qquad (2)$$

A_t is absorbance at 5 hours; A_0 is absorbance at 0 hours

2.9 Coaggregation activity

Coaggregation of *Lactobacillus fermentum* InaCC B1295 with or without CMF encapsulation of empty oil palm fruit bunches was carried out according to the method of [27]. *Lactobacillus fermentum* InaCC B1295, either with or without CMF hydrogel encapsulation derived from empty oil palm bunches, or *Pediococcus pentosaceus* strain 2397, was inoculated at a volume of 0.5 ml each into 50 ml of MRSB medium. *Escherichia coli* FNCC-19 and *S. aureus* FNCC-15 were introduced in a quantity of 0.5 ml each into 50 ml of NB medium. The bacterial coaggregation mixture was subjected to incubation at a temperature of 37°C for 20 hours. The cells were subjected to centrifugation at a speed of 4500 revolutions per minute for 15 minutes at a temperature of 4 degrees Celsius in order to acquire the cell biomass. The cellular biomass was subjected to two rounds of washing with phosphate-buffered saline (PBS) at a pH of 7.2. Subsequently, each bacterium was re-suspended in 60 ml of PBS. Two millilitres of each bacterial cell solution were combined and agitated using a vortex for 10 seconds. A control was prepared by taking 4 ml of each bacterial solution. The suspension was kept at a temperature of 37°C for 5 hours. Spectrophotometric measurements were conducted at a wavelength of 600 nm at both 0 hours and 5 hours of incubation time. The coaggregation percentage is calculated using the following equation:

$$\text{Coaggregation (\%)} = \frac{\frac{A_x+A_y}{2} - A(x+y)}{\frac{A_x+A_y}{2}} \text{ x100} \qquad (3)$$

Ax is the absorbance of bacterial suspension x; Ay is the absorbance of bacterial suspension y, and A(x + y) is the absorbance of a mixture of suspensions of 2 bacteria.

2.10 Hidrofobicity activity

Surface cell hydrophobicity was determined according to the method of [28]. Cells of *Pediococcus pentosaceus* strain 2397 and *Lactobacillus fermentum* InaCC B1295 were cultivated in MRSB medium at 30°C for 18 hours after being encapsulated with oil palm leaf CMF hydrogel. Following this, the cells were centrifuged at 10,000 rpm for 5 minutes. The cell particle underwent two washes utilising a pH 7.1 phosphate urea magnesium (PUM) buffer. In 60 ml of PUM buffer, the particle (cell biomass) that had been rinsed was reconstituted. After determining the volume of the cell suspension to be 3.0 mL, 1.0 mL of xylene was introduced and thoroughly agitated with a vortex. The samples were incubated at 30°C for 10 minutes, followed by 1 minute of vortex homogenization. It was then incubated for one hour at 30°C to facilitate phase separation. Following the meticulous removal of the water phase, the absorbance was measured at 600 nanometers. Surface cell hydrophobicity is calculated using the following equation:

$$\text{Surface cell hydrophobicity (\%)} = (1 - \frac{A_{\text{after}}}{A_{\text{before}}}) \text{ x100} \quad (4)$$

An $_{after}$ is the absorbance of the initial suspension before the addition of xylene; A $_{before}$ is the absorbance of the initial suspension after the addition of xylene.

2.11 Data analysis

The scavenging ability of DPPH radicals and the measurement of γ-aminobutyric acid (GABA) in encapsulated *L. fermentum* InaCC B129 from palm oil solid waste (OPT, OPF, OPEFB, and OPL) were analyzed at room temperature and refrigerated temperatures (4°C) for 0, 14, and 28 days. The results are presented descriptively.

3 Result and discussion

DPPH radical scavenging activity was analyzed to estimate the antioxidant activity of encapsulated *L. fermentum* InaCC B129 from palm oil solid waste (OPT, OPF, OPEFB, and OPL) during storage (0, 14, and 28 days) at room temperature and refrigerated temperatures (4°C). The results are shown in Figure 1. DPPH radical scavenging activity of *L. fermentum* InaCC B129 was in the range of 26.02–251.01 ppm. This study found that encapsulated *Lactobacillus fermentum* InaCC B129 demonstrates excellent ability to scavenge DPPH free radicals at room and refrigerated temperature on the initial day (26.02–87.30 ppm). CMFH of oil palm solid waste (OPT, OPF, OPEFB, and OPL) as encapsulant of bacteria do not show a difference in the ability to scavenge DPPH free radicals at 0-day storage time. CMFH of palm oil solid waste (OPT, OPF, OPEFB, and OPL) as a bacterial encapsulant showed no difference in the ability to scavenge DPPH free radicals.

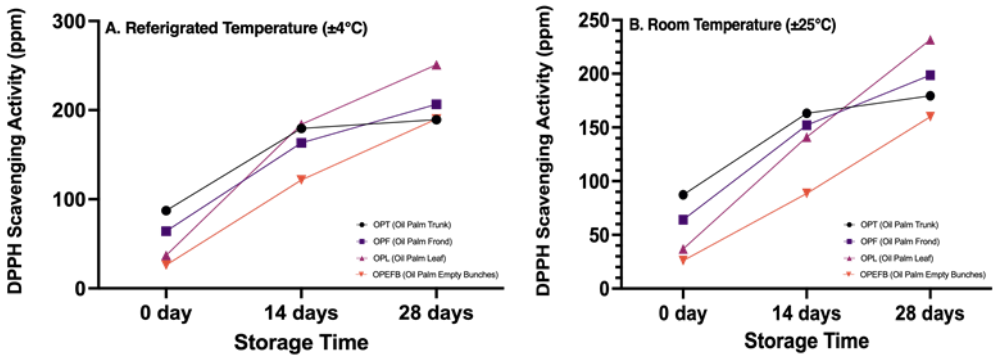


Fig. 1. Scavenging activity of DPPH Radicals by *L. Fermentum* InaCC B1295.

However, during storage, both at room temperature and at a cold temperature, the ability of bacteria to prevent the DPPH free radicals decreased. During time storage, DPPH scavenging ability decreased in *Lactobacillus fermentum* InaCC B129 encapsulated with OPT, OPF, OPEFB, and OPL. However, cold storage temperatures helped maintain the antioxidant activity produced by bacteria compared to room temperature during storage for 14 and 28 days. The treatment with encapsulation with oil palm empty bunches (OPEFB) at refrigeration temperature after storage for 14 days, which reached 88.56 ppm, shows strong antioxidant activity. Meanwhile, at room temperature, the scavenging DPPH free radicals of *L. fermentum* InaCC B129 encapsulated by CMFH-OPEFB increase to 121.56 ppm. According to [29], the natural compounds have powerful antioxidants if the IC_{50} value is <50 ppm, strong 50–100 ppm, moderate 100–150 ppm, and weak 150–200 ppm. Hence, the study of [30] showed that the antioxidant activity of probiotic *L. casei* at refrigeration temperature storage (4°C) had higher antioxidant activity than storage at room temperature. The decrease in antioxidant activity is from 59% to 32% in refrigeration storage and 59% to 28% in room-temperature storage after 14 days of storage.

Several antioxidant assays using stable non-biological radicals determine probiotic free radical scavenging activity. *Lactobacillus fermentum* InaCC B1295 is a safe LAB that can make various compounds, including organic acids and bacteriocins. Furthermore, due to its nutritional value, safety, and probiotic function, LAB is recognized as a potent natural xenobiotic antioxidant. Different LABs have been tested to verify their antioxidant activity, including *Leuconostoc mesenteroides*, *Leuconostoc citreum*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Weissella cibaria*, *Levilactobacillus brevis*, *Latilactobacillus curvatus*, and *Latilactobacillus sakei* [31], *Lactobacillus fermentum* have been shown to have antioxidant properties [32]. Probiotics produce antioxidant activity by producing antioxidant enzymes, including superoxide dismutase (SOD), antioxidant glutathione (GSH), and exopolysaccharide (EPS) [33]. Enzymatic antioxidants are a group of reductase enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx), and catalase, along with their cofactors. They work to limit the concentration of free radicals in cells, preventing excessive oxidative damage. These enzymes act as oxidase inhibitors [34].

Screening for lactic acid bacteria with the ability to synthesize GABA is crucial for the food industry. The LAB enables the production of natural GABA, a bioactive compound that can regulate health functions and create novel and appealing food products for consumers. Gamma-aminobutyric acid (GABA) production of encapsulated *L. fermentum* InaCC B129 from palm oil solid waste (OPT, OPF, OPEFB, and OPL) during storage (0, 14, and 28 days) at room temperature and refrigerated temperatures (4°C) was analyzed in this study. The results are shown in Figure 2. GABA production of *L. fermentum* InaCC B129 ranged from

0.435–2.215 mg/L. The study found that the encapsulated *Lactobacillus fermentum* InaCC B129 effectively produced GABA at room and refrigerated temperatures on the initial day (1.053–2.215 mg/L). The analysis compared the use of oil palm solid wastes (OPT, OPF, OPEFB and OPL) as encapsulating agents for *L. fermentum* InaCC B129 and found no significant difference in their ability to produce GABA. However, GABA production of *L. fermentum* InaCC B129 encapsulated by OPT and OPL resulted in a higher amount of 2.168 mg/L and 2.215 mg/L, respectively. During time storage, most of the encapsulated *L. fermentum* InaCC B129 with oil palm solid wastes (OPT, OPF, OPEFB and OPL) show decreased GABA production at 14 days and 28 days (0.435–1.505 mg/L). However, the *L. fermentum* InaCC B129 encapsulated with CMFH-OPT showed a different pattern at 28 days of storage, demonstrating increased GABA production at room and refrigerated temperatures.

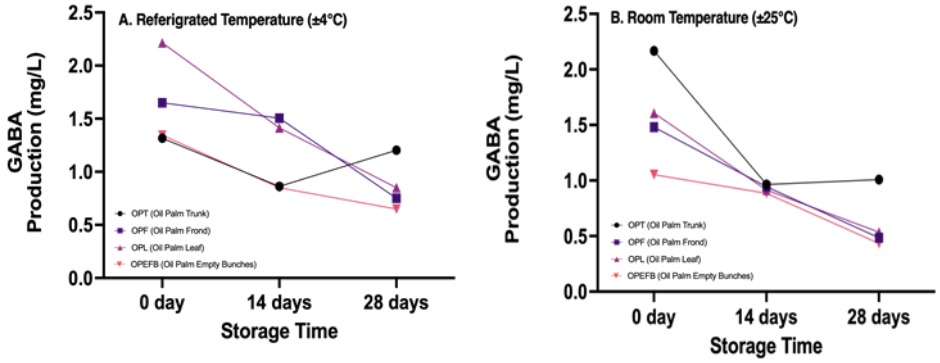


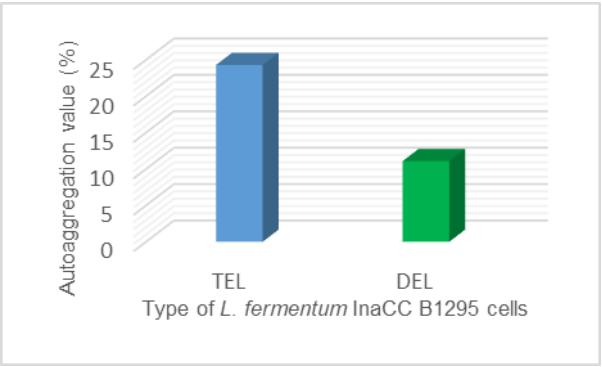
Fig 2. GABA Production of *L. Fermentum* InaCC B1295.

γ-aminobutyric acid (GABA) production is produced through the enzymatic conversion of glutamate by glutamate decarboxylase (GAD; EC 4.1.1.15). GAD is an intracellular enzyme that relies on pyridoxal 5′-phosphate (PLP) to facilitate the decarboxylation of L-glutamate. This process occurs after L-glutamate is transported into cells by the glutamate GABA antiporter, producing GABA [35]. GABA-producing bacteria can maintain a low pH inside their cells by eliminating protons while converting glutamate into GABA. Naturally, foods rich in GABA can serve as a valuable source for cultivating LAB that produces high levels of GABA [36]. Therefore, the food source could potentially impact the extent of GABA synthesis. It has been shown that acidic foods can serve as a suitable environment for organisms that produce significant levels of GABA.

Nevertheless, the existence and activity of enzymes, rather than microbes, are thought to be responsible for the production of GABA. In addition, lactic acid bacteria (LAB) are frequently extracted from food sources by selectively culturing them on a medium supplemented with monosodium glutamate (MSG) to enhance their ability to produce high levels of gamma-aminobutyric acid (GABA). It was observed that the yield of GABA gradually increased as the amount of MSG added increased from 0 to 270 mM [37]. Furthermore, according to [38], *Lactobacillus brevis* RK03 growth in optimal medium with different MSG concentrations (250–850 mM), 1×10^9 CFU/mL initial inoculum, and pH 4.5 at 30°C for 88 h, produced a maximum GABA yield of 62,523 mg/L. Hence, the choice of detection technology (such as enzyme test, chromatography, or automated amino acid analyzer) significantly impacts the measurement of GABA levels.

Based on the data in Figures 1 and 2, *Lactobacillus fermentum* InaCC B1295 encapsulated with CMF hydrogel from oil palm empty bunches produces the best antioxidant activity and GABA production. Therefore, the colonization potential test was continued, including autoaggregation, coaggregation and hydrophobicity using only CMF hydrogel from oil palm

empty bunches. Microorganisms, such as LAB, can interact and form groups or aggregates, a process known as autoaggregation. This interaction is a phenomenon where larger clumps or groups can grow through interactions between bacteria belonging to the same species. LAB may enable attachment to their hosts in specific situations by using their autoaggregation abilities. By doing this, pathogenic bacteria cannot invade the digestive tract, aiding LAB. The autoaggregation values of *Lactobacillus fermentum* InaCC B1295 cells encapsulated, or without encapsulation with CMF hydrogel from oil palm empty bunches are presented in Figure 3.

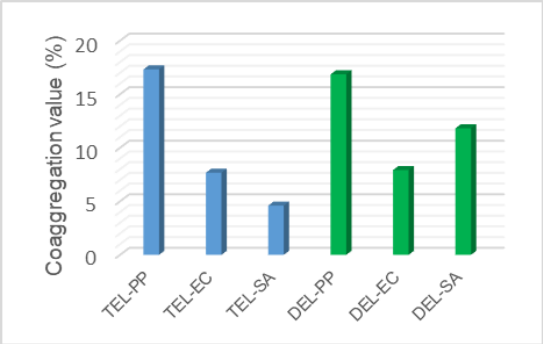


TEL is unencapsulated or free cells of *L. fermentum* InaCC B1295;
DEL is encapsulated cells of *L. fermentum* InaCC B1295

Fig 3. Autoaggregation values by *Lactobacillus fermentum* InaCC B1295 cells encapsulated with or without CMF hydrogel from oil palm empty bunches.

The data shown in Figure 3 indicates that *Lactobacillus fermentum* InaCC B1295 cells that are not encapsulated exhibit a higher autoagregation value than those encapsulated using CMF hydrogel from oil palm empty bunches. This fact is probably because liberated cells can proliferate more quickly in the medium and form aggregates with other *Lactobacillus fermentum* InaCC B1295 cells. In contrast to free cells, encapsulated cells cannot proliferate and aggregate from one cell to another without first emerging from the CMF hydrogel layer. The study found that the autoaggregation value of *Lactobacillus fermentum* InaCC B1295 cells varied between 11.08 and 24.29%. The findings of this investigation closely resemble the autoaggregation values for *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus acidophilus* found in earlier investigations [39]. This range was less than the autoaggregation value of many strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* ranged between 45.02 and 93.09% [40]. The autoaggregation ability of Lactobacillus depends on the species. This statement is supported by [41], who reported autoaggregation values for several Lactobacillus species in the range of 21–97%.

When it comes to adhering to the epithelial cells of the human digestive tract, probiotics can rival harmful bacteria. Probiotics may work this way to prevent harmful bacteria from adhering to the necessary locations to create infection. The coaggregation values of *Lactobacillus fermentum* InaCC B1295 cells from OPEFB, either encapsulated or not, using CMF hydrogel, are displayed in Figure 4.

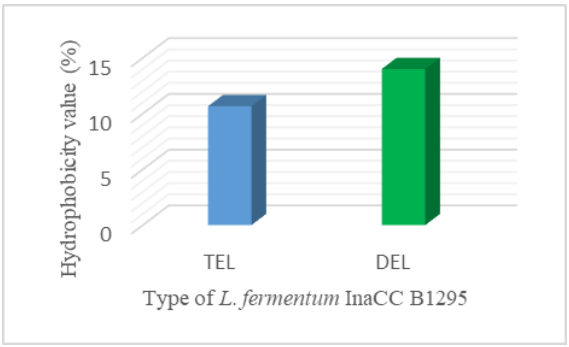


TEL-PP is unencapsulated or free cells of *L. fermentum* InaCC B1295 and *P. pentosaceus*; TEL-EC is unencapsulated or free cells of *L. fermentum* InaCC B1295 and *E. coli*, TEL-SA is unencapsulated or free cells of *L. fermentum* InaCC B1295 and *S. aureus*; DEL-PP is encapsulated cells of *Lactobacillus fermentum* InaCC B1295 and *P. pentosaceus*; DEL-EC is encapsulated cells of *L. fermentum* InaCC B1295 and *E. coli*, DEL-SA is encapsulated cells of *L. fermentum* InaCC B1295 and *S. aureus*.

Fig 4. Coaggregation values by *Lactobacillus fermentum* InaCC B1295 cells encapsulated with or without CMF hydrogel from oil palm empty bunches.

The outcomes demonstrated that *P. pentosaceus* cells and encapsulated and unencapsulated *Lactobacillus fermentum* InaCC B1295 cells had similar coaggregation values. Nonetheless, the coaggregation value between free *Lactobacillus fermentum* InaCC B1295 cells and *S. aureus* or *E. coli* cells is comparatively lower than the coaggregation value between *Lactobacillus fermentum* InaCC B1295 cells encapsulated in CMF hydrogel from empty oil palm fruit bunches. The coaggregation ability of *Lactobacillus fermentum* InaCC B1295 cells with a range of 4.60–17.32% is slightly lower than several previously reported Lactobacillus strains [39, 42]. However, the coaggregation value of Lactobacillus fermentum InaCC B1295 cells was almost the same as *that of Lactobacillus delbrueckii* subsp. *bulgaricu*s strains strain 22, as reported by [40].

The human digestive tract is a water-rich environment, and many molecules that enter the digestive system must interact with water to be digested, absorbed, and used by the body. Therefore, the hydrophobic nature of molecules in the digestive tract can significantly affect how much they can function. The hydrophobicity values of *Lactobacillus fermentum* InaCC B1295 cells encapsulated, or without encapsulation with CMF hydrogel from oil palm empty bunches are presented in Figure 5.



TEL is unencapsulated or free cells of *Lactobacillus fermentum* InaCC B1295;
DEL is encapsulated cells of *Lactobacillus fermentum* InaCC B1295

Fig 5. Hydrophobicity values by *Lactobacillus fermentum* InaCC B1295 cells encapsulated with or without CMF hydrogel from oil palm empty bunches.

Lactobacillus fermentum InaCC B1295 cells encapsulated in CMF hydrogel from oil palm empty bunches have a higher hydrophobicity value than cells without encapsulation (free cells). The autoaggregation value of encapsulated and unencapsulated *Lactobacillus fermentum* InaCC B1295 cells was 10.66-14.00% in this study, lower than the autoaggregation value of several strains of *Lactobacillus plantarum, L. casei, L. rhamnosus, L. paracasei* and *L. acidophilus* [39, 42].

4 Conclusion

The strain InaCC B1295 showed a high scavenging ability against DPPH (1,1-diphenyl-2-picrylhydrazyl) with a value of 88.56 ppm after encapsulation with oil palm empty bunches (OPEFB) and storage at refrigeration temperature for 14 days. Additionally, GABA production ranged from 0.435 to 2.215 mg/L. The highest concentration of GABA, 2.215 mg/L, was produced by *L. fermentum* InaCC B 1295 after encapsulation with oil palm trunk (OPT) for 28 days, ranging from 1.008 to 1.203 mg/L. In contrast, the concentration of GABA was decreased during storage for all treatments. Free cells demonstrated a higher autoaggregation value than *Lactobacillus fermentum* InaCC B1295 cells encased in CMF hydrogel from empty oil palm fruit bunches. However, unlike free cells (unencapsulated cells), cells encapsulated with CMF hydrogel from OPEFB had higher coaggregation and hydrophobicity values.

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