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SUSTAINABLE CELLULOSE MICROFIBER FROM OIL PALM SOLID WASTE AS ENCAPSULANT: SAFETY AND PROBIOTIC PROPERTIES OF *PEDIOCOCCUS PENTOSACEUS* STRAIN 2397

SUMMARY

Pediococcus pentosaceus is a lactic acid bacterium with probiotic potential, but its functionality is strain-dependent and influenced by delivery systems. This study evaluated the safety and probiotic properties of *P. pentosaceus* strain 2397 in both free and encapsulated forms, using a cellulose microfiber hydrogel (CMFH) derived from OPSW. Safety was assessed through hemolysis, enzyme activity, and biogenic amine production, while probiotic properties included aggregation, hydrophobicity, coaggregation, and bile salt deconjugation. All free and encapsulated cells were negative for hemolysis, cytolysin, gelatinase, and biogenic amine production, confirming safety. Encapsulation significantly affected probiotic properties, with leaf-derived CMFH enhancing autoaggregation and hydrophobicity. Coaggregation varied depending on the CMFH source. All treatments exhibited bile salt hydrolase activity, showing a preference for glycine-conjugated bile salts. Overall, *P. pentosaceus* strain 2397 is safe, and OPSW-derived CMFH represents a promising sustainable probiotic delivery system.

Keywords: safety, probiotics, *Pediococcus pentosaceus*, cellulose microfiber, oil palm solid waste

INTRODUCTION

Probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (WHO/FAO, 2002). Lactic acid bacteria (LAB) are among the most extensively studied probiotics due to their long history of safe use in fermented foods and their well-documented health benefits, including modulation of gut microbiota, enhancement of

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intestinal barrier function, immunomodulatory effects, and cholesterol reduction (Mazziotta *et al.*, 2023). However, probiotic effects are highly strain-specific, necessitating thorough safety and functional evaluation before application in food or nutraceutical products (Pradhan *et al.*, 2020).

P. pentosaceus is a Gram-positive, homofermentative LAB frequently isolated from traditional fermented foods. Several strains have demonstrated tolerance to gastrointestinal conditions, antimicrobial activity, and the ability to produce bioactive compounds, highlighting their probiotic potential (Jiang *et al.*, 2021). Nevertheless, safety assessment remains essential and must include evaluation of hemolytic activity, production of virulence-related enzymes, and biogenic amine formation (EFSA, 2020). In addition to safety, functional characteristics such as autoaggregation, coaggregation, cell surface hydrophobicity, and bile salt hydrolase (BSH) activity are critical determinants of probiotic efficacy. These properties contribute to intestinal adhesion and cholesterol metabolism, thereby supporting probiotic survival and functionality in the gastrointestinal tract (Kreve and Reis, 2021).

However, probiotic viability and functional stability can be compromised during processing, storage, and gastrointestinal transit. Microencapsulation has therefore gained attention as a strategy to enhance probiotic protection and performance. Biopolymer-based encapsulation systems can improve survival, maintain metabolic activity, and modulate cell surface properties (Far *et al.*, 2024). Cellulose-based materials are particularly attractive due to their biocompatibility, mechanical strength, and resistance to harsh gastrointestinal conditions (Yang *et al.*, 2024). Oil palm solid waste (OPSW), abundantly generated by the palm oil industry, is a sustainable and underutilized lignocellulosic biomass source. Indonesia, the world's largest palm oil producer, generates millions of tons of OPSW annually, which is rich in cellulose and suitable for conversion into cellulose microfibers (CMF) (Finalis *et al.*, 2020). CMF derived from OPSW has shown promise as a low-cost and renewable encapsulation matrix for probiotics (Meziane *et al.*, 2024).

Although *Pediococcus pentosaceus* has been widely reported to possess probiotic potential and cellulose-based materials are recognized as promising encapsulation matrices, three key limitations remain namely 1) Strain-specific safety and functionality are insufficiently characterized, particularly for *P. pentosaceus* strain 2397, whose virulence traits, aggregation behavior, hydrophobicity, and bile salt hydrolase activity have not been comprehensively evaluated, 2) Limited evidence exists on how microencapsulation using cellulose derived from OPSW affects probiotic performance, especially regarding functional traits linked to intestinal adhesion and cholesterol metabolism, 3) Comparative effects of CMF obtained from different oil palm residues (trunks, fronds, leaves, and empty fruit bunches) on probiotic safety and functionality have not been systematically investigated. This study introduces several original contributions and novelties. First comprehensive safety and functional evaluation of *P. pentosaceus* strain 2397 in both free and encapsulated states, including

virulence screening, aggregation properties, hydrophobicity, and bile salt deconjugation. Application of cellulose microfiber hydrogel derived from OPSW as a sustainable probiotic encapsulant, linking probiotic delivery technology with agricultural waste valorization and circular bioeconomy principles. Comparative assessment of CMF from multiple oil palm residues, demonstrating that leaf-derived CMFH uniquely enhances autoaggregation and surface.

Despite this potential, limited information is available on how CMF derived from different oil palm residues influences both the safety and probiotic functionality of *P. pentosaceus*. Therefore, this study aimed to evaluate the safety profile of *P. pentosaceus* Strain 2397 and to investigate the effects of encapsulation with OPSW-derived CMF hydrogels on its in vitro probiotic properties.

MATERIAL AND METHODS

Materials and bacterial strain

OPSW was obtained from PT Multi Plasma Sejahtera, Pelalawan Regency, Riau Province, Indonesia. *P. pentosaceus* Strain 2397 was obtained from the Indonesian Culture Collection, National Research Agency, Cibinong, Indonesia.

Bacterial propagation and cell preparation

Propagation and cell separation were then performed (Pato *et al.*, 2021). *P. pentosaceus* Strain 2397 was cultured in sterile MRS broth at 37 °C for 24 h. Cells were harvested by centrifugation at 4,500 rpm for 15 min at 4 °C, washed twice with sterile distilled water, and resuspended in phosphate buffer (pH 7) at a 1:1 ratio. Cell suspensions were stored at refrigeration temperature until use.

Preparation of cellulose microfiber (CMF)

CMF was prepared from OPSW as described by Pato *et al.*, (2021). OPSW was cut (0.5–1 cm), oven-dried at 60 °C for 4 h, and soaked in 6% KOH (12 h). Fibers were washed, treated with hypochlorite for 5 h, rinsed to neutral pH, dried, pulverized, and sieved (80 mesh). The material was further processed using a planetary ball mill (8,000 rpm, 60 min) and passed through a 100-mesh sieve to obtain CMF.

Preparation of sterile cellulose microfiber hydrogel (CMFH)

Sterile CMFH was prepared as described by Fung *et al.*, (2011). The PVA was weighed to 96 g, then combined with 1104 mL of distilled water and heated to 100 °C with a hot magnetic stirrer until dissolved. The solution was allowed to be refrigerated to room temperature. Next, 250 g of PVA 8% was mixed with 250 mL of CMF from OPSW and heated to 60 °C until fully dissolved, yielding a CMF hydrogel (CMFH). The pH and viscosity of the CMFH were then measured using a pH meter and a viscometer. The hydrogel was autoclaved at 121 °C for 15 min. After cooling to room temperature, sterile CMFH was ready for use as a LAB encapsulant.

Encapsulation of lactic acid bacteria

Encapsulation was performed as described by Pato *et al.*, (2021). The encapsulation process involved adding 40 mL of *P. pentosaceus* strain 2397 cell

biomass (about 4×10^{10} log CFU/mL) to 40 mL of sterile CMF hydrogel from OPSW, then stirring until well-blended with a stirring rod. Storage of *P. pentosaceus* Strain 2397 was carried out by inserting 2 mL of the encapsulated LAB into a 5 mL cryovial, and the probiotic properties of the encapsulated *P. pentosaceus* Strain 2397 were assessed.

Safety evaluation of probiotics

Safety assessment included hemolytic activity, cytotoxin production, gelatinase activity, and biogenic amine production.

Hemolytic activity was evaluated by streaking free or encapsulated *P. pentosaceus* Strain 2397 on Columbia blood agar containing 5% sheep blood and incubating at 37 °C for 24 h (Han *et al.*, 2024). Clear zones indicated hemolysis.

Cytotoxin production was assessed on brain heart infusion agar supplemented with 5% sheep blood and incubated at 37 °C for 24 h. Clear zones around colonies indicated positive activity (Han *et al.*, 2024).

Gelatinase activity was tested on BHIA containing 1.5% skim milk, incubated at 37 °C for 24 h. Clear zones indicated gelatinase production (Han *et al.*, 2024).

Biogenic amine production followed (Han *et al.*, 2024). PP2397 was subcultured twice in MRS broth containing 1% amino acids and 0.005% pyridoxal-5-phosphate. Cultures were streaked on decarboxylase agar containing bromocresol purple and incubated at 37 °C for 24 h. A color change from brown to purple indicated biogenic amine production.

Autoaggregation assay

Autoaggregation was determined according to Han *et al.*, (2024). *P. pentosaceus* strain 2397 encapsulated with CMFH-OPSW was inoculated as much as 0.5 mL into 50 mL MRSB and incubated at 37 °C for 20 h. The cells were centrifuged at 4500 rpm for 15 min 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 sec and evaluated at 0 and 5 h of incubation at 37 °C. 0.1 mL of the upper suspension was transferred to a tube containing 3.9 mL PBS. The absorbance was measured at 600 nm using a spectrophotometer. The autoaggregation percentage is calculated using the following equation:

$$\text{Autoaggregation (\%)} = (1 - (At/Ao)) \times 100 \quad (1)$$

At is the absorbance at 5 h; Ao is the absorbance at 0 h.

Coaggregation assay

Coaggregation with *Staphylococcus aureus* and *Escherichia coli* was evaluated as described by Han *et al.*, (2024). *P. pentosaceus* strain 2397 encapsulated with CMFH-OPSW was inoculated with 0.5 mL into 50 mL MRSB medium. *S. aureus* FNCC-15, *E. coli* FNCC-19, and *L. fermentum* InaCC B1295 were inoculated with as much as 0.5 mL into 50 mL of NB medium. The bacterial

coaggregation mixture was incubated at 37 °C for 20 h. The cells were centrifuged at 4500 rpm for 15 min at 4 °C to obtain cell biomass. The cell biomass was washed twice with phosphate-buffered saline (PBS), pH 7.2, and each bacterium was resuspended in 60 mL of PBS. Each 2 mL cell suspension of each bacterium was mixed in pairs using a vortex for 10 seconds. 4 mL of each bacterial suspension was taken as a control. This suspension was incubated for 5 h at 37 °C. Absorbance measurements were carried out using a spectrophotometer at 600 nm at 0 and 5 h of incubation. The coaggregation percentage is calculated using the following equation:

$$\text{Coaggregation (\%)} = \frac{\frac{A_x + A_y}{2} - A(x+y)}{\frac{A_x + A_y}{2}} \times 100 \quad (2)$$

A_x is the absorbance of bacterial suspension x ; A_y is the absorbance of bacterial suspension y , and $A(x+y)$ is the absorbance of a mixture of the suspensions of the two bacteria.

Cell surface hydrophobicity

Hydrophobicity was measured using the xylene partition method of Abdulla *et al.*, (2014). *P. pentosaceus* strain 2397 cells encapsulated with CMFH-OPSW were grown in MRSB medium at 30 °C for 18 h and then centrifuged at 10,000 rpm for 5 min. The cell pellet was rinsed twice with a phosphate-urea-magnesium (PUM) buffer at pH 7.1. The washed pellet (cell biomass) was resuspended in 60 mL of PUM buffer. The cell suspension was measured as 3.0 mL, and then 1.0 mL of xylene was added and mixed evenly using a vortex. Incubation was carried out at 30 °C for 10 min, then the sample was homogenized again using a vortex for 1 minute. Next, it was incubated at 30 °C for 1 h for phase separation. The water phase was carefully removed, and the absorbance was measured at 600 nm. Cell surface hydrophobicity is calculated using the following equation:

$$\text{Cell surface hydrophobicity (100\%)} = \left(1 - \frac{A_{\text{after}}}{A_{\text{before}}}\right) \times 100 \quad (3)$$

A_{after} is the absorbance of the initial suspension before adding xylene; A_{before} is the absorbance of the initial suspension after adding xylene.

Data analysis

Safety evaluation data were analyzed descriptively. Autoaggregation, coaggregation, and hydrophobicity data were analyzed using ANOVA (IBM SPSS version 26). Significant differences were further evaluated using Duncan's multiple-range test at a 5% significance level.

RESULTS AND DISCUSSION

Safety assessment

The safety-related phenotypic characteristics of *P. pentosaceus* strain 2397, evaluated for free cells and cells encapsulated with CMFH derived from OPSW, are summarized in Table 1. All treatments showed negative results for hemolytic activity, cytolysin, gelatinase, and biogenic amine formation. The absence of hemolysis on Columbia Blood Agar confirms that *P. pentosaceus* strain 2397 did not induce erythrocyte lysis, a fundamental requirement for probiotic safety. Likewise, the lack of cytolysin and gelatinase production indicates the absence of virulence-associated extracellular enzymes linked to tissue damage and opportunistic infections. The strain also did not produce biogenic amines, eliminating concerns related to the formation of toxic metabolites such as histamine or tyramine in fermented foods. Importantly, encapsulation with CMFH from different OPSW sources did not induce or modify any virulence-related traits, demonstrating that the encapsulation process and cellulose-based carriers are biologically inert.

Table 1. Hemolytic activity, production of cytolysin, gelatinase, and biogenic amines by *P. pentosaceus* strain 2397 cells encapsulated with or without CMFH from OPSW

Treatments	Hemolytic activity*	Production of cytolysin**	Production of gelatinase***	Production of biogenic amines****
Free cells	No	No	No	No
ET cells	No	No	No	No
EF cells	No	No	No	No
EL cells	No	No	No	No
EE cells	No	No	No	No

ET cells=cells encapsulated with CMFH of trunks, *EF cells*=cells encapsulated with CMFH of fronds, *EL cells*=cells encapsulated with CMFH of leaves, *EE cells*=cells encapsulated with CMFH of empty fruit bunches. No clear zone around LAB colonies on *CBA, **BHIA, ***BHIA containing skim milk, and ****DA media

Hemolysis and cytolysin production are key virulence determinants in lactic acid bacteria (LAB), particularly in some *Enterococcus* species. The absence of hemolysis indicates that *P. pentosaceus* strain 2397 does not lyse erythrocytes and therefore lacks β -hemolytic properties, an essential safety requirement for probiotic candidates. Similar findings have been reported for several *P. pentosaceus* strains isolated from fermented foods, which generally show γ -hemolytic (non-hemolytic) characteristics (Borges *et al.*, 2013; Zommiti *et al.*, 2020; Argyri *et al.*, 2013). Cytolysin production, often associated with pathogenicity, was also not detected. This finding supports the Generally Recognized as Safe (GRAS) status of the genus *Pediococcus*, especially strains intended for food and feed applications. Recent genomic-based safety assessments have confirmed that most food-derived *P. pentosaceus* strains lack

genes encoding cytolysin and other major virulence factors (Wang *et al.*, 2023). Importantly, encapsulation with CMFH from OPSW did not induce stress-related expression of virulence traits. This indicates that the encapsulation matrix is biologically inert and does not trigger pathogenic behavior, consistent with reports that cellulose-based hydrogels are non-toxic and biocompatible carriers for probiotics (Liu *et al.*, 2021; Singh *et al.*, 2024).

Gelatinase is an extracellular metalloprotease associated with tissue degradation and virulence in certain Gram-positive bacteria. The absence of gelatinase activity in both free and encapsulated cells indicates that strain 2397 lacks proteolytic traits associated with pathogenicity. Previous studies have shown that probiotic *P. pentosaceus* strains typically lack gelatinase production, distinguishing them from opportunistic pathogens such as *Enterococcus faecalis* (Arief *et al.*, 2021; FAO/WHO probiotic safety guidelines updates, 2023). The current findings align with those reports and further confirm the strain's safety profile. Encapsulation with CMFH from different OPSW sources (trunks, fronds, leaves, empty fruit bunches) did not alter enzyme expression. This finding suggests that the microenvironment created by CMFH does not activate extracellular protease systems, reinforcing its suitability as a delivery system.

Biogenic amines (e.g., histamine, tyramine, and putrescine) are produced by amino acid decarboxylation and pose food safety concerns due to their toxic effects. The absence of halo formation on decarboxylase agar indicates that *P. pentosaceus* strain 2397 lacks amino acid decarboxylase activity under the tested conditions. This is a critical safety characteristic, as some LAB strains can produce tyramine or histamine during fermentation. Recent investigations (EFSA, 2023; Lee *et al.*, 2022) have emphasized that probiotic strains must be screened for decarboxylase genes to prevent the accumulation of harmful amines. The present findings are consistent with reports that most *P. pentosaceus* strains from plant-based fermentations are non-amine producers (Zhang *et al.*, 2020; Zhang *et al.*, 2021; Wang *et al.*, 2023). Notably, encapsulation with CMFH from OPSW did not induce metabolic shifts toward amino acid decarboxylation. This finding indicates that the strain remains metabolically stable after immobilization, which is advantageous for functional food applications.

Autoaggregation ability

Autoaggregation values for *P. pentosaceus* strain 2397 were significantly influenced by the encapsulation material (Table 2).

Table 2. Autoaggregation value by *P. pentosaceus* strain 2397 cells encapsulated with or without CMFH from OPSW

Treatments	Autoaggregation values (%)
Free cells	29.88±4.01 ^b
ET cells	19.82±2.32 ^c
EF cells	10.29±0.78 ^e
EL cells	35.41±4.93 ^a
EE cells	13.30±1.07 ^d

Means followed by different letters indicate significant ($P < 0.05$)

Among all treatments, cells encapsulated with CMFH from leaves (EL) exhibited the highest autoaggregation (35.41%), significantly exceeding free cells (29.88%) and other encapsulated treatments. In contrast, CMFH from fronds (EF) and empty fruit bunches (EE) resulted in markedly lower autoaggregation values. Enhanced autoaggregation in EL cells may be attributed to the physicochemical properties of leaf-derived CMFH, including surface roughness, functional hydroxyl groups, and lignocellulosic composition, which can promote cell–cell interactions and aggregation-promoting factors. Conversely, reduced autoaggregation in EF and EE cells suggests that certain CMFH characteristics may partially mask bacterial surface proteins involved in aggregation. The autoaggregation value of free cells falls within the typical range reported for LAB (20–40%). It is comparable to that of *Pediococcus acidilactici* (Abbasiliasi *et al.*, 2017), although lower than that of highly aggregative *P. pentosaceus* strains reported by Vidhyasagar and Jeevaratnam (2012). Similar ranges have been reported for other LAB such as *Lactobacillus plantarum* (30–50%) and *Pediococcus acidilactici* (20–45%) (Collado *et al.*, 2008; Zommiti *et al.*, 2020). Overall, these findings demonstrate that the CMFH source critically determines the impact of encapsulation on adhesion-related probiotic traits.

Coaggregation with pathogenic and beneficial bacteria

Coaggregation results of *P. pentosaceus* strain 2397 with *S. aureus*, *E. coli*, and *L. fermentum* InaCC B1295 are presented in Table 3.

Table 3. Coaggregation values by *P. pentosaceus* strain 2397 encapsulated with or without HCMF from OPSW

Treatments	Coaggregation values (%)
Free cells and SA	9.41±0.78 ^e
ET cells and SA	11.45±1.02 ^d
EF cells and SA	12.52±1.54 ^{cd}
EL cells and SA	8.29±0.59 ^{bc}
EE cells and SA	7.12±0.75 ^f
Free cells and EC	12.67±1.57 ^{cd}
ET cells and EC	12.76±2.03 ^{cd}
EF cells and EC	13.37±2.98 ^c
EL cells and EC	7.09±0.76 ^f
EE cells and EC	8.40±0.69 ^{ef}
Free cells and LF	17.32±3.04 ^a
ET cells and LF	15.57±2.86 ^b
EF cells and LF	15.21±2.56 ^b
EL cells and LF	15.06±1.09 ^b
EE cells and LF	13.75±0.88 ^c

Means followed by different letters indicate significant ($P < 0.05$); SA=*S. aureus*, EC=*E. coli*, LF=*L. fermentum* InaCC 1295

Coaggregation varied significantly depending on the encapsulation matrix and interacting microorganism. Encapsulation with CMFH from trunks (ET) and

fronds (EF) significantly enhanced coaggregation with *S. aureus* and *E. coli* compared to free cells, suggesting improved heterotypic interactions that may support pathogen exclusion. In contrast, EL and EE cells showed lower coaggregation with these pathogens, possibly because their higher lignin content limited surface accessibility. All treatments exhibited substantially higher coaggregation with *L. fermentum* than with pathogenic bacteria. Free cells showed the highest interaction, while encapsulated cells retained moderate but significant coaggregation capacity. This preferential probiotic–probiotic interaction is advantageous for multispecies probiotic formulations and supports microbial stability in the gut. These findings align with previous studies demonstrating that dietary fiber-based encapsulants enhance probiotic aggregation and pathogen exclusion by providing functional surface groups that facilitate electrostatic and hydrophobic interactions (Rojek *et al.*, 2022). Reduced coaggregation associated with high lignin content has also been reported for other lignocellulosic matrices (Li *et al.*, 2022).

Coaggregation is considered an important probiotic trait because it contributes to competitive exclusion of pathogens, biofilm formation, and enhanced delivery of antimicrobial compounds. Recent studies have shown that encapsulation can either enhance or reduce probiotic adhesion depending on the material composition and its effect on cell surface hydrophobicity and charge (Diaz-Vega *et al.*, 2022; Sun *et al.*, 2023). Moreover, Ku *et al.*, (2023) reported that stronger coaggregation correlates with improved pathogen exclusion in vitro, supporting the functional relevance of the higher EF and ET values observed in this study. Similarly, Mohan *et al.*, (2024) demonstrated that polysaccharide-based encapsulation modifies surface adhesin exposure, which may explain the reduced coaggregation observed in EL and EE treatments. Compared with previous findings, the coaggregation values in this study (7–17%) fall within the moderate range commonly reported for *P. pentosaceus* strains (Kos *et al.*, 2020; Singh and Sharma, 2024), confirming that *P. pentosaceus* strain 2397 possesses acceptable probiotic interaction capacity. Importantly, the data indicate that encapsulation using selected HCMF types (particularly EF and ET) may enhance pathogen-targeted coaggregation without completely compromising interactions with beneficial LAB.

Cell surface hydrophobicity

Hydrophobicity values of *P. pentosaceus* strain 2397 ranged from 12.93% to 19.12% across treatments (Table 4).

Table 4. Hydrophobicity value by *P. pentosaceus* strain 2397 encapsulated with or without HMF from OPSW

Treatments	Hydrophobicity value (%)
Free cells	12.93±2.54 ^d
ET cells	15.16±1.69 ^b
EF cells	14.22±1.76 ^c
EL cells	18.08±2.98 ^a
EE cells	19.12±3.05 ^a

Means followed by different letters indicate significant ($P < 0.05$)

Free cells exhibited the lowest hydrophobicity, while encapsulated cells showed significantly higher values. The highest hydrophobicity was observed in EE and EL cells, followed by ET and EF cells. Cell surface hydrophobicity is a key determinant of probiotic adhesion to intestinal epithelial cells (Collado *et al.*, 2009). The increased hydrophobicity of encapsulated cells suggests enhanced adhesion potential, likely resulting from interactions between bacterial cell envelopes and cellulose microfibrils that modify surface charge distribution and expose hydrophobic domains. Variations among OPSW sources may reflect differences in fiber morphology, crystallinity, and surface chemistry. These findings are consistent with previous reports showing that cellulose- and fiber-based encapsulation enhances hydrophobicity and adhesion-related properties of LAB (Burgain *et al.*, 2011). Thus, CMFH from OPSW effectively improves surface-associated probiotic functionality.

BSH activity

The BSH ability of *P. pentosaceus* strain 2397 toward sodium taurocholate and glycocholic acid is shown in Table 5. Both free and encapsulated cells exhibited measurable BSH activity, confirming an important functional probiotic trait. For taurocholate, free cells and ET cells showed the highest activity, indicating that trunk-derived CMFH preserved BSH functionality. EF, EL, and EE cells showed reduced activity, possibly due to diffusion limitations or to differences in matrix density. In contrast, for glycocholic acid, free cells, EL cells, and EE cells exhibited significantly higher activity than ET and EF cells, suggesting better preservation or enhancement of BSH activity toward glycine-conjugated bile salts.

Table 5. Deconjugation of bile salt value by *P. pentosaceus* strain 2397 encapsulated with or without CMFH from OPSW

Treatments	Precipitation zone (cm)	
	Sodium taurocholate	Glycocholate acid
Free cells	0.63±0.06 ^a	0.77±0.04 ^a
ET cells	0.63±0.04 ^a	0.63±0.03 ^b
EF cells	0.45±0.03 ^b	0.61±0.07 ^b
EL cells	0.52±0.02 ^b	0.78±0.06 ^a
EE cells	0.57±0.05 ^b	0.71±0.03 ^a

Means in the same column followed by different letters indicate significant ($P<0.05$)

Encapsulation with CMFH derived from OPSW affected BSH activity differently depending on the biomass source. ET cells maintained taurocholate activity similar to that of free cells (0.63 cm), suggesting that encapsulation with trunk-derived CMFH did not hinder enzyme expression or diffusion. However, EF, EL, and EE cells showed reduced taurocholate precipitation zones (0.45–0.57 cm), indicating that encapsulation may partially restrict enzyme–substrate interaction or bile salt diffusion through the hydrogel matrix. For glycocholic acid, EL (0.78 cm) and EE (0.71 cm) cells showed activity comparable to that of

free cells, whereas ET and EF treatments showed significantly lower values (0.63 and 0.61 cm, respectively). This suggests that the encapsulation matrix composition influences substrate accessibility and possibly enzyme specificity toward glycine- versus taurine-conjugated bile salts. Previous studies have reported that BSH enzymes often exhibit substrate preference, with some LAB strains showing a higher affinity for glycoconjugated bile salts than tauroconjugates (Begley *et al.*, 2006; Jones *et al.*, 2008). The reduction observed in some encapsulated treatments may be explained by mass-transfer limitations imposed by the cellulose microfiber hydrogel (CMFH). Encapsulation systems can either protect probiotic cells or reduce immediate metabolic interaction with the surrounding medium, depending on matrix porosity and crosslink density (Krasaekoopt *et al.*, 2003). However, maintaining measurable BSH activity after encapsulation indicates that CMFH does not completely inhibit functional expression, which is crucial for in vivo probiotic efficacy. Compared with previous findings on *P. pentosaceus*, the precipitation zones in this study (0.45–0.78 cm) fall within the range reported for other LAB species showing moderate to strong BSH activity (Patel *et al.*, 2012; Argyri *et al.*, 2013). Some studies have shown even higher BSH activity in *Lactobacillus plantarum*, suggesting species- and strain-specific variability (Begley *et al.*, 2006). Therefore, Strain 2397 can be categorized as having moderate-to-high BSH activity, particularly in free and EL treatments.

CONCLUSIONS

P. pentosaceus strain 2397 is a safe probiotic candidate, showing no hemolytic activity or production of cytolysin, gelatinase, or biogenic amines. Microencapsulation using CMFH derived from OPSW effectively enhanced probiotic performance. Leaf-derived CMFH significantly improved autoaggregation and surface hydrophobicity, which are important for intestinal adhesion and colonization. The CMFH materials are biologically inert and did not introduce virulence factors, confirming their safety. Overall, valorizing oil palm waste into functional encapsulants provided a sustainable and eco-friendly strategy to improve probiotic delivery and support a circular bioeconomy.

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REFERENCES

- Abbasiliasi, S., Tan, J.S., Bashokouh, F., Ibrahim, T.A.T., et al. (2017). In vitro assessment of *Pediococcus acidilactici* Kp10 for its potential use in the food industry. *BMC Microbiology*, 17(1):1–12.
- Abdulla, A.A., Abed, T.A., & Saeed, A.M. (2014). Adhesion, autoaggregation and hydrophobicity of six *Lactobacillus* strains. *Microbiology Research Journal International* 4(4): 381–391.

- Argyri, A.A., Zoumpopoulou, G., Karatzas, K.A.G., et al. (2013). Selection of potential probiotic lactic acid bacteria from fermented olives. *Food Microbiology*, 34, 116–124.
- Arief, I.I., Jenie, B.S.L., Astawan, M., & Fujiyama, K. (2021). Safety assessment of probiotic lactic acid bacteria isolated from Indonesian fermented foods. *International Journal of Food Microbiology*, 343, 109090.
- Begley, M., Hill, C., & Gahan, C.G.M. (2006). Bile salt hydrolase activity in probiotics. *Applied and Environmental Microbiology*, 72, 1729–1738.
- Borges, S., Barbosa, J., Silva, J., P. Teixeira, P. (2013). Evaluation of characteristics of *Pediococcus* spp. to be used as a vaginal probiotic. *Journal of Applied Microbiology*, 115(2): 527–538.
- Burgain, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering*, 104(4): 467–483.
- Collado, M. C., Isolauri, E., Salminen, S., Sanz, Y. (2009). The impact of probiotic on gut health. *Current Drug Metabolism*, 10(1): 68–78.
- Collado, M. C., Meriluoto, J., & Salminen, S. (2008). Adhesion and aggregation properties of probiotic and pathogen strains. *European Food Research and Technology*, 226, 1065–1073.
- European Food Safety Authority (EFSA). (2020). EFSA Strategy 2020. Trusted science for safe food: Protecting consumers' health with independent scientific advice on the food chain. 1–30.
- European Food Safety Authority (EFSA). (2023). Scientific opinion on the safety evaluation of microorganisms used in food and feed production (QPS update 2023). *EFSA Journal*, 21(7), e08012.
- FAO/WHO (2002) Guidelines for the Evaluation of Probiotics in Food. Food and Agriculture Organization of the United Nations/World Health Organization, London, Ontario.
- FAO/WHO. (2023). Guidelines for the evaluation of probiotics in food – Updated recommendations. Food and Agriculture Organization of the United Nations & World Health Organization.
- Far, B. F., Safaei, M., Nahavandi, R., Gholami, A., et al. (2024). Hydrogel Encapsulation techniques and its clinical applications in drug delivery and regenerative medicine : A Systematic review. *ACS Omega*, 9(27):29139–29158.
- Finalis, E.R., Sri Djangkung, S.M., Arfiana, Noor, I., et al. (2020). Development of carbon based NPK slow release fertilizer using biochar from oil palm empty fruits. *Indonesian Journal of Energy*, 3(1): 19–24.
- Fung, W.Y., Yuen, K.H. and Liong, M.T. (2011). Agrowaste-based nanofibers as a probiotic encapsulant: Fabrication and characterization. *Journal of Agricultural and Food Chemistry*, 59(15): 8140–8147.
- Han, K.I., Shin, H.D., Lee, Y., Baek, S., et al. (2024). Probiotic and postbiotic potentials of *Enterococcus faecalis* EF-2001 : A Safety Assessment. *Pharmaceuticals*, 17(10): 1383.
- Jiang, S., Lingzhi, C., Longxian, L., Lanjuan, L. (2021). *Pediococcus pentosaceus*, a future additive or probiotic candidate. *Microbial Cell Factories*, 20(1), pp. 1–14.
- Jones, M.L., Tomaro-Duchesneau, C., & Prakash, S. (2008). The gut microbiome, probiotics, bile acids and cholesterol metabolism. *Gut Microbes*, 5, 1–9.
- Kos, B., Šušković, J., Vuković, S., Sikora, S., & Matošić, S. (2020). Adhesion and coaggregation ability of probiotic lactic acid bacteria against pathogens: Mechanisms and applications. *International Journal of Food Microbiology*, 319, 108496.

- Krasaekoopt, W., Bhandari, B., & Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*, 13, 3–13.
- Kreve, S. and Reis, A.C.D. (2021). Bacterial adhesion to biomaterials: What regulates this attachment? A review. *Japanese Dental Science Review*, 57(4): 85–96.
- Ku, S., Park, M.S., Ji, G.E., & You, H.J. (2023). Coaggregation and competitive exclusion of pathogens by probiotic lactic acid bacteria: Functional implications for gut health. *Journal of Applied Microbiology*, 134(2), 123–129.
- Lee, Y.K., Ow, D.S.W., & Oh, S. (2022). Biogenic amine production by lactic acid bacteria: Mechanisms and safety implications in fermented foods. *Critical Reviews in Food Science and Nutrition*, 62(18), 4971–4986.
- Li, L., Zhao, Y., Li, J., Ban, L., et al. (2022). The adhesion of the gut microbiota to insoluble dietary fiber from soy hulls promoted the proliferation of probiotics in vitro. *LWT- Food Science and Technology*, 153: 112560.
- Liu, H., Wang, L., Li, Y., & Zhang, X. (2021). Cellulose-based hydrogels as encapsulation matrices for probiotic delivery: Structure, functionality and safety aspects. *Carbohydrate Polymers*, 259, 117735.
- Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E., Rotondo, J.C. (2023). Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*, 12(1): 184.
- Meziane, H., Laita, M., Azzaoui, K., Boulouiz, A., et al. (2024). Nanocellulose fibers : A Review of preparation methods, characterization techniques, and reinforcement applications. *Moroccan Journal of Chemistry*, 12(1): 305–343.
- Mohan, S., Tomar, S. K., & Singh, R. (2024). Influence of polysaccharide-based encapsulation on probiotic surface hydrophobicity and adhesion-related properties. *Carbohydrate Polymers*, 322, 121330.
- Patel, A.K., Singhanian, R.R., et al. (2012). Probiotic properties of *Pediococcus* spp. and their applications. *Food Research International*, 45, 59–68.
- Pato, U., Ayu, D.F., Riftyan, E., Restuhadi, F., et al. (2021). Physicochemical property of oil palm leaves and utilization of cellulose microfibril as probiotic encapsulant. *Biodiversitas*, 22(7): 2937–2944.
- Pradhan, D., Mallappa, R. H. and Grover, S. (2020). Comprehensive approaches for assessing the safety of probiotic bacteria. *Food Control*, 108: 106872.
- Rojek, A. Z., Kośmider, A., Stępień, K. and Tyski, S. (2022). Adhesion and aggregation properties of Lactobacillaceae strains as protection ways against enteropathogenic bacteria. *Archives of Microbiology*, 204(5): 1–13.
- Singh, J., & Sharma, M. (2024). Coaggregation and pathogen exclusion potential of probiotic strains: Recent advances and mechanistic insights. *Probiotics and Antimicrobial Proteins*, 16, 145–158.
- Singh, R., Shitiz, K., & Singh, A. (2024). Advances in biopolymer-based microencapsulation systems for probiotic stabilization. *Food Hydrocolloids*, 146, 109273.
- Sun, G., Li, X., Chen, Y., & Zhang, H. (2023). Polysaccharide microencapsulation enhances probiotic functionality by modulating cell surface properties. *Food Hydrocolloids*, 139, 108547.
- Vidhyasagar, V. and Jeevaratnam, K. (2012). Evaluation of *Pediococcus pentosaceus* strains isolated from Idly batter for probiotic properties in vitro. *Journal of Functional Foods*, 5(1): 235–243.

- Wang, G., Chen, S., Zhao, X., & Zhang, H. (2023). Genomic safety assessment of *Pediococcus pentosaceus* strains intended for probiotic applications. *Frontiers in Microbiology*, 14, 1189023.
- Yang, Y., Zhang, J. and Li, C. (2024). Delivery of probiotics with cellulose-based films and their food applications. *Polymers*, 16(6): 794.
- Zhang, L., Li, Y., & Chen, X. (2021). Safety evaluation of biogenic amine production in lactic acid bacteria from plant-based fermented foods. *Food Control*, 123, 107723.
- Zhang, Y., Hu, P., Xie, Y. and Wang, X (2020). Co-fermentation with *Lactobacillus curvatus* LAB26 and *Pediococcus pentosaceus* SWU73571 for improving quality and safety of sour meat. *Meat Science*, 170(1): 1–10.
- Zommiti, M., Almohammed, H., Ferchichi, M., & Feuilloley, M.G.J. (2020). Evaluation of probiotic properties and safety of *Pediococcus pentosaceus* strains isolated from fermented foods. *Probiotics and Antimicrobial Proteins*, 12, 148–159.