Research Article

Isolation and Identification of Cellulase-Producing Lactic Acid Bacteria from Water Hyacinth for Fermentation Applications

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Abstract | The utilization of water hyacinth can help mitigate environmental issues caused by its accumulation in water bodies. Fermentation with cellulolytic lactic acid bacteria (LAB) isolated from water hyacinth can enhance its use as poultry feed. This study aimed to isolate cellulase-producing LAB from fermented water hyacinth to be used as a fermentation starter. The research involved two stages: first, isolation, selection, enzyme activity measurement, and identification of LAB; second, fermentation of water hyacinth using selected LAB in a 3×4 Completely Randomized Design (CRD) with 3 replications. Factor A was inoculum dose (1%, 2%, and 3%), and Factor B was fermentation duration (3, 5, 7, and 9 days). Variables measured included crude fiber content, gross energy, and fiber structure. The results showed that 1,130 bacterial colonies were isolated, with 47 LAB isolates. The cellulase-producing LAB selection revealed 46 isolates producing clear zones, with EG10FCC showing the highest cellulase enzyme (0.94 U/ml). Molecular identification of EG10FCC as *Lactiplantibacillus plantarum* (91% similarity) showed the best results. Fermentation of water hyacinth with *Lactiplantibacillus plantarum* reduced crude fiber by 17.98% and increased gross energy by 3870.19 KKal/kg with a 3% inoculum dose and 9 days of fermentation. Treatment A4B4 resulted in a looser fiber structure. In conclusion, isolate EG10FCC is *Lactiplantibacillus plantarum*, producing the highest cellulase enzyme. Fermentation reduces crude fiber by 35.58%, increases gross energy by 10.21%, and loosens fiber structure.

Keywords | Crude Fiber, Enzyme, Fermentation, Isolation, Lactic acid bacteria, Water hyacinth

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INTRODUCTION

The poultry farming sector in Indonesia faces significant challenges in ensuring a sustainable feed supply. This is because the country still depends heavily on imported feed ingredients. According to data from the Directorate General of Animal Husbandry and Animal Health (2023) and GPMT (2019), 42% of poultry feed ingredients in Indonesia are still reliant on imports. This dependency on imported feed ingredients affects the cost of poultry feed production. Therefore, finding alternative feed ingredients based on local resources that are more sustainable is of great importance. One such resource that can be utilized as poultry feed is *Eichhornia crassipes* (water hyacinth), which is often considered a nuisance weed in various water bodies.

Water hyacinth is an invasive plant species that grows abundantly in Indonesia's water bodies. In Lombok Island, around 267 hectares of water hyacinth grow in the Batujai Reservoir (Muazzasari *et al*., 2023). The invasive growth of water hyacinth causes significant environmental problems. This plant is considered one of the worst aquatic weeds in the

world (FAO, 2002). Water hyacinth grows rapidly and can cover the entire water surface with a dense layer in just two weeks or more (Sharma, 2016). It can disrupt the stability of freshwater ecosystems by outcompeting all surrounding species, ultimately threatening aquatic biodiversity and degrading water quality (Gichuki *et al*., 2012; Patel, 2012; Dagaga, 2018; Gezie *et al*., 2018). Additionally, water hyacinth can hinder community activities (Enyew *et al*., 2019; Honlah *et al*., 2019a) and become a breeding ground for diseases (Honlah *et al*., 2019b).

The utilization of water hyacinth as poultry feed is an effective solution for meeting feed ingredient needs while simultaneously addressing environmental issues. Water hyacinth is nutritionally rich, particularly in energy content, especially in its leaves, making it a good source of energy for animal feed. Water hyacinth contains 3700 Kcal of metabolizable energy (21.23%), crude protein (13%), crude fat (0.023%), phosphorus (0.0011%), calcium (12.8%), and crude fiber (20.6%) (Kusrinah *et al*., 2016; Maslami *et al*., 2014). The high crude fiber content (CF) is a challenge in using water hyacinth as poultry feed. Poultry has a certain tolerance limit for CF in their feed. According to the Indonesian National Standard (SNI, 2016), the maximum CF limit in poultry feed rations is 3-7%. Reducing CF in water hyacinth can be achieved through fermentation.

The fermentation of water hyacinth using various microbes has been studied by previous researchers, but its effectiveness in reducing CF content remains limited. Fermentation of water hyacinth with *Phanerochaete chrysosporium* and *Trichoderma reesei* reduced CF by up to 14.35% (Kumajas and Onibala, 2022), while silage fermentation of water hyacinth reduced CF by 16.32% (Irawati *et al*., 2019). Other fermentations also showed CF reduction, including with molasses (16.3%), rumen fermentation (17.3%), yeast fermentation (16.2%), and *Trichoderma harzianum* fermentation, which reduced CF by 21.81% (Saha *et al*., 2011). As posited by Maslami *et al*. (2024), the fermentation of water hyacinth using *Neurospora crassa* at a 2% dose and a 5-day fermentation period resulted in a crude fibre (CF) content of 23.57%, which is a reduction of 16.91% compared to the initial crude fibre value. However, these results indicate that the CF content remains relatively high for poultry feed. Therefore, there is a need to identify more effective microbes for CF reduction. One approach is isolating microbes directly from water hyacinth that has undergone natural decomposition (fermentation).

One effective microbe for reducing CF is cellulolytic lactic acid bacteria (LAB), which is known for its high ability to produce cellulase enzymes. LAB is a gram-positive bacterium considered safe and capable of producing cellulose enzymes (Khalid, 2011; Krabi *et al*., 2015). LAB derives energy by converting cellulose into sugars for metabolism and growth (Vlasova *et al*., 2016). Compared to fungi, LAB is superior as a fermentation starter due to its faster growth rate and ability to produce cellulase enzymes in a shorter time (Barzkar and Sohail, 2020; Moseri *et al*., 2023). It has been demonstrated that the fermentation of water hyacinth leaf meal with *B. subtilis* Cy5 and *Lactobacillus acidophilus* can reduce crude fibre to 13.5% (Saha and Ray, 2011), while fermentation with *Trichoderma harzianum* results in a crude fibre reduction of 21.81% (El-Sayed). LAB with high cellulase activity can be directly isolated from water hyacinth with high crude fiber content, as microorganisms from natural environments tend to adapt to their growth substrate, directing metabolism to produce the required enzymes (Lata *et al*., 2013).

The isolation of microbes from water hyacinth has been shown to produce cellulase enzymes in significant amounts. Several microorganisms isolated from water hyacinth have been found to produce cellulase enzymes. Trichoderma reesei isolated from water hyacinth has been reported to produce cellulase at a rate of 5% per hour per liter (Murti *et al*., 2018). *Trichoderma harzianum* from the roots of water hyacinth and *Aspergillus oryzae* from compost exhibit high activity, with clear zones up to 35 mm (Sharma *et al*., 2020; Nhah *et al*., 2021). As a fermentation substrate, water hyacinth also produces significant cellulase, such as 6.4 IU/g DM by *T. harzianum* and 647.51 U/g DM by *Penicillium crustosum* (Arana-Cuenca *et al*., 2019). Lactic acid bacteria (LAB) producing cellulase enzymes are expected to reduce the crude fiber content of water hyacinth, making its nutrients more digestible and increasing its percentage in poultry feed. The aim of this study is to isolate cellulolytic LAB from water hyacinth as a fermentation starter capable of reducing CF.

MATERIALS AND METHODS

Time and location of the study

The research was conducted from June 1 to September 15, 2025, at the Biotechnology Laboratory of the Faculty of Animal Science, University of Mataram.

Isolation and selection of lactic acid bacteria

The study began with the natural fermentation of water hyacinth. The fermentation process entails the submergence of water hyacinth leaves and stems in water, followed by a seven-day fermentation period in anaerobic conditions at ambient temperature. This duration is considered optimal for the fermentation process.Isolation was carried out by mixing 1 gram of fermented water hyacinth into 100 mL of distilled water (Shifani *et al*., 2023), followed by shaking and serial dilution (10^2 to 10^{10}). Inoculation on MRS Agar was performed using the pour plate method with 100 µL of the suspension, repeated three times, and incubated for

24 hours at 37°C. Further purification was conducted on MRS Agar containing 1% $CaCO_3$, and the cultures were incubated for another 24 hours at the same temperature (Herdiana *et al.*, 2016). The formation of clear zones indicated the presence of lactic acid bacteria.

Selection of cellulase-producing lactic acid bacteria

The cellulase activity of lactic acid bacteria (LAB) was tested following the procedure described by Srifani *et al.* (2023). The medium consisted of 0.02 g MgSO4·7H2O, 0.075 g KNO3, 1 g CMC, 0.05 g K2HPO4, 0.002 g FeSO4, 0.004 g CaCl2, 0.2 g nutrient agar, and 0.1 g glucose in 100 mL of distilled water. The medium was sterilized by autoclaving at 121°C for 15 minutes, then poured into petri dishes and inoculated with bacterial isolates. The petri dishes were incubated at 30°C for 24 hours and examined for clear zones. After incubation, the petri dishes were stained with 0.1% Congo Red for 15 minutes, washed with 1M NaCl for 15-20 minutes. The areas that remained unstained indicated CMC hydrolysis, and cellulolytic isolates were selected based on the size of the clear zone.

Cellulase enzyme measurement

Cellulase enzyme activity was measured quantitatively using the 3,5-dinitrosalicylic acid (DNS) method (Mullings *et al.*, 1985; Ghose, 1987). The isolates were incubated overnight at room temperature. After incubation, centrifugation was performed at full speed, and the supernatant was collected as crude enzyme. Carboxymethyl cellulose (CMC) substrate was prepared by dissolving 1% CMC in Phosphate Buffered Saline (PBS). One milliliter of CMC was mixed with 100 µL of crude cellulase enzyme and incubated at 45°C for 30 minutes. The reaction was stopped by adding 1-2 mL of DNS reagent, and the mixture was incubated at 70-100°C for 10 minutes. After cooling, the absorbance was measured at a wavelength of 540 nm to determine the release of reducing sugars, which was used to calculate the quantitative cellulase activity. The cellulase activity was calculated using a previously established formula. The reduction of sugar is determined using a standard curve.

$$Enzyme\ activity\ (U/ml) = \frac{Reducing\ sugar\ (product\ conventration \times 1000 \times dilution\ factor}{moleculer\ weght\ of\ glucose \times incubation\ time\ (minute)}$$

Molecular identification of cellulase-producing lactic acid bacteria (LAB)

The identification of lactic acid bacteria (LAB) was performed molecularly by amplifying the 16S ribosomal DNA (16S rDNA) region. Ten LAB isolates identified as high cellulase producers were selected. Genomic DNA isolation was carried out following the Clarridge protocol (Jennifer and Thirunelakandan, 2015) using the Invitrogen DNA extraction kit (Tamura *et al.*, 2011). Sequencing was performed through the 1st BASE service (Malaysia). The sequencing results were compared with GenBank using BLASTN (NCBI) (http://www.ncbi.nih.gov), and sequence alignment as well as phylogenetic tree construction were conducted using MEGA 5.05 software (Promega, 2016), employing the neighbor-joining tree method and a bootstrap value of 1000×.

Fermentation of water hyacinth with selected lactic acid bacteria

A total of 300 grams of water hyacinth samples were weighed and placed in plastic bags, then distilled water was added until the moisture content reached 50%. The water hyacinth was then autoclaved at 121°C for 15 minutes. Selected LAB was inoculated, mixed thoroughly, and allowed to ferment according to the treatment time. This study used a Completely Randomized Design (CRD) with a 3 × 4 factorial pattern and 3 replications. Factor A was the LAB inoculum dose, with A1 = 1%, A2 = 2%, A3 = 3%, and Factor B was the fermentation duration, with B1 = 3 days, B2 = 5 days, B3 = 7 days, B4 = 9 days. Data were analyzed using factorial CRD analysis (Steel and Torrie, 1991), followed by Duncan's multiple range test. The parameters observed included crude fiber (AOAC, 2019), fiber structure using Scanning Electron Microscopy (SEM), and gross energy.

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria (LAB) from fermented water hyacinth

The isolation of LAB from fermented water hyacinth was carried out by plating cultures from dilutions of 10^5 to 10^7 on MRS agar to obtain single colonies. The results showed that 1,130 colonies were obtained at a dilution of 10^7. The single colonies were subsequently subcultured on MRS agar supplemented with 2% CaCO3 to assess their ability to produce lactic acid. The study identified 47 LAB isolates from the fermented water hyacinth. The LAB grown on MRS agar supplemented with 2% CaCO3 are shown in Figure 1.

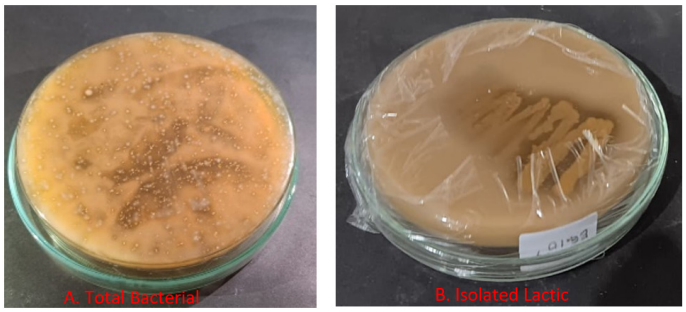

Figure 1: Isolation and selection of lactic acid bacteria.

The results of the study showed that 47 LAB isolates were obtained. The bacterial isolation process involved taking

samples of fermented water hyacinth, which were then inoculated onto MRS Agar for the growth of LAB. MRS Agar media supports the growth of lactic acid bacteria, such as *Lactobacillus, Enterococcus,* and *Pediococcus* (Khushboo *et al*., 2023). The LAB colonies exhibited morphological characteristics such as being round, cream-colored, and smooth. In line with Chammas *et al*. (2006), LAB colonies are generally round or oval in shape, white, cream, or yellowish in color, with a smooth or sticky texture.

The isolated LAB were then characterized using Gram staining and catalase testing. The results showed that 47 isolates were Gram-positive with a *bacillus* morphology. Furthermore, the catalase test results showed a negative reaction, as no bubbles were formed when the H_2O_2 solution was applied. This further confirms that the isolates are lactic acid bacteria. The morphological characteristics are shown in Figure 2.

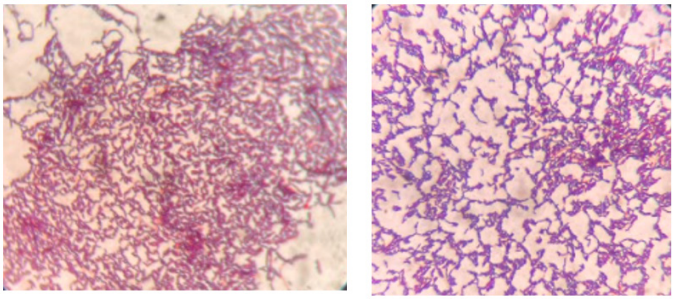

Figure 2: Morphological shape of lactic acid bacteria.

Gram-positive bacteria have a thick cell wall primarily composed of peptidoglycan, which allows them to retain the purple color from crystal violet dye after the staining process and washing with alcohol. In contrast, Gram-negative bacteria have a thinner cell wall with less peptidoglycan, but are surrounded by an outer membrane containing lipopolysaccharides. As a result, after alcohol washing, Gram-negative bacteria lose the purple color and take on the pink color from the safranin dye, which is used as a counterstain (Adjoudj *et al*., 2020). The catalase test is a biochemical test used to identify bacteria based on their ability to produce the catalase enzyme. The LAB isolates were unable to produce catalase. The catalase enzyme functions to convert hydrogen peroxide (H_2O_2), a harmful byproduct of oxygen metabolism, into water (H_2O) and oxygen (O_2) (Anwar *et al*., 2024).

Selection of cellulase-producing lactic acid bacteria (LAB) The results of the study showed that 46 isolates produced clear zones, while 1 isolate did not produce a clear zone. The isolate that produced the largest clear zone was isolate EG10FCC, with a zone size of 10 mm. The clear zone results produced by each isolate are presented in Table 1.

Cellulase enzyme activity in hydrolyzing CMC was observed using Congo Red staining, indicated by the formation of a clear zone around the growing LAB colonies. The results showed that the EGFC6 isolate produced the largest clear zone, with a diameter of 10 mm. The clear zone indicates the hydrolytic activity of the extracellular cellulase enzyme secreted by the LAB isolates, with a specific diameter. The larger the clear zone, the higher the cellulase enzyme activity produced by the isolate. CMC degradation involves a consortium of three enzymes: endo-β-(1,4)-glucanase, exo-β-(1,4)-glucanase, and β-(1,4)-glucosidase. These enzymes work together to cleave the CMC chain, leading to a reduction in the liquid viscosity (Scheffer *et al*., 2022). Endo-glucanase hydrolyzes the polymer backbone, releasing cellobiose, cellotriose, and other oligomers (Scheffer *et al*., 2021). Differences in the cellulolytic activity indices are suspected to be due to the cellulase secretion by each bacterial isolate, which have varying potentials to break down the substrate in their growth media (Sudiana *et al*., 2001). The higher the cellulase index in an isolate, the greater the cellulolytic activity it produces (Apun *et al*., 2000). The size of the clear zones produced by the LAB isolates is shown in Figure 3.

Table 1: Qualitative selection of cellulase-producing lactic acid bacteria.

Isolate	Clear zone (mm)	Isolat	Clear zone (mm)	Isolate	Clear zone (mm)	Isolate	Clear zone (mm)
EGA1	3	EGE12	1	EGI26	4	EGN21	2
EGA45	5	EGE8	8	EGI10	2	EGN31	2
EGA6	3	EGF1	9	EGK33	10	EGO35	1
EGB4	5	EGF38	5	EGK11	8	EGO25	7
EGB23	5	EGF32	3	EGK9	1	EGO30	7
EGE6	5	EG10FCC	10	EGL39	2	EGP22	7
EGC6	2	EGG41	4	EGL29	9	EGP44	7
EGC17	0	EGG15	4	EGL43	2	EGP40	5
EGC42	1	EGG5	3	EGM19	3	EGPI3	8
EGD28	4	EGH20	5	EGM24	1	EGP36	1
EGD34	1	EGH1	2	EGM18	2	EGP37	3
EGD35	2	EGI16	3	EGN7	2		

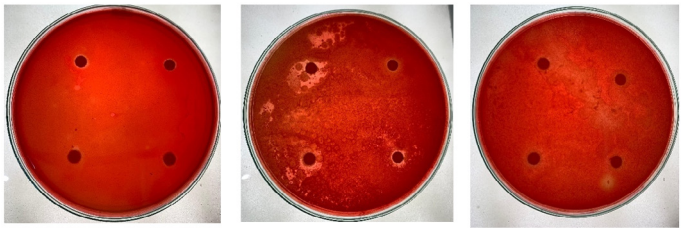

Figure 3: Qualitative test of cellulase-producing lactic acid bacteria.

Cellulase enzyme activity

Quantitative enzyme activity measurements were performed on isolates demonstrating the highest capacity to produce clear zones. Eleven LAB isolates with the highest clear zone production were selected for further measurement of their enzyme activity. The results of the quantitative cellulase enzyme activity are shown in Figure 4.

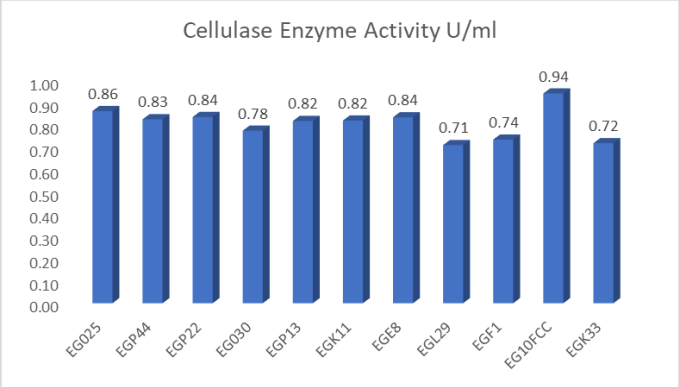

Figure 4: Cellulase enzyme activity of each isolate.

Based on the graph showing cellulase enzyme activity (U/mL) across various isolates, it is evident that the EG10FCC isolate exhibits the highest enzyme activity, at 0.94 U/mL. This measured enzyme activity indicates the bacterium's ability to produce cellulase enzymes capable of hydrolyzing CMC-based substrates into simpler products. Cellulase enzymes can hydrolyze cellulose into glucose or oligosaccharides (Ranganathan *et al.*, 2022). The degradation process of CMC by cellulase-producing bacteria begins with the binding of CMC to the bacterial cell surface. CMC, which contains carboxymethyl groups, is soluble in water, and the bacteria bind it through adhesin proteins on the cell surface (Pulyala *et al.*, 2025). Subsequently, the bacteria produce cellulase enzymes, which consist of several types, including endo-β-glucanase, exoglucosidase, and β-glucosidase. These enzymes work together to break the β-1,4-glycosidic bonds in the CMC chain. Endo-β-glucanase cleaves CMC randomly, generating smaller fragments, while exoglucosidase works at the chain ends to produce disaccharides or monosaccharides. β-glucosidase then hydrolyzes these products into glucose (Datta, 2024; Jiménez-Leyva *et al.*, 2017).

The differences in enzyme activity between isolates are attributed to the specific characteristics and genetic factors of each isolate. Variations in enzyme activity among isolates are influenced by genetic differences, enzyme expression regulation, as well as the conditions under which microorganisms are cultured during isolation and fermentation (Cross *et al.*, 2006). Similar enzyme activity results were reported by Demissie *et al.* (2024), who showed that the cellulase enzyme activity produced by *Bacillus bacteria* isolated from the forest reached approximately 1 U/ml after a 24-hour incubation period. The production of cellulase by Bacillus sp. isolated from a mangrove forest, after optimization, reached 1.510 U/ml (Bamrungpanichtavorn *et al.*, 2023). Lower results were reported by Baharuddin *et al.* (2021), who reported cellulase enzyme activity from bacteria of 0.1308 U/ml.

Molecular identification of lactic acid bacteria (LAB)

From the 11 isolates with the highest cellulase enzyme production, molecular identification was performed using the 16S rRNA method to determine the strain of LAB that produced the highest cellulase enzyme. The results of the BLAST analysis of the EG10FCC bacterial isolates obtained from GenBank are illustrated in Figure 5.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Lactobacillus plantarum strain CQ99-1 16S ribosomal RNA gene, partial sequence	2619	2619	100%	0.0	100.00%	MG646872.1
Lactiplantibacillus plantarum strain TMPC 33322 16S ribosomal RNA gene, partial sequence	2619	2619	100%	0.0	100.00%	OM265412.1
Lactiplantibacillus plantarum strain Lp01 16S ribosomal RNA gene, partial sequence	2619	2619	100%	0.0	100.00%	MZ674414.1
Lactiplantibacillus plantarum strain M3 16S ribosomal RNA gene, partial sequence	2619	2619	100%	0.0	100.00%	MW925128.1
Lactobacillus plantarum strain LP84 16S ribosomal RNA gene, partial sequence	2617	2617	100%	0.0	100.00%	KU710491.1
Lactobacillus plantarum strain LP59 16S ribosomal RNA gene, partial sequence	2617	2617	100%	0.0	100.00%	KU710489.1
Lactobacillus plantarum strain 3304 16S ribosomal RNA gene, partial sequence	2614	2614	100%	0.0	99.93%	MT613605.1
Lactobacillus plantarum strain 4619 16S ribosomal RNA gene, partial sequence	2614	2614	100%	0.0	99.93%	MT545111.1
Lactobacillus plantarum strain 4594 16S ribosomal RNA gene, partial sequence	2614	2614	100%	0.0	99.93%	MT545092.1
Lactobacillus plantarum strain 4506 16S ribosomal RNA gene, partial sequence	2614	2614	100%	0.0	99.93%	MT545027.1

Figure 5: BLAST analysis results of EG10FCC bacteria isolates found in GenBank.

The identification results showed that the EG10FCC isolate was *Lactiplantibacillus plantarum*. The phylogenetic tree is shown in Figure 6.

The EG10FCC isolate in the phylogenetic tree shows a significant closeness to the *Lactiplantibacillus* group, particularly to *Lactiplantibacillus plantarum*, with a bootstrap value of 100%, indicating a high level of confidence in the phylogenetic relationship. Several other researchers have also reported cellulase-producing LAB, including *Lactobacillus* sp. (Srifani *et al.*, 2023), *Raoultella terrigena* (Zhang *et al.*, 2023), *Lactiplantibacillus plantarum,* and *Levilactobacillus brevis* (Haokok *et al.*, 2023).

Fermentation of water hyacinth with selected *Lactiplantibacillus plantarum* isolate

Lactiplantibacillus plantarum bacteria, which produce the highest cellulase enzyme activity, were then used as a fermentation starter for water hyacinth. The nutritional

content of water hyacinth before fermentation is shown in Table 3. The fermentation of water hyacinth with *Lactiplantibacillus plantarum* at different inoculum doses and fermentation times is also presented in Table 4.

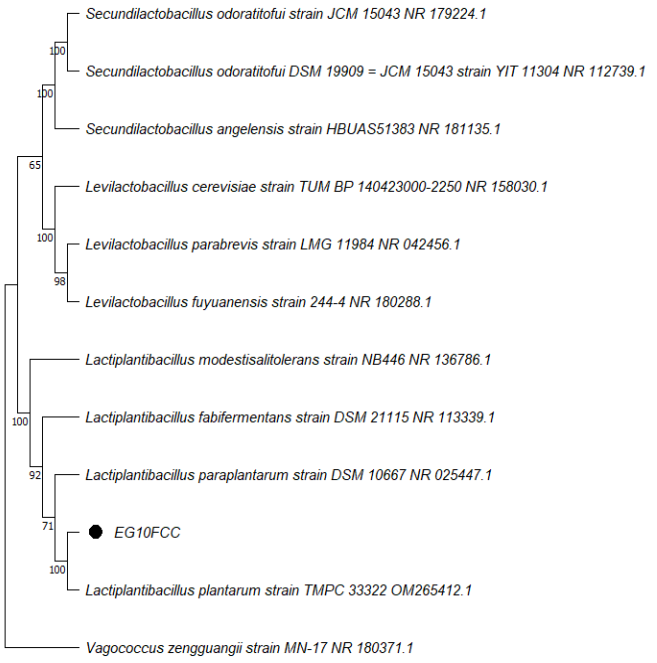

Figure 6: Phylogenetic tree of EG10FCC isolate.

Table 2: Nutritional content of water hyacinth before fermentation.

Nutritional content of water hyacinth	Dry matter content
Crude protein (%)	8.03
Crude fat (%)	0.59
Crude fiber (%)	27.91
Gross energy (Kkal/kg)	3474.95
Ca (%)	2.86
P (%)	0.09
Organic matter (%)	87.01

Crude fiber

In a study investigating the effect of *Lactiplantibacillus plantarum* fermentation on the crude fiber content of water hyacinth, the different treatments significantly affected the crude fiber composition. As demonstrated by the DMRT follow-up test, a significant difference ($p<0.05$) was observed, the interaction between inoculum dose and fermentation duration in treatment A3B4 resulted in the most significant reduction in crude fibre, with a value of 17.98%. Compared to the pre-fermentation stage, the crude fiber content of water hyacinth decreased by 35.58%. This reduction in crude fiber was attributed to the high activity of cellulase enzymes, which resulted from the optimal inoculum dose and fermentation duration. The cellulase enzyme penetrates the protective layer of cellulose and interacts with other components such as hemicellulose and lignin that encapsulate the cellulose. Subsequently, the cellulase enzyme binds and positions the β-1,4-glycosidic bonds at its active site for cleavage. This cleavage process results in oligosaccharides (short glucose chains) and, eventually, glucose (Ejaz *et al*., 2021). Cellulase consists of three types of enzymes: β-glucosidase, endo-1,4-β-D-glucanase (endoglucanase), and exo-1,4-β-D-glucanase (exoglucanase). These three enzymes work synergistically to achieve effective cellulose hydrolysis (Patel *et al*., 2019). Endoglucanase acts on the internal oligosaccharides found in carboxymethylcellulose, cellooligosaccharides, or amorphous cellulose. Exoglucanase hydrolyzes the non-reducing end of crystalline cellulose, producing cellobiose or glucose as the primary product. Meanwhile, β-glucosidase acts on the non-reducing end of cellobiose and cellodextrin (Sakka *et al*., 2000).

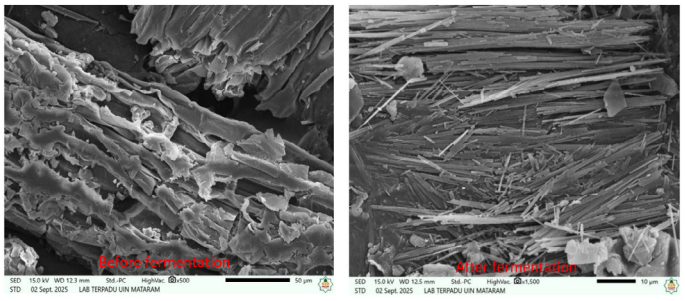

Figure 7: Structure of water hyacinth fiber before and after fermentation.

Table 3: Nutritional content of water hyacinth after fermentation.

Nutritional content	Factor A (Dose)	Factor B (fermentation duration)				Average
		B1 (3 Days)	B2 (5 Days)	B3 (7 Days)	B4 (9 Days)	
Crude fiber	A1 (1%)	23.53±0,94^{a}	22.55±1,17^{ab}	21.49±0,40^{bc}	20.31±0,61^{c}	21.97±1,20^{a}
	A2 (2%)	21.40±1,00^{bc}	21.19±0,05^{bc}	20.99±0,26^{bc}	19.96±1,23^{c}	20.89±0,55^{ab}
	A3 (3%)	21.17±1,07^{bc}	21.02±0,16^{bc}	19.83±0,25^{c}	17.98±0,25^{d}	20.00±1,00^{b}
	Average	22.03±1,06^{a}	21.59±0,69^{a}	20.77±0,69^{ab}	19.42±1,02^{b}	
Gross energy	A1 (1%)	3462.38±75,59^{cd}	3512.92±47,69^{bc}	3412.39±7,44^{cd}	3665.64±5,11^{bc}	3513.33±94,84ab
	A2 (2%)	3344.01±45,04^{d}	3577.36±96,42^{bc}	3444.07±10,28^{cd}	3524.02±38,25^{bc}	3472.36±87,99^{b}
	A3 (3%)	3442.04±32,82^{cd}	3464.86±111,06^{cd}	3712.92±97,91^{b}	3870.19±127,9^{0}a	3622.50±178,14^{a}
	Average	3416.14±51,68^{b}	3518.38±46,09^{b}	3523.13±134,83^{b}	3686.61±142,10^{a}	

Note: Different in superscripts at the same rows and columns of each variable indicate significantly different effects ($p < 0.05$).

The results indicate that an increase in the inoculum dose leads to a reduction in crude fiber content. This is because a higher dose provides more *Lactiplantibacillus plantarum* bacteria that produce degrading enzymes, which directly contribute to an increased rate of crude fiber hydrolysis. A higher inoculum dose tends to accelerate the fermentation process, as a greater number of microorganisms accelerates the breakdown of organic material. According to Sun *et al*. (2025), increasing the inoculum dose of *L. plantarum* can speed up the fermentation process and result in a more significant reduction in crude fiber content, as microorganisms degrade fiber components more quickly with increased cellulase enzyme production. In contrast, a lower inoculum dose may not be sufficient to produce effective fermentation, thus hindering the breakdown of crude fiber and slowing down the reduction in crude fiber content.

The increase in fermentation time in this study significantly reduced the crude fiber content of water hyacinth. The fermentation process requires time to degrade crude fiber. This process takes time because the enzymes produced by Lactiplantibacillus plantarum work gradually, breaking down the more complex crude fiber structure into compounds that are easier to digest. The breakdown of crude fiber by cellulase enzymes is a complex biochemical reaction that involves several steps, requiring time and a combination of specific enzymes to hydrolyze cellulose into simple sugars that can be digested (Lynd *et al*., 2002). This gradual breakdown allows the bacteria to convert complex and external cellulose materials into soluble compounds, which can then be absorbed and used for their metabolism.

Gross energy

Fermentation of water hyacinth with *Lactiplantibacillus plantarum* significantly influenced the gross energy content, which was affected by two main factors: fermentation duration and inoculum dose. As indicated by the DMRT follow-up test, a significant difference ($p<0.05$) was observed, indicating an interaction between inoculum dose and fermentation duration. The highest gross energy content in this experiment was observed in treatment A3B4, with a value of 3870.19 kcal/kg. The results demonstrate a 17.98% increase in gross energy content following fermentation in comparison with the pre-fermentation state.The increase in the gross energy content of fermented water hyacinth was 10.21% compared to its pre-fermentation content. The increase in gross energy (GE) in fermented water hyacinth occurred because Lactiplantibacillus plantarum breaks down crude fiber into simpler sugars, such as glucose, which are easier to digest. Fermentation is a process that breaks down fibre into simpler compounds, such as glucose, which can be absorbed by poultry and used as an energy source (Al-Aboudi and Hamodi, 2023). The increase in fermentation time can enhance the gross energy of fermented water hyacinth. A longer fermentation period allows *Lactiplantibacillus plantarum* to hydrolyze more components of water hyacinth into simpler, more digestible compounds, which ultimately results in more energy. In line with Kitessa *et al*. (2024), microorganisms work more efficiently in breaking down feed components and producing higher energy with a longer fermentation time.

Structure of water hyacinth fiber

Water hyacinth with the best treatment was observed for the structure of its fibers and compared to water hyacinth before fermentation. The images depicting the structure of water hyacinth are displayed in Figure 7.

The structure of water hyacinth fiber underwent significant changes during the fermentation process, which can be observed by comparing the images of the fibers before and after fermentation. In the pre-fermentation image, the water hyacinth fibers appear dense and compact, with strong lignocellulosic bonds. The high presence of lignin in the fibers makes them rigid and difficult to degrade. This structure indicates that the components of the cell wall are still intact and have not yet undergone degradation. As a result, the fibers tend to be stiff and are not easily processed (Ishaya *et al*., 2024).

After undergoing the process, the images clearly show noticeable changes. The fibers have become more loosely arranged, with small cavities visible and a rougher, fragmented surface. This morphological change occurs due to the activity of microorganisms producing cellulase enzymes that break down the complex bonds in water hyacinth fibers. This process reduces the stiffness of the fibers and increases the availability of organic substances that are more easily accessed by microbes or other organisms (Sanchez-Torres *et al*., 2025). With this structural change, the water hyacinth fibers after fermentation become more digestible and more efficient in their use as poultry feed. The more open fiber degradation allows better access for enzymes to digest carbohydrate components, thus enhancing the efficiency of using water hyacinth as a feed ingredient. This is consistent with studies showing that fermentation can improve the availability of nutrients (Knez *et al*., 2023).

CONCLUSION

Forty-seven lactic acid bacteria (LAB) isolates, isolated from fermented water hyacinth, produced clear zones on the test medium. The EG10FCC isolate was the best in enzyme production, with an activity of 0.94 U/ml. After

molecular identification using 16S rRNA, this isolate showed a 100% similarity to *Lactiplantibacillus plantarum*. Fermentation of water hyacinth with *Lactiplantibacillus plantarum* resulted in a 35.58% reduction in crude fiber, a 10.21% increase in gross energy, and caused the structure of the fibers to become more loosened.

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NOVELTY STATEMENT

The present study is distinctive in its isolation and identification of cellulase-producing lactic acid bacteria (LAB) from water hyacinth (*Eichhornia crassipes*), an abundant aquatic plant that has not yet been fully utilized. The distinguishing aspect of this study is the identification of LAB strains that exhibit cellulolytic activity, thereby providing a sustainable solution for cellulose degradation in the fermentation process. This research underscores the promise of water hyacinth as a bio-resource for biotechnology applications, which significantly contributes to water hyacinth fermentation for use as poultry feed and environmental sustainability.

AUTHOR'S CONTRIBUTION

The first author conducted the research and wrote the paper. The second author assisted with data analysis, and the third author contributed to the research and paper writing.

Generative AI and AI-assisted technology statement

This research employs generative AI and AI-assisted technologies to enhance the efficiency of data analysis and model optimization in the study of enzyme production by microorganisms. The integration of machine learning algorithms has enabled the accurate prediction of microbial behavior and enzyme activity, significantly enhancing the efficiency of experimental designs. Generative AI models further assist in generating hypotheses and suggesting novel experimental approaches based on previous data patterns, fostering innovation in the field of biotechnological research. This collaborative synergy between human expertise and AI technologies is paving the way for more efficient and sustainable advancements in microbial biotechnology and industrial applications.

Conflict of interest

The authors have declared no conflict of interest.

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