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Research Article

Harnessing *Bacillus subtilis* C6 as a bioprotective starter: A strategy for histamine mitigation and product quality in fermented *Parkia biglobosa*

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Abstract

The accumulation of histamine, a biogenic amine, in fermented foods poses health risk. Therefore, there is a need to reduce the quantity in food. Alongside existing interventions, starter cultures, capable of degrading and/or incapable of producing these amines have been proposed for mitigation. This study investigated the potential of *Bacillus* sp. starter culture, in reducing histamine formation in fermented locust beans (*Parkia biglobosa*). Isolated bacteria were screened for safe use as starter and decarboxylase activity. Molecular identification of the selected isolates was done using standard methods. Process optimization was estimated by spectrophotometric method. Tyramine content was quantified with high-performance liquid chromatography while proximate estimation was done on fermented product. Selected *Bacillus subtilis* C6 (MW 287212.1) was histidine decarboxylase-negative, non-hemolytic, DNase and gelatinase negative. The isolate showed optimal growth at pH 10 (0.712), 72 h (1.650), 35°C (1.648), 0.1% NaCl (0.298), sucrose (0.731) carbon source, beef extract (2.047) and $NaNO_3$ (1.746) as organic and inorganic nitrogen sources at a wavelength of 600 nm. Histamine content in unfermented locust bean was 6.35 mg/mL, while the spontaneously-fermented and starter-fermented samples had 6.81 mg/mL and 6.67 mg/mL, respectively. Fermentation with *Bacillus subtilis* C6 elevated the ash (1.01 ± 0.02%), fat (10.33 ± 0.02%), crude fibre (8.00 ± 0.01%), and carbohydrate (9.30 ± 0.07%) contents, with slight reduction in moisture (63.60 ± 0.02%) and protein (15.76 ± 0.05%), compared to the unfermented sample. These findings established *Bacillus subtilis* C6 as a safe and functional starter culture for reducing the histamine content of fermented locust beans, while retaining their overall nutritional properties, thus, proving its effectiveness in mitigating the accumulation of histamine.

1. Introduction

Biogenic amines are low molecular weight organic compounds that are generally produced as a result of the decarboxylation of amino acids. Exposure to excessive amounts in food can be harmful to health [1]. Histamine is a biogenic amine that plays an essential role in immune response, gastric acid secretion, and in neurotransmission. However, excessive histamine accumulation in food can lead to histamine intoxication, also known as scombroid poisoning. Symptoms of histamine poisoning include headaches, nausea, vomiting, diarrhea, skin flushing, and respiratory distress, particularly in individuals with low histamine metabolism due to deficiencies in the enzyme diamine oxidase (DAO) [2]. Histamine formation in food is largely attributed to the bacterial decarboxylation of histidine, catalyzed by histidine decarboxylase enzymes [3]. Several bacterial species, including *Lactobacillus*, *Enterobacter*, *Pseudomonas*, and

Bacillus, have been identified as histamine producers, particularly in fermented foods [4-6]. While fermentation enhances the nutritional quality, flavor, and preservation, it also increases the risk of histamine buildup, making food safety a major concern [7].

Fermented locust bean (*Parkia biglobosa*) is a widely consumed traditional condiment in West Africa, known as *iru*, *soumbala*, and *dawadawa*. It is an important source of protein, essential amino acids, and probiotics [8]. However, spontaneous fermentation involves uncontrolled microbial activity, allowing the growth of histamine-producing bacteria alongside beneficial fermentative organisms [9]. The absence of standardized fermentation can, be linked to variability in histamine levels, which poses food safety risks.

Unlike fermented fish and dairy products, which have strict regulatory limits for histamine, fermented African foods lack official safety standards for histamine content. Studies have shown that histamine levels in some fermented plant-based foods fluctuate, with some reaching elevated levels, as many factors contribute to their formation [10-12].

Studies have suggested that biogenic amine-degrading bacteria can serve as starter cultures to regulate fermentation and reduce histamine accumulation in fermented foods [13, 14]. Certain strains of *Bacillus* and *Lactobacillus* have demonstrated the ability to inhibit histamine-producing bacteria and enzymatically degrade histamine, leading to safer, low-histamine fermented products [15, 16]. Incorporating such functional starter cultures into locust bean fermentation could enhance food safety, consistency, and commercial viability. This study was designed to investigate the potential of *Bacillus* sp. as a fermentation starter, to migrate histamine accumulation in fermented locust bean (*Parkia biglobosa*).

2. Materials and methods

2.1. Sample collection and preparation

Locust bean (*Parkia biglobosa*) seeds were purchased from the Bodija market in Ibadan, Ibadan North Local Government Area. Oyo State, Nigeria. The seeds were aseptically placed in sterile Ziploc bags, properly labelled and transported immediately to the Microbial Physiology and Biochemistry Laboratory, Department of Microbiology, University of Ibadan, for further analysis. Ten (10) grams of healthy, cleaned seeds were weighed using an analytical weighing balance and homogenized in 100 mL of sterile peptone physiological saline solution. The mixture was allowed to ferment for 72 h.

2.2. Isolation and maintenance of pure cultures

Fermentation mixture (1 mL) was serially diluted in sterile distilled water to a dilution of 10^{-6}. Aliquots (0.1mL) from 10^{-4} and 10^{-6} diluents were collected using sterile pipettes and aseptically inoculated onto solidified Nutrient Agar (NA) (LABM, UK), de Man Rogosa and Sharpe (MRS) agar (LABM, UK) plates. Sterile glass rods were used to spread the culture evenly, and the plates were incubated at 37°C for 24–48 h. Distinct colonies were sub-cultured on fresh agar plates until pure cultures were obtained.

2.3. Screening for histidine decarboxylase activity

Isolates were screened for histidine decarboxylase activity in histidine-supplemented broth (0.5% L-histidine, 0.5% NaCl, 1% glucose, and 0.1% yeast extract, pH 6.5) [17, 18]. Isolates were inoculated into the respective broth and incubated at 37°C for 48 h. Change from yellow to purple indicated histamine production. However, the intensity of the purple coloration indicated the level of decarboxylase activity.

2.4. Safety assessment of potential starter culture

To evaluate safety, hemolytic test was conducted by inoculating a single streak of the isolate on a Blood Agar plate. The incubation was allowed at 35°C for 24 h, after which the surrounding area of the colony was assessed for clear zones of total or partial hemolysis [19]. The DNase test was conducted by inoculating a single streak of the isolate on DNase agar plate and then incubated at 35°C for 24 h. Hydrochloric acid (1N) was added, and a positive result was evident from a clear zone surrounding the colony, indicating DNA degradation [20]. A gelatinase test [21] was performed on the selected isolates. The isolate was inoculated into a tube containing nutrient gelatin medium and incubated at 35°C for 24 h. The tube was placed in a refrigerator for 10-15 minutes, after which gelatin liquefaction was observed.

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2.5. Identification of selected potential starter culture

The GeneJet genomic DNA purification kit (Thermo) was used for deoxyribonucleic acid (DNA) extraction, design of specific primers (5′- AGA GTT TGA TCM TGG CTC AG-3′ and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′) and sequencing. Polymerase chain reaction (PCR) was carried out using a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA). The cycle was performed at an initial denaturation of 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 50 °C for 60 s and 72 °C for 90 s; then cycle termination at 72 °C for 10 min with subsequent chilling at 4 °C. The PCR mixture (4 mL) was transferred onto a 1% agarose gel to examine the product against a 1 Kb plus ladder (Thermo). Genetic Analyzer 3130xl sequencer (Applied Biosystems) and sequencing kit (BigDye Terminator v3.1) were used to sequence the amplified fragments. The resultant sequences were compared with the 16S rRNA gene sequence database, using the NCBI Blast and further submitted to the Genbank for Accession number [22].

2.6. Optimization of growth parameters of selected starter.

2.6.1. Effect of incubation temperature, pH and NaCl concentration

The impact of incubation temperature, pH and NaCl concentration on the growth of the selected isolates were assessed at different temperatures (25 °C, 30 °C, 35 °C and 40 °C), pH (6, 7, 8, 9, and 10) and NaCl concentrations (1%, 2%, 3%, and 4%). Subsequent estimation was carried out with UV Spectrophotometer (Cecil CE 1011, Cambridge, England) at 600 nm [23].

2.6.2. *Effects of different nitrogen and carbon sources*

The impact of organic nitrogen (peptone, beef extract, and yeast extract) and carbon (glucose, maltose, and sucrose) sources on the growth of selected isolates were analysed. The estimation was carried out using UV Spectrophotometer (Cecil CE 1011, Cambridge, England) at 600 nm [23].

2.7. Fermentation of locust bean seeds with selected starter culture

The selected starter was cultured in a modified nutrient broth, using the optimal growth parameters. The broth was centrifuged (Himac CR21GII, Japan) after incubation, at 6000 rpm for 10 min. The cell pellet was washed with normal saline solution and used as an inoculum [24].

Parkia biglobosa seeds were sorted to remove debris and defective seeds, then washed in sterile distilled water and boiled at 100 °C to soften the seed coat. They were then cooled and de-hulled manually. The seeds were subsequently washed with sterile distilled water, drained, and divided into three batches: unfermented locust bean ULB (control 1); spontaneously-fermented locust bean FLB (control 2), and starter-fermented locust bean SFLB. All three samples (500 g each) were introduced into sterile fermentation containers, with only SFLB aseptically inoculated with the starter (1.0 mL of 0.5 MacFarland standard equivalent). Fermentation was allowed at ambient temperature for 72 h.

2.8. Histamine quantification in fermented samples

The histamine concentration in the samples, after fermentation, was measured using high performance liquid chromatography (HPLC) according to the modified method of Marcobal et al. [25]. A stock solution of 1 mg/mL histamine was prepared by diluting 100 mg of histamine (Sigma Aldrich) with 0.1 M HCl in a 100 mL volumetric flask. Calibration standard histamine solutions (0.8, 1.2, 1.6, 2.0, and 2.4 µM) were prepared from the stock solution by dilution with water. Each standard solution (0.5 mL) was transferred to a 15 mL tube. The reconstituted sample was prepared by addition of 0.5 mL water to a vial, which was then transferred to a 15 mL tube.

Chromatographic analyses were performed on a Shimadzu model, SCL-10AVP apparatus equipped with two LC-10AD analytical pumps connected to an SPD-M10AVP diode drag detector and an SIL-9A automatic injector controlled by a communication module SCL-10AVP. The analyses were performed on a Phenomenex R reverse phase C-18 column (Luna C-18 150 × 4.6 mm, 5 µm), and the data were analyzed using the Class-VP version 6.10 program. All samples were mixed with (1:1 vol./vol.) and filtered through a 0.45 µm filter (Acrodisc CRPTFE). The C18 column was kept on a column oven at 55°C and the autosampler thermostat was set at 4°C. Mobile phase solutions A and B were 0.1% TFA in water and 0.1% TFA in acetonitrile, respectively. The flow rate was 0.35 mL/min with 4 µL of sample injection (total

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50 min/run). The elution peaks were monitored at 260 nm.

2.9. Proximate analysis of fermented samples

Moisture, ash, crude fat, crude fibre, crude protein and carbohydrate contents in the fermented samples were estimated [26, 27].

2.10. Data analysis

Mean values and standard deviations of replicate data were determined and mean comparisons were conducted using Analysis of Variance (ANOVA), with significance set at $p < 0.05$.

3. Results

Eighteen pure bacterial isolates were obtained from the fermented locust bean samples. Six bacterial isolates exhibited histidine decarboxylase activity. Among all isolates, C6 showed the least activity, which was reflected in its faint purple colour reaction in the histidine decarboxylase test, as shown in Fig. 1.

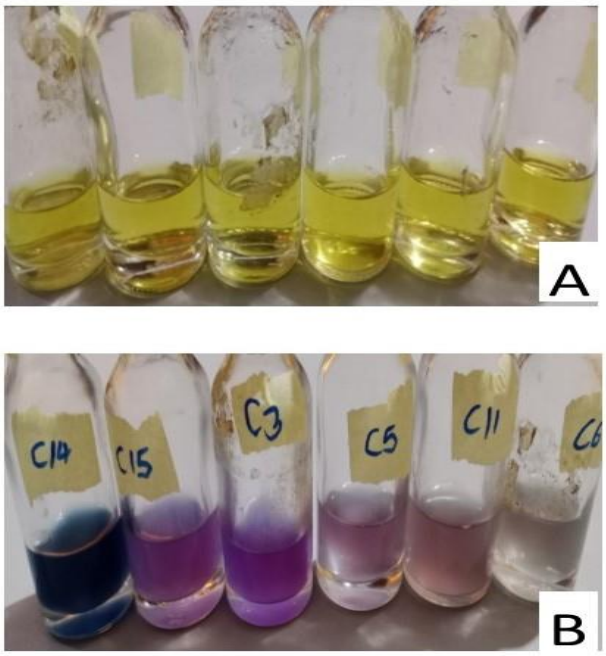

Figure 1. Qualitative determination of histidine decarboxylase activity at 0 h (A) and 96 h (B) of incubation.

As shown in Table 1, the safety evaluation of isolate C6 indicates that it can be regarded as conditionally safe, as it tested negative for both gelatinase and DNase assays and exhibited γ-haemolysis.

Table 2 outlines information from the National Centre for Biotechnology Information (NCBI) indicating that isolate C6 belonged to *Bacilliaceae*, genus *Bacillus* and species *subtilis* with a 99.69% match to the *Bacillus subtilis* strain IAM (NR 112116.2). The nucleotide sequence was deposited in the GenBank as *Bacillus subtilis* C6, with Accession number MW 287212.1 and the phylogenetic relationship with closely related organisms is shown in Fig. 2.

Table 1. Safety assessment of selected isolate C6 with least decarboxylase activity.

Safety evaluation test	Reaction
Hemolytic	ɣ
Gelatinase	Negative
DNase	Negative

The effects of temperature, pH, NaCl concentration, carbon, and nitrogen sources on the growth of *Bacillus subtilis* C6 are presented in Table 3**.** The optimal growth conditions for the isolate were at pH 10 (0.712), incubation period of 72 h (1.650), 35°C incubation temperature (1.648), with 0.1% NaCl concentration (0.298). Sucrose (0.731), beef extract (2.047) and $NaNO_3$ (1.746) were the optimal carbon, organic and inorganic nitrogen sources, respectively.

Histamine content, as calculated from the chromatogram in Figs. 3-5, revealed that unfermented soya bean had 6.35 ± 0.01 mg/mL, while the spontaneously-fermented and starter-fermented samples had 6.81 ± 0.02 mg/mL and 6.67 ± 0.01 mg/mL, respectively. This revealed that although, fermentation generally led to increase in histamine content, using *Bacillus subtilis* C6 as a starter culture showed a detectable reduction, compared to spontaneously-fermented soya bean.

Proximate analysis of the unfermented locust bean sample (LB), spontaneously-fermented (FLB) and starter-fermented (SFLB) samples, presented in Table 4, indicated that the moisture content was highest (66.56%) in LB. Fermentation reduced moisture availability in the other samples, ranging from 62.99% to 63.60%. LB had the highest protein content (18.21%), followed closely by FLB (17.82%), whereas SFLB (15.76%) had the. lowest. Ash content, which is indicative of mineral presence was slightly higher in FLB (1.03%) and SFLB (1.01%) compared to LB (0.83%), whereas the starter-fermented sample recorded the highest total carbohydrate (9.30%).

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Table 2. Molecular Identification of isolate C6.

Isolate code	Closely Related Specie (Accession number)	Percentage Similarity	Base Pair Analyzed	Identification (Accession number)
C6	*Bacillus subtilis* strain IAM (NR 112116.2)	99.69%	981bp	*Bacillus subtilis* C6 (MW 287212.1)

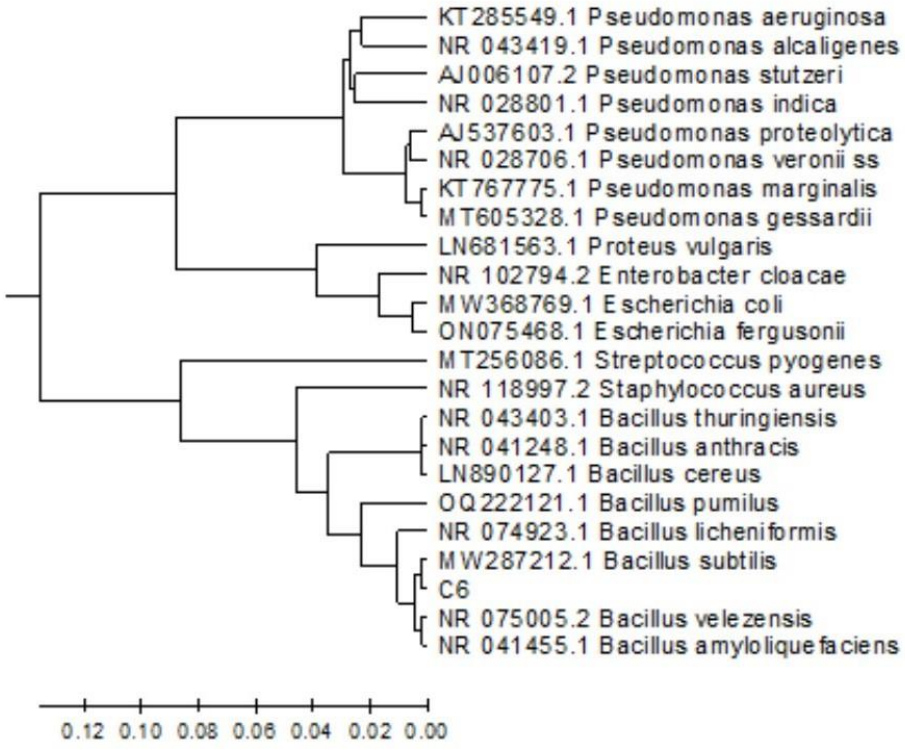

Figure 2. Phylogenetic relationship of *Bacillus subtilis* C6 with closely related strains.

Table 3. Optimal growth conditions for *Bacillus subtilis* C6.

Parameters	Optimal growth condition	Optical density (600 nm)	Least growth condition	Optical density (600 nm)
pH	10	0.712 ± 0.00^{a}	8	0.111 ± 0.00^{b}
Temperature	35°C	1.648 ± 0.01^{a}	25°C	0.755 ± 0.10^{b}
NaCl	0.1%	0.298 ± 0.01^{a}	0.4%	0.205 ± 0.07^{a}
Incubation period	72 hours	1.650 ± 0.06^{a}	24 hours	0.943 ± 0.00^{b}
Carbon source	Sucrose	0.731 ± 0.00^{a}	Lactose	0.349 ± 0.12^{a}
Organic N_2 source	Beef extract	2.047 ± 0.07^{a}	Yeast extract	0.464 ± 0.03^{b}
Inorganic N_2 source	$NaNO_3$	1.746 ± 0.00^{a}	NH_4Cl	0.297 ± 0.00^{b}

Values are Means ± Standard deviations of duplicate observations. Means with different superscript alphabet across each row are significantly different from each other at $p \leq 0.05$.

4. Discussion

The screening results showed that none of the 18 isolates from the fermented locust beans produced histamine on Niven's agar. This indicated that the isolates were not histamine-producing bacteria. It has been proposed that strain screening methods or strategies that not only facilitate degradation but also inhibit the producing of bacteria are important [28]. Hemolytic activity has been described as an important criterion for assessing the safety of bacteria of food interest [29]. Most of the isolates exhibited γ-hemolysis (non-hemolytic), indicating their non-pathogenicity and safety for food use. However, four isolates showed β-hemolysis, suggesting the presence

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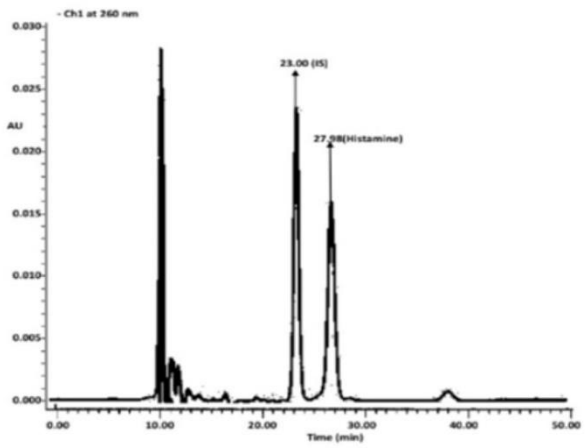

Figure 3. Chromatogram of unfermented locust bean (ULB) showing histamine (6.35 mg/mL) detection at 27:98 min.

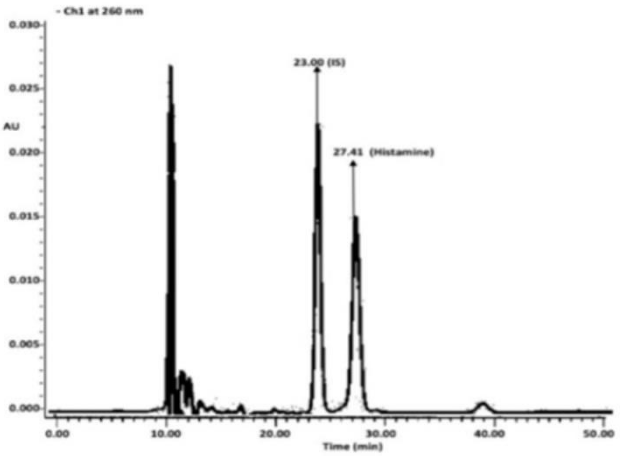

Figure 4. Chromatogram of spontaneously-fermented locust bean (FLB) showing histamine (6.81 mg/mL) detection at 27:41 min.

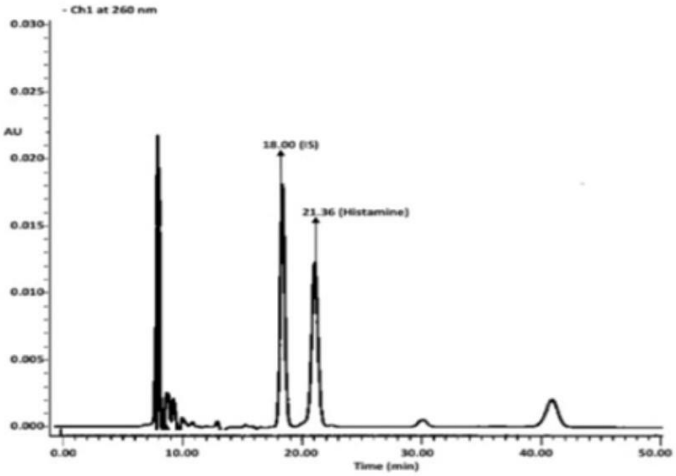

Figure 5. Chromatogram of starter-fermented (SFLB) locust bean showing histamine (6.67 mg/mL) detection at 23:36 min.

Table 4. Proximate composition of locust bean samples.

Samples	Moisture content (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Total carbohydrate (%)
LB	66.56±0.02^{a}	18.21±0.05^{a}	7.49±0.01^{c}	5.27±0.01^{c}	0.83±0.01^{ab}	6.92±0.05^{b}
FLB	62.99±0.04^{c}	17.82±0.06^{ab}	11.24±0.02^{a}	7.20±0.01^{ab}	1.03±0.02^{a}	6.92±0.07^{b}
SFLB	63.60±0.02^{bc}	15.76±0.05^{bc}	10.33±0.02^{ab}	8.00±0.01^{a}	1.01±0.02^{a}	9.30±0.07^{a}

Values are Means ± Standard deviations of duplicate observations. Means with different superscript alphabet down each column are significantly different from each other at $p \leq 0.05$.

of hemolysin that destroys red blood cells, making it unsafe. Occasionally, β-hemolytic *Bacillus* strains have been detected in fermented foods, highlighting the importance of controlled fermentation to reduce contamination risks. Several studies have reported the isolation of both γ and β-hemolytic *Bacillus* strains in foods [29-31]. DNase and gelatinase tests further confirmed that only a few isolates exhibited potential virulence factors, supporting the general safety of the fermentation process.

The cultural and biochemical characteristics of the bacterial isolates were consistent with the known profiles of *Bacillus* and *Lactobacillus* species. The presence of oxidase and catalase-positive isolates supports their aerobic and facultative anaerobic nature. The ability of some isolates to utilize citrate

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and exhibit urease activity further suggests metabolic diversity, which may influence the fermentation efficiency and product quality.

According to Lee et al. [9], histamine production in fermented foods is closely linked to the presence of bacteria with strong histidine decarboxylase activity, which can lead to the accumulation of histamine. The absence of this enzyme activity in the selected potential starter culture suggested that it is a safe candidate for fermentation without contributing to histamine formation. The variation in decarboxylase activity among the other isolates aligned with previous findings which reported that not all bacterial strains in fermented foods possess the enzymatic ability for biogenic amine degradation [6, 28, 32]. This reinforces the importance of selecting a suitable starter culture, to control histamine levels in fermented products. The results also indicated that spontaneous fermentation could involve strains with varying decarboxylase activity, potentially leading to inconsistent histamine levels in the final product.

The molecular identification of the selected isolate confirmed *Bacillus subtilis* C6 as the best candidate starter culture, with 99.69% homology to *Bacillus subtilis* strain IAM (NR 112116.2) based on database information available on the National Centre for Biotechnology Information (NCBI). *Bacillus* species, such as *Bacillus subtilis* and *Bacillus licheniformis* have been widely reported in locust bean fermentation although the microbial diversity in locust bean fermentation may be broader than reported values. Studies on the controlled fermentation of African locust bean (*Parkia biglobosa*) have focused on *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus licheniformis*, which are known for their proteolytic activity and ability to improve sensory properties [33, 34].

The highest growth rate of *Bacillus subtilis* C6 at 35°C, with a significantly lower growth at 20°C confirms that the strain thrives best at moderate temperatures, aligning with previous findings that reported *Bacillus species* growth at temperature ranges between 25–37 °C in fermented food systems [35-37]. The optimal pH was recorded at pH 10, which indicates that the strain would perform efficiently under alkaline conditions, making it suitable for alkaline-fermented foods like locust beans. Similar pH-dependent growth trends have been observed in *Bacillus subtilis*, a well-known starter culture for fermented legumes [35]. Optimal incubation up to 72 h suggested that extended fermentation enhances bacterial activity, leading to improved fermentation outcomes. A similar finding was reported with peak microbial activity at 72 h in legume fermentations [25]. Excessive salt concentration has been reported to inhibit the growth of *Bacillus* species [24]. *Bacillus subtilis* C6 was tolerant to 1% NaCl concentration, however, it did not thrive in a highly saline environment (4%), in the present study.

Histamine levels varied among the fermentation trials, with the lowest concentration observed in the starter-fermented locust bean sample, even though values obtained were less than 50 mg/kg, which is regarded as a No Observed Adverse Effect Level (NOAEL) in healthy individuals. However, for individuals with increased sensitivity i.e. histamine intolerance, 5 mg/kg is the threshold [2]. As such, the histamine concentrations in this study may likely cause harm to individuals with high sensitivity. However, it should be noted that controlled fermentation with a carefully selected starter culture limited histamine accumulation, possibly due to low decarboxylase activity as well as non-histamine producing ability of the culture. Some bacteria do not have amino decarboxylase activity, and can therefore act as good alternatives for reducing BAs formation, especially in fermented foods. They have been used as starter cultures for the fermentation of foods, thereby degrading the content of BA [13, 14]. The effect of starter utilization resulted in a 2% reduction in histamine content, compared to the spontaneously-fermented *iru.* A 27.7% histamine reduction has been previously reported in fish sauce, inoculated with *B. polymyxa D05* [15]. This disparity me be due to strain specificity among *Bacillus spp.* and the level of histidine decarboxylase activity.

Proximate analysis of the unfermented locust bean (LB), spontaneously-fermented (FLB) and starter-fermented (SFLB) samples showed clear effects of fermentation on moisture, macronutrients and ash. Moisture content was highest in the unfermented sample (LB = 66.56%) and was reduced by

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fermentation (FLB 62.99% and SFLB 63.60%), a pattern consistent with many fermentation processes, where microbial metabolic activity reduces free water. This trend aligns with recent reviews reporting reduced moisture content and improved shelf stability for many fermented legumes and condiments [38, 39].

The crude protein content was highest in unfermented locust bean (LB) (18.21%) but decreased progressively in FLB (17.82%) and SFLB (15.76%). This decline suggests proteolysis with the conversion of proteins into peptides and free amino acids that are metabolized by the starter strain, a trend also observed in previous studies on fermented legumes [28]. The breakdown of proteins into amino acids during fermentation contributes not only to flavor development but also increases the digestibility and bioavailability of peptides and free amino acids that are more readily absorbed by the body. Microbial hydrolysis of complex lipids into measurable crude fat fractions may be responsible for the increased crude fat content in the fermented samples. Recent studies on locust bean fermentation have confirmed that fat content can increase after fermentation and the magnitude depends on fermentation parameters and starter selection [39]. The higher fibre content in SFLB suggests either selective removal/metabolism of soluble components or concentration of insoluble fractions. This is nutritionally beneficial because higher fibre content is associated with improved gut health and glycemic modulation, which may increase the functional value of SFLB as a dietary condiment or ingredient [38]. Increase in ash, a proxy for mineral concentration, after fermentation from 0.83% to 1.01% and 1.03% suggests that fermentation may enhance mineral bioavailability. The total carbohydrate content was highest in the starter-fermented sample (9.30%) compared with LB and FLB (both 6.92%). This result reflects the breakdown of complex carbohydrates into measurable soluble sugars. Given that the protein content in SFLB is lower, a relative increase in the carbohydrate percentage is expected if the total solids shift [40].

5. Conclusions

Bacillus subtilis C6 (MW 287212.1) as a starter culture for locust bean fermentation influenced the histamine concentration and nutritional composition. The non-hemolytic, DNase and gelatinase-negative organism, had low histidine decarboxylase activity, which minimally converted histidine to histamine, thus, it is safe for use in food. The isolate played a role in limiting histamine accumulation, when used as starter culture during fermentation, which is beneficial for food safety. Proximate analysis revealed that fermentation with starter culture enhanced digestibility and shelf-life while optimization studies confirmed that *Bacillus subtilis* C6 grows best at 35°C, pH 10, 72 h of incubation, and 1% NaCl concentration, with sucrose, $NaNO_3$ and beef extract, being the best carbon and nitrogen sources. These parameters provide a foundation for improving fermentation conditions for scale-up. Overall, the controlled fermentation of locust bean with *Bacillus subtilis* C6 offers a promising approach to enhance the safety, nutritional quality, and standardization of fermented *Parkia biglobosa* products.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, K.A.O.; methodology, K.A.O., A.J.; validation, K.A.O.; formal analysis, K.A.O., A.J.; Investigation, A.J.; resources, K.A.O., A.J.; data curation, A.J.; writing – original draft preparation, K.A.O., A.J.; writing – review & editing, K.A.O.; supervision, K.A.O.; project administration, K.A.O.

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Availability of data and materials

All data will be made available on request according to the journal policy.

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Conflicts of interest

The authors declare no conflict of interest.

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