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Original Research

Enhancement of Cassava (*Manihot esculenta* Crantz) Retting Efficiency Using Pectinolytic and Cellulolytic *Streptomyces* sp. in the Traditional Starter Sta_96

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Abstract

Background: This study aimed to improve the cassava retting efficiency of starter Sta_96, a fermented cassava flour obtained after four days of fermentation, by incorporating bacteria of the genus *Streptomyces*. **Methods**: *Streptomyces* isolates were obtained from Sta_96, farm soils, and a cassava peel dump. The subsequent pectinolytic and cellulolytic activities, along with the associated ability to soften cassava roots, were evaluated. The most effective isolates were incorporated into Sta_96 at a concentration of 10^6 CFU/g, and the associated retting and detoxification efficiencies were assessed. **Results**: Of the 33 *Streptomyces* isolates recovered, 24 produced pectinases, with hydrolysis halos ranging from 11.0 ± 1.4 mm to 42.5 ± 3.5 mm. Sixteen of these displayed cellulase activity, with halos ranging from 16.0 ± 0.1 mm to 40.0 ± 0.1 mm. STF 16 showed the highest softening efficiency, achieving a retting time of 38.6 ± 0.4 h, and significantly improved the performance of Sta_96 by reducing the retting duration from 44.5 ± 0.6 h to 24.3 ± 0.4 h, representing a 45.39% reduction. STF 16 also demonstrated substantial detoxification efficiency (~85%). **Conclusions**: This is the first report demonstrating that *Streptomyces* species can act as efficient cassava retting agents. Incorporation of STF 16 into the Sta_96 starter markedly accelerated root softening to about 24 hours while ensuring complete elimination of cyanide. This approach represents a promising strategy for improving fermentation efficiency and product safety in cassava-processing industries.

Keywords: cassava; fermentation; *Streptomyces* sp.; pectinase; cellulase; cyanides

1. Introduction

Cassava is one of the most important tuberous roots cultivated in tropical regions, serving as the staple food for nearly 800 million people worldwide [1]. In Cameroon, this tuberous root is the primary source of calories [2], representing a valuable alternative to imported cereals such as rice, sorghum, and wheat [3]. Its high carbohydrate content and adaptability to marginal soils make cassava a critical crop for food security and rural livelihoods. Despite these benefits, roots from high-yielding varieties contain elevated levels of cyanogenic glycosides, mainly linamarin and lotaustralin [4]. Their enzymatic hydrolysis releases cyanide, which is associated with severe health disorders, including goiter, cretinism, ataxic neuropathy, and xerophthalmia [5]. Effective detoxification during processing is therefore essential, highlighting the critical role of cassava retting. Traditional retting practices used by smallholder farmers in Central and West Africa reduce cyanide levels while contributing to the characteristic sensory attributes of fermented cassava products [6,7]. However, fermentation remains slow, typically lasting 3–7 days, depending on environmental conditions, cassava variety, root maturity, and the composition of microbial communities [8]. Prolonged retting increases postharvest losses due to spoilage, limits production capacity, and constrains the revenue of processors—most of whom are women working in small-scale industries. Additionally, the lack of standardized processing conditions leads to variability in product safety and quality. To shorten fermentation time and improve product consistency, the use of defined microbial starters has been proposed [9]. Starters based on lactic acid bacteria, yeasts, and fungi have shown promise under controlled laboratory settings [10,11], yet often perform inconsistently in field conditions due to microbial competition and substrate heterogeneity. In response, the traditional starter Sta_96—comprising a naturally selected microbial consortium derived from pre-fermented cassava chips—has gained traction as a more robust option [12]. Sta_96 reduces retting time by approximately 50% while maintaining residual cyanide below recommended safety thresholds (20 mg/kg) [12]. Nevertheless, further improvement is necessary to meet increasing consumer demand and industrial requirements for rapid and reliable processing cycles. Enzymatic degradation of cassava root cell walls is essential for efficient retting, suggesting that enrichment of starter cultures with microbes capable of producing strong hy-

drolytic enzymes could further enhance fermentation performance. *Streptomyces* species are well-recognized soil bacteria known for secreting diverse extracellular enzymes, including cellulases, xylanases, proteases, and pectinases [13], which contribute to plant tissue softening and have wide applications in agriculture and food biotechnology [14]. Despite this enzymatic potential, the use of *Streptomyces* as direct retting agents for cassava has not yet been investigated, representing a significant knowledge gap and an untapped biotechnological opportunity. Therefore, the objective of this study is to evaluate whether incorporating pectinolytic and cellulolytic *Streptomyces* strains into the Sta_96 starter can enhance its retting efficiency. By improving cell wall degradation and potentially accelerating detoxification, this approach aims to standardize fermentation duration, ensure product safety, and promote industrial-scale cassava processing. Ultimately, such advancements could strengthen food security, reduce postharvest losses, and support the economic empowerment of local producers by increasing both the quality and availability of fermented cassava products in Central and West African markets.

2. Materials and Methods

2.1 Isolation of Streptomyces sp.

Streptomyces spp. were isolated following the protocol described by [15] from three distinct sources: the traditional starter Sta_96 produced in the laboratory according to [9], farm soils, and a cassava peel dump soil. For soil samples collection, 100 g of soil was collected from three different farms located in Yaoundé, Cameroon (3.848° N and 11.502° E). Soil samples were taken at a depth of 10–15 cm below the surface using sterile trowels to minimize contamination and ensure consistency. Sampling was conducted during the rainy season (April–May), when microbial activity is typically highest due to increased moisture and nutrient availability. At the time of collection, environmental conditions were recorded: average temperature ranged from 24–28 °C, relative humidity was between 75–85%, and the soil was moderately moist with visible organic matter. Samples were immediately placed in sterile plastic bags, labeled, and transported to the laboratory for processing within 24 hours to preserve microbial viability. The cassava peel dump sample was collected from a site with visible microbial colonization and was processed similarly to the soil samples. Once in the laboratory, samples were mixed with calcium carbonate (ACS reagent grade, ≥99.0% purity- Sigma-Aldrich) at a ratio of 1/10 (g/g) and kept in a dry, ventilated location for 48 h. Subsequently, 25 g of each sample was transferred into 225 mL of sterile physiological saline, vortexed for 5 min, and allowed to settle for an additional 5 min. The supernatants were serially diluted, and 0.1 mL of each dilution was spread onto Inorganic Salt Starch Agar (ISP4) previously poured into Petri dishes. Plates were incubated at 28 °C ± 2 for 7 days. After incubation, colonies exhibiting typical *Streptomyces* morphology (round and powdery, slightly embedded in agar, Gram-positive filamentous cells, catalase-positive) were presumptively identified as *Streptomyces* spp. These colonies underwent five successive subcultures to ensure purity, and culture purity was verified by Gram staining followed by microscopic examination, by confirming consistent colony morphology on solid medium, and by the absence of growth on non-selective contamination control plates. Following purity confirmation, biochemical characterization—including salt tolerance and sugar degradation—was systematically performed on all isolates (results for selected strains are presented in **Supplementary Table 2).** After this, these strains were then preserved in glycerol stocks (20%, v/v) at –80 °C and maintained on nutrient agar slants at 4 °C for short-term storage.

2.2 Evaluation of the Cellulolytic and Pectinolytic Activity of the Isolates

Cellulolytic and pectinolytic activities were assessed as described by [16] using mineral media supplemented with either Sodium-carboxymethyl cellulose (CMC 10 g/L, Sigma Aldrich CAS Number: 9004-32-4, Saint-Quentin-Fallavier, Isère, France) or pectin (10 g/L, Sigma Aldrich CAS Number: 9000-69-5, Saint-Quentin-Fallavier, Isère, France) as the sole carbon source. Isolates were first grown on ISP4 medium to obtain a confluent microbial lawn. Cylindrical plugs (6 mm diameter) were aseptically removed and placed onto CMC agar or pectin agar. Plates were incubated at 37 °C for 96 h, then flooded with 1% (w/v) potassium iodide solution and stained for 5 minutes. Clear halos surrounding the inoculum discs indicated extracellular cellulase or pectinase production. Hydrolysis halo diameters were measured using a caliper following the standard method described by [16]. All enzymatic activity tests were performed in triplicate for each isolate to ensure reproducibility. Only isolates exhibiting at least one of the enzymatic activities were retained for subsequent experiments. It is important to emphasize that, this study evaluates whole microbial retting performance rather than purified enzyme kinetics, reflecting real fermentation conditions where enzymatic cocktails, microbial succession, and matrix-substrate interactions determine efficacy.

2.3 Evaluation of the Cassava Roots Softening Capacity of the Isolates

Cassava roots from a 9-month-old bitter local variety (“Six-mois”) [17] selected for its prevalence in Cameroonian fermentation processes, highly cyanogenic glycoside content (400~655 mg HCN/kg fresh weight), and high starch content (70–74% dry matter basis), were peeled, washed, and cut into 1-cm-thick cylinders. Batches of 100 g were inoculated with *Streptomyces* isolates at 10^6 CFU/g of root tissue, submerged in 100 mL of tap water, and in-

cubated for natural fermentation. A non-inoculated batch was included as a negative control. Retting progression was monitored over 4 days. Every 3 h, six randomly selected cylinders from each batch were tested for firmness using a penetrometer. The softening level was expressed as the mean penetration depth (six measurements per cylinder) [14]. The penetration measurements were performed using the Penetrometer RPN10 Berlin model; the pressure was applied at a controlled rate of 10 mm/s to insure reproducibility and accuracy of the measurements. Penetration data obtained from each time point were compiled in Microsoft Excel 2016 and fitted using DM-fit software (https://browser.combase.cc/DMFit.aspx). The resulting sigmoid kinetics were modeled according to the equation described by [18], thereby, enabling calculation of the retting time as follows:

$$D_r = \text{Lag} + \left(\frac{L}{\mu}\right)$$

Here, D_r, Lag, L and μ are respectively the retting time (h), the time required to initiate fermentation (h), the critical penetrometry index (cm/h) and the softening speed (cm/h).

The percentage reduction of the retting time of each strain was calculated as follows:

$$\text{Percentage of retting time reduction (\%)} = \frac{\text{Retting time of strain}}{\text{Retting time of control}} \times 100$$

2.4 Improvement of the Retting Activity of the Sta_96 Starter

Isolates demonstrating the shortest retting times were selected for further testing. They were incorporated into cassava roots at a concentration of 10^6 CFU/g either individually or in combination with the traditional Sta_96 starter. The effectiveness of the enriched starters in accelerating retting was evaluated by comparing their retting duration with that of the non-enriched Sta_96 starter. Cassava roots were peeled, washed with tap water, and cut into 1-cm-thick cylinders. Batches of 100 g were prepared, and each selected isolate was mixed with Sta_96 starter at a load corresponding to 10^6 CFU/g of starter. The mixtures were then applied to each batch, except for the controls (spontaneous fermentation and Sta_96-only control), and submerged in 100 mL of tap water. Where relevant, isolates showing retting-enhancing properties were also tested in combination with the best-performing isolate to further improve the efficiency of the Sta_96 starter. All treatments were incubated under laboratory conditions (28 °C ± 2), and retting progression was monitored as described in Section 2.3.

2.5 Determination of Residual Cyanogen Content

The effect of *Streptomyces* enrichment on the detoxification activity of Sta_96 was assessed by quantifying the total cyanide content of the retted cassava paste using the method of [11]. Briefly, 0.1 g of fermented cassava paste was placed into screw-cap vials containing 1 mL of distilled water and a strip of picrate paper. Vials were sealed and incubated at 30 °C for 18 h to allow cyanide interaction with the picrate paper. Thereafter, the picrate papers were transferred into test tubes containing 5 mL of distilled water and boiled for 5 min. Following boiling, the strips were removed and the solution was allowed to cool to room temperature (28 °C ± 3) before further analysis. The absorbance of the cooled solution was measured at 510 nm using a UV-Vis spectrophotometer Jenway 7305 model (Fisher Scientific SAS, Illkirch Cedex, France). The blank control consisted of the boiled solution from a picrate paper strip that had not been exposed to cyanide to ensure accurate baseline correction. Total cyanide content (ppm) was calculated by multiplying the absorbance by 396, as described by [19]. Cyanide reduction efficiency was expressed as the ratio between the cyanide concentration in retted paste and that of fresh roots.

2.6 Statistical Analysis

All statistical analyses were performed using the IBM SPSS Statistics version 26.0 (IBM Corp., Chicago, IL, USA). The Duncan range test was applied to compare differences in pectinolytic and cellulolytic activities among *Streptomyces* isolates, retting times of Sta_96–*Streptomyces* combinations, and cyanide detoxification among treatments. Significant differences are represented by distinct letters in figures and tables. All data are expressed as arithmetic means ± standard deviations (Mean ± SD).

3. Results

3.1 Streptomyces Strains Obtained From Different Sources

To investigate the phenotypic characterization of all 33 *Streptomyces* isolates, the macroscopic and microscopic characteristics of strains from different sources were investigated. The summary reported in Table 1 shows that, among isolates originating from the starter Sta_96, 66.66% produced orange to brown pigments. In contrast, 58.82% of isolates from cassava field soils exhibited pigment production, predominantly pink. Additionally, 53.84% of isolates obtained from cassava peel soil produced pigments ranging from orange to brown.

3.2 Pectinolytic and Cellulolytic Activities of Streptomyces spp Isolates

Table 2 summarizes the pectinolytic and cellulolytic activities of the strains. A total of 24 out of 33 (72.7%) demonstrated pectinolytic activity, indicated by clear zones of pectin hydrolysis varying from 11.0 ± 1.4 mm (isolate

Table 1. Macroscopic and microscopic characteristics of *Streptomyces* strains from different sources.

Sources	Isolates	Mycelium color	Gram strain	Shape	Pigment coloration
Sta_96	STF 16	Cream	+	Filamentous	Orange
	STF 28	Wheat	+	Filamentous	Brown
	STF 33	Pink	+	Filamentous	None
Cassava cultivated soil	CCS 4	White	+	Filamentous	None
	CCS 8	Brown	+	Filamentous	None
	CCS 17	Wheat	+	Filamentous	None
	CCS 18	Cream	+	Filamentous	None
	CCS 20	Gray	+	Filamentous	None
	CCS 21	Brown	+	Filamentous	White
	CCS 6	Gray	+	Filamentous	Green
	CCS 9	White	+	Filamentous	None
	CCS 11	Cream	+	Filamentous	Pink
	CCS 22	Wheat	+	Filamentous	Wheat
	CCS 29	Brown	+	Filamentous	pink
	CCS 30	Brown	+	Filamentous	Brown
	CCS 13	Gray	+	Filamentous	Gris
	CCS 19	Pink	+	Filamentous	Pink
	CCS 23	Pink	+	Filamentous	None
	CCS 26	Green	+	Filamentous	Brown
	CCSl27	Gray	+	Filamentous	Pink
Cassava dumping soil	CDS 2	White	+	Filamentous	Orange
	CDS 7	Light-gray	+	Filamentous	None
	CDS 14	Gray	+	Filamentous	White
	CDS 15	Gray	+	Filamentous	None
	CDS 24	Blue	+	Filamentous	None
	CDS 25	Blue	+	Filamentous	Brown
	CDS 31	Gray	+	Filamentous	Orange
	CDS 32	Brown	+	Filamentous	Brown
	CDS 1	White	+	Filamentous	None
	CDS 3	Wheat	+	Filamentous	Orange
	CDS 5	White	+	Filamentous	None
	CDS 10	Orange	+	Filamentous	None
	CDS 12	Pink	+	Filamentous	Pink

STF, Strain from Sta_96 Flour; CCS, Strain from Cassava Soil; CDS, Strains from Cassava Dumping Soil.

CCS 3 from cultivated soil) to 42.5 $\pm$ 3.5 mm (isolate CDS 2 from cassava peel soil). Among these 24 pectinolytic isolates, 16 (66.7%) also produced cellulase, with hydrolysis halos ranging from 16.0 $\pm$ 0.1 mm (CDS 1) to 39.0 $\pm$ 0.1 mm (CCS 4). CCS 4 was the only cellulolytic isolate that lacked detectable pectinolytic activity.

3.3 Retting Capacity of Streptomyces spp. Isolates

The ability of the isolates to soften cassava roots presented in Fig. 1, was compared with that of the control, using rating time as evaluating evaluation metric. Retting time, ranged from 38.6 $\pm$ 0.4 h to 82.0 $\pm$ 10.4 h for STF 16 and CDS 1, as shown in Table 3 and Fig. 2, respectively. The 24 isolates can thus be classified into the following two main categories: isolates with the ability to soften cassava roots (*i.e.*, category 1) and those without (*i.e.*, category 2). Further, category 1, could subsequently subdivided into fast, moderately fast and slow, and respectively composed of isolates with the ability to reduce retting time by more than 50% (STF 16, CCS 4, CCS 20 and CDS 24); between 35 and 45% (CDS 31, CDS 15, CCS 21, CCS 6, STF 33 and CDS 2) and less than 30% (CDS 32, CCS 22 and CDS 12). Additionally, the duration of retting time under CDS 1 isolate did not differ significantly ($p < 0.05$) from that of the control (natural retting). Category 2 on the other hand includes isolates that do not have the capacity to soften cassava roots.

3.4 Activity of the Starter Enriched With Streptomyces spp. Isolates

Retting trials using Sta_96 enriched with the five most effective isolates (STF 16, CCS 4, CCS 20, CDS 24, and CDS 31), either individually or in combination, are presented in Table 4. Enrichment significantly reduced retting

Table 2. Pectinolytic and cellulolytic activities of *Streptomyces* strains isolated from different sources (n = 33).

Sources	Strains	Pectinolytic activity	cellulolytic activity
Sta_96	STF 16	30.0 ± 0.0^{ef}	29.5 ± 0.1^{def}
	STF 33	31.0 ± 1.4^{efg}	30.0 ± 0.0^{def}
	STF 28	27.5 ± 3.5^{de}	<LOD
Cassava cultivated soil	CCS 4	<LOD	39.0 ± 0.1^{hi}
	CCS 20	37.5 ± 3.5^{hijklm}	33.0 ± 0.1^{fgh}
	CCS 21	41.5 ± 0.7^{klm}	21.5 ± 0.2^{bc}
	CCS 6	30.0 ± 0.0^{ef}	22.0 ± 0.1^{bcd}
	CCS 22	42.5 ± 3.5^{lm}	25.0 ± 0.0^{cde}
	CCS 8	32.0 ± 0.0^{efgh}	20 ± 0.0^{ab}
	CCS 17	38.5 ± 2.1^{ijklm}	<LOD
	CCS 18	<LOD	<LOD
	CCS 9	<LOD	<LOD
	CCS 11	30.0 ± 0.0^{ef}	<LOD
	CCS 29	34.5 ± 0.7^{fghij}	<LOD
	CCS 30	11.0 ± 1.4^{b}	<LOD
	CCS 13	37.5 ± 3.5^{hijklm}	<LOD
	CCS 19	30.0 ± 0.0^{ef}	<LOD
	CCS 23	<LOD	<LOD
	CCS 26	<LOD	<LOD
	CCS 27	<LOD	<LOD
Cassava dumping soil	CDS 2	42.5 ± 3.5^{lm}	27.5 ± 0.3^{de}
	CDS 15	41 ± 1.4^{klm}	28.5 ± 0.2^{def}
	CDS 24	36.0 ± 0.0^{fghijk}	35.0 ± 0.0^{gh}
	CDS 31	34.0 ± 1.4^{fghi}	34.5 ± 0.0^{gh}
	CDS 32	38.0 ± 0.0^{hijklm}	25.0 ± 0.0^{cde}
	CDS 12	27.5 ± 3.5^{de}	17.5 ± 0.0^{ab}
	CDS 1	40.5 ± 2.1^{jklm}	16.0 ± 0.1^{a}
	CDS 7	<LOD	40 ± 0.0^{i}
	CDS 14	43 ± 2.8^{m}	35 ± 0.0^{gh}
	CDS 25	23 ± 2.1^{cd}	21.5 ± 0.21^{bc}
	CDS 3	19.5 ± 2.1^{c}	<LOD
	CDS 5	36.5 ± 2.1^{ghijkl}	32 ± 0.14^{efg}
	CDS 10	27.5 ± 10.6^{de}	<LOD

The results are presented as the means ± standard deviations. Values followed by different lowercase letters (a, b, c, d...) indicate significant differences at $p < 0.05$ via Duncan's multiple range test. LOD: Limit of Detection indicating below detection threshold (<6 mm). STF, Strain from Sta_96 Flour; CCS, Strain from Cassava Soil; CDS, Strains from Cassava Dumping Soil.

times ($p < 0.05$), from 44.5 ± 0.6 h (Sta_96 alone) to 24.3 ± 0.4 h (Sta_96 + STF 16), corresponding to reductions of 45.5%, 40.5%, 39.6%, 38.1%, and 31.9% for STF 16, CDS 31, CCS 4, CCS 20, and CDS 24, respectively. STF 16 was the most efficient strain in enhancing the activity of Sta_96. When combined with other isolates, a reduction in its performance was observed, except with CCS 20, which did not significantly alter STF 16 retting efficiency ($p < 0.05$).

3.5 Residual Total Cyanide Content

A study of the detoxification efficiency of cassava roots retted with the best isolate yielded the data shown in Fig. 3. Fresh cassava roots contained 471.5 ± 7.5 mg/kg of total cyanide. Following spontaneous fermentation, cyanide levels decreased by 96.64%, reaching 15.4 ± 2.5 mg/kg in the retted paste. When Sta_96 was added, either alone or enriched with STF 16, cyanide was reduced to non-detectable levels.

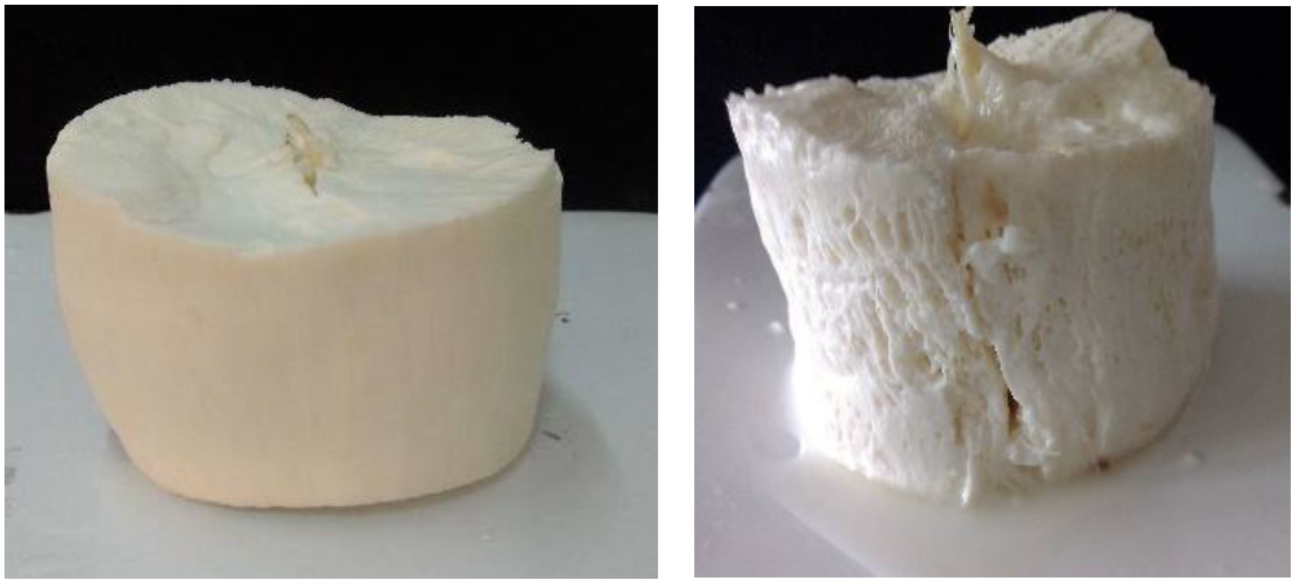

Fig. 1. Cassava roots: (left) non-retted cassava root and (right) retted-softened.

4. Discussion

Despite the socioeconomic importance of cassava and its role as a staple food in many tropical regions, processing cassava roots into edible and marketable products remains constrained by the long duration of retting, a key unit operation in fermentation. Although various strategies have been explored to shorten retting times, current solutions remain insufficient to support growing consumer and industrial demands. In this context, this study aimed to enhance the retting efficiency of a traditional starter (Sta_96), previously reported to reduce retting duration [9], through supplementation with selected *Streptomyces* strains.

A total of thirty-three *Streptomyces* isolates displaying diverse morpho-cultural and microscopic characteristics were collected from Sta_96 flour samples, field soil and a cassava peel dump soil. This diversity reinforces the well-documented ecological variability of the genus and its capacity to produce a broad spectrum of bioactive molecules, including hydrolytic enzymes [20–22]. Of the isolates obtained, three originated from the traditional starter, seventeen from cultivated soils, and thirteen from cassava peel disposal sites.

The unequal distribution likely reflects differences in nutrient availability and environmental selective pressures across sampling habitats. Functional screening showed that fourteen isolates demonstrated the ability to soften cassava roots, although to varying extents. This variability may be attributed to differences in microbial physiology, including enzyme synthesis potential, extracellular enzyme concentrations, and environmental tolerance factors such as temperature and pH [23]. Because each microorganism possesses specific optimal growth conditions, cultivating all isolates under identical physicochemical conditions may have favored those better adapted to the assay environment. Additionally, the initial microbial load influences the lag phase duration and, consequently, the overall fermentation kinetics. This may explain why addition of STF 16 to Sta_96 markedly accelerated softening, reducing retting to about 24 hours. One plausible explanation of the notably outperformance of strain STF compared to other isolates in accelerating root softening lies its enhanced enzyme production profile. STF 16 may synthesize higher quantities or more efficient forms of key enzymes—such as pectinases, cellulases, and hemicellulases—that directly degrade plant cell wall components integral to cassava tissue structure. Moreover, STF 16 might possess enzymes with greater catalytic efficiency or broader activity ranges under the assay conditions, enabling more rapid and effective hydrolysis of the matrix.

Evaluation of enzymatic activity revealed that all retting-efficient isolates produced both pectinases and cellulases to varying degrees, except isolate CCS 4, which exhibited only cellulolytic activity. The ability of CDS 4 to soften cassava roots despite lacking detectable pectinolytic activity suggests that softening is not exclusively mediated by pectinases, contrary to earlier assumptions [6,13]. Cellulose hydrolysis also contributes significantly to tissue disintegration, as cellulose is a key structural component involved in maintaining cell wall rigidity [24,25]. Furthermore, specific enzymes such as pectate lyases and endoglucanases play pivotal roles in plant cell wall degradation by depolymerizing complex pectin chains and hydrolyzing cellulose microfibrils, respectively [26]. The hypothesis of synergistic enzymatic yield is supported by studies demonstrating that microbial co-fermentation enhances substrate accessibility through sequential release of cell wall polymers, thereby optimizing overall retting performance [27,28]. Therefore, synergistic action of cellulases

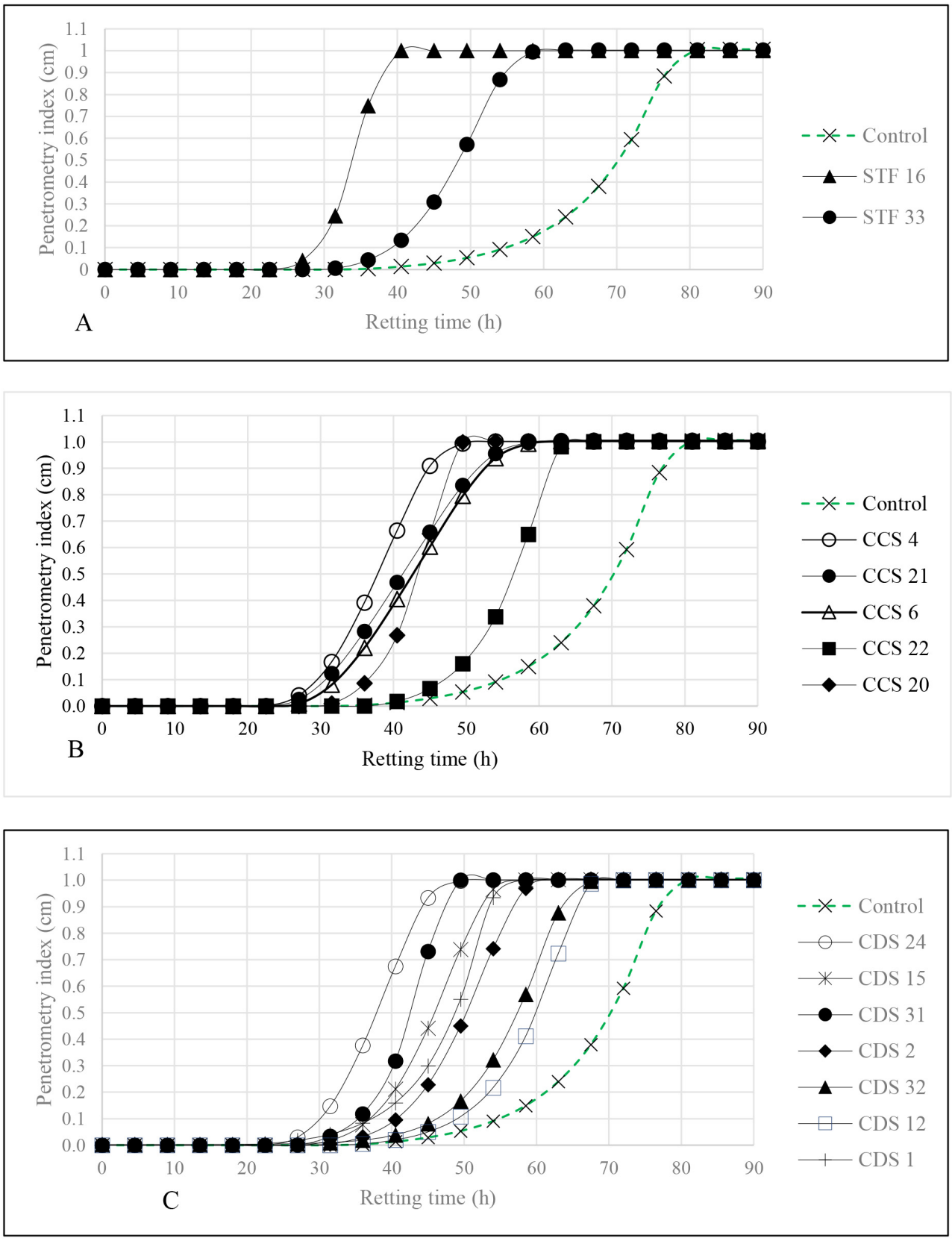

Fig. 2. Kinetics of cassava roots softening by: starter isolates (A), soil from a cassava field (B) and soil from cassava peels (C) Control defined as sterile water-inoculated group.

Table 3. Retting times for different *Streptomyces* spp. isolated from different sources (n = 33).

Sources	Strains	Retting time (h)	Retting time percentage Compared to control	Retting time of control (h)
Sta_96	STF 16	38.6 ± 0.4^{a}	57.4%	90.5 ± 16.6^{f}
	STF 33	55.3 ± 0.1^{bcd}	38.9%	
	STF 28	$\geq 90.5 \pm 16.6^{f}$	0.0%	
Cassava cultivated soil	CCS 4	45.2 ± 0.2^{ab}	50.1%	
	CCS 20	44.7 ± 0.2^{ab}	50.6%	
	CCS 21	52.9 ± 0.1^{bc}	41.6%	
	CCS 6	53.1 ± 0.1^{bc}	41.3%	
	CCS 22	67.3 ± 0.1^{de}	25.6%	
	CCS 8	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 17	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 18	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 9	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 11	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 29	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 30	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 13	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 19	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 23	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 26	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CCS 27	$\geq 90.5 \pm 16.6^{f}$	0.0%	
Cassava dumping soil	CDS 2	57.7 ± 0.3^{bcde}	36.3%	
	CDS 15	52.2 ± 0.1^{bc}	42.%	
	CDS 24	44.7 ± 0.2^{ab}	50.8%	
	CDS 31	47.9 ± 3.1^{ab}	47.1%	
	CDS 32	65.0 ± 0.3^{cde}	28.2%	
	CDS 12	69.2 ± 0.4^{e}	23.6%	
	CDS 1	82.0 ± 10.4^{f}	9.4%	
	CDS 7	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CDS 14	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CDS 25	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CDS 3	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CDS 5	$\geq 90.5 \pm 16.6^{f}$	0.0%	
	CDS 10	$\geq 90.5 \pm 16.6^{f}$	0.0%	

The results are presented as the means ± standard deviations. Values followed by different letters (a, b, c, d…) are significantly different ($p < 0.05$). STF, Strain from Sta_96 Flour; CCS, Strain from Cassava Soil; CDS, Strains from Cassava Dumping Soil.

and pectinases is likely necessary for optimal retting performance.

Furthermore, the reduction of total cyanide content to non-detectable levels following fermentation with Sta_96 alone or in combination with STF 16 may be explained by both the sensitivity of the quantification assay and the metabolic activity of microorganisms in the retting environment. Enzymatic degradation of structural polysaccharides during fermentation promotes cell wall breakdown, enabling contact between linamarin and endogenous linamarase, thus facilitating cyanogenic glycoside hydrolysis and detoxification of cassava tissues [17]. While this study primarily targeted high-cyanide, bitter cassava varieties—predominant in fermentation processes in Cameroon and across Africa—the detoxification mechanism achieved through enhanced microbial retting is expected to be transferable to sweet varieties, which, despite their lower cyanogenic potential and higher sugar content, may require re-validation of microbial consortium compatibility and process optimization. Industrial parameters for the composite inoculum (Sta_96 supplemented with STF16) are under optimization, and its good storage performance—e.g., 30 days at 4–8 °C—has been preliminarily observed in practical use.

5. Limitations

This study demonstrates the potential of pectinolytic and cellulolytic Streptomyces sp. to enhance cassava retting efficiency via the traditional starter Sta_96. Nevertheless, three key limitations temper these conclusions. First, it ignores microbial interactions within Sta_96 and lacks profiling of fermentation end-products. This could poten-

Table 4. Retting time and improvement frequencies of the starter Sta_96 in various combination (n = 10).

Combination	RT (h)	Percentage of improvement compared to Sta_96	Percentage of improvement compared to Control
Control	90.5 ± 16.6^{e}	0	0
Sta_96	44.5 ± 0.6^{d}	0	50.8%
Sta_96 + CCS 4	26.9 ± 0.8^{b}	39.6%	70.3%
Sta_96 + CDS 31	26.5 ± 0.0^{b}	40.5%	70.7%
Sta_96 + CCS 20	27.6 ± 0.0^{b}	38.1%	69.5%
Sta_96 + CDS 24	30.3 ± 0.8^{c}	31.9%	66.5%
Sta_96 + STF 16	24.3 ± 0.4^{a}	45.5%	73.2%
Sta_96 + CCS 4 + STF 16	30.2 ± 0.5^{c}	32.2%	66.6%
Sta_96 + CDS 31 + STF 16	26.2 ± 0.1^{b}	41.2%	71.1%
Sta_96 + CCS 20 + STF 16	24.5 ± 0.3^{a}	45.1%	72.9%
Sta_96 + CDS 24 + STF 16	26.8 ± 1.3^{b}	39.7%	70.4%

The results are presented as the means ± standard deviations. The values followed by different letters (a, b, c, d…) are significantly different ($p < 0.05$), RT: retting time; *Sta_96: traditional* starter. STF, Strain from Sta_96 Flour; CCS, Strain from Cassava Soil; CDS, Strains from Cassava Dumping Soil. Control defined as sterile water-inoculated group.

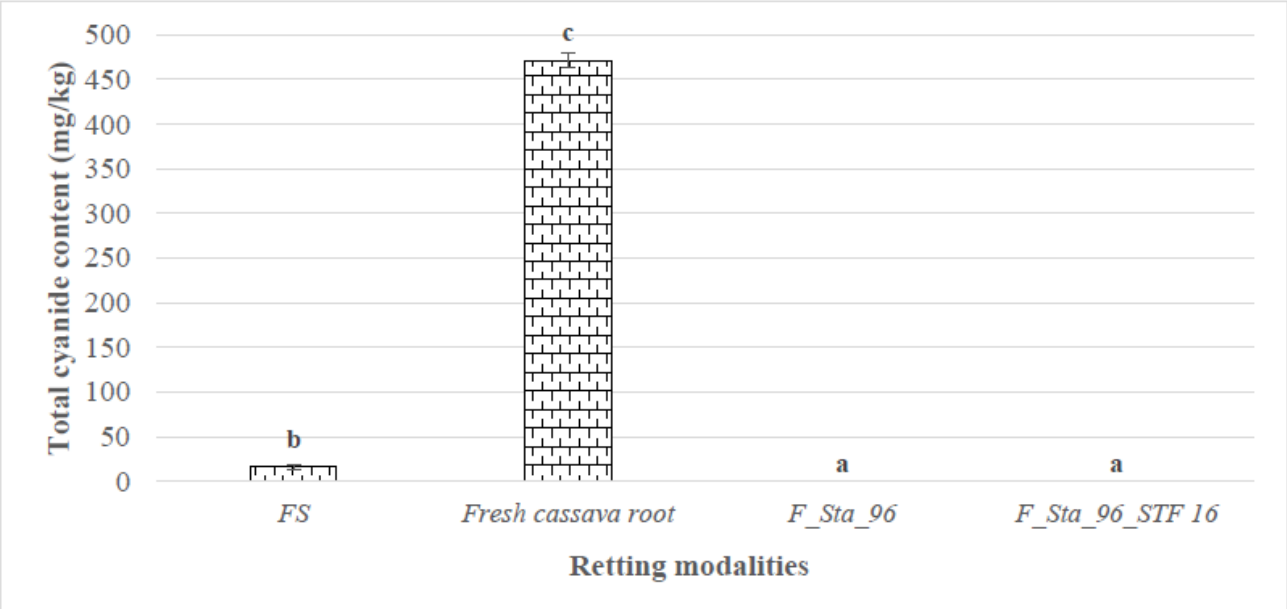

Fig. 3. Residual total cyanide content of fermented cassava. (FS) in the absence of the traditional starter Sta_96; (F_Sta_96) in the presence of the traditional starter Sta_96; (F_Sta_96_STF16) in the presence of Sta_96 with added Streptomyces. Sample size for each group is n = 3. Histograms represent the mean ± standard deviation. Averages followed by different letters (a, b, c) are significantly different at the $p < 0.05$ level. The detection limit for undetectable levels is <0.4 mg/kg.

tially overattribute the 85% detoxification to *Streptomyces* alone. Second, screening only 33 *Streptomyces* isolates from narrow sources risks selection bias and overlooks superior strains. Finally, the absence of long-term stability, sensory data, and economic comparisons weakens claims of a promising strategy for cassava processing. However, the results of this work remain highly worthy of interest.

6. Conclusions

In this study, we demonstrated that among the *Streptomyces* isolates tested, 24 exhibited pectinolytic activity, while 16 showed both cellulolytic and pectinolytic activities. Notably, the isolate STF 16 achieved the shortest retting time (38.6 ± 0.4 h), reducing processing time by 45.5%. When incorporated into the Sta_96 starter at 10^{6} CFU/g of cassava root, STF 16 effectively lowered

cyanide levels by 96.64%, reducing concentrations from 471.5 ± 7.5 mg/kg to undetectable levels in the retted cassava paste. STF_16 alone demonstrated substantial detoxification (~85% cyanide reduction in preliminary trials (data none shown), yet the composite Sta_96-STF16 treatment achieved superior efficacy (>96% reduction), highlighting synergistic microbial interactions that enhance enzymatic hydrolysis and linamarin degradation. These results indicate that the combination of STF 16 with Sta_96 can reduce cassava root softening time to about 24 hours. By addressing the major limitation of prolonged retting duration and facilitating more efficient cassava processing, the finding in the proposed study could be employed to pave the way to promising application at the industrial scale.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

BBDO and BAN performed the research and analyzed the data, NZN designed the research study and curated, analyzed and validated the data. EEF participated in experiments, provided help and advice on biochemistry analysis. BBDO, BAN and NZN wrote the manuscript. JJEN conceptualized and supervised the study. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBE45577.

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