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Research Article

Bacillus Species Consortium as a New Starter in the Optimization of Cassava Tuber Retting

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Abstract

This work aims to contribute to the elaboration of a stater consortium of performing *Bacillus* Spp capable of significantly standardizing the retting of cassava tubers. We monitored the retting, and the changes in multiple parameters, including pH, titrable acidity, bacterial level, texture profiles, volatile flavor compounds, and sensory quality. We proceeded with the isolation of bacteria of the genus *Bacillus* which were further characterized by classical microbiology techniques. In total, fifty-seven bacteria were obtained. Some of them were confirmed by FibE multiplex PCR. The identified organisms belonged to three *Bacillus* species: *B. subtilis*, *B. pumilus*, and *B. safensis*. Based on the Penetrometry Indices after 24, 48 and 72 hours (PI48) and enzymatic profiles, 24.5% (14) from Mokiki presented interesting fermentation potential, these were selected to realize seventy-seven *Bacillus* spp consortia in duo. 12% could easily soften cassava tubers after 24 and 48 hours (PI24≥6 and PI48≥8). These consortia allowed the retting of cassava with a shorter fermentation time of two days. *Bacillus* constituting the consortia also showed the ability to produce a range of biomolecules potentially involved in their fermentative capacity including Pectinase, Amylase, Protease and Biosurfactant.

Keywords

Bacillus, Consortium, Cassava Tuber, Retting, Starter, Fermentation, Consortium, *Bacillus*

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1. Introduction

Throughout Africa, traditional fermented foods are an important source of consumption and increasingly occupy a prime choice in the diet of populations [1, 2]. Fermented foods are produced using artisanal processes generally with raw materials needed for them to be grown and produced. In Republic of Congo, there is a lot of diversity of fermented foods [1], resulting from the metabolic activity orchestrated by various microorganisms (bacteria, yeasts, and molds) [2]. Among the fermented foods found in the Republic of Congo, we included those made from cassava tubers. This is highly consumed by the population from the twelve departments [3]. However, the difficulties linked to the main traditional slow process of production and the duration, and production capacity well below demand influences the cost of sale and consumption, costs linked to imports; which is far from meeting the needs of the population. Moreover, the westernization of our eating habits resulted in a decline in consumption of traditionally fermented healthy foods.

Indeed, to overcome these difficulties and satisfy the ever-increasing demand for fermented foods, in many Congolese homes, various projects relating to the valorization of fermented foods have been developed over the years to reduce the cost of importing foods. This necessarily involves the development of new processes and new starter bacteria to increase the productivity of fermented foods without altering and compromising the nutritional and hygienic qualities that are so appreciated. *Bacillus* Spp have been used as *starter for a long period of time* [3-8]. The commercially used *Bacillus* Spp in probiotics are *B. subtilis*, *B. polyfermenticus*, *B. clausii*, *B. cereus*, *B. coagulans*, *B. pumilus*, and *B. licheniformis* [2] and has demonstrated its ability to be used as a starter [9].

Cassava is the staple food in the Republic of Congo. Fermentation of cassava tubers is often carried out within four days [3]. High demand does not allow cassava producers and manufacturers to satisfy local consumers. It is necessary to create optimum conditions for rapid and qualitative fermentation to reduce the cost of imports and increase local production of cassava tubers based fermented foods to meet the needs of the Congolese population.

It is necessary to develop a starter consortium of microorganisms to improve the quality and reduce the production time of the retting process of cassava tubers; as the traditional method of manufacturing fermented foods from cassava tubers is a long, arduous work with low yield.

2. Methods

2.1. Isolation and Characterization of Isolates

10g of fermented tuber samples were aseptically transferred into sterile falcon tubes. Using sterile water (0.85%) the sample was homogenized and distributed into ten sterile falcontubes. Dilutions were done and the bacterial suspension was streaked on Mossel agar base medium supplemented with 4.2 mL of polymyxin B for promoting the growth of *Bacillus* species. Enumeration of colonies was done in triplicate on medium. The plates were incubated at 37°C for 24 h to 48°C in aerobic and anaerobic conditions in the incubator.

Standard biochemical and microbiological methods were used to characterize each isolate. The shape, size, and color of bacterial colonies on Mossel agar were studied post 24 hours of incubation. The morphological characterization was done using a light microscope (OPTIKA, Italie). Further, the Gram status of the bacterial isolates has been done using 3% of potassium hydroxide (KOH) [22] and a sporulation test was performed to determine the ability of isolates to form endospores. Oxidase and catalase tests were also conducted for all bacterial strains. Bacterial isolates were also tested for their ability to swarm, as most Bacillus species are motile.

2.2. Ability to Ferment Tubers

Three pieces of freshly cut cassava tubers around 2 cm^3 in size each weighing 12.30 g were added to the jars under aseptic conditions containing 100 mL of autoclaved distilled water 3 mL of overnight culture was inoculated into each jars. The optical density was taken before and after seeding. Physicochemical parameters such as softening, O.D. and pH were monitored using a graduated penetrometer, a spectrophotometer, and a pH meter. After 16 hours of incubation at room temperature, physicochemical parameters were read three times per day at a regular interval of 3 hours, during the 5 days period of fermentation. The isolates were selected based on the complete softening time of the pieces of cassava tubers, which must be less than 72 hours for which the values of the O.D. and the pH are closely associated. At the end of the selection, 3 classes of isolates were retained; namely the classes of isolates which fermented cassava tubers in 24, 48 and 72 hours.

Tuber penetrometer resistance is an effective and reliable method for evaluating the strength of cassava tubers. A mechanic penetrometer has been introduced in fermented tubers. The penetrometry Index (PI) has been assessed during the fermentation process. The values were established according to the texture of the fermented cassava tubers. A score of ten was associated with the tuber whose penetrometer was completely penetrated and broke the tubers. An index of seven to eight was associated with the tubers with penetration of the stem creating cracks. An index of five was associated with the tubers where the penetrometer entered but remained unbroken. Zero was associated with tubers with maximum resistance observed.

2.3. Enzymatic Activities

Bacillus strains are known for their ability to degrade ca-

sein and starch (should be written in the introduction and abstract to make the paper more attractive). Casein hemolysis and amylolytic activities were evaluated for all isolates as previously demonstrated by Kayath et al. 2019 [10]. For the casein hemolysis activity, 1 g of agarose was added to an Erlenmeyer flask containing 100 mL of PBS (Phosphate Buffer Saline). After heating the mixture until the agarose is completely dissolved, it was cooled off in a water bath at 50 ℃. 10 mL of skimmed milk was added. The mixture was poured into Petri dishes and allowed it to solidify. Wells were aseptically made using sterile tips. 50 µL of supernatant of the overnight culture was placed into the wells. After incubation at 37 ℃ for 24 hours, the halo formed around the colony was measured for its enzyme lytic activity. The tests were done in triplicates for each isolate.

On the other hand, for the amylolytic activity, a 24-hour old colony was deposited on the surface of LB agar containing 1% starch. Petri dishes were incubated at 37 ℃ for 24–72 hours. Iodine was used as a revelatory. The halo around the colony was measured [10].

2.4. Setting up Consortia

For each group of selected isolates, consortia of two isolates (duo) were developed.

To do this, 1.5 mL of each isolate from the previous overnight culture was added to 100 mL of distilled water, then 3 pieces of cassava tubers of 2 cm^3 in size and 12.30g of mass was added in different jars to start the fermentation. After 72 h, the duos which had softened the tubers significantly were selected taking into account the softening capacity of the cassava tubers in reduced time.

At the end of the selection, the selected consortia were divided into 3 groups; namely the consortia group having fermented the cassava tubers in 24 hours (D1), those in 48 hours (D2) and those in 72 hours (D3).

2.5. Biosurfactant Production Assay

The emulsifying activity of a biosurfactant is its capability of retaining the emulsion of hydrocarbons or oils in water. 5 mL of a washing cell and 5 mL of acellular supernatant of each isolate was poured into a test tube containing 5 mL (v/v) of gasoline or fuel. The mixture was vigorously shaken for 3 minutes using a vortex mixer (VELP Scientifica, Italy). The tubes were then incubated at room temperature for 24 hours. The height of the emulsion layer and the total height of the mixture were then measured. All the experiments were performed in triplicates and the emulsification index (E24%) was calculated using the standard formula E24% (He/Ht) 100, with He being the emulsion height, Ht the total height of the mixture, and E24% the emulsification percentage after 24 h.

2.6. Molecular Identification of *Bacillus* Isolates

Extraction and purification of genomic DNA of the isolates were performed according to the NucleoSpin Microbial DNA (Macherey-NAGEL) kit. DNA purity was assessed by electrophoresis on 1% agarose gel and by the ratio of optical densities of 260/280 nm. The genomic DNA obtained was used as a template for all PCR amplification experiments. To target amplify the genes of *B. amyloliquefaciens*, *B. subtilis*, *B. pumilus*, *B. licheniformis*, and *B. safensis*, a PCR multiplex reaction mix for the *fibE* gene encoding fibrinolytic enzyme was used [11].

3. Results

3.1. Screening of and Consortium with the Ability to Ferment Cassava Tubers

Isolation of colonies Zero was associated with tubers with maximum resistance observed from Mossel medium supplemented with polymyxin B made it possible to enrich *Bacillus* isolates presenting different phenotypes from the samples used. Following this, only colonies having a yellowish appearance on Mossel medium, considered as *Bacillus* spp. wereused for the subsequent studies..

A total of fifty-seven (57) isolates were obtained from the different samples of fermented foods and beverages, including fourteen (14) isolates from ginger wine, twenty-five (25) from Mokiki, seven (7) isolates from Ntoba mbodi and eleven (11) isolates from palm wine (Table 1).

Table 1. *Isolation of Bacillus spp. isolates. in Mossel.*

Samples	Ginger Wine	*Mokiki*	*Ntoba mbodi*	Palm Wine
Isolates	14	25	7	11
Total	57			

The characteristics highlighted by the fifty-seven (57) isolates obtained after purification (Table 1), shows that all the isolates presented are Gram-positive, Catalase-positive, rod-shaped cells capable of developing endospores, under conditions of environmental distress. More or less significant variations were seen depending on the case concerning size, shape, contours, pigmentation, arrangement and mobility.

3.2. Evaluation of the Fermentative Capacity of Isolates

The controlled fermentation of the fifty-seven (57) isolates in the presence of cassava tubers for five (5) days, monitor-

ing parameters such as O.D., pH, and PI, provided the data shown in the Table 2. From these data, out of fifty-seven (57) isolates, 24.5% (14) from Mokiki isolates were selected after 48 hours. No isolates were obtained from Ginger Wine, Mokiki, Ntoba mbodi, or Palm Wine. The MK3, MK9, MK11, MK13, MK14, MK16, MK19, MK21, MK24 and MK25 strains presented penetrometry indices after 48h (PI48) above eight (PI48≥8), i.e. a percentage of 70% among the fourteen isolated from Mokiki (Table 2). These isolates were then randomly chosen and designed by the consortia. It is important to note that beyond 72 hours, the isolates were able to fully ferment cassava tubers with indices greater than eight (PI72≥8) except for MK26.

Table 2. *Evaluation of the fermentative capacity of isolates.*

Origin of isolates	Isolates	Softening Test													
		0h		Group I 24 h			Group II 48 h			Group III 72 h			Group IV 96 h			Results
		D.O	pH	D.O	pH	PI	D.O	pH	PI	D.O	pH	PI	D.O	pH	PI	
Mokiki	MK3	0,13	6	0,664	6	0	0,63	6	8	0,742	5	10	1,041	5	10	+
	MK9	0,822	6	0,691	6	0	0,637	6	10	0,633	6	10	0,788	5	10	+
	MK10	0,5	6	0,488	6	0	0,539	6	0	0,562	6	8	0,796	6	8	+
	MK11	0,42	6	0,406	6	0	0,759	6	10	0,7	6	10	0,941	6	10	+
	MK12	0,254	6	0,22	6	0	0,773	6	0	0,787	6	10	0,62	6	10	+
	MK13	0,88	6	0,385	6	0	0,691	6	8	0,697	6	8	0,702	6	8	+
	MK14	0,858	6	0,198	6	0	0,568	5	8	0,82	5	8	1,36	5	8	+
	MK15	0,958	6	0,226	6	0	0,641	6	0	0,56	6	8	0,772	5	8	+
	MK16	0,186	6	0,233	6	0	0,855	6	8	0,793	6	8	0,869	6	8	+
	MK19	0,915	6	0,294	6	0	0,52	6	8	1,169	5	8	0,653	5	8	+
	MK21	0,458	6	0,127	6	0	0,567	6	10	0,401	6	10	1,116	6	10	+
	MK24	0,829	6	0,178	6	0	0,572	6	10	0,76	6	10	0,596	5	10	+
	MK25	0,651	6	0,095	6	0	0,789	6	8	0,587	6	8	0,477	6	8	+
	MK27	/	6	0,127	6	0	0,263	6	0	0,259	6	0	0,458	5	5	+

3.3. The Ability of Isolates to Produce Cellulase, Protease, Amylase and Biosurfactants

MK3, MK9, MK11, MK13, MK14, MK16, MK19, MK21, MK24 and MK25 isolates exhibiting a good penetrometer index after 48h (PI48≥8) were subjected to cellulolytic, amylolytic, proteolytic tests and their capacity to secrete biosurfactants. All five (5) isolates were positive for enzymatic activities, the lysis percentages of the different isolates after 48h (PA48, AA48 and CA48) are illustrated below as well as their EI24 (Table 3).

Table 3. *Lysis percentages of Bacillus sp. isolate. for their cellulolytic (CA48), amylolytic (AA48), proteolytic activities (PA48) and their ability to secrete biosurfactants (E24).*

	PA48 Diameter (cm)	CA48 Percentage (%)	AA48 Percentage (%)	EI24 Percentage (%)
MK3	2.3±0.5	54.7%	60%	96%
MK9	2.2±0.1	76.2%	57%	65%

	PA48 Diameter (cm)	CA48 Percentage (%)	AA48 Percentage (%)	EI24 Percentage (%)
MK11	2.3±0.1	86.2%	60%	70%
MK13	2.1±0.1	35.5%	32%	65%
MK14	1.6±0.5	14.7%	47%	80%
MK16	1.8±0.5	54.3%	67%	100%
MK19	2.8±0.5	60.2%	67%	100%
MK21	2.3±0.2	55%	44%	100%
MK24	1.7±0.4	62%	63,15%	100%
MK25	1.8±0.4	67%	65,15%	80%

3.4. Molecular Identification of Isolates

MK3, MK9, MK14, MK21 and MK24 isolates present better profiles based on the penetrometry index from amplifications carried out using multiplex PCR of fibE gene primers specific to the species *B. subtillis*, *B. pumilus*, and *B. safensis*. The following table shows the results obtained after multiplex PCR. MK3 as *Bacillus subtilis* MK9 and MK21 were associated with *Bacillus pumilus* and MK24 as *Bacillus safensis*.

Table 4. *Multiplex PCR for isolate identifications.*

Isolates	Amplification of different genes		
	fib*E-Bp for *Bacillus pumilus	***fib*E-Bsa for *Bacillus safensis***	***fib*E-Bs *Bacillus subtilis***
	Bp.id.Ma-F Bp.id.Ma-R	**Bsa.id.Ma-F Bsa id.Ma-R**	**BS.id.Ma-F BS.id.Ma-R**
MK3	-	-	+
MK9	+	-	-
MK14	-	-	-
MK21	+	-	-
MK24	-	+	-

3.5. Experimentation of Consortia

As indicated in the methodology, the isolates selected at the end of the simplex fermentation at occurrence thirteen (13), made it possible to develop duet consortia by highlighting the aforementioned parameters over three (3) days, for a total of over seventy-seven (77) consortia (duo) tested. Indeed, the selection of consortia was made mainly based on the observation of the PI which must be greater than or equal to 5 as a function of time (h); to which we have also associated the corresponding D.O. and pH values.

Although presenting Penetrometry indices equal to zero after 24 hours, the isolates of group I were constituted in consortiums C1, C2, C3 and C4 to see if consortia could complement each other. Surprisingly we showed that this consortia could ferment the cassava tubers after 24 hours (Table 5).

Table 5. *Selected Group I consortium of Bacillus spp. at the end of controlled fermentation after 24 hours (O.D. Optical density, pH: potential hydrogen, PI: Penetropetry Indices).*

Consortia	Isolates	Softening Test					
		0h			24h		
		D.O	pH	PI	D.O	pH	PI
C1	MK3 MK14	0,158	6	0	0,379	5	5
C2	MK3 MK24	0,18	6	0	0,502	6	5
C3	MK9 MK14	0,117	6	0	0,157	6	3
C4	MK9 MK24	0,152	6	0	0,203	5	8

The group II isolates (PI48) were also formed into a consortium named from C4 to C33. The results show that the consortia could ferment the cassava tubers after 48 hours. 33% of consortia including C4, C8, C9, C13, C14, C20, C22, C25, C26 and C28 with penetrometry indices equal to 10 (PI48=10), (Table 6). 13% of isolates including C4, C8, C13 and C31 consortia were able to ferment cassava tubers only after 24 hours (PI=5) (Table 6).

Table 6. *Selected Group II consortium of Bacillus spp. at the end of controlled fermentation after 48 hours (O.D. Optical density, pH: potential hydrogen, PI: Penetrometry Indices).*

Consortia CODE	Isolates	Softening Test								
		24h			48h			72h		
		Do	pH	PI	Do	pH	PI	Do	pH	PI
C4	MK3 MK11	0,266	6	5	0,483	5	10	0,565	5	10
C5	MK3 MK13	0,233	6	0	0,345	5	5	0,486	5	5
C6	MK3 MK16	0,423	6	0	0,469	5	8	0,649	5	8
C7	MK3 MK21	0,25	6	0	0,258	6	0	0,395	6	8
C8	MK3 MK25	0,338	6	5	0,448	6	10	0,714	5	10
C9	MK3 MK10	0,394	6	0	0,452	6	10	0,547	5	10
C10	MK3 MK12	0,444	6	0	0,276	5	5	0,471	5	5
C11	MK9 MK13	0,146	6	0	0,304	5	5	0,297	5	5

Consortia CODE	Isolates	Softening Test								
		24h			48h			72h		
		Do	pH	PI	Do	pH	PI	Do	pH	PI
C12	MK9 MK16	0,168	6	0	0,359	5	8	0,471	5	8
C13	MK9 MK21	0,186	6	5	0,33	6	10	0,34	5	10
C14	MK9 MK25	0,177	6	0	0,499	5	10	0,456	6	10
C15	MK9 MK10	0,201	6	0	0,349	5	5	1,245	5	5
C16	MK9 MK12	0,151	6	0	0,261	5	5	0,285	5	5
C17	MK11 MK13	0,225	6	0	0,477	6	5	0,533	6	5
C18	MK11 MK14	0,179	6	0	0,57	6	5	0,643	6	5
C19	MK11 MK16	0,244	6	0	0,528	6	5	0,672	6	5
C20	MK11 MK20	0,254	6	0	0,566	6	10	0,591	6	10
C21	MK11 MK24	0,282	6	0	0,637	6	8	0,498	6	8
C22	MK11 MK25	0,215	6	0	0,265	6	10	0,541	6	10
C23	MK11 MK10	0,293	6	0	0,641	6	8	0,803	6	8
C24	MK11 MK12	0,304	6	0	0,501	6	5	0,755	6	5
C25	MK11 MK15	0,242	6	0	0,561	6	10	0,533	6	10
C26	MK13 MK14	0,268	6	0	0,606	6	10	0,588	6	10
C27	MK13 MK21	0,151	6	0	0,414	6	5	0,63	6	5
C28	MK13 MK25	0,226	6	0	0,447	6	10	0,58	6	10
C29	MK13 MK10	0,291	6	0	0,432	6	5	0,668	6	5
C30	MK13 MK12	0,268	6	0	0,335	6	5	0,439	5	5
C31	MK13	0,19	6	5	0,48	6	10	0,451	6	8

Consortia CODE	Isolates	Softening Test								
		24h			48h			72h		
		Do	pH	PI	Do	pH	PI	Do	pH	PI
C32	MK15 MK14 MK16	0,193	6	0	0,494	6	8	0,544	6	8
C33	MK14 MK25	0,256	6	0	0,681	6	5	0,573	6	5

These isolates were not able to soften the cassava tubers after 24 and 48 hours. Penetrometry indices were observed after 72 hours. These did not represent a good choice for the future of work (Table 7).

Table 7. *Selected Group III consortium of Bacillus spp. at the end of controlled fermentation after 72 hours (O.D. Optical density, pH: potential hydrogen, PI: Penetropetry Indices) based on the table.*

Consortia CODE	Isolates	Softening Test								
		24h			48h			72h		
		DO	pH	PI	Do	pH	PI	Do	pH	PI
C33	MK3 MK21	0,25	6	0	0,258	6	0	0,395	6	8
C34	MK9 MK19	0,148	6	0	0,435	5	0	0,46	5	8
C35	MK11 MK21	0,153	6	0	0,446	6	0	0,407	6	8
C36	MK13 MK16	0,235	6	0	0,489	6	0	0,751	6	5
C37	MK13 MK24	0,233	6	0	0,439	6	0	0,407	6	10
C38	MK14 MK19	0,222	6	0	0,658	6	0	0,547	6	8
C39	MK14 MK21	0,22	6	0	0,518	6	0	0,634	6	5
C40	MK14 MK24	0,282	6	0	0,648	6	0	0,668	6	8
C41	MK14 MK10	0,186	6	0	0,533	6	0	0,342	6	5
C42	MK14 MK12	0,21	6	0	0,551	6	0	0,418	6	5
C43	MK14 MK15	0,214	6	0	0,507	6	0	0,346	6	8

Based on the results obtained, 85 consortia were tested based on the penetrometry indices. 12% (10/85) (C43, C44, C45, C46, C47, C48, C49, C50, C51 and C52) could easily soften cassava tubers after 24 and 48 hours (Table 8).

Table 8. *Selected consortium of Bacillus spp. at the end of controlled fermentation after (O.D. Optical density, pH: potential hydrogen, PI: Penetropetry Indices).*

Consortia CODE	Isolates	Softening Test								
		24h			30			48h		
		DO	pH	PI	DO	pH	PI	DO	pH	PI
C43	MK9 MK11	0,155	6	8	0,25	6	10	0,395	6	10
C44	MK9 MK12	0,148	6	7	0,42	5	8	0,46	5	8
C45	MK9 MK21	0,153	6	8	0,406	6	10	0,407	6	10
C46	MK9 MK24	0,135	6	7	0,489	6	10	0,751	6	10
C47	MK11 MK12	0,133	6	6	0,409	6	8	0,407	6	8
C48	MK11 MK21	0,122	6	7	0,628	6	10	0,547	6	10
C49	MK11 MK24	0,22	6	8	0,508	6	10	0,634	6	10
C50	MK12 MK21	0,182	6	7	0,608	6	10	0,668	6	10
C51	MK12 MK21	0,186	6	8	0,503	6	08	0,342	6	08
C52	MK21 MK24	0,121	6	8	0,501	6	10	0,418	6	10

4. Discussion

This work aims to develop a consortium based on *Bacillus* to show in vitro the microbiological and biotechnological interest of bacteria of the *Bacillus* genus in the fermentation and optimization of retting of cassava tubers.

In this work, we first assumed that the *Bacillus* genus is capable of constituting a starter culture for the retting of cassava tubers. This led us to isolate these bacteria from fermented foods [12-14].

We isolated a total of fifty-seven (57) bacterial isolates of the Bacillus genus. Only bacteria isolated from Mokiki, which is a fermented cassava product, presented interesting penetrometry indices. Mokiki is a fermented cassava food from the Republic of Congo made and is from red tubers. We could therefore infer that these bacteria coming from this environment have already acquired skills to easily ferment the tubers [15].

Isolates exhibiting good fermentation and enzymatic profiles were the subjects of molecular identification by using direct PCR amplification of the fibE gene. In total, four isolates were identified, two as *B. pumilus*, one as *B. safensis* and the other as *B. subtilis*. In sum, a consortium of *Bacillus* strains including species of the genus *Bacillus pumilus*, *Bacillus safensis* and *B. subtilis* could be used to trigger fermentation and retting of cassava.

We further showed that these isolates were capable of secreting enzymes such as amylases, cellulases and proteases

as has already been demonstrated by the work in our laboratory [16]. Production of alpha-amylase under solid state fermentation by *Bacillus subtilis* CM3 has been showed using cassava fibrous residue [17]. In addition, exo-polygalacturonase production by Bacillus subtilis CM5 in solid-state fermentation has also been demonstrated [18].

The *Bacillus* genus is known for its ability to produce extracellular enzymes such as amylases [19], pectinases [18, 20, 21], cellulases, proteases and many other biomolecules of interest [22-28]. We have shown in this work that isolates of Bacillus spp. selected are endowed with these enzymatic activities among other proteolytic, amylolytic and cellulolytic activity. For each test performed, the isolates were positive with lysis diameters ranging from 1.6 to 2.2 cm showing the proteolytic activity, for cellulose activity the lysis percentage ranges from 14.28 to 76 %, and from 46.89- 63.15% for amylolytic activity. Indeed, these enzymatic activities such as proteases and amylases induce the degradation of starch (the main carbohydrate compound present in cassava tubers), thuspromoting the processing of certain foods [18]. Likewise, cellulase would induce cell lysis by degradation of the pectocellulosic wall and promote the release and making available of biomolecules [25, 29]. Also, we showed that isolates of *Bacillus* sp. are capable of emulsifying hydrocarbons such as gasoline, this has been tested by obtaining an emulsion index of 96%. This not only explains the absence of biosurfactants in cassava tubers before fermentation and their presence in post fermentation but also attests that isolates of the Bacillus genus capable of secreting biosurfactants [16]. In addition, this production of biomolecules brings significant added value, significant both biotechnological and food, which could be said to justify the organoleptic quality of the fermented products which become improved.

Bacillus species such as *Bacillus subtilis*, *Bacillus coagulans* GBI-30, *Bacillus polymyxa*, *Bacillus pumilus* and *Bacillus amyloliquefaciens* are widely used to produce fermented foods in Asian and West African countries [4-8, 30, 31]. These were determined as the best starter combination because of rapid growth, high amylolytic and proteolytic activities, high levels of polyglutamic acid production [6]. However, it should be noted a few studies have tested the capacity of these bacteria to ferment cassava tubers in consortium and above all that biodiversity in the wild implies the presence of several bacterial species [6]. The consortiums tested as a duo were able to reduce the fermentation time. Cassava tubers could be fermented in just two days as evidenced by penetrometry indices (PI48). Our starter consists of bacteria of the Bacillus genus isolated in their biotope which is represented here by Mokiki.

This work added value to the search for identifying starters to optimize the fermentation of foods in Africa. Fermentation could represent an incredible future in the field of nutrition in Africa.

5. Conclusion

With the aim of contributing to the development of a highly efficient consortium of bacteria of the genus Bacillus in order to reduce the retting time of cassava, bacteria of the genus Bacillus obtained from local fermented foods (palm wine, wine ginger, Ntoba mbodi, Mokiki) were characterized. These bacteria showed the ability to ferment cassava tubers, alone and then in consortium in less time. Likewise, these isolates have the capacity to produce biomolecules that can play a role in fermentation, including biosurfactants, proteases, cellulases and amylases. In this sense, this work made it possible to obtain thirteen (13) isolates of the Bacillus genus capable of softening cassava tubers in less than 3 days. The Bacillus genus consortia were also capable of ensuring the softening of cassava tubers. in two days.

Abbreviations

PI Penetrometry Indices

Author Contributions

Josabeth Ickofa: Investigation, Resources, Writing – original draft

Christian Aimé Kayath: Methodology, Supervision, Validation, Writing – review & editing

Jean Mathurin Nzikou: Software, Supervision, Validation, Visualization, Writing – review & editing

Michel Dzondo Gadet: Formal Analysis, Project administration, Software, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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