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Isolation and Identification of Lactic Acid Bacteria and Acetic Acid Bacteria Playing a Lead Role in the Fermentation of Cocoa in Fako Division of Cameroon

Lewis D. Levai[1,2*], Rauwitta O. Afoh[3], Yannick Tah[3], Ekwa Y. Monono[1], Lewis Enow[1], Fossi B.Tatsinkou[2], Jane-Francis K. Akoachere[2] and Vincent P. K. Titanji[4]

[1]Laboratory of JPJ Biotechnology, Institute of Agricultural Research for Development (IRAD) Ekona, PMB 25 Buea, Cameroon.
[2]Department of Microbiology and Parasitology, Faculty of Science, University of Buea. P.O.Box 63 Buea, Cameroon.
[3]Laboratory of Food Technology, Institute of Agricultural Research for Development (IRAD), P.O.Box 2123 Nkolbisson Yaoundé, Cameroon.
[4]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P.O.Box 63 Buea, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Author LDL designed the study, supervised laboratory analyses and wrote draft manuscript. Authors ROA, YT and LE collected samples and performed laboratory analyses. Author EYM collated data and performed statistical analyses. Author FBT supervised laboratory work and preparation of manuscript. Authors JFKA and VPKT read and edited manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Heaps of cocoa beans and mucilage placed within plantain leaves in Ekona, Fako Division of the South West Region of Cameroon was studied to isolate and identify bacteria playing a lead role during natural fermentation process. All experiments were performed at JP Johnson Biotechnology

**Corresponding author: E-mail: doplevai@yahoo.co.uk;*

Laboratory at IRAD Ekona, Fako Division, South West Region, Cameroon between March – October, 2020. Heaps of 10kg were placed on and covered with plantain leaves and allowed to ferment naturally. Before every sample was collected the temperature and pH were measured. Samples were inoculated into de Mann Rogosa and Sharpe (MRS) agar and Caar agar for the isolation of lactic acid bacteria (LAB) and acetic acid bacteria (AAB) respectively. Standard microbiological procedures for obtaining bacteria counts and isolation of pure cultures were performed and isolated bacteria were identified following morphological and phenotypic characterization on API50CHL for LAB and Enrichment Media for AAB. The fermentation temperature rose to 45^{o}C after 4 days while the pH fluctuated with a peak at 4.82 after 108 hours. Maximum counts of Acetic Acid Bacteria (AAB) (5.8x10^{8} Colony Forming Units (CFU)/ mL) and Lactic Acid Bacteria (LAB) (8.9x8x10^{8} (CFU)/mL were obtained at 2 days and 5 days respectively. Twelve isolates each of AAB and LAB were identified on growth media. After phenotypic characterization, 03 *Lactobacillus species* and 02 *Acetobacter* species were identified. The most abundant bacteria were *L. plantarum* (41.77%) and *Acetobacter tropicalis* (24.11%) on MRS agar and Caar Agar respectively.
L. plantarum and *A. tropicalis* have been identified as the bacteria playing a lead role in the fermentation of cocoa in Fako Division of the South West Region of Cameroon. These organisms may be used as good candidates in a consortium for use as a starter culture.

Keywords: Cocoa; bacteria; fermentation; leading role; starter.

1. INTRODUCTION

Fermentation is an essential processing step to develop precursor compounds for aroma and flavour characteristics of chocolate, as well as preventing germination of the cocoa bean [1,2,3,4]. Two major events occur during fermentation: Firstly, microbial action on the mucilaginous pulp produces alcohols and acids as well as heat. Secondly, micro-organisms produce metabolites that diffuse into cotyledons and trigger complex biochemical reactions [5,2,6].

Cocoa beans are mainly fermented in heaps (Fig. 1A) enveloped in plantain leaves (Fig. 1C) or in wooden trays, baskets and perforated vessels [7,8,9,10]. In some parts of Cameroon, after the pods are opened, farmers place the beans in 50 kg rice-bags (Fig. 1B) where fermentation takes place. Placing the scooped-out beans in bags facilitates transportation from the farms to their backyards where fermentation is carried out and thereafter to a Samoa oven for drying.

Due to the benefits derived from their activities, microbes have contributed to the development of characteristic properties such as taste, aroma, visual appearance, texture, shelf life and safety [11]. The maximum average fermentation temperature reaches 42 - 52°C after 48 h and the pH reached 4.5 after 144 h of fermentation [12]. The heat produced kills the seed embryo and subsequently the fruit tissue and makes bean drying easier [4,7]. Indeed, Acetic Acid Bacteria (AAB) performed the best and produced the largest amounts of acetate in mixed culture experiments when lactic acid bacteria and yeasts were both present [13,14].

Fermentation is influenced by the state of maturity in which the pod is harvested, time that it is left stored prior to fermentation, type of cocoa, climatic factors, fermentation method and bean removal frequency [15,16]. A higher frequency enables better fermentation. In a study conducted to compare fermentation masses ranging from 10 – 100 kg, fermentation with 60kg masses was recommended because it provides the highest concentration of flavour precursors, such as total reducing sugars [15].

Natural fermentation is spontaneous and usually dominated by autochthonous microbes and/or those erroneously introduced by tools and workers. Fermentation is usually on-farm or in nearby small-scale processing units and is poorly controlled leading to variable and/or poor-quality cocoa beans [16]. Therefore, microbes that are best adapted to the conditions during the fermentation process will dominate. Based on their activity, these microbes can contribute both positively and negatively to the overall process [17,18].

The microbial succession during the fermentation process has been established [5,10,16,19,20]. At the onset of fermentation, the presence of the seed pulp reduces oxygen diffusion within the

fermenting seed mass, creating anaerobic conditions [5]. In particular, yeasts are the first to grow on the pulp surrounding the beans. Insects, such as the fruit-fly (*Drosophila melanogaster*) are probably responsible for the transfer of micro-organisms to the heaps. Other sources include inoculums present on the pod surface, utensils (machete, knives, baskets, buckets, etc) used during pod opening, hands of farmers and leaves used in covering the heaps [20]. Yeasts are the largest producers of esters and higher alcohols, which may contribute to the complex mixture of volatile compounds that characterizes chocolate's aroma [1,5,21,22,23].

During fermentation, yeasts and LAB consume pulp sugars and organic acids, producing ethanol and lactate [9,19,16,20,24]. Pectinolytic yeasts start to break down mucilaginous pulp during the second day and cause the draining of sweatings from the heap. This increases aeration, favouring the establishment of AAB [19,20,24]. The increase in temperature may be caused by the energy released in the exothermic reaction from the oxidation of ethanol to acetic acid by AAB [19,20].

Bacteria, especially lactic acid bacteria then oxidise the ethanol to lactic acid and then to carbon dioxide and water, producing more heat and raising the temperature. The oxidation of ethanol into acetic acid is catalysed by two sequential catalytic reactions of membrane bound, pyrroloquinoline quinone (PQQ)-dependent alcohol dehydrogenase, which oxidizes ethanol into acetaldehyde, and aldehyde dehydrogenase, which oxidizes acetaldehyde into acetic acid [5,25,26]. The growth of AAB actively oxidises the alcohol to acetic acid and makes the conditions to become more aerobic [27].

The importance of bacteria in fermented foods has promoted the application of different strategies to analyse the bacterial diversity and their role during the elaboration process [28,29]. The microbial dynamics of several African foods have been studied and shown to contain micro-organisms that play leading roles in the fermentation process for such foods. In Cote d'Ivoire for example, palm wine has been shown to contain lots of yeasts and lactobacillus throughout the tapping process [30].

Fig. 1: Constituted heap for fermentation (A). Fermentation in bags (B) and on leaves covered with plantain leaves (C)

The key microorganisms for successful cocoa bean fermentation processes are yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) [8,9,31,32]. Failure of fermentation processes can result in spoilage and/or the survival of pathogens, thereby creating unexpected health risks in food products [33,34]. Microorganisms best adapted to the conditions during the fermentation process will eventually dominate at the various stages [35]. This study thus aimed at isolating and identifying the microorganisms that play a leading role in the fermentation of cocoa in the major cocoa production basin of the South West Region of Cameroon.

2. MATERIAL AND METHODS

2.1 Study Area

The study was carried out in Fako Division, South West Region of Cameroon. It is located within one of the major cocoa production basins.

2.2 Preparation of Heap and Sampling

Pods were harvested from research farm of the Institute of Agricultural Research for Development (IRAD) Barombi Kang – Kumba, South West Region. They were taken to the JP Johnson Biotechnology Laboratory at IRAD Ekona where all laboratory experiments were performed. Pods were opened using a sterile knife and the beans and surrounding mucilage scooped out. Heaps of 10Kg of beans with surrounding mucilage formed on plantain leaves and natural fermentations carried out. Samples were collected for morphological and phenotypical characterization to identify microbes that play a leading role in fermentation.

Sampling of fermenting cocoa was done following the methodology of Eijlander et al., [36] and Moreira et al., [19]. Two samples were collected 20 cm apart on the surface and one sample collected 20 cm deep from the centre of the fermenting mass. The temperature and pH were measured before sample collection. Each sample composed of at least twenty cocoa beans surrounded with pulp (weighing about 100 g), withdrawn using a sterile spatula and placed in sterile falcon tubes, capped and labelled appropriately.

2.3 Isolation and Morphological Characterisation of Microorganisms

Fundamental microbiological techniques were used for the isolation of lactic acid bacteria (LAB) and acetic acid bacteria (AAB). Enumeration of microorganisms and relative abundance of different morphologically distinct types was carried on Caar Agar for AAB and de Mann Rogosa Sharpe (MRS) agar for LAB [31]. Pure cultures of all colonies presumptively identified as different were obtained by subculturing several times on the same media used for isolation.

2.4 Phenotypic Characterisation of Microorganism

Preliminary identification of microorganisms followed the methods of Ndip et al., [37] and Schwan and Fleet [31]. This was done by studying both macroscopic and microscopic characteristics of pure colonies. Pure colony parameters recorded include; colour, shape, form and elevation. Enrichment media (Table 1) as described by Lisdiyanti et al., [38] for AAB and the Analytic Profile Index (API) 50 CHL kit for LAB were used for phenotypic characterization.

Table 1. Composition of enrichment media for identification of AAB

Composition	Enrichment media at pH 3.5				
	I	II	III	IV	V
D-Glucose	1.0	0.0	0.0	0.0	0.15
D-Sorbitol	–	2.0	–	–	–
D-Mannitol	–	–	2.0	–	–
Dulcitol	–	–	–	2.0	–
Methanol	–	–	–	–	2.0
Ethanol	0.5	–	–	–	–
Peptone	1.5	0.5	0.5	0.5	0.5
Yeast extract	0.8	0.3	0.3	0.3	0.3
Acetic acid	0.3	–	0.2	–	–
Cycloheximide	0.1	0.1	0.1	0.1	0.1

Source: Lisdiyanti et al. [38]

2.7 Data Analysis

Data was analyzed using a one-way analysis of variance (ANOVA) using Minitab statistical software package version 16 at $p < 0.5$. The treatment means were compared and separated using Tukey's method at 5% probability level.

3. RESULTS

3.1 Measurement of Temperature and pH

During sampling the pH and temperature of the fermentation masses were measured to evaluate their variation in the course of the fermentation process. Three main pH peaks were observed during the fermentation that lasted 6 days. Three main peaks were observed at 36 hours (4.41), 60 hours (4.2) and 108 hours (4.82) into the fermentation process (Fig. 2).

The temperature rose from 25°C on the first day to 45°C on day 4, and then almost levelled till day 6 at 45°C (Fig. 3).

3.2 Mean Bacteria Count and Relative Abundance of Isolates

Counts of AAB on Caar medium peaked after 2 days reaching 5.8x10^{8}Colony Forming Units (CFU)/mL and declined gradually to 4.5 x10^{8}CFU/mL at the end of fermentation on day 6 (Fig. 4). Lactic Acid Bacteria counts rose from 5.4 x10^{8}CFU/mL and peaked after 5 days at 8.9 x 10^{8}CFU/mL and then dropped sharply to 6.0 x10^{8}CFU/mL.

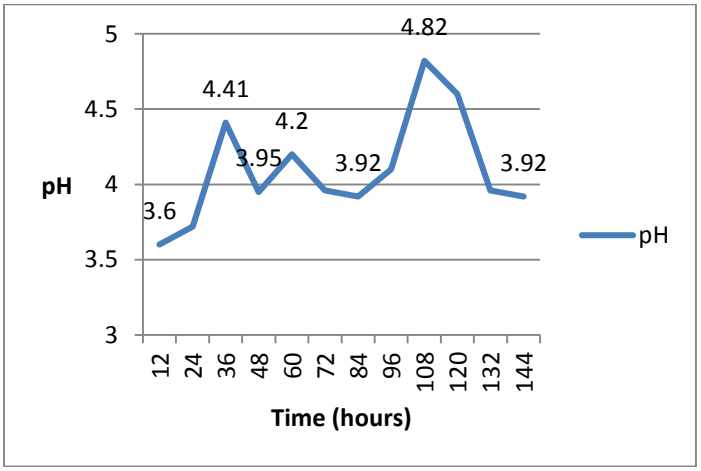

Fig. 2. pH values during primary natural fermentation

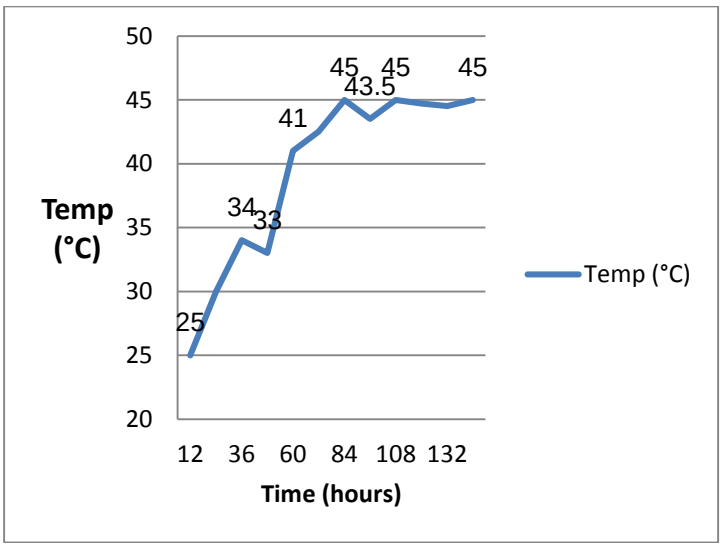

Fig. 2. Temperature variation during indigenous fermentation

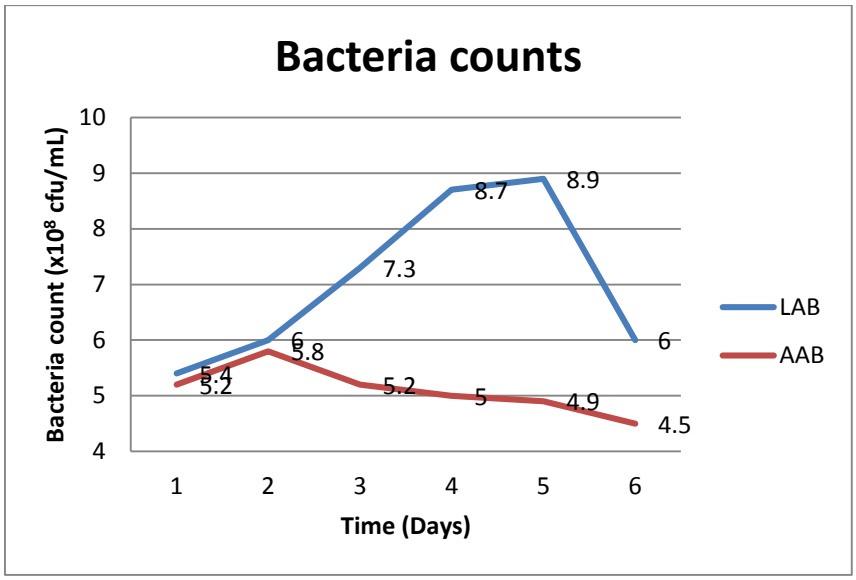

Fig. 4. Counts of lactic acid bacteria and acetic acid bacteria of fermentation heap

The relative abundance of AAB and LAB isolates is as shown on Table 2. Highest relative abundance 33(41.77%) was observed for Isolate P1 and lowest (1.27%) for PK1 and PK2 growing on MRS. For colonies growing on Caar agar, TK3 recorded the highest relative abundance 27(24.11%) while the lowest was observed with isolate AA1. These high values for P1 and TK3 were respectively statistically different (P=0.05) from the rest of the counts on the same medium. Based on their relative abundance, 3 LAB isolates (P1, P2 and C3) and 2 AAB isolates (TK3 and TK6) where selected for phenotypic characterisation.

3.3 Identification of Lactic Acid Bacteria (LAB) Isolates

3.3.1 Lactic Acid Bacteria (LAB)

Colonial morphology of isolates was recorded as well as Gram reaction test and catalase test. Based on these results, 12 presumptive isolates of LAB were identified as shown on Table 3. Considering their relative abundance on MRS agar, 03 isolates (P2, P1 and C3) were subjected to biochemical testing using the Analytic Profile Index (API50 CHL) kit. These 3 isolates were all Gram positive, catalase positive, cream, single rods. The fermentative profile of these isolates is presented on Table 4.

Only C3 could ferment D-xylose and β methyl-D-xyloside, P2 alone will ferment Adonitol, ducitol, xylitol and D-tagatose while P1 alone will ferment sorbose, Inulin, Raffinose and L-fucose. These isolates P2, P1 and C3 identified as *Lactobacillus pentosus*, *L. plantarum* and *L. brevis* respectively.

3.3.2 Acetic acid bacteria (AAB) isolates

Isolation of AAB was done on Caar agar which is a differential medium for AAB. Colonies surrounded by a yellow clearing were picked for characterisation. The results of Gram reaction, cellular and colonial morphology and catalase test of AAB isolates are presented on Table 5. Of the 12 isolates 6 were Gram negative cocco-bacilli, 4 were Gram negative bacilli and 2 were Gram negative cocci.

Due to their relative abundance on CARR medium, TK3 and TK6 were chosen for further characterisation and grown on enrichment medium (EM) (Table 6) for identification. While there was no growth on enrichment media II, IV and V, growth was observed on Enrichment medium I and III.

4. DISCUSSION

The succession of microorganisms in cocoa bean fermentation has been studied elaborately [13,30,31,39,40]. During the last decade, knowledge about the spontaneous cocoa bean fermentation process has increased [3,10,19,27,39,40,41,42,43]. It has been well established that the yeasts *Hanseniaspora opuntiae, S. cerevisiae, Pichia pijperi* dominate during the first 2 days of fermentation followed by lactic acid bacteria (LAB) and acetic acid bacteria

(AAB) [3,10,11,13,16,19,39,42]. *Acetobacter pasteurianus*, and *Lactobacillus fermentum* are the most abundant operational taxonomic units during cocoa fermentation processes [27,30,42]. In this study, we did not investigate the dynamics in the population of microorganisms. However, we isolated and identified microorganisms playing a leading role during indigenous fermentation.

Acetic acid bacteria are a large group of obligate aerobic catalase positive Gram-negative bacteria, commonly found in association with various kinds of sugary material [38]. Presently, there are 19 genera and 92 species of AAB that have been identified [44,45]. These genera include; *Acetobacter*, *Acidomonas*, *Ameyamaea*, *Asaia*, *Bombella*, *Commensalibacter*, *Endobacter*, *Gluconacetobacter*, *Gluconobacter*, *Granulibacter*, *Komagataeibacter*, *Kozakia*, *Neoasaia*, *Neokomagataea*, *Nguyenibacter*, *Saccharibacter*, *Swaminathania*, *Swingsia* and *Tanticharoenia*. Acetobacter and *Gluconobacter* species are most commonly found associated with cocoa bean fermentations [32,42].

We isolated 12 acetic acid bacteria during indigenous bean fermentations and observed that their population was higher earlier (12–36h) rather than later in the fermentation process. Our data was consistent with those of [40] who isolated AAB early with a peak after 48h. Acetic acid bacteria are aerobic in their metabolism and are not very tolerant to high temperatures. Thus, higher temperatures (>40^{o}C) after 48h hindered their growth. It has also been reported previously that acetic acid bacteria can survive quite well and possibly grow under anaerobic conditions. Our fermentation temperatures (maximum of 45^{o}C) were a little lower than the average of 50^{o}C as reported by Castro-Alayo *et al*., [20] and Kouame et al., [42]. This could be attributed to the fact at laboratory scale the fermentation mass was much smaller. This has equally been reported elsewhere in experiments with ≤20 Kg fermentation mass with temperatures averaging 35^{o}C [39]. As obligate aerobic bacteria, AAB consume oxygen during oxidation of ethanol and lactic acid into acetic acid and/or acetoin. This is the reason aerobic conditions have to be maintained during fermentations with AAB, which is generally accomplished by continuous dispersion of oxygen into the fermentation medium by turning of the heap.

Many reports have also addressed the usefulness of the enrichment culture technique in selective isolation of targeted microorganisms [38,46,47,48]. In this study, Caar agar was used for the selective isolation of AAB and identification on 5 different enrichment media. Based on their relative abundances (Table 2) on plate cultures, we selected two isolates for identification TK3 and TK6. When grown on enrichment media, growth was observed only on EM I and EM III inoculated with TK6 and TK3 respectively (Table 6). These were identified as *Acetobacter tropicalis* and *A. pasteurianus*. Studies have shown that these AAB are present in fermentation sites with substrates like cocoa and palm wine [38]. While *A. pasteurianus* is not found associated with fruits, *A. tropicalis* has been found associated with the fermentation of citrus fruits and guava [38]. These fruits being almost always around cocoa farms could have influenced abundant growth of *A. tropicalis* in this area. In their study Pereira et al., [39] equally identified this microbe as the dominant AAB.

Table 2. Mean counts and relative abundance (%) of bacteria

LAB isolates on MRS agar	Mean Counts	Relative abundance (%)	AAB Isolates on Caar Agar	Mean Counts	Relative Abundance (%)
PK1	1.00^{f}	1.27	TK1	8.00^{e}	7.14
PK2	1.00^{f}	1.27	T6	3.00^{g}	2.68
PkM5	3.00^{def}	3.80	**TK3***	**27.00^{a}**	**24.11**
PK3	5.00^{cd}	5.06	TK5	9.00^{e}	8.04
PK4	4.00^{cde}	6.33	TK6*	19.00^{b}	16.96
PK5	5.00^{cd}	6.33	TK7	11.00^{d}	9.82
P2	2.00^{ef}	2.53	T7	6.00^{f}	5.36
B1	3.00^{def}	3.80	AA9	13.00^{c}	11.61
P1*	**33.00^{a}**	**41.77**	AA10	8.00^{e}	7.14
PM1	4.00^{cde}	5.06	AA1	1.00^{h}	0.89
P2*	12.00^{b}	15.19	AA4	3.00^{g}	2.68
C3*	6.00^{c}	7.59	AA	4.00^{g}	3.57
Total	**79**	**100.00**		**112**	**100.00**

**Isolates selected for further phenotypic characterisation. Mean count values followed by the same superscript are statistically not different at p=0.05*

Table 3. Morphological characteristics of presumptive LAB isolates on MRS agar, microscopy and catalase test results

Code	Macroscopy				Microscopy		Gram Reaction	Catalase	Conclusion
	Colour	Form	Size	Elevation	Shape	Arrangement			
PK1	Brown	Circular	Large	Umbonate	Cocci	Clusters	+ve	-ve	G+ve cocci
PK2	Brown	Circular	Medium	Convex	Cocci	Single	+ve	-ve	G+ve cocci
PkM5	Cream	Irregular	Small	Raised	Coco-bacilli	Chains	+ve	-ve	G+ve coco-bacilli
PK3	Cream	Irregular	Small	Convex	Bacilli	Single	+ve	-ve	G+ve cocci
PK4	Cream	Irregular	Medium	Raised	Coco-bacilli	Single	+ve	-ve	G+ve coco-bacilli
PK5	Brown	Irregular	Medium	Convex	Bacilli	Single	+ve	-ve	G+ve bacilli
P3	Cream	Irregular	Medium	Raised	Rods	Single	+ve	-ve	G+ve rod
B1	Cream	Irregular	Small	Convex	Bacilli	Chains	+ve	-ve	G+ve bacilli
P1*	Cream	Irregular	Medium	Raised	Rod	Single	+ve	-ve	G+ve rod
PM1	Cream	Irregular	Medium	Raised	Cocci	Single	+ve	-ve	G+ve cocci
P2*	Cream	Smooth	Small	Convex	Rods	Single	+ve	-ve	G+ve rod
C3*	Cream	Circular	Medium	Raised	Rods	Single	+ve	-ve	G+ve rod

*+ve, positive; -ve, negative; G+ve, Gram positive; *, Isolates selected for further characterisation using API 50 CHL kit*

Table 4. Analytic Profile Index (API) test results after 48 hours of incubation

Number	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Strains Code	**Control**	**Glycerol**	**Erythritol**	**D-arabinose**	**L-arabinose**	**Ribose**	**D-xylose**	**L-xylose**	**Adonitol**	**ß methyl-D-Xyloside**	**Galactose**	**Glucose**	**Fructose**	**Mannose**	**Sorbose**	**Rhamnose**	**Dulcitol**	**Inositol**	**Mannitol**	**Sorbitol**	**Methyl-D-mannoside**	**Methyl-D-glucoside**	**N-Acetyl-Glucosamine**	**Amygdalin**	**Arbutin**	**Esculin**	**Salicin**	**Cellobiose**
P2	-	-	-	-	-	+	-	-	+		+	+	+	+	-	-	+	-	+	+	-	-	+	+	+	-	+	+
P1	-	-	?	-	+	+	-	-	-	-	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	-	+	+
C3	-	-	-	-	+	+	+	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	-	+	+

Number	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	
Strains code	**Maltose**	**Lactose**	**Melibiose**	**Sucrose**	**Trehalose**	**Inulin**	**Melezitose**	**Raffinose**	**Starch**	**Glycogen**	**Xylitol**	**ß Gentiobiose**	**D-turanose**	**D-lyxose**	**D-tagatose**	**D-fucose**	**L-fucose**	**D-arabitol**	**L-arabitol**	**Gluconate**	**2-Keto-Gluconate**	**5-Keto-Gluconate**	**Identified species**
P2	+	-	-	?	+	-	+	-	?	?	+	+	+	-	+	-	-	-	-	+	-	?	***Lactobacillus pentosus***
P1	+	+	+	+	-	+	+	+	-	-	-	-	-	-		-	+	-	-	+	-	-	***Lactobacillus plantarum***
C3	+	+	+	+	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	?	-	?	***Lactobacillus brevis 1***

+, Positive; –, Negative;?, non-conclusive

Table 5. Morphological characteristics, Gram reaction and catalase test results of presumptive AAB isolates

Code	Macroscopy				Microscopy			Catalase test	Conclusion
	Colour	Form	Size	Elevation	Shape	Arrangement	Gram Reaction		
TK1	Green	Irregular	Small	Flat	Cocco-bacilli	Single	-ve	+ve	G-vecocco-bacilli
T6	Yellow	Irregular	Medium	Flat	Bacilli	Single	-ve	+ve	G -ve bacilli
TK3*	Yellow	Circular	Medium	Raised	Cocco-bacilli	single	-ve	+ve	G-ve coco-bacilli
TK5	Green	Irregular	Medium	Convex	Cocci	Single	-ve	+ve	G-ve cocci
TK6*	Yellow	Irregular	Medium	Convex	Bacilli	single	-ve	+ve	G-ve bacilli
TK7	Green	Circular	Small	Raised	Bacilli	Single	-ve	+ve	G-ve bacilli
T7	Yellow	Irregular	Medium	Raised	Cocco-bacilli	Single	-ve	+ve	G-vecocco-bacilli
AA9	Green	Irregular	Medium	Flat	Bacilli	Single	-ve	+ve	G-vecocco-bacilli
AA10	Green	Irregular	Small	Flat	Bacilli	Single	-ve	+ve	G-ve bacilli
AA1	Yellow	Irregular	Small	Convex	Cocco-bacilli	Single	-ve	+ve	G-vecocco-bacilli
AA4	Green	Irregular	Medium	Flat	Cocci	cluster	-ve	+ve	G-ve cocci
AA	Yellow	Circular	Large	Convex	Cocco-bacilli	Single	-ve	+ve	G-vecocco-bacilli

*+ve, positive; -ve, negative; G-ve, Gram negative; *, Isolates select for further characterization using enrichment medium*

Table 6. Growth on enrichment media

Isolates	Enrichment media at pH 3.5					Identity of Microbe
	I	II	III	IV	V	
TK3	-	-	+	-	-	*A. tropicalis*
TK6	+	-	-	-	-	*A. pasteurianus*

-, no growth; +, growth

TK6 grew well on EM I with 30% D-glucose and thus was identified as A. pasteurianus. The isolate TK3 grew well on EM III was thus identified as A. tropicalis

Growing on MRS, 12 isolates of LAB were differentiated based on morphological evaluation (Table 02). Based on their high relative abundance (Table 2) of 33(41.77%), 12(15.19%) and 6(7.59) respectively 03 isolates (P1, P2 and C3) were chosen for further characterization using the API kit. They were identified as *Lactobacillus pentosus*, *L. Plantarum* and *L. Brevis* (Table 3). These species have equally been identified elsewhere as being part of the LAB that contributes to the fermentation of cocoa bean in the natural process [49,50]. *L. fermentum*, *L. plantarum*, *L. mesenteroides*, and *Lactococcus lactis* have been described as the predominant LAB species isolated from cocoa fermentations [39]. [9] isolated *L. casei*, *L. plantarum*, *L. delbrueckii*, *L. acidophilus*, *L. brevis*, *P. dextrinicus*, and *P. acidilactici* from cocoa fermentations in Brazil. Furthermore, in most studies on the bacterial diversity from cocoa fermentation, *L. plantarum* has been described as the main representative [10,19,39,49,51].

Previous studies have linked the variation of LAB species to the type of cocoa, country, farm, pod ripeness, postharvest pod storage and pod diseases, variations in pulp/bean ratio, fermentation method, size of the batch, season and weather conditions, turning frequency, and fermentation time [31]. In their study, Moreira et al., [19] showed that species diversity was more linked to turning than to the environment.

5. CONCLUSION

In this study, we were able to use morphology and phenotypic characterisation to isolate and Identify *L. plantarum* and *Acetobacter tropicalis* as the bacteria playing a lead role in the fermentation of cocoa based on their relative abundance. These findings are in agreement with those of other authors in different regions. These bacteria can be used in a consortium as a starter culture for a controlled fermentation of cocoa.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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