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ORIGINAL ARTICLE

Screening, isolation and characterization of lactic acid bacteria strains in fermenting cocoa heaps from the Eastern Region of Ghana

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Abstract

Cocoa fermentation is the primary process of producing a quality chocolate taste and flavour. Lactic acid bacteria (LAB) are one of the key microorganisms in this heterogeneous fermentation process. They break down the sugars and citric acid in the cocoa pulp to produce lactic acid, acetic acid and mannitol that influence the quality of the fermented bean. LAB strains as monoculture or co-culture could be an essential component of starter culture aimed at the control of cocoa bean fermentation process. This study screened, isolated and characterized LAB strains in cocoa heap fermentation to compare the amount of lactic acid produced by the various strains. Cocoa beans were fermented and enumeration of microorganisms was carried out. Microbial colonies were randomly picked and sub-cultured on MRS medium. Pure isolates were screened for LAB by carrying out catalase test and gram reaction. The isolated pure LAB strains were subjected to morphological, physiological and biochemical characterization. The amounts of lactic acid produced by the various strains were determined. There were two presumptive *Lactococcus* species as well as two presumptive *Lactobacillus* species. Different NaCl concentrations as well as citrate hydrolysis test were used to subdivide both species. C16 was identified to be presumptive *Lactobacillus fermentum,* C17 was identified to be *Lactococcus lactis* subsp. *cremoris,* C20 was identified to be presumptive *Lactobacillus brevis* and C23 was identified to be *Lactococcus lactis subsp. lactis* biovar *diacetylactis. Lactococcus lactis* subsp. *cremoris* produced the highest amount of lactic acid (185g lactic acid / L of culture medium) with *Lactococcus lactis* subsp. *lactis biovar diacetylactis* producing the least amount of lactic acid (95g lactic acid / L of culture medium). The above presumptive LAB species especially one with the highest amount of lactic acid produced could be a potential strain in producing a starter culture for a controlled cocoa fermentation.

Keywords: Cocoa fermentation; Microbial colonies; Lactic Acid Bacteria strains; Starter culture

Introduction

Lactic Acid Bacteria are members of phylogenetically distinct divisions of Gram-positive and catalase–negative bacteria which grow under micro aero-tolerant to strictly anaerobic conditions [1]. The use of Lactic Acid Bacteria (LAB) has been of great importance in food technology. It is basically used to improve aroma, texture and flavour in the production of fermented food products and beverages such as dairy foods, fermented sausages, sourdough's and fermented ketchups [2]. Lactic Acid Bacteria can carry out these various functions because they synthesize a broad range of compounds including organic acids, hydrogen peroxide, antimicrobial agents, aromatic compounds and exopolysaccharides (EPS) among others [3].

An essential crop in West Africa is the Cocoa Plant (*Theobroma spp.*). Ghana is currently the second largest world producers of cocoa bean after Cote d'Ivoire with an annual production level of over 696,000 tonnes [4]. Cocoa fermentation is a natural heterogeneous process which is one of the basic key components to get rid of astringency in cocoa bean for the development of quality chocolate. The fermentation process is spontaneously carried out

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in heaps, boxes, basket, tray or on platforms which normally last for about 7 days [5]. Cocoa bean fermentation normally requires a lot of successive microbial activities basically involving yeast, Acetic Acid Bacteria and Lactic Acid Bacteria. The action of these microorganisms on the cocoa pulp leads to the reaction of endogenous enzymes on carbohydrates, proteins and polyphenols in the cocoa beans.

The yeast uses pulp carbohydrates under both aerobic and anaerobic condition to produce ethanol and liquefies the pulp through depectinization, hence reducing the viscosity of the pulp [6]. The role of LAB is to convert fermentable sugars and organic acids in the pulp mainly into lactic acid as well as other product such as acetic acid, gas (CO_2) and ethanol [7]. As more air comes in, AAB start to grow and oxidizes the ethanol, initially produced by the yeasts, to acetic acid. These results in development of flavour precursors [8].

During cocoa fermentation, LAB strains undergo either the homo-fermentative or the hetero-fermentative pathway. LAB strains like *L. lactis, L. delbrueckii,* and *L. casei* uses the homo-fermentative pathway to metabolize one molecule of hexose sugar such as glucose to two molecules of lactic acid and two molecules of ATP whiles, strains like *L. amylovorus, L. reuteri,* and *L. manihotivorans* ferments one molecule of glucose to one molecule of lactic acid, one molecule of ethanol/acetate, one molecule of CO_2, and only one molecule of ATP.

The control of cocoa fermentation process has been a very challenging task for farmers in Ghana. This is because cocoa fermentation has always been a natural process and this sometimes results in poorly fermented cocoa arising from variations of microorganism and metabolite concentration. However, studies suggest the use of starter microbial culture as the best approach to improve fermentation process. Also, studies have shown the role of LAB in producing quality cocoa bean but little has been done in identifying and developing the potential cocoa-specific LAB strains as starter culture. LAB strains as monoculture or co-culture would be essential component of starter culture aimed at the control of cocoa bean fermentation process to obtain well-fermented dry cocoa beans for improved standard and superior tasting chocolate and other food production [9, 10].

With starter culture development, farmers will not have to depend on wholly natural fermentation which is an unpredictable process rather a better controlled cocoa fermentation will be achieved. This will be very beneficial especially to large scale farmers.

More recently, it was demonstrated that a mixed starter culture encompassing strains of *Lactobacillus fermentum*, *Acetobacter pasteurianus* and *Saccharomysces cerevisiae* produced cocoa of a superior quality compared to fermentations inoculated with a pure LAB/AAB bacterial starter culture [11]. However, world largest producers of cocoa such as Barry Callebaut has been using yeast starter culture (*Saccharomyces* strains) to produce highly flavour chocolate quality for five years now [12].

The role of Lactic Acid Bacteria as one of the key microorganism in this process has been studied and possesses the characteristics to be a potential starter culture. This merit the need to isolate, screen and characterize LAB strains as an important step to selecting strains that may positively influence the quality of a controlled cocoa fermentation. Information on LAB strains producing high amounts of lactic acid would be obtained, which could also be useful for developing a cocktail of starter culture.

Methodology

Sample collection

Samples of fermented cocoa were collected from 3 different heaps on daily basis from the first day to the sixth day of fermentation at the Cocoa Research Institute of Ghana (CRIG) in the Eastern Region of Ghana. The samples were taken aseptically and packaged into zip-lock bags, then stored at 4°C.

Study site

The isolation, screening and characterization of Lactic Acid Bacteria strains were carried out at CAN Lab and the Department of Biochemistry and Biotechnology's Laboratory, KNUST.

Isolation and screening of Lactic Acid Bacteria

MRS (de Man, Rogosa, and Sharpe)-agar plates were prepared by weighing 31g in 500 ml distilled water into a conical flask and 15 mins autoclaving was done at 121^{0}C. After cooling for 45 min, L-cysteine and cyclohexamide were added to the media to create an anaerobic condition and prevent growth of yeast/moulds respectively. This was distributed into sterile petri dishes and allowed to solidify. Ten grams of the fermented cocoa seeds from the 3 different heaps for each day were suspended into bottles containing 90 ml sterile peptone water (0.1% w/v) each respectively and rapidly shook for 2 min. The suspension was used to make serial dilutions of 10^{-3} up to 10^{-6} by incorporating 1ml into 9 ml of sterile peptone water (0.1% w/v). Sample of diluted solutions were cultured by spread plating on MRS plates and incubated for 2 days at 36^{0}C. After incubation, various colonies were obtained on the media and enumerated. Then 24 desired glistening colonies were randomly picked up from MRS agar using a sterile platinum loop and sub-cultured on the above medium by streak plating and incubated at 36^{0}C for 2 days. After 2 days of incubation, the pure colonies were stored at 4^{0}C for further use.

The isolates were identified to strain level according to the methodology and characteristics given by [13]; [14] with modifications.

Morphological, Physiological and Biochemical Characterization of LAB Strains

The pure cultures were characterized using cell morphology, catalase reaction and Gram reaction as described by [15]. Strains with catalase negative reactions were further used for various biochemical test [16].

Sugar Test (2% w/v)

MRS broth devoid of glucose and beef extract was prepared and distributed into test tubes, covered with cotton wool and autoclaved for 15 min at 121^{0}C. After cooling, the different sterile sugars were added to the broth respectively and 1 ml inoculum of each pure isolates was pipetted into the tubes labelled accordingly. A drop of 1% Bromocresol purple was added to each tube as an indicator and sterile Durham tube was inverted in each tube. Incubation was done at 30^{0}C for 2 days. Results were then observed and recorded [15]

NaCl Tolerance

Concentrations of 2%, 4%, 6.5% and 1% NaCl was prepared using a basal MRS broth and 10 ml each was pipetted into sterile tubes and labelled accordingly. A volume of 1 ml each of pure colonies

was pipetted into the labelled tubes respectively and a drop of 1% bromocresol purple dye (BPC) was added as an indicator. This was incubated for 2 days at 30^{0}C. Results were then observed and recorded [15].

Temperature tolerance test

A 100 ml basal MRS broth was prepared and distributed into sterile text tubes (10 ml each).

A sterile loop was use to inoculate the pure isolates into the broth and labelled accordingly. Few drops of BCP dye was added to each tube as an indicator. The labelled tubes were subjected to growth at temperatures of 10^{0}C and 45^{0}C for 48 hour [17]. Change of purple colour to yellow confirms growth.

Arginine and Citrate Hydrolysis

For the citrate hydrolysis, 10 ml of MRS broth devoid of glucose with 2% citrate was prepared and 1 ml each of the pure isolate was pipetted into the tubes respectively and labelled accordingly. Sterile Durham tubes were inverted into the tubes respectively to detect gas production [17].

For the arginine hydrolysis, 0.3% arginine and 0.2% sodium citrate was added to a basal MRS broth, 1 ml of each pure isolate was pipetted into the tubes containing 5 ml of the broth respectively. This was incubated for 3 days at 30^{0}C. After the incubation, ammonia production was looked out for by adding 3 drops of Nessler reagent per the method of [18].

Lactic Acid Estimation

Lactic acid production by fermentation process was carried out by the method according to [19] with modification. Each test organism was inoculated into 20 ml MRS broth and incubated at 37°C for 72 hours. The LAB cultures were transferred to centrifuge tubes and centrifugation was done at 3000 rpm for 15 minutes. The pH of the supernatant in each tube was recorded. To 20 ml of the supernatant of the test organism, 5 drops of phenolphthalein were added as indicator and titration was done using a burette containing 0.1M NaOH. This was slowly added to the samples until a pink colour appeared. Each ml of 0.1M NaOH is equivalent to 90.08 mg of lactic acid. The amount of lactic acid produced by each LAB strains was calculated and recorded as g lactic acid per litre of culture medium.

Result

Screening and isolation

Microbial growths were observed on most of the MRS plate in which the fermented cocoa samples were grown. Observations made after 72 hours revealed the result in Table 1.

Isolation of unique colonies

Unique colonies were isolated based on their different physical and morphological characteristics as observed under white light with the naked eye. The morphological characteristics used in the isolation included margin, colour, form, elevation and optical characteristics. A total of twenty-four (24) colonies were isolated. The results are given in Table 2.

Isolation for pure colonies of Lactic Acid Bacteria

Pure colonies of isolates as identified by morphology above were sub-cultures by streaking on fresh MRS plates to obtain pure colonies for further work. After 24 hours of inoculation, all isolates grew on the fresh plates as pure colonies.

Table 1 – Enumeration of Microorganisms after incubation

Fermentation Days	Serial dilutions			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}
	10^4 CFU/ml	10^5 CFU/ml	10^6 CFU/ml	10^7 CFU/ml
1	22	16	0	0
2	20	1	0	0
3	66	12	0	2
4	TNTC	TNTC	60	20
5	30	6	0	0
6	TNTC	TNTC	70	20

Table 2 – Screening for LAB strains using catalase test

Fermentation Days	Microbial Colonies	Catalase Test
1	C1	+
	C2	+
2	C3	+
	C4	+
3	C5	+
	C6	+
	C7	+
4	C8	+
	C9	+
	C10	+
	C11	+
	C12	+
	C13	+
	C14	+
5	C15	+
	C16	-
	C17	-
	C18	+
	C19	+
	C20	-
	C21	+
6	C22	+
	C23	-
	C24	+

+: Positive -: Negative C1-C24: Colonies 1 to Colonies 24

Catalase test carried out on the pure colonies revealed four (4) of them being catalase negative whiles the other twenty (20) were catalase positive. The results are given in Table 2 above.

Phenotypic and morphological Characterization

The general properties of four LAB strains isolated from the fermented Cocoa were ascertained by phenotypic and morphological characterization by using the microscope, Gram reaction and catalase test. The results are given in Table 3.

Biochemical Characterization and Carbohydrate Fermentation of LAB strains

The four isolated LAB strains were further subdivided by original characterization based on: production of gas from glucose, hydrolysis of arginine and citrate, growth at different temperatures and at different levels of salt concentration. Species were arranged in groups by carbohydrates fermentation profiles as shown in Table 4 and Table 5.

Table 3 – Phenotypic and Morphological characterization of LAB strains

Colonies	C16	C17	C20	C23
Cell Morphology	Rods	Cocci	Rods	Cocci
Gram Reaction	Positive	Positive	Positive	Positive
Catalase Test	Negative	Negative	Negative	Negative
Shape	Circular	Circular	Irregular	Circular
Colour	white	White	Cream	white

Table 4 – Biochemical Characterization

Colonies	Acid from Glucose	Gas from Glucose	Hydrolysis of:		Growth at Different Temperatures		Growth in the Presence of NaCl			
			Arginine	Citrate	10^0C	45^0C	2.0%	4.0%	6.5%	10.0%
C23	+	-	-	+	V	+	+	V	-	V
C16	+	+	+	+	-	+	V	V	-	-
C17	+	-	-	-	V	+	+	+	-	-
C20	+	+	+	-	-	+	+	V	V	-

+: Positive -: Negative V: Variable

Table 5 – Carbohydrate Fermentation of LAB strains

Colonies	Acid from Galactose	Gas from Galactose	Acid from Fructose	Gas from Fructose	Acid from Xylose	Gas from Xylose	Acid from Sorbitol	Gas from Sorbitol	Acid from Mannose	Gas from Mannose	Acid from Sucrose	Gas from Sucrose
C23	V	V	+	V	V	+	-	-	+	-	+	+
C16	V	V	+	+	V	-	-	-	+	-	+	-
C17	V	V	V	V	+	+	-	-	+	-	+	+
C20	V	V	+	+	V	-	-	-	+	-	+	-

+: Positive -: Negative V: Variable

Lactic Acid Estimation

The four isolated LAB strains were screened for quantitative production of lactic acid using basal MRS broth by titration and using a pH meter to check for pH. The results are given in Table 6.

Table 6 – Lactic Acid Estimation

Colonies	g lactic acid / L of culture medium	pH
23	95	5.06
16	120	5.08
17	185	4.45
20	155	4.90

Discussion

Screening and Isolation of LAB

Lactic Acid Bacteria are normally seen in decomposing plants and milk products, producing lactic acid as the major metabolic end-product of carbohydrate fermentation. This characteristic, throughout history, has contributed to the use of LAB in food fermentation [3]. According to [1], LAB group involved in food fermentation includes *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus and Tetragenococcus* genera.

In most cases shown in Table 1, more than one colony was observed on the MRS plates and majority of them being catalase positive microorganisms. As shown in Table 1, more bacterial growth was observed on the plates for the various dilutions from the third day of fermentation to the last day. This confirms the defined microbial succession for cocoa fermentation that yeast

activity predominates the first two days of the process then followed by the activities of bacteria [20].

Pure Colonies were identified according to their morphological and physiological characteristics. As shown in Table 3, four colonies out of the 24 colonies tested positive to Gram-reaction and negative to catalase activity. According to [21], a group of bacteria that are Gram-positive and catalase negative belong to the Lactic Acid Bacteria group. Hence it can be said that the four colonies belong to the Lactic Acid Bacteria group. Various biochemical tests were performed on the four colonies to aid in further classifying them into genus and species level.

Characterization of LAB strains

As shown in Table 4.3, there are 2 cocci-shaped, colony 23 (C23) and colony 17 (C17) and 2 rod-shaped, colony 16 (C16) and colony 20 (C20) LAB strains. According to Saranraj *et al.*, 2013, rod-shaped LAB strains include *Lactobacillus spp*. and cocci-shaped LAB strains include *Enterococci*, *Lactococci*, *Pedicocci* and *Streptococci*, among others. The four isolated LAB strains (C16, C17, C20 and C23) were subjected to various biochemical tests according to [13]; [14] with modifications: Production of gas from glucose, arginine and citrate hydrolysis, growth in the presence of different salt concentrations and growth at different temperatures. After the biochemical tests, C16 being a rod-shaped LAB, produced gas from glucose hence a hetero-fermentative organism. It also grew in the presence of 2.0% NaCl and 4.0% NaCl as well as a positive test to citrate hydrolysis and a positive test to arginine hydrolysis hence presumptive *Lactobacillus fermentum* according to [22]. C20 being a rod-shaped LAB, produced gas from glucose hence a hetero-fermentative organism. It also grew in the presence of 2% NaCl and was variable for 4.0% and 6.5% NaCl as well as a being positive for arginine hydrolysis and tested negative to citrate hydrolysis hence a presumptive *Lactobacillus brevis* according to findings from [23].

C17 being a cocci-shaped LAB did not produce gas from glucose hence a homo-fermentative organism. It did grow at 2% and 4% NaCl as well as 45^{0}C and negative tests to arginine and citrate hydrolysis hence can be classified to be presumptive *Lactococcus lactis subsp. cremoris* according to [24].

C23 did not produce gas from glucose a homo-fermentative organism. It did grow at 2% and 4% and 10% NaCl as well as 45^{0}C, although it gave negative tests to arginine and positive for citrate hydrolysis hence can be classified to be a presumptive *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* according to [25].

In this study, *Lactococcus* and *Lactobacillus* species were the only LAB species isolated from fermented cocoa bean which corresponds to findings from [26]. stating that both homo-fermentative and hetero-fermentative LAB occur in cocoa fermentations, however the majority are homo-fermentative.

The genus *Lactobacillus* comprises of about 200 species and plays a great role as functional starter culture in wide variety of fermented food products, including cheese, fermented plant-derived foods, fermented meats, wine and beer production, as well as sourdoughs [27]. However, they have been found to play an important role during the cocoa bean fermentation process [28]. Pyruvate, a by-product of yeast fermentation is metabolized by hetero-fermentative *Lactobacilli* to various acids helping in flavour development [29]. Belonging to this genus is hetero-fermentative *L. fermentum* which is one of the specie that is mainly found in this process. It is one of the first group of LAB strains that is seen at the initial stage of the fermentation process [30; 31; 32]. From the Table 4 and Table 5 it can be seen that *L. fermentum* produced acid and gas from fructose and citric acid. This may probably be due to the fact *L. fermentum* being a hetero-fermentative LAB is able to convert citric acid and fructose to alternative external electron acceptors enhancing their competitiveness and resulting in the production of mannitol and succinate, or lactate and flavour-active compounds such as 2,3-butanediol or acetoin, respectively [33; 9]. This makes *Lactobacillus fermentum* a potential starter culture for flavour enhancement. *Lactobacillus fermentum* has been found to possess enzymes such as citrate lyase, α-acetolactate synthase, α-acetolactate decarboxylase and 2, 3-butanediol dehydrogenase in their genome sequence [34; 35].

L. brevis is a rod with round ends and are non-motile hetero-fermentative. They are normally isolated from milk, cheese, sauerkraut, sourdough, cow manure among others. It has the ability to ferment arabinose, fructose, glucose, maltose as well as ribose and can also hydrolyse arginine to obtain ammonia [36].

Lactococcus species are Gram positive LAB that are homo-fermentative, catalase negative, non-sporulating and non-motile. They are one of the most important microorganisms in the food industry [37]. They can be used in formulating single-strain starter cultures or in mixed-strain starter cultures with other LAB such as *Lactobacillus* to aid in developing the flavour of the final product of cocoa bean when used in cocoa fermentation [17]. *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *Lactis biovar* diacetylactis are two subspecies belonging to the *Lactococcus* species. They use enzymes such as β-galactosidase to convert lactose to energy molecules (ATP) whiles yielding lactic acid as by-product. The lactic acid produced can then increase acidity of the cocoa beans during fermentation and contribute to the characteristic taste of the cocoa bean [38; 29]. Hence they can be helpful starter cultures for flavour development in cocoa beans. It is reported that *L. lactis* subsp. *Lactis biovar* diacetylactis have higher salt tolerance (4-10%) compared to *L. lactis* subsp. *cremoris* (2%) [16; 39; 40] corresponding to findings from this study.

From Table 6, it can be seen that *L. lactis* subsp. *cremoris* (C17) produced a higher amount of lactic acid (185g lactic acid / L of culture medium) compared to *L. lactis* subsp. *lactis* biovar *diacetylactis* (C23) which produced the lowest (95g lactic acid / L of culture medium). However, they are both homo-fermentative LAB strains. This corresponds to the findings of [41], that *L. lactis* subsp. *lactis* biovar *diacetylactis* produces more of acetaldehyde whiles *L. lactis* subsp. *cremoris* produces more acid.

For the *Lactobacillus* species, it can be seen that *L. fermentum* produced lower amount of lactic acid (120g lactic acid / L of culture medium) as compared to the amount of lactic acid produced by *L. brevis* (155g lactic acid / L of culture medium) as shown in Table 4.5. This is due to the ability of *L. brevis* to utilize mannitol to produce more of lactic acid as a major metabolite whiles *L. fermentum* utilizes more of citric acid and fructose to produce mannitol and succinate, or lactate and flavour-active compounds such as 2,3-butanediol or acetoin [42; 33; 9].

The study showed less microbial growth on the plate for the various dilutions on day 1 and day 2. Lactic Acid Bacteria were screened for and isolated. The strains were identified to belong to

Lactococcus and *Lactobacillus* species based on their morphological, physiological and biochemical characteristics. *Lactococcus* species identified were presumed to be *lactococcus lactis* subsp *lactis* biovar *diacetylactis and lactococcus lactis* subsp. c*remoris*. Also, the *lactobacillus* species identified were presumed to be *lactobacillus fermentum* and *lactobacillus brevis*. The amounts of lactic acid produced by the various LAB strains were determined by measuring pH and titrating with 0.1M NaOH. *Lactococcus lactis subsp. cremoris* produced the highest amount of lactic acid (185g lactic acid / L of culture medium) with *Lactococcus lactis subsp. lactis biovar diacetylactis* producing the least amount of lactic acid (95g lactic acid / L of culture medium). Lactic acid amounts of was 120g lactic acid / L of culture medium and 155g lactic acid / L of culture medium were produced by *Lactobacillus fermentum* and *Lactobacillus brevis* respectively. The above presumptive LAB species can be used in starter culture formulation for controlled cocoa fermentation to improve cocoa bean quality and flavour.

Conclusion

The study showed less microbial growth on the plate for the various dilutions on day 1 and day 2. Lactic Acid Bacteria were screened for and isolated. The strains were identified to belong to *Lactococcus* and *Lactobacillus* species based on their morphological, physiological and biochemical characteristics. *Lactococcus* species identified were presumed to be *lactococcus lactis* subsp *lactis* biovar *diacetylactis and lactococcus lactis* subsp. c*remoris*. Also, the *lactobacillus* species identified were presumed to be *lactobacillus fermentum* and *lactobacillus brevis*. The amounts of lactic acid produced by the various LAB strains were determined by measuring pH and titrating with 0.1M NaOH. *Lactococcus lactis subsp. cremoris* produced the highest amount of lactic acid (185g lactic acid / L of culture medium) with *Lactococcus lactis subsp. lactis biovar diacetylactis* producing the least amount of lactic acid (95g lactic acid / L of culture medium). Lactic acid amounts of was 120g lactic acid / L of culture medium and 155g lactic acid / L of culture medium were produced by *Lactobacillus fermentum* and *Lactobacillus brevis* respectively. The above presumptive LAB species can be used in starter culture formulation for controlled cocoa fermentation to improve cocoa bean quality and flavour.

List of Abbreviations

AAB: Acetic Acid Bacteria
BCP: Bromocresol Purple
CAN Lab: Clinical Analysis Laboratory
CFU: Coliform Unit
C16, 17, 20, 23: Colony 16, 17, 20, 23
CRIG: Cocoa Research Institute of Ghana
EPS: Exopolysaccharides
KNUST: Kwame Nkrumah University of Science and Technology
L: Lactococcus
LAB: Lactic Acid Bacteria
MRS: de Man, Rogosa and Sharpe
SPP: Species
W/v: Weight per volume

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