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Analysis of Environmental Conditions Affecting Acids Production in Lactic Acid Bacteria Involved in Ivorian Cocoa Fermentation

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Abstract | **Original Research Article**

Acetic and lactic acids produced during cocoa fermentation impact strongly the quality of fermented cocoa beans and chocolate. In this study, acid production from various carbon source and environmental conditions was analyzed in five lactic acid bacteria previously characterized as citrate lyase producers. It appears that production of acetic and lactic acids by these strains is strongly dependent on environmental conditions such as pH and temperature. Indeed, maximum yield of acids occurred at 30°C and pH 4 in *Lactobacillus plantarum* strain T11G3 and *Lactobacillus casei* strain T10G5. On the other hand acid production was maximum at 40°C and pH 4 in *Lactobacillus plantarum* strains T8N10, T10AG26 and at 40°C and pH 4.5 in *Leuconostoc mesenteroides* strain T8AB6. These strains also presented variable acid production regarding the sugar tested with sucrose allowing the highest acid yield (38.12 mg/mL) comparatively to that obtained from glucose (23.91 mg/mL) and fructose (26.28 mg/mL). Moreover, maximum acid production in all the strains was rapidly achieved, after 36-48h fermentation with sucrose, whereas a delayed production (around 72 h) was observed with glucose or fructose. Alcoholic conditions were found to particularly enhance acid production in the strains studied (*Leuconostoc mesenteroides* strain T8AB6). The maximum yield of acetic and lactic acids was 12.7 mg/mL and 14.4 mg/mL respectively in *Leuconostoc mesenteroides* strain T8AB6 and *Lactobacillus plantarum* strain T8N10 under citric acid condition. Overall, this study highlights that carbon source and environnemental condition impact on the production of acidic compounds by LAB from fermenting cocoa. Furthermore, variability in acid production was observed between the studied strains in most of the considered conditions. Thus, the different LAB strains studied probably behave as complementary consortium to continuously achieve the process of natural cocoa fermentation.

Keywords: Acetic acid production, lactic acid production; lactic acid bacteria; starter; cocoa fermentation; Ivory Coast.

INTRODUCTION

Fermentation is an indispensable step in the transformation process of cocoa into chocolate, particularly on the flavor, color and aroma [4, 22]. During the fermentation process, cocoa pulp is degraded following complex biochemical reactions in which take part micro-organisms mainly yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and *Bacillus* [3, 17, 18]. Yeasts degrade the viscous pectin contained in cocoa pulp and produce ethanol from carbohydrates [7, 19]. LABs consume carbohydrates (glucose and fructose) and citric acid to produce lactic acid, acetic acid, and acetoin [13, 16]. In addition, lactic acid and ethanol serve as substrates for acetic acid production by AAB [5, 13]. The penetration of acids and heat deep into beans lead to activation of hydrolysis reaction and a quick degradation of the storage proteins and carbohydrates by seed-derived enzymes into peptides, free amino acids and reducing sugars, that are the precursors of chocolate flavor [10, 22, 23]. Moreover, the phenolic compounds undergo browning reactions resulting in a decrease in astringency and in the formation of the typical brown colour of the well-fermented cocoa beans [2, 12, 23].

Finally, organic acids produced by the microbiota during fermentation are highly important to develop the characteristic features notably the chocolate aroma. Recently, we reported the implication of LAB in the production of citric acid degrading enzymes (citrate lyase) during cocoa fermentation, as a fundamental property for a successful cocoa fermentation process [16]. However, the potential of acidification of these bacteria remains to be elucidated since they could be used as valuable starter strains to improve cocoa fermentation.

In this paper we report the influence of environmental conditions on lactic and acetic acids

production in LAB isolated from cocoa fermenting beans.

MATERIALS AND METHODS

Bacterial strains

Previously we isolated from fermenting cocoa and characterized five LAB strains belonging to *Lactobacillus plantarum* (strains T11G3, T10AG26 and T8N10), *Leuconostoc mesenteroides* (strain T8AB6) and *Lactobacillus casei* (strain T10G5). These strains capable to breakdown citric acid during the process of cocoa fermentation [16] were used in this study as model to analyze the potential of acid production in LAB under different culture conditions.

Acidification capability of LAB strains from various carbon substrates

A qualitative analysis was first performed to assess the capability of strains to produce acid by catabolizing different sugars such as arabinose, glucose, fructose, saccharose, galactose, lactose, maltose, mannitol, mannose, rhamnose, xylose, mélibiose and raffinose. This study was performed in a liquid medium A, containing 1% casein peptone; 0.4% yeast extract; 0.2% K_2HPO_4; 0.02% $MgSO_4$; 0.004% $MnSO_4$; 2 % appropriate carbohydrate and 0.005 % of bromocresol purple as a pH indicator. The medium A was spread into 20 mL tubes, each tube containing 10 mL of medium and then sterilized for 15 min at 121 °C. LAB cells were first grown on MRS (De Man Rogosa Sharpe) plate medium (Oxoid, Abidjan) for 24 h, at 30 °C. Then cells were pelleted by centrifugation (6000 ×g) and resuspended tryptone salt to obtain a microbial suspension of OD_{600}= 0.5. An aliquot (100 µL) of this suspension was then used to inoculate 10 mL of medium A described above. A negative control was carried out in the same conditions but not inoculated with strains. The ability of each strain to produce acid from each sugar is assessed by the change of the medium color that becomes yellow comparatively to the negative control that remains purple.

Effects of culture conditions on acids production by LAB

This study consisted in analyzing the yield of acids from LAB strains cultured under various pH and temperature and different content of compounds such as ethanol and citric acid. These culture conditions are that mainly encountered in natural cocoa fermentation [21]. Strains were grown in a standard liquid medium containing 0.8% citric acid; 0.2% ammonium citrate; 1% casein peptone; 0.4% yeast extract; 1% glucose; 0.2% K_2HPO_4; 0.02% $MgSO_4$ and 0.004% $MnSO_4$. To analyze the influence of temperature on acids production in lactic acid bacteria, ten milliliters of standard liquid medium contained in a 50 mL test tube, were inoculated with 100 µL of bacterial, suspension OD_{600}= 0.5 (described above). Then, cultures were incubated for 72 h at different temperatures (30; 35; 40 and 45 °C). Effects of pH variations on lactic and acetic acids production were analyzed in the same medium buffered alternatively with HCl or KOH at the following pH values 3.5; 4; 4.5; 5 and 6, incubated at-single temperature (30 °C). To analyze the influence of ethanol and citric acid contents on lactic and acetic acids production, bacteria were grown in the standard medium containing different ethanol concentrations (2; 4; 6; 8; 10; 12 and 15%) and citric acid (0.2; 0.5; 0.8; 1; 1.5; 2 and 2.5) incubated at 30 °C. For the influence of temperature and ethanol the pH of the liquid medium was maintained at 3.8 using KOH. After incubation, the bacterial growth was measured via absorbance at 600 nm against the medium. The cell extract was clarified by centrifugation at 7830 rpm for 30 min and the resulting supernatant was used to quantify by HPLC the lactic and acetic acids production.

Quantification of lactic and acetic acids using high performance liquid chromatography

The supernatant previously obtained, was used for the determination of lactic and acetic acids using a new approach based on separation by High Performance Liquid Chromatography (HPLC). The column used is a Hypersyl GOLD aQ (250 × 4.6 mm), with a particle size of 5 µm (Thermo Fisher, France). Lactic and acetic acids were separated using the following elution protocol, with solvents A and B composed of 50 mM KH2PO4 MilliQ water made up to pH 2.65 with H2PO4 and acetonitrile (or methanol), all of HPLC grade. Separation was carried out at 25 °C with a flow rate of 1 mL/min and under the following conditions: pre-equilibration for 10 min in buffer A prior to injection; 0 min, 100% A; 8 min, 100% A; 10 min, 100% B; 12 min, 100% B; 13 min 100% A; 15 min, 100% A before a new injection, as described. The separation procedure was performed with an Alliance HT Waters 2795 separation module and for detection a Waters 2996 photodiode array detector (PDA) (Waters, France) was used. A wavelength of 210 nm and a spectrum from 210 to 400 nm were employed for detection of the lactic and acetic acids peaks. Under our conditions, lactic and acetic acids were eluted at retention times of 4.70 and 5 min, respectively. In culture media runs, the identity of these compounds was evaluated by co-injection with authentic standards. Quantification was performed by measuring peak areas using the Mass-Link 4.1 software (Waters, France). Peak areas were then compared with standard curves, realized for each acid in order to measure the amount in each sample in mg/ml. Linear standard curves were obtained by increasing the injected quantity of each acid to determine the slope as a correcting factor. Slopes of 1.E+7 (R2 of 0.99) and 6.E+6 (R2 of 0.98) were determined, respectively, for lactic and acetic acids.

Carbohydrate consumption and acid production in LAB strains

The carbohydrate consumption and metabolite production in each strain were carried out in medium A, containing 2% of glucose or fructose or sucrose as substrate, purple bromocresol free, and adjusted to pH 7. Then 100 mL of this medium were distributed in a 250 mL Erlenmeyer flask and autoclaved at 121°C for 20 min. The medium was inoculated with 1 mL of suspension medium described above (OD_{600} = 0.5). The inoculated medium was then incubated at 30°C for 72 h. At 12 h intervals, 10 mL of sample are withdrawn to monitor biomass evolution by measuring absorbance at 600 nm and quantify the residual carbohydrate (glucose, fructose or sucrose) and lactic and acetic acids in the supernatant after centrifugation at 7830 trs/min for 15 min. Concentration of lactic and acetic acids was determined by HPLC (as described in the section 2.4).

RESULTS

Acidification capacity of lactic acid bacteria (LAB)

To investigate the acidification spectrum of the LAB strains (*Lactobacillus plantarum* strains T11G3, T10AG26, T8N10, *Lactobacillus casei* strain T10G5 and *Leuconostoc mesenteroides* strain T8AB6), a wide range of sugars including the main cocoa pulp sugars (glucose, fructose and sucrose) and others substrate such as arabinose, galactose, lactose, maltose, mannitol, mannose, rhamnose, xylose, melibiose and raffinose were tested. The results showed that the five strains were able to utilize most of the sugars tested regarding the color change of the culture medium into acidic range that was considered as positive test (Table 1). Almost all the sugars tested were fermented by LAB strains analyzed. However some slight metabolic differences were observed. Hence *Leuconostoc mesenteroides* strain T8AB6 and *Lactobacillus casei* strain T10G5 were not able to ferment mannitol and raffinose respectively. Moreover, rhamnose was only fermented by *Lactobacillus plantarum* strains T11G3 and T8N10, while xylose was only fermented by *Leuconostoc mesenteroides* strain T8AB6 (Table 1).

Moreover the resuts show that among the main cocoa pulp sugars (glucose, fructose and sucrose), sucrose is preferentially fermented by most of the strains tested based on the high quantity of acid yielded from this sugar (Table 2). Indeed, the quantity of lactic acid obtained from sucrose is greater than that obtained from glucose and fructose in the five strains. For instance, in *Leuconostoc mesenteroides* strain T8AB6, maximum acids production from sucrose was approximately two-folds more important comparatively to maximum acid production from glucose (Table 2). Lactic acid yield was lower with fructose used as subtrate for bacterial growth (Table 2). However, the time course of acid yield show that maximum acids production occured at different time depending on the strain (data not shown). Hence, maximum acids production occured rapidly with sucrose as subtrate, after 36 to 60 h of incubation time whereas fructose allowed the slowest acid production (Table 2). More, *Lactobacillus plantarum* strain T10AG26 reached maximum acid production (32.15 mg/mL) after 36 h of sucrose fermentation, whiles maximum acids production with glucose and fructose as subtrate occured at 48 h and 72 h of fermentation, respectively.

Effect of temperature on acids production by LAB strains

High Performance Liquid Chromatography (HPLC) was used to identify the products obtained when the five strains were subjected to thermal stress under temperatures ranging from 30 to 45 °C with regards to the conditions occuring in the natural fermentation of the cocoa. The results indicate that, the analyzed LAB strains produce mainly two acids from glucose, notably lactic acid as major product and acetic acid in less important quantity. Lactic acid yield was approximately 31-fold higher than that of acetic acid (Fig. 1). The strains gather in two distinct groups depending on acid production pattern at variable temperatures. The first group including *Lactobacillus plantarum* strains T10AG26, T8N10 and *Leuconostoc mesenteroides* strain T8AB6 showed an increasing production of lactic acid from 6.10 to 7.69 mg/mL when the growth temperature increase from 30 to 40°C. The maximum production of lactic acid in these strains (*Lactobacillus plantarum* strains T10AG26, T8N10 and *Leuconostoc mesenteroides* strain T8AB6) occurs at 40°C and reaches a level of 7.33, 7.58 and 7.69 mg/mL, respectively. Accordingly, a sharp decrease of lactic acid yield occured above 40°C, dropping to 0.42 - 1.04 mg/mL at 45°C (Fig. 1). The other group of LAB including *Lactobacillus casei* strain T10G5 and *Lactobacillus plantarum* strain T11G3 showed a continuous and regular decrease of acid yield as the temperature increases from 30 to 45°C. Indeed in this group, maximum production of lactic acid reached 6.68 and 7.45 mg/mL, obtained at 30°C respectively for *Lactobacillus casei* strain T10G5 and *Lactobacillus plantarum* strain T11G3. Between 30 and 40°C, a 2 to 8-folds decrease of this production was observed for *Lactobacillus plantarum* strain T11G3 and *Lactobacillus casei* strain T10G5, respectively. Likewise, a strong decrease of acid production from 4.03 mg/mL at 40°C to 0.35 mg/mL at 45°C was observed with the *Lactobacillus plantarum* strain T11G3 (Fig. 1). The most thermosensitive *Lactobacillus casei* strain T10G5, almost failed to produce lactic acid at 40°C. With regard to the acetic acid, the production is low and varies very little under the thermal stress in the five strains studied.

pH Influence on acids production in LAB strains

pH variation is one of the most important parameters contributing to the dynamic conditions occurring during the fermentation process of cocoa. Regarding these conditions, LAB strains were grown in different pH and the resulting acid production was analyzed (see section 2.3 material and methods). The

results show that the analyzed LAB strains produce two acids, notably lactic acid and acetic acid (Fig. 2). The strains gather in three groups; the first group including *Lactobacillus casei* strain T10G5 and *Lactobacillus plantarum* strain T11G3 show a production of lactic acid between 5.26 mg/mL and 6.43 mg/mL in pH 4-5 range. Out of this pH interval, these strains present a low production of lactic acid. The second group of LAB including *Lactobacillus plantarum* strains T8N10 and T10G26 yields maximum amount (6.97 mg/mL) of lactic acid at pH 3.5, this yield decrease gradually with the increase of pH, to reach approximately 3.54 mg/mL at pH 6 (Fig. 2). In both groups the bacteria failed to produce acetic acid. *Leuconostoc mesenteroides* strain T8AB6 classified in the third group has a similar pattern of lactic acid production as those of the second group in the pH 3.5-5 range. Maximum lactic acid production was around 6.015 mg/mL at pH 3.5, decreasing to 2.425 mg/mL at pH 5. Strains of this group were particularly able to produce acetic acid at 3.61 mg/ mL. However, this strain also failed to produce acetic acid at pH 7. The decrease of acids production in increasing pH range appears to be the common feature for all the strains analyzed (Fig. 2).

Acids production in LAB from citric acid as substrate

Due to the relatively high content of citric acid in the cocoa pulp the ability of the five strains to produce lactic and acetic acids under different concentrations of this acid was investigated. Three profiles emerge from this study. *Lactobacillus plantarum* strains T10AG26 and T8N10 appear as strict homofermentative since they mainly produce lactic acid from citric acid (Fig. 3). The maximum yields of lactic acid in these strains reached 14.4 mg/mL from 1 % citric acid. On the other hand, *Lactobacillus plantarum* strain T11G3 and *Lactobacillus casei* strain T10G5 rather displayed heterofermentative type with increasing production of acetic acid when the concentration of citric acid is scaled up in the medium. Lactic and acetic acids produced by these strains increased respectively from 6.08 to 13.65 mg/mL and from 2.93 to 10.3 mg/mL (Fig. 3). These concentrations of acids are mainly produced in the strains growing in 0.8-1 % citric acid. *Lactobacillus plantarum* strain T11G3 and *Lactobacillus casei* strain T10G5 express maximum yields of lactic and acetic acid with 0.8 and 2.5 % of citric acid. However, in *Lactobacillus plantarum* strains T11G3 and T10AG26, the production of these metabolites (lactic and acetic acids) is inhibited by 2 % of citric acid. For *Leuconostoc mesenteroides* strain T8AB6, heterofermentative, the maximum concentration of lactic (9.97 mg/mL) and acetic acids (12.7 mg/mL) produced is obtained with 1.5% citric acid (Fig. 3).

Acids production in LAB growing in alcoholic conditions

Alcohol is the main metabolite produced by yeasts during the anaerobic phase of the cocoa fermentation process [9, 20]. Since alcohol concentration can reach up to 7.1 % in the fermenting cocoa, acids production by LAB in different concentration of alcohol was therefore analysed. The results indicated that acids secretion by LAB increase from 4.3 to 14.55 mg/mL for lactic acid and 0.95 to 21.29 mg/mL for acetic acid in ethanol contained medium ranging from 2 to 8 % alcohol (Fig. 4). Concentrations of ethanol from 6 to 8% promote maximum production of acetic acids. At these concentrations, acetic acid is obtained predominantly with values ranging from 14.2 to 21.29 mg/mL. Beyond 8 % of ethanol, the amounts of acetic acid formed decreased sharply and the strains could not produce acetic acids at 15 % ethanol. At this concentration only lactic acid is produced. The maximum production of lactic acid (8.93 to 15.53 mg/mL) is obtained with 4 to 10 of ethanol (Fig. 4).

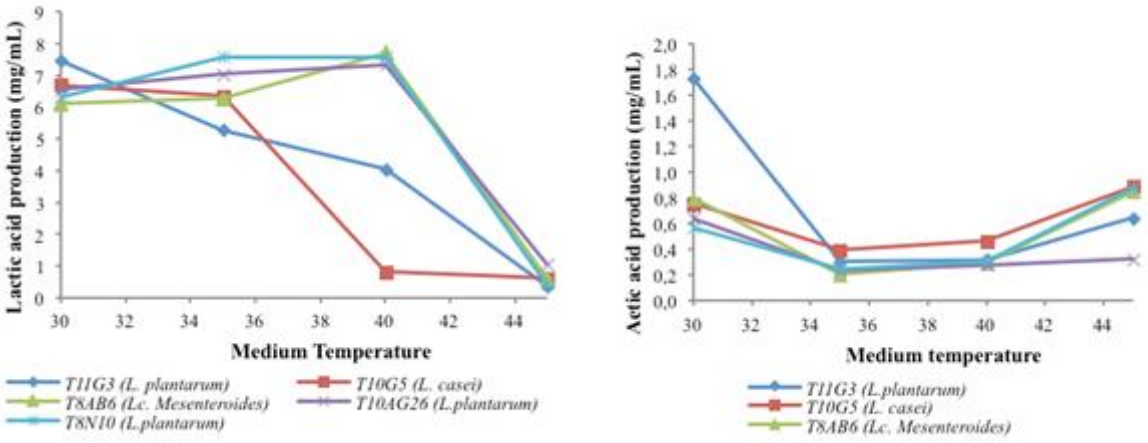

Fig-1: Influence of temperature on acetic and lactic acids production in lactic acid bacteria

Cells were grown in liquid medium containing 0.8% citric acid; 0.2% ammonium citrate; 1% casein peptone; 0.4% yeast extract; 1% glucose; 0.2% K_2HPO_4; 0.02% $MgSO_4$; 0.004% $MnSO_4$; pH 3.8, and incubated at differents temperatures from 30°C to 45°C for 72h.

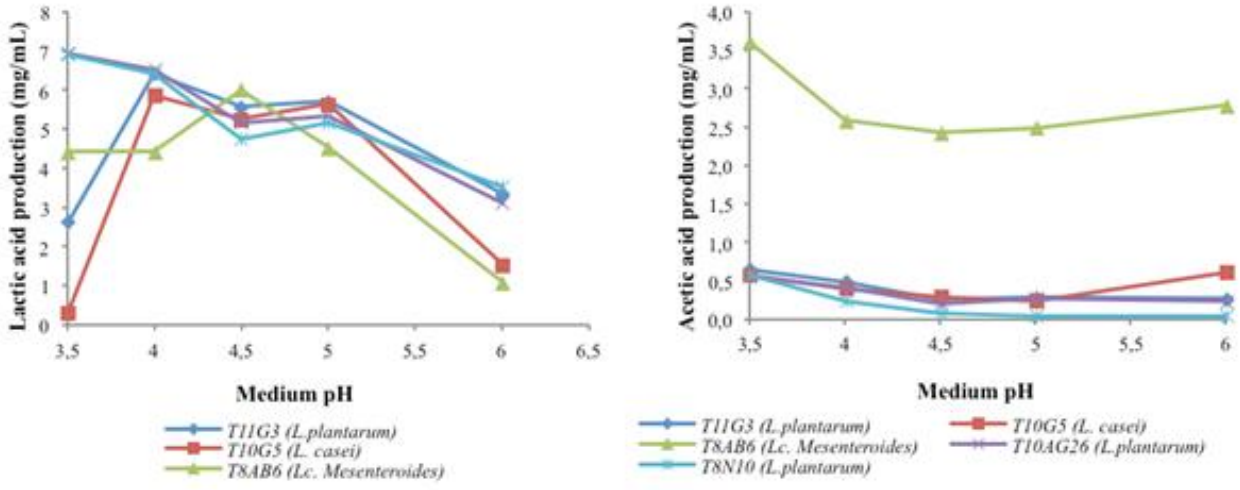

Fig-2: Influence of pH on acetic and lactic acids production in lactic acid bacteria at 30°C for 72h.

Cells were grown in liquid medium containing 0.8% citric acid; 0.2% ammonium citrate; 1% casein peptone; 0.4% yeast extract; 1% glucose; 0.2% K_2HPO_4; 0.02% $MgSO_4$; 0.004% $MnSO_4$; different pH from pH 3.5 to pH 6 and incubated.

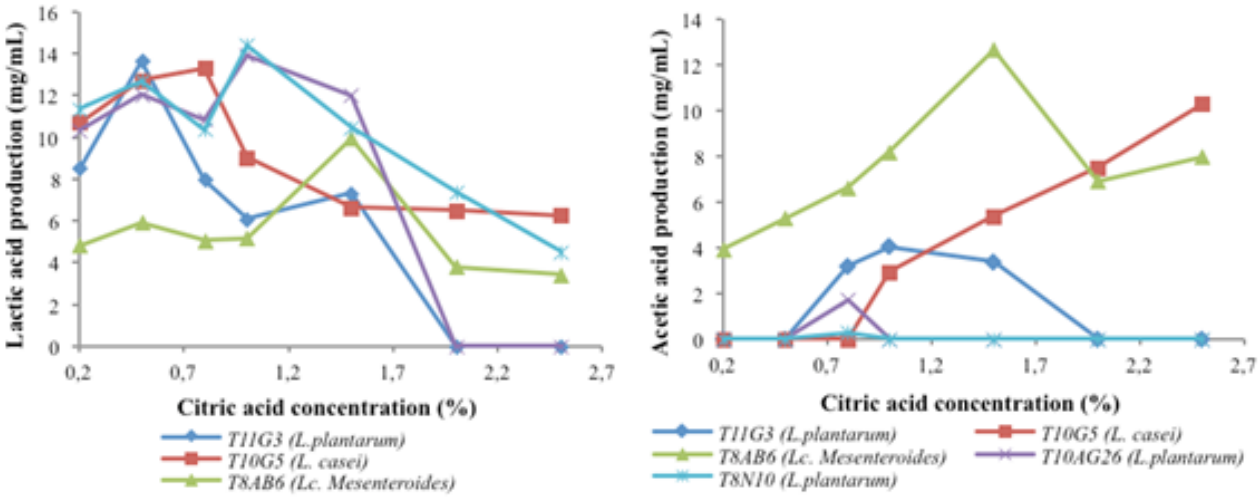

Fig-3: Influence of citric acid on acetic and lactic acids production in lactic acid bacteria

Cells were grown in liquid medium containing 0.2% to 2.5% citric acid; 0.2% ammonium citrate; 1% casein peptone; 0.4% yeast extract; 1% glucose; 0.2% K_2HPO_4; 0.02% $MgSO_4$; 0.004% $MnSO_4$ and incubated at 30°C for 72h.

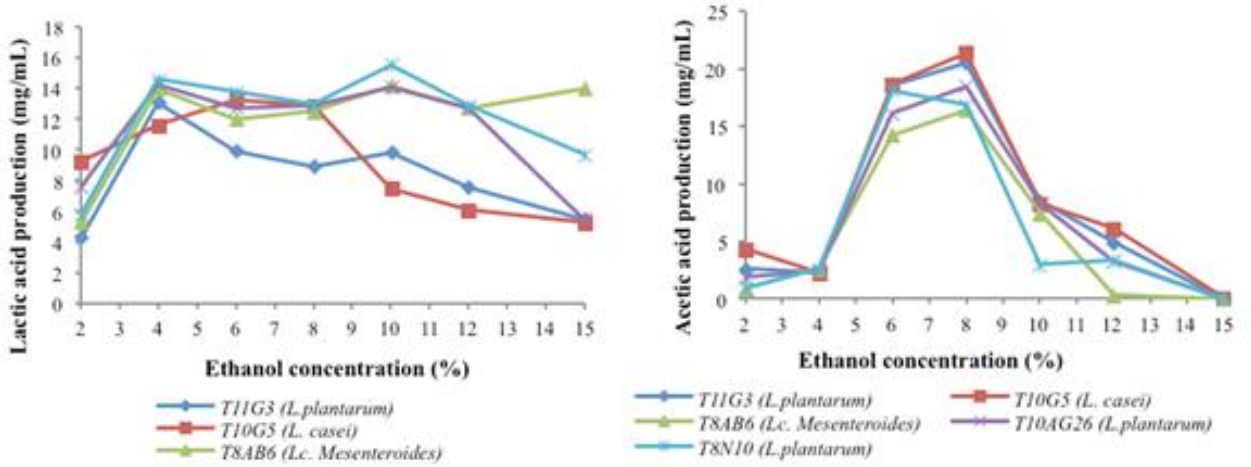

Fig-4: Influence of ethanol on acetic and lactic acids production in lactic acid bacteria

Cells were grown in liquid medium containing 0.8% citric acid; 0.2% ammonium citrate; 1% casein peptone; 0.4% yeast extract; 1% glucose; 0.2% K_2HPO_4; 0.02% $MgSO_4$; 0.004% $MnSO_4$; 2% to 15% ethanol and incubated at 30°C for 72h.

Table-1: Metabolism of various sugars in studied lactic acid bacteria

Strains	Suc	Fru	Glu	Gal	Lac	Mal	Mtl	Man	Rha	Ara	Xyl	Mel	Raf
L. plantarum (T11G3)	+	+	+	+	+	+	+	+	+	+	-	+	+
L. casei (T10G5)	+	+	+	+	+	+	+	+	-	+	-	+	-
Lc. Mesenteroides (T8AB6)	+	+	+	+	+	+	-	+	-	+	+	+	+
L. Plantarum (T10AG26)	+	+	+	+	+	+	+	+	-	+	-	+	+
L. plantarum (T8N10)	+	+	+	+	+	+	+	+	+	+	-	+	+

Cells were cultivated in liquid medium containing 1% casein peptone; 0.4% yeast extract; 0.2% K_2HPO_4; 0.02% $MgSO_4$; 0.004% $MnSO_4$; each carbohydrate (2% final concentration) and supplemented with 0.005 % of bromocresol purple as a color indicator pH and incubated at 30°C for 48 h. In this table «+» indicates that the strain metabolizes the carbonaceous substrate while «-» indicates that the strain does not metabolize the substrate. Ara: arabinose; Glu: glucose; Fru: fructose, Suc: sucrose; Gal: galactose; Lac: lactose; Mal: maltose; Mtl: mannitol; Man: mannose; Rha: rhamnose; Xyl: xylose; Mel: melibiose; Raf: raffinose.

Table-2: Lactic acid production from various sugars in studied lactic acid bacteria

Strains		Maximum acid production (mg/mL)		
		Substrates		
		Glucose	Fructose	Sucrose
L. plantarum (T11G3)	Lactic acid	23.91	21.47	30.94
	Time (h)	72	72	36
L. casei (T10G5)	Lactic acid	21.78	14.42	25.74
	Time (h)	72	72	60
Lc. Mesenteroides (T8AB6)	Lactic acid	20.65	17.36	38.12
	Time (h)	60	72	60
L. plantarum (T10AG26)	Lactic acid	20.58	13.14	32.15
	Time (h)	48	72	36
L. plantarum (T8N10)	Lactic acid	21.73	26.28	32.22
	Time (h)	60	72	60

Cells were cultivated in liquid medium containing 1% casein peptone; 0.4% yeast extract; 0.2% K_2HPO_4; 0.02% $MgSO_4$; 0.004% $MnSO_4$; 2% glucose, fructose or sucrose, pH 7 and incubated at 30°C for 72h.

DISCUSSION

Acid production during fermentation is crucial functional property for a quality chocolate [2, 5, 12]. This study investigated the variation of acid production in regard to environmental conditions in Lactic Acid Bacteria (LAB) isolated from fermenting cocoa. Five strains were used as model. The results show that the strains behave differently in response to temperature variations. Hence, maximum acid production occur at 30°C in some strains (*Lactobacillus casei* strain T10G5 and *Lactobacillus plantarum* strain T11G3) and at 40°C in other strains (*Lactobacillus plantarum* strains T10AG26, T8N10 and *Leuconostoc mesenteroides* strain T8AB6) indicating a functional diversity of LAB analyzed. A mixture of the different groups of strain during cocoa fermentation may ensure a continuous yield of acids since temperature variation was reported during the cocoa bean fermentation process [6, 21]. For instance, *Lactobacillus plantarum* strain T11G3 and *Lactobacillus casei* strain T10G5 could initiate fermentation at low temperature round 30°C, then as the fermenting cocoa temperature is rising [6, 21]. *Lactobacillus plantarum* strains T10AG26, T8N10 and *Leuconostoc mesenteroides* strain T8AB6, more thermotolerant, could relay this production.

Likewise, the strains also responded differently to pH variation with a maximum acid production at pH 4 for *Lactobacillus plantarum* strains T8N10, T10AG26; T11G3, *Lactobacillus casei* strain T10G5 and pH 4.5 for *Leuconostoc mesenteroides* strain T8AB6. Cocoa pulp contains many sugars that were found to influence lactic acid production in LAB with sucrose allowing maximum yield of lactic acid. So the content of pulp in sugar, particularly sucrose that is known to have a genetic background [8] may be one of the main factors modulating lactic acid production from LAB during cocoa fermentation. This suggests that strain performance for acid production during fermentation is also related to cocoa genotype. Moreover, citric acid is a natural component of cocoa pulp and the breakdown of this acid is assumed to modulate the pH of the fermenting cocoa pulp and influence the microbial growth [1, 14] that may in turn impact the final quality of cocoa product. In this study, citric acid was found to be metabolized differently since it was transformed into lactic and acetic acids by *Lactobacillus plantarum* strain T11G3 and *Lactobacillus casei* strain T10G5 while *Lactobacillus plantarum* strains T10AG26 and T8N10 produced only lactic acid from citric acid as substrate. One of the most interesting features was the ability of *Lactobacillus plantarum* strains T10AG26 and T8N10 to breakdown citric acid at high concentration into lactic acid and acetic acid indicating that they could play a particular role in decreasing the acidity of fermenting cocoa presenting very acidic pulp. Furthermore, the breakdown of citric acid in these strains is accompanied by an increase of acids that generally penetrate into beans and/or undergo subsequent reactions [9, 22], leading to their deasapearance; so acids produced are not intended to acidify the cocoa pulp. On the other hand, this study also showed the occurrence of LAB strains (*Lactobacillus plantarum* strains T10AG26 and T8N10) that failed to degrade citric acid at high concentration. In the cocoa ecosystem, the balance between LAB able to breakdown high concentration citric acid and LAB degrading citric acid at low concentration may take an important place in the homeostasis and regulation of acids yield from citric acid metabolism. Indeed, at high concentrations, acids from microbial metabolism are likely to impair cocoa quality [11].

Cocoa fermentation is characterized by a successive involvement of various microbes during which maximum growth of LAB occurs after yeasts action that yields important quantity of ethanol [3, 19, 22]. In the present study, alcoholic conditions were found to promote lactic and acetic acids yields from LAB. The ability of LAB to metabolize ethanol into acetic acid was previously reported by [15] suggesting that the increase of acids yield in the observed alcoholic conditions may result from direct oxidization of ethanol into acetic acid by LAB. This result also point out the relevance of yeasts growth that prepare alcoholic conditions for optimized acids production from LAB in fermenting cocoa. A probable existence of interaction between yeasts and LAB as a key factor for acids yields during on-farm processing of cocoa is therefore hypothesized.

As a conclusion, the variabilities observed in these five strains show they are certainly reflect specific but complementary involvement in the cocoa bean fermentation process. This complementarity could contribute to achieving an effective microbial cocktail for a well processed fermentation of cocoa.

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