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Lactic Acid Bacteria (LAB) strains are actively involved in the occurrence of aroma compounds during cocoa fermentation

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Abstract

Fermentation is a crucial step in the processing of cocoa into chocolate. The role of microorganisms involved in this process is not well understood. In this study, we investigated the production of aroma compounds by Lactic acid bacteria (LAB) strains during fermentation in pulp simulation medium. LABs were isolated from fermenting cocoa in the region of Nawa (Côte d'Ivoire) and identified at species level using 16S RNA gene sequence phylogeny. The aroma compounds from microbial cultures were extracted using headspace microextration (SPME) method and quantified by Gas Chromatography-Mass Spectroscopy (GC-MS). The identified LAB species were mainly dominated by *Lactobacillus plantarum* and *Leuconostoc mesenteroides* with *Weissella paramesenteroides*, *Lactobacillus curieae* and Fructobacillu ssp as minor microbiota. The main aroma compounds produced by these LAB strains belonged to the class of alcohol namely methyl butanols (3.190 g/L) and 1,3-butanediols (2.582 g/L). Additionally, Lactobacillus plantarum unlike the other strains, produced ketones such as 2, 3-butadione (0.471 g/L) and 3 -hydroxy, 2-butanone (0.604 g/L). These compounds known to be responsible for desirable flavor note were yielded in quantities beyond their odor perception threshold. The maximum production of aroma compounds occurred in the pH range 4-5 and temperature around 35 °C. Taken together, these results indicated the implication of LAB strains in the production of aroma compounds during cocoa natural fermentation.

Keywords: Lactic acid bacteria, aroma compounds, cocoa fermentation, chocolate quality, role .

Résumé

La fermentation est une étape cruciale dans le processus de transformation technologique du cacao en chocolat. Le rôle des différents microorganismes impliqués dans le processus fermentaire reste encore à élucider. Dans cette étude, nous avons analysé l'implication de souches de Bactéries Lactiques (BL) dans la production d'aromes au cours de la fermentation. Les BL ont été isolées du cacao en fermentation dans la région du Nawa par la méthode standard sur gélose sélective MRS, et identifiées au niveau de l'espèce par séquençage du gène de l'ARN 16S. Les aromes produits en culture ont été extraits par la méthode de microextraction en phase solide, utilisant une microfibre et identifiés par chromatographie en phase gazeuse. Les résultats ont montré que les BL isolées incluaient des espèces dominantes comme *Lactobacillus plantarum* et *Leuconostoc mesenteroides*, et des espèces mineures comme *Weissella paramesenteroides*, *Lactobacillus curieae* et *Fructobacillu ssp*. Les principaux aromes produits par les BL appartenaient à la classe des alcools methyl butanols (3.190 g/L) et 1,3-butanediols (2.582 g/L) des cétones 2, 3-butadione (0.471 g/L) et 3-hydroxy, 2-butanone (0.604 g/L). Ces aromes qui sont connus pour être responsables de flaveurs désirables ont été produits à des quantités au delà du seuil de perception. La production maximale d'aromes par les BL survient à pH 4-5 et à des températures autour de 35 °C correspondant à la période 48-72 h de fermentation naturelle de cacao. L'ensemble des résultats de cette étude indique l'implication des BL dans la production d'aromes au cours de la fermentation du cacao.

Mots clés : Bactéries lactiques, aromes, fermentation du cacao, qualité du chocolat, rôle

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Introduction

Cocoa fermentation is a natural process, indispensible for beans transformation into chocolate (Samagaci, et al., 2016; Beckett, 2009). Processed entirely at farm level, cocoa fermentation is mainly performed by microbial strains namely yeasts, lactic acid bacteria, acetic acid bacteria and Bacillus (Ouattara, et al., 2017; Samagaci, et al., 2014; Soumahoro et al., 2015; Lefeber et al., 2011) The action of this microbiota is crucial for the development of the typical traits characteristic of a quality chocolate. Basically, yeasts transform the sugars into ethanol that is further oxidized into acetic acid by acetic acid bacteria (AAB) (Ho et al., 2018), whereas lactic acid bacteria metabolize sugar into lactic acid (Ouattara et al., 2017). These reactions happening in the pulp, the outer part of the bean, constitute the exogenous reactions.

Then, the products from exogenous reactions, particularly acids penetrate deep into the beans and activate endogenous hydrolytic reactions (Voigt et al., 2018) leading principally to the breakdown of proteins into amino acids and peptides known as the precursors of chocolate aroma (Voigt et al., 1994). This also leads, to the development of the brown color characteristic of a well-fermented cocoa bean as well as the reduction of their bitterness and astringency (Kongor et al., 2016; Eskes et al., 2012).

The role of acids (acetic acids and lactic acid) is crucial for chocolate flavor since they are the main activator of the pH-dependent hydrolytic enzymes namely aspartic endoprotease and serine carboxypeptidase that are responsible for protein hydrolysis into peptides (Voigt et al., 2018; Janek, et al., 2016). Hence, acids producing bacteria (LAB and AAB) are important for a well-processed fermentation of cocoa via they indirect action in the chocolate aroma development (Figueroa-Hernández et al., 2019).

However, another role of the microbiota, consisting in the direct production of aroma compounds in the fermenting cocoa was recently hypothesized. As to confirm this hypothesis, yeasts strains involved in cocoa fermentation were shown to produce aroma compounds that where found in the final chocolate (Koné et al., 2015; Meersman et al., 2016). This assess that microorganisms are capable of yielding aroma compounds susceptible to significantly influence the aroma profile of the final chocolate (Meersman et al., 2016). However, the contribution of acids producing bacteria to the occurrence of such aroma compound during cocoa fermentation remains unknown.

In this paper, we identified the aroma compounds yielded by the main LAB species involved in Ivorian cocoa fermentation.

Materials and Methods

Cocoa fermentation conditions, strains isolation and identification

Cocoa pods were collected from three areas of Côte d'Ivoire notably Agboville (geographic coordinates 5°59' North 4°28'West), Divo (5°55' North 5°37'West) and Aboisso (5°28'06' North 3°12'25' West). Heap fermentations of cocoa beans were conducted using local traditional methods processed for six days as described by Ouattara et al. (2017). During fermentation, the bacterial growth was monitored at 12 h intervals by withdrawing 100 g sample of fermenting cocoa collected in Stomacher bag for analysis purpose, using decimal dilution method. Then 25 g were homogenized in 225 mL of 0.1% (w/v) buffered (pH 7.2) peptone water (Oxoid, Basingstoke, United Kingdom) contained in a 500 mL sterile flask, and shaken for 2 to 5 min at room temperature to obtain an homogenous sample containing the bacteria. A serial dilution was then made from this suspension up to 108 and 0.1 mL of each diluted suspension was plated onto Mans De Rogosa-Sharpe (MRS) agar medium (Oxoid) supplemented with 50 µg·mL−1 of nystatin to inhibit fungal growth. Plates were incubated at 30 °C for 48–72 h, under anaerobic conditions. Then a maximum of 15 isolates were randomly selected from two successive dilutions medium for identification. The isolated bacteria were characterized using Gram reaction as well as oxidase, catalase and sporulation tests.

Molecular identification of isolated bacteria

Bacterial strains were further identified using genotypic approach. This consisted in targeting the 16S RNA gene in a PCR using the forward F27 (5-GGYRTGCCTAATACATGCAAGT-3) and reverse R1492 (5'-CCCGGGAACGTATTCACCGCG-3') primers previously

used by Ouattara et al. (2017). PCR reactions were run in a final volume of 50 µL containing 1 µL of bacterial suspension, 1.25 U of Taq DNA polymerase (Biolabs, Lyon, France), 5 µL of 10× standard buffer; 1 µL deoxynucleoside triphosphate (10 mM), 2 µL of each primer (10 µM) (Eurofins Genomics, Allemagne) and 38.75 µL of water. The template was composed of a bacterial suspension made with 24 h preculture in sterile water that was added (1 mL) to the reaction mixture.

PCR amplification was carried out in a Sensoquest Labcycler using the following program: After an initial denaturation at 95 °C for 4 min, reactions were run for 35 cycles, each cycle comprising: denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min and extension at 72 °C for 1 min. Finally, a 10 min extension at 72 °C was carried out. The presence and yield of specific PCR products were monitored using agarose 0.8% (w/v) gel electrophoresis at 70 V, for 2 h, in 1× Tris Borate EDTA buffer and visualized with ethidium bromide staining and UV transillumination. PCR products were purified using the nucleospinâ extract II kit (Macherey-Nagel, Germany) and sequenced by Eurofins Genomics (Germany) using the primer F27.

The basic local alignment search tool (BLAST, blastN) from the NCBI database site (blast.ncbi.nlm.nih.gov/) was used to find the closest sequences relative to the amplified 16S RNA genes in order to identify the species of our LAB. The phylogenetic tree was constructed from the partial 16S RNA gene sequences alignment using the maximum likelihood method (Tamura et al., 2013).

Culture conditions and volatile compound extraction

Bacterial strains were cultured in cocoa pulp simulation medium (PSM) containing 3 % fructose; 2 % glucose; 1 % sucrose; 1 % pectin; 1 % citric acid; 0.6 % yeast extracts; 0.6 % soya peptone; 0.2 % potassium sulphate; 0.2 % magnesium sulphate, 0.04 % manganese sulphate and 0.1 % Tween 80, adjusted to pH 6.0. The concentration for each compound (Sigma Aldrich, Pennsylvania, USA) is indicated as w/v.

Strains were grown at 30°C for 18-24 h in MRS broth, respectively. Colonies were picked and re-suspended in sterile distilled water to an OD600 = 0.05. This suspension was used to inoculate to 1% (v/v), 5 mL of PSM medium contained within a 20 mL sterilized vial suitable for GC-MS analysis (Restek, USA). The vial was sealed with an 18 mm cap with a thermo-resistant silicon septum, and was incubated at 30°C for 48 h. The negative control was non-inoculated medium incubated under the same conditions. Cultures were prepared in triplicate.

The extraction of the volatile compounds was performed using solid phase microextraction of the vial headspace (SPME-HS) using a 50/30 µm divinylbenzene/ carboxene/ polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco/Sigma Aldrich, Bellefonte, PA, USA) as previously described by Koné et al. (2015).

The fiber was first conditioned in the chromatograph injector at 250 °C for 3 min and then was exposed to the sample headspace at 50 °C for 45 min. Extracted volatile compounds sticked to the fiber were analyzed using an Agilent Technologies 7890A gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA) equipped with a Gerstel MPS2 multipurpose autosampler (Gerstel GmbH & Co. KG. Mülheim an der Ruhr, Germany) was coupled to an Agilent 5975C mass selective detector that was a Hewlett Packard DBWAX capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) (Agilent, Palo Alto, CA, USA). Agilent MassHunter GC/MS Acquisition software (Version B.07.00 SP2.1654) and Gerstel Maestro 1 software (Version 1.3.20.41) were used to control the instrument and autosampler, respectively.

The GC oven temperature was initially set at 40 °C, was held for 5 min, and then increased to 140 °C at a rate of 2 °C/min. Helium was used as the carrier gas at a flow rate of 1 mL min−1. Headspace samples were injected in splitless mode over the course of 2 min and the injector temperature was held at 250 °C.

Identification and quantification of the volatile compounds

The extracted volatile compounds were putatively identified by probability based matching of their mass spectra with those obtained from a commercial database (Wiley275.L, HP product) and by matching the Kovac index (KI) of the compounds with literature values reported for equivalent columns. Whenever possible, the identification was confirmed by using pure reference standards of the components.

Relative quantification was performed from peak areas integration of well-identified compounds using the MSD Chemstation software (version E.02.02.1431, Agilent Technologies). Absolute quantification was performed using external and internal standards. External standard curves were obtained with good linearity over the relevant concentration range of the sample volatiles (R^2 0.997-0.999). Internal standards consisting of isotopically labeled forms of the compounds were used at constant concentrations to normalize the differences in the response of the GC detector in the vials (see supplemental material Table S1). Volatile concentrations were calculated based on linear regression equations derived from their standard curves.

Statistical analysis

When required, a one-way analysis of variance (ANOVA) using StatPlus software version 5.4 at 95 % confidence was performed to determine whether or not there were any significant differences between the means of independent group of replicates.

Results

LAB Diversity and time course of species growth during cocoa fermentation

From this fermenting cocoa, 138 LAB were isolated and identified at biochemical level, and then the species were identified using PCR-amplified 16S RNA gene sequencing. The results showed that the LAB species involved in Nawa cocoa fermentation were *Lactobacillus plantarum, Leuconostoc mesenteroides, Weissella paramesenteroides, Lactobacillus curieae* and Fructobacillu ssp. (Fig.1). This LAB microbiota was dominated by *Lactobacillus* plantarum and *Leuconostoc mesenteroides* representing 93 % of total isolates, while *Weissella paramesenteroides*, *Lactobacillus curieae* and Fructobacillu ssp were the minor species (Table 1). The time course of LAB growth during the fermentation shows a rapid increase in bacterial population during the first three days, reaching a peak at 72 h (Fig 2) corresponding to pH 5.2 and 45 °C of temperature (Fig 3). Then a sharp decrease of LAB viable cells was observed after 72 h, dropping to 5.4 log (UFC/mL) (Fig 1). These species, namely *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were found throughout the fermentation process but *Lactobacillus plantarum* peaked at 72 h. The peak of the other major species *Leuconostoc mesenteroides* occurred at 48 h (Table 2) corresponding to a temperature of 43 °C and a pH of 4.8 (Fig 2). All the five LAB species could be isolated at 72 h, whereas only certain species where present at a particular time of fermentation (Table 2).

As the widest diversity of LAB species was isolated at 72 h of fermentation, the strains used for further analysis of aroma production were screened at this period. Thus the *Lactobacillus plantarum* strain T6N19, *Leuconostoc mesenteroides* strain T6N6, and *Weissella paramesenteroides* T6N2 were used for further study.

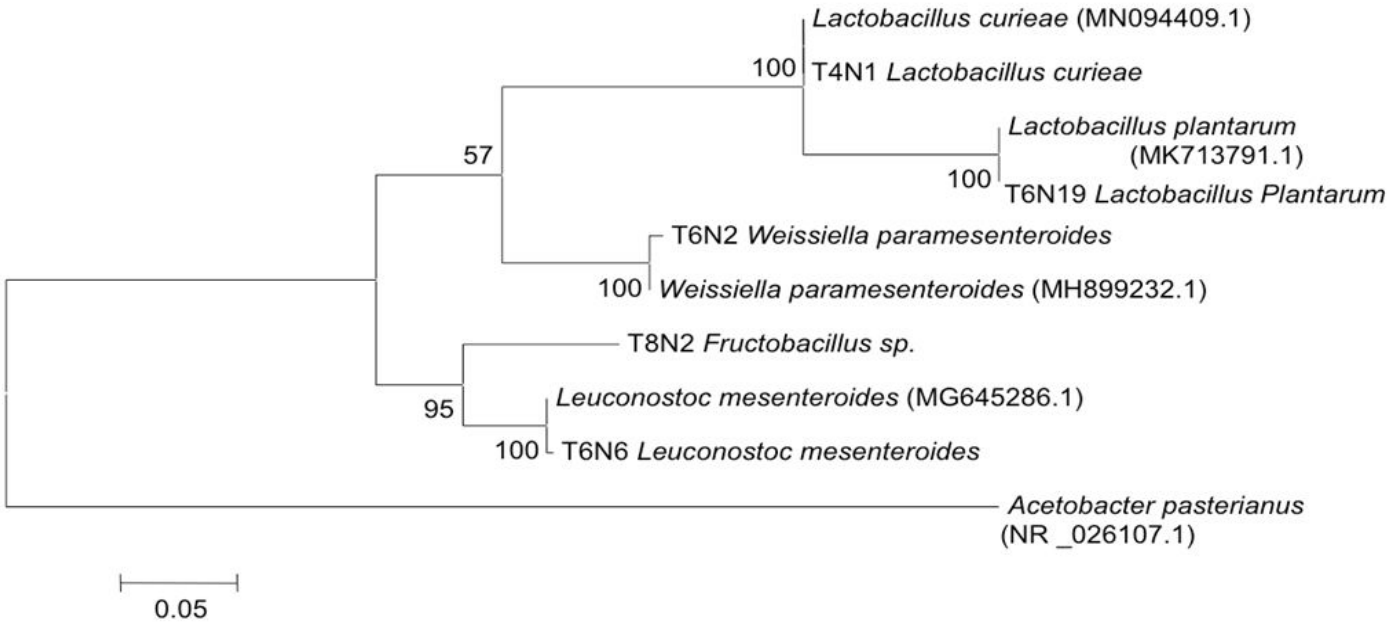

Figure 1. Phylogenetic identification of LAB strains from fermenting cocoa in Nawa region.

Strains from the main species were those used for aroma compounds production.

The program MEGA6 (Tamura *et al*., 2013) was used to align the 16S rRNA gene sequences. The tree was inferred by using the Maximum Likelihood method. Numbers in parenthesis are the accession numbers of the closest relative from NCBI genbank. *Acetobacter pasteurianus* was used as outgroup.

Table 1. Species distribution of isolated LAB strains isolated

Species isolated	Nawa
Lactobacillus plantarum	89
Leuconostoc mesenteroides	41
Weissella paramesenteroides	3
Lactobacillus curieae	2
Fructobacillu ssp.	3

Table 2. LAB species isolated in the course of cocoa fermentation

	Number of strains at different time							
LAB species isolated	0 h	24 h	48 h	72 h	96 h	120 h	144 h	Total strains
Lactobacillus plantarum	7	12	22	25	15	6	2	89
Leuconostoc mesenteroides	4	7	15	9	4	2	0	41
Weissella paramesenteroides	0	0	0	2	0	1	0	3
Lactobacillus curieae	0	0	1	1	0	0	0	2
Fructobacillu ssp.	0	0	0	1	1	0	1	3

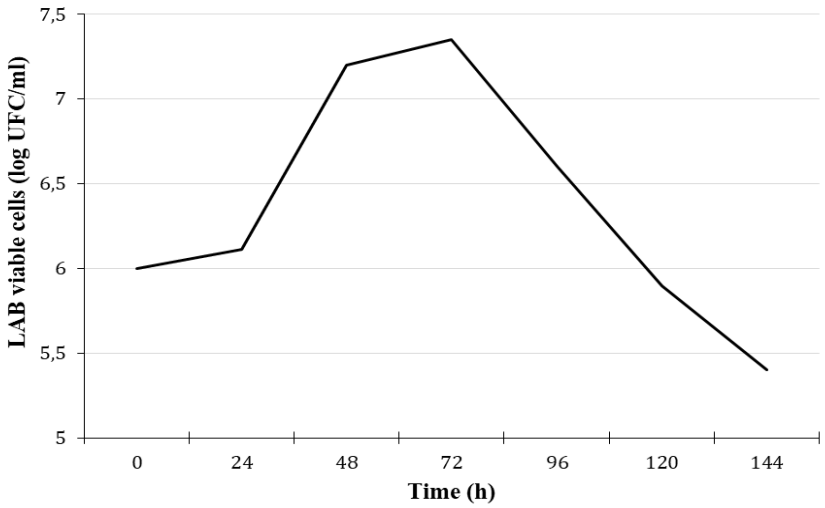

Figure 2. Growth dynamic of LAB population during natural cocoa fermentation

Aroma compounds produced by LAB during culture

The analyzed LAB strains produced an array of aroma compounds including butanediol, methyl butanol, acetic acid, methyl butanoic acid and 3-hydroxy 2-butanone (acetoin) that belong to the class of alcohols, ketones and acids (Table 3). All the LAB strains produced generally the same aroma compounds (Table 3). However, slight variations of aroma compounds produced by different LAB species were observed. Hence, the strain *Lactobacillus plantarum* T6N19, produced acetoin, butadione and methyl butanoic acid that were not detected in the cultures with *Leuconostoc mesenteroides* T6N6 and *Weissella paramesenteroides* T6N2. The most abundant aroma compounds produced by LAB strains were .

alcohols that represented 83 % of total quantities of aroma compounds produced by L. plantarum T6N6. Moreover, these alcohols represented up to 99 % of aroma compound yielded by L. mesenteroides and W. paramesenteroidesThe strain of Lactobacillus plantarum (T6N19) yielded more butanol 2-methyl, whereas Weissiella paramesenteroides (T6N2) reached the highest production of butanol 3-methyl. However, the productions of butanediols and acetic acid were similar in at least two strains (Table 3).

Influence of temperature on the yield of aroma compounds by LAB

As a general trend, we observed that, the maximum aroma production occurred at 35 ° C, but this production decreased rapidly at 40° C **(Table 4)**. in the pH ranges 4-5 with *Lactobacillus plantarum* strains **(Fig 4)**. The same observation was made with the others strains that also produced maximum aroma compounds in pH ranging from 4 to 5.

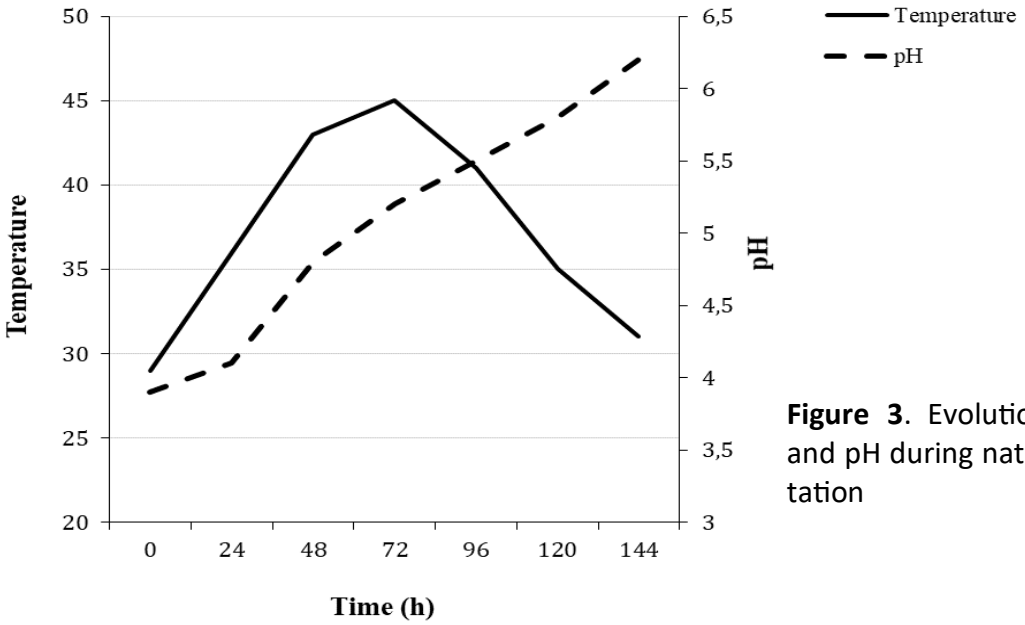

Figure 3. Evolution of temperature and pH during natural cocoa fermentation

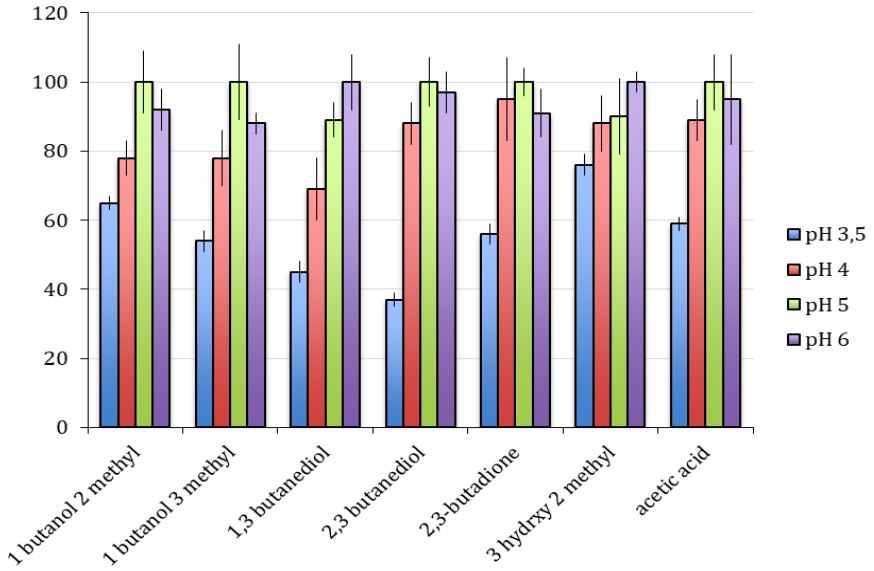

Figure 4. Influence of pH on aroma production during fermentation in pulp simulation medium

Table 3. Aroma compounds yielded by LAB strains analyzed

Aroma compounds production (g/L)						
Class	Aroma compounds produced	*Lactobacillus plantarum* (T6N19)	*Leuconostoc mesenteroides* (T6N6)	*Weissiella paramesenteroides* (T6N2)	Odor perception	Odor threshold (g/L)
Alcohols	1-Butanol, 3 methyl	2.233 (±0.454) [a]	1.992 (±0.141) [a]	3.190 (±0.554) [b]	malty, chocolate	$0.3x10^{-5}$
	1-Butanol, 2 methyl	0.570 (±0.166) [a]	0.317 (±0.049) [b]	0.343 (±0.056) [b]	Fruity	$12x10^{-5}$
	1,3-Butanediol	1.696 (±0.209) [a]	1.841 (±0.371) [a]	2.582 (±0.633) [b]	-	-
	2,3-Butanediol	3.221 (±0.0185) [a]	2.651 (±0.770) [a]	2.878 (±0.477) [a]	sweet, chocolate	-
Ketones	2,3-Butadione	0.471 (±0.073)	-	-	buttery	-
	3-Hydroxy, 2 butanone	0.604 (±0.050)	-	-	buttery, creamy	-
Acids	Acetic acid	0.0434 (±0.015) [a]	0.051 (±0.014) [a]	0.024 (±0.006) [b]	sour, vinegar	0.032
	Butanoic acid, 3 methyl	0.461 (±0.042)	-	-	Rancid, cheesy	$7x10^{-4}$

Table 4. Influence of temperature on the aroma production in LAB strains studied

Aroma production (g/L)									
	Lactobacillus plantarum			*Leuconostoc mesenteroides*			*Weissiella paramesenteroides*		
Aroma compounds	**30 °C**	**35°C**	**40°C**	**30°C**	**35°C**	**40°C**	**30°C**	**35°C**	**40°C**
1-Butanol, 3 methyl	1.873	2.129	3.429	2.142	3.205	1.428	2.705	6.106	2.073
1-Butanol, 2 methyl	0.72	0.524	0.113	0.374	0.369	0.174	0.299	1.064	0.843
1,3- Butanediol	1.991	2.886	1.275	2.364	3.002	1.034	1.864	2.203	1.731
2,3-Butanediol	3.196	5.927	4.321	1.947	2.264	1.225	2.493	5.68	3.274
Acetic acid	0.0632	0.170	0.098	0.038	0.016	0.010	0.033	0.028	0.042

However, the strains particularly *Lactobacillus plantarum* (T9N19) reached the maximum production of 1 butanol, 3 methyl at 40°C. Likewise, maximum production of acetic acid was also observed at 40°C with *Weissiella paramesenteroides (T6N2).*

Concerning the influence of pH on aroma compounds produced by the identified LAB it was shown that highest productions occurred

Discussion

In this study we identified LAB strains isolated from fermenting cocoa in Nawa region and their capacity to produce aroma was analyzed. All the 139 isolated strains presented a restricted diversity with only 5 LAB species identified namely *Lactobacillus plantarum* and *Leuconostoc mesenteroides* the dominant species and *Weissella paramesenteroides, Lactobacillus curieae* and *Fructobacillu ssp* as minors microbiota.

These species were those, which were commonly isolated from fermenting cocoa in Côte d'Ivoire as reported by Ouattara et al., 2017. The isolated species were also reported in many countries notably in Brazil, Nigeria and Côte d'Ivoire (Kostinek et al., 2008; Papalexandratou, Vrancken, De Bruyne, Vandamme, & De Vuyst, 2011; Visintin et al., 2017).

The time course of LAB species growth during cocoa fermentation showed that the dominant species *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were present throughout the fermentative process at all stages, while the minor species grew at a specific period of the fermentation, generally towards the advanced stage of the process. This suggested that the dominant species could undoubtedly be the key LAB players in the fermenting cocoa and might have among the LAB species, a great impact on the fermentation process.

The strains belonging to the different species *Lactobacillus plantarum*, *Leuconostoc mesenteroides* and *Weissella paramesenteroides,* were then further analyzed for their capacity to produce aroma compounds. It was shown that the main aroma produced by all strains were alcohol particularly 1-Butanol, 2 methyl that has a fruity note (Ramos, et al., 2014) and 1-Butanol, 3-methyl responsible for malty or chocolate odor (Rodriguez-Campos et al., 2012) as well as butanediol bearing sweet and chocolate odor perception (Ramos et al., 2014). Additionally, unlike the two other strains, the *Lactobacillus plantarum* strain produced ketones specifically 2,3-Butadione and 3-Hydroxy, 2 butanone that have buttery and creamy note (Rodriguez-Campos et al., 2012). This evidenceds –that each strain might produce a particular aroma profile depending on intrinsic physiological properties. This highlighted the relevance of screening performant strains.

These compounds from LAB strains may have an impact on the total flavor of the resulting chocolate since they all were produced beyond the odor perception threshold. However, the capability of the microbial compounds to remain in the beans during the fermentation and the drying processes remains a scientific debate among researchers worldwide. Meanwhile, Meersman et al., (2016), used *Saccharomyces cerevisiae* starter culture to tune the final flavor of chocolate, assuming the influence of microbial aroma on the final chocolate quality.

In the present study, LAB strains produced generally the maximum aroma compounds at 35 °C and pH range 4-5, corresponding to the conditions reached at 48-72 h of natural cocoa fermentation. These conditions were also described by many studies as a general trend of conditions occurring during natural cocoa fermentation (Schwan & Wheals, 2004; Yao et al., 2014). The period 48-72h of natural cocoa fermentation can be targeted as the optimal period of aroma compound production by LAB strains.

Conclusion

This study showed that LAB strains are liable to produce aroma compounds during cocoa natural fermentation notably methyl butanol, butanediol, butadione, 3-hydroxy, 2 butanone, that are known to be responsible for desirable flavor note. The impact of LAB producers of such aroma compounds on the final chocolate aroma profile remains to be elucidated.

References

Beckett ST (2009). Conching, p. 192–222. In: Beckett, S.T. (Ed.), Industrial chocolate manufacture and use. John Wiley & Sons Ltd..

Eskes B, Ahnert D, Carrion LG, Seguine E, Assemat S, Guarda D, Garcia P (2012). Evidence on the Effect of the Cocoa Pulp Flavour Environment during Fermentation on the Flavour Profile of Chocolates. Improving the Profitability of Small and Medium-Sized Farms : The Principal Key to a Global Sustainbale Cocoa Economy, 1–20. Retrieved from http://www.copal-cpa.org/icrc.php

Figueroa-Hernández C, Mota-Gutierrez J, Ferrocino I, Hernández-Estrada ZJ, González-Ríos O, Cocolin L, Suárez-Quiroz ML (2019). The challenges and perspectives of the selection of starter cultures for fermented cocoa beans. International Journal of Food Microbiology, 301:41–50. https://doi.org/10.1016/J.IJFOODMICRO.2019.05.002

Ho VTT, Fleet GH, Zhao J (2018). Unravelling the contribution of lactic acid bacteria and acetic acid bacteria to cocoa fermentation using inoculated organisms. International Journal of Food Microbiology 279:43–56. https://doi.org/10.1016/j.ijfoodmicro.2018.04.040

Janek K, Niewienda A, Wöstemeyer J, Voigt J (2016). The cleavage specificity of the aspartic protease of cocoa beans involved in the generation of the cocoa-specific aroma precursors. Food Chemistry 211: 320–328. ttps://doi.org/10.1016/j.foodchem.2016.05.033

Koné MK, Guéhi ST, Durand N, Ban-Koffi L, Berthiot L, Tachon AF, Kouakou B, Renaud B, Montet D (2015). Contribution of predominant yeasts to the occurrence of aroma compounds during cocoa bean fermentation. Food Research International 89: 910-917. https://doi.org/10.1016/j.foodres.2016.04.010

Kongor JE, Hinneh M, de Walle DV, Afoakwa EO, Boeckx P, Dewettinck K (2016). Factors influencing quality variation in cocoa (Theobroma cacao) bean flavour profile - A review. Food Research International. https://doi.org/10.1016/j.foodres.2016.01.012

Kostinek M, Ban-Koffi L, Ottah-Atikpo M, Teniola D, Schillinger U, Holzapfel WH, Franz CMAP (2008). Diversity of Predominant Lactic Acid Bacteria Associated with Cocoa Fermentation in Nigeria. Current Microbiology 56: 306–314. https://doi.org/10.1007/s00284-008-9097-9.

Lefeber T, Gobert W, Vrancken G, Camu N, De Vuyst L (2011). Dynamics and species diversity of communities of lactic acid bacteria and acetic acid bacteria during spontaneous cocoa bean fermentation in vessels. Food Microbiology 28: 457–464. https://doi.org/10.1016/j.fm.2010.10.010

Meersman E, Steensels J, Struyf N, Paulus T, Saels V, Mathawan M, Allegaert L, Vrancken G,Verstrepen KJ (2016). Tuning chocolate flavor through development of thermotolerant Saccharomyces cerevisiae starter cultures with increased acetate ester production. Applied and Environmental Microbiology 82: 732–746. https://doi.org/10.1128/AEM.02556-15

Ouattara HD, Ouattara HG, Droux M, Reverchon S, Nasser W, Niamke SL (2017). Lactic acid bacteria involved in cocoa beans fermentation from Ivory Coast: Species diversity and citrate lyase production. International Journal of Food Microbiology, 256: 1 1–19. https://doi.org/10.1016/j.ijfoodmicro.2017.05.008

Ouattara HG, Reverchon S, Niamke SL, Nasser W (2017). Regulation of the synthesis of pulp degrading enzymes in Bacillus isolated from cocoa fermentation. Food Microbiology 63: 255–262. https://doi.org/10.1016/j.fm.2016.12.004.

Papalexandratou Z, Vrancken G, De Bruyne K, Vandamme P, De Vuyst L (2011). Spontaneous organic cocoa bean box fermentations in Brazil are characterized by a restricted species diversity of lactic acid bacteria and acetic acid bacteria. Food Microbiology, 28: 1326–1338. https://doi.org/10.1016/j.fm.2011.06.003

Ramos CL, Dias DR, Miguel MG, Da CP, Schwan RF (2014). Impact of different cocoa hybrids (Theobroma cacao L.) and S. cerevisiae UFLA CA11 inoculation on microbial communities and volatile compounds of cocoa fermentation. Food Research International, 64: 908–918. https://doi.org/10.1016/j.foodres.2014.08.033

Rodriguez-Campos J, Escalona-Buendía HB, Contreras-Ramos SM, Orozco-Avila I, Jaramillo-Flores E, Lugo-Cervantes E (2012). Effect of fermentation time and drying temperature on volatile compounds in cocoa. Food Chemistry, 132: 277–288. https://doi.org/10.1016/j.foodchem.2011.10.078

Samagaci L, Ouattara HG, Goualié BG, Niamke SL (2014). Growth capacity of yeasts potential starter strains under cocoa fermentation stress conditions in Ivory Coast. Emirates Journal of Food and Agriculture, 26: 861–870. https://doi.org/10.9755/ejfa.v26i10.18114

Samagaci L, Ouattara H, Niamke S, Lemaire M (2016). Pichia kudrazevii and Candida nitrativorans are the most well-adapted and relevant yeast species fermenting cocoa in Agneby-Tiassa, a local Ivorian cocoa producing region. Food Research International, 89: 773–780. https://doi.org/10.1016/j.foodres.2016.10.007

Schwan RF, Wheals AE (2004). The microbiology of cocoa fermentation and its role in chocolate quality. Critical Reviews in Food Science and Nutrition, 44: 205–221. https://doi.org/10.1080/10408690490464104.

Soumahoro S, Ouattara HG, Goualié BG, Koua G, Doue G, Niamke SL (2015). Occurrence of High Acetic Acid-Producing Bacteria in Ivorian Cocoa Fermentation and Analysis of Their Response to Fermentative Stress. American Journal of BioScience 3: 70–79. https://doi.org/10.11648/j.ajbio.20150303.12

Visintin S, Ramos L, Batista N, Dolci P, Schwan F, Cocolin L (2017). Impact of Saccharomyces cerevisiae and Torulaspora delbrueckii starter cultures on cocoa beans fermentation. International Journal of Food Microbiology 257: 31–40. https://doi.org/10.1016/j.ijfoodmicro.2017.06.004

Voigt J, Heinrichs H, Voigt G, Biehl B (1994). Cocoa-specific aroma precursors are generated by proteolytic digestion of the vicilin-like globulin of cocoa seeds. Food Chemistry 50: 177–184. https://doi.org/10.1016/0308-8146(94)90117-1

Voigt J, Textoris-Taube K, Wöstemeyer J (2018). pH-Dependency of the proteolytic formation of cocoa- and nutty-specific aroma precursors. Food Chemistry 255: 209–215. https://doi.org/10.1016/J.FOODCHEM.2018.02.045

Yao W, Ouattara HG, Goualie B, Soumahoro S (2014). Analysis of some functional properties of acetic acid bacteria involved in Ivorian cocoa fermentation, Journal of Applied Biosciences. 75: 6282–6290. https://doi.org/10.4314/jab.v75i1.13