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Review

The challenges and perspectives of the selection of starter cultures for fermented cocoa beans

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ABSTRACT

Fermentation is an essential process step to develop precursor compounds for aroma and flavour characteristics of chocolate, as well as preventing germination of the cocoa bean. Despite the importance of the role of microorganisms during the chocolate production, to date, there are some discrepancies of the *"cocobiota"* community found during fermentation and the impact of starter culture in fermented cocoa beans. This review provides both a detailed overview of the starter cultures used in fermented cocoa beans and the microbial diversity involved during this process, and an in-depth discussion of the methods used to identify these microorganisms. In this review, we included only published articles from 2008 to 2018 in English language. A total of forty-seven studies contributed to the description of the cocobiota from 13 different countries. In detail, we observed that the most common fermentation method used is the wooden box, followed by heap. Interestingly, 37% of the studies cited in this review did not mention the type of cocoa variety studied. Most of the techniques used to identify the microbiota are fingerprinting based (DGGE); however, few studies have been using next-generation technologies to elucidate the possible functions and interactions among microbes. Our results showed a greater diversity of yeasts if compared with bacterial involved in the fermentation. This review will help researchers seeking to design starter cultures to drive cocoa bean fermentation, and thus achieve a homogenous mass of fermented cocoa beans as well as serve as a guide for assessing methodologies for the identification of microorganisms.

1. Introduction

Fermentation is one of the oldest food-manufacturing methodologies used to add value to the raw materials. This process involves a metabolic route in order to obtain energy from organic compounds, without the participation of an oxidising agent of exogenous origin (Bourdichon et al., 2012). Some of the advantages of a successful fermentation are that it allows perishable products such as milk, meat, and vegetables to have higher microbiological stability and safety (Cocolin et al., 2016). Besides the bio-preservation of foodstuff, the microbes involved during fermentation have a significant impact on the physicochemical, microbiological, safety and sensorial quality of the products (Bortolini et al., 2016). In food production, three kinds of fermentations are used such as lactic, alcoholic and acetic fermentations (Steinkraus, 2002). These can be performed simultaneously in some food matrices, such as cocoa beans for the production of chocolate (De Vuyst and Weckx, 2016; Ho et al., 2015; Lima et al., 2011; Pereira et al., 2016; Schwan and Wheals, 2004; Schwan et al., 2015).

The identification of microorganisms present on fermented cocoa beans has been studied since the '60s. However, the use of DNA sequencing technologies to profile the microbial ecosystems of foodstuff has been conducted only during the last 10 years, while DNA based fingerprinting such as DGGE or RFLP have been widely used to identify and quantify microbial species involved in food matrices (Cocolin and Ercolini, 2015). This review showed data regarding the main microbial population belonging to yeasts, lactic acid bacteria (LAB), acetic acid

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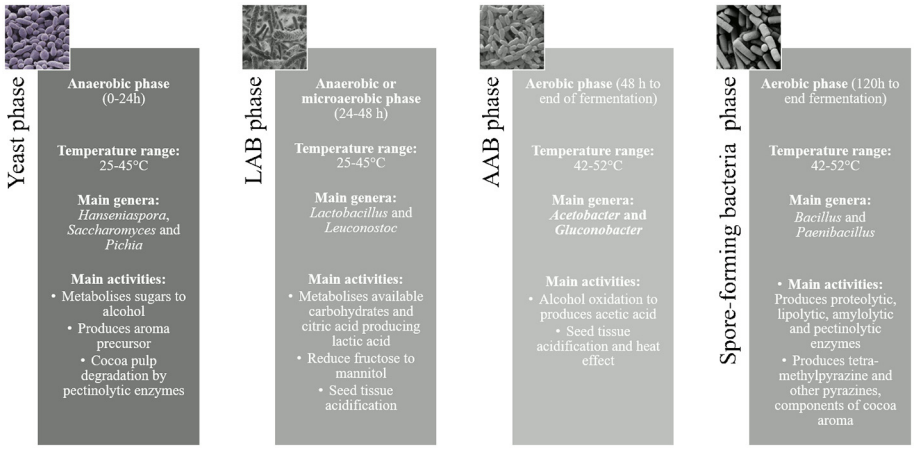

Fig. 1. Physical requirements and biochemical characteristics of the microbial growth of yeast, LAB, AAB and *Bacillaceae* genus during cocoa bean fermentation. Adapted from (Kadow et al., 2015).

bacteria (AAB) and *Bacillacellae* involved in different varieties of fermented cocoa beans from different origins, fermented by using different methods, mainly box, heap or tray. In addition, a brief description of the methods that have been used to identified microbial communities in spontaneous fermentations to give new insights for standardising microbial communities as inoculum.

2. From cocoa tree to cocoa bean

Theobroma cacao L. is a perennial tree native to the South American tropical region and is cultivated only in the equatorial zone. Nowadays, this agricultural product serves as one of the main incomes for farmers in world tropical countries. In general, *Forastero*, *Criollo,* and *Trinitario* are the three main varieties of cocoa, and its pods are approximately 12 to 30 cm in length and composed of 30 to 40 seeds cover by a mucilaginous white pulp. Interestingly, 95% of the world's cocoa production comes from the *Forastero* and its varietal hybrids cross (International Cocoa Organization, 2013).

Cocoa trees have been intensively grown in various part of the globe. According to recently published figures, in 2015–2016 the annual production of cocoa of the eight main producing countries, Ivory Coast, Ghana, Indonesia, Cameroon, Brazil, Nigeria, Ecuador and Peru, was estimated at 3985 thousand tonnes, of which 37% of the total cocoa was produced in Ivory Coast. Besides the most important cocoa producing countries, cocoa beans are also produced in Uganda, Malaysia, India, Mexico, Venezuela and Colombia (FAO, 2018).

2.1. Nutritional composition of cocoa beans

Cocoa bean is composed of two cotyledons and an embryo enclosed by a seed coat, enveloped in a sweet, white mucilaginous pulp. This pulp is approximately 40% of the weight of the fresh seed and holds 10–15% sugars, mainly glucose, fructose, and sucrose (Ardhana and Fleet, 2003; Camu et al., 2008a). However, when cocoa is unripe, the pulp has a higher proportion of sucrose compared to a ripe pulp, which is composed mainly of fructose and glucose (Packiyasothy et al., 1981). Besides sugars, the cocoa pulp contains about 1–2% of pectin and other polysaccharides, proteins, amino acids, minerals, and vitamins, mainly vitamin C, as well as 0.5–2% of citric acid (Camu et al., 2008b).

From the nutritional point of view, cocoa beans are composed mainly by water (32–39%), total fat (30–32%, of which 65% is saturated fat), proteins (8–10%), cellulose (2–3%), starch (4–6%), pentoses (4–6%), sucrose (2–3%), polyphenols (5–6%), organic acids, mainly citric, oxalic, and malic (1%), theobromine (1–3%) and caffeine (0.2–0.1%) (Bucheli et al., 2001; Schwan et al., 2015). This chemical composition of cocoa beans and pulp can be considered an appropriate medium for microbial growth (Ardhana and Fleet, 2003; Ozturk and Young, 2017). Once the pulp and the cocoa beans are removed from the pod (mechanically or manually), they interact with a vast variety of microorganisms from the environment like the tools used to open them or worker's hands.

3. Microbial dynamics during cocoa beans fermentation

Cocoa bean fermentation is one of the post-harvest operations that have the most significant impact on the final quality of chocolate. This process is regulated mainly by yeast, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and members of the *Bacillaceae* (especially *Bacillus*) that use the pulp that covers the cocoa bean as a growing substrate. During the microbial cocoa fermentation, several molecules are released, which give to the chocolate its characteristic aromatic profile, reducing the bitter taste and astringency and finally kill the embryo to avoid its germination (Kadow et al., 2013, 2015). Thus, most efforts have been focused on the identification of the microbiota involved during fermentation in the last decade. In this review, a total of 47 available published studies are discussed in deep to describe the microbial composition of fermented cocoa beans of different varieties on cocoa plants, fermented by different process, and from different cocoa producing countries (Afoakwa et al., 2013; Bortolini et al., 2016; Camu et al., 2008a).

3.1. Microbial succession and biochemical changes in fermented cocoa beans

Fermentation is a complex process involving autochthonous microorganisms (Crafack et al., 2013; Jespersen et al., 2005) originated from post-harvest procedures (from knives, banana or plantain leaves), pods surfaces, worker's hands and from the surface of the containers where the fermentation process is carried out and were yeasts, LAB and AAB play the leading role (Adler et al., 2014; Camu et al., 2007, 2008b; De Vuyst and Weckx, 2016; Jespersen et al., 2005; Ostovar and Keeney, 1973; Schwan et al., 2015).

During cocoa bean fermentation, three main stages can be distinguished. An overview of the microbial and biochemical reactions involved in cocoa bean fermentation is shown in Fig. 1. The first stage involves the growth of yeasts which mainly belongs to *Saccharomycetaceae* family, including *Hanseniaspora*, *Saccharomyces*, *Kluyveromyces* and *Pichia* (Dujon and Louis, 2017), these genera, that produce ethanol, are favoured by the higher concentration of glucose and citric acid and the low availability of oxygen. However, yeasts can also produce other compounds such as carbon dioxide, organic acids (acetic and succinic acid) and glycerol (Ardhana and Fleet, 2003; Camu et al.,

Table 1
Overview of the microbial diversity involved in spontaneous cocoa fermentation from main studies performed over the last decade.

Publication year	Australia	Bolivia	Brazil	Cameroon	Cuba	Ecuador	Ghana	Honduras	Ivory Coast	Malaysia	Mexico	Nigeria	Philippines	Total
2008			1				1					1		3
2009							2							2
2010			1				1							2
2011			3			2			3	1				9
2012			1						1	1				3
2013			3				1			2				6
2014	1													1
2015	1		1						1		1		1	5
2016		1	3	1	1		1		3					10
2017			2						1					3
2018			1	1				1						3
Total	2	1	16	2	1	2	6	1	9	4	1	1	1	47

2008b; Nielsen et al., 2005, 2007; Papalexandratou et al., 2011a, 2011b, 2011c, 2013). Yeasts are also involved in the degradation of pectin found in the cocoa pulp, and in the production of a large number of aroma compounds precursors, such as higher alcohols and esters, that contribute significantly to the development of chocolate aroma profile (Crafack et al., 2013; Ho et al., 2014).

The second stage is characterised by the increment of lactic acid concentrations, as a consequence of the increase of LAB populations and the decrease of yeasts. However, the importance of the role of LAB during the fermentation of cocoa has been controversial. According to Ho et al. (2015) LAB may not be essential in cocoa bean fermentation. However, several studies prove the presence of a limited diversity of LAB, mainly *Lactobacillus fermentum*, which is heterofermentative and produced volatile compounds such as diacetyl, acetoin and 2,3-butanediol to support bacterial growth and allowed a slight increase of pH in cocoa pulp (Adler et al., 2013; Camu et al., 2007, 2008b; Lefeber et al., 2010, 2011; Papalexandratou et al., 2013).

Finally, in the third stage, LAB population begins to decrease and simultaneously increases the population of AAB, which are responsible for the simultaneous oxidation of ethanol produced by yeasts and the conversion of lactic acid produced by LAB to acetic acid and acetoin (Adler et al., 2014; Moens et al., 2014). Subsequently, acetic acid can be overoxidized to carbon dioxide and water. The rise in temperature, the decrease in pH from 6.5 to 4.8 and the penetration of acetic acid and ethanol to the cocoa bean are the cause of the death of the embryo (Ardhana and Fleet, 2003; Camu et al., 2008b; Cleenwerck et al., 2008; Garcia-Armisen et al., 2010; Lefeber et al., 2012; Nielsen et al., 2005). Besides the well-known bacteria described above, *Bacillus* has been often isolated from fermented cocoa beans, however, its contribution to this process remains to be elucidated. According to Ouattara et al. (2011), *Bacillus subtilis*, *B. pumilus* and *B. fusiformis* isolated from cocoa fermentation were the main producers of pectin lyase which may play an important role during cocoa fermentation. In addition, it seems that *Bacillus* could be implicated in the production of other pectinolytic enzymes such as polygalacturonase during cocoa fermentation (Ouattara et al., 2008). However, it should be pointed out that members of the *Bacillus* genus can release off- flavour on fermented cocoa beans due to the production of short- chain fatty acids (Schwan and Wheals, 2004; Schwan et al., 2015).

Despite the biochemical changes described above, a cell disruption occurs inside the cocoa bean. This phenomenon causes the release of cellular components of the cotyledon and produced favourable environments to develop taste precursors and pigment degradation by the enzymatic action of invertases, glycosidases, and proteases as endoproteinases, carboxylases, aminopeptidases and polyphenol oxidases (Rabadan-Chávez and Lugo-Cervantes, 2018). Also, enzymatic hydrolysis of cocoa bean proteins, mainly vicilin and albumins, occurs during this process. These biochemical reactions can produce peptides with a wide range of molecular weights, amino acids and reducing sugars that are substrates needed for Maillard reactions which are necessary for the full development of aromas and flavours during drying and roasting of cocoa bean (Caligiani et al., 2016; D'Souza et al., 2018; Kongor et al., 2016).

3.2. Main microorganisms involved in cocoa bean fermentation

The study of microbial diversity during the fermentation process of cocoa has been addressed in many studies using culture dependent and independent methods. Microbial diversity of fermented cocoa beans in the last ten years has been carried out in Ivory Coast (Bortolini et al., 2016; Hamdouche et al., 2015; Koné et al., 2016; Lefeber et al., 2012; Ouattara et al., 2008, 2011, 2017; Papalexandratou et al., 2011a; Papalexandratou and Vuyst, 2011; Samagaci et al., 2016), Ghana (Bortolini et al., 2016; Cleenwerck et al., 2008; Crafack et al., 2013; Daniel et al., 2009; Garcia-Armisen et al., 2010), Brazil (Bastos et al., 2018; Batista et al., 2015; Garcia-Armisen et al., 2010; Illeghems et al., 2012; Leal et al., 2008; Menezes et al., 2016; Moreira et al., 2013, 2016; Papalexandratou et al., 2011a, 2011c; Papalexandratou and Vuyst, 2011; Pedroso Da Cruz et al., 2017; Pereira et al., 2013a, 2013b), Malaysia (Lefeber et al., 2012; Meersman et al., 2013; Papalexandratou et al., 2013; Papalexandratou and Vuyst, 2011), Mexico (Arana-Sánchez et al., 2015), Honduras (Romanens et al., 2018) Ecuador (Papalexandratou et al., 2011b; Papalexandratou and Vuyst, 2011), Cuba (Fernández- Maura et al., 2016), Cameroon (Bortolini et al., 2016; Mota-Gutierrez et al., 2018), Bolivia (Miescher Schwenninger et al., 2016), Nigeria (Kostinek et al., 2008), Philippines (Gibe and Pangan, 2015), and Australia (Ho et al., 2014, 2015) as shown in Table 1.

In detail, the most representative microbial species of fermented cocoa beans from 13 different geographical locations include a total of 274 microbes, comprising 132 yeasts, 59 LAB and 46 AAB and 11 *Bacillus* as shown in Table 2. One notes, that the yeast community showed greater diversity compared with bacteria (Table 2). Differences between the main microorganisms found during the process according to the type of fermentation used as well as the variety of cocoa used were observed (Table S1). Overall, the most commonly identified microbial species involved in fermented cocoa include yeasts (*Pichia kudriavzevii*, *Hanseniaspora opuntiae*, and *Saccharomyces cerevisiae*), LAB (*Lactobacillus fermentum*, and *Lactobacillus plantarum*), and AAB (*Acetobacter pasterianus*). Additionally, in some studies, the presence of member of the *Bacillus* genus, *Enterobacteriaceae*, and filamentous fungi have also been reported (Copetti et al., 2011; Garcia-Armisen et al., 2010; Mounjouenpou et al., 2008). Interestingly, it appears that the dominating microbial species are not at all present in every country studied, as observed in Fig. 2. The following results indicate that microbial composition can be discriminated by geographical location. In detail, Malaysia and Brazil reported the presence of all the most commonly identified microorganisms involved in cocoa bean fermentation (*P. kudriavzevii*, *H. opuntiae*, *H. uvarum*, *S. cerevisiae*, *Lb. fermentum*, *Lb. plantarum*, and *A. pasterianus*), while Cuba and Mexico reported only the presence of yeasts. In general, further studies are needed to

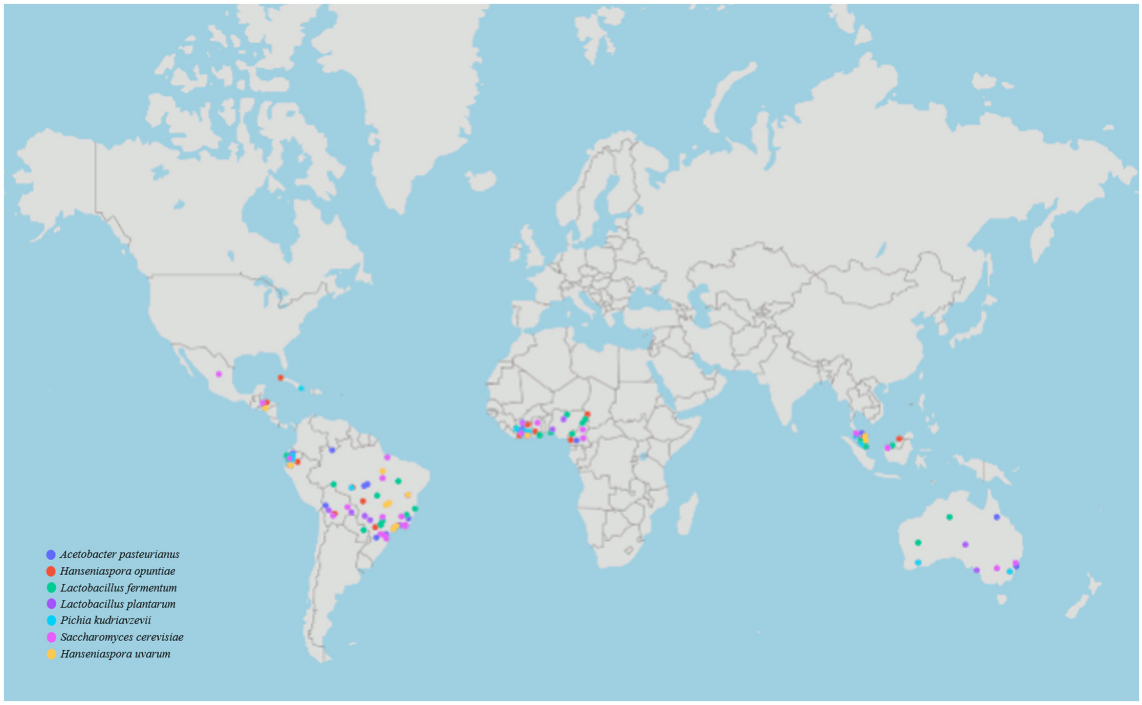

Fig. 2. Occurrence of dominating microbial species over the last decade in fermented cocoa beans.

elucidate the bacterial ecology in fermented cocoa beans from the American continent to provide a better knowledge of the cocobiota in this region.

3.3. Fermented cocoa varieties studied in food microbiology

Thus, fermented cocoa beans have been extensively studied; it is alarming that 37% of the total amount of studies published do not mention the type of cocoa variety investigated as shown in Fig. 3. Excluding the studies where the cocoa variety used was not mention, the mixed of cocoa varieties (*Trinitario, Criollo* and/or *Forastero*) have been more investigated rather than single varieties (Fig. 3). Clearly, there is a lack of studies exploring the fermentation of single cocoa varieties. In addition, we also strongly encourage researchers to explicitly state in their research the type of cocoa variety investigates.

3.4. Geographical location of microbial fermented cocoa communities

Over the last decade, there has been a growing interest in studying fermented cocoa beans from leading producing countries, except Indonesia and Peru, from which to our best knowledge, no update or none information has been generated concerning microbial diversity. In contrast, we observed that over the last decade the microbial community of fermented cocoa beans of Brazil has been extensively studied, followed by Ivory Coast, and Malaysia (Table 1). It is expected that the minor and main producing cocoa countries will begin to close these gaps. Concerning the microbial fermented cocoa beans species found by country, interesting data are driving to the conclusion that minor microbial species discriminate cocoa beans by origin (Table 2).

3.5. Fermentation method and duration

There are several methods of fermentation employed for cocoa bean, and these can vary according to the region where the cocoa is fermented as shown in Table 3. The most frequent methods studied over the last decade are the following: (i) heap (Daniel et al., 2009; Samagaci et al., 2016), (ii) trays (Crafack et al., 2013, 2014; Kostinek et al., 2008), (iii) box (Arana-Sánchez et al., 2015; Papalexandratou et al., 2011c, 2013), (iv) basket (Schwan et al., 2015), and (v) platform (Papalexandratou et al., 2011b). However, as shown in Fig. 4 most of the fermented cocoa in the world is produced by using the box fermentation method and the quantity of cocoa beans processed varies between 5 and 2000 kg (Schwan et al., 2015). However, this amount of beans depends on the production capacity of the local cocoa farm.

4. Culture-dependent and independent methods used to identify the diversity of microorganisms in fermented cocoa beans

Methods for investigating the cocobiota have been either studied through culture-dependent or culture-independent methods. Traditional microbial culturing approach has been extensively used for enumeration and identification of microbial communities in fermented cocoa beans (Kostinek et al., 2008; Mota-Gutierrez et al., 2018; Papalexandratou et al., 2011c; Papalexandratou and Vuyst, 2011). However, this technique fails to reproduce the ecological niches and symbiotic relationships encountered in a complex natural ecosystem (Besnard et al., 2000). Through culture-independent methods molecular techniques including direct cloning or sequencing of DNA fragments of fingerprinting methods (restriction fragment polymorphism (RFLP), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), single strand conformation polymorphism (SSCP) and denaturing high-performance liquid chromatography (DHPLC)) has been used to identified the microbial community (Nocker et al., 2007).

Over the last decade, we observed that the microbial communities of fermented cocoa beans have been mostly assessed through culture-independent technique like PCR-DGGE (Arana-Sánchez et al., 2015; Hamdouche et al., 2015; Ho et al., 2014, 2015; Moreira et al., 2013; Papalexandratou et al., 2011b, 2011c, 2013; Papalexandratou and Vuyst, 2011), while only few studies have been using amplicon based next generation sequencing to analyse microbial communities as shown in Fig. 5 (Bortolini et al., 2016; Daniel et al., 2009; Fernández- Maura et al., 2016; Meersman et al., 2013; Mota-Gutierrez et al., 2018). However, misidentification of microbial species is a limiting factor that also influences in all independent and dependent techniques (Mota-Gutierrez et al., 2019; Ozturk and Young, 2017). Therefore, the

Table 2
Frequency distribution of main microbial species found in fermented cocoa beans.

Main microbes	Australia	Bolivia	Brazil	Cameroon	Cuba	Ecuador	Ghana	Honduras	Ivory Coast	Malaysia	Mexico	Nigeria	Philippines	Total
Acetobacter calcoacetis								1						1
Acetobacter cerevisiae								1						1
Acetobacter fabarum		1	2					1						4
Acetobacter ghanensis		1	2				2	1						6
Acetobacter lovaniensis			1											1
Acetobacter pasteurianus	2	1	7	1		2		1	1	3				18
Acetobacter pomorum			1							1				2
Acetobacter senegalensis		1	2					1						4
Acetobacter sp.			1						1					2
Acetobacter syzygii			1	1			2		1					5
Acetobacter tropicalis			1											1
Acinetobacter sp.			1											1
Bacillus cereus			3											3
Bacillus fusiformis									1					1
Bacillus pumilus									1				1	2
Bacillus sp.				2										2
Bacillus subtilis			2						1					3
Candida krusei			1											1
Candida magnoliae			1											1
Candida nitrativorans									1					1
Candida pseudotropicalis			1											1
Candida sorbosivorans						2								2
Candida tropicalis			1						3					4
Enterobacteriaceae									1					1
Enterococcus italicus										1				1
Fructobacillus sp.										1				1
Fructobacillus pseudoficulneus			1											1
Fructobacillus tropaeoli						2								2
Galactomyces geotrichum									3					3
Gluconobacter frateurii	2													2
Gluconobacter oxydans			2											2
Hanseniaspora guilliermondii	2		1			1		1	1	2				8
Hanseniaspora opuntiae		1	3	2	1	1	1	1	2	2				14
Hanseniaspora sp.			2								1			3
Hanseniaspora thailandica							1							1
Hanseniaspora uvarum			7			1		1	1	2				12
Klebsiella sp.				1										1
Kluyveromyces marxianus	2			2										4
Lactobacillus brevis												2		2
Lactobacillus cacaonum								1						1
Lactobacillus casei			1											1
Lactobacillus curieae									1					1
Lactobacillus durianis			1											1
Lactobacillus fermentum	2	1	10	3		2	2	1	1	3		2		27
Lactobacillus mali			1											1
Lactobacillus nagelii			2											2
Lactobacillus pentosus	2									1				3
Lactobacillus plantarum	2	1	9					1	1	2		2		18
Lactobacillus rhamnosus			1											1
Lactobacillus sp.								1						1
Leuconostoc mesenteroides									1					1
Leuconostoc pseudomesenteroides			1			2			1	1				5
Lysinibacillus fusiformis			3											3
Pediococcus acidilactici		1	2									2		5
Pichia caribbica			3											3
Pichia galeiforms									3					3
Pichia kluyveri			4											4
Pichia kudriavzevii	2		2		1	3			6	2				16
Pichia manshurica					1	2	1							4
Pichia membranifaciens			1											1
Pichia mexicana													1	1
Pichia pijperi				2										2
Pichia sp.			2											2
Saccharomyces cerevisiae	2	1	12	2		2	1	1	4	4	1			30
Schizosaccharomyces pombe										1				1
Tatumella punctata						2								2
Tatumella saanichensis						2								2
Torulaspora delbrueckii			2											2
Wickerhamomyces anomalus									3					3
Total	18	9	101	16	3	24	10	14	39	28	2	8	2	274

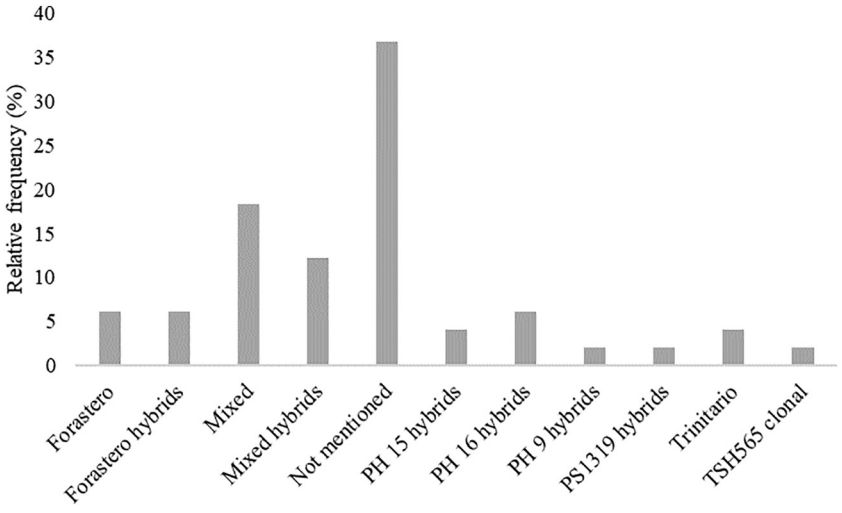

Fig. 3. Relative frequency of the cocoa varieties studied from 2008 to 2018.

application of both dependent and independent culture techniques has been extensively used to reveal the microbial ecology in fermented cocoa beans (Camu et al., 2007, 2008a; Daniel et al., 2009; Jespersen et al., 2005; Lagunes- Gálvez et al., 2007; Meersman et al., 2013; Mota-Gutierrez et al., 2018; Nielsen et al., 2005; Papalexandratou et al., 2011b, 2011c; Papalexandratou and Vuyst, 2011). Overall, the study of microbial diversity through culture-dependent methodologies is an essential step to select potential starter culture with relevant biochemical or physiological features as high aromatic compounds producing, heat-tolerant, acid-tolerant, ethanol-tolerant, phenolic compounds-tolerant, pectin, and citric acid strains. These two methodologies complement and provide a better understanding of the complexity of the cocoa bean fermentation process, as well as the growth kinetics and presence of the microorganisms involved. Therefore, it is highly recommended to use a polyphasic approach to study the microbial ecology to decrease the risk of misidentification of microorganisms.

Besides molecular biological techniques, the identification of microbes using either intact cells or cell extracts have been also detected by Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and Biolog system. In addition to these techniques, conventional morphological and biochemical studies, have been used to profile the microbial diversity in fermented cocoa beans (Pedroso Da Cruz et al., 2017).

5. Use of starter cultures in the cocoa bean fermentation process

Several attempts on designing a microbial starter culture have been addressed for cocoa bean fermentation process (Batista et al., 2015; Crafack et al., 2013, 2014; Leal et al., 2008; Lefeber et al., 2010, 2011, 2012; Meersman et al., 2016; Menezes et al., 2016; Mota-Gutierrez et al., 2018; Schwan, 1998; Visintin et al., 2016, 2017). Interestingly, the first evaluation of the performance of a mixed starter culture composed of *S. cerevisiae* var. *chevalieri*, *L. lactis*, *Lb. plantarum*, *A. aceti*, and *G. oxydans* subsp. *suboxydans* for fermented cocoa beans was done by Schwan (1998). According to Schwan (1998), it was concluded that starter cultures could be a viable option to obtain a reliable fermentation process and consequently desirable characteristics of chocolate. However, it should be pointed out that member of *Gluconobacter* genus has also been known as producers of acids and off-flavours and caused a late yeast development into cocoa fermentation (Adler et al., 2014; De Vuyst and Weckx, 2016; Moens et al., 2014; Papalexandratou et al., 2011c).

Over the last decade, *K. marxianus*, *P. kluyveri*, *S. cerevisiae*, *T. delbrueckii*, *Lb. fermentum*, and *A. pasteurianus* have also been extensively studied., Leal et al., 2008 evaluated the capacity of a hybrid strain of *K. marxianus*, as a starter culture, finding that the chocolate produced with these strain were more accepted by the sensorial panellist in comparison with those obtained from a spontaneous fermentation process. In addition, the cocoa beans previously inoculated with *K. marxianus* increased the volume of the sweating and modified the microorganism's dynamics and protein degradation.

P. kluyveri a recognised strain for its high production capacity of aromatic compounds has also been evaluated as inoculum to conduct this process. The use of this yeast had pronounced differences in the composition of volatile organic compounds (VOCs) in the roasted cocoa liquors and chocolates when compared with those obtained from a spontaneous fermentation process. The chocolates produced with the inoculated cocoa beans were described as fruity, acid, yoghurt and balsamic flavours, whereas the spontaneously fermented chocolates were described as sweet, cocoa and caramel flavours. However, despite

Table 3
Type of cocoa fermentation used over the last decade.

Fermentation type	Australia	Bolivia	Brazil	Cameroon	Cuba	Ecuador	Ghana	Honduras	Ivory Coast	Malaysia	Mexico	Nigeria	Philippines	Total
Box			4			1			1	3				9
Heap				2	1		3		7	2		1		16
Mixed			1											1
Not mentioned													1	1
Plastic box	1		1						1	1				4
Plastic bucket								1						1
Platform						2								2
Stainless tank			1											1
Tray							1					1		2
Wooden box	1	1	14	1		1	1		3		1			23
Total	2	1	21	3	1	4	5	1	12	6	1	2	1	60

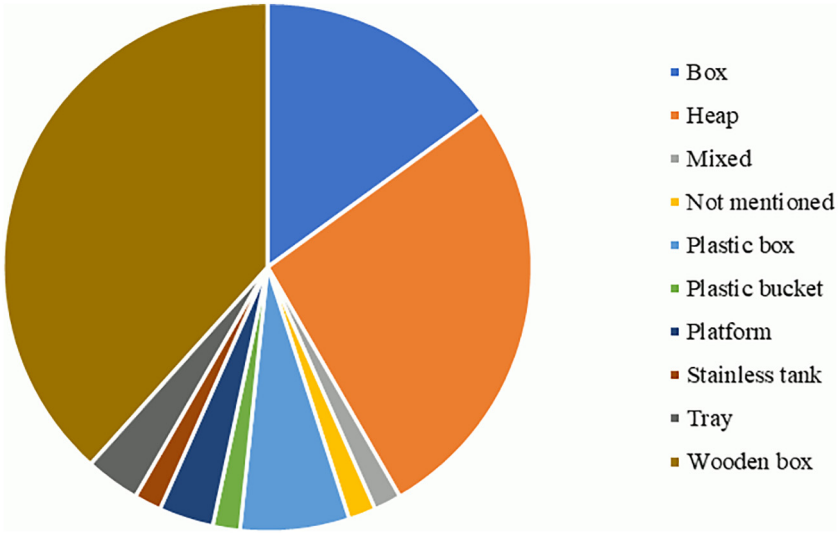

Fig. 4. Relative frequency of the type of cocoa fermentation method used.

these differences, the perception by consumers did not show a significant change (Crafack et al., 2014).

The differences between the composition of VOCs between inoculated and non-inoculated cocoa beans is also supported by Visintin et al. (2017). According to this study, a positive impact on the aromatic profile of the chocolates produced with beans inoculated with a mixed starter culture composed by *S. cerevisiae* and *T. delbrueckii* and a monoculture of *T. delbrueckii* was observed in comparison to those obtained through spontaneous fermentation. However, a recent study observed no significant difference between the composition of VOCs between inoculated and non-inoculated cocoa beans using the same inoculated yeast strains at the end of the fermentation (Mota-Gutierrez et al., 2018).

On the other hand, Crafack et al. (2013), studied the potential impact of *P. kluyveri* (aromatic) and *K. marxianus* (pectinolytic) on cocoa flavour during fermentation coupled with *Lb. fermentum* L18 or with *A. pasteurianus* A149. They concluded that the starter culture composed by *P. kluyveri* CH/*Lb. fermentum* L18/*A. pasteurianus* A149 seemed to have a positive effect on the flavour profile compared with spontaneously fermented bean by obtaining the highest scores for sensorial descriptors such as sweetness, fruitiness, cocoa aroma and general liking from a panel of an un-trained panellist. Besides the most studied microorganisms also *Bacillus pumilus* and *Pichia mexicana* have been shown to provide an acceptable level or degree of cocoa fermentation (Gibe and Pangan, 2015).

Based on knowledge of bacterial metabolism and physiology as on the microbial diversity within fermented cocoa beans, two bacterial species, *Lb. fermentum* and *A. pasteurianus* can be considered good candidates for starter cultures. In detail, the heterofermentative metabolism, fructose-growth capacity, citrate conversion, mannitol production, and acid-heat, and ethanol tolerance of *Lb. fermentum* are suitable characteristics of a starter strains in cocoa fermentation (Adler et al., 2013; Camu et al., 2007; De Vuyst and Weckx, 2016; Lefeber et al., 2010, 2011), while *A. pasteurianus*, a relevant AAB in the cocoa bean fermentation process, shows ethanol-, mannitol-, and lactic acid- oxidising capacity and heat-tolerance (Ardhana and Fleet, 2003; De Vuyst and Weckx, 2016; Moens et al., 2014; Papalexandratou et al., 2013). Finally, the great diversity of yeasts involved in the cocoa bean fermentation process has a pronounced effect on the fermentation efficiency and the quality of the fermented cocoa bean. The most outstanding yeast species, such as *Saccharomyces*, *Pichia*, and *Hanseniaspora* have suitable characteristics, such as the production of aromatic

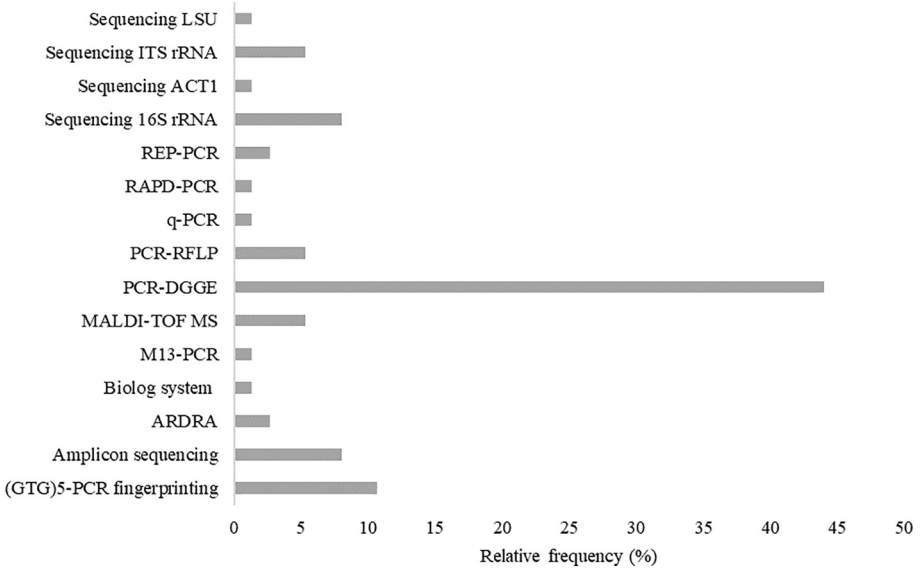

Fig. 5. Relative percentage of the type of independent methodology used to identify fermented cocoa microbial diversity.

compounds, high growth capacity and pectinolytic activity (De Vuyst and Weckx, 2016; Ho et al., 2014; Meersman et al., 2013). In general, we can conclude that a proper starter culture can direct the cocoa fermentation process and must be composed of at least one strain of each microorganism's group (yeast, LAB, and AAB). However, it is necessary to select the strain of LAB properly to avoid overproduction of lactic acid during this process and therefore having a negative impact on cocoa bean quality.

Regarding the application of starter cultures at an industrial level, only yeasts cultures have been employed for cocoa fermentation. In detail, the company Barry Callebaut executed a project called "Cocoa Horizons" in Ivory Coast promoting the use of a yeast starter culture, mainly *Saccharomyces* strains, to conduct the fermentation process of cocoa to obtain a chocolate with a high content of aromatic compounds Terra cacao®, in which more than 8000 cocoa beneficiaries participated (Nielburg, 2014). Also, a starter culture company Lallemand, a French chocolate producer Cémoi together with an agriculture research centre (CIRAD) launched a research program in 2016 focused on conducting the cocoa bean fermentation process by using starter cultures to obtain a consistent quality of chocolate. To achieve this objective, commercial yeast strains used in wine production, as well as commercial AAB, were used. However, during the execution of this five-year project, it is also intended to isolate and characterise the wild yeasts strains during this process to also be considered as potential starter cultures strains (Nielburg, 2016).

6. Challenges and perspectives

The use of genomics and metabolomics techniques as well as bioinformatic tools have generated a great amount of knowledge to elucidated the cocobiota ecosystem. Current challenges -omics analysis are related to accurate identification and the microbial community and functions. These methods are characterised to be computationally intensive and are again challenged by sequencing bias as well as under/overestimation (Mota-Gutierrez et al., 2019). Despite these challenges, culture-independent methods have revealed greater richness and numbers of bacteria and yeast than found by culturing (Arana-Sánchez et al., 2015; Ho et al., 2014; Mota-Gutierrez et al., 2019; Papalexandratou et al., 2011b; Pedroso Da Cruz et al., 2017) and have been providing novel insights into many food ecosystems and hold great promising data access. However, culture dependent studies are necessary to achieve the proper selection of the strains that will be part of starter culture. In addition, it is highly recommended to combine a methodological strategy that allows us to achieve the identification at strain level with analytic and sensorial data to evaluate the potential of each microorganism (yeast and bacteria) as starter cultures to conduct the cocoa bean fermentation.

On the other hand, the success of the starter culture is highly influenced by on the environment and/or agricultural practices of the country where the cocoa is produced (Mota-Gutierrez et al., 2018). For this reason, it is recommended that each cocoa producing region characterise the autochthonous microbial diversity involved in this process. In this regard, most studies of microbial diversity in fermented cocoa bean have been conducted in Africa (Ghana, Cameroon, Nigeria and Ivory Coast), Southeast Asia (Malaysia, Philippines, Indonesia), South America (Brazil, Bolivia and Ecuador), North America (Mexico), Central America (Cuba, and Honduras) and Oceania (Australia). Interestingly, in Mexico, a country with a deep-rooted tradition of production and consumption of cocoa, we found only one study describing the yeast diversity in fermented cocoa beans. Therefore, more studies are needed to characterise the cocoa microbial diversity during fermentation to design a suitable mixed starter culture to conduct the fermentation process and to obtain a fermented cocoa bean with competitive quality in the international market.

Starter cultures will not be able to standardise the whole fermentation process alone. Thus, the design and construction of a fermentation system that allows this process to be carried out under controlled conditions and thus avoiding microbial contamination with a probable reduction of the fermentation time need to be considered. A fermentation system could allow us to have a standardised, mechanised, and reproducible cocoa bean fermentation process. The design of stainless steel bioreactors has been proposed to carry out this process (Pereira et al., 2013b). However, other process conditions must be taken into consideration, such as a specific rate of agitation, the adequate control of temperature and pH following the microbial succession pattern and they must have a system that allows the elimination of waste from the process. It should be noted that the design of the starter culture and a suitable fermentation system will allow us to control and conduct this process.

Lastly, the -omics sciences seem to be an excellent alternative to understand the diversity and functionality of the microorganisms involved in the cocoa ecosystem. Illeghems et al. (2015) reconstructed the microbial meta-pathways based on metagenomic data of a single representative sample of Brazilian spontaneous cocoa bean fermentation (30 h). The analysis of bacterial functionality showed that the central metabolic pathways associated with LAB were heterolactic fermentation and citrate assimilation, while AAB, was only partially reconstructed and was involved in responses toward stress. However, although the data used in this study were representative for this fermentation point since the sample was analysed several times by different techniques, it is necessary to perform more research with different fermentation cocoa bean samples in order to achieve a full panorama of the complex microbial functionality of the cocoa bean fermentation ecosystem. Challenges and opportunities in understanding the complexity of microbial diversity and interactions in fermented cocoa beans under a control fermentation system guided by a dominated microbial species might help us to obtain high-quality chocolate.

7. Conclusions

In conclusion, the available knowledge highlights the lack of characterising autochthonous microbial diversity by region of all or key producing cocoa countries. As consequences, the conduction of cocoa fermentative process by autochthonous starter cultures has not been able to be industrialised yet. However, chocolate companies together with research centres have started using commercial yeast starter cultures or autochthonous microorganisms to direct this fermentation process. It is imperative to select appropriately the microorganisms that will be part of this starter culture, based on the functional, physiological, and biochemical criteria of each of the microbial strains.

This review also highlights the limited availability of -omics analysis to date, to compare microbial diversity and functionality involved in cocoa bean fermentation ecosystem around the world. From a molecular perspective, concerning the identification of especially yeast, there is an evident need for more extensive identification of microbial species to ensure innocuous fermented cocoa bean with homogenous quality.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.05.002.

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References

Adler, P., Bolten, C.J., Dohnt, K., Hansen, C.E., Wittmann, C., 2013. Core fluxome and metafluxome of lactic acid bacteria under simulated cocoa pulp fermentation conditions. Appl. Environ. Microbiol. 79, 5670–5681. https://doi.org/10.1128/aem.01483-13.

Adler, P., Frey, L.J., Berger, A., Bolten, C.J., Hansen, C.E., Wittmann, C., 2014. The key to acetate: metabolic fluxes of acetic acid bacteria under cocoa pulp fermentation-

simulating conditions. Appl. Environ. Microbiol. 80, 4702–4716. https://doi.org/10.1128/aem.01048-14.
Afoakwa, E.O., Quao, J., Takrama, J., Budu, A.S., Saalia, F.K., 2013. Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. J. Food Sci. Technol. 50, 1097–1105. https://doi.org/10.1007/s13197-011-0446-5.
Arana-Sánchez, A., Segura-García, L.E., Kirchmayr, M., Orozco-Ávila, I., Lugo-Cervantes, E., Gschaedler-Mathis, A., 2015. Identification of predominant yeasts associated with artisan Mexican cocoa fermentations using culture-dependent and culture-independent approaches. World J. Microbiol. Biotechnol. 31, 359–369. https://doi.org/10.1007/s11274-014-1788-8.
Ardhana, M.M., Fleet, G.H., 2003. The microbial ecology of cocoa bean fermentations in Indonesia. Int. J. Food Microbiol. 86, 87–99. https://doi.org/10.1016/S0168-1605(03)00081-3.
Bastos, V.S., Santos, M.F., Gomes, L.P., Leite, A.M., Flosi Paschoalin, V.M., Del Aguila, E.M., 2018. Analysis of the cocobiota and metabolites of *Moniliophthora perniciosa* -resistant *Theobroma cacao* beans during spontaneous fermentation in southern Brazil. J. Sci. Food Agric. 98, 4963–4970. https://doi.org/10.1002/jsfa.9029.
Batista, N.N., Ramos, C.L., Ribeiro, D.D., Pinheiro, A.C.M., Schwan, R.F., 2015. Dynamic behavior of *Saccharomyces cerevisiae*, *Pichia kluyveri* and *Hanseniaspora uvarum* during spontaneous and inoculated cocoa fermentations and their effect on sensory characteristics of chocolate. LWT - Food Sci. Technol. 63, 221–227. https://doi.org/10.1016/j.lwt.2015.03.051.
Besnard, V., Federighi, M., Cappelier, J.M., 2000. Development of a direct viable count procedure for the investigation of VBNC state in *Listeria monocytogenes*. Lett. Appl. Microbiol. 31, 77–81. https://doi.org/10.1046/j.1472-765x.2000.00771.x.
Bortolini, C., Patrone, V., Puglisi, E., Morelli, L., 2016. Detailed analyses of the bacterial populations in processed cocoa beans of different geographic origin, subject to varied fermentation conditions. Int. J. Food Microbiol. 236, 98–106. https://doi.org/10.1016/j.ijfoodmicro.2016.07.004.
Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I.B., Prajapati, J.B., Seto, Y., Ter Schure, E., Van Boven, A., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S., Hansen, E.B., 2012. Food fermentations: microorganisms with technological beneficial use. Int. J. Food Microbiol. https://doi.org/10.1016/j.ijfoodmicro.2011.12.030.
Bucheli, P., Rousseau, G., Alvarez, M., Laloi, M., McCarthy, J., 2001. Developmental variation of sugars, carboxylic acids, purine alkaloids, fatty acids, and endoproteinase activity during maturation of *Theobroma cacao* L. seeds. J. Agric. Food Chem. 49, 5046–5051. https://doi.org/10.1021/jf010620z.
Caligiani, A., Marseglia, A., Prandi, B., Palla, G., Sforza, S., 2016. Influence of fermentation level and geographical origin on cocoa bean oligopeptide pattern. Food Chem. 211, 431–439. https://doi.org/10.1016/j.foodchem.2016.05.072.
Camu, N., De Winter, T., Verbrugghe, K., Cleenwerck, I., Vandamme, P., Takrama, J.S., Vancanneyt, M., De Vuyst, L., 2007. Dynamics and biodiversity of populations of lactic acid bacteria and acetic acid bacteria involved in spontaneous heap fermentation of cocoa beans in Ghana. Appl. Environ. Microbiol. 73, 1809–1824. https://doi.org/10.1128/aem.02189-06.
Camu, N., De Winter, T., Addo, S.K., Takrama, J.S., Bernaert, H., De Vuyst, L., 2008a. Fermentation of cocoa beans: influence of microbial activities and polyphenol concentrations on the flavour of chocolate. J. Sci. Food Agric. 88, 2288–2297. https://doi.org/10.1002/jsfa.3349.
Camu, N., González, A., De Winter, T., Van Schoor, A., De Bruyne, K., Vandamme, P., Takrama, J.S., Addo, S.K., De Vuyst, L., 2008b. Influence of turning and environmental contamination on the dynamics of populations of lactic acid and acetic acid bacteria involved in spontaneous cocoa bean heap fermentation in Ghana. Appl. Environ. Microbiol. 74, 86–98. https://doi.org/10.1128/aem.01512-07.
Cleenwerck, I., Gonzalez, A., Camu, N., Engelbeen, K., De Vos, P., De Vuyst, L., 2008. *Acetobacter fabarum* sp. nov., an acetic acid bacterium from a Ghanaian cocoa bean heap fermentation. Int. J. Syst. Evol. Microbiol. 58, 2180–2185. https://doi.org/10.1099/ijs.0.65778-0.
Cocolin, L., Ercolini, D., 2015. Zooming into food-associated microbial consortia: a 'cultural' evolution. Curr. Opin. Food Sci. 2, 43–50. https://doi.org/10.1016/j.cofs.2015.01.003.
Cocolin, L., Gobbetti, M., Neviani, E., Daffonchio, D., 2016. Ensuring safety in artisanal food microbiology. Nat. Microbiol. 1, 16171. https://doi.org/10.1038/nmicrobiol.2016.171.
Copetti, M.V., Iamanaka, B.T., Pereira, J.L., Fungaro, M.H., Taniwaki, M.H., 2011. Aflatoxigenic fungi and aflatoxin in cocoa. Int. J. Food Microbiol. 148, 141–144. https://doi.org/10.1016/j.ijfoodmicro.2011.05.020.
Crafack, M., Mikkelsen, M.B., Saerens, S., Knudsen, M., Blennow, A., Lowor, S., Takrama, J., Swiegers, J.H., Petersen, G.B., Heimdal, H., Nielsen, D.S., 2013. Influencing cocoa flavour using *Pichia kluyveri* and *Kluyveromyces marxianus* in a defined mixed starter culture for cocoa fermentation. Int. J. Food Microbiol. 167, 103–116. https://doi.org/10.1016/j.ijfoodmicro.2013.06.024.
Crafack, M., Keul, H., Eskildsen, C.E., Petersen, M.A., Saerens, S., Blennow, A., Skovmand-Larsen, M., Swiegers, J.H., Petersen, G.B., Heimdal, H., Nielsen, D.S., 2014. Impact of starter cultures and fermentation techniques on the volatile aroma and sensory profile of chocolate. Food Res. Int. 63, 306–316. https://doi.org/10.1016/j.foodres.2014.04.032.
Daniel, H.-M., Vrancken, G., Takrama, J.F., Camu, N., De Vos, P., De Vuyst, L., 2009. Yeast diversity of Ghanaian cocoa bean heap fermentations. FEMS Yeast Res. 9, 774–783. https://doi.org/10.1111/j.1567-1364.2009.00520.x.
De Vuyst, L., Weckx, S., 2016. The cocoa bean fermentation process: from ecosystem analysis to starter culture development. J. Appl. Microbiol. 121, 5–17. https://doi.org/10.1111/jam.13045.
D'Souza, R.N., Grimbs, A., Grimbs, S., Behrends, B., Corno, M., Ullrich, M.S., Kuhnert, N., 2018. Degradation of cocoa proteins into oligopeptides during spontaneous fermentation of cocoa beans. Food Res. Int. 109, 506–516. https://doi.org/10.1016/j.foodres.2018.04.068.
Dujon, B.A., Louis, E.J., 2017. Genome diversity and evolution in the budding yeasts (*Saccharomycotina*). Genetics 206, 717–750. https://doi.org/10.1534/genetics.116.199216.
Fernández- Maura, Y., Balzarini, T., Clapé Borges, P., Evrard, P., De Vuyst, L., Daniel, H.-M., 2016. The environmental and intrinsic yeast diversity of Cuban cocoa bean heap fermentations. Int. J. Food Microbiol. 233, 34–43. https://doi.org/10.1016/j.ijfoodmicro.2016.06.012.
Food and Agriculture Organization of the United Nations (FAO), 2018. FAOSTAT Database. Available in. http://www.fao.org/faostat/en/#data/QC/visualize, Accessed date: 5 January 2019.
Garcia-Armisen, T., Papalexandratou, Z., Hendryckx, H., Camu, N., Vrancken, G., De Vuyst, L., Cornelis, P., 2010. Diversity of the total bacterial community associated with Ghanaian and Brazilian cocoa bean fermentation samples as revealed by a 16S rRNA gene clone library. Appl. Microbiol. Biotechnol. 87, 2281–2292. https://doi.org/10.1007/s00253-010-2698-9.
Gibe, A.J.G., Pangan, J.O., 2015. Evaluation of *Pichia* mexicana and *Bacillus pumilus* in hastening fermentation process and its resulting effect of the quality of dried cacao beans. Asian J. Appl. Sci. 3, 1–7.
Hamdouche, Y., Guehi, T., Durand, N., Kedjebo, K.B.D., Montet, D., Meile, J.C., 2015. Dynamics of microbial ecology during cocoa fermentation and drying: towards the identification of molecular markers. Food Control 48, 117–122. https://doi.org/10.1016/j.foodcont.2014.05.031.
Ho, V.T.T., Zhao, J., Fleet, G., 2014. Yeasts are essential for cocoa bean fermentation. Int. J. Food Microbiol. 174, 72–87. https://doi.org/10.1016/j.ijfoodmicro.2013.12.014.
Ho, V.T.T., Zhao, J., Fleet, G., 2015. The effect of lactic acid bacteria on cocoa bean fermentation. Int. J. Food Microbiol. 205, 54–67. https://doi.org/10.1016/j.ijfoodmicro.2015.03.031.
Illeghems, K., De Vuyst, L., Papalexandratou, Z., Weckx, S., 2012. Phylogenetic analysis of a spontaneous cocoa bean fermentation metagenome reveals new insights into its bacterial and fungal community diversity. PLoS One 7, 1–11. https://doi.org/10.1371/journal.pone.0038040.
Illeghems, K., Weckx, S., De Vuyst, L., 2015. Applying meta-pathway analyses through metagenomics to identify the functional properties of the major bacterial communities of a single spontaneous cocoa bean fermentation process sample. Food Microbiol. 50, 54–63. https://doi.org/10.1016/j.fm.2015.03.005.
International Cocoa Organization, 2013. Growing cocoa. Available in. https://www.icco.org/about-cocoa/growing-cocoa.html, Accessed date: 5 January 2019.
Jespersen, L., Nielsen, D., Honholt, S., Jakobsen, M., 2005. Occurrence and diversity of yeasts involved in fermentation of west African cocoa beans. FEMS Yeast Res. 5, 441–453. https://doi.org/10.1016/j.femsyr.2004.11.002.
Kadow, D., Bohlmann, J., Phillips-Mora, W., Lieberei, R., 2013. Identification of main fine or flavour components in two genotypes of the cocoa tree (*Theobroma cacao* L.). J. Appl. Bot. Food Qual. 86, 90–98. https://doi.org/10.5073/jabfq.2013.086.013.
Kadow, D., Niemenak, N., Rohn, S., Lieberei, R., 2015. Fermentation-like incubation of cocoa seeds (*Theobroma cacao* L.) – reconstruction and guidance of the fermentation process. LWT - Food Sci. Technol. 62, 357–361. https://doi.org/10.1016/j.lwt.2015.01.015.
Koné, M.K., Guéhi, S.T., Durand, N., Ban-Koffi, L., Berthiot, L., Tachon, A.F., Brou, K., Boulanger, R., Montet, D., 2016. Contribution of predominant yeasts to the occurrence of aroma compounds during cocoa bean fermentation. Food Res. Int. 89, 910–917. https://doi.org/10.1016/j.foodres.2016.04.010.
Kongor, J.E., Hinneh, M., Walle, de, Van, D., Afoakwa, E.O., Boeckx, P., Dewettinck, K., 2016. Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavour profile — a review. Food Res. Int. 82, 44–52. https://doi.org/10.1016/j.foodres.2016.01.012.
Kostinek, M., Ban-Koffi, L., Ottah-Atikpo, M., Teniola, D., Schillinger, U., Holzapfel, W.H., Franz, C.M.A.P., 2008. Diversity of predominant lactic acid bacteria associated with cocoa fermentation in Nigeria. Curr. Microbiol. 56, 306–314. https://doi.org/10.1007/s00284-008-9097-9.
Lagunes- Gálvez, S., Loiseau, G., Paredes, J.L., Barel, M., Guiraud, J.-P., 2007. Study on the microflora and biochemistry of cocoa fermentation in the Dominican Republic. Int. J. Food Microbiol. 114, 124–130. https://doi.org/10.1016/j.ijfoodmicro.2006.10.041.
Leal, G.A., Gomes, L.H., Efraim, P., de Almeida Tavares, F.C., Figueira, A., 2008. Fermentation of cacao (*Theobroma cacao* L.) seeds with a hybrid *Kluyveromyces marxianus* strain improved product quality attributes. FEMS Yeast Res. 8, 788–798. https://doi.org/10.1111/j.1567-1364.2008.00405.x.
Lefeber, T., Janssens, M., Camu, N., De Vuyst, L., 2010. Kinetic analysis of strains of lactic acid bacteria and acetic acid bacteria in cocoa pulp simulation media toward development of a starter culture for cocoa bean fermentation. Appl. Environ. Microbiol. 76, 7708–7716. https://doi.org/10.1128/aem.01206-10.
Lefeber, T., Janssens, M., Moens, F., Gobert, W., De Vuyst, L., 2011. Interesting starter culture strains for controlled cocoa bean fermentation revealed by simulated cocoa pulp fermentations of cocoa-specific lactic acid bacteria. Appl. Environ. Microbiol. 77, 6694–6698. https://doi.org/10.1128/aem.00594-11.
Lefeber, T., Papalexandratou, Z., Gobert, W., Camu, N., De Vuyst, L., 2012. On-farm implementation of a starter culture for improved cocoa bean fermentation and its influence on the flavour of chocolates produced thereof. Food Microbiol. 30, 379–392. https://doi.org/10.1016/j.fm.2011.12.021.
Lima, L.J.R., Almeida, M.H., Nout, M.J.R., Zwietering, M.H., 2011. *Theobroma cacao* L., "The food of the gods": quality determinants of commercial cocoa beans, with particular reference to the impact of fermentation. Crit. Rev. Food Sci. Nutr. 51, 731–761. https://doi.org/10.1080/10408391003799913.

Meersman, E., Steensels, J., Mathawan, M., Wittocx, P.-J., Saels, V., Struyf, N., Bernaert, H., Vrancken, G., Verstrepen, K.J., 2013. Detailed analysis of the microbial population in Malaysian spontaneous cocoa pulp fermentations reveals a core and variable microbiota. PLoS One 8, 1–10. https://doi.org/10.1371/journal.pone.0081559.

Meersman, E., Steensels, J., Struyf, N., Paulus, T., Saels, V., Mathawan, M., Allegaert, L., Vrancken, G., Verstrepen, K.J., 2016. Tuning chocolate flavor through development of thermotolerant *Saccharomyces cerevisiae* starter cultures with increased acetate ester production. Appl. Environ. Microbiol. 82, 732–746. https://doi.org/10.1128/aem.02556-15.

Menezes, A.G., Batista, N.N., Ramos, C.L., de Andrade e Silva, A.R., Efraim, P., Pinheiro, A.C.M., Schwan, R.F., 2016. Investigation of chocolate produced from four different Brazilian varieties of cocoa (*Theobroma cacao* L.) inoculated with *Saccharomyces cerevisiae*. Food Res. Int. 81, 83–90. doi:https://doi.org/10.1016/j.foodres.2015.12.036.

Miescher Schwenninger, S., Freimüller Leischtfeld, S., Gantenbein-Demarchi, C., 2016. High-throughput identification of the microbial biodiversity of cocoa bean fermentation by MALDI-TOF MS. Lett. Appl. Microbiol. 63, 347–355. https://doi.org/10.1111/lam.12621.

Moens, F., Lefeber, T., De Vuyst, L., 2014. Oxidation of metabolites highlights the microbial interactions and role of *Acetobacter pasteurianus* during cocoa bean fermentation. Appl. Environ. Microbiol. 80, 1848–1857. https://doi.org/10.1128/aem.03344-13.

Moreira, I.M. da V., Miguel, M.G. da C.P., Duarte, W.F., Dias, D.R., Schwan, R.F., 2013. Microbial succession and the dynamics of metabolites and sugars during the fermentation of three different cocoa (*Theobroma cacao* L.) hybrids. Food Res. Int. 54, 9–17. https://doi.org/10.1016/j.foodres.2013.06.001.

Moreira, I., Pedrozo Miguel, G.M., Lacerda Ramos, C., Ferreira Duarte, W., Efraim, P., Freitas Schwan, R., 2016. Influence of cocoa hybrids on volatile compounds of fermented beans, microbial diversity during fermentation and sensory characteristics and acceptance of chocolates. J. Food Qual. 39, 839–849. https://doi.org/10.1111/jfq.12238.

Mota-Gutierrez, J., Botta, C., Ferrocino, I., Giordano, M., Bertolino, M., Dolci, P., Cannoni, M., Cocolin, L., 2018. Dynamics and biodiversity of bacterial and yeast communities during fermentation of cocoa beans. Appl. Environ. Microbiol. 84, e01164-18. https://doi.org/10.1128/aem.01164-18.

Mota-Gutierrez, J., Ferrocino, I., Rantsiou, K., Cocolin, L., 2019. Metataxonomic comparison between internal transcribed spacer and 26S ribosomal large subunit (LSU) rDNA gene. Int. J. Food Microbiol. 290, 132–140. https://doi.org/10.1016/j.ijfoodmicro.2018.10.010.

Mounjouenpou, P., Gueule, D., Fontana-Tachon, A., Guyot, B., Tondje, P.R., Guiraud, J.-P., 2008. Filamentous fungi producing ochratoxin a during cocoa processing in Cameroon. Int. J. Food Microbiol. 121, 234–241. https://doi.org/10.1016/j.ijfoodmicro.2007.11.017.

Nielburg, O., 2014. Improved chocolate quality with controlled cocoa fermentation, says Barry Callebaut. Available in Confect. news.comhttps://www.confectionerynews.com/Article/2014/02/04/Controlled-cocoa-fermentation-with-starter-cultures-Barry-Callebaut.Acessed:, Accessed date: 10 January 2019.

Nielburg O, 2016. Cémoi brings wine science to cocoa in chocolate research project. In Confect. news.com. Available in https://www.confectionerynews.com/Article/2016/04/12/Cemoi-brings-wine-science-to-cocoa-in-chocolate-research-project. Acessed: 2019-January-9.

Nielsen, D.S., Hønholt, S., Tano-Debrah, K., Jespersen, L., 2005. Yeast populations associated with Ghanaian cocoa fermentations analysed using denaturing gradient gel electrophoresis (DGGE). Yeast 22, 271–284. https://doi.org/10.1002/yea.1207.

Nielsen, D.S., Teniola, O.D., Ban-Koffi, L., Owusu, M., Andersson, T.S., Holzapfel, W.H., 2007. The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. Int. J. Food Microbiol. 114, 168–186. https://doi.org/10.1016/j.ijfoodmicro.2006.09.010.

Nocker, A., Burr, M., Camper, A.K., 2007. Genotypic microbial community profiling: a critical technical review. Microb. Ecol. https://doi.org/10.1007/s00248-006-9199-5.

Ostovar, K., Keeney, P.G., 1973. Isolation and characterization of microorganisms involved in the fermentation of Trinidad's cacao beans. J. Food Sci. 38, 611–617. https://doi.org/10.1111/j.1365-2621.1973.tb02826.x.

Ouattara, H.G., Koffi, B.L., Karou, G.T., Sangaré, A., Niamke, S.L., Diopoh, J.K., 2008. Implication of *Bacillus* sp. in the production of pectinolytic enzymes during cocoa fermentation. World J. Microbiol. Biotechnol. 24, 1753–1760. https://doi.org/10.1007/s11274-008-9683-9.

Ouattara, H.G., Reverchon, S., Niamke, S.L., Nasser, W., 2011. Molecular identification and pectate lyase production by *Bacillus* strains involved in cocoa fermentation. Food Microbiol. 28, 1–8. https://doi.org/10.1016/j.fm.2010.07.020.

Ouattara, H.D., Ouattara, H.G., Droux, M., Reverchon, S., Nasser, W., Niamke, S.L., 2017. Lactic acid bacteria involved in cocoa beans fermentation from Ivory Coast: species diversity and citrate lyase production. Int. J. Food Microbiol. 256, 11–19. https://doi.org/10.1016/j.ijfoodmicro.2017.05.008.

Ozturk, G., Young, G.M., 2017. Food evolution: the impact of society and science on the fermentation of cocoa beans. Compr. Rev. Food Sci. Food Saf. 16, 431–455. https://doi.org/10.1111/1541-4337.12264.

Packiyasothy, E.V., Jansz, E.R., Senanayake, U.M., Wijesundara, R.C., Wickremasinghe, P., 1981. Effect of maturity on some chemical components of cocoa. J. Sci. Food Agric. 32, 873–876. https://doi.org/10.1002/jsfa.2740320906.

Papalexandratou, Z., Vuyst, L., 2011. Assessment of the yeast species composition of cocoa bean fermentations in different cocoa-producing regions using denaturing gradient gel electrophoresis. FEMS Yeast Res. 11, 564–574. https://doi.org/10.1111/j.1567-1364.2011.00747.x.

Papalexandratou, Z., Camu, N., Falony, G., De Vuyst, L., 2011a. Comparison of the bacterial species diversity of spontaneous cocoa bean fermentations carried out at selected farms in Ivory Coast and Brazil. Food Microbiol. 28, 964–973. https://doi.org/10.1016/j.fm.2011.01.010.

Papalexandratou, Z., Falony, G., Romanens, E., Jimenez, J.C., Amores, F., Daniel, H.-M., De Vuyst, L., 2011b. Species diversity, community dynamics, and metabolite kinetics of the microbiota associated with traditional ecuadorian spontaneous cocoa bean fermentations. Appl. Environ. Microbiol. 77, 7698–7714. https://doi.org/10.1128/aem.05523-11.

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

Papalexandratou, Z., Lefeber, T., Bahrim, B., Lee, O.S., Daniel, H.-M., De Vuyst, L., 2013. *Hanseniaspora opuntiae*, *Saccharomyces cerevisiae*, *Lactobacillus fermentum*, and *Acetobacter pasteurianus* predominate during well-performed Malaysian cocoa bean box fermentations, underlining the importance of these microbial species for a successful cocoa. Food Microbiol. 35, 73–85. https://doi.org/10.1016/j.fm.2013.02.015.

Pedroso Da Cruz, M., Viana De Castro Reis, L., Efraim, P., Santos, C., Lima, N., Schwan, R.F., 2017. Cocoa fermentation: microbial identification by MALDI-TOF MS, and sensory evaluation of produced chocolate. LWT - Food Sci. Technol. 77, 362–369. https://doi.org/10.1016/j.lwt.2016.11.076.

Pereira, G., Magalhães-Guedes, K.T., Schwan, R.F., 2013a. RDNA-based DGGE analysis and electron microscopic observation of cocoa beans to monitor microbial diversity and distribution during the fermentation process. Food Res. Int. 53, 482–486. https://doi.org/10.1016/j.foodres.2013.05.030.

Pereira, G., Magalhães, K.T., de Almeida, E.G., da Silva Coelho, I., Schwan, R.F., 2013b. Spontaneous cocoa bean fermentation carried out in a novel-design stainless steel tank: influence on the dynamics of microbial populations and physical–chemical properties. Int. J. Food Microbiol. 161, 121–133. https://doi.org/10.1016/j.ijfoodmicro.2012.11.018.

Pereira, G.V. de M., Soccol, V.T., Soccol, C.R., 2016. Current state of research on cocoa and coffee fermentations. Curr. Opin. Food Sci. 7, 50–57. https://doi.org/10.1016/j.cofs.2015.11.001.

Rabadan-Chávez, G., Lugo-Cervantes, E., 2018. Phenolic compounds in cocoa and chocolate. In: Nollet, L.M.L., Gutierrez-Uribe, J.A. (Eds.), Phenolic Compounds in Food. vol. 2018. CRC Press, Taylor & Francis Group, CRC Press, Boca Raton, pp. 375–393. https://doi.org/10.1021/bk-1992-0506.ch001.

Romanens, E., Näf, R., Lobmaier, T., Pedan, V., Leischtfeld, S.F., Meile, L., Schwenninger, S.M., 2018. A lab-scale model system for cocoa bean fermentation. Appl. Microbiol. Biotechnol. 102, 3349–3362. https://doi.org/10.1007/s00253-018-8835-6.

Samagaci, L., Ouattara, H., Niamké, 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 Res. Int. 89, 773–780. https://doi.org/10.1016/j.foodres.2016.10.007.

Schwan, R., 1998. Cocoa fermentations conducted with a defined microbial cocktail inoculum. Appl. Environ. Microbiol. 64, 1477–1483.

Schwan, R., Wheals, A.E., 2004. The microbiology of cocoa fermentation and its role in chocolate quality. Crit. Rev. Food Sci. Nutr. 44, 205–221. https://doi.org/10.1080/10408690490464104.

Schwan, R.F., Fleet, G.H., Afoakwa, E.O., 2015. Cocoa and Coffee Fermentations. CRC Press, Boca Raton.

Steinkraus, K.H., 2002. Fermentations in world food processing. Compr. Rev. Food Sci. Food Saf. 1, 23–32. https://doi.org/10.1111/j.1541-4337.2002.tb00004.x.

Visintin, S., Alessandria, V., Valente, A., Dolci, P., Cocolin, L., 2016. Molecular identification and physiological characterization of yeasts, lactic acid bacteria and acetic acid bacteria isolated from heap and box cocoa bean fermentations in West Africa. Int. J. Food Microbiol. 216, 69–78. https://doi.org/10.1016/j.ijfoodmicro.2015.09.004.

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. Int. J. Food Microbiol. 257, 31–40. https://doi.org/10.1016/j.ijfoodmicro.2017.06.004.