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Emphasis on Techno-functional Properties of *Bacillus* Strains Involved in Ivorian Cocoa Fermentation towards their Use as Potential Starter

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SLN and HGO designed the study. Authors SLN, HGO and WY managed the draft of the manuscript. Authors HGO, WY, BGG wrote the protocol. Authors WY, BGG, GAK and GD managed the literature searches and performed the analysis. All authors red and approved the final manuscript.

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ABSTRACT

Aims: To investigate in *Bacillus* strains some functional properties potentially interesting for cocoa fermentation processing
Place and Duration of Study: Laboratory of Biotechnology, UFR Biosciences, University Félix Houphouet-Boigny (Côte d'Ivoire), between May 2014 and March 2015.
Methodology: Spontaneous heaps fermentation were conducted in three cocoa producing regions. *Bacillus* were isolated from cocoa fermentation using plate agar on nutrient medium and analyzed for pectinolytic enzymes production, citric acid breakdown, acidification and thermotolerance: these properties are known as essential for cocoa fermentation.
Results: A total of 600 *Bacillus* strains were isolated and 42.50% of them produced pectinolytic activity with different levels of enzymes production. The production of these enzymes is influenced by sugars content and ethanol beyond 2%, while the influence of acid was sharper with 0.15% as limit of tolerance. A large proportion of *Bacillus* population (67.16%) exhibited acidifying capacity,

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this property was accompanied by gas production in some strains. The total acidity yield ranged from 30 to 300 mg/L and strains presenting gas production tend to produce more acid. Additionally, more than 75% of acidifying strains presented the ability to breakdown citric acid. Moreover, all the strains exhibited a strong thermotolerance at 45ºC with a level of bacterial growth round 80% of that reached at 35ºC, the optimal growth temperature. The distribution of these properties in the *Bacillus* population was not uniform in the different regions.

Conclusion: This study indicates that *Bacillus* strains involved in Ivorian cocoa fermentation possess some properties such as ability to produce pectinolytic enzymes, capacity to breakdown citric acid and strong thermotolerance at 45ºC, essential for a well-fermented cocoa. These strains may play a more important role in cocoa fermentation, than it is believed at date. Taken together, these results show that *Bacillus* studied should be potential candidate as starter for cocoa fermentation control.

Keywords: Cocoa fermentation; techno functional properties; Bacillus; starter; Côte d'Ivoire.

1. INTRODUCTION

Cocoa beans as the major raw material for chocolate production need to be fermented and dried before the transformation process. Cocoa fermentation takes place at farm level and constitute a spontaneous microbial process [1].

In fact, at the opening of cocoa pods, the fresh beans are immediately contaminated by numerous microbial strains mainly yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and *Bacillus* sp., which lead the fermentation and impact strongly the quality of fermented bean and chocolate [1-4]. The microbial activity in cocoa fermentation occurring in the pulp present in the outer part of the beans, begins with yeasts which oxidize the sugars contained in the pulp to produce ethanol, organic acid and volatile organic compounds; these strains also breakdown the mucilaginous pulp (viscous and sticky mater due to pectin) by producing pectinolytic enzymes that are known to be indispensable for normal course of the fermentation process and for the development of the quality of fermented cocoa [5].

Furthermore, via the citrate metabolism, yeasts are responsible for rising the pH of the fermentative mass which is initially acid (due to citric acid), allowing the growth of desired bacterial strains. Lactic acid bacteria (LAB) play also an important role in the citrate metabolism, contributing more than yeasts to pH modulation during cocoa fermentation [6]. Furthermore, LAB converts sugars and citrate to lactic acid, acetic acid, ethanol and mannitol that migrate into beans to trigger further reactions that develop the aromatic potential of chocolate [7].

Acetic acid bacteria (AAB) grow through oxidation of ethanol into acetic acid [6,8]. Ethanol and acetic acid diffuse into the beans, and this, in combination with the heat produced by this exothermic bioconversion, causes the death of the seed embryo as well as the end of fermentation. Acidification initiates biochemical changes in beans, leading to the formation of precursor molecules for the development of characteristic flavor and color of the beans [6].

However, *Bacillus* constitute the major microflora in cocoa fermentation with the less known role. For instance, *Bacillus* are thought to be responsible for off flavor of fermented cocoa without any study demonstrating that. Hence, *Bacillus* are believed to have no crucial role in cocoa fermentation, and yet, their have never been included in a microbial cocktail as starter in assay aiming to control and standardize cocoa fermentation. However, more recently, Ouattara et al. [9] reported their implication in the production of pectinolytic enzymes and assumed that these bacterial strains may act as complementary partner to yeast strains in the depectinization of pulp during cocoa fermentation [10]. Furthermore, the results achieved by Schwan clearly showed that chocolate obtained with a high presence of *Bacillus* gave less off-flavor comparatively to the chocolate obtained without *Bacillus* strains [11]. Several studies have showed that *Bacillus* are present throughout the spontaneous and natural cocoa fermentation at higher level than yeasts, LAB and AAB [12]. Obviously, the role of *Bacillus* strains in cocoa fermentation is not well elucidated at date. These strains may have an important and positive impact on cocoa fermentative process.

At date, cocoa fermentation remains a difficult process to control leading to variable quality of product and very often to low crop value for farmer. The control of this process requires a well understanding of cocoa microbiota

physiology and screening of strains with high biochemical performances.

A deep understanding of *Bacillus* role in the cocoa fermentation, notably their technological properties, is indispensible for their use as starter in microbial cocktails for control of the fermentation process. The aim of this paper is to explore some technological properties such as the production of pectinolytic enzymes, the acidification capacity and the citrate metabolism in *Bacillus* strains involved in Ivorian cocoa fermentation.

2. MATERIALS AND METHODS

2.1 Fermentation Conditions

Cocoa pods were harvested from 3 cultivars (Forastero, Trinitario, and Criollo cultivars) in the regions of Agnéby-Tiassa (geographic coordinates 5°59' North 4°28'West), Lôh-Djiboua (5°55' North 5°37'West) and Sud-Comoé (5°28'06' North 3°12'25' West). Approximately 100 kg of cocoa beans were removed from pods and fermented traditionally by heap fermentation for six days on banana leaves. Cocoa beans of different regions were fermented separately and were turned each 12 h.

2.2 Isolation of Bacterial Strains

Isolation was performed according to the standard method described by Nielsen et al. [3]. Fermenting samples, about 200 g were withdrawn every 12 h during the fermentation process and placed in sterile bag then transferred to laboratory. An amount of 25 g of fermenting cocoa beans was homogenized in 225 mL sterile peptone water (pH 7.0) to obtain a range of serial decimal dilutions spanning from 10^{-1} to 10^{-8}. The bacteria were isolated on plate nutrient agar supplemented with 0.1% nystatin to inhibit fungal growth, after incubating culture at 30ºC for 48 h. *Bacillus* strains were characterized as Gram- and catalase-positive rods, spore-forming and able to grow aerobically. The strains isolated were stored at -80ºC in nutrient broth supplemented with glycerol 20% in Eppendorf tubes, for further studies.

2.3 Analysis of *Bacillus* Strains for Pectinolytic Activity

2.3.1 Screening of pectinolytic strains

Pectinolytic strains were screened using the method described by Soares et al. [13] and adapted by Ouattara et al. [9]. Basal mineral medium was prepared with 0.28% $(NH_4)_2SO_4$, 0.6% K_2HPO_4, 0.01% $MgSO_4$, 0.2% KH_2PO_4, 0.02% yeast extract, 1% pectin and 1.7% agar, pH 6. Four wells of 0.5 cm in diameter and 2 to 3 mm in depth were made aseptically in the medium. Then pure *Bacillus* culture was suspended in saline tryptone to have an optical density of 1 at 600 nm. The wells were subsequently loaded with 7 µL of the suspension. All the wells of the same plate were inoculated with a single suspension and incubated at 30ºC for 48 h.

After, incubation, the solid culture medium was flooded with a solution of iodine and potassium iodide (5 g potassium iodide + 1 g iodine + 330 ml distilled water) to reveal the clear zones around the wells indicating pectinolytic activity and halo diameter was measured.

2.3.2 Influence of fermentation conditions on pectinolytic enzymes production in Bacillus

Sugars (glucose, fructose and sucrose), ethanol, organics acids (acetic acid, lactic acid and citric acid) and pectin present in the fermenting cocoa pulp constitute together with the temperature, the main conditions influencing microbial growth during cocoa fermentation [9]. The influence of these conditions on the production of pectinolytic enzymes in *Bacillus* strains was evaluated. The modulation of enzymes production by temperature was studied on solid medium described above. Cultures were incubated for 48 h at different temperatures ranging between 30 and 50ºC.

To evaluate the effect of sugars, ethanol, organic acids and pectin, the different compounds were added to the basal mineral medium at concentration 0.5 to 6% (v/v) for sugar, 0.5 to 4% (v/v) for pectin, 0.5 to 6% for ethanol and 0.025 to 0.5% for organic acids. Halo diameter was measured after incubation.

2.4 Analysis of *Bacillus* Strains for Acidification Capacity

Acidification of the fermenting cocoa beans is one of the most important factors indispensable for obtaining cocoa of good quality [14]. *Bacillus* strains isolated were subjected to analysis for their ability to acidify the medium according to the method described by Aydin and Aksoy [15] with slight modification. Calcium carbonate agar

containing 1% D-glucose, 0.5% yeast extract, 0.3% casein peptone and 1.5% agar was prepared and supplemented with 0.0016% bromocresol green, pH 6.2. A volume of 3 mL of medium was put in a 5 mL tube and then sterilized for 15 min at 121ºC.

Each strain was inoculated by central sting in the medium with pure 24 h pre-culture of bacterial strain and incubated at 30ºC for 48 h. A negative control was prepared in the same conditions but was not inoculated with microbial culture. Acid production was assessed by formation of yellow area in the tube with or not gas production and acid yielded was quantified by the method described by Nanda et al. [16]. Nutrient broth, pH 6.3, containing 1% D-glucose, D-fructose and D-saccharose was prepared. Five (5) mL of this medium contained in a 25 mL tube were sterilized for 15 min at 121ºC and inoculated with 100 µL of pure 24 hours pre-culture (OD600 = 1) of bacterial strains then incubated at 30ºC. After 24 hours, liquid medium was taken and analyzed for determination of growth rate, pH of medium and quantification of acid produced.

The growth rate was directly determinate by measurement of culture medium absorbance at 600 nm. The pH was measured in cell free supernatant after centrifugation of the culture medium at 4500 rpm for 10 min. Acid yield was quantified in cell free supernatants by titration with NaOH (0.1 N) and phenolphthalein as a color indicator. Acid production was expressed as mg/mL of acid produced using the following relation:

$$\text{acid quantity } \left(\frac{\text{mg}}{\text{mL}}\right) = \frac{\text{volume of NaOH} \times 0.1 \times \text{PM of acid}}{\text{volume of culture medium}}$$

2.5 Analysis of *Bacillus* Strains for Citrate Metabolism

The breakdown of citric acid contained in the cocoa pulp is very useful for raising the pH and allowing the growth of other bacterial strains during cocoa fermentation [17]. Citrate metabolism constitute a desired and relevant property in cocoa fermentation. The capacity of *Bacillus* sp to metabolize citrate was carried out on Simmons citrate medium. The medium was inoculated with pure pre-culture and incubated at 30°C for 48 h. After incubation, the colonies which appear blue on the medium are those able to metabolize citrate [18].

2.6 Thermotolerance Analysis of *Bacillus* Strains

The effect of temperature on the growth of *Bacillus* strains was analyzed in nutrient broth as described by Sow et al. [19]. Pure culture of *Bacillus* was suspended in a sterile saline solution to give an optical density of 1 at 600 nm. Then 200 µL of this suspension were added in 5 mL of nutrient broth and the cultures were incubated at different temperatures from 30 to 50°C. After 24 h incubation at 30°C, the absorbance was measured at 600 nm against the sterile nutrient broth to determine the turbidity, using a spectrophotometer PIOWAY Medical Lab - UV 752.

3. RESULTS AND DISCUSSION

3.1 Pectinolytic Activity of *Bacillus* Strains

A total number of 600 isolates were isolated from fermenting cocoa bean in three regions and analyzed for pectinolytic activity. The Table 1 shows that a proportion of *Bacillus* isolates ranging between 33 and 65% exhibit pectinolytic activity, depending on the region.

The region of Sud-Comoé recorded the most important rate of pectinolytic isolates with 64.60%. Moreover, different levels of enzyme production were observed as assessed by halo diameter which varied from weak enzyme producers (1 cm) to high enzyme producer (3.4 cm). Although the region of Agnéby-Tiassa presented the higher number of isolates analyzed (314), a weak proportion of high producing pectinolytic (4.8%) was observed in this region comparatively to the region of Lôh-Djiboua where 15 isolates (19.23%) were high producer (Table 1).

At date, very few study on *Bacillus* pectinolytic strains involved in cocoa fermentation have been reported. Previous study reported that more than 90% of *Bacillus* strains isolated from Ivoirian cocoa fermentation were pectinolytic strains [9]. Comparatively, the proportion of pectinolytic isolates analyzed in this study appears to be less important. However, Samagaci et al. [20] found that among yeasts population isolated from fermenting cocoa beans, pectinolytic strains represented only 17%. Hence, *Bacillus* pectinolytic strains may be present in larger proportion than yeasts in cocoa fermentation pointing out the probable role of *Bacillus* as

mains pectinolytic producers in this process. It was also observed that the distribution of *Bacillus* pectinolytic isolates is not uniform indicating the local geographic area as an important factor influencing the composition of the microflora involved in cocoa fermentation as reported by Schwan and Wheals [6].

Pectinolytic activity, breakdown the cocoa pulp, allow the aeration of cocoa fermenting mass and the growth of aerobic strains such as acetic acid bacteria that are the main actors responsible for the raise of temperature, the death of embryos and the generation of molecule precursors of cocoa aroma. From pectin degradation, pectinolytic activity may also provide carbon source from pectin for the dynamic growth of the microflora. Furthermore, one of the crucial role of *Bacillus* in cocoa fermentation may be their complementarity with yeasts. In fact, Ouattara et al. [10] suggested that pectinolytic enzyme produced by yeasts and those produced by *Bacillus* should have complementary characteristics that may allow a synergistic effect for an efficient degradation of cocoa pulp during fermentation. To this regards, an absence of *Bacillus* may be a limiting factor for pulp degradation.

3.2 Influence of Fermentation Conditions on Pectinolytic Enzymes Production in *Bacillus*

The production of these enzymes in *Bacillus* isolates is influenced differently by fermentation conditions. The sugar content revealed to have an influence on pectinolytic enzymes production in *Bacillus* (Figs. 1A, B, C). In general, at sugar concentrations inferior to 2% *Bacillus* isolates retain more than 60% of their capacity to produce pectinolytic enzymes. However, beyond 2% of sugar content in the medium the decrease of enzymes production is sharper. Among the sugars tested, glucose seems to have the most hindering effect on enzymes production in *Bacillus* which failed to produce enzymes at 6% of glucose, while at the same concentration of fructose and sucrose, *Bacillus* keep 20 to 60% of enzymes production (Figs. 1A, B, C). Ethanol tends to have the same effect as sugars on enzymes production in *Bacillus* studied (Fig. 1D).

In acid conditions induced by lactic and acetic acid, *Bacillus* isolates proved to be able to express their potential of enzymes production but at a strict range of concentrations of these acid. Hence, enzymes production in *Bacillus* occurred in medium containing acetic acid concentration in the range 0-0.1% and lactic acid in the range 0-0.25% (Figs. 2A, B). Beyond these acid concentrations, *Bacillus* isolates were not able to yield enzymes in the medium. The influence of citric acid on enzyme production in *Bacillus* is less sharp than the others acids studied. Enzymes production decreases progressively with increasing concentrations of citric acid until this production is nulled at 1% of citric acid (Fig. 2C).

Increasing temperature from 30 to 45°C seems to promote enzymes production in *Bacillus* studied, since a slight increase of enzymatic yield was observed (Fig. 2D). However, *Bacillus* isolates were not able to produce enzymes at 50°C. Meanwhile, the strain S40 presented another pattern of enzymes production unlike the others isolates. With S40, although there is a continuous decrease of enzymes production with the raise of temperature, this strain was able to keep round 20% of its potential of enzymes production at 50°C (Fig. 2D). This type of strain should be particularly interesting as starter, since the expression of pectinolytic enzymes in this strain is strongly stable at high temperature.

Table 1. Distribution of pectinolytic *Bacillus* isolates

Regions	Total isolates	Pectinolytic isolates	Non pectinolytic isolates	Halo diameters (cm)	High producing pectinolytic isolates
Lôh-Djiboua	173	78 (44.32%)	95 (55.68%)	1-3.4	15 (19.23%)
Sud-Comoé	113	73 (64.60%)	40 (35.40%)	1-3.4	4 (6.75%)
Agnéby-Tiassa	314	104 (33.17%)	210 (66.87%)	1-3.4	5 (4.8%)

Isolates yielding a halo diameter superior to 75% of the maximum halo diameter were considered as high producing pectinolytic isolates

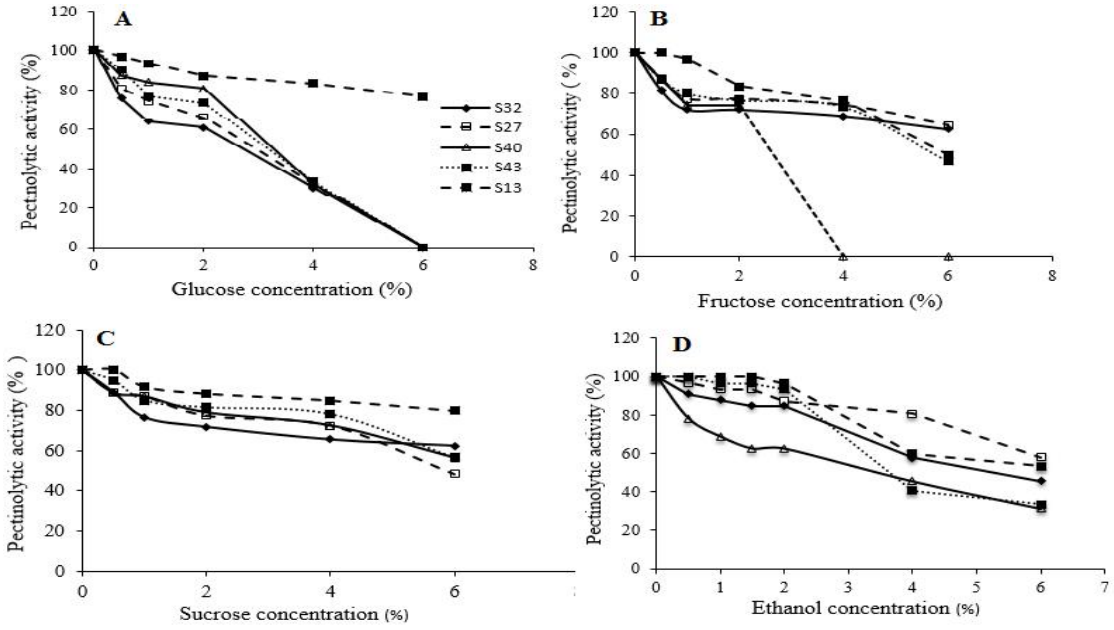

Fig. 1. Influence of sugars and ethanol concentration on pectinolytic enzymes production by *Bacillus*

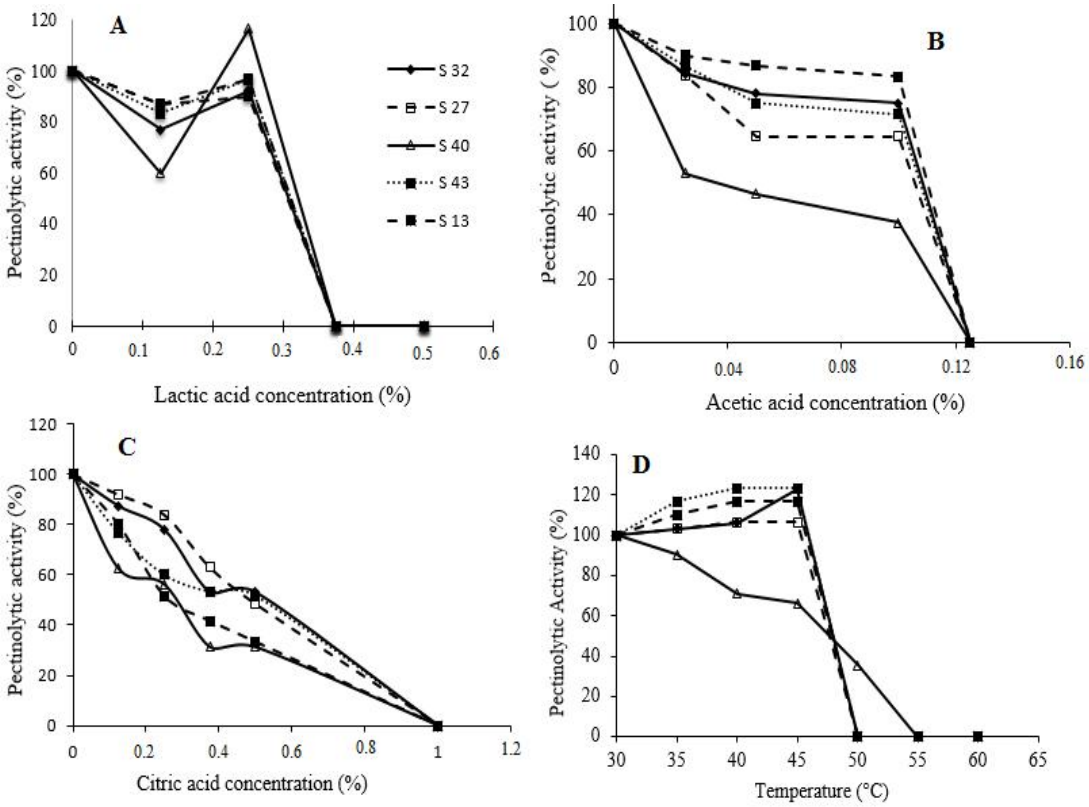

Fig. 2. Influence of acid and temperature conditions on pectinolytic enzymes production by *Bacillus*

The sugars influence on enzymes production may be due to osmotic pressure exerted on cells by sugars, limiting their growth and subsequently affecting their general metabolism involving enzymes production [21] whereas the actions of acid result in a reduction of cytoplasmic pH that is likely to impair enzymes production [22]. This indicates that, compounds present at variable concentrations in the cocoa pulp such as sugars and acids may sometime be factors limiting enzymes production in *Bacillus* during cocoa fermentation. Due to the high sugars content of the pulp at the beginning of fermentation [23], pectinolytic enzymes production in *Bacillus* may be more favorable in the advanced stage of cocoa fermentation when sugars content is low.

Furthermore, it has been previously reported that acetic and lactic acid together with ethanol content trend to considerably reduce beyond 84 h of fermentation [24], reaching scarcely 0.15% in the fermenting pulp [11]. Occurrence of these factors may impose a timing in which *Bacillus* should express their maximum potential of pectinolytic enzymes production, preferentially in the advanced stage of fermentation when these compounds are present in small quantities.

3.3 Acidification Capacity of *Bacillus* Isolates

Acidification stands one of the most relevant properties desired in cocoa fermentation. Acidity produced by microbial metabolism contributes to activate several enzymatic activities which leads to the formations of characteristic aroma and flavor of cocoa and chocolate [6,25]. The results show that *Bacillus* isolates were able to lower the pH of the medium due to acid production as indicated by the color change. Table 2, shows that, a large proportion of *Bacillus* isolates up to 85% and possesses acidifying property. The region of Sud-Comoé provided the most important number of acidifying isolates with a proportion of 86.71% while the region of Agnéby-Tiassa recorded the weaker proportion of acidifying isolates with 55.73%. The results also show that in some isolates the acidifying activity was accompanied by gas production (data not shown). The most acidifying strain yielded titrable acidity of 300 mg/L while the weaker acid production was 30 mg/L. Acidifying isolates presenting gas production trend to produce more acid with more pH reduction (Table 3).

It was also observed that acidification from sucrose tended to be more pronounced than glucose and fructose. The yield of acids by *Bacillus* studied (up to 300 mg/L) remains very low comparatively to acetic acid bacteria involved in cocoa fermentation which were able to yield up to 38 g/L of acid [26], representing a production about 200 folds superior to that of Bacillus. The strains of *Bacillus* involved in cocoa fermentation may not play a significant role in the acidification process of fermenting beans since they exhibit a very weak activity comparatively to acidifying strains in cocoa fermentation such as acetic acid bacteria. However, it was observed that most of *Bacillus* acidifying isolates were able to metabolize citrate.

3.4 Citrate Metabolism of *Bacillus* Isolates

The citrate metabolism is known to have a benefic effect on fermentation process since it allows to modulate pH variation and bacterial growth during this process [6]. A wide proportion of *Bacillus* isolates were able to metabolize citrate as indicated by the growth of colony on the medium and the color change. In the region of Lôh-Djiboua, 64 isolates out of 173 representing a rate of 36.99% were able to metabolize citrate, while the region of Sud-Comoé recorded a proportion of 33.62% (38/113) of isolates with the phenotype citrate positive. In the region of Agnéby-Tiassa, 314 *Bacillus* isolates were tested for citrate metabolism, among them, 149 were citrate positive which represent a proportion of 47.45%. We observed that, very generally the isolates presenting citrate metabolism were also acid producers. Hence 71% of *Bacillus* citrate positive from Lôh-Djiboua and 94.73% of *Bacillus* citrate positive from Sud-Comoé were acid producers. To a lesser extend 56.37% of isolates citrate positive from Agnéby-Tiassa exhibited acidifying property. The results indicate that this essential property is widely distributed into the population of *Bacillus* studied in the three regions constituting an advantage.

At date only yeasts and lactic acid bacteria were assumed to be responsible for the breakdown of citric acid during cocoa fermentation process. This study indicates that *Bacillus* may also be another actor contributing together with lactic acid bacteria and yeasts to citric acid consumption. Owing the proportion of citrate positive among *Bacillus* population studied, these bacteria may strongly contribute to the production of well-fermented cocoa.

Table 2. Distribution of acidifying *Bacillus* isolates

Regions	Total isolates	Acidifying isolates with gas production	Acidifying isolates without gas production	Non acidifying isolates
Lôh-Djiboua	173	63 (36.41%)	67 (38.72%)	43 (24.85%)
Sud-Comoé	113	40 (35.39%)	58 (51.32%)	15 (13.27%)
Agnéby-Tiassa	314	111 (35.35%)	64 (20.38%)	139 (44.26%)

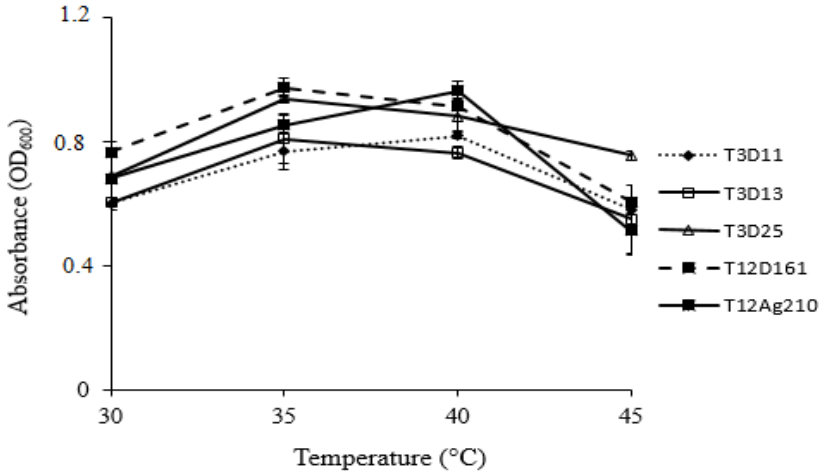

Fig. 3. *Bacillus* growth at different temperatures

Citrate metabolism is also liable to produce certain molecules with desirable flavor and aroma such as acetoin [27]. *Bacillus* strains capable to breakdown citrate may also be involved in cocoa aroma production. At date *Bacillus* are believed to be involved in off flavor of fermented cocoa, but this hypothesis has never been confirmed in any study. In contrast the presence of *Bacillus* has been correlated to the development of desired semibitter flavor of chocolate [11]. These strains were also reported to be implicated in the production of tetramethylpyrazine [28] now known as the major component of chocolate aroma [29].

Owing to the proportion of citrate positive among *Bacillus* population studied, these bacteria may strongly contribute to the production of well-fermented cocoa and chocolate with desirable flavor.

3.5 Thermotolerance Analysis

Fig. 3 shows that *Bacillus* strains isolated present the capacity to grow at temperatures up to 45°C. The maximum growth occurs at 35-40°C, but beyond and below these temperatures, *Bacillus* still keep more than 80 of their growth capacity (data not shown).

Table 3. Acidity produced by *Bacillus* isolates

Isolates	Absorbance (OD600)	pH	Acidity (mg/L)
33 A	1.22±0.12	5.64±0.06	130±0.00
39 A	1.75±0.05	5.68±0.01	140±0.00
46 A	1.97±0.05	5.82±0.01	140±0.01
113 A	0.85±0.09	5.07±0.08	260±0.09
88 A	2.08±0.30	6.08±0.33	50±0.030
01 Ag	1.26±0.09	4.74±0.02	300±0.01
17Ag	1.81±0.95	5.33±0.24	210±0.04
71 Ag	1.97±0.13	6.56±0.04	30±0.01
108 Ag	1.89±0.23	6.54±0.05	80±0.01
171 Ag	1.97±0.24	5.83±0.04	140±0.00
T2D79	1.83±0.02	6.29±0.04	70±0.00
T2D61	1.41±0.33	5.57±0.05	50±0.03
T12D142	1.24±0.10	6.32±0.07	30±0.01
T11D130	1.09±0.07	5.93±0.05	70±0.00

Cocoa fermentation is assumed to be generally characterized by high temperature up to 45°C occurring at 48-72 h of fermentation [4,30]. This high temperature is in part responsible for the decline of several groups of microorganisms such as yeast, acetic acid bacteria and lactic acid bacteria [13,17,20]. Since temperature is susceptible to strongly limit the microbial growth during fermentation, the thermotolerance constitutes an important property to be

considered for efficient microbial activity and production of cocoa and chocolate of quality. The results indicate that *Bacillus* isolates studied present a high tolerance to temperature at 45°C. *Bacillus* strains are reported to be the only bacterial flora that grow throughout cocoa fermentation while the other groups of microorganisms decrease [17]. To this point of view, due to their ability to growth under thermic stress at 45ºC, *Bacillus* population may be the dominant microflora in cocoa fermentation and therefore with some important techno-functional properties, may takes a more important place as key players for a proper processing of fermentation.

4. CONCLUSION

Bacillus constitute the major flora with the less known role in cocoa fermentation. This study pointed out that *Bacillus* involved in cocoa fermentation possess ability to produce pectinolytic enzymes, capacity to breakdown citric acid, strong thermotolerance at 45°C and to a lesser extent acidifying properties. All these properties are very relevant and necessary for a fine and well-performed process of cocoa fermentation and production of high quality of chocolate. *Bacillus* strains should play a more important role in cocoa fermentation than it is believed at date. The properties evidenced in this study, may make them some candidate as starter for cocoa fermentation control.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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