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The Contribution of Microorganisms to the Fermentation of Cassava Dough during *Attiéké* Processing in Côte d'Ivoire

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ABSTRACT

Biochemical properties of fermentative microorganisms of *attiéké* traditional starters were evaluated *in vitro* for highlighting the contribution of each of them in cassava dough fermentation and selection of potential starter strains. A total of 345 isolates from three types of inocula (Adjoukrou, Alladjan and Ebrié) were screening for their abilities to produce α-amylase, β-glucosidase, pectinases and their acidification rate. Lactic acid bacteria (LAB) and at a lesser extent *Bacillus sp.* have been recognized as high acidifying microorganisms. Yeasts isolates were most involved in α-amylase (47.56%) production. But, LAB were the most involved in the detoxication of cassava by the high rate of isolates producing β-glucosidase (34.66%), while *Bacillus* sp showed high pectinases producing isolates (43.75%). A proportion of 7 to 10% of moulds contributes to the production of α amylase, linamarase and pectinase. A total of 42 strains with one or more desirable biochemical properties were pre-selected for the development as starter cultures for *attiéké* production.

Keywords: *attiéké*, cassava fermentation, enzymes producing microorganisms, starter cultures, technological properties

INTRODUCTION

Solid-state fermentation is often employed in the preparation of a variety of traditional African cassava foods and plays an important role in the food supply system of Côte d'Ivoire by contributing to the curtailment of the post-harvest loss of the highly perishable root crop.

Attiéké (steamed cassava fermented semolina) is one such fermented cassava product and is of significant importance for an increasing number of people in Côte d'Ivoire (Assanvo *et al.* 2006) and other countries in the world. It was originally prepared and consumed exclusively in a restricted ethno-cultural setting among the ethnic groups (Adjoukrou, Alladjan, Ebrié, Avikam, Ahizi), living in the Laguna area in the south of Côte d'Ivoire. But in recent years, its preparation has extended throughout the country. Aboua *et al.* (1990) estimated the consumption of *attiéké* between 28,000 and 34,000 tons per year, the equivalent of 40 000-50,000 tons of fresh cassava. The popularity of *attiéké* to urban dwellers in recent years has been associated with its cheapness, lower bulk (as compared to other cassava product) and its characteristic of ready to eat food.

Attiéké is however given less attention with respect to process optimization and quality standardization. The production, laborious and time consuming covers a combination of steps among which roots peeling, grating, fermentation, pressing, granulation, sundrying and steaming. It is one of the few products, whose fermentation is not spontaneous but involves the use of an inoculum. This inoculum is obtained after 2 to 3 days of spontaneous fermentation of cassava roots, thus colonized by a wide variety of microorganisms which constitutes the main source of microbial activities during the cassava dough fermentation (Djeni *et al*. 2008). Many different methods exist for the processing of the traditional starter. The main purpose for using this inoculum is to shorten the fermentation time to 15 h compared with other fermented products like *gari* (dish of fermented and roasted cassava root) or *chickwangue* (heavy type of dough with a light elastic texture made out 100% of cassava), where the fermentation lasts for two to five days (Heuberger 2005), and to improve the texture and flavour of the final product. Thus, the extent to which the inoculum enhances these attributes constitutes a measure of its performance.

Previous studies by Amoa-Awua and Jakobsen (1995) and Amoa-Awua *et al*. (1997) have shown that four principal activities occur when traditional inoculum is used to ferment cassava dough into *agbelima* (traditional fermented cassava dough of Ghana): (i) the breaking down of the coarse texture of cassava dough, (ii) the souring of cassava dough, (iii) the reduction in the level of cyanogenic glucosides and (iv) the synthesis of volatile aroma compounds. All the microbial and biochemical activities which occur during the fermentation of cassava dough into *agbelima* in the presence of traditional inoculum are also observed in the fermentation of cassava dough into *attiéké* (Djeni 2009). The selection of microorganisms, with such interesting activities could lead to the improvement and the optimization of the traditional fermentation of cassava into *attiéké*.

Thus, this study was achieved in order to highlight the contribution of each group of microorganisms during cassava dough fermentation for *attiéké* production and select some (*Bacillus* spp., lactic acid bacteria (LAB), yeasts and moulds) with particular metabolic activities, which inoculation could allow improving the fermentation.

MATERIALS AND METHODS

Material and sampling

The biological material used in this study was constituted by three different types of ready to use traditional cassava inocula (Ebrié, Adjoukrou and Alladjan), taken in small-scale *attiéké* production in three intense processing zones (Abidjan, Dabou and Jacqueville). These inocula were prepared in each processing zone with boiled cassava roots packed in an ancient fermenting bag and left to ferment during 3 days by natural microorganisms present in the bag. On each processing zone, 6 samples were collected from 6

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different proccessors, thus making a total of 18 samples. All samples were collected in plastic bags (Stomacher, Laboratoire Humeau, Rennes, France) and immediately transported in an ice-box to the laboratory for microbial analysis.

Isolation of fermentative microorganisms

Preparation of stock solutions, inoculation of agar plates and cultivation of the various microorganisms in the inocula were carried out according to Coulin *et al*. (2006). For all determinations, 10 g of the samples were homogenized in a plastic bag "stomacher" with 90 ml of sterile peptone buffered water (AES Laboratoire, Combourg, France). Tenfold serial dilutions of stomacher fluid were prepared. Isolation of LAB was carried out using plates of DeMan, Rogosa and Sharp agar (MRS, Merck 10660, Merck, Darmstadt, Germany) which were incubated anaerobically at 30°C for 3 days. Yeasts and moulds were isolated on plates of Sabouraud chloramphenicol agar (Fluka, Bochemica 89579, Sigma-Aldrich Chemie GmbH, India) incubated at 30°C for 4 days. *Bacillus* were enumerated on plates of Plate Count Agar (PCA Oxoid LTD, Basingstore, UK) with 2% soluble starch and incubated at 32°C for 2 days.

After incubation, different looking colonies from highest dilutions on each specific medium were subcultured on selective media and analyze for morphological tests (microscopic examination of living cells, Gram staining, motility) and catalase determined with the appearance of bubbles after mixing a portion of colony with Hydrogen peroxide. This allows separation between LAB (negative for catalase), *Bacillus* genus (positive for catalase) and yeasts and moulds. Representative microorganisms of each group were purified by repeated streaking on appropriate agar and stored in appropriate liquid culture media with 30% glycerol at – 80°C until further analysis. A total of 345 fermentative microorganisms were isolated from three types of inocula, constituted for each inoculum by 50 LAB, 15 *Bacillus* spp., 20 moulds and 30 yeasts.

Biochemical properties of isolates

1. Rate of acidification of cassava broth

The rate of acidification of isolates was assessed following the method used by Sawadogo-Lingani *et al*. (2008), but with a slight modification. Triplicate trial fermentations were realized with each isolate by using an autoclaved cassava broth as substrate and the change in total titratable acidity (TTA) during time was measured.

For the preparation of the cassava broth, an aqueous suspension of 15% (w/v) cassava dough was prepared with distilled water, shaken for 10 min and let to settle for 1 h.

The aqueous suspension was then centrifuged, distributed in flasks (200 ml/flask), autoclaved at 121°C for 20 min and cooled at room temperature (30°C). Isolate cultures used as inoculum were obtained as following: a loopful of each isolate collected from appropriate media agar plate was cultured in 10 ml of cassava broth and incubated at 30°C for 24 h for LAB and *Bacillus* sp, and 32°C for 24-48 h for yeasts and mould. After incubation, 2 ml were sampled for viable cells count on appropriate agar media. Flasks containing 200 ml of autoclaved cassava broth were inoculated in triplicate with 2 ml of the isolate inoculum, and incubated at 37°C for 24 h. Every 4 h, 10 ml of broth were aseptically sampled for TTA determination by titration with 0.1 N NaOH, using 1% phenophtalein as indicator. 200 ml of autoclaved cassava broth without inoculum was used as negative control. The rate of acidification was calculated as ΔTTA by following the method of Ayad *et al*. (2004) with a slight modification: ΔTTA = TTA at time – TTA at zero time. Then, the isolates were grouped according to their rates of acidification. For each group of isolates, the average rates of acidification and the standard deviation were calculated.

2. Screening of amylase-producing microorganisms

The ability of the isolates to produce amylase was determined according to the method described by Sanni *et al.* (2002). Many spots of each isolate were made with an inoculation needle on modified MRS, PCA and Sabouraud chloramphenicol agar without glucose but with 2% (w/v) potato-soluble starch (Prolabo, Paris, France) as the only carbon source respectively for LAB, *Bacillus* species and yeasts and moulds. The composition of these media were [1% (w/v) tryptone, 1% (w/v) meat extract, 0.5% (w/v) yeast extract, 2% (w/v) potato-soluble starch, 0.2% (w/v) $K_2HPO_4 \cdot 3H_2O$, 0.5% (w/v) sodium acetate, 0.2% (w/v) triammonium citrate, 0.02% (w/v) $MgSO_4 \cdot 7H_2O$, 0.005% (w/v) manganese sulfate, 1% (w/v) Tween 80, pH 5.0 ± 0.2] for modified MRS agar [1% (w/v) tryptone, 0.5% (w/v) yeast extract, 2% (w/v) potato-soluble starch, pH 7.0 ± 0.2] for modified PCA and [1% (w/v) meat peptone, yeast extract, 2% (w/v) potato-soluble starch, chloramphenicol, pH 5.0 ± 0.2] for modified Sabouraud chloramphenicol. The plates were incubated at 37°C for 48 h in an anaerobic jar with Anaerocult A (Merck, Darmstadt, Germany) for LAB, at 37°C and 30 for 48 h respectively for *Bacillus* spp. and yeasts and moulds. After incubation, the culture plates were covered by spraying with Lugol's iodine [0.33% (w/v) iodine (Prolabo), 0.66% (w/v) potassium iodide (Labosi, Paris, France)] to detect starch hydrolysis.

3. Screening of linamarase-producing microorganisms

The selection of isolates with linamarase activity was determined according to the method described by Djouldé (2004) by using modified MRS, PCA and Sabouraud agar without glucose but with 1% (w/v) linamarine as the only carbon source and 1% of alkaline picrate as cyanide sensor. 15 ml of each medium were introduced into screw tubes, sterilized by autoclaving at 121°C for 15 min and cooled at room temperature until solidification. Isolates were then inoculated into the medium by central sticking. After 48 h of incubation at 32°C for LAB and *Bacillus* sp., and 72 h at 30°C for yeasts and moulds, tubes with a development of black colour around the perimeter of the injection were considered as positive for linamarase activity.

4. Screening of pectinase-producing microorganisms

The ability of the isolates to produce pectinase enzyme was determined according to the method described by Jaafar *et al*. (2006). Pectinase medium (15 g agar in 516 ml distilled water, 1 g of yeast extract in 20 ml distilled water, 5 ml $(NH_4)_2SO_4$ 20%, 5 ml of aqueous 87% glycerol, 250 ml aqueous solution of 2% polygalacturonic acid, 200 ml 0.1 M phosphate buffer at pH 8, 100 ml of distilled water, 1 ml $MgSO_4 \cdot 7H_2O$ 1 M) enabled the highlighting of the pectinolytic activity of isolates. Inoculation of isolates was realized by line streak on the medium. After 24 h of incubation at 37°C, the plates were flooded with an aqueous solution of copper acetate ($CuSO_4 \cdot 5H_2O$) 7.5% and the presence of a white halo indicates the presence of pectinolytic activity.

Statistical analysis

All trials were repeated three times. The different sample treatments were compared by performing one-way analysis of variance on the replicates at a 95% level of significance using the Statistica (99th Edn, Alabama, USA) statistical programme. Unless otherwise stated, significant results refer to $P < 0.05$. This software was also used to calculate mean values and standard deviations of the trials and to classify isolates according their biochemical activities.

RESULTS AND DISCUSSION

Rate of acidification of isolates

A total of 345 isolates including LAB, *Bacillus*, yeasts and moulds were screened. The TTA of the cassava broth used to evaluate the acidifying activity of the isolates was 0.0045% and its total soluble matter was 4.0 ± 0.2 °Brix. Among these isolates, yeasts and moulds showed very weak rate of acidification (data not showed) compared to that of LAB and *Bacillus* species. The very low rate of acidification of yeasts and moulds demonstrated that these microorganisms were not involved in the acidification of cassava dough during *attiéké* processing.

Fig. 1A-F presents the distribution of LAB according to the rate of acidification. As it can be illustrated in these

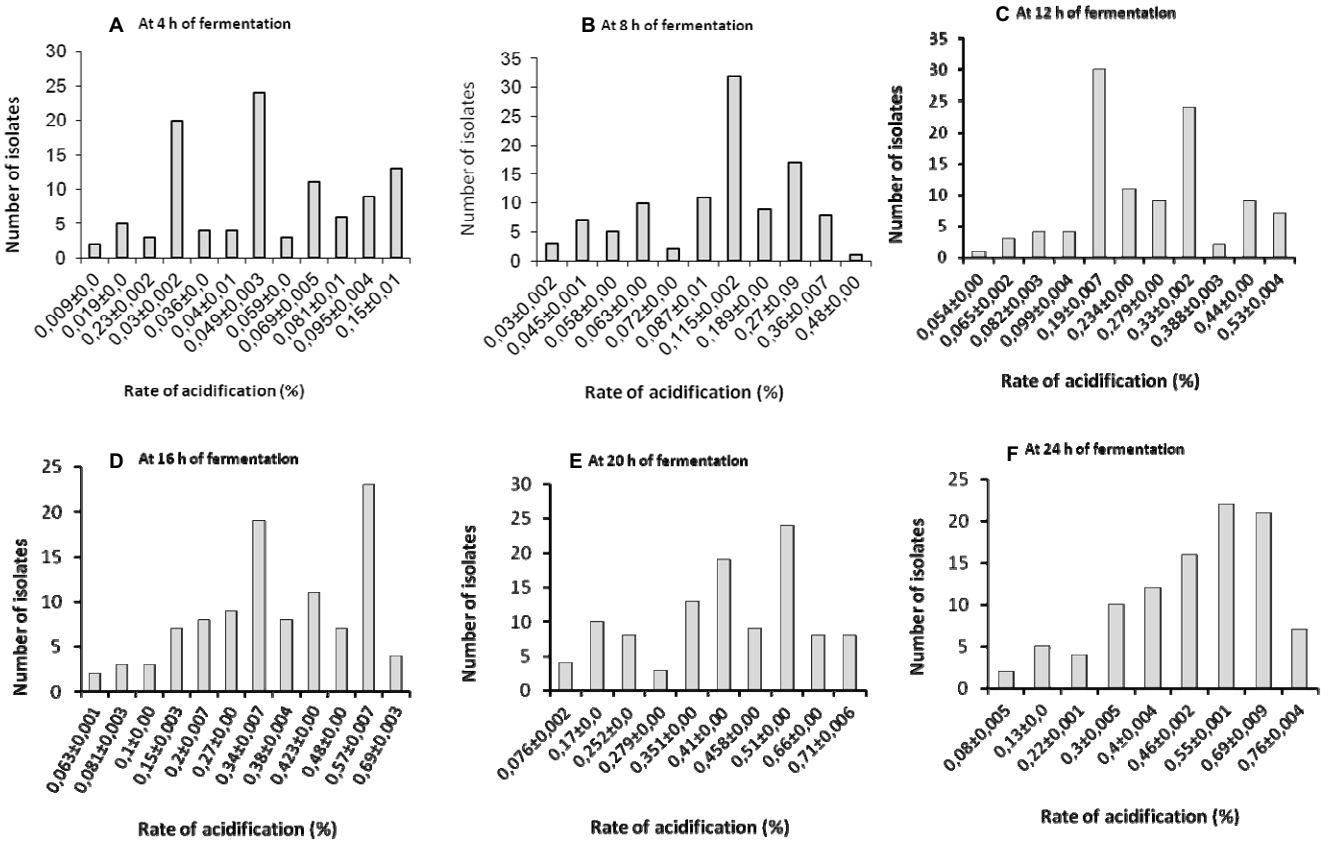

Fig. 1 Distribution of lactic acid bacteria isolates according to their rates of acidification during the trial fermentations of cassava broth.

figures, at the sampling times of the fermentation of cassava broth, a similarity was observed between the LAB isolates, which were clustered in different groups according to their rates of acidification, calculated as ΔTTA. At 4 h of fermentation (**Fig. 1A**), the 150 isolates of LAB were clustered in 11 groups with rates of acidification ranging from 0.01% (2 isolates) to 0.16% ± 0.001 (10 isolates) with the two largest clusters containing 20 and 24 isolates. At 8 h of fermentation (**Fig. 1B**), 10 clusters (groups) were observed with rates of acidification ranging from 0.03% ± 0.002 (3 isolates) to 0.48% ± 0.0 with one largest cluster observed and containing 32 with rates of acidification of 0.12% ± 0.002. **Fig. 1C** shows isolates clustered in 11 groups at 12 h of fermentation, with rates of acidification ranging from 0.05% ± 0.00 to 0.53% ± 0.004; at this sampling time, two largest clusters containing 30 and 24 isolates were observed with rates of acidification of 0.19% ± 0.007 and 0.33% ± 0.002, respectively. As is illustrated in **Fig. 1D**, the isolates clustered in 12 groups with acidification rates ranging from 0.06 ± 0.001 (2 isolates) to 0.7% ± 0.003 at 16 h of fermentation; a few of them (2 isolates) had rates of acidification of < 0.08% ± 0.03. At 20 h of fermentation, no isolate had a rate of acidification less than 0.08% ± 0.002. Similar results were recorded at 20 h and 24 h of fermentation with rates of acidification ranging from 0.08% ± 0.005 to 0.8% ± 0 (**Fig. 1E-F**). The value of 0.08% constitutes after 24 h of fermentation, the lowest value of rate of acidification. Thus, this value was taken as reference to compare the properties of isolates and to classify them in groups (faster, medium and lower) according their acidification rate. **Table 1** shows the different groups of LAB and *Bacillus* according to their acidifying activity during the trial fermentations of cassava broth. After 4 h of fermentation, 27 of the LAB isolates showed a rate of acidification ≥ 0.08%. These isolates constituted the first faster group of LAB and were referred as group 1 (G1). Then at 8h of fermentation, 51 isolates (34%) showed rates of acidification ≥ 0.08%. they constituted the second faster group of isolates and were referred to as

Table 1 Grouping of 195 isolates of LAB and *Bacillus sp* according to their acidifying activity during the trial fermentations of cassava broth, mean total titratable acidity (TTA) 0.0045%.

	Fermentation Time (h)	Total number of isolates having rates of acidification ≥ 0.08 %*	New isolates having rates of acidification ≥ 0.08 % compared to the previous sampling step	
			Number	Percentage
LAB isolates	4	27	27 (first group of isolates, G1) †	18
	8	78	51 (second group of isolates, G2)	34
	12	146	68 (third group of isolates, G3)	45.33
	16	148	2 (fourth group of isolates, G4)	1.33
	20	150	2 (fifth group of isolates, G5)	1.33
	24	150	0	0
Bacillus spp. isolates	4	0	0	0
	8	4	4 (first group of isolates, G1)	8.88
	12	6	2 (second group of isolates, G2)	4.44
	16	25	19 (third group of isolates, G3)	42.22
	20	36	17 (fourth group of isolates, G4)	37.77
	24	45	3 (fifth group of isolates, G5)	6.66

*The rate of acidification was calculated as ΔTTA (TTA at time - TTA zero time).
†Data given in parentheses are groups of isolates according to their acidifying activity.

group 2 (G2). At 12h, a total of 146 isolates containing 68 new isolates (45.33%) had rates of acidification ≥ 0.08%. Those constituted the third faster group of isolates and were referred to as group3 (G3). At 16 h and 20 h, only 2 isolates (1.33%) were respectively obtained and referred as the fourth (G4) and the fifth (G5) groups. While at 24 h of fermentation, none of the isolates showed a rate of acidification ≥ 0.08%.

These results revealed that an interesting proportion of LAB isolates examined showed important capacities of acidification. As many LAB isolates were found to be faster acidifying strains, there is a good reason to believe that the acidification is their major role in the processing *attiéké* (Djeni *et al*. (2008). They acidify the dough by lowering its pH and producing many organic acids. The decreasing of pH has been well demonstrated during the spontaneous fermentation of cassava-based products where LAB were involved (Ben Omar *et al.* 2000; Kimaryo *et al*. 2000). These authors have followed the succession of microorganisms during fermentation, and indicated that the predominant microorganisms at the beginning of fermentation are LAB. According to other authors, sourdough is a unique food ecosystem in that it (i) selects for LAB strains that are adapted to their environment, and (ii) harbours LAB species specific for sourdough (De Vuyst and Neysens 2005; Gobbetti *et al*. 2005; Dal Bello *et al*. 2005). Adaptations of certain LAB to a sourdough environment include (i) a unique central metabolism and/or transport of sourdough-specific carbohydrates such as maltose and fructose, maltose being the most abundant fermentable carbohydrate and fructose being an important alternative electron acceptor; (ii) an activated proteolytic activity and/or arginine deiminase pathway; (iii) particular stress responses; and (iv) production of antimicrobial compounds. For instance, dough acidification is a prerequisite for rye baking to inhibit the flour a-amylase. Acidification also promotes a solubilization of pentosans and thus enhances water-binding of the dough (De Vuysta and Vancanneyt 2007).

Besides these adaptations, dominance of sourdough LAB may depend on the technology used for sourdough production, for instance through the selective pressure exerted by the environmental conditions, as in the case of *L. sanfranciscensis* in type I sourdoughs, in particular in San Francisco bread (Gobbetti and Corsetti 1997; Picozzi *et al*. 2006). Also, the production of specific inhibitory substances, such as reutericyclin in the case of *L. reuteri*, may favour the dominance of sourdough LAB, for instance in German, type II sourdoughs (Gänzle and Vogel 2002; Corsetti *et al*. 2004; Dal Bello *et al*. 2005). This often results in stable sourdough ecosystems, of which the composition remained stable over a period of at least two decades (Böcker *et al*. 1990; Gänzle *et al*. 1998).

Finally, several sourdough LAB contribute to food product aroma (for instance through the production of ornithine as aroma precursor), dough rheology (amino acid metabolism), texture (production of exopolysaccharides), and delay of spoilage (production of acetic acid) (Gobbetti *et al*. 2005; Latifa *et al*. 2006).

The rate of acidification of *Bacillus* isolates allowed classifying them into 5 groups as done for LAB isolates (**Table 1**), with a rate of acids ≥ 0.23% only after 8 h of fermentation, particularly for strains isolated from Alladjan starters. These results were consistent with those of Ofuya and Najiorfor (1989) that indicated an important rate of acidification for *Bacillus subtilis* during the cassava fermentation

The evolution of the rates of acidification of each of the five groups of LAB isolates during the trial fermentations of cassava broth can be illustrated in **Fig. 2A**. For the faster group of isolates (G1), the average rates of acidification recorded were 0.11 ± 0.09, 0.23 ± 0.015, 0.34 ± 0.014, 0.41 ± 0.013, 0.47 ± 0.012 and 0.52 ± 0.01%, respectively at 4, 8, 12, 16, 20 and 24 h (**Fig. 2A**). The isolates belonging to group G2 had average rates of acidification varying between 0.044 ± 0.007 and 0.46 ± 0.009%. While those of

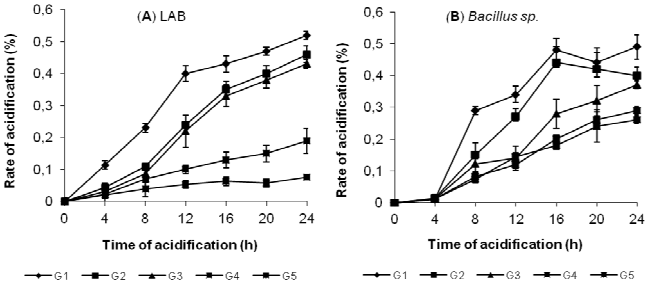

Fig. 2 Evolution of the rates of acidification of isolates clustered groups during the trial fermentations of cassava broth. (**A**) Groups of LAB, (**B**) groups of *Bacillus*.

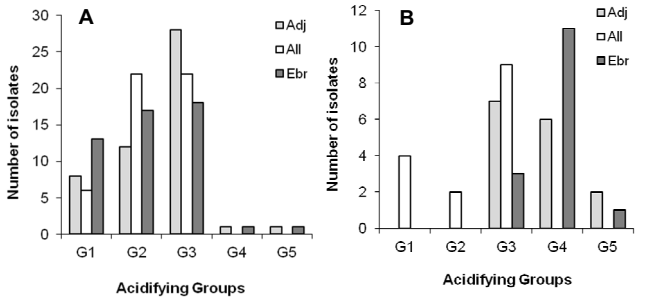

Fig. 3 Distribution of isolates belonging to the different groups (G1, G2, G3, G4 and G5) according to the production zones. (**A**) Groups of LAB, (**B**) groups of *Bacillus*.

the three other groups (G3, G4 and G5) were between 0.03 ± 0.002 and 0.43 ± 0.01, 0.023 ± 0.003 and 0.13 ± 0.01 and 0.027 ± 0.002 and 0.08 ± 0.002%, respectively (**Fig. 2A**). For the *Bacillus* isolates, the most acidifying components of group I had a rate of acidification ranging from 0.0045 ± 0.00 to 0.47% ± 0.003 during 24 h of fermentation. The lowest acidifying group (group 5) had rates of acidification ranging from 0.0045 to 0.26 ± 0.006% after 24 h of fermentation (**Fig. 2B**). Some species of lactic acid bacteria and *Bacillus* sp. have been found to be acid-tolerant, well adapted to acid conditions that develop during natural fermentation of sourdough (Calderon *et al*. 2003). This acid tolerance may partly explain the dominance of such microorganisms in various traditional African sourdoughs.

Fig. 3 shows the distribution of the LAB and *Bacillus* isolates according to their origins, i.e. their production zone and their rates of acidification. These results demonstrate a diversity of isolates with regard to their acidifying activity. The faster acidifying LAB groups was constituted by isolates of Ebrié (13 isolates), Adjoukrou (8 isolates) and Alladjan (6 isolates) starters. The isolates of group 2 were more abundant in Alladjan starter (22 isolates), while Adjoukrou starters contained more isolates (28) of group 3. Isolates of group 4 and 5 were very fairly represented (only 2 isolates) and found only in Adjoukrou and Ebrié starters. Considering their origins, the occurrence of LAB with high rate of acidification varied from one starter to another. Indeed, the rate of high power acidifying LAB were similar for isolates from Adjoukrou and Alladjan traditional starter, but lower than this of Ebrié starters. This could explain the diversity of *attiéké* taste as established by Djeni *et al*. (2011). These authors demonstrated the similarities in the physic-chemical and sensorial characteristics of both *attiéké* Adjoukrou and Alladjan and the divergence with Ebrié's one which have the sourest taste.

Enzyme-producing isolates

All groups of microorganisms isolated from the different *attiéké* traditional starters contained strains with α-amylase activity, expressed as a clear zone around the colony on the culture medium (**Fig. 4A**), with ability to degrade linamarin

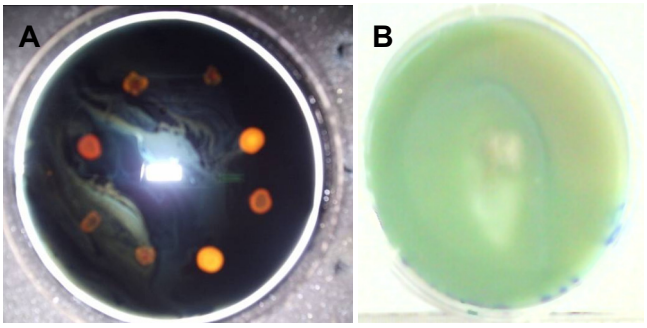

Fig. 4 (**A**) LAB producing α-amylase on modified MRS agar supplemented with starch (2%) translated by a clear zone around the colonies after pulverization of iodine solution. (**B**) Pectinase-producing mould on pectin agar revealed after spraying solution of copper acetate on the medium.

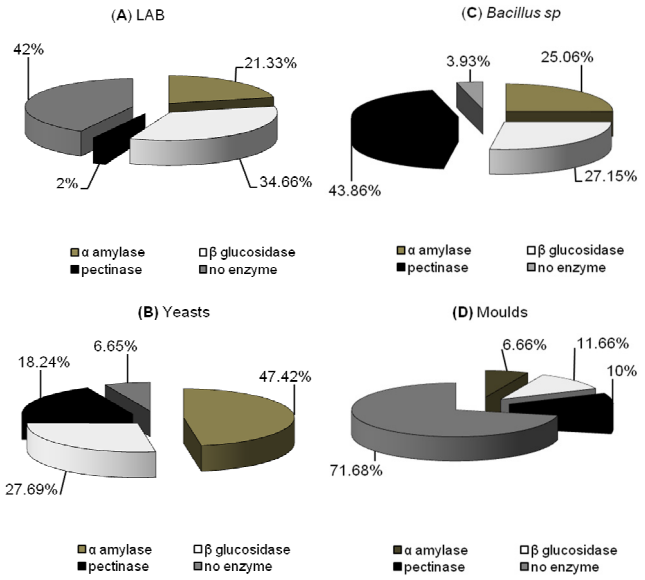

Fig. 5 Qualitative production of enzymes by the isolates of *attiéké* traditional starters. (**A**) LAB, (**B**) yeasts, (**C**) *Bacillus* (**D**) moulds.

(black coloration in the culture tube) and pectin (white halo around the colony on the medium; see **Fig. 4B**). The overall production of enzymes shows that the isolates were involved in the production of enzymes in varying proportions. The global rates of strains with positive tested enzyme abilities were about 58% for lactic acid bacteria, 96% for *Bacillus* species, 93% for yeasts and only 29% for the moulds.

The results showed that a halo surrounding the amylase-positive colonies was more present for the yeasts (47.42%) than the other microorganisms with rates of 21.33, 25.06 and 6.66%, respectively for LAB, *Bacillus* spp. and moulds. Their involvement in starch degradation has been previously demonstrated by Djouldé *et al*. (2005). The high occurrence of yeasts with positive amylolytic activity in these *attiéké* starters could be related to the fact that sugars of cassava was essentially constituted of starch (about 90%), according to Chuzel (1990).

However, in our study, production of α-amylase was a rare trait among LAB and *Bacillus* species, except a few isolates. This was quite surprising, as cassava contains essentially starch as carbohydrates (Ketiku and Oyenuga 1972), representing an important potential energy source also for all the microorganisms isolated (LAB, yeasts, moulds and *Bacillus* spp.). Only a few amylolytic LAB have been isolated from starchy fermented foods in Africa (Sanni et *al*. 2002). Thus, it could be suggested a co-metabolism between yeasts and the other microorganism particularly the lactic acid bacteria; whereby the bacteria provide the acid environment, which selects for the growth of yeasts and, the yeasts provide vitamins and other growth factors to the bacteria. Similar observations were provided by Gobbetti *et al*. (1994); Steinkraus (1996) on sourdoughs and indigenous products. Yeasts have also been reported to make a useful contribution to the improvement of flavour and acceptability of fermented cereal gruels (Banigo *et al*. 1974; Odunfa and Adeyele 1985).

In the fermentation of cassava dough during *attiéké* processing, in addition to the role of acidification, LAB are specialized in the degradation of cyanogenic compounds as seen by the high rate (34.66%) of linamarase positive activities in this group of microorganisms. Similar observations were made by Djeni (2009), Kostinek *et al*. (2007), who showed that all groups of LAB (heterofrementative, homofermentative rods or cocci) were able to produce linamarase, so were generally involved in the detoxification of cassava. Linamarase positive activity was also characteristic of 27.15 and 27.69%, respectively of *Bacillus* spp. and yeasts. Adjoukrou starters contained the highest rates of linamarase-positive isolates (42%), indicating that a high level of detoxication could be reach while this type of starter or its positive isolates would be used for fermentation. However, the cassava tuber itself contains an endogenous linamarase, which hydrolyses linamarin when the plant cells are disrupted (Kimaryo *et al*. 2000).

Another biochemical property investigated concerned the production pectinase enzymes. Possession of pectinase activity was critical to the ability of isolates to disintegrate cassava tissue. Possession of pectinase activity was also sufficient to cause the breakdown of the texture of cassava dough as shown by Amoa-Awua and Jakobsen (1995) and Djouldé (2004). Among all the isolates, *Bacillus* species were the most involved in the production of pectinases with a high proportion of 43.75% coming from Adjoukrou starters, against 37.5% and 36.66% respectively for Alladjan and Ebrié starters. That could be at the basis of the difference of texture of the derivate *attiéké*.

Moreover, the occurrence of *Bacillus* spp. producing pectinase in high numbers in the microflora of traditional *attiéké* starters is of considerable interest since published information on the involvement of *Bacillus* spp. in *attiéké* processing does not exist or is very limited. Thus, these microorganisms would attack the skeletal structures of the cellular partition (pectin) of cassava tubers and thus contribute to soften quickly cassava dough during fermentation. Stutzenberger (1990) describes *Bacillus* spp. as being ubiquitous and with an acknowledged ability to secrete a wide variety of depolymerizing enzymes suggesting that their primary ecological role is to degrade biomass polymers. This observation gave an indication that *Bacillus* isolates from *attiéké* starters were likely to be the agents responsible for the softening of cassava dough. This result is consistent with previous studies (Yandju 1989; Amoa-Awua and Jakobsen 1995). The other microorganisms (yeasts, moulds and LAB), at a lesser extent showed also ability to produced pectinases enzymes during fermentation, thus contributing to yield a smooth textured dough.

The contribution of yeasts to the modification of cassava texture in comparison to the moulds has been assigned a secondary role by Amoa-Awua *et al*. (1997). Tudor and Board (1993), in their review, list some yeasts such as *C. tropicalis*, *C. krusei* and several species of *Zygosaccharomyces* as food spoilage yeasts but, in the work by Amoa-Awua *et al*. (1997), yeasts have been shown to play a positive role in cassava fermentation by contributing to the modification of the texture of the product. Moreover, it is important to note that 71.68% of moulds, 42% of LAB, 3.93% of *Bacillus* and 6.65% of yeasts did not show enzyme activities (**Fig. 5**).

All the microorganisms with positive enzyme activities originated from the three traditional starters studied. The biggest number of LAB with amylase (36%) and linamarase (42%) activities was isolated from the Adjoukrou traditional starters (**Fig. 6**), while the rate of *Bacillus* species with pectinases activities reached 50% in Ebrié starters and 43.75%

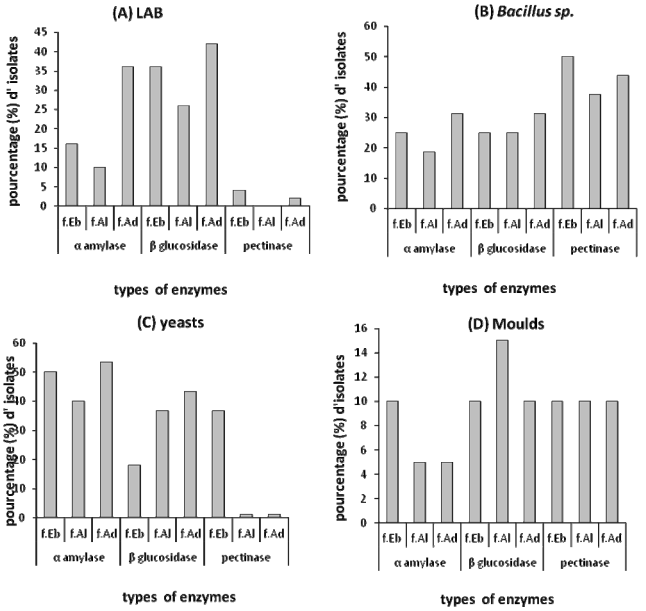

Fig. 6 Distribution of enzymes producing microorganisms according to traditional starters production zones. (**A**) LAB, (**B**) *Bacillus*, (**C**) yeasts, (**D**) moulds.

in these of Adjoukrou. But most of the yeasts able to produce amylase were isolated from Ebrié (50%) and Adjoukrou (50.33%) starters. However, there were more yeasts with linamarase activity in Adjoukrou starters (43.33%) than in the others. Very few LAB and yeasts with pectinases activities (up to 2 isolated) were found in both Alladjan and Adjoukrou starters. The number of moulds with enzyme activities was the lowest among all isolates (at least 15% of moulds isolated) and was similar for the three starters (**Fig. 6**).

Selection of starter cultures

On the basis of their biochemical properties, 42 (15 LAB, 9 *Bacillus* spp., 9 yeasts and 9 moulds) of the 345 isolates of *attiéké* traditional starters were preselected as possible starter cultures for *attiéké* production. Desirable biochemical characteristics were considered to be α-amylase activity, β-glucosidase activity, pectinases activity and rapid acid production. The results of all biochemical characteristics of the pre-selected LAB and *Bacillus* spp. strains are shown in **Table 2**. The 42 pre-selected strains could be inoculated singly or mixed for a controlled fermentation during *attiéké* production. The use of starter cultures for a controlled fermentation cassava dough should improve the processing by shortening its duration, and improve also the shelf-life as well as the quality of the final products.

CONCLUSION

The main conclusion of this work is that the microflora of traditional starter used to initiate the fermentation of cassava dough during *attiéké* processing are dominated by microorganisms (LAB, *Bacillus* spp., yeasts and moulds) with interesting technological potentialities. Therefore, representative isolates associated with the fermentation of cassava for the production of *attiéké* were selected as starter strains. The biochemical traits of these selected strains, however, were mostly investigated *in vitro*. Therefore, to assess the performance of the strains in establishing themselves as dominant starter cultures in actual fermentations, and to validate whether these biochemical traits are indeed expressed and have a positive impact on product quality, the strains are currently being developed as starter cultures on an industrial scale. These strains will then be used in pilot plant fermentations, where their performance will be evaluated.

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Table 2 Biochemical properties as pertaining to production of α-amylase, β-glucosidase, pectinases and acidification of the 32 pre-selected strains determined *in vitro*.

Microorganisms	α-amylase	β-glucosidase	pectinase	ΔAT ≤ 0.4% after 24 h	ΔAT ≥ 0.4% after 24 h
LABX1	+	++	+-	+	+
LABX2	+	+++	+-	+	+
LABX3	+-	+	+-	+	+
LABX11	+-	++	+-	+	+
LABX21	+	+-	+-	+	+
LABY9	+-	+++	+-	+	+
LABY14	+	+	+	+	+
LABY15	+-	+	+	+	+
LABY38	+-	+	+	+	+
LABY39	+-	+	+	+	+
LABZ4	+	+++	+-	+	+
LABZ44	+-	+++	+	+	+
LABZ46	++	+++	+	+	+
LABZ48	+-	++	+	+	+
LABZ7	+-	++	+	+	+
BX3	+-	+-	++	-	+
BX5	+	+-	+++	-	+
BX8	+-	+-	++	-	+
BY4	+	+-	+++	-	+
BY9	+-	+-	+	-	+
BY11	+-	+--	+	-	+
BZ5	+	+	++	-	+
BZ14	+-	+	+	-	+
BZ15	+-	+	+ ++	-	+

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