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CONTRIBUTION TO THE BIOTECHNOLOGICAL POTENTIAL STUDY OF ISOLATED LACTIC LEVAINS OF FERMENTED CASSAVA FOR THE PRODUCTION OF *ADJOUKROU, AHIZI* AND *EBRIÉ ATTIÉKÉ*

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ABSTRACT
The current work aims to study the typology of the lactic starter isolated from the fermented cassava for the production of *Adjoukrou, Ahizi* and *Ebrié* attiéké. The objective is to highlight the contribution of autochthone strain selected empirically by local know-how, to the development of the originality of the *attiéké* produced in the *Adjoukrou, Ahizi* and *Ebrié* areas. The leaven is defined by as a culture of selected microorganisms which are introduced into the products to be fermented. In this work, the lactic leaven of *attiéké* was defined as the set of species of lactic acid bacteria detected during the fermentation of the cassava paste initiated by an *attiéké* inoculum. In order to identify the biotechnological parameters which, characterize each lactic starter, a number of five to seven strains of lactic acid bacteria, representative of the species competing during the fermentation, were selected for each area. These descriptors are: the proportion of the alkaline phosphatase, cysteine arylamidase, valine arylamidase, Lipase esterase C8 and N-acetyl-β-glucoaminidase positive strains. The originality of the lactic leaven involved in the production of *Adjoukrou attiéké* is the prevalence of positive alkaline phosphatase bacteria and the deficiency in exo enzymes positive bacteria. *Ahizi attiéké* leaven from Abraco sites is characterized by the quantitative importance of cysteine arylamidase, esterase lipase C8, valine arylamidase, N-acetyl-β-glucoaminidase producing bacteria and by a deficiency of bacteriocin producing bacteria. *Ebrié attiéké* leaven of Abidjan sites similar to that of *Ahizi* origin by, inter alia, the quantitative extent of osidases producing bacteria and then *Adjoukrou* one by bacteria with antibacterial activity (bacteriocins producers).

KEYWORDS: *Attieké*, Enzymes Activity, Lactic Starter.

INTRODUCTION
In Côte d'Ivoire, traditional cassava manufacturing lead to about ten local meals (**Yéboué et *al.*, 2017**) whose the well-known are *placali*, *attoukpou* and *attiéké*. A*ttiéké* is the main manufacturing form of cassava (**Djeni et *al.*, 2014**). Originally, this dish constituted the staple food for some lagoon people in the southern of Côte d'Ivoire (e.g. *Ebrié, Adjoukrou, Alladian, Avikam, Attié, Ahizi*) (**Assanvo *et al.*, 2006**). Nowadays, the production and the consumption of *attiéké* have conquered all the ivorian territory. Even, they were spread in the bordering countries (**Assanvo *et al.*, 2006; Djeni *et al.*, 2011**) and overseas (**Djeni *et al.*, 2014**). However, it remains that *attiéké* constitutes a food typically of Côte d'Ivoire, produced with the fermented cassava at the domestic or artisanal level.

The manufacturing technology of cassava in *attiéké* starts by peeling of the roots towards the steaming of the pulp, by passing numerous operations including fermentation (**Djeni et *al.*, 2008**). The fermentation of cassava is not initiated by selected species, but by a biological starter called starter of *attiéké* (**Assanvo *et al.*, 2006**). This starter is itself a product of the spontaneous fermentation of the cassava root by the microorganisms of the environment (**Djeni et *al.*, 2008**). A lot of works have shown that lactic bacteria are involved in this fermentation (**Djeni et *al.*, 2015; Bouatenin et *al.*, 2012; Assanvo et *al.*, 2006; Coulin et *al.*, 2006**) and contributed to the development of the organoleptic characteristics of the final product (**Soro-Yao *et al.*, 2013**).

The current work aims to study the typology of the lactic starter isolated from the fermented cassava for the production of the *Adjoukrou, Ahizi* and *Ebrié attiéké*. The objective is to highlight the contribution of autochthone strains selected empirically by local know-how, to the development of the originality of the *attiéké* produced in the *Adjoukrou, Ahizi* and *Ebrié* areas.

MATERIALS AND METHODS

Materials and sampling

Isolation were made from samples of ready to use traditional cassava inocula and fermenting cassava dough obtained from three small-scale women's enterprise in *Adjoukrou, Ahizi* and *Ebrié* people villages, South of Côte d'Ivoire. 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. The cassava dough was incubated at 35°C for fermentation monitored over for 12 to 16 hours. A quantity of 500 g of inoculum and fermenting dough samples were aseptically taken for different analyses at the beginning (0 hour), 4, 8 hours and at the end of the fermentation. On each processing site, 15 samples (3 samples of cassava inocula and 12 samples of cassava dough) were collected, thus making a total of 45 samples for three passages. In total, 27 samples of inocula and 108 samples of cassava dough were taken from the nines sites from the three zones. All samples were collected in plastic Stomacher bags (Laboratoire Humeau, Rennes, France) and immediately transported in an icebox to the laboratory for microbiological analysis.

Microbiological analysis

Enumeration and isolation of lactic acid bacteria

Microbiological analysis were carried out to determine lactic acid bacteria (LAB) loads in inocula and cassava fermenting dough samples. Preparation of stock solutions, inoculation of agar plates, cultivation and quantification LAB were carried out according to **Coulin *et al.*, (2006)**. For all determinations, 10 g of samples were homogenized in a stomacher bag with 90 mL of sterile peptoned buffered water (AES Laboratoire, COMBOURG France). Tenfold serial dilutions of stomacher fluid were prepared and spread plated for determination of microorganism counts. Enumeration of LAB was carried out using plates of de Man, Rogosa and Sharpe (MRS) agar (Merck, Darmastadt, Germany) which were incubated under anaerobic conditions (Anaerocult A, Merck) at 30 °C for 72 h.

Isolation of LAB was performed from traditional inocula and fermenting dough samples by picking colonies from plates of highest dilutions showing growth. Each isolate was then characterized for Gram and catalase reactions. Sixty-six (*Adjoukrou*), Seventy-eight (*Ahizi*) and Sixty-six (*Ebrié*), gram positive and catalase negative isolates were obtained and purified twice on De Man, Rogosa and Sharpe (MRS) agar (Merck, Darmastadt, Germany).

Molecular characterization of LAB

Total DNA was extracted from traditional cassava inocula and fermenting cassava dough Gram-positive and catalase-negative isolates, by the rapid cold shock method of **Gaya *et al.* (1999)**. Rep-PCR was used first to classify and then to type the LAB. Primer GTG-5 (5'-GTGGTGGTGGTGGTG-3') (Invitrogen, Cergy Pontoise, France) (Versalovic *et al.*, 1991), was used for Rep-PCR amplification. PCR amplification was performed in a final volume of 25 mL containing 1X PCR buffer (Bioline, Spain), 2.5 mM $MgCl_2$, 200 mM each dNTP, 2 U Taq polymerase (Bioline, Spain) and 2 mL of extracted DNA. PCR reactions were carried out in a thermal cycler (Techne® Prime Thermal Cycler Range, USA) programmed as described by **Berthier et *al.* (2001)**. The reactions were resolved on horizontal 1.5% agarose (Pronadise, Madrid, Spain) gels by applying a current of 120 V for 5 h. A DNA molecular mass marker (Reddy Run Superladder low 1 Kb, Thermo Scientific) was used as standard. The resulting fingerprints were analyzed by the BioNumerics software, version 7.1 (Applied Maths, 1998 to 2013, Kortrijk, Belgium). Calculation of similarity between fingerprints was based on the Pearson correlation coefficient. A dendrogram was deduced from the similarity matrix by the Unweighted Pair Group Method with Arithmetic Averages (UGPMA) algorithm. Fingerprints with a similarity coefficient of 97% and visually identical were deemed to be fingerprints of the same strain.

Amplification of the 16S rDNA genes was performed by PCR as described by **Li *et al.*, (2007)** using the specific primers 16F27 and 16R1522. PCR products were then sent to GATC-biotech (Germany) for sequence determination. The resulting sequences were assembled into a unique contig with BioEdit sequence alignment software and then submitted to the NCBI database (NCBI, Bethesda, USA, http://www.ncbi.nlm.nih.gov/) for representation of sequence and similarity searches in the GenBank database.

Technological capacity characterization of LAB

Enzyme activity

A total of eighteen (18) isolates of the dominant genera and species were further characterized, in terms of their enzymatic profile, using the API-ZYM kit strips (BioMerieux, Marcy-l'Etoile, France). The API ZYM system is a semi-quantitative micromethod research of enzymatic activities applicable to various environments, tissues, cells, body fluids, organisms ... (Laviolette, 1977). The device is in the form of a twenty microtanks gallery or wells of 9 mm in diameter and 3 mm in height, the bottom consists of a specially prepared medium containing the appropriate substrate in buffer solution (Tris-maleate 0.05 M to pH 5.4, 0.05 M Tris-HCl pH 7.5 to 8.5). The support fibers ensure a homogeneous distribution of the insoluble or sparingly soluble molecules in aqueous medium. The enzymatic activity is detected by color development. Miniaturization and simplicity of the device used to perform many tests in a

short time and it is possible to study a large number of strains. No aseptic precautions are necessary because the incubation time is limited and the load microbial cells inoculated per well must be important. The gallery used allows research nineteen enzyme systems.

Statistical analysis

One-way analyses of variance based on Newman-Keuls multiple tests with significant level a ¼ 0.05 were performed in order to compare biochemical and microbial characteristics samples and also to determine significant differences between inoculum types. The software used for the statistical evaluation was XLSTAT (Addinosoft Inc.).

RESULTS

Lactic bacteria involved in the fermentation of cassava for the production Adjoukrou, Ahizi and Ebrié of attiéké

The bacteria strains were identified as *Enterococcus italicus, Leuconostoc mesenteroides* PON 259, *Leuconostoc fallax, Leuconostoc mesenteroides subsp mesenteroides Weissella confusa, Weissella cibaria, Weissella sp, Lactobacillus fermentum, lactobacillus plantarum, Enterococcus italicus* ATC BAA-780, *Weissella paramesenteroide* (Table 1).

Table. 1: Distribution, by origin, of the species of lactic acid bacteria identified in fermented cassava.

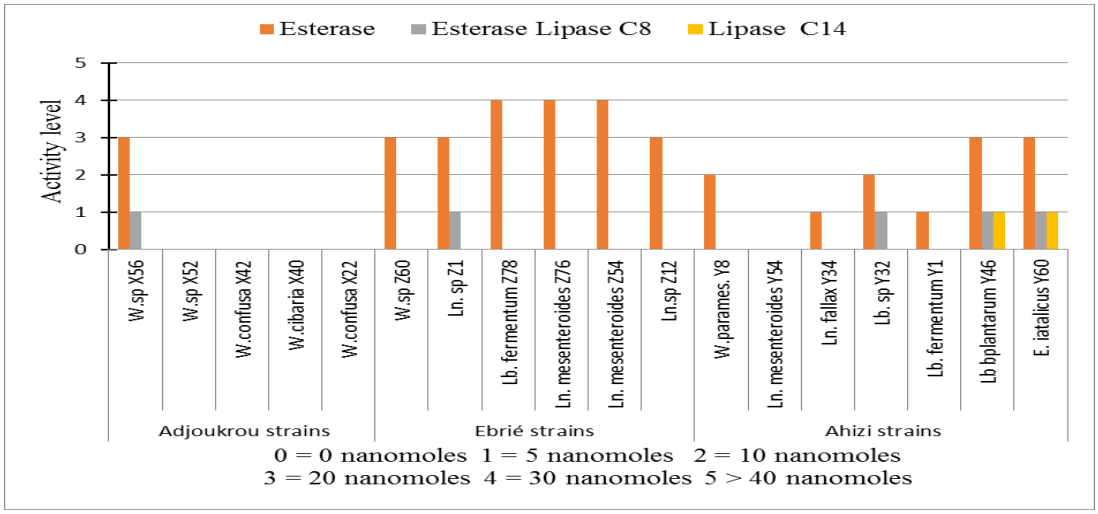
Original species *Adjoukrou*	Original species *Ahizi*	Original species *Ebrié*
Weissella confusa AB13	*Weissella* sp.NBRC 107245	*Weissella cibaria*
Weissella cibaria PON 10032	*Lactobacillus sp*	*Lactobacillus plantarum*
*Weissella cibaria I*MAU10280	*Lactobacillus plantarum*	*Weissella sp*
Weissella sp.	*W. paramesenteroïdes*	*Leuconostoc mesenteroides* PON259
Weissella confusa	*Leuconostoc fallax*	*Leuconostoc sp.*
*Weissella sp.*NBRC 107245	*Lactobacillus fermentum*	*Lactobacillus fermentum*
Lactobacillus fermentum	*Enterococcus italicus* ATC BAA-780	*Leuconostoc Mesenteroides subsp mesenteroides*

Enzymatic profile and antimicrobial activity

Lipolytic activities

Three lipase activities (esterase, esterase Lipase C8 and Lipase C14) were tested on 18 strains. Positive strains for at least one of the evaluated activities accounted for 66.67% of the strains tested. Of the three activities tested, esterase activity (66.66% of all strains) was the most expressed (**Fig. 1**) and only 11.11% of the strains tested positive esterase lipase C14.

Adjoukrou strains did not show any lipolytic activity except *Weissella sp* X56 which weakly expressed esterase and esterase lipase C14 at respective levels of 3 and 1. The strains of the *Ahizi* zone expressed variously the three enzymes: the esterase activity is expressed moderately (level 3 to 4) by 85.71% of the *Ahizi* strains tested; the C8 esterase lipase was weakly expressed by 43% of the strains. C14 lipase was detected only in *Lactobacillus plantarum* Y46 and *Enterococcus italicus* ATC BAA-780 Y60 which had the broadest spectrum of enzymes tested. All *Ebrie* strains produced only the esterase except *Leuconostoc sp* Z1, which in addition weakly expressed esterase lipase C8. The *Ebrie* strains expressed strong esterase activity. Ahizi strains are moderately active. As for the *Adjoukrou* strains, they are characterized by a deficiency in lipase activities.

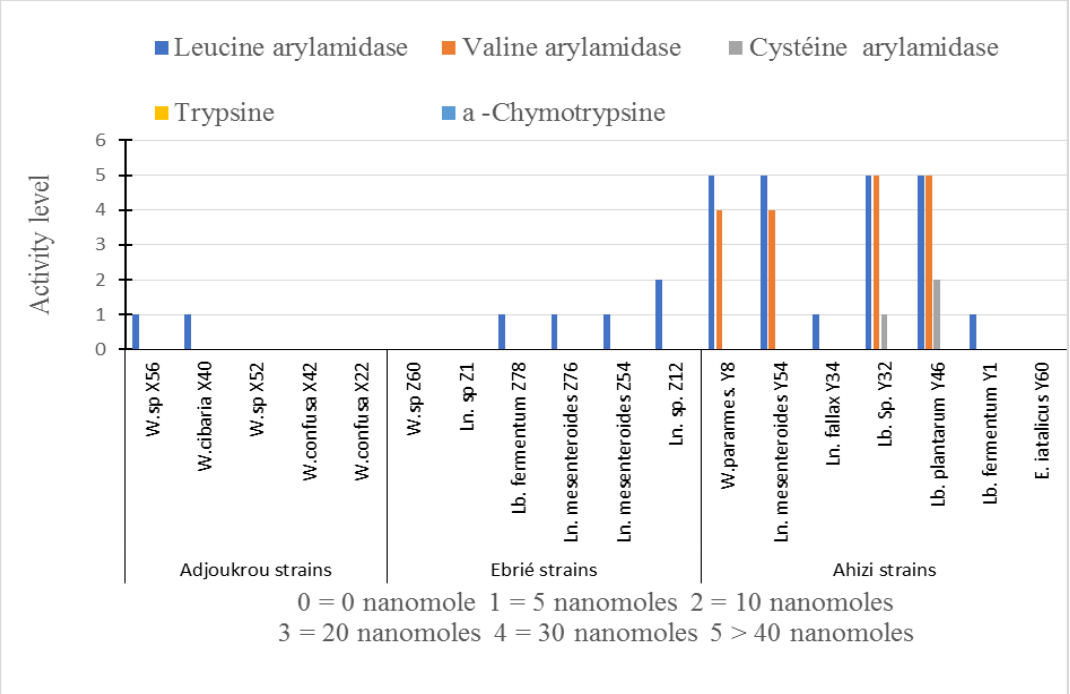


E. : *Enterococcus ;* Lb *: Lactobacillus ;* Ln *: Leuconostoc;* W. *: Weissella ; parames.: paramesenteroides.*

Fig 1: Lipasique activities of lactic acid bacteria isolated from *Adjoukrou, Ahizi* and *Ebrié* fermented cassava (attiéké inocula and cassava dough).

Proteolytic activities

Proteolytic activities were tested on 18 strains (5 strains *Adjoukrou*, 6 strains *Ebrié* and 7 strains *Ahizi*). The activities tested are leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin and α-chymotrypsin. Trypsin and α-Chymotrypsin were not expressed by any of the strains tested under the conditions of this study (**Fig 2**). As for leucine arylamidase, it was expressed by 66.67% of the strains and mostly at low levels. This enzyme is the only one that has been expressed by two *Adjoukrou* strains (*Weissella sp*. and *Weissella cibaria*) on the five strains tested. The other *Adjoukrou* strains showed total deficiency in proteolytic enzymes. The proteolytic activity of the *Ebrié* strains is also low. It concerns only valine arylamidase which is expressed by 66.67% of the strains, the remainder being inactivated. In contrast, the bacteria of the *Ahizi* fermented cassava showed significant proteolytic activities. Most bacteria (85.67%) showed large arylamidasic leucine activities; 57.14% of these microorganisms produced valine arylamidase. The strains of *Ahizi Lactobacillus sp*. Y32 and *Lactobacillus plantarum* Y46 showed in addition a low production of cysteine arylamidase. *Enterococus italiccus* ATC BAA-780 Y60 showed no proteolytic activity. *Ahizi* strains showed the best proteolytic activity in quantitative terms and number of producing strains. The majority (66.66%) of the strains of *Ebrié* origin showed a low leucine arylamidase activity generally level 1. As for the bacterial species isolated from the fermented cassava Adjoukrou, they are practically devoid of proteolytic activities.

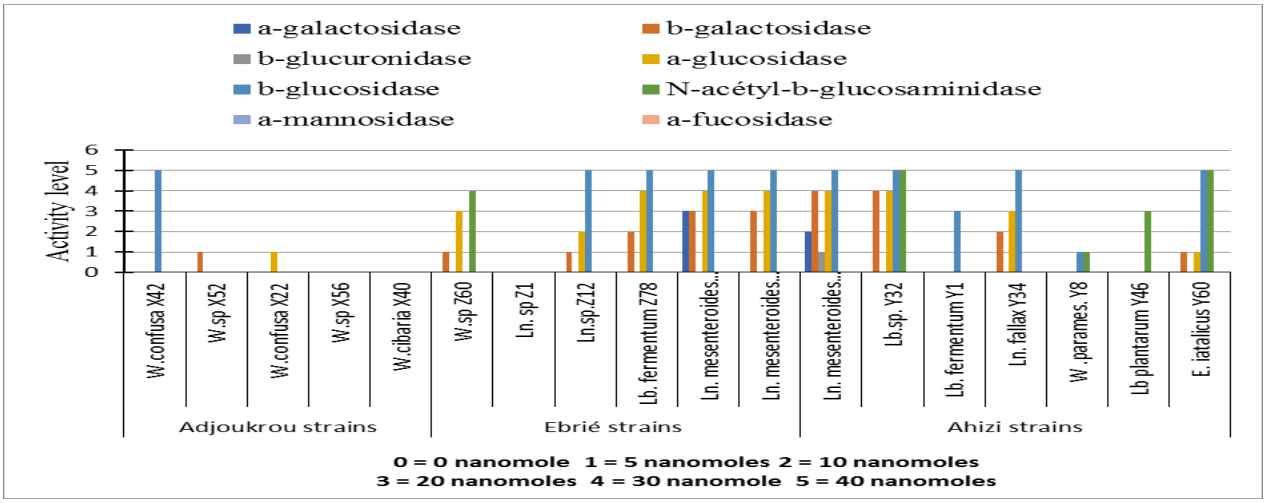


E. : *Enterococcus ;* Lb *: Lactobacillus ;* Ln *: Leuconostoc;* W. *: Weissella ; parames.: paramesenteroides.*

Fig. 2 : Proteolytic activities of lactic acid bacteria isolated from *Adjoukrou, Ahizi* and *Ebrié* fermented cassava (attiéké inocula and cassava dough).

Osidasic activities: Eight osidasic activities were tested on 18 strains. The activities tested are: α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-fucosidase, α-mannosidase, N- acetyl-β-glucosaminidase. The Adjoukrou strains are practically devoid of osidasic activities, except for the only Weissella confusa strain X42 which showed a high β-glucosidase activity. The lactic acid bacteria of the *Ahizi* fermented cassava expressed five different five-fold osidasic activities out of the eight studied: 85.71% of the strains expressed β-glucosidase, 57.14% α-glucosidase, 57.14% α and β Galactosidase, 28.57% N-Acetyl-b-glucosaminidase and only 14.28% showed glucuronisidase activity. However, α and β - glucosidase are produced in a detectable amount by 43% of the strains. The strain Leuconostoc mesenteroides Y54 differs from all other strains in its broader spectrum of activities, which highlights five osidasic activities (**Fig. 3**). It is the only strain to express β-glucuronidase. The Lactobacillus sp. Y32 strain is characterized by a spectrum of four enzyme activities of levels 4 to 5. The Y60 strain is one of a few which has N-Acetyl-b-glucosaminidase activity, but it expresses weakly the other enzymes, unlike *Lactobacillus sp.* Y32 which shows a significant activity for four enzymes. The *Ebrié* strains expressed five enzymes. The α-glucosidase was expressed by 83.33%, β-glucosidase by 66.66%, α and β-galactosidase by 83.33%, α-galactosidase by 16.66% and N-Acetyl-b-glucosaminidase by 16.66% of the strains tested. Most of the *Ebrié* strains tested (66.66%) showed strong α-glucosidase and β-glucosidase activities. The strains with high α-glucosidase and β-glucosidase

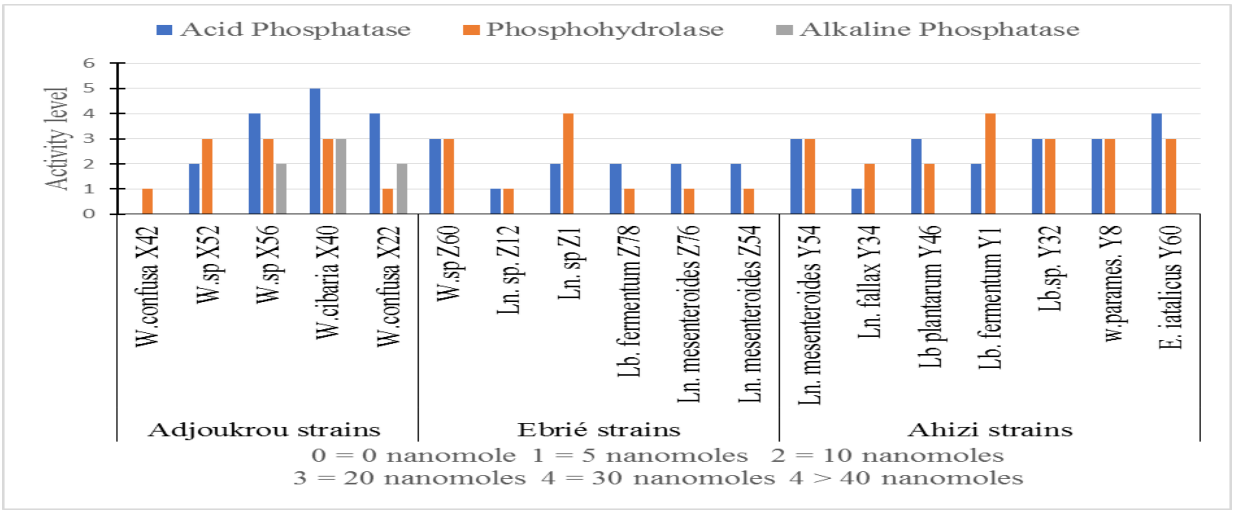
activity belong to the genus Lactobacillus (*Lactobacillus fermentum* Z78) and Leuconostoc (*Lactobacillus fermentum* Z78, *Leuconostoc mesenteroides* Z76, *Leuconostoc sp*. Z12, *Leuconostoc mesenteroides* Z54). No strain was tested positive for α-manosidase and α-fucosidase. All strains tested, except for *Leuconostoc mesenteroides* Z54, did not produce α-galactosidase. The best expression of the osidasic activities was observed in the lactic bacteria of the *Ebrié* zone. These bacteria are good β-glucosidase producers. In contrast, the *Adjoukrou* strains are virtually devoid of this enzyme.



E. : *Enterococcus ;* Lb *: Lactobacillus ;* Ln *: Leuconostoc;* W. *: Weissella ; parames.: paramesenteroides.*

Fig. 3: Osidasic activities of lactic acid bacteria isolated from *Adjoukrou, Ahizi* and *Ebrié* fermented cassava (attiéké inocula and cassava dough).

Phosphatase activities: Three phosphatase activities (acid phosphatase, phosphohydrolase and alkaline phosphatase) were tested on 18 strains. All strains expressed phosphohydrolase activity (**Fig. 4**). This activity is expressed differently by the strains: 44.44% of the strains express it at levels 1 to 2 of the Api-Zym scale. In the other strains (55.55%), the activity was quantified at higher levels of 3 to 4. All strains expressed acid phosphatase with the exception of *Weissella confusa* X42, of *Adjoukrou* origin. The strains (50%) of this zone are the only ones to express the alkaline phosphatase. The *Adjoukrou* strains were the only ones expressing the three phosphatase activities. All *Ahizi* strains showed acid phosphatase production generally at level 3. On the other hand, they are devoid of alkaline phosphatase. The majority (71.43%) of these strains expressed acid phosphatase and phosphohydrolase at levels of 3 to 4. All of the *Ebrié* strains tested showed low production (level 1 to 2) of acid phosphatase and of the phosphohydrolase, with the exception of *Leuconostoc sp.* Z1 and *Weissella sp.* Z60 richer in these enzymes level 4 and level 3 respectively). The *Adjoukrou* strains showed the best phosphatase performance before the lactic acid bacteria of the *Ahizi* zone. The *Adjoukrou Weissella cibaria* X40 strain with three enzymatic activities of levels 3 to 5 seems the most interesting of all.

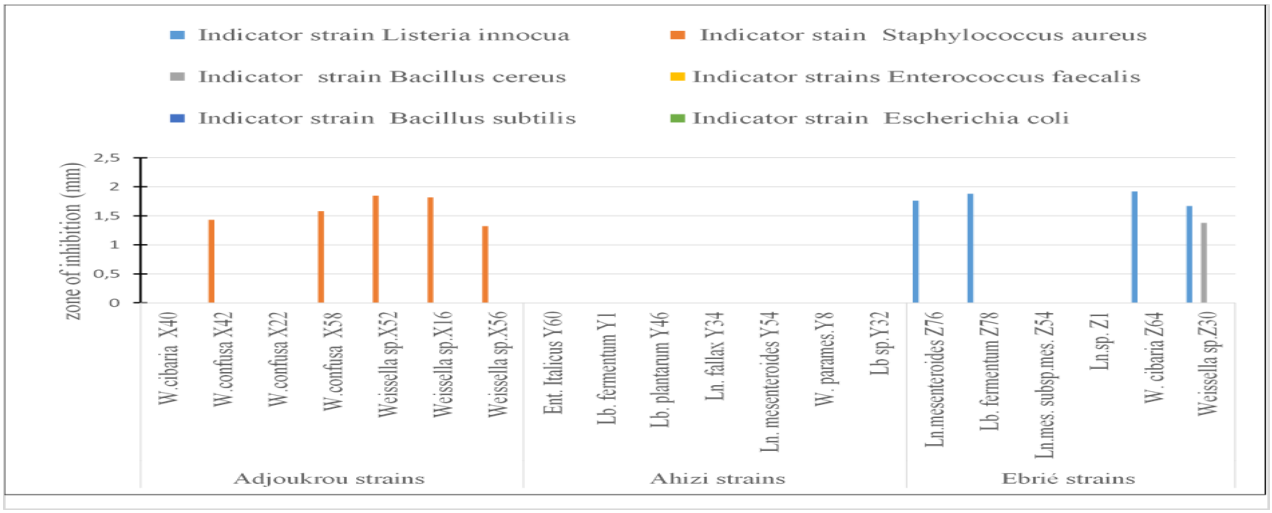


E. : *Enterococcus ;* Lb *: Lactobacillus ;* Ln *: Leuconostoc ;* W. *: Weissella ; parames. : paramesenteroides.*

Fig. 4: Phosphatase activities of lactic acid bacteria isolated from *Adjoukrou, Ahizi* and *Ebrié* fermented cassava (attiéké inocula and cassava dough).

Antibacterial activities

A total of 20 strains were tested for seven strains per Adjoukrou and Ahizi production area and six strains for for Ebrié area. Of the 20 strains, ten showed antibacterial activity on indicator strains. Among these strains having antibacterial activity, five strains (75%) originate from the seven strains of *Adjoukrou* origin. These strains exhibited an inhibitory action on Staphylococcus aureus. The imposition of the zones of inhibition varies from 0.32 to 1.85 cm. Concerning the *Ebrié* strains, 67% of the strains showed an inhibitory activity on the indicator strain *Listeria innocua*. None of the seven strains of *Ahizi* origin showed detectable inhibitory activity (**Fig. 5**). The positive strains of Adjoukrou origin belong to the genus Weissella, including two *Weissella confusa* and two *Weissella sp*. At the level of strains of *Ebrie* origin, *Weissella cibaria* Z64 and *Weissella sp*.Z30 are distinguished from the other Weissella by a spectrum of inhibition which inhibits *Listeria innocua* and *Bacillus cereus*. No strain had activity on *Enterococcus, Bacillus subtilis* and *E. coli*.



E. : *Enterococcus ;* Lb *: Lactobacillus ;* Ln *: Leuconostoc ;* W. *: Weissella ; parames.: paramesenteroides.*

Fig. 5: Inhibitory activities of lactic acid bacteria isolated from *Adjoukrou, Ahizi* and *Ebrié* fermented cassava (attiéké inocula and cassava dough).

DISCUSSION

The leaven is defined by **Lortal (2015)** as a culture of selected microorganisms which are introduced into the products to be fermented. In this work, the lactic leaven of *attiéké* was defined as the set of species of lactic acid bacteria detected during the fermentation of the cassava paste initiated by an *attiéké* inoculum. In order to identify the biotechnological parameters which, characterize each lactic inoculum, a number of five to seven strains of lactic acid bacteria, representative of the species competing during the fermentation, were selected. These descriptors are: the proportion of the phosphatase (acid and alkaline), cysteine arylamidase, valine arylamidase, Lipase esterase C8 and N-acetyl-β-glucoaminidase positive strains. The lactic leaven of Adjoukrou attiéké from the three sites of Debrimou differs from the others in the prevalence of strains producing acid and alkaline phosphatases. **Akuzawa and Fox (2004)** reported that the combined action of acid phosphatases and proteolytic enzymes is often required for the intensive production of peptides and free amino acids. These compounds contribute to the formation of flavors in certain products such as cheeses. None of the Weissella strains of Adjoukrou origin tested showed proteolytic activity at a detectable level. Accordingly, the production of flavorings, according to the principle described above, could not be carried out by the lactic leaven of Adjoukrou origin. Complementation with other associated microorganisms that can express proteases would be necessary. Bacillus sp strains, one of the main microorganisms of *attiéké* inocula (**Bouatenin *et al.*, 2013**), would play this complement function by their capacity to produce extracellular proteases (**Rajkumar *et al.*, 2011**). Another major characteristic of Adjoukorou leaven is the presence of Weissella (71%) with antibacterial activity on Staphylococcus aureus. The antibiotic activity of Weissella has been extensively studied and demonstrated by numerous authors (**Emerenini et *al.*, 2014; Serna-Cock et *al.*, 2013; Yoshiyama *et al.*, 2013; Papagianni and Papamichael 2012**) **Vitali et *al.*, 2012**). This antibacterial activity could disfavor, at the beginning of fermentation, the rapid proliferation of Staphylococcus whose presence in the ferments has been reported by **Kouamé *et al.*, (2017)**. On the other hand, the Weissella of the Adjoukrou leaven are deficient in osidases, in particular the glucosidases, as obseve by **Assamoi *et al.*, (2016)**. The absence of osidasic activity in the lactic leavening of the three Adjoukrou production sites could be compensated by a symbiosis with other fermentative microorganisms which would supply the fermentable sugars to the lactic acid bacteria. The majority of microorganisms isolated from *attiéké* inocula by **Bouatenin *et al.*, (2013)** produce amylases.

As for the lactic leaven of Ahizi *attiéké*, it is characterized by the quantitative importance of lactic acid bacteria producing Lipase Esterase C8, cysteine arylamidase, valine arylamidase and N-acetyl-β-glucoaminidase. The N-acetyl-β-glucoaminidase activity could compensate for the deficiency of lactic bacteria producing bacteriocins. According to **Hussain *et al.*, (1992)**, N-acetyl-β-glucoaminidase has broad spectrum bactericidal activity. Although the indicator strains tested are not sensitive to enzyme activity under the conditions of this study, this N-acetyl-β-glucoaminidase activity may justify the elimination of Gram + coccobacilli (GC +) bacteria during the fermentation of the manioc pulp assured by the *Ahizi* sourdough. No biotechnological characteristics specific to the leaven involved in the production of Ebrie *Attiéké* in the three study sites in Abidjan were observed. This leaven approximates that of the Ahizi sites by, inter alia, the quantitative importance of osidase-producing bacteria. The importance of the osidase-producing lactic acid bacteria in the *attiéké* leaven of the Abraco (Ahizi) and Abidjan (Ebrie) sites could be explained by an adaptation linked to the same process of environmental selection as l 'have shown the work of **Tra Bi *et al.*, (2016)** on the production of osidases by strains of saccharomyces cerevisiae. According to these authors, strains of saccharomyces cerevisiae involved in the alcoholic fermentation of starchy raw materials produce exo-osidases, unlike those in non-starchy media. The lactic leaven Ebrie also consists of a high proportion of lactic acid bacteria with antibacterial activity. One of the strains, Weissella sp. Z30, was found to be active on Listeria innocua and Bacillus cereus, as observed by previous similar work (**Leong *et al.*, 2013; Srionnual *et al.*, 2007**). These two pathogens detected in all ferments analyzed by **Kouamé *et al.*, (2017)** can be eliminated during fermentation by the combined action of antibacterial activities and acidification. The presence of Bacillus spores in the *attiéké* sold in the markets of Abidjan (**Yobouet *et al.*, 2016; Kouamé-Sina 2013**) has also been noted. The expression of antibacterial activities associated with acidification is undoubtedly a microbiological safety factor of the finished product.

CONCLUSION

Adjoukrou, *Ahizi* and *Ebrie* lactic leaven were differentiated from each other by six enzymatic activities. The lactic leaven of *attiéké* Ahizi is distinguished from the others by the quantitative importance of bacteria producing cysteine arylamidase, Lipase Esterase C8, valine arylamidase and N-acetyl-β-glucoaminidase. The specificity of the lactic leaven of Adjoukrou *attiéké* is related to the quantitative importance of the alkaline phosphatase producing bacteria and to a deficiency in bacteria producing osidases. No biotechnological characteristics specific to the lactic leaven of Ebrie *attiéké* were observed. This leaven is similar to that of *Ahizi* origin by, among other things, the quantitative importance of bacteria producing osidases and that of origin *Adjoukrou* by bacteria endowed with antibacterial activity (producing bacteriocins).

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