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Technological Characterization of Acetic Bacteria Derived from the Pulp of Mango of Kent Variety in the City of Korhogo

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Abstract | **Original Research Article**

Acetic bacteria are of great interest to the biotechnology industry for their ability to oxidize ethanol into acetic acid leading to vinegar production. The aim of this study was to characterize and select potential starters of acetic bacteria strains from the pulp of the Kent variety mango. This study was conducted in the laboratory of Peleforo Gon Coulibaly University in Korhogo, with a view to recovering mango waste from processing and export plants. First, the physico-chemical parameters of the pulp of the Kent mango were determined. Then, microbiological analyses were carried out to isolate and identify the acetic bacteria of the Kent variety mango. In addition, the capacity of acidification in solid medium of these acetic bacteria was determined. Afterwards, they were subjected to stress and finally they were put in a liquid medium to test their ability to produce acetic acid. The results of physico-chemical parameters revealed that the pH of mango is acidic with a high moisture content, a high vitamin C content and a soluble dry extract of 14°B content. Then, microbiological analyses allowed to isolate thirty (30) strains of acetic bacteria belonging to the genus *Gluconobacter* and *Acetobacter*. Five (5) of the thirty (30) strains showed high acidifying power and three (3) had better growths compared to the different tests with acetic acid productions between 1.40 and 5.60 mg/ml. These three (3) isolates, BAK 20, BAK 22 and BAK 24 are the potential starters and could be used for vinegar production.

Keywords: Acetic bacteria, Korhogo, Kent variety, titratable acidity, acidifying ability.

1. INTRODUCTION

Fruits and vegetables play a very important role in the fight against poverty (through marketing) and malnutrition in the countries of the South. Indeed, their consumption is highly recommended by the FAO, the PNNS and the WHO for their beneficial effect on health, as they contribute to the important contribution of micronutrients (vitamins, minerals), dietary fiber and secondary compounds (carotenoids, polyphenols) necessary for the proper functioning of the organism [Lauricella *et al*., 2017]. In addition, they provide a protective effect against chronic diseases such as cardiovascular disease. Today, tropical fruits, such as mango, pineapple, and banana, are among the most important fruits worldwide and their market share at the international level continues to grow in order to meet increased consumer demand [Silué, 2022].

Mango (Mangifera indica L.,) is one of the five (5) most produced and consumed fruits in the world after orange, banana, grape and apple. More than eighty (80) tropical and subtropical countries around the world are mango producers and share the global market. Its annual global production is estimated at 55,026,131 tons and 54,831,104 tons in 2019 and 2020, respectively [FAOSTAT, 2022].

The mango (Mangifera indica L.) is a climacteric fruit with high nutritional and economic potential. Its cultivation is adapted to different agroecological zones ranging from subhumid to semi-

arid areas [Grant *et al.,* 2015]. Its production represents 50% of the production of tropical fruits. Mango is the second most important fruit in terms of area and production in the sequence of the following tropical fruits: banana (37.6%), mango (19.6%), pineapple (12.1%), avocado (9.8%), papaya (5.4%), oranges (4.6%), watermelon (4.2%) and passion fruit (3.7%). Its production is largely dominated by India, which alone accounts for 41% of global production [Cissé, 2012].

Mango is among the three most exported fruits from Cote d'Ivoire with banana and pineapple. These three crops alone contribute nearly 4% of the national gross domestic product (GDP) and 10% of the country's agricultural GDP.

Côte d'Ivoire is the leading mango exporting country in West Africa and is the 3rd largest supplier to the European market, with more than 30,000 shavings exported each year with an estimated annual production of 150,000 tons, 20% of which are destined for export. The fresh mango occupies 60% in terms of fruit market share. Generating more than 10 million Euros in income in the northern areas where it is grown, it is a flagship product of Côte d'Ivoire, attracting growing interest for the potential diversification of exports [FRUITROP, 2024].

Domestic consumption is estimated at about 50,000 t/year on a production exceeding 100,000 t/year with post-harvest losses estimated between 30,000 and 40,000 t/year. The production of mango for export is located in the northern zone of the country, covering the regions of Bagoue, of the Poro, the Tchologo and the Hambol. Part of the production is marketed in the Ivorian territory; some is intended for export and another solely for local consumption without any resulting profit-making activity. But a large quantity is lost because the mango is a very perishable fruit. It is even rejected by export organizations because of its condition when it becomes too ripe and is considered a waste [FRUITROP, 2018].

About 20-40% of the total mango production is lost each year due to various problems especially the lack of post-harvest infrastructure, such as processing units. In 2022, the country exported about 40,000 tons of mangoes to Europe, but the substantial losses due to shortages of processing units represent a significant economic loss for local producers and the country's economy. Although some alternatives to guide consumption have already been implemented (jams, fruit concentrates, fruit juices, nectars, purees, dried mangoes, etc.), they are not sufficient to reduce post-harvest losses. These post-harvest losses constitute a fraction of the mango production that cannot be exported due to certain factors called defects. These losses are related to mechanical phenomena such as injuries on mangoes, biological phenomena such as fruit fly bites and fungi (fungal infections) generally caused by handling problems, the inadequate storage conditions and the perishable nature of the mango [AIP, 2022].

The valorization of post-harvest losses of mangoes can be achieved by obtaining various by-products, which could provide significant added value. Several mango by-products exist, among which we find vinegar and wine from the mango obtained after fermentation of it by so-called acetic bacteria and other microorganisms.

Acetic bacteria are a group of microorganisms widespread in nature, present in fruits and vegetables [Erkmen et Bozoglu, 2016]. They are isolated from very varied sources: fermented cocoa beans, tropical fruits, flowers, rice, beers, ciders, wine, etc... They have a great interest for the biotechnological industry, but their most important application lies in their ability to oxidize ethanol into acetic acid leading to the production of vinegar [Kourouma *al., 2021*].

Post-harvest losses are a major challenge for the agricultural industry, severely affecting producers' profitability and the availability of fruits for consumers. However, these losses can be valued through industrial processing. The mango is suitable for various transformations to obtain products such as canned mango in syrup, mango paste, vinegar, etc. [Traoré *et al*.,2017]. It is in this context that the present study was conducted with the aim of contributing to the valuation of post-harvest losses of the Kent variety mango.

2. MATERIAL AND METHODS

2.1. Biological material

The biological material used in this study was mango (Mangifera indica L.), more precisely of the variety Kent (Figure 1). These were mangoes that could not be exported but used for processing. The pulp of these mangoes was used for laboratory analyses.

Figure 1: Mangoes of the variety Kent

2.2 METHODS

2.2.1. Determination of physico-chemical parameters

❖ Moisture and dry matter content

The method used to determine moisture and dry matter content is that described by [AOAC, 1990]. It is based on dehydration by drying the samples in an oven until a constant weight is obtained. Five (5) grams of mango pulp are weighed in a glass capsule of known mass (m0). The capsule containing the sample (total mass m1) is placed in the incubator at 105 ^{0}C for 24 hours and then placed in the desiccator to be cooled. The set (sample + capsule) is weighed (m2) after cooling with a desiccator.

The moisture content (H) expressed as a percentage by mass of wet samples is determined by the following relationship:

$$H(\%)=\frac{(m1-m2)}{m1-m0}\times 100$$

The dry matter content (DM) expressed as a percentage of wet sample mass is determined by the following relationship:

DM (%)= 100 – H

❖ **pH**

The pH is determined using a pH meter that measures the electromotive force, thanks to its electrode sensitive to hydrogen ions (H+). This determination is made in accordance with [AOAC, 1990] method. A mass of ten (10) grams of fresh mango pulp is ground and homogenized in 100 ml of distilled water and the glass electrode of the pH meter (HANNA) is immersed in the filtrate. The pH value is displayed on the pre-calibrated pH meter display.

❖ **Titratable acidity**

The titratable acidity is determined according to the method proposed by [AOAC, 1990]. The principle of this method is to measure the titratable acidity of a product with a standard solution of sodium hydroxide (NaOH) in the presence of phenophthalein serving as a color indicator. Ten (10) grams (me) of fresh mango pulp are ground and homogenized in 100 mL of distilled water. A volume (Vo) of 10 mL of the filtrate using Whatman paper is recovered in an Erlenmeyer flask to which three (03) drops of phenophthalein are added. A solution of NaOH (0.1N) contained in a burette was then added drop by drop to the mixture until a persistent pink color was obtained. The volume of NaOH (V_1) added up to the turning point has been noted on the burette scale. This volume allowed the acid concentration in the initial sample to be calculated, expressed as a percentage of the corresponding acid.

The titratable acidity is expressed in milliequivalent (meq) per 100 g of fresh material by the following relationship:

$$Tac = (N\times V_1\times 10^4) / (mp\times V_0)$$

N: concentration of NaOH; V_1: volume of the NaOH solution; me: mass of the sample; V_0: sample volume

❖ **Soluble dry extract**

The soluble solids expressed in brix were measured with an ATC digital refractometer according to the recommendations of the device manufacturer. Ten (10) grams of mango pulp were crushed in the extractor. The crushed allowed to obtain the juice from the mango. A drop of this juice was placed on the plate of the refractometer prism. The values are directly read.

❖ **Vitamin C content**

The method used to determine vitamin C content was that described by [Pelletier, 1985]. The principle of this method consists in stabilizing vitamin C with metaphosphoric acid/acetic acid 2, then oxidizing it with 2,6-dichlorophenol indolphenol which is reduced. A mass of 10 g of mango pulp (me) was solubilized in 40 mL metaphosphoric acid/acetic acid (2%; w/v). The mixture was centrifuged at 3000 rpm for 20 min. The supernatant is recovered in a 50 mL flask and completed to the mark with boiled distilled water and cooled out of air. A volume of 10 mL of the contents of the vial was taken and then introduced into an Erlenmeyer flask (test sample). The test sample is titrated with a 2,6-DCPIP (2,6-dichlorophenol indophenol) solution at 0.5 g/L, until it turns pink persistently for 30 seconds. The 2,6-DCPIP solution is previously calibrated with a vitamin C solution at 0.5 g/L. Let V (mL), the volume of 2.6 DCPIP paid to equivalence. The vitamin C content as a percentage of fresh sample mass is determined by the following relationship:

$$\text{Vitamine C content} = [2(V_C-V_O) / (V_E-V_O)] *100$$

V_O: volume (mL) of 2,6-DCPIP solution poured into the blank; V_E: volume (mL) of solution used for 2.6 DCPIP solution calibration; V_C: volume (mL) of 2,6-DCPIP solution used for the test portion.

❖ **Ethanol content**

The percentage ethanol content was measured with a digital refractometer as recommended by the device manufacturer. Ten (10) grams of fruit pulp were crushed in the extractor. The regrind was used to obtain the pulp juice. A drop of this juice was placed on the plate of the prism of the refractometer. The value is obtained directly.

2.2.2. Isolation and identification of acetic bacteria

❖ **Isolation**

25 g of the mango pulp taken under aseptic conditions into a stomacher bag to which 225 ml of buffered peptonic water (BPW) are added. The whole was carefully mixed by manual agitation thus giving the mother solution. This agitation aims to make the germs pass into solution. In the test tubes containing 9 mL of Tryptone Sel (TS), decimal dilutions of the stock solution were carried out from 10^{-1} to 10^{-5} under aseptic

conditions. To do this, 1mL of the stock solution was taken and placed in a tube containing 9 mL of TS, which allowed for the 10^{-1} dilution. Subsequently, 1mL of this dilution was taken and put into another tube containing 9 mL of TS, which corresponds to the 10^{-2} dilution. The dilutions are thus carried out in the same way, one after the other. 100µL of each dilute from the dilution range were seeded by spreading on the agar surface. The seeded media were incubated in an oven set at 30°C for 72 hours under aerobic conditions.

The biochemical tests carried out for the identification of acetic bacteria are Gram staining, catalase test, oxidase test and respiratory type.

❖ **Group distinction test for acetic bacteria**

The group distinction test was performed according to the method described by [Soumahoro *et al.*, 2015]. The purpose of this test is to subdivide isolates into two groups: those that oxidize the acetic acid into $CO_2 + H_2O$ and those that are unable to do it.

The principle of this group distinction test is based on the ability of strains to deacidify the medium containing acetic acid. Thus, the HS broth was prepared with bromocresol green and placed in a flask. After sterilization at 121°C and cooling to 45°C in a water bath, an amount of acetic acid (1%) as the carbon source was added to the broth. The addition of acetic acid causes the colored pH indicator to turn from green to yellow. The HS broth is distributed in 5 mL hemolysis tubes at a rate of 3 mL per tube. Seeding a colony of the strain to be tested is done in broth with a sterile Pasteur pipette. The seeded tubes are then incubated for 5 days in aerobic conditions at 30°C. The ability of the strain to use acetic acid results in a deacidification of the broth, causing the medium to go from yellow to green.

❖ **Acetic acid bacteria identification using the MALDI-TOF MS approach**

Using MALDI-TOF MS whole-cell analysis, the potential starters of acetic acid bacteria were identified (Andrés-Barrao *et al*., 2013). This technique distinguishes between bacteria based on the screening of observed peaks as protein biomarkers for bacterial identification. This strategy is enhanced by the use of one or more reference strains for each species to be included in the database. Identification was performed using the SARAMISTM software package (Spectral Archive and Microbial Identification System; Anagnostec Gmbh, Germany).

2.2.3. Study of the potential technological properties of isolated acetic bacteria isolates

❖ **Analysis of the acidification ability**

The ability of isolates to acidify the medium was demonstrated using the method proposed by [Aydin and Aksoy, 2009]. The purpose of this method is to evaluate the acidification power of isolated strains. Thus, colonies were inoculated by spot on pre-poured agar in petri dishes. The seeded media were incubated for 5 days at 30°C in aerobic conditions. During the growth of acetic bacteria, acidification of the medium results in the presence of a clear halo around the colony. Two trials for each isolated strain were carried out on the same container.

❖ **Influence of ethanol on isolates growth**

To evaluate the effect of ethanol resistance, 100 µL of a suspension of acetic acid bacteria OD_{600}=0.5, optical density at 600 nm) previously prepared in Tryptone Salt (TS) were inoculated into 10 mL of HS broth, then the culture broths were incubated at 30°C for 48 hours. Bacterial growth was determined by reading the turbidity in the culture broth with a spectrophotometer at 600nm.

❖ **Influence of temperature on isolates growth**

100 µL of a suspension of acetic bacteria of OD_{600} = 0.5 previously prepared in Tryptone Salt (TS) were inoculated into each tube. Then the tubes were incubated for 48 hours under aerobic conditions at different temperatures (30°C, 35°C, 40°C, 45°C). Bacterial growth was determined by reading the turbidity in the culture broth using a spectrophotometer at 600nm.

❖ **Influence of pH on isolates growth**

This method is based on the ability of the strains to resist changes in pH. The evaluation of resistance to pH makes it possible to select strains capable of resisting the influence of pH. Eight hundred (800) mL of HS broth were prepared and divided into 8 vials due to 100 mL per vial. Sterilization at 121°C and cooling to 45°C were performed, ethanol was added at a final concentration of 4% in each of the 100 mL vials. This influence was realized but the HS broth was adjusted to different pH: 2, 3, 4, 5, 7, 8, 10 and 12. Bacterial growth was determined at 600nm using a spectrophotometer.

❖ **Influence of glucose on isolates growth**

The principle of this method is based on the ability of the strains to resist osmotic stress. The evaluation of osmotic stress resistance allows selecting the strains capable of growing under the influence of a raised concentration of glucose. Six hundred (600) mL of HS broth were prepared and divided into 5 vials due to 100 mL per vial. Sterilization at 121°C and cooling to 45°C is performed. Each flask containing the medium is poured into test tubes at a rate of 10 mL per tube. This influence was carried out as described above but the HS broth was adjusted to the following different glucose concentrations: 0%, 4%, 6%, 8%, 10% and 12%. Bacterial growth was determined at 600nm using a spectrophotometer.

❖ **Production of acetic acid in liquid medium**

The acidity produced by selected strains was quantified. This assay was done by titration with sodium hydroxide solution, 0.1 N (NaOH 0.1 N) and phenolphthalein as a color indicator [Nanda *et al.,* 2001].

For this, the HS medium was used. A 24h bacterial culture of all selected isolate was previously carried out. Then, for each of them, 10 mL of culture medium was introduced into a test tube and inoculated with 100 µL of the different suspensions. Incubation was carried out under agitation at 160 tr/min. Every day, acetic acid production was quantified until constant values are observed while growth was determined by measuring the optical density (OD) at 600nm and pH evolution using pH meter. To determine the amount of acid produced, 3 drops of phenolphthalein were added to a beaker containing 2ml of culture medium. A volumetric dosage with NaOH 0.1 N contained in a burette, made it possible to quantify the production of acetic acid. Acetic acid production (AAP) per liter is given by the following relation: Acetic acid production (AAP) per liter is given by the following relationship:

$$AAP = (N \times V_b \times 1000 \times M) / Va$$

Va: Test volume (mL); N = 0.1: NAOH Normality; Vb: NAOH Volume (mL); M: Acetic acid molar mass

2.2.5. Data processing

The analysis of variances (ANOVA) followed by the Tukey test with a 5% level of significance was carried out with the 2006 version of the XLSTAT software. This software made it possible to calculate the means, the standard deviations of the microbiological parameters. It also made it possible to compare the sample parameter means and determine if the differences observed in the microbiological parameter means were significant at the 5% threshold. The Principal Component Analysis (PCA) was used to compare the strains of acetic bacteria from the variables measured. Hierarchical Ascending Classification (HAC) was used to agglomerate strains of acetic bacteria by similarity of the analyzed parameters. HAC consists of aggregating the isolates according to their resemblance measured using a similarity or dissimilarity index. It produces a sequence of partitions embedded in the set of isolates to be classified. The algorithm begins by gathering the most similar pairs of acetic acid bacteria, then gradually aggregating the other isolates according to their resemblance, until all the acetic acid bacteria form a single group.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Physico-chemical parameters

The results of Table III show that the variety of Kent mango studied has a very acidic pH (3.74) and a titratable acidity of 5.33; with a dry matter content of 15.72% and a moisture content of 84,28%. The value of the soluble dry extract is 14°Brix with an alcohol content of 8% and a high concentration of vitamin C (36.35%).

Table III: Physico-chemical parameters of the Kent mango

PARAMETERS	VALUES
pH	3,74±0,03
Titratable acidity (meq/ 100g)	5,33±0.58
Dry matter (%)	15,72±0,42
Moisture content (%)	84,28±0,42
Soluble dry extract (%)	14±0,00
Alcohol content (%)	8±0,00
Vitamin C content (%)	36,35±5,87

3.1.2. Isolated and identified bacteria from the Kent variety mango

❖ **Isolated bacteria**

The macroscopic and microscopic observations made it possible to select the acetic bacteria. The acetic bacteria are small flat colonies with a smooth outline of beige color. They are also short bacilli. The biochemical tests carried out highlighted short Gram-negative bacilli, strict aerobic bacteria, positive catalase and negative oxidase. A total of thirty (30) strains were isolated and named with codes ranging from BAK 1 to BAK 30.

❖ **Bacteria groups Groups identified**

The group distinction test was intended to subdivide the 30 isolates of acetic bacteria into two groups, those capable of oxidizing acetic acid and those unable to do so. The results obtained show that out of 30 isolates of acetic bacteria, 18 isolates (60%) turned yellow to green indicating that these isolates have the ability to oxidize acetic acid. However, 12 isolates (40%) could not oxidize acetic acid. The isolates capable of degrading acetic acid belong to the group of *Acetobacte*r and those that do not have this ability belong to the group of *Gluconobacter*.

3.1.3. Technological potentials of highly acidifying bacteria

❖ **Acetic bacteria isolated with Acidifying ability**

The results show that of the 30 isolates of acetic bacteria only 13 (43.33%) were able to acidify solid medium and that 17 (56.67%) of the isolates were not able to. Isolates with the ability to acidize the environment showed transparent halos around the spots and those without the ability to acidify the environment did not show transparent halos around the spots. The 13 isolates capable of acidifying the environment had different acidifying powers, 5 strains (38.46%) had a high acidifying power with a diameter greater than 1.5 cm while 8 strains (61.53%) had a low acidifying power with a diameter less than 1.5 cm. The 5 strains were selected for further testing.

❖ **Acetic bacteria identified using the MALDI-TOF MS approach**

MALDI-TOF MS approach used to identify the five best acidifiers species revealed that all these strains were *Acetobacter pasteurianus*.

❖ **Ethanol influence on bacterial growth**

Five (5) isolates were selected from the 13 isolates capable of acidifying the environment. The ethanol resistance was intended to evaluate these 5 isolates of acetic bacteria capable of tolerating high alcohol concentrations. The culture media were prepared with varying proportions of ethanol (4, 8, 10 and 12%). The p-value of each ethanol concentration is less than 0.05, so there is a significant difference between the growth of the strains.

The results show that all the strains studied had their best growths when the medium was supplemented with 4% ethanol. From 4 to 8% ethanol in the medium, the growth of isolates BAK 17, BAK 20 and BAK 22 is stationary while the growth of isolates BAK 24 and BAK 29 decreases considerably. From 8 to 10%, the growth of all isolates decreases and that of isolates BAK 17 and BAK 20 cancels. Finally, from 10 to 12% ethanol, the growth of the three other isolates namely BAK 22, BAK 24 and BAK 29 remains constant (Figure 2).

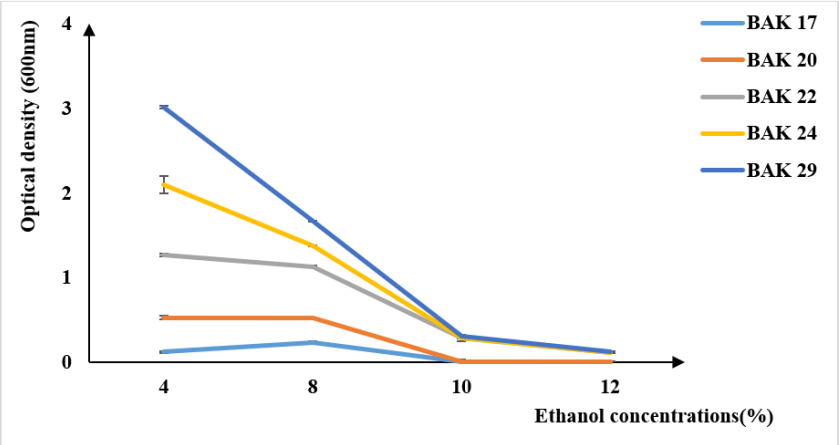

Figure 2: Ethanol influence on bacterial growth

❖ **Temperature influence on bacterial growth**

The observed results show that generally the isolates of acetic bacteria have a growth which varied at different temperatures. The p-value of each temperature is less than 0.05, so there is a significant difference between the growth of the strains. Generally, the growth of all studied strains increase until reaching a growth peak (optimal temperature) at 37°C. At 30°C, the temperature varies from 0,2 to 0.4 and it is isolate BAK 29 that had the best growth while isolate BAK 17 had the weakest growth. Above 37°C, the temperature decreases for all the strains studied and this growth cancels out at 50°C for isolates BAK 17 and BAK 20. At 50°C, the other three isolates had good growth with ODs ranging from 0.2 to 0.31 (Figure 3).

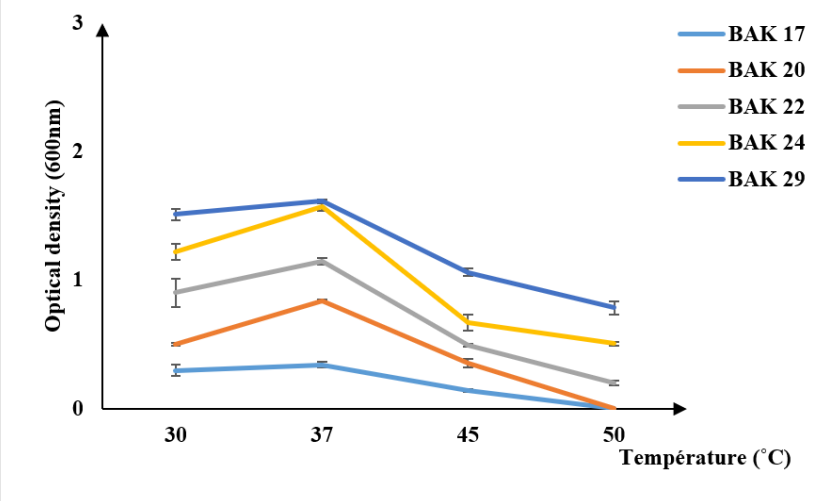

Figure 3: Temperature influence on bacterial growth

❖ **pH influence on bacterial growth**

From a general point of view, growth increases until reaching an optimum beyond which growth decreases. The p-value of pH 2 is greater than 0.05 with a value of 0.199 so there is no significant difference between the growth of strains at pH 2 and the p-value of other pH is less than 0.05 so there is a significant difference between the growth of strains. From pH 2 to pH 3, a latency time is observed at the level of strains BAK 17 and BAK 20 while an exponential growth is observed for the other three strains. Beyond pH 3, all strains reach their peak growth at pH 4 (optimum pH). From pH 4 to pH 8, the growth of all strains stabilizes with the exception of strain BAK 29 which shows slight growth. Thus, at pH 8, growth ranged from 0.474 to 0.617 and strain BAK 20 had the highest growth and BAK 17 the lowest growth (figure 4).

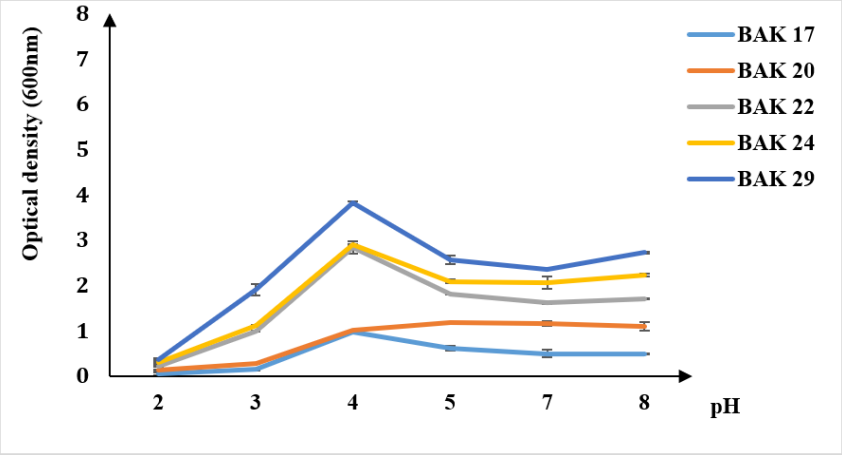

Figure 4: pH influence on bacterial growth

❖ **Glucose influence on bacterial growth**

Glucose has a significant influence on the growth of isolates of acetic bacteria according to what presents the results obtained. Indeed, the selected isolates of acetic bacteria had a good growth at different glucose concentrations. The p-value of the following glucose concentrations (0, 4, 8, 10 and 12%) is lower than 0.05 so there is a significant difference between strain growth at these concentrations. While at 6% concentration the p-value is greater than 0.05 then there is no significant difference between the growth of sources at 6% glucose. The results show a decrease in growth from 0 to 4% of glucose. From 4 to 8% glucose in the medium, growth increases until reaching a peak at 10%. Thus, growth varies from 1.361 to 1.844 and isolate BAK 17 had the highest growth while isolate BAK 22 had the lowest growth. Above 10% glucose in the medium, growth decreases for all strains and isolate BAK 29 adapts better to osmotic stress (figure 5).

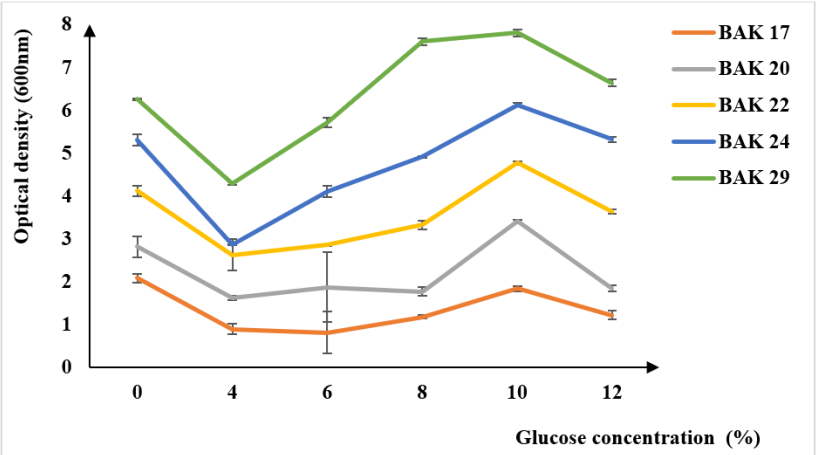

Figure 5: Glucose influence on bacterial growth

❖ **Influence of acetic acid on bacterial growth**

The results show that the more one increases the concentration of acetic acid, the growth of isolates of acetic bacteria is weak and tends to cancel out. The p-value of acetic acid concentrations is less than 0.05 for all strains, so there is a significant difference between the growth of all strains.

When the medium does not contain acetic acid (0%), all strains show their best growth ranging from

0.203 to 0.849 with BAK 22 showing the best growth while BAK 20 has the lowest growth.

At 1%, the growth of isolates of acetic bacteria is decreasing ranging from 0.058 to 0.320. From 2% onwards, growth gradually decreases for all strains and even tends to cancel out for BAK 17 and BAK 20.

From 3 to 4%, growth continues to gradually decrease for all strains (Figure 6).

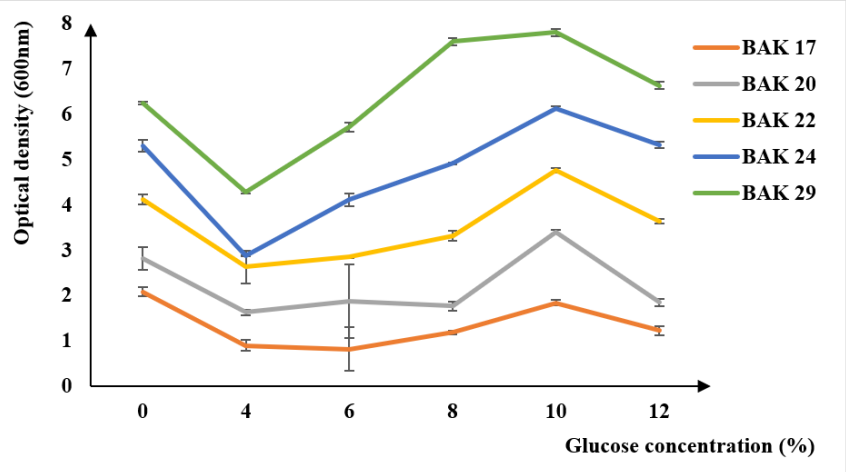

Figure 6: Influence of acetic on bacterial growth

❖ **Production of acetic acid in liquid medium**

The evaluation of acetic acid production in liquid medium showed an acetic acid production by the five (05) isolates studied. Production varies depending on the isolates (figure 7). This study made it possible to follow the evolution of the pH and the growth of the isolates during 120 h. The statistical analysis shows that on the 1st day and the 2nd day, the p-value is greater than 0,05 then there is no significant difference between the production of acetic acid between the 1st and the 2nd day. Unlike on the 3rd, 4th and 5th day where the p-value is less than 0.05 so there is a significant difference between the acid productions of the isolates.

The results obtained show that from 24 to 72 h, acetic acid production is low for all strains. From 72 h, the production of acetic acid by the isolates increases except for the production of isolates BAK 17 and BAK 29. The quantity of acetic acid produced by isolates BAK 20, BAK 22 and BAK 24 is maximal at 120 h. This quantity ranges between 1.4 and 5.6 mg/mL and isolate BAK 22 has the lowest production while isolate BAK 20 gives the highest production (5.6 mg/mL).

The evolution of the growth of acetic bacteria isolates during the production of acetic acid is shown in figure 8. The growth of isolates varies with time. From 24 h to 96 h, the isolates exhibit high growth until they reach a peak. Isolate BAK 17 obtained the lowest peak while isolate BAK 29 gave the highest peak. From 96 to 120 h, the growth of isolates decreases. Isolate BAK 17 has the lowest DO, unlike isolate BAK 29 which has the highest growth.

Figure 9 presents the evolution of the pH of acetic bacteria during the production of acetic acid as a function of time. Indeed, the pH of the fermentation medium decreases for all the isolates tested. At 24 h, the pH varies between 5.75 and 6.87. From 24 to 96 h, the pH of the different fermentation media decreases. Between 96 and 120 h, the pH of the different fermentation media is constant. The fermentation medium containing isolate BAK 29 has the lowest pH (2.51) while the fermented medium with isolate BAK 22 has the highest pH (4.44). At the end of fermentation, the media containing isolates BAK 17 and BAK 29 have the lowest pH (2.20) while the fermented medium with isolate BAK 22 has the highest pH (4.14).

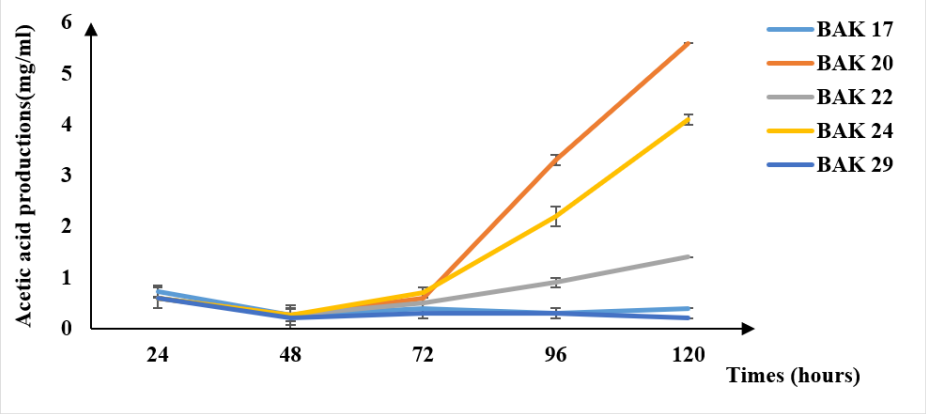

Figure 7: Acetic acid production during bacterial growth

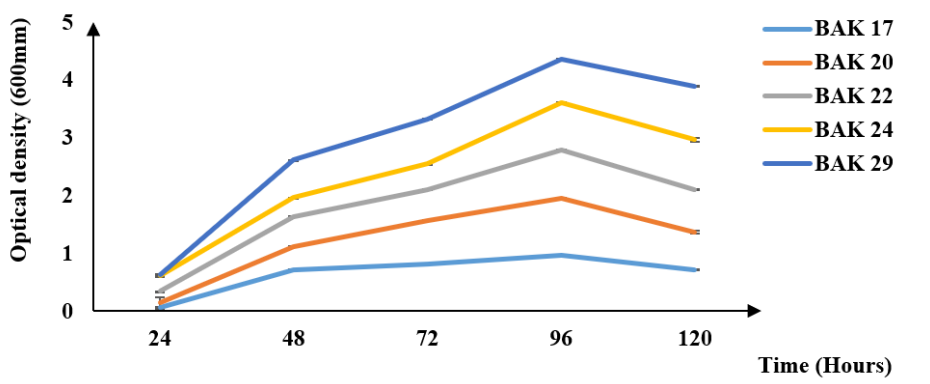

Figure 8: Growth of bacteria during acetic acid production

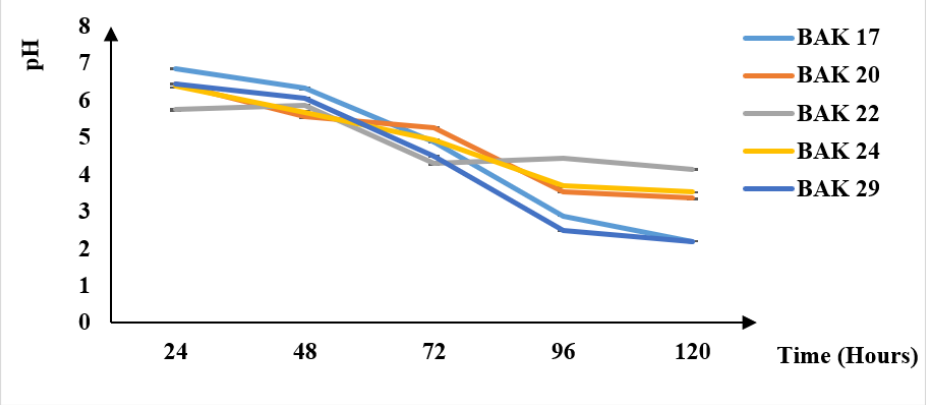

Figure 9: Evolution of the pH during bacterial growth

❖ **Principal component analysis and hierarchical ascending classification of acetic bacteria isolates**

The PCA and the CAH reduced the measured variables to two virtual main components (F1, F2) of which F1 and F2 explain 65.79% of the total variation. The first main component F1 accounted for 37.27% of the observed variations and the second component F2 accounted for 28.53%. The distribution of the isolates of acetic bacteria from the F1 and F2 axes allowed them to be classified into three (3) groups. The first group consisted of isolates BAK 17, BAK 22 and BAK 29. The second group included isolate BAK 20. The third group was composed of isolate BAK 24 (Figure 10). The dendrogram created from the Hierarchical Ascending Classification allows grouping isolates based on their characteristics. Thus, the dendrogram confirmed the classification into 3 groups (Figure 11). Isolates of acetic bacteria previously shown by Principal Component Analysis with the following groups: group 1 (BAK 17, BAK 22 and BAK 29), group 2 (BAK 20) and group 3 (BAK 24). The isolates selected as potential starters are BAK 20, BAK 22 and BAK 24.

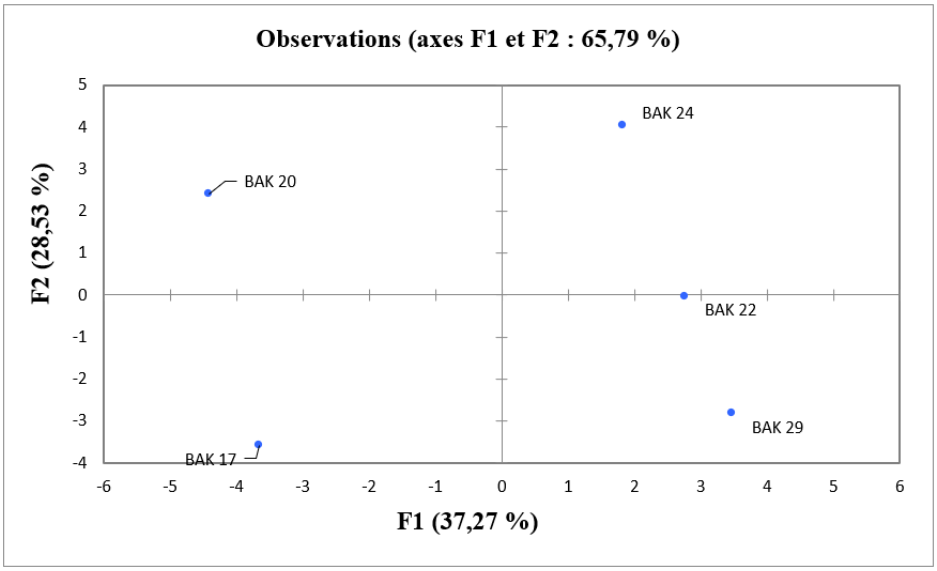

Figure 10: Biplot of analysis in main components

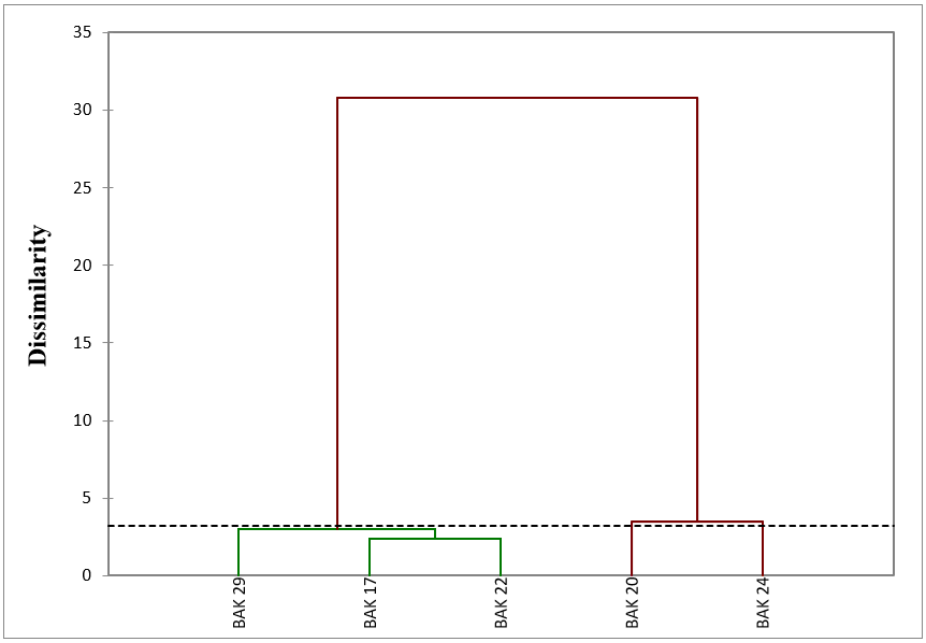

Figure 11: Dendrogram of acetic bacteria

3.2. DISCUSSION

The study conducted first made it possible to determine the physico-chemical parameters of the mango. As a result, the pH of the samples from the Kent mango is acidic with an average of 3,74 ± 0,03. This is due to the presence of organic acid responsible for its acidity and plays several biological roles that are defense against pathogens, helps regulate fruit ripening, influences taste and helps in fruit preservation. The results obtained are in agreement with those of [Kaméni *et al.*, 2003] on which the chemical elements of the mango vary depending on the variety and the level of maturity. The pH increases with the level of maturity and is between 3 and 4.1. Then, the titratable acidity was studied as the second parameter, whose obtained value is 5.33. There is a close relationship between pH and titratable acidity, the results obtained converge with those of [Plancher, 2011] which show that the acidity of media such as sweet fruit juices ranges from 7.10 ± 0.004% to 2.733 ± 0.305% and that it is a function of pH, the higher the titratable acidity, the lower the pH. Subsequently, the humidity rate gave a high value of 84.28 ± 0.42%, indeed, the Kent mango is a fleshy fruit very rich in water, essential for the development of its tissues and the transport of nutrients through the different parts of it. During its maturation, the mango accumulates a lot of sugar and water, which improves its flavor and juicy texture. The results obtained join those of [Ekué *et al.*, 2008]), who showed that most fleshy fruits have a rather high moisture content which can reach 79.19 ± 0.29%. Moreover, the soluble dry extract gave a value of 14°Brix which highlights the sweet taste of mango and also which is a criterion of choice in the appreciation of the sweet taste of fruits. The results obtained are similar

to those of [Koné *et al*., 2018] who obtained values of 18.57 ± 0.25°B for black plum pulp in the Poro region and indicating that black plum is very rich in sugar. Finally, the vitamin c content gave a value of 36.35 ± 5.87. Vitamin C is important because it helps to maintain the normal functioning of the immune system, protects cells against oxidative stress and increases the absorption of iron by the body.

Regarding the group distinction test, two groups of acetic bacteria were obtained, those capable of oxidizing acetic acid and those unable to do so. Namely the genus Acetobacter and the genus Gluconobacter. These results are consistent with those of [Hamdi, 2021], who indicates that the majority of the genera of acetic bacteria belong to the genera Acetobacter and Gluconobacter. In this study, 60% of the isolates were able to use acetic acid, identified as Acetobacter and 40% of the isolates could not oxidize acetic acid and were identified as Gluconobacter.

The acidifying power test revealed that of the 30 isolated strains, 5 showed high acidifying power with halos greater than 1.5 cm and were selected for further testing. Our results are in line with those of [Soumahoro *et al.*, 2019] who mention that only strains producing a halo greater than 1.7 have been arbitrarily considered as the best producers of acetic acid. MALDI-TOF MS approach used to identify the five best acidifiers species revealed that all these strains were *Acetobacter pasteurianus*. They belong to the 60% of isolates that had been able to oxidize acetic acid, thus confirming the group distinction test.

In addition, the ethanol influence test revealed that the isolates exhibited growths at only 4% and 8% while ethanol is the preferred substrate for acetic acids. This is explained by the fact that a high concentration of ethanol can become a significant stress for acetic bacteria, leading to the inhibition of their growth and therefore a decrease in acetic productivity [Gullo *et al.*, 2008]. However, obtaining high acetic acid concentrations requires the presence of acetic bacteria capable of tolerating high ethanol concentrations [Gullo *et al.*, 2014]. The results obtained are similar to those of [Vaughn *et al.*, 1958], which indicate that the maximum concentration of alcohol tolerated by acetic bacteria is between 14 and 15%.

The influence of temperature on the selected isolates showed low growth as the temperature increased. The isolates showed their best growth at 30 and 37° while strains BAK 22, BAK 24 and BAK 29 qualified as thermo-tolerant strains with growths at 50°C, they could be interesting for the vinegar industry. Indeed, acetic bacteria are of the mesophilic type and their optimal growth temperature varies between 25 and 30°C [Ory *et al.*, 1998]. However, the isolation of acetic bacteria capable of growing at high temperatures is of paramount importance for the vinegar industry [Chen *et al*., 2016]. The results are better than those of [Kourouma *et al.,* 2021] where the optimal growth temperatures obtained in this study range between 27°C and 40°C.

Subsequently, the results showed that the best growths of acetic bacteria were between pH 4 and pH 7. However, growth was significant at pH 3 and pH 8, the strains develop as well at acidic pH as at neutral pH. Indeed, the optimum pH for the growth of acetic bacteria is between 5 and 6.5 and they can nevertheless grow, at lower pH values [Mamlouk and Gullo, 2013] or at higher pH values [Soumahoro *et al*., 2015]. The results obtained are similar to those of [Kourouma *et al*., 2021], in this study, the pH optima vary between 5 and 7.

The glucose influence test showed good growth of the acetic acid bacteria isolates at all concentrations. This is explained by the fact that acetic bacteria proceed with an ability to oxidize glucose to produce gluconic acid and acetic acid which differ in functions from the genus of acetic bacteria. Gluconic acid production from glucose and ketogenic activity from glycerol are low to negligible in Acetobacter, but significant in Gluconobacter [Adachi *et al*., 1978].

The results of the acetic acid influence test showed a decreasing growth in all strains. Acetic acid has an effect on the pH of the medium, so it can lower the pH of the medium. Too acidic a pH can inhibit the growth of acetic bacteria because they are not adapted to extremely acidic environments. Indeed, acidity can harm the cell by decreasing internal pH, a key parameter for cellular metabolism, which affects cell growth [Alatou, 2022].

Finally, the solid-state acid production test revealed good production for strains BAK 20, BAK 22 and BAK 24, unlike strains BAK 17 and BAK 29. The BAK 20 strain is the best producer with a production of 5.6 mg/ml. The results are different from those of [Soumahoro *et al*., 2015] who obtained 38.1 g/L acid production by their best strain during cocoa fermentation.

The Principal Component Analysis (PCA) and those of the Hierarchical Ascending Classification (HAC) confirm that the strains having produced the most significant quantities of acetic acid and having the most resistant to different stresses are the isolates BAK 20, BAK 22 and BAK 24 called potential starters.

CONCLUSION

According to our study, physico-chemical analyses revealed that the pH of the 'Kent' mango was acidic and its humidity level was high. Its vitamin C content was high and the soluble dry extract was 14°Brix. A total of thirty (30) strains of acetic bacteria were isolated following the microbiological analyses, eighteen (18) of which are Acetobacter and twelve (12) of *Gluconobacter*. Among these thirty (30), five (5) had high acidifying power and were identified as *Acetobacter*

pasteurianus. They were selected to be subjected to stress in order to choose the most resistant ones. The results showed that all strains tolerate alcohol up to 8%. They show also that the growth optima of acetic bacteria were between 30°C and 37°C respectively for temperature and between 4 and 7 for pH. Finally, the acid production test led to the choice of three (03) potential starters namely BAK 20, BAK 22 and BAK 24, because they had a high capacity for acetic acid production in liquid medium and also showed good growth under the influence of different variations. These acetic bacteria with high acidifying power and good resistance to stress could be used in vinegar production.

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