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Production of acetic acid by acetic acid bacteria using mango juice in Burkina Faso

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ABSTRACT

The present study focused on isolation and selection of acetic bacteria of genus *Acetobacter* for acetic acid production throughout sugar of mango as carbohydrates source. Physicochemical parameters of mango were determined using AOAC standards method. Methods of microbiology were used for selection, phenotypical identification and physiological study of targeted strains. Acetic acid production was realized through batch fermentation process. Physicochemical parameters results showed that pH, reducing and total sugars, moisture and ash were ranged respectively 4.68, 32.11% (w/w), 43% (w/w), 84, 35% (w/w) and 1, 87% (w/w). Fifteen (15) strains were identified as belonging to *Acetobacter*. Four (04) targeted strains have presented maximum rate of growth ranged from 0.28 to 0.34 h^{-1}. Acetic acid obtained by four strains varied respectively from 1.30 to 4.26% (v/v). These results demonstrated the possible use of mango juice as carbohydrate source to produce vinegar.

Keywords: Acetic bacteria, carbohydrate, mango, fermentation, vinegar.

INTRODUCTION

Vinegar is defined as a 4% acetic acid solution that is obtained from double stage fermentation, alcoholic and acetic, performed, respectively, by yeasts and acetic acid bacteria (Johnston and Gaas, 2006). Recently, the vinegar industry has been developed to produce several vinegar types using various qualified native or engineered acetic acid bacteria (Kocher et al., 2006). Acetic acid (Vinegar) is an aqueous solution produced by acetic acid bacteria (AAB) from a dilute ethanol using carbohydrates substances (Kersters et al., 2006). It is determined by distinctive sour taste and pungent smell (Awad et al., 2011). The microorganisms that oxidize ethanol to acetic acid were commonly called acetic acid bacteria (Zahoor et al., 2006). In the past, acetic acid bacteria were classified into two genera, *Acetobacter* and *Gluconobacter* but at present there are twelve genera which are in the Family

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Acetobactaceae that are *Acetobacter*, *Gluconobacter*, *Acidomonas*, *Gluconacetobacter*, *Asaia*, *Kozakia*, *Swaminathania*, *Saccharibacter*, *Neoasaia*, *Granulibacter*, *Tanticharoenia* and *Ameyamaea* (Sengun and Karabiyikli, 2010). The genus *Acetobacter* is generally involved in vinegar production (Kadere et al., 2008).

There are several factors that affect the growth and survival of AAB that amongst, ethanol concentration, acetic acid concentration, oxygen, temperature and nutrient availability are the most important factors that can affect the survival of AAB.

Acetobacter strains have been isolated from several natural origins such as grape, date and palm resources, coconut, fruits and especially in damaged fruits (Kadere et al., 2008, Gullo et Giudici, 2008) and have been applied for production of several vinegar types from various substrates as sugarcane (Kocher et al., 2006), rice (Nanda et al., 2001), balsam and fruits (Giudici and Rinaldi, 2007; Falcone and Giudici, 2008).

Fruits production in Burkina Faso is dominated by mango with two hundred thousand tonnes/an (APROMAB, 2016). Mango fruit is one of main sources of money income for producers. An important proportion of post-harvest fruit is lost due to a lack of conservation. Essential of its transformation is drying and juice production. Mango contains tannins, carbohydrates as starch, pectins, cellulose and fructose with significant concentration of glucose. Due to its important carbohydrates rate, mango can be a valuable fermented substrate for vinegar production (Somda et al., 2017). So valorization of mango fruit by bioconversion to acetic acid could allow to reduce post-harvest losses. This approach may contribute to limit environment pollution. This study aimed at selecting acetic acid bacteria in biotope of mango waste and using them to produce high levels of acetic acid.

MATERIALS AND METHODS

Sampling

Twenty samples of four varieties (Amelia, Kent, Irwin and wild) of mangoes were purchased in different in markets of Ouagadougou. These samples were transported to the laboratory for analysis.

Physico-chemical analysis of mango fruit

The pH sample was measured directly with a pHmeter calibrated with buffer solutions pH4 and pH7 at 25 °C (Nout et al., 1989).The moisture and ash were determined by drying respectively at 105 °C and 550 °C according to AOAC (1990).Total and reducing sugar were estimated by Fox et Robyt (1991) method.

Isolation of *Acetobacter* spp. strains in raw source

For isolation of indigenous culture, mango wastes were used as raw source materials. The method of Zahoor et al. (2006) and Sharafi et al. (2010) was adapted for strains isolation. Wastes samples of mango were subsequently crushed and incubated at 30 °C for fermentation during 7 days. After fermentation, a volume of 100 µl was inoculated on GYC medium (10% glucose, 1.0% yeast extract, 2.0% calcium carbonate, 1.5% agar, pH 6.8) supplemented with 100 mg. l^{-1} of Pimaricin to inhibit the growth of yeasts and moulds. Culture was incubated at 30 °C for 48 hours. Acetic bacteria strains were selected by using of the presence of bromocresol green in medium.

Characterization of phenotypical properties of strains

Morphological examination

Strains isolated and purified on GYC medium were used to make suspensions olded from 16 to 24 h. An optical microscope observation was made at G x 40 to determine the shape, clustering pattern, and mobility. Also Gram staining was done and optical observation was made at G x 100.

Biochemical tests

The biochemical tests were performed to further confirm the culture isolates.

The test of catalase and oxydase was realized by the method of Holt et al. (1994). The capacity of sugar fermentation was tested using method of Kowser et al. (2015).

Tests of physiological properties

The physiological properties of selected strains were studied by testing their tolerance to alcohol and glucose uptake. The kinetics growth was monitored by using method of Obire (2005).The concentrations of alcohol and glucose tested were ranged respectively from 0% to 10% (v/v) and 0% to 15% (w/v).

Production of acetic acid from mango juice

The production of acetic acid was carried out according to Sharafi et al. (2010). The variety of mango which contained higher concentration of glucose was served for experiment. Acetic fermentation of mango juice was done at 30 °C for 15 days (Klawpiyapamornkun et al**.,** 2015). The monitoring of the acetic acid production was carried out each 24 h. The concentration of acetic acid was determined by titration using method of Sharafi et al. (2010).

Statistical analysis

XLSTAT software was used to determine average, standard deviation and significant difference between the values. The difference between maximum growth rate of the bacteria Strains was examined by the ANOVA test. The difference between means is considered significant at $p < 0.05$.

RESULTS

Physico-chemical characteristics of mango

Results of physico-chemical parameters are represented in Table 1. They were obtained from 04 varieties of mango. Total and reducing sugars and pH of the mango samples varied from 39.32 to 46.62% (m/m), 25.13 to 39.09(m/m) and 3.87 to 5.49. Moisture and ash values varied from 80.03 ±0.39 to 88.67 ± 0.50% (m/m), and 1.35± 0.36 to 2.4± 0.43% (m/m).

Morphology and biochemistry of strains

Fifteen bacterial strains were obtained after isolation and purification. The appearance of colonies after purification and morphological characterization of cell after Gram die were illustrated in Figure 1 and 2. It shown that colonies were round white and pink color. Biochemical and morphological characteristics allowed to retain 4 strains from 15 for analysis. Characteristics of selected strains were presented in Table 2 who shows the capacity of these strains to metabolize some sugars and also the morphology of their cells that are the form of small stick. Sugars as glucose, saccharose, mannitol and melibiose where degraded by all the four strains whereas the lactose, maltose, and arabinose did not degraded.

Physiological properties of strains

The evolution of µmax values in the Figure3 and 4 revealed the influence of ethanol and glucose on kinectic growth of the targeted strains. Indeed, the maximum growth rate varied with strains and the nature of the substrate. The highest maximum rate was obtained with the CRSBAN-BVK1 strain and the lowest with the CRSBAN-BVI1 and CRSBAN-BVA1 strains for the ethanol and glucose concentrations.

Maximum growth rate and yield of biomass cell were presented in Table 3. The highest maximum speed of growth and the greatest quantity of produced biomass were obtained with the CRSBAN-BVI1 strain and lowest maximum speed with the CRSBAN-BVA1 strain.

Production of acetic acid

The results of Table 4 showed the concentration of acetic acid moduced by the targeted strains using juice of mango. All strains had the ability to produce acetic acid but at different concentrations. The maximum content of acetic acid was obtained with the concentration 10% of glucose with all strains. The highest concentration was 4.26 obtained with the strain CRSBAN-BVA1.

Table 1: Physico-chemical characteristics of mango.

Mangos	pH	ash (g/g)	Moisture (g/g)	Reducing sugar (g/g)	Total sugar (g/g)
Kent	5.49± 0.11	1.91 ± 0.20	84.36 ± 0.20	39.09± 0.12	46.62±0.05
Irwin	5.22± 0.31	2.01 ± 0.50	80.03 ±0.39	25.13± 0.28	39.32 ±0.08
Sauvage	4.85± 0.30	2.4± 0.43	84.51 ± 0.17	25.54± 0.08	41.30± 0.08
Amelia	3.87± 0.23	1.35± 0.36	88.67 ± 0.50	26.31± 0.57	44.51± 0.03

Figure1: Colonies of acetic bacteria.

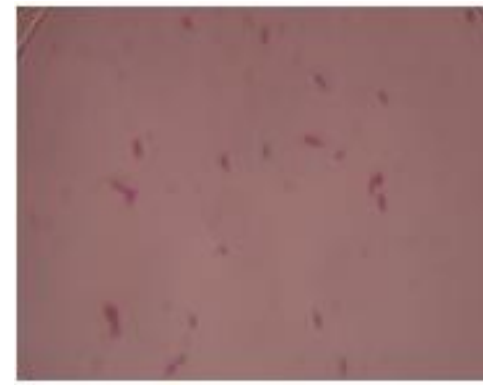

Figure2: Gram die of bacteria.

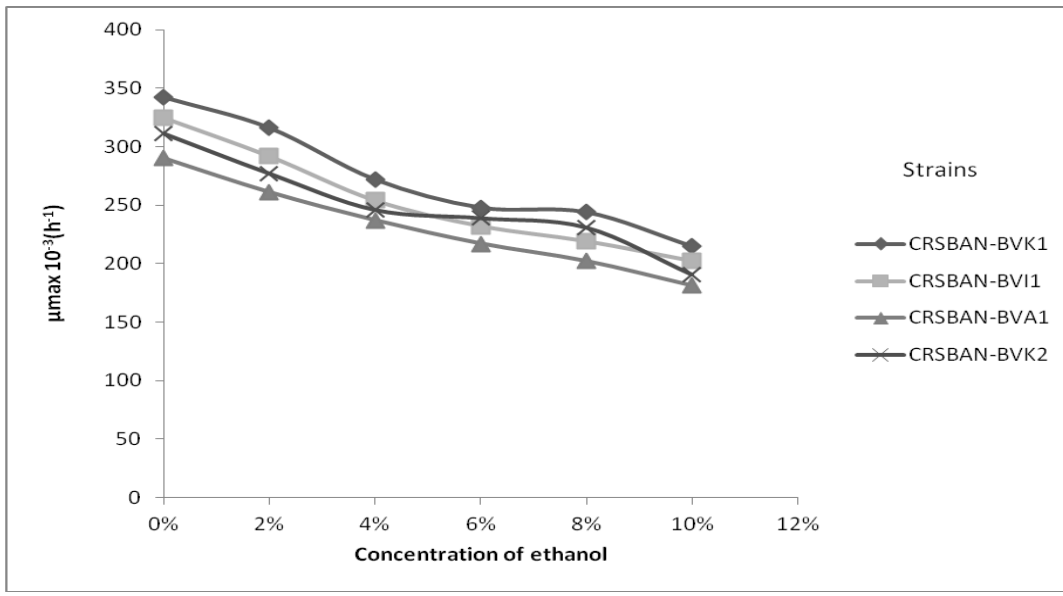

Figure 3: Effect of ethanol concentration on growth of strains.

Table 2: Morphological, biochemical and characteristics of selected strains.

Strains	**Gram**	**Form**	**Catalase**	**Oxidase**	**Citrate**	**Glucose**	**Lactose**	**Mannitol**	**Gaz**	**H_2S**	**Mobilité**	**Ure**	**Indol**	**Saccharose**	**Arabinose**	**Melibiose**	**Maltose**	**Cellulose**	**Ac Sodium**	**Presumption**
CRSBAN-BVA1	-	Bacille	+	-	-	+	-	+	+	-	+	-	-	+	-	+	-	+	-	*Acetobacter sp*
CRSBAN-BK1	-	Bacille	+	-	-	+	-	+	+	-	+	-	-	+	-	+	-	+	+	*Acetobacter sp*
CRSBAN-BVK2	-	Bacille	+	-	-	+	-	+	+	-	+	-	-	+	-	+	-	-	+	*Acetobacter sp*
CRSBAN-BVI1	-	Bacille	+	-	-	+	-	+	+	-	+	-	-	+	-	+	-	-	-	*Acetobacter sp*

+ = positive test; - = negative test; Ac = acetate.

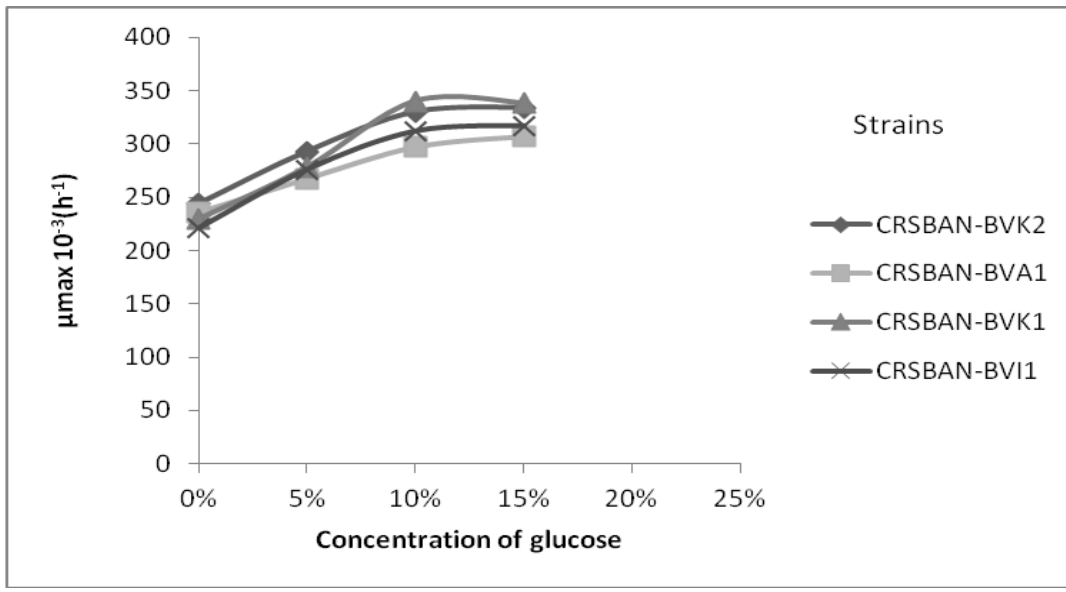

Figure 4: Effect of glucose concentration on growth of strains.

Table3: maximum rate of growth (µmax h-1) and yield of biomass cell.

Strains	**µmax (h^{-1})**	**$Y_{X/S}$ (g/g)**
CRSBAN-BVK1	0.331	0.22
CRSBAN-BVK2	0.285	0.15
CRSBAN-BVI1	0.347	0.27
CRSBAN-BVA1	0.283	0.17

Yx/s= yield of biomass cell, µmax = maximum growth rate

Table 4: Production of acetic acid.

Strains	**Glucose concentration from mango juice (m/v)**			
	2%	6%	10%	12%
	Concentration of acetic acid produced (m/v)			
CRSBAN-BVK1	2.46± 0.12	2.7± 0.12	2.89± 0.05	1.92± 0.01
CRSBAN-BVA1	2.76±0.08	3.48± 0.01	4.26± 0.00	1.68± 0.04
CRSBAN-BVK2	3.22± 0.00	3.24± 0.03	3.72± 0.02	1.38± 0.02
CRSBAN-BVI1	3.24± 0.04	3.3± 0.02	3.64± 0.00	1.08± 0.00

DISCUSSION

Physico-chemical characteristics of mango

The pH of the different mango varieties was ranged from 3.87 to 5.49. These values are closed to those obtained by Somda et al. (2010) which found from 3.61 to 5.20. That is related to the acidity of acid mango favoring the proliferation of acidophile microorganisms.

Total and reducing sugar contained was ranged respectively 39.32 to 46.62% (m/m) and 25.13 to 39.09(m/m). These values were higher than those (18.70 to 26.85) obtained by Hossain et al. (2001) then Somda et al. (2010) as 13.12 to 25.78%. This difference could be due to varieties of mango. The composition of ash and moisture who vary from 8.03 ±0.39 to 88.67 ± 0.50% (m/m), and 1.35± 0.36 to 2.4± 0.43% (m/m) are slightly higher than those obtained on the mango by Somda et al. (2010) (83,10 ± 0,5% to 76,36 ± 0,2% and 1,60 ± 0,3% to 3,01± 0,16%). These values show the high water content predisposing fresh mango to microbial growth.

Morphology and biochemistry of strains

The colonies were further isolated while resolving on the basis of morphological and microscopic examination. They were small, white, spherical, pinpoint, raised, off-white and showed a clear halo on GYC agar, revealing their ability to dissolve calcium carbonate by producing acid (Ouoba et al., 2012).

Strains were biochemically negative oxidase, positive catalase and negative Gram. The characteristics found were in agreement with those of Zahoo et al. (2006) and Mamlouk et Gullo.(2013) on Acetic acid bacteria. All strains were motile and also had the ability to degrade glucose, saccharose, mannitol and melibiose. Lactose, maltose, arabinose and citrate were not metabolized. This result was similar to that of Aydin et Aksoy (2009) who had found that the lactose, maltose did not fermented by Acetic acid bacteria. The strains CRSBAN-BVA1 and CRSBAN-BVK1 metabolized the cellulose. Sodium acetate was metabolized by the strain CRSBAN-BVK1 and CRSBAN-BVK2. These same characteristics have been observed by Romero and al. (2011) in the characterization of acetic bacteria isolated from fermented cocoa. Hydrolysis of urea indole was negative for all strains. These results were emphasized with those of Hwan et al. (2004).The different characteristics of the selected strains demonstrated that it could be belonging to *Acetobacter* genus.

The rate of maximum growth decreased proportionally to increasing of ethanol. Concentration yet concerning glucose uptake the growth rate was raised-up simultaneously to the increase of glucose concentration until 10% (w/w) before stabilization. Bacteria cells were affected by the concentration of alcohol. The increase of growth rate proportionally to glucose concentration explained by strains tolerance of glucose. According to Awad et al. (2011), Acetic bacteria (*Acetobacter*) could uptake more than 10% of glucose.

The maximum growth rate and yield of biomass cell were ranged respectively from $0.283h^{-1}$ to $0.347h^{-1}$ and 0.15 to 0.27 (w/w). This shows the conversion capacity of glucose to reducible sugars for these strains.

The results of Table 4 showed the concentration of acetic acid moduced by the targeted strains using juice of mango. The highest concentration was 4.26 w/v at the 10% glucose concentration with the CRSBAN-BVA1 strain, which was closed of concentration of classic vinegar (8%). This value was slightly superior that found by Bovonsombut et al. (2015) who was founded a production of acetic acid of 4.06% starting acetic acid Bacteria isolated from fruits.

Conclusion

This study shows the possibility of isolated from acetic acid bacteria starting from mango with high performances. Four (04) strains exhibited a better maximum growth rate and optimal production in acetic acid. The results obtained during this study show that the mango pulp contained carbohydrates necessary to be transformed into acetic acid. It would be necessary to develop pure culture of the vinegar from this local fruits to avoid the

production of the synthetic vinegar. That will also contribute to the depollution of the environment.

COMPETING INTERESTS

There is no competing interest for this article.

AUTHOR'S CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author AO was the field investigator and drafted the manuscript. Author MKS designed the study and supervised the work. CATO advised the external research. Authors AST and ASO revised the manuscript. All authors read and approved the final manuscript.

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