Heliyon 7 (2021) e07079

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Lactic acid production ability of *Lactobacillus* sp. from four tropical fruits using their by-products as carbon source

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ARTICLE INFO

Keywords:
Tropical fruits
Lactic acid bacteria
Technological properties
By-products
Lactic acid

ABSTRACT

The present work was aimed at studying the technological properties of lactobacilli isolated from four tropical fruits (banana, papaya, pineapple, and orange) sold in Dschang (a city of the Menoua Division, West-Cameroon), as well as their ability to produce lactic acid (LA) from by-products of these fruits. After isolation and preliminary identification, homofermentative isolates were investigated for acidifying, amylolytic, cellulolytic activities as well as exopolysaccharides production. The chemical composition of the by-products was determined prior to fermentation assays and the most promising isolates were identified by 16S rRNA gene sequencing. From the 54 homofermentative lactobacilli obtained, 9 isolates were pre-selected based on their higher acidifying activity in MRS-Glucose medium. They all showed amylolytic activity, with the most important activity (54.26 ± 0.10 µg of reducing sugar/ml/min) recorded by isolate O31. Relatively to their cellulolytic activity, isolate 1B9 showed the best activity, displaying a production rate of 7.98 ± 0.40 µg glucose/ml/min, while none of them produced exopolysaccharides. The proximate analysis showed that the fruit-derived by-products contained proteins (0.40 ± 0.06% DM to 1.54 ± 0.06% DM), carbohydrates (61.75 ± 0.75% DM to 71.94 ± 2.02% DM) that are main nutrient needs for bacterial growth. Banana-derived and pineapple-derived by-products showed the highest LA production rates with values, 26.37 ± 0.05 g/l (isolate 3A5) and 26.29 ± 0.38 g/l (isolate 1B9) respectively after 16 h of fermentation. Based on the principal component analysis, isolates O31, 1B9, 3A5, 3A9 and 4O8 were selected as the most promising isolates and were identified as *Lactobacillus plantarum* strains. According to the obtained results, lactobacilli from tropical fruits displayed properties of commerical interest and can be promising candidates in the valorisation of by-products from tropical fruits through LA production.

1. Introduction

The use of lactic acid bacteria (LAB) in the agri-food industry has evolved exponentially over the years as they possess technological properties that can be exploited at the industrial level. These include proteolytic, cellulolytic, flavoring, amylolytic, texturizing, but also their ability to produce lactic acid (Elmaged et al., 2015; Herdian et al., 2018; Galli et al., 2020). Indeed, lactic acid (LA) is an organic compound that plays a role in multiple biochemical processes; it has various applications cosmetic, pharmaceutical, chemical and food industries (Mohan et al., 2015; Battula et al., 2018; Abedi and Hashemi, 2020). The production of LA by microorganisms has well been developed over the years because this fermentation process can yield an optically pure form of lactic acid or racemate, depending on microorganisms, substrates and fermentation conditions employed in the production process. In addition, the high demand of naturally produced LA for the synthesis of biodegradable compounds has boosted the lactic acid's market (Coelho et al., 2011; Agata et al., 2020). However, this fermentation process involves relatively expensive substrates such as glucose, sucrose, lactose and maltose, hence the need for research for raw materials that are less expensive to be used in its production (Richard, 2015; Battula et al., 2018). Thus it is in this context that efficient LA production has been reported from various

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https://doi.org/10.1016/j.heliyon.2021.e07079
Received 31 December 2020; Received in revised form 25 February 2021; Accepted 12 May 2021

alternative substrates such as molasses, hydrolysate newspaper, whey, Cassava bagasse, starch, sweet sorghum juice and lignocellulose biomass (Wee et al., 2006; Zhang and Vadlani, 2014; Abedi and Hashemi, 2020). As well as the derived by-products mentioned above, by-products from households and tropical fruit (orange, mango, papaya, banana, and pineapple) processing industries, due to their high carbohydrate composition (Phatcharaporn et al., 2009; Chukwuka et al., 2013; Maria et al., 2014), could be potential substrates for LA production by appropriate microbial strains and thus reducing the cost linked to the LA production by this fermentation pathway.

In several developing countries such as Cameroon, the processing and marketing chain for tropical fruits (pineapple, papaya and banana) and households dump large quantities of by-products that pollute the environment. Moreover, in terms of their composition, these derived by-products represent a significant loss of biomass and nutrients (Phatcharaporn et al., 2009; Chukwuka et al., 2013; Maria et al., 2014). The judicious use of these by-products to produce LA could be a means of controlling this pollution while making use of all this nutrient-rich biomass.

Several authors have been interested in the exploitation of pineapple, papaya and banana by-products for the production of LA in India (Pushparani et al., 2012; Mridul and Preethi, 2014; Ranjit and Srividya, 2014). However, they did not priorily determine the proximate composition of these by-products, which could give a nutritional overview of the fermentation medium and hence influence the LA production. In addition, these authors did not isolate the fermentative strains of the fruits from which these by-products are obtained but rather use strains from research institutes. Yet these fruits are potential niches for LAB. Previous work on the lactic flora of these fruits has shown that they contain LAB with interesting properties such as antimicrobial, proteolytic, probiotic and antioxidant properties (Maryam et al., 2012; Ram et al., 2013; Maryam and Wedad, 2017). To the best of our knowledge, no study at the current state has been carried out on the exploitation of the technological properties of the lactic flora of these biotopes (pineapple, papaya, orange and banana) to produce lactic acid from their derived by-products. Thus, exploring the microbiota of the aforementioned fruits would increase the chances of finding lactic acid bacteria strains able to better ensure the bioconversion of the by-products of these fruits into metabolites of interest, especially lactic acid.

The present study was aimed to determine the technological properties (acidifying, amylolytic and cellulolytic activities) of *Lactobacillus* sp. isolated from four rotting tropical fruits. Also, the proximate composition of the by-products derived from these fruits as well as the ability of the strains to produce LA therein were assessed.

2. Materials and methods

Graphical methodology

2.1. Sample collection and isolation of LAB

Fifty-six samples of overripe fruits (orange, banana, papaya and pineapple) were aseptically collected from the main market of the city of Dschang (Menoua Division, West Cameroon region) from March to June 2018 and stored in sterile bags. Immediately after collection, these samples were transported to the laboratory where they were used as matrices for isolating lactobacilli. Ten-fold dilutions were prepared using crushed fruit in 0.9% NaCl solution and aliquots (100 µl) of chosen dilutions were swabbed onto MRS-agar (TM MEDIA, Titan Biotech Ltd, India), and subsequently incubated under anaerobic conditions at 30 °C for 48 h. Using morphological, phenotypic and biochemical methods as described by Sharpe (1979), identification of the isolates at genus level was carried out. In addition, all isolates were tested for CO2 production from glucose and for growth at 10 °C for 10 days, 45 °C for 48 h. All these tests were carried out in triplicates. Only homofermentative LAB were used in further work.

2.2. Screening of acidifying isolates

The modified method of Larpent (1997) was used to evaluate the acidifying activity. Young cultures (12 h-old cultures) of LAB isolates were used to inoculate MRS broth (5% v/v) and the pH was recorded after 6 h of incubation at 30 °C. At the end of this set of experiment, the isolates that showed best acidifying activities were further investigated for their acidifying activity in MRS-Fructose broth and MRS-Sucrose broth. All the experiments were carried out in triplicate.

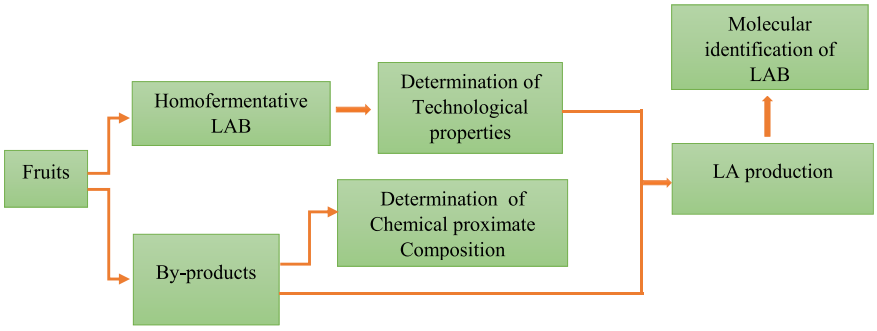
2.3. Screening for exopolysaccharides production

Young LAB cultures were streaked on MRS agar supplemented with various carbohydrate sources: sucrose (50 g/l), glucose (20 g/l), lactose and fructose (20 g/l). After incubation at 30 °C for 48 h, the plates were checked for the presence of large, sticky or mucoid colonies and as well as those forming a long visible filament as soon as they were touched and lifted by a sterile loop (Silvia-Simona and Medana, 2014). The experiment was carried out in triplicate.

2.4. Assessment of amylolytic activity

2.4.1. Screening of amylolytic bacteria

The method of Anthony et al. (2017) was used to evaluate the amylolytic activity of different LAB isolates on solid media. The young cultures (12 h-old cultures) of the LAB isolates were spotted MRS-Starch agar (on 2% w/v soluble starch) followed by incubation at 30 °C for 48 h upon which a solution of Lugol was sprayed on the surface of the entire Petri dish. The presence of clear halo surrounding colonies was considered as amylase production by the isolate. All the experiments were repeated three times.



2.4.2. Evaluation of amylase activity

The amylase activity was also determined through the production of reducing sugars by crude enzyme using the modified method of Anteneh and Prapulla (2015). Briefly, after growing LAB isolates in MRS-starch broth (2% w/v) in which starch was the sole carbon source for 48 h, the supernatant was collected (5000 rpm/30 min/4 °C) and used as a source of crude enzyme. Then reducing sugars produce by crude enzyme in a soluble solution of starch (2%) prepared in a sterile phosphate buffer (pH 7; 0,1M) after 30 min of incubation at 30 °C were quantified by DNS (3, 5-dinitro salicylic acid) colorimetric method. Reading the OD was done at 540 nm, the reducing sugar was calculated based on a previously plotted calibration curve. The results obtained were the average of three experiments.

2.5. Assessment of cellulase activity

2.5.1. Evaluation of cellulase activity in a solid medium

It was evaluated according to the diffusion method described by Aaisha and Barate (2016). After growing LAB isolates at 30 °C for 48 h in MRS-Carboxymethylcellulose (CMC) broth (5% w/v) in which glucose was substituted by CMC, the supernatant was collected (5000 rpm/30 min/4 °C) and used as a source of crude enzyme. Then, wells of 5 mm diameter were made in sterile Petri dish containing CMC-agar (1%). Subsequently, 50 µl of the previously obtained raw enzyme source was introduced into the various wells; after diffusion of the supernatants, the Petri dish were incubated at 30 °C for 24 h. Finally, a 1% (w/v) Congo red solution was sprayed on the surface of the Petri dish and after 20 min, it was rinsed with NaCl (1 M) to reveal the wells surrounded by a clear halo.

2.5.2. Evaluation of cellulase activity in a liquid medium

The protocol described previously was used to evaluate cellulase activity in liquid media. However, the sterile CMC solution (1%) used in this case as a substrate for cellulase was prepared in phosphate buffer (pH 7; 0.05M) (Saowapar et al., 2014). All the experiments were repeated three times.

2.6. Lactic acid production using a tropical fruit-derived by-products

A total of three different fruit-derived by-products (peels of papaya, banana and pineapple) was collected in the main market of the city of Dschang (Menoua Division, West Cameroon region) and they were used in the further work.

2.6.1. Determination of chemical proximate composition of fruit-derived by-products

It was assess according to the methods described by IUPAC (1979) and AOAC (1980).

2.6.2. Preparation of hydrolysates for fermentation

The modified method of Pushparani et al. (2012) was used for substrate hydrolysate preparation and their supplementation. About 500 g of each by-product was autoclaved (121 °C for 20 min). Sterile water was added to the wet pretreated material to make the volume of 1l and boiled at 80 °C for 30 min followed by filtration with cheese cloth. Acid hydrolysis of the filtrate was carried out by autoclaving at 121 °C with 1% HCl (2M) v/v for 30 min. The pH of the hydrolysate after hydrolysis was adjusted with CaO to 6.6 and the $CaSO_4$ precipitate formed was removed by filtration with Whatman filter paper No.1. The synthetic medium for the fermentation was prepared as follow: Hydrolysate: 1000 ml; yeast extract: 30g; sodium acetate: 5g; $MgS0_4.7H20$: 0.6 g; $MnSO_4.H_20$: 0.05 g; K_2HPO_4: 0.8g; KH_2PO_4: 0.8 g; $FeSO_4$: 0.05g.

2.6.3. Inoculum preparation

Inoculum preparation was performed using Herdian et al. (2018) method with modification. Young cultures (12 h-old cultures) were streaked on previously cast and solidified MRS-Agar. The seeded medium was incubated for 48 h, after which the bacteria were collected aseptically from the surface of the agar using a platinum loop and suspended in 10 ml of a sterile (0.9%) NaCl solution contained in a glass tube. The whole was then vortexed and its opacity compared to Mc Farland scale 2. The cell suspension corresponding to Mc Farland's scale 2 (approximately 6×10^8 CFU/ml) was used as inoculum for fermentation.

2.6.4. Fermentation

Batch cultures were carried out in 250 ml Erlenmeyer units with a useful volume of 100 ml of the supplemented and sterile hydrolysate. Then this fermentation medium was inoculated at a rate of 5% (v/v) (Pushparani et al., 2012) and kept for incubation at 30 °C on a incubator under agitation after every 2 h for 2 days. Substrate consumption and lactic acid production were determined on a regular time range (0, 8, 16, 24, 32, and 48 h).

2.6.5. Estimation of lactic acid production and reducing sugars consumption

2.6.5.1. Estimation of LA production. Lactic acid was quantified by the spectrophotometric method as described by Borshchevskaya et al. (2016). One ml of the fermentation broth was centrifuged (4500 rpm for 30 min) and the supernatant was collected and diluted 10 times. Subsequently, 0.1ml of the previous dilution was added to 4 ml of a $FeCl_3$ solution (0.2%); after homogenization, the optical density was measured with a spectrophotometer (Thermo Scientific BioMate 3S UV-Visible spectrophotometer, Thermo Scientific, USA) at a wavelength of 390 nm. The amount of lactic acid was calculated based on of a previously drawn calibration curve. The results obtained were the mean of three experiments.

2.6.5.2. Estimation of residual sugar. The residual reducing sugar content of the fermentation broth was estimated on regular basis spectrophotometrically using DNS (3, 5-dinitro salicylic acid) method as described by Fisher and Stein (1961).

2.7. Molecular identification of LAB isolates by 16S rRNA gene sequencing

- DNA extraction

Genomic DNA was prepared using 1 ml of stationary phase cultures. Then bacterial cell lysis was completed by incubation first in 0.05 N NaOH (15 min at room temperature), then in TES buffer (25% saccharose, 10 mM EDTA, 0.1 M Tris-HCl, pH 8.0) with 1 mg ml^{-1} lysozyme and 100 U ml^{-1} mutanolysin for 1 h at 37 °C. Genomic DNA was purified using the High Pure PCR Template Preparation Kit (Roche) and resuspended in 200 µl final volume. The purity of the extracted DNA was estimated using a NANODROP (ND 1000) Spectrophotometer (Thermo Scientific, USA).

2.8. PCR amplification and sequencing

Amplifications of 25 µl were performed using 1 µl of purified DNA solution with the AmpliTaq Gold PCR Master Mix (Roche). After preincubation at 95 °C for 8 min, amplifications were carried out in a GeneAmp PCR system 2400 (Applied Biosystems) for 35 cycles, each with 30 s denaturation at 95 °C, 1 min annealing at 55 °C and 30 s extension at 72 °C. The final elongation step at 72 °C was for 10 min. Bacterial universal primers (27F -AGAGTTTGATCCTGGCTCAG 1492R-GGTTACCTTGTTACGACTT) was used (Lane, 1991). The amplification product was separated on a 1.5% agarose gel and electrophoresis at 80 V for 120 min. The DNA bands were visualized by ethidium bromide staining. All PCR products (fragments of about 1400g bp on the agarose gel) were purified using the QIAquick PCR Purification Kit (QIAGEN®) and sent for sequencing. After sequencing was completed, the chimeras within the DNA sequences were trimmed by ChromasPro 2.1.8 software.

The remaining DNA sequences were identified by the FASTA search (Pearson, 1990) in the NCBI GenBank database where the 16S rRNA gene sequences of all the strains were deposited and their accession numbers obtained (http://blast.ncbi.nlm.nih.gov/blast.cgi).

2.9. Statistical analysis

The results obtained were expressed as a mean ± standard deviation and then analysed by the Analysis of Variance (ANOVA) test using Minitab 18 software, followed by comparisons of the means between them by the Fisher test at the 0.05 probability threshold. Using the XLSTAT 2007 software, the principal component analysis (PCA) was carried out to determine which isolates of LAB showed the best activities for all the evaluated quantitative parameters.

3. Results

3.1. Generic identification

A total of One hundred and sixteen rod-shape, gram-positive and catalase-negative bacteria were isolated from 56 samples of fruits (banana, papaya, orange and pineapple). Then 62 of them have shown their ability to produce CO_2 and they were classified in the genus *Lactobacillus* group III. The rest of 54 LAB isolates were unable to produce CO_2 and they showed growth at temperatures of 10 °C and 45 °C. Thus, the latters are similar to optional facultative heterofermentative LAB and have been classified in the genus *Lactobacillus* group II according to Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986).

3.2. Screening of acidifying isolates

Among the 54 homofermentative LAB isolates tested, only 9 showed the best activities (ΔpH greater than 1.5 after 6 h of incubation) represented in Table 1. It appears that acidification varies from one isolate to another. In fact, in the MRS-glucose medium, the 4O2 isolate has the largest ΔpH which is 2.035 ± 0.00; a significantly higher value than those recorded with other cultures ($p < 0.05$). However, in MRS-Fructose broth, isolate 4O8 is in the lead with a ΔpH of 1.57 and is especially significantly different from those of other crops ($p < 0.05$). In the MRS-Sucrose medium, the most important ΔpH (2.285 ± 0.01) was that resulting from the activity of 4O2 and O22; moreover, no significant difference was observed between this value and those of isolates O31 and O25 ($p > 0.05$).

3.3. Exopolysaccharide production

The production of EPS by lactic acid bacteria is a phenomenon that is favorable to many industrial food processes. As part of our work, all LAB isolates were able to develop on MRS-Glucose, MRS-Fructose, MRS-Sucrose, MRS-Lactose agar. However, there was a total absence of large, sticky or mucoid colonies, but also an absence of colonies that form a long visible filament as soon as they were touched and lifted with a sterile loop. These isolates do not produce EPS in the form of extracellular mud.

Table 1. pH variation caused by LAB isolates in MRS-Fructose, MRS-Sucrose, and MRS-Glucose broth.

Isolates	MRS-Fructose	MRS-Sucrose	MRS-Glucose
	ΔpH (pH_{t0h}–pH_{t6h})		
1B9	1.53 ± 0.01^{d}	2.225 ± 0.02^{cd}	1.935 ± 0.00^{c}
4O2	1.425 ± 0.00^{f}	2.285 ± 0.01^{a}	2.035 ± 0.00^{a}
3A5	1.545 ± 0.00^{c}	2.240 ± 0.01^{bc}	1.89 ± 0.01^{e}
4O8	1.575 ± 0.00^{a}	2.250 ± 0.01^{bc}	1.875 ± 0.00^{f}
O23	1.545 ± 0.00^{c}	2.230 ± 0.03^{bcd}	1.895 ± 0.00^{e}
3A9	1.505 ± 0.00^{e}	2.200 ± 0.00^{d}	1.785 ± 0.00^{g}
O25	1.555 ± 0.00^{b}	2.260 ± 0.01^{ab}	1.955 ± 0.00^{b}
O31	1.505 ± 0.00^{e}	2.255 ± 0.01^{abc}	1.950 ± 0.01^{b}
O22	1.545 ± 0.00^{c}	2.285 ± 0.01^{a}	1.910 ± 0.01^{d}

^{a,b,c,d,e,f,g} The values with different letters on the same column differ significantly each other ($p < 0.05$).

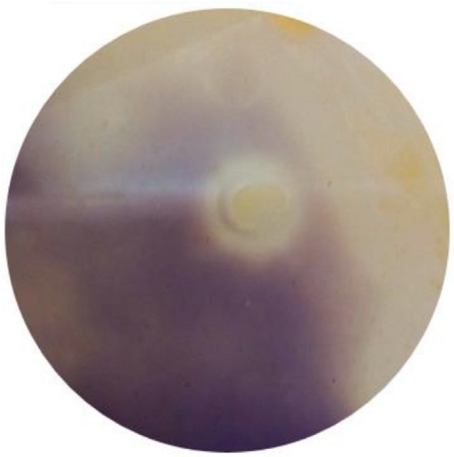


Figure 1. Starch hydrolysis on MRS-starch Agar plate showing clear zones.

3.4. Amylolytic activity

The results of the amylolytic activity on agar media are presented in Table 2. It appears that all the isolates were able to grow on an MRS-Starch medium. The presence of a clear halo around the colonies as illustrated in Figure 1 indicates their amylase production. The 4O2 isolate showed the greatest amylolytic activity on agar medium with a clear zone diameter of about 3.9 ± 0.10 mm; significantly different from those of the other isolates ($p < 0.05$). Also, the results obtained during the evaluation of amylase activity of enzymes from the different LAB isolates are grouped in Table 2. It appears that amylase from isolate O31 showed the most important activity with a production of 54.26 ± 0.10 µg of maltose per ml of solution for one minute: values significantly different from those of other amylases ($p < 0.05$).

3.5. Cellulolytic activity

The ability to degrade cellulose compound in a solid medium (Figure 2) was observed in all 9 isolates tested. Indeed, the activity (Table 3) varied between 0.5 ± 0.35 mm and 3 ± 0.35 mm. The results obtained during the evaluation of the cellulase activity of enzymes from the different LAB isolates are grouped in Table 3. It shows that the amylase activity varies from one LAB isolation biotope to another. Indeed cellulase from isolate 1B9 showed the most significant activity with a production of 7.98 ± 0.40 µg of glucose per ml of solution for one minute. This value was significantly different from those obtained by cellulase from other isolates ($p > 0.05$).

3.6. Determination of the chemical proximate composition of fruits-derived by-products

The results obtained when determining the chemical proximate composition of the different fruits by-products are summarized in Table 4. The results show that the levels of dry matter (DM), fat, protein, carbohydrate, ash and crude fibre vary from one sample to another. The banana peels have the highest fat (16.08 ± 0.02%), and protein content (1.54 ± 0.06%) which are significantly different from those of the other samples ($p < 0.05$). As for ash, the most important value was observed in banana-derived by-products although no significant difference was observed between this value and that of pineapple peels ($p > 0.05$). Also,

Table 2. Diameter of clear areas around colonies and enzymatic activity.

Isolates	Diameter of clear areas (mm)	Enzymatic activity (µg of reducing sugars/ml solution/min)
1B9	3.00 ± 0.00[b]	10.99 ± 0.10[c]
4O2	3.90 ± 0.10[a]	9.9 ± 0.00[c]
3A5	1.50 ± 0.00[d]	7.01 ± 0.00[d]
4O8	2.00 ± 0.00[c]	9.62 ± 1.94[c]
O23	2.30 ± 0.20[c]	1.94 ± 0.10[b]
3A9	1.95 ± 0.07[c]	3.02 ± 0.10[e]
O25	2.70 ± 0.20[b]	3.43 ± 0.10[e]
O31	2.20 ± 0.10[c]	54.26 ± 0.10[a]
O22	3.05 ± 0.07[b]	6.46 ± 0.10[d]

[a,b,c,d,e,f,g] The values with different letters on the same column differ significantly each other ($p < 0.05$).

Figure 2. Clear zone around the wells reflecting activity of 1B9 isolatecellulase on CMC -agar.

pineapple peels has the highest carbohydrate content (71.94 ± 2.02%), which is significantly different from that of other fruit peels ($p < 0.05$), and the same applies to the crude fibre content.

3.7. Lactic acid production from fruit-derived by-products

The production of lactic acid by homofermentative LAB is one of their main activities when they are in the presence of a useable carbohydrate source. In the present study where homofermentative LAB isolated from tropical fruits were used to perform batch fermentation over a 48 h period from by-products, the LA production kinetics (Figures 3A, 4A, 5A) varied from one by-product to another and from one isolate to another. In general, the kinetics showed a peak at 16h. Indeed the evolutions of the reducing sugars content in the different production mediums during 48 h are represented by Figures 3B, 4B and 5B and there is a progressive decrease in the concentration of reducing sugars over time.

The highest lactic acid concentrations were obtained in the production media consisting of banana (26.37 ± 0.05 g/l) and pineapple (26.29 ± 0.38 g/l) by-products. In fact, the isolate 1B9 recorded the best production of LA in media consisting of banana by-products with a volumetric productivity of about 1.65 ± 0.00 g/l.h (Table 5) which was not significantly different from those obtained with the isolate O31 ($p > 0.05$). However, in the fermentation broth made from pineapple by-products, the 3A5 isolate was the best with a volumetric production of 1.64 ± 0.02 g/l.h (Table 5). In addition, no significant difference was observed between the LA production of the latter and that of the 3A9 and 1B9 isolates ($p > 0.05$). In our study, using papaya by-products for fermentation, the isolate O23 stood out from the others with a volumetric production of LA of 1.61 ± 0.00 g/l.h (Table 5), it's production was significantly different from that of all other isolates ($p < 0.05$). The present study also shows that the production medium made from pineapple by-products is still the best because in the latter we recorded average volumetric productivity of 1.59 ± 0.34 g/l.h although it is not significantly different from that obtained in the fermentation broth made from banana by-products (1.55 ± 0.05 g/l.h) ($p < 0.05$).

3.8. Principal component analysis (PCA) of technological characteristics of isolates

Eight variables were considered during PCA, included cellulase activity (CA), amylase activity (AMA), LA volumetric productivity in the production media made from pineapple by-products (PVMPI), LA volumetric productivity in the production media made from papaya by-products (PVMPA), LA volumetric productivity in the production media made from banana by-products (PVMB), LA yield in the production media made from pineapple by-products (YMPI); LA yield in the production media made from papaya by-products (YMPA) and LA yield in the production media made from banana by-products (YMB). In the case of our study, 2 components F1 and F2, representing the axes, were chosen according to Kaiser's Guttman rules. The contribution of the variables for the formation of the main axes is presented in Table 6. It can be seen that the YMB, PVMB, PVMPI and YMPI contributed the most to the formation of the F1 (explaining 43.43% variability). On the other hand, axis F2 is formed by the contributions of the PVMPA, AMA, YMPI and the CA (explaining 24.67% variability). However, YMPA and AMA contributed the most to the formation of the F1 and F3 (explaining 14.9% variability). This relationship is clearly represented on the projection of the variables onto the plane formed by the two selected axes (Figure 6). Thus, axis F1 is related to the technological characteristics as well as

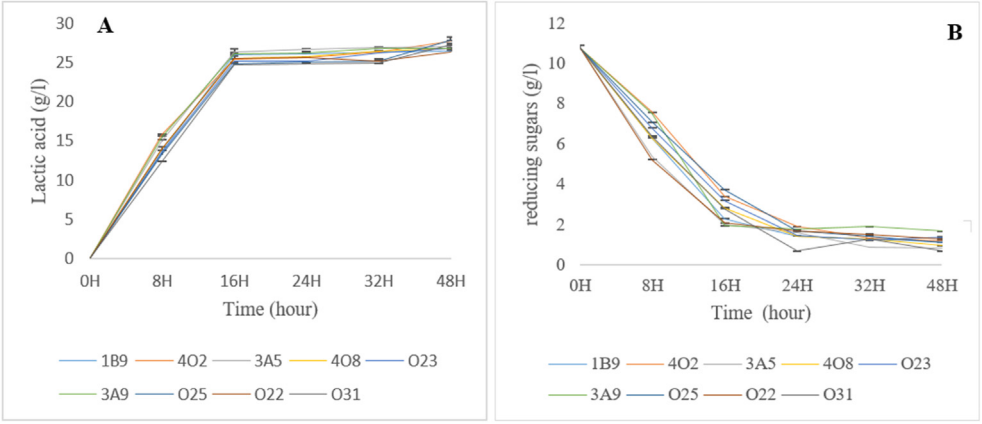


Figure 3. Time course of LA production (A) and reducing sugars consumption (B) of isolates in the broth medium containing pineapple by-products as carbon source.

Table 3. Diameter of lysis zone around wells and enzymatic activity.

Isolates	Diameter of clear areas (mm)	Enzymatic activity (µg of reducing sugars/ml solution/min)
1B9	3.00 ± 0.35^{a}	7.98 ± 0.40^{a}
4O2	0.50 ± 0.00^{e}	0.14 ± 0.00^{g}
3A5	1.50 ± 0.71^{cd}	4.95 ± 0.00^{c}
4O8	2.70 ± 0.42^{ab}	6.47 ± 0.00^{b}
O23	1.75 ± 0.00^{c}	1.80 ± 0.00^{e}
3A9	2.00 ± 0.00^{bc}	0.62 ± 0.10^{f}
O25	1.00 ± 0.00^{de}	0.34 ± 0.10^{fg}
O31	2.70 ± 0.42^{ab}	0.21 ± 0.10^{g}
O22	2.00 ± 0.00^{bc}	2.5 ± 0.00^{d}

^{a,b,c,d,e,f,g} The values with different letters on the same column differ significantly each other ($p < 0.05$).

PVMB while axis F2 is related to PVMPA. Indeed, this figure also shows that the PCA allowed the separation of the observations into 4 main groups. The first group consisting of the O31 isolate is positioned on the positive side of the F1 and F 2 axes and it is characterized by high values of AMA, PVMB and YMB. The second group contains the isolates 1B9, 3A5, 4O8 and 3A9 and these are found on the positive side of the F1 axis and the negative side of the F2 axis; they are characterized by high values of CA, PVMPI, YMPI, PVMPA and YMPA. However, the 4O2, O22 and O23 isolates that are combined in group 3 are on the negative side of the F1 and F2 axis. In group 4 only the isolate O25 is present and this is positioned on the negative side of the F1 axis and on the positive side of the F2 axis.

3.9. Molecular identification of selected isolates

Following PCA, the best isolates were (O31, 1B9, 3A5, 3A9 and 4O8) were identified at the molecular level using the 16S rRNA gene sequencing (Table 7). The latter belong to the *Lactobacillus plantarum* species with more than 98.62% identify. The accession numbers of the nucleotides assigned by the NCBI GenBank database goes from MW164845 to MW164849.

4. Discussion

In our study, 54 homofermentative lactobacilli were obtained from fruits (banana, papaya, orange and pineapple) they were classified in the genus *Lactobacillus* group II according to Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986). In fact, group II Lactobacilli can be heterofermentative depending on the substrate and they only produce lactic acid in the presence of sugars with 6 carbons atoms (Paul et al., 2009) especially glucose and fructose which are widely represented in the vegetal kingdom such as fruits (Phatcharaporn et al., 2009; Chukwuka et al., 2013; Maria et al., 2014). Indeed tropical fruits, because of its composition in carbohydrates, minerals, vitamins and nitrogen compound, is a favorable environment for the growth of microorganisms. Several scientific works have already revealed the presence of microorganisms, in particular lactic acid bacteria (Lactobacilli) in tropical fruits (Ram et al., 2013; Todorov et al., 2011; Maryam and Wedad, 2017). It is therefore to maximize the yield of lactic acid production on the one hand and to avoid multiple purifications on the other hand that the choice was made on homofermentative isolates for further work.

The ability of lactic acid bacteria to metabolize different carbohydrates present into a by-product which will be used to produce lactic acid through fermentation is of paramount importance. All 9 preselected isolates showed greater acidifying activity (ΔpH) in MRS-Sucrose compared to the other two media (MRS and MRS-Fructose). This could be explained by their ability to produce sucrase that allows them to obtain two moles of reducing sugars (glucose and fructose) from one mole of sucrose and it leads to four moles of lactic acid (José et al., 2020). These observations are similar to those that obtained by Yağmur et al. (2014) who reported in their work that the most important quantity of LA produced by *Lactobacillus brevis* was obtained when the carbon source in the fermentation medium was sucrose. Thus, the acidifying activity of lactic acid bacteria would not only be dependent on the strain but would also depend on the sugar present in the environment. The high acidifying activity can also be linked to the absence of EPS production.

In fact, the main advantage of using EPS-producing LAB, particularly those in the form of extracellular sludge (high molecular weight EPS) detached from the bacterial membrane in the manufacture of fermented dairy products is the improvement of texture, the reduction of the

Table 4. Chemical proximate composition of fruits peels.

Samples	Dry matter (% DM)	Fat (% DM)	Protein (% DM)	Ash (% DM)	Carbohydrate (% DM)	Crude Fibre (% DM)
Pineapple peels	93.48 ± 0.09^{a}	8.65 ± 0.16^{c}	0.40 ± 0.06^{c}	12.5 ± 2.12^{a}	71.94 ± 2.02^{a}	32.98 ± 0.62^{a}
Papaya peels	83.00 ± 0.26^{c}	15.12 ± 0.5^{b}	0.57 ± 0.06^{b}	4.5 ± 0.71^{b}	62.81 ± 0.15^{b}	25.34 ± 1.38^{b}
Banana peels	91.87 ± 0.24^{b}	16.08 ± 0.02^{a}	1.54 ± 0.06^{a}	12.5 ± 0.71^{a}	61.75 ± 0.79^{b}	24.57 ± 1.01^{b}

^{a,b,c,} The values with different letters on the same column differ significantly each other ($p < 0.05$).

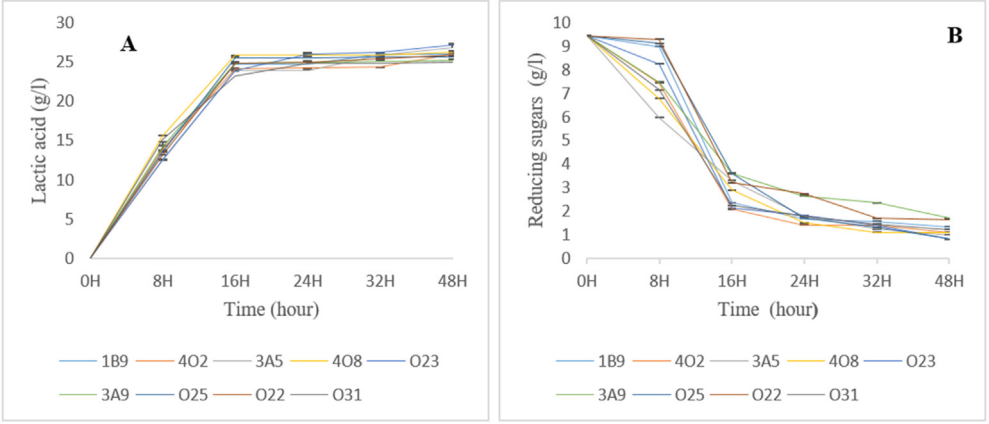


Figure 4. Time course of LA production (A) and reducing sugars consumption (B) of isolates in the broth medium containing papaya by-products as carbon source.

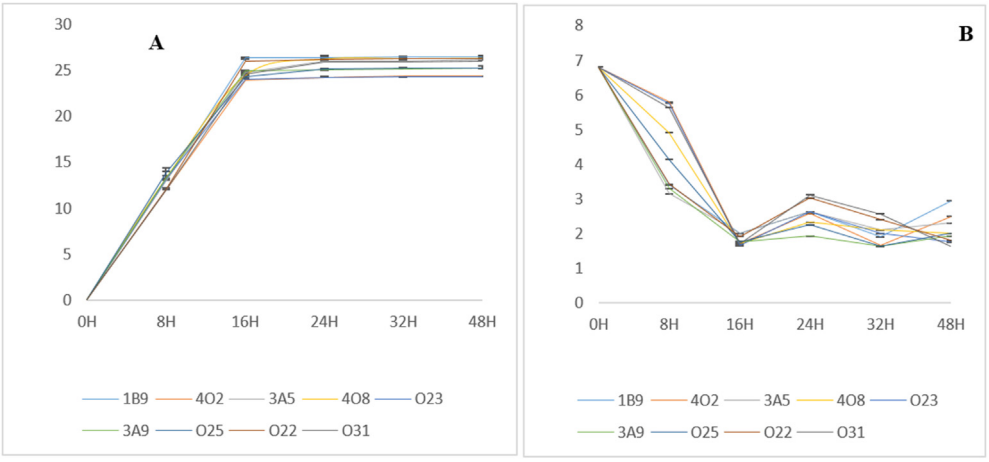


Figure 5. Time course of LA production (A) and reducing sugars consumption (B) of isolates in the broth medium containing banana by-products as carbon source.

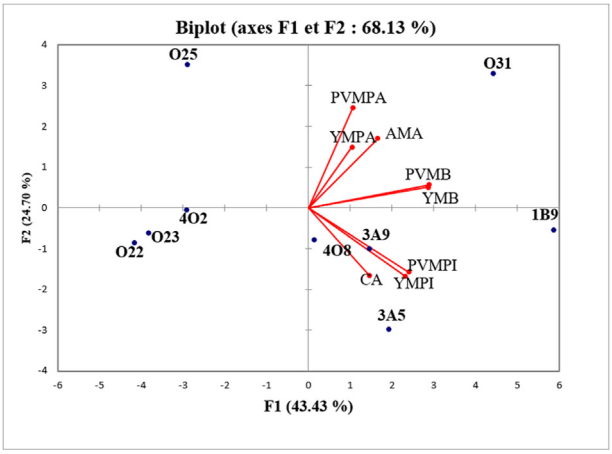


Figure 6. Projection of the variables and LAB isolates onto the plane formed by the two factors obtained from PCA.

syneresis and increased product viscosity (Silvia and Medana, 2011). However, the use of LAB isolates capable to produce EPS in the form of extracellular mud is not appropriate for industrial lactic acid production. Because during their metabolism, these LAB isolates use a significant part of sugars in particular the sucrose (Krajl et al., 2003; Paul et al., 2009) present in the fermentation medium for the production of EPS and it leads to the reduction of the LA yield. So, that's why in the context of our work, this property was not sought after. Indeed, all our 9 preselected isolates do not produce EPS in the form of extracellular mud. These results are in agreement with those of Silvia-Simona and Medana (2014) who showed in their work that among 21 isolates of lactic acid bacteria isolated from vegetables, only five of them were able of producing EPS in MRS-gelose in which glucose had been substituted by sucrose (50 g/l). This lack of activity could be explained either by the absence of the genes involved in the synthesis of the enzymes responsible for this activity or by a total absence of expression of these different genes. Indeed, Roel et al. (2007) showed that several lactic acid bacteria possess the genes involved in the production of EPS; however, they do not express them.

In ripe fruit such as banana, sucrose is found in the majority and starch in a small proportion (Thomas et al., 2008). The optimal exploitation of the by-products from this fruit for the production of LA requires LAB isolates that can transform these sugars into LA. This is the reason why in our study LAB isolates were evaluated for their ability to metabolize starch. As part of our work, amylase activity varies from one isolate to another. The same observation has been reported by Hattingh et al. (2015) in their studies. Amylase from isolate O31 showed the most important activity which was the production of 54.26 µg maltose per ml of solution for one minute. Indeed, this activity is higher than that obtained by Anteneh and Prapulla (2015) as part of their work, which was 15 µg/ml/min. The differences in amylase activity observed between the different isolates tested could be due to the amount of amylase produced by each isolate as reported by Liang et al. (2014), but also to physico-chemical conditions (pH, temperature) that were not favorable to the optimal activity of the amylase produced. Indeed, Tchekessi et al. (2014)

Table 5. Lactate, volumetric productivity and lactic acid yield after 16 h of fermentation.

Isolates	PIB			PAB			BAB		
	Lactate (g/l)	VP (g/l.h)	Lactic acid Yield (mg/g)	Lactate (g/l)	VP (g/l.h)	Lactic acid yield (mg/g)	Lactate (g/l)	VP (g/l.h)	Lactic acid yield (mg/g)
1B9	25.91 ± 0.1^{ab}	1.62 ± 0.00^{ab}	51.82 ± 0.14^{ab}	24.71 ± 0.01^{c}	1.54 ± 0.00^{c}	49.41 ± 0.014^{c}	26.37 ± 0.05^{a}	1.65 ± 0.00^{a}	52.73 ± 0.10^{a}
4O2	25.37 ± 0.1^{c}	1.59 ± 0.0^{c}	50.74 ± 0.14^{c}	24.13 ± 0.04^{d}	1.51 ± 0.00^{d}	48.26 ± 0.1^{d}	23.93 ± 0.25^{f}	1.5 ± 0.02^{f}	47.85 ± 0.5^{f}
3A5	26.29 ± 0.38^{a}	1.64 ± 0.02^{a}	52.58 ± 0.74^{a}	23.9 ± 0.14^{d}	1.49 ± 0.01^{d}	47.80 ± 0.3^{d}	24.8 ± 0.00^{bc}	1.55 ± 0.00^{bc}	49.6 ± 0.00^{bc}
4O8	$25.55 \pm 0.04b^{c}$	1.6 ± 0.002^{c}	51.10 ± 0.10^{c}	25.81 ± 0.01^{a}	1.61 ± 0.00^{a}	51.61 ± 0.01^{a}	24.4 ± 0.14^{cde}	1.525 ± 0.01^{cde}	48.8 ± 0.30^{cde}
O23	25.16 ± 0.1^{cd}	1.57 ± 0.00^{cd}	50.32 ± 0.14^{cd}	23.9 ± 0.00^{d}	1.49 ± 0.00^{d}	47.8 ± 0.00^{d}	24.05 ± 0.01^{ef}	1.50 ± 0.00^{ef}	48.10 ± 0.14^{ef}
3A9	26.13 ± 0.11^{a}	1.63 ± 0.01^{a}	52.26 ± 0.23^{a}	24.77 ± 0.04^{c}	1.51 ± 0.00^{c}	49.54 ± 0.1^{c}	24.92 ± 0.12^{b}	1.56 ± 0.01	49.83 ± 0.24
O25	24.77 ± 0.2^{de}	1.55 ± 0.01^{de}	49.54 ± 0.4^{de}	25.5 ± 0.14^{b}	1.59 ± 0.01^{b}	51.00 ± 0.3^{b}	24.28 ± 0.2^{def}	1.52 ± 0.012^{def}	48.56 ± 0.4^{def}
O31	25.52 ± 0.27^{bc}	1.6 ± 0.02^{bc}	51.04 ± 0.54^{bc}	24.85 ± 0.21^{c}	1.55 ± 0.01^{c}	49.70 ± 0.42^{c}	26.02 ± 0.25^{a}	1.63 ± 0.02^{a}	52.04 ± 0.51^{a}
O22	24.75 ± 0.1^{e}	1.55 ± 0.00^{e}	49.5 ± 0.14^{e}	23.2 ± 0.14^{e}	1.45 ± 0.01^{e}	46.40 ± 0.28^{e}	24.55 ± 0.28^{bcd}	1.53 ± 0.02^{bcd}	49.09 ± 0.55^{bcd}
Mean	**25.49 ± 0.55**	**1.59 ± 0.034**	**51 ± 1.10**	**24.53 ± 0.83**	**1.53 ± 0.05**	**49.06 ± 1.66**	**24.81 ± 0.85**	**1.55 ± 0.05**	**49.62 ± 1.70**

[a,b,c,d,e,f,g] The values with different letters on the same column differ significantly each other ($p < 0.05$).**VP**: Volumetric productivity; **PIB:** Pineapple by-product broth; **PAB**: Papaya by-product broth; **BAB**: Banana by-product broth.

Table 6. Contribution of the variables to the factors in the PCA based on correlation.

Variables	F1	F2	F3	F4	F5
AMA	0.274	0.291	0.228	0.029	0.163
CA	0.212	0.276	0.055	0.421	0.015
PVMPI	0.576	0.247	0.028	0.147	0.000
PVMPA	0.112	0.601	0.147	0.006	0.108
PVMB	0.837	0.033	0.068	0.036	0.019
YMPI	0.534	0.280	0.033	0.151	0.001
YMPA	0.109	0.223	0.562	0.003	0.086
YMB	0.819	0.025	0.079	0.048	0.018

Table 7. The 16S rRNA sequencing identification of benefit LAB isolates.

Isolates code	Origin of isolates	16S rRNA sequencing identification	Sequence length (bp)	% query coverage	% max identity	Accession numbers
O31	Orange	*Lactobacillus plantarum*	200	100%	100%	MW164846
4O8	Orange	*Lactobacillus plantarum*	488	100%	100%	MW164849
3A5	Pineapple	*Lactobacillus plantarum*	587	100%	100%	MW164845
3A9	Pineapple	*Lactobacillus plantarum*	500	100%	98.62%	MW164848
1B9	Banana	*Lactobacillus plantarum*	574	100%	100%	MW164847

showed in their work that pH and temperature had a definite influence on the amylase activity of lactic acid bacteria; they highlighted the reality that each amylase could only have an optimal amylase activity at its own pH and temperature. However, the isolation biotope of the LAB would also have a definite influence on its amylase activity (Essozimna et al., 2017). Just like starch, cellulose can be exploited for LA production if it is in the presence of a LAB able to metabolize it.

Saowapar et al. (2014) and Herdian et al. (2018) in the course of their work showed that the cellulase activity of LAB not only varied between isolates from different biotopes but also varied between isolates from the same biotope. Similar data were observed in our study and cellulase from isolate1B9 showed the most significant activity with a production of 7.98 μg of glucose per ml of solution for one minute. The differences in cellulase activity observed between the isolates tested could be due to the nature and the origin of LAB isolates, to the quantity of cellulase produced, but also to the physicochemical conditions. In fact, the same observation had been reported in several previous works (Basavaraj et al., 2014; Saowapar et al., 2014; Tchekessi et al., 2014). Cellulase activity, amylase activity and non-production of EPS are useable properties of lactic acid bacteria that can be useful in the production of LA using by-products coming from fruits.

In the context of our study, the chemical proximate composition of the different fruits (papaya, banana and pineapple) by-products varied from one sample to another; Feumba et al. (2016) have reported the same data. In addition, Maria et al. (2014), showed that 100 g of pineapple (*Ananas comosus*) peels from Mexico contain on average 2 g of crude fat; 0.75 g of protein; 1.5 g of ash; 65 g of crude fibre. The fat and ash contents obtained in this study are higher than those in the literature, while the protein and fibre contents are respectively close to and lower than those obtained by these authors. The peels of the *Carica papaya L* variety were the subject of a study conducted by Chukwuka et al. (2013) in Nigeria. It revealed that 100 g of this substrate contained an average of 13.67 g of crude fiber; 4.84 g of ash; 9.04 g of protein; 0.31 g of lipid and 27.87 g of carbohydrate. The ash (4.5%) and crude fibre (25.3%) contents obtained during this work on papaya (*Carica papaya L)* peels are relatively close to those obtained by these authors. However, the protein content (0.57%) is lower than that reported by these authors; indeed, the lipid (15.12%) and total sugar (62.81%) contents obtained during our work are higher. Within the framework of our work, bromatological analysis of the banana (*Musa Sapientium*) peels showed that it contains an average of 16.08% fat and 12.5% of ash. These results are very close to those obtained by Phatcharaporn et al. (2009) who revealed in their work that 100g of banana peels of the *Musa ABB* variety in Thailand is made up of 13.1g of lipid and 15.25g of ash. Furthermore, the protein (1.54%) and fibre (24.57%) contents reported by our analyses are much lower than those revealed by these authors in their work, which was 8.6% for protein and 50.25% for fibre respectively. The differences in the contents of these above-mentioned components in fruit peels could be because the chemical composition of these substrates is dependent on the environment and therefore on geographical location. Furthermore, the chemical composition of these substrates would vary from one fruit variety to another. Similarly, the cultivation practices employed, which vary from one farmer to another, could have a significant influence on the levels of these elements in these matrices (Leterme et al., 2006; Feumba et al., 2016). With regard to the composition of these by-products from fruits, they can thus be exploited in the LA production.

The present work also aimed to assess the ability of lactic acid production of *Lactobacillus* Sp. from fruits using their by-products. In fact, our study showed that LA production kinetics varied from one by-product to another and from one isolate to another. Similar results were reported by Pushparani et al. (2012) after 6 days of fermentation. In the framework of our study the LA production was generally constant after 16h; this was probably due to the decrease in the quantity of reducing sugars in the fermentation medium as reported by Ranjit and Srividya, and Xiang-Yang et al. (2014). Also, excess of LA in the medium during fermentation, the production of lactic acid resulting from the transformation of sugars induces the lowering of the pH of the medium. In fact, if the pH of the medium is too acidic, and if the membrane of the LAB is made permeable to protons, the internal pH of the LAB decreases causes the stop of growth and metabolism. This pH is called critical pH and in Lactobacilli it is 4 (Kandler and Weiss, 1986). The maximum concentrations of LA obtained during fermentation are much higher than the amount of reducing sugars present in the production medium at the start of fermentation. This is due to the saccharification of carbohydrate substrates by the LAB isolatesespecially oligosides and polysaccharides still present in the production medium, as revealed by the work of Yáñez et al. (2003). Indeed, fruits such as banana and papaya contain significant amounts of sucrose, which coming from the processing of starch during the ripening of these fruits (Thomas et al., 2008), so this carbohydrate would logically be found in the by-products of these fruits. Thus the consumption of one mole of this sugar present in the production medium by the LAB isolates would lead to four moles of lactic acid (José et al., 2020). However, the presence of sucrose in the production medium after acid hydrolysis implies evidence that the concentration of 2N HCl (1%) used in the acid treatment was not sufficient to hydrolyze all the sucrose to release glucose and fructose. Also, the LAB isolate used to carry out

these fermentations have shown their ability to hydrolyze certain holosides, particularly cellulose, starch and sucrose. In the present study, the best LA production (26.37 g/l) was obtained in the production medium where banana by-products were used as carbon source; indeed, this activity was recorded by a strain isolated from banana, namely *Lactobacillus plantarum* 1B9. Thus, a strain of lactic acid bacteria isolated from a specific biotope would better ensure the bioconversion of by-products of this biotope into metabolites of interest (Naresh et al., 2019). The maximum concentrations recorded in media consisting of pineapple (26.29 g/l) and banana (26.37 g/l) by-products are significantly higher than those obtained by Mridul and Preethi (2014) who, in their studies, used a *Lactobacillus plantarum* strain and were able to produce 4.68 g/l of LA from these two by-products; and yet they could have even supplemented their production media with a significant amount of MRS and fermented in bioreactors over a period of 6 days. Using papaya fruit by-products, we obtained in our work a volumetric production of LA of 1.61 g/l.h (O23). Moreover, this production is much higher than that obtained by Ranjit and Srividya (2014) where in the framework of their work were able to obtain volumetric productivity of about 0.94 g/l.h from papaya by-products by optimizing the production in bioreactor over 72h and by using a strain of fungus (*Rhizopus oryzae* MTCC 8784) as fermentation agent. In the present study, the best VP (1.64 g/l.h; 1.65 g/l.h and 1.65 g/l.h) obtained in the PIB, PAB and BAB respectively are much important than the one that obtained (0.121 g/l.h) by Ali et al. (2012) who, in the course of their work, produced LA from mango peel waste using a factorial experiment. From these observations, it follows that the acidifying power could be dependent on the strain and experimental conditions; similarly, it would be equally influenced by the duration of fermentation and the nature of the substrate present in the production medium (Wee et al., 2006; Battula et al., 2018; Abedi and Hashemi, 2020). However, the chemical composition of the fruit by-products used during fermentation, the nature of the treatments to which the different carbohydrate substrates are subjected, the composition of the fermentation medium, would influence the amount of lactic acid produced and would be responsible for the differences observed between our acidity values and those in the literature.

Our study shows that the production medium made from pineapple by-products was still the best because in the latter we recorded average volumetric productivity of 1.59 g/l.h; this can be simply explained by the concentration of reducing sugars present in the fermentation broth at the beginning of the fermentation. In fact, beginning of fermentation, the production medium made from pineapple contained the most important concentration of reducing sugars. This observation could also have its origin in the total sugars content of this matrix. Also, the analysis of the chemical proximate composition of the different substrates used to carry out the fermentation during our study revealed that pineapple by-products had the highest total sugar content (71.94%). Similar observations were reported by Coelho et al. (2011) who found that the amount of lactic acid produced during the fermentation was relative to the concentration of sugars present in the production medium at the beginning of the fermentation process.

In the present research, PCA was useful to select the most promising LAB tested according to technological characteristics. LAB isolate O31 was retained as the isolate with the best technological characteristics, followed by LAB isolates 1B9, 3A5, 3A9 and 4O8, then LAB isolate O25 and finally, LAB isolates 4O2, O23 and O22. As the result of this selection, the best LAB isolates were identified at the molecular level.

LAB are an integral part of the microbial flora of tropical fruits. In fact Bacteria of the genus *Lactobacillus pentosus*, *Lactobacillus plantarum* ST16Pa had been respectively isolated from banana and papaya (Maryam et al., 2017; Todorov et al., 2011). In addition, a study conducted on the microflora of pineapple and orange also showed that the latter were ecological niches of BAL (Maria, 2012; Ram et al., 2013). In the context of our study, the molecular identification of the five best isolates (O31, 1B9, 3A5, 3A9 and 4O8) showed that they belong to the species *Lactobacillus plantarum*. All these fives strains belong to the genus *Lactobacillus* group II and it confirms results that we previously obtained during generic identification of pre-selected LAB isolates. These results are in agreement with the literature, indeed, the lactic acid bacteria of the *plantarum* species belong to the plant microbiota, hence the name *plantarum* (Sneath et al., 1986).

5. Conclusion

Based on our investigation, it appears that the tropical fruits collected in the city of Dschang (West Cameroon) contained lactobacilli belonging to the species *Lactobacillus plantarum*. They have properties of commerical interest including amylase, cellulase and acidifying activities which vary from one stain to another. Regarding the production of LA, this not only depended on the strain considered but also on the composition of the production medium which was closely linked to the nature of carbohydrate substrate used. In addition, the best production medium was the one containing pineapple by-products as carbon source as the highest average VP was recorded there. Based on PCA, the most promising strains for the production of LA are in order *Lactobacillus plantarum* O 31, *Lactobacillus plantarum* 1B9, *Lactobacillus plantarum* 3A5, *Lactobacillus plantarum* 3A9 *and Lactobacillus plantarum* 4O8Consequently, these LAB strains can be exploited in the valorization of by-products from tropical fruits through the production of lactic acid at the industrial level. However, for a large scale production of LA from these by-products, further investigations should be oriented towards the screening of inexpensive nitrogen sources to replace yeast extract, coupled with the optimization of the fermentation process in a bioreactor.

Declarations

Author contribution statement

Joel Romial Ngouénam, Chancel Hector Kenfack Momo, Edith Marius Kouam Foko, Pierre Marie Kaktcham, Rukesh Maharjan, François Zambou Ngoufack: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the Third World Academy of Sciences (TWAS) and the International Center of Chemical and Biological Sciences (ICCBS) (University of Karachi, Pakistan) grant FR number: 3240299154.

Data availability statement

Data will be made available on request.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors express their gratitude to Miss Bilkissou Japndounke, Mr. Laverdure Piame Tchamani and Mr. Ulrich Daquain Fotso for their contribution during the work in the laboratory as well as during the writing, analysis and discussion of the results.

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