Effective biorefinery approach for lactic acid production based on co-fermentation of mixed organic wastes by *Enterococcus durans* BP130

Saad El-Din Hassan[a,1], Mohamed Ali Abdel-Rahman[a,*,1], Mohamed M. Roushdy[a,b], Mohamed Salah Azab[a], Mahmoud Ali Gaber[a]

[a] *Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, P.N.:11884, Nasr City, Cairo, Egypt*
[b] *Department of Biology, Faculty of Sciences and Arts-Khulais, University of Jeddah, Jeddah, Saudi Arabia*

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ABSTRACT

Various challenges facing the industrial manufacture of biobased lactic acid (LA) have been occurred. These include substrate and/or pre-treatments cost, medium cost, and contamination risk as a result of utilizing neutral pH and mesophilic conditions. Therefore, this study focused on development of consolidated bioprocess for LA production from organic wastes under harsh conditions. A newly isolated thermo-alkali-tolerant lactic acid bacterium, *Enterococcus durans* BP130, was firstly used for direct LA production from banana peels (BP). Increasing the solid concentration of BP from 4.0% to 6.7% [w-dry matter/v] have resulted in increased fermentation efficiency with the highest LA production ($19.2 \, g \, L^{-1}$) at 6.7% [w-dry matter/v]. Besides, co-fermentation of BP and food waste (FW) materials resulted in 13% improvements in LA concentration and 12% in LA productivity at 0.5:1 of FW:BP (w/w). Increasing of food waste concentrations could replace the nutritional components of the media and achieved increased LA production. Without any nutrients supplementation, food waste substituted the medium components and resulted in $14.3 \, g \, L^{-1}$ of LA produced using mixed substrates of FW:BP at 3:1 (w/w). The highest LA concentration of 28.8 $g \, L^{-1}$ with a yield of $0.85 \, g \, g^{-1}$-consumed sugar and productivity of $0.24 \, g \, L^{-1} \, h^{-1}$ was achieved in batch fermentation using $CaCO_3$ as a neutralizing agent. This study introduced effective mixed waste-based biorefinery process for direct LA production in an inexpensive fermentation system.

1. Introduction

In the recent decades, bioconversion of organic-rich wastes such as lignocelluloses and food wastes into organic acids has gained great interest. This is mainly attributed to its availability, sustainability, cost-effectiveness processes, as well as its environmental impacts (Campanhol et al., 2019; Cizeikiene et al., 2018; Liu et al., 2018). Of these products, lactic acid (LA) is an important biorefinery platform chemical that has also gained a lot of interest and increased worldwide demand. It has various applications in food, cosmetic, medical and pharmaceutical industries, in addition to its use a precursor for manufacturing of eco-friendly biodegradable plastic materials (poly-lactic acid) as substitute to petrochemical plastics (Wang et al., 2014; Whulanza et al., 2018).

Microbial fermentation processes are considered to be the most effective methods for LA production compared to chemical methods. This is due to obtaining optically pure LA and utilization of renewable cheap substrates (Abdel-Rahman et al., 2013; Tan et al., 2017). Lactic acid bacteria (LAB) are the most frequently used for LA production among the main bacterial producers [LAB, *Bacillus* strains, *Escherichia* coli, and *Corynebacterium glutamicum*] (Abdel-Rahman et al., 2013; Cizeikiene et al., 2018). LAB constitute a diverse group of Gram-positive microorganisms that can be used safely as probiotics or for LA production with high yield and high productivity (Abdel-Rahman et al., 2019a; Dawood et al., 2018). However, most LAB reported so far exhibited optimal growth conditions at 30–37 °C and pH 6–7 that might facilitate contamination risk and retard conduction of open [non-sterilized] fermentations (Abdel-Rahman et al., 2015a; Tan et al., 2017). Consequently, searching for potential LAB strains tolerating extreme conditions is of outstanding importance that would greatly rescue such limitation.

Besides, the potentiality of LA production using cheap and renewable materials such as lignocellulosic materials (Abdel-Rahman et al., 2011a; Germec et al., 2018), and food wastes (Kim et al., 2016) is

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growing to overcome substarte cost as one of the main challenges in economics of LA production. However, direct LA fermentation using lignocellulosic materials as sole carbon source is poor. This is due to lack of several macro and micronutrients for the bioconversion process, and requirements for pre-treatments to be effectively utilized by microbes (Abdel-Rahman and Sonomoto, 2016; Cizeikiene et al., 2018). On the other hand, food waste materials account for a considerable proportion of municipal solid waste all over the world that contains large amount of easily degradable organic materials and sugars (Zheng et al., 2017); however, the LA fermentations from only food waste have shown a low yield (Akao et al., 2007). Therefore, the co-fermentation of food waste with other organic waste such as lignocellulosic materials could improve the efficiency of fermentation processes due to optimization of C/N ratio, achieving nutrient balance, and dilution of toxic compounds (Zhang et al., 2017).

In the tropical and the sub-tropical regions of the world, banana is one of the most important fruit crops with high global production yield. It is richer in valuable macromolecules such as carbohydrates, proteins, fibres and low amount of lignin (Alagesan et al., 2018). Considering its huge amount, peels are dried and ground for sale with low price to feed manufacturers that is not a good exploit for such carbohydrate substances (Mamma et al., 2008). Alternatively, it could be serving as attractive feedstock for production of value-added chemicals (Hidayat et al., 2019; Nadar and Rathod, 2019). However, the main limitation of using such materials is the requirement of costly chemical and/or enzymatic treatments that might also result in release of microbial inhibitors due to the sugar and lignin degradations (Abdel-Rahman and Sonomoto, 2016). Thereby, there is an urgent need for cost effective system by direct utilization of food and agricultural wastes into LA production.

Therefore, this study focused on establishing effecient system for co-fermentation of waste materials to produce lactic acid. Isolation of lactic acid bacterial strains and investigation of their potential for effective LA production from lignocellulosic wastes under harsh fermentation (thermo-alkaline) conditions will be studied. We also aimed to optimize the fermentation process for high LA titre and yield based on co-fermentation of food waste with plant wastes without any nutritional supplementation.

2. Materials and methods

2.1. Banana peels and food waste materials

Banana peels were collected from local markets in Nasr city, Cairo, Egypt. Food wastes were obtained from student city kitchen of Al-Azhar University, Cairo, Egypt. These wastes were oven dried at 80 °C, milled and sieved to < 5.0 mm in diameter. Protein content was estimated by Lowry method (Lowery et al., 1951) and total sugars was estimated using phenol sulphuric acid method (Herbert et al., 1971).

2.2. Isolation and screening of potent lactic acid producers

Modified PYD (mPYD) medium was used. It contained g L^{-1}: yeast extract, 10; peptone, 20 supplemented with dried BP and/or FW [as carbon source] at concentration indicated in each experiment. mPYD media were adjusted to pH 8.0 or 9.0 (as indicated in each experiment) using 5N NaOH solution, autoclaved at 121 °C for 20 min. All chemicals used in this study were purchased from Sigma Aldrich.

Twenty-five samples of dairy products (solid and liquid) were collected from different governorates in Egypt. One gram or 1 mL from each sample was placed in a 250 ml Erlenmeyer flask containing 100 ml working volume of the selective mPYD broth media containing 4.0% [w-$_{dry\ matter}$/v] of untreated BP. The media were incubated at 50 °C for 48 h. From all fermentation media, 100 μL of bacterial solutions were then streaked on PYD agar medium and incubated at 50 °C for 24 h. Purification protocol was conducted on agar paltes and the purified

isolates were kept in glycerol (30%) at −80 °C.

A total of 59 obtained pure isolates were subjected to secondary screening test for LA production on mPYD broth media containing 4.0% [w-$_{dry\ matter}$/v] of untreated BP as the sole carbon source at pH 8.0 and 50 °C. Isolates that produced less than 10 g L^{-1} of LA were excluded. Tertiary screening tests were conducted for the selected 9 isolates by investigating LA production at various harsh fermentation conditions *viz.*, pH 8.0/60 °C, pH 9.0 at 50 °C or 60 °C.

2.3. Identification of bacterial isolate BP130

Morphological characterization of isolate coded BP130 (colony color, cell shape, and arrangement), Grams reaction, and biochemical characterization (KOH and utilization of different sugars) were investigated. Genetic identification was conducted by the extraction of genomic DNA according to modified method by Miller et al. (1999). 16S rRNA gene was amplified using 1 μL of genomic DNA as template and bacterial universal primers [27f (5-GAGTTTGATCACTGGCTCAG-3) and 1492r (5-TACGGCTACCTTGTTACGACTT-3)] (Lane, 1991) in polymerase chain reaction (PCR). The PCR mixture which contained 1 × PCR buffer, 0.25 mM dNTP, 0.5 mM MgCl$_2$, 0.5 μM of each primer, 2.5 U Taq DNA polymerase (QIAGEN), and 1 μL of genomic DNA. PCR was conducted in a DNA Engine Thermal Cycler (PTC-200, BIO-RAD, USA) at the following conditions: hot starting performed for 3 min at 94 °C, followed by 30 cycles of 94 °C for 0.5 min, 55 °C for 0.5 min, and 72 °C for 1 min, with an extension performed for 10 min at 72 °C. PCR product was sequenced at Sigma Company using ABI 3730xl DNA sequencer. The sequence was then compared with those in the GenBank database by using BLASTN. Multiple sequence alignment was performed using ClustalX 1.8 software package and the phylogenetic tree was established with neighbour-joining method using MEGA (Version 6.1) software. By bootstrap analysis, the level of confidence for each branch at 1000 repeats was tested. 16S rRNA gene sequence of this isolate was deposited in GenBank with accession number of MK744074.

2.4. Optimization of fermentation conditions

Preparation of inoculum: Inoculum was prepared by transferring a colony from the plate agar to 10 ml mPYD medium in test tube [incubated at 50 °C for 24 h] before inoculation into 250 ml Erlenmeyer flask with 100 ml PYD broth media then incubated at 50 °C for 24 h.

Effect of solid waste (BP) concentration on LA-fermentation: To investigate the optimal BP concentration for LA production by *Enterococcus durans* BP130, different concentrations of untreated banana peels (4.0–20.0% [w-$_{dry\ matter}$/v]) were supplemented to mPYD media. Main batch fermentations were conducted in 250 mL Erlenmeyer flasks containing 100 mL working volume. pH was adjusted at 9.0 using 5N NaOH solution and medium was inoculated at 10% (v/v) from the inoculume seed culture and then incubated at 50 °C for 48 h. Different samples were taken at different time intervals and analysed for cell growth, sugar concentration, and LA concentration.

Effect of co-substrate utilization on LA-fermentation: To investigate the optimal substrate mixture for LA fermentation, different mixtures of FW:BP were used at different ratios of 0.5:1, 1:1, 1.5:1, 2:1, and 3:1 (w/w) based on optimal carbon source concentration at 6.7% [w-$_{dry\ matter}$/v]. FW only (1:0 of FW:BP [w/w]) and BP only (0:1 of FW:BP w/w) were also tested as sole carbon source. This experiment was performed using mPYD broth media as liquid part prepared at pH 9.0 and incubated at 50 °C for 48 h. Samples were withdrawn periodically for investigating fermentation bacterial growth, sugar content and fermentation product. This experiment was also conducted at the same conditions mentioned above but after excluding of nutrients (20 g L^{-1} peptone and 10 g L^{-1} yeast extract) presented in mPYD media. Batch fermentations were conducted using the wastes and tape water only.

Effect of neutralizing agents on LA-fermentation: By using 6.7% [w-$_{dry\ matter}$/v] of substrates with FW:BP, at 3:1 (w/w) without nutrients,

sodium hydroxide and calcium carbonate were tested as neutralizing agents. The fermentations were conducted at 50 °C and pH 9.0 for 120 h. 5N NaOH was added at different times along the fermentation for controlling the pH at 9.0, while CaCO$_3$ was added with the fermentation media at 25% (*w/w*) of dry substrates. Intermittent samples were withdrawn at different intervals for analysing growth, sugar concentration, and LA concentration.

2.5. Cell growth, sugars and lactic acid analysis

Cell growth was measured as total viable cell count using modified plate count agar media that contained (g L^{-1}); peptone, 5.0; yeast extract, 2.5; glucose, 1.0; agar, 15. The pH adjusted to 9.0 ± 0.2 before sterilization (Atlas, 2004). The serially diluted samples were inoculated on plate count agar media, incubated at 50 °C for 24–48 h, then colonies were counted. Sugar concentrations were estimated using phenol sulfuric acid method (Herbert et al., 1971). Lactic acid concentration was determined after centrifugation of the samples at 6000 rpm for 10 min by modified Barker and Summerson method (Barker and Summerson, 1941).

3. Results and discussions

3.1. Isolation of banana peels-utilizing lactic acid bacteria

In order to address some problems associated with LA fermentations including costly substrates, high utilization of neutralizing agents and contamination risk, isolation of LAB was conducted at thermo-alkaline conditions on lignocellulose (Banana peels) containing media. mPYD media contained 4.0% [*w*-dry matter/*v*] of untreated BP was used as selective media. Protein content of BP used in this study was 6.93 ± 0.53% while total sugars concentration was 46.7 ± 1.95%. Using different dairy products as an isolation sources collected from Egypt, 59 BP-utilizing bacterial isolates for LA production were obtained. From screening protocol on broth media, only 9 potent isolates were selected which produced greater than 10 g L^{-1} of LA at pH 8.0 and 50 °C after 48 h.

To investigate the stability of obtained isolates under stress fermentation conditions, the nine potent isolates were investigated for LA production at higher pH and/or higher temperature as indicated in Fig. 1. Interestingly, all 9 isolates could grow at 60 °C and pH 9.0. Amongst all isolates, BP130 exhibited high stability under all conditions and gave LA values of 14.3, 10.3, 16.0 and 11.2 g L^{-1} under pH 8.0/ 50 °C, pH 8.0/60 °C, pH 9.0/50 °C and pH 9.0/60 °C, respectively.

Table 1
Morphological and biochemical characterization of bacterial isolate BP130.

Character	Result	Character	Result
Cell shape	Cocci	Tolerance to NaCl %:	
Colony Color	White	1.5–7.5	+
Colony shape	Convex	10.0	−
Gram stain	+	Sugar Fermentation:	+
Catalase activity	−	Fructose	+
Fermentation type	Homo	Sucrose	+
Growth temperature		lactose	+
20 °C–60 °C	+	Maltose	+
Growth at different pH values		Raffinose	+
4.0–10.0	+	Starch	+
11.0	−	Cellulose	+

+, Positive reaction; −, Negative reaction.

Therefore, isolate BP130 was selected as the most potent isolate for characterization and further studies. The highest LA concentration (16.0 g L^{-1}) was obtained at pH 9.0 and 50 °C and therefore these conditions were considered the optimal for LA production by this isolate.

The morphological and biochemical identification tests for isolate BP130 are shown in Table 1. Isolate BP130 appeared on the PYD agar media as small in colony shape and white color. Microscopic examination showed that isolate BP130 is coccal shape and Gram positive. It is catalase and KOH negative. This isolate could tolerate high concentration of NaCl solution (Up to 75 g L^{-1}), wide range of pH (4–10) and temperature (20–60 °C). Interestingly, isolate BP130 could utilize a wide range of sugars whether monosaccharide, disaccharide or polysaccharides (glucose, fructose, sucrose, lactose, maltose, raffinose, starch and cellulose) (Table .1). By using molecular identification, isolate BP130 showed similarity at 99% with *Enterococcus durans* JCM8725 and 98D strains. Phylogenetic tree indicated that isolate BP130 was clustered with different isolates of *Enterococcus durans* (Fig. 2). Therefore, this isolate was identified as *Enterococcus durans* BP130.

Enterococcus species was isolated naturally which preferred than genetically modified one as previously recorded (Abdel-Rahman et al., 2011b, 2019b). *E. durans* strains were previously reported for production of valuable products utilizing various substrates at different pH and temperature conditions. For example, *Enterococcus durans* IPLA 655 was used for tyramine production at pH 5.0 and temperature 30 °C from pure sugars (Fernández et al., 2007) and at pH 6.5 and 37 °C for bacteriocin production from lactose (Renye et al., 2009). On the other
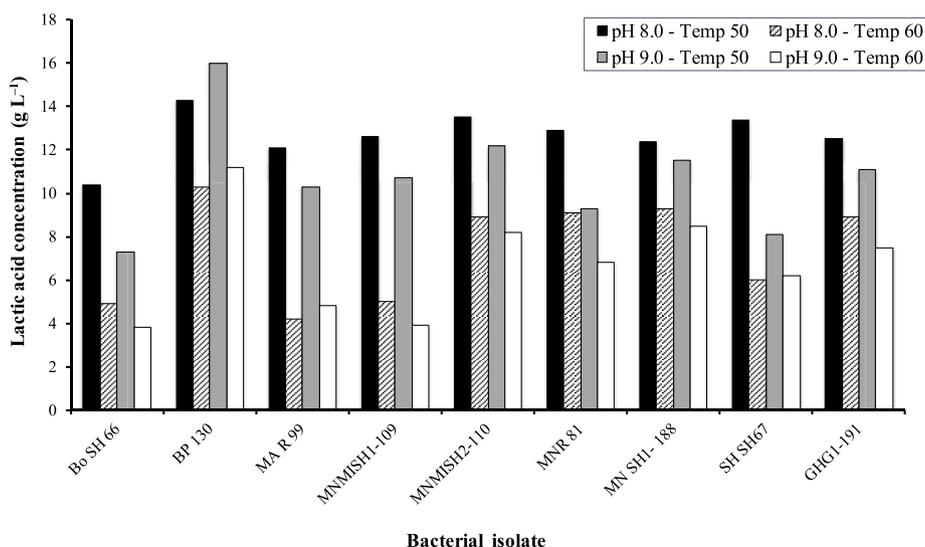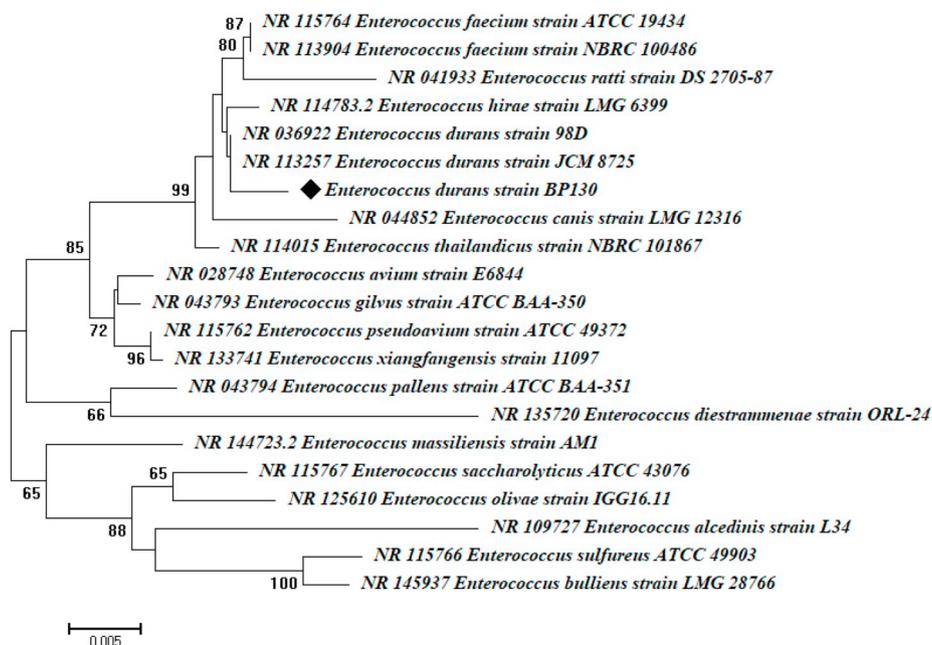

Fig. 1. Lactic acid production from banana peels materials under different conditions of pH and temperatures (°C) of the most potent nine bacterial isolates.

Fig. 2. Phylogenetic analysis of 16S rRNA sequences of the bacterial isolate with the sequences from NCBI. Symbol ♦ refers to 16S rRNA gene fragments retrieved from this study. The analysis was conducted with MEGA 6 using neighbour-joining method.

hand, there is no reports for LA production by thermo-alkaliphilic *E. durans* strains. To the best of authors knowledge, there are only two reports in literature describing thermophilic LAB stains at 50 °C. Abdel-Rahman et al. (2015a, b) reported *E. faecium* QU 50 that produced LA at 50 °C and pH 7.0 and more recently *E. faecium* FW26 that produced LA at 50 °C and pH 9.0 (Abdel-Rahman et al., 2019c). Data in Table 2 showed the potentiality of our reported strain in term of substrate utilization and fermentation conditions (pH and temperature) compared to other *Enterococcs* spp. reported so far.

3.2. Optimization of solid waste (banana peels) concentration

High initial substrate tolerance is very important for obtaining high product concentration, productivity and for reduction of downstreaming processing cost (Wang et al., 2014). Different concentrations of untreated BP were used ranged 4.0–20.0% [w-$_{\text{dry matter}}/v$]. As shown in Table 3, the total viable cells exhibited its maximal value of $346 \pm 18 \times 10^{10}$ cfu/mL at 4.0% [w-$_{\text{dry matter}}/v$] of carbon source while became decreased gradually after that. The minimal value for growth ($91.5 \pm 11 \times 10^{10}$ cfu/mL) was obtained at high concentration of banana peels of 20.0% [w-$_{\text{dry matter}}/v$] that may attributed to substrate inhibition (Abdel-Rahman et al., 2011a; Ding and tan, 2006).

Sugar consumption was increased from $13.9 \pm 0.8 \,\text{g L}^{-1}$ at 4.0% [w-$_{\text{dry matter}}/v$] giving its maximal value of $21.8 \pm 1.0 \,\text{g L}^{-1}$ at 10.0% [w-$_{\text{dry matter}}/v$]. On the other hand, the maximum LA concentration of $19.2 \pm 0.4 \,\text{g L}^{-1}$ was achieved when using 6.7% [w-$_{\text{dry matter}}/v$] of BP. Interestingly, LA yield showed high range values of 0.75–0.90 g g^{-1} of sugar consumed, in the range of 4.0–16.7% [w-$_{\text{dry matter}}/v$] of BP indicating homofermentative LA production by this strain at these concentrations. However, at higher concentration of BP (20.0%, w-$_{\text{dry matter}}/v$), LA yield was drastically decreased to 0.62 g g^{-1} of sugar consumed. The highest LA yield of 0.90 g g^{-1} of sugar consumed was obtained at 6.7% (w-$_{\text{dry matter}}/v$) of BP. Also, the total LA productivity and maximum LA productivity gave their maximum values of 0.40 and 1.2 g L^{-1} h^{-1}, respectively, at 6.7% (w-$_{\text{dry matter}}/v$) of BP. Therefore, 6.7% [w-$_{\text{dry matter}}/v$] was considered as the optimal concentration for LA production by *Enterococcus durans* BP130 and was selected for further studies.

Interestingly, the cellulolytic activity for *Enterococcus durans* BP130

facilitated direct utilization of banana peels without any physical, chemical or enzymatic treatments. *Enterococcus durans* species was described previously to produce high amount of cellulase enzymes (Ho and Yin, 2018). To the best of author's knowledge, there is no report on direct LA production from BP. Banana peels were used as good substrate for production of various platform chemicals due to their components of proteins, carbohydrates, lipids and various minerals (Anhwange et al., 2008). For example, BP was used for ethanol production at 10% [w-$_{\text{dry matter}}/v$] (Oberoi et al., 2011). Kareem, and Rahman (2013) reported citric acid production from banana peels in solid state fermentation at 60% [w-$_{\text{dry matter}}/v$]. Also, 10% [w-$_{\text{dry matter}}/v$] of banana peels was used for production of ethanol using *Saccharomyces cerevisae* (Gebregergs et al., 2016). *Sophora flavescens* residues were used for LA production by *Lactobacillus casei* 6106 at 11.1% [w-$_{\text{dry matter}}/v$] (Zheng et al., 2017; Wang et al., 2016).

3.3. Co-fermentation of mixed waste materials

Co-fermentation of plant wastes with food waste is advantageous in synergistic effect, pH control, and optimizing C/N ratio. This is because food waste materials might contain feasible organic matters that affect pH of the fermentation media and lowering the yield of LA (Zhang et al., 2017). Therefore, co-fermentation by *E. durans* BP130 was investigated at different ratios of solid wastes at final solid waste concentration of 6.7% (w/v). The results were compared to fermentation of food waste or banana peels as sole carbon source separately. Protein content of FW used in this study is $13.0 \pm 0.70\%$ while total sugars concentration is $62.48 \pm 3.16\%$. This experiment was conducted in the presence and absence of nutritional components of medium as have been summarized in Table 4.

Fermentation of banana peels as a sole carbon source, in the presence of medium components, exhibited higher LA concentration of $19.7 \pm 0.6 \,\text{g L}^{-1}$ than obtained food waste of LA $16.1 \pm 0.2 \,\text{g L}^{-1}$ (Fig. 3A and C). However, utilization of BP only, in the absent of nutrients, yielding the lowest LA fermentation parameters (Fig. 3B and D), with cell growth, sugar consumption, LA-production, productivity, and maximum productivity of $26.5 \pm 4.9 \times 10^{10}$ cfu/mL, 8.5 ± 0.4, $6.08 \pm 0.2 \,\text{g L}^{-1}$, $0.14 \,\text{g L}^{-1} \,\text{h}^{-1}$ and $0.48 \,\text{g L}^{-1} \,\text{h}^{-1}$, respectively. On the other hand, growth and fermentation parameters were improved

Table 2
Summary of previous studies showing substrates and fermentation conditions (pH and temperate) used for lactic acid production by Enterococs spp.

Strain	Fermentative Substrate	Fermentation pH	Fermentation temperature (°C)	Reference
Enterococcus faecalis CBRD01	Glucose	7.0	37	Subramanian et al. (2015)
Enterococcus faecalis SI	Steam explosion (AISE)-treated plywood chips	7.0	42	Yuan et al. (2018)
Enterococcus faecalis QU 11	Glycerol	6.5	30	Murakami et al. (2016)
Enterococcus faecalis RKY1	Hydrol, soybean curd residues (SCR), and malt.	7.0	38	Reddy et al. (2016)
Enterococcus faecium LLAA-1	Glucose, fructose, cellobiose and xylose	6.9	37	Pessione et al. (2014)
Enterococcus faecium No. 78	Starch	6.5	30	Shibata et al. (2007)
Enterococcus faecium QU 50	Xylose and simulated energy cane hydrolysate	6.5	50	Abdel-Rahman et al. (2015a); Tan et al. (2017)
Enterococcus faecium strain FW26	Banana peels plus food waste	9.0	50	Abdel-Rahman et al. (2019c)
Enterococcus mundtii QU 25	Simulated energy cane hydrolysate; cellobiose; xylose, and glucose	7.0	42	Wang et al. (2014); Abdel-Rahman et al. (2011a); Abdel-Rahman et al. (2015b)
Enterococcus durans BP130	Banana peels plus food wastes	9.0	50	This study

when food waste used as a sole carbon source without nutrient supplementation, with final LA concentration of $12.2 \pm 0.7\,\mathrm{g\,L^{-1}}$ and LA yield of $0.82\,\mathrm{g\,g^{-1}}$ of sugar consumed. The decrease in fermentation efficiency using BP only is attributed to substrate deficiency for several macro and micronutrients. These nutrients are urgently required for microbial growth and consequently LA production (Zheng et al., 2017). In contrast, food waste is nutrient–rich material containing high amount of lipids (10–40%), proteins (5–10%) and several nutrients (Kiran et al., 2014).

Interestingly, LA fermentation parameters were enhanced when the food wastes were mixed with banana peels even in the absence of medium components (Fig. 3). In the presence of medium component, the total viable cells were enhanced giving values ranged $312–347 \times 10^{10}$ cfu/mL and the maximum value was obtained at ratio of 1.5:1 FW:BP, (w/w) as compared to 236×10^{10} cfu/mL obtained with fermentation of FW as single substrate. Also, total viable cells were clearly increased as compared to fermentation of BP only in the absence of nutrients; while it was almost comparable with that obtained from fermentation of FW only at values ranged $53.0–68.4 \times 10^{10}$ cfu/mL with the maximum value at 3:1 FW:BP, (w/w).

Consumed sugars as well as lactic acid concentrations were also enhanced by the co-fermentation with food waste achieving the highest values at 0.5:1 of FW:BP (w/w) at 24.6 and $22.2\,\mathrm{g\,L^{-1}}$, respectively (Fig. 3A). At this ratio, the yield of LA, total productivity and maximum productivity exhibited the highest values of $0.90\,\mathrm{g\,g^{-1}}$ of sugar consumed, $0.46\,\mathrm{g\,L^{-1}\,h^{-1}}$ and $1.2\,\mathrm{g\,L^{-1}\,h^{-1}}$, with 10.0, 11.0 and 20.0% improvements than utilizing BP as sole substrate, respectively. Similarly, positive synergistic effect was obtained in LA concentration pattern by co-fermentation of FW and BP in the absence of medium supplementation. The final LA concentration was increased with an increase of FW content in the media giving the highest value of $14.3\,\mathrm{g\,L^{-1}}$ at 3:1 of FW:BP, (w/w) with 17 and 135% improvements than utilizing FW or BP as a single substrate, respectively (Fig. 3C and D). At 3:1 ratio, maximum LA yield and productivity ($0.91\,\mathrm{g\,g^{-1}}$ of sugar consumed; and $0.30\,\mathrm{g\,L^{-1}\,h^{-1}}$) were attained which were improved by 11% and 20% than fermentations with FW only.

The increased fermentation parameters after mixing with food waste is mainly attributed to its high contents of lipids, proteins, carbohydrates (Oberoi et al., 2011) and minerals (Anhwange et al., 2008) that support bacterial growth. In additions; it may also attributed to the optimization of C/N ratio, an important factor affecting fermentation (Zheng et al., 2017). It was found that, maximum hydrogen yield was attained when the C/N ratio was adjusted to 25 (Kalil et al., 2008). Also, during mixed acid fermentation, the microbial metabolic activities were enhanced by the C/N ratio when pre-treated bagasse was mixed with wastewater sludge (Rughoonundun et al., 2012). Therefore, co-fermentation of two-substrates might improve LA fermentation due to nutrient balance, the addition of biodegradable organic matter, pH control, dilution of toxic compounds, and synergistic effects (RedCom and Engelberth, 2016).

Positive synergistic effect using mixed substrates was previously reported under various conditions. The maximum LA concentration was obtained when FW was mixed with *Sophora flavescens* residues at 1.5:1 ratio (Zheng et al., 2017). The improvements of biogas yield reached 19% when de-oiled grease trap waste mixed with food waste (Wu et al., 2016). RedCom and Engelberth (2016) reported co-digestion of food waste and primary sludge achieving the 97% of the theoretical yield of LA.

Although total viable cells were decreased when the nutrients were excluded, but LA fermentation parameters were not much affected as compared to that obtained in the presence of peptone ($20\,\mathrm{g\,L^{-1}}$) and yeast extract ($10\,\mathrm{g\,L^{-1}}$) (Table 4). The final LA concentration (14.3 $\mathrm{g\,L^{-1}}$) obtained at ratio 3:1 of FW:BP (w/w) was almost comparable to that obtained in the presence of all medium components at $16.9\,\mathrm{g\,L^{-1}}$. Interestingly, *E. durans* BP130 achieved higher LA yield ($0.91\,\mathrm{g\,g^{-1}}$ of sugar consumed) when excluding all nitrogen sources from the

Table 3

Effect of banana peels concentrations on growth, sugar consumption and lactic acid production by *E. durans* BP130.

Conc. of banana peels % (w-$_{\text{dry matter}}$/v)	Total Viable Cells $\times 10^{10}$ (cfu/ml)	Consumed sugar (g L^{-1})	LA conc. (g L^{-1})[a]	LA yield (g g^{-1})[b]	P_{LA} (g L^{-1} h^{-1})[c]	Max P_{LA} (g L^{-1} h^{-1})[d]
4.0	346 ± 18	13.9 ± 0.8	11.2 ± 0.4	0.80	0.23	0.47 (12 h)
5.0	323 ± 9.2	16.8 ± 1.5	12.9 ± 0.1	0.77	0.27	0.53 (12 h)
6.7	338 ± 9.9	21.2 ± 0.6	19.2 ± 0.4	0.90	0.40	1.2 (12 h)
10.0	250 ± 16	21.8 ± 1.0	18.8 ± 0.6	0.86	0.39	1.0 12 h)
11.1	218 ± 9.2	19.5 ± 0.5	16.4 ± 0.5	0.84	0.34	1.1 (12 h)
12.5	167 ± 23	13.1 ± 0.3	11.3 ± 0.5	0.87	0.24	0.51 (6 h)
14.3	129 ± 3.5	10.9 ± 0.1	8.20 ± 0.1	0.75	0.17	0.41 (6 h)
16.7	118 ± 7.8	7.40 ± 0.2	6.10 ± 0.1	0.82	0.13	0.36 (6 h)
20.0	91.5 ± 11	5.10 ± 0.1	3.20 ± 0.1	0.62	0.07	0.35 (6 h)

[a] Maximum lactic acid concentration after 48 h.
[b] Lactic acid yield.
[c] Lactic acid productivity at the end of fermentation time.
[d] Maximum lactic acid productivity at indicated time.

fermentation medium at 3:1 ratio of FW:BP (w/w) as compared to that obtained in the presence of medium components (0.84 g g^{-1} of sugar consumed).

Enterococcus durans BP130 strain utilized food waste as well as banana peels directly without any treatments for starch in food waste or cellulose in banana peels owing to the amylolytic and cellulytic activities that were detected by this strain. Several researches had also reported amylolytic and cellulytic activity for *E. durans* strains and other *Enterococcus* species (Ho and Yin, 2018; Ryu et al., 2010). Several studies has been reported to utilize different wastes but after various pre-treatment rather than using of expensive nutrient supplementation in the fermentation media. Wheat straw, sugarcane bagasse and bagasse sulfite pulp were utilized for LA fermentation but after ionic liquid, acidic and/or enzymatic treatments and supplementation various expensive nutrients in media components (Grewal and Khare, 2018; Zhou et al., 2016). Date palm wastes were utilized for LA production after alkaline and enzymatic treatments and using 60 g L^{-1} of the expensive yeast extract and other minerals (Alrumman, 2016). Also, food waste was used for LA production after enzymatic treatments (Kitpreechavanich et al., 2016). Utilization of food waste mixed with *Sophora flavescens* residues for LA production was also achieved but after alkaline and enzymatic treatments (Zheng et al., 2017). Zhang et al. (2017) reported that, waste activated sludge and FW were utilized for LA production either under mesophilic fermentation of weak alkaline or thermophilic fermentation of neutral condition but after chemical and thermal treatments.

3.4. Effect of neutralizing agents on biorefinery of mixed wastes into lactic acid

In further attempt to increase the final LA concentration and decreasing the fermentation costs, different neutralizing agents were investigated for pH control during fermentation. As indicated from Fig. 4, utilizing CaCO$_3$ as neutralizing agent greatly improved LA fermentation by *Enterococcus durans* BP130 in comparison with utilizing NaOH solution or without neutralizing agents. As shown in Table 5, higher cell growth, consumed sugar, LA concentration, yield, volumetric productivity and maximum productivity were obtained when using CaCO$_3$ [163.3 $\times 10^{10}$ cfu/mL, 34.0, 28.8 g L^{-1}, 0.85 g g^{-1} of sugar consumed, 0.24 and 1.1 g L^{-1} h^{-1}, respectively] compared to that obtained with NaOH [81.5 $\times 10^{10}$ cfu/mL, 27.2, 22.3 g L^{-1}, 0.82 g g^{-1} of sugar consumed, 0.19 g L^{-1} h^{-1} and 0.98 g L^{-1} h^{-1}, respectively]. It is clear that, final LA concentration was two-fold with the pH control (about 201% increases) than that in fermentation system without pH control. Also, maximum LA productivity was increased by 67% than that in the previous experiment.

Use of neutralizing agents counteract the pH reduction during LA fermentation, preventing the progressive acidification of the medium, and consequently improve LA production as previously reported (Abdel-Rahman et al., 2015 a; b). Yao and Toda (1990) investigated the effects of sodium hydroxide and calcium carbonate on the inhibition of *Lactobacillus delbrueckii* strain YPX as compared to non pH-controlled
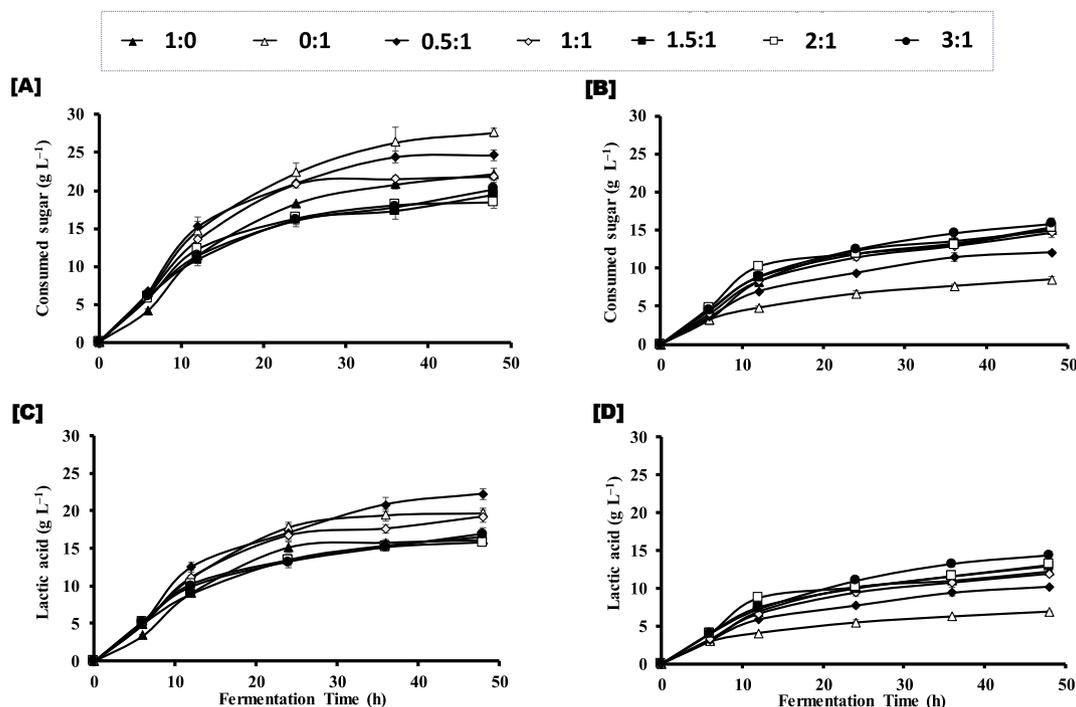
Table 4

Effect of co-fermentation of banana peels and food waste materials (at final concentration of 6.7%, w-$_{\text{dry matter}}$/v) on lactic acid production by *E. durans* BP130 with/without media components.

FW: BP ratio (w/w)	With/without media component	Total Viable Cells $\times 10^{10}$ (cfu/ml)	Consumed sugar (g L^{-1})	LA conc. (g L^{-1})[a]	LA yield (g g^{-1})[b]	P_{LA} (g L^{-1} $^{-1}$h)[c]	Max P_{LA} (g L^{-1} h^{-1})[d]
1:0	with	236 ± 21	19.6 ± 0.8	16.1 ± 0.2	0.820	0.340	0.940 (12 h)
	without	67.0 ± 5.6	15.0 ± 0.2	12.2 ± 0.7	0.820	0.250	0.640 (12 h)
0:1	with	292 ± 8.5	25.6 ± 0.5	19.7 ± 0.6	0.790	0.410	1.00 (12 h)
	without	26.5 ± 4.9	8.50 ± 0.4	6.08 ± 0.2	0.800	0.140	0.480 (6 h)
0.5:1	with	346 ± 8.5	24.6 ± 0.7	22.2 ± 0.7	0.900	0.460	1.20 (12 h)
	without	60.0 ± 1.4	12.1 ± 0.2	10.2 ± 0.2	0.840	0.210	0.520 (6 h)
1:1	with	343 ± 26.0	21.8 ± 0.4	19.2 ± 0.8	0.880	0.400	1.10 (12 h)
	without	56.0 ± 4.2	14.7 ± 0.7	11.9 ± 0.1	0.810	0.250	0.560 (12 h)
1.5:1	with	347 ± 18	19.4 ± 0.6	16.5 ± 0.7	0.850	0.340	0.830 (6 h)
	without	53.0 ± 4.2	15.4 ± 0.6	12.9 ± 0.1	0.840	0.270	0.660 (6 h)
2:1	with	315 ± 15	18.4 ± 0.8	15.8 ± 0.4	0.860	0.330	0.870 (6 h)
	without	57.5 ± 4.8	15.3 ± 0.6	13.1 ± 0.8	0.860	0.270	0.780 (12 h)
3:1	with	312 ± 3.5	20.2 ± 0.9	16.9 ± 0.9	0.840	0.350	0.880 (6 h)
	without	68.4 ± 7.9	15.8 ± 0.8	14.3 ± 0.5	0.910	0.300	0.660 (6 h)

[a] Maximum lactic acid concentration after 48 h.
[b] Lactic acid yield.
[c] Lactic acid productivity at the end of fermentation time.
[d] Maximum lactic acid productivity at indicated time.

Fig. 3. Effect of mixed waste materials at different ratios on consumed sugars and LA production by *E. durans* BP130 at pH 9.0 and 50 °C for 48 h. [A] and [B], consumed sugars in fermentations with and without nutrient supplementation, respectively. [C] and [D], lactic acid production in fermentations with and without nutrient supplementation, respectively. Symbols: ▲, 1:0; Δ, 0:1; ◆, 0.5:1; ◊, 1:1; ■, 1.5:1; □ 2:1; and ●, 3:1 (FW:BP; *w/w*).
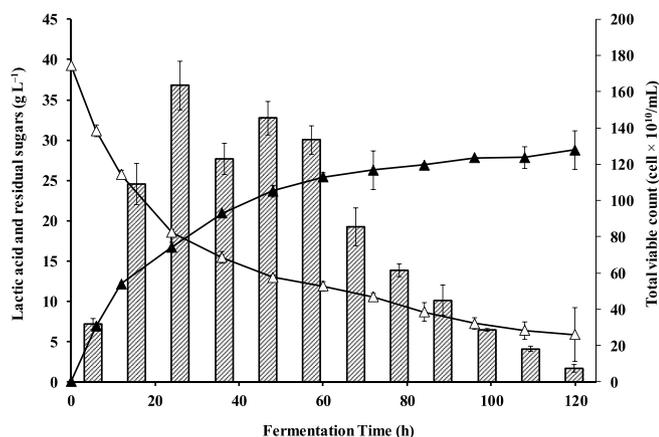

Fig. 4. Lactic acid production from mixed food wastes and banana peels at 3:1 (*w/w*) with final solid matter concentration of 6.7% (*w/v*) using *Enterococcus durans* BP130. Batch fermentation was carried out without nutrient supplementation at 50 °C and pH 9.0 using $CaCO_3$ as neutralizing agent. Symbols: ▲, lactic acid concentration [g L^{-1}]; Δ, residual sugars [g L^{-1}]; open bar, total viable count of strain BP130 [cells × 10^{10} cfu/mL].

fermentation. They reported that inhibition by Ca-lactate is the weakest as the degree of product inhibition was in the next order; lactic acid » Na-lactate » Ca-lactate. In addition to as one of the best neutralizing agent, $CaCO_3$ has been reported to protect the cells from inhibition by lactic acid as have been confirmed by viable cell count (Marković et al., 2011) and to enhance the cells to adsorb nutrients (Sonomoto et al., 2000).

4. Conclusion

This study attained cost effective system for lactic acid production from untreated wastes at high temperature and pH values. Different concentrations of untreated banana peels were investigated for LA production by *Enterococcus durans* BP130 which showed high utilization and LA production at 6.7% [w-dry matter/v]. Positive synergistic effects in term of LA concentration and productivity were obtained by co-fermentations of food waste and banana peels. Interestingly, strain BP130 facilitated LA fermentation without medium supplementation (after removal of all nitrogen sources from the media). Without any treatments, 28.8 g L^{-1} of lactic acid was produced with the media consisted only of untreated mixed wastes and neutralizing agent with tap water under thermoalkaline conditions.

Table 5
Effect of neutralizing agent on fermentation parameters for lactic acid production by *E. durans* BP130.

Neutralizing Agents	Total Viable Cells × 10^{10} (cfu/ml)	Consumed sugar (g L^{-1})	LA conc. (g L^{-1})[a]	LA yield (g g^{-1})[b]	P_{LA} (g L^{-1} h^{-1})[c]	Max P_{LA} (g L^{-1}/h)[d]
NaOH	81.5 ± 10.6	27.2 ± 0.3	22.3 ± 2.5	0.82	0.19	0.98 (6 h)
CaCO$_3$	163.5 ± 13.4	34.0 ± 3.3	28.8 ± 2.4	0.85	0.24	1.1 (6 h)

[a] Maximum lactic acid concentration after 5d.

[b] Lactic acid yield.

[c] Lactic acid productivity at the end of fermentation time.

[d] Maximum lactic acid productivity at indicated time.

Conflicts of interest

Authors declare that there is no conflict of interest.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101203.

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