Potential Utilization of Sap from Oil Palm (*Elaeis guineensis*) for Lactic Acid Production by *Lactobacillus casei*

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Abstract: Lactic acid is a product that has several applications in food, cosmetic, pharmaceutical and chemical industries. The main objective of this work was to evaluate the potential use of sap of oil palm (*Elaeis guineensis*) as a substrate for lactic acid production by *Lactobacillus casei* TISTR 1500. The effects of constant pH at 5.5 in a 2 L bioreactor and supplementary de Man, Rogosa, Sharpe (MRS) medium with oil palm sap as a carbon source on fermentation performance were investigated. In this report, when oil palm sap was used as a carbon source for *L. casei* TISTR 1500, constant pH at 5.5 did not significantly affect lactic acid production. The addition of MRS medium improved the biomass and the product yield of oil palm sap. Fermentation runs pH-unfixed gave an improved productivity of 0.55 g L^{-1} h^{-1} during the fermentation containing 100 mL of oil palm sap (20 g L^{-1} of total sugars) supplemented MRS medium in flasks under static condition at 37°C. Oil palm sap could serve as a good potential source of raw materials for the efficient production of lactic acid by *L. casei* TISTR 1500.

Keywords: Fermentation; Lactic acid; *Lactobacillus casei*; Oil palm sap; Palm Oil.

1. Introduction

Lactic acid is a versatile chemical, used as an acidant, flavor and preservative in food, and in pharmaceutical, leather and textile industries. It is also used for production of base chemicals, and can be polymerize to produce biodegradable polylactic acid (PLA) [1]. PLA could be a good substitute for synthetic plastic derived from petroleum feedstock. Being highly reactive due to the presence of carboxylic and hydroxyl groups, lactic acid can undergo a variety of chemical conversions into potentially useful chemicals such as propylene oxide, propylene glycol, acrylic acid, 2,3 pentanedione and lactate ester [2]. Lactic acid is produced commercially either by chemical synthesis or by microbial fermentation. Approximately 90% of the total lactic acid produced worldwide is by bacterial fermentation and the rest is produced synthetically by hydrolysis of lactonitrile. The chemical synthesis of lactic acid always results in racemic mixture D/L lactic acid, which is a major disadvantage. Fermentative production of lactic acid offers the advantage of both utilization of renewable carbohydrates and production of optically pure L- or D-lactic acid depending on the strain of microorganism selected [3].

Lactobacillus casei strains are lactic acid bacteria with remarkable phenotypic and genotypic variability [4] that colonize diverse ecological niches, including the human gastrointestinal tract [5], and which have broad commercial applications. The homofermentative *L. casei* is known to be an L(+)-lactic acid producer. Furthermore, *L. casei* is acidotolerant with an optimum pH of 5.5 and is relatively insensitive to product inhibition by lactic acid [6]. *L. casei* is also an anaerobic microorganism and consequently grows better in a static culture where the fermentation conditions are anaerobic [7].

The efficiency and economics of the ultimate lactic acid fermentation is however still a problem from many points of view and media composition play a vital role in the improvement of such a process. In recent years research effort has focused on looking for new and effective nutritional sources and new progressive fermentation techniques enabling the achievement of both high substrate conversion rates and high production yields [8]. A number of different substrates have been used for biotechnological production of lactic acid, including glucose, sucrose, lactose, maltose, mannose, xylose, and galactose. The most pure product is obtained when pure sugar is fermented, resulting in lower purification costs. However, this is economically unfavorable, because pure sugars are expensive and lactic acid is a relatively cheap product. To replace these refined and costly raw materials, the use of agricultural resources provides an attractive alternative because of their low prices [9-10]. Using cheap raw materials as a fermentation substrate for lactic acid is an alternative way to reduce the cost of lactic acid production.

Oil palm (*Elaeis guineensis*) is widely planted for its edible oil in tropical countries such as Malaysia, Indonesia and Thailand. Palm oil is the most produced plant oil, with a worldwide production of 4.3 million tons in 2008. Oil palm sap contains large quantities of high glucose content sap. Glucose was found to be the dominant sugar in all parts, accounting for approximately 86.9%, 86.3% and 65.2% of the total free sugars contained in the inner, middle and outer parts of the sap, respectively [11]. However, for other species of oil palm, Eze and Organ [12] reported that oil palm sap collected in Nigeria, by tapping at the base of the inflorescence, contained sucrose as the dominant sugar (10%, w/v). Similar results have been reported in the sap of Raphia palm (*Raphia hookeri*) in Nigeria, with sucrose as the dominant sugar [13]. The variations in composition may be due to the difference in species and/or cultivation conditions. The other possibility is that the sugar composition of sap collected from fallen palm trunks differs from that of sap collected by tapping the base of the inflorescence. Oil palm sap has been found to be rich in various kinds of amino acids, organic acids, minerals and vitamins. Based on these findings, the sap was fermented to produce ethanol using the sake brewing yeast strain, *Saccharomyces cerrevisiae* Kyokai no.7, and produced lactic acid using the homolactic acid bacterium, *Lactobacillus lactis* ATCC19435 [11].

In this study, we determined the physical and chemical composition of oil palm sap (*E. guineensis*) to evaluate its suitability as a substrate for the production of lactic acid by *L. casei* TISTR 1500. The effects of pH control and nutrient supplementation of MRS in the oil palm sap were investigated for maximizing biomass and lactic acid production. The influence of pH on lactic acid production was studied by comparing fermentations between two conditions: with initial pH 5.5 that was allowed to vary, and with constant pH at 5.5 in a 2 L bioreactor.

2. Experimental

2.1 Microorganism and inoculum

L. casei TISTR 1500, obtained from the Department of

Biotechnology, Faculty of Agro-Industry, Rajamangala University of Technology Srivijaya, Thailand, was the microorganism used in these experiments. The strain was provided by the Thailand Institute of Scientific and Technological Research (TISTR). It was maintained at 4°C in plate culture on MRS (de Man, Rogosa, Sharpe) agar media with the following composition (in g L^{-1}): proteose peptone, 10; beef extract, 10; yeast extract, 5; glucose, 20; polysorbate 80, 1; ammonium citrate, 2; sodium acetate, 5; magnesium sulphate, 0.1; manganese sulphate, 0.05; dipotassium phosphate, 2; and agar, 15. The inoculum was prepared by transferring a loopful of cells to 250 mL conical flasks containing 50 mL sterile MRS broth (the same composition of MRS agar, but without agar). The flasks were incubated at 37°C for 24 h. Ten milliliters of this culture was then transferred to a 250 mL Erlnemeyer flask containing 90 mL MRS broth, and incubated under the same conditions. Finally, the cells were harvested by centrifugation (8,000 rpm, 15 min) and directly resuspended in the fermentation medium to obtain a cell concentration of 1.0 g L^{-1} at the beginning of the fermentation.

2.2 Raw materials and characterization

Oil palm sap from the inner part of oil palm trunks was collected by using a laboratory-scale hydraulic press. The sap was centrifuged at 6,000 rpm for 15 min and the supernatant was stored at -20°C before use. The physical and chemical properties of oil palm sap from the inner part of oil palm trunks are: pH 7.49, moisture content 97.06%, total sugars 19.17 g L^{-1} (glucose 16.58 g L^{-1} and fructose 2.59 g L^{-1}), total soluble solid 3.4°Brix, and total nitrogen 0.06 g L^{-1}. The average compositions of the samples are given in Table 1. The oil palm sap contained 19.17 g L^{-1} of total sugars which was concentrated using an evaporator, to remove excess water and produce concentrated oil palm containing 20.0 g L^{-1} total sugars.

Table 1. Physical and chemical properties of oil palm sap.

Parameters	Value	Unit
pH	7.49	-
Moisture	97.06	%
Total sugar	19.17	g L^{-1}
glucose	16.58	g L^{-1}
fructose	2.59	g L^{-1}
sucrose	-	g L^{-1}
Total soluble solid	3.40	°Brix
Total nitrogen	0.06	g L^{-1}

2.3 Lactic acid fermentation

L. casei TISTR 1500 was used for lactic acid fermentation experiments. The bacterium was pre-cultured on MRS medium. After reactivation on MRS under microaerophilic conditions (without shaking), 10% (vv^{-1}) of this culture (exponential growth phase) was used to inoculate 90 mL of oil palm sap (total sugars concentration of 20.0 g L^{-1}) supplemented with MRS medium and the medium was adjusted to pH 5.5. After formulation, the mediums were transferred to 250 mL Erlenmeyer flasks and sterilized at 121°C for 15 min. The flasks were then statically incubated at 37°C for 72 h. Uncontrolled pH fermentations were performed in a final working volume of 100 mL in static flasks. Batch experiments, with controlled pH, were performed in a 2 L bioreactor with a final working volume of 1 L, equipped with a temperature and pH control unit; temperature was controlled at 37°C and the pH was set at 5.5 using 2.0 N NaOH. Due to the fact that *L. casei* is anaerobic, there was no need for agitation. However, when the pH dropped during fermentation, the pH control unit fed 2.0 N NaOH in fermentation broth, and after that low speed agitation was implemented to allow dispersion of pH agent the fermentation broth. Samples were taken every 12 h and the dry cell weight, total sugars consumption, and lactic acid production and productivity were compared to evaluate the process efficiency under different fermentation conditions. Reactor fermentation under each condition was carried out in triplicate and data shown on Table 2.

2.4 Analytical methods

Cell growth was measured by diluting the culture broth with distilled water to obtain optimum dilution. After mixing, the absorbance was measured using a UV-spectrophotometer (UV-1601, Shimadzu, Japan) at 660 nm [14]. Dry cell weight was determined by centrifugation of the culture broth (2 mL) at 8,000 rpm for 15 min. The cell sediments were dried for 24 h at 105°C and then weighed to constant weight after cooling in a desiccator [15]. Lactic acid and acetic acid concentrations in the supernatant were conducted by means of GC analysis. Gas chromatography (GC-14A, Shimadzu, Japan) was equipped with a BP-20 GC column (30 m × 0.53 mm) using a flame ionization detector [16]. Residual sugar (sucrose, glucose and fructose) in the supernatant was determined by HPLC analysis (System controller: SCL-10A *VP*, Liquid chromatograph: LC-10AD *VP*, Degasser: DGU-12A, RI detector: RID-10A, Auto injector: SIL-10AD *VP*, Column oven: CTO-10AS *VP*, Shimadzu, Japan), adapted from [17]. Total sugars concentrations were analyzed by the Dubois method using phenol and sulphuric acid [18]. Total nitrogen content was determined according to the Kjeldahl method [19].

2.5 Fermentative parameters

The fermentation parameters determined were: the specific growth rate (μ, h^{-1}), defined as the ratio of logarithm of biomass concentration produced to elapsed time (h); cellular yield coefficient ($Y_{X/S}$, g g^{-1}), defined as the ratio of the total cell mass present in the medium to sugar consumed; conversion yield of substrate to product ($Y_{P/S}$, g g^{-1}), defined as the ratio of lactic acid produced to sugar consumed; and maximum productivity (R_M, g L^{-1} h^{-1}), calculated as the ratio of lactic acid concentration to the fermentation time [20].

3. Results and Discussion

3.1 Effect of medium supplementation on lactic acid production

Experiments were initially carried out to investigate the influence of fermentation performance with and without MRS medium addition to the oil palm sap for lactic acid production. The results, shown in Figure 1, clearly show that *L. casei* TISTR 1500 could grow slightly in the without-addition MRS medium of the oil palm sap. This might be due to the insufficient nutrients. Products from fermentation were increased over time and were relatively constant at 42 h. The highest two product concentrations detected were lactic acid and acetic acid.

Table 2. Kinetic parameters of lactic acid production by *L. casei* TISTR 1500 from different fermentation media.

Fermentation Media	Residual sugar (g L^{-1})	Lactic acid (g L^{-1})	Yield (g g^{-1})	Maximum DCW (g L^{-1})	Productivity (42h) (g $L^{-1}h^{-1}$)	Enhancement of product yield (%)
OPS	12.10±0.28	8.85±0.12	0.70±0.02	2.20±0.05	0.21±0.004	-
OPS+MRS	0.13±0.03	22.90±0.15	0.95±0.01	3.65±0.04	0.55±0.005	35.71

Batch fermentations were performed on 250 mL static flask with working volume of 100 mL at pH 5.5, 37°C for 42 h. Results are the average of data from triplicate experiments.
OPS: Oil Palm Sap; MRS: de Man Rogosa and Sharpe.

Figure 1 shows a strong *L. casei* TISTR 1500 growth. The results clearly show that the oil palm sap with the addition MRS medium improved the fermentation by *L. casaei* TISTR 1500 compared to the without-addition MRS medium. Lactic acid bacteria are considered fastidious microorganisms and have complex nutrient requirements due to their limited ability to biosynthesize B-vitamins and amino acids. Therefore, to achieve optimal cultivation conditions, the fermentation medium must contain minerals, B-vitamins, amino acids, fatty acids, purines and pyrimidines for bacteria growth and biological activity. Hofvendahl and Hahn-Hagerdal [1] compared several studies concerning lactic acid production in fermentation media supplemented with different kinds of nutrients and reported that the addition of MRS broth components promotes a better fermentation performance when compared with the addition of yeast extract. This could be explained by considering that yeast extract is also present in the MRS medium composition together with other nutrients such as meat extract, peptone and some salts.

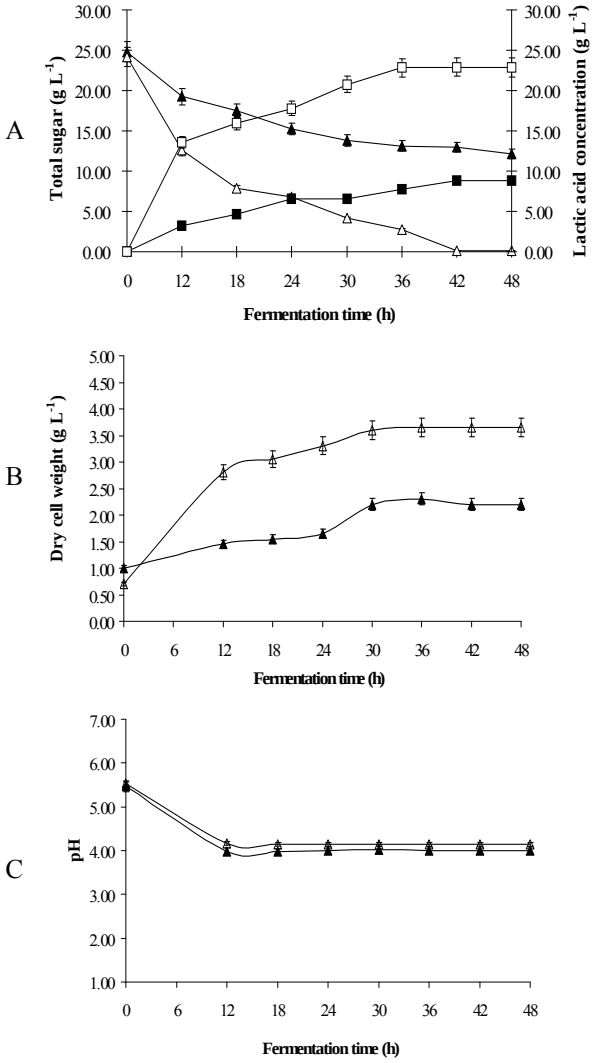

Figure 1. Kinetic profiles of total sugars consumption (Δ: OPS+MRS; ▲: OPS) and lactic acid production (□: OPS+MRS; ■: OPS) A, Dry cell weight B, and pH C over time of fermentation for lactic acid production by *L. casei* TISTR 1500 with▲: OPS and Δ: OPS+MRS.

Figure 1A shows sugar consumption in with and without addition MRS medium to the oil palm sap. The consumption of total sugars (99.38%) in the with-addition MRS medium of the oil palm sap was higher than that in the without-addition MRS medium (46.93%). The total sugars uptake in the with-addition MRS medium to the oil palm sap continued until the end of fermentation (42 h).

Cell growth was higher in the oil palm sap with the addition MRS medium (Figure 1B). The bacteria grew rapidly for 36 h and then ceased to grow. This was correlated with the rapid decline of pH due to the sugar having been metabolized by the cells to form acidic metabolites. This caused growth inhibition and lactic acid production.

It is worth emphasizing that all these assays were performed without pH control which clearly affected the fermentation performance. As shown in Figure 1C, at the beginning of the process (the first 12 h), pH decreased from 5.46 to 4.00 in all fermentation media as a consequence of lactic acid production by the microorganisms. This affected the microorganisms' metabolism which preformed better in a pH range between 5.0 and 7.0 [21]. Moreover, pH 5.5 has been used for lactic acid production using *L. helveticus* [22]. Hydrogen ion concentration of a medium has the maximum influence on microbial growth. The pH affects at least two aspects of microbial cells; the functioning of its enzymes and the transport of nutrients into the cell. It limits the synthesis of metabolic enzymes responsible for the synthesis of new protoplasm. The pH values also affect RNA and protein synthesis. When microorganisms are grown on either side of their optimum pH range, there may be an increased the cell growth in lag phase.

The kinetic parameters of batch cultivation in a static flask are given in Table 2. Kinetic values obtained from cultivation with MRS medium in the oil palm sap were higher than from the oil palm sap without the MRS medium. Lactic acid yields, based on total sugars consumed, were obtained at 95.00%. Moreover, the maximum lactic acid productivity (0.55 g L^{-1} h^{-1}) and dry cell weight (3.65 g L^{-1}) were found in the MRS-contained oil palm sap. The addition of MRS medium enhanced the product yield of oil palm sap by 35.71% (Table 2).

3.2 Effect of pH control on fermentation

The pH level is one of the most important environmental parameters affecting cell growth and product formation. In general, the effects of pH on cell growth and product accumulation vary with different microorganisms, medium compositions, and operational conditions. Some literature [1,23-24] dealing with conditions of *lactobacillus* strain reported the optimal pH varies between 5.0 to 7.0 for cell growth and lactic acid production. To date, no reports have been found about the effects of pH control on cell growth and lactic acid production in a lab-scale fermentation with oil palm sap as the substrate.

Lactic acid-producing bacteria (LAB) are constantly confronted with acidified environments, making acid stress part of their life cycle due to their ability to ferment sugars into lactate. The purpose of this experiment was to evaluate the inhibitory effect on growth and lactic acid production by *L. casei* TISTR 1500 exposed to conditions of stress caused by acidification of the medium. The results are displayed in Figure 2.

Firstly, it is important to note that microorganisms were able to grow and produce lactic acid in both culture media tested (with, and without pH control). There was a similar growth pattern within both media reaching a stationary phase after 48 h of fermentation. During the initial 12 h, a similar performance was observed in fermentations with and without pH control. However, the consumption of total sugars, lactic acid production and cell growth were influenced by the fermentation pH (Figure 2).

According to some authors [1,25], weak acids, *e.g.* lactic acid, inhibit bacterial growth because as the external pH declines, the acid is protonized as soon as it is exported out of the bacteria. Lactic acid production in pH-controlled oil palm sap supplemented with MRS components was 26.89 g L^{-1} whereas in the medium without pH control only 22.90 g L^{-1} was obtained (Figure 2).

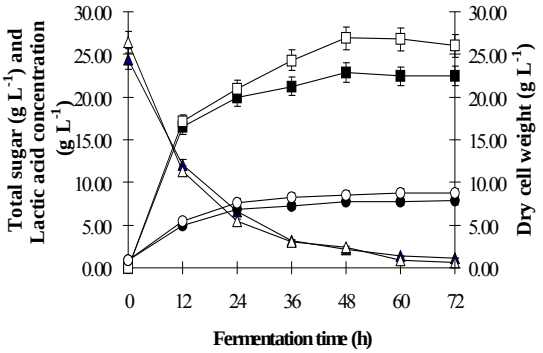

Figure 2. Effect of pH control on total sugars consumption (Δ: pH control; ▲: without pH control), Lactic acid production (□: pH control; ■: without pH control) and Dry cell weight of *L. casei* TISTR 1500 (O: pH control; ●: without pH control) in media: oil palm sap with MRS components.

These values represent an increase in lactic acid concentration of 17% when the pH of the MRS-supplemented oil palm sap was controlled. This result was similar to that acheived by Wee et al. [26] *Enterococcus faecalis* RKY1 grown on molasses preferred neutral or alkali conditions for lactic acid fermentation. When acidic conditions (pH 5.0) were used in lactic acid production, cell growth ceased after 10 h. Microorganisms were able to grow and produce lactic acid with the highest efficiency in the MRS-supplemented oil palm sap, which could have favored the bioconversion process since the higher the cell concentration the larger the amount of substrate which could be consumed and converted into product (Figure 2).

According to Idris and Suzana [23] lactic acid production depends on microbial growth, thus an increase in microbial growth promotes an increase in the lactic acid production. Mussatto et al. [27] found that after 60 h fermentation lactic acid production by *L. delbrueckii* UFV H2B20 in brewer's grain cellulosic hydrolysate supplemented with MRS components, and pH controlled at 6.0, $Y_{P/S}$ and R_M values of 0.99 g g^{-1} and 0.59 g L^{-1} h^{-1}, respectively, were obtained.

Table 3 summarizes the fermentative parameters obtained in the fermentation runs with and without pH control. It was found that the kinetic parameters of pH control of the MRS-supplemented oil palm sap were highest with the following results: conversion yield of substrate to product ($Y_{P/S}$) 1.04 g lactic acid g^{-1} total sugars, cellular yield coefficient ($Y_{X/S}$) 0.31 cells g^{-1} total sugars, and the maximum productivity (R_M) 0.56 g lactic acid L^{-1} h^{-1}. In the culture, both for the uncontrolled and controlled pH, not only lactic acid but also acetic acid (0.26 g L^{-1}) were the main products in the fermentation broth. These results indicated that glucose, the main sugar in the oil palm sap, was metabolized to lactic acid via the heterolactic fermentation pathway or mixed acid fermentation.

As tabulated in Table 3, with pH control of oil palm sap, product yield increases were 6.12%. This indicated that the effect of pH control of oil palm sap rendered only a slight significant increase in lactic acid production. Therefore, pH control from oil palm sap may not be required. Cost-effectiveness of controlled versus uncontrolled pH for lactic acid production should be further studied.

Table 3. Fermentation parameters of lactic acid production by *L. casei* TISTR 1500 in different fermentation media.

	OPS+MRS	
	Without pH control	With pH control
Lactic acid (g L^{-1})	22.90±0.240	26.89±0.400
Enhance of product yield (%)	-	6.12
Acetic acid (g L^{-1})	0.25±0.120	0.26±0.080
$Y_{P/S}$ (g g^{-1})	0.98±0.049	1.04±0.001
$Y_{X/S}$ (g g^{-1})	0.30±0.005	0.31±0.020
R_M (g L^{-1} h^{-1})	0.48±0.007	0.56±0.012

Batch fermentations were performed on a 2.0 L stirred tank bioreactor with 1 L working volume at pH 5.5, 37°C for 48 h. Results are the average of data from triplicate experiments.

Different results between the fermentation of the MRS-supplemented oil palm sap in flask (Table 2) and in fermenter (Table 3) were evaluated with kinetic parameters. For flask cultivation without pH control, pH steadily dropped during the time course of cultivation. This caused growth inhibition and lactic acid production. Lactic acid production in a fermenter with controlled pH 5.5 using oil palm sap as a substrate was investigated. Comparing lactic acid production in flask and fermenter cultures, lactic acid concentrations (26.89 g L^{-1}), lactic acid yield (1.04), and productivity (0.56) were higher than those in flasks. These results were similar to those obtained by Wee et al. [26], and Prachamon et al. [28] in that lactic acid production using sugar cane juice as a carbon source by *L. casei* TISTR 390 in a fermenter increase lactic acid yield and productivity more than in flask cultivation.

L. casei is an anaerobic microorganism. Consequently, the microorganism grows better in a static culture where the fermentation conditions are anaerobic. Panesar et al. [29] used *L. casei* for L(+)lactic acid production. They found that no difference was observed for lactic acid production with agitation. Moreover, Gandhi et al. [30] used stationary conditions for the lactic acid production using different lactobacilli cultures (*L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus, L. casei* etc.).

With increasing interests in producing biotechnological products from low-cost and renewable biomass, production of lactic acid from various raw agricultural materials has gained considerable attention recently. Many microorganisms, such as lactic acid bacteria (LAB), have been investigated for the production of lactic acid. Some examples of microbial lactic acid production from agricultural resources by LAB are shown in Table 4.

Table 4. Data reported on batch fermentations for lactic acid from agricultural resources.

Microorganism	Raw material	Initial sugar (g L^{-1})	Lactic acid (g L^{-1})	Productivity (g L^{-1} h^{-1})	Reference
Lactobacillus delbrueckii	Hydrolyzed cane sugar	150.00 (sucrose)	128.50	3.20	[2]
Lactobacillus lactis ATCC19435	Oil palm sap	18.95 (total sugars)	17.04	0.24	[7]
Lactobacillus delbrueckii	Brewer's spent grain	50.00 (glucose)	35.54	0.59	[30]
Lactobacillus lactis IO-1	Sugarcane bagasse	30.00 (xylose)	10.85	0.17	[31]
Lactobacillus rhamnosus	Wheat bran	25.00 (wheat bran hydrolysate)	75.00	3.75	[32]
Lactobacillus delbrueckii NCIMB 8130	Molasses	100.00 (molasses sugar)	90.00	3.80	[33]
Lactobacillus delbrueckii Uc-3	Molasses	148.00 (molasses sugar)	129.00	4.30	[34]
Lactobacillus delbrueckii NCIMB 8130	Molasses	190.00 (molasses sugar)	166.00	4.15	[34]
Lactobacillus bulgaricus	Whey	50.00 (lactose)	20.80	0.30	[35]
Lactobacillus casei B-442	Cashew apple juice	50 (reducing sugar)	47.37	2.36	[36]
Lactobacillus casei TISTR 1500	Oil palm sap	20.00 (total sugars)	22.90	0.55	This study

Relatively low lactic acid concentrations were obtained when oil palm sap [7], sugarcane bagasse [31], and whey [35] were used for lactic acid production. However, higher concentrations of lactic acid were reported when using hydrolyzed cane sugar [2], brewer's spent grain [30], wheat bran [32], molasses [33-34], and cashew apple juice [36]. In the present study, high productivity of lactic acid could be obtained by using oil palm sap. Hence, oil palm sap is potentially more feasible and more efficient in lactic acid production using *L. casei* TISTR 1500.

4. Conclusion

This study has shown the potential use of *L. casei* TISTR 1500 for the bioconversion of oil palm sap into lactic acid. Lactic acid production by *L. casei* TISTR 1500 in oil palm sap was influenced by MRS supplementation. The highest values of production yield and maximum productivity were 0.95 g g^{-1} and 0.55 g L^{-1} h^{-1}, respectively, when using flasks under static conditions without controlled pH at 37°C and 20 g L^{-1} of total sugars. Oil palm sap was proven to be a great potential raw material for lactic acid production by *L. casei* TISTR 1500.

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References

[1] Hofvendahl K, Hahn-Hagerdal B, Factors affecting the fermentative lactic acid production from renewable resources, *Enzyme Micro Technol* 26 (2000) 87-107.

[2] Kadam SR, Patil SS, Bastawde KB, Khire JM, Gokhale DV, Strain improvement of *Lactobacillus delbrueckii* NCIM 2365 for lactic acid production, *Process Biochem* 41 (2006) 120-126.

[3] Patil SS, Kadam SR, Bastawde KB, Khire JM, Gokhale DV, Production of lactic acid and fructose from media with cane sugar using mutant of *Lactobacillus delbrueckii* NCIM 2365, *Lett Appl Microbiol* 43 (2006) 53-57.

[4] Cai H, Thompson R, Budinich MF, Broadbent, JR, Steele JL, Genome sequence and comparative genome analysis of *Lactobacillus casei*: insights into their niche-associated evolution, *Genome Biol Evol* 1 (2009) 239-257.

[5] Kinoshita H, Uchida H, Kawai Y, Kitazawa H, Miura K, Shiba K, Horii A, Saito T, Quantitative evaluation of adhesion of Lactobacilli isolated from human intestinal tissue to human colonic mucin using surface plasmon resonance (BIACORE assay), *J Appl Microbiol* 102 (2007) 116-123.

[6] Bruno Barcena JM, Ragout AL, Cordoba PR, Sineriz F, Continuous production of L(+)-lactic acid by *Lactobacillus casei* in two-stage systems, *Appl Microbiol Biotechnol* 51 (1999) 316-324.

[7] Vaccari G, Gonalez-Varay RA, Campi AL, Dosi E, Brigidi P, Matteuzzi D, Fermentative production of L-lactic acid by *Lactobacillus casei* DSM 20011 and product recovery using ion exchange resins, *Appl Microbiol Biotechnol* 40 (1993) 23-27.

[8] Bulut S, Elibol M, Ozer D, Effect of different carbon sources on L(+)-lactic acid production by *Rhizopus oryzae, Biochem Eng J* 21 (2004) 33-37.

[9] John RP, Nampoothiri KM, Pandey A, Solid-state fermentation for L-lactic acid production from agro wastes using *Lactobacillus delbrueckii*, *Process Biochem* 41 (2006) 759-763.

[10] Tanaka T, Hoshina M, Tanabe S, Sakai K, Ohtsubo S, Taniguchi M, Production of D-lactic acid from defatted rice bran by simultaneous saccharification and fermentation, *Bioresour Technol* 97 (2006) 211-217.

[11] Kosugi A, Tanaka R, Magara K, Murata Y, Arai T, Sulaiman O, Hashim R, Hamid ZAA, Yahya MKA, Yusof MNM, Ibrahim WA, Mori Y, Ethanol and lactic acid production using sap squeezed from old oil palm trunks felled for replanting, *J Biosci Bioeng* 110 (2010) 322-325.

[12] Eze OM, Organ U, Sugars of unfermentated sap and the wine from the oil palm, *Elaeis quinensis*, tree, *Plant Food Hum Nutr* 38 (1988) 121-126.

[13] Obahiagbon FI, Osagie AU, Sugar and macrominerals composition of sap produced by *Raphia hookeri* palms, *Afr J Biotechnol* 6 (2007) 744-750.

[14] Kurane R, Hatamochi K, Kakuno T, Kiyoshara M, Hirano M, Taniguchi Y, Production of a bioflocculant by *Rhodococcus erythropolis* S-1 grown on alcohols, *Biosci Biotechnol Biochem* 58/2 (1994) 428-429.

[15] Dermlim W, Prasertsan P, Doelle HW, Screening and characterization of bioflocculant produced by isolated *Klebsiella sp*, *Appl Microbiol Biotechnol* 52 (1999) 698-703.

[16] Sura-apinan P, Development of chromatographic methods to investigation the purification process of lactic acid from lactic acid bacteria, M.Sc Thesis, Prince of Songkla University, Thailand (2010).

[17] Liu S, Steinbuchel A, Investigation of poly (β-L-malic acid) production by strains of *Aureobasidium pullulans*, *Appl Microbiol Biotechnol* 46 (1997) 273-278.

[18] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F, Colormetric method for determination of sugars and related substances, *Analy Chem* 28 (1956) 350-356.

[19] AOAC International, Official methods of analysis, AOAC International, Arlington, VA.

[20] Pirt SJ, *Principles of microbe and cell cultivation*, London: Blackwell Scientific Publications (1975).

[21] Lasekan O, Buettner A, Christlbaure M, Investigation of important odorant of palm wine (*Elaeis guineesis*), *Food Chem* 105 (2007) 15-23.

[22] Ghaly AE, Tango MSA, Mahmood NS, Avery AC, Batch propagation of *Lactobacillus helveticus* for production of lactic acid from lactose concentrated cheese whey with microaeration and nutrient supplementation, *World J Microbiol Biotechnol* 20 (2004) 65-75.

[23] Idris A, Suzana W, Effect of sodium alginate concentration, bead diameter, initial pH and temperature on lactic acid production pineapple waste using immobilized *Lactobacillus delbrueckii, Process Biochem* 41 (2006) 1117-1123.

[24] Chang CE, Kim SC, So JS, Yun HS, Cultivation of *Lactobacillus crispatus* KLB46 isolated from human vagina, *Biotechnol Bioprocess Eng* 6 (2001) 128-132.

[25] Kashket ER, Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance, *FEMS Microb* 46 (1987) 233-244.

[26] Wee YJ, Kim JN, Yun JS, Ryu HW, Utilization of sugar molasses for economical L(+)-lactic acid production by batch fermentation of *Enterococcus faecalis*, *J Microb Technol* 35 (2004) 568-570.

[27] Mussatto SI, Fernandes M, Mancilha IM, Roberto I, Effects of medium supplementation and pH control on lactic acid production from brewer's spent grain, *Biochem Eng J* 40 (2008) 437-444.

[28] Prachamon T, Boonmee M, Hamsupo K, Lactic acid production using sugar cane juice as a substrate, *KKU Res J* 8 (2008) 1-8.

[29] Panesar PS, Kennedy JF, Knill CJ, Kosseva M, Production of L(+)-lactic acid using *Lactobacillus casei* from whey, *Braz Arch Biol Technol* 53 (2010) 219-226.

[30] Gandhi DN, Patel RS, Wadhla BK, Bansal N, Kaur M, Kumar G, Effect of agro-based by-products on production of lactic acid in whey permeat medium, *J Food Sci Technol* 37 (2000) 292-295.

[31] Laopaiboon P, Thani A, Leelavatcharamas V, Laopaiboon L, Acid hydrolysis of sugarcane bagasse for lactic acid production, *Bioresour Technol* 101 (2010) 1036-1043.

[32] Li Z, Han L, Ji Y, Wang X, Tan T, Fermentative production of L-lactic acid from hydrolysate of wheat bran by *Lactobacillus rhamnosus*, *Biochem Eng J* 49 (2010) 138-142.

[33] Kotzamanidis C, Roukas T, Skaracis G, Optimization of lactic acid production from beet molasses by *Lactobacillus delbrueckii* NCIMB 1830, *World J Microbiol Biotechnol* 18 (2002) 441-448.

[34] Dumbrepatil A, Adsul M, Chaudhari S, Khire J, Gokhale D, Utilization of molasses sugar for lactic acid production by *Lactobacillus delbrueckii* subsp. *delbrueckii* mutant uc-3 in batch fermentation, *Appl Environ Microbiol* 74 (2008) 333-335.

[35] Ghasemi M, Najafpour G, Rahimnejad M, Beigi PA, Sedighi M, Hashemiyeh B, Effect of different media on production of lactic acid from whey by *Lactobacillus bulgaricus*, *Afr J Biotechnol* 8 (2009) 81-84.

[36] Silveira MS, Fontes CPM, Guilherme AA, Fernandes AN, Rodrigues S, Cashew apple juice as substrate for lactic acid production, *Food Bioprocess Technol* (2010).