Lactic Acid Production Using Sugar Cane Juice as a Substrate
การผลิตกรดแลกติกโดยใช้น้ำอ้อยเป็นสารตั้งต้น

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ABSTRACT

To study lactic acid (LA) production from sucrose, five strains of lactic acid bacteria, namely *Lactobacillus plantarum* SP1-3, *Lactobacillus pentosus* KUB-ST 10-1, *Lactobacillus casei* TISTR 390, *Lactobacillus salivarius* ssp. *salivarius* TISTR 1112 and *Lactobacillus delbrueckii* ssp. *bulgaricus* TISTR 895 were investigated. Variation of 20-25 g l^{-1} carbon sources (glucose, sucrose and sugar cane juice) was carried out in flasks under static condition without controlled pH at 37˚C for 24 h. No significant differences were observed in LA production of all strains in the presence of glucose and sucrose. LA concentrations of 17-20 g l^{-1} were obtained from the cultivated cultures with glucose and sucrose except *Lb. salivarius* ssp. *salivarius* TISTR 1112 (11-12 g l^{-1}). The ability of the strains to produce LA was decreased when using sugar cane juice and supplemented with 5 g l^{-1} yeast extract as a substrate. However, higher LA concentrations (15-23 g l^{-1}) were noticed when the cultures were cultivated in a fermenter with controlled pH 6 at 37˚C and 150 rpm using 20-25 g l^{-1} sugar cane juice and supplemented with 5 g l^{-1} yeast extract. In addition, the cultivation time in the fermenter was shorter than that in the flask and higher productivity of LA was obtained.

บทคัดย่อ

งานวิจัยนี้ได้ศึกษาการผลิตกรดแลกติกโดยแบคทีเรียกรดแลกติก 5 สายพันธุ์ ที่ใช้น้ำตาลซูโครสได้ คือ *Lactobacillus plantarum* SP1-3, *Lactobacillus pentosus* KUB-ST 10-1, *Lactobacillus casei* TISTR 390, *Lactobacillus salivarius* ssp. *salivarius* TISTR 1112 และ *Lactobacillus delbrueckii* ssp. *bulgaricus* TISTR 895 ซึ่งเพาะเลี้ยงเชื้อแบบไม่เขย่าในระดับฟลาสก์ เป็นเวลา 24 ชม. ที่อุณหภูมิ 37˚C และไม่ควบคุมค่า pH โดยการแปรผันแหล่งคาร์บอน (20-25 กรัมต่อลิตร) คือ น้ำตาลกลูโคส น้ำตาลซูโครส และน้ำอ้อย ผลการทดลองพบว่าเมื่อใช้น้ำตาลกลูโคส และน้ำตาลซูโครสเป็นแหล่งคาร์บอน ผลผลิตกรดแลกติกไม่แตกต่างกัน ซึ่ง *Lb. plantarum* SP1-3, *Lb. pentosus* KUB-ST 10-1, *Lb. casei* TISTR 390 และ *Lb. delbrueckii* ssp. *bulgaricus* TISTR 895 สามารถผลิตกรดแลกติกได้ 17-20 กรัมต่อลิตร ส่วน *Lb. salivarius* ssp. *salivarius* TISTR 1112 ผลิตกรดแลกติกได้เพียง 11-12 กรัมต่อลิตร เท่านั้น

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ส่วนการใช้น้ำอ้อยที่เติมยีสต์สกัด 5 กรัมต่อลิตร เป็นสารตั้งต้นนั้น พบว่าเชื้อทั้ง 5 สายพันธุ์ ผลิตกรดแลกติกได้ลดลง แต่เมื่อนำแบคทีเรียกรดแลกติกทั้ง 5 สายพันธุ์นี้ มาเพาะเลี้ยงในถังปฏิกรณ์ชีวภาพโดยใช้น้ำอ้อย 20-25 กรัมต่อลิตร ที่เติมยีสต์สกัด 5 กรัมต่อลิตร เป็นสารตั้งต้น ด้วยการควบคุมค่าพีเอช 6 ที่อุณหภูมิ 37 °C และมีอัตราการกวน 150 rpm พบว่าแบคทีเรียกรดแลกติกทั้งหมดที่นำมาศึกษาสามารถผลิตกรดแลกติกได้ในปริมาณที่สูงขึ้น (15-23 กรัมต่อลิตร) และใช้ระยะเวลาในการเพาะเลี้ยงสั้นกว่าการเพาะเลี้ยงในระดับฟลาสก์ จึงให้อัตราผลผลิตกรดแลกติกสูงกว่าอีกด้วย

Key Words : Lactic acid, Lactic acid bacteria, Sugar cane juice, Sucrose
คำสำคัญ : กรดแลกติก แบคทีเรียกรดแลกติก น้ำอ้อย น้ำตาลซูโครส

Introduction

Lactic acid has been widely used in food, pharmaceuticals, cosmetics and chemical industries (VickRoy, 1985). The continuous increase in the demand of lactic acid has been due to its increasing applications in preparation of biodegradable polymers, medical sutures and green solvents (Datta *et al.*, 1995; Litchfield, 1996). Approximately 90% of lactic acid produced worldwide is made by bacterial fermentation. Microbiological fermentation of lactic acid offers the advantages in both utilization of renewable carbohydrates and production of optically pure L- or D-lactic acid depending on the strain selected (Amass *et al.*, 1998; Lunt, 1998).

Manufacturing cost of lactic acid is greatly influenced by the cost of raw materials; especially pure substrates such as glucose, sucrose and lactose (Anjana and Kumar, 2007). Hence, using cheap raw materials as a fermentation substrate for lactic acid is an alternative to reduce the cost of lactic acid production. Since, Thailand has renewable and abundant agricultural resources which are mainly composed of sucrose such as sugar cane juice (Timbuntam *et al.*, 2006), selection of lactic acid bacteria strains using sucrose-based substrate is of primary interest.

The aim of this study is to evaluate the possibility of using sucrose as a substrate, especially sugar cane juice for lactic acid bacteria strains to produce lactic acid.

Materials and methods

Microorganisms

Lactobacillus plantarum SP1-3 and *Lb. pentosus* KUB-ST 10-1 were kindly provided by the collection of Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University. *Lb. salivarius* ssp. *salivarius* TISTR 1112, *Lb. delbrueckii* ssp. *bulgaricus* TISTR 895 and *Lb. casei* TISTR 390 were purchased from Thailand Institute of Scientific and Technological Research (TISTR). The strains were preserved in MRS medium (de Man *et al.*, 1960) containing 20% vv^{-1} glycerol at -20 °C. The culture was propagated twice in MRS medium (initial pH 6.5, 18-24 h, 37°C) prior to use as an inoculum.

Cultures preparation

Ten percent of inoculum (vv^{-1}) were propagated in MRS broth or sugar cane juice which was obtained from Mitr Phol Sugar, Phuvieng, Thailand. The cultures were cultivated at 37 °C for 24 h. The composition of media were shown in Table 1.

For medium 3, sugar cane juice was diluted from its initial total sugar concentration of 136 g l-1 to approximately the same concentration as that in MRS medium. Five g l^{-1} yeast extract was supplemented to the diluted sugar cane juice to support growth as the juice did not contain significant amount of nitrogen (Table 2). The pH of all media was adjusted to 6.5 with 5 M HCl prior to sterilization at 121°C for 15 min.

Effect of carbon sources on lactic acid production

The 10% inoculum of culture was added into medium containing various carbon sources (glucose, sucrose and sugar cane juice) and incubated at 37°C for 24 h. The samples were withdrawn aseptically from the fermentation flasks at time intervals. Experiments were performed in duplicate.

Lactic acid production in fermenter using sugar cane juice as a substrate

The strains were cultivated in 1 L fermenter with working volume of 0.5 L at 37°C and 150 rpm agitation. pH was maintained constant at 6.0 during fermentation by the automatic addition of 5 N NaOH. The samples were withdrawn aseptically from the fermenter at time intervals.

Analytical methods

Lactic acid concentration was determined by HPLC (Thani, 2005; Timbuntam et al., 2006). RI detector and Inertsil ODS-3V column (250x4.6 mm I.D., GL Sciences, U.S.A) were used and controlled at 40°C with 0.1 M $NH_4H_2PO_4$ as a mobile phase at flow rate of 1 ml min^{-1}. Residual sugar concentration in fermentation broths was measured by phenol sulfuric method (Dubois *et al.*,1956).

Results and discussion

Five strains of lactic acid bacteria (*Lb. plantarum* SP1-3, *Lb. pentosus* KUB-ST 10-1, *Lb. casei* TISTR 390, *Lb. salivarius* ssp. *salivarius* TISTR 1112 and *Lb. delbrueckii* ssp. *bulgaricus* TISTR 895) were cultivated in static flasks with different carbon sources (i.e. glucose, sucrose and sugar cane juice). The results showed that there is small difference in lactic acid concentration when using glucose and sucrose as substrates (Table 3). Lactic acid concentration of 18.19-20.63 g l^{-1} and 17.78-20.27 g l^{-1}, productivity of 0.71-0.77 g l^{-1} and 0.66-0.70 g l^{-1} h^{-1} were obtained at glucose and sucrose, respectively. *Lb. salivarius* ssp. *salivarius* TISTR 1112 was the most inferior strain in lactic acid production. Lactic acid concentration of 5.56-10.03 g l^{-1} and productivity of 0.19-0.34 g l^{-1} h^{-1} were decreased when using sugar cane juice as substrate except *Lb. salivarius* ssp. *salivarius* TISTR 1112. From Fig. 1, it was found that *Lb. casei* TISTR 390 and *Lb. salivarius* ssp. *salivarius* TISTR 1112 showed the lowest lactic acid produced in sugar cane juice.

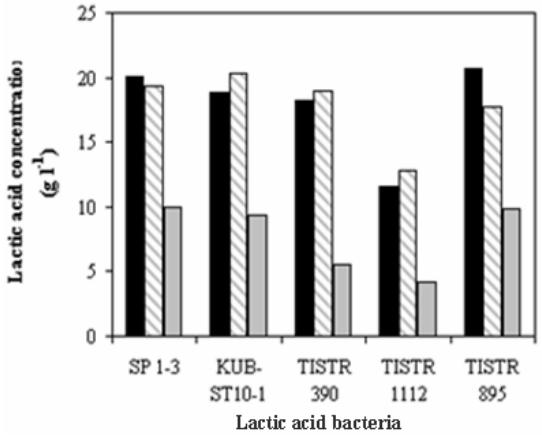

Fig. 1 Lactic acid production by five strains of lactic acid bacteria in static flasks with different carbon sources (■, glucose; ▨, sucrose; ■, sugar cane juice)

Regardless of amount of acid produced, the selected 5 strains are able to use sucrose as a substrate for lactic acid production. For flask cultivation without pH control, pH steadily dropped during the time course of cultivation. This causes growth inhibition and lactic acid production. Therefore, lactic acid production in fermenter with controlled pH 6.0 using sugar cane juice as substrate was investigated.

As shown in Table 4, *Lb. salivarius* ssp. *salivarius* TISTR 1112 and *Lb. casei* TISTR 390 gave the lowest lactic acid concentration and the longest cultivation time, respectively. Comparing lactic acid production in flask and fermenter cultures, lactic acid concentrations produced in fermenter were higher than those in flasks (Table 5). This indicates the importance of pH control (Fig. 2). The result was obtained similarly by Wee et al., (2004). They reported that *Enterococcus faecalis* RKY1 grown on molasses preferred neutral or alkali conditions for lactic acid fermentation. When acidic conditions (pH 5.0) was used in lactic acid production, cell growth was ceased after 10 h of fermentation.

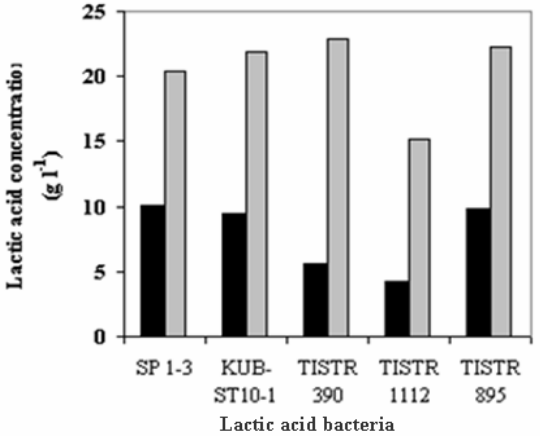

Fig. 2 Lactic acid production by lactic acid bacteria in flask and fermenter cultures using sugar cane juice as a carbon source (■, flask; ■, fermenter)

Moreover, the cultivation times in fermenter were shorter than those in the flask culture except for *Lb. casei* TISTR 390. Considering the yield of lactic acid, all strains used in this study are homofermentative with the yield approximately over 90%.

Conclusions

From the results of lactic acid concentration and productivity, *Lb. plantarum* SP1-3, Lb. *pentosus* KUB-ST 10-1 and *Lb. delbrueckii* ssp. *bulgaricus* TISTR 895 are potential for lactic acid fermentation using sugar cane juice as substrate. However, pH and substrate concentration should be optimized in fermenter cultivation.

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Table 1 Composition of the media for lactic acid production

Compositions	Medium 1 ($g\ l^{-1}$)	Medium 2 ($g\ l^{-1}$)	Medium 3 ($g\ l^{-1}$)
C-source	20 (Glucose)	20 (Sucrose)	20-25 (SCJ*)
Casein peptone	10	10	-
Meat extract	10	10	-
Yeast extract	5	5	5
Tween 80	1 ml	1 ml	-
K_2HPO_4	2	2	-
Sodium acetate	5	5	-
$(NH_4)_3$ citrate	2	2	-
$MgSO_4 \cdot 7H_2O$	0.2	0.2	-
$MnSO_4 \cdot H_2O$	0.05	0.05	-

***** SCJ = sugar cane juice

Table 2 Composition of sugar cane juice

Properties	Values	Method
pH	6.81*	pH meter
Total solid	15 °Brix*	Hand refractometer
Total sugar	136 $g\ l^{-1}$*	Phenol sulfuric method
Nitrogen	0.01 $g\ l^{-1}$*	Phenol-hyperchloric method

* Average values from the experiments

Table 3 Lactic acid concentration and productivity of five strains of lactic acid bacteria under different carbon sources

Lactic acid bacteria*	Lactic acid concentration** ($g\ l^{-1}$)			LA Productivity** ($g\ l^{-1}h^{-1}$)		
	Glucose	Sucrose	Sugar cane juice	Glucose	Sucrose	Sugar cane juice
Lactobacillus plantarum SP1-3	20.02	19.34	10.03	0.72	0.68	0.32
Lb. pentosus KUB-ST 10-1	18.86	20.27	9.40	0.75	0.70	0.32
Lb. casei TISTR 390	18.19	19.00	5.56	0.71	0.66	0.19
Lb. salivarius ssp. *salivarius* TISTR 1112	11.59	12.78	4.17	0.36	0.45	0.09
Lb. delbrueckii ssp. *bulgaricus* TISTR 895	20.63	17.78	9.83	0.77	0.68	0.34

* The cultures were grown under static condition at 37 °C for 24 h

** The results were calculated at 24 h

Table 4 Lactic acid production of the cultures in fermenter using sugar cane juice as carbon source

Lactic acid bacteria *	Lactic acid ($g\ l^{-1}$)	Sugar consumption ($g\ l^{-1}$)	Cultivation time (h)	Lactic acid yield ($Y_{P/S}$)	LA Productivity ($g\ l^{-1}h^{-1}$)**
Lactobacillus plantarum SP1-3	20.35	20.78	10	0.98	2.20 (10)**
Lb. pentosus KUB-ST 10-1	21.91	22.77	14	0.96	1.66 (14)**
Lb. casei TISTR 390	22.88	22.95	37	1.00	0.75 (31)**
Lb. salivarius ssp. *salivarius* TISTR 1112	15.19	16.64	12	0.91	1.48 (11)**
Lb. delbrueckii ssp. *bulgaricus* TISTR 895	22.28	25.1	16	0.89	1.63 (14)**

* The cultures were grown on 20-25 $g\ l^{-1}$ sugar cane juice in a fermenter

** Culture time at the highest lactic acid concentration

Table 5 Comparison of lactic acid production in flask and fermenter using sugar cane juice as carbon source

Lactic acid bacteria	Cultivations							
	Flask*				Fermenter**			
	LA ($g\ l^{-1}$)	Time (h)	LA yield ($Y_{P/S}$)	LA Productivity ($g\ l^{-1}h^{-1}$)***	LA ($g\ l^{-1}$)	Time (h)	LA yield ($Y_{P/S}$)	LA Productivity ($g\ l^{-1}h^{-1}$)
Lactobacillus plantarum SP1-3	10.03	24	0.85	0.32	20.35	10	0.98	2.20 (10)****
Lb. pentosus KUB-ST 10-1	9.40	24	0.79	0.32	21.91	14	0.96	1.66 (14)****
Lb. casei TISTR 390	5.56	24	0.65	0.19	22.88	37	1.00	0.75 (31)****
Lb. salivarius ssp. *salivarius* TISTR 1112	4.17	24	0.36	0.09	15.19	12	0.91	1.48 (11)****
Lb. delbrueckii ssp. *bulgaricus* TISTR 895	9.83	24	0.75	0.34	22.28	16	0.89	1.63 (14)****

* The cultures were grown under static condition at 37 °C for 24 h

** The cultures were grown on 20-25 $g\ l^{-1}$ sugar cane juice in a fermenter

*** The results were calculated at 24 h

**** Culture time at the highest lactic acid concentration