การหมักน้ำตาลผสมระหว่างกลูโคสและไซโลสโดย *Lactococcus lactis* IO-1 เพื่อผลิตกรดแลคติค

Fermentation of glucose and xylose mixtures by *Lactococcus lactic* IO-1 for lactic acid production

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บทคัดย่อ

Lactococcus lactis IO-1 ซึ่งเป็นแบคทีเรียกรดแลคติคพวกโฮโมเฟอร์เมนเททีฟ เมื่อเลี้ยงแบบกะในน้ำตาลผสมระหว่างกลูโคสและไซโลสในปริมาณที่เท่ากัน ที่ความเข้มข้นต่าง ๆ พบว่า *L. lactis* IO-1 ผลิตกรดแลคติคเป็นผลิตภัณฑ์หลัก และเกิดการเจริญแบบไดออกสิค นอกจากนี้ยังพบว่า แบคทีเรียชนิดนี้ผลิตกรดอะซิติค กรดฟอร์มิค และเอทานอลเป็นผลพลอยได้ เมื่อเลี้ยง *L. lactis* IO-1 ในน้ำตาลผสมที่ความเข้มข้นต่ำคือ 5 และ 10 กรัมต่อลิตร พบว่า กลูโคสและไซโลสถูกใช้หมด ในขณะที่เมื่อเลี้ยงในน้ำตาลผสมที่ความเข้มข้นสูงคือ 30 และ 50 กรัมต่อลิตร พบว่า กลูโคสถูกใช้หมด ส่วนไซโลสถูกใช้เพียง 25.6 และ 32.9 กรัมต่อลิตร ตามลำดับ การสังเกตนี้อาจเกิดจากกลูโคสเหนี่ยวนำการกดการใช้ไซโลส และการผลิตกรดแลคติคที่สูงอาจยับยั้งการใช้ไซโลส ส่วนผลได้โมลาร์ของกรดแลคติคเมื่อ *L. lactis* IO-1 ใช้น้ำตาลกลูโคส สูงกว่าผลได้โมลาร์ของกรดแลคติคเมื่อใช้น้ำตาลไซโลสอย่างมีนัยสำคัญ เมื่อเปรียบเทียบที่ความเข้มข้นของน้ำตาลที่เท่ากัน นอกจากนี้ยังพบว่าผลได้โมลาร์ของกรดแลคติคเมื่อใช้น้ำตาลผสมขึ้นอยู่กับความเข้มข้นของน้ำตาลไซโลสเป็นหลัก

Abstract

Lactococcus lactis IO-1, a homofermentative lactic acid bacterium, was grown on mixtures of the same amount of glucose and xylose at various concentrations in batch culture. The results showed that *L. lactis* IO-1 produced lactic acid as a main product and a diauxic growth was observed. In addition it produced acetic acid, formic acid and ethanol as by-products. When *L. lactis* IO-1 was grown on the mixtures of sugars at low concentrations (5 and 10 g l^{-1}), glucose and xylose were completely utilised. At high concentrations (30 and 50 g l^{-1}), glucose was completely consumed whereas xylose was utilised only 25.6 and 32.9 g l^{-1} respectively. This was possible due to glucose induces the repression of xylose utilisation and high lactic acid production may inhibit xylose utilisation. At the same sugar concentrations, molar yields of lactic acid during glucose utilisation were significantly higher than those during xylose utilisation. In addition the molar yields from glucose and xylose mixtures mainly depends on xylose concentration.

คำสำคัญ : กรดแลคติค น้ำตาลผสม *Lactococcus lactis*

Keywords : Lactic acid, mixed sugar, *Lactococcus lactis*,

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INTRODUCTION

Lactic acid has been produced commercially by fermentation process since 1881 and its fermentative production accounts for half of the world production (Vickroy, 1985). Lactic acid is used as acidulant, flavour and preservative in food, pharmaceutical, leather and textile industries. It is also used for polymerization to biodegradable polylactic acid (PLA) which is used for medical applications such as sutures and clips for wound closure or posthetic devices. Additionally, it is used for the production of basic chemicals (Hofvendahl and Hahn-Hägerdal, 2000).

Lactic acid bacteria (LAB) are believed to be facultative or microaerophilic microorganisms, obtaining their energy through the Embden-Meyerhof-Parnas pathway or phosphoketolase pathway (Sakamoto and Komagata, 1996). There are many evidences indicating that products occurred from carbohydrate metabolism depend on species of LAB, substrates and cultured conditions (Axelsson, 1993).

Hemicellulose is a major constituent of plant cell wall materials and makes up 30-40 % of many agricultural residues (Amartey and Jeffries, 1994). After pretreatment and hydrolysis of lignocellulosic materials, the hemicellulose fraction is liquefied to make sugars (xylose and glucose) accessible to fermenting microorganisms (Lynd et al., 1999; Aristidou and Penttila, 2000). However not all fermenting microorganisms can utilize xylose (5-atom carbon). There are many evidences indicating that *Lactococcus lactis* IO-1 is one of fermenting microorganisms which can utilise both carbon substrates for lactic acid production (Kanagachandran et al. 1997). The aim of this work is to study the effect of dual sugar i.e. glucose and xylose on lactic acid production in batch cultures by *Lactococcus lactis* IO-1. Molar product yields of glucose and xylose utilisation of the microorganism are also investigated.

Materials and methods

Microorganism

Lactococcus lactis IO-1 (JCM 7638) was donated by Peter F. Stanbury, University of Hertfordshire, Hatfield, UK. The microorganism from stock culture was transplanted into a sterile Borosilicate culture tube containing 9 ml of sterile thioglycolate medium (Difco, USA) at two weeks intervals and stored in a refrigerator at 4°C.

Medium

The basal medium was composed of (per litre of distilled water) 5.0 g yeast extract (Oxoid, England), 5.0 g peptone (Oxoid, England) and 5.0 g NaCl (BDH, England). The medium was supplemented with 5, 10, 30 and 50 g glucose (Fluka, Switzerland) and the same amount of xylose (Fluka, Switzerland) and adjusted to pH 6.0 with 2 mol l^{-1} of HCl.

Inoculum

The stock culture was rejuvenated by incubation in 10 ml of thioglycolate medium for 18 h at 37°C in a static incubator. The 18-h culture (10 ml) was then transferred into 100 ml of basal medium containing mixtures of glucose and xylose (5, 10, 30 and 50 g l^{-1}) and incubated at 37°C with agitation 150 rpm for 3 h. An inoculum (5% by volume) was used to initiate the batch cultures.

Culture conditions

All batch fermentations throughout this work were conducted in a Biostat B (B.Braun, Germany)

2.5-litre fermenter with the working volume of one litre. The culture was agitated at 200 rpm and the temperature was controlled at 37 ?C. The pH of the cultures was monitored using an in situ pH electrode and maintained at pH 6.0 by automatic addition of 2.5 mol l^{-1} of NaOH. Steady-state conditions were indicated by stable of biomass, substrates and product levels.

Analytical methods

Bacterial growth was monitored by spectrophotometric measurement at 562 nm (UV-1601 spectrophotometer, Shimadzu, Japan) and converted to cell dry weight from standard calibration curve. Determination of sugars (glucose and xylose) and metabolic end products (lactic acid, formic acid, acetic acid and ethanol) from fermentation supernatant was performed by HPLC with a refractometer detector (RID-6A, Shimadzu, Japan) using an Aminex HPX $87H^{+}$ column (300 mm X 7.8 mm, Bio - Rad Lab, CA, USA) under the following conditions: a temperature of 45 ^{o}C and 5 mmol l^{-1} of H_2SO_4 as a mobile phase at a flow rate of 0.6 ml min^{-1}.

Molar yields of product were calculated as mole of product produced per mole of sugar utilised.

Results

During the batch growth of *L. lactis* IO-1 at various concentrations of glucose and xylose, mixtures of lactic acid, formic acid, acetic acid and ethanol were produced (Fig. 1). When *L. lactis* IO-1 was grown in the medium containing a mixture between 5 g glucose l^{-1} and 5 g xylose l^{-1} (Fig. 1A), initially only glucose was utilised and xylose utilisation started after glucose was completely consumed. Completion of both glucose and xylose utilisation was observed at 23 h. At this time, biomass, lactic acid, formic acid, acetic acid and ethanol concentrations were 0.52, 5.02, 1.12, 1.50 and 0.52 g l^{-1} respectively. When *L. lactis* IO-1 was grown in the medium containing 10 g glucose l^{-1} and 10 g xylose l^{-1}, complete utilisation of the dual substrates was observed at 31 h (Fig. 1B). The result showed that the profiles of glucose and xylose utilisation and product formation were similar to those on the medium containing 5 g glucose l^{-1} and 5 g xylose l^{-1}. At 31 h, biomass, lactic acid, formic acid, acetic acid and ethanol concentrations were 0.91, 10.95, 3.34, 4.01 and 1.04 g l^{-1} respectively.

During the batch growth of *L. lactis* IO-1 on the medium containing 30 g glucose l^{-1} and 30 g xylose l^{-1} (Fig. 1C), only glucose was completely utilised whereas xylose was utilised only 25.6 g l^{-1}. At the beginning of steady state (93 h), biomass, lactic acid, formic acid, acetic acid and ethanol concentrations were 1.00, 45.57, 2.62, 6.18 and 2.35 g l^{-1} respectively. When *L. lactis* IO-1 was grown in the presence of 50 g glucose l^{-1} and 50 g xylose l^{-1}, complete utilisation of glucose was also observed whereas xylose was utilised only 32.9 g l^{-1} (Fig. 1D). The system reached steady state at 133 h. At this time, biomass, lactic acid, formic acid, acetic acid and ethanol concentrations were 1.65, 58.15, 0.98, 1.96 and 0.79 g l^{-1} respectively.

Molar product yields calculated from the batch culture profiles of the dual sugars (Fig. 1) are summarised in Table 1. At glucose consumption phase, the molar product yields of each product were similar at all glucose concentrations. High molar yields of lactic acid (1.52 - 1.75 mol mol^{-1} glucose utilised) and low molar yields of the by-products

(0.04 – 0.13 mol mol^{-1} glucose utilised) were observed at this phase. At xylose consumption phase, the molar yields of lactic acid increased whereas the molar yields of the by-products decreased with increasing xylose concentration. At the second phase, lower molar yields of lactic acid and higher molar yields of the by-products were observed when compared to those of the first phase at the same sugar concentrations.

Discussion

Co-fermentation of glucose and xylose was carried out by using *L. lactis* IO-1. The experiments indicated that a classic diauxic profile was exhibited. Sequential utilisation resulting in diauxic growth has often been observed (Brandt et al., 2004). Acetic acid, formic acid and ethanol were markedly produced as a result of xylose utilisation and, thus, appeared late in the batch cultures. Preferential utilisation of glucose over xylose and catabolite repression of xylose uptake has been described in various studies. When *Pichia stipitis* Y 7124 was grown in multiple substrates, sequential substrate consumption was observed. Initially, *Pichia stipitis* Y 7124 assimilated glucose and xylose utilisation occurred immediately after glucose depletion (Delgenes et al., 1989). Tyree et al. (1990) investigated the substrate utilisation and product formation of *Lactobacillus xylosus* on glucose-xylose mixtures in batch cultures. They found that glucose completely prevented xylose utilisation when *L. xylosus* was grown on 5 g glucose l^{-1} and 30 g xylose l^{-1}. Bothast et al. (1994) reported the ability of *Klebsiella oxytoca* strain P2 to ferment glucose, xylose and arabinose mixtures in batch cultures. The strain preferred glucose to xylose. Approximately 29-49 % of supplied xylose was left unutilised in the fermented broth.

In this study, the time to reach steady state in the batch cultures increased with increasing sugar concentration (Fig. 1). Similar observations were reported in fructose batch cultures of *Acetobacter xylinum* subsp. *sucrofermentans* BPR 3001A and BPR 2001 (Naritomi *et al.*, 1998).

Ishizaki and Ueda (1995) revealed that the levels of acetic acid produced in xylose batch culture did not inhibit growth of *L. lactis* IO-1 whereas lactic acid was responsible for end-product inhibition. Growth inhibition in other LAB such as *Lactobacillus casei* (Gonzàlez-Vara et al., 2000) and *L. delbrueckii* (Tohyama et al., 2000) by lactic acid has also been demonstrated.

At the high concentrations of the substrates i.e. 30 and 50 g l^{-1}, glucose was completely utilised but xylose was utilised only 85 and 66 % respectively. The observation might be due to glucose induces the repression of xylose utilisation and lactic acid produced during glucose consumption may inhibit xylose utilisation. A similar observation was reported by Bibal et al. (1988) who studied lactic acid production of from lactose by *Streptococcus cremoris* at an initial sugar concentration of 50 g l^{-1}.

Lactic acid production during glucose fermentation indicates that glucose is metabolised to lactic acid via Embden-Meyerhof-Parnas pathway (EMP) (Axelsson, 1993; Erten, 1998; Laopaiboon, 2001). At xylose consumption phase, the molar yields of lactic acid increased and there was a decrease in the molar yields of formic acid, acetic acid and ethanol when xylose concentration increased. This suggests

that xylose may be metabolised to lactic acid via the pathway of heterolactic fermentation or mixed acid fermentation (Cocaign-Bousquet *et al*., 1996). In addition ethanol was produced during the batch growth of *L. lactis* IO-1 at all mixed sugar concentrations indicating that the reducing equivalent equilibrium necessitated the reduction of acetyl-Co A to ethanol (Laopaiboon, 2003). However, ethanol production in the medium containing 50 g glucose l^{-1} and 50 g xylose l^{-1} was less than those at the lower dual sugar concentrations suggesting that the reducing equivalent equilibrium during batch growth of *L. lactis* IO-1 at 50 g glucose l^{-1} and 50 g xylose l^{-1} necessitated the reduction of less acetyl-Co A to ethanol than those at the lower mixed sugar concentrations. These results are in agreement with those of Brown and Collin (1977) and Nov?k et al. (1997) using glucose as a substrate for *Streptococcus sp*. and *L. lactis* respectively.

Lactic acid production and its molar yield from glucose and xylose mixtures in batch culture of *L. lactis* IO-1 highly depends on xylose concentration. Therefore, an optimisation of xylose utilisation by the microorganism has to be investigated to maximise xylose conversion to product and to minimise the difficulties of effluent treatment.

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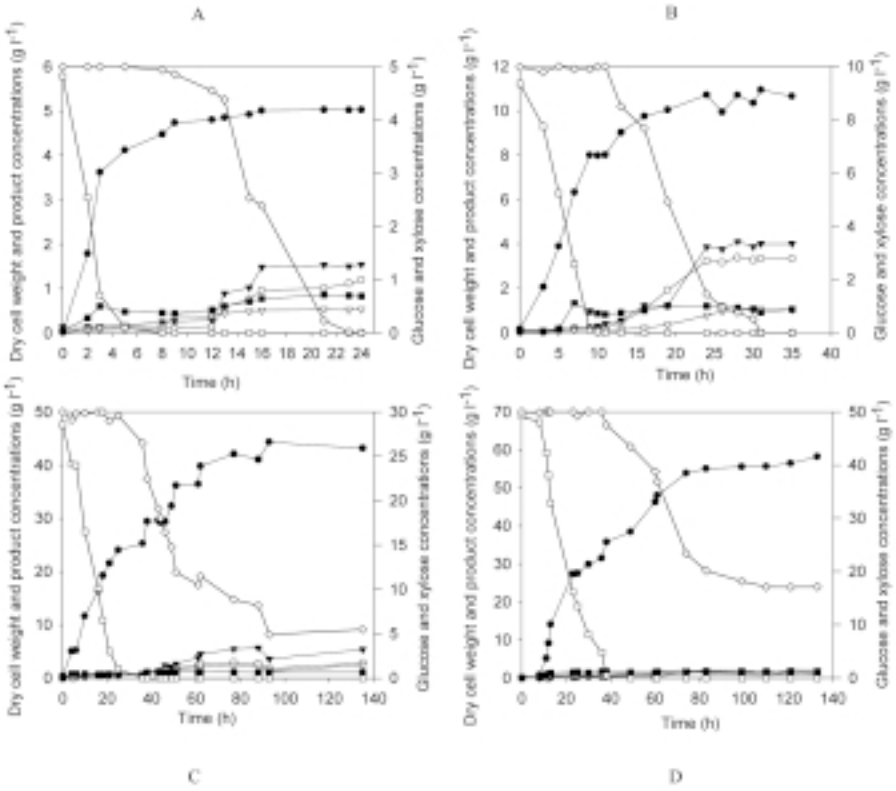

Fig. 1 Batch culture profiles of *L. lactis* IO-1 grown on glucose and xylose mixtures (A) 5 g, (B) 10 g, (C) 30 g and (D) 50 g l^{-1} : lactic acid (●), formic acid (▽), acetic acid (▼), ethanol (○), dry cell weight (■), glucose (□), xylose (◇).

Table 1 Effect of initial glucose and xylose concentrations on molar product yields in batch cultures of *Lactococcus lactis* IO-1.

Initial glucose concentrations (g l^{-1})	Molar product yields (mol product mol^{-1} glucose utilised)			
	Lactic acid	Formic acid	Acetic acid	Ethanol
5	1.75	0.05	0.13	0.11
10	1.62	0.07	0.09	0.10
30	1.61	0.09	0.05	0.11
50	1.52	0.10	0.04	0.10
Initial xylose concentrations (g l^{-1})	Molar product yields (mol product mol^{-1} xylose utilised)			
	Lactic acid	Formic acid	Acetic acid	Ethanol
5	0.12	1.03	1.18	0.53
10	0.22	0.97	0.90	0.31
30	0.54	0.32	058	0.24
50	0.91	0.12	0.08	0.13