ผลของการให้อากาศต่อการหมักน้ำตาลไซโลสแบบกะโดย *Lactococcus lactis*

Effect of Aeration on Xylose Fermentation in Batch Cultures by *Lactococcus lactis*

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บทคัดย่อ

Lactococcus lactis IO - 1 (JCM 7638) ถูกเลี้ยงในอาหารพื้นฐานที่มีน้ำตาลไซโลส (5 กรัมต่อลิตร) เป็นแหล่งคาร์บอนในสภาวะที่มีการให้อากาศและไม่ให้อากาศ เพื่อศึกษาถึงอิทธิพลของการให้อากาศต่อไพรูเวทเมตาบอลิซึม ผลการทดลองพบว่า ภายใต้สภาวะที่มีการให้อากาศ *L. lactis* IO-1 ผลิตแลคเตทและอะซิเตท 0.14 และ 0.69 โมลต่อโมลไซโลสที่ใช้ ตามลำดับ ในขณะที่เมื่อเลี้ยงภายใต้สภาวะที่ไม่ให้อากาศจุลินทรีย์นี้ผลิตแลคเตท อะซิเตท ฟอร์เมท และเอทานอล 0.15, 0.73, 0.92 และ 0.43 โมลต่อโมลไซโลสที่ใช้ตามลำดับ ภายใต้สภาวะที่มีการให้อากาศฟอร์เมทไม่ถูกผลิตอาจเนื่องจากเกิดการยับยั้งไพรูเวทฟอร์เมทไลเอสโดยออกซิเจน ในขณะที่ฟอร์เมทและเอทานอลถูกพบภายใต้สภาวะที่ไม่ให้อากาศ แสดงให้เห็นว่ามีการทำงานของไพรูเวทฟอร์เมทไลเอส และ/หรือ ไพรูเวทดีไฮโดรจีเนส

Abstract

Lactococcus lactis IO-1 (JCM 7638) was anaerobically and aerobically cultured in basal medium implemented with xylose (5 g l^{-1}) as a carbon source to study the influence of aeration on pyruvate metabolism. The results showed that under aerobic conditions *L. lactis* IO-1 produced lactate and acetate by 0.14 and 0.69 mol mol^{-1} xylose utilized respectively whereas it produced lactate, acetate, formate and ethanol by 0.15, 0.73, 0.92 and 0.43 mol mol^{-1} xylose utilized respectively under anaerobic conditions. Under aerobic conditions formate was not produced possibly due to the inhibition of pyruvate formate lyase by oxygen. Formate and ethanol were detected under anaerobic conditions, suggesting that pyruvate formate lyase and/or pyruvate dehydrogenase were operational.

คำสำคัญ: การให้อากาศ, ไพรูเวทเมตาบอลิซึม

Keywords: Aeration, Pyruvate metabolism, *Lactococcus lactis*

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Introduction

Lactic acid bacteria (LAB) play an important role in the manufacture of fermented foods, not only because the end-products of these fermentations confer protection against spoilage, but also some of them contribute to the flavour and texture of the fermented products (Cocaign-Bousquet et al., 1996; de Vos et al., 1998).

Lactic acid bacteria are believed to be facultative anaerobic or microaerophilic microorganisms, obtaining their energy through the Embden-Meyerhof-Parnas pathway or phosphoketolase pathway (Sakamoto and Komagata, 1996). There are many evidences which showed that products occurred from carbohydrate metabolism mainly depending on species of lactic acid bacteria, substrates and cultured conditions. Some strains, e.g. *Lactobacillus plantarum*, grow well under aerobic conditions and produce a large amount of acetate in glucose medium (Murphy and Condon, 1984). Some hetero-fermenters utilize oxygen and produce lactate and acetate as main end products from glucose (Sakamoto and Komagata, 1996). Cogan ;Walsh and Codon. (1989) reported that in galactose batch cultures lactate, formate, acetate and ethanol were produced by *Streptococcus lactis* under anaerobic conditions, whereas acetate and acetoin were formed under aerobic conditions.

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 the sugar (glucose and xylose) accessible to fermenting microorganism (Lynd, Wyman and Gerngross., 1999; Aristidou and Penttila, 2000). However not all fermenting microorganisms can utilize xylose (5-atom carbon). Therefore, it will be very useful if there is a fermenting microorganism which can utilize both carbon substrates to maximize carbon conversion to the product and to minimize the difficulties of effluent treatment.

Lactococcus lactis IO-1 is a bacterium capable of producing L-lactate at the expense of both glucose and xylose (Ishizaki et al., 1993; Kanagachandran et al., 1997). Many researchers have investigated the feasibility of glucose and/or xylose utilization for lactic acid production by *Lactococcus lactis* under unaerated conditions (Cocaign-Bousquet et al., 1996; Hols et al., 1999; Erlandson et al., 2000) but no studies under aerated conditions. Hence, the aim of this work was to study the influence of aeration on metabolic end products in pyruvate metabolism of *L. lactis* IO-1 in xylose batch cultures.

Materials and methods

Microorganism

Lactococcus lactis IO-1 (JCM 7638) from stock culture was transplanted into a sterile Borosilicate culture tube containing 9 ml of sterile thioglycolated medium (Difco, USA) at two week 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, Basingstoke, England), 5.0 g peptone (Oxoid) and 5.0 g NaCl (BDH, Lutterworth, England) . The medium was supplemented with 5 g xylose l^{-1}.

Inoculum

The stock culture was revived by incubation in 10 ml thioglycolated 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 5 g xylose l^{-1} and incubated at 37oC 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 Bioflow III (New Braunswick, USA) 2.5-litre fermenter with the working volume of 1000 ml. The culture was agitated at 400 rpm and a temperature of 37°C. The medium was sparged with air at a flow rate of 0.2 l h^{-1} for 2 h before inoculation

and was continually sparged throughout the cultivation under aerobic conditions. Under anaerobic conditions the medium was sparged with nitrogen gas at a flow rate of 0.2 l h^{-1} for 2 h before inoculation and was continually sparged throughout the cultivation. The pH of the cultures was monitored using an in situ pH probe 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, substrate and product levels.

Analytical methods

Bacterial growth was monitored by spectrophotometric measurements at 562 nm (Unicam 5625 spectrophotometer, UK) and converted to cell dry weight from a standard calibration curve. Determination of xylose and metabolic end products (lactate, formate, acetate and ethanol) from fermentation supernatant was performed by HPLC with a refractometer detector (refracto Monitor II, Milton Roy, England) using an Aminex HPX 87H' column (300 mm X 78 mm, Bio-Rad Lab, CA, USA) under the following conditions: a temperature of 50 °C, mobile phase 5 mmol l^{-1} of H_2SO_4 and a flow rate of 0.57 ml min^{-1}.

Results

L. lactis IO-1 was grown in the medium containing 5.0 g xylose l^{-1} in batch culture under aerobic and anaerobic conditions. The results showed that the profiles of xylose utilization and product formation in the conditions were similar (Fig. 1). However, the time to reach the steady state condition and the metabolic products occurring were different. Under aerobic conditions, the system reached the steady state condition at 24 h and a completion of xylose utilization was observed. At this time, biomass, lactate and acetate concentrations were 0.83 g l^{-1}, 0.43 g l^{-1} and 1.38 g l^{-1} respectively (Fig. 1a) . Under anaerobic conditions, xylose was completely utilized at only 11 h with 0.94 g l^{-1} of biomass, 0.37 g l^{-1} of lactate, 1.36 g l^{-1} of formate, 1.50 g l^{-1} of acetate and 0.59 g l^{-1} of ethanol were detected in the fermentation medium (Fig. 1b).

At the steady state conditions of both conditions biomass yield, molar product yields, and percentage of carbon recovery were calculated as shown in Table 1. The molar lactate yields were similar under both conditions, while the biomass and molar acetate yields under anaerobic conditions were higher than those under aerobic conditions approximately 19 and 6% respectively.

Discussion

Batch fermentations of xylose under aerobic and anaerobic conditions were carried out by using *L. lactis* IO-1. The results showed that *L. lactis* IO-1 produced a mixture of lactate and acetate under aerobic conditions. A continuous increase in lactate concentration during the growth on xylose batch culture under aerobic conditions (Fig. 1a) was reflected by an increased lactate dehydrogenase (LDH) pathway (Fig. 2). Formate was not detected under this condition possibly due to the inhibition of PFL (Fig. 2) by oxygen (Abbe et al., 1982; Takahashi et al., 1982; Higuchi, 1984; Cogan et al., 1989). The molar yield of acetate was observed under this condition indicating the generation of additional ATP via the acetate kinase pathway (Fig. 2). This also implied that pyruvate dehydrogenase (PDH) pathway was operational.

Under anaerobic conditions, *L. lactis* IO-1 produced a mixture of lactate, formate, acetate and ethanol suggesting that, in addition to LDH pathway, PFL and/or PDH were also operational (Fig. 2). The production of acetate would have contributed to the generation of extra energy (Fig. 2). Ethanol was detected under this condition indicating the generation of NAD via alcohol dehydrogenase pathway in addition to via LDH pathway.

The results indicated that the molar yield of acetate produced affected biomass yield. An increase in biomass yield under anaerobic conditions (Table 1) could be explained by an enhancement of ATP

production via the acetate kinase pathway. The results were similar to those of *Leuconostoc mesenteroides* subsp. cremoris in glucose and citrate batch cultures (Schmitt; et. al., 1992). Another reason was proposed by Archibald and Fridovich (cited in Fu and Mathews, 1999), who suggested that lower cell growth rate under aerobic conditions could be attributed to oxygen inhibition associated with superoxide (O_2^-).

The experiments indicated that the presence of oxygen increased the time to reach the steady-state condition in xylose batch cultures of *L. lactis* IO-1. The result was consistent with that of Fu and Mathews (1999), who studied lactose metabolism by *L. plantarum*. They found that the complete lactose utilization was extended from 33 hours for anaerobic condition to 53 hours for aerobic condition. The difference in the rate of sugar consumption could be attributed to differences in metabolic pathways under aerobic and anaerobic conditions (Murphy and Condon, 1984). In this study, the molar yields of lactate under aerobic and anaerobic conditions were not significantly different (Table 1). This implied that LDH pathway was not affected by oxygen. The result obtained was similar to that of *Leuc. mesenteroides* subsp. *mesenteroides* grown in lactose batch culture (Plihon et al., 1995; 1996) and that of *L. fermentum* Y6, *Pediococcus pentosaceus* NRIC 1105, *Leuc. lactis* NRIC 1540^T, *Leuc. mesenteroides* subsp. *cremoris* NRIC 1538^T, *Leuc. mesenteroides* subsp. *mesenteroides* NRIC 1541^T and *Enterococcus faecium* NRIC 1140 grown in glucose batch cultures (Sakamoto and Komagata, 1996).

During the batch growth of *L. lactis* IO-1 on xylose about 36.5% and 73.7% of the utilized carbon was recovered as the products in the presence of oxygen (lactate and acetate) and absence of oxygen (lactate, formate, acetate and ethanol) respectively (Table 1). The carbon recovery was less than 100% which could possibly be due to parts of the utilized carbon have been converted to other products of pyruvate metabolism such as CO_2, diacetyl, acetoin, acetolactate and 2,3-butanediol (Fig. 2) which were not detected in this study.

In conclusion, comparison of the results from aerobic and anaerobic fermentation studies showed that aeration had an influence on pyruvate metabolism of *L. lactis* IO-1 in xylose batch cultures resulting in difference in metabolic end products. The main end products of aerobic xylose catabolism were lactate and acetate whereas, for anaerobic catabolism the main end products were lactate, acetate, formate and ethanol. Under aerobic conditions, formate was not produced possibly due to the inhibition of PFL by oxygen and ethanol was not observed suggesting that alcohol dehydrogenase was not operational. Incomplete conversion yield and mixture product formation in both conditions imply that the use of this organism in xylose fermentation for the industrial production of lactic acid has to be questioned.

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Table 1 Effect of aeration on biomass yield, molar product yields and carbon recovery in 5.0 g xylose l^{-1} batch culture of *Lactococcus lactis* IO-1.

Growth Condition	Biomass yield (g dry cell weight g^{-1} xylose utilized)	Molar product yields (mol product mol^{-1} xylose utilized)				Carbon recovery (%)
		Lactate	Formate	Acetate	Ethanol	
Aerobic	0.16	0.14	0	0.69	0	36.5
Anaerobic	0.19	0.15	0.92	0.73	0.43	73.7

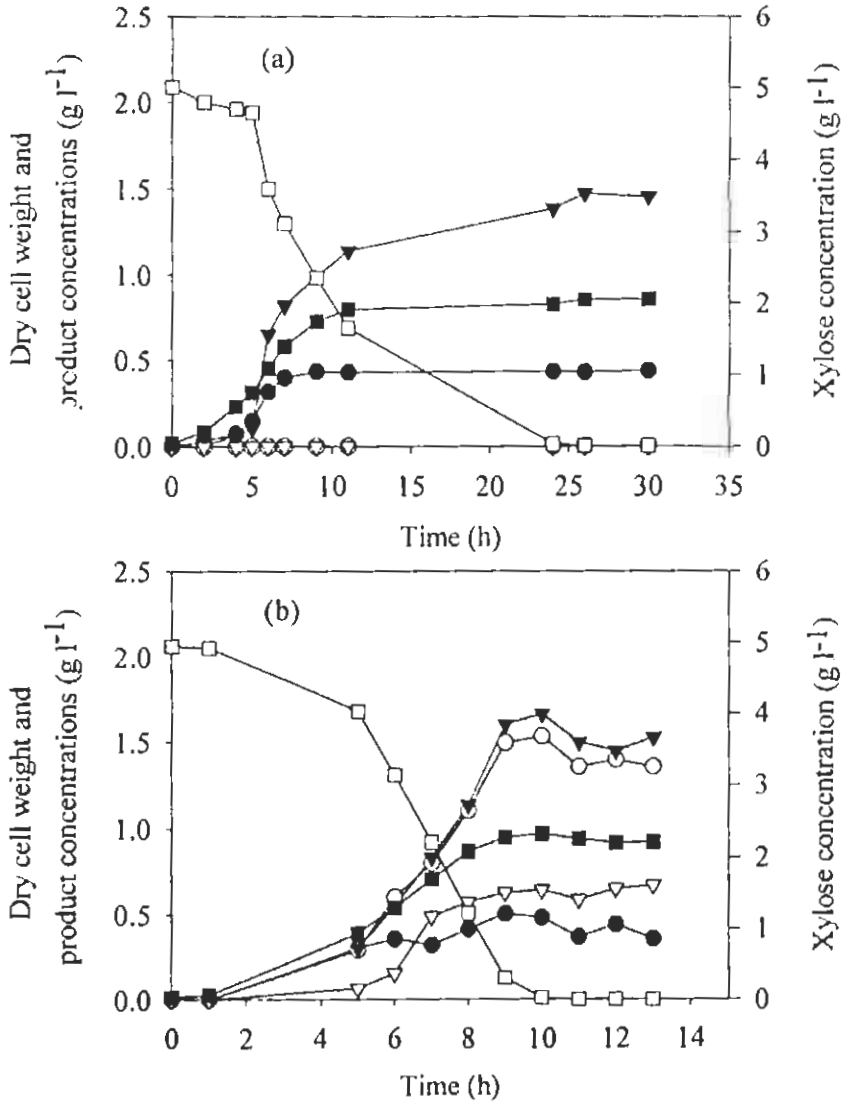

Fig. 1 Batch culture of *Lactococcus lactis* IO-1 on medium containing 5 g xylose l^{-1} under aerobic (a) and anaerobic (b) conditions : lactate (●), formate (○), acetate (▼), ethanol (▽), dry cell weight (■) and xylose (□).

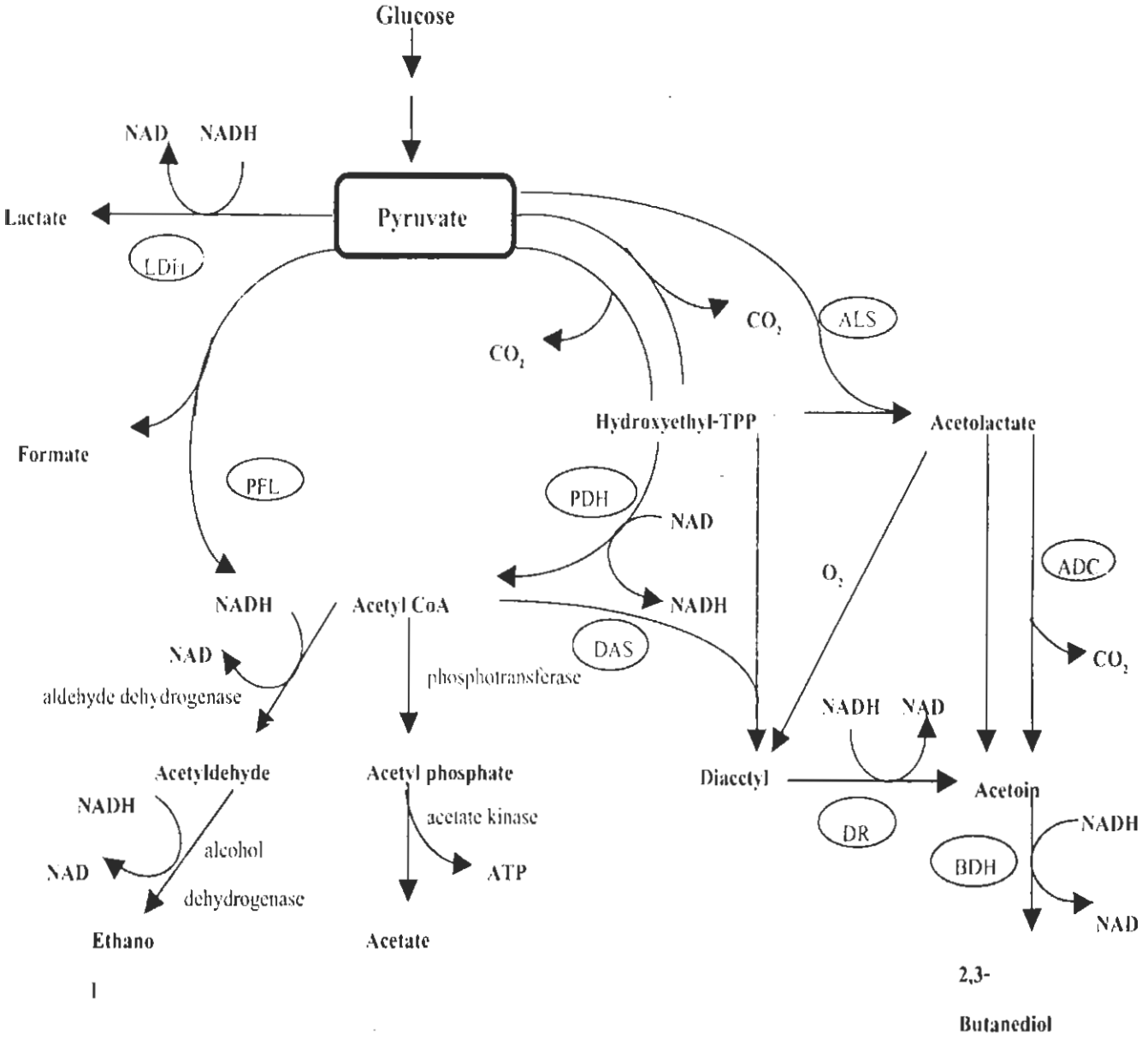

Fig. 2 Pyruvate metabolism in *Lactococcus lactis* IO-1. LDH: lactate dehydrogenase; PFL: pyruvate formate lyase; PDH: pyruvate dehydrogenase; ALS: acetolactate synthase; ADC: acetolactate decarboxylase; BDH: butanediol dehydrogenase; DAS: diacetyl synthase; DR: diacetyl reductase; TPP: thiamine pyrophosphate (Modified from Hugenholtz and Starrenburg, 1992; Platteeuw et al., 1995; Cocaign-Bouaquet et al., 1996).