IUFS Journal of Biology
IUFS J Biol 2014, 73(1): 1-7

Research Article

Lactic Acid Production by *Lactobacillus brevis* Isolated from Oral Microbiota

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Abstract

Lactic acid is used in various industrial areas such as the food, pharmaceutical, textile and other chemical industries. In this study, 49 lactic acid bacteria were isolated from oral microbiota of voluntary patients. These isolates were screened for their lactic acid production abilities. The isolate which has a high potential for lactic acid production was selected. The optimal conditions for lactic acid production by *Lactobacillus brevis* were determined. According to the results obtained, sucrose as carbon source, 50 g/L of sucrose amount, diammonium hydrogen citrate as nitrogen source, 8 g/L of nitrogen amount, 42 °C as temperature, 4.5 x 108 CFU/mL of inoculum amount and 72 hours of incubation time were found to be optimal values.

Keywords: Oral lactic acid bacteria, lactic acid production, *Lactobacillus brevis*.
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(Received: 18.07.2013 Accepted: 18.04.2014)

Ağız Mikrobiyotasından İzole Edilen *Lactobacillus brevis* ile Laktik Asit Üretimi

Özet

Laktik asit; gıda, eczacılık, tekstil ve diğer kimyasal endüstriler gibi çeşitli endüstrilerde kullanılmaktadır. Bu çalışmada, gönüllü hastaların oral mikrobiyotalarından 49 tane laktik asit üreticisi izolat izole edilmiştir. Bu izolatlar, laktik asit üretim yetenekleri açısından taranmıştır. İzole edilen suşlardan yüksek üretim potansiyeline sahip olan *Lactobacillus brevis* ile laktik asit üretimi için optimum koşullar belirlenmiştir. Optimum koşullar; karbon kaynağı sükroz, karbon kaynağı miktarı 50 g/l, azot kaynağı diamonyum hidrojen sitrat, azot miktarı 8 g/l, sıcaklık 42 °C, inokulum miktarı 4,5x108 CFU/ml, inkübasyon süresi 72 saat olarak belirlenmiştir.

Anahtar kelimeler: Oral laktik asit bakterileri, laktik asit üretimi, *Lactobacillus brevis*.

Introduction

Lactic acid is an organic acid with a wide range of applications in the food, pharmaceutical and cosmetic industries (Datta et al. 1995). Recent information indicates approximately 90 % of all lactic acid is produced by bacterial fermentation (Zhou et al. 2006). Microbial fermentation by lactic acid bacteria (LAB) has the advantage of producing only one of the isomers. Synthetic production always results in a racemic mixture of lactic acid (Litchfield 1996; Lunt 1998).

LAB need to complex nutrient requirements during their biosynthesize B-vitamins and amino acids (Fitzpatrick and Keeffe 2001). LAB perform lactic acid fermentation by two different pathways; homo-fermentative and heterofermentative (Drinan et al. 1976). *Lactobacillus brevis* is heterofermentative LAB which produces lactic acid by phosphoketolase pathways. Heterofermentative LAB convert 6-carbon sugars (hexoses) to 5-carbon sugars (pentoses) and form carbon dioxide catalyzed by several enzymes (Abdel-Rahman et al. 2011).

Most LAB such as *Lactobacilli*, are thought to be safe for large scale industrial lactic acid production without adverse human health effects (Abdel-Rahman et al. 2013).

Several factors were reported to increase lactic acid production efficiency such as carbon sources, nitrogen sources, temperature, fermentation mode and period, pH, neutralizing agents and aeration (Abdel-Rahman et al. 2013).

The aim of this study is to determine potential lactic acid producer and to optimize the medium components and environmental parameters considered to affect lactic acid production. Forty nine isolates from dental plaque were isolated and their lactic acid production abilities were tested. A good producer among forty nine isolates was selected for the optimization of lactic acid production.

Materials and methods

Microorganism and fermentation medium

Forty nine *Lactobacillus* isolates were isolated from dental plaques of the patients suffering from dental caries at the hospital of Gazi University Faculty of Dentistry. The organisms were take from Gazi University Faculty of Dentistry. According to the ethics committee report 09.06.2009/21 of the Faculty of Dentistry, Gazi University and rules of Helsinki declaration, the samples were take from voluntary patients who signed a patient form.

The isolates were tested for their abilities of lactic acid production at 42 °C for 24 h, with 5 % CO_2 atmosphere. The fermentation medium contains (g/L) 10.0 of casein peptone, 8.0 of meat extract, 4.0 of yeast extract, 50.0 of glucose, 1.0 of tween-80, 2.0 of K_2HPO_4, 5.0 of sodium acetate, 2.0 of di-ammonium hydrogen citrate, 0.2 of $MgSO_4.7H_2O$, 0.04 of $MnSO_4.4H_2O$, 25.0 of $CaCO_3$ in 1000 mL of distilled water.

Measurement of lactic acid

The amount of lactic acid was determined by a colorimetric method (Kimberley and Taylor 1996). Six milliliters of concentrated H_2SO_4 (96 %) in borosilicate tubes were added to 1ml of fermented samples and mixed in a vortex mixer. The mixed solutions were incubated at 95-100 °C for 10 min in a water bath. The tubes were cooled to room temperature, subsequently, 100 mL $CuSO_4$ (4 %) reagent and then 200 mL ρ-phenylphenol (1.5 %) reagent were added, and mixed well using a vortex mixer. The tubes were kept at 20 °C for at least 30 min and the absorbance level recorded in 570 nm (Madrid et al. 1999).

Optimization of process parameters

In this study, lactic acid production was performed by using a carbon source such as glucose, lactose, xylose, sucrose. Furthermore, the amount of carbon source varying from 25 to 100 g/L was investigated. The effect of nitrogen source was investigated by using NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$ and di-ammonium hydrogen citrate and yeast extract. The amount of nitrogen source (1 to 8 g/L) was investigated after determining the nitrogen source. Different process parameters such as temperature (37 to 45 °C), amount of inoculum (0.75×10^8- 6.0×10^8 CFU/mL) and incubation period (16 to 96 h) were optimized by varying the respective parameters. Experiments were carried out in ErlenMayer flasks containing fermentation medium in CO_2 (5%) incubator. All experiments were repeated three times.

Results and Discussion

In this study, LAB were isolated for lactic acid production from dental plaque. Since LAB are required for complex nutrient components such as B-vitamins and amino acids, the mouth was chosen as an isolation source due to it being a habitat with rich nutrients (Viniegra-Gonzalez and Gomez 1984). 28# isolate with characteristics of Gram positive, non-motile and rod shaped was selected because it had higher lactic acid production ability. 28# isolate was identified as *Lactobacillus brevis,* a heterofermentative strain, according to API CHL 50 and biochemical test results. Although the lactic acid yield of *L. brevis* was lower compared with the homofermentative strains, the lactic acid yield can be enhanced by strain improvement and fermentation technology development.

L.brevis, a heterofermentative strain, is known to produce lactic acid. There are several reports in the literature. *L. brevis* is generally regarded as an obligate heterofermentative bacterium, but the high yield of 0.86 g/g from glucose indicated that glucose was not metabolized through the phosphoketolase pathway, according to which only 0.5 g of lactic acid should be produced from 1 g of glucose. This result is in accordance with that of the study of Garde et al. (2002) who reported the lactic acid yield of 89% by *L. brevis* from glucose.

Producer type, carbon and nitrogen sources, the amount of carbon and nitrogen sources, type of fermentation, pH, temperature, inoculum amount, the incubation period are parameters significantly affecting the fermentative lactic acid production (Hofvendahl and Hahn-Hagerdal 2000).

Carbon source and the amount of carbon source have important roles on the lactic acid fermentation (Wee et al. 2006). According to the study of Kotzamanidis et al. (2002), 88.0 g/L of lactic acid concentration was obtained by using sucrose as carbon source. Consequently, in this study, the experimental results relating to utilization of different sugars are shown in Fig.1. 50.0 g/L of sucrose was an optimum amount for lactic acid production (Fig. 2).

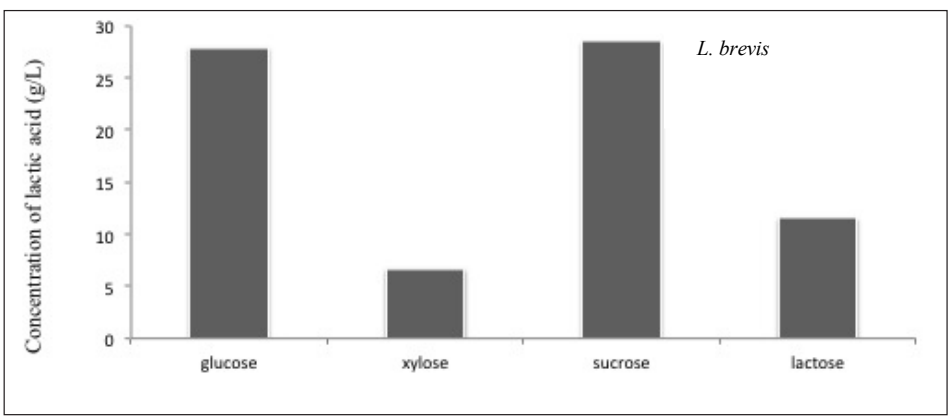

Figure 1. The effect of different carbon sources on lactic acid fermentation

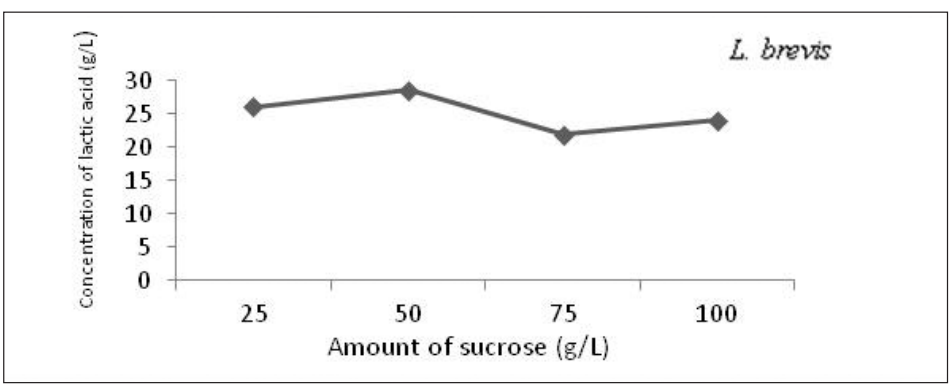

Figure 2. The effect of the amount of sucrose on lactic fermentation

Lactic acid synthesis as a result of fermentation is associated with cell growth, so product formation does not occur in the absence of a sufficient concentration of nitrogen (Pritchard and Coolbear 1993). On the other hand, a high concentration of nitrogen can lead to the death of the cells and/or causing catabolic repression (Bolner de Lima et al 2009). The experimental results for nitrogen sources were shown in Fig. 3, and that relating to nitrogen amount were shown in Fig. 4. According to these results, 30.2 g/L of lactic acid concentration was obtained when di-ammonium hydrogen citrate was used as the nitrogen source, and 36.9 g/L of lactic acid concentration was provided when 8.0 g/L of di-ammonium hydrogen citrate was used. Although, in many fermentation studies, yeast extract is used as nitrogen sources, due to the high cost, it was not preferred in large-scale productions (Yoo et al. 1997). Liu et al. (2010) investigated the effect of different nitrogen sources such as NH_4Cl, NH_4NO_3, corn steep liquor, di-amine citrate, malt sprout. According to these findings, it was reported that they determined high lactic acid concentration in 12 g/L corn steep liquor and 16 g/L malt sprout (Liu et al. 2010).

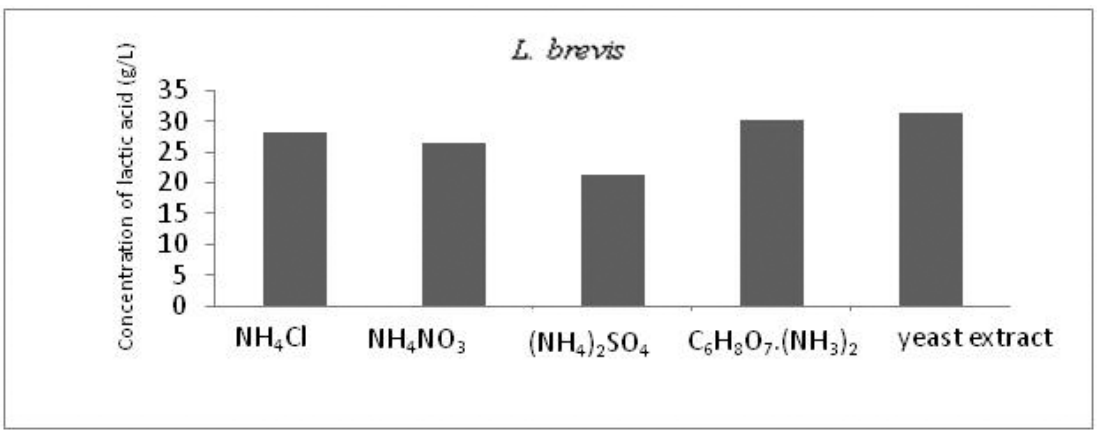

Figure 3. The effect of different nitrogen sources on lactic acid fermentation

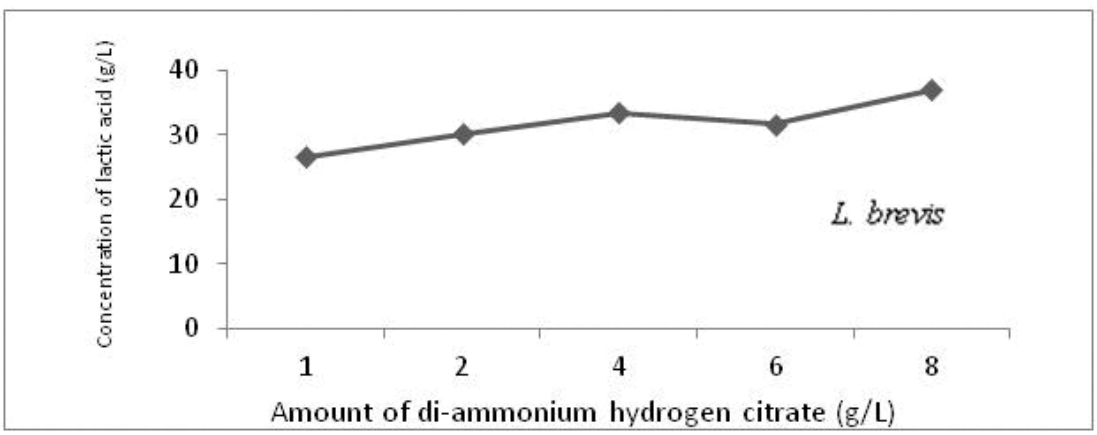

Figure 4. The effect of the amount of di-ammonium hydrogen citrate on lactic acid fermentation

Temperature is also an important environmental parameter on the lactic acid fermentation process. Generally, LAB can grow in psycrophilic to mesophilic conditions such as 5 to 45 °C (Bolner de Lima et al. 2010). Therefore, it is vital to determine the optimum temperature for microbial growth. To the best knowledge, *Lactobacillus amylophilus* that

grow at 15 °C could produce lactic acid at 35 °C (Yumoto and Ikeda 1995). In this study, the optimum temperature for microbial growth and lactic acid production of the isolate were found as 37 and 42 ºC, respectively (Fig. 5). In another study, lactic acid production ability between 30-50 ºC was investigated, and it was observed that the lactic acid profile increases when temperature increases from 30 to 45 ºC, but it rapidly decreases after 45 ºC (Trontel 2010).

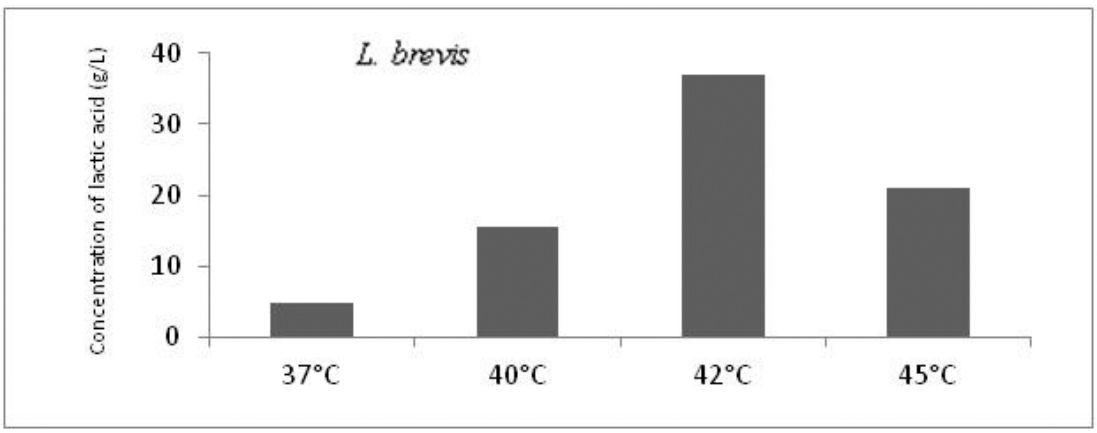

Figure 5. The effect of temperature on lactic acid fermentation

One of the factors affecting the production of lactic acid is the amount of the inoculum. The amount of inoculum can allow the use of sugar as a substrate. The high amount of inoculum accelerated oligosaccharide consumption, significantly reduced the concentration of reducing sugar (Yu et al. 2008). In this study, 4.5×10^8 CFU/mL was found as the best result for the inoculum amount (Fig. 6). According to the results of Gupta et al. (2010) experiments, lactic acid production started to decrease in 3.02×10^{10} cell density at the end of the 8 hours period of fermentation.

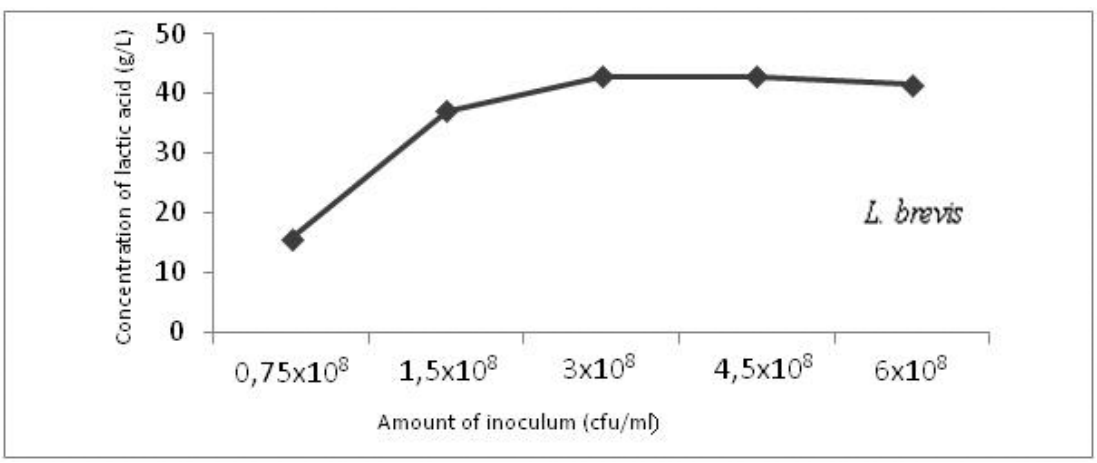

Figure 6. The effect of amount of inoculum on lactic acid fermentation

The incubation period is also another important parameter on lactic acid production. Bustos et al. (2004) in their study, found the maximum amount of lactic acid 58.9 g/L at 96th hour. In our study, we found 45.3 g/L of lactic acid concentration at the end 72nd hour (Fig. 7).

Fermentation type is also important for the production of lactic acid. Generally, batch manufacturing is preferred but one can see continuous fermentation in the literature.

Comparing batch and continuous fermentation types, more efficient lactic acid production was obtained through batch fermentation in many studies (Hoshino et al. 1991).

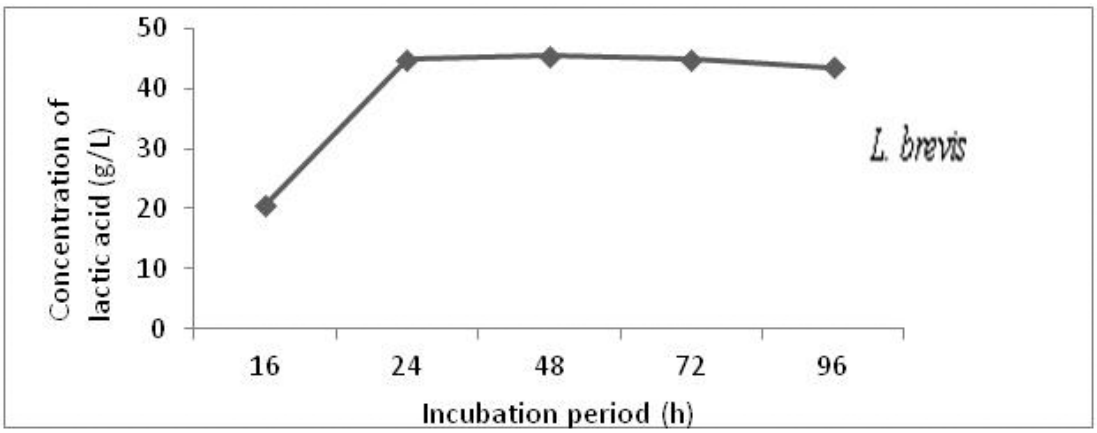

Figure 7. The effect of incubation period on lactic acid fermentation

The results obtained in this study demonstrated that *L. brevis* is a good microbial producer of lactic acid from sucrose. Due to its high potentiality in the conversion of sucrose to lactic acid, this selected isolate may be exploited industrially to develop a new technology.

Acknowledgement

This study is based on the MSc Thesis by Yağmur Toptaş. Also, this study was presented at 15th European Congress on Biotechnology, İstanbul, Turkey as a poster section of Industrial Biotechnology, Session: Biobased Chemicals and Materials.

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