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Production of Lactic Acid from 1, 2-Propanediol by Yeast

Ki Soo Chae and Jung Hwn Seu

Department of Agricultural Chemistry, College of Agriculture,
Kyungpook National University, Daegu, Korea

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1, 2-Propanediol로 부터 Lactic acid의 生成

채 기 수·서 정 훈

경북대학교 농과대학 농화학과

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Abstract

1, 2-propanediol-utilizing yeast, Y-1-4, was isolated from sludge sample by the enrichment culture technique. The product produced from 1, 2-propanediol by the selected strain was identified as lactic acid by paper chromatography and infrared absorption spectrum. The strain assimilated ethanol, 1, 2-propanediol, glycerine and glucose, but it produced lactic acid from 1, 2-propanediol used as the sole carbon source. Under optimal conditions, the strain Y-1-4 was cultured with shaking at 30°C for 4 days in the medium containing 1, 2-propanediol 20.0g, NH_4Cl 5.0g, KH_2PO_4 1.0g, $MgSO_4 \cdot 7H_2O$ 0.5g, $FeSO_4 \cdot 7H_2O$ 0.25g, yeast extract 0.4g, $CaCO_3$ 3.0g and tap water to one liter, and then the yield of lactic acid was about 12.1g per liter of the culture broth

Introduction

Petrochemical industries have developed recently and many petrochemicals have been studied as new fermentative sources. 1, 2-propanediol is available on a large scale as one of the products of the petrochemical industry, This compound is relatively cheap, water-miscible, non-volatile and non-toxic to animal and human beings. Furthermore, 1, 2-propanediol was found to be susceptible to the microbial attack.

The studies concerned with microbial metabolism of 1, 2-propanediol were carried out by several groups of workers[1-6]. Several microbial products from 1, 2-propanediol have already been reported. These products include pyruvic acid[7], propionic acid[8], glutamic acid[9], 0-2-hydroxypropylhomoserine[10,11], polysaccharide[12,13], cell mass[14], oligoglucuronides[15], vitamin B_{12}[16-19] and lactic acid[20-24].

Among them, lactic acid is used in such field as food, drinks, leather and textile industries. And lactic acid is thought to be useful as a monomer of the synthetic substance becauce of two functional groups in the molecule.

Because lactic acid is presently being made by chemical synthesis or glucose fermentation, its price is fairly high. From an economic point of view, the lactic acid production from 1, 2-propanediol by microorganisms is of great advantage.

This report describes the isolation of one of microorganisms which can utilize 1, 2-propanediol as the sole carbon source, the identification of the fermented product and the production condition of lactic acid from 1, 2-propanediol.

Materials and Methods

Microorganism

Three hundred and twenty strains of 1, 2-propanediol utilizing microorganisms were isolated from soil and sludge. One strain of yeast(Y-1-4) having the highest lactic acid productivity was used thorughout this work.

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The composition of medium to select microorganisms utilizing 1, 2-propanediol and to produce lactic acid was as follows; 1, 2-propanediol 20g, NH_4Cl 3g, KH_2PO_4 1g, $MgSO_4 \cdot 7H_2O$ 0.5g, $FeSO_4 \cdot 7H_2O$ 0.25g, yeast extract 0.2g per liter of tap water(pH5.0). Solid medium for stock culture was prepared by adding 1.8% of agar to the above medium.

Cultivation

One loop of the cells grown on agar medium was inoculated to 20m*l* of the 1, 2-propanediol medium in a 100m*l* Erlenmeyer flask, and cultivated on a reciprocal shaker(80 strokes per min., 7cm) at 30°C for 4 days.

Cell concentration

Gell growth was determined by measuring optical density of the culture broth with a Shimadzu spectronic 20 photometer at 610nm. One unit of optical density was corresponded to 0.56g of dry cells per liter by means of the reference curve.

Ion exchange column chromatography

Column chromatography was carried out using an ion exchange resin, Amberlite IRA-410 (OH^-) to separate lactic acid from the culture broth. After the culture broth was centrifuged to remove yeast cells, the clear supernatant was applied to the column and washed with distilled water. Acidic compounds such as lactic acid was then eluted with 0.1 N-HCl, and this effluent was assayed colorimetrically for lactic acid.

Determination of lactic acid

Lactic acid was quantitatively determined by the *p*-hydroxybiphenyl method of Barker and Summerson[25] One m*l* of the sample eluted from ion exchanger and 0.05m*l* of 4% cupric sulfate solution were transferred to a test tube, and 6.0m*l* of concentrated sulfuric acid was added slowly. It was shaken in the ice bath, and then placed in the boiling water for 5 min. After it was cooled below 20°C in cold water, 0.1m*l* of 1.5% *p*-hydroxybiphenyl solution was added, and then placed at 30°C for 30min to develope full color intensity. In order to dissolve the excess of *p*-hydroxybiphenyl, it was placed in the boiling water bath for 90 seconds. After cooling to room temperature, the absorbancy of the reaction mixture was measured at 560nm.

Identification of the product

Paper chromatography and infrared spectroscopy were carried out for the identification of the product. The developing solvent systems for paper chromatography were the mixture of n-butanol: aceticacid: water (4:1:1, v/v/v), n-butanol: formic acid: water(8:3:2, v/v/v) and n-propanol: 2N-NH_4OH (7:3, v/v). The spot was visualized by spraying bromophenol blue or bromocresol green. For obtaining an infrared absorption spectrum, the product eluted from ion exchanger was crystalized as calcium salt.

Results and Discussion

Assimilation of various alcoholic compounds by the strain of Y-1-4

One percent of alcoholic substances was added to the previous medium as the sole carbon source. The cell growth and the production of acidic substances were indicated in Table I. The strain Y-1-4 grew well in the medium containing ethanol, glycerine or glucose, though each *Rf* value of their acidic substances was different from that of lactic acid. A large amount of lactic acid was produced only when 1, 2-propanediol was added to the medium as carbon source.

Separation and identification of the product

Table I. Assimilation of alcoholic compounds

Alcohols (1%)	Growth*	Production of acid**	Production of lactic acid
methanol	—	—	—
ethanol	##	+	—
n-propanol	—	—	—
iso-propanol	—	—	—
n-butanol	—	—	—
n-pentanal	—	—	—
iso-pentanol	—	—	—
1, 2-propanediol	##	+	+
glycerine	##	+	—
glucose	##	+	—

*By visual estimation: — not grown, # good, ## very good

**production of acid: + below pH5.0

After 4 day culture in the 1, 2-propanediol medium, the cells were separated from the culture broth by centrifugation. The supernatant was applied to the column of Amberlite IRA 410(OH^-) type. The acidic adsorbate was eluted with 0.1N-HCl and the effluent was evaporated off under the vacuum. The residue was dissolved in 0.1N-HCl solution and subjected to paper chromatography using three kinds of solvent systems.

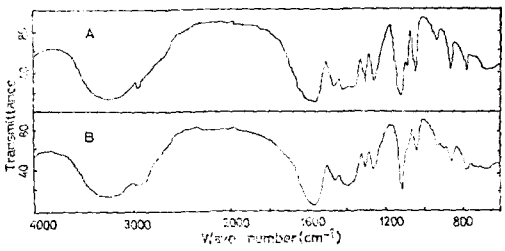

Fig. 1. Infrared spectrum of product from 1, 2-propanediol by strain Y-1-4
A: the product (Ca salt)
B: authentic Ca-lactate

Table II. Rf values of the product on paper chromatography

Solvent system	Rf value of the product	Rf value of lactic acid
n-butanol: acetic acid: water	0.60	0.60
n-butanol: formic acid: water	0.71	0.72
n-butanol: 2N-NH_4OH	0.69	0.69

The 1, 2-propanediol utilizing yeast Y-1-4 was observed to convert 1, 2-propanediol into a compound with the same *Rf* value of lactic acid from the paper chromatographic system shown in Table II. As shown in Fig 1. the infrared spectrum of the prepared product, calcium salt, showed that it was well agreed to that of the authentic calcium lactate.

Time course of the cell growth and the lactic acid production

The growth of strain Y-1-4 in the 1, 2-propanediol medium was studied with the shaking culture. As shown in Fig 2, the growth rate increased with the gradually increased production of lactic acid corresponding to yielding amount of 5.5g per liter for 4 day cultivation.

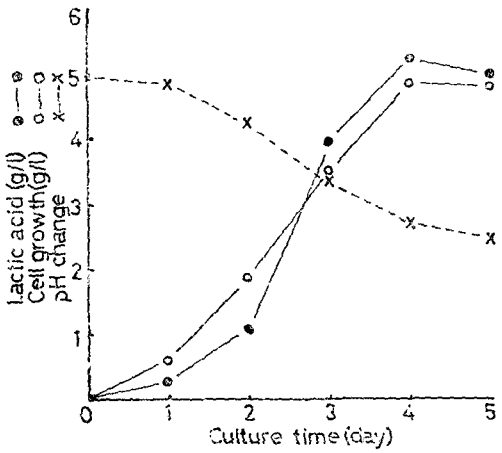

Fig. 2. Time course of lactic acid production from 1, 2-propanediol by strain Y-1-4

Effect of concentration of 1, 2-propanediol

The growth of the strain Y-1-4 on various concentrations of 1, 2-propanediol was studied using the substrate as the sole carbon source. As 1, 2-propanediol was added to 2%, the lactic acid could be increased up as shown in Fig 3. When the concentration of 1, 2-propanediol was above 2%, the growth rate of cells was gradually decreased because of its toxicity.

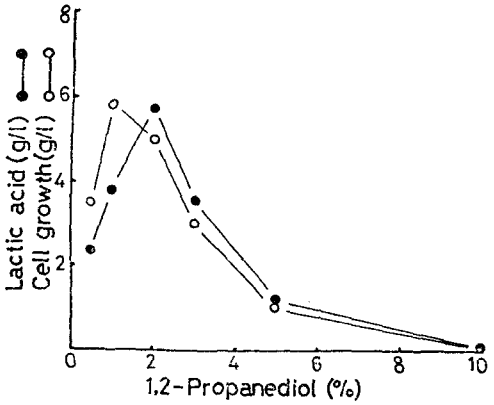

Fig. 3. Effect of concentration of 1, 2-propediol on lactic acid production

Effect of nitrogen source

In order to find suitable nitrogen source for lactic acid production, various kinds of nitrogen sources were tested at the concentration of 0.3%. For the production of lactic acid and the growth of cells, ammonium chloride was superior to any other nitrogen source in Table III. In batch culture with the initial concentration of 2% of 1, 2-propanediol, the reasonable concentration of ammonium chloride was 0.5% as shown in Fig4.

Table III. Effect of various nitrogen sources

N-sources (0.3%)	Growth (g-dry cell/liter)	Lactic acid (g/liter)
NH_4NO_3	3.05	2.90
NH_4Cl	5.37	5.05
$(NH_4)_2SO_4$	3.62	3.75
$(NH_4)_2HPO_4$	4.82	3.70
$NaNO_3$	2.75	2.05
Urea	1.79	1.28
Peptone	6.96	2.70

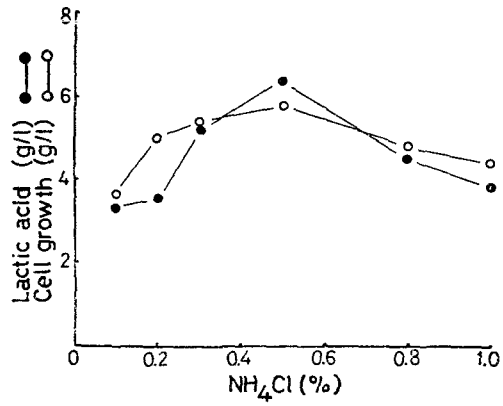

Fig. 4. Effect of ammonium chloride on lactic acid production

Effect of yeast extract

The organismcould not grow on the 1, 2-propanediol medium without yeast extract as growth factor. As shown in Fig 5, yeast extract gave an efficient influence on both the production of lactic acid and the role as growth factor of cells at the concentration of 0.04%.

Effect of metal ions

It has been reported that a bacterial culture which could be utilized methanol as carbon source

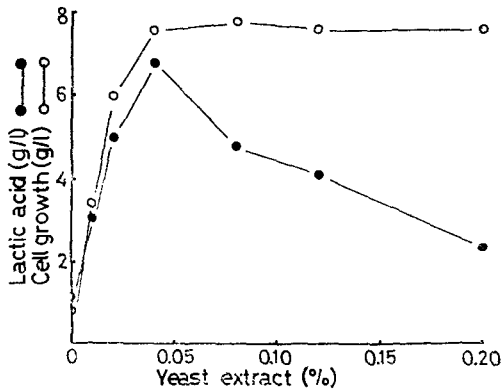

Fig. 5. Effect of yeast extract on lactic acid production

Table IV. Effect of divalent cations

Metal ions (10^{-3}M)	Growth (g-dry cell/liter)	Lactic acid (g/liter)
Ba^{++}	5.97	3.71
Ca^{++}	5.50	4.50
Co^{++}	0.40	0
Cu^{++}	0.18	0
Fe^{++}	6.02	5.23
Hg^{++}	0.36	0
Mg^{++}	4.09	5.29
Mn^{++}	1.86	2.15
Ni^{++}	1.18	0.85
Zn^{++}	3.35	2.00
none	3.39	4.60

requires ferrous and magnesiumions for cell growth, and catalase also requires ferrous ion as the prosthetic group[26]. The effect of metal ions at a concentration of 10^{-3}mole was investigated here. As the result followed in Table IV, ferrous and magnesium ions significantly enhanced both the cell growth and the lactic acid production, but barium and calcium ions increased only the cell growth.

Effect of addition of calcium carbonate

To know the effect of addition of calcium carbonate on the production of lactic acid, various concentrations of calcium carbonate were added to the medium. As shown in Fig 6, the 0.3% of calcium carbonate was very effective on its production. At this optimal concentration of cacium carbonate, the pH of the culture broth was approached to optimal pH for its production. The result implies that the external pH adjustment for optimal condition throughout the fermentation process seems to be useful for higher production of lactic acid.

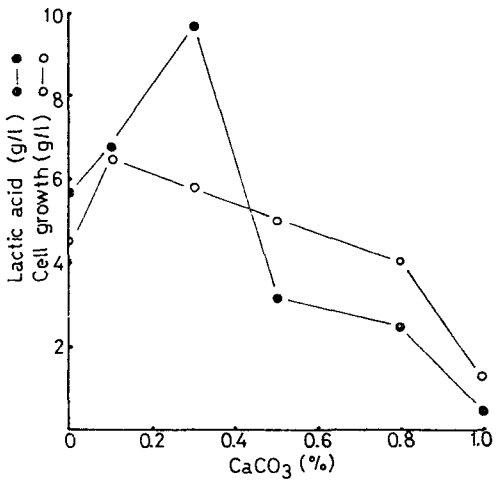

Fig. 6. Effect of addition of calcium carbonate on lactic acid production

Effect of aeration

To ascertain the fact that the fermentation of lactic acid from 1, 2-propanediol is an oxidative process, the effect of aeration on the lactic acid production by yeast Y-1-4 was studied by varying the volume of the medium in a 500m*l* shaking flask. Production of lactic acid was shown to be stimulated by aeration from this result in Fig 7, and the optimal volume of medium was 50m*l* in a 500m*l* shaking flask.

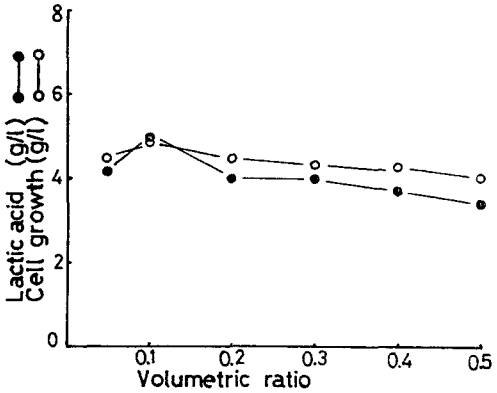

Fig. 7. Effect of aeration

Effect of initial pH of the medium

This was determined in the medium with the initial pH ranging from 2.0 to 7.0. As the result shown in Fig. 8, the organisms grew well and also produced lactic acid at the pH range of 4.0 to 5.0 with the optimum of pH 4.5.

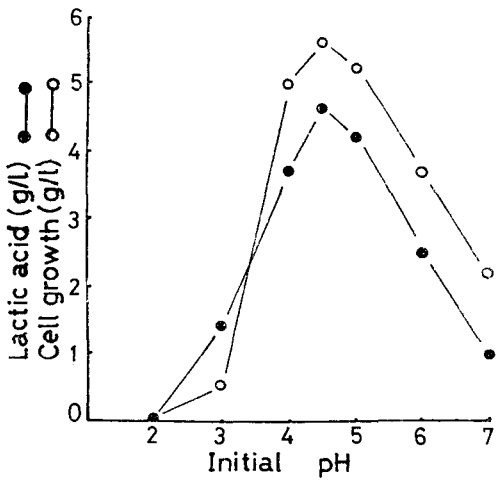

Fig. 8. Effect of initial pH of medium on lactic acid production

Effect of temperature

The yeast Y-1-4 grew well and produced lactic acid at the temperature range of 25°C to 30°C as

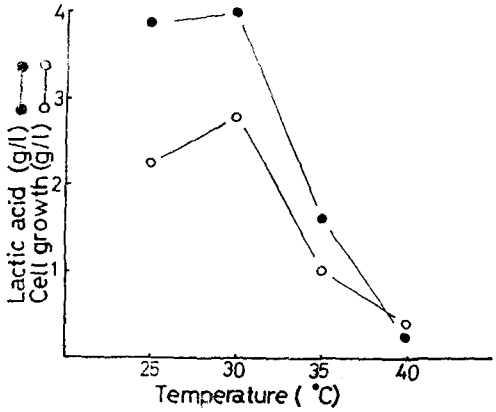

Fig. 9. Effect of temperature on lactic acid production

shown in Fig 9. The cells did not grow over 40°C at all.

Production of lactic acid under optimal conditions

For optimal conditions of culture systems to produce lactic acid from 1, 2-propanediol, the composition of the medium was as follows; 1, 2-propanediol 20g, NH_4Cl 5g, KH_2PO_4 1g, $MgSO_4$ $7H_2O$ 0.5g, $FeSO_4 \cdot 7H_2O$ 0.25g, yeast extract 0.4g, $CaCO_3$ 3g and tap water to make one liter. The temperature of the fermentation process was 30°C, and the volume of the medium was 50m*l* in a 500m*l* shaking flask.

Under these conditions, the yield of lactic acid was 12. 1g per liter after 4 day culture. Yagi et al reported that lactic acid from 1, 2-propanediol by the batch culture of *Arthrobacter oxydans* (PG 21-1) was produced 9.02g per liter[20], though 17.8g per liter by jar culture[24] It was therefore expected that more yield of lactic acid from this strain would be increased by jar fermentation and pH adjustment throughout the fermentation process.

요 약

토양과 sludge 에서 1, 2-propanediol 을 자화할 수 있는 미생물을 분리하여 이들중 가장 많은 젖산을 생성하는 효모 한 균주(Y-1-4)를 선정하였다. 배양액에 생성된 산은 paper chromatography 와 IR spectrum 에 의해서 젖산으로 확인되었다. 이 효모는 탄소원으로서 ethanol, glycerin, glucose 및 1, 2-propanediol 등을 자화할수 있었는데 1, 2-propanediol 으로부터만 젖산을 생성했다.

1, 2-propanediol 에서부터 젖산생성의 최적 배지 및 최적 배양조건을 검토해서 1, 2-propanediol 2.0 %, NH_4Cl 0.5%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, $FeSO_4 \cdot 7H_2O$ 0.025%, yeast extract 0.04 %와 $CaCO_3$ 0.3%를 함유하는 배지를 30°C에서 4일간 진탕배양한 결과 젖산 생성량은 liter 당 12.1gram 이었다.

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