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NOTE

Effect of pH on the Production of Lactic Acid and Secondary Products in Batch Cultures of *Lactobacillus casei*

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Batch fermentations of lactic acid were performed with *Lactobacillus casei* to investigate the effect of pH on cell growth and production of lactic acid and by-products. Maximum productivity of lactic acid increased with increasing pH from 5.0 to 6.5, and the extent of D-lactate production was different at each pH. Acetate and D-lactate concentrations increased even after the complete consumption of glucose in the medium. While a pH range of 6.0-6.5 was optimal for cell growth and lactic acid production, superior results were achieved at pH 6.0 when both maximum lactic acid productivity and minimum by-product formation were considered.

Lactic acid is an organic acid that can be produced both biologically and chemically. In recent years, lactic acid has been highlighted as a raw material for making biodegradable polylactic acid polymer, besides its traditional uses in the food industry (3). Physical properties of this polymer, such as crystallinity and degradability, can be adjusted by manipulating the D/L isomeric ratio of monomeric lactic acid (8, 18). Chemical synthesis of lactic acid has a drawback that the product obtained is a racemic mixture of D- and L-lactic acid (12). Since L-lactic acid of high optical purity is required to obtain desired properties of polymer, the appropriate method for producing L-lactic acid is not chemical synthesis but rather fermentation using defined strains. Although there are numerous reports on lactic acid fermentation (15, 17, 21), few groups have studied the production of lactic acid as a monomer for polymer synthesis. Tsai *et al.* (18) showed that a mixed culture of lactic acid bacteria exhibited a product stereospecificity of higher than 98%. Ohara (11) noted that the formation of by-products requiring removal during the purification process of lactic acid, such as acetic acid, formic acid and ethanol, should be minimized during fermentation and used as a screening criterion for lactic acid bacteria. Ohara (11) also reported that fermentation conditions should be optimized because the optical purity of lactic acid and the formation of acetic acid could be affected by the culture environment.

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Several groups have examined the interactions between pH and lactic acid concentration, and the resultant effect on the growth rate during the time-courses of batch cultures (1, 6, 9, 14, 19). In these reports, the optimal range of pH for cell growth and lactic acid production was between 5.5 and 6.5. However, none of these studies showed the effect of pH on the pattern of product formation or the optical purity of lactic acid. We believe that the fermentation pH should be controlled to optimize product quality as well as the productivity of lactic acid. The aim of this study was to investigate the effect of pH on the growth characteristics and by-product formation in batch cultures of *Lactobacillus casei*, and to find an optimum pH for both lactic acid production and by-products minimization.

Lactobacillus casei subsp. *rhamnosus* (ATCC 10863) was kept as a stock culture in glycerol (20% v/v) at -76°C. Inocula were prepared by transferring glycerol stock culture to 50 ml of the MRS medium (Difco Co., Detroit, USA) and incubating for 12 h in a rotary shaker at 42°C. The production medium contained, per liter: glucose, 100 g; yeast extract, 15 g; Na-acetate·$3H_2O$, 1 g; K_2HPO_4, 0.5 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $MnSO_4 \cdot H_2O$, 0.03 g; and $FeSO_4 \cdot 7H_2O$, 0.03 g. All medium components were autoclaved together except glucose, $MgSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, and $FeSO_4 \cdot 7H_2O$.

A 5% seed inoculum grown in the MRS medium was used in all fermentations. Batch experiments were car-

ried out in a jar fermentor (2.5 liter: Korea Fermentor Co., Incheon, Korea) containing 1 liter of medium at 42°C. The pH was controlled using 10 N NaOH. During the first 2 h of fermentation, N_2 gas was sparged through the fermentor to maintain anaerobic conditions.

Cell mass was measured in terms of optical density at 620 nm. Dry weight determination was made after centrifuging cells, washing with saline water, and drying at 105°C. Glucose concentration was measured enzymatically using the glucose oxidase-peroxidase method (Glucose-E kits, Youngdong Pharm. Co., Seoul, Korea). Total lactic acid and acetate were analyzed by using a HPLC equipped with an RI detector (Hitachi L-6000, Tokyo, Japan) and an Aminex HPX-87H column (Bio-Rad Co., CA, USA). The column temperature was 50°C, the flow rate was 0.5 ml/min, and the mobile phase was 0.01 N H_2SO_4. D-lactate, formate, and acetate concentrations were analyzed by using the enzyme test kits (Boehringer Mannheim Co., Mannheim, Germany).

Fig. 1 shows cell mass and lactic acid concentration in batch fermentations at a controlled pH varying from 5.0 to 6.5. Except for pH 5.0, the growth curves were similar indicating that cell growth was not seriously affected by pH in the range of 5.5 to 6.5. However, there was a significant difference in lactic acid production rate between pH 5.5 and pH 6.0, although the final concentrations of lactic acid were similar above pH 5.5. At pH 5.0, glucose available in the medium was not completely utilized and so lactate production was considerably low compared with other pH values. In order to compare the degree of inhibition by lactic acid at different pH, the specific growth rate and specific production rate were expressed against the concentration of lactic acid produced during batch fermentation (Fig. 2). The experimental data shown in Fig. 1 were processed to obtain the instantaneous values of specific growth rate and specific production rate by using a numerical differentiation procedure suggested by LeDuy and Zajic (7). As can be seen in Fig. 2, the decreasing slope of both specific rates was steeper at lower pH values. The drop in specific rates varied exponentially with increasing lactic acid concentration implying that lactic acid produced inhibited cell growth significantly. These observations are in good agreement with earlier reports for other homofermentative lactic acid strains, such as *Streptococcus cremoris* (1) and *Lactobacillus bulgaricus* (19).

Growth characteristics and lactic acid production for various pH values are summarized in Table 1. The difference in maximum growth rates at different pHs was not significant even though a slightly lower value of growth

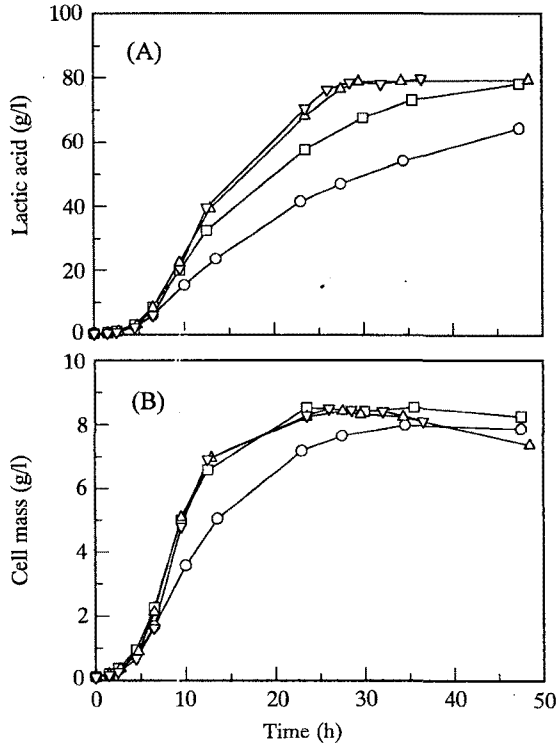

Fig. 1. Cell mass (A) and lactic acid concentration (B) during batch fermentation at different pH.
(○), pH 5.0; (□), pH 5.5; (△), pH 6.0; (▽), pH 6.5.

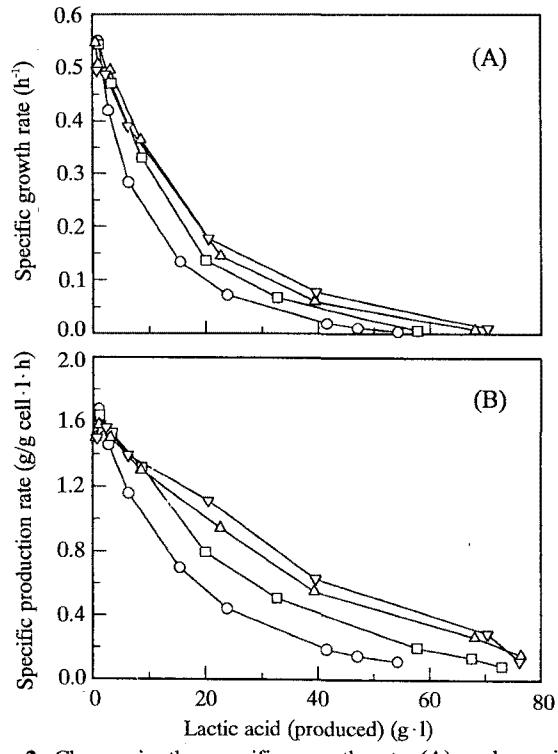

Fig. 2. Change in the specific growth rate (A) and specific production rate (B) at different pH.
(○), pH 5.0; (□), pH 5.5; (△), pH 6.0; (▽), pH 6.5.

Table 1. Growth characteristics and lactic acid production for various pH values.

pH	μ_{max}[1] (h^{-1})	X_{max}[2] (g dry wt./l)	ν_{max}[3] (g/g cell ·l·h)	Maximum productivity of lactic acid	Lactic acid conc. (g/l) after 48 h of culture
5.0	0.551	7.98	1.65	1.79	64.5
5.5	0.544	8.52	1.64	2.59	78.1
6.0	0.548	8.40	1.58	3.03	79.0
6.5	0.510	8.48	1.56	3.10	78.3

[1]Maximum specific growth rate. [2]Maximum cell concentration. [3]Maximum specific production rate.

rate (0.510 h^{-1}) was obtained at pH 6.5. However, the final cell mass obtained at pH 5.0 was significantly lower than that at pH values higher than 5.5. This means that the growth rate was not influenced by pH during the initial growth phase. In a study performed by Yabannavar and Wang (20), the value of μ_{max} was similarly constant at all pH values. The difference in final cell mass resulted from the inhibitory effect of lactate due to higher concentrations of undissociated lactic acid during batch fermentation at lower pH. The equilibrium dissociation reaction $C_3H_8O_3 \leftrightarrow C_3H_7O_3^- + H^+$ is described by $[Pu]=[P]/(1+10^{(pH-pK)})$, where [P] is the total lactic acid concentration, [Pu] is the concentration of undissociated lactic acid, and pK is the negative common logarithm of the dissociation constant of lactic acid. For lactic acid, pK is 3.86 at 37°C. Lactic acid productivity was also influenced by pH. Maximum productivity increased with an increase in pH from 5.0 to 6.5, but the difference between pH 6.0 and 6.5 was not significant. Normally intracellular pH is kept higher than culture pH, while the difference being greater at a lower culture pH. The greater pH difference causes to increase ATP consumption in order to maintain intracellular pH by proton pump within cell membranes (10).

From the above results, it may be concluded that the optimum pH for lactic acid production is between 6.0 and 6.5 when considering both cell growth rate and lactic acid productivity. However, changes in the pH of the medium may alter the pattern of products formation as reported by Rhee and Pack (13). Changes in fermentation products must be regarded as important because the optical purity of lactic acid is of increasing interest (3, 8, 12, 18). Therefore, we analyzed the fermentation products obtained at different pHs from 5.0 to 6.5. As shown in Fig. 3, the concentrations of various products were expressed against the concentration of lactic acid produced during batch culture in order to determine the product ratio for various pH values. The percentage of D-form lactic acid generally increased with an increase in total lactic acid produced. In an earlier study, it was found that the optimum pHs for L-lactate dehydrogenase and D-lactate dehydrogenase are 7.5 and 8.5, respectively (4). So it can be predicted that L-lactate dehydrogenase is more active than D-lactate dehydrogenase at a pH lower than 7.5. Indeed Fig. 3 shows that a higher culture pH leads to higher percentage of D-lactate with an exemption at pH 5.0 where cell growth was severely inhibited. Maximum D-lactate obtained was about 3.2% when the pH was controlled at 6.5, the highest culture pH tested in this study. It has been reported that heterolactic fermentation by a homofermentative *Lactobacillus* sp. may occur due to glucose limitation in a complete cell recycle operation (2). In this study, a significant increase in acetate concentration was observed during the final stage of culture, when lactic acid concentration varied from 75 to 80 g/l. Maximum acetate was obtained at pH 6.5. An interesting finding was that formate production did not increase during the medium and final stages of culture. In a previous section of this paper, we showed an optimum pH range of 6.0-6.5 for lactic acid production. It can now be seen that because of the increased formation of by-products, such as D-lactate, acetate, and formate at pH 6.5, lactic acid fermentation should be carried out at pH 6.0 if the fermented lactic acid is to be used as a raw material for polymer synthesis.

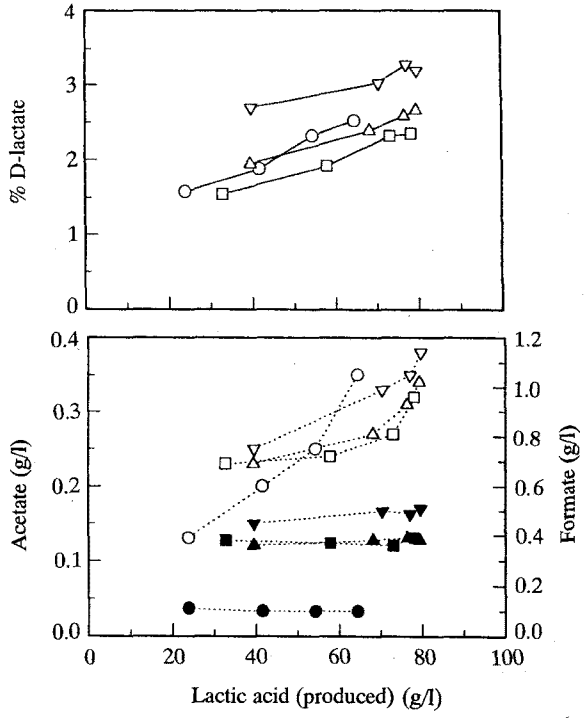

Fig. 3. The influence of pH on the fermentation products of *Lactobacillus casei*.
(○), pH 5.0; (□), pH 5.5; (△), pH 6.0; (▽), pH 6.5; closed symbols, formate; open symbols, acetate.

Fig. 4 shows the time course of product formation for a batch fermentation controlled at pH 6. There was a sig-

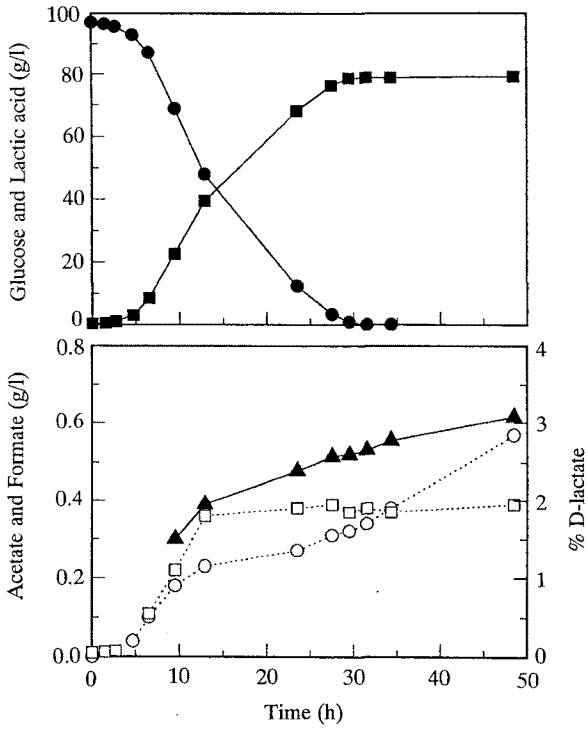

Fig. 4. Time profile of products formation for batch fermentation at pH 6.
(●), glucose; (■), lactic acid; (▲), % D-lactate; (○), acetate; (□), formate.

nificant increase in formate concentration during the initial stage of fermentation. Acetate and D-lactate concentrations increased even after glucose available in the medium was completely consumed. It seems that glucose depletion can influence the product formation pattern. As reported by some authors (5, 16), acetate production can be metabolically advantageous under glucose limitation since extra ATP can be formed in the final step of acetate formation catalyzed by acetate kinase.

In conclusion, the pH of lactic acid fermentation should be controlled at 6 rather than 6.5 when both lactic acid productivity and by-product minimization were considered.

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