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DIRECT FERMENTATION OF STARCH TO L-(+)-LACTIC ACID USING *LACTOBACILLUS AMYLOPHILUS*

Isao Yumoto and Koji Ikeda

Hokkaido National Industrial Research Institute, Tsukisamu-Higashi, Toyohira-ku Sapporo 062, Japan

Summary

Fermentation of L-(+)-lactic acid from soluble starch by *Lactobacillus amylophilus* was studied. The bacterium produced about 30 g of L-(+)-lactic acid from 50 g of soluble starch when the pH of the culture was ranging from pH 5 to pH 6.8 at 28°C. 53.4 g of L-(+)-lactic acid was produced when 100 g of starch was added in the medium. The fermentation procedures will reduce the cost of complete hydrolysis of starch to glucose prior to fermentation.

Introduction

Lactic acid can be used in food technology as preservative or taste enhancing additive, and is the source of polylactic acid, a polymer used as biodegradable plastic (Naude 1989). Lactic acid can be produced by lactic acid bacteria in the form of D-(-)-isomer, L-(+)-isomer, or racemic mixture; or by chemical synthesis as a racemic mixture. The racemic polymer and L-(+)-lactate polymer differ from each other in physical properties (Kulkarni *et al* 1971). For instance, lactate polymer will possess a higher melting point and crystallinity when L-(+)-lactic acid with higher optical purity is present in the lactate polymer.

Refined glucose and sucrose are the most commonly used substrate for producing lactic acid in fermentation process. However, production cost can be reduced if starch is used, as the saccharification process can be eliminated. Several studies on the fermentation of lactic acid using starch as the fermentation substrate, have been carried out recently with various microorganisms such as DL-lactic acid producing *Lactobacillus amylovorus* (Cheng *et al* 1991, Zang and Cheryan 1991); two kinds of microorganisms: amylolytic enzyme producing *Aspergillus awamori* and L-(+)-lactic acid producing *Streptococcus lactis* (Kurosawa *et al* 1988); and L-(+)-lactic acid producing *Rhizopus oryzae* (Hang 1989; Suntornsuk and Hang 1994).

In purification processes, it is very difficult and cost inefficient to purify L-(+)-lactic acid from broth medium when various other acids are contained in it. A broth medium with no acid other than L-(+)-lactic acid is therefore required.

In this study, the productivity of L-(+)-lactic acid from liquefied starch using *L. amylophilus* (Nakamura and Crowell 1979) in various conditions has been investigated, and the presence of possible acids in the broth medium has been analyzed.

Materials and Methods

Microorganism and medium;

The *Lactobacillus amylophilus* JCM 1125, used in the present investigation was obtained from The Institute of Physical and Chemical Research (Saitama, Japan). The culture medium contained polypeptone (Nihon Pharmaceutical, Tokyo; 10 g), yeast extract (Kyokuto, Tokyo; 5 g), soluble starch (Wako Pure Chemical, Osaka; 50 g), Tween 80 (1 g), sodium acetate (0.5 g), ammonium citrate (0.5 g), K_2HPO_4 (0.5 g), $MgSO_4 \cdot H_2O$ (0.2 g), $MnSO_4 \cdot H_2O$ (0.05 g) in 1 liter distilled water.

Cultivation;

Cultivation was performed in 1 liter of culture medium with 2-liter fermenter. The temperature was maintained at 28°C with agitation speed of 100 rpm. The pH was controlled at pH 6.8 by adding 20% Na_2CO_3 during cultivation via a pH controller. Nitrogen gas was introduced in order to keep an anaerobic condition.

Analysis;

The concentration of lactic acid and analysis of acids in broth medium were determined by post-column reaction system HPLC (CCPM-II, TOSOH) with spectrophotomeric detector (450 nm). The analytical conditions: column, TSKgel OApak (7.8 mm x 30 cm x 2) (TOSOH); column temperature, 60°C; solvent for elution, 0.75 mM H_2SO_4; solvent for reaction, 0.2 mM BTB-15 mM Na_2HPO_4 solution (pH 8.6); flow late, solvent for elution, 0.8 ml/min; solvent for reaction, 0.8 ml/min.

The optical purity of L-(+)-lactic acid was determined by HPLC (655A-12, HITACHI) with spectrophotomeric detector (254 nm). The analytical conditions; column, TSKgel ENANTIO L1 (4.6 mm x 25 cm) (TOSOH); column temperature, room temperature; solvent for elution, 0.5 mM $CuSO_4$ solution; flow late, 0.8 ml/min.

Results and Discussion

Figure 1 shows the production of L-(+)-lactic acid from liquefied starch at various temperatures. The concentration of L-(+)-lactic acid produced was in an increasing order at temperatures from 25°C to 35°C, but at 40°C, the production was much reduced. Thus the optimum temperature for the production of L-(+)-lactic acid is between 28°C and 35°C, comparable to the optimum growth temperature range reported previously (Nakamura and Crowell 1979).

In order to determine the optimum pH for the production of L-(+)-lactic acid, the cultivation was performed at pH 5.0, pH 6.0, and pH 6.8. The pH was continuously controlled at each pH with 20% Na_2CO_3. As shown in Fig. 2, there is no remarkable difference in the production from pH 5.0 to pH 6.8.

Figure 3 indicates the relationship between the concentration of substrate used and the concentration of L-(+)-lactic acid produced. The production rate was broadly similar for 3 different concentrations of substrate. The final L-(+)-lactic acid concentration in the medium was higher when higher initial substrate concentration was used. The highest concentration of lactic acid (53.4 g/l) was observed at 100 g/l of initial concentration of liquefied starch. However, the production rate became low when the concentration of the product attained 2.8% and above.

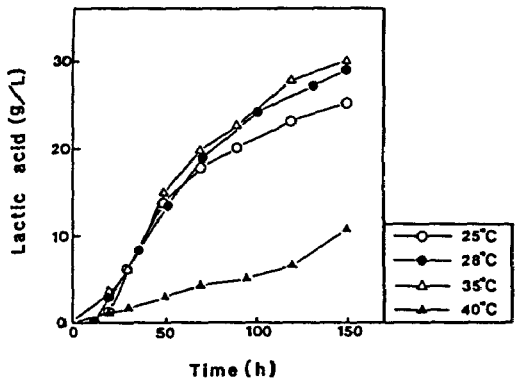

Fig. 1 Effect of pH and fermentation period on L-(+)-lactic acid production by *L. amylophilus*.

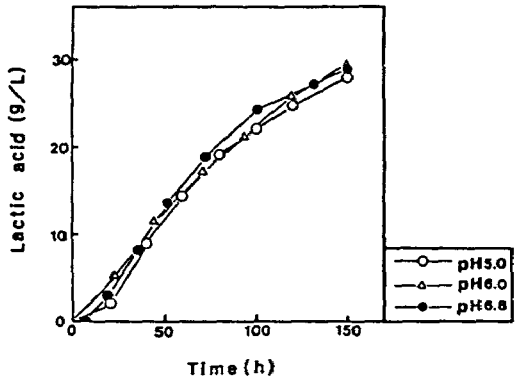

Fig. 2 Effect of temperature and fermentation period on L-(+)-lactic acid production by *L. amylophilus*.

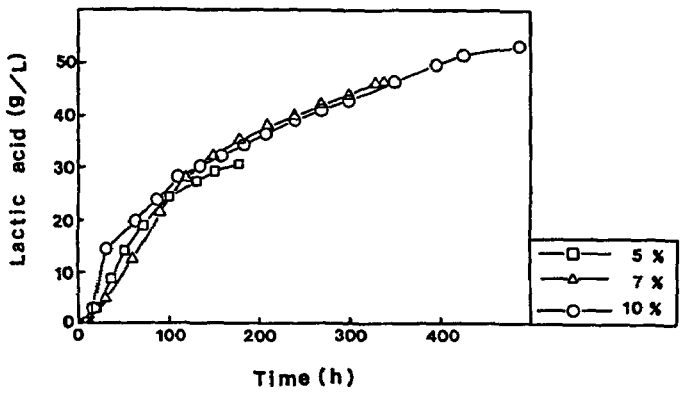

Fig. 3 Effect of starch concentration and fermentation period on L-(+)-lactic acid production by *L. amylophilus*.

Analysis by HPLC (results not shown) indicated that the bacteria produce no other acids besides lactic acid.

The optical purity of L-(+)-lactic acid was found to be 92.5% as shown in Figure 4 indicating that the bacterium produced L-(+)-lactic acid as the main acid and D-(+)-lactic as the minor.

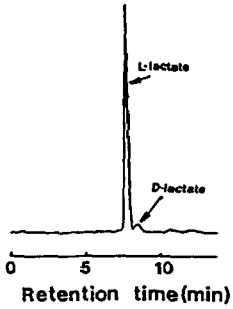

Fig.4 Analysis of optical purity of lactic acid in the cultured medium of *L. amylophilus* by HPLC. The analytical procedure was described in "materials and methods".

In conclusion, a direct fermentation of starch for the production of more than 30% of L-(+)-lactic acid, was first reported to be the genus *Lactobacillus*. The fermentation procedures will reduce the cost of complete hydrolysis of starch to glucose.

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