COMMUNICATION TO THE EDITOR

Production of Mannitol and Lactic Acid by Fermentation With *Lactobacillus intermedius* NRRL B-3693†

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Abstract: *Lactobacillus intermedius* B-3693 was selected as a good producer of mannitol from fructose after screening 72 bacterial strains. The bacterium produced mannitol, lactic acid, and acetic acid from fructose in pH-controlled batch fermentation. Typical yields of mannitol, lactic acid, and acetic acid from 250 g/L fructose were 0.70, 0.16, and 0.12 g, respectively per g of fructose. The fermentation time was greatly dependent on fructose concentration but the product yields were not dependent on fructose level. Fed-batch fermentation decreased the time of maximum mannitol production from fructose (300 g/L) from 136 to 92 h. One-third of fructose could be replaced with glucose, maltose, galactose, mannose, raffinose, or starch with glucoamylase (simultaneous saccharification and fermentation), and two-thirds of fructose could be replaced with sucrose. *L. intermedius* B-3693 did not co-utilize lactose, cellobiose, glycerol, or xylose with fructose. It produced lactic acid and ethanol but no acetic acid from glucose. The bacterium produced 21.3 ± 0.6 g lactic acid, 10.5 ± 0.3 g acetic acid, and 4.7 ± 0.0 g ethanol per L of fermentation broth from dilute acid (15% solids, 0.5% H_2SO_4, 121°C, 1 h) pretreated enzyme (cellulase, β-glucosidase) saccharified corn fiber hydrolyzate. **Biotechnol Bioeng* **82**: 864–871, 2003.

Keywords: *Lactobacillus intermedius;* mannitol production; lactic acid production; ethanol production; mixed acid production; fructose fermentation; batch fermentation; fed-batch fermentation; pH-controlled fermentation

INTRODUCTION

Mannitol, a naturally occurring polyol, is widely used in the food, pharmaceutical, medical, and chemical industries (Soetaert et al., 1995). It is used as a sweet-tasting bodying and texturing agent. Mannitol reduces the crystallization tendency of sugars and is used as such to increase the shelf-life of foodstuffs. Crystalline mannitol exhibits a very low hygroscopicity, making it useful in products that are stable at high humidity. It is extensively used in chewing gum. Because of its desirable properties, mannitol is commonly used in the pharmaceutical formulation of chewable tablets and granulated powders. It prevents moisture absorption from the air, exhibits excellent mechanical compressing properties, does not interact with the active components, and has a sweet cool taste that masks the unpleasant taste of many drugs (Debord et al., 1987). The complex of boric acid with mannitol is used in the production of dry electrolytic capacitors. It is an extensively used polyol for production of resins and surfactants. Mannitol is used in medicine as a powerful osmotic diuretic and in many types of surgery for the prevention of kidney failure and to reduce dye and brain edema. Mannitol hexanitrate is a well-known vasodilator, used in the treatment of hypertension.

Mannitol is currently produced industrially by high-pressure hydrogenation of fructose/glucose mixtures in aqueous solution at high temperature (120–160°C) with Raney nickel as catalyst (Makkee et al., 1985). Typically, the hydrogenation of a 50/50 fructose/glucose mixture results in an approximately 25/75 mixture of mannitol and sorbitol. This means that about half of the fructose is converted to mannitol and half of it to sorbitol. The glucose is hydrogenated exclusively to sorbitol. As a consequence, the commercial production of mannitol is always accompanied by the production of sorbitol thus resulting in a less efficient process (Soetaert et al., 1995).

In recent years, research efforts have been directed towards production of polyols by fermentation and enzymatic means (Vandamme and Soetaert, 1995). We recently screened 72 bacterial cultures from ARS Culture Collection for production of mannitol from glucose or fructose. While

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† Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

none of the cultures produced mannitol from glucose, nine cultures produced mannitol from fructose. We selected *Lactobacillus intermedius* B-3693 as a good producer of mannitol. To our knowledge, the production of mannitol by this organism has not been reported.

In this paper, we describe the production of mannitol and lactic acid by fermentation with *L. intermedius* B-3693.

MATERIALS AND METHODS

Materials

Aminex HPX-87P (300 × 7.8 mm) and Aminex HPX-87H (300 × 7.8 mm) columns were purchased from Bio-Rad Laboratories (Hercules, CA). An Astec CLC-D (150 × 4.6 mm) column was obtained from Advanced Separation Technologies, Inc. (Wippany, NJ). Corn fiber from a corn wet milling facility was supplied by Williams Bioenergy (Pekin, IL). Celluclast (cellulase) and Novozyme 188 (β-glucosidase) were from Novozymes (Franklinton, NC). Both D- and L-lactic acids were purchased from Sigma Chemical Company (St. Louis, MO).

Bacterial Strains

Seventy-two bacterial strains were obtained from the ARS Culture Collection (National Center for Agricultural Utilization Research, Peoria, IL) These strains (with NRRL numbers) were: *L. acidophilus* B-4495, *L. amylophilus* B-4436, *L. amylovorus* B-4545, *L. animalis* B14177, *L. arabinosus* B-787, *L. brevis* B-1836, *L. buchneri* B-1860, *L. bulgaricus* B-548, *L. casei* B-1922, *L. cellobiosus* B-1840, *L. coryniformis* B-4391, *L. delbrueckii* B-763, *L. fermentum* B-1915, *L. fructivorans* B-4000, *L. gasseri* B-14168, *L. gramminis* B-14857, *L. helveticus* B-1935, *L. intermedius* B-3693, *L. jensenii* B-4550, *L. leichmanii* B-4525, *L. mali* B-4565, *L. paracasei* B-4564, *L. pentosus* B-473, *L. plantarum* B-4496, *L. reuteri* B-14172, *L. rhamnosus* B-442, *L. salivarius* B-1949, *Leuconostoc amelibiosum* B-742, *L. citrovorum* B-1147, *L. mesenteroides* subsp. *dextranicum* B-1120, *L. mesenteroides* subsp. *mesenteroides* B-1209, *L. paramesenteroides* B-3471, *L. oenos* B-3474, *L. lactis* B-3468, *Pediococcus acidilactici* B-1153, *P. pentosaceus* B-14009, *Lactococcus lactis* B-1821, *Streptococcus dysgalactiae* B-688, *Enterococcus faecalis* B-537, *E. faecium* B-1295, *E. casseliflavus* B-3502, *E. hirae* B-14926, *Bacillus subtilis* NRS-744, *B. cereus* B-3711, *B. licheniformis* NRS-1264, *B. megaterium* B-14308, *B. pumilus* B-14292, *B. coagulans* NRS-609, *B. smithii* NRS-173, *B. amyloliquefaciens* B-14394, *B. mycoides* NRS-273, *Paenibacillus polymyxa* B-367, *P. peoriae* B-14750, *P. amylolyticus* B-377, *P. illinoisensis* NRS-1356, *P. chondroitinus* B-14420, *P. alginolyticus* NRS-1347, *P. pulvifaciens* B-14166, *P. lautus* NRS-666, *P. validus* NRS-1000, *P. pabuli* B-14213, *P. thiaminolyticus* B-14605, *P. macerans* B-172, *P. glucanolyticus* B-14680, *P. curdlanolyticus* B-23243, *P. apiarius* NRS-1438, *Micrococcus luteus* B-287, *M. kristinae* B-14845, *Brevibacillus brevis* NRS-604, *B. agri* B-1158, *B. choshinensis* B-23247, and *B. reuszeri* NRS-1206.

Screening Medium and Culture Conditions

The screening medium designated as MRS was based on that of de Man et al. (1960) and contained 10 g peptone, 10 g beef extract, 5 g yeast extract, 1.0 mL Tween 80, 2 g ammonium citrate, 5 g sodium acetate, 0.1 g magnesium sulfate, 0.05 g manganese sulfate, and 2 g disodium phosphate per liter (final pH 6.5). The medium and the substrate (glucose or fructose 5%, w/v) were sterilized separately at 121°C for 15 min. A 125-mL Erlenmeyer flask containing 50 mL MRS medium with substrate was inoculated with a loopful of cells taken from a stock slant and incubated at 30°C on a rotary shaker (130 rpm). Samples were withdrawn at a specific time to determine cell growth, residual substrate, and product yield.

Fermentation Experiments

The fermentation experiments were carried out in simplified MRS medium (without beef extract and Tween 80). For seed culture, a 250-mL Erlenmeyer flask containing 100 mL of the medium with fructose (2%, w/v) was inoculated with a loopful of cells taken from a stock slant and incubated at 37°C on a rotary shaker (130 rpm) for 24 h. Batch culture experiments were performed in pH-controlled 500-mL fleakers with an initial medium volume of 300 mL at either 30 or 37°C essentially as described by Bothast et al. (1994). The pH was maintained at 5.0 by adding 2, 4, or 8 *N* NaOH. Cultures were stirred magnetically using 1.5-inch stir bars, at 130 rev/min. The experimental set-up is shown in Fig. 1. Samples were withdrawn periodically to determine cell growth, sugar utilization, and production yield.

Preparation of Corn Fiber Hydrolyzate

Corn fiber was pretreated and saccharified with enzymes by the procedure described previously (Saha and Bothast, 1999).

Analytical Methods

Cell growth was monitored by measuring optical density of the appropriately diluted culture broth at 660 nm. Sugar utilization and product analysis were performed by high-pressure liquid chromatography (HPLC) (Spectra-Physics, San Jose, CA). For quantification of sugar and mannitol, an Aminex HPX-87P column was used. The column was maintained at 85°C, and the sugars were eluted with deionized water (Milli-Q water, Millipore Corp., Bedford, MA) at a flow rate of 0.6 mL/min. For organic acid and ethanol analyses, an Aminex HPX-87H column was used. The column was maintained at 65°C, and the organic acids were eluted with 5 m*M* H_2SO_4 or 10 m*M* HNO_3 at a flow rate of

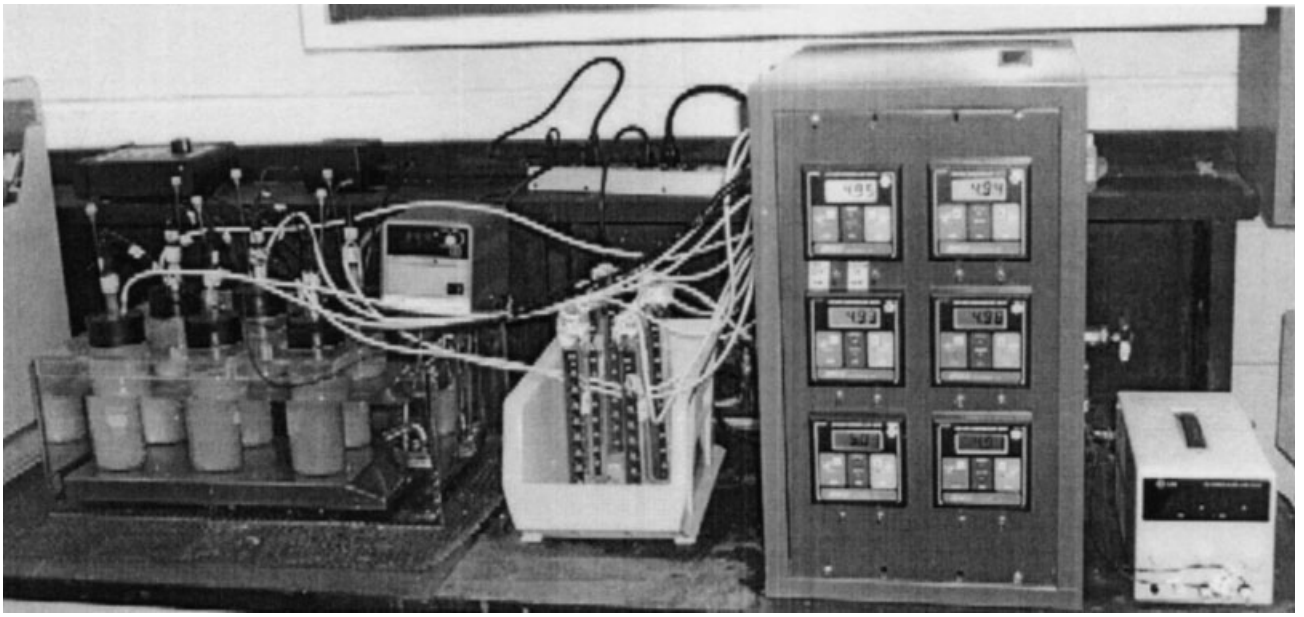

Figure 1. pH-controlled fleaker fermentation system set-up.

0.6 mL/min. Peaks were detected by refractive index and identified and quantified by comparison to retention times of authentic standards. Both D-(−)- and L-(+)-lactic acid were measured quantitatively by HPLC. An Astec CLC-D column was used at room temperature, and the mobile phase was 5 m*M* $CuSO_4$ at a flow rate of 1.0 mL/min. Peaks were detected by UV detector at 240 nm and identified and quantified by comparison to retention times of authentic standards.

RESULTS

Screening for Mannitol Production

The strains that produced mannitol from fructose were *L. brevis* B-1836, *L. buchneri* B-1860, *L. cellobiosus* B-1840, *L. fermentum* B-1915, *L. intermedius* B-3693, *Leuconostoc amelibiosum* B-742, *L. citrovorum* B-1147, *L. mesenteroides* subsp. *dextranicum* B-1120, and *L. paramesenteroides* B-3471. In addition, all these strains produced lactic acid and acetic acid. Among these nine strains, the strain *L. intermedius* B-3693 was selected for further study on mannitol production based on its ability to produce mannitol more rapidly than the other strains (data not shown).

Batch Production of Mannitol by *L. intermedius* B-3693

Effect of Fructose Concentration

Strain B-3693 produced mannitol, lactic acid, and acetic acid when grown on fructose in pH-controlled fermentation. The effect of fructose concentration ranging from 150 to 300 g/L on mannitol production by *L. intermedius* B-3693 is presented in Table I. The mannitol yields were 107.6 ± 0.5, 138.6 ± 6.9, 175.6 ± 5.9, and 198.3 ± 11.0 g/L at 150, 200, 250, and 300 g/L fructose, respectively. Small white needle-like crystals of mannitol appeared upon refrigeration of the cell-free fermentation broth of 300 g/L fructose at 4°C (Fig. 2). Typical time courses of fructose utilization and mannitol, lactic acid. and acetic acid production at 150, 200, 250, and 300 g/L substrate concentration are shown in Fig. 3A–D. The time of maximum mannitol yield varied greatly from 15 h at 150 g/L fructose to 136 h at 300 g/L fructose concentration. Also, there was a long lag period of about 72 h in growth and fructose utilization at 300 g/L fructose concentration in comparison to the lag period of about 16 h at 250 g/L fructose. However, the product patterns and yields were not greatly influenced by fructose concentration. The bacterium transformed fructose to mannitol from the early growth stage, and it did not consume mannitol even when all supplied fructose had been utilized. Moreover, the product (mannitol, lactic acid, and acetic acid) concentration continued to increase slightly upon further continuation of

Table I. Mannitol production from fructose by *L. intermedius* NRRL B-3693 in pH-controlled batch fermentation.[a]

Fructose (g/L)	Time (h)	Mannitol (g/g)[b]	Lactic acid (g/g)[b]	Acetic acid (g/g)[b]
150	15	0.72 ± 0.00	0.17 ± 0.00	0.12 ± 0.00
200	40	0.69 ± 0.03	0.17 ± 0.00	0.13 ± 0.00
250	64	0.70 ± 0.02	0.16 ± 0.00	0.12 ± 0.00
300	136	0.66 ± 0.03	0.15 ± 0.01	0.11 ± 0.00

[a]At 37°C, 130 rpm, initial pH 6.5, pH controlled at 5.0, 500-mL fleaker with 300 mL of medium.

[b]In g product per g fructose provided.

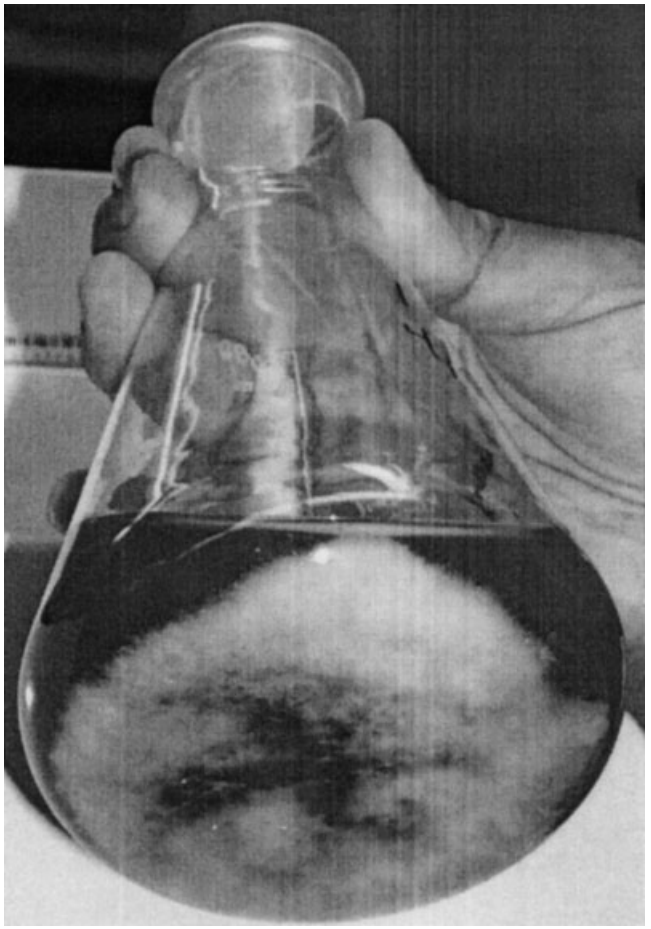

Figure 2. Mannitol crystals formed in cell-free fermentation broth at 4°C. Fructose used: 300 g/L.

the fermentation in most cases. The maximum cell growth (A_{660} of 8.7 ± 0.5 in 15 h) was obtained at fructose concentration of 150 g/L. The average maximum cell densities (A_{660}) were 4.7 ± 0.4 in 24 h, 5.3 ± 1.0 in 64 h, and 6.5 ± 0.8 in 136 h at fructose concentrations of 200, 250, and 300 g/L, respectively.

Substitution of Fructose With Other Energy Sources

In order to investigate the role of other sugars in mannitol production, one-third of the fructose was replaced with other substrates such as glucose, maltose, starch plus glucoamylase (simultaneous saccharification and fermentation, SSF), mannose, galactose, xylose, arabinose, cellobiose, and glycerol. Two-thirds of the fructose were also replaced by sucrose. The results of mannitol production by the two-substrate system are presented in Table II. It is clear that one-third of the fructose can be replaced with glucose, starch with glucoamylase, maltose, mannose, and galactose. Two-thirds of the fructose can also be replaced by sucrose. Even though arabinose was co-utilized with fructose very well by the bacterium, it did not contribute to net mannitol production. However, the production of lactic acid and acetic acid increased considerably. The bacterium was not able to co-utilize lactose, glycerol, cellobiose, and xylose with fructose. A time course of fructose (100 g/L) and glucose (50 g/L) co-fermentation is shown in Fig. 4. The bacterium co-utilized fructose and glucose simultaneously and produced very similar quantities of mannitol, lactic acid, and acetic acid in comparison with fructose only. The conversion efficiency of fructose to mannitol was 96%. The glucose was converted to lactic acid and acetic acid, which were partially neutralized during fermentation by adding NaOH to control the pH at 5.0.

Fed-Batch Production of Mannitol by *L. intermedius* B-3693

In order to decrease the fermentation time required to complete 300 g/L fructose utilization, the fed-batch culture technique was used. The results of fed batch culture with 300 g/L fructose are shown in Fig. 5. The fermentation time decreased considerably from 136 to 92 h by feeding equal amounts of substrate and medium four times. The yields of mannitol, lactic acid, and acetic acid were 202.5 ± 4.3, 52.6 ± 0.96, and 38.5 ± 0.7 g/L, respectively. The maximum cell growth (cell density, A_{660} of 6.9 ± 0.2) occurred in 64 h. The yields of mannitol, lactic acid, and acetic acid from co-fermentation of fructose and glucose (2:1) at 300 g/L total substrate concentration were 179.4 ± 9.3, 44.1 ± 0.0, and 33.4 ± 0.6 g, respectively in 160 h. The maximum cell growth (A_{660} of 3.1 ± 0.3) was observed at 88 h.

Fermentation of Glucose by *L. intermedius* B-3693

The bacterium utilized glucose and produced lactic acid and ethanol. During the entire fermentation, no formation of acetic acid was observed. As shown in Fig. 6, the bacterium utilized all glucose provided (150 g/L) and produced lactic acid (70.4 ± 0.6 g/L) and ethanol (36.2 ± 1.0 g/L) in 40 h. The maximum cell growth (A_{660} of 7.3 ± 0.1) was obtained at 24 h. At 200 g/L glucose, the bacterium utilized 80% substrate and produced 70.4 ± 2.1 g lactic acid and 38.0 ± 0.9 g ethanol in 64 h. There was a lag of growth and glucose utilization for about 24 h and maximum cell growth (A_{660} of 8.3 ± 0.3) was observed at 48 h. Use of the fed-batch approach to decrease the fermentation time or increase the glucose concentration did not improve these fermentation parameters. HPLC analysis of the fermentation broth with an Astec CLC-D column indicates that the bacterium produced both D- and L-lactic acid in equal proportions (1:1 ratio) throughout the time course studied. In this column, the L-form eluted first and was well separated from the D-form (Fig. 7). The retention times of L- and D-lactic acids were 22.5 and 30.2 min, respectively, under the specified conditions as mentioned in the section Materials and Methods.

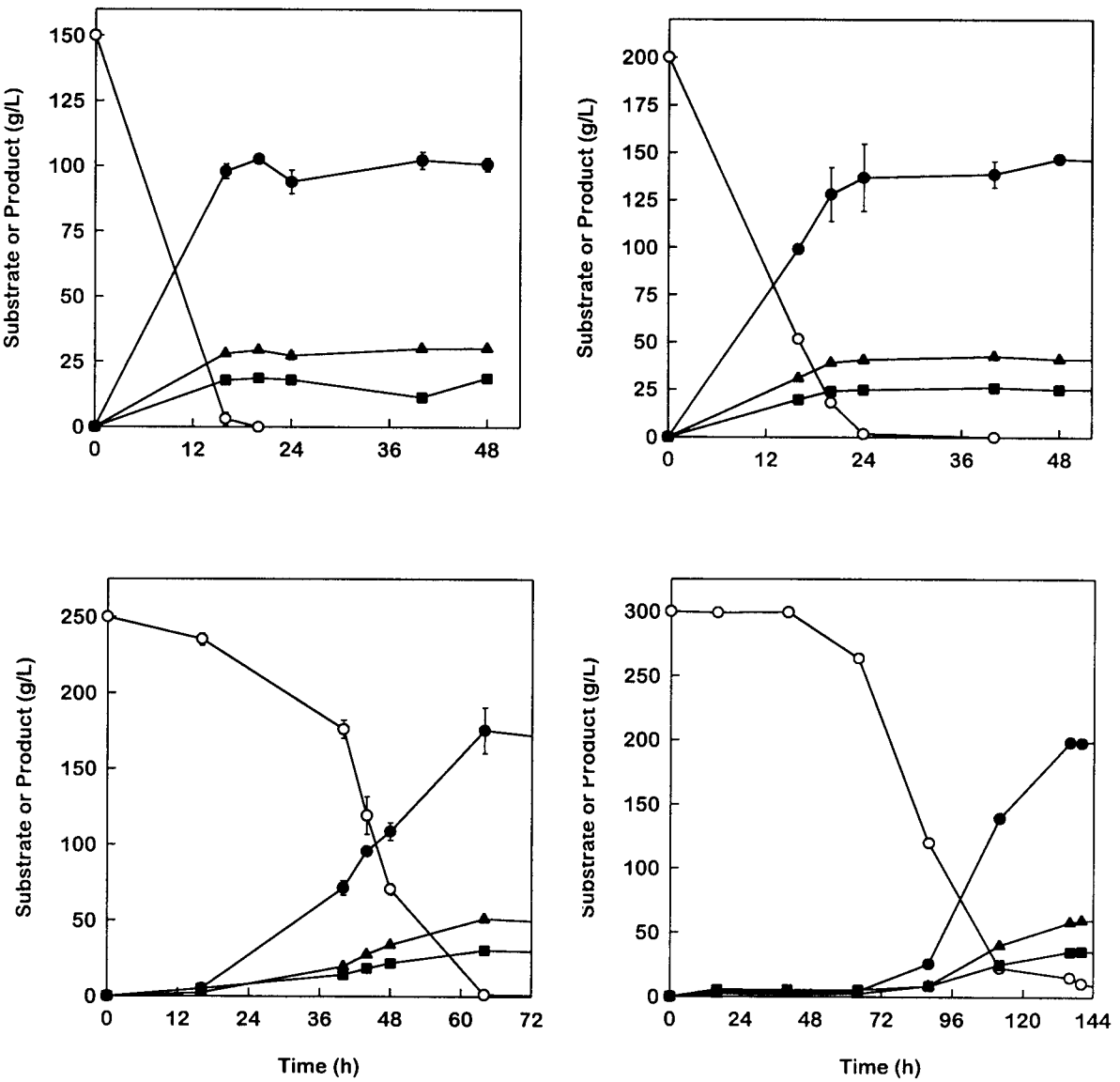

Figure 3. Time courses of fructose (150–300 g/L) utilization and mannitol production by *L. intermedius* B-3693 in pH-controlled batch fermentation at 37°C: (**A**) 150 g/L; (**B**) 200 g/L; (**C**) 250 g/L; (**D**) 300 g/L. Symbols: (○) fructose; (●) mannitol; (▲) lactic acid; (■) acetic acid.

Fermentation of Corn Fiber Hydrolyzate by *L. intermedius* B-3693

Corn fiber (15% solids) was pretreated with 0.5% H_2SO_4 at 121°C for 1 h, treated with Celluclast and Novozyme 188 (1 mL each for 100 g corn fiber) at pH 5.0 and 45°C for 72 h, overlimed with $Ca(OH)_2$ to pH 10.5, neutralized back to pH 6.5 with H_2SO_4, and centrifuged (8,000 rpm, 20 min) to remove the solids. The liquid portion of corn fiber hydro-

Table II. Mannitol production using two substrate system (fructose and another sugar) by *L. intermedius* NRRL B-3693 in pH-controlled batch fermentation.[a]

Substrate (g/L)	Time (h)	Mannitol (g/L)	Lactic acid (g/L)	Acetic acid (g/L)
Fructose (100) plus glucose (50)	20	97.3 ± 2.6	23.2 ± 0.5	15.8 ± 0.4
Fructose (50) plus sucrose (100)	64	84.5 ± 0.7	23.6 ± 1.6	13.6 ± 0.3
Fructose (100) plus starch (50) and glucoamylase	24	86.6 ± 1.2	25.7 ± 0.5	13.8 ± 0.1
Fructose (100) plus maltose (50)	15	95.9 ± 0.8	20.9 ± 0.2	14.2 ± 0.4
Fructose (100) plus mannose (50)	89	89.1 ± 1.9	18.4 ± 2.6	14.6 ± 1.9
Fructose (100) plus galactose (50)	15	82.3 ± 0.7	16.7 ± 0.7	13.2 ± 0.2
Fructose (100) plus affinose (50)	40	94.1 ± 0.7	24.8 ± 0.3	15.3 ± 0.1
Fructose (100) plus arabinose (50)	64	61.6 ± 0.9	41.1 ± 1.1	27.3 ± 1.2

[a]At 30°C, initial pH 6.5, pH maintained at 5.0, 130 rpm, 500-mL fleaker with 300 mL of medium.

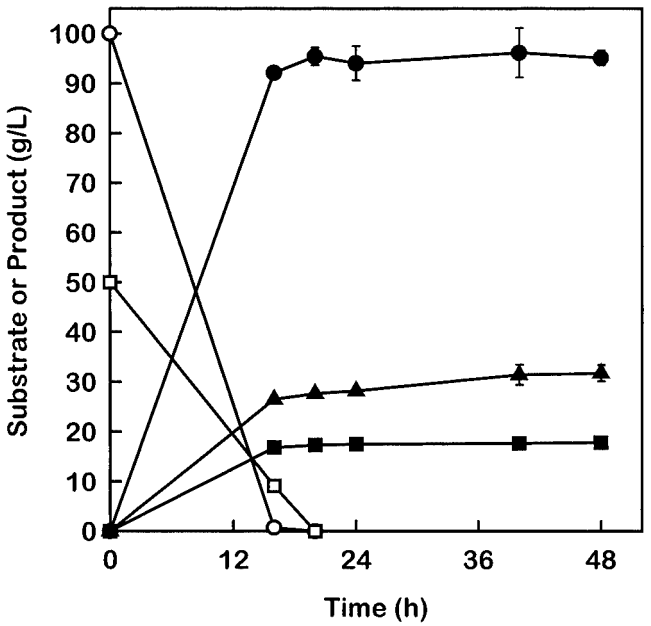

Figure 4. Time courses of fructose (100 g/L) and glucose (50 g/L) co-utilization and mannitol production by *L. intermedius* NRRL B-3693 in pH-controlled batch fermentation at 35°C. Symbols: (○) fructose; (□), glucose; (●) mannitol; (▲) lactic acid; (■) acetic acid.

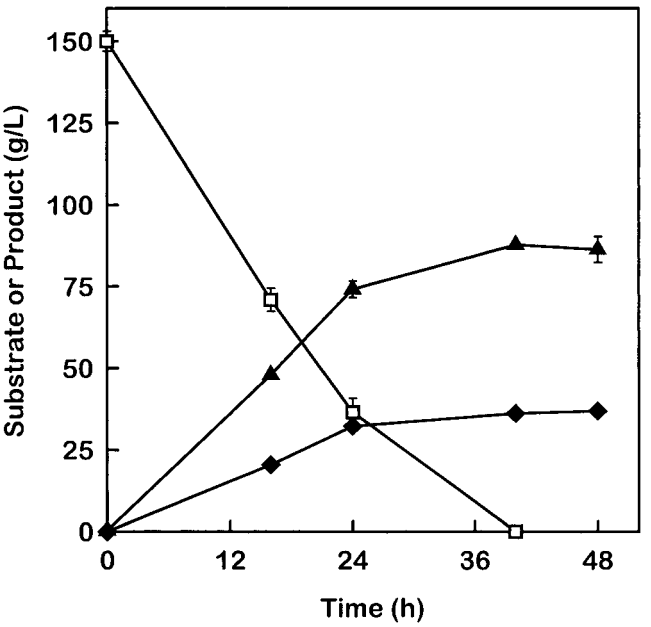

Figure 6. Time course of glucose (150 g/L) utilization, and lactic acid and ethanol production by *L. intermedius* NRRL B-3693 in pH-controlled batch fermentation at 37°C. Symbols: (□) glucose; (▲) lactic acid; (◆) ethanol.

lyzate (total sugar content, 50.6 ± 0.1 g) was used as substrate. The bacterium was able to grow well in this overlimed corn fiber hydrolyzate and produced 21.3 ± 0.6 g lactic acid,10.5 ± 0.3 g acetic acid, and 4.7 ± 0.0 g ethanol per L of fermentation broth.

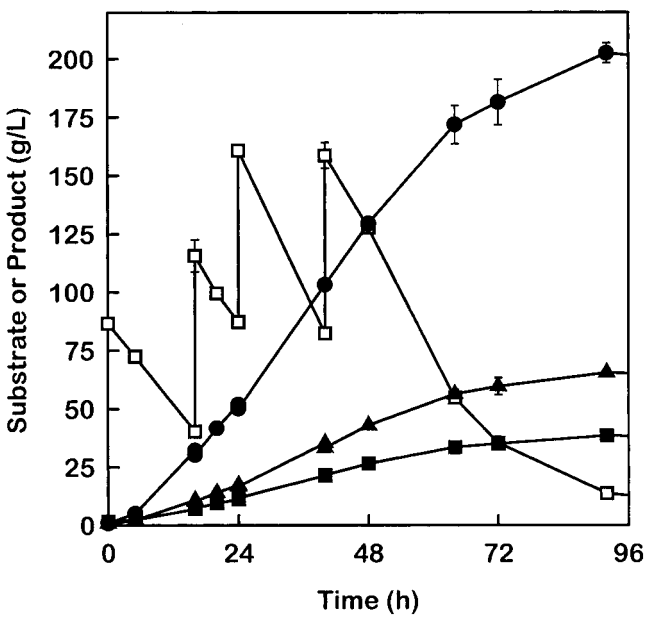

Figure 5. Time courses of fructose utilization and mannitol production by *L. intermedius* NRRL B-3693 in pH-controlled fed-batch fermentation at 37°C. Fructose used: 300 g/L (final concentration). Symbols: (○) fructose; (●) mannitol; (▲) lactic acid; (■) acetic acid.

Fructose and Glucose Metabolism by *L. intermedius* B-3693 Under Anaerobic Conditions

The bacterium was grown in MRS medium under anaerobic condition using 2% fructose or 2% glucose as carbon source. The product patterns were analyzed by HPLC. It utilized both fructose and glucose well and produced mannitol, lactic acid, and acetic acid with fructose as growth substrate. The bacterium produced lactic acid and ethanol but no acetic acid from glucose. There was practically no difference in product profiles as well as in product ratios in both cases of fructose and glucose metabolism under aerobic and anaerobic conditions.

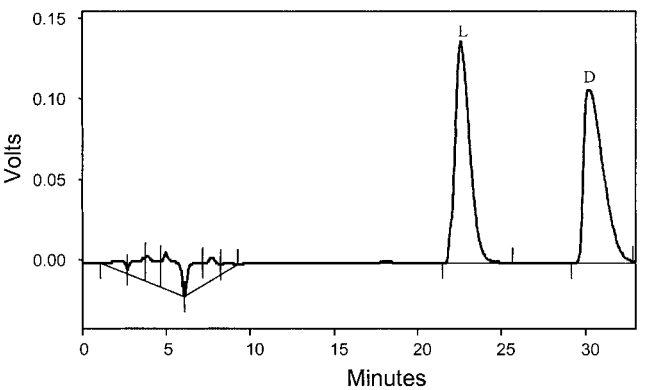

Figure 7. HPLC pattern of fermentation broth analysis of *L. intermedius* NRRL B-3693 grown on glucose (150 g/L). An Astec CLC-D column was used at room temperature. The mobile phase was 5 m*M* $CuSO_4$ at a flow rate of 1.0 mL/min. Peaks were detected by UV detector. L, L-(+)-lactic acid; D, D-(−)-lactic acid.

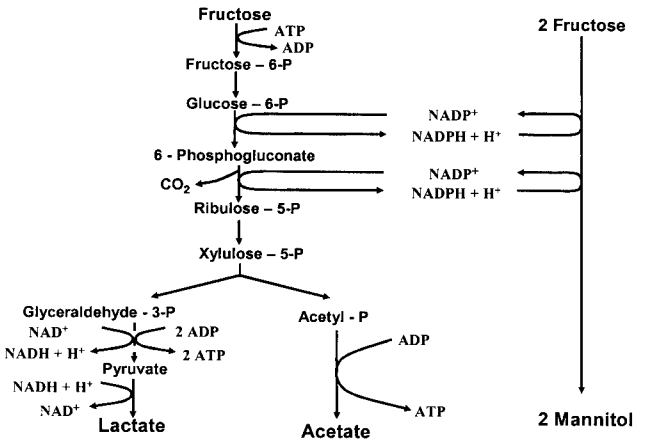

Figure 8. Overview of the pathway of fructose utilization by *L. intermedius* NRRL B-3693.

DISCUSSION

This is the first study of mannitol production by *L. intermedius* B-3693. This is also the first detailed production formation study for a mannitol producing *Lactobacillus* sp. It appears that mannitol produced by *L. intermedius* was derived from hexose phosphate pathway like other mannitol-producing bacteria, such as *Lactobacillus* sp. Y-107, *Leuconostoc* sp. Y-002, and *Leucononostoc mesenteroides* (Soetaert et al., 1995; Yun and Kim, 1996, 1998). The process makes use of the capability of *L. intermedius* to utilize fructose as an alternative electron acceptor, thereby reducing it to mannitol with the enzyme mannitol dehydrogenase. In this process, the reducing equivalents are generated by conversion of about one-third of the fructose to lactic acid and acetic acid. It is thought that enzyme reaction proceeds according to the following (theoretical) equation:

$$3\ \text{Fructose} \rightarrow 2\ \text{Mannitol} + \text{Lactic Acid} + \text{Acetic Acid} + CO_2.$$

For fructose and glucose (2:1) co-fermentation, the equation becomes:

$$2\ \text{Fructose} + \text{Glucose} \rightarrow 2\ \text{Mannitol} + \text{Lactic Acid} + \text{Acetic Acid} + CO_2.$$

An overview of the proposed pathway of fructose utilization by *L. intermedius* NRRL B-3693 is presented in Fig. 8. Several heterofermentative lactic acid bacteria produce mannitol in large amounts, using fructose as an electron acceptor (Grobben et al., 2001; Wisselink et al., 2002).

There was no formation of sorbitol, xylitol, or arabitol in the cases of glucose, xylose, and arabinose co-fermentation with fructose, respectively. Glucose can be replaced with starch plus glucoamylase (simultaneous saccharification to glucose and fermentation to lactic acid and acetic acid), maltose, galactose, mannose, and raffinose (Table II).

At fructose concentration at 300 g/L, the final mannitol concentration in the fermentation broth exceeded the solubility limit of 180 g/L at 25°C. Thus a desired mannitol concentration in the fermentation broth can be achieved by using 300 g/L substrate concentration by both batch and fed-batch fermentation. The fermentation time decreased considerably from 136 to 92 h by using the fed-batch approach. This offers an extra advantage for downstream processing of fermentation broth because mannitol can be crystallized out by simple cooling of the fermentation broth

Table III. Comparison of mannitol production by *L. intermedius* B-3693 with results of earlier workers.

Microorganism	Substrate (g/L)	Time[a] (h)	Yield[b] (%)	Reference
Bacteria				
Lactobacillus intermedius B-3693	Fructose (150)	15	72	This work
	Fructose (200)	40	69	This work
	Fructose (250)	64	70	This work
	Fructose (300)	136	66	This work
	Fructose (300)	92	67	This work (fed-batch)
	Fructose (100) + glucose (50)	20	65	This work
Lactobacillus sp. B001	Fructose (100) + glucose (50)	24	65	Itoh et al., 1992
Lactobacillus sp. Y-107	Fructose (100)	120	73	Yun and Kim, 1998
Lactobacillus sanfranciscensis	Fructose (?)	120	60 g/L	Korakli et al., 2000
Leuconostoc mesenteroides	Fructose (100) + glucose (50)	35	60	Soetart et al., 1995
	Fructose (08)	—	30–40	Erten, 1998
Leuconostoc sp. Y-002	Fructose (50)	25	40	Yun and Kim, 1998
Yeast				
Candida magnoliae	Fructose (150)	168	45	Song et al., 2002
Candida zeylannoides	*n*-Paraffin (100)	100	52	Hattori and Suzuki, 1974
Torulopsis versalitis	Glucose (194)	240	28	Onishi and Suzuku, 1968
Torulopsis mannitofaciens	Glycerol (100)	168	31	Onishi and Suzuki, 1970
Fungi				
Aspergillus candidus	Glucose (32)	288	69	Smiley et al., 1967
Penicillium scabrosum	Sucrose (150)	288	40	Hendriksen et al., 1988

[a]Time required to reach maximum mannitol yield.
[b]Mannitol yields were calculated on the basis of initial sugars employed.

(Fig. 2). Lactic acid and acetic acid can also be recovered by electrodialysis (Datta and Tsai, 1997).

The heterofermentative lactic acid bacterium produced lactic acid (0.47 g/g) and no acetic acid from glucose (150 g/L). However, it also produced ethanol in good yield (0.24 g/g glucose). It is not clear why acetic acid was not produced. Further, it produced both D- and L-lactic acid in equal amounts. This finding is quite different from the reported D-lactic acid production from *Leuconostoc mesenteroides* (Soetaert et al., 1995). To the authors' knowledge, these authors did not present any experimental evidence for the D-lactic acid production. The bacterium was able to utilize sugars (glucose, xylose, arabinose, and galactose) in corn fiber acid hydrolyzate and produced lactic acid, acetic acid, and ethanol. It has the potential to be used for making lactic acid, acetic acid, and ethanol from waste and underutilized agricultural residues such as corn fiber hydrolyzate. A comparative study of mannitol production by *L. intermedius* B-3693 with those of the earlier workers is presented in Table III. The data presented in this table with other bacteria are with enriched MRS medium. A simplified MRS medium without beef extract and Tween was used throughout this study. The reported fermentation time for *L. intermedius* B-30560 might be shortened by using the enriched MRS medium.

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