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Full Length Research Paper

Biomass production of *Lactobacillus plantarum* LP02 isolated from infant feces with potential cholesterol-lowering ability

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The potentially hypocholesterolemic strain, designated PL02, of *Lactobacillus plantarum*, was isolated from infant feces. The aim of this study was to characterize and to cultivate this isolate for biomass production in a 5 L fermentor by batch or fed-batch fermentation. A modified medium composition without peptone was used to produce more viable cells with a 150 rpm agitation speed and 0.5 vvm of aeration during incubation. A cell concentration of 2.2 g dry cell weight (DCW) per liter in fermented broth was reached in a 5 L fermentor after the glucose was consumed completely during batch fermentation. In addition, biomass was significantly improved at 28 h of fed-batch fermentation (9.45 g DCW/l) over a constant feeding rate of 20 ml/h of feeding solution since the glucose was consumed at batch process. A much shorter fermentation time (15 h) and greater biomass (10.12 g DCW/l) was obtained by using multistep feeding rates to maintain glucose concentration of 1 to 5 g/l during fed-batch process. The biomass of *L. plantarum* PL02 produced by fed-batch fermentation was greater than that achieved by batch fermentation and the fed-batch method might also be suitable for other lactic acid bacteria fermentation.

Key words: *Lactobacillus plantarum*, fermentation, fed-batch fermentation, cholesterol

INTRODUCTION

Probiotics, microorganisms beneficial to human or animals, have recently gained greater interest for use in the industry. Numerous papers have described their physiological functions, such as inhibiting of pathogenic microorganisms, lowering cholesterol levels, enhancing immunity, alleviating lactose intolerance, improving nutrition and digestion, and conferring anticancer effects (Hirayama and Rafter, 2000; Leroy and Vuyst, 2004; Wollowski et al., 2001).

Some lactic acid bacteria (LAB) have been reported to possess a cholesterol-lowering ability (Buck and Gilliland, 1994; Lye et al., 2010). The possible mechanisms for this ability have been reported by Lye et al. (2010) under conditions that mimic that of the human gastrointestinal tract. Oral administration of sufficient amounts of fermented *Lactobacillus acidophilus* products has been claimed to decrease the concentration of blood cholesterol in humans (Schaafsma et al., 1998). Gilliland et al. (1985) proposed that the cholesterol might be adhered to or absorbed into the LAB cells when these strains are cultured with cholesterol. LAB might absorb cholesterol in the intestinal canal to prevent absorption back into the body in enterohepatic circulation (Grill et al., 2000).

Production of lactic acid and its derivates from batch or fed-batch fermentation of LAB has been studied extensively (Peng et al., 2006; Racine and Saha, 2007; Tan and Ding, 2006; Wee and Ryu, 2009; Xu et al., 2003). Jiang et al. (2009) improved phenyllactic acid (PLA) production from the strain *Lactobacillus* sp. SK007 by intermittent fed-batch fermentation supplemented with

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a suitable precursor, phenylpyruvic acid. Production of bacteriocins, such as nisin or pediocin, has also been studied in fed-batch fermentation (Castro et al., 2007; Papagianni et al., 2007). LAB metabolite production is generally studied in batch or fed-batch fermentation. However, medium formulations and fermentation conditions for LAB industrial production are rarely reported due to the commercial advantage for the company marketing the strains.

L. plantarum is one of the most common LAB found in fermented foods. Survival, functional and probiotic properties of *L. plantarum* in the human intestinal tract have been reviewed (de Vries et al., 2006). Recently, Jeun et al. (2010) reported a strain of *L. plantarum* with hypocholesterolemic effects in mice. The effects on growth and kinetics of *L. plantarum* produced in different conditions during the fermentation of edible sea weeds was studied by Gupta et al. (2010).

Many LAB related papers described the production of lactic acid or other metabolites by batch or fed-batch fermentation; however, there are limited papers describing LAB biomass production for human, animal or industrial use. The purpose of this study was to develop a suitable industrial medium and a set of applicable conditions for producing biomass of *L. plantarum* LP02 isolated from infant feces with potential cholesterol-lowering ability by batch and fed-batch fermentation. Biomass production from the fed-batch fermentation of the isolated strain was carried out by controlling the glucose concentration of the media at 1 to 5 g/l during the feeding process.

MATERIALS AND METHODS

Isolation and identification of potential cholesterol-lowering strains

Strains were isolated from healthy babies as previously reported by Kimura et al. (1997). Fresh feces (1 g) from newborn babies at local hospitals in Taiwan was suspended and sequentially diluted in 9 ml sterile water. Aliquots of 100 µl of the diluted samples were spread onto Rogosa agar plates and incubated at 37°C for 24 to 48 h. The colonies that displayed sufficient growth were cultivated in MRS medium at 37°C for 24 to 48 h and preserved at -70°C in a 10% glycerol solution. The isolated strains were assayed for characteristics, such as catalase activity, Gram stain type, morphology, mobility and acid/oxgall tolerance.

The isolated strains were further screened for potential cholesterol-lowering strains according to the procedure in Ahn et al. (2003). The MRS agar plates containing 0.5% taurocholic acid (TCA), 0.5% taurodeoxycholic acid (TDCA) and 0.37 g/l $CaCl_2$ were made holes by an aseptic glass tube with a diameter of 5 mm. Aliquots of 50 µl supernatant from centrifuging the fresh culture of isolated strains were inoculated into the holes in the agar plate and incubated at 37°C for 48 h. The precipitate halo that formed around some of the holes was considered indicative of a cholesterol-lowering strain. During the screening process, the cholesterol-lowering strain *Lactobacillus acidophilus* ATCC 43121 was used as a positive control.

Strain identification was performed by the Biosource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan). The design of the carbohydrate fermentation was determined using the API 50CHL kit and 16S rRNA sequencing was performed using total DNA from the experimental isolate and the reference strain *Lactobacillus plantarum* BCRC 10069.

Medium composition

The compositions of culture media used in batch and fed-batch fermentations are as follows. MRS broth supplemented with 0.05% L-cysteine was used for strain activation and cultivation of seed culture. The base medium used for both batch and fed-batch fermentations of *L. plantarum* LP02 in 5 L fermentors consisted of (per liter) glucose, 10 g; yeast extract, 40 g; mono sodium glutamate (MSG), 2 g; L-cysteine, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; KH_2PO_4, 0.2 g; $FeCl_3$, 0.05 g; and $MnSO_4 \cdot 4H_2O$, 0.05 g. All the compounds were first dissolved in appropriate amounts of distilled water and then sterilized at 121°C for 20 min. The feed medium composition (1 L) was composed of (per liter) glucose, 500 g; yeast extract, 50 g; MSG, 10 g; KH_2PO_4, 0.2 g; $MgSO_4.7H_2O$, 0.2 g; L-cysteine, 0.2 g; $MnSO_4.4H_2O$, 0.05 g; and $FeCl_3$, 0.05 g. The glucose was dissolved in 700 ml deionized water and sterilized separately before it was mixed with other compounds, which were also dissolved and sterilized in 300 ml of water before use.

Strain activation, cultivation and characterization

The isolated strains were incubated on a MRS agar plate for 24 to 48 h and then grown in MRS broth to produce a seed culture. For Gram-staining, a loop of fresh culture was spread on a glass slice and then stained with crystal violet solution for 1 min. The slide was then washed and iodine solution was added. Then, the slide was washed again with ethanol and stained finally with safranin for 30 s. After the stained slide dried in the air, it was examined by microscopy. Cells with a deep blue color were identified as Gram-positive, while a red color indicated Gram-negative cells. Catalase activity was measured by spreading one loop of fresh culture on a slide and then applying H_2O_2 (35% concentration) to the culture; the production of bubbles indicated positive activity, while no bubbles meant a negative activity. To determine the strain's motility, 50 ul of fresh culture was inoculated onto the concave side of a slide with a glass cover slip to observe the motility. To evaluate tolerance to acids and bile salts, 1 ml of fresh culture was mixed with 9 ml of phosphate-buffered saline (PBS) solution (pH 2, 2.5, 3.2 adjusted by HCl) and incubated at 37°C, 80 rpm for 3 h. Cell viability was determined by counting the number of colony forming units (cfu) produced after inoculation of serially diluted culture onto MRS agar plates. The viable colony count for the control was obtained by serially diluting the cells with acid-free PBS buffer control at pH 7.2 PBS buffer and counting the cfu produced in agar plates. Two milliliter aliquots of the acid-treated samples (pH 2.0 PBS for 3 h) were centrifuged at 6,000 rpm for 10 min to test for bile salt tolerance. The cell pellet was resuspended in pH 7.2 PBS and added to 10 ml MRS broth containing 0.3% (W/V) of oxgall and the cultures were grown at 37°C for 24 h. Samples were taken at time intervals (3, 12 and 24 h) to determine viable cell counts on MRS agar plates. All cultures were performed in triplicate in 50 ml Erlenmeyer flasks containing 10 ml of the corresponding medium at 37°C for 24 h.

Incubation of strains in a solution of cholesterol-phosphatidylcholine micelles

A cholesterol-phosphatidylcholine micelle solution was prepared

Table 1. Characteristics of *L. plantarum* LP02 used in this study.

Acid tolerance				Bile salt tolerance	
Control	**pH 2.0**	**pH 2.5**	**pH 3.2**	**3 h**	**12 h**
8.40±0.14*	5.55±0.12^{c}	7.05±0.07^{b}	8.35±0.07^{a}	8.03±0.02	8.35±0.07
Others: Gram-positive, catalase-negative, rod-shape, non-mobility					

*Counts are converted to $\log_{10}$ CFU/ml.
Each value represents mean ± SD from three different experiments. Data in the same row with different letters are significantly different at $p < 0.05$.

according to the method of Gilliland et al. (1985). First, 10 mg of cholesterol and 22 mg of egg phosphatidylcholine were dissolved in chloroform and dried by nitrogen gas. Then, 10 ml of a sucrose solution (0.4 M) was added to the dried material, and the mixture was vortexed for 15 min total, with a pause every 5 min. Finally, a mixture of 10 ml of freshly prepared cholesterol-phosphatidylcholine micelles solution and 10 ml of MRS-thio broth, containing 0.2% sodium thioglycollate and 0.3% oxgall, was inoculated with 0.2 ml of fresh culture (incubated 16 h). After 24 h of cultivation at 37°C and centrifugation, the cell pellet was washed twice and resuspended in the same volume of sterile water. Both the supernatant and the cell suspension solution were used for further analysis of the cholesterol concentration.

Batch and fed-batch fermentations

Batch fermentation was conducted in a 5 L fermentor (Biotop BTF-A5L, Taiwan). Three liters of the base medium was sterilized in the fermentor at 121°C for 30 min. Fermentation was carried out at 37°C in media with pH 6.25 (achieved by the addition of 5 N NaOH), using the previously determined agitation and aeration parameters. A 1% solution of seed culture, cultivated in MRS medium for 16 h, was used as the inoculum. Samples were taken every two hours for analysis of cell density and glucose concentration. Fed-batch fermentation was carried out in a 5 L fermentor with 2 L of initial working base medium. When the glucose consumed reached approximately 1 g/l, feeding began at a constant rate of 20 ml/h until the end of fermentation or at multi stages of feeding rates to maintain glucose concentration of 1 to 5 g/l by measuring the glucose level and adjusting the feeding rates immediately after each sampled time point. The conditions of pH, temp, agitation and aeration parameters were the same as in the batch fermentation. Volume productivity was calculated based on the biomass production rate per liter, per hour. During the fermentation, the cell density and glucose concentration were measured in triplicate.

Analytical methods

The glucose concentration was determined by the DNS method. The DNS reagent contained the following: 3, 5-dinitrosalicyclic acid, 1 g; potassium sodium, 30 g; and sodium hydroxide, 1.6 g in 100 ml deionized water. A mixture of 1 ml of appropriately diluted supernatant and 1 ml of DNS reagent was boiled for 5 min. Before measuring the cell density at OD_{550} nm, 3 ml of distilled water were added to the reaction solution. The glucose concentration was calculated by comparing the experimental value obtained to a glucose standard curve. The concentration of lactic acid was measured by high-pressure liquid chromatography (HPLC) (LC-10AT, Shimadzu, Japan) with a Sypergi 4 µ Fursion-RP80A column (250 x 4.6 mm, Phenomenex, USA) and a micro-guard column maintained at 25°C. Cell growth was measured dry cell weight (DCW) per liter. To determine the DCW, cells of known optical density were pelleted from a 10 ml sample and washed once with water. The washed cell pellet was dried at 80°C for 24 h and weighed. A conversion factor of 3.20 ± 0.08 g DCW/l-OD was determined and used to estimate the biomass from the optical density measured at 600 nm. Viable counts (cfu/ml) were conducted by the spread plate method on MRS agar supplemented with 0.05% L-cysteine.

Cholesterol concentration was determined according to the protocol reported in Gilliland et al. (1985). A mixture containing 0.5 ml of supernatant or pellet suspension, 3 ml 95% ethanol and 2 ml 50% NaOH was heated in a 60°C water bath for 10 min. Then, 5 ml n-hexane and 3 ml distilled water were added to the solution, shaken for 20 s and incubated for 15 min at room temperature to allow for separation. The solution in the organic layer was dried by blowing N_2 gas during incubation in a 60°C water bath. Next, 4 ml of o-phthalaldehyde reagent was added to the dry sample, gently mixed with 2 ml concentrated sulfuric acid and incubated for 10 min at room temperature. The absorbance at 550 nm was measured to calculate the cholesterol concentration by comparing the experimental value with a range (0 to 50 ppm) of standard cholesterol solutions.

Data were expressed as mean ± standard error of the mean. Treatments were compared using one-way analysis of variance followed by Tukey's tests for multiple comparisons between means. P-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Characterization of the isolated strain

The isolated strain was rod-shaped, Gram-positive, catalase-negative, non motile, and acid/oxgall tolerant as shown in Table 1. Based on these results, the strain was identified as lactic acid bacteria. The acid tolerance of this strain was similar to those strains reported by Havenaar et al. (1992) and Marteau et al. (1997) which were analyzed *in vitro* and *in vivo* in conditions that mimic the environment of the digestive tract. This strain exhibited satisfactory growth at pH values of 2.0, 2.5 and 3.2 for 3 h. An average of 0.05 to 2.8 log cfu/ml decrease also showed that the strain was not greatly affected by the acid. To simulate an intestinal environment, the cell pellet that was acid tolerant to pH 2.0 was next inoculated in MRS medium supplemented with 0.3% oxgall and incubated for 24 h. The isolated strain is also tolerant to bile salts, as evidenced by the similar cell viability count obtained between the experiment and control conditions after incubation for 12 h.

Table 2. Cholesterol concentration in the cells and supernatant after incubation of different lactic acid bacteria with a cholesterol-containing solution for 24 h.

Strain	Cholesterol (µg/ml)	
	Cell	Supernatant
Lactobacillus plantarum LP02	42.59±1.37[a]	31.11±1.12[d]
Lactobacillus acidophilus BCRC 10695	37.40±1.29[b]	34.44±1.28[d]
Lactobacillus casei BCRC 10697	2.59±0.32[d]	65.18±3.09[b]
Lactobacillus rhamnosus GG	3.33±0.21[d]	68.18±2.46[b]
Bifidobacterium lactis Bb12	14.07±0.67[c]	57.29±1.86[c]
Lactobacillus paracasei 33	0.74±0.14[e]	73.70±3.06[a]
Lactobacillus bulgaricus BCRC 10696	0.51±0.12[e]	71.48±2.95[ab]
Bifidobacterium longum BCRC 11847	3.33±0.19[d]	68.18±2.16[b]

*Initial cholesterol concentration in culture broth was around 100 µg/ml which was estimated using the concentration of cholesterol-phosphatidlycholine mycelles added to the media.
Each value represents the mean ± SD from three different experiments. Data in the same column with different letters are significantly different at $p < 0.05$.

Strain identification reported from FIRDI showed that partial sequencing of the 16S rRNA from the isolate had a 99% identity with that of *L. plantarum* BCRC10069. In addition, results from a biochemical assay using the API 50CHL kit also suggested that the isolate was a strain of *L. plantarum*. Therefore, the isolate was confirmed as a strain of *L. plantarum* and was designated *L. plantarum* LP02.

Cholesterol concentration in the supernatant and the cells

The hypocholesterolemic effects of LAB in various animals and human are strain specific. Gilliland et al. (1985) isolated strains with the ability to reduce cholesterol concentration in serum by screening for strains with the ability to lower the cholesterol concentration in broth. To evaluate the cholesterol-lowering ability of the isolated LAB, the concentration of cholesterol in both the cells and supernatant after incubation with cholesterol-phosphatidylcholine micelles solution was determined and compared to that of a known cholesterol-lowering strain, *L. acidophilus* BCRC17010. As shown in Table 2, final cholesterol concentrations in the cells and supernatant of the *L. plantarum* LP02 culture were 42.59 µg/ml and 31.11 µg/ml, respectively when the strain was incubated for 24 h in a solution with an initial concentration of approximately 100 µg/ml of cholesterol estimated using the concentration of cholesterol-phosphatidlycholine mycelles added to the solution. Table 2 also indicated that strains *Lactobacillus rhamnosus* GG, *Lactobacillus paracasei* 33, *Lactobacillus bulgaricus*, and *Bifidobacterium longum* exhibited relatively low concentration of cholesterol in cells. During incubation of this isolated strain, the cholesterol concentration gradually decreased in the supernatant and increased in the cells (Figure 1); this finding supported the hypothesis that the isolated strain had potent cholesterol-reducing ability. The reduction of cholesterol in the supernatant might be explained by absorption of cholesterol onto the cell surface or into the cells. Some LAB can modulate bile acid excretion which can cause a lowering of plasma cholesterol levels. The cholesterol-decreasing ability may result from LAB's co-precipitation with the deconjugated bile salts by strains of bacteria that produce bile salt hydrolase (Pereira et al., 2003). Some *Lactobacillus* species have been proven to reduce blood cholesterol levels and are used as probiotics (de Vries et al., 2006).

However, there are few reports about a decrease of cholesterol concentration or absorption of cholesterol by the strain *L. plantarum*. Recent studies have demonstrated that *L. plantarum* may exert beneficial cholesterol reducing effects of increasing bile acid excretion in mice (Jeun et al., 2010). In addition, the strain *L. plantarum* PH04 isolated from infant feces was also evaluated for its potential as a cholesterol-reducing probiotic in mice (Nguyen et al., 2007). There are five possible mechanisms for the removal of cholesterol from media by lactobacilli (Lye et al., 2010). In this result, cholesterol-lowering effects might be due to the deconjugation of bile salts by the enzyme bile salt hydrolase (Ahn et al., 2003) or the cholesterol might be removed by either binding to the bacterial cellular surface or by being absorbed into the cells.

Medium composition

To produce *L. plantarum* LP02 biomass for industrial use, composition of the commercial medium, for example, the source of carbon and nitrogen sources should be studied beforehand. Different levels of glucose concentration (0 to 50 g/l), yeast extract (0 to 50 g/l) and sodium glutamate (0 to 10 g/l) were tested to find the most

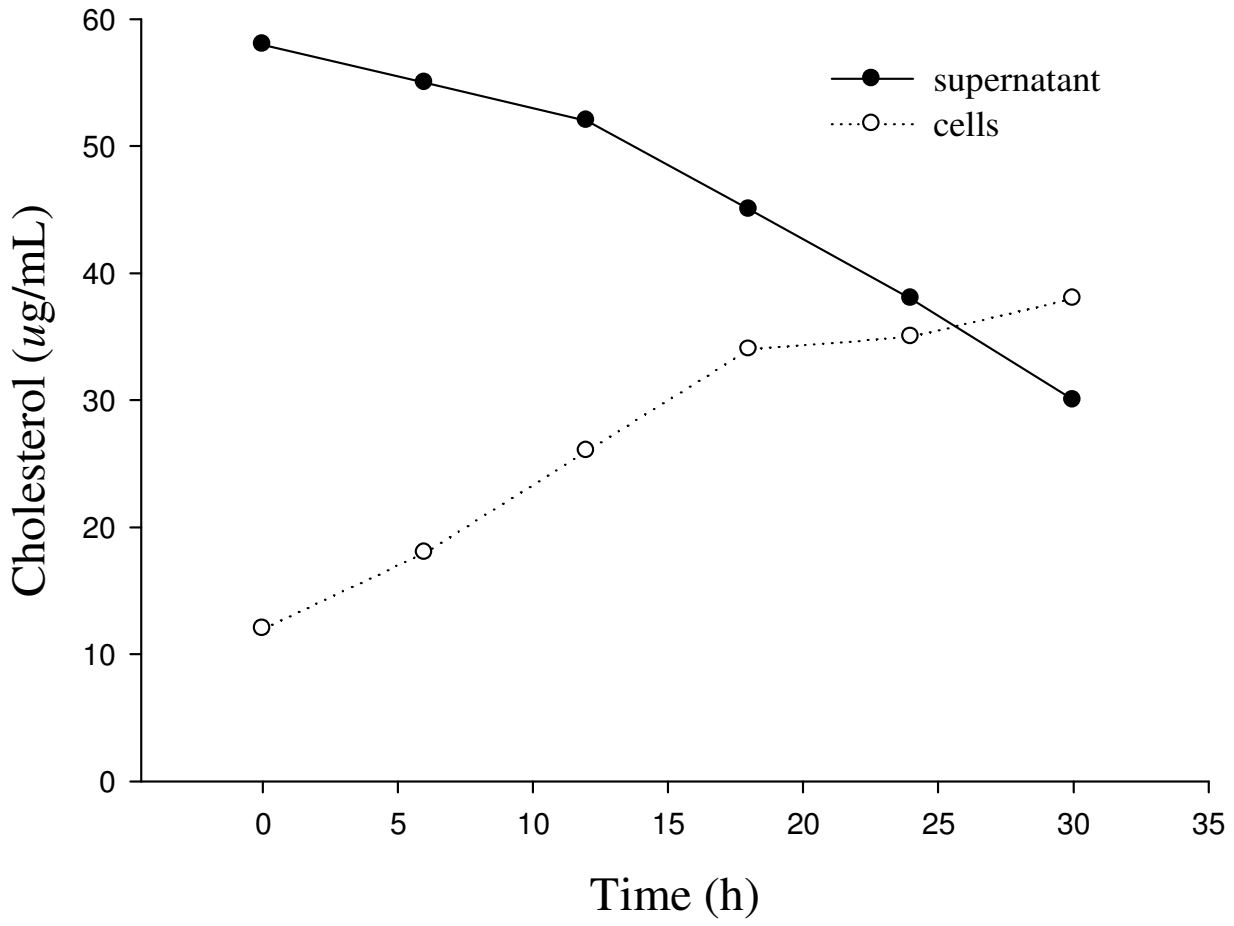

Figure 1. Cholesterol concentration in the cells and supernatant of the strain *L. plantarum* LP02 during incubation.

suitable media composition for obtaining a greater amount of biomass. Figure 2 shows the effects of different levels of glucose, yeast extract and sodium glutamate on the cell density of the strain *L. plantarum* for 24 h incubation. Results from the use of more suitable medium compositions are outlined in Table 3. The media composition with 10 g/l of glucose, 40 g/l of yeast extract and 2 g/l of sodium glutamate could reach the highest cell density of 1.6 to 2 g (DCW)/l which was similar to that of using the commercial MRS medium. This medium contained the cheaper, industrial quality of yeast extract and sodium glutamate, without using the peptone, a more expensive nitrogen source. Factors known to affect the production of LAB metabolites, such as lactic acid or bacteriocin, during fermentation by LAB include medium composition (carbohydrate source, sugar concentration and growth factors), the presence of oxygen, level of pH and concentration of the product (Burgos-Rubioetal et al., 2000; Todorov and Dicks, 2006). However, there are few reports describing the influences of medium composition on viable cells production for commercial purpose. An example elucidated the growth medium, free from animal-derived ingredients, with potential for commercial cultivation of probiotics strains for application in vegetarian foods (Heenan et al., 2002). However, the optimized medium compositions for commercial production of LAB biomass are different depending on the various characteristics of the strains.

Batch fermentation

Optimization of the fermentation process improved the development of higher biomass production to ensure its economical viability. Biomass production from the strain *L. plantarum* LP02, cultivated both by batch and fed-batch fermentations in 5 L fermentors, depended on the procedural conditions, such as agitation speed, aeration, and pH values. Figure 3 shows that agitation speeds of 150 or 300 rpm during batch fermentation without aeration enhanced the cell density when compared with no agitation. This result suggested that the isolated strain might be oxygen tolerance. However, biomass production with an agitation speed of 150 rpm and aeration at 1 vvm exhibited a significantly lower cell density (Figure 4). Biomass production with 0.5 vvm was similar to that

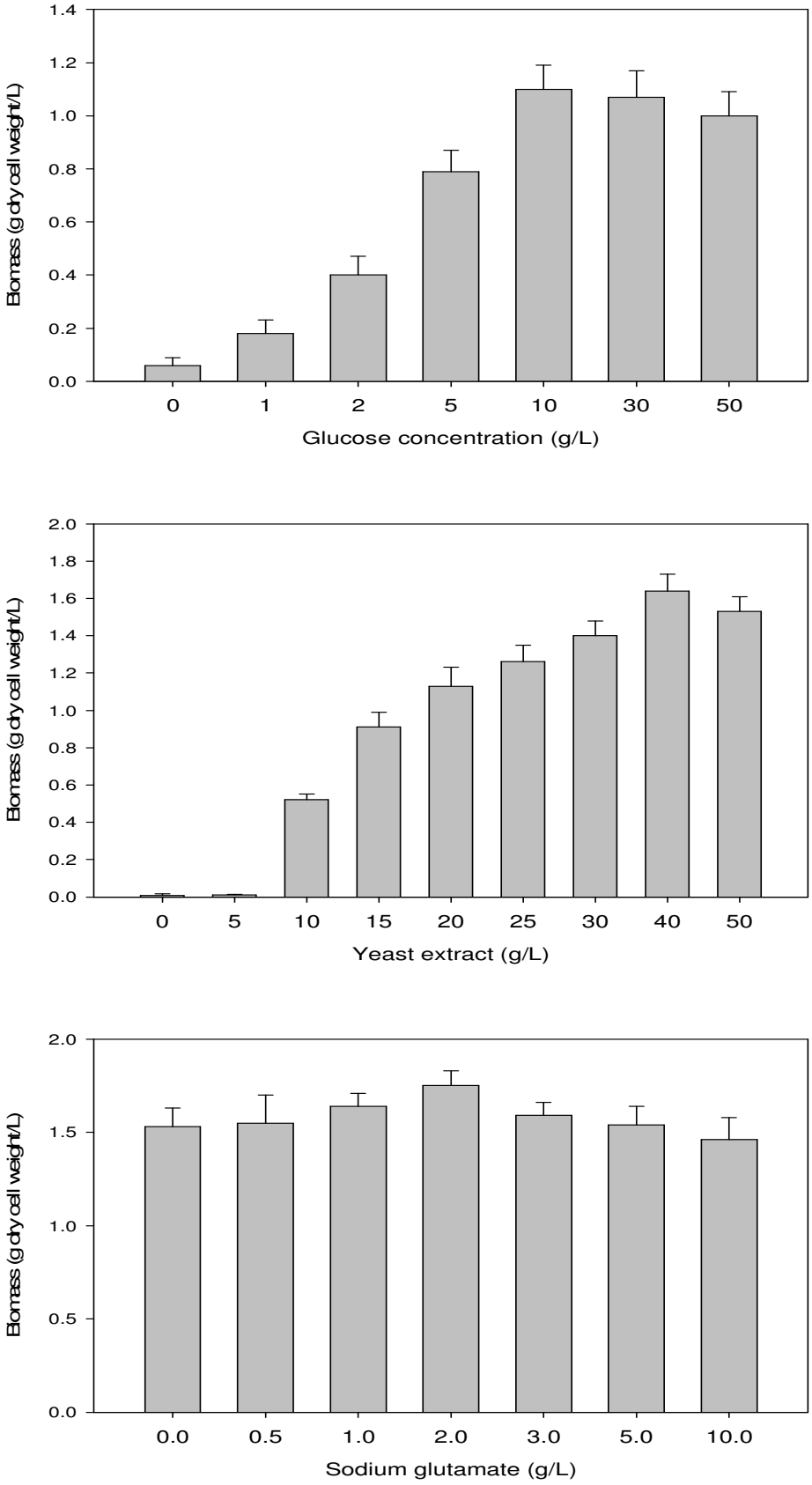

Figure 2. Effect of glucose, yeast extract, and sodium glutamate concentration on biomass production of the strain *L. plantarum* LP02 for 24 h incubation.

Table 3. Medium composition for production of biomass determined by this study.

Item	Concentration (g/l)
Glucose	10
Yeast extract	40
Sodium glutamate	2
$MgSO_4$. $7H_2O$	0.2
KH_2PO_4	0.2
$FeCl_3$	0.05
$MnSO_4.4H_2O$	0.05

without aeration. Higher cell densities were observed in the initial period of fermentation when the aeration was 1.0 vvm; however, the final cell density was lower than that without aeration or at 0.5 vvm. These data showed that the strain is microaerophilic, because the higher oxygen concentrations hindered cell growth. Fu and Mathews (1999) found that when *L. plantarum* was cultivated for lactic acid production from lactose, the cell yield was higher under aerobic conditions, but the lactic acid production was higher under anaerobic conditions. Appropriate control of aeration rate during the process of batch or fed-batch fermentation is important for the *L. plantarum* biomass production.

A time course for *L. plantarum* LP02 cell growth by batch fermentation was carried out in a 5 L fermentor at 37°C, at 150 rpm, pH 6.2 and without aeration. As shown in Figure 5, cell density and biomass productivity reached the highest levels of 2.53 g/l and 0.43 g/l-h, respectively, at 8 h of fermentation. The glucose was almost completely consumed at 8 h, a significantly shorter time period than that of significantly shaking flask fermentation, which took 16 h to accomplish the same level of glucose depletion (data not shown). In addition, the biomass yield based on consumed glucose reached its highest level of 0.30 g DCW/g at 4 h. All the data showed that biomass production and glucose consumption in the 5 L fermentor was faster than cultivating in flask. After 8 h of fermentation, cell density, biomass yield and productivity significantly decreased due to the limited glucose concentration in the fermented culture.

Fed-batch fermentation

A high concentration of medium composition (500 g/l glucose and 50 g/l yeast extract) was added at a constant rate of 20 ml/min or at multi-stage rates to maintain an appropriate glucose concentration. A constant rate (20 ml/min) of feeding was started at 8 h of fermentation when the glucose concentration was very low (1.12 g/l) and cell density was at 7 OD_{600nm} (2.5 g DCW/l) (Figure 6). Cell density significantly increased to 28 OD_{600nm} (9.45 g DCW/l) at 28 h of fermentation after maintaining the glucose concentration at an average value of 1.32 g/l. The highest productivity, 1 g/h-l, was obtained at 9 h of fermentation, in the initial period of feeding. Biomass yield based on the consumed glucose was found to have the highest yield, 0.30 g/g at 4 h of fermentation. The yield dropped closely to 0 at 8 h and sharply increased to 0.10 g/g at 9 h, one hour after feeding and then gradually reduced during the rest of the fermentation process. When comparing batch and fed-batch fermentations, a significantly greater amount of biomass and a higher productivity rate were observed during fed-batch fermentation, although, longer incubation time and greater amounts of materials were required.

To investigate the effect of glucose on biomass production, different concentration of glucose were maintained in the cultures during fermentation. Biomass production was conducted by increasing the feeding rate to adapt the glucose concentration between 1 and 5 g/l by manually changing the feeding rate at every sampling time after 8 h of fermentation. Results showed in Figure 7 indicated that cell density reached the highest biomass of 31 OD (around 10.12 g DCW/l) at 15 h and the highest volume productivity of 1.72 g/l-h for 10 h. The average productivity after feeding was 1.11 g/l-h and was higher than that achieved with a constant feeding rate (Figure 6). The highest biomass yield (0.24 g/g) was recorded at 4 and 7.5 h of sampling times and then gradually decreased over time. Changing the feeding rates to maintain glucose level at 1 to 5 g/l significantly shortened the fermentation time required to reach the same biomass concentration comparing with that at a constant feeding rate and the highest productivity was concurrently obtained.

Biomass production could be further improved by modifying fed-batch fermentation. Hayakawa et al. (1990) obtained a large dried biomass (40 g/l) at 34 h of continuous fed-batch fermentation by recycling cells through cross-filters in fermentors to remove lactic acid. Four strains of LAB were produced by fed-batch fermentation and evaluated as feed additives for weaned piglets (Gurrea et al., 2007). A mathematic model was created to produce lactic acid in membrane bioreactor through fermentation of *Lactobacillus casei* at a high cell density (98.7 OD at 600 nm) (Boudrant et al., 2005); this density was seven-times higher than the cell density achieved in fed-batch fermentation. A cell-recycle fermentation system was also established for the production of lactic acid (Xu et al., 2006) and in the future, could be developed for the production of not only metabolites but also biomass.

Conclusion

Characterization of *L. plantarum* LP02 showed that it is acid and bile tolerant, a characteristic important for the LAB to survive gastrointestinal ingestion. The potential hypocholesterolemic effects of the isolated strain *L.*

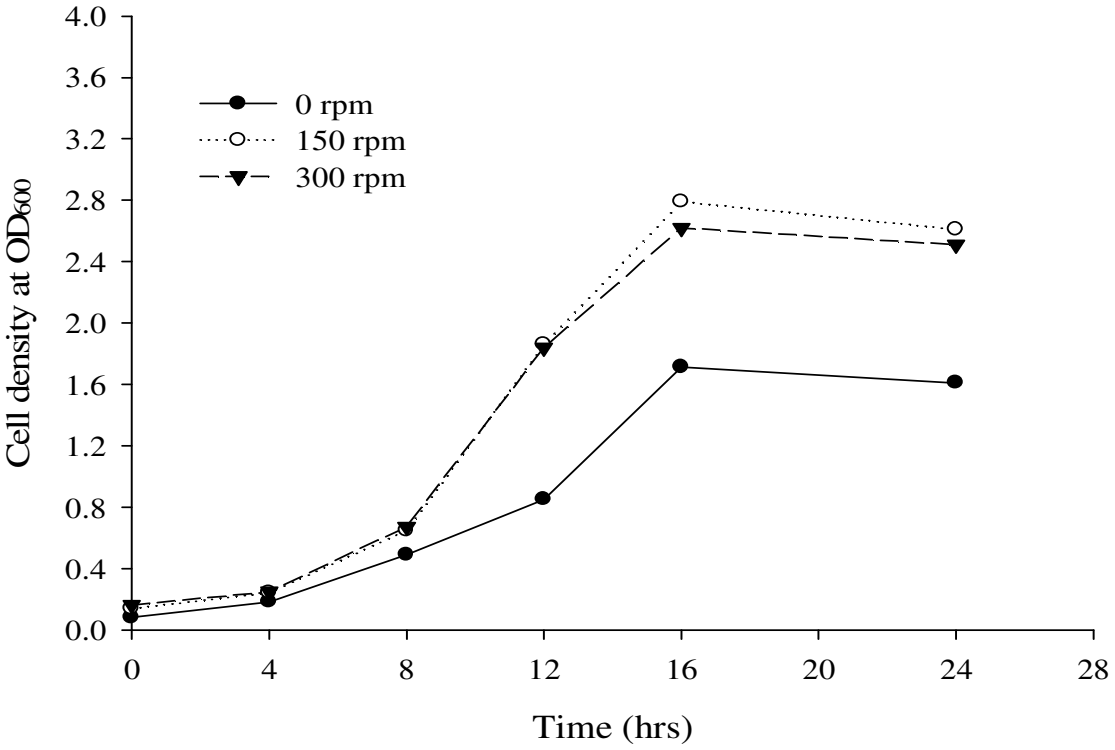

Figure 3. Effect of the stirring speeds on the cell density of *L. plantarum* LP02 in a 5 L fermentor grown at 37℃, without areation.

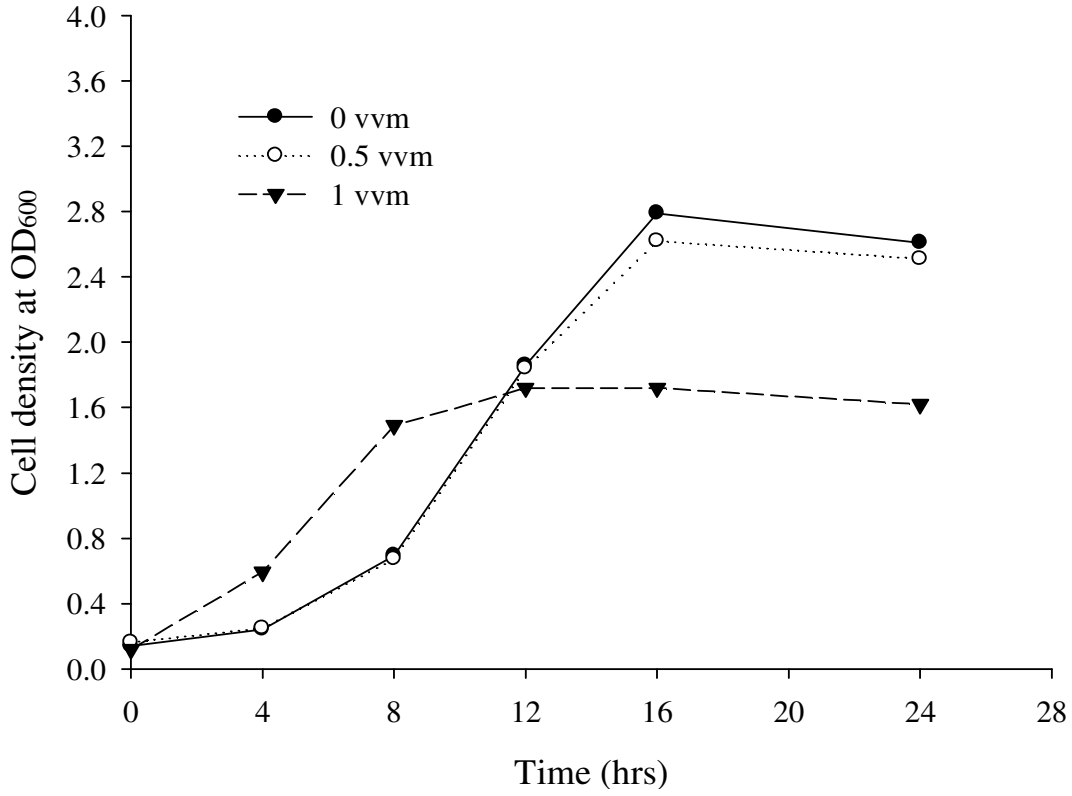

Figure 4. Effect of the aeration rate on the cell density of *L. plantarum* LP02 in a 5 L fermentor grown at 37℃ and 150 rpm.

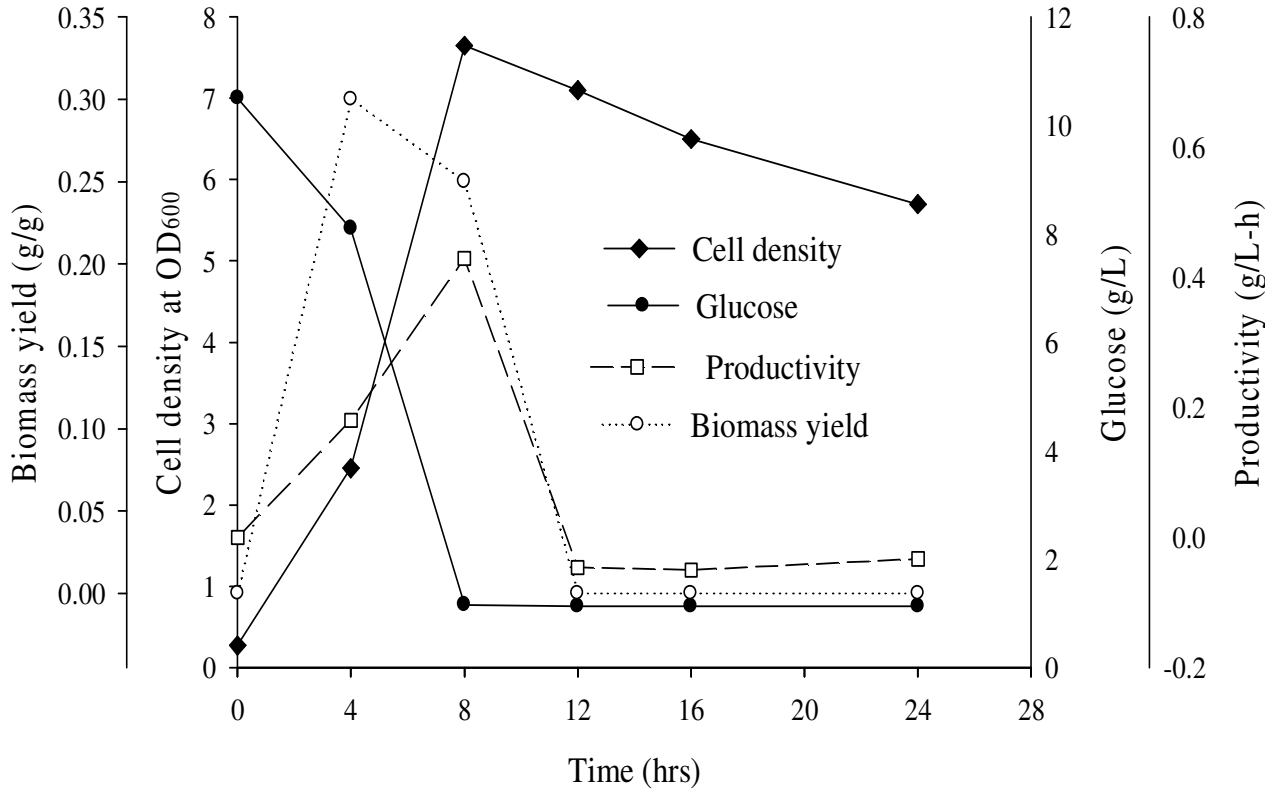

Figure 5. Time course of *L. plantarum* LP02 batch fermentation in a 5 L fermentor. Biomass yield ($Y_{X/S}$) = (Biomass produced/Glucose consumed); Volumetric biomass productivity = (Biomass increased/h-l).

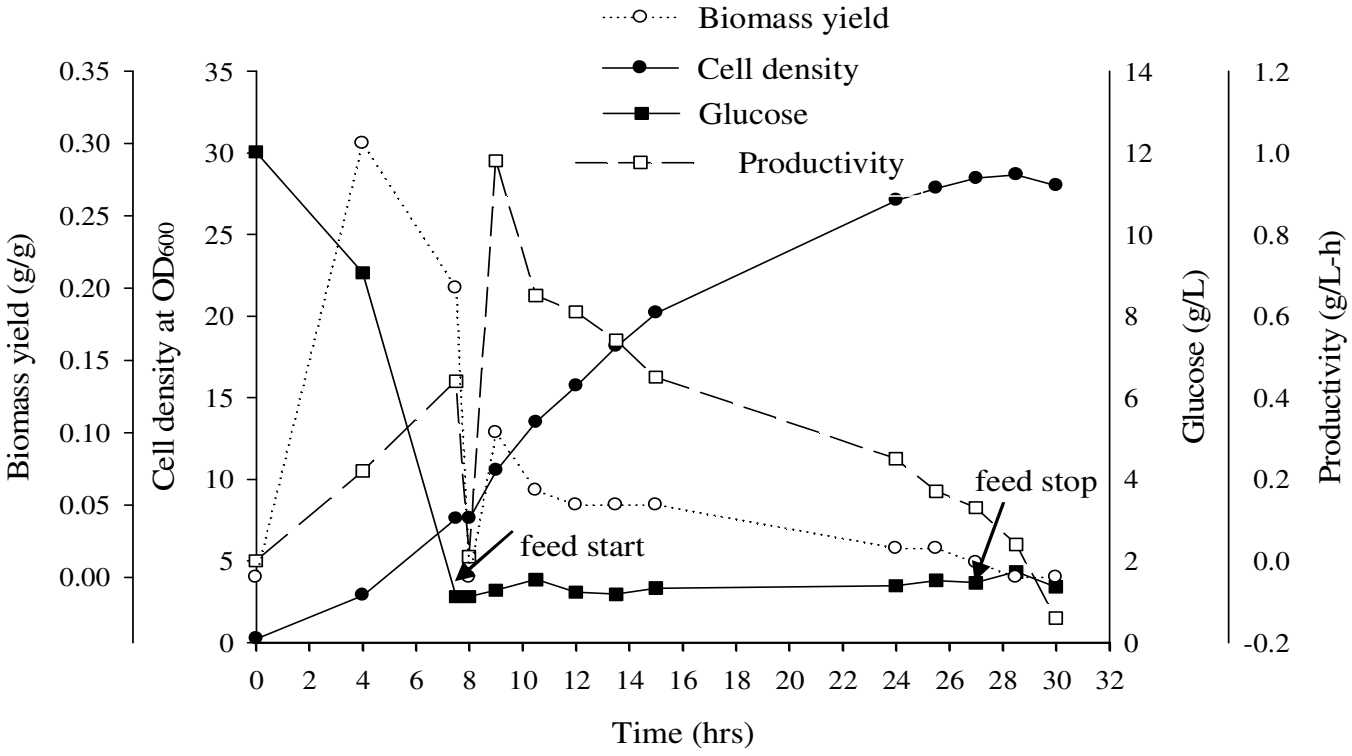

Figure 6. Fed-batch fermentation of *L. plantarum* LP02 with a constant feeding rate of 20 ml/h in a 5 L fermentor. Biomass yield ($Y_{X/S}$) = (Biomass produced/Glucose consumed); Volumetric biomass productivity (Biomass increased/h-l).

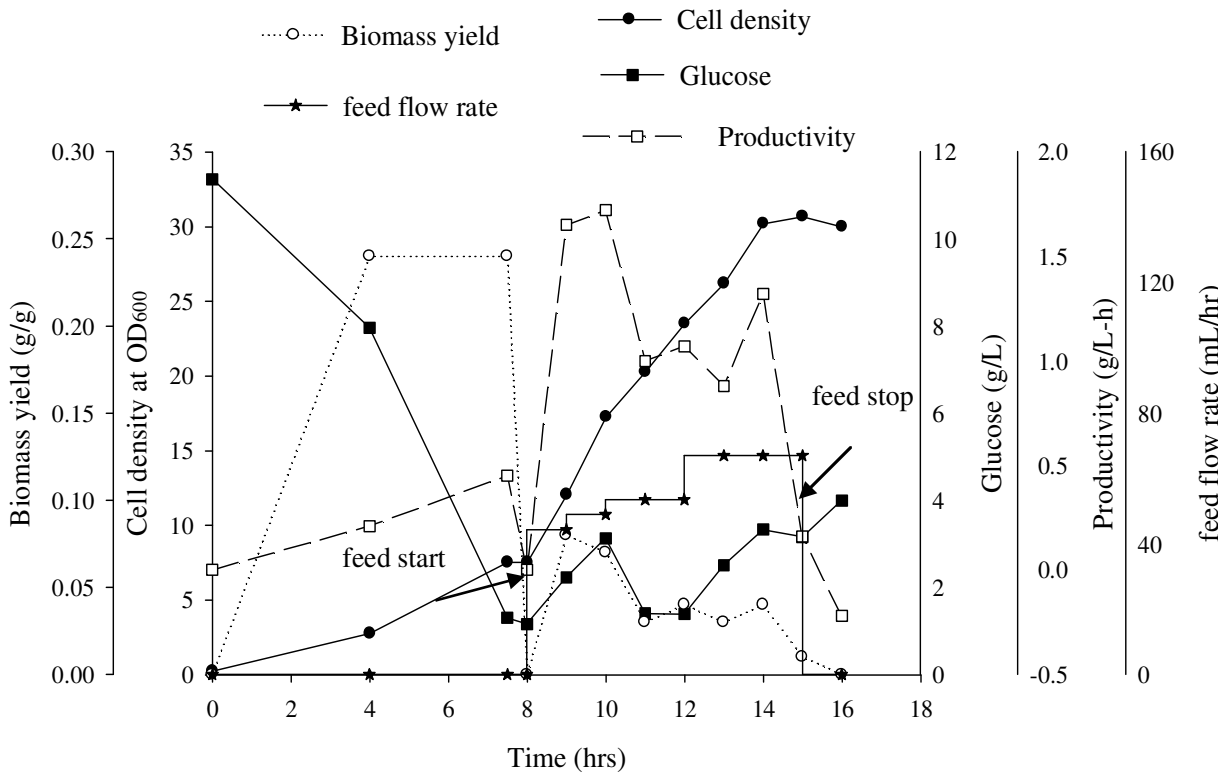

Figure 7. Fed-batch fermentation of *L. plantarum* LP02 in a 5 L fermentor with glucose concentrations between 1 to 5 g/l maintained through changing the feeding rates. Biomass yield ($Y_{X/S}$) = (Biomass produced/Glucose consumed); Volumetric biomass productivity = (Biomass increased/h-l).

plantarum LP02 require the support of additional research before application, especially in industrial or human use. The isolated strain was definitively identified as *L. plantarum* by the report of FIRDI to match the sequence of the 16S RNA gene of the isolate, as well as being identified by an API 50 CHL kit. Strains with cholesterol-lowering properties as functional starter cultures may enhance quality of end product besides probiotic characteristics. The microaerobic characteristic of this strain is beneficial for biomass production in fermentors because agitation is needed during the process of pH control or feeding. Cell density was significantly improved from 2.53 g DCW/l, with batch fermentation to 10.12 g DCW/l with fed-batch fermentation, while controlling the feeding rate to maintain an appropriate glucose concentration. The lactic acid concentration should be monitored during the process because high concentrations can slow cell growth during the batch or fed-batch fermentation. Modified continuous fermentation or fed-batch fermentation with cell recycle through a membrane to remove lactic acid might enhance the cell density. Besides, cell density and biomass yield might be further improved through other fed-batch strategies based on exponential feeding or with feedback control, such as DO stat. The potential cholesterol-reducing strain isolated in this study may be added to fermented dairy products to achieve hypocholesterolemic properties. The success of using a functional starter culture in a particular food is strongly strain dependent and is crucial for rational selection. The applied strains will be adapted to the process conditions and the intrinsic factors inherent to the food.

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