Journal of Applied Pharmaceutical Science Vol. 4 (11), pp. 008-014, November, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.4112
ISSN 2231-3354

Improvement of cell mass production of *Lactobacillus delbrueckii* sp. *bulgaricus* WICC-B-02: A newly isolated probiotic strain from mother's milk

Elsayed A. Elsayed[1,2], Nor Zalina Othman[3], Roslinda Malek[3],Tebbie Tang[3], and HeshamA. El Enshasy[3,4*]

[1]Bioproducts Research Chair, Zoology Department, Faculty of Science, King Saud University, Kingdom of Saudi Arabia.
[2]Natural and Microbial Products Department, National Research Centre, Dokki, Cairo, Egypt.
[3]Institute of Bioproduct Development (IBD), UniversitiTeknologi Malaysia (UTM), 81130 UTM, Skudai, Malaysia.
[4]City of Scientific Research and Technology Application, New Burg Al Arab, Alexandria, Egypt.

ARTICLE INFO

Article history:
Received on: 19/05/2014
Revised on: 09/06/2014
Accepted on: 03/07/2014
Available online: 27/11/2014

Key words:
Probiotics, Process development, Mother's milk, *Lactobacillus delbrueckii.*

ABSTRACT

Lactobacillus delbrueckii sp. *bulgaricus* WICC-B-02 is new probiotic strain which was initially isolated from the mother's milk. As lactic acid bacterium, it's known as a highly efficient probiotic microorganism with a wide range of benefits on the human health. This study was conducted to design and establish industrial platform for high cell mass production of *L. delbrueckii* sp. *bulgaricus* for pharmaceutical and food industries application. However, due to low cell mass production caused by the accumulation of lactic acid during the cultivation of lactic acid bacteria, therefore, the optimization of cell mass production with low lactic acid production was developed in this study. The new formulation of the production medium was developed by optimizing the main components such as glucose (30 $g.L^{-1}$), yeast extract (6.0 $g.L^{-1}$) and peptone (6.0 $g.L^{-1}$)in shake flask cultivation. The cell mass production was 3.14 $g.L^{-1}$, and it increased to 6.08$g.L^{-1}$ with the lactic acid production being reduced by about 33%, compared to the un-optimized medium.

INTRODUCTION

By definition, probiotics are living microorganisms, which upon ingestion in certain numbers exert various health benefits beyond the inherent basic nutrition (Arora *et al.*, 2013; Tamminen *et al.*, 2013; Guarner and Schaafsma, 1998). It is recommended that probiotic products contain at least 10^7 living microorganisms per g or per mL (Sarmidi and El Enshasy, 2012; Ishibashi and Shimamura, 1993). Probiotics products usually incorporate intestinal species of *Lactobacillus* because of their long tradition of safe use in the dairy industry as well as the fact that some strains of lactic acid bacteria (LAB) are capable of exerting their beneficial effects by balancing the intestinal flora and eventually competing with pathogens for gut colonization (Schiraldi *et al.*, 2002). Studies have shown that the probiotic potential of *Lactobacilli* isolated from milk of healthy mothers is similar to that of the strains commonly used in commercial probiotic products. The functional foods containing probiotic in certain amounts exert health beneficial effects on human such as enhancement of the immune system (Wadher *et al.*, 2010; Sanders *et al.*, 1994), production of antibiotics, including bacteriocin (Sugita *et al.*, 1998), and prevention of mucosal infectionin children (Villena *et al.*, 2012). Moreover, the nutritional requirements for *Lactobacilli* to grow include carbohydrates, peptides, fatty acid esters, salts and nucleic acid derivatives. It has limited biosynthetic abilities and therefore vitamins and amino acids are often added to the medium (Lebeer *et al* 2008; Bernardeau *et al.*, 2006). Nevertheless, the possibility of obtaining high biomass from a low cost medium is very challenging due to the production of lactic acid concomitant with the cell growth (Vodnar *et al.*, 2010; Othman *et al.*, 2009, Elmarzugi *et al.*, 2010; Malek *et al.*, 2012). For industrial applications, *Lactobacillus* bacteria are not simple to grow in large scale bioreactors, therefore high cell density cultivations are more important as their biomass is becoming very valuable (Hun *et al.*, 2013).

* *Corresponding Author*
Hesham Ali El Enshasy, Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Johor Bahru, Johor, Malaysia.
Tel.: 006-07-5531573 , E.-Mail: henshasy@ibd.utm.my

Accordingly, from a commercial point of view, an inexpensive method for large-scale production of cultures containing high levels of viable probiotic cells in a form suitable for product applications is highly desirable. In the present investigation, the probiotic strain, *L. delbrueckii sp. Bulgaricus* WICC-B-02, isolated from mother's milk was used. This strain showed high potential application as probiotic strain based on its high acid and bile salt tolerance in addition to resistance to antibiotics (Othman *et al.*, 2009).

Cultivations were conducted in submerged culture to study the effects of the main medium components on the kinetics of cell growth in shake flask level. Furthermore, the growth kinetics of this strain in semi industrial scale 16-L stirred tank bioreactor was studied under controlled and uncontrolled pH for high cell mass and lactic acid production.

MATERIALS AND METHODS

Microorganism

The probiotic strain used in this research, *Lactobacillus delbrueckii sp. Bulgaricus* WICC-B-02 obtained from Wellness Industries Culture Collection (WICC, Institute of Bioproduct Development, Universiti Teknologi Malaysia, Johor Bahru, Johor, Malaysia). This strain was initially isolated from mother's milk, fully characterized by microbiologcal and biochemical method using API Kit "API CHI 50 Kit" and deposited under WICC Culture Collection.

Cells were obtained in frozen glyceol culture and were activated initially on MRS agar medium, DeMan, Rogosa, Sharpe Agar. MRS medium was composed of ($g.L^{-1}$): Glucose, 20.0; Yeast extract, 5.0; Peptone, 10.0; Beef extract, 10.00; Polysorbate 80, 1.0; Ammonium citrate, 2.0; Sodium acetate, 5.0; Magnesium sulphate, 0.1 and Dipotassium phosphate, 2.0. The pH was adjusted to 6.5 before autoclaving.

The inoculated plates were incubated at 37 °C. After 24 hours of incubation, the cells on the solid medium were dissolved with glycerol. Stock cultures were preserved in glycerol (50%, $v.v^{-1}$) at -80°C as working cell bank which was used as starting culture for inoculum preparation for each experiment.

Inoculum preparation

For shake flask cultures, 250 mL Erleymyer flasks with 50 mL working volume of MRS medium were inoculated. The flasks were incubated at 37 °C for 24 h. Then, 1.0 mL of the inoculum was used to inoculate the 250 mL shake flasks, and the flasks were incubated on a rotary shaker (Innova 4080, New Brunswick, NJ, USA) at 200 rpm at 37 °C for 24 hours for biomass production.

Screening of production medium

Different cultivation media have been screened for the production of cell mass (Table 1). The inoculated flasks were incubated at 200 rpm and 37 °C for 24 hours in a rotary shaker (Innova 4080 New Brunswick Scientific CO., NJ, USA).

Table 1: Screening of production medium for the growth of lactic acid bacteria from literatures review.

No.	Components ($g\ L^{-1}$)	Reference
1	Soy peptone, 25; Glucose,25; Yeast extract, 25; pH 6.5	Heenan *et al*., 2002
2	Glucose, 45; Yeast extract, 20; NaCl, 0.01; Sodium acetate, 0.5; Tri-ammonium citrate, 0.2; KH_2PO_4, 0.2; $MgSO4.7H_2O$, 0.2; $MnSO_4.7H_2O$, 0.05; pH 6.25	Li *et al*.,2006
3	Glucose, 22; Yeast extract, 5.0, Peptone, 10.0; $MgSO_4.7H_2O$,0.1; $MnSO_4.7H_2O$,0.038; Ammonium citrate, 8.29; Sodium acetate, 8.29; KH_2PO_4, 2.0; pH 6.5	Schiraldi *et al.*, 2002
4	Glucose, 19.8; Yeast extract, 5.0, Citric acid, 0.826; $MgSO_4.7H_2O$,0.197; $MnSO_4.7H_2O$,0.045; Sodium acetate, 18.57; KH_2PO_4, 1.007; Na_2HPO_4, 2.002; pH 6.0	Petry *et al*., 2000
5	Glucose, 19.8; Citric acid, 0.826;$MgSO_4.7H_2O$,0.197;$MnSO_4.7H_2O$,0.045; Sodium acetate,18.57; KH_2PO_4, 1.007; Na_2HPO_4, 2.002; Vitamins (g/100ml): L-alanine, 1.1; L-arginine, 0.5; L-asparagine, 0.7; L-cysteine, 2.5; L-isoleucine, 0.8; L-lysine,0.6; L-methionine,0.7; L-phenylalanine,0.6; L-proline,0.9; L-threonine,0.8; L-tryptophan,0.5; L-tyrosine, 0.6; L-valine, 0.9; riboflavin, 0.27; pH 6.0	Grobben *et al*., 1998
6	Glucose, 11; Yeast extract, 6.0, Peptone, 30;$MgSO_4.7H_2O$,0.197; $MnSO_4.7H_2O$,0.05; Sodium acetate,8.29; KH_2PO_4, 1.007; Na_2HPO_4, 2; Ammonium citrate, 2; pH 6.5	Avonts *et al*., 2004
7	Glucose, 11; Yeast extract, 6.0;Peptone, 30;$MgSO_4.7H_2O$,0.197; $MnSO_4.7H_2O$,0.05; Sodium acetate,8.29; KH_2PO_4, 1.007; Na_2HPO_4, 2; Ammonium citrate, 2; Skimmed milk powder; pH 6.5	Avonts *et al*., 2004

Optimization of the production medium

From the initial screening of the production medium, SPY was chosen as the best medium for the growth of *Lactobacillus delbrueckii* sp. *Bulgaricus*. The medium contained glucose, yeast extract and peptone ($25g.L^{-1}$,each). The medium composition has been modified and optimized according to Table 2.

Optimization of the key nutrients in shake flask level

The SPY 10 was the medium selected for further optimization. For optimization experiments, the key nutrients (glucose, yeast extract, and peptone) were prepared at different concentrations to investigate their effect on biomass production. The carbon source, glucose was prepared and autoclaved separately and was added to the flasks prior to inoculation.

Bioreactor Cultivation

After the optimization of the cultivation medium, the new optimized medium, which promoted the production of high cell mass, was then chosen for the cultivation in semi-scale 16-L bioreactor(BioEngineering, Wald, Switzerland), with a working volume of 8-L. The bioreactor is equipped with a

pH probe, oxygen probe, foam sensor, and stirrer with four Rushton turbines. The cultivation was run at 400 rpm and 37 °C for. In controlled pH batch cultures, the pH was controlled at 6.5 by the addition of 2.5 M NaOH and 2.5 M HCl throughout the whole fermentation process.

The dissolved oxygen was initially adjusted at 100% saturated with airflow of 1 $v.v^{-1}$. min^{-1}. Antifoam reagent was added to suppress the foaming when necessary.

Analytical Methods

Sample Preparation

For both shake flasks and bioreactors, 10 mL samples were taken at different time intervals for the measurement of OD and pH. Glucose concentration and lactic acid determination were performed separately.

Optical density determination

The optical density was measured by using spectrophotometer (Model DR/2500, Hach Company, Lovel and, CO, USA) at 600 nm. 1 OD_{600} was almost equivalent to 0.3 $g.L^{-1}$.

Lactic Acid and Glucose Determination

Lactic acid and glucose concentrations were determined by HPLC. For lactic acid, a 250 mm X 4.6 mm ID Spherisob Octyl Column (Waters) and a UV detector (210nm)were used (Vodnar *et al*., 2010). The adsorbed substances were eluted with 0.2 M H_3PO_4 at a flow rate of 0.8 $mL.min^{-1}$ at room temperature. For glucose, a 4 mm diameter, 300 mm long ID µ Bondapak/Carbohydrate column (Waters) with RI detector was used. The carrier solution was acetonitrile : water (80:20) at a flow rate of 1.0 $mL.min^{-1}$ at room temperature (Malek *et al*., 2010).

pH Determination

The pH of medium was determined using pH probe (TOLEDO, Delta 320 pH Meter).

RESULTS AND DISCUSSION

Screening of different production mediafor cell growth and lactic acid production

Seven cultivation media were selected based on the previous studies performed with lactic acid bacteria (LAB). As shown in Figure (1), it can be observed that all tested media can support cell growth and lactic acid production, but in different extents. Heenan *et al*. (2001) have grown probiotic bacteria in a basal medium containing soy peptone, yeast extract and glucose monohydrate. This medium has the greatest potential as an industrial growth medium for probiotics. In this experiment, medium No.1 (SPY 1) can produce the highest dry cell mass among others after 24 hours cultivation (about 2.84 $g.L^{-1}$). However, medium No.7 gave the highest dry cell mass after 12 hours of cultivation (about 2.68 $g.L^{-1}$), which remained more or less constant for the rest of the cultivation. Accordingly, medium No.1 (SPY 1) was chosen for further optimization steps.

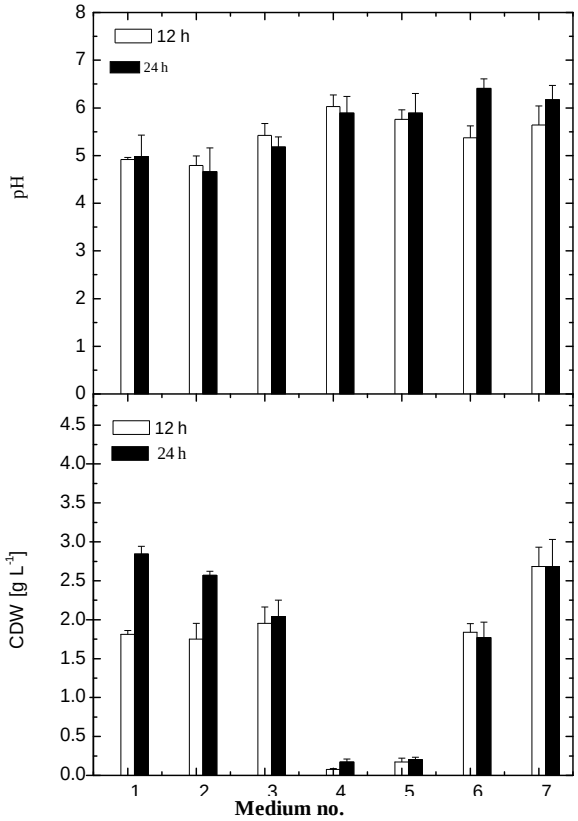

Fig. 1: Effect of different cultivation media on cell growth and pH profile of *L. delbrueckii sp. bulgaricus* WICC B-02.

Optimization of SPY medium

In this experiment, medium No. 1, SPY medium, was further modified based on the components of medium No.7 (Table 2).

Table 2: Modification of SPY medium components (Modified from Heenan *et al*., 2002).

Media	Components ($g\ L^{-1}$)
SPY 1	Soy peptone, 25; Glucose,25; Yeast extract,25
SPY 5	Glucose, 25; Yeast extract, 5; Peptone, 10;Tween 80, 1;Sodium acetate, 1;KH_2PO_4, 1.5; $MgSO_4.7H_2O$,0.4;$MnSO_4.7H_2O$, 0.05; Ammonia citrate dibasic, 1; Citric acid, 0.5
SPY10	Glucose, 25; Yeast extract, 10; Peptone, 10;Tween 80, 1;Sodium acetate, 1;KH_2PO_4, 1.5; $MgSO_4.7H_2O$,0.4;$MnSO_4.7H_2O$, 0.05; Ammonia citrate dibasic, 1; Citric acid, 0.5

It was observed that *L.delbrueckii* sp. *bulgaricus* grew well with the addition of trace elements. Accordingly, sodium acetate, $MgSO_4$, and $MnSO_4$ can be considered as essential elements for cell growth. The obtained results showed that SPY 1 gave the highest dry cell mass and lactic acid production, where they reached 3.25 $g.L^{-1}$ and 2.45 $g.L^{-1}$, respectively. Meanwhile, SPY 5 and SPY 10 exhibited non-significant differences oncell mass compared with SPY 1, which were 2.79 $g.L^{-1}$ and 2.84 $g.L^{-1}$,

respectively after 24 hours cultivation. However, upon comparing lactic acid production, SPY 10 gave the lowest acid concentration (only 1.38 $g.L^{-1}$), which is considered to be good for cell growth in order to prevent end-product-inhibition.

Kinetics of cell growth and lactic acid production by *L.delbrueckii* sp. *bulgaricus* in un-optimized medium

The kinetics of cell growth of *L. delbrueckii sp. bulgaricus* were studied in shake flask level. The cells grew at 37°C for 24 hours in un-optimized SPY 10 medium, where the cell growth, lactic acid production and the change in the pH of the culture were recorded hourly (figure 2). The cells grew slowly in the first 2 hours, and then entered the exponential phase with a specific growth rate of 0.47 h^{-1}, and the maximal cell mass of 2.96 $g.L^{-1}$ was obtained after 10 hours of cultivation. As reported by Schiraldi *et al.* (2002), the production of lactic acid as a primary metabolite is strictly dependent on cell growth. Therefore, after 10 hours of cultivation, the pH dropped from 6.5 to about 5.0 with the production of 1.49 $g.L^{-1}$ lactic acid and a production rate of 0.05$g.L^{-1}.hr^{-1}$(Table 3). Such concentration is considered to beacidic for the cells.

Table 3: Kinetic parameters of cell growth, glucose consumption and lactate production during submerged cultivations of *L. delbrueckii* sp. *Bulgaricus* WICC B-02 in the shake flask and in bioreactor.

Parameters	Un-optimized medium	Optimized medium	Controlled pH	Uncontrolled pH
X_{max} ($g\ L^{-1}$)	2.96	3.16	6.4	4.88
μ(h^{-1})	0.47	0.42	0.32	0.18
$Lact_{max}$ ($g\ L^{-1}$)	1.49	0.99	1.42	1.94
Q_{lact}($g\ L^{-1}\ h^{-1}$)	0.06	0.10	0.12	0.14

X_{max}: maximal cell dry weight;; dx/dt: growth rate; μ: specific growth rate; $Lact_{max}$: maximal lactate production; Q_{lact}: lactic acid production rate.

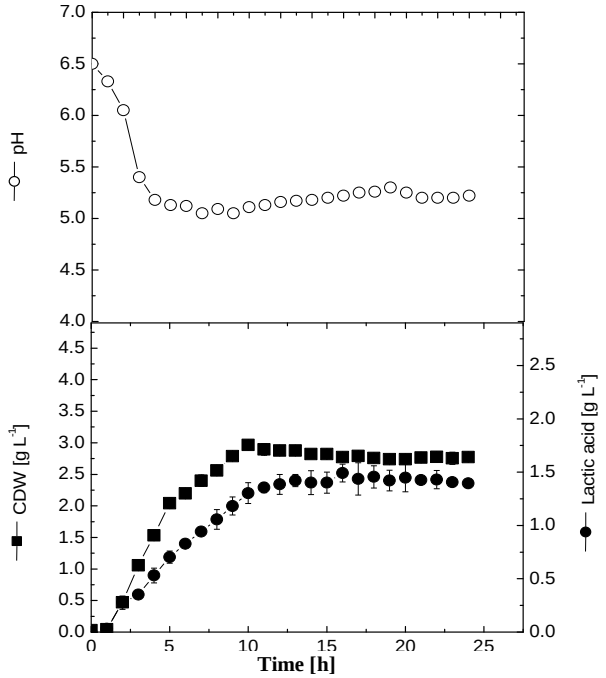

Fig. 2: Cell growth, lactic acid production and pH profile as a function of time during the cultivation of *L. delbrueckii* sp. *bulgaricus* WICC B-02 at 37°C in the un-optimized production medium in shake flask level.

Optimization of the cultivation medium for high cell mass production

Cell growth is normally supported by the best medium, which should in turn contain the key nutrients. Of these key nutrients, glucose, yeast extract and peptone were the main components for cell mass production. Therefore, different sets of experiments were designed for cultivating the cells in different ratios of these three main nutrients. The other nutrients were kept constant as ($g.L^{-1}$): KH_2PO_4, 1.5; $MgSO_4.7H_2O$, 0.4; $MnSO_4.7H_2O$, 0.05; sodium acetate, 1.0; citric acid, 0.5; ammonium citrate dibasic, 1.0 and Tween 80, 1mL. All the experiments were conducted at 37 °C in shake flask level. At the end of the experiments, the finally optimized production medium was used for high cell mass production of *L. delbrueckii* sp. *bulgaricus.*

Effect of different concentrations of glucose as the carbon source on cell growth and lactic acid production

According to Chervaux *et al* (2000), glucose is the most preferred carbon source for bacterial strains. As shown in Figure 3, the maximal dry cell weight of 2.95 $g.L^{-1}$ was achieved at 30 $g.L^{-1}$ glucose. Then, the cell growth decreased slowly with the increasing glucose concentration from 35 to 80 $g.L^{-1}$ concomitant with an increase in lactic acid production up to 2.5 $g.L^{-1}$at 40 $g.L^{-1}$. High glucose concentration favours lactic acid production, which indicates metabolic regulation not only by oxygen, but also by the concentration of carbon source indicating by drop of pH value. Meanwhile, the pH for the cultivation at different glucose concentration was in a range from 5.0 to 5.43. In conclusion, 30 $g.L^{-1}$ was chosen as the best glucose concentration for further optimization.

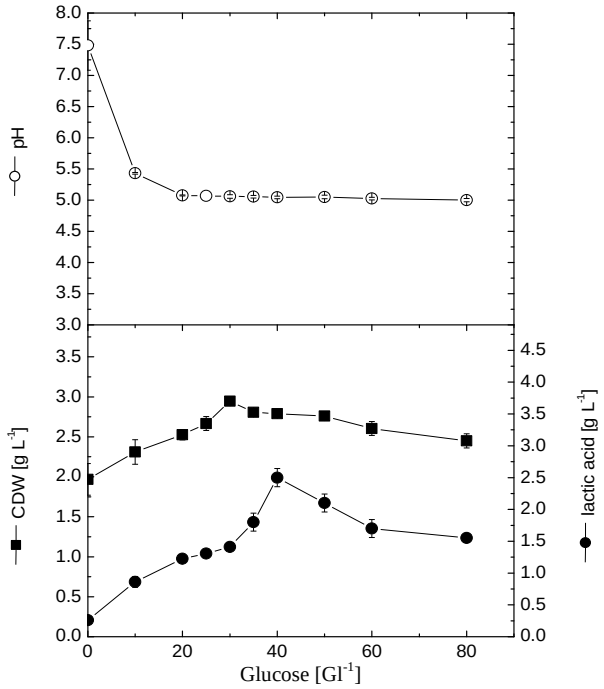

Fig. 3: Effect of different glucose concentrations on cell growth, lactic acid production and pH of L.delbrueckii sp. bulgaricus WICC B-02 in shake flask level.

Effect of different concentrations of yeast extract on cell growth and lactic acid production

Most of the cultivation media used for LAB is generally supplemented with a nitrogenous source, which has great influence on the biomass production. In the present work, different concentrations of yeast extract ranging from 0.0 to 14 g.L^{-1} were studied for their suitability to promote cell growth and lactic acid production (figure 3). As reported by Thi *et al.* (2001), 5g.L^{-1} yeast extract gave the highest OD value of 1.5 in their work with LAB cultivation. Yeast extract is the most commonly used nitrogen source that provides vitamin B complex content in addition to organic nitrogen to LAB (Dumbrepatil *et al.*, 2008). As shown from Figure (5), the maximal cell mass of about 3.07 g.L^{-1} was achieved at 6 g.L^{-1} with about 0.75 g.L^{-1} lactic acid. The higher the concentration of yeast extract, the higher the production of lactic acid, which almost reached 2.5 g.L^{-1}. Therefore, the dry cell mass dropped to 2.48 g.L^{-1} upon using 14 g.L^{-1} of yeast extract, which decreased the pH to 5.08. It is well reported that the pH can inhibit the growth of LAB (Thi *et al.*, 2001).Meanwhile, the pH dropped from 6.5 to about 5.2 for all concentrations of yeast extract. Accordingly, 6 g.L^{-1} was chosen as the best concentration of yeast extract for obtaining the highest biomass with the lowest lactic acid production (0.75 g.L^{-1}).

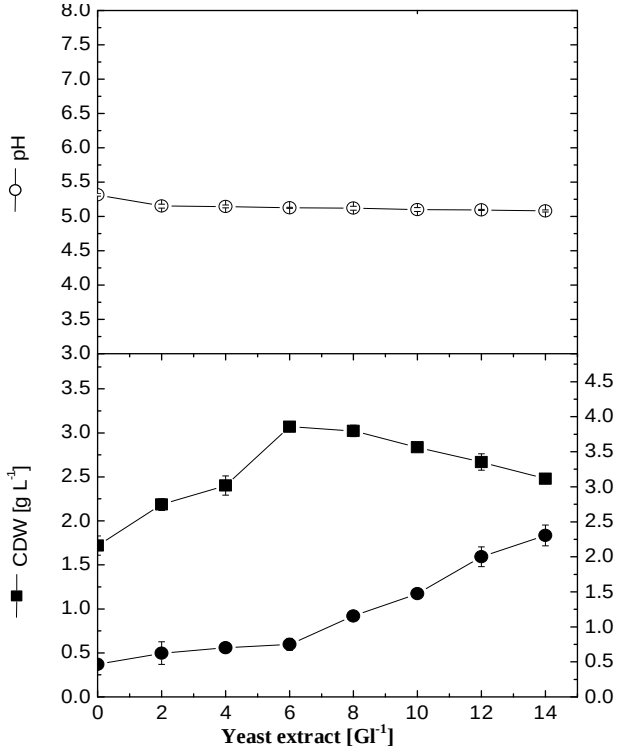

Fig. 4: Effect of different yeast extract concentrations on cell growth, lactic acid production and pH of *L. delbrueckii* sp. *Bulgaricus* WICC B-02 in shake flask level.

Effect of different concentrations of peptone on cell growth and lactic acid production

Peptone is considered to be a good nitrogen and carbon source as well (Amrane and Prigent, 1997). Thi *et al.* (2003) proved that a combination of meat peptone with other nitrogen sources such as yeast extract resulted in a good bacterial growth. Yeast extract was chosen as 6 g.L^{-1}, and different concentrations of peptone ranging from 0.0 to 14.0 g.L^{-1} were studied. It was observed that the addition of peptone to yeast extract increased the dry cell weight up to 26.4 %, where it reached about 3.16 g.L^{-1}, with only 0.79 g.L^{-1} lactic acid. Peptone-free medium gave the lowest dry cell weight (1.4 g.L^{-1}). However, increasing the peptone concentration (7.0-14.0 g.L^{-1}) was accompanied by an increased production of lactic acid up to 1.35 g.L^{-1}.

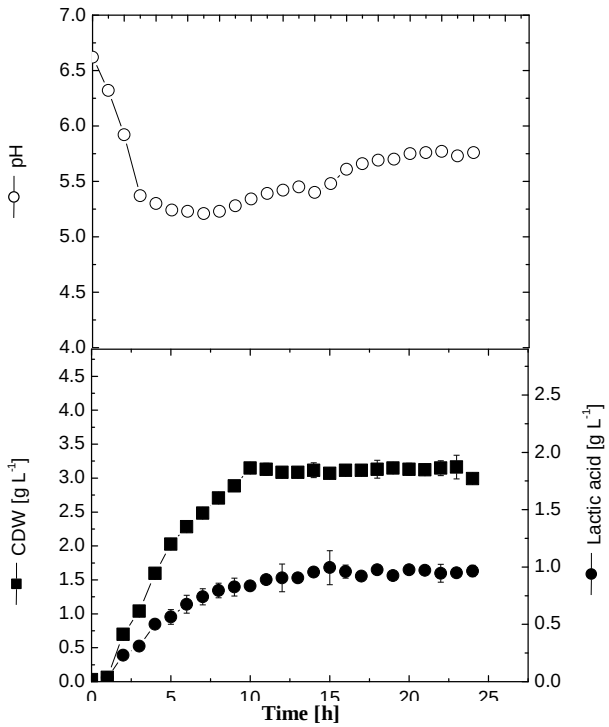

Fig. 5: Cell growth, lactic acid production and pH profile as a function of time during the cultivation of *L. delbrueckii sp. bulgaricus* WICC B-02 at 37°C in the optimized production medium in shake flask level.

Kinetics of cell growth and lactic acid production by *L. delbrueckii sp. bulgaricus*WICC B-02 cultivated in optimized medium

The optimized medium containing the three main components glucose (30 g.L^{-1}), yeast extract (6.0 g.L^{-1}) and peptone (6.0 g.L^{-1}) will be used for high cell mass production of *L. delbrueckii sp. bulgaricus* in the next part of the work. The growth profile, change in the pH and lactic acid production as a function of time were investigated in shake flask cultivation at 37°C. As can be shown in Figure (5), cells entered a lag phase for the first 2 hours of cultivation, and then the cells started to grow exponentially, where they reached a maximal cell dry weight of about 3.14g.L^{-1} before entering the stationary phase after 10 hours of cultivation. The pH of the medium decreased gradually and reached a minimal of about 5.28. Meanwhile, the lactic acid production reached its maximal of about 0.99 gL^{-1} after 15 hours of cultivation as the production of lactic acid was directly proportional to the cells growth. However, the pH increased after

that gradually to reach about 5.76 by the end of cultivation. This may be attributed to the formation of free ammonium ions from the reaction of lactic acid and amino acids by *L. delbrueckii sp. bulgaricus*, and thus explaining the increase in the pH of the medium. The growth was limited due to the accumulation of the by- products (lactic acid) and nutrient limitation (Leroy and Vuyst, 2001).These results revealed that the optimized production medium was able to support better growth of *L. delbrueckii sp. bulgaricus* reaching maximal cell dry weight of about 3.14 $g.L^{-1}$, with an increase of about 6.08% when compared to the un-optimized medium. Although the specific growth rate of both caseswas more or less the same (in the range of $0.40h^{-1}$), the lactic acid production for the optimized medium was reduced by about 33%.

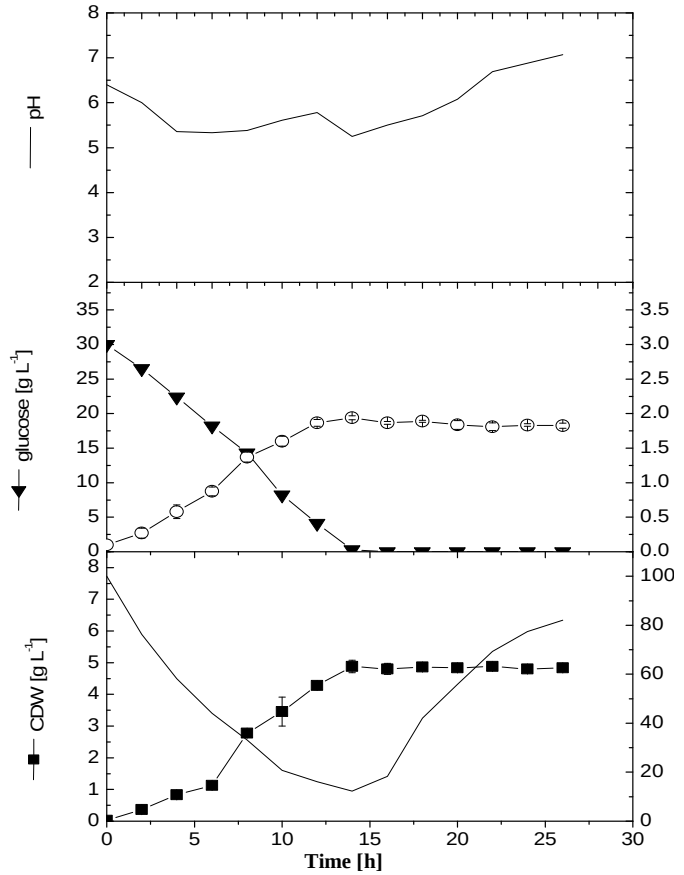

Fig. 6: Cell growth, lactic acid production, glucose consumption and dissolve oxygen as a function of time during the cultivation of *L. delbrueckii sp. Bulgaricus* WICC B-02 in semi industrial scale 16-L stirred tank bioreactor under uncontrolled pH conditions.

Kinetics of cell growth and lactic acid production in batch cultivation in semi industrial scale 16-L stirred tank bioreactor under uncontrolled pH

The growth of *L. delbrueckii* sp. *bulgaricus* was evaluated in stirred tank semi-scale 16-L bioreactor using the optimized medium (figure 6). The bioreactor was run under uncontrolled pH conditions, where the pH was initially set at 6.5. Fig. 8 showed that the pH of the medium drastically dropped to about 5.25 after 14 hours of cultivation and then increased gradually up to 7.0 by the end of the cultivation. The specific growth rate of *L. delbrueckii* sp. *bulgaricus* was 0.18 h^{-1} and the accumulated lactic acid reached 2.0 $g.L^{-1}$ after 12 hours of cultivation with a production rate of almost 0.12 $g.L^{-1}.h^{-1}$(Table 3).This production can be explained due to the effect of uncontrolled pH which does not support cell growth. The cell mass increased gradually and reached its maximum (4.88 $g.L^{-1}$) after 12 hours of cultivation. Under uncontrolled pH cultivation, the dissolved oxygen level decreased to 12.3% after 14 hours cultivation. Unfortunately, anaerobic condition scan promote high lactic acid production on the expense of cell growth (Schiraldi *et al.*, 2002). After 14 hours of cultivation, the dissolved oxygen level increased gradually, indicating that the cells are physiologically inactive due to the low pH (Shuler and Kargi, 2002).

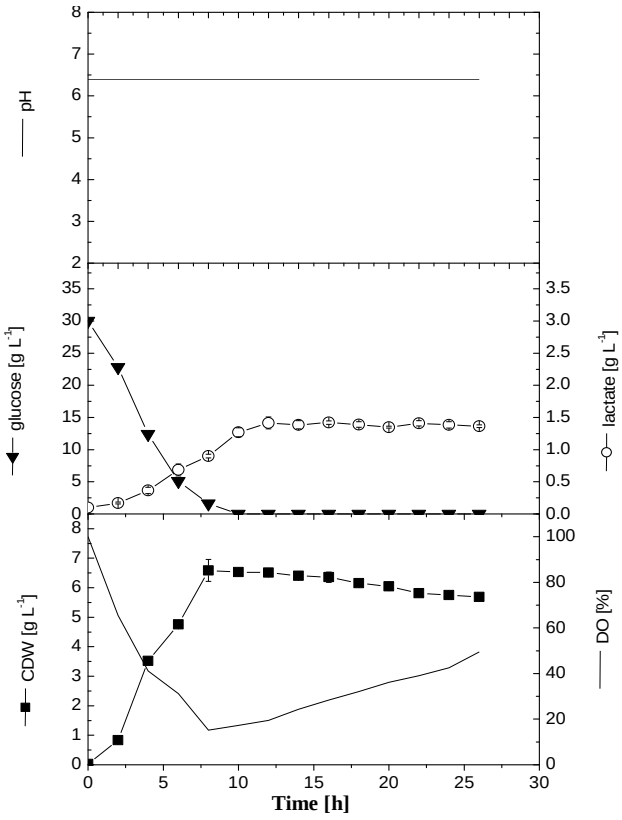

Fig. 7: Cell growth, lactic acid production, glucose consumption and dissolve oxygen as a function of time during the cultivation of *L. delbrueckii sp. bulgaricus*WICC B-02 in semi industrial scale 16-L stirred tank bioreactor under controlled pH conditions.

Kinetics of cell growth and lactic acid production in batch cultivation in semi industrial scale 16-L stirred tank bioreactor under controlled pH

The cultivation of *L. delbrueckii sp. bulgaricus* was then evaluated under controlled pH conditions (figure 7). The pH was set at 6.5, and was maintained throughout the cultivation by the addition of 2.5M NaOH and 2.5M HCl. During the cultivation, the dissolved oxygen decreased rapidly after 8 hours of cultivation. However, the production of lactic acid was less than 1.42 $g.L^{-1}$,

although lactic acid was produced with the same production rate as in the uncontrolled pH conditions (0.14 $g.L^{-1}.h^{-1}$) (Table 3). In the early phase of fermentation, the bacterial cells grew exponentially with a significant increase in the cell mass, where they reached their maximal cell growth of 6.59 $g.L^{-1}$ after 8 hours of cultivation, corresponding to a 31.14% increase. However, the cells entered the stationary phase after 8 hours, where the biomass remained more or less constant for the rest of the fermentation. This may attributed to the consumption of the carbon source. The glucose was completely consumed after 8 hours of cultivation, where the glucose concentration reached its minimum (0.0 $g.L^{-1}$) with a substrate consumption rate of 3.73 $g.L^{-1}.h^{-1}$ (Figure 9).

CONCLUSION

The optimization of the production medium resulted in an increase in the cell mass produced by *L. delbrueckii sp. bulgaricus* accompanied with a minimal production of lactic acid. Moreover, the batch cultivation in semi-scale 16-L bioreactor under controlled pH conditions showed significant improvement in terms of cell mass and lactic acid production.

REFERENCES

Amrane A,Prigent Y. Influence of yeast extract concentration on batch cultures of *Lactobacillus heltivicus:* Growth and production coupling. World J Microbiol Biotechnol, 1998; 14: 529-534.

Arora M, Sharma S, Baldi A. Comparative insight of regulatory guidelines for probiotics in USA, India and Malaysia: A critical review. Inter J Biotechnol Wellness Industry, 2013; 2: 51-54.

Avonts L, Uytven EV, Vuyst LD. Cell growth and bacteriocin production of probiotic lactobacillus strains in different media. Inter Dairy J, 2004; 14: 947-955.

Bernardeau M, Guguen M, VernouxJP . Beneficial *lactobacilli* in food and feed: long term use, biodiversity and proposals for specific and realistic safety assessments. FEMS Microbiol, 2006; 30: 487-513.

Chervaux C, Dusco Ehrlich S, Maguin, E. Physiological study of *Lactobacillus delbrueckii subsp. bulgaricus*strains in a novel chemically defined medium. Appl Environ Microbiol, 2000; 66(12): 5306-5311.

El Marzugi N, El Enshasy HA, AbdMalek R, Othman Z, Sarmidi MR, Abdul Aziz R. 2010. Optimization of cell mass production of the probiotic strain *Lactobacillus lactis* in batch and fed-batch culture in pilot scale levels. In: Médez-Vilas A, ed. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Vol. 2, Spain: Formatex Research Centre, Badajoz 873-79.

Guarner F, Schaafsma GJ. Probiotics. Inter J Food Microbiol, 1998; 39: 237-238.

Grobben GJ, Chin-Joe I, Kitzen VA, Boels IC, Boer F, Sikkema J, Smith MR, deBont JAM.Enhancement of exopolysaccharide production by *Lactobacillus delbruekii Subsp. Bulgaricus* NCFB 2772 with a simplified defined medium. J Appl Environ Microbiol, 1998; 64: 1333-1337.

Heenan CN, Adams MC, Hosken RW, Fleet GH. Growth medium for culturing probiotic bacteria for applications in vegetarian food products. Lebensm-Wiss Technol, 2002; 35: 171-176.

Hun CH, MohdSueb MS, Malek A, Othman Z,, Elsayed EA, Ramili S, Elmarzugi NA, Sarmidi MR, Aziz R, El Enshasy HA. Bioprocess development for high cell mass production of the probiotic yeast *Kluyveromyceslactis*. IOSR J Pharceut Biol Sci, 2013; 8: 49-59.

Ishibashi N, Shimamura S. *Bifidobacteria*: Research and development in Japan. Food Technol, 1993; 47(6): 129-134.

Lebeer S, Vanderleyden J, De Keersmaecker SC. Genes and molecules of *lactobacilli* support in probiotic action. Microbiol Mol Biol, 2008; 72: 728-64.

Leroy F, De Vuyst L. Growth of the bacteriocin-producing *Lactobacillus sakei* strain CTC 494 in MRS broth is strongly reduced due to nutrient exhaustion: a nutrient depletion model for the growth of lactic acid bacteria. Appl Environ Microbiol, 2001; 67(10): 4407-4413.

Li Z, Ding S, Li Z, Tan T. L-Lactic acid production by *lactobacillus casei* fermentation with corn steep liquor-supplemented acid-hydrolysate of soybean meal. Biotechnol J, 2006; 1(12): 1453-1458.

Malek RA, Ishak AF, El Sayed S, El badry I, Othman NZ, Sarmidi MR, Aziz RA, El Enshasy H.2012. Semi-industrial production of probiotic/biotherapeutic yeast *Saccharomyces boulardii* in high cell density culture using different cultivation strategies. AIChE, Annual Meeting, Paper No. 594d. USA: Pittsburgh.

Malek RA, Hamdan S, El Enshasy H, Othman NZ, Zainol NA, Sarmidi MR, Aziz R. 2010. Production of *Lactobacillus salivarius*, a new probiotic strain isolated from human breast milk, in semi-industrial scale and studies on its functional characterization. In: Médez-Vilas A, ed. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Vol. 2, Spain: Formatex Research Centre, Badajoz1196-1204.

Petry S, Furlan S, Crepeau MJ, Cerning J, Desmazeaud M. Factors affecting exocellular polysaccharide production by *Lactobacillus Delbruekiii Subsp. Bulgaricus* grown in a chemically defined medium. Appl Environ Microbiol, 2000; 66(8): 3427-3431.

Othman NZ, El Enshasy HA, Abdul Malek R, Sarmidi MR, Aziz RA. Kinetics of cell growth and functional characterization of probiotic strains *Lactobacillus delbrueckii and Lactobacillus paracasei* isolated from breast milk. Deutche Lebensmittel Rundschau, 2009; 105: 444-450.

Sanders ME.Lactic acid bacteria as promoters of human health. 1994. In: Goldberg I, ed. Functional Foods, London: Chapman & Hall 294-322.

Sarmidi MR, El Enshasy HA. Biotechnology for wellness industry: Concepts and biofactories. Inter J Biotechnol Wellness Industry, 2012; 1: 3-28.

Schiraldi C, Adduci V, Valli V, Maresca C, Giuliano M, Lamberti M, Carteni M, Rosa MD. High cell density cultivations of probiotics and lactic acid production. Biotechnol Bioeng. 2002; 82: 213-222.

Sugita H, Hirose Y, Matsuo N, Deguchi Y. Production of the antibacterial substance by *Bacillus species* strain NM12, an intestinal bacterium of Japanese coastal fish. Aquaculture, 1998; 165: 269-280.

Tammainen M, Salminen S, Ouwehand AC. Fermentation of carrot juice by probiotics: Viability and preservation of adhesion. Inter J Biotechnol Wellness Industry, 2013; 2: 10-15.

Thi LN, Champagnea CP, Lee BH, Gouletb J. Growth of *Lactobacillus paracasei ssp. paracasei* on tofu whey. Inter J Food Microbiol, 2003; 89(1): 67-75.

Villena J, Salva S,, Núñez M Corzo J, Tolaba R .Probiotics for Everyone! The novel immunobiotic *Lactobacillus rhamnosus* CRL1505 and the beginning of social probiotic programs in Argentina. Inter J Biotechnol Wellnes Industry. 2012; 1(3):189-198.

Vodnar DC, Paucean, A, Dulf FC, Socaciu, C. HPLC characterization of lactic acid formation and FTIR fingerprint of probiotic bacteria during fermentation processes. Not Bot Hort Agrobot Cluj, 2010;38(1): 109-113.

Wadher KJ, Mahore JG, Umekar MJ. Probiotics: Living medicines in health maintenance and disease prevention. Inter J Pharma Bio Sci, 2010; 1(3): 1-9.

How to cite this article:

Elsayed A. Elsayed, Nor Zalina Othman, Roslinda Malek, Tebbie Tang, HeshamA. El Enshasy. Improvement of cell mass production of *Lactobacillus delbrueckii* sp. *bulgaricus* WICC-B-02: A newly isolated probiotic strain from mother's milk. J App Pharm Sci, 2014; 4 (11): 008-014.