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A low-cost *Lactobacillus salivarius* L29 growth medium containing molasses and corn steep liquor allows the attainment of high levels of cell mass and lactic acid production

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The aim of the present work was to formulate a *Lactobacillus salivarius* L29 industrial fermentation medium. High cell numbers and good levels of lactic acid by a *L. salivarius* L29 were obtained after shake flask fermentation using molasses as the sole carbon source and corn steep liquor (CSL (industrial grade); an organic source of N) as the principal nitrogen source. The optimum concentrations of molasses and CSL facilitating good cell growth and high-level lactic acid production were found to be 6 and 6% (both v/v), respectively. The maximum cell yield was 2.02 × 10^9 CFU/mL, thus about 15% lower than that obtained when MRS broth was employed for 5-L fermenters culture. Lactic acid production upon growth in industrial broth was 105 g/L; the total sugar content of the medium was 118 g/L (sucrose: glucose: fructose 68:14:18; w/w/w). Upon growth in De Man, Rogosa and Sharpe (MRS) broth (the total sugar content of which was 127 g/L, all of which was glucose), the lactic acid yield was 120 g/L. The optimized industrial growth medium was significantly more economical than were conventional broths.

Key words: *Lactobacillus salivarius* L29, molasses, corn steep liquor, culture medium optimization, lactic acid.

INTRODUCTION

Probiotics are defined as viable microorganisms that when consumed in adequate amounts, confer health benefits by improving the properties of indigenous microflora (Guillot, 1998). Antimicrobial polypeptides synthesized by bacteria can be admixed with live probiotic bacteria, thus increasing antimicrobial activity against pathogens (Dover et al., 2007). Probiotics have been used as an alternative to antibiotics in animals and humans. The bacteria most commonly associated with probiotic activity are lactobacilli and bifidobacteria (Gionchetti et al., 2003). The development of live microbial feed supplements currently attracts much research attention. Any probiotic benefiting human or animal health must fulfill several criteria (Gaurav et al., 2012). First, the probiotic must be amenable to large scale production. Second, the probiotic must be stable in food, in adverse environmental conditions associated with mixing, and in the gastrointestinal tract of the host.

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Lactobacillus strains are the most widely used probiotic bacteria. Lactobacilli produce lactic acid as the sole or principal end-product of carbohydrate metabolism. However, *Lactobacillus* strains are nutritionally fastidious (Holt et al., 1994) and culture can be difficult, because such microbes have limited biosynthetic capacities and it is necessary that expensive nutrients (carbohydrates, amino acids, peptides, nucleic acid derivatives, and vitamins) be added to culture media. De Man, Rogosa and Sharpe (MRS) broth is the medium most commonly employed for the growth of lactobacilli.

However, the medium is complex and is too costly for industrial use. Therefore, it is important to reduce the cost of growth medium; low-cost (but effective) medium components must be identified. Molasses is the most important world-wide raw material for lactic acid production (Rashid and Altaf 2008; Ortiz et al., 2012). It provides a cheap source of fermentable sugars and contains some fermentive nutrients as well (Aslam, 1999). Corn steep liquor (CSL), a major by-product of the corn wet-milling industry, is also an inexpensive source of nutrients available on a large scale (Parekh et al., 1999; Altaf et al., 2007; Salgado et al., 2009).

L. salivarius L29, isolated from pig feces, is a probiotic bacterium that resides in the gastrointestinal tracts of animals (Yun et al., 2009). Earlier studies showed that this microorganism exhibited some valuable characteristics (Yun et al., 2009). It inhibited the growth of various pathogens including *Enterococcus faecalis*, *E. faecium, Escherichia coli, Staphylococcus aureus,* and *S.agalactiae*. The low pH created upon lactobacterial growth, and secretion by lactobacilli of a bacteriocin termed salivaricin (Ocana et al., 1999; Vera Pingitore et al., 2007; Juarez 2003; O' Shea et al., 2012) was considered to explain the observed beneficial effects. Thus, *L. salivarius* L29 is potentially a health-promoting probiotic.

For this reason, industry interest in production of *L. salivarius* L29 and products thereof has grown. The objective of the present work was to develop a low-cost culture medium that nonetheless facilitated efficient production of both *L. salivarius* L29 biomass, and lactic acid.

MATERIALS AND METHODS

Bacterial strain and preparation of inocula

A strain of *L. salivarius* L29 isolated from a pig farm in Korea (Yun et al., 2009) was used in the present study. A stock culture of *L. salivarius* L29 was maintained at -80°C in MRS broth containing 40% (v/v) glycerol. Each inoculum was prepared as follows. A 1 mL amount of frozen stock was inoculated into 50 mL MRS broth and grown at 37°C for 16 h. Aliquots (2.5 mL) of such cultures were transferred to 50 mL amounts of broth in 250 mL Erlenmeyer flasks and incubated at 37°C for 16 h on a rotary shaker operating at 150 rpm. The broth composition varied with the experimental design.

Culture media and growth conditions

Molasses-plus-CSL-based media supplemented with yeast extract (3.1 g/L), $MnSO_4$ (0.04 g/L), sodium citrate (0.01 g/L), K_2HPO_4 (4 g/L), and Tween-80 (0.2 g/L), containing different amounts with specified increments in concentration of both molasses and CSL, were prepared. Medium (50 mL aliquots) in 250 mL Erlenmeyer flasks was autoclaved at 121°C for 15 min and next inoculated to 5% (v/v) using a 16 h seed culture, prepared as described above. Incubation at 37°C followed. Sixteen hours later, the viable cell count and the lactate level of each culture were measured. Viability was determined by counting of colony-forming units (CFUs).

Optimization of industrial medium

The levels of molasses (the carbon source) and CSL (an organic nitrogen source) in the fermentation medium varied in the following manner. Initially, the molasses levels ranged from 2 to 10% (v/v) increments in the medium described above. Upon optimization of the molasses level, the CSL level varied between 2 to 10% (v/v) increments, again in the medium described above, but now containing the optimum concentration of molasses. The experiments were repeated in the reverse order (that is, vary CSL levels first; optimize; and next test molasses levels).

Bioreactor fermentation

Scale-up employed 5-L fermenters (Fermentec Inc. Co. Daejeon, Korea) containing 3-L working volumes of optimized medium. The control medium was MRS medium. In parallel, the experimental medium was: 6% (v/v) molasses, 6% (v/v) CSL, yeast extract (3.1 g/L), $MnSO_4$ (0.04 g/L), sodium citrate (0.01 g/L), K_2HPO_4 (4 g/L), and Tween-80 (0.2 g/L). Fermentation media were inoculated with flask cultures to 5% (v/v). Fermentation was conducted at 37±1°C with agitation of 150 rpm/min. The pH was maintained at pH 5.5 by automatic addition of 10 M NaOH. The total fermentation time was approximately 24 h. All experiments were performed at least in triplicate. Culture broth samples were taken periodically and analyzed in terms of lactate and sugar content, pH, and viable cell numbers.

Analytical methods

Cell growth was measured spectrophotometrically employing a UV-visible spectrometer (Beckman-Coulter Inc. Co. Fullerton, USA) operating at 600 nm. Sugar and lactic acid concentrations were determined via high- performance liquid chromatography (HPLC). An Agilent Technologies 1200 series column equipped with an RI detector was used (Agilent Technologies Inc. Co. Santa Clara, USA). The ion exclusion column (Aminex HPX-87H, Biorad, Hercules, CA, USA) was operated at a temperature of 40°C; the mobile phase was 0.06 N H_2SO_4; and the flow rate was 0.6 mL/min. The sample injection volume was 20 µL.

RESULTS AND DISCUSSION

Flask fermentation

MRS broth is commonly used for small-scale cultivation of *L.salivarius* L29. However, if industrial-scale growth of *L. salivarius* L29 in bioreactors is contemplated, it is necessary to develop a new low-cost medium. We sought

Table 1. Optimization of molasses concentration for the growth of *L. salivarius* L29.

Molasses concentration (%)	Maximum number of viable cells (CFU/mL)
2.0	$1.25 \pm 0.00 \times 10^6$
4.0	$1.95 \pm 0.01 \times 10^6$
6.0	$3.70 \pm 0.05 \times 10^6$
8.0	$4.20 \pm 0.01 \times 10^6$
10.0	$4.00 \pm 0.01 \times 10^6$

Shake-flask cultivation, 37°C, 150 rpm, 5% (v/v) inoculums, 16 h. Each number represents the mean ± SD of three replicates.

Table 2. Optimization of CSL concentration for the growth of *L. salivarius* L29 in medium containing 8.0% (v/v) molasses.

CSL concentration (%)	Maximum number of viable cells (CFU/mL)
2.0	$0.57 \pm 0.01 \times 10^9$
4.0	$0.81 \pm 0.02 \times 10^9$
6.0	$1.26 \pm 0.01 \times 10^9$
8.0	$1.09 \pm 0.00 \times 10^9$
10.0	$0.75 \pm 0.00 \times 10^9$

to optimize bacterial yield and lactic acid production and reduce production cost, by evaluating the utility of various media upon shake flask culture (250 mL flasks). We tested inexpensive growth media containing molasses and CSL. Refined sugars (glucose or sucrose) are commonly used as a carbon source for the growth of probiotics, but these are economically not feasible due to high cost of pure sugars whereas the product (lactic acid) is relatively cheap (Oh et al., 2005). Molasses, a food industrial waste, high in moisture and rich in carbon source have been considered as attractive nutrient source for industrial lactic acid production. The large amount of inorganic salts in molasses, exerting high osmotic pressure and the toxicity to cells are important factor during fermentation (Maiorella et al., 1984).

First, we explored the effects of various concentrations of molasses (2 to 10%, v/v) on the maximum yield of viable *L. salivarius* L29 cells. Cell levels were highest (4.20×10^6 CFU/mL) when 8% (v/v) molasses was employed (Table 1). The low number of viable cells yielded upon culture in medium containing 2% molasses was presumably attributable to a paucity of nutrients. Higher levels of molasses (ca. 10%, v/v) appeared to inhibit cell growth, possibly because of the presence of high levels of salt (Mendes et al., 2011). Yeast extract is commonly used as a nitrogen source for the growth of probiotics, but CSL, an agricultural waste product, may be more cost-effective when industrial fermentation is contemplated. Although CSL is a good source of nutrients and elements, certain required growth components are lacking, and yeast extract (3.1 g/L), $MnSO_4$ (0.04 g/L), sodium citrate (0.01 g/L), K_2HPO_4 (4 g/L), and Tween-80 (0.2 g/L) must be added as supplements. We also measured the numbers of viable cells in cultures grown with different concentrations of CSL in the presence of 8% (v/v) molasses. The highest numbers of viable cells (ca. 1.26×10^9 CFU/mL) were obtained in cultures grown with 6% (v/v) CSL (Table 2). Viable cell numbers did not rise when the CSL level was further increased. Higher CSL levels may be inhibitory because of distortion of the C: N ratio and impurities remaining in CSL during lactic acid fermentation (Underwood et al., 2004).

To better understand the effects of 8% (v/v) molasses and 6% (v/v) CSL on growth and lactic acid production by *L. salivarius* L29, CSL was added at 6% (v/v) to cultures growing in the presence of molasses at 2 to 10% (v/v); growth proceeded for 16 h. The optimum concentration of molasses was 6% (v/v); viable cell numbers and lactic acid yield were 2.0×10^9CFU/ mL and 20 g/L, respectively (Table 3). These findings indicate that when molasses is used as sole (main) carbon source, *L. salivarius* L29 may require relatively high levels of the molasses, possibly because of a shortage of other nutrients. However, CSL is also a carbon source; hence, the optimal concentration of molasses upon the addition of CSL appeared to be 6% (v/v). The adverse effects of high molasses concentrations (over 6% [v/v]) may be attributable to osmotic effects and the toxicity. Beyond a critical substrate concentration, a reduction in water activity combined with plasmolysis can lower the fermentation rate. To reconfirm the optimal concentrations identified, we measured the effects of different concentrations of CSL on growth and lactic acid production by *L. salivarius* L29 in the media containing 6% (v/v) molasses. In all instances, both cell growth and lactic acid yield were maximal at 6% (v/v) CSL (Table 4). Thus, we found that optimal conditions for the industrial growth of *L. salivarius*, in a medium containing inexpensive nutrients, are 6% (v/v) molasses and 6% (v/v) CSL.

Cultivation of *L. salivarius* L29 in an optimal medium in 5-L fermenters

To explore the ability of *L. salivarius* L29 to utilize sugars contained in molasses, batch fermentation was performed in 5-L fermenters, accompanied by pH control. Cell growth was dependent on the culture medium employed (data not shown). The cell number attained 9.57[OD_{600nm}] in MRS broth; the figure upon growth in industrial broth was 7.71[OD_{600nm}]. In both broths, *L. salivarius* L29 grew exponentially for the first 10 h and then entered a typical stationary phase. Growth between 10 and 24 h was probably limited by the presence of inhibitory compounds including organic acids, aldehydes,

Table 3. Optimization of molasses concentration for the growth of *L. salivarius* L 29 in medium containing 6% (v/v) CSL.

Molasses concentration (%)	Maximum number of viable cells (CFU/mL)	Lactic acid yield (g/L)
2.0	$1.02 \pm 0.00 \times 10^9$	16.1 ± 0.08
4.0	$1.29 \pm 0.04 \times 10^9$	18.6 ± 0.04
6.0	$2.00 \pm 0.02 \times 10^9$	20.0 ± 0.06
8.0	$1.20 \pm 0.04 \times 10^9$	17.83 ± 0.15
10.0	$1.12 \pm 0.04 \times 10^9$	16.88 ± 0.03

Table 4. Optimization of CSL level for the growth of *L. salivarius* L29 in medium containing 6% (v/v) molasses.

CSL concentration (%)	Maximum number of viable cells (CFU/mL)	Lactic acid yield (g/L)
2.0	$1.20 \pm 0.02 \times 10^9$	15.52 ± 0.14
4.0	$1.26 \pm 0.04 \times 10^9$	16.93 ± 0.17
6.0	$1.97 \pm 0.01 \times 10^9$	19.76 ± 0.19
8.0	$1.29 \pm 0.04 \times 10^9$	17.19 ± 0.08
10.0	$1.29 \pm 0.03 \times 10^9$	17.19 ± 0.07

phenols, and heavy metals (Roukas, 1998). We also measured medium pH values during growth (the initial pH was 6.0). Similar decreases in pH, from pH 6.0 to 5.5, were seen in the earlier stages of growth in either broth. This may be attributable to formation of organic acids via both deamination of medium amino acids and production of organic acids during growth. Later, the pH was held relatively constant at 5.5 via pH control.

The nutritional requirements of lactic acid bacteria are complex (Dalié et al., 2010), and only some available peptides are metabolized. To improve cell growth and lactic acid production, basic culture media, usually high in protein, are commonly supplemented with yeast extract, CSL, molasses, minerals and inorganic supplements (Amrane and Prigent, 1998; Amrane, 2000; Ha et al., 2003; Liu et al., 2004; Panesar et al., 2007). The sugar utilization and lactic acid production profiles are shown in Figure 1. Sugars in both MRS and industrial broth were directly fermented to lactic acid by *L. salivarius* L29. However, a lag in lactic acid production was evident between 12 to 24 h of fermentation. This may be attributable to temporary inactivation of *L. salivarius* L29 because the undissociated form of lactic acid can inhibit cell membrane functions (Ariyapitipun et al., 1999), thus compromising both nutrient transport and growth. The levels of lactic acid produced from MRS broth (127 g total sugar/L) were 120 g/L whereas that produced when the industrial medium (118 g total sugar/L) was employed was 105 g/L.

The industrial broth contained 80 g sucrose/L, 21.8 g glucose/L, and 16.3 g fructose/L. *L. salivarius* L29 can use both the di- and mono-saccharides of molasses as carbon sources for lactic acid fermentation. *L. salivarius* L29 is homofermentative and efficiently produces lactic acid from sucrose. Table 5 summarizes our data on fermentation by *L. salivarius* L29 of MRS and industrial broth. The maximum viable cell counts obtained were 2.38×10^9 and 2.02×10^9/mL in MRS and industrial broth, respectively. The lactic acid yield was considerably higher (by 94%) when MRS broth was employed. This is attributable to the high nutritive value of MRS broth, which contains glucose, proteins, amino acids, and vitamins. The lower lactic acid yield observed when industrial broth was used may be attributable to the presence of inhibitory compounds such as inorganic salts. The lactic acid production rate was as 5 g/L/h when MRS broth was used, and 4.38 g/L/h when the industrial inhibitory compounds. Further, when molasses and CSL are used together in fermentation medium, serious problems in product purification may become evident (Timbuntam et al., 2006). These problems can be medium was employed. The lower production evident in industrial medium may again reflect the presence of overcome by the use of ultra filtration and electrodialysis.

In conclusion, we assessed the ability of *L. salivarius* L29 (in terms of both bacterial growth and lactic acid production) to grow in an inexpensive industrial broth containing molasses as the carbon source and CSL as the principal nitrogen source; minor nutrients were also added. The optimal medium composition featured 6% (v/v) molasses and 6% (v/v) CSL. Yeast extract (3.1 g/L), $MnSO_4$ (0.04 g/L), sodium citrate (0.01 g/L), K_2HPO_4 (4 g/L), and Tween-80 (0.2 g/L) were also required. We have thus shown that high numbers of viable cells and good levels of lactic acid may be achieved via fermentation in an industrial broth. This medium is simpler and less expensive than MRS medium. We have thus laid the foundation for the development of cost-

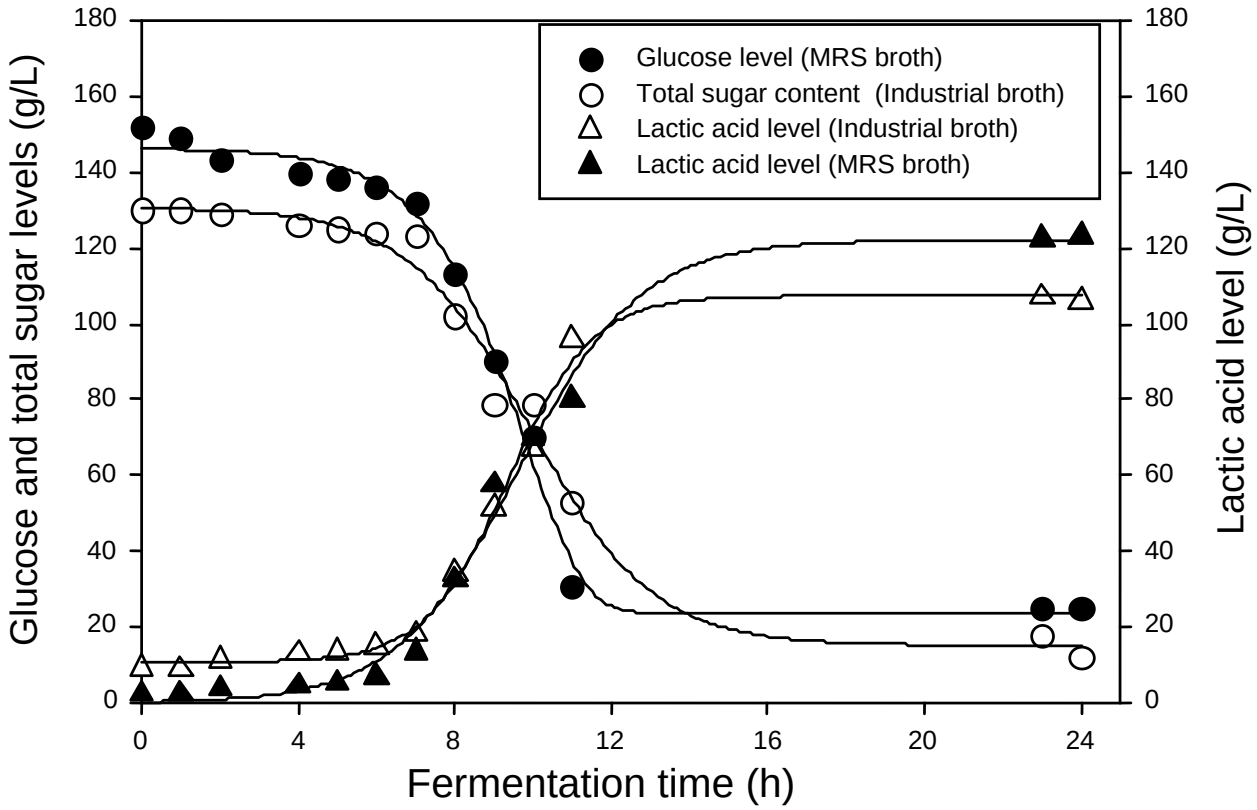

Figure 1. Glucose and total sugar consumption levels, and lactic acid yield, by *L. salivarius* L29 grown in batch fermentations using MRS and industrial broth. Each point represents the mean value of data obtained in three independent experiments.

Table 5. Summary of fermentation parameters derived from experiments using MRS and industrial medium for the growth of *L. salivarius* L29.

Parameter	MRS broth	Industrial broth
Maximum CFU ($\times 10^9$)	2.38 ± 0.01	2.02 ± 0.02
Initial sugar level (g/L)	152	130
Sucrose	0	88
Glucose	152	18
Fructose	0	24
Residual sugar level (g/L)	25	12
Sucrose	0	6.1
Glucose	25	2.9
Fructose	0	2.9
Sugar consumption (g/L)	127	118
Total lactic acid yield (g/L)	120	105
% Yield $_{p/s}$	94	89
Productivity after 24 h (g/L/h)	5	4.38

The fermentation time was 24 h.

effective lactic acid fermentation.

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REFERENCES

Altaf M, Venkateshwar M, Srijana M, Reddy G (2007). An economic approach for L-(+) lactic acid fermentation by *Lactobacillus amylophilus* GV6 using inexpensive carbon and nitrogen sources. J. Appl. Microbiol. 103:372-380.

Amrane A (2000). Effect of inorganic phosphate on lactate production by *Lactobacillus helveticus* grown on supplemented whey permeate. J. Chem. Technol. Biotechnol. 75:223-228.

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

Ariyapitipun T, Mustapha A, Clarke AD (1999). Microbial shelf life determination of vacuum-packaged fresh beef treated with polylactic acid, lactic acid, and nisin solutions. J. Food Prot. 62(8):913-920.

Aslam M (1999). Ethanol production for motor vehicles and chemical feedback. Pakistan Academy of Science, lslamabad, pp. 3-37.

Dalié DKD, Deschamps AM, Richard-Forget F (2010). Lactic acid bacteria-Potential for control of mould growth and mycotoxins: A review. Food Control 21(4):370-380.

Dover SE, Aroutcheva AA, Faro S, Chikindas ML (2007). Safety study of an antimicrobial peptide lactocin 160, produced by the vaginal *Lactobacillus rhamnosus*. Infect. Dis. Obstet. Gynecol. 2007:78248.

Gaurav T, Ruchi T, Saurabh P, Preeti P (2012). Promising future of probiotics for human health: Current scenario. Chron. Young Sci. 3:17-28.

Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M (2003). Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology 124(5):1202-1209.

Guillot JF (1998). Les probiotiques en alimentation animale. Cahiers Agricultures 7:49-54.

Ha MY, Kim SW, Lee YW, Kim MJ, Kim SJ (2003). Kinetics analysis of growth and lactic acid production in pH-controlled batch cultures of *Lactobacillus casei* KH-1 using yeast extract/corn steep liquor/glucose medium. J. Biosci. Bioeng. 96:134-140.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). Group 19. Regular, nonsporing Gram-positive rods. In: Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (Eds.), Bergey's manual of determinative bacteriology, Williams & Wilkins Co., Baltimore, pp. 565-570.

Juarez Tomas MS, Ocana V, Wiese B, Nader-Macias ME (2003). Growth and lactic acid production by vaginal *Lactobacillus adidophilus* CRL 1259. Inhibition of uropathogenic *Escherichia coli*. J. Med. Microbiol. 52:1117-1124.

Liu C, Liu Y, Liao W, Wen Z, Chen S (2004). Simultaneous production of nisin and lactic acid from cheese whey: optimization of fermentation conditions through statistically based experimental designs. Appl. Biochem. Biotechnol. 114:627-638.

Maiorella BL, Blanch HW, Wilke CR (1984). Feed component inhibition in ethanolic fermentation by *Saccharomyces cerevisiae*. Biotechnol. Bioeng. 24:1155-1166.

Mendes FS, Pajuelo MG, Cordier H, Francois JM, Vasconcelos I (2011). 1,3-Propanediol production in a two-step process fermentation from renewable feedstock. Appl. Microbiol. Biotechnol. 92(3):519-527.

Ocana VS, De Ruiz Holgado AP, Nader-Macias ME (1999). Characterization of a bacteriocin-like substance produced by a vaginal *Lactobacillus salivarius* strain. Appl. Environ. Microbiol. 65:5631-5635.

Oh HR, Wee YJ, Yun JS, Han SH, Jung SW, Ryu HW (2005). Lactic acid production from agaricultural resources as cheap raw materials. Biores. Technol. 96:1492-1498.

Ortiz ME, Fornaguera MJ, Raya RR, Mozzi F (2012). Lactobacillus reuteri CRL 1101 highly produces mannitol from sugarcane molasses as carbon source. Appl. Microbiol.Biotechnol. 95:991-999.

O' Shea EF, O' Connor PM, Raftis EJ, O' Toole PW, Stanton C, Cotter PD, Ross RP, Hill C (2012). Subspecies diversity in bacteriocin production by intestinal *Lactobacillus salivarius* strains. Gut Microbes. 3(5):468-473.

Panesar PS, Kennedy JF, Gandhi DN, Bunko K (2007). Bioutilisation of whey for lactic acid production. Food Chem. 105:1-14.

Parekh M, Formanek J, Blaschek HP (1999). Pilot-scale production of butanol by *Clostridium beijerinckii* BA101 using a low-cost fermentation medium based on corn steep water. Appl. Microbiol. Biotechnol. 51:152-157.

Rashid T, Altaf Z (2008). Potential and environmental concerns of ethanol production from sugarcane molasses in Pakistan. Nature Precedings. hdl.10101/npre. 1499.1.

Roukas T (1998). Pretreatment of beet molasses to increase pullulan production. Proc. Biochem. 33:805-810.

Salgado JM, Rodriguez N, Cortés S, Dominguez JM (2009). Development of cost-effective media to increase the economic potential for larger-scale bioproduction of natural food additives by *Lactobacillus rhamnousus*, *Debaryomyces hansenii*, and *Aspergillus niger*. J. Agric. Food Chem. 11:10414-10428.

Timbuntam W, Sriroth K, Tokiwa Y (2006). Lactic acid production from sugar-cane juice by a newly isolated *Lactobacillus* sp. Biotechnol. Lett. 28:811-814.

Underwood SA, Buszko ML, Shanmugam KT, Ingram LO (2004). Lack of protective osmolytes limits final cell density and volumetric productivity of ethanologenic, *Escherichia coli* k011 during xylose fermentation. Appl. Environ. Microbiol. 70:2734-2740.

Vera Pingitore E, Salvucci E, Sesma F, Nader-Macias ME (2007). Different strategies for purification of antimicrobial peptides from Lactic Acid Bacteria (LAB), in communicating current research and educational topics and trends in applied microbiology, In: Mendez-Vilas A (Ed), Formatex, Badajoz pp. 557-568.

Yun JH, Lee KB, Sung YK, Kim EB, Lee HG, Choi YJ (2009). Isolation and characterization of potential probiotic lactobacilli from pig feces. J. Basic. Microbiol. 49:220-226.