Full Length Research Paper

Medium component improvement for β-galactosidase production by a probiotic strain *Lactobacillus fermentum* CM33

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Plackett and Burman statistical design was applied to screen the nutritional factors affecting β-galactosidase production by *Lactobacillus fermentum* CM33. Accordingly, lactose, tryptone and Tween80 were found to be the positive effective factors at the significant level above 80%. Central composite design (CCD) and response surface plot predicted that the maximum enzyme activity of 49.81 U/100 ml would be obtained at 3.23 (w/v) lactose, 4.91% (w/v) tryptone and 0.62% (w/v) Tween80 and a trial experiment validated the activity total at 114% at 24 h of cultivation. However, the maximum activity of 63.31 U/100 ml culture was found at 16 h. The initial pH range 6.0 to 6.5 was found to be the most suitable for enzyme production. The final stage of optimization increased the productivity by 14.60 folds over that obtained with the non-optimized medium. The replacing of the expensive ingredients such as yeast extract, peptone and beef extract composed in the optimized medium by skimmed milk, did not significantly alter the enzyme productivity.

Key words: Medium optimization, *Lactobacillus* sp., β-galactosidase, experimental design

INTRODUCTION

The lactose-hydrolysing enzyme, β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) has been long accepted as an important enzyme for both the dairy industry and food related products in general, while some applications have been in the treatment of cheese whey to reduce environmental pollution (Rouwenhorst et al., 1989). The recent application of β-galactosidase has been extensively investigated toward the transgalactosylation reaction resulting in the formation of di-, tri-, or higher galacto-oligosaccharides (GOS) (Rabiu et al., 2001; Chen et al., 2003; Gosling et al., 2010; Patel and Goyal, 2011). GOS have been proven to be effective as a probiotic food ingredient for promoting the growth of health promoting bacteria known as *Bifidobacteria and Lactobacilli* (Kim et al., 2006; Splechtna et al., 2006; Depeint et al., 2008) and this, therefore, has led to the development of functional foods which target the prevention of colon cancer (Saulnier et al., 2009).

Lactobacillus fermentum CM33, a probiotic strain isolated from healthy infants, has been selected, based on its capability of β-galactosidase production. This bacterium clearly demonstrated the properties of a human probiotic and produced β-galactosidase with transgalactosylation activity to generate GOS, which was further expected to combine with the live cells of *L. fermentum* CM33 in the formulation of a symbiotic neutraceutical (Sriphannam et al., 2012). However, intracellular β-galactosidase produced by this bacterium was found to yield only 0.42 U/100 ml culture when cultivated at 37°C in the expensive medium de Man, Rogosa and Sharpe (MRS) broth for 24 h, and then

increased to 4.5 units/100 ml culture, when the normal carbon source at 2% (w/v) glucose was replaced by 1% (w/v) lactose (Sriphannam et al., 2012). The high cost of the culture medium and the low rate of productivity are the main tasks in large-scale preparation, as well as in the application of this enzyme. Hence, either the medium optimization for maximal enzyme yield or the search of the low cost medium is necessary.

Some previous reports have investigated the medium optimization for β-galactosidase production by lactic acid bacteria, particularly *Lactobacilli* and *Bifidobacteria* (Vasiljevic and Jelen, 2001; Hsu et al., 2005). These, most often use MRS broth with some modifications and are usually composed of costly complex ingredients such as peptone, yeast extract and beef extract. However, the β-galactosidase production media compositions for other microbes have also included *Sirobasidium magnum* (Onishi and Tanaka, 1995), *Kluyveromyces maxianus* (Furlan et al., 2000) and *Bacillus* sp. MTCC 3088 (Chakraborti et al., 2000). The traditional method used to optimize the medium composition for maximal enzyme productivity is commonly carried out by single factor optimization, while other factors are held constant. However, this strategy is time consuming and involves a large number of experiments, which directly increases the complexity along an increase of the factor numbers (Charyulu and Gnanamani, 2010). In addition, single factor optimizing may result in a lack of acknowledgement of the interaction between the two factors, and the experiment will therefore become incapable of reaching a true optimum. Differing from the single factor optimization, the central composite design helps to analyze the interaction of the factors, along with the effect of significant variables on the response value (Oliveira et al., 2009). Recently, medium optimization by experimental design has been accepted as the efficacious method and has been widely reviewed by both theoretical and practical researchers (Ren et al., 2008). Production of aquaculture probiotic *Micrococcus* MCCB 104 was succeeded, using central composite design (Kim et al., 2004). Moreover, Preetha et al. (2007) also described the optimization of medium composition for growth of *Leuconostoc citreum*.

In this study, the medium optimization for β-galactosidase production by *L. fermentum* CM33 was performed sequentially, using Plackett and Burman design, central composite design (CCD) and response surface methodology. Modification of the optimized medium composition to achieve simpler and cheaper enzyme production medium has also been described.

MATERIALS AND METHODS

Microorganism and inoculum preparation

L. fermentum CM33, a probiotic strain isolated from healthy infants by Sriphannam et al. (2012), was used throughout this study. The strain was maintained in glycerol and stored at -85°C and reactivated by being transferred to the fresh MRS broth and incubated at 37°C for 24 h. Seed inoculums were prepared by inoculating a single colony of bacterial strain into MRS broth and incubating it at 37°C for 18 h without agitation.

Screening for factors affected on β-galactosidase production using Plackett and Burman design

Based on the medium compositions for β-galactosidase production reported by Onishi and Tanaka (1995) and MRS medium, the medium used in this experiment was modified to 11 ingredients including lactose, yeast extract, peptone, beef extract, tryptone, $(NH_4)_2SO_4 \cdot 7H_2O$, KH_2PO_4, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, L-cysteine and Tween80 (pH 7.0). The Plackett and Burman statistical design, based on equation (1), were created to evaluate the most important medium compositions affected on β-galactosidase production.

$$Y = \beta_0 + \sum \beta_i X_i \ (i = 1, \dots . k) \tag{1}$$

Where, Y is the estimated target function, β_0 is a constant, β_1 is regression coefficient, X is independent variable and k is number of variables. The 11 variables of medium compositions were investigated by the two levels (-1 and +1) of Plackett and Burman experimental design (Table 1). All cultures of which each medium were static, incubated at 37°C for 24 h. Bacterial cells were harvested by centrifugation and disrupted for β-galactosidase activity determination. The important factors affecting β-galactosidase production were evaluated by regression analysis, using the Statistix program version 7.0 (Analytical Solfware, USA).

Enzyme preparation and activity assay

Cells of lactic acid bacteria (LAB), which were grown in the culture, were harvested by centrifugation at 4200 × g for 10 min under 4°C, after washing twice with 0.1 M sodium phosphate buffer (pH 7.0), then being re-suspended in the appropriate volume of the same buffer. Cell suspension maintained on an ice bath was disrupted by sonication with 40% amplitude for 30 min (Vibra-Cell, Sonics), and centrifuged with 7200 × g for 10 min. The precipitated cell debris was discarded and the supernatant obtained was kept as the enzyme solution. The β-galactosidase activity was assayed according to the method described by Konsoula and Liakopoulou-Kyriakides (2007). The substrate of 0.15 ml of 4 mM *ortho*-nitrophenyl-β-D-galactopyronoside (ONPG), in 0.1 M sodium phosphate buffer, pH 7.0, was mixed with 0.15 ml of enzyme solution and incubated at 37°C for 30 min. The reaction was stopped by adding 0.7 ml of 0.5 M sodium carbonate solution (pH 10). The amount of *o*-nitrophenol released in the reaction mixture was measured by the absorbance at 420 nm, using a UV-1700 spectrophotometer (Shimadzu, Japan). One unit of enzyme was defined as the amount of enzyme that released 1 micromole of *o*-nitrophenol from the substrate per minute. Protein was determined according to the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Determination of lactose, tryptone and Tween80 concentrations by central composite design and response surface methodology

As lactose, tryptone and Tween80 were three factors positively

Table 1. The concentration of each variable at high and low levels used in the Plackett and Burman design for investigation of the factors affecting β-galactosidase production.

Variable	Concentration % (w/v)	
	High (+1)	Low (-1)
Lactose	2.000	0.200
Yeast extract	2.000	0.200
Peptone	2.000	0.200
Beef extract	2.000	0.200
Tryptone	2.000	0.200
$(NH_4)_2SO_4.7H_2O$	1.000	0.050
K_2HPO_4	0.600	0.060
KH_2PO_4	0.200	0.020
$MgSO_4.7H_2O$	0.100	0.005
L-cysteine	0.060	0.010
Tween80	0.500	0.050

Table 2. The least square linear regression analysis of Plackett and Burman statistical design to screen for important factors influencing β-galactosidase production.

Variable	Coefficient	P-value
Constant	0.44500	0.0000
Lactose	0.23375	0.0000
Yeast extract	0.02625	0.5010
Peptone	-0.04667	0.2347
Beef extract	-0.11375	0.0056
Tryptone	0.26583	0.0000
$(NH_4)_2SO_4·7H_2O$	-0.08625	0.0318
K_2HPO_4	-0.07125	0.0732
KH_2PO_4	-0.16500	0.0001
$MgSO_4·7H_2O$	0.02917	0.4550
Tween80	-0.02708	0.4876
Tween-80	0.11000	0.0072
$R^2 = 0.9238$		0.0000

affected on β-galactosidase production regarding the result from Plackett and Burman, the CCD was then applied to determine the optimal quantity of these factors. The central composite rotatable design, containing 20 runs, including six replicates of the center points, one replicate of factorial points and one replicate of axial star points, were used to develop a second order model of response surface methodology that could find the optimum point of two independent variables. The multiple regression analysis gene-rated a quadratic equation (2) as follows:

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_i^2 + \sum b_{ij} x_i x_j \qquad (2)$$

Where, Y is predicted value (β-galactosidase activity); b_0 is the offset term; b_i is the linear effect; b_{ii} is the squared effect; b_{ij} is the interaction effect; x_i and x_j is the independent variable.

Lactose, 2 to 4% (w/v), tryptone, 2 to 6% (w/v) and Tween80, 0.2 to 1.0% (w/v) were added to the culture medium containing percentage weight by volume of 2.0 yeast extract, 0.2 peptone, 0.2 beef extract, 0.05 $(NH_4)_2SO_4·7H_2O$, 0.02 KH_2PO_4, 0.06 K_2HPO_4, 0.1 $MgSO_4·7H_2O$ and 0.01 L-cysteine. After 24 h, at 37°C, bacterial cells were harvested by centrifugation and disrupted for enzyme preparation as described previously, and β-galactosidase activity was determined along with the fermentation period. Total data were analyzed, using the Design expert 7.0.0 (StatEase, USA).

Effect of initial pH on enzyme production

L. fermentum CM33 was inoculated in the optimal production medium, in the presence of various pH values: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. The culture was incubated at 37°C for 16 h. Cells were harvested and disrupted for determination of enzyme activity. Viable cell enumeration was investigated by spread plate technique on MRS agar.

Modification of optimized enzyme production medium

To achieve a more simple medium composition of enzyme production medium, some medium components in the optimized medium were deleted to investigate their effect on enzyme

production. Treatment 1 (T-1: dBYP), beef extract, yeast extract and peptone were deleted, treatment 2 (T-2: dBYPA) was T-1 with ammonium sulfate deletion, treatment 3 (T-3: dBYPcA) was T-1 with the total nitrogen compensated by ammonium sulfate and treatment 4 (T-4: dBYPAcS) was T-2 with compensation of total nitrogen with skimmed milk. The culture in the optimized medium, obtained from the previous experimental design, was used as a control treatment (T-5). Seed inoculum was prepared and inoculated to 100 ml of medium broth and statically incubated at 37°C. Sampling was carried out with 4 h intervals and determined for β-galactosidase.

RESULTS

Screening of factors affecting β-galactosidase production using Plackett and Burman design

The effect of the nutritional factors on β-galactosidase production by *L. fermentum* CM33 was considered for the values obtained by the least square linear regression analysis (Table 2). Factors show that P-values of less than 0.2 were classified as the significant effecters on the β-galactosidase production, whereas, the plus and minus values represent the positive and negative effect on enzyme production, respectively. Lactose, tryptone and Tween80 were found to be significant factors positively affecting β-galactosidase production, but beef extract, $(NH_4)_2SO_4.7H_2O$, KH_2PO_4 and K_2HPO_4 were the signi-ficant factors negatively affecting β-galactosidase pro-duction. Peptone, yeast extract, $MgSO_4.7H_2O$ and L-cysteine were classified as non-significant factors. Thus, three of the significantly positive factors, lactose, tryptone and Tween80 were further investigated to predict their optimal quantities using CCD, while the amounts of negative affectivity and the non-significant factors were

Table 3. The experimental results of β-galactosidase activity produced by *L. fermentum* CM33 cultivated at 37°C in various medium compositions designed by CCD.

Run	Lactose (X_1) %(w/v)	Tryptone (X_2) %(w/v)	Tween80 (X_3) %(w/v)	β-galactosidase activity (U/100 ml)
1	3.00	4.00	0.60	47.42
2	3.00	4.00	0.60	47.67
3	2.00	4.00	0.60	38.85
4	3.00	4.00	0.60	48.45
5	3.59	2.81	0.36	38.73
6	2.41	2.81	0.84	35.55
7	2.41	5.19	0.84	46.31
8	3.00	4.00	1.00	41.90
9	4.00	4.00	0.60	45.65
10	3.00	4.00	0.60	48.66
11	3.59	5.19	0.84	48.26
12	3.59	2.81	0.84	36.39
13	3.00	6.00	0.60	44.20
14	2.41	2.81	0.36	37.65
15	3.00	2.00	0.60	37.96
16	3.59	5.19	0.36	47.42
17	3.00	4.00	0.20	43.19
18	2.41	5.19	0.36	44.79
19	3.00	4.00	0.60	50.00
20	3.00	4.00	0.60	47.03

fixed at their low sitting.

Optimizing lactose, tryptone and Tween80 quantities by central composite design (CCD)

The variables tested were 2 to 4% (w/v) lactose, 2 to 4% (w/v) tryptone and 0.2 to 1.0% (w/v) Tween80. The regression analysis expressed the relationship between β-galactosidase activity (Y) that depend on the amounts of lactose (X_1), tryptone (X_2) and Tween80 (X_3). The model obtained from regression analysis is presented as the following equation:

$$Y = -50.33763 + 35.44244X_1 + 13.68270X_2 + 30.18126X_3 - 5.77111X_1^2 - 1.73528X_2^2 - 34.22569X_3^2 + 0.47023X_1X_2 - 0.81317X_1X_3 + 3.00520X_2X_3$$

The β-galactosidase productivities from all treatments (Table 3) were processed by the design expert program to find the optimal concentration of three factors, to maximize enzyme activity. The response surface plot clearly demonstrated the interactions to each other between three positive nutritional factors (Figure 1). Interaction between tryptone and lactose is demonstrated in Figure 1A, while Figure 1B shows that of Tween80 versus lactose. The interaction of Tween80 and tryptone is demonstrated in Figure 1C. Calculation by the design expert program predicted the highest β-galactosidase

productivity of 49.81 U/100 ml culture when 3.23% (w/v) lactose, 4.91% (w/v) tryptone and 0.62% (w/v) Tween80 were applied. To validate the prediction of the CCD experiment, β-galactosidase production, using the optimized medium containing percentage weight by volume of 3.23 lactose, 4.91 tryptone, 0.62 Tween80, 2.0 yeast extract, 0.2 beef extract, 0.2 peptone, 0.05 $(NH_4)_2SO_4.7H_2O$, 0.06 K_2HPO_4, 0.02 KH_2PO_4, 0.1 $MgSO_4.7H_2O$ and 0.01 L-cysteine was subsequently investtigated at 24 h of cultivation at 37°C. The enzyme productivity was found at 56.78 U/100 ml culture (Figure 2), which was calculated to 114% of validation, based on the predicted value. However, the highest activity of 63.31 U/100 ml culture was found at 16 h cultivation.

Effect of initial pH on β-galactosidase production

The effect of initial pH on β-galactosidase production by *L. fermentum* CM33 was investigated at pH values ranging from 4.0 to 8.0. The maximum β-galactosidase activity was obtained as 65.50 U/100 ml culture, at pH 6.5 (Figure 3). The strain CM33 showed the general characteristic of responding to the initial pH of 6.0 to 6.5 of the selected medium for lactic acid bacteria as MRS. Moreover, our results indicated that the β-galactosidase activity clearly depends on bacterial growth, as almost the same ratio of activity to the viable cell number was
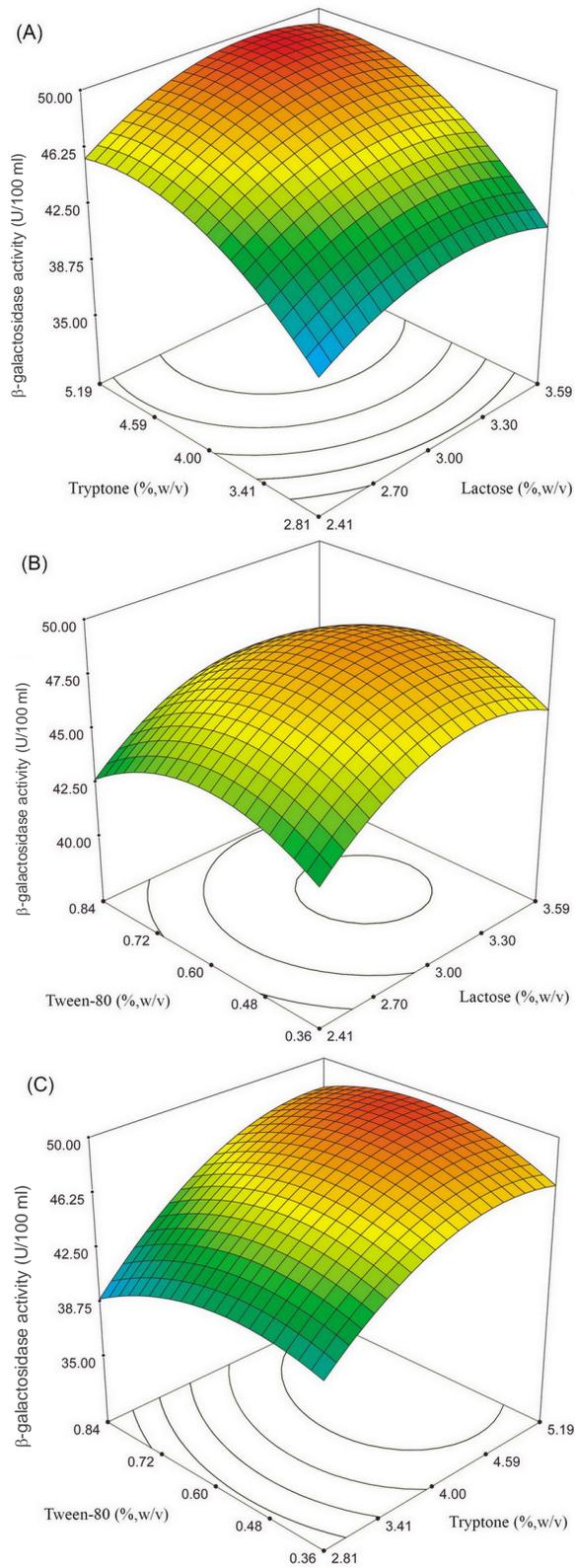
(A)

(B)

(C)

Figure 1. Response surface plots demonstrating interactions of significantly influencing factors on β-galactosidase production by *L. fermentum* CM33 at 37°C.
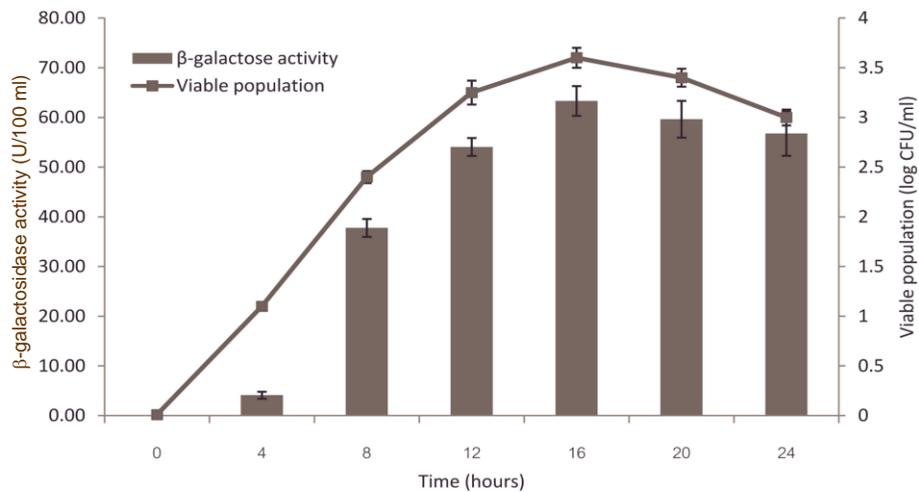
Figure 2. Time course of β-galactosidase production and the viable cell number of *L. fermentum* CM33 cultured in an optimized medium at 37°C. Each point represents the mean ± standard deviation (SD) of three replicates.
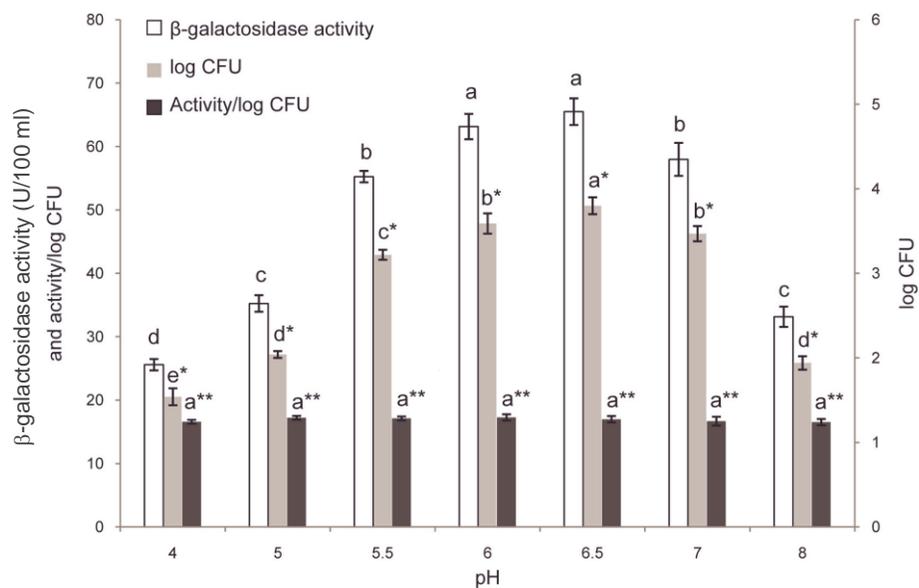

Figure 3. Effect of initial pH on β-galactosidase production by *L. fermentum* CM33 in optimized medium at 37°C for 16 h. Each point represents the mean ± SD of three replicates. Different small letters above the bars indicate statistical comparison between groups using ANOVA and Turkey honestly significant difference (HSD's) test.

observed.

Modification of the optimized medium for β-galactosidase production

As described previously, low cost and simple culture medium benefit the enzyme preparation on the large scale, and deletion and replacement of some expensive medium components were investigated. Time course production of β-galactosidase activity is demonstrated in Figure 4. The differences in enzyme productivity by five medium formulations were observed, however all medium formulations showed the maximum activity at the same cultivation time of 16 h. After that, a decrease of enzyme activities were markedly observed (Figure 4A).
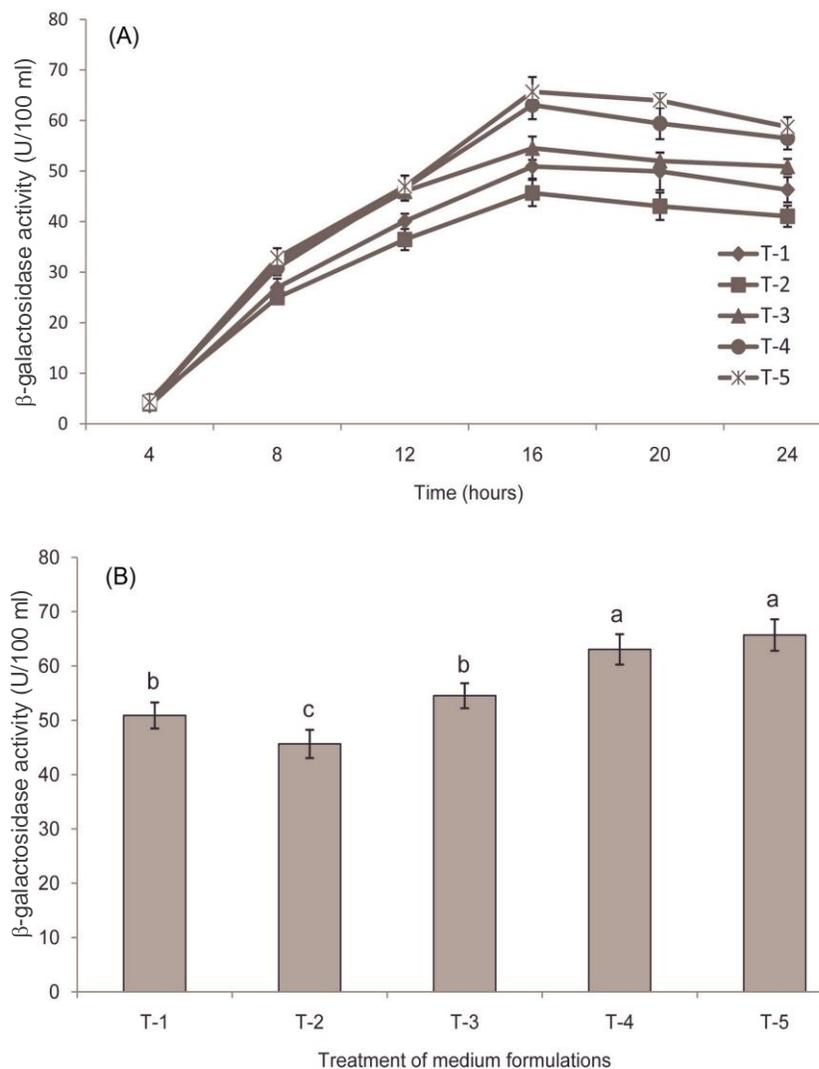
Figure 4. Effects of different medium formulations on β-galactosidase productivity from *L. fermentum* CM33 cultured at 37°C. (A) Enzyme activity at various cultivation time. (B) Comparison of enzyme activity at 16 h. Each point represents the mean ± SD of three replicates. Different small letters above the bars indicate statistical comparison between groups using ANOVA and Turkey honestly significant difference (HSD's) test.

The deletion of organic nitrogen sources (T-1: dYEP), and both organic nitrogen and inorganic nitrogen sources (T-2: dYEPA), without any compensation of total nitrogen significantly decreased the β-galactosidase productivity, compared to 65.71 U/100 ml of the control experiment (T-5). Interestingly, compensation of deleted organic nitrogen sources with skimmed milk (T-4: dYEPcS) recovered the β-galactosidase productivity of 63.07 U/100 ml culture, respectively, which is statistically analyzed at the same level with the control experiment (Figure 4B). In the event of ammonium sulfate compensation (T-3: dYEPcA), the enzyme productivity was recovered to 54.53 U/100 ml

culture, but was not found to be as good as that from skimmed milk.

From this result, we concluded that it is possible to replace the expensive organic nitrogen sources of yeast extract, peptone and beef extract with a low cost organic nitrogen source as skimmed milk, without the significant reduction in β-galactosidase productivity. From this study, the most simple medium composition for β-galactosidase, by L. fermentum CM33, was comprised of 3.23% (w/v) lactose, 4.91% (w/v) tryptone, 2.4% (w/v) skimmed milk (w/v), 0.62% Tween80, 0.05 $(NH4)2SO4$, 0.06 K_2HPO_4, 0.02 KH_2PO_4, 0.1 $MgSO_4 \cdot 7H_2O$ and 0.01 L-cysteine.

DISCUSSION

Our result clearly demonstrates the efficiency of experimental design to screen the effective factor and find the optimal medium composition for β-galactosidase production as its productivity was improved by 14.60 folds, compared to non-optimized medium. This paper is one of several research works that confirms the usefulness of experimental design in facilitating medium optimization during the past decade (Altaf et al., 2006; Coelho et al., 2011; Bhatt and Srivastava, 2008; Hsu et al., 2005; Ren et al., 2008).

Lactose has been reported to be the most preferable substrate for β-galactosidase production by various microorganisms, and the response on lactose concentration in β-galactosidase production by *L. fermentum* CM33 is not out of expectation. The maximum productivity was obtained at lactose concentration of 3.23% (w/v) and the lower enzyme activity was found at either the lower and higher concentration. Hsu et al. (2005) reported that 4% (w/v) lactose gave the highest β-galactosidase productivity by *Bifidobacterium longum* CCRC15708, while the excess amounts of 10% (w/v) lactose caused the reduction of enzyme activity. Culturing the strain in a medium containing lactose of 5% (w/v) or higher, possibly repressed the β-galactosidase biosynthesis by *Kluyveromyces fragilis*, due to the increased concentration of internally released glucose (Fiedurek and Szczodrak, 1994).

Considering the organic nitrogen sources used in this experiment, tryptone is the only organic nitrogen source that significantly exhibited the positive effect on β-galactosidase production, while beef extract and yeast extract showed a significant negative influence, and peptone was assumed to be a non-significant effective nutritional factor. This result confirms the specific requirement of tryptone as nitrogen source for *L. fermentum* CM33 and corresponds with many previous studies which stated that nitrogen sources affect the microbial biosynthesis of β-galactosidase, and different types of nitrogen source generally show the effectiveness in different microbial strains (Shaikh et al., 1997). Tryptone is specially formulated of enzymatically digested casein and it has been used as an organic nitrogen source for various investigations on microbial enzyme production. The higher productivity of thermostable β-amylase and β-galactosidase by *Bacillus* sp. JF strain (Jin et al., 2001) and *Bacillus subtilis* (Konsoula and Liakopoulou-Kyriakides, 2007) was obtained, when using tryptone as an organic nitrogen source. In addition, the supplementation of tryptone in soymilk enabled *Bifidobacterium infantis* to grow faster and reach its maximum population in a shorter cultivation time of 24 h (Chou and Hou, 2000).

In the case of Tween80, this component clearly exhibited the positive effecter in β-galactosidase production by *L. fermentum* CM33 and the predicted optimal concentration is 0.6% (w/v). Tweens are well known, non-ionic surfactants, widely used in pharmaceutical industries, due to their unique characteristics, including their effectiveness at low concentrations, relative low toxicity levels and the fact that they usually do not interact with active ingredients. All of the above have made them almost indispensable in the biotechnological industry over the past two decades (Wang et al., 2008). It has been suggested that this surfactant is capable of promoting cell uptake and the exit of compounds through the modification of the plasma membrane permeability (Huot et al., 1996), and has been included in the composition of MRS medium (a selective medium for lactic acid bacteria) (De Man et al., 1960). Our result confirms the essential requirement of Tween80 for the growth of lactic acid bacteria, particularly in *Lactobacilli*. Tween80 is reported as the positive effecter on growth and lactic acid production by *Lactobacillus plantarum* LMISM6 (Coelho et al., 2011) and *Lactobacillus delbrueckii* NCIM 2025 (Bhatt and Srivastava, 2008). Besides this, surfactant also positively influenced the production of extracellular enzymes, such as extracellular proteases produced by *Bacillus* sp. L21 (Tari et al., 2006) and alkalostable lipase by *Burkhoderia cenocepacia* ST8 (Lau et al., 2011). However, the addition of Tween80 to rice bran-soybean milk medium did not affect the phytase production by *Mitsuokella jalaludinii*. It has been suggested that the different effect of Tween80 may be caused from the differences in the cell wall structure of fungi and bacteria (Lan et al., 2002).

The optimal pH for β-galactosidase production by *L. fermentum* CM33 was proven to be 6.5, and our result indicates that the β-galactosidase activity clearly depends on bacterial growth. The previous report support the strong influence of pH on the growth of *L. fermentum* IMDO 130101 at 30°C, an optimal pH was 5.5, while the lower cell densities were observed at pH 3.5 and 7.5 (Vrancken et al., 2008). The initial pH of 6.5 was also reported to be an optimal pH for β-galactosidase production at ca 15.87 U/ml by *B. longum* CCRC 15708; however, the relationship to bacterial biomass was not mentioned (Hsu et al., 2005).

Cost reduction of the optimized culture medium used in *L. fermentum* CM33 β-galactosidase production by deletion of some expensive organic nitrogen sources, and replacing with cheaper organic nitrogen source as skimmed milk was achieved. There are some previous reports which demonstrate the replacing of high cost ingredients in culture medium especially yeast extract, peptone and beef extract with low cost ingredient such as the replacement of yeast extract by corn steep liquor in culture medium for lactic acid production by *Enterococcus faecalis* RKY1 (Wee et al., 2008), and replaced peptone and yeast extract by red lentil flour in the medium for single step fermentation of starch to L(+) lactic acid by *Lactobacillus amylophilus* GV6 (Altaf et al.,

2006). A new discovered medium formulation obtained from this work will be very useful for application in large scale production of enzyme.

Conclusion

The experimental design was proven to be successful in its application for the optimization of the medium composition used in β-galactosidase production by *L. fermentum* CM33, and improved 10.60 times, when compared to the non-optimized medium. The cheaper and simpler medium was also achieved. The higher production of β-galactosidase by the strain CM33 is deemed to be useful in the preparation of enzymes for further studies and also for the application in prebiotic galactooligosaccharide synthesis.

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