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Full Length Research Paper

Effect of saccharides on growth, sporulation rate and δ-endotoxin synthesis of *Bacillus thuringiensis*

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Effect of monosaccharides (glucose, fructose and galactose) and disaccharides (sucrose, lactose and maltose) as carbon sources on growth and sporulation rate of *Bacillus thuringiensis* MPK13 as well as δ-endotoxin production was investigated using 400 ml shake flasks culture. Substantially high growth and sporulation rate were obtained in cultivation using glucose and sucrose. Fructose, galactose, lactose and maltose were also able to support growth, but failed to enhance high sporulation rate. In general, high sporulation rate was related with high growth rate and high viable cell count (>1.5 x 10^{12} cfu/ml). However, the presence of appropriate quantity of glucose was required to trigger δ-endotoxin formation. The presence of δ-endotoxin with molecular weight of 130 kD assayed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), was only detected after 48 h of cultivation using 8 g/L glucose as the sole carbon source. Although good growth with high spore production was obtained in cultivation using a mixture of glucose (4 g/L) with other sugars, the spores produced contained no endotoxin. The existence of δ-endotoxin was also observed in cultivation using a mixture of various sugars (fructose, sucrose, maltose and lactose) with 8 g/L glucose, indicating that high glucose (> 8 g/L) must be present in the culture to trigger δ-endotoxin formation in the spores of *Bacillus thuringiensis* MPK13.

Key words: *Bacillus thuringiensis,* saccharides, cell growth, sporulation rate, δ-endotoxin.

INTRODUCTION

Bacillus thuringiensis is a soil Gram-positive and spore-forming bacterium distinguishable from the closely related species *Bacillus cereus* by its capability to form large proteinaceous crystalline inclusions that emerge during sporulation (Buchanan and Gibbon, 1974). The parasporal insecticidal crystal proteins (ICPs) known as δ-endotoxin and produced by *B. thuringiensis* had been proven to be effective in controlling some agricultural pests and vectors of diseases (Li-Ming et al., 2008; de Maagd et al., 2003; Schnepf et al., 1998; Aronson, 1993).

The medium for industrial production of *B. thuringiensis* is basically composed of complex carbon and nitrogen sources. Complex medium containing fishmeal and corn steep liquor (Kumar et al., 2000; Saalma et al., 1983), gruel based medium (Zouari and Jaoua, 1999), fish meal (Zouari et al., 2002) and broiler litter extracts (Adams et al., 1999) have been reported for the production of *B. thuringiensis* spores for subsequent use as bio-insecticides. Recently, the use of seawater as medium for the production of *B. thuringiensis* bioinsecticides has also been reported (Ghribi et al., 2007). In order to formulate a complex medium for *B. thuringiensis*, the basic knowledge of *B. thuringiensis* growth behaviour on different monosaccharides and disaccharides as carbon sources and also their effect on cell morphology shall be generated.The effects of glucose on *B. thuringiensis* production have been reported by many researchers

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(Berbert-Molina et al., 2008; Keshavarzi et al., 2005; Bhatnagar, 1996; Mignone and Avignone-Rossa, 1996; Muhammad and David, 1987; Maruyama et al., 1980; Sherrer et al., 1973; Yousten and Rogoff, 1969). However, information on *B. thuringiensis* metabolism, cell growth, sporulation rate and toxin production during cultivation using various sugars are lacking. The effect of basic sugars as carbon sources is crucial as carbon catabolite repression is shown to be one of the major limitations of δ-endotoxin production by fermentation (Ghribi et al., 2007; Brückner and Titgemeyer, 2002; Zouari et al., 1998, 2002).

The objective of this study was to investigate the effect of monosaccharides and disaccharides in cultivation medium on growth and sporulation rate of *B. thuringiensis* MPK13. Variation in cell metabolism, cell morphology and toxin production in relation to the utilization of different sugars, individual and as a mixture, was also studied.

MATERIALS AND METHODS

Microorganism and inoculum preparation

The bacterium, *B. thuringiensis* MPK13, obtained from the culture collection unit at Malaysian Palm Oil Board (MPOB), Bangi, Selangor, Malaysia, was used throughout this study. This bacterium was isolated from soil in oil palm plantation and was cultured three times from the dead lepidopteran larvae. The bacterium was grown on nutrient agar and stored at 4°C as a stock culture prior to use in inoculum preparation. A loop of colony from the stock was inoculated into 20 ml of nutrient broth in 100 ml Erlenmeyer flask. The flask was incubated at 30°C in rotary orbital shaker agitated at 150 rpm for 12 h. This culture was used as a standard inoculum for all fermentations.

Medium and fermentation

The basal medium proposed by Yousten and Rogoff (1969), which has been used to enhance growth and sporulation rate of *B. thuringiensis* (Içgen et al., 2002; Jaquet et al., 1987), was used in this study. This basal medium consisted of (g/L): $(NH_4)_2SO_4$, 2.0; $K_2HPO_4.3H_2O$, 0.5; $MgSO_4.7H_2O$, 0.2; $MnSO_4.4H_2O$, 0.05; $CaCl_2.2H_2O$, 0.08; yeast extract, 2.0. To investigate the effect of individual sugar, different types of sugar (glucose, fructose, lactose, sucrose, maltose and galactose) at a concentration of 8.0 g/L were added to the basal medium as a carbon source. The effect of using a mixture of sugar was also performed in two sets of experiment. The first experiment was conducted by adding 4 g/L sugar (fructose, lactose, sucrose, maltose or galactose) with 4 g/L glucose. In the second experiment, sugar and glucose concentrations was increased to 8 g/L, respectively. The initial pH of the medium was adjusted in the range from 6.5 to 7.0 using 1 M HCl. The sterile culture medium (180 ml) in a 1000 ml Erlenmeyer flask was inoculated with 20 ml of inoculums to give the inoculum size of 11% (v/v). The flasks were incubated at 30°C for 48 h on a rotary shaker agitated at 200 rpm.

Analytical procedures

During the cultivation, culture samples were collected at 0, 4, 8, 12, 24, 36, 40 and 48 h for analysis. The culture samples were serially diluted using 0.85% (v/v) saline buffers and plated on nutrient agar (NA) plates. The plates were incubated at 30°C for 48 h and the number of single colony developed was counted and expressed in CFU/ml. For spore count, the culture samples were heated at 80°C for 15 min to kill the vegetative cells before serially diluted and plated on NA plates. The plates were incubated at 30°C for 48 h and the number of single colony developed was counted and expressed as spore/ml.

A phase contrast microscope (Leica DM 3000) was used to examine the cells morphology. Vegetative cells and spores were distinguished by the refractive nature of the spore. The cells length was measured using the Leica microscopic software (Morphology 5.1). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, conducted using the method proposed by Laemmli (1970), was used for the detection of 130 kD δ-endotoxin formed in the spores.

Kinetic data analysis

The following simplified batch fermentation kinetic models for cell growth based on logistic equation, which have been described elsewhere (Ariff et al., 1997), were used to evaluate the growth kinetics of *B. thuringiensis* MPK13; cell growth was estimated using the formula:

$$dX/dt = [\mu_{max}(1-X/X_{max})] \quad (1)$$

Where, X is the cell concentration (g/L), X_{max} is the maximum cell concentration (g/L), μ_{max} is maximum specific growth rate (1/h) and t is the fermentation time (h).

The growth kinetic model (Equation 1) was fitted to the experimental data by non-linear regression with a Marquadt algorithm using SIGMA PLOT 10 computer software. The model parameter values were first evaluated by solving Equation 1 and then the computer program was used as a search method to minimize the sum of squares of the differences between the predicted and measured values (Mohamed et al, 2012; Weiss and Ollis, 1980).. The predicted values were then used to simulate the profiles of cell during the fermentation.

Determination of saccharides

High performance liquid chromatography (HPLC) with carbohydrate analysis column (BioRad) was used for the determination of saccharides (glucose, fructose, galactose, sucrose, maltose and lactose) in the culture as described elsewhere (Victorita et al., 2008). The mobile phase used was 75% CH_3CN in water at a flow rate of 0.6 mL/min. The column temperature was set at 85°C. A series of sugar calibration standards at 0.5%, 1.0%, 1.5% and 2.0% (w/v) in HPLC grade water was get prepared to acquire a calibration curve for each sugar of interest. Retention time for peaks of glucose, fructose, galactose, sucrose, maltose and lactose were obtained at 6.8 min, 5.5 min, 6.7 min, 9.4 min, 11.29 min and 10.9 min, respectively.

RESULTS

Time course of *B. thuringiensis* MPK13 cultivation in mono and disaccharides

The profile of cell growth and sporulation during *B. thuringiensis* MPK13 cultivation in two different types of saccharides, monosaccharides (glucose) and disaccharides (sucrose) are shown in Figures 1 and 2.

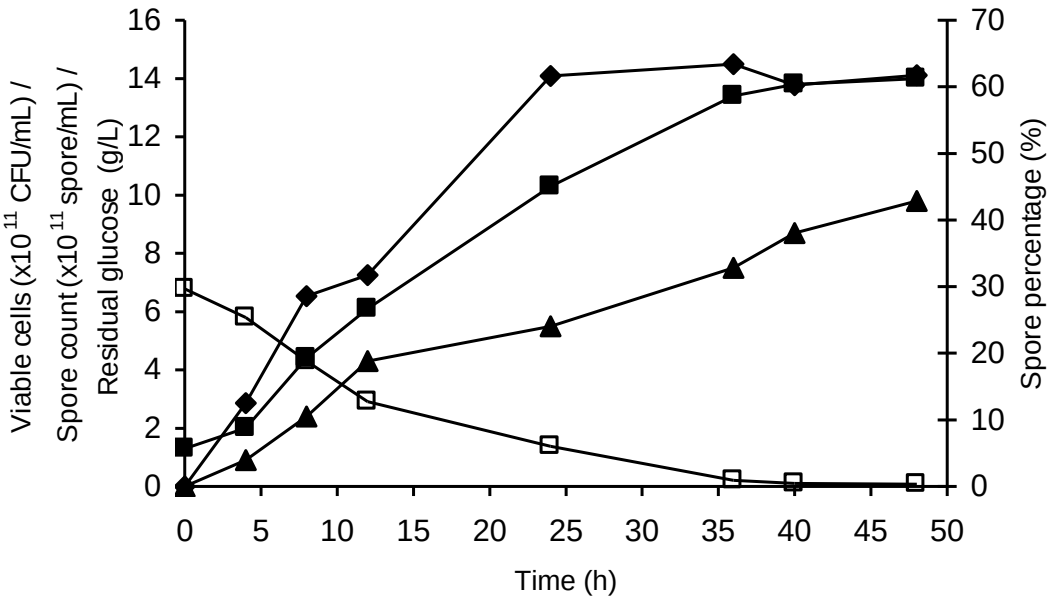

Figure 1. Typical time course of *B. thuringiensis* MPK13 cultivation in medium containing glucose. ■, viable cell; ▲, spore count; □, residual glucose; ◆, percentage of sporulation (%).

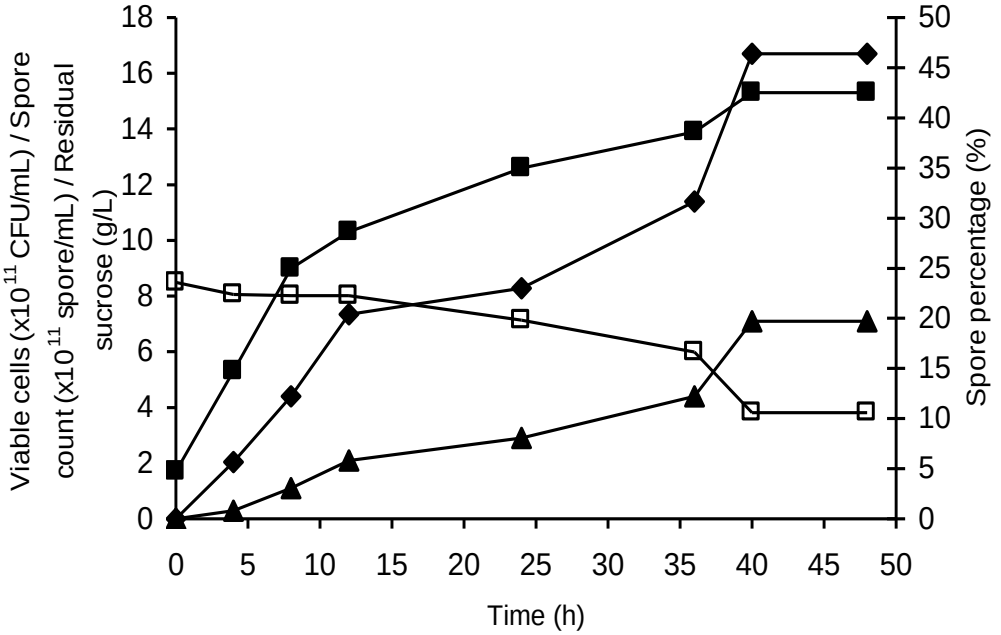

Figure 2. Typical time course of *B. thuringiensis* MPK13 cultivation in medium containing sucrose. ■, viable cell; ▲, spore count; □, residual sucrose; ◆, percentage of sporulation (%).

During the cultivation in 8 g/L glucose (Figure 1), cell concentration increased drastically from 4 to 24 h and a stationary growth phase was achieved after 24 h of cultivation. During active growth (0 to 24 h), a drastic reduction in residual glucose concentration was also observed. Spore formation rate was initially slow from 0 to 12 h and increased rapidly from 12 to 36 h. In cultivation using sucrose, growth was also rapidly increased from 0 to 24 h of cultivation using sucrose and reached a stationary phase after 36 h. Only a slight increase in spore count (26%) was observed during the stationary growth phase (Figure 2). However, residual glucose concentration in the culture for cultivation using sucrose was very low (< 0.1 g/L).

Table 1. Effect of different saccharides, individual and a mixture, on growth and sporulation rate of *B. thuringiensis.*

Saccharide	Final cell concentration (CFU/ml)	Final spore concentration (spore/ml)	Percentage of sporulation (%)	Specific growth rate (h^{-1})
Glucose (8 g/L)	1.4×10^{12}	9.8×10^{11}	70	0.17
Fructose (8 g/L)	1.1×10^{12}	4.3×10^{11}	39	0.1
Galactose (8 g/L)	4.6×10^{11}	2.0×10^{11}	43	0.07
Sucrose (8 g/L)	1.5×10^{12}	7.1×10^{11}	47	0.31
Lactose (8 g/L)	1.1×10^{12}	3.3×10^{11}	30	0.09
Maltose (8 g/L)	5.9×10^{11}	2.8×10^{11}	47	0.08
Glucose (4 g/L) + Galactose (4 g/L)	1.0×10^{12}	4.4×10^{11}	44	0.13
Glucose (4 g/L) + Fructose (4 g/L)	1.3×10^{12}	4.5×10^{11}	35	0.12
Glucose (4 g/L) + Sucrose (4 g/L)	1.6×10^{12}	7.3×10^{11}	46	0.1
Glucose (4 g/L) + Lactose (4 g/L)	1.2×10^{12}	5.4×10^{11}	45	0.11
Glucose (4 g/L) + Maltose (4 g/L)	1.4×10^{12}	6.7×10^{11}	48	0.19
Glucose (8 g/L) + Galactose (8 g/L)	1.1×10^{12}	4.5×10^{11}	41	0.1
Glucose (8 g/L) + Fructose (8 g/L)	1.6×10^{12}	5.7×10^{11}	35	0.12
Glucose (8 g/L) + Sucrose (8 g/L)	2.0×10^{12}	1.2×10^{12}	60	0.2
Glucose (8 g/L) + Lactose (8 g/L)	1.3×10^{12}	5.7×10^{11}	44	0.11
Glucose (8 g/L) + Maltose (8 g/L)	1.5×10^{12}	7.0×10^{11}	47	0.11
Basic medium (without saccharides)	4.9×10^{11}	0.3×10^{11}	6	0.07

Effect of different individual saccharides on growth and sporulation of *B. thuringiensis* MPK13

Effect of different saccharides (glucose, fructose, galactose, sucrose, lactose and maltose) as individual and a mixture, on growth and sporulation performance of *B. thuringiensis* MPK13 are summarized in Table 1. Growth of *B. thuringiensis* MPK13 and percentage of sporulation was greatly influenced by the different types of saccharides used in the medium formulation. For individual saccharides, the highest cell number (1.5×10^{12} cfu/ml) was obtained at 48 h of cultivation in medium containing sucrose, followed by cultivation with glucose (1.4×10^{12} cfu/ml). Medium containing fructose and lactose recorded equivalent cell number with values of about 1.1×10^{12} cfu/ml. Reduced growth was observed in cultivation with maltose and galactose, with a final cell concentration of 5.9×10^{11} cfu/ml and 4.6×10^{11} cfu/ml, respectively.

Contrary to growth, final spore number obtained during the cultivation was higher in medium containing glucose (9.8×10^{11} spore/ml) as compared to cultivation with sucrose (7.1×10^{11} spore/ml). Reduced sporulation ($<3.5 \times 10^{11}$ spore/ml) was observed in cultivation with galactose, lactose and maltose. Consequently, high number of spore obtained in cultivation with glucose contributed to the highest percentage of sporulation (70%). For other individual carbon sources, sporulation rate was not more than 50%. Significant growth (4.9×10^{10} cfu/ml) was also obtained in cultivation using basal medium without the addition of sugar. The present of high amount of yeast extract as nitrogen source may also contain significant amount of carbohydrates to support growth of *B. thuringiensis* MPK 13 cells (Table 1).

The residual sugars remaining after 48 h of fermentation in various saccharides are also shown in Table 2. In the cultivation using glucose as the sole carbon source, residual glucose was not been detected after 48 h. From Figure 1A, it can also be seen that 76% of glucose supplied to the medium was consumed after 12 h and all glucose was consumed after 24 h. In cultivation using fructose with an initial concentration of 9.15 g/L, only 0.56 g/L fructose still remained in the culture after 48 h. Similar to cultivation with sucrose, residual glucose (0.07 g/L) was also detected in cultivation using maltose at 48 h. All maltose (8.69 g/L) supplied was hydrolysed and consumed by B*. thuringiensis* MPK13 after 48 h, indicating that the bacterium was capable to break maltose into glucose. Galactose and lactose were hardly used by *B. thuringiensis* MPK13 for growth, as about 8 g/L of these sugars still remained in the culture after 48 h. The highest specific growth rate (0.31 h^{-1}) was obtained in cultivation using sucrose, followed by cultivation using glucose (0.17 h^{-1}). Reduced specific growth rate (0.10 h^{-1}) was obtained in cultivation using fructose and the specific growth rate for cultivation using other individual sugar

Table 2. Residual sugar (analysed by HPLC) in the culture of *B. thuringiensis* MPK 13 after 48 h of cultivation.

Saccharide	Residual concentration in the culture after 48 h of cultivation (g/L)					
	Glucose	Fructose	Galactose	Sucrose	Lactose	Maltose
Glucose (8 g/L)	0	-	-	-	-	-
Fructose (8 g/L)	-	0.56	-	-	-	-
Galactose (8 g/L)	-	-	8.03	-	-	-
Sucrose (8 g/L)	0.02	-	-	3.81	-	-
Maltose (8 g/L)	0.07	-	-	-	-	0.17
Lactose (8 g/L)	-	-	-	-	6.91	-
Glucose (4 g/L) + Fructose (4 g/L)	0	1.34	-	-	-	-
Glucose (4 g/L) + Galactose (4 g/L)	0	3.43	-	-	-	-
Glucose (4 g/L) + Sucrose (4 g/L)	0	-	-	2.93	-	-
Glucose (4 g/L) + Maltose (4 g/L)	0	-	-	-	-	2.37
Glucose (4 g/L) + Lactose (4 g/L)	0	-	-	-	2.54	-
Glucose (8 g/L) + Fructose (8 g/L)	0	3.98	-	-	-	-
Glucose (8 g/L) + Galactose (8 g/L)	0	-	7.98	-	-	-
Glucose (8 g/L) + Sucrose (8 g/L)	0	-	-	3.24	-	-
Glucose (8 g/L) + Maltose (8 g/L)	0	-	-	-	-	5.35
Glucose (8 g/L) + Lactose (8 g/L)	0	-	-	-	6.09	-

was less than 0.10 h^{-1} (Table 1).

Effect of a mixture of different saccharides and glucose on growth and sporulation rate of *B. thuringiensis* MPK13

Table 1 also shows the effect of a mixture of different saccharides and glucose on growth and sporulation rate of *B. thuringiensis* MPK13. In general, among the mixture of saccharides investigated, the highest growth and sporulation rate was obtained in a mixture of glucose and sucrose. In cultivation using a mixture of saccharides, growth and sporulation rate in higher saccharide concentration (8 g/L) was increased by about 20 and 65% as compared to low saccharide concentration (4 g/L), respectively. Significant increased in growth and sporulation rate were also observed in cultivation using saccharides with the addition of glucose. In all media containing glucose, the final cell concentration obtained was fairly high (>1.0 × 10^{12} cfu/ml). Although the use of galactose and lactose as the sole carbon source did not support growth of *B. thuringiensis* MPK13, the addition of glucose into these saccharides greatly enhanced cell growth. However, the final spore concentration and percentage of sporulation were not improved.

Table 2 shows the residual sugar after 48 h of cultivation of *B. thuringiensis* using a mixture of various saccharides and glucose. In all cases, glucose has been consumed after 48 h of cultivation. Similar with cultivations using individual sugar, galactose and lactose were hardly used by the bacterium. For other saccharides, substantial residual sugar concentration still remained in the culture after 48 h of cultivation, indicating that glucose was the preferred saccharide and was consumed at higher rates than other saccharides. This means that the readily utilizable monosaccharide is required to enhance growth and sporulation of *B. thuringiensis*. The use of glucose as individual carbon source at a concentration of 8 g/L was found sufficient to support growth at an intensive energy consuming growth phase, which is exponential phase. Limitation of energy supply, as early as 12 h of cultivation, was observed in cultivation with low initial glucose concentration (4 g/L). Thus, growth at log phase was restricted and sporulation rate was inhibited.

Effect of different saccharides on cell size and growth morphology of *B. thuringiensis*

Observation under phase contrast microscope showed that cultivation with lactose, sucrose, galactose and maltose contributed to shrivelled cells shape after 24 h (Figure 3). Table 3 shows the effect of different saccharides on cell length of *B. thuringiensis* MPK 3 and the existence of crystal protein. The length of *B. thuringiensis* MPK13 cells was increased by about two-fold during the cultivation in glucose and fructose from 24 to 48 h. Reduction in cell length by about 50%, from 24 to 48 h, was observed during cultivation lactose and galactose. During the cultivation in sucrose and maltose, the cell length was reduced by about 23 and 18%, respectively. In basal medium without the addition of carbon source, no increase in cell length was recorded. Similar to

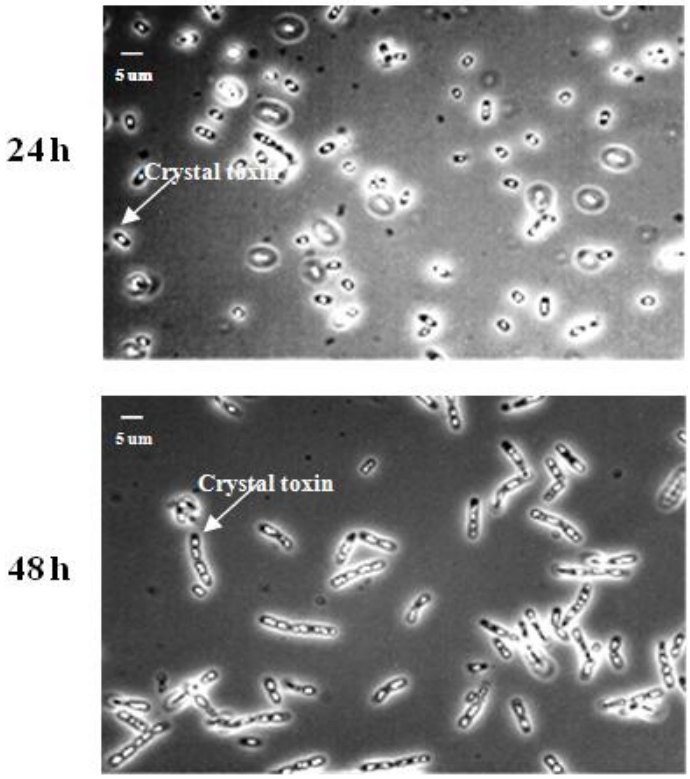

Figure 3. Microscopic observation of *B. thuringiensis* MPK13 cell in culture medium containing glucose.

Table 3. Changes in cell length of *B. thuringiensis* and the existence of crystal protein in the cell during cultivation in different sugars.

Carbon source	Average cell length (μM)		Increment rate (%)	Decrement rate (%)	Crystal protein
	24 h	48 h			
Glucose (8 g/L)	5.59 ± 1.46	8.08 ± 2.35	31	-	Yes
Fructose (8 g/L)	3.15 ± 0.9	5.59 ± 0.94	38	-	No
Lactose (8 g/L)	9.17 ± 2.24	4.52 ± 1.04	-	51	No
Sucrose (8 g/L)	4.02 ± 1.38	3.11 ± 0.70	-	23	No
Galactose (8 g/L)	11.79 ± 6.04	5.82 ± 5.82	-	51	No
Maltose (8 g/L)	6.73 ± 1.56	5.51 ± 2.1	-	18	No
Without carbon source	10.46 ± 0.7	10.97 ± 0.1	5	-	No
Nutrient Broth	8.24 ± 3.26	4.12 ± 1.67	-	50	No

cultivation in lactose and galactose, 50% reduction in cell length was observed during cultivation using nutrient broth. However, high production of mature spores and crystal protein was observed only in cultivation using glucose.

δ-Endotoxin synthesis during the fermentation

A typical example of SDS-PAGE analysis of crude protein extracted from the culture of *B. thuringiensis* MPK13 is shown in Figures 3 and 4, which contained protein with molecular size ranging from 17 to 170 kDa. For cultivation using individual saccharides, only the protein extracted from the cells cultivated in medium containing glucose harvested at 48 h of cultivation showed band of 130 kDa, which has similar size with cry genes (Figure 4). Cry genes with the size of about 130 kDa were the most common reported δ-endotoxin in *B. thuringiensis* (Sarrafzadeh et al., 2007; Andrews et al., 1985).

It is important to note that the protein extracted from cells cultivated using fructose, sucrose, lactose,

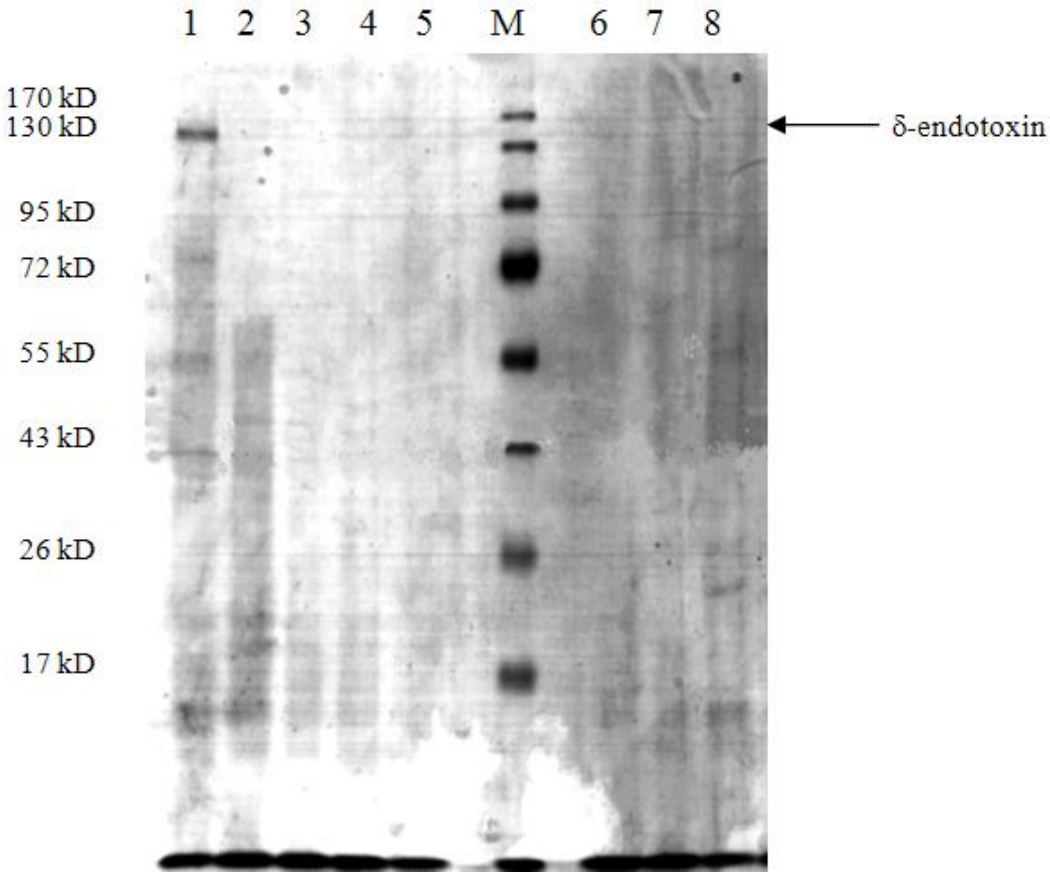

Figure 4. Determination of protein profiles of 130 kD δ-endotoxin *B. thuringiensis* MPK13 samples in individual saccharides culture by SDS-PAGE. Samples were applied to 10% polyacrylamide gel and stained with Coomassie brilliant blue. M, Standard marker; 1, medium with glucose; 2, medium with fructose; 3, medium with lactose; 4, medium with sucrose; 5, medium with galactose; 6, medium with maltose; 7, medium without any sugar; 8, nutrient broth.

galactose and maltose as an individual carbon source did not exhibit any toxin production. Toxin was also not detected in *B. thuringiensis* MPK13 cells obtained from the cultivation in basal medium without the addition of sugars. In medium containing a mixture of 4 g/L glucose and 4 g/L of other saccharides, δ-endotoxin was not detected in the cells of *B. thuringiensis* MPK13. However, when the concentration of glucose was increased to 8 g/L, a thick band of δ-endotoxin was recorded in medium containing a mixture of glucose and sucrose, glucose and maltose, and also glucose and fructose (Figure 5). δ-Endotoxin was not detected in the cultivation using a mixture of glucose and galactose, as well as glucose and lactose. These results indicate that the presence of glucose in the culture is required for the formation of δ-endotoxin. However, the presence of glucose in the medium using a mixture of saccharides at concentration lower than 8 g/L did not guarantee the formation of δ-endotoxin. Glucose at a sufficient level might be required to trigger δ-endotoxin formation in the spores of *B. thuringiensis* MPK13.

DISCUSSION

Results from this study demonstrate that the type of saccharides used as carbon source greatly influences the growth of *B. thuringiensis*, sporulation rate and toxin production. The importance of suitable carbohydrate sources and their concentration in promoting growth and sporulation in *B. thuringiensis* has also been reported by several researchers (Keshavarzi et al., 2005; Zuoari et al., 2002; Sherrer, 1973). Among the saccharides tested, glucose was the preferred carbon source to support growth of *B. thuringiensis* MPK 13 cells and spores as well as toxin formation. Glucose is needed at the very early stage of spore germination as the endogenous energy sources for 3-phosphoglycerate, which contribute to the formation of ATP required for germination of spores (Setlow and Konberg, 1970). The breakdown of glucose through the Kreb's cycle and oxidative phosphorylation produce ATP. Spore formation in *B. thuringiensis* is an elaborate and energy intensive process and this is the possible explanation for higher sporulation rate obtained in cultivation using glucose.

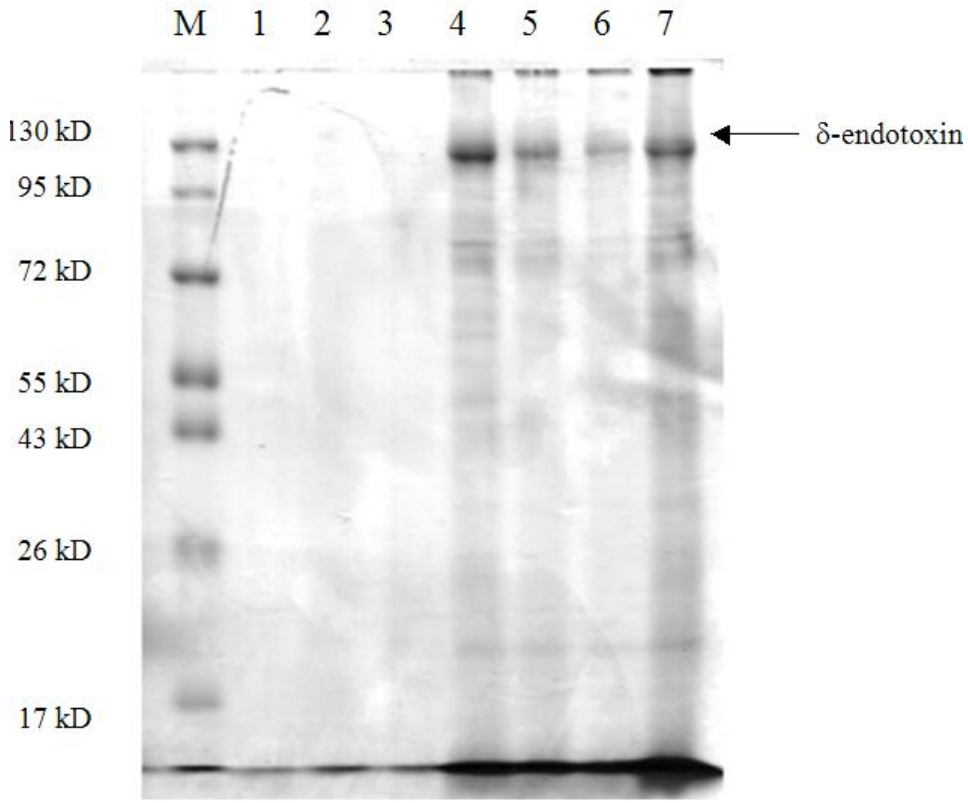

Figure 5. Determination of protein profiles of 130 kD δ-endotoxin *B. thuringiensis* MPK13 samples in mixture of glucose and saccharides at 8 g/L by SDS-PAGE. Samples were applied to 10% polyacrylamide gel and stained with Coomassie brilliant blue. M, Standard marker; 1, nutrient broth; 2, basal medium without saccharides; 3, glucose + galactose; 4, glucose + sucrose; 5, glucose + fructose; 6, glucose + lactose; 7, glucose + maltose.

Appropriate glucose concentration in the culture medium, however, needs to be selected to avoid occurrence of catabolite repression. Catabolite repression refers to inactivation of certain sugar-metabolizing operons, such as lac, in favour of glucose utilization when glucose is the predominant carbon source in the environment of the cell. Excess carbon source in the medium will not only cause catabolite repression, but also repress the expression of the spo0A fusion gene which triggers the spore production. It is likely that reduced expression of the spo0A gene in the presence of excess glucose at an early stage of sporulation causes the repression of sporulation (Yamashita et al., 1989). The importance of appropriate glucose concentration for the production of δ-endotoxin has been reported by several researchers (Scherrer et al., 1973; Yousten and Rogoff 1969). They claimed that the optimal glucose concentration for enhancement of δ-endotoxin ranged from 6 to 8 g/L, which corresponded well to the concentration used in this study (8 g/L). SDS-PAGE analysis to confirm the existence of 130 kD obtained in medium containing glucose is in agreement with high sporulation rate and larger size of *B. thuringiensis* cell. This is also related with the existence of crystal protein. The production of crystalline protein in the culture gives early indication of the production of δ-endotoxin. Since the insecticidal activity of *B. thuringiensis* is based on the crystalline protein or the parasporal bodies, high yields of δ-endotoxin is desirable (Scherrer et al., 1973).

Although sucrose individually enhanced growth of *B. thuringiensis* MPK13 as shown by high yield and high specific growth rate, it did not contribute to high sporulation rate and δ-endotoxin production. Zuoari et al. (2002) claimed that improvement in sporulation rate of *B. thuringiensis* and δ-endotoxin production did not directly correlate with the enhancement of cell growth rate. High growth rate has a synergistic effect with energy utilisation in *Bacillus* spp. (Dephamphilis and Hanson, 1969). However, the relationship between high specific growth rate and sporulation has not been elucidated in detail. High maximum specific growth rate (0.31 h^{-1}) of *B. thuringiensis* MPK13 obtained in cultivation with sucrose did not enhance sporulation rate and production of δ-endotoxin. The ability of *B. thuringiensis* to grow in disaccharides (sucrose and maltose) indicated that the bacterium might secrete an enzyme that is capable to break disaccharide into monosaccharide. This result indicated that *B. thuringiensis* MPK13 may have the

ability to produce enzyme sucrase for the degradation of sucrose to glucose**.**

Galactose was reported to inhibit the binding of *B. thuringiensis* crystal toxin with glycolipids which acts as receptors for activation of the toxin *in vivo* (Joel et al., 2005). This phenomenon may be one of the possible explanations for reduced sporulation rate and inability to form toxin in the cultivation of *B. thuringiensis* in galactose. The information obtained from this study is therefore, very useful in the formulation of medium for large-scale production of *B. thuringiensis* spore with endotoxin for use as biopesticides. It is very clear that glucose at appropriate concentrations must be present in the medium formulation to enhance growth, sporulation rate and the formation of toxin. Other carbon sources may be added into the medium to support growth and partly to enhance sporulation, but glucose must be present to trigger endotoxin formation.

Conclusion

Results obtained from this study demonstrate the importance of glucose for growth, sporulation, cell morphology and also on the production of δ-endotoxin during the cultivation of *B. thuringiensis*. Adequate quantity of glucose supplied in the culture medium is required to promote high cell growth, sporulation and formation of δ-endotoxin in spore. Glucose also contributes to better cell morphology and crystalline protein production. However, additional or less amount of glucose in the culture medium may lead to the repressive regulation of δ-endotoxin caused by an increase in the concentration of readily assimilated carbon sources.

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