Acta Scientiarum
http://www.uem.br/acta
ISSN printed: 1806-2563
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v38i4.28704

Utilization of low-cost substrates for the production of nystose by *Bacillus subtilis* natto cct 7712

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ABSTRACT. Current analysis describes the capacity of *Bacillus subtilis* natto CCT 7712 to produce high amounts of nystose by low-cost substrates available in Brazil, such as commercial sucrose, sugarcane molasses and sugarcane juice. Optimization resulted in a maximum production of 179.77 g L^{-1} of nystose, averaging 7.49 g L^{-1} $hour^{-1}$ of productivity and a 71.73% yield in a medium with 400 g $L^{-1}$of commercial sucrose and 0.8 g L^{-1} of $MnSO_4$. Fermentations with sugarcane molasses and sugarcane juice also resulted in a satisfactory production reaching 97.93 and 42.58 g L^{-1} nystose, respectively. High nystose production in a medium with sugarcane derivatives suggests submerged fermentation with *Bacillus subtillis* natto CT 7712 as a promising strategy to produce nystose at industrial level.

Keywords: *Bacillus subtilis* natto CT 7712, commercial sucrose, nystose, sugarcane juice, sugarcane molasses.

Utilização de substratos de baixo custo para produção de nistose por *Bacillus subtilis* natto CCT 7712

RESUMO. Este estudo descreve a capacidade do *Bacillus subtilis natto* CCT 7712 para produzir quantidades elevadas de nistose usando sacarose comercial, melaço de cana-de-açúcar e caldo de cana-de-açúcar que constituem substratos de baixo custo e gerados em grande quantidade no Brasil. A optimização resultou na produção máxima de 179,77 g L^{-1} de nistose, correspondente a 7,49 g L^{-1} $hour^{-1}$ de produtividade e um rendimento de 71,73%, em meio contendo 400 g L^{-1} de sacarose comercial e 0,8 g L^{-1} de $MnSO_4$. As fermentações utilizando melaço de cana e caldo de cana também resultaram em produção satisfatória atingindo 97,93 e 42,58 g L^{-1} de nistose, respectivamente. A produção elevada de nistose obtida em meio contendo derivados da cana-de-açúcar sugere a fermentação submersa utilizando o *Bacillus subtilis* natto CT 7712 como uma estratégia promissora para sintetizar nistose para uso industrial.

Palavras-chave: *Bacillus subtilis* nattoCT 7712, sacarose comercial, nistose, caldo de cana, melaço de cana.

Introduction

Owing to growing demands for natural, healthy and low-calorie food, large numbers of alternative sweeteners emerged since the early 1980s, among which fructooligosaccharides (FOS) may be underscored. They represent an important source of prebiotic compounds widely used as ingredients in functional foods (Patel & Goyal, 2011). Several studies have shown that FOS may stimulate the *Bifidobacterium* growth in the human colon, help gut absorption of calcium and magnesium and decrease the plasma levels of phospholipids, triglycerides and cholesterol. Indeed, FOS have low caloric rates and anticariogenia properties and are useful in the formulation of diabetic products (Mussatto & Mancilha, 2007). Further, since 295.5 million euro were earned on the prebiotics market in 2008 and 766.9 million euro are estimated for 2015, an extraordinary market growth for prebiotics foods has been predicted (Morris & Morris, 2012). In the case of nystose, the commercial value is 39 dollars for 25 mg, corresponding to US$ 1.56 g^{-1}.

FOS are oligosaccharides of fructose containing a single glucose moiety in which fructosyl units are bound at the β (2→1) position of a sucrose molecule (Yun, 1996). Indeed, a particular branched structure in which fructosyl units are bound at β (2→6) position of sucrose molecule could be also found in FOS (Lim, Lee, Kang, Park, & Kim, 2007). Nystose is a tetrasaccharide formed by two fructosyl units linked in position β (2→1) of sucrose. Due to its chemical structure, nystose has anticariogenic properties and approximately possess about 30% the sweetness of sucrose and are largely applied in the food industry in replacement of conventional sugar

(Ikeda, Kurita, Hidaka, Michalek, & Hirasawa, 1990).

FOS are industrially produced from sucrose by microbial fructosyltransferases derived from several fungi and bacteria (Nemukula, Mutanda, Wilhelmi, & Whiteley, 2009; Prata, Mussatto, Rodrigues, & Teixeira, 2010; Patel & Goyal, 2011; Belghith, Dahecha, Belghithb, & Mejdouba, 2012; Chen, Sheu, & Duan, 2014; Ganaie, Rawatb, Wania, Gupta, & Kango,2014). *Bacillus subtilis* natto is a member of *B. subtilis* species used in the manufacture of natto, a traditional food in Japan. The microorganism has proved to be a good producer of levan and FOS by expressing large amounts of enzymes involved in the sucrose metabolism (Shih,Chen, & Wu, 2010; Gonçalves, Mantovan, Ribeiro, Borsato, & Celligoi, 2013; Silva, Borsato, & Celligoi, 2014).The production of FOS by microbial fermentation occurs through the activity of β-fructosyltransferase and β-frutofuranosidases with three enzymatic reactions: (1) polymerization, in which fructose units are linked to a fructan polymer; (2) hydrolysis, in which sucrose is split into fructose and glucose; and (3) transfers of fructose to an acceptor forming an oligosaccharide (Hijum, 2003).

Low yield (55–60%) is themain problem of commercial FOS production by transfructosylation (Yun, 1996).Consequently, the search for new substrates and economically viable biotechnological processes for the industrial production of FOS is still necessary to obtain higher yield and productivity. Further, the political priorities of a sustainable society have pointed towards the use of renewable resources, such as residual agricultural biomass and wastes, which may be transformed into valuable biomolecules (Angenent, Karim, Al-Dahhan, & Domiguez-Espinosa, 2004). These aspects have enhanced studies on alternative media in fermentation processes. Furthermore, resources are very cheap and allow the production of valuable main components and supplements of culture media metabolized as carbon and energy sources bymicroorganisms (Thomsen, 2005).

In this sense, the aim of this study was to evaluate the production of nystose by *Bacillus subtilis* natto CCT 7712 using low-cost substrates such as commercial sucrose, sugarcane molasses and sugarcane juice. These compounds are highly available in Brazil, which is the biggest producer of sugarcane in the world. To our knowledge, current analysis is one of the few to describe the high production of nystose by *Bacillus subtilis* nattoCCT 7712 using sugarcane derivatives.

Material and methods

Microorganism

Bacillus subtilis natto CCT 7712 was isolated from fermented soybeans, a Japanese food called 'natto', at the Department of Biochemistry and Biotechnology of the State University of Londrina, Londrina, Paraná State, Brazil, and identified by the Fundação André Tosello, Campinas SP Brazil. The strain was maintained in a medium containing (g L^{-1}): peptone 50, meat extract 30 and agar 20, and subcultured every 45 days and preserved at 4 °C.

Preparation of inoculum and fermentation of medium

The inoculum was prepared from stock culture by transferring two wire loops from solid medium to 125 mL Erlenmeyer flasks containing 25 mL of medium (g L^{-1}): sucrose 100; yeast extract 2; KH_2PO_4 2, $(NH_4)2SO_4$ 1 and $MgSO_4(7H_2O)$ 0.5. After incubation (150 rpm, 37°C, 48 hours), the medium was centrifuged at 9056 xg and cells were re-suspended in saline solution 0.9% (w v^{-1}). The inoculum containing 0.2 g L^{-1}cells was used in all fermentations. The fermentation occurred in 125mL Erlenmeyer flasks with 25 mL of medium (g L^{-1}): sucrose 2.0, yeast extract 2.0; KH_2PO_4 1.0, $(NH_4)2SO_4$ 3.0, $MgSO_4(7H_2O)$ 0.6, $MnSO_4$ 0.2. The pH was set at 7.7 and the flasks were incubated under orbital shaking at 230 rpm, as previously described (Silva et al., 2014). Temperature, incubation period and concentration of sucrose were adjusted following statistical design (Table 1). In experiments with molasses and sugarcane juice, the total sugar of the compounds was previously quantified by Phenol-Sulfuric Method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), with total sugar respectively at 166 and 570 g L^{-1}. Total sugar concentrations were set at 200, 300 and 400 g L^{-1} for comparison. In the case of medium composed of molasses commercial sucrose was added to adjust the final concentration. The pH was set at 7.7 and the flasks were incubated under orbital shaking at 230 rpm, for 24 hours. All fermentations were interrupted by centrifugation at 9056 × g for 15 min, at 4°C and the supernatant was used to determine nystose production.

Optimization of nystose production

Two experimental designs optimized the production of nystose by *Bacillus subtilis* natto CCT 7712. In the first one, the influence of sucrose (X_1), temperature (X_2) and incubation period (X_3) on nystose production was evaluated by Box-Behnken design (Hill & Lewicki, 2006). with three repetitions on the central point (Table 1). In the second experiment, the effect of concentration $(NH_4)_2SO_4$

(X_1), $MnSO_4$ (X_2), $ZnSO_4$ (X_3) and NaCl (X_4) was studied by experimental mixture design, with four repetitions on the central point (Table 3). The concentration of nystose was analyzed by HPLC (Shimadzu RID-10A) coupled to a refractive index detector, with Aminex Carbohydrate HPX-87C (300 x 7,8 mm, Biorad) column. The mobile phase was Milli-Q™, water at a flow rate of 0.6 mL min^{-1}. The column temperature was kept constant at 80°C. The nystose concentration of the supernatant was estimated by a nystose analytical standard (GP_3-666.58 Da- Sigma-Aldrich).

Table 1. Effect of sucrose concentration, temperature and incubation period on nystose production by *Bacillus subtilis* natto CCT 7712.

	Variables			Response
Assays	X_1 Sucrose (g L^{-1})	X_2 Temperature (°C)	X_3 Incubation period (hour)	Y_1 Nystose (g L^{-1})
1	400 (+)	55 (+)	24 (0)	121.60
2	400 (+)	35 (-)	24 (0)	142.97
3	200 (-)	55 (+)	24 (0)	37.63
4	200 (-)	35 (-)	24 (0)	42.57
5	400 (+)	45 (0)	36 (+)	107.29
6	400 (+)	45 (0)	12 (-)	109.54
7	200 (-)	45 (0)	36 (+)	27.33
8	200 (-)	45 (0)	12 (-)	23.56
9	300 (0)	55 (+)	36 (+)	72.61
10	300 (0)	55 (+)	12 (-)	69.20
11	300(0)	35 (-)	36 (+)	98.46
12	300 (0)	35 (-)	12 (-)	112.33
13	300 (0)	45 (0)	24 (0)	65.70
14	300 (0)	45 (0)	24 (0)	66.39
15	300 (0)	45 (0)	24 (0)	69.32

Determination of fermentation yield

Fermentation yield was determined by global mass balance based on the determination of nystose produced and depending on the weight of sucrose. Yield was calculated by considering the production of nystose as a function of the maximum theoretical yield, calculated by sucrose consumption.

Statistical analysis

Analysis of variance (ANOVA) and multiple regression were carried out at 5% probability ($p < 0.05$) by Statistica 9.0.

Results

The influence of sucrose concentration, temperature and incubation period for the production of nystose by *Bacillus subtilis* natto CT 7712 was evaluated by Box-Behnken design (Hill & Lewicki, 2006). The production of nystose ranged from 23.56 (run 8) to 142.97 g L^{-1} (run 2), indicating that the evaluated parameters affected the production of nystose (Table 1). The highest production (142.97 g L^{-1}) was obtained under the conditions of 400 g L^{-1} sucrose, 35°C and 24 hours of fermentation. Analysis of variance showed that sucrose (X_1) ($p = 0.0002$), temperature (X_2) ($p=0.0032$) and the interaction of temperature and incubation period (X_2X_3) ($p = 0.0460$) had a significant effect on nystose production (Table 2). Statistical analysis showed that sucrose concentration affected profoundly nystose production (Table 2). When runs 2 and 4 were compared (Table 1), the production of nystose was three times lower at 35°C during a 24 hours incubation period, and sucrose concentration was reduced from 400 (run 2) to 200 g L^{-1} (run 4).

Coefficient of determination (R^2) at 0.983 implied that 98% of sample variation could be explained by the model. The lack of fit was significant ($p = 0.0372$). However, the analysis of variance showed that the model is significant at 5% level, whilst the high rate of R^2 suggested that the model might be used for predictive purposes and was valid to describe the variations in production nystose. Results were used to fit a second-order polynomial equation (Equation 1). Only significant factors were considered to estimate the nystose production.

$$Y_1 = 67.1367 + 43.7888\, x_1 - 11.9113\, x_2 + 20.1379\, x_2^2 + 4.32\, x_2\, x_3 \quad (1)$$

where:
Y_1: nystose production,
x_1: sucrose concentration,
x_2: temperature and
x_3: incubation period.

Table 2. Analysis of variance of the effect of sucrose concentration, temperature and incubation period on nystose production by *Bacillus subtilis* natto CCT 7712.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	*F*-test	p value
(X_1) Sucrose (L)	15339.64	1	15339.64	4152.31	0.0002
Sucrose (Q)	4.32	1	4.32	1.17	0.3924
(X_2) Temperature (L)	1135.02	1	1135.02	307.24	0.0032
Temperature (Q)	1497.36	1	1497.36	405.32	0.0024
(X_3) Incubation period (L)	9.99	1	9.99	2.70	0.2418
Incubation period (Q)	2.83	1	2.83	0.76	0.4737
X_1X_2	67.49	1	67.49	18.26	0.0506
X_1X_3	9.06	1	9.06	2.45	0.2578
X_2X_3	74.65	1	74.65	20.20	0.0460
Lack of fit	287.99	3	96.00	25.98	0.0372
Pure error	7.39	2	3.69		
Total	18455.18	14			

Table 3. Experimental mixture design to evaluate the production of nystose by *Bacillus subtilis* natto CCT 7712.

Assays	Mixtures	Components				Responses	
		X_1 $(NH_4)_2$ SO_4 (g L^{-1})	X_2 $MnSO_4$ (g L^{-1})	X_3 $ZnSO_4$ (g L^{-1})	X_4 NaCl (g L^{-1})	Y_1 Nystose (g L^{-1})	Y_2 Nystose (g L^{-1} $hour^{-1}$)
1	(1; 0; 0; 0)	12	0	0	0	18.77	0.78
2	(0; 1; 0; 0)	0	0.8	0	0	179.77	7.49
3	(0; 0; 1; 0)	0	0	0.025	0	125.10	5.21
4	(0; 0; 0; 1)	0	0	0	35	31.72	1.32
5	(0.5; 0.5; 0; 0)	6	0.4	0	0	125.93	5.25
6	(0.5; 0; 0.5; 0)	6	0	0.012	0	119.99	5.00
7	(0.5; 0; 0; 0.5)	6	0	0	17.5	30.17	1.26
8	(0; 0.5; 0.5; 0)	0	0.4	0.012	0	125.23	5.22
9	(0; 0.5; 0; 0.5)	0	0.4	0	17.5	126.86	5.29
10	(0; 0; 0.5; 0.5)	0	0	0.012	17.5	51.98	2.17
11	(0.25; 0.25; 0.25; 0.25)	3	0.2	0.006	8.75	126.23	5.26
12	(0.25; 0.25; 0.25; 0.25)	3	0.2	0.006	8.75	131.32	5.47
13	(0.25; 0.25; 0.25; 0.25)	3	0.2	0.006	8.75	135.77	5.66
14	(0.25; 0.25; 0.25; 0.25)	3	0.2	0.006	8.75	133.28	5.55

The predicted rate for nystose production was 146 g L^{-1}(400 g L^{-1} sucrose, 35°C and 24 hours of fermentation). Three repetitions of the predicted conditions were performed to confirm the validity of the statistical model; average production was 140.06 g L^{-1} (Figure 1). This result did not show any significant difference from the predicted optimum rate ($p<0.05$). In summary, results indicated that the best condition for nystose production was 400 g L^{-1} of sucrose, 35°C and 24 hours of fermentation.

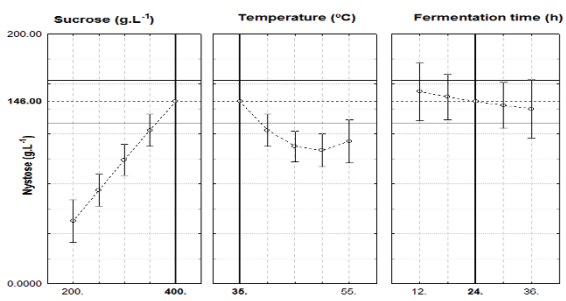

Figure 1. Optimum conditions for nystose production by *Bacillus subtilis* natto CCT 7712.

For the next step, the effect of $(NH_4)_2SO_4$, $MnSO_4$, $ZnSO_4$ and NaCl on nystose production was evaluated by using optimized conditions of the first experimental design, i.e., 400 g L^{-1} of sucrose, 35°C and 24 hours of fermentation. Table 3 shows production of nystose at 179.77 g L^{-1} (run 2) obtained when $MnSO_4$ was added to the fermentation medium, with a 71.73% yield. A satisfactory production was also observed with the addition of $ZnSO_4$, with 125.10 g. L^{-1} of nystose. On the other hand, the addition of $(NH_4)_2SO_4$ and NaCl significantly decreased the nystose production (Table 3). A 25% mixture of each salt produced an average 133.45 g L^{-1}, with a 53.17% yield (run 11-14). However, the yield obtained with $MnSO_4$ (run 2) alone was approximately 8 times higher when compared to $(NH4)_2SO_4$ (run 1), and 5 times higher when compared to NaCl (run 4).

Regression analysis revealed that, on a single basis, all tested salts had a positive effect on nystose production, with $MnSO_4$ featuring the strongest positive effect (Table 4). When the interaction of salts was analyzed, the mixture $(NH_4)_2SO_4/MnSO_4$ and $(NH_4)_2SO_4/ ZnSO_4$ had a strong positive effect, although mixtures $MnSO_4/ ZnSO_4$ and $ZnSO_4/$ NaCl had a negative effect on nystose production.

Table 4. Regression analysis for the effects of salts on nystose production by *Bacillus subtilis* natto CCT 7712.

Components	Coefficient	Error	*t*-test	p value
$(NH_4)_2$ $SO_4(X_1)$	18.77	4.04	4.63	0.01887
$MnSO_4$ (X_2)	179.77	4.04	44.42	0.00002
$ZnSO_4$ (X_3)	125.10	4.04	30.91	0.00007
NaCl (X_4)	31.72	4.04	7.83	0.00432
X_1X_2	106.64	19.82	5.37	0.01257
X_1X_3	192.22	19.82	9.69	0.00232
X_1X_4	19.70	19.82	0.99	0.39356
X_2X_3	-108.82	19.82	-5.48	0.01189
X_2X_4	84.46	19.82	4.26	0.02371
X_3X_4	-105.72	19.82	-5.33	0.01288
$X_1X_2X_3$	1985.92	214.71	9.24	0.00267

Based on experimental results, a canonical equation was developed to estimate nystose production (Equation 2):

$$y_1 = 18.77x_1 + 179.77x_2 + 125.1x_3 + 31.72x_4 + \\ +106.64x_1x_2 + 192.22x_1x_3 - 108.82x_2x_3 + \\ +84.46x_2x_4 - 105.72x_3x_4 + \\ +1985.92x_1x_2x_3 \quad (2)$$

where:

Y_1 is the response (nystose production) and x_1, x_2, x_3 and x_4 are $(NH_4)_2SO_4$, $MnSO_4$, $ZnSO_4$ and NaCl, respectively. Since the coefficient of determination (R^2) was 0.998, the proposed model may be used for prediction.

Figure 2 shows the contour plots for the interaction of salt effect on nystose production by

Bacillus subtilis natto CCT 7712 are showed on Figure 2. The interaction of equal proportions of $ZnSO_4$, $(NH_4)_2SO_4$ and $MnSO_4$ resulted in an estimated produced 200 g L^{-1} of nystose, while a production of 160 g L^{-1} was estimated using $MnSO_4$ to 100% of the mixture (Figure 2).

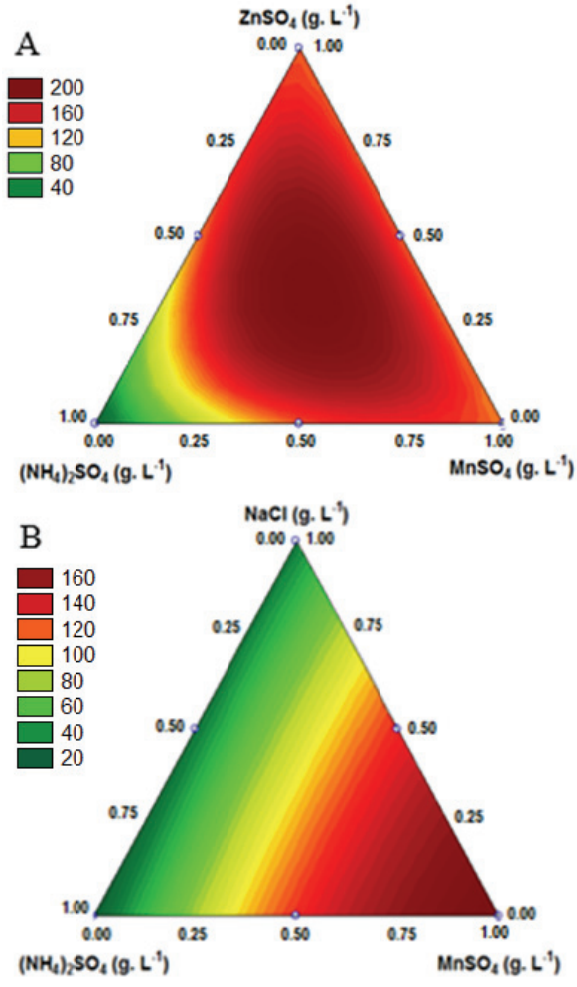

Figure 2. Effect of salt interaction on nystose production by *Bacillus subtilis* natto CCT 7712; (a) $ZnSO_4$, $(NH_4)_2$ SO_4 and $MnSO_4$ interaction; (b) NaCl, $(NH_4)_2$ SO_4 and $MnSO_4$ interaction.

Consequently, the optimization of nystose production by *Bacillus subtilis* natto CCT 7712 provided a maximum production of 179.77 g L^{-1}, corresponding to 7.49 g L^{-1} $hour^{-1}$ of productivity and a 71.73% yield in a medium with 400 g $L^{-1}$of commercial sucrose and 0.8 g L^{-1} of $MnSO_4$, at 35°C and 24 hours of fermentation.

The optimized conditions obtained from the experiments using commercial sucrose evaluated the nystose production in a medium of sugarcane molasses or sugarcane juice as carbon source. The highest nystose production was obtained when sugarcane juice was employed at a production of 97.93 g L^{-1}.In fermentations with sugarcane molasses, the highest production was 42.58 g $L^{-1}$of nystose. The concentration of total sugar in the medium influenced the production of nystose. When total sugar concentration was increased from 200 to 400 g L^{-1}in a medium with molasses, the nystose production was reduced approximately 16 times. In the case of sugarcane juice-based medium, the production was reduced approximately 20 times when the concentration of total sugarwas increased from 300 to 400 g L^{-1}.

Discussion

Current analysis describes the high production of nystose by *Bacillus subtilis* natto CCT7712 with sugarcane derivatives. The optimization of fermentation parameters showed that sucrose concentration was the factor that most influenced nystose production. According to Ghazi et al. (2006), the synthesis of FOS from sucrose is a kinetically controlled reaction that involves a fructosyl-enzyme intermediate. The nucleophiles H_2O and sucrose compete for the fructosyl-enzyme intermediate. When H_2O is the nucleophile, the enzyme acts as a hydrolase (releasing glucose and fructose); when sucrose is the nucleophile, the enzyme acts as a transfructosidase. The first condensation product (1-kestose) may also be hydrolyzed by the enzyme. Therefore, maximum FOS yield depends on the concentration of sucrose and the intrinsic transferase-hydrolase ratio of the enzyme. Consequently, the yield of transfructosy lating products could be increased by using high sucrose concentration (Ghazi et al., 2006; Ning et al., 2010) also tested the effect of sucrose concentration and temperature on biotransformation of sucrose by *Xanthophyllomyces dendrorhous* and observed the highest production of neo-FOS (227.72 g L^{-1}) with 400 g L^{-1} of sucrose at 30°C. When Silva, Fai, Santos, Basso, and Pastore (2011) evaluated sucrose concentration, temperature and incubation period for FOS production by *Aureobasium pullulans*, they reported a 54.7% yield after 48 hours of fermentation in a medium with 400 g L^{-1} of sucrose. Moreover, Nemukula, Mutanda, Wilhelmi, and Whiteley (2009) studied the synthesis of FOS by fructosyltransferase from *Aspergillus aculeatus*, and verified that transfructosylation and hydrolysis activities increased by 88% in a medium with sucrose at 600 g L^{-1}. Interestingly, the nystose yields obtained in current study (71.73%) were higher than the rates reported in the literature.

The addition of mineral salts to the fermentation media may influence the biosynthesis of oligosaccharides and polysaccharides (Bekers et al., 2000; Ammar et al., 2002; Arundhati et al., 2011). The results obtained in this study showed the strongest positive effect of $MnSO_4$ on nystose production. Similar results were obtained by Silva et al. (2011), who also reported that $MnSO_4$ increased the production of FOS by *Aureobasium pullulans*.The increase of FOS production after salt addition could be due to the osmoprotection mechanism developed by the microorganism for regulating the medium´s osmolarity (Bekers et al., 2000). On the other hand, the addition of $(NH_4)_2SO_4$ and NaCl resulted in a significant reduction in nystose production. According to Bekers et al. (2000), the salts inhibited FOS production by *Zymomonas mobilis* in a medium with high sucrose concentration.

Concerns to reduce environmental pollution have encouraged the use of industrial waste and by products through bioprocessing. Besides decreasing the environmental impact, the utilization of the substrates also reduces the cost of production of biomolecules (Bicas, Silva, Dionísio, & Pastore, 2010). Current assay reports that the nystose production in a medium composed of sugarcane juice was 56% higher than the production in molasses, which may be due to the high concentration of carbohydrates, reaction products, minerals and metals in molasses that may have negatively influenced production. However, it should be underscored that, while the sugarcane juice needed to be supplemented with sucrose to achieve maximum concentration of total sugars, the molasses required dilution to reach such concentration. Thus, sugarcane molasses seems to be an interesting substrate to nystose production by *Bacillus subtilis* natto CCT7712even at a lower production of nystose.

Molasses is a good source of nutrients for the production of enzymes and microorganisms for fermentation and direct production of compounds such as FOS. In fact, 166 g $L^{-1}$of FOS were produced from 360 g L^{-1} of sugar molasses as sucrose equivalent at 55°C and pH 5.5, after 24 hour sof fermentation by *A. pullulans* cells (Shin et al., 2004). Interestingly, sugar syrup and molasses from beet were tested as low-cost substrates for the enzymatic synthesis of FOS. After 30 hours, the FOS concentration reached a maximum of 388 mg mL^{-1} when syrup was employed. When the molasses was used, 235 mg mL^{-1} of FOS were obtained in 65 hours of fermentation. The above rates corresponded to approximately 56 (syrup) and 49% (molasses) of the total amount of carbohydrates in the mixture (Ghazi et al., 2006). In summary, the obtained results demonstrated the capacity of *Bacillus subtilis* natto CCT 7712 to produce high amounts of nystose with low-cost substrates such as commercial sucrose, sugarcane molasses and sugarcane juice. The development of new biotechnological processes proposing alternatives for FOS production as the utilization of industrial byproducts are essential to increase the productivity and to reduce the production cost of FOS.

Acknowledgements

The authors thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes/PNPD) and Fundação Araucaria, Brazil for financial support.

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Received on July 30, 2015.
Accepted on October23, 2015.