Vol.47, n. 1 : pp. 135-138, March 2004
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Studies on Alkalophilic CGTase-Producing Bacteria and Effect of Starch on Cyclodextrin-Glycosyltransferase Activity

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ABSTRACT

The production of β- and γ-CD by CGTase from Bacillus firmus was studied, in regard to the effect of the source and concentration of starch on the yield of CD and CGTase activity. A cyclic activity of accumulation and consumption of β-CD and γ-CD occurred during the bacterial growth. CGTase was more active when citrate buffer, pH 5.5 was used. No differences were found for production of γ-CD with the use of commercial starch flour when compared with corn starch ($p>0.05$). The use of commercial starch flour resulted in decreased conversion of starch to ß-CD (all the 4 starch sources were statistically different, $p<0.05$). The best results for β-CD production were obtained with the use of corn starch. The specific activity of CGTase was not affected by starch concentration, different source of starch nor by the presence of glutamic acid, a CD-complexing agent.

Key words: Cyclodextrin glycosyltransferase, *Bacillus firmus*, starch

INTRODUCTION

Cyclodextrin glycosyltranferase (CGTase EC 2.4.1.19) is a bacterial enzyme that converts starch and other 1,4-linked α-glucans to cyclodextrins (α, β and γ-CDs in varying proportions) (French, 1957; Thoma and Stewart, 1965). They have the ability to form inclusion complexes with organic and inorganic compounds, which have numerous applications in the food and pharmaceutical industries (Pszczola, 1988). Several attempts have been made to obtain an optimal growth of bacteria and maximum CGTase synthesis, with the use of process optimization of various fermentation parameters (Jamuna, et al., 1993; Gawande and Patkar, 1999; Stefanova et al., 1999).

We are searching for isolates of alkalophilic microorganisms from Brazilian soils which have a high CGTase activity. CGTase production profiles were studied with different sources and concentrations of industrial starch in a basal medium in order to optimize CGTase activity.

MATERIAL AND METHODS

Microorganisms

Isolate number 31 of strain of alkalophilic CGTase-producing bacteria, identified as *Bacillus firmus* by morphological, physiological and biochemical tests (Higuti et al., 2003), was used. It was grown for 6-7 days at 28 °C and 120 rpm in a New Brunswick rotary shaker. The sterilized control

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culture medium contained 2% soluble starch (corn starch Sigma), 0.5% peptone, 0.5% yeast extract, 0.02% $MgSO_4.7H_2O$, 0.1% K_2HPO_4, pH 10.5, (Salva et al., 1997). The cells were removed by spinning, and the supernatant that contained the enzyme, substrates and products was used for further assays. Starch of corn, cassava and potato at 2-15% concentrations were used in the assay experiments.

Enzyme assay

Dextrinizing activity was assayed using soluble corn starch as substrate and by measurement of the decrease in iodine-staining power (Salva et al., 1997). The reaction medium contained 0.1 ml of the enzyme solution, 0.5 ml of 1% starch solution, 0.4 ml of 0.1 M citrate buffer, pH 5.5, and incubated in a water-bath at 50°C for 10 min. The reaction was terminated with 0.5 ml of 1 M HCl. To this, 0.1 ml of 4 mM iodine in 30 mM potassium iodide was added and then diluted to 10 ml with water. The starch-iodine complex absorption was monitored at 620 nm. Protein concentration was analyzed with the method of Lowry et al. (1951).

Measurement of β and γ-CD

The concentration of β-CD was analyzed by decrease in absorbance at 550 nm due to phenolphtalein-CD complex (Makela *et al*, 1988, modified from Vikmon, 1981). The concentration of γ-CD was analyzed by absorption increase at 620 nm due to bromocresol-CD complex (Kato and Horikoshi, 1984).

Statistical analysis

The significance among the groups of the experimental data was analyzed using the ANOVA test. A p value ≤ 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Fig. 1 illustrates the production of β- and γ - CD, starch consumption and CGTase activity during the growth of *B. firmus*. Higher CGTase activity was observed at 96 hr, followed by a drop that was due to decrease in the ß-CD yield and protein. The starch at 2% concentration disappeared in 24 hr, when β-CD production was maximum.

Fig. 2 illustrates the *B. firmus* growth profile using ß-CD as carbon source. ß-CD has been termed as good carbon source for CGTase producing bacteria (Gawande et al., 1999). The effect of starch concentration on β-CD production is illustrated in Fig. 3. β-CD production showed two peaks, at 24 and 96 hr. The culture produced and consumed the β-CD during growth.

Approximately the same phenomenon occurred with γ- CD. After 24 hr the same amount of β-CD was formed ($p>0.05$), but after 120 hr much more β-CD was formed when the initial concentration was 10 or 15% starch ($p<0.05$).

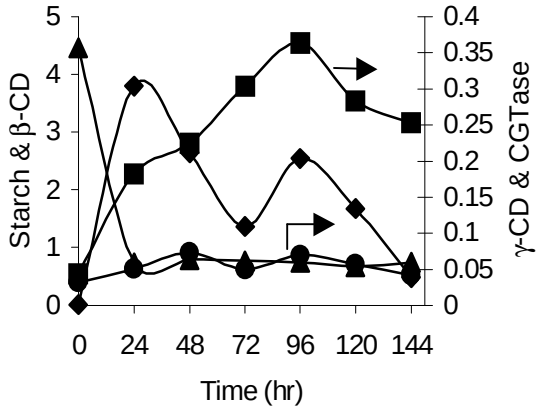

Figure 1 - Production of β and γ - CD, starch consumption and CGTase activity during growth of *Bacillus firmus* in culture medium. ■ – CGTase (EA mg/min/mg); ▲- Starch (mg/ml); ● – γ-CD (mM) ♦ - β-CD (mM)

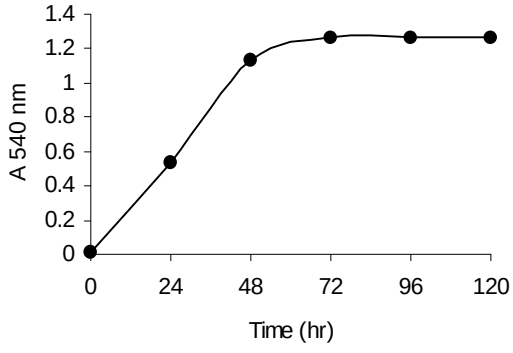

Figure 2 - Growth profile of *Bacillus firmus* in culture medium. β-CD 2% was used as carbon source

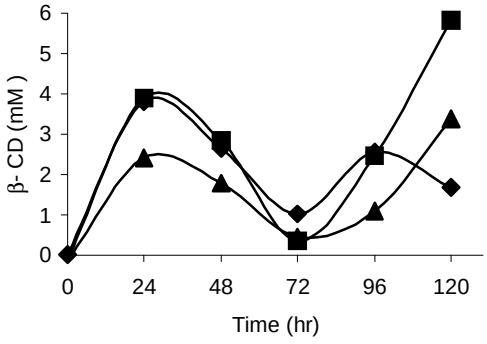

Figure 3 - Effect of starch concentration on β-CD production during growth of *Bacillus firmus* in culture medium ♦ Starch 2%; ▲ starch 10%; ■ starch 15%
Corn starch Sigma was used

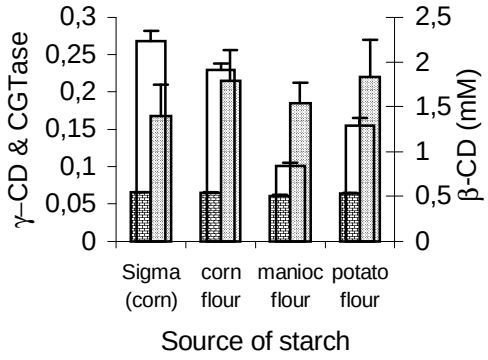

Figure 4 - Commercial flours, β- and γ-CD and CGTase productions during growth of *Bacillus firmus* in culture medium. Empty – β-CD (mM); - CGTase activity (EA mg/min/mg); - γ-CD (mM).

No difference was found in the activity of the CGTase formed with different starch concentrations (not shown). The ability of the bacteria to grow in commercial starch flour was analyzed (Fig. 4).

No differences were found for the activity of CGTase or production of γ-CD with the use of commercial starch flour when compared with corn starch from Sigma ($p>0.05$). Differences were found for β-CD production (all the 4 starch sources were statistically different, $p<0.05$). The best results for β-CD production were obtained with the use of corn starch-Sigma.

Glutamic acid forms complex with CDs and can be used to increase CD productions (Stefanova et al., 1999). Fig. 5 illustrated the assay of CGTase activity in the absence and in the presence of 0.2 % glutamic acid. It could be observed that glutamic acid has no effect on CGTase activity, nor was able to suppress the activity drop after 96 hr incubation. However, the use of 0.05 - 0.2 % glutamic acid in the culture medium decreased free ß-CD (Table 1).

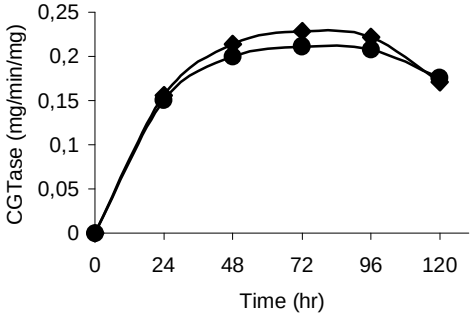

Figure 5 - Effect of glutamic acid (0.2%) on CGTase activity during growth of *Bacillus firmus* in liquid medium. ♦ - Control; ● – glutamic acid 0.2%. Corn starch Sigma was used.

Table 1 - Effect of glutamic acid on ß-CD production during growth of *Bacillus firmus* in liquid medium

Time		**Glutamic ac. (%)**		
(hr)	0	0.05	0.1	0.2
48	5,91 ± 0,29	2,34 ± 0,15	2,10 ± 0,24	1,46 ± 0,10
96	3,97 ± 0,09	1,85 ± 0,07	1,82 ± 0,04	1,26 ± 0,05

The data show ß-CD (mM) as average of 3 independent determinations and standard deviation. Corn starch Sigma was used.

Although glutamic acid did not increase the CGTase yield, it showed the potential use in sequestering ß-CD during the fermentation process.
The use of commercial starch flour resulted in decreased conversion of starch to ß-CD, probably due to the presence of some enzyme inhibitor.

RESUMO

A produção de β- e γ-CD pela CGTase de *Bacillus firmus* foi estudada com respeito ao efeito de diferentes fontes e concentrações de amido. A CGTase utilizada neste estudo foi obtida da cepa 31 isolada em estudos anteriores. Foram observadas atividades cíclicas de acúmulo da produção e consumo de β-CD e γ-CD pela bactéria. A atividade enzimática ensaiada em solução tampão citrato (pH 5,5) foi maior que em solução tampão Tris-HCl (pH 8,5). Não foi observada diferença na produção de γ-CD em diferentes fontes comerciais de amido (milho, mandioca e batata), comparados com amido Sigma ($p>0,05$). Foram encontradas diferenças para a produção de β-CD em todas as fontes de amido utilizadas. O uso de féculas de amido comerciais diminuiu a produção de β-CD comparados com amido Sigma. As concentrações de β-CD e γ-CD apresentaram uma variação ao longo do tempo, em função da concentração de amido no meio de cultivo (2, 5, 10 e 15%). Nem as diferentes fontes de amido nem as suas diferentes concentrações alteraram a atividade específica da CGTase. A atividade dextrinizante da enzima em presença de ácido glutâmico, complexante de CD, não apresentou diferença significativa em relação ao controle.

ACKNOWLEGMENT

The authors wish to thank CNPq and FUNPAR-UFPR for the financial support, and to Marcondes, A.A. for the kindly supply of ß-CD.

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Received: March 20, 2002;
Revised: October 09, 2002;
Accepted: April 29, 2003.