2011 International Conference on Food Engineering and Biotechnology
IPCBEE vol.9 (2011) © (2011)IACSIT Press, Singapore

Standardization of optimum conditions for cyclodextrin glycosyltransferase production

Nallusamy Sivakumar[1 +] and Shakila Banu[2]

[1] Department of Biology, College of Science, Sultan Qaboos University, Muscat, Sultanate of Oman
[2] Department of Microbiology, J. J. College of Arts and Science, Pudukkottai, India

Abstract. The cyclodextrin glucosyltransferase producing bacteria were isolated from soil. The efficient strain was isolated and identified as *Bacillus licheniformis*. Fermentation conditions for CGTase production by *B. licheniformis* were standardized. Maximum CGTase production was obtained at 37°C at pH 8. CGTase production was significantly high when the medium was supplemented with soluble starch. Glucose and cassava supplementation in the media resulted in low level of CGTase production. Yeast extract was found as the best nitrogen source. The enzyme production was inhibited by Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+} and Zn^{2+}.

Key words: CGTase, B. licheniformis, optimum conditions, metal ions

1. Introduction

The enzyme cyclodextrin glycosyl transferase (CGTase E.C 2.4.1.19) is a bacterial enzyme which converts starch into cyclic maltooligosaccharides known as cyclodextrins which are non-reducing, cyclic oligosaccharides composed of D-glucose units linked by α-1,4 glycosidic bonds. CGTase can synthesis all forms of cyclodextrins which are cyclomaltohexaose (α-CD), cyclomaltoheptaose (β-CD) and cyclomaltooctaose (γ-CD) consisting of six, seven and eight glucose molecules respectively (Illias *et al*., 2003). Among the three types of CDs, β-CD is more suitable for practical use. The main difference among them is the apolar cavity size and solubility in water (Allegre *et al*., 1994). Due to their apolar cavity they are able to form complexes with molecules and change the physico-chemical properties such as solubility and stability of guest compounds. Cyclodextrins have the ability to form inclusion complexes with organic and inorganic compounds, which have numerous applications in agricultural, food, cosmetics, chemical and pharmaceutical industries (Pszczola, 1998; Szejtli, 2004).

In food industry, cyclodextrins are used for the production of low-cholesterol butter, where the cyclodextrin is used to specifically remove the cholesterol from the milk fat. They are utilized for flavour stabilization and delivery in chewing gum, flavored tea, cinnamon flavored apples, lemon and grapes fruit candies, mints and lemon flavored sugar (Pszczola, 1998). Cyclodextrin is used in fruit juice beverages to mask vitamin odor and to mask bitterness in alone containing beverages. It is used to convert acetic acid to powder. It also increases the solubility of mustard sauce. The main objective of this work was to isolate CGTase producing *Bacillus* sp. from soil and standardization of its production conditions.

2. Materials and methods

2.1 Isolation of CGTase producing organisms

1 g of soil sample was suspended in 50 ml of sterile water and plated on a medium containing soluble starch 2%,

peptone 0.5%, yeast extract 0.5%, K_2HPO_4 0.1%, $MgSO_4.7H_2O$ 0.02%, Na_2CO_3 1%, phenolphthalein 0.03%,

methyl orange 0.01% and agar 2% and incubated at 37°C. CGTase positive colonies were identified by a clearing zone around them in the phenolphthalein containing media. Colony with a higher zone diameter was selected as an efficient strain and used for further studies.

2.2 CGTase production

The inoculum prepared using the isolated culture was inoculated in to production medium containing 1.5% soluble starch, 0.4% yeast extract, 0.15g of KH_2PO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, 0.12g of 20% KCl, and 0.9% NaCl and incubated at 37°C. Samples were withdrawn at specific intervals of time and analyzed for CGTase activity.

2.3 Enzyme assay (Goel and Nene, 1995)

To measure the enzyme activity cell free culture medium was centrifuged at 10000 rpm for 20 minutes at room temperature and supernatant containing cyclodextrin glycosyltransferase was collected. 5ml of the supernatant and 5ml of 1% starch solution (0.1g of soluble starch, 1ml of 0.05mM $CaCl_2$ and pure water for a total volume of 10 ml) were mixed in a thermostatic reactor at 50°C. Samples were taken periodically from the reactors and inactivated in water at 100°C for 5 minutes. The concentration of cyclodextrin was measured by the addition of 2.5ml of 3mM phenolphthalein solution (5ml of 0.6mM Na_2CO_3 buffer, pH 10.5 and the volume completed with 2.5ml of distilled water in a volumetric flask) to 0.5ml of inactivated samples. The absorbance of the final solution was analyzed in spectrophotometer at 550nm. A unit of enzymatic activity was defined as the quantity of enzyme that produces one μmol of cyclodextrin per minute under standard conditions.

2.4 Standardization of optimum conditions for CGTase production

Factors like carbon, nitrogen sources, microelements, temperature and pH affecting the production of CGTase were optimized by adopting the search technique by varying one factor at a time. The experiments were conducted in 500ml Erlenmeyer flasks. All the experiments were carried out in three sets with a control.

The optimum temperature and pH for the CGTase production were standardized. Different carbon sources like cassava, starch, maltose and glucose were tried to increase the enzyme production. Different nitrogen sources such as ammonium salts, tryptone, urea and yeast extract was supplemented in the production media to analyze their influence on enzyme production. Effect of cation concentration on CGTase production was carried out by the addition of $CaSO_4$, $MgSO_4$, $MnSO_4$, FeSO4 and $ZnSO_4$ to the production media.

2.5 Statistical analysis

The mean values and standard deviations were calculated from the data obtained from three different experiments. Analysis of variance was performed by one way ANOVA procedures followed by Tukey HSD Post Hoc tests using SPSS 11.5. Statistical differences at $p < 0.05$ were considered to be significant

3. Results and discussion

Ten CGTase positive bacteria (BS 1-BS 10) were isolated from soil sample (Fig. 1). Among them one bacteria showing highest activity (BS 7) was selected and identified as *Bacillus licheniformis*.

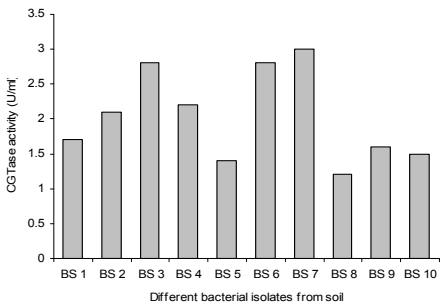

Figure 1. CGTase activity exhibited by different bacteria isolated from soil sample

At 37°C the CGTase activity was significantly higher than 47 & 27°C during 16 to 36 h of incubation. At 47°C the CGTase activity was significantly less than 37 & 27°C. pH 8 showed maximum CGTase activity at

24h of incubation. But the difference in CGTase activity among the different pH ranges is insignificant (Fig. 2&3). The CGTase activity of *Bacillus* sp G1 was found to be optimal at pH 6 (Illias *et al*., 2003). Larsen *et al*., (1998) observed that the pH optima of the enzyme were 7.5 for the cyclisation activity and 8.0 for the hydrolysis activity. The temperature optima for the cyclisation and hydrolysis activity were 50°C and 60°C respectively. Single optimum pH range for CGtase activity suggests a different degree of ionization of the enzyme catalytic site in order to produce different types of cyclodextrins. A broad range of temperature between 45°C and 70°C was already reported (Salva et al., 1997; Yim et al., 1997; Higuti et al., 2003).

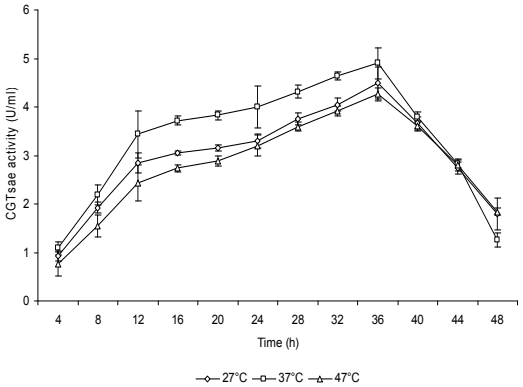

Figure 2. Effect of different temperature ranges on CGTase activity

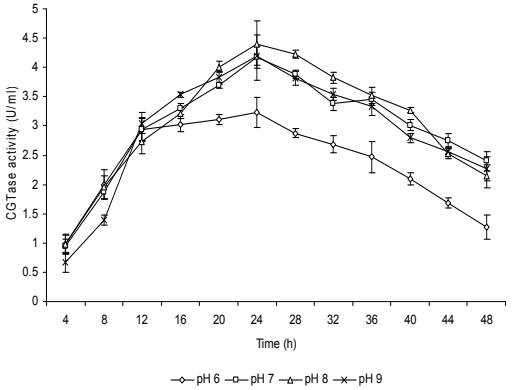

Figure 3. Effect of different pH ranges on CGTase activity

Significantly high CGTase activity was observed at 32 h of incubation when soluble starch was supplemented as a carbon source. But with maltose significantly less activity was obtained (Fig. 4). Although cassava supplementation produces a high growth rate for *B. licheniformis*, it induces low CGTase activity. The supplemented starch in the medium serves as an inducer for CGTase production. Soluble starch was found as a best carbon source for *B. firmus* and *B. macerans* (Posci *et al.*, 1998). Glucose supplementation has given only low yields of CGTase production. Varavinit *et al*., (1998) reported that the production of CGTase by *Bacillus* sp. MP 523 was repressed significantly by glucose. However studies carried out by Stefanova *et al*., (1999) showed that 0.5% (w/v) glucose was found to be the most suitable substrate for CGTase production.

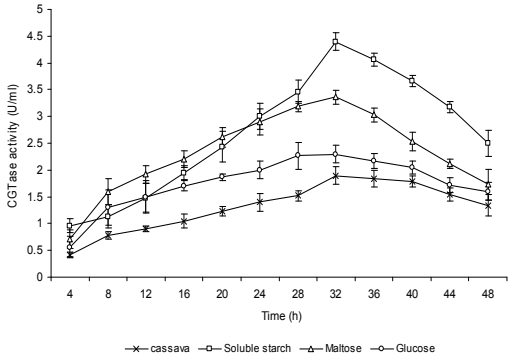

Figure 4. Influence of different carbon sources on CGTase production

Among the four different nitrogen sources tested yeast extract influenced significantly the productivity of CGTase than urea, tryptone and ammonium salt (Fig. 5). Maximum growth of *B. licheniformis* was also observed in yeast extract containing media. CGTase production was higher when an organic nitrogen source was present in the medium. Enzyme production using inorganic nitrogen sources was found to be low when compared with organic nitrogen source. It was reported that the best nitrogen source for the production of CGTase was peptone (Illias *et al*., 2002). But Mahat et al (2004) observed that sago starch and yeast extract have a significant effect on CGTase production.

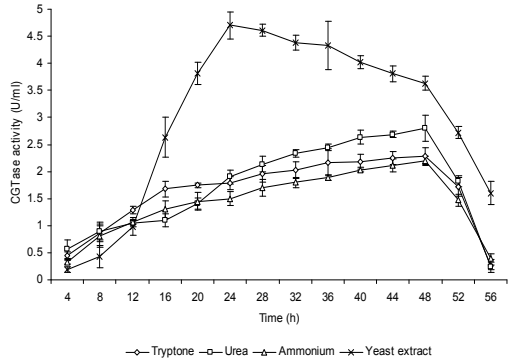

Figure 5. Influence of different nitrogen sources on CGTase production

The effect of cations on the production of CGTase was studied by substituting different metal ions in the media (Fig. 6). All metal ions inhibited the activity of CGTase. Mg^{2+} showed moderate inhibition but Fe^{2+} and Zn^{2+} were the strong inhibitors of CGTase activity (Table 1). This is in accordance with Higuti et al.,(2003). But it was also reported that Mg^{2+} and Ca^{2+} showed little activation on CGTase activity (Freitas et al., 2004). The effect of metal ions on enzyme activity seems to depend on the enzyme source (Higuti et al., 2003).

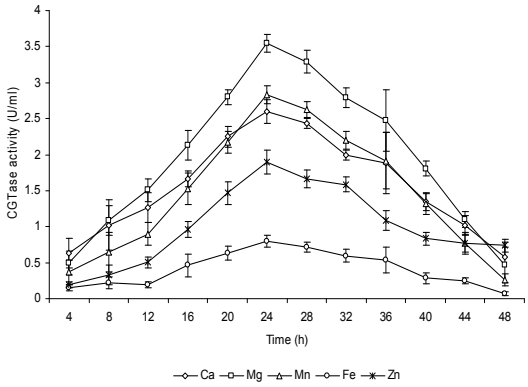

Figure 6: Effect of different metal ions on CGTase production

Table 1: Effect of metal ions on the activity of CGTase

Metal ions	Relative activity (%)
Control	100
Ca^{2+}	55.3
Mg^{2+}	75.5
Mn^{2+}	60.2
Fe^{2+}	17
Zn^{2+}	40.4

4. References

[1] A. Goel and S. N. Nene. Modifications in the phenolphthalein method for spectrophotometric estimation of β-cyclodextrin, Starch/ Starke. 1995, 47 (10) : 339 - 400.

[2] D. G. Yim, H. H. Sato, Y. H. Park and Y. K. Park. Production of cyclodextrin from starch by cyclodextrin glycosyltransferase from *Bacillus firmus*and characterization of purified enzyme. *J. Ind. Microbiol. Biotechnol.*, 1997, **18**, 402-405.

[3] D.E. Pszczola. Production and Potential food application of cyclodextrins, *Food Tech*. 1998, **42**: 96 – 100.

[4] I. H. Higuti, S. W. Grande, R. Sacco and A. J. Nascimento.Isolation of Alkalophilic CGTase-Producing Bacteria and Characterization of Cyclodextrin-Glycosyltransferase *Braz. Arch. Boil. Technol*., 2003, **46**(2) : 183-186.

[5] J. Posci, N. Nogyrady, A. Liptak, and A. Szentiramai. Cyclodextrins are likely to induced cyclodextrin glycosyl transferase production in *Bacillus macerans*, *Folia Microbiol*. 1998, **43**: 71 – 74.

[6] J. Szejtli. Past, Present and future of cyclodextrin research, *Pure Appl. Chem*. 2004, **76**(10): 1825 – 1845. K. I. Larsen, L. H. Duedahl and W. Zimmermann. Purification and characterization of cyclodextrin glycosyltransferase from *Paenibacillus* sp. F. 8, *Carbohydr. Res*. 1998, **310**: 211-219.

[7] M. Allegre and A. Deratani. Cyclodextrin uses: from concept to industrial reality, *Agro Food Industry Hitech.* 1994, pp 9 – 17.

[8] M. K. Mahat, R. M. Illias, R. A. Rahman, N. A. A. Rashid, N. Mahmood, O. Hassan, S. A. Aziz and K. Kamaruddin. Production of cyclodextrin glucanotransferase (CGTase) from alkalophilic *Bacillus* sp. TS1-1: media optimization using experinmental design. *Enz. Microbial. Technol.,*2004, **35**: 467-473.

[9] M. R. Illias, T. S. Fen, N. A. Abdul Rashid, M. W. Yusoff, A. A. Hamid, O. Hassan and K. Kamaruddin. Application of factorial design to study the effect of temperature, initial PH and agitation on the production of cyclodextrin glucosyl tranferase from alkalophilic *Bacillus* spp. *GI. Science Asia.* 2003, **29**: 135 – 140.

[10] S. Varavint, V. Sanguanpong and S. Shobsngob. Isolation from starch waste and partial characterization of a new cyclodextrin glycosyl transferase producing *Bacillus* sp, foods *Food Ingred J. Jap*. 1997, **173**: 84 – 89.

[11] T. J. G. Salva, V. B. Lima and A. P. Pagan. Screening of alkalophilic bacteria for cyclodextrin glycosyltransferase production. *Rev.Microbiol.*, 1997, **28**, 157-164.

[12] T. L. Freitas, R. Monti and J. Contiero. Production of CGTase by a *Bacillus* Alkalophilic CGII strain isolated from waste water of a manioc flour industry. *Braz. J. Microbiol*., 2004, **35**: 255-260.