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Studies on production of Cyclodextrin glycosyl transferase (CGTase) enzyme using alkaliphilic bacteria

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Studies on production of Cyclodextrin glycosyl transferase (CGTase) enzyme using alkaliphilic bacteria

Cyclodextrin glycosyl transferase (CGTase; EC 2.4.1.19) is the enzyme that converts starch into cyclodextrins (CD’s) which are closed-ring structures having six or more glucose units joined by means of \( \alpha \)-1, 4 glucosidic bonds. CGTases are classified in the \( \alpha \)-amylase family and are known to catalyze four different transferase reactions: cyclisation, coupling, disproportionation, and hydrolysis. Three major types of Cyclodextrins are produced by CGTases depending on number of glucose units, \( \alpha \)– CD, \( \beta \)- CD and \( \gamma \)- CD. CGTase is used for industrial production of cyclodextrin and for biotransformation. Cyclodextrins have a variety of applications in food, pharmaceutical and cosmetic industry. Cyclodextrins are used as drug carriers and tableting vehicles.

It is evident from the above mentioned applications that CGTase enzyme is industrially a very important enzyme because of its application in production of CD’s. However, its application is still significantly limited because of its low yield and extravagant price. CGTase from alkaliphilic Bacillus sp. overcome all these problems and lead to mass production of \( \alpha \)– CD, \( \beta \)- CD and \( \gamma \)- CD. Since then it is known that alkaliphiles are candidate organisms for CGTase production.

In India, Lonar Lake (Buldhana Dist., Maharashtra State) is a well known alkaline lake formed by meteorite impact in basalt. The cultivable bacterial diversity of Lonar lake and screening of organisms for industrially important compounds with biotechnological applications (PHA, EPS, proteases) has been studied previously but there is no report on the occurrence of CGTase in organisms isolated from Lonar Lake. Thus studies on alkaliphilic bacteria from Lonar Lake for production of CGTase may pave way for discovery of this extremozyme with huge biotechnological potential and application for production of CD’s.
The present investigation resulted in following findings:

Part 1

Aerobic alkaliphilic bacteria previously isolated and identified from Lonar Lake were screened for starch hydrolysis using starch medium and for production of CGTase on phenolphthalein methyl orange medium. 15 isolates from Lonar lake showed starch hydrolytic activity and 6 were positive for CGTase production on phenolphthalein methyl orange medium. The results of CGTase production were confirmed by phenolphthalein assay. The six isolates showing positive CGTase activity were *Bacillus firmus*, *Bacillus fusiformis*, *Bacillus licheniformis*, *Paenibacillus sp L55*, *Exiguobacterium aurantiacum* and Lake Bogoria isolate 25 B1. To the best of our knowledge, this is the first report of alkaliphilic *Exiguobacterium aurantiacum*, Lake Bogoria isolate 25 B1 and *Paenibacillus sp*. *Bacillus* sp is a common producer of CGTase hence *Exiguobacterium aurantiacum* and *Paenibacillus sp*. L55 were selected for production of CGTase.

Part 2

The preliminary studies on optimization of fermentation parameters using one parameter at a time and two parameters at a time at multi levels were performed. Taguchi Design of Experiments (DOE) was used for optimization of medium composition. In Taguchi DOE, a standard L9 orthogonal array was used to examine 4 factors at 3 levels. Taguchi DOE revealed that starch is the most significant factor for the CGTase production by *Paenibacillus sp*. and *E. aurantiacum* followed by peptone and yeast extract. pH showed the least impact among the factors studied with the assigned variance of values. The error observed was very low which indicated the accuracy of the experimentation. Based on the equation for prediction, the CGTase activity by *E. aurantiacum* can be increased from 4.19 U/ml (which is average activity obtained from the nine trials) in an optimized batch submerged shake flask level fermentation to 6.15 U/ml. CGTase activity of 6.15 U/ml which is 96% of the predicted >6.4 U/ml with the modified culture conditions. Similarly the CGTase activity by *Paenibacillus sp*. was enhanced from 3.5 U/ml to 5.15 U/ml.

*Exiguobacterium aurantiacum*
demonstrated higher activity of CGTase with a faster growth rate and was selected for scale up studies.

Part 3

After optimization of production medium in shake flask, the production was scaled up in 3 L fermentor where aeration, agitation and aspect ratio were optimized. The production was further scaled up in a 14 L SS stirred tank in-situ sterilizable Fermentor. The yield of CGTase was enhanced from 6.15 U/ml in 250 ml shake flask to 7.7 U/ml in 3 L and upto 8.2 U/ml in 14 L fermentor. The CGTase production was attained in 4 hours in 14 L fermentor which demonstrates that the production process is of a remarkably short time which is industrially significant. An optimized and rapid fermentative process for production of CGTase by E. aurantiacum was thus developed successfully using starch based medium.

Part 4

CGTase produced by Exiguobacterium aurantiacum was successfully purified using starch adsorption chromatography with 25 fold purification. A single band of 77.84 kDa on SDS PAGE indicates the homogeneity and possible molecular weight. CGTase demonstrated pH Optimum 9, pH stability8 – 9, temperature optimum 50°C and temperature Stability 50 – 70 °C. CGTase was strongly inhibited with complete loss in activity by Fe+3 and partially inhibited by Mn+2, Zn+2, Cu+2, DTT and PMSF. The $K_m$, $V_{max}$ and $K_{cat}$ value for CGTase produced by E. aurantiacum was calculated as 0.0225 g/L, 57 µg/ml/min and 316.7 s-1. MALDI TOF –MS revealed its homology to glycosylase produced by Colwellia spp. The isoelectric potential was predicted as 9.7 by MALDI TOF-MS.

Part 5

Cyclodextrins were produced by action of CGTase from E. aurantiacum on raw potato starch, corn starch, soluble potato starch, wheat starch, rice starch and sago starch. The cyclodextrins were detected by HPLC and FEG-SEM.

CGTase was produced by immobilized cells of E. aurantiacum entrapped in calcium alginate beads in a packed bed reactor and was compared to CGTase produced by free cells. For economical production of CGTase, agro-waste like
potato-peel waste was used as the sole carbon substrate. The immobilized cells were effectively used for the production of β -Cyclodextrins from potato peel waste with an activity of 5 .1 U/ml as compared to 6 U/ml for free cells. The β – Cyclodextrins produced were detected by HPLC, microscopy and colorimetric estimation. Thus immobilized whole cells can be effectively used for economical production of cyclodextrins from agro –waste based materials.

CGTase produced by *E .aurantiacum* demonstrated moderate thermostability due to which it can be used as an antistaling enzyme in bread making. The firmness and textural properties of bread containing CGTase were studied. It was observed that loaf volume and texture of bread with CGTase was greater than control bread. The parameters like hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience of bread were studied using TA XT2i Texture Profile analyzer. The pore size analysis of bread was also studied. CGTase from *E. aurantiacum* can thus be used to enhance texture and freshness of bread and has a potential application in bakery industry.

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