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Optimization of Cyclodextrin Glucano-Transferase by *Bacillus Cereus* Isolated from Semi Arid Soil, Kuchchh, Gujarat

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Abstract

Cyclodextrin glucanotransferase (CG-Tase) is a commercially important enzyme which catalyses the formation of cyclodextrins (CDs) from starch belonging to the amylase family. The optimization enzyme cyclomaltodextrin glucano transferase was carried out from semi arid region of Kachchh Gujarat different enzyme activities and optimization of cyclodextrin glucano-transferase, with different physicochemical Parameter were Temperature effect was observed 35°C with 42.02 U/ml enzyme activity, were the most active at pH 7.0 reported 44.28 U/ml, at highest incubation at 96 hrs, activity was 39.68 U/ml. stood with different inoculum at 3% of inoculum, 54.38 U/ml activity verified highest. Different cations zinc express highest activity 36.98 U/ml. In case of different carbon source starch prompt highest activity at 39.28 U/ml. while Response surface methodology and standardization error to estimate to the mean value of observed response.

Keywords
Cyclodextrin glycosyltransferase (CGTase), Optimization, carbon source. physicochemical parameters, RSM.

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INTRODUCTION

Cyclodextrin glucanotransferase is an enzyme which catalyzes intramolecular and intermolecular transglycosylation as blooming a hydrolytic action on starch and cyclodextrins (Tonkova, 1998). CDs are cyclic oligosaccharides consisting of a-1, 4-linked 6, 7, or 8-glucopyranose units, usually referred to as a, ß, or γ-CDs respectively. (Kamble and Gupte, 2014)., cyclodextrins, beta and gamma, which could be of interest for their easy separation and industrial production. (Atanasova *et al*., 2008). Cyclodextrin glycosyltransferase (CGTase EC.3.2.1.19) was immobilized on chitosan through covalent bonding using glutaratdehyde. (Yang & Su 1989). The enzyme

converts starch and related α-1, 4-glucans to cyclodextrins which are widely utilized in food, pharmaceutical and chemical industries (Tonkova, 1998). However, continued developments in immobilization technology have led to more sophisticated and specialized applications of the process. (Cowan & Fernandez, 2011.). Various species such as *Bacillus, Klebsiella, micrococcus, Brevibacterium, Thermoactinomycetes* and Thermophilicarchea are able to Produce major CGTase. Growth cultural conditions providing optimal enzyme biosynthesis in batches, repeated batch and continuous cultivation of free and immobilized cells, as well as some physicochemical and biochemical characteristics of the enzyme, CGTase immobilization, and enzyme structure. (Tonkova,1998) (Yampayont *et al*., 2006). Can be used as a sole source of carbon for the production of CD with the sago starch as a potential alternative substrate. (Charoenlap *et al.,* 2004). They reflected the differences in the enzyme activities found among the soils (Acosta et al., 2003). Analysis revealed a relationship between enzyme activities and the age and the management of fallow. (Badiane*et al.,* 2001). The present work carried optimization of media composition for better enzyme production and activity.

MATERIAL AND METHODS:

Screening and isolation of the strain:

The alkaliphilic CGTase producer was isolated from a soil sample was collected from Kachchh located in Gujarat, India. Soil (1 g) was suspended in 10 ml of sterile D/W and was allowed to settle down. Supernatant (0.1 ml) was placed on agar medium. Plates were incubated at 37°C for 24 hours. Isolated 12 bacterial colonies were observed and microscopic scopic observation carried out. (Atanasova *et al.,* 2008)

Characterization and identification of Bacteria:

The morphological characteristics of the isolate were examined nutrient agar. The size, shape, presence of endospores and motility of the cells were determined. The biochemical characterization was done according to the method of Bergey's Manual of Determinative Bacteriology. 16S rRNA sequencing of the strain was carried out for further identification and classification by NCCS (National Centre of Cell Science, Pune).

Preparation of bacterial inoculums:

The selected bacterial strain was grown in Soil extract agar containing 2% soluble starch, 5% peptone, 5% yeast extract, 1% KH_2PO_4, 0.02% MgSO4.7H2O and 1% Na2CO3. The culture was incubated at 37°C for 24 h on orbital shaker at 120rpm. The cells were harvested at 5,000 rpm for 15 min, washed with normal saline (0.85% w/v NaCl) and were then suspended in normal saline to give an optical density reading of 0.5 at 550 nm which was utilized as the inoculum.

Fermentation/Enzyme Production

In fermentation 10% (v/v) of *bacillus cereus* actively growing culture as inoculum for the production media at 370 C for 24hrs with continuous shaking in conical flask containing 100 ml of medium. The production inoculums. Salts were autoclaved separately and added after cooling to avoid precipitation. After cultivation cells were removed by centrifugation at 4000 rpm for 4min. The supernatant was used as crude enzyme for assaying CGTase activity.

CGTase assay:

CGTase activity was measured by using phenolphthalein method. Reaction mixture containing 40 mg of soluble starch in 1.0 ml of 0.1M sodium phosphate buffer (pH 6.0). After sufficient growth fermentation medium was centrifuged at 4000rpm for 4 min and supernatant was used as a crude enzyme. Addition of 0.5 ml crude enzyme in each test tube was incubated at 90°C for 10 minutes. After incubation the reaction was stopped by adding 30 ml of 30 mM NaOH and 0.5 ml of 0.02% (w/v) phenolphthalein in 5 Mm Na2CO3 solutions. After standing at room temperature for 15 minutes, the colour intensity was measured at 550 nm. (Park*et al.,* 1989)

Effect of physicochemical condition.

Effect of initial pH:

To study the effect of pH on the CGTase activity various pH values studied by varying Glacial acetic acid or 1N HCL.1% NaOH. The concentrations correspond to initial pH 5, 7, 9, and 11were tested. Checked pH of the medium was set or pH of assay system was set. The cells were harvested and the supernatant was analyzed for CGTase activity.

Effect of carbon and nitrogen source:

Effect of carbon sources on growth and CGTase production was investigated by Nutrient agar medium with different types of carbon sources such as glucose, lactose, maltose, dextrin, potato starch, similarly effect of nitrogen sources was evaluated by NH_4CL, $NaNO_3$, Na_2SO_4 and Yeast Extract. The most

suitable carbon and nitrogen source for CGTase production were chosen and incorporated in basal media for further studies.

Effect of cations:

Different types of Cation are representing to classify the MgSO4, CaSO4, FeSO4, ZnSO4 reaction toward the Bacilus and explore activity.

Gowth Kinetics:

The growth kinetics for the isolated bacterial culture was studied and compared by inoculating the alkaline Horikoshi (II) medium (basal medium), pH 10.5, containing 1% soluble starch, 0.5% peptone, 0.5% yeast extract, 0.1% KH2PO4, 0.02% MgSO4.7H2O, 1% Na2CO3 and the optimized alkaline media containing 1% tapioca starch, 0.5 % peptone, 0.5% yeast extract, 0.14% Magnesium Sulphate, 0.2% ammonium dihydrogen phosphate and 1% Na2CO3. (Mahat*et al.,* 2004, Nakamura & Horikoshi, 1976).

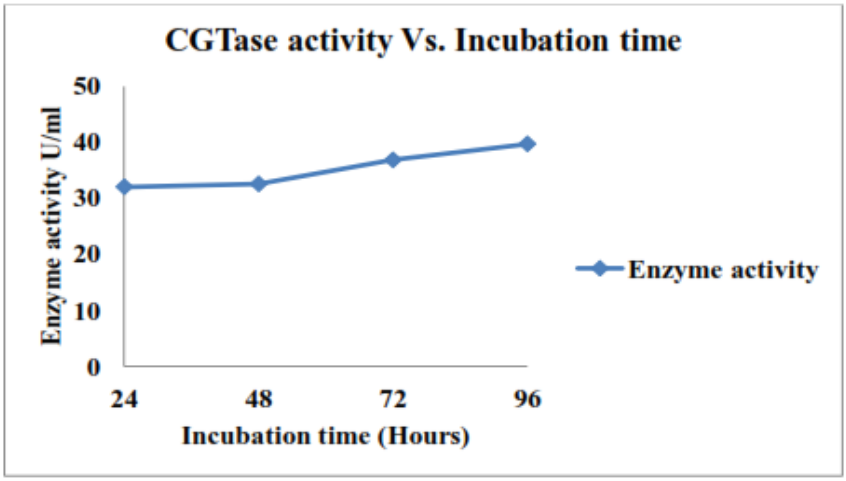
RESULTS AND DISCUSSION

Isolation, identification and characterization of the Isolate

The strain was isolate from soil sample and characterized for various physiological properties. It found to be Gram Positive rods. In biochemical characteristics hydrolyze starch and ability to produce acid using carbohydrates were investigated according to Bergey's manual of Bacteriology. Catalase, Ammonia production, Citrate utilization were representing positive. While MRVP, Indole production, H2S production, Urea hydrolysis, Nitrate reduction, Citrate utilization were represent Negative .Based on the morphological and biochemical characterization the strain was identified as *Bacillus cereus*. The additional characterization by 16S rRNA with accession number CM000729.1.

Physiochemical characteristics:

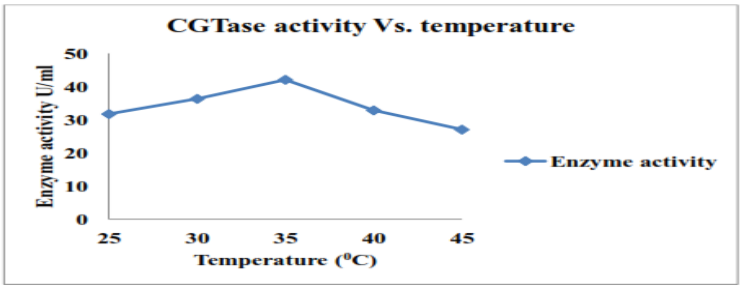
ACTIVITY Vs INCUBATION TIME



Effect of incubation time Vs. CGTase Activity (Fig-1)

Effect of incubation on enzyme activity was measured at various incubation times (24hrs, 48hrs, 72hrs and 96hrs). Result of effect of incubation time on CGTase production indicates that at incubation time 96 hrs more amount of enzyme production is also increased at longer incubation time. After incubation of 96 hrs, CGTase activity was 39.68 U/ml. (Fig- 1)

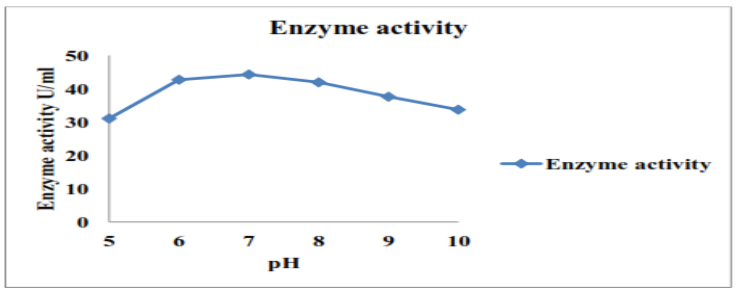
ACTIVITY Vs TEMPERATURE



Effect of temperature Vs. CGTase activity (Fig- 2)

Effect of Temperature on enzyme activity was measured at various temperatures (250C, 300C, 350C, 400C, 450C). Results of effect of temperature on CGTase production indicates that at temperature 35C more amount of enzyme was produced besides this enzyme production is also increased at longer incubation period. CGTase activity was **42.02 U/ml. (Fig-2)**

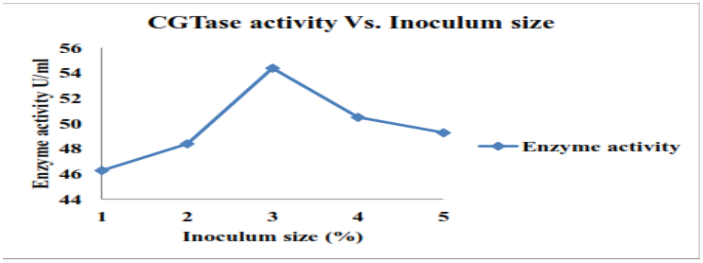
ACTIVITY Vs pH



Effect of pH Vs. CGTase activity (Fig-3)

Effect of pH on enzyme activity was measured at various pH (5, 6, 7, 8, 9 and 10). Results of effect of pH on CGTase production indicates that at pH 7.0 more amount of enzyme is produced beside this enzyme production is also increased at longer period. CGTase activity was 44.28 U/ml. Result indicates that CGTase is active of neutral pH. Alkaline and acidic pH affects stability of enzyme. (Fig-3)

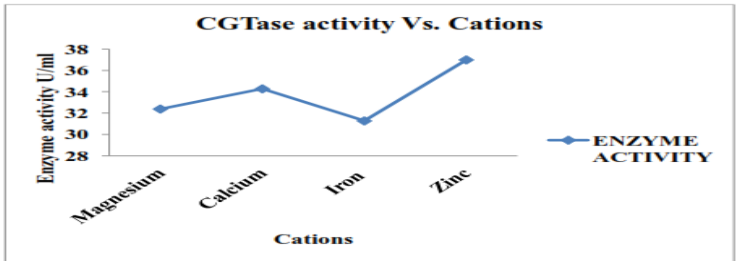
ACTIVITY Vs INOCULUM SIZE



Effect of inoculum size Vs. CGTaseactivity (Fig-4)

Effect of Inoculum size on enzyme activity was measured at various Inoculum sizes (1%, 2%, 3%, 4% and 5%). Results of effect of Inoculum size on CGTase production indicates that at 3% more amount of enzyme is produced beside this enzyme production is also increased at longer period. CGTase activity was **54.38 U/ml.** (Fig-4)

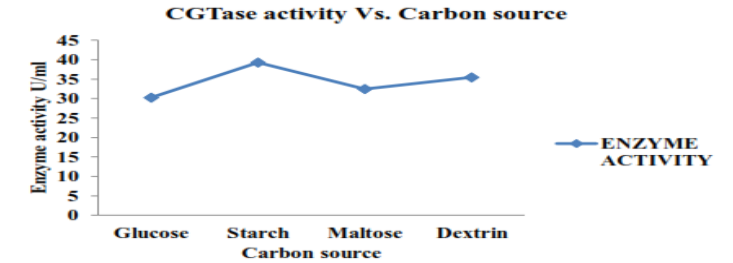
ACTIVITY Vs CATIONS



Effect of cations Vs. CGTase activity (Fig-5)

Effect of cations on enzyme activity was measured at various cations (magnesium, calcium, iron and zinc). Results of effect of cations on CGTase production indicates that zinc produced more amount of enzyme beside this enzyme production is also increased at longer incubation period. CGTase activity was **36.98 U/ml. (**Fig-5)

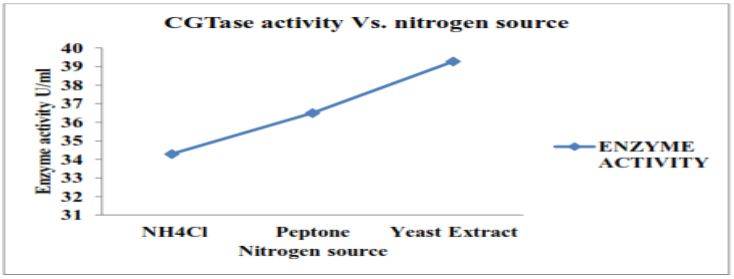
ACTIVITY Vs CARBON SOURCE



Effect of carbon source Vs. CGTase activity (Fig -6)

Effect of carbon source on enzyme activity was measured at various carbon sources (glucose, starch, maltose and dextrin). Results of effect of carbon source on CGTase production indicates that starch produced more amount of enzyme beside this enzyme production is also increased at longer incubation period. CGTase activity was **39.28 U/ml. (Fig -6)**

ACTIVITY Vs NITROGEN SOURCE



Effect of nitrogen source Vs. CGTase activity (Fig-7)

Effect of nitrogen source on enzyme activity was measured at various nitrogen sources (ammonium chloride, peptone and yeast extract). Results of effect of nitrogen source on CGTase production indicates that yeast extract produced more amount of enzyme beside this enzyme production is also increased at longer incubation period. CGTase activity was **39.28 U/ml. (Fig-7).**

CONCLUSION

From Semi-arid region *Bacillus cereus* was isolated identification carried out by biochemical characterization and 16s rRNA with accession no. CM000729.1. with different effect of physico-chemical parameters on enzyme activity indicates this enzyme is more stable at neutral. Longer period incubation affects not only temperature but also other parameters. Incubation of enzymes at different temperature affects CGTaseactivity. Higher enzyme activity was obtained at 35°C which indicates enzyme is mesophile not only this but low temperature affect it's stability as indicated in results. The effect of incubation time on CGTase activity showed at 96 hrs more amount of enzyme production is also increased at longer incubation time. The effect of Inoculum size on CGTase production obtained at 3% more amount of enzyme is produced beside this enzyme production is also increased at longer period. The effect different carbon sources on CGTase production showed that enzyme production was highest when starch was used as carbon source. Use of glucose, maltose and dextrin gave low yield of CGTase activity. The effect different cations on CGTase production showed that enzyme production was highest when zinc was used as cations. Use of calcium, magnesium and iron gave low yield of CGTase activity. The effect different nitrogen sources on CGTase production showed that enzyme production was highest when yeast extract was used as nitrogen source. Use of NH CL and peptone gave low yield of CGTase activity. Response surface analysis was useful to determine the optimum levels of medium concentrations and factors that significantly influence of CGTase from *Bacillus cereus* the final composition of the defined medium to produce CGTase after the optimization step was as follows: 2% starch, **7** pH, 374 0C temperature, 48 hours incubation time and agitation speed of 120 rpm. The production of CGTase was at 75.23 U/ml. results from the experiment done shown that CGTase from Bacillus cereus were able to produce ß-cyclodextrin

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