Cloning of α-Amylase enzyme producing gene into nonα-amylase producing organism and its Expression

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***Abstract*—** In this work, the gene of α-amylase from B. subtilis was cloned and expressed in Non-amylase produsing E.coli. B. subtilis was isolated by performing various biochemical tests. The organism producing α-amylase enzyme was successfully isolated. The isolation of genomic DNA followed by amplification of Gene Of Interest using α-amylase primer and annealing temperature 56°C was done using PCR technique. The amplified product was eluted from electrophoresis gel and subjected to restriction digestion (EcoRI) and then ligation with the vector (Plasmid DNA pUC18). A competent cell was prepared using chemical method (CaCl2). The ligation mixture was allowed to react with the competent cells in order to produce transformed cells producing α-amylase enzyme. The mixture was spread over the starch plates. The successfully transformed E.coli cells were observed over the Starch agar plate.

***Keywords*—** Bacillus subtilis, E.coli, PCR, pUC18, EcoRI, etc.

I. INTRODUCTION

"Transformation" describes the insertion of new genetic material into nonbacterial cells, including animal and plant cells; Introduction of foreign DNA into eukaryotic cells is often called "transfection"(Alberts, Bruce; et al., 2002). The bacteria belonging to the genus Bacillus produce extracellular amylases with different specificities. Some strains have already been developed for massive production of particular amylase for industrial purpose. The cloning of one gene directing the synthesis of amylase in a well characterized host like E. coli, B. subtilus should help greatly for the characterization of new amylases, cloned and studied the expression of glucoamylase gene from A.nigerin E.coliusing pBR322 as vector. Using derivatives of bacteriophage lamda as vector have already been cloned two different amylase gene from B.megabacteriumand B.coagulans. α-Amylases (1, 4-a-D-glucan glucanohydrolase; EC 3.2.1.1) are endo-acting enzymes that hydrolyze starch by cleaving a-1, 4-glucosidic linkages (Wang N, 2006), and have been widely used in food, textile, and pharmaceutical industries (Malhotra R, et al., 2000, Murakami S, et al.,2008) The alkaline a-amylases have high catalyticefficiency and stability at the alkaline pH ranging from 9 to 11 (hKuilderd H, et al.,2008) and have potential applications for hydrolysing starch under high pH conditions in the starch and textile industries and as ingredients in detergents for automatic dishwashers and laundries. While the alkaline a-amylases have potential applications in textile and detergent industries, relatively few efforts have been made to improve the yield of alkaline a-amylases. The current studies mainly focus on the strain screening, enzyme purification, and properties characterization (Chen DL,et al., 2010, Hagihara H, et al.,2001).

II. MATERIALS AND METHODS

SAMPLE COLLECTION

In this study, *Bacillus subtilis* was isolated from soil sample using serial dilution method and identified by various Biochemical test.

DNA EXTRACTION

The genomic DNA was isolated from Bacterial species using Phenol: Chloroform extraction method and presence of DNA was analyzed on 0.8% Agarose Gel. Concentration of DNA was determined by double beam spectrophotometer at 260/280nm.

AMPLIFICATION BY PCR

The PCR reactions were performed using a 15µl reaction mixture containing 10X Taq DNA Polymerase buffer with 2mM $MgCl_2$, 2.5 Mm dNTPs, 5µM primers, 4µl Taq DNA polymerase, 30 to 40 ng templates DNA and sterile distilled water. After completion samples were stored at 4 ºC. This PCR amplified products were separated by electrophoresis on 1.2% agarose gel with 1X TBE buffer containing Ethidium Bromide (EtBr) and then visualized.

RESTRICTION DIGESTION

Amplified DNA product and Vector DNA was digested with EcoRI. Incubate the reaction mixture at 37°C (optional temperature recommended for maximal for enzyme activity) for 1 hr. Reaction was stopped by incubating the reaction mixture at 72°C for 5 minutes.

LIGATION

Ligation of the amplified DNA with the vector digest was done by T4 DNA ligase and Incubate at 16°C (maintain at 16°C in an ice bucket and keep the ligation sample in float rack) for 1 hr. Stop the reaction by incubating the reaction mixture at 72°C for

5 minutes. Store the ligated sample at -20°C for further use.

COMPETENT CELL PREPARATION

The isolated colonies of E.coli was plated from the plate and inoculated in to the LB broth and inoculated for overnight at 37°C. From the overnight culture a 2-3 hour fresh culture was prepared. 2ml of broth was taken in an eppendrof tube and centrifuge at 10000 rpm for 5 minutes at 4°C. Supernatant was discarded and the pellet was suspended in 1ml of 0.1M calcium chloride. The tubes were kept in ice for 20minutes. It was then centrifuged at 6000 rpm for 10 minutes at 4°C. Supernatant was discarded and pellet was resuspended gently in 100µl of 0.1M calcium chloride and 16µl of 40% glycerol. The competent cell prepared was stored at 4°C.

TRANFORMATION

Three no. of 100µl of the competent cell was taken and labeled as 1,2 and 3. The tube labeled as 1 is the control for the competent cell to check the viability and contamination. 2µl of pUC plasmid was added to the tube 2 and mixed gently. 2µl of the ligated sample is loaded in the tube 3. The tubes were kept in ice for 30 minutes. It was then subjected to heat shock at 42°C for 90 seconds by keeping in water bath. This was then kept on ice for 5 minutes. 600µl of LB broth was added and inoculated at 37°C for 1 hour. Now transfer 300µl broth over solidified Agar with 1% starch plates. Incubate at 37°C and observe the plates for transformant for 3 consecutive days.

RESULT AND DISCUSSION

Bacillus subtilis was isolated from the soil sample by serial dilution method and screened for the starch production by starch hydrolysis test. The genomic DNA from the *Bacillus subtilis* was isolated and amplified by PCR. The amplified DNA fragments was transformed into the non-amylase producing bacteria *E.coli* using $CaCl_2$ and screened on Starch plates.

Table

S. No.	Biochemical test	Results
1	Grams stain	+ve
2	Catalase test	+ve
3	Gelatine test	-ve
4	Indole test	-ve
5	MR test	+ve
6	VP test	-ve
7	Citrate test	-ve

Figures

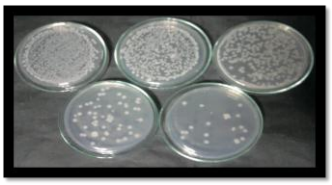

Fig. 1 Spread plates of Isolated Bacterial Colony

Fig. 2 Pure Colony of *Bacillus subtilis*

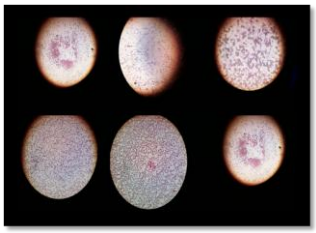

Fig. 3 Gram's Staining

Fig. 4 Starch Hydrolysis Test

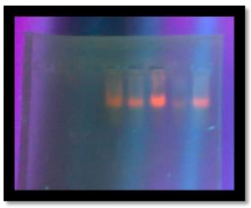

Fig. 5 Genomic DNA of *Bacillus subtilis*

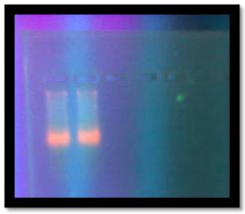

Fig. 6 Bands of Isolated DNA after PCR amplification

Fig. 7 Transformed cells on Agar with 1% starch solution

Conclusions

The gene of α-amylase from *B. subtilis* was cloned and expressed in Non-amylase producing *E.coli*. *B. subtilis* was isolated by performing various biochemical tests. The organism producing α-amylase enzyme was successfully isolated. The isolation of genomic DNA followed by amplification of Gene of Interest using α-amylase primer and annealing temperature 56°C was done using PCR technique. The amplified product was eluted from electrophoresis gel and subjected to restriction digestion (EcoRI) and then ligation with the vector (Plasmid DNA pUC18). A competent cell was prepared using chemical method ($CaCl_2$). The ligation mixture was allowed to react with the competent cells in order to produce transformed cells producing α-amylase enzyme. The mixture was spread over the starch plates. The successfully transformed E.coli cells were observed over the Starch agar plate.

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