development indicates the presence of GST

Results:

Isolated E.coli PGEX-3X host by alkali lysis method and was resolved on 0.8% agarose along with λ Hind III molecular weight marker, The PGEX 3X plasmid DNA was measured as 4900bp approximately. Quantification of DNA was done with ratio of absorbance at 260nm / 280 nm is 1.825 i.e. between 0.555/0.304

DNA concentration = 50*A260*dilution factor µg/ml

Dilution factor = total volume of the sample /volume of DNA preparation used

= 2000 µl/10 µl

= 200 µl

DNA concentration = 50*0.555*200 µg/ml

= 5550 µg/ml

Quantification of pure DNA was done in the ratio of absorbance at 1.825 and recovered. The absorbance ratio less or above was discarded as protein or RNA contamination.

The transformed PGEX 3X vector showed growth in ampicillin (100 µg/ml) containing LB agar plates. This inferred the presence of transformed PGEX-3X in E.coli BL21.IPTG induced GST was collected by cell lysis method from both BI and AI and cell lysate was analyzed through SDS PAGE. By protein specific for anti GST antibody [11].

performing SDS PAGE with both BI and AI reveals the presence of induced protein only in AI but not in BI. Before subjecting to the SDS PAGE the cell lysates were mixed with extraction buffer and centrifuged at 10k for 20minutes under 4°c to pellet out the insoluble proteins. The crude AI cell lysate was purified in glutathione cross linked agarose column .The trapped GST protein was eluted by elution buffer by checking with the absorbance 0.05 at 280nm indicates the glutathione CL agarose column contained only the expressed GST protein.

The CDNB assay values are calculated by the following equation

Δ A340/min/ml = A340 (t_0) - A340 (t_1)/(t_1-t_0) (ml of sample added)

A340 (t_0) = Absorbance at 340nm at time t_0 in minutes

A340 (t_1) = Absorbance at 340nm at time t_1 in minutes

(t_1-t_0) = time interval between the reading in minutes

Δ A_{340}/min/ml = $A_{340}(t_0)$- A_{340} (t_1)/(t_1-t_0) (ml of sample added)

= 0.009-0.012/1*0.05

=0.06

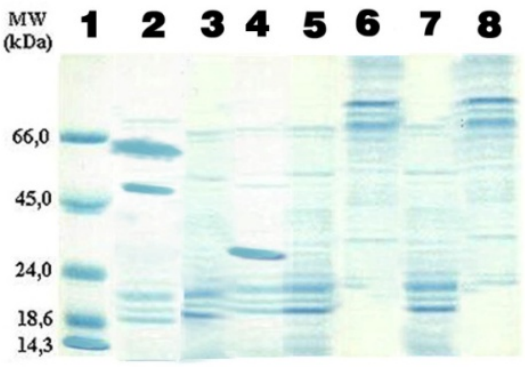

Figure 1

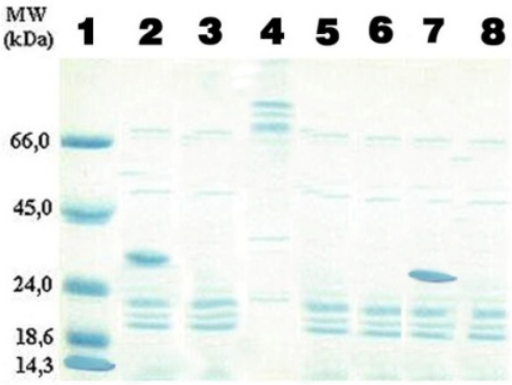

Figure 2

Immunodetection: The nitrocellulose membrane was carefully taken and subjected for immunodetection. The non specific sites in membrane are blocked with blocking buffer for two hours, which blocks all the proteins except GST. Later incubation with anti-GST HRP conjugate antibody bind only to the GST in the membrane. On washing with the wash buffer it washes out all the The GST activity is found to be increasing when the binding between glutathione and CDNB increases, and it was found to be decreasing gradually when binding decreases. (Table -1)

SDS PAGE GEL : In figure 1 lane1 shows protein molecular weight marker , lane 2 shows AI (After induction) with thick band indicating that IPTG has induced GST, lanes 3 contains BI(Before induction) sample which has no band, lane 4,5,6 were loaded with equilibrated sample EQ_1,EQ_2 and EQ_3 and no bands were seen, which indicates the absence of GST and lane 7 were loaded with Eluate (E_2) which shows sharp thin band measuring about 26KDa approximately.

SDS PAGE GEL In figure 2 the lane1 shows protein molecular weight marker, lane 2 were loaded with crude lysate (L) which contains all bacterial proteins except GST and lanes 3,4,5,6 ,7 and 8 were loaded with eluate samples E_2,E_3,E_4,E_5 and E_6 (E7was not shown). E_2 shows sharp thin band and other samples E_1, E3, E4, E5 and E_6 show faint bands (not 26kDa GST).E_2 was confirmed with immunodetection.

Western blotting :In figure 3 the lane 1 with protein molecular weight marker, lane2 shows 26kDa GST Eluate (E_2), lane3 were loaded with after induction sample(AI) containing induced protein and lane 4 contains crude cell lysate(BI) containing all other bacterial proteins except GST.

Immunodetection: In figure 4 the lane MW shows protein molecular weight marker detected by specific antibodies (Promega, unbound proteins in the membrane. The substrate TMB H_2O_2 specific for this HRP was added in dark condition, the GST protein formed complex with anti GST HRP conjugate antibody when reacted with substrate solution blue colour bands were observed on nitrocellulose membrane and hence inferred that isolated proteins were GST proteins.

USA), lane E2 shows sharp thin band approximately 26kDa GST protein detected by anti GST HRP conjugate antibody. Lane L was loaded with BI sample which has no band indicating the absence of GST.

Discussion:

Because of vast fund of knowledge about its genetics, biochemistry, and molecular biology, E.coli is the system of first choice for expression of many heterologous proteins. Genetic manipulation is straight forward and many foreign proteins were well tolerated and may be expressed at high levels. Expression of fused reading frames generates hybrid proteins in which the protein of interest is attached to carrier protein [12&13].Fusion proteins expressed from PGEX 3X contains the GST moiety and can therefore be purified to near homogeneity by affinity chromatography by glutathione agarose matrix. Bound GST proteins are readily displaced from the column by elution with buffers containing free glutathione.

In order to further elucidate the mechanism of IPTG induced GST protein in PGEX3X vector within the expression host BL21 and to monitor for its presence in the transformed cells. BI cell lysate reveals the absence of GST protein whereas SDS PAGE reveals the presence of expressed GST as a 26KDa thin sharp band was visualized again in SDS PAGE and its enzymatic activity was confirmed by CDNB method. Immuno blotting analysis with anti GST HRP conjugate confirms the presence of GST protein in the blotted membrane.

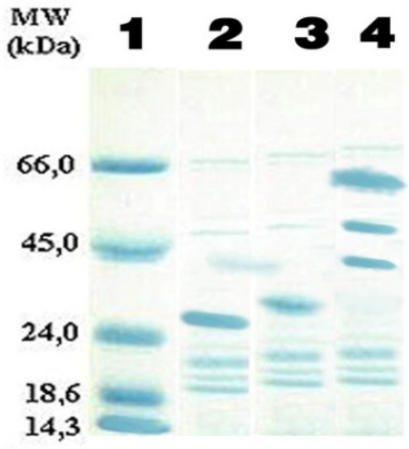

Figure 3

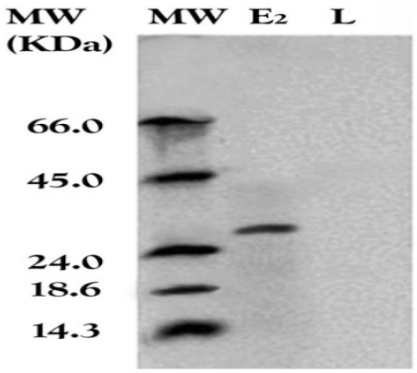

Figure 4

Any fusion protein with the GST can be cleaved and can obtain protein in the native and a biologically active form the fusion proteins cellular localization can be detected either by immuno electron microscopy and its targeting to the nucleus will be studied through transgenic approaches either by purifying a fusion proteins tagged to fluorescent marker like GFP are creating transitional fusion of the protein to β-glucoronidase (GUS) reporter protein followed by histochemical and biochemical analysis of GUS activity can be done. Invitro phosphorylation assay of the purified protein with different kinases will also assess whether phosphorylation mediates protein accumulation and function in different cellular compartments.

Table 1: The GST activity of isolated Protein.

TIME	OD AT 340nm	GST activity
t_0	0.009	-
t_1	0.012	0.06
t_2	0.016	0.08
t_3	0.020	0.08
t_4	0.022	0.04
t_5	0.023	0.02
t_6	0.024	0.02

Humans are exposed regularly to atleast 100 different chemicals which are naturally occurring which includes scientific pollutants that are highly mutagenic and carcinogens catalysed via conjugation of glutathione (GSH) to the electrophilic center of various carcinogens and mutagens. These belong to super family of multigene and multi functional dimeric proteins ubiquitously distributed in most of life forms i.e, animals, plants, insects, parasites, yeast, fungi, and bacteria. They occur in cytoplasm mitochondria microsomes and nuclei of each organ which possess a unique profile of GST.

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