



Role of Microbial Triose Phosphate Isomerase in Regulating Human Lung Cancer

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Abstract:

The current work aims to study the role of Microbial TPI in regulating the Human Lung cancer. Studies showed that there was a relative increase in the activity of the TPI in the patients suffering with cancer. The protein showed an increased expression in colon and bladder carcinomas. The study was undertaken to un reveal the molecular basis of this relation and also to prove the same. The article involved the extraction of DNA from microbe lactobacillus and isolation and amplification of TPI gene using molecular techniques. Transformation of the gene into the compatible cells of the E Coli Dh5 alpha cells. The transformed cells are used as a source f TPI to act against the Human cancer Cell lines. The effect of these TPI cells on the cancer cell lines can be read using Colorimeter. The overall observation of the work was that there was a substantial effect of TPI gene on the cell line growth rate.

KeyWords:

Triose Phosphate Isomerase, Transformation, Comptable, Carcinomas, Colorimeter

Introduction:

Triose Phosphate Isomerase is a dimeric enzyme that catalyzes the conversion of dihydroxy acetone phosphate (DHAP) and glyceraldehydes-3-phosphate (GAP) an important step in the glycolytic pathway^[1]. It has been referred to as a “perfect Enzyme” because the chemical steps of the reaction are accelerated sufficiently so the diffusion steps are rate limiting.^[2]. TPI deficiency is a recessive loss-of-function human disease resulting from missense mutations in the TPI gene. TPI deficiency is clinically characterized by symptoms such as hemolytic anemia, cardiomyopathy, neurological dysfunction and degeneration, and premature death^[3]. Pathogenic TPI deficiency mutations can affect the promoter or coding sequence and all have been reported to dramatically reduced TPI activity owing to changes in catalysis and/or enzyme stability^[4]. TPI deficiency has a very poor genotype-phenotype correlation and studies to elucidate pathogenesis are extremely limited, especially in animal systems. An inherited

deficiency occurs in humans and causes a rise in dihydroxyacetone phosphate. Clinical signs are hemolytic anemia, early death, and neurodegeneration^[5].

The results of some researchers, finding an absence of triosephosphate isomerase in most lymphoma cases, are in contrast with previous findings of other investigators. Two-dimensional electrophoretic analysis of lung adeno carcinoma revealed an increase of trios phosphate isomerase in 60% of adenocarcinomas compared with uninvolved lung^[6]. Similarly, increased expression of triosephosphate isomerase was found in lung squamous cell carcinomas^[7]. Triosephosphate isomerase has also been found to be increased in urinary cancers^[8]. Possibly, the down-regulation of triose phosphate isomerase in the lymphoma samples represents underlying cell type specificity, but overall, it seems that mis regulation of triosephosphate isomerase is a common feature of cancer^[9].

Materials and Methods:

Sample Collection for the isolation of the required organism:

To isolate the TPI gene the best organism selected was Lactobacillus. To isolate these organisms milk sample has been chosen. The sample was used to pick the inoculums plate it onto the Nutrient agar medium. After the incubation and culture growth the sample was used for the identification and pure culture preparation. Finally the pure culture of Lactobacillus was obtained that was used for broth production.

Extraction of the DNA from the Lactobacillus cells and purification:

The lactobacillus cells were processed for DNA Extraction followed by purification. The purification was done by Ammonium sulphate precipitation, and Dialysis. For the Extraction of DNA , MRS broth was inoculated with the Bacteria for 24-48hrs. The Broth is used for the DNA extraction by PCI method. Qualitative Analysis of DNA was done UV-VIS spectrophotometer to determine the amount, concentration and purity of the given DNA sample.

Amplification of the gene using Specific primers:

The pure DNA was later used for amplification of the TPI Gene using PCR. The specific primers were synthesized and used for amplification. The amplified gene is further visualized on Gel Electrophoresis.

Preparation of Competent cells:

Competence is the ability of a cell to take up extra cellular DNA from its environment. Competency can be artificially induced by treating the cells with CaCl₂ prior to adding DNA. The calcium destabilizes the cell membrane and adheres to the cell surface favoring the formation of the pores for the entry of DNA. The colonies were seen on the plates. Growth of the colonies indicates the successful competent cell produced.

Transformation:

The Amplified TPI gene product is used for transforming the E Coli DH5alpha cells that are competent and can take up the naked DNA. The transformed cells are detected using the Blue White Screening test for Transformation efficiency, using X gal media. Transformed cells are taken up and can be used for testing on cancer cell lines.

Screening For the Transformation Efficiency:

Competent cells were taken out from -80°C freezer and thawed in the ice. 5ul of the ligated product was mixed with the competent cells and incubated in ice for 30 min. The mixture was then subjected to heat shock at 42°C for 45 seconds. About 500ul of LB broth was added in a sterile condition and incubated under shaking conditions at 37°C for 1 hr. Meanwhile, 40µl of IPTG and 40µl of X-gal were plated on top of the LB AMP plates, and the plates were dried. About 100ul of the inoculum was plated on LB AMP plates containing and IPTG / X-gal, incubated overnight at 37 degrees C for 16 hrs. About 100ul of untransformed cells were plated on a LB AMP plates containing and IPTG / X-gal as a negative control. Blue colonies indicated the self ligated product and the white colonies indicated the recombinant products.

Extraction of the TPI Protein from EColi:

After the successful transformation the Ecoli cells starts producing the intracellular Protein TPI. The Incubation time enables the accumulation of the intracellular TPI. The next step in the series is to extract the TPI from the cells and use it in the further analysis All extraction procedures were carried out at 4_C.

Escherichia coli cell pellets were lysed using Bug Buster protein extraction reagent(Novagen, CA)

supplemented with a proteinase inhibitors cocktail (Rathinasabapathi et al. 2001).

Purification of the Protein:

The Purification of the above extracted crude protein is done using various molecular techniques like Salt Precipitation, Dialysis etc. The pure protein was further used for analysis.

Regulation of TPI on Cell Line Proliferation:

The human lung cancer cell line BT-20 was maintained at 37°C in 5% CO₂/95% air and passaged every 3 days in RPMI 1640 culture medium supplemented with 10% heat-inactivated FCS, 1% glutamine, and 1% penicillin/streptomycin. To 100ul of this cell suspension 100ul of each of the elutes 1 and 5 are added in 2 different eppendorffs while one eppendorff was kept as control in which no TPI was added. 1ml of Culture medium was added in all of them. These were incubated at 37°C in 5% CO₂ and 90% relative humidity in CO₂ incubator. O D was measured at 540nm daily for 4 consecutive days.

Results and Discussion:

**Isolation of *Lactobacillus delbrueckii* from milk:
The figure 1 shows the colonies obtained in the Master Plate:**



The figure shows the colonies selected for pure culture preparation

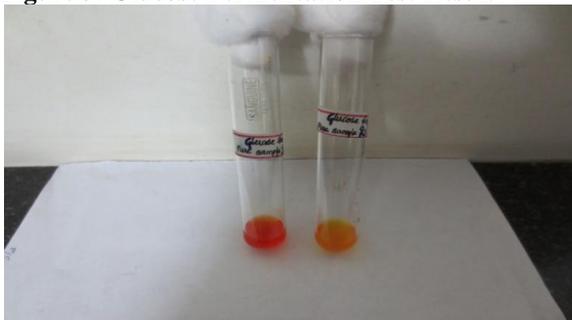
Figure 2: Showing the Pure culture Obtained after Plating:



Biochemical Tests for the confirmation of Bacteria
Table 1: Biochemical tests performed with their results to confirm the identity of the organism.

S.N	TEST	RESULT
1	Grams staining	Gram +ve rods
2	Spore Forming	-ve
3	Acid Fast Test	-ve
4	Catalase	-ve
5	Glucose Fermentation	+ve for Acid Production
6	Manitol Test	-ve

Figure 3: Glucose Fermentation Test Result



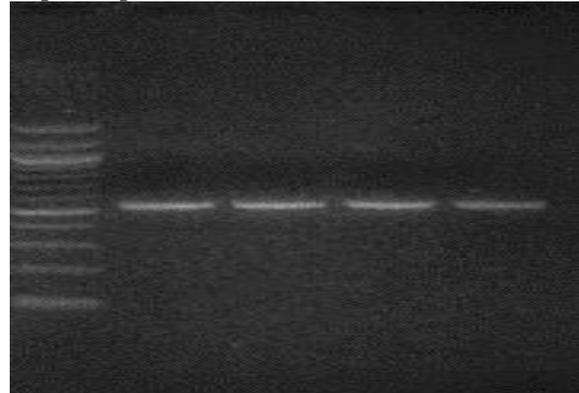
The above picture shows the negative result of glucose fermentation test as exhibited by the bacterial sample. Based on the above results the bacteria are confirmed to be *Lactobacillus delbrueckii*.

Table 2: Primer Designing for TPI gene for PCR Amplification:

Type	Primer
Forward primer	GGTAAGCTGCCAGATCCAAG
Reverse primer	ACCGATAGCCCAATTG GTT

Using the above specified primers and the extracted DNA from E Coli the amplification was performed using PCR.

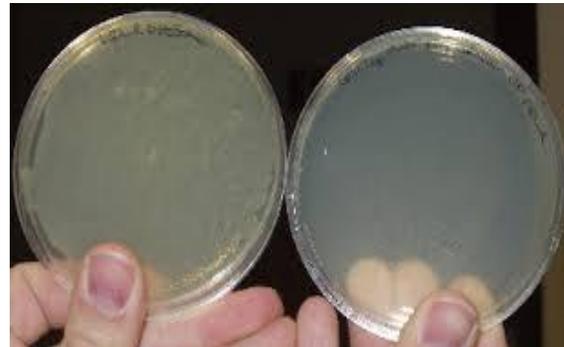
Figure 4: Gel Electrophoresis for the TPI gene amplified product after PCR



Transformation of E Coli cells with TPI gene:

The competent E Coli cells were used to transform with the TPI gene obtained from *Lactobacillus*. The efficacy of transformation was screened using X Gal medium based on Blue – White coloration. The colonies colored white indicates successful transformation and the blue color colonies indicate untransformed.

Figure 5: Blue White screening for identification of Transformed cells



Effect of Extracted TPI on Lung Cancer Cell Lines:

The TPI extracted is now added in various to the cell lines that were ordered and brought. The effect of this TPI on the regulation of the cells in the Cell lines is assayed using colorimetric study. The OD value of the cell lines inoculated with TPI at various intervals of time is taken and the results are analyzed. The results are shown below in the Table 3

Table 3: Showing the Colorimetric values obtained for different samples of Cell lines with TPI

Time (in	Control cell lines	Cell lines
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hrs.)	(Without TPI)	(With TPI)
0	0.18	0.18
24	0.23	0.26
48	0.34	0.40
72	0.39	0.44
96	0.43	0.49

From the above colorimetric results it can be noted that with the increased in the incubation time the OD value increases. This indicates a simultaneous increase in the cell concentration with the addition of TPI

Conclusion:

Isolation and identification of *Lactobacillus delbrukii* was done from milk and curd source following several biochemical tests and Gram's staining. A successful transformation of *Lactobacillus delbrukii* TPI into mutated E coli strain was also accomplished in this work by virtue of which the strains became able to grow in glucose media. The TPI gene was incorporated into E Coli cells thereby transforming them. The Transformed cell would synthesize the internal TPI protein which was later extracted and used to test against Cell Lines. The effect of TPI on the cell lines was analyzed using Colorimetric estimations. The colorimetric study showed that the addition of TPI would increase the cell proliferation rate to a great extent. This proliferation increased with an increase in the incubation time. This is due to the activation of glycolysis steps with the induction of TPI. This work concludes that the TPI has a positive influence on the cell proliferation and division rate.

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