CHAPTER 6
SUMMARY

The science of today is the technology of tomorrow.

--Edward Teller

Experimentations were carried out on the production, purification, molecular profiling and biotechnological industrial applications of the glucose isomerase enzyme from the soil samples and were presented and discussed in detail in the respective chapters of this thesis. The salient features of the present investigation are summarized as follows:

Isolation and primary screening of glucose isomerase producing bacteria was carried out from different soil samples collected from various sites of Udaipur district. Forty two isolates were obtained from these sources out of which thirteen showed isomerization ability. The cultures obtained were spotted on all three formulated media and their growth and ability to isomerize glucose to fructose was checked by plate assay method. The organisms which gave better growth were considered to be glucose isomerase producers. The enzymatic activity was measured for 13 bacterial isolates which was ranged 0.1-1.0 U/ml. Among thirteen, 4 isolates namely GIUC-1, GINM-3, GIBD-9 and GIBG-5 showed maximum production of glucose isomerase. These four bacterial isolates were selected for further studies.

A total of four isolates were profiled by studying their cultural, morphological, biochemical and molecular characteristics. All four isolates showed different cultural characteristics on nutrient agar. All four isolates GIUC-1, GINM-3, GIBD-9 and GIBG-5 reacted with different biochemical test and matched with Bergey’s manual. All four isolates namely isolate GIUC-1, GINM-3, GIBD-9 and GIBG-5 were identified through 16S rRNA gene sequencing as Bacillus megaterium GIUC-1 (KY568090), Bacillus licheniformis GINM-3 (KY492396), Bacillus sp. GIBD-9 and Bacillus pumilus GIBG-5 (KY744701) respectively.
Optimization for enhanced production of bacterial glucose isomerase for all four isolates was carried out. All four isolate showed highest yield in the formulated medium with initial pH 7.0. The maximum production (0.795 U/ml) of glucose isomerase was observed at in the formulated medium with initial pH 7.0 by Bacillus megaterium GIUC-1.

For all four isolates 40°C was found to be optimal temperature. The maximum production (0.709 U/ml) of glucose isomerase was observed at in the formulated medium with initial pH 7.0 at 40°C by Bacillus megaterium GIUC-1. The maximum reaction time required for elevated values was found after 48h of incubation by all four isolates. Among the various carbon sources used in the study xylose was found to be the best as Bacillus megaterium GIUC-1 showed maximum production (0.796 U/ml) in the formulated medium containing xylose at initial pH 7.0 at 40°C after 48h. The minimum concentration of xylose at which maximum production (0.835 U/ml) of glucose isomerase was recorded for Bacillus megaterium GIUC-1 was 2000 µg/ml.

The Plackett-Burman design for Bacillus megaterium GIUC-1 has maximum glucose isomerase activity of 0.865 U/ml was obtained in the medium containing 2.5% (w/v) xylose, 1% (w/v) MgSO₄·7H₂O, and 2% peptone having pH 7 with inoculum of 1% (v/v) and incubated at 40°C at 250 rpm (Run No. 7). The Analysis of Variance (ANOVA) demonstrated that the model was significant with p-value < 0.001 with Model F-value of 9634.14, having p value of 0.04 indicating that the model terms were significant. From the CCD model study, xylose (A), peptone (B), MgSO₄·7H₂O (C), AB, BC, A², B² and C² were observed as significant to enzyme production.

Partial purification of glucose isomerase from Bacillus megaterium GIUC-1 was carried out by salt and solvent precipitation method. Acetone precipitation was carried in varying ratios and it was found that 1:4 ratio of enzyme: acetone yielded maximum glucose isomerase activity. However, acetone precipitation was not effective process to concentrate the enzyme as compared to ammonium sulphate precipitation. Ammonium sulphate salt precipitation was carried for salting-in process to partially purify the enzyme. The saltingout process was carried out by dialysis membrane of 30kDa MWCO
against buffer. Extracellular glucose isomerase was precipitated at 70% saturation with 0.768 U/ml of enzyme activity. The partially purified enzyme was subjected to gel-filtration chromatography using Sephadex G-50 column with elution flow rate of 1 ml/min. The 20th fraction gave the highest specific activity of 10.58 U/ml.

The glucose isomerase enzyme obtained from *Bacillus megaterium* GIUC-1 is a tetramer of four polypeptide chains of 43,000 D. The band of partially purified GI observed on SDS-PAGE gel near the marker band of 43,500 D. It produced a thick band, which indicated that the protein is polymeric in nature. The purified enzyme revealed highest enzyme activity at 40°C with pH 7.0. Magnesium sulphate, cobalt chloride and sodium chloride showed stimulatory effect on glucose isomerase activity, while all the other metal salts inhibited enzyme activity. The enzyme kinetics was studied using glucose as substrate where $K_m$ and $V_{max}$ values were recorded. The value of $V_{max}$ was 41.62 and the $K_m$ value so obtained was 24.17 which is slightly higher than the half of the $V_{max}$.

Verification of isomerized products from various treated enzyme samples like crude, partially purified and purified enzyme was carried out against reference sample through analytical techniques such as TLC, HPTLC and FTIR for detection of isomerized product fructose. The results obtained from the above techniques confirmed the presence of fructose.

The glucose isomerase obtained from four bacterial isolates (*Bacillus megaterium* GIUC-1, *Bacillus licheniformis* GINM-3, *Bacillus* sp. GIBD-9 and *Bacillus pumilus* GIBG-5) was evaluated and checked for production of amylase. Out of all the four GI producing bacterial isolates, *Bacillus licheniformis* GINM-3 showed maximum amylase (specific activity 23.96 mg/ml) production potential.

Various substrates have been provided to glucose isomerase obtained from *Bacillus megaterium* GIUC-1 to validate its property in term of fructose production. Glucose served to be the best substrate with maximum fructose production *i.e.* 0.30 mg/ml. But subsequent production of fructose as 0.28mg/ml with the use of corn seeds is taken into
consideration with higher application value as corn seeds are cheaper, easily available agro waste. Therefore, glucose isomerase obtained from *Bacillus megaterium* GIUC-1 showed promising potential in multi-disciplinary fields which can be exploited for commercial purposes.