Name: Razique Anwer

Supervisor: Prof. Tasneem Fatma

Department: Biosciences

Title: "Investigation of Insulin like protein from *Spirulina*"

ABSTRACT

Objectives

1. Standardization of protocol for insulin like protein isolation for the screening of Indian *Spirulina*
2. Isolation, purification and characterization [SDS PAGE, Western blot, RP-HPLC, Circular Dichroism and MALDI-TOF (Sequences)] of insulin like protein from best strain
3. Correlation between growth phases and insulin like protein production
4. Culture condition manipulation for enhancement of insulin like protein
5. Testing of antidiabetic potential of isolated protein in streptozotocin-induced diabetic rats

Results

1. 16 out of 23 strains of *Spirulina* showed positive for insulin like protein by ELISA.
2. Characterization was done by SDS-PAGE and Western Blotting, an approximately 6-kDa protein band similar to bovine insulin marker and also showed similar antigenicity.
3. Retention time of extracted insulin like protein from *Spirulina* same as the bovine insulin.
4. Qualitative characterization by Circular Dichroism, spectrum of both *Spirulina*'s insulin like protein and bovine insulin showed α-helical structure in far UV region.
5. Amino acid sequencing of *Spirulina's* insulin like protein was done and found only one blast hit on the query sequence.
6. To check the efficacy of insulin like protein animal studies was performed.
   i. Body weight of diabetic rats was increased after treated with crude, aqueous extract, ethanolic extract and insulin-like protein of *Spirulina.*
   ii. Raised levels of blood glucose in diabetic rats declined sharply after oral feeding of crude (126.37 mg/dl) and insulin-like protein of *Spirulina* (131.57 mg/dL).

iii. HbA_{1C} levels decreased after administration of insulin-like protein of *Spirulina.*

iv. SGOT, SGPT, Alkaline phosphatase and Bilrubin total were found decreased in diabetic rats after treated with crude, aqueous, ethanolic and insulin-like protein of *Spirulina*.

7. After treatment with crude, aqueous, ethanolic extract and insulin-like protein of *Spirulina*, creatinine serum, uric acid serum and urea nitrogen were found to be reduced diabetic rats.
8. The growth was measured in terms of protein every ^{3rd} day till 18 days, along with insulin determination.
9. *S. platensis* CFTRI Mysore which yielded maximum amount of insulin like protein was grown in Zarrouk's medium for culture conditions manipulation effect on insulin yield.
   i. Highest growth and insulin like protein was found at 55mM $NaNO_3$ on 12^{th} day as compared to control.
   ii. K_2HPO_4 supplement there was slight increase in protein as well as in insulin like protein content (3.70%) at 5.5mM conc.
   iii. The maximum growth and insulin like protein was noticed in 180 mM $NaHCO_3$.
   iv. In the case of sulphates protein as well as insulin like protein increased to 6% and 26% respectively as compared to control.
   v. Increased in growth and insulin like protein was noticed in 1.2mM of calcium on 12^{th} day.
   vi. Highest growth as well as insulin like protein at 10mM glucose on 12^{th} .
   vii. In selenium suplimentation maximum growth and insulin like protein were observed at 0.30mM.
   viii. Varying in pH, growth and insulin like protein inceased at pH 10.

Presence of insulin like protein in plant system shows parallelism between plant and animal physiology for glucose metabolism. My results as well as findings of many investigators suggest that insulin is a molecule present in all organisms, from unicellular to multi-cellular organisms. Immuno-recognition with anti-bovine or human insulin antibodies in *Spirulina* suggested that the insulin-like peptide is present in many organisms, other than animal pancreas and their conserved nature during evolution.