Integration of SPC-insulin plasmid into E.coli BL21 expression system to develop a long-lasting effect of insulin and reducing dosages in a cost-effective manner

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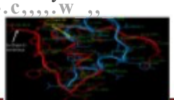
Background

The WHO listed insulin as an essential medicine, however the current medication is short-lasting(6-8 hrs) which causes people to take multiple dosages throughout theday

Another concern is that in order to produce insulin it takes two steps which makes it expensive, but if cost effective expression systems are used such as E. coli or yeast in combination with the SPC-insulin plasmid it will decrease production to one step

The SPC-insulin plasmid is a novel developmentthat would increase the lastingeffectivity of insulin to 24-48 hrs.

This is possible due to thestructure of insulin in which linking thea-chain N terminus and IH:hain C- terminus would create an active single chain that would delay theactive insulinfrom reaching the inactive a and b chains (Fig A) -.c,,,,.w ,,



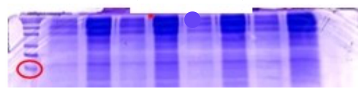
Methodology

- First, I ceU cultured E. coli BL21 that contains theSPC-insulin plasmid in Ampicillin (Amp) selected ag 1 es using the spread plate method
- Then, selectedabout 12colonies from the Amp(+) plate to determine where/which colony has theexpressed SPC-insulin plasmid
- For each corresponding colony, thedilution was induced with IP'TG to increase expression of the SPC-insulin
- Later, I an bromophenol blue SOS- PAGE gel and analyzed theelectrophoresis to determine thecolonythat expresses SPC-insulin around the 5808 Da band, which was colony 2
- The next stepis cell culturing and amplifyingcolony2 with LB broth/ Amp on Amp(+) agar plate to be used later for inductionand re-examination with SOS. PAGE gel

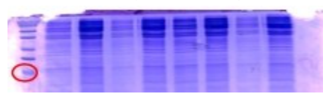
Results

Bromophenol blue SOS-PAGE gel results for the 12 colonies and induced correspondence

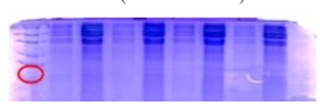
Gel I (colonies 1-4)



Gel 2 (colonies 5-8)



Gel 3 (colonies 9-12)



Gels: Column one is the standard to be compared. Then every two columns represent first the non induced then the induced.For example, column twois colony I non-induced, column three colony I induced and colony 2,3,4,5,6,7,8,9,10,11, and 12 follows the same pattern. The red circle represents where an insulin protein would be located which indicates in which colony the SPC-insulin is expressed.

After careful analysis of the gels, it was determined that colony two (•) showed the best expression of the SPC-insulin.

Conclusion

Since colony two was determined to be the best colony for expression so colony two wiU be cell cultured,amplifiedand induced with IP'TG and will rerun SOS gel to ensure presence of the inulin protein

Procedures to extract and purify the insulin protein for further experimentation to proceed with the second aim of this project which is creating an appropriate"prototype" bioreactor for theexpression of SPC-insulin

Acknowledgment

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