P2.122 – High level soluble expression of human Glucagon-Like Peptide-1 (GLP-1) analogue as a recombinant peptide in *E. coli*

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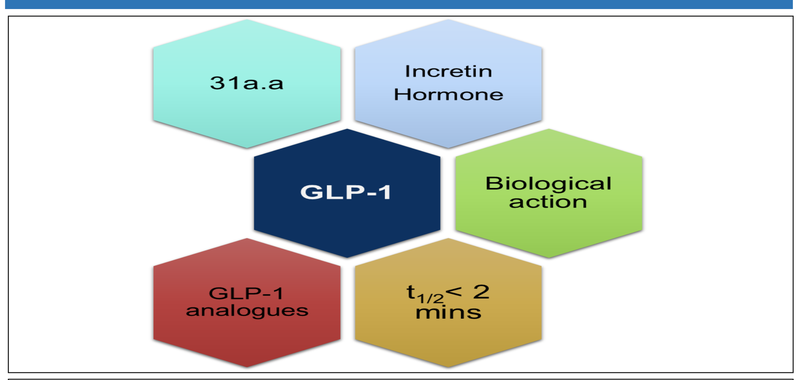
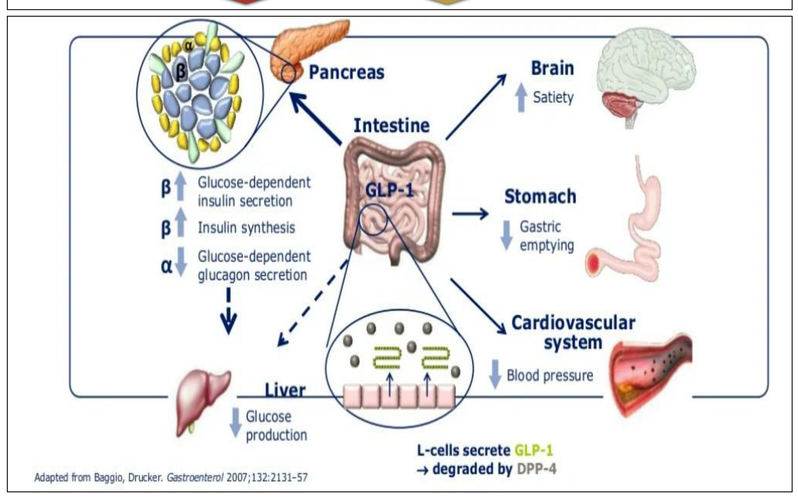
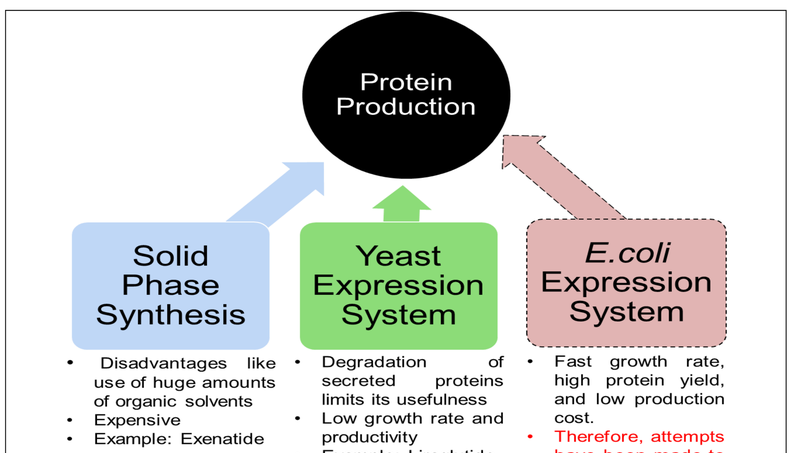
Abstract

Human GLP-1 is an incretin gut peptide hormone produced by proteolytic cleavage of proglucagon. The 31 amino acid peptide GLP-1 stimulates postprandial insulin secretion and treats Type II diabetes mellitus. This drug is currently marketed as Liraglutide, an acetylated GLP-1 analogue produced in *S. cerevisiae*. The goal of this study is to develop a bioprocess for producing GLP-1 analogues for Type II diabetics that are affordable. It has been attempted to express GLP-1 analogues on an *E. coli* host platform due to its advantages in yield and productivity. An expression construct has been designed in which an analogue of GLP-1 is fused with GST and an enterokinase (EK) site is sandwiched between them. Using a medium, inducer concentration, temperature, pH, and strains, we optimized expression in shake flask experiments to improve fusion protein expression in soluble form. In a series of batch fermenters, the kinetics of GLP-1 analogue production have been studied. We investigated how the growth stage before induction affects GLP-1 expression. We induced cultures during early, mid, and late log phases of growth, which were measured at optical densities (at 600nm) of 1, 10, and 20 respectively. We then monitored the expression for 12 hours after induction. Using information from previous batches, the fed-batch was designed to maintain a pre-induction specific growth rate (μ) of 0.35 h^{-1} and a post-induction specific growth rate (μ) of 0.15 h^{-1}. Fed-batch fermentation resulted in approximately 88.9 g/L dry cell weight at 8 hours post induction with GST-GLP-1 analogue. A volumetric yield of 10.3 g/L was obtained at an OD600 of 180. Purification of the GLP-1 analogue from the fusion tag was achieved by affinity chromatography followed by enzymatic cleavage with enterokinase. Affinity chromatography was used to purify the cleaved GLP 1 analogue, which was then identified by western blotting and mass spectrometry.

Introduction

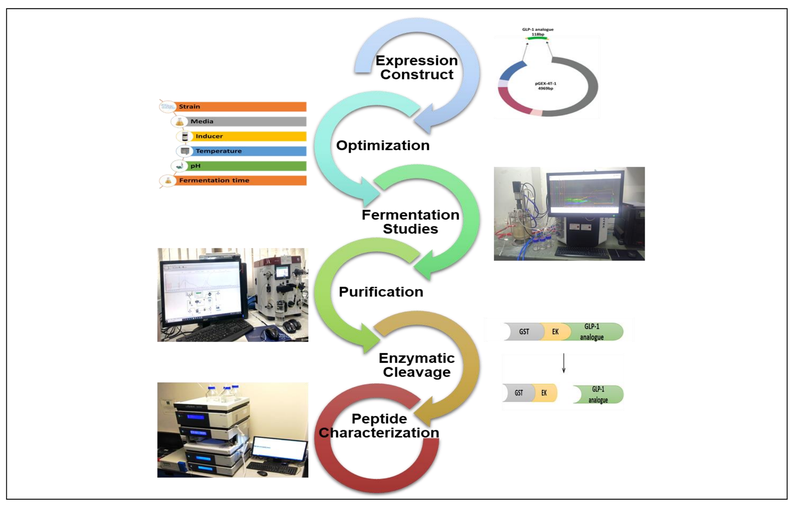






Methodology

Workflow



Results

(A) Growth kinetics and expression of GST-GLP-1 analogue in Batch fermentation

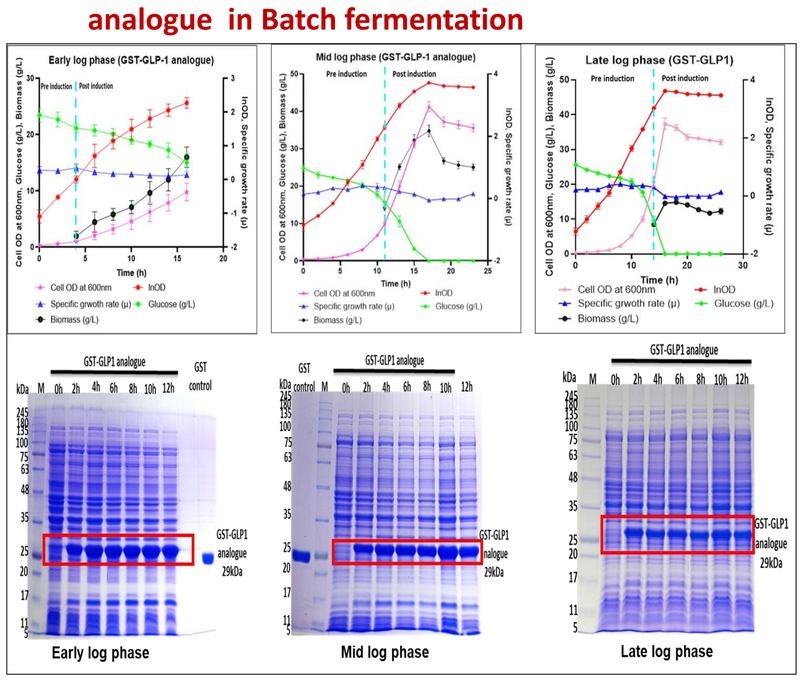
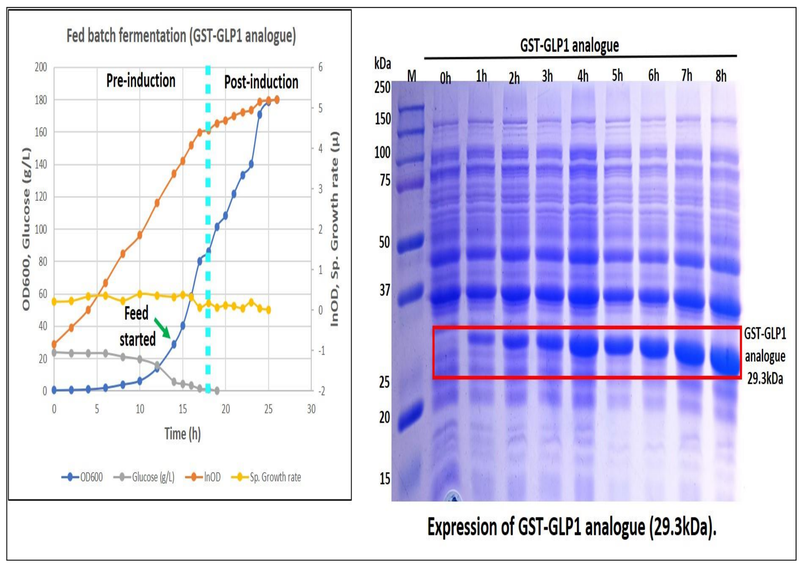


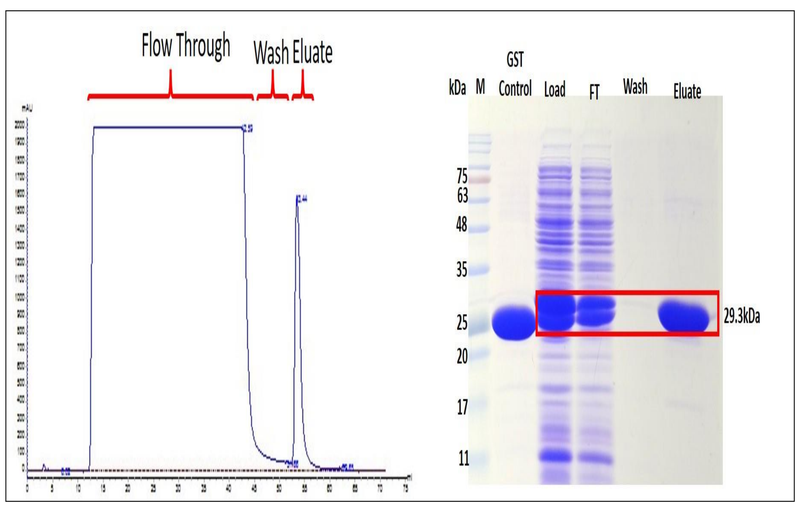
Table showing the specific product yield and volumetric yield of GST-GLP-1 analogue in Batch mode fermentation

S.No	Log phase	Induction at OD_{600} nm	Final Biomass (OD at 600nm)	Total GST-GLP1 analogue yield (mg/L)	Specific GST-GLP1 analogue yield (mg/g)
1	Early log phase	1	~10	~1800	~110
2	Mid log phase	10	~35	~2100	~90
3	Late log phase	20	~32	~650	~52
4	Uninduced culture	-	~35	-	-

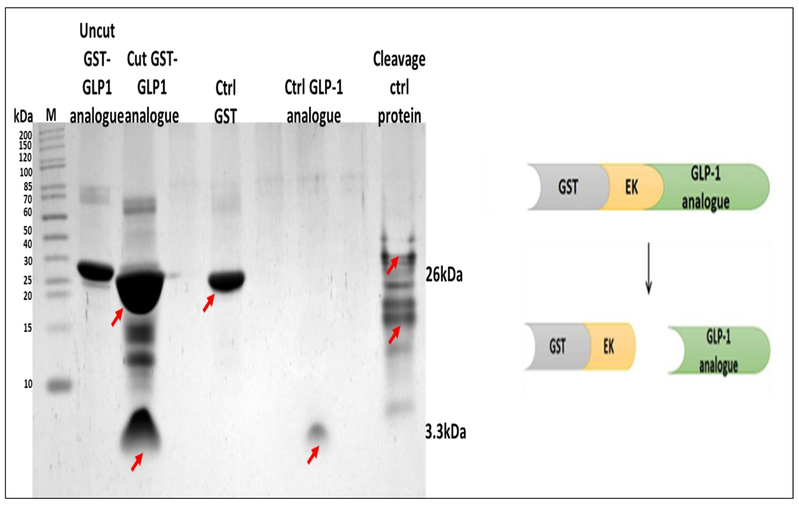
(B) Growth kinetics and expression of GST-GLP-1 analogue in Batch fermentation



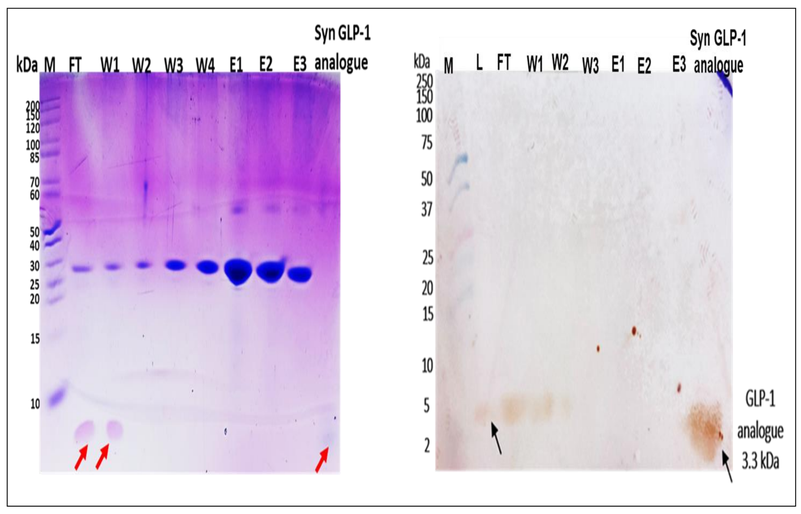
Batch	Time (h)	Induction point	Final OD	Biomass (g/L)	Volumetric yield (g/L)	Specific yield (g/g)
Fed Batch	8	86	180.3	88.9	10.37641	0.11672



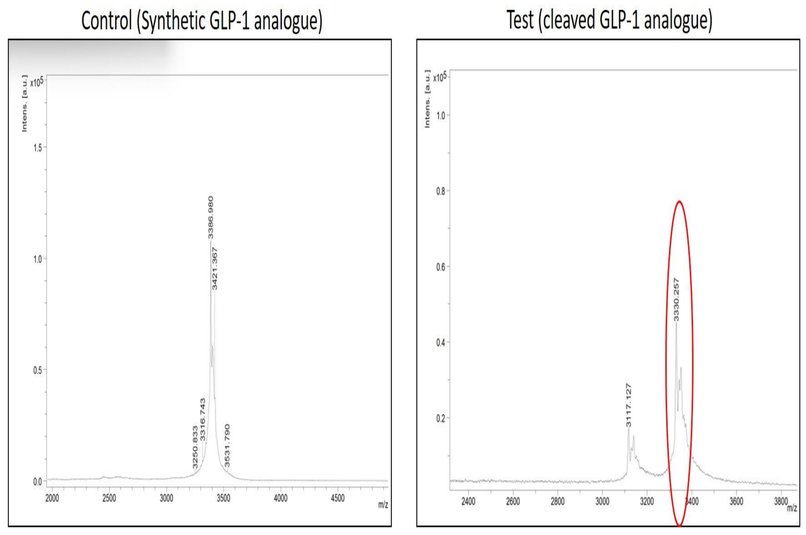
(D) Enzymatic cleavage of GST-GLP1 analogue and its identity confirmation wtih western blot



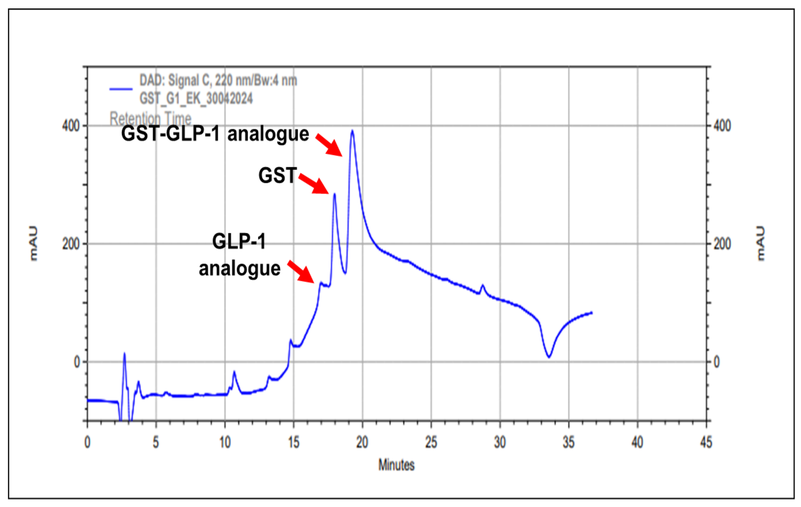
(E) Purification of cleaved GLP-1 analogue



(F) Confirmation of Molecular weight of cleaved GLP-1 analogue by mass spectrometry



(G) Confirmation of cleaved GLP-1 analogue by RP-HPLC



Conclusion

- GLP-1 analogue was successfully cloned in pGEX4T1 vector with N-terminal GST tag for expression. High expression of GST-GLP1 was achieved in BL21(DE3) strain in the synthetic medium at temperature of 22°C and after induction with 0.5mM IPTG.
- In Batch fermentations, high specific activity and volumetric yield are observed when the culture was induced at mid-log phase that is OD_{600} of 10.
- In fed-batch fermentation, the volumetric yield of GST-GLP1 analogue was 6270 mg/L and specific GST-GLP-1 analogue yield was 116 mg/g.
- Fusion protein GST-GLP-1 was purified by affinity chromatography and cleaved with r-Enterokinase enzyme to confirm the peptide identity with western blot.
- Intact mass of the cleaved GLP-1 analogue was confirmed.
- Confirmation of cleaved GLP-1 was confirmed by RP-HPLC.

References

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Acknowledgments

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