探討使用發酵槽培養大腸桿菌並以自動誘導法生產重組酵素的條件

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Outline

- **Introduction**
- **An efficient approach for overproduction of DNA polymerase from *Pyrococcus furiosus* using an optimized autoinduction system in *Escherichia coli***
- **Production of recombinant D-allulose 3-epimerase utilizing an auto-induction approach in fermenter cultures suitable for industrial application**
- **Conclusion**

Abstract

High fidelity DNA polymerase from Pyrococcus furiosus (Pfupol) is in great demand for biotechnological applications, optimizing Pfupol production is essential to supplying the industry's expanding demand.

D-Allulose 3-epimerase (DAE) is the key enzyme catalyzing D-fructose into D-allulose, a rare sugar in foods, which has lately drawn increasing worldwide attention owing to its possible health advantages and application as a substitute of sucrose.

This work focused on the development of an economical, scalable production method of enzyme by using the Escherichia coli BL21 star™ (DE3) as expression host. T7 expression systems is used to express recombinant enzyme in *Escherichia coli*; optimize the production of the enzyme through an auto-induction strategy in chemically defined media by using lactose as a natural inducer, thereby overcoming various limitations of conventional IPTG induction methods. In 5 L bioreactor auto-induction-based strategy demonstrated its potential for large-scale production of enzyme in a cost-effective manner with enhanced reproducibility, which makes it an economically viable and practically useful approach.

Keyword: bioreactor, auto-induction-based, D-allulose 3-epimerase (DAE),

Pyrococcus furiosus (Pfupol)

References

Briand L, Marcion G, Kriznik A, Heydel JM, Artur Y, Garrido C, Seigneuric R, Neiers F (2016) A self-inducible heterologous protein expression system in *Escherichia coli*. Sci Rep.

Blommel PG, Becker KJ, Duvnjak P, Fox BG (2007) Enhanced bacterial protein expression during auto-induction obtained by alteration of lac repressor dosage and medium composition. Biotechnol Prog.

Bu Z, Biehl R, Monkenbusch M, Richter D, Callaway DJE (2005) Coupled protein domain motion in Taq polymerase revealed by neutron spin-echo spectroscopy. Proc Natl Acad Sci USA.

Din RU, Khan MI, Jan A, Khan SA, Ali I (2020) A novel approach for high-level expression and purification of GST-fused highly thermostable Taq DNA polymerase in *Escherichia coli*. Arch Microbiol.

Golayj S, Tolami HF, Riahifar V, Toulami S, Jorshari S, Nazemi A (2014) Expression and simple purification of cold sensitive I707L modified Taq DNA polymerase sequence in *Escherichia coli*. Adv Stud Biol 6:137–148.

Khani MH, Bagheri M (2020) Skimmed milk as an alternative for IPTG in induction of recombinant protein expression. Protein Expr Purif.

Laksmi FA, Nirwantono R, Nuryana I, Agustriana E. (2022)Expression and characterization of thermostable D-allulose 3-epimerase from Arthrobacter psychrolactophilus (Ap DAEase) with potential catalytic activity for bioconversion of D-allulose from d-fructose. Int J Biol Macromol.;214:426–38.

Lee SY (1996) High cell-density culture of *Escherichia coli*. Trends Biotechnol.

Lu C, Erickson HP (1997) Expression in *Escherichia coil* of the thermostable DNA polymerase from *Pyrococcus furiosus*. Protein Expr Purif.

Studier FW (2005) Protein production by auto-induction in high density shaking cultures. Protein Expr Purif.

Zhang J, Reddy J, Buckland B, Greasham R (2003) Toward consistent and productive complex media for industrial fermentations: studies on yeast extract for a recombinant yeast fermentation process. Biotechnol Bioeng.