E. coli Fermentation Optimization for Production of an Active Enzyme

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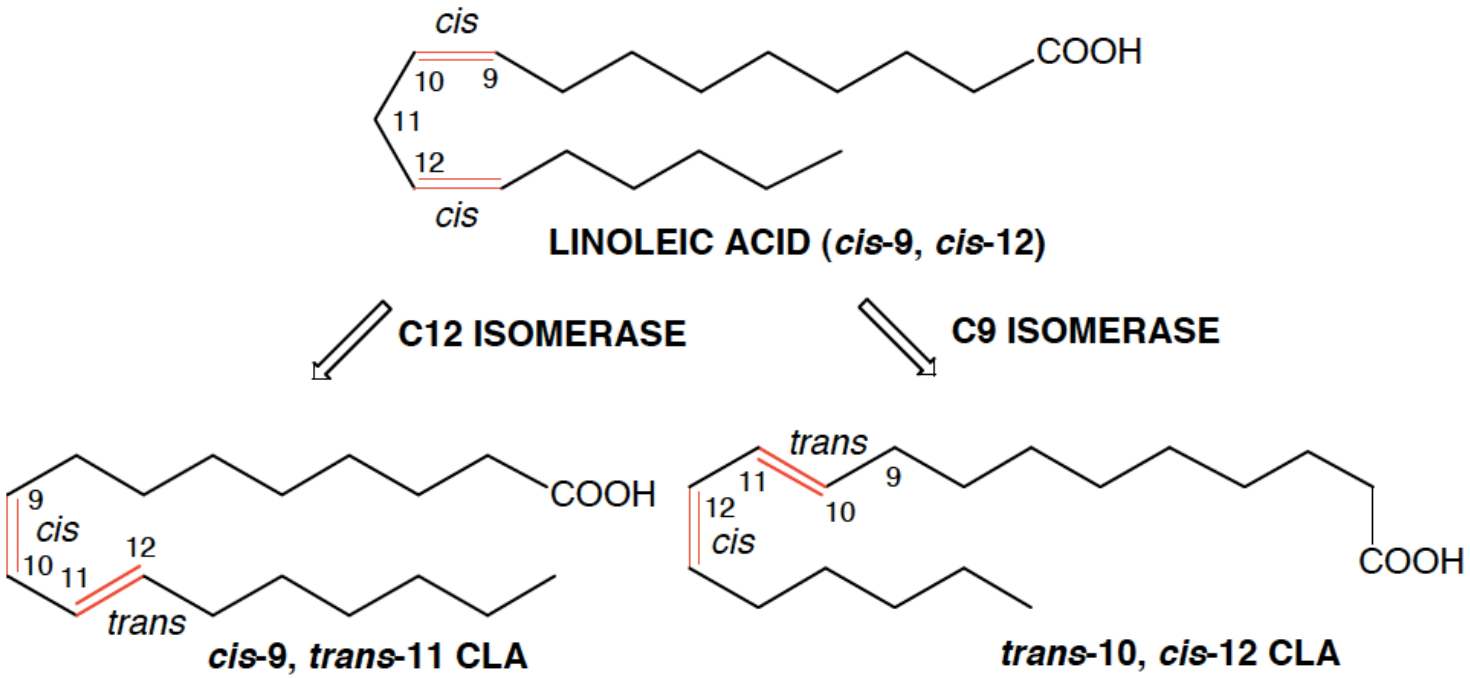
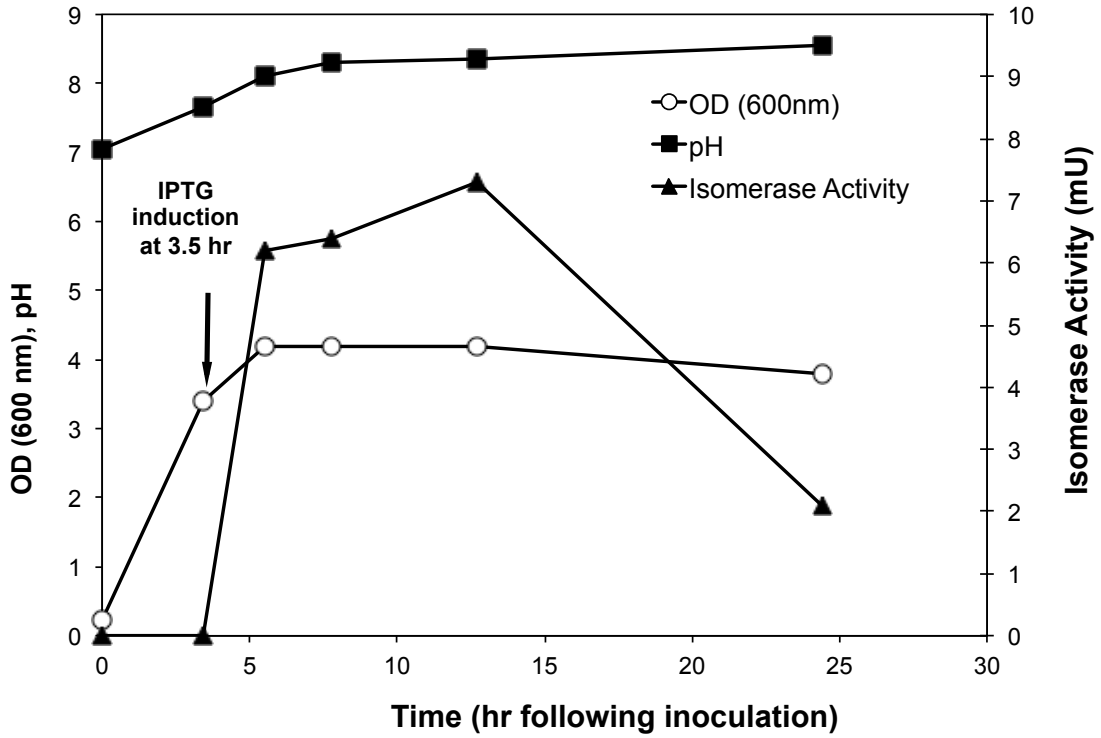
POI: Linoleic Acid C9 Isomerase



- Linoleic acid isomerase purified and gene cloned from *Propionibacterium acnes*
- Conversion of linoleic acid (*c*9,*c*12, 18:2) to conjugated linoleic acid (*t*10,*c*12, 18:2)
- Recombinant isomerase (55 kDa) expressed at high level in *E. coli*, but almost all in inclusion bodies (IB) in shake flask cultures
- IB solubilization & refolding: results not satisfactory

Poor Expression of Soluble Isomerase in Fermentation Using LB Medium

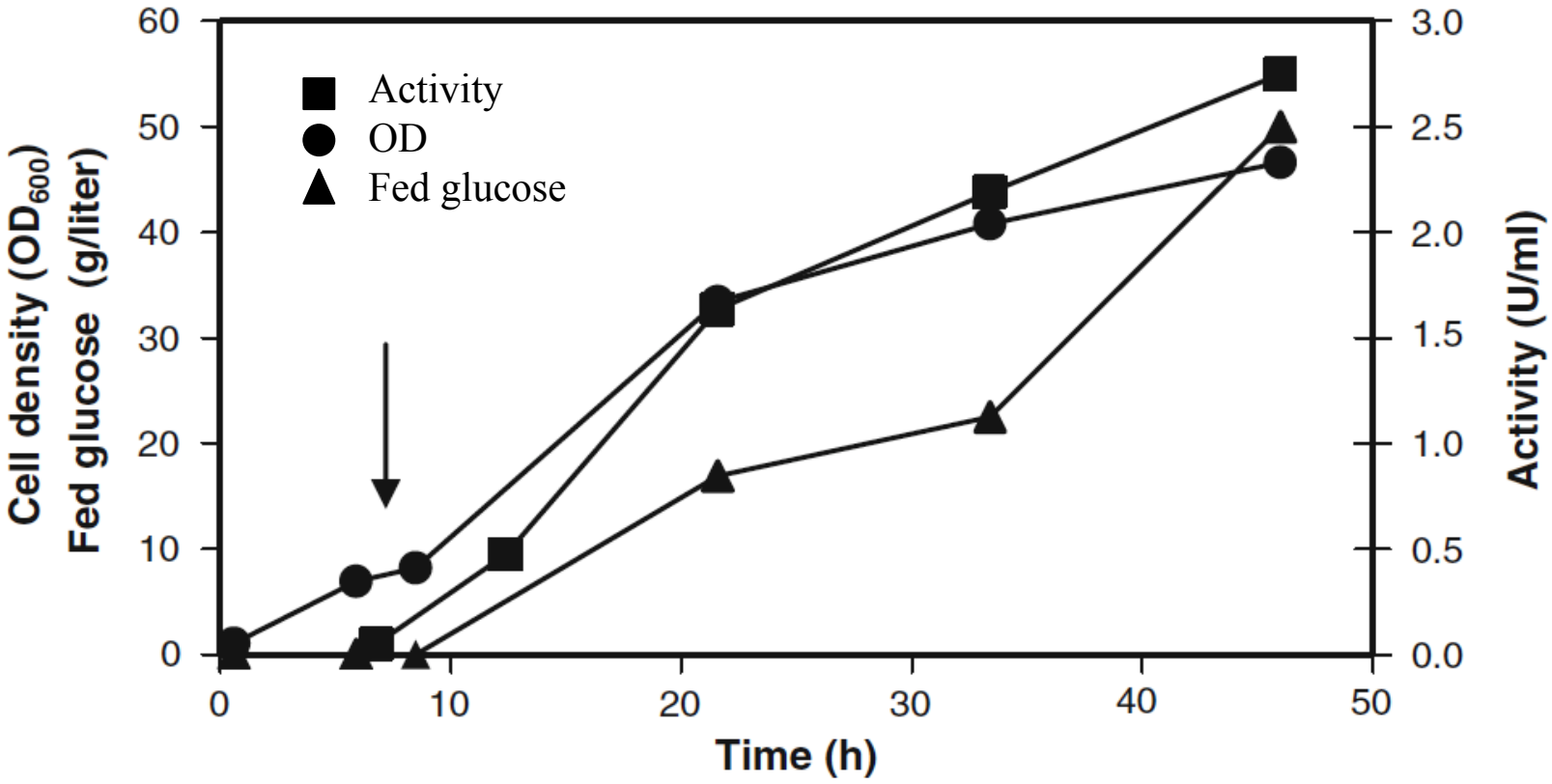


- Cells were grown in LB at 37 C for 3.5 hr, and induced with 0.4 mM IPTG.
- Isomerase activity research 37 mU after 2-hr induction, increased slightly to 43 mU. Activity declined to 12 mU at the end of experiment (21 hr post induction).
- Again, almost all isomerase in inclusion bodies.

Fermentation Process Development

- 2 Mineral salts-based media evaluated
- Supplemented with N-Z amine and glucose (immediately 10 fold improvement vs LB)
- IPTG: concentration, timing, temperature, pH
- Dissolved oxygen (DO) levels
- N-Z amine levels
- Amount of glucose and feeding rate

Optimized Fermentation Process



- Mineral salts-based medium, supplemented with N-Z amine, fed with glucose
- Growth phase (6 hr) at 37 C)
- Induction phase: started by adding 1 mM IPTG (26 C)

Outcome of the Development Program

- Fermentation conditions optimized:10-fold higher cell density than LB; 376-fold higher soluble isomerase
- Several 14-liter fermentations for production
- Down-stream processing: cell harvest and homogenization
- DEAE Column chromatography for partial purification
- Successfully prepared 50 to 60 g partially purified enzyme (at ~80% purity) for bioconversion studies

Reference

Deng MD et al, Linoleic acid isomerase from *Propionibacterium acnes*: purification, characterization, molecular coning, and heterologous expression. Applied Biochem and Biotechnol 2007, 143:199-211