

Chaperone 첨가에 의한 LipA와 LplA 발현시 α -Lipoic acid 생산 증가

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Chaperone-aided Expression of LipA and LplA Followed by the Increase in α -Lipoic Acid Production

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Objectives

To improvement α -lipoic acid production by chaperone-aided expression of LipA and LplA

Materials and Methods

This is metabolic engineering study for higher LA production with the aid of chaperone plasmids, *DnaKJE* and trigger factor(*Tf*). The *lipA* and *lplA* genes encoding lipoate synthase and lipoate protein ligase in *Pseudomonas fluorescens*, respectively, were cloned and transformed into *E. coli* K12.

Results

It were overexpressed in *E. coli*, both LipA and LplA were expressed as inclusion bodies leading to no increase in LA production. However, when chaperone plasmids *DnaKJE* and *Tf* were coexpressed with *lipA* and *lplA*, the resulting recombinant *E. coli* strains showed higher LA production than the wild-type *E. coli* by 32% - 70%. This result describes the first metabolic engineering study using the enzymes involved in LA biosynthesis.

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시험성적

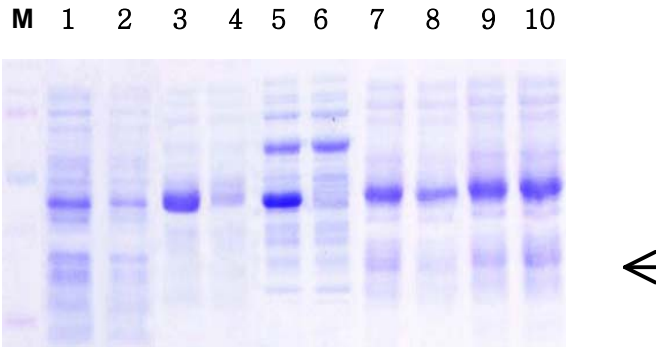


Figure 1. SDS-PAGE analysis of *E. coli* harboring pQE80L-*lipA* and chaperone plasmids. Lane: M, Molecular weight marker; 1, Total fraction of *E. coli* harboring pQE80L; 2, Soluble fraction of *E. coli* harboring pQE80L; 3, Total fraction of *E. coli* harboring pQE80L-*lipA*; 4, Soluble fraction of *E. coli* harboring pQE80L-*lipA*; 5, Total fraction of *E. coli* harboring pQE80L-*lipA* and GroELS; 6, Soluble fraction of *E. coli* harboring pQE80L-*lipA* and GroELS; 7, Total fraction of *E. coli* harboring pQE80L-*lipA* and DnaKJE; 8, Soluble fraction of *E. coli* harboring pQE80L-*lipA* and DnaKJE; 9, Total fraction of *E. coli* harboring pColdTF-*lipA* and DnaKJE; 10, Soluble fraction of *E. coli* harboring pColdTF-*lipA* and DnaKJE. The arrow at 40kDa indicates the LipA protein.

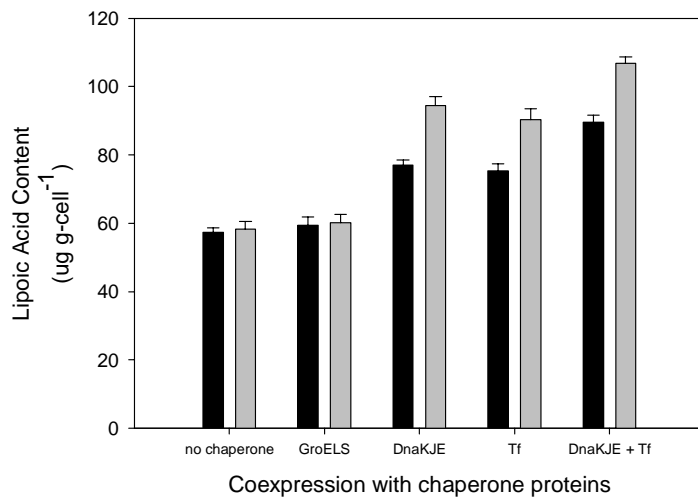


Figure 4. Effect of chaperon plasmid coexpression on the production of lipoic acid in *E. coli*. With the coexpression of chaperone plasmids (groELS, dnaKJE, Tf, and dnaKJE+Tf), the specific content of α -lipoic acid in *E. coli* harboring pQE80L-*lipA* and pQE80L-*lplA* are shown in black and gray bar, respectively. Data represent the mean of three separate experiments and error bars represent standard deviation.