Fine-tuning of *atoB* expression for optimization of hexanoic acid production in recombinant *Escherichia coli*

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In recombinant *E. coli* strains, hexanoic acid is generally synthesized from artificial pathways using acetyl-CoA and butyryl-CoA as precursors. Here, we demonstrate that rebalancing of metabolic fluxes at acetyl-CoA can substantially improve the titer and productivity of hexanoic acid. First, the expression of *mhact* coding ACT increased hexanoic acid production by 47% compared to that with an *E. coli* thioesterase, TesB. Next, metabolic flux was optimized at the acetyl-CoA branch point by fine-tuning the expression level of the gene for acetyl-CoA acetyltransferase (AtoB). Four different synthetic 5'-untranslated regions (UTRs) were designed for *atoB* using UTR Degisner to modulate the expression level of the gene. The flux-optimized variant produced 528 mg/L hexanoic acid in 36 h, and this titer represents an 8.7-fold increase compared to that of the non-optimized parental strain. Notably, the productivity of the optimized strain was 14.7 mg/L/h and this value was the highest among the recombinant *E. coli* strains when using a similar inoculum size for fermentation. Our results show that fine-tuning the expression level of atoB is critical for production of hexanoic acid.