Metabolic engineering of *E. coli* for the sustainable production of short-chain esters

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With the advent of environmental safety policies and development of sustainable industrialization practices, global research has been geared towards the development and use of biodegradable solvents like acetate and lactate esters from renewable feedstocks. While majority of the biological esterification studies involve the use of acyltransferase enzymes, exploring esterases for esterification have significant advantages. Esterases would be advantageous in two ways; their precursors (organic acid and alcohol) can be synthesized in relatively high titres and is one step short as compared to the acyltransferase pathway. To verify our approach, *E. coli* strains were constructed for the heterologous expression of seven different esterases and alcohol-acyl transferases, sourced from various microbial sources. Bioinformatics analysis was performed to curate distinct gene sequences with inappropriate nucleotides from *Brettanomyces bruxellensis* AWRI1499, followed by their codon optimization.

Preliminary substrate doping studies on the *E. coli* strains constructed showed that higher concentration of ethanol is indispensable for the formation of corresponding esters. Based on high-cell density fermentation of these strains four candidates were selected for further optimizations *viz*. AAT, CE, EstA, tEstA (truncated EstA). Amongst them, EstA (from *Pseudomonas aeruginosa*) and CE (from *Brettanomyces*), despite being esterases, exhibited higher ethyl lactate and ethyl acetate titres, hence were explored through bioreactor studies. Batch fermentation studies showed EstA to be the efficient producer with ethyl lactate (5±1.5 mg/L) and while the one possessing CE yielded 45±2 mg/L of ethyl acetate. Hence, choice of enzyme becomes mandatory for improving the yields of the desired product.

Further studies are still ongoing with fed-batch fermentation of these candidates to increase the titre and rate of ester production. Our research serves as the first founding study to employ esterases for esterification reaction. It reveals commercial potential of these underexplored esterases for industrial esterification reactions.