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"Enhancing Propionic Acid Production in Metabolically Engineered E.coli with Acrylate Pathway Genes from Clostridium Propionicum"

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Abstract Propionic acid and its derivatives are one of the best and safest mold and bacterial preservatives in animal feed and food preservatives. The recent market of the global propionic acid and derivatives is $935.7 million (2012) and is estimated to reach $1608.8 million by 2018.

There has always been an increasing demand for eco-friendly processes for the manufacture of fine chemicals implying fermentation derived products are more preferred compared to chemical synthesis. Propionic acid synthesis occurs mainly via 2 pathways, acrylate pathway (in *Clostridium propionicum, Megaspheara elsdenii*) and dicarboxylate pathway(*Propionibacterium acidipropioni)*. The synthesis of propionic acid by native microbes like *C.propionicum, Megaspheara elsdeni* are limited by factors like slow growth, product inhibition and expensive product recovery making it unsuitable for industrial production. This poses a requirement of alternative host for producing propionic acid. In our previous work, a metabolically engineered *E. coli* with genes (from *Clostridium propionicum*) encoding acrylate pathway enzymes was constructed for conversion of D-lactic acid to propionic acid. The engineered strain synthesized ample amount of precursor of the pathway, D-lactate, with yield reaching upto 0.9 g/g of glucose consumed. In contrast the product, propionic acid was synthesized in low levels, 3.7 mM. So, the current work focuses on the complete conversion of the precursor, D-Lactate, to the desired product. Also the work involves identification of the primary bottlenecks affecting propionic acid production.

The suggested bottlenecks to be overcome include redox imbalance caused by additional consumption of NADH in metabolically engineered *E. coli*, lower activities of heterologous enzymes especially acryloyl CoA reductase in the acrylate pathway. Hence the current work aims to confront the above limitations by using reduced substrates like sorbitol and enzyme activity measurements followed by kinetic modeling to determine the optimum enzyme activities for maximum product conversion.

Keywords: Propionic acid, Acrylate pathway, E.coli, Lactic acid