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Investigation of itaconate metabolism in *Cupriavidus Necator* H16

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Recent challenges of pollution and climate change in our environment stems from the over-dependence on fossil fuel through the extraction, processing, and exploitation for petrochemical-based products. This has caused severe havoc to the environment and its natural habitats, leading to deaths and displacements into unfavorable conditions. Researchers in the US Department of Energy (DoE) in 2004 identified itaconate, one of the twelve attractive platform chemicals, as a potential chemical suitable for bio-based industrial products using biological routes. Previous research has also shown that itaconate has the potential to replace petroleum-based products such as petrochemical-based acrylic and methacrylic acid; and detergents, surface active agents and biosynthesized plastics for industrial applications with bio-based products. This can be achieved through biological or chemical conversions and be subsequently converted into several high-value bio-based chemicals and materials from biomass. Research also discovered that itaconate is naturally produced by microorganisms such as *Candida* sp., *Ustilago madis* and *Aspergillus terreus* although many microorganisms have been genetically engineered for the biosynthesis of itaconate. It is, therefore, necessary for the current generation to identify various sustainable and cleaner processes for chemical, fuel and energy production. HPLC was used to estimate the concentration of itaconate consumed. The purpose of this research was to identify the genes involved in itaconate metabolism and abolish its metabolism. To investigate itaconate metabolism on host organism *Cupriavidus necator* H16, the growth of mutants was observed using itaconate as a sole carbon source. Single, double and triple knock-outs of ict genes involved in itaconate conversion to itaconyl-CoA (itaconate-CoA transferase activity) were generated. Growth and itaconate consumption assays were performed establishing that only H16_RS22140 gene is clearly involved in itaconate metabolism. This study revealed that other genes can be involved in itaconate degradation and therefore further research to investigate the function of these genes is required.

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