

# Investigation of Itaconate Metabolism in *Cupriavidus Necator* H16

Suresh Singh\*

Head of Department, Department of Surgical Oncology, SMS Medical College and Hospital, Jaipur, India

## Introduction

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. *Cupriavidus necator* has gone through a series of name changes. In the first half of the 20th century, many micro-organisms were isolated for their ability to use hydrogen. Hydrogen-metabolizing chemolithotrophic organisms were clustered into the group *Hydrogenomonas* C.

*necator* was originally named *Hydrogenomonas eutrophus* because it fell under the *Hydrogenomonas* classification and was “well-nourished and robust”. Some of the original *H. eutrophus* cultures isolated were by Bovell and Wilde.[4][5] After characterizing cell morphology, metabolism and GC content, the *Hydrogenomonas* nomenclature was disbanded because it comprised many species of microorganisms. *H. eutrophus* was then renamed *Alcaligenes eutropha* because it was a micro-organism with degenerated peritrichous flagellation. Upon further study of the genus, *Ralstonia* was found to comprise two phenotypically distinct clusters. The new genus *Wautersia* was created from one of these clusters which included *R. eutropha*. In turn *R. eutropha* was renamed *Wautersia eutropha*.

## Metabolism

*Cupriavidus necator* is a hydrogen-oxidizing bacterium (“knallgas” bacterium) capable of growing at the interface of anaerobic and aerobic environments. It can easily adapt between heterotrophic and autotrophic lifestyles. Both organic compounds and hydrogen can be used as a source of energy. *C. necator* can perform aerobic or anaerobic respiration by denitrification of nitrate and/or nitrite to nitrogen gas. When growing under autotrophic conditions, *C. necator* fixes carbon through the reductive pentose phosphate pathway. It is known to produce and sequester Polyhydroxyalkanoate (PHA) plastics when exposed to excess amounts of sugar substrate. PHA can accumulate to levels around 90% of the cell's dry weight. To better characterize the lifestyle of *C. necator*, the genomes of two strains have been sequenced.

## Regulatory Hydrogenase

The first hydrogenase is a Regulatory Hydrogenase (RH) that signals to the cell hydrogen is present. The RH is a protein containing large and small [Ni-Fe] hydrogenase subunits attached to a histidine protein kinase subunit. The hydrogen gas is oxidized at the [Ni-Fe] center in the large subunit and in turn reduces the [Fe-S] clusters in the small subunit. It is unknown whether the electrons are transferred from the [Fe-S] clusters to the protein kinase domain the histidine

\*Corresponding author: Suresh Singh, Head of Department, Department of Surgical Oncology, SMS Medical College and Hospital, Jaipur, India

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protein kinase activates a response regulator. The response regulator is active in the dephosphorylated form. The dephosphorylated response regulator promotes the transcription of the membrane bound hydrogenase and soluble hydrogenase. The Membrane-Bound Hydrogenase (MBH) is linked to the respiratory chain through a specific cytochrome b-related protein in *C. necator*. Hydrogen gas is oxidized at the [Ni-Fe] active site in the large subunit and the electrons are shuttled through the [Fe-S] clusters in the small subunit to the cytochrome b-like protein. The MBH is located on the outer cytoplasmic membrane. It recovers energy for the cell by funneling

electrons into the respiratory chain and by increasing the proton gradient. The MBH in *C. necator* is not inhibited by CO and is tolerant to oxygen.

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