Product Secretion and Induced Mechanism of Cytochrome P450 by H_2O_2 in Long-Chain Dicarboxylic Acid Fermentation

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Abstract

A new experimental method was established to investigate 1,11-dicarboxylic acid (DCA_{13}) secretion in alkane-assimulating yeast, *Candida tropicalis*. By this method, phenomenon of acid production in resting cells was observed. The whole process can be divided into two phases: the acid secretion phase and the coupling phase of both acid secretion and alkane bioconversion. Extracellular pH and DCA_{13}^{2-} played important roles in this process. High extracellular pH and low DCA_{13}^{2-} can facilitate acid secretion. A chemical structured model was proposed to analyze these two factors detailedly. Model parameters were simulated by the random search method. Simulated data fited the experimental results so well that the maxium error between them was less than 5%. This kind of experimental method and kinetic analysis could also be used in other organic acid fermentation.

It has been established the alkane degradation pathway which exists in various microorganisms, notably the yeast *Candida*.. Following transport into the cell, *n*-alkane is hydroxylated to fatty alcohols by a specific cytochrome P450 system, which is the key enzyme of the α-oxidation and ω-oxidation. Two further oxidation steps catalyzed by alcohol oxidase and aldehyde dehydrogenase, lead to the corresponding fatty acid (α-oxidation). The fatty acids can be further oxidized through the same pathway (ω-oxidation) to the corresponding dicarboxylic acid.

The system of producing DCA is an aerobic, multi-phase fermentation system which is consisted of *Candida tropicalis* cell, alkane (oil), water and gas. In this system, oxygen supply is of crucial importance because insufficient oxygen supply can lead to suboptimal productivity as well as product of low quality. A method to overcome gas-liquid transport resistance and increasing oxygen supply is by adding hydrogen peroxide (H_2O_2) in the fermentation system. Although it is commonly known that H_2O_2 is toxic to cells at high concentrations, but at lower concentration H_2O_2 can increase the DCA production. Furthermore, the mechanisms of this promoting function are also studied in this paper.

The studies showed that the mechanisms of H_2O_2 stimulating DCA production included 3 factors:

1. H_2O_2, as molecular oxygen substrate, reduces the gas-liquid transport resistance and increase molecular oxygen supply;
2. H_2O_2 is able to induce higher activity of cytochrome P450, the key enzyme of α , ω -oxidation pathway;
3. When H_2O_2 exists, Alkane can be oxidized directly by an effective peroxide shunt of cytochrome P450.