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The Impact of Proton Motive Force on Energy Metabolism and Hydrogenase Activity of *Cupriavidus Necator* H16

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ABSTRACT

The facultative chemolithoautotroph *Cupriavidus necator* H16 capable to utilize H_2 as a main energy source through the O_2-tolerant [NiFe]-hydrogenases (Hyds), such as membrane-bound (MBH), soluble (SH), actinobacterial (AH) and regulatory (RH) Hyds . These enzymes are key components of autotrophic growth, and are an important part of energy and H_2 homeostasis of bacterial cells. The aim of this study was to investigate the impact of inhibitors of proton motive force generation (sodium azide (NaN_3), carbonyl cyanide m-chlorophenyl hydrazone (CCCP)), and F_OF_1-ATPase (N,N′-dicyclohexylcarbodiimide (DCCD)) on the growth parameters and Hyds activity of *C. necator* H16. Bacteria were cultivated in glycerol-fructose-nitrogen (GFN) medium with addition of 50μM CCCP and DCCD, 1.5mM of NaN_3 under microaerobic conditions. Heterotrophic growth of the culture was monitored over a period of 7 days at 30 °C, at 150 rpm. H_2-oxidizing Hyd activity was assessed by methylene blue reduction at 570 nm, 30 °C, using a spectrophotometer (Cary 60 UV–vis, Agilent Technologies, USA). Growth inhibition by 2.5- to 6-fold was observed starting from the first day of cultivation upon the addition of inhibitors, however the inhibitory effect of the studied substances was overcome on the 4th day of growth. A slight decrease in oxidation-reduction potential (ORP) and pH was recorded across all samples. The H_2-oxidizing Hyd activity in GFN samples remained consistently high throughout the cultivation period of *C. necator*, ranging from 12 to 35 ± 0.5 U/mg(CDW)/min. The addition of inhibitors resulted in a significant reduction of Hyd activity, specifically, DCCD reduced Hyd activity by 32–48%, CCCP by 32–66%, and NaN_3 by 40–70%. Thereby, the extent of inhibition depends on both the bacterial growth phase and the specific inhibitor is used. These results provide insight into the cellular bioenergetics underlying H_2 metabolism.

Keywords: *Cupriavidus necator* H16, H_2-oxidizing hydrogenases, metabolic Inhibitors, CCCP, DCCD, NaN_3

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