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Conversion of *Corynebacterium glutamicum* from an aerobic respiring to an aerobic fermenting bacterium by inactivation of the respiratory chain

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Abstract:

In this study a comparative analysis of three *Corynebacterium glutamicum* ATCC 13032 respiratory chain mutants lacking either the cytochrome *bd* branch ($\Delta cydAB$), or the cytochrome *bc₁-aa₃* branch (Δqcr), or both branches was performed. The lack of cytochrome *bd* oxidase was inhibitory only under conditions of oxygen limitation, whereas the absence of a functional cytochrome *bc₁-aa₃* supercomplex led to decreases in growth rate, biomass yields, respiration and proton-motive force (pmf) and a strongly increased maintenance coefficient under oxygen excess. These results show that the *bc₁-aa₃* supercomplex is of major importance for aerobic respiration. For the first time, a *C. glutamicum* strain with a completely inactivated aerobic respiratory chain was obtained ($\Delta cydAD\Delta qcr$), named DOOR (devoid of oxygen respiration), which was able to grow aerobically in BHI (brain-heart infusion) glucose complex medium with a 70 % reduced biomass yield compared to the wild type. Surprisingly, reasonable aerobic growth was also possible in glucose minimal medium after supplementation with peptone. Under these conditions, the DOOR strain displayed a fermentative type of catabolism with L-lactate as major and acetate and succinate as minor products. The DOOR strain had about 2 % of the oxygen consumption rate of the wild type, showing the absence of additional terminal oxidases. The pmf of the DOOR mutant was reduced by about 30 % compared to the wild type. Candidates for pmf generation in the DOOR strain are succinate:menaquinone oxidoreductase, which probably can generate pmf in the direction of fumarate reduction, and F_1F_0 -ATP synthase, which can couple ATP hydrolysis to the export of protons.

Keywords: *Corynebacterium glutamicum*, respiration, Cytochrome *bd* oxidase, Cytochrome *bc₁* complex, Cytochrome *aa₃* oxidase, proton-motive force, fermentation, maintenance coefficient