MUTATIONAL ANALYSIS OF RESPIRATION AND OXIDATIVE PHOSPHORYLATION IN CORYNEBACTERIUM GLUTAMICUM

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Introduction. *Corynebacterium glutamicum* is a major player in industrial biotechnology as the most important producer of amino acids and as platform organism capable of producing a wide variety of other products such as organic acids, diamines, biofuels, or proteins. It uses a respiratory type of energy metabolism with oxygen or nitrate as terminal electron acceptors and an F_{1}F_{0}-ATP synthase driving ATP synthesis (Fig.1, [1]). In contrast to lacking either the cytochrome oxidase, the branch of major importance for aerobic respiration, while the absence of a functional cytochrome *bc1*-aa3 supercomplex led to decreases in growth rate, biomass yield, respiration and pmf. For the first time, a *C. glutamicum* strain with a completely inactivated aerobic respiratory chain was obtained (ΔacydABΔqcr), named DOOR (devoid of oxygen respiration), which was able to grow in BHI glucose complex medium with a 70% reduced biomass yield compared to the wild type. In glucose minimal medium, reasonable growth was only possible after peptone supplementation. The DOOR strain showed a fermentative type of catabolism with L-lactate as major and acetate and succinate as minor products. A residual oxygen consumption rate of only 2% of the wild type rate indicated 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_{1}F_{0}-ATP synthase, which can couple ATP hydrolysis to the export of protons.

A mutant of *C. glutamicum* ATCC 13032 with a deletion of the *atpBEF1HAGDC* genes encoding F_{1}F_{0}-ATP synthase was also characterized [4]. This mutant can synthesize ATP only via substrate level phosphorylation. Whereas no growth was observed with acetate as sole carbon source, the ΔF_{1}F_{0} mutant reached 47% of the growth rate and 65% of the biomass of the wild type during shake-flask cultivation in glucose minimal medium. The ΔF_{1}F_{0} mutant had increased levels of *b*- and *d*-type cytochromes and a significantly increased pmf.

Transcriptome analysis of the mutants described above was performed to obtain a global view on expression changes. In ΔacydAB, Δqcr, DOOR and ΔF_{1}F_{0} the number of genes with an at least 2-fold changed mRNA level (either up or down) were 57, 221, 939, and 290, respectively. Cluster analysis revealed a number of common traits in several of these strains, such as genes involved in the oxidative stress response.

Conclusions. The results show that the *bc1*-aa3 complex is of major importance for aerobic respiration, while cytochrome *bd* oxidase is an ancillary enzyme. The DOOR mutant shows the potential of *C. glutamicum* for mixed-acid fermentation under aerobic conditions. The results obtained for the ΔF_{1}F_{0} mutant prove for the first time that F_{1}F_{0}-ATP synthase and oxidative phosphorylation are in general not essential for growth of *C. glutamicum*.

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References.