MUTATIONAL ANALYSIS OF RESPIRATION AND OXIDATIVE PHOSPHORYLATION IN *CORYNEBACTERIUM GLUTAMICUM*

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Corynebacterium glutamicum, respiratory mutants, ATP synthase mutant

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_1F_O-ATP synthase driving ATP synthesis (Fig.1, [1]). In contrast to vigorous aerobic growth, anaerobic growth by nitrate respiration is very limited due to the accumulation of nitrite. The aerobic respiratory chain is composed of two branches formed by the cytochrome bc_1-aa_3 supercomplex [2] and cytochrome *bd* oxidase. The proton-motive force (pmf) generated by these complexes drive ATP synthesis via F_1F_O-ATP synthase.
We have analyzed the importance of enzymes involved in respiratory energy metabolism by a detailed analysis of defined mutants.

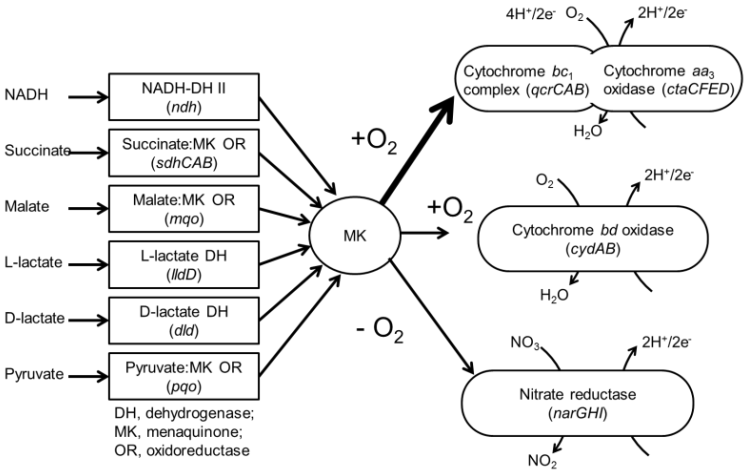

Fig.1. The branched respiratory chain of *C. glutamicum*.

Methods. Methods are described in the references.

Results. A comparative analyses of three *C. glutamicum* ATCC 13032 respiratory chain mutants was performed lacking either the cytochrome *bd* branch (Δ*cydAB*), or the cytochrome bc_1-aa_3 branch (Δ*qcr*), or both branches [3]. The lack of cytochrome *bd* oxidase was inhibitory only under conditions of oxygen limitation, whereas the absence of a functional cytochrome bc_1-aa_3 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 (Δ*cydAB*Δ*qcr)*, 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_1F_O-ATP synthase, which can couple ATP hydrolysis to the export of protons.
A mutant of *C. glutamicum* ATCC 13032 with a deletion of the *atpBEFHAGDC* genes encoding F_1F_O-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_1F_O 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_1F_O 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 Δ*cydAB*, Δ*qcr*, DOOR and ΔF_1F_O 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 bc_1-aa_3complex 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_1F_O mutant prove for the first time that F_1F_O-ATP synthase and oxidative phosphorylation are in general not essential for growth of *C. glutamicum*.

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References.

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