Importance of cytochrome c maturation genes in iron metabolism and oxidative stress in Pseudomonas aeruginosa.

Submitted in fulfillement of the requirement for the degree of Doctor (Ph.D) of Applied Biological Sciences

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Abstract

Pseudomonas aeruginosa is a highly pathogenic species and the cause of high mortality in immunosuppressed patients. Especially those patients who suffer from burn wounds, cancer, aids or cystic fibrosis are very susceptible to this pathogen. Bacterial infections are combatted by administration of antibiotics but the widespread use and misuse renders them resistant in time. For this reason new antibiotics and especially new targets in the bacterial cell have to be found to fight Pseudomonas infections.

The biogenesis of type-c cytochromes in γ-Proteobacteria is dependent on the products of the ccmABCDEFGH genes, which encode different inner-membrane proteins involved in the maturation of the cytochrome c. In short, heme is transported from the cytoplasm to the periplasmic space and shuttled towards the apocytochrome to be covalently bound to it. Deletion of some genes, especially ccmC, has a pleiotropic effect involving different processes not immediately linked to the loss of c-type cytochromes, such as siderophore production and utilisation. The mutants were constructed via apolar inactivation using the pKnockout-system. These mutants were phenotypically characterised and different phenotypes were compared.

The effect was very explicit in the ccmC mutant and, to a lesser extent, in ccmF. The production of the siderophore pyoverdine was very low and growth under the condition of iron limitation was severely restricted although production of the secondary siderophore pyochelin was increased.

Other interesting results concerned the production of pyocyanin, swarming, swimming and twitching motility, and rhamnolipid production. The production of the quorum sensing molecules N-acyl homoserine lactones and the Pseudomonas quinolone signal (PQS) was however not affected by the mutation. In the ccmC mutant we also observed the accumulation
of protoporphyrin IX, a strongly reduced production of heme-containing proteins, such as catalase, and as a result of this, an increased sensitivity to hydrogen peroxide. Finally, in both $ccmC$ and $ccmF$ mutants the content of Fe-S clusters was reduced.

To study the global physiological impact of this $ccmC$ mutation more deeply, we decided to perform a proteomic analysis of the mutant and this under conditions of iron-limitation. This was done on periplasmic and cytoplasmic proteins and the resulting protein spots were analysed via mass spectrometry. The results showed clearly that the mutant was subjected to oxidative stress and confirmed that its metabolic activity was disturbed.

In conclusion, we have demonstrated that CcmC is a key determinant for cytochrome $c$ biogenesis, pyoverdine maturation, expression of some quorum sensing-regulated traits (but not signal production) and oxidative stress defence.

The fact that CcmC clearly plays different roles next to the maturation of type $c$ cytochromes makes it an interesting target for the development of new antibiotics.