Studies on the Oxidative Stress Response of *Porphyromonas gingivalis*

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A thesis submitted in fulfillment of the requirements for admission to the degree of Doctor of Philosophy

By

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Signed Statement

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Summary

Porphyromonas gingivalis is a Gram-negative anaerobic cocco-bacillus strongly implicated in the aetiology of adult periodontitis. During the colonisation of the oral cavity it is likely that *P. gingivalis* encounters different sources of oxidative stress. Adaptation to this challenge is necessary for the microorganism to survive and establish in the periodontal environment. The aim of the present study was, therefore, to investigate the oxidative stress survival mechanisms of *P. gingivalis*.

P. gingivalis was grown under different oxygenated environments in a continuous culture system under conditions of haemin-limitation and excess. Steady state was achieved under moderately oxygenated atmospheres, although a decrease in cell viability was observed as the oxygen concentration in the gas mixture increased. Haemin-excess conditions seemed to increase the ability of a culture to cope with a determined oxygen concentration. The main change in fermentation end-products characterising oxygen stressed cultures was an increase in the production of acetate. Scanning electron micrographs showed that oxygen triggers a change in the cell shape from a cocco-bacillary to a short rod.

The effect of oxygen on the expression of cysteine proteinases, critical virulence factors of *P. gingivalis*, was assayed in supernatants and cell fractions and further analysed by 2-D gel electrophoresis. Both evaluations showed an increase in the cell-associated Arg-gingipain and a decrease in Lys-gingipain in oxygen stressed cells.

Assays for NADH oxidase, NADH peroxidase and superoxide dismutase in cell extracts showed an increase in their activities as the environment became more

oxygenated. The NADH oxidase activity was partially purified and characterised and, surprisingly, the isolated protein was identified as a 4-hydroxybutyryl-CoA dehydratase. This is the first report of NADH oxidase activity associated with this enzyme.

The existence of other open reading frames in the *P. gingivalis* genome sequence that would encode for proteins with the potential for NADH oxidase/peroxidase activity was further investigated. The transcription product of the identified ORFs, encoding for a possible NADH oxidase (Nox) and an alkyl hydroperoxide reductase (AhpF-C), was analysed under anaerobic and oxygenated environments by northern blot hybridization. No mRNA for Nox was detected but AhpF-C was expressed constitutively in anaerobic cells and slightly increased under oxygenated conditions.

Furthermore, the possibility of the existence of a common transcriptional switch for oxidative stress-related proteins was investigated. A homologue of OxyR, a redox-sensitive transcriptional activator, was identified in the *P. gingivalis* genome sequence and an OxyR-disrupted mutant was constructed. Mutants exhibited decreased tolerance to air and hydrogen peroxide and were characterised by the absence of alkyl hydroperoxide reductase mRNA, suggesting a control of OxyR over its expression.

The second part of this research project consisted of studies of *P. gingivalis* in co-culture with *F. nucleatum*, under oxygenated environments. These studies showed that not only does *F. nucleatum* have a much higher tolerance to oxygen than *P. gingivalis* but, in co-culture, it can protect the latter organism and increase its ability to survive under oxygenated conditions. Additionally, it was observed

that *F. nucleatum* is able to generate a capnophilic environment essential for the growth of *P. gingivalis*.

In conclusion, this study showed that *P. gingivalis* possesses basic mechanisms to cope with moderate or transient oxidative stress while it probably relies on the protection of other organisms, like *F. nucleatum*, to survive and replicate in highly oxygenated atmospheres.

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