Protein Expression in *Fusobacterium nucleatum* ATCC 10953 Biofilm Cells Induced by an Alkaline Environment

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

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December 2010

Signed Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference has been made in the text.

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Acknowledgements

I would like to thank a number of people who provided assistance during the course of this work.

Dr. Neville Gully, Dr. Peter Zilm and Dr. Janet Fuss, who acted as my supervisors. This study would not have been possible without their guidance, advice and encouragement.

Dr. Tracy Fitzsimmons, Mr. Krzyztof Mrozik, Mr.Victor Marino, Ms. Lyn Waterhouse (Adelaide Microscopy Centre) and the staff at the Adelaide Proteomic Centre for their advice and technical assistance on many occasions.

My friends, for their support and encouragement.

My parents, Da-Jie, Thien, Han and Yen, for their understanding, continual support and faith.

The University of Adelaide, for awarding me a scholarship (ASI).

Abstract

Fusobacterium nucleatum is a Gram negative anaerobic bacterium, frequently isolated from both healthy and diseased dental plaque and has been implicated in the aetiology of periodontal diseases. Studies have shown that this organism increases in number and proportion in diseased periodontal pockets suggesting that changes in the environment favour the growth of the organism. One of the physico-chemical changes that occurs in the diseased periodontal sulcus is an increased environmental pH, which may be as high as 8.5. In our laboratory, *F. nucleatum* subspecies *polymorphum* ATCC 10953 formed mono-culture biofilms at pH 8.2. The formation of biofilms in nature is a survival strategy for bacteria, often associated with altered physiology and increased virulence. The aim of this study was to investigate changes in *F. nucleatum* protein expression associated with these alkaline induced biofilms.

A chemostat was used to produce *F. nucleatum* planktonic and biofilm cells grown at pH 7.4 and 8.2, respectively. The bacterial cells were separated into cell envelope and cytoplasmic fractions. The soluble proteins were extracted from each fraction and resolved by two-dimensional gel electrophoresis. Fifty five differentially expressed proteins were identified using mass spectrometry and database searching. These proteins were classified according to functional class, including metabolic, transport and stress proteins.

One of the most interesting findings was the significant up-regulation of Fusobacterial Outer Membrane Protein A (FomA), a porin that is responsible for the coaggregation between *F. nucleatum* and periodontopathogens. It has been suggested that this protein may be a target for future vaccination against periodontal diseases.

Other proteomic findings showed that transport proteins such as ATP binding cassette (ABC) transporter binding protein was down-regulated while tripartite ATP-independent (TRAP) transporter binding protein was enhanced in the alkaline environment. ABC transporters require ATP hydrolysis for solute transport. In contrast, TRAP transporter is ATP independent and co-transports protons into the cytoplasm, which may help maintain a neutral intracellular pH under alkaline conditions. The regulation of these transport proteins reflected the conservation of energy and maintenance of pH homeostasis in biofilm cells.

In addition, *F. nucleatum* regulated key enzymes in energy-producing pathways. A combination of proteomic and other methods confirmed that biofilm cells increased their glucose uptake and storage as intracellular polyglucose in comparison to their planktonic counterparts. Furthermore, the increased energy requirement of these cells was associated with changes in metabolism resulting in both qualitative and quantitative shifts in acidic end-products.

Bacterial biofilm formation can be triggered by adverse environmental conditions and the expression of stress proteins help to protect cellular functions. The stress proteins, GroEL and RecA, were up-regulated in biofilm cells and the expression of these proteins was verified using Western-blotting and quantitative real-time polymerase chain reaction techniques.

In conclusion, this investigation successfully identified *F. nucleatum* proteins that were regulated in response to alkaline conditions, similar to those that are thought to exist in diseased periodontal pockets. The physiological adaptations observed, to the

changed ecology, provide evidence at the molecular level supporting the key role of *F. nucleatum* in the establishment of mature dental plaque.

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2DE	– Two-dimensional gel electrophoresis
ABC transporter	– ATP-binding cassette transporter
BAT	– Butanoate: acetoacetate Coenzyme-A transferase
BHI	– Brain Heart Infusion
CDFF	– Constant depth film fermentor
CDM	– Chemically Defined Media
CID	– Collision-induced dissociation
DTT	– Dithiothreitol
EC	– Enzyme Commission
EF	– Elongation factor
Eh	– Redox potential
EPS	– Extracellular polymeric substance
ExPASy	– Expert Protein Assay Systems
FomA	– Fusobacterial outer membrane protein A
GCF	– Gingival crevicular fluid
HGEC	– Human gingival epithelial cell
HGT	– Horizontal gene transfer
HPLC	– High Performance Liquid Chromatography
HSR	– Heat shock response
IEF	– Isoelectric focusing
IP	– Intracellular polyglucose
IPG	– Immobilised pH gradient
LC-ESI	– Liquid Chromatography – Electrospray Ionisation
LDH	– Lactate dehydrogenase
LPS	– Lipopolysaccharides
MALDI-TOF	– Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight
MMP	– Matrix metalloproteinase
MRD	– Modified Robbins Device
MS	– Mass spectrometry
MW	– Molecular weight
NAD^+-GDH	– NAD^+-specific glutamate dehydrogenase
NCBI	– National Center for Biotechnology Information
OMP	– Outer membrane protein
OmpIP	– Outer membrane protein Insertional Porin
pI	– Isoelectric point
PSORTb	– Subcellular localisation prediction tool for bacterial cells
PTM	– Post-translational modifications
RCDC	– ReduCing agent and Detergent Compatible
RND antiporter	– Resistance-Nodulation-Cell Division antiporter
RT-PCR	– Real Time-Polymerase Chain Reaction
SDS	– Sodium dodecyl sulphate
SEM	– Scanning Electron Microscopy
TBP	– Tributyl phosphine
TRAP-transporter	– Tripartite ATP-independent transporter
TTT transporter	– Tripartite tricarboxylate transporter