Proteomic analysis of *Enterococcus faecalis* cell membrane proteins under alkaline stress conditions

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

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Abstract

Background: *Enterococcus faecalis* is able to survive in a number of biological niches, which are often nutrient limited and in which the pH can vary greatly. Endodontic (root canal) treatment of a tooth with a severely inflamed, infected or necrotic pulp usually involves the chemo-mechanical debridement of the canal(s) using metal files, irrigants such as sodium hypochlorite and often inter-appointment medicaments such as calcium hydroxide (~pH 12.5 to 12.8) placed in the main root canal to help in the elimination of surviving bacteria. *E. faecalis* is commonly recovered from endodontic infections that have persisted following treatment with this highly alkaline medicament. The expression of the cell membrane proteins under alkaline conditions at a biologically relevant growth rate may increase our understanding of how *E. faecalis* can adapt and persist.

Aims:

1. To determine the phenotypic changes of *E. faecalis* V583 when grown at a slow growth rate at pH 11.
2. To investigate and quantify cell membrane protein expression of *E. faecalis* V583, at pH 11 compared to pH 8, at an imposed growth rate using continuous culture.

Methods: *E. faecalis* ATCC V583 was grown in a chemostat at pH 8 (control) and pH 11. Under each pH condition, the maximum growth rates were determined and an imposed growth rate of one-tenth the organism's maximum growth rate (μ_{rel}) was used for growth at pH 8 or 11. After steady state had been achieved, cells were harvested, lysed and membrane proteins were fractionated by ultracentrifugation, homogenisation in carbonate buffer, and membrane shaving. Following chymotrypsin digest (in the presence of RapiGest®) of the membrane fraction, heavy- or light-isotope-coding protein labels (ICPL) were added to samples from pH 8 or 11. Heavy-labelled (pH 11) and light-labelled (pH 8) samples were combined and the relative proportion of membrane proteins were identified using Liquid chromatography, electrospray ionisation (LC-ESI) mass spectrometry and MaxQuant analysis. The MaxQuant labelled ratios of membrane associated proteins were $\log_2$ transformed, and the proteins that deviated by more than one standard deviation (SD) from the mean were considered to be up- or down-regulated.

Results: The mean generation time at pH 8 was 1.16 hours and 7.7 hours at pH 11. One-tenth of the maximum growth rate (0.1 μ_{rel}) was determined and set at 0.059 h^{-1} for pH 8 and

0.009 h^{-1} for pH 11. The extreme alkaline conditions produced co-aggregation of the cells into flocs (a variant of biofilm formation) with the appearance of an extracellular matrix. These observations are consistent with a shift towards spontaneous biofilm formation.

Six proteins had a $\log_2$ H/L ratio (pH 11/pH 8) greater than one SD of the mean including: Polysaccharide biosynthesis family protein EF0669 (2SD), Glycosyl hydrolase, family 20 EF0114 (4SD), Glycerol uptake facilitator protein EF1927 (1SD), whilst five proteins had a $\log_2$ ratio one SD less of the mean: PTS system IIC component EF1838 (1D), PTS system IID component EF0456 (2SD), C4-dicarboxylate transporter EF0108 (1SD), PTS system mannose-specific IID component EF0022 (1SD).

Conclusion: When cultured at an imposed slow growth rate, extreme alkaline conditions resulted in a reduced mean generation time and altered expression of several membrane proteins. Collectively these membrane proteins appear to be involved in the transition to biofilm formation seen at pH 11. It was hypothesised that the capsule observed at pH 11 protects the cell from destructive OH- ions whilst concentrating H+ ions and substrates required for the electrochemical gradient close to the cell membrane.

Keywords: *Enterococcus faecalis*, isotope-coding protein labels (ICPL), alkaline pH, membrane shaving.

Statement of Authorship

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 accept where due reference has been made in the text.

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Signed:

Peter Cathro Date:

Acknowledgements

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

Dr Peter Zilm, who has been a mentor, colleague and friend over many years and without whose support this thesis and our other joint research endeavours would not have been possible.

Dr Stephen Kidd, for his dedication and willingness to supervise this project and his wonderful insights into the world of bioinformatics.

Associate Prof Neville Gully, for his help in guiding this and other research projects over the years.

Dr Peter McCarthy, for his expert research skills and "rescuing" my precious samples.

Prof Peter Hoffmann, for his support and generous help facilitating the proteomic components of the project.

Camilla, Samuel, Emily & Alfred, my wonderful family, I cannot thank them enough for their ongoing love and care of me.

This study was supported by several grants from the Australian Dental Research Foundation, The Dental School University of Adelaide and the Australian Society of Endodontology.

I sincerely thank the staff at the Adelaide Proteomic Centre for their excellent technical assistance.