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# **Microarray analysis of *Acidovorax temperans***

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A thesis submitted in fulfilment of the requirements for the degree of  
Doctor of Philosophy, The University of Auckland

2008

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# Abstract

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Bacteria belonging to the genus *Acidovorax* have been shown to be a consistent member of the activated sludge microbial community. Two phenotypic variants of *A. temperans* CB2 isolated locally from activated sludge exhibit noteworthy characteristics, such as the ability to form biofilms and flocs, which are critical microbial processes underlying all modern wastewater treatment systems. Gene expression microarray technology is a functional genomics platform that enables the simultaneous interrogation of all expressed transcripts during normal cell ontogeny, or in response to specific environmental stimuli. Microarray technology offers the opportunity to investigate gene expression changes relevant to key processes in wastewater treatment, using *A. temperans* as a model organism.

The aims of this research were to develop a full genome microarray platform for *A. temperans* CB2 and to use this microarray platform to investigate major differences in gene expression between the Hpos and Hneg phenotypic variants. An optimised gene expression microarray platform was established through the assessment of various experimental methods, such as RNA extraction, RNA amplification, microarray probe design, and quantitative PCR. Using the microarray platform, gene expression comparisons were obtained for planktonic broth cultures, static biofilms and bacterial colonies.

Gene expression analyses have provided insights into the complex developmental processes involved in the transition from planktonic cells to stages of initial attachment, cell proliferation, biofilm maturation and nutrient limitation during the formation of *A. temperans* biofilms. Factors that have been identified in other bacterial systems such as type IV pili and activation of stress responses were also observed in *A. temperans* biofilms. In addition, several intriguing classes of genes, such as transcriptional regulators, a toxin-antitoxin gene cassette, and nitrate metabolism were also found to be differentially expressed during the formation of *A. temperans* biofilm. The incorporation of microarray technology with other functional genomics techniques to investigate molecular mechanisms underlying the complex processes occurring in wastewater treatment will provide a scientific basis to improve the reliability of current wastewater treatment strategies and for the development of new treatment technologies.

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# Abbreviations

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aRNA	amplified RNA
AU	Absorbance Unit
$\beta$ -ME	beta-Mercaptoethanol
bp	Base pair/s
cDNA	complimentary DNA
CFU	Colony Forming Units
COG	Clusters of Orthologous Groups
$C_t$	Threshold cycle
CTAB	Ceyltrimethylammonium bromide
DEPC	Diethylpyrocarbonate
DGGE	Denaturing Gradient Gel Electrophoresis
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
dNTP	deoxy Nucleotide Triphosphate
DTT	Dithiothreitol
<i>E</i>	Amplification Efficiency
EDTA	Ethylenediaminetetraacetic Acid
EPS	Extracellular Polymeric Substances
ERC	External RNA Controls
EST	Expressed Sequence Tag
FISH	Fluorescent <i>in situ</i> Hybridisation
GAL	GenePix Array List
GPR	GenePix Results
h	Hour/s
HS	Heat Shock
IVET	<i>in vivo</i> Expression Technology
KO	KEGG Orthology
min	Minute/s
mRNA	messenger RNA
NHS	Non-Heat Shock
OD	Optical Density
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PMT	Photomultiplier
PVP	polyvinyl pyrrolidone
<i>Q</i>	Raw transcript expression levels or quantities
qPCR	Real Time quantitative PCR
RFU	Relative Fluorescent Units
RIN	RNA Integrity Number
RNA	ribonucleic acid
rpm	Revolutions per Minute
rRNA	ribosomal RNA
RT	Room Temp
s	Second/s
SDS	Sodium Dodecyl Sulphate
TB	Terrific Broth
$T_m$	Melting Temperature