Extracellular polymers of *Acidovorax temperans*

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Abstract

All modern wastewater treatment systems rely on microbial processes for water clarification, with some 70% of systems utilising some form of the activated sludge process. This thesis describes an investigation into the extracellular polymers of a consistent member of the activated sludge microbial community: *Acidovorax temperans.*

Several types of polymers are present on the cellular surface of *A. temperans* which were hypothesised to play a role in cellular and cell-surface interactions. The two morphotypes of *A. temperans* (Hpos and Hneg) were proposed to enable the study of these extracellular polymers in wastewater treatment related processes, such as biofilm formation and flocculation. The initial aim of this research was to chemically characterise and compare the exopolysaccharides produced by the two morphotypes. The results are described in Chapter Three; it was found the Hpos strain consistently produced exopolysaccharides in greater abundance, although exopolysaccharides from both strains shared a similar monosaccharide composition, which in turn, was nutrient dependent.

Due to subsequent findings of large amounts of extracellular DNA in EPS fractions, it was further hypothesised that extracellular DNA is utilised as a structural element in biofilm formation by *A. temperans*. This is described in Chapter Four; enzymatic breakdown of extracellular DNA resulted in impaired biofilm formation. In addition, the presence of Type IV pili was also essential for cellular attachment to surfaces, while the underlying binding mechanism was concluded to be electrostatic. These results add further to the recently emerging notion that extracellular DNA could function as a structural molecule outside the cell.

To date, this is the first reported study on an extracellular DNA and Type IV pili combined attachment mechanism. In addition, this thesis describes the most detailed study to date on exopolysaccharides from a member of the *Comamonadaceae*. An obvious function for *Acidovorax* in the activated sludge environment was not indicated

by these studies on the presence, function and composition of extracellular polymers. However the discovery that eDNA and Type IV pili mediate cell-cell and cell-surface attachment in this organism provides a fertile starting point for investigating the contribution of these interactions to initiation and promotion of flocculation in activated sludge systems.

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Abbreviations

AS	Activated Sludge
AHL	Acylhomoserine Lactone
NTHI	Nontypeable *Haemophilus Influenzae*
AFM	Atomic Force Microscopy
AHL	Acylhomoserine Lactones
Bcc	*Burkholderia Cepacia* Complex
BOD	Biological Oxygen Demand
CAZY	Carbohydrate Active Enzyme Database
CF	Cystic Fibrosis
CFU	Colony Forming Units
CLSM	Confocal Laser Scanning Microscopy
DLVO	Derjaguin-Landau-Verweij-Overbeek Theory
DNase	Deoxyribonuclease
DO	Dissolved oxygen
eDNA	Extracellular DNA
EDTA	Ethylenediaminetetraacetic Acid
EI	Electron Impact
EPS	Exopolysaccharide / Extracellular Polysaccharides
ERMA	New Zealand Environmental Risk Management Authority
FID	Flame Ionisation Detector
FISH	Fluorescent *in situ* hybridisation
GalA	Galacturonic acid
Galf	Galactofuranose
GalM	Galactose Mutarotase
GalN	Galactosamine
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionisation Detector
GC-MS	Gas Chromatography-Mass Spectrometry
GLC	Gas-Liquid Chromatography
GLC	Gas-Liquid Chromatography
GlcN	Glucosamine
GT	Glycosyltransferase
GW	Glass Wool

Hneg	Halo negative
Hpos	Halo positive
JGI	Joint Genome Institute
KDO	3-deoxy-D-manno-octulosonic acid
LPS	Lipopolysaccharide
MALDI-ToF MS	Matrix Assisted Laser Desorption Time-of-Flight Mass Spectrometry
MS	Mass Spectrometry
MurNAc	N-acetylmuramic acid
NETs	Neutrophil extracellular traps
NMR	Nuclear Magnetic Resonance
NTHI	Nontypeable Haemophilus Influenzae
OM	Outer Membrane
p	Pyranose
PG	Peptidoglycan
pH	Potenz Hydrogen
PHA	Polyhydroxylalkanoates
PM	Plasma membrane
RAS	Returned Activated Sludge
RNase	Ribonuclease
rRNA	Ribosomal Ribonucleic Acid
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis
SEM	Scanning Electron Microscopy
SN	Supernatant
TCA	Tricarboxylic Acid
TEM	Transmission Electron Microscopy
TFA	Trifluoroacetic Acid
TLC	Thin Layer Chromatography
TMS	O-trimethyl silyl ethers
TMV	Tobacco Mosaic Virus
und-P	undecaprenyl Phosphate
WWTP	Wastewater Treatment Plant

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