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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	<u>A</u> ctivated <u>S</u> ludge
AHL	<u>A</u> cy <u>l</u> homoserine <u>L</u> actone
NTHI	<u>N</u> ontypeable <u>H</u> aemophilus <u>I</u> nfluenzae
AFM	<u>A</u> tom <u>i</u> c <u>F</u> orce <u>M</u> icroscopy
AHL	<u>A</u> cy <u>l</u> homoserine <u>L</u> actones
Bcc	<u>B</u> urkholderia <u>C</u> epacia <u>C</u> omplex
BOD	<u>B</u> iological <u>O</u> xygen <u>D</u> emand
CAZY	<u>C</u> arbohydrate <u>A</u> ctive <u>E</u> nzyme <u>D</u> atabase
CF	<u>C</u> ystic <u>F</u> ibrosis
CFU	<u>C</u> olony <u>F</u> orming <u>U</u> nits
CLSM	<u>C</u> onfocal <u>L</u> aser <u>S</u> canning <u>M</u> icroscopy
DLVO	<u>D</u> erjaguin- <u>L</u> andau- <u>V</u> erweij- <u>O</u> verbeek Theory
DNase	<u>D</u> eoxyribo <u>n</u> uclease
DO	<u>D</u> issolved <u>o</u> xygen
eDNA	<u>E</u> xtracellular <u>D</u> N <u>A</u>
EDTA	<u>E</u> thylenediaminetetraacetic <u>A</u> cid
EI	<u>E</u> lectron <u>I</u> mpact
EPS	<u>E</u> xopolysaccharide / <u>E</u> xtracellular <u>P</u> olysaccharides
ERMA	New Zealand <u>E</u> nvironmental <u>R</u> isk <u>M</u> anagement <u>A</u> uthority
FID	<u>F</u> lame <u>I</u> onisation <u>D</u> etector
FISH	<u>F</u> luorescent <i>in situ</i> hybridisation
GalA	<u>G</u> alacturonic <u>a</u> cid
Galf	<u>G</u> alactofuranose
GalM	<u>G</u> alactose <u>M</u> utarotase
GalN	<u>G</u> alactosamine
GC	<u>G</u> as <u>C</u> hromatography
GC-FID	<u>G</u> as <u>C</u> hromatography- <u>F</u> lame <u>I</u> onisation <u>D</u> etector
GC-MS	<u>G</u> as <u>C</u> hromatography- <u>M</u> ass <u>S</u> pectrometry
GLC	<u>G</u> as- <u>L</u> iquid <u>C</u> hromatography
GLC	<u>G</u> as- <u>L</u> iquid <u>C</u> hromatography
GlcN	<u>G</u> lucosamine
GT	<u>G</u> lycosyltransferase
GW	<u>G</u> lass <u>W</u> ool

Introduction: Abbreviations

Hneg	<u>H</u> alo <u>n</u> egative
Hpos	<u>H</u> alo <u>p</u> ositive
JGI	<u>J</u> oint <u>G</u> enome <u>I</u> nstitute
KDO	3-deoxy-D-manno-octulosonic acid
LPS	<u>L</u> ipopolys <u>a</u> ccaride
MALDI-ToF MS	<u>M</u> atrix <u>A</u> ssisted <u>L</u> aser <u>D</u> esorption <u>T</u> ime-of- <u>F</u> light <u>M</u> ass <u>S</u> pectrometry
MS	<u>M</u> ass <u>S</u> pectrometry
MurNAc	<u>N</u> -acetyl <u>m</u> uramic acid
NETs	<u>N</u> eutrophil <u>e</u> xtracellular <u>t</u> raps
NMR	<u>N</u> uclear <u>M</u> agnetic <u>R</u> esonance
NTHI	<u>N</u> ontypeable <u>H</u> aemophilus <u>I</u> nfluenzae
OM	<u>O</u> uter <u>M</u> embrane
p	<u>P</u> yranose
PG	<u>P</u> eptidoglycan
pH	<u>P</u> otenz <u>H</u> ydrogen
PHA	<u>P</u> oly <u>h</u> ydroxyl <u>a</u> lkanoates
PM	<u>P</u> lasma <u>m</u> embrane
RAS	<u>R</u> eturned <u>A</u> ctivated <u>S</u> ludge
RNase	<u>R</u> ibon <u>u</u> clease
rRNA	<u>R</u> ibosomal <u>R</u> ibon <u>u</u> cleic <u>A</u> cid
SDS	<u>S</u> odium <u>D</u> odecyl <u>S</u> ulfate
SDS-PAGE	<u>S</u> odium <u>D</u> odecyl <u>S</u> ulphate- <u>P</u> oly <u>a</u> crylamide <u>G</u> el <u>E</u> lectrophoresis
SEM	<u>S</u> canning <u>E</u> lectron <u>M</u> icroscopy
SN	<u>S</u> upernatant
TCA	<u>T</u> ricarboxylic <u>A</u> cid
TEM	<u>T</u> ransmission <u>E</u> lectron <u>M</u> icroscopy
TFA	<u>T</u> ri <u>f</u> luoroacetic <u>A</u> cid
TLC	<u>T</u> hin <u>L</u> ayer <u>C</u> hromatography
TMS	<u>O</u> - <u>t</u> rimethyl <u>s</u> ilyl ethers
TMV	<u>T</u> obacco <u>M</u> osaic <u>V</u> irus
und-P	<u>u</u> ndecaprenyl <u>P</u> hosphate
WWTP	<u>W</u> astewater <u>T</u> reatment <u>P</u> lant

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