Purification and Characterisation of a Secreted Glycosidase, from the Extreme Xerophile *Wallemia ichthyophaga*

A thesis presented in partial fulfilment of the requirement for the degree in

Master of Science

in

Biochemistry

at Massey University Palmerston North,

New Zealand

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2014

Acknowledgments

Firstly, I would like to thank my supervisor Gillian Norris for encouraging me to always strive for my best, for always believing in me, your irreplaceable advice, and for the countless hours spent editing. Your mentoring throughout my masters has helped me develop as a person and a scientist, and I will always be grateful.

I would also like to thank my co-supervisor Mark Patchett for always being available to bounce ideas off, for his invaluable advice, and for editing my thesis.

Thank you to Trever Loo, our lab guru, whose wealth of knowledge and character has helped me with my masters in so many ways. Thank you especially with all the advice and time spent on the chromatography and mass spectrometry experiments.

Also, thank you to Meekyung Ann for never giving up on me. Thank you for all the advice, encouragement, comfort food, and company throughout my journey. I could not have done it without you, and I am a stronger person because of you.

Thank you to my amazing mum and dad. I am so blessed to have such loving and caring parents, and I wouldn't have gotten where I have or be the person that I am if it wasn't for all their encouragement, support, and belief in me.

I would also like to thank the rest of my family as well as all my Auckland and Palmerston North friends for all the encouragement, cups of tea, and for believing in me every step of the way, even when I didn't think I could do it. I would like to specifically thank my brother Gareth, Auntie Anita, Uncle Leon, Auntie Ida, Tracey Cropp, Andrew West, Rhiannon Moloney, Mackenna Dent, Fiona Given, Sarah Richardson, and Mark Thomas.

Special thanks to Natalie Gardner for always being there for me. Thank you for the support, for being a shoulder to cry on, and the many hours of entertainment spent in the decon room and tea room. Your friendship has been a constant source of inspiration.

Finally, thank you to all the other X-lab and IFS/IMBS people who have helped me throughout my thesis, whether it be through giving advice or assisting in an experiment.

Also, thank you to Massey University and LASRA for the opportunity and funding.

Abstract

With recent pressure to reduce the environmental impact of leather production, research has been focused on the development of an alternative depilation method, as the conventional method for depilation contributes up to 60% of the total pollution produced. Contaminated salted ovine pelts stored at LASRA were easily depilated when drum washed, and the resultant leather was of good quality. The pelts were visibly contaminated with microorganisms, and it was thought that these may be secreting enzymes that loosened the wool fibre without damaging key structural skin components. Identification of the enzyme or enzymes was thus of interest.

The microorganism/s responsible for the secretion of the depilation enzyme/s were isolated and identified through sequencing the 16S/18S ribosomal RNA genes. Depilation, using the crude secretome solutions, was then assessed using fresh ovine skin as well as SACPIC, a micro scale staining method used to assess skin structure. Unfortunately, none of the secretomes from either a single or a combination of the microorganisms isolated, had depilation activity.

The secretome of *W.ichthyophaga,* a xerophilic filamentous fungus, which was consistently isolated from the contaminated pelts, was chosen to be characterised using proteomic methods. 1D SDS-PAGE gel/CHIP separation of the proteins in the secretome showed it contained mainly glycosidases, with no lipases, esterases, or proteases identified. Some of the proteins identified had suggested roles in resistance to osmotic pressure, while the remaining proteins were intracellular. Overall, 21 proteins were identified.

A purification procedure involving AEX and SEC was successfully developed for the isolation of one of the glycosidases from the secretome. The resultant purified fractions formed a doublet band when analysed by SDS-PAGE. The reason for this remains unknown, but was shown not to be due to an impurity or heterodimerisation.

The purified glycosidase was identifed as belonging to the GH3 family by mass spectrometry. It was found to have a pH optimum of pH 6.0, was optimally active at 10% NaCl, and was itself glycosylated. The glycosidase was able to hydrolyse both α- and β- linked glycosidic bonds in di- and polysaccharides. Interestingly, both the disaccharide and artifical *p*-nitrophenol forms of galactose were not cleaved by the enzyme.

Table of Contents

2.2 Methods

List of Figures

1.0 Introduction and Aims

1.1 Introduction

1.2 Aims

3.0 Results and Discussion

3.1 Identification of microorganism from the contaminated salted ovine pelts

3.2 Analyse of the protein composition of the secretome of *W.ichthyophaga* using in-gel tryptic digest and EIS-Q-TOF mass spectrometry.

3.3 Purification of a novel glycosidase enzyme from the secretome of *Wallemia ichthyophaga*

3.4 Characterisation of a secreted glycosidase from *W.ichthyophaga*

List of Tables

2.0 Materials and Methods

2.2 Methods

3.0 Results and Discussion

3.1 Identification of microorganism from the contaminated salted ovine pelts

3.2 Analyse of the protein composition of the secretome of *W.ichthyophaga* using in-gel tryptic digest and EIS-Q-TOF mass spectrometry

3.3 Purification of a novel glycosidase enzyme from the secretome of *Wallemia ichthyophaga*.

3.4 Characterisation of a secreted glycosidase from *W.ichthyophaga*

List of Abbreviations

Listed in alphabetical and then numeral order

a.a	Amino Acid
AEX	Anion Exchange Chromatography
Abs	Absorbance
APS	Ammonium persulfate
Asn	Asparagine
AU	Absorbance Units
BLAST	Basic Local Alignment Search Tool
BSA	Bovine Serum Albumin
CEX	Cation Exchange Chromatography
cRAP	Common Repository of Adventitious Proteins
CV	Column Volume
DC	Direct Current
DNA	Deoxyribonucleic acid
DPX	Distrene, Plasticiser, Xylene
EDTA	Ethylenediaminetetraacetic acid
Endo H	Endoglycosidase H
EPS	Extracellular Polymeric Substrates
ESI	Electrospray Ionisation
ExPASy	Expert Protein Analysis System
FAS	Faciclin
Gdp1	Glycerol-3-phosphate Dehydrogenase
GHA	Glycosidase Clan A
GH2	Glycosidase Family 2
GlcNac	N-acetlyglucosamine
GMC	Glucose-methanol-choline
GOSs	Galactooligosacchride
HIC	Hydrophobic Chromatography
IEX	Ion Exchange Chromatography
KDa	Kilo Dalton

LASRA	Leather and Shoe Research Association
LB	Luria Broth
M	Molar
MES	2-(N-morpholino)ethanesulfonic acid
MIB	Sodium Malonate, Imidazole, and Boric acid
MS/MS	Tandem Mass Spectrometry
M/Z	Mass to Charge Ratio
PCR	Polymerase Chain Reaction
PNGase F	Peptide -N-Glycosidase F
ProDH	Proline Dehydrogenase
P5CDH	Pyrroline-5-carboxylate Dehydrogenase
Q-TOF	Quadruple Time-of-Flight
RF	Retardation Factor
RNA	Ribonucleic Acid
RPLC	Reverse-Phase Liquid Chromatography
SACPIC	SAfranine Celestin blue Picric acid
SAGE	Serial analysis of gene expression
SDS-PAGE	Sodium Dodecyl Sulfate Poylacrylaminde Gel Electrophoresis
SEC	Size Exclusion Chromatography
SSP	Small Secreted Proteins
TCA	Trichloroacetic acid
TEMED	N,N,N’,N’- Tetramethylethylenediamine
TGFBIp	Transforming Growth Factor-Beta-Induced Protein
UniProt	Universal Protein Resource
UV	Ultra Violet
w/v	Weight to Volume
w/w	Weight to Weight
1D	One Dimension
2D	Two Dimensions