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

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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 identified 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 artificial *p*-nitrophenol forms of galactose were not cleaved by the enzyme.

Table of Contents

Acknowledgements	i
Abstract	iii
List of Figures	ix
List of Tables	xiii
List of Abbreviations	xv

1.0 Introduction and Aims

1.1 Introduction

1.1.1 Conventional leather process.....	1
1.1.2 Re-evaluating the conventional leather process.....	2
1.1.3 Using enzymes as an alternative method for dewooling.....	2
1.1.4 Skin structure	3
1.1.5 Hair follicle.....	4
1.1.6 Factors effecting leather quality.....	6
1.1.7 Contaminated sheep pelts.....	6
1.1.8 Wallemia ichthyophaga.....	7
1.1.9 Fungal secretomes.....	11
1.1.10 Secretome analysis using a proteomic approach.....	14
1.1.11 Mass spectrometry.....	15
1.1.12 Glycosidases.....	16
1.1.13 Glycoside hydrolase family 3.....	17

1.2 Aims

1.2.1 Aims.....	19
1.2.2 Overview of the methodology.....	19

2.0 Materials and Methods

2.1 Material	21
---------------------------	-----------

2.2 Methods

2.2.1 MQ water.....	25
2.2.2 Media.....	25
2.2.3 Isolation by direct transfer.....	25
2.2.4 Isolation by liquid culture.....	25
2.2.5 PCR.....	26
2.2.6 PCR primers.....	27
2.2.7 Agarose gel electrophoresis.....	27
2.2.8 DNA sequencing.....	28
2.2.9 Database searches.....	28
2.2.10 Depilation: fresh skin.....	28
2.2.11 Depilation: Single section depilation method SACPIC visualisation....	28
2.2.12 Secretome preparation for mass spectrometry.....	30
2.2.13 SDS-PAGE.....	30
2.2.14 Coomassie staining of SDS-PAGE gels.....	31
2.2.15 Colloidal Coomassie staining of SDS-PAGE gels.....	32
2.2.16 In-gel tryptic digest.....	32
2.2.17 Mass spectrometry.....	33
2.2.18 Database searches.....	33
2.2.19 Secretome preparation for chromatography.....	34
2.2.20 Chromatograph preparation.....	34
2.2.21 Chromatography fraction standardisation.....	35
2.2.22 Anion exchange chromatography conditions.....	35
2.2.23 Cation exchange chromatography conditions.....	35
2.2.24 Generalised AEX and CEX parameters	36
2.2.25 Batch chromatography.....	36
2.2.26 Size exclusion conditions	36
2.2.27 SEC standard curve.....	37
2.2.28 Protease activity assay.....	37
2.2.29 Glycosidase activity assay for purification.....	37
2.2.30 Lipase activity assay.....	37
2.2.31 Bradford assay.....	38

2.2.32 Assay conditions for characterisation of glycosidase.....	38
2.2.33 Stability of the glycosidase.....	38
2.2.34 Substrate specificity assays.....	38
2.2.35 PNGase F and Endo H deglycosylation of glycosidase.....	39

3.0 Results and Discussion

3.1 Identification of microorganism from the contaminated salted ovine pelts

3.1.1 Isolation of microorganisms from the contaminated ovine pelts.....	41
3.1.2 Isolation of microorganisms from the contaminated ovine pelts using agar plates.....	44
3.1.3 Isolation of microorganisms from the contaminated ovine pelts using broths.....	45
3.1.4 Depilation activity.....	47
3.1.5 Addition of dried ground ovine skin to Wilson's media.....	49
3.1.6 Origin of the depilation activity from <i>W. ichthyophaga</i>	49

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

3.2.1 Secretome concentration via precipitation.....	51
3.2.2 Secretome concentration via ultrafiltration.....	52
3.2.3 Addition of dried ground ovine skin.....	54
3.2.4 Protein identification through mass spectrometry.....	54
3.2.5 Molecular weight of secreted proteins using SDS-PAGE.....	60
3.2.6 Mass spectrometry results of the secreted proteins from <i>W.ichthyophaga</i>	62

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

3.3.1 Anionic and cationic chromatography.....	69
3.3.2 Optimisation of anion exchange chromatography.....	70
3.3.3 Hydrophobic chromatography.....	80
3.3.4 Size exclusion chromatography.....	81

3.3.5 Final purification method using chromatography.....	83
3.4 Characterisation of a secreted glycosidase from <i>W.ichthyophaga</i>	
3.4.1 Quaternary structure of the glycosidase.....	97
3.4.2 Deglycosylation of the glycosidase using PNGase F and Endo H.....	98
3.4.3 Mass spectrometry of the final purified glycosidase fraction.....	102
3.4.4 Characterisation of the glycosidase.....	104
3.4.5 Optimum pH.....	105
3.4.6 The effect of NaCl on the activity of the glycosidase.....	107
3.4.7 The effect of metal ions on the activity of the glycosidase.....	108
3.4.8 Stability at 25°C.....	109
3.4.9 Inactivation of the glycosidase.....	110
3.4.10 Mini scale glucose test.....	111
3.4.11 Substrate specificity.....	111
4.0 Conclusion and Future Direction	
4.1 Conclusion.....	115
4.2 Future direction.....	119
5.0 Reference List.....	121

List of Figures

1.0 Introduction and Aims

1.1 Introduction

Figure 1.1.1: Summary of the conventional leather process.....	1
Figure 1.1.2: Diagram of the different layers and structures present in human skin.....	4
Figure 1.1.3: Diagram of the structure of a human hair follicle and pore.....	5
Figure 1.1.4: Phylogenetic tree of <i>Wallemiomycetes</i> in the phylum Basidiomycota.....	7
Figure 1.1.5: Morphological responses of the three <i>Walleimia</i> species to different NaCl concentrations.....	10
Figure 1.1.6: Classical and non-classical secretory mechanisms used by fungi.....	13
Figure 1.1.7: General CAZyme mechanism.....	16

1.2 Aims

Figure 1.2.1: Summary of the methodology used to isolate and identify the enzyme/s responsible from the depilation activity observed from the contaminated pelts.....	19
---	----

3.0 Results and Discussion

3.1 Identification of microorganism from the contaminated salted ovine pelts

Figure 3.1.1: Methodology used to isolate the microorganisms from the contaminated salted ovine pelts. Cultures in broths were done in duplicate with a different pelt used for each replicate.....	42
Figure 3.1.2: Examples of the different morphologies of colonies isolated from the contaminated pelts using both agar plates and broths of the three different media.....	43
Figure 3.1.3: SACPIC staining method.....	48

Figure 3.1.4: Fresh skin incubated in the secretome of *W. ichthyophaga* for 72 hours.....48

Figure 3.1.5: An example of a plate that may contain the isolates responsible for the initial depilation detected.....50

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

Figure 3.2.1. 7.5% acrylamide gel of the supernatant and pellet from the TCA-acetone precipitation of the secretome of *W.ichthyophaga*.....52

Figure 3.2.2: SDS-PAGE gel of a secretome from a 2 month old *W.ichthyophaga* culture grown in Wilson’s media made 20% with NaCl, and desalted by ultrafiltration.....53

Figure 3.2.3: *Wallemia ichthyophaga* secretome composition from cultures started from 3 individual colonies.....56

Figure 3.2.4: 7.5% acrylamide gel of the secretome of a *W.ichthyophaga* culture grown from colony.....57

Figure 3.2.5: Methodology used to analyse each band from the SDS-PAGE gel of the whole secretome of *W. ichthyophaga*.....58

Figure 3.2.6: Methodology for the analysis of the proteins present in the whole secretome of *W. ichthyophaga*.....59

Figure 3.2.7: Standard curve of the size marker from the SDS-PAGE of the secretome of *W.ichthyophaga*.....62

Figure 3.2.8 Structural diagram of phytate.....66

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

Figure 3.3.1: Trial 1, AEX of the secretome of *Wallemia ichthyophaga* using Q sephadex resin.....72

Figure 3.3.2: Trial 2: AEX of the secretome of *Wallemia ichthyophaga* using Q sephadex resin.....73

Figure 3.3.3: Protein concentration and enzymatic activity in fractions obtained from AEX of the secretome of *Wallemia ichthyophaga*.....75

Figure 3.3.4: Bradford standard curves using 90µL of reagent to 10µL of sample.....	76
Figure 3.3.5: 7.5% acrylamide gel of fractions obtained from AEX, and enzymatic activities.....	77
Figure 3.3.6: 7.5% acrylamide gel of the fractions obtained from the separation of the secretome of <i>Wallemia ichthyophaga</i> using AEX.....	79
Figure 3.3.7: The 3 step gradient elution profile of the secretome of <i>Wallemia ichthyophaga</i> with 10% glycerol using Q sephadex resin.....	80
Figure 3.3.8: The elution profile of fraction F13, fractionated by superdex 200 column.....	81
Figure 3.3.9: 7.5% acrylamide gel of fractions separated by SEC.....	82
Figure 3.3.10: Bradford assays across fractions obtained from AEX.....	83
Figure 3.3.11: The elution profile of the secretome of <i>Wallemia ichthyophaga</i> with 10% glycerol from Q sephadex resin.....	84
Figure 3.3.12: Bradford assays of concentrated fractions obtained from AEX.....	85
Figure 3.3.13: Glycosidase activity across fractions obtained from AEX.....	86
Figure 3.3.14: 7.5% acrylamide gel of fractions obtained from AEX, and specific activity.....	87
Figure 3.3.15: SEC of the fractions C9 and C11 from AEX.....	88
Figure 3.3.16: Bradford assays of fractions obtained from SEC.....	89
Figure 3.3.17: The average protein concentration, measured by Bradford assays, on fractions obtained from SEC.....	90
Figure 3.3.18: Glycosidase activity of fractions obtained from SEC.....	91
Figure 3.3.19: 7.5% SDS-PAGE analysing the concentrated fractions obtained from SEC and specific activity.....	92
3.4 Characterisation of a secreted glycosidase from <i>W.ichthyophaga</i>	
Figure 3.4.1: Size exclusion calibration curve using a sephadex 200 column.....	98
Figure 3.4.2: Cleavage sites of endoglycosidases PNGase F and Endo H.....	99
Figure 3.4.3: 7.5% SDS-PAGE of a PNGase digest of the glycosidase from <i>Wallemia ichthyophaga</i>	100

Figure 3.4.4: 7.5% SDS-PAGE of a Endo H digest of the glycosidase from <i>Wallemia ichthyophaga</i>	101
Figure 3.4.5: Graphical representation of the gene from the glycosidase purified from the secretome of <i>W.ichthyophaga</i> that was identified by mass spectrometry.....	103
Figure 3.4.6: Graphical representation of the possible protein domains present in the glycosidase purified from the secretome of <i>W.ichthyophaga</i>	104
Figure 3.4.7: Activity of the glycosidase from <i>Wallemia ichthyophaga</i> at different pH.....	106
Figure 3.4.8: Activity of the glycosidase from <i>Wallemia ichthyophaga</i> using 100mM MES.....	106
Figure 3.4.9: Activity of the glycosidase from <i>Wallemia ichthyophaga</i> over different pH ranges using 100mM citric acid:sodium phosphate.....	107
Figure 3.4.10: Activity of the glycosidase from <i>Wallemia ichthyophaga</i> at different NaCl concentrations in 100mM citric acid: sodium phosphate at pH 6.0.....	108
Figure 3.4.11: Activity of the secreted glycosidase from <i>Wallemia ichthyophaga</i> at different concentrations of EDTA in 100mM citric acid: sodium phosphate.....	109
Figure 3.4.12: The stability of glycosidase from <i>Wallemia ichthyophaga</i> at 25°C.....	110
Figure 3.4.13 Glucose assay kit reaction.....	110
Figure 3.4.14: Standard curve of different glucose concentrations using 1mL reactions from Glucose (GOD) assay kit (Sigma).....	111

List of Tables

2.0 Materials and Methods

2.2 Methods

Table 2.2.1: PCR reaction mixture for rRNA gene amplification.....	26
Table 2.2.2: SDS-PAGE stacking gel components and volumes.....	31
Table 2.2.3: SDS-PAGE separation gel components and volumes.....	31
Table 2.2.4: Reaction mixtures for the PNGase F and Endo H treatment of the purified glycosidase.....	39

3.0 Results and Discussion

3.1 Identification of microorganism from the contaminated salted ovine pelts

Table 3.1.1: Summary of the microorganisms isolated from the contaminated salted ovine pelts using agar plates of various media incubated at 25°C.....	44
Table 3.1.2: Summary of the microorganisms isolated from the contaminated salted ovine pelts by culturing in broths of different media incubated at 25°C.....	46

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

Table 3.2.1: Molecular weight of each band of the SDS-PAGE of the secretome of <i>W.ichthyophaga</i> using a standard curve.....	61
Table 3.2.2: Proteins identified in the secretome of <i>W.ichthyophaga</i>	62

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

Table 3.3.1: Protein concentrations of unbound fractions from CEX and AEX fractionation.....	70
Table 3.3.2: The gradient profile for anion exchange chromatography; trial one.....	71

Table 3.3.3: The two-step gradient profile run for the anion exchange chromatography; trial two.....	73
Table 3.3.4: The three-step gradient profile run for the anion exchange chromatography.....	78
Table 3.3.5: Glycosidase purification summary.....	93
Table 3.3.6: The overall percentage yield and purification fold for the purification of the glycosidase from <i>W.ichthyophaga</i>	94

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

Table 3.4.1: Protein identification of bands obtained from SEC and Endo H digestion gels using in-gel tryptic digestion and mass spectrometry.....	102
Table 3.4.2: Domains predicted to be present in the isolated glycosidase protein.....	105
Table 3.4.3: Substrate specificity of the glycosidase secreted from <i>Wallemia ichthyophaga</i>	112

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-acetylglucosamine
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	Pyrraline-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