Enzyme activities of fungi isolated from koala faeces

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Table of contents

Abstract

Filamentous fungi secrete enzymes to break down complex substances in the environment into smaller molecules they can use for nutrition. Investigation of the enzymes secreted by a fungus can lead to a better understanding of how it survives in a natural habitat; furthermore, new enzymes with potential for industrial applications can be revealed. In this work, the enzyme activities of fungi from koala faeces were investigated. As a result of a diet of *Eucalyptus* leaves, koala faeces are composed of recalcitrant plant cell wall polymers (cellulose, hemicellulose, pectin and lignin). Consequently, fungi that grow on koala faeces hold high potential as sources of enzymes for efficient degradation of plant biomass.

Thirty-seven fungal strains were isolated from koala faeces, identified, and screened for enzyme activities using agar plate assays; over two-thirds of the isolates secreted xylanases, endoglucanases, ligninases and proteases, and over one-third secreted amylases, mannanases and tannases. The enzyme activities of seven isolates were comprehensively characterised using liquid cultures, liquid enzyme assays and zymography. Two isolates, *Gelasinospora cratophora* A10 and *Trichoderma atroviride* A2, were high secretors of protein and heat-tolerant enzymes. The lipase(s) from *Mariannaea camptospora* A11 sustained activity at cool temperatures. The xylanase(s), mannanase(s), endoglucanase(s) and β-glucosidase(s) of *Doratomyces stemonitis* C8 displayed optimal activities under neutral to alkaline conditions. Some of the enzymes hold potential for application in the production of paper, textiles, detergents and ethanol-based biofuels.

Finally, the secretome of *D. stemonitis* C8 was studied by gel electrophoresis and mass spectrometry. As the genome of *D. stemonitis* has not been sequenced, the secretome analysis required cross-species identification and *de novo* sequencing; furthermore, a new technique was developed to identify proteins directly from zymogram gels by mass spectrometry. In the

first secretome analysis of a coprophilous fungus, a complex array of enzymes integral to plant biomass degradation was identified, including enzymes that could be of value to industry in the future.

Declaration

I certify that the research presented in this thesis is original work carried out by the author. The work has not been presented for a higher degree to any university or institution other than Macquarie University, and contains no material previously published or written by any other person except where due reference is made in the text.

Robyn Peterson

Publications

The following publications resulted from the work carried out for this thesis. Each of the publications are introduced, presented and discussed in turn throughout the thesis chapters. Additional work, not included in the publications, is also described.

Publication 1:

Peterson, R.A., Bradner, J.R., Roberts, T.H., Nevalainen, K.M.H., 2009. Fungi from koala (*Phascolarctos cinereus*) faeces exhibit a broad range of enzyme activities against recalcitrant substrates. Letters in Applied Microbiology. 48, 218-225.

Publication 2:

Peterson, R., Grinyer, J., Nevalainen, H., 2011. Extracellular hydrolase profiles of fungi isolated from koala faeces invite biotechnological interest. Mycological Progress. 10, 207-218.

Publication 3:

Peterson, R., Grinyer, J., Joss, J., Khan, A., Nevalainen, H., 2009. Fungal proteins with mannanase activity identified directly from a Congo Red stained zymogram by mass spectrometry. Journal of Microbiological Methods. 79, 374-377.

Publication 4:

Peterson, R., Grinyer, J., Nevalainen, H., 2011. Secretome of the coprophilous fungus *Doratomyces stemonitis* C8, isolated from koala faeces. Applied and Environmental Microbiology. 77, 3793-3801.

Abbreviations

BLAST	Basic Local Alignment Search Tool
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
IPG	immobilised pH gradient
ITS	internal transcribed spacer
Kb	kilobase pair
kDa	kilodaltons
LC	liquid chromatography
MALDI	matrix assisted laser desorption ionisation
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NCBI	National Center for Biotechnology Information
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PDA	potato dextrose agar
PMF	peptide mass fingerprinting
Q-TOF	quadrupole time-of-flight
rDNA	ribosomal DNA
SDS	sodium dodecyl sulphate
v/v	volume/volume
w/v	weight/volume

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