Investigating Mechanisms of Toxicity and Detoxification of BMAA in *Escherichia coli*

by Carly Jade Italiano

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy (Science)

under the supervision of Associate Professor Kenneth Rodgers and Associate Professor Iain Duggin

University of Technology Sydney
Faculty of Science

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Certificate of Original Authorship

I, Carly Jade Italiano declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy in the School of Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

Signature:

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The pursuit of this PhD has been a difficult one and am deeply thankful to the many people in my life who have helped me get to the finish line. I would like to thank Ken and Iain for giving me the opportunity to undertake this project, and for supervising me through both my honours year and now this PhD. Their advice and feedback have helped shape this project in many ways. Additionally, support from everyone in the Rodgers and Duggin lab groups has been wonderful. Special mentions must go to Joel, Jake, Lisa, Connor, and Rodger's lab alumni, Kate, and Brendan. Even though my *E. coli* work was a little different from the rest of the research group, it was always embraced and appreciated.

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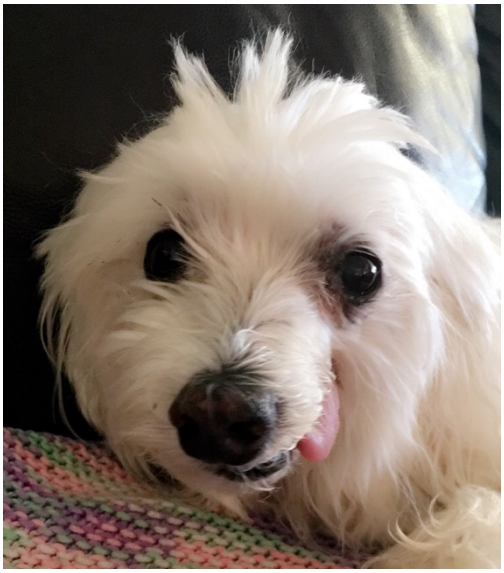
For Spud
2005 -2020



COVID-19 Impact Statement

The latter part of this thesis was conducted during the COVID-19 pandemic and as such, a number of adaptations to the project were necessary.

Travel restrictions during 2020 resulted in disruptions and delays to ongoing experiments. The continuing delays in sourcing required laboratory supplies due to global changes to transport extended experiment timelines beyond anticipation. Reduced access to facilities required for data analysis meant much processing was conducted in a home setting. Resulting analysis using limited internet services in a rural area meant delays in data analysis, particularly for Aim 3.
Despite the various delays and difficulties experienced due to COVID-19, a solid body of work has been produced, culminating in this thesis.

Out of the four Aims covered in this thesis, Aims 3 and 4 were particularly impacted by difficulties that arose due to COVID-19 impacts. As a result, the number of experiments able to be completed was somewhat less than initially planned prior to COVID-19. Despite this, each Aim has still been covered satisfactorily and represents a meaningful contribution to the research field. I ask that the examiners please take this into consideration during their assessment.

Publications

Steele JR, Italiano CJ, Phillips CR, Violi JP, Pu L, Rodgers KJ, and Padula MP, *Misincorporation Proteomics Technologies: A Review.* Proteomes 2021;**9**(1).

Main BJ, Italiano CJ, and Rodgers KJ, *Investigation of the interaction of beta-methylamino-L-alanine with eukaryotic and prokaryotic proteins.* Amino Acids 2018;**50**(3-4):397-407.

Under Review:

Italiano CJ, Pu L, Violi JP, Duggin IG, and Rodgers KJ, *Tolerance towards Beta-methylamino-L-alanine in Escherichia coli requires cysteine biosynthesis genes.* Research in Microbiology 2021.

Accepted version:

Italiano CJ, Pu L, Violi JP, Duggin IG, and Rodgers KJ, *Cysteine biosynthesis contributes to β-methylamino-l-alanine tolerance in Escherichia coli.* Research in Microbiology 2021;**172**(6):103852.

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List of Abbreviations

AARS - aminoacyl-tRNA synthetase

ALS - Amyotrophic Lateral Sclerosis

ALS-PDC - Amyotrophic Lateral Sclerosis – parkinsonism/dementia complex

AQC - 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate

BCA - bicinchoninic acid protein quantification

BMAA - β-Methylamino-L-alanine

CFU/mL - colony forming units per millilitre

CGSC - Coli Genetic Stock Centre

Cm - Chloramphenicol

CmR - Chloramphenicol Resistant

DNA - deoxyribonucleic acid

DTT - Dithiothreitol

E. coli - *Escherichia coli*

EDTA - Ethylenediaminetetraacetic acid

FA - Formic acid

FC – fold change

Flp - Flippase recombinase

FRT - Flippase recognition target

GSH - glutathione

GSSG - glutathione disulfide

HCl - hydrochloric acid

IGV - integrative genomics viewer

OD_{600} - Optical density at 600 nanometres

IPTG - Isopropyl β-D-1-thiogalactopyranoside

Km - Kanamycin

KmR - Kanamycin Resistant

L-DOPA - L-3,4-dihydroxyphenylalanine

LB - Luria Bertani medium

LC-MS/MS - Liquid chromatography with tandem mass spectrometry

LOD - limit of detection

MND - Motor Neurone Disease

MRM - Multiple reaction monitoring

LOQ - limit of quantification

NADPH - reduced form of nicotinamide adenine dinucleotide phosphate

NAS - N-Acetyl-L-serine

NCBI - National Center for Biotechnology Information.

NMDA - N-methyl-D-aspartate

NPAA - Non-protein amino acid

OAS - O-acetyl-L-Serine

OASase - O-acetylserine sulfhydrylase

PAGE - polyacrylamide gel electrophoresis

PBS - phosphate buffered saline

PCR - Polymerase Chain Reaction

PLP - pyridoxal-5’ phosphate

RNA - Ribonucleic acid

ROS - reactive oxygen species

Rpm – revolutions per minute

RSD - relative standard deviation

SAT - Serine O-Acetyltransferase

SDS - sodium dodecyl sulfate

Snp - single nucleotide polymorphism

STRING - Search Tool for the Retrieval of Interacting Genes/Proteins

TIC - Total ion chromatogram

TQMS - Triple quadrupole mass spectrometry

UHPLC - ultra high-performance liquid chromatography

UTS - University of Technology Sydney

Vo - Initial velocity

β-PA - β-(Pyrazol-1-yl)-L-alanine

Abstract

Incurable neurodegenerative diseases are becoming an increasingly common problem affecting our population. Research into environmental triggers which contribute to development of these conditions is vital for understanding disease pathogenesis and prevention. Non-protein amino acids are a class of naturally occurring molecules that can interfere with metabolic processes in cells to cause toxicity. Exposure to the neurotoxic non-protein amino acid BMAA has been linked to the development of neurodegenerative disease. BMAA is known to be produced by cyanobacteria, and their wide distribution globally increases the likelihood of human contact with this toxin. Additionally, BMAA can bioaccumulate in ecosystems, compounding the risk of human exposure. While there are a variety of studies on the neurotoxic effects of BMAA in mammalian systems, less work has been conducted in prokaryotic species such as bacteria. Previous work has indicated that the bacterium *Escherichia coli* exhibits tolerance towards BMAA that is in contrast to the toxicity seen in mammalian cells and animal models. However, there has been no studies into the mechanism behind this tolerance.

In this project, screening of *Escherichia coli* mutants with disruptions to various amino acid biosynthesis pathways indicated that cysteine biosynthesis genes (*cysE, cysK,* and *cysM*) are important for BMAA tolerance. Disruption to any of these genes resulted in susceptibility to BMAA and growth inhibition, which manifested as a substantial delay in the onset of logarithmic growth. Severity of delay was dependent on BMAA concentration. However, following a period of BMAA-induced growth delay, a recovery in growth was observed. The possibility that additional genetic mutations spontaneously arise in cultures and suppress sensitivity to BMAA in *Escherichia coli* (*cysE* mutant) was also investigated. Results showed that genetic changes were not responsible for the restoration of growth that occurs following BMAA-induced inhibition. Analysis of gene expression changes in *Escherichia coli* exposed to BMAA showed a pattern indicative of an iron starvation response. There were also changes to sulfur homeostasis and amino acid metabolism (cysteine and threonine).

O-acetylserine sulfhydrylase (*cysM*) from the cysteine biosynthetic pathway was tested for ability to enzymatically degrade BMAA, which would explain the requirement of

cysteine biosynthetic genes for BMAA tolerance. While the enzyme did not degrade BMAA, the cofactor pyridoxal-5'phosphate which the enzyme uses to catalyse cysteine biosynthesis reactions did, resulting in the production of methylamine. Results from this project have explored different possibilities for *Escherichia coli* tolerance towards BMAA and provided insights into BMAA metabolism. This work has opened up many future directions of BMAA research including the importance of cysteine metabolism in BMAA toxicity, enzymatic degradation of BMAA via pyridoxal-5'phosphate dependent enzymes, and exploration of iron chelating properties of BMAA.