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**GENETICS AND PHYSIOLOGY  
OF RESPIRATION  
IN *ASPERGILLUS NIDULANS***

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
DOCTOR IN PHILOSOPHY  
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# ABSTRACT

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Obligate aerobes such as *Aspergillus nidulans* primarily use the classical respiratory pathway for ATP production. However, the use of alternative energy-producing processes in *A. nidulans* was first speculated upon when cytochrome *c*-deficient strains were observed to be viable upon fermentable carbon sources. It was therefore postulated that the *cytA* strains of *A. nidulans* may use fermentation and an alternative respiratory pathway to compensate for the non-functioning cytochrome *c*-dependent pathway.

Characterisation of the *A. nidulans* cytochrome *c*-deficient strains was carried out. The growth parameters for strain A68 were consistent with other cytochrome *c* mutants; the strain grew more slowly than the corresponding wildtype strain on fermentable carbon sources, and produced higher levels of ethanol. Spectral analysis confirmed the lack of detectable levels of cytochrome *c* in the *cytA* strains, and decreased levels of cytochrome *c* oxidase, consistent with the non-functioning cytochrome *c*-dependent respiratory pathway. The presence of a hemoglobin-like molecule in the *cytA* and *cytA*<sup>+</sup> strains was determined by CO binding assays.

Inhibitor studies determined the presence of an alternative respiratory pathway in *cytA* and *cytA*<sup>+</sup> strains of *A. nidulans*. An active cytochrome *c*-dependent pathway was found to be present in the *cytA*<sup>+</sup> strains, yet absent from the *cytA* strains. Results also suggested the presence of a putative third terminal oxidase in the *cytA* and *cytA*<sup>+</sup> strains. Increased levels of *b*-type heme observed in the redox spectra of the *cytA* strains were suggested to be associated with the putative third terminal oxidase.

Therefore, the *cytA* strains of *A. nidulans* appear to use fermentation and the alternative respiratory pathway to compensate for the non-functioning cytochrome *c*-dependent pathway. The putative hemoglobin molecule identified in these strains may also function as a terminal oxidase, in addition to the putative third terminal oxidase.

PCR amplification with degenerate primers was carried out to confirm the presence of an *AOX* gene in *cytA*<sup>+</sup> and *cytA* strains of *A. nidulans*. The product of the *AOX* gene is likely to function as a terminal oxidase in the alternative pathway. A comparison of fungal and plant AOX protein sequences was carried out. A conserved cysteine residue which has

been implicated in dimer formation and pyruvate regulation was found to be absent from the fungal sequences.

A preliminary expression study of the *A. nidulans* *AOX* gene was carried out by RT-PCR. The putative regulatory elements identified within the *A. nidulans* *AOX* gene promoter are also located within the promoters of other respiratory-related genes (eg. *A. nidulans* *cycA*) and genes involved in reducing the formation of reactive oxygen species (ROS) (eg. *A. nidulans* *catA-C* and *sod1*). This implies similar mechanisms of regulation, which may be controlled in a coordinated manner.

A functional analysis of the *A. nidulans* *cycA* gene promoter was carried out to identify important regulatory elements. Reporter constructs containing *cycA-lacZ* fusion genes were transformed into *A. nidulans*. Although integrated at the *argB* locus, the constructs had very low levels of *lacZ* expression (with the exception of the positive control). A number of parameters were investigated, and a 'promoter switch' experiment commenced, however the cause of the faulty *cycA-lacZ* expression system was not determined.

Functional expression of the *A. nidulans* *cycA* gene promoter in yeast was also attempted, as regulatory mechanisms controlling cytochrome *c* expression are believed to be analogous in *A. nidulans* and *Saccharomyces cerevisiae*. Reporter constructs containing *cycA-lacZ* fusion genes were transformed into *S. cerevisiae*, with resulting low  $\beta$ -galactosidase activity, similar to the results in *A. nidulans*. The wildtype strain of *A. nidulans* from which the *cycA* gene promoter fragment was amplified was shown by spectral analysis to have low levels of cytochrome *c*, in comparison to other wildtype strains of *A. nidulans*. Therefore, it is possible that the low levels of *lacZ* expression from the *cycA-lacZ* fusion genes may be representative of the level of *cycA* expression in that strain. However, low levels of *lacZ* expression were also observed for the *S. cerevisiae* positive control, indicating that the expression system was not working properly. Therefore, the expression of the *A. nidulans* *cycA* gene promoter in *S. cerevisiae* could not be assessed in this study.

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## ABBREVIATIONS

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ANCF	<i>Aspergillus nidulans</i> CCAAT binding factor
AOX	alternative oxidase
ATP	adenosine triphosphate
bp	base pair
cAMP	cyclic adenosine monophosphate
CCR	carbon catabolite repression
CCHL	cytochrome <i>c</i> heme lyase
CO	carbon monoxide
COX	cytochrome <i>c</i> oxidase
<i>CycA</i>	<i>A. nidulans</i> gene encoding cytochrome <i>c</i>
<i>CYC1</i>	<i>Saccharomyces cerevisiae</i> gene encoding iso-1-cytochrome <i>c</i>
<i>CYC7</i>	<i>S. cerevisiae</i> gene encoding iso-2-cytochrome <i>c</i>
DNA	deoxyribonucleic acid
EMP	Embden-Meyerhof-Parnas pathway
ETC	electron transport chain
HAP1	<i>S. cerevisiae</i> heme activated protein, mediating oxygen regulation
HAP2/3/4/5	<i>S. cerevisiae</i> CCAAT binding factor, mediating carbon regulation
HMC	high molecular weight complex
hr	hour(s)
Kb	kilobase pair(s)
KCN	potassium cyanide
MAI	maximum amount of inhibition
min	minute(s)
NADH	nicotinamide adenine dinucleotide
ORF	open reading frame
PCR	polymerase chain reaction
RPM	repression modules
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcribed PCR
SHAM	salicyl hydroxamic acid
SOD	Superoxide dismutase
TCA	tricarboxylic acid cycle
<i>tsp</i>	transcriptional start site

# TABLE OF CONTENTS

---

Abstract	i
Acknowledgements	iii
Abbreviations	iv
Table of Contents	v
List of Figures	xii
List of Tables	xiv
List of Appendices	xv
<b>Chapter 1</b>	<b>Introduction</b>
	1
<b>1.1</b>	<b>General introduction</b>
	1
1.1.1	Commerical applications of <i>Aspergillus</i>
	1
1.1.2	Adverse effects of <i>Aspergillus</i>
	2
1.1.3	Control of environmental conditions
	3
1.1.4	<i>A. nidulans</i>
	3
<b>1.2</b>	<b>Energy transduction</b>
	4
1.2.1	Brief overview
	4
1.2.2	Aerobic respiration
	6
1.2.3	Anaerobic respiration
	8
<b>1.3</b>	<b>The classical respiratory pathway</b>
	9
1.3.1	The cytochrome <i>c</i> protein
	10
	Translocation of the cytochrome <i>c</i> protein
	10
	The role of the cytochrome <i>c</i> protein in apoptosis
	11
<b>1.4</b>	<b>The alternative respiratory pathway</b>
	11
1.4.1	Activity of the pathway
	13
	Inducing conditions
	13
1.4.2	Functions of the pathway
	14
1.4.3	The alternative oxidase protein
	16
	Structure of the AOX protein
	17
1.4.4	Regulation of the alternative respiratory pathway
	18
<b>1.5</b>	<b>Transcriptional regulation of eukaryotic gene expression</b>
	20
1.5.3	Control of gene expression in filamentous fungi
	21
<b>1.6</b>	<b>The cytochrome <i>c</i> gene (<i>cycA</i>) in <i>Aspergillus nidulans</i></b>
	22
1.6.1	The presence of putative regulatory elements within the <i>cycA</i>
	gene promoter
	22
1.6.2	Transcriptional regulation of the <i>cycA</i> gene
	23
	Regulation by oxygen
	24
	Regulation by carbon source
	25
	Heat shock regulation
	25
<b>1.7</b>	<b>Regulation of cytochrome <i>c</i> expression in <i>Saccharomyces cerevisiae</i></b>
	26
1.7.1	Transcriptional regulation of <i>CYC1</i> & <i>CYC7</i>
	27
	Regulation by heme & oxygen
	27
	Regulation by carbon source
	29
<b>1.8</b>	<b>The yeast transcriptional activator, HAP1</b>
	30
1.8.1	Structure of the HAP1 protein
	31
	HAP1 target sequences
	32
	The dimerisation domain
	34
	The heme-responsive motifs
	35
	The transcriptional activation domain
	35

1.8.2	Activation & repression of the HAP1 protein	35
<b>1.9</b>	<b>The yeast transcriptional activator, the heteromeric HAP2/3/4/5 complex</b>	<b>36</b>
1.9.1	Homologues of the HAP2/3/4/5 complex	36
1.9.2	Activity of the HAP2/3/4/5 complex	37
1.9.3	The subunits of the HAP2/3/4/5 complex	37
1.9.4	The subunits of the ANCF	38
1.9.4	Assembly of the HAPB/C/E complex (ANCF)	38
<b>1.10</b>	<b>Aims of the project</b>	<b>40</b>
<b>Chapter 2</b>	<b>Materials and methods</b>	<b>42</b>
<b>2.1</b>	<b>Fungal strains, bacterial strains and plasmids</b>	<b>42</b>
<b>2.2</b>	<b>Water supply and sterilisation</b>	<b>42</b>
<b>2.3</b>	<b>Media</b>	<b>42</b>
2.3.1	Bacterial media	42
	Liquid media	43
	Solid media	43
	Media supplements	43
2.3.2	Fungal media	43
	Liquid media for <i>A. nidulans</i>	43
	Liquid media for <i>S. cerevisiae</i>	43
	Solid media	43
	Solid media for <i>S. cerevisiae</i>	44
	Media supplements for <i>A. nidulans</i>	44
	Media supplements for <i>S. cerevisiae</i>	47
<b>2.4</b>	<b>Growth and maintenance of cultures</b>	<b>47</b>
2.4.1	Bacterial cultures	47
	Long-term storage	47
2.4.2	Fungal cultures	48
	Purification of <i>A. nidulans</i> strains	48
	Preparation of <i>A. nidulans</i> spore suspensions	48
	Long-term storage of <i>A. nidulans</i>	49
	Long-term storage of <i>S. cerevisiae</i>	49
	Short-term storage of <i>A. nidulans</i>	49
	Short-term storage of <i>S. cerevisiae</i>	49
<b>2.5</b>	<b>Plasmid DNA isolation</b>	<b>50</b>
2.5.1	Small scale plasmid DNA isolation	50
	Small scale plasmid DNA isolation by the rapid boil method	50
	Small scale alkaline lysis plasmid preparation	50
	Small scale plasmid DNA isolation using the concert kit	51
2.5.2	Purification of small scale plasmid DNA by peg precipitation	51
	Large scale plasmid DNA preparation	51
	Large scale alkaline lysis plasmid preparation	52
	Large scale plasmid isolation using the Qiagen midi plasmid preparation kit	52
<b>2.6</b>	<b>Fungal DNA extraction</b>	<b>52</b>
2.6.1	Small scale DNA preparation	53
2.6.2	Large scale DNA preparation	53
<b>2.7</b>	<b>DNA manipulation procedures</b>	<b>54</b>
2.7.1	Enzymatic digestion of DNA	54
2.7.2	Electrophoresis	54

2.7.3	Determination of molecular weights	55
2.7.4	Isolation of DNA fragments	55
	Extraction of DNA from agarose	55
2.7.5	Quantification of DNA concentration	56
	Estimation of DNA concentration using electrophoresis	56
	Estimation of DNA concentration by fluorometry	56
<b>2.8</b>	<b>Polymerase chain reaction (PCR)</b>	<b>56</b>
2.8.1	Standard PCR protocol	56
2.8.2	Gel-stab PCR	57
2.8.3	Purification of PCR products	59
<b>2.9</b>	<b>Cloning</b>	<b>59</b>
2.9.1	Phosphatase treatment of vectors	59
2.9.2	DNA ligation	59
	General ligation reactions	59
	Ligations with the pGEM system	60
<b>2.10</b>	<b>Transformation of genetic material into cells</b>	<b>60</b>
2.10.1	Bacterial transformation	60
	Preparation of competent cells	60
	Electroporation of competent cells	61
2.10.2	<i>Aspergillus</i> transformation	61
	Preparation of fungal protoplasts from liquid culture	61
	Preparation of fungal protoplasts from solid media	62
	Protoplast flotation	62
	Transformation of competent cells by peg precipitation	62
2.10.3	Yeast transformation	63
	Preparation of competent cells	63
	Lithium acetate transformation	64
<b>2.11</b>	<b>Sequencing</b>	<b>64</b>
2.11.1	Automated sequencing	64
2.11.2	Manual sequencing	64
	Preparation of the DNA samples	64
	Preparation of the sequencing gel	65
	Electrophoresis of the sequencing samples	65
2.11.3	Analysis of DNA or amino acid sequence	66
<b>2.12</b>	<b>Southern blotting &amp; probe hybridisation</b>	<b>66</b>
<b>2.13</b>	<b>Quantitative assays</b>	<b>67</b>
2.13.1	$\beta$ -galactosidase assays	67
	<i>Aspergillus</i> $\beta$ -galactosidase assays	68
	Yeast $\beta$ -galactosidase assays	69
2.13.2	Protein assays	69
<b>2.14</b>	<b>Growth measurements of the fungal strains</b>	<b>70</b>
2.14.1	Growth on various carbon sources	70
2.14.2	Radial growth measurements	70
<b>2.15</b>	<b>Respiratory measurements of the fungal strains</b>	<b>70</b>
2.15.1	Growth of cultures	70
2.15.2	Preparation of mycelial samples	71
2.15.3	Oxygen consumption assays	71
<b>2.16</b>	<b>Ethanol assays</b>	<b>71</b>
<b>2.17</b>	<b>Cytochrome spectra &amp; CO binding assay</b>	<b>72</b>
<b>2.18</b>	<b>RNA work</b>	<b>72</b>
2.18.1	RNA extraction	72

2.18.2	Estimation of RNA concentration	73
2.18.3	DNase treatment	73
	Ampligrade DNase kit	74
	DNase treatment	74
2.18.4	cDNA synthesis	74
	The expand reverse transcriptase (Rt) kit	75
	The Geneamp Gold RNA PCR reagent kit (one-step)	75
2.18.5	Rt-PCR	76
	Standard PCR protocol	76
	The Geneamp Gold RNA PCR reagent kit (two-step)	77
<b>Chapter 3</b>	<b>Characterisation of the mutant strains</b>	<b>78</b>
<b>3.1</b>	<b>Introduction</b>	<b>78</b>
<b>3.2</b>	<b>Growth studies of the mutant strain</b>	<b>79</b>
3.2.1	Growth on various carbon sources	79
3.2.2	Radial growth and spore germination	79
3.2.3	Growth curve for the <i>ycA</i> - strain	81
3.2.4	Discussion	83
<b>3.3</b>	<b>Levels of cytochrome <i>c</i> in the <i>ycA</i>- strains</b>	<b>84</b>
3.3.1	Redox spectra results	84
3.3.2	Discussion	86
<b>3.4</b>	<b>Fermentation in <i>A. nidulans</i></b>	<b>88</b>
3.4.1	Results	88
3.4.2	Discussion	89
<b>3.5</b>	<b>Oxygen consumption assays and inhibitor studies</b>	<b>89</b>
3.5.1	Activity of the alternative respiratory pathway	89
3.5.2	Results	91
	Strain A67 ( <i>ycA</i> <sup>+</sup> )	91
	Strain A68 ( <i>ycA</i> <sup>-</sup> )	95
	Strains A57 ( <i>ycA</i> <sup>+</sup> ) and A58 ( <i>ycA</i> <sup>-</sup> )	97
3.5.3	Discussion	97
	Strains A57 and A67 ( <i>ycA</i> <sup>+</sup> )	97
	Strains A58 and A68 ( <i>ycA</i> <sup>-</sup> )	98
	Putative third terminal oxidase	98
	The validity of inhibitor studies	99
	Techniques for ascertaining electron partitioning	101
3.5.4	Conclusion	102
<b>3.6</b>	<b>Carbon monoxide binding assay</b>	<b>103</b>
3.6.1	Results	103
3.6.2	Discussion	105
	The role of hemoglobin in other organisms	105
	Expression of hemoglobin	106
	Possible roles of hemoglobin in <i>A. nidulans</i>	107
<b>3.7</b>	<b>Conclusion</b>	<b>107</b>
<b>Chapter 4</b>	<b>The <i>AOX</i> gene of <i>A. nidulans</i></b>	<b>109</b>
<b>4.1</b>	<b>Introduction</b>	<b>109</b>
4.1.1	The alternative oxidase protein	109
4.1.2	Putative regulatory elements involved in <i>AOX</i> transcriptional regulation	111

<b>4.2</b>	<b>PCR amplification of the alternative oxidase gene from <i>A. nidulans</i></b>	112
4.2.1	Design of the degenerate PCR primers	112
4.2.2	PCR amplification of fragments of the <i>A. nidulans</i> alternative oxidase gene	113
<b>4.3</b>	<b>Cloning and sequencing of the <i>A. nidulans</i> AOX fragments</b>	115
4.3.1	Sequence results	117
<b>4.4</b>	<b>Discussion of sequence results</b>	117
4.4.1	Alignment of the complete <i>A. nidulans</i> AOX nucleotide sequence with the partial fragment	117
4.4.2	Alignment of the complete <i>A. nidulans</i> AOX nucleotide sequence with other fungal AOX genes	121
	Presence of introns	121
4.4.3	Conserved sequences within the coding region	121
4.4.4	Analysis of the <i>A. nidulans</i> AOX gene promoter	124
<b>4.5</b>	<b>Expression study of the <i>A. nidulans</i> alternative oxidase gene</b>	127
4.5.1	Preparation of RNA samples	129
4.5.2	Primers for Rt-PCR amplification	129
4.5.3	Results from Rt-PCR amplification	129
<b>4.6</b>	<b>Conclusion</b>	130
<b>Chapter 5</b>	<b>Functional analysis of the <i>A. nidulans cycA</i> gene promoter</b>	132
<b>5.1</b>	<b>Introduction</b>	132
<b>5.2</b>	<b>The components of the reporter constructs</b>	133
5.2.1	The reporter vector	133
5.2.2	The <i>A. nidulans cycA</i> gene promoter fragments	135
	PCR amplification of the <i>cycA</i> promoter fragments	135
<b>5.3</b>	<b>Preparation of the reporter constructs</b>	138
5.3.1	Overview of the cloning strategy	138
5.3.2	Methodology	139
5.3.3	The reporter construct	139
5.3.4	The <i>A. nidulans cycA</i> gene promoter fragments	139
<b>5.4</b>	<b>Transformation of the reporter constructs into <i>A. nidulans</i></b>	140
<b>5.5</b>	<b>Analysis by southern blotting</b>	141
5.5.1	Strategy for southern blotting analysis	141
5.5.2	Southern blotting results	143
	Determination of single copy integration	143
	Determination of the presence of the <i>A. nidulans cycA</i> gene promoter fragment	146
<b>5.6</b>	<b>Beta-galactosidase assays</b>	148
5.6.1	Results	149
5.6.3	Discussion	151
<b>5.7</b>	<b>Experiment to determine the cause of the faulty expression system</b>	151
5.7.1	The <i>argB2</i> mutant strain	151
5.7.2	Translational fusions	151
5.7.3	Sequencing of the <i>lacZ</i> reporter gene	152
5.7.4	The promoter switch experiment	152
	Results	153
<b>5.8</b>	<b>Discussion</b>	154

5.9	Conclusion	157
Chapter 6	Functional expression of the <i>A. nidulans cycA</i> gene promoter in <i>S. cerevisiae</i>	158
6.1	Introduction	158
6.2	The components of the reporter constructs	158
6.2.1	The reporter constructs	158
6.2.2	The <i>A. nidulans cycA</i> gene promoter fragment	160
6.3	Preparation of the reporter constructs	160
6.3.1	Methodology	160
6.3.2	The reporter constructs	161
6.4	Transformation of the constructs into <i>S. cerevisiae</i>	161
6.5	$\beta$ -galactosidase assays	162
6.5.1	Results & discussion	162
6.6	Conclusion	166
	Conclusion	167
	Future directions	170
	Appendices	175
	References	198

## LIST OF FIGURES

<b>Figure 1.1</b>	Simplistic model showing the interconnection of the three pathways involved in energy transduction	6
<b>Figure 1.2</b>	The classical respiratory pathway	7
<b>Figure 1.3</b>	Fermentative metabolism	9
<b>Figure 1.4</b>	Entry point of the alternative respiratory pathway into the classical respiratory pathway	12
<b>Figure 1.5</b>	Alignment of putative HAP1 binding sites located within the <i>A. nidulans cycA</i> promoter fragment, with 'consensus' yeast HAP1 binding site	23
<b>Figure 1.6</b>	Regulation of aerobic and hypoxic genes in yeast	28
<b>Figure 1.7</b>	Schematic representation of the HAP1 protein	31
<b>Figure 1.8</b>	A Binding of the HAP1 protein B Alignment of known yeast HAP1 UASs with the consensus HAP1 target sequence	33
<b>Figure 1.9</b>	Assembly of the AnCF	39
<b>Figure 3.1</b>	Growth of the <i>cycA</i> <sup>-</sup> strain on fermentable & non-fermentable carbon sources	80
<b>Figure 3.2</b>	Growth curves of strains A67 ( <i>cycA</i> <sup>+</sup> ) and A68 ( <i>cycA</i> <sup>-</sup> )	82
<b>Figure 3.3</b>	Redox spectra for <i>cycA</i> mutant strains A58 and A68, and <i>cycA</i> <sup>+</sup> strain A67	85
<b>Figure 3.4</b>	Relative activity of strain A67 ( <i>cycA</i> <sup>+</sup> ) following the addition of inhibitors, late-exponential and stationary phase samples	93
<b>Figure 3.5</b>	Representative oxygen consumption assay traces of the <i>A. nidulans</i> strains	94
<b>Figure 3.6</b>	Relative activity of strain A68 ( <i>cycA</i> ) following the addition of SHAM, mid-exponential and stationary phase samples	96
<b>Figure 3.7</b>	Carbon monoxide spectra of <i>A. nidulans</i> and <i>S. cerevisiae</i> strains	104
<b>Figure 4.1</b>	Alignment of alternative oxidase amino acid sequences	110
<b>Figure 4.2</b>	Fragments of the <i>A. nidulans</i> alternative oxidase gene, generated by PCR amplification using degenerate primers (annealing temperature of 47°C)	114
<b>Figure 4.3</b>	Re-amplification of PCR products, using degenerate primers to the <i>A. nidulans</i> alternative oxidase gene	116
<b>Figure 4.4</b>	A <i>A. nidulans</i> AOX gene promoter B <i>A. nidulans</i> AOX gene coding region	118 119
<b>Figure 4.5</b>	Alignment of the Nucleotide Sequence of the Partial <i>A. nidulans</i> AOX fragment with AOX Sequences from <i>A. niger</i> and <i>N. crassa</i>	120
<b>Figure 4.6</b>	Alignment of fungal and plant AOX protein sequences	122
<b>Figure 4.7</b>	Fungal AOX gene promoter sequences	126
<b>Figure 4.8</b>	<i>A. nidulans</i> gene promoter sequences	128
<b>Figure 5.1</b>	The pAN923-42B <sub>B<sub>g</sub>A1</sub> plasmid (R117), containing the <i>lacZ</i> reporter gene	134
<b>Figure 5.2</b>	The promoter region of the <i>A. nidulans cycA</i> gene	136
<b>Figure 5.3</b>	Putative regulatory elements contained within the <i>cycA</i> promoter	137

	fragments	
<b>Figure 5.4</b>	Diagrammatic representation of homologous recombination of the reporter constructs at the <i>argB</i> locus	142
	A <i>Bam</i> HI digested samples, probed with <i>argB</i>	
	B <i>Sph</i> I digested samples, probed with <i>cyxA</i>	
<b>Figure 5.5</b>	Southern blot to determine integration of the reporter constructs at the <i>argB</i> locus	145
<b>Figure 5.6</b>	Southern blot to determine the presence of the <i>cyxA</i> promoter fragment	147
<b>Figure 5.7</b>	Determination of single copy integration at the <i>argB</i> locus	155
<b>Figure 6.1</b>	Reporter constructs R141 and R142	159

## LIST OF TABLES

---

<b>Table 2.1</b>	Strains & plasmids	45
<b>Table 2.2</b>	PCR & sequencing primers	58
<b>Table 3.1</b>	Radial growth and % spore viability for strains A67 ( <i>ycyA</i> <sup>+</sup> ) and A68 ( <i>ycyA</i> )	81
<b>Table 3.2</b>	Ethanol production in strains A67 ( <i>ycyA</i> <sup>+</sup> ) and A68 ( <i>ycyA</i> )	88
<b>Table 3.3</b>	Respiratory measurements of <i>A. nidulans</i>	92
<b>Table 5.1</b>	Transformation of the <i>argB2</i> strain of <i>A. nidulans</i>	140
<b>Table 5.2</b>	Southern analysis results	144
<b>Table 5.3</b>	Results for <i>A. nidulans</i> $\beta$ -galactosidase assays	150
<b>Table 6.1</b>	Results for <i>S. cerevisiae</i> $\beta$ -galactosidase assays	163

## LIST OF APPENDICES

---

<b>Appendix 1.1</b>	Vector map of pGM32	175
<b>Appendix 1.2</b>	Growth curve data for <i>A. nidulans</i> strains A67 and A68	176
<b>Appendix 1.3</b>	Estimation of cytochrome <i>c</i> amount	177
<b>Appendix 1.4</b>	Measurement of respiratory rate following the addition of inhibitors	
	<b>A</b> Late-exponential phase	178
	<b>A</b> Stationary phase	179
<b>Appendix 1.5</b>	Raw data for inhibitor studies	180
<b>Appendix 2.1</b>	Design of degenerate primers for PCR amplification of <i>A. nidulans</i> AOX gene	181
<b>Appendix 2.2</b>	Codon usage table for <i>A. nidulans</i>	184
<b>Appendix 2.3</b>	Vector map of pGEM-T	185
<b>Appendix 3.1</b>	Vector map of R12	186
<b>Appendix 3.2</b>	Sequence of the translational fusion of the <i>ycA</i> promoter fragment with the <i>lacZ</i> reporter gene	187
<b>Appendix 3.3</b>	Transformation of strain A71 with reporter constructs	188
<b>Appendix 3.4</b>	Raw data for <i>A. nidulans</i> $\beta$ -galactosidase assays	189
<b>Appendix 3.5</b>	Alignment of the <i>lacZ</i> reporter gene sequences	190
<b>Appendix 4.1</b>	Sequencing over the <i>ycA</i> - <i>CYC1</i> fusion	194
<b>Appendix 4.2</b>	Raw data for the <i>S. cerevisiae</i> $\beta$ -galactosidase assays	195
<b>Appendix 4.3</b>	ANOVA analysis of <i>S. cerevisiae</i> $\beta$ -galactosidase activities	196
<b>Appendix 4.4</b>	T-test analysis of <i>S. cerevisiae</i> $\beta$ -galactosidase activities	197