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**CLONING, CHARACTERISATION AND EVOLUTIONARY
RELATIONSHIPS OF TWO *PYR4* GENES FROM AN *ACREMONIUM*
ENDOPHYTE OF PERENNIAL RYEGRASS**

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy In Molecular Genetics
at Massey University, Palmerston North
New Zealand

Michael Anthony Collett

1994

ABSTRACT

A fragment of the *Claviceps purpurea pyr4* gene, encoding the enzyme orotidine-5'-monophosphate decarboxylase (OMPdecarboxylase) was used to screen a genomic library to an isolate (designated Lp1) of an *Acremonium* sp. which grows as an endophyte in perennial ryegrass (*Lolium perenne*). Four positive clones, λ MC11, λ MC12, λ MC14 and λ MC20 were isolated. Three of these clones, λ MC12, λ MC14 and λ MC20 were overlapping clones from the same locus, while λ MC11 was from a different locus.

Fragments of these clones which hybridised with *C. purpurea pyr4* were sequenced and found to have similarity with *pyr4* from other fungi of the Pyrenomycetes and related Deuteromycetes, suggesting that Lp1 has evolved from a sexual Pyrenomycetes species. The *pyr4* from λ MC12, λ MC14 and λ MC20 was designated *pyr4-1* and that from λ MC11 was designated *pyr4-2*. The predicted ORFs of the two genes were highly conserved and the 5' non-coding nucleotide sequences were the least conserved regions.

RT-PCR and northern analysis of total RNA from Lp1 demonstrated that transcripts approximately 1.4 kb in length were produced from the two genes and present at similar levels. Genomic fragments containing *pyr4-1* or *pyr4-2* were transformed into a strain of *Aspergillus nidulans* which has a mutation in the *pyrG* gene (encoding OMPdecarboxylase). Both of the Lp1 *pyr4* complemented a *pyrG* mutation in *Aspergillus nidulans*, confirming that both *pyr4-1* and *pyr4-2* encode functional OMPdecarboxylases.

Comparisons of *pyr4* restriction fragment length polymorphisms (RFLPs) from Lp1 and isolates of *Epichloë typhina*, *E. festucae*, *A. lolii*, *A. uncinatum*, and three endophyte taxonomic groupings from *Festuca arundinacea*: FaTG-1 (= *A. coenophialum*), FaTG-2 and FaTG-3 suggested that *pyr4-1* originated from *E. typhina*, the ryegrass choke pathogen, and *pyr4-2* originated from *A. lolii*, another endophyte from perennial ryegrass. This suggested that Lp1 is an interspecific hybrid, between *E. typhina* and *A. lolii*. Comparisons of the variable 5' non-coding nucleotide sequences from *pyr4* of Lp1 and other isolates demonstrated that *E. typhina*, and *A. lolii* or *E. festucae* were the most likely ancestors of the two *pyr4* found in Lp1. The *A. lolii* and *E. festucae* sequences were very similar, suggesting they are closely related. *A. lolii* has most probably evolved from an *E. festucae*, and in the process lost the sexual cycle.

Analysis of single spore purified isolates of Lp1 revealed that Lp1 was a homokaryon for *pyr4*. A Southern blot of a CHEF gel of Lp1 and these single spored

isolates was hybridised to a *pyr4* probe and demonstrated that *pyr4-1* and *pyr4-2* were present on either two chromosomes of similar size, or one chromosome.

The hybridisation that gave rise to *Lpl* was concluded to have been a relatively recent event, given the similarity of *pyr4-1* and *pyr4-2* nucleotide sequences to those of their probable ancestors, and the fact that both genes are expressed and functional. Interspecific hybridisation is probably widespread in the asexual endophytes, and may be an important event in their evolution, and the evolution of other fungal species.

ACKNOWLEDGEMENTS

I would like to first offer my sincerest appreciation to my supervisor, Barry Scott, for excellent supervision and encouragement throughout all stages of this project. I would also like to thank my co-supervisors, Rosie Bradshaw for assistance and advice, particularly with RNA work, and Gary Latch for encouragement. Thanks also to the staff and students of the Department of Microbiology and Genetics, too numerous to mention here, for their assistance and encouragement. In addition I would also like to thank all members of the Molecular Genetics Unit, past and present, who have assisted me during my time here, thanks to all of you for making the Department and MGU an enjoyable (and interesting!) place to work. In particular I would like to thank Carolyn "bladder infection" Young and the other "Carolyn" (while the real one "recovered" from her infection), Dianne Watt for excellent technical assistance and advice on all things molecular and more besides during the course of this research. I would also like to thank Richard "rapid boil" Johnson, Yasuo "it just can not be helped" Itoh, Hale Nicholson, Austin "I'm not quite sure" Ganley, Sirinda "we're just friends" Yunchalard, Chris "PCR" Schardl, Celia Berkahn, Kim Gan, Robert Hickson, Grant Hotter and Cherie Stayner, for their much welcome and varied advice, encouragement and distractions offered over the course of this thesis. Thanks also to all tramping partners, for helping to keep me sane and alive (just). I would also like to offer my most grateful thanks to my family and friends for all their encouragement and help during this, often trying, three and a half years, I'm sorry if I've been notably inconspicuous during this time, but a PhD is like that some times. In particular I would like to thank Kate for all her love and support. It is not easy expressing my gratitude to all of you, but hopefully this goes some way towards that.

Thanks are also due to AgResearch, Grasslands (formerly DSIR Plant Protection) for generous financial assistance over the period of this thesis, and Massey Graduate Research Fund, for financial assistance received. Approval for this research was obtained from the Massey University Ethics Committee for Genetic Experimentation.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xii
Chapter 1.0 INTRODUCTION.....	1
1.1 THE BIOLOGICAL IMPORTANCE OF FUNGI.....	1
1.2 ENDOPHYTES OF GRASSES.....	2
1.2.1 Evolutionary Relationships	6
1.2.2 Biologically Active Secondary Metabolites	7
1.3 OROTIDINE-5'-MONOPHOSPHATE DECARBOXYLASE	13
1.4 AIMS AND BACKGROUND OF THIS STUDY	16
Chapter 2.0 MATERIALS AND METHODS.....	17
2.1 FUNGAL AND BACTERIAL STRAINS, λ CLONES AND PLASMIDS	17
2.2 GROWTH OF CULTURES.....	17
2.3 MEDIA	17
2.3.1 Potato Dextrose Agar and Potato Dextrose Broth (PDA and PDB)	20
2.3.2 <i>Aspergillus</i> Complete Medium (ACM)	20
2.3.3 LB Media.....	20
2.3.4 Top Agarose	20
2.3.5 SOC medium	20
2.4 BUFFERS AND SOLUTIONS.....	21
2.4.1 1 x TBE Buffer.....	21
2.4.2 STET Buffer.....	21
2.4.3 <i>Hae</i> III (Universal) Buffer.....	21
2.4.4 SDS Loading Buffer.....	21

2.4.5	TE Buffer	21
2.4.6	Hybridisation Buffer.....	21
2.4.7	Tris-Equilibrated Phenol	21
2.4.8	20 x SSC.....	21
2.4.9	2 x SSC	21
2.4.10	TES (10/1/100).....	21
2.4.11	SM buffer.....	22
2.4.12	Acrylamide mix.....	22
2.4.13	OM Buffer	22
2.4.14	STC buffer.....	22
2.4.15	DNase free RNaseA	22
2.4.16	10 x Sequencing TBE Buffer.....	22
2.5	DNA ISOLATION.....	22
2.5.1	Maxiprep DNA Isolation from Fungal Cultures.....	22
2.5.2	Miniprep DNA Isolation from Fungal Cultures	22
2.5.3	Plasmid Isolation by the Rapid Boiling Method.....	23
2.5.4	Plasmid Isolation by a Small Scale Alkaline Lysis Method.....	23
2.5.5	Plasmid Isolation by a Large Scale Alkaline Lysis Method.....	23
2.5.6	Purification of Plasmid DNA by Cesium Chloride-Ethidium Bromide Density Gradient.....	24
2.5.7	Purification of Plasmid DNA by PEG Precipitation	24
2.5.8	Purification of DNA from Phage λ	25
2.6	PURIFICATION OF DNA BY PHENOL/CHLOROFORM EXTRACTION.....	25
2.7	PRECIPITATION OF DNA WITH ETHANOL OR ISOPROPANOL.....	25
2.8	DNA QUANTIFICATION	26
2.8.1	Spectrophotometric Determination of DNA Concentration.....	26
2.8.2	Fluorometric Quantitation of DNA.....	26
2.8.3	Minigel Method for Determination of DNA Concentration.....	26
2.9	RESTRICTION ENDONUCLEASE DIGESTION OF DNA	27
2.10	AGAROSE GEL ELECTROPHORESIS OF DNA.....	27
2.11	SUBCLONING.....	28
2.11.1	Recovery of DNA from Agarose Gels.....	28

2.11.2	DNA Ligations	29
2.11.2.1	CAP-Treatment of Vector DNA	29
2.11.2.2	Ligation.....	29
2.11.3	Transformation of <i>E. coli</i> by Electroporation.....	29
2.11.3.1	Preparation of Electro-Competent <i>E. coli</i> Cells.....	29
2.11.3.2	Electroporation.....	29
2.12	SOUTHERN BLOTTING AND HYBRIDISATION	30
2.12.1	Southern (Capillary) Blotting.....	30
2.12.2	Preparation of [α - ³² P]dCTP-Labelled Probe with the Ready-To-Go DNA Labelling Kit	31
2.12.3	Preparation of [α - ³² P]dCTP-Labelled Probe DNA with Individual Reagents.....	31
2.12.4	Separation of Unincorporated Nucleotides by Minispin Column Chromatography.....	32
2.12.5	Hybridisation of Probe DNA to Southern Blots.....	32
2.12.6	Stripping Hybridised DNA off Southern Blots.....	32
2.13	LIBRARY SCREENING BY PLAQUE HYBRIDISATION	33
2.13.1	Plating Phage λ	33
2.13.2	Filter Lifts.....	33
2.13.3	Hybridisation of phage λ DNA to [α - ³² P]dCTP labelled DNA probe	33
2.14	RESTRICTION MAPPING λ CLONES WITH A λ MAPPING SYSTEM	34
2.14.1	5' End Labelling of Oligonucleotides.....	34
2.14.2	Partial Restriction Digestion of λ Clones.....	34
2.14.3	Hybridisation of End Labelled Primers to Partially Digested DNA and Separation by Gel Electrophoresis.....	34
2.15	AMPLIFICATION OF DNA BY THE POLYMERASE CHAIN REACTION (PCR).....	35
2.16	DNA SEQUENCING	35
2.16.1	Purification of PCR Products for Sequencing.....	36
2.16.2	fmol DNA Sequencing Protocol.....	36
2.16.3	Sequenase Version 2.0 Sequencing Protocol.....	36
2.16.4	PAGE Gel Electrophoresis of Sequencing Reactions.....	37
2.17	TECHNIQUE FOR WORKING WITH RNA	37

2.18	ISOLATION OF RNA FROM FUNGAL CULTURES	38
2.19	NORTHERN BLOTTING OF RNA AND HYBRIDISATION TO [α - ³² P]dCTP-LABELLED PROBES	38
2.19.1	Glyoxylation of RNA.....	38
2.19.2	Glyoxal Gel.....	38
2.19.3	Northern Blotting and Hybridisation	39
2.20	RT-PCR ANALYSIS OF RNA	39
2.20.1	Reverse Transcriptase Synthesis of cDNA.....	39
2.20.2	Amplification of cDNA by PCR.....	39
2.21	PREPARATION OF FUNGAL PROTOPLASTS.....	40
2.22	TRANSFORMATION OF <i>ASPERGILLUS NIDULANS</i>	40
2.23	SEPARATION OF FUNGAL CHROMOSOMAL DNA BY PULSE FIELD GEL ELECTROPHORESIS	41
2.23.1	Preparation of Fungal DNA for Pulse Field Gel Electrophoresis	41
2.23.2	Pulse Field Gel Electrophoresis	41
Chapter 3.0	RESULTS	42
3.1	DETERMINING CONDITIONS FOR LIBRARY SCREENING	42
3.1.1	Determining Hybridisation and Washing Temperature for Library Screening	42
3.1.2	Determining Post Hybridisation Washing Conditions for Library Screening	42
3.2	LIBRARY SCREENING AND MAPPING POSITIVE CLONES.....	42
3.2.1	Library Screening.....	42
3.2.2	Mapping Positive λ Clones	45
3.2.2.1	Restriction Digestion of λ Clones and Southern Hybridisation to <i>C. purpurea pyr4</i>	45
3.2.2.2	Mapping λ Clones with <i>Bam</i> HI and <i>Sal</i> I Using a λ Mapping System.....	45
3.2.2.3	Mapping λ Clones Further	55
3.2.3	Identification of two <i>pyr4</i> Genes in Lp1 Genomic DNA.....	59
3.2.4	Confirmation that the λ Clones Isolated Correspond to two Separate Genetic Regions of the Lp1 Genome.....	59

3.3	SEQUENCE ANALYSIS OF CLONED DNA HYBRIDISING TO THE <i>C. PURPUREA</i> <i>PYR4</i> GENE	59
3.3.1	Subcloning of Regions Required for Sequencing.....	59
3.3.2	Sequencing and Identification of two <i>pyr4</i> Sequences from Lp1, <i>pyr4-1</i> and <i>pyr4-2</i>	64
3.3.3	Identification of Proposed Sites for Translational Start and Transcriptional Initiation of <i>pyr4-1</i> and <i>pyr4-2</i>	64
3.3.4	Comparisons of <i>pyr4-1</i> , <i>pyr4-2</i> , OMPD-1 and OMPD-2.....	67
3.3.4.1	Sequence Comparisons.....	67
3.3.4.2	Comparisons of GC Content of <i>pyr4-1</i> and <i>pyr4-2</i>	67
3.3.5	Comparisons of OMPD-1 and OMPD-2 to Other OMPdecarboxylase Sequences	68
3.3.5.1	Similarities of OMPD-1 and OMPD-2 to OMPdecarboxylase Sequences from the Pyrenomycetes and their Imperfect Relatives.....	68
3.3.5.2	Comparisons of OMPD-1 and OMPD-2 to OMPdecarboxylase Sequences from Filamentous Ascomycetes	68
3.3.5.3	Sequence Features Common to OMPD-1, OMPD-2 and Other OMPdecarboxylase Sequences	72
3.3.6	Codon Usage of <i>pyr4-1</i> and <i>pyr4-2</i>	72
3.4	EXPRESSION OF <i>PYR4-1</i> AND <i>PYR4-2</i>	72
3.4.1	RT-PCR Analysis of <i>pyr4</i> Transcripts	72
3.4.2	Northern Analysis of <i>pyr4</i> Transcripts	74
3.5	COMPLEMENTATION OF AN <i>ASPERGILLUS NIDULANS</i> URACIL AUXOTROPH BY <i>PYR4-1</i> AND <i>PYR4-2</i>	77
3.5.1	Subcloning Suitable Genomic Fragments for Complementation	77
3.5.2	Transformation of <i>A. nidulans</i> with <i>pyr4-1</i> and <i>pyr4-2</i>	77
3.5.3	Molecular Analysis of URA ⁺ Transformants of <i>A. nidulans</i>	77
3.6	EVOLUTIONARY ORIGINS OF <i>PYR4-1</i> AND <i>PYR4-2</i>	87
3.6.1	Polymorphisms of <i>pyr4</i> in <i>Acremonium</i> and <i>Epichloë</i> Species	87
3.6.2	Demonstrating that Lp1 is Homokaryotic for <i>pyr4-1</i> and <i>pyr4-2</i>	91
3.6.3	Sequence Comparisons of the 5' Non-Coding Sequences of <i>pyr4</i> from <i>Acremonium</i> and <i>Epichloë</i> Species	96
3.7	CHEF GEL ANALYSIS OF THE CHROMOSOMAL LOCATION OF <i>PYR4-1</i> AND <i>PYR4-2</i>	98

Chapter 4.0	DISCUSSION	101
4.1	IMPLICATIONS OF DUPLICATE <i>PYR4</i> IN LP1	101
4.2	SEQUENCE FEATURES OF OROTIDINE-5'- MONOPHOSPHATE DECARBOXYLASES	107
4.3	POTENTIAL USES OF <i>PYR4</i>	108
Chapter 5.0	SUMMARY AND CONCLUSIONS	110
Appendix 1.0	LIBRARY CONSTRUCTION.....	113
A1.1	CONSTRUCTION OF LP1 GENOMIC LIBRARY IN λ EMBL3A	113
A1.2	CONSTRUCTION OF LP1 GENOMIC LIBRARY IN COSMID pAN7-2	113
Appendix 2.0	VECTOR MAPS.....	114
Appendix 3.0	SEQUENCE DATA	119
A3.1	BIGPICTURE FROM <i>PYR4</i> -1 CONTIG	119
A3.2	PRETTYOUT FROM <i>PYR4</i> -1 CONTIG.....	121
A3.3	BIGPICTURE FROM <i>PYR4</i> -2 CONTIG	126
A3.4	PRETTYOUT FROM <i>PYR4</i> -2 CONTIG.....	127
APPENDIX 4.0	Published paper	132
REFERENCES	133

LIST OF TABLES

Table 1	Strains, λ clones and plasmids.	18
Table 2	Data from restriction mapping of λ clones from Fig. 7 and Fig. 8.....	49
Table 3	Data from λ mapping of clones.....	56
Table 4	Combined codon bias table for <i>pyr4-1</i> and <i>pyr4-2</i>	73
Table 5	Sizes of pMC7 and pMC11 restriction fragments.....	78
Table 6	Transformation frequencies of <i>Aspergillus nidulans</i> strain 1-85 protoplasts to uracil prototrophy with plasmid constructs containing three different <i>pyr4</i> genes.	82
Table 7	Sizes of fragments hybridising to pMC11 in untransformed and transformed cultures of <i>Aspergillus nidulans</i> strain 1-85.	86

LIST OF FIGURES

- Fig. 1. Life cycle of an *Acremonium* endophyte and an *Epichloë festucae* growing symbiotically with tall fescue (*Festuca arundinacea*). 3
- Fig. 2. Structures of biologically active secondary metabolites thought to play an important role in grass-endophyte associations. 8
- Fig. 3. Relationship of primary metabolism to the biologically active secondary metabolites thought to play an important role in grass-endophyte associations. 10
- Fig. 4. The pyrimidine biosynthetic pathway. 14
- Fig. 5. Establishing optimal temperature for hybridisation of *Claviceps purpurea pyr4* to Lp1 total DNA. 43
- Fig. 6. Establishing salt concentration for post-hybridisation washing of *Claviceps purpurea pyr4* off Lp1 total DNA. 44
- Fig. 7A-F. Restriction digestion profiles of λ MC11, λ MC12, λ MC14 and λ MC20. 46
- Fig. 8A-F. Mapping the position of *pyr4* on λ MC11, λ MC12, λ MC14 and λ MC20. 51
- Fig. 9. Profiles of λ MC11, λ MC12, λ MC14 and λ MC20 partially digested with *Bam*HI or *Sal*I and hybridised to ON-L or ON-R. 54
- Fig. 10. Physical maps of λ clones isolated from an Lp1 genomic library that hybridised to *Claviceps purpurea pyr4*. 57
- Fig. 11A-B. Demonstration that the inserts present in λ clones correspond to regions of the Lp1 genome. 60
- Fig. 11C-D. Demonstration that the inserts present in λ clones correspond to regions of the Lp1 genome. 62
- Fig. 12. Sequences of two *pyr4* genes from Lp1. 65
- Fig. 13. Comparisons of OMPD-1 and OMPD-2 to other OMPD amino acid sequences. 69

Fig. 14.	Dendogram showing relatedness of OMPD-1 and OMPD-2 to OMPdecarboxylase encoding genes from other filamentous Ascomycetes.	71
Fig. 15.	Identification of transcripts from <i>pyr4-1</i> and <i>pyr4-2</i> in total RNA.	75
Fig. 16	Identification of transcripts from <i>pyr4-1</i> and <i>pyr4-2</i>	
Fig. 17A-B.	Restriction maps of pMC7 and pMC11.	79
Fig. 18A-B.	Identification of restriction fragments from λ MC11 suitable for subcloning the complete <i>pyr4</i> gene.	80
Fig. 19A-B.	Analysis of pMC7 and pMC11 transformants complementing an <i>Aspergillus nidulans pyrG</i> ⁻ mutation.	84
Fig. 20A.	Demonstration that <i>pyr4-1</i> and <i>pyr4-2</i> from Lp1 have different evolutionary origins.	88
Fig. 20B-C.	Demonstration that <i>pyr4-1</i> and <i>pyr4-2</i> from Lp1 have different evolutionary origins.	89
Fig. 21A-B.	Further evidence for the origins of <i>pyr4-1</i> and <i>pyr4-2</i> in Lp1.	92
Fig. 22.	Polymorphisms of <i>pyr4</i> genes in a range of <i>Acremonium</i> and <i>Epichloë</i> species.	94
Fig. 23.	Demonstration that Lp1 is homokaryotic for <i>pyr4-1</i> and <i>pyr4-2</i> .	95
Fig. 24.	Sequence alignment demonstrating that <i>pyr4-1</i> and <i>pyr4-2</i> sequences are more similar to sequences from <i>Epichloë typhina</i> and <i>Acremonium lolii</i> than to each other.	97
Fig. 25A-B.	CHEF gel showing chromosomal position of <i>pyr4-1</i> and <i>pyr4-2</i> and that Lp1 is a homokaryon.	99