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CLONING, CHARACTERISATION AND EVOLUTIONARY RELATIONSHIPS OF TWO *PYR*4 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

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ABSTRACT

A fragment of the *Claviceps purpurea pyr*4 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, λMC11, λMC12, λMC14 and λMC20 were isolated. Three of these clones, λMC12, λMC14 and λMC20 were overlapping clones from the same locus, while λMC11 was from a different locus.

Fragments of these clones which hybridised with *C. purpurea pyr*4 were sequenced and found to have similarity with *pyr*4 from other fungi of the Pyrenomycetes and related Deuteromycetes, suggesting that Lp1 has evolved from a sexual Pyrenomycetes species. The *pyr*4 from λMC12, λMC14 and λMC20 was designated *pyr*4-1 and that from λMC11 was designated *pyr*4-2. 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 *pyr*4-1 or *pyr*4-2 were transformed into a strain of *Aspergillus nidulans* which has a mutation in the *pyrG* gene (encoding OMPdecarboxylase). Both of the Lp1 *pyr*4 complemented a *pyrG* mutation in *Aspergillus nidulans*, confirming that both *pyr*4-1 and *pyr*4-2 encode functional OMPdecarboxylases.

Comparisons of *pyr*4 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 *pyr*4-1 originated from *E. typhina*, the ryegrass choke pathogen, and *pyr*4-2 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 *pyr*4 of Lp1 and other isolates demonstrated that *E. typhina*, and *A. lolii* or *E. festucae* were the most likely ancestors of the two *pyr*4 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 *pyr*4. A Southern blot of a CHEF gel of Lp1 and these single spored

isolates was hybridised to a *pyr*4 probe and demonstrated that *pyr*4-1 and *pyr*4-2 were present on either two chromosomes of similar size, or one chromosome.

The hybridisation that gave rise to Lp1 was concluded to have been a relatively recent event, given the similarity of *pyr*4-1 and *pyr*4-2 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.

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