Isolation and characterisation of novel BTB domain protein encoding genes from fungal grass endophytes

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Introduction Pasture grasses belonging to the Pooideae sub-family of the Poaceae family frequently host symbiotic fungal endophytes. These include the sexual *Epichloë* species and the anamorphic asexual *Neotyphodium* species, which are thought to have evolved from *Epichloë* species either by the direct loss of sexual reproduction or by interspecific hybridisation. The two key temperate pasture grasses, tall fescue (*Festuca arundinacea* Schreb) and perennial ryegrass (*Lolium perenne* L.) interact with the fungal endophytes *N. coenophialum* and *N. lolii*, respectively. Large insert genomic DNA libraries are valuable resources for the discovery and isolation of genes and their regulatory sequences, for physical mapping, map-based cloning of target genes as well as for whole genome sequencing. BTB (Bric-a-brac, tram-track, broad complex) domains are highly conserved motifs of 120 amino acids in length. The domains are rich in hydrophobic amino acids, and mediate protein-protein interaction that lead to homomeric dimerisation and in some cases heteromeric dimerisation of a large number of functionally diverse proteins. The presence of BTB domains defines a large family of genes involved in various biological processes, such as the regulation of transcription, DNA binding activity and structural organisation of macromolecular structures. Genes encoding BTB domain proteins (BDP) have previously been described in viruses, yeasts, plants, nematodes, insects, fish and mammals. However, BDP genes have not as yet been described for filamentous fungi.

Materials and methods Four related sequences encoding BDPs that are transcribed at high levels were isolated and characterised from cDNA libraries of the grass endophytes *N. lolii*, *N. coenophialum* and *E. festucae*.

Results and conclusions DNA sequence and Southern hybridisation analyses demonstrated that two distinct BDP gene variants are present in *N. coenophialum*, while a single gene is present in each of *N. lolii* and *E. festucae*. To assist in gene and promoter discovery in these fungal endophytes of grasses, large-insert genomic DNA libraries of *E. festucae*, *N. coenophialum* and *N. lolii* were generated using phage lambda, as well as a bacterial artificial chromosome (BAC) library was constructed for *N. lolii* with a 120 kb average insert size and 15-fold genome coverage. The genomic library from *N. lolii* was screened using the relevant cDNA (*Nl*BDP) and the resulting genomic sequence (6,982 bp) was shown to contain a BDP open reading frame (858 bp) lacking introns (Figure 1). The transcriptional activity of the *N. lolii* BDP gene was determined in cultured endophytes by northern hybridisation analysis and *in planta* by real time (RT) PCR.

Genes related to the grass fungal endophyte BDP genes were also identified *in silico* within genomic and cDNA sequences from other fungal species. This analysis has defined a grass endophyte-derived BTB domain that has not been previously characterised in filamentous fungi, and consequently a discrete class of BDP genes.

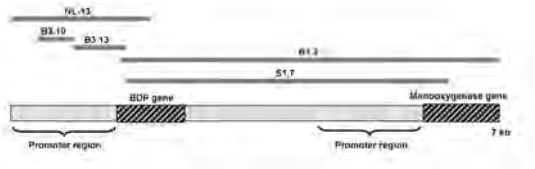

Figure 1 Characterisation of an *N. lolii* genomic clone containing a BTB domain protein (BDP) gene and a monooxygenase gene. Subcloned and sequenced fragments are shown. Upstream regulatory promoter (1,504 bp) and coding (858 bp) regions of the NlBDP gene were isolated and used for gene functional analysis in the grass-endophyte association