CLONING AND CHARACTERISATION OF TWO SUBTILISIN-LIKE PROTEASE GENES FROM NEOTYPHODIUM LOLII

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ABSTRACT

PCR amplification of *Neotyphodium lolii* genomic DNA with degenerate primers detected two different sequences with homology to subtilisin-like proteases. These two PCR products were used to screen a *N. lolii* Lp19 genomic library.

The *prt1* gene was isolated by screening the genomic library with the GH30 PCR product. This gene encodes a putative peptide of 434 amino acids that is most similar to subtilisin-like proteases from *Aspergillus* sp. The *prt1* gene contained a single intron, which was in a position conserved with other fungal genes. 3'RACE was used to determine the polyadenylation site for the *prt1* gene.

Repetitive DNA was a feature of both the 3' untranslated region (UTR) and sequences downstream of the *prt1* gene. Within the 3' UTR, a complex microsatellite was found extending over 50 base pairs. Downstream of the gene, a minisatellite locus of 360 base pairs in size was found, consisting of 40 copies of a 9 base pair AT-rich repeat.

Expression of *prt1* was examined in cultures with various types of carbon and nitrogen sources. Although no conclusive results could be drawn, the type of carbon and nitrogen available did have some effect on *prt1* expression. Repression of *prt1* expression was only observed in media supplemented with sucrose and glutamate.

A 500 bp fragment from the *prt1* promoter was introduced into the vector pFunGus to create a translational fusion with gusA. This vector, pMM9, was transformed into *Penicillium paxilli*. Although transformation frequencies were low, the transformants obtained appeared to be stable for hygromycin resistance. Expression of GUS was observed in seven out of twelve of the stable transformants. This showed that the promoter fragment in pMM9 was sufficient for expression of GUS in a heterologous system.

The *prt2* gene was isolated by screening a genomic library with the GH3 PCR product. Partial sequence has been obtained for the *prt2* gene. The *prt2* gene contains at least three introns, the first of which is conserved with *prt1*. From the sequence obtained, *prt2* encodes a peptide with strong similarity to subtilisin-like proteases from *Metarhizium anisopliae*, a fungal pathogen of insects.

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