Title Cloning, characterization and expression of an exo-1,3-β-glucanase gene from the antagonistic yeast, *Pichia guilliermondii* strain M8 against grey mold on apples

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Abstract

The strain M8 of *Pichia guillermondii* isolated from the carbosphere of apples (cv. Golden Delicious) showed a high efficacy in controlling grey mold, caused by *Botrytis cinerea*, on apples under semi-commercial conditions. Moreover, *Pichia guilliermondii* M8 produced high amounts of active exo-1,3-β-glucanase in Lilly-Barnett minimal salt medium with different carbon sources, which greatly inhibited *B. cinereain vitro* and *in vivo*tests. Therefore, an exo-1,3-β-glucanase gene, named as *PgExg1* (GenBank accession number HQ113463) was cloned from the genomic DNA of the strain M8 by genome walking. The sequencing and the nucleotide BLAST analysis indicates that no introns are present inside the gene, which was confirmed by amplifying the full gene from complementary DNA (cDNA) of the yeast. An open reading frame of 1224 bp encoding a 408-amino acid (aa) protein with a calculated molecular weight (M_r) of 46.9 kDa and an isoelectric point (p*I*) of 4.5 was characterized. Protein BLAST and phylogenetic tree analysis of the deduced amino acid sequences from the *PgExg1* gene suggested that the glucanase produced by *PgExg1* gene belongs to the Glycoside Hydrolase Family 5. Expression of *PgExg1* in *Escherichia coli* BL21 (DE3), followed by identification with Western-blotting, purification with Ni-NTA and analysis with enzyme assay, yielded homogeneous recombinant PgExg1. At its optimal pH of 5.0 and its optimal temperature of 40 °C, the recombinant enzyme protein showed the highest activity towards laminarin, while the highest stability was obtained when the enzyme was stored at pH of 7.0 and temperature of 4 °C.