Title The cel4 gene of *Agaricus bisporus* encodes a β-mannanase

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Abstract

Mannases have industrial uses in food and pulp industries, and their regulation may influence development of the mushrooms of commercially important basidiomycetes. We expressed an *Agaricus bisporus cel4* cDNA, which encodes a mannanase, in*Saccharomyces cerevisiae* and *Pichia pastoris*. CEL4 had no detectable activity on cellulose or xylan. This gene is the first isolated from this economically important fungus to encode a mannanase. *P. pastoris* secreted about three times more CEL4 than *S. cerevisiae*. The removal of the cellulose-binding domain of CEL4 lowered the secreted specific activity by *P. pastoris* by approximately 97%. The genomic sequence of*cel4* was isolated by screening a cosmid library of *A. bisporus* C54-*carb8*. The open reading frame was interrupted by 12 introns. The level of extracellular CEL4 increases dramatically at the postharvest stage in compost extracts of *A. bisporus* fruiting cultures. In laboratory liquid cultures of*A. bisporus*, the activity of CEL4 detected in the culture filtrate reached a maximum after 21 days. The levels of CEL4 broadly mirrored the levels of enzyme activity. In the Solka floc-bound mycelium, CEL4 protein showed a maximum after 2 to 3 weeks of culture and then declined. Changes in CEL4 activity during fruiting-body development suggest that hemicellulose utilization plays an important role in sporophore formation. The availability of the cloned gene will further studies of compost decomposition and the extracellular enzymes that fungi deploy in this process.