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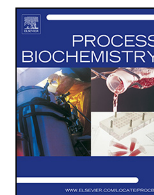

Development of a novel *Aspergillus* uracil deficient expression system and its application in expressing a cold-adapted α-amylase gene from Antarctic fungi *Geomyces pannorum*

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ABSTRACT

Filamentous fungi have increasingly been used as hosts for heterologous protein production because of their high secretion capability and ability to add eukaryotic post-translational modifications. In this study, a novel uracil-deficient *Aspergillus* transformation system, which was based on an orotate phosphoribosyltransferase (*pyrF*) nutritional selection marker, was discovered. Additionally, a universal, purify-able vector that directed genes into the *Aspergillus* host strain was engineered. A genomic DNA segment encoding a novel α-amylase was isolated from the psychrotolerant fungus *Geomyces pannorum* and the open reading frame was determined, deduced 497 amino acids. *G. pannorum* α-amylase was then expressed in the newly constructed *Aspergillus oryzae* system, with an amylase activity reaching 958 U/ml. It was purified to electrophoretic homogeneity and has a molecular mass of approximately 54 kDa. The enzyme exhibited an optimal activity at pH 5.0 and 40 °C and retained over 20% of maximal activity over the temperature range 0–20 °C. To our knowledge, this report is the first of the heterologous expression of a cold-adapted enzyme in filamentous fungi. *G. pannorum* α-amylase is an economical amylase with many potential applications.

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1. Introduction

The filamentous fungus *Aspergillus oryzae*, for which the complete genome sequence is known [1], is used extensively in the manufacture of fermented foods because of its proven safety record in the food industry and being generally recognized as safe (GRAS). *A. oryzae* has also been considered a favourable host for enzyme production (e.g. amylases, proteases, phytases, lipases, etc.) because of its ability to secrete proteins into the extracellular medium and introduce eukaryotic post-translational modifications [2]. Indeed, for many years, classical genetic strategies and recombinant DNA techniques have been applied to the construction of excellent host strains, providing for a high level of gene expression and protein secretion [3]. However, *A. oryzae* is not used as a host to express cold-adapted enzymes thus far. Like most fungi, the Aspergilli lack natural extra-chromosomally replicating DNA elements. Accordingly, researchers have attempted to construct artificial plasmids and expression systems for *Aspergillus* [4]. Since the initial report by Boeke [5], genetic methods requiring the loss of function of orotidine 5′-monophosphate decarboxylase (URA3) or its orthologue (*pyrG*) by selection on a 5-fluoroorotic acid (5-FOA) agar plate have been used with a number of mycelial fungus, including *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus parasiticus*, *Trichoderma reesei* and *Monascus aurantiacus*. By the mid-1990s, a range of Aspergilli transformation systems and expression vectors had been developed, besides the *pyrG* described above, most based on nutritional selection markers: *niaD* (encoding nitrate reductase), *sC* (encoding ATP sulfurylase), *adeA* (encoding N-succinyl-5-aminoimidazole-4-carboxamide ribotide synthetase), *argB* (encoding ornithine carbamoyltransferase), etc. Recently, the polyauxotroph mutants have become popular in genetic engineering [6]. Nevertheless, the further development of new transformation selection markers is still required so that increased amounts of more important proteins can be produced in *A. oryzae*.

In the pyrimidine biosynthetic pathway, the final two steps in the de novo biosynthesis of uridine 5′-monophosphate (UMP)

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