Isolation and characterization of the 3-phosphoglycerate kinase gene (pgk) from the filamentous fungus \textit{Trichoderma reesei}

S. Vanhanen, M. Penttilä, P. Lehtovaara, and J. Knowles
Biotechnical Laboratory, VTT, Tietotie 2, SF-02150 Espoo, Finland

Summary. The 3-phosphoglycerate kinase gene (pgk) from \textit{Trichoderma reesei} was isolated by hybridization with the corresponding \textit{Saccharomyces cerevisiae} PGK gene. The 1,545 nt long nucleotide sequence of the cloned gene codes for a 416 amino acid protein. The coding sequence contains two introns of 219 and 75 nt, respectively, at positions identical to those corresponding genes from the other filamentous fungi \textit{Aspergillus nidulans} and \textit{Penicillium chrysogenum}. This gene codes for two mRNAs of about 1.65 kb and 1.85 kb. The PGK protein of \textit{Trichoderma} shows extensive homology to the PGKs of other fungi \textit{A. nidulans} (77%), \textit{P. chrysogenum} (73%) and \textit{Saccharomyces cerevisiae} (69%). However, the PGKs of the two other filamentous fungi, \textit{A. nidulans} and \textit{P. chrysogenum}, seem to be more closely related to each other than to the \textit{T. reesei} enzyme.

Key words: \textit{Trichoderma reesei} - Filamentous fungus - 3-Phosphoglycerate kinase - Gene structure

Introduction

\textit{Trichoderma reesei} is an industrially important filamentous fungus that secretes large amounts of cellulolytic enzymes into the culture medium (Enari 1983; Mountenecourt 1983; Knowles et al. 1987). Recently, Penttilä et al. (1987) reported a transformation system for this organism that enables the expression of both homologous and heterologous genes. \textit{Trichoderma} has already been shown to have potential as a production organism for heterologous proteins such as calf chymosin, which is secreted in an active form into the fungal culture medium (Knowles et al. 1987; Harkki et al. 1987, 1989 in press). The study of highly expressed genes involved in basic metabolism provides not only important information on the molecular biology of fungi, and is also useful in the further development of \textit{Trichoderma} as an efficient production organism.

3-Phosphoglycerate kinase (PGK; ATP:3-phospho-D-glycerate 1-phosphotransferase; EC 2.7.2.3) is a key enzyme in ATP generation by glycolysis. It catalyses the transfer of a phosphoryl group from the acyl phosphate of 1,3-diphosphoglycerate to ADP, thus forming ATP and 3-phosphoglycerate. Amino acid sequence studies and X-ray studies of horse (Blake and Evans 1974; Banks et al. 1979) and yeast (Watson et al. 1982) PGK have shown that the protein has a highly conserved primary structure composed of two globular domains connected by a hinge. This conservation of protein structure even enables a chimeric yeast-human PGK protein to be expressed in \textit{S. cerevisiae} at approximately the same level as the homologous PGK protein (Chen and Hitzeman 1987). The previously isolated and characterized fungal genes coding for PGK from \textit{Saccharomyces cerevisiae} (Hitzeman et al. 1982), \textit{Aspergillus nidulans} (Clements and Roberts 1985, 1986) and \textit{Penicillium chrysogenum} (van Solingen et al. 1988) show extensive homology to each other.

In the yeast \textit{S. cerevisiae}, the PGK protein constitutes about 1% of the total cell protein, and its mRNA is correspondingly abundant (Holland and Holland 1978). Vectors carrying the PGK promoter are among the most efficient expression systems developed for yeast (eg. Tuite et al. 1982; Mellor et al. 1983, 1985; Dobson et al. 1984; Verbakel et al. 1987).

In this paper, we report the molecular cloning of the 3-phosphoglycerate kinase gene (pgk) of \textit{T. reesei} using the PGK gene of \textit{S. cerevisiae} as a hybridization probe and the complete nucleotide sequence of this gene.
Fig. 1. Nucleotide sequence of the *T. reesei* *pgk* gene and the deduced amino acid sequence of the PGK protein. Putative introns are written in lower-case letters. The suggested splicing signals are underlined. Localization of the coding region and positions of the introns have been predicted from the sequence homology with the *pgk* gene of *S. cerevisiae*, *A. nidulans* and *P. chrysogenum*.

**Materials and methods**

*Preparation of the gene bank*. *T. reesei* strain QM9414 (VTT-D-74075) was grown for 19 h in minimal medium containing (per ml)

- 20 mg glucose
- 2 mg bacto-peptone (Difco)
- 5 mg (NH₄)₂SO₄
- 15 mg KH₂PO₄
- 0.5 mg MgSO₄
- 0.5 mg CaCl₂
- 0.005 mg FeSO₄·7H₂O
- 0.0016 mg MnSO₄·H₂O
- 0.0014 mg ZnSO₄·7H₂O
- 0.002 mg CoCl₂·6H₂O

Chromosomal DNA was isolated according to Raeder and Broda (1985) except that the sample was treated for 30 min at 38°C with 130 Iμg/ml RNAaseA before phenol-chloroform extraction. DNA was partially digested with MboI, and the resulting DNA fragments of 10-22 kb were isolated by sucrose gradient centrifugation (Maniatis et al. 1982) and then ligated to a *K* EMBL3 replacement vector (Frischauf et al. 1983) cut with BamHI (Boehringer Mannheim, FRG). DNA was packaged into *K* particles in vitro and transfected into *E. coli* NM 538 (supE hsdR). The extract for in vitro packaging was prepared from *E. coli* strains BHB2690 and BHB2688 by using the method described by Arber et al. (1983).

**Screening of the gene bank**. Plaques of the *Trichoderma* gene bank were transferred onto nitrocellulose membrane filters (Schleicher & Schuell BA 85) and hybridized with a 2.95 kb HindIII fragment of the vector pMA1 (Mellor et al. 1983) containing the yeast PGK gene with its 5' and 3' flanking areas. The probe was nick-translated to a specific activity of 10⁷ cpm/μg DNA using a nick-translation kit (Boehringer Mannheim) with [32P]dCTP (Amersham). Hybridization was carried out at 48°C in 50 mM Tris·HCl pH 7.5, 10 mM EDTA, 1 MNaCl, 0.5% SDS, 0.1% sodium pyrophosphate, 10× Denhardt’s...