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Short communication

Transformation of *Penicillium chrysogenum* using the *Aspergillus nidulans amdS* gene as a dominant selective marker

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Summary. The *Aspergillus nidulans* acetamidase gene (*amdS*) has been used to transform *Penicillium chrysogenum* at low frequency. Several transformants were tested and shown to be mitotically stable. Southern blot analysis indicated that transforming DNA had integrated into the chromosomal DNA, possibly at multiple sites.

Key words: Penicillium chrysogenum – Transformation – Acetamidase gene

Introduction

Transformation systems in which a nutritional mutant is transformed by the equivalent wild-type gene have been described for *Neurospora crassa* (Case et al. 1979) and *Aspergillus nidulans* (Ballance et al. 1983; Tilburn et al. 1983; Yelton et al. 1984). One of the difficulties in developing similar transformation systems for fungi of industrial importance has been the unavailability of mutants which are genetically well characterised. However, it has been reported that *Cephalosporium acremonium* can be transformed using G418 resistance as a genetic marker (Penalva et al. 1985).

An alternative approach, which also requires no isolation or characterisation of mutants has demonstrated that a wild-type strain of *Aspergillus niger* can be transformed using the *amdS* gene (encoding acetamidase) of *A. nidulans* (Kelly and Hynes 1985). Development of this system followed the observation that a wild-type strain of *A. niger* grows poorly on acetamide and in addition, lacks sequences homologous to the *A. nidulans amdS* gene (Kelly and Hynes 1985). Furthermore, even $AmdS^+$ *A. nidulans*, which grows fairly weakly on acetamide, can be transformed to vigorous growth by introducing extra copies of the *amdS* gene (Kelly and Hynes 1985). Therefore, it is possible that the method described by Kelly and Hynes (1985) is applicable to other fungal strains of commercial interest, the requirements being poor growth of the recipient on acetamide and expression of the *A. nidulans amdS* gene. In this communication we report transformation of *Penicillium chrysogenum* using the *amdS* gene of *A. nidulans*.

Materials and methods

Several strains of *P. chrysogenum* were tested for growth on acetamide and strain HP60 (a nicotinamide requiring strain), which showed the least growth, was selected for transformation experiments. Essentially, *P. chrysogenum* strain HP60 was grown overnight on *Aspergillus* complete medium (Rowlands and Turner 1973) and protoplasts were prepared as described for *A. nidulans* (Ballance and Turner 1985) except for the following modifications:

(1) 0.6 M KCl was replaced by 0.9 M NaCl as the osmotic stabiliser;

(2) the final protoplast pellet was washed in 1 M sorbitol/10 mM $CaCl_2$ and resuspended in the same buffer.

For transformation, 10–15 μg of plasmid p3SR2 (Hynes et al. 1983), which contains the coding and *cis*-acting regions of the *A. nidulans amdS* gene, was added to approximately 10^7 viable *P. chrysogenum* protoplasts. Media and selection of transformants were as for *A. nidulans* (Tilburn et al. 1983) except that plates were incubated at 30 °C. As with *A. nidulans* (Tilburn et al. 1983) and *A. niger* (Kelly and Hynes 1985) inclusion of caesium chloride in the selection medium was found to be effective in reducing background growth of *P. chrysogenum* and thereby allowing selection of $AmdS^+$ transformants. DNA was isolated

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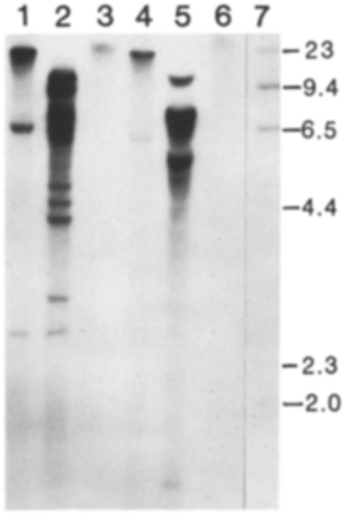

Fig. 1. Autoradiogram showing hybridisation of [α-^{32}P]-labelled pBR322 to total DNA from *P. chrysogenum* AmdS$^+$ transformants. *Lanes 1, 2 and 3* contain *Xho*I digested, *Bam*HI digested and undigested DNA from transformant T1 (approximately 2 μg. DNA per lane). Similarly, *lanes 4, 5 and 6* contain *Xho*I digested, *Bam*HI digested and undigested DNA from transformant T3. *Lane 7* contains marker lambda DNA digested with *Hind*III

from *P. chrysogenum* as described previously for *A. nidulans* (Ballance et al. 1983). Restriction enzyme digestion, transfer of DNA to nitrocellulose and nick translation were by standard procedures (Maniatis et al. 1982).

Results and discussion

Following incubation for 5–8 days, strongly growing colonies of *P. chrysogenum* were observed against a weak background growth. The efficiency of protoplast regeneration was 1–20% and the frequency of transformation was 2–20 transformants/μg DNA in three independent experiments. Several transformants were further tested on a variety of nitrogen sources and as with *A. nidulans* and *A. niger* (Tilburn et al. 1983; Kelly and Hynes 1985), acrylamide served as an efficient indicator of amide utilization. The transformants were mitotically stable as judged by growth on acrylamide following several generations of growth on non-selective medium.

To determine if transformation is the result of plasmid DNA integration into the genome, two stable transformants (T1 and T3) were further analysed by Southern hybridisation. DNA from transformants was electrophoresed either uncut or digested with *Bam*HI (single site in p3SR2) or *Xho*I (no site in p3SR2) and probed with pBR322. In undigested lanes, no free plasmid band was observed which suggests that transforming DNA has integrated into the genome (Fig. 1). Poor transfer of high molecular weight DNA may account for the low intensity seen in these lanes. However, results from other Southern blots (data not shown) clearly showed that hybridisation of pBR322 occured only to high molecular weight DNA.

Multiple band patterns using *Bam*HI and *Xho*I suggest that plasmid DNA has integrated at multiple sites and may have undergone rearrangement. Blots of *Xho*I digested DNA show a simpler hybridisation pattern and the observation of a large band approximately 20 kilobases (kb) in size may indicate tandem integration of at least two copies of p3SR2. The presence of additional fainter bands presumably indicates rearrangement of inserted DNA. No hybridisation of pBR322 to DNA from untransformed *P. chrysogenum* was detected at the level of stringency used (data not shown).

The *A. nidulans amdS* gene has been used to transform an *A. nidulans amdS* mutant to prototrophy (Tilburn et al. 1983) and in addition, it has been possible to transform wild-type (AmdS$^+$) strains of *A. nidulans* and *A. niger* using the same gene (Kelly and Hynes 1985). We have shown that a wild-type strain of *P. chrysogenum* can be transformed using the *A. nidulans amdS* gene as a dominant selective marker, even though no sequences homologous to the *amdS* gene were apparent in *P. chrysogenum*. Although the transformation frequency is fairly low, it might be possible to increase this by optimization of transformation conditions (Ballance and Turner 1985) or through use of fungal ARS (autonomously replicating sequences) elements functional in yeast.

Transformation of *P. chrysogenum* further demonstrates the applicability of the method described by Kelly and Hynes (1985) and should obviate the necessity of isolating mutants in fungi which are genetically not well characterised. Recently, the fungal pathogen *Cochliobolus heterostrophus* has also been transformed using the procedures described (Turgeon et al. 1985). To our knowledge, three examples of heterologous gene expression have been reported in wild-type or commercially important filamentous fungi using the *amdS* gene as selective marker. It may be possible to use this system to transform a wide range of filamentous ascomycetes or related organisms.

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