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Some factors affecting transformation of Aspergillus nidulans - Problems and progress

The high frequency transformation vector pDJB3 (Ballance and Turner 1985 Gene 36:321-331) has already proved successful in the cloning of genes affecting both carbon metabolism and spore development (Ballance and Turner M.G.G. 202:271-275). We are currently attempting to clone genes affecting DNA repair in *Aspergillus nidulans* using this plasmid to generate genomic DNA banks. Initial problems encountered in poor consistency of transformation have allowed us to identify some of the variables in this system.

Crucial to the transformation of *A. nidulans* is the production of viable protoplasts for introduction to plasmid vectors in the presence of polyethylene glycol (PEG). Using the standard technique of Ballance and Turner (1985) extensive vacuolation of protoplasts was often observed and regeneration frequencies were low (frequency <1%). Increasing the molarity of buffering KCl from 0.6 M to 0.9 M in protoplasting and regeneration media did not, however, markedly improve protoplast regeneration. Protoplasts released by enzymatic digestion of hyphal "mats" vary considerably in size. Size fractionation using "Milipore" filters indicated that protoplasts in the size range 5-8 um (diameter) showed higher regeneration frequencies than those smaller or larger.

Table I illustrates some of the techniques used to improve protoplast regeneration. Clearly, while consistent improvements in protoplast regeneration can be achieved, individual experiments still show great variation.

Protoplast suspensions of the standard *A. nidulans* strain G191 prepared on different occasions using the standard procedure showed variable efficiencies of transformation- the maximum achieved being 9000 per ug DNA (13.4% viable protoplasts transformed) down to a negligible frequency, with around 400 per ug DNA (0.29% viable protoplasts transformed) being a more typical value.

Individual plasmid preparations prepared in an *E. coli* (*rec*^-) host (HB101) differed markedly in transformation efficiency of G191 even though each preparation was "clean" enough to be linearized with *Eco*RI. An additional phenol extraction of proteins followed by an extra CsCl gradient step may be advisable to rigorously purify plasmid DNA.

Table 1: A Summary of Protoplast Regeneration Techniques

Treatment	% Regeneration (range, 3 or 4 replicates)
Medium, buffered with 0.6 M KCl + 50 mM CaCl2 (standard method)	0.5 - 1.0
Use of cultures grown from regenerated protoplasts	5.0 - 8.0
Pre-incubation in 100 ug/ml 2 deoxy-D-glucose after growth on complete medium	39.0 - 64.0
Growth of mycelial mat on medium containing 100 ug/ml 2 deoxy-D-glucose*	41.0 - 48.0
Growth of myceliel mat on medium containing 0.4 M NH4Cl	60.0 - 77.0

* Foury and Goffeau 1973 J. Gen. Micro. 75:227-229

Figure 1 demonstrates that PEG 6000 is more effective than PEG 4000 (both supplied by BDH Chemicals Ltd. Poole, England) at transforming each of two strains, G191 and M, a recombinant strain containing pyrG (for selection of pyrimidine prototrophic transformants) and two mutations increasing sensitivity to alkylating agents. There is no significant difference in the transforming ability of these two strains. Effective screening and sampling procedures for complementation tests with cloned genes should take into account the asynchronous development of transformed colonies.

Further experiments revealed that transformed protoplasts could regenerate efficiently on the surfaces of agar plates (containing 0.6 M KCl) although regeneration occurred at higher frequencies in agar overlays. Prolonged treatments in PEG 6000 (up to an extra 20 minutes) appeared not to alter transformation efficiency.

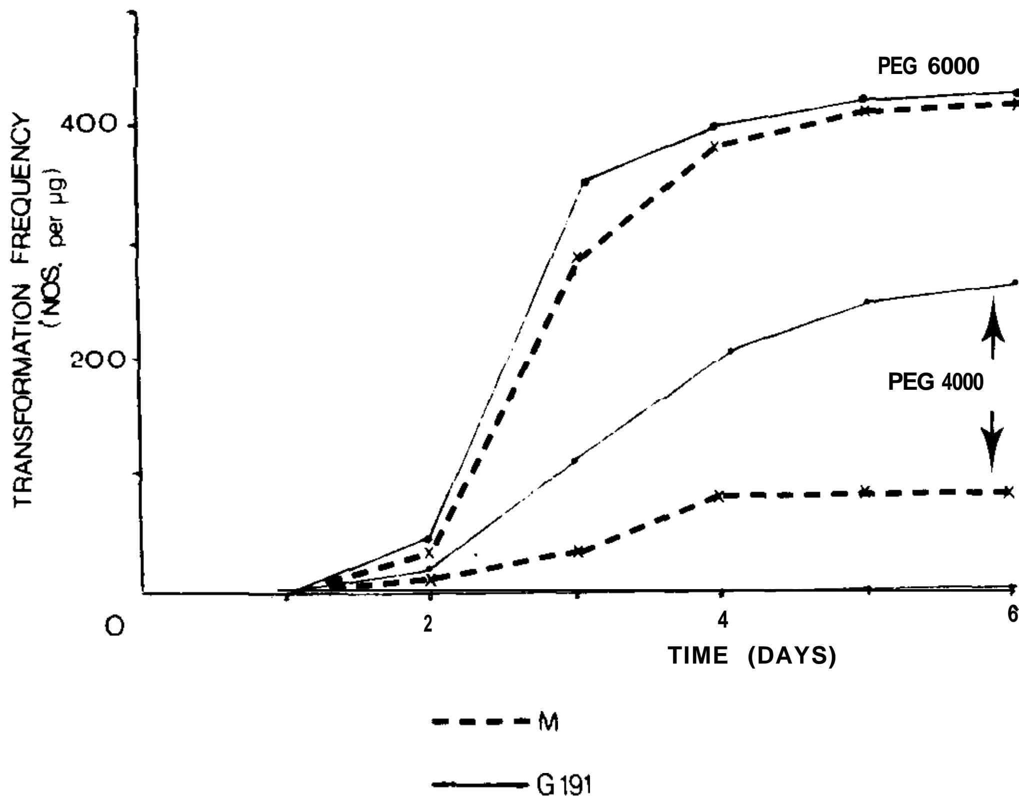

Figure 1. Transformation frequencies of two strains of A. nidulans with pDJB3 using two molecular weights of PEG.

When problems are experienced in achieving transformation of *A. nidulans,* it seems likely that further variables will be identified. For example, different batches of PEG vary in their toxicity towards streptomycete protoplasts (Hopwood *et al*. 1985 Genetic manipulation of Streptomyces - a laboratory manual, John Innes Foundation). The efficiency of transformation of linearized plasmids (Dhawale and Marzluf 1985 Curr. Genet. **10**:205-212) or higher forms of pDJB3 may be worth investigation. We have frequently observed that highest transformation efficiencies are not necessarily correlated with highest protoplast regeneration frequencies. Protoplasts that regenerate more easily may partially retain cell wall materials which inhibit DNA uptake. Phase contrast microscopy may reveal if this is so. Low melting point agarose may reduce heat shock of protoplasts in agar overlays (Shirahama *et al*. Agric. and Biol. Chem. **45**:1271-1273). - - - Dept. of Genetics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX U.K.