Some factors affecting transformation of Aspergillus nidulans - Problems and progress.

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Abstract
Some factors affecting transformation of Aspergillus nidulans - Problems and progress.
VII. Linked csp-2 (4%)
Perithecia are barren when pen-1 is used as female, even if the fertilizing parent is pen^+. An ostiole is present, but no beak. No ascospores are extruded. A few croziers and asci are present within the beakless perithecia, and a few mature ascospores may be formed. Perithecia are fully fertile and morphologically normal in the reciprocal cross, where pen-1 is used as male to fertilize pen^+. Development is about equally abnormal in pen female x pen^+ male and in pen x pen. Perithecia are normal and fertile when the female parent is heterokaryotic for pen in the cross (pen-1;un-3 ad-3 nit-2 A + pen^+; a^m1 ad-3B cyh-1) female x pen-1 a male, even though all asci are homozygous for the mutant pen-1 allele. Allele DL413 was obtained and described by DeLange and Griffiths 1980 Genetics 96:367-378. Cytology by N.B. Raju. Mapping by Perkins, Pollard and F.M. Delagi.

ufa(P73B118): unsaturated fatty acids
IVR. Linked to met-5 (7%). (Likely a recurrence of ufa-1/ufa-2, which were mapped to IV or V by Scott 1977, J. Bacteriol. 130:1144-1148)
Requirement and stability similar to those described for ufa-1 and ufa-2, stocks of which have been lost. Responds strongly to oleic acid and Tween 80 (oleic + palmitic), not to palmitic, Tween 40 (palmitic), stearic or linolenic acid, and weakly to linoleic (S. Brody, personal communication). Grows well on 1% Tween 80 supplement, but vegetatively unstable. Reversions produce conidiating sectors. Maintained reliably on minimal medium in heterokaryon with a^m1 ad-3B cyh-1. Unripe, light colored ascospores are probably enriched for revertants. Reliable mapping should be based on ufa^- progeny. Perithecia are barren in ufa x ufa crosses (made using heterokaryons).

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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 EcoRI. 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 mycelial 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 transformat- mants) 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).

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Linkage group assignments for two Neurospora crassa catalase genes: the Metzenberg RFLP mapping kit applied to an enzyme polymorphism

The enzyme catalase, as a scavenger of hydrogen peroxide, is considered one of the primary defenses against the toxic effects of oxygen. The genetic control of *N. crassa* catalase is of special interest to us, because this enzyme is induced by the superoxide-generating compound paraquat to a greater degree than has been reported for any other organism (unpublished results). Extracts from stationary-phase *Neurospora* mycelia (N broth) typically exhibit three distinct catalase bands on stained (Harris and Hopkinson 1976 Handbook of Enzyme Electrophoresis in Human Genetics) polyacrylamide gels (Fig. 1). These consist of 8 major band and two minor bands, one faster and one slower than the major band. A survey of wild-collected strains revealed natural polymorphism among catalases. In crossing experiments, we demonstrated that variant forms of major and minor catalases exhibit Mendelian segregation. We have employed two strains that exhibit altered catalase mobilities with reference to standard Oak Ridge strains to establish tentative genetic map positions for major and minor catalase loci, which appear to reside on separate chromosomes. One strain, FGSC 2225 (Mauriceville, Texas), possesses a minor band that is "slower" relative to the corresponding band of Oak Ridge. The linkage group assignment for the minor catalase gene was determined by analysis of strains in the Metzenberg et al. (1984 Neurospora Newsl. 31:35-39) large RFLP mapping kit, multicent-2. The second strain, FGSC 2230 (Welsh, Louisiana) possesses a major band that is "fast" relative to Oak Ridge. Mapping of this major catalase gene was accomplished by analysis of crosses between strain 2230 and alcoy strains (Perkins et al. 1969 Genetica 40:247-278). Our results are summarized below. Values for p represent a $\chi^2$ test of the hypothesis that two loci exhibit random assortment.

![Fig. 1 Catalalase bands on acrylamide gels.](image)

A. Major (cat-1) and minor (cat-2 and uppermost arrow) bands from *multicent-2* progeny. Note the faster minor band in strain 4450 (Oak Ridge type). B. Fast (first three lanes) and slow major catalase bands in selected strains. Strains 117, 121 and 123 are progeny from the 2230 x 1207 cross.