Evidence for Two Control Genes Regulating Expression of the Quinic Acid Utilization (qut) Gene Cluster in Aspergillus nidulans

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The first three steps in quinic acid degradation in Aspergillus nidulans are catalysed by highly inducible enzymes encoded by a gene cluster regulated by an adjacent control region. Analysis of two non-inducible mutants has been done in diploid strains, where qutA8 is recessive and all three enzyme activities are fully induced in heterozygous qutA8/qutA+ diploids. In contrast, qutA4/qutA+ heterozygous diploids show semi-dominance of the mutant allele, giving markedly diminished growth on quinic acid and 30-40% decrease of enzyme induction. Strikingly, the qutA4/qutA8 heterozygous diploid grows to the same degree on quinic acid as the qutA4/qutA+ heterozygote and shows the same level of enzyme induction, whereas both the homozygous mutant diploids do not grow on quinic acid and show no enzyme induction. Therefore the two mutant genomes complement, identifying two distinct regulatory gene functions. A genetic model is proposed of a negatively acting gene (qutA) repressing expression of a positively acting gene (qutD, previously designated qutA8+) whose product is in turn required for expression of the three structural genes. The qutA4 mutation is interpreted to produce an altered repressor insensitive to quinic acid, and the qutD8 mutation the loss of activator protein. Close similarity in the regulation of the quinic acid gene cluster in Neurospora crassa suggests that the two types of control mutation, qa1S and qa1F, described for N. crassa may also reflect two regulatory genes.

INTRODUCTION

Quinic acid is a good carbon source for Aspergillus nidulans, inducing high activities of the three enzymes required for catabolism to protocatechunic acid. These enzymes are: (1) quinate:NAD oxidoreductase (dehydrogenase) EC 1.1.1.24; (2) dehydroquinate hydro-lyase (dehydroquinase) EC 4.2.1.10; (3) a dehydratase converting 3-dehydroshikimate to protocatechuate. Quinic acid non-utilizing mutants (designated qut) lacking dehydrogenase (qutB), dehydratase (qutC), or all three enzyme activities (qutA), define genes tightly linked on chromosome VIII (Hawkins et al., 1982). The pleiotropic non-inducible qutA mutants identify a control region which acts positively to induce the enzyme activities in the presence of quinic acid, in a similar fashion to the qa1 region described in Neurospora crassa (Case & Giles, 1975).

Further analysis of pleiotropic qutA non-inducible mutants combined in heterozygous diploid strains of A. nidulans strongly suggests that the control region comprises two distinct complementing genes. One acts negatively, repressing expression of the second, positive, activator gene required for expression of each of the structural genes. Quinic acid relieves repression, allowing expression of the activator gene and thus enzyme formation.

METHODS

Strains. Representative qut mutants (Hawkins et al., 1982) in the R21 genotype (pabaA1 yA2) were crossed with strain R153 (wA3; pyroA4) to isolate R153 qut recombinants. In each case the mutant phenotype segregated as a

Abbreviations: PMSF, phenylmethylsulphonyl fluoride; MA, minimal agar.
single gene difference. Heterokaryons and heterozygous diploid strains were constructed by conventional procedures (Pontecorvo et al., 1953) and maintained on minimal agar (MA) media with glucose as carbon source.

Growth tests were done by transferring small blocks of agar containing heterokaryotic mycelium to MA plus quinic acid (test) or glucose (control), or by inoculating conidiospores of the diploid strains onto these two media.

**Media.** Defined minimal medium and addition of appropriate nutritional requirements for auxotrophic strains were those described previously (Armitt et al., 1976). Media for plates were solidified with 1-5% (w/v) agar (Oxoid no. 3). Media for liquid culture contained the wetting agent Tween 80, diluted $10^{-5}$ (w/v), and sterile MgSO$_4$ solution was added after autoclaving.

Carbon sources were supplied from sterile stock solutions in distilled water. Glucose was added to give final concentrations of 40 mM for solid media and 10 mM for liquid culture. Quinic acid [10% (w/v)] stock solutions brought to pH 6-5 with NaOH] was added to 1% for agar media and 0-1% for enzyme induction in liquid culture.

**Growth of mycelium for enzyme assays.** Mycelium was grown in liquid culture using 2-1 Erlenmeyer flasks with vertical baffles, formed by indenting the walls of the flasks, and treated with water-repellent silicone 6lm (Armitt et al., 1976). Each flask contained 250 ml minimal salts medium and was inoculated with a suspension of conidiospores to yield $10^6$ spores ml$^{-1}$. Cultures were grown with glucose as carbon source for 16 h at 30°C on a gyratory shaker. Mycelium was harvested by filtration, washed with salts medium at 30°C and resuspended in growth medium with either glucose (non-induced culture) or quinic acid (induced culture) as carbon source, and incubated for a further 4 h. It was then harvested by filtration, washed thoroughly on the filter with buffer (0-1 M-potassium phosphate pH 7-2, 10 mM-EDTA, 1 mM-DTT) and stored at $18^\circ$C.

**Preparation of cell-free extracts.** A thin pad of frozen mycelium (about 10 g) was immersed in liquid nitrogen in a pre-cooled mortar and ground to a fine powder with a cold pestle. The excess liquid nitrogen was decanted and the frozen powdered mycelium resuspended in 1 ml extraction buffer (0-1 M-potassium phosphate buffer pH 7-2, 10 mM-EDTA, 1 mM-DTT, 1 mM-PMSF). After thawing, the mycelium was extracted by gently shaking the slurry on ice for 1 h, after which cell debris was removed by centrifugation for 15 min in an Eppendorf microfuge in a cold-room. The supernatant was decanted, stored on ice and the enzymes assayed within 6 h of extraction. A sample of the supernatant was heated at 71°C for 10 min to denature biosynthetic dehydroquinase activity (Kinghorn & Hawkins, 1982). The heated sample was cooled on ice andcentrifuged to pellet denatured proteins. Protein concentrations were determined by the Folin reagent, using bovine serum albumin (Sigma) as standard.

**Enzyme assays.** Each of the three enzymes was assayed in a Unicam (Cambridge) SP1800 recording spectrophotometer at 37°C in 1 ml reaction mixtures, taking 5 and 10$\mu$l samples of the cell-free extracts.

1. Quinate dehydrogenase was assayed by following the reduction of NAD at 340 nm in a reaction mixture containing 10 mM-Tris/HCl (pH 8-6), 1 mM-quinic acid and 2-5$\mu$M-NAD.
2. Catabolic dehydroquinase was assayed by monitoring the increase in $A_{220}$ in a reaction mixture containing 10 mM-Tris/HCl (pH 7-2), 1 mM-EDTA and 5$\mu$l dehydroquinase solution. Preparation of the substrate is described below; the volume added gives $A_{220}$ of 0-2 before addition of cell-free extract. Thermolabile biosynthetic dehydroquinase had been inactivated by heating (see above).
3. 3-Dehydroshikimate dehydratase was assayed by measuring the increase in $A_{290}$ in a reaction mixture containing 0-1 mM-Tris/HCl (pH 7-5), 2-5 mM-MgCl$_2$ and 5$\mu$l dehydroshikimate solution. Dehydroshikimate was freshly prepared enzymically from dehydroquinate (5$\mu$l, $A_{220}$ 0-2) using purified *A. nidulans* catabolic dehydroquinase (Hawkins et al., 1982). Dehydroquinate was prepared by the catalytic dehydrogenation of quinate using Adams' catalyst. 1 $g$ PtO$_2$ was hydrogenated and stirred overnight with 5 $g$ quinate (Sigma) whilst bubbling with oxygen. The catalyst was removed by filtration and cations removed by passing the solution through a Dowex AG 50-X8 column equilibrated with water. The solution was loaded onto a Dowex AG 1-X8 (acetate form) column, which had been equilibrated with water, and eluted with a 0-5 to 6-0 M-acetic acid gradient. Fractions (7 ml) were collected and assayed by adding 20$\mu$l samples to a 1 ml reaction mixture containing 0-1 M-phosphate (pH 7-2) and 5$\mu$l (10 U) of purified *A. nidulans* catabolic dehydroquinase (Hawkins et al., 1982). Active fractions were pooled, concentrated by vacuum distillation to a yellow oil and mixed with 50 ml acetone. The solution was concentrated from acetone three times and the resulting oil dried with a vacuum pump. The resulting sticky solid was mixed with 50 ml acetone to yield a pale yellow supernatant and a white precipitate; the supernatant was recovered by filtration and passed over activated charcoal. Enzyme assays showed that the dehydroquinate was in the supernatant. The filtered supernatant was cooled to 4°C, mixed with 75 ml cold chloroform and held at 4°C. After 4 d a white, non-crystalline precipitate formed which was recovered by decanting the chloroform and drying with a vacuum pump. The white solid was dissolved in 30 ml water and shown to contain the dehydroquinate by assays with purified catabolic dehydroquinase.

The assay for each enzyme activity in each cell-free extract was repeated once, and the average value taken when compiling the enzyme specific activities shown in Tables 2 and 3. An analysis of variance was done on the replicate values for each of the enzyme specific activities for different diploid strains in each separate induction experiment. The standard errors of the mean ranged overall from 1-3 to 5-6% of the mean enzyme specific activities, with average values of 3-1%, 3-4% and 3-2% for enzymes (1), (2) and (3) respectively.
Two qut control genes in Aspergillus nidulans

Table 1. Growth on quinic acid

The amount of growth on quinic acid minimal agar was scored visually on a scale from 0 (no growth) to 5 (normal growth) in comparison to the wild-type combination (qut+/qut+). All heterokaryons and diploid strains grow normally on glucose minimal agar. Component strains were in the R21 genotype (vertical columns) and the R153 genotype (horizontal rows).

<table>
<thead>
<tr>
<th>(a) Heterokaryons</th>
<th>qut+</th>
<th>qutA4</th>
<th>qutA8</th>
<th>qutB42</th>
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<td>qutB42</td>
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RESULTS AND DISCUSSION

Growth tests in heterokaryons and heterozygous diploid strains

Growth on quinic acid as sole carbon source of heterokaryons and heterozygous diploid strains synthesized between the quinic acid non-utilizing (qut) mutants is shown in Table 1. All heterokaryons and diploid strains grow as wild-type on glucose. Growth of the homozygous mutant combinations (qutA/qutA; qutA8/qutA8; qutB42/qutB42) on quinic acid was in each case drastically reduced compared to that of the wild-type control (qut+/qut+). The observed 'sparse' residual growth is typical of that seen in other classes of mutant deficient in carbon source utilization and represents the metabolism of small amounts of alternative carbon sources present in agar plates (Payton et al., 1976). The haploid mutant strains also show similar residual growth on quinic acid plates.

Comparison of the results for reciprocal pairs of strains in growth tests with heterokaryons (Table 1a) shows minor differences for combinations of qutA4 and qutA8 mutations. On the other hand, growth tests with the corresponding heterozygous diploid strains yield identical results for all reciprocal combinations (Table 1b). Differences observed in the heterokaryon tests could reflect the segregation of modifying genes in the crosses from which the R153 qut recombinant strains were isolated. However, heterokaryon formation in A. nidulans is unstable, requiring constant selection for prototrophic growth to maintain a low proportion of heterokaryotic hyphal-tip cells against segregation of homokaryotic hyphae (Clutterbuck & Roper, 1966). Thus the ratio of component nuclei in the heterokaryon is unstable and subject to wide fluctuation. For these reasons, and also because it is not possible to grow heterokaryotic mycelium in liquid culture to do enzyme assays, we choose to base our analysis upon the use of diploid strains.

The mutant qutB42 lacks dehydrogenase activity (Hawkins et al., 1982). When combined with a wild-type qutB+ strain, growth on quinic acid is fully restored, indicating that the mutant allele is fully recessive, as might be expected of a structural gene loss-mutation. The two mutant strains qutA4 and qutA8 are non-inducible for all three enzyme activities (Hawkins et al., 1982) and are more complex and interesting in their behaviour. When combined with wild-type qutA+ strains in heterokaryons, growth on quinic acid is drastically reduced, indicating apparent dominance of the mutant alleles. However, in heterozygous diploid strains qutA8/qutA+ (and also qutA8 qutB+/qutA+ qutB42) growth on quinic acid is normal, indicating that the qutA8 mutation is fully recessive. In contrast, heterozygous diploid strains qutA4/qutA+ (and qutA4 qutB+/qutA4 qutB42) show growth on quinic acid intermediate between that of the homozygous mutant diploid strain (qutA/qutA4) and wild-type, indicating that the qutA4 mutation is semi-dominant to the wild-type allele.

Most importantly, combination of these two mutations in heterozygous diploids qutA4/qutA8 yields strains which grow on quinic acid to a lesser extent than the wild-type but far better than each of the homozygous mutant diploids (qutA4/qutA4; qutA8/qutA8). Thus, the qutA4 and qutA8 mutant genomes complement in heterozygous diploids, indicating that each mutation affects different gene functions, and that two regulatory genes control expression of the three structural genes.
In the previous study qutA4 and qutA8 were reported not to complement in heterozygous diploid strains (Table IV of Hawkins et al., 1982). This discrepancy may have resulted from the use of Vogel's salts medium and unidentified nutritional factors in the original qutA8 strain which we noted in preliminary crosses to isolate R21 qutA8 recombinants. A third non-inducible mutant strain qutA7 reported by Hawkins et al. (1982) proved to be sterile upon attempting crosses, and was not included in the analysis.

**Enzyme activities in heterozygous diploid strains**

Specific activities of each of the three enzymes for the initial steps in quinic acid metabolism were measured in glucose-grown uninduced and quinic acid-induced mycelium of the diploid strain in order to extend the observations made on the properties of the qut mutant genes in the growth tests. Dehydrogenase (1) and dehydratase (3) activities were not detectable in uninduced mycelium (data not shown). Very low activities of dehydroquinase (2) were occasionally found after heating extracts to inactivate the biosynthetic isoenzyme (Kinghorn & Hawkins, 1982). The amounts of activity are generally insignificant and could represent residual biosynthetic enzyme surviving heat treatment. It is noticeable that somewhat greater amounts of activity were detected in qutA8/qutA+ heterozygous strains and especially the qutA4/qutA8 heterozygous diploid. The absolute enzyme activities induced in the wild-type control (qut+/qut+) on different occasions (Table 2, compare the values in the fifth block) show variation between experiments. Data for each of the three induced enzyme activities are therefore shown relative to the values observed in the wild-type control for each induction experiment (represented in horizontal rows in Table 2). The results for the homozygous mutant diploid strains are consistent with expectation. The homozgyous qutB42 strain totally lacks dehydrogenase (1) activity and the non-inducible qutA4 and qutA8 homozygous diploids each lack all three enzymes. Agreement between results for reciprocal pairs of heterozygous diploid strains is good, the only major discrepancy, repeatable in two separate experiments, being for dehydrogenase (1) in the qutA4/qutB42 pair of diploid strains. We are investigating the possibility that the basis for this difference may lie in the segregation of modifying genes in the crosses done to isolate the recombinant strains used in constructing the heterozygous diploid strains.

Inspection of Table 2 shows that the induction of enzyme activities in the heterozygous diploid strains presents several important features which are summarized in the following points.

(a) The mutant qutB42 which lack dehydrogenase (1) activity and is recessive to wild-type in growth of heterozygous diploid strains on quinic acid (Table 1b) shows gene-dosage with respect to enzyme activity, producing some 60–70% of the wild-type dehydrogenase activity in the heterozygote.

(b) The non-inducible qutA8 mutant which lacks all three enzyme activities and is recessive to wild-type growth of heterozygous diploid strains on quinic acid is also fully recessive with respect to enzyme induction, and all three activities are found at the same levels as the wild-type. In addition, the qutA8 mutant is fully complemented in trans by the qutB42 dehydrogenase mutant with respect to enzyme induction. Thus the most economical interpretation of qutA8 is of a recessive mutation in a regulatory gene whose product is actively required for the expression of all three structural genes.

(c) In contrast, the second non-inducible mutant qutA4, which is semi-dominant to wild-type in growth of heterozygous diploid strains on quinic acid, shows a corresponding intermediate phenotype in the induction of all three enzymes to 60–80% levels of wild-type. In addition, the qutA4 mutant in trans with the dehydrogenase (1) qutB42 mutant in heterozygous diploids also shows some 60–80% of the enzyme levels seen in the wild-type control. Taken together, these observations suggest that the qutA4 mutant strain identifies a regulatory gene repressing expression of all three structural genes, the qutA4 mutation conferring an uninducible phenotype.

(d) Most importantly, complementation of the qutA4 and qutA8 mutant genomes in trans heterozygous diploid strains in growth on quinic acid (Table 1b) clearly reflects induction of all three enzyme activities not observed in each of the homozygous diploid strains. This result confirms that the qutA4 and qutA8 mutations define two separate regulatory gene functions and,
Table 2. *Enzyme activities in diploid strains relative to the wild-type (qut\*+/qut\*+) diploid strain*

Specific activities of the enzymes in each of the diploid strains induced by growth in the presence of quinic acid are shown relative to the activity of each of the three enzymes found in the wild-type (qut\*+/qut\*+) diploid strain. Component strains in the diploids were R21 (vertical columns) and R153 (horizontal rows). The diploid strains in each horizontal row were grown and treated at one time. Therefore for sets R153 \textit{qut}A4, \textit{qut}A8 and \textit{qut}B42 a separate control of wild-type (qut+/qut+) diploid was included to establish levels of induction since the absolute level of induction varies between experiments. Specific enzyme activities observed in the controls are shown on the right of the Table. Duplicate figures for diploids \textit{qut}A4/\textit{qut}B42 and \textit{qut}B42/\textit{qut}A4 are from separate experiments. Enzymes: 1, dehydrogenase (\text{umol product min}^{-1} \text{mg}^{-1}); 2, dehydroquinase, \text{A}_{220} \text{min}^{-1} \text{mg}^{-1} \text{ (1 unit is } \frac{\Delta \text{A}_{220}}{0.1 \text{ min}} \text{ at } 37^\circ \text{C}); 3, dehydratase (\text{umol product min}^{-1} \text{mg}^{-1}). Details of the growth of mycelium and procedures for enzyme induction, extraction and assay are given in Methods.

![Table 2](image)

Table 3. *Catabolite repression of enzyme induction by quinic acid in Aspergillus nidulans*

Mycelium of haploid strain R21 was grown in liquid culture with glucose as carbon source as described in Methods. Mycelium was collected and washed on a filter and resuspended in liquid medium with the carbon sources indicated for a further 4 h before harvesting and preparation of cell-free extracts for enzyme assay. For comparison, enzyme assays were also done on cell-free extracts prepared from mycelium grown for 20 h on 1\% (w/v) quinic acid. Units of specific activity are the same as in Table 2. ND, Enzyme activity not detectable.

![Table 3](image)
therefore, two regulatory genes controlling expression of the structural genes and enzyme induction. In the light of this conclusion, and to simplify description of the genetic model developed below, we now propose that *qutA*8 defines a second regulatory gene *qutD*, and designate the mutant allele *qutD*8.

**Glucose catabolite repression of enzyme induction by quinic acid**

Further information on regulation of the three enzymes for quinic acid catabolism in *A. nidulans* is shown in Table 3, which demonstrates that enzyme induction by quinic acid in a wild-type haploid strain is subject to carbon catabolite repression when glucose is supplied with quinic acid. For comparison, enzyme activities in mycelium grown on quinic acid alone are also shown.

**Genetic model for regulation of the quinic acid utilization (qut) gene cluster of *A. nidulans***

A basic model explaining how the two regulatory genes interact to control expression of the structural genes is shown in Fig. 1. We propose that the negatively acting repressor gene *qutA* is transcribed constitutively, and the repressor protein inhibits transcription of the positive activator gene *qutD* by binding at a 5′ control region for *qutD*. In the presence of quinic acid the repressor protein is either inactivated, allowing transcription of *qutD*, or binding quinic acid may convert the repressor protein into a positive signal for *qutD* transcription. Transcription of *qutD* results in the synthesis of an activator protein which binds to the 5′ regions of each of the structural genes, stimulating their transcription.

The data suggest that the concentration of activator protein alone does not limit structural gene expression, since diploids with only one active *qutD* gene show full enzyme induction (Table 2, *qutA*8/*qutA*8+ diploid strains). Diploids homozygous for the repressor gene mutation (*qutA*4/*qutA*4) are totally non-inducible, while diploids heterozygous for this gene (*qutA*4/ *qutA*+) show intermediate levels of enzyme induction approximating to the amount of an
enzyme produced in a diploid heterozygous for a recessive (loss) structural gene mutation (qutB42/qutB*). These observations suggest that the qutA4 mutation determines a repressor protein that does not bind quinic acid and thus prevents transcription of the activator gene qutD. In diploid strains heterozygous for the qutA4 mutation and with two wild-type activator genes (qutA4 qutD* /qutA* qutD*), enzymes are induced to about 60% normal levels. However, in qutA4 heterozygous diploid strains containing only one functional activator gene (qutA4 qutD* /qutA* qutD8), essentially the same levels of induction are observed. This contrasts with a further 50% decrease of induction to 30% normal levels, which might be expected if the concentration of activator protein alone determined the expression of the structural genes. This observation suggests that the basic genetic model proposed is over-simplified. Two levels of interaction between quinic acid and the repressor protein may occur. First, to release transcription of the activator gene by altering the binding of repressor protein to the qutD 5' control region, and secondly, an interaction between the repressor protein–quinic acid complex and the qutD protein product, to produce a true structural gene–activator protein complex. This stage of interaction could function in a concerted fashion to amplify control signals.

We are aware that other genetic models can be constructed from the data available. For example, the repressor protein may act directly to prevent transcription of each of the structural genes. In this case the qutD (activator) gene product would convert quinic acid to an effective inducer, or be required together with quinic acid to inactivate the repressor protein. Non-inducible regulatory mutants are a significant proportion of the quinic acid non-utilizing mutants isolated. Hawkins et al. (1982) recovered three from ten qut mutant strains and we have found another apparently leaky non-inducible mutant in five new qut strains. It is desirable that further regulatory mutants are recovered and studied to establish the phenotypes conferred by the mutations and the number of genes represented. However, we think the simplest model available is the most useful in addressing the central issue that control is exerted at the level of transcription, and particularly to establish whether transcription of qutA and qutD is constitutive or inducible. Similar genetic models involving cascades of negative- and positive-acting regulatory genes have been proposed previously for the galactose utilization and phosphatase systems in yeast (reviewed by Oshima, 1982) and the phosphatases in N. crassa (Littlewood et al., 1975).

If our model for control of the quinic acid gene cluster in A. nidulans is essentially correct, a number of predictions follow which are amenable to experimental test. At the genetic level, two further classes of regulatory mutation should occur. Recessive constitutive mutants in the repressor gene (qutA) defective in repressor protein or its binding to the activator gene (qutD) control region; and cis-acting dominant constitutive mutations in this control region which do not bind repressor protein. At the level of transcription control and analysis of mRNA coded by the various qut genes, the model predicts a single constitutively expressed mRNA transcript from the qutA (repressor) gene and four further transcripts induced by quinic acid and corresponding to the qutD (activator) gene and the three structural genes.

Analysis of mRNA populations has already been undertaken in the equivalent quinic acid qa system in N. crassa (Patel et al., 1981), and provides striking parallel evidence for the two-gene model. Thus, the constitutively expressed qa-1 mRNA in N. crassa may be equivalent to the proposed qutA transcript in A. nidulans and the unascrued mRNA species qa-y seen in N. crassa equivalent to the qutD gene transcript in A. nidulans. Following those parallels, the two classes of qa-1 mutation found in N. crassa and characterized by slow and fast complementation with biosynthetic dehydroquinase mutant strains (Case & Giles, 1975) may in fact define two regulatory genes equivalent to qutA and qutD respectively. Limited complementation is observed between some pairs of regulatory qa-1 gene mutants and structural gene mutants in heterokaryons of N. crassa (Case & Giles, 1975), a further parallel to the observations reported here for A. nidulans heterokaryons. Thus, present information on the quinic acid system in A. nidulans presents fascinating similarities with that in N. crassa where genetic mapping and gene cloning are at an advanced stage of analysis (Patel et al., 1981). The major advantage of A. nidulans is the use of diploid strains, not available in N. crassa, to enable ready analysis of gene interactions in terms of growth response, enzyme activity and gene transcription products.
We have isolated a recombinant λ phage containing approximately 11.9 kb \textit{A. nidulans} DNA, from a gene bank generated by ligating sized \textit{Sau3A} partially digested DNA into appropriate λ \textit{BamHI} sites. This phage was identified by cross-hybridization with the \textit{N. crassa} insert DNA contained in plasmid pVK57 (Kushner \textit{et al.}, 1977) and contains at least the \textit{qutE} catabolic dehydroquinase gene of \textit{A. nidulans}. This recombinant phage will enable us to clone the entire \textit{qut} cluster and analyse the \textit{qut} mRNA species in haploid and diploid strains.

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