Characteristics of six new para-fluorophenylalanine resistant loci of Aspergillus nidulans.

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Characteristics of six new para-fluorophenylalanine resistant loci of Aspergillus nidulans.

Abstract
Characteristics of six new para-fluorophenylalanine resistant loci of Aspergillus nidulans.
Major catalase (cat-1)

Strain 2230 (mt=a, fast catalase) was crossed to the alboc translocation strain 3361 (mt=A, slow catalase) which carries genetic markers for ylo-2, cot-1, csp-2 and al-1 (Perkins and Bjorkman 1979 Neurospora Newsl. 26:9). Thirty-nine ascospore-derived progeny were scored for these markers (only non-albino progeny can be scored for ylo-1), and mycelial extracts were examined for catalase mobility. Results indicated linkage to either chromosome III or VI (data for non-albino progeny: 5 slow, yellow; 20 fast, wild type; 1 slow, wild type; p<0.01). A second cross of strain 2230 was made to the alboc follow-up tester strain 1207 which carries non-translocation markers for ylo-1 (L.G. VI) and trp-1 (L.G. III). Progeny scoring from this cross indicated linkage to trp-1 (17% recombination) and therefore chromosome III (13 trp^+, fast; 3 trp^-, fast; 2 trp^+, slow; 11 trp^-, slow; p<0.025).

We have designated this major catalase locus cat-1.

Minor catalase (cat-2)

A cross of wild-collected Mauriceville strain to Oak Ridge-derived strain 4488, which carries genetic markers for all linkage groups, was performed by Metzenberg et al. (op cit). Progeny were scored and deposited with FGSC to facilitate the mapping of cloned DNA sequences using restriction-fragment polymorphisms. We scored mycelial extracts from 37 of the 38 progeny from the Metzenberg et al. cross # 2 for the presence of Mauriceville (slow) or Oak Ridge (fast) minor catalase. The best matches were with loci nic-3 (24/37 matches) and ars-1 (27/37 matches, p<0.01) indicating that this second catalase locus maps to chromosome VII. Assuming that cat-2 is on the right arm of chromosome VII, our data indicate 23/37 parental progeny, 13/37 single recombinants and 1/37 double recombinants (therefore 38% cat-2/nic-3 recombination and 27% cat-2/ars-1 recombination; data not presented).

We have designated this minor catalase cat-2.

Our assumption that the mapped loci represent structural catalase genes is consistent with genetic analyses of catalases from other organisms (e.g. Scandalios 1968 Ann. NY Acad. Sci. 151:272-293). A catalase purified from N. crassa mycelium was comprised of four identical subunits of MW 80,000, making this catalase somewhat larger than, but otherwise similar to, catalases from diverse organisms (Jacob and Orme-Johnson 1979 Biochemistry 18:2967-2974 and references therein). Because there does not appear to be biochemical or genetic evidence for strain-specific post-translational modification of catalases, we tentatively conclude that cat-1 and cat-2 do not represent genes for minor subunits or protein modifiers.

We note that the second minor catalase band (uppermost band on gels) exhibits a reduced migration in strain 2225 that appears to correspond with that of the cat-2 band. This suggests that the two minor catalases are products of the same gene or linked genes. This conclusion is tentative, however, because the uppermost band migrates close to the major band, and it has not been possible to score this upper band consistently on our gels. - - - Dept. of Biology, University of New Mexico, Albuquerque, NM 87131

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Characteristics of six new para-fluorophenylalanine-resistant loci of Aspergillus nidulans

We have identified six new loci in Aspergillus nidulans, at which mutations confer resistance to para-fluorophenylalanine (FPA), a toxic analogue of the aromatic amino acid phenylalanine. Spontaneous mutations at these loci in the presence of FPA have led to the following genotypic and phenotypic alterations. Studies based upon the utilization of amino acids as the sole source of nitrogen by mutants as described by Kinghorn and Pateman (1975 J. Gen. Microbiol. 86:174-184) and interaction between different fpa markers as reported by Srivastava and Sinha (1975 Genet. Res. Camb. 25:29-38) were carried out to characterize the mutants in addition to their formal genetics.
Mutants fpa-74 and fpa-75 were unable to utilize acidic (aspartic acid and glutamic acid), neutral (alanine, serine, leucine and valine) and basic (arginine, glutamine, asparagine and methionine) amino acids as the sole sources of nitrogen. Both were recessive in heterozygous diploids, allelic and mapped at the locus fpaV on linkage group I in the luA - proA interval, about 32 map units left of proA. The distance between luA and fpaV could not be taken into consideration because there was some interaction between the leucine requirement of the auxotroph and FPA resistance and only 3 of 386 colonies analysed were poorly growing luA fpaV recombinants. It has been suggested that mutation at the fpaV locus leads to a defect in the uptake of leucine and other acidic, neutral and basic amino acids.

fpa-77 is another recessive mutant mapping at the locus fpaP on linkage group II, about 7 units distal to riboE. The fpaP locus interacts with the earlier known amino uptake locus fpaK reported by Srivastava and Sinha (1975 Genet. Res. Camb. 25:29-38) by yielding 50% FPA-sensitive recombinants. Included is the genotype fpaP77;fpaK69 which, on outcrossing to a wild type strain, segregates FPA resistant progeny. This mutant utilizes all the amino acids as the sole source of nitrogen. The fpaP locus might be involved in the synthesis of a transcription regulator.

fpa-79 and fpa-80 were characterized as semi-dominant and dominant, respectively. Linkage data showed that the two mutants can be mapped to a single locus, henceforth assigned the symbol fpaQ on linkage group II in the adH - AcrA interval, about 14 map units right of adH and 19 map units left of AcrA. Both of these mutants interact with the earlier known fpaD locus (Sinha, 1969) in a way similar to the interaction of fpaP77 and fpaK69. fpaQ79 is unable to utilize any amino acid as the sole source of carbon whereas fpaQ80 utilizes basic amino acids. None of the mutants could utilize aromatic amino acids as the source of nitrogen. The difference in the degree of dominance and amino acid utilization pattern indicates that the genes coding for the 'general permease' in A. nidulans are overlapped and the two mutations are contained within the overlapped region.

fpa-76 was found to be dominant and mapped about 25 units distal to the biA1 marker on linkage group I. It has been assigned the locus symbol fpaR. This mutant possesses a normal ability to utilize all the amino acids as the sole source of carbon and nitrogen and a high degree of resistance to FPA.

fpa-78 and fpa-81, the two dominant mutants, have been assigned to linkage group II and I, respectively, only by analyzing the haploids obtained from the heterozygous diploids synthesized with MSG of McCully and Forbes (1965 Genet. Res. Camb. 6:353-359). Mapping by meiotic analysis has not been successful due to non-recovery of hybrid perithecia, the reason for which is not understood. The data of complementation analysis showed that these two mutants are different from those previously isolated and therefore were assigned the locus symbols fpaS78 and fpaT81. Both of these mutants utilize all the amino acids as the sole source of nitrogen.

A seventh class of FPA-resistant mutants defining the fpaU locus is described by Tiwary et al. (1987 Mol. Gen. Genet., in press). The locus has been mapped on linkage group V in the facA - riboD interval, equidistant (30 map units) from both markers. Our results suggest that mutation in the fpaU gene alters the binding site of phenylalanyl-tRNA synthetase in such a way that it effectively binds phenylalanine and discriminates against FPA.

A list of parental and derived strains is given in Table 1 and the map position of four fpa loci mapped on linkage group I and II is shown in figure 1. The standard map is taken from Clutterbuck (1981 Genetic Maps. ed. S.J. O'Brien).

Table 1. List of new FPA-resistant mutants of Aspergillus nidulans

| Parental Strain I | Derived Strains | Parental Strain II | Derived Strains |
|-------------------|----------------|-------------------|----------------|
| proA1 pabaA1 yA2  | 1. proA1 pabaA1 yA2 fpa074 | biA1;phenA3 | 1. biA1;fpaU82 |
|                   | 2. proA1 pabaA1 yA2 fpaP77 |                | 2. phenA3;fpaU85 |
|                   | 3. proA1 pabaA1 yA2 fpaQ79 |                |                |
|                   | 4. proA1 pabaA1 yA2 fpaQ80 |                |                |
|                   | 5. proA1 pabaA1 yA2 fpaR76 |                |                |
|                   | 6. proA1 pabaA1 yA2 fpaS78 |                |                |
|                   | 7. proA1 pabaA1 yA2 fpaT81 |                |                |
Figure 1. Map of linkage groups I and II of A. nidulans showing the locations of the fpaP, fpaQ, fpaR and fpaV genes

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