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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.

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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. Sponaneous 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.

<u>fpa-77</u> is another recessive mutant mapping at the locus <u>fpaP</u> on linkage group II, about 7 units distal to <u>riboE</u>. The <u>fpaP</u> locus interacts with the earlier known amino uptake locus <u>fpaK</u> reported by Srivastava and Sinha (1975 Genet. Res. Camb. 25:29-38) by yielding 50% FPA-sensitive recombinants. Included is the genotype <u>fpaP77;fpaK69</u> 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 <u>fpaP</u> locus might be involved in the synthesis of a transcription regulator.

<u>fpa-79</u> and <u>fpa-80</u> 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 <u>fpa0</u> on linkage group II in the <u>adH</u> - <u>AcrA</u> interval, about 14 map units right of <u>adH</u> and 19 map units left of <u>AcrA</u>. Both of these mutants interact with the earlier known <u>fpaD</u> locus (Sinha, 1969) in a way similar to the interaction of <u>fpaP77</u> and <u>fpaK69</u>. <u>fpa079</u> is unable to utilize any amino acid as the sole source of carbon whereas <u>fpa080</u> 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 <u>A</u>. <u>nidulans</u> are overlapped and the two mutations are contained within the overlapped region.

<u>fpa-76</u> was found to be dominant and mapped about 25 units distal to the <u>biA1</u> marker on linkage group I. It has been assigned the locus symbol <u>fpaR</u>. 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.

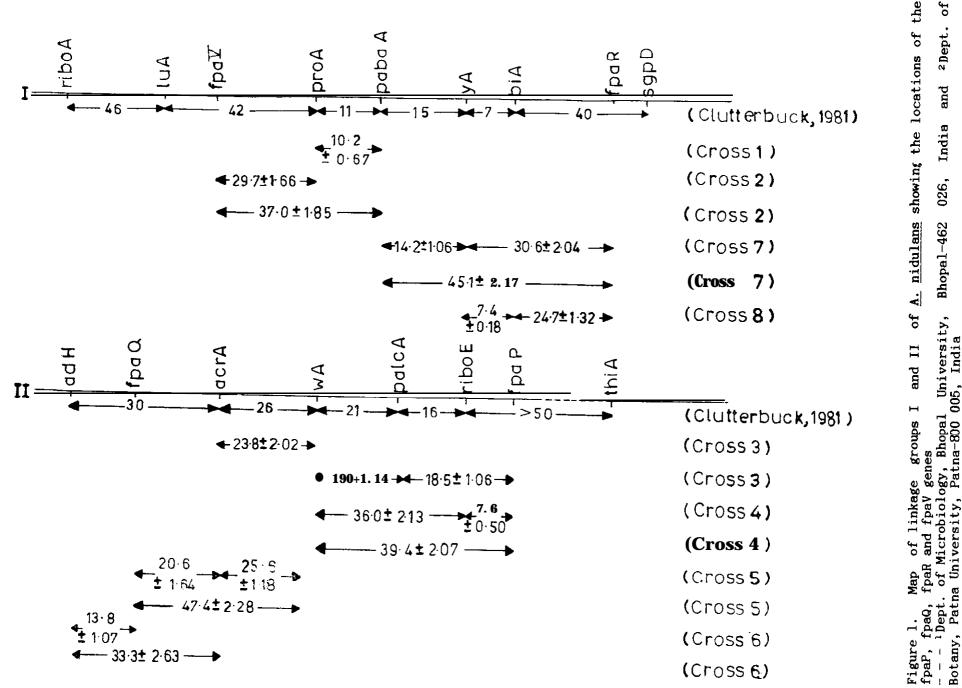
<u>fpa-78</u> and <u>fpa-81</u>, the two dominant mutants, have been assigned to linkage group II and I, respectively, only by analyzing the haploids obtained from the heterozygous diploids synthesized with MSG of McCully and Forbes (1965 Genet. Res. Camb. <u>6</u>:353-359). Mapping by meiotic analysis 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 <u>fpaS78</u> and <u>fpaT81</u>. Both of these mutants utilize all the amino acids as the sole source of nitrogen.

A seventh class of FPA-resistant mutants defining the <u>fpaU</u> locus is described by Tiwary et al. (1987 Mol. Gen. Genet., in press). The locus has been mapped on linkage group V in the <u>facA</u> - <u>riboD</u> interval, equidistant (30 map units) from both markers. Our results suggest that mutation in the <u>fpaU</u> gene alters the binding site of phenylalanyltRNA 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
proAl pabaAl yA2	<ol> <li>proAl pabaAl yA2 fpa074</li> <li>proAl pabaAl yA2 fpaP77</li> <li>proAl pabaAl yA2 fpaP77</li> <li>proAl pabaAl yA2 fpaQ79</li> <li>proAl pabaAl yA2 fpaQ80</li> <li>proAl pabaAl yA2 fpaR76</li> <li>proAl pabaAl yA2 fpaS78</li> <li>proAl pabaAl yA2 fpaT81</li> </ol>	<u>biAl;phenA3</u>	1. <u>biAl;fpaU82</u> 2. <u>phenA3;fpaU85</u>



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