PREFERENTIAL SELECTION AND GENETIC CHARACTERIZATION OF AN AMINO ACID UPTAKE MUTANT IN *ASPERGILLUS*

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SELECTION for resistance to the structural analogues of metabolites as a means of obtaining mutants deficient defective in specific as well as general permeases, especially of amino acids, has been used in a variety of microorganisms[1–7]. Such mutants provide information regarding the genetic control and specificity of transport mechanisms. In *Aspergillus nidulans*, so far four dominant *p*-fluorophenylalanine (FPA)-resistant loci are known, mutations at which lead to the loss or reduction in the transport of various amino acids. Recombinants between the auxotrophs for the affected uptake systems and FPA-resistance are either not recoverable or grow very poorly[2]. The mutants identified so far had been selected by either using glucose as the source of carbon in combination with amino acid analogue(s) (*fpa* D11, *fpa* K 69, *nap*)[1,3,4] or by using glutamate as the sole source of carbon and nitrogen (*aau* C, *aau* D)[5]. We report here the selection and genetic characterization of a new class of recessive amino acid uptake deficient mutants using acetate as the sole source of carbon in the presence of FPA and ethionine.

A proline and para-aminobenzoic acid requiring yellow conidial (*pro* A 1, *paba* A 1, *y* A 1) strain was used as the starting strain. Mutants resistant to two amino acid analogues, FPA (0.0007 M) and ethionine (0.003 M), were isolated on a medium containing sodium acetate (1% w/v) as the sole source of carbon[8,9]. The use of acetate in place of glucose was based on the finding that on a poor source of carbon, internal synthesis of amino acids becomes limited[10], which in turn leads to increased sensitivity of the organism to the analogue/s. In such circumstances only those nuclei which have a mutation in the amino acid permease or utilisation system would maintain their resistance. Two isolates thus obtained were purified by single colony isolations and given the isolate numbers *fpa* 74 and *fpa* 75.

The growth behaviour of the mutants was monitored on different amino acids as sole sources of nitrogen. The amino acids phenylalanine, tyrosine, tryptophan, aspartic acid, glutamic acid, glutamine, asparagine, ornithine, arginine, serine, leucine, valine and alanine were used at a final concentration of 10 mM[5]. Both the isolates (*fpa* 74 and *fpa* 75) grew well on phenylalanine and tyrosine (aromatic amino acids) but not at all on any of the other tested amino acids, indicating thereby that these two isolates could be defective in the transport of acidic, basic and neutral amino acids.

By mitotic haploidization of the heterozygous diploids synthesized between the mutants and the Master Strain 'G'[11], using chloral hydrate as the haploidizing agent[12,13], both *fpa* 74 and *fpa* 75 were assigned to linkage group I of *A. nidulans*. These mutants turned out to be recessive to their wild type alleles. Allelism tests with the previously known recessive loci of linkage group I (*fpa* A, *fpa* B and *fpa* M) revealed that both the new mutants were non-allelic to the pre-

Table 1 *Segregation of genetic workers with respect to the fpa 0 locus in two crosses in Aspergillus nidulans. Figures in parentheses indicate the recombinant class.*

Number of progeny analysed	Pairs of markers considered	Segregation class ++	+−	−+	−−	Recombination fraction (%)
Cross 1: *pro* A 1, *y* A 1, *fpa* 0 74 × *gal* A 1; *pyro* A 4; *fac* A 303; *s* B 3; *nic* B 8; *ribo* B 2						
124	*pro* A 1-*fpa* 0 74	80	(05)	(21)	18	20.9
	paba A 1-*fpa* 0 74	77	(05)	(23)	19	22.6
	y A 1-*fpa* 0 74	75	(09)	(26)	14	28.2
Cross 2: *pro* A 1, *paba* A 1, *y* A 1, *fpa* 0 74 × *lu* A 1, *bi* A 1; *phen* A 3						
386	*lu* A 1-*fpa* 0 74	(269)	67	47	(03)	50 0
	pro A 1-*fpa* 0 74	222	(34)	(94)	36	33.1
	paba A 1-*fpa* 0 74	211	(38)	(105)	32	37.5
	y A 1-*fpa* 0 74	190	(36)	(119)	41	40.2
	bi A 1-*fpa* 0 74	(146)	40	170	(30)	45 6

viously defined loci. Since both the mutants behaved in the same way, only one of them (*fpa* 74) was subjected to meiotic analysis. It was mapped on the left arm of linkage group I, distal to the *pro* A locus (table 1). The locus symbol 0 was assigned to this new isolate.

From the results of the two crosses presented in the table it is evident that there is some interaction between FPA-resistance of a mutant at the locus *fpa* 0 and leucine requirement of the auxotroph. However, there is no interaction between FPA-resistance on the one hand and proline, *p*-amino-benzoic acid or biotin requirement on the other. In the second cross, very few (only 3 out of 386 progeny tested) *lu* A 1, *fpa* 0 74 recombinants were recovered. The recombinants, which could be detected, formed very small and poorly conidiating colonies even on minimal medium supplemented with all the growth factors, indicating thereby that *fpa* 0 74 is able to utilize leucine of the medium only sparingly.

Based on the growth behaviour on limited media and genetic interaction, it can be concluded that the recessive mutant *fpa* 0 74 is defective in the uptake of acidic, basic and neutral amino acids.

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1. Sinha, U., *Ph.D. Thesis, University of Glasgow*, U.K. 1967.
2. Sinha, U., *Genetics*, 1969, **62**, 495.
3. Srivastava, S. and Sinha, U., *Genet. Res. Camb.* 1975, **25**, 29.
4. Piotrowska, M., Stepién, P. P., Bartnik, E. and Zarkzewska, E., *J. Gen. Microbiol.*, 1976, **92**, 89.
5. Kinghorn, J. R. and Pateman, J. A., *J. Gen. Microbiol.*, 1975, **86**, 174.
6. Jacobson, E. S. and Metzenberg, R. L., *Biochim. Biophys. Acta*, 1968, **156**, 140.
7. Surdin, Y., Sly, W., Sire, J., Bordes, A. M. and Robichonschzultzmaster, H. De., *Biochim. Biophys. Acta*, 1965, **107**, 546.
8. Singh, M. and Sinha, U., *Genet. Res. Camb.*, 1979, **34**, 121.
9. Sinha, U. and Tiwary, B. N., In: *Aspects of plant sciences*, (ed.), S. S. Bir. Today and Tomorrow Printers and Publishers, New Delhi (in press) 1984.
10. Calhoun, D. H. and Jensen, R. A., *J. Bacteriol.*, 1972, **109**, 365.
11. McCully, K. S. and Forbes, E., *Genet. Res. Camb.*, 1965, **6**, 352.
12. Singh, M. and Sinha, U., *Experientia*, 1976, **32**, 1144.
13. Singh, M. and Sinha, U., In: *Current approaches in cytogenetics*, (eds), R. P. Sinha and U. Sinha Spectrum Publishing House, Patna and Delhi, 1983, p. 37.

FOLIAR VENATION IN *GLOSSOSTIGMA SPATHULATUM* ARN. EX BENTH.

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GLOSSOSTIGMA SPATHULATUM Arn. ex Benth. is a minute, tufted herb belonging to the family Scrophulariaceae. During a survey of the aquatic vegetation of the district Mainpuri its plants were found growing on the wetland, both in field and submerged state in the water which collected in a depression in the same vicinity. The former population has been referred to as the wetland-form and the latter water-form. Morphologically, the two were different in that the wetland-form was in copious flowering whereas the water-form was sterile.

That the environment causes marked influence on the leaf venation is borne out from a number of reports in the literature[1-3]. Besides, it has also been opined by some authors that aquatic medium alters the organization of venation to variable degrees. The present study was, therefore, undertaken to examine and compare the foliar venation in a form which shows luxuriant growth in two conditions, from the point of view of availability of water.

The material was collected from the fields near village Ujrai in the district Mainpuri, U. P. The plants were fixed in FAA and then stored in 70% alcohol. The leaves were cleared following the method outlined by Paliwal & Kakkar[4]. The sketches of the venation pattern were made using a camera lucida under low power of the compound microscope.

The major venation pattern of leaves of both the exposed as well as submerged specimens is of hypodromous type. The minor venation pattern, on the