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SHORT PAPERS

Nitrite toxicity in *Aspergillus nidulans*: a new locus in a *proA1 pabaA6 yA2* strain

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SUMMARY

This report describes the existence of a mutation in a *proA1 pabaA6 yA2* strain of *Aspergillus nidulans*, which prevents it growing on elevated concentrations of nitrite. The nitrite toxicity marker (*nihA*) resulted from a mutation in a single gene representing a new locus in linkage group I, and behaved as a recessive character. The observed recombination frequencies between *nihA* and *proA1* and between *nihA* and *pabaA6* were about 1 and 11%, respectively. Nitrite toxicity was not due to a defect in nitrite reductase, because the mutant grew well on nitrate. Furthermore, ammonium protected both *nis-5* and *nii*A$^-$ strains against nitrite toxicity, which was not observed for the *nihA* strain. Since growth of the mutant was inhibited more by a mixture of ammonium and nitrite, compared to nitrite alone, it is proposed that the *proA1 pabaA6 yA2* strain has a mutation leading to increased efficiency in nitrite uptake, which is not repressed by ammonium.

1. INTRODUCTION

The genetic control of nitrate assimilation is a well characterized phenomenon in the ascomycete fungus *Aspergillus nidulans*. In this fungus the enzymes nitrate and nitrite reductases are specified, respectively, by the *nia*D (Pateman, Rever & Cove, 1967; MacDonald & Cove, 1974; Cove, 1979) and *nii*A (Pateman *et al.* 1967; Rand & Arst, 1977; Cove, 1979) genes, both regulated by two positively acting control genes (*nir*A and *are*A) which mediate, respectively, induction by nitrate or nitrite (Pateman & Cove, 1967; Cove, 1970) and repression by ammonium (Arst & Cove, 1973). Although the two structural genes are closely linked in linkage group VIII (Cove, 1970, 1979) and both are subject to nitrate induction and ammonium repression, they are not co-ordinatelly regulated (Arst, Rand & Bailey, 1979; Tomsett & Cove, 1979). This arises from the fact that a mutation, designed *nis-5* (Rand & Arst, 1977), specifically affects *nii*A expression. For this reason, *nis-5* single mutants grew poorly on nitrate and nitrite (Rand & Arst, 1977).

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This paper reports the existence of a new locus in a yellow conidia *p*-aminobenzoic acid and proline-requiring strain of *Aspergillus nidulans* (which grows well on nitrate) which prevents it growing on elevated concentrations of nitrite.

2. MATERIALS AND METHODS

(i) *Strains*

The strains of *Aspergillus nidulans* were originally obtained from the Department of Genetics, Glasgow University. The three haploid strains used were: wild-type, a yellow conidia *p*-aminobenzoic acid and proline-requiring strain (*proA1 pabaA6 yA2*) and Master Strain E (MSE) which carries markers on all eight linkage groups (McCully & Forbes, 1965).

(ii) *Media and growth conditions*

The nitrogen-less minimal medium (MM) was prepared as described by Pontecorvo *et al.* (1953). Iron and zinc were added as sulphate salts to give final concentrations of 66 and 62 μM, respectively. The various nitrogen sources were added to give the final concentration desired. Nitrate and nitrite were added as sodium salts. The complete medium (CM) contained yeast extract, hydrolysed casein, yeast nucleic acids, vitamins, etc. (Pontecorvo *et al.* 1953). Solid medium contained 1·5% agar. Growth was estimated by measurement of the colony diameter of strains inoculated into the centre of MM dishes containing different nitrogen sources. All incubations were at 37 °C for 3 days.

(iii) *Genetic analysis*

Genetic analysis was performed as described by Pontecorvo *et al.* (1953). Diploids were obtained according to the technique of Roper (1952). Mitotic analysis was done by haploidization (Forbes, 1959), using *p*-fluorophenylalanine (Lhoas, 1961; Morpurgo, 1961). Replication was carried out with a 26 point nickel-chrome wire replicator (Azevedo, Oliveira & Campos, 1976).

3. RESULTS AND DISCUSSION

The data presented in Table 1 show that the *proA1 pabaA6 yA2* strain of *Aspergillus nidulans* did not grow on media supplemented with elevated concentrations of nitrite as the sole nitrogen source, but it grew well on other nitrogen sources, including nitrate. This result suggested that the enzymes for nitrate assimilation, i.e. nitrate and nitrite reductases (Cove, 1970; Pateman & Cove, 1967), are present in this mutant. It was also observed that the wild-type strain and MSE grew well on media supplemented with the same concentration of nitrite (28 and 24 mm colony diameters, respectively, after incubation for 3 days at 37 °C).

Ascospores from hybrid cleistothecia obtained from cross between the mutant and MSE showed a segregation of 113 nitrite$^+$ to 129 nitrite$^-$ (about a 1:1 ratio), suggesting that the nitrite toxicity marker was due to mutation in a single gene, designated *nihA*. The observed recombination frequency between *nihA* and *proA1* and between *nihA* and *pabaA6* was about 1 and 11%, respectively. These are all markers located in linkage group I. The heterozygous diploid (MSE//*nihA*) grew well on an elevated concentration of nitrite, indicating that this marker behaved as a recessive character.

The results presented in Table 2 show that the mutant strain grew better on media supplemented with low concentrations of nitrite as the sole nitrogen source. Despite this fact, sporulation and colony morphology were not fully normal, when compared to the mutant strain grown on nitrate (not shown). From these results it could be supposed that growth is inhibited by the accumulation of toxic levels of nitrite. However, the accumulation of toxic levels of nitrite could not be explained by a defect in the expression

Table 1. *Colony diameter (mm) of the* proA1 pabaA6 yA2 *and wild-type strains of* Aspergillus nidulans *after 3 days incubation at 37 °C on different nitrogen sources*

Nitrogen source	Final concentration (mM)	Colony diameter (mm)	
		proA1 pabaA6 yA2	Wild-type
Sodium nitrate	70·6	30	30
Sodium nitrite	69·6	0	28
Ammonium chloride	71·0	33	32
Proline	43·5	23	29
Xanthine	3·3	26	28
Hypoxanthine	3·7	27	25
Uric acid	3·0	27	29
Urea	5·0	28	28

Table 2. *Colony diameter (mm) of the* proA1 pabaA6 yA2 *and wild-type strains of* Aspergillus nidulans, *after incubation for 3 days at 37 °C on various concentrations of sodium nitrite*

Sodium nitrite (mM)	Colony diameter (mm)	
	proA1 pabaA6 yA2	Wild-type
69·6	0	28
34·8	5	29
29·0	9	27
23·2	25	28
17·4	26	30
11·6	27	29

of the structural gene for nitrite reductase (for review, see Marzluf, 1981), since the mutant grew well on nitrate (Table 1). Furthermore, it is well known that ammonium protects both *nis-5* and *nii*A$^-$ strains against nitrite toxicity (Rand & Arst, 1977), but this was not true with the *nihA* mutant (Table 3). It was also observed that growth of the *nihA* strain was inhibited more on media supplemented with a mixture of ammonium and nitrite, than with nitrite (Table 2) or nitrite plus nitrate (Table 3). The ammonium effect, which was also observed in *mea*B strains (Arst & Cove, 1969; Arst & Page, 1973), could be due to repression of the synthesis of nitrite reductase leading to an enhanced nitrite toxicity (Rand & Arst, 1977). This effect was not observed in the wild-type strain and MSE (Table 3), suggesting that ammonium repression could also affect the uptake system for nitrite. Therefore, it is possible that the *nihA* mutation leads to an increased efficiency of nitrite uptake, which is not repressed by ammonium. A similar situation was demonstrated for a uric acid-xanthine permease in *Aspergillus nidulans* (Arst & Scazzocchio, 1975).

Table 3. *Colony diameter (mm) of* Aspergillus nidulans *strains after 3 days incubation at 37 °C, on different combinations of nitrogen sources*

Strain	Sodium nitrate (70·6 mM) + sodium nitrite (mM)				
	0	17·4	23·2	29·0	69·6
Wild-type	30	29	31	28	31
proA1 pabaA6 yA2	30	29	29	5	0
MSE//*nihA*	34	32	33	30	31

	Ammonium chloride (71·0 mM) + sodium nitrite (mM)						
	0	2·9	7·2	17·4	23·2	29·0	69·6
Wild-type	32	30	31	32	29	30	31
proA1 pabaA6 yA2	33	31	29	6	5	0	0
MSE//*nihA*	34	30	32	30	29	31	30

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REFERENCES

ARST, H. N., JR. & COVE, D. J. (1969). Methylammonium resistance in *Aspergillus nidulans*. *Journal of Bacteriology* **98**, 1284–1293.

ARST, H. N., JR. & COVE, D. J. (1973). Nitrogen metabolite repression in *Aspergillus nidulans*. *Molecular and General Genetics* **126**, 111–141.

ARST, H. N., JR. & PAGE, M. M. (1973). Mutants of *Aspergillus nidulans* altered in the transport of methylammonium and ammonium. *Molecular and General Genetics* **121**, 239–245.

ARST, H. N., JR., RAND, K. N. & BAILEY, C. R. (1979). Do the tightly linked structural genes for nitrate and nitrite reductases in *Aspergillus nidulans* form an Operon? Evidence from an insertional translocation which separates them. *Molecular and General Genetics* **174**, 89–100.

ARST, H. N., JR. & SCAZZOCCHIO, C. (1975). Initiator constitutive mutation with an 'up-promoter' effect in *Aspergillus nidulans*. *Nature* **254**, 31–34.

AZEVEDO, J. L., OLIVEIRA, A. & CAMPOS, A. J. R. (1976). Replicador múltiplo para transferência de esporos de fungos filamentosos. *Summa Phytopathológica* **2**, 237–241.

COVE, D. J. (1970). Control of gene action in *Aspergillus nidulans*. *Proceedings of the Royal Society, London* B **176**, 267–275.

COVE, D. J. (1979). Genetic studies of nitrate assimilation in *Aspergillus nidulans*. *Biological Reviews* **54**, 291–327.

FORBES, E. (1959). Use of mitotic segregation for assigning genes to linkage groups in *Aspergillus nidulans*. *Heredity* **13**, 67–80.

LHOAS, P. (1961). Mitotic haploidization by treatment of *Aspergillus niger* diploids with *p*-fluorophenylalanine. *Nature* **190**, 744.

MACDONALD, D. W. & COVE, D. J. (1974). Studies on temperature-sensitive mutants affecting the assimilatory nitrate reductase of *Aspergillus nidulans*. *European Journal of Biochemistry* **47**, 107–110.

MARZLUF, G. A. (1981). Regulation of nitrogen metabolism and gene expression in fungi. *Microbiological Reviews* **45**(3), 437–461.

MCCULLY, K. S. & FORBES, E. (1965). The use of *p*-fluorophenylalanine with 'master' strains of *Aspergillus nidulans* for assigning genes to linkage groups. *Genetical Research* **6**, 352–359.

MORPURGO, G. (1961). Somatic segregation induced by *p*-fluorophenylalanine. *Aspergillus Newsletter* **2**, 10.

PATEMAN, J. A. & COVE, D. J. (1967). Regulation of nitrate reduction in *Aspergillus nidulans*. *Nature* **215**, 1234–1237.

PATEMAN, J. A., REVER, B. M. & COVE, D. J. (1967). Genetic and biochemical studies of nitrate reduction in *Aspergillus nidulans*. *Biochemical Journal* **104**, 103–111.

PONTECORVO, G., ROPER, J. A., HEMMONS, L. M., MACDONALD, K. D. & BUFTON, A. W. J. (1953). The genetics of *Aspergillus nidulans*. *Advances in Genetics* **5**, 141–238.

RAND, K. N. & ARST, H. N., JR. (1977). A mutation in *Aspergillus nidulans* which affects the regulation of nitrite reductase and is tightly linked to its structural gene. *Molecular and General Genetics* **155**, 67–75.

ROPER, J. A. (1952). Production of heterozygous diploids in filamentous fungi. *Experientia* **8**(1), 14–15.

TOMSETT, A. B. & COVE, D. J. (1979). Deletion mapping of the *nii*A *nia*D gene region of *Aspergillus nidulans*. *Genetical Research* **34**, 19–32.