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 niiA− 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 niaD (Pateman, Rever & Cove, 1967; MacDonald & Cove, 1974; Cove, 1979) and niiA (Pateman et al. 1967; Rand & Arst, 1977; Cove, 1979) genes, both regulated by two positively acting control genes (nirA and areA) 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-ordinately regulated (Arst, Rand & Bailey, 1979; Tomsett & Cove, 1979). This arises from the fact that a mutation, designed nis-5 (Rand & Arst, 1977), specifically affects niiA 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 (*proAl 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 *proAl 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 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 *proAl* 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 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 niiA~ 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 meaB 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 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        |
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) |
|-------------------------|---------------------------|---|---------------------|
| Wild-type               | 30                        | 29| 31                  |
| proAl pabaA6 yA2        | 30                        | 29| 29                  |
| MSE//nihA               | 34                        | 32| 33                  |

| Ammonium chloride (71.0 mm) | + | sodium nitrite (mm) |
|-----------------------------|---|---------------------|
| Wild-type                   | 32| 30                  |
| proAl pabaA6 yA2            | 33| 31                  |
| MSE//nihA                   | 34| 32                  |

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