SHORT COMMUNICATIONS

The Occurrence in Nature of a Diploid Strain of *Aspergillus niger*

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INTRODUCTION

Diversity of phenotype and vegetative instability are often encountered with isolates from nature of the genus *Aspergillus* (Raper & Fennel, 1965). When conidia from strains previously cloned by plating for isolated colonies are cultivated on agar, variants frequently appear as distinct sectors, over-growths or localized areas of changed appearance. They may or may not retain their distinguishing characteristics when isolated. Yuill (1939) isolated a buff-coloured conidial variant which arose spontaneously in a culture of *Aspergillus fumigatus*, and a white variant from a culture of *Aspergillus nidulans*.

Aspergillus niger has uninucleate conidia (Yuill, 1950). The form of colonies is characteristic, and variants occurring in vegetative growth can readily be observed. We report observations on such variation in a strain of *A. niger* isolated from the wild, and which has the properties expected of a heterozygous diploid. To test whether it was heterozygous, we sought answers to the following questions: Is the spontaneous variation in the strain under genetic control? Is this variation affected by the incorporation of the amino acid analogue *p*-fluorophenylalanine (*p*FA) in the growth medium? Is the effect of *p*FA on the strain consistent with its known effect as an agent facilitating the haploidization of diploid strains?

METHODS

Media. Beef-glucose agar, containing 2 g Lemco beef extract, 4 g bacteriological peptone, 10 g glucose, 15 g Bacto-agar and 1 l H₂O, was used to maintain the stock cultures. Minimal medium (MM) was Czapek Dox medium with 1 % (w/v) glucose. Complete medium (CM) was a complex medium containing yeast extract, hydrolysed casein, vitamins, etc. (Pontecorvo *et al.* 1953). Solid medium contained 2 % agar. Incubation was at 34 °C.

Methods. Synthesis of diploids and mitotic haploidization by the *p*FA technique were by the methods of Lhoas (1961).

Organisms. The conidia of *A. niger* strain B, obtained from a soil isolate, were cultivated on beef-glucose agar. The strain was cloned by plating a suspension of conidia to yield isolated colonies. Strains B1 and B2 were vegetative sectors which arose from strain B.

Mutagenesis. Conidia were treated with u.v. radiation, to give a survival of 1.5 %.

RESULTS

Conidia of *A. niger* strain B were inoculated at the centre of 25 Petri dishes of beef-glucose agar and incubated at 34 °C for 7 days. The colonies had a regular outline, but four poorly-conidiating sectors were readily distinguished. Two of these sectors, called B1 and B2, were isolated for further examination.

Table 1. *Classification of haploid segregants derived from pFA treatment of A. niger strains B, B1, and B2*

Parent strain	Number of segregants	
	Conidiating	Non-conidiating
B	14	6 (2 <i>thi</i>)
B1	0	12 (1 <i>thi</i>)
B2	0	12

Table 2. *Mutant strains induced by u.v. irradiation of putative haploid strains from A. niger strains B and B1*

Haploid strain	No. of conidial isolates	No. of conidial colour mutants	No. of nutritional mutants*
BH1	304	4	3 (1 <i>thi</i> , 1 <i>bi</i> , 1 <i>nic</i>)
BH2	330	3	3 (1 <i>rib</i> , 1 <i>bi</i> , 1 <i>leu</i>)
B1H1	284	3	2 (1 <i>nic</i> , 1 <i>bi</i>)

* *bi*, *leu*, *nic*, *rib* and *thi*, requirements for biotin, leucine, nicotinic acid, riboflavine and thiamine, respectively.

The vegetative instability of *A. niger* B could be explained by a high rate of spontaneous mutation to the particular mutant phenotype, or alternatively strain B is a heterozygous diploid. The second possibility was tested by the use of *pFA*, shown by Lhoas (1961) to induce haploid segregants from diploids in *A. niger*.

Conidia of strains B, B1 and B2 were inoculated at the centre of each of 40 Petri dishes of CM containing *pFA* (0.1 g/l). After two weeks' incubation the original inocula showed little growth but one or two vigorously-growing sectors were formed on each colony. When the concentration of *pFA* was 0.07 g/l, sectors occurred after 3 to 4 days' incubation. Most of the segregants from *pFA* treatment showed normal growth on CM, but a few grew slowly, producing irregular colonies. The conidia of the segregants were of various sizes, with an average diameter of 4.0 μ m. The mean diameters of conidia of B, B1 and B2 were 4.0, 4.2 and 4.8 μ m, respectively. In a report by Lhoas (1967), the mean diameters of diploid conidia ranged from 3.88 to 4.08 μ m, and those of haploid conidia from 2.72 to 3.36 μ m. Pontecorvo, Roper & Forbes (1953) reported a much wider range of diameter.

Slow-growing segregants of strains B, B1 and B2 from *pFA* treatment gave further sectors when cultivated on CM. The slow-growing colony of strain B, which had good conidiation, gave faster-growing sectors, some with good and others with poor conidiation.

In general, the faster-growing sectors from strains B, B1 and B2 were also either well or poorly conidiating. Both types were stable in further growth on CM and on media with *pFA*, and may thus now be haploid strains. The average diameter of conidia of the putative haploid strains was 3.8 μ m, with a range of 3.4 to 4.0 μ m.

The putative haploid sectors from strains B and B1 were tested for possible nutritional requirements. Two nutritionally deficient haploids were obtained from 20 isolates of B, and 1 in 12 for sectors from B1 (Table 1). All three showed thiamine requirement. These results further confirmed the diploid status of B and B1 and their heterozygosity for the thiamine (*thi*) marker.

No nutritional mutants could be isolated in strains B, B1 and B2 after treatment with u.v. radiation. Some 550 colonies of strain B were tested and no auxotrophic or conidial colour variants were obtained.

On the other hand, u.v. treatment of two *thi*⁺ putative haploid strains from strain B (Bh1 and Bh2) and one from strain B1 (B1h1) produced a number of nutritional mutants (Table 2). These results are consistent with the interpretation that strains B, B1 and B2 are diploid and that some of the segregants isolated from them after *p*FA treatment are haploid.

Two new diploid strains were synthesized from two pairs of auxotrophic haploid strains, *thi* and *nic*, and *rib* and *leu*. These diploids, which were prototrophic, were haploidized by *p*FA treatment. Classification of 18 haploid segregants from the first diploid showed only two classes of segregant, *thi nic*⁺ or *thi*⁺*nic*, suggesting that these markers are on the same pair of homologous chromosomes. From the second diploid, haploids of the following classes were obtained: *rib*, *leu*, *rib*⁺*leu*⁺, *rib leu*; this indicates that these markers are on different chromosomes.

DISCUSSION

The relative stability of strains B, B1 and B2 on CM and the occurrence of unstable putative aneuploids from *p*FA treatment suggest that B, B1 and B2 are likely to be diploid. In *A. nidulans*, Käfer (1960) showed that aneuploids and hyperdiploids were highly unstable in vegetative culture. The recovery of *thi*⁻ haploid segregants from strains B and B1 and the production of nutritional mutants by mutagenic treatment of putative haploids support this conclusion.

Answers can now be given to the questions posed earlier. Diploid B is heterozygous for nuclear markers affecting conidiation. The origin of strains B1 and B2 from B could be by mitotic crossing-over or by mitotic non-disjunction. *p*FA facilitated the recovery of haploid sectors from the diploids B, B1 and B2. Heterozygosity of the marker, *thi*, in B and B1 was indicated by the recovery of haploids which were thiamine-requiring.

In an investigation with *Penicillium cyclopium*, Jinks (1952) reported the occurrence of heterokaryons in nature. It seems likely that strain B of *A. niger* may have arisen in nature either by heterokaryon formation between two different haploid strains followed by nuclear fusion, or by nuclear fusion in a homokaryon followed by accumulation of recessive mutations in the diploid clone.

These observations indicate that the parasexual cycle (Pontecorvo, 1956) may well occur in natural populations of fungi.

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