SHORT COMMUNICATION

Spontaneous Auxotrophs in *Agaricus bisporus*

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(Received 9 July 1979)

Single spores from commercial strains of *Agaricus bisporus* were screened for spontaneous auxotrophs. Methionine auxotrophs were recovered from three strains. The auxotrophs were of two complementary groups and one group was common to two strains. Proline auxotrophy was confirmed in a broad-stipe strain.

INTRODUCTION

The commercial mushroom *Agaricus bisporus* (Lange) Imbach differs from most other heterothallic homobasidiomycetes that have been studied in that heterokaryon formation is very restricted, even in compatible matings (Elliott, 1972; Raper *et al.*, 1972). Genetic markers are useful in the establishment and subsequent recognition of heterokaryons but few are known in *A. bisporus*. Two markers affecting sporophore morphology have been identified and shown to be monogenically inherited; broad-stipe (Miller *et al.*, 1976) and frilly-gill (Elliott, 1979*a*). A third marker, resulting in the constitutive production of fruit-body initials in axenic culture has been described (Elliott & Wood, 1978). Auxotrophic markers have also been reported (Pelham, 1967; Raper *et al.*, 1972; Wang, 1972). These are of more immediate use than fruit-body characters because they enable heterokaryons to be recognized in Petri-dish culture.

Raper *et al.* (1972) proposed the use of auxotrophs in a breeding programme for *A. bisporus* and suggested that auxotrophic nuclei might be present in most commercial mushroom spawns. This note reports the results of screening single spore cultures derived from commercial spawns for auxotrophy.

METHODS

Single spore isolates were obtained either by micromanipulation from two-, three- and four-spored basidia or by dilution plating. Spores were incubated and germinated as described previously (Elliott, 1978). Germ-lings from dilution plates were isolated under the microscope to ensure that colonies originated from individual spores.

Commercial spawns are indicated by a 'C' coding and single spores isolated by micromanipulation by a 'B' coding.

Isolates were tested for auxotrophy using the complete (CYM) and minimal (MM) media described by Raper *et al.* (1972). The colony diameters on both CYM and MM were assessed and used to classify the isolates as prototrophic, intermediate (growth on MM markedly less than on CYM) or auxotrophic. In an initial screening, MM was supplemented separately by peptone, nucleic acids or a vitamin mixture. Suspected amino-acid auxotrophs were then screened on MM supplemented by different amino-acid mixtures according to the technique of Holliday (1956).

Table 1. *Recovery of auxotrophs from single spore isolates of nine commercial spawns*

Spawn	Single spore colonies tested	Potential auxotrophs	Confirmed auxotrophs	Phenotype	Genotype
C10	136	0	0	—	—
C13	584	8	5	Methionine-requiring	<i>met.1</i>
C20	418	37	0	—	—
C25	416	16	0	—	—
C26	243	32	18	Methionine-requiring	<i>met.2</i>
C35	742	18	0	—	—
C44	565	12	0	—	—
C46	524	0	0	—	—
C52	403	11	0	—	—

RESULTS

A total of 242 isolates, mainly derived from aberrant three- and four-spored basidia of six spawns, were tested. Of these, 91 were prototrophic and 73 were intermediate. Another 77 showed such marked differences in growth on CYM and MM that they were retested: 50 of these were then classified as intermediate. When the remaining isolates were grown on MM with supplements, 15 were confirmed as auxotrophs: 12 were methionine-requiring and 3 were proline-requiring. Another 12 showed a clear response to peptone supplementation but their requirements were either non-specific or not recognized by the methods used.

The 12 methionine auxotrophs could be separated into two groups on the basis of their growth response to precursors of methionine; these groupings were consistent with parentage. Ten auxotrophs, designated as belonging to the *met.1* group, grew on MM supplemented with cystathionine; all were derived from spawn C6. The other two, belonging to the *met.2* group, responded only to supplementation with methionine itself and both were derived from three-spored basidia of spawn C26. This division into two groups was confirmed by complementation tests. The majority of the *met.1* isolates were obtained from B131 (ATCC 36974), a single spore culture from spawn C6, which tends to produce higher numbers of aberrant basidia than is usual in *A. bisporus* (Elliott, 1976). A total of 29 isolates representing 22 tetrads were obtained from this strain by micromanipulation. Of these, 22 were dwarf (Elliott & Wood, 1978), and of the remaining seven isolates, five were methionine-requiring and two were prototrophic.

Raper *et al.* (1972) had reported a methionine auxotroph which also responded to phenylalanine so the *met.1* and *met.2* isolates were tested for this response. However, none of them grew on MM supplemented with phenylalanine.

The *met.1* auxotrophs had a characteristic colony morphology on CYM. There was vigorous fluffy growth at first but colonies never reached more than 10 to 20 mm in diameter. The *met.2* auxotrophs also grew poorly on CYM but they could not be distinguished on the basis of colony morphology. The *met.1* auxotrophs were non-leaky and did not grow on MM; the *met.2* auxotrophs grew slightly on MM.

The proline auxotrophs originated from four-spored basidia of the broad-stipe strain 14A-WT (ATCC 36979). They grew better on MM than some of the isolates with a non-specific requirement. They did, however, give a clear growth response to supplementation with proline. Such mutants have previously been detected amongst hyphal fragments of this strain (Raper *et al.*, 1972; Wang, 1972).

Isolates were collected from nine different commercial spawns (Table 1). Spawn C26 was included because it had earlier been shown to be a *met.2/met.2+* heterokaryon and its use enabled the efficiency of dilution plating for the detection of auxotrophs to be judged. Germination in *A. bisporus* is very variable and spores generally require an incubation period of between 5 and 15 d. A distinction was therefore made between early and late

germinators in three spawns, C44, C46 and C52, in case auxotrophy delayed germination. The majority of colonies representing early germination were picked off as soon as they were apparent and a second batch was taken 14 d later.

The results of the screening are shown in Table 1. Some germlings were dwarf and others produced only micro-colonies (< 5 mm diameter). Isolates from all but two spawns showed sufficient differences in growth on CYM and MM to warrant further testing, but auxotrophs were confirmed only in spawns C26 and C13. The methionine auxotrophs recovered from C26 represented 7% of the C26 isolates screened. Complementation with *met. 1* and *met. 2* testers confirmed that the isolates were *met. 2*. The methionine auxotrophs recovered from C13 represented about 1% of the C13 colonies screened. They showed the typical colony morphology of *met. 1* and this genotype was confirmed by complementation tests.

DISCUSSION

The presence of auxotrophic nuclei in normal commercial spawns has been demonstrated. Presumably these mutants were spontaneous in origin like those affecting sporophore morphology (Miller *et al.*, 1976; Elliott, 1979*b*). The 'two-sporedness' of *A. bisporus* and the multinucleate nature of its mycelial cells ensure that such markers are maintained though not normally expressed.

Previous work has shown that 70 to 80% of spores from two-spored basidia are heterokaryotic but those from three- and four-spored basidia are usually homokaryotic (Elliott, 1972). Auxotrophs, when present, should therefore be detected more readily in single spores from aberrant basidia than from random samples in which the majority of spores originate from two-spored basidia. This was confirmed in the study. All of the auxotrophs which were recovered by micromanipulation originated from aberrant basidia.

After dilution plating of basidiospores the level of recovery of auxotrophs was low. This may have resulted either from an effect of auxotrophy on viability or germinability or more probably from the non-random orientation of the meiotic spindles, demonstrated by Evans (1959), which tends to favour heterozygosity. Raper *et al.* (1972) have also reported low recoveries of auxotrophic markers.

Auxotrophs were readily detected in C26 which confirmed the efficiency of the dilution plating screening procedure. It was disappointing that only methionine auxotrophs were recovered though they were found for the first time in C13. Sufficient numbers of spores were screened for other requirements for them to have been detected if present. This view is consistent with the finding of Raper *et al.* (1972) who detected methionine/phenylalanine-requirers five times in a sample of only 100 monosporous cultures. The ready recovery of microcolonies and dwarfs further suggests that even if auxotrophy did result in late germination and poor growth it would still have been detected. It therefore seems likely that the failure to recover auxotrophs from seven of the spawns tested genuinely reflects their absence from those spawns.

The presence of auxotrophic nuclei may be useful in demonstrating relationships between spawns. It seems likely that C6 and C13, which were obtained from different spawn makers, have a common ancestry for both carry a *met. 1* component and both produce sporophores with brown caps.

Auxotrophs have great value when making crosses in *A. bisporus* since the formation of heterokaryons between prototrophs is not evident in culture. However, on the basis of this present work they are apparently not as widespread in commercial spawns as might have been expected. Attempts to induce and recover such mutants using ultraviolet and gamma irradiation have not been particularly successful due to the multinucleate nature of hyphal cells and spores (Pelham, 1967; Raper *et al.*, 1972; Wang, 1972). When hyphal fragments were used most of the mutants obtained were spontaneous in origin (Raper *et al.*, 1972; Wang, 1972). An alternative approach to the problem of making defined crosses when breeding *A. bisporus* may be to use dominant resistance markers (Elliott, 1979*b*).

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