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SHORT PAPERS

A simple and rapid technique for obtaining a high proportion of hybrid cleistothecia in *Aspergillus nidulans*

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SUMMARY

A technique is reported for crossing strains of *Aspergillus nidulans* which produces hybrid cleistothecia in 7 days. The method depends on the partial inhibition of cleistothecium formation of auxotrophic mutants by growth-factor limitation. Cleistothecia form at the junction of two strains inoculated on to complete medium. The method has a number of advantages over standard techniques.

The standard techniques for crossing strains of *Aspergillus nidulans* usually involve the isolation of heterokaryons and the production of mature hybrid cleistothecia takes at least 10 days and sometimes up to 16 days (Pontecorvo *et al.* 1953; Foley, Giles & Roberts, 1965). A method is reported here which can produce hybrid cleistothecia on complete medium in 7 days. The technique is simple to use and has a number of other advantages over the traditional methods. A preliminary note on this technique has already been published (Bainbridge, 1965).

The strains used were isolated from crosses between strains originating in the Genetics Institute in Glasgow. Strain numbers and genotypes are shown in Table 1. The technique has been shown to work for *argA*1 and *argB*2 (arginine requiring mutants) and for *lysB*5 (a lysine requiring mutant). Media were basically those used by Pontecorvo *et al.* (1953) but MgSO4 was omitted from the basic minimal medium (MM) and added in a trace element solution (Trinci, 1969). The complete medium was that of MacKintosh & Pritchard (1963) to which was added 10 ml/l. trace element solution. Media were supplemented with excess arginine (10^{-2} M) or lysine (5×10^{-3} M) to obtain good conidiation of the respective auxotrophs. For experimental media the concentrations of arginine and lysine were as indicated.

An *argA* or an *argB* strain and a second strain were streaked on unsupplemented complete medium (CM) about 1 mm apart with occasional regions overlapping. Abundant mature cleistothecia formed within 7 days at the junction between the two strains (Plate 1, fig. 1). No cleistothecia resulted if the arginine-requiring mutants were replaced with the lysine-requiring strain on the same medium (Plate 1, fig. 2). Two explanations for this phenomenon were investigated. Firstly that the effect with the arginine strains was due to an inhibitory balance of lysine and arginine in CM. Alternatively the phenomenon could be due to the presence of a limiting concentration of arginine. The latter was shown to be correct because the cleistothecial effect could also be produced on MM to which only arginine had been added. Less cleistothecia were produced at the junction but the effect was still well marked. Cleistothecia were most abundant on MM plus 5×10^{-4} M and 10^{-3} M arginine. The lysine-requiring mutant showed similar effects on MM plus 5×10^{-5} M and 10^{-4} M lysine. Arginine would need to be added to CM to

produce this effective concentration of lysine to use the technique for the *lysB* strain. Hybrid cleistothecia were usually formed when small white immature cleistothecia were produced on the auxotrophic mutant indicating partial nutrient limitation. The percentage hybrids varied between 24 and 100 % but was usually above 70 % (Table 1).

The effects of CM could be simulated on MM containing different ratios of lysine and arginine. Hybrid products were produced at lysine:arginine ratios of 2:1 for the arginine mutants and between 1:1 and 2:1 for the lysine mutant (Table 1). These results correlated with the report by Cybis & Weglenski (1969) that arginine is a strong antagonist of lysine uptake but that lysine is a less strong antagonist of arginine.

Table 1. *Frequency of hybrid cleistothecia in crosses on unsupplemented CM and on MM with various concentrations of lysine and arginine*

	Strains crossed*	Medium	Supplement†		Cleistothecia		Hybrid (%)
			Lysine	Arginine	Hybrid	Total	
1	BWB140 *yA; argA1* × BWB141 *anA1 adE20 biA1; AcrA1 wA*	CM	—	—	18	21	85·7
2	BWB157 *biA1; argA1* × BWB224 *yA; veA*	CM	—	—	9	12	75
3	BWB151 *pabaA1 yA; argA1* × BWB149 *adE20 biA1*	CM	—	—	11	11	100
4	BWB140 *yA; argA1* × BWB272 *veA*	MM	—	5×10^{-4} M	16	68	23·6
5	BWB157 *biA1; argB2* × BWB224 *yA; veA*	MM	—	10^{-3} M	10	21	47·6
6	BWB140 *yA; argA1* × BWB272 *veA*	MM	10^{-2} M	5×10^{-3} M	9	10	90
7	BWB408 *biA1; lysB5; chaA* × BWB224 *yA; veA*	MM	5×10^{-5} M	—	13	13	100
8	BWB408 *biA1; lysB5; chaA* × BWB272 *veA*	MM	10^{-2} M	5×10^{-3} M	3	10	30

* BWB strains held at Queen Elizabeth College. See Clutterbuck (1973) for a complete list of gene symbols.

† MM was supplemented with biotin where necessary.

This crossing technique has several advantages over the methods normally used. Large predominantly hybrid cleistothecia can be obtained more quickly with less media and manipulations. It is possible to carry out crosses with prototrophic strains or strains with very similar genotypes as long as one strain has an *arg* or *lys* marker. The technique is particularly useful for crossing unstable strains or strains with a high reversion rate as the amount of growth before nuclear fusion is minimal. Analyses of this type were done on the relatively unstable duplication types resulting from crosses heterozygous for an unequal translocation (Bainbridge & Roper, 1966). There are also possible applications in a teaching situation as strains can be crossed quickly and easily. In addition, the omission of arginine from the supplements to CM would allow the visual classification of the arginine marker without the use of further media.

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