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SOMATIC RECOMBINATION IN COMMON-*A* HETEROKARYONS OF *SCHIZOPHYLLUM COMMUNE*

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Abstract

Common-*A* heterokaryons of *Schizophyllum commune* developed from auxotrophic parents were used for studying somatic recombination and, if possible, isolating vegetative diploid strains. A simple method was devised for this purpose, which comprised the growth of the heterokaryons first in liquid minimal medium, then the fragmentation of the resultant mycelia and subsequently the plating of the fragments on minimal agar to select colonies differing from the 'flat' heterokaryon types. The selected colonies were isolated and analysed both for nutritional requirements and mating type factors. Genotypes resulting from recombination between two parental nuclei of a heterokaryon could be detected. The probable mechanism of their formation and the usefulness of the technique in selecting them have been discussed.

Introduction

Schizophyllum commune is a heterothallic fungus in which the pattern of mating is controlled by two unlinked mating factors *A* and *B* each with a series of alternative states, viz : A_1, A_2, A_3.....A_n and B_1, B_2, B_3.....B_n. Crossing between two monokaryotic strains with dissimilar *A* and *B* factors (i.e., $A_1B_1 \times A_2B_2$) usually results in the formation of a fertile dikaryon. When two strains with similar *A* or *B* factors are mated together a common-*A* or a common-*B* heterokaryon is respectively formed. However, mating between two strains with similar *A* and *B* factors results in the formation of a common-*AB* heterokaryon (Middleton 1964a).

Somatic recombination has been studied in dikaryons (Ellingboe and Raper 1962, Ellingboe 1964) and common-*AB* heterokaryons (Middleton 1964b) of *S. commune*. Vegetative diploid strains have been isolated and somatic recombination studied in common-*AB* (Mills and Ellingboe 1969) and common-*B* (Parag and Nachman 1966) heterokaryons of the same fungus. In every study it required special methods for the selection of recombinants and the isolation of diploids occurring in the vegetative mycelia. It is necessary to isolate and analyse a good number of recombinants in order to understand the mechanism(s) underlying their formation. However, the occurrence of vegetative recombinants or diploids is quite infrequent and sometimes they cannot be detected without using selective techniques.

This paper reports the isolation of somatic recombinants from two common-*A* heterokaryons of *S. commune* and also describes a simple method which may be employed in selecting vegetative diploids or recombinants from heterokaryons.

Materials and Methods

Two common-*A* heterokaryons were used, the genotypes of which are as follows :

Heterokaryon (1)	A_{41}	ad_5	B_{42}	+	+	+	+
	A_{41}	+	B_{41}	arg_7	+	+	+
,, (2)	A_{42}	+	B_{41}	+	ad_2	ur_2	+
	A_{42}	+	B_{51}	+	+	+	arg_6

The heterokaryons, each of which carried nuclei with complementing auxotrophic alleles, showed the characteristic 'flat' morphology and slower growth on minimal agar medium. None of the monokaryotic parents forming the heterokaryons was able to grow on minimal medium because of nutritional deficiency.

Both complete and minimal media used in these experiments were similar to those described by Snider and Raper (1958). Fruiting medium consisted of the complete medium containing, in addition, 0.3–0.4% yeast extract.

The heterokaryons were first developed on complete agar medium and then were grown on minimal agar medium. Inocula from each of the heterokaryons growing on minimal agar were transferred to 250 ml conical flasks each containing 100 ml liquid minimal medium and were incubated at 28 C for a week. The resultant mycelium of each heterokaryon was fragmented by an M.S.E. homogeniser using the fine blade for 60 sec at a speed of 10,000 r.p.m. The beaker containing the mycelium was held in a small chamber with ice. Appropriate dilutions of the fragments were spread on minimal agar medium in Petri dishes so as to obtain 24-30 visible colonies in each plate. Following 5-7 days' incubation at 28 C, the plates were observed for the presence of any colony showing morphological characteristics and growth rate markedly different from those of the heterokaryon concerned. When such colonies were found they were isolated, further tested on minimal medium and their mating type factors determined by making appropriate crosses.

Results and Discussion

Colonies with characteristic features (Fig. 1) resulted on minimal medium from the fragments of each heterokaryon at a frequency of 2-6/100. These colonies were not 'flat' and showed denser and faster growth compared to the respective heterokaryon. Hyphal tips from such colonies were isolated and grown on fresh minimal agar medium to confirm their prototrophic nature and observe the pattern of growth. The mating type factors carried by each of the isolates were also determined. The results given in Table 1 show that two classes of prototrophic isolates were isolated from the fragments of a common-*A* heterokaryon plated on minimal agar medium ; (a) those which showed normal growth and carried the mating type factors similar to those of one or the other parent monokaryon (auxotrophic) of the heterokaryon concerned and (b) those which showed normal growth with 'crusty' colony surface and carried the mating type factors similar to those of the heterokaryon concerned but did not conform to the morphological characteristics of the typically 'flat' common-*A* heterokaryon. Such results may be interpreted to mean that the class 'a' type isolates arose as a result of somatic recombination between two nuclei each carrying auxotrophic alleles in the common-*A* heterokaryon and the class 'b' type isolates were either common-*A* diploids or aneuploid strains carrying disomic mating factors. The role of mutation resulting in the production of protorophic strains from either of the auxtrophic parents of a heterokaryon seemed highly unlikely in this case since prototrophic isolates carrying the mating type factors of different parent monokaryons were obtained and hence a single mutational event involving one locus could not account for all the prototrophs isolated from any one heterokaryon.

When the isolates were grown on agar medium, distinguishable sectors (Figs. 2a & b) occurred from some of the class 'a' and all of the class 'b' type isolates. Such sectoring was more common when the isolates were grown on complete medium. Inocula taken from these sectors mostly showed very poor growth on both minimal and complete medium. No auxotrophic sector from any of the prototrophic isolates could be obtained. The sectors were not further analysed.

Crossing of the isolates with a third monokaryon

Each of the two prototrophic isolates 1A and 1G showing 'crusty' appearance and conforming to the mating type factors of the common-*A* heterokaryon (1) but not to its morphological features was crossed with another monokaryon requiring uracil (*ur* 2) and carrying the mating type factors A_{42} B_{41}. The resultant dikaryons from the two crosses were grown on fruiting medium. Basidiospores

Fig. 1. Characteristic non-'flat' colonies with 'crusty' surface and denser growth occurring in a plate.

Fig. 2a. Smooth sector is developing from an isolate with 'crusty' surface.

Fig. 2b. Poorly growing but distinguishable sector is arising from an isolate with 'smooth' surface.

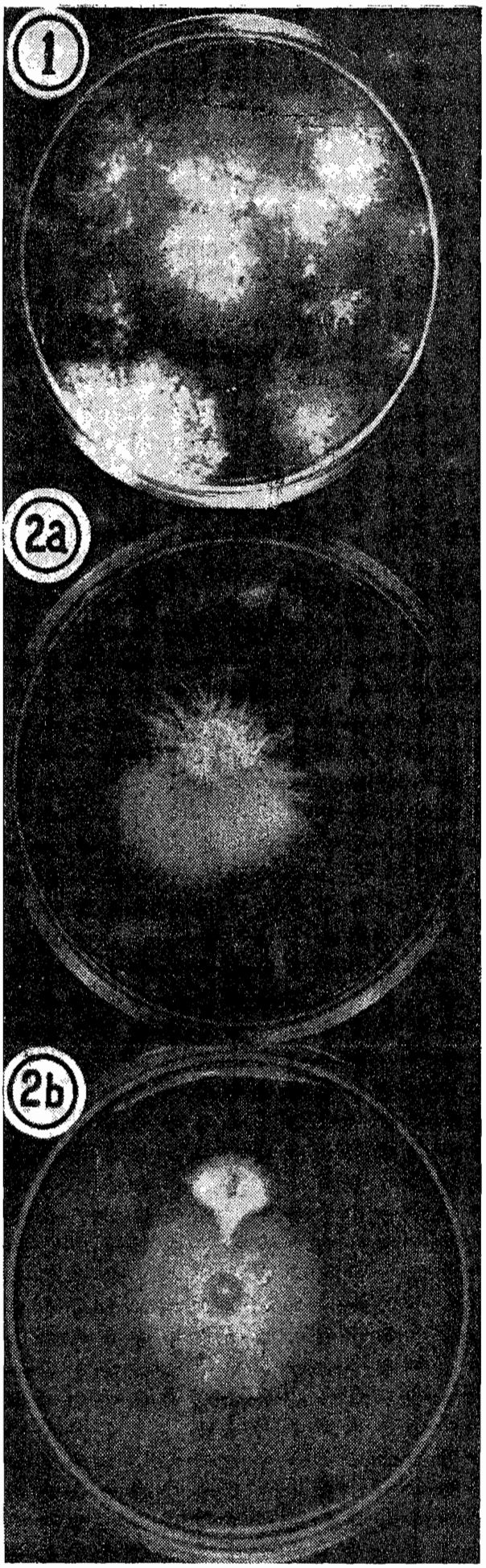

Table 1

Nutritional requirements, morphological characteristics and mating type factors of the isolates obtained from common-A heterokaryons (1) and (2)

Heterokaryons from which isolates were obtained	Nos. of isolates tested	Growth on minimal medium	Morphological feature of colony	Mating type factors	Mating reaction	Remarks
1	2	3	4	5	6	7
(1)	1A	+	'Crusty' surface	A_{41} B_{42}/A_{41} B_{41}	Delayed	Prototrophic, carries dissimilar *B* factors but morphologically unlike common-*A* heterokaryon
	1B	+	,,	A_{41} B_{42} A_{41} B_{41}	,,	,,
	1C	+	'Smooth' surface	A_{41} B_{42}	Normal	Prototrophic, monokaryotic type
	1D	+	,,	A_{41} B_{42}	,,	,,
	1E	+	,,	A_{41} B_{41}	,,	,,
	1F	+	,,	A_{41} B_{41}	,,	,,
	1G	+	'Crusty' surface	A_{41} B_{42}/A_{41} B_{41}	,,	Prototrophic, carries dissimilar *B* factors but morphologically unlike common-*A* heterokaryon
	Parent heterokaryon (1) as 'control'	+ (slower growth)	'Flat'	A_{41} B_{42}/A_{41} B_{41}	,,	Prototrophic, carries dissimilar *B* factors and typically, 'flat' like common-*A* heterokaryon
(2)	2A	+	'Crusty' surface	A_{42} B_{41}/A_{42} B_{51}	Delayed	Prototrophic, carries dissimilar *B* factors but morphologically unlike common-*A* heterokaryon
	2B	+	,	A_{42} B_{41}	Normal	Prototrophic, monokaryotic type
	2C	+	'Smooth' surface	A_{42} B_{41}	,,	,,
	2D	+	,,	A_{42} B_{41}	,,	,,
	2E	+	'Fluffy' surface	A_{42} B_{41}	,,	,,
	2F	+	'Crusty' surface	A_{42} B_{51}	,,	,,
	Parent heterokaryon (2) as 'control'	+ (slower growth)	'Flat'	A_{42} B_{41}/A_{42} B_{51}	Normal	Prototrophic, carries dissimilar *B* factors and typically 'flat' like common-*A* heterokaryon

from three different fruit bodies resulting from each cross were analysed for nutritional requirements. The results given in Table 2 showed decreased viability of spores in some fruit bodies, notably in the case of the fruit body 1 of the cross 1. From the same cross six arginine requiring progeny were obtained indicating that the prototrophic isolate 1A had carried the chromosome containing arg_7 locus which was linked to B_{41} mating type factor of a parent monokaryon of the heterokaryon (1). The recovery of the adenine requiring progeny from the cross 2 involving the isolate 1G also indicated that the apparently prototrophic isolate 1G had carried the chromosome containing ad_5 locus linked with A_{41} mating type factor of the other parent of the heterokaryon (1). Such results, however, did not show any evidence of triploid segregation suggesting that three nuclear genomes participated in meiosis in the above crosses. Thus, it is only fair to conclude that the isolates 1A and 1G which were analysed could be either aneuploids carrying disomic mating factors or unstable diploids rapidly undergoing haploidisation prior to fruit body formation and meiosis in the crosses, unlike those diploid strains obtained from common-*B* and common-*AB* heterokaryons of *S. commune* (Parag and Nachman 1966, Mills and Ellingboe 1969). Further, these results could also mean that the unstable diploid nuclei formed in the mycelia of common-*A* heterokaryons underwent haploidization through aneuploidy (Middleton 1964, Cowan and Lewis 1966). The instability of the diploid nuclei, however, could be due to some undefined factors involved in the present investigations.

In any case, further studies using more markers in different linkage groups are necessary to confirm whether or not stable common-*A* diploid strains could be isolated by using similar selective methods.

The simple technique used in the present studies may prove to be fruitful in isolating either vegetative diploids or recombinants from heterokaryons of *S. commune*.

It is found that in diploid and dikaryotic strains allelic complementation is stronger than in heterokaryons (Casselton and Lewis 1967). Thus, a diploid strain grows faster on minimal medium than a heterokaryon carrying the similar alleles in different nuclei. A wild type haploid would also grow faster than a heterokaryon carrying complementing auxotrophic alleles. On this basis it was assumed that the prototrophic diploid nuclei or recombinant nuclei, either haploid or aneuploid, arising from heterokaryons (1) and (2) would be in an advantageous position to grow quickly in liquid minimal medium. This would allow the multiplication of the diploid or recombinant nuclei and thus many of them would outgrow the poorly growing common-*A* heterokaryon mycelium and be never missed when the fragments were plated on minimal agar. This simple

Table 2

The analysis of basidiospores from crosses involving each of isolates 1A and 1G, and an uracil requiring monokaryon of mating types A_{42} B_{41}

Cross	Isolated involved	Fruit bodies formed in	No. of fruit bodies analysed	No. of spores		% germination	Segregation of alleles					
				Isolated	Germinated		*ad* : *ad*$^+$		*ur* : *ur*$^-$		*arg* : *arg*$^+$	
1	1A × uracil requiring monokaryon of A_{42} B_{41} mating types	13 days	1	80	19	23.7	5	14	7	12	*6	13
			2	100	59	59.0	15	44	28	31	0	59
			3	50	36	72.0	16	20	17	19	0	36
2	1G × uracil requiring monokaryon of A_{42} B_{41} mating types	19 days	1	40	29	72.5	7	22	11	18	0	29
			2	50	41	82.0	10	31	18	23	0	41
			3	100	89	89.0	41	48	40	49	0	89

* Arginine requiring progeny occurred from the prototrophic isolate 1A.

technique seemed to have worked but it remains to be seen whether the recombinant nuclei isolated, represented those which had originally occurred or there had been already much changes in their nuclear constitution in between the period of their occurrence and isolation. Further, the isolates obtained from the common-*A* heterokaryons in no way provided an indication of the frequency of the occurrence of recombinant nuclei since many of them would merely be the result of multiplication of the original ones.

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References

Casselton, L.A. and D. Lewis. 1967. Dilution of gene products in the cytoplasm of heterokaryons in *Coprinus lagopus*. Genet. Res. (Camb.) **9** : 63-71.

Cowan, J.W. and D. Lewis. 1966. Somatic recombination in the dikaryon of *Coprinus lagopus*. Ibid **7** : 235-244.

Ellingboe, A.H. 1964. Somatic recombination in dikaryon-*K* of *Schizophyllum commune*. Genetics **49** : 247-251.

———and J.R. Raper. 1962. Somatic recombination in *Schizophyllum commune*. Ibid **47** : 85-98.

Middleton, R.B. 1964a. Evidence of common-*AB* heterokaryosis in *Schizophyllum commune*. Amer. J. Bot. **51** : 379-387.

———1964b. Sexual and somatic recombination in common-*AB* heterokaryons of *Schizophyllum commune*. Genetics **50** : 701-710.

Mills, D.I. and A.H. Ellingboe. 1969. A common-*AB* diploid of *Schizophyllum commune*. Ibid **62** : 271-279.

Parag, Y. and B. Nachman. 1966. Diploidy in the tertapolar heterothallic basidiomycete *Schizophyllum commune*. Heredity **21** : 151-154.

Snider, P.J. and J.R. Raper. 1958. Nuclear migration in the basidiomycete *Schizophyllum commune*. Amer. J. Bot. **45** : 538-546.