ISOLATION OF PROTOTROPHIC HYPHAL TIPS FROM SPECIFIC HETEROCARYONS IN *CEPHALOSPORIUM MYCOPHILUM*

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ABSTRACT. — Specific combinations of mutant strains in *Cephalosporium mycophilum* form "balanced" heterocaryons from which heterocaryotic and prototrophic hyphal tips may be isolated. The competitive advantage exhibited by somatic diploid mycelium in previously investigated "unbalanced" heterocaryons was also observed in the "balanced" heterocaryons suggesting ineffective complementation within both "balanced" and "unbalanced" heterocaryons. The results further suggest some functional difference between genetic elements combined in a single nucleus and the same genetic elements within separate nuclei, but combined into a single cytoplasmic unit. How nuclear fusion might enhance complementation in this species is not obvious.

It has been reported previously that it was not possible to isolate prototrophic hyphal tips from specific heterocaryons in *Cephalosporium mycophilum* (Cda.) Tubaki (Tuveson and Coy, 1963; Tuveson 1964). With a single exception, hyphal tips isolated from the heterocaryons and transferred to complex medium were homocaryotic for one of the nuclear components in the heterocaryon. Hyphal tips from the exceptional heterocaryon (HET VI-C) when transferred to complex medium were homocaryotic for one or the other of the component nuclei in the heterocaryon. However, not a single heterocaryotic hyphal tip was isolated from this exceptional heterocaryon. It was postulated that the ability to isolate heterocaryotic hyphal tips from this exceptional heterocaryon may simply have been a result of the small number of tips analyzed. This paper presents evidence which suggests that heterocaryotic and prototrophic hyphal tips can be isolated from HET VI-C when a sufficiently large sample of tips is isolated. Further, evidence is presented showing that heterocaryotic and prototrophic hyphal tips can be isolated from another heterocaryon (HET XI-C) which differs from HET VI-C in that one of its components has an additional nutritional requirement.

MATERIALS AND METHODS

The source of the wild type culture, the medium used and the methods for the induction, isolation and characterization of mutants have been presented (Tuveson and Coy, 1961). The mutant strains used in this investigation and in previous investigations (Tuveson, 1964) were: adenineless, methionineless, orange (-ade, -met, ora); cysteineless, leucineless, green (-cys, -leu, gr); cysteineless, leucineless, argininеless, green (-cys, -leu, -arg, gr); argininеless, orange (-arg, ora). Retesting of the arginine requirement in the -arg, ora strain revealed that this mutant also responded to ornithine or citrulline. The arginine requirement in the green strain (-cys, -leu, -arg, gr) was satisfied specifically by arginine indicating that the arginine requirements in the orange and green strains involved different genes.

Methods for the synthesis of heterocaryons and "mat controls" have been described (Tuveson and Coy, 1961; Tuveson, 1964).

The input proportions of the two nuclear types for the heterocaryons were

determined by viable counts after initial adjustments based on haemocytometer counts. A mean viable count was calculated from ten replicates.

The concentrations of supplements added to minimal medium for the identification of the nutritional requirements of strains and techniques for the isolation of hyphal tips have been presented (Tuveson and Coy, 1961; 1963).

The methods used for the identification of the nutritional requirements of the colonies derived from hyphal tips were identical to those described previously (Tuveson, 1964).

All cultures were incubated at 25 ± 2C.

Experimental Results and Discussion

In previous experiments, it had been demonstrated that it was not possible to isolate prototrophic hyphal tips from HET IV-C (Tuveson and Coy, 1963; Tuveson, 1964). As a check on previous experiments, hyphal tips were isolated from mycelial mat fragments of HET IV-C (-ade, -met, ora + -cys, -leu, -arg, gr) growing on minimal medium (Table 1). As was expected, all the hyphal tips isolated and transferred to complex medium were homocaryotic for the orange component (-ade, -met, ora). None of the tips transferred to minimal medium formed colonies after 28 days of incubation. These results substantiate previous observations. Clearly, maintaining the mutant stocks for three years in the laboratory had not resulted in any significant alterations of these stocks.

Hyphal tips were isolated from mat fragments of HET VI-C (-arg, ora + -cys, -leu, gr) after 5 and 13 days of incubatiaon on minimal medium. The results are in substantial

Table 1.—Growth of Hyphal Tips from Heterocaryons Grown on Minimal Medium

Heterocaryon and component strains	Input nuclear ratio[a] orange component : green component	Age in days of colony from which tips were isolated	Medium to which isolated	Total no. of tips	No. of colonies	Genetic Constitution
IV-C -ade, -met, ora + -cys, -leu, -arg, gr	2 : 1	13	complex	25	25	-ade, -met, ora
			minimal	25	0	—
VI-C -arg, ora + -cys, -leu, gr	7 : 1	5	complex	26	26	25 -cys, -leu, gr 1 heterocaryon
			minimal	24	0	- -
		13	complex	50	49	30 -cys, -leu, gr 14 -arg, ora 5 heterocaryons
			minimal	50	2	2 heterocaryons
XI-C -arg, ora + -cys, -leu, -arg, gr	1 : 1	13	complex	27	25	7 -cys, -leu, gr 14 -arg, ora 4 heterocaryons
			minimal	25	2	2 heterocaryons

[a]Input nuclear ratios are based on viable counts.

agreement with those reported previously (Tuveson, 1964) with the notable exception that a single and five heterocaryotic hyphal tips were observed among the tips transferred to complex medium from 5 and 13 day old colonies respectively. In addition, two of the tips isolated from the 13 day old cultures and transferred to minimal medium formed small colonies after 21 days of incubation on minimal medium. These colonies were transferred to fresh minimal medium plates to prevent desiccation and incubated for 21 additional days at which time it was possible to analyze the conidia produced by the colonies. Both nuclear components were recovered among the conidia establishing that these colonies were heterocaryotic and prototrophic. HET VI-C differs from the five heterocaryons previously investigated in that when growing on minimal medium one of the nuclear components does not predominate resulting in the colonies developing a homocaryotic periphery. Ineffective complementation had been postulated to account for the results with the five previous heterocaryons in which one of the nuclear components predominated in the heterocaryons growing on minimal medium (Tuveson and Coy, 1963; Tuveson, 1964). It had been postulated that as a result of ineffective complementation, the heterocaryons became homocaryotic at the periphery, stopped growing following which fans of somatic diploid mycelium emerged from the heterocaryotic colonies. When HET VI-C was grown on minimal medium, both component strains were easily recoverable among the hyphal tips isolated from a 13 day old colony. In contrast to the five previously investigated heterocaryons, HET VI-C appeared to be a "balanced" heterocaryon (neither nuclear component predominates in the heterocaryotic mycelium). Despite the fact that HET VI-C appeared to be a "balanced" heterocaryon, somatic diploid fans emerged from these colonies when growing on minimal medium in a manner equivalent to that observed with other heterocaryons in this species. It is also of interest to note that heterocaryotic hyphal tips isolated from HET VI-C and growing on minimal medium required six weeks to form a colony large enough for the isolation of conidia for the re-isolation of the original component homocaryons. Heterocaryotic hyphal tips isolated from HET VI-C and growing on complex medium required 18-21 days to form colonies large enough such that conidia might be isolated to recover the original component strains. This observation suggests that complementation between the component strains in HET VI-C is ineffective (or inefficient) although it would seem to be a "balanced" heterocaryon. It must be concluded that complementation is much more effective when the component nuclei have fused to form a somatic diploid rather than when unfused nuclei are present in the same cytoplasmic unit (heterocaryon). Roberts (1964) has reported that complementation for certain sorbitol markers in *Aspergillus nidulans* differs in heterocaryons and somatic diploids. These observations suggest some functional significance

to the association of genetic elements within a single nucleus as opposed to the same genetic elements in separate nuclei within a single cytoplasm. How fusion of nuclei might enhance complementation is not obvious.

The results obtained with HET XI-C were equivalent to those obtained with HET VI-C (Table 1). The only difference between these two heterocaryons was that the green component in HET XI-C carried an additional nutritional marker (-arg). Clearly, the additional marker had little influence on the complementation relationship between the components.

The results reported here differ from those reported previously (Tuveson and Coy, 1963; Tuveson 1964) in that it was possible to find specific combinations of mutant strains which when incorporated into heterocaryons allowed for the isolation of prototrophic hyphal tips. This leads to the conclusion that heterocaryons may have differing properties depending, in very large part, on the mutants employed in the investigation. Recently, it has been reported that the yield of prototrophic hyphal tips from specific heterocaryons in the plant pathogenic fungus, *Ascochyta imperfecta*, is dependent on the combination of mutants used to synthesize the specific heterocaryons (Sanderson and Srb, 1965). It would appear that conclusions may be drawn concerning the properties of specific heterocaryons within a species, but it seems doubtful that general conclusions concerning heterocaryosis as a phenomenon can be drawn from the examination of a limited number of heterocaryons within a species.

Acknowledgment

This investigation was aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

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Manuscript received March 18, 1965.