HOMOTHALLISM IN THANATEPHORUS CUCUMERIS

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Summary

Evidence is given to support the claim made by several workers that T. cucumeris is homothallic. In some isolates the evidence for homothallism is conclusive. However, in other isolates sterility factors prevent fruiting in otherwise self-fertile progeny and in still other isolates self-fertility of progeny can be accounted for by the frequency of binucleate spores in which both nuclei are immediate meiotic products. The importance of accompanying genetical studies with cytological information is therefore stressed.

I. INTRODUCTION

The occurrence of specialized pathogenic strains within Thanatephorus cucumeris (Frank) Donk presents an opportunity for genetical studies on the inheritance of pathogenicity, and concomitantly on the physiological and biochemical factors involved. However, prior to any genetical studies it is important that the factors affecting sexuality are understood. Such understanding is complicated by the changing species concept in T. cucumeris. Previous work on sexual factors has been done on both T. cucumeris and T. praticola (Kotila) Flentje but the validity of separating these species has been repeatedly questioned. At a symposium on Rhizoctonia solani and related forms, organized by the American Phytopathological Society and held at Miami in 1965, it was agreed that the grounds for separating T. cucumeris, T. praticola, and some other species were insufficient and that they should be included in the collective species T. cucumeris. It was further agreed that it would be convenient to indicate which type of isolate was being used by referring to a “solani” type or a “praticola” type, etc. This terminology is therefore adopted in this paper.

T. cucumeris has been reported by a number of investigators as being homothallic (Müller 1924; Kotila 1929; Hawn and Vanterpool 1953; Sakseña 1961; Whitney and Parmeter 1963; Flentje and Stretton 1964), because of the production of self-fertile single-basidiospore cultures among its progeny. If all basidiospores were uninucleate the above investigations would provide substantial evidence of homothallism. However, Flentje, Stretton, and Hawn (1963) have shown that in some “solani” and some “praticola” type isolates of T. cucumeris up to 35% of binucleate spores occur in which both nuclei are probably immediate meiotic products. A pseudo-homothallic condition may sometimes appear in an organism which is basically heterothallic, if two nuclei of opposite mating type are included within a sexual spore. This condition, termed secondary homothallism, occurs in

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the Hymenomycetes with varying degrees of efficiency (Whitehouse 1949), resulting
in the production of both self-sterile and self-fertile progeny. Thus, while in T. cucumeris the occurrence of self-fertile progeny excludes the possibility of true heterothallism operating, cytological data are required to determine whether or not the isolates are homothallic. The lack of cytological information accompanying some of the earlier work on this organism presents difficulties in interpretation of some of these data. In view of this uncertainty four "solani" type isolates of T. cucumeris were more thoroughly investigated and this paper presents cytological and genetical data concerning homothallism in this species.

II. MATERIALS AND METHODS

The following isolates of T. cucumeris "solani" type (cf. Flentje and Saksena 1957; Flentje, Dodman, and Kerr 1963), were used:

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Origin</th>
<th>Pathogenic Specificity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>Wheat root, Cungena, S. Aust.</td>
<td>Roots; not host specific</td>
</tr>
<tr>
<td>48</td>
<td>Soil, Waite Institute, Adelaide</td>
<td>Stems of Cruciferae</td>
</tr>
<tr>
<td>69</td>
<td>Cabbage stem, Clare, S. Aust.</td>
<td>Stems of Cruciferae</td>
</tr>
<tr>
<td>82</td>
<td>Soil, Cungena, S. Aust.</td>
<td>Non-pathogenic to all hosts tested</td>
</tr>
</tbody>
</table>

* Representatives of the families Cruciferae, Compositae, Solanaceae, and Gramineae were tested as hosts.

First, second, and third generation single-basidiospore cultures are designated by the isolation number for each generation in series. The isolation numbers have no particular significance, but are used for brevity. Further, for brevity, G1, G2, etc. are used to designate the particular generation, e.g. 48–11 is a G1 culture.

Fructifications were obtained either on low-nutrient agar (with isolate 82), or on the surface of aerated steam-treated soil (Stretton et al. 1964).

Single-basidiospore cultures were obtained from spore showers on agar (Flentje and Stretton 1964), or from spores shed on Cellophane over agar. The latter method enabled spores to be removed easily on small pieces of agar held on glass needles. The agar pieces, each with a single spore, were then plated on agar.

The HCl-Giemsa method was used for fixing and staining all material in which counts of nuclei were made.

Prebasidial cells were isolated from clumps of immature basidia on soil or agar by teasing the clumps apart under a film of water. The distinctly curved prebasidial cells, shorter and more branched than ordinary vegetative cells, were then isolated using fine glass needles, and cultured on agar. Under these conditions, the prebasidial cells discontinued the reproductive phase before the formation of diploid nuclei, resulting in the growth of vegetative cells containing (4)–8–(10) nuclei per cell.

Anastomosis was determined by apposition of isolates on Cellophane overlying agar (Flentje and Stretton 1964).
III. Experimental

(a) Isolate 82

Earlier studies with this isolate (Flentje and Stretton 1964) showed a lack of variation in single-basidiospore cultures. These studies were extended through five successive generations, and results indicated that stable homokaryotic lines were developed from single-basidiospore cultures. Ready formation of fertile hymenia by both the parent field isolate and subsequent progeny through five generations coupled with a consistent uninucleate condition of basidiospores, demonstrated that the isolate is homothallic with little evidence of mutation affecting fertility.

Opposition of pairs of single-basidiospore cultures, within each generation, resulted in successful anastomoses.

(b) Isolate 48

Twelve G1 basidiospore cultures of this isolate were tested singly for ability to fruit. Culture 48–4 fruited as regularly as the parent isolate 48, but in 12 separate experiments none of the other 11 G1 single-spore cultures could be induced to fruit under the test conditions. G2 single-spore cultures obtained from 48–4 did not vary significantly from one another or from the original 48–4 in either cultural characteristics or pathogenicity, suggesting that this culture was homokaryotic and that self-fertility was not due to a binucleate spore condition. When inoculated into soil, approximately 70% of the G2 cultures from 48–4 fruited prolifically (Flentje and Stretton 1964). Twelve months later, after more than 24 attempts to induce the other 11 G1 single-basidiospore cultures to fruit, no fruiting had occurred and furthermore 48–4 could no longer be induced to fruit. However, after 26 months of repeated fruiting experiments, 8 of the 12 G1 single-basidiospore cultures had been induced to fruit, although in some the fructifications were sparse and formed only once. One of the earliest of these single-spore cultures to fruit, 48–11, formed a mass of basidia in one localized area on the soil. Prebasidial cells were isolated from this area and basidiospores were collected. The former were cultured on agar to give 10 separate isolates, all of which were indistinguishable from 48–11 in appearance and all of which fruited prolifically in 3½ days or less, from the time soil was added to the cultures; this represents the most rapid development of basidia that we have observed in this species. These prebasidial cell isolates continued to fruit readily after repeated subculturing. However, further subcultures from the original stock culture of 48–11 failed to fruit.

Among the basidiospores collected from 48–11, germination was variable, and many spores, which germinated initially, later died. Only 30% of the shed spores established colonies, and 83% of these were self-fertile. Similar results were obtained with other G1 cultures which fruited, the percentage viability of spores and the percentage self-fertility of the single-spore cultures varying considerably. A single-basidiospore culture 48–11–14 was selected for further study and 82% of its spores established colonies, 94% of these eventually fruiting after 4 months of repeated experimenting. Again a single-spore culture, 48–11–14–56, was selected, and 99% of the spores from this culture were uninucleate, 99% established colonies, and 100%
of these subsequently fruited. These results are tabulated in Table 1. As the G4 spores were 99% uninnucleate and as the resulting cultures were indistinguishable and all self-fertile, it was concluded that 48–11–14–56 was a homokaryotic, homothallic culture.

Table 1
CHARACTERISTICS OF BASIDIOSPORES AND BASIDIOSPORE PROGENY FROM T. CUCUMERIS CRUCIFER ISOLATE 48

<table>
<thead>
<tr>
<th>Generation</th>
<th>Isolation No.</th>
<th>No. of Four-spored Basidia* out of 100 (%)</th>
<th>Basidiospores</th>
<th>Basidiospore Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. Uninnucleate out of 400 (%)</td>
<td>No. Viable out of 200 (%)</td>
</tr>
<tr>
<td>P</td>
<td>48</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>G1</td>
<td>48–11</td>
<td>100</td>
<td>–</td>
<td>30†</td>
</tr>
<tr>
<td>G2</td>
<td>48–14</td>
<td>97</td>
<td>–</td>
<td>82</td>
</tr>
<tr>
<td>G3</td>
<td>48–56</td>
<td>100</td>
<td>99</td>
<td>99</td>
</tr>
</tbody>
</table>

* Remainder were five-spored basidia. † 48–11 count of 100.

The successive generations of progeny of isolate 48 provided an opportunity of studying anastomosis reactions between closely related cultures including the progeny of a homokaryotic, homothallic culture. The results of these anastomosis studies are outlined in the following scheme:
From these results it appears that completely successful anastomosis occurs between a homokaryotic parent and its progeny or between the progeny. Between other closely related isolates which are not genetically identical some killing reaction occurred except between 48 and the 48–11 prebasidial cell isolates. There is no evidence from this study to indicate whether the killing reaction would completely prevent heterokaryon formation.

(c) Isolate 69

Successive generations of single basidiospores were cultured and examined as for isolate 48. Cultural variation and sectoring was much greater in G1 cultures than had been the case in isolate 48. Also a greater percentage of G1 cultures than in the case of isolate 48 fruited immediately. Otherwise the results were similar to those obtained with isolate 48. Fertility increased with each successive generation, although some G2 progeny produced basidia but no spores and others which produced basidia and spores completely failed to shed the spores. However, two G2 single-spore cultures selected for further study produced basidiospores which were 99% uni-nucleate and the resulting G3 single-basidiospore cultures from each parent were identical in cultural appearance and uniformly fertile.

It was concluded that these two G2 single-spore cultures were homothallic and homokaryotic.

(d) Isolate 16

The G1 single-basidiospore cultures of this isolate showed comparable variation and occurrence of sectors to those in isolate 69. This general variation is attributed to the heterokaryotic condition of the parent isolate, but is probably enhanced by the heterokaryotic condition of a small percentage of the progeny which develop from binucleate spores—12% are binucleate owing to migration of two nuclei from the basidium (Flentje and Stretton 1964).
When the G1 progeny were tested singly for ability to fruit, 27% produced basidia and spores. Culture 16–8 was selected, and on fruiting, produced two distinct cultural types, so-called "smooth" and "ringed". This suggests that the 16–8 culture may have originated from a binucleate spore. Of the G2 progeny 45% established colonies. When one of these G2 cultures obtained from a tetrad 16–8–28c was fruiting and the progeny analysed, the G3 cultural variation was also wide. The procedure was repeated through to G5, where single-basidiospore cultures continued to exhibit wide variation. The viability of spores decreased and self-fertility remained low. A relatively high percentage of binucleate spores persisted throughout each generation. Cytological studies revealed that only 50–60% of the basidia in each generation produced four spores per basidium, the remaining basidia producing only three spores. These results are set out in Table 2. Generally, this isolate appears to be much more variable than those previously discussed, and even after five generations of selfing, a homokaryotic line was not produced. Other G1 cultures were investigated, but the results were even less satisfactory as percentage fertility decreased in subsequent generations and variation among progeny was as great as in the 16–8 line.

IV. Discussion

The above studies on four isolates of T. cucumeris give evidence that homothallism may be a feature of the "solani" type within this species. Fertile homothallic homokaryotic lines were selected from each of the isolates 82, 48, and 69.

However, there appear to be significant differences between these isolates with respect to fertility. Isolate 82 is stable and uniformly self-fertile in that almost 100% of the G1 cultures fruited readily. Isolates 48 and 69 appear to contain sterility factors which segregate through the sexual stage, resulting in a high percentage of non-fertile progeny and a masking of the homothallic nature. In this investigation selections were made away from the sterility factors to obtain fertile homothallic homokaryotic cultures. The fruiting of 48–11 in one localized area after months of infertility and the prolific fruiting of all prebasidial cell cultures derived from the localized hymenium, is evidence that a mutation occurred which in some way overcame the sterility factors. The fact that subcultures from the original 48–11 failed to fruit under the same conditions as the prebasidial cell cultures discounts the likelihood that fruiting was simply a matter of environmental difference. Also the fact that only 83% of the G2 cultures fruited over several experiments suggests that segregation of sterility factors occurred in this generation. It might well have been expected, however, that 48–11–14 would prove to be a fertile homothallic, homokaryotic culture. It anastomosed successfully with its progeny and the progeny anastomosed successfully with each other. However, only 94% of these progeny fruited, indicating there was still some variation in fertility. This could be explained on the basis either that 48–11–14 came from a binucleate spore or more likely that a mutation had occurred in the culture before it was fruited. It also seems likely that 48–4, which fruited readily at first, was a fertile homothallic homokaryotic culture, but that a mutation later occurred causing the blockage of subsequent basidium formation.
The pattern of development and selection in isolate 69 was similar to that for isolate 48, but fertile, homothallic, homokaryotic cultures were obtained a generation earlier than in isolate 48.

The results with isolate 16 are more difficult to interpret because of the occurrence in successive generations of 10% or more of binucleate spores and spore viability of less than 50%. If the viability of binucleate spores is considerably greater than that of uninucleate spores the percentage fertility in each generation, except the fourth, could be attributed wholly to secondary homothallism and this could explain the wide variation between progeny within each successive generation. Alternatively, there may be a much higher mutation rate in the nuclei of this isolate than in those of the other three isolates. Although there is no conclusive evidence, we would suggest that isolate 16 is basically homothallic, but is less stable than isolates 48 and 69 with a greater occurrence of sterility factors. This together with the high percentage of binucleate spores and low percentage spore viability may have prevented selection of a fertile, homothallic, homokaryotic line. The behaviour of isolate 16, however, should not be regarded as typical of all “solani” type isolates which attack roots. Subsequent investigations in this laboratory (Flentje, unpublished data) with another root-attacking isolate have yielded a fertile, homothallic, homokaryotic culture.

It appears likely, then, from the above results that homothallism may be common in T. cucumeris as we have no evidence of mating-type nuclei. In view of this, although critical cytological data are missing, it is likely that the isolates used by earlier workers were in fact homothallic. Nevertheless if T. cucumeris is accepted as a collective species it would be unsatisfactory to regard the species as a whole as homothallic on the basis of the limited number of isolates so far investigated.

The study of anastomosis between related progeny from isolate 48 shows that while cultures which are identical genetically will anastomose successfully, anastomosis between other closely related but not identical progeny frequently results in the death of participating and neighbouring cells. It is important, then, to determine whether this reaction prevents heterokaryon formation. If it does, it may represent a most important mechanism for biological isolation of any new strains which occur.

V. Acknowledgments

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VI. References


