Reversible Change of Mating Type in *Phytophthora parasitica*

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(Received 17 November 1980; revised 17 February 1981)

In addition to the original mating type, the opposite mating type appeared in the zoospore population of single isolates of *Phytophthora parasitica* after long-term storage or chloroneb treatment. Occasionally self-fertile zoospores were also detected, but their self-induction nature was transitory and unstable. Both hormone production and hormone reception were changed in the sexual variants. The results explain the apparent change from cross-induction ('heterothallic') to self-induction ('homothallic') in *P. parasitica* after long-term storage and chloroneb treatment, and suggest one possible evolutionary origin of sex in the lower fungi.

**INTRODUCTION**

Previous reports (Brasier, 1972; Kamjaipai & Ui, 1978; Tsao et al., 1980) showed that after long-term storage, certain isolates of cross-inducing ('heterothallic') species of *Phytophthora* behaved like a self-inducing ('homothallic') type by producing oospores in sectors or around the initial inocula in single cultures. A similar phenomenon was observed when the A², but not A¹, mating type of *Phytophthora capsici* was grown for 21 d on agar medium containing chloroneb (Noon & Hickman, 1974). This paper reports the isolation of the opposite mating types from aged or chloroneb-treated *Phytophthora parasitica* and provides an explanation for the apparent change from cross-induction to self-induction after long-term storage and chloroneb treatment.

**METHODS**

**Organisms.** Isolates P991 and P731 of *Phytophthora parasitica* Dast. were supplied by G. A. Zentmyer, and isolates P174 and P191 by M. Aragaki. These isolates were selected for study because of their ability to produce self-fertile sectors on subcultures after treatment.

**Single propagule isolation and mating type determination.** Sporangia of *Phytophthora parasitica* were induced by incubating pieces (about 10 x 5 x 2 mm) of agar culture for 2 d in 20 ml sterile distilled water in a Petri plate at 25 °C under fluorescent light. Zoospores were released when the sporangia were chilled at 5 °C for 15 min. Zoospores were induced to encyst by agitating zoospore suspensions in a test tube for 1 min with a Vortex mixer. About 0.2 ml of encysted zoospore suspension (about 100–300 zoospores ml⁻¹) was placed on 2% (w/v) Bacto agar (Difco) in a Petri plate and incubated at 25 °C for 2 d. Only those colonies originating from single zoospores were transferred to V-8 agar plates [10% (v/v) V-8 juice, 0-02% (w/v) CaCO₃ and 2% Bacto-agar] and incubated at 25 °C for 2 d. Only those colonies originating from single zoospores were transferred to V-8 agar plates [10% (v/v) V-8 juice, 0-02% (w/v) CaCO₃ and 2% Bacto-agar]. Five colonies were evenly distributed around the edge of each plate. After 2 d, the mating type of each single-zoospore isolate was determined by pairing with an A¹ type (P991) or an A² type (P731) of *P. parasitica* on V-8 agar plates. To detect the presence of self-inducing isolates (A¹A²), culture blocks (15 x 10 x 2 mm) of individual isolates were incubated for 4 d in sterile Petri plates.

For hyphal tip culture, single hyphal tips were cut from the margin of young colonies in 2% Bacto agar and transferred to V-8 agar plates. The mating type of each single hyphal-tip culture was determined as described above.
RESULTS

Effect of long-term storage

Isolates P991 (A'), P174 (A') and P191 (A2) of P. parasitica that had been stored in distilled water or V-8 agar in test tubes for 24–26 months produced oospores in sectors when grown on V-8 agar for 6 d. All except two single-zoospore cultures obtained from oospore sectors were self-sterile, behaving as A' or A2, but none of them were neuter (A0) (Table 1). In addition to the original mating type, the opposite mating type also appeared in the zoospore population of both A' and A2 isolates tested after long-term storage. The two exceptions, both from A' isolate P991, were self-fertile (A'A2), forming oospores in sectors, but their self-induction nature was transitory and unstable. After two transfers to V-8 agar, both became self-sterile and behaved as an A' mating type. When single-zoospore cultures from oospore sectors of these two self-fertile isolates were analysed, all 49 cultures from one isolate were found to be A' type. The other isolate produced 49 A' zoospores and 1 A'A2 zoospore. The A'A2 character of the latter was again unstable: the culture gave rise to 9 A' single hyphal-tip cultures and 1 A2 single hyphal-tip culture. Two single-zoospore isolates derived from an aged culture of P174 (A') were examined, namely P174-SZ1 (parental type: A') and P174-SZ15 (sexual variant: A2). Without treatment, these two isolates gave only A' or A2 types, respectively (Table 1).

Origin of mating type change

To determine when the change of mating type occurs, a piece of agar culture (5 x 5 x 2 mm) of isolate P174 (A') stored in distilled water in a test tube for 25 months was placed in a sterile Petri plate, covered with a sterile cover slide and pressed gently. The broken pieces of agar culture were mixed with 1 ml sterile distilled water and spread on five plates of 2% Bacto water agar. The origin of each colony was observed under a microscope after 2 d at 25 °C. Most colonies originated from small spherical propagules (12–16 μm diam.) similar to encysted zoospores. Some colonies originated from sporangia. Colonies originating from single propagules were transferred to V-8 agar plates, and the mating type of each colony was determined. Among 11 isolates obtained, 3 were the original A', 4 were A2 and the other 4 were A1A2 type. The A1A2 isolates were again transitory, and all 10 single hyphal-tip cultures from each isolate were self-sterile and behaved as A' type. These results show that change of sexuality from A' to A2 type and vice versa occurs during the storage period.

Effect of chloroneb treatment

The method of Noon & Hickman (1974) was used to prepare medium containing chloroneb (1,4-dichloro-2,5-dimethoxybenzene, 88-6% active). Isolates P731 (A2) and P991-SZ18 (A2) of P. parasitica produced oospores in sectors on V-8 agar supplemented with 5 p.p.m. chloroneb in 14 d. The latter isolate was a single-zoospore isolate representing the sexual variant from aged isolate P991 (A'). Both isolates remained self-fertile after being transferred to V-8 agar without chloroneb. Single-zoospore cultures were obtained from oospore sectors and the mating type of each culture was determined. Both isolates gave rise to the new A1 mating type in addition to the parental A2 type (Table 1). The only A1A2 culture from isolate P991-SZ18 was unstable like those described above: all 10 single hyphal-tip cultures derived from it were self-sterile and behaved as A2 type. These results show that the A2 mating type of some P. parasitica isolates can be converted to A' type by chloroneb in a relatively short period of time.

Hormone production and reception in sexual variants

Previous reports (Ko, 1978, 1980) showed that the A' mating type of P. parasitica is capable of inducing sexual reproduction of the A2 mating type (production of hormone α')
Table 1. Mating type distribution in single-zoospore cultures of Phytophthora parasitica isolates after long-term storage or chloroneb treatment

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Mating type</th>
<th>Treatment</th>
<th>Total scored</th>
<th>Mating type</th>
</tr>
</thead>
<tbody>
<tr>
<td>P991</td>
<td>A¹</td>
<td>Stored in water for 26 months</td>
<td>50</td>
<td>A¹</td>
</tr>
<tr>
<td>P174</td>
<td>A¹</td>
<td>Stored in water for 25 months</td>
<td>48</td>
<td>A¹</td>
</tr>
<tr>
<td>P191</td>
<td>A²</td>
<td>Stored in V-8 agar for 24 months</td>
<td>50</td>
<td>A¹</td>
</tr>
<tr>
<td>P731</td>
<td>A²</td>
<td>Grown on chloroneb medium for 14 d</td>
<td>44</td>
<td>A²</td>
</tr>
<tr>
<td>P991-SZ18*</td>
<td>A²</td>
<td>Grown on chloroneb medium for 14 d</td>
<td>50</td>
<td>A¹</td>
</tr>
<tr>
<td>P174-SZ1*</td>
<td>A¹</td>
<td>None</td>
<td>56</td>
<td>A¹</td>
</tr>
<tr>
<td>P174-SZ15*</td>
<td>A²</td>
<td>None</td>
<td>50</td>
<td>A²</td>
</tr>
</tbody>
</table>

*Isolate P991-SZ18 and isolates P174-SZ1 and P174-SZ15 were single-zoospore isolates recently derived from aged cultures of isolates P991 and P174, respectively.

and forming oospores after being stimulated by an A² mating type (presence of hormone α² receptor), and vice versa. To determine if both hormone production and reception were changed in the sexual variants induced by long-term storage or chloroneb treatment, each parent isolate and sexual variant was mated with an A¹ mating type (P991) and an A² mating type (P731) of P. parasitica, using the polycarbonate membrane technique (Ko, 1978). The results showed that both hormone production and reception were changed in every sexual variant. For example, the parent isolate of P174 (A¹) was able to stimulate and be stimulated by A² but not A¹ mating type to form oospores, while its sexual variant (A²) could stimulate and be stimulated only by A¹ type. The new mating type derived from a parental culture was, therefore, similar in its hormone production and reception to a wild type of that new mating type.

**Interaction between A¹ and A² mating types**

Parent isolate P174-SZ1 (A¹) and its sexual variant P174-SZ15 (A²) were used to study their interaction in a mixed culture. A block (about 6×10×2 mm) of P174-SZ1 culture with a small piece (about 1×1×1 mm) of P174-SZ15 culture embedded in the centre was placed on the centre of a V-8 agar plate. Oospores were formed only underneath the inoculum. When the oospore-forming area (about 3×3×2 mm) was transferred to a fresh V-8 agar plate, no oospores were formed in 5 d and the culture behaved as an A¹ mating type. When the small piece of P174-SZ15 culture was placed on the edge of a P174-SZ1 culture block, an oospore sector was formed on the V-8 agar plate near the inoculum of P174-SZ15 in 5 d. When the oospore-forming area was transferred to a fresh V-8 agar plate, oospores were formed in sectors in the second transfer but not in the third transfer. The culture of the third transfer behaved as an A¹ mating type in one test. In another test, an oospore sector was formed in the culture of the third transfer; however, the mycelia obtained from the edge of the colony were self-sterile and behaved as an A² mating type.

**DISCUSSION**

The appearance of a new mating type explains the apparent change from cross-induction to self-induction in subcultures after long-term storage and chloroneb treatment of P. parasitica cultures. This may also explain, at least in part, the appearance of oospores in certain old cultures of cross-inducing species of Phytophthora (Ashby, 1929; Chee & Newhook, 1965; Kreutzer et al., 1940; Royle & Hickman, 1964; Savage et al., 1968; Stamps, 1953; Tucker, 1931). Brasier (1972) showed that the ability of aged cultures of certain self-sterile isolates of Phytophthora to form oospores around the inoculum or in sectors in subcultures usually
disappeared after one or two transfers. Such oospore-forming ability could be extended if inoculum was obtained from an area containing oospores (Savage et al., 1968; Tsao et al., 1980). A similar phenomenon was observed in this study when the A\textsuperscript{1} and A\textsuperscript{2} mating types of 

P. parasitica were grown on the same plate in different ratios.

The fact that an A\textsuperscript{2} type (P991-SZ18) that was derived from an A\textsuperscript{1} type (P991) through ageing gave rise to A\textsuperscript{1} type zoospores after chloroneb treatment clearly demonstrates the reversibility of mating type change in P. parasitica. It is suggested that the unstable A\textsuperscript{1}A\textsuperscript{2} type is a transitional state in the process of change in both directions. The behaviour of the A\textsuperscript{1}A\textsuperscript{2} type was similar to that of certain self-inducing cultures of Phytophthora drechsleri that originated from germinated oospores (Mortimer et al., 1977). The latter were also unstable, giving rise to the A\textsuperscript{1} or A\textsuperscript{2} mating type, and occasionally the A\textsuperscript{1}A\textsuperscript{2} type.

Both hormone production and hormone reception were changed in all sexual variants resulting from long-term storage and chloroneb treatment that were tested. This suggests the possibility that production of hormone $\alpha$\textsuperscript{1} and the existence of hormone $\alpha$\textsuperscript{2} receptor in A\textsuperscript{1} mating type of P. parasitica are controlled by two linked genes (P'R\textsuperscript{2}), and that production of hormone $\alpha$\textsuperscript{2} and the possession of hormone $\alpha$\textsuperscript{1} receptor in A\textsuperscript{2} mating type are controlled by another linked pair (P'R\textsuperscript{1}). To account for the reversible conversion of mating types, it is postulated that the transcription of such linked genes is regulated by a repressor which represses the expression of A\textsuperscript{1} mating type (P'R\textsuperscript{2}) with one molecular configuration, and A\textsuperscript{2} type (P'R\textsuperscript{1}) with another configuration. Based on this hypothesis, ageing and chloroneb treatment would have changed the mating type of P. parasitica through alteration of the molecular configuration of the repressor.

Initiation of sexual reproduction in a population of a self-sterile species by the transformation of certain individuals to an opposite mating type, as shown in this work, is suggested as a possible evolutionary origin of sex in lower fungi.

The author gratefully acknowledges a generous gift of chloroneb from E. I. DuPont de Nemours & Co., Wilmington, Delaware, U.S.A. Journal Series Paper No. 2561 of the Hawaii Institute of Tropical Agriculture and Human Resources.

REFERENCES


