

Intra-Isolate Heterokaryosis in *Pyricularia oryzae*

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ABSTRACT

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Conidial progeny of some isolates of *Pyricularia oryzae* differing in colony morphology and/or color produced heterokaryons on polished rice agar plus rose bengal when compatible progeny of the same isolate were paired. Heterokaryotic hyphae appeared as a solid line at the point of contact, consisting of mycelial tufts. A mycelial fragment was taken from each of many tufts and hyphal-tip isolates were obtained from the resulting colonies. Fifty or more monoconidial isolates were obtained from each hyphal-tip isolate.

Additional key words: rice blast, genetic variation.

Both parental types and some nonparental types were recovered in most instances, demonstrating that the mycelial tufts were heterokaryotic. Three of six isolates tested produced heterokaryons including isolate 301 (Race IG-1, Texas), isolate 310 (Race IC-17, Pakistan), and isolate 314. Heterokaryons were formed at a frequency of 0.75, 3.2, and 15.4% for isolates 301, 310, and 314, respectively. Conidial progeny of isolates 304 (Race IG-1, Louisiana), 307 (Peru), and 351 (Race ID-13, Texas) did not produce heterokaryons.

The term heterokaryosis has been used to cover all conditions in which two or more genetically different nuclei are associated in a common cytoplasm (1). Heterokaryosis confers on a haploid organism many of the advantages of heterozygosity enjoyed by a diploid.

Biochemically deficient mutants frequently have been used to force heterokaryon formation, although the use of such artificial pressure is thought to have little reference to nature (1, 6). The use of naturally occurring markers or differences among isolates may be more appropriate than using auxotrophs in studying the phenomenon of heterokaryosis.

Blast, which is caused by *Pyricularia oryzae* Cav., is one of the most important diseases of rice and occurs in almost all countries where rice is grown. The world-wide importance of the disease is due to the occurrence of numerous races of the pathogen. Heterokaryosis is assumed to be an important factor contributing to the immense variation within the pathogen.

Suzuki (12, 13) first reported heterokaryosis in *P. oryzae*. He used an appressorial type as a genetic marker for investigating what he called persistent heterokaryosis. He reported that conidia, appressoria, and mycelial cells are in a persistent heterokaryotic state. In addition, he reported that conidial and mycelial cells were multinucleate. However, recent investigations have shown that mycelial and conidial cells are uninucleate (5, 15, 16). Consequently, cultures originating from single conidia are considered to be genetically pure (15, 16). Based on these findings Suzuki's proof of heterokaryosis may not be generally acceptable.

Yamasaki and Niizeki (16) used auxotrophic strains, some differing also in colony morphology, to synthesize heterokaryons. They recovered parental types and new types, including diploids, and, in one case, 69.0% of the single spores isolated differed from either parent. Genovesi (3) synthesized heterokaryons between auxotrophs derived from the same strain. He reported that the occurrence of a high number of prototrophic colonies arising from individual conidia suggested that diploids occur frequently.

This investigation was undertaken to find a simple method for the synthesis and detection of heterokaryons of *P. oryzae* in vitro and to determine the potential and frequency of heterokaryon formation among progeny of the same isolate.

MATERIALS AND METHODS

Single-spore progeny of six isolates representing several races were used in this investigation. Isolates 301 (Race IG-1, Texas), 304 (Race IG-1, Louisiana), 307 (Peru), and 310 (Race IC-17, Pakistan) were obtained from T. T. Hebert of the North Carolina State University. Isolate 314 was obtained from F. M. Latterell, Frederick, Maryland, and isolate 351 (Race ID-13, Arkansas) from M. A. Marchetti, Beaumont, Texas. Single-spore cultures were maintained on potato-dextrose agar (PDA) in test tubes and petri dishes during the course of the investigation.

Thirty monoconidial cultures were obtained from each of the six field isolates. There were distinctive differences among the monoconidial cultures in colony morphology and/or color, which will be illustrated in a subsequent

Figure. These differences suggested that the field isolates were heterokaryotic. Monoconidial isolates derived from monoconidial cultures essentially were indistinguishable from one another, further suggesting that the monoconidial cultures were homokaryotic.

Several pairings between monoconidial cultures from the same isolate were made initially on nine different media in search of suitable substrates for the synthesis of heterokaryons. Pairings were made by placing small pieces of the cultures on opposite sides of a petri dish containing the medium. Inoculated plates were placed in brown paper bags and incubated for two weeks at room temperature (21 ± 1 C). Certain pairings of cultures on polished rice agar plus rose bengal (polished rice, 20 g; agar, 15 g; rose bengal, 33 mg; and distilled water, 1 liter) produced a solid line of hyphae in the form of mycelial tufts (Fig. 1). A small mycelial fragment from the tufted zone was transferred to petri dishes containing PDA. After some growth, hyphal-tip transfers were made to petri dishes containing PDA. When the hyphal-tip cultures were sporulating, a minimum of 50 single conidial cultures were isolated and plated on PDA in petri dishes. The resulting cultures were compared with the two "parental" isolates that had produced the tufted reaction. We believed that we would have demonstrated the phenomenon of heterokaryosis if different monoconidial isolates from hyphal-tip cultures were representative of both "parental" types and/or if new colony morphologies were recovered.

RESULTS

The 30 monoconidial cultures obtained from each of the six field isolates were paired among themselves in all of the possible 435 combinations, resulting in 2,610 pairings (435 for each of the six isolates).

Certain paired combinations of monoconidial cultures derived from field isolates 301, 310, and 314 produced the tufted reaction suggestive of heterokaryon formation. The tufted reaction was observed in 0.68, 3.2, and 15.4 percent of the 435 pairings for isolates 301, 310, and 314, respectively. Progeny from isolates 304, 307, and 351

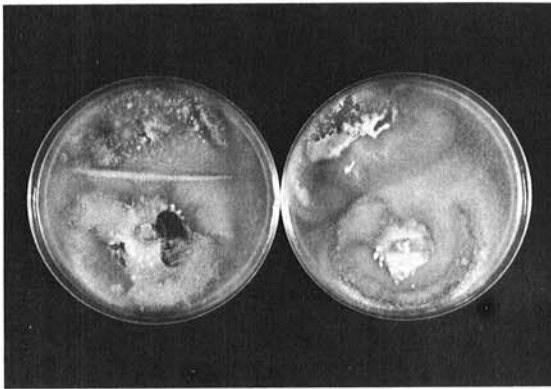


Fig. 1. Positive heterokaryon reaction between isolates of *Pyricularia oryzae* is shown in the left culture dish. No observable interaction occurred between paired isolates on the right.

failed to produce mycelial tufts.

Mycelial fragments were transferred from numerous tufts produced in pairings of isolates exhibiting distinct differences in colony morphology and/or color. Hyphal-tip isolates were obtained from the resulting colonies and a minimum of 50 monoconidial isolates were obtained from each hyphal-tip isolate.

Inspection of the colony types of the individual sets of 50 or more monoconidial isolates derived from hyphal-tip cultures revealed a number of different results. In most cases, both parental types were recovered with varying frequencies of one parental type. In a few instances, one or both parental types and one or more nonparental types were recovered (Fig. 2). Only one parental type was recovered in a very few cases. The colony morphology of all monoconidial isolates tested was not recorded. We were interested only in the general presence of parental and nonparental colony types within each set of 50 or more monoconidial isolates, since our sole objective was to determine whether we could demonstrate intra-isolate heterokaryosis in *P. oryzae*.

Two types of behavior were observed between paired isolates that did not produce mycelial tufts at points of contact. A zone of inhibition occurred in some pairings in which no hyphae were observed. In other pairings, the hyphae of both isolates intermingled with no noticeable reaction. No anastomosis between these isolates was observed. When mycelial fragments were taken from the zone of contact and processed as previously described, all monoconidial isolates were of one parental type.

Anastomosis was observed frequently between hyphae originating from the two opposed parental colonies in pairings that produced the tuft reaction. However, anas-

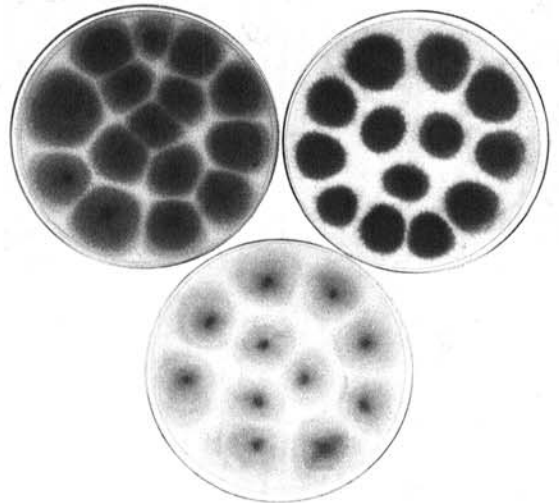


Fig. 2. Two parental types (above) and a nonparental type (below) obtained by taking a mycelial fragment from a mycelial tuft produced in a pairing of two parental isolates of *Pyricularia oryzae*. Hyphal-tip isolates were obtained from the colony produced from the mycelial fragment and monoconidial isolates were obtained from the hyphal-tip cultures. Each small colony represents a monoconidial isolate.

tomosis in itself is not sufficient proof of heterokaryosis since hyphae of the same mating type sometimes anastomose.

Supportive evidence for heterokaryosis was found in the fact that colonies originating from a mycelial fragment taken from the mycelial tufts frequently were different from either parental type.

DISCUSSION

This investigation indicated that naturally occurring differences in colony morphology may be used as markers to synthesize heterokaryons between monoconidial cultures of *P. oryzae*. Heterokaryons can be detected on a medium on which both homokaryons are able to grow.

Although our research suggests that the mycelial tufts formed at the point of interaction between isolates are heterokaryotic, cultures derived from the heterokaryon occasionally are representative of a single morphological type. This phenomenon might reflect uneven distribution of the nuclei as reported for *Verticillium* spp. (9, 10) which also have uninucleate cells as in *P. oryzae*. Some part of the heterokaryon may contain a single nuclear type and thus a single genetic component is recovered when hyphae are cut and conidia isolated. A second explanation may be the instability of the heterokaryon as reported by Garnjobst (2) and Hastie (7, 8). Sanderson and Srb (11) reported that 44% of hyphal tips isolated from a heterokaryon of *Ascochyta imperfecta* did not grow on minimal medium. Heterokaryotic strains of *F. oxysporum* f. *pisi* also yielded spores bearing one or predominantly one of the component strains (14). Both of these findings lend some support to either or both of our tentative explanations.

Although our studies suggested that progeny of some races of *P. oryzae* produce heterokaryons when mated among themselves, as reported by Genovesi (3), the frequency varies considerably among races. Frequency of heterokaryon formation is especially important in fungi such as *Verticillium* and *Pyricularia* spp. in which the homokaryotic condition is perpetuated by formation of homokaryotic conidia. The heterokaryotic condition apparently is established by anastomosis between homokaryons and formation of new heterokaryons. The fact that conidia isolated from a single field isolate can be categorized into several pathological (4) and morphological groups supports the belief that heterokaryosis is operative in *P. oryzae*.

The existence of some races whose conidial progeny do not form heterokaryons among themselves (isolate 304), whereas those of the same race from a different location do (isolate 301), suggests the genetic complexity of races. It is possible that all single conidial isolates of isolate 304 were of the same compatibility type or that some field isolates are homokaryotic.

The initiation of the heterokaryotic condition in races

the progeny of which do not form heterokaryons in intra-isolate matings could be accomplished by mutation and perpetuation of the new nucleus or by formation of heterokaryons with progeny of another race. Studies to determine the potential and frequency of inter-isolate heterokaryon formation in *P. oryzae* are in progress.

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