

A Medium for Heterokaryon Formation in *Rhizoctonia solani*

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ABSTRACT

The formation of heterokaryons by *Rhizoctonia solani* was greatly enhanced by pairing compatible isolates on potato-dextrose agar containing 1% charcoal. On this medium, a thick, dense zone of heterokaryotic hyphae consistently formed between colonies. Thus, pairings between homokaryons, once thought to be incompatible, were found to be compatible.

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The pairing of compatible homokaryons of the praticola-type of *Rhizoctonia solani* Kuehn [= *Thanatephorus cucumeris* (Frank) Donk] leads to the formation of heterokaryotic hyphae at the juncture where the two mycelia meet (1, 4, 5). This phenomenon was first shown by Whitney & Parmeter (5) on water agar; they reported that ".....individual mycelial tufts were formed at the line of contact". The tufts were small patches of heterokaryotic hyphae sparsely scattered at the juncture of the colonies. In our experiments, water agar was found to be unsatisfactory as a substrate for heterokaryon formation; the results were erratic and the amount of heterokaryotic mycelium was very sparse. Other workers (4, 5) have employed different natural and synthetic media for the formation of heterokaryons in *R. solani*; however, there are no data to indicate the reliability of any of these media. In this connection we have employed potato-dextrose agar (PDA) and asparagine-dextrose agar (AGA) and observed patterns of tuft formation similar to those on water agar.

Recently, we obtained outstanding results using PDA containing 1% charcoal (grade G 60, Atlas Powder Co., Wilmington, Del.). PDA was prepared from the aqueous extract of 200 g potato, 10 g glucose, and distilled water to make 1 liter; charcoal was added prior to autoclaving at 121 C for 20 min. On this medium, very striking zones of heterokaryotic mycelium developed where compatible colonies met, and the isolated tufts characteristically formed on other media were absent (Fig. 1). More than 100 different combinations of compatible homokaryons were mated, including material from different geographical areas. All matings produced heterokaryotic hyphae in less time than required on PDA, AGA, or water agar, but the most significant observations were that: (i) a thick, dense zone of heterokaryotic hyphae (Fig. 1) formed between colonies instead of the scattered, sparse, tufts of mycelium formed on other media; (ii) the results of pairing homokaryons were consistently

repeatable. Charcoal-PDA is at present the most reliable medium for indicating compatibility between homokaryons of *R. solani*. In this regard, we have observed that some combinations of homokaryons form heterokaryotic hyphae on this medium, but not on water agar, PDA, or AGA. When two incompatible isolates are paired on PDA-charcoal medium, colonies meet without any production of whitish heterokaryotic mycelium (Fig. 1).

To obtain proof that the hyphae formed at the juncture of two compatible homokaryons were heterokaryotic, we transferred potentially heterokaryotic mycelium to water agar, allowed it to grow 24 hr, then made 20 hyphal tip transfers to PDA. Each of the colonies, presumably heterokaryons, arising from the hyphal tips were morphologically different from either parent. One was induced to sporulate and 100 single basidiospores were isolated; parental phenotypes and the two parental mating types were recovered from the progeny.

Charcoal medium was previously used by Day & Anagnostakis (2, 3) to induce the dikaryon in *Ustilago maydis*; they suggested that charcoal may remove a substance which ordinarily produces a yeastlike growth (3). Factors leading to the dikaryon of *U. maydis* may be quite different from factors controlling heterokaryon formation in *R. solani*. Nevertheless, the most obvious explanation for the action of charcoal is that it removes substances inhibitory to the formation of the heterokaryon. In any case, the use of charcoal-PDA may lead to valid data from which the potential role of heterokaryosis in *R. solani* can be evaluated.

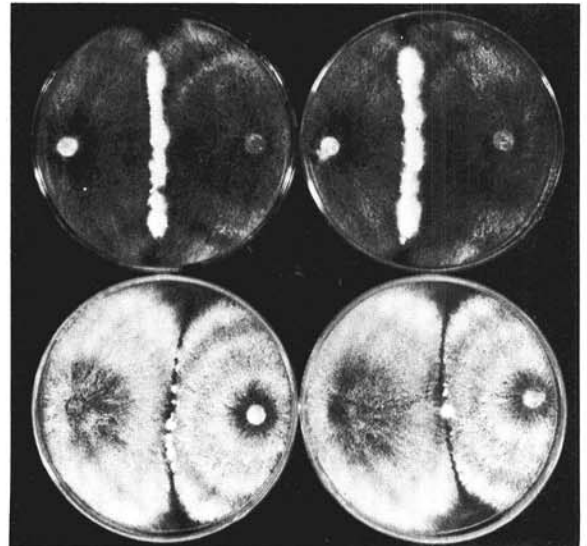


Fig. 1. *Rhizoctonia solani*. Formation of heterokaryotic mycelium at the line of contact between two different pairs of compatible homokaryons. Top row: potato-dextrose agar (PDA) + 1% charcoal. Bottom row: PDA without charcoal.

LITERATURE CITED

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