

Use of Mycelial Suspension for Increasing
Puccinia graminis f. sp. *tritici* in
Axenic Culture

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ABSTRACT

Axenic cultures of *Puccinia graminis* f. sp. *tritici* race 126-ANZ-6,7 were initiated using suspensions of mycelium prepared by blending stroma of the fungus in a liquid culture medium. Over a 5-month period, cultures derived from one 50-mg section of fungal stroma yielded 1,325 vigorously growing colonies. The fungus was capable of infecting wheat plants after three successive subcultures using mycelial suspensions. Phytopathology 63:653-654

The establishment of axenic cultures of *Puccinia graminis* (Pers.) f. sp. *tritici* Eriks & E. Henn (1, 5, 6) has facilitated investigations of the physiology and nutrition of this pathogen (2, 4). However, these studies have been limited by the length of time required to increase these cultures by sectioning and subculturing stromata of the fungus. In order to establish large numbers of uniform-aged colonies, we have investigated the potential of using aseptically prepared mycelial suspensions for increasing the fungus in axenic culture.

MATERIALS AND METHODS.—Approximately 50 mg of stroma from axenic cultures of *P. graminis* f. sp. *tritici* race 126-ANZ-6,7 were blended in a 50 ml container for 30 sec using a Sorvall Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.). Twenty-five ml of modified Czapek-Dox salts (3) containing 3% glucose and 0.6% vitamin-free casamino acids (GCA medium) was used as the blending medium. One-ml portions of the resulting mycelial suspension were transferred to 9-cm petri dishes containing GCA medium solidified with 1% agar. These "seed cultures" were sealed with paper tape and incubated in darkness at 16 C. At 30-day intervals, seed cultures were opened and all colonies over 1 mm in diam were transferred, three per dish, to 5 cm petri dishes containing GCA medium, sealed, and incubated as above. Sixty days after the above transfer, the small dishes were opened and fresh weights were obtained by transferring the colonies directly to an analytical balance.

For comparison of growth rates and yields, 50-mg sections from the same stromas used for blending were transferred directly to the agar medium and subcultured by sectioning the colonies every 30 days.

Two tests for growth in liquid culture were also

performed. One-ml quantities of mycelial suspension were inoculated into 25 ml of GCA liquid medium in 125 ml DeLong culture flasks and incubated as above. Also, a 25-ml portion of mycelial suspension was inoculated into 3 liters of liquid medium in a 5-liter vessel in a MicroFerm laboratory fermenter (New Brunswick Scientific Co., New Brunswick, N.J.). The fermenter was operated at 16 C and stirred at 200 rpm with an aeration rate of 2.5 liters per min. Still liquid cultures were evaluated visually, and fresh weight of the mycelium produced in the fermenter was taken 3 weeks after initiation of the culture.

Colonies obtained after three successive subcultures from mycelial suspension were blended as above, and 0.5-ml portions were inoculated into wheat, *Triticum aestivum* L. 'Little Club' by injection into the center of the developing leaf roll. These plants were isolated from other infected wheat and maintained in a greenhouse with a 16-hr photoperiod for 4 weeks after inoculation.

RESULTS AND DISCUSSION.—Growth from mycelial fragments plated on agar medium was evident microscopically 4 to 8 days after inoculation and colony development was macroscopically evident in 2 to 3 weeks. Numerous colonies were formed in the seed cultures (Fig. 1). The developing colonies exhibited a wide range of morphology, from white, vegetative types with abundant aerial mycelium to compact, orange colonies, frequently bearing uredospores. Although most colonies developed on the agar surface, 2 to 5% of the colonies developed into the medium. An average of 30 colonies per month could be transferred from each seed culture

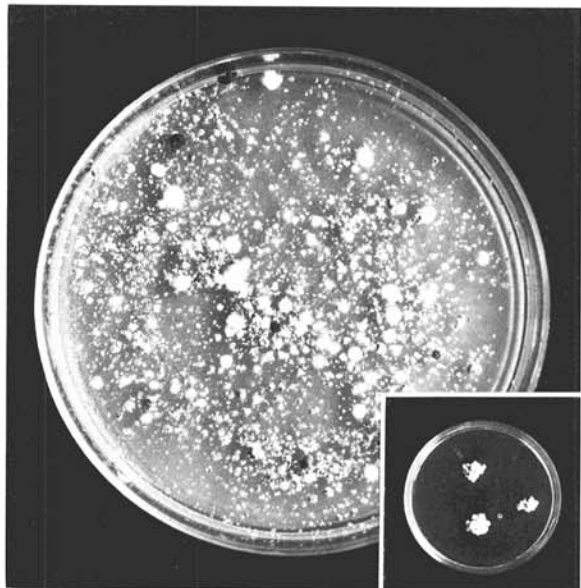


Fig. 1. "Seed culture" of *Puccinia graminis* var. *tritici* race 126-ANZ-6,7 60 days after initiation from a mycelial suspension. Insert: Three individual colonies 60 days after transfer from the seed culture.

TABLE 1. Colonies obtained by successive harvests of seed cultures of *Puccinia graminis* f. sp. *tritici*. The seed cultures were initiated with a mycelial suspension from a single stroma of the fungus

| Age of seed cultures (days) | No. seed cultures harvested | No. colonies transferred ^a |
|-----------------------------|-----------------------------|---------------------------------------|
| 30 | 12 | 380 |
| 60 | 11 ^b | 284 |
| 90 | 10 ^b | 264 |
| 120 | 10 | 270 |
| 150 | 10 | 127 |

^a All colonies over 1-mm diam were transferred.

^b One seed culture lost to contamination during previous 30 days.

for the first 4 months (Table 1). In the fifth month, colony yields declined to 13 per seed culture. Subsequently, yields were not considered sufficient to warrant further maintenance of the seed cultures.

Growth on agar medium was enhanced if the surface of the agar was dried thoroughly prior to application of the mycelial suspension. For maximum growth rates, the surface had to be sufficiently dry to absorb the water from the inoculum suspension in 2 to 4 hours. On media that had not been previously dried, acceptable yields eventually developed; however, new growth was not apparent until after the surface of the medium had dried (2 to 4 weeks).

Sixty days after transfer from seed cultures, individual colonies had an average fresh weight of 56 mg with a standard deviation of 22%. In one direct comparison, colonies harvested over a 5-month period from an initial suspension consisting of 50 mg mycelium produced 74.1 g fresh wt of mycelium, while a single stroma subcultured by sectioning yielded 2.1 g fresh wt.

In still liquid culture the fungus developed rapidly for 2 to 4 days, apparently by budding of the hyphal fragments. The cultures were then quiescent for 2 to 3 weeks, after which segments of compact mycelium became apparent in the liquid. These continued to develop for a further 4 to 6 weeks. After 12 weeks, colonies growing in liquid culture had developed to approximately one-third the size of colonies growing on agar. No further size increases were observed in liquid culture, but colonies transferred from liquid to solid medium at 12 weeks continued to grow, and

were morphologically indistinguishable from colonies initiated on agar. Growth in the fermenter was poor, producing an increase of only 28 mg fresh wt after 3 weeks. An equal volume of mycelial suspension inoculated into 25 agar plates had increased by 290 mg fresh wt in 3 weeks.

Inoculation of Little Club wheat with mycelial suspensions obtained after three subcultures using our methods resulted in leaf flecks appearing 15 days after inoculation. In another 5 days, these flecks developed into pustules containing typical uredospores of *P. graminis*. These inoculations resulted in only two to four pustules per plant; however, we feel that this is sufficient to demonstrate that cultures of *P. graminis* f. sp. *tritici* derived from mycelial suspensions retain their pathogenicity. The slow development of these pustules was probably because the mycelial fragments were placed on intact leaf surfaces rather than directly in contact with leaf mesophyll tissue (6).

We have now maintained cultures of race 126-ANZ-6,7 for more than 1 year (12 subcultures) without decline in either viability or growth rate of the fungus, and have successfully used these techniques with both fast- and slow-growing isolates of the fungus. These methods made it possible to rapidly increase axenic cultures of *P. graminis* f. sp. *tritici* and provide a source of uniform cultures for growth, nutrition, and physiology studies with this pathogen.

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