Improved Methods for the Axenic Culture of Puccinia recondita f. sp. tritici

S. A. Raymundo and H. C. Young, Jr.

Instructor and Professor, respectively, Department of Botany and Plant Pathology, Oklahoma State University, Stillwater 74074.

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ABSTRACT

Improved methods for obtaining axenic growth of *Puccinia recondita* f. sp. *tritici* are described. Details of techniques for production of sterile uredospore inoculum, seeding of culture medium, and maintenance and care of established cultures, are presented. The improved methods consistently yielded 75 to 90% successful seedings in each trial, compared to erratic results yielding 0 to 40% obtained with previous methods.

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A common observation by workers attempting axenic culture of rust fungi is the relatively low percentage of success and the divergent results between experiments performed under apparently identical conditions. Scott and Maclean (1) stated that some possible causes of these variations were differences in growth potential of germ tubes from different batches of spores, degree of asepsis in obtaining spores for inoculum, age of spores, and presence of inhibitors.

A study on axenic culture of the wheat leaf rust fungus, *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks. & Henn., following previously described methods (2) for culture of rust fungi resulted in a very low percentage of successful culture and poor growth. In addition, considerable variation was noted in the rate and extent of growth among some cultures that were seeded using spores from a common source. Modification of the methods used were tried in order to increase the number of successful cultures and to yield more consistent results. After several trials, the following variations in methods and procedures were developed. These methods and procedures increased the percentage of successful axenic cultures and gave more predictable results. More extensive, and markedly more differentiated, growth also resulted when these new methods were used.

Production of aseptic uredospore inoculum.—Detached wheat leaves infected with *P. recondita* to the point of visible flecks were dipped instantaneously into 75% ethyl alcohol. This was followed by suspending the leaves for 1 min in a solution containing 1.5 g $HgCl_2$ in 1 liter distilled water. They were then transferred to the surface of potato-dextrose agar (PDA) in petri dishes with the excised ends of the leaves slightly buried in the medium. Four leaves per plate were arranged side by side with the abaxial side in contact with the medium. These were incubated with continuous fluorescent light 5,380 lx (500 ft-c) inside a growth chamber (Percival Model E-30, Boone, Io.) until the uredosori broke through the leaf epidermis.

Seeding of medium.—Thirty ml of modified Czapek's Mineral Salt Medium (2 g $NaNO_3$, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 g KH_2PO_4, 10 mg $FeSO_4 \cdot 7H_2O$, 30 g Glucose, 4 g Evan's Peptone, 15 g Bacto-Difco Agar, plus glass-distilled water to 1 liter), autoclaved for 20 min at 121 C, and adjusted to pH 6, was dispensed in 125-ml Erlenmeyer flasks and allowed to congeal. A thin film, 2-4 mm thick, of liquid medium (the same medium solution without agar), was dispersed over the surface of the congealed medium. Seedling was accomplished by holding a leaf bearing sterile spores slightly above the surface of the medium and tapping the leaf gently to dislodge the spores. During the seeding process, no contact took place between the spore-bearing leaves and the medium. Spores were allowed to drop freely on the thin liquid film on the surface of the congealed growth medium. Seeded flasks were plugged with sterile cotton and incubated without light in the growth chamber at 17 C.

The use of flasks instead of petri dishes facilitated easier handling of seeded cultures with a liquid film on the surface. Cotton-plugged flasks also eliminated condensation, which was a problem with taped petri dishes.

Care of cultures.—Care was taken to prevent the medium from becoming dry. The film of liquid medium was maintained on the surface by using a pipette to place small amount of the liquid onto the older cultures as needed.

The percent of successful cultures was raised from 0 to 40% with the old method to from 75 to 90% when the new methods were used. In addition, more extensive growth of the cultures obtained by the modified methods was readily apparent when compared with the growth obtained by the older methods.

DISCUSSION.—The extensive and highly differentiated axenic growth obtained with these methods may have been more successful for a number of reasons, but certainly it was likely that the inoculum consisted primarily of uredospores that were mature and more or less of uniform age. Other methods may have included in the inoculum spores that were of sufficiently different ages that germination and growth would be erratic. It was also possible that the aseptic condition of the inoculum source gave less problems with inhibitory substances or organisms that could significantly alter the results.

The presence of a liquid film on the surface of the solidified agar medium contributed immensely to improved growth. The film enabled the fungus to form active and turgid germ tubes that were useful in establishing a stable and effective contact with the growth medium resulting in the formation of very extensive and highly differentiated growth.

LITERATURE CITED

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