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OBSERVATIONS ON NUCLEAR DIVISION IN VEGETATIVE HYPHAE OF CERATOCYSTIS FAGACEARUM

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INTRODUCTION

There has been considerable controversy in recent years concerning nuclear division in vegetative hyphae of various fungi (1-9). Much of the controversy centers around a failure to find distinct metaphase plates. Ward and Ciurysek (6) contend that this may be due to staining of chromosome matrix as well as chromosomes. More refined staining techniques are needed, since the nuclei of most fungi are relatively small, and details of nuclear divisions are hard to demonstrate with existing techniques.

The major emphasis of this paper is on the techniques used in preparing material for study of nuclear division. A unique type of vegetative nuclear division is also described.

MATERIALS AND METHODS

The fungus used in this study was an isolate of Ceratocystis fagacearum (Bretz) Hunt which was maintained at 26°C on 10% V-8 Juice agar medium.

Material for staining was prepared by inoculating cover slips with a spore suspension or with an agar block cut from the periphery of a growing culture.

Dilute (3:1), sterile V-8 Juice filtrate was used in preparing spore suspensions in order to increase the growth rate and size of the hyphae on the cover slips. The filtrate was added to the agar block with an inoculating loop which was also used to spread the filtrate evenly over the surface of the cover slip. Sterile water was added to the filter paper prior to incubation to insure 100% relative humidity. The material was incubated at 26°C for 3-10 days. Cover slips for inoculation were prepared as follows: three or four filter papers were placed in a Petri dish and moistened with water. A microscope slide was then placed on the paper, and three 22 x 40 mm No. 1 cover slips were spaced across the slide. The Petri dishes were then autoclaved.

Preparations used for photographs were stained by the HC1-Giemsa method (schedule below).

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2Mr. Aist is an undergraduate research participant and Dr. Wilson is Associate Professor in the Department of Plant Pathology.
Nuclear Division in Ceratocystis fagacearum

Staining Schedule Used for the HC1-Giemsa and Feulgen Techniques*

(Cover Slip Cultures)

1. Carnoy's fluid — 10-90 min.
2. 95% alcohol — 15 min.
3. Acetone — 20 min.
4. 95% alcohol — 15 min.
5. 70% alcohol — 15 min.
6. 50% alcohol — 15 min.
7. 1 Normal HCL (room temp.) — 10 min.
8. 1 Normal HCL (60° C) — 10 min.
9. H₂O, 3 changes — 5 min.
10. ½ buffer-½ H₂O, 2 changes — 5 min. (for Giemsa only)
11. Buffer (all buffers pH 7±.02) — 10 min. (for Giemsa only)
12. Giemsa stain (2 drops Giemsa per ml. buffer) — 30 min., or Feul- gen reag. — at least 90 min.
13. Buffer — Rinse (Giemsa) or, Water — Rinse, 3 changes (Feulgen)
14. Acetone — Xylene (20-1) — 1 sec.
15. Acetone — Xylene (14-6) — 5 sec.
16. Acetone — Xylene (6-14) — 10 sec.
17. Xylene, mount in balsam.

RESULTS

Material obtained from agar block inoculations was superior to that obtained from spore suspensions. The hyphae from agar blocks often fanned out evenly over the cover slip, with no aerial growth which could subsequently interfere with staining. Hyphae growing from agar blocks often attained a diameter of about 8μ whereas hyphae obtained from spore suspensions did not exceed 4μ. Addition of V-8 Juice filtrate greatly facilitated growth and resulted in an increased size of the hyphae. Although nuclei in the larger hyphae were approximately the same size as those in the smaller hyphae, details of prophase and anaphase were more distinct, perhaps because the chromosomes were not as confined.

Giemsa stained material was superior to both basic fuchsin and iron-alum haematoxylin stained material. Chromosomes could often be resolved in Giemsa preparations, but not in Feulgen or iron-alum haematoxylin preparations. All divisional stages observed in Giemsa-stained material were also observed in Feulgen preparations.

Acetone was a very useful addition to the staining schedule (3). Treating cover slip cultures with acetone prior to staining caused the nuclei to stain more differentially and removed certain cytoplasmic interference (Figs. 1, 2).

These techniques have also been used in staining vegetative nuclei of Aspergillus nidulans (Eidam) Wint. and three other members of the

*Adapted from a staining schedule obtained from Dr. James Dale, Department of Plant Pathology, University of Arkansas
Fig. 1 — Vegetative nucleus of *C. fagacearum* at anaphase. This was an unusually well synchronized anaphase. Lower nucleus had already begun to migrate into branch. 2600X.

Fig. 2 — Vegetative nucleus of *C. fagacearum* at telophase of division perpendicular to the longitudinal axis of the hypha. Note the spindle, particulate nuclei, and the absence of cytoplasmic interference. 2600X.
Nuclear Division in *Ceratocystis* fagacearum

The genus *Ceratocystis*: *Ceratocystis ulmi* (Buisman), *Ceratocystis virescens* (Münch) Bakshi, and *Ceratocystis ambrosia* (Bakshi). In each case the quality of staining was similar to that obtained with *C. fagacearum*, with no changes in the staining schedule being necessary.

Division of nuclei in vegetative hyphae of *C. fagacearum* was unique in that it occurred perpendicular to the longitudinal axes of the hyphae (Fig. 1). Anaphase movement was not synchronized and spindles were usually seen only between chromatids or groups of chromatids which had already separated. Metaphase chromosomes occurred against hyphal walls and showed end-to-end association.

Observations of nuclei in vegetative hyphae of *C. ulmi*, *C. virescens*, and *C. ambrosia* indicated that nuclear division in these related species may be similar to that found in *C. fagacearum*.

Other aspects of the behavior of these nuclei, such as nuclear migration and chromosome associations, as well as a detailed description of the division process, will be presented in another publication.

**DISCUSSION**

The use of cover slip cultures for the study of fungus nuclei should reduce nuclear distortion. The hyphae are less likely to be physically distorted, as is the case when cellophane strips or homogenized hyphae are used. Study of the material is also facilitated, since the hyphae tend to be fixed in one plane on the cover slip.

The type of growth exhibited by the fungus is important. A suppressed type of growth (as opposed to aerial growth) is to be preferred, since aerial growth may result in a thick mat of tangled hyphae that interferes with dehydration. Study of nuclear behavior in fungi which normally exhibit aerial growth may be facilitated by using a mutant strain (natural or induced) which has suppressed growth. Such a mutant strain of *Aspergillus nidulans* was used to test the applicability of the HC1-Giemsa technique as stated above.

The significance of divisions perpendicular to the longitudinal axes of hyphae of *C. fagacearum* is not known. If later detailed investigations show similar divisions in several related fungi, this may prove to be of taxonomic value.

Association of metaphase chromosomes to hyphal walls, unilateral unsynchronized anaphase movement, and appearance of spindle—usually between separated chromatids only, also distinguish asexual nuclear division in *C. fagacearum* from classical mitosis. Since metaphases with distinct chromosomes, and spindles are involved, this type of division appears to be an evolutionary form of classical mitosis.

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