Effect of Storage on the Rate of Incorporation of Uridine into Ribonucleic Acid by Germinating Uredospores

L. Ramakrishnan and R. C. Staples

Boyce Thompson Institute, Yonkers, New York 10701.

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ABSTRACT

Uredospores of the bean rust fungus virtually lost their capacity to incorporate exogenous uridine into ribonucleic acid during germination when the spores had been stored longer than 4 days in the refrigerator. The decline in uridine incorporation is not related to infectivity of the spores or to their capacity to germinate or differentiate. Phytopathology 61:244-245.

Additional key words: Density gradient analysis, *Uromyces phaseoli.*

Wynn et al. (7) previously described the physiology of uredospores during storage. Spores stored at 4 C did not lose their viability for at least 5 weeks, and the amounts of various compounds such as amino acids and organic acids did not change greatly. The present studies were part of an effort to describe the synthesis of ribonucleic acid (RNA) by germinating spores. The work was prompted by futile attempts to reproduce data on incorporation of labeled nucleotides into RNA when different lots of spores were used which had been stored for various periods of time. The observations are not yet explained, but may be a useful guide to other workers who are investigating uredospore behavior.

Uredospores of the bean rust fungus (*Uromyces phaseoli* [Pers.] Wint.) were obtained from infected leaves of bean (*Phaseolus vulgaris* L. 'Pinto') plants grown in controlled environment chambers as described previously (4). The spores were hydrated in a cold room at 4 C for 16 hr before use in germination experiments (3).

After dusting the collodion membranes with hydrated spores, the membrane surface was sprayed with water. When required, 1 ml of water containing 50 μc of uridine-5-^{3}H (specific activity 25.2 c/mmole) was sprayed directly on the membrane surface, and the spores were germinated in the dark at 20 C (6). The spores (1 g) on 20 membranes were pulverized in liquid nitrogen (2), and the RNA was extracted with phenol and sodium lauryl sulfate and fractionated on 5-20% gradients as described previously (2). Radioactivity in the RNA was determined by the procedure of Trewavas (5), in which the RNA was precipitated by cetyltrimethylammonium bromide (CTA), collected on glass fiber filters, and counted by liquid scintillation.

The incorporation of ^{3}H-uridine into RNA by germinating spores varied according to the number of days that the spores were stored (Fig. 1). This was true whether or not the spores produced appressoria during germination. It was found that incorporation of uridine into RNA by germinating spores declined rapidly during the first 4 days of storage, then stabilized. During this time, the capacity of the spores to germinate and differentiate was unchanged. Uridine incorporation declined to much lower values if the spores failed to form infection structures.

To determine if storage changed the pattern of synthesis of the various nucleic acid fractions during germination, RNA was extracted from spores exposed to ^{3}H-uridine during the 4th-5th hr of germination (Fig. 2), the time chosen because that is when formation of infection structures occurs (6). It was found that uridine was incorporated into each of the ribonucleic acid fractions, but the amount of label incorporated was dramatically reduced by storage.

Thus, a very brief storage of 4 days caused these rust spores virtually to lose their capacity to incorporate exogenous uridine into RNA during germination. Studies failed to reveal if changed pool sizes of precursors could account for the results. Adenosine triphosphate did increase during germination at the expense of adenosine diphosphate and adenosine monophosphate (1), but the uridine nucleotides were too low for accurate determination by the very sensitive procedure employed. The decline in uridine incorporation is not related to infectivity of the spores or their capacity to germinate or differentiate.

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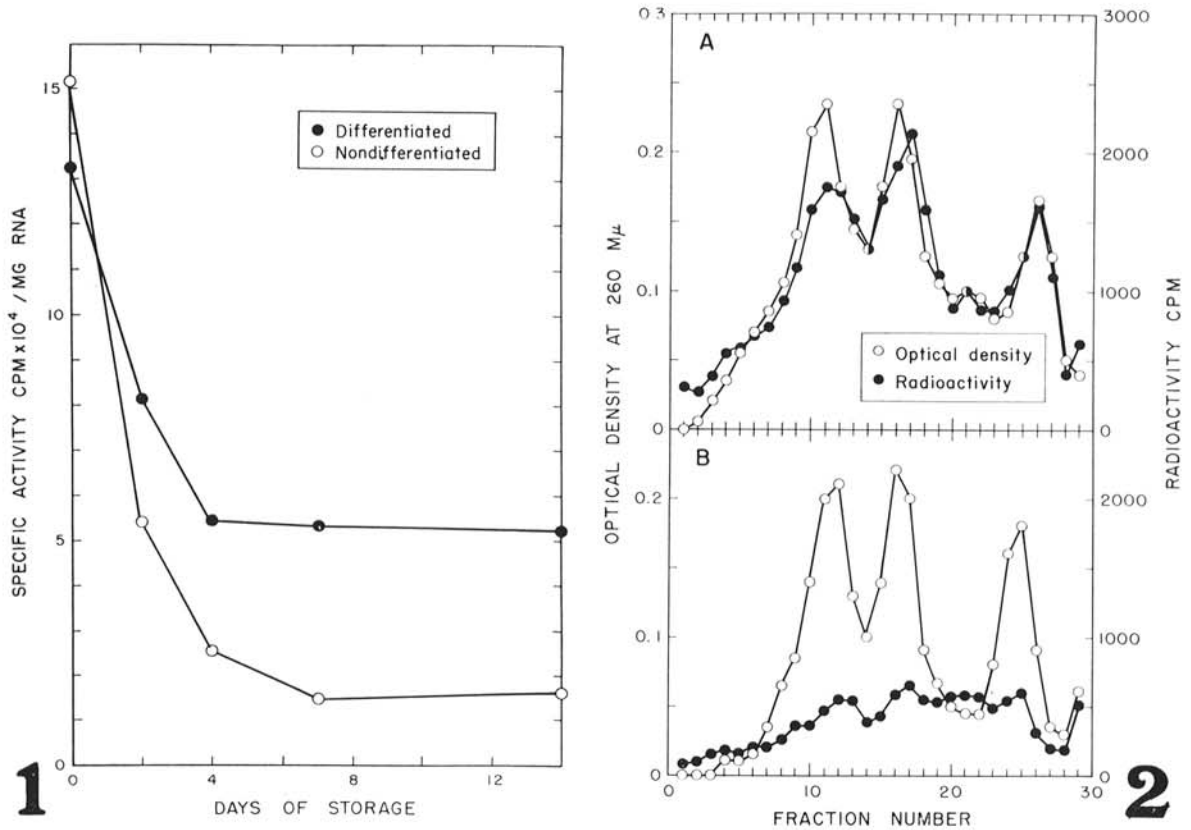

Fig. 1-2. **1)** Effect of storage on the rate of incorporation of ^{3}H-uridine into ribonucleic acid (RNA) from germinated spores. The spores were labeled during the 4th-8th hr of germination, and the specific activity of the RNA determined. One g of spores (20 membranes) was used for each assay. **2)** Sucrose gradient analyses of RNA from fresh spores (A) and spores stored for 2 weeks (B). The spores were germinated for 4 hr until appressoria had formed, then germinated for 1 hr in the presence of ^{3}H-uridine. The labeled RNA was fractionated by sucrose density gradient centrifugation. One g of spores (20 membranes) was used for each assay. The RNA was layered on a 5-20% sucrose gradient and centrifuged for 2.5 hr at 65,000 rpm.