Fungal Genetics Reports

Volume 2 Article 16

Viability of Neurospora crassa ascospores after heat activation

H. E. Brockman

Viability of Neurospora crassa ascospores after heat activation

Abstract

Viability of *Neurospora crassa* ascospores after heat activation

Brockman, H.E.* Viability of Neurospora crassa ascospores after heat activation.

We have been investigating various factors which influence the viability of Neurospora crassa conidia and ascospores in sorbose-supplemented media (de Serres, Kølmark, and Brockman, Nature 193: 556, 1962; and unpublished results). In an experiment designed to test the effect of autoclaving time of the media on ascospore viability, there was an unexpected decrease in viability at 45 min autoclaving (Figure 1). The ascospores were from a cross of a, arg-3, hist-3, +, nic-2, X A, +, +, ad-3, + and had been heat activated 30-40 min at 60°C in 0.1% agar prior to plating in (A) Fries' basal medium, 0.1% sucrose, 1.0% L-sorbose, 1.5% agar, and 100 µg L-histidine, 100 µg adenine sulphate, and 10 µg nicotinamide/cc, or (B) the same medium without sorbose followed by overplating 9 hr later with the same concentration of sorbose (Newmeyer, Genetics 39: 604, 1954), or (C) the same conditions as (A) except that 0.05% glucose and 0.05% fructose were substituted for the 0.1% sucrose. The media were autoclaved in five lots corresponding to the five autoclaving times, and the ascospores were held, therefore, in 0.1% agar at approximately 24°C for varying periods of time between heat activation and plating.

A second experiment was performed to determine whether the decrease in viability was due to the 45 min autoclaving of the media or to the time of ascospore incubation in 0.1% agar. Ascospores from the same cross were plated immediately after heat activation and at various subsequent times using the same conditions as in (C) of the previous experiment except that 1.5% sorbose and a constant 10 min autoclaving time were used. There was a sharp decrease in ascospore viability at 4 hr and again after 24 hr incubation at approximately 24°C (Figure 2). The reasons for the decrease in viability at 4 hr has not been determined, but it may be due to a heat sensitivity of the ascospores (Lingappa and Sussman, Am. J. Botany 46: 671, 1959) at a particular stage of germination, as the ascospores were plated in 43°C media. This idea is supported by the observation that this decrease does not occur when the ascospores are heat activated and then stored at a temperature (2-4°C) at which germination would not occur (Figure 2).

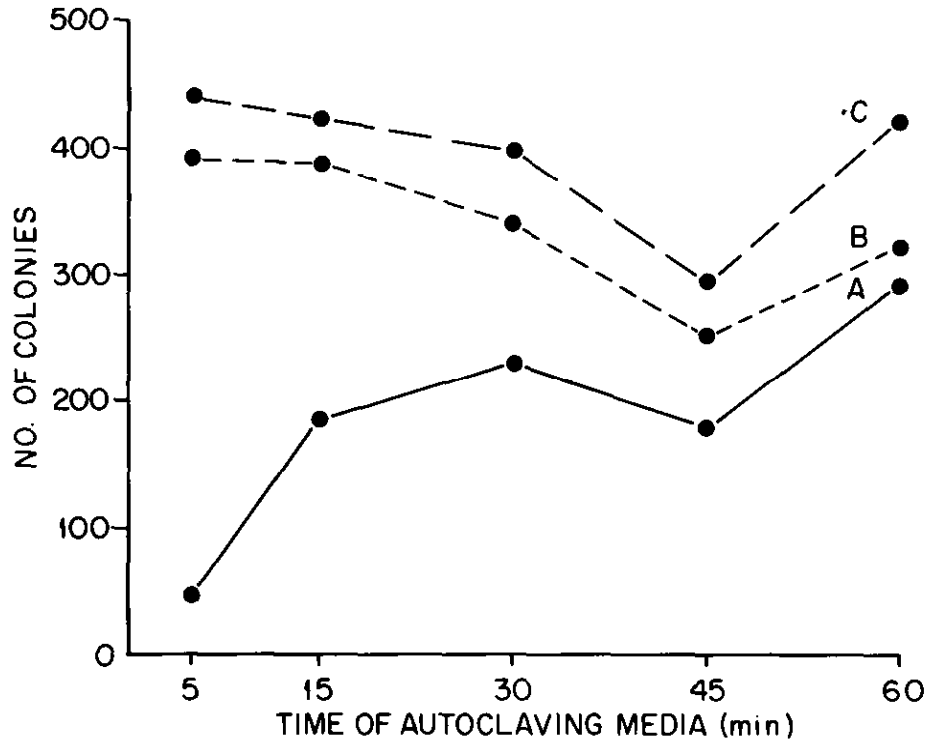

Figure 1. Effect of autoclaving time on ascospore viability.

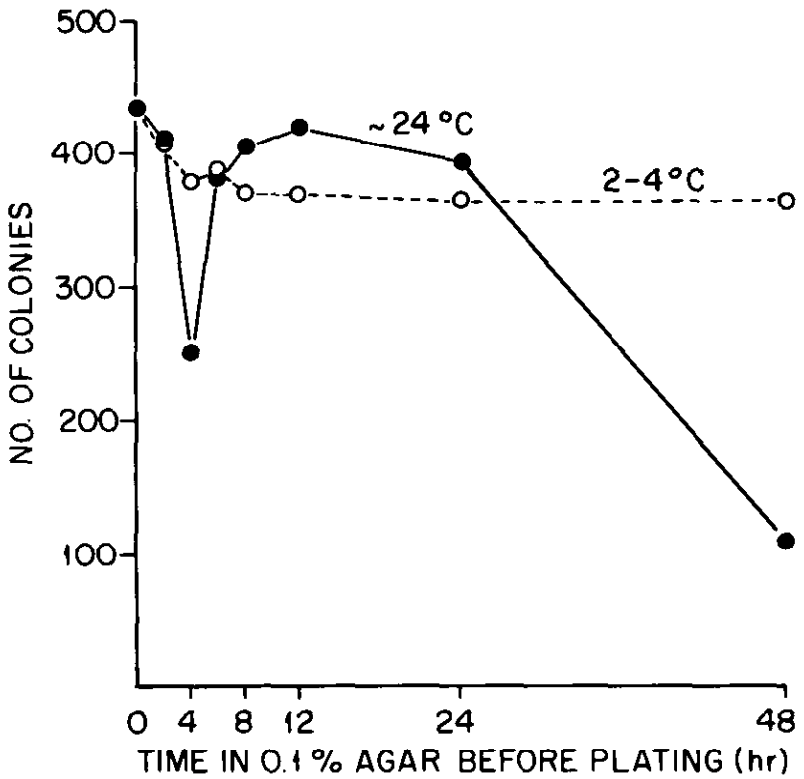

Figure 2. Effect of storage at two different temperatures in 0.1% agar between heat activation and plating on ascospore viability.

If these plating conditions are used, it appears necessary either to plate the ascospores immediately after heat activation or to hold them at 0-4°C prior to plating in order to obtain a constant viability.

---*Postdoctoral Fellow supported by the Air Research and Development Command and the National Academy of Sciences—National Research Council. Contribution from Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.