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Abstract

Effect of age on relative plating efficiency of conidia

Hoard, K. Effect of age on relative plating efficiency of *Neurospora* conidia.

The early work of Ryan (1948 Am. J. Bot. 35: 497) showed that the age of conidia and their concentration were critical factors in controlling rate of germination. Since both the rate and the percent of conidial germination seemed to be the highest for 6-8-day-old

cultures, we have routinely used week-old cultures whenever possible for plating experiments with conidia. However, in some recent routine plating experiments on Vogel's sorbose-glucose medium we found that the proportion of conidia which formed countable colonies was much higher than the proportion of conidia which germinated in non-sorbose liquid medium in a 6-8 hr. period. Moreover the age of the cultures (all were less than 10 days old) had little effect on plating efficiency. Since in many experiments it is often more convenient to be able to use cultures of different ages for plating, and since the investigator is usually more interested in colony-forming ability than in either the rate of germination or percent of conidia which simply germinate, the effect of age was rechecked using several additional strains.

In some typical experiments reported below, the following strains were used: *pan-2* (allele B3) a, *tryp-1* (allele A9) in Em a background, Em o, 74a, and a heterocaryon (*pan-2* a + *ad-4* a). The *ad-4* allele employed was F4. The cultures were inoculated in cotton-stoppered slants an minimal or appropriately supplemented Vogel's medium each day for periods up to 20 days. At the time of plating cultures were available ranging in age up to 20 days. The cultures obviously differed considerably in regard to the number of conidia formed and older cultures were assumed to have been quite heterogeneous in terms of the age of the conidia present. Following inoculation the cultures were placed at 30°C for two days and thereafter were maintained at 25°C. Conidia were collected for plating in sterile distilled water, filtered through glass wool, and adjusted to concentrations of approximately 1.5 to 2×10^6 conidia/ml. To determine conidial concentrations, four counts were made of each suspension using a hemocytometer. A 10^{-4} dilution was made in two milk dilution battles each containing 99 ml of water. Conidia were then plated on five plates of Vogel's medium containing 0.01% glucose, 1% sorbose and 2% agar along with the appropriate growth supplements. The results of the effect of age on relative plating efficiency are shown in Table 1.

Table 1. % of conidia of 5 different cultures of different ages forming colonies on Vogel's medium supplemented with .01% glucose, 1% sorbose and 2% agar.

Age of culture (in days)	% Viability				
	<i>pan-2</i>	74A	Em a	<i>tryp-1</i>	<i>pan-2</i> + <i>ad-4</i>
1	51	55	65	48	57
2	78	77	58	70	80
3	76	70	89	91	81
4	76	80	80	72	80
5	74	76	82	76	80
6	77	72	65	83	100
7	75	77	63	66	71
a	72	74	64	90	78
9	76	74	63	77	82
10	74	60			
11		74			
12		56			
13		52			
14		57			
15		47			
16		38			
17		33			
18		45			
19					

Table 2. Comparison of % germination of *pan-2* conidia of different ages after 8 hr incubation in liquid Fries #3 medium and % conidia forming colonies on the same medium supplemented with .01% glucose, 1% sorbose and 2% agar.

Age of culture (in days)	% conidia forming colonies on agar	% germination medium in liquid medium
1	65	81
2	80	89
3	90	85
4	--	
5	--	
6	--	
7	87	87
8	--	
9	91	91
10	78	81
11	94	82
12	80	85
13	81	83
14	66	73
15	73	72
16	68	67
17	63	62
18	76	65

A second experiment, using pan-2 cultures ranging in age up to 18 days, was done using Fries #3 medium throughout. Conidia were incubated in liquid Fries using the method of Ryan (loc. cit.) and the percent of conidial germination was determined after 8 hours. The same suspensions were also plated as described in the previous experiment to determine the relative plating efficiencies. These two values are compared in Table 2.

Although we realize that the Plating efficiencies given in the two tables will vary from experiment to experiment and from culture to culture, we were impressed with the fact that although the plating efficiency is only about 50% for one-day-old cultures, it increases to a maximum on the second day and remains fairly constant for periods of up to ten days or longer. For our own purposes it seems clear that once a culture has enough conidia so that they can be easily collected, the viability is as good in a two-day-old culture as it is in a week-old culture. Furthermore, in growth tubes, where conidia in the proximal and distal ends are of different ages, the viability of the conidia from opposite ends is comparable.

In the second experiment using the medium of Fries, there was a good correlation between the colony counts on the plates and the percent of conidia which had germinated after 8 hours' incubation in liquid medium. This was surprising since we had found, although the data are not given here, that the percent of germination in liquid Vogel's medium after 8 hours was significantly lower than the percent of conidia which formed colonies. * * * Department of Agronomy, Kansas State University, Manhattan, Kansas 66504.