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Germination and enzyme activities by the aging of Neurospora conidia in water

Abstract

Effect of storage in water on germination and enzyme activities

Stine, G. J. Germination and enzyme activities affected by the aging of Neurospora conidio in water.

In a recent investigation (Stine 1966 Ph.D. Thesis, University of Delaware), four enzymes (NAD- and NADP-dependent glutamic acid dehydrogenases, succinic dehydrogenase and nicotinamide-adenine dinucleotidase) were assayed throughout the asexual cycle of N. crassa. As a part of this investigation, conidio were stored aseptically at 4°C for 81 doys in distilled deionized water, which was changed every 3rd doy in order to determine: the effect of repeated washing on conidiol germination and subsequent mycelial growth, the time of onset of autolysis, and the stability of the activity of the four enzymes under these conditions.

Approximately 5 x 10^6 conidio of wild type strain STA4 (FGSC#262), grown on slants of Vogel's minimal medium N (Vogel 1956 Microbial Gene+. Bull. 13: 42), were used to inoculate solidified medium N in petri dishes. The dishes were incubated for 48 hours and the conidio were harvested. The conidio were then used to inoculate a second ret of petri dishes. After 48 hours' incubation, conidio from the second set of dishes were harvested and used to inoculate a third ret. Conidio from the third se+ were used to prepare the experiment.1 suspension.

The conidia were harvested by adding 30 ml of water to each dish, swirling for a few minutes and scraping the myceiial surface with a gloss probe. The resulting suspension from each petri dish was poured into a centrifuge bottle through a double layer of absorbent cotton and centrifuged at 3000 x g for 10 minutes. The conidiol pellet was resuspended in 100 ml of water and the conidio were counted in a Neubauer hemocytometer. 3 x 10^{10} conidio from the third set of dishes were placed in a 250 ml centrifuge bottle containing 100 ml of water and stored at 4°C. This suspension was centrifuged every third doy (3,000 x g, IO min.) and each time the pellet of conidia war resuspended in 100 ml of water and counted. The dimensions of the conidia were determined by use of on ocular micrometer in a light microscope. The results of measurements on 200 conidio were: mean length 6.5 μ (range 4.7-10 μ); mean width 5.7 μ (range 3.8-8.7 μ). Dry weight determinations were mode on four samples of co. 4x 10^9 conidio. The conidio were placed in pre-weighed viols ond dried in an oven set at 90°C for 48 hours. An overage dry weight of 1.39 x 10^{-11} grams per conidium was obtained.

The ability of the conidio to germinate was determined by removing a sample from the stored suspension and incubating it in medium N plus 2% sucrose (50 ml/250 ml Erlenmeyer flork; 2 x 10^6 conidia/ml) on a Burrell wrist action shaker at 320 stroker per minute. At the intervals indicated in Table 1, samples were removed and counted and scored for germination and lysis. A conidium was scored as germinated if a germ tubs was visible. Lyrd conidio were recognized by their low refractility and the obvious absence of their contents. The total yield of mycelium from these flasks was determined at 48 hours (as wet weigh+).

Considering the number of times the stored suspension was washed (every 3rd day), the conidio showed surprising stability, as con be seen in Table 1. After 21 days storage and 7 washings, no lysed conidia were observed. After 81 days and 27 washings, 30% of the conidia hod lysed. Of those conidia which had not lysed, 90% germinated within 24 hours after 30 days of storage, and 70% germinated within 48 hours after 81 days of storage. A very pronounced delay in germination and decrease in total yield of mycelia from the germination flasks was apparent after 30 ond 66 days of storage. After 81 days of storage the conidio showed on extreme lag in germination ond foiled to show any significant growth beyond the formation of a germ tube a few microns long.

Table 1. Germination of aging Neurospora conidio.

Time of conidio in water	No. of lysed conidia per sample of 10^8	Time in hours						48 hour wet weight' mycelial growth
		0	7	12	16	24	48	
		% germination**						
1 hour		0	90					2.8
3 days		0	96					2.8
21 days	none	0	42	92				2.6
30 days	3 x 10^4	0	7	26		90		1.9
66 days	3% 10^7	0	5	7		40		1
81 days	3 x 10^7	0	0		<1		70	none***

Culture flasks (250 ml) contained 50 ml of Vogel's medium N + 2% sucrose + 2 x 10^6 conidio per ml as inoculum. Cultures were aerated at 25°C. ** Germination was determined by the presence of a germ tube. % germination was bored on non-lysed conidia as determined by light microscopy. • Wet weight of mycelia obtained by pressing harvested mycelia between sheets of filter paper to reduce residual moisture. • ** None means that growth beyond germ tube was insignificant.

Enzyme assays were performed on sampler removed directly from the storage bottle. The conidio were disintegrated by treating for 2 minutes in a Nossal disintegrater. The capsule contained 6 ml of cysteine-HCl carbonate buffer, pH 8.4, 2 g alundum and 3 x 10^9 conidio. Preliminary experiments showed that 3 x 10^8 conidio represented a sufficient sample for performing the enzyme assays, whereas 1 x 10^8 conidio were insufficient. As a measure of safety, the assays were performed on a sample of 3 x 10^9 conidio. The methods used for performing the enzyme assays hove been described by Barratt (1963 J. Gen. Microbiol. 33: 33), Kaplan et al. (1951 J. Biol. Chem. 191: 473) and Singer et al. (1957 Methods of Biochemical Analysis 4: 307).

Table 2. Enzyme activities in Neurospora conidia.

Time of conidio in water	NAD-glutamic dehydrogenase		NADP-glutamic dehydrogenase		Succinic dehydrogenase		Nicotinamide-adenine dinucleotidase	
	TU	SPA	TU	SPA	TU	SPA	TU	SPA
1 hour	80	20	200	50	4	1	5034	1678
30 days	105	45	14	6	0	0	5742	2468
66 days	30	10	0	0	0	0	2400	800
81 days	0	0	0	0	0	0	233 1	850

3×10^9 conidia were withdrawn from stored sample, washed twice and used for enzyme assays. TU = total units; SPA = specific activity (units/mg protein).

As can be seen from Table 2, the NAD-dependent glutamic dehydrogenase doubled in specific activity while the total units increased by approximately 25% between the first hour after conidial harvest and 30 days of storage. The enzyme nicotinamide-adenine nucleotidase (NADase) also increased in both total units and specific activity between 1 hour and day 30. Although NADase declined appreciably after day 30, this enzyme was still measurable after 81 days of storage, in contrast to the other 3 enzymes which could not be detected at this time. By day 30 the activity of NADP-dependent glutamic dehydrogenase had declined to 12% of the specific activity present 1 hour after harvest. Succinic dehydrogenase was no longer measurable at day 30. The increase in the total units of both enzymes during the same period may be due to the activation of the separate enzymes or, less likely, to enzyme synthesis.

It con be concluded that storage at 4°C in distilled water for a long period with repeated washing drastically affected conidial germination, subsequent mycelial growth and the levels and specific activities of the four enzymes measured. No conclusions as to the roles of these enzymes in conidiol germination can be drawn from the dab presented here. Further work has shown that succinic dehydrogenase and NAD- and NADP-glutamic dehydrogenases do not appear to be necessary for conidial germination, whereas nicotinamide-adenine dinucleotidase may be directly involved in the germination processes. These results will be presented elsewhere.

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