CHEMICAL ACTIVATION OF ASCOSPORE GERMINATION IN NEUROSPORA CRASSA[1]

MARY R. EMERSON

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California

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In the red bread mold *Neurospora crassa* as in other pyrenomycetes the sexual spores, the ascospores, germinate only after the application of heat (Shear and Dodge, 1927; Goddard, 1935, 1938, 1939). When treated at a temperature of from 50 to 60 C for periods varying from 10 to 60 minutes the spores will germinate up to 100 per cent. Germination may occur without this treatment, the percentage depending on the condition of the spores and on the medium used. The spontaneous germination rate is usually higher on media fortified with malt and yeast extracts, hay infusion, etc., and may occasionally go as high as for treated spores.

Recently, while a synthetic minimal medium was being used in which *d*-xylose was the carbon source, a consistently high percentage of spontaneous germination was observed. A number of experiments were then conducted in an attempt to determine the active agent involved. From these it appeared that xylose was much more effective after being autoclaved than after being filter sterilized. On the assumption that in the mineral solution (Fries no. 3, having a pH of 5.5) used in the medium and under the pressure and temperature of autoclaving there might be a slight conversion of the pentose into furfural (C_4H_3OCHO), the latter was tried alone and proved very effective.

MATERIALS AND METHODS

The ascospores used in the germination tests were obtained from repeated crosses of wild-type strains E-5256A and E-5297a unless otherwise indicated. They were collected in small lots, as they were needed, from the tops of petri dishes in which the crosses had been made, usually from 1 to 4 weeks from the time the perithecia started to shed. For germination counts the spores were individually transferred to small blocks of 4 per cent agar in distilled water, flooded with 1.5 per cent sodium hypochlorite solution ("purex"), allowed to stand for a short time until most of the "purex" had drained off, and then transferred to petri dishes containing the medium to be tested. The hypochlorite is used to kill any conidia clinging to the ascospores, since conidial germination might obscure that of the ascospores. Media used in germination tests consisted of 1.5 per cent agar, 2 per cent sucrose, Fries no. 3, and biotin, plus the substance to be tested, which for the sake of uniformity was added after the rest had been autoclaved. Experience showed that heat sterilization did not appreciably affect the activity of furfural. Controls on the same medium without the added substance were used to measure the germination percentages of each

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lot of spores before and after heat activation. In some cases the chemically treated spores were heat treated, after being counted, to see whether further germination would occur.

TABLE 1

Effect of furfural concentration on spontaneous germination of ascospores

FURFURAL CONCENTRATION	SPORES TESTED	GERMINATION WITHOUT HEAT		FURTHER GERMINATION ON HEAT TREATMENT	TOTAL GERMINATION		GERMINATED WITHOUT HEAT AS PERCENTAGE OF TOTAL GERMINATED
		no.	%		*no.*	%	
0	40	1	2.5	37	38	95.0	2.6
1/1,000	40	38*	95.0				
1/10,000	40	38	95.0				
0	40	0	0	39	39	97.5	0
1/500,000	80	73	91.2				
1/5,000,000	80	12	15.0	66	78	98.7	15.4
1/50,000,000	80	0	0	77	77	96.2	0
1/500,000	100	90	90.0	4	94	94.0	95.7
1/1,000,000	100	85	85.0	13	98	98.0	86.7
1/2,000,000	100	94	94.0	4	98	98.0	95.9
1/4,000,000	100	96	96.0	2	98	98.0	98.0
0	72	0	0	54	54	75.0	0
1/500,000	157	145	92.4	0	145	92.4	100
0	220	2	0.9	182	184	83.6	1.1
1/500,000	139	136	97.8				
1/500,000	100	92	92				
1/1,000,000	100	93	93				
1/2,000,000	100	87	87				
1/4,000,000	100	40	40				
1/8,000,000	100	31	31				
1/16,000,000	100	24	24				
0	320	62	19.4	247	309	96.6	20.1
1/10,000	210	206	98.1				
1/100,000	188	185	97.9				
0	180	1	0.6	173	174	96.7	0.6
1/250,000	204	193	94.6	7	200	98.0	96.5
0	106	11	10.4	82	93	87.7	11.8
1/500,000	100	100	100.				100

* Germination indicated by germinal buds only. At concentrations greater than 1/40,000 furfural is increasingly poisonous to mycelial growth; at 1/1,000 growth is completely inhibited.

RESULTS

Data from a series of typical experiments are summarized in table 1. A number of trials show that the same degree of activation is brought about by allowing spores to stand from 10 to 15 minutes in a solution of furfural in distilled water, as when a solid medium is used. As can be seen from the table, the germination rate remains consistently high up to dilutions of 1/1,000,000 or more and is in most cases very close to that of the heat-treated spores. With

TABLE 2

Effect of degree of maturity of spores upon response to furfural

CROSS	TYPE AND NUMBER OF SPORES	MEDIUM	GERMINATION WITHOUT HEAT		FURTHER GERMINATION ON HEAT TREATMENT	TOTAL AS PER CENT
			no.	%		
Abb-4A	135 shed spores	1/100,000 furfural	122	90.4		
and						
E-5297a	8 asci (59 spores)	1/100,000 furfural	6	10.2		
Abb-4A	145 shed spores	1/100,000 furfural	115	79.3		
and						
25a	6 asci (42 spores)	1/100,000 furfural	5	11.9		
Abb-4A	50 shed spores	1/100,000 furfural	44	88.0	3	94.0
and						
E-5297a	9 asci (65 spores)	1/100,000 furfural	16	24.6	39	84.6
E-5256A	96 shed spores	Minimal	9	9.4	71	83.3
	106 shed spores	1/500,000 furfural	92	86.9	7	93.2
and						
E-5297a	12 asci (88 spores)	1/500,000 furfural	25	28.4	48	83.0

greater dilutions the rate drops fairly fast, and there is greater variation between spore lots. The latter may be due partially to age differences, since the age or ripeness of spores is of more critical importance in chemical than in heat activation. This is most noticeable in the case of spores dissected from asci. Table 2 gives a comparison in several crosses of the difference in germination rate on furfural between spores already shed and those from ripe asci (as judged by the color of the spores and the fragility of the ascal sac). There would be very little or no difference if the two sets were activated by heat.

Goddard has shown that in *Neurospora tetrasperma* heat activation of asco-

spores is reversible. This does not seem to be true of chemical activation by furfural. Neither furfural activation nor heat activation is sensitive to cyanide (M/1,000 or M/500), but the respiration of the activated spore is; and prolonged immersion in cyanide not only inhibits nearly all growth but reverses the activation process. The originally heat-activated spores can be again activated by further heating, and this cycle can be repeated as desired. On the other hand, spores that have once been activated by furfural and then induced to return to dormancy by the cyanide treatment become refractory to further furfural treatment but remain sensitive to heat activation. In one experiment spores that had been returned to dormancy after heat treatment were reactivated by furfural, but this could not be repeated in two other trials.

Other furan derivatives and other aldehydes are being tested for activity. Of the substances in the first group so far tested (furan, furfuryl alcohol, furoic acid, furoamide, furoin, β-furfural dioxime) only furfuryl alcohol was active. When used immediately after purification, it seems to be about 75 per cent as effective as furfural. On standing, a considerable portion is converted to the aldehyde and the test loses meaning.

No definite report can yet be made on other aldehydes except to say that the one that might most easily be expected to be the naturally occurring agent, acetaldehyde, has been entirely negative in every test conducted so far. Nor has it been possible to demonstrate the presence of furfural during heat activation, though chemical tests are sensitive for concentrations as low as 1 part in 5 to 10 million.

It must remain an open question whether furfural is a natural agent in inducing ascospore germination. The environments in which *Neurospora* is usually found might easily have traces of furfural, and possible precursors of furfural are doubtless present within the ascospores, but there is no direct evidence that either plays a role in ascospore germination in nature.

SUMMARY

Furfural is shown to be an effective chemical agent for activating the dormant ascospores of *Neurospora crassa*. The germination rate of chemically activated spores approximates that brought about by heat treatment up to dilutions of 1/1,000,000; with greater dilutions the rate drops sharply, but variably. The high germination rates apply only to spores already shed; those dissected from asci are recalcitrant to chemical activation.

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