Indian J. mycol. Res., 23(2), 43-47 (1985)
Printed in Innia

RESPIRATORY METABOLISM OF GERMINATING CONIDIA OF *DRECHSLERA ORYZAE*

BY

GITASREE GHOSH AND SUBHENDU CHAUDHURI

Department of Plant Pathology
Bidhan Chandra Krishi Viswavidyalaya
Kalyani, Nadia, 741235, West Bengal, India

High endogenous respiratory rate during initial hours of germination was characteristic of the conidia of *Drechslera oryzae*. Leaching of conidia in water substantially lowered their respiratory rate and germinability under endogenous metabolism. Oxidizable, simple carbohydrates applied exogenously activated both respiration and germination of such leached conidia. Studies with spceific enzyme inhibitors showed that aerobic endogenous respiratory metabolism of germinating conidia proceeds through the normal EMP-TCA-and cytochrome mediated electron transport system. But an alternative energy production mechanism other than the cytochrome dependent one in terminal electron transport may also operate in the germinating conidia. Energy produced by incomplete oxidation of carbohydrates by the EMP sequance was not sufficient to sustatin germination.

INTRODUCTION

Drechslera oryzae (Breda de Haan) Sub. & Jain *(Cochliobolus miyabeanus)* (Ito et Kurib.) Drechs. et Dastur is a virulent necrotrophic pathogen of rice causing brown (leaf) spot disease. Conidia of the fungus are phragmosporic porospores which germinate from two polar cells in presence of water. Respiratory metabolism of germinating conidia of the fungus was studied to elucidate the endogenous respiratory pathways operating during germination.

MATERIALS AND METHODS

A monoconidial isolate of *Drechslera oryzae* (H/39 C) obtained from leaf lesions of rice was used. Bulk conidia were produced in filter paper cultures over nutrient medium and were preserved dry at 20°C. Leaching of conidia was done by repeated suspension and low speed (3000 rpm) centrifugation in cold water (4°C). Conidia were germinated by standard glass slide germination technique. Data on germination percentage including that in presence of stimulators and inhibitors were recorded from ca. 10^4 conidia/ml at 6-10 hours of incubation. Respirometry was done with Warburg respirometer following standard manometric techniques Umbreitt *et al* (1964). Data on

effects of inhibitors on respiration was recorded by measuring 0_2 uptake by a sample of dry conidia between 1-3 hours.

RESULTS

Endogenous respiration of germinating conidia was very high. $Q0_2$ (μl 0_2 uptake per mg dry conidia per hour) rose to about 17 during first three hours of germination sequence. The rate increased further thereafter ($Q0_2$ 24.5 between 3-4 hours), when germ tubes differentiated in most spores. Conidia leached for 24 hours had a very low endogenous respiratory rate ($Q0_2$ 1.5) and failed to germinate appreciably during initial hours. Exogenous soluble sugars enhanced the respiratory rate of such conidia which germinated equally as unleached conidia. TCA cycle intermediates did not stimulate either respiration or germination of the leached conidia. Incubation for longer duration resulted in higher germination rate of the leached conidia (Table 1).

The non-specific metal chelator, 8-oxyquinoline inhibited both 0_2 uptake and germination. EDTA did not have much effect on either respiration or germination. Iodoacetate, which inhibits NAD linked triose phosphate dehydrogenase

TABLE 1. *Endogenous respiration and germination of conidia of* Drechslera oryzae *under various treatments*

Treatments	μl. 0_2 uptake per mg/hour 0-3 hours	Percent Germination ± S. Em		Explanations
		6h	12h	
Fresh. unleached spores in germination medium	16.8	90.2±3.4	—	Germination medium : 0.01M PO_4 buffer, PH 6.5.
24 hours leached spores	1.6	14.2±4.6	53.4±5.2	(a) Soluble sugars : D. glucose, D-Fructose. D-Mannose, maltose and sucrose @ 0.2% in germination medium
Soluble sugars (a)				(b) TCA cycle intermediates : pyruvate, citrate, acetate, succinate, malate. 0.01M in germination medium
leached spores	18.0	88.7±3.5	—	
unleached spores	23.4	92.6±2.8	—	
TCA cycle intermediates (b)				
leached spores	4.3	16.8+4.2	48.3±3.7	Respiration and germination data are average of all sources at (a) and (b)
unleached spores	21.6	90.5±2.7	—	

TABLE 2. *Effect of respiratory inhibitors on endogenous respiration and germination of conidia* Drechslera oryzae

Inhibitors	Concentration (molarity)	µl. O_2 uptake per mg dry spores per hour (a)	Percent germination ± S. Em	Explanations
None (b)	—	21.2	96.5±3.2	(a) Conidia were allowed to germinate blank for 1 h and then added to Warburg flasks ; O_2 uptake data for 1-3 hours.
8-oxyquinoline	$5x10^{-4}$	2.1	26.3±3.4	
EDTA	$5x10^{-4}$	14.4	73.4±2.8	
Iodoacetate	$5x10^{-4}$	3.0	12.6±4.1	
Arsenate	$5x10^{-4}$	12.8	56.0±2.4	
	10^{-3}	4.7	26.2±3.3	
Fluoride	$5x10^{-4}$	6.4	23.5±2.8	(b) Germination medium 0.01 m PO_4 buffer PH 6.5 ; for fluoride, buffer PH was 5.5.
Malonate	$5x10^{-4}$	17.5	82.8±4.2	
	10^{-3}	16.0	83.5±3.7	
Arsenite	$5x10^{-4}$	10.2	61.4±3.2	
	10^{-3}	3.2	13.2±5.1	
Cyanide	$5x10^{-4}$	6.2	73.2±2.7	
	10^{-3}	2.9	59.4±3.5	
Azide	$5x10^{-4}$	7.5	79.3±4.2	
	10^{-3}	2.4	63.2±3.7	
Diohyldithiocarbamate	$5x10^{-4}$	13.6	74.5±2.8	

in the EMP sequence of glycolysis strongly inhibited both respiration and germination of conidia. Arsenate, an uncoupler of phosphorylation at this stage of oxidation, was not equally inhibitory as iodoacetate in inhibiting either germination or respiration. Fluoride, which inhibits subsequent enolase reaction in the EMP sequence strongly inhibited both respiration and germination of conidia. Malonate, the inhibitor of succinic dehydrogenase in the TCA cycle did not effect either germination or respiration. Arsenite, however had significant effect on both (Table 2).

Inhibitors of cytochrome oxidase in the mitochondrial electron transport system azide and cyanide, strongly inhibited oxygen uptake, But, even higher concentrations of these inhibitors did not inhibit germination appreciably ; only the germination sequence was slightly retarded. Diethyldithiocarbamate which inhibits copper containing oxidases did not have much effect on either germination or respiration.

DISCUSSION

Drechslera oryzae conidia with very high endogenous respiratory rate during germination are characteristically similar to those fungal spores which germinate upon activation of endogenous respiration in presence of water (Cochrane, 1966). Energy substrates are available and enzymes necessary for energy metabolism are activated very quickly in the germinating conidia of the fungus. The primary substrate(s) for germination was highly leachable—may be a soluble carbohydrate. Oku (1971) suggested that an alkali stable carbohydrate was probably the substrate for germination of the same fungus. But, an alkali stable carbohydrate fraction of spores should be of high molecular weight which is not expected to leach so esaily. It is possible that besides such reserve fraction(s), soluble and low molecular weight substances serve as primary substrates for germination. Under conditions of limiting availability or exhaustion of such preferential substrates, high molecular reserve fractions may be utilized.

The generally observed nutritional independence of germination of spores of such fungi may not be an absolute character. Leaching may be one of the many factors which might make them nutritionally dependent for germination in nature. Ko and Lockwood (1967) had previously shown a similar phenomenon in *Neurospora* and *Helminthosporium victoriae* conidia.

The results have shown that endogenous respiratory metabolism of germinating *D. oryzae* conidia proceeds through the normal E. M. P. sequence. The sequence is connected to the TCA cycle, only partial depression of which by arsenite caused germination inhibition. This would suggest that energy and intermediates produced by incomplete oxidation of carbohydrates by the E.M.P. sequence are not sufficient to sustain germination. Failure of malonate to inhibit either germination or respiration in this context may be due to impermeability as explained earlier for similar spores (Townsley and Bell, 1965). Inhibition of respiration by azide and cyanide indicated that the terminal electron transport in the germinating conidia is mediated through the normal mitochondrial cytochrome system. But, insensitivity of germination to the same while oxygen uptake is prevened, suggested the possibility of presence of some alternative system. Conclusion regarding participation of copper containing oxidases, like polyphenol oxidase in electron transport could not, however, be made from the data. Kuroda (1957) showed that conidia of the same fungus could germinate under complete anaerobic condition. Oku (1971) supported the observation from indirect evidences. These and the present presumtive evidences of inadequacy of glycolytic sequence alone and indispensibility of TCA cycle for germination would suggest the operation of an alternative electron transport mechanism orher than the cytochrome mediated

one. Although, electron transport in the germinating fungal spores is mostly mediated through the cytochrome system, presence of alternative mechanisms are also not discounted (Brambl, 1981). It is possible that endogenous energy metabolism in the germinating conidia of *D. oryzae* proceeds through the EMP-TCA and normal cytochrome dependent electron transport system. But an alternative electron transport mechanism not requiring molecular oxygen, may also operate or is activated under limiting conditions for the normal cytochrome dependent system.

REFERENCES

Brambl, R. (1981). Respiration and mitochondrial biogenesis during fungal spore germination, pp. 585-604. In G. Turian & H. R. Hohl (ed.) *The fungal spore-morphogenetic controls*, Acad. Press, London.

Cochrane, V. W. (1966). Respiration and spore germination, p. 210-215. In M. F. Madelin, (ed). *The fungus spore*, Butterworths, London.

Ko, W. and Lockwood J. L. (1967). Soil fungistasis : relation to fungal spore nutrition. *Phytopathology* **57** : 894-901.

Kuroda, S. (1957). The mode of respiration of *Cochliobolus miyabeanus* during conidial germination. *Ann. Phytopath. Soc.* (Japan) **22** : 88-92.

Oku, H. (1971). Physiological changes during spore germination, p. 90-106, In S. Akai and S. Ouchi (ed) *Morphological and biochemical events in plant parasite interaction*. Phytopath. Soc., Tokyo, Japan.

Townsley, W. M. and Bell, A. A. (1965). Respiratory metabolism of germinating conidia of *Curvularia geniculata*. *Univ. Maryland Agric. Exptl. Sta. Bull.* (A) **140** : 1-20.

Umbreitt, W. W., Burris, R. H. and Stauffer J. F. (1964). *Manometric Techniques*, Burgess Publishing Co., Minnesota, 305 p.