

METABOLIC REGULATION OF MORPHOGENESIS DURING GERMINATION OF *PHYTOPHTHORA* *INFESTANS* ZOOSPORANGIA

BY

GITASREE GHOSH (DUTTA) AND S. CHAUDHURI

Department of Plant Pathology, Bidhan Chandra

Krishi Viswavidyalaya, Kalyani-741 235,

Nadia, West Bengal

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Circumstantial evidence have shown that the phenomenon of temperature dependent differential morphogenesis during germination of *Phytophthora infestans* zoosporangia (zoosporangogenesis and germ tube differentiation at different temperatures) are regulated by different molecular mechanisms. Essential proteins for zoosporogenesis are synthesized during first 30 minutes of incubation on template activity of stored m-RNA. Synthesis of fresh m-RNA appeared necessary for continued synthesis of proteins in the zoosporangia for direct germ tube differentiation.

INTRODUCTION

Germination differentiation of zoosporangia of some *Phytophthora* spp. is dependent upon temperature. Crosier (1934) first observed that zoosporangia of *Phytophthora infestans* differentiate to zoospores at or below 15°C but at temperatures above 15°C zoosporangia directly differentiates to hyphae through germ tubes. Direct germ tube differentiation at elevated temperature or due to ageing has been reported for other species of *Phytophthora* as well (Vujicic and Colhoun, 1966; King *et al.*, 1968; Hemmes and Hohl, 1969). Metabolic regulation of such temperature dependent differential morphogenesis of germinating *P. infestans* zoosporangia is reported here.

MATERIALS AND METHODS

Zoosporangia of *Phytophthora infestans* were collected in glass distilled water from infected potato leaves obtained from experimental fields of the University. Infected leaves after surface sterilization with dilute hypochlorite solution were incubated in darkness for 16-18h in moist chambers at 15°C. Fresh sporangia formed at the periphery of the lesions were harvested in 4°C sterile water by

brushing with soft camel hair brush. Appropriate dilutions of sporangial suspension (5×10^3 - 1×10^4 /ml) were incubated in thin films on clean glass slides, in moist chambers, separately at $15 \pm 1^\circ\text{C}$ and $24 \pm 1^\circ\text{C}$ in presence of different concentrations of inhibitors of macromolecular synthesis. Data on zoospore and germ tube differentiation were recorded at 4 h and 8 h respectively from 15°C and 24°C incubated slides. Vacuolated zoosporangia were considered to have germinated by zoospore differentiation. Germ tubes equal to sporangial length indicated direct germination. In another set, cycloheximide ($50 \mu\text{g/ml}$) was added to sporangial suspension at intervals of 10 minutes between 0 to 90 minutes of germination incubation.

RESULTS

Both zoosporogenesis at 15°C and germ tube formation at 25°C was markedly inhibited by low concentrations of the antibiotic cycloheximide (Actidione) (Table 1). Considering 95% inhibition of germination as an index of sensitivity, zoosporogenesis was sensitive to cycloheximide only upto first 30 minutes of incubation. Sensitivity of direct germination at elevated temperature to the same

Table 1. Effect of inhibitors of macromolecular synthesis on germination of *phytophthora infestans* zoosporangia

Inhibitors	MIC ($\mu\text{g/ml}$ for 95% inhibition)	
	Zoosporogenesis (15°C)	Germ tube formation (24°C)
Cycloheximide	22.2	28.5
Streptomycin	68.4	76.2
Actinomycin D	160.0	160.0
Proflavin	83.2	32.4
Ethidium bromide	96.6	41.8
6-mercaptopurine	127.0	53.3

Based on log. probit analysis of percent inhibition data of 300 sporangial count for each treatment.

concentration of cycloheximide prolonged upto 55 minutes of incubation (Table 2). Streptomycin inhibited direct germination more strongly than zoosporogenesis. Proflavin and ethidium bromide strongly inhibited germ tube differentiation at higher temperature. Zoosporogenesis, on the other hand, was not affected equally. Concentrations required for inhibition of zoospore formation were relatively high. 6-mercaptopurine, the purine analogue had similar effects. Actinomycin D failed to have any significant effect on either type of germination.

Table 2. Effect of time lapse addition of cycloheximide on germination of *Phytophthora infestans* zoosporangia

Addition Time (minutes of incubation)	Percentage inhibition of	
	Zoosporogenesis (15°C)	Germ tube formation (24°C)
0	100	100
10	100	100
20	100	100
30	94.3	100
40	71.2	100
50	46.5	100
60	29.0	88.3
70	16.4	66.4
80	5.3	42.8
90	—	28.4

Based on germination count of minimum 300 zoosporangia at 4 h of incubation.

DISCUSSION

Molecular events during zoosporogenesis in lower fungi have not been investigated in much detail (Olson *et al.*, 1981). Limited evidences in this respect suggest that in some fungi like *Blastocladiella* and *Allomyces* (Burke *et al.*, 1972; Fahnrich, 1976), synthesis of necessary m-RNA for zoospore differentiation takes place early in the vegetative stage during sporangium/gametangium differentiation and protein synthesis occurs throughout sporogenesis on template activity of such stored m-RNA. In some other fungi like *Achlya* (Griffin and Breuker, 1969; Timberlake *et al.*, 1973) continued m-RNA and protein synthesis are necessary for zoospore formation. Molecular events during temperature dependent differential morphogenesis of *Phytophthora* zoosporangia during germination are interesting in this context.

Essentiality of continued protein synthesis for either zoosporogenesis or direct germ tube differentiation of *Phytophthora* zoosporangia as suggested by pronounced cycloheximide sensitivity of both, fits into the general pattern of molecular events of fungal spore germination. The inhibitory role of streptomycin on zoosporogenesis or germ tube formation needs further study for revealing the underlying mechanisms.

Insensitivity of zoospore differentiation at low temperature to relatively low concentrations of both proflavin and ethium bromide, suggests the possibility of presence of stored m-RNA for template activity for protein synthesis during zoosporogenesis. Higher concentrations of proflavin to which zoosporogenesis was sensitive is known to directly inhibit protein synthesis in similar fungi

(Hollomon, 1969). Sensitivity of direct differentiation of germ tubes to particularly very low concentrations of proflavin and ethium bromide suggested that such differentiation was dependent upon fresh m-RNA synthesis and different proteins were synthesized for and during direct germination on such new template activity. Essentiality of synthesis of some RNA species for direct germination was proved further by its high sensitivity to 6 mercaptopurine. Results with Actinomycin D were not conclusive as relatively high concentrations were necessary for inhibition of germ tube differentiation.

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